Vibrating Membrane Filtration: Microfiltration Performance During the Processing of Biological Feedstreams.

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By

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Abstract

Conventional crossflow microfiltration systems rely on high liquid velocities to generate shear at the liquid–membrane interface. Shear is necessary in order to maintain acceptable flux and product transmission levels especially when processing fluids with a high solids loading. PallSep is a new technology that uses mechanical energy generated by vibration to create high intermittent shear rates at the membrane surface thus decoupling shear and liquid crossflow velocity. High permeate fluxes can then be maintained over extended periods of operation at low retentate flow-rates. This work considers the use of a PallSep PS10 (0.2 m² membrane area) for the recovery of both proteins and low molecular weight molecules from complex biological feedstreams.

For the optimisation of protein recovery it is necessary to understand how flux and transmission levels vary as a function of membrane operation. In this work, a model system of baker's yeast (*Saccharomyces cerevisiae*) and BSA is used to study the effect of membrane operation on permeate flux and protein transmission. Similarly, the recovery of polyketide antibiotics from whole fermentation broths is a particularly challenging application for membrane technologies. Such broths typically have both a high viscosity and solids loading and the economics of the process require >95 % w/w product recovery with a minimum of diafiltration. In this work the interactions between fermentation and microfiltration operations are investigated, examining the recovery of the polyketide antibiotic erythromycin from *Saccharopolyspora erythraea* fermentation broths.

The results for both systems indicate that flux and transmission levels are independent of liquid crossflow velocity but critically dependant on membrane head amplitude and hence shear rate, membrane spacing, transmembrane pressure and the solids concentration of the process stream. The study has shown PallSep technology to be a viable alternative to conventional filtration technology for the processing of feed streams particularly those with a viscous nature or containing high levels of suspended solids.
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Experimental conditions: Membrane head amplitude 19.5 mm, crossflow rate in the range of 0.3 to 2 Lmin$^{-1}$ and membrane gap width 1.4 mm.

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<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
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<tr>
<td>A1 and A2</td>
<td>Amplitudes of mass A and mass B (Hz)</td>
</tr>
<tr>
<td>AC</td>
<td>Alternating current</td>
</tr>
<tr>
<td>b</td>
<td>Channel height (m)</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>C_b</td>
<td>Bulk concentration of cells in the feed stream (% w/w)</td>
</tr>
<tr>
<td>CER</td>
<td>Carbon dioxide evolution rate (mmolL(^{-1})hr(^{-1}))</td>
</tr>
<tr>
<td>cTMP</td>
<td>Critical transmembrane pressure</td>
</tr>
<tr>
<td>C_w</td>
<td>Wall concentration of cells (% w/w)</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion coefficient (m(^2)s(^{-1}))</td>
</tr>
<tr>
<td>d</td>
<td>Membrane head displacement (m)</td>
</tr>
<tr>
<td>d(_{50})</td>
<td>Mean particle diameter</td>
</tr>
<tr>
<td>DCW</td>
<td>Dry cell weight</td>
</tr>
<tr>
<td>DMF</td>
<td>Dynamic membrane filtration</td>
</tr>
<tr>
<td>DOT</td>
<td>Dissolved oxygen tension (%)</td>
</tr>
<tr>
<td>DSP</td>
<td>Down stream processing</td>
</tr>
<tr>
<td>EFD</td>
<td>Enhanced flow device</td>
</tr>
<tr>
<td>F</td>
<td>Frequency of oscillation (Hz)</td>
</tr>
<tr>
<td>h</td>
<td>Distance between membrane discs (m)</td>
</tr>
<tr>
<td>HSA</td>
<td>Human serum albumin</td>
</tr>
<tr>
<td>IA</td>
<td>Image analysis</td>
</tr>
<tr>
<td>J</td>
<td>Permeate flux (Lm(^{-2})hr(^{-1}))</td>
</tr>
<tr>
<td>J1 and J2</td>
<td>Mass moment of inertia of mass A and mass B</td>
</tr>
<tr>
<td>k</td>
<td>Torsional spring constant</td>
</tr>
<tr>
<td>K</td>
<td>Mass transfer coefficient</td>
</tr>
<tr>
<td>K_b</td>
<td>Boltzman constant</td>
</tr>
<tr>
<td>K_p</td>
<td>Proportionality constant</td>
</tr>
<tr>
<td>L</td>
<td>Channel length (m)</td>
</tr>
<tr>
<td>L_BL</td>
<td>Boundary layer thickness</td>
</tr>
<tr>
<td>MF</td>
<td>Microfiltration</td>
</tr>
<tr>
<td>n</td>
<td>Flow behaviour index</td>
</tr>
<tr>
<td>NB</td>
<td>Nutrient broth</td>
</tr>
<tr>
<td>NFF</td>
<td>Normal flow filtration</td>
</tr>
<tr>
<td>OBM</td>
<td>Oil based media</td>
</tr>
<tr>
<td>OUR</td>
<td>Oxygen uptake rate (mmolL(^{-1})hr(^{-1}))</td>
</tr>
</tbody>
</table>
$P_1$ Pressure at the feed inlet
$P_2$ Pressure at the retentate outlet
$P_3$ Pressure at the permeate outlet
$Q$ Volumetric flow rate ($m^3 s^{-1}$)
$r$ Radius (m)
$R_b$ Resistance due to the blocking of the pores
$R_c$ Cake resistance
$R_m$ Membrane resistance
$RO$ Reverse osmosis
$r_p$ Radius of the diffusing particle (m)
$RQ$ Respiratory quotient
$RSM$ Response surface methodology
$R_t$ Total hydrodynamic resistance
$R_1$ and $R_2$ Membrane inner and outer radii respectively (m)
RDVF Rotary vacuum filtration
SCM Soluble complex media
STR Stirred tank reactor
$t$ Time (sec)
TFF Tangential flow filtration
$U$ Average velocity of the fluid ($m s^{-1}$)
UF Ultrafiltration
$V$ Azimuthal velocity ($m s^{-1}$)
VCF Volumetric concentration factor
VMF Vibrating membrane filtration
VOC Volatile organic compound
$w$ Channel width (m)
**Greek symbols**

\[ \mu \] Viscosity (Pa.s)

\[ \Delta P_{TM} \] Transmembrane pressure

\[ \Omega \] Angular velocity amplitude (radians s\(^{-1}\))

\[ \gamma \] Shear rate (s\(^{-1}\))

\[ \gamma_{w_{\text{max}}} \] Maximum shear rate (s\(^{-1}\))

\[ \nu \] Kinematic viscosity of the fluid (m\(^2\)s\(^{-1}\))

\[ \bar{\gamma}_w \] Mean shear rate (s\(^{-1}\))

\[ \omega \] Frequency (radians s\(^{-1}\))

\[ \rho \] Process fluid density (kgm\(^{-3}\)).
1. INTRODUCTION

1.0 Socio-economic context

Infectious diseases account for half of the deaths in developing countries, more than 13 million deaths each year world-wide. In developing countries, about one third of the population (1.3 billion people) live on less than $1 per day, almost one in three children are malnourished, one in five are not immunised by their first birthday and lack access to essential drugs (www.who.int. December 1999). This list of appalling statistics is compounded by the huge increase in world population movements spreading infectious disease into new areas. The growth of densely populated cities with their poor sanitation and widespread poverty has created an ideal environment for the growth and spread of disease. The outbreak of war in the former Yugoslavia in the 1990s led to hundreds of thousands of people fleeing their homes for the refugee camps of Albania, Greece and the former Yugoslavia. The ensuing outbreak of Polio showed how easy it is for disease to be introduced once standards of living and health care are allowed to drop.

This problem is amplified by the growing resistance of organisms to existing antimicrobial drugs. In the early 1940s, it was estimated that more than 90% of the strains of S. aureus were susceptible to penicillin. Almost 50% of the strains showed some degree of resistance by the late 1950s and resistance in the strains approached 90% in the 1990s (Kotra. 2000).

The containment of infectious disease through programmes of treatment and immunisation has long been the aim of organisations such as the World Health Organisation, and with the cost to the tax payer of treating these diseases estimated at more than $20 billion annually in the United States alone (Persidis. 1999), concerted and systematic programmes of drug discovery and development are in place. In 1998 there were 27 antibiotics, 12 antifungals and 14 vaccines in various stages of development in the US alone (Persidis. 1999), emphasising the efforts being made to develop novel treatments to the vast array of infectious microbial diseases in the world.

Despite the increasing market demand for antimicrobial drugs, the past twenty years has witnessed a slow and steady decline in productivity bought about by a consolidation in the European antibiotics industry (Barber, 1999). From around 1980...
onwards, the number of antibiotic producers has declined rapidly. Table 1.1 illustrates this clearly.

<table>
<thead>
<tr>
<th>Companies Producing</th>
<th>Total 1976</th>
<th>Europe 1976</th>
<th>Europe 1999</th>
<th>Reduction 99/76</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>38</td>
<td>24</td>
<td>4</td>
<td>20</td>
<td>-85</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>18</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>-60</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>12</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>-80</td>
</tr>
<tr>
<td>Totals</td>
<td>&gt;200</td>
<td>118</td>
<td>35</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1 - The decline in antibiotic producing companies (Table adapted from Barber, 1999).

Despite this apparent decline in the antibiotic production industry research into the development of novel antibiotics has not stagnated. The traditional method of combating microbial resistance has been to develop new drugs. There are currently seven major classes of antibacterials in clinical use; β-lactams, aminoglycosides, sulphonamides, tetracyclins, macrolides, glycopeptides and quinolones (Kotra et al. 2000). One new class of antibiotics is the oxazolidinones. Linezolid, developed by Pharmacia and Upjohn, was the first member of this class to be approved by the FDA in May 2000. Even before Linezolid was approved however, some degree of bacterial resistance over time was observed (Henry. 2000).

With the high demand for anti-microbial drugs, and the sequencing of the human genome completed, the number of new drugs entering clinical trials has never been higher. The cost of putting a new drug through clinical trials is so expensive that producers are constantly searching for new and improved production regimes to go with the new drugs, in an attempt to keep costs down whilst improving product yields.

1.1 *Saccharopolyspora erythraea* and the production of polyketide antibiotics

One of the most widely used families of antimicrobials are the polyketide antibiotics, the erythromycins. They are produced as secondary metabolites by *Streptomyces* spp. of which *Saccharopolyspora erythraea* is an example (Minas et al. 1998).
1.1.1 Erythromycin – a polyketide antibiotic

The erythromycins are known to be among some of the safest antibiotics used in human medicine. The WHO is currently investigating the use of erythromycins in the treatment of pneumonia in the USA, Cholera in Somalia and Shigella in Guinea (www.who.int). Erythromycin A is the main product of the fermentation with erythromycins B, C, D, E and F produced as minor components (10 – 15 % w/w). All the components display antimicrobial properties although that of Erythromycin A is by far the strongest. Erythromycin A is a white or slightly yellow crystalline powder slightly soluble in water, soluble in alcohol, in chloroform, and in ether. It is practically odourless, is active in a pH range between 8.0 and 10.5 and has a molecular weight in the region of 718 – 750 (Hahn. 1972).

1.1.2 S. erythraea

*S. erythraea* is a member of the actinomycetes group and grows through the formation of long multi-cellular hyphae with a diameter of 0.4 – 0.8 μm, developed as branched septate (Labeda. 1987). Like other filamentous actinomycetes the submerged culture media of *S. erythraea* requires considerable aeration and agitation for adequate oxygen transfer and maximum growth (Davies. 2003). In order to avoid pellet formation and achieve heavy dispersed growth, it is necessary to inoculate the shake flask or fermenter with high numbers of spores or hyphal fragments (Davies. 2003).

1.1.3 Choice of fermentation media for the growth of *S. erythraea*

Fermentation media can be divided into two broad categories; chemically defined (synthetic) or undefined (natural, complex) (Zhang *et al.* 1999). Chemically defined media are often preferred in the research laboratory as they allow media development trials for a specific organism. It is possible to examine the limiting effects of individual components with minimal complicated medium interactions and reproducible culture conditions. In contrast, the media used to support high antibiotic productivities in commercial fermentations are predominantly formulated using readily available, inexpensive complex carbon and nitrogen sources such as oils and flours (Miller *et al.* 1986).

For obvious reasons, antibiotic producers are unwilling to divulge information relating to the composition of their media or the antibiotic titres produced. In a study by Davies *et al* (2000), the characteristics of *S. erythraea* fermentations in two types of media, an undefined soluble complex media (SCM), and an oil based media (OBM),
and their consequent affects on downstream processing (microfiltration) were examined. The authors showed a maximum titre of erythromycin A of 700 mgL\(^{-1}\) in the OBM, three times higher than that seen using the SCM. Although the product titres were seen to be higher, it was observed that this was achieved at some cost, namely longer fermentation times and higher broth viscosities.

The authors also looked at the subsequent down stream processing of the fermentation broths, using microfiltration to recover the erythromycin and separate the biomass and undissolved solids. The authors showed that when using the OBM, permeate flux and erythromycin transmission were significantly lower (12.8 Lm\(^{2}\)hr\(^{-1}\) and 89.6 % w/w respectively) than those seen when using the SCM (35.9 Lm\(^{2}\)hr\(^{-1}\) and 96.7 % respectively). This was attributed to the higher solids loading and apparent viscosity of the OBM.

From this it is clear that in terms of antibiotic titres, less well defined media are preferable, hence their use in industrial fermentations. The increased fermentation times and subsequent problems with down stream processing however present serious draw backs to their use.

1.2 Selection of clarification operations

During the fermentation of S. erythraea, raw materials are altered by reactions occurring within the vessel to give the desired product, erythromycin. Once this reaction is completed or run to a desired end point, the antibiotic product needs to be isolated and removed from the fermentation liquor. This isolation and the subsequent purification is know as downstream processing or DSP. In order for a process to be economically viable, production costs must be kept to a minimum. DSP operations account for a significant proportion of the production costs and can be reflected as a percentage of the selling price, in the case of bulk penicillin production for example this has been estimated at 30 % and for enzyme production greater than 60 % (Hacking. 1986). For this reason it is imperative that the correct unit operations are chosen.

One of the earliest steps used in the recovery of erythromycin is the clarification of the fermentation broth and the removal of any unwanted cells and associated cell debris together with insoluble medium components. This is traditionally performed by either centrifugation, membrane filtration or a combination of the two (Doran 1998).
In producing the maximum yield of product, the fermentation also produces a large fraction of biomass which has an affect on the rheology of the fermentation liquor (Warren et al. 1994; Karsheva et al. 1996). During the production of polyketide antibiotics such as erythromycin, a large amount of filamentous biomass (S. erythraea) is produced. This broth is both biochemically and rheologically complex with broth viscosities that range from 100 cp to several thousand cp (Warren et al. 1995; Karsheva et al. 1997). As a result of this, the broth is often very difficult to process leading many manufacturers to use a dilution and centrifugation step followed by a dead-end rotary vacuum filtration (RVDF) step to recover the product in sufficiently high yield (Leach. 2000).

A preliminary economic analysis by Sheehan et al (1988) has indicated that microfiltration is twice as cost effective as centrifugation. However, advances in filtration technology have been held back by a number of challenges limiting their use on a wider scale; unreliability and low permeate fluxes to name but two.

1.3 Membrane separation and its use as a clarification operation
Membrane separation is a pressure driven process during which a solid-liquid separation is achieved via a selectively permeable membrane. The feed is pumped either towards or across the membrane depending upon the mode of operation employed.

1.3.1 Normal flow filtration - NFF
In normal flow filtration (NFF) or dead-end filtration, a membrane is placed directly in the path of the feedstream flow as shown in Figure 1.1. As the liquid phase passes through the membrane, a cake of solid particles builds up on the membrane. This cake build up causes a decrease in the permeate flux so higher pressures are often required to allow an effective process to be run. NFF is not the subject of the current study and is described in detail elsewhere (Davies and Grant. 1992; Bacchin et al. 2002; Viadero and Noblet. 2002; Gwon et al. 2003;).

1.3.2 Tangential flow filtration - TFF
In tangential flow filtration (TFF) or crossflow filtration, the feed flows tangentially to the membrane surface as shown in Figure 1.2 (Reismeier et al. 1989; Hernandez – Pinzon and Bautista. 1992; Parnham and Davis. 1995). TFF systems employ high crossflow velocities which, through mechanisms described in Section 1.7.2, reduce the accumulation of materials on the membrane surface, allowing the filtration
process to continue beyond the point that would be possible using NFF (Lin and Coller. 1998; Ravetkar et al. 2001).

Figure 1.1 – Schematic of flow patterns in normal flow filtration (NFF). Figure adapted from Davies. 2003.

Figure 1.2 – Schematic of flow patterns in tangential flow filtration (TFF). Figure adapted from Davies. 2003.

1.4 Membrane classification based on pore size
Dependant on the application, there are three classes of membrane which are widely employed in the bioprocess industry: Reverse osmosis (RO), ultrafiltration (UF) and microfiltration (MF).
1.4.1 Reverse osmosis
Reverse osmosis is a separation process that utilises semi-permeable membranes to achieve the extraction of low molecular weight solutes from a solvent (Hoornaert. 1984). The process works using elevated system pressures in excess of the osmotic pressure of the solution being treated.

Like most membranes, a typical reverse osmosis membrane consists of a very thin skin layer supported by a thicker more porous layer. This asymmetric structure makes it possible to combine good salt rejection with a high water flux. The active skin layer is dense and responsible for the salt rejection, but also very thin to allow a reasonable water flux to be maintained. The thicker more porous layer provides the mechanical support needed to withstand the high pressures used in the separation process.

Reverse osmosis has its main use in the desalination of sea water and the preparation of RO water for laboratory work. In recent years, the pressure on industry to clean up industrial effluent prior to discharge has seen the use of reverse osmosis become more popular. The technology provides a means by which the amount of industrial effluent discharged from a site can be reduced whilst also reducing the water demands of the site. (Winston Ho and Sirkar. 1992).

1.4.2 Nanofiltration
Nanofiltration (NF) membranes display excellent rejection of divalent ions while allowing a majority of monovalent ions to pass. Organic molecules in the 200 – 300 molecular weight range are also highly rejected. The unique separation capability of NF provides the opportunity to selectively concentrate either valuable or undesirable substances from a process stream with greater effectiveness, consistency, reliability and economy (www.membraneonline.com. October 2003). NF is particularly well established for the removal of ions associated with scaling from water as well as for cheese – whey desalting in the dairy industry. Other growing markets are in RO pretreatment; pharmaceutical concentration; kidney dialysis units and maple sugar concentration.

1.4.3 Ultrafiltration
Ultrafiltration (UF) is a low pressure fractionation of selected components by size. The technique is used mainly in the separation of macromolecular materials, such as proteins, that are smaller than 0.1 μm. It is often used as a concentration step, during
which time the volume of process solution is decreased by removing fluid but retaining the required product. (Winston Ho and Sirkar. 1992).

1.4.4 **Microfiltration**

Microfiltration (MF) is essentially the separation of a solid – liquid mixture and is used to recover particles in the range of 0.05 – 10 µm. MF processes can be operated in a number of ways, the choice of which will depend on the nature of the product, the characteristics of the feed and the desired process objectives. They include batch, fed-batch, feed and bleed, continuous and diafiltration. Typical materials removed include starch, bacteria, moulds, yeast and emulsified oils. (Winston Ho and Sirkar. 1992).

1.5 **Membrane system operating techniques**

As stated in Section 1.4.3, the most common configurations used in filtration processes are batch concentration, feed and bleed and diafiltration (Winston Ho and Sirkar. 1992).

1.5.1 **Batch concentration**

Batch concentration is the simplest configuration and requires the least membrane area to achieve a given separation. The removal of the permeate causes an increase in the concentration of the retained species in the retentate stream. This is particularly useful during the processing of large volumes of feed, such as antibiotic fermentations, in order to reduce diafiltration buffer requirements later in the purification process (Leach. 2000).

1.5.2 **Feed and bleed**

The feed and bleed configuration is commonly used for continuous operation especially in the food industries (Winston Ho and Sirkar. 1992; Leach. 2003). Permeate is removed from the system, as is a small portion of the retentate, referred to as the bleed. Most of the retentate is recycled however to maintain a high crossflow velocity across the membrane surface.

1.5.3 **Diafiltration**

Diafiltration involves the addition of buffer to the retentate and continuing filtration in order to overcome low fluxes at high concentrations or to get better removal of permeable species. As the permeate is removed during concentration, the concentration of the retained species within the retentate increases, causing changes
in the process stream viscosity which has implications for the filtration performance (as discussed in Section 1.7.3). Diafiltration involves replacing the removed permeate with buffer thus keeping process stream viscosity at levels acceptable for processing (Sessa et al. 2003; Goulas et al. 2003).

1.6 Microfiltration models

A number of models have been developed to predict the flux in typical membrane systems and are used to estimate membrane areas required for particular duties. Two of the more important models are the concentration polarisation model and the fouling model.

1.6.1 Concentration polarisation

In the concentration polarisation model, material is transported towards the membrane by convection. A concentration gradient at the membrane is developed as soon as filtration starts, and as filtration progresses, a boundary layer, or gel layer, of high concentrations of solutes, including proteins, builds up on the membrane surface. Porter (1972) assumed that this gel layer forms the limiting resistance to flow and that it is possible to calculate the transport rate of water through the membrane (flux) on the basis of the mass transfer of membrane retained species from the membrane surface back into the bulk stream. He stated that this was so because the gel layer is assumed to have a fixed concentration \( C_g \) but is free to vary in terms of thickness and porosity. In such a system, the flux is independent of the membrane permeability since the dynamic boundary layer resistance to permeate flow will adjust itself until the convective transport of material to the membrane surface equals the back transport to the bulk stream. He proposed that permeate flux can be predicted using Equation [1.1].

\[
J = k \left( \ln \frac{C_w}{C_b} \right)
\]

[1.1]

Where \( J \) is the permeate flux (Lm\(^{-2}\)hr\(^{-1}\)), \( k \) is the mass transfer coefficient, \( C_w \) is the wall concentration of cells (% w/w) and \( C_b \) is the bulk concentration of cells in the feed stream (% w/w). Taken from Porter (1972).

The mass transfer coefficient can be calculated using the heat-transfer correlation described by Dittus and Boelter (1930) and is shown in Equation [1.2].
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\[ k = K_p \left( \frac{Q}{bwL} \right)^{0.5} \frac{D^{0.66}}{v^{0.17}} \]  

[1.2]

Where \( K_p \) is the proportionality constant, \( Q \) is the volumetric flow rate \((m^3 s^{-1})\), \( D \) is the diffusion coefficient \((m^2 s^{-1})\), \( b \) is the channel height \((m)\), \( w \) is the channel width \((m)\), \( L \) is the channel length \((m)\) and \( v \) is the kinematic viscosity of the broth \((m^2 s^{-1})\). Taken from Dittus and Boelter (1930).

The diffusion coefficient is described by the Stokes-Einstein relationship for diffusivity shown in Equation [1.3].

\[ D = \frac{k_b T}{6\pi\mu r_p} \]  

[1.3]

Where \( k_b \) is the Boltzmann constant, \( \mu \) is the viscosity \((Pa.s)\) and \( r_p \) is the radius of the diffusing particle \((m)\). Taken from Davies (2003).

Porter (1972) stated that by assuming that the cells at the membrane surface resemble a layer of closely packed spheres, having 65 – 75 % solids by volume, the experimental values to calculate \( K_p \) can be used to predict the steady state permeate flux. The concentration polarisation theory has been used by many investigators in an attempt to describe microfiltration behaviour (Wakeman and Williams, 2002, Mignard and Glass, 2001). Mikulasek (1994) reported that permeate flux may be as low as 2 – 10 % of that of pure water as a result of concentration polarisation.

1.6.2 Membrane fouling

In membrane fouling, molecules from the feed stream physically block the membrane pores. The resulting total hydrodynamic resistance can be used to predict the flux using Equation [1.4].

\[ J = \frac{\Delta P_{TM}}{\mu R_t} \]  

[1.4]

Where \( \Delta P_{TM} \) is the transmembrane pressure \((Pa)\) and \( R_t \) is the total hydrodynamic resistance defined for a given membrane and a pure solvent (water). Taken from Davies (2003).
Davies (2003) states that $R_t$ is defined as the sum of the membrane resistance ($R_m$), the cake resistance ($R_c$) and the resistance due to the blocking of the pores ($R_b$) which are related by Equation [1.5].

\[ R_t = R_m + R_c + R_b \]  \hspace{1cm} [1.5]

Davies (2003) goes on to state that membrane resistance can be measured using clean water flux data. When using pure water, Equation [1.4] can be rewritten as Equation [1.6].

\[ J = \frac{\Delta P_{TM}}{\mu R_m} \]  \hspace{1cm} [1.6]

The resistance of the membrane can then calculated by plotting flux against $\Delta P_{TM}$, the gradient of the graph being $1/R_m\mu$.

1.7 Factors influencing conventional TFF performance

A number of factors influence the performance of any filtration system.

1.7.1 Transmembrane Pressure

As stated in Section 1.3, membrane filtration is a pressure driven process. Any increase in pressure on the upstream side of the membrane with reference to the downstream side, will result in process fluid being forced through the membrane (permeate). This pressure difference is known as the transmembrane pressure ($\Delta P_{TM}$ or TMP) and for crossflow filtration is defined by Equation [1.7].

\[ \Delta P_{TM} = \frac{P_1 + P_2}{2} - P_3 \]  \hspace{1cm} [1.7]

Where $P_1$ is the pressure at the feed inlet, $P_2$ is the pressure at the retentate outlet and $P_3$ is the pressure at the permeate outlet. Taken from Davies (2003).

The process stream contains a solid phase as well, so as the fluid is forced through the membrane, these solid particles will either pass through the membrane or will be retained on the membrane surface, depending on the membrane pore size. At the same time as material is deposited on the membrane, it is being removed by back transport (mechanisms that have been proposed include Brownian diffusion and
inertial lift) (Davies et al. 2000). As the $\Delta P_{TM}$ is increased, the rate of solute convection towards the membrane will eventually exceed the rate of back transport away from it. At this point the flux will be unchanged by any further increases in $\Delta P_{TM}$ and the membrane is said to be concentration polarised. This point is described as the critical transmembrane pressure or cTMP. This effect was first noticed in UF (Porter. 1972) but has been reported in MF applications (Frenander et al. 1996; Davies et al. 2000; Gesan-Guiziou et al. 2000).

### 1.7.2 Crossflow velocity

The flow of feed tangential to the membrane surface in TFF can be used to minimise the build up of any fouling layer deposited by the mechanisms described in section 1.6. Increased crossflow velocity creates the shear necessary to remove the deposited material and maintain permeate flux at an acceptable level. Removal of the fouling layer by increasing crossflow has been demonstrated by a number of investigators (Mikulasek. 1994; Culkin et al. 1998; Su et al. 2000).

Mikulasek (1994) reports that in the turbulent regime, flow rate is proportional to the pressure drop across the length of the membrane squared. Higher velocities bring about higher pressure drops through the membrane module, which in turn bring about lower pressures available for the filtration process. In a study of the fouling of ceramic membranes by albumins, Su et al (2000) demonstrated that permeate flux dropped faster at elevated cross flow velocities, although the extent of protein deposition inside the membrane pores was found to be similar to that exhibited at lower velocities. The authors concluded that pore blockage at the front, outer surface of the membrane is intensified by a higher flow velocity. Higher flow velocities also necessitate bigger, and thus more expensive, circulation pumps and higher energy consumption. An important factor with regards to the processing of biological molecules is the incidence of shear damage bought about by increased flow rates. This is dealt with in more detail in Section 1.11.

### 1.7.3 Process stream viscosity

D’arcy’s filtration law states that permeate flux is proportional to $\Delta P_{TM}$ and inversely proportional to liquid/broth viscosity and is shown in Equation [1.8].

$$J_\alpha = \frac{\Delta P_{TM}}{\mu}$$  

[1.8]
Viscosity changes will occur as a result of biomass concentration changes (Warren et al. 1994; Karsheva et al. 1997; Davies et al. 2000) with implications for the downstream processing of the fermentation broth.

1.7.4 Modes of operation
Regardless of the type of membrane used or the system that it is employed in, there are two main modes of operation employed in the bioprocess industry; constant pressure mode and constant flux mode.

1.7.4.1 Constant pressure mode
In constant pressure mode, flux is allowed to decline over time. Pump speed is varied to maintain constant $\Delta P_{TM}$ (Defrance and Jaffrin. 1999).

1.7.4.2 Constant flux mode
In constant flux mode, a pump is placed on the permeate line and permeate is drawn through the membrane at a constant flux. $\Delta P_{TM}$ is allowed to vary within specified limits. It is this later method that seems to be more popular in industrial processes as it minimises membrane fouling thus reducing operating costs (Field et al. 1995; Defrance and Jaffrin. 1999).

1.7.5 Membrane types and surface chemistry interactions
Membrane surface chemistry determines such important properties as hydrophilicity or hydrophobicity, presence or absence of ionic charges, chemical and thermal resistance, binding affinity for solutes or particles and bio-compatibility all important in determining the performance of the membrane application. The interactions between the surface of the membrane and the process feed stream play an important part in determining the flux characteristics of the membrane, and as such, membrane surface chemistry can be modified to improve filtration performance in targeted applications. Childress et al (1998) reported that the alteration of the ionic charge of reverse osmosis membranes had a significant influence on the salt rejection of the membrane at low pH. A study by Ma et al (2000) emphasised the importance of membrane surface charge. Using modified polypropylene membranes (0.22 μm), the authors studied permeate fluxes during the MF of 0.14 gL$^{-1}$ (DCW) E. coli. They showed that permeate volumes were greater using hydrophilic, charged membranes than for hydrophobic neutral polypropylene membranes, but less than those for hydrophilic neutral membranes. This work is supported by several other studies.
which have shown the importance of using hydrophilic membranes to reduce protein adsorption and thus prevent flux decline (Kroner et al. 1984; Fane et al. 1987). Bowen et al. (1999) have reported that electrostatic double-layer interactions can have a strong influence on rejection at the pores of both MF and UF membranes.

The importance of process stream pH and the resulting interactions with the membrane has been emphasised by Su et al. (2000). In a study on the fouling of ceramic membranes (nominal pore diameter 200 Å) by the albumins bovine serum albumin (BSA) and human serum albumin (HSA), the authors reported a rapid decline in permeate flux at pH 5 and a slower decline at pH 3 and pH 7 associated with increased levels of protein deposition observed within the membrane pores at pH 5 and lower levels at pH 3 and pH 7. The authors conclude that as the pH is shifted away from the iso-electric point of BSA and HSA (pH 4.8 for both), the extent of adsorption decreases as does the level of protein deposition observed within the membrane. A similar pattern of fouling was observed for membranes with different pore diameters. The authors also concluded that this may be true for different membrane types although the levels of adsorption and deposition would vary.

<table>
<thead>
<tr>
<th>Membrane material</th>
<th>Product example</th>
<th>Membrane properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysulphone</td>
<td>Pall Gelman HT</td>
<td>Hydrophilic</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>Pall Gelman GH</td>
<td>Hydrophilic, Low protein binding</td>
</tr>
<tr>
<td>PTFE</td>
<td>Pall Gelman TF</td>
<td>Hydrophobic, Good resistance to solvents</td>
</tr>
<tr>
<td>PVDF</td>
<td>Millipore GVHP</td>
<td>Can be modified to render surface hydrophilic, Good resistance to solvents</td>
</tr>
<tr>
<td>Nylon</td>
<td>Pall Ultipor</td>
<td>Hydrophilic</td>
</tr>
</tbody>
</table>

Table 1.2 – Some properties of different microfiltration membranes (Information from Pall Gelman and Millipore).

1.8 Use of membrane design to combat membrane fouling

As described in section 1.3, conventional TFF systems rely on feed stream crossflow velocity to provide the shear forces necessary to combat membrane fouling. The size of the pumps required to maintain these cross flow rates has obvious implications for
power consumption and hence overall running costs of the process. As a result of
this, many membrane system manufacturers and academic research groups are
looking at ways of adapting both the membrane module design and the method of
operation of the membrane separation processes (NFF and TFF) in an attempt to
minimise membrane fouling, whilst reducing crossflow velocity, hence keeping power
requirements to a minimum.

1.8.1 Conventional TFF membrane module designs
Generally, TFF systems consist of membrane elements placed in a plate and frame,
tubular or spiral wound cartridge assembly through which the feed stream is pumped
at a high velocity. Forces generated by these systems shear liquid near the surface
of the membrane and, thus, remove trapped particles tending to keep the membrane
clean for longer periods of time (Doran. 1998).

Due to the solids loading of most fermentation broths, the use of narrow channel
devices such as hollow fibres is not possible. Crossflow filtration of most fermentation
broths is usually performed using open channel or polymeric flat sheet membranes
with high cross-flow rates. Shear rates at the membrane surface in the range of 30 -
50,000 s⁻¹ have been reported for these TFF systems (Culkin et al. 1998).

Whilst standard TFF systems can delay membrane fouling, for many bioprocess
streams, the level of membrane fouling is still unacceptable. For example, in some
protein and enzyme separation processes, where products are being isolated from
lysed cells, high concentrations of organic material are still found to collect on the
membrane surface (Parnham and Davis. 1995; Sousa et al. 2002; Czekaj et al.
2000). This accumulation leads to restricted flow of the product through the
membrane. With the increased crossflow velocities required to prevent this fouling,
the cost of separation becomes dominated by the amount of energy needed to
maintain acceptable flux and transmission levels (Culkin et al.1998).

1.8.2 Systems using altered membrane geometry and diverted fluid flow
The need for membrane filtration systems allowing large volume throughput of feed
at sufficiently high flux rates, lead to the development of membrane separation
technologies utilising localised high shear fields generated by altering the membrane
geometry and thus the fluid flow across it (Doran. 1998).
If the solids loading of the broth is not too high, then spiral wound membrane systems may be used, such as the enhanced flow device (EFD) system being developed by Millipore. This system makes use of Dean vortices to create the shear forces necessary to prevent membrane fouling. A number of authors have reported that fluid flow around a curved channel at a sufficient rate produces centrifugal instabilities (Dean vortices). These vortices have been shown to depolarise the build-up of suspended particles at the membrane - solution interface and allow for increased membrane permeation rates (Brewster et al. 1993; Mallubhotla et al. 1995; Mallubhotla et al. 1996; Costgan et al. 2002). In other words, Dean vortices reduce concentration polarisation and increase observed fluxes and transmission levels.

A study by Scott et al (2000) showed that corrugating the membrane improves the overall performance of the system. During the crossflow membrane filtration of a 30 % w/w water in oil emulsion, an increase in the crossflow velocity resulted in an improved permeate flux caused by increased shear effects at the membrane surface. The use of corrugated membranes, however, enhanced the flux in a more efficient way by preferentially promoting turbulence near the membrane wall region, repeatedly mixing the boundary layer and hence reducing concentration polarisation. It was illustrated that corrugations with angles of 45 and 90 degrees can lead to a reduction in energy consumption of up to 80 and 88 % respectively (Scott et al. 2000).

1.8.3 Dynamic filtration systems

There are two main types of dynamic filtration systems, dynamic membrane filtration systems (DMF) and vibrating membrane filtration systems (VMF).

1.8.3.1 DMF systems

DMF systems tend to consist of a rotating disk suspended above the membrane, providing the shear necessary to reduce membrane fouling. Lee et al (1995) showed that by using a laboratory scale (0.147 ft² membrane), novel rotating disk dynamic filtration system for the concentration of recombinant yeast cells, significant improvements in flux could be achieved by creating high shear rates (up to 120,000 s⁻¹) on the membrane surface when compared with the existing TFF method used for the same process. Among the many factors investigated by the authors, disk rotation speed was shown to be the most important adjustable parameter. The results showed that flux increased with disk rotation speed, which determines shear rates...
and fluid flow patterns. The authors attributed the increased fluxes observed to minimal cake build up and reduced membrane fouling.

During the ultrafiltration of UHT skimmed milk using a smooth disc and a disc equipped with 6 mm high vanes rotating at high angular velocity, Ding et al (2002) showed that the addition of vanes to the disc increased the permeate flux by 56%. The authors attributed this to a higher fluid core velocity resulting in higher shear rates at the membrane surface bought about by the addition of the vanes to the rotating disc. Similarly, Brou et al (2002) showed that the addition of vanes to the rotating disc brings about a gain in performance obtained during the microfiltration of baker's yeast suspensions. The authors showed that by adding the vanes to the disc, a large increase in wall shear stress is produced leading to an increase in permeate flux.

Whilst rotating disk technology tends to be the realm of academic research groups, a few industrial filtration system manufacturers have attempted to commercialise the technology with varying degrees of success. The DMF system developed by Pall (Pall Corporation, East Hills, NY, USA) consists of membrane elements stacked between rotors in 180° sectors. The stainless steel rotors are then rotated at speeds of up to 1800 rpm. This high speed rotation causes high shear in the gap between the solid rotating disk and the static membrane element. At the maximum speed of rotation, the shear rate is estimated to be greater than 2x10⁵ s⁻¹ (Alex and Haughney, 1998). The competition between dynamic and viscous forces in the fluid results in rotation of the bulk of the liquid as a nearly rigid disk. Lee et al (1995) compared the DMF system with a traditional cross-flow device for the separation of an industrial recombinant yeast cell harvest. The authors found that a concentration factor of six could be achieved in excess of eight hours using the conventional TFF device which exhibited a gradual flux decline to 25 Lm²hr⁻¹. A similar concentration factor could be achieved using the DMF within 100 min and at an average flux rate of greater than 200 Lm²hr⁻¹. The DMF system was also shown to allow effective decoupling of liquid velocity from system pressure, thus, it was concluded, the DMF unit may operate at low ΔP_TM leading to high filtrate flow rates, high product yields and minimum fouling at the membrane surface. DMF systems have been successfully employed in the treatment of wastewater streams containing low levels of suspended solids, although it has been reported that the system is not suitable for more viscous suspensions due to limitations of membrane area per machine and the high energy consumption (Leach, 2000).
Although this system is no longer commercially available from Pall, other companies are now marketing the technology. Spintek™ have recently started to produce the ST-II system. Similar in design to the Pall DMF system, Spintek state that the membrane disks rotate at speeds of up to 60 ft sec\(^{-1}\) allowing the processing of very viscous solutions (www.spintek.com).

1.8.3.2 VMF systems

In recent times, the Pall DMF system has been replaced by the PallSep VMF system. Originally developed by New Logic (New Logic International, Emeryville, CA, USA) and still marketed by them as the VSEP, VMF systems have been reported to be a more energy efficient device than the DMF systems. The PallSep VMF system is described in greater detail in section 1.9.

VMF systems have been used in many different applications such as water and wastewater treatment (Huuhiolo et al. 2001), recovery and concentration of insect killing nematodes (Wilson and Postlethwaite. 2003), volatile organic compound (VOC) treatment (Vane et al. 1999; Vane and Alvarez. 2002), pulp and paper processing (Nuortila-Jokinen et al. 1998), processing of skimmed milk (Al Akoum et al. 2002a; Al Akoum et al. 2002b), yeast cell processing (Buckland and Cheung. 1996) and paint and pigment concentration and clarification, oil production, processing and recycling and mining and related processes (www.vsep.com).

In a study performed by Al Akoum et al (2002), during a concentration test, a yeast concentration of 300 gL\(^{-1}\) was achieved whilst using a VMF system, evidence of the systems high solids handling ability. In the same study, the effect of mean shear rate on permeate flux was investigated. The authors observed a drop in permeate flux when the frequency of vibration was lowered. Bian et al (2000) studied the effect of shear rate on controlling concentration polarisation and membrane fouling. The authors showed that humic substances with a molecular weight of more than 6000 Da are transported away from the membrane surface by shear induced diffusion. Vane et al (1999) showed that during the processing of VOCs, the vibrations of the VMF system greatly reduced concentration polarisation in the system.

1.8.4 Gas sparging

Numerous authors have shown that the introduction of bubbles of gas into the process stream increases permeate flux in both MF and UF systems using a variety of membranes. Vera et al (2000) showed that the MF fluxes of both a ferric hydroxide
suspension and biologically treated waste water could be increased by the use of air sparging through a tubular membrane. Majewskanowak et al (1999) showed that air sparging during the hollow fibre UF of both an organic dye and the mineral kaolin increased the permeate flux by 25 %. The authors also showed that the rate of air flow was of negligible importance. Li et al (1998) showed that during the flat sheet UF of both HSA and human IgG, gas sparging led to increased fluxes and increased protein transmission levels using both polysulphone and polyethersulphone membranes. A number of authors have shown that gas sparging increases permeate fluxes of HSA by 10 – 60 % and dextran by 20 – 50 % in both tubular membrane and hollow fibre systems (Li et al. 1997; Bellara et al. 1996). Li et al (1997) showed that the flux increases observed where dependant upon bubble frequency and not bubble size. In a study by Cui and Wright (1996), flux enhancements of up to 320 % were observed after the introduction of gas bubbles into the feed. The authors had previously reported flux increases of up to 250 % in air sparged ultrafiltration of dextrans and BSA using vertical tubular membranes (Cui and Wright. 1994). The authors reported that the damaging effects of the air bubbles on macromolecules, particularly proteins, in solution in such an operation was insignificant due to the low gas flow rates used.

1.8.5 Collapsible – tube pulse generators
The efficiency of crossflow microfiltration can be improved if the crossflow includes a pulsatile component (Hadzismajlovic and Bertram. 1999). The introduction of a crossflow induced oscillation of an externally pressurised flexible tube, upstream of the membrane module, has been shown to increase by 102 %, the flux of a 5 gL⁻¹ DCW suspension of yeast in water when filtered through a 0.2 μm unbaffled tubular ceramic membrane (Hadzismajlovic and Bertram. 1999).

1.8.6 Backflushing
Backflushing involves the use of bursts of retrofiltration, induced by pressurising the permeate above the retentate pressure by the use of a pressurised tank (Mikulasek. 1994). This effectively forces the permeate back through the membrane, unclogging the pores and removing the cake build up. The process can be repeated frequently if required although no permeate can be collected during the backflush. A compromise must be found between the frequency required for keeping the membrane clean and the amount of fluid retrofiltered which decreased the overall efficiency of the process.
A study by Quan (1999) showed that the use of backflushing effectively increased the permeate flux observed during the ceramic membrane microfiltration of primary municipal sewage effluent. The author does state however that the increased total dissolved solids concentration in the permeate has a profound implication on how the backflushing should be operated. Hargrove et al (1999) have shown that backflushing during the ultrafiltration of BSA using a hollow fibre membrane module produced a marked increase in permeate flux when compared with runs without flow reversal. Kuberkar et al (1998) have shown that using very short backpulses (0.1 – 1.0 seconds) during crossflow microfiltration increased net fluxes of washed bacterial suspensions and whole bacterial fermentation broths. Under optimum backflushing conditions for the washed bacteria, fluxes where shown to be ten fold higher than those obtained during normal crossflow microfiltration, while two fold increases where observed for the fluxes of fermentation broths. An economic analysis by the authors proved that crossflow microfiltration with backflushing has lower costs than centrifugation, rotary vacuum filtration, and crossflow microfiltration without backflushing. Although numerous authors have shown the benefits of backflushing, a study by Ma et al (2000) showed that recovered flux after a long backwash of the membranes fouled with backflushing during the MF of 0.14 gL⁻¹ (DCW) E. coli, is 20 – 40 % lower than that of the membrane fouled without backflushing. The authors concluded that this was due to the greater adhesive internal fouling when the membrane surface is frequently exposed by rapid backflushing.

A different method of backflushing, electro-osmotic backflushing, has been reported by Bowen and Sabuni (1994). The authors reported that water flux was restored to 97 % of the initial value following the application of electric fields to a microfiltration membrane which had become severely fouled by the in-pore deposition of proteins. The authors reported that electro-osmotic backflushing is more effective than pressure driven backflushing.

1.8.7 Ultrasonic filtration

Ultrasonic filtration involves exposing the filtration membrane to high intensity ultrasonic waves in an attempt to dislodge the fouling layer that has built up on it. A study by Matsumoto et al (1996) showed a four to six fold increase in the steady state permeate flux obtained during the cross flow microfiltration of baker's yeast and BSA when the permeate side of the membrane was exposed to intermittent ultrasonic bursts. It was concluded that the method was very effective for removing the cake layer and preventing the plugging of the membrane pores. Whilst this
technology may generate the required results, it is both energy intensive and difficult to construct on an industrial scale. Table 1.3 shows a summary of the technologies described in this section.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Application</th>
<th>Advantage/Disadvantage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>Containing moderate to high solids. Also animal cells</td>
<td>High Shear, High energy requirements</td>
<td>Sellick. 1997, Vogel. 1998</td>
</tr>
<tr>
<td>Dean vortices</td>
<td>Viscous Soln. Containing low solids</td>
<td>High shear, Low energy</td>
<td>Millipore. 2000</td>
</tr>
<tr>
<td>Gas sparging</td>
<td>Ultrafiltration of BSA and dextrin Soln. Waste water treatment</td>
<td>Shear at air-liquid interface, Cheap process</td>
<td>Cui. 1994, Cui. 1996</td>
</tr>
<tr>
<td>Collapsible tube pulse</td>
<td>5 gL⁻¹ DCW yeast in water suspension (MF)</td>
<td>Increased flux by 60%, Poor in turbulent conditions</td>
<td>Hadzismajlovic. 1999</td>
</tr>
<tr>
<td>Backflushing</td>
<td>Treatment of waste water</td>
<td>Process must be halted</td>
<td>Mikulasek. 1994</td>
</tr>
</tbody>
</table>

Table 1.3 - Summary of a number of filtration systems making use of high or intermittent shear fields.

1.9 PallSep – Theory and operation

Commercially, PallSep is produced on three scales; PS400 (Figure 1.3a), PS1000 (Figure 1.3b) and PS10 (Figure 1.4). The PallSep PS400 has a membrane surface area up to 40 m² and is used in the industrial recovery of antibiotics from fermentation broths and other applications in the food industry (Alex and Haughney. 1998). A smaller pilot scale version of the PS400, the PS10, has a maximum membrane surface area of 1 m² and is more commonly used in pilot plant development trials (Wilson and Postlethwaite. 2003). The larger PS1000 is used in similar applications to the PS400 (Leach. 2000) but has a larger surface area (up to 100 m² membrane area). PallSep technology is currently used in the processing of
gelatine, egg white, sugar and yeast extract (Sellick. 1997) as well as beer bottoms, antibiotics and therapeutic enzymes (Leach. 2000).

VMF technology utilises mechanical energy and a torsion spring mechanism (described in more detail in Section 1.9.1) to create vibrational shear, which is distributed through the thin boundary layer that exists at the membrane surface (the calculation of the shear rate and the boundary layer thickness are described in Section 1.9.2). The frequency of the motor is adjusted so that the system vibrates near its' own natural frequency of around 55 - 60 Hz, producing a controlled amplitude of around 19.5 mm. This vibration allows the shear necessary to prevent membrane fouling to be decoupled from liquid crossflow velocity, thus helping to reduce running costs as a result of minimal energy input into the system. Sellick (1997) reported that for a PS400 system concentrating a yeast slurry to a 22 % dry weight paste, the energy requirements might be in the order of 2.5 kW, considerably lower than that required for a similar result using TFF technology.

Leach (2000) states that the PallSep is able to achieve a high concentration factor with a stable flux for the majority of the run. Leach also states that the PallSep may be successful in processing some broths that may be un-filterable due to the fouling or poor product transmission with conventional TFF systems.
Figure 1.4 – PallSep PS10 (membrane area 0.1 – 1 m²).
Figures 1.5 to 1.7 show various views of the PallSep membrane head assembly, the fluid flow patterns through it and the individual membrane design.

**Figure 1.5** – Cross section of individual PallSep membrane disc

**Figure 1.6** – Plan view of an individual PallSep membrane disc.
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1.9.1 The torsion spring mechanism

In common with the VSEP system, PallSep uses an oscillating disc filter stack vibrating at approximately 55 – 60 Hz about a vertical axis. The filter stack moves back and forth with a maximum vibrational peak to peak amplitude of 19.5 mm as shown in Figure 1.8.

The torsion spring/mass system consists of two masses, the membrane head stack (mass B) and a seismic mass (mass A) connected by a torsion bar as shown in Figure 1.9. If mass A is rotated slightly in one direction, then mass B will move in the opposite direction. This occurs because the torsion spring is "twisted" by the motion of mass A. The twisted spring will exert a torque on mass B, in the opposite direction. To create an oscillation of the masses, one of the masses is rotated back and forth with a specific amplitude and this motion is translated through the torsion spring to the second mass. The motion is an angular simple harmonic motion.
Figure 1.8 – Sinusoidal motion of PallSep membrane head stack. The y axis shows distance moved from the central point, 0, with negative values showing movement to the left of this central point and positive values showing motion to the right. Amplitude is defined as total peak to peak head amplitude, in this case 19.5 mm. Data shown for operation at a frequency of 56 Hz.

The size, shape and weight of the masses are important, because the equation of motion for this type of harmonic motion depends upon the mass moment of inertia. A system such as this would be governed by Equation [1.9].

\[ F = k^{1/2} \left( \frac{1}{J_1} + \frac{1}{J_2} \right) \]  

[1.9]

were F is the frequency of the oscillation (Hz), J1 and J2 are the mass moment of inertia of the masses and k is the torsional spring constant. Taken from PallSep PS10 instruction manual.
The spring constant depends upon the specific material properties and geometry of the torsion bar. The ratio of amplitudes of the two masses are inversely related to the ratio of their mass moment of inertia as per Equation [1.10].

\[
\frac{J_1}{J_2} = \frac{A_2}{A_1}
\]

where \(A_1\) and \(A_2\) are the amplitudes of mass A and mass B respectively. Taken from PallSep PS10 instruction manual.

**Figure 1.9** - Schematic representation of the PallSep PS10 VMF torsion spring system. If mass A (seismic mass) is rotated slightly in one direction, then mass B (filter housing) will move in the opposite direction. This occurs because the torsion bar is "twisted" by the motion of mass A. The twisted spring will thus exert a torque on mass B, in the opposite direction to rotation. To create an oscillation of the masses, mass A is rotated back and forth by the motor with a specific amplitude and this motion is translated through the torsion spring to the filter housing. The motion established in this way is angular simple harmonic.

In the case of the PallSep PS10 VMF system, the actual torsion spring, and the two masses are indicated in Figure. 1.4. The mass at the bottom (mass A) is the seismic
mass and the mass at the top of the torsion bar (mass B) is the membrane head assembly.

The goal of the design is to maximise the amplitude for the membrane element assembly to provide shear at the membrane surface. Thus from Equation [1.10] the seismic mass is designed to have a much larger moment of inertia than the membrane stack, so that the amplitude of the seismic mass is at a minimum when the membrane assembly is at its operational amplitude.

In order to initiate and maintain the oscillatory motion, the torsion spring needs to be excited. An alternating current (AC) motor controlled by a variable frequency, solid state speed controller is used to produce the excitation by spinning an eccentric weight attached to the seismic mass.

1.10 Flow, calculation of shear rate and boundary layer thickness in a VMF system
Due to the oscillatory motion of the PallSep head, defining the flow, shear rates and boundary layer thickness is complicated.

1.10.1 Flow induced by torsional oscillations of two parallel disks
The flow induced by torsional oscillations of two parallel disks has been addressed by a number of investigators, namely Rosenblat (1960) for Newtonian fluids and extended to the case of non-Newtonian fluids with suction by Sharma and Gupta (1983). Al Akoum et al (2002) state that the azimuthal (transverse) velocity is given as a function of radius and oscillation frequency as shown in Equation [1.11].

\[ V = r\Omega e^{2\pi Ft} \text{ on } z = 0, \ h \]

[1.11]

Where \( V \) is the azimuthal velocity (ms\(^{-1}\)), \( r \) is the radius (m), \( \Omega \) is the angular velocity amplitude (radians s\(^{-1}\)), \( F \) is the frequency of oscillation (Hz), \( h \) is the distance between the discs (m) and \( t \) is the time (sec). Taken from Al Akoum et al (2002).
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The total displacement at the membrane periphery, is given by Equation [1.12].

\[ d = \frac{R_2 \Omega}{\pi F} \]  

[1.12]

Where \( d \) is the total displacement (m) and \( R_2 \) is the membrane outer radii (m), \( \Omega \) is the angular velocity amplitude (radians s\(^{-1}\)) and \( F \) is the frequency of oscillation (Hz). Taken from Al Akoum et al (2002).

1.10.2 Calculation of boundary layer thickness

PallSep VMF membranes are mounted onto either side of a stainless steel, circular plate on top of a drainage support layer as shown in Figure 1.5. These membrane discs are then stacked together with spacer elements (each 1.4 mm thick) to form the membrane head stack. Within the flow channel created by two adjacent membrane plates, the liquid hydrodynamics are complex. The oscillation of the membranes creates separate liquid boundary layers on the surface of the upper and lower membranes. In theory, so long as the gap between the membranes is larger than the sum of the two boundary layer thicknesses, the boundary layer thickness is not influenced by the gap width. This is not an assumption but a result of the hydrodynamics. Within each boundary layer, close to the membrane plate surface, the primary fluid motion is a rotational vibration set up by the plate oscillation.

The definition of the boundary layer thickness is somewhat arbitrary because transition from the liquid velocity at the membrane surface to that in the bulk fluid is asymptotic. The velocity of the fluid in the boundary layer will however attain a value which is very close to the velocity of the bulk fluid, a small distance from the membrane plate, as described by Schlichting (1979). The boundary layer thickness can therefore be calculated from the angular velocity profile of the fluid, which is governed by the Navier-Stokes equations. The solution is expected to be similar to that set up by a linear oscillation as in Stokes Second Problem (Ramos et al. 2001). The boundary layer thickness is calculated using Equation [1.13]. The derivation of this equation is discussed elsewhere (Postlethwaite et al. 2003).
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\[ L_{BL} = -\ln(0.1) \cdot \sqrt[2]{\frac{2\mu}{\omega\rho}} \]  

[1.13]

Where \( L_{BL} \) is the boundary layer thickness, \( \mu \) is the process stream viscosity (Pa.s), \( \omega \) is the frequency (radians s\(^{-1}\)) and \( \rho \) is the fluid density (kgm\(^{-3}\)). The derivation of this equation is discussed elsewhere (Postlethwaite et al. 2003).

1.10.3 Calculation of shear rate

Shear rate can be calculated in a number of ways, depending on the configuration and operation of the membrane system.

1.10.3.1 Calculation of shear rate in a conventional TFF system

In conventional flat sheet TFF systems, the shear at the membrane surface can be described by Equation [1.14] (Porter 1972).

\[ \gamma = \frac{6U}{h} \]  

[1.14]

Where \( \gamma \) is the shear rate (s\(^{-1}\)), \( U \) is the average velocity of the fluid (ms\(^{-1}\)) and \( h \) is the distance between the membrane discs (m). Taken from Porter (1972).

1.10.3.2 Calculation of shear rate in the PallSep VMF system

As stated in Section 1.10.2, PallSep VMF membrane plates are stacked on top of each other separated by spacer elements, each 1.4 mm thick. The oscillation of the membranes creates separate liquid boundary layers on the surface of the upper and lower membranes. Given the low crossflow rates at which the VMF unit is operated, typically 1.0 – 5.0 Lmin\(^{-1}\), the shear rates in this direction are considered to be negligible compared to that created by the membrane vibration. Fluid flow between the two boundary layers will be independent of the plate oscillation and is perpendicular to the direction of vibration. For this reason it can be assumed that the main area of shear is within these boundary layers.

A number of different methods exist for calculating the shear rate within these boundary layers, two of which are described here.

Al Akoum et al (2002) showed that the shear rates on one disk are independent of the motion of the other disks in the stack because of thin boundary layers. They state that by using the method of calculating the maximum shear rate they describe in Equation [1.15], exactly the same value is achieved as when using the equations described by Sclichting (1979) in the case of a single oscillating plate.

\[ \gamma_{\text{max}} = 2^{1/2} d \left( \pi F \right)^{3/2} \nu^{-1/2} \]

[1.15]

Where \( \gamma_{\text{max}} \) is the maximum shear rate (s\(^{-1}\)) at the periphery of the membrane disc, \( d \) is the membrane head displacement (m), \( F \) is the frequency of oscillation (Hz) and \( \nu \) is the fluid kinematic viscosity (m\(^2\) s\(^{-1}\)). Taken from Al Akoum et al (2002).

The authors also define a "mean" shear rate by averaging the absolute value of the shear rate over a period and over the membrane area which for the PallSep VMF system, is annular and limited by radii \( R_1 \) and \( R_2 \).

\[ \bar{\gamma}_w = \frac{2^{3/2} \left( R_2^3 - R_1^3 \right)}{3 \pi R_2 \left( R_2^2 - R_1^2 \right)} \gamma_{\text{max}} \]

[1.16]

Where \( \bar{\gamma}_w \) is the mean shear rate (s\(^{-1}\)) over the membrane area, \( R_1 \) is the membrane inner radii (m), \( R_2 \) is the membrane outer radii (m) and \( \gamma_{\text{max}} \) is the maximum shear rate (s\(^{-1}\)) at the periphery of the membrane disc. Taken from Al Akoum et al (2002).

Method 2 – Hurwitz 2001

A second method of calculating shear rates in the VMF system has been suggested by Hurwitz (2001) and is shown in Equation [1.17]. The full derivation of this equation is described in Appendix III.
\[ \gamma_w(0,t) = \omega r \Delta \theta \sqrt{\frac{\rho \rho}{\mu}} \]  

[1.17]

Where \( \gamma_w \) is the shear rate (s\(^{-1}\)) over the membrane area, \( t \) is time (sec), \( \omega \) is the frequency (radians s\(^{-1}\)), \( r \) is the radius (m), \( \Delta \theta \) is the amplitude of angular amplitude (rad), \( \rho \) is the process fluid density (kgm\(^{-3}\)) and \( \mu \) is the viscosity (Pa.s). The full derivation of this equation is described in Appendix III.

1.10.3.3 Effect of shear rate on permeate flux in VMF systems

Using a VSEP system, AI Akoum et al (2002) have suggested that permeate flux can be represented by two different functions of shear rate depending on the value of \( \bar{\gamma}_w \) when processing \( S. \) \textit{cerevisae} at 20 gL\(^{-1}\) DCW, at a \( \Delta P_{TM} \) of 30 kPa and using a 0.2 \( \mu \)m nylon membrane.

\[ J = 6.62 \bar{\gamma}_w^{0.19} \text{ for } \bar{\gamma}_w < 12,000 \text{ s}^{-1}, \text{i.e. } F < 59.7 \text{Hz} \]  

[1.18]

\[ J = 0.35 \bar{\gamma}_w^{0.50} \text{ for } \bar{\gamma}_w > 12,000 \text{ s}^{-1}, \text{i.e. } F > 59.7 \text{Hz} \]  

[1.19]

Where \( \bar{\gamma}_w \) is the mean shear rate (s\(^{-1}\)) over the membrane area.

1.11 Sensitivity of cells to shear damage

As described in Section 1.2, one of the purposes of filtration, like any DSP unit operation, is the recovery and initial purification of the required product of a fermentation. The shear forces necessary to prevent membrane blockage and maintain flux at an acceptable level may well be sufficiently high to damage the cells themselves. The effect of shear on a variety of cells has shown this to be the case.

In a study by Vogel and Kroner (1997), the effect of shear force and cell stability on filtration performance was investigated. The authors showed a loss of viability in mammalian rBHK cells at high shear rates with implications for membrane clogging. A study by Mardiker et al (2000) showed that a variety of animal cells were sensitive to damage when exposed to shear fields in the range of 1 Pa – 100 Pa in a concentric cylinders viscometer.
A study on filtration of sea microalgae by Vandanjon et al (1999) showed that the shear forces seen in TFF systems had a detrimental affect on the viability of cells. In a study by Shimizu et al (1994), the influence of cell breakage due to shear stress on filtration flux was examined. It was found that shear stress seen during cross-flow filtration broke baker’s yeast cells and induced a discharge of granulated matter such as glycogen and cell wall fragments. It was concluded that in cross-flow filtration, cell breakage due to shear stress reduced the filtration flux because of the increase of the hydraulic resistance of the filtration cake formed on the membrane during filtration (Sousa et al. 2002; Czekaj et al. 2000; Parnham and Davis. 1995).

Filamentous microorganisms such as Streptomyces and Penicillium are industrially important, being widely used for antibiotic production (Minas et al. 1998). As described in Section 1.1.2, Streptomyces are prokaryotic actinomycetes with systems of branched hyphae with a typical diameter of 0.5 – 1.5 μm and mycelial length of up to several hundred microns. In submerged culture, filamentous mycelia can result in broths of high viscosities due to hyphal entanglement (Sarra et al. 1996; Karsheva et al. 1997). Filtration of very viscous broths is traditionally a very difficult task due to the low flux rates achieved. The very nature of the culture lends itself to membrane fouling. Studies of the effects of cross-flow filtration induced shear on filamentous micro-organisms, are very rare although much work has been carried out on the effects of shear on the organism in stirred tank reactors. A study by Ayazi Shamlou et al (1994) showed that intense agitation in the fermenter environment can cause the hyphae of Penicillium chrysogenum to break into fragments. They concluded that this damage was due to shear stresses acting on the hyphae rather than mechanical collision with the rotating blades of the impeller. A study by van Suijdam and Metz (1981), proposed that hyphal breakage occurs only after repeated exposure to a sufficiently high level of stress. A study using Streptomyces fradiae by Tamura et al (1997), showed that the average areas of pellets and mycelia in an air-lift reactor were respectively one hundred and twenty fold higher than those seen in the high shear environment of a stirred tank reactor.

The breakdown of the hyphal structure and reduction in hyphal length seen in conditions of high shear leads to the release of intracellular proteins with subsequent consequences on further DSP steps, e.g. Stable emulsion formation in liquid-liquid extraction, as well as contributing to membrane fouling leading to the poor flux rates can be seen under these conditions. A membrane filtration system allowing large volume throughput of feed at sufficiently high shear rates to prevent membrane
fouling, and yet sufficiently low enough to prevent cellular damage, would lead to the high flux rates required to make membrane separation a viable alternative to centrifugation.

1.12 Aims of this work

The main aim of this work is to provide a novel addition to the pool of academic knowledge available on filtration. This will be done by addressing the following aims.

- To obtain a better understanding of the performance of the PallSep PS10 VMF system in terms of permeate flux and product transmission and how this relates to key operating variables such as frequency and amplitude of vibration.

- To compare the VMF system with a conventional static TFF system in terms of filtration performance.

- To add to the limited knowledge base currently available on VMF systems by focusing on the processing of biological feedstreams, specifically whole yeast cells *Saccharomyces cerevisae* and the filamentous actinomycete *Saccharopolyspora erythraea* CA340.

The specific objectives of each chapter are as follows:

**Chapter 3** - A series of key operational variables are identified. The influence of these variables on the performance of the PallSep PS10 is investigated. Microfiltration experiments were performed using whole yeast cell suspensions.

**Chapter 4** - The interactions of the key operational variables identified in Chapter 3 are investigated using high concentration whole yeast cell suspensions. Models are developed for predicting the optimum processing conditions in terms of permeate flux and protein transmission. These models are verified experimentally.

**Chapter 5** - The effect of increasing the between membrane spacing is investigated using both whole yeast cell suspensions and real industrial fermentation broth. Within this chapter a model for prediction of the maximum solids handling ability of the PS10 is proposed.
Chapter 6 - The performance of the PallSep PS10 whilst processing *Saccharopolyspora erythraea* CA340 fermentation broths is investigated.

Chapter 7 – General discussion and concluding remarks.
2. Materials and methods

2.1 Materials and micro-organisms
All chemicals were supplied by Sigma-Aldrich Company (Dorset, UK) unless otherwise stated and were of analytical grade. All fermentation media components were supplied by Oxoid Ltd. (Basingstoke, UK.) while the water used throughout the study was prepared by reverse osmosis (RO). Two micro-organisms were used in this study, *Saccharomyces cerevisiae* and *Saccharopolyspora erythraea* CA340 as described in Sections 2.2 and 2.3 respectively.

2.2 *Saccharomyces cerevisiae* suspensions
*S. cerevisiae* in the form of 1 kg blocks of packed baker’s yeast (JW Pike Ltd., Surrey, UK) was used as a model system for certain microfiltration experiments due to ease of preparation. Feed solutions were typically prepared by suspending a known mass of yeast in 20 l of 50 mM KH$_2$PO$_4$ buffer, pH 6.5, using an homogeniser (Silverson, Chesham, Buckinghamshire, UK). All biomass concentrations referred to in this work are wet weight values unless otherwise specified although it should be noted that the yeast blocks are composed of water as well as cells so the wet weight value does not represent a true wet weight value. Bovine serum albumin (BSA) fraction V powder was added at a fixed concentration of 0.75 gL$^{-1}$ as required.

2.3 *Saccharopolyspora erythraea* CA340 fermentations

2.3.1 Fermentation media
*S. erythraea* fermentations were carried out in a 75 L stirred tank reactor (STR) using either 2 L baffled shake flasks or a 7 L STR for the preparation of the inoculum as described in Sections 2.4.2 and 2.4.3 respectively. A chemically defined soluble complex medium (SCM) was used in all cases. This media type has been previously used within the department (Davies *et al.* 2000) and was made up according to the recipe outlined in Table 2.1. Polypropylene glycol (PPG) was used as an antifoam.

2.3.2 Fermentation equipment
All pilot scale fermentations were carried out in an LH series 75 L fermenter (Inceltech Ltd., Pangbourne, UK). The vessel was constructed from stainless steel with a bottom driven impeller. The vessel was fitted with a three stage 6-bladed Rushton turbine impeller system and four equally spaced baffles. Vessel temperature
was controlled by an electric heating element and by passing cooled process water through the vessel jacket. Dissolved oxygen tension (DOT) was measured by an Ingold Messtechnik AG (Urdorf, Switzerland) DOT probe, sterilised in situ and calibrated for zero and 100% with nitrogen and air respectively. pH was measured using a Mettler Toledo pH probe (Mettler Toledo GmbH, Urdorf, Switzerland), calibrated using standards at pH 4.0 and 7.0. The probe was then sterilised in situ.

<table>
<thead>
<tr>
<th>Soluble complex Medium (SCM)</th>
<th>Concentration (gL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>30.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>6.0</td>
</tr>
<tr>
<td>Bactopeptone</td>
<td>4.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.0</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.68</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>PPG</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Table 2.1** - Soluble complex medium preparation.

Inoculum preparation for the 75 L vessel was performed in either a series of 2 L baffled shake flasks or in an LH series 7 L fermenter (as described further in Section 2.4). The 7 L vessel was glass with stainless steel top and bottom plates with a top driven impeller. Vessel temperature was controlled by passing steam or cooled process water through coils within the vessel. It was fitted with a two stage, 6-bladed Rushton turbine impeller and four equally spaced baffles. DOT was measured by an Ingold Messtechnik AG DOT probe, sterilised in situ and calibrated for zero and 100% with nitrogen and air respectively. pH was measured using a Mettler Toledo pH probe, calibrated using standards at pH 4.0 and 7.0. The probe was then sterilised in situ.

Both fermenters were controlled by Adaptive Biosystems Ltd. (Luton, UK) software. Data logging was carried out by RT DAS (Acquisition Systems Ltd., Sandhurst, UK). On line exit gas composition was measured using an MM8-808S mass spectrometer (VG Gas Analysis Ltd, Winsworth, UK).
The complete dimensions of the two vessels used in this study are shown in Table 2.2.

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>LH7L #02</th>
<th>LH75L #01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume (L)</td>
<td>7.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Working volume (L)</td>
<td>5.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Vessel height (mm)</td>
<td>400.0</td>
<td>925.0</td>
</tr>
<tr>
<td>Vessel diameter (mm)</td>
<td>160.0</td>
<td>327.0</td>
</tr>
<tr>
<td>Shaft height (mm)</td>
<td>385.0</td>
<td>595.0</td>
</tr>
<tr>
<td>Shaft diameter (mm)</td>
<td>12.9</td>
<td>26.0</td>
</tr>
<tr>
<td>No. of impellers</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Impeller diameter (mm)</td>
<td>62.3</td>
<td>105.5</td>
</tr>
<tr>
<td>Tip length (mm)</td>
<td>16.0</td>
<td>26.7</td>
</tr>
<tr>
<td>Tip width (mm)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Tip height (mm)</td>
<td>13.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Sparger height (mm)</td>
<td>400.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Sparger diameter (mm)</td>
<td>9.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Baffle height (mm)</td>
<td>305</td>
<td>612.0</td>
</tr>
<tr>
<td>Baffle width (mm)</td>
<td>20.8</td>
<td>32.0</td>
</tr>
<tr>
<td>Baffle thickness (mm)</td>
<td>1.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Impeller distance from base (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>109.0</td>
<td>109.0</td>
</tr>
<tr>
<td>2</td>
<td>197.0</td>
<td>308.0</td>
</tr>
<tr>
<td>3</td>
<td>N/A</td>
<td>506.0</td>
</tr>
</tbody>
</table>

Table 2.2 - Dimensions of the 7 and 75 L vessels used in this study.

2.4 *Saccharopolyspora erythrea* CA340 inoculum chain

2.4.1 *Saccharopolyspora erythrea* CA340 seed stock preparation

Seed stocks were initially prepared from liquid cultures of *S. erythrea* CA340 grown in nutrient broth (NB) for 72 hours. Cells were harvested by spinning down the culture in a bench top centrifuge (Beckman Instruments, Palo Alto, California, USA) at 13,000 rpm and 10 °C for 10 minutes. The supernatant was then removed using a sterile pipette and discarded. The cells were then re-suspended in an aqueous
mixture of 20 % (v/v) glycerol and 0.1 % (v/v) Tween 80 and aliquoted into 1 ml eppendorff tubes. Cell stocks were then stored at −80 °C until required.

2.4.2 Saccharopolyspora erythraea CA340 inoculum preparation

An appropriate number of baffled conical flasks containing 50 ml of nutrient broth were prepared according to the manufacturer’s instructions and autoclaved at 121 °C for 20 min at 1 bar. Following sterilisation, a 1 ml seed culture of S. erythraea CA340 (prepared as described in Section 2.4.1) was defrosted and added to each flask under aseptic conditions. The flasks were then incubated with shaking for 24 hr at 28 °C and 200 rpm.

After 24 hr, each of the 50 ml volumes of culture was added to 450 ml of sterile nutrient broth in 2 L baffled conical flasks. These flasks were then incubated for either a further 24 or 41 hours with shaking at 28 °C and 200 rpm, depending upon the next step in the inoculum chain.

If the 75 L vessel was to be inoculated using shake flasks, after 41 hr 50 ml of the NB culture was transferred to 450 ml of sterile SCM in each of the twelve 2 L baffled conical flasks used and incubated with shaking at 200 rpm and 28 °C for a further 30h.

2.4.3 7 L fermentations

4.5 L of SCM media was made up as described in Table 2.1 and sterilised in situ. Fermenter ancillaries (inoculation flask and addition vessels) and additional solutions (MgSO₄·7H₂O, acid, base and antifoam) were sterilised separately by autoclaving for 20 min at 121 °C and 1 bar. Prior to inoculation, the addition vessels were attached aseptically, the pH of the media adjusted to 7.0 and the temperature allowed to stabilise at 28 °C. The inoculum was then checked for sterility (as defined by the absence of contaminating organisms) by visual examination under a microscope. Assuming no contamination was present, 500 ml of inoculum was then added to the vessel aseptically and the agitation and aeration rates set. The vessel was aerated at a rate of 0.5 - 1.0 vvm and at impeller speeds of 500 -1000 rpm depending on the DOT. After 26 hr, the broth was used to inoculate the 75 L vessel by means of a sterile transfer line.
2. Materials and methods

2.4.4 75 L fermentations

45 L of SCM fermentation media was first prepared as detailed in Table 2.1 and sterilised in situ at 121 °C and 1 bar for 20 min. Fermenter ancillaries (inoculation flask and addition vessels) and additional solutions (MgSO₄·7H₂O, acid, base and antifoam) were sterilised by autoclaving for 20 min at 121 °C and 1 bar. Prior to inoculation, the addition vessels were attached aseptically, the pH of the media adjusted to 7.0 and the temperature allowed to stabilise at 28 °C. The inoculum was then checked for sterility as described in Section 2.4.3. 5 L of inoculum was then added to the vessel aseptically either from the shake flasks (Section 2.4.2) or via a sterile transfer line from the 7 L inoculum vessel (Section 2.4.3) and the agitation and aeration rates set. The vessel was aerated by a ring sparger at a rate of 0.5 - 1.0 vvm and at impeller speeds of 500 - 1000 rpm depending on DOT.

2.5 Analytical Techniques

2.5.1 Determination of biomass concentration

Biomass concentrations of fermentation broth samples were measured by determining the dry cell weight. Prior to assaying the sample, Whatman GF/F filter papers (Whatman Ind. Ltd., Maidstone, U.K.) were dried in a HG53 Halogen Moisture Analyser (Mettler-Toledo, UK) to constant weight. For each sample, 3 ml of broth were filtered under vacuum and washed through with an equal volume of RO water. The filter paper was then dried to constant weight in the moisture analyser and re-weighed to ascertain dry cell weight. All measurements were made in triplicate with the maximum coefficient of variance of the assay being 4.2 %.

2.5.2 Ferric ion assay for measuring erythromycin

In order to measure total antibiotic transmission, erythromycin concentration in both broth samples and permeate samples was ascertained using the ferric ion assay described by Gallagher and Danielson (1995). The macrolide ring of antibiotics such as erythromycin and spiramycin have been shown to react with Fe³⁺ in the presence of an acetic acid – sulphuric acid mixture to form a coloured compound having an absorption maximum at 592 nm. The assay described by Gallagher and Danielson permits the analysis of fermentation broths containing erythromycin without sample pre-treatment (other than drying).

An erythromycin calibration curve was first constructed using fermentation broth as diluent in order to account for any interference by the broth components. A 0.5 gL⁻¹
2. Materials and methods

Erythromycin solution was made up by dissolving 0.001 g of erythromycin in 20 ml of S. erythraea CA340 fermentation broth previously clarified by centrifugation at 4000 rpm for 10 min. An Fe\(^{3+}\) solution was made up by dissolving 0.08 g of FeCl\(_3\) in 2 ml of H\(_2\)SO\(_4\) and 2 ml of RO water. This solution was made up to 100 ml with glacial acetic acid. Dilutions of the 0.5 gL\(^{-1}\) erythromycin solution were made up in glass vials and were then evaporated to dryness under vacuum at low temperature for 4 hr. To each of these samples, 0.75 ml of glacial acetic acid was added and the solids allowed to re-suspend. The solutions were then transferred to 2.5 ml eppendorf tubes and placed into a water bath at 45 °C for 45 min. To this solution, 0.75 ml of the Fe\(^{3+}\) solution was added and placed into a water bath at 50 °C for 15 min. The tubes were then placed on ice and centrifuged at 13,000 rpm for 5 min. The absorbance was read at 592 nm and a standard curve constructed.

Fermentation samples were subsequently assayed by drying 0.15 ml of broth sample under vacuum and treating with glacial acetic acid and Fe\(^{3+}\) solution as described above and reading the absorbance at 592 nm. All measurements were made in triplicate with the maximum coefficient of variance of the assay being 1.5 %.

2.5.3 Bio-Rad protein assay

In order to measure protein levels, a Bio-Rad protein assay kit (Bio-Rad laboratories, Hemel Hempstead, Herts., UK) was used for measurement of soluble protein in both broth supernatant and permeate samples. This method is based on the Bradford assay (Bradford 1976) and relies on the shift in the maximum absorbance of a dye, Coomassie Brilliant Blue G-250, when it binds protein under acidic conditions.

A calibration curve was first constructed by assaying known concentrations of bovine serum albumin (BSA). A 10 mgmL\(^{-1}\) stock solution of BSA was made by dissolving 10 mg of BSA in 1 ml of 50 mM Tris buffer (pH 8). A set of standards was then made by dilution in Tris buffer. 100 \(\mu\)l of each of these BSA standards was then added to 2.5 ml of BioRad reagent (diluted 1:5 in RO water) and left to stand for 5 min. The absorbance was then read at 595 nm. Samples of fermentation broth supernatant and permeate were assayed following centrifugation as necessary by addition of 100 \(\mu\)l of sample to 2.5 ml of BioRad reagent diluted as previously described. All the measurements were made in triplicate with the maximum coefficient of variance of the assay being 3.3 %.
2.5.4 Rheological measurement of fermentation broth

Rheological measurements were carried out using a Rheomat 115 rotational viscometer (Contraves AG, Zurich, Switzerland) with a plug in 7/7 module operating system and a concentric cylinder (MS-0/115) measuring unit with quick release coupling. Measurements were carried out at 20 °C (Haake DC1 Circulator and K15 Bath, Haake, Sussex, UK) immediately after removal from the fermenter.

The device was filled with 20 ml of broth, installed into the rheometer and rotated for 10 sec at the lowest speed (step 1, shear rate = 24.3 s⁻¹). The torque was then recorded as the speed was increased to step 15 (shear rate = 3680 s⁻¹). Readings were allowed to settle before moving onto the next step. Speed was translated into shear stress using the table supplied by Contraves.

Fungal fermentations have been reported to exhibit Non Newtonian behaviour (Karsheva et al. 1996; Warren et al. 1994) and adhere to the pseudoplastic Power Law or "Shear-thinning" model of viscosity (Atkinson and Mavituna. 1991) shown in Equation [2.1].

\[ \tau = K \gamma^n \]  

[2.1]

Shear stress (\(\tau\)) was plotted against shear rate (\(\gamma\)) in a ln-ln plot and the slope and intercept were considered as values of flow behaviour index (n) and consistency index (K) respectively. From the values of K and n, the apparent viscosity (\(\mu_a\)) can be calculated.

\[ \mu_a = K \gamma^n \]  

[2.2]

2.5.5 Particle size analysis

Particle size analysis was carried out using the Malvern Mastersizer 2000 laser sizer (Malvern, Worcestershire, U.K.). Laser diffraction more accurately called Low Angle Laser Light Scattering (LALLS), measures particles in the range of 0.1 to 2000 \(\mu\)m.

Drops of Yeast suspension were added into the re-circulating cell, which contained 50 mM \(\text{KH}_2\text{PO}_4\) buffer at pH 6.5 to ensure that the conditions within the instrument
matched that of the sample. Each sample was analysed three times in order to obtain a mean value with the maximum coefficient of variance of the assay being 2.8%.

2.6 Microfiltration rig and operation
A PallSep PS10 (Pall Filtration, East Hills, N.Y., USA) VMF unit, shown in Figure 1.3, was used throughout this work fitted with two membrane discs covered on both sides with a proprietary hydrophobic PTFE membrane (0.45 μm pore size) giving a total membrane surface area of 0.2 m². Figures 1.5 and 1.6 show a schematic cross section and a plan view of a single membrane disc. The flow paths of feed, retentate and permeate streams are shown in Figure 1.7. The feed is delivered to the first membrane disc through a feed distribution channel in the bottom endplate of the VMF membrane assembly. The feed is then distributed around the outer circumference of each membrane through 12 equally spaced, 8.5 mm diameter holes, located on the outer edge (270 mm) of each membrane disc. The fluid then flows inwards tangential to the membrane surface towards 6 equally spaced, 8.5 mm diameter retentate openings located near the inner diameter (26 mm) of the membrane discs. The retentate exits the VMF system through the top end plate retentate ports. Permeate is collected in a series of grooves running from the outer to the inner diameter of the stainless steel support disc, on the down-stream side of the membrane, and exits the VMF system through the top end plate permeate port.

The PS10 unit was attached to a custom built pumping and data collection rig, shown in Figure 2.1, by means of 9 mm i.d. PTFE sanitary hoses. The rig was constructed of stainless steel tubing of 9 mm i.d. and operated in a one-pump configuration. The crossflow rate of the process fluid was controlled by a Fristam SK 90L/4 lobe pump (Fristam Pumps, Hailsham, UK) linked to an AC Tech mc series controller (AC Technology Corporation, Uxbridge, MA, USA). Crossflow rate was measured by a digital Promag flowmeter (Endress & Hauser, Reinach, Switzerland).

2.6.1 Microfiltration system operation
Prior to each experimental run, the MF rig and PallSep filter housing were flushed with excess RO water to remove the 0.1 M NaOH solution used for membrane storage and prevention of microbial growth. The PallSep was then switched on to allow the membrane head amplitude to stabilise before each experimental run commenced, (membrane head amplitude is defined as the total peak to peak amplitude and is illustrated in Figure 3.6). Pure water flux was measured at this point to ensure the cleanliness of the membranes. Experiments were performed in either
total recycle or concentration mode. In both cases, permeate flux was measured by collecting permeate in a measuring cylinder over three one minute intervals. This volume measurement was subsequently converted to a flux having units of $\text{L} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$. The maximum coefficient of variance was 1.1%. Certain key experiments were performed in triplicate to ensure reproducibility of the results obtained.

Following each experiment, the rig was washed with RO water to remove any solids from the membrane surface. The rig was then flushed with a 1 % (w/v) caustic solution, Ultracil 41 (Henkel Ecolab, Swindon, Wiltshire, UK.), at 50 °C for 30 min with vibration. The membrane was then rinsed with excess RO water and the clean water flux measured. The cleaning step was repeated if necessary. The system was then filled with a 0.1 M NaOH storage solution as described previously.

![Figure 2.1 - Schematic layout of the PallSep PS10 pumping and data collection rig. The inlet, outlet and permeate pressure gauges are denoted by $P_1$, $P_2$ and $P_3$ respectively. Retentate and permeate flow meters are denoted by $F_2$ and $F_1$ respectively.](image-url)
2.7 Factorial design experiments

In Chapter 4 of this work, a series of incomplete factorial design experiments, specifically Box-Behnken, are used to examine the interactions of certain variables and their influence on microfiltration processes.

For the design of the experiments, Design-Expert 5 (Statease, Minneapolis, USA) software was used. For each of the experimental variables, a desired high (+1) and low (-1) value was chosen and entered into the programme. The software then calculates the midpoint, or 0 value, and lists each individual experiment to be carried out. The final list contains far fewer individual experiments than if the interactions of all variables were to be investigated without the programme. Following completion of the experiments, the software was used to create response surface models and perturbation plots allowing analysis.
3. Flux and transmission characteristics of the PallSep PS10

3.0 Introduction and aims

Conventional, static tangential flow filtration systems rely on high liquid crossflow velocities to generate shear at the liquid-membrane interface as described in Section 1.3.2. Shear is necessary in order to keep the membrane clear of foulants, thus maintaining acceptable permeate flux and product transmission levels. VMF systems use mechanical energy, generated by vibration, to create high intermittent shear fields at the membrane surface, thus decoupling shear and liquid crossflow velocity. Theoretically, this should allow the maintenance of permeate flux and product transmissions at lower crossflow velocities and perhaps higher solids levels than conventional static TFF systems. Lower liquid crossflow velocities would mean that much smaller pumps and pipes are required thus reducing the hold up volume of the system. This may be critical in some of the smaller volume and high value processes involved in the biotech industry.

Apart from the introduction of shear by vibration, VMF systems are essentially still operated as tangential flow filtration systems and as such their performance should be affected by the same factors that govern the performance of conventional TFF systems, e.g. $\Delta P_{\text{TM}}$, process stream viscosity and solids load, mode of operation and membrane type. These factors were discussed in detail in Section 1.7. In this chapter, the basic performance of the PallSep PS10 is investigated using a number of key variables identified on the basis of their likely influence on the performance of the VMF system. These variables and the reasons for their choice are outlined below.

- **Membrane head amplitude** - VMF technology works by using mechanical energy in the form of vibration to create high intensity localised shear fields at the membrane surface that help to prevent the build up of any fouling layer. The shear rate is directly linked to membrane head amplitude (as will be described in Section 3.4), so by varying the head amplitude and thus the shear rate, its influence may be studied. The affect of membrane head amplitude on permeate flux and BSA transmission is described in Section 3.5.
3. Flux and transmission characteristics of the PallSep PS10 Postlethwaite 2003

• **Feedstream solids loading** - By their very nature, conventional TFF systems are only able to handle feedstreams with limited solids levels and hence viscosity (the relationship between solids loading and viscosity is described in Section 1.7.3). The elevated levels of shear observed at the membrane surface of the VMF systems would suggest that such systems are better able to process broths with higher solids loads and viscosities. The affect of feedstream solids loading on permeate flux and BSA transmission is described in Section 3.6.

• **Transmembrane pressure** ($A P_{TM}$) - The influence of $A P_{TM}$ on microfiltration performance is well documented as outlined in Section 1.7.1. The affect of $A P_{TM}$ on permeate flux and BSA transmission is described in Section 3.7.

• **Membrane gap width** - During the assembly of the membrane head, a single spacer and gasket are normally inserted between each membrane plate in the head stack to give a gap of 1.4 mm between each membrane plate. The significance of this gap width in terms of microfiltration performance is unclear and will thus be studied. The affect of membrane gap width on permeate flux and BSA transmission is described in Section 3.8.

• **Crossflow rate** - The influence of crossflow rate on conventional TFF systems is well documented and as outlined in Section 1.7.2. As the shear necessary to prevent fouling of the VMF system is provided by mechanical vibration, the need for high crossflow velocities is theoretically diminished. The affect of crossflow rate on permeate flux and BSA transmission is described in Section 3.9.

Much of the work presented within this chapter has been published as Postlethwaite J, Lamping S, Hurwitz M, Leach G and Lye G. Flux and transmission characteristics of a vibrating microfiltration system operated at high biomass loading. Journal of Membrane Science (accepted for publication 2003).

3.1 **Experimental methods**

For the work presented in this chapter, *Saccharomyces cerevisae* suspensions with added BSA, prepared as described in Section 2.2, were used as a model biological feedstream. Microfiltration experiments were performed in either concentration or total
3. Flux and transmission characteristics of the PallSep PS10 recycle mode as outlined in Section 2.6.1. All analytical techniques were carried out as detailed in Section 2.5.

3.2 Characterisation of *Saccharomyces cerevisae* suspensions

Prior to filtration experiments, *S. cerevisae* feedstreams were first characterised in terms of their particle size and rheology. Figure 3.1 shows a light microscope image of *S. cerevisae* cells at 400x magnification. The figure shows the cells to be roughly spherical in shape and of approximately uniform size.

![Light microscope image of *Saccharomyces cerevisae* cells.](image)

**Figure 3.1** – *Saccharomyces cerevisae* cells dispersed in phosphate buffer (50 mM KH₂PO₄ at pH 6.5) suspension prepared as described in Section 2.2 and image obtained at 400x magnification. Bar represents ~40 μm.

Analysis of the cell size distribution was performed using the Malvern Mastersizer 2000 laser sizer as described in Section 2.5.5. Typical results are shown in Figure 3.2.
3. Flux and transmission characteristics of the PallSep PS10

Figure 3.2 – Size distribution of *Saccharomyces cerevisae* cells dispersed in phosphate buffer (50mM KH$_2$PO$_4$ at pH 6.5). Suspension prepared as described in Section 2.2 and size distribution measured as described in Section 2.5.5. The distribution represents the average of triplicate readings.

Analysis of the figure shows the cells to have a narrow size distribution with a mean diameter ($d_{50}$) of 5.3 ± 3 μm. The lack of any peaks at particle sizes below 1 μm suggests an absence of cellular debris or undissolved media components in the yeast block used to prepare the suspension.

D'Arcy's filtration law states that permeate flux is inversely proportional to liquid/broth viscosity as described in Section 1.7.3. The viscosity of *S. cerevisae* suspensions across the range of concentrations used in this study is shown in Figure 3.3.
3. Flux and transmission characteristics of the PallSep PS10 Postlethwaite 2003

Figure 3.3 – Rheology of *Saccharomyces cerevisae* suspensions in phosphate buffer (50mM KH$_2$PO$_4$ at pH 6.5) across the range of concentrations used in this study. Suspensions prepared as described in Section 2.2. Rheological measurements made using the technique described in Section 2.5.4. Error bars represent standard error of the mean.

The figure shows a steady rise in apparent viscosity with increasing yeast concentration up to 700 gL$^{-1}$. After this, a rapid rise in apparent viscosity is observed at 800 gL$^{-1}$. In all cases the relationship between shear rate and shear stress was linear indicating Newtonian rheology (Doran. 1998).

3.3 Microfiltration characteristics of *Saccharomyces cerevisae* and BSA suspensions

In this section, initial experiments designed to assess the influence of *S. cerevisae* and BSA on VMF performance are described. Figure 3.4 shows the influence of BSA (0.75 gL$^{-1}$) on permeate flux during the processing of *S. cerevisae* (500 gL$^{-1}$) in total recycle mode of operation.
Figure 3.4 – Influence of BSA on permeate flux during the processing of S. cerevisiae at 500 gL⁻¹ in total recycle mode. Permeate flux (■) with and (●) without BSA present. Experimental conditions: BSA concentration 0.75 gL⁻¹, membrane head amplitude 19.5 mm, ΔP<sub>tm</sub> 0.65 bar, crossflow rate 1 Lmin⁻¹ and membrane gap width 1.4 mm.

In the absence of BSA, a steady state is immediately established at a permeate flux of 94 Lm⁻²hr⁻¹, suggesting a lack of membrane fouling. This is perhaps due to the lack of smaller fouling molecules such as BSA or undissolved media components. In the presence of BSA however, permeate flux can be seen to decline steadily from 115 Lm⁻²hr⁻¹ to 97 Lm⁻²hr⁻¹ over a period of 90 minutes. It is unclear whether the flux levels off at this point or continues to fall. This steady decline in permeate flux in the presence of BSA would suggest a gradual fouling of the membrane by the BSA molecules. Whilst the steady state flux of each experimental run is approximately the same (94 and 97 Lm⁻²hr⁻¹), the reason for the higher initial flux in the presence of BSA is unclear.
Figure 3.5 shows the influence of *S. cerevisae* on BSA transmission. In the absence of cells, BSA transmission is seen to rise slightly from an initial value of 77 % w/w after 18 minutes to 85 % w/w after 30 minutes although this rise can possibly be explained by error within the assay. In the presence of *S. cerevisae* however, transmission can be seen to fall gradually from an initial value of 86 % w/w after 18 minutes to 68 % w/w after 90 minutes, the same decline observed in Figure 3.4. The lower flux observed at the end of the experimental run would perhaps suggest that steady state had not been reached in Figure 3.4.

![Figure 3.5 - Influence of *S. cerevisae* on BSA transmission during the processing of BSA at 0.75 gL⁻¹ in total recycle mode. Transmission (■) with and (●) without *S. cerevisae*. Experimental conditions: yeast concentration 500 gL⁻¹, membrane head amplitude 19.5 mm, ΔP_{TM} 0.65 bar, crossflow rate 1 Lmin⁻¹ and membrane gap width 1.4 mm.](image-url)
Figures 3.4 and 3.5 together show that when both *S. cerevisae* and BSA are present, permeate flux and BSA transmission take substantially longer to reach steady state. A possible explanation for this is that BSA molecules are known to form aggregates (Maruyama *et al.* 2001; Huisman *et al.* 2000). If the aggregation of BSA molecules was somehow greater in the presence of the *S. cerevisae* cells, then the absorption of the larger BSA aggregates into the membrane pores or their deposition on the membrane surface will be sufficient to cause the gradual decline in permeate flux and transmission observed in the presence of both *S. cerevisae* and BSA.

### 3.4 Oscillation of the membrane head and calculation of the corresponding shear rates

![Graph showing sinusoidal motion of PallSep membrane head stack](image)

**Figure 3.6** – Sinusoidal motion of PallSep membrane head stack. The y axis shows distance moved from the central point, 0, with negative values showing movement to the left of this central point and positive values showing motion to the right. Amplitude is defined as total peak to peak head amplitude throughout this work, in this case 19.5 mm, and is indicated by the arrow. Data shown for operation at a frequency of 56 Hz.
As described in Section 1.9.1, PallSep motion is oscillatory and sinusoidal as shown in Figure 3.6. This sinusoidal vibration creates an intermittent boundary layer at the top and bottom surface of each membrane plate. The uplifting forces generated by the vibrational shear keeps all solids away from the membrane meaning that the boundary layers contain liquid only. The exact nature of these boundary layers and the rationale behind this statement is described in detail in Section 5.2.1.

3.4.1 Calculation of shear rate without vibration

The method used to calculate shear rate in conventional TFF systems is described in Section 1.10.3.1. Using Equation [1.14], average shear rates in the range of $0.7 \times 10^2$ s$^{-1}$ at the feed inlet to $1.7 \times 10^2$ s$^{-1}$ at the feed outlet are calculated across the membrane surface of the PallSep PS10 when operated without vibration during the processing of a $S.\ cerevisae$ suspension of 500 gL$^{-1}$. The calculation assumes an average flow rate of 1 Lmin$^{-1}$ and a membrane gap width of 1.4 mm. A sample of this calculation is shown in Appendix 1.

3.4.2 Calculation of shear rate in VMF systems

A number of different methods can be used to calculate the shear rate in the PallSep VMF system as described in Section 1.10.3.2.

**Method 1** - The first method of calculation was based on that used by Al Akoum et al. (2002) to calculate the shear rate in a VSEP Series L. Using equations [1.15] and [1.16], values for the maximum and average shear rates in the PallSep PS10 were calculated at amplitudes between 6.5 – 26 mm. The results are shown in Table 3.1 and a sample calculation is shown in Appendix 1.

**Method 2** – The second method used to calculate the mean shear rate involves using Equation [1.17] as suggested by Hurwitz (2001). Again, calculated values for amplitudes between 6.5 – 26 mm are shown Table 3.1 and a sample calculation is shown in Appendix 1.
Table 3.1 - Maximum and average shear rates for the PallSep PS10 system operating with water at 20°C. Where $\gamma_{w,\text{max}}$ is the maximum shear rate s$^{-1}$ and $\bar{\gamma}_w$ is the mean shear rate s$^{-1}$. Values calculated using the methods suggested by Al Akoum et al. (2002) in Equations [1.15] and [1.16] and Hurwitz (2001) in Equation [1.17].

The table shows a gradual increase in maximum shear rate, using Method 1, from $2.1 \times 10^4$ s$^{-1}$ to $8.5 \times 10^4$ s$^{-1}$ and in mean shear rates from $6.8 \times 10^3$ s$^{-1}$ to $2.8 \times 10^4$ s$^{-1}$ as head amplitude is increased from 6.5 mm up to 26 mm. Maximum shear rates for the VSEP series L have been reported as $1.1 \times 10^5$ s$^{-1}$ and mean shear rates as $3.6 \times 10^4$ s$^{-1}$ (Al Akoum et al, 2002), slightly higher than those observed here. This is due to the higher maximum amplitude (30 mm) used in the other studies. Using Method 2, significantly higher average shear rates are calculated compared to Method 1, although they are within the same order of magnitude.

Figure 3.7 shows the sinusoidal motion of the PallSep membrane head stack onto which the corresponding shear rates have been superimposed. The figure shows that the notional shear rate changes as the head vibrates and is seen to be at its maximum when the head is furthest away from the origin as would be expected. Due to the sinusoidal motion of the membrane head, the direction of the shear changes during each oscillation although it still remains tangential to the direction of the process stream crossflow (as shown in Figure 1.7). The shear due to crossflow is significantly lower than that generated by vibration as described in Section 3.4.1.
3. Flux and transmission characteristics of the PallSep PS10

Fig. 3.7 – Sinusoidal motion of PallSep PS10 membrane head stack with corresponding shear rates. (■) maximum shear rate as calculated using Equation [1.16], (○) average shear rate as calculated using Equation [1.17], (▼) average shear rate as calculated using Equation [1.18]. Shear rates are calculated at a frequency of approx. 56Hz corresponding to a PallSep PS10 head amplitude of 26 mm, the maximum amplitude used in this work.

3.4.3 Comparison of VMF shear rates with static TFF systems

The shear rates experienced at the membrane surface in the PallSep are higher than those reported for conventional static TFF systems. Using a Minitan II TFF system, Davies et al. (2002) have reported average shear rates in the region of $1.1 \times 10^4$ s$^{-1}$. Using equation [1.14], an average shear rate of approximately $1.7 \times 10^5$ s$^{-1}$ is observed at the membrane surface of the PallSep PS10 when operated without vibration. This value is of little use in a comparison however. The flow pattern in the VMF system, described in Section 2.6, is very different to that in a conventional TFF system. At the recommended
operational amplitude of 19.5 mm however, the average and maximum shear rates are $2.1 \times 10^4 \text{ s}^{-1}$ and $6.3 \times 10^4 \text{ s}^{-1}$ as calculated using Equations [1.16] and [1.17].

3.5 Influence of membrane head amplitude on VMF performance

VMF systems rely on mechanical vibration bought about by an oscillating membrane head stack to create the shear necessary to prevent membrane fouling. The frequency of this oscillation dictates the head amplitude and hence the shear rate. It should be noted however that this is true only for a given torsion spring system. If the spring coefficient of the torsion bar or the mass of either the membrane head or the seismic mass is altered then this relationship will change as described in Section 1.9.1. The effect of vibration on permeate flux is shown in Figure 3.8.

![Figure 3.8](image_url)

**Figure 3.8** – Influence of vibration during the processing of 200 gL$^{-1}$ S. *cerevisae* in total recycle mode. Permeate flux (▲) with and (■) without vibration. Experimental conditions: Membrane head amplitude 19.5 mm, Δ$P_{TM}$ 0.9 bar, crossflow rate 1 Lmin$^{-1}$ and membrane gap width 1.4 mm.
The figure shows that vibration of the membrane head is crucial in maintaining permeate flux. During the processing of 200 gL$^{-1}$ yeast without vibration, permeate flux is seen to drop from around 32 Lm$^{-2}$hr$^{-1}$ to zero within 10 minutes as the membrane becomes completely fouled. When the same yeast suspension is processed with vibration at an amplitude of 19.5 mm (the operational amplitude recommended by Pall for reasons related to the operational life of key machine components) an initial decline in permeate flux from approximately 77 Lm$^{-2}$hr$^{-1}$ is observed followed by the establishment of an apparent steady state after 10 – 12 minutes of 45 – 50 Lm$^{-2}$hr$^{-1}$.

![Figure 3.9 - Permeate flux and BSA transmission as a function of PS10 head amplitude during processing of 400 gL$^{-1}$ *S. cerevisae* in total recycle mode. (■) BSA transmission, (▼) experimental permeate flux, (—) predicted permeate flux obtained using Equations [1.15] and [1.16]. Dashed line represents the line used to obtain Equation [3.1]. Experimental conditions: $\Delta P_{\text{TM}}$ 0.9 bar, crossflow rate 1 Lmin$^{-1}$ and membrane gap width 1.4 mm. Error bars represent standard error of the mean.](image-url)
Figure 3.9 shows how both permeate flux and protein transmission (BSA) vary with head amplitude. The figure shows a steady rise in both permeate flux and BSA transmission levels as head amplitude is increased from 6.5 mm up to a maximum of 26 mm. At an amplitude of 6.5 mm, the permeate flux is $35 \pm 0.64 \text{ Lm}^2\text{hr}^{-1}$ and BSA transmission is $46.9 \pm 6.72 \% \text{ w/w}$. As amplitude is increased to 26 mm, permeate flux is seen to increase to a maximum of $47 \pm 0.35 \text{ Lm}^2\text{hr}^{-1}$ and BSA transmission to $75.3 \pm 5.23 \% \text{ w/w}$.

Figure 3.9 also shows the predicted permeate flux calculated using Equations [1.18] and [1.19] described in Section 1.10.3.3. The figure shows good agreement between the predicted and experimental permeate flux results at the lowest head amplitude of 6.5 mm. As head amplitude is increased however, experimental results vary significantly from the predictions and noticeably the predicted inflection at a head amplitude of 13 mm is not found experimentally. The experimental results reported here can be described by Equation [3.1].

$$J = 5.94 \tilde{f}^{0.2}$$

[3.1]

The difference in these results from those reported by Al Akoum et al. (2002) can be explained, certainly in part, by the differences in yeast concentration used during the testing of the different systems. In this study a 400 gL$^{-1}$ yeast suspension is used whilst Al Akoum et al. (2002) used only a 20 gL$^{-1}$ yeast suspension. The membrane rigs themselves are remarkably similar. The dimensions of the membrane disks used in the Al Akoum study are identical to those used in this study as described in Section 2.6. The construction of the membrane head is slightly different in each system which again may contribute to the difference in the observed results.

As discussed in Section 3.0, unlike conventional TFF systems which rely on crossflow velocity to provide the shear necessary to prevent membrane fouling, VMF uses mechanical vibration to create intermittent shear fields at the membrane surface. Figure 3.9 clearly demonstrates increases in amplitude cause an increase in permeate flux and BSA transmission, presumably bought about by a decrease in membrane fouling caused by the intermittent shear fields bought about by mechanical vibration.
Influence of feedstream solids loading on VMF performance

In an attempt to maximise product titre, pharmaceutical companies spend a considerable amount of time and money on process optimisation, the results of which are, with very few exceptions, closely guarded secrets. Regardless of whether the desired product is an extracellular molecule, an intracellular aggregate or the organism itself, the objective of the fermentation process is usually to maximise the biomass levels (Bader. 1986). The high viscosities bought about as a result of either the media composition (Liew et al. 1997; Conrad and Lee. 1998) or the high biomass levels (Das et al. 2002), have implications for microfiltration performance as outlined in Section 1.7.3.

*S. cerevisiae* suspensions have been used as model systems to assess microfiltration performance and as such, literature values are available for comparison. Table 3.2 summarises some of the studies performed with this model feed stream for a range of conventional and shear enhanced membrane systems as described in Section 1.8. The most striking feature of the table is the very low solids levels used in all cases, well below those expected in commercial applications. Permeate fluxes are generally in the range of 20 - 50 Lm²hr⁻¹.

<table>
<thead>
<tr>
<th>Yeast concentration gL⁻¹ (wet weight)</th>
<th>Membrane pore size (µm)</th>
<th>System type</th>
<th>Permeate flux (Lm²hr⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.45</td>
<td>TFF</td>
<td>30 - 50</td>
<td>Russotti et al (1995)</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>TFF</td>
<td>20</td>
<td>Kluge et al (1999)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.02</td>
<td>TFF</td>
<td>20</td>
<td>Li et al (1998)</td>
</tr>
<tr>
<td>280</td>
<td>0.2</td>
<td>TFF</td>
<td>20 - 30</td>
<td>Okec (1998)</td>
</tr>
<tr>
<td>202</td>
<td>0.2</td>
<td>VMF</td>
<td>20</td>
<td>Al Akoum et al (2002)</td>
</tr>
<tr>
<td>200 - 500</td>
<td>0.45</td>
<td>VMF</td>
<td>40 - 75</td>
<td>This work</td>
</tr>
</tbody>
</table>

Table 3.2 - Summary of previous microfiltration studies using model systems comprised of *S. cerevisiae* and BSA. Note the difference in yeast concentrations used in this work to all the previous studies.

Figure 3.10 shows how permeate flux and protein transmission levels vary with increasing biomass concentration in the feed stream. The figure clearly shows that VMF
systems are well able to cope with process fluids containing high levels of biomass. At 300 gL\(^{-1}\) \textit{S. cerevisae} concentration, a flux of 74 Lm\(^2\)hr\(^{-1}\) and a protein transmission level of around 80 \% w/w are observed. The figure shows a decline in both flux and transmission as biomass levels are increased, in agreement with Equation [1.4] which indicates that permeate flux is proportional to process stream viscosity.

![Graph](image)

**Figure 3.10** – Influence of solids concentration during the processing of \textit{S. cerevisae} and BSA suspensions in total recycle mode. Steady state (■) permeate flux and (▼) BSA transmission. Experimental conditions: BSA concentration 0.75 gL\(^{-1}\), head amplitude 19.5 mm, \(\Delta P_{\text{Tm}}\) 0.65 bar, crossflow rate 1 Lmin\(^{-1}\) and membrane gap width 1.4 mm.

An important feature of Figure 3.10 is that even at 500 gL\(^{-1}\) \textit{S. cerevisae} concentration, the VMF system is still able to achieve a permeate flux of around 37 Lm\(^2\)hr\(^{-1}\) and a protein transmission level of 69 \% w/w. One explanation for the fall in protein
transmission observed at a yeast concentration of 600 gL\(^{-1}\) is the possibility of some form of association of BSA aggregates with yeast cells or increased aggregation in the presence of yeast cells as discussed in Section 3.3. At higher yeast concentrations, more BSA associates with the cells thus preventing their transmission through the membrane.

Comparing these results with the data presented in Table 3.2 shows how VMF systems are able to process broths with much higher solids loads than conventional static TFF systems. The highest \(S.\ cerevisae\) concentration used in the cited studies is that used by Okec (1998). Using a conventional TFF system, the author obtained a permeate flux of 10 Lm\(^{-2}\)hr\(^{-1}\) and a total protein transmission level of 15.6 % w/w whilst processing \(S.\ cerevisae\) (280 gL\(^{-1}\) wet weight) at a \(\Delta P_{\text{TM}}\) of 0.5 bar. Flux and transmission levels were observed to increase to 30 Lm\(^{-2}\)hr\(^{-1}\) and 73.5 % w/w respectively however, following an increase in operating \(\Delta P_{\text{TM}}\) to 1.1 bar. At a similar yeast concentration (300 gL\(^{-1}\)) a permeate flux of 75 Lm\(^{-2}\)hr\(^{-1}\) and protein transmission levels of around 80 % w/w are achieved using the VMF system described here. The difference in membrane pore size between the two studies should be noted however.

3.7 Influence of \(\Delta P_{\text{TM}}\) on VMF performance

As stated in Section 3.0, the importance of \(\Delta P_{\text{TM}}\) on microfiltration performance is well documented (Porter, 1972). Traditional flux against \(\Delta P_{\text{TM}}\) curves show a linear increase in permeate flux with ascending \(\Delta P_{\text{TM}}\) until a pressure is reached beyond which any increase does not bring about a corresponding increase in permeate flux (Gesan - Guiziou et al. 2000). This pressure is known as the critical \(\Delta P_{\text{TM}}\) or cTMP and is discussed in further detail in Section 1.7.1. In order to determine the influence of \(\Delta P_{\text{TM}}\) on PS10 performance, a series of experiments to ascertain the cTMP values for the PS10 at different solids loadings were performed. \(S.\ cerevisae\) suspensions of 300, 400 and 500 gL\(^{-1}\) were prepared as outlined in Section 2.2. These suspensions were then processed using the PallSep in total recycle mode of operation at increasing \(\Delta P_{\text{TM}}\). Flux measurements were taken at steady state. The results are shown in Figure 3.11.

The figure shows a linear increase in permeate flux with \(\Delta P_{\text{TM}}\) over the range of pressures investigated for 300 and 400 gL\(^{-1}\) \(S.\ cerevisae\) suspensions. This suggests
that for these yeast concentrations, the cTMP value is somewhere above 1.9 bar. Experiments did not go higher than this value due to the pressure limitations of the feed hoses connecting the pumping rig to the VMF unit. When the yeast concentration was increased to 500 gL⁻¹ however, a drop in permeate flux was observed after 1.3 bar suggesting that the cTMP is around this value.

![Figure 3.11](image)

**Figure 3.11** – Variation of steady state permeate flux with increasing ΔPₜm during the microfiltration of *S. cerevisae* suspensions (■) 300gL⁻¹, (●) 400gL⁻¹ and (▲) 500gL⁻¹ in total recycle mode. Experimental conditions: Membrane head amplitude 19.5 mm and 1.4 mm membrane gap width.

Comparing the data presented in Figure 3.10 to that in Figure 3.11 shows the importance of BSA on permeate flux. At a yeast concentration of 300 gL⁻¹, Figure 3.11 shows a flux of around 87 Lm⁻²hr⁻¹ is achieved. When the experiment is repeated with BSA present and at the same ΔPₜm, as shown in Figure 3.10, permeate flux is observed to fall to around 75 Lm⁻²hr⁻¹, a fall of around 14 %. At the higher yeast concentration of
500 gL\(^{-1}\) however, the introduction of BSA causes a fall in permeate flux from around 65 Lm\(^2\)hr\(^{-1}\) to around 37 Lm\(^2\)hr\(^{-1}\), a fall of around 43%. These results would suggest that BSA has more of a detrimental effect on permeate flux at higher yeast concentrations. This would perhaps back up the theory of increased BSA - yeast interactions at high solids concentrations alluded to in Section 3.6.

Figure 3.12 shows the influence of \(\Delta P_{\text{TM}}\) on protein transmission. The figure shows no apparent trend in transmission with increasing \(\Delta P_{\text{TM}}\) at the pressures investigated although this may due to the fact that all the pressure investigated were under the cTMP values for the \textit{S. cerevisae} concentrations used as illustrated in Figure 3.11.

![Graph](image)

**Figure 3.12** – Variation of steady state BSA transmission with increasing \(\Delta P_{\text{TM}}\) during the microfiltration of \textit{S. cerevisae} suspensions in total recycle mode. Yeast concentration (■) 300 gL\(^{-1}\), (●) 400 gL\(^{-1}\) and (▲) 500 gL\(^{-1}\) and BSA concentration 0.75 gL\(^{-1}\). Experimental conditions: Membrane head amplitude 19.5 mm, crossflow rate 1 Lmin\(^{-1}\) and membrane gap width 1.4 mm.
3.8 Influence of between membrane gap width on VMF performance

During the assembly of the VMF membrane head stack, a spacer – gasket combination is inserted between each membrane disc giving a gap of 1.4 mm between each membrane disc in the head stack. The work described in this section was designed to test the influence of this membrane gap width on VMF performance in terms of permeate flux and product transmission. A 500 gL⁻¹ suspension of *S. cerevisae* was processed in concentration mode at membrane gap widths of 1.4 mm and 4.2 mm. The broth was processed in concentration mode so the maximum solids handling ability of the VMF system could be assessed. The results are shown in Figure 3.13 as a function of permeate flux against volumetric concentration factor (VCF). VCF is defined here as the biomass concentration of the feed compared to that of the retentate.

![Figure 3.13 - Permeate flux as a function of volumetric concentration factor during the processing of 500 gL⁻¹ *S. cerevisae* (initial concentration) in concentration mode. Permeate flux (■ and ●) 1.4mm gap width (▲ and ▼) 4.2mm gap width. Experimental conditions: Membrane head amplitude 19.5mm, ΔP<sub>TM</sub> 0.7 bar and crossflow rate in the range of 0.3 to 2 Lmin⁻¹.](image-url)
The figure clearly demonstrates that increasing the gap width between the membrane discs results in an improvement in permeate flux. The mechanism behind these improvements is examined in more detail and a model to predict the maximum solids handling capacity of the PS10 is described in Chapter 5.

Experiments designed to assess the impact of membrane spacing on BSA transmission during the processing of 500 gL\(^{-1}\) \textit{S. cerevisae} with 0.75 gL\(^{-1}\) BSA in total recycle mode were also performed (head amplitude 19.5 mm and \(\Delta P_{TM}\) maintained at 0.7 bar throughout). The results showed a very slight improvement in transmission with increasing gap width from 66 \(\pm\) 3.3 % w/w at a gap width of 1.4 mm to 68 \(\pm\) 3.4 % w/w at 2.8 mm and 71 \(\pm\) 3.6 % w/w at 4.2 mm.

### 3.9 Influence of crossflow rate on VMF performance

As stated in Section 1.7.2, conventional TFF systems rely on process fluid crossflow rate to create the shear forces necessary to minimise membrane fouling. Due to the high level of shear generated by the mechanical vibration of VMF systems, the need for elevated crossflow rates is theoretically diminished.

Figure 3.14 shows how permeate flux and BSA transmission vary with crossflow rate during the processing of \textit{S. cerevisae} suspensions at 300 and 500 gL\(^{-1}\). At both of the yeast concentrations used here, permeate flux and protein transmission values can be seen to remain constant as crossflow rate is increased from <1.0 Lmin\(^{-1}\) up to 5.5 Lmin\(^{-1}\). This clearly demonstrates that in the VMF system used, permeate flux and BSA transmission are independent of crossflow rate within the ranges tested.

Operation at crossflow rates in excess of 5 Lmin\(^{-1}\) has been observed to bring about larger pressure drops along the membrane between the retentate inlet and outlet. At a crossflow rate of 1 Lmin\(^{-1}\), a pressure drop of 0.2 bar was observed during the processing of \textit{S. cerevisae} at 500 gL\(^{-1}\) in total recycle mode compared with a drop of 0.7 bar during the processing of the same feed at a crossflow rate of 5 Lmin\(^{-1}\).
3. Flux and transmission characteristics of the PallSep PS10 Postlethwaite 2003

Figure 3.14 – Influence of crossflow rate during the processing of *S. cerevisae* in total recycle mode. Steady state (■) permeate flux and (▲) BSA transmission at 300 gL\(^{-1}\) and (●) permeate flux and (▼) BSA transmission at 500 gL\(^{-1}\). Experimental conditions: Membrane head amplitude 19.5 mm, △*P*\(_{TM}\) at 0.65 bar and membrane gap width 1.4 mm.

3.10 Discussion and summary

In this chapter a number of key operational variables have been identified and their influence on VMF performance assessed in order to give a better understanding of how the PallSep PS10 system works. The main findings are summarised and discussed here.

Initial studies looking at the microfiltration characteristics of *Saccharomyces cerevisae* and BSA suspensions suggest a correlation between solids load and protein transmission. During the processing of yeast and BSA individually, permeate flux and
protein transmission are observed to remain constant for the duration suggesting that steady state is quickly established. This is perhaps unsurprising as at ~66 KD, BSA molecules are known to be considerably smaller than the pores of the membrane used (0.45 μm). The importance of vibrational shear in maintaining permeate flux levels during the processing of *S. cerevisiae* has also been shown (Figure 3.8). When these two components are combined however, both permeate flux and protein transmission are observed to decline steadily for the duration of the experiment. These results would suggest that yeast and BSA somehow interact in each other's presence to have a detrimental effect on both flux and transmission. Figure 3.10 shows how protein transmission falls with increasing yeast concentration, suggesting an increasing level of interaction. The aggregation of BSA molecules is well documented (Maruyama et al. 2001; Huisman et al. 2000). If this aggregation was somehow increased in the presence of yeast cells, the formation of large BSA complexes would perhaps be enough to cause the fouling necessary to bring about the observed fall in permeate flux and protein transmission. Whilst this only a theory, it does seem to provide a logical explanation for the results observed.

Membrane head frequency and amplitude of vibration have been shown to be directly related to the shear rates observed at the membrane surface. A number of different methods for calculating the shear rate in VMF systems have been reported (Al Akoum et al. 2002; Hurwitz. 2001). Comparison of these calculated shear rates with those calculated at the membrane surface of a conventional TFF system (Davies et al., 2002), shows that the mechanical vibration of VMF systems brings about higher shear rates. The shear generated in the VMF system due to liquid crossflow velocity (Section 3.4.1) has also been shown to be negligible compared to the shear generated due to vibration. The importance of this vibration induced shear in maintaining permeate flux and product transmission levels has subsequently been demonstrated (Figure 3.9). Permeate flux has also been correlated to shear rate and is described by Equation [3.1].

Experimental data has shown that the PallSep PS10 is able to maintain a permeate flux of 80 Lm⁻²hr⁻¹ (Figure 3.11) and a protein transmission level of ~80 % w/w (Figure 3.12) during the processing of *S. cerevisiae* (500 gL⁻¹) at a ΔP_TM of 1.0 bar. It should be noted however that it is not always necessary to run a process at such a high ΔP_TM. Leach (2003) has reported that in some industrial applications, microfiltration processes are run
at as low a ΔP_{TM} as 0.2 bar to achieve in excess of 90% w/w protein transmission even if this means operating at a lower permeate flux. The solids loads considered in this work are considerably higher than those in the literature for conventional TFF systems. Okec (1998) reported permeate fluxes in the region of 20 – 30 Lm⁻²hr⁻¹ during the processing of S. cerevisiae at 280 gL⁻¹ using a static TFF system, values similar to those demonstrated using the VMF system at over double the solids concentration. Given that the deposition of solids on the membrane is increased during the processing of broths with a higher solids concentration (Porter, 1972), permeate flux would be expected to be considerably lower than that at the lower solids concentration. It is safe to conclude that the elevated levels of shear at the membrane surface of the VMF system allow the processing of broths at much higher solids loads than conventional static TFF systems.

ΔP_{TM} has been shown to exert a similar influence on VMF performance as on conventional static TFF systems, in that as the solids load of the process stream increases the cTMP value falls for the range of yeast concentrations tested (Figure 3.11). For identical feedstreams, the cTMP is likely to be considerably higher than that of a conventional TFF system. In static TFF systems, at the cTMP, the downward pressure forcing solids onto the membrane surface becomes greater than the uplifting forces generated by the crossflow velocity. At this point any further increase in pressure will only result in the further compaction of solids onto the membrane surface. In vibrating TFF systems the same principle applies. However, as the uplifting forces generated by the mechanical vibration are higher than those generated by static TFF systems, the cTMP value will be higher. In theory this will allow the VMF systems to be operated at higher solids loads and ΔP_{TM} than conventional TFF systems. In this study it has been observed that at the pressures and yeast concentrations studied, ΔP_{TM} has little influence on protein transmission although values are considerably lower than the 100% w/w that would be anticipated considering the relative size differences between the protein molecules and the pore size of the membrane. Hernandez-Pinzon and Bautista (1992) however, have described how, even in MF processes where the pore size is much greater than that of the protein, the transmission of the molecule is less than 100% w/w due to the charge and hydrophobicity of the molecule as well as the formation of aggregates. The reason for the lack of an effect of ΔP_{TM} on protein transmission is unclear.
Experiments have shown how an improvement in VMF performance can be bought about by increasing the gap width between the membrane elements in the PallSep PS10 head (Figure 3.13). The mechanism behind this improvement will be examined in more detail in Chapter 5.

Crossflow rate, an important factor in maintaining acceptable permeate flux and product transmission levels in TFF systems, has been shown to have no influence on the performance of the system over crossflow rates between 0.2 to 5.5 Lmin\(^{-1}\) (Figure 3.14). The influence of crossflow rate on the performance of static TFF systems is well documented with both Fradin and Field (1999) and Porter (1972) reporting an increase in permeate flux with increased crossflow rate. Reismeier \textit{et al} (1989) and Gyure (1992) have also described how permeate flux and transmission decline can be minimised by increasing crossflow rate. The elevated shear rates generated by the mechanical vibration in VMF systems, negate the demands for high crossflow rates to keep the membrane surface clear of foulants. This has important implications for processing costs as with no need for high crossflow rates, both the equipment cost (in terms of large system pumps, pipe-work, valves and inline instrumentation) and the power costs of the process are greatly reduced. Nagata \textit{et al}. (1989) showed that by increasing crossflow rate an increase in both trans-cartridge pressure and \(\Delta P_{TM}\) resulted causing a compaction of the fouling layer and a reduction in permeate flux during the processing of \textit{Bacillus polymyxa} broths. These pressure changes have also been observed during the operation of the PallSep at high crossflow rates (> 5 Lmin\(^{-1}\)). Whilst no data is available on the effect of these pressure changes on fouling levels across the membrane, it can be assumed that with \(\Delta P_{TM}\) gradients being established on the upstream side of the membrane, increased levels of membrane fouling will occur, with subsequent consequences for permeate flux (Figure 3.11) and product transmission (Figure 3.12) levels. In order to minimise these effects, operation of the VMF system at low crossflow rates (< 3 Lmin\(^{-1}\)) would be recommended.

The results in this chapter clearly demonstrate that VMF technology can provide a suitable alternative to conventional static TFF systems. The mechanical vibration generated by PallSep allows the shear forces necessary to prevent membrane fouling to be de-coupled from process stream crossflow rate. This allows permeate fluxes and product transmission levels to be maintained at acceptable levels whilst minimising
costs due to low energy requirements with lower system hold up volumes. These elevated shear rates will of course have implications for the process stream in terms of shear damage as discussed in Section 1.11. During the processing of *S. cerevisiae* however, the mean particle diameters ($d_{50}$) determined at the end of the experiments was $\sim 5 \, \mu m \pm 3 \, \mu m$, the same as at the start of the experiments (Figure 3.2). This unaltered size distribution indicates that no mechanical damage was caused to the yeast cells by the high, but intermittent, shear rates generated by the VMF system. The level of shear damage to the cells will depend on a number of things, only one of which is the susceptibility of the cells themselves to shear damage (Section 1.11). Another factor is how much of the process fluid is subjected to shearing conditions in the flow channel of the membrane head. This in turn is dependant on the shear boundary layer profile. This is described fully in Section 5.2.1.

In the next chapter, the interactions of a selection of the operational variables described in this chapter will be examined using a series of factorial design experiments. These will enable the synergistic effects to be identified and also provide a model by which either permeate flux or protein transmission can be optimised.
4. Interaction of operating variables and process optimisation using response surface methodology

4.0 Introduction and aims

In Chapter 3, a series of operational variables were identified and their effect on the performance of the PallSep PS10 investigated using permeate flux and protein transmission as indicators. In this chapter, the interactions between some of these variables and their statistical significance, during the processing of S. cerevisae and BSA suspensions, are investigated using response surface methodology (RSM). Response surface methodology quantifies relationships among one or more measured responses and a number of input factors. It provides a practical means by which multivariate experiments may be designed and the results analysed statistically in order to assess the impact of a set of independent factors, whose values may be altered in a defined manner (Myers and Montgomery. 1995; Mount et al. 2003). To examine the interactions of the operational variables described in Chapter 3, a series of Box–Behnken factorial experiments were used. Box-Behnken designs have been used extensively in optimisation studies in fields ranging from industrial chemicals (Fisher. 1949) to bioprocess design (Kalil et al. 2000) and are designed to identify important effects and interactions responsible for producing a particular result whilst significantly reducing the experimental load of the investigator. They are described in more detail in Appendix II.

Also in this chapter, a model is proposed that predicts the experimental conditions necessary to maximise permeate flux and protein transmission either independently or in parallel. The model's accuracy is then tested experimentally.

4.1 Choice of operational variables

In this study, the interactions of a number of operational variables were investigated using permeate flux and protein transmission as measured responses. In Section 3.3, a series of operational variables were identified and the rationale behind their selection discussed. For the purpose of this study, only three of these variables were chosen, these being membrane head amplitude, solids concentration and \( \Delta P_{TM} \). Crossflow rate and membrane spacing were excluded for the reasons outlined below.
Crossflow rate - Results presented in Section 3.9 clearly show that crossflow rate has no influence on permeate flux or BSA transmission during the processing of \textit{S. cerevisiae} suspensions.

Membrane spacing – In order to minimise the number of experiments, a decision was taken to look at the operation of the PallSep PS10 in a normal configuration, namely with a single membrane spacing of 1.4 mm.

### 4.1.1 Choice of operational variable values

The Design Expert 5 software (Section 2.7) used in this study offers Box-Behnken designs for three to seven factors, in this case three were used; head amplitude, solids load and $\Delta P_{\text{TM}}$. Experimental designs require each operational variable to be coded at three levels, designated -1, 0 and +1. The 0 values were chosen as described below and then -1 and +1 values assigned accordingly.

- **Membrane head amplitude** – 19.5 mm, or \( \frac{3}{4} \) inch, is the operational head amplitude recommended by the manufacturer.

- **Solids loading** – 400 gL\(^{-1}\) \textit{S. cerevisiae} concentration is considerably higher than existing literature values shown in Table 3.2

- **$\Delta P_{\text{TM}}$** – Preliminary experiments to determine the cTMP at yeast concentrations of 300, 400 and 500 gL\(^{-1}\) were performed, the results of which are shown in Figure 3.11. As a result of this, a value of 1.0 bar was selected as this was below the cTMP for all yeast concentrations used in this study.

For the three experimental variables used in this study, the software created 17 experiments, the details of which are shown in Table 4.1. The data obtained from experiment No 5 in Table 4.1 is shown in Figure 4.1. The plot shows that permeate flux and BSA transmission reached a steady state after approximately 75 min. The experiment was run for a further 30 min to confirm that steady state had indeed been reached.
### Table 4.1 – The experimental design created by Design-Expert 5 software. Zero values selected based on experimental results from Chapter 3. -1 and +1 values selected by the software as described in Section 2.7. Experiments were carried out in total recycle mode to steady state using *S. cerevisae* suspensions prepared as described in Section 2.2.

#### 4.2 Experimental methods

*S. cerevisae* feed streams were prepared as per Section 2.2. Microfiltration experiments were performed in total recycle mode as described in Section 2.6.1. All analytical techniques were carried out as outlined in Section 2.5. Experimental design and analysis of experiments was carried out using Design Expert 5 software as described in Section 2.7. All experiments were run until a steady state for both permeate flux and BSA transmission had been reached as shown in Figure 4.1.
4.4 Interactions of operational variables

After completion of all the experiments outlined in Table 4.1, the software created quadratic models to described the behaviour of the variables and their affect on the measured responses. Using these models, a series of response surfaces and perturbation curves were created for each measured response.

**Figure 4.1** – Data from experimental run No. 5 as detailed in Table 4.1. (■) Permeate flux and (▲) BSA transmission. Experiment run in total recycle mode to steady state. Experimental conditions: Membrane head amplitude 19.5 mm, ΔP_{TM} at 0.7 bar, S. cerevisae concentration 500 gL⁻¹, crossflow rate 1 Lmin⁻¹ and membrane gap width 1.4 mm. Experiment designed by Design Expert 5 software as described in Section 2.7.
4. Interactions of operational variables

4.3.1 Permeate flux

The first of the measured responses was permeate flux. The model generated by the software to described the behaviour of the variables and their affect on permeate flux is shown as Equation [4.1].

\[
\text{Flux} = -65.71 + 331.94 \times \Delta P_{TM} - 3.43 \times \text{Head amplitude} + 0.18 \times \text{Solids} - 153.06 \times \Delta P_{TM}^2 - 0.12 \times \text{Head amplitude}^2 - 4.275 \times 10^{-4} \times \text{Solids}^2 + 6.03 \times \Delta P_{TM} \times \text{Head amplitude} - 0.22 \times \Delta P_{TM} \times \text{Solids} + 8.077 \times 10^{-3} \times \text{Head amplitude} \times \text{Solids}
\]  

[4.1]

Analysis of variance (ANOVA) showed that the model was highly significant for the response of permeate flux (F value 41.10, Prob >F <0.0001). Table 4.2 shows the regression coefficients and |t| values for the design factors and the quadratic model shown in Equation [4.1] used to predict permeate flux.

| Factor          | Coefficient estimate | Prob >|t|   |
|-----------------|----------------------|-------|
| Intercept       | 93.8                 |       |
| A - \Delta P_{TM} | 16                   | <0.0001|
| B - Head amplitude | 6.25                 | 0.0076 |
| C - Solids load | - 22.75              | <0.0001|
|              | A^2                  | 0.0006 |
|              | B^2                  | 0.0576 |
|              | C^2                  | 0.1085 |
|              | AB                   | 0.0017 |
|              | AC                   | 0.0254 |
|              | BC                   | 0.0636 |

Table 4.2 - Regression coefficients and |t| values for the design factors for the response of permeate flux. Model generated by Design Expert 5 software using experimental data presented in Table 4.1.
Figure 4.2 shows experimental flux plotted against the flux predicted using the model in Equation [4.1]. The figure shows good agreement between the experimental data and the predicted results \( R^2 = 0.98 \).

Figure 4.2 – Experimental permeate flux data against predicted permeate flux data. Experiments designed by Design Expert 5 software as outlined in Table 4.1 and run to steady state as described in Section 4.2. Predicted data calculated using the engineering model described by Equation [4.1].

Figure 4.3 shows response surfaces demonstrating the influence of the three operational variables on permeate flux. In response surfaces, the influence of only two variables can be assessed at a time. In all of the response surfaces presented in this section, the affect of varying two factors on the measured response is displayed whilst keeping the third variable constant at its 0 value as given in Table 4.1.
Figure 4.3 – Influence of operational variables on steady state permeate flux. (a) shows the influence of head amplitude and $\Delta P_{TM}$ at 400 gL$^{-1}$ yeast. (b) shows the influence of solids loading and $\Delta P_{TM}$ at membrane head amplitude of 19.5 mm. (c) shows the influence of head amplitude and solids loading at $\Delta P_{TM}$ of 1.0 bar. Membrane spacing 1.4 mm and BSA 0.75 gL$^{-1}$ throughout. Data to construct response surfaces taken from Table 4.1.
In Figure 4.3, response surface A shows two effects of $\Delta P_{TM}$ at high and low head amplitudes. At an amplitude of 13.0 mm, permeate flux is seen to rise steadily until a $\Delta P_{TM}$ of approximately 1.0 bar. After this, flux is seen to level off and finally start to decrease with further increases in $\Delta P_{TM}$. At the higher amplitude of 26 mm however, permeate flux can be seen to increase with $\Delta P_{TM}$ before levelling off. The figure also shows a general increase in permeate flux with head amplitude especially at higher $\Delta P_{TM}$ values.

Response surface B shows the same rise in flux with $\Delta P_{TM}$, again following two different trends. At the lower solids load of 300 gL$^{-1}$, permeate flux is observed to increase with $\Delta P_{TM}$ before levelling off. At the higher solids load of 500 gL$^{-1}$, flux is observed to increase to a maximum at between 1.0 and 1.1 bar before decreasing to 63 Lm$^{-2}$hr$^{-1}$ at 1.3 bar. The figure also shows a fall in permeate flux with increasing solids load.

Response surface C shows that head amplitude has a mixed effect on permeate flux. At the lower solids load of 300 gL$^{-1}$, head amplitude appears to have no real effect on permeate flux. As the solids load is increased to 500 gL$^{-1}$ however, an increase in head amplitude from 13 mm to 26 mm brings about an improvement in flux. The figure also shows the same effect of solids load as observed in Figure 4.3 B.

In all three response surfaces, the curvature of the contours indicates significant interactions between the variables. The narrowing of the gap between the contours indicates a region where sharp changes in the measured responses would be expected following only small changes to the operational variables. The larger gaps between the contours shows areas where changes in the operational variables would be predicted to bring about very small changes in the measured response. Identification of this region of operation, where the process is considered to be most robust, is often considered to be a major aim of RSM (Mount et al. 2003). In Figure 4.3, it can be observed that the preferred area of operation, the region in which permeate flux is expected to be at its most stable, is the area where flux is maximised, i.e. when solids are at their $-1$ value and $\Delta P_{TM}$ and head amplitude are at their $+1$ value.
4.3.2 BSA transmission

The second of the measured responses was protein (BSA) transmission. The model generated by the software to described the behaviour of the variables and their affect on protein transmission is shown as Equation [4.2].

\[
\text{Transmission} = 75.15 - 56.39 \times \Delta P_{TM} + 0.50 \times \text{Head amplitude} + \\
0.22 \times \text{Solids} - 1.39 \times \Delta P_{TM}^2 + 8.876 \times 10^{-3} \times \text{Head amplitude}^2 - \\
2.625 \times 10^{-4} \times \text{Solids}^2 + 1.15 \times \Delta P_{TM} \times \text{Head amplitude} + 0.058 \times \\
\Delta P_{TM} \times \text{Solids} - 5.769 \times 10^{-3} \times \text{Head amplitude} \times \text{Solids} 
\]  

[4.2]

Analysis of variance (ANOVA) showed that the model was not highly significant for the response of BSA transmission (F value 0.83, Prob >F 0.6104). The unpredictable nature of protein aggregation (Huisman et al. 2000; Maruyama et al. 2001) as well as the probable interactions between the protein molecules and the membrane may have contributed to the poor fit of the model to the experimental data. Table 4.3 shows the regression coefficients and [t] values for the design factors and the quadratic model shown in Equation [4.2] used to predict BSA transmission.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient estimate</th>
<th>Prob &gt;[t]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>A - \Delta P_{TM}</td>
<td>-4</td>
<td>0.1758</td>
</tr>
<tr>
<td>B - Head amplitude</td>
<td>-2</td>
<td>0.476</td>
</tr>
<tr>
<td>C - Solids load</td>
<td>-4.25</td>
<td>0.1536</td>
</tr>
<tr>
<td>A^2</td>
<td>-0.125</td>
<td>0.9737</td>
</tr>
<tr>
<td>B^2</td>
<td>0.375</td>
<td>0.9213</td>
</tr>
<tr>
<td>C^2</td>
<td>-2.625</td>
<td>0.4966</td>
</tr>
<tr>
<td>AB</td>
<td>2.25</td>
<td>0.568</td>
</tr>
<tr>
<td>AC</td>
<td>1.75</td>
<td>0.6554</td>
</tr>
<tr>
<td>BC</td>
<td>-3.75</td>
<td>0.3513</td>
</tr>
</tbody>
</table>

Table 4.3 - Regression coefficients and [t] values for the design factors for the response of BSA transmission. Model generated by Design Expert 5 software using experimental data presented in Table 4.1.
Figure 4.4 shows the experimental BSA transmission against the transmission predicted using the model in Equation [4.2]. The figure does not show good agreement between the experimental data and the predicted results ($R^2 = 0.52$).

![Graph showing experimental BSA transmission against predicted BSA transmission.](image)

**Figure 4.4** – Experimental BSA transmission data against predicted BSA transmission data. Experiments designed by Design Expert 5 software as outlined in Table 4.1 and run to steady state as described in Section 4.2. Predicted data calculated using the engineering model described by Equation [4.2].

Figure 4.5 shows response surfaces demonstrating the influence of the three operational variables tested on protein transmission. As before, in the response surfaces the influence of only two variables can be assessed at a time. In all of the response surfaces presented in this section, the affect of varying two factors on the measured response is displayed whilst keeping the third variable constant at its 0 value as given in Table 4.1.
Figure 4.5 – Influence of operational variables on steady state BSA transmission (w/w). (a) shows the influence of head amplitude and $\Delta P_{TM}$ at 400 gL$^{-1}$ yeast. (b) shows the influence of solids loading and $\Delta P_{TM}$ at membrane head amplitude of 19.5 mm. (c) shows the influence of head amplitude and solids loading at $\Delta P_{TM}$ of 1.0 bar. Membrane spacing 1.4 mm and BSA 0.75 gL$^{-1}$ throughout. Data to construct response surfaces taken from Table 4.1.
In Figure 4.5, response surface A shows two different affects of $\Delta P_{TM}$ on transmission. At the lower head amplitude of 13 mm, increases in $\Delta P_{TM}$ are observed to bring about a fall in transmission. At the higher head amplitude of 26 mm, transmission is still observed to decline with increases in $\Delta P_{TM}$ although at the higher head amplitude the rate of transmission decline with increasing $\Delta P_{TM}$ is lower. The figure also shows that at the lower $\Delta P_{TM}$ of 0.7 bar, increases in amplitude from 13 mm to 26 mm brings about a fall in transmission. At the higher $\Delta P_{TM}$ of 1.3 bar, however, increases in head amplitude have very little affect on transmission levels.

Response surface B again shows a fall in protein transmission with increasing $\Delta P_{TM}$ at high medium and low solids levels. The figure also shows a fall in transmission with increasing solids at both high and low $\Delta P_{TM}$.

Response surface C shows two different affects of head amplitude on protein transmission. At the lower solids load of 300 gL$^{-1}$, an increase in head amplitude from 13 mm to 26 mm brings about a rise in transmission. At the higher solids load of 500 gL$^{-1}$, an increase in head amplitude from 13 mm to 26 mm brings about a fall in transmission. The figure also shows two different effects of solids load on protein transmission. At the higher head amplitude of 26 mm, a rise in solids load from 300 – 500 gL$^{-1}$, brings about a fall in transmission. At the lower head amplitude of 13 mm however, a rise in solids load from 300 – 500 gL$^{-1}$ brings about an initial rise in transmission from around 78% w/w up to around 81% w/w at a solids load of around 400 gL$^{-1}$ followed by a gradual decline in transmission back to around 78% w/w at 500 gL$^{-1}$.

As in Figure 4.3, the curvature of all three response surfaces in Figure 4.5 indicate significant interactions between the variables. Unlike Figure 4.2 however, Figure 4.5 shows the preferred area of operation to be unclear with no one set of operational variables appearing to give the most stable transmission levels.

4.3.3 Analysis of interaction of operational variables

Perturbation plots can be used to show the impact of each factor by plotting changes in only one factor over its experimental range (coded -1 to +1) whilst holding both the other factors constant at the reference point (coded 0). In this section perturbation plots
summarising the response surfaces shown as Figures 4.3 and 4.5 are presented and their implications discussed.

In the series of response surfaces shown as Figure 4.3, the influence of the three operational variables on permeate flux is observed. The perturbation plot shown as Figure 4.6 is just one of a series of plots that can be drawn from the data presented in Figure 4.3. This particular plot was chosen as it shows the effect of varying all three variables from their 0 point as defined by Table 4.1.

![Perturbation Plot](image)

**Figure 4.6** – Perturbation plot showing the influence of the individual operational variables on permeate flux whilst holding the others at their 0 value. (A) $\Delta P_{TM}$ (bar), (B) membrane head amplitude (mm), (C) solids load (gL$^{-1}$). The reference point refers to the zero value displayed in Table 4.1. Figure constructed from response surfaces displayed as Figure 4.3.
Line A of the perturbation plot summarises the effect of $\Delta P_{\text{TM}}$ on permeate flux. The line in Figure 4.6 shows an increase in flux with increasing $\Delta P_{\text{TM}}$ up to about 1.1 bar when a levelling off is observed followed by a drop at 1.3 bar. This line clearly illustrates the concept of cTMP described in Section 3.7. The results show that at a $S. \text{cerevisae}$ concentration of 400 gL$^{-1}$, head amplitude of 19.5 mm and membrane spacing of 2.8 mm, the cTMP for the system is in the region of 1.0 to 1.1 bar. Figure 3.11 shows the cTMP value to be well above this however, perhaps due to the presence of BSA in the suspension used in Figure 3.11.

Line B in Figure 4.6 shows the influence of head amplitude on permeate flux. The figure shows that a rise in amplitude from the -1 value to the 0 value (13 mm to 19.5 mm) causes a rise in permeate flux. An increase in amplitude from 0 to +1 (19.5 mm to 26 mm) however, causes a much smaller change in permeate flux. From Table 3.1 it can be seen that these increases in head amplitude correspond to a rise in $\dot{f}_{\text{w}}$ from $1.4 \times 10^4$ s$^{-1}$ at 13 mm to $2.1 \times 10^4$ s$^{-1}$ at 19.5 mm and $2.8 \times 10^4$ s$^{-1}$ at 26 mm. Line B in Figure 4.2 would suggest that permeate flux can be described by two different functions of shear rate as stated by Al Akoum et al (2002) and described in Section 3.4. Data presented in Figure 3.9 shows how when using the PS10 to process 400 gL$^{-1}$ $S. \text{cerevisae}$ in total recycle mode, permeate flux is represented by only one function of shear rate.

Line C in Figure 4.6 shows the influence of solids load on permeate flux. The line shows how increases in $S. \text{cerevisae}$ concentration bring about large decreases in permeate flux. Increases in solids load and hence viscosity would be expected to have an effect on permeate flux as described in Section 3.6.

Figure 4.6 shows that in the case of permeate flux, solids load is the most significant factor closely followed by $\Delta P_{\text{TM}}$ up to the cTMP value, above which the significance of the factor is greatly reduced. The plot also shows that whilst membrane head amplitude has a significant influence on permeate flux, its effect is less than the other two variables and becomes greatly diminished above the 0 value.

In the series of response surfaces shown as Figure 4.5, the influence of the three operational variables on protein transmission is observed. The perturbation plot shown as Figure 4.7 is just one of a series of plots that can be drawn from the data presented in
Figure 4.5. As for Figure 4.6, this particular plot was chosen as it shows the effect of varying all three variables from their 0 point as defined by Table 4.1.

![Perturbation](image)

**Figure 4.7** – Perturbation plot showing the influence of the individual operational variables on BSA transmission whilst holding the others at their 0 value. (A) $\Delta P_{TM}$ (bar), (B) membrane head amplitude (mm), (C) solids load (gL$^{-1}$). Reference point refers to the zero value displayed in Table 4.1. Figure constructed from response surfaces displayed as Figure 4.5.

Line A of the perturbation plot summarises the effect of $\Delta P_{TM}$ on protein transmission. The line clearly shows a fall in protein transmission observed with increasing $\Delta P_{TM}$. Line B also shows a decline in transmission with increasing head amplitude although considering the poor fit of Equation [4.2] to the data, the significance of this decline must be questioned. Line C shows no initial effect of solids load on transmission but then a
sharp decrease is observed as the solids load gets above 400 gL⁻¹. As described in Section 4.5.2, analysis of the model generated to predict protein transmission levels showed a poor fit of the model to the experimental data. The large amount of "noise" within the model will certainly have contributed to these results so their validity is therefore questionable.

Figure 4.5 shows that in the case of protein transmission, $\Delta P_{TM}$ is the most significant factor influencing performance. The plot also shows that the influence of solids load is minimal up to the 0 value after which it becomes the most significant factor.

4.4 Process optimisation using RSM

RSM is commonly used in the optimisation of bioprocess design (Mount et al. 2003). In fermentation and cell culture, RSM has been applied to establish the optimum growing conditions for the organism by looking at pH, aeration and agitation rate and media composition (Kalil et al. 2000; Li et al. 2002; Adinarayana et al. 2003; Chun et al. 2003). In filtration, RSM has been used to study the interactions of operating parameters (Sung and Parekh. 1996) process optimisation (Dharmappa et al. 1992; Benitez et al. 1994; Ruegger and Tauk Tornisielo. 1996). Further examples of the use of RSM for process optimisation are described elsewhere (Li et al. 2002).

In this study, the applicability of RSM as a mathematical tool to identify the optimum processing conditions of S. cerevisiae and BSA suspensions using the VMF system was assessed. The Design Expert 5 software was used to predict the processing conditions that would give the optimum VMF performance in terms of permeate flux and protein transmission independently as well as both parameters together.

4.4.1 Optimisation of permeate flux

During the processing of some biological feedstreams by microfiltration, it may be necessary to optimise the process in terms of permeate flux. During the processing of wastewater for example, optimising permeate flux would lead to a reduction in processing time (Tazi-Pain et al. 2002).

Using the data from the experiments described in Section 4.1 and Equation [4.1], the Design Expert 5 software was used to predict the experimental conditions needed to
give the optimum permeate flux during the processing of *S. cerevisae* and BSA suspensions over the range of operational variables investigated. Figure 4.8 illustrates these conditions as a ramp display. Ramp displays are used to give a visual representation of the range of the operational variables used and show the factor settings to give the optimum response required as predicted by the Design Expert 5 software. In this case, the factor settings can be observed as dots on the ramp display.

![Ramp Display](image)

**Figure 4.8** – Ramp display showing the factor settings and response predictions for optimisation of permeate flux as predicted by Design Expert 5 software. The figure shows a ΔP<sub>TM</sub> of 1.3 bar, head amplitude of 26 mm and solids load of 300 gL<sup>-1</sup> giving a predicted permeate flux of 129 Lm<sup>-2</sup>hr<sup>-1</sup>.

This predicted optimum was then verified over 3 repeated experiments and the results are shown in Figure 4.9. The figure shows a gradual decline in permeate flux to an apparent steady state of 132 ± 6 Lm<sup>-2</sup>hr<sup>-1</sup> after 90 minutes. This is in good agreement with the experimental model which predicts a permeate flux of 129 Lm<sup>-2</sup>hr<sup>-1</sup> under these experimental conditions.
4.4.2 Optimisation of protein transmission

In many industrial bioprocesses, choosing the correct unit operation for a primary separation step is critical. If a membrane separation process is employed, high product transmission across the membrane is vital (Leach. 2003; van Reis and Saksena. 1997). High product transmission is not always associated with high permeate flux however. Maximising permeate flux in order to reach a concentration suitable for a diafiltration step may lead to membrane fouling over time, causing the subsequent diafiltration step to take too long and the product transmission levels to be too low (Leach. 2003). For this

Figure 4.9 – Permeate flux data obtained during the processing of S. cerevisae and BSA in total recycle mode of operation. Experiment performed at the conditions predicted for the optimisation of permeate flux as given in Figure 4.8. Figure shows a steady state permeate flux of $132 \pm 6 \text{Lm}^{-2}\text{hr}^{-1}$. Experimental conditions: 300 gL$^{-1}$ yeast, $\Delta P _{\text{m}}$ 1.3 bar, membrane head amplitude 26 mm, 0.75 gL$^{-1}$ BSA and membrane spacing 1.4 mm.
reason, the Design Expert 5 software was used to predict the operating conditions for optimising protein transmission whilst ignoring permeate flux, during the processing of S. cerevisae and BSA suspensions. Figure 4.10 illustrates these conditions as a ramp display.

Figure 4.10 – Ramp display showing the factor settings and response predictions for the optimisation of protein transmission as predicted by the Design Expert 5 software. Figure shows a $\Delta P_{\text{M}}$ of 0.7 bar, membrane head amplitude of 13 mm and solids load of 357 gL$^{-1}$ giving a predicted protein transmission level of 87 % w/w.

This predicted optimum was then verified over three repeated experiments and the results are shown in Figure 4.11. The figure shows a steady decline in protein transmission over time to an apparent steady state of around 81 ± 3 % w/w after 60 minutes. This result does not show good agreement with the model prediction of 87 % w/w.
4. Interactions of operational variables

Figure 4.11 – Protein transmission data obtained during the processing of S. cerevisiae and BSA in total recycle mode of operation. Experiment performed at the conditions predicted for the optimisation of protein transmission as given in Figure 4.10. Figure shows a steady state protein transmission of around 81 ± 3 % w/w. Experimental conditions: 357 gL⁻¹ yeast, ΔP₀m 0.7 bar, membrane head amplitude 13 mm, 0.75 gL⁻¹ BSA and membrane spacing 1.4 mm.

4.4.3 Optimisation of permeate flux and protein transmission

Frequently during the processing of fermentations containing high levels of solids, optimising both permeate flux and product transmission is desirable as this will allow maximum product transmission whilst keeping the diafiltration step as short as possible (most of the product yield is recovered in the diafiltration step) (Leach. 2003). For this reason, the design Expert 5 software was used to predict the operating conditions for optimising both permeate flux and protein transmission during the processing of S.
cerevisae and BSA suspensions. Figure 4.12 illustrates these conditions as a ramp display.

**Figure 4.12** – Ramp display showing the factor settings and response predictions for optimisation of permeate flux and protein transmission as predicted by the Design Expert 5 software. Figure shows a $\Delta P_{TM}$ of 1.3 bar, membrane head amplitude of 26 mm and solids load of 300 gL$^{-1}$ giving a predicted permeate flux of 129 Lm$^{-2}$hr$^{-1}$ and a predicted protein transmission level of 87 % w/w.

These predicted optima were then verified over three repeated experiments and the results are shown in Figure 4.13. The figure shows a steady decline in both permeate flux and protein transmission over time to an apparent steady state of around 132 ± 6 Lm$^{-2}$hr$^{-1}$ and 84 ± 3 % w/w respectively. The results show good agreement with the predicted permeate flux value of 129 Lm$^{-2}$hr$^{-1}$ but not with the predicted transmission level of 78 % w/w.
4. Interactions of operational variables

Figure 4.13 – (■) Permeate flux and (●) protein transmission data obtained during the processing of *S. cerevisae* and BSA in total recycle mode of operation. Experiment performed at the conditions predicted for the optimisation of both permeate flux and protein transmission given by Figure 4.12. Figure shows a steady state permeate flux of $132 \pm 6 \text{ Lm}^{-2}\text{h}^{-1}$ and protein transmission of around $84 \pm 3 \% \text{ w/w}$. Experimental conditions: $300 \text{ gL}^{-1}$ yeast, $\Delta P_{\text{TM}} 1.3$ bar, membrane head amplitude 26 mm, $0.75 \text{ gL}^{-1}$ BSA and membrane spacing 1.4 mm.

4.5 Discussion and summary

In this chapter RSM is used to study the effects and interactions of a series of operational variables on VMF performance in terms of permeate flux and protein transmission. Engineering equations are proposed to describe the relationship between these variables and the two responses. Also in this chapter, a model is proposed that predicts the experimental conditions necessary to maximise permeate flux and protein...
transmission either independently or in parallel. The main findings and conclusions of the work are summarised here.

Figure 4.6 shows solids load to be the most important factor affecting permeate flux. The figure shows a sharp fall in observed flux levels with increasing solids load, as expected according to D'Arcy's filtration law described in Section 1.7.3. The figure also clearly demonstrates the concept of cTMP. As \( \Delta P_{\text{TM}} \) is increased, permeate flux can be seen rising up to about 0.8 bar after which no further increase is observed. Membrane head amplitude is observed to have two different effects. Permeate flux is observed to rise with increasing amplitude up to around 19.5 mm corresponding to an average shear rate of \( 2.1 \times 10^4 \text{ s}^{-1} \) according to Table 3.1. As amplitude is increased further, flux is observed to continue increasing, but at a slower rate however. These findings would suggest that permeate flux can be represented by two different functions of membrane head amplitude and hence shear rate. This backs up the findings of Al Akoum et al (2002) described in Section 3.5 but is in conflict with those described as part of this study also described in Section 3.5 which state that permeate flux can be described by only one function of shear rate.

Figure 4.7 shows that solids load has the most important effect on protein transmission levels. As the level of suspended solids is increased, protein transmission is observed to fall, slowly at first but at an increasing rate as the concentration is increased. This would suggest a degree of interaction between the solid yeast cells and the BSA molecules. This interaction is also observed in Section 3.3 and is described in more detail there. The figure also shows increases in \( \Delta P_{\text{TM}} \) to bring about a fall in protein transmission levels. This is in disagreement with data presented in Figure 3.12 which shows changes in \( \Delta P_{\text{TM}} \) to have no effect on protein transmission. As Figure 4.7 shows the total decrease in transmission to be only around 8 % w/w, this result should not be relied upon to be significant. Figure 4.7 also shows increasing membrane head amplitude to bring about a fall in transmission levels, contradicting the results observed in Figure 3.9. As for the influence of \( \Delta P_{\text{TM}} \) however, the significance of this result should be questioned.

Equations [4.1] and [4.2] show the models generated by the Design Expert 5 software to describe the behaviour of the operational variables and their affect on permeate flux and
protein transmission. Using these equations, predicted values for both responses where generated for all experiments listed in Table 4.1. Figure 4.2 shows a plot of predicted permeate flux results plotted against actual permeate flux results obtained experimentally. The figure shows the model to be very accurate ($R^2 = 0.98$). This backs up the ANOVA results for the equation stated in Section 4.3.1. Figure 4.4 shows a plot of predicted protein transmission results plotted against actual transmission results obtained experimentally. The figure shows the model described in Equation [4.2] to be not very accurate ($R^2 = 0.52$). This again backs up the ANOVA results described in Section 4.3.2. This is not entirely surprising however considering the accuracy of the two techniques used to measure permeate flux ($\pm 1.1 \%$) and protein transmission ($\pm 3.3 \%$) as described in Sections 2.6.1 and 2.5.3 respectively.

Process optimisation is an important step in the design of any bioprocess and is described in numerous studies as reported in Section 4.4. Results presented in Sections 4.4.1 and 4.4.2 have described how the Design Expert 5 software can be used to predict the operating conditions necessary to give the optimum performance in terms of permeate flux and protein transmission independently as well as both parameters together.

The optimisation ramp displays shown as Figures 4.8, 4.10 and 4.12 show the experimental conditions predicted to optimise permeate flux at $129 \text{ Lm}^2\text{hr}^{-1}$, protein transmission at $87 \% \text{ w/w}$ and permeate flux and protein transmission at $129 \text{ Lm}^2\text{hr}^{-1}$ and $78 \% \text{ w/w}$ respectively. Table 4.4 summarises the set point of each variable for the specific optimisation target. The table also shows the desirability value for each solution. The desirability is a numerical measure (from 0 to 1) of how well the data fits the optimisation criteria outlined in each of the ramp displays.

The table shows the operating conditions predicted to optimise permeate flux are high $\Delta P_{\text{TM}}$, high head amplitude and low solids load giving a desirability of 0.919. High $\Delta P_{\text{TM}}$ would be expected to provide the driving force to push the permeate through the membrane. The elevated level of membrane fouling bought about by this would be countered by the high head amplitude giving a high intermittent shear rate. The low solids levels would minimise the membrane fouling and reduce the process stream viscosity. The table also shows the operating conditions predicted to optimise protein...
transmission are low $\Delta P_{TM}$, low head amplitude and low solids giving a desirability of 0.871. The lack of fit of the model described by Equation [4.2] however casts doubt onto the accuracy of these predicted values however although Section 3.10 does state that in some industrial applications, microfiltration processes are run at as low a $\Delta P_{TM}$ as 0.2 bar to achieve in excess of 90 % w/w protein transmission even if this means operating at a lower permeate flux. This would support the use of low $\Delta P_{TM}$ to optimise protein transmission. Finally, the table shows the operating conditions predicted to optimise both permeate flux and protein transmission are high $\Delta P_{TM}$, high amplitude and low solids giving a desirability of 0.848. Again however, the lack of fit of the model described by Equation [4.2] casts doubt into the accuracy of these predicted values.

<table>
<thead>
<tr>
<th>Optimisation target</th>
<th>$\Delta P_{TM}$ (Bar)</th>
<th>Amplitude (mm)</th>
<th>Solids (gL⁻¹)</th>
<th>Desirability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeate flux</td>
<td>1.3</td>
<td>26</td>
<td>300</td>
<td>0.919</td>
</tr>
<tr>
<td>Protein transmission</td>
<td>0.7</td>
<td>13</td>
<td>356</td>
<td>0.871</td>
</tr>
<tr>
<td>Permeate flux &amp; protein transmission</td>
<td>1.3</td>
<td>26</td>
<td>300</td>
<td>0.848</td>
</tr>
</tbody>
</table>

Table 4.4 - Set points of each experimental variable for the specific optimisation target. The table also shows the desirability value for each solution. Desirability is a numerical measure (from 0 to 1) of how well the data fits the optimisation criteria outlined in each of the ramp displays shown as Figures 4.5, 4.10 and 4.15.

Experimental verification of these predicted optima has shown them to exhibit differing levels of accuracy. Figure 4.9 shows how permeate flux varies with time during the processing of *S. cerevisae* under the experimental conditions shown in Table 4.4. As anticipated, permeate flux is observed to decline steadily over time, reaching an apparent steady state of around $132 \pm 6$ Lm⁻²hr⁻¹ after ~90 minutes of processing in total recycle mode. This is in very good agreement with the predicted value of $129$ Lm⁻²hr⁻¹ as would be expected considering the accuracy of the model described in Equation [4.1]. Figure 4.11 shows how protein transmission varies with time during processing of *S.
cerevisae under the experimental conditions shown in Table 4.4. Again, transmission levels are observed to decline slowly, in agreement with the findings described in Section 3.3, to an apparent steady state of around 81 ± 3 % w/w after 70 minutes of processing in total recycle mode. This does not agree with the predicted optimum value of 87 % w/w although considering the lack of accuracy in the model described in Equation [4.2], this was to be expected. Finally, Figure 4.13 shows how both permeate flux and protein transmission vary with time during processing of S. cerevisae under the experimental conditions shown in Table 4.4. Both figures show the same steady decline in flux and transmission levels observed previously before steady state is reached at 132 ± 6 Lm²hr⁻¹ and 84 ± 3 % w/w. Both of these values are in good agreement with the predicted optima of 129 Lm²hr⁻¹ and 87 % w/w.

The results in this chapter clearly demonstrate that the Design Expert 5 software can be used to enhance our understanding of microfiltration operations, and hence lead to process improvements via optimisation thus helping to reduce overall costs. In the next chapter the influence of membrane gap width on the performance of a VMF system operated at high solids loading is examined.
5. Prediction of the maximum solids handling capacity of the PallSep PS10

5.0 Introduction and aims

In Chapter 3 a series of operational variables were identified and their influences on the performance of the PallSep PS10 were examined. One of the key variables identified was membrane gap width (Section 3.0). The head of the VMF unit is essentially a stack of flat, double sided, membrane discs separated by spacer elements located at the inner and outer radii of each disc. In order to increase membrane surface area, more membrane elements are added up to a maximum of 10 giving a total surface area of 1 m\(^2\) in the PallSep PS10. The assembly of the head in a standard configuration is shown in more detail in Figure 1.7. The manufacturers standard operating conditions recommend that during assembly of the head, one spacer and gasket combination be inserted between each membrane disc giving a spacing or gap between each membrane of 1.4 mm.

Results described previously in Section 3.8, showed how when processing \textit{S. cerevisae} at a starting concentration of 500 gL\(^{-1}\), increasing the gap width between the membranes from 1.4 mm (1 spacer) to 4.2 mm (3 spacers), brought about an improvement in microfiltration performance in terms of permeate flux and protein transmission.

The aim of this chapter is to examine and understand the influence of membrane gap width on the performance of a VMF system operated at high solids loading. The work seeks to establish the maximum solids handling capacity of the particular PallSep PS10 unit used here and how that is affected by varying the membrane spacing. A theory is also proposed that allows prediction of the maximum solids handling capacity of the system. The gap width theory and some of the results presented in this chapter have been published as Postlethwaite J, Lamping S, Hurwitz M, Leach G and Lye G. Flux and transmission characteristics of a vibrating microfiltration system operated at high biomass loading. Journal of Membrane Science (accepted for publication 2003).
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5.1 Experimental methods

Both model yeast suspensions (S. cerevisiae) and real fermentation broths (S. erythraea CA340, Aspergillus spp. and Bacillus spp.) were used in this work. S. cerevisiae feed streams and S. erythraea CA340 fermentations were prepared as described in Section 2.2 and Section 2.3 respectively. Aspergillus spp. and Bacillus spp. fermentation broths were kindly provided by Novozymes A/S, Bagsvaerd, Copenhagen, Denmark. Microfiltration experiments were performed in concentration mode as described in Section 2.6. All analytical techniques were carried out as outlined in Section 2.5.

5.2 Affect of membrane gap width on VMF performance

The PallSep membrane head is constructed such that there is normally a 1.4 mm gap in between each membrane (Figure 1.7). The oscillation of the membrane plates through a fluid creates separate liquid boundary layers on the surface of the upper and lower membranes. These boundary layers are not distinct layers with defined limits but are gradual decreases in the relative speed of the process fluid compared to the speed of movement of the membrane plates (the exact definition of the boundary layer thickness is described in Section 5.2.1. For the purposes of this work however and for ease of calculation, it is assumed that the boundary layers do have a defined limit thus establishing a central "core" region between the adjacent membrane elements. In order to predict the maximum solids handling capacity of the VMF unit, it is assumed that the lifting forces generated by the vibrational shear at the membrane surface exclude all solids from the membrane boundary layers and therefore the fluid in this region has physical properties similar to water. (Data presented in Section 3.4 showed that mechanical vibration is responsible for the high shear rates observed at the membrane surface and Figure 3.14 has shown the influence of crossflow rate to be negligible and can therefore be excluded from consideration). Figure 5.1 shows a schematic of the solids in the feedstream entering the membrane head. As the solids enter the core region between each pair of membrane plates, their effective concentration increases as they are repelled from the liquid boundary layers. As the feedstream leaves the membrane head, it becomes more dilute as the solids and the liquid from the boundary layers are mixed together again.

It is hypothesised that as the quantity of solids in the feedstream increases, a maximum concentration of solids in the central core region will eventually be reached. In the case
of approximately spherical yeast cells, this maximum concentration will correspond to a value somewhere between that of random close packed spheres (64 % solids) and closest packed spheres (74 % solids) (Torquato et al. 2000). As this limit is reached, the boundary layers will become less well defined as cells are forced into them. As the solids concentration increases further, more and more solids will enter the boundary layer region until the entire membrane channel is filled with solids. At this point no boundary layers will exist and there will be a dramatic decrease in permeate flux due to membrane fouling. This process is shown schematically in Fig 5.2.

Figure 5.1 - Schematic diagram of the feedstream entering the PallSep PS10 membrane head showing vibration induced liquid boundary layers and solids being retained in the proposed central core region.

Furthermore, if the width of the boundary layers is assumed to be constant then an increase in the membrane gap width should result in an increase in the volume of the core region between the membranes. Since the gap width can be varied by changing the number of spacer elements, a theoretical value for the maximum solids handling level of the VMF system can also be calculated as a function of membrane gap width.
Figure 5.2 - Schematic of feedstream entering PallSep PS10 membrane head. (a) At a medium solids concentration, solids concentration in the core region is increasing. Solids remain in the core region between the liquid boundary layers. (b) At a high solids concentration, solids concentration in the core region has exceeded the value of random close packed spheres (64 % solids) or closest packed spheres (74 % solids). Solids have leaked into the boundary layers causing a drop in permeate flux due to membrane fouling.
5.2.1 Estimation of fluid boundary layer thickness

When calculating the thickness of the liquid boundary layer on the upper and lower surfaces of each membrane disc, it is assumed that the edge of the boundary layer is at a distance from the surface of the membrane where the fluid velocity due to the membrane plate oscillation is small (the method used to calculate boundary layer thickness was introduced in Section 1.10.2). A velocity profile was therefore constructed using Equation [1.13] to identify the distance from the membrane plate at which the magnitude of the angular velocity of the fluid was zero.

Figure 5.3 shows the calculated velocity profile for vibration at an amplitude of 19.5 mm. The figure shows that as the distance from the plate is increased, a sharp reduction in the magnitude of the angular velocity from 10 % to 0 is observed. For this reason it was decided to define the thickness of the boundary layer as the distance from the membrane at which the magnitude of the angular velocity of the fluid was 10 % of the velocity of the plate. Using Equation [1.13], this gives a boundary layer thickness of 0.168 mm at an amplitude of 19.5 mm. A sample calculation is shown in Appendix 1.

As stated in Section 5.2, the oscillation of the membrane plates creates separate liquid boundary layers on the surface of the upper and lower membranes. This means that for an amplitude of 19.5 mm, within the 1.4 mm gap width between the membrane plates created by the spacer elements, 0.336 mm or 24 % is taken up by the fluid boundary layers. According to the model described in Section 5.2, this leaves a central core region of 1.064 mm, 76 % of the gap width between the membranes, which contains any solids present in the feed stream.
5. Prediction of the maximum solids handling capacity of the PallSep PS10 Postlethwaite 2003

Figure 5.3 – Estimated velocity profile showing the speed of movement of the fluid across the membrane as a fraction of the membrane plate oscillation as the distance from the membrane plate is increased. Velocities were calculated using Equation [1.13] and assuming a membrane head amplitude of 19.5 mm.

5.2.2 Influence of membrane head amplitude on boundary layer thickness
Throughout this work, the VMF system has been operated at different amplitudes, bought about by changes in the frequency of vibration. The influence of this change in amplitude on permeate flux and protein transmission have previously been discussed in Section 3.5 and have been shown to be related to notional shear rates. Figure 5.4 now shows how the fluid boundary layer thickness is influenced by changes in the frequency of vibration.
5. Prediction of the maximum solids handling capacity of the PallSep PS10

As discussed in Section 3.2, *S. cerevisae* cells are of an approximately uniform size and shape. As the concentration of the spherical yeast cells in the central core region increases due to the operation of the VMF system in concentration mode, the cells will become packed closer together. When the core region is full of cells, a certain proportion of the core will be filled with solid cells and a certain proportion will be filled with the liquid buffer solution the cells are suspended in. Depending on how the cells pack together, these proportions will vary. When spheres pack together randomly, the volume taken up by solids is 64 % (Torquato *et al*, 2000) and when they pack together as efficiently as possible, the volume taken up by solids is 74 % (Torquato *et al*, 2000).

Figure 5.4 – Estimated boundary layer thickness as a function of frequency of vibration of the PallSep PS10 membrane head. Boundary layer thickness calculated using Equation [1.13] and defined as the distance from the membrane at which the magnitude of the angular velocity of the fluid was 10 % of the velocity of the plate.
Assuming that the yeast cells will pack together randomly, this corresponds to a maximum concentration in the core region of 640 gL\(^{-1}\) of yeast cells. Alternatively, assuming that cells pack together as efficiently as possible, this will correspond to a maximum concentration in the core region of 740 gL\(^{-1}\). As explained in Section 5.2, the absence of cells in the liquid boundary layer will bring about a higher concentration of solids in the core region than in the feed. Table 5.1 shows the feed solids concentrations that will bring about random packed spheres and closest packed spheres in the core region. Now, the theoretical model described earlier in Section 5.2 states that as the concentration of cells in the core region exceeds that of random or close packed spheres, the solids will leak into the boundary layers as shown in Figure 5.2(b). The values shown in Table 5.1 therefore correspond to the likely range of maximum theoretical solids concentrations that can be processed by the PallSep PS10. Whilst the cells are not expected to be closest packed, the slight compressibility of yeast cells (Mashmoushy et al. 1998; Smith et al. 2000) will allow concentrations higher than the random packed spheres model predicts.

<table>
<thead>
<tr>
<th>Membrane gap width (mm)</th>
<th>Maximum Solids Loading (gL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Random packed spheres model</td>
</tr>
<tr>
<td>1.4</td>
<td>480</td>
</tr>
<tr>
<td>4.2</td>
<td>589</td>
</tr>
</tbody>
</table>

**Table 5.1** – Theoretical maximum solids concentration that can be processed by the PallSep PS10. Values calculated assuming an individual boundary layer thickness of 0.168 mm at a membrane head amplitude of 19.5 mm calculated using Equation [1.13] and defined as the distance from the membrane at which the magnitude of the angular velocity of the fluid was 10% of the velocity of the plate.

### 5.3 Experimentally determined maximum solids handling capacity

In order to test the theory described in Section 5.2, the maximum solids handling capacity of the VMF system was evaluated at two different gap widths. A solution of 300 gL\(^{-1}\) *S. cerevisae* was processed in concentration mode using a membrane head stack containing either 1 or 3 spacer elements between two membrane discs, corresponding
to gap widths of 1.4 mm and 4.2 mm respectively. Permeate flux was recorded as well as permeate volume over time. $\Delta P_{TM}$ and membrane head amplitude were kept constant throughout the experimental runs. Experiments were run up to a limiting concentration factor determined by the difficulty in controlling system pressure with the rapidly rising viscosity. The results are shown in Figure 5.5 as a function of membrane permeability against VCF. Membrane permeability is determined by dividing the measured flux by the appropriate $\Delta P_{TM}$ value at any given time point and thus has units of Lm$^{-2}$hr$^{-1}$bar$^{-1}$. It gives an indication of how permeate flux changes independently of $\Delta P_{TM}$ and is thus a useful indicator of membrane fouling. The results were shown to be reproducible over five repeat experiments.

![Figure 5.5](image)

**Figure 5.5** – Influence of membrane gap width during the processing of 300 gL$^{-1}$ *S. cerevisae* in concentration mode. Membrane gap width (▲) 1.4 mm (■) 4.2 mm. Experimental conditions: Membrane head amplitude 19.5 mm and $\Delta P_{TM}$ 0.65 bar. The arrows indicate the range of solids concentrations at which cells in the core region would be expected to be forced into the liquid boundary layers assuming both (→) random and (←) closest packed spheres models apply.
The results presented in Figure 5.5 show that during the concentration of *S. cerevisae* suspensions, increasing the membrane gap width brings about a significant improvement in the performance of the VMF system used, both in terms of membrane permeability (and hence permeate flux) and final VCF achieved. For a gap width of 1.4 mm, membrane permeability is seen to remain approximately constant at 90 Lm\(^2\)hr\(^{-1}\)bar\(^{-1}\) until at a VCF of 1.82 (± 0.04) at a solids concentration of 561 gL\(^{-1}\) when a rapid decline in permeability is observed until a maximum VCF of 2.37 (± 0.09) is achieved at a solids concentration of 711 gL\(^{-1}\). With a gap width of 4.2 mm however, an approximately 20 % higher membrane permeability is achieved and this is maintained for longer until a VCF of 2.11 (± 0.02) or 633 gL\(^{-1}\). As found with the smaller gap width, the membrane permeability then falls rapidly until a VCF of 2.58 (± 0.04) or 774 gL\(^{-1}\) is achieved. In both cases, as ΔP\(_{TM}\) was maintained, the decline in permeability can be assumed to be due to the solids concentration of the feed solution.

The arrows in Figure 5.5 indicate, for each membrane gap width, the range of solids concentration at which, according to the theory described in Section 5.2, the cells in the core region of the membrane channel begin to be forced into the liquid boundary layers. As predicted by the model, this is the range within which a decrease in permeability should occur, bought about by membrane fouling. Table 5.1 show that the maximum solids concentration obtained experimentally falls with the range of predicted maximum solids concentrations calculated for both random and closest packed spheres. If the theory was correct then this result would be anticipated. The closest packed spheres model assumes that the completely spherical, uncompressed cells are packed together in the most efficient manner possible. In practice this would never occur. The random packing of the yeast cells is much more likely to occur as the movement of the fluid through the membrane head of the VMF system continuously mixes the feed solution. As the yeast cells are only approximately spherical and have been shown to be compressible (Mashmoushy *et al.* 1998; Smith *et al.* 2000), a maximum solids concentration somewhere above the value for random packed spheres but below that of closest packed spheres would be anticipated.

### 5.4 Application of gap width theory to industrial fermentation broths

The results up to this point show that an improvement in VMF system performance, in terms of membrane permeability and final VCF achieved, is observed during the
5. Prediction of the maximum solids handling capacity of the PallSep PS10 — Postlethwaite 2003

processing of feed streams containing high concentrations of \textit{S. cerevisiae} when the distance between the two adjacent membrane discs is increased from 1.4 mm to 4.2 mm.

In order to demonstrate the applicability of this finding to industrial fermentation broths, similar experiments were performed as described in Section 5.3 using both \textit{Bacillus} and \textit{Aspergillus} fermentation broths supplied by Novozymes A/S ( Bagsvaerd, Copenhagen, Denmark) and \textit{S. erythraea} CA340 broth grown at UCL as described in Section 2.3. During the processing of these real broths, experiments were run up to a limiting concentration factor determined by either the low limit of measurable permeate flow in the case of the \textit{Bacillus} broths, or the difficulty in controlling the system pressure with the rapidly rising viscosity observed in the case of the \textit{Aspergillus} and \textit{S. erythraea} CA340 broths.

Figure 5.6 shows data from the concentration of both the Novozymes broths. In this case the permeability data is normalised for commercial reason against the initial flux value of \textit{Aspergillus} or \textit{Bacillus} when processed with a membrane spacing of 4.2 mm. During the concentration of the \textit{Bacillus} broth, a pronounced increase in performance is seen when the membrane gap width is increased from 1.4 mm to 4.2 mm. At a gap width of 1.4 mm, a VCF of 2.44 is achieved compared to a VCF of 2.98 with a gap width of 4.2 mm. During the processing of the \textit{Aspergillus} broth, an improvement as a result of increasing the gap width is also observed. With a gap width of 1.4 mm, a VCF of 1.51 is achieved, whilst with a gap width 4.2 mm, a VCF of 1.73 is achieved but at a considerably higher membrane permeability.

At the starting concentrations of the two broth types, a significant difference in permeability is observed at a gap width of 4.2 mm compared to that at 1.4 mm with \textit{Aspergillus} broth, whereas a negligible difference is observed with \textit{Bacillus} broth. This would imply that the \textit{Aspergillus} broth is already at a critical condition where the core region is affected by the solids concentration. Also with the \textit{Aspergillus} broth, increasing the concentration factor is shown to cause a significant further divergence in performance between the two gap width settings with a rapid decline or “crash” in permeability with increasing concentration factor observed with a gap width of 1.4 mm compared with apparent stabilisation at a gap width of 4.2 mm. At the higher gap width it
was not possible to go beyond a VCF of 1.75 due to the difficulty in pumping such a high viscosity broth. The maximum VCF achieved for broth processed at a gap width of 1.4 mm was 1.5 due to a decline in permeability. The \textit{Bacillus} broth was less viscous however and could be concentrated to higher volumetric concentration factors.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.6.png}
\caption{Influence of membrane gap width during the processing of \textit{Bacillus} and \textit{Aspergillus} fermentation broths in concentration mode. Permeability of \textit{Bacillus} broth with membrane gap width (■) 1.4 mm (▲) 4.2 mm and an \textit{Aspergillus} broth with membrane gap width (●) 1.4 mm (◇) 4.2 mm. Experimental conditions: Membrane head amplitude 19.5 mm and $\Delta P_{\text{JM}}$ 0.4 bar.}
\end{figure}

Unlike the \textit{Aspergillus} broth, the \textit{Bacillus} broth shows a continuous decline in permeability with increasing VCF during concentration at both gap widths. As VCF increases, a significant divergence in performance is observed in favour of the greater
5. Prediction of the maximum solids handling capacity of the PallSep PS10 Postlethwaite 2003

gap width, however this was not as significant as observed during the processing of Aspergillus.

Industrial fermentations often use inexpensive yet complex insoluble carbon and nitrogen sources such as oils and flours to support high productivities (Miller et al. 1986). These insoluble media components have been shown to dominate filtration performance (Davies et al. 2000). The fermentation of the different organisms used here will have been optimised to ensure maximum product titre is achieved. As a result both organisms will be grown in very different fermentation media and under different conditions. This coupled with the different morphology of the Bacillus and Aspergillus cells, rods and mycelia respectively, and the consequent differences in the packing of the cells in the core region between the membrane discs, as well as potential difference in permeate viscosity, will mean that the microfiltration characteristics of each broth type will be different.

Figure 5.7 shows a similar concentration experiment using S. erythraea CA340 fermentation broth processed using membrane gap widths of 1.4 mm and 4.2 mm. Fermentations were performed as described in Section 2.3. As for the processing of Aspergillus broths, experiments were run up to a limiting concentration factor determined by the difficulty in controlling the system pressure with the rapidly rising viscosity.

The figure shows that S. erythraea CA340 broths show similar properties to Bacillus broths in that a continuous decline in permeability is observed with increasing VCF during their processing at different membrane gap widths. As VCF increases, a significant divergence in performance is observed in favour of the greater gap width. This is somewhat surprising as the morphology of S. erythraea CA340 is known to be similar to that of Aspergillus, so would be expected to follow similar behaviour to that exhibited by the filamentous organism shown in Figure 5.6. Unlike S. erythraea CA340, Aspergillus is a filamentous fungus and not an actinomycete. Whilst the morphologies of the organisms may be very similar, their cellular structure is different. Exposure of the two organisms to the high intermittent shear fields generated by the mechanical vibration of the VMF system (discussed in Section 3.4) may have caused damage to the hyphae to different degrees thus affecting the microfiltration performance. The affect of S. erythraea CA340 morphology on microfiltration performance is discussed elsewhere
5. Prediction of the maximum solids handling capacity of the PallSep PS10 Postlethwaite 2003

(Davies. 2003). This, coupled with differences in the media composition between the two broths, will have contributed to the differences in the observed behaviour.

![Graph](image_url)

**Figure 5.7** – Influence of membrane gap width during the processing of *S. erythraea* CA340 fermentation broth (11.55 gL⁻¹ DCW) in concentration mode. Permeability with membrane gap width (■) 1.4 mm (●) 4.2 mm. Experimental conditions: Membrane head amplitude 19.5 mm and ΔP\text{TM} 0.65 bar.

An alternative explanation is that the experiment was stopped at too low a VCF. Figure 5.6 shows a sharp decline in permeability with increasing VCF during the processing of *Aspergillus* broth at a membrane gap width of 1.4 mm. Figure 5.7 may be showing the start of a similar rapid decline. As previously mentioned however, the experiment was run up to a limiting concentration factor determined by the difficulty in controlling the system pressure with the rapidly rising viscosity. In order to run to a higher VCF, a different type of system pump would be required as well as substantial alterations to the pumping and data collection rig.
Figure 5.8 shows the influence of membrane spacing on total protein and total erythromycin transmission during the processing of *S. erythraea* CA340 in concentration mode. The figure shows no real change in erythromycin transmission with increased membrane spacing. At a gap width of 1.4 mm, 95.3 % w/w transmission is achieved at a VCF of 1.97 after processing for 70 min whilst at a gap width of 4.2 mm, 96.9 % w/w transmission at a VCF of 2.03 is achieved after the same period of time. The figure does however show an increase in protein transmission over the same period although by the end of the concentration run, the difference in protein transmission levels is very small. At the start of the run and at a gap width of 1.4 mm, 66.9 % w/w transmission is achieved at a VCF of 1.09 after 10 min. processing whilst at a gap width of 4.2 mm, 78.5 % w/w transmission is achieved also at a VCF of 1.09 after 10 min. processing. At the end of the run and at a gap width of 1.4 mm, 92.4 % w/w transmission is achieved at a VCF of 1.97 after 70 min. processing whilst at a gap width of 4.2 mm, 91.3 % w/w transmission is achieved at a VCF of 2.03 after 70 min. processing. Figure 5.8 is interesting as it shows erythromycin transmission to remain constant as VCF increases whilst total protein transmission is observed to rise as VCF increases.

5.5 Discussion and summary

The results described in this chapter clearly show that an improvement in the overall performance of the VMF system can be achieved by increasing the spacing between the membrane elements. This improvement has been demonstrated in a model system and real fermentation broths and whilst the degree of improvement varies with broth type, the overall trend is the same for all broth types tested. In a study on the influence of membrane channel height on permeate flux during the nanofiltration of various colloidal suspensions, Hoek *et al.* (2002) showed a marked improvement in normalised permeate flux following an increase in filtration channel height from 0.864 mm to 1.727 mm. The authors tested four different types of membrane and found similar results with all of them. The authors attributed this improvement in performance to increased shear rates bought about as a result of the increase in channel height. It is unlikely that the improvement in VMF performance is bought about as a result of increased shear rates however. Results presented in Section 3.4 and 3.5 have shown that the mechanical vibration is the main source of the shear generated at the membrane surface of VMF systems. Any increase in shear rate bought about by an increase in membrane gap width will be negligible. Conversely, Wiley *et al.* (1985) showed that during the
desalination of brackish water using spiral wound, tubular and flat plate membrane geometries, the optimum module design is one where the channel height is small.

**Figure 5.8** – Influence of membrane gap width on protein and erythromycin transmission during the processing of *S. erythraea* CA340 fermentation broth (11.55 g L\(^{-1}\) wet weight harvested 91 hours after inoculation) in concentration mode. Total protein transmission (■) 1.4 mm (●) 4.2 mm and erythromycin transmission (▲) 1.4 mm (▼) 4.2 mm. Experimental conditions: Membrane head amplitude 19.5 mm and ΔP\(_{TM}\) 0.65 bar.

Results have also shown an improvement in protein transmission during processing at a higher membrane gap width. Interestingly no improvement in erythromycin transmission was observed. If the theoretical model described in Section 5.2 is correct, then the transmission of molecules should not be affected by the concentration of the feed stream as the liquid boundary layers prevent any fouling layer building up. With no solids in the boundary layers, small molecules such as erythromycin are free to pass through and into the membrane pores. Theoretically the same should apply to larger molecules such as
proteins although their larger size means that they are more likely to get caught on the membrane surface or within the membrane pores. It follows then that over time, a gradual decrease in protein transmission should be observed not, as is the case here, a gradual increase.

The construction of the PallSep head is, at present, governed to a certain extent by the length of the bolts used to fasten it together. In the PS10, a maximum of ten membrane discs can be fitted into the membrane head with 1 spacer element between each disc as described in Section 1.10.2. At present, in order to allow the inclusion of more spacer elements, thus increasing the between membrane gap width, membrane area must be sacrificed. A reduced installed membrane area bought about by the increase in process efficiency reported here, may result in a reduction in membrane replacement costs and hence improve the economics of this particular unit operation within the purification process. An obvious way of getting around this problem of limited membrane surface area is to increase the length of the bolts holding the head together. This may not be as easy as it sounds however. The forces acting on the individual bolts will be very high as the membrane oscillates back and forth. Increasing the length of these bolts may have implications for their strength and therefore the mechanical reliability of the VMF system. Secondly, as mentioned in Section 3.5, the frequency of oscillation of the membrane head dictates the head amplitude and hence the shear rate. This is only true however for a given torsion spring system. If the spring coefficient of the torsion bar or the mass of either the membrane head or the seismic mass is altered then this relationship will change as described in Section 1.9.1 (Alex and Haughney. 1998). By increasing the gap width between the membranes, the mass of the membrane head will be increased thus affecting the relationship described above. In order to achieve the same shear rate it may be necessary to run the VMF system at a higher frequency perhaps putting increased mechanical strain on the system. All of these points would require further investigation before any changes are made to the recommended configuration of the membrane head.

The results presented this chapter have built upon those presented in Chapter 3, showing that in an industrial process, increasing the gap width between the membrane plates could be beneficial when processing broths with high solids loads such as \textit{P. pastoris} fermentations or broths with high viscosities such as fungal fermentations. The
model presented here shows a method of calculating the maximum solids load achievable in the PallSep PS10 using a model yeast system. The model itself does have limitations however. Much work has been done looking at the concept of closest packed spheres in the field of crystallography (Sofo and Mahan. 2000; Dong et al. 2003) making the study of yeast cells an easy option. When broths of a more complex morphology are processed however, it becomes more difficult to predict the maximum solids capacity due to the lack of data on the packing of such cells. Assumptions made within the model are also recognised to affect its validity. The model assumes that the boundary layer thickness has a defined cut off point. As discussed in Section 1.10.2 however, the definition of the boundary layer thickness is somewhat arbitrary because transition from the liquid velocity at the membrane surface to that in the bulk fluid is asymptotic. The velocity of the fluid in the boundary layer will however attain a value which is very close to the velocity of the bulk fluid, a small distance from the membrane plate, as described by Schlichting (1979). The velocity profile used to calculate boundary layer thickness has been discussed in Section 5.4. Despite these limitations, the model has been proven to be accurate for predicting the maximum solids capacity of the VMF system during the processing of S. cerevisae suspensions. With more work to understand the packing of cells of different morphologies, it is anticipated that similar predictions could be made for the maximum solids capacity of the VMF system during the processing of cells with differing morphologies.

Apart from the results presented in Figures 5.6, 5.7 and 5.8, the work thus far has concentrated on the microfiltration of model yeast suspensions. The next chapter of this thesis will look at a real system and examine the recovery of the polyketide antibiotic erythromycin from S. erythraea CA340 fermentations by VMF.
6. Erythromycin recovery from \textit{Saccharopolyspora erythraea} CA340 fermentation broths

6.0 Introduction and aims

As described in Section 1.1.2, \textit{S. erythraea} CA340 differs from \textit{S. cerevisae}, the organism used so far in this work, in that it is a filamentous actinomycete and grows in submerged culture through the formation of long multi-cellular hyphae developed as branched septae. High levels of biomass within the fermentation broth, as well as the entangling of the hyphae, have implications for the recovery of erythromycin by microfiltration and will be discussed later in Section 6.4.4. The interactions between \textit{S. erythraea} CA340 fermentations and conventional TFF operations are covered elsewhere (Davies \textit{et al.} 2000).

In this chapter, the recovery of the polyketide antibiotic erythromycin from \textit{S. erythraea} CA340 fermentations by VMF is investigated. Microfiltration performance in terms of permeate flux and product transmission is investigated as a function of membrane head amplitude, $\Delta P_{TM}$, crossflow rate and fermentation harvest time. Where appropriate parallels will be drawn with the data reported so far on model \textit{S. cerevisae} systems (Chapters 3 and 4) and also with previously published results on erythromycin recovery from \textit{S. erythraea} CA340 broths using conventional, static TFF systems (Davies \textit{et al.} 2000).

6.1 Experimental methods

Fermentations were performed as described in section 2.3 using a soluble, glucose based medium. Microfiltration experiments were performed in either concentration or total recycle mode as outlined in Section 2.6.1. All analytical techniques were carried out as described in Section 2.5.

6.2 \textit{Saccharopolyspora erythraea} CA340 fermentation and broth characteristics

6.2.1 \textit{S. erythraea} CA340 fermentation characteristics

\textit{S. erythraea} has been used extensively within this department (Davies \textit{et al.} 2000; Davies. 2003; Heydarian \textit{et al.} 1998; Heydarian. 1998a), and as such much data on its
growth is available. A typical fermentation profile for a 20 L S. erythraea CA340 fermentation is shown in Figure 6.1. The Figure shows biomass levels increasing during the log phase of the fermentation, reaching a maximum concentration of 12 gL⁻¹ (DCW) after approximately 40 hours. The glucose carbon source shows a corresponding decrease in concentration over this period. Dissolved oxygen tension (DOT) can be seen to reach a minimum of approximately 20% after 20 hours, at which point the airflow was increased from 5 Lmin⁻¹ to 7 Lmin⁻¹ and then to 10 Lmin⁻¹. This ensured that sufficient oxygen was available to the organism for continued growth. Both the oxygen uptake rate (OUR) and the carbon dioxide evolution rate (CER) can be observed to peak at 35 hours (~28 mmolL⁻¹hr⁻¹). The respiratory quotient (RQ) remains at around 1.0 for the majority of the fermentation.

Erythromycin A synthesis exhibits a peak approximately 55 hours into the fermentation at a concentration of 230 mgL⁻¹. OUR and CER can be seen to decrease rapidly after the maximum biomass concentration is achieved, whereas the cell mass decreases slowly showing a physiological change in the organism during the production of erythromycin. Finally the RQ decreases at 70 hours, which can be attributed to the utilisation of carbon sources other than carbohydrates after this time (Davies, 2003).

In this work S. erythraea CA340 fermentations were performed at 75 L scale (50 L working volume) in order to provide two identical 25 L batches of broth with which to perform microfiltration experiments. Table 6.1 gives details of each fermentation performed and how the broth was used. The first seven fermentations (JP75L #2.1 to JP75L #2.7 inclusive) were performed using the inoculum chain described in Section 2.4.2, using 2 L baffled shake flasks to grow up the inoculum. Subsequent fermentations were performed using the inoculum chain described in Section 2.4.3, using the 7 L vessel to grow up the inoculum. This change was bought about by a number of contaminated fermentations, the cause of which was identified as poor aseptic technique during the handling of the large number of 2 L baffled shake flasks. Changing the inoculum chain to include the 7 L fermenter resulted in no further contamination.
Figure 6.1 – Typical fermentation profile for a 20L *S. erythraea* CA340 fermentation grown in soluble complex media. (a) — DOT, — air flow rate. (b) — OUR, — CER, — RQ. (c) □ Biomass, ○ glucose, △ erythromycin A. Error bars show the standard deviation of three replicate measurements. Data taken from Davies (2003).
Table 6.1 – Details of *S. erythraea* CA340 fermentations carried out and the corresponding microfiltration experiments. Fermentations performed as described in Section 2.3. Microfiltration experiments performed as described in Section 2.6.1.

In order to minimise the risk of contamination, no samples were withdrawn for analysis during the fermentations. Consequently, no details of biomass growth, erythromycin production or glucose utilisation are available during the time course of the fermentation although as described in Section 6.2.2, the composition and properties of the broth at harvest time were fully characterised. Throughout the fermentation, off gas analysis was possible as well as on line measurements of OUR and CER, allowing limited fermentation profiles to be constructed.

Figure 6.2 shows a typical fermentation profile for a 7L *S. erythraea* CA340 fermentation (JP 7L # 1.02) as used for the inoculation for JP75L # 2.09 and JP75L # 2.10. In Figure 6.2 (a), DOT can be seen to reach a minimum of approximately 72 % after 26 hours, at which point the fermentation was transferred to the 75L vessel as described in Section 2.4.3. The impeller speed remains constant throughout the fermentation with the exception of the unexplained drop after around 23 hours which can be seen to bring about a slight, and unexplained, rise in DOT. When the speed returns to the set point after approximately 25 hours, DOT can be seen to drop to expected levels. Figure 6.2
(b) shows both the OUR and the CER rising throughout the fermentation to a peak at 23 hours (~19 mmol·L⁻¹·hr⁻¹). The RQ can also be seen to rise throughout to a peak of around 1.0 at the end of the fermentation. DOT, CER, OUR and RQ data would suggest that the fermentation was harvested during exponential phase.

Figure 6.2 – Typical fermentation profile for a 7L *S. erythraea* CA340 fermentation grown in Soluble complex media (JP 7L # 1.02). (a) — impeller speed, — DOT. (b) — OUR, — CER, — RQ. Fermentation performed as described in Section 2.3.
Figure 6.3 shows a typical fermentation profile for a 75L *S. erythraea* CA340 fermentation (JP75L # 2.06) inoculated using 2L baffled shake flasks. In Figure 6.3 (a), DOT can be seen to reach a minimum of approximately 76% after 35 hours whilst the impeller speed remains constant throughout the fermentation. Figure 6.3 (b) shows both the OUR and the CER rising throughout the fermentation to a peak at 35 hours (~16 mmolL⁻¹hr⁻¹). The RQ can also be seen to rise to a peak of around 1.0 after 15 hours.

**Figure 6.3** – Typical fermentation profile for a 75L *S. erythraea* CA340 fermentation grown in Soluble complex media (JP75L # 2.01). (a) — impeller speed, — DOT. (b) — OUR, — CER, — RQ. Fermentation performed as described in Section 2.3.
6.2.2 S. erythraea CA340 fermentation broth characterisation

Prior to microfiltration studies, the harvested broth at the end of each fermentation was characterised in order to define its composition and rheological properties. The details of these studies are shown in Table 6.2.

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Biomass (gL⁻¹ DCW)</th>
<th>Erythromycin (mgL⁻¹)</th>
<th>Protein (gL⁻¹)</th>
<th>Viscosity (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JP75L # 2.01</td>
<td>12.3</td>
<td>170</td>
<td>0.83</td>
<td>N/D</td>
</tr>
<tr>
<td>JP75L # 2.02</td>
<td>9.7</td>
<td>185</td>
<td>0.54</td>
<td>N/D</td>
</tr>
<tr>
<td>JP75L # 2.03</td>
<td>10.2</td>
<td>180</td>
<td>0.73</td>
<td>N/D</td>
</tr>
<tr>
<td>JP75L # 2.04</td>
<td>11.55</td>
<td>175</td>
<td>0.64</td>
<td>0.0017</td>
</tr>
<tr>
<td>JP75L # 2.05</td>
<td>12.5</td>
<td>280</td>
<td>1.13</td>
<td>0.0019</td>
</tr>
<tr>
<td>JP75L # 2.06</td>
<td>7.8</td>
<td>140</td>
<td>0.31</td>
<td>0.0013</td>
</tr>
<tr>
<td>JP75L # 2.07</td>
<td>12.5</td>
<td>190</td>
<td>0.82</td>
<td>0.0018</td>
</tr>
<tr>
<td>JP75L # 2.09</td>
<td>11.1</td>
<td>185</td>
<td>0.77</td>
<td>0.0016</td>
</tr>
<tr>
<td>JP75L # 2.10</td>
<td>12.5</td>
<td>180</td>
<td>0.66</td>
<td>0.0017</td>
</tr>
<tr>
<td>JP 7L # 1.01</td>
<td>9.8</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>JP 7L # 1.02</td>
<td>10.2</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
</tr>
</tbody>
</table>

Table 6.2 – Details of fermentation broth characterisation. Fermentations performed as described in Section 2.3. Analysis of broth samples performed as described in Section 2.5. N/D: Not determined.

The table shows the biomass levels of the fermentations to range from 7.8 gL⁻¹ DCW to 12.5 gL⁻¹ DCW. These biomass levels are well below those used in the S. cerevisae studies described in Chapters 3 and 4 as these fermentations have not been optimised. For reasons of confidentiality, companies are unwilling to divulge details of their fermentation regimes and their results but Leach (2003) has reported that by using richer media and fed batch strategies, higher biomass levels and product titres can be reached.
Figure 6.4 shows an image of *S. erythraea* CA340 cells during the early stages of growth at 400x magnification. The figure clearly shows the filamentous nature of the organism. Although the influence of cellular morphology is not studied here, it is known to influence microfiltration performance (Nakanishi et al. 1987; Oolman and Liu. 1991; Tanaka et al. 1994; McCarthy et al. 1998; Davies. 2003). The use of image analysis technology would allow the exact morphology of the organism to be established, and a measure of its impact on VMF performance to be assessed.

![Image of S. erythraea CA340 cells](image)

**Figure 6.4** – *Saccharopolyspora erythraea* CA340 cells at 400x magnification.

Figure 6.5 shows the relationship between shear stress and shear rate for broth harvested 48 hours and 72 hours after inoculation. Table 6.2 shows the apparent viscosities of the broth used in this study to be comparable to those achieved by other investigators using the same fermentation system (Davies *et al.* 2000; Davies 2003). The figure shows the broth to exhibit shear thinning properties, as the shear rate increases, the apparent viscosity is reduced. This is in line with previous findings (Davies, 2003).
The inversely proportional relationship between permeate flux and apparent viscosity is well documented and is discussed in Section 1.7.3.

**Figure 6.5** - Rheology of a typical SCM fermentation. Figure displays the relationship between shear rate and shear stress for broth harvested at (●) 48 hours and (■) 72 hours. The figure shows *S. erythraea* CA340 broths to exhibit shear thinning properties (Doran. 1998; Davies. 2003).

### 6.3 Flux and transmission characteristics of the PallSep PS10

In this section the basic performance of the VMF system during the processing of *S. erythraea* CA340 broths in total recycle mode is examined and compared to similar experiments described previously in Chapter 3 using *S. cerevisae*. Figure 6.6 shows permeate flux as well as erythromycin and protein transmission during the microfiltration of *S. erythraea* CA340 (12.5 gL\(^{-1}\) DCW) in total recycle mode. The figure shows a rapid decline in permeate flux during the first 5 minutes of processing from 108 Lm\(^{-2}\)hr\(^{-1}\) to a steady state value of 95 Lm\(^{-2}\)hr\(^{-1}\). Following this initial decline, a steady state appears to
be established. This same trend can be seen in the protein transmission data presented in the same figure. A rapid decline in protein transmission is observed in the first 3 – 4 minutes of processing from an initial value of 95 % w/w to what appears to be a steady state at around 87 % w/w. In contrast, there is no real decline in erythromycin transmission over time with values remaining constant at around 95 – 98 % w/w.

**Figure 6.6** - Permeate flux and erythromycin and protein transmission during the processing of *S. erythraea* CA340 (12.5 g L\(^{-1}\) DCW) in total recycle mode. (■) Permeate flux, (●) total erythromycin transmission, (∆) total protein transmission. Experimental conditions: Membrane head amplitude 19.5 mm, \(\Delta P_{TM}\) 0.6 bar, crossflow rate 0.1 - 2 L min\(^{-1}\), membrane gap width 1.4 mm.

The low biomass levels of the fermentation broth (12.5 g L\(^{-1}\) DCW) compared to those of the *S. cerevisiae* suspensions used in the work described in Chapter 3 and Chapter 4 (300 to 500 g L\(^{-1}\)) explain the higher steady state permeate fluxes observed in Figure 6.6. The initial decline in permeate flux and protein transmission followed by the establishment of a steady state as observed in Figures 6.6, is most likely due to protein fouling of the membrane. With the higher permeate flux observed, the elevated levels of
protein in the fermentation broth will be transported to the membrane surface causing the rapid fouling of the membrane observed in the first five minutes of processing in Figure 6.6. The lower permeate flux observed after this initial decline, will then cause a reduction in the flow of protein foulants to the membrane surface. This would explain the reduction in the rate of permeate flux decline observed after 5 minutes. The relative size of the erythromycin molecules (the erythromycins have a molecular weight of around 718 – 750, whilst that of the protein BSA is approximately 66,000 Da, approximately 90 times higher) allows them to pass unhindered through the membrane pores explaining the lack of any decline in transmission levels.

6.4 Influence of operational variables

In Section 3.0, a series of key operational variables were identified and their influence on the performance of the VMF unit in terms of permeate flux and BSA transmission during the processing of S. cerevisae was investigated. The influence of these variables is assessed here using S. erythraea CA340 fermentation broths in order to establish their influence during the processing of real fermentations. The influence of membrane spacing on VMF performance during the processing of S. erythraea CA340 was described previously in Section 5.4.

6.4.1 Influence of membrane head amplitude on VMF performance

In Section 3.5, the influence of membrane head amplitude on PallSep performance whilst processing S. cerevisae was described in some detail and it was shown that increases in amplitude cause an increase in permeate flux and BSA transmission, presumably brought about by a decrease in membrane fouling caused by the intermittent shear fields brought about by mechanical vibration. Figure 6.7 shows the influence of membrane head amplitude on permeate flux during the processing of S. erythraea CA340 (11.1 gL\(^{-1}\) DCW) broth in total recycle mode. The figure shows a gradual rise in steady state permeate flux and both total protein and erythromycin transmission levels as head amplitude is increased from 6.5 mm up to a maximum of 26 mm. At an amplitude of 6.5 mm, permeate flux is 45.5 ± 0.45 Lm\(^{-2}\)hr\(^{-1}\), total protein transmission is 45 ± 6.13 % w/w and total erythromycin transmission is 91 ± 1.2 % w/w. As the head amplitude is increased, permeate flux is seen to increase gradually to a maximum of 54 ± 0.32 Lm\(^{-2}\)hr\(^{-1}\), total protein transmission increases to 65 ± 4.67 % w/w and erythromycin transmission increases to 98 ± 2.1 % w/w at a maximum amplitude of 26
mm. This increase in permeate flux and transmission levels follows the same trend as that seen in Section 3.5 during the processing of 400 gL\(^{-1}\) \textit{S. cerevisae}. The differences in the values observed during the processing of the two organisms are perhaps not as great as would be anticipated considering the large difference in biomass concentrations. The smaller than anticipated difference are most likely due to the presence of other solids such as un-dissolved media components present in the \textit{S. erythraea} CA340 broth.

![Figure 6.7](image)

**Figure 6.7** - Permeate flux and total protein and erythromycin transmission as a function of PS10 head amplitude during processing of \textit{S. erythraea} CA340 (11.1 gL\(^{-1}\) DCW) in total recycle mode. (●) Total protein transmission, (▼) total erythromycin transmission, (■) permeate flux. The dashed line represents the line used to obtain Equation [6.1]. Experimental conditions: \(\Delta P_{TM}\) 0.9 bar, crossflow rate 1 Lmin\(^{-1}\) and membrane gap width 1.4 mm. Error bars represent standard error of the mean.
Figure 6.7 also shows the same relationship between permeate flux and head amplitude first described by Al Akoum et al (2002) and discussed in Section 3.5. Experimental results reported here can be described by Equation [6.1].

\[ J = 15.78 \bar{y}_w^{0.12} \]

[Eq. 6.1]

The difference in these results from those reported by Al Akoum et al (2002) and those presented in Section 3.5, can be explained, certainly in part, by the differences in cell morphology between the two systems tested, although the difference in solids concentrations between the three models as well as the presence of other solids, proteins and undissolved media components may be a more likely cause.

6.4.2 Influence of ΔP_{TM} on VMF performance

The concept of critical transmembrane pressure, or cTMP, is well documented in the literature and is described in Section 3.7. Figure 6.8 shows the influence of ΔP_{TM} on permeate flux and protein and erythromycin transmission during the processing of S. erythraea CA340 fermentation broth (12.5 gL^{-1} DCW) in total recycle mode.

The results show a steady rise in permeate flux with ΔP_{TM} up to 72 Lm^{-2}hr^{-1} at 0.8 bar. Past this point any further increases in ΔP_{TM} can be seen to bring about a fall in permeate flux, indicating a cTMP value in the region of 0.9 bar during the processing of S. erythraea CA340 (12.5 gL^{-1} DCW). The figure also shows a fall in the transmission of both total protein and total erythromycin as the ΔP_{TM} is increased. At 0.4 bar, total erythromycin transmission is observed at 97 % w/w and total protein at 70 % w/w. As pressure is increased, a gradual decline in the transmission of both molecules is observed. At the maximum ΔP_{TM} used here (1.05 bar), total erythromycin transmission is observed to have fallen to 87 % w/w whilst total protein transmission has fallen to 45 % w/w. The differences observed in the levels of transmission between the protein molecules and the erythromycin molecules can be explained by the differences in their relative molecular size as discussed in Section 6.3.
6. Erythromycin recovery from S. erythraea CA340 fermentation broth Postlethwaite 2003

Figure 6.8 – Variation of permeate flux with increasing $\Delta P_{\text{TM}}$ during the microfiltration of S. erythraea CA340 (12.5 gL$^{-1}$ DCW) in total recycle mode. (■) permeate flux, (●) protein and (▲) erythromycin transmission. Experimental conditions: Membrane head amplitude 19.5 mm, crossflow rate 0.1 - 2 Lmin$^{-1}$ and membrane gap width 1.4 mm.

Section 5.2 describes the formation of boundary layers within which no cells are assumed to be present due to the uplift forces of the vibrational shear at the membrane surface. The results presented here however would suggest that the smaller solids and soluble protein molecules present in the feed stream are not transported away from the membrane surface. As the operating pressure is increased, a fouling layer is formed either on or within the membrane, limiting the transmission of both the protein and erythromycin molecules, but not the fluid, across the membrane, hence permeate flux continues to rise with increasing pressure. Independently of this, as the pressure continues to rise, the forces compressing the cells onto the membrane increase above the uplifting forces generated by the shear within the boundary layers causing a cake to build up, decreasing the permeate flux as observed.
Comparing the results in Figure 6.8 with those presented in Figure 3.11, it can be seen that cTMP value obtained when processing S. erythraea CA340 at 12.5 gL\(^{-1}\) (DCW) is much lower than those observed during the processing of S. cerevisiae suspensions at 300 – 500 gL\(^{-1}\). The lower cTMP value observed is most likely due to the differences in broth composition. The S. cerevisiae suspensions used in Section 3.7 contain only cells, BSA and buffer whereas the S. erythraea CA340 broth used in this section of work contains cells, proteins, undissolved media components as well as other unidentified components. As a result of these broth components, membrane fouling is more likely to occur.

6.4.3 Influence of crossflow rate on VMF performance

The influence of crossflow rate on conventional TFF performance, described in Section 1.6.2, is well documented and as such is commonly used as a means of maintaining permeate flux and product transmission. In Section 3.9, the effect of crossflow rate on VMF performance was examined during the processing of S. cerevisiae suspensions at 300 and 500 gL\(^{-1}\). Figure 3.14 clearly demonstrates that in the VMF system used, permeate flux and BSA transmission are independent of crossflow rate within the ranges tested. In this section, this influence is examined for the recovery of S. erythraea CA340 fermentation broth.

Figure 6.9 shows the influence of crossflow rate on permeate flux and total protein and erythromycin transmission during the processing of S. erythraea CA340 broth (12.5 gL\(^{-1}\) DCW). The figure shows permeate flux remaining constant at 63 – 65 Lm\(^2\)hr\(^{-1}\) with varying crossflow rate from 0.2 – 6 Lmin\(^{-1}\). Across the same range of flow rates, total protein and erythromycin transmission are seen to remain constant at around 60 % w/w and 98 % w/w respectively. Like Figure 3.14, Figure 6.9 shows that varying crossflow rate has no effect on the performance of the VMF system, confirming that the increase in microfiltration performance observed is a result of the vibrational shear generated at the membrane surface.
6.4.4 Influence of fermentation harvest time (solids loading)

The influence of solids loading on VMF performance using *S. cerevisae* suspensions was described in Section 3.6. In this section, the influence of fermentation harvest time, and hence broth solids loading, on the recovery of erythromycin and protein from *S. erythraea* CA340 fermentations is described. *S. erythraea* CA340 fermentations were harvested 48 hours and 72 hours after inoculation at final biomass concentrations of 7.8 gL\(^{-1}\) DCW (JP75L #2.05) and 12.5 gL\(^{-1}\) DCW (JP75L # 2.06) respectively. Broth was then processed in concentration mode of operation using the PallSep PS10 to simulate a real industrial process.

Figure 6.10 shows a plot of membrane permeability as a function of VCF. During the processing of broth harvested after 48 hours, membrane permeability is seen to drop
from around 94 Lm⁻²hr⁻¹bar⁻¹ at the start of the run to 87 Lm⁻²hr⁻¹bar⁻¹ after a doubling in concentration. A final VCF of around 3.3 was achieved after 70 minutes of processing. During the processing of broth harvested after 72 hours however, a rapid decline in membrane permeability is observed from 76 Lm⁻²hr⁻¹bar⁻¹ at the start of the run to 67 Lm⁻²hr⁻¹bar⁻¹ at a final VCF of 2.0 again after 70 minutes of processing. In both cases the experiments were run up to a limiting concentration factor determined by the difficulty in controlling the system pressure with the rapidly rising viscosity.

**Figure 6.10** – Membrane permeability as a function of fermentation harvest time during the processing of *S. erythraea* CA340 in concentration mode. Broth harvested after (■) 48 hours (7.8 gL⁻¹ DCW) and broth harvested after (●) 72 hours (12.5 gL⁻¹ DCW). Experimental conditions: Membrane head amplitude 19.5 mm, ΔPₘ at 0.65 bar, crossflow rate in the range of 2 to 0.3 Lmin⁻¹ and membrane gap width 1.4mm. Figure shows the VCF achieved after 70 minutes processing for both fermentations.
Figure 6.11 shows total protein transmission as a function of VCF during the processing of the two broths in concentration mode. The figure shows a gradual fall in protein transmission during the processing of broth harvested after 48 hours. During the experiment, transmission is observed to drop from around 47 % w/w at the start of the processing run to just under 30 % w/w after 70 minutes. During the processing of broth harvested after 72 hours however, an increase in transmission is observed from 67 % w/w to around 90 % w/w. The same trend is observed in Figure 5.8 during the concentration of *S. erythraea* CA340 at a membrane spacing of 4.2 mm.

![Figure 6.11](image.png)

**Figure 6.11** – Total protein transmission as a function of fermentation harvest time during the processing of *S. erythraea* CA340 in concentration mode. Broth harvested after (■) 48 hours (7.8 gL⁻¹ DCW) and broth harvested after (○) 72 hours (12.5 gL⁻¹ DCW). Experimental conditions: Membrane head amplitude 19.5 mm, ΔP_TM at 0.65 bar, crossflow rate in the range of 0.3 to 2 Lmin⁻¹ and membrane gap width 1.4mm.
Figure 6.12 – Influence of $\Delta P_{tm}$ during the processing of *S. erythraea* CA340 in total recycle mode. Variation of steady state (■) permeate flux, (●) total protein transmission and (▲) total erythromycin transmission. (a) broth harvested after 48 hours (7.8 gL$^{-1}$ DCW). (b) broth harvested after 72 hours (12.5 gL$^{-1}$ DCW). Experimental conditions: Membrane head amplitude 19.5 mm, crossflow rate in the range of 0.3 to 2 Lmin$^{-1}$ and membrane gap width 1.4mm.
Figure 6.12 shows the influence of fermentation harvest time on permeate flux and total protein and erythromycin recovery as a function of $\Delta P_{TM}$. The figure shows that in the broth harvested after 48 hours, $\Delta P_{TM}$, has less of an effect on permeate flux than on broth harvested after 72 hours. At the early harvest point, permeate fluxes are almost 3 times higher than those under the same conditions for the later harvest point. With increasing $\Delta P_{TM}$, however, a much steeper rise in permeate flux is observed for broth harvested after 72 hours. Both broths appear to have a cTMP value of around 0.9 bar, giving a maximum permeate flux of around 71 Lm$^{-2}$hr$^{-1}$. The figure shows $\Delta P_{TM}$ to have no apparent effect on erythromycin transmission for broth harvested after 48 hours with transmission values remaining stable at around 96 – 98 % w/w. At the higher $\Delta P_{TM}$ tested during the processing of broth harvested after 72 hours however, a slight decrease in erythromycin transmission is observed from a steady state of around 96 % w/w up to 0.8 bar to around 89 % w/w at 1.05 bar. This decrease in erythromycin transmission corresponds to the fall in permeate flux observed at the same $\Delta P_{TM}$. Protein transmission values show a similar trend for both broth ages, namely a fall in transmission with increasing $\Delta P_{TM}$. Transmission values can be seen to fall from 95 % w/w at 0.3 bar to 80 % w/w at 1.05 bar for broth aged 48 hours and from 69 % w/w at 0.4 bar to 45 % w/w at 1.05 bar for broth aged 72 hours. There are however differences in the initial protein transmission levels at each harvest time. At the lower $\Delta P_{TM}$ used (0.4 bar), Figure 6.12 shows there to be a ~20 % w/w difference in protein transmission levels between the two broths. At the higher $\Delta P_{TM}$ used (1.05 bar) however, this difference has increased to ~35 % w/w. At the higher $\Delta P_{TM}$, there is more biomass at the membrane surface. Protein association with this biomass means that less protein can now permeate the membrane. At higher biomass levels this effect is obviously more pronounced causing the observed differences.

6.5 Comparison with conventional static TFF systems

Thus far, the results presented and discussed describe the use of a VMF system to process *S. erythraea* CA340 fermentation broths. In this section, the influence of *S. erythraea* CA340 fermentation harvest time on the performance of a conventional TFF system compared to that of the VMF system will be examined. All TFF data is taken from Davies (2003). In his work, Davies used a Minitan II rig (Millipore, UK.) fitted with a 0.2 $\mu$m hydrophilic Durapore membrane with a total surface area of $6 \times 10^{-3}$ m$^2$. The
differences in the membrane chemistry and pore size between the VMF system and the static TFF system are acknowledged, however no other data on the microfiltration performance of this organism is available in the literature. Shear rates in the region of 1.1×10⁴ s⁻¹ have been reported for the Minitan II TFF system (Davies, 2003) The calculation of this value and how it compares to the shear rates generated by the mechanical vibration of the VMF system is discussed fully in Section 3.4. No details of the biomass levels of the fermentations used to study the effect of broth age on static TFF performance are available, however close examination of the available fermentation profiles showed them to have the same growth characteristics as those for the fermentations performed as part of this work. This would suggest that biomass levels were in the same range for both studies.

Figure 6.13 shows the influence of fermentation harvest time on steady state permeate flux and total protein transmission during the processing of S. erythraea CA340 using TFF and VMF systems. Figure 6.13 (a) shows that using the TFF system, no obvious trend can be drawn from the permeate flux data. With the VMF system however, an obvious decline in permeate flux is observed albeit with a limited number of data points. When processing younger broths, the VMF system seems better able to cope giving a permeate flux of 56 ± 4.6 Lm⁻²hr⁻¹ at 48 hours compared with 34.5 ± 2.1 Lm⁻²hr⁻¹ at 40 hours processed using the TFF system. Towards the middle of the fermentation, the difference in performance between the two filtration systems becomes less pronounced. Using the VMF system to process broth aged 72 hours gave a permeate flux of around 39 ± 5.3 Lm⁻²hr⁻¹ whilst processing broths aged 60 hours and 90 hours using the TFF system gave permeate fluxes of 35.7 ± 8.1 Lm⁻²hr⁻¹ and 32.3 ± 13 Lm⁻²hr⁻¹ respectively.

Figure 6.13 (b) shows a tendency for protein transmission to be unaffected by fermentation age when using the TFF system. With the VMF system however, a decrease in protein transmission is observed as the age of the broth increases. This can be explained by increasing biomass and protein concentration over time causing a decrease in the protein transmission through cake formation and gel layer polarisation respectively. Erythromycin transmission is observed to be unaffected by the age of the broth remaining constant at around 95 – 98 % w/w in both the VMF and TFF systems tested. As a result of the low molecular weight of the molecule, the observed transmission is high using both filtration systems.
Figure 6.13 – Influence of fermentation harvest time during the processing of *S. erythraea* CA340 in total recycle mode. Variation of steady state (a) permeate flux and (b) total protein transmission. (■) MF runs carried out using Minitan TFF system, (●) MF runs carried out using PallSep VMF system at membrane head amplitude 19.5 mm, $\Delta P_{TM}$ at 0.65 bar, crossflow rate 1 Lmin$^{-1}$ and membrane gap width 1.4 mm. Minitan data adapted from Davies (2003) $\Delta P_{TM}$ in the range of 1.25 to 7.6 bar.
6. Erythromycin recovery from S. erythraea CA340 fermentation broth

6.6 Discussion and summary

In this Chapter the key experimental variables identified in Chapter 3 have been examined and their influence on the performance of a VMF system during the processing of S. erythraea CA340 examined. Results presented in this chapter have shown that membrane head amplitude, $\Delta P_{tm}$ and crossflow rate exert similar influences on VMF performance during the processing of real fermentation broths.

Work presented in this chapter has shown how the recovery of erythromycin and protein from S. erythraea CA340 broth is affected not only by the processing conditions but also the nature of the fermentation itself. Figure 6.1 shows the production of erythromycin as a function of fermentation time. The figure shows how the erythromycin titre is maximised during the later stages of the fermentation, specifically late exponential phase and death phase, data supported by other studies (Davies et al. 2000; Bushell et al. 1997). This presents problems for the processing of the erythromycin molecule however. During death phase of the fermentation, as the name suggests, the fermentation is dying with increases in the extracellular concentrations of DNA, membrane components and free amino acids indicating massive cell lysis (Chartrain et al. 1991). The presence of these components is known to have a detrimental effect on conventional TFF performance by causing a massive increase in membrane fouling by concentration polarisation (Sousa et al. 2002; Czekaj et al. 2000; Parnham and Davis. 1995). This can be observed in the data presented in Figure 6.10 where during the concentration of S. erythraea CA340 broth aged 48 hours, VMF performance is significantly better than that achieved during the processing of broth aged 72 hours. This difference in performance will also be related to broth viscosity however. Table 6.2 shows the viscosity of the broth aged 48 hours to be 0.0013 Pa.s at 7.8 gL$^{-1}$ DCW compared to 0.0018 Pa.s at 12.5 gL$^{-1}$ DCW for that aged 72 hours (the relationship between viscosity and permeate flux is described in Section 1.7.3). At 72 hours however, the fermentation is well into death phase so the drop in VMF performance can be attributed to both the higher viscosity observed as well as increase in the level of cellular debris leading to membrane fouling.

Whilst Figure 6.10 shows a fall in membrane permeability as a result of increased broth age, Figure 6.11 shows an increase in protein transmission levels during the processing of broth aged 72 hours. This would appear to contradict the hypothesis that the older broth will contain lysed cells thus causing a release of protein into the broth leading to
membrane fouling. A possible explanation for this lies in the increasing transmission levels observed. With the broth aged 48 hours, the cells are young and robust and are unaffected by the high shear rates generated by the mechanical vibration of the VMF system as discussed in Section 3.4. During the processing of the older broth however, prolonged exposure of the older, weaker cells to the high shear generated in the VMF system causes an increase in the levels of cellular damage observed. Release of cellular components into the broth causes the fall in permeate flux observed whilst the intracellular proteins such as DNA are now exposed to the high levels of shear. This causes them to break into smaller molecules (Levy et al. 1998; Levy et al. 1999; Lengsfeld and Anchordoquy. 2002) which are able to permeate through the membrane and, whilst perhaps not being viable protein molecules, they are still detected by the total protein assay used (described in Section 2.5.3).

The use of an image analysis (IA) system would allow better understanding of the influence of broth age on VMF performance. IA systems vary enormously but they are essentially a microscope fitted with a camera (for image capture) linked to a computer with software capable of analysing the image. Such systems have been used within this department (Davies et al. 2000; Davies. 2003; Heydarian. 1996; Heydarian. 1998) as well as elsewhere (Packer and Thomas. 1990; Paul and Thomas 1998) for the characterisation of filamentous fermentation broths.

This study looks at the use of VMF technology for the purification of erythromycin from fermentation broths and compares the results to those obtained using a conventional static TFF system. The results in Figure 6.13 show that during the processing of fermentations at an age when erythromycin titre would be expected to be at its highest, namely around 72 hours according to Figure 6.1, there is very little difference between the performance of VMF technology and TFF technology. The data presented in Figure 6.13 is summarised in Table 6.3. Although the performance of the two systems is observed to be very similar under the conditions tested, very few significant conclusions can be drawn as the two systems compared are both fundamentally different in design and the way they are operated. The table is meant as a very basic comparison. Evidence has shown that the VMF system is better able to cope with broths of a higher solids load (Section 3.6). It follows therefore that during any concentration step, prior to a diafiltration step, VMF technology would offer advantages in terms of performance over
conventional TFF systems. In order to fully compare VMF technology with conventional TFF however, a full economic analysis of the process would be necessary.

<table>
<thead>
<tr>
<th>Harvest time (Hours)</th>
<th>Membrane system</th>
<th>Flux (Lm⁻³hr⁻¹)</th>
<th>Erythromycin transmission (% w/w)</th>
<th>Protein transmission (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>34.5 ± 2.1</td>
<td>95 ± 7.1</td>
<td>60 ± 1.4</td>
</tr>
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<td>98.7 ± 3.8</td>
<td>43 ± 10.1</td>
</tr>
<tr>
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<td>&quot;</td>
<td>32.3 ± 13</td>
<td>93 ± 9.8</td>
<td>44 ± 4.6</td>
</tr>
<tr>
<td>110</td>
<td>&quot;</td>
<td>29 ± 4.2</td>
<td>102 ± 8.5</td>
<td>49.5 ± 12</td>
</tr>
<tr>
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<td>56 ± 4.6</td>
<td>95 ± 7.2</td>
<td>97 ± 9.6</td>
</tr>
<tr>
<td>72</td>
<td>&quot;</td>
<td>39 ± 5.3</td>
<td>97 ± 8.6</td>
<td>65 ± 8.2</td>
</tr>
</tbody>
</table>

Table 6.3 - Comparison of the performance of a Minitan II TFF system and a PallSep PS10 VMF system in terms of permeate flux and protein and erythromycin transmission during the processing of S. erythraea CA340 broth harvested at various ages.

The work presented in this chapter highlights the critical importance of understanding the interactions between microfiltration and fermentation raised by numerous authors (Harscoat et al. 1999; Davies et al. 2000; Eagles and Wakeman. 2002; Davies. 2003; Meireles et al. 2003). In order to maximise the efficiency of any erythromycin recovery process, it is critical that the trade off between maximising the erythromycin titre and the effect that the older fermentation broth will have on the primary recovery step is well understood. Data presented in this chapter has shown how running a fermentation for longer to maximise the product titre means that the broth is in such a condition as to hamper the primary recovery. The results also show how critical it is to select the correct operating conditions of the VMF system in order to maximise erythromycin recovery. The importance of process optimisation, discussed in Section 4.4, is highlighted here using a real fermentation system.
7. General discussion and future work

7.0 General discussion

Filtration is one of a series of unit operations available to the biochemical engineer during the recovery of fermentation products such as proteins or antibiotics. Conventional TFF is more often than not the mode of operation employed. In order to maximise product titres whilst minimising production costs, the media used to support high antibiotic productivities in commercial fermentations are predominantly formulated using readily available, inexpensive complex carbon and nitrogen sources such as oils and flours (Miller et al. 1986). These media components, whilst increasing product titres, have been shown to have serious implications for microfiltration performance in terms of reduced permeate flux and product transmission bought about by increased levels of membrane fouling (Davies et al. 2000). One of the methods used to combat membrane fouling in TFF is to increase the broth recirculation rate, hence increasing the flow rate across the membrane surface. By elevating the crossflow rate, the shear forces generated cause uplifting of particles from the membrane surface thus allowing permeate fluxes to be maintained. Problems can arise at larger scales however. Increased crossflow rate can lead to pressure drops across the membrane surface as well as increased power requirements for the process pump. For these reasons, filtration is often overlooked as a primary separation step in favour of techniques such as centrifugation. VMF systems provide a novel means of solving this dilemma by using mechanical vibration to create intermittent shear fields at the membrane surface thus decoupling the shear forces necessary to minimise membrane fouling from process fluid crossflow rate. In this thesis the performance of a VMF system, the PallSep PS10, has been investigated during the processing of a model S. cerevisiae feedstream as well as during the processing of real fermentation broths.

Initial investigations sought to identify the key operational variables that affected PallSep performance in terms of permeate flux and product transmission. Five operational variables were identified in Section 3.0; membrane head amplitude, feedstream solids loading, \( \Delta P_{TM} \), membrane gap width and crossflow rate, each one having a different influence on the system performance. Results presented in Figure 3.8 show that the elevated levels of shear observed at the membrane surface (described in Section 3.4) allow the PallSep to operate under much more testing conditions than would otherwise be attempted using TFF. Figure 3.9 shows how
permeate flux can be represented as a function of shear rate. This finding is in slight
disagreement with the findings of another study (Al Akoum et al. 2002) which showed
how permeate flux can be represented by two different functions of shear rate. Figure
3.9 does however show an apparent inflection in the permeate flux data at a
membrane head amplitude of 19.5 mm suggesting that with the addition of further
data points to the figure, the permeate flux model presented here may indeed be in
agreement with the model of Al Akoum et al (2002). As a result of the elevated shear,
it is possible to process feed streams with very high solids loads whilst maintaining
permeate flux and product transmission at acceptable levels. Figure 3.10 shows that
at a yeast concentration of 500 gL⁻¹, a permeate flux of ~40 Lm⁻²hr⁻¹ and a protein
transmission of ~69% was achieved. The ability to operate at solids loads higher
than traditional TFF systems allows the VMF system to achieve much higher
concentration factors, thus reducing the volume of process fluid to be passed on to
the next unit operation in the processing chain. The high intermittent shear fields also
reduce the need for elevated crossflow rates. Figure 3.14 shows VMF performance
to be unaffected by crossflow rates across the range of > 1 to 5.5 Lmin⁻¹. With the
need for high crossflow rates to maintain permeate fluxes and product transmission
levels removed, filtration becomes a more economically viable choice as a unit
operation. Experiments have shown that PallSep performance is independent of
crossflow velocity (Figure 3.14) but critically dependent on solids concentration
(Figure 3.10), operating ΔP_TM (Figure 3.11) and head amplitude and hence shear rate
(Figure 3.9). Results have also shown how the construction of the membrane head
stack plays a very important role in the performance of the VMF system (Figure 3.13).

The interactions of the operational variables identified in Chapter 3 have also been
considered. The use of RSM to study the interactions of operating variables is well
documented (Kalil et al. 2000; Kennedy et al. 1999; Mount et al. 2003). Each of the
operational variables described and tested in this work has been observed to interact
with others to produce a result on VMF performance (Figure 4.3 and Figure 4.5).
Models were generated to describe the influence of the operational variables on both
permeate flux and protein transmission. The model for prediction of permeate flux is
shown to be very accurate (Figure 4.2, R² = 0.98) whilst the model for prediction of
protein transmission is shown to be less so (Figure 4.4, R² = 0.52). The design
Expert 5 software used to study the interactions of the operational variables was also
used to predict the operating conditions necessary to give optimum permeate flux
(Figure 4.8) and protein transmission (Figure 4.10) individually as well as both
parameters together (Figure 4.12). These predictions were then verified
experimentally. Results showed that the model generated for the prediction of optimum permeate flux was very accurate (Figure 4.9) with an experimental flux of $132 \pm 6 \text{ Lm}^2\text{hr}^{-1}$ being achieved compared to a predicted flux of $129 \text{ Lm}^2\text{hr}^{-1}$. The model generated for protein transmission was less accurate (Figure 4.11) with an experimental transmission level of $81 \pm 3\% \text{ w/w}$ being achieved compared to a predicted transmission level of $87\% \text{ w/w}$. The model generated for the prediction of optimum permeate flux and protein transmission was reasonably accurate (Figure 4.13) with an experimental flux and transmission of $132 \pm 6 \text{ Lm}^2\text{hr}^{-1}$ and $84 \pm 3\% \text{ w/w}$ respectively being achieved compared to predicted levels of $129 \text{ Lm}^2\text{hr}^{-1}$ and $78\% \text{ w/w}$ respectively. The results have shown how powerful a tool RSM is in both understanding process interactions and also optimising the process.

In Chapter 5 a model is proposed for the improved performance observed following an increase in the number of spacer elements between each membrane plate. Figure 3.13 shows that one of the factors limiting the solids handling ability of the PallSep PS10 system is the gap between the membrane plates. Within this gap, liquid filled boundary layers are shown to exist, creating a central core region within which all the solids are contained (Figure 5.2a). Once this core region is filled the solids leak into the boundary layer (Figure 5.2b), observed as a dramatic fall in VMF performance (Figure 5.5). As the thickness of the boundary layer can be calculated (described in Section 5.2.1) and the distance between the membrane plates is known (1.4 mm with 1 spacer element), a theoretical maximum solids handling concentration for each gap width can be calculated as shown in Table 5.1. These predicted values were then verified experimentally. Figure 5.5 shows a rapid decline in permeate flux during the processing of *S. cerevisiae* in concentration mode at membrane gap widths of 1.4 mm and 4.2 mm. These experimental values compare well with the predicted maximum solids handling values (Table 5.1 and Figure 5.5) suggesting the model is correct. Similar improvements in permeate flux are observed during the processing of real industrial *Aspergillus, Bacillus* (Figure 5.6) and *S. erythraea CA340* (Figure 5.7) fermentation broths although no predictions of the maximum solids load are possible due the lack of available data on the packing of cells of rod and mycelial morphology.

The impact of the operational variables identified in Chapter 3 on VMF performance during the processing of *S. erythraea CA340* broth is considered. These broths have a different morphology to the *S. cerevisiae* systems used in the study up to this point. The fermentation profiles presented as Figure 6.2 (7L scale) and Figure 6.3
(75L scale) show the organism following a similar growth pattern to previous studies using the same fermentation system (Figure 6.1 adapted from Davies. 2003). Figure 6.7 shows the same relationship between permeate flux and membrane head amplitude presented in Figure 3.9. Unlike Figure 3.9 however, no apparent inflection is observed in the data in Figure 6.7. $\Delta P_{\text{TM}}$ (Figure 6.8) and crossflow rate (Figure 6.9) are shown to exert similar influences on VMF performance as those seen when using the model yeast system described in Sections 3.7 and 3.9 respectively.

Fermentation – microfiltration interactions are studied by looking at the influence of *S. erythraea* fermentation harvest time on VMF performance. The results are compared with those obtained whilst using a conventional TFF system. The results show that broth harvest time has a significant effect on microfiltration performance in terms of membrane permeability (Figure 6.10) with much better results being achieved with the younger broth aged 48 hours (7.8 gL$^{-1}$ DCW) than with the older broth aged 72 hours (12.5 gL$^{-1}$ DCW). Total protein transmission is observed to increase with broth age however (Figure 6.11), perhaps due to the mechanical vibration breaking open the older cells and exposing their contents to the damaging shear forces. The increased level of cellular debris can be observed to cause a decrease in permeate flux in Figure 4.10 and has been reported by other investigators (Sousa *et al.* 2002; Czekaj *et al.* 2000; Parnham and Davis. 1995). The release of cellular components into the broth causes intracellular proteins such as DNA to be exposed to the high levels of shear. This causes them to break into smaller molecules (Levy *et al.* 1998; Levy *et al.* 1999; Lengsfeld and Anchordoquy. 2002) which are able to permeate through the membrane and, whilst perhaps not being viable protein molecules, they are still detected by the total protein assay used (described in Section 2.5.3). This would explain the rise in protein transmission observed with increasing broth age.

Although, in industry, *S. erythraea* CA340 broth would not be harvested 48 hours into the fermentation due to the low erythromycin titres observed (Figure 6.1), the results presented in Section 6.4.4 show that there is a trade off between product concentration and processing results. Harvesting the fermentation later on may give a higher product titre, but it is harder to process due to the elevated levels of biomass and protein levels in the broth. Using the VMF system, this drop in overall performance can be minimised when compared to conventional TFF, but it still exists. In practice, most manufacturers would choose to maximise product titre and the deal with the consequences due to the large drop in yield achieved at each processing step. Maximising product titre would reduce the overall effect of this. However, this
said, manufacturers need to be aware that changes to the fermentation will have consequences for the unit operation steps used in the purification of the product.

7.1 Industrial Implications and applications of this work

The VMF system used throughout this work is commercially available and as such the results described here will have implications for industry.

A model yeast suspension was used during the process optimisation experiments described in Section 4.4 because of its ease of preparation. In an industrial context, this method of optimisation could be more easily employed as fermentation broth may be more readily available from the sites production facilities. The drawback of this however, is that a lack of fermentation broth uniformity from batch to batch would affect the results. The yeast solutions used in this work were all made up using the same type of yeast blocks as described in Section 2.2 and as such all test solutions can be assumed to be identical. Fermentations however, vary from batch to batch even though the operating conditions are kept the same. This variation will have consequences for the validity of any optimisation study carried out using the method described here.

In Chapter 5, a method for improving the solids handling ability of the PallSep PS10 system is proposed. The improved performance has obvious implications for an industrial process. Within the unit operation of clarification, a high concentration factor is advantageous, provided product transmission is maintained, as less diafiltration will be needed to achieve an acceptable product yield. This will result in either a faster processing time or a lower installed membrane area. Taking a view of an integral series of unit operations, a reduced number of diafiltration volumes means a less dilute final product concentration is passed on to the subsequent downstream unit operations (Nabais and Cardoso. 1999). In the 1970’s, Philips Petroleum Company developed media and protocols for growing the methylotrophic yeast *Pichia Pastoris* on methanol in continuous culture at high cell densities (> 130 gL⁻¹ DCW). In the following decade the *P. pastoris* system was developed as an organism for heterologous protein expression producing surprisingly high levels of foreign protein expression (Cereghino and Cregg. 2000). The yeast is currently used for the production of numerous foreign proteins at high levels due to the very high cell densities achievable in submerged culture. Bacterial proteins that are currently produced include *Clostridium botulinum* neurotoxin (BoNT) serotype A and B at 78 mgL⁻¹ and *Streptococcus equisimilis* streptokinase at 77 mgL⁻¹. The organism is also
used for the production of human insulin like growth factor - 1 (IGF - 1) at 600 mgL\(^{-1}\) and Fibrinogen 143 – 411, 143 – 427 at 110 mgL\(^{-1}\) (for a full review see Cereghino and Cregg. 2000). The versatility of the organism in terms of the level of protein expression as well as the high cell densities achievable in submerged culture, have made it a very popular system in biotechnology. At such high cell densities however, harvesting the broth is often a problem with conventional primary separation techniques more often than not unable to cope. With their ability to handle solids at very high levels, coupled with the improved performance observed with increased membrane spacing, VMF systems offer a real alternative to conventional unit operations for the harvesting of the yeast. As previously stated in Section 5.4, within the unit operation of clarification, a high concentration factor is advantageous, provided product transmission is maintained, as less diafiltration will be needed to achieve an acceptable product yield. At such high cell densities, VMF systems will offer obvious advantages over other conventional unit operations.

Rotary drum vacuum filtration (RDVF) is currently the most widely used method of filtration for the purification of antibiotics from fermentation broths (Davies. 2003; Doran. 1998). As a result of the biochemically and rheologically complex broth, it is often very difficult to process leading many manufacturers to use a dilution and centrifugation step followed by a RDVF step to recover the product in sufficiently high yield. In an industrial RDVF system the cake must be continuously removed in order to maintain permeate flux and transmission levels. This is achieved by the presence of a scrapper positioned just above the surface of the membrane. As the drum rotates through the broth, the solids on the membrane are removed. RVDF is a costly process however, this coupled with the cost factors associated with dealing with in excess of 100 m\(^3\) of dilute product in the solvent extraction step and a maximum yield typically in the region of 90% and the process starts to be uneconomical (Leach. 2000). Results presented in Chapter 6 show that VMF technology is a viable alternative to conventional processing methods for the recovery of erythromycin. Data presented in Figure 6.13 and summarised in Table 6.3 shows that the performance of the VMF system is very similar to that of a conventional TFF system. The cost of running the VMF system is known to be considerably lower than that of a TFF system (Sellick. 1997). This alone makes the VMF system a more appealing choice as a primary separation technique, although a fully optimised recovery process involving a VMF step would be expected to give better performance in terms of product recovery, permeate flux and lower overall operating costs when compared to a conventional TFF system.
7.2 Conclusions

In Section 1.1.2 the aims of this work are laid out. In this section, these aims will be readdressed and concluding remarks made as to how effectively they have been met.

- A good understanding of the performance of the PallSep PS10 VMF system in terms of permeate flux and product transmission has been obtained. How this relates to key operating variables such as frequency and amplitude of vibration is now well understood.

- The VMF system has been compared with a conventional static TFF system in terms of filtration performance. The results have shown that in certain applications, VMF systems may be considered more suitable than conventional TFF systems.

- The limited knowledge base currently available on VMF systems has been improved upon by focusing on the processing of biological feedstreams, specifically whole yeast cells *Saccharomyces cerevisiae* and the filamentous actinomycete *Saccharopolyspora erythraea* CA340. The publication of this thesis as well as journal articles and conference presentations has added to the existing knowledge base.

7.3 Future work

This study has focused on the basic operation of the PallSep PS10 and its performance during the processing of a model biological feedstream and real fermentation broths. It is advised that any future work should expand upon these foundations. The proposed future work can be divided into two broad categories.

7.3.1 Future work based on fundamentals

Examination of the flow patterns through the membrane head by CFD as well as a study of the exact nature of the shear rate at different places within the membrane head would allow better understanding of the VMF system. The construction of a rig to allow visualisation of the inside of the membrane head during operation would allow the CFD predictions to be assessed.

Currently, broth volumes in excess of 15 L are required in order to gain meaningful data from the PS10. The design of a scale down mimic allowing faster evaluation of broth type and operating conditions would be beneficial.
7.3.2 Future work based on industrial applications

A more in depth study of fermentation microfiltration interactions should focus on the effect of membrane material on microfiltration performance in terms of protein transmission and permeate flux. The influence of fermentation media type on VMF performance should also be considered. The next generation of antibiotics will come from genetically modified organisms. Preliminary work on a triketide lactone producing strain of one of these organisms has shown it to be less mechanically stable than the unmodified version. The implications of this for microfiltration performance should be examined.

The design of the membrane head should be assessed. This work has shown that by increasing the spacing between the membranes, and improvement in filtration performance results suggesting that the current membrane head geometry may not be the most efficient design. The effect of varying the radius of the membranes themselves as well as the frequency at which they are operated should be investigated.
8 References


8. References


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Leach G C. 2003. Personal communication.


Nabais A M A and Cardoso J P. **1999.** Purification of benzylpenicillin filtered broths by ultrafiltration and effect on solvent extraction. *Bioproc. Eng.* **21:** 157 – 163.


8. References


Appendix I: Calculations

I.1 Calculation of shear rate without vibration

Using the equation of Porter (1972), shear at the membrane surface without vibration can be calculated using Equation [I.1].

\[ \gamma = \frac{6U}{b} \]  

[I.1]

Where \( \gamma \) is the shear rate (s\(^{-1}\)), \( U \) is the average velocity of the fluid (0.2 m s\(^{-1}\) at the entry to the membrane head and 0.04 m s\(^{-1}\) at the exit of the membrane head) and \( b \) is the channel height (1.4 \( \times 10^{-3} \) m with one membrane spacer element).


Using the equations of Al Akoum et al. (2002), maximum and average shear at the membrane surface can be calculated using Equations [I.2] and [I.3] respectively.

\[ \gamma_{w_{\text{max}}} = 2^{3/2} d \left( \frac{\pi F}{2} \right)^{3/2} \nu^{-1/2} \]  

[I.2]

Where \( \gamma_{w_{\text{max}}} \) is the maximum shear rate (s\(^{-1}\)) at the periphery of the membrane disc, \( d \) is the membrane head displacement (0.0195 m), \( F \) is the frequency of vibration (55.71 Hz) and \( \nu \) is the fluid kinematic viscosity (1.01\( \times 10^{-6} \) m\(^2\) s\(^{-1}\)).

\[ \bar{\gamma}_w = \frac{2^{3/2} \left( R_2^3 - R_1^3 \right)}{3\pi R_2 \left( R_2^2 - R_1^2 \right)} \gamma_{w_{\text{max}}} \]  

[I.3]

Where \( \bar{\gamma}_w \) is the mean shear rate (s\(^{-1}\)) over the membrane area, \( R_1 \) is the membrane inner radii (0.047 m), \( R_2 \) is the membrane outer radii (0.135 m) and \( \gamma_{w_{\text{max}}} \) is the maximum shear rate at the periphery of the membrane disc (6.3\( \times 10^4 \) s\(^{-1}\)).
I.3 Calculation of shear rate with vibration by Method 2 - Hurwitz (2001)

Using the equation of Hurwitz (2001), the average shear at the membrane surface can be calculated using Equation [1.4] assuming a membrane head amplitude of 19.5 mm at 55.51 Hz.

\[ \dot{\gamma}(0,t) = \omega r \Omega \sqrt{\frac{\omega \rho}{\mu}} \]

[1.4]

Where \( \dot{\gamma} \) is the shear rate (s\(^{-1}\)), \( \omega \) is the frequency (0.14 radians s\(^{-1}\)), \( r \) is the radius (0.135 m), \( \Omega \) is the angular velocity amplitude (348 radians s\(^{-1}\)) and \( \rho \) is the process fluid density (1x10\(^3\) kgm\(^{-3}\)).

I.4 Calculation of boundary layer thickness

The boundary layer thickness can be calculated using Equation [1.5].

\[ L_{BL} = -\ln(0.1) \cdot \frac{2\mu}{\omega \rho} \]

[1.5]

Where \( L_{BL} \) is the boundary layer thickness, \( \mu \) is the fluid viscosity (1x10\(^3\) Pa.s), \( \omega \) is the frequency (348 radians s\(^{-1}\)) and \( \rho \) is the fluid density (1x10\(^3\) kgm\(^{-3}\)).
Appendix II: Box - Behnken design

Box - Behnken designs were developed in 1960 by Box and Behnken as a means of fitting second order response surfaces. The design is based on the construction of balanced incomplete block designs. For example, a balanced incomplete block design with three treatments and three blocks is given by Table II.1.

<table>
<thead>
<tr>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>Block 1</td>
</tr>
<tr>
<td>Block 2</td>
</tr>
<tr>
<td>Block 3</td>
</tr>
</tbody>
</table>

Table II.1 – Representation of a balanced incomplete block design with three treatments and three blocks. An example of such a design is given in Section 4.1.1.

The pairing together of treatments 1 and 2 in Table II.1 symbolically implies, in the response surface setting, that design variables $X_1$ and $X_2$ are paired together in a $2^2$ factorial (scaling ±1) while $X_3$ remains fixed at the centre ($X_3 = 0$). The same applies for blocks 2 and 3, with a $2^2$ factorial being represented by each pair of treatments while the third factor remains fixed at 0. As a result the K=3 Box - Behnken design, such as the one described in Section 4.11, is given by Table II.2.

<table>
<thead>
<tr>
<th>$X_1$</th>
<th>$X_2$</th>
<th>$X_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>-1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>-1</td>
<td>0</td>
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<tr>
<td>1</td>
<td>1</td>
<td>0</td>
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<tr>
<td>-1</td>
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<td>-1</td>
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<td>1</td>
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<td>1</td>
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<tr>
<td>0</td>
<td>1</td>
<td>-1</td>
</tr>
</tbody>
</table>
Table II.2 – Coded factor settings for a Box – Behnken design where \( k=3 \). The last row in the design matrix implies a vector of centre runs. An example of such a design is given in Section 4.1.1. In that case five replicate centre runs \( (X_1, X_2 \text{ and } X_3 = 0) \) are included in the design.

The design matrix shown in Table II.2 can be represented diagrammatically by Figure II.1. Notice that the design is a spherical design with all points lying on a sphere of radius \( \sqrt{2} \).

![Diagram of Box-Behnken design space (K=3)](image)

**Figure II.1** – Diagrammatic representation of the Box – Behnken design space \( (K=3) \) described by Table II.2.

In designing the experiments, the Design Expert software looks at the number of design variables being included by the operator and produces the appropriate design matrix following the rules illustrated in Figure II.2. If more than 3 design variables are required, then exactly the same procedure is followed although it is not possible to illustrate this diagrammatically.
Appendix III: Derivation of Equation [1.17]

Equation [1.17] was obtained from an internal report entitled Vibrating Boundary Layers published by Pall Life Sciences on 15th October 2001 by Dr Mark Hurwitz. The paper was passed on as a personal communication.

In the paper the author defines the shear rate $\gamma_w$ as:

$$\gamma_w = r \frac{\partial V}{\partial z}$$  \[III.1\]

Where a cylindrical coordinate system is used with coordinates $r$, $\theta$ and $z$ where the origin occupies the centre of rotation of a plate on the $z = 0$ surface and $V$ is the azimuthal velocity (m/s).

Using the formula for $V$ as:

$$V = -\omega \Delta \theta e^{-\frac{\omega \rho}{2 \mu} z} \sin \left( \omega t - \sqrt{\frac{\omega \rho}{2 \mu}} z \right)$$  \[III.2\]

Where $\omega$ is the frequency (radians s$^{-1}$), $\Delta \theta$ is the amplitude of angular amplitude (rad), $\rho$ is the process fluid density (kg/m$^3$), $\mu$ is the viscosity (Pa.s) and $t$ is time (sec).

Taken from Postlethwaite et al (2003).

Now by substituting Equation III.2 into III.1 we get:

$$\gamma_w(z,t) = -\omega r \Delta \theta \frac{\partial}{\partial z} \left[ e^{-\frac{\omega \rho}{2 \mu} z} \sin \left( \omega t - \sqrt{\frac{\omega \rho}{2 \mu}} z \right) \right]$$  \[III.3\]
After the partial differentiation we get:

\[
\gamma_w(z, t) = \omega r \Delta \theta \sqrt{\frac{\sigma_p}{2 \mu}} \left( \sin \left( \omega t - \frac{\sigma_p}{2 \mu} z \right) - \cos \left( \omega t - \frac{\sigma_p}{2 \mu} z \right) \right)
\]

[III.4]

At the surface of the plate, \( z = 0 \):

\[
\gamma_w(0, t) = \omega r \Delta \theta \sqrt{\frac{\sigma_p}{2 \mu}} \left( \sin(\omega t) - \cos(\omega t) \right)
\]

[III.5]

Now it may be shown that:

\[
\sin(\omega t) - \cos(\omega t) = \sqrt{2} \cos(\omega t + \pi / 4)
\]

[III.6]

So:

\[
\gamma_w(0, t) = \omega r \Delta \theta \sqrt{\frac{\sigma_p}{\mu}} \cos(\omega t + \pi / 4)
\]

[III.7]

Therefore the magnitude of the shear rate can be described as:

\[
|\gamma_w(0, t)| = \omega r \Delta \theta \sqrt{\frac{\sigma_p}{\mu}}
\]

[III.8]