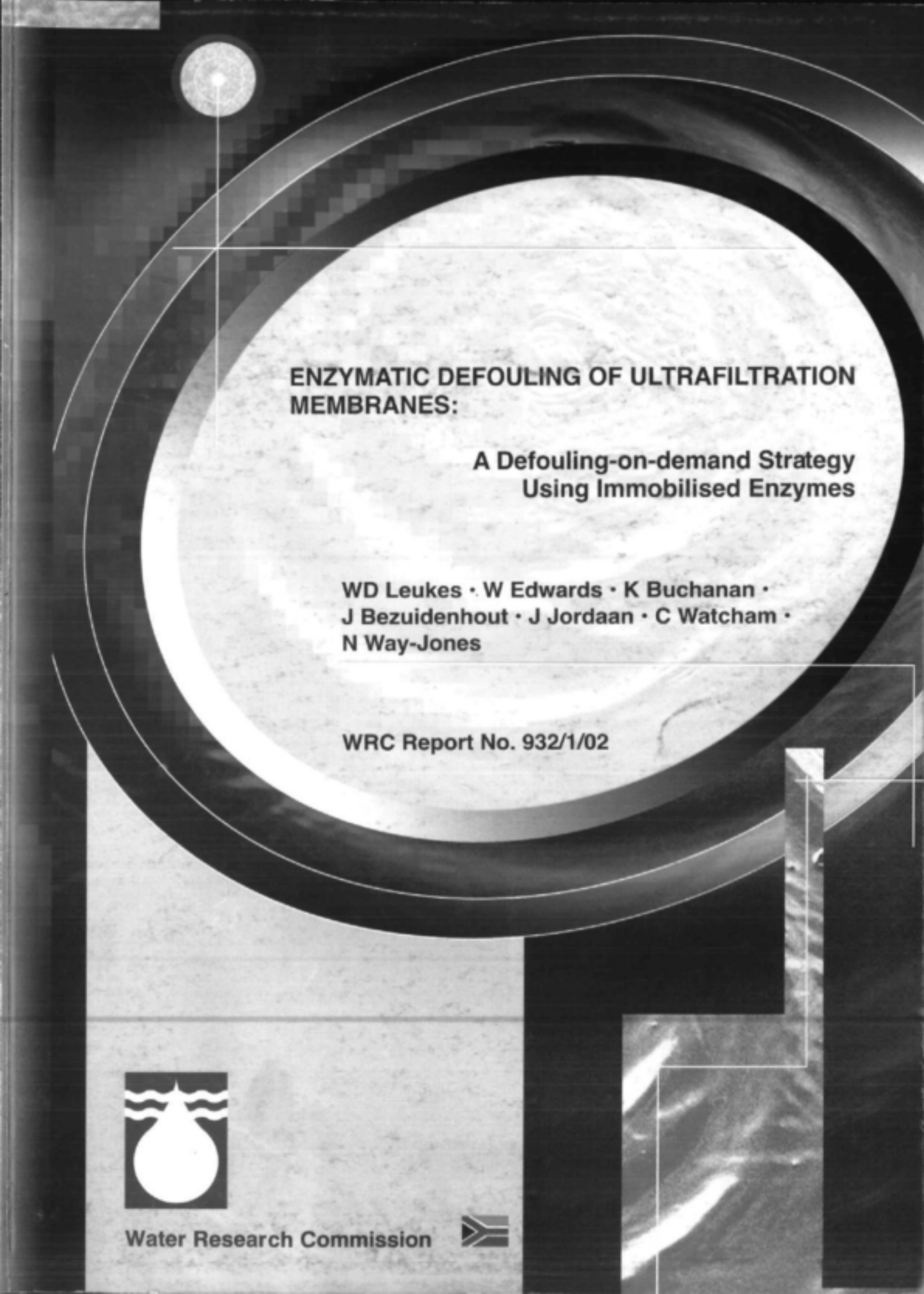

ENZYMATIC DEFOULING OF ULTRAFILTRATION MEMBRANES:

A Defouling-on-demand Strategy Using Immobilised Enzymes

WD Leukes · W Edwards · K Buchanan ·
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WRC Report No. 932/1/02

Water Research Commission

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A Defouling-on-Demand Strategy Using Immobilised Enzymes

Final Report to the
Water Research Commission

by

W Leukes, W Edwards*, K Buchanan, J Bezuidenhout, J Jordaan,
C Watcham and N Way-Jones

Department of Biochemistry, Microbiology and Biotechnology
Rhodes University
Grahamstown

*Department of Microbiology, School of Environmental Science
Potchefstroom University for CHE
Potchefstroom

ISBN 1 86845 881 4
WRC Report No 932/1/02

SEPTEMBER 2002

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LIST OF ABREVIATIONS AND GLOSSARY

ABTS – 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)

Activator (Activator Solution) – A solution of compounds which activates the defouling system. In this case, these compounds are enzyme inducers

Aggregation (macromolecular aggregation) – coagulation of macromolecules to form larger, often insoluble aggregates

Breakthrough analysis – a study, during which an adsorbent molecule (in this case, phenolic compounds) is allowed to adsorb to a sorbate (in this case, a membrane) until saturation point is reached, thereafter, further addition of phenolic compound will result in break through into the permeate.

Brown Water (organically coloured water, naturally brown water) – water originating from rivers in the Western cape with a high content of humic substances which impart a dark brown colour.

DCP – di-chloro phenol

De-polymerisation – hydrolysis or degradation of polymeric compounds into their substituent monomers

DMAB – 3- (dimethylamino) benzoic acid

DMP – di-methyl phenol

Dynamic membrane: During filtration, a layer of filtered material accumulates at the membrane surface due to concentration polarization and/or cake layer formation. This layer of material, called a dynamic membrane, since its formation depends on process conditions, enhances the filtration effectiveness of the membrane by excluding particles through depth filtration, ion exchange or adsorption.

EDTA – ethylene diamino tetraacetic acid

Humic substances (tannins) – polyphenolic compounds derived from plant material which imparts a brown colour to water. The major constituents, in general, are tannic- gallic- and fulvic acids.

Inducer (enzyme inducer) – achemical that is required by an enzyme to make it catalytically active.

Irreversible fouling – during operation of a filtration process, 2 types of fouling predominate, viz. concentration polarization, an increase in concentration of filtered material near the surface, which can be alleviated through back-flushing, and irreversible fouling, which relates to materials which have adsorbed to the membrane. This cannot be remedied through backflushing, and requires a cleaning regime.

J – Flux (L.m^{-2}.h^{-1})

K. – proportionality constant between flux and pressure

MBTH – 3-methyl-benzothiazolinone hydrazone

MnP – Manganese Peroxidase

NBW – Natural Brown Water – Brown water

NOM – natural organic matter – material, typically organic, which is found dissolved or in suspension in surface water. A major component of the NOM in brown water in the humic substance group.

P – Pressure (kPa)

PAGE – polyacrylamide gel electrophoresis

Polymerisation – covalent bonding of monomers into repeating units of a polymer.

RRW – Raw River Water – see NBW

RTD – Residence Time Distribution

SEC – Size Exclusion Chromatography

SEM – Scanning Electron Microscopy

TA – Technology Assessment.

TGA – thioglycolic acid

ACKNOWLEDGEMENTS

The authors would like to thank the following people for their input into the project:

Dr. E. P. Jacobs of the University of Stellenbosch for supply of test membranes and advice on ultrafiltration.

Mr. P.J. Jansen van Rensburg and Prof. K.J. Riedel of the University of Potchefstroom for CHE for assistance with analyses and advice, respectively.

Ms. S. Pinchuck and Mr. R. Cross from the Electron Microscopy Unit at Rhodes University.

Mr. B. Kemp from the Physics and Electronics Unit at Rhodes University for assistance with data capture.

The steering committee, Mr. M. Pryor, Prof. C. Buckley, Prof. P. Swart, Ms. M. Santa, Prof. T.E. Cloete and Dr. G. Offringa for valuable assistance.

SASOL for providing effluent and the George Municipality for providing brown river water.

Prof. P. Rose for ideas and support.

The WRC for their support.

EXECUTIVE SUMMARY

Motivation

Membranes have found increasingly greater application for water purification, and this trend is expected to continue in the future. A particularly challenging application is the ultrafiltration (UF) of natural organic matter (NOM) for the removal of polyphenolic compounds derived from plant matter. These polyphenolic compounds, mostly humic substances, contribute significantly to membrane fouling, resulting in the requirement for frequent chemical cleaning. Chemical cleaning methods have proven highly effective, but the chemicals used tend to be aggressive, often causing damage to the membranes. The chemicals tend to be toxic, which makes their discharge into the environment unacceptable.

Enzymatic cleaning has been offered as an alternative to chemical cleaning since enzymes are biodegradable and do not cause additional pollution problems. In addition, enzymes operate under mild conditions of pH and temperature, and would not contribute to membrane damage. However, slow action and high cost makes the use of enzyme-based cleaning methods unattractive. In this work, a new approach is presented for the cleaning of UF membranes used for NOM decolorisation. With this approach, fungal enzymes capable of oxidising humic substances are immobilised onto UF membranes. These enzymes require co-factors for their catalytic action, so are inactive during the UF (water purification) process. When the membrane is fouled the enzymes are "switched on" by addition of the co-factors. The enzymes then act on the humic substances that contribute to membrane fouling, thereby restoring flux by disengagement of the foulant layer. This approach has several benefits over conventional UF for water purification, including 1) The enzymes are immobilised and therefore re-usable, thereby considerably reducing the cost of the enzymes, 2) Because the enzymes are inactive during the UF process, a controllable dynamic layer of humic substances can be used to further improve the water purification efficiency, 3) Because the enzymes are located between the membrane and the foulant layer, the entire foulant layer need not be oxidised. This considerably reduces the cleaning time compared to enzyme washing, where the entire foulant layer has to be digested.

Objectives

Previous work proved the concept of using the enzyme manganese peroxidase (MnP) for defouling of membranes used for the ultrafiltration of river water with high humic substance content (Leukes *et al.* 1999). The objectives of this work were to analyse factors affecting the performance of MnP for defouling; determination of the fouling and defouling mechanisms; development of the process for application to capillary membranes for effluent treatment and brown water purification and engagement in technology assessment with the emphasis on technology development for community use.

Approach

The enzyme Manganese Peroxidase (MnP), produced by the fungus *Phanerochaete chrysosporium*, was initially used for enzymatic defouling. Flat sheet membranes were used with a Stirred UF Cell to study the mechanism of defouling of the enzyme and the factors affecting the performance of the system. Single fibre capillary membranes were used to determine the applicability of the system for capillary UF membranes. Based on analysis of shortcomings of the process (by Technology Assessment tools), a new enzyme, a stable laccase with broad substrate range, was evaluated. Research involved the screening of the natural isolates of local fungi for strains that produce suitable enzymes. Sufficient enzyme was then produced by the isolated fungus for evaluation on capillary membranes. The process was evaluated for the purification of natural brown river water. Preliminary tests were also performed to evaluate the system for the treatment of a polyphenol-rich industrial effluent.

Results

Initially, the factors affecting performance of the defouling system on flat sheet membranes was investigated. It was found that enzyme concentration, H_2O_2 and Mn^{2+} concentrations and the presence of organic acids affected the performance of the system. It was found that 400 μM $MnSO_4$, 5 mM H_2O_2 and 200 mM lactate offered adequate defouling, but that for large scale application this would result in considerable expenditure on reagents. Concerns were also raised about the safety implications of stored H_2O_2 and the potential negative effects of manganese and H_2O_2 carry-over into product water.

Studies were then undertaken to determine the mechanism of fouling and defouling in an attempt to either make dramatic improvements in the current system or to identify alternative enzyme-activator systems. The results of this study showed that the dominant fouling mechanism was pore blocking as opposed to pore constriction. It was also shown that the process conditions affected the nature of the cake layer formed, implying that the physical conditions of the process as well as the chemistry of the enzyme and foulants needed to be considered.

The mechanism of defouling was investigated using size exclusion chromatography to determine the dominant mechanism of enzyme action on the foulant layer and electron microscopy to determine the physical nature of the defouling action. It was found that the process defouled the membrane by polymerisation or aggregation of components of the foulant layer. Sufficient shear was required to ensure lifting of the de-stabilised cake layer generated by the enzyme action.

The above study allowed the identification of another enzyme system which would have better potential application for brown water treatment. This approach involved the use of a laccase enzyme, known to polymerise polyphenolic compounds, which requires only oxygen as a co-factor for activation. This is much less expensive and safer than the use of the chemical activators required for use of MnP as a defouling enzyme. A new laccase enzyme with good stability and an extended substrate range was thus investigated for application to the defouling of river water. This proved to be successful when used with capillary membranes operated under a constant flux regime. Effective restoration of specific flux was obtained by daily oxygenation of the system. This could be maintained for several days making it competitive with backflushing regimes and applicable to systems currently used for brown water ultrafiltration.

The process was also applied to effluent treatment. An aggressive combined waste from the petrochemical industry was used as an example. The system proved useful for the elimination of low-molecular weight aromatic compounds, but not for membrane defouling. Useful methodology was developed for the identification and handling of enzyme inhibitors present in such effluents.

Technology Assessment protocols were used to identify the problems associated with the use of MnP and to guide the project into the new direction taken, ie. the use of a new enzyme system, as opposed to improvement and scale-up of the MnP system.

Conclusion

A different approach is offered to dealing with the problem of membrane defouling during the UF of natural brown water. By using "activatable" enzymes the defouling technology is integrated with the operation of the system, offering improvements in quality of product water as well as a convenient method of membrane defouling. The implication of this approach is that an easy-to-operate, safe system can be developed for peri-urban and rural water supply. The system also offers a unique solution to the treatment of industrial effluents containing aromatic pollutants since the enzymes could potentially be used to both detoxify the effluent as well as defoul the membranes. Since the system allows the application of a dynamic layer of foulant molecules, the potential exists for use of this system on membranes that have reduced clarification performance because of age or wear.

For the system to realise the above potential benefits, large-scale, low-cost production of the thermostable laccase should be developed for provision of sufficient enzyme for pilot-scale testing. Also, effective process design needs to be done to formulate this technology into a usable operating system.

CHAPTER 1: INTRODUCTION

Organic colour is usually associated with soft surface waters with little or no alkalinity (Swartz and de Villiers, 1998). 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, 1998; 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, 1998).

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 Platinum (Swartz and de Villiers, 1998).

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, 1998). 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, 1998; Owen *et al*., 1995).

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

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 pre-filtration with metal strainers to remove particulate matter, a single step clarification and decontamination 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 municipal 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.*, 1995). 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).

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 and reverse osmosis constitute 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 solutions, 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).

MEMBRANE FOULING

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 only 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 of fouling, there is a decrease in the effective driving force for ultrafiltration or an increase in the resistance against the transport of the permeating solvent during the filtration process.

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).

Adsorption and precipitation of matter onto the membrane surface 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 when hydrophobic membranes are used, polar interactions and charge transfer in the case of hydrophilic membranes, which result 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 affect 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 (Dequin, 1987). This will, over time, 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. 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; Parnhaim 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.

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 (Liberage *et al.*, 1994; Bowden *et al.*, 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).

REDUCTION OF FOULING

Optimization 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.

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 hydrophobic 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).

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:

Hydraulic and mechanical cleaning 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.

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 methods, however, increases the complexity of membrane filtration since the system has to be halted to restore flux (Song, 1998).

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 100 000s^{-1} compared with rates of 30000-50000s^{-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).

A recent method is the application of a pulsed electric field, which results in the movement of charged particles or molecules away from the membrane. This method can be carried out without interrupting the process. One of the main problems with this method is that it requires an electrically conducting membrane and special module design, which increase the capital costs of a given process (Mulder, 1995).

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.

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.

THE USE OF IMMOBILISED ENZYMES IN UF

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). 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 were modelled 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 were 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).

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 immobilized 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:

- ***Immobilization 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 enzyme would be retained by the membrane. 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 macromolecules (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 Immobilized 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.

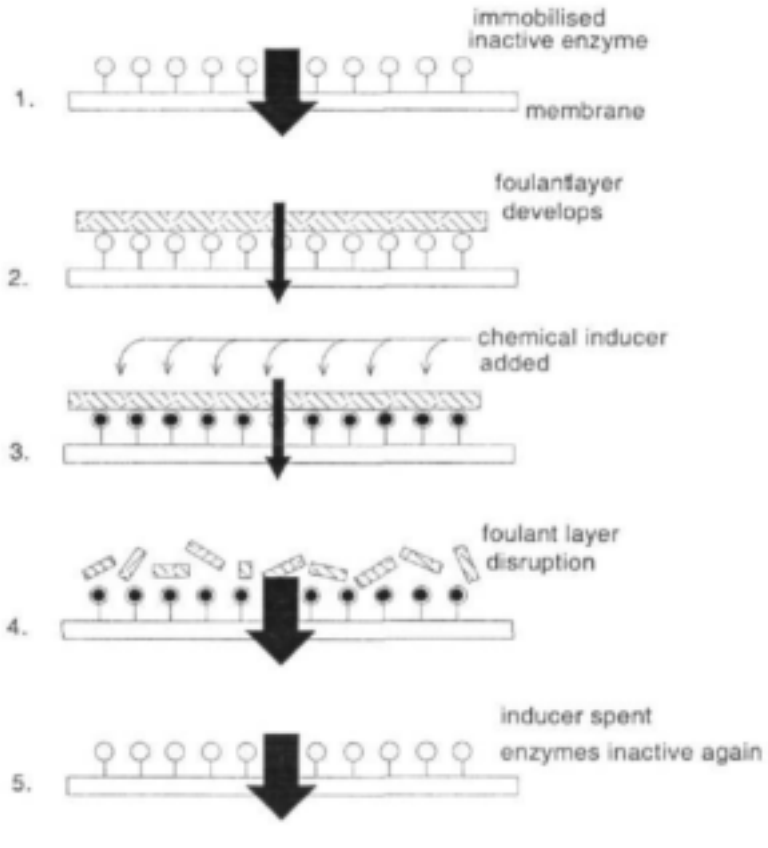

Figure 1: Schematic depiction of the 'defouling on demand' strategy.

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: This work was reported in a previous report (Leukes. *et al.* 1999). The important criteria 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. It was shown that the enzymes manganese peroxidase and soybean peroxidase showed the most promise (Leukes, *et al.* 1999)

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. From previous work it was found that the enzyme manganese peroxidase (MnP) was a promising candidate for application to the defouling system. The objectives of this work were to:

- Determine the factors affecting performance of the enzymatic defouling system and thereby evaluate the feasibility of the process.
- Analyse the mechanisms of fouling and defouling to make rational judgements on improvements and scale-up of the process.
- Adapt the process for application to capillary membranes.
- Assess the application of the process for treatment of industrial effluents.
- Perform a preliminary technology assessment to determine the impacts of the technology in a social context.

CHAPTER 2: EVALUATION OF MANGANESE PEROXIDASE IN DEFOULING ULTRAFILTRATION MEMBRANES

INTRODUCTION

Of the enzymes evaluated, MnP was found to be the most effective peroxidase enzyme in restoring flux to the ultafiltration system. MnP is a glycosylated haem protein containing protoporphyrin IX (Mino *et al.*, 1988; Bonnarme and Jeffries, 1990; Brown *et al.*, 1993; Leukes *et al.*, 1999). The enzyme exists as a series of isozymes with pI values 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).

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, focuses 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 can be established.

The objectives of this work were to ascertain that the defouling effects are 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.

METHODS AND MATERIALS

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.

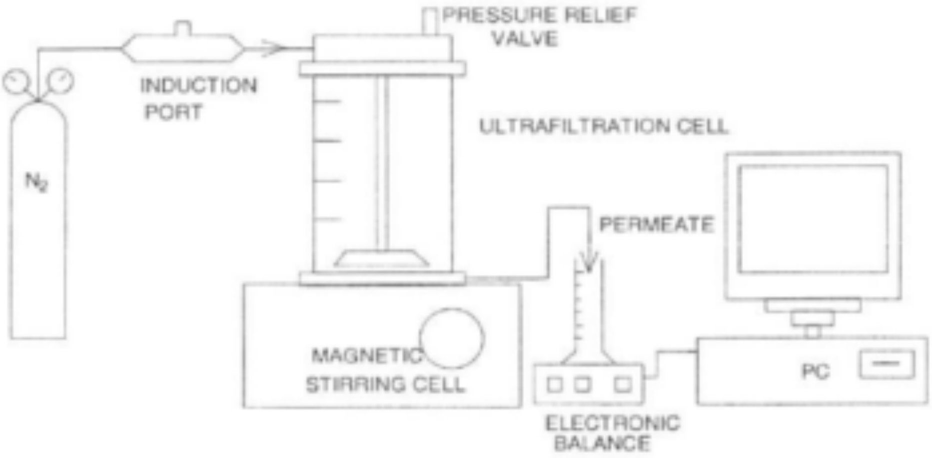

Figure 2: Experimental stirred cell ultrafiltration system

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). Flat sheet polysulphone membranes (MWCO 30 000 Da., supplied by the Institute of Polymer Science, Stellenbosch) with a total surface area of 27.5 cm^2 was used for the experiment to validate the role of MnP in defouling. Polysulphone membranes used in all subsequent experiments were purchased from Sartorius (Asymmetric Ultrafilters). 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 stirred ultrafiltration cell was modified by adding a glass induction 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.

Enzyme Preparation

MnP was produced by the fungus *P. chrysosporium* BKM-F 1767 (ATCC 24725). Stock cultures of *P. chrysosporium* were maintained on yeast extract-malt extract-peptone-glucose (YMPG) slants, in one litre Roux bottles. 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.

MnP was produced either in static flask culture (Bonnarme and Jeffries, 1990), agitated cultures (Perez and Jeffries, 1992; Bonnarme and Jeffries, 1990) or membrane bioreactors (Burton, *et al.* 1997) .

After induction MnP activity peaked after 24 hours, after which 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.

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 initially 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, by subtraction from the original concentration, the amount of enzyme immobilised.

Chemical Activation

The activator solution used for the induction of MnP was 0.475 mg. L^{-1} $MnSO_4$ and 0.05%(v/v) H_2O_2 in 5mL. deionised water. The activator solution used for both HRP and SBP was 1 mL. 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.

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 (Chapter 3).

Confirmation of the action of Manganese Peroxidase in Defouling

Once the concept had been proven (Leukes, *et al.* 1999) 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, (21 U.) MnP was immobilised and induced with hydrogen peroxide (100mM), except no manganous sulphate was added.

Control experiments were used to determine if the flux restoration 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 (0.02 U. cm^{-2}) of enzyme were immobilised unless otherwise stated.

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.

The Effect of Organic Acids

The effect of lactate and oxalate on the catalytic activity of MnP was established. 2.5mL 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.

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.

Statistical Validity

To determine to statistical validity of these results the experiments were performed in triplicate. A mean is represented in all the results presented.

RESULTS AND DISCUSSION

Control experiments

Table 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 1: Flux values obtained upon induction for the control experiments (values given in L. m $^{-2}$. h $^{-1}$). Summary of Controls: 1. Denatured enzyme, 2. No enzyme, only activator solution, 3. Pressure control, only water added, 4. Only H_2O_2 added.

	MnP (0.5U) immobilised (not induced)	**Control 1**	**Control 2**	**Control 3**	**Control 4**
Initial Flux (± Std. Deviation)	**213.8** ± 10.8	**381.78** ± 35.9	**263.91** ± 14.9	**410.84** ± 76.5	**252.89** ± 26.3
Flux before induction	**98.17** ± 1.3	**113.44** ± 5.8	**102.54** ± 2.6	**106.89** ± 2.1	**104.72** ± 3.1
Flux after induction	**135.26** ± 11.2	**113.44** ± 4.5	**104.72** ± 2.9	**95.99** ± 1.6	**98.17** ± 1.5
Flux 5 min after induction	**104.71** ± 1.1	**102.54** ± 4.6	**95.99** ± 1.8	**95.99** ± 1.6	**95.99** ± 2.7
Flux at end of experiment	**81.26** ± 5.5	**63.26** ± 5.6	**71.14** ± 9.4	**52.35** ± 7.8	**63.26** ± 6.3

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 4. The fouled membranes were removed from the stirred cell reactor and photographed.

Figure 3: 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 (4.5U.).

Distinct differences in the density, colour and dispersion of the foulant layer was observed when the enzyme was immobilised and activated (Figure 3). 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.

The Effect of Manganese Peroxidase Concentration on defouling

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

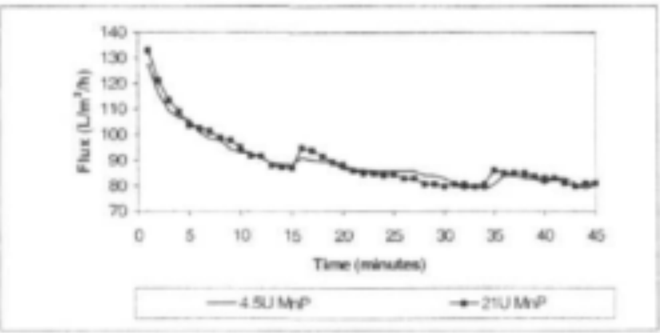

Figure 4: Effect of increasing manganese peroxidase concentration on transmembrane flux restoration.

Figure 4 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 (0.76 U. cm^{-2}) 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 (0.16 U. cm^{-2}) but an increase of 36.96 L/m^2/h compared to an enzyme concentration of 0.5U (0.02 U. cm^{-2}). 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 4 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 to compensate for enzyme leaching from the membrane. The low flux improvement seen with higher enzyme concentrations indicates that several other parameters maybe required in order to enhance enzyme activity.

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, 1992; 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.

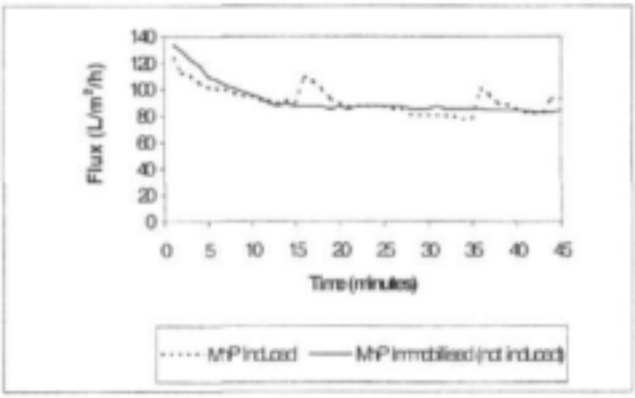

Figure 5: Effect on manganese peroxidase activity and flux restoration with the addition of lactate in the activator solution.

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 5). 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 (compared to the control). 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. The effect of the presence of oxalate in the activator solution is presented in Figure 6.

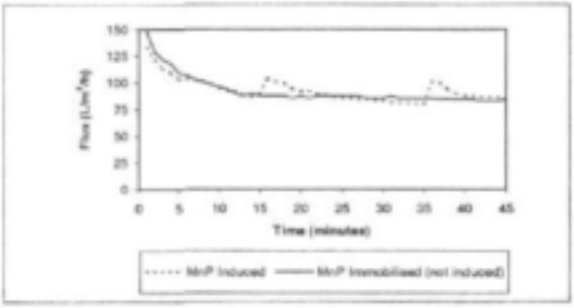

Figure 6: Effect on manganese peroxidase activity and flux restoration with the addition of oxalate in the activator solution.

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. Flux improvement to 88% of the pre-fouling value 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 increased from 89.26 L/m^2/h to 102.53 L/m^2/h, a flux restoration of 77% 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 L/m^2/h to 100.35 L/m^2/h, a 75% flux restoration. It should also be noted that the flux improvement was significantly increased 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 .

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 are likely to have decreased until no more substrate (aromatic compounds) 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.

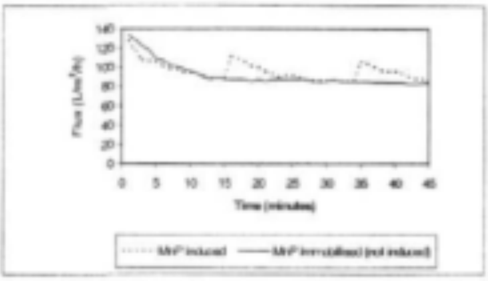

Figure 7: Effect on manganese peroxidase activity and flux restoration by increasing the concentration of manganous sulphate and hydrogen peroxide (400 μM $MnSO_4$, 5 mM H_2O_2 and 200 mM lactate was added to the ultrafiltration system).

In the first experiment, when the concentration was increased to 400μM $MnSO_4$, 5mM H_2O_2 and 200mM lactacte (Figure 7). An 89 % flux restoration was observed under these conditions. Flux increased from 88.52 L/m^2/h to 111.36 L/m^2/h on induction. 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% restoration.

When the concentration of the inducer was increased further to 800μM $MnSO_4$, 100mM H_2O_2 and 200mM lactate (Figure 8) 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 restoration on the first and second induction respectively. The flux improvement lasted 15 minutes before returning to the steady state flux seen when the MnP was immobilised but not induced.

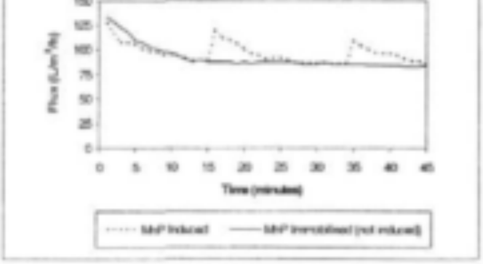

Figure 8: Effect on manganese peroxidase activity and flux restoration by increasing the concentration of manganous sulphate and hydrogen peroxide (800 μM $MnSO_4$, 100mM H_2O_2 and 200 mM lactate was added to the ultrafiltration system).

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 compounds. 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 the membrane 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 0.005 mg. L^{-1} .

CHAPTER 3: MECHANISMS OF MEMBRANE DEFOULING

Introduction

It was found, from a study in which UF data was fitted to constant pressure blocking filtration equations described by Kim, *et al.* (1993), as well as Scanning Electron Microscope analysis of foulant layers (results not shown), that the predominant mechanism of fouling was pore blocking by cake layer deposition and that this deposition was influenced by the process conditions. The next step was to determine the mechanism of defouling by the enzyme system. First, the mechanism of enzyme action on the humic substances was determined. This was done conveniently using organic acid chelated Mn(III), and then confirmed using suspended and immobilised MnP. Given the non-specific nature of MnP and the mixed chemical nature of the effluent, the proposed dominant mechanism was either de-polymerisation, polymerisation (which includes macromolecular aggregation) or transformation of the polyphenolic compounds.

The reaction end-products were analysed by relative size-exclusion chromatography (SEC) and the effect on the foulant layer was observed by scanning electron microscopy (SEM).

Materials and Methods

Size Exclusion Chromatography

Sephadex G150 was used for size exclusion chromatography. The gel was packed into a glass column (22mm OD; 450mm long; bed volume 22 cm^3). 1 g of powder was suspended in 40 ml Milli-Q$^®$ water and packed by passing 4-times the bed volume of water through the gel.

The sample size to be passed through the column was 5 ml. This was kept standard throughout all SEC experiments. The column that was assembled, operated on the principle of controlled or set flow rate. This involved the attachment of a pump to the column that controlled the flow of sample and then water through the column. The pump used was a peristaltic pump, and the flow rate was set at 0.23 ml per min. A Gilson FC 204 fraction collector was also attached to the column to minute automatically collect 30 equal fractions over time. The fraction collector was run in drop mode collecting 40 drops per tube which was equivalent to 1 ml. The fractions were analysed spectrophotometrically and raw river water profiles established.

Manganese (III)-Lactate Complex Reactions

In the absence of enzymes, Mn^{3+} has been reported to depolymerise high molecular weight chlorolignin when complexed with lactate, causing colour to decrease (Lackner *et al*. 1991). Based on this an experiment was set up to determine if a Mn(III)-Lactate complex would have any effect on the tannins in the river water.

Manganese(III)-acetate (Aldrich) was dissolved in 2 M Na-lactate , pH 4.5, to a final concentration of 4mM (Glenn *et al*., 1986). The experiments were carried out in 150 ml Erlenmeyer flasks. 50% (v/v) of the Mn(III)-Lactate complex was the added directly to the river water (25 ml) and incubated at 37°C for 12 hours, based on experimental work done by Lackner *et al.* (1991). UV Absorbance readings were taken every 4 hours. The controls included were, a) 50% complex + 50% de-ionised water, and b) 50% de-ionised water + 50% river water.

Free Enzyme Reactions

Experiments were carried out where freely suspended (non-immobilised) enzyme was added to raw river water. This was to establish what effect MnP has on the tannins in the river water. Experiments were based on those carried out by Lackner et al (1991). Experiments were carried out in test tubes holding 9 ml of raw river water, 1 ml of inducer and 10 μl of H_2O_2. The inducer was made up of Na-Lactate, Na-Succinate and $MnSO_4$. Controls were included as a) no H_2O_2, b) no inducer, c) no enzyme and d) heat-inactivated enzyme. The test tubes were incubated at 37°C for 1 hour after which the contents were spectrophotometrically assessed. Scans were done on all samples from 285 nm to 665 nm.

Results

The profiles of the raw river water samples show that the tannins in the water contributing to its brown colour eluted in one major peak at fractions 35 and 36 (Figures 9 and 10). The tannins had relatively high molecular weights since they are collected fairly early in the SEC run. The profile of the raw river water obtained from SEC, indicated that the river water contained aromatic compounds that were being detected at 285 nm. Being so distinct in the profile meant that detection of change for these compounds after enzyme action would be made easier.

Raw River Water Profiles

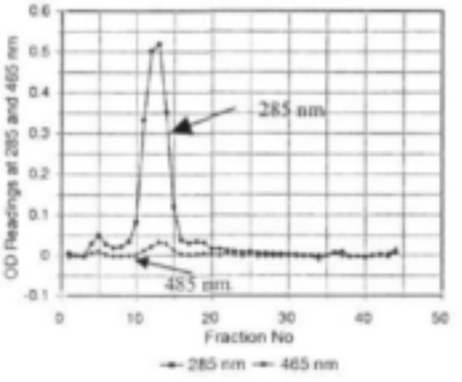

Figure 9: Raw River Water Profile after passing through Sephadex G150 gel SEC column. OD readings at 285 nm and 465 nm for 40 fractions collected

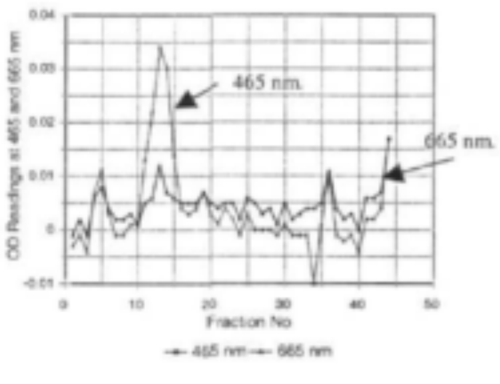

Figure 10: Profile of Raw River water after passing through Sephadex G150 gel SEC column. OD readings at 465 nm and 665 nm of 40 fractions collected.

Oxidised River Water Profiles

Profiles for the river water samples reacted with MnIII-Lactate were generated (Figures 11 and 12). These two profiles were then plotted together to establish if any change had occurred in the river water due to the MnIII-Lactate complex reaction (Figure 13). Optical density readings were adjusted to account for different dilutions required to attain sufficient resolution. A shift in the profile to either side of the elution fraction of the peaks observed in the raw river water profile would suggest a change in molecular weight of the tannins. An increase or decrease in absorbance which would lead to a larger or smaller peak than those exhibited in the river water profile would suggest that a change had occurred in the structure of the humic substances in the river water.

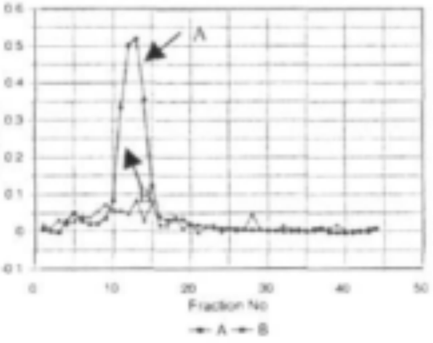

Figure 11: Profile of RRW (A) and RRW Reacted with MnIII-Lactate (B). OD readings at 285 nm for 44 Samples Collected from SEC

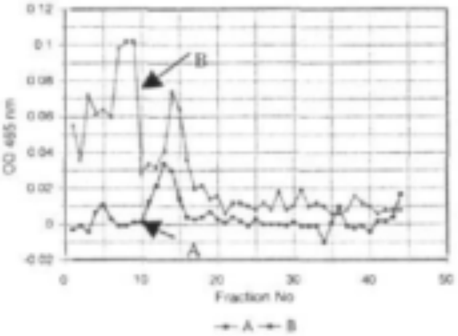

Figure 12: profile of RRW (A) and RRW Reacted with MnIII-Lactate complex (B). OD Readings at 465 nm for 44 fractions collected from SEC.

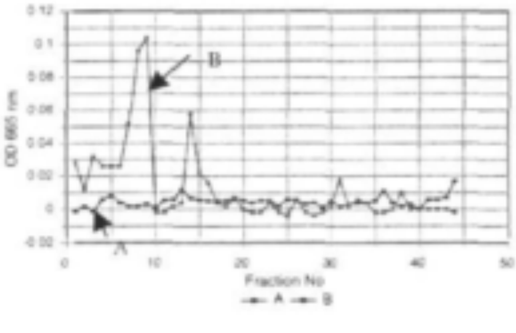

Figure 13: Profile of RRW (A) and RRW reacted with MnIII-Lactate complex (B). OD Readings at 665 nm for 44 fractions collected from SEC.

Figure 11 shows that the MnIII-lactate complex appears to lower the concentration of the aromatic compounds in the raw river water. This is indicated by the observation that the peak for the RRW sample is considerably larger than that of the MnIII-Lactate complex reacted sample. At 465 nm the tannins responsible for colour in the water are being detected. The MnIII-Lactate complex has increased the concentration of colour forming complexes. These are in the range of high molecular weight particles. It therefore appears as though polymerisation or macromolecular aggregation is taking place. This is indicated by the fact that the peak for the MnIII-Lactate complex sample is larger than that of the RRW sample and is shifted to the left (Figure 12).

At 665 nm turbidity is detected in the sample (Figure 13). The turbidity may be due to the presence of high molecular weight molecules within the sample. Very little contamination or presence of carbohydrate or proteins were observed in this water (Prof. P Swart. - Stellenbosch University - Pers. comm.). This would explain the increase in absorbance for the RRW reacted with MnIII-lactate complex sample. This would back up the suggestion that the complex is in fact polymerising the humic substances in the river water.

A definite change in the absorbances between the raw river water samples and the MnIII-Lactate samples was observed. Most of the changes occurred in the 285 nm range, which was not expected. It was thought that if any effect occurred that it would involve mostly the colour producing tannins. The MnIII-lactate did not remove the colour from the river water, nor did it significantly increase or reduce the amount of colour in the river water.

From this experiment it can be deduced that the MnIII-Lactate complex which simulates MnP activity, is in fact both polymerising and depolymerising the tannins in the raw river water, with the dominant mechanism being polymerisation or macromolecular aggregation. It could be that the river water fractions that are being detected in the 285 nm range are being removed from that range by the polymerisation (aggregation) of the tannins. This would account for the drop in absorbance after the raw river water has been treated with the MnIII-Lactate complex.

Mode of Action of Enzyme

An experiment was performed investigating the action of MnP to support up the MnIII-Lactate complex experiment and confirm the mode of action of the enzyme. The results of this experiment confirm that the major mode of action of the enzyme is to polymerise and aggregate the tannins in the raw river water.

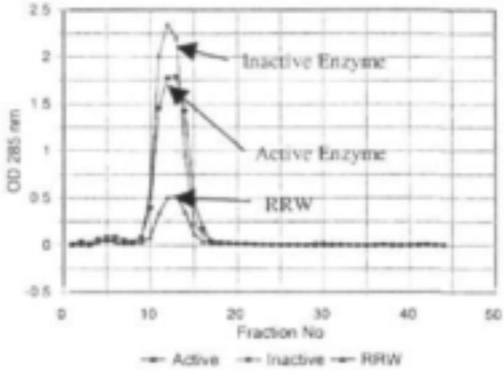

Figure 14: Showing the profiles of RRW, RRW reacted with Active enzyme (MnP) and RRW reacted with Inactive enzyme (heat-inactivated MnP). Profiles established from OD 285 nm readings on 44 fractions collected from SEC.

Figure 14 shows that the MnP is having an effect on the tannins in the river water. It can be seen from the graph the RRW reacted with Inactive (heat-inactivated) enzyme has the highest absorbance at 285 nm. The RRW sample in comparison has the lowest absorbance. The fact that the sample where RRW was reacted with active enzyme, lies between these two samples suggests that the active enzyme is acting on the aromatic compounds detected at 285 nm. The aromatic component is being removed from the water by the enzyme and what is being detected is due to the presence of the enzyme in the sample.

The profiles obtained at 465 nm where the colour of the water is measured, show that the sample where RRW reacted with Active enzyme has the highest absorbance (Figures 15 and 16). This suggests that the MnP is polymerising the humic substances in the river water. The polymerisation or aggregation occurs mainly for the largest tannins which are being eluted first. This is shown in the graph when the first peak at fraction 5 is observed. Although some polymerisation or aggregation occurs for the second group of tannins being eluted at fractions 11 and 12, most of the increase in absorption is due to the presence of the enzyme in the sample. Some changes are occurring for the fractions being eluted later on but the changes are minimal and are not discussed.

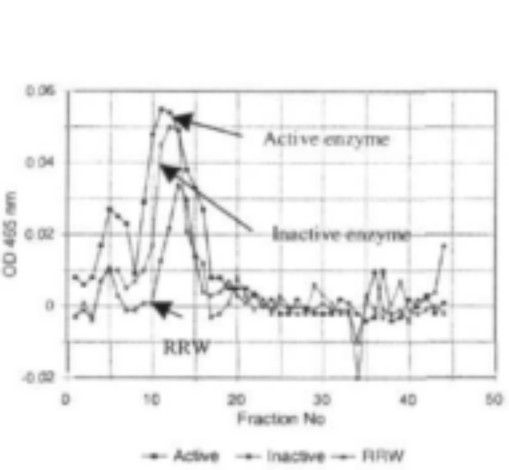

Figure 15: Profiles of RRW, RRW reacted with Active enzyme (MnP) and RRW reacted with Inactive(heat-inactivated) enzyme. OD readings of 44 fractions collected from SEC

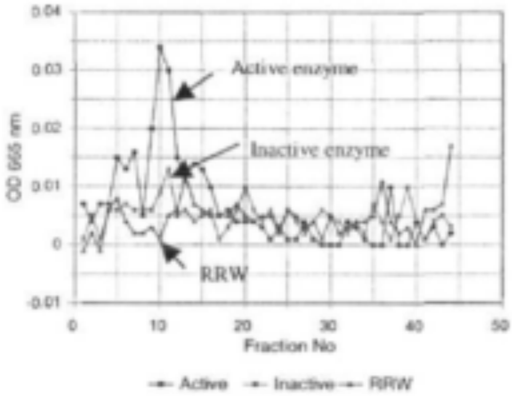

Figure 16: Profiles for RRW, RRW reacted with Active enzyme (MnP), and RRW reacted with Inactive enzyme (heat-inactivated). OD readings at 665 nm for 44 fractions collected from SEC.

SEM Analysis

To determine the effect of the enzyme on the cake layer during defouling, SEM analysis was performed on the foulant layer after enzyme activation during stirred and unstirred conditions. Figure 17 and 18 clearly show disruption of the cake layer and that the nature of this disruption depends on the nature of the process. Analysis of the foulant layer after enzyme activation in stirred and unstirred systems indicated that the cake layer dispruption followed the force field of the system.

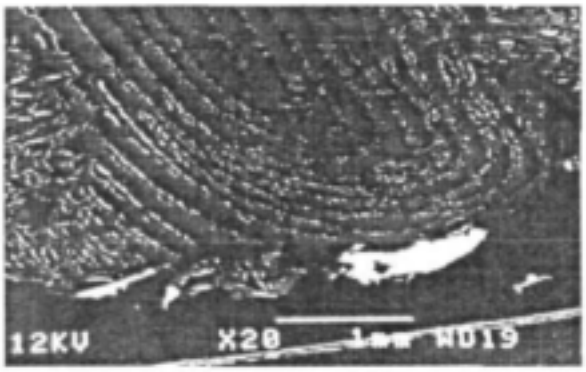

Figure 17: SEM analysis of the foulant layer after MnP induction. The system was operated with stirring at 100rpm and 100kPa. Concentric Elliptical patterns were observed, indicating that the cake layer lifting followed the rotational forces of the system. The cake layer is completely removed at the outer edges of the membrane, where the enzyme concentration is expected to be highest.

Figure 18: SEM analysis of the membrane in an unstirred reactor. Clear disruption of the biofilm can be seen, without the presence of the concentric patterns observed in the stirred system.

Conclusion

The effect of catalysis with an MnIII-Lactate Complex (which simulates the action of pure MnP) or a crude extract of MnP on the relative size exclusion profiles of raw river water was evaluated. Due to the low resolution offered by SEC, results were inconclusive, however, holistic interpretation of all the data presented in this chapter led to the conclusion that the dominant mode of action of manganese peroxidase on the humic substances found in the George river water is that of polymerisation/ aggregation. After reaction of the river water with the MnIII-lactate complex, a decrease in absorbance at 285 nm and an increase in absorbance at 465nm. and 665 nm was observed. Also, shifts in the elution profiles to the left (higher molecular weight range) of the original profile were observed. This indicated that the molar quantity of free aromatic compounds had been reduced by the MnIII action and that this resulted in an increase in brown colour and turbidity. This is best explained as a molecular aggregation event in which low-molecular weight polyphenolic chains are fused to form larger aggregates.

SEM analysis showed the influence of lift forces on the nature of the cake layer disruption. From the above information a mechanism for enzyme defouling was proposed:

After enzyme induction, the lower molecular weight aromatic compounds are polymerised and possibly linked to the high molecular weight fractions.

These changes affect the physical structure of the foulant layer, thereby destabilising the cake layer. This could occur through a change in density or solubility of the cake layer or through changes in morphology of the substructures.

Shear forces in the system therefore have more purchase on the cake layer, resulting in easier lifting of the cake layer for defouling.

CHAPTER 4: APPLICATION OF THE ENZYME DEFOULING SYSTEM TO CAPILLARY MEMBRANES OPERATED IN DEAD-END MODE

Introduction

The concept and fundamental insights into the operation of the enzymatic defouling system were done on flat-sheet membranes.

The next step was to gain some experience on the utility of the system on capillary membranes. Although the enzymatic process might have more utility in spiral wrap and other systems where back-flushing is difficult, capillary membranes were chosen for this component of the study because:

1) Experience was already available in the use of capillaries for UF of NBW (University of Stellenbosch).
2) Capillary membranes were available, courtesy of the University of Stellenbosch, for testing.
3) Pilot facilities were available.

Materials and Methods

$MnSO_4$ was obtained from NT Laboratory Supplies (Pty) Ltd. H_2O_2 and Sodium Lactate were obtained from Saarchem (Pty) Ltd. Untreated water was obtained from a water purification plant in George (RSA). The water was brown in colour and had a low turbidity (70-80 NTU), low COD (88 $mg.L^{-1}$), and a colour index ranging from 400-1200 Hazen units (variation due to seasonal fluctuations). The humic acid fraction that had the highest concentration was *ca.* 48 kDa as determined from MW markers using a Sephadex G150 SEC column. Prior to use, the raw water was filtered through Whatman No. 1 filter paper (Whatman International (Ltd), Maidstone, U.K.) to remove particulate matter. Anisotropic internally- and externally-skinned poly(ether sulphone) capillary membranes were obtained from the Institute for Polymer Science (Stellenbosch University, South Africa).

Enzyme Immobilisation

Membranes were prewashed for a minimum of 1 hour and flushed with Milli-Q® water to remove residual storage solution (glycerol). The lyophilised crude MnP extract was suspended in 5-7mL Milli-Q® water. This solution was then injected through the induction port and filtered under normal dead-end filtration operation for 1 hour to ensure that the enzyme was forced to compact against the ultrafiltration skin of the membrane.

Enzyme induction

The activator solution used for the induction of MnP was 0.475mg.L^{-1} $MnSO_4$, 0.05%(v/v) H_2O_2, and Na Lactate (200mM.) in 5mL Milli-Q® water. The chemical activator solution was added to the membrane reactors through the induction port 60 minutes after initiation of the experiment.

Membrane reactor configuration

Operating conditions

The membrane reactor system comprised two / three single capillary membranes reactors in parallel with each membrane reactor operating under the same hydraulic conditions. This was done in order to statistically validate the data obtained due to the possibility that the membranes within each reactor are not identical and may display slightly different characteristics due to slight perturbations occurring during the manufacturing process. The membrane reactors were operated at a pressure of 150 - 200 kPa giving a PWF_i of 60 - 80 L.m^{-2}.h^{-1}

Residence time distribution determination

To ascertain the length of time required within the reactor before enzyme-inducer contact was facilitated, an inert pulse tracer study was done. This was effected with the injection of 5mL of 3M NaCl solution in the induction port and the percentage NaCl in the exit stream (permeate) was determined using a salinity refractometer. The residence time distributions was determined for both a nascent capillary membrane and one after 24 hours of NBW filtration.

Colour removal due to ultrafiltration

Due to the presence of unsaturated compounds in the NBW the characterisation and quantitation of the colour removal efficiency of the ultrafiltration treatment of the NBW was achieved using UV-vis spectrophotometry. Changes in absorbance at 285nm were used to monitor the changes in the humic acid content (NOM concentration). Samples of the permeate and feed were collected and analysed spectrophotometrically (Shimadzu UV-160A, Shimadzu Corporation, Japan). The percentage colour retention was determined according to the following equation:

$$100 - (NBW_p/NBW_f \times 100)$$

NBW_p and NBW_f represent the absorbance at 285nm of the permeate samples and feed solution respectively.

Control experiments

A number of control experiments were performed to verify that the flux increase was due to the catalytic action of MnP. To determine any influence the immobilised protein layer might effect, the enzyme was first denatured, immobilised, and then induced. To determine if the activator solution had an oxidative effect on the foulant layer, the inducer was added in the absence of immobilised enzyme. Any effects as a result of dilution or pressure change when the activator solution was added, was determined by substituting Milli-Q® water in exchange for the activator solution.

Results and Discussion

Residence time distribution

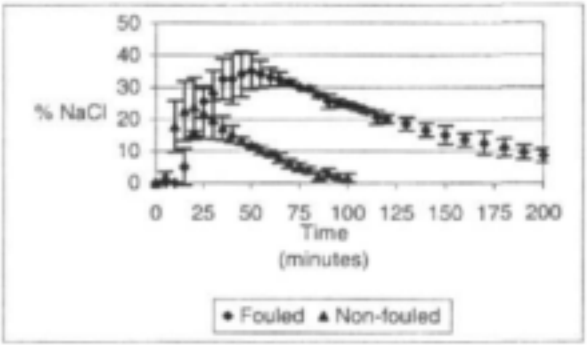

Figure 19: Residence time distribution for under fouling and non-fouling conditions

From the RTD experiments (figure 19) it was evident that tracer exited in the permeate stream within 30-50 minutes after injection. This indicated a time estimate for contact between the immobilised enzyme and the inducer solutions when added through the inducer port. This gave a downstream window where flux restoration after inducer addition should occur. This window was therefore closely monitored during the enzyme-immobilised experiments. This information is important for module design for rapid enzyme activator contact.

Control experiments

No increase in flux was observed upon addition of the individual inducer solutions. Figure 20, Figure 21, and Figure 22 show that no increase in flux was observed upon addition of $MnSO_4$, H_2O_2, and NaLactate respectively, 60 minutes after the initiation of the NBW filtration.

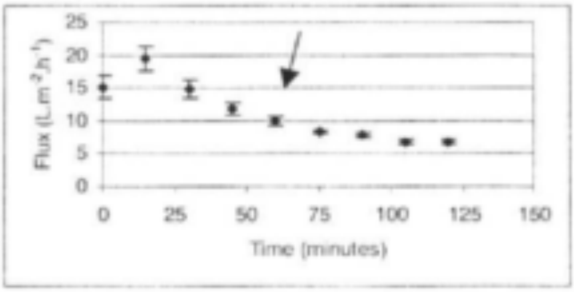

Figure 20: Control experiment with $MnSO_4$ added as the inducer 60 minutes after initiation of the experiment (arrow indicates when inducer was added). No flux increase is observed within the 30-50 minute 'window'.

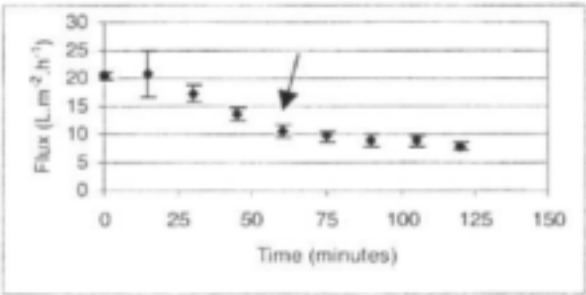

Figure 21: Control experiment with H_2O_2 added as the inducer 60 minutes (arrow indicates when inducer was added) after initiation of the experiment. No flux increase is observed within the 30-50 minute 'window'.

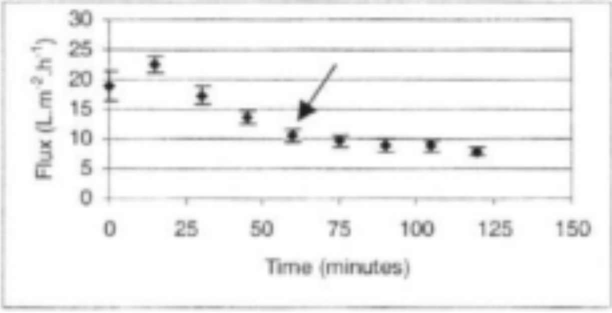

Figure 22: Control experiment with NaLactate added as the inducer 60 minutes (arrow indicates when inducer was added) after initiation of the experiment. No flux increase is observed within the 30-50 minute 'window'.

Defouling using immobilised MnP

Long-term studies were initiated to determine the activity of immobilised MnP over an extended period of time. Figure 23 indicates the total time of the experiment and the different times of induction.

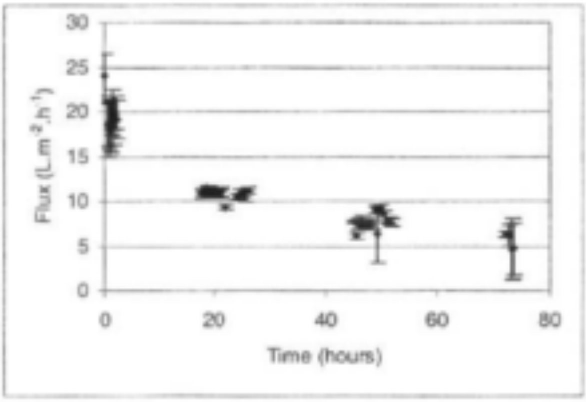

Figure 23: Long-term ultrafiltration of NBW in the presence of immobilised MnP

Figure 24 shows a close-up view of the first induction event showing minimal flux restoration .

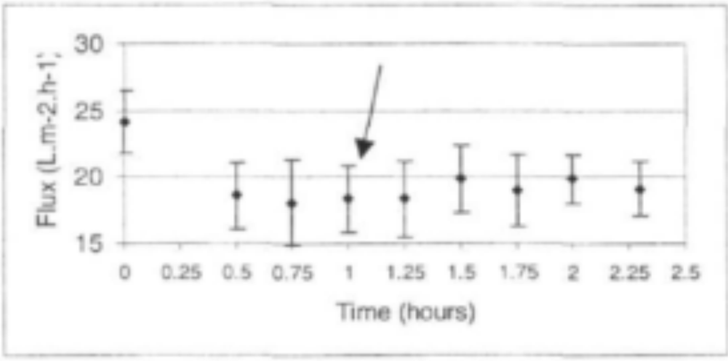

Figure 24: Initial induction after the first hour of ultrafiltration (arrow indicates when inducer was added)

Enzyme distribution

Due to the lack of significant flux increases with immobilised MnP, it was envisaged that it was possible that uneven enzyme distribution was occurring during the immobilisation step/procedure. The protein distribution within the reactor was therefore determined using the BCA protein test kit (Pierce).

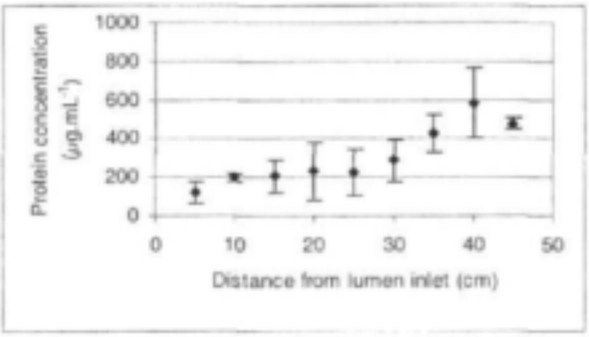

Figure 25: BSA protein distribution as a function of distance from the lumen inlet

From the results (Figure 25) it was evident that uneven distribution was occurring with the highest concentration of protein (and therefore enzyme) was immobilised on a relatively small fraction of the ultrafiltration surface. Thus the observed lack of significant flux restoration was attributed to uneven enzyme distribution and significant flux decline as a function of the entire ultrafiltration surface.

Conclusions

The enzymatic defouling approach was evaluated for application to capillary membranes operated in dead-end mode. Moderate flux improvement was obtained after enzyme induction. The flux improvement was observed several minutes after addition of the enzyme inducing solution was added as opposed to the immediate response observed when using flat sheet membranes in a stirred cell. This was explained by the fluid dynamics of the system described by residence time distribution. The poor performance of the system was ascribed to uneven distribution of the enzyme and also to the fact that it was operated in dead-end mode, given that the defouling mechanism required shear forces to lift the disrupted cake layer.. At this stage concerns were also raised about the use chemical inducers, given the cost and safety implications involved with the use of H_2O_2 as an inducer.

CHAPTER 5: ISOLATION OF A THERMOSTABLE LACCASE

Introduction

Based on the outcomes of the Technology Assessment and the information from the investigation of MnP as a candidate enzyme, new requirements for the defouling system emerged.

- For the technology to be economically competitive an enzyme is required that uses co-factors other than H_2O_2 and organic acids, unstable compounds which would need to be stored for extended periods and are relatively expensive.
- The major advantage of the enzymatic system over the chemical CIP system for dealing with irreversible fouling is potential reduction of waste and improvement of safety. Thus, Mn^{2+} and H_2O_2 were not good candidates as co-factors.
- Based on published results from the Monvilla project (Jacobs, *et al.*1999), cleaning is only required weekly, and hence a robust and stable enzyme would be required.

Given the above considerations and the knowledge that the defouling mechanism is predominantly a polymerisation or macromolecular aggregation reaction, it was decided that the enzyme laccase should be evaluated for the defouling system.

Laccase is produced by several fungi, and is able to decrease the toxicity of phenolic compounds by employing a polymerisation reaction. Owing to it's non-specificity it can cause the cross-coupling of pollutant phenols with natural phenols. Laccase is able to oxidise phenolic compounds to their anionic free radicals which are extremely reactive.
(Karam and Nicell, 1997)

A simple example of what type of reaction laccase catalyses is given below.

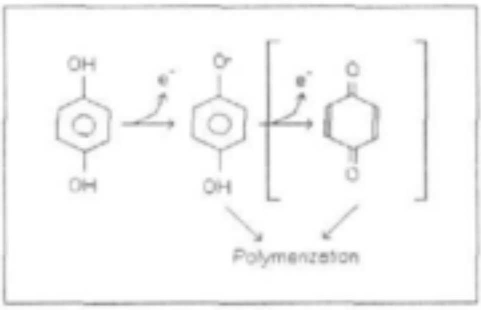

Figure 26: A typical laccase reaction, where a diphenol undergoes a one-electron oxidation to form an oxygen-centred free radical. This can be in turn converted to a quinone in a second enzyme-catalysed reaction, or it may be spontaneous. Quinone and free radical products undergo polymerisation. (Thurston, 1994)

Laccase requires oxygen as a co-factor, which would have considerably improved cost implications in comparison to cleaning agents such as H_2O_2, Mn^{2+} and organic acids required for the use of MnP. The use of oxygen is much safer and generates no waste products.

A further criterion for a suitable candidate enzyme considered was long-term stability. Laccase, however, does not have as wide a substrate range as MnP, so a further requirement was that a laccase be found with extended substrate range, similar to that of MnP.

It is known that enzymes with improved thermostability also tend to have improved mechanical stability (Prof. K. Kieslich, pers. comm.). On this basis a screening strategy was devised to find a suitable candidate laccase enzyme for the defouling system. Natural isolates of white rot fungi would be screened for the presence of a thermostable laccase with the ability to transform MnP unique substrates.

Materials and Methods

Isolation

The fungi were isolated from the wild as fruiting bodies, and were generally all basidiomycetes, with a single ascomycete being isolated. The fruiting bodies were surface sterilised using 70 % ethanol . A small piece of carpophore, approximately 4 mm^3, was cut from the middle using a sterile scalpel and placed on selective medium (de Jong *et al.*, 1992). The medium was supplemented with yeast extract and the hemp stem wood was not added.

Once pure cultures were obtained, they were incubated at 25°C in a Nitrogen limited medium (Tien and Kirk, 1988) for 14 days. The extracellular fluid was then harvested and tested for thermostable laccase production with Mnp activity.

Enzyme Assays

The assay performed for the determination of Laccase and Manganese peroxidase is the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-(dimethylamino)benzoic acid (DMAB). In the presence of manganese peroxidase (or laccase) the coupling results in a purple chromophore with an absorption maximum at 590 nm, and an extinction coefficient of 53000 $M^{-1}cm^{-1}$. (Castillo *et a.*, 1994)

Enzyme assays, unless otherwise specified, were performed in the PowerWave Microtitre Plate Reader in hexaplate.

To determine joint manganese peroxidase and laccase activity in the DMAB and MBTH assay, manganese sulphate and hydrogen peroxide were required, since Mn^{2+} and H_2O_2 are essential for manganese peroxidase to be active. To determine laccase activity EDTA was added to the assay reagents and manganese sulphate and hydrogen peroxide are added . This negates the effects of manganese peroxidase in the assay, since these 2 chemicals are required in the assay reagents to activate the manganese peroxidase. The effect of EDTA is to remove the residual Mn^{2+} from the assay medium. (adapted from Castillo *et al*, 1994)

Using this enzyme assay there is no way to determine manganese peroxidase activity directly, thus one subtracts the laccase activity determination (using EDTA) from the laccase and manganese peroxidase joint activity as determined by the assay (using hydrogen peroxide and manganese sulphate). The resultant activity is the activity of the manganese peroxidase.

Laccase is a metalloprotein and contains copper centers within its structure. Thus, EDTA could have an inhibitory effect on the enzyme. To determine whether this was the case, a control was performed that contained the MBTH and DMAB, omitting the manganese sulphate, hydrogen peroxide, and EDTA, and these were replaced with catalase (Boehringer Ingelheim). The activity of catalase is such that the enzyme removes residual hydrogen peroxide, and thus ensures the specificity for laccase, without the addition of EDTA. The results indicated an extremely small difference in the enzyme activity determined using these two different techniques (results not shown) and thus it was determined that the EDTA had a small effect on the laccase activity, but the effect could be considered negligible, and thus the EDTA assay could be used to determine the laccase activity. Catalase is an expensive chemical and this also contributed to the use of EDTA as the selective ingredient for laccase activity determination.

The usual assay performed for the determination of manganese peroxidase and laccase, employing the same additional reagents as mentioned above is the ABTS method. This method has gained acclaim and is more often used than the MBTH and DMAB method, but owing to the fact that it is not very accurate, and it is very difficult to perform, the MBTH and DMAB assay method was employed. (Gold and Glenn, 1988)

Confirmation of Enzyme Type

To confirm that the enzyme being dealt with was indeed laccase and not manganese peroxidase, several non-denaturing polyacrylamide gels were electrophoresed and stained using different reagents in the presence and absence of manganese sulphate and hydrogen peroxide. The stains were prepared as outlined in the table below. These were added to the gels and allowed to stand for an hour.

Table 2: Use of stains used with the non-denaturing gels to confirm the activity of laccase

Stain Label	Substrate	MnSO4 and H2O2	Catalase
A	ABTS	Yes	No
B	ABTS	No	Yes
C	Guaiacol	Yes	No
D	Guaiacol	No	Yes
E	DMAB + MBTH	Yes	No
F	DMAB + MBTH	No	Yes

The explanation for the use of the various stains mentioned above and what they in turn would indicate in the protein gel with respect to type of enzyme is elaborated on here. Stain A would indicate all the manganese peroxidase and laccase activity bands. Stain B would then determine which bands had laccase activity, and from derivation (the bands that don't show up) those that indicate the presence of manganese peroxidase. Guaiacol is considered to be a laccase specific substrate, and was used as a control substrate. Stain C should only show those bands with apparent laccase activity, and stain D should only have shown those bands expressing the same activity. Since guaiacol is laccase specific, no manganese peroxidase bands should have been located on the gel, even in the presence of manganese sulphate and hydrogen peroxide. The DMAB and MBTH assay was also performed with and without manganese sulphate and hydrogen peroxide. Stain E should indicate only those proteins that exhibit manganese peroxidase activity, since these reagents were meant to be a manganese peroxidase specific substrate. Stain F should not react with any proteins present in the gel, since the manganese peroxidase should be inactive, owing to the absence of manganese sulphate and hydrogen peroxide. Laccase bands should not appear, since the substrate should exhibit specificity for manganese peroxidase only. Catalase was added (200U./l) (as mentioned above) to remove any residual hydrogen peroxide present in the reagents, and thus add validity and accuracy to the results obtained for this experiment.

pH Profile

Optimum pH was determined using universal buffer adjusted to various pH's. This method was determined to be the best for the determination of the optimum pH owing to the fact that universal buffer contains many salts with diverse buffering capacities and may be adjusted to any pH. Laccase is known to be affected by the specific buffer salts used, therefore by using a single buffer (universal buffer) with a wide pH range the effect of the use of different buffer salts to attain the desired pH range is avoided.

Optimum Temperature Profile

The assays were performed using a Shimadzu spectrophotometer, connected to a waterbath, which was set at the temperature of assay and pumped, using a peristaltic pump at maximum speed, through the temperature cell of the spectrophotometer. This was then allowed to run until the temperature of the waterbath no longer fluctuated. The temperature of the chamber was also measured to ensure that the chamber was at the correct temperature. The samples and reagents were pre-heated in the waterbath for 1 minute and placed together in a quartz cuvette (also preheated) into the heated chamber of the Shimadzu spectrophotmeter.

Results and Discussion

Sixteen strains of fungi were isolated from dry, hot areas in the Eastern Cape. These were purified and grown in pure culture to produce extracts which were tested for MnP and laccase activity using the ABTS assay. Only one isolate had MnP activity (based on DMAB and MBTH transformation) in the laccase specific assay (presence of catalase and EDTA, but no H_2O_2 and Mn^{2+}). The temperature profiles of the various extracts were then determined.

Temperature profiles

The results yielded an enzyme, thought to be manganese peroxidase from an isolate from Sam Knott Nature Reserve with an optimum temperature of approximately 70°C (Figure 27). The control experiments using the manganese peroxidase from *Trametes versicolor* (Figure 28) yielded an optimum temperature within the theoretical values of between 50 and 55 °C, from literature, indicating that the technique used for the temperature profile was accurate.

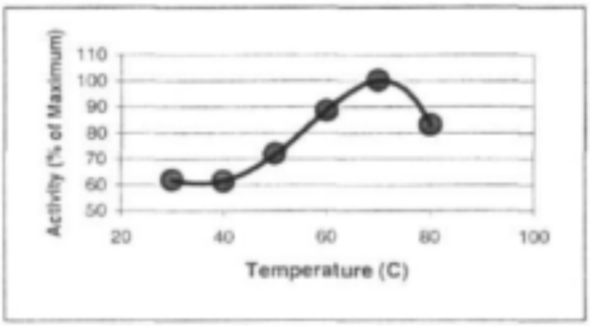

Figure 27: Temperature profile obtained for the laccase from isolate 3WJ, indicating an optimum temperature of approximately 70 °C.

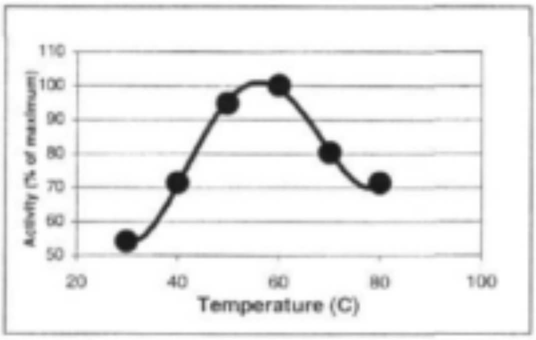

Figure 28: Temperature profile of laccase from *Trametes versicolor* indicating an optimum temperature of approximately 55 °C.

Laccase Thermostability Assay

To determine the thermostability of the isolated laccase, it was incubated in a 60 °C waterbath for 9 hours and a sample was extracted at 1 hour intervals for 9 hours and assayed for laccase activity. Within 9 hours of being placed in the waterbath, the laccase from *Trametes versicolor* had almost lost 50 % of its activity (Figure 29), whereas the laccase from the isolate 3WJ hadn't lost any activity (Figure 30).

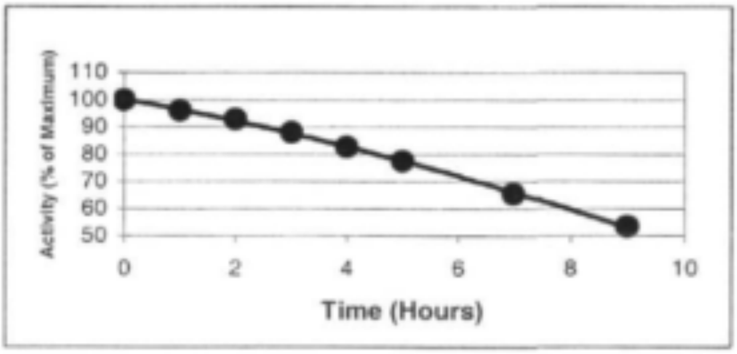

Figure 29: Thermostability assay for the laccase from *Trametes versicolor* indicating an almost 50 % decrease in enzyme activity over a period of 9 hours at 60 °C.

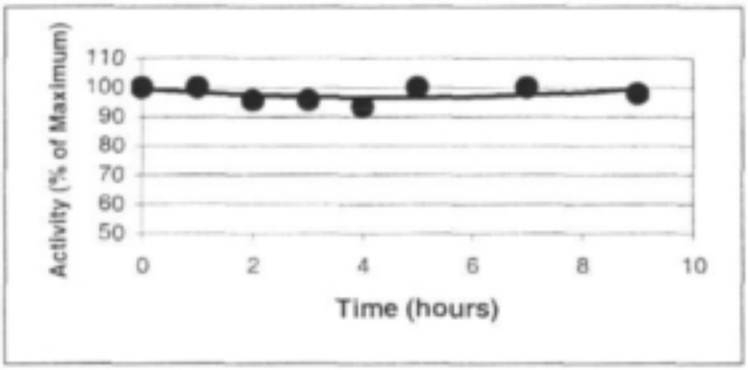

Figure 30: Graph showing that over a nine hour period at 60 °C, laccase from isolate 3WJ loses no apparent activity.

Non-Denaturing Polyacrylamide Gel Electrophoresis Results

The thermostable enzyme described above was then shown to be a thermostable laccase with a substrate range similar to MnP based on the differential enzyme assays. Zymograms (non-denaturing gels with enzyme substrates) were used for further confirmation that this was not an artifact due to mixtures of enzymes.

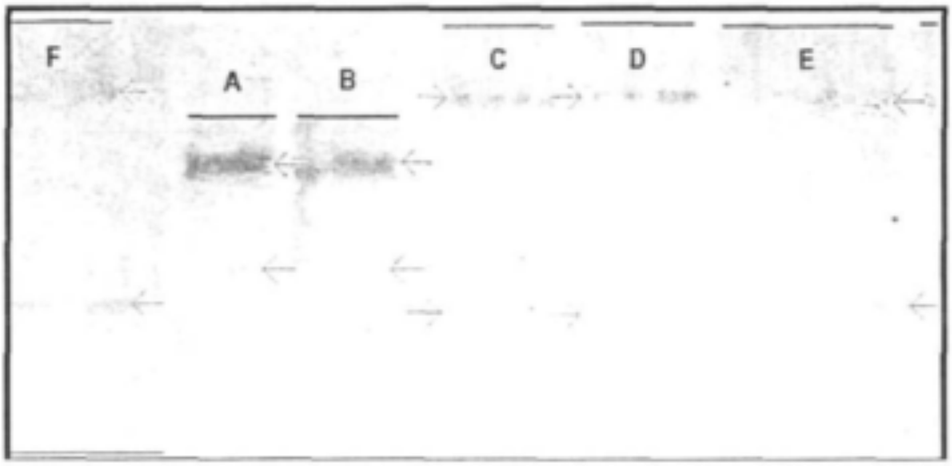

Figure 31: Photograph of the PAGE gels, indicating clearly where the bands appear, and also their relative positions, the gel indicated on the right (indicated by letters A and B), is a mini-gel preparation, run on a Tall-Mighty-Small gel apparatus. The four gel fragments on the right (C, D, E, and F), are fragments of a larger gel (20 cm²). The Black lines indicate the interface between the stacking gel and resolving gel. The blue arrows indicate the position of the tracking dye (bromophenol blue), and the red arrows indicate the positions of the bands that have reacted with the reagents in question.

The results above (Figure 31) indicate the presence of only one enzyme as the ratio of the tracking dye to the protein band movement was determined and was a constant for all the lanes. This indicates the rate of migration is the same for the protein. Only one band was seen in all the lanes, and it exhibited enzyme activity in all the tested conditions. Thus the band corresponds to laccase since it was able to convert all the reagents with and without the presence of manganese sulphate and hydrogen peroxide.

pH profile of the isolated Laccase with MnP activity

Below (Figure 32) is the graph indicating the average values obtained for the enzyme activity at varying pH's. The optimum pH as determined by this method indicates a value of approximately pH 5 for optimum enzymatic activity. Since the brown water and most phenolic effluents have a pH range between 4 and 6, this enzyme would be ideal for application to such waters.

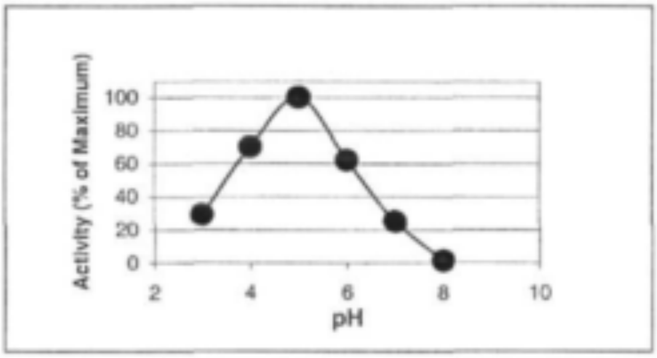

Figure 32: Graph showing the average values obtained for the pH profile for Laccase from isolate 3WJ

Conclusions

From the above experiments it can be determined that this particular fungal isolate produces a thermostable laccase. This laccase has enormous potential in all fields where laccase is used. Thermostable enzymes are highly sought after, owing to their enhanced capabilities and potential use in industry. The mechanical stability, which usually accompanies thermostability should confer extended operation to the enzymatic defouling process as well as allow the use of oxygen as an inducer of activity.

The fact that the laccase is also able to convert DMAB and MBTH to the purple coloured end product indicates that the enzyme may exhibit a broad substrate range, since this assay is used to determine manganese peroxidase activity and was unaffected by the commercially available laccase, as determined by the authors of the paper which describes this assay. (Castillo *et al.*, 1994). The enzyme was thus tested for its action on brown river water.

CHAPTER 6: APPLICATION OF ENZYMATIC DEFOULING USING A THERMOSTABLE LACCASE TO CAPILLARY MEMBRANES OPERATED IN CROSS FLOW MODE

Introduction

Enzymatic defouling of capillary membranes used for cross flow, constant flux ultrafiltration of brown water was investigated. The thermostable laccase was used. Two approaches were considered for removal of the fouling layer:

1. Immobilisation of enzymes on the membranes and using oxygen as enzyme activator when defouling is required.
2. Periodic washing of the membranes with a freely suspended activated enzyme solution

Materials and Methods

System and operation

Single hollow fiber membranes were potted in glass manifolds as shown in Figure 43. The system was configured for constant flux operation by the use of inlet and outlet peristaltic pumps with adjustable flow rates (see Figure 23). Based on a critical flux analysis (results not shown), an operational regime was chosen that results in rapid fouling but not excessive cake layer formation. The outlet pump was used to regulate the horizontal velocity of the fluid in the membrane, this was maintained at approximately 0.3 $m.s^{-1}$. The inlet pump was used to control the permeate flux, which was maintained at approximately 50 $L.m^{-2}.hr^{-1}$. Inlet and outlet pressures were monitored with pressure gauges. Fouling was manifest as an increase in transmembrane pressure. Permeate was collected in a volumetric flask in order to measure transmembrane flux.

Membrane fouling was measured as a decrease in specific flux. Darcy's equation for flux states:

$$J = K.\Delta P \qquad (5)$$

where J = transmembrane flux ($L.m^{-2}.hr^{-1}$), K = specific flux ($L.m^{-2}.hr^{-1}.kPa^{-1}$) and ΔP = transmembrane pressure (kPa). Here K is a lumped parameter and is the inverse of the membrane resistance. This was done so as to overcome inaccuracy arising from slight fluctuations of pressure and flux, which were not constant over time. All specific flux measurements are reported as a percentage of the initial Pure Water Flux (PWF), so as to compensate for membrane inconsistency.

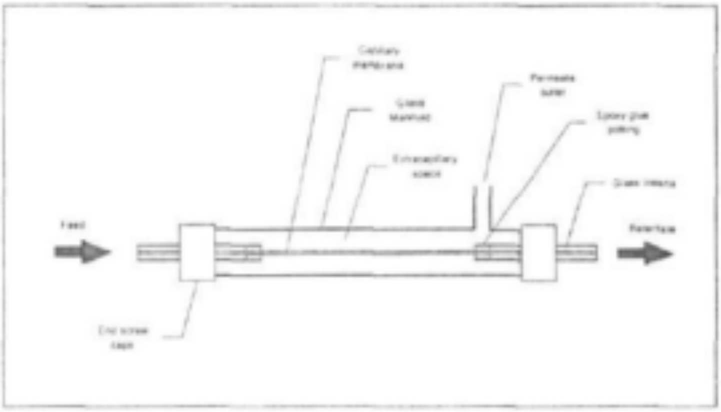

Figure 33: Single hollow fiber membrane manifold

Before enzyme immobilisation and operation the membranes were rinsed with water to remove the glycerol which is used to preserve the membranes. Active membrane length was 450 mm, giving an active surface filtration area of 1.7×10^{-3} m^2. All experiments were performed at 20 ± 2 °C. Brown water was obtained from George Municipality.

Immobilisation experiment

After membrane rinsing the initial PWF was measured, thereafter the enzymes were immobilised and the PWF measured again. The system was then operated at constant flux with brown water. Specific flux measurements were taken periodically and plotted as a percentage of initial PWF.

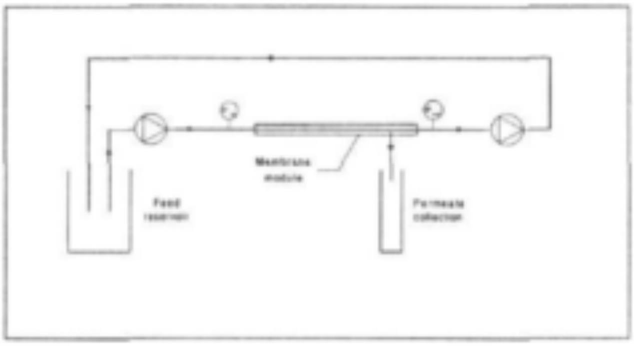

Figure 34: Schematic depiction of the experimental system

5 units of enzyme (enzyme activity determined using DMAB and MBTH as substrates) was immobilised per cm^2 membrane surface area. Immobilisation was performed by filtering the enzyme solution through the membrane at standard operating conditions to ensure a uniform distribution.

To activate the enzymes, the brown water feed was replaced by a 500 ml reservoir of pure water (Figure 34) which was vigorously sparged with technical grade oxygen (Afrox). The outlet pump was bypassed; the saturated water mixture was recycled with no back pressure (and thus no flux). This was performed for 45 to 90 minutes. PWF measurements were taken after enzyme activation so as to compare flux recovery to the initial PWF.

Enzymatic washing experiment

Again the initial PWF was measured, then the system was immediately switched to constant flux fouling with brown water. Specific flux was measured periodically and reported as a percentage of initial PWF.

Defouling was attempted by recycling an activated enzyme solution (sparged with O_2) through the membrane lumen with no back pressure for one hour. PWF was then compared to the initial value.

The membrane was also rinsed periodically (for 1 hour) with pure water sparged with O_2 (as in the immobilised enzyme experiment) in order to act as a control, i.e. to compare flux recovery due to oxygenation only against that for the immobilised enzymes. It must be noted that considerably less time than 1 hour would be necessary for sparging in the final design.

Results and Discussion

Figures 35 and 36 show graphs of the specific flux decline over time. The membranes were fouled for a total of 60 hours. Flux was maintained, by regular adjustment of pump speed as close as possible to 50 $L.m^{-2}.hr^{-1}$. Typical operational flux (Jacobs *et al.* 1999) is around 30 $L.m^{-2}.hr^{-1}$, however fouling was found to be very gradual at this flux during preliminary experiments, thus a higher value was used in the experiments in order to promote more rapid fouling for experimental purposes.

Figure 35 shows data for the enzyme washing experiment. It can be seen that there is a rapid initial flux decline over the first few hours. Specific flux reached a pseudo steady-state of ~ 0.4 $L.m^{-2}.hr^{-1}.kPa^{-1}$. Enzyme washing was performed at 62 hours. PWF was measured thereafter, the specific flux was found to have *decreased* slightly.

Figure 36 shows flux decline, and then flux recovery at 18, 43 and 54 hours. This corresponds to enzyme activation. Flux recovered to 87%, 67% and 67 % of the initial pure water flux respectively. The control was also oxygenated (at 49 hrs, Figure 35) to ascertain whether the increase in flux could be a result of oxygenation only. This however resulted in only a 3% flux recovery (compared to 31%, 21% and 21% recovery for the activated enzymes), indicating that the flux recovery is due to the action of the enzymes.

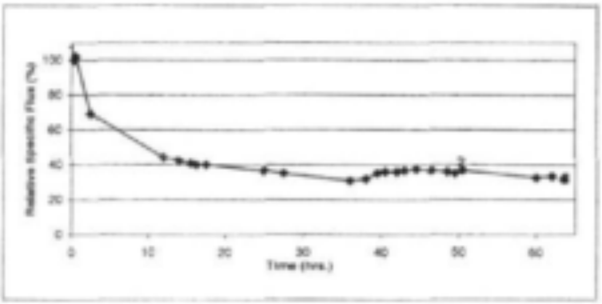

Figure 35: Decline of relative specific flux over time during fouling with brown water with no immobilised membranes. 1, 2 and 3 are pure water fluxes, with 3 the flux after enzyme washing.

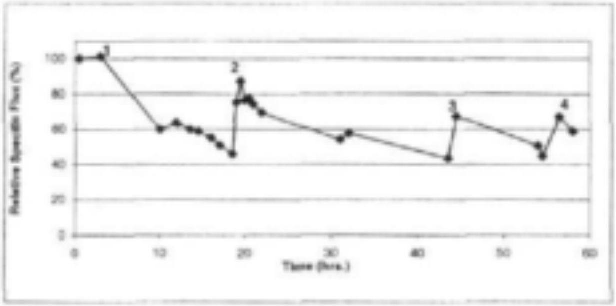

Figure 36: Flux decline and recovery with immobilised enzymes. 1 is PWF after immobilisation, 2, 3 and 4 are PWF's after enzyme activation

Conclusion

It was shown that the thermostable laccase with the extended substrate range proved effective in defouling UF membranes used for brown river water purification. Oxygenated water was used to induce the action of the enzyme, obviating the need for expensive and hazardous cleaning chemicals. This process could be extended for 10 days before enzyme washout and blockage of membrane inlets (due to the presence of suspended solids in the water) required enzyme regeneration. This indicated that pre-treatment technology would have to be incorporated with the process to remove suspended solids to obtain significant length of operation. The results hold good promise for application of the defouling process for application to small-scale treatment plants. Further work needs to be done to develop effective systems for application of the process.

Unfortunately little success was obtained in the use of the enzyme as a cleaning reagent for restoring flux to fouled membranes in conventional UF (enzyme not immobilised). This is not surprising, given the mechanism of action of the enzyme. De-polymerising enzymes (such as tannase) could be ore effective for this application.

CHAPTER 7: APPLICATION OF THE ENZYMATIC DEFOULING SYSTEM TO INDUSTRIAL EFFLUENTS

Introduction

Phenolic pollutants are considered to be a major pollutant in the aqueous effluents from petrochemical and coal-conversion industries (Greminger *et al.*, 1982), as well as being major components of wood-preservatives and phenoxy-herbicides (Bollag *et al.*, 1988). Phenols are also present as waste products from wood pulp bleaching (Kondo *et al.*, 1994) but may also enter the environment through manure, soil, sewage and vegetation (Rowe, 1983). The phenolic pollutants also exhibit varying degrees of toxicity and it is therefore essential that phenols must be removed from wastewaters (Klibanov *et al.*, 1983).

Due to the oxidising capability of white rot fungal enzyme suites on xenobiotics such as PAHs and phenolic compounds, ultrafiltration in combination with immobilised enzymes appears to be an attractive approach for the treatment of industrial wastewater due to the fact that removal can be combined with detoxification (Leukes *et al.*, 1999). It was therefore hypothesised that if a mixture of MnP and laccase were immobilised on a UF membrane, that the laccase could be used to polymerise low-molecular weight aromatic compounds. These polymerisation/ aggregation products, together with polyphenolic compounds already present in the effluents would eventually result in fouling of the membrane. The MnP could then be activated to defoul the membrane.

In the first part of this study the kinetics of laccase will be investigated with the following goals in mind:

1. To investigate the kinetic parameters of laccase and manganese peroxidase for the selected phenolic substrates: phenol, 2,5-dimethylphenol and 2,4-dichlorophenol present in a petrochemical industrially derived effluent.
2. An investigation into the inhibition kinetic parameters of laccase for sodium azide, sodium fluoride and sodium thioglycolate was also performed to evaluate known kinetic inhibition mechanisms with that of components of the petrochemical effluent.

The second part of this study focussed on characterisation of the membrane system and the following objectives were planned:

1. Characterisation of the membrane system through residence time distribution analysis to determine the expected impact of the MnP inducer solution, as well as expected activity outcomes and length of activation period. Attempts were made to correlate the RTD with the pollutant removal capacity of the system.
2. Characterisation of interactions between the membrane system and pollutants studied in this particular study by means of breakthrough analysis and adsorption isotherms.

In the last part of this study the following was evaluated:

1. Defouling of the membrane as a detectable increase in flux in comparison to controls.
2. Membrane interactions in terms of adsorption of phenolic pollutants from an industrial and synthetic effluent.
3. Efficacy of the immobilised laccase and manganese peroxidase in removal of phenolic pollutants from an industrial and synthetic effluent.
4. Effect of inhibitors on the above mentioned immobilised enzyme system.

Free Enzyme Kinetics and Inhibition Studies

The objective of this work was to investigate the kinetic parameters of laccase and manganese peroxidase for the selected phenolic substrates: phenol, 2,5-dimethylphenol and 2,4-dichlorophenol present in a petrochemical industrially derived effluent. An investigation into the inhibition kinetic parameters of laccase for sodium azide, sodium fluoride and sodium thioglycolate was also performed to evaluate known kinetic inhibition mechanisms with that of components of the petrochemical effluent.

MATERIALS AND METHODS

Culture maintenance and enzyme production

Trametes versicolor was maintained on malt agar plates and incubated at 25-28°C. Enzyme production was facilitated using the method described by Tien & Kirk (1988). Strips of *Trametes versicolor* were transferred to the broth and incubated at 28°C. After seven to eight days the enzymes were harvested by separating the culture from the broth. The supernatant was freeze dried to obtain the crude enzyme extract used for the studies. The freeze-dried crude enzyme extract was resuspended in 10 ml distilled water, aliquoted, and stored at –20°C until further use.

Enzyme quantification (Protein Determination)

Protein determination was performed using BCA protein determination kits (BCA Protein Assay Reagent, Catalogue number 23225T, Pierce Chemical Company) according to Heinzkill *et al.*, (1998).

Enzyme assays

All the mentioned assays were performed using an Ultrospec 1000 UV/Vis spectrophotometer (Pharmacia Biotech). The substrate ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) is an electron-rich, non-phenolic substrate. (Childs & Bardsley, 1975: cited in: Johannes & Majcherzyk, 2000). As a result no quinones form during this assay, and the oxidation potential of ABTS is pH independent in the range 2-11. The reaction proceeds in a single step and yields a coloured radical cation. (Johannes & Majcherzyk, 2000). The reaction can be followed spectrophotometrically at 420 nm with an extinction coefficient (ε) of $3.6 \times 10^4\ M^{-1}\ cm^{-1}$.

Activity is determined as 1 μmol of ABTS converted per unit time being representative of 1 unit. Laccase activity was determined using the method described by Paice *et al.* (1993) with ABTS as substrate. Manganese peroxidase activity was determined with ABTS as substrate according to Gold & Glenn (1988). As the crude enzyme extract contained both laccase and manganese peroxidase, the manganese peroxidase activity was masked. To overcome this problem inhibition studies were performed on laccase to determine the appropriate amount of inhibitor to block any laccase activity and thus make it possible to measure the activity of the manganese peroxidase present in the crude extract.

Inhibition studies

For the inhibition studies sodium azide, sodium flouride and thioglycollic acid (Johannes & Majcherczyk, 2000) were used as reference inhibitors for laccase. Fluoride and azide act by binding to the copper ions thus interfering this electron transfers within the enzyme. Fluoride in particular acts by binding to the dioxygen reduction site. Sulfhydryl compounds such as thioglycolic acid (TGA) are not laccase specific. Their application to phenoloxidases resulted from studies with other metalloenzymes where the sulfhydryl compound chelate the metal ions thus affecting enzyme function. Of all the inhibitors considered literature stated that only sodium azide can be considered as a true laccase inhibitor (Johannes & Majcherczyk, 2000).

Inhibition studies using the effluent at various concentrations were also performed. This was accomplished by substituting a known percentage of the water in the assay mix with filtered effluent. Due to the makeup of the assay mixture, the highest "purity" of effluent that could be used in the assays was 80% (v/v). Non-incubated enzyme kinetic studies involved addition of the inhibitor to the reaction mixture prior to the assay, while incubated inhibition studies involved addition of the inhibitor to the crude enzyme extract to match the required concentration in the aliquot volume and then incubating for 20 to 30 minutes. The incubated extract was then used in the assays.

Enzyme assays versus phenolic substrates

Enzyme activity against the phenolic substrates phenol, 2,5DMP and 2,4DCP was also determined. Data from the inhibition studies of laccase versus sodium azide and thioglycollic acid were used to select the best concentration to inhibit laccase's activity to make it possible to measure the masked manganese peroxidase activity.

RESULTS AND DISCUSSION

Inhibition studies using thioglycollic acid

From the results obtained the inhibition mechanism for thioglycollic acid (TGA) appears to be partial non-competitive, however the reliability of the model ($R^2 = 0.7920$) indicates that though the data approximates the model, it can't be considered as certainty. This is in agreement with the work of Johannes & Majcherzyk (2000) which showed that thioglycollic acid is not a true inhibitor of laccase, but rather serves to decolour the cation thereby giving false spectrophotomeric results which appear to indicate inhibition. For this reason the enzyme was not incubated with this particular inhibitor in any further experiments. Figures 37 and 38 indicate lag phases characteristic of inhibition by TGA i.e. the product is continually being decolourised until the TGA is depleted after which the reaction appear to proceed normally.

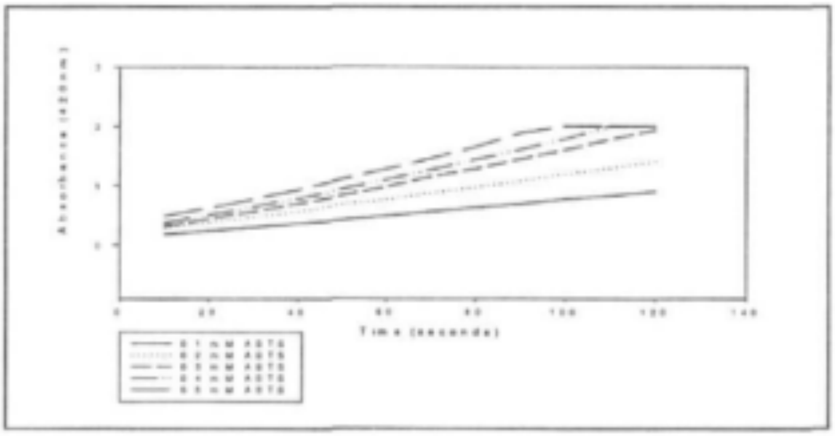

Figure 37: Absorbance vs time for control laccase assay.

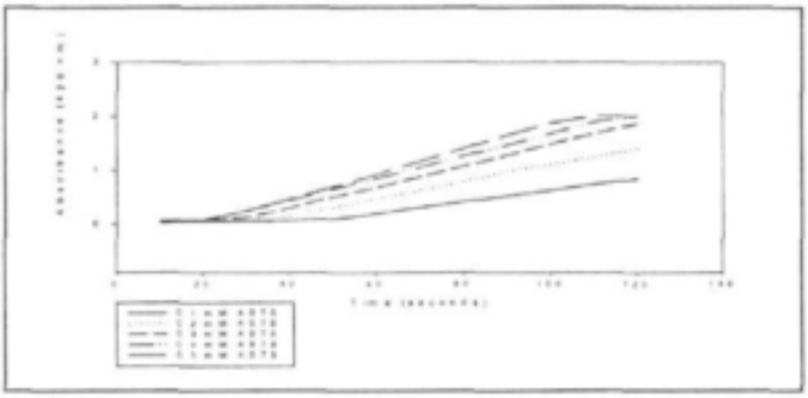

Figure 38. Absorbance vs time for laccase assay with 10 mM thioglycolate as inhibitor.

Lag times increase as the thioglycollic acid concentration is increased for any particular substrate concentration, while an increase in the substrate concentration will shorten the observed lag phase at a particular inhibitor concentration. After the lag phase the reaction rates are more or less equal as can be observed from the reaction slopes. The Hanes-Woolf plot (Figure 39) shows an apparent decrease in enzyme activity with an increase in the TGA concentration. A more plausible explanation would be that the rate of decolouration of the cation increases with the increasing TGA concentration until the TGA is depleted. These observed tendencies are in accordance with studies performed by Johannes and Majcherczyk, (2000).

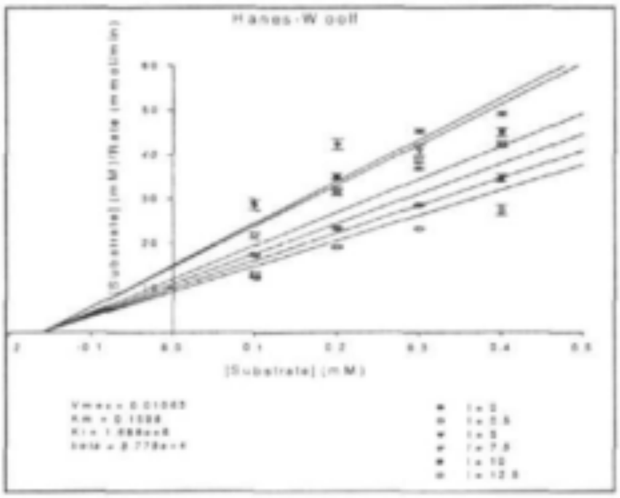

Figure 39: Hanes-Woolf plot for non-competitive inhibition of laccase by thioglycolic acid.

Inhibition studies using sodium azide

In both the non-incubated and incubated experiments, the inhibition mechanism appeared to be mixed inhibition (as indicated by the reliability of the fitted model (R^2 in table)). Compared to sodium fluoride, sodium azide is a far more potent inhibitor of laccase. Incubation in the case of sodium azide shows little effect on the V_{max} and K_m, but a significant increase in K_i is observed with incubation of the enzyme (Table 3 and Figure 40).

Table 3: Table summarising kinetic parameters for incubated (I) and non-incubated (NI) sodium azide experiments. (Refer to Tables 9 and 10 for a combined summary and equations.)

Inhibitor	Probable mechanism	Reliability of model (R^2)	V_{max} (mmol per min)	K_m (mmol)	K_i	Alpha	Beta
NaN_3 (NI)	Mixed (Full)	0.99417	0.0167	0.4901	0.01954	0.4001	N/A
NaN_3 (I)	Mixed (Full)	0.99154	0.01666	0.4883	0.7281	0.0449	N/A

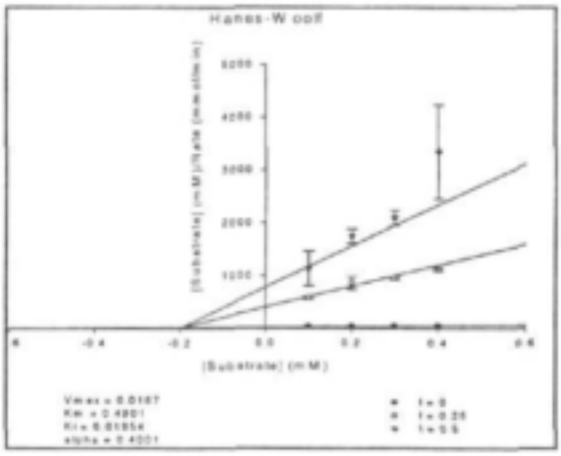

Figure 40: Hanes-Woolf plot for inhibition by nonincubated sodium azide (Inhibitor concentrations in mM)

Inhibition studies using sodium fluoride

In both incubated and non-incubated experiments the inhibition mechanism appears to be mixed inhibition (as indicated by the reliability of the fitted model (R^2 in table), as was the case with sodium azide. This is not surprising as both inhibitors function by binding with the copper ions within the enzyme. However, incubation of the enzyme with the inhibitor results in a more pronounced level of inhibition. Incubation has a significant effect on V_{max}, while only slightly affecting K_m (See Table 4).

Table 4: Table summarising kinetic parameters for incubated (I) and non-incubated (NI) sodium fluoride experiments (Refer to Table 6 and 7 for a combined summary and equations.)

Inhibitor	Probable mechanism	Reliability of model (R^2)	V_{max} (mmol per min)	K_m (mmol)	K_i	Alpha	Beta
NaF (NI)	Mixed (Partial)	0.99600	0.00507	0.1465	0.1712	1.3	0.1205
NaF (I)	Mixed (Partial)	0.99160	0.9003	0.1221	0.4963	1.6	0.06615

Inhibition studies using effluent

From the results it is apparent that the inhibition mechanism can either be competitive; full mixed inhibition or partial mixed inhibition (plots not shown). This is attributed to the results not fitting any of the model equations available. However, the presence of sodium and fluoride ions in the effluent suggests that the inhibition may partly due to the presence of sodium fluoride.

Since the NaF inhibition data most closely fits the model represented here by mixed inhibition, the presence of fluoride as well as hitherto unknown potential inhibitory compounds in the effluent and the combinations thereof make it difficult to accurately ascertain the exact mechanism of inhibition associated with the industrial effluent hence the low correlation factor with any of the models ($R^2 = 0.88$).

Table 5: Table summarising kinetic parameters for laccase inhibition by non-incubated industrial effluent. (Refer to Table 9 and 10 for a combined summary and equations.)

Inhibitor	Probable mechanism	Reliability of model (R^2)	V_{max} (mmol per min)	K_m (mmol)	K_i (mmol)	Alpha	Beta
Effluent	Mixed (Full)	0.85902	0.01239	0.0962	1.3	N/A	N/A

CONCLUSION

The kinetic parameters obtained corresponded with the expected results (Refer to Table 6). NaN_3 and NaF represent actual inhibitors of laccase according to their mechanisms, while literature only reports NaN_3 as a true inhibitor. As both fluoride and azide bind to the copper ions and not the complete reaction centre, mixed inhibition would be the most probable mechanism to expect. During true uncompetitive inhibition the inhibitor would only bind to the enzyme-substrate-complex and not the free enzyme, whilst during true non-competitive inhibition the formation of a dead-end complex between the enzyme and inhibitor regardless of whether the substrate has bound or not would have resulted (Palmer, 1995). (Refer to Table 7 for equations for the various models.) Thioglycolic acid can not be regarded as a true inhibitor and this is supported by the non-competitive inhibition obtained as well as the weak correlation of the model to the experimental data obtained ($R^2 = 0.70551$). The mixed inhibition model for the inhibitory effect of the effluent is somewhat not unexpected as other compounds present in the effluent can act as inhibitors. However, screening for these particular inhibitors would be inconclusive because, as already stated, inhibition of the immobilised enzyme can be attributed to a variety of factors.

Table 6: Summary of kinetic parameters for various inhibition studies.

Inhibitor	Probable mechanism	Reliability of model (R^2)	Vmax (mmol per min)	Km (mmol)	Ki (mmol)	Alpha	Beta
NaF (NI)	Mixed (Partial)	0.99600	0.005071	0.1465	0.1712	1.3	0.1205
NaF (I)	Mixed (Partial)	0.99160	0.9003	0.1221	0.4963	1.6	0.06615
NaN3 (NI)	Mixed (Full)	0.99417	0.0167	0.4901	0.01954	0.4001	N/A
NaN3 (I)	Mixed (Full)	0.99154	0.01666	0.4883	0.7281	0.0449	N/A
TGA (NI)	Non-competitive (Partial)	0.70551	0.1063	0.1598	$1.68\text{x}10^6$	N/A	$8.778\text{x}10^4$
Effluent	Competitive (Full)	0.85902	0.01239	0.0962	1.3	N/A	N/A

(NI) = Non-incubated

(I) = Incubated

TGA = Thioglycolic acid

Table 7: Summary of equations for the various inhibition models

Model	Equation
Competitive (Full)	$V = V_{max}/(1+(K_m/S)*(1+I/K_i))$
Competitive (Partial)	$V = V_{max}/(1+(K_m/S)*(1+I/K_i1)/(1+I/K_i2))$
Non-competitive (Full)	$v = V_{max}/((1+I/K_i)*(1+K_m/S))$
Non-competitive (Partial)	$v = V_{max}/((1+K_m/S)*(1+I/K_i)/(1+I*beta/K_i))$
Mixed (Full)	$V = V_{max}/((K_m/S)*(1+I/K_i))/(1+I/(alpha/K_i)))$
Mixed (Partial)	$V = V_{max}*((1+beta*I/(alpha*K_i))/(1+I/(alpha*K_i)))/(1+K_m/S)*(1+I/K_i)/(1+I/(alpha*K_i)))$
Uncompetitive (Full)	$v = V_{max}/(1+I/K_i+K_m/S)$
Uncompetitive (Partial)	$v = V_{max}*(1+beta*(I/K_i))/(1+I/K_i+K_m/S)$

CHAPTER 8: MEMBRANE/SUBSTRATE INTERACTIONS

In the operation of any given tubular-flow reactor system, it is considered ideal if reactants and products are transported in a state of *plug flow* with no backmixing of substances present in the system occurring. During plug flow conditions it is not only assumed that the mass flow rate is uniform through out the system, but that fluid properties (temperature, pressure, and composition) also remain uniform. In practice, reactors rarely satisfy this ideal condition of true plug flow and thus any deviations from this ideal flow should be taken into account. These deviations can be the result of channelling of the fluid through the reactor, stagnant pockets or internal recycling of the fluid inside the reactor. By characterising the flow dynamics within the system, it is possible to estimate the behaviour of a fluid within the reactor. In the scale-up of any reactor-based process this non-ideal flow is closely associated with problems associated with industrial-scale plant commissioning (Levenspiel, 1972).

Besides the molecular weight cut-off properties of a membrane, physicochemical interactions also affect separation processes. This is particularly true as the membranes' chemical properties and the chemical properties of the pollutant/substance to be filtered need to be considered when selecting membranes for a particular application. The adsorption isotherm can supply valuable information for the affinity between sorbent and sorbate and aid to clarify breakthrough points and removal patterns that may be observed.

The following objectives were planned:

- Characterisation of the membrane system through residence time distribution analysis to determine the expected impact of the MnP inducer solution, as well as expected activity outcomes and length of activation period. Attempts were made to correlate the RTD with the pollutant removal capacity of the system.
- Characterisation of interactions between the membrane system and pollutants studied in this particular study by means of breakthrough analysis and adsorption isotherms.

MATERIALS AND METHODS

Residence time distribution (RTD)

A tracer solution containing 5 ml of a 3 M NaCl solution and 1 ml of a 3 M NaCl solution was introduced at the injection port of the membrane system. The feed solution of the system consisted of Milli-RO® H_2O and was not recirculated. The NaCl solution exiting the system was measured as a function of conductivity using a HANNA HI8733 portable conductivity meter (HANNAH Instruments, Woonsocket, USA). Conductivity measurements were taken as soon as a sufficient volume of permeate was obtained to immerse the probe (approximately 8 ml accumulated every 5 minutes).

Breakthrough analysis

For the breakthrough analysis a feed solution was prepared containing each of the phenolic compounds at a concentration of 50 ppm. The system was operated in dead-end filtration mode, with no recirculation. Samples were collected on an hourly basis and analysed using HPLC. During the first breakthrough experiment the system was run for 8 hours. As not all the phenolic compounds in the permeate reached the equivalent respective feed concentrations the breakthrough experiment was repeated with a time extension to 80 hours.

Adsorption isotherms

For the adsorption analysis isotherm a Langmiur model for adsorption was used as it was assumed that monolayer adsorption of the organics would be the dominant mechanism involved. For this method a specific weight of membrane (membrane length of 1 cm corresponding to an average weight of 0.885 g) was soaked overnight in deionised water. Solutions containing the phenolic substances in a mixture were prepared with a concentration range maximum of 50 mg. L^{-1} to represent the concentrations used in the feed solutions. The membrane segments were added to the phenolic solutions and sufficient contact time allowed in order for the system to reach equilibrium. At equilibrium, samples were analysed by HPLC to quantify the amounts of non-adsorbed phenolic compounds. Experiments were performed at pH 5, pH 7, and pH 9. The three pH points were selected to flank the effluent's native pH and thus provide boundaries to allow a measure of prediction of adsorption behaviour at pH levels in between.

HPLC analysis of samples

Analysis of the samples were performed on a HP1100 HPLC analyser (Hewlett-Packard, Palo Alto, California) with a Phenomenex EnviroSep PP (125 x 4.6 mm) column. A methanol/water gradient was used at a flow rate of 1.5 ml per minute. The initial ratio of MeOH:H_2O was 30:70. The MeOH:H_2O ratio was increased to 56% over 6 minutes. The gradient was then increased from 56% to 100% over the period 6 to 12 minutes. Detection was achieved using a G1365A detector as 280 nm with a reference wavelength of 550 nm. The system was controlled by HP Chemstation for LC software. An injection volume of 30 μl was used.

RESULTS AND DISCUSSION

Reactor fluid dynamics

From the conductivity response curve it is apparent that the tracer solution peaked in concentration 15 minutes after addition and remained detectable for a period of 60 minutes thereafter (See Figures 41 and 42). After this period the detectable response was negligible. The residence time is thus not volume dependent. The implication of this for the membrane reactor with immobilised enzymes is that the inducer will induce MnP maximally after 15 minutes and decrease over a period of approximately 45 minutes. MnP activity declined to the resting level during the period 15 to 60 minutes. The MnP should therefore be completely inactive after a period of 60 minutes. As a result of this response, defouling or flux restoration would be evident within 60 minutes after induction. The dispersion number describes as indicated in Table 8 the movement of the inducer solution through the system, i.e. either as a plug through the system (ideal plug flow) where the dispersion number will approach a value of 0 or as non-ideal dispersed flow as indicated by dispersion values ranging from 0.002 to 0.2 (representing small to large amounts of dispersion). A value approaching infinity would indicate mixed flow in the system. From these results it can be concluded that plug-flow does not occur in this system and as a result a significant degree of backward mixing or dispersion occurs which is what is expected from a dead-end flow system (Levenspiel, 1972).

From the results obtained in the stimulus response experiments and the calculated dispersion numbers the addition of the MnP inducer solution to the system would facilitate maximal contact time of the inducer with the immobilised MnP. A typical plug flow response would imply very short activation or induction periods resulting in negligible periods of MnP activity. Due to the non-ideal dispersed flow occurring in *this* system however, greater phenolic removal can be anticipated in comparison to what would be expected in an ideal plug flow reactor.

Table 8: Summary of reactor dispersion numbers for the membrane modules

Membrane batch	Reactor dispersion number (dimensionless)
New membranes	0.108 ± 0.00142

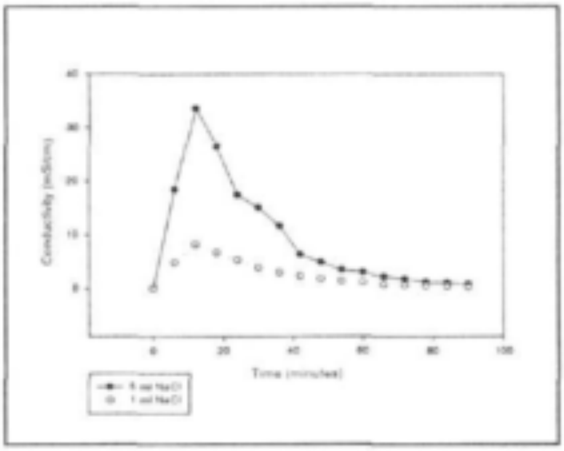

Figure 41: Conductivity response vs time at various tracer volumes

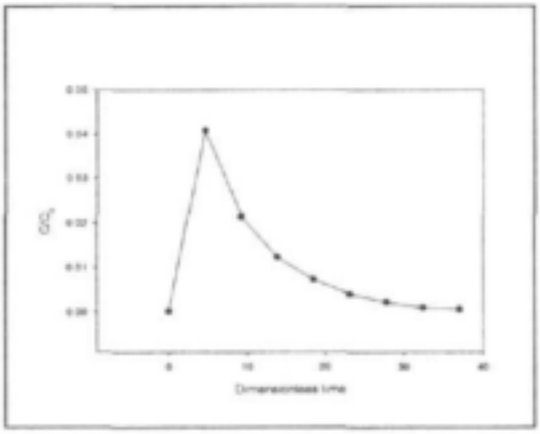

Figure 42: Normalised residence time distribution membrane

Breakthrough points

Breakthrough experiments were done to determine the time frame required for each effluent phenolic component in the permeate to reach its maximum exit concentration (Figures 43 and 44). This was necessary in order to determine any enzyme-substrate concentration effects in terms of inhibition, saturation, and long-term activity loss.

From the 8-hour breakthrough experiment (Figure 44) it can be seen that phenol is not significantly retained by the membrane system, while 2,5DMP and 2,4DCP do not seem to reach saturation levels within the same period. During the 80-hour breakthrough experiment 2,5DMP reaches saturation levels after approximately 25 hours (1500 minutes), while 2,4DCP reached saturation levels after 70 hours (4200 minutes). It is important to note that although all the phenolic compounds in the feed were present at a concentration of 50 ppm, corresponding to mM concentrations were 0.53 mM for phenol, 0.41 mM for 2,5DMP, and 0.31 mM for 2,4DCP. The feed concentrations (mM) of the individual phenolic compounds more or less correlated with the saturation concentrations achieved at 8 hours for phenol, 25 hours for 2,5DMP, and 70 hours for 2,4DCP. This implies that the enzyme will only experience local micro-environment concentrations approaching feed concentrations 8, 25 and 70 hours after start-up of the system for phenol, 2,5DMP and 2,4DCP respectively during continuous operation. This has implications in terms of any concentration-dependent inhibition that might occur. This implies that the expected reactor efficacy would be high initially and then decrease with time. This was observed to occur. The decrease in reactor performance can be attributed to the concentrations of phenolic compounds that the enzyme is experiencing increasing. Inhibition studies on the system therefore indicate best-case scenarios for the systems behaviour under ever increasing inhibitory conditions.

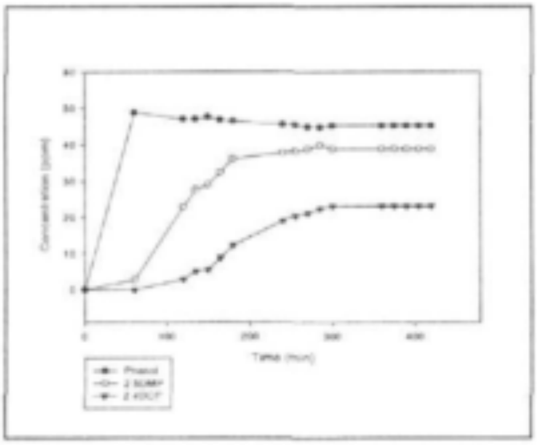

Figure 43: Breakthrough analysis after 8 hours

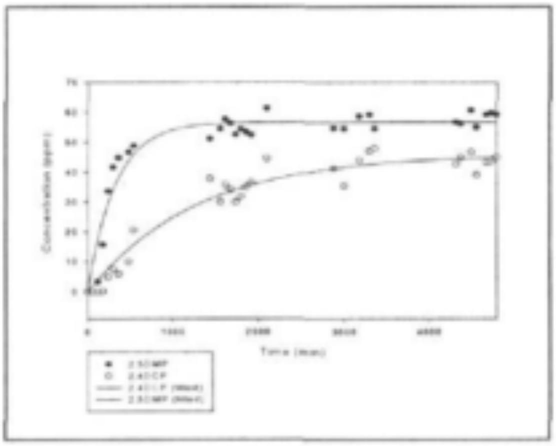

Figure 44: Breakthrough analysis after 80 hours

Adsorption isotherms

Adsorption isotherm experiments were done to correlate the physical and chemical properties of the selected phenolic pollutants to the breakthrough and saturation phenomena observed.

In Table 9 the parameter b refers to the affinity of the compound to the membrane and q*max* represents a calculated adsorption maxima. When different sorbents are compared, q*max* is useful for interpretation. As this experiment was performed at non-saturating concentrations the actual *qmax* may be higher than stated (See Table 10 for water solubility for each compound). The affinity of the compounds for the membrane is thus regarded as a more useful parameter for interpretation.

From the Langmuir isotherms plotted (results not shown) and Table 9 it is evident that phenol shows the lowest affinity for the membrane. When comparing the affinity for the phenolic compounds using pH 7 at 30 minutes exposure time as the reference point the increase to pH 9 and decrease to pH 5 results in an increase in the affinity of the phenolic compounds to the membrane. When the exposure period was increased to 60 minutes, phenol's affinity declined at the respective pHs while a slight increase in *qmax* was observed. The slight increase in the affinity (b) was also observed in the breakthrough curves as a very gradual decrease in the phenol concentrations in the permeate. The difference in affinity for each of the phenolic compounds was also reflected in the time each compound required to reach saturation levels. The adsorption maximum (*qmax*) for phenol increased when the pH was altered to pH 5 and pH 9, while a decrease was *qmax* was observed for 2,4DCP and 2,5DMP when the pH was altered to 5 and 9.

The data for pH 7 should be regarded at most relevant as the pH of the synthetic effluent and industrial effluent ranges from pH 6.9 to pH 7.12. From the difference in the parameters from 30 to 60 minutes, the equilibrium appears to be dynamic with time. At pH 7 the affinity increases significantly for all the phenols, and while the adsorption maximum for phenol and 2,5DMP increased the 2,4DCP's adsorption maximum only increased slightly. At pH 9 a similar tendency as to pH 7 was observed with the exception of phenol where an increase in *qmax* is observed. At pH 5 the *qmax* of all the phenolic compounds increased over time, while the affinity increased with the exception of phenol.

These tendencies can possibly be related to the chemical structure and properties of molecules. Phenol is a benzene ring with a hydroxyl group, which should induce a charge on the molecule, thus allowing for a high degree of water solubility compared to 2,4DCP and 2,5DMP. On the other hand, 2,4DCP with chlorine substitution in the *ortho*- and *para*-positions could induce a larger degree of charge on the molecule resulting in increased interaction with, and adsorption to, the membrane. Lastly, 2,5DMP has methyl substitutions in the *ortho*- and *meta*-positions and these serve to stabilise the charge on the molecule thus making it weakly hydrophilic, yet also weakly interactive with the membrane. (Refer to Table 10) These results correlate with observations obtained for the breakthrough curves.

Table 9: Summary of adsorption parameters (affinity (b) and adsorption maximum (q*max*) for phenol, 2,5DMP and 2,4DCP after 30 minutes and 60 minutes exposure.

Compound	30 minutes exposure		60 minutes exposure	
	b	q*max* (mg/g)	b	Q*max* (mg/g)
At pH 5				
Phenol	0.058	0.213	0.045	0.331
2,5DMP	0.023	0.722	0.041	0.937
2,4DCP	0.036	0.847	0.0642	1.160
At pH 7				
Phenol	0.029	0.233	0.119	0.180
2,5DMP	0.0002	33.288	0.0003	52.100
2,4DCP	0.0002	53.142	0.0005	43.466
At pH 9				
Phenol	0.041	0.210	0.090	0.215
2,5DMP	0.121	0.431	0.197	0.600
2,4DCP	0.0009	13.145	0.0160	2.190

Table 10: Selected chemical properties of phenol, 2,5DMP and 2,4DCP.

Properties	Phenol	2,5DMP	2.4DCP
Molecular weight	94.11	122.17	163.0
Water solubility (at 25°C)	84g/L	78-223 mg. L^{-1}	4.5 g/L
Acid dissociation constant (pKa)	9.9	10.22	7.68
Log K_{ow}	1.46	3.15	3.06

CHAPTER 9 : MEMBRANE IMMOBILISED ENZYME – CHARACTERISATION AND EVALUATION

One major concern in the use of enzymes in processes is their lack of reusability (Vishwanath *et al*., 1995). One of the solutions to help ensure enzyme stability is to immobilise the enzymes onto a solid support material such as ion exchange resins, gel entrapment and polymeric membranes. Non site-directed (random) immobilisation however can result in changes in the characteristics of the immobilised enzyme such as decreasing the K_m as well as masking the active site (Boy *et al*., 1999; Douglas, 1994).

In this study the following was evaluated:

- Defouling of the membrane as a detectable increase in flux in comparison to controls.
- Membrane interactions in terms of adsorption of phenolic pollutants from an industrial and synthetic effluent.
- Efficacy of the immobilised laccase and manganese peroxidase in removal of phenolic pollutants from an industrial and synthetic effluent.
- Effect of inhibitors on the above mentioned immobilised enzyme system.

MATERIALS AND METHODS

Membrane Module

For the purposes of these experiments a single-capillary reactor (SCR) set-up was configured. This configuration consists of a single capillary membrane in a PVC shell. The SCR was assembled by threading the membrane through the shell and bonding it at each end with Epoxy resin. The length of the reactor was 0.58 m giving an effective membrane area of 0.0018 m^2. Polysulphone membranes obtained from the Institute for Polymer Science, Stellenbosch University were used for each experiment. Some variations between various membranes were anticipated due to perturbations in the manufacturing process. An injection port was included in the system's design to facilitate immobilisation of the enzyme fraction and addition of the inducer solution.

Operation

Hydraulic delivery was facilitated using Tygon® tubing. A pressure vessel maintained at 55-60 kPa facilitated substrate delivery at an inlet pressure of 45-50 kPa. Both the feed system and the SCR were held at room temperature during operation. The reactor was run in dead-end filtration mode with no recirculation of the permeate. All control experiments were run with no immobilised enzyme added to the system through the injection port, but the inducer solution was added, where specified (at intervals 2, 4, and 6 hours of operation). As the residence time of the system is approximately 60 minutes, the system was run for an additional hour after the last induction (at 6 hours) giving a total of seven hours operation time per experiment. Prior to any experiment, the membrane reactor was operated for about thirty minutes using de-ionised water until the pure water flux (PWF) stabilised. After the PWF was determined, the water in the pressure vessel was replaced with the effluent. Flux was normalised to % of the initial PWF to circumvent the problems of membrane inconsistency.

Preparation of effluent

HPLC analysis of the industrial effluent indicated no significant mono-phenolic content. As a result, the effluent was supplemented with 50 mg/L phenol, 2,5DMP and 2,4DCP. The synthetic effluent consisted of distilled water supplemented with 50 mg/L phenol, 2,5DMP and 2,4DCP.

For the studies on the effect of inhibitors on the process the effluent was supplemented to a final concentration of 1 mM NaN_3 and 20 mM NaF respectively. These concentrations were selected as a result of the inhibition studies where these concentrations resulted in complete inhibition of enzyme activity in the assays.

RESULTS AND DISCUSSION

Synthetic effluent (phenol-, 2,5DMP, 2,4DCP supplemented de-ionised water) – Uninhibited
Observation of the ultrafiltration flux decline (See Figure 45), shows up to 30% decline in flux in the control. Adsorption of the phenolic compounds can explain this significant decline in flux. The reduced loss of flux in the immobilised enzyme experiment is probably due to polymerisation and conversion of the phenolic compounds by the enzyme laccase and thus less adsorption of the phenolic compounds on the membrane. Addition of the MnP induction solution at 120 minutes, 240 minutes, and 360 minutes seems to have no effect in the phenol removal in either experiment.

Most trends discussed here are based on the performance of the system after approximately 120 minutes as this was the time the system required to stabilise (with regard to phenol and 2,5DMP, with 2,4DCP taking at least 240 minutes to stabilise). Phenol content in the permeate shows a 6 % adsorption of phenol in the control. In contrast to this, the immobilised enzyme reactor shows an average removal of 25 % (results summarised in Table 11). Compared to this, the substituted phenols showed a lesser degree of removal. In the case of 2,5DMP, membrane adsorption of 2,5DMP amounts to an average percentage adsorption of 1.8 % while enzymatic removal amounts to an average removal of 6.7 %. In the case of 2,4DCP membrane adsorption of 2,4DCP amounts to 6.6 % adsorption while enzymatic removal amounts to an average of 13 % removal. Removal results for 2,4DCP should be interpreted with caution as 2,4DCP does not reach saturation levels within the 7 hour period per experiment, and as a result of this may appear deceptively high. It is important to note that removal patterns are observed and discussed when the permeate levels stabilise at a level nearing saturation. Also important is the time it takes for the system to stabilise. In the case of phenol, levels in the permeate reached saturation approximately 1 hour after switching to the supplemented synthetic effluent, compared to 2,5DMP and 2,4DCP which takes 120 and 300 minutes respectively. This phenomenon correlates with the breakthrough curve data. As a result, 2,4DCP removal seems to be adsorption-based in the time period before saturation levels are achieved and beyond the point of saturation, enzymatic removal takes precedence as is evident from the system's performance in the time period 0 to 300 minutes. When comparing the removal of phenol and 2,5DMP by the membrane and the immobilised enzyme (results summarised in Table 11) 2,5DMP shows the lowest removal followed by phenol with 2,4DCP removal being the highest. On the immobilised enzyme system, removal of the phenolic compounds is almost double that of adsorption of the phenolic compounds by the membrane system. These tendencies can be attributed to chemical and structural properties of phenol, 2,5DMP and 2,4DCP.

Table 11: Summary of amounts of phenol, 2,5DMP and 2,4DCP removed by various mechanisms from the synthetic effluent

	% Adsorbed	% removed by enzyme	Total % removal Adsorption and enzymatic
Phenol	6	19	25
2,5DMP	1.8	4.9	6.7
2,4DCP	6.5	6.5	13

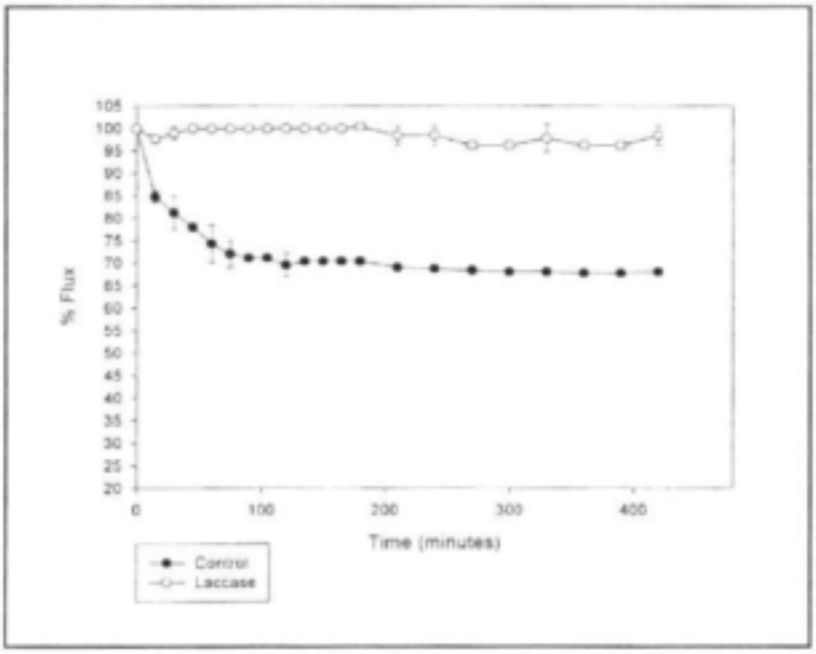

Figure 45: Ultrafiltration of phenolic supplemented deionised water

Phenol-, 2,5DMP, 2,4DCP supplemented industrial effluent – Uninhibited

When referring to the ultrafiltration flux data, immobilisation of the enzyme results in a slower rate of fouling in the period 0 to 120 minutes, with a 5% better flux rate over extent of the experiment (See Figure 46). As with the supplemented effluent, addition of the MnP inducer solution results in no flux restoration in the control as is expected. However in the experimental run the expected flux restoration also does not occur. This was attributed to masking of MnP activity by laccase activity and confirmed by non-immobilised inhibition studies.

As was anticipated from the residence time data, the period lasts approximately 60 minutes and is characterised by increased phenolic compound removal (results summarised in Table 12). In this regard, 2,5DMP removal showed the best response to MnP induction, followed by phenol. 2,4DCP showed the weakest response in terms of enhanced removal.

Phenol in the permeate reached a level of 48 mg/L within 60 minutes and decreased to 45 mg/L during the remainder of the experiment corresponding to an average adsorption to the membrane of 3 %. As with the phenolic supplemented de-ionised water, the immobilised membrane system shows a higher degree of phenol removal i.e. an average of 54 %. 2,5DMP shows a higher degree of adsorption than with the phenolic supplemented de-ionised water. Adsorption of 2,5DMP by the membrane amounts to an average of 30 % while conversion/removal by the immobilised enzyme system amounts to an average of 48 % . Compared to the phenolic supplemented DE-IONISED WATER 2,4DCP showed a much higher degree of adsorption by the membrane. Adsorption of 2,4DCP amounts to an average 68 %. Compared to this the immobilised enzyme system is far more efficient, removing an average of 93%. This very high initial removal of 2,4DCP by the immobilised enzyme system can be attributed to a combination of adsorption and enzymatic conversion.

When comparing removal of the phenolic compounds by adsorption due to the membrane system alone, phenol removal is the lowest. 2,4DCP and 2,5DMP removal is initially high but after 300 minutes 2,4DCP is removed more efficiently than 2,5DMP. This is more or less opposite that of the removal pattern observed in the phenolic supplemented de-ionised water. The above results are summarised in Table 12.

Table 12: Summary of amounts of phenol, 2,5DMP and 2,4DCP removed by various mechanisms from phenolic non-inhibited supplemented industrial effluent

	% Adsorbed	% removed by enzyme	Total % removal Adsorption and enzymatic
Phenol	3	51	51
2,5DMP	30	18	18
2,4DCP	68	93	25

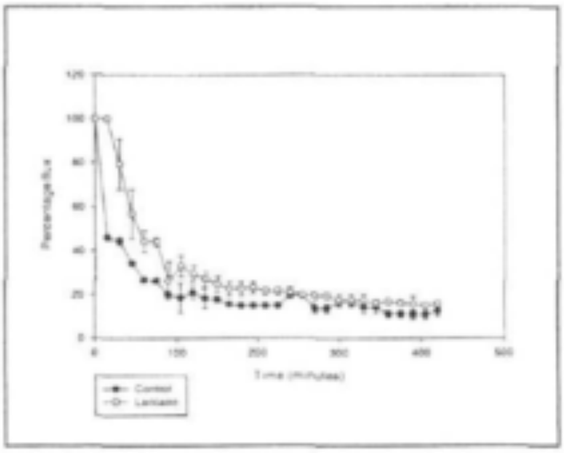

Figure 46. Ultrafiltration of phenolic supplemented industrial effluent.

Inhibition studies on immobilised enzymes

Studies wre also performed using NaN_3 and NaF as inhibitors. Several interesting trends were observed, which will not, however be discussed in detail here. In summary, the effluent appeared to show inhibition most similar to NaF.

NaF concentration of the industrial effluent was therefore determined and compared with the exiting permeate fluoride concentrations. Fluoride accumulation would imply higher localised fluoride concentrations at the immobilised enzyme/membrane microenvironment. This would offer some explanation as to the inhibitory effect of the industrial effluent, however, although other compounds present in the effluent may have contributed to the inhibition, it was not possible to screen and characterise all of these. Screening of all the compounds would also have been inconclusive as inhibition of the immobilised enzyme layer could be attributed to a combination of a number of components. However, due to the nature of NaF inhibition, it was concluded that high fluoride concentration at the immobilised enzyme/membrane interface would have had a significant contribution to the overall inhibition of the laccase in the industrial effluent in comparison to the phenolic supplemented de-ionised water as indicated in the preceding results.

There is a significant difference between the fluoride concentration in the feed and the permeate of the system (Figure 47). This suggests accumulation/concentration of fluoride ions at the membrane surface as anticipated. The decrease in enzyme activity can therefore be partly attributed to inhibition by fluoride (particularly sodium fluoride present in the uninhibited industrial effluent). Other physical factors contributing to the decrease in the immobilised enzyme performance may have been the suspended solids concentrated at the membrane surface limiting mass transfer of substrate to the enzyme. This phenomenon was not investigated in any great depth.

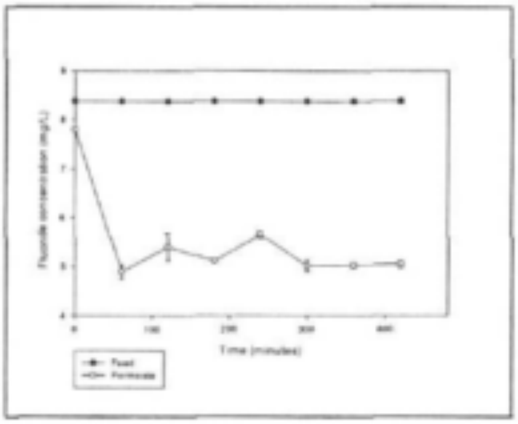

Figure 47: Fluoride concentration feed and permeate from industrial effluent.

Conclusion

In evaluating the feasibility of the system the following conclusions were made. Firstly, defouling of the membrane in the presence of the immobilised enzyme appeared to be a continuous process, manifesting as a significantly lower degree of flux decline during the non-inhibited studies. This was attributed to the activity of laccase within the crude enzyme extract. Activation of the immobilised MnP, however, was not observed as periods of increased flux, but rather as periods of enhanced phenolic compound removal from the effluent. This observed behaviour may be attributed to the suspended solids not being phenolic in nature and thus not substrates for the immobilised enzymes hence the low levels of flux restoration observed both under non-induced MnP laccase-mediated and induced MnP and laccase mediated operation.

Secondly, interactions between the membrane (in the presence and absence of immobilised enzyme) and the phenolic pollutants in terms of adsorption correlated well with the isotherm data in terms of affinity of the various phenolic compounds for the membrane. The only significant departure from this observed trend occurred in the case of the industrial effluent where slightly longer periods were required to reach saturation levels with an associated increase in adsorption onto the membrane. This might be explained by retention of the suspended solids by the membrane thus forming a retained layer, resulting in increased removal of the suspended solids during the control experiments. Possible binding of the phenolic compounds onto the suspended solids might explain the observed enhanced removal of phenolic compounds from the industrial effluent. Furthermore, the difference in phenolic removal from the industrial effluent may be attributed to possible reaction mediators present in the effluent, however, these were not screened for. In both the synthetic and industrial effluents, addition of the MnP inducer solution resulted in enhanced phenolic compound removal as was anticipated.

Furthermore, the inhibition studies on the immobilised enzyme system can be considered valuable in predicting the effect of variable inhibitory conditions on the efficacy of the intended process. Complete inhibition of the enzyme would manifest as removal trends correlating with that of an enzyme-free system, while partial or no inhibition would exhibit trends similar to that of an uninhibited system. Complete inhibition was observed with NaN_3 in the feed, while NaF in either the feed or the inducer solution resembled partial inhibition. NaN_3 in the inducer exhibited phenolic removal approximating that of non-inhibited immobilised enzyme. MnP activity appeared to be unaffected be these types of inhibitors as was evident by the response to addition of the inducer solution. Furthermore, NaF appeared to affect overall removal in the sense that it precipitated the suspended solids and effected greatest removal of 2,5-DMP, followed by phenol, while 2,4-DCP showing decreased removal. In studies where NaF was not used as inhibitor, 2,5-DMP was usually the least adsorbed and/or removed. No valid explanation was reached in terms of these observations. Flouride accumulation at the membrane/enzyme interface suggested that flouride may have contributed to the overall inhibitory effect. From the observed inhibition of laccase in the free enzyme studies, it was anticipated that phenolic removal and defouling did not occur at optimal levels.

To summarise:

- Laccase activity in both the free-form and immobilised form is affected by a number of inhibitory compounds. Of these, high fluoride concentrations in the effluent was of considerable concern and therefore the inhibitory effects of which were determined.
- Membrane/substrate interactions are influenced by the physicochemical properties of both the target pollutants and the membrane polymer selected for the intended application.

- The non-inhibited immobilised enzyme was more efficient at removal of the phenolic pollutants in the industrial effluent when compared to that of the synthetic effluent.
- Inhibition studies indicated complete inhibition of the immobilised enzyme manifesting as phenolic removal and flux decline similar to that of an enzyme-free system.

Evaluating the targeting of specific effluents using enzyme inhibition studies to determine the effectiveness of enzyme-based treatment of these effluents has been shown here to provide effective evaluation criteria in terms of choice of method for bioremediation purposes.

CHAPTER 10: CONCLUSION AND FUTURE WORK

Brown Water Treatment

Use of MnP on Flat Sheet Membranes

Initial Investigations on the characterisation of the defouling technology involved the use of MnP as a candidate enzyme on flat sheet membranes. MnP is an enzyme known to be active on humic substances and requires H_2O_2 and $MnSO_4$ as co-factors, thereby fulfilling the requirement of being activatable. Flat sheet membranes were used as they were a convenient format for analysis.

The research showed that good flux restoration (up to 93% of pre-fouling flux) could be obtained when the inducer solution was optimised for chelator (organic acid), Mn^{2+} and H_2O_2 concentration. This system was also used to study the mechanism of defouling. From this research, it was found that the predominant mechanism of defouling was aggregation of the compounds of the NOM forming the cake layer. This occurred after induction of the enzyme. With the flat sheet membrane system, the defouling action was rapid due to good mass transfer of inducer compounds to the enzyme.

MnP and Capillary membranes

The process was then adapted to use capillary membranes, since these were a more feasible scale-up option. Single-fibre capillary modules were operated with dead-end filtration according to methods adopted from methods adapted from the experience gained from flat sheet membranes. Very little flux restoration was attained using this system, and there was a significant delay between addition of the enzyme inducer solution and flux restoration. The reason for the poor defouling performanve was found to be uneven distribution of the immobilised enzyme on the membrane surface. The dead-end operational mode did not allow sufficient shear to lift the cake layer that had been de-stabilised by the enzyme action. The delay in defouling was found, following RTD analysis, to be due to poor mass transfer of the inducer solution to the enzyme.

Laccase Enzyme and Cross-flow operation of Capillary Membranes

The next step was to evaluate the defouling process for in capillary membranes under cross-flow conditions. It was also decided that a different enzyme system was required to replace MnP. It was found that the MnP used was not sufficiently stable for long term operation, and that the requirement for H_2O_2, Mn^{2+} and organic acids was not desirable for potable water generation. A laccase enzyme was chosen, since it has a similar substrate range to MnP, but requires only oxygen (In the form of air) as an inducer. The use of air as an inducer is far less expensive, and safer than the use of H_2O_2 and $MnSO_4$. To ensure greater stability of enzyme, the strategy of choosing a thermostable enzyme was used. This was based on the rationale that enzymes with greater thermostability also have greater mechanical stability. Hence, a thermostable laccase preparation derived from a locally isolated fungal strain, was evaluated. This enzyme also had an extended substrate range, and was therefore active on a larger proportion of NOM components.

The process incorporating the use of the thermostable laccase with an extended substrate range proved successful for application to the defouling of membranes used for the ultrafiltration of brown river water from the George area.

Using a constant flux operating regime, the immobilised enzyme layer offered no increase in membrane resistance, so will not increase energy expenditure. Significant specific flux restoration was achieved by recirculation of oxygenated water to activate the enzyme. This process could be extended for significant periods due to the stability of the enzyme. However, enzyme washout does require regeneration of the enzyme layer.

The use of oxygen as an activator considerably reduces the cost of cleaning (in comparison with the chemical activators required for MnP use) and requires minimal infrastructure compared to use of a backflushing regime. The system also allows for the use of potentially more compact (densely packed) membrane modules since it obviates the momentum transfer considerations associated with backflushing.

The next step in the development of the process is to consult with systems engineering specialists about integrating the knowledge obtained about the enzyme action with appropriate membrane technology and operating regimes. The process can then be optimised after more detailed analysis of the biochemical and physical mechanism of defouling. Attention is at present focussed on large scale production of the thermostable laccase at a sufficiently low cost to make the system commercially viable.

The community participatory research and technology assessment program should then be re-introduced to established new strategic objectives and opportunities for the process.

Effluent Treatment

Valuable experience was gained in the application of this technology to improve the removal of dissolved aromatic pollutants and to potentially disengage the polymers produced in the process. Although the defouling process was relatively unsuccessful with the effluent chosen, important methodologies were developed to identify pollutant-enzyme, pollutant-substrate and pollutant-membrane interactions. Valuable knowledge was also obtained about the mechanism and impact of enzyme inhibitors that might be present in the effluent. These techniques can be used to analyse harsh effluents so that enzyme cocktails can be formulated with minimal sensitivity to inhibitors and maximal interaction with pollutants for effective treatment of the effluents with the enzyme membrane system.

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APPENDIX A: TECHNOLOGY ASSESSMENT

Introduction

This research aimed at establishing the suitability of the membrane ultrafiltration technologies in terms of environmental costs/ impacts and in terms of the social context. The TA is also expected to inform research and development of new membrane water treatment technologies, identify alternative methods to achieve the goals of brown water treatment and identify mitigatory measures to reduce or eliminate negative impacts. The process will also identify further studies to remove any uncertainties surrounding the technology impacts, and provide existing information to interested and affected parties.

Research objectives

- To apply chosen TA methodology to the membrane bioreactor systems and determine all associated impacts and their significance;
- To use the results of the TA process to evaluate the competitiveness AND sustainability of the membrane systems against conventional defouling and brown water treatment methods.

Approach to the study

The outline developed for the technology assessment and identification of potential impacts follows the 1992 Integrated Environmental Management guidelines for the assessment of a proposed technology, outlined in the 1998 National Environmental Management Act, NEMA. The method calls for investigation into the possible modification of a technology to mitigate to significantly enhance the positive impacts, to ensure that all social costs are accounted for, and to be sustainable and in the interests of future generations. It emphasises the involvement and notification of the affected communities through community participation, and the use of social impact assessment to identify key players or social groups.

The focal issues for this TA micro assessment were identified as: definition of problem (nature of brown water and its effects on the environment), assessment of the impacts and presentation of results in a clear manner, so as to facilitate a further comprehensive assessment. The assessment was performed using a hybrid method for TA, incorporating the checklist approach and network approach to define the problem and identify inter-related impacts, the Leopold matrix to evaluate impacts and identify major impacts, and the interpretive structural modelling approach to present the results of the TA.

The Enzymatic Defouling System

The costs and stability of the enzymes are the major limitation on this system for use in poor, outlying communities. Installation of the treatment plant would require 20 square metres, although the area required is determined by the capacity of the plant and the costs of acquiring land, as well as the need for supporting technology, such as debris filtration devices. The plants are expected to be transient treatment facilities and to operate for about a year in the communities before conventional treatment plants are installed. The overall lifespan of a plant is estimated at 5 years, when plant is operated at full capacity. The output volume is estimated at 12 cubic metres per day, which is sufficient for farming purposes, or the needs of a community. The system is still in the laboratory-testing phase, an ideal period to reap the benefits from such a technology assessment process

Established Low Pressure UF System

An alternative, comparable system, developed by the Institute of Polymer Science at the University of Stellenbosch, South Africa is already in operation in various outlying communities. This membrane bioreactor does not make use of the dynamic filtration layer; instead clean-in-place methods are used to remove the foulant layer, when flux has been significantly reduced by the formation of a foulant layer. These methods include periodic back flushing where the foulant layer is allowed to accumulate over a 6-12 hour period. The cross flow velocity is maintained at between 0.3 and 1.0 m/s, causing high shear rates, and resulting in gradual removal of the foulant layer. Clean water is required for this process (0.5-5L per module), and is forced back through the membrane under pressure. This process reduces resistance to flow of the product (permeate) caused by the cake or foulant layer. The process of back flushing requires increased energy, as the recirculation pumps are kept in operation throughout to loosen debris and pump the water through a recirculation strainer and back into the feed water tank. In the case of irreversible fouling compounds become adsorbed onto the membrane surface and the pressure in the system reaches an unacceptably high level. Flux is reduced by 35%. A secondary skin of metal ions forms on the UF membranes, especially where the brown water contains high levels of iron. Fungi and algae are trapped in this secondary layer. When the layer is dislodged during back flushing these complexes form tubes in the system, which can be dissolved using a combination of a sequestrant, emulsifying agent and an alkali. In water with higher algal content, it was found that chlor-alkali was more effective. This process was required once every 1-3 weeks of operation.

The chemical mixtures added to the systems in the case of irreversible fouling, are composed of sodium hydroxide, or potassium hydroxide, SLS detergent and EDTA. According to Maartens *et al* (1999), chemical cleaning is expensive due to the costs of the chemical chelators and detergents. The detergents may also alter the membrane retention properties, and may cause membrane damage over time. The chemicals may also be added in excess of the organic foulants, and may then contaminate the feed water, increasing treatment costs. The SLS and EDTA are non-biodegradable and pose a problem in terms of waste treatment.

Assessment and Evaluation and impacts of proposed Treatment Technology

The technologies for treating brown water are expected to impact on labour, population, land, energy, water, and materials and the aquatic ecosystem through stream flow reduction. .

In this case, the stakeholders in the technology were identified as:

- The technology developers
- The associated institutions (Water Research Commission, Rhodes University Biotechnology Department and the Institute of Polymer Science at Stellenbosch University)
- The targeted communities in the Western Cape and Natal
- The municipal water suppliers
- Relevant Government departments involved in some aspect of environmental management, including The Department of Environmental Affairs and Tourism, Department of Water Affairs and Forestry, Department of Land Affairs.
- Peri-urban communities.
- Water service providers

Checklist of potential impacts

The proposed technology will impact on:

1. Level of aquatic humic substances and associated THM potential

2. Colour/turbidity of water
3. Employment opportunities for skilled and unskilled workers
4. Land value
5. Need for government provision of water treatment infrastructure
6. Need for conventional brown water treatment methods
7. Public perception of water resource
8. Human use of water resource
9. Level of infrastructure development in community (pipelines, electricity supply)
10. *Stream flow levels of river*
11. Ability to use chlorination for disinfection
12. Levels of water-related illness

Network of impacts

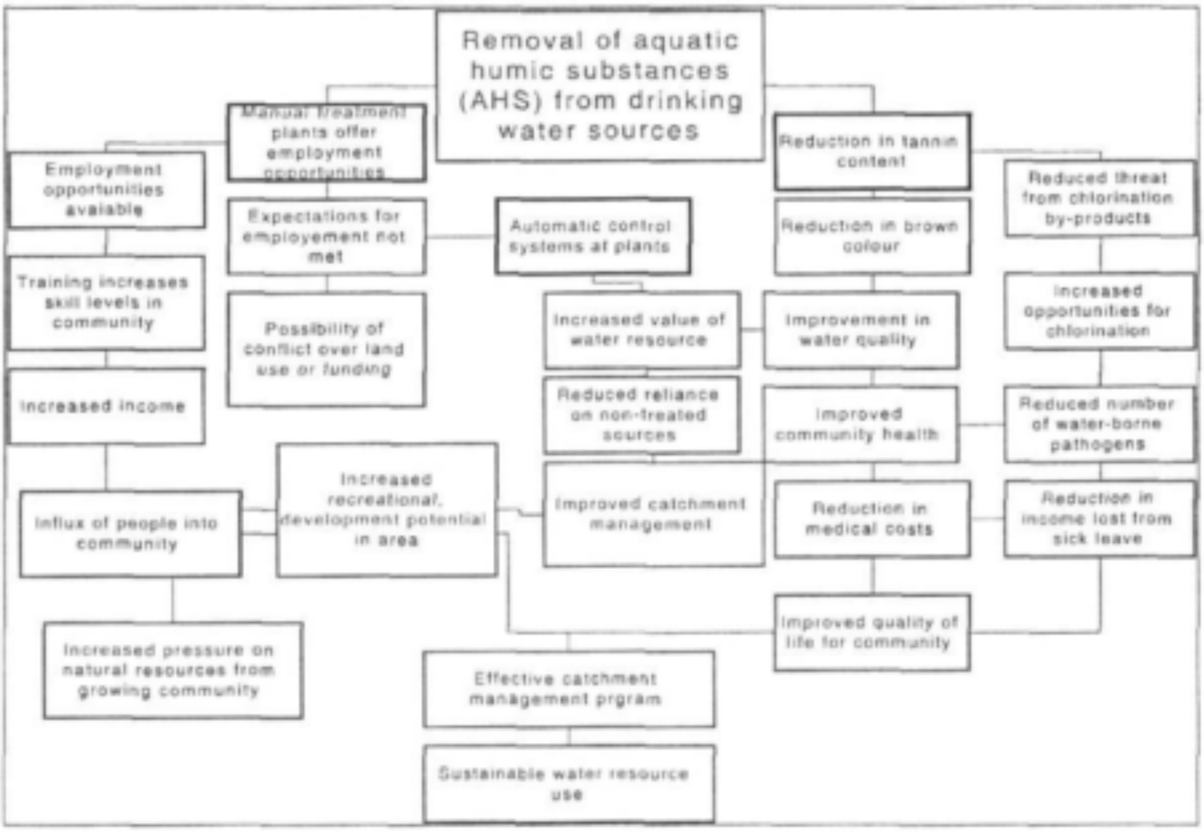

Figure 48: Network analysis of the generalised impacts associated with removal of AHS (aquatic humic substances) from potable water

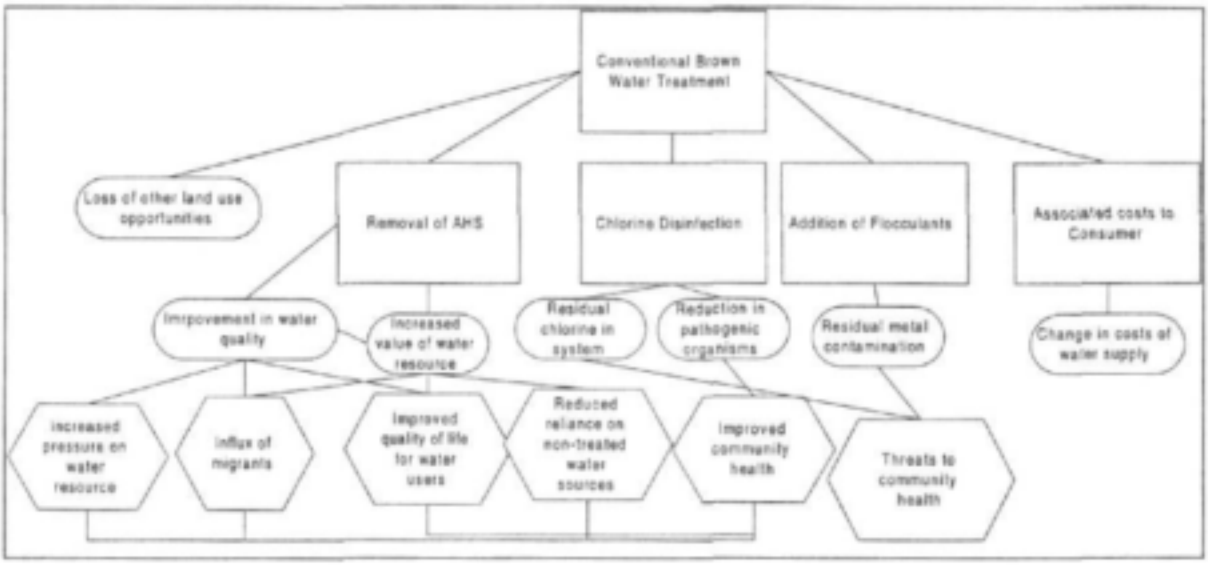

Figure 49: Network analysis of impacts associated with conventional brown water treatment involving addition of metal flocculants and chlorine disinfection.

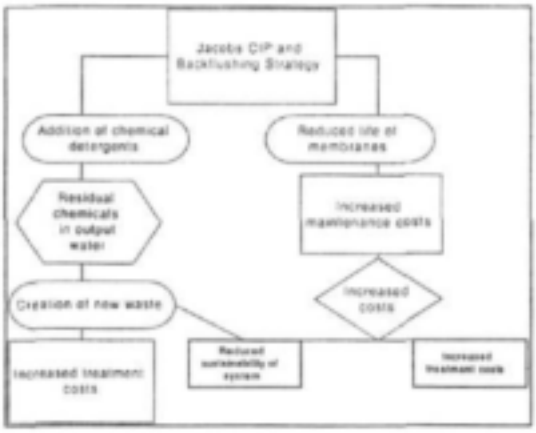

Figure 50: Network analysis of the impacts associated with the use of chemical detergents and backflushing for cleaning in place (CIP) of ultrafiltration membranes during treatment of brown water. (Jacobs et al, 1999)

Table 13: Summary Matrixof the likelihood, benefit and order of impact associated with each brown water treatment technology.

	Likelihood			Benefit			Order of impact		
Impact	CT	UF1	UF2	CT	UF1	UF2	CT	UF1	UF2
Water resource									
Increased value	4	4	4	√	√	√	2nd	2nd	2nd
Improved water quality	3	3	4	√	√	√	1st	1st	1st
Increased pressure from community	3	3	3	X	X	X	3rd	3rd	3rd
Community quality of life									
Improvement in health	3	4	4	√	√	√	4th	4th	4th
Reduction of pathogenic microorganisms	4	3	3	√	√	√	4th	4th	4th
Loss of land use opportunities	4	2	2	X	X	X	1st	1st	1st
Opportunity for employment	3	1	2	√	√	√	2nd	2nd	2nd

Key

CT **Conventional Treatment**

UF1 **Ultrafiltration system: Jacobs *et al*, 1999**

UF2 **Ultrafiltration system: Leukes *et al*, 2000**

In the summary matrix (Table 13), the impacts identified by the checklist and network analysis methods are combined and given numerical values. Each treatment alternative is rated in terms of the likelihood of the impacts, benefits realised by the impacts and order of the impacts. In the likelihood column, the scale ranges from 1-5, with 5 denoting an impact of high likelihood, and 1 denoting an impact of low likelihood. In the benefits column, a tick indicates potential benefit for the community, while a cross indicates an adverse effect. 0 denotes no real effect. In the final column, the impacts are rated according to their order i.e. 1st-5th order, depending on the time scale involved. A 1st order impact is immediately obvious, while lower order impacts are indirect, and may only become apparent much later. The results of the network analyses were used to determine the order of the impacts i.e. the lower order impacts occur lower in the network. An adverse impact of high likelihood is more significant than an adverse impact of low likelihood. In accordance with the literature, a lower order impact carries more significance than higher order impacts (1st order impact is less significant than a 4th order impact)

A 4th order impact carries a significance of 4. Likelihood of 4 gives a score of 4. Thus a 4th order impact of a likelihood of 4, carries a total significance of 8 out of a potential score of 10.

From this significance analysis framework, scores of significance were given to each impact associated with each technology.

Interpretive Structural Modelling (ISM)

From the above impact evaluations, it is possible to create an interpretive structural model of the technologies for brown water treatment. Where the overall impact is beneficial to the community, the score is positive (+) and where the impact is not beneficial, the score is made negative (-).

Table 14: Significance score for the alternative brown water treatment technologies.

	CT	UF1	UF2
Increased value	6	6	6
Improved water quality	4	4	5
Increased pressure from community	-6	-6	-6
Improvement in health	7	8	8
Loss of land use opportunities	-5	-3	-3
Opportunity for employment	2	3	5
TOTAL RELATIVE SIGNIFICANCE	***8***	***12***	***15***

Through the use of the checklist, network analysis and interpretive structural modelling approaches, it was possible to convert essentially qualitative data into quantitative data. The results are however, entirely subjective and are based on the assumptions outlined in the first section of the TA. The use of a comprehensive TA approach and primary operational and economic data could produce a new set of results. This assessment is intended merely to steer future assessments towards impacts of higher significance, and set a framework for such an assessment.

CONCLUSIONS AND RECOMMENDATIONS

Through the use of a combination of existing TA methods, it was possible to investigate the potential impacts of the water treatment technology in relation to conventional treatment methods. The key impacts identified included:

- Impact on the value of the targeted water resource
- Impact on the quality of the potable water in the community
- Increased pressure from the growing community on the water resource
- Improvement in the quality of life on the affected community, through removal of AHS and pathogens
- Opportunities for employment offered by the treatment plants

These key impacts were shared by the various technologies. The most significant impact identified was the improvements to community health associated with treatment of the brown water supplies.

Major Impacts and Mitigation

The use of both membrane technology systems would result in an improvement in the overall water quality, and associated improvements in human health if used in communities while municipal infrastructure is not available.

The objective of community participation should not be to subversively involve people or to force compliance with the needs of the technology developer. In terms of the TA for the water treatment systems, the process should identify whether the community has been involved in the management of their water resources in the past, and should identify any expectations the community have with regards to income or employment opportunities when the water treatment plants are installed. The process should empower the communities to receive benefits form the installation of the technology. Ultimately the goal should be a reconciliation of the needs of both the primary stakeholders: the research and development team and the local communities. Without meaningful local community participation, the technology, no matter how effective or efficient, will be inappropriate and possibly poorly accepted by the communities.

After informal discussion with farmers in the Eastern Cape region, as well as various community leaders, it was found that considerable interest was shown in the potential for job creation resulting from operation of small plants for groups of people, however, familiarity with the technology was limited.

The current system would therefore have to be modified for improved safety, simplicity and effluent handling. It was suggested by the TA team, though, that full end-user participation should be incorporated when pilot plant experience was gained and economic data became available. However, the developers were made aware that further development should be made bearing in mind the competence and aspirations of the end-users.

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Research into polymeric and ceramic-based membranes for use in electromembrane reactors

VM Linkov

The fouling of ion-exchange membranes by large organic anions together with their low chemical, mechanical and thermal stability, impose serious limitations on electromembrane separation processes. Chemical and electrochemical pretreatment techniques are promising means by which to reduce these problems. A novel procedure for the coating of standard (Ionics) polymeric-electrodialysis membranes was developed by the project team in conjunction with Eskom. This coating renders the membranes more resistant to fouling by organic material. The modified membranes will be very useful in applications where organic material is present together with inorganic salts, such as the situation that exists in many industrial water and effluents from Sasol, Eskom and others. As another product from the project, a significant step was taken toward the development of ceramic membranes possessing conductive and catalytic properties for the oxidation of unwanted organic material. These properties of ceramic membranes, together with their high stability in aggressive media, allow their potential use for plating-effluent treatment, extraction of non-ferrous metals by electrodialysis, treatment of mine waters, electrochemical synthesis of acids and alkalis, and for the preparation of sodium hypochlorite disinfectant.

Report Number 844/1/99 **ISBN 1 86845 539 4**

The development of characterising and cleaning techniques to classify foulants and to remove them from ultra- and microfiltration membranes by biochemical means.

P Swart, A Maartens, AC Swart and EP Jacobs

The main objectives of this research project were the identification and classification of membrane foulants occurring in abattoir, wool-scouring and other industrial effluents. Two further aims were the development of methods for the biological removal of such foulants from membrane surfaces and the cloning and large-scale preparation of specialised enzymes to degrade specific foulants.

Proteins and lipids were found to be the main foulants in abattoir effluents, whilst proteins and waxes were predominant foulants in wool-scouring effluents. Lipases and esterases were successful enzymes for the removal of foulants from abattoir and wool-scouring effluents, respectively, and restored the fluxes in fouled membranes.

These promising results indicated that enzyme-based biological cleaning regimes hold great promise for the restoration of fouled ultrafiltration membranes.

Report Number 531/1/96 **ISBN 1 86845 209 3**

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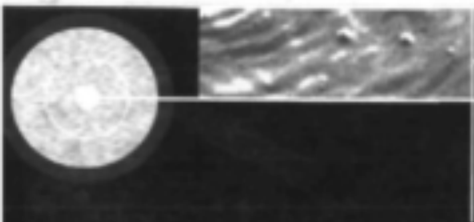

Water Research Commission
Private Bag X03, Gezina, 0031, South Africa
Tel: +27 12 330 0340, Fax: +27 12 331 2565
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