Extraction of amaranth starch from an aqueous medium using microfiltration

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PAUL GREGORY MIDDLEWOOD

Hamilton, New Zealand

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ABSTRACT

Amaranth starch granules are very small, which makes them suitable for a range of specialty applications. This starch is difficult to extract by wet milling due to the strong association between the starch and protein, the high protein content of the seed, and the small granule size. At the time of this research, no commercial amaranth starch extraction methods existed. The recently developed Al-Hakkak process has been successfully used to extract amaranth starch on a laboratory-scale. The work reported here forms part of the Al-Hakkak process scale-up investigations being performed by AgResearch Ltd, for the Biopolymer Network Ltd.

During the Al-Hakkak process, an aqueous stream (known as starch-milk) containing insoluble starch granules, soluble carbohydrates, soluble proteins, and lipids, is produced. On the laboratory-scale the starch is recovered from the starch-milk using a high-speed centrifuge. However, at pilot and industrial scales density-based processes, such as centrifuges, settling tables, or hydrocyclones, may not be practical due to the small size, and low mass, of the amaranth starch granules.

The research reported here investigated microfiltration as an alternative to density-based processes for separating the amaranth starch-milk into (i) a starch-rich concentrate and (ii) an aqueous stream containing the soluble proteins and carbohydrates. A Millipore ProFlux M12 Tangential Filtration System, fitted with a 1000 kDa regenerated cellulose membrane, was used as the experimental apparatus.

It was shown that microfiltration has the potential to recover amaranth starch from the starch-milk produced during the pilot-scale Al-Hakkak process. The selected membrane retained all the starch granules, but also retained more protein than desired (protein retention was 67 % and the starch-rich concentrate had a dry-basis protein content of 12 %). Diafiltration was used to decrease the protein content of the starch-rich concentrate and after six washes the protein content had stabilised at 4 %, which was significantly higher than the 0.1 %

previously reported for the laboratory-scale Al-Hakkak process. Analysis of the feed liquor, and diafiltered concentrate, revealed the presence of some non-starch insoluble material. This material, which may have been protein-based, was present in the starch-milk produced using the pilot-scale method but not the laboratory-scale method, and its presence determined the final protein content of the diafiltered concentrate.

During processing to reach steady-state conditions membrane flux declined from 60 to 15 L m^{-2} h^{-1} over the first 45 minutes. This decrease was predominantly caused by the soluble components of the feed stream, and to a lesser extent by the starch granules. During concentration, flux had a three stage relationship with volumetric concentration (VCF). During the first stage flux decreased almost linearly with increasing VCF, in the second stage flux increased with increasing VCF, and in the third stage flux was independent of VCF. The second stage (flux increase) is unusual, and could form the subject of a separate study.

The optimal transmembrane pressure was approximately 100 – 150 kPa, above which flux increased non-linearly with pressure. However, the flux-pressure relationship was weak, suggesting that higher operating pressures may be sustainable.

The membrane proved very difficult to clean. A multi-step cleaning cycle was developed which adequately cleaned the membrane between runs. Key cleaning steps were: a cold water rinse to remove loosely bound material, a protease wash to remove protein, a sodium hydroxide wash to “pre-treat” any remaining starch granules, an amylase wash to degrade the starch granules, and a final sodium hydroxide wash to remove residues from the previous step.

Additional research is needed to determine why the starch-milk from the pilot-scale process contains insoluble non-starch material, and to improve the process to prevent its inclusion, or remove it. Microfiltration should then be re-evaluated as a potential starch recovery process. An alternative membrane material, and larger pore size, should be trialled with the aim of decreasing protein retention and improving cleanability.

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NOMENCLATURE

Concentration, *C*, (% g g^{-1}). The mass concentration of a particular component in a particular stream.

C_F = Feed concentration, C_R = Retentate concentration, C_p = Permeate concentration.

Diavolume, *DV*, (unitless). A measure of the extent of washing that has been performed during a diafiltration step.

$$DV = \frac{Total\ buffer\ volume\ added}{Retentate\ volume}$$

Membrane area, *A*, (m^2). The area of the filtration surface of the membrane.

Permeate flow rate, Q_P, (L h^{-1}). The rate liquor is permeating the membrane.

Permeate Flux, *J*, (L m^{-2} h^{-1}). The permeate flow rate, normalised for the area of the membrane.

$$J = \frac{Q_P}{A}$$

Pressure, *P*, (kPa). P_F = Feed pressure, P_R = Retentate pressure, P_p = Permeate pressure.

Pressure drop, ΔP, (kPa). The difference in pressure along the feed channel of the membrane from the inlet to the outlet.

$$\Delta P = P_F - P_R$$

Product recovery, *PR*, (%). The fraction of the product in the feed that is recovered in the retentate at the end of a run.

$$PR = \frac{V_R . C_R}{V_F . C_F} \times 100$$

Retention, *R*, (unitless). The fraction of a particular feed component that is retained by the membrane. Retention is also called rejection.

$$R = 1 - \frac{C_P}{C_R}$$

Transmembrane Pressure, *TMP*, (kPa). The average applied pressure from the feed to the filtrate side of the membrane.

$$TMP = \frac{P_F - P_R}{2} - P_P$$

Volume, *V*, (L). V_F = Feed volume, V_R = Retentate volume, V_p = Permeate volume.

Volumetric concentration factor, *VCF*, (unitless). The amount the initial feed

stream has been reduced in volume.

$$VCF = \frac{Initial\ feed\ volume}{Current\ retentate\ volume}$$

1 INTRODUCTION

Starch is extracted from cereal grains for use in a large variety of both food and non-food applications. In food applications, as well as being a source of energy, starch is added for its functional properties which include: providing texture, gelatinisation, altering viscosity, stabilising emulsions, improving mouthfeel, film-forming, and adhesiveness. Non-food uses for starch include: as an ingredient in glues and adhesives, as a binding agent, for surface sizing in paper making, fabric finishing, and as a dusting powder and carrier in pharmaceuticals.

The two major components of starch are amylose and amylopectin, both of which are made up of glucose units linked together (Hoseney, 1994). The size of the starch granule, and the relative amounts of amylose and amylopectin, vary between plant species and affect many functional and physicochemical properties of the starch, and hence its potential uses. The majority of commercially available starches have a medium (10 – 25 μm) or large (> 25 μm) granule size, amaranth seed is one of the few sources of small granule starch. It is unique due to its very small (typically 1 – 3 μm micrometers in diameter) and regular granule size. At the time of this research, no commercial amaranth starch extraction methods exist.

The Al-Hakkak process (Al-Hakkak & Al-Hakkak, 2007) has been used to extract starch from amaranth seed on a laboratory-scale, and work is currently underway to scale-up this process. The Al-Hakkak process produces an intermediate product stream known as starch-milk, which contains the insoluble starch granules and other seed components such as water extractable carbohydrates, protein, and fat. On the laboratory-scale the starch is recovered from the starch-milk using a high-speed centrifuge. However, at pilot and commercial scales the use of density-based processes such as centrifuges, settling tables, or hydrocyclones may not be practical as the small granule size either reduces the efficiency of, or completely excludes, density-based separations (Coulson & Richardson, 1993).

An alternative to density-based separations is tangential flow filtration (TFF). To be technically viable in an industrial application a TFF process must achieve the

desired separation, perform the separation using a realistic membrane area, and any fouling that occurs must be removable. Unfortunately it cannot be assumed that selecting a membrane with an apparently suitable pore size will perform the desired separation since a membrane pore size, as stated by the manufacturer, is a guide only. Small-scale trials are recommended to confirm that the selected membrane achieves the desired separation because of the many factors that can affect the actual separation (Zeman & Zydney, 1996). A key parameter that determines the required membrane area is the permeate flow rate, which is dependent on a number of factors. At present no mathematical models exist that can accurately predict, from first principles, the TFF performance of complex solutions. Therefore, data must to be collected to determine the membrane selectivity, permeate flow rate, and optimal operating conditions.

Membrane fouling, which causes a reduction in permeate flow rate, can be a significant problem in TFF processes. The degree of fouling is very difficult to predict as it is the result of specific interactions between the membrane and various solutes in the feed stream, and sometimes between the adsorbed solute and other solutes in the feed. Membrane cleaning has been described as being more of an art than a science (Liu et al., 2006). Although the initial selection of cleaning agents can be based on the composition of the feed stream, trial work is usually required to determine the optimal cleaning conditions and, if multiple cleaning steps are required, the best order of applying the various cleaning steps.

The objective of this research was to perform a preliminary assessment of the suitability of using TFF for recovering amaranth starch from the aqueous starch-milk produced by the pilot-scale Al-Hakkak process. The key goals were to:

- Propose a suitable membrane, and determine whether it retained the starch granules while passing the soluble carbohydrates and proteins.
- Determine the permeate flow rate, and its relationship with operating conditions and liquor concentration.
- Investigate the severity of membrane fouling, and identify the main fouling mechanism.
- Assess how well the selected membrane could be cleaned.

2 BACKGROUND

2.1 Amaranth

2.1.1 Introduction

Amaranth is a seed bearing plant that has been grown as a food source for over 4000 years (Wilhelm et al., 2002). More than sixty species of amaranth exist. These are classified into four varieties: grain, wild leaf, ornamental, and weed (Belton & Taylor, 2002). The grain varieties are considered pseudocereals (true cereals are grasses) as they yield high amounts of small, starch-rich, seeds that can be used in the same way as the grain from true cereals. Although the term "amaranth grain" is often used, technically, amaranth produces a seed and not a grain.

Amaranth seeds are lentil-shaped (Figure 2.1), and compared to cereal grains, they are very small (approximately 1 mm diameter) and light (1000 seeds weigh 0.5 – 1.0 g) (Belton & Taylor, 2002).

Figure 2.1. SEM image of an amaranth seed.

Like all other grains and seeds, amaranth seeds have three basic anatomical parts: a seed coat (or bran) to protect the seed from the outside environment, an embryo (or germ) which will grow into a new plant, and food storage tissue to nourish the growing embryo (Hoseney, 1994). In amaranth the main food storage tissue is the perisperm, while in grains (e.g. wheat) the main food storage tissue is the endosperm; both are composed of starch granules embedded in a protein matrix.

The location and relative size of the bran, germ, and perisperm of an amaranth seed are shown in Figure 2.2. The seed coat is thin, while the germ is relatively large (accounts for approximately 25 % of the seed weight) and forms a ring that surrounds the perisperm (Belton & Taylor, 2002).

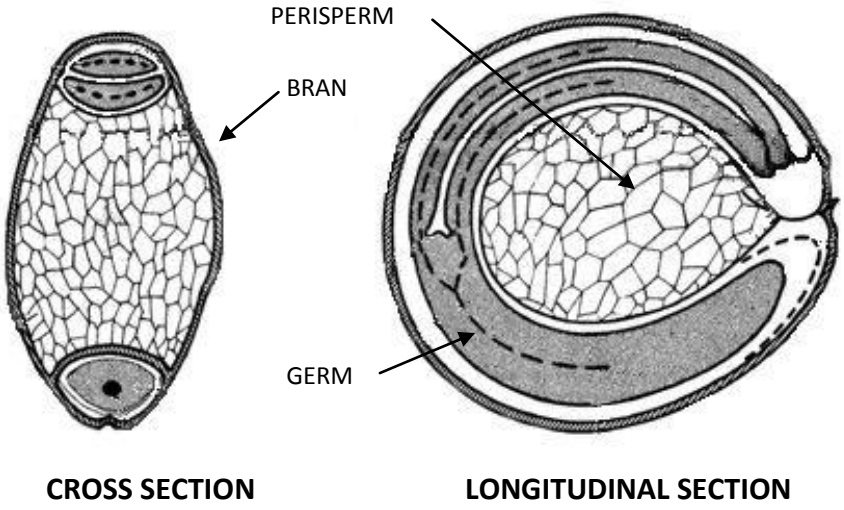

Figure 2.2. Diagram of longitudinal and cross sections of an amaranth seed.

(Source: Picture adapted from Irving, 1981, pp. 1171, used by permission).

2.1.2 Seed Composition

Starch is the main component of the seed. It is mainly located in the perisperm, where it is present as very small granules embedded in a protein matrix (Belton & Taylor, 2002). Depending on variety, the amount of starch varies from 48 % to 69 % (Resio et al., 2009), and the average starch granule diameter ranges from 1 to 3 µm (Wilhelm et al., 2002). Amaranth starch granules are polygonal in shape and very uniform (Figure 2.3). As well as starch, small amounts of low molecular

weight carbohydrates are also present. These include sucrose (1.08 – 2.26 %), raffinose (0.45 – 1.23 %), stachyose (0.02 – 0.15 %), and maltose (0.02 – 0.36 %). Their concentrations vary with variety.

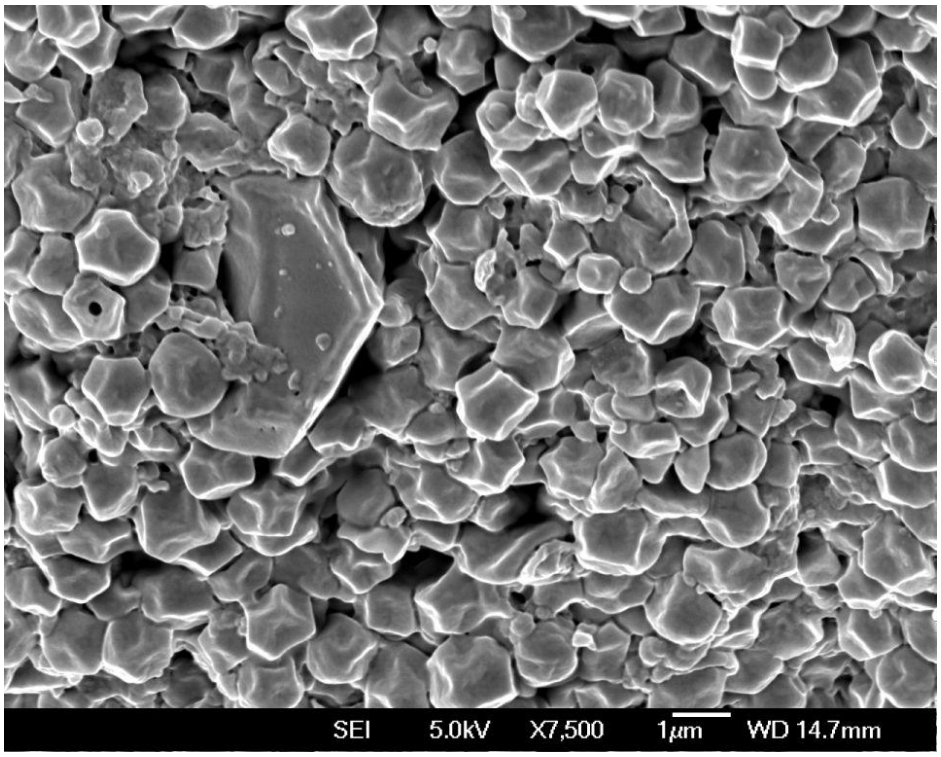

Figure 2.3. SEM image of amaranth starch granules.

The protein content of amaranth seed ranges from 11.7 to 18.4 % (Belton & Taylor, 2002). Of this, 65 % is located in the germ and bran, and 35 % in the perisperm. Bressani & Garcia-Vela (1990) used the Osborne classification system to test three different amaranth grain species and found that, on average, the proteins were comprised of 20.7 % albumins (water soluble), 19.2 % globulins (soluble in dilute salt solutions), 2.2 % prolamines (soluble in 70% ethanol), 44.4 % glutelins (soluble in dilute acids or bases), with 13.4 % residue (not extracted). Further information on amaranth seed protein classification, including amino acid content, has been presented by Marcone et al. (1994).

Amaranth seeds also contain 5 – 7 % fat, 3 – 4 % minerals (Burisova et al., 2000) and 9 – 16 % dietary fibre (Tosi et al., 2001).

The average composition of seed from three amaranth varieties is presented in Table 2.1.

Table 2.1. Chemical composition of amaranth seed (A. caudatus, A. cruentus, A. hypochondriacus).

Parameter	*A. caudatus*	*A. cruentus*	*A. hypochondriacus*
Protein (%)	14.7	15.5	15.9
Fat (%)	8.1	7.7	6.1
Starch (%)	63.9	58.3	62.4
Crude fibre (%)	3.7	4.4	5.0
Dietary fibre (%)	8.1	16.3	No data
Ash (%)	3.2	3.3	3.3

(Source: Belton & Taylor, 2002)

2.1.3 Modern Uses

Amaranth has become a subject of renewed interest due to the nutritional value of its seed, and the potential for using the various seed components (especially starch and proteins) as functional ingredients in both food, and non-food (e.g. cosmetic) applications.

Nutritionally, amaranth seed has a higher protein content, higher digestibility, higher protein utilisation, and a higher protein efficiency ratio than traditional cereals such as corn and wheat (Salcedo-Chavez et al., 2009). The protein has an amino acid profile that is well balanced, approximates the World Health Organisation standard protein, and includes lysine (an essential amino acid that most cereals lack, or have in small amounts) (Bressani & Garcia-Vela, 1990). Amaranth does not contain gluten, which makes it a suitable food for those with celiac disease. Amaranth seeds are also a good source of dietary fibre (Tosi et al., 2001, and Repo-Carrasco-Valencia et al., 2009).

Outside of nutrition, research has focused on investigating the properties, isolation methods, and uses, of amaranth starch, proteins, and oil.

Amaranth starch granules are smaller than the commercially produced starches (see Table 2.2) and, since many starch physicochemical properties are

determined by granule size (Lindeboom et al., 2004), have the potential to be used in different applications. Physicochemical properties influenced by granule size include: gelatinization properties, pasting properties, enzyme susceptibility, crystallinity, swelling, and solubility. Potential uses for small granule starches have been reviewed by Lindeboom et al. (2004) and include: as a fat replacer in food, a component in biodegradable films and plastic sheets, as a binder with orally active ingredients, and as a carrier material in cosmetics. Small granule starches may also have potential use as thin coatings in the cosmetics, paper, textile, and photographic industries (Lindeboom et al., 2004). Wilhelm et al. (2002) noted that freeze drying in water caused no harm to the native starch structure, indicating high stability of the granule structure, which may open new fields of application. Amaranth starch also has good freeze-thaw stability, making it suitable for use in frozen foods (Bello-Perez et al., 1998), and good resistance to mechanical shear.

Table 2.2. Granule size of different starches.

Source	Granule diameter
Amaranth	1 – 3 μm
Barley	Bimodal, 20 – 25 μm and 2 – 6 μm
Corn	15 μm
Oats	3 – 10 μm
Rice	3 – 8 μm
Sorghum	25 μm
Wheat	Bimodal, 20 – 35 μm and 2 – 10 μm

(Source: Hoseney, 1994)

A number of researchers have investigated the properties, and uses, of amaranth seed proteins. Fidantis & Doxastakis (2001) found that amaranth protein isolates act as effective foaming agents and enhance emulsion stability. Konisihi & Yoshimoto (1989) suggested that, as some amaranth proteins have excellent heat-stable emulsification properties, they have potential uses in thermally processed foods.

Amaranth oil can be extracted using hexane (Lyon & Becker, 1987) and supercritical carbon dioxide (Westerman et al., 2006). The oil is of interest as it is high in squalene, which is an important cosmetic ingredient due to its photo-protective role, and it has been suggested to have other health promoting attributes including decreasing the risk to certain cancers, and decreasing serum cholesterol levels (He et al., 2002).

2.2 Starch Extraction from Grains and Seeds

2.2.1 Overview

Grains can be separated into their anatomical parts (bran, germ, and endosperm) by dry milling. Wet milling goes a step further and separates a grain into its chemical components; starch and protein (Hoseney, 1994). Traditional wet milling is used to extract starch from most commercial grains except wheat. The dough process, or similar batter processes, are preferred for commercial wheat-starch production as they offer two advantages; firstly, wheat gluten is produced as a co-product, and secondly, the complications caused by the tendency of the wheat gluten proteins to agglomerate during traditional wet milling are avoided (Van Der Borght et al., 2005).

2.2.2 Wet Milling

A very basic overview of the wet milling process is presented here, a more detailed description is provided by Hoseney (1994) and Sayaslan (2004).

In wet milling, grain is milled in the presence of water to release the starch, which is subsequently recovered from the water, purified, and dried. Similar to dry milling, the first step is to separate the bran, germ, and endosperm. In some cases the grain is conditioned before wet milling to improve separation. For example, corn is conditioned by steeping in a 0.1 – 0.2 % sulphur dioxide solution. Steeping alters the characteristics of the corn proteins, making the grains softer, and the germ swollen and rubbery; this improves separation and the release of starch from the protein matrix (Hoseney, 1994). Once broken away from the bran and germ, the endosperm is recovered by density or size-based

processes, and then ground in water to extract the starch and protein. With some grains (e.g. rice) the protein-starch association is very strong and chemical treatments (e.g. soaking in sodium hydroxide) are needed to solubilise the protein to allow the release of starch (Hoseney, 1994). To obtain a pure starch product, the starch and protein must be separated i.e. the aqueous stream containing the starch and protein is split into two streams, one starch-rich and the other protein-rich. In commercial processes this task is performed by hydrocyclones, centrifuges, or settling tables, all of which utilise the density difference between the starch and protein. To further purify the starch, several washing steps are used which involve adding water to the starch and then using hydrocyclones to separate the clean starch from the wash-water.

2.2.3 Dough Process

The dough process (and related processes such as the batter process) take advantage of the agglomerate forming ability of wheat gluten proteins to form a matrix which binds the proteins together and allows the starch to be washed out (Van Der borght et al., 2005).

In the dough process (also known as the Martin and dough-ball process) wheat flour is made into a stiff dough which is allowed to rest and form a gluten matrix. The dough is then kneaded with added water, which washes out the starch and other water-extractable components. The gluten matrix remains in large pieces (relative to the starch) and is typically recovered by gyrating sieves. The liquor containing the starch and water-extractable components passes through the sieves while the gluten matrix is retained (Sayaslan, 2004). The starch is separated from the other water-extractable components using the same density-based processes as wet milling (centrifuges, hyrocyclones, and settling tables). The starch is then washed (again density-based separation processes are used to remove the wash-water) and dried. The gluten matrix is washed and dried as a separate product stream. More detailed descriptions of the dough and other wheat starch extraction processes have been given by Van Der Borght et al. (2005) and Sayaslan (2004).

2.3 Amaranth Starch Extraction

Amaranth starch is difficult to extract by wet milling due to the strong association between the starch and protein (Zhao & Whistler, 1994), the high protein content of the seed, and the small starch granule size (Resio et al., 2009). A number of laboratory-scale wet milling methods have been developed to extract small amounts of amaranth starch. Most of these methods use either alkali treatment (Zhao & Whistler, 1994, Bello-Perez et al., 1993), or an enzyme treatment (Radosavljevic et al., 1998), to remove the protein from the starch. At the time of this research, no commercial amaranth starch extraction methods exist (Resio et al., 2009, Al-Hakkak & Al-Hakkak, 2007).

2.3.1 The Al-Hakkak Process

The Al-Hakkak process is a dough-based starch extraction method that has been developed to extract starch from plant materials that do not contain gluten (Al-Hakkak & Al-Hakkak, 2007). The innovative step in the Al-Hakkak process is the addition of vital wheat gluten; this enables a dough to be formed as the wheat gluten proteins form a protein matrix with some of the amaranth proteins. The Al-Hakkak process does not use alkaline conditions or enzymes that could denature the proteins; as a result the water soluble seed components (proteins and carbohydrates) remain a potential co-product.

An overview of the Al-Hakkak process follows; a more detailed description has been presented by Al-Hakkak & Al-Hakkak (2007). Amaranth flour (that has been sieved to remove the bran fraction), wheat gluten flour, salt, and water are mixed to form a stiff dough. The dough is allowed to rest so that the protein network has time to develop, and then the starch is washed out, purified, and dried. In the laboratory method, starch is extracted from the dough by adding water and gently hand-massaging the dough to release the starch; a total of six washes are used. The wash-water is filtered through a 20 µm screen to remove particulates, and then the filtrate is centrifuged to recover the starch. The starch pellet has a very thin top layer of proteinaceous material which is scraped off. In the pilot-scale process the dough is mechanically agitated in water to release the

starch; during this step the dough breaks into small fragments. The wash-water is screened through a 40 µm vibrating sieve which produces two streams. The material retained by the sieve (dough fragments) is returned to the mixing vessel for the next wash, while the starch-rich stream (known as starch-milk) passes through the sieve, ready for further processing to recover the starch.

2.4 Starch Extraction from Aqueous Process Streams

Regardless of the starch extraction method used (wet milling, dough, batter, or Al-Hakkak) an intermediate aqueous stream containing both starch and protein is produced – this stream needs to be processed to recover the starch in an acceptably pure (protein free) form.

Traditional starch-protein separation methods rely on density-based processes such as settling tables, hydrocyclones, and centrifuges.

2.4.1 Settling

Preliminary investigations (MacManus & Macdonald, 2009) have shown that the starch-milk from the Al-Hakkak process settles very slowly (0.44 – 0.85 mm min^{-1}) and Stokes' law (Perry & Green, 1997) predicts the settling velocity of amaranth starch will be one to two orders of magnitude lower than that of corn starch or wheat starch. This suggests that settling tables would be impractical. Not only would a long settling time and/or large settling tables be required but, as the starch-milk is a nutritious growth medium, there is also a high chance microbial contamination would occur. Using settling additives (e.g. flocculants), or altering (e.g. acidifying) the starch-milk to improve settling are not desirable solutions as flocculating agents are an unwanted contaminant that may have to be removed later, whilst acidifying the starch-milk may reduce starch quality (MacManus & Macdonald, 2009).

2.4.2 Hydrocyclones

Hydrocyclones use centrifugal force to separate materials of different density. In brief, a solution is fed into the hydrocyclone at a high velocity and the geometry of the hydrocyclone creates a flow pattern that splits the feed into two streams.

The heavier material migrates to the outside of the hydrocyclone and exits the bottom of the hydrocyclone in what is termed the underflow, while the lighter material exits the top of the hydrocyclone in what is called the overflow (Coulson & Richardson, 1993). In commercial starch processes hydrocyclones are used instead of centrifuges as they are low cost, contain no moving parts, and result in faster separation (Lindeboom et al., 2004). However, hydrocyclones have a relatively low efficiency in recovering small granule starches (Lindeboom et al., 2004). The hydrocyclone diameter is chosen in relation to the particles that need to be separated; the smaller the particles the smaller the required diameter. But, there is a limit to what size particles can be separated using hydrocyclones; decreasing the diameter below 10 mm does not lead to the separation of finer particles (van Esch, 1991). Coulson & Richardson (1993) suggest that hydrocyclones will not be effective in removing particles smaller than 2 – 3 μm, and van Esch (1991) reported that hydrocyclones are not suitable for washing rice starch (3 – 8 μm) or wheat B starch (2 – 10 μm).

2.4.3 Centrifuges

There are many different types of centrifuge, but they all work on the principle of using a rapidly rotating chamber to subject the feed liquor to a high g-force. Centrifuges are expensive, their separation efficiency decreases with decreasing particle size (Ladisch, 2001), and a number of authors have suggested that tangential flow microfiltration could provide a means to replace or augment them in the starch industry (Rausch, 2002, Lutin et al., 2002).

2.5 Tangential Flow Filtration

2.5.1 Overview

Tangential flow filtration (TFF) offers a viable alternative to density-based separation processes. TFF is a pressure driven separation process that uses a semi-permeable membrane to separate components in a liquid solution, or suspension, based primarily on their size differences. Pressure is used to force the feed solution against a semi-permeable membrane; components smaller than the membrane pores pass through it in what is termed the permeate

stream, while components larger than the membrane pores are retained in the retentate stream. Although the primary basis for separation is size, the permeability of the membrane can be affected by the chemical, molecular or electrostatic properties of the feed and membrane (Zeman & Zydney, 1996).

TFF differs from normal dead-end filtration in that the feed liquor flows tangentially to the filter medium (or membrane) surface (see Figure 2.4). In conventional dead-end filtration liquor flow is perpendicular to the filter medium and retained particles continuously accumulate on the filter medium, forming a filter-cake. As the cake thickness increases, or if the cake compresses, the resistance to flow increases which can rapidly reduce the filtration rate to a near zero value (Coulson & Richardson, 1993). By maintaining a relatively high liquor flow tangential to the membrane surface (i.e. TFF) the build-up of solids on the membrane surface is greatly reduced as the liquor flow limits the amount of material that can accumulate. Reducing the build-up of material on the membrane reduces the resistance to filtration, enabling a higher, and more consistent, filtration rate. As the retentate flow is much higher than the permeate flow the retentate is continuously recycled across the membrane until the desired separation has occurred. To be recycled the retentate must remain pumpable. As a result, in instances where the feed contains particulate material, the concentrated solids take the form of a slurry rather than a solid cake.

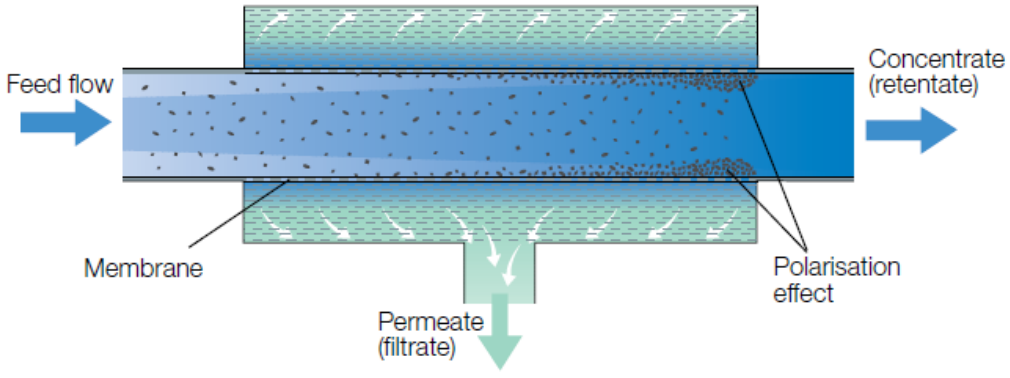

Figure 2.4. Tangential flow filtration schematic.

(Source: Dairy Processing Handbook, 1995, pp. 124)

Advantages of TFF over conventional filtration have been summarised by Coulson & Richardson (1993) and Matteson & Orr (1987) and include:

- TFF can be used on materials that are difficult, expensive, or impossible to separate using other methods. These materials may be finely dispersed solids, especially those that are compressible, or have a density close to that of the liquid phase, or biological materials which are sensitive to their physical and chemical environment.
- A higher overall liquid removal rate is achieved by preventing the formation of an extensive filter cake.
- The process feed remains in the form of a liquid concentrate, or mobile slurry, suitable for further processing.
- The solids content of the product slurry may be varied over a wide range.
- It may be possible to fractionate particles of different sizes.

2.5.2 Types of TFF

There are four types of TFF: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). Each is designed to operate over a different separation size range, as shown in Figure 2.5. Microfiltration generally refers to processes used to retain particles that measure 0.1 to 10 µm in diameter (e.g. colloidal particles, bacteria). Ultrafiltration is used to filter dissolved macromolecules, such as proteins, from solutions. Reverse osmosis is used for ionic separations, e.g. water purification applications in which the water molecules pass through the membrane but the contaminating ions do not (Matteson & Orr, 1987). Nanofiltration slots in between UF and RO i.e. separates solvent, monovalent salts, and small organics from divalent ions and larger species (van Reis & Zydney, 2007). There is some variation in the published separation limits of the four TFF process. For example, Matteson & Orr (1987) use 0.02 µm as the transition size from UF to MF, while Perry & Green (1997) uses 0.2 µm. In practice the processes have other differences (such as operating pressure) and their applications can overlap. For example, “loose” UF processes can overlap “tight” MF processes (see Figure 2.5).

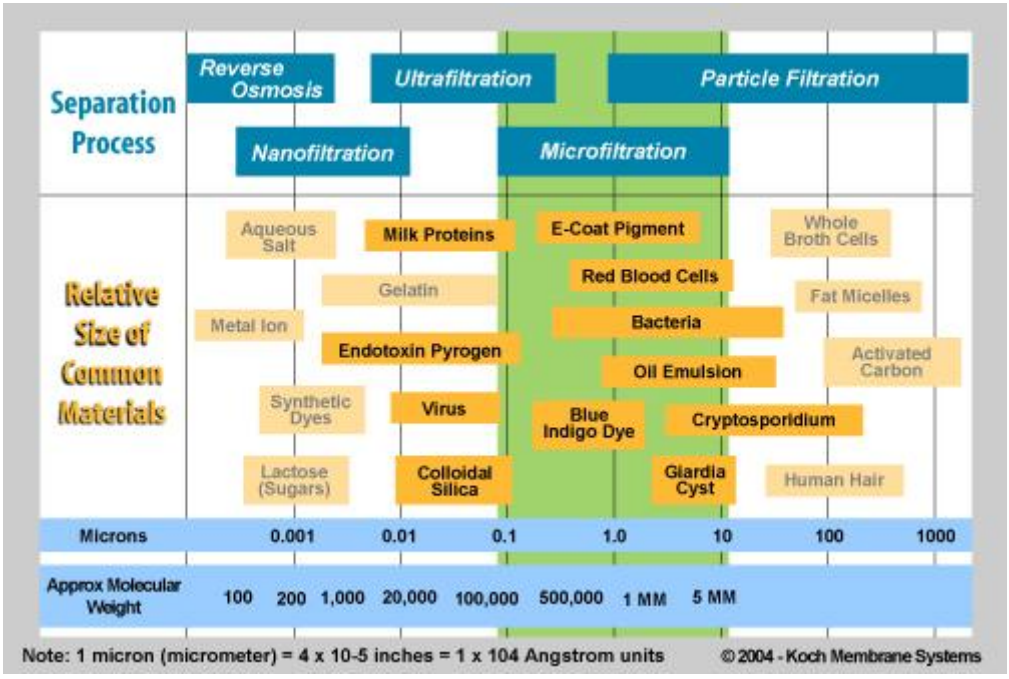

Figure 2.5. Tangential flow filtration separation processes.

(Source: open access, http://www.kochmembrane.com/sep_mf.html, 9 November 2009)

2.5.3 Applications

The three main applications of TFF are concentration, purification, and diafiltration. These applications are well summarized by Shuler & Kargi (2002). In concentration, the desired component is larger than the membrane pore size; during processing, water, and components smaller than the pore size, are removed thereby concentrating the desired (retained) component. In purification, the desired component is smaller than, and passes through, the membrane. Unwanted feed components are retained by the membrane and thus removed from the product stream. Diafiltration is a means to purify a product stream when the target material is larger than the membrane pores, and the unwanted material is smaller than the membrane pores. Basically, the process is run as per concentration, but the feed volume is kept constant by replacing the permeate with pure water (or another suitable solvent). As processing continues the unwanted material is washed through the membrane. Diafiltration is also used for buffer exchange, in which case the diafiltration water is replaced by the appropriate buffer solution.

2.5.4 Membranes

Key membrane properties include pore size, selectivity, permeability, mechanical strength, chemical resistance, fouling characteristics, capacity, and cost (Perry & Green, 1997). Most of these properties are determined by the membrane material and configuration.

The thickness of the membrane's selective layer is a major variable in determining the membrane permeability. In order to achieve maximum permeability, multi-layer membranes, consisting of a very thin selective layer (skin layer) attached to an open porous support structure, are used. This style of membrane is called an asymmetric membrane; the thickness of a typical skin layer is less than 1 µm, while the support structure thickness is typically 150 – 250 µm (Wagner, 2001).

The most common commercially available membrane materials are cellulose acetate (CA), polysulphone (PSO), polyvinylidenedifluoride (PVDF), ceramics, and sintered metal (Coulson & Richardson, 1993, and Wagner, 2001).

The chemical resistance, mechanical strength, and characteristics of the membrane pores vary widely with membrane material. Important pore-related properties that differ with material (and method of manufacture) include: open area, pore size distribution, and pore-path length and geometry. At one extreme are cellulose acetate membranes, which have a relatively open structure, large pore size distribution, and tortuous pores (i.e. a long and winding pore path). At the other extreme are track etched polycarbonate membranes which have less open area and a narrow pore size distribution (see Figure 2.6).

Each membrane material has its own advantages and disadvantages. For example, cellulose acetate is hydrophilic, which makes it less prone to fouling, however it has relatively poor resistance to high or low pH and high temperatures. PSO on the other hand, has good resistance to pH but does not tolerate oils and polar solvents (Wagner, 2001). In some instances, membranes can be surface modified to reduce fouling (Stopka et al., 1997), or increase chemical resistance (Singh et al., 2008, van Reis et al., 1999, Perry & Green, 1997).

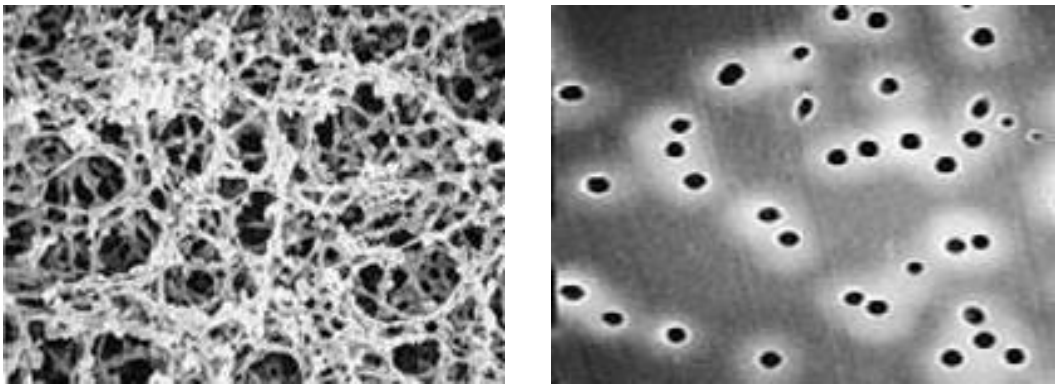

Figure 2.6. SEM images of a cellulose acetate membrane (left) and a track etched polycarbonate membrane (right).

(Source: open access, http://www.membrane-solutions.com, viewed 22 June 10)

Membranes are available in different configurations and come with various physical set-ups. Common configurations include flat sheets, spiral wound cartridges, hollow fibre tubes, and tubular membranes. The key physical parameters differ with membrane configuration but include: flow channel depth, flow path length, presence of turbulence promoters, and surface roughness. A more comprehensive list is provided by Wagner (2001). The configuration and physical set-up have a significant effect on process performance as they influence turbulence within the membrane, the maximum pressure that can be used, and the cross-flow rate per unit area of membrane.

Further information on membrane materials, properties, and configurations, has been presented by Wagner (2001), Perry & Green (1997), and Zeman & Zydney (1996).

2.5.5 TFF Process Performance

The three key indicators of TFF viability are retention, permeate flux, and membrane cleanability.

2.5.5.1 Retention

Membrane retention refers to the fraction of a particular component that is retained by the membrane. The retention at any point during the process can be calculated with the following formula:

$$R = 1 - \frac{C_P}{C_R} \qquad \textbf{Equation 2.1}$$

Where: R is the rejection coefficient, C_P the concentration in the permeate, and C_R the concentration in the retentate.

An additional equation, which takes the change in volume into consideration, is used to calculate the average rejection of a membrane. This equation is:

$$R_{av} = \frac{ln(C_f/C_o)}{ln(V_o/V_f)} \qquad \textbf{Equation 2.2}$$

Where: C_f is the final concentration, C_o the initial concentration, V_o the original process volume, and V_f the final process volume.

The ideal concentration or diafiltration process would have a product retention of 1 i.e. all the product would remain in the retentate. In contrast, in the ideal purification process the desired components would have a retention of 0, and the undesired components would have a retention of 1. Unfortunately, in practice retention usually falls somewhere between 0 and 1. Matteson & Orr (1987) and Coulson & Richardson (1993) list the main factors affecting retention as product lost by membrane fouling (see Section 2.5.5.5), and product passing through the membrane.

Reasons product may be lost through a membrane are: the membrane pore size is too large, the membrane pore size has a large size distribution, and the membrane has defects (holes).

In general, it is not possible to select the ideal membrane pore size based solely on the manufacturer's stated pore size. Membrane manufacturers label their membranes with a Nominal Molecular Weight Limit (NMWL), and not an actual pore size. NMWL is a number, expressed in Dalton for UF membranes and micron for MF membranes, indicating that 90 % of material larger than the NMWL will be retained by the membrane (Wagner, 2001). The stated NMWL of a membrane depends on the product on which the membrane was tested. This is because many component-specific characteristics (shape, ability to deform, interactions with other components, interactions with the membrane, etc) affect what molecules will pass through a membrane. Therefore, the stated NMWL must be considered a label rather than a specification, and trials need to be run

to determine the retention of a particular material through a particular membrane.

2.5.5.2 Permeate Flux

Permeate flux (also called filtrate flux, or flux) is the flow rate of the liquor permeating the membrane. The value is normalised to a standard membrane area, the symbol is *J*, and typical units are L m^{-2} h^{-1}.

Ideally, permeate flux during TFF microfiltration would be constant. However, practically all TFF processes exhibit a decrease in flux with time. This decrease varies widely in magnitude, from less than 10 % to greater than 80 % of the start-up flux (Perry & Green, 1997). The main reasons for this flux decrease, as stated by Zeman & Zydney (1996), are:

- Concentration polarisation resulting in the formation of a boundary, or gel, layer on the membrane surface.
- The accumulation of particles on the membrane surface.
- Membrane fouling.
- A combination of the above.

2.5.5.3 Concentration Polarisation

Concentration polarisation is common during ultrafiltration separations. It is less common, but can occur, during microfiltration separations (depending on the composition of the feed, and the characteristics of the membrane being used).

As fluid is drawn through the membrane, the solute concentration is elevated on the retentate side of the membrane surface (Figure 2.7). This local elevation in concentration can form a physical barrier to permeate flow, which is referred to as the boundary layer (Zeman & Zydney, 1996). If the concentration becomes high enough a gel layer can form at the membrane surface. The resistance of the gel layer is significantly greater than that of the membrane and flux becomes independent of membrane permeability and transmembrane pressure. The presence of a boundary layer can also increase membrane selectivity and alter retention, as the boundary layer acts as a second, tighter, membrane. The topic

of concentration polarization is covered in greater detail by Zeman & Zydney (1996), and Coulson & Richardson (1993).

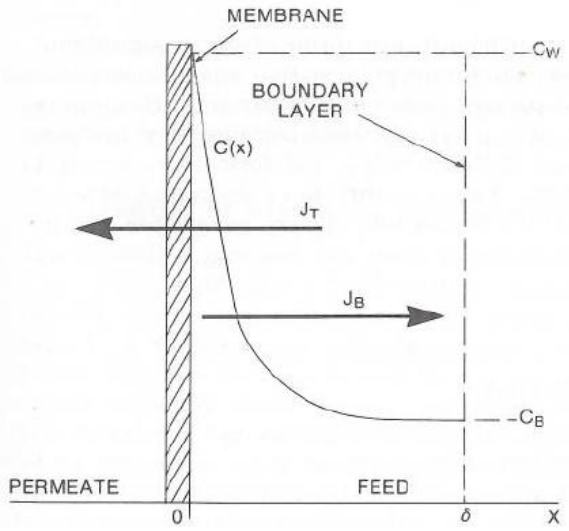

Figure 2.7. Schematic of a solute concentration profile in a boundary layer during ultrafiltration. C_W = solute concentration at the wall, C_B = solute concentration of the bulk solution, J_T = solute flux towards the membrane, and J_B = solute flux away from the membrane.

(Source: Matteson & Orr, 1987, pp. 429, used by permission)

2.5.5.4 Particle Accumulation

If the feed solution contains insoluble particles, which is the case in microfiltration applications, these particles accumulate at the membrane surface and form a layer, or cake, of material. The presence of this cake increases the total resistance to permeate flow, which reduces flux. Coulson & Richardson (1993) have described how the generalised cake filtration theory (as used in conventional filtration) can be manipulated for use in calculating flux under these circumstances. The resulting equation predicts that a steady state (constant flux) should be achieved; more often than not this is not the case as fouling, and or boundary layer effects, are also occurring.

During microfiltration, the layer of caked particles is analogous to the gel layer in ultrafiltration. In theory, the cake thickness reaches equilibrium when the rate of particle back-transport equals the rate of particle deposition. Three models are commonly used to predict membrane flux, these are: Brownian diffusion, shear-induced diffusion, and inertial lift. These models have been reviewed in depth by Davis (1992), who concluded that Brownian diffusion is important for submicron

particles, inertial lift is important for particles larger than ten micron, and shear-induced diffusion is dominant for intermediate sized particles.

2.5.5.5 Fouling

One of the major drawbacks of TFF is membrane fouling. This has been described by Matteson & Orr (1987) as “the accumulation, entrapment, or adsorption of material on the membrane”. Fouling manifests as a reduction in flux, which can continue until the flux is so low the process must be stopped and the membrane cleaned or replaced. In some cases, the foulant also acts as a secondary membrane and alters the selectivity of the overall process i.e. the foulant layer retains material that the membrane would pass.

There is some debate over what does, and does not constitute fouling. Some authors (Matteson & Orr, 1987) include the presence of a gel layer, and or caked material, as fouling; others (Perry & Green, 1997) divide fouling into reversible and irreversible, where the gel layer and cake are considered reversible fouling. Zeman & Zydney (1996) do not consider the gel layer, or cake, as a foulant, as it can be prevented, or removed, by altering process conditions.

Fouling can be further described as either internal or external (Ousman & Bennasar, 1995). External fouling is the accumulation of rejected material on the top surface of the membrane, during which the pore entrances become totally or partially obstructed. Internal fouling occurs when small particles or macromolecules deposit or adsorb within the internal pore structure (Figure 2.8). Adsorption refers to molecules in direct contact with the membrane, while deposition refers to all material forming a cake on the membrane due to protein-protein interactions, convection driven sieving, and further growth of the initial adsorption layers (D’Souza & Mawson, 2005). Internal and external fouling are both undesirable, and often occur together.

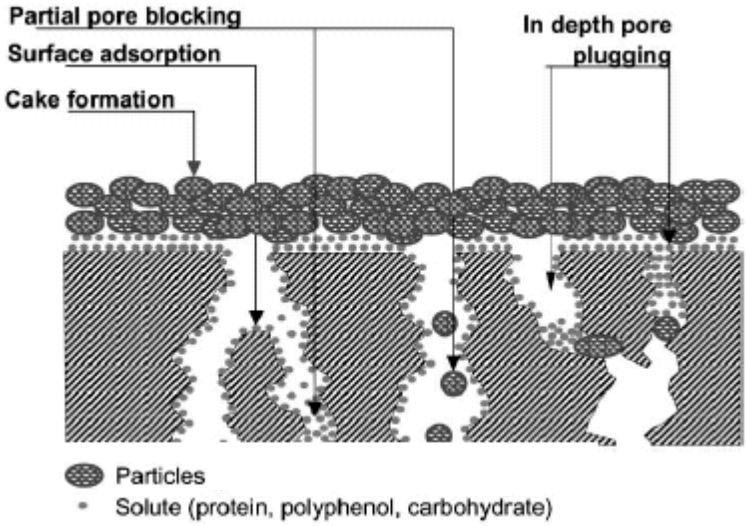

Figure 2.8. Diagram of the various fouling methods.

(Source: Fillaudeau & Carrère, 2002, pp. 42, used by permission)

Two different models can be used to describe internal fouling. The first, known as the standard blocking model (SBM), assumes that flux reduction is due to the effective pore radius being reduced by protein adsorption. The second, called the pore blocking model (PBM), assumes flux decline is caused by complete blocking of some of the pores. External fouling can be described by the cake filtration model (CFM), which assumes cake resistance increases with time due to the deposition of rejected material on the membrane surface (Guell & Davis, 1996). It is possible to distinguish between internal and external fouling by observing the slope of resistance versus time; the CFM model (external fouling) yields a total resistance versus time curve that increases with decreasing gradient. Whereas for both the SBM and PBM (internal fouling) the total resistance increases with increasing gradient (Guell & Davis, 1996, and Zeman & Zydney, 1996).

The flux decline of microporous membranes can generally be described by the following equation (Jonsson et al., 1996):

$$J(t) = \frac{J_0}{(1+\alpha t)^n} \qquad \textbf{Equation 2.3}$$

Where: $J(t)$ is the flux at time t, J_0 is the initial flux (t = 0), α is a system characteristic constant, and n has different values for each fouling

mechanism (1 for surface pore blocking, 2 for internal fouling, and 0.5 when a solute gel layer or cake is present).

Fouling is very difficult to predict as it is the result of specific interactions between the membrane and various solutes in the feed stream, and sometimes between the adsorbed solute and other solutes in the feed stream. The “foulant” is typically a sparingly soluble, hydrophobic or surface-active component, sometimes with a specific affinity for the membrane, and often only present at very low levels in the feed solution (Zeman & Zydney, 1996). In UF and MF applications, proteins and polysaccharides are well known to cause membrane fouling, even though they are much smaller than the typical microfiltration membrane pore size (Guell & Davis, 1996). Operating parameters (cross-flow velocity, pressure, and temperature) can also influence the rate and severity of fouling.

The generally accepted mechanism of UF and MF fouling, when processing solutions containing proteins, starts with an initial rapid adsorption of protein at the membrane surface, which provides an ideal surface for the further adsorption of proteins and other material (e.g. lipids and polysaccharides). As a result a multilayer adsorption occurs, which, with each successive layer, increases the thickness and resistance of the foulant.

Much work has been undertaken attempting to prevent, minimise, and predict membrane fouling. Methods of reducing or preventing fouling include the use of charged membranes, modifying membrane materials, changes to the operational mode (e.g. increasing cross-flow or reducing TMP), and pre-treatment of the feed by pH adjustment, sequestering agents, coagulation, or pre-filtration. Wagner (2001) provides good coverage of various pre-treatments and operational modes, van Reis et al. (1999) discusses the use of charged membranes, and various authors report on backflushing using air (Qaisrani & Samhaber, 2008), micro pulsing (Williams & Wakeman, 2000), and ultrasound (Chai et al., 1998).

2.5.6 Effect of Operating Conditions on Flux

A number of operating conditions have a large influence on flux.

2.5.6.1 Transmembrane Pressure (TMP)

During tangential flow microfiltration processes a characteristic flux-TMP relationship usually exists. Initially flux increases linearly with TMP, but as TMP is further increased the increase in flux reduces, and in some cases (e.g. if the cake is compressible) flux may even decrease. The ideal operating TMP is at the end of the linear section. Due to the complexities of microfiltration systems this relationship, and the ideal operating pressure, can only be determined experimentally.

2.5.6.2 Cross-flow Velocity

The accumulation of particulate material, and boundary layer formation, at the membrane surface are affected by the cross-flow velocity. Increasing the cross-flow velocity reduces the thickness of these layers, which gives an increased flux (at a given TMP). However, the relationship is often not linear and must be determined experimentally. Limitations to the maximum TMP exist due to equipment limitations, and the shear sensitivity of the feed (Zeman & Zydney, 1996).

2.5.7 Membrane Cleaning

2.5.7.1 Introduction

As discussed in Section 2.5.5.5 *(Fouling)*, all membranes foul during operation resulting in loss of performance. Membrane cleaning is needed to restore this lost performance. Within the industry there are different definitions of “clean”; a physically clean membrane is free of foreign matter (providing adequate flux and separation in subsequent batches), a chemically clean membrane is free of residues that could contaminate subsequent batches of product, while a biologically clean membrane has an acceptably low microbial load (D’Souza & Mawson, 2005).

Membrane cleaning has been described as being as much an art as a science, with the optimal cleaning cycle often determined in large part by trial and error (Zeman & Zydney, 1996, Liu et al., 2006). This is because developing an efficient cleaning cycle requires knowledge of the foulant, membrane material, and the exact interactions causing the fouling. These interactions are often unknown or not fully understood. The key foulant may only be present in low levels in the feed solution, and may initially be overlooked as a potential foulant. In addition, more than one type of foulant, and fouling mechanism, often occur simultaneously, requiring a multi-step cleaning process to systematically remove the individual foulants. In many of these cases, not only is the choice of cleaning agents critical, but so also is the order in which they are applied and the conditions used e.g. flowrate, TMP, temperature, time, concentration. For example, membranes fouled by high-calcium whey are more thoroughly cleaned by an acid wash followed by a caustic wash, than by a caustic wash followed by an acid wash (McCray & Glater, 1985, as sited by Zeman & Zydney, 1996).

2.5.7.2 Cleaning Requirements

The ideal cleaning cycle would fully restore the membrane flux, leave the membrane with acceptably low levels or microbes and residual chemicals, be cost effective (not require expensive reagents), be fast (minimising process downtime), not use conditions that shorten the membrane life, and not use environmentally unfriendly cleaning materials.

2.5.7.3 Assessing Membrane Cleanness

Membrane cleanliness is usually assessed indirectly by evaluating the water flux after cleaning, the rinse water composition and/or appearance, or the flux during the subsequent production run. The most common technique is to measure the pure water flux before the process and compare it to the pure water flux after the membrane has been cleaned (D'Souza & Mawson, 2005).

2.5.7.4 Cleaning Frequency

The optimal cleaning frequency is diverse. In some applications membranes are only cleaned once flux drops below an unacceptable value (which can take from

a few hours to a number of months, e.g. water purification), in other applications membranes are routinely cleaned on a daily basis, and in remote cases the membranes are not cleaned at all, but are used once and then disposed of. This expensive option is only seen in the pharmaceutical industry (Rathore et al., 2007) where the product selling price can absorb the replacement cost of the membranes, when the cost of validating that the membrane has been properly cleaned is relatively high, or when the risk of product failure, or recall, due to an improperly cleaned membrane is severe.

2.5.7.5 Cleaning Methods

Physical Cleaning

Membranes can be cleaned by physical or chemical means, or a combination of both. The simplest form of physical cleaning is to remove the TMP and recirculate the feed stream. This type of cleaning is only effective on very loosely adhered foulants. For more strongly adhered foulants the process liquor is replaced with a rinse solution which is recirculated at a high flow rate (so there is a higher shear force acting on the foulant) and zero TMP (to prevent the redeposition of material on the membrane). The next level of physical cleaning is backflushing; this involves forcing the permeate, or a rinse/cleaning solution, through the membrane in the opposite direction to normal permeate flow i.e. a negative TMP is applied. Backflushing can be performed periodically during the actual filtration process, or performed separately as part of a stand-alone cleaning cycle. Not all membranes can be backflushed without damage e.g. delaminating of the membrane from the support material. Some membranes, e.g. those with tubular modules, can be physically cleaned by forcing sponge rubber balls through the tubes. This type of cleaning can remove material from the surface of the membrane but cannot remove material from within the membrane pores.

In general, physical cleaning alone is not satisfactory so it is supplemented with one or more chemical cleaning steps.

Chemical Cleaning

Depending on the foulant, many different chemicals can be used to clean membranes. When choosing a cleaning solution, the decision should not be made solely on the type of foulant; the compatibility of the membrane with the cleaning solution, and the required temperature and chemical strength must also be considered (Williams, 2000).

Chemical cleaning agents remove foulants by one, or a combination, of the following mechanisms:

- Displacement of the foulants (e.g. by competitive adsorption of appropriate surface-active agents).
- Solubilisation of the foulants (e.g. dispersing, emulsifying, hydrolysing).
- Chemical modification of the foulant (e.g. oxidation of protein, saponification of oils, chelation of divalent cations).

The main types of chemical cleaners, their method of operation, and the type(s) of foulant they remove are summarised in Table 2.3.

While single component cleaners can be used, in many cases using a combination of cleaners has a better effect than using the same cleaners individually. For example, adding Triton X100 to a solution of sodium hydroxide reduces the required cleaning time by improving the penetration of the cleaning solution (Chen & Ko, 1997). Similarly, chlorine has been shown to improve sodium hydroxide performance when removing proteins and organic matter. The chlorine reacts with the foulant layer making it more porous, which enables deeper and faster penetration by the hydroxide (Chen & Ko, 1997). Other common combinations include urea-SDS, and various blends of enzyme-chelation-dispersant (Whittaker et al., 1984). Most industrially available membrane cleaners are formulated from several cleaning solutions and typically consist of a mixture of alkalis, phosphates, sequestering agents, and surfactants (D'Souza & Mawson, 2005).

Table 2.3. Overview of different cleaning agents.

Chemical	Foulant	Method of operation
Alkalis e.g. NaOH	• Protein • Fats and oils • Acidic material (e.g. fatty acids, humic acid • Colloidal material	• Hydrolysis • Saponification • Neutralisation • Dispersion/emulsification
Acids e.g. Nitric	• Calcium • Metal oxides • Some proteins	• Foulant is dissolved by the acid to form a soluble salt • Some acids have a chelating ability • Hydrolysis
Surfactants e.g. Triton X100	• Fats and oils • Organic foulants	• Displacing foulants from the membrane surface • Emulsifying • Solubilising hydrophobic foulants
Sequestrants e.g. EDTA	• Mineral deposits	• Chelating
Enzymes e.g. Protease	• Protein • Starch • Fats / oils • Cellulose	• Hydrolysis • Hydrolysis • Esterification, hydrolysis • Hydrolysis
Oxidiser e.g. Chlorine	• Protein	• Oxidising
Chaotropic e.g. Urea	• Protein	• Denature and solubilise

Cleaning Conditions

The conditions under which the cleaning solutions are used can have a significant effect on cleaning effectiveness. Increasing the temperature gives better cleaning by improving diffusion, increasing the solubility of foulants and cleaning agents, increasing reaction rates, and melting fats. Although the upper temperature is limited by the membrane material, and the optimum

temperature for cleaning is likely to depend upon the foulant, D'Souza & Mawson (2005) suggest that a temperature of 50 – 55°C should be used for chemical cleaning involving protein fouling; above this temperature there is a change in the nature of the foulant making it less amenable to loosening and breaking up. The pH of the cleaning solution can also influence cleaning effectiveness, particularly with proteins whose charge, solubility, and structure, can alter with pH. For example, some proteins (e.g. albumin) are more easily removed if the pH is at their isoelectric point (Zeman & Zydney, 1996). Again, the membrane material may limit the pH range that can be used during cleaning. Increasing the cross-flow velocity during cleaning will increase the shear forces acting on the foulants and increase turbulence; thereby improving soil dispersion and the cleaning solutions' soil carrying properties. A low (preferably zero) TMP during cleaning prevents material being redeposited on the membrane, or forced into the membrane pores. In practice, the amount that cross-flow can be increased is limited by pump capacity and membrane pressure limits; as pressure drop increases with cross-flow velocity, a trade-off is sometimes needed between maintaining a low TMP and having a high cross-flow velocity.

2.5.8 General Membrane Equation

No mathematical models that allow prediction, from first principles, of membrane flux or solute rejection, for a real microfiltration separation presently exist. As stated by Coulson & Richardson (1993), the physical properties of the membrane and solute are too complex for such analysis.

Zeman & Andrew (1996) provide a detailed review of the various models used to predict flux through a microfiltration membrane. While these models explain the individual mechanisms that occur (viscous flow, diffusion, mass transfer), none manage to combine all the mechanisms and accurately predict the performance of a real process where the feed stream is a multi-component biological solution.

The general membrane equation is used to state factors that may be important in determining flux for a pressure driven membrane system. The equation, also known as the resistance-in-series model, is given by:

$$J = \frac{|\Delta P| - |\Delta \Pi|}{(R_m + R_c + R_f)\mu}$$ **Equation 2.4**

Where: J is the membrane permeation rate (or flux, $m^3\ m^{-2}\ s^{-1}$), ΔP the pressure difference across the membrane (TMP, Pa), $\Delta \Pi$ the difference in osmotic pressure across the membrane (Pa); this usually negligible during microfiltration processes, R_m the resistance of the membrane, R_c the resistance of material deposited on the membrane, R_f the resistance of the film layer (all resistances m^{-1}), and μ the viscosity of the permeate (Pa s).

This equation is useful for illustrating what factors contribute to membrane flux; however, its limitation is that the resistance values are not readily calculable.

2.6 Starch Microfiltration

Literature considered relevant to this project includes (i) research relating specifically to the microfiltration of starch granules, (ii) investigations into the microfiltration of suspensions in general, (iii) research into the microfiltration of similar feed streams.

Research into starch microfiltration and ultrafiltration falls into one of four areas:

- Using starch granules as a model foulant to develop models for predicting flux (e.g. Ousman & Bennasar, 1995, and Lee et al., 2004).
- As a means to replace conventional commercial starch granule separation processes (e.g. Hinkova et al., 2005, and Shukla et al., 2000).
- A method of processing starch hydrolysates and syrups (e.g. Singh & Cheryan, 1998, and Amar-Rekik et al., 1994).
- Concentrating effluents from starch processing plants to produce a starch and protein rich concentrate suitable for use as an animal feed, fermentation medium, or for human consumption (e.g. Boykin et al., 2005).

While research has been performed into using microfiltration to process starch from wheat, corn, and rice starch, only a single reference to the microfiltration of amaranth starch was found. In this work Hinkova et al. (2005) investigated the

suitability of an inorganic membrane for the purification and concentration of three different food based materials, one of which was amaranth starch. The feed material was a suspension containing 3 % starch and a maximum of 3 % protein (no detailed information on the protein was given). The membrane was tubular ceramic-alumina with a pore size of 0.1 µm, and operated at 40˚C, 5 m s^{-1} cross-flow, and a feed-side pressure of 150 kPa. The starch suspension was concentrated 5 times, during which the flux reduced slightly from 44 to 42 L m^{-2} h^{-1}. The cause of the flux decline (fouling or concentration) was not stated, and no results regarding the retention of the membrane (e.g. the starch and protein content of the retentate and permeate) were presented.

The microfiltration and ultrafiltration of similar feed streams is considered relevant as it gives an insight into the possible relationship between flux and operating conditions, and interactions between the different feed components (Boykin et al., 2005, and Stopka et al., 2001), provides information on the membrane fouling encountered with these feed streams, and identifies possible cleaning methods (Fillaudeau & Carrere, 2002, Chen & Ko, 1997, and Sayed-Razavi et al., 1996).

A selection of relevant papers is summarised in Table 2.4.

Table 2.4. Overview of literature concerning microfiltration of solutions containing starch granules.

Author	Details	Comments
Boykin et al. (2005)		
Feed stream	Rice cooker waste water.	Optimum TMP and velocity were 275 kPa and 5 m s^{-1} respectively.
Membrane	MF 0.1 µm tubular stainless steel-titanium dioxide.	Key findings were a logarithmic increase in flux with both TMP and velocity, and a logarithmic decrease in flux with concentration.
Operating conditions	82°C, TMP 0 – 500 kPa, 5.5 m s^{-1}.	The permeate contained no suspended solids (i.e. 0.1 µm pore size retained all suspended solids).
Chen & Ko (1997)		
Feed stream	Waste water from mungbean starch processing.	Flux rapidly declined as the VCF increased from 1 to 2, and then remained almost constant from a VCF of 2 to 10.
Membrane	PES, UF 30 kDa, spiral wound, 0.46 m^2.	No single cleaning agent was 100 % effective. The successful cleaning procedure had the following steps, a water rinse, sodium hydroxide with surfactant wash, hydrochloric acid wash, and protease detergent wash (with a water rinse after each step).
Operating conditions	30°C, TMP 380 kPa.	
Hinkova et al. (2005)		
Feed stream	Amaranth starch suspension (3 % starch and 3 % protein).	Achieved a VCF of 5 (i.e. concentrated from 3 to 15 % starch). Stopped at a VCF of 5 due to the low retentate volume causing foaming.

Table 2.4 continued.

Author	Details	Comments
Membrane	MF 0.1 µm Membralox inorganic membrane.	A very small decrease in flux from VCF of 1 to a VCF of 5 (44.2 to 42.8 L m^{-2} h^{-1}). No reference to bringing the membrane to steady-state before starting the concentration – flux decline could be due to fouling not concentration.
Operating conditions	40°C, TMP 150 kPa, 5 m s^{-1}.	No data on starch or protein retention.
Ousman & Bennasar (1995)		
Feed stream	Starch suspension (type not disclosed).	For the starch suspension trialled, the main parameter responsible for flux decrease was fouling caused by deposit on the membrane. Membrane resistance was negligible, total resistance decreased with cross-flow velocity and increased with pressure or concentration, and increasing pore size did not significantly improve performance.
Membrane	MF (0.1, 0.2, 0.5, 0.8 µm) composite inorganic membranes.	
Operating conditions	40°C, TMP 200 kPa, 1.8 m s^{-1}	SEM observations revealed that the foulant was contained (i) a thin film formed by the agglomeration of small particles, (ii) starch granule fragments, (iii) starch granules of various shapes and sizes.
		Cleaning methods normally used for these membranes were insufficient. The membrane cleaned by back-flushing, followed by a hot acid bath (50 % HCl).
Razavi et al. (1996)		
Feed stream	Aqueous extract of soy flour	Primary focus was to Investigate membrane fouling and cleaning.

Table 2.4 continued.

Author	Details	Comments
	(suspension of proteins, lipids and carbohydrates).	SEM revealed a difference in the thickness and appearance of the foulant from the two membranes. The thinner foulant was a polymer-like coating, while the thicker foulant had a more permeable globular structure.
Membrane	PES UF 50 kDa and UF 100 kDa, 2.25 m^2.	Lipids in the form of spherical globules, as well as proteins and polysaccharides, were incorporated in the foulant.
Operating conditions	50°C, TMP 300 kPa, 100 L min^{-1}.	The successful cleaning procedure was a water rinse, sodium hydroxide wash, protease wash, sodium hypochlorite wash, and final water rinse.
Shukla et al. (2000)		
Feed stream	Corn-starch suspension.	They found that mass-transfer models were not applicable and instead based their work on the resistance in series model.
Membrane	MF 0.1 µm tubular stainless steel-titania composite, 0.35 m^2.	Low pressures (< 150 kPa) and high cross-flow (> 5 m s^{-1}) minimised fouling.
		Flux was almost independent of TMP above 150 kPa.
Operating conditions	49°C, TMP 100 – 450 kPa, 2, 3.5 and 5 m s^{-1}.	Corn-starch retention was 100 %, protein retention was high (60 – 70 %).
		The successful cleaning procedure was a water rinse, sodium hydroxide wash, sodium hypochlorite plus chlorine wash, glucoamylase wash, and sodium hypochlorite plus chlorine wash. The order of the steps was important.

2.7 Summary

This literature review has confirmed that tangential flow filtration is a potential method for separating the starch-milk produced by the pilot-scale Al-Hakkak process into a starch-rich stream and a stream containing the soluble components. Based on the size of the starch granules the separation process will be in the transition zone between ultrafiltration and microfiltration.

Interactions between the membrane and feed components, and between the individual feed components, are expected. These interactions may affect the ability of the soluble components to pass through the membrane. No mathematical models exist that can accurately predict, for complex feed streams, what interactions will occur and how they will affect the separation process; as such trial work is needed to confirm membrane performance. These interactions, which can be influenced by operating conditions, also result in membrane fouling, resulting in a reduced flux and altered selectivity. The review of papers detailing the ultrafiltration or microfiltration of similar complex feed streams highlighted that membrane fouling and cleaning could be an issue.

The technical feasibility of using tangential flow filtration to perform the desired separation will be investigated in two parts. The first will investigate the selectivity of the membrane and the relationships between flux and key process variables (TMP, cross-flow, and concentration). The second will focus on membrane cleaning.

3 SEPARATION CHARACTERISATION

3.1 Methods

3.1.1 Feed Liquor Preparation

3.1.1.1 Materials

Organic amaranth whole flour was purchased from Chantal Organic Wholesalers (Napier). The amaranth seeds were of the White Oscar variety, and the whole flour contained 12.7 % protein, 5.08 % fat, 5.1 % fibre, and 59.4 % carbohydrate (composition data provided by manufacturer). The whole flour was sieved through a pilot-scale vibrating screen (fitted with a 100 µm screen) to obtain the fraction less than 100 µm. Healtheries Fine Ground Gluten Flour was purchased from a local supermarket, salt (sodium chloride) was obtained from Sigma-Aldrich.

3.1.1.2 Standard Feed Liquor

Feed liquor (starch-milk) was produced using the pilot-scale Al-Hakkak method described by MacManus & Macdonald (2009b). This method was completed in two parts. Firstly, a stiff dough was made by combining amaranth flour (2485 g), vital wheat gluten (620 g), 1 % salt solution (185 g), and water (1710 g, 25˚C) using a Varimixer AR40 planetary mixer. Once formed, the dough was allowed to rest for 90 minutes. Secondly, two successive “washes” were used to wash the starch from the dough. Water (20 L, 25˚C) was added to the dough and mixed for 90 minutes. The solution was passed through a pilot-scale vibrating screen (40 µm) to separate the starch-rich liquor from the solid dough residue. The dough residue was returned to the mixer and a second wash performed (20 L of water, 40 minutes). As per the first wash, a pilot-scale vibrating screen was used to separate the starch-rich liquor from the solid dough residue. The two lots of starch-milk were combined to make a bulk batch of feed liquor. This bulk liquor was divided into 2 L lots and frozen until required.

The day before use, the starch-milk was defrosted by standing at room temperature. Despite the starch-milk being screened before freezing, the defrosted starch-milk had some small (2 – 5mm) lumps. These lumps were brown in colour, resembling the spent dough retained by the vibrating screen during the starch-milk preparation. Prior to use the starch-milk was filtered through a 30 µm screen, and heated to 25˚C on an IKAmag Ret-G hot plate.

3.1.1.3 Feed A1 and A2

Additional feed solutions (A1 and A2) were prepared by splitting a sample of the standard feed into its soluble and insoluble components. These feed solutions were used to investigate how the soluble and insoluble components contributed to membrane performance (refer to Section 3.2.5.2 for further details).

Four litres of the standard feed liquor was divided into 200 ml lots and centrifuged (Beckman Avanti J-301 laboratory centrifuge, 2500 g, 15 minutes). The supernatant was decanted off and set aside as Feed A1. The pellets were washed three times. For each wash, the pellet was re-suspended in 200 ml of distilled water, mixed for 5 minutes, and re-centrifuged. The washed pellets were then combined, made up to the original starting volume (4 L) with distilled water, and set aside as Feed A2. Feed A1 was used the same day that it was produced; Feed A2 was stored in a fridge and used two days later.

3.1.2 Filtration Equipment

All experiments were performed using a Millipore ProFlux M12 Tangential Filtration System. This unit has a 3 L feed tank with heating/cooling coil, a variable speed feed pump, inlet and outlet pressure sensors, permeate and retentate flowmeters, and a back-pressure control valve. The unit can be used with a variety of different membranes, and can be configured to operate in concentration mode, or recycle mode (Figure 3.1).

A Techne water bath was used to supply hot, or cold, water to the heating/cooling coil, a Mettler-PM34 balance was used to record process masses, and a Center-305 thermometer fitted with a K-type thermo-couple was used to measure temperatures.

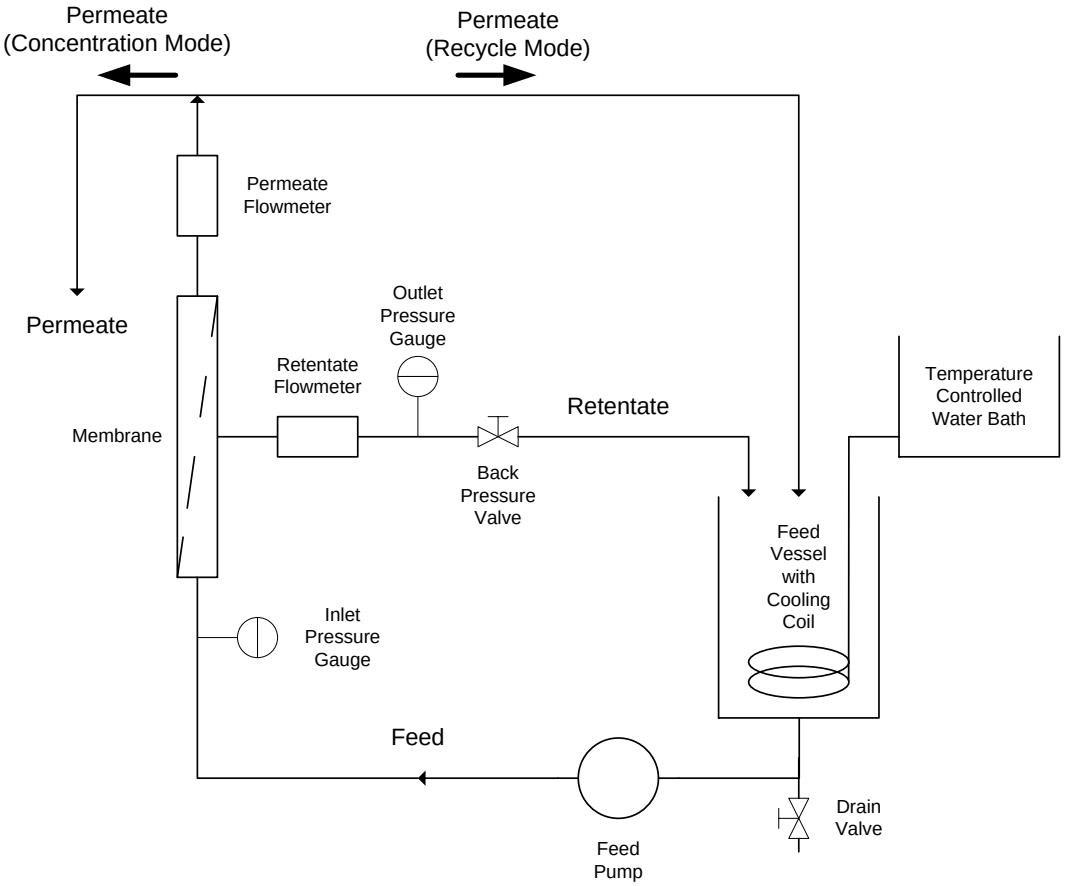

Figure 3.1. Schematic flow diagram of the microfiltration equipment.

The membrane used for all trial work was a Pellicon 2 Ultracel PLCXK membrane (purchased from Millipore, through their New Zealand agent Bio-Logic Solutions Limited). The PLCXK membrane had a flat plate (cassette) configuration, a filtration area of 0.1 m^2, a Nominal Molecular Weight Limit (NMWL) of 1000 kDa, and was constructed of regenerated cellulose. Full membrane details may be found in Appendix A.

Regenerated cellulose was chosen as the membrane material because it is hydrophilic, which minimises non-specific protein binding. This offers two advantages (i) protein losses should be low, which is important as the protein-rich permeate is a potential co-product, (ii) protein-based membrane fouling should be minimised. One constraining issue is that regenerated cellulose membranes have a low tolerance to pH extremes, high temperature, and some chemicals (Wagner, 2001). This reduces the range and severity of steps that can be used to clean the membrane. Polyethersulfone was considered as an alternative membrane material. This material has a better chemical resistance

than regenerated cellulose, but was ruled out as it is higher fouling, and does not tolerate fats or oils (which are present in low amounts in the feed).

A NMWL of 1000 kDa was chosen as it was the closest available size to the range needed to pass the soluble proteins while retaining the starch granules. As mentioned in Section 2.5.5.1 (*Retention*), a membrane's NMWL is a label and not a specification. For retention applications, membrane suppliers recommend using a NMWL that is 1/3 the size of the material to be retained. Amaranth starch granules may be as small as 0.5 µm (Kong et al., 2009); therefore the recommended pore size is less than 0.2 µm. For passage applications, a factor of 5 is recommended. As the largest protein present in the starch-milk is approximately 80 kDa (MacManus & Macdonald, 2009a), a NMWL greater than 400 kDa is required to ensure all the protein passes through the membrane pores. A slight complication is that microfiltration membranes are rated by 2-dimensional physical size (µm), while ultrafiltration membranes are rated by molecular weight (kDa), and the present application is in the transition area (i.e. the range where loose UF overlaps tight MF). There is no direct correlation or conversion between µm and kDa. However, Millipore claim their NMWL 1000 kDa membrane will retain greater than 99 % of molecules larger than 0.03 µm (Millipore BioProcess Division, n.d.). As such, this NMWL should retain the starch granules and pass the soluble proteins.

3.1.3 Trial Procedures

3.1.3.1 Flux versus Time

Flux versus time data were collected while running in recycle mode, with constant cross-flow, TMP, and temperature (typically 20 L h^{-1}, 50 – 100 kPa, and 25°C respectively).

To begin a run, the desired mass of feed liquor was transferred into the feed hopper, and the feed pump started and set to give the required feed rate. The back-pressure valve was then adjusted to give the required TMP. Temperature was controlled by altering the temperature of the water bath that fed the heating/cooling coil, and cross-flow rate was controlled by adjusting the feed

pump speed. Flux was measured at 5 minute intervals for the first 30 minutes, and at 15 minute intervals for the remainder of the run (typically 3 to 4 hours). As the flux was generally below the readable scale of the permeate flowmeter, permeate flux rates were determined by measuring the mass of permeate collected over a 1 – 2 minute period.

Data sheets compiled during the membrane characterisation runs may be found in Appendix B.

3.1.3.2 Flux versus TMP

The system was brought to steady state by operating in recycle mode, at a low TMP (50 kPa), for 2 to 3 hours (as described Section 3.1.3.1). Once at steady state the baseline flux was measured, and the operating conditions (inlet and outlet pressure, cross-flow rate, pump speed, and temperature) recorded. The TMP was increased by approximately 50 kPa by closing the back-pressure valve. If required the feed pump speed was increased to compensate for any decrease in cross-flow resulting from the increased back-pressure. The system was allowed 15 to 20 minutes to stabilise, and then the flux and operating conditions recorded. This was repeated for increments of 50 kPa, up to a maximum TMP of 200 kPa. The sequence was then performed in reverse (incrementally decreasing TMP) to check for hysteresis.

3.1.3.3 Flux versus Concentration

The system was brought to steady state by operating in recycle mode for 2 to 3 hours (as described in Section 3.1.3.1). Concentration was then started by diverting the permeate away from the feed tank and into a collection vessel. The operating conditions were kept constant using the methods described in Section 3.1.3.1. The mass of permeate against time was recorded, as were the key process variables. Retentate and permeate samples were taken each time the volumetric concentration factor doubled (i.e. VCF1, VCF2, VCF4, VCF8 or VCF final).

3.1.3.4 Diafiltration

The retentate solutions from two concentration runs, which had been stored in a frozen state (approx. -20°C), were combined and used as the feed for the diafiltration trial. This combined feed liquor had 11 % total solids, 0.12 % ash, 1.24 % protein, 9.2 % starch, and 0.2 % fat.

The system was operated in recycle mode (cross-flow 20 L h^{-1}, TMP 100 kPa, temperature 25°C) until a steady flux was obtained. Batch-wise diafiltration was then performed by adding RO water to the feed (at a ratio of 1:1), and running in concentration mode until the original feed volume was reached. This sequence was repeated six times i.e. six diavolumes were performed. Retentate samples were taken after the first, second, forth, and sixth diavolumes, and permeate samples were taken after each of the six diavolumes.

3.1.4 Membrane Cleaning

After each run the membrane was cleaned as described in Section 4 (*Membrane Cleaning*). Between runs the membrane was stored in 0.1 M sodium hydroxide, in a fridge at approximately 4˚C (as per the manufacturers' recommendations).

3.1.5 Sample Analysis

3.1.5.1 Ash

Ash content was determined using ISO 3593:1981 Starch – Determination of ash.

3.1.5.2 Fat

Fat content was measured using acidified organic solvents, including diethyl ether and petroleum ether acidified with a dilute solution of hydrochloric acid (ACC Method 30-10).

3.1.5.3 Gel Electrophoresis

Samples were diluted 1:1 with the 1DE sample buffer (8 M urea, 62 mM Tris, 5 % 2-mercaptoethanol, 10 % glycerol, 2 % sodium dodecylsulphate (SDS), 0.001 % Bromophenol Blue), boiled for 5 minutes and run on 4 – 20 % T linear gradient Criterion Gels (Bio-Rad Ltd), at 200 V, 80 mA and 15 W for 1 hour, in a running

gel buffer composed of 0.182 M glycine, 0.25 M Tris and 1 % SDS. The gels were stained with Colloidal Coomassie Blue G250. The molecular weight standards were Bio-Rad Precision Plus Protein Standards.

3.1.5.4 Moisture

Moisture content was determined by drying to constant weight following test method ISO 1666 Starch – Determination of moisture content, oven-drying method.

3.1.5.5 Non-starch Polysaccharides (NSP's)

Non-starch polysaccharides were calculated by difference, using Equation 3.1.

$$NSP\ (\%) = Total\ Solids\ (\%) - Starch\ (\%) - Protein\ (\%) - Fat\ (\%) - Ash\ (\%)$$

Equation 3.1

Any fibre present in the samples would be included as NSP.

3.1.5.6 Protein

Protein was defined as nitrogen content multiplied by 6.25 (Resio et al., 2009). Nitrogen content was measured by the Dumas Combustion Method, using an LECO CNS-2000 Elemental Analyser.

3.1.5.7 SEM

Samples were mounted onto brass stubs using conductive carbon adhesive tape and sputter coated from a gold/palladium leaf source to impart conductivity to the surface of the sample. The thickness of the gold coating is approximately 100 Angstroms.

Samples were studied using a Jeol JSM 7000F Field Emission Gun Scanning Electron Microscope (SEM). The microscope was operated at 5 kV and samples were viewed at a working distance of 15 mm.

3.1.5.8 Starch (iodine test)

Two drops of 0.1 M potassium iodide (KI) were added to a 100 ml sample and mixed for 10 seconds. The sample was then observed for colour change, a change from brown to blue-black indicates starch is present.

3.1.5.9 Starch Assay

The Megazyme starch assay procedure was used. This procedure follows AOAC Method 996.11 which is also AACC Method 76.13 and ICC Standard Method 168 (Megazyme, n.d.).

3.1.5.10 Suspended Solids (SS)

Suspended solids (mass of material retained by a filter paper) were tested following the method described in the WRONZ Scour Tech Lab Manual (March 1992). A known mass of sample was filtered through a pre-dried and weighed Whatman GF/C filter paper. The paper and retained solids were rinsed with distilled water, and dried for two hours at 105°C, the resulting dry mass is expressed as a percentage of the original sample mass (Equation 3.2).

$$SS\ (\%) = \frac{(weight\ of\ dry\ filter\ paper + SS)(g) - weight\ of\ dry\ paper\ (g)}{Sample\ mass\ (g)} \times 100$$

Equation 3.2

3.1.5.11 Total solids (TS)

$$TS\ (\%) = 100 - Moisture\ (\%)$$

Equation 3.3

3.1.5.12 Viscosity

Viscosity was measured using a Cannon-Ubbelohde 50 M649 tube viscometer. The instructions for its use can be found in Appendix C.

3.2 Results and Discussion

3.2.1 Feed Liquor Composition

The feed liquor (starch-milk) contains a range of seed components including starch granules, protein, fat, and soluble carbohydrates. The concentrations of these components are shown in Table 3.1.

Table 3.1. Starch-milk composition.

Component	Concentration	
	(% g g^{-1})	(% DB)
Starch (insoluble granules)	1.10	51
NSP (soluble carbohydrate)	0.54 [A]	25 [A]
Protein	0.33	15
Fat	0.09	4
Ash	0.10	5
Total Solids	2.16	100

[A] calculated by difference, DB = Dry-weigh basis

A sample of the starch-milk was separated into its "soluble fraction" (starch-free) and "insoluble fraction" (starch-rich) using a laboratory centrifuge (Section 3.1.1.3). The soluble fraction was tested for TS, ash, and protein, while the insoluble fraction was washed in distilled water, and then subjected to the same tests. Results for the soluble fraction (Table 3.2), and insoluble fraction (Table 3.3), show that most of the protein present in the feed went into the soluble fraction, but a small amount also remained with the insoluble fraction. Insoluble material will not generally pass through a 1000 kDa regenerated cellulose membrane as it is usually too large to enter the pores; if it could enter the pores it would be unlikely to pass all the way through due to the tortuous pore path. Identifying the presence of protein in the insoluble fraction was a key finding as it indicated that some of the protein may have been in a form, or associated with

the starch in such away, that microfiltration could not remove it from the starch-milk.

Table 3.2. Composition of the starch-milk soluble fraction (Feed A1).

Component	Concentration	
	(% g g^{-1})	(% DB)
NSP (soluble carbohydrate)	0.48 [A]	57 [A]
Protein	0.27	32
Ash	0.10	11
Total Solids	0.84	100

[A] calculated by difference (if fat or fibre is present it will be included here)

Table 3.3. Composition of the starch-milk insoluble fraction (Feed A2).

Component	Concentration	
	(% g g^{-1})	(% DB)
Starch (insoluble granules)	1.07 [A]	95 [A]
Protein	0.05	4
Ash	0.01	1
Total Solids	1.13	100

[A] calculated by difference (if fat or fibre is present it will be included here)

The dry-basis (DB) protein content of the starch-rich stream (Table 3.3) was 4 %, which is considerably higher than the 0.1 % achieved by Al-Hakkak & Al-Hakkak (2007) during their laboratory-based study. Investigating reasons for this difference was outside the scope of this project. However, a possible explanation is that the Al-Hakkak laboratory process included a step to scrape a thin proteinaceous layer off the starch pellet; this step was not replicated during the preliminary pilot-scale process used to generate the starch-milk for the present study. An alternative explanation is; in the laboratory process the dough was very gently hand-massaged to release the starch, and the dough remained

mostly intact, whereas in the pilot-scale process the dough was vigorously mechanically agitated to release the starch. During agitation the dough disintegrated into small fragments, some of which may have been small enough to pass through the fine mesh used to separate the starch-milk from the spent dough, and hence contaminate the starch-milk.

The insoluble fraction of the starch-milk was examined using SEM. The resulting image (Figure 3.2) shows that, in addition to the polygonal shaped starch granules, some other insoluble material is present. This "other" material was not specifically separated and analysed for protein content.

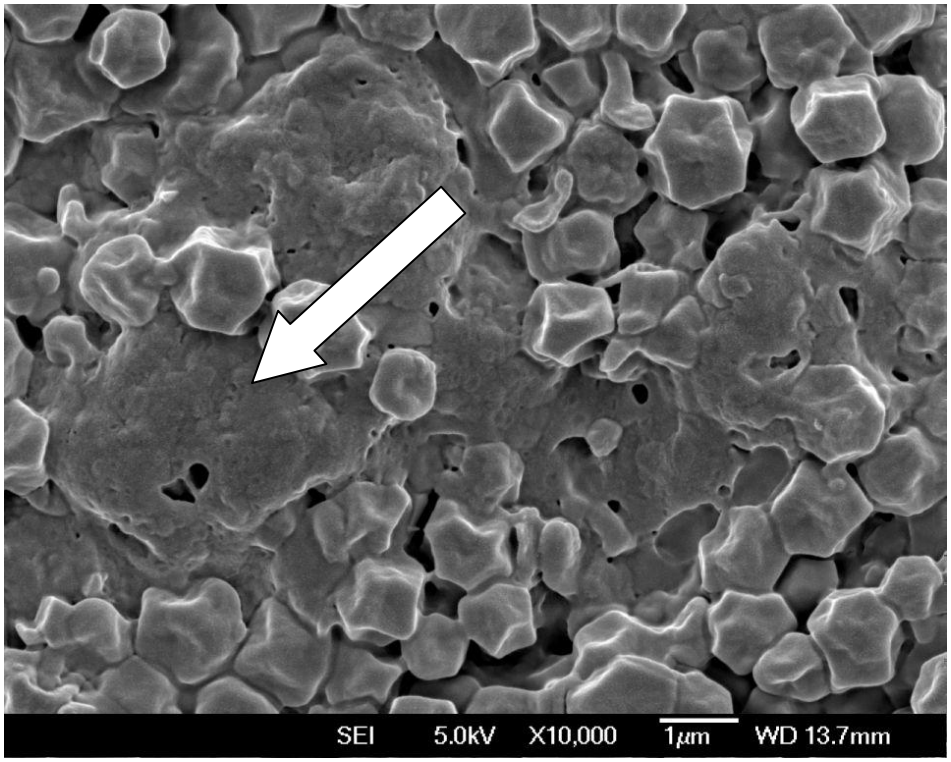

Figure 3.2. SEM image of the starch-milk insoluble fraction. The arrow highlights an area of insoluble non-starch material.

3.2.2 Membrane Characterisation

3.2.2.1 Retentate and Permeate Concentration and Retention

The change in composition of the retentate and permeate streams was measured, and plotted, across a concentration run. The retentate data are shown in Figure 3.3, and the permeate data in Figure 3.4. The measured values were used to calculate the initial retention, and average retention, of the membrane (Table 3.4).

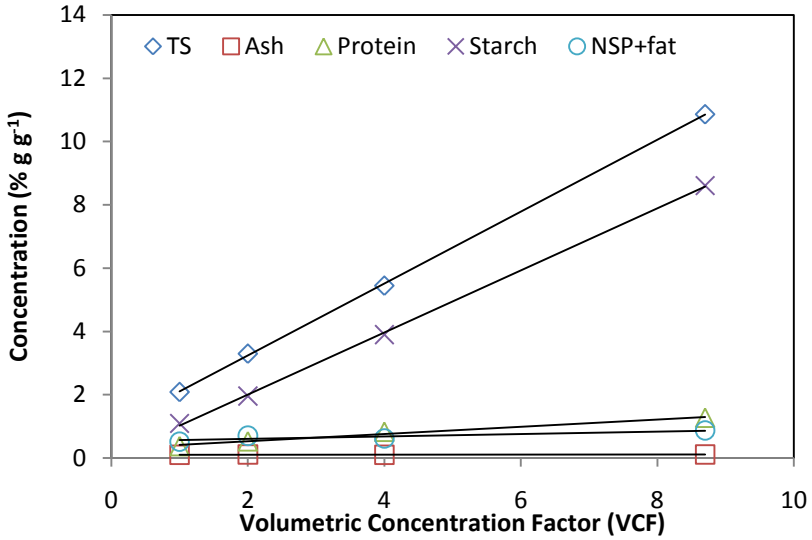

Figure 3.3. Plot of retention composition during concentration.

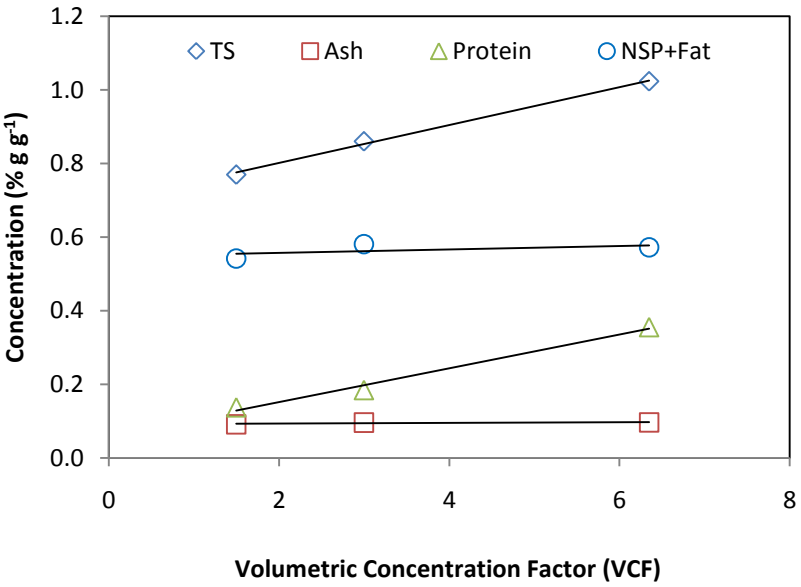

Figure 3.4. Plot of permeate composition during concentration.

Table 3.4. Retention and average retention of the various feed components.

Component	Initial Retention [A]	Average Retention [B]
Starch	1.00	0.98
Protein	0.67	0.63
NSP	*nm*	0.14
Ash	0.05	0.12
Fat	*nm*	0.37

[A] calculated using Equation 2.1, [B] calculated using Equation 2.2, *nm* = not measured.

3.2.2.2 Starch Retention and Yield

Selected permeate samples were analysed for the presence of starch using the iodine test, and SEM. In all cases the result was negative, indicating 100 % retention of the starch granules.

High retention does not always result in a high process yield. This is because product can be lost by membrane fouling and system "hold up" (product remaining in the membrane and associated equipment after draining). The overall starch yield was measured across a concentration run (nine-fold volume reduction) and a diafiltration run (six diavolumes). The concentration run had a starch yield of 94 %, and the diafiltration run a starch yield of 98 % (Appendix D). A combined concentration-diafiltration run is therefore expected to have an approximate starch yield of 92 %. Yield could be increased by altering how product is removed from the membrane. For example, using an air purge to completely drain the system, or using a water flush and adding the flush water to the next batch of feed. No such investigations were undertaken as part of this project.

3.2.2.3 Protein Retention

The protein retention was 0.67, indicating that while some protein passed through the membrane, some protein was also retained by the membrane. This is evident in Figure 3.3, in which the protein content increases with volumetric concentration. If all the protein was passing through the membrane (i.e. a retention of 1) the plot would be a horizontal line equal to the feed protein level.

Although the protein concentration increased with volumetric concentration, because some protein was passing through the membrane, and the starch was fully retained, the protein content on a dry basis decreased from 16 to 12 % (Figure 3.5).

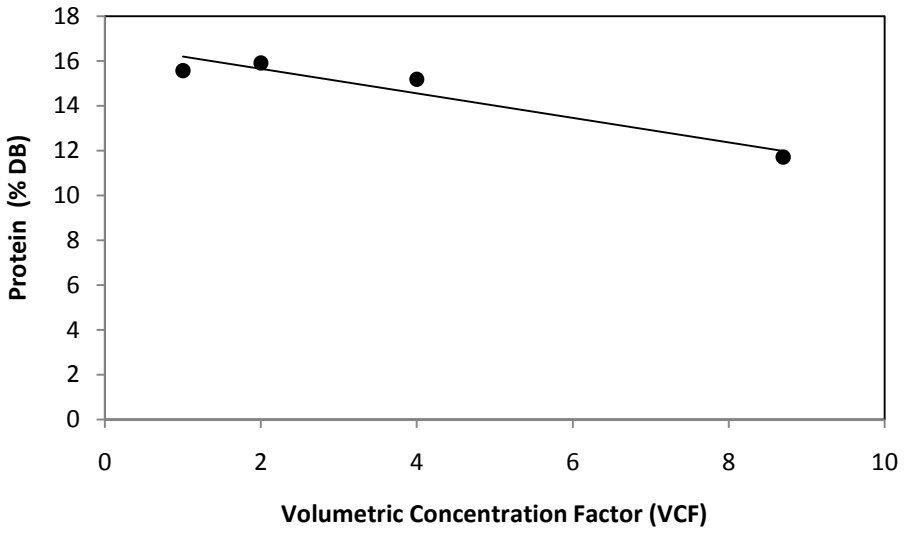

Figure 3.5. Dry-basis protein content during concentration.

The measured retention (0.67) was higher than expected, given that the membrane NMWL was ten times larger than the molecular weight of the largest protein. Possible reasons for the high retention are:

- The proteins interacted with themselves, or other feed components (NSP, lipids) to form complexes that were too large to permeate the membrane.
- A gel layer formed on the membrane surface, and acted as a secondary membrane.
- The three-dimensional shape of some of the proteins prevented them from passing through the membrane pores e.g. they were rod or sheet shaped, which can bridge the pores instead of passing through them.
- Any combination of the above.

The high protein retention has two negative consequences. Firstly, assuming all the protein can permeate the membrane, it increases the amount of “washing” that will be needed to ensure the final product (a dry starch powder) has an acceptably low protein level. Secondly, the protein content of the permeate will

be reduced. As the permeate is a potential co-product, it would be advantageous if it had as high a protein level as possible. Assuming this co-product will take the form of a protein concentrate or powder, a high permeate protein content should mean a greater mass of product, and a lower cost of downstream processing (e.g. concentration and drying). Additionally, the "missing" proteins (i.e. those retained by the membrane) could have good functionality, which would result in a higher value co-product if they were included.

3.2.2.4 NSP Retention

The fat content of the permeate was not specifically measured, which prevented the NSP content from being calculated by difference, and therefore the NSP retention could not be calculated. Instead, the average retention was calculated, and found to be 0.14. This indicates that most, but not all, NSP's were able to permeate the membrane pores. Reasons why some NSP's were retained are the same as those listed for the high protein retention.

3.2.2.5 Fat Retention

The fat content of the permeate was not specifically measured (a prohibitive 500 ml permeate sample would be needed to provide enough dry-matter to perform the test), which prevented the fat retention in the permeate from being calculated. Instead, the average fat retention was calculated, and found to be 0.37. It is possible that instead of permeating the membrane the fat formed part of the fouling layer, and as such was removed from the retentate. This was observed by Sayed-Razavi (1996) when concentrating a similar feed stream (soy flour extract).

3.2.2.6 Ash Retention

The ash level of the permeate was almost equal to the ash level of the retentate; as such the ash retention was very low (0.05). This was expected as the ash component is comprised of metal salts, all of which are many orders of magnitude smaller than the membrane pore size. The retained starch and protein contain small amounts of minerals, which explains why a small fraction of the ash was retained.

3.2.2.7 Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Figure 3.6) was used to further characterise the feed stream, investigate the high protein retention, and provide an insight into the nature of the membrane foulant.

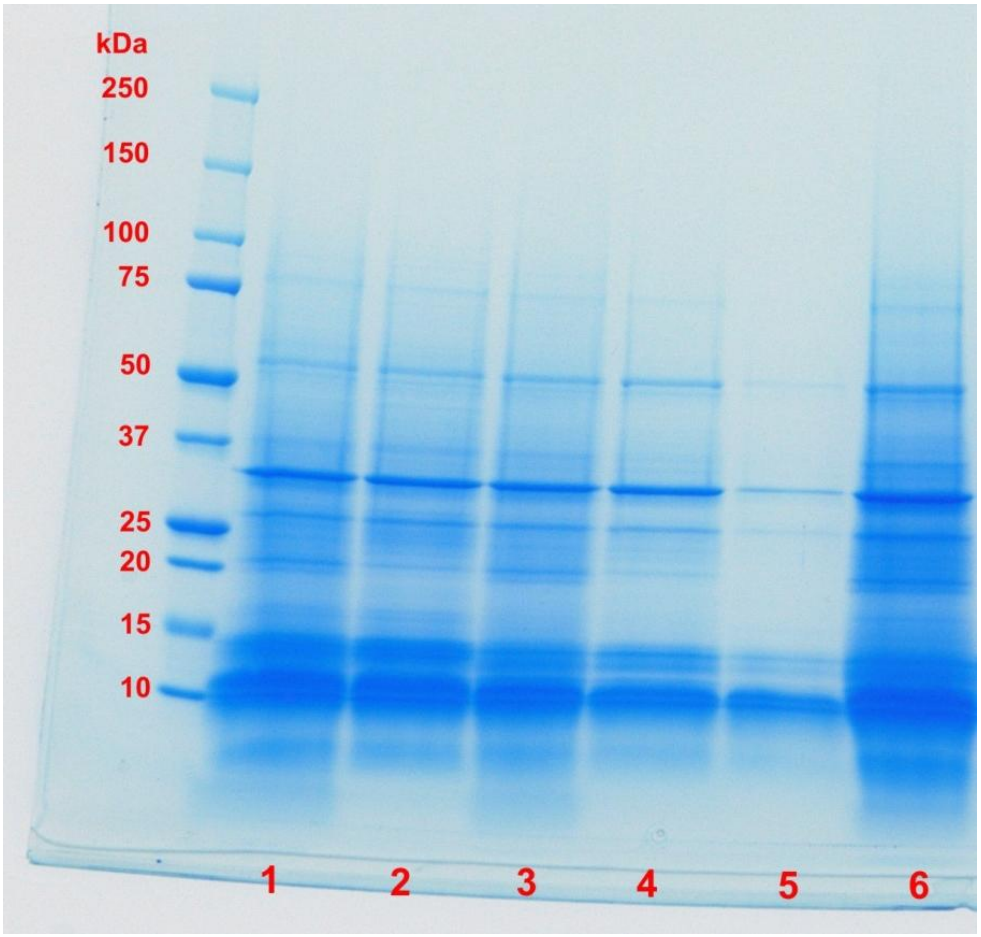

Figure 3.6. SDS-PAGE, lanes: 1 = feed, 2 = feed soluble, 3 = retentate, 4 = retentate soluble, 5 = permeate, 6 = retentate concentrated 2 times.

The following observations were made:

- Insoluble protein. The feed material was run as a whole (lane 1) and after removing the insoluble material by centrifuging (lane 2). These lanes are not different enough to indicate that any bands were removed with the insoluble fraction of the feed.
- Retention. Comparing the soluble fraction of the steady-state retentate (lane 4) and the permeate (lane 5), gives a good indication of the overall protein retention. If all the proteins were passing through the membrane these two

lanes would be identical. Instead, the permeate (lane 5) is lighter in colour, indicating it contains less protein, and has a number of bands missing. Although it could be said that these bands are missing because the proteins represented by them are not passing through the membrane, it is more likely that the missing bands are due to lane 5 being loaded with less protein than the other lanes.

- Membrane foulant. The steady-state retentate (lanes 3 & 4) differs from the feed (lanes 1 & 2); the most noticeable difference is that some bands in the 15 – 17 kDa range are missing from the steady-state retentate. It is possible that this material adsorbed to the membrane and become a foulant, but also possible the difference was due to subtle differences in resolution between the lanes. The presence of bands in both samples does not exclude them from also being possible foulants, as the abundance of the protein will determine if it is completely or only partially removed from the liquor stream by fouling.

3.2.3 Diafiltration

The concentrated retentate from the membrane characterisation trials had a protein content of 12 % DB, which was much higher than typical commercial starches (0.5 % DB). By comparison, starch that was recovered from the starch-milk feed stream by centrifuging, and then washed in distilled water (Feed A2), had a protein content of 4 % DB. This latter value was considered the lowest protein level obtainable from the pilot-scale Al-Hakkak process (at this stage in its development), and the target of the diafiltration trial; even though it was higher than the 0.1 % DB achieved by Al-Hakkak & Al-Hakkak (2007) during their laboratory-based studies. Possible reasons for this difference in protein concentrations were discussed in Section 3.2.1 (*Feed Liquor Composition*).

Based on the protein retention coefficient of 0.67, five diavolumes should lower the protein content from 12 to 4 % DB. As a “safety-factor” an extra diavolume was added, the resulting protein contents are plotted in Figure 3.7.

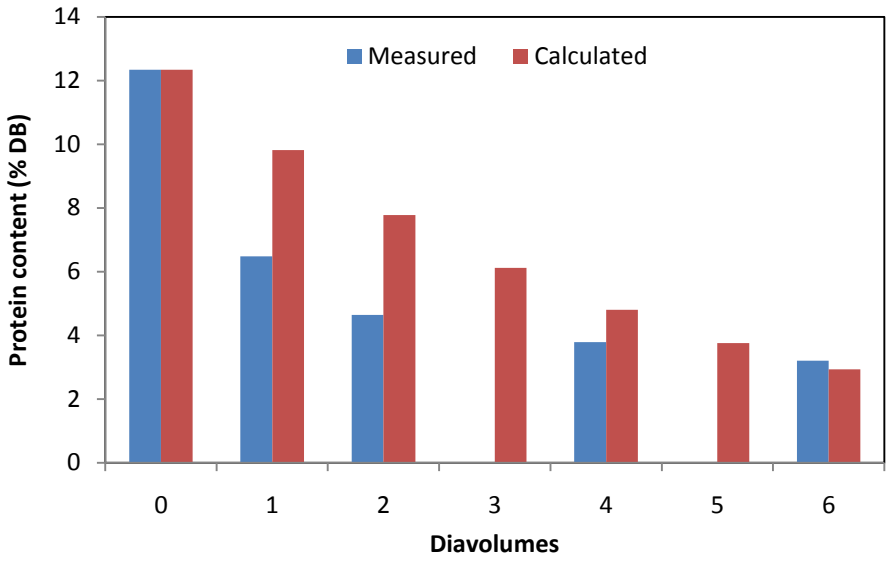

Figure 3.7. Dry-basis protein content after each diavolume.

Six diavolumes reduced the protein content of the concentrated retentate to 3.3 % DB, which matches the calculated value. However, although the end point was correct, the protein contents after the intermediate diavolumes were lower than the calculated values. The differences could be due to protein adsorbing to the membrane, which had been cleaned between the concentration and diafiltration trials. Assuming the protein adsorbs to the membrane more quickly than it is washed through the membrane during diafiltration, the protein level will decrease faster than predicted. A protein mass balance performed over the diafiltration showed 8 % of the starting protein was unaccounted for (i.e. not in the retentate or wash liquor). If this protein rapidly adsorbed to the membrane, it would account for the rapid decrease in protein content (compared to the calculated value). The protein mass balance can be found in Appendix E.

The levelling-off of the actual protein content could indicate that some of the proteins have a retention closer to 1 than the measured value of 0.67. The retention calculation treats all the different proteins in the starch-milk as one, and gives them a single retention coefficient. In actual fact, the starch-milk has a range of proteins (Figure 3.6) which may have quite different retentions. The levelling-off trend shows that continued washing (more diavolumes) will not significantly further decrease the protein content.

The insoluble fraction of the diafiltered starch-milk was isolated by centrifuging, and examined for non-starch material using SEM. The resulting image (Figure 3.8) is similar to that taken of the insoluble fraction of the original feed liquor (Figure 3.2). Both show that, in addition to the starch granules, there is some non-starch material. Assuming this material is the source of the protein contamination, a method to remove it, or prevent it initially entering the starch-milk, should be investigated as a follow-on project.

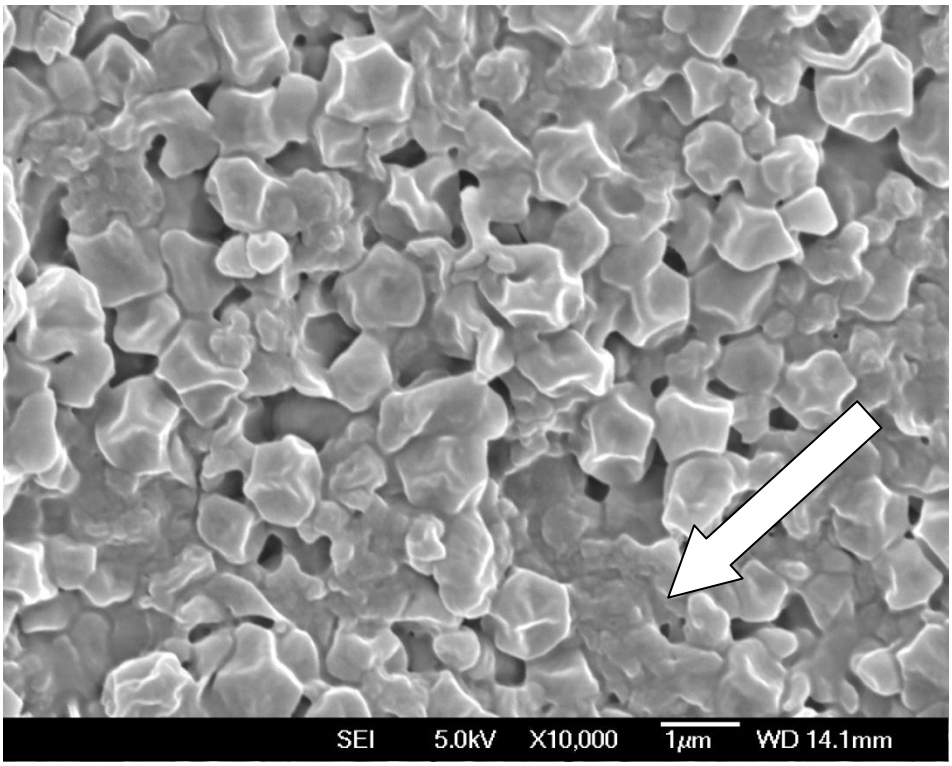

Figure 3.8. SEM image of the insoluble starch-milk fraction after six diavolumes. The arrow highlights an area of insoluble non-starch material.

3.2.4 Summary of Dry-basis Composition

The dry-basis composition of the starch-milk before and after the concentration and diafiltration steps, and the composition of the permeate generated during the concentration step, are summarised in (Table 3.5).

Table 3.5. Dry-basis composition.

Sample	Protein (% DB)	Ash (% DB)	Fat (% DB)	NSP [A] (% DB)	Starch (% DB)
Feed	15.8	4.9	4.4	24.2	50.6
Concentrate	11.7	1.0	1.8	6.2	79.2
Permeate	22	11.2	*nm*	66.8	0.0
Diafiltration feed	12.3	1.1	1.8	1.2	83.4
Diafiltered liquor	3.3	0.3	1.4	(3.0)	98.0

[A] calculated by difference

nm = permeate fat level not tested, any fat present will be included with the NSP

Key data from Table 3.7 are the protein and fat content of the diafiltered liquor, both of which are higher than typical values for commercially available starches indicating that the final starch product would not be considered satisfactorily pure (a summary of the composition of some commercially available starches can be found in Appendix F).

3.2.5 Flux Characteristics

3.2.5.1 Flux versus Time

Plotting flux against time (while running in recycle mode) gives an indication of the fouling potential of the membrane. The data from five trials is presented in Figure 3.9. The flux can be approximated (solid line on Figure 3.9) using Equation 2.3 with values of 60 L m^{-2} h^{-1} for the initial flux, 240 for α, and 0.27 for *n.*

The initial flux decrease is common during tangential flow filtration processes. It is generally caused by concentration polarisation in ultrafiltration processes, and cake formation in microfiltration processes. The extent and time dependence of the flux decrease are largely influenced by the interactions between the feed stream and membrane; as such they vary from process to process. For the process studied here, the quasi-steady state flux occurs after 2 to 3 hours, and is significantly lower than the start-up flux. Past the one hour mark the flux continues to decline, but much more gradually. Davis (1992) has attributed this

gradual decrease, for feed stocks containing particulates, to cake consolidation, compaction, or fouling.

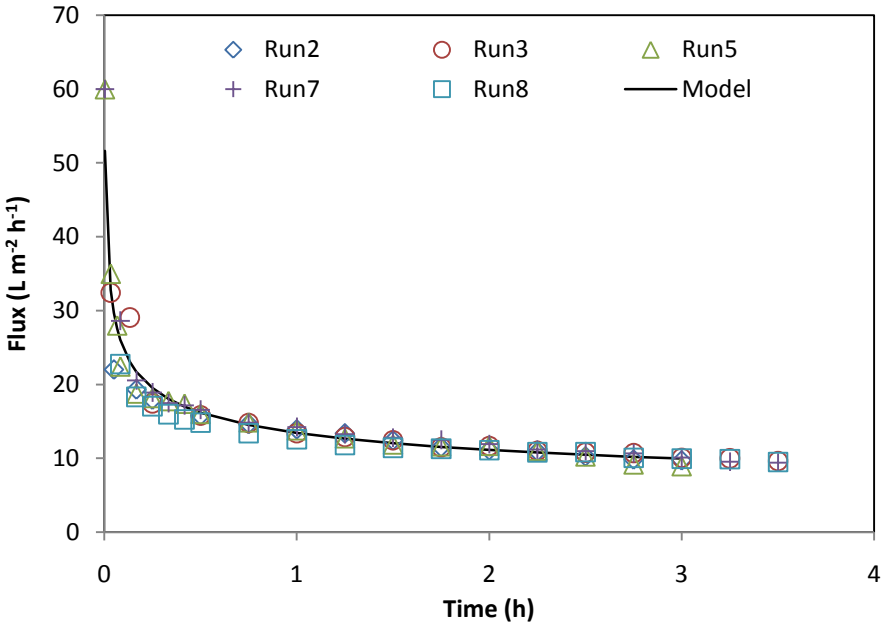

Figure 3.9. Flux versus time while running in recycle mode. TMP 100 kPa, feed 20 L h^{-1}, temperature 25°C.

The combination of a large first-stage flux decrease, plus a continual second-stage flux decrease, indicates that fouling is significant.

The steady-state flux (10 L m^{-2} h^{-1}) is low when compared to the values (40 – 70 L m^{-2} h^{-1}) reported by other authors (Hinkova et al., 2005, Shukla et al.,2000, Singh et al., 2008, and Sayed-Razavi et al., 1996), albeit they used different membranes and different starch solutions.

3.2.5.2 Flux versus Feed Composition

As the feed stream has many different components, it is possible that multiple fouling mechanisms are taking place, in particular, cake formation by the starch granules, and gel formation by the soluble feed components. To gain an insight into which of these two fouling mechanisms was dominating the flux decline, three different feed solutions were compared. These were:

- The standard feed.
- The soluble fraction of the standard feed (Feed A1). This contained no starch granules or insoluble material. The soluble protein profile of this stream is the same as that shown on lane 2 of the SDS-PAGE (Figure 3.6).

- The insoluble (starch granule) fraction of the standard feed (Feed A2). This stream contained no soluble components, and was made up to its original volume with distilled water.

The flux versus time relationships of these three feed streams are shown by Figure 3.10. The viscosity of the permeate from each run was compared, to ensure viscosity differences weren't having an effect, and the differences were indeed found to be insignificant.

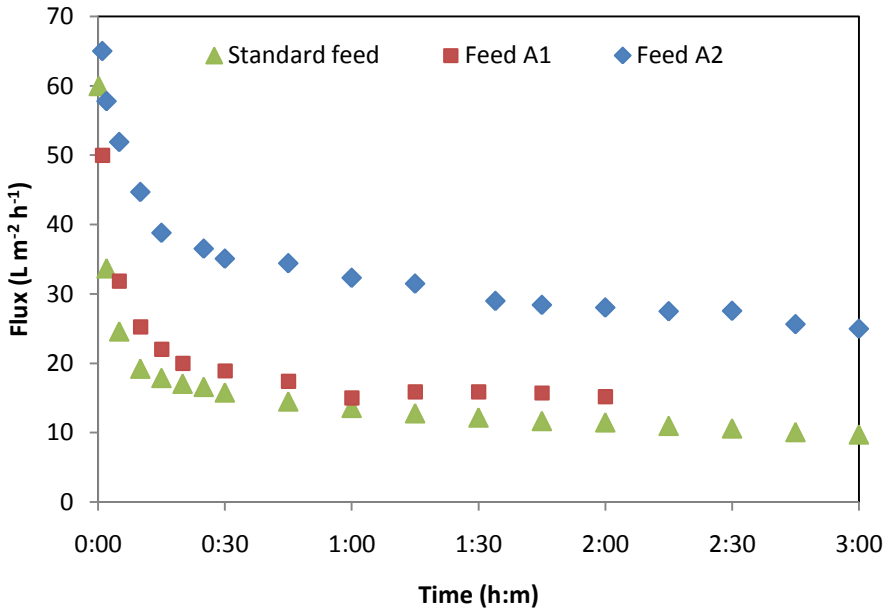

Figure 3.10. Flux versus time profile for the standard feed, Feed A1, and Feed A2. TMP 100 kPa, feed 20 L h^{-1}, temperature 25˚C.

The data presented in Figure 3.10 shows that the flux versus time relationship of Feed A1 is similar to a standard run, while Feed A2 has a quasi-steady-state flux that is approximately twice as high as the other two feed streams.

The flux data from Figure 3.10 were used to calculate the increase in resistance (R_c + R_f, as derived from Equation 2.4), which is plotted against time in Figure 3.11. Key points are (i) the standard run resistance increases a little faster than Feed A1, suggesting that the first-stage of the standard feed resistance increase is largely due to the soluble components in the feed, although the insoluble components also make a contribution, (ii) Feed A1 levels off to a constant resistance, while Feed A2 and the standard feed both continue to increase

slightly with time. This indicates that the second-phase of the resistance increase is due to the insoluble fraction of the feed.

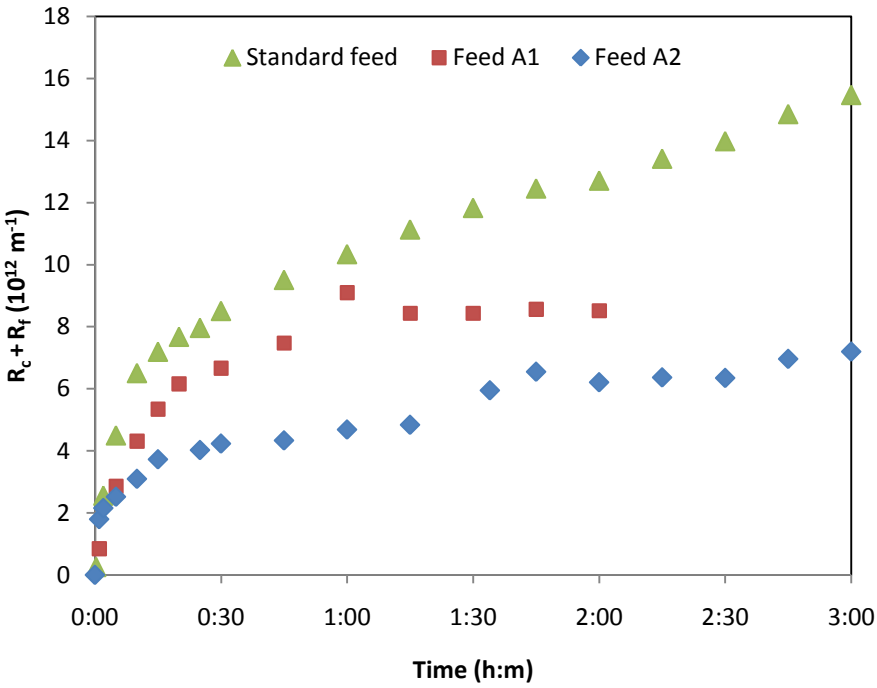

Figure 3.11. Plot of the increase in resistance against time for the three different feed streams.

3.2.5.3 Flux versus Transmembrane Pressure

The effect of TMP on flux, for a typical run, is shown by Figure 3.12. Data was collected for the unconcentrated (VCF1) starch-milk, and after a two-fold concentration (VCF2). For the unconcentrated starch-milk, flux increases non-linearly with TMP. This indicates the resistance of the gel-layer, or caked material, increases with increasing pressure i.e. its thickness increases (due to a decrease in back-transport), or the gel, or caked material, is compressible.

Further evidence that compression was occurring is the hysteresis between the "a" (increasing pressure) and "b" (decreasing pressure) measurements, particularly on the first set of trials (VCF1). When the TMP was lowered to a previous value (e.g. from 200 to 150 kPa) the flux did not return to the value recorded previously at that TMP. This suggests the foulant had compressed and did not relax when the pressure was removed. It is also possible that increasing the TMP accelerated fouling by forcing material into the membrane pores.

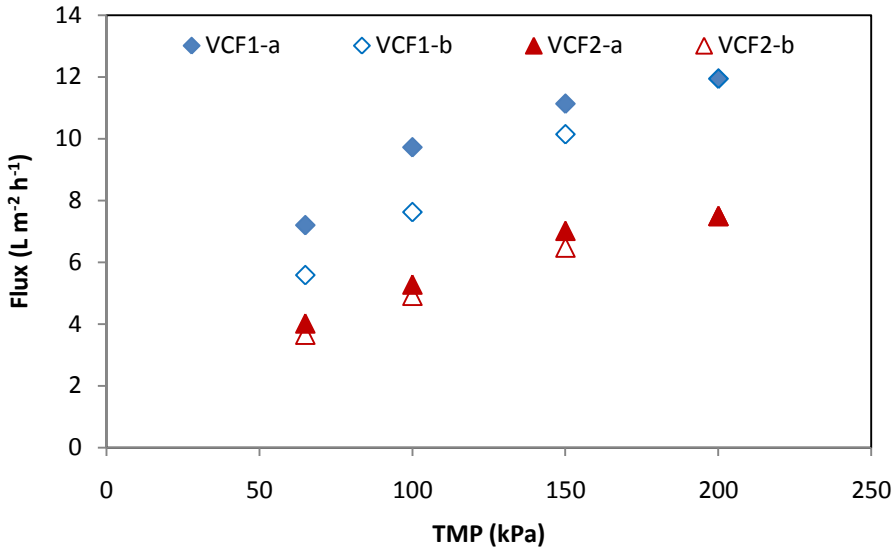

Figure 3.12. Influence of TMP on flux. Run 8, feed 20 L h^{-1}, temperature 25˚C.

The flux versus TMP relationship for liquor that had been concentrated two-fold (VCF2) was closer to linearity, and had less hysteresis. However, the irreversible cake compression, or accelerated fouling, during the VCF1 evaluation could have influenced the shape of the subsequent VCF2 curves. Literature suggests flux should decrease with increasing concentration and if a non-linear flux-TMP relationship is present at a low concentration a similar relationship should exist at a higher concentration (Zeman & Zydney, 1996).

The flux versus TMP data collected over four runs are shown in Figure 3.13. It can be seen that the data can be approximated (solid line) using the resistance in series model,

$$J = \frac{\Delta P}{\mu(R_m + R_c)}$$

Where R_m was determined experimentally, and R_c was approximated using the following power-law function (Davis, 1992),

$$R_c = \alpha_o(\Delta P)^s$$

Where α_o (a constant related primarily to the size and shape of the particles forming the cake), and s (the cake compressibility) were determined by fitting a power-law trend to the flux versus TMP data collected during the trials. These calculations can be found in Appendix G.

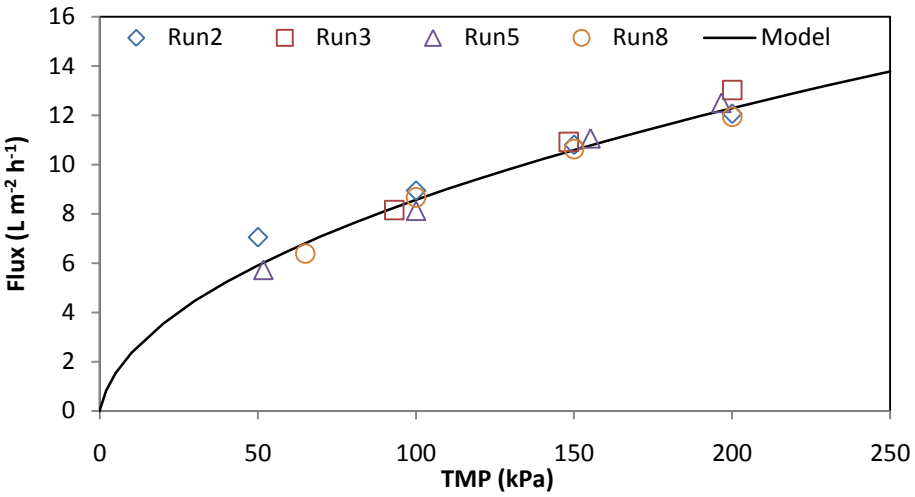

Figure 3.13. Flux versus TMP, VCF1, feed 20 L h^{-1}, temperature 25°C, steady state flux reached before taking measurements.

3.2.5.4 Flux versus Feed Rate

The effect of feed rate on flux was evaluated over a range of TMP's (Figure 3.14). Due to equipment pressure limitations it was not possible to test the flux at each TMP for all the feed rates. Details on the pressure limitations can be found in Appendix H.

Flux was almost independent of feed rate. If anything it decreased slightly with increasing feed rate, which contradicts typical flux-feed rate trends.

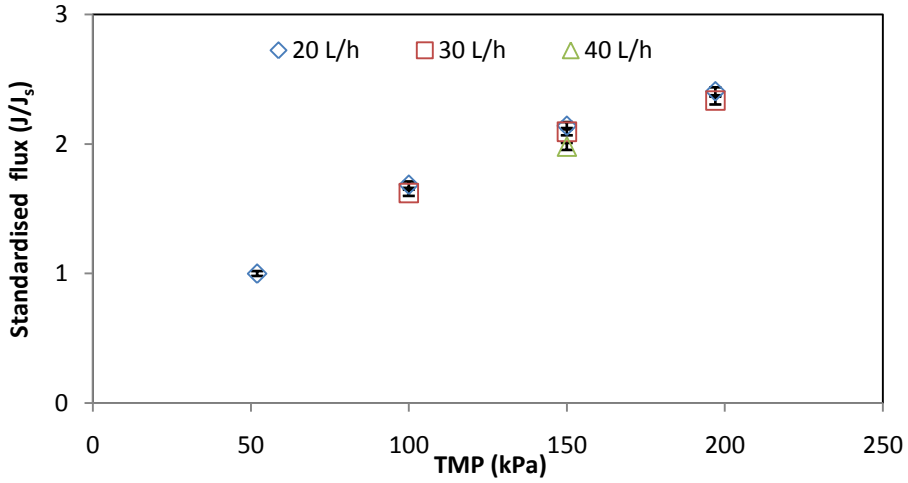

Figure 3.14. Influence of feed rate on flux. Data averaged from two trials. J_s = starting flux (TMP 50 kPa, feed 20 L h^{-1}), temperature 25°C, error bars represent the experimental uncertainty estimate.

The reason flux was higher at a lower feed rate could be because pressure drop increases with feed rate. This means, to obtain the same TMP at different feed

rates, the feed and retentate pressures must be altered. For example, when the feed rate is increased the inlet pressure increases, as a result the retentate pressure must be lowered to maintain the same TMP. Since, in most cases flux is not linearly proportional to TMP, when there is a high pressure drop regions near the membrane inlet and outlet can have a TMP that is outside the optimal pressure range, and therefore a reduced flux. The higher the feed rate, the more likely, and larger, these non-optimal regions will be.

The fact that flux did not increase with feed rate could also indicate:

- The increase in velocity was not enough to reduce the thickness of the caked material. During the centrifuging step performed when preparing feed A1 (Section 3.1.1.3), the starch granules were observed to exhibit non-Newtonian behaviour i.e. the settled starch formed a pseudo-solid. A velocity higher than what was possible using the current equipment may be required to re-suspend any settled starch granules.
- The cake thickness was reduced, but it did not result in an increased flux as the gel-layer (assumed to be closer to the membrane surface) was undisturbed by the increased velocity.

3.2.5.5 Flux versus Concentration

The flux versus volumetric concentration data collected over two runs (Run 6 and Run 8) are plotted in Figure 3.15. The flux versus VCF relationships for the two runs have similar shapes, although the values are a little different (e.g. the bottom-out VCF for Run 6 was 2.3, while for Run 8 it was 1.7). The feed liquor used for each trial had a slightly different total solids content (2.05 % for Run 6, 2.30 % for Run 8), which could account for the offset between the two datasets.

When the flux data is plotted against the retentate total solids content (Figure 3.16), with the exception of the starting value (first point), the two sets of data are very similar. The difference between the starting values can be easily explained. For both runs the system was run in recycle mode to obtain a quasi-steady-state flux prior to starting the concentration, but for Run 8 a set of flux versus TMP trials was run between reaching steady state and starting the concentration trial. These TMP trials accelerated membrane fouling, resulting in

a lower flux at the start of the concentration trial. A plot of flux versus time (for Runs 6 and 8) can be found in Appendix I (Figure I.1).

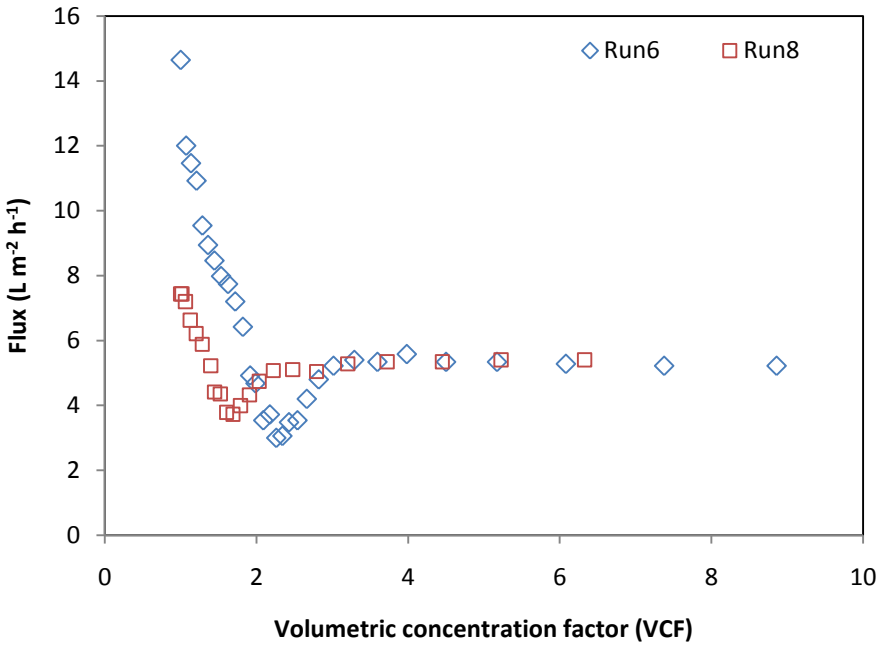

Figure 3.15. Plot of flux versus VCF. TMP 100 kPa, feed 20 L h^{-1}, temperature 25˚C.

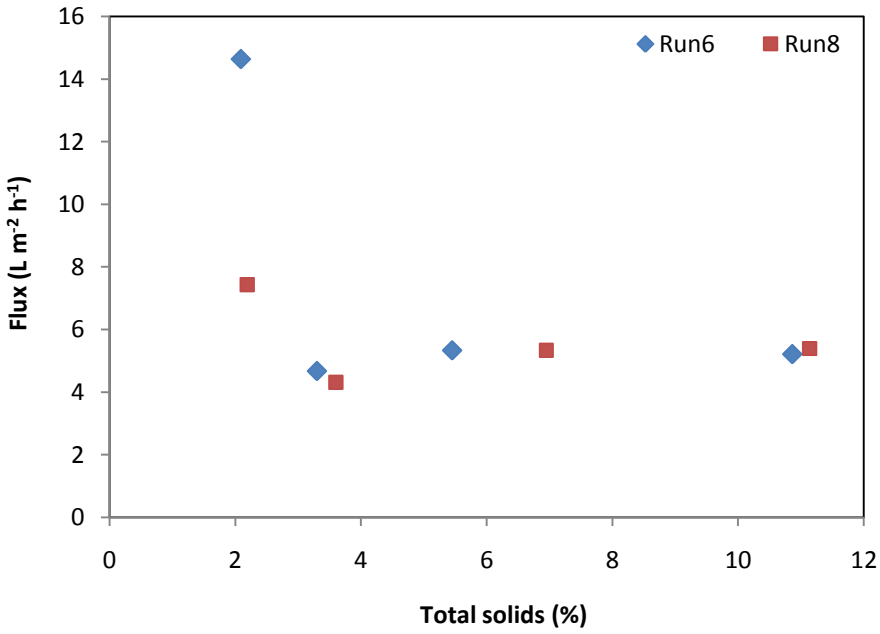

Figure 3.16. Plot of flux versus retentate total solids. TMP 100 kPa, feed 20 L h^{-1}, temperature 25˚C.

It is common for flux to decrease with concentration as the equilibrium gel-layer, or cake thickness, increases with concentration (Zeman & Zydney, 1996). The pattern of a rapid flux decrease between a VCF of 1 and 2, followed by a plateau

is less common, but has been observed (Chen & Ko, 1997). Hinkova et al. (2005) noted a continuous plateau when concentrating an amaranth starch suspension from 3 % to 15 % (i.e. flux was independent of concentration). The small increase in flux observed prior to the plateau during the present study is unusual. Investigating the reason for this flux increase was outside the scope of this project, however, the resistance in series model allows some preliminary deductions to be made. In order for flux to increase one of the following must occur:

- An increase in TMP. The trial data sheets show TMP was constant throughout the runs. Copies of these data sheets can be found in Appendix B (Tables B.6 and B.8).
- A decrease in viscosity.
    - An increase in temperature would cause a decrease in viscosity; however, the trial data sheets show temperature was constant throughout both runs.
    - The viscosities of the permeates collected while concentrating from VCF1 to 2, VCF2 to 4, and VCF4 to VCF8 were tested and found to increase slightly with concentration (1.05, 1.06, and 1.24 cSt respectively). Therefore, the increase in flux was not due to a decrease in viscosity.
- A decrease in membrane resistance (R_m).
    - R_m can change with time if the membrane compacts or relaxes. A plot of flux versus time revealed that the flux increase for each run occurred after different run times (7 hours for Run 6, and 6 hours for Run 8), indicating that the flux increase was not related to the time the filtration had been running (which might occur if the membrane relaxed with time). A plot of actual flux versus time can be found in Appendix I (Figure I.1).
    - The measured R_m is specific for the feed material (e.g. for the same membrane, R_m for water is generally lower than the R_m for product). Therefore, it is possible that if the feed changes during processing R_m will also change. During concentration the ash

retention was very low; this means that ions were free to permeate the membrane, which would have affected the ionic strength and/or pH of the retentate. Protein shape, charge, apparent molecular weight, and intermolecular interactions are all influenced by pH and ionic strength; changing any one of these could have resulted in the increase in flux.

- A decrease in the resistance of the foulant ($R_{c,}$). A plot of flux versus VCF for Feed A1 and A2 reveals that, like the flux versus time profile, the flux versus VCF is dominated by the soluble component of the feed stream. This plot is included in Appendix I (Figure I.2). Neither Feed A1 or A2 exhibited the increase in flux that occurred with the standard feed. This suggests that the interactions between the gel material and caked material changed, and resulted in an increased flux. This area needs further investigation.

3.3 Separation Characterisation Results Summary

Key results from the membrane separation and characterisation trials are:

- Retention.
    - The selected membrane successfully retained the starch granules.
    - The retention of the non-starch polysaccharides and ash components of the feed was acceptably low.
    - The retention of protein and fat was higher than desired.
- Diafiltration lowered the protein and fat content of the retentate, but not far enough to reach commercially acceptable levels.
- The high protein retention, and inability of diafiltration to reach an acceptably low protein level, was in part due to the presence of some insoluble protein in the starch-milk.
- Flux.
    - While operating in recycle mode flux declined significantly with time; indicating severe membrane fouling. This fouling was mainly caused by the soluble feed components.

- Flux increased with increasing TMP, but the increase was non-linear and showed that the foulant (or caked material) was compressible. The optimum TMP was 100 – 150 kPa.
- Flux was almost independent of feed rate across the range trialled. However, equipment pressure limitations prevented a thorough investigation into using higher feed rates.
- Flux had a unique three stage relationship with volumetric concentration. During the first stage flux reduced linearly with increasing concentration, in the second stage flux increased with increasing concentration, and in the third stage flux was independent of concentration.

4 MEMBRANE CLEANING

The membrane manufacturer's "recommended" method for cleaning a regenerated cellulose membrane, that has been fouled by a feed stream containing protein and polysaccharide, consists of a water rinse followed by a hot sodium hydroxide wash (Millipore BioProcess Division, n.d.). During this study the recommended cleaning method was ineffective and developing a suitable cleaning procedure became a major focus of this research.

4.1 Methods

After its first use the membrane was cleaned using the two-step cleaning cycle recommended by the membrane manufacturer. This cleaning cycle was ineffective, so trials were run to evaluate alternative cleaning chemicals and develop an effective cleaning procedure. Ideally, a supply of identically fouled membranes would be available for use during a cleaning trial; this would allow a direct comparison of the various cleaning chemicals. During this trial only one membrane was available, this constrained the experimental design that could be applied to the cleaning trial. The cleaning trial was in part empirical, with the various cleaning chemicals consecutively trialled until a satisfactory level of cleanliness was achieved. The membrane was then re-fouled (during the subsequent characterisation run) and the cleaning steps that had shown positive cleaning effects were further investigated. This process was repeated until an acceptable and repeatable cleaning method had been developed.

4.1.1 Selection of Cleaning Chemicals

The starch-milk feed stream was known to contain the following components from the amaranth flour: soluble protein, soluble carbohydrate, insoluble carbohydrate (starch granules), and fat. Given the other three raw materials used in the Al-Hakkak process (wheat gluten, water, salt) the starch-milk may also contain wheat proteins and carbohydrates (e.g. gluten, pentosans, beta-glucans), and impurities from the water (e.g. calcium, iron, bacteria).

It was hypothesised that the fouling was multi-layer, consisting of (1) a thin layer of protein-carbohydrate-lipid gel adsorbed to the membrane surface, (2) an outer layer of caked starch granules that are relatively clean and un-compacted, (3) a middle transition layer that contains starch granules partially embedded in the gel layer, and starch granules glued together by, or covered with, small amounts of the protein-carbohydrate-lipid gel. This hypothesis was based on text book descriptions of the steps involved in protein fouling (Zeman & Zydney, 1995), and observations made by various authors when examining the foulant layer that developed while membrane filtrating similar complex biological feed streams (Ousman & Bennasar, 1995, Sayed-Razavi et al., 1996, and Fillaudeau & Carrère, 2002).

4.1.1.1 Initial Cleaning Chemicals

The initial cleaning chemicals were chosen by attempting to anticipate what the foulant would be composed of, selecting an appropriate cleaning chemical (based on data from the literature review), and then cross-checking the selected cleaning chemical against the membrane manufacturer's recommendations.

The feed components that were thought to contribute to the fouling, and the membrane manufacturer's recommended "first choice" cleaning chemical for removing these components, are summarised in Table 4.1. The manufacturer's full membrane cleaning solution recommendations, and the recommended cleaning conditions (concentration, temperature, time), may be found in Appendix J.

Table 4.1. Summary of recommended cleaning solutions.

Foulant	Recommended cleaning solutions
Protein	Sodium hydroxide (0.1 M)
Polysaccharides (including starch)	Sodium hydroxide (0.1 M)
Fat	Triton® X100 (0.1 %) Sodium dodecyl sulfate (SDS) (0.1 %)
Minerals	Phosphoric acid (0.03 M)

4.1.1.2 Additional Cleaning Chemicals

The additional cleaning chemicals trialled, and the reason for their selection, are summarised in Table 4.2.

Table 4.2. Additional cleaning chemicals.

Cleaning solution	Details
Citric acid	Removes metal scale, but can also be effective at removing protein fouling, especially when the fouling contains calcium salt-bridges, which the citric acid breaks down by chelating the calcium (Liu et al., 2006)
Glucoamylase	An enzyme that coverts starch to glucose. Shukla et al. (2000) used a glucoamylase to clean a stainless steel-titania membrane fouled by a cornstarch suspension. They found that although the glucoamylase wash improved the NWP, a final sodium hydroxide plus chlorine wash was needed to complete the clean, and the order of the cleaning steps was important.
NaOH plus chlorine	The combined cleaning power of NaOH with added chlorine has been well documented. Also, Zondervan & Roffel (2007) found oxidisers had a positive effect when cleaning starch from ultrafiltration membranes.
Enzidase® PXT6L	A protease that has been used to solubilise the dough residue produced during the Al-Hakkak process (Paulik & MacManus, 2009).
Tergazyme®	A protease detergent recommended by Millipore as an alternative to sodium hydroxide. As well as a protease, it contains active ingredients to remove polysaccharides and lipids. Chen & Ko (1997) used Tergazyme as part of a cycle to clean mungbean protein from a polysulfone ultrafiltration membrane, and Sayed-Razavi et al. (1996) used it to clean a polysulfone membrane fouled by a soy flour extract.
Urea	A chaotropic agent (disrupts the three dimensional structure in macromolecules) that was shown to be effective at removing starch fouling from cellulose acetate RO membranes (Whittaker et al., 1984).

4.1.2 Materials

Materials used during the cleaning trials were:

- Technical grade sodium hydroxide pearl from Sigma Aldrich.
- Sodium-dodecyl-sulphate (SDS) from Sigma Aldrich.
- Triton X-100 from Sigma Aldrich.
- Technical grade citric acid from Jasol NZ.
- Sodium hypochlorite (Hypostat 135) from Jasol NZ.
- Urea from BDH Laboratory Supplies.
- Phosphoric acid from BDH Laboratory Supplies.
- A protease detergent (Tergazyme®) manufactured by Alconox.
- A protease (Enzidase® PTX6L) from Zymus International Ltd.
- A glucoamylase (MagiZyme® X4) from Zymus International Ltd.

4.1.3 Cleaning Solution Preparation

All cleaning solutions were made up with reverse-osmosis (RO) water to the concentrations recommended by Millipore (Table 4.3), and heated to 45 – 50°C before use.

Table 4.3. Concentration of cleaning solutions.

Chemical	Strength	pH
Blend 1	0.2 % g g^{-1} Tergazyme + 0.2 % g g^{-1} PTX6L	8 – 9
Blend 2	0.1 M NaOH + 50 ppm chlorine	12
Citric acid	1 % g g^{-1}, adjusted to pH 3 with NH_4OH	3
MagiZyme X4 [A]	0.2 g g^{-1}	4 – 5
Phosphoric Acid	0.03 M	2
PTX6L [A]	0.2 % g g^{-1}	8 – 9
SDS	0.1 % g g^{-1}	5 – 8
Sodium hydroxide	0.1 M	12
Tergazyme	0.2 % g g^{-1}	8 – 9
Urea	7 M	8

[A] There were no data from Millipore regarding this chemical so it was made up to the same concentration as the Tergazyme.

4.1.4 Membrane Cleaning Procedure

The membrane was flushed with RO water until the exiting flush-water appeared clear. This typically required 2 – 3 litres of water and was performed at a cross-flow rate of 20 – 30 L h^{-1}, and a TMP of 70 – 100 kPa. During this rinse step the system was configured so that all the rinse water (permeate and retentate) exited to drain after a single pass i.e. no rinse water was recycled. After rinsing, the membrane was washed with one or more of the cleaning reagents from Tables 4.1 and 4.2. Typical conditions were: 45 – 50°C, cross-flow 20 – 30 L h^{-1}, TMP 35 – 70 kPa, and duration 60 minutes. After each chemical wash the membrane was rinsed by passing 2 – 5 litres of RO water through the retentate side of the membrane, and 3.5 – 7 litres of RO water through the permeate side.

Due to time constraints it was not possible to perform the entire washing sequence on the same day as the trial. Therefore, at the end of the first day the cleaning cycle was paused overnight. Depending on how far the cleaning cycle had progressed, the membrane was left overnight full of RO water, 0.1 M sodium hydroxide, or a cleaning solution.

Data sheets complied during the cleaning runs may be found in Appendix K.

4.1.5 Measuring Normalised Water Permeability

The normalised water permeability (NWP) was measured before and after each cleaning step. The NWP measurements were performed by following the method described in the Millipore Maintenance Procedures (Appendix L). In brief, the membrane was flushed with RO water to remove all storage or cleaning solution, the pump speed and back-pressure valve were then adjusted to give an inlet pressure of 70 kPa and an outlet pressure of 35 kPa (TMP 52 kPa). The system was left to stabilise for 5 minutes and then the permeate flow rate was measured. The temperature of the feed water was measured so that a temperature compensation factor could be used to normalise the permeate flow to 25°C. The NWP was calculated using Equation 4.1.

$$NWP = \frac{J \times f}{A \times TMP} \qquad \textbf{Equation 4.1}$$

Where; *NWP* = Normalised Water Permeability (L m^{-2} h^{-1} kPa^{-1})

J = Permeate flux (L h^{-1})

f = Temperature correction factor (dimensionless)

A = Membrane area (m^2)

TMP = Transmembrane pressure (kPa)

4.2 Results and Discussion

For the purpose of this discussion the cleaning trials have been grouped into four sets, where:

- Set 1 trialled the initial cleaning cycle (3 Runs).
- Set 2 tested a number of alternative cleaning chemicals (11 Runs).
- Set 3 further investigated the findings from Set 1 and 2, and proposed an optimal cleaning cycle (7 Runs).
- Set 4 evaluated the proposed cleaning cycle (3 Runs).

The results from the cleaning investigation will be discussed on a set by set basis, in chronological order; this way the key results, and their implications for the following trials, will be most easily followed.

The effectiveness of the membrane cleaning chemicals was assessed by comparing the NWP of the clean membrane with the NWP of the membrane before its very first use (i.e. the virgin membrane). A rule of thumb is that after the first use and clean, the NWP should return to between 60 and 80 % of the virgin NWP, and after repeated use the NWP should not vary by more than 10 % from run to run (Millipore BioProcess Division, n.d.).

4.2.1 Set 1 – Initial Cleaning

The first cleaning method was based on a simple two step approach consisting of (1) a water flush to remove loosely bound material, (2) a hot sodium hydroxide wash to remove adsorbed organic material (protein, lipid, carbohydrate).

This simple two-step method was ineffective. The water rinse increased the NWP by 48 %, but surprisingly, the hot sodium hydroxide wash caused a 7 % decrease in NWP; the resulting NWP was only 43 % of the virgin NWP (see Figure 4.1). Following the two-step cleaning cycle the membrane was stored overnight in 0.1 M sodium hydroxide, and then the cleaning method was extended by adding a surfactant (Triton X-100) wash, and a phosphoric acid wash. The overnight storage in sodium hydroxide improved the NWP to 66 %, and the surfactant and acid washes each further improved the NWP by 5 %. The final NWP was 76 % of the virgin NWP, which was considered an acceptable overall NWP recovery.

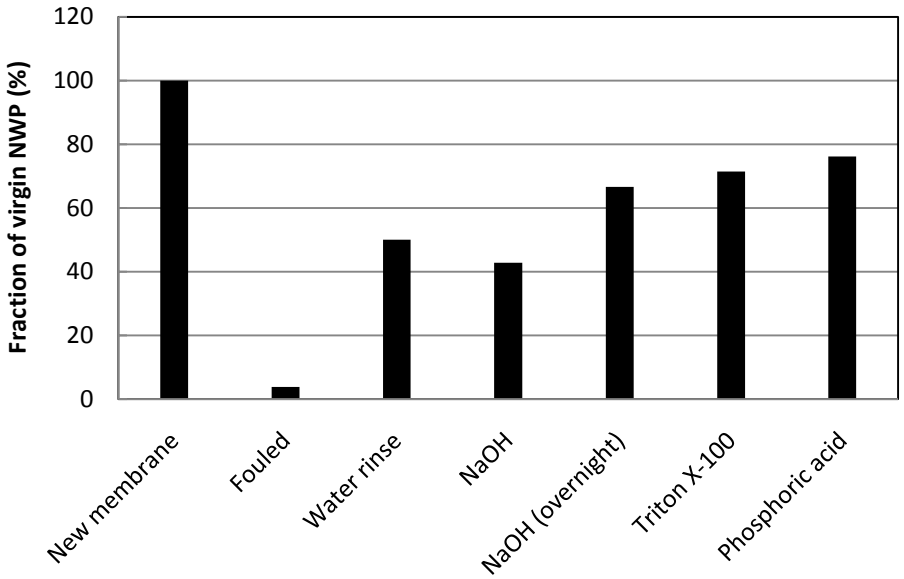

Figure 4.1. Set 1 Run 1, NWP after each step of the cleaning cycle.

During the rinse the water turned slightly cloudy, and on standing a thin layer of white solids settled. These solids were examined by SEM, which showed they were predominantly starch granules, and a small amount of non-starch material (Figure 4.2).

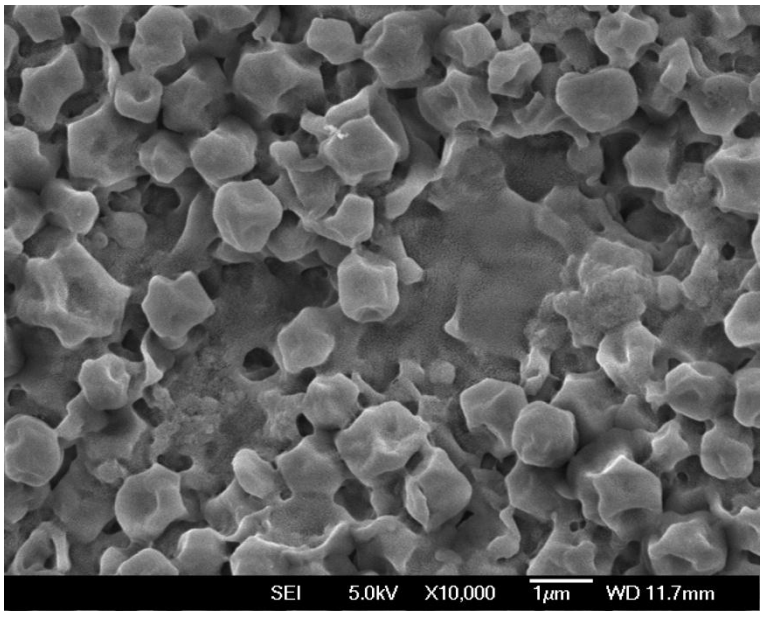

Figure 4.2. SEM image of solids recovered from the rinse water.

It is interesting that the hot sodium hydroxide wash decreased the NWP, while storage in the same strength sodium hydroxide, at room temperature, improved it (a hot wash is generally more effective than a static soak). A possible

explanation as to why the hot sodium hydroxide wash reduced the NWP was proposed after examining work by Roberts & Cameron (2002) that investigated the effect of sodium hydroxide on potato starch gelatinisation. They observed that potato starch granules swell on contact with sodium hydroxide solution, and that if the solution is strong enough, or if heat is applied, the swelling continues until the granules rupture. If it is assumed that (a) the fouling material contains a layer of amaranth starch granules, and (b), amaranth starch will behave in a similar way as potato starch when it is contacted with sodium hydroxide solution, then it is possible that instead of removing the fouling material the hot sodium hydroxide caused the starch granules to swell, and some to rupture. The swollen granules would have less void space between them, which would restrict permeate flow, while the polysaccharides and granule fragments released by the ruptured granules could increase the degree of fouling by adsorbing to the membrane, adding to the already existing gel layer, or entering and blocking some membrane pores.

During one of the membrane characterisation trials performed during this study, the starch-milk feed liquor was centrifuged to remove the starch granules, and the starch-granule-free liquor was concentrated using the microfiltration membrane. Considerable fouling occurred, and a hot sodium hydroxide wash was used as one of the cleaning steps (see Appendix M, Figure M.1). In this starch-granule-free case the hot sodium hydroxide wash did not result in a decrease in NWP. This adds weight to the possibility that the starch granules were interacting with the hot sodium hydroxide and decreasing the NWP.

With respect to the room temperature sodium hydroxide soak increasing the NWP, it was assumed that the absence of any TMP during this step was linked to it being more effective than the hot sodium hydroxide wash, and that the foulant removed was different to the foulant interacting with the hot sodium hydroxide (i.e. the cold sodium hydroxide wash was not acting on the starch granules, but on the protein, non-starch polysaccharides, and lipid). If the assumption that the foulant is multi-layered, consisting of an inner gel layer and an outer starch granule layer is correct, then the presence of some TMP during the hot sodium

hydroxide wash could compress the foulant layer, forcing the swollen outer layer of starch granules closer together, which would reduce the void space between them and restrict the sodium hydroxide from fully interacting with, and removing, the inner gel layer. Removing the TMP, as was the case during the soak, could allow the sodium hydroxide to permeate past the now less compacted starch granules to the gel layer, and to also reach the gel layer from the permeate side of the membrane. The long soak time would partially compensate for any decrease in cleaning effectiveness caused by the lower temperature and absence of shear-inducing cross-flow.

The second membrane cleaning (Set 1 Run 2) was a repeat of Set 1 Run 1, with two small changes. Firstly, an anionic detergent (SDS) was used instead if the non-ionic Triton X-100, and secondly, due to time constraints, the overnight soak occurred after, instead of before, the detergent wash. The resulting NWP profiles were very similar to those from Set 1 Run 1 (see Appendix M, Figure M.2).

During the third, and final, wash cycle of Set 1 (Set 1 Run 3), the NWP-decreasing hot sodium hydroxide wash was omitted, but the overall NWP recovery was still not acceptable. This can be seen in Figure 4.3, which shows a continued, and significant, decrease in NWP after each of the first three uses and cleans. The full NWP profile from Set 1 Run 3 is included in Appendix M (Figure M.3).

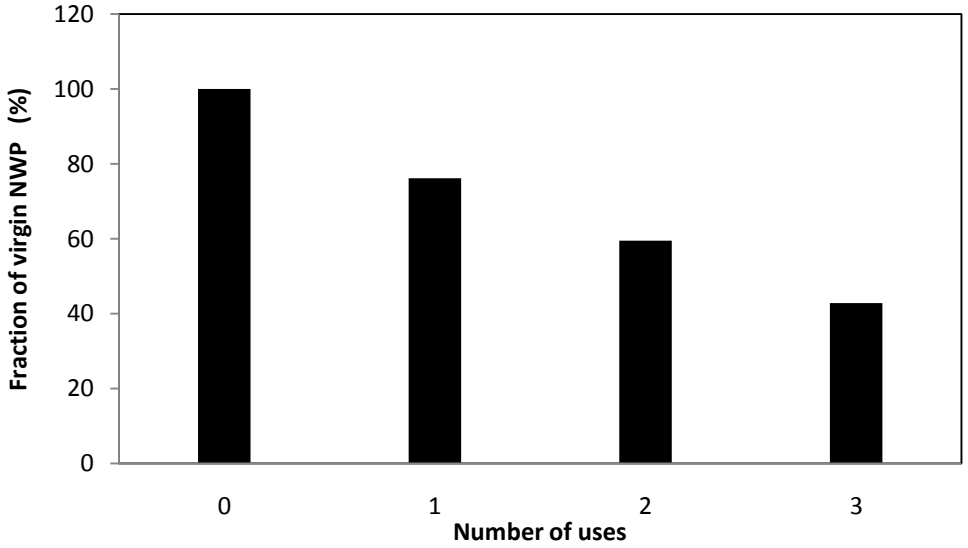

Figure 4.3. Decrease in virgin NWP with use. 0 = virgin membrane, 1 = after Set 1 Run 1, 2 = after Set 1 Run2, 3 = after Set 1 Run 3.

When the NWP continues to decrease with each successive use, as is the case in Figure 4.3, three options can be investigated to improve membrane cleaning. These are (i) pre-treatment of the feed to alter the foulant composition and/or fouling mechanism, (ii) use of an alternative cleaning method, (iii) use of an alternative membrane material. In the present study, developing a better cleaning method was the logical way forward. Altering the feed material was not an option as this would involve either modifying the Al-Hakkak process (which is outside the scope of this project), or altering the starch-milk (adjusting pH, adding flocculating agents, etc); this was not desirable as an aim of the overall research programme is to extract the various biopolymers (starch and protein) in their native form. Trialling an alternative membrane material was considered a last resort. Aside from the additional cost, and the need to repeat the trials already performed using the new membrane, without more detailed knowledge on what was causing the fouling there would be no guarantee that the new material would foul any less, or be any easier to clean (although a more resilient membrane, enabling the use of harsher cleaning conditions, would have an advantage).

4.2.2 Set 2 – Screening of Additional Cleaning Chemicals

In an effort to restore the NWP to at least 80 % of its virgin value, a number of alternative cleaning chemicals were trialled. These are shown, in the order they were used, in Table 4.4.

Table 4.4. Cleaning chemicals trialled during Set 2.

Run	Cleaning agent	Strength	Duration	Temperature
1	Glucoamylase	0.2 %	1 h	45 – 50˚C
2	Sodium hydroxide	0.1 M	1 h	45 – 50˚C
3	Tergazyme (protease)	0.2 %	1 h	45 – 50˚C
4	Sodium hydroxide + chlorine	0.1 M, 50 ppm [A]	1 h	45 – 50˚C
5	Sodium hydroxide (soak)	0.1 M	18 h	10 – 20˚C [B]
6	Citric acid	1 % pH 3	1 h	45 – 50˚C
7	Tergazyme (protease, soak)	0.2 %	18 h	10 – 20˚C [B]
8	Urea	7 M	1 h	45 – 50˚C
9	Sodium hydroxide (soak)	0.1 M	18 h	10 – 20˚C [B]
10	Glucoamylase	0.2 %	3 h	45 – 50˚C
11	Sodium hydroxide	0.1 M	1 h	45 – 50˚C

[A] A higher chlorine level (250 – 500 ppm) is generally used for cleaning, but 50 ppm is the maximum allowable for the membrane material used.
[B] Ambient temperature.

The effectiveness of these cleaning solutions is shown in Figure 4.4. When evaluating these results it must be kept in mind that the membrane had already been partially cleaned during Set 1, and the cleaning steps used in Set 2 were performed sequentially i.e. the membrane was not re-fouled between cleaning steps. It can be seen that only four steps improved the NWP. The protease detergent (Set 2 Run 7), storage in sodium hydroxide (Set 2 Run 9), and the glucoamylase wash (Set 2 Run 10), each resulted in a small (5 – 7 %) increase in NWP, whereas the sodium hydroxide wash (Set 2 Run 11) had a dramatic effect, returning the NWP almost to its virgin value.

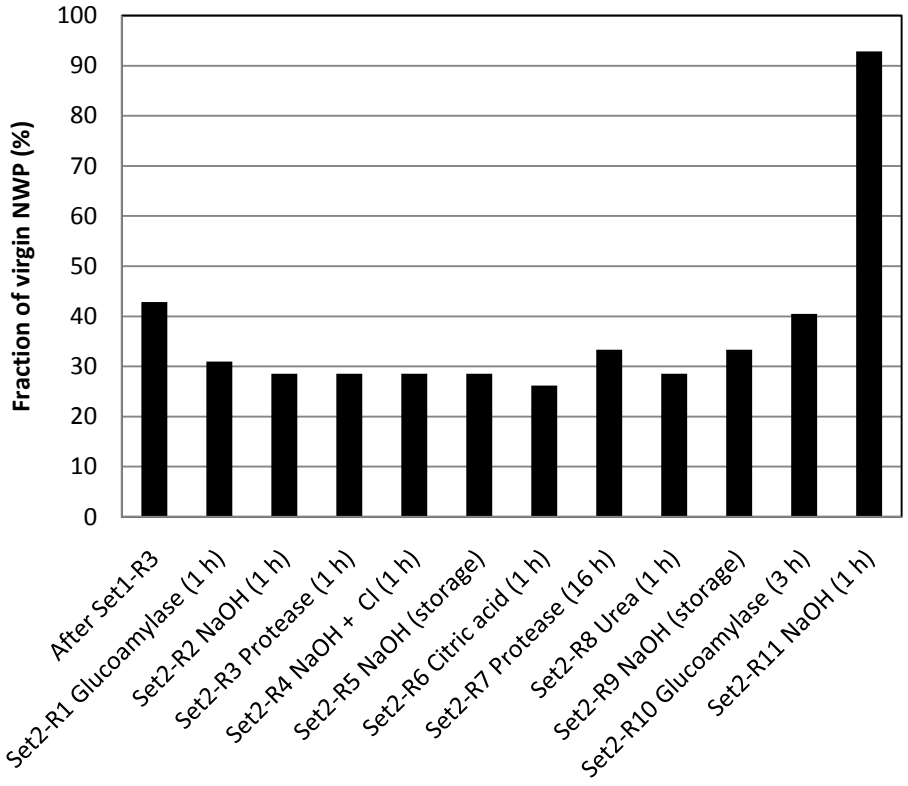

Figure 4.4. Set 2, NWP after each cleaning step.

The success of the sodium hydroxide wash (Set 2 Run 11) contradicts earlier observations that showed a hot sodium hydroxide wash was ineffective (see Set 1 Run 1, and Set 2 Run 2). It was postulated that the success of Run 11 was due to the preceding glucoamylase wash (Set 2 Run 10) altering the starch component of the foulant in such a way that the subsequent sodium hydroxide wash was more effective. Similarly, the first glucoamylase wash (Set 2 Run 1) was ineffective (it actually reduced the NWP), while the second glucoamylase wash (Run 10) was effective, suggesting that a step was needed to pre-treat the starch prior to the glucoamylase wash.

It must be noted that during the second glucoamylase wash (Set 2 Run 10) an equipment malfunction occurred that could have improved the cleaning effectiveness. At some time during the wash, the rubber hose that returns the wash liquor from the permeate side of the membrane to the feed reservoir kinked and shut off the permeate flow. This caused the pressure to increase on the permeate side of the membrane, which could have resulted in some unintentional backflushing. While backflushing is a well practised membrane

cleaning technique, the membrane used during this work is not designed to be backflushed; it has a low maximum allowable reverse TMP (35 kPa), above this pressure the membrane may delaminate. Following the cleaning run which may have included some unintentional backflushing the membrane was given an integrity test (see Appendix N) and did not show any signs of delaminating.

The main conclusions drawn from Set 2 were:

- A glucoamylase wash, followed by a sodium hydroxide wash, gave an acceptable increase in NWP, but, a pre-treatment step is needed prior to the glucoamylase wash.
- The positive result may have been partially attributed to some unintentional backflushing.
- The protease detergent had a small positive effect.

4.2.3 Set 3 – Further Investigations

Set 3 was performed to further investigate the main findings from Set 2, as such the aims were to:

i) Confirm which step was acting as a pre-treatment to the glucoamylase wash.
ii) Investigate backflushing.
iii) Assess proteases as a cleaning agent.

4.2.3.1 Pre-treatment

Cleaning steps performed during Set 2 that could have acted as a pre-treatment to the glucoamylase wash were:

- *Protease detergent wash.* This would remove any protein, polysaccharides, or lipids that may have coated the starch granule, and therefore provide improved contact between the starch granules and glucoamylase. In addition, this wash may remove material that could inhibit the enzyme.
- *Hot sodium hydroxide*. If the earlier postulation that a hot sodium hydroxide wash partially solubilises, or gelatinises, the starch granules is correct, then this would improve the glucoamylase wash. Although

glucoamylase is effective on intact starch granules, the reaction is slow; if the granules are solubilised first the hydrolysis is much more rapid (Oates, 1997).

- *Hot sodium hydroxide with chlorine*. The effects of this wash are the same as for the hot sodium hydroxide, with the added benefit that chlorine can oxidise starch. However, due to the low chemical tolerance of the membrane only a very low level of chlorine can be used, which means the full potential of the chlorine could not be realised.
- *Urea wash*. Urea is a chaotropic agent (i.e. it disrupts the structure of macromolecules). If it effectively disrupts the starch granules, it will result in a larger surface area and faster enzyme action. Urea has also been shown to gelatinise starch (Hebeish et al., 1981), but it is unknown if the concentration and time used during the wash were enough to cause such an effect.
- *Acid wash*. Acid is known to modify starch granules, and is used intentionally to alter the characteristics of starch. During starch acid-modification, acid penetrates the amorphous parts of the starch granule and hydrolyses glucosidic bonds (Hoseney, 1994); this could make the granules more susceptible to enzyme hydrolysis. However, as an acid wash was used as part of Set 1 Run 3, if it was an effective pre-treatment a positive cleaning result should have been obtained with the first glucoamylase and sodium hydroxide wash (Set 2 Run 1 & Run2). The likely explanation as to why a positive cleaning effect did not result from Set 2 Run 1 & Run 2 is that the acid strength (which is limited by the chemical resistance of the membrane), and contact time, were lower than what is used during starch acid-modification processes.

Three trials were performed to determine which step was most effective as a pre-treatment to the glucoamylase wash. A protease wash was included in all trials, and the difference in NWP between the protease wash and final sodium hydroxide wash was used to assess cleaning effectiveness. Due to the low tolerance of the membrane to chlorine the sodium-hydroxide-plus-chlorine wash

was considered a last resort, only to be trialled if the other options were unsuccessful. The cleaning steps used in each trial are shown by Table 4.5.

Table 4.5. Cleaning cycles used during the pre-treatment trials.

Run	Water	Protease	Pre-treatment[A]	Glucoamylase	Sodium hydroxide
Set 3 Run 1	✓		Protease	✓	✓
Set 3 Run 2	✓	✓	Urea	✓	✓
Set 3 Run 3	✓	✓	Sodium hydroxide	✓	✓

[A] All pre-treatments performed at 40 – 50°C, for 1 h.

The results are summarised in Table 4.6, and a chart showing the NWP after each step is included in Appendix M (Figure M.4). The sodium hydroxide wash was the most effective as a pre-treatment, resulting in a 14 % increase in NWP after the subsequent glucoamylase and sodium hydroxide washes. These results are considered indicative only, as there was some variation in how fouled the membrane was, and the effectiveness of the initial water flush and protease wash (see Appendix M, Figure M.4).

Table 4.6. Effectiveness of a glucoamylase, then sodium hydroxide wash, after various pre-treatments.

Pre-treatment	Improvement in NWP after the subsequent glucoamylase and sodium hydroxide washes (% of virgin NWP)
Protease wash	2
Urea wash	7
Sodium hydroxide wash	14

Although an effective pre-treatment step was found during Set 3, the NWP did not return to the “almost virgin value” obtained during Set 2. The NWP of the clean membrane was typically 60 – 70 % of the virgin NWP. A possible reason is

that some beneficial backflushing occurred during Set 2 (Set 2 R10) but not during Set 3.

4.2.3.2 Backflushing

A one-off backflushing trial (Set 3 Run 4) was performed by circulating reverse osmosis water through the permeate side of the membrane for thirty minutes, while restricting the outlet flow to provide a backpressure of 28 kPa. The NWP before and after backflushing did not change, which shows that backflushing under these conditions was ineffective.

This does not mean that no beneficial backflushing occurred during Set 2 Run 10, but it does suggest that any backflushing that did occur was at a pressure higher than the manufacturer's recommendations. Repeating these conditions was not attempted due to the risk of damaging the membrane.

4.2.3.3 Protease Wash

During Set 2 the protease detergent (Tergazyme) showed some cleaning effect, even though by the time it was used the membrane had already undergone a number of wash steps, many of which could have reduced the amount of protein-based fouling present. The effectiveness of a protease wash was further investigated by using Tergazyme and an alternative protease (PTX6L) to clean a fouled membrane. The two different proteases were used successively (Tergazyme then PTX6L) in Set 3 Run 5, successively in the reverse order (PTX6L then Tergazyme) in Set 3 Run 6, and then as a mix containing equal parts Tergazyme and PTX6L in Set 3 Run 7. The results (Figure 4.5) show that both Tergazyme and PTX6L had a positive cleaning effect, and that using them both gave a better clean than using just one of them i.e. when combined into one solution their cleaning effects were summed.

Combining the two proteases has the advantages of removing a cleaning step, which reduces the overall cleaning time, and removing the need to monitor and control the pH during the PTX6L wash (as the Tergazyme is buffered to pH 9, which is within the operating range of the PTX6L).

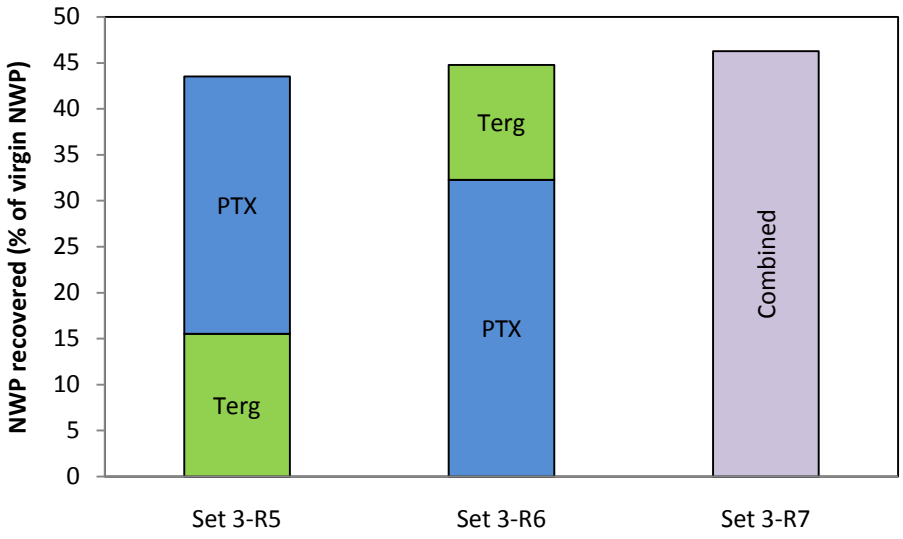

Figure 4.5. Set 3, evaluation of Tergazyme and PTX6L.

4.2.3.4 Proposed Cleaning Cycle

Based on the results from Set 1 – Set 3, the following five-step cleaning cycle was proposed:

1) A water rinse to remove loosely bound material.
2) A wash with a solution containing equal parts Tergazyme and PTX6L to remove protein, lipid, and carbohydrate.
3) A sodium hydroxide wash as a pre-treatment to the glucoamylase wash.
4) A glucoamylase wash to breakdown the starch.
5) A sodium hydroxide wash to remove products of the enzymatic process and residual enzyme.

The recommended operating conditions of the proposed cleaning cycle are shown in Table 4.7.

Table 4.7. Recommended cleaning conditions.

Step	Description	Details	Temperature	Time
1	Water flush	RO water	Ambient	N/A
2	Protease wash	0.2 % Tergazyme, 0.2 % PTX6L, pH 8 – 9	45 – 50°C	1 h
3	Sodium hydroxide	0.1 M	45 – 50°C	1 h
4	Glucoamylase	0.2 %, pH 4 – 5	45 – 50°C	3 h
5	Sodium hydroxide	0.1 M	45 – 50°C	1 h

4.2.4 Set 4 – Confirmation of the Proposed Cleaning Cycle

The proposed cleaning method was used to clean the membrane after each of the next three microfiltration trials. The overall results are shown in Figure 4.6, and the normalised NWP after each step is shown in Figure 4.7. Note, in these figures the vertical axis represents the fraction of the "before use" NWP, not the fraction of the virgin NWP.

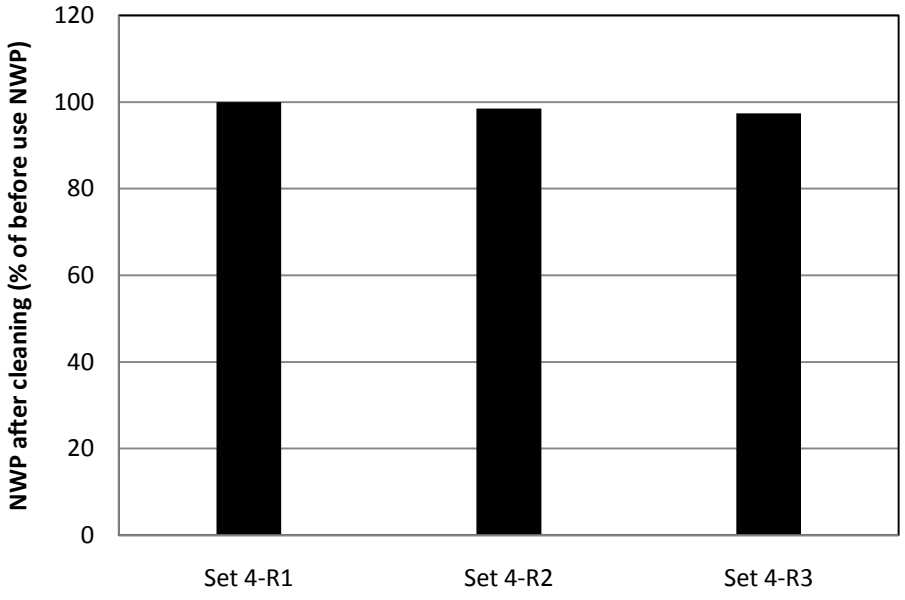

Figure 4.6. Effectiveness of the proposed cleaning cycle.

The proposed cleaning cycle consistently resulted in an NWP that was greater than 97 % of the pre-use value. Therefore, the cleaning method was considered satisfactory. The slight decrease in NWP that occurred may be to due to small

amounts of enzyme-resistant starch becoming entrapped in the membrane. Ideally the membrane would be dissected, and its interior surface examined to identify residual foulant. Such an examination could also reveal why the “before use” NWP stabilised at 60 % (and not 100 %) of the virgin NWP. This examination was not possible during this project, as the membrane was need for additional trials.

The NWP profile of the various steps (Figure 4.7) shows that:

- The water rinse was less effective than in the earlier trials (Set 1 and Set 2). This could be because a higher TMP was used during the runs that fouled the membrane. The foulant layer would therefore be more tightly compressed and tightly bound.
- The protease wash performs the majority of the cleaning. This is not surprising as during the membrane characterisation work it was shown that the soluble feed components were the major foulant, and not the starch granules (see Section 3.2.5.2).
- The NaOH-glucoamylase-NaOH sequence is needed to finish the cleaning.

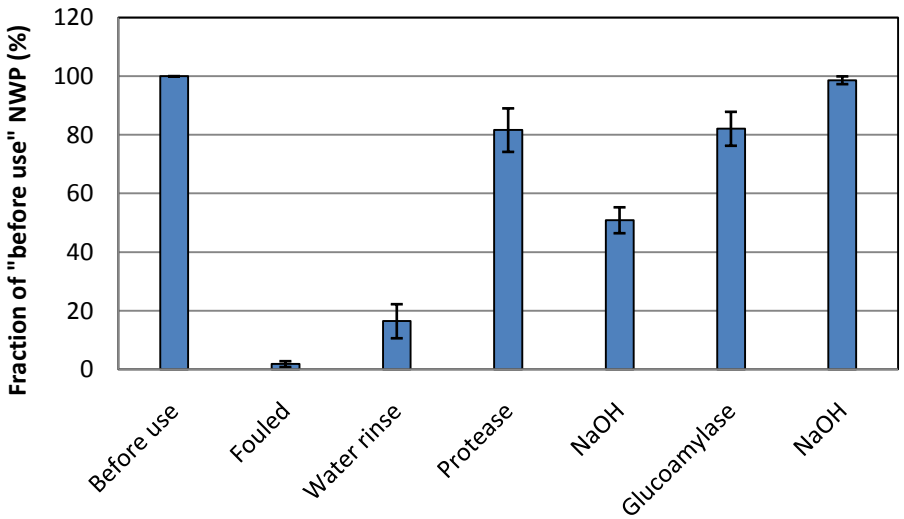

Figure 4.7. NWP profile of the improved cleaning cycle. Error bars are +/- one standard deviation.

The combined results from all the cleaning trials appear to support the proposed fouling mechanism. Analysis of the rinse water showed that it predominantly removed starch granules (Figure 4.2). The high NWP recovery achieved by the protease wash (Figure 4.7) shows that a significant proportion of the foulant was

protein based, and the fact that a glucoamylase wash also improved the NWP indicates that some tightly bound starch granules formed part of the foulant.

These cleaning trials were performed so that the membrane could be returned to an acceptable, and repeatable, NWP between membrane characterisation runs; they were not performed to develop a fully optimised cleaning method. As such, although the cleaning method met the needs of this research, there is room for further improvement. For example, further investigations could be performed into the optimal concentrations, temperatures, and duration of the steps used, or into using different cleaning chemicals (e.g. formulated membrane cleaners).

4.3 Membrane Cleaning Results Summary

The membrane proved very difficult to clean. The "recommended" sodium hydroxide based cleaning method was ineffective, necessitating the need to develop an improved procedure. A number of different cleaning chemicals were trialled, and although no single chemical was completely effective, when a number of cleaning chemicals were used in succession the membrane was adequately cleaned. The following cleaning steps were required, and the order of the steps was important:

- A water rinse to remove loosely bound material.
- A wash with a solution containing equal parts Tergazyme and PTX6L to remove protein, lipid, and carbohydrate.
- A sodium hydroxide wash as a pre-treatment to the glucoamylase wash.
- A glucoamylase wash to breakdown the starch.
- A sodium hydroxide wash to remove products of the enzymatic process and residual enzyme.

Although adequate for the needs of this work there is room to optimise the cleaning cycle, particularly the concentration of the wash solutions and duration of the individual cleaning steps.

5 CONCLUSIONS

The 1000 kDa regenerated cellulose membrane used in this investigation successfully retained the starch granules, and had an acceptably low retention for the non-starch polysaccharide and ash components of the feed. However, the protein and fat retentions were higher than desired. Diafiltration lowered the protein and fat content of the starch-rich retentate, but not far enough to reach commercially acceptable levels. Examination of the starch-milk produced using the pilot-scale Al-Hakkak process showed it contained some insoluble proteinaceous material, the presence of which largely determined the lowest protein content achievable after diafiltration.

The flux-time profile showed the starch-milk was high-fouling. Comparisons between the standard feed, soluble fraction of the feed, and starch fraction of the feed, showed that fouling was dominated by the soluble feed components. As such, the major underlying mechanism of flux reduction was interpreted as gel formation on the membrane surface.

Flux had a three stage relationship with volumetric concentration (VCF). During the first stage flux reduced almost linearly with increasing VCF, in the second stage flux increased slightly with increasing VCF, and in the third stage flux was independent of VCF. The second stage flux increase is unusual, and could be the subject of a separate study. Understanding why the flux increased could provide insight into methods to achieve a higher flux.

Flux increased with increasing TMP. The optimal TMP was approximately 100 – 150 kPa, above this pressure flux increased non-linearly with increasing pressure, following a power-law trend which indicated the foulant, or caked material, was compressible. Increasing the feed rate (within the limitations of the equipment used) did not result in an increase in flux.

The membrane proved very difficult to clean. A multi-step cleaning cycle was developed to adequately clean the membrane between runs; key cleaning steps were a cold water rinse to remove loosely bound material, a protease wash to remove protein, a sodium hydroxide wash to “pre-treat” any remaining starch

granules, an amylase wash to degrade the starch granules, and a final sodium hydroxide wash to remove residues from the previous step.

This cleaning method should be applicable to all membrane materials (that have been fouled by the feed stream, or similar feed streams to that used in this research) as it uses conditions suitable for regenerated cellulose, which is the least tolerable membrane material as far as chemical resistance and temperature are concerned.

6 RECOMMENDATIONS

This study has shown that microfiltration can be used to generate a starch-rich concentrate from the starch-milk produced by the pilot-scale Al-Hakkak process, but additional work is required to lower the protein, and fat, content of the starch-rich concentrate. Research into reducing membrane fouling and increasing membrane flux would also be advantageous. The following recommendations are made:

- Investigate options for eliminating the insoluble protein from the starch-milk. Ideally this would be done by modifying the pilot-scale Al-Hakkak process to prevent the inclusion of insoluble protein in the starch-milk. If this is not possible, a process to remove the insoluble protein should be developed (e.g. adding an alkali or protease wash). Where this step is added depends on whether the soluble protein is going to be separated as a co-product (as the treatment will degrade the soluble, as well as the insoluble, protein). If the soluble protein is desired, the additional step will need to be added after the soluble protein has been separated (e.g. between the microfiltration and diafiltration steps). If the soluble protein is not desired, the starch-milk could be treated before the microfiltration. Regardless of the where the step is added, or the method used, research will be needed to determine if the properties of the starch are altered by the protein removal step. The performance and fouling characteristics of the membrane are likely to change with the altered feed-stock, requiring a re-evaluation of the membrane selectivity, flux relationships, and cleanability.
- Research to gain an in-depth understanding of the mechanisms contributing to the membrane fouling would be valuable. This would help identify the most suitable membrane material, shed light on techniques that could improve membrane cleaning, and identify if desired soluble proteins are being lost in the fouling layer.
- Investigate if protein retention can be decreased by using a membrane with a larger pore size. The starch retention of the 1000 kDa membrane

used in this trial was 100 %. It is possible a membrane with a larger pore size would retain as much starch, but also allow more protein to pass through into the permeate.

- Trial a membrane that can be more easily, and thoroughly, cleaned. This would reduce the time required for membrane cleaning, and may improve the level of cleanliness (i.e. the NWP of the clean membrane may be closer to the NWP of the virgin membrane). Possible membranes include those that can be back-flushed, or cleaned using harsher conditions. For example, tubular ceramic membranes can be back-flushed, withstand temperatures high enough to gelatinise starch, and can be cleaned using chlorine.
- Optimise the cleaning cycle. Although suitable for the needs of this research, the cleaning cycle was not fully optimised. Further research is recommended to fully optimise the temperature, duration, and chemical concentration of each step.
- The microfiltration equipment was run in constant-TMP mode, and a low TMP was found to be optimal. It may be possible to increase flux (which would reduce the required membrane area and/or processing time) by operating in constant-flux mode, or operating with frequent back-flushing. Further research is into these areas is recommended.
- Increasing the operating temperature generally increases flux, and there is room to increase the operating temperature without exceeding the gelatinisation temperature of the starch. Trials could be run, at 40°C for example, to determine if a higher flux can be obtained without any detrimental effects (e.g. denaturing the soluble proteins, or Maillard reactions between the proteins and carbohydrates).

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APPENDICES

Appendix A: PLCXK Pellicon 2 Mini Ultrafiltration Module

Table A.1. Pellicon 2 PLCXK Mini Ultrafiltration Module

Description	Value
Trade Name	Pellicon
Prefiltration Requirement	100 µm
Screen Material	Polypropylene
Operating Temperature Range	4 – 50˚C
Recirculation Rate	5 – 35 L/min/m² @ 0.4 bar (6 psi)
Length	21 cm (8.3 in)
Configuration	Cassette
Filter Material	Composite Regenerated Cellulose
pH Range	2 – 13
Filter Brand Name	Ultracel
Filtration Area	0.1 m²
Height	1.5 cm (0.6 in)
Filter Type	Ultrafiltration
NMWL	1000 kDa
Width	5.6 cm (2.2 in)
Ultrafiltration Product Type	Cassettes
Adhesive Material	Polyurethane
Filter Code	PLCXK
Max Transmembrane Pressure	3.5 bar (50 psig) @ 30˚C

(Table sourced from Millipore, http://www.millipore.com/catalogue/item/P2C01MC01, viewed 13/11/09)

Appendix B: Membrane Characterisation Trial Data Sheets

Table B.1. Trial Data Sheet Run 1

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Run 1 - 1.0 L (W1 and W2 mixed)	14:30	00:00	30/4	0.8	0.0	19.1	4.12	22.4	0	0.8	0.4
	14:35	00:05	30/4	0.9	0.0	18.4	3.24	22.5	0	0.9	0.5
	14:40	00:10	30/4	0.9	0.0	17.9	2.77	22.7	0	0.9	0.5
	14:45	00:15	30/4	0.9	0.0	17.4	2.34	23.0	0	0.9	0.5
	15:00	00:30	31/4	0.9	0.0	17.8	2.09	23.2	0	0.9	0.5
	15:15	00:45	31/4	0.9	0.0	17.2	1.79	23.1	0	0.9	0.5
	15:30	01:00	31/4	0.9	0.0	17.2	1.59	22.7	0	0.9	0.5
Increse feed rate	15:40	00:00	50/4	1.6	0.1	30	2.08	22.0	0	1.5	0.9
	15:50	00:10	50/4	1.6	0.1	30	2.10	21.9	0	1.5	0.9
	16:00	00:20	50/4	1.6	0.1	30	2.15	22.2	0	1.5	0.9

Table B.2. Trial Data Sheet Run 2

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Start, R20 P0.6	10:25	00:01	44/4	1.0	0.1	(26)	(4)		0	0.9	0.6
	10:28	00:03	44/4	1.0	0.1	21.7	2.20	26.2	0	0.9	0.6
	10:35	00:10	44/4	1.0	0.1	21.3	1.93	25.4	0	0.9	0.6
	10:40	00:15	44/4	1.0	0.1	21.0	1.79	25.3	0	0.9	0.6
	10:55	00:30	44/4	1.0	0.1	20.4	1.58	25.3	0	0.9	0.6
	11:10	00:45	45/4	1.0	0.1	19.7	1.46	25.6	0	0.9	0.6
	11:25	01:00	45/5	1.0	0.1	20.1	1.39	25.1	0	0.9	0.6
	11:40	01:15	45/5	1.0	0.1	19.8	1.34	25.2	0	0.9	0.6
	11:55	01:30	45/5	1.0	0.1	19.7	1.24	25.1	0	0.9	0.6
	12:10	01:45	45/5	1.0	0.1	19.2	1.15	25.3	0	0.9	0.6
	12:25	02:00	45/5	1.0	0.1	19.2	1.11	25.4	0	0.9	0.6
	12:40	02:15	45/5	1.0	0.1	18.6	1.09	25.1	0	0.9	0.6
	12:55	02:30	45/5	1.0	0.1	18.4	1.03	25.2	0	0.9	0.6
	13:10	02:45	45/5	1.0	0.1	18.6	0.98	25.3	0	0.9	0.6
	13:25	03:00	45/5	1.0	0.1	18.2	0.94	25.1	0	0.9	0.6
R20 P1.0	01:30		53/4	1.5	0.5	(20)			0	1.0	1.0
	01:40		53/4	1.5	0.5	18.7	1.10	25.0	0	1.0	1.0
	01:45		53/4	1.5	0.5	18.7	1.10	25.0	0	1.0	1.0
R20 P1.5	01:48		63/4	2.0	1.0	18.2			0	1.0	1.5
	01:58		63/4	2.0	1.0		1.23	25.3	0	1.0	1.5
	02:03		63/4	2.0	1.0		1.19	25.1	0	1.0	1.5
R20 P2.0	02:07		81/4	2.5	1.5	18.2			0	1.0	2.0
	02:12		81/4	2.5	1.5		1.27	25.3	0	1.0	2.0
	02:17		81/4	2.5	1.5		1.24	25.1	0	1.0	2.0
	02:22		81/4	2.5	1.5		1.21	24.9	0	1.0	2.0

Table B.2 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
R20 P1.5	02:25		71/4	2.0	1.0	{20)			0	1.0	1.5
	02:30		71/4	2.0	1.0	17.9			0	1.0	1.5
	02:35		71/4	2.0	1.0		1.00	25.1	0	1.0	1.5
	02:40		71/4	2.0	1.0		1.00	25.4	0	1.0	1.5
R20 P1.0	02:43		56/4	1.5	0.5	(20}		25.2	0	1.0	1.0
	02:48		56/4	1.5	0.5	18.0			0	1.0	1.0
	02:53		56/4	1.5	0.5		0.70	25.2	0	1.0	1.0
	02:58		56/4	1.5	0.5		0.69	25.1	0	1.0	1.0
R20 P0.5	03:02		47/4	1.1	0.1	(19)		25.3	0	1.0	0.6
	03:07		47/4	1.1	0.1	17.0			0	1.0	0.6
	03:12		47/4	1.1	0.1		0.47	25.2	0	1.0	0.6
	03:17		47/4	1.1	0.1		0.47	25.1	0	1.0	0.6
R30 P1.0	03:30		77/4	2.1	0.1	(30)		25.2	0	2.0	1.1
	03:38		77/4	2.1	0.1	27.8	0.69	25.7	0	2.0	1.1
	03:45		77/4	2.1	0.1		0.69	25.5	0	2.0	1.1
R30 P1.5	03:50		81/4	2.5	0.5	27.5		25.3	0	2.0	1.5
	04:00		81/4	2.5	0.5		0.88	25.2	0	2.0	1.5
	04:15		81/4	2.5	0.5		0.87	25.5	0	2.0	1.5
R30 P2.0	04:25		77/4.5	2.9	0.9	(30)		25.3	0	2.0	1.9
	04:28		77/4.5	2.9	0.9		1.16	25.5	0	2.0	1.9
	04:30		77/4.5	2.9	0.9		1.11	25.8	0	2.0	1.9
	04:32		77/4.5	2.9	0.9		1.03	25.9	0	2.0	1.9
	04:35		77/4.5	2.9	0.9		1.07	26.1	0	2.0	1.9

Table B.2 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
R30 P 1.5	04:37		69/4.5	2.6	0.4	(30)		25.8	0	2.2	1.5
	04:40		69/4.5	2.6	0.4		0.94	25.9	0	2.2	1.5
	04:43		69/4.5	2.6	0.4		0.85	26.0	0	2.2	1.5
	04:47		69/4.5	2.6	0.4		0.89	25.9	0	2.2	1.5
R30 P1.0	04:52		82/4	2.1	0.1	(30)		26.1	0	2.0	1.1
	04:55		82/4	2.1	0.1	26.4			0	2.0	1.1
	04:57		82/4	2.1	0.1		0.72	26.1	0	2.0	1.1
	05:02		82/4	2.1	0.1		0.72	25.3	0	2.0	1.1
R20 P0.6 (compare with earlier)	05:03		57/4	1.1	0.1	(20)		25.1	0	1.0	0.6
	05:06		57/4	1.1	0.1				0	1.0	0.6
	05:10		57/4	1.1	0.1		0.45		0	1.0	0.6
	05:13		57/4	1.1	0.1		0.45	24.6	0	1.0	0.6

Table B.3. Trial Data Sheet Run 3

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
	09:35	00:00	33/4	1.4	0.1	30	6	25.7	0	1.4	0.8
	09:37	00:02	33/4	1.4	0.1	26.2	3.24		0	1.4	0.8
	09:45	00:10	37/4	1.7	0.1	27.2	2.90	25.0	0	1.7	0.8
	10:05	00:30	37/4	1.7	0.1	24.4	1.58	25.5	0	1.7	0.9
	10:20	00:45	39/4	1.8	0.1	24.4	1.48	25.3	0	1.7	0.9
	10:35	01:00	40/4	1.8	0.1	24.0	1.34	25.6	0	1.7	0.9
	10:50	01:15	40/4	1.8	0.1	23.3	1.28	25.2	0	1.7	0.9
	11:05	01:30	40/4	1.8	0.1		1.24	25.3	0	1.7	0.9
	11:20	01:45	40/4	1.7	0.1	21.5	1.15	24.8	0	1.7	0.9
	11:35	02:00	40/4	1.8	0.1		1.16	25.3	0	1.7	0.9
	11:50	02:15	40/4	1.8	0.1		1.10	25.1	0	1.7	0.9
	12:05	02:30	41/4	1.8	0.1	21.2	1.07	25.0	0	1.7	0.9
	12:20	02:45	42/4	1.8	0.1		1.07	25.1	0	1.7	0.9
	12:35	03:00	42/4	1.8	0.1	21.0	1.00	25.2	0	1.7	0.9
	12:50	03:15	42/4	1.8	0.1	19.9	1.00	25.4	0	1.7	0.9
	13:05	03:30	43/3	1.8	0.1		0.96	24.7	0	1.7	0.9
	13:20	03:45	43/4	1.8	0.1		0.95	25.0	0	1.7	0.9

Table B.4. Trial Data Sheet Run 4

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Steady state	09:55	00:00	36/3.5	1.0	0.1	(20)	(4)	24.6	0	0.9	0.5
	09:56	00:01	36/3.5	1.0	0.1	(20)	2.69		0	0.9	0.5
	10:00	00:05	36/3.5	1.0	0.1	(21)	2.20	24.4	0	0.9	0.5
	10:05	00:10	36/3.5	1.0	0.1	(21)	1.93	24.4	0	0.9	0.5
	10:10	00:15	35/3.5	1.0	0.1	20.7	1.88	24.6	0	0.9	0.5
	10:16	00:21	35/3.5	1.0	0.1	(22)	1.74	24.7	0	1.0	0.6
	10:20	00:25	34/3.5	1.0	0.1	21.1	1.57	24.5	0	1.0	0.6
	10:26	00:31	34/3.5	1.0	0.1	(22)	1.44	24.2	0	0.9	0.5
	10:40	00:45	34/3.5	1.0	0.1	19.3	1.25	24.9	0	0.9	0.5
	10:55	01:00	35/3.5	1.0	0.1	(21)	1.22	25.3	0	0.9	0.5
	11:10	01:15	35/3.5	0.9	0.1	18.4	1.15	25.4	0	0.8	0.5
	11:25	01:30	37/3.5	1.0	0.1	19.1	1.12	25.6	0	0.9	0.5
	11:40	01:45	37/3.5	0.9	0.1	18.9	1.00	24.4	0	0.8	0.5
	11:55	02:00	38/3.5	1.0	0.1	(20)	0.99	24.9	0	0.9	0.5
	12:10	02:15	38/3.5	1.0	0.1	18.5	0.90	25.5	0	0.9	0.5
	12:25	02:30	39/3.5	1.0	0.1	18.9	0.89	24.8	0	0.9	0.5
	12:40	02:45	39/3.5	1.0	0.1	(21)	0.76	24.9	0	0.9	0.5
	12:55	03:00	40/3.5	1.0	0.1	(21)	0.71	25.1	0	0.9	0.5
	13:10	03:15	40/3.5	1.0	0.1	19.4	0.62	25.6	0	0.9	0.5
	13:25	03:30	40/3.5	1.0	0.1	(20)	0.60	25.2	0	0.9	0.5
Increase TMP (1 bar)	13:26	00:00	51/3.5	1.4	0.6	19.6	0.82	24.9	0	0.9	1.0
	13:36	00:10	47/3.5	1.5	0.6	20	0.79	25.0	0	1.0	1.0
	13:46	00:20	47/3.5	1.5	0.6	20	0.74	25.1	0	1.0	1.0
Increase TMP (1.5 bar)	13:47	00:00	59/3.5	2.1	1.0	20	0.93	25.2	0	1.0	1.6
	13:57	00:10	59/3.5	2.0	1.0	(21)	0.94	25.8	0	1.0	1.5

Table B.4 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
	14:07	00:20	59/3.5	2.0	1.0	(21)	0.89	25.8	0	1.0	1.5
Increase feed rate (30 L/h)	14:12	00:00	88/3.5	1.8	0.2	30.3	0.70	25.6	0	1.6	1.0
	14:22	00:10	88/3.5	1.8	0.2	(30)	0.68	25.0	0	1.6	1.0
	14:32	00:20	88/3.5	1.8	0.2	(30)	0.74	25.0	0	1.6	1.0
Increaase TMP	14:33	00:00	70/3.5	2.3	0.6	29.6	1.01	24.9	0	1.7	1.5
	14:43	00:10	64/3.5	2.3	0.7	(30)	0.97	25.1	0	1.7	1.5
	14:53	00:20	64/3.5	2.3	0.7	(30)	1.01	25.1	0	1.7	1.5
Check base-line	14:56	00:00	39/3.5	1.0	0.1	(20)	0.49	25.0	0	0.9	0.5
	15:06	00:10	39/3.5	1.0	0.1	(20)	0.55	24.9	0	0.9	0.5
	15:16	00:20	40/3.5	1.0	0.1	(20)	0.62	24.7	0	0.9	0.5
Increase feed rate (40 L/h)	15:22	00:00	69/4	2.8	0.3	(41)	1.30	25.0	0	2.6	1.6
	15:32	00:10	70/4	2.8	0.3	(40)	1.28	25.8	0	2.4	1.6
	15:42	00:20	70/4	2.8	0.3	(40)	1.30	25.3	0	2.4	1.6
Decrease TMP	15:45	00:00	64/4	2.3	0.1	(40)	0.99	24.8	0	2.3	1.2
	15:55	00:10	68/4	2.4	0.1	(40)	1.03	24.5	0	2.3	1.2
Decrease TMP	15:59	00:00	65/4	1.8	0.2	(30)	0.95	24.1	0	1.6	1.0
	16:10	00:11	65/4	1.8	0.2	(30)	1.07	25.2	0	1.6	1.0
	16:20	00:21	65/4	1.8	0.2	(30)	1.02	24.5	0	1.6	1.0
Increaase TMP	16:21	00:00	65/4	2.3	0.7	(30)	1.34	25.0	0	1.7	1.5
	16:36	00:15	65/4	2.3	0.7	(30)	1.37	25.9	0	1.6	1.5
Increase TMP	16:37	00:00	76/4	2.8	1.1	(30)	1.58	25.7	0	1.7	1.9
	16:47	00:10	76/4	2.8	1.1	(30)	1.53	24.7	0	1.7	1.9
	16:52	00:15	76/4	2.8	1.1	(30)	1.52	25.9	0	1.7	1.9
Check base-line	16:54	00:00	41/3.5	1.0	0.1	(20)	0.60	26.0	0	0.9	0.5
	17:04	00:10	41/3.5	1.0	0.1	(20)	0.62	24.2	0	0.9	0.5

Table B.4 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Increaase TMP	17:06	00:00	56/3.5	1.4	0.6	(20)	1.18	25.0	0	0.9	1.0
	17:16	00:10	56/3.5	1.4	0.6	(20)	1.19	25.2	0	0.9	1.0
Increaase TMP	17:18	00:00	69/3.5	2.1	1.0	(20)	1.54	26.0	0	1.0	1.6
	17:28	00:10	70/3.5	2.1	1.0	(20)	1.43	25.2	0	1.0	1.6
	17:33	00:15	70/3.5	2.1	1.0	(20)	1.41	25.2	0	1.0	1.6
Increaase TMP	17:37	00:00	70/4	2.5	1.4	(20)	1.60	24.6	0	1.0	2.0
	17:47	00:10	70/4	2.5	1.4	(20)	1.55	25.0	0	1.0	2.0
	17:52	00:15	70/4	2.4	1.4	(20)	1.57	25.1	0	1.0	1.9

Table B.5. Trial Data Sheet Run 5

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Steady state	09:30	00:00	53/3.5	1.4	0.6	20	(6)	24.5	0	0.8	1.0
	09:32	00:02	51/3.5	1.4	0.6	20	3.26		0	0.8	1.0
	09:35	00:05	51/3.5	1.4	0.6	20	2.44	24.1	0	0.8	1.0
	09:40	00:10	51/3.5	1.4	0.6	20	2.14	24.3	0	0.8	1.0
	09:45	00:15	51/3.5	1.4	0.6	20	2.01	24.4	0	0.8	1.0
	09:50	00:20	51/3.5	1.4	0.6	21	1.85	24.4	0	0.8	1.0
	09:55	00:25	51/3.5	1.4	0.6	20	1.81	24.5	0	0.8	1.0
	10:00	00:30	51/3.5	1.4	0.6	20	1.77	24.9	0	0.8	1.0
	10:15	00:45	51/3.5	1.4	0.6	20	1.64	24.9	0	0.8	1.0
	10:30	01:00	53/3.5	1.4	0.6	20	1.58	25.5	0	0.8	1.0
	10:45	01:15	55/3.5	1.4	0.6	20	1.49	24.4	0	0.8	1.0
	11:00	01:30	56/3.5	1.4	0.6	20	1.46	25.0	0	0.8	1.0
	11:15	01:45	57/3.5	1.4	0.6	20	1.52	25.5	0	0.8	1.0
Sample R1	11:30	02:00	57/3.5	1.4	0.6	20	1.46	25.0	0	0.8	1.0
	11:45	02:15	58/3.5	1.4	0.6	20	1.20	24.2	0	0.8	1.0
	12:00	02:30	59/3.5	1.4	0.6	20	1.15	25.2	0	0.8	1.0
	12:15	02:45	59/3.5	1.4	0.6	20	1.09	25.3	0	0.9	1.0
	12:30	03:00	59/3.5	1.4	0.6	20	0.95	24.9	0	0.8	1.0
	12:45	03:15	59/3.5	1.4	0.5	20	0.89	24.1	0	0.9	0.9
	13:00	03:30	60/3.5	1.4	0.6	20	0.85	24.8	0	0.8	1.0
	13:15	03:45	59/3.5	1.4	0.6	20	0.80	25.3	0	0.8	1.0
	13:30	04:00	61/3.5	1.4	0.6	20	0.77	24.8	0	0.9	1.0
	13:45	04:15	62/3.5	1.4	0.6	20	0.72	25.1	0	0.9	1.0
	14:00	04:30	62/3.5	1.4	0.6	20	0.64	25.4	0	0.9	1.0
	14:15	04:45	62/3.5	1.4	0.5	20	0.49	24.2	0	1.0	1.0

Table B.5 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Sample R2 and P1	14:30	05:00	62/3.5	1.4	0.6	20	0.468	24.1	0	0.9	1.0
	14:45	05:15	62/3.5	1.4	0.6	20	0.35	25.4	0	0.9	1.0
	15:00	05:30	62/3.5	1.5	0.6	20	0.37	25.0	1	1.0	1.0
	15:15	05:45	62/3.5	1.5	0.6	20	0.30	25.5	2	1.0	1.0
	15:30	06:00	64/3.5	1.6	0.5	20	0.31	24.1	3	1.1	1.0
	15:45	06:15	64/3.5	1.6	0.5	20	0.35	25.0	4	1.1	1.0
	16:00	06:30	64/3.5	1.6	0.5	20	0.35	24.2	5	1.1	1.0
	16:15	06:45	64/3.5	1.6	0.5	20	0.42	25.6	6	1.1	1.0
	16:30	07:00	64/3.5	1.6	0.5	20	0.48	24.8	7	1.1	1.0
	16:45	07:15	64/3.5	1.6	0.5	20	0.52	25.4	8	1.1	1.0
	17:00	07:30	64/3.5	1.6	0.5	20	0.54	24.9	9	1.1	1.0
	17:15	07:45	64/3.5	1.6	0.5	20	0.53	24.5	10	1.1	1.0
Sample R3 and P2	17:28	07:58	64/3.5	1.6	0.5	20			11	1.1	1.0
	17:30	08:00	64/3.5	1.6	0.5	20	0.56	25.1	12	1.1	1.0
	17:45	08:15	64/3.5	1.6	0.5	20	0.53	24.5	13	1.1	1.0
	18:00	08:30	64/3.5	1.6	0.5	20	0.53	25.2	14	1.1	1.0
	18:15	08:45	64/3.5	1.6	0.5	20	0.53	25.3	15	1.1	1.0
	18:30	09:00	64/3.5	1.6	0.5	20	0.52	25.3	16	1.1	1.0
Sample R4 and P3	18:43	09:13	64/3.5	1.6	0.5	20	0.52	25.2	17	1.1	1.0

Table B.6. Trial Data Sheet Run 6

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Steady state	09:30	00:00	53/3.5	1.4	0.6	20	(6)	24.5	0	0.8	1.0
	09:32	00:02	51/3.5	1.4	0.6	20	3.26		0	0.8	1.0
	09:35	00:05	51/3.5	1.4	0.6	20	2.44	24.1	0	0.8	1.0
	09:40	00:10	51/3.5	1.4	0.6	20	2.14	24.3	0	0.8	1.0
	09:45	00:15	51/3.5	1.4	0.6	20	2.01	24.4	0	0.8	1.0
	09:50	00:20	51/3.5	1.4	0.6	21	1.85	24.4	0	0.8	1.0
	09:55	00:25	51/3.5	1.4	0.6	20	1.81	24.5	0	0.8	1.0
	10:00	00:30	51/3.5	1.4	0.6	20	1.77	24.9	0	0.8	1.0
	10:15	00:45	51/3.5	1.4	0.6	20	1.64	24.9	0	0.8	1.0
	10:30	01:00	53/3.5	1.4	0.6	20	1.58	25.5	0	0.8	1.0
	10:45	01:15	55/3.5	1.4	0.6	20	1.49	24.4	0	0.8	1.0
	11:00	01:30	56/3.5	1.4	0.6	20	1.46	25.0	0	0.8	1.0
	11:15	01:45	57/3.5	1.4	0.6	20	1.52	25.5	0	0.8	1.0
Start concentrating. Sample R1	11:30	02:00	57/3.5	1.4	0.6	20	1.46	25.0	0.00	0.8	1.0
	11:45	02:15	58/3.5	1.4	0.6	20	1.20	24.2	0.37	0.8	1.0
	12:00	02:30	59/3.5	1.4	0.6	20	1.15	25.2	0.65	0.8	1.0
	12:15	02:45	59/3.5	1.4	0.6	20	1.09	25.3	0.94	0.9	1.0
	12:30	03:00	59/3.5	1.4	0.6	20	0.95	24.9	1.21	0.8	1.0
	12:45	03:15	59/3.5	1.4	0.5	20	0.89	24.1	1.44	0.9	0.9
	13:00	03:30	60/3.5	1.4	0.6	20	0.85	24.8	1.68	0.8	1.0
	13:15	03:45	59/3.5	1.4	0.6	20	0.80	25.3	1.89	0.8	1.0
	13:30	04:00	61/3.5	1.4	0.6	20	0.77	24.8	2.09	0.9	1.0
	13:45	04:15	62/3.5	1.4	0.6	20	0.72	25.1	2.28	0.9	1.0
	14:00	04:30	62/3.5	1.4	0.6	20	0.64	25.4	2.45	0.9	1.0
	14:15	04:45	62/3.5	1.4	0.5	20	0.49	24.2	2.60	1.0	1.0

Table B.6 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Sample R2 and P1	14:30	05:00	62/3.5	1.4	0.6	20	0.468	24.1	2.70	0.9	1.0
	14:45	05:15	62/3.5	1.4	0.6	20	0.35	25.4	2.83	0.9	1.0
	15:00	05:30	62/3.5	1.5	0.6	20	0.37	25.0	2.93	1.0	1.0
	15:15	05:45	62/3.5	1.5	0.6	20	0.30	25.5	3.02	1.0	1.0
	15:30	06:00	64/3.5	1.6	0.5	20	0.31	24.1	3.10	1.1	1.0
	15:45	06:15	64/3.5	1.6	0.5	20	0.35	25.0	3.18	1.1	1.0
	16:00	06:30	64/3.5	1.6	0.5	20	0.35	24.2	3.28	1.1	1.0
	16:15	06:45	64/3.5	1.6	0.5	20	0.42	25.6	3.37	1.1	1.0
	16:30	07:00	64/3.5	1.6	0.5	20	0.48	24.8	3.48	1.1	1.0
	16:45	07:15	64/3.5	1.6	0.5	20	0.52	25.4	3.60	1.1	1.0
	17:00	07:30	64/3.5	1.6	0.5	20	0.54	24.9	3.75	1.1	1.0
	17:15	07:45	64/3.5	1.6	0.5	20	0.53	24.5	3.88	1.1	1.0
Sample R3 and P2	17:28	07:58	64/3.5	1.6	0.5	20			4.00	1.1	1.0
	17:30	08:00	64/3.5	1.6	0.5	20	0.56	25.1	4.02	1.1	1.0
	17:45	08:15	64/3.5	1.6	0.5	20	0.53	24.5	4.16	1.1	1.0
	18:00	08:30	64/3.5	1.6	0.5	20	0.53	25.2	4.30	1.1	1.0
	18:15	08:45	64/3.5	1.6	0.5	20	0.53	25.3	4.44	1.1	1.0
	18:30	09:00	64/3.5	1.6	0.5	20	0.52	25.3	4.58	1.1	1.0
Sample R4 and P3	18:43	09:13	64/3.5	1.6	0.5	20	0.52	25.2	4.69	1.1	1.0

Table B.7. Trial Data Sheet Run 7

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Start steady state	09:25	00:00	41/3.5	1.5	0.6	21	6	25.2	0	1.0	1.0
	09:30	00:05	41/3.5	1.5	0.6	20	2.86	24.8	0	1.0	1.0
	09:35	00:10	41/3.5	1.5	0.6	20	2.05	24.5	0	1.0	1.0
	09:40	00:15	41/3.5	1.5	0.6	20	1.89	24.3	0	1.0	1.0
	09:45	00:20	41/3.5	1.5	0.6	20	1.75	24.9	0	1.0	1.0
	09:50	00:25	41/3.5	1.5	0.6	20	1.72	25.0	0	1.0	1.0
	09:55	00:30	41/3.5	1.5	0.6	20	1.66	25.3	0	1.0	1.0
	10:10	00:45	41/3.5	1.5	0.6	20	1.48	25.3	0	1.0	1.0
	10:25	01:00	44/3.5	1.5	0.6	20	1.42	25.4	0	1.0	1.0
	10:40	01:15	44/3.5	1.4	0.6	20	1.33	25.5	0	0.9	1.0
	10:55	01:30	48/3.5	1.5	0.6	20	1.28	25.3	0	1.0	1.0
	11:10	01:45	48/3.5	1.5	0.6	20	1.25	25.0	0	1.0	1.0
	11:25	02:00	49/3.5	1.5	0.6	20	1.19	24.9	0	1.0	1.0
	11:40	02:15	50/3.5	1.5	0.6	20	1.12	25.0	0	1.0	1.0
	11:55	02:30	50/3.5	1.5	0.6	20	1.10	25.0	0	1.0	1.0
	12:10	02:45	51/3.5	1.5	0.6	20	1.07	25.0	0	1.0	1.0
	12:25	03:00	51/3.5	1.5	0.6	20	1.01	25.0	0	1.0	1.0
	12:40	03:15	52/3.5	1.5	0.6	20	0.95	25.3	0	1.0	1.0
Sample 500 g retentate	12:55	03:30	52/3.5	1.5	0.6	20	0.94	25.2	0	1.0	1.0
Decrease TMP (0,5 bar)	13:00	00:00	40/3.5	1.0	0.1	20		25.2	0	1.0	0.6
	13:10	00:10	40/3.5	1.0	0.1	20	0.76	25.0	0	0.9	0.5
	13:15	00:15	40/3.5	1.0	0.1	20	0.71	25.0	0	0.9	0.5
Increase tMP (1 bar)	13:20	00:00	53/3.5	1.5	0.6	20	1.05	25.0	0	1.0	1.0
	13:30	00:10	53/3.5	1.5	0.6	20	0.88	25.3	0	1.0	1.0
	13:35	00:15	53/3.5	1.5	0.6	20	0.83	24.9	0	1.0	1.0

Table B.7 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Increase TMP (1.5 bar)	13:40	00:00	65/3.5	2.0	1.0	20	1.00	24.6	0.0	1.0	1.5
	13:50	00:10	61/3.5	2.1	1.0	20	0.85	24.9	0.0	1.0	1.6
	13:55	00:15	61/3.5	2.1	1.0	20	0.79	24.5	0.0	1.0	1.6
Increase TMP (2 bar)	14:00	00:00	56/4	2.4	1.5	20	0.92	25.5	0.0	0.9	2.0
	14:10	00:10	56/4	2.5	1.5	20	0.84	25.3	0.0	1.0	2.0
	14:15	00:15	57/4	2.6	1.4	20	0.82	25.0	0.0	1.1	2.0
Decreaase TMP (1.5 bar)	14:20	00:00	61/3.5	2.0	1.1	20	0.71	25.0	0.0	0.9	1.6
	14:30	00:10	61/3.5	2.0	1.1	20	0.71	24.9	0.0	0.9	1.6
Decrease TMP (1 bar)	14:35	00:00	52/3.5	1.5	0.6	20	0.55	25.0	0.0	1.0	1.0
	14:45	00:10	52/3.5	1.5	0.6	20	0.59	24.8	0.0	1.0	1.0
	14:50	00:15	52/3.5	1.5	0.6	20	0.61	25.5	0.0	1.0	1.0
Decrease TMP (0,5 bar)	14:55	00:00	41/3.5	1.0	0.1	20	0.39	25.5	0.0	1.0	0.6
	15:05	00:10	41/3.5	1.0	0.1	20	0.44	24.8	0.0	1.0	0.6
	15:15	00:20	41/3.5	1.0	0.1	20	0.47	24.5	0.0	1.0	0.6
Increase TMP (1 bar)	15:20	00:00	52/3.5	1.5	0.5	20	0.73	25.2	0.0	1.0	1.0
Start concentration	15:30	00:00	52/3.5	1.5	0.5	20	0.71	25.5	0.00	1.0	1.0
	15:47	00:17	52/3.5	1.5	0.5	20	0.75	25.2	0.22	1.0	1.0
	15:50	00:20	52/3.5	1.5	0.5	20			0.25	1.0	1.0
	15:55	00:25	52/3.5	1.5	0.5	20	0.00		0.31	1.0	1.0
	16:00	00:30	52/3.5	1.5	0.5	20	0.77	25.7	0.38	1.0	1.0
	16:05	00:35	52/3.5	1.5	0.5	20	0.00		0.45	1.0	1.0
	16:09	00:39	52/3.5	1.5	0.5	20	0.00		0.50	1.0	1.0
J v TMP at CV2	16:10	00:40	52/3.5	1.5	0.5	20	0.80	26.1	0.0	1.0	1.0
Decrease TMP (0.5 bar)	16:15	00:00	41/3.5	1.0	0.1	20	0.55	25.5	0.0	1.0	0.6
	16:20	00:05	41/3.5	1.0	0.1	20	0.56	25.0	0.0	1.0	0.6

Table B.7 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Increase TMP (1.5 bar)	16:42	00:00	67/3.5	2.1	1.0	20	1.16	27.0	0	1.0	1.6
	16:47	00:05	67/3.5	2.1	1.0	20	1.17	26.7	0	1.0	1.6
Decrease TMP (1 bar)	16:49	00:00	56/3.5	1.5	0.5	20	0.87	27.8	0	1.0	1.0
	16:54	00:12	56/3.5	1.5	0.5	20	0.90	26.8	0	1.0	1.0
Decrease TMP (0.5 bar)	16:56	00:00	44/3.5	1.0	0.1	20	0.60	26.8	0	1.0	0.6
	17:01	00:19	44/3.5	1.0	0.1	20	0.61	26.7	0	1.0	0.6

Table B.8. Trial Data Sheet Run 8

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Start steady state	07:20	00:00	43/3.5	1.5	0.5	20	8	24.1	0	1.0	1.0
	07:25	00:05	43/3.5	1.5	0.5	20	2.27	23.8	0	1.0	1.0
	07:30	00:10	46/3.5	1.5	0.5	20	1.83	23.8	0	1.0	1.0
	07:35	00:15	49/3.5	1.5	0.4	20	1.70	24.2	0	1.1	1.0
	07:40	00:20	52/3.5	1.6	0.4	20	1.60	24.4	0	1.2	1.0
	07:45	00:25	52/3.5	1.6	0.4	20	1.52	24.8	0	1.2	1.0
	07:50	00:30	52/3.5	1.5	0.4	20	1.48	25.3	0	1.1	1.0
	08:05	00:45	56/3.5	1.6	0.4	20	1.34	25.0	0	1.2	1.0
	08:20	01:00	56/3.5	1.5	0.4	20	1.25	24.8	0	1.1	1.0
	08:35	01:15	58/3.5	1.6	0.4	20	1.18	25.0	0	1.2	1.0
	08:50	01:30	58/3.5	1.6	0.4	20	1.14	24.8	0	1.2	1.0
	09:05	01:45	59/3.5	1.6	0.4	20	1.13	24.9	0	1.2	1.0
	09:20	02:00	59/3.5	1.6	0.4	20	1.11	25.0	0	1.2	1.0
	09:35	02:15	59/3.5	1.6	0.4	20	1.08	25.2	0	1.2	1.0
	09:50	02:30	59/3.5	1.6	0.4	20	1.08	25.9	0	1.2	1.0
	10:05	02:45	59/3.5	1.6	0.4	20	1.01	24.8	0	1.2	1.0
	10:20	03:00	60/3.5	1.6	0.4	20	0.99	24.6	0	1.2	1.0
	10:35	03:15	60/3.5	1.6	0.4	20	0.99	25.0	0	1.2	1.0
	10:50	03:30	61/3.5	1.6	0.4	20	0.95	24.5	0	1.2	1.0
Start TMP runs (0.5 bar)	10:55	00:00	55/3.5	1.3	0.1	20	0.68	24.6	0	1.2	0.7
	11:00	00:05	55/3.5	1.3	0.1	20	0.72	25.0	0	1.2	0.7
	11:05	00:10	55/3.5	1.3	0.1	20	0.72	25.0	0	1.2	0.7
Increase TMP (1 bar)	11:07	00:00	61/3.5	1.6	0.4	20	1.02	25.0	0	1.2	1.0
	11:12	00:05	61/3.5	1.6	0.4	20	0.98	25.0	0	1.2	1.0
	11:17	00:10	61/3.5	1.6	0.4	20	0.97	25.1	0	1.2	1.0

Table B.8 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Increase TMP (1.5 bar)	11:20	00:13	79/3.5	2.3	1.0	20	1.25	24.9	0	1.3	1.6
	11:25	00:18	76/3.5	2.3	0.9	20	1.11	24.0	0	1.4	1.6
	11:30	00:23	76/3.5	2.3	0.9	20	1.12	24.1	0	1.4	1.6
Increase TMP (2 bar)	11:33	00:26	76/3.7	2.6	1.4	20	1.27	24.3	0	1.2	2.0
	11:38	00:31	76/3.7	2.6	1.4	20	1.19	24.1	0	1.2	2.0
	11:43	00:36	79/3.7	2.6	1.4	20	1.19	24.9	0	1.2	2.0
Decrease TMP (1.5 bar)	11:45	00:38	74/3.7	2.3	0.9	20	1.05	25.0	0	1.4	1.6
	11:50	00:43	74/3.7	2.3	0.9	20	1.06	25.4	0	1.4	1.6
	11:55	00:48	74/3.7	2.3	0.9	20	1.01	25.2	0	1.4	1.6
Decrease TMP (1 bar)	11:58	00:51	59/3.5	1.6	0.4	20	0.75	25.0	0	1.2	1.0
	12:02	00:55	59/3.5	1.6	0.4	20	0.77	25.1	0	1.2	1.0
	12:08	01:01	59/3.5	1.6	0.4	20	0.76	24.9	0	1.2	1.0
Decrease TMP (0.5 bar)	12:10	01:03	52/3.5	1.3	0.1	20	0.53	25.0	0	1.2	0.7
	12:15	01:08	52/3.5	1.3	0.1	20	0.56	25.3	0	1.2	0.7
	12:20	01:13	52/3.5	1.3	0.1	20	0.55	25.3	0	1.2	0.7
Increase TMP (1 bar)	12:23	01:16	61/3.5	1.7	0.3	20	0.80	24.2	0	1.4	1.0
Start Concentrating, Sample R1	12:25	01:18	61/3.5	1.7	0.3	20	0.74	24.2	0.000	1.4	1.0
	12:30	01:23	61/3.5	1.7	0.3	20	0.74	24.8	0.076	1.4	1.0
	12:42	01:35	61/3.5	1.7	0.3	20	0.72	25.4	0.205	1.4	1.0
	12:55	01:48	61/3.5	1.7	0.3	20	0.66	25.2	0.365	1.4	1.0
	13:10	02:03	61/3.5	1.7	0.3	20	0.62	25.2	0.536	1.4	1.0
	13:20	02:13	61/3.5	1.7	0.3	20	0.00	25.3	0.640	1.4	1.0
	13:25	02:18	61/3.5	1.7	0.3	20	0.59	25.5	0.692	1.4	1.0
	13:45	02:38	61/3.5	1.7	0.3	20	0.52	25.5	0.877	1.4	1.0
	13:55	02:48	61/3.5	1.7	0.3	20	0.44	24.5	0.952	1.4	1.0

Table B.8 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
	14:10	03:03	62/3.5	1.8	0.3	20	0.44	24.8	1.056	1.4	1.1
	14:25	03:18	62/3.5	1.8	0.3	20	0.38	25.4	1.157	1.4	1.1
	14:40	03:33	62/3.5	1.8	0.3	19	0.37	24.7	1.251	1.5	1.0
	14:55	03:48	62/3.5	1.8	0.3	19	0.40	24.9	1.348	1.5	1.0
	15:10	04:03	62/3.5	1.8	0.3	19	0.43	25.0	1.453	1.5	1.0
Sample R2	15:17	04:10							1.500		
	15:25	04:18	62/3.5	1.8	0.3	19	0.47	24.5	1.552	1.5	1.0
	15:40	04:33	62/3.5	1.8	0.3	19	0.51	25.0	1.673	1.5	1.0
	15:55	04:48	62/3.5	1.8	0.3	19	0.51	24.5	1.810	1.5	1.0
	16:10	05:03	63/3.5	1.9	0.2	20	0.50	24.4	1.944	1.7	1.0
	16:25	05:18	63/3.5	1.9	0.2	20	0.53	24.1	2.080	1.7	1.0
	16:40	05:33	64/3.5	1.9	0.2	20	0.53	24.9	2.209	1.7	1.0
Sample R3	16:43	05:36							2.265		
	16:55	05:48	64/3.5	1.9	0.2	20	0.53	25.0	2.361	1.7	1.0
	17:10	06:03	64/3.5	1.9	0.2	20	0.54	24.5	2.492	1.7	1.0
Sample R4, End	17:19	06:12	00:00						2.565		

Table B.9. Trial Data Sheet Run 9

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Start steady state	09:50	00:00	34/3.5	0.8	0.1	20		25.0	0	0.8	0.4
	09:51	00:01	38/3.5	1.0	0.1	20	6.50	24.0	0	0.9	0.5
	09:53	00:03	38/3.5	1.0	0.1	20	6.65		0	0.9	0.5
	09:55	00:05	38/3.5	1.0	0.1	20	6.62	23.7	0	0.9	0.5
	10:00	00:10	38/3.5	1.0	0.1	20	7.08	25.0	0	1.0	0.6
	10:05	00:15	38/3.5	1.0	0.1	20	7.04	25.0	0	1.0	0.6
	10:10	00:20	38/3.5	1.0	0.1	20	7.04	24.9	0	1.0	0.6
	10:15	00:25	38/3.5	1.0	0.1	20	7.00	25.0	0	1.0	0.6
	10:20	00:30	38/3.5	1.0	0.1	20	6.95	24.9	0	1.0	0.6
	10:35	00:45	38/3.5	1.0	0.1	20	6.76	25.0	0	1.0	0.6
	10:50	01:00	38/3.5	1.0	0.1	20	6.73	25.5	0	1.0	0.6
	11:05	01:15	39/3.5	1.0	0.1	20	6.43	25.0	0	1.0	0.6
	11:20	01:30	39/3.5	1.0	0.1	20	6.17	24.9	0	1.0	0.6
	11:35	01:45	39/3.5	1.0	0.1	20	5.97	25.0	0	1.0	0.6
	11:50	02:00	39/3.5	1.0	0.1	20	5.91	25.3	0	1.0	0.6
	12:05	02:15	39/3.5	1.0	0.1	20	5.68	25.0	0	0.9	0.5
	12:20	02:30	39/3.5	1.0	0.1	20	5.68	25.2	0	0.9	0.5
	12:35	02:45	39/3.5	1.0	0.1	20	5.66	25.0	0	0.9	0.5
Increase TMP (1 bar)	12:38	00:00	50/3.8	1.5	0.5	20	8.48	25.0	0	1.0	1.0
	12:45	00:07	50/3.8	1.5	0.5	20	7.65	24.5	0	1.0	1.0
	12:48	00:10	50/3.8	1.5	0.5	20	7.57	24.8	0	1.0	1.0
	12:54	00:16	50/3.8	1.5	0.5	20	7.47	25.3	0	1.0	1.0
Increase TMP (1.5 bar)	12:57	00:00	60/3.5	2.0	1.0	20	8.35	25.3	0	1.0	1.5
	13:07	00:10	60/3.5	2.0	1.0	20	7.69	25.0	0	1.0	1.5
	13:12	00:15	60/3.5	2.0	1.0	20	7.61	25.3	0	1.0	1.5

Table B.9 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Increase TMP (2 bar)	13:15	00:00	69/3.5	2.5	1.4	20	8.35	25.0	0	1.0	2.0
	13:25	00:10	69/3.5	2.5	1.5	20	7.85	25.4	0	1.0	2.0
	13:30	00:15	69/3.5	2.5	1.5	20	7.49	25.3	0	1.0	2.0
Decrease TMP (1.5 bar)	13:34	00:00	59/3.5	2.0	1.0	20	6.71	25.2	0	1.0	1.5
	13:44	00:10	59/3.5	1.9	1.0	20	6.72	25.9	0	0.9	1.5
	13:49	00:15	60/3,5	2.0	1.1	20	6.83	25.5	0	0.9	1.6
Decrease TMP (1 bar)	13:53	00:00	51/3.5	1.4	0.6	20	5.71	25.4	0	0.8	1.0
	14:03	00:10	51/3.5	1.4	0.6	20	5.70	25.3	0	0.8	1.0
Decrease TMP (0.5 bar)	14:06	00:00	40/3.5	1.0	0.1	20	4.11	25.2	0	0.9	0.5
	14:16	00:10	41/3.5	1.0	0.1	20	4.39	25.0	0	0.9	0.5
	14:21	00:15	41/3.5	1.0	0.1	20	4.29	25.3	0	0.9	0.5
Increase TMP (1 bar)	14:23	00:00	54/3.5	1.4	0.6	20	6.39	25.3	0	0.9	1.0
	14:33	00:10	54/3.5	1.4	0.6	20	5.92	25.2	0	0.9	1.0
	14:38	00:15	54/3.5	1.4	0.6	20	5.92	24.6	0	0.9	1.0
Start concentrating	14:41	00:00	52/3.5	1.4	0.6	20		25.0	0.00	0.9	1.0
	14:43	00:02	52/3.5	1.4	0.6	20		25.0	0.21	0.9	1.0
	14:45	00:04	52/3.5	1.4	0.6	20		24.8	0.40	0.9	1.0
	14:47	00:06	51/3.5	1.5	0.6	21		24.5	0.58	1.0	1.0
	14:49	00:08	51/3.5	1.5	0.6	21	5.59	0.0	0.75	1.0	1.0
	14:50	00:09	51/3.5	1.5	0.6	21		25.0	0.87	1.0	1.0
	14:52	00:11	51/3.5	1.5	0.6	21		25.5	1.05	1.0	1.0
	14:53	00:12	51/3.6	1.5	0.6	21		0.0	1.13	1.0	1.0
Recirculate at low TMP	14:55	00:00	51/3.5	1.4	0.6	20	5.00	24.9		0.9	1.0
End	15:00	00:05	51/3.5	1.4	0.6	20	4.99	24.8		0.9	1.0

Table B.10. Trial Data Sheet Run 10

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Start steady state	09:10	00:00	0.0	0.0	0.0		10.67		0	0.0	0.0
	09:11	00:02	38/3.5	1.0	0.1	18	5.34	25.5	0	0.9	0.5
	09:15	00:05	38/3.5	1.0	0.1	20	3.19	25.3	0	0.9	0.5
	09:20	00:10	38/3.5	1.0	0.1	21	2.53	24.6	0	0.9	0.5
	09:25	00:15	38/3.5	1.0	0.1	21	2.20	24.4	0	0.9	0.5
	09:30	00:20	38/3.5	1.0	0.1	21	2.00	24.3	0	0.9	0.5
	09:35	00:25	38/3.5	1.0	0.1	21	1.89	24.5	0	0.9	0.5
	09:40	00:30	38/3.5	0.9	0.1	20	1.74	24.6	0	0.8	0.5
	09:55	00:45	38/3.5	0.9	0.1	20	1.50	25.0	0	0.8	0.5
	10:10	01:00	39/3.5	0.9	0.1	20	1.59	25.4	0	0.8	0.5
	10:25	01:15	39/3.5	1.0	0.1	20	1.59	24.9	0	0.9	0.5
	10:40	01:30	39/3.5	0.9	0.1	20	1.57	25.0	0	0.8	0.5
	10:55	01:45	40/3.5	0.8	0.1	20	1.52	25.0	0	0.8	0.4
	11:10	02:00	41/3.5	0.9	0.1	20	1.58	24.9	0	0.8	0.5
Increase TMP (1 bar)	11:13	00:00	56/3.5	1.4	0.6	20	2.26	25.0	0	0.8	1.0
	11:23	00:10	56/3.5	1.4	0.6	20	2.00	25.2	0	0.8	1.0
	11:28	00:15	56/3.5	1.4	0.6	20	1.96	25.2	0	0.8	1.0
	11:33	00:20	56/3.5	1.4	0.6	20	1.93	25.0	0	0.8	1.0
Increase TMP (1.5 bar)	11:37	00:00	71/3.7	1.9	1.1	20	2.35	25.0	0	0.8	1.5
	11:47	00:10	71/3.7	1.9	1.1	20	2.15	25.2	0	0.8	1.5
	11:52	00:15	71/3.7	1.9	1.1	20	2.11	25.5	0	0.8	1.5
	11:57	00:20	71/3.7	1.9	1.1	20	2.09	25.5	0	0.8	1.5
Increase TMP (2 bar)	12:00	00:00	71/4.2	2.3	1.5	20	2.41	25.6	0	0.8	1.9
	12:10	00:10	73/4.2	2.3	1.5	20	2.10	24.9	0	0.8	1.9
	12:15	00:15	73/4.2	2.3	1.5	20	2.12	24.8	0	0.8	1.9

Table B.10 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
	12:20	00:20	73/4.2	2.3	1.5	20	2.12	24.9	0	0.8	1.9
Decrease TMP (1.5 bar)	12:23	00:00	71/3.7	1.9	1.1	20	1.87	25.0	0	0.8	1.5
	12:33	00:10	71/3.7	1.9	1.1	20	1.78	24.8	0	0.8	1.5
	12:38	00:15	71/3.7	1.9	1.1	20	1.75	24.6	0	0.8	1.5
Decrease TMP (1 bar)	12:40	00:00	58/3.7	1.4	0.6	20	1.39	24.6	0	0.8	1.0
	12:50	00:10	58/3.7	1.4	0.6	20	1.46	24.8	0	0.8	1.0
	12:55	00:15	58/3.7	1.4	0.6	20	1.48	24.8	0	0.8	1.0
Decrease TMP (0.5 bar)	12:58	00:00	47/3.5	0.9	0.1	21	0.97	24.8	0	0.8	0.5
	13:08	00:10	47/3.5	0.9	0.1	21	1.12	24.8	0	0.8	0.5
	13:13	00:15	47/3.5	0.9	0.1	21	1.15	24.5	0	0.8	0.5
	13:18	00:20	47/3.5	0.9	0.1	21	1.17	24.6	0	0.8	0.5
Increase TMP (1 bar)	13:20	00:00	61/3.5	1.4	0.6	20	1.68	24.6	0	0.8	1.0
	13:35	00:15	61/3.5	1.4	0.6	20	1.51	24.9	0	0.8	1.0
	13:45	00:25	61/3.5	1.4	0.6	20	1.47	25.0	0	0.8	1.0
	13:55	00:35	61/3.5	1.4	0.6	20	1.45	25.0	0	0.8	1.0
Sample R1	13:56	00:36	0.0	0.0	0.0				0	0.0	0.0
Start concentration	14:00	00:00	62/3.5	1.4	0.6	20	1.45	25.0	0.00	0.8	1.0
	14:10	00:10	62/3.5	1.4	0.6	20	1.28	25.3	0.23	0.8	1.0
	14:20	00:20	62/3.5	1.4	0.6	20	1.30	25.3	0.46	0.8	1.0
	14:30	00:30	62/3.5	1.4	0.6	20	1.27	24.6	0.68	0.8	1.0
	14:40	00:40	62/3.5	1.4	0.6	20	1.27	24.9	0.89	0.8	1.0
	14:45	00:45	62/3.5	1.4	0.6	20			0.99	0.8	1.0
	14:50	00:50	62/3.5	1.4	0.6	20	1.24	25.3	1.11	0.8	1.0
	14:55	00:55	62/3.5	1.4	0.6	20			1.19	0.8	1.0
	15:00	01:00	62/3.5	1.4	0.6	20	1.27	25.0	1.29	0.8	1.0

Table B.10 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Sample R2	15:12	01:12	62/3.5	1.4	0.6	20			1.52	0.8	1.0
	15:20	01:20	62/3.5	1.4	0.6	20	1.06	24.9	1.65	0.8	1.0
	15:30	01:30	62/3.5	1.4	0.6	20	0.88	24.9	1.81	0.8	1.0
	15:40	01:40	62/3.5	1.4	0.6	20	0.75	24.9	1.95	0.8	1.0
	15:50	01:50	62/3.5	1.4	0.6	20	0.58	24.9	2.06	0.8	1.0
	16:00	02:00	62/3.5	1.4	0.6	20	0.50	24.6	2.15	0.8	1.0
Sample R3	16:10	02:10	62/3.5	1.4	0.6	21	0.41	25.3	2.23	0.8	1.0
	16:20	02:20	62/3.5	1.4	0.6	21	0.36	25.0	2.30	0.8	1.0
	16:30	02:30	62/3.5	1.4	0.6	21	0.30	24.5	2.35	0.9	1.0
	16:40	02:40	62/3.5	1.4	0.6	21	0.22	24.9	2.40	0.8	1.0
	16:50	02:50	62/3.5	1.4	0.6	22	0.22	24.6	2.43	0.9	1.0
	16:55	02:55	62/3.5	1.4	0.6	22	0.21	24.8	2.47	0.9	1.0
END - Sample R4	17:00	03:00	0.0	1.4	0.6					0.9	1.0

Table B.11. Trial Data Sheet Run 11

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Start steady state	09:00	00:00	39/3.5	1.0	0.1	17.0			0	0.9	0.5
	09:01	00:02	39/3.5	1.0	0.1	17.0	6.50		0	0.9	0.5
	09:02	00:05	39/3.5	1.0	0.1	18.0	5.78	25.9	0	0.9	0.5
	09:05	00:10	39/3.5	1.0	0.1	18.0	5.19	25.5	0	0.9	0.5
	09:10	00:15	39/3.5	1.0	0.1	18.0	4.47	25.4	0	0.9	0.5
	09:15	00:20	40/3.5	0.9	0.1	18.0	3.88	25.2	0	0.8	0.5
	09:20	00:25	40/3.5	0.8	0.1	18.0	3.65	25.2	0	0.8	0.4
	09:25	00:30	40/3.5	0.8	0.1	18.0	3.51	25.2	0	0.8	0.4
	09:30	00:45	40/3.5	0.8	0.1	18.0	3.44	25.0	0	0.8	0.4
	09:45	01:00	40/3.5	0.8	0.1	18.0	3.23	25.4	0	0.8	0.4
	10:00	01:15	41/3.5	0.8	0.1	18.0	3.15	25.3	0	0.8	0.4
	10:15	01:30	41/3.5	0.8	0.1	18.0	2.90		0	0.8	0.4
	10:34	01:45	41/3.5	0.8	0.1	18.0	2.84	25.5	0	0.8	0.4
	10:45	02:00	44/3.5	0.9	0.1	18.0	2.81	25.0	0	0.8	0.5
	11:00	02:15	48/3.5	0.9	0.1	18.0	2.75	24.8	0	0.8	0.5
	11:15	02:30	50/3.5	1.0	0.1	18.0	2.76	25.3	0	0.9	0.5
	11:30	02:45	50/3.5	1.0	0.1	18.0	2.57	25.0	0	0.9	0.5
	11:45	03:00	51/3.5	1.0	0.1	18.0	2.50	24.8	0	0.9	0.5
	12:00	03:15	52/3.5	1.0	0.1	18.0	2.49	24.9	0	0.9	0.5
Increase TMP (1 bar)	12:03	03:30	65/3.5	1.7	0.6	19.0	3.40	24.8	0	1.1	1.1
	12:06	03:45	60/3.5	1.4	0.4	18.0	2.89		0	1.0	0.9
	12:10	04:00	64/3.5	1.5	0.6	18.0	2.98	24.9	0	1.0	1.0
	12:13	04:15	64/3.5	1.4	0.5	18.0	2.83	25.0	0	1.0	1.0
	12:18	04:30	64/3.5	1.4	0.5	18.0	2.69	25.0	0	1.0	1.0
	12:23	04:45	64/3.5	1.4	0.5	18.0	2.60	24.9	0	1.0	1.0

Table B.11 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Increase TMP (1.5 bar)	12:26	05:00	78/3.6	1.9	1.0	18.0	3.15	25.0	0	1.0	1.4
	12:36	05:15	76/3.6	1.9	1.0	18.0	2.75	25.1	0	1.0	1.4
	12:41	05:30	76/3.6	1.9	1.0	18.0	2.59	25.0	0	0.9	1.4
	12:46	05:45	76/3.6	1.9	1.0	17.0	2.52	25.0	0	0.9	1.4
Increase TMP (2 bar)	12:50	06:00	70/4	2.3	1.3	18.0	2.73	25.2	0	1.0	1.8
	13:00	06:15	78/4	2.4	1.4	18.0	2.54	24.4	0	1.0	1.9
	13:05	06:30	78/4	2.4	1.4	18.0	2.47	25.0	0	1.0	1.9
Decrease TMP (1.5 bar)	13:08	06:45	75/3.6	1.9	1.0	18.0	2.09	25.0	0	0.9	1.4
	13:18	07:00	75/3.6	1.9	1.0	18.0	2.07	25.0	0	0.9	1.4
	13:23	07:15	75/3.6	1.9	1.0	18.0	2.04	25.0	0	0.9	1.4
Decrease TMP (1 bar)	13:26	07:30	68/3.5	1.5	0.5	19.0	1.66	25.4	0	1.0	1.0
	13:36	07:45	68/3.5	1.5	0.5	19.0	1.66	24.9	0	1.0	1.0
Decrease TMP (0.5 bar)	13:39	07:58	55/3.5	1.0	0.1	18.0	1.11	25.0	0	1.0	0.6
	13:44	08:00	55/3.5	1.1	0.1	18.0	1.18	25.0	0	1.0	0.6
	13:49	08:15	55/3.5	1.1	0.1	18.0	1.19	25.0	0	1.0	0.6
Increase TMP (1.5 bar ready for conc.)	13:52	08:30	65/3.5	1.5	0.5	19.0	1.73	25.0	0	1.0	1.0
	14:00	08:45	65/3.5	1.5	0.5	19.0	1.68	25.0	0	1.0	1.0
	14:07	09:00	65/3.5	1.5	0.5	19.0	1.65	24.5	0	1.0	1.0
Start concentrating, sample R1	14:10	09:13	65/3.5	1.5	0.5	19.0	1.68	24.1	0.00	1.0	1.0
	14:15	10:13	65/3.5	1.5	0.5	19.0		24.8	0.14	1.0	1.0
	14:20	11:13	65/3.5	1.5	0.5	19.0		25.0	0.27	1.0	1.0
	14:25	12:13	65/3.5	1.5	0.5	19.0	1.60	25.0	0.40	1.0	1.0
	14:32	13:13	65/3.5	1.5	0.5	19.0			0.59	1.0	1.0
	14:35	14:13	65/3.5	1.5	0.5	19.0			0.67	1.0	1.0
	14:40	15:13	65/3.5	1.5	0.5	19.0	1.52	25.0	0.79	1.0	1.0

Table B.11 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
	14:45	15:13	65/3.5	1.5	0.5	19.0		25.2	0.92	1.0	1.0
	14:50	16:13	66/3.5	1.5	0.5	18.0			1.05	1.0	1.0
	14:55	17:13	66/3.5	1.5	0.5	18.0	1.50	25.1	1.17	1.0	1.0
	15:00	18:13	66/3.5	1.5	0.5	18.0			1.30	1.0	1.0
	15:10	19:13	66/3.5	1.6	0.5	18.0	1.46	25.2	1.54	1.1	1.0
Sample R2	15:12	20:13	66/3.5	1.6	0.5	18.0			1.60	1.1	1.0
	15:15	21:13	66/3.5	1.6	0.5	18.0			1.67	1.1	1.0
	15:20	22:13	66/3.5	1.6	0.5	18.0	1.42	24.9	1.78	1.1	1.0
	15:25	23:13	66/3.5	1.6	0.5	18.0			1.90	1.1	1.0
	15:30	00:13	66/3.5	1.6	0.5	18.0	1.38	25.0	2.01	1.1	1.0
	15:35	01:13	66/3.5	1.6	0.5	18.0			2.13	1.1	1.0
	15:40	02:13	66/3.5	1.6	0.5	18.0	1.34	25.3	2.24	1.1	1.0
	15:45	03:13	66/3.5	1.6	0.5	18.0			2.35	1.1	1.0
Sample R3	15:46	04:13	66/3.5	1.6	0.5	18.0			2.37	1.1	1.0
	15:50	05:13	66/3.5	1.6	0.5	18.0	1.27	25.0	0.00	1.1	1.0
	15:55	06:13	66/3.5	1.6	0.5	18.0			2.55	1.1	1.0
	16:00	07:13	66/3.5	1.6	0.5	18.0	1.22	25.5	2.66	1.1	1.0
Sample R4	16:03	08:13	66/3.5	1.6	0.5	18.0	1.16		2.72	1.1	1.0

Table B.12. Trial Data Sheet Run 12

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Start steady state	10:04	00:00	40/3.5	1.4	0.1	20	2.00	25.2	0	1.2	0.8
	10:09	00:05	40/3.5	1.3	0.1	21	1.27		0	1.2	0.7
	10:14	00:10	35/3.5	1.1	0.1	22	0.94	24.5	0	1.0	0.6
	10:20	00:16	35/3.5	1.2	0.1	23	0.78	25.3	0	1.1	0.6
	10:24	00:20	34/3.5	1.1	0.1	22	0.73	25.2	0	1.0	0.6
	10:29	00:25	34/3.5	1.1	0.1	22	0.67	25.2	0	1.0	0.6
	10:34	00:30	34/3.5	1.1	0.1	22	0.65	25.6	0	1.0	0.6
Power failure	10:49	00:45	34/3.5	1.1	0.1	23	0.55	25.5	0	1.0	0.6
Restart after power failure (DV water added)	12:58	00:00	40/3.5	1.2	0.1	20	3.00	25.9	0	1.2	0.7
	13:00	00:02	40/3.5	1.2	0.1	21	2.23	0.0	0	1.1	0.6
	13:03	00:05	40/3.5	1.2	0.1	20	1.97	25.7	0	1.1	0.6
	13:08	00:10	40/3.5	1.2	0.1	19	1.84	25.3	0	1.1	0.6
	13:13	00:15	40/3.5	1.2	0.1	19	1.73	25.0	0	1.1	0.6
	13:18	00:20	40/3.5	1.2	0.1	19	1.67	25.8	0	1.1	0.6
	13:23	00:25	40/3.5	1.1	0.1	19	1.64	25.4	0	1.0	0.6
	13:28	00:30	40/3.5	1.1	0.1	19	1.61	24.9	0	1.0	0.6
Start DV1	13:30	00:00	40/3.5	1.1	0.1	19			0.000	1.0	0.6
	13:36	00:06	40/3.5	1.1	0.1	19		25.2	0.158	1.0	0.6
	13:40	00:10	40/3.5	1.1	0.1	19		25.0	0.260	1.0	0.6
	13:45	00:15	40/3.5	1.1	0.1	20		24.5	0.384	1.0	0.6
End DV1	13:50	00:20							0.492		
Start DV2	13:53	00:00	40/3.5	1.1	0.1	18		25.7	0.000	1.0	0.6
	13:57	00:04	40/3.5	1.1	0.1	18	1.28			1.0	0.6
	15:58	02:05	40/3.5	1.1	0.1	18		25.0	0.112	1.0	0.6
	14:03	00:10	40/3.5	1.1	0.1	18		24.9	0.216	1.0	0.6
	14:08	00:15	40/3.5	1.1	0.1	18		24.7	0.321	1.0	0.6
	14:13	00:20	40/3.5	1.1	0.1	18			0.425	1.0	0.6
End DV2	14:14	00:21	40/3.5	1.1	0.1	18			0.446	1.0	0.6

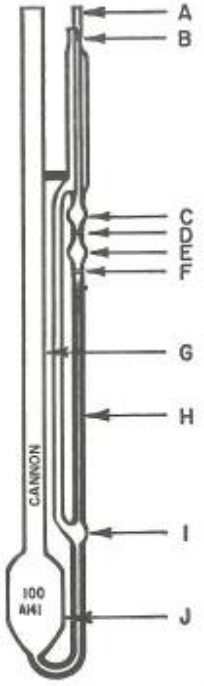
Table B.12 cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	Permeate (L)	ΔP (bar)	TMP (bar)
Start DV3	14:19	00:00	45/3.5	1.2	0.1	18			0.000	1.1	0.6
	14:21	00:02	45/3.5	1.2	0.1	18		25.2	0.046	1.1	0.6
	14:25	00:06	45/3.5	1.2	0.1	18		25.5	0.119	1.1	0.6
	14:29	00:10	45/3.5	1.2	0.1	18		25.3	0.202	1.1	0.6
	14:32	00:13	45/3.5	1.2	0.1	18		25.2	0.268	1.1	0.6
	14:34	00:15	45/3.5	1.2	0.1	18			0.303	1.1	0.6
	14:37	00:18	45/3.5	1.2	0.1	19		25.0	0.364	1.1	0.6
End DV3	14:38	00:19	45/3.5	0.0	0.0				0.400		
Start DV4	14:39	00:00	45/3.5	1.2	0.1	18			0.000	1.1	0.6
	14:41	00:02	45/3.5	1.2	0.1	18		25.0	0.044	1.1	0.6
	14:45	00:06	45/3.5	1.2	0.1	18		24.9	0.122	1.1	0.6
	14:50	00:11	45/3.5	1.2	0.1	18		24.9	0.219	1.1	0.6
	14:53	00:14	45/3.5	1.2	0.1	18		24.8	0.276	1.1	0.6
	14:57	00:18	45/3.5	1.2	0.1	18		25.4	0.352	1.1	0.6
End DV4	14:59	00:20							0.400		
Start DV5	15:03	00:00	46/3.5	1.1	0.1	18		25.0	0.000	1.0	0.6
	15:09	00:06	46/3.5	1.1	0.1	18		25.0	0.119	1.0	0.6
	15:12	00:09	46/3.5	1.1	0.1	18		25.0	0.169	1.0	0.6
	15:15	00:12	46/3.5	1.1	0.1	18		0.0	0.225	1.0	0.6
	15:20	00:17	46/3.5	1.1	0.1	18		24.8	0.317	1.0	0.6
End DV5	15:21	00:18	46/3.5	1.1	0.1	18			0.353	1.0	0.6
Start DV6	15:22	00:00	46/3.5	1.1	0.1	18		25.6	0.000	1.0	0.6
	15:26	00:04	46/3.5	1.1	0.1	18			0.078	1.0	0.6
	15:29	00:07	46/3.5	1.1	0.1	18		25.3	0.133	1.0	0.6
	15:34	00:12	46/3.5	1.1	0.1	18		25.0	0.225	1.0	0.6
	15:39	00:17	46/3.5	1.1	0.1	18		24.9	0.318	1.0	0.6
	15:41	00:19							0.353		

Appendix C: Cannon-Ubbelohde Viscometer

Instructions for the use of
*The Cannon-Ubbelohde Semi-Micro Viscometer**
*The Cannon-Ubbelohde Semi-Micro Dilution Viscometer**

See also ASTM D 445 and ASTM D 2515



Cannon-Ubbelohde Semi-Micro Type and Cannon-Ubbelohde Semi-Micro Dilution Type for Transparent Liquids

1. Clean the viscometer using suitable solvents and dry by passing clean, dry filtered air through the instrument to remove the final traces of solvents. Periodically, traces of organic deposits should be removed with chromic acid.

2. If there is a possibility of lint, dust, or other solid material in the liquid sample, filter the sample through a fritted glass filter or fine mesh screen.

3. For dilution work charge a measured volume of sample (1.00 to 3.00 ml) directly from the pipette through tube G into the lower reservoir of the viscometer. If dilutions are not to be made, it is not necessary to measure the volume of the sample.

4. Place the viscometer into the holder** and insert it into the constant temperature bath. Vertically align the viscometer in the bath if a self aligning holder has not been used.

5. Allow approximately 20 minutes for the sample to come to bath temperature.

6. Place a finger over tube B and apply suction to tube A until the liquid reaches the center of bulb C. Remove suction from tube A. Remove finger from tube B, and immediately place it over tube A until the sample drops away from the lower end of the capillary into bulb I. Then remove finger and measure the efflux time.

7. To measure the efflux time, allow the liquid sample to flow freely down past etch mark D, measuring the time for the meniscus to pass from etch mark D to etch mark F to the nearest 0.1 second.

8. Calculate the viscosity of the sample by multiplying the efflux time by the viscometer constant.

9. Without recharging the viscometer, make check determinations by repeating steps 6 to 8.

10. Dilute sample by adding measured quantity of solvent from pipette directly into the lower reservoir of the viscometer. Mix the original sample and the solvent by applying slight pressure to tube B several times, and shaking the viscometer.

11. Repeat steps 5 to 9. Additional dilution may be made if necessary.

* U.S. Patent 2,805,570
** Excellent inexpensive holders are listed in our Viscometer Bulletin.

RECOMMENDED VISCOSITY RANGES FOR THE CANNON-UBBELOHDE SEMI-MICRO AND CANNON-UBBELOHDE SEMI-MICRO DILUTION VISCOMETERS

Size	*Approximate Constant Centistokes/Second*	*Viscosity Range Centistokes*
25	0.002	0.4 to 2
50	0.004	0.8 to 4
75	0.008	1.6 to 8
100	0.015	3 to 15
150	0.035	7 to 35
200	0.1	20 to 100
300	0.25	50 to 250
350	0.5	100 to 500
400	1.2	240 to 1200
450	2.5	500 to 2500
500	8	1600 to 8000
600	20	4000 to 20000

For information for other sizes and viscometers, write to us.

CANNON INSTRUMENT CO. P. O. BOX 16 STATE COLLEGE, PA 16801

Appendix D: Trial Yield Data

Table D.1. Yield over a concentration run.

Description	Mass (g)	SS (%)	Starch* (g)
IN			
Feed	5537	1.0	57.5
Total In			*57.5*
OUT			
Retentate sample 1	98	1.1	1.1
Retentate sample 2	104	2.0	2.0
Retentate sample 3	102	3.9	4.0
Retentate sample 4	100	8.6	8.6
Retentate	444	8.6	38.3
Total out			*54.0*
*Assumes starch = suspended solids (SS).			
Yield (Total out/Total in)			**93.8 %**

Table D.2. Yield over a diafiltration run.

Description	Mass (g)	SS (%)	Starch* (g)
IN			
Feed	534	9.2	48.8
Total In			*48.8*
OUT			
Retentate sample 1	97	11.1	10.7
Retentate sample 2	93	11.8	11.0
Retentate sample 3	97	4.9	4.7
Retentate sample 4	304	7.1	21.6
Total out			*48.0*
*Assumes starch = suspended solids (SS).			
Yield (Total out/Total in)			**98.3 %**

Appendix E: Diafiltration - Protein Mass Balance

Table E.1. Protein mass balance across a diafiltration run.

Description	Mass	Protein	
	(g)	(%)	(g)
IN			
Feed	492	1.4	6.7
Total In			*6.7*
OUT			
Retentate sample 1 (DV1)	97	0.67	0.65
Retentate sample 2 (DV2)	93	0.47	0.44
Retentate sample 3 (DV4)	97	0.38	0.37
Retentate sample 4 (DV6)	304	0.32	0.97
Wash liquor DV1 -DV6	2444	0.15	3.67
Total out			*6.1*
error (Total In - Total out)			*0.6*
Yield (Total out/Total in)			**91 %**

Appendix F: Composition of Commercial Starches

Source	Manufacturer/Retailer	Moisture %	Protein %	Fat %	Ash %
Corn	ARASCO [1]	< 13	< 0.4	< 0.2	< 0.2
Corn	Huge Roc Enterprises Co., Ltd [2]	< 13.5	< 0.4	< 0.2	< 0.2
Potato	Manitoba Starch Products [3]	16.4	0.4	0.3	< 0.5
Wheat	Can AM Ingredients Inc. [4]	11	< 0.4	1	< 0.4
Wheat	Qingdao Hisea Importers & Exporters Co., Ltd.[5]	< 14	< 0.4	< 0.1	< 0.3

[1] www.arasco.com/en/products/glucose_starch/food_grade.asp

[2] www.ecplaza.net/tradeleads/seller/6668105/corn_starch.html

[3] www.manitobastarch.com/MSP%20Potato%20Starch%20Specification%20Sheet.pdf

[4] www.canamingredients.com/products/WheatStarch_Spec.pdf

[5] hisea.en.alibaba.com/product/228789879-200746765/Wheat_Starch.html

Appendix G: Cake Resistance Calculations

Resistance calculation

Membrane resistance (R_m)

$$R_m = \frac{\Delta P}{J \times \eta_o}$$

Eqn. 1

Data collected while passing permeate through a clean membrane (Run 9)

ΔP	Pa	55000
n_o	Pa s	0.0011
J	$m^3 m^{-2} s^{-1}$	19.4E-6
R_m	m^{-1}	2.7E+12

Total resistance (R_t)

$$R_t = (R_m + R_c) = \frac{\Delta P}{J \times \eta_o}$$

Eqn. 2

Flux versus TMP data collected over various runs

Run	ΔP	J	R_t (from Eqn 2)	R_c ($R_t - R_m$)
	kPa	$m^3 m^{-2} s^{-1}$	m^{-1}	m^{-1}
2	50	2.0E-6	24.3E+12	21.6E+12
	100	2.5E-6	38.3E+12	35.6E+12
	150	3.0E-6	47.5E+12	44.8E+12
	200	3.4E-6	56.7E+12	54.1E+12
3	93	2.3E-6	39.0E+12	36.4E+12
	148	3.0E-6	46.5E+12	43.8E+12
	200	3.6E-6	52.6E+12	49.9E+12
5	52	1.6E-6	30.9E+12	28.2E+12
	100	2.3E-6	42.1E+12	39.4E+12
	155	3.1E-6	48.0E+12	45.3E+12
	197	3.5E-6	53.8E+12	51.1E+12
8	65	1.8E-6	34.8E+12	32.1E+12
	100	2.4E-6	39.5E+12	36.8E+12
	150	3.0E-6	48.3E+12	45.6E+12
	200	3.3E-6	57.3E+12	54.6E+12

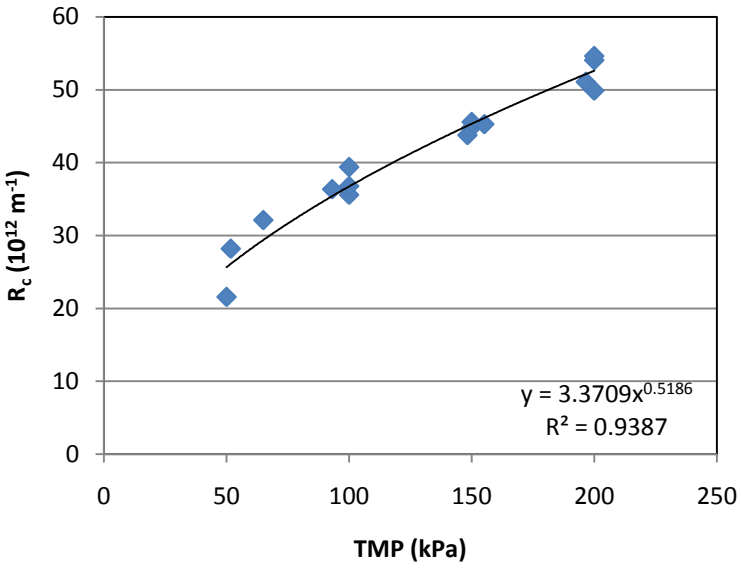


Figure G.1. Plot of calculated Rc versus TMP, solid line is the fitted power-law trend.

Appendix H: Membrane Pressure Limitations

Pressure limitations

Maximum membrane pressure = 350 kPa (50 psi)

Maximum continuous pump tubing pressure = 170 kPa (25 psi)

Maximum intermittent pump tubing pressure = 280 kPa (40 psi)

20 L h^{-1} feed

Measured pressure drop (ΔP) at 20 L h^{-1} = 100 kPa (14.5 psi)

Inlet Pressure (P_{in}) (kPa)	**Outlet Pressure (P_{out}) (kPa)** P_{in}-ΔP	**TMP (kPa)** $(P_{in}-P_{out})/2$	**Comments**
100	0	50	Allowable
150	50	100	Allowable
200	100	150	Short duration only
250	150	200	Short duration only
300	200	250	Not recommended
350	250	300	Not recommended

30 L h^{-1} feed

Measured pressure drop (ΔP)at 30 L h^{-1} = 172 kPa (25 psi)

Inlet Pressure (P_{in}) (kPa)	**Outlet Pressure (P_{out}) (kPa)** P_{in}-ΔP	**TMP (kPa)** $(P_{in}-P_{out})/2$	**Comments**
170	-70	50	Not possible
170	0	90	Allowable
190	10	100	Short duration only
240	60	150	Short duration only
290	110	200	Not recommended
340	160	250	Not recommended

Appendix I: Selected Results

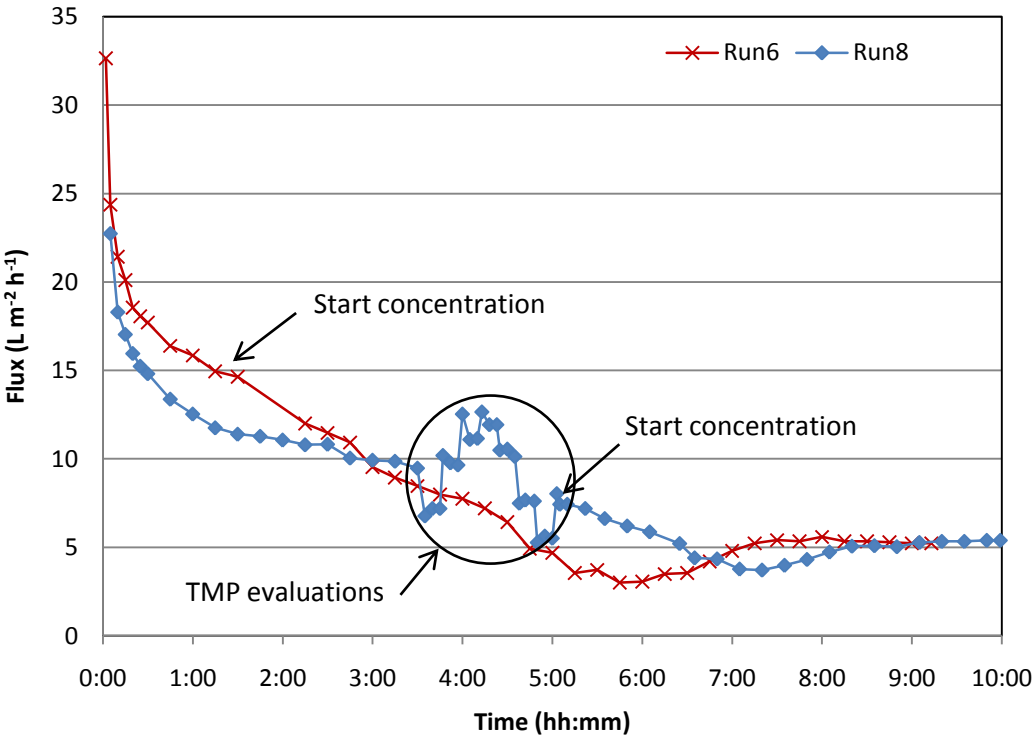


Figure I.1. Flux versus time during the two concentration runs. TMP 100 kPa, Feed 20 L h^{-1}, 25°C.

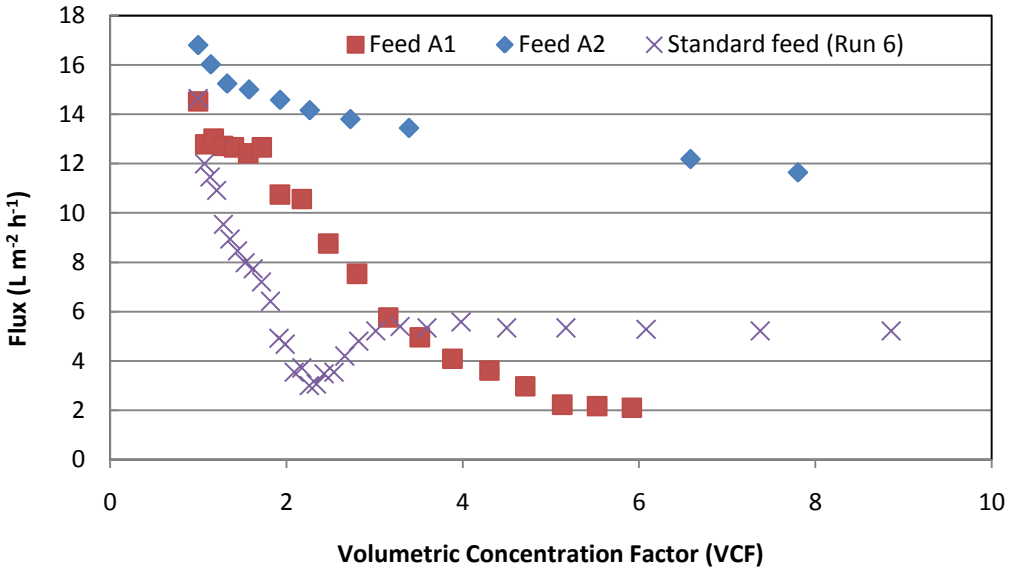


Figure I.2. Flux versus VCF for different feed streams. TMP 100 kPa, feed 20 L h^{-1}, temperature 25°C.

Appendix J: Millipore Cleaning Agent Selection Guide

Table J.1. Cleaning agent selection guide for Pellicon and Pellicon-2 ultrafiltration membranes.

Process Fluid	Probable Foulants	Cleaning Agents	
		Recommended	Alternative
PROTEIN SOLUTIONS Blood & Serum products Bacterial & Mammalian Products Enzymes Vaccines Viruses	Adsorbed Protein	NaOH Tergazyme® NaOCl*	Triton® X-100
BACTERIAL CELL WHOLE BROTHS E. Coli Bacillus Other	Adsorbed Protein, Antifoams, Cell Debris, Lipids, Polysaccharides	NaOH followed by NaOCl	Triton X-100 or SDS followed by NaOCl
BACTERIAL LYSATES	Protein, Lipopolysaccharides, Cell Debris	NaOH followed by H_3PO_4 NaOCl followed by H_2PO_4	Tween®-80 Triton X-100 or SDS followed by NaOCl
NUCLEIC ACIDS		H_3PO_4	
POLYSACCHARIDES Lipopolysaccharides Dextrins Pectins Starches Mucopolysaccharides	Adsorbed Polysaccharide	NaOH NaOCl* Tergazyme	Triton X-100 or SDS followed by NaOCl
DEPYROGENATION Radio-opaque imaging agents Antibiotics Low molecular weight solutions	Residual Organic Colloids, Lipopolysaccharides	NaOH	
FOOD & BEVERAGE STREAMS Food Wine Proteins Vinegar and Juice	Protein, Tannins, Phenolics, Organic Colloids, Humic Acids	NaOH followed by NaOCl Tergazyme followed by NaOCl	Tergazyme
WATER TREATMENT	Iron Complexes	Citric Acid	HNO_3
WATER TREATMENT	Mineral Scale Inorganic Deposits	HNO_3 H_3PO_4	Citric Acid

*Not for use with PL series membranes.

Table J.2. Pellcon-2 cleaning conditions.

Foulants	Membrane	Cleaning Agents	Conc.	Temp. °C	pH	Time (min.)
Organics, Biofilms, Biopolymer, Proteins, Polyphenolic	PT Series Biomax	NaOH	0.1-0.5N	40-50	13-13.7	30-60
same as above	PLAC, PLBC, PLCC	NaOH	0.1N	20-25	13	30-60
same as above	PLGC, PLCGC, PLTK, PLCTK, PLCHK, PLCMK, PLCXK	NaOH	0.1N	25-40	13	30-60
Proteins, Biopolymers Polysaccharides	PT Series Biomax Durapore	NaOCl	250 ppm Active Chlorine	40-50	10-11	30-60
Biopolymers Proteins, Colloidal Deposits, Polyphenolic Fats, Oils, Grease, Antifoams, Scale	All Membranes	Tergazyme	0.2%	40-50	9-10	30-60
Proteins, Lipids, Lipopolysaccarides, Oils, Antifoams	All Membranes	Triton-X 100 SDS Tween 80	0.1% 0.1% 0.1%	40-50 40-50 40-50	5-8 5-8 5-8	30-60 30-60 30-60
Proteins, Protein precipitates	All Membranes	Urea	7M	40-50	8	60
Mineral Scale, Nucleic Acids	All Membranes	HNO_3 H_3PO_4	0.1N 0.1N	40-50 40-50	1.0 1.0	30 30
Iron, Manganese, Scale	All Membranes	Citric Acid (Adjust to pH 3 with NH_4OH)	1%	40-50	3.0	60

Appendix K: Membrane Cleaning Trial Data Sheets

Table K.1. Cleaning Data Sheet Set 1 Run 1

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
RO water flush - 3L Ret side	16:30										
NWP	16:45		36/4	0.7	0.3	5	14.2	19.9		0.3	0.5
0.1N NaOH - 30 mns	16:50		60/4	1.4	0.1	40	11	45	11.0	1.2	0.8
RO water flush - 2L Ret side, 7L perm	17:30										
NWP	18:00		46/4	0.7	0.3	12	12	20		0.3	0.5
Store 0.1N NaOH - overnight	18:05										
RO water flush - 2L Ret side, 7L perm	08:40		38/4	0.7	0.0	18	15			0.7	0.3
NWP	09:10		37/4	0.7	0.3	10	18	18.3		0.3	0.5
Triton X-100	09:35	00:00	36/4	0.7	0.1	20	12.5	50		0.6	0.4
	09:50	00:15	56/3	0.7	0.1	20	12.5	40		0.6	0.4
	10:05	00:30	56/3	0.7	0.1	20	12.5	40		0.6	0.4
	10:20	00:45	56/3	0.7	0.1	20	12.5	39		0.6	0.4
	11:20	01:45	56/3	0.7	0.1	20	12.5	38		0.6	0.4
RO water flush - 2L Ret side, 7L perm	11:25		45/4	0.8	0.1	18	14			0.7	0.4
NWP	11:30		37/4	0.7	0.3	8	19.8	21.7		0.3	0.5
NWP (reverse feed direction)	11:35		38/4	0.7	0.3	8	21.6	22.7		0.3	0.5
0.1N phosphoric acid	13:15	00:00	37/4	0.7	0.1	21	15	45	2.2	0.6	0.4
	13:30	00:15	37/4	0.7	0.1	21	15	42		0.6	0.4
	13:45	00:30	37/4	0.7	0.1	21	15	44		0.6	0.4
	14:00	00:45	37/4	0.7	0.1	21	14.5	42		0.6	0.4
RO water flush - 2L Ret side, 7L perm	14:05		66/4	1.4	0.0	35	>25	21		1.4	0.7
NWP	14:30		40/4	0.7	0.3	9	21.1	22.2		0.3	0.5

Table K.2. Cleaning Data Sheet Set 1 Run 2

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
RO water flush 2.5L Ret, 7L perm			74/4	1.7	0.1	28	23			1.6	0.9
NWP			39/4	0.7	0.3	5	14.9	23.3		0.3	0.5
0.1N NaOH		00:00	40/4	0.7	0.0				11.0	0.7	0.3
		00:15	40/4	0.7	0.0	27	7.5	40.0		0.7	0.3
		01:00	40/4	0.7	0.0					0.7	0.3
RO water flush 2L Ret, 7L perm			68/4	1.4	0.0	20	13			1.4	0.7
NWP			39/4	0.7	0.3	5	11.4	22.5		0.3	0.5
0.1% SDS		00:00	37/4	0.7	0.1	18	7.8	39.3		0.6	0.4
		00:30	37/4	0.7	0.1	18	8.2	39.7		0.6	0.4
		00:45	37/4	0.7	0.1					0.6	0.4
RO water flush 2L Ret, 2L perm			66/4	1.5	0.1	19	18.5			1.4	0.8
NWP			40/4	0.7	0.3	5	12.6	21.4		0.3	0.5
Store 0.1N NaOH - overnight											
RO water flush 2L Ret, 7L perm			68/4	1.4	0.1	26	>25			1.3	0.7
NWP			42/4	0.7	0.3	8	17.8	19.4		0.3	0.5
NWP (reverse feed direction)			40/4	0.7	0.3	5	16	20.0		0.3	0.5
0.1N phosphoric acid		00:00	86/3	1.4	0.1	22	24	37.1	2.2	1.3	0.7
		00:20	86/3	1.4	0.1	22	24	41		1.3	0.7
		00:45	86/3	1.4	0.1	22	24.8	39.5		1.3	0.7
RO water flush 2L Ret			22/3	0.9	0.0	20	13			0.9	0.4
RO water flush 7L perm			67/4	1.4	0.5	23	20			0.9	0.9
NWP			38/4	0.7	0.3	5	16.9	20.4		0.3	0.5
NWP (reverse feed direction)			38/4	0.7	0.3	5	17	20.8		0.3	0.5

Table K.3. Cleaning Data Sheet Set 1 Run 3

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
RO water flush 2.5L ret, 1.0 L perm.			46/4	1.0	0.1	15	2.0			0.9	0.5
NWP			37/4	0.7	0.3	8	3.6	23.5		0.3	0.5
Store 0.1N NaOH - overnight				0.0	0.0					0.0	0.0
RO water flush 2 L ret.			39/4	1.0	0.0	15	11.0			1.0	0.5
RO water flush 4 L perm.			50/4	1.4	0.0	18	14.0			1.4	0.7
NWP			37/4	0.7	0.3	8	10.8	21.1		0.3	0.5
Triton X-100	09:45	00:00	31/4	0.7	0.1	14	8.0	42		0.6	0.4
	10:00	00:15	31/4	0.7	0.1	13	9.0	44		0.6	0.4
	10:45	01:00	31/4	0.7	0.1	13	8.5	42		0.6	0.4
RO water flush 2 L ret.			44/4	1.0	0.0	18	12.0			1.0	0.5
RO water flush 4 L perm.			54/4	1.4	0.0	21	15.5			1.4	0.7
NWP			33/4	0.7	0.3	5	13.5	23.2		0.3	0.5
Phosphoric acid	11:20	00:00	31/4	0.7	0.1	14	8.50	48	2.2	0.6	0.4
	11:50	00:30	31/4	0.7	0.1	15	8.50	42		0.6	0.4
	12:20	01:00	31/4	0.7	0.1	15	8.00	41		0.6	0.4
RO water flush 2 L ret.			44/4	0.8	0.1	16	10.5			0.8	0.4
RO water flush 4 L perm.			62/4	1.4	0.1	21	14.0			1.3	0.7
NWP			39/4	0.7	0.3	4	13.6	24.5		0.3	0.5

Table K.4. Cleaning Data Sheet Set 2 Run 1 to 3

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 1: Glucoamylase wash	12:45	00:00	40/4	0.7	0.1	18	9.0	44.1	5.0	0.6	0.4
	13:05	00:20	40/4	0.8	0.1	17	10.0	44.2	4.9	0.7	0.4
	13:20	00:35	40/4	0.8	0.1	16	9.0	44.7	4.3	0.8	0.4
	13:35	00:50	40/4	0.9	0.1	16	8.5	43.8	4.2	0.8	0.5
	13:45	01:00	40/4	0.8	0.1	16	8.5	42.5	4.1	0.8	0.4
Increase TMP	14:00	01:15	57/4	1.3	0.1	20	14.0	40.4	4.0	1.2	0.7
	14:15	01:30	57/4	1.3	0.1	20	13.5	39.2	4.1	1.2	0.7
RO water flush 2 L ret.			44/4	1.0	0.0	18	7.5			1.0	0.5
RO water flush 4 L perm.			58/4	1.4	0.0	25	9.0			1.4	0.7
NWP			35/4	0.7	0.3	6	9.6	25.8		0.3	0.5
Run 2: NaOH	14:55	00:00	38/4	0.9	0.1	17	7.0	50.5	11.0	0.8	0.5
	15:05	00:10	38/4	0.9	0.1	17	7.0	43.5		0.8	0.5
	15:40	00:45	38/4	1.0	0.1	18	6.5	43.7		0.9	0.5
	15:55	01:00	38/4	1.0	0.1	18	6.0	42.1		0.9	0.5
RO water flush 2 L ret.			44/4	1.0	0.0	17	6.0			1.0	0.5
RO water flush 5 L perm.			62/4	1.4	0.0	23	9.0			1.4	0.7
NWP			35/4	0.7	0.3	5	8.8	25.1		0.3	0.5
Run 3: Tergazyme	10:05	00:00	30/3.5	0.7	0.0	15	6.5	48.1		0.7	0.3
	10:25	00:20	56/4.5	2.8	0.1	47	19.0	39.5		2.7	1.4
	10:40	00:35	56/4.5	2.7	0.1	47	19.0	37.3		2.6	1.4
	11:00	00:55	56/4.5	2.7	0.1	50	20.0			2.6	1.4
RO water flush 2 L ret.			29/4	0.7	0.0	20	6.5			0.7	0.3
RO water flush 7 L perm.			51/4	1.4	0.1					1.4	0.8
NWP			26/3.5	0.7	0.3	8	7.9	19.7		0.3	0.5
NWP (opposite direction)			26/3.5	0.7	0.3	8	7.55	18.7		0.3	0.5

Table K.5. Cleaning Data Sheet Set 2 Run 4 to 7

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 4: NaOH + CL	12:20	00:00	31/4	0.7	0.0	20	7.0	47.8	11.0	0.7	0.3
	12:30	00:10	51/5	2.7	2.0	10	30.0	38.2		0.7	2.3
	12:55	00:35	51/5	2.7	2.2	5	30.0	39.2		0.5	2.4
	13:20	01:00	51/5	2.6	2.1	5	30.0	39.1		0.6	2.3
RO water flush 2 L ret.			29/4	0.7	0.0	20	6.5	19.5		0.7	0.3
RO water flush 7 L perm.			51/4	1.4	0.1	32	9.5	18.8		1.4	0.8
NWP			32/4	0.7	0.3	12	7.8	20.2		0.3	0.5
Run 5: NaOH storage (overnight)				0.0	0.0					0.0	0.0
RO water flush 2.5 L ret.			28/4	0.7	0.1	21	6.0			0.6	0.4
RO water flush 3.5 L perm.			42/4	1.4	1.0	13	15.0			0.4	1.2
NWP			35/3.5	0.7	0.3	12	8.1	19.9		0.3	0.5
Run 6: Citric acid	15:45	00:00	26/3.5	0.3	0.0	15	4.0	47.1	3	0.3	0.2
Increase TMP	15:50	00:05	42/4	1.4	1.0	5	18.0	41.8		0.4	1.2
	16:05	00:20	61/4	2.1	1.7	6	22.0	35.8		0.4	1.9
	16:20	00:35	75/4	2.7	2.3	5	>25	37.2		0.4	2.5
	16:30	00:45	75/4	2.7	2.3	5	>25			0.4	2.5
RO water flush 2 L ret.			39/3.5	0.7	0.0	23	4.5			0.7	0.3
RO water flush 3.5 L perm.			52/3.5	1.4	1.2	6	14.5			0.2	1.3
NWP			37/3.5	0.7	0.3	12	7.5	20.5		0.3	0.5
Run 7: Tergazyme (overnight)				0.0	0.0					0.0	0.0
RO water flush 2.5 L ret.			42/4	1.0	0.0	30	8.0			1.0	0.5
RO water flush 5 L perm.			55/4	1.7	1.4	5	19.0			0.3	1.5
NWP			36/3.5	0.7	0.3	12	8.6	18.5		0.3	0.5

Table K.6. Cleaning Data Sheet Set 2 Run 8 to 10

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 8: Urea	10:15	00:00	45/3.5	1.2	0.3	20	11.0	36.9	8.0	0.9	0.8
	11:15	01:00	45/3.5	1.2	0.4	21	12.0	40.1		0.8	0.8
	11:30	01:15	45/3.5	1.2	0.6	19	15.0	47.5		0.6	0.9
Increase TMP	11:35	01:20	47/4.5	2.8	2.3	5	>25	47.5		0.4	2.6
	11:55	01:40	47/4.5	2.6	2.1	5	>25	45.2		0.4	2.3
RO water flush 2 L ret.			42/3.5	0.4	0.0	20	6.0			0.4	0.2
RO water flush 5 L perm.			54/4	1.7	1.4	10	21.0			0.3	1.6
NWP			49/3.5	0.7	0.3	5	8.5	20.7		0.3	0.5
Run 9: NaOH (overnight)				0.0	0.0				11.0	0.0	0.0
RO water flush 2 L ret.			42/3.5	0.8	0.0	20	8.5			0.8	0.4
RO water flush 5 L perm.			54/4	1.7	1.4	8	18.0			0.3	1.6
NWP			39/3.5	0.7	0.3	12	9.5	20.6		0.3	0.5
Run 10: Glucoamylase	09:40	00:00	39/3.5	0.7	0.1	20	10.0	47.8	5.2	0.6	0.4
	10:00	00:20	39/3.5	0.6	0.1	20	12.5	48.4	5.4	0.6	0.3
Increase TMP	10:12	00:32	63/4	1.7	0.7	5	>25	51.3		1.0	1.2
	10:35	00:55	63/4	1.7	0.7	5	>25	42.1	5.7	1.0	1.2
	11:00	01:20	63/4	1.7	0.7	5	>25	47.1	5.7	1.0	1.2
Decrease TMP	11:30	01:50	44/3.5	0.7	0.3	9	21.0	46.6	5.4	0.3	0.5
Reverse direction	11:35	01:55	44/3.5	0.7	0.0	16	21.0	46.3		0.7	0.3
Increase TMP	12:00	02:20	66/3.5	1.4	0.1	20	>25	48.5	5.2	1.3	0.7
	12:40	03:00	63/4	1.8	0.8	17	>25	49.2	5.4	1.0	1.3
	13:10	03:30	59/4	1.7	1.3	5	>25	50.1	5.2	0.4	1.5
RO water flush 2 L ret.			71/3.5	0.8	0.0	23	18.0			0.8	0.4
RO water flush 7 L perm.			83/4	1.6	1.2	5	>25			0.4	1.4
NWP			44/4	0.7	0.3	9	12.1	22.7		0.3	0.5

Table K.7. Cleaning Data Sheet Set 2 Run 11

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 11: NaOH	14:45	00:00	45/4	0.8	0.0	21	7.0	46.1	11.0	0.8	0.4
	14:55	00:10	66/4	1.4	0.1	30	21.0	36.5		1.3	0.7
	15:05	00:20	70/4	1.7	0.7	0	>25	40.3		1.0	1.2
	15:25	00:40	70/4	1.6	0.6	0	>25	41.4		1.0	1.1
	15:30	00:45	85/4	2.0	0.8	0	>25			1.2	1.4
	15:40	00:55	66/4	1.4	0.1	2	>25	40.3		1.2	0.8
Reverse direction	15:45	01:00	46/4	0.7	0.0	16	23.0	40.2		0.7	0.3
	15:50	01:05	69/4	1.4	0.1	26	>25			1.3	0.7
	16:00	01:15	69/4	1.5	0.8	0	>25	40.3		0.8	1.1
	16:30	01:45	87/4	2.1	1.0	0	>25	41.8		1.2	1.6
RO water flush 2 L ret.			56/4	0.8	0.0	20	25.0			0.8	0.4
RO water flush 7 L perm.			79/4	1.4	0.7	0	>25			0.8	1.1
NWP			48/4	0.7	0.3	5	27.2	18.2		0.3	0.5
NWP reverse direction			46/4	0.7	0.3	5	24.1	20.0		0.3	0.5

Table K.8. Cleaning Data Sheet Set 3 Run 1

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 1a: RO water rinse (2L ret)			60/4	2.1	0.0	20	1.1			2.1	1.1
Run 1b: Tergazyme	17:30	00:00	47/4	1.7	0.1	20	13.0	48	9.1	1.7	0.9
	17:45	00:15	47/4	1.7	0.1	27	10.0	46		1.6	0.9
	18:00	00:30	47/4	1.8	1.2	14	9.0	47		0.6	1.5
	18:15	00:45	47/4	1.6	0.1	30	7.5	48		1.5	0.8
RO water flush 2 L ret.			49/3.5	1.0	0.0	20	13.0			1.0	0.5
RO water flush 2 L perm.			71/3.5	1.9	1.4	7	17.0			0.6	1.7
NWP			44/3.5	0.7	0.3	8	8.6	22.2		0.3	0.5
Run 1c: Glucoamylase	09:20	00:00	44/3.5	1.0	0.0	20	15.0	50.0	5.5	1.0	0.5
	09:30	00:10	44/3.5	0.8	0.0	20	15.0	48.2		0.8	0.4
	09:40	00:20	44/3.5	0.8	0.0	20	15.0	44.5		0.8	0.4
	10:00	00:40	71/3.5	1.7	0.2	29	23.5	50.1		1.5	1.0
	10:30	01:10	73/4	2.3	1.7	5	17.0	45.9		0.6	2.0
RO water flush 2.5 L ret.			65/3.5	0.8	0.0	30	6.0			0.8	0.4
RO water flush 4 L perm.			54/4	1.8	1.4	10	15.0			0.4	1.6
NWP			44/3.5	0.7	0.3	12	5.7	23.8		0.3	0.5
Run 1d: NaOH	14:20	00:00	52/3.5	1.1	0.0	20	14.0	44.7	11.0	1.1	0.6
	14:40	00:20	52/3.5	1.1	0.2	22	14.5			0.9	0.7
	14:50	00:30	62/4	2.1	1.4	0	>25	48.2		0.7	1.7
	15:00	00:40	55/4	1.1	0.0	20	19.0	48.2		1.1	0.6
Increase TMP	15:10	00:50	73/4	2.1	1.4	5	>25	48.5		0.6	1.8
	15:20	01:00	73/4	2.1	1.4	5	>25	50.3		0.6	1.8
RO water flush 2.5 L ret.			65/3.5	1.0	0.0	24	13.5			1.0	0.5
RO water flush 5 L perm.			74/4	1.7	1.2	5	>25			0.5	1.5
NWP			45/3.5	0.7	0.3	11	11.1	23.2		0.3	0.5

Table K.9. Cleaning Data Sheet Set 3 Run 2

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	$\Delta\Pi$ ($\beta\alpha\rho$)	TMP (bar)
Run 2a: RO water rinse (2 L ret)			49/3.5	0.7	0.0	16	2.5			0.7	0.3
NWP			38/3.5	0.7	0.3	10	4.4	21.6		0.3	0.5
Run 2b: Tergazyme	09:15	00:00	40/3.5	0.7	0.0	16	9.0	45.0	9.3	0.7	0.3
	10:00	00:45	40/3.5	0.7	0.0	16	9.0	46.1		0.7	0.3
	10:35	01:20	69/3.5	1.4	0.1	23	17.0	46.8		1.3	0.7
RO water flush 2 L ret.			48/3.5	0.9	0.1	15	14.0			0.8	0.5
RO water flush 5 L perm.			66/4	1.7	0.7	5	>25			1.0	1.2
NWP			46/3.5	0.7	0.3	5	18.2	22.5		0.3	0.5
Run 2c: Urea	11:45	00:00	36/3.5	0.7	0.0	13	4.5	49.8	8	0.7	0.3
	12:15	00:30	37/3.5	0.7	0.0	18	4.0	46.3		0.7	0.3
	12:26	00:41	55/3.5	1.5	0.8	10	21.0	46.2		0.8	1.1
	12:45	01:00	60/3.5	1.5	0.9	11	17.0	46.1		0.6	1.2
RO water flush 2 L ret.			41/3.5	0.8	0.0	17	6.0			0.8	0.4
RO water flush 3.5 L perm.			72/3.5	1.7	1.1	10	18.0			0.6	1.4
NWP			43/3.5	0.7	0.3	9	9.2	21.0		0.3	0.5
Run 2d: Glucoamylase	13:00	00:00	43/3.5	0.7	0.0	18	8.5	45.9	4.9	0.7	0.3
	14:00	01:00	43/3.5	0.8	0.1	18	12.0	46.2	5.3	0.7	0.4
	15:30	02:30	43/3.5	0.6	0.3	8	20.0	46.2	5.2	0.3	0.4
RO water flush 2 L ret.			44/3.5	0.7	0.0	12	14.0			0.7	0.3
RO water flush 5 L perm.			83/3.5	1.8	0.6	10	.>25			1.2	1.2
NWP			42/3.5	0.7	0.3	6	14.7	20.7		0.3	0.5
Run 2e: NaOH	17:05	00:00	48/3.5	0.8	0.0	18	19.0	44.1	11	0.8	0.4
	17:10	00:05	57/4	1.4	0.3	14	>25			1.0	0.9
	17:25	00:20	57/4	1.4	0.4	13	>25	44.4		1.0	0.9
	17:35	00:30	57/4	1.4	0.4	13	>25	44.8		1.0	0.9
RO water flush 2 L ret.			41/3.5	0.7	0.0	12	15.0			0.7	0.3
RO water flush 5 L perm.			76/3.5	1.8	0.6	10	>25			1.2	1.2
NWP			42/3.5	0.7	0.3	5	17.5	16.5		0.3	0.5

Table K.10. Cleaning Data Sheet Set 3 Run 3

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 3a: RO water rinse (2 L ret)			40/3.5	1.0	0.0	20	< 2			1.0	0.5
NWP			39/3.5	0.7	0.3	11	2.5	22.0		0.3	0.5
Run 3b: Tergazyme + PTX6L	07:00	00:00	46/3.5	0.8	0.1	17	13	42.3	9.3	0.8	0.4
	08:40	01:40	46/3.5	0.8	0.1	17	12	46.7		0.7	0.4
	09:00	02:00	33/3.5	0.8	0.1	15	11	45.8		0.6	0.4
RO water flush 2 L ret.			51/3.5	0.7	0.0	16	12			0.7	0.3
RO water flush 3.5 L perm.			69/3.5	1.4	0.6	10	24			0.8	1.0
NWP			39/3.5	0.7	0.3	5	13.5	18.9		0.3	0.5
Run 3c: NaOH	09:30	00:00	45/3.5	0.8	0.1	12	15	42.7	11.0	0.6	0.4
	10:00	00:30	45/3.5	0.8	0.1	15	10	45.7		0.6	0.4
	10:35	01:05	42/3.5	0.7	0.1	16	8	46.4		0.6	0.4
RO water flush 2 L ret.			45/3.5	0.8	0.0	17	7			0.8	0.4
RO water flush 3.5 L perm.			72/3.5	1.5	0.8	12	18			0.7	1.2
NWP			38/3.5	0.7	0.3	10	9.5	23.2		0.3	0.5
Run 3d: Glucoamylase	11:00	00:00	38/3.5	0.7	0.1	18	5	47.5	5.1	0.6	0.4
	12:15	01:15	38/3.5	0.6	0.1	15	13	46.5	5.1	0.6	0.3
	14:15	03:15	38/3.5	0.7	0.1	13	13	46.8	4.6	0.6	0.4
RO water flush 2 L ret.			39/3.5	0.7	0.0	15	6			0.7	0.3
RO water flush 2 L perm.			65/3.5	1.5	0.8	27	11			0.7	1.2
NWP			39/3.5	0.7	0.3	10	8.2	22.7		0.3	0.5
Run 3e: NaOH	15:25	00:00	39/3.5	0.7	0.1	19	6	47.5	11.0	0.6	0.4
	15:55	00:30	39/3.5	0.8	0.1	17	12	47.2		0.6	0.4
	16:35	01:10	54/3.5	1.4	0.6	10	> 25	47.7		0.8	1.0
RO water flush 2 L ret.			40/3.5	0.6	0.0	12	16			0.6	0.3
RO water flush 3.5 L perm.			74/3.5	1.7	0.6	10	> 25			1.1	1.2
NWP			42/3.5	0.7	0.3	5	18.6	26.5		0.3	0.5

Table K.11. Cleaning Data Sheet Set 3 Run 4

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
RO water flush 2 L ret.			33/3	0.7	0.0	17	0.0			0.7	0.3
RO water flush 7 L perm.			58/3	1.4	0.3	20	11.2			1.1	0.8
NWP			36/3	0.7	0.3	5	6.5	18.9		0.3	0.5
Run 4: Back-flush	16:50	00:00	30/2.5	0.3	0.1	-	-			0.2	0.2
Change direction	17:05	00:15	32/2.5	0.3	0.1	-	-			0.2	0.2
	17:20	00:30	32/2.5	0.3	0.1	-	-			0.2	0.2
NWP			40/3	0.7	0.3	5	6.5	19.1		0.3	0.5

Table K.12. Cleaning Data Sheet Set 3 Run 5 & 6

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 5a: RO water flush 2.5 L ret.			54/3.5	0.6	0.0	20	7.0			0.6	0.3
RO water flush 2 L perm.			73/4	1.8	1.3	10	16.0			0.5	1.6
NWP			46/3.5	0.7	0.3	10	7.7	22.7		0.3	0.5
Run 5b: Tergazyme	10:40	00:00	44/3.5	0.7	0.1	17	6.5	42.5	9.0	0.6	0.4
	12:40	02:00	44/3.5	0.8	0.3	17	14.0	42.5		0.6	0.6
RO water flush 2.5 L ret.			59/3.5	1.0	0.1	20	12.0			1.0	0.6
RO water flush 3.5 L perm.			66/4	1.8	1.0	13	23.0			0.8	1.4
NWP			45/3.5	0.7	0.3	9	11.6	19.7		0.3	0.5
Run 5c: PTX6L	13:10	00:00	43/3.5	0.7	0.0	18	8.0	43.3	9.0	0.7	0.3
	13:55	00:45	60/3.5	1.2	0.5	8	>25	45.0	8.9	0.7	0.8
	15:10	02:00	60/3.5	1.2	0.6	10	>25	44.2	8.5	0.6	0.9
RO water flush 2.5 L ret.			56/3.5	0.7	0.0	20	20.0			0.7	0.3
RO water flush 5 L perm.			65/4	1.9	0.7	9	>25			1.2	1.3
NWP			45/3.5	0.7	0.3	5	19.7	21.1		0.3	0.5
Run 6a: RO water flush 2 L ret.			73/3.5	1.4	0.0	30	3.0			1.4	0.7
NWP			42/3.5	0.7	0.3	10	4.1	18.8		0.3	0.5
Run 6b: PTX6L	10:15	00:00	40/3.5	0.7	0.0	14	9.0	41.2	9.0	0.7	0.3
	10:45	00:30	40/3.5	0.7	0.0	14	10.0	44.7		0.7	0.3
	11:20	01:05	40/3.5	0.7	0.1	10	15.0	46.3		0.6	0.4
	12:00	01:45	58/3.5	1.2	0.4	10	25.0			0.8	0.8
	12:20	02:05	58/3.5	1.2	0.5	10	24.0	47.5		0.7	0.8
RO water flush 2 L ret.			56/3.5	0.8	0.1	20	13.0			0.8	0.4
RO water flush 3.5 L perm.			65/4	1.7	0.8	10	>25			0.9	1.3
NWP			41/3.5	0.7	0.3	5	13.7	20.5		0.3	0.5
Run 6c: Tergazyme	12:45	00:00	49/3.5	0.9	0.1	15	14.0	44.5	9.0	0.8	0.5
	14:10	01:25	49/3.5	0.9	0.2	15	17.0	46.5		0.7	0.6
	14:40	01:55	63/3.5	1.4	0.6	9	>25	47.5		0.9	1.0
RO water flush 2 L ret.			49/3.5	0.8	0.1	15	14.0			0.8	0.4
RO water flush 3.5 L perm.			78/3.5	1.6	0.6	10	>25			1.0	1.1
NWP			47/3.5	0.7	0.3	5	17.3	21.6		0.3	0.5

Table K.13. Cleaning Data Sheet Set 3 Run 7

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 7a: RO water rinse (3 L ret)			56/3	1.0	0.0	20	4.0			1.0	0.5
RO water flush 3 L perm.			68/3.5	1.7	1.1	10	13.0			0.6	1.4
NWP			39/3.5	0.7	0.3	10	5.6	20.8		0.3	0.5
Run 7b: Tergazyme + PTX6L	09:15	00:00	40/3.5	0.7	0.0	16	9.0	45.1	9.3	0.7	0.3
	10:00	00:45	40/3.5	0.7	0.0	16	9.0	46.1	9.2	0.7	0.3
	10:35	01:20	73/3.5	1.3	0.1	23	17.0	46.8	9.2	1.2	0.7
	11:15	02:00	73/3.5	1.7	0.7	10	>25	46.5	9.2	1.0	1.2
RO water flush 2 L ret.			43/3.5	0.7	0.1	12	12.0			0.6	0.4
RO water flush 5 L perm.			66/4	1.7	0.7	5	>25			1.0	1.2
NWP			46/3.5	0.7	0.3	5	18.2	22.5		0.3	0.5

Table K.14. Cleaning Data Sheet Set 4 Run 1

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 1a: RO water rinse (3 L ret)			56/3	1.0	0.0	20	4.0			1.0	0.5
RO water flush 3 L perm.			68/3.5	1.7	1.1	10	13.0			0.6	1.4
NWP			39/3.5	0.7	0.3	10	5.6	20.8		0.3	0.5
Run 1b: Tergazyme + PTX6L	09:15	00:00	40/3.5	0.7	0.0	16	9.0	45.1	9.3	0.7	0.3
	10:00	00:45	40/3.5	0.7	0.0	16	9.0	46.1	9.2	0.7	0.3
	10:35	01:20	73/3.5	1.3	0.1	23	17.0	46.8	9.2	1.2	0.7
	11:15	02:00	73/3.5	1.7	0.7	10	>25	46.5	9.2	1.0	1.2
RO water flush 2 L ret.			43/3.5	0.7	0.1	12	12.0			0.6	0.4
RO water flush 5 L perm.			66/4	1.7	0.7	5	>25			1.0	1.2
NWP			46/3.5	0.7	0.3	5	18.2	22.5		0.3	0.5
Run 1c: NaOH	11:45	00:00	36/3.5	0.7	0.0	13	4.5	49.8	11	0.7	0.3
	12:15	00:30	37/3.5	0.7	0.0	18	4.0	46.3		0.7	0.3
	12:26	00:41	55/3.5	1.5	0.8	10	21.0	46.2		0.8	1.1
	12:45	01:00	60/3.5	1.5	0.9	11	17.0	46.1		0.6	1.2
RO water flush 2 L ret.			41/3.5	0.8	0.0	17	6.0			0.8	0.4
RO water flush 3.5 L perm.			72/3.5	1.7	1.1	10	18.0			0.6	1.4
NWP			43/3.5	0.7	0.3	9	9.2	21.0		0.3	0.5
Run 1d: Glucoamylase	13:00	00:00	43/3.5	0.7	0.0	18	8.5	45.9	4.9	0.7	0.3
	14:00	01:00	43/3.5	0.8	0.1	18	12.0	46.2	5.3	0.7	0.4
	15:30	02:30	41/3.5	0.6	0.3	8	20.0	46.2	5.2	0.3	0.4
	16:30	03:30	41/3.5	0.7	0.3	8	21.0	45.5	5.3	0.4	0.5
RO water flush 2 L ret.			44/3.5	0.7	0.0	12	14.0			0.7	0.3
RO water flush 5 L perm.			83/3.5	1.8	0.6	10	.>25			1.2	1.2
NWP			42/3.5	0.7	0.3	6	14.7	20.7		0.3	0.5

Table K.14. cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 1e: NaOH	17:05	00:00	48/3.5	0.8	0.0	18	19.0	44.1	11	0.8	0.4
	17:10	00:05	57/4	1.4	0.3	14	>25			1.0	0.9
	17:25	00:20	57/4	1.4	0.4	13	>25	44.4		1.0	0.9
	17:35	00:30	57/4	1.4	0.4	13	>25	44.8		1.0	0.9
RO water flush 2 L ret.			41/3.5	0.7	0.0	12	15.0			0.7	0.3
RO water flush 5 L perm.			76/3.5	1.8	0.6	10	>25			1.2	1.2
NWP			42/3.5	0.7	0.3	5	17.5	16.5		0.3	0.5

Table K.15. Cleaning Data Sheet Set 4 Run 2

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 3a: RO water rinse (2 L ret)			40/3.5	1.0	0.0	20	< 2			1.0	0.5
NWP			39/3.5	0.7	0.3	11	2.5	22.0		0.3	0.5
Run 3b: Tergazyme + PTX6L	07:00	00:00	46/3.5	0.8	0.1	17	13	42.3	9.3	0.8	0.4
	08:40	01:40	46/3.5	0.8	0.1	17	12	46.7		0.7	0.4
	09:00	02:00	33/3.5	0.8	0.1	15	11	45.8		0.6	0.4
RO water flush 2 L ret.			51/3.5	0.7	0.0	16	12			0.7	0.3
RO water flush 3.5 L perm.			69/3.5	1.4	0.6	10	24			0.8	1.0
NWP			39/3.5	0.7	0.3	5	13.5	18.9		0.3	0.5
Run 3c: NaOH	09:30	00:00	45/3.5	0.8	0.1	12	15	42.7	11.0	0.6	0.4
	10:00	00:30	45/3.5	0.8	0.1	15	10	45.7		0.6	0.4
	10:35	01:05	42/3.5	0.7	0.1	16	8	46.4		0.6	0.4
RO water flush 2 L ret.			45/3.5	0.8	0.0	17	7			0.8	0.4
RO water flush 3.5 L perm.			72/3.5	1.5	0.8	12	18			0.7	1.2
NWP			38/3.5	0.7	0.3	10	9.5	23.2		0.3	0.5
Run 3d: Glucoamylase	11:00	00:00	38/3.5	0.7	0.1	18	5	47.5	5.1	0.6	0.4
	12:15	01:15	38/3.5	0.6	0.1	15	13	46.5	5.1	0.6	0.3
	14:15	03:15	38/3.5	0.7	0.1	13	13	46.8	4.6	0.6	0.4
RO water flush 2 L ret.			39/3.5	0.7	0.0	15	6			0.7	0.3
RO water flush 2 L perm.			65/3.5	1.5	0.8	27	11			0.7	1.2
NWP			39/3.5	0.7	0.3	10	8.2	22.7		0.3	0.5
Run 3e: NaOH	15:25	00:00	39/3.5	0.7	0.1	19	6	47.5	11.0	0.6	0.4
	15:55	00:30	39/3.5	0.8	0.1	17	12	47.2		0.6	0.4
	16:35	01:10	54/3.5	1.4	0.6	10	> 25	47.7		0.8	1.0
RO water flush 2 L ret.			40/3.5	0.6	0.0	12	16			0.6	0.3
RO water flush 3.5 L perm.			74/3.5	1.7	0.6	10	> 25			1.1	1.2
NWP			42/3.5	0.7	0.3	5	18.6	26.5		0.3	0.5

Table K.16. Cleaning Data Sheet Set 4 Run 3

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 3a: RO water rinse (2 L ret)			61/3.5	1.4	0.0	15	2.0			1.4	0.7
NWP			45/3.5	0.7	0.3	10	2.3	21.2		0.3	0.5
Run 3b: Tergazyme + PTX6L	09:10	00:00	60/3.5	1.4	0.0	22	12.0	44.1	9.2	1.4	0.7
	10:10	01:00	60/3.5	1.3	0.1	23	11.0	45.4		1.2	0.7
Increase TMP	10:13	01:03	62/3.5	1.4	0.6	10	25.0	47.1		0.8	1.0
	11:50	02:40	62/3.5	1.4	0.6	8	24.0	45.7		0.8	1.0
RO water flush 3 L ret.			43/3.5	0.7	0.0	13	10.0			0.7	0.3
RO water flush 5 L perm.			84/3.5	1.9	0.8	10	>25			1.0	1.3
NWP			48/3.5	0.7	0.3	5	13.7	20.0		0.3	0.5
Run 3c: NaOH	16:25	00:00	63/3.5	1.5	0.1	26	10.0	46.3	11	1.4	0.8
	16:35	00:10	63/3.5	1.5	0.1	23	15.0	44.8		1.4	0.8
	17:10	00:45	63/3.5	1.5	0.8	10	20.0			0.8	1.1
RO water flush 3 L ret.			45/3.5	0.7	0.0	17	12.0			0.7	0.3
RO water flush 2 L perm.			73/3.5	1.4	0.8	10	20.0			0.6	1.1
NWP			44/3.5	0.7	0.3	8	10.1	21.0		0.3	0.5
Run 3d: Glucoamylase	08:40	00:00	45/3.5	1.0	0.1	20	11.0	45.3	5.0	1.0	0.6
	09:40	01:00	45/3.5	1.0	0.1	18	19.0	45.0		0.8	0.6
	10:10	01:30	46/3.5	1.0	0.1	18	23.0	47.1		0.8	0.6
	10:40	02:00	46/3.5	1.0	0.1	18	23.0	47.5	5.0	0.8	0.6
	11:10	02:30	46/3.5	1.0	0.2	17	22.0	48.1	5.2	0.8	0.6
	12:00	03:20	50/3.5	1.0	0.2	13	20.0	48.5	5.2	0.8	0.6
RO water flush 3 L ret.			49/3.5	0.7	0.1	13	14.0			0.6	0.4
RO water flush 2 L perm.			73/3.5	1.4	0.6	10	>25			0.8	1.0
NWP			43/3.5	0.7	0.3	5	14.7	17.8		0.3	0.5

Table K.16. cont.

Step	Time (hh:mm)	Run Time (hh:mm)	Feed Rate (%/Oc)	P In (bar)	P out (bar)	Ret (L/h)	Flux (L/h)	Temp (°C)	pH	ΔP (bar)	TMP (bar)
Run 3e: NaOH	12:25	00:00	68/3.5	1.7	0.1	24	>25	45.2	11	1.7	0.9
	13:05	00:40	68/3.5	1.7	0.1	24	>25	47.6		1.5	0.9
	13:25	01:00	68/3.5	1.5	0.1	26	24.0	47.5		1.4	0.8
RO water flush 3 L ret.			47/3.5	0.7	0.0	14	11.0			0.7	0.3
RO water flush 3.5 L perm.			68/3.5	1.4	0.6	10	>25			0.9	1.0
NWP			42/3.5	0.7	0.3	5	16.2	18.1		0.3	0.5

Appendix L: Measurement of Normalised Water Permeability

Millipore Maintenance Procedures

MEASUREMENT OF NORMALIZED WATER PERMEABILITY (NWP)

The normalized water permeability (NWP) for Pellicon and Pellicon-2 Cassette Filter membranes should be established prior to the first use of each filter. New membranes should be cleaned and flushed before measuring NWP. The NWP measured at this point is used as a benchmark against which subsequent water permeability measurements are compared to. These subsequent NWP measurement are used to determine cleaning efficacy.

PROCEDURE

1. Direct the permeate and retentate lines back to the cleaning tank.
2. Close the tank drain valve and open the retentate line or valve. Open the permeate valve if present.
3. If the system has a variable speed pump, set it to the lowest speed and flow rate. If the system has a centrifugal pump, close the discharge valve.
4. Fill the tank with clean water, preferably 25 °C. For accurate NWP measurement, water quality must satisfy the criteria noted on page 13.
5. Start the recirculation pump and adjust it to the following pressure conditions:

   Feed inlet pressure: 10 psi (0.7 bar)

   Retentate outlet pressure: 5 psi (0.35 bar)
6. Recirculate the water for 5-10 minutes. Ensure that the pressure and the temperature conditions are stable.
7. Record the permeate flow rate, inlet and outlet pressures, and the temperature of the water.
8. After the cycle is complete, shut down the recirculation pump and drain the system.
9. Calculate the NWP with the equation below. Compare the results to the original NWP measured when the filter was new.

$$NWP = \frac{R \bullet F}{A \bullet \left\{ \left[\frac{P_{in} + P_{out}}{2} \right] - P_p \right\}}$$

These units yield LMH/psi [liters/m2 • hours • psi]

Calculate:

R = Permeate Flow Rate in L/hour

P_{in} = Feed Inlet Pressure in psi

P_{out} = Retentate Discharge Pressure in psi

Pp = Permeate Discharge Pressure (if non-zero) in psi

T = Water Temperatures in °C

A = Total Filter area in m^2

F = Temperature correction factor from table on page 13.

10. After the first use of the module, the normal water permeability should be 60 to 80% of the original NWP. After repeated use (more than 5 times) the normalized water permeability should not vary more than 10% from run-to-run.

If the NWP decreases from run-to-run, cleaning procedures may be inadequate. Alternative cleaning agents and procedures should be investigated. Contact your Millipore Technical Service Representative for assistance.

NORMALIZED WATER PERMEABILITY TEMPERATURE CORRECTION FACTOR (F)*

T (°F)	T (°C)	F	T (°F)	T (°C)	F	T (°F)	T (°C)	F
125.6	52	0.595	96.8	36	0.793	68.0	20	1.125
123.8	51	0.605	95.0	35	0.808	66.2	19	1.152
122.0	50	0.615	93.2	34	0.825	64.4	18	1.181
120.2	49	0.625	91.4	33	0.842	62.6	17	1.212
118.4	48	0.636	89.6	32	0.859	60.8	16	1.243
116.6	47	0.647	87.8	31	0.877	59.0	15	1.276
114.8	46	0.658	86.0	30	0.896	57.2	14	1.310
113.0	45	0.670	84.2	29	0.915	55.4	13	1.346
111.2	44	0.682	82.4	28	0.935	53.6	12	1.383
109.4	43	0.694	80.6	27	0.956	51.8	11	1.422
107.6	42	0.707	78.8	26	0.978	50.0	10	1.463
105.8	41	0.720	77.0	25	1.000	48.2	9	1.506
104.0	40	0.734	75.2	24	1.023	46.4	8	1.551
102.2	39	0.748	73.4	23	1.047	44.6	7	1.598
100.4	38	0.762	71.6	22	1.072	42.8	6	1.648
98.6	37	0.777	69.8	21	1.098	41.0	5	1.699

*Based on Water Fluidity Relative to 25°C (77°F) Fluidity Value $F = (\mu_{T°C}/\mu_{25°C})$ or $(\mu_{T°F}/\mu_{77°F})$

WATER QUALITY

The re-establishment of the clean water permeability of the membrane is the key criterion for the determination of cleaning effectiveness. Therefore, high quality water must be used for cleaning and flushing.

WATER QUALITY

Constituent	Acceptable Concentration	Constituent	Acceptable Concentration
Fe	<0.05 ppm	Ca, Mg	<25 ppm
Mn	<0.05 ppm	Turbidity	<1.0 JTU
Al	<0.5 ppm	SDI 15 (fouling index)	preferably <3.0
Reactive Silica	<2 ppm	Particulate Matter	None
Colloidal Silica	Nil	Oil, Grease, etc.	None

Reverse osmosis permeate or water for injection is recommended whenever possible.

Appendix M: Membrane Cleaning Trial Charts

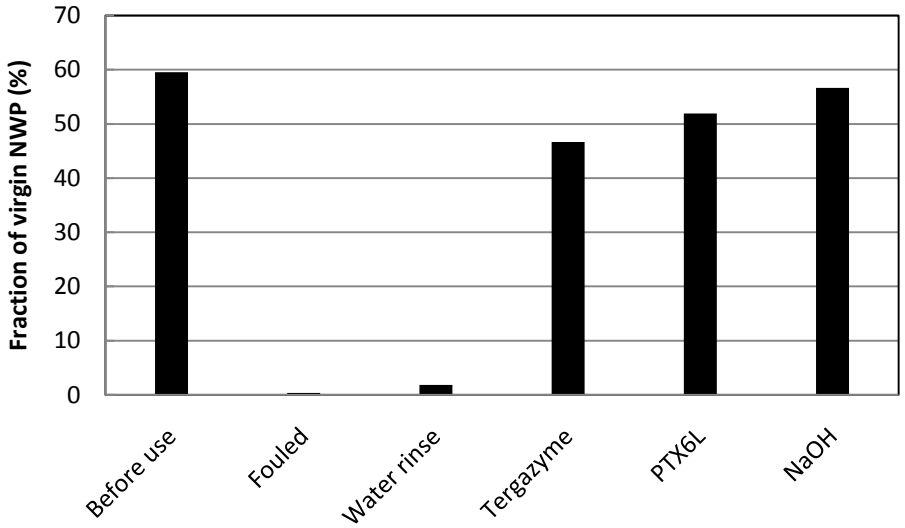


Figure M.1. NWP during membrane cleaning – membrane fouled by a starch-free feed stream.

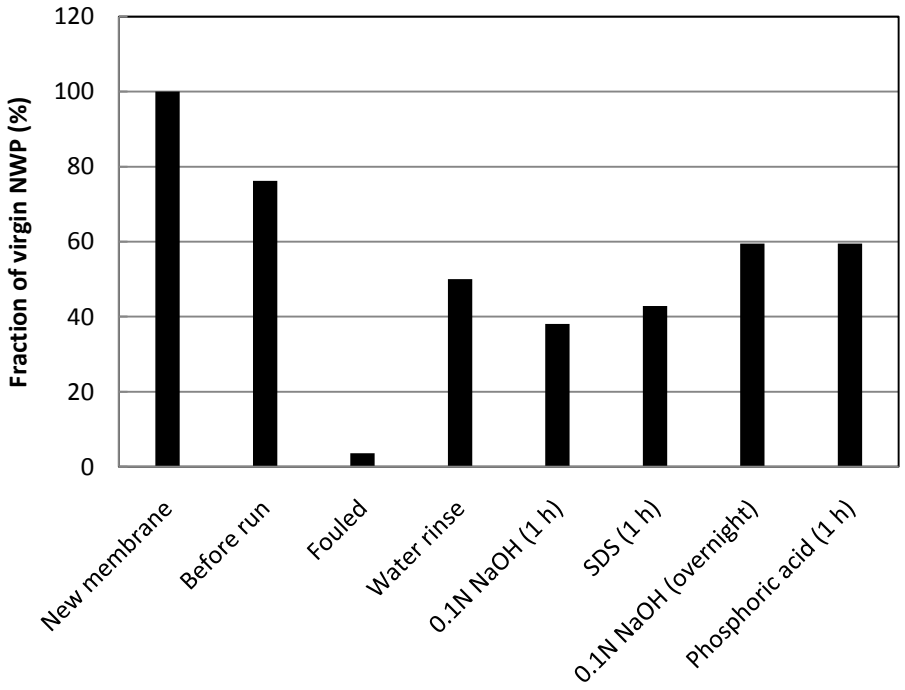


Figure M.2. Set 1 Run 2, NWP after each step of the cleaning cycle.

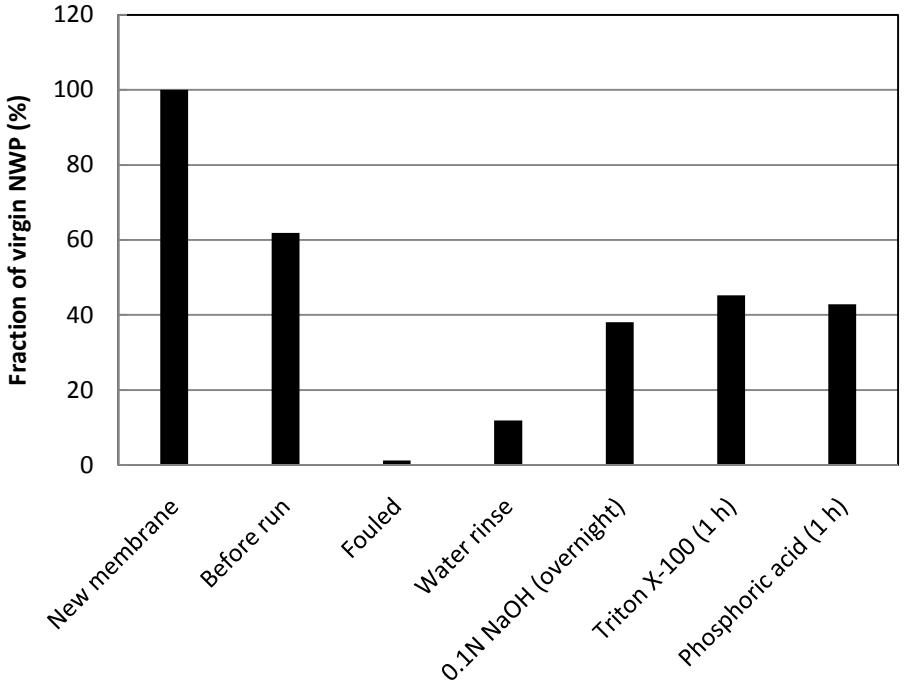


Figure M.3. Set 1 Run 3, NWP after each step of the cleaning cycle.

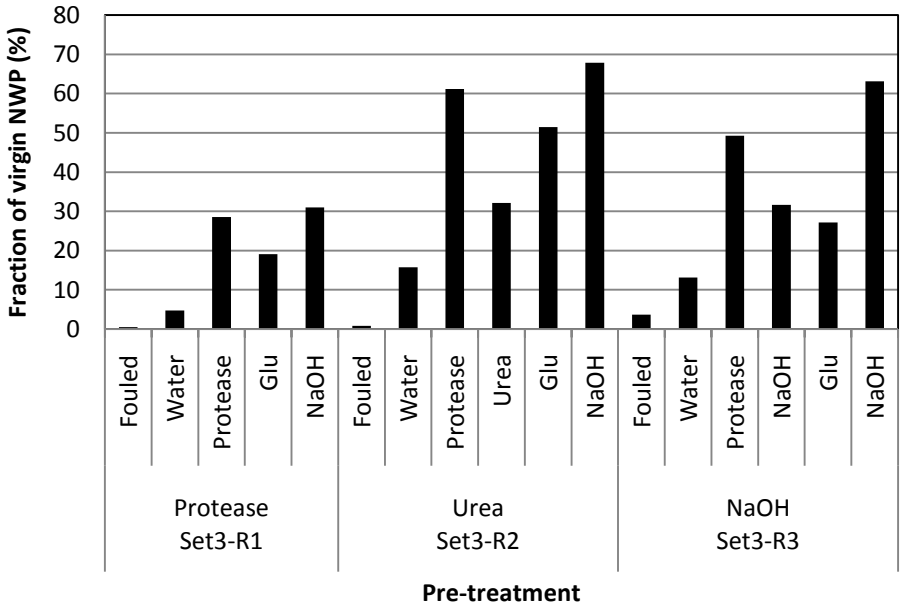


Figure M.4. Effectiveness of a glucoamylase-sodium hydroxide wash after various pre-treatments

Appendix N: Membrane Integrity Test Method

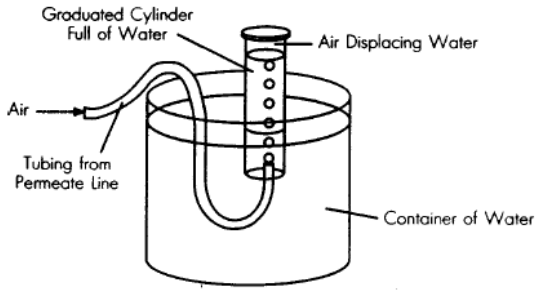
Millipore Maintenance Procedures

MODULE INTEGRITY TEST PROCEDURE

The module integrity should be tested on a cleaned and thoroughly flushed system. The presence of residual cleaning agents can significantly alter integrity test results.

1. Ensure that the system is thoroughly cleaned and that the membrane is thoroughly wetted. Thorough wetting can be ensured by recirculating water at 30 psi (2.4 bar) TMP for 5 minutes.
2. Drain the system of water. **It is important to drain the retentate side of the system as thoroughly as possible.**
3. Attach a regulated and filtered air supply to the feed or retentate side of the holder, preferably to the more elevated end.
4. Isolate either the feed or the retentate manifold by closing a valve or capping the manifold if there is no valve on it.
5. The permeate line should be open at all times
6. Slowly raise the air pressure to the recommended value and wait 5 minutes to purge residual water in the permeate line.

   Do not exceed the recommended air pressure, which will displace water out of the wettedpores. This will result in excessively high air flow. Rewet the membrane if this occurs.
7. Measure and record the air pressure, temperature and the air flow rate exiting the permeate line. The air flow rate may be measured with an air flow meter or by measuring the air displaced into a submerged and inverted volumetric cylinder as shown in the figure below.



8. Compare the measured air flow rate to the specified flow value in the following table. If the measured flow rate exceeds the specified flow value, check to confirm that the Pellicon or Pellicon-2 Cassette Filter has been installed correctly according to the operating manual. If the filter has been installed correctly and the air flow remains in excess of the specified value, contact your local Millipore Applications Specialist, or call Millipore Technical Services.

RECOMMENDED AIR FLOWS TO CONFIRM INTEGRITY Of Pellicon Cassette Filters

AIR FLOW THROUGH FULLY WETTED MEMBRANE OF AN INTEGRAL FILTER (CC/MIN)

Membrane Designation	NMWL or Pore Size Mini	per 1.0 ft² (0.1 m²) Cassette	per 5.0 ft² (0.5 m²) Maxi	per 25.0 ft² (2.5 m²) PSI (BAR)	Test Pressure
PT SERIES					
PTGC	10,000	<3	<12	<60	5 (0.34)
PHSA-G	10,000	N/A	<12	N/A	5 (0.34)
PTTK	30,000	<3	<12	<60	5 (0.34)
PHSA-T	HI-FLUX 10,000	N/A	<24	N/A	10 (0.68)
PTHK	100,000	<3	<12	<60	5 (0.34)
PTMK*	300,000	<12	<60	<300	5 (0.34)
PL SERIES					
PLAC	1,000	<3	N/A	N/A	5 (0.34)
PLBC	3,000	<3	N/A	N/A	5 (0.34)
PLCC	5,000	<3	<12	N/A	5 (0.34)
PLGC	10,000	<10	<46	N/A	5 (0.34)
PLTK	30,000	<12	<60	N/A	5 (0.34)
PLHK	100,000	<12	<60	N/A	5 (0.34)
PLMK	300,000	<72	<360	N/A	5 (0.34)
DURAPORE					
VVPP	0.10 um	<3	<12	N/A	10 (0.68)
GVPP	0.22 um	<3	<12	N/A	10 (0.68)
HVMP	0.45 um	<3	<12	<60	10 (0.68)
DVPP	0.65 um	<3	N/A	N/A	10 (0.68)

*PES = POLYETHERSULFONE * POLYSULFONE*

RECOMMENDED AIR FLOWS TO CONFIRM INTEGRITY Of Pellicon-2 Cassette Filters

AIR FLOW THROUGH FULLY WETTED MEMBRANE OF AN INTEGRAL FILTER (CC/MIN)

Membrane Designation	NMWL or Pore Size	per 1.0 ft² per (0.1 m²) Mini	5.0 ft² (0.5 m²) Cassette	per 25.0 ft² (2.5 m²) Maxi	Test Pressure PSI (BAR)
Biomax					
Biomax 5	5,000	<4	<18	<90[1]	10 (0.68)
Biomax 8	8,000	<4	<18	<90[1]	10 (0.68)
Biomax 10	10,000	<4	<18	<90	30 (2.4)
Biomax 30	30,000	<4	<18	<90	10 (0.68)
Biomax 50	50,000	<4	<18	<90	10 (0.68)
Biomax 100	100,000	<4	<18	<90	10 (0.68)
Biomax 300	300,000	<12	<60	<300	10 (0.68)
Biomax 500	500,000	<12	<60	<300	10 (0.68)
Biomax 1,000	1,000,000	<12	<60	<300	10 (0.68)
PL SERIES					
PLAC	1,000	<10	<48	<240[1]	30 (2.4)
PLBC	3,000	<10	<48	<240[1]	30 (2.4)
PLCC	5,000	<10	<48	<240[1]	30 (2.4)
PLGC	10,000	<6	<30	<150	5 (0.34)
PLTK	30,000	<12	<60	<300	5 (0.34)
PLC SERIES (Composite)					
PLCCC (PC005)	5,000	<7	<35	<175	30 (2.4)
PLCGC (PC010)	10,000	<7	<35	<175	30 (2.4)
PLCTK (PC030)	30,000	<4	<18	<90	10 (0.68)
PLCHK (PC100)	100,000	<3	<12	<60[1]	10 (0.68)
PLCMK (PC300)	300,000	<4	<18	<90[1]	10 (0.68)
PLCXK (PC01M)	1,000,000	<4	<18	<90[1]	10 (0.68)

[1] *Contact Millipore Technical Services for availability of Maxi cassettes.*