EVALUATION OF A 'DEFOULING ON DEMAND' STRATEGY FOR THE ULTRAFILTRATION OF BROWN WATER USING ACTIVATABLE ENZYMES

Thesis

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ABSTRACT

New approaches to the application of membranes for the production of potable water are constantly being sought after in anticipation of future demands for increasingly rigorous water quality standards and reduced environmental impact. A major limitation, however, is membrane fouling, which manifests itself as a continual reduction in flux over time and thus restricts the practical implementation to restore flux. Mechanical and chemical methods have been implemented to restore flux to ultrafiltration systems, but these either result in a break in the process operation or lead to membrane damage or additional pollution problems. This project was aimed to develop a 'defouling on demand' stategy for cleaning membranes used during brown water ultrafiltration. The process involves the use of activatable peroxidase enzymes, which were immobilised onto flat sheet polysulphone membranes. Following flux decline which reaches a critical level with the build-up of the foulant layer, the immobilised enzyme layer was activated by the addition of a chemical activator solution, in this case hydrogen peroxidase and manganous sulphate. Manganese peroxidase was found to be the most effective enzyme at alleviating fouling by degrading the foulant layer formed on the membrane surface and hence restored flux to the ultrafiltration system. A 93% flux improvement was observed when manganese peroxidase was activated when 800μM manganous sulphate, 100mM hydrogen peroxide were added in the presence of a manganese chelator, lactate. The concept and the potential benefits this system holds will be discussed in further detail.

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ABBREVIATIONS

A = membrane area (m^2)
C = volume of solid particles retained per unit filtrate
L = membrane thickness
$\mathbf{K_S}$ = Standard blocking model
$\mathbf{K_C}$ =Cake filtration model
m = mass ratio of wet to dry cake
$\mathbf{Q_o}$ = initial flow rate
$\mathbf{R_m}$ = membrane resistance
s = mass fraction of solids in slurry
t = time (s)
V =Volume (m^3)

k= proportionality constant
n= proportionality constant
α = cake specific resistance (m/kg)
γ = filtrate density (kg/m^3)

BOD- Biological Oxygen Demand

COD- Chemical Oxygen Demand

DOC- Dissolved Organic Carbon

FA- Fulvic Acids

HA- Humic Acids

HRP- Horseradish Peroxidase

$\mathbf{H_2O_2}$- Hydrogen Peroxide

MnP- Manganese Peroxidase

$\mathbf{MnSO_4}$- Manganese Sulphate

MWCO- Molecular Weight Cut-Off

SBP- Soybean Peroxidase

SEM- Scanning Electron Microscope

P. chrysosporium - *Phanerochaete chrysosporium*

PM-30- Polysulphone Membrane (molecular weight cut-off 30 000)

PM-100- Polysulphone Membrane (molecular weight cut-off 100 000)

PM-300- Polysulphone Membrane (molecular weight cut-off 300 000)

TOC- Total Organic Carbon

PUBLICATIONS

Patents

- RSA patent application No. 97/7805 in the name of Water Research Commission- 'Defouling membranes' Completed under No. 98/7763.

- RSA patent application No. 98/7763 pending.

- Austrialian patent application No. 81964/98 request for exam filed.

Reports

Leukes W.D., Buchanan K. and Rose P.D. (1999). The development of a 'defouling on demand' strategy for operation of ultrafiltration membranes. Interim Report. Project No. 932. Water Research Commission, Pretoria.

Publications

Leukes W.D. (1998). Defouling on demand- a new strategy for potable water production. Internation Newsletter Membrane Technol. 102: 11-12.

Conference Proceedings

Buchanan K., Jacobs E.P., Rose P.D. and Leukes W.D. (1997). Evaluation of a 'defouling on demand' strategy for the ultrafiltration of river water using immobilised activatable enzymes. 2nd WISA Mtd Workshop. Badplaas, Mpumalanga, South Africa.

Buchanan K., Jacobs E.P., Rose P.D. and Leukes W.D. (1998). Defouling on demand: The application of immobilised activatable enzymes and their defouling potential during river water ultrafiltration. WISA'98 Proceedings. Cape Town, South Africa.

CHAPTER 1

WATER PURIFICATION AND MEMBRANE TECHNOLOGY

1.1 INTRODUCTION

Although water covers 70% of the surface of the earth, only a small percentage is directly available for use by man, either directly or after simple treatment. The general consensus defined by the resolution from the United Nations Conference at Mar del Plate (1977) was that "All people have the right to have access to drinking water in quantity and of a quality equal to their basic needs". Despite the efforts which have been made, this goal has not been reached. Today a third of the world's population does not yet have access to safe drinking water and some 50 000 deaths occur every day from water-borne diseases (Giacasso, 1996).

The rapidly expanding population of the world is placing a great strain on available water supplies (Singh, 1996a). During the next 30 years, the world population is projected to escalate, to over 8 billion people, which is expected to result in an increase in the demand for water of more than 650% (Giacasso, 1996). As water consumption for domestic and industrial use increases, the quantity of treated water entering the environment will also increase. The past decade witnessed rising environmental pollution together with substantial progress in membrane technology designed to address this problem. The constant contamination of surface water resources by industrial and agricultural organic

pollutants, and the increasingly stringent water quality standards now being imposed for both domestic and industrial purposes, has placed a greater emphasis and need for advanced water treatment techniques (Aim *et al.*, 1993; Pervov *et al.*, 1996; Singh, 1996a).

1.2 ORGANICALLY COLOURED WATER

Organic colour is usually associated with soft surface waters with little or no alkalinity (Swartz and de Villiers, 1996). Organically coloured water is mainly found in mountainous regions where water streams pass through the soil and over the decaying vegetation, resulting in the accumulation of tannins and humic substances in the water (Swartz and de Villiers, 1996; Nystrom *et al.*, 1996). Tannins are complex, water soluble, naturally occurring polyphenols which are found extensively in higher plants (Makkar *et al.*, 1993). Tannins can be divided into two distinct groupings: hydrostable and condensed tannins. Condensed tannins, or non-hydrostable tannins, are polymers of catechins, leukoanthocyanidins or anthocyanidins, whereas hydrostable tannins are polyphenolic compounds which upon acid hydrolysis form gallic acid and/or a hexahydroxydiphenic acid (Archambault *et al.*, 1996; Lorusso *et al.*, 1996). Condensed tannins are the most naturally abundant tannins found in plant material and in the aquatic environment and are a major problem in river pollution (Mahaderan and Muthukumar, 1980).

Humic substances constitute between 50 and 80% of the total organic carbon in aquatic systems (Fustec *et al.*, 1989; Jucker and Clark, 1994). Humic substances in general are complex, hardly separable mixtures of similar organic macromolecules with a broad spectrum of functional groups, substructures and molecular weight distributions which strongly depend on the nature of origin (Haslam, 1993; Aster *et al.*, 1996).

Humic substances are important with respect to water treatment due to their role as precursors in the formation of chlorination by-products such as trihalomethanes (THM's) as well as their role in the concentration and transport of inorganic and organic pollutants (Collins *et al.*, 1986). In addition to this, if humic substances are present in surface waters in high concentrations, they give water a yellowish brown colour (Nystrom *et al.*, 1996).

Colour, taste, odour and turbidity are a primary concern when water is supplied from a raw water source for human consumption. Clear and odourless water is generally considered to be a minimum requirement when water is to be used for drinking purposes. It is therefore essential for the organic colour to be removed from drinking water as it is found to be visually unacceptable and results in aesthetic pollution (Mittar *et al.*, 1992; Wnoroski, 1992; Swartz and de Villiers, 1996).

The amount of humic substances, and therefore organic colour present in surface waters, is associated with the soil conditions and the type of vegetation, such as fynbos, which is prevalent in the region concerned. This constitutes a problem especially in the Western and Southern Cape. The quantities of humic substances will also vary according to the season (Nystrom *et al.*, 1996). In South Africa humic substances have been recorded in amounts as high as 1000 mg/L as Pt, while general guidelines issued from the World Health Organisation recommends that water which is intended for human consumption should not have a humic concentration exceeding 15 mg/L as Pt (Swartz and de Villiers, 1996).

1.3 CONVENTIONAL TREATMENT OF COLOURED WATER

Conventional methods for the treatment of water containing organic colour consists of the following main processes: the removal of colour by chemical precipitation, consisting of coagulation with pH adjustment, flocculation, settling and sand filtration, with disinfection and stabilization of the treated water. Depending on the quality of the water

to be treated it may also include the removal of iron and /or manganese (Swart *et al.*, 1996).

There are several disadvantages associated with conventional methods of treatment for potable water production. These include the variability of the raw water quality, which results in periodic poor colour removal, or overdosing of chemicals when dosing control is inadequate. Alkalinity adjustments may be needed, as with most potable water production in southern Cape regions where low carbonate alkalinity levels lead to leaching of concrete pipes, resulting in pipe fractures and high replacement costs. Over-stabilization of the treated waters lead to deposits in the pipes, resulting in higher pumping costs (Osborn, 1989; Jacobs *et al.*, 1996; Swartz and de Villiers, 1996). Filtration of organically coloured water, and in particular backwashing of these filters, has not been optimised, therefore, large quantities of water and energy for satisfactory filter operation are required. In addition, the filters do not serve as an absolute barrier for the removal of bacteria and suspended solids (Jacobs *et al.*, 1996). Chlorination of water that contains colour (as a result of inadequate colour removal upstream) results in the formation of halogen compounds, such as chloroform, that may be carcinogenic to humans. These compounds would have to be removed if chlorination is needed for disinfection of the water (Swartz and de Villiers, 1996; Owen *et al.*, 1995).

There are several other treatment processes currently used to remove natural organic material from brown water. They include the following:

- Coagulation of humic substances using aluminium salts. This is the most common method used by the water industry to remove colour and turbidity from water supplies. This process has several disadvantages in that the dosing is difficult and a large amount of sludge is produced. In addition, there is a health risk associated with the aluminium salt itself.

- ❖ Adsorption by activated carbon. This has shown only limited effectiveness in the removal of humic acids.

- ❖ Biosorption by immobilised fungi such as *Rhizopus arrhizus* is used. This method suggests that the binding of the humic acid to the fungal wall, most probably to the chitin/ chitosan components, is mediated.

- ❖ Ultrafiltration, nanofiltration and reverse osmosis is one of the more modern techniques to remove natural organic matter from coloured water supplies. (A. Maartens, 1998, pers. comm.).

1.4 MEMBRANE TREATMENT AS AN ALTERNATIVE

There are numerous applications in industry where conventional processes such as sedimentation, conventional filtration and centrifugation do not give the required particle separation (Rencken and Strohwald, 1996). In considering the provision of potable water to small developing communities, smaller water treatment units offer a significant advance over conventional water treatment plants (Pillay *et al.*, 1996). Increasing attention has been directed to membrane processes for low volume applications, such as water treatment. This has been fueled by several factors including anticipation of higher standards for disinfection, potential lower capital costs for membrane treatment facilities, continual improvements in membrane technology and increased marketing of membranes by manufacturers (Laine *et al.*, 1989).

Membrane filtration processes offer an advanced technology for water treatment in terms of simplicity of operation, maintenance and lower energy requirements (Wetterau *et al.*, 1996). A typical procedure would include prefiltration with metal strainers to remove particulate matter, a single step clarification and sterilisation operation by membrane filtration and finally, residual-level chlorination as a precaution against contamination of

the distribution stream. The need to investigate alternative water treatment methods is essential, especially in developing, rural and farming communities that do not have access to municiple water supplies (Jacobs *et al.*, 1996).

The advantages membrane technology possesses over conventional methods includes superior quality of treated water, reduced environmental impact of effluents, reduced land requirements and the potential for mobile treatment plants (Owen *et al.*, 1993). Higher productivity leads to a more compact system, easier control of operation and maintenance and the use of fewer chemicals. In addition, there is less sludge produced (Nakatsuka *et al.*, 1996). The application of cost-effective and energy efficient water treatment such as membrane separation processes are currently being utilised on an increasing scale (Singh, 1996b).

1.5 ULTRAFILTRATION

Ultrafiltration has become an important industrial operation in several areas of application (Opong and Zydney, 1991). Desalination and water treatment by reverse osmosis is probably the earliest and best known application. The use of membrane processes for drinking water production has, until recently, been limited to desalination of sea and brackish water (Baker *et al.*, 1995). Today membrane units by their capital and operational costs have become more competitive and have been determined effective treatment to provide safe drinking water for general public consumption (Wetterau *et al.*, 1996). For this reason membrane filtration originally used for producing ultra-pure water, is now replacing conventional processes in potable water treatment, where it clarifies and disinfects in one step. (Gandini *et al.*, 1996; Kaiya *et al.*, 1996).

Ultrafiltration is defined as a pressure driven membrane separation process in which the retention of the molecules is defined primarily by the shape and size of the solutes relative to the pores in the membrane (Tutunjian, 1988; Nilsson *et al.*, 1988). In

ultrafiltration the size of the particles to be rejected are in the range of 1-50nm with a membrane pore size in the range of 0.001-0.02μm (Himelstein and Amjad, 1985). Ultrafiltration and reverse osmosis constitutes the first of the continuous processes for water purification which do not involve phase change or interphase mass transfer (Porter, 1986). Ultrafiltation can be regarded as a method for simultaneously purifying, concentrating and fractioning of macromolecules or fine colloidal suspensions, and it readily removes components that contribute to colour and turbidity in process water (Jacobs *et al.*, 1997). The process of ultrafiltration is regarded as a relatively simple and an efficient process which requires no chemical addition and only requires small energy inputs (Tutunjian, 1988; Vigneswaran *et al.*, 1996).

Although filtration has been considered to be a mature technology, many limitations of the process persist (Miller *et al.*, 1993). The flux of pure water through a membrane is directly proportional to the applied pressure (Mulder, 1995). For other liquids, however, this proportionality does not exist, and the permeate flow may be as low as 20% of that of pure water. The flux rate usually declines very rapidly in the initial stages, eventually reaching a near stable flux where the rate of decline is much lower (Scott *et al.*, 1994). The steady state flux values are a function of the nature of the feed (viscosity, solute concentration, diffusivity, pH and ionic strength), operating conditions such as fluid shear rate at the membrane surface and transmembrane pressure drop (Robertson and Zydney, 1990; Singh, 1996b). One of the most serious operational constraints limiting the widespread application of ultrafiltration membranes, especially in multi-component feed streams, is what is collectively known as membrane fouling (Turker and Hubble, 1987; Song, 1998). Membrane fouling is characterized by low productivity which manifests itself in a continual reduction in flux of the process fluid. This flux decline can not be reversed by altering the operating conditions and is, therefore, considered to be the most significant explanation for why ultrafiltration has not yet fulfilled its promise as an efficient and economical method for water and wastewater treatment (Laine *et al.*, 1989; Bhattacharjee and Bhattacharya, 1993; Wetterau *et al.*, 1996).

1.6 MEMBRANE FOULING

Fouling occurs when rejected solids are not transported from the surface of the membrane back into the bulk stream. As a result, both the dissolved salts and suspended solids accumulate at the membrane surface. The deposition of solids on the membrane causes an increase in resistance of water flow to the membrane and inhibits diffusion of dissolved solids from the membrane surface. The causes of fouling vary depending on the nature of the solute and solute-membrane interactions (Fane and Fell, 1987).

Fouling is a major obstacle to the economic implementation of ultrafiltration for the purification and recovery of water from biological effluents, as it causes a decrease in the separation efficiency and quality, and prolongs the operating time of a given process (Graham *et al.*, 1989; Pervov *et al.*, 1996). This results in higher capital costs as premature membrane replacement costs in the region of 10-30% of the installed costs for reverse osmosis plants and 75% for ultrafiltration and microfiltration plants (Singh, 1996b).

The process performance of ultrafiltration systems is not related to the intrinsic membrane properties, but the efficiency is restricted by one or more of the following mechanisms:

- Accumulation of solute near the membrane surface (gel polarization);
- Gradual irreversible changes to the polarised layer (such as cake formation);

❖ Adsorption/ deposition of solutes on the membrane surface. (Dejmek and Nilsson, 1989; van den Berg and Smolders, 1990; Carlsson *et al.*, 1998).

As a result there is a decrease in the driving force for ultrafiltration or an increase in the resistance against the transport of the permeating solvent during the filtration process.

1.6.1 Concentration Polarisation

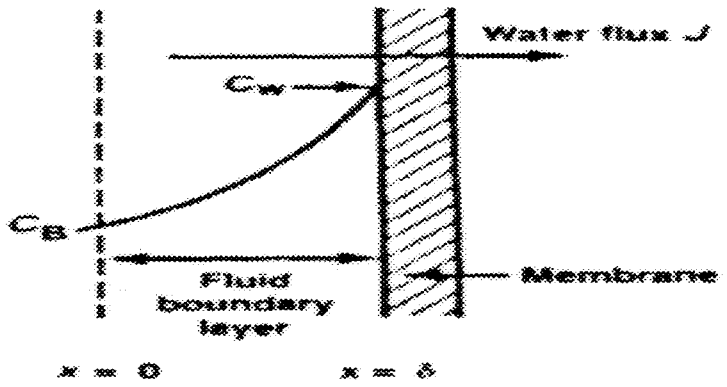

Figure 1.1: Concentration Polarisation during ultrafiltration. C_w is the solute concentration at the membrane surface and C_B is the bulk solute at the membrane surface. δ is the boundary layer thickness.

Membranes are porous, therefore, under pressure which is applied to the system, there is a continuous convective flow of solvents towards the membrane surface. The macromolecular solutes are retained by the membrane and accumulate at the upstream surface of the membrane where the concentration and resistance gradually increases (Fane *et al.*, 1981; Serra *et al.*, 1998). The variation of solute concentration across the concentration polarisation layer implies the existence of a density variation so that the solution density at the membrane surface is higher than in the bulk solution (Song and

Elimelech, 1995; Youm *et al.*, 1996). The concentration gradient creates additional resistance to the fluid flow through the membrane.

The severity of concentration polarisation depends on the distribution of the feed flow over the membrane surface, the geometry of the membrane module and the nature of the solutes and other variables such as pH, ionic strength, solution temperature and operating pressure (Jonsson and Tragardh, 1990; Bader and Veenstra, 1996).

1.6.2 Adsorption and Precipitation of Solute on the Membrane Surface.

Adsorption begins before the pressure has been applied and as soon as the top surface of the membrane is in contact with the macromolecular solution. Solute molecules adsorb to the membrane surface by physico-chemical interactions, such as: hydrophobic interactions, polar interactions and charge transfer, which results in the blockage of membrane pores (Nilsson, 1988; Mulder, 1995). The extent of the adsorption depends on the affinity between solute molecules and the material of the membrane surface. The process generally occurs within the first 10 minutes after which a slow flux decline is evident as a result of the gel-layer formation (Chen *et al.*, 1992). If the build-up of solutes on the membrane is significant, it may act as a secondary membrane and change the net transport and sieving properties of the system and affects filtration yields (Scott *et al.*, 1994).

Concentration polarisation and fouling are not completely independent of each other since fouling can occur as a result of the polarisation phenomenon. Concentration polarisation occurs immediately once the process has begun but is a reversible occurrence (Deqian, 1987). This will eventually reach a point of equilibrium. Concentration polarisation plays an important role as it is often the first step towards irreversible fouling (Airey *et al.*, 1998). Several methods have been developed to reduce the phenomenon of concentration polarisation. This includes pretreatment, modifications of surface

characteristics of the membrane and provision of good fluid management by increasing cross flow velocity (Youm *et al.*, 1996). As an example of good fluid management Youm *et al.* (1996) found that by changing the gravitational orientation or angle of the membrane module, the density inversion in which the higher density solutions overlay lower density solutions was obtained. In this way the density inversion creates an unstable fluid behaviour or 'natural convection' flow which was effective in reducing concentration polarisation and fouling as it promotes mass transfer from the membrane surface to the bulk solution.

In ultrafiltration for drinking water, the pressure and feed concentrations are low and the build up of a gel layer is not considered to be significant. The irreversible fouling is therefore due primarily to the formation of a cake layer on the membrane surface (Jones *et al.*, 1993). In ultrafiltation, two modes of operation exist. The process can either be performed in a dead-end mode (Figure 1.2) or a cross-flow mode (Figure1.3).

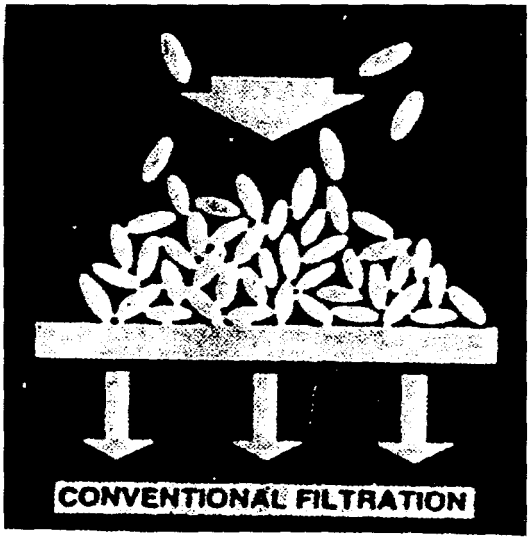

Figure 1.2: Dead-end mode of ultrafiltration showing the build up of retained particles due to the perpendicular flow of suspended particles.

In dead-end filtration the feed flow is perpendicular to the membrane surface. As a result the rejected solutes tend to accumulate at the upstream surface of the membrane surface which offers additional hydraulic resistance to the fluid flow through the membrane (Porter, 1972; Parnham and Davis, 1995).

The thickness of the cake layer increases with filtration time and consequently the permeation rate decreases. The pressure drop across the layer similarly increases with time (Serra *et al.*, 1998). The problem may be overcome by stirring the feed solution to reduce this build-up, although this is not always possible in the apparatus used. Dead-end filtration is, therefore, more suited for the treatment of clean liquids or for the disposal of solids.

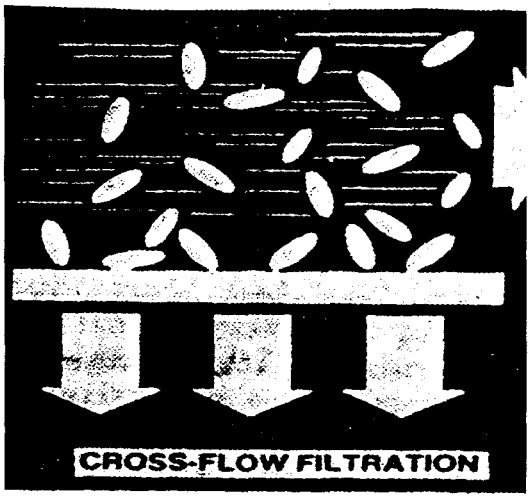

Figure 1.3: Cross-flow mode of ultrafiltration where the retained particles are recirculated over the membrane surface, minimising the accumulation of retained particles at the membrane surface.

In cross-flow filtration the accumulation of retained particles at the membrane surface does not occur to the same extent. The cross-flow mode is a better method to reduce the possibility of compounds clogging the membrane, since the feed solution is pumped at a high speed parallel to the membrane surface, and at right angles to the permeate flow direction (Liberge *et al.*, 1994; Bowden, 1993). Solids deposited on the membrane surface are thus sheared off and carried with the feed solution (Eykamp, 1995). The tangential movement of the process fluid, in the cross-flow motion, removes most of the rejected material that accumulates at the membrane surface. As a result the thickness, to which the gel-layer can build up, is minimised (Lojkine *et al.*, 1992). Initially, the flux will decrease rapidly, but a steady-state gel-layer thickness is rapidly attained. The pressure drop follows the same trend if secondary resistance due to adsorption does not prove to be predominant. The thickness of this initial layer is a function of the membrane permeability, cross-flow velocity and process fluid (Dornier *et al.*, 1995).

Even in the cross-flow mode, ultrafiltration membranes can and do become clogged when high load effluents are filtered. Once this deposition of compounds on the membrane surface becomes irreversible, this phenomenon is known as membrane fouling (Fane, 1986). Due to the high replacement costs of ultrafiltration membranes, fouled membranes have to be cleaned. If a foulant is permitted to accumulate beyond established guidelines, irreparable damage may be done to the membrane. A primary foulant layer can inhibit back diffusion of soluble ions and lead to secondary fouling that has even more serious consequences. Formation of a cake layer shows compaction which causes progressive decrease in the porosity of the cake layer. In many cases the foulant layer determines the cut-off of the separation instead of the membrane itself, thus there is an increase in the cleaning efficiency of the water that passes through the membrane as it acts as an additional filter in the system (Scott *et al.*, 1994). Periodic cleaning of a membrane to remove accumulated foulants is essential to maximise membrane life and to maintain the permeability and selectivity of the membrane process (Graham *et al.*, 1989; Bartlett *et al.*, 1995).

1.7 REDUCTION OF FOULING

Optimisation of membrane cleaning procedures requires an understanding of the complex interactions between foulants and the membrane (Kim *et al.*, 1993a). The most effective way to minimise fouling will depend on the nature of the fouling process. Research on fouling has always been a primary concern in membrane science and the focus has been centered around the prevention of fouling (van Boxtel and Otten, 1993; Dekker and Boom, 1995).

Research has been approached from several directions including pretreatment of the effluent. This has been found to be the most effective way of minimising fouling which results from specific membrane-solute interactions. Pretreatment can be mechanical, thermal or chemical (Tragardh, 1989). Additional methods used to reduce fouling include the production of modified membranes, optimisation of operational conditions, membrane characteristics and the use of high-quality rinse-water (Maartens *et al.*, 1996a). These have yielded reasonably successful results but at fairly high costs. An alternative approach to reducing the problem of fouling to acceptable levels is to introduce extensive, but simple cleaning protocols.

1.7.1 Membrane Properties

Modification of the membrane surface to minimise the protein/ surface interaction is one option available to minimise fouling (Brink and Romijn, 1990). Membrane properties can be altered by modification before filtration. Important results of this research concern the development of new membrane materials and membrane coating with a low affinity for product components (van Boxtel and Otten, 1993). These effects may be permanent, with techniques such as polymer blending and surface modifications, or reversible by creating dynamic membranes (Rucka *et al*, 1996). Hydrophilic membranes, such as regenerated cellulose acetate, are less susceptible to irreversible fouling than hydrophobic

membranes, such as polyvinylidene fluoride (PVDF), polysulfone, polyethersulfone and polypropylene (Jonsson and Tragardh, 1990; van den Berg and Smolders, 1990). The influence of the membrane material on the fouling of the membrane shows that the flux of hydrophilic membranes is marginally reduced, whereas the flux reduction of hydrophobic membranes is significant. Hydrophilic membranes have superior properties with regards to fouling, but hydrophobic membranes are still commonly used in ultrafiltration installations. This is because of the higher chemical resistance of hydrophobic membranes (Jonsson and Jonsson, 1995). Cellulose acetate membranes are less durable than noncellulose membranes and operate within a very narrow pH range of 3-8 and a maximum temperature range of 30-40°C while hydrophobic polysulfone membranes have a broad pH range 1-13 and can withstand temperatures of up to 80°C (Tragardh, 1989; Jacobs *et al.*, 1993). In general, hydrophilic polymers have restricted chemical and thermal stability (Jacobs *et al.*, 1993).

Membranes have been grafted and coated with hydrophilic polymers or surfactants. However, changes in the membrane structure and integrity occur during these processes. Another alternative for improving membrane properties is the use of polymer blending of the original polymer and a modified, more hydrophilic one (Rucka *et al.*, 1996). A blend of hydrophobic polysulfone and polyethersulfone polymers, which give the membrane sufficient stability, and the hydrophilic polymer polyvinylpyrrolidone (PVP), which renders the membrane more hydrophilic, has been used to prepare stable ultrafiltration hydropholic membranes (Singh, 1996b).

When choosing a suitable membrane for a particular application, it is essential to choose a membrane which is resistant to the pH, temperature and chemical composition of the feed. Choice of membrane properties such as hydrophobic and hydrophilic characteristics, depending on the nature of the feed, are essential to minimise fouling (Tragardh, 1989; Mulder, 1995).

1.8 MEMBRANE CLEANING

Membrane cleaning has been achieved by mechanical, chemical and biological methods or a combination of these. The type of cleaning agent will depend on the type of foulant. It is essential to know what components of the feed stream are causing the fouling in order to develop an effective cleaning strategy. Due to the lack of information on the nature and extent of foulants on the membranes, and the lack of appropriate cleaning agents, the degree of membrane cleaning has thus been variable.

The choice of cleaning methods used depends mainly on the module configuration, the chemical configuration of the membrane and the type of foulant encountered (Mulder, 1995). In using the most appropriate and successful cleaning techniques it is essential to consider the economic aspects of the cleaning procedures including the costs of the cleaning process and the effect of the procedures on membrane life-time and efficiency (Munoz-Aguado *et al.*, 1996). The cleaning methods currently employed have been grouped as follows:

1.8.1 Hydraulic and Mechanical Cleaning

This includes backflushing, back shock treatment, foam ball swabbing, gas-liquid flushing, alternative pressuring and depressuring and by reversing the flow direction at a given frequency (Tragardh, 1989). These methods are costly and they require the membrane system to be shut down for a period of time which incurs further costs (Song, 1998). In order to reduce fouling, several declogging techniques have been investigated. The two most common methods employed will be discussed in more detail.

1.8.1.1 Backflushing

Periodical backflushing occurs when the pressure on the feed side of the membrane is released and a back pressure is applied to the support side to cause a backflow of liquid through the membrane (Aim *et al.*, 1993). Research has shown that backflushing is an effective declogging technique to remove foulants concentrated on the feed side without altering the pore structure of the membrane. The efficiency of this type of cleaning depends entirely on the type of suspension to be treated and the type of fouling it causes, but also depends on the frequency and amplitude of the pulses of reverse pressure (Tragardh, 1989). This method, however, increases the complexity of membrane filtration since the system has to be halted to restore flux (Song, 1998).

1.8.1.2 High Shear Rate Velocity

Various design features, such as cross flow filtration and careful matching of the membrane type to the feed characteristics have been found to lessen the degree of fouling (Fane and Fell, 1987; Akhtar, 1995). Cross flow filtration is a technically efficient process for the separation of colloidal and suspended particles but its application is presently limited as all systems will eventually suffer a decline in performance due to a gradual build up of foulants (Kim *et al.*, 1993c; Akhtar *et al.*, 1995; Vigneswaran *et al.*, 1996). High velocity crossflow, which increases the hydrodynamic shear, does prevent fouling but is fairly costly. If, however, fluid turbulence and shear are focussed at the membrane surface, foulants would be dispersed more effectively and the need for high-velocity flow that consumes large amounts of energy would be reduced. High-shear systems, which do this, have been developed. These include vibration, spinning disc, and spinning cylinders. These systems produce shear at rates greater than $100000s^{-1}$ compared with rates of $30000\text{-}50000s^{-1}$ for traditional crossflow systems. The new systems can be used in applications ranging from wastewater treatment to more sensitive biotechnological separations (Culkin *et al.*, 1998).

1.8.2 Electric Cleaning

A recently developed method for cleaning is the utilisation of a pulsed electric field, which results in the movement of charged particles or molecules away from the membrane. This method can be applied without interrupting the process. One of the main problems with this method is that it requires a membrane capable of conducting electrical charges or a metal membrane with a special module design. This increases the capital costs of a given process (Mulder, 1995).

1.8.3 Chemical Cleaning

Chemical cleaning agents with hydrolytic, oxidative or detergent properties are widely implemented for reducing fouling, with a number of chemicals being used separately or in combinations (Kavitskaya, 1990; Ebrahim and Malik, 1987). Acids, alkali detergents, complexing agents such as ethylenediamine tetra acetic acid (EDTA) and disinfectants such as hydrogen peroxide have been used (Deqian, 1987; Hwang *et al.*, 1998). The success of cleaning solutions relies on several parameters relating to the concentration of the cleaning solution, the temperature, pH, flow rate, circulation time which affects the overall cleaning procedure and in addition are very important in relation to the chemical resistance of the membrane (Eykamp, 1995). These methods are normally highly effective, but tend to be highly aggressive, which limits the life expectancy of the membrane. Harsh chemical cleaning agents have been found to damage membrane morphology (pore shape, pore length) and has an adverse effect on the chemical and electrical surface properties of the membrane (Swart *et al.*, 1996). They also tend to be toxic and their discharge is normally unacceptable. There is also the risk of carry over into the product water, thus their use for producing potable water for human consumption could be harmful.

1.8.4 Enzymatic Cleaning

Biological cleaning has been described as the use of cleaning mixtures that contain bioactive agents to enhance the removal of foulants (Jacobs *et al.*, 1996). Enzyme based detergents due to their substrate specificity and environmental friendliness, have found increasing application, especially on cellulose acetate membranes which cannot withstand high temperature or pH. In certain cases enzymatic cleaning agents with good dispersion and emulsifying agents, which are effective at low temperatures, are required to remove fats or to break down proteins and other high molecular weight compounds (Tragardh, 1989). The enzyme based detergents, however, have been found to have several drawbacks. Enzymes are costly and formulating them into effective cleaning agents are expensive. The catalytic activity of certain enzymes are slow and as a result long process downtime during cleaning is experienced. One of the main concerns with enzymatic cleaning is that deleterious enzyme activity or proteolytic products may be carried over into the material being processed (Deqian, 1987; Coolbear *et al.*, 1992).

Enzymes hold numerous advantages over conventional methods as they are biodegradable and do not cause additional pollution problems. Enzymes are highly specific for reactions that they catalyse and with substances with which they interact. In addition, enzymes function under mild conditions of pH, temperature and ionic strength and will not damage the membrane surface properties (Maartens *et al.*, 1996b). It is for this reason that researchers in membrane technology have re-examined the use of enzymes as possible cleaning agents for fouled ultrafiltration membranes and for the prevention of fouling by the adsorption of enzymes onto membranes.

1.9 IMMOBILISED ENZYMES IN ULTRAFILTRATION

Enzymatic cleaning has shown considerable promise in alleviating the problems associated with membrane cleaning. However, due to the cost of these enzymes, enzyme washing is not economically viable. A solution to this is to immobilise the enzyme onto the active filtration surface of the membrane so that they can be used for extended periods of time. In addition enzyme immobilisation has been found to increase enzyme stability, and due to the cost of enzymes it is a definite process advantage for their industrial application (Jenq *et al.*, 1980).

The concept of immobilising enzymes onto the membrane surface to restore flux and reduce fouling was first reported by Velicangil and Howell (1977). In this study they immobilised enzymes onto the membrane surface to hydrolyse the gel layer, thereby reducing the overall resistance to permeate flux. Howell and Velicangil (1982) developed a method to immobilise food grade proteases onto ultrafiltration membranes, thereby producing a primary adsorbed layer of enzymes, which functioned to retard the rate of gel layer formation on the ultrafiltration membrane. Results showed a 25-78% improvement in the cumulative yield. The enhanced flux observed with immobilised enzymes was modeled mathematically to incorporate the enzymatic layer counteracting the deposition of a gel layer on the membrane surface. Harris and Dobos (1989) researched the effect of enzymatic hydrolysis in protein recovery from wheat starch effluents. They found that the enzymatic hydrolysis reduced the viscosity of the gel layer, which was as a result more easily removed by shear stress.

Chen *et al.* (1992) found that the immobilisation of proteases on ultrafiltration membranes was effective in reducing fouling by the protein feed through the hydrolytic cleaning action of the enzyme and/or the hydrophobic repulsion of the enzymatic layer. The existence of the enzymatic layer formed an intermediate layer, which increased the resistance to a certain extent. They found that even when small amounts of protein

molecules precipitated onto or penetrated the immobilised enzyme layer, it was decomposed by the enzyme molecules, and the protein fractions passed through the membrane or diffused back into the bulk solution driven by the pressure of the liquid feed. In this way further adsorption of proteins onto the membrane surface and pore plugging could essentially be avoided and thus the possibility of gel formation was reduced. It was for this reason that it was felt that an enzymatic layer immobilised onto the membrane surface could ultimately improve the ultrafiltration transmembrane permeate flux (Chen *et al.*, 1992).

1.10 RESEARCH HYPOTHESIS

With the drive to cleaner technology, the use of enzymes for cleaning fouled membranes has found widespread application due to the efficiency and mild operating conditions of these biological catalysts. Immobilisation of enzymes onto the active filtration surface of the membrane is beneficial in that the enzyme can be used for extended periods. By this means expensive biocatalysts become reusable (Hicke *et al.*, 1996). The added advantage of such a system is that the foulant layer is degraded at the point of attachment, obviating the need to digest the whole foulant layer. The disadvantage of using immobilised enzymes in this manner is that they degrade the foulant layer from the membrane side and hence degrade the very macromolecules which are to be retained by the filtration membrane (Jenq *et al.*, 1980).

It was hypothesised that if activatable enzymes could be immobilised onto the surface of ultrafiltration membranes, then an ultrafiltration process could be devised in which enzymes remain attached to the membrane surface during the procedure, but remain dormant, allowing dynamic layer formation to aid the process. The enzymes can then be activated when cleaning is required to restore flux to a system. This would be a new approach in enzymatic cleaning for the regeneration of ultrafiltration membranes fouled during water purification. The process envisioned would operate as follows:

- ***Immobilisation of the Inactive Enzyme.*** Many immobilisation techniques exist which could be employed to attach the enzyme to the membrane surface. Covalent enzyme immobilisation onto a membrane pore surface is favoured if operation at high fluxes is desired. However, activity decay due to chemical inactivation of the enzyme may occur (Hicke *et al.*, 1996). For the purpose of this investigation the enzyme of choice would be immobilised by physical adsorption onto the membrane surface. Pressure can be applied during enzyme immobilisation to improve the efficiency of adsorption. Membranes with a low molecular weight cut-off would be used to ensure that the membrane would retain the enzyme. It is possible that a small portion of the enzyme adsorbed to the membrane would penetrate the pores due to the shape of the enzyme, but this was expected to be negligible.

- ***Operation of the Ultrafiltration Process***. The water purification process can be operated in either dead-end or cross-flow filtration. The ultrafiltration membrane retains microbes and large marcomolecules (mostly humic substances). A cake layer of humic substances forms over the layer of immobilised enzymes, aiding the filtration process by adsorption and entrapment of smaller molecules. Eventually the hydraulic resistance of the cake layer increases to an extent where it causes a decline in flux, resulting in unacceptably low process productivity.

- ***Activation of the Immobilised Enzymatic Layer.*** When the flux decline due to excess cake layer formation reaches a critical level, the enzymes can be activated on demand by the addition of an activator solution, containing the co-substrate, added to the feed stream. Once the enzymatic layer is activated flux restoration should theoretically occur rapidly due to the catalytic efficiency of the enzyme. The flux restoration would occur as a result of the transformation of the humic substances (and other organic polymers which are found in the river water being purified) by the enzyme action. This would allow the foulant layer to be degraded at the point of attachment and lift

off the membrane surface. Once the activator solution is exhausted, the enzymes would then return to an inactive state and the process can begin again.

The envisioned process is depicted schematically in Figure 1.4.

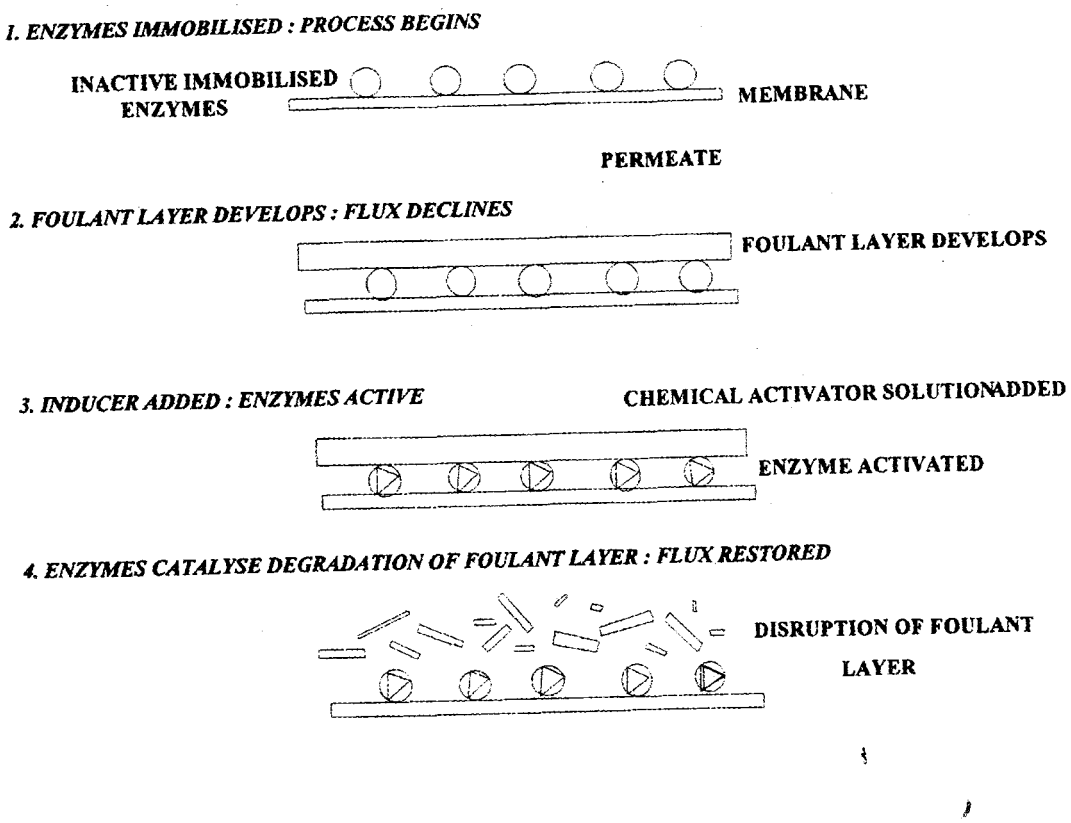

Figure 1.4: Schematic depiction of the 'defouling on demand' strategy.

The project presented here represents a novel application where activatable enzymes are utilized for the development of a 'defouling on demand' strategy. This would be an advancement on the current use of immobilised enzyme systems and given the simplicity and non-toxic nature of the process, should be considered as an enabling development in technology for the production of membrane water purification systems.

1.11 RESEARCH OBJECTIVES

The research objectives were focussed on demonstrating this hypothesis by the development of a 'defouling on demand' strategy as listed below:

- ❖ To evaluate different peroxidase enzymes in terms of availability and their ability to degrade the foulant layer.

- ❖ To perform a feasibility study of the system developed, using the most appropriate enzyme in terms of flux restoration.

- ❖ To investigate the impact of this process on various mechanisms of fouling. A study would be performed on the usefulness of this system in dealing with various forms of irreversible fouling:
  - pore plugging,
  - pore constriction,
  - gel/cake layer formation,
  - selective plugging of larger pores.

- ❖ To study the improvement of clarification performance offered by the dynamic layer formed by the immobilised layer and the deposited foulants present in the feed stream. The impact of this layer on the improvement of filtration characteristics of the membrane would also be evaluated.

CHAPTER 2

EXAMINATION OF PEROXIDASE ENZYMES IN DEGRADING THE FOULANT LAYER

2.1 INTRODUCTION

2.1.1 Choice of Enzymes

The first step in the development of the 'defouling on demand' process required the establishment of critieria for the choice of enzyme to be used. These are listed below:

- The enzyme of choice needed to be capable of degrading humic substances which were identified in earlier work as the major contributors to fouling by brown river water (Jacobs, 1997, pers. comm). It should preferentially have a broad enough substrate range to degrade organic materials that contribute to fouling, e.g. polysaccharides, lipids, and proteins.

- It needed to be inactive under the physio-chemical conditions at which the ultra-filtration process operates and needs to be activated (i.e. heat or chemical activation) by some means for defouling.

- The enzyme would have to be fairly stable for extended periods of time.

❖ It needed to be readily available.

Peroxidase enzymes were considered to be ideally suited, since they are known to have a wide substrate range, which includes phenolic compounds (such as tannins and other humic substances). They also require hydrogen peroxide as a co-factor for their activation, hence they would remain inactive during the normal operating procedure.

2.1.2 Manganese Peroxidase and Lignin Peroxidase

White rot fungi have become the focus of attention over the past 20 years because of their ability to degrade lignin. Lignin is a complex, optically active, heterogenous and random phenylpropanoid (Mino *et al.*, 1988; Gold and Alic, 1993). The irregular and recalcitrant nature of lignin and the fact that it contains a variety of bonds which are commonly present in primary aromatic pollutants, led scientists to study the lignin degradation system, which the fungi possess, for bioremediation (Barr and Aust, 1994; Paszczyski and Crawford, 1995; Bogan *et al.*, 1996a/b).

It has been found that these fungi possess enzymes which are non-specific, oxidative and have the ability to degrade a broad spectrum of structurally diverse aromatic pollutants (Tuisel *et al.*, 1990; Moreira *et al.*, 1997). Many enzymes are involved in the oxidative degradation of lignin. The most significant of these are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Bonnarme *et al,* 1993; Mayfield *et al.*, 1994).

Mechanism of action of peroxidases

The catalytic cycle of the action of plant peroxidases is depicted in Figure 2.1.

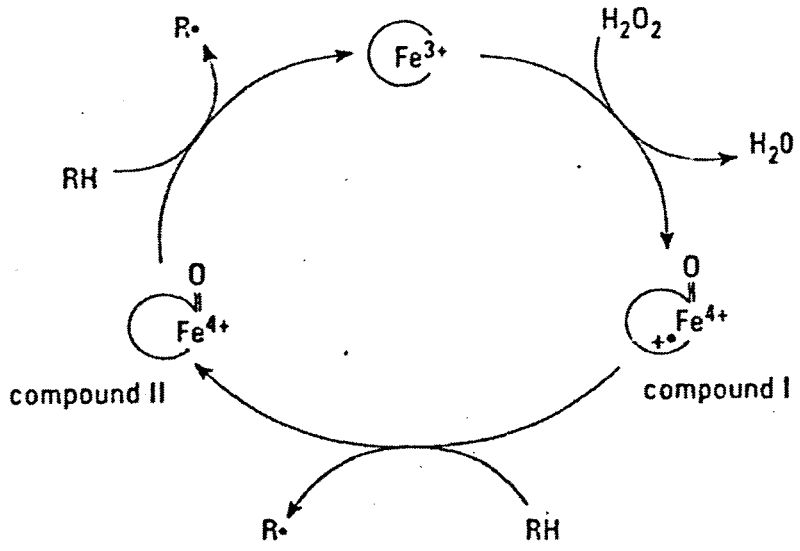

Figure 2.1: Catalytic cycle for peroxidases. The iron is in haem, photoporphyrin IX, form (Barr and Aust, 1994).

The ferric form of the enzyme, the resting form, is initially oxidised by two electrons from hydrogen peroxide to produce a form of peroxidases known as compound I. Compound I can be reduced by one electron from chemicals such as aromatic pollutants having a suitable reduction potential. The enzyme is reduced to a form called compound II, whereas the reactant chemical pollutant is oxidised by one electron to form a radical. A second molecule of the reactant chemical then donates a further electron to compound II to return the enzyme to its resting state (Aust, 1995). In this process, the aromatic reducing substrate is oxidised to an aryl cation radical (Gold and Alic, 1993). The mechanism of oxidation generally applicable to most peroxidases is shown in Figure 2.1.

LiP was discovered in 1982 as a result of a search for lignin degradation enzymes. It was the first enzyme known capable of the oxidative cleavage of C_α -C_β bonds of the non phenolic propyl side chain of certain aromatic lignin models (Haapala and Linko, 1993; Jensen *et al.*, 1994). Subsequently the extracellular enzymes secreted from the white rot Basidiomycete *Phanerochaete chrysosporium* have been reported to degrade several aromatic pollutants including DDT, polychlorinated biphenols, benzo[a]pyrene and 2,3,7-8-TCDD which are considered to be environmental health hazards and are resistant to biodegradation by most other microorganisms (Bumpus and Aust, 1990; Lamar, 1992; Sayadi and Ellouz, 1995). Humic substances have been shown to cause significant fouling to ultrafiltration membranes (Fukui, 1992; Clark and Lucas, 1998; Yoon *et al.*, 1998). The extracellular enzymes produced by *P. chrysosporium* have been found to decolourise humic acids at acidic pH (Michel *et al.*, 1991; Hofrichter and Fritsch, 1997).

P. chysosporium produced two classes of extracellular haem protein peroxidase which are involved in the degradation of lignin: LiP and MnP. LiP and MnP are actually families of isozymes, highly homologous in structure and function. LiP catalyses the one electron oxidation of various aromatic compounds. LiP is a strong oxidant and is capable of the degradation of 90% of the most recalcitrant of wood (lignin) substructures (Kersten *et al.*, 1990). The unique feature of this enzyme is that it is able to oxidise aromatic compounds with redox potentials beyond the reach of most other peroxidases (Gold and Alic, 1993).

MnP catalyses the oxidation of Mn(II) to Mn(III) which in turn can oxidize several more labile phenolic substrates and plays a significant role in the depolymerisation of lignin (Lackner *et al.*, 1991; Wariishi *et al.*, 1991; Jensen *et al.*, 1996). The manganese ion participates in the reaction as a redox couple rather than acting as an enzyme binding activator (Wariishi *et al.*, 1989).

2.1.3 Horseradish Peroxidase

Horseradish peroxidase (HRP) is one of the most thoroughly characterised of all enzyme peroxidases and is widely used in medical applications. It has been evaluated for the bioremediation of foundry effluents which have high phenolic contents (Dunford, 1991; Nicell *et al.*, 1993). HRP has been shown to oxidise phenols and aromatic amines as well as low oxidation potential methoxybenzenes and non-aromatic reductants such as dihydrofumaric acid, NADH, glutathione, cysteine and dithiothreitol (McEldoon *et al.*, 1995). HRP is readily available and acts on a wider range of substrates than other oxidative enzymes. It is for this reason that HRP has found potential application in the treatment of water and waste waters which are contaminated with aromatic pollutants (Nicell *et al.*, 1993; Joo *et al.*, 1997). HRP has also been shown to effect the decolourisation of Kraft effluent, hence should be applicable to degrade humic substances present in brown river water (Ferrer *et al.*, 1991).

The catalytic cycle of both LiP and MnP are similar to that of HRP (Wariishi *et al.*, 1989; Stewart *et al.*, 1996; Matsubara *et al.*, 1996). HRP holds several advantages over MnP and LiP in that the enzyme is less costly to produce, it is commercially available and inactivates less rapidly than other plant peroxidases (Buchanan and Nicell, 1996). The main problem is that HRP has been shown to be a poor oxidant, unlike LiP excreted from *P. chrysosporium* (McEldoon, 1995).

2.1.4 Soybean Peroxidase

Soybean peroxidase (SBP) is easy to isolate and purify. It is extracted from soybean hulls which are a byproduct of the food industry and is, therefore, economically viable to produce. SBP has a molecular weight of 37 kDa and exists as a single acidic isozyme. The presence of a single isozyme simplifies the elucidation of the substrate specificity of the enzyme. SBP is also capable of direct peroxidase oxidation (McEldoon *et al.*, 1995). SBP is highly stable and has shown extended catalytic activity even in very acidic

conditions but has a lower catalytic activity when compared to LiP. This is thought to be due to the fact that it possesses a lower oxidation potential than LiP (McEldoon *et al*, 1995).

2.1.5 Research Objectives

An ideal peroxidase for large scale biocatalysis would be one that is already abundant, possesses a wide substrate specificity, along with a high oxidation potential in order to oxidise non-phenolic compounds (such as tannins) and one which remains stable over a wide pH range. It was believed that the four enzymes mentioned above, in terms of the availability and stability, could potentially degrade the foulant layer thereby restoring flux to UF systems. The important difference between peroxidase enzymes is the nature of the reducing substate but due to availability and enzyme stability HRP, MnP, and SBP were chosen for the initial enzyme screening. LiP has been found to be unstable and difficult to isolate and was, therefore, not evaluated in this study. This chapter focussed on the screening of HRP, SBP and MnP to determine if, upon activation, the enzyme could restore flux to ultrafiltration systems.

2.2 METHODS AND MATERIALS

2.2.1 Stirred Ultrafiltration Cell Reactor

Membrane experiments were performed in a stirred ultrafiltration cell (Amicon Corp, Lexington, Model 202). A schematic depiction of the stirred cell ultrafiltration reactor system is represented in Figure 2.2.

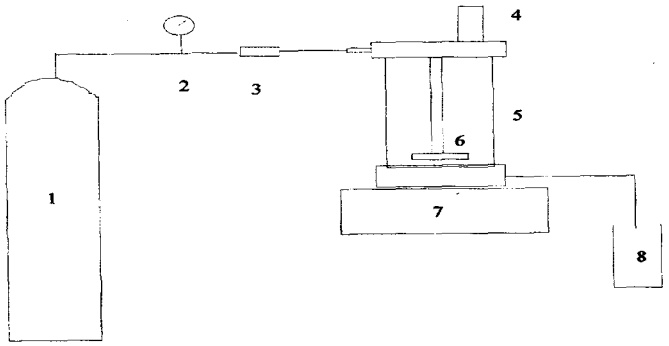

Figure 2.2: Experimental stirred cell ultrafiltration system (1. Nitrogen gas. 2. Pressure gauge. 3. Induction port. 4. Pressure release value. 5. Polycarbonate vessel (200ml capacity). 6. Agitator. 7. Magnetic stirrer table. 8. Permeation collection device).

The ultrafiltration cell is a polycarbonate cylinder with a working volume of 200mL and was capable of withstanding a pressure up to 100psi. The cell housed an internal magnetic stirrer bar (49,4mm and 8.8mm in diameter) suspended close to the upper surface of the membrane (~4mm above the membrane). A flat sheet polysulphone membrane (MWCO 30 000, supplied by the Institute of Polymer Science, Stellenbosch) with a total surface area of 27.5 cm^2 was used for each experiment. The hydraulic

pressure for ultrafiltration was supplied by nitrogen gas. All ultrafiltration batch experiments were carried out at 160kPa, stirring at 400 rpm and a constant temperature of 22°C. The permeate flow rate was measured volumetrically, every minute, for the duration of the experiment. The units of all measurements are in litres/ m^2 of membrane surface area/ hour.

2.2.2 Enzyme Preparation

2.2.2.1a Production of Manganese Peroxidase and Maintenance of Phanerochaete Chrysosporium

MnP was produced by the fungus *P. chrysosporium* BKM-F 1767 (ATCC 24745). Stock cultures of *P. chrysosporium* were maintained on yeast extract-malt extract-peptone-glucose (YMPG) slants, in one litre Roux bottles as previously described by Gold and Glenn, 1988 [Appendix 1]. The slants were incubated at 37°C for 10 to 12 days. Sufficient growth has been reported after 5 days when grown at 39°C (Tien and Kirk, 1988). The slants were then stored at 4°C.

2.2.2.1b Stationary Flask Cultures

100mL stationary cultures were grown up in one litre Fernbach flasks. 80mL growth medium and 20 m*l* spore suspension was added to each flask to make the total volume 100m*l*. The growth medium with 2% glucose as the carbon source, 1.2mM ammonium tartrate as the limiting nitrogen source and 20mM sodium 2,2-dimethylsuccinate (pH 4.5) as the buffer medium (Bonnarme and Jeffries, 1990; Brown *et al.*, 1990; Brown *et al.*, 1991) [Appendix 2] except Tween-20 was omitted. These stationary cultures were incubated at 37°C, for 48 hours, until a solid white mycelial mat was visible on the surface of the growth medium. No oxygen was supplied during incubation. The

mycelium was then blended for one minute in a Sorvall Omni-Mixer (Du-Pont instruments), and used as an inoculum for the agitated cultures.

2.2.2.1c Agitated Flask Cultures

Agitated cultures were grown in 1-litre Erlenmeyer flasks containing 375m*l* basal medium. Cultures were inoculated with a spore suspension of *P. chrysosporium* and incubated at 37°C on an agitated flat bed shaker at 180rpm (Perez and Jeffries, 1992). After 24 hours of growth, cultures were flushed daily with pure oxygen (gas flow rate, 2.5 *l*/min). The innoculum established itself as small pellets as a result of the shear, thereby increasing the surface area for oxygen exchange. MnP production is stimulated by high concentrations of manganous ions which also functions to suppress LiP production. The agitated cultures were, therefore, induced with manganous sulphate (40ppm) after 72 hours (Bonnarme and Jeffries, 1990). MnP activity peaked within 24-48 hours thereafter.

MnP activity was determined colourmetrically using a Shimadzu UV-160A spectrophotometer at 420nm where ABTS was used as the substrate as described by Gold and Glenn (1988) [Appendix 3]. One unit of activity was defined as 1 µmol of substrate oxidized in 1 minute. The protein content was determined according to the method of Bradford (1976). In each experiment 0.5U of enzyme was immobilised onto the membrane surface, by adsorption, unless otherwise stated.

After induction MnP activity peaked and the cultures were harvested. The mycelial pellets were filtered and extracellular fluid was concentrated by freeze drying. The crude extract of MnP was initally dialysed against deionised water (Milli-Q, Millipore) but a loss of activity was noted. The crude extract of MnP was, therefore, dialysed against 10mM sodium acetate (pH6) (Perez and Jeffries, 1992). After dialysis the crude extract was freeze dried and stored at -20°C.

2.2.2.2 Extraction of Soybean Peroxidase

A crude extract of SBP was prepared as follows: 20 g Soybean seed coats were soaked and homogenised in 200m*l* of 25mM potassium phosphate buffer, pH 7.5. Another 300m*l* of the above extraction buffer was added to the homogenate and stirred for 2-3 hours at room temperature. This was then filtered through a sieve to separate the plant material, then filtered through 0.45μ Whatman no.1 filter. The filtrate was subsequently precipitated with 0-30% ammonium sulphate, stirred for 30 minutes in an ice bath, and thereafter centrifuged for 15 minutes at 1 800g. The supernatant was then added to 30-80% ammonium sulphate, stirred for 2 hours in an ice bath and centrifuged for 15 minutes.

SBP activity was measured colourmetrically at 436nm using a guanicol-based assay as previously described by Bergmeyer (1974) [Appendix 4]. In each experiment 20U of enzyme was immobilised onto the membrane surface by passive adsorption.

2.2.2.3 Horseradish Peroxidase

A commercial preparation of HRP was used (Serevac, Fine Chemicals). The peroxidase activity was given by the manufacturer as 275.8 units per mg solid. In each experiment 25U of enzyme was immobilised onto the membrane surface by passive adsorption. The enzyme activity was measured as above.

2.2.3 Enzyme Immobilisation

The polysulfone membranes contained glycerol which was removed by immersing the membrane in pure water for 1 hour. Washed membranes where placed in the stirred batch ultrafitration cell and were initally flushed with deionised water to remove any residual storage solution. The crude enzyme extract was suspended in 10mL deionised water and

centrifuged (1 200g), for 1 minute, to remove suspended polysaccharides and proteins. The enzyme solution was poured into the vessel and immobilised at low pressure, 100kPa, by passive adsorption. The permeate obtained after immobilisation were assayed for enzyme activity to determine the amount of enzyme immobilised.

2.2.4 Chemical Activation

The activator solution used for the induction of MnP was 0.475mg/*l* $MnSO_4$ and 0.05%(v/v) H_2O_2 in 5m*l* deionised water. The activator solution used for both HRP and SBP was 1 m*l* of 30% H_2O_2 in 120mL of water adjusted to 0.4 absorbance units at 240nm.

A chemical activator solution was added to the ultrafiltration system to activate the enzymatic layer. The pressure applied to the stirred cell reactor was dropped and the activator solution (5m*l*) was added through the induction port after 15 and 35 minutes in each experiment.

2.2.5 Brown Water Decolourisation

The water used in these experiments was obtained from the water purification plant in George (Southern Cape, RSA). The brown water obtained had a low turbidity (70-80 NTU), a COD of 88mg/*l*, and a colour index which ranged from 400-1200 Hazen units which varied according to the season (Pretorius, 1997, per. comm.). A water profile was obtained by passing the brown water through a Sephadex G150 SEC column. The profile was taken at 285nm and 465nm. It was found that the highest concentration of humic compounds corresponded to a molecular weight marker of 46 000 Da (Claire Watcham, 1998, B.Sc. (Hons), Rhodes University).

2.3 RESULTS AND DISCUSSION

Figure 2.3 shows the typical flux decline which occurs during the ultrafiltration of brown water compared to the effect enzyme immobilisation has on flux.

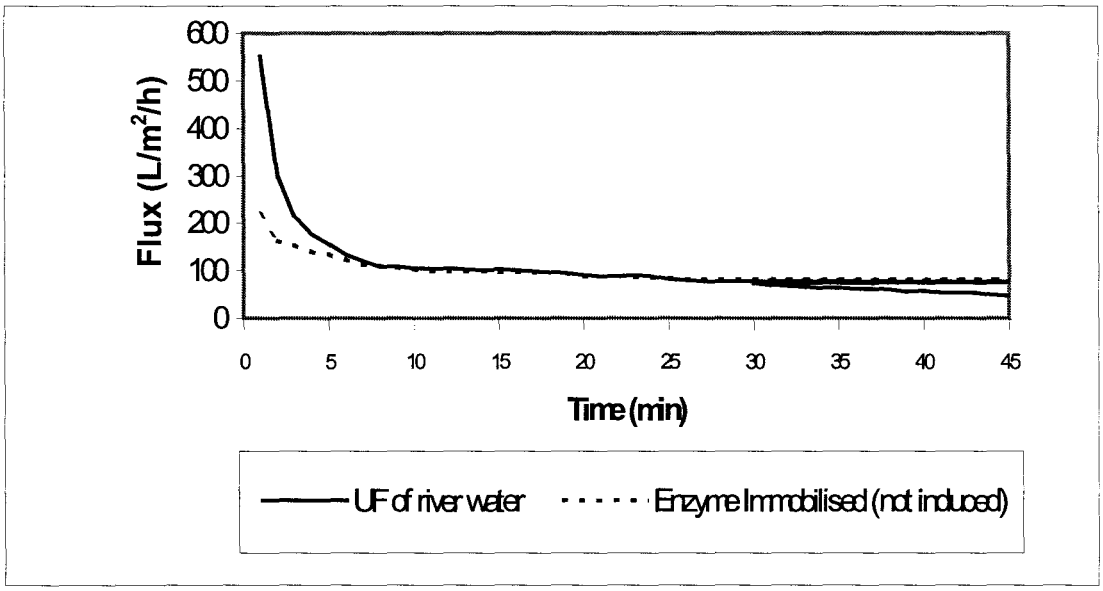

Figure 2.3: Flux decline during river water ultrafiltration comparing the effect on transmembrane flux before and after enzyme immobilisation.

The flux decline can be divided into three separate stages. It begins with a rapid initial drop in transmembrane flux, followed by a long term gradual flux decrease and ending with a steady state flux. The nature of ultrafiltration membranes, particulary low surface porosity and pore size distribution, makes them sensitive to fouling by pore blocking (Brink *et al.*, 1993). The flux decline observed during membrane filtration was a result of the increase in the membrane resistance and the development of the resistance layer, which can be described in terms of pore blocking and cake formation, respectively.

In order to compare the 'defouling on demand' strategy, it was first necessary to determine the impact of the immobilised layer on the performance of batch ultrafiltration of brown water. Table 2.1 shows a set of experiments where the enzyme was not induced.

Table 2.1: Flux profile for the ultrafiltration of brown water compared with the flux obtained when an immobilised, non-induced enzymatic layer was present (standard deviation represented, units of values given are in L/m^2/h).

TIME	Ultrafiltration of brown water	Effect of enzyme immobilisation (MnP not induced)
Initial Flux	550.8 ±168.95	217.19 ± 14.16
Flux during experiment (15 min)	95.99 ± 1.258	95.99 ± 1.137
Flux at end of experiment (45 min)	46.9 ± 8.15	78.35 ± 6.68

The table shows the flux decline profile of an ultrafiltration system for brown water decolourisation and the effect that enzyme immobilisation has on transmembrane flux. It would be expected that the enzymatic layer would provide resistance to flux therefore reducing the initial flux to 217.19 *l*/m^2/h compared to 550.8 *l*/m^2/h when no enzyme was immobilised. The presence of the immobilised enzymes results in the formation of an intermediate layer, increasing the resistance to transmembrane permeability. The initial difference in flux only lasted for approximately 5 minutes.

It should also be noted that the steady state flux values, after 15 minutes, are the same (95.99 *l*/m^2/h) and that, in fact, an improvement in flux was obtained at the end of the experiment, where the immobilised MnP layer was present 78.35 *l*/m^2/h compared to a lower flux (46.9 *l*/m^2/h) at the end of the experiment in the case where the enzyme layer was absent.

The data for flux restoration using HRP, SBP and MnP is shown in Figure 2.4, 2.5 and 2.6 respectively. In each of these, flux decline over time in the experimental system described was shown with the enzyme immobilised without induction and compared to flux values when the enzyme was induced with the appropriate activator solution after 15 and 35 minutes.

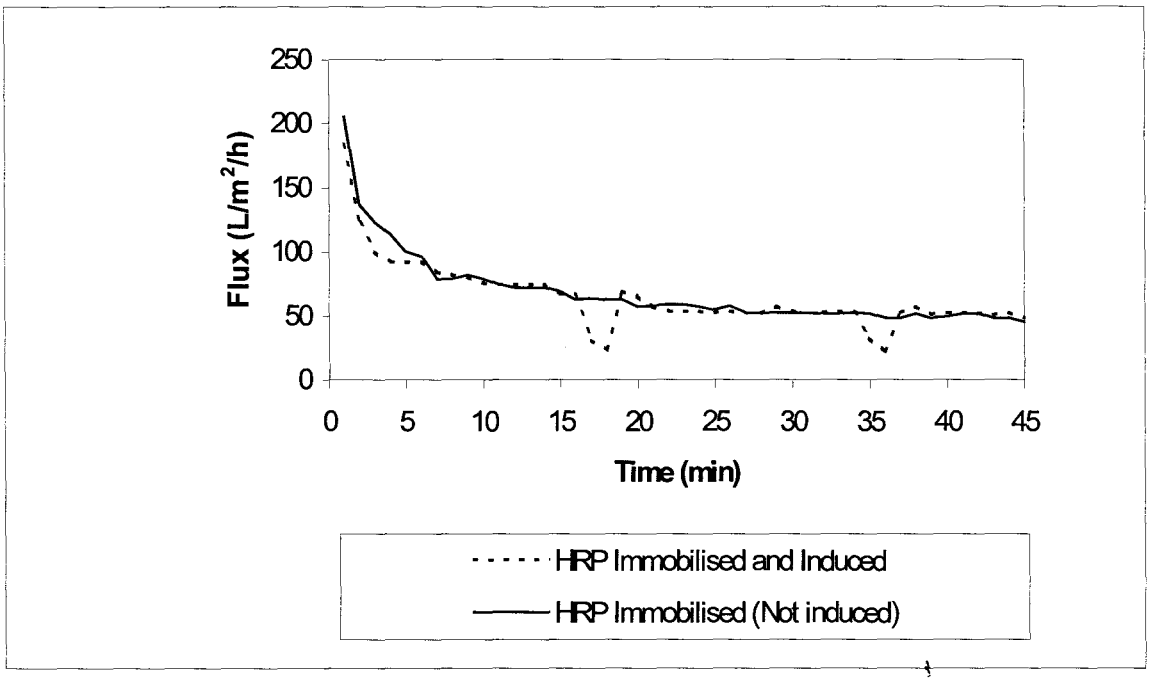

Figure 2.4: Flux recovery obtained when horseradish peroxidase was immobilised and induced.

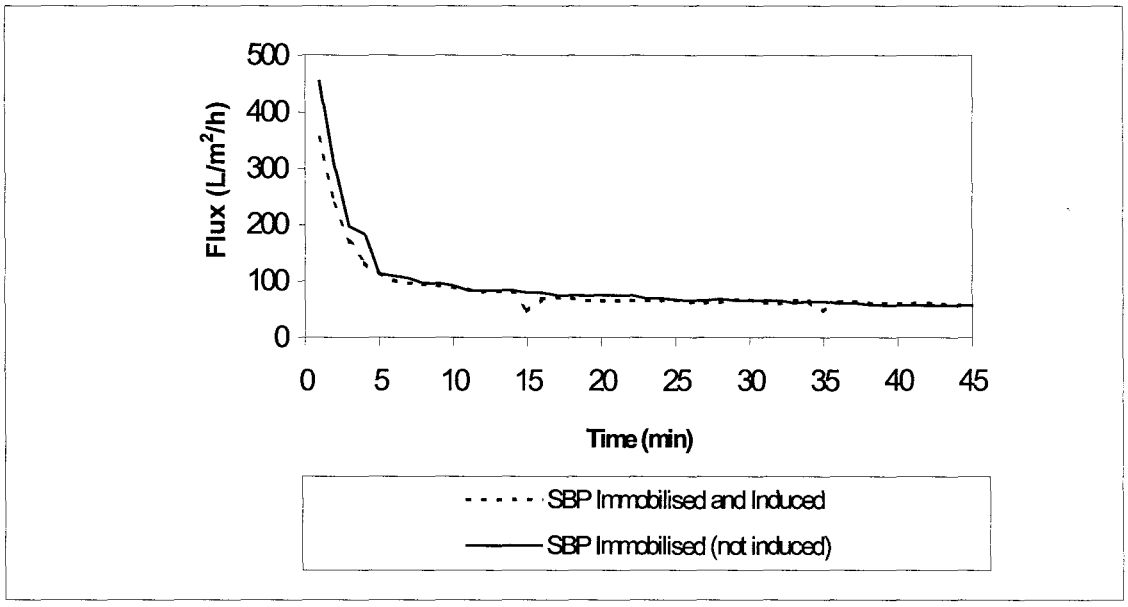

Figure 2.5: Flux recovery obtained when soybean peroxidase was immobilised and induced.

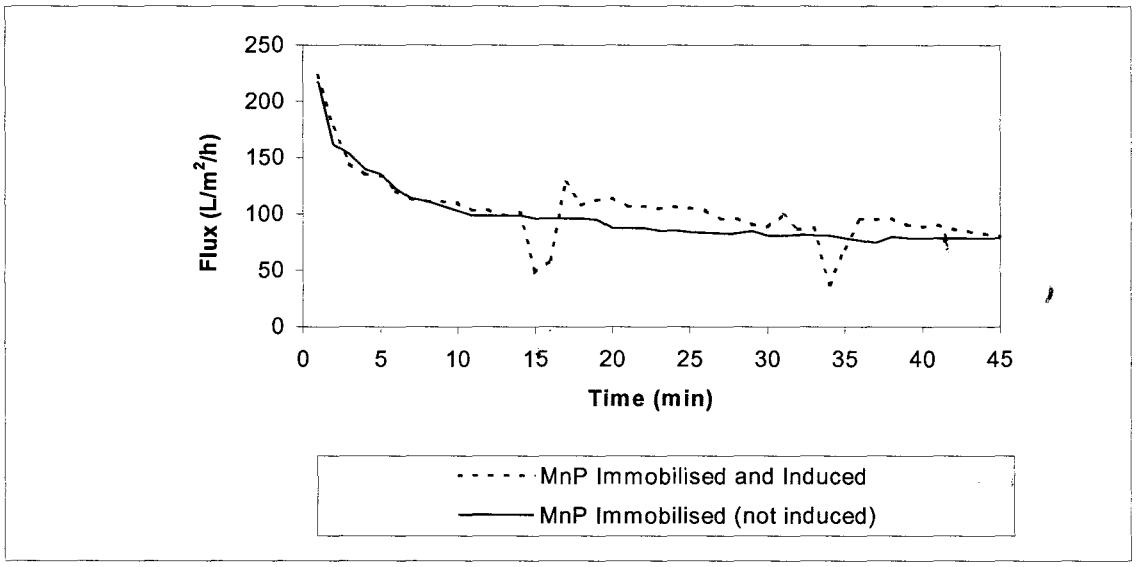

Figure 2.6: Flux recovery obtained when manganese peroxidase from *P.chrysosporium* was immobilised and induced.

Figure 2.6 represents the effect of MnP induction on flux. The rapid decline in flux seen after 15 and 35 minutes was a result of a drop in pressure. This occurred with the addition of the chemical activator solution. The subsequent action of MnP on the foulant layer was rapidly obtained, shown by the immediate flux improvement. This indicates that the activator penetrates the foulant layer quickly and was rapidly utilised by the enzyme to transform compounds of the primary foulant layer. The results show a 25% flux improvement after the first induction.

Greater improvement was observed after the first induction (15 minutes) in comparison to the second induction (35 minutes). This was expected as the retentate concentration increases during batch ultrafiltration. In this experiment only 0.5U of enzyme was immobilised onto the membrane surface. This was a small enzyme concentration yet a promising improvement in flux was observed. MnP was shown to give the best flux recovery. HRP provides a barely noticable improvement (Figure 2.4), while no flux improvement was observed using SBP (Figure 2.5).

Spectrophotometric analysis was performed on the retentate and permeate immediately after enzyme induction. Further evidence for the difference in activity was obtained from the spectrophotometric evaluation of the retentate (Figure 2.7) and permeate (Figure 2.8). The results shown in Figure 2.7 represent the analysis of the retentate after enzyme induction. Absorbance at 465nm was represented, in Figure 2.7, as the samples showed the greatest variation in this region and 465nm is the wavelength which shows the removal of brown colour according to the concentration of phenolic compounds present in the sample (Davis and Burns, 1990).

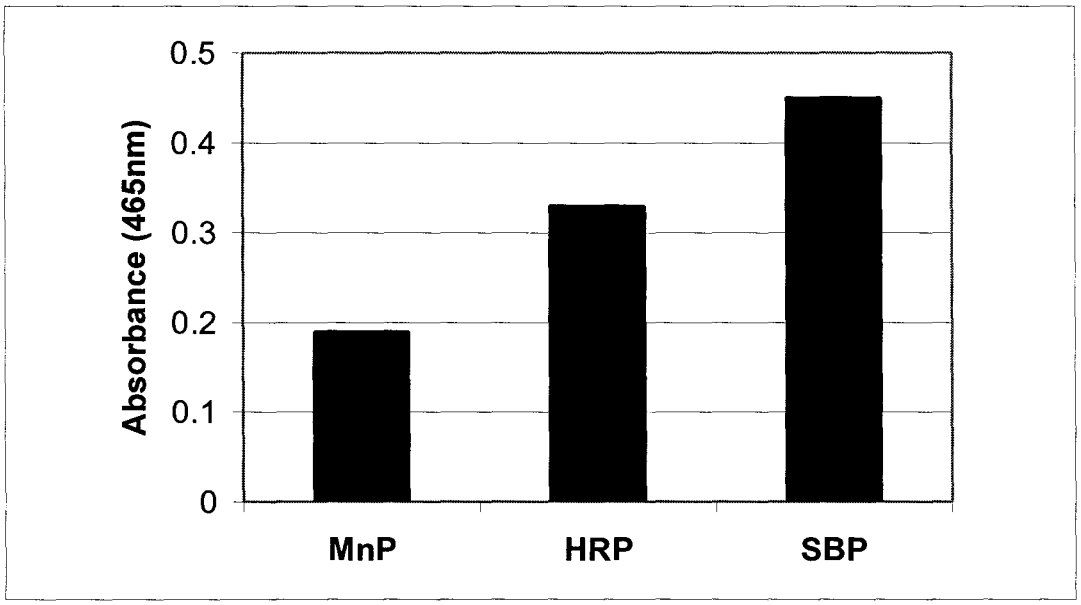

Figure 2.7: Spectrophotometric analysis of the retentate at 465nm after enzyme induction.

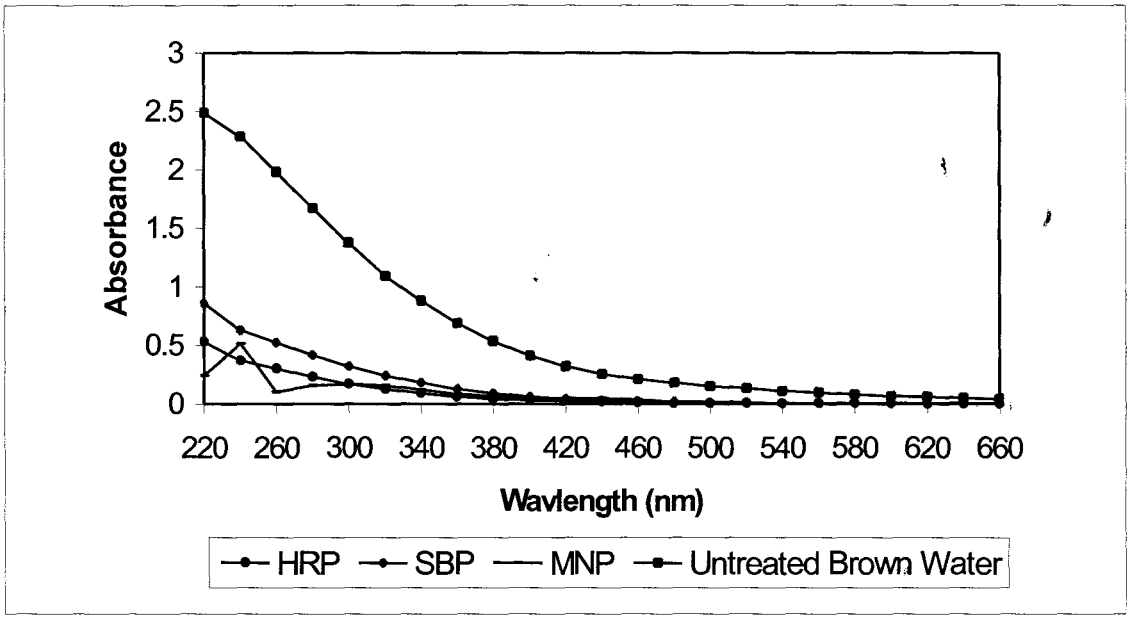

Figure 2.8: Spectrohotometric analysis of the permeate after enzyme induction.

From the spectrophotometric analysis of the retentate and permeate it was evident that the colour component of the brown water was reduced by the action of MnP and to a lesser extent by HRP. This indicates that the humic and phenolic substances, which were present in the brown water, were degraded or transformed by enzyme action. The nature of this action could be the result of a polymerisation reaction or a cleavage reaction. If the polymers were cleaved into smaller oligomers or monomers, these would no longer be retained by the membrane and an increase in absorbance at lower wavelengths would be observed. If the humic polymers were further polymerised; larger, less soluble and possibly more stable particulate matter would be formed which would improve the retention of such compounds.

2.4 CONCLUSION

The screening of three peroxidase enzymes has indicated that MnP activity, directed at the transformation of the foulant layer during the ultrafiltration of brown water, was the most effective enzyme in restoring transmembrane flux. MnP and HRP have similar catalytic activities (Wariishi *et al.*, 1988; Kersten *et al.*, 1990) and, therefore, should affect the substrates similarly, yet a noticeable difference in flux restoration was only seen when MnP was induced. The flux verses time curves gave higher overall flux yields when MnP was immobilised and induced.

CHAPTER 3

EVALUATION OF MANGANESE PEROXIDASE IN DEFOULING ULTRAFILTRATION MEMBRANES

3.1 INTRODUCTION

Of the enzymes evaluated, MnP was found to be the most effective peroxidase enzyme in restoring flux to the ultafiltration system. However, the main problem concerning its applicability is that MnP is not readily available. MnP is a glycosylated haem protein containing protoporphyrin IX (Mino *et al.*, 1988; Bonnarme and Jeffries, 1990; Brown *et al.*, 1990). The enzyme exists as a series of isozymes with pI's ranging from 4.2 to 4.9 and has a molecular weight of approximately 46 000 daltons (Pease and Tien, 1992; Godfrey *et al.*, 1994; Sutherland *et al.*, 1996). Each isozyme contains 1 mol. of iron haem per mol. of protein (Gold and Alic, 1993).

A number of problems are, however, associated with the economic production of enzymes on a large scale. One of the main limitations of using lignolytic enzymes commercially is that they are produced in limited quantities as they are expressed during fungal secondary metabolism in response to carbon and nitrogen starvation (Gold *et al.*, 1982; Matsubara *et al.*, 1996). The growth conditions for the production of MnP and LiP have been well reported, including the carbon and nitrogen regulation, oxygen tension, and especially the effects of Mn(II) (Bonnarme and Jeffries, 1990, Bono *et al.*, 1990;

Kerem and Hadar, 1995). Three main problems arise with for the continuous production of MnP. These include:

- Enzyme production occurs under specific physiological conditions corresponding to carbon and nitrogen limitation, high oxygen tension and adequate Mn(II) concentrations (Tien, 1987).

- The enzymes may be partially inactivated, destabilised or proteolytically hydrolysed by extracellular proteases during the production phase (Tien, 1987).

- Excessive growth of the mycellial pellets block effective oxygen transfer. Microbial enzyme production systems may be sensitive to shear forces, especially in the case of LiP and MnP production (Moreira *et al.*, 1997). Maintenance of sufficient and optimal aeration and mixing rates may be difficult.

Shear stress, biofilm thickness and aeration have been shown to be the key parameters for the control of peroxidase production (Bosco *et al.*, 1996). Immobilisation of the production organism on a solid matrix has been investigated to solve such problems (Zhao *et al*, 1996), as well as using the immobilised biocatalyst for enzyme production in appropriately modified conventional bioreactor methods (Bonnarme *et al.*, 1993; Bosco *et al.*, 1996; Moreira *et al.*, 1997). Bonnarme *et al.* (1993) found that the pneumatic bioreactors stimulated a high mycelial growth, and oxygen transfer was significantly improved, which resulted in the production of high peroxidase titres. The use of pneumatic devices, for the production of extracellular peroxidases, avoids shear stress affects due to the turbine agitation found in conventional fermentors and provides a good alternative for the production of shear sensitive metabolites such as those of *P. chrysosporium* (Bonnarme *et al.*, 1993). Moreira *et al.* (1996) examined enzyme production when MnP was immobilised on polyurethane foam in a pulsed bed reactor. This was a continuous reactor while others have been batch cultures. This method of

producing peroxidases yielded a maximum activity of 250U/ *l* with a productivity rating of 202 U/ *l* /d.

MnP production has been found difficult for the reasons mentioned above. In addition systems for the production of MnP are not yet commercially available. It has been found that the oxidation of Mn(II) to Mn(III) does require a manganese chelator to support the steady state turnover of the enzyme by forming complexes with Mn(II) and facilitating the movement of Mn(III) away from the enzyme (Kuan *et al.*, 1993; Timofeevski and Aust, 1997). This chapter, therefore, focusses on factors which would best enhance enzyme activity hence improving the application of MnP in defouling ultrafiltration membranes so that methods for the economical use of the enzyme could be established.

3.1.1 Research Objectives

To ascertain that the defouling effects were due to enzymatic action alone and to evaluate potential factors affecting enzymatic performance. The factors chosen included the effect of a manganese chelator, manganous ion concentration, hydrogen peroxide concentration and enzyme concentration.

3.2 METHODS AND MATERIALS

3.2.1 Modifications to the Stirred Cell Reactor

The experimental procedure for membrane fouling analysis was followed as in section 2.2.1, except a few alterations were made to the stirred cell reactor. The stirred ultrafiltration cell was modified by adding a glass port to the reactor so that the pressure did not have to be lowered to add the activator solution. In addition, the permeate flux was measured gravimetrically with the aid of an electronic balance (Precisa, 300 SCS series) which was linked to a computer. This monitored the permeate flux continually

with a higher degree of accuracy with specially developed data capture software. Polysulphone membranes used in all subsequent experiments were purchased from Sartorius (Asymmetric Ultrafilters).

3.2.2 Confirmation of Manganese Peroxidase Activity in Defouling

Once the concept had been proven it was important to verify the results and, therefore, certain control experiments were performed. The control experiments included :

- Control 1: To determine if an increase in flux was a result of the catalytic action of MnP and not just the presence of a protein layer, the enzyme was denatured, immobilised onto the membrane surface and induced as normal.

- Control 2: To determine if the activator solution had an oxidative effect on the foulant layer, the inducer was added to the system but no MnP was immobilised onto the membrane surface.

- Control 3: In the normal operating procedure, the pressure was dropped to add the activator solution, thus to test if this had an effect on the flux, deionised water was added to the system in place of the activator solution to determine the effect of the dilution and pressure change.

- Control 4: To determine if hydrogen peroxide had an effect on transmembrane flux, (21U) MnP was immobilised and induced with hydrogen peroxide (100mM), except no manganous sulphate was added.

Control experiments were used to determine if the effect noted in Figure 2.6 was a direct result of enzymatic action rather than due to experimental procedure or the effect of the

inducer on membrane porosity. In all control experiments 0.5U of enzyme were immobilised unless otherwise stated.

3.2.3 Factor Analysis in the 'Defouling on Demand' Strategy

In order to determine the factors that influence the enhancement of enzymatic activation and therefore flux restoration certain variables including enzyme concentration, the effect of chelating agents and the effect of higher concentration of activator solution were examined.

3.2.3.1 The Effect of Organic Acids

The effect of lactate and oxalate on the catalytic activity of MnP was established. 2.5m *l* lactate or oxalate (200mM), was added to the activator solution. The pH of lactate was adjusted to pH 4.5 with sodium hydroxide. The total volume of activator solution, added to the stirred cell reactor, was 10mL.

3.2.3.2 The Effect of Increasing the Concentration of H_2O_2 and $MnSO_4$

In the initial experiments 200μM $MnSO_4$ and 0.1 mM H_2O_2 was added to the activator solution. In order to determine the effect of the concentration of activator solution, increasing concentrations of $MnSO_4$ and H_2O_2 were added to the stirred cell reactor. In the first experiment the concentration was increased to 400μM $MnSO_4$ and 5mM H_2O_2. Subsequently the concentration was increased further to 800μM $MnSO_4$ and 100mM H_2O_2.

3.2.4 Statistical Validity

To determine the statistical validity of these results the experiments were performed in triplicate. A mean is represented in all the results presented. The mean and standard deviation is given in Table 3.1.

3.3 RESULTS AND DISCUSSION

3.3.1 Control experiments

Table 3.1 shows the initial experiments where MnP was immobilised onto the membrane surface and induced with the activator solution after 15 minutes. At the end of each experimental run the flux was significantly higher than any of the control experiments. In all four control experiments no flux increase was observed after induction indicating than any increase in flux was a result of the enzymatic action on the foulant layer.

Table 3.1: Flux values obtained upon induction for the control experiments (as described in section 3.3.2, values given in $l/m^2/h$).

	MnP (0.5U) immobilised (not induced)	Control 1	Control 2	Control 3	Control 4
Initial Flux	**213.8** ± 10.76	**381.78** ± 35.89	**263.91** ± 14.85	**410.84** ± 76.46	**252.89** ± 26.33
Flux before induction	**98.17** ± 1.25	**113.44** ± 5.82	**102.54** ± 2.56	**106.89** ± 2.085	**104.72** ± 3.12
Flux after induction	**135.26** ± 11.19	**113.44** ± 4.54	**104.72** ± 2.89	**95.99** ± 1.55	**98.17** ± 1.47
Flux 5 min after induction	**104.71** ± 1.12	**102.54** ± 4.54	**95.99** ± 1.79	**95.99** ± 1.53	**95.99** ± 2.65
Flux at end of experiment	**81.26** ± 5.46	**63.26** ± 5.59	**71.14** ± 9.43	**52.35** ± 7.71	**63.26** ± 6.29

In addition a higher enzyme concentration, 4.5U, was used. The enzyme was immobilised and induced to compare the effect of enzyme concentration on flux restoration. Flux improvement is shown in Figure 3.2. The fouled membranes were removed from the stirred cell reactor and photographed.

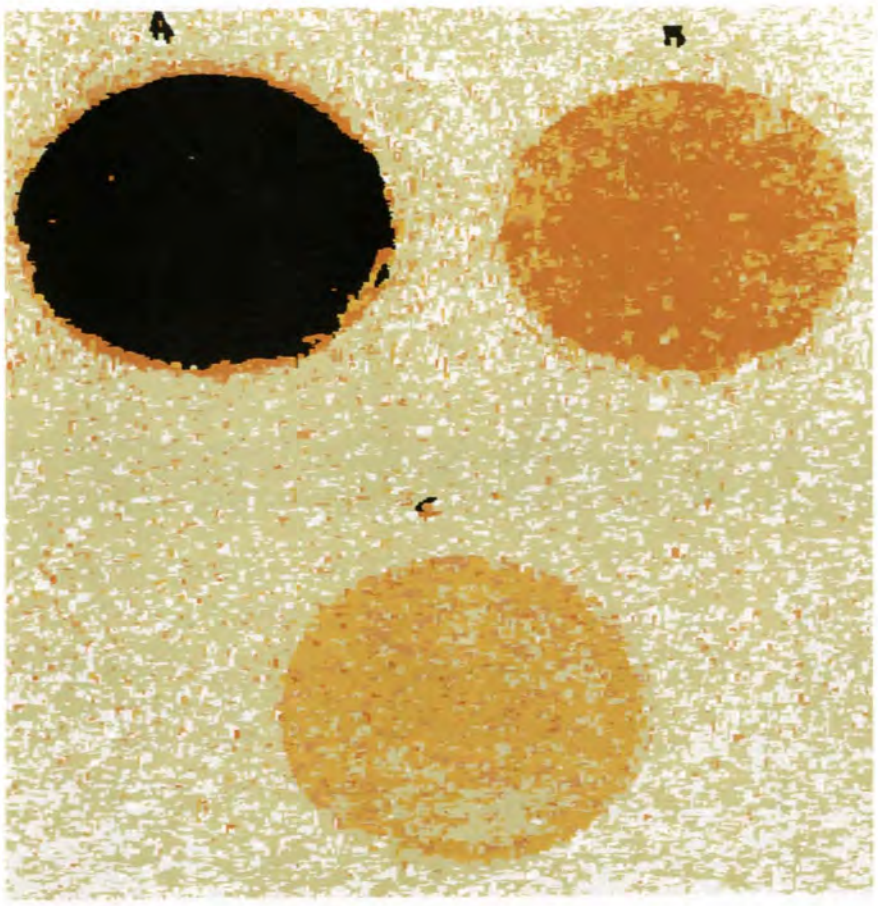

Figure 3.1: Photographic depiction of the defouling of polysulphone membranes after ultrafiltration of brown water. (A) shows an evenly distributed dense foulant layer resulting from brown water ultrafiltration. (B) shows a reduced foulant layer following induction of MnP (0.5U). (C) shows an even more reduced layer following the induction of MnP (4.5 U).

Distinct differences in the density, colour and dispersion of the foulant layer was observed when the enzyme was immobilised and activated (Figure 3.1). This method of cleaning using activatable enzymes has shown potential to be effective and upon further development could be an effective biological agent for defouling polysulphone membranes during brown water decolourisation.

3.3.2 The Effect of Increasing Concentrations of Manganese Peroxidase

To determine the effectiveness of MnP in restoring flux to ultrafiltration membranes, the effect of increasing the enzyme concentration was examined.

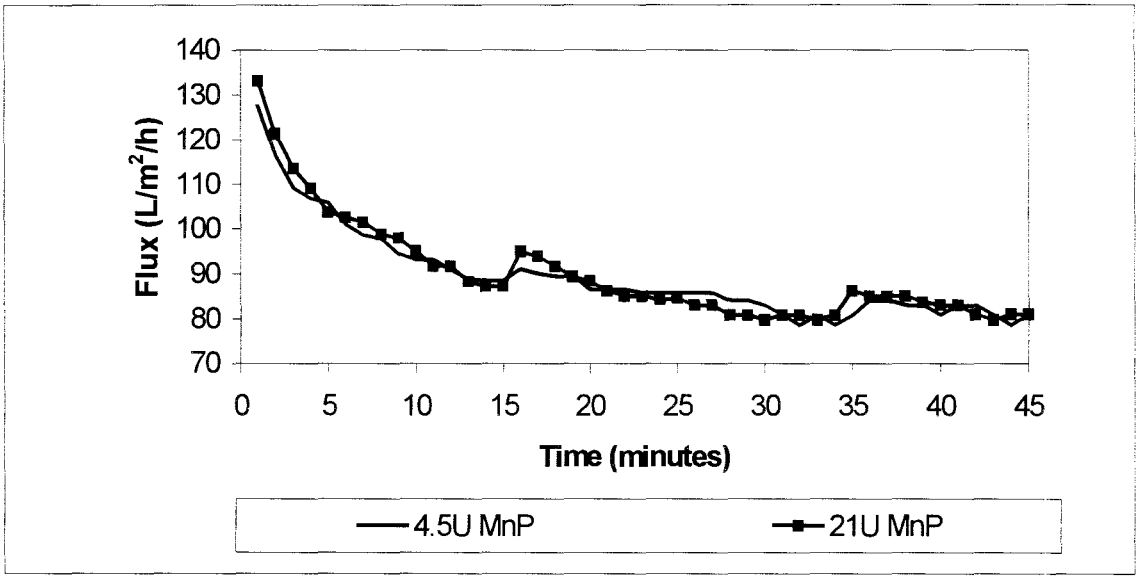

Figure 3.2: The effect of increasing manganese peroxidase concentration on transmembrane flux restoration.

Figure 3.2 shows that only a small improvement in flux was noticed upon induction regardless of the enzyme concentration used. Flux improved from 87.26 to 94.99 l /m^2 /h upon induction when 21U of enzyme was immobilised. This was only an increase of 9.76 l /m^2 /h when 21U was used compared to an enzyme concentration of 4.5U but an increase of 36,96 l /m^2/h compared to an enzyme concentration of 0.5U. Flux improvement occurs immediately following induction but declines rapidly returning to the steady state flux. Replicate experiments presented an identical trend and similar levels of flux restoration.

Figure 3.2 shows that a higher concentration of MnP, above 4.5U, did not increase the enzymatic cleaning efficiency. Although only a small increase in flux was obtained this was a relevant result as it suggests that once the system is optimised only a small enzyme concentration would be needed. In light of the difficulties in producing MnP, lower concentrations of enzyme needed to restore flux would be beneficial.

After determining the effect of increasing concentration of MnP the enzyme concentration for the remainder of the experiments was standardised to 21U. The low flux improvement seen with higher enzyme concentrations indicates that several other parameters maybe required in order to enhance enzyme activity.

3.3.3 The Effect of Organic Acids on the Catalytic Activity of Manganese Peroxidase

Organic acids such as oxalate and malonate, which are produced by *P. chrysosporium*, stimulate the MnP system by chelating Mn(II) to form stable complexes with high redox potentials (200mV) by facilitating the dissociation of Mn(III) from the enzyme to act as a diffusible oxidant (Wariishi *et al.*, 1989; Perez and Jeffries, 1993; Gold and Alic, 1993; Mayfield *et al.*, 1994). The effect of organic acids was, therefore, tested for their ability to enhance enzyme activity and to promote flux restoration during filtration.

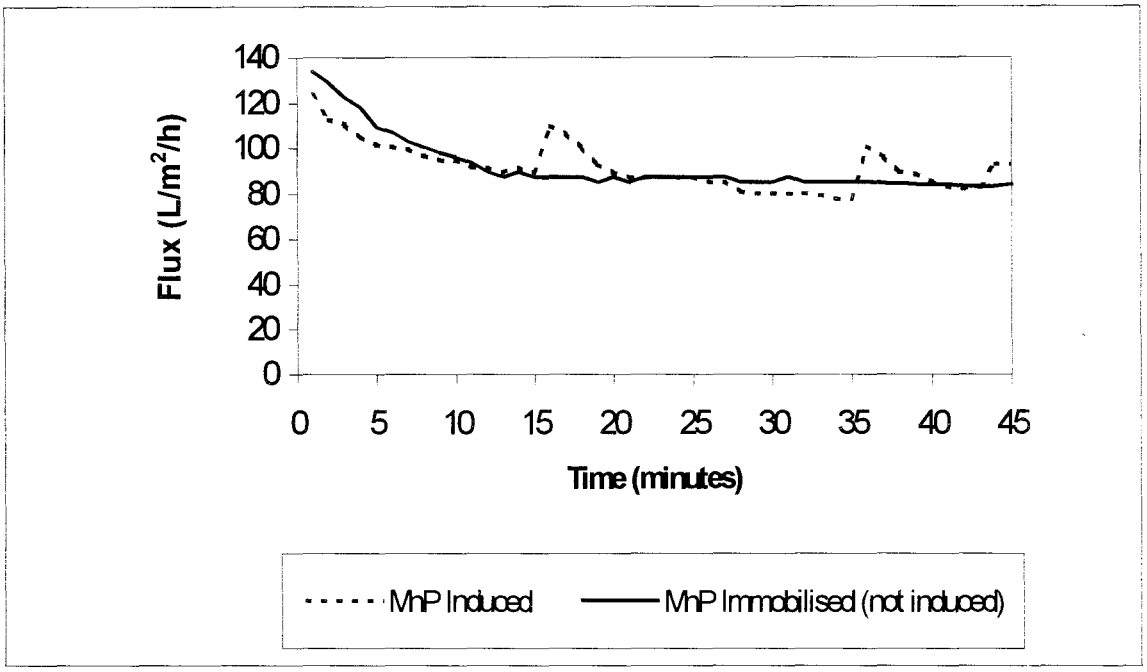

Figure 3.3: The effect on manganese peroxidase activity and flux restoration with the addition of lactate in the activator solution.

The data shows than when lactate was introduced, with H_2O_2 and $MnSO_4$ in the activator solution, flux was restored to 88% of the initial prefouling value (Figure 3.3). On the first induction the flux increased from 89.44 *l* /m^2/h to 109.63 *l* m^2/h. The improvement in flux was observed for 4 minutes before returning to the steady state flux observed when the enzyme was immobilised but not induced. On the second induction flux increases from 77.44 *l* /m^2/h to 106.23 *l* /m^2/h, taking 6 minutes to return to the steady state flux. On the second induction an 86% flux reduction was observed. The effect of the presence of oxalate in the activator solution is presented in Figure 3.4.

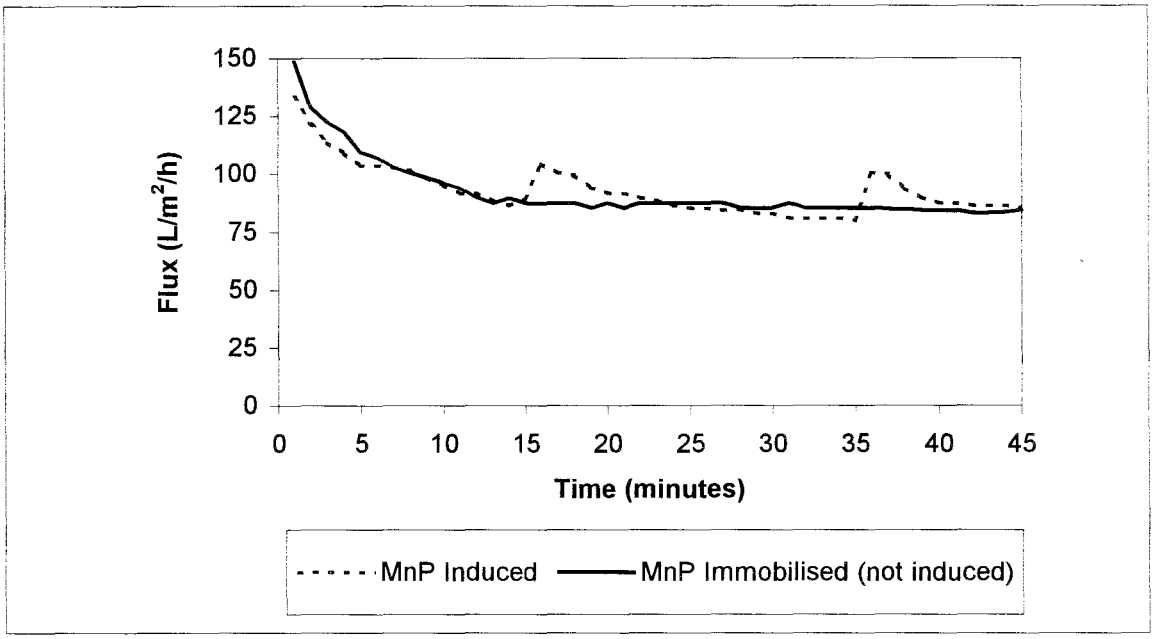

Figure 3.4: The effect on manganese peroxidase activity and flux restoration with the addition of oxalate in the activator solution.

Oxalate is a metabolite of *P. chrysosporium* (Popp *et al.*, 1990), but was found to be less effective at restoring flux when compared to the Mn(II) chelator lactate. An 88% flux improvement was seen when lactate was added to the activator solution compared to a 77% improvement seen with oxalate. When oxalate was added to the activator solution flux increases from 89.26 *l*/m^2/h to 102.53 *l*/m^2/h, a 77% flux improvement compared to the initial flux values obtained at the start of the experiment. On the second induction the increase in flux observed was from 79.63 /m^2/h to 100.35 *l*/m^2/h, a 75% flux improvement. It should also be noted that the flux improvement was significantly improved when an organic acid was included in the activator solution and the duration for which the flux improvement lasted was extended.

The improvement is in accordance with the findings of Wariishi *et al.* (1989) who showed high reaction rates when lactate buffer was added. Oxalate is also toxic and,

therefore, the use of lactate would be beneficial if treated water is to be used for human consumption. Kuan *et al.* (1993) proved that MnP does not readily oxidise free (hexa-aqua) Mn(II) as previously reported (Wariishi *et al.*, 1992), but the Mn(II) has to be chelated to support a steady state turnover.

Mn(II) binding studies performed by Wariishi *et al* (1992) demonstrated that MnP has a single manganese binding site near the haem, and two Mn(III) equivalents are formed at the expense of one H_2O_2 equivalent. Timofeevski and Aust (1997) showed that the enzyme catalyses a manganese dependant disproportionation of hydrogen peroxide when a chelator was not included. They also showed that the catalytic activity of the enzyme may be protected from inactivation, by high concentrations of hydrogen peroxide in an aqueous environment, where chelating agents are present (Timofeesvki and Aust, 1997). A higher catalytic turnover was produced when a manganese chelator was present, which functions to explain the relatively low flux improvement seen when a chelator was not added to the ultrafiltration system (Figure 2.6).

3.3.4 The Effect of Increasing the Concentration of H_2O_2 and $MnSO_4$

During the treatment of fouled membranes with MnP, the H_2O_2 donates a single electron to MnP to form an active enzyme complex. As a result, the H_2O_2 levels decreased constantly until no more substrate was available for the enzyme or the H_2O_2 was depleted. The action of MnP cannot, therefore, be described as the accumulative effect of the enzyme and H_2O_2, but as the effect of the active enzyme complex. The effect of increasing concentration of H_2O_2 and $MnSO_4$ was therefore determined.

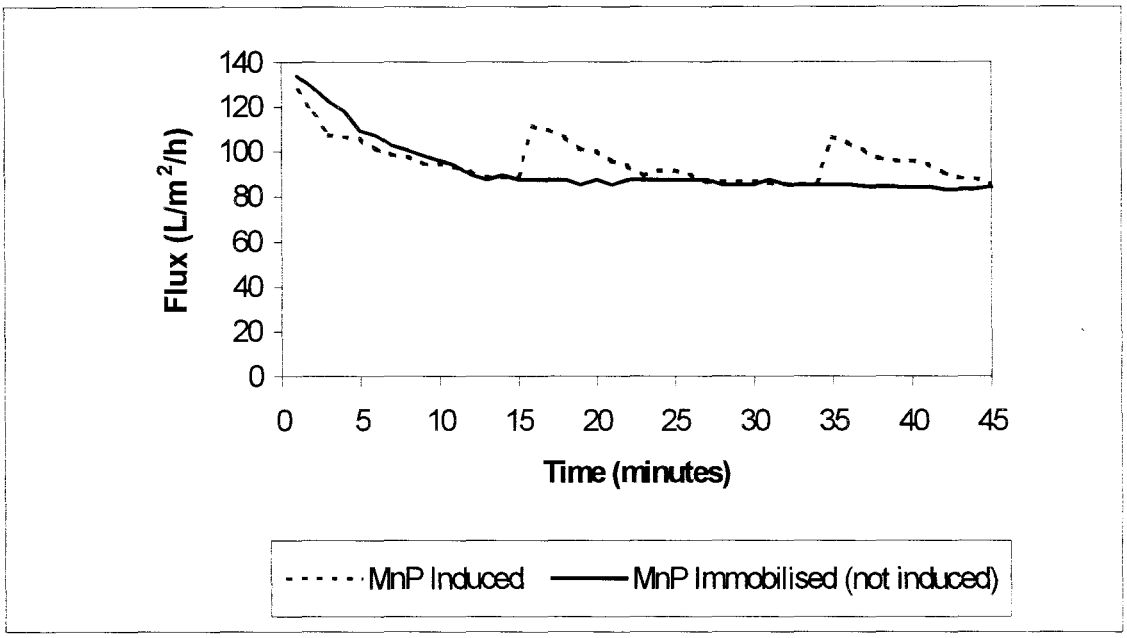

Figure 3.5: The effect on manganese peroxidase activity and flux restoration by increasing the concentration of manganous sulphate and hydrogen peroxide (400 µM $MnSO_4$, 5 mM H_2O_2 and 200 mM lactate was added to the ultrafiltration system).

In the first experiment, when the concentration was increased to 400µM $MnSO_4$, 5mM H_2O_2 and 200mM lactacte (Figure 3.5). An 89 % flux improvement was observed under these conditions. Flux increased from 88.52 *l* /m^2/h to 111.36 *l*/m^2/h. The flux improvement was maintained for 11 minutes before returning to the steady state flux. On the second induction flux increased from 85.8 *l* /m^2/h to 106.23 *l* /m^2/h, an 83% improvement.

When the concentration of the inducer was increased further to 800µM $MnSO_4$, 100mM H_2O_2 and 200mM lactate (Figure 3.6) flux increased from 88.52 *l* /m^2/h to 119.36 *l* /m^2/h and on the second induction from 85.8 *l* /m^2/h to 109.23 *l* /m^2/h. There was a 93% and 88% flux improvement on the first and second induction respectively. The duration of the improvement lasted 15 minutes before returning to the steady state flux seen when the MnP was immobilised but not induced.

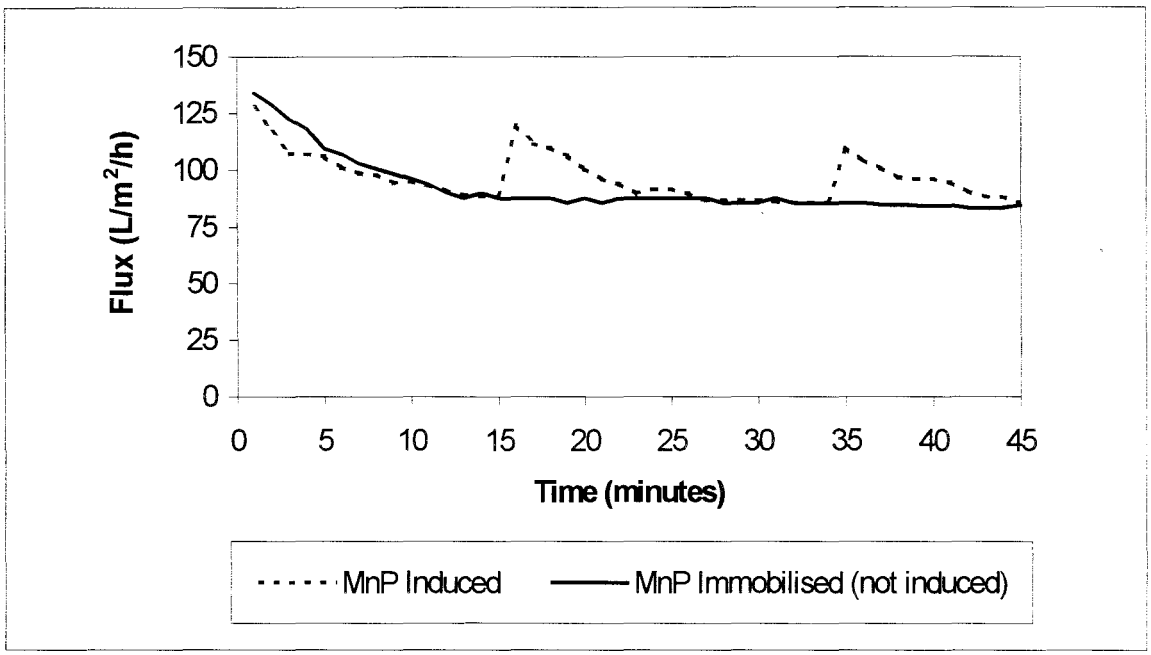

Figure 3.6: The effect on manganese peroxidase activity and flux restoration by increasing the concentration of manganous sulphate and hydrogen peroxide (800 μM $MnSO_4$, 100mM H_2O_2 and 200 mM lactate was added to the ultrafiltration system).

3.4 CONCLUSION

Control experiments showed that any increase in flux was a direct result of enzymatic action and not due to the experimental procedure or due to the effect of the inducer on membrane porosity. The concentration of hydrogen peroxide, manganous ions and the presence of organic acids were shown to be factors affecting MnP activity and the performance of the defouling system. Activation of the enzyme and its subsequent action occurs rapidly. The inducer appeared to have rapidly penetrated the foulant layer to react with the enzyme. The results showed that high concentrations of hydrogen peroxide and manganous ions used resulted in increased flux and these levels were maintained for extended periods of time.

With regards to the enzyme concentration it was found that above 4.5U of MnP, no substantial increase in flux was observed and thus MnP concentration, above 4.5U, was not found to be a factor affecting the performance of the defouling system. A low concentration of MnP can be used but it needs to be determined to which extent the enzyme can tolerate a high peroxide environment before inactivation. Higher levels of hydrogen peroxide may result in damage to membrane morphology or changes in the membrane porosity. Furthermore, higher levels of hydrogen peroxide and manganese may also cause additional pollution problems by penetrating the membrane pores and entering the product water. The optimum concentration level of the activator solution that can be added to ensure that it was utilised completely by the enzyme layer needs to be established. As a result a compromise in terms of flux restoration and penetration of the activator solution through the membrane into the product water would be essential as water guidelines recommend that levels of manganese should not exceed 0.005mg/ *l* (Offringa, 1997).

CHAPTER 4

FLUX, REJECTION AND FOULING CHARACTERISTICS

4.1 INTRODUCTION

In all membrane operations fouling can be measured by the amount of material deposited and by changes in the membrane retention. Membrane fouling is a complex process involving convective deposition of aggregated materials and adsorption (Dejmek and Nilsson, 1989). Flux during UF can be divided into three separate parts. The initial solvent decline which is a result of fouling and compaction. The second, flux decline, which is related to the boundary layer formation, plugging and adsorption. The last stage is the long term flux decline which is related to fouling deposition/adsorption and cake consolidation (Dejmek and Nilsson, 1989).

There is a widespread belief that small particles and organic materials in natural waters will result in irreversible fouling of ultrafiltration membranes, leading to a decrease in permeate flux and the need for frequent membrane replacement (Laine *et al.*, 1989). The literature extensively covers fouling of ultrafiltration membranes in many other industries, yet little literature seems to be available on membrane fouling during water treatment. Most studies of ultrafiltration and microfiltration of colloids have used completely retentive membranes and focussed on factors determining concentration

polarisation. Furthermore, in most cases, the concentration of the colloidal suspensions have been significant, inevitably leading to a "cake-controlled" process independent of membrane properties (Kim *et al.*, 1993b).

These above conditions have placed most emphasis on cross-flow or stirred operations to provide hydrodynamic control of cake thickness. There are, however, numerous applications where the feed streams are very dilute, such in the purification of process waters, beverage clarification, and water sterilisation. Under these conditions the membrane properties, such as pore size and pore distribution, porosity, and morphology, could be important (Kim *et al.*, 1993b). In addition, the need for crossflow or stirring is less obvious.

4.1.1 Research Objectives

There is still a large degree of uncertainty regarding a detailed mechanism by which adsorbed foulants alter the membrane. For example pore blockage vs pore constriction or how these effects are related to the size of particles to be filtered and the membrane pores (Mochizuki and Zydney, 1992). For UF and MF membranes contain 'pores', and for most membranes, they are not all the same size. Fouling affects pores differently. Researchers disagree on whether the primary mode of organic fouling is by surface or pore deposition (Laine *et al.*, 1989).

In order to understand the physical nature of the defouling process developed, so that predictions can be made about process applications, an understanding of the mechanism of fouling in brown water ultrafiltration had to be established. This was achieved by:

- Observation of the fouling of membranes with varying pore sizes.
- Examination of the influence of stirring on fouling.

- ❖ Microscopic analysis of the foulant layer.

- ❖ Fitting filtration data into established mathematical models for different fouling mechanisms.

4.2 METHODS AND MATERIALS

4.2.1 Experimental Procedure

To compare the formation of the foulant layer three different polysulphone membranes were used. The membranes varied with pore size or molecular weight cut-off (MWCO), these included PM-30 (30 000 MWCO), PM-100 (100 000 MWCO) and PM-300 (300 000 MWCO). The stirred cell reactor was filled with a fixed volume of brown water (200m *l*) for each experiment. The instantaneous flux was measured gravimetrically with the aid of a Precisa electronic balance. At four different time intervals the concentration of the bulk and the concentration of the permeate was measured by COD. The concentrations were used to determine the percentage rejection given by:

$$\mathbf{R(\%) = 100[\ 1 - \ (Cp/Cb)]} \quad \textbf{4.1}$$

At each time interval the membranes were removed from the stirred cell reactor, freeze dried and stored at -4°C for further analysis. The membranes, thin-coated with gold, were viewed under high resolution by a scanning electron microscope (SEM)(JEOL JSM 840) to show the gradual build up of the foulant layer.

The transmembrane pressure used for each experiment was 100 kPa, with stirring speeds of either 100 or 400 rpm. This was compared to data from a non-stirred cell reactor in order to determine the effect of stirring of the build up of the foulant layer.

4.3 RESULTS AND DISCUSSION

4.3.1 The Effect of Stirring on Ultrafiltration Flux

As solutes accumulate near or within the membrane they may reduce the permeability of the membrane by blocking or constricting pores and forming an additional layer of resistance to the flow across the membrane (Chen *et al.*, 1995). Reduction in flux over time may be substantial and represent a loss in the capacity of the membrane. Figure 4.1, 4.2 and 4.3 shows the effect of stirring on the rate of flux decline for membranes with increasing MWCO.

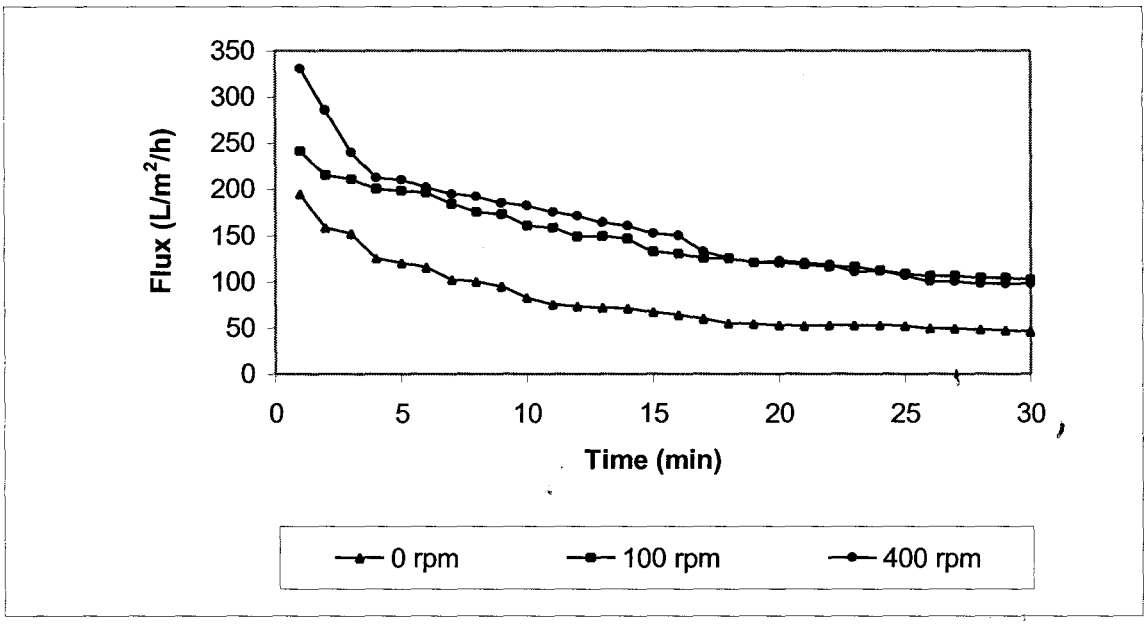

Figure 4.1: Ultrafiltration flux profiles, the effect of stirring on flux decline (PM-30).

Figure 4.1 shows a significantly lower flux when the system was operated without stirring. Higher fluxes were observed with PM-30, with increasing stirring speeds. In unstirred ultrafiltration, solutes are carried by convective flow to the membrane surface where it accumulates and provides an increasing barrier to the solvent flow (Chudacek and Fane, 1984).

An increase in flux would be expected as stirring facilitates the solute diffusion back from the membrane into solution. This moderates the fouling effect by reducing the net transport of the solute to the membrane. The rate of deposition or gel-layer formation was, therefore, minimised with higher stirring speeds, where it has little or no effect on membranes with large pores.

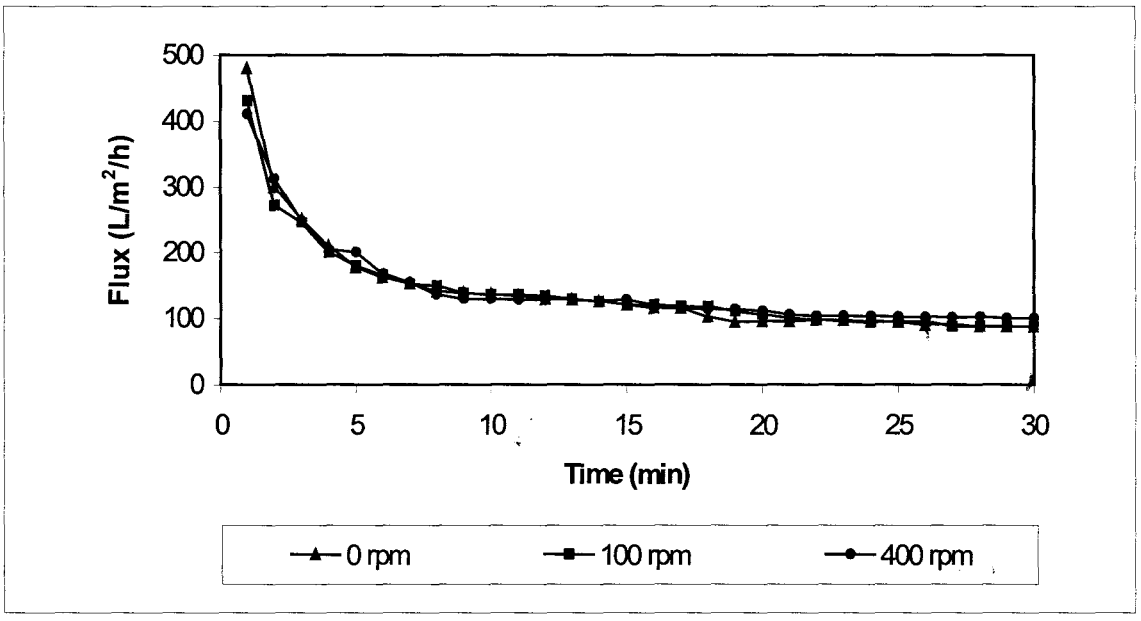

Figure 4.2: Ultrafiltration flux profiles, the effect of stirring on flux decline (PM- 100).

Figure 4.2 and 4.3 shows the effect of stirring speed when the MWCO of the membrane was increased. Flux reduction, therefore, becomes less pronounced with increasing pore size, regardless of whether the system was stirred or not. When comparing the flux

decline comparing all three membranes, higher relative fluxes were seen when the MWCO was far greater (PM-100, PM-300) than the particles to be filtered. It was concluded, as expected, that pore constriction could be the major cause of fouling.

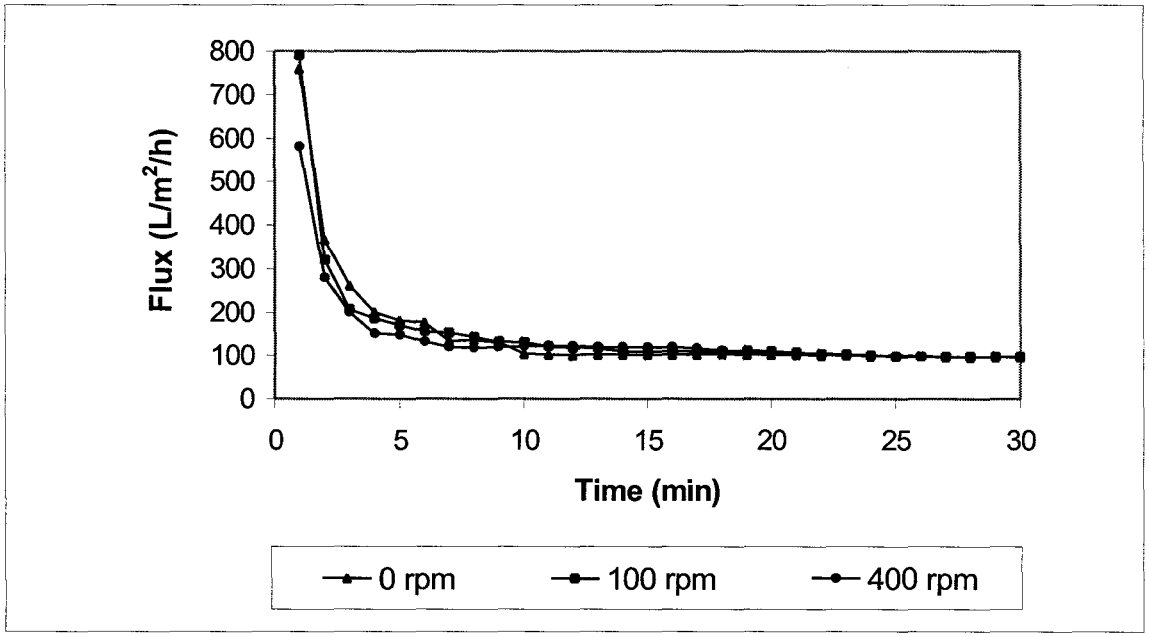

Figure 4.3: Ultrafiltration flux profiles, the effect of stirring on flux decline (PM-300).

4.3.2 Ultrafiltration Rejection Profiles

The results to follow were obtained from the filtration experiments (section 4.2.1) where the stirring speeds were increased from 100 to 400 rpm, from which the values for percentage rejection were calculated. These are presented in terms of permeation flux and the percentage rejections of macromolecules. The rejection coefficient of the membrane is the fraction of the solute present in the upstream solution which is retained by the membrane.

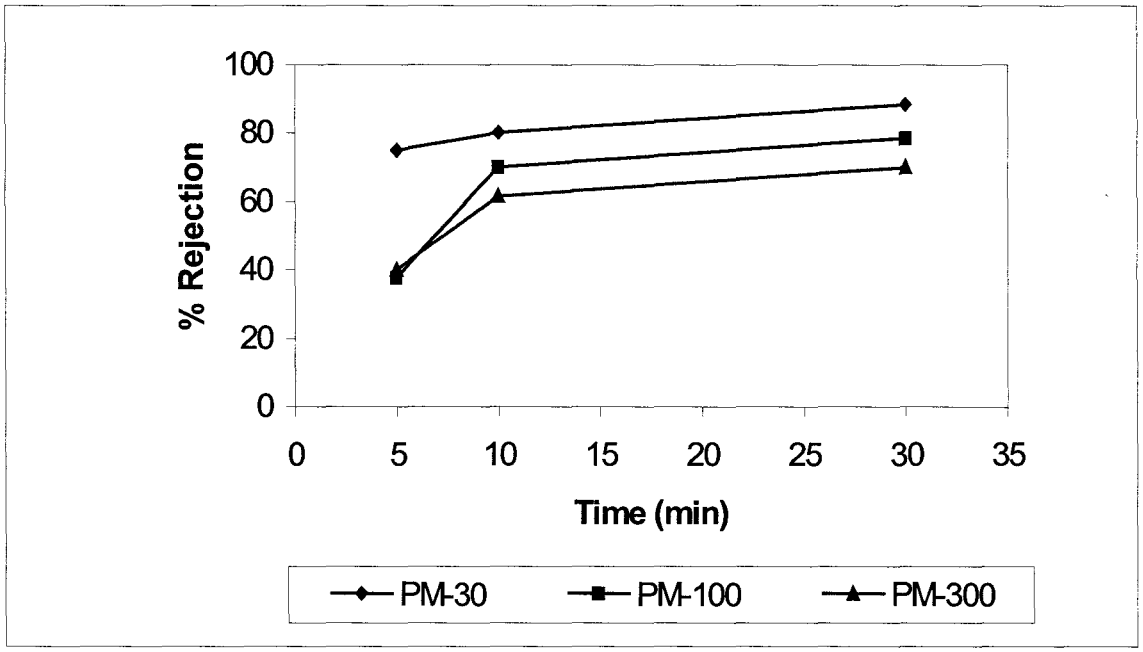

Figure 4.4: Rejection profiles with time for various membranes in a stirred batch system (100kPa, 100 rpm).

Figure 4.4 and 4.5 shows the percentage rejections as a function of time and stirring speeds for the membrane with increasing MWCO. An initial 75% rejection was obtained with PM-30. This increased with time to 88%. The rejection for PM-100 and PM-300 membranes were initially low at 37% and 40% respectively. This can be explained by the fact that the particles, present in the brown water, were smaller than the pore size of the membranes used. The initial rejections of the macromolecules were, therefore, relatively low. This increased with time to 78% and 70% for the PM-100 and PM-300 membranes respectively.

It is believed that the interaction between macromolecules and the membrane and the macromolecules themselves are responsible for rejection (Jiraratananon *et al.*, 1997). The increase in percentage rejection seen with the membranes with high MWCO was not unexpected when the particles to be filtered are smaller than the pore size of the

membranes used (Jonsson and Jonsson, 1995).

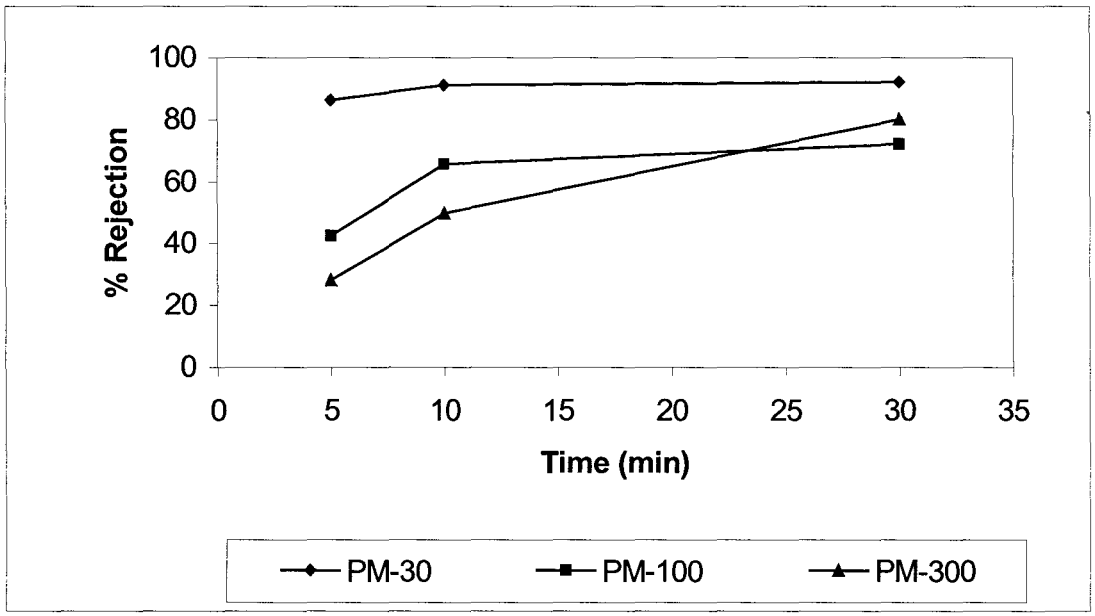

Figure 4.5: Rejection profiles with time for various membranes in a stirred batch system (100kPa, 400 rpm).

Figure 4.5 shows the comparison of percentage rejection profiles with time for various membranes in a stirred batch system (100kPa, 400 rpm). It is evident from the results that the percentage rejection increased with increasing stirring speeds. For PM-30 the initial percentage rejection, after 5 minutes, was 86% which increases to 92% after 30 minutes. For PM-100 and PM-300 the percentage rejection was again initially very low, 43% and 27%, respectively after 5 minutes. These values increased significantly to 72% and 80% after 30 minutes, for the PM-100 and PM-300 respectively. This increase in percentage rejection with time was considered to be a result of transport of the macromolecules into the membrane pores. These adsorb onto the membrane pores and pore diameters are reduced such that more molecules are retained. The development of the dynamic layer on the membrane surface, therefore, functioned to increase the percentage rejection.

Fouling has been shown to be strongly dependant on the average pore diameter of the membrane used. A decrease in pore diameter (decrease in MWCO) results in considerably increased fouling of ultrafiltration membranes. This can be accounted for by pore blocking which has a marked effect on flux when the pores are relatively small. Larger pores, therefore, are presumably fouled by a different mechanism. Pore blocking has been found to be the major mechanism of fouling in the case of membranes with smaller pores (Brink *et al.*, 1993), while pore constriction and pore narrowing through internal deposition (protein adsorption) would be dominant in larger pores (Mulder, 1995).

Assuming that the initial deposition predominately blocks the larger pores of the membrane, it would be likely that PM-30 will suffer the most, having pores the same magnitude as the particles to be filtered. Thus for the PM-30 it was proposed that the initial deposition closes off the most permeable regions of the surface porosity, therefore, the membrane resistance and the initial percentage rejection would be high. Figure 4.1 indicates that PM-30 shows a greater sensitivity to changes in stirring speeds compared to PM-100 and PM-300. According to Kim *et al.* (1988) this suggests that the particles creating the foulant layer in PM-30 are not firmly bound onto the membrane surface. Fouling of PM-30 was, therefore, considered to be a cumulative effect of adsorption and concentration polarisation or gel-layer formation.

4.3.3 Electron Microscopic Analysis of Filtration and Fouling

Thin section SEM micrographs are shown in Figure 4.6 to Figure 4.17 for PM-30, PM-100 and PM-300 membranes, showing the build up of the foulant layer over time, and the effect of stirring speeds. Figure 4.6 and 4.7 shows the build up of the foulant layer on the PM-30 membrane over time in stirred conditions. This was compared to unstirred conditions, PM-30, in Figure 4.8 and 4.9. In Figure 4.6 to Figure 4.9 which compares the build up of the foulant layer over time for PM-30 under stirred and unstirred conditions, it

can be seen that the foulant layer shows a smooth surface morphology whether or not the system was stirred. This was not seen with PM-100 or PM-300.

When one compares this to PM-100 (Figure 4.10 to Figure 4.13), and PM 300 (Figure 4.14 to Figure 4.17) under stirrred and unstirred conditions it can be seen that there were distinct differences in the surface morphology over time. The comparison between the foulant morphology between the smooth foulant layer morphology for PM-30 and the rough foulant layer morphology of PM-100 and PM-300 reiterates that PM-30 was fouled by a different mechanism.

For PM-100 and PM-300 a thick, rough surface morphology was observed in both stirred and unstirred conditions. The SEM micrographs for PM-100 and PM-300 shows strong evidence for cake layer formation and/or cake compaction.

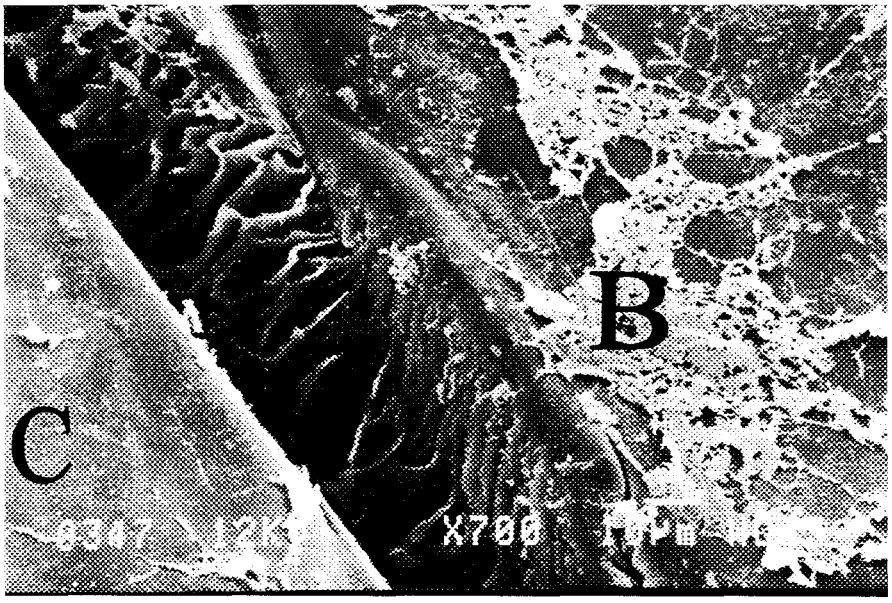

Figure 4.6: SEM micrograph of the foulant layer after 5 minutes of brown water ultrafiltration, PM-30, 100kPa, 100rpm. (A) crack in the foulant layer, (B) polysaccharides, (C) smooth foulant layer. Mag=700X.

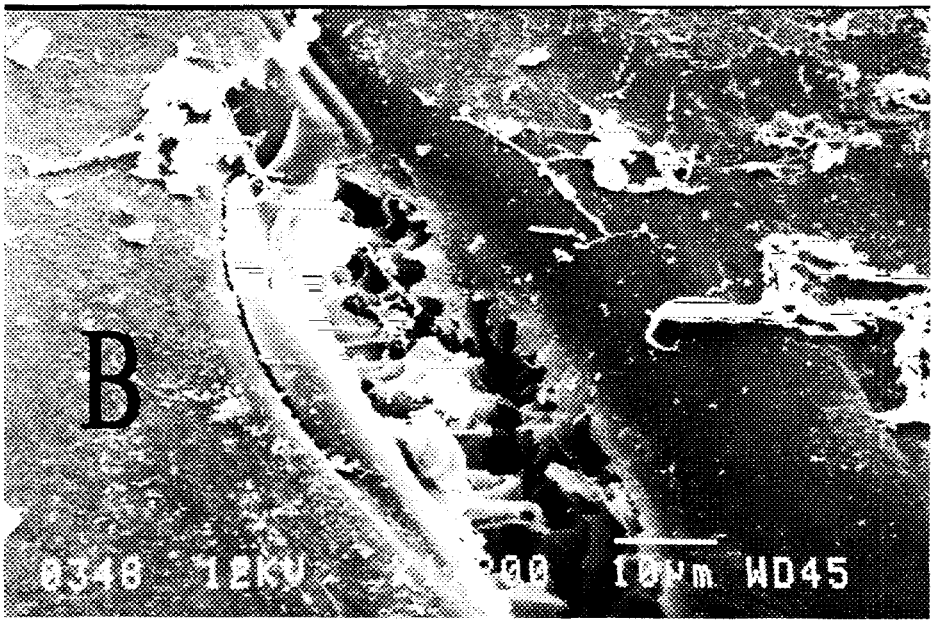

Figure 4.7: SEM micrograph of the foulant layer after 30 minutes of brown water ultrafiltration, PM-30, 100kPa, 100rpm. (A) crack in the foulant layer, (B) smooth surface morphology. Mag=1 200X.

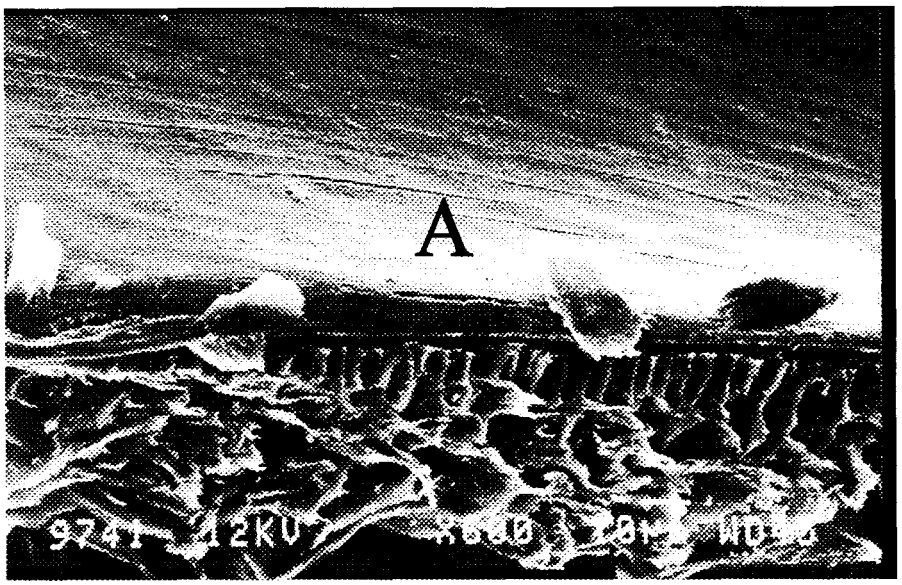

Figure 4.8: SEM micrograph of the foulant layer after 5 minutes of brown water ultrafiltration, PM-30, 100kPa , without stirring. (A) a smooth foulant layer, (B) the polysulphone membrane. Mag= 600X.

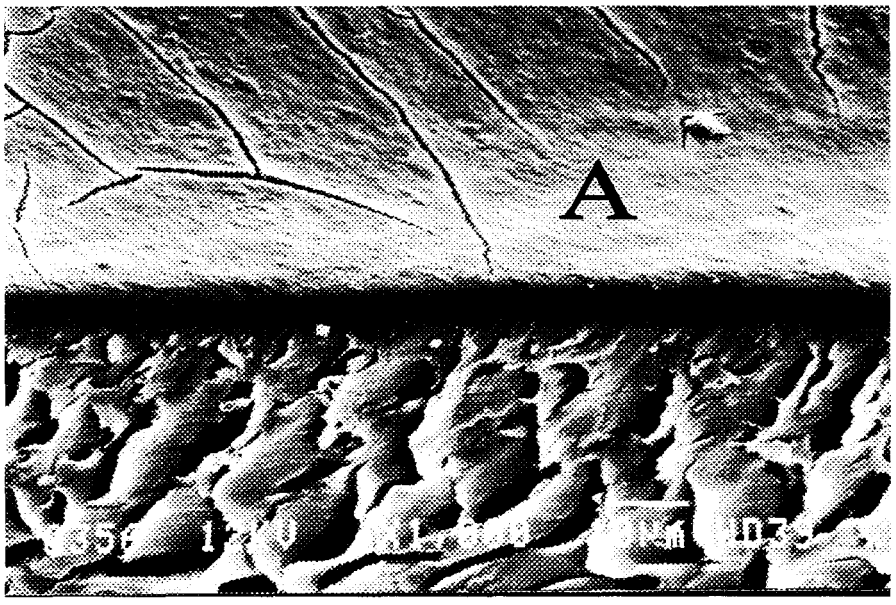

Figure 4.9: SEM micrograph of the foulant layer after 30 minutes of brown water ultrafiltration, PM-30, 100kPa, without stirring. (A) a smooth foulant layer, the cracks visible are probably a result of the freeze-drying technique, (B) the polysulphone membrane support. Mag= 1 000X.

Figure 4.10: SEM micrograph of the folant layer after 5 minutes of brown water ultrafiltration, PM-100, 100kPa, 100rpm. A rough foulant layer can be observed (A). Mag= 1 500X.

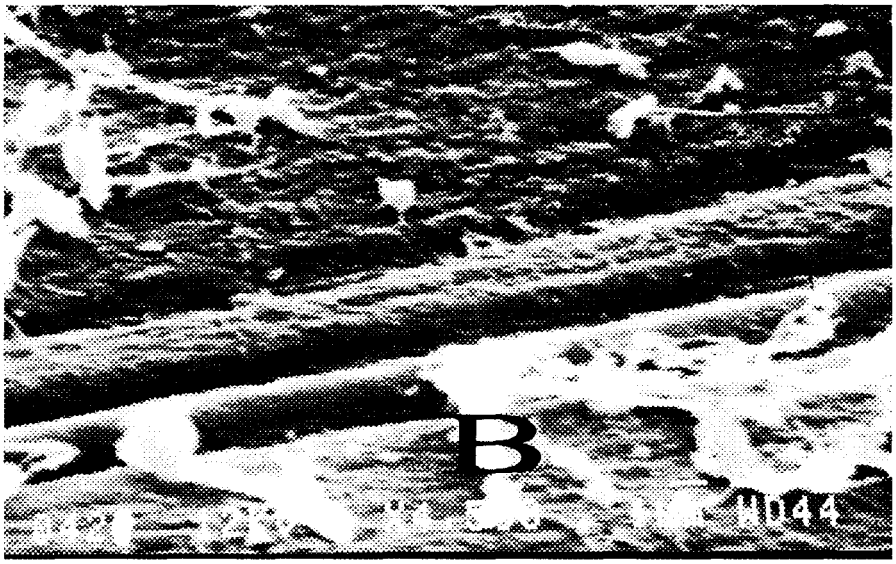

Figure 4.11: SEM micrograph of the foulant layer after 30 minutes of brown water ultrafiltration, PM-100, 100kPa, 100rpm. (A) indicates a rough foulant layer with distinct ridges in the surface morphology, (B) polysaccharides on the surface. Mag= 4 500X

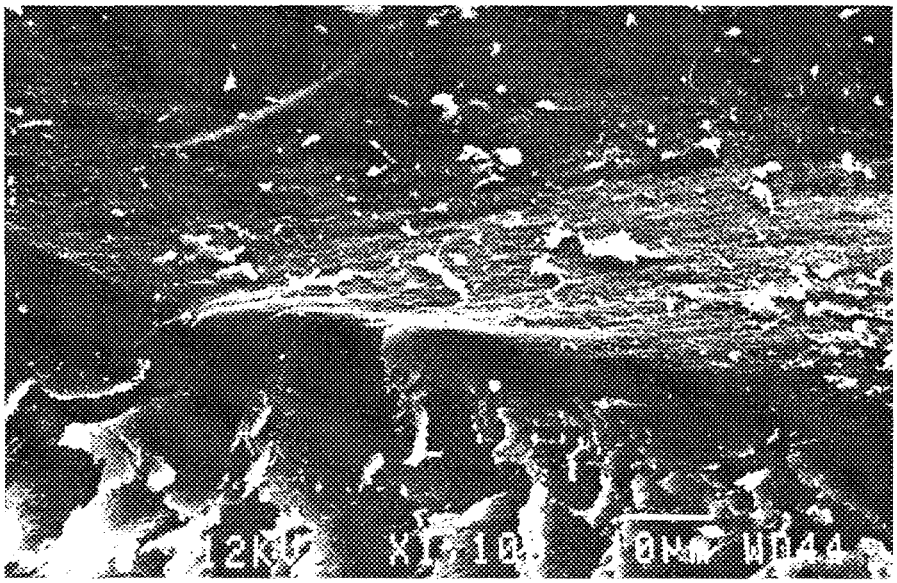

Figure 4.12: SEM micrograph of the foulant layer after 5 minutes of brown water ultrafiltration, PM-100, 100kPa, without stirring. (A) foulant layer, (B) polysulphone membrane support. Mag= 1 100X.

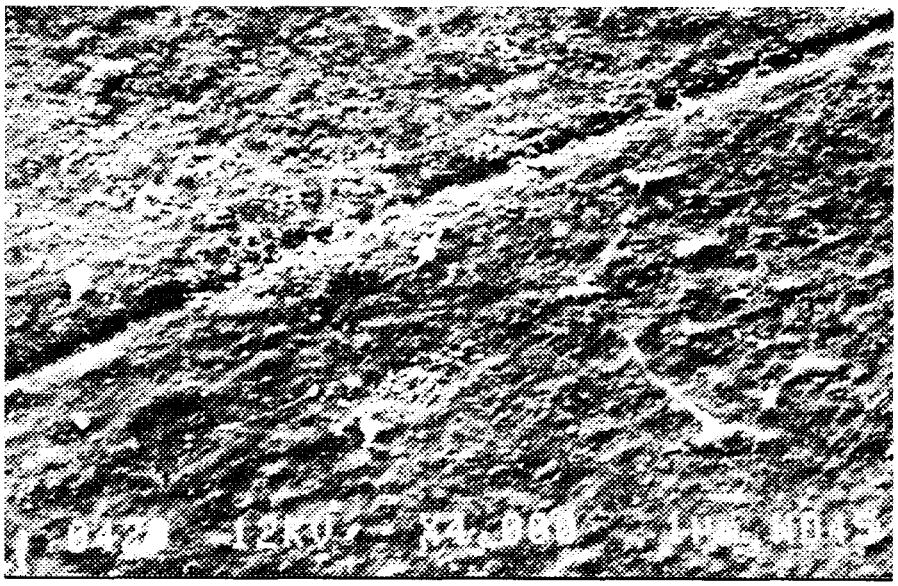

Figure 4.13: SEM micrograph of the foulant layer after 30 minutes of brown water ultrafiltration, PM-100, 100kPa, without stirring. A rough surface morphology can be seen. Mag=4 000X

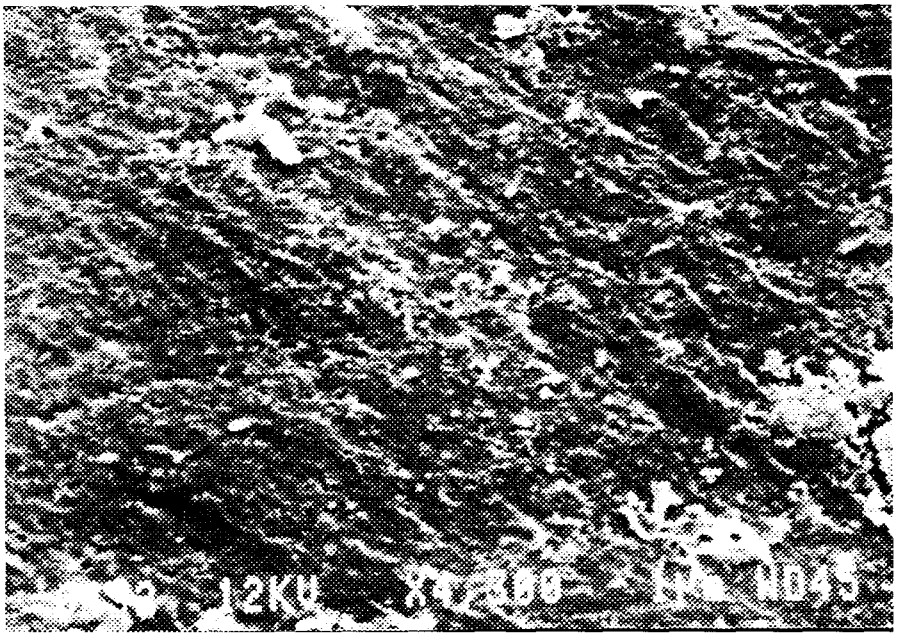

Figure 4.14: SEM micrograph of the foulant layer after 5 minutes of brown water ultrafiltration, PM-300, 100kPa, 100rpm. A rough surface morphology is evident. Mag= 4 300X

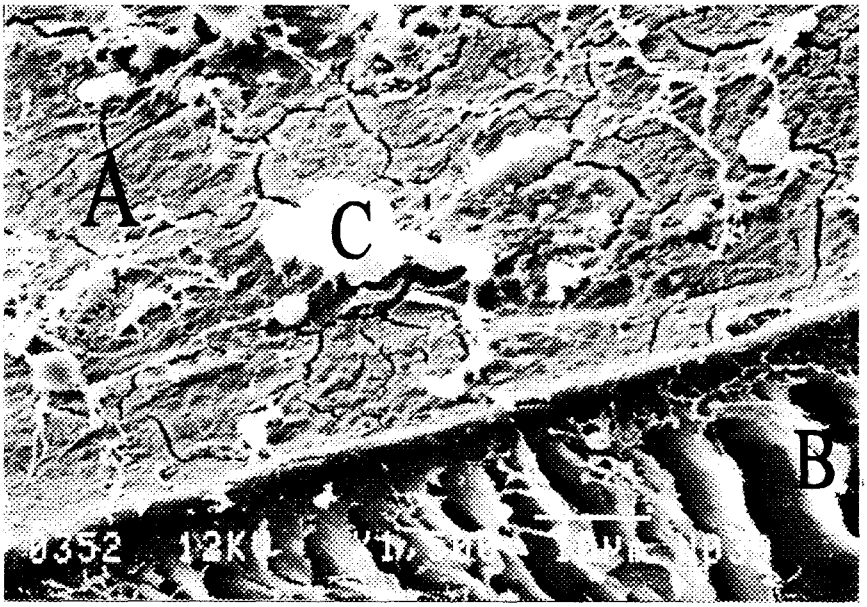

Figure 4.15: SEM micrograph of the foualnt layer after 30 minutes of brown water ultrafiltration, PM-300, 100kPa, 100rpm. (A) a rough surface morphology, (B) polysulphone membrane support, (C) polysaccharides. Mag= 1 500X.

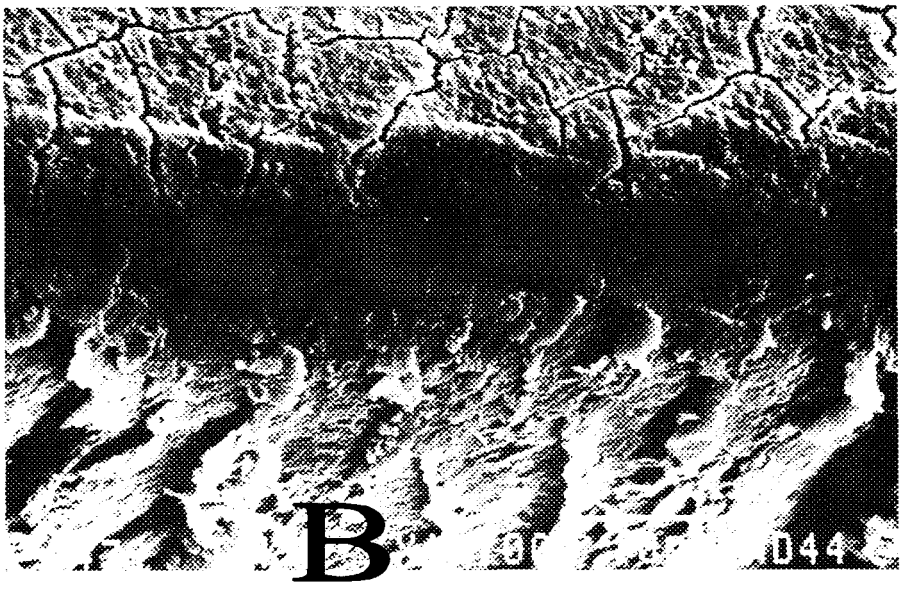

Figure 4.16: SEM micrograph of the foulant layer after 5 minutes of brown water ultrafiltration, PM-300, 100kPa, without stirring. (A) thick, rough foulant layer (B) polysulphone membrane support. Mag= 1 700X.

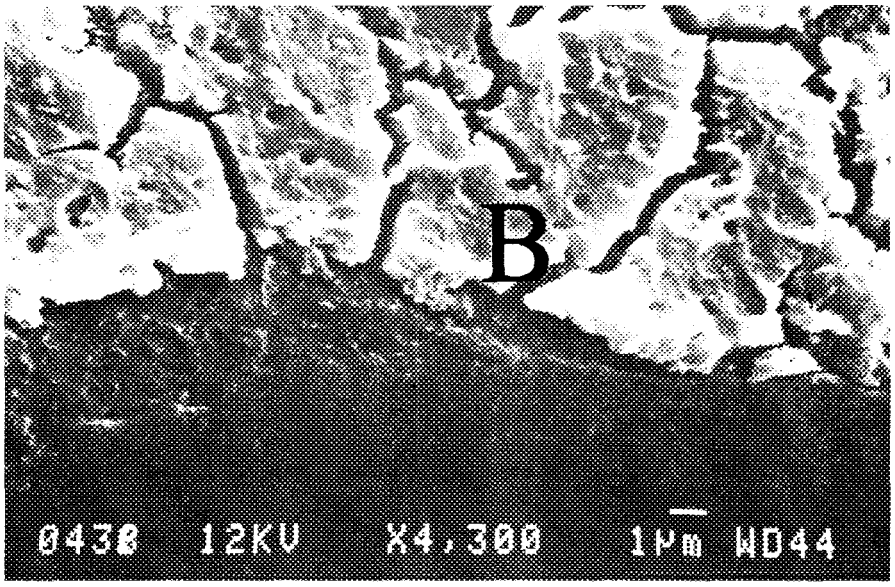

Figure 4.17: SEM microgarph of the foulant layer after 30 minutes of brown water ultrafiltration, PM-300, 100kPa, without stirring. (A) a rough surface layer morphology, (B) shows an additional more, compact layer indicating that the mechanism of fouling may be a result of cake compaction. Mag= 4 300X.

4.3.4 Fitting of Filtration Data to Established Fouling Models

Table 4.1: Equations for the constant pressure blocking filtration laws (Kim *et al.*, 1993b).

Model	κ	*n*	*Intregated equation*
Standard blocking (equation A)	$Ks = \left(\frac{2C}{LA_o}\right) Q_0^{0.5}$	1.5	$K_s t = \frac{2t}{V} - \frac{2}{Q_o}$
Cake Filtration (equation B)	$Kc = \frac{\alpha \gamma s}{AR_m Q_o (1 - ms)}$	0	$K_c V = \frac{2t}{V} - \frac{2}{Q_o}$

Abrreviations

A = membrane area (m^2)
C = volume of solid particles retained per unit filtrate
L = membrane thickness
K_S = Standard blocking model
K_C =Cake filtration model
m = mass ratio of wet to dry cake
Q_o = initial flow rate
R_m = membrane resistance
s = mass fraction of solids in slurry
t = time (s)
V = Volume (m^3)

k= proportionality constant
n= proportionality constant
α = cake specific resistance (m/kg)
γ = filtrate density (kg/m^3)

Table 4.1 shows two equations, relating to the filtration time and filtration volume, known as the constant-pressure blocking filtration laws.

These were obtained from the integration of the general equation (Kim *et al.*, 1993b):

$$\frac{d^2t}{dV^2} = \kappa\left(\frac{dt}{dV}\right)^n \qquad \text{.....4.2}$$

Where κ and n vary with the different physical models of the filtration process.

The standard blocking equation was derived from an assumption that particles reaching the membrane not only obstruct the pores but can also deposit on other particles. For the standard blocking equation, it was assumed that pore volume decreases proportionally with filtrate volume by particles being deposited on the pore wall. Cake filtration assumes that the increase in resistance was due to an accumulation of particles as a cake on the membrane surface (Kim *et al.*, 1993b).

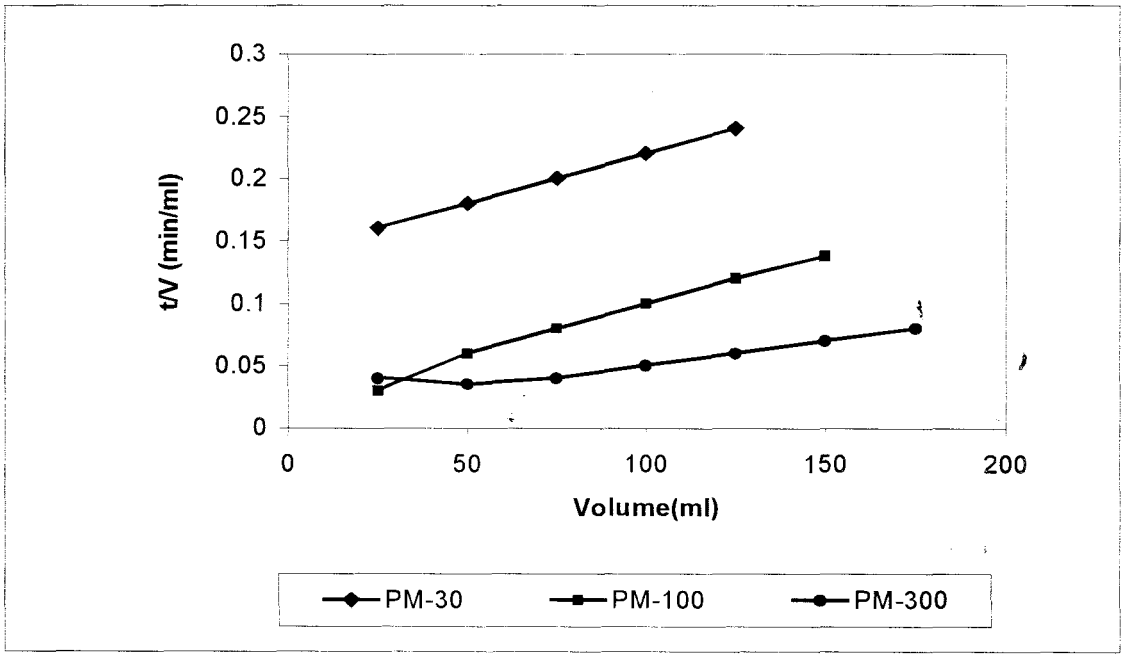

Figure 4.18: Analysis of the filtration data, according to the standard blocking model.

Figure 4.18 and Figure 4.19 displays the unstirred ultrafiltration data plotted according to the constant blocking filtration laws in order to identify the dominant mechanism of

fouling for the different membranes used in this investigation. Figure 4.18 shows a linear relationship for the standard blocking model and cake filtration model. The relationship between t/V and V should be linear if pore blocking was the dominant mechanism of fouling according to the equation of Table 4.1a. The results show that PM-30 after 25mL, PM-100 and PM-300 after 50mL approaches a linear plot indicating that the membranes were fouled according to the the standard filtration model (Figure 4.18). Values obtained were also calculated according to the equation given for the cake filtration model (Table 4.1, equation b). A linear relationship was obtained for PM-100 and PM-300 only. These approached a linear relationship at V=50mL, shown in Figure 4.19. This indicates that the most accessible pores for PM-30, PM-100 and PM-300 are blocked initially and the prevailing mechanism changes to either standard blocking in the case of PM-30 or cake filtration in the case of PM-100 and PM-300.

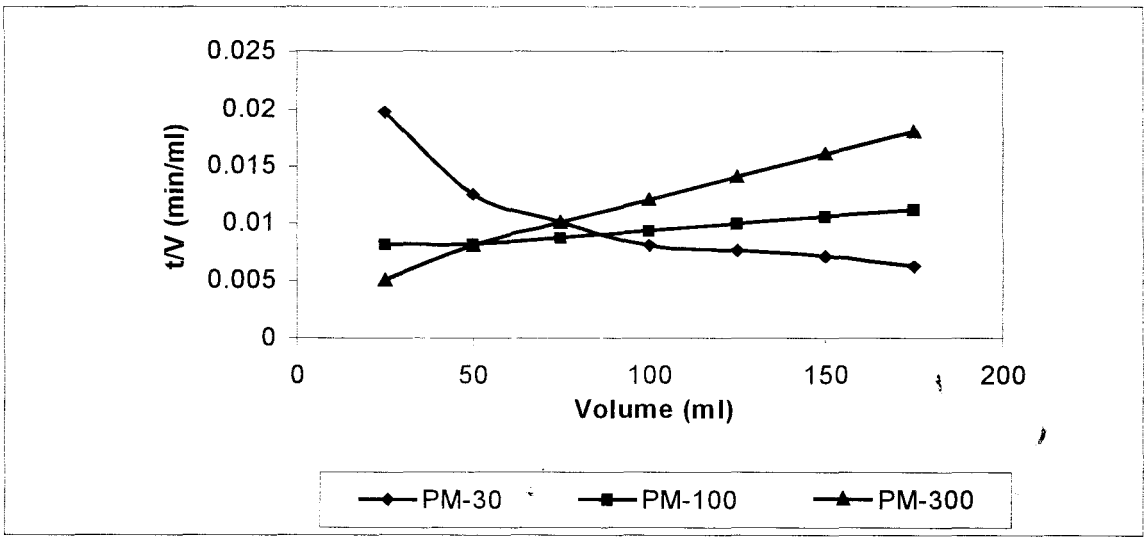

Figure 4.19: Analysis of the filtration data, according to the cake filtration model.

4.4 CONCLUSION

The data obtained for PM-100 and PM-300 during the course of the experiments suggests that there was a two step deposition, where initally pores are blocked or filled with particles being filtered. This was shown by the increase in the percentage rejection profiles over time. Subsequently the second stage would be a result of cake accumulation on the surface, for which evidence was provided by the SEM micrographs. In fouling adsorbed materials (surface and pore attachment) reduce flux and functions to improve retention in ultrafiltration.

PM-30 was used in all further immobilisation experiments to ensure the enzyme was retained by the membrane and to ensure that a high percentage rejection was obtained. The data suggests that the mechanism of fouling of PM-30 was due to adsorption according to the standard blocking filtration model. PM-30 was shown to be sensitive to changes in the stirring speed, which indicates that concentration polarisation or gel-layer formation played a role in flux decline. This gel-layer could be an extension of the adsorbed material due to hydrostatic or other attractive forces. The gel-layer could be non-invasive in a sense that the component particles do not remain within the boundary layer because their diffusivities are low due to high molecular masses. The gel-layer could be reduced further by increasing the linear-crossflow velocity at the expense, however, of increased energy consumption.

CHAPTER 5

MANGANESE PEROXIDASE INDUCTION: THE EFFECT ON THE FOULANT LAYER AND CLARIFICATION EFFICIENCIES

5.1 INTRODUCTION

Since a physical mechanism of fouling of ultrafiltration membranes by brown water had been established, the next step would be to characterise the defouling step. This was studied by visual inspection of the foulant layer and by chemical analysis of brown water clarification.

Several techniques exist to characterise organic carbon, in the case of colour producing humic substances, in water. The parameter, dissolved organic carbon (DOC) or total organic carbon (TOC), are among the most fundamental for the characterisation of organic matter in natural waters. In combination with these two parameters, the chemical oxygen demand (COD) and the biological degradability as determined by the biological oxygen demand (BOD) may also be used to assess the organic content of natural water sources.

The presence of unsaturated compounds (substances with delocalised electrons) usually imparts a distinct colour to the contaminated water. In the case UV-VIS spectroscopy and fluorescence are also used to get an overview of the organic carbon content of these types

of waters (Adham *et al.*, 1991; Huber and Frimmel, 1992). UV-VIS spectroscopy is an attractive technique to study the humic substance in feed water and permeate during ultrafiltration of naturally occurring brown water. Previous results reported by Huber and Frimmel (1992) have shown that a correlation exists between the UV absorbance and the DOC in water with high humic content.

Additional information from UV-VIS spectroscopy can be obtained by measuring the ratio between the absorbance at 465nm and 665nm. The ratio of optical densities or absorbances of dilute, aqueous humic acid (HA) and fulvic acid (FA) solutions at 465nm and 665nm (E_4/E_6) are widely used to characterise these materials in solution (Chen *et al.*, 1977). Chen *et al.* (1977) stated that the (E_4/E_6) ratios of HA and FA are governed by the particle size (or particle molecular weight), affected by pH, correlated with the free radical concentration, -O and -C (content), and -COOH (content and total acidity) in as far as these parameters are also functions of the particle size or particle molecular weight.

The ratio of absorbances at 465nm and 665nm is, therefore, an indication of the molecular size and the degree of chromophore conjunction or intramolecular complexation (Gressel *et al.*, 1995). Although the independent absorbance of HA and FA at 465nm and 665nm are influenced by the concentration of the substances (Baes and Bloom, 1989), the (E_4/E_6) ratio is independent of the concentration of HA and FA in the solution.

In addition, the magnitude of the (E_4/E_6) ratio is related to the degree of condensation of the aromatic carbon network, with a low ratio indicative of a relatively high degree of aromatic humic constituents. Conversely, a high (E_4/E_6) ratio reflects a low degree of aromatic condensation and infers the presence of relatively large proportions of aliphatic structures. The light absorption of aqueous HA and FA in the visible region of the aliphatic sidechains is indicative of the total carbon content and the molecular weight. All

of these factors are likely to influence the magnitude of the (E_4/E_6) ratio, but no rigorous experimental evidence has been presented in confirmation thereof (Chen *et al.*, 1977).

5.1.1 Research Objectives

The objectives of this chapter were to chemically and physically characterise the effects that MnP immobilisation and induction had on the clarification efficiency of the given process.

5.2 METHODS AND MATERIALS

5.2.1 Membrane Characteristics after Induction

In order to determine if there were any structural changes in the topography of the foulant layer after induction, ultrafiltration experiments were performed according to section 2.2.3. MnP was induced with the appropriate activator solution and the membranes were removed from the stirred cell reactor. 1cm^2 fragments were excised from the membrane, freeze-dried and gold coated. These were then viewed with the SEM.

5.2.2 Water Characteristics

Brown water absorbs light strongly in the UV range and this characterisatic can be used to measure the concentration of the humic substances that gives rise to the brown water colour. The UV absorbance of the brown water can therefore be used to determine the efficiency of colour removal. UV-VIS characteristics were measured continually through-out the experiment to determine the effect of enzyme immobilisation and enzyme induction on permeate quality. Wavelength scans of the feed and permeate were determined using a Shimadzu UV-VIS spectrophotometer. The absorbance, at 245nm, of

the permeate and feed are indicative of the percentage humic substances rejected from the feed (Chen *et al.*, 1977).

The percentage colour rejection was therefore determined from these values, given by the equation:

$$\%\, colour\ rejection = 100 - \left(\frac{A_{245nm} permeate}{A_{245nm} concentrate} x100 \right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 5.1$$

The absorbance at 465nm and 665nm were used to determine the degree of condensation of the aromatic carbon-network and to gain information on the particle size. Due to the sensitivity of the (E_4/E_6) ratios to changes to pH all samples were standardised to pH 6.

Throughout the experiment, the permeate and feed were removed (2m*l*) for sampling. The COD, TOC, pH, turbidity and colour were measured to determine if the product water changed after enzyme immobilisation, induction and flux restoration. Colour (Hazen units), turbidity (NTU) and COD (mg/*l*) were measured colorimetrically using the spectroquant™ system (Merck SQ118). TOC was measured by injecting 50µl samples into a TOC boat sampler and measured using a Total Organic Carbon Analyser (Dohrmann division, Rosemount Analytical Inc., Model 183) [Appendix 5]. Throughout the TOC analysis standard TOC solutions were analysed to ensure accuracy.

5.3 RESULTS AND DISCUSSION

5.3.1 Changes in Permeate Quality with Enzyme Immobilisation and Induction

In order to chemically characterise the defouling action of MnP, ultrafiltration of brown water was performed using PM-30 membranes. Table 5.1 compares the (E_4/E_6) ratio, percentage rejection and percentage colour removal during ultrafiltration before and after enzyme immobilisation.

Table 5.1: Analysis of the permeate showing the (E_4/E_6) ratio, % rejection, % colour rejection before and after immobilisation of manganese peroxidase (Values given after 30 minutes of filtration).

	Filtration of Brown Water (MnP not immobilised)	MnP Immobilised (MnP not induced)
E_4/E_6 ratio	4.87	3.3
% Rejection	88	93
% Colour Rejection	63.97	68.2

In both cases, the (E_4/E_6) ratio before and after enzyme immobilisation were relatively low (Figure 5.1). This indicated a relatively low degree of condensation of aromatic humic constituents. According to the literature, molecules of this nature have a high carbon-content, but relatively low -O, -COOH group content and low total acidity (Chen *et al.*, 1977). Humic substances are considered to be the primary foulant during the filtration of brown water (Swart, 1998, pers.comm.). Adsorption of humic substances onto the membrane surface probably occurs to form an extra foulant layer which subsequently causes changes in the membrane surface, pore characteristics and, therefore, permeate quality.

The (E_4/E_6) ratio decreased from 4.75 to 3.3 when MnP was immobilised onto the membrane surface (Table 5.1). This indicates that only smaller molecules are moving through the membrane pores when MnP was immobilised.

When MnP was immobilised the percentage rejection increases to 93%, compared to 88% when MnP was not immobilised. The percentage colour rejection as a result increases from 63.97% to 68.2% when MnP was immobilised. The percentage rejection and percentage colour rejection during filtration was higher after MnP immobilisation which indicates that the existence of the immobilised enzyme layer formed offered additional permeability constraints during the filtration process. The existence of the immobilised enzyme forms an intermediate layer which increased the membrane resistance and therefore the percentage rejection. In addition, the dynamic layer during ultrafiltration, as well as the immobilisation of the enzyme helped improve the clarification of the filtration process. This is considered advantageous since start-up time to obtain a good quality permeate would be reduced, and the process could become more controllable.

Table 5.2: Analysis of the permeate showing the (E_4/E_6) ratio, % rejection, % colour rejection before and after activation of manganese peroxidase (Induction occurred after 30 minutes).

	MnP Immobilised	
	Before Induction	**After Induction**
E_4/E_6 ratio	**3.3**	**2.8**
% Rejection	**93**	**74**
% Colour Rejection	**68.2**	**53.21**

The precise chemical mechanism of action of MnP on brown water humic substances is presently unknown, but after induction it is suspected that a conformational change in the foulant layer occurs. The conformational change may result in the lifting of the foulant layer creating a 'non-fouled' area which allows water to flow more freely through the

membrane. During the defouling process some of the fouling substances were removed resulting in an increase in the effective permeability. This can be seen by the decrease in the percentage rejection from 93 to 74 after MnP induction (Figure 5.2). The percentage rejection decreased and as a result the percentage colour rejection decreased, from 68.21 to 53.21 after MnP induction, indicating increased permeation of humic substances.

The slight reduction observed in the (E_4/E_6) ratio from 3.3 before MnP induction to 2.8 after MnP induction was unexpected (Table 5.2). This may be an indication that the humic substances are being transformed as a result of the chemical mechanism of action of MnP on the foulant layer. The decrease in the (E_4/E_6) ratio may indicate that the humic substances are being polymerised by the action of MnP and therefore retained in the retentate or depolymerised into smaller fractions which permeated through the membrane.

Table 5.3: Organic and particle fractionation by PM-30 before and after MnP induction.

WATER ANALYSIS	MnP immobilised Before induction		MnP immobilsed After induction (30 min)	
	Permeate	Retentate	Permeate	Retentate
COD (mg/L)	52	289	69	230
TOC (ppm)	58	69	65	72
Colour (Hazen units)	30	315	103	242
Turbidity (NTU)	9	81	35	53

If the values shown in table 5.3 are compared before and after enzyme induction, it can be seen that there was a substantial increase in the concentration of carbon content of the permeate and a decrease in the retentate after induction. This indicated that substances,

which were once retained by the membrane, permeated freely after MnP induction due to the disruption of the foulant layer. The increase in the permeate COD and TOC was not as dramatic as the increase observed with the colour and turbidity. COD values increased from 52 to 69 mg/L, while the TOC increased from 58 to 65ppm. The most dramatic changes were the increase in colour from 30 to 103 Hazen units, and turbidity which increased from 9 to 35 NTU. A maximum of 15 Hazen units (Swartz and de Villiers, 1996) and 0.1 NTU (Offringa, 1997) are required if water is directed at human consumption. There was a marked decrease in the clarification efficiency after enzyme activation which suggests that the defouling step decreases the process efficiency while increasing productivity. This needs to be born in mind when optimising the process.

5.3.2 SEM Micrographs of the Foulant Layer Before and After Induction

For analysis of the physical effect of MnP on the foulant layer a series of experiments were performed to visualise the disruption of the foulant layer after MnP induction. The SEM micrographs are shown in Figure 5.1 to Figure 5.5.

Figure 5.1 and 5.2 shows the foulant layer after filtering 100mL brown water through the membrane after MnP has been immobilised and not induced. The foulant layer has a rough surface morphology, a distinct difference when compared to the SEM micrographs of the foulant layer when no enzyme was immobilsed (Figure 4.8 and Figure 4.9). Evidence exists which indicates a relationship between the morphology and physical characteristics of fouling (Riedl *et al.*, 1998).

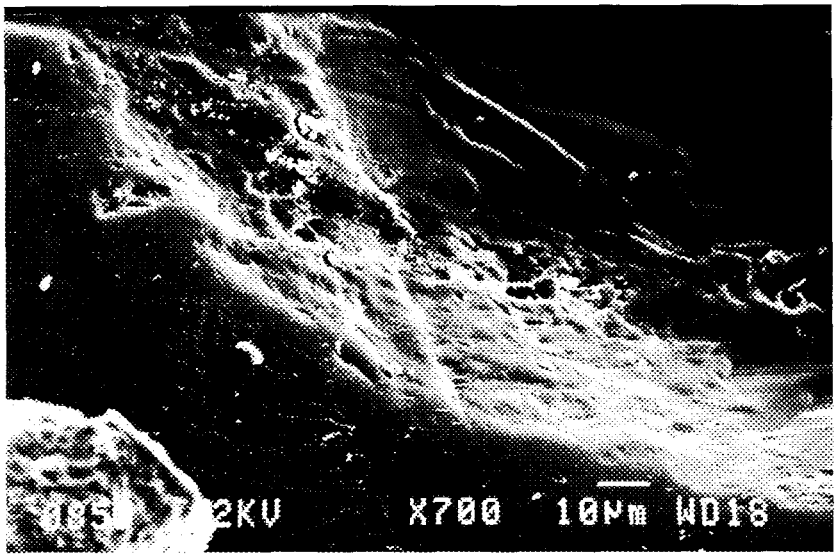

Figure 5.1: SEM micrograph of the foulant layer after 30 minutes of brown water ultrafiltration at 100rpm, 100kPa. MnP was immobilised but not induced. It can be seen that the foulant layer has a rough surface morphology. Mag=700X.

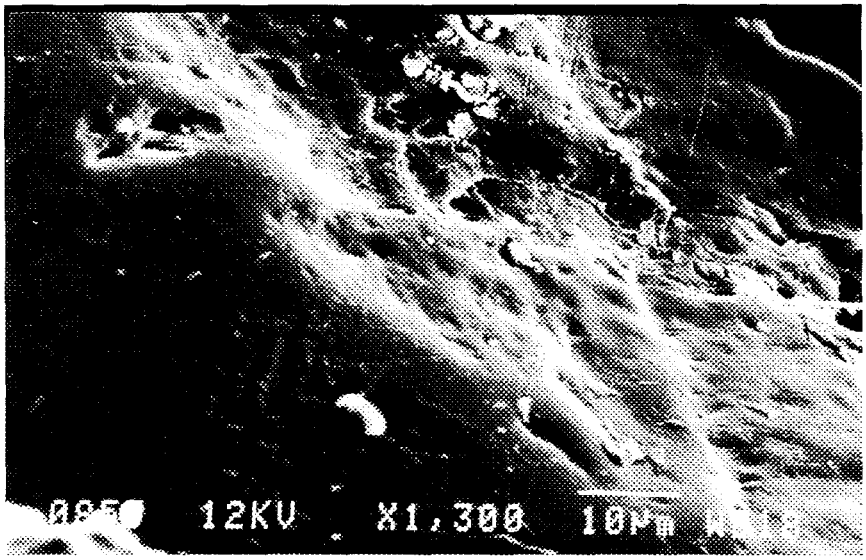

Figure 5.2: SEM micrograph of the foulant layer after 30 minutes of brown water ultrafiltration at 100rpm, 100kPa. MnP was immobilised but not induced and shows again that the foulant layer has a rough surface morphology. Mag= 1 300X.

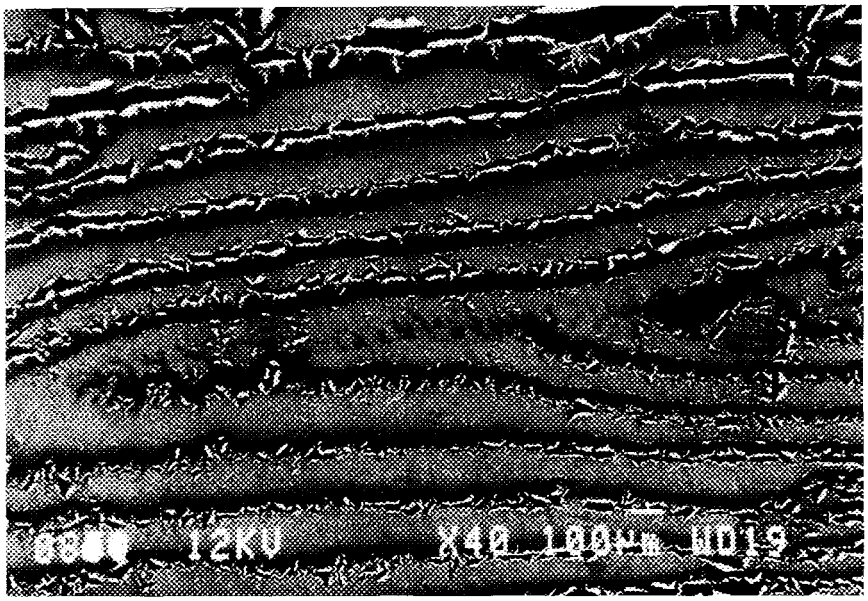

Figure 5.3: SEM micrograph of the foulant layer after MnP was induced. (A) the 'grey areas' indicates the foulant layer, (B) the 'black areas' indicates the region where Mn(III) has disrupted the foulant layer. Mag=40X.

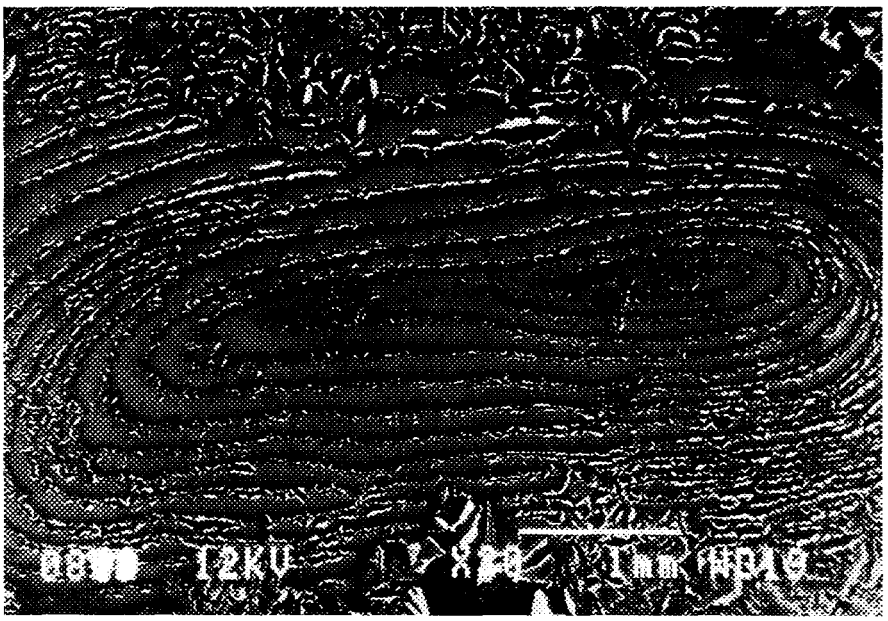

Figure 5.4: SEM micrograph of the foulant layer after MnP was induced. Distinct concentric elliptical patterns can be seen. Mag=20X.

Figure 5.3 to 5.5 shows the conformational change in the morphology of the foulant layer after MnP induction. The black areas show the 'non-fouled' area or the area where the foulant layer has lifted. The SEM micrographs shows that the disruption of the foulant layer occurs in distinct concentric elliptical patterns. In order to determine the cause of these patterns the disruption of the foulant layer was compared to unstirred conditions. Figure 5.6 shows the SEM micrograph of the disruption of the foulant layer upon induction, during unstirred conditions.

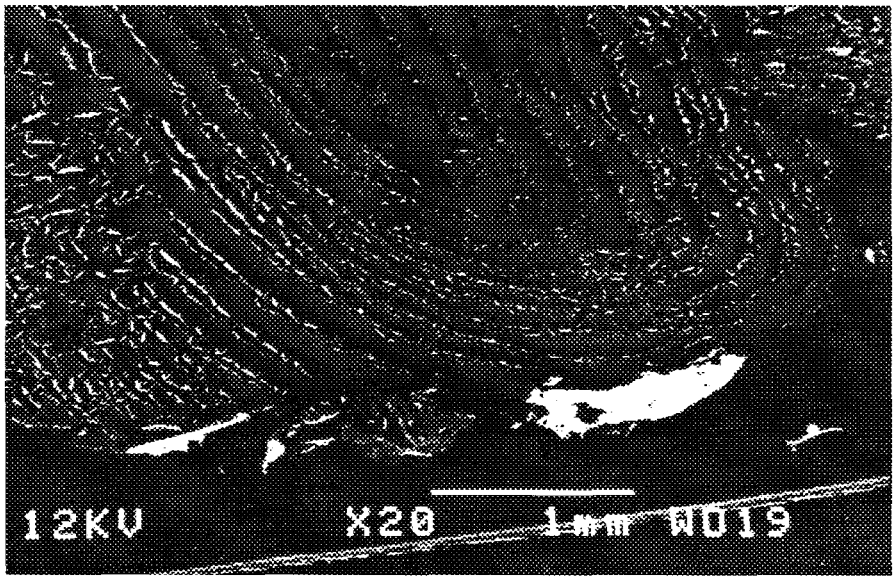

Figure 5.5: SEM micrograph of the foulant layer after MnP induction, 100rpm and 100kPa (T=30 min). (A) shows the intermittent grey and black areas showing the regions of the disruption in the foulant layer. (B) shows a large black region where the foulant layer has been removed completely. This was found on the outer edges of the membrane and was expected as MnP would be concentrated around the perimeter during the immobilisation technique. Mag= 20X.

Figure 5.6: SEM micrograph of the foulant layer after MnP induction (30 minutes), 100kPa, without stirring. (A) the foulant layer, (B) shows the the disruption of the foulant layer. No distinct concentric eliptical patterns are seen in un unstirred system. Mag=35X.

Figure 5.6 shows a similar surface morphology of the disruption of the foulant layer. The black areas represent the 'non-fouled' areas. The main difference was that, the concentric patterns seen which were visible under stirred conditions were not present under unstirred conditions. This indicates that the concentric rings which are seen under stirred conditions are a result of the Mn(III) disassociating from MnP which are then carried by the fluid force fields created by the stirring action in the ultrafiltration cell used.

5.4 CONCLUSION

From the evidence presented thus far, it has been shown that upon induction of MnP flux was restored and the clarification efficiency decreases indicating that MnP causes disruption of the foulant layer. SEM micrographs show clearly the disruption of the foulant layer and 'non-fouled' areas which explains the flux increase and the decrease in the permeate quality.

The chemical mechanism of action of MnP is uncertain. MnP could depolymerise, polymerize or merely transform the foulant layer. In a preliminary study performed by Claire Watcham (B.Sc.(Hons), 1998, Rhodes University) it was suggested that the humic substances in the water were being polymerised, however, there is no rigorous experimental evidence to prove this. Further experimentation is required to attain the mechanism of action of MnP. Experiments involving mass spectrometry are required to ascertain the mechanism of MnP. This will need to be undertaken in follow-up studies.

CHAPTER 6

CONCLUSION AND FUTURE RESEARCH

6.1 DEFOULING ULTRAFILTRATION MEMBRANES

The screening of three peroxidase enzymes revealed that MnP activity, directed at the degradation of the foulant layer during ultrafiltration of brown water, was the most effective enzyme at restoring transmembrane flux. MnP activity, related to defouling, was dependant on the concentration of hydrogen peroxide and manganous ions and was stimulated by organic acids such as lactate and oxalate. A 93% flux improvement was obtained when 800 μM manganous sulphate, 100mM hydrogen peroxide were added in the presence of a manganese chelator, lactate.

Upon further investigation results showed that humic substances, that contribute to colour in brown water, were the major cause of fouling which resulted in a decrease in the permeability of the membrane. PM-30 membranes were fouled according to the standard blocking model. The impact of the foulant layer together with the immobilised layer resulted in an increased clarification efficiency. The defouling mechanism was visually detected, with the use of SEM, which showed that there was a conformational change in the foulant layer. The action of MnP caused a transformation of the foulant layer creating a non-fouled area which allowed the process water to flow more freely through the membrane.

A novel approach to the problem of membrane fouling and the use of immobilised MnP was therefore introduced by this investigation.

The economic impact of any cleaning procedure, including the costs of the cleaning process itself and the effect of the membrane lifetime and efficiency, is an important consideration. The treatment costs, however, must relate to the direct benefits obtained from such a system. This system has several strengths making it attractive for certain applications. These are based on the following criteria:

❖ ***Speed of Action***

Due to the fact that the enzymes act on the upstream side of the foulant layer, the need to digest the entire foulant layer was obviated resulting in rapid defouling. In addition, enzymes have a high catalytic efficiency and act with reasonable specificity on the substrate to be degraded.

❖ ***Ease of Operation***

Low concentrations of activator solution are needed to alleviate fouling, as opposed to chemical methods, where sufficient washing is required to remove the entire foulant layer.

❖ ***Clean, Effective Operation***

Efficient operation results from less frequent backflushing and chemical cleaning. Although this system could operate without backflushing and chemical cleaning for extended periods, it is anticipated that these will still be required in some applications. The minimisation of backflushing means that less of the product would be needed for the

procedure, thereby improving the inherent productivity of the process. This is of particular relevance when comparatively small batches of water are to be clarified. Less frequent chemical cleaning means that large quantities of these chemicals are not released into the environment, making the process safer, more environmentally friendly and reducing overall chemical costs. The membrane's life expectancy would therefore also be extended since aggressive cleaning chemicals are known to damage membranes and change their overall properties. The effect of the action of MnP on the membrane integrity still needs to be determined.

❖ ***Benefits of the Dynamic Layer Filtration***

At present, ultrafiltration membranes routinely applied to the clarification of brown water are efficient at colour removal and retention of microbes and viruses. However, small unwanted impurities like pesticides and toxic heavy metals can find their way into the permeate quite easily. Unlike several other ultrafiltration processes, dynamic layer formation can be encouraged with this process since the cake layer is easily detached by enzyme action, and the humic acids have very good adsorbant properties. Although the permeate quality is not expected to match that of reverse osmosis systems, the energy requirements are expected to be much lower using this approach.

The initial anticipated application of this technology included portable/compact units for the provision of potable water to small, rural communities. The speed and simplicity of this process makes it very attractive for small-scale systems for brown water purification. The production of portable units, however, may not be feasible as enzymes have to be kept under controlled conditions and it may be difficult in producing enzyme immobilised commercial membranes which are easily preserved and transported under normal conditions and have good repeatability and stability. A more practical application is a large scale unit which would function to supply water to rural communities. A mechanical dosing pump can be added to moderate the addition of the activator solution, during the process. The system would, therefore, be able to operate in most locations with reduced manpower, power costs and require a low level of technical proficiency.

Given the potential of this concept a range of applications could potentially be developed, involving various membrane processes (not only ultrafiltration). In addition, with the drive towards greener production technologies, it is anticipated that the 'defouling on demand' strategy may be suitable for an increasing number of applications in the food industry. In fact, it can be applied to any form of filtration application where fouling of organic complexes are a problem. Future applications would depend on the provision of appropriate activatable enzymes and their effect on the major fouling component in the feed stream.

Two critical factors which need to be addressed for the application of this technology are the capital cost and stability of the enzymes used. Immobilisation of MnP reduces the amount of enzyme required for cleaning. In addition, MnP is not continually active, thereby increasing the enzyme half-life, which in turn ultimately reduces the capital cost for the enzyme. With respect to enzyme production, several advances have been made which ensures high production rates of MnP under optimal conditions. Shear stress, biofilm thickness and agitation have been shown to be the key parameters. The application of enzymes in industrial processes on a large scale requires the use of an efficient production system. Thus it is important to select a suitable bioreactor configuration and the correct environmental and operating conditions which will affect both the enzyme stability and enzyme production. Results have also shown that a low concentration of a crude extract of MnP is required to restore flux to ultrafiltration membranes.

For this type of practical application envisioned, it is likely that a crude extract rather than a purified enzyme system would be more effective due to the high costs of enzyme purification. In addition, the entire complement of homologous extracellular enzymes, which are secreted by *P. chrysosporium,* may be necessary for this type of application. It

was for this reason that a crude extract of MnP rather than a purified one was used for the duration of the investigation.

A fundamental criterion of this system which still needs to be addressed is enzyme stability. For enzymes immobilised onto membranes by physical adsorption, the structure of the enzyme layer may change continually under the action of pressure, enzyme activity may be affected by pressure and shear effects.

The concept of the 'defouling on demand' strategy using activatable enzymes has been shown to be practically viable and characterisation of the process shows it to have considerable potential for this type of application. Further studies will be directed towards the establishment of the feasibilty of the 'defouling on demand' system in terms of enzyme stability, cost and the operational practicality of the ultrafiltration models produced for rural water purification. The proper characterisation of the mechanism of action of MnP under experimental conditions needs to be assessed. Finally the evaluation of this technology developed for large-scale membrane defouling applications would be required before an economic assessment can be made.

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APPENDIX 1

Maintenance and Storage of Phanerochaete chrysosporium ATCC 24575.

A) 2% w/v Malt Extract Agar (MEA) Slants

MEA	4.5g
Agar	0.8g

B) Maintenance and Spore Production

Glucose	10g
Malt Extract	10g
Yeast Extract	2g
L-Asparagine	2g
KH_2PO_4	2g
$MgSO_4.7H_2O$	1g
Thiamin-HCl	1mg
Agar	20g

- Make up to 1L with deionised water.
- Autoclave and and dispense into 1L Roux bottles
- Inoculate and incubate for 5-7days at 39°C.
- Store at 4 °C

APPENDIX 2

Shaking Culture Medium

Basal Medium	100mL
10% Glucose	100mL
0.1M 2,2 dimethylsuccinate (pH 4.2)	100mL
Thiamin (500mg/L stock, filter sterilised)	2mL
Ammonium Tartrate	25mL
Veratyl Alcohol (0.02M stock, filter sterilised)	100mL
Trace Elements	70mL
Spores	50mL

-Make up to 1L with deionised water.

Trace Element Solution

$MgSO_4$	30g
$MnSO_4$	0.5g
NaCl	1g
$FeSO_4.7H_2O$	0.1g
$CoCl_2$	0.1g
$ZnSO_4.7H_2O$	0.1g
$CuSO_4.7H2O$	0.1g
$AlK(SO_4)_2.12H_2O$	10mg
H_3BO_3	10mg
$NaMoO_4.2H_2O$	10mg
Nitrilo acetic acid	1.5g

Dissolve nitrilotriacetate in 800mL H_2O, adjust pH-6.5 with 1M KOH, add each trace element, and then bring the volume to 1L.

Basal Medium III Stock Solution

KH_2PO_4	20g
$MgSO_4.7H_2O$	10,236g
$CaCl_2$	1g

- Make up to 1L with deionised water
- Autoclave

APPENDIX 3

MnP Activity- Oxidation of ABTS

Reagent A

Make up fresh, centrifuge before used to give a clear supernatant

Egg Albumin	150g (6mg/m*l*)
Sodium lactate buffer	2.5ml (100mM)
Sodium succinate buffer	2.5ml (100mM)
$MnSO_4$ solution	1.0ml (200mM)
ABTS solution	1.0ml (80 μg/m*l*)

- Add reagent to a 25m*l* volumetric flask, make up volume with deionised water.

Reagent B

100 μM hydrogen peroxide. Make up fresh, store in dark container.

- Measure 1.0 μ*l* of 30% hydrogen peroxide into 100m*l* volumetric flask, make up volume with deionised water.

ASSAY

Into a 1m*l* quartz cuvette place:

Reagent A	450 μ*l*
Reagent B	450 μ*l*
Enzyme sample	100 μ*l*
Total volume	1000 μ*l*

Invert, mix and assay immediately

Read at 420nm

$E = 3.6 \times 10^{-4}$

APPENDIX 4

HRP and SBP Activity

Reagent A

0.1M K_2HPO_4 and citric acid, pH5.5. Store at 25°C.

Reagent B

0.018 M Guanicol. Store on ice.

Reagent C

Dilute 0.1m*l* 30% H_2O_2 with deionised water to 120m*l*, and adjust A_{240nm} in 1cm light path to 0.4 -0.41. Make up fresh, store in dark container.

ASSAY

Into a 3mL quartz cuvette place:

Reagent A	2.8m*l*
Reagent B	50µ*l*
Reagent C	50µ*l*
Enzyme sample	100µ*l*
Total volume	3000µ*l*

Invert, mix and assay immediately

Read at 435nm

E=3.6 x 10^{-4}

APPENDIX 5

TOC Analysis

Prepare a 200ppm C stock standard by weighing 425mg of reagent grade Potassium Hydrogen Phthalate ($C_8H_5O_4K$), KHP, dried to a constant weight. Throughly transfer the KHP to 100m*l* volumetric flask, and dissolve with 75m*l* reagent water. Add 0.1m*l* reagent grade concentrated phosphoric acid, and fill to the 100m*l* mark with reagent water.

Store at 4°C in a dark glass
Dilute this solution to a concentration that is appropriate for the application.

100ppm C standard

Add 5.0mL of 2000ppm standard to 100m*l* with deionized water to obtain a 100ppm standard.