THE DEVELOPMENT AND IMPLEMENTATION OF BIOLOGICAL CLEANING TECHNIQUES FOR ULTRAFILTRATION AND REVERSE OSMOSIS MEMBRANES FOULED BY ORGANIC SUBSTANCES

Final Report to the

Water Research Commission

by

P Swart[1], A Maartens[1], J Engelbrecht[1], Z Allie[1]
and EP Jacobs[2]

[1]Department of Biochemistry and
the [2]Institute for Polymer Science, University of Stellenbosch
Private Bag X1, Matieland, 7600

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EXECUTIVE SUMMARY

Background and Motivation

The use of pressure-driven cross-flow filtration processes such as reverse osmosis (RO), ultrafiltration (UF) and microfiltration (MF) for the purification of industrial effluents, has gained enormous popularity in the past decade. The ease of incorporation of these systems into existing plants and the high quality of water that can be obtained by pressure-driven filtration processes are the most outstanding features of this modern technology. In addition to industrial applications, the use of polymer based membrane filters for the supply of potable water on a small, yet economical scale from eutrophic surface waters, is attractive.

The most challenging obstacle to full implementation of modern pressure-driven filtration processes, apart from the production of chemically inert high-efficiency membranes, is the fouling phenomenon, an inherent consequence of any high pressure effluent or polluted water filtration process. Fouling, characterised by the irreversible adsorption of foulants onto the membrane surface, causes a rapid decline of flux through the membrane, (productivity loss) which can be reversed only by effective foulant removal. The problem of fouling becomes more acute when high pressure filtration processes are applied to effluents of biologically related industrial processes such as in the abattoir and paper and pulp industries, which produce large amounts of hydrophobic foulants with a large affinity for the typically non-polar UF and MF membranes.

The classical approach to foulant removal in the past has been the use of harsh chemical treatment procedures in combination with mechanical cleaning operations. The repeated use of physical and chemical foulant-removal techniques, however, drastically shortens membrane life in systems used in high organic foulant producing industries. In addition the traditional chemical cleaning regimes such as chlorine and alkaline treatment, have added significantly to industrial pollution. These factors have significantly curtailed the implementation of membrane technology on a large scale.

In recent years most research have been directed towards membrane and membrane module development while comparatively little research has gone into solving and preventing the problem of fouling. Milder and environmentally more friendly cleaning regimes are, however, available for the removal of biologically derived foulants from polymer membranes. The use of enzymes, in combination with biodegradable detergents, is an attractive alternative to the classical cleaning regimes used to date. Previous studies by the membrane fouling research group at the department of Biochemistry at the University of Stellenbosch, on abattoir and wool scouring effluents, have shown that enzymes, as biocatalysts, can be used effectively in combination with detergents to reduce fouling and restore flux through previously fouled membranes (Jacobs *et al.*, 1992; [a]Maartens *et al.*, 1996; [b]Maartens *et al.*, 1996; Maartens *et al.*, 1998). Foulant characterisation, however, is of the utmost importance for any membrane cleaning regime to be efficient and economical. Most researchers agree that foulant characterisation in effluent and on fouled membranes is one of the most important areas of research to be focused on in order to reduce fouling and to make UF a feasible alternative for the large-scale purification of industrial effluents. This view was recently echoed by the majority of delegates at a South African conference on membrane technology (MTD Membrane Technology Division Conference, October 1997, Badplaas, South Africa).

The investigation described in this report follows on a previous WRC project (WRC No. 531) in which characterisation and enzyme-based cleaning techniques were developed using abattoir and wool-scouring effluents as model systems. That study showed that enzymes could be effectively applied for the removal of organic foulants from membranes fouled by these effluents. This report deals with extensions of the previous study. Methods of foulant characterisation, developed in the previous investigation, were applied and further developed for membranes fouled by Cape brown water and by the effluent from the paper and pulp mills at Piet Retief. Cleaning methods as well as membrane pretreatment procedures were subsequently developed that increased flux without damaging the membranes. These methods are now available for implementation. In addition the most efficient enzymes (proteases and lipases) for foulant removal from abattoir effluent fouled membranes were identified and used to establish the most efficient membrane cleaning regime for these membranes under operational conditions.

Objectives

The original goals for this project were:

1. The implementation of enzyme-based cleaning regimes at an abattoir pilot plant to refine cleaning techniques for implementation in the industry.
2. Characterisation of foulants in paper and pulp effluent using established techniques as reference.
3. Identification of enzymes or detergents that will remove fouling agents (characterised in 2). This would be preceded by a literature survey to investigate current cleaning techniques.
4. Cloning and production of genes that code for enzymes which could remove foulants (abattoir and paper and pulp) for expression in *Eschericia coli* or *Saccharomyces cereviseae.* These organisms would be used for subsequent large-scale enzyme production.
5. Implementation of biological cleaning techniques on membranes fouled in the paper and pulp industry.

At the first Steering Committee meeting of the project it was decided to change the original objectives of the project. It was decided that the paper and pulp effluent should be given priority over the abattoir effluent and that Cape brown water should be included as an important part of the project. These changes were necessitated for the following reasons:

Abacor had decided not to go ahead with the introduction of a pilot UF plant on abattoir effluent in Port Elizabeth, and although the abattoir would still serve as an important model for fouling studies, its priority for practical implementation was not as high as in the past.

The Institute of Polymer Science at the University of Stellenbosch was running a pilot plant on Cape brown water at Mon Villa and the installation of a pilot plant at Suurbraak was imminent. These applications made the characterisation of the foulants in Cape brown water and their removal from fouled membranes a priority.

Envig (Pty) Ltd, involved in the manufacture and installation of the membrane plant, reported that there were fouling problems at Mondi's Piet Retief plant and requested that the paper and pulp effluent characterisation and foulant removal be given priority.

The new objectives established by the steering committee were therefore:

1. A characterisation of foulants in Cape brown water and an investigation of the fouling potential of this water in UF systems.
2. A characterisation of foulants in paper and pulp effluent from Mondi's the Piet Retief plant and the development of environmentally friendly cleaning techniques for membranes fouled in this effluent.
3. The identification of the most suitable enzymes (proteases and lipases) for the cleaning of UF membranes fouled in abattoir effluent and the formulation of cleaning regimes that could be tested on pilot scale.

Results and Conclusions

Cape Brown Water

Brown water from the municipal reservoir at Suurbraak were analysed before and after ultrafiltration using UV-spectroscopy, electrospray mass spectrometry and total organic carbon. Of these techniques UV-spectroscopy proved to be the most practical and reliable method of analysis, especially at remote locations and water sources. In addition, membranes fouled during ultrafiltration of this water, were examined for adsorbed foulants and the effect of fouling on flux was investigated. Fouled membranes were subsequently cleaned with various techniques and the cleaning regimes were evaluated. Finally membranes as well as the feed water was pre-treated to reduce fouling. The following conclusions could be drawn from the results obtained.

Feed characterisation: Brown water from Suurbraak absorbed light strongly in the UV-range. This characteristic was used to measure the concentration of natural organic matter that gives rise to the brownness of the water colour. In addition, the results were compared with UV-analyses of commercial humic acid. The ratio of the absorbance at 465 and 656 nm (E_4/E_6) was used to estimate the relative sizes of the molecules that constitute natural organic matter in Cape brown water.

Dynamic membrane fouling: During the filtration of natural brown water both the operational and pure-water fluxes declined due to the irreversible adsorption of substances from the water solutions onto/into the membranes. This type of adsorption necessitated the development of effective cleaning strategies that would restore the transmembrane flux without membrane damage.

Characterisation of product water: The use of ultraviolet-visible spectrophotometry, to monitor the changes in the permeate quality, proved to be of great value. It provided an effective and facile method to monitor water quality for this specific brown water and membrane system.

Membrane cleaning: The pure-water flux through membranes fouled by natural organic matter in the brown water, could be restored by the use of alkaline solutions and detergents such as Triton X100. The colour rejection of the membranes was, however, negatively influenced by these cleaning trials. After cleaning, the membranes had a more open structure that permitted larger molecules to pass through them. This effect of the cleaning solutions was, however, temporary, and during the first hour of prolonged filtration the original membrane retention properties were restored. These trials showed that colour removal from Cape brown water with capillary polysulphone membranes was effective and that the process can be operated successfully and economically with the right choice of membrane configuration and operating and cleaning conditions.

Fouling prevention: Membrane fouling could be reduced, but not prevented, by the adjustment of the pH of the feed solution. Water pre-treatment with metal-ions to reduce the concentration of natural organic matter in the feed solutions, did not prevent foulant adsorption. On the contrary, results showed that metal-ion-treated feed-water caused more extensive fouling than untreated feed. Pre-coating of membranes with Triton® X100 and Pluronic® RPE both reduced foulant adsorption, but influenced the colour retention and flux of water through the membranes. Triton® X100 reduced fouling slightly, increased flux, but had a negative effect on colour retention. Pluronic® RPE treatment reduced fouling significantly, reduced flux and improved colour rejection. These results clearly showed that membrane pre-treatment with a hydrophilic polymer such as Pluronic ® RPE, was the most effective method of preventing fouling of polysulphone membranes fouled during the ultrafiltration of Cape Brown Water.

Paper and Pulp Effluent

In this study compounds that adsorbed onto polysulphone membranes, used to treat paper and pulp effluent were partially characterised. After the nature of these compounds had been established, a choice was made of enzymes that might remove these compounds from the membrane surface in order to reduce fouling. Subsequently, a system was developed to foul polysulphone membranes statically under controlled conditions. The resulting fouled membranes were treated with the chosen enzyme preparations. Ligninase and pectinase preparations proved to be the most effective in remaining the polyphenolic foulants in these experiments. This preliminary study paved the way for the evaluation of these enzymes in a continuous pilot plant system for cleaning tubular membranes fouled in real paper and pulp effluent under operational conditions.

Under dynamic conditions the pure-water flux was reduced by 53 % over a 20 h period, indicating irreversible fouling. Early evidence of irreversible fouling could not be detected by monitoring only the product flux. Pretreatment of the membranes with Pluronic® RPE decreased the flux of pure-water through the membrane, but prevented or reduced the extent of foulant adsorption onto the membranes. The product flux through coated membranes was in the same order as that of an untreated membrane. Chemical cleaning in combination with mechanical cleaning could restore the flux to values above those of unfouled membranes. Pluronic® RPE coated membranes were more easily cleaned with Triton® X100, but the coating material was also removed during these cleaning trials. The results show that precoating of polysulphone ultrafiltration membranes with a non-covalent surface modifier could reduce fouling significantly and facilitate foulant removal after extended filtration periods. Future research will have to focus on a search for ideal precoating agents and cleaning regimes that could be used in conjunction with these agents.

None of the enzymes evaluated in flat-sheet trials increased the pure-water flux more efficiently than Triton® X100 did in dynamic studies. This phenomenon, which will need further investigation, was possibly caused by denaturation of the enzymes during recirculation over fouled membranes.

Abattoir effluent

Previous investigations showed that proteins and lipids are the major foulants that adsorb onto polysulphone membranes fouled during treatment of abattoir effluent by ultrafiltration. These foulants severely reduce the effectiveness of ultrafiltration in abattoir effluent purification and therefore necessitated the development of effective enzyme-based cleaning protocols. Enzymes specific for lipid and protein removal (lipases and proteases) were evaluated in membrane cleaning trials. The enzymes (three lipases and three proteases) were selected on the basis of their activity, which was determined by standard methods. Cleaning with lipases alone effectively reduced the amount of membrane foulants (proteins and lipids) adsorbed onto the membranes. A combination of lipases with proteases was, however, more effective than lipases alone. After enzymatic cleaning an increase in pure-water flux through the cleaned membranes was obtained. This flux increase correlated well with a reduction in the protein and lipid adsorbed onto the membranes.

The results obtained in this study show that enzymes are effective as cleaning agents for ultrafiltration membranes fouled in abattoir effluent. It is, however, doubtful if raw abattoir effluent directly can be ultrafiltered and pre-treatment of effluent should be investigated. If ultrafiltration is used to purify the effluent, the enzyme-cleaning protocols developed in this study wil play an important role in combination with pulsatile and mechanical cleaning methods.

Recommendations for future research

After five years of study of membrane fouling which included foulant removal and fouling prevention, it is apparent that research and development efforts in this area of membrane research is very much problem-driven. Even though it is inevitable that membranes will foul during modern pressure-driven filtration processes, research is carried out only after the fouling problem has arisen. Like membrane development, fouling prevention should be investigated and developed to obtain general skills in foulant removal from membranes as well as fouling prevention. In this regard important areas for future research will be:

1. Identifying, characterising and quantifying possible foulants in water sources

2. Developing methods for identifying, characterising and quantifying of foulants adsorbed onto membranes

3. Developing non-covalent membrane pre-treatment strategies for minimising fouling and prolonging membrane life

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The steering committee responsible for this project consisted of the following persons:

Drs. H. Saayman and G. Offringa	Water Research Commission (Chairpersons)
Dr. J.J Schoeman	CSIR/ Environmentek
Dr. J. Duncan	Dept. Microbiology and Biochemistry Rhodes University
Dr. E.P. Jacobs	Institute of Polymer Science, University of Stellenbosch
Mr. N.K.H. Strohwald	ENVIG (Pty)LTD, Paarl

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INTRODUCTION

The past two decades have seen a significant increase in the use of modern pressure driven membrane filtering processes for the purification of industrial effluents with a high organic content. These systems are readily incorporated into existing plants and the high quality of water that can be obtained by pressure driven filtration processes is an outstanding feature of this modern technology. Development through its application in industrial effluent streams, has also made polymer based membrane filters an attractive option for the supply of potable water on a small, yet economical scale from eutrophic surface waters.

The fouling phenomenon, an inherent consequence of any high pressure effluent or polluted water filtration process, is currently one of the most challenging obstacles in the way of full implementation of modern pressure driven membrane filtration processes. Fouling, characterised by the irreversible adsorption of foulants onto the membrane surface, causes a rapid flux decline through the membrane (productivity loss) which can be reversed only by effective foulant desorption from the membrane surface. The problem of fouling becomes more acute when high pressure filtration processes are applied to effluents of biologically related industries such as the abattoir and paper and pulp industries. These industries produce large amounts of hydrophobic foulants with a large affinity for the typically non-polar ultrafiltration and microfiltration membranes.

Harsh chemical treatment procedures, in combination with mechanical cleaning techniques, have in the past been the standard cleaning regimes used for foulant removal from fouled membranes. The repeated use of these foulant removal techniques, however, drastically shortens membrane life. In addition the traditional chemical cleaning regimes (chlorine and alkaline treatment to name two) add significantly to the industrial pollution process. These factors have significantly curtailed the implementation of membrane filtration technology on a large scale.

In recent years most research has been directed towards membrane and membrane module development while comparatively little research effort went into fouling and fouling prevention. Milder and environmentally more friendly cleaning regimes are, however, available for the removal of biologically derived foulants from polymer membranes. The use of enzymes, in combination with biodegradable detergents, is an attractive alternative to the classical cleaning regimes used to date. Previous studies by this group, on abattoir and wool-scouring effluents, have shown that enzymes, as biocatalysts, can be used effectively in combination with detergents to selectively reduce fouling and restore flux through previously fouled membranes (Jacobs *et al.*, 1992; [a]Maartens *et al.*, 1996; [b]Maartens *et al.*, 1996; Maartens *et al.*, 1998). Foulant characterisation, however, is of utmost importance for any membrane cleaning regime to be efficient and economical.

This project continues on a previous WRC project (WRC No. 531) in which characterisation and enzyme based cleaning techniques were developed using abattoir and wool scouring effluents as model systems. The study showed that enzymes were effective for the removal of organic foulants from membranes fouled in these effluents. This report deals with the extensions of the previous study.

The goals of this investigation were:

1. Characterisation of foulants in Cape brown water and an investigation of the fouling potential of this water in ultrafiltration systems.
2. Characterisation of foulants in paper and pulp effluent from Mondi's the Piet Retief plant and the development of environmentally friendly cleaning techniques for membranes fouled in this effluent.
3. Identification of the most suitable enzymes (proteases and lipases) for the cleaning of ultrafiltration membranes fouled in abattoir effluent and the formulation of cleaning regimes that could be tested on pilot scale.

In this study methods of foulant characterisation, developed in the previous investigation, were applied and further developed for membranes fouled in Cape brown water and the effluent from the paper and pulp mills at Piet Retief. Cleaning methods as well as membrane pre-treatment procedures were subsequently developed that could increase flux without damaging the membranes. These methods are now available for implementation. In addition the most efficient enzymes (proteases and lipases) for foulant removal from abattoir effluent fouled membranes were identified and used to establish the most efficient membrane cleaning regime for these membranes under operational conditions.

This report is divided into three parts. Part one deals with the characterisation and quantification of membrane foulants in Cape brown water, the development of techniques for the removal of these foulants, an investigation of fouling prevention methods and the evaluation of these processes. In Part two an investigation of organic foulants in effluent from the Mondi Paper and Pulp Mills at Piet Retief is described. Static and dynamic fouling of polysulphone membranes were carried out, adsorbed foulants were characterised and enzymatic as well as mild chemical and mechanical cleaning systems were evaluated. Part three describes the use of specialised enzyme mixtures for the removal of proteins and lipids from membranes fouled in abattoir effluent.

CAPE BROWN WATER

1.1 INTRODUCTION

Natural organic matter represent a major portion of organic pollution of natural surface waters. It does not have a precisely defined structure, and the composition is dependent on the nature of the natural material that was decomposed in its formation (Stárek *et al.*, 1994). Only about 20% of the dissolved organic carbon consists of identifiable compounds that include carbohydrates, carboxylic acids, amino acids and hydrocarbons. Natural organic matter, consisting of complex environmentally altered plant residues of bacterial and fungal origin, represent the remaining 80% of the dissolved organic carbon of natural waters (Higgo *et al.*, 1993; Jucker and Clark, 1994 and Leenheer, 1994). This natural organic matter comprises polyphenolic molecules with a moderate molecular mass (500-50000 dalton) that give rise to the colour in Cape brown water. The colour is not only aesthetically undesirable, but polyphenolic molecules complex with heavy metals and pesticides (Senesi, 1992; Banks and Parkinson, 1992; Stefanova *et al.*, 1993; Österberg *et al.*, 1993; Leenheer, 1994). In addition, natural organic matter is considered to be the precursor of organic halogenated compounds (potential carcinogenic substances) formed by chlorination in the disinfection of drinking water (Agui *et al.*, 1992). Furthermore, ozonation, another disinfection technique, can also cause some pesticides or heavy metals formerly bound in humate complexes, to be released (Stárek *et al.*, 1994). Against this background, studies on the removal of natural organic matter from water have been energetically carried out in order to produce safer drinking water. A number of studies have been reported on the removal of natural organic matter from water (Zau and Banks, 1992; Higgo *et al.*, 1993; Jucker and Clark, 1994). Before colour-producing natural organic matter can be efficiently removed from coloured water, these substances should be characterised and quantified to determine the efficiency of the removal technique(s). This is a complicated task because the composition of natural organic matter depends on the nature of the natural material that was decomposed during its formation (Higgo *et al.*, 1993; Leenheer, 1994; Jucker and Clark, 1994; and Stárek *et al.*, 1994).

1.1.1 CHARACTERISATION OF ORGANIC CARBON IN AQUEOUS SYSTEMS

There are a number of techniques by which to characterise organic carbon, in this case the colour producing humic substances (natural organic matter), in water. Dissolved organic carbon or total organic carbon, are among the most important components by which organic matter in natural and human influenced aquatic systems is characterised. The chemical oxidizability and the biological degradability of these components may also be used to assess the organic content of natural water sources. Due to the difficulty involved in the direct measurement of aquatic humus, limnologists and oceanographers have used assays that measure properties of the humic substances instead, such as colour, phenol content, or tannin and lignin content (Guthbert and del Giorgio, 1992).

Unsaturated compounds (substances with delocalized electrons) usually impart a distinct colour to the contaminated waters and in this case ultraviolet-visible light spectroscopy and fluorescence are also used for the detection of the organic carbon content of these waters (Huber and Frimmel, 1992). The ease of use and relative low cost of ultraviolet-visible light spectroscopy make this technique attractive to study the natural organic matter in the feed water and permeate during ultrafiltration of naturally occurring brown water. Previous results have shown that a correlation exists between the ultraviolet-visible light-absorbance and the dissolved organic carbon in water with a high natural organic matter content (Huber and Frimmel, 1992). Size exclusion chromatography, followed by ultraviolet-visible light detection at 210 nm, showed that an increase in ultraviolet light absorption indicated an increase in the dissolved organic carbon content.

Baes and Bloom, (1990) showed that, for fulvic acid up to a concentration of 80 mg/l at pH 5, a linear relationship existed between absorbance and concentration of fulvic acid in solution at 260 and 280 nm and that the Beer-Lambert law was obeyed under these conditions. This fact is of great value in determining the concentration of humic acid or fulvic acid in solution. Jucker and Clark, (1994) used calibration curves based on the ultra-violet absorbance at 254 nm, total organic carbon and fluorescence measurements to determine natural organic matter concentrations. Stárek *et al.* (1994) used spectrophotometric methods in the ultra-violet region at 254 and 280 nm to determine humic acid concentrations. It was, however, observed that the ultraviolet-visible light spectra of natural organic matter in aqueous solutions are pH dependent and that the pH of the solution under investigation should be the same for comparative studies. This was due to the ionisation of carboxyl groups and that the relevant chromophoric systems were -OH-, and -COOH-substituted benzene rings. Additional information from ultraviolet-visible light spectroscopy can be obtained by measuring the ratio between the absorbance at 465 nm and 656 nm (E_4/E_6 ratio).

E_4/E_6 ratios: The E_4/E_6 ratio of dilute, aqueous humic acid and fulvic acid solutions is widely used to characterise these materials in solution. Chen *et al.* (1977) stated that the E_4/E_6 ratios of humic and fulvic acid solutions are mainly governed by the particle size (or particle molecular mass). The ratio is affected by pH, correlates with the free radical concentration, O, C and the CO_2H content as well as the total acidity of the sample. The ratio of absorbances at 465 and 665 nm therefore provides an indication of molecular size and the degree of chromophore conjunction or intramolecular aggregation (Gressel *et al.*, 1995). In addition, the E_4/E_6 ratio is independent of the concentration of the humic acid and fulvic acid in the solution (Baes and Bloom, 1990) even though the absorbances of the humic and fulvic acids at 465 and 656 nm are concentration dependent. A low E_4/E_6 ratio indicates a relatively high degree of condensation of aromatic humic constituents. Conversely, a high E_4/E_6 ratio reflects a low degree of aromatic condensation and implies the presence of relatively large quantities of aliphatic structures. High molecular mass fractions (large molecules) are therefore associated with a relatively low E_4/E_6 ratio. These molecules have a high C-content, but a relatively low number of O-, and $-CO_2H$-groups and subsequently a low total acidity. Lower molecular mass fractions (smaller molecules), give higher E_4/E_6 ratios. Such molecules are smaller, contain less C, but more O- and $-CO_2H$-groups and their total acidity is higher (Chen *et al.*, 1977). The E_4/E_6 ratios are especially sensitive to changes in pH, apparently because the size and conformation of the molecules are influenced by the pH. Fulvic acid particles, for instance, become bigger as the pH is raised to approximately 7. This increase in particle size is reflected by decreases in the E_4/E_6 ratios.

Information gained from the literature provided the background for establishing methods by which to determine the concentrations of natural organic matter in brown water solutions. These techniques could be used to determine and monitor the performance of any colour removal protocol including ultrafiltration.

1.1.2 COLOUR REMOVAL FROM BROWN WATER

There is an increasing need for effective alternatives to the current colour removal technology. Methods that are currently being used for colour removal from drinking water include:

1. Coagulation using aluminium salts. This is the most common method used by the water industry to remove colour and turbidity from water. The process has, however, certain disadvantages such as problems with dosing automation, the high amounts of coagulant sludge that are produced and the health risk of the aluminium salt itself (Zhou and Banks, 1992).
2. Oxidation by chlorine and ozone.
3. Adsorption by activated carbon. This technique has only limited effectiveness for the removal of humic acids (Zhou and Banks, 1992 and Stárek *et al.*, 1994).
4. Biosorption to immobilised fungi, such as *Rhizopus arrhizus.* This method involves the binding of humic acid to the fungal cell wall, most probably to the chitin/chitosan components, mediated by the presence of a positively-charged bridging ion. (Banks and Parkinson, 1992; Zau and Banks, 1992).
5. Ultrafiltration, nanofiltration and reverse osmosis. These are three of the more modern techniques used to remove natural organic matter from coloured water (Jucker and Clark, 1994 Agui *et al.*, 1992).

The aim of this study was to develop techniques to characterise natural organic matter in brown water and to use these techniques to evaluate the performance of ultrafiltration as a technique for removing colour from Cape brown water. Further discussions will therefore concentrate on colour removal by means of ultrafiltration membranes.

During the ultrafiltration of natural waters for general drinking purposes, flux normally declines with time because of: (a) accumulation of solute near the membrane surface (gel polarisation), (b) gradual irreversible changes to the polarised layer (such as cake formation) and (c) adsorption. Flux decline can therefore be divided into a reversible and an irreversible component. Reversible flux decline can be recovered by backwash techniques whereas the irreversible flux decline cannot. Specialised cleaning techniques are required to remove foulants irreversibly adsorbed onto the membrane surface (Jucker and Clark, 1994). In addition, if the adsorption process of natural organic matter is understood, preventative measures can be combined with cleaning agents to reduce or prevent fouling.

In this study naturally occurring brown water and commercial humic acid, characterised by ultraviolet-visible light spectroscopy, were ultrafiltered to determine the efficiency of ultrafiltration in retaining colour from natural brown water. Standard curves, obtained by ultraviolet-visible light measurements of natural organic matter and commercial humic acid samples, were used to determine the concentration of organic substances in the feed and in the permeate solutions. The natural organic matter concentrations were measured continuously and the results were used to determine the extent of membrane fouling and the efficiency of colour-removal techniques. These values were compared with standard Hazen colour measurements. The E_4/E_6 ratios, a measure of molecular size for humic substances, were used to monitor changes in the relative sizes of the molecules in the permeate and in the feed solutions. As a final measure, the magnitude and effects of membrane fouling were determined by determining the flux of water through the membranes.

Fouled membranes were subsequently cleaned with alkaline agents normally used to extract natural organic matter from soil samples. The cleaning efficiency and the effect of these cleaning solutions on the membrane selectivity were also monitored by the methods referred to above.

The effects of adsorbed substances and the amount of material adsorbed, can be minimised in two ways namely, effective chemical cleaning of membranes or water and/or membrane pre-treatment. Chemical cleaning agents are usually expensive, influence the retention properties of the membranes and can lead to severe membrane damage after lengthy use. The second approach, to reduce or prevent the adsorption of foulants from natural brown water onto ultrafiltration membranes, will reduce the frequency of scabrous chemical cleaning and prolong membrane life.

Previous studies have shown that the adsorption of natural organic matter onto ultrafiltration membranes is influenced by factors such as the solubility (dependent on pH), ionic strength, concentration of natural organic matter in the feed water and the membrane properties (Agui *et al.*, 1992; Jucker and Clark, 1994).

Natural organic matter is a mixture of complex, heterogeneous molecules which are normally defined on the basis of solubility. The mixture includes humin, humic acid and fulvic acid. Humin is the fraction not soluble in water at any pH, humic acid is not soluble under acidic conditions (precipitates at pH < 2), but becomes soluble at a higher pH and fulvic acid is soluble at all pH conditions (Leenheer, 1994). The division between humic and fulvic acid, however, is not precise and for each there will be a solubility spectrum (Kipton *et al.*, 1992; Hiraide, 1992). The solubility of natural organic matter is influenced by the pH of the solution. The solubility in turn influences the adsorptive properties of natural organic matter during ultrafiltration. When solubility is at a minimum, adsorption will occur readily, but there will be less adsorption when the natural organic matter is more soluble. Given the pH-dependence of the solubility of natural organic matter, it can therefore be assumed that, at a certain pH, solubility will be high and membrane fouling will be minimal. In addition to the solubility, the pH of the solution also influences the charge on the molecules in natural organic matter. Natural organic matter becomes less negatively charged as the pH decreases resulting in the reduction of intermolecular repulsion as well as a reduction in the repulsion between the negatively charged membranes and the natural organic matter molecules thereby increasing adsorption (Jucker and Clark, 1994).

Because of the large number of functional groups on humic acid and fulvic acid molecules the concentration of natural organic matter can be reduced by interaction with coagulants such as metal-ions (Jucker and Clark, 1994). Therefore pre-flocculation with metal-ions appears to be an effective method for reducing adsorption of natural organic matter onto membranes.

Membrane surface properties are the last factors that influence membrane fouling. Membranes with a lower contact angle (more hydrophilic) have been found to be more resistant to fouling by natural organic matter (Jucker and Clark, 1994). The most suitable membrane for an aqueous feed would be hydrophilic and homogeneously permeable (Trägårdh, 1989). Hydrophilic membranes, however, do not have thermal stability and are susceptible to chemical degradation. Membranes used for most ultrafiltration processes are therefore usually hydrophobic (Toyomoto and Higuchi, 1992) and therefor more susceptible to the adsorption of foulants. Fane and Fell, (1987) showed that foulant adsorption of hydrophobic membranes can be reduced by forming a hydrophilic monolayer (so-called Langmuir-Blodgett layer) on the membrane surface. This monolayer makes membranes hydrophilic with fewer hydrophobic sites for foulant adhesion (Fane and Fell, 1987).

In this study the effect of feed-water and membrane pre-treatment on the adsorptive behaviour of natural organic matter was investigated under dynamic conditions. The pH of the feed-water was adjusted prior to filtration; coagulants were introduced into the feed-water mixture 24 h before filtration; and membranes were pre coated with hydrophilic agents. Characterisation techniques modified from Jucker and Clarke, (1994) were used to determine the magnitude of natural organic matter adsorption. To study the effects induced on the permeability of capillary ultrafiltration membranes, changes in the pure-water and product fluxes was measured. The quality of the resultant permeate solutions, after the different pre-treatment conditions, was monitored by ultraviolet-visible light spectroscopy. These data provided important information for future ultrafiltration treatment of natural brown water.

1.2 EXPERIMENTAL

Materials. All chemicals and cleaning agents used were obtained from BDH Chemicals Ltd., Poole, UK, unless otherwise stated. Ultraviolet-visible light spectra were recorded with a Beckman DU640 spectrophotometer, analytical grade water was used as a reference for all water samples. commercial humic acid was obtained from Fluka, Chemika, Switzerland.

Natural brown water, used in characterisation and filtration experiments, was collected at Suurbraak in the Southern Cape region of South Africa. After measurements of pH, samples were preserved in dark containers with sodium azide (1mg/l) to prevent alteration to the chemical character of organic matter by ultraviolet radiation or micro-organism growth. The pH of water samples was adjusted with HCl or NaOH or NH_4OH (27% NH_3^+) as required. A typical example of South Cape brown-water are summarised in Table 1.1.

Table 1.1 Typical water analyses of coloured water from the South Cape (Swartz and de Villiers).

Variable	Values
Colour (apparent) mg/l as Pt	**160 - 650**
Turbidity (NTU)	**2 - 400**
Conductivity (mS/m)	**8 - 70**
pH	**4.1 - 6.5**
Total alkalinity (mg/l as $CaCO_3$	**2 - 28**
Magnesium (mg/l as Mg)	**4 - 30**
Total hardness (mg/l as $CaCO_3$)	**8 - 64**
Iron (mg/l as Fe)	**0.46 - 1.42**
Manganese (mg/l as Mn)	**0.07 - 0.13**
Aluminium (mg/l as Al)	**0.04 - 0.16**

Capillary membranes, spun from a solution of Udel P3500 polysulphone in *N*-methyl-2-pyrrolidone and dried with glycerol at room temperature were used in filtration experiments. The ultrafiltration module comprised ten capillary polysulphone membranes from the same batch in a feed-and-bleed system with a feed capacity of 25l. The operating pressure was 150 kPa and the feed solution temperature was 20°C unless otherwise mentioned. A constant linear flow velocity was maintained for all experiments. Experiments were done in triplicate and the results are presented as the mathematical means of all determinations ±SD.

1.2.1 ULTRAVIOLET-VISIBLE LIGHT SPECTROSCOPY OF NATURAL BROWN WATER AND COMMERCIAL HUMIC ACID SOLUTIONS

The spectrophotometric characteristics of natural brown water and commercial humic acid solutions were determined at wavelengths between 200-800 nm. To minimise the effect of pH on the ultraviolet-visible light spectroscopic character of natural organic matter and to ensure total solubility of the natural organic matter particles, the pH of all samples was adjusted to 8 before analyses. For both the natural brown water and commercial humic acid samples, E_4/E_6 ratios were calculated from absorption values measured at 465 and 656 nm.

Baes and Bloom (1989) showed that there is a linear relationship between the concentration of humic substances in solution and the ultraviolet-visible-absorbance at 254 nm of the solutions. The relationship between the natural organic matter concentration in Suurbraak natural brown water and commercial humic acid samples, and the ultraviolet-visible light absorbance at 254 nm was subsequently determined. Natural brown water samples were lyophilised and 80 mg of the resultant powder was dissolved in 1 000 ml analytical grade water. To ensure total solubility of the lyophilised substances, the pH of the solution was adjusted to 8 with NH_4OH. For comparative studies commercial humic acid was treated in exactly the same manner. A dilution range of 0-80 mg/l was prepared from both water samples using analytical grade water as diluent. The ultraviolet-visible light absorbances of these solutions was measured between 200 to 800 nm. The absorbances at 465 and 656 nm were used to calculate and monitor the effects of different natural organic matter concentrations on the E_4/E_6 ratios. The ultraviolet-visible-absorbance at 254 nm was used to set up and compare standard curves for the same solutions. In addition, the colour of these dilution ranges were also measured in Hazen units.

1.2.2 COLOUR REMOVAL BY ULTRAFILTRATION

Before brown water was ultrafiltered through capillary polysulphone membranes, membranes were compacted with pure-water for a period of 2 h. During this operation pure-water was pumped through the membranes at 800 kPa and 20°C. Compaction ensured that all solvents used during the manufacturing and drying processes were removed from the membrane surface and pores, and that any further decline in the pure-water flux during the filtration of a solution containing particulate matter could be ascribed only to the irreversible adsorption of these foulants onto and into the membrane. After compaction of the membranes, the initial flux of pure-water (PWF_{in}) was measured at 150 kPa and 20°C. This flux value was used as a reference before any solute filtration or membrane fouling took place.

The pure-water feed was replaced with natural brown water (25 l) which was ultrafiltered through the compacted membranes at 150 kPa and 20°C. The natural brown water was ultrafiltered at its natural pH (4.5) and concentration (85 mg/l). The product flux was measured immediately at the start of filtration of the natural brown water and regularly thereafter throughout the experiment. Product flux measurements were used in combination with the pure-water flux measurements to monitor flux decline caused by concentration polarisation and membrane fouling. After a filtration period of 300 min with natural brown water the system was flushed thoroughly with pure-water to remove all loosely or reversibly adsorbed natural organic matter from the membrane surface and pores. After 300 min a second pure-water flux (PWF_b) was measured and the difference between the PWF_{in} and PWF_b was used to determine the degree of irreversible fouling. The filtration process was continued with fresh natural brown water for two further 300 min periods to give a total filtration period of 1200 min. In a parallel study, the processes were repeated with a commercial humic acid solution (80 mg/l), prepared by dissolving 2 g commercial humic acid powder in 25l pure water at pH 8. Before ultrafiltration the pH of the commercial humic acid feed solution was adjusted to 4.5, the same as that of natural brown water.

Samples (5 ml) from both feed and permeate solutions were collected with each product flux measurement. The pH of these water samples was adjusted to 8 before further analyses. The resultant water mixtures were analysed by ultraviolet-visible light spectroscopy between 200 and 800 nm. The natural organic matter concentrations in the feed solutions, as well as in the resultant permeate solutions, were calculated from the ultraviolet-visible light absorbance at 254 nm and previously prepared standard curves. With this technique concentration changes in the feed solutions, due to adsorption onto and into the membranes, the quality of the resultant permeate solutions and the colour-removal efficiency were determined for the duration of the filtration process. The percentage colour-retention (%R) was determined by equation 1:

$$\%R = 100 - (NBW_p/NBW_f \times 100) \qquad (1)$$

where **NBW_p** and **NBW_f** are the natural organic matter concentrations in the permeate and feed solutions at each specific filtration time respectively. When commercial humic acid was used as the feed solution, **NBW_p** and **NBW_f** were replaced by **CHA_p** and **CHA_f**. In addition, the changes in the E_4/E_6 ratios of the two different water samples, were calculated initially and again after 300 min of filtration.

1.2.3 MEMBRANE CLEANING

Pure-water flux measurements showed that natural organic matter was adsorbed irreversibly onto and into polysulphone membranes during the ultrafiltration of natural brown water solutions. This induced changes in the permeability of the membranes. Membrane cleaning trials were subsequently carried out to determine the effect of cleaning agents on the permeability of polysulphone membranes.

Different aqueous alkaline cleaning solutions, in various combinations and under various conditions were used in cleaning trials under identical experimental conditions. Membranes, compacted with pure-water, were fouled for a period of 300 minutes with natural brown water at 20°C, at constant operating pressure and flow velocity. After the fouling period membranes were washed thoroughly with pure-water and the PFW_{fouled} was measured. The pure-water in the feed tank was then replaced by a cleaning solution which was pumped through the membrane at 100 kPa and 30°C for various periods of time depending on the cleaning regime. The cleaning solutions were subsequently replaced with pure-water and the membranes flushed thoroughly to remove all loosely adsorbed substances and residual cleaning substances. After thorough removal of the cleaning solution, the PWF_{clean} were determined and the performance of the different cleaning agents calculated as a percentage of the original PFW_{in}. After cleaning, filtration of natural brown water was continued for an additional 60 min to determine the extent and consequences of re-fouling.

Information on the effects of these cleaning trials on the selectivity of the membranes was acquired from spectrophotometric analyses of the permeate before and after cleaning. The selectivities of the membranes were monitored by calculating the colour retention with equation 1. Measurements were taken initially within the first 5 min, after 300 min of filtration, after each specific cleaning trial and again after 60 min of re-fouling.

1.2.4 FEED-WATER AND MEMBRANE PRE-TREATMENT: METHODS TO REDUCE FOULING BY NATURAL ORGANIC MATTER

pH variation: The pH of natural brown water, collected from Suurbraak in the Southern Cape, was adjusted by the addition of concentrated HCl or NaOH as required. The effect of different pH values on light absorbance by natural brown water was determined by measuring absorbance at 254 nm. In addition the E_4/E_6 ratios were calculated to determine the effect of pH on the degree of condensation of the aromatic C-network and to monitor any changes in the average particle size of natural organic matter.

Concentration variation: Precipitation of natural organic matter with metal-ions can reduce the concentration of these substances in natural brown water. In some large water treatment processes metal-ions, such as Al^{3+}, $Ca^{2+}$and Fe^{3+}, are being used to form large precipitating complexes with the humic acid and fulvic acid in brown water (Jucker and Clark, 1994). The aqueous solution containing less colour producing organic material is then removed from the precipitated complexes. The effect of metal-ions on natural brown water was investigated by ultraviolet-visible light analyses. Due to the interference of the Fe^{3+} complexes with spectrophotometric readings, natural brown water was treated only with $AlCl_3$ and $CaCO_3$. Alkaline salts of these metal-ions were dissolved in natural brown water solutions (1 g/l), and the resultant mixtures left for 8 h at 4°C. Supernatants were then decanted and analysed spectrophotometrically. The colour removal ability of such treatments was compared with results obtained after filtration through capillary ultrafiltration membranes.

The concentration of natural organic matter in natural brown water was determined from standard curves set up in previous sections.

1.2.5 ULTRAFILTRATION OF NATURAL AND PRE-TREATED BROWN WATER

Capillary ultrafiltration membranes were pre-compacted as described in section 1.2.3. After pre-compaction the first flux of pure-water (PWF_{in}) through the membrane was measured at an operating pressure of 150 kPa and 20°C. This flux measurement was used as a reference value before any solute filtration or membrane fouling took place.

Pure-water in the feed tank was replaced with natural brown water (25 l), at different pH values, and ultrafiltered at 150 kPa and 20°C. Twenty four h prior to ultrafiltration experiments the pH of five natural brown water solutions were adjusted from pH 4.5 to 2, 3, 7, 8 and 9 respectively. The resultant mixtures were kept at room temperature until ultrafiltration. During ultrafiltration of these samples the product flux was measured immediately after each filtration process started and thereafter at regular time intervals throughout the experiment. Product flux measurements were used in combination with the pure-water flux measurements to monitor and compare the flux decline caused by natural organic matter at its natural, higher and lower pH values. After a filtration period of 300 min the system was flushed thoroughly with pure-water to remove all loosely or reversibly adsorbed natural organic matter from the membrane surface and pores. A second pure-water flux (PWF_b) was measured and the difference between the PWF_{in} and PWF_b was used to calculate the extent of irreversible fouling. Ultraviolet-visible light spectroscopy was used to monitor changes in the feed solution, permeate quality and colour removal efficiency for the duration of the filtration experiment. Samples of the feed water and the permeate (5 ml) were collected for analyses with each product flux determination.

To determine the long term flux decline, of natural brown water solutions at pH 2 and 7, the filtration period was increased from 300 min to 1000 min. The experiments were performed at these pH values which represented the conditions for maximum and minimum fouling respectively. After these filtration periode the feed solutions were replaced with fresh natural brown water. To determine the effects of changing the pH of the feed solution after a prolonged period of filtration at a specific pH, the pH of the acidic run was increased from 2 to 7 and that of the neutral run was lowered from 7 to 2. These new feed solutions were filtered for an additional period of 80 min.

The natural organic matter concentration in natural brown water (pH 4.5) was reduced by the addition of $AlCl_3$ or $CaCO_3$ (1 g/l) respectively. The supernatant was decanted and the resultant solutions ultrafiltered through pre-compacted membranes as previously described. For both solutions the flux decline was monitored throughout the filtration experiment and permeate and feed samples were analysed spectrophotometrically.

The anti-fouling effects of two surfactants namely, Triton X100® and a Pluronic® F108, were investigated in this study. Capillary polysulphone membranes were pre compacted with 0,5% solutions of Triton X100® and Pluronic® F108 respectively for 1 h. After this 1 h period, the different coating solutions were replaced with pure-water and the system flushed thoroughly to remove all excess coating material. The feed solution, again, was replaced with a pure-water solution and the pure-water flux measured. Natural brown water was the ultrafiltered through the membranes. Exactly the same experimental procedure was followed and results recorded as with natural brown water.

1.3 RESULTS

1.3.1 CHARACTERISATION AND REMOVAL OF NATURAL ORGANIC MATTER FROM CAPE BROWN WATER

As expected, the pH of the Suurbraak natural brown water samples were acidic with an average pH of 4.5(±0.25) for at least ten different samples. The light absorbance of natural brown water and commercial humic acid samples, measured by ultraviolet-visible light spectroscopy at 200 to 700 nm, are presented in Figure 1.1. Both samples showed strong light absorption in the UV-range and almost no absorption in the visible-light region. E_4/E_6 ratios of the natural brown water and commercial humic acid samples, calculated from these results, were 6.06±0.1 and 5.12±0.18, respectively.

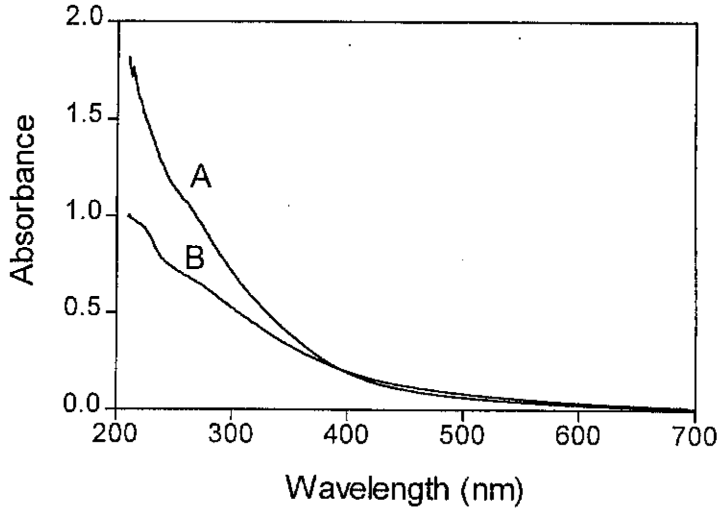

Figure 1.1 Comparison between the ultraviolet-visible light absorbance of natural brown water and a commercial humic acid solution at pH 8. Samples: [A] natural brown water from Suurbraak [B] commercial humic acid from Fluka® (80 mg/l).

The correlations between the concentrations of natural organic matter in these samples and the ultraviolet-visible-absorbance measured at 254 nm are shown in Figure 1.2. At 254 nm the solutions of natural organic matter in both samples obeyed Beer's law, and linear curves, with R-squared values of 0.998, were obtained up to the maximum concentration used. Also included in this figure is the Hazen colour values. According to these results 80 mg/l natural organic matter gave a Hazen value of 300mg/l as Pt 350.

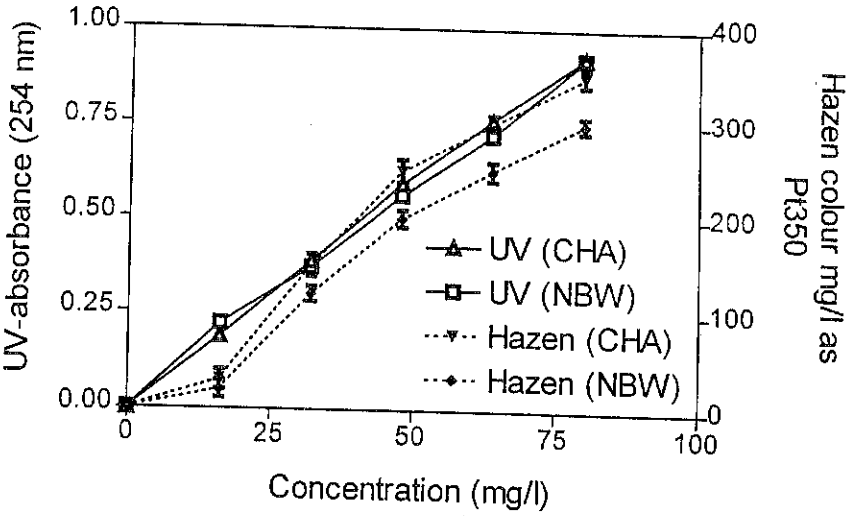

Figure 1.2 Natural organic matter concentration vs. absorbance at 254 nm for natural brown water and commercial humic acid at pH 8. Values are the mathematical means of four determinations.

The Hazen values obtained with commercial humic acid were slightly higer than those with natural brown water from Suurbraak. As shown in Figure 1.3, variation of the natural organic matter concentration had no effect on the E_4/E_6 ratios. In both cases the E_4/E_6 ratios were constant, with the average value for commercial humic acid lower than that for natural brown water.

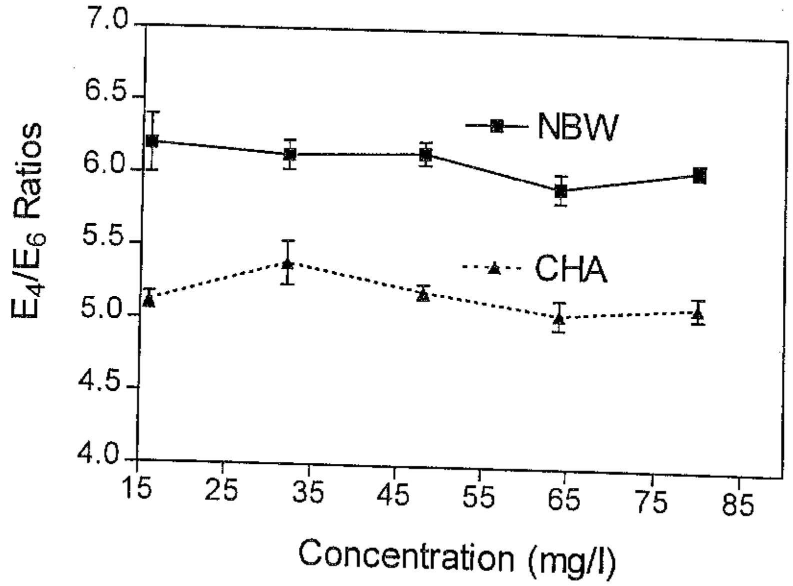

Figure 1.3 E_4/E_6 ratios vs. concentration (mg/l) for natural brown water and commercial humic acid at pH 8. Values presented are the mathematical means of at least three determinations ± SD.

1.3.2 COLOUR REMOVAL WITH ULTRAFILTRATION

Flux decline data for both the natural brown water and the commercial humic acid samples are presented as percentages of the original flux before any solution other than pure-water was filtered. The product flux and pure-water flux values for natural brown water and commercial humic acid are shown in Figures 1.4 and 1.5, respectively.

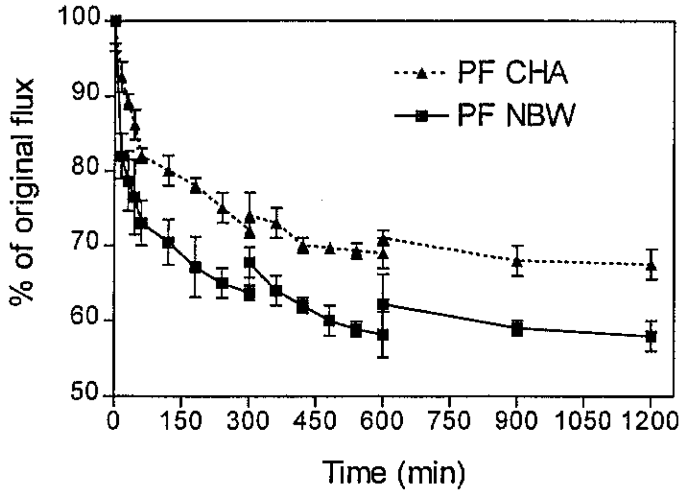

Figure 1.4 Time dependent operational flux decline with natural brown water (NBW) and a commercial humic solution (CHA) as feed solutions. Values presented are the mathematical means of at least three determinations ± SD.

The product flux, measured for both filtration media, was characterised by a sharp decline within the first few minutes of filtration. This was followed by a more gradual decline during the later stages of the filtration process. After 60 min of filtration, the product fluxes measured for natural brown water and commercial humic acid were, respectively, 27% and 20 % lower than that of the original flux. These product flux values declined further to 63% and 72% of the original flux after 300 min, and stabilised at 58% and 67% after 1 200 min of filtration. The decline of the pure-water flux values was the same as that of the product flux. The percentages of the original pure-water flux after 300 min of filtration with natural brown water and commercial humic acid were 54% and 74%, respectively and stabilised at 50% and 67%, respectively, after 1 200 min.

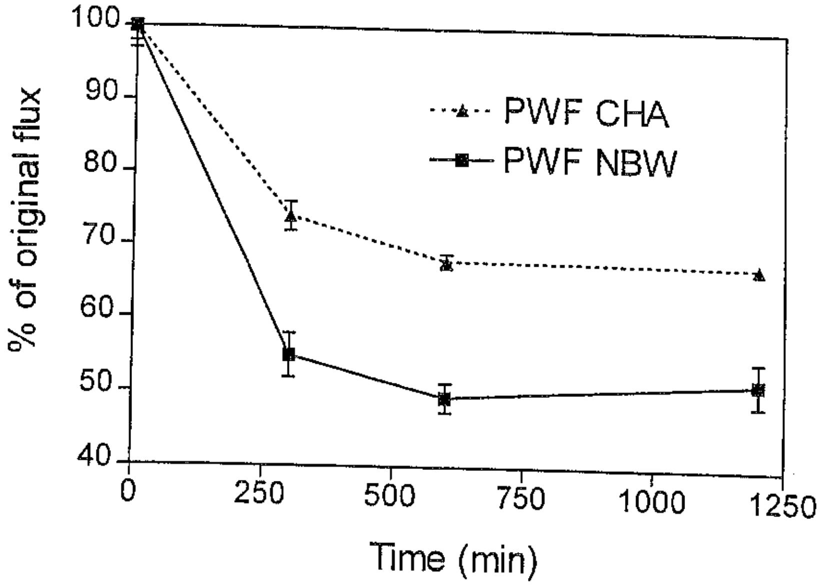

Figure 1.5 Time dependent pure-water flux decline with natural brown water (NBW) and a commercial humic solution (CHA) as feed solutions. Values presented are the mathematical means of at least three determinations ± SD.

During the entire filtration period changes were observed in the ultraviolet-visible-absorbances of the feed and the permeate solutions of both natural brown water and commercial humic acid solutions, measured at a wavelength of 254 nm. These changes were converted to concentration (mg/l) using the standard curve regression data. The changes in the natural organic matter concentrations of natural brown water and commercial humic acid in the feed and permeate solutions are shown in Figures 1.6 and 1.7. The natural organic matter concentrations of the feed-water solutions decreased rapidly within the first minutes of filtration, followed by a more gradual decline towards the end of the filtration run. After 300 and 600 min of filtration the pure-water flux was measured, and prolonged filtration runs were started with fresh natural brown water and commercial humic acid feed-water solutions. With both feed-water solutions, the natural organic matter concentration decreased by approximately 24% during the first 300 min of filtration. The decline in the natural organic matter concentration in both the feed solutions in filtration periods from 300 to 600 and 600 to 1 200 minutes were 12% and 9%, respectively.

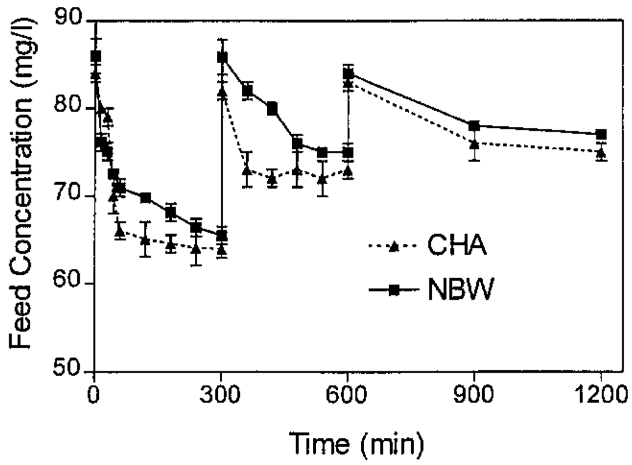

Figure 1.6 Comparison between the concentration changes in the feed solutions during the filtration of natural brown water (NBW) and commercial humic acid (CHA). Values presented are the mathematical means of at least three determinations ± SD.

The natural organic matter concentrations of the permeate solutions also decreased rapidly within the first few minutes of filtration, followed by more gradual decreases towards the end of the experiment. The natural organic matter concentrations in the natural brown water and commercial humic acid permeate solution decreased by 74% and 71%, respectively, within the first 300 min. Natural organic matter concentration in these permeate solutions stabilised at approximately 83% and 73%, respectively, of the original concentrations after 1 200 min of filtration.

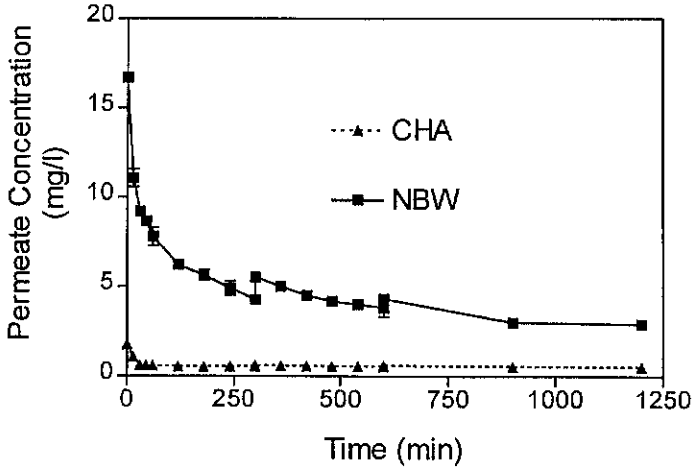

Figure 1.7 Comparison between the concentration changes in the permeate solutions during the filtration of natural brown water (NBW) and commercial humic acid (CHA). Values presented are the mathematical means of at least three determinations ± SD.

The percentage natural organic matter retained by the polysulphone membranes, as determined by equation 1, is presented in Figure 1.8. The retention of natural organic matter from the natural brown water solution was initially 80%, but increased gradually up to 99% over the filtration period of 1 200 min. The colour retention with commercial humic acid as feed solution was initially 97% and increased within the first 60 min to 99%.

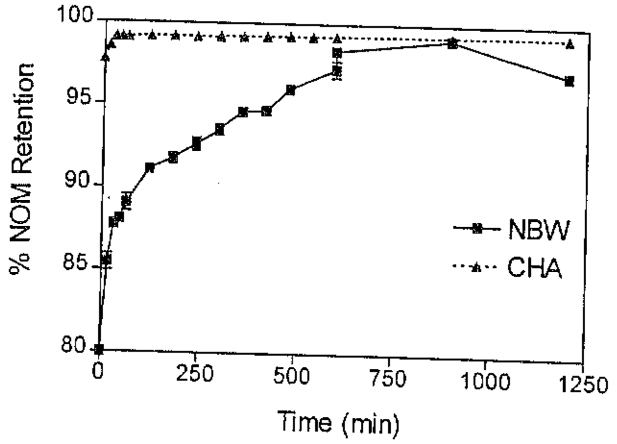

Figure 1.8 Percentage natural organic matter retention from natural brown water (NBW) and commercial humic acid (CHA) solutions over a 1200 min of filtration period. Values presented are the mathematical means of at least three determinations ± SD.

The E_4/E_6 ratios of the feed and permeate solutions for both samples tested at time zero and after 300 min, are presented in Table 1.2.

Table 1.2 E_4/E_6 ratios of the feed and permeate initially and after 300 min of filtration. Operating conditions included an operating pressure of 150 kPa and a feed solution temperature of 20°C. Values presented are the mathematical means of at least three determinations ± SD.

Sample Description	Permeate (NBW)	Feed (NBW)	Permeate (CHA)	Feed (CHA)
E_4/E_6 Ratio [0 min]	12.98 ± 0.23	6.06 ± 0.1	*	5.12 ± 0.2
E_4/E_6 Ratio [300 min]	24 ± 0.87	8.74 ± 0.34	*	7.8 ± 0.56

The E_4/E_6 ratios calculated from the feed solutions, derived from natural brown water and commercial humic acid, increased slightly, whereas the ratio of the natural brown water permeate solutions increased dramatically. The E_4/E_6 ratio of the permeate solution derived from the commercial humic acid sample could not be determined as the absorbance at 656 nm was too low for accurate measurement.

1.3.3 MEMBRANE CLEANING

The efficiency of cleaning procedures is usually measured by the increase in the flux through fouled membranes. The changes in the pure-water flux values, after cleaning with different cleaning solutions and combinations of cleaning solutions, are presented as percentages of the original pure-water flux in Table 1.2. The initial pure-water flux was measured before any fouling occurred and could thus be taken as 100%. After 300 min of fouling the pure-water flux declined to approximately 55% of the original flux. All the cleaning solutions increased the pure-water flux substantially. This increase in the pure-water flux, however, depended on the efficiency of the cleaning procedure. Cleaning with a specific aqueous solution, consisting of 1% NH_4OH (27% NH_3) for 1 h, increased the flux values to 78% of the original pure-water flux whereas cleaning with aqueous solution containing 1.5% NH_4OH (27% NH_3) and 0.1% Triton X100® for a period of 1 h increased the pure-water flux to 200 % of the original flux. After application of each cleaning solution, membranes were refouled, and the pure-water flux was again measured after 60 min of filtration with natural brown water as feed solution. During the refouling, the pure-water flux declined dramatically irrespective of the cleaning agent used. The rate of pure-water flux decline, however, depended on the type of cleaning solution that was used in the trial. For instance, during the refouling of membranes the pure-water flux declined by 40% after treatment with a 1.5% NH_4OH (27% NH_3) and 0.1% Triton X100® solution and only 15% after treatment with 1% NH_4OH (27% NH_3).

The colour retention of natural brown water by an unfouled membrane was about 80%, but increased to approximately 95% after 300 min of filtration because of pore restriction due to fouling. Cleaning agents, however, removed some or all of the foulants on the membrane surface and in the pores, to give a decrease in colour retention immediately after cleaning. The colour retentions by fouled membranes treated with different chemical cleaning agents, immediately after cleaning, are also presented in Table 1.3. Cleaning regimes caused the colour retention to decrease dramatically.

Table 1.3 Cleaning efficiency of chemical cleaning agents used to clean membranes, fouled by natural brown water. Cleaning efficiency is presented as the % of the original pure-water flux and the change in the permeate quality. Values presented are the mathematical means of at least three determinations ± SD.

Cleaning Solution	% of PWF (directly after cleaning)	% of PWF (after 60 min refouling)	% Colour Retention (after cleaning)	% Colour retention (60 min refouling)	E_4/E_6 Ratio (directly after cleaning)	E_4/E_6 Ratio (after 60 min refouling)
1) 1% NH_4OH for 1 h	78.6 ± 2.1	66 ± 0.78	58 ± 0.45	80 ± 0.87	19.08 ± 0.9	22.5 ± 1
2) 1%NH_4OH+ 1% $CaSO_4$ (1hr)	83.05 ± 0.85	65 ± 1.8	60 ± 0.2	89 ± 0.34	17.02 ± 0.9	23 ± 0.8
3) 2% NH_4OH 2 h	88.56 ± 1.3	67 ± 1.23	59 ± 0.3	84 ± 0.35	16.2 ± 0.4	not determined
Repeat washing with solution 3 for 1 h	95.56 ± 1.0	66 ± 0.5	60 ± 1.0	78 ± 0.45	18.8 ± 0.6	22.04 ± 1.02
4) 1.5%NH_4OH + 0.1% Triton X100® for 1 h	200 ± 3.04	not determined	not determined	not determined	not determined	not determined
Solution 4 flushed thoroughly with pure-water (120 min)	151 ± 2.16	80 ± 0.67	49 ± 0.56	84 ± 0.77	8.1 ± 0.4	19.8 ± 0.9
5) NaOH + SDS + EDTA (1g/l solutions) for 1 h	111.1 ± 2.0	70 ± 1.2	62 ± 0.7	89 ± 0.7	13 ± 0.76	22.3 ± 0.9
6) 2% N_H4OH + EDTA 1 h followed by 1% Biotex® 1 h	150 ± 1.5	78 ± 0.1	59 ± 0.34	87 ± 0.45	12.5 ± 1	21.7 ± 0.75

Decrease in the colour retention, after chemical cleaning, however, depended on the type of cleaning agent used. Colour-retention values were as low as 49% after cleaning with a solution containing Triton X100®. In conjunction with the colour retention, E_4/E_6 ratios of the permeate solutions were determined directly after cleaning. All cleaning agents, some more than others, caused the ratios to decrease. The most notable change again occurred with Triton X100® included in the cleaning solution. The E_4/E_6 ratio of the permeate decreased from 24 before cleaning, to 8.1 after treatment with 1.5% NH_4OH (27% NH_3) and 0.1% Triton X100®.

The colour retention and E_4/E_6 ratios of the cleaned membranes were measured after 60 min of refouling with natural brown water. As with unfouled membranes, the colour retention and the E_4/E_6 ratios again increased markedly with prolonged filtration. E_4/E_6 ratios of some of the permeate solutions could not be calculated as light absorption at 656 nm could not be measured accurately.

1.3.4 FEED-WATER AND MEMBRANE PRE-TREATMENT: METHODS TO REDUCE FOULING BY NATURAL ORGANIC MATTER

The natural pH of Suurbraak brown-water, as expected because of the carboxylic functional groups of humic acid and fulvic acid, was acidic with an average pH of 4.5 for all native samples measured. All natural brown water samples showed strong light absorption in the UV-range. This spectrophotometric characteristic of natural brown water was influenced by the pH of the solution under investigation. Changes induced by different pH values on ultraviolet-visible-absorbance at 254 nm and E_4/E_6 calculated from wavelength scans, are shown in Figure 1.9. The ultraviolet-visible-absorbance at 254 nm increased from 0.5 at pH 2 to 1.18 at pH 11 while E_4/E_6 ratios decreased from 9 at pH 2, to 3 at pH 11.

Changes in the natural organic matter concentration and E_4/E_6 ratios, induced by the complexation of natural organic matter with metal-ions, are presented in Table. 1.4. The natural organic matter concentration was reduced from 85 mg/l in natural brown water to 49 and 23 mg/l, respectively, by the addition of $AlCl_3$ and $CaCO_3$. For both solutions, after the addition of $AlCl_3$ or $CaCO_3$ to the natural brown water solution, there were decreases in the calculated E_4/E_6 ratios.

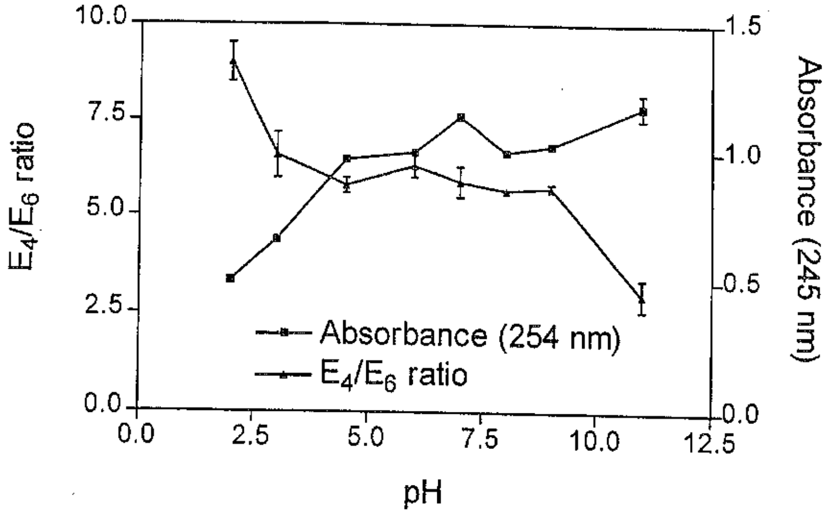

Figure 1.9 Effect of different pH values on the ultraviolet-visible light spectrophotometric characteristics of Suurbraak brown water. Values presented are the mathematical means of at least three determinations ± STD.

Table 1.4 Changes in the natural organic matter concentration and E_4/E_6 ratios induced by pre-treatment with metal-ions. Values presented are the mathematical means of at least three determinations ± STD.

Pre-treatment	Absorbance at 254 nm	Concentration (mg/l)	E_4/E_6 ratios
None	0.98 ± 0.03	85 ± 2.4	6.06 ± 0.3
$CaCO_3$ (1 g/l)	0.56 ± 0.019	49 ± 3.4	5.03 ± 0.23
$AlCl_3$ (5 g /l)	0.264 ± 0.025	23 ± 0.8	3.21 ± 0.4

1.3.5 ULTRAFILTRATION OF NATURAL AND PRE-TREATED BROWN WATER

Flux declines are presented as the percentages of the original pure-water or operational fluxes, measured with pure-water or natural brown water, respectively. There was a decline in both the pure-water flux and product flux, at all pH values investigated because of the adsorption of natural organic matter onto the membranes. The adsorptive behaviour of natural organic matter, however, was influenced by the pH of the natural brown water solution in contact with the membrane. Therefore, by adjusting the pH of natural brown water before filtration, the magnitude of foulant adsorption and flux decline could be increased or reduced.

The decline in the product flux over a period of time as a function of the pH of natural brown water, is presented in Figure 1.10. At pH 2 and 9 the product flux declined by 61% and 35%, respectively, within the first 15 minutes of filtration. At pH 7 the decline over the same period was only 11%. After 300 minutes of filtration the product flux at pH 2 and 9 were 33% and 50% of the original flux respectively, compared with 69% at pH 7.

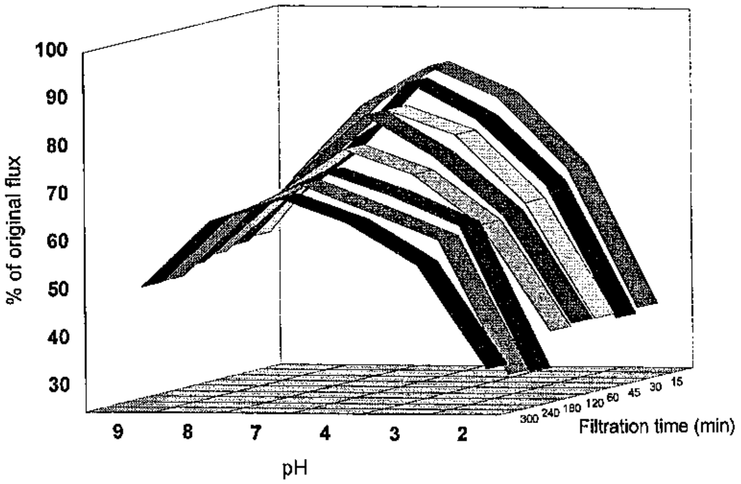

Figure 1.10 Effect of pH on the product fluxes during the ultrafiltration of natural brown water. Values presented are the mathematical means of at least three determinations (STD < 4.66).

To determine the effects of different feed pH values on the irreversibility of membrane fouling and flux decline, the pure-water flux was measured after 300 min of filtration. Again the proportion of irreversible foulant adsorption was influenced by the pH of the natural brown water feed solution. Results are shown in Figure 1.11. The pure-water flux is presented as a percentage of the original pure-water flux and is also compared with the product flux measured after 300 min of filtration. The decline in the pure-water flux, after 300 min of filtration, was of the same order as that observed with the product flux. In addition, changes in the feed solution pH resulted in the same trend of higher fouling rates and more marked flux decline at pH 2 and 9 when compared with declines other pH values.

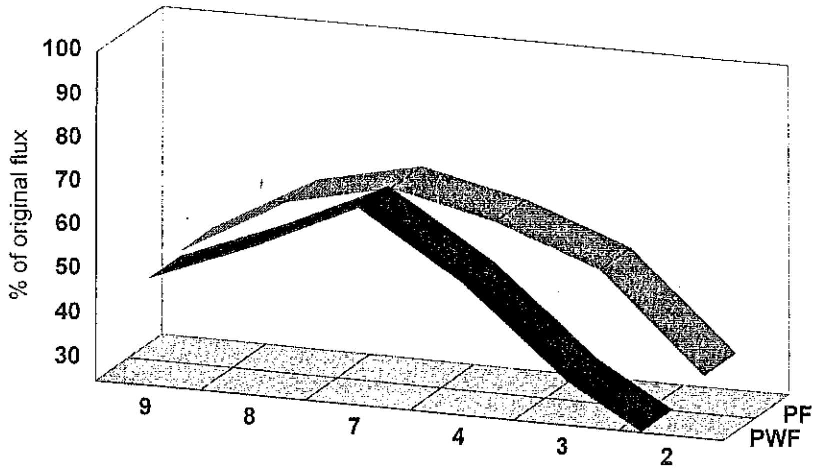

Figure 1.11 Effect of pH changes on the pure-water and product flux observed after 300 min of filtration. Values presented are the mathematical means of three determinations (STD < 5.36)

Changes in the absorbances of six natural brown water feed solutions of different acidities measured at 254 nm, throughout a 300 min filtration period, are presented in Figure 1.12. At all pH values, the ultraviolet-visible absorbance which represents the natural organic matter concentration in the feed solution, decreased over the filtration period of 300 min. The decline, however, was more pronounced at pH 9 and 2 than at other pH values. At pH 9 and 2 the absorbance decreased by 57% and 99 %, respectively, while only a 1% decrease was observed at pH 7.

Changes in the absorbance at 254 nm of the resultant permeate solutions, filtered at the six different pH values, are presented in Figure 1.13. At all pH values, except at pH 2 and 4, the initial absorbance of the permeate at 254 nm was of the order of 0.21. After a few minutes of filtration the absorbance of the permeate solutions started to decrease gradually. The most pronounced decline in permeate absorbance was observed at pH 7 where a drastic decline occurred within the first 15 minutes of filtration followed by a more gradual decrease for the duration of the experiment.

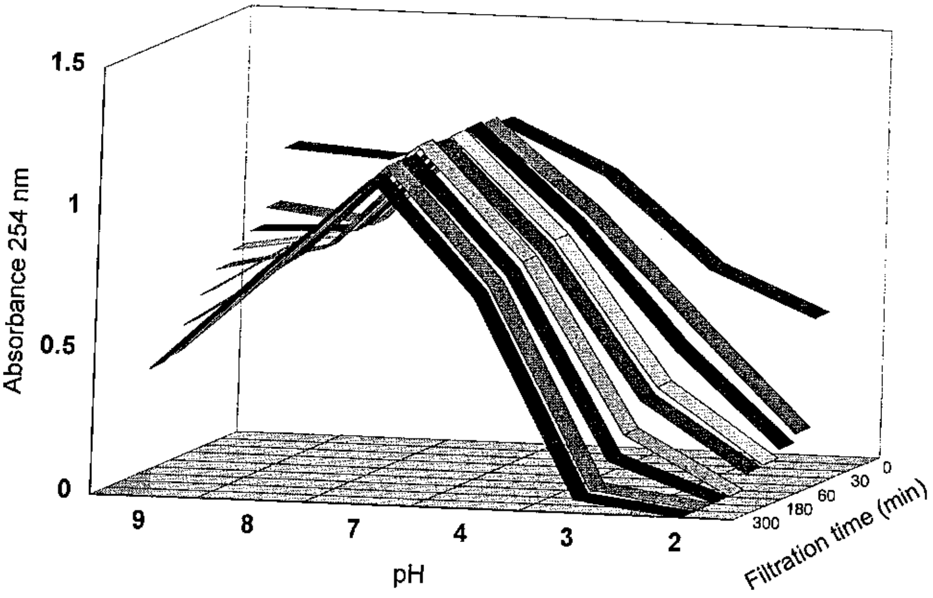

Figure 1.12 Changes in the ultraviolet-visible-absorbance of feed-water at different pH values during the ultrafiltration of natural brown water. Values presented are the mathematical means of at least three determinations (STD < 0.016).

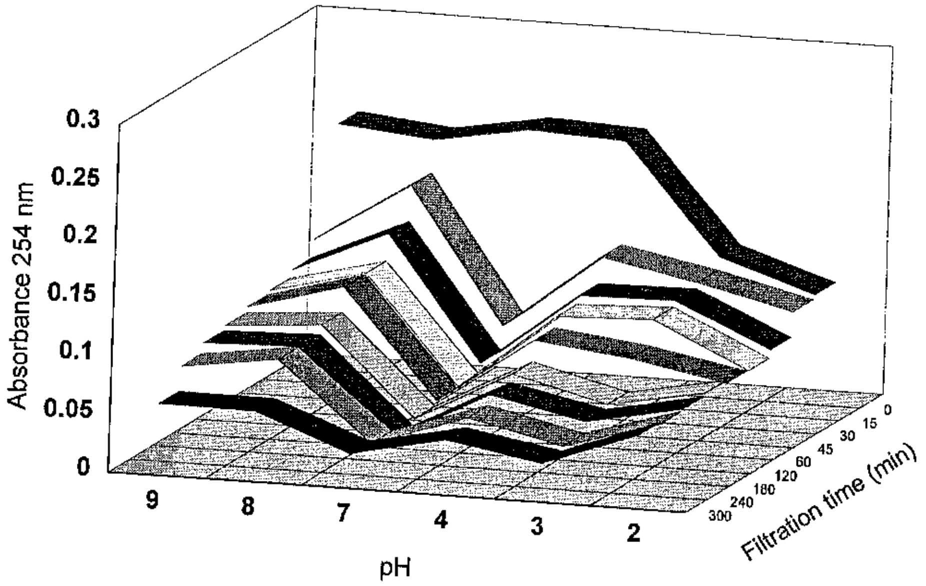

Figure 1.13 Changes in the ultraviolet-visible-absorbance of permeate at different pH values during the ultrafiltration of natural brown water. Values presented are the mathematical means of three determinations (STD < 0.006)

The percentages of natural organic matter rejected from the feed water solutions by capillary polysulphone membranes, as a function of feed water pH, are presented in Figure 1.14. Retention (%R) was calculated using equation [2]:

$$\%R = 100 - (NBW_p/nNBW_f \times 100) \qquad (2)$$

where **NBW**$_p$ and **nNBW**$_f$ are the absorbance of the permeate and the initial native brown water feed solutions, measured at 254 nm, respectively. The initial colour retention at all pH values, except at pH 2 and 3, was 80%. After 300 minutes of filtration the colour retention, at all pH values, increased to above 94%.

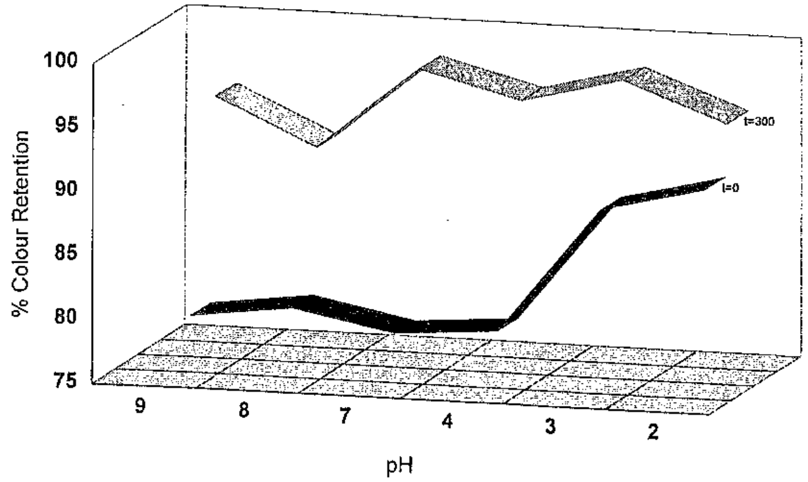

Figure 1.14 Effect of pH and filtration time on the colour retention of capillary polysulphone membranes. Values presented are the mathematical means of at least three determinations. (STD < 4.36).

Additional information on the specrophotometric characteristics of the different natural brown water feed solutions was obtained by comparing changes in the E_4/E_6 ratios, over the 300 min filtration period. Results are shown in Figure 1.15. At pH 9, 8, 7 and 4.5 the ratios of the feed solutions increased but decreased at pH 3 and 2 was observed. Calculation of the E_4/E_6 ratios of the resultant permeate solutions other than at pH 8 and 4 was prevented because of low light absorbance at 656 nm.

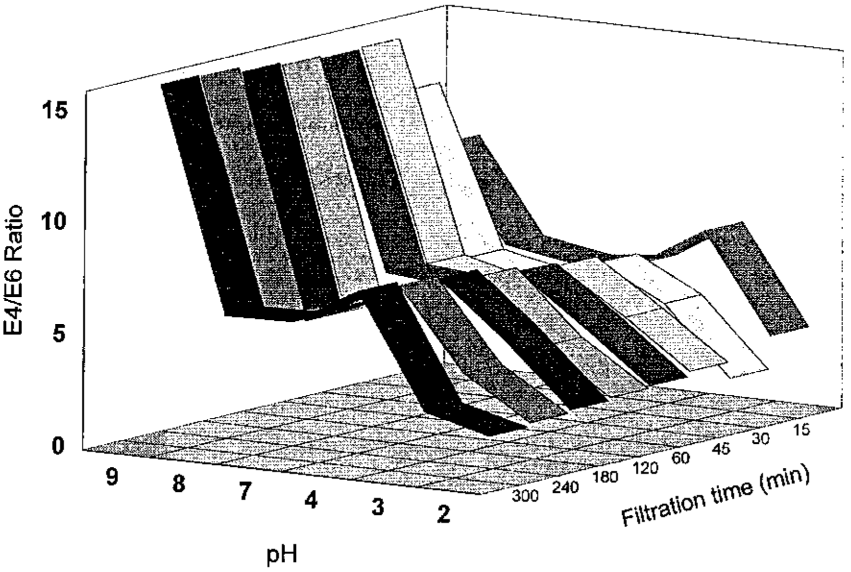

Figure 1.15 Changes in the E_4/E_6 ratios of the feed solutions at different pH values over a 300 min filtration period. Values presented are the mathematical means of at least three determinations (STD < 0.96).

Long term flux decline data at pH 2 and 7, as well as the effects induced by changing the pH after 1000 min filtration, are presented in Figure 1.16.

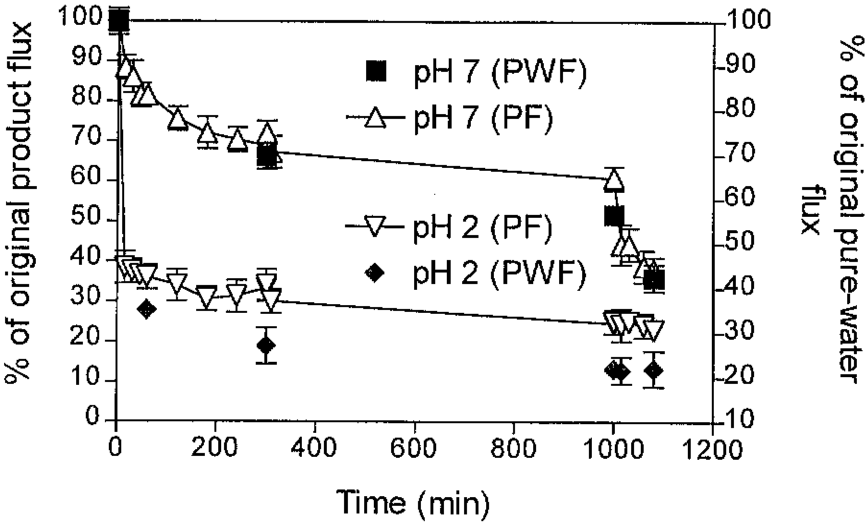

Figure 1.16 Flux decline during the ultrafiltration of natural brown water at pH 7 and 2 over a period of 1100 min. After 1000 min the pH of the two feed solution was lowered to 2 or increased to 7 respectively. Values presented are the mathematical means of at least three determinations ± STD.

For both pH values the product flux decline stabilised after 300 min. The product and pure-water flux of the filtration run at neutral pH, however, dropped drastically within the first minutes after changing the pH of the feed solution from 7 to 2. No noticeable change in the product flux or pure-water flux for the pH 2- filtration run was, however, observed after changing the pH of the feed solution to 7.

Pre-treatment of natural brown water with metal-ions prior to ultrafiltration influenced the rate of flux decline. The influence of metal ion pre-treatment on the percentage flux decline over a 300 min filtration period, is presented in Figure 1.17. Different flux decline patterns were observed depending on whether native or metal-ion pre-treated natural brown water feed solutions were used. Within the first 15 minutes these differences were insignificant, but they became more pronounced after 60 min. After 300 min of filtration there was a difference of 20 % percent between water pre-treated with metal-ions and native natural brown water. The pure-water flux was affected in the same manner, with flux after 300 min were 36%; 30% and 55% of the original values for $AlCl_3$ and $CaCO_3$ pre-treated and native natural brown water, respectively.

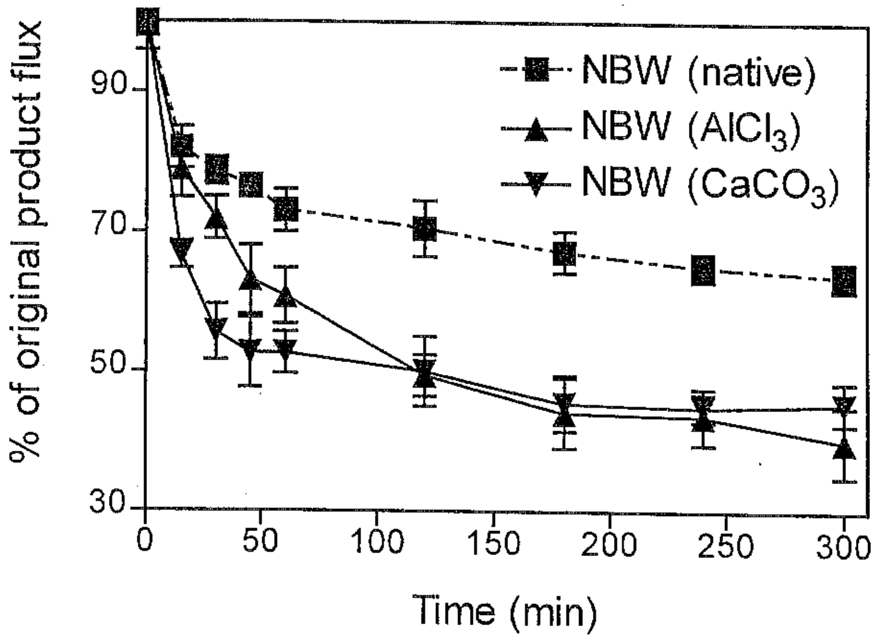

Figure 1.17 Comparison between the flux decline patterns of native and metal-ion pre-treated natural brown water. Natural brown water was pre-treated with $AlCl_3$ or $CaCO_3$ (1 g/l). Values presented are the mathematical means of at least three determinations ± STD.

Changes in the natural organic matter concentrations and other spectrophotometric characteristics of natural brown water after the addition of metal-ions are shown in Table 1.3. Changes during the ultrafiltration of the native and pre-treated natural brown water solutions are summarised in Table 1.5. During a filtration period of 300 min, the natural organic matter concentrations of $AlCl_3$ and $CaCO_3$ pre-treated natural brown water solutions decreased further by 69% and 51%, respectively, while the absorbances of the resultant permeate solutions decreased by 71% and

60%, respectively.

Table 1.5 Changes observed in the natural organic matter concentration and other spectrophotometric characteristics during the ultrafiltration of native and metal-ion pre-treated natural brown water. Natural brown water was pre-treated with $AlCl_3$ or $CaCO_3$ (1 g/l). Values presented are the mathematical means of at least three determinations ± STD.

Spectrophotometric analyses	Native NBW	NBW + $AlCl_3$ (1 g/l)	NBW + $CaCO_3$ (1 g/l)
Feed Time 0 min (mg/l)	86.72 ± 2.1	23.36 ± 1.5	49.5 ± 1.19
Feed Time 300 min (mg/l)	65.49 ± 3.0	7.3 ± 0.9	21.6 ± 3.2
Permeate Time 0 min (mg/l)	19.03 ± 1.5	14.7 ± 2.0	13.27 ± 2.3
Permeate Time 300 min (mg/l)	3.98 ± 0.75	4.16 ± 0.7	5.31 ± 0.87
E_4/E_6 ratio feed (0 min)	6.06 ± 0.3	3.3 ± 0.4	5.03 ± 0.23
E_4/E_6 ratio feed (300 min)	7.8 ± 0.8	*	5.23 ± 0.76
E_4/E_6 ratio permeate (0 min)	12.9 ± 0.9	*	12 ± 1.2
E_4/E_6 ratio permeate (300 min)	24 ± 0.9	*	16 ± 1.3

***Ratio could not be calculated accurately due to low absorbance at 656nm.**

The E_4/E_6 ratios for natural brown water were also influenced by pre-treatment with metal-ions. Unfortunately, low absorbance at 656 nm prevented calculation of the E_4/E_6 ratios for the $AlCl_3$ pre-treated feed-water after 300 min of filtration. The same problem arose for all of the permeate samples. The E_4/E_6 ratios of the $CaCO_3$ treated feed-water stayed almost constant after 300 min of filtration while that of the permeate increased from 12 to 16.

Coating of precompacted capillary polysulphone membranes with 0.5% Triton X100®, for a period of 1 h, increased the pure-water flux from 74% to 174%. Flushing these Triton treated membranes with pure-water for another hour resulted in a 37% decrease in the pure-water flux to 137%. Processing with Pluronic® F108, on the other hand, decreased the pure-water flux to 22% of that of an untreated membrane. Results are shown in Figure 1.18. After thorough flushing with pure-water, the pure-water flux increased to 24% of the original value.

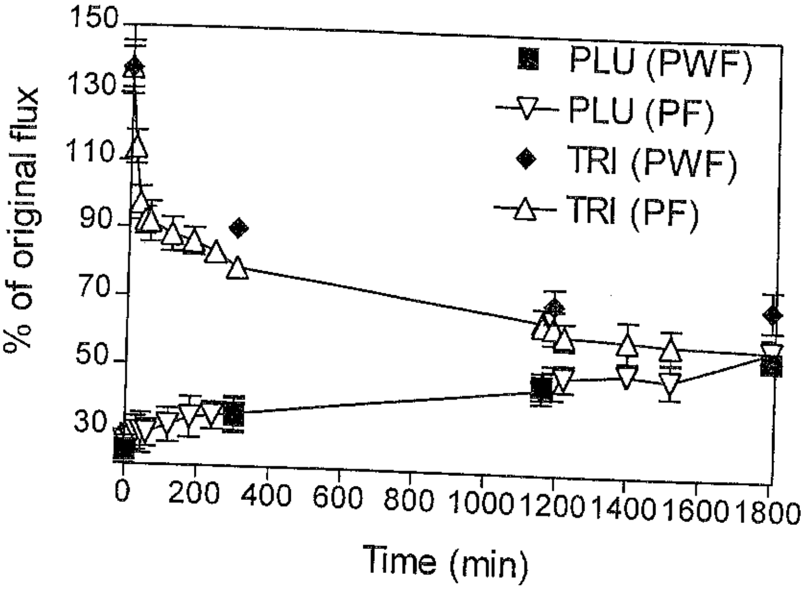

Figure 1.18 Effect of membrane pre-treatment on the ultrafiltration of natural brown water. Capillary polysulphone membranes were precompacted with 0,5% Triton X100® and Pluronic® F108 respectively for one h. Values presented are the mathematical means of at least three determinations ± STD.

pure-water flux and product flux decline profiles of Triton X100® treated membranes were similar to those for other filtration experiments with untreated membranes. The initial product flux was high, but decreased rapidly from 137% to 92% of the original product flux of an untreated membrane, during the first 60 minutes of filtration. After this drastic decline the product flux decreased more gradually for the rest of the filtration period of 1800 min. The decline of pure-water flux for Triton X100® pre-coated membranes, followed the same pattern as that of the product flux confirming irreversible fouling.

Product flux measurements, with the Pluronic® F108 pre-coated membranes, were initially much lower than those for an uncoated membrane. Unlike previous experiments, however, the product flux increased throughout the filtration trial. After 300 min of filtration the product flux increased from 26% to 35% of the original and increased further to 55% after 1800 min of filtration. The pure-water flux increased from 24%, of the original flux before natural brown water filtration, to 58% after 1800 min.

Natural brown water was used in both filtration trials with Triton X100® or Pluronic® F108 pre-coated membrane. The ultraviolet-visible light-absorbance of the feed water used with the Triton X100® pre-coated membranes decreased from approximately 1 to 0.76 while the absorbance of the feed water, filtered with the Pluronic® F108 pre-coated membranes, stayed constant throughout the experiment.

Spectrophotometric changes in permeate solutions, that passed through the pre-coated membranes, are shown in Figure 1.19. The ultraviolet-visible-absorbance of the permeate, associated with the Triton X100® pre-coated membranes, were initially rather high, but decreased throughout the experiment. The ultraviolet-visible-absorbance decreased from the initial 0.29 to 0.06 after 1800 min. The ultraviolet-visible-absorbance of the permeate, associated with the Pluronic® F108 pre coated membranes, increased from 0.032 initially to 0.15 after 1800 minutes of filtration.

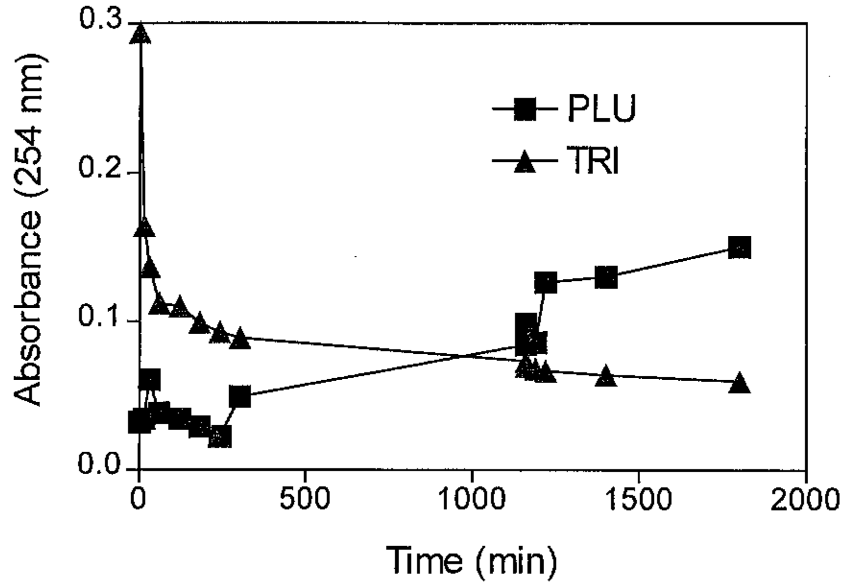

Figure 1.19 Colour retention of Pluronic® F108 (PLU) and Triton X100® (TRI) coated membranes during the filtration of natural brown water for a prolonged period. Values presented are the mathematical means of at least three determinations ± STD.

In addition, changes in the E_4/E_6 ratios of the above-mentioned solutions were: Ratios of the feed solutions stayed constant throughout the filtration period. Ratios of the permeate associated with the Triton X100® coated membranes increased from 4 to 9 while that of the Pluronic® F108 pre-treated membranes decreased from 23 to 13.

The percentage colour retentions by pre-treated and untreated capillary polysulphone membranes, as determined by equation 2, are summarised in Table 1.6. The colour retention by membranes pre coated with Triton X100® was initially 70%, but increased to 94% after 1800 min of filtration. The colour retention of the Pluronic® F108 precasted membranes, on the other hand, started off high at 97% and decreased to 83% after 1800 min of filtration.

Table 1.6 Percentage colour removal by pre-coated and untreated polysulphone membranes. Capillary polysulphone membranes were pre compacted with 0,5% Triton X100® and Pluronic® F108, respectively, for 1 h. Values presented are the mathematical means of at least three determinations ± STD.

Membrane Type	% Colour retention (initially)	% Colour retention (300 min)	% Colour retention (1500 min)
Untreated Membranes	80 ± 0.9	94 ± 3.24	96.4 ± 4.3
Triton X100® Pre-coated	70.2 ± 2.0	91 ± 1.0	94 ± 3.3
Pluronic® F108 pre-coated	96.7 ± 1.6	95.2 ± 3.2	83 ± 2.4

1.4 DISCUSSION

1.4.1 CHARACTERISATION AND REMOVAL

The pH measurements of natural brown water clearly indicated the acidic nature of the water caused by the presence of carboxylic acid residues in the natural organic matter structure and the absence of alkaline metals.

Ultraviolet-visible light spectra of natural brown water and commercial humic acid samples indicated that the natural organic matter in these samples were closely related. In both samples, light was strongly absorbed in the UV-range with less or almost no absorption in the visible-light range. This strong light absorption at the short wavelengths (e.g. 210-350 nm) can be attributed to the benzonoid bands of carboxyphenols. E_4/E_6 ratios, calculated from the above-mentioned wavelength scans, were lower than those for fulvic acid solutions obtained from the literature (Baes and bloom, 1990). This can be attributed to the fact that the natural brown water and commercial humic acid samples are mixtures containing both large humic and small fulvic acid molecules. Low E_4/E_6 ratios obtained from these two samples therefore indicated the presence of large molecules, with high carbon and low oxygen contents, small amounts of CO_2H groups, and low acidity (Chen *et al.*,1977).

Results indicated that there were linear relationships between the ultraviolet-visible-absorbance of the natural brown water and commercial humic acid solutions at a wavelength of 254 nm, and that Beer's law was obeyed in the concentration range of 0 to 80 mg/l. These characteristics of natural brown water and commercial humic acid samples were used in further filtration experiments to determine the natural organic matter concentration changes in the feed and permeate. This application was, however, pH dependent and it was important that the pH of all solutions were adjusted to the same values as the solutions used to obtain the calibration curves. The values of the E_4/E_6 ratios, determined at the different concentrations, were independent of the natural organic matter concentration, which confirmed previous results that light scattering does not contribute to the ultraviolet-visible light absorption, and that the E_4/E_6 ratios are the result of photon absorption by chromophoric groups (Baes and Bloom, 1990).

1.4.2 COLOUR REMOVAL BY ULTRAFILTRATION

Flux of feed solutions of natural brown water and commercial humic acid, followed the same pattern of rapid decline in the initial stages, followed by a more gradual decline, as has been previously observed with other solutions such as proteins (Suki *et al.*, 1984 and Maartens *et al.* (1998). The decline in the flux could have been caused by concentration polarisation and/or foulant adsorption from the filtration solution onto the membrane surface and pores. By measuring only the product flux it was difficult to determine the reversibility of foulant adsorption. Sometimes the foulant adsorption was reversible and the product flux could be rectified by a simple pure-water wash. On the other hand, when foulant adsorption was irreversible, an appropriate cleaning protocol was needed to restore the water flux through the membrane. The product flux was therefore used to indicate the extent of membrane fouling and to determine the quality of the permeate produced by these membranes. Pure-water flux measurements were used to determine whether or not the flux decline was a result of irreversible adsorption of substances onto or into the membranes. Pure-water flux measurements, after thorough flushing of the membrane with pure-water, confirmed that the flux decline was the result of irreversible membrane fouling. Comparison of the flux decline with natural brown water and a solution of commercial humic acid as feed solutions, yielded almost similar results. The overall decline in the pure-water flux after filtration of natural brown water was approximately 10% higher than that measured with commercial humic acid. This was because natural brown water was a heterogeneous mixture of small and large molecules, whereas the commercial humic acid had a more homogeneous content of large molecules only. Membranes were therefore fouled more rapidly by substances contained in natural brown water. With both solutions the adsorption of substances were, however, irreversible and specific cleaning protocols were needed to restore the flux.

Changes in the natural organic matter concentrations of the natural brown water and commercial humic acid feed solutions during filtration could be ascribed only to adsorption onto or into the membranes as a feed-and-bleed system was used, and adsorption of natural organic matter onto the system without the membranes was negligible. Changes in natural organic matter concentrations in the feed solutions were measured to monitor the magnitude of membrane fouling/adsorption. It was, however, taken into account that a small fraction of natural organic matter adsorbed reversibly onto the membranes and that the exact amount of foulant adsorption was therefore slightly less than calculat. This technique was, however, a valuable tool for measuring and comparing the extent of commercial humic acid and natural organic matter adsorption onto the membranes. The decline of the natural organic matter concentration in the feed solutions over a period of 300 min indicated that approximately 24% of the natural organic matter in the natural brown water and commercial humic acid feed solutions had been adsorbed onto the membranes. During the second and third filtration periods from 300 to 600 and 600 to 1 200 min, changes in the feed concentrations were much smaller indicating that foulant adsorption was saturating and mainly occurred during the initial stages of filtration. During the initial period substances adsorbed onto the membrane to form a foulant layer. The thickness of this fouling layer depended on the shear stresses imposed by cross-flow velocity which prevented an increase in the foulant layer after a certain stage.

The natural organic matter concentration in the permeate solutions also decreased throughout the filtration period of 1200 min. This occurred because the membrane pores were initially unfouled and allowed certain molecules in the feed solutions to pass through. The adsorption of foulants onto the membrane surface and into the pores, however, caused the average size of the pores to decrease, which reduced the quantity of substances from the feed-water solutions that passed through the membranes. This observation, that colour retention by membranes was enhanced by fouling, with colour retention initialy being lower than that measured at the end of the experiment, was important. From these results it could be concluded that there was a critical membrane pore size that would ensure effective removal of colour from natural brown water.

Flushing with pure-water before each measurement of pure-water flux increased the natural organic matter concentration in the permeate slightly; an indication of the small effect of the reversibly adsorbed substances on the permeability of the membrane. An increase in the natural organic matter concentration of the feed solutions, after 300 min of filtration, showed no effect on the levels of natural organic matter in the permeate. Again, from these results it was clear that the crucial changes to the membrane, caused by irreversible fouling, occurred during the first few minutes of filtration. These effects were more marked with commercial humic acid as feed solution than with natural brown water. The reason for this was that the average molecular mass of commercial humic acids (as indicated by the E_4/E_6 ratio), was larger than that of the humic substances in natural brown water.

The assumption that the pore size of the membranes decreased as a result of the adsorption natural organic matter, was supported by the E_4/E_6 ratios of the natural brown water permeate solutions initially, and after 300 min of ultrafiltration. The ratio increased significantly during the filtration period from 0 to 300 min which indicated that, towards the end of the filtration period, only smaller molecules passed through the membrane. E_4/E_6 ratios for permeate solutions arriving from the commercial humic acid feed solution could not be calculated accurately due to extremely low absorption of light at 656 nm in these solutions. This confirmed assumptions that the molecules in the commercial humic acid samples were larger and had a more uniform molecular mass distribution than that of natural brown water. The large molecules were almost totally rejected by the membrane which accounted for the low ultraviolet-visible light absorbance by the permeate solution. The E_4/E_6 ratios of both the feed solutions increased slightly, which indicated that the large molecules or large molecular complexes were adsorbed onto the membranes first and the smaller molecules remained in solution.

1.4.3 MEMBRANE CLEANING

Membrane cleaning can be approached in a number of different ways. One technique is to minimise the adsorption of substances onto the membrane, thereby reducing the amount of cleaning agent required as well as the frequency of cleaning. There is, however, no technique that can prevent certain substances, such as proteins, lipids and phenolic substances, from adsorbing onto membranes. In most membrane filtration processes some kind of cleaning protocol is therefore required.

The aim of any cleaning protocol is to remove the adsorbed substances from the membrane surface and pores, so as to increase the flux of water through the membrane.

Pure-water flux measurements showed that the natural organic matter present in brown water solutions, adsorbed irreversibly onto or into the membranes. This changed the permeability of the membranes and led to an irreversible decline in the flux. The adsorption of substances from these solutions, however, had the advantage that colour retention by the fouled membranes increased significantly with the reduction in the pore size by adsorbing substances. These two factors could give rise to some controversy as to whether or not membranes should be cleaned regularly. From one aspect, regular membrane cleaning is necessary to remove foulants before the adsorbed layers become too thick and compressed. Under high operating pressures foulant layers which are not removed regularly, can form impenetrable layers that can later be removed only with high risk of membrane damage. Regular membrane cleaning will increase the overall water flux, but will reduce colour retention. Despite the advantage of higher colour retention by natural organic matter fouled membranes, foulant removal is necessary to restore and maintain flux.

From the literature it is apparent that alkaline cleaning solutions can be used to extract natural organic matter from soil samples (Kipton *et al.*, 1992). Consequently, alkaline cleaning solutions were used with great success to remove the natural organic matter foulants on ultrafiltration membranes. Solutions containing NH_3 could remove some of the foulants, but it was clear that such solutions alone could not totally restore the flux. A 0.1% Triton X100® solution was therefore combined with NH_3, and as previously observed by Maartens *et al.* (1996^b), the pure-water flux increased to levels higher than those for unfouled membranes. A possible explanation for this was that the Triton X100® not only removed the foulants, but also adsorbed onto the membrane and caused a more open or more flexible membrane structure which resulted in a higher pure-water flux. This explanation was supported by observations of steady decline in flux after lengthy flushing with pure-water. As the Triton X100® was removed the swelling effect, or some of the membrane flexibility induced by the detergent, decreased with a reduction in the pure-water flux. The pure-water flux, after prolonged flushing, however, remained higher than that of an unfouled membrane. The same result was obtained with other detergent-based cleaning methods.

With an increase in the pure-water flux after cleaning there was also a decrease in colour retention which clearly indicated that adsorbed substances were removed by the cleaning agents to allow more and larger molecules to pass through the membrane. Some of the detergent solutions, such as Triton X100®, not only increased the pure-water flux values to above normal levels, but also decreased the colour removal efficiency of the membranes. This was confirmed by the lower E_4/E_6 ratios of permeates after cleaning. A decrease in the ratios indicated an increase in the average size of the natural organic matter molecules that passed through the membrane. Again, this effect of the cleaning solutions was more marked when Triton X100® was present in the cleaning agent indicating that detergents, such as Triton X100®, increased the permeability of the polysulphone ultrafiltration membranes.

Refouling of cleaned membranes occurred within 1 h after the start of natural brown water filtration. Colour retention and E_4/E_6 ratios of the permeate increased in parallel with the re-adsorption of foulants onto the membrane. Although the pure-water flux values after some of the cleaning trials were much higher than others, colour retention by all cleaned membranes was about the same.

1.4.4 FEED-WATER AND MEMBRANE PRE-TREATMENT: METHODS TO REDUCE FOULING BY NATURAL ORGANIC MATTER

The presence of unsaturated compounds (substances with delocalised electrons) in natural brown water provided an easy method to study natural organic matter in solution with the aid of ultraviolet-visible light spectroscopy. Ultraviolet-visible light spectroscopy can be used to determine changes in the concentration and average size of soluble natural organic matter molecules in the water. The pH of the solution, however, influences the solubility and the particle size of these natural organic compounds, so that the pH of the solution also affects the ultraviolet-visible-absorbance (Gressel *et al.*, 1995). Ultraviolet-visible light analyses of natural brown water showed an increase in light absorbance with an increase in the pH of the solution. At low pH values large complexes form between protonated natural organic matter molecules. These complexes are less soluble and precipitated from the solution to give a subsequent 66% reduction in the ultraviolet-visible-absorption. The precipitated complexes represent the humic fraction of natural organic matter. With the humic acid fraction removed from solution, most of the resultant ultraviolet-visible-absorption can be ascribed to the soluble fulvic fraction of natural organic matter. Some humic acid, however, did not precipitate from the solution and therefore could contribute to the ultraviolet-visible-absorption. At higher pH values the carboxyl functional groups of the humic acid and fulvic acid ionised, were more soluble and absorbed more light.

E_4/E_6 ratios are also sensitive to changes in the pH, apparently because the size of the molecules are influenced by the pH (Chen *et al.*, 1977). This influence is particularly noticeable at very low and high pH values. At low pH values the large complexes precipitated from the solution leaving only the smaller molecules with higher E_4/E_6 ratios. At high pH values intra molecular repulsion, due to ionisation, resulted in very soluble large molecules with low E_4/E_6 ratios. At pH values ranging from 4.5 to 9 there was statistically no difference in the E_4/E_6 ratios.

Complexation and precipitation with metal-ions were used as pre-treatment to reduce the concentration of natural organic matter in solution. The natural organic matter concentration was reduced by 73 % and 43 % after the addition of $AlCl_3$ and $CaCO_3$ respectively. These results proved that the addition of metal-ions can be used to reduce the natural organic matter concentration prior to ultrafiltration.

The E_4/E_6 ratios decreased after the addition of metal-ions because not all the large natural organic matter-metal complexes precipitated from the solution. These complexes increased the average size of the natural organic matter molecules and subsequently increased the light absorption at 465 nm. In both cases the addition of alkaline metal salts increased the pH of the natural brown water solution from 4.5 to 6 which could also have contributed to the decrease in the E_4/E_6 ratios. Natural organic matter molecules are more compact in a solution of high ionic strength. In addition, Jucker and Clark, (1994) showed that calcium ions screen lateral electrostatic repulsion in the adsorbed layer and it was shown that calcium is adsorbed in proportion to the amount of humic acid adsorbed. The calcium acts as a bridge between the membrane surface and the negatively charged humic acid molecules.

1.4.5 ULTRAFILTRATION OF NATURAL AND TREATED BROWN WATER

The flux of water through capillary polysulphone membranes, measured during the filtration of natural brown water, followed a pattern of rapid flux decline in the initial stages followed by more gradual decline. The same trend has been experienced with other filtration solutions such as proteins (Fane *et al.*, 1987). In this study it was proved that the pH of the natural brown water feed solution played an important role in the amount of foulants that adsorbed onto the membrane and the resultant flux decline. Dissimilar flux decline patterns were observed at the different pH values. The flux decline measured at pH 7 was much lower than that at pH 2 and 9.

The decline in the product flux, within the first 15 minutes of filtration, at pH 2 can be ascribed to the precipitation effect of humic acid. At pH 2 and below protonated humic acid molecules form large, less soluble, complexes. Most of these complexes precipitated from the natural brown water, leaving the small more soluble fulvic acid molecules in solution. The large humic acid complexes were, however, not precipitated quantitatively from the feed solution. During ultrafiltration the effect of concentration polarisation concentrated these remaining large humic acid particles near the membrane surface to such an extent that it precipitated. These precipitating complexes adsorbed onto/into the membrane surface and pores and subsequently increased the flux decline. The greater flux decline at pH 9 was caused by increases in the average size of the molecules and charge repulsion forces. At pH 7 natural organic matter molecules contain approximately equal numbers of COO- and COOH groups and act as pH buffers. This stable conformation of natural organic matter lowered the interactive forces between the membrane surface and the foulant molecules.

It is often difficult to prove whether observed changes in the product flux are induced by irreversible foulant adsorption. When using product flux measurements, other factors such as reversible fouling, concentration polarisation and the effect of different pH values on the membranes should be taken into account. The flux of pure-water through the membrane, before and after fouling, was therefore used to measure and compare the extent of irreversible fouling at different pH values. Pure-water flux measurements, after filtration at any pH and thorough pure-water flushing, will be influenced only by the extent of irreversible foulant adsorption. Any temporary effects induced on the membrane permeability, by concentration polarisation and the pH of the feed solution during filtration, are ruled out by pure-water flux measurements. Changes in the pure-water flux after 300 minutes of filtration were of the same order as that of the product flux. This observation identified irreversible foulant adsorption as the principal reason for flux decline. Pure-water flux values further indicated that effects induced by pH changes, were caused by the dissimilar adsorptive character, conformation and behaviour of natural organic matter at different pH values.

Water colour and natural organic matter concentration are often measured by means of ultraviolet-visible-absorbance at 245 nm, a thecnique used in previous filtration studies to measure the natural organic matter concentration of both the natural brown water feed and permeate solutions (section 3.1). Experiments, using a feed and bleed filtration system, showed changes in the absorbance at 254 nm of both the feed and permeate solutions. The changes in the feed water absorbance, which is also a measure of the natural organic matter concentration, can be attributed to adsorption onto/into the membrane surface and pores. Adsorption onto the membrane test equipment when there were no membranes present, was found to be minimal. Changes in the permeate absorbance or natural organic matter concentration were attributed to changes in the pore size of the membranes. Membrane pore sizes decreased due to blockage by adsorbed foulants allowing only smaller and therefore less, natural organic matter molecules to pass through the membrane.

In this study, changes in the UV absorbance at 254 nm of the feed and resultant permeate solutions were measured at different pH values. The variation in the spectrophotometric character of natural organic matter at different pH values, however, made it difficult to determine the exact amount of natural organic matter adsorbed onto the membranes. The amount of foulant adsorption or changes in the membrane pore size, at different pH values, were therefore determined from the changes in the ultraviolet-visible-absorbance of the feed and permeates. At pH 7 the absorbance of the natural brown water feed decreased by 1.5% over a 300 min filtration period. This value, compared with 98%, 92% and 60% reduction at pH 2, 3 and 9, respectively, indicated a totally different adsorption pattern at different pH values. These results correlated well with earlier findings of dissimilar pure-water flux and product flux decline rates at different pH values. Diverse quantities of natural organic matter adsorbs onto/into the membrane at different pH values under the same operational conditions.

During filtration, absorbance values of permeate solutions decreased significantly at all pH values. Changes in the permeate absorption at 254 nm are explained as follows: substances from the feed water adsorbs or precipitates onto the membrane surface and into the membrane pores during filtration. The size of the adsorbing molecules determined whether substances adsorbed into the pores or only onto the membrane surface. The sizes of these molecules, on the other hand, are influenced by the pH of the solution. At a low pH the small molecules caused blocked the pores while larger molecules, at higher pH values, induced surface fouling. Adsorbed foulants caused membrane pores to become smaller allowing only smaller molecules to pass through. Pure-water flux measurements showed foulant adsorption to be irreversible at all pH values. The magnitudes of these effects depended on the amount of foulant adsorption which was in turn depended on the pH.

The rate of the permeate absorbance decline at 254 nm is explained by two different yet intervening ways. Firstly, at some pH values the rate of fouling and amount of foulant adsorption were higher, and blocked membrane pores much faster. Second, smaller amounts of small particles adsorbed into membrane pores causing a swift decrease in the membrane pore diameter with an immediate effect on the flux of water through the membrane.

At pH 7, no substantial adsorption of natural organic matter from the feed solution was observed and the pure-water flux decreased only by 30% over a 300 min filtration period. This does not explain the 73% decrease in the permeate adsorption within the initial minutes. During the filtration of natural brown water at pH 7 additional factors were, therefore involved that prevented natural organic matter to adsorb onto or passing through membranes. These factors included repulsion between positively or negatively charged carboxyl groups of the neutral natural organic matter molecules and the membrane, increased solubility of natural organic matter that reduced the adsorption potential, temporary changes in the membrane character and changes in the conformational structure of natural organic matter at pH 7.

Substances that adsorbed onto/into the membrane affected the permeate concentration and thereby caused changes in the colour rejection. Use of equation 1 to calculate the percentage colour removal, showed that the initial retention of 80% increased to above 90% irrespectable of the feed pH. If, therefore foulant adsorption and flux decline are not taken into account removal of colour will be effective at any feed-water pH. If, however, fouling is taken into account, some of the pH values investigated will drastically reduce the economical potential of the membrane filtration operation.

The increase in the E_4/E_6 ratios of the feed-water during the 300 min filtration period, at pH 9, 8, 7 and 4, indicated that the larger molecules in these feed solutions adsorbed onto the membranes. The increase in the E_4/E_6 ratios were, however, pH dependent with the largest increase at pH 9. At pH 8, 7, and 4 the ratios increased only slightly. The decreases in the E_4/E_6 ratios at pH 2 and 3 are explained by one of the following arguments. First, smaller molecules in the feed solution adsorbed into the membrane pores, causing an increase in the average size of the remaining molecules. Second, at low pH values natural organic matter molecules in the feed-water formed large complexes with a subsequent increase in the average molecule size.

The light-absorbance at 656 nm was high enough only for filtration runs at pH 8 and 4, to enable changes in E_4/E_6 ratios of the permeate to be calculated. The calculated E_4/E_6 ratios confirmed the assumption that membrane pore size decreased with the adsorption of natural organic matter. The increase in the ratios at pH 8 and 4, over a 300 min filtration period, could be ascribed to the increased selectivity of the membrane caused by the adsorbed foulants. In addition, adsorbed material reduced the molecular mass cut off point of the membrane.

Filtration with feed-water solutions at pH 2 and 7 for an additional 700 min resulted in almost no further decrease in the product flux or pure-water flux. This confirmed previous results that the majority of foulant adsorption and flux decline occurred within the first minutes of filtration.

To determine the reversibility of the effects of the feed pH after 1000 min of filtration, the pH values of the feed solutions were changed from 2 to 7 and from 7 to 2, respectively, and filtration continued for an additional period. Changing the pH from 2 to 7 induced little changes in the product flux or pure water flux, proving the irreversible nature of the initial fouling at pH 2. Reduction of the feed pH from 7 to 2 caused a drastic decrease in the product flux and pure-water flux measurements. This indicated that at pH 7 only a small amount of foulants adsorbed onto the membrane. When the adsorptive properties of fouling substances was increased, by changing the pH of the feed from 7 to 2, foulants adsorbed onto the membranes with marked effects on the product flux and the pure-water flux.

There were no changes in the spectrophotometric character of the permeate after the pH of the feed was changed from 2 to 7. Thus no further foulant adsorption or any changes in the membrane permeability character occurred. In contrast, when the pH of the feed water was changed from 7 to 2 the ultraviolet-visible-absorbance of the feed solution decreased indicating natural organic matter adsorption. There were no changes in the spectrophotometric character of the permeate when the pH of the feed was changed to 2.

1.4.6 EFFECT OF WATER TREATMENT ON ULTRAFILTRATION

The complexation of metal-ions with natural organic matter was used as a pre-treatment to reduce the natural organic matter concentration in the feed water prior to ultrafiltration. By comparing the ultraviolet-visible-absorbance of natural brown water solutions before and after metal-ion pre-treatment, the effectiveness of these pre-treatment steps was measured. Reduction of the natural organic matter concentration with $CaCO_3$ and $AlCl_3$ was 43% and 73%, respectively. The E_4/E_6 ratios of natural brown water were also influenced by pre-treatment with metal-ions.

A reduction in the natural organic matter concentration, by pre coagulation with metal-ions before filtration, could not reduce or prevent membrane fouling. The percentage declines in the pure-water flux were 64% and 70% with the $CaCO_3$ and the $AlCl_3$ pre-treated solutions, respectively. This indicated more serious irreversible membrane fouling when compared with the 45% decline when untreated natural brown water was filtered. Large changes in the natural organic matter concentration of the metal-ion pre-treated water, over the 300 min period, indicated that more substances adsorbed onto the membranes when the water was pre-treated. Higher natural organic matter adsorption could be explained by complexation of the metal-ions that remained in solution with the natural organic matter in the feed. During filtration, the increase in the natural organic matter concentration near the membrane surface by concentration polarisation increased the potential for complex formation. Complexes were adsorbed onto the membranes with a subsequent decrease in the flux and an increase in selectivity. The colour retention, after metal-ion pre-treatment, was greater than 90%. It can therefore be concluded that heavy metal pre-treatment of brown water has no significant advantage for ultrafiltration applications.

1.4.7 EFFECT OF MEMBRANE COATING ON ULTRAFILTRATION

Membrane pre-coating can increase or reduce the flux of pure-water through a membrane. These changes are induced by changing the hydrophilicity and/or heterogeneity and/or selectivity of the membrane. Pre-coating of capillary polysulphone membranes with Triton X100®, increased the pure-water flux. Triton X100® adsorbed onto/into the membrane made it more flexible, and increased hydrophilicity (Maartens *et al.*, 1996[b]). These effects enhanced the passage of water through the membranes. The adsorption effect of Triton X100®, however, was not completely irreversible as was proved by the decrease in the pure-water flux after a pure-water wash. Pre-coating with Pluronic® F108, on the other hand, reduced the pure-water flux indicating that Pluronic® F108, in contrast with Triton X100®, restricted membrane pores. The 2% increase in the pure-water flux after thorough flushing of the membrane with pure-water, also indicated reversible adsorption of Pluronic® F108.

Triton X100® pre-coating of membranes, however, did not prevent substances from the natural brown water feed solution to adsorb irreversibly onto/into the membrane causing a decline in both the product flux and pure-water flux. The 24% decrease in the natural organic matter concentration of the feed solution confirmed that membranes, pre-treated with Triton X100®, performed better and fouled less than untreated membranes.

In conjunction with the enhanced performance, the quality of the permeate resulting from natural brown water filtered through membranes treated with Triton X-100, has to be considered. The ultraviolet-visible-absorbance of the permeate solution decreased by 78% over a filtration period of 1800 min. This data converted to percentage colour retention by equation 1, indicated that filtration with the Triton X100® pre coated membranes initially removed 71% of the colour producing natural organic matter. After 1800 min of filtration, the colour retention was 91% (see Table 1.5). Triton X100®, therefore, increased the membrane permeability and the water flux through the membrane, but decreased the initial selectivity of the membrane. More and larger substances were allowed to pass through the membrane during the early stages of filtration.

Pre-treatment with Pluronic® F108 produced the opposite effect in flux and colour retention. Initial flux was low, but increased throughout the experiment. A large portion of the Pluronic® F108 molecules adsorbed reversibly onto the membrane to reduce pore size. After a while Pluronic® F108 molecules started to desorb from the membrane with a subsequent increase in the pore size and an increase in flux. The coating effect of Pluronic® F108, however, was not lost during this steady desorption process and the flux remained lower than that for an untreated membrane.

The ultraviolet-visible-absorption of the feed solution, filtered through Pluronic F108 treated membranes, stayed constant throughout the experiment, indicating little or no natural organic matter adsorption. It thus seems that pre-coating with Pluronic® F108 prevented natural organic matter from adsorbing onto the membranes. The fact that less organic matter adsorbed onto membranes, pre coated with Pluronic® F108, indicated that the fouling substances in natural brown water were hydrophobic. These hydrophobic substances could not adsorb onto the more hydrophilic surface of the membranes pre-treated with Pluronic® F108.

The same contrasting effect was observed in the spectrophotometric changes of the permeate solutions associated with the Pluronic® F108 pre coated membranes. The ultraviolet-visible-absorbance of the resultant permeate solution increased as the pores became less clogged due to the desorption of the Pluronic® F108. The increase in the average molecular size of molecules in the permeate was confirmed by a slight decrease in the E_4/E_6 ratios of the permeate.

1.5 CONCLUSIONS

1. Brown water strongly absorbs ultraviolet light; a characteristic that can be used to measure the concentration of natural organic matter in brown water.
2. The ratio of the absorbance at 465 and 656 nm (E_4/E_6) is a useful technique to estimate the relative sizes of the molecules that constitute natural organic matter in brown water.
3. The ultraviolet-visible-absorbance of natural organic matter in brown water can be used to measure the efficiency of colour reduction and pre-treatment protocols. The ultraviolet-visible-absorbance is, however, pH-dependent and therefore the pH of samples should be the same for comparative studies.
4. Colour removal with capillary polysulphone membranes was effective and with the right choice of membrane configuration, operating and cleaning conditions the process can be applied successfully and economically for the purification of brown water.
5. Membrane fouling negatively influenced filtration performance and membrane cleaning and fouling prevention were essential for optimal quality product water delivery.
6. The pure-water flux through a membrane, fouled by natural organic matter, could be restored by the use of alkaline solutions and detergents such as Triton X100® in cleaning regimes.
7. Membrane fouling could be reduced, but not prevented, by adjustment of the pH of the feed solution.
8. Water pre-treatment with metal-ions, to reduce the natural organic matter concentration in the feed solutions, did not prevent foulant adsorption. On the contrary, results obtained in this study showed that metal-ion treated feed-water caused more extensive fouling than untreated feed solutions.
9. Pre-coating of membranes with Triton X100® and Pluronic® F108 effectively

reduced foulant adsorption, but influenced the colour retention and flux of water through the membranes.

1.6 RECOMMENDATIONS

1. The development of an online ultraviolet detector to monitor colour removal and membrane integrity continuously during filtration.
2. Evaluation of cleaning protocols for membranes fouled during brown water filtration on an industrial scale.
3. Further development of membrane coating systems to prevent irreversible fouling.

PULP AND PAPER EFFLUENT

2.1 INTRODUCTION

Pulp and paper mills, as many other industries, consume large amounts of water during the manufacturing process. In the pulp and paper industry this large volume of water is converted to a highly polluting effluent. Disposing of such a large volume of polluting effluent is difficult and costly. An obvious cost effective and environmentally friendly alternative would be to recycle the effluent water back into the plant. This can, however, only be achieved after extensive treatment of the effluent.

A modern technique for effluent purification is ultrafiltration through specially designed polymer membranes. Due to the high capital cost involved in the manufacture of specially designed polymer membranes, the use of membrane technology for effluent purification is, however, only economically feasible if a long membrane life span can be ensured. The harsh nature and complexity of pulp and paper effluent causes clogging of ultrafiltration membranes which reduces the applicability of this water purification technique in the pulp and paper industry. If the fouling of membranes can be prevented or redeemed in a satisfactory way, ultrafiltration through polysulphone membranes will remain an environmentally friendly and economically feasible option for effluent treatment in the pulp and paper industry.

The obvious advantages ultrafiltration offers for industrial effluent purification prompted the Water Research Commission to initiate research into possible cleaning regimes for ultrafiltration membranes, since current cleaning techniques are ineffective, damaging to membranes and sometimes pose further pollution problems. The use of enzymes as cleaning agents for membranes, fouled in biological effluent, is an attractive option as enzymes are highly specific catalysts, will not harm the inert polymer membranes and do not constitute additional pollution problems.

This part of the study served as a case study in which the Membratek® effluent purification plant was used to determine if membranes, fouled during ultrafiltration treatment of effluent from the Mondi Kraft paper mill near Piet Retief, could be cleaned using enzymes as the major foulant removing agents.

It is important to note that in this study conditions at one specific purification plant were investigated and results obtained are directly applicable only to this plant. The indications are, however, that the experimental strategy used here could be applied to investigate the situation at any other polymer membrane purification plant.

2.1.1 THE USE OF ULTRAFILTRATION MEMBRANES FOR THE TREATMENT OF EFFLUENT FROM THE PULP AND PAPER INDUSTRY

Chemical pulping of wood is mostly carried out either according to the Kraft (sulphate) process or the sulphite process. The Kraft process is presently the most popular method in the paper industry. The purpose of both these pulping methods is to remove lignin to facilitate fibre separation and to improve the paper making properties of the fibres.

A schematic diagram of the pulping process at Mondi Kraft in Piet Retief is shown in Figure 2.1 (E. Dlamini, Mondi Kraft, Piet Retief). In the main processing chamber (digester), wood chips are boiled in concentrated sodium hydroxide, sodium sulphite and sodium carbonate (40% consistency[1]).

This process leads to the partial solubilisation of the lignin and hemicellulose fractions. The aqueous phase is subsequently drawn off, leaving the cellulose fibres as a pulp that can then be used for the manufacture of paper and paper products.

Neither the Kraft nor the sulphite process removes all the lignin from the pulp. About 5-10% of the original lignin remains in the pulp and the presence of this residual lignin calls for a multistage bleaching process for the manufacturing of white paper. The manufacturing of brown paper, cardboard and other paper products does not necessitate bleaching and therefore these processes are not shown in Figure 2.1.

For softwood Kraft pulps, bleaching is normally accomplished by successive treatments with chlorine (C1), alkali (E1), chlorine dioxide (D1), alkali (E2) and chlorine dioxide (D2); a process known as the CEDED bleaching sequence. Often a hypochlorite stage (H) may be inserted between the E1 and D1 stages, giving a CEHDED sequence (Kringstad and Lindström, 1984).

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[1] The estimated fluidity of the wood chips and concentrated alkali mixture is expressed by the paper industry in terms of consistency. It is not necessary to measure the exact mass per volume ratio of wood chips to alkali in this industrial process.

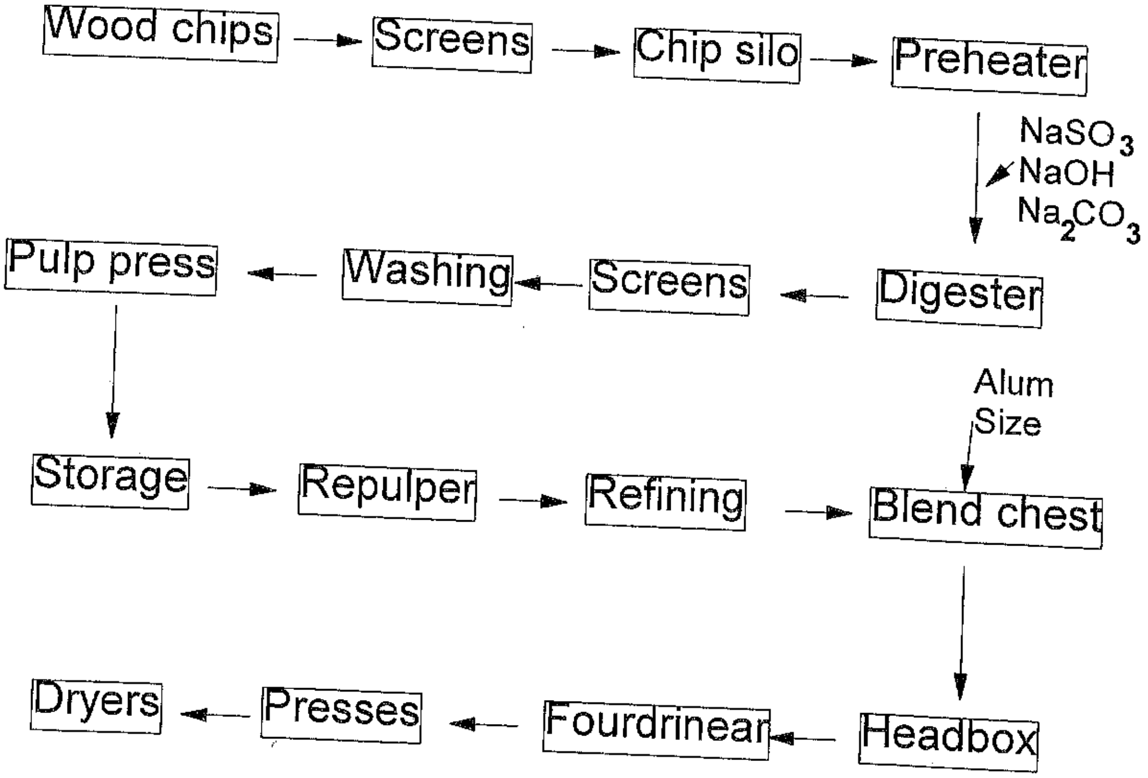

Figure 2.1 Process used at Mondi Kraft for the manufacturing of pulp and paper products E. Dlamini, Mondi Kraft). At this paper mill, wood chips made from several *Pinus* and six *Eucalyptus* species are used. These include *Eucalyptus grandis*, *E. saligna*, *E. nitens*, *E. smithii*, *E. fastigata* and *E. elata*. Approximately equal volumes of Eucalyptus and Pine wood are used. The upper aqueous phase is drawn off just after the digester and pumped into maturation ponds. All the processes after the pulp press are involved only in the paper making process and does not contribute to the formation of effluent

The upper aqueous phase which is drawn off from the pulp, is neutralised by the addition of sulphuric acid and pumped into maturation ponds. During neutralisation of the upper phase some of the lignin and hemicellulose compounds precipitate and settle, forming a thick sludge at the bottom of the maturation ponds. Precipitation of certain fractions of the neutralised effluent resulted in a distinction being made between raw effluent and matured effluent. Matured effluent refers to the upper aqueous phase that is drawn off the maturation ponds after precipitation and is the only part of the raw effluent that is treated in the purification plant. The sludge is burned in incinerators, serving as a heat source for the alkali extraction process.

Neutralisation of the effluent with sulphuric acid also leads to the formation of organo-sulphur compounds, generating a pungent smelling effluent and increasing the pressure on the pulp and paper industry to develop effective effluent treatment strategies.

Another factor that adds to the increased need for efficient effluent treatment, is the chemical oxygen demand and biological oxygen demand of pulp and paper effluent. These values give an indication of the amount of oxygen needed to break down or inactivate the compounds present in a solution. Environmental problems arising from the discharge of mixtures with high chemical oxygen demand and biological oxygen demand values into river systems are that these mixtures decrease the amount of oxygen available for other processes, resulting in the suffocation of organisms such as fish and plants.

Approximately 10 m^3 of E-stage effluent is produced per ton of pulp. The pulp and paper industry is therefore faced with the challenge of substantially reducing the discharge of conventional and toxic pollutants to the receiving environment, without compromising competitiveness. Most pollutants are present in the first sodium hydroxide extraction filtrate (E-stage effluent). These compounds are usually combined chemically with the sodium hydroxide to form sodium salts of resinous and other organic acids (Bhattacharjee and Bhattacharya, 1993). If a paper mill performs bleaching processes, the majority of the pollutants occur in the first chlorination filtrate (C-stage effluent), while only minor pollutants originate from the other bleaching stages.

2.1.2 PULP EFFLUENT COMPOSITION

More than 250 different substances have been identified in bleached Kraft mill effluent and 200 of these are chlorinated compounds. The composition of Kraft mill and bleached Kraft mill effluent varies from mill to mill, due to variations in the clean water available in each geographical area, as well as the tree species being used at a given mill at a particular time. It is, however, possible to make some generalisations, based on numerous reports. Some of the chlorinated organic compounds identified include: chlorinated resin acids, chlorophenolics, chloroguaiacols, chlorocatechols, chlorinated dioxins and chlorinated furans (Hodson *et al.*, 1992).

Chemical oxygen demand values of solutions describe some aspects of the impact of these mixtures on the receiving environment. Other characteristics of solutions that also give an indication of the detrimental effects of these solutions to the environment, include the sodium content, the sulphate content, the total amount of solids, the concentration of substances causing colour or bad odour and the total organic content. When chlorine is present in or added to a solution, the chlorine binds to the organic compounds in the solution. These organo-chlorine complexes can be measured and expressed as the amount of adsorbed organic halogens (Jönsson, 1987). According to Jönsson (1987), the adsorbed organic halogens values of bleached Kraft effluent are equivalent to what the total amount of organics would have been in non-bleached Kraft effluent. Like the effluent composition, the values of these pollution indicators vary from mill to mill. Previously reported values are summarised in Table 2.1.

From the chemical oxygen demand values in Table 2.1 it is clear that the effluent treated by the Membratek purification plant is comparable to effluents commonly produced by the pulp industry and that this facility can be used as a model for investigating membrane fouling phenomena as well as cleaning techniques applicable to the pulp and paper industry.

Table 2.1 Important pollution related values reported for paper mill effluents.

Reference	**Paper mill**	**COD (mg/l)**	**Sodium (mg/l)**	**Total solids (mg/l)**	**AOX* (mg/l)**	**Colorants (mg/l)**	**Sulphate (mg/l)**
Dorica *et al.* (1986)		6200	3000	N/A	N/A	N/A	N/A
Zaidi *et al.* (1992)	Performing bleaching	2150	N/A	N/A.	85	N/A	N/A
Muratore et al. (1983)		2500	N/A	N/A	N/A	20 000	N/A
Bhattacharyee and Bhattacharya (1993)	Surat, India	N/A	8420	N/A	N/A	N/A	N/A
Inspectorate Mondi PTY Ltd	Piet Retief	8800	2285	8615	N/A	N/A	250

* Adsorbed organic halogen values are equivalent to amounts of organically bound chlorine in bleached Kraft effluents. N/A (not available) indicates that those values were not supplied by the authors.

Kringstad and Lindström (1984) divided the pollutants in bleached Kraft effluent into high- and low-molecular-mass compounds.

It appears that in spent chlorination liquor (C-stage), about 70% of the organically bound chlorine is present as high molecular mass material (Mr > 1000). In the case of alkali extraction (E-stage) liquor, about 95% of the organically bound chlorine belongs to this class (Kringstad and Lingström, 1984). Determination of the elemental composition of these substances in C-stage liquor yielded $C_9H_9O_4Cl$, $C_9H_{10}O_8Cl$ and $C_{10}H_{14}O_7Cl$ as the representative formulae, while $C_{14}H_{15}O_8Cl$ and $C_{14}H_{10}O_9Cl$ were the most abundant species in E-stage effluent (Kringstad and Lingström, 1984).

About 30% of the organically bound chlorine in C-stage liquor and approximately 5% in E-stage liquor is of relative low molecular mass (Mr < 1000) (Kringstad and Lindström, 1984).

Phenolic compounds may also be formed from carbohydrates that are present in black liquor. Chlorinated catechols occur primarily in C-stage liquor, whereas chlorinated guaiacols and vanillins are found in E-stage liquor (Kringstad and Lindström, 1984).

Both high- and low-molecular mass compounds can further be classified as acidic, phenolic or neutral. The acidic compounds consist of five categories of acids, including fatty, hydroxy, dibasic, aromatic and resin acids. Formic and acetic acids are quantitatively the most important fatty acids, while glyceric acid predominates the hydroxy acids. The hydroxy acids are mostly oxidation products of carbohydrates. Dibasic acids like oxalic, malonic, succinic and malic acid are present in considerable quantities in both types of spent liquor. Three kinds of aromatic acids are formed from residual lignin, i.e. phenolic, catecholic and guaiacolic acids (Kringstad and Lindström, 1984).

Methanol and various hemicelluloses are quantitatively the most dominant neutral compounds in spent liquor. Rani and Nand (1996) reported that hemicelluloses, including the plant structural polymer xylan, constitute 20-40% of black liquor.

2.1.3 TREATMENT OF PAPER MILL EFFLUENT

There are two main approaches to this problem i.e.; the prevention of toxic effluent production and innovative end-of-pipe treatment of effluent.

Preventive techniques include the treatment of the pulp before entering the paper mill to reduce the amount of effluent produced and the integration of physical/chemical separation processes into the pulp mill. Implementing new processes in an existing plant is, however, not always practical or economically viable. In addition, the production of effluent can only be reduced and cannot be prevented altogether. Disposing of the reduced volume effluent is, however, more practical and cheaper (Zaidi *et al.*, 1992).

Various end-of-pipe treatment strategies exist for pulp and paper effluent, with the possibility of recycling process water to the plant. These include aerobic and anaerobic treatments (Tirsch, 1990), lime and alum precipitation, adsorption, oxidation with hydrogen peroxide, with sodium hypochlorite and with chlorine, ion exchange resins, rapid infiltration ultrafiltration (Jönsson, 1987; Dorica *et al.*, 1986) and coagulation based processes (Dixon *et al.*, 1992).

The use of membrane-based processes have proved to be a very attractive alternative to the above-mentioned treatment methods, because it removes the need for adding additional expensive chemicals and it facilitates the recycling of water to the plant.

2.1.4 THE USE OF MEMBRANE BASED PROCESSES IN THE TREATMENT OF PAPER MILL EFFLUENT

The membrane processes most often used for the treatment of industrial effluents, such as paper mill effluent, are: (i) microfiltration, (ii) ultrafiltration, (iii) nanofiltration and (iv) reverse osmosis (Zaidi *et al.*, 1992).

Microfiltration is used for the filtration of particles in the 0.1 to 10 micrometer range which includes bacteria. Viruses and compounds causing bad odour, colour or taste of water can, however, pass through these membranes. Since Kraft black liquor contains colorants in fairly large concentrations, microfiltration is not used frequently in this application.

Due to the rapid progress made with the development of ultrafiltration and reverse osmosis purification systems, nanofiltration is also not often used in the purification of paper mill effluent.

Reverse osmosis membranes can retain particles as small as metal ions and aqueous salt solutions and are frequently used for water desalination. These membranes can, however, take only a limited load of particles in the feed solution, since membrane clogging occurs very easily. Although reverse osmosis alone is not an ideal treatment method for raw paper mill effluent, it has been used with great success in combination with ultrafiltration.

Specific attention will now be given to the use of ultrafiltration in the treatment of paper mill effluents, since this is the first membrane based method used at the Membratek effluent purification plant near Piet Retief. At this purification plant the effluent passes through an ultrafiltration process, followed by an ion exchange step and is subsequently purified through reverse osmosis membranes. The ultrafiltration membranes come into contact with the raw feed solution and consequently are fouled more severely than the reverse osmosis membranes that come into contact with a much cleaner feed stream. The fouling of the ultrafiltration membranes, as well as cleaning methods that can be used to reduce the fouling of these membranes, were therefore investigated

Ultrafiltration is a pressure driven process where a fluid is forced through a semipermeable membrane by the application of pressure on the feed solution. The selective passage of compounds through the membrane is made possible by pores of a given size. Ultrafiltration membranes are usually characterised by the molecular mass of compounds that will be retained by the membrane. In ultrafiltration these compounds range in size from 0.001 to 0.1 micrometers, or approximate molecular masses of 200 to 200 000.

The E-stage effluent is well suited for ultrafiltration treatment as most of the polluting substances are high molecular mass compounds which are readily removed by ultrafiltration (Jönsson, 1987).

Results obtained at various purification plants, using ultrafiltration for the treatment of E-stage paper mill effluent, are summarised in Table 2.2.

Table 2.2 Results obtained with the treatment of paper mill effluent by ultrafiltration alone, or a combination of ultrafiltration and reverse osmosis.

Authors or paper mill	Process	Colour removal	COD removal	BOD removal	AOX removal	Reduction in suspended solids	Reduction in dissolved solids
Zaidi *et al.*(1992), Dorica *et al.* (1986), Muratore *et al.* (1983)	Ultrafiltration alone	70-98%	55-87%	35-44%	85-91%	N/A*	N/A
Mondi Kraft at Piet Retief	Ultrafiltration alone	N/A	53%	N/A	N/A	85%	30%
Mondi Kraft at Piet Retief	Ultrafiltration and reverse osmosis	N/A	99%	N/A	N/A	100%	98%

* N/A indicates values that were not supplied by the specific authors.

Values obtained for the Membratek purification plant compare well with these values. Ekengen and co-workers (1991) also observed a decrease in toxicity of about 50% and a 90% removal of substances with a bio-accumulation potential after ultrafiltration (Ekengen *et al.*, 1991). High removal efficiencies of pollutants were obtained with a combination of ultrafiltration and reverse osmosis processes at various purification plants (Jönsson, 1987). This is also clear from the values reported in Table 2.2 for the effluent purification plant at the Mondi Kraft paper mill.

2.1.5 THE ROLE OF LIGNIN AND CARBOHYDRATES IN THE FOULING PROCESS

A well known phenomenon during membrane filtration is the initial flux decline during the first hours of operation (Jönsson, 1987; Ko and Pellegrino, 1992; Bhattacharjee and Bhattacharya, 1993).

The earlier work done on the use of ultrafiltration treatments of pulp mill effluents concentrated on the removal of colour rather than on the removal of specific toxins. High molecular mass chlorolignins from E-stage effluent were considered to be both the main source of colour as well as the most important foulants. As a result, only a few studies were conducted at the time to investigate the nature of foulants that cause the flux decline:

- Dorica *et al. (*1986) showed that the flux decline observed in the treatment of first-stage effluent (caustic bleach effluent) was caused by calcium deposits and possibly an oil-based defoamer used in the bleach plant during the trial period. Membrane regeneration was achieved by using a detergent. The flux increased, but could not be restored to the initial value. Further analysis of the deposits on the fouled membrane indicated that magnesium and calcium carbonate salts were the dominant substances, which were easily removed with 5 N HCl.
- Zaidi *et al.* (1992) indicated that organic species, such as chlorocatechols, may also partly be responsible for the observed flux decline. Negligible flux decline was observed during tests in which dehydroabietic acid were used as a model foulant, whereas significant flux decline was observed with sulphonated lignin and 4,5-dichlorocatechol.

An extensive literature search showed that the possibility of carbohydrates being major foulants has not been investigated to date. However, Ziobro raised the possibility that both carbohydrates and lignin derivatives could play a role in the production of Kraft colour (Ziobro, 1990). This opened the discussion on the role of carbohydrates in the fouling process and various researchers now consider carbohydrates as possible foulants (T.

Jeffreys from USDA, Forest Products Laboratory, Madison, Wisconsin, personal communication). Due to a lack of results published on the role of carbohydrates in the fouling of ultrafiltration membranes used in black liquor treatment, a summary of the role of carbohydrates in the production of pulp colour is given in the following section.

2.1.6 THE SOURCE OF COLOUR OF KRAFT PULP EFFLUENT

The colour of Kraft pulp is of environmental importance since it necessitates bleaching. Bleaching is usually achieved with chlorine. Toxic chlorinated organics are formed in the bleaching process and can be released into the environment. Although the compounds responsible for the colour of pulp plant effluent are probably biologically inactive because of their high molecular mass, they are still of environmental importance because they impart light-absorbing qualities to receiving waters which has an impact on the light-penetration properties of the water and affects the organisms that live in it (Kringstad and Lindström, 1984).

The high molecular mass compounds contain chromophoric groups which absorb light of certain wavelengths and cause the compounds to have a certain colour. Kraft effluents are usually brown to dark brown or black due to the presence of these chromophores and has consequently been named black liquor.

It is a well known property of aromatic nuclei to act as chromophores. It has therefore always been assumed that the colour of Kraft pulp and effluent must be due to aromatic nuclei. Although lignin and its degradation products are a good source of aromatic nuclei, Forsskahl *et al.* (reviewed by Ziobro, 1990) showed that they are not the sole source of aromatic residues in pulp effluent. Alen and Sjöström (reviewed by Ziobro, 1990) have isolated various condensation products of glycolic, lactic and 2-hydroxybutanoic acids from Kraft black liquor. Ziobro and co-workers analysed Kraft cooked glucose, Kraft cooked cellobiose and Kraft cooked glucose and xylose (1:1 mixture). All of the carbohydrates tested produced dark brown liquors and dark brown precipitates after the pH was adjusted to 3 (Ziobro, 1990). The aromatic compounds, phenol, cresol and ethylphenol were identified as chromophores in these liquors. This finding lead to the conclusion by Ziobro that the colour of Kraft effluent originated, primarily, from degradation products of carbohydrates and not only from lignin as was always assumed (Ziobro, 1990). This fact does, however, not exclude the possibility that the chemically stable lignin extracts may be responsible for the fouling of ultrafiltration membranes used in the purification of paper mill effluent.

2.2 EXPERIMENTAL

In order to address the fouling problems experienced by membrane water purification plants in the pulp and paper industry, an experimental strategy was required that would include an investigation of the compounds in the effluent that could be potential clogging agents of the membranes. Due to the very complex nature of the effluent a complete and exact analysis of the effluent was both impractical and of little value as not all of the approximately 250 compounds that could be present in the effluent, would be responsible for fouling (Hodson *et al.*, 1992). Furthermore, the composition of the effluent is subject to seasonal changes, as well as sporadic changes in the composition of the original clean water stream.

These factors led to our decision to characterise partially the pulp and paper effluent by ultraviolet-visible-spectrophotometry. This analytical technique was used to characterise both the matured effluent at the intake port of the water purification plant, as well as compounds that could be extracted off membranes that were fouled in the operational plant at Mondi Kraft in Piet Retief. The first experiment would indicate the nature of compounds in the effluent, while the second experiment would indicate the nature of the foulants adsorbed onto the membranes under operational conditions.

Since the exact conditions prevailing in the 500 m long membrane plant cannot be determined, we supplemented these experiments on commercially used membranes, with experiments on membranes fouled under controlled conditions. These conditions included a constant temperature, fixed contact angles with the effluent stream, constant flow rates, as well as the oppotunity to study static fouling as oposed to the dynamic fouling process found in the industrial plant.

During static fouling of a membrane, only the characteristics of the compounds and the surface characteristics of the membrane determine the adsorption of compounds that lead to fouling, allowing a study of fouling independent of the pressure used in a specific ultrafiltration process. During dynamic adsorption (where pressure is applied to force the fluid through the membrane), a process called the concentration polarisation effect comes into play. This effect is defined by Weber (1992) as: "... a phenomenon involving upgradient diffusive transport of solute away from the membrane in a direction opposite to permeate flow, due to the build-up of solute concentration at the membrane surface." The polarisation concentration effect makes the quantitative analysis of the dynamic fouling of tubular membranes more difficult than the analysis of static fouling of flat sheet membranes.

Membranes fouled under controlled conditions in a static adsorption process were used to study the effects of different enzymes as cleaning agents. Results from experiments carried out to characterise effluent composition as well as foulants adsorbed onto the membrane, led to the decision to evaluate the following enzymes in cleaning regimes: ligninases, cellulases, xylanases, pectinases and peroxidases.

Ligninases are unstable in solution and quite difficult to isolate and are therefore not commercially available. Consequently these enzymes had to be produced before they could be evaluated in membrane cleaning techniques.

2.2.1 CHARACTERISATION OF FOULANTS IN PAPER AND PULP EFFLUENT

Materials and methods. Matured pulp and paper effluent, obtained from Mondi Kraft, was diluted with distilled water until a spectrophotometric reading of less than 1 absorbance unit was recorded at a wavelength of 280 nm. The absorbance of the diluted effluent (500 µl) was recorded between 200-700 nm on a Beckman DU650 spectrophotometer.

Results: The spectrophotometric analysis of matured effluent is shown in Figure 2.2.

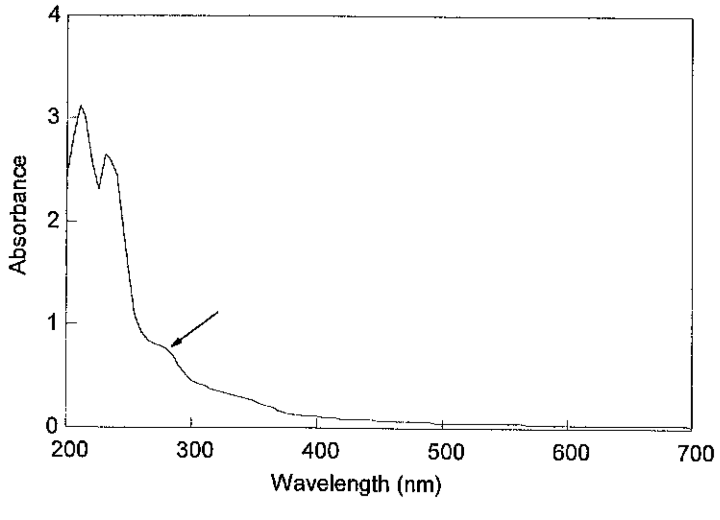

Figure 2.2 Spectrophotometric analysis of diluted paper mill effluent. The arrow indicates 280 nm.

Most of the substances in the effluent absorbed light of shorter wavelengths, in the ultraviolet light region. Absorbance maxima at 210 nm, 230 nm and 280 nm can be seen in Figure 2.5. Absorbances in the lower wavelength region (200 to 245 nm) can be attributed to a number of small molecules such as water, methanol, ethanol, dichloromethane and chloroform (Pasto and Johnson, 1969). The absorbance maximum at 280 nm, marked with an arrow, indicated that the nature of certain foulants are aromatic. The complexity of paper mill effluent resulted in a very complex absorbance pattern and no further direct deductions could be made from these results.

2.2.2 ALKALINE-SDS EXTRACTIONS OF MEMBRANE ADSORBENTS

Materials and methods. Tubular membranes, fouled in the industrial ultrafiltration process at the Mondi Kraft paper mill, were obtained from Membratek. Foulants were extracted by incubating membrane sections with a NaOH solution (50 ml, 0.5 M, 5% SDS). Samples were collected at 12 hour intervals, diluted ten times and analysed by ultraviolet-visible-spectrophotometry. In a pilot study the wavelengths between 200 and 700 nm were monitored and it was found that the greatest increase in absorbance occurred at 280 nm. The change in absorbance at 280 nm was used in subsequent experiments to follow the extraction of adsorbents from the fouled membranes.

Results. The absorbance at 280 nm of foulants extracted from tubular membranes is shown in Figure 2.3. The increase in absorbance at 280 nm indicated that the substances extracted from the fouled membranes contain aromatic groups, possibly in the form of phenolic compounds. When the extraction fluid was cooled below 30°C a pink precipitate formed, which was further indication of the phenolic nature of the extractives. The results, shown in Figure 2.3, indicate that most foulants were extracted with the sodium hydroxide-SDS solution from tubular membranes in the first 60 h. This very harsh method for extracting foulants from ultrafiltration membranes would not be used as a cleaning method for these membranes. It did, however, allow for the removal and subsequent characterisation of most of the foulants on industrially fouled membranes and also gave an indication of the time required to remove the foulants from the membranes quantitatively. This extraction method could therefore be used to evaluate biological cleaning methods of membranes fouled and pulp and paper effluent. It was interesting to note that the absorbance spectrum of the extracted foulants corresponded with that of the diluted effluent and experiments were subsequently carried out to establish if the chromogenic compounds in the effluent as well as membrane adsorbents were phenol derivatives.

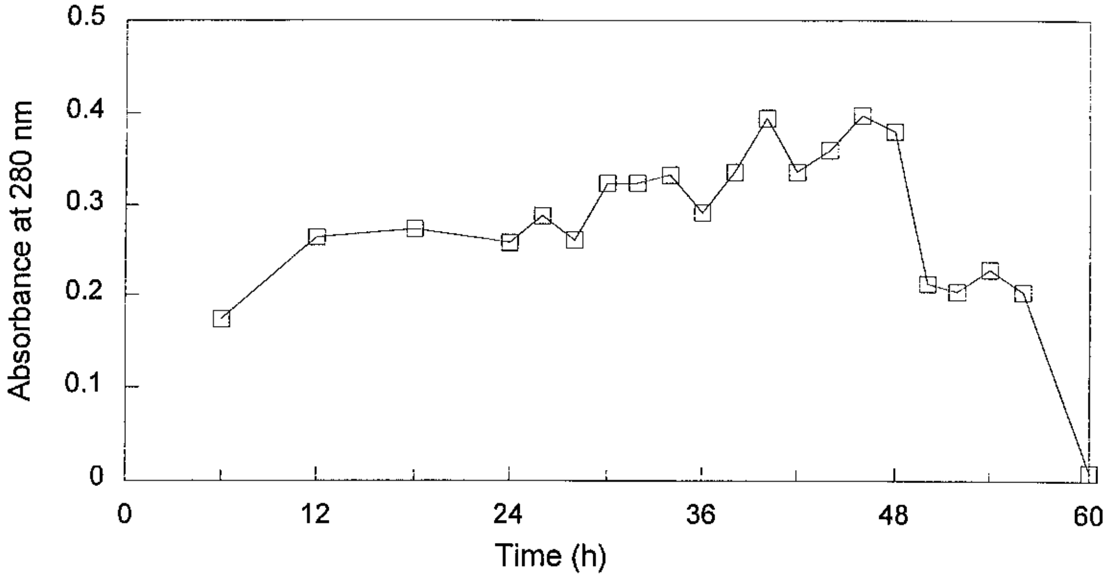

Figure 2.3 Absorbance at 280 nm of compounds extracted from tubular membranes, fouled in paper mill effluent at the Mondi Kraft paper mill, Piet Retief.

2.2.3 PHENOLIC STAINING METHOD FOR FOULANT CHARACTERISATION

Materials and methods: A stain, specific for phenolic functional groups, was used to determine the nature of foulants that adsorbed onto statically fouled flat sheet polysulphone membranes as well as on fouled tubular membranes obtained from Membratek. The stain consisted of toluidine blue (Gurr, London) (0.05 % w/v) in phosphate buffer (0.1 M, pH 6.8) (O'Brien *et al.,* 1965). Membranes fouled under controlled conditions for 48 h and tubular membranes fouled in the industrial process were stained. An unfouled strip of flat sheet membrane was included as a negative control. These membranes were soaked in the solution for 1.5 min and then washed repeatedly in distilled water. Toluidine blue stained the lignin dark blue. Non-lignified plant material stained reddish purple, whereas starch and cellulose did not stain at all.

Results: The results of phenolic staining of flat sheet and tubular membranes are shown in Figure 2.4. The control membrane stained only slightly, while the flat sheet and tubular membrane pieces stained dark blue. This staining method proved to be a reliable, quick, qualitative test to evaluate the extent of membrane fouling and could be used in qualitative studies to determine the effect of a specific enzyme solution on the foulants adsorbed onto the membranes.

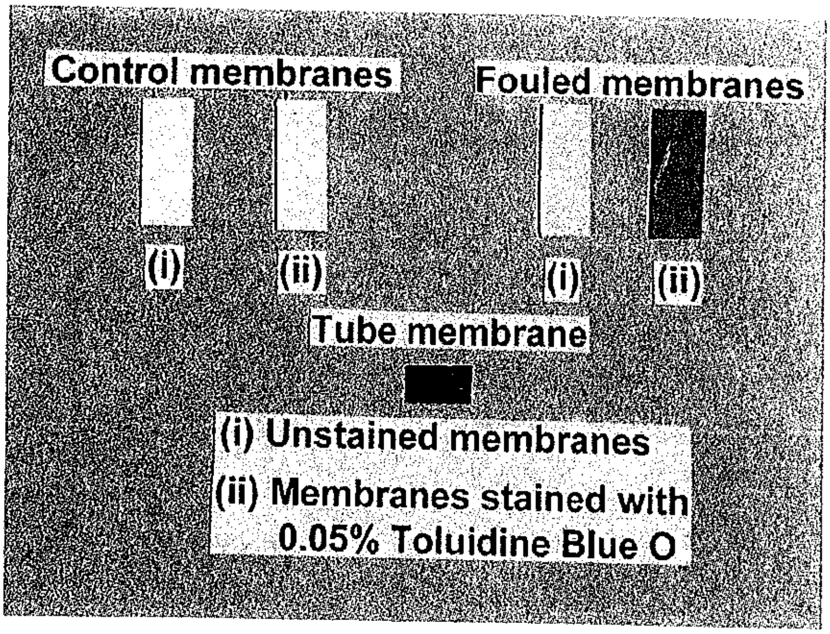

Figure 2.4 Phenolic staining of membranes fouled in paper mill effluent. The control membranes are sections from an unfouled flat sheet membrane. Section (i) was not stained, while section (ii) was stained with Toluidine Blue O. Fouled membranes (i) and (ii) are sections from a flat sheet membrane statically fouled in real paper mill effluent for 48 h. Section (i) is not stained, while section (ii) is stained with Toluidine Blue O. The tubular membrane was obtained from Membratek after it was fouled in the effluent purification plant at Mondi Kraft, Piet Retief. The section shown was stained with Toluidine Blue O in the same manner as sections (ii) of the control and fouled membranes.

2.2.4 MEMBRANE FOULING UNDER CONTROLLED CONDITIONS

A schematic diagram of the static flat sheet membrane fouling chamber is shown in Figure 2.5. It consists of three main components: a stainless steel water bath, with a heating element and thermostat to control the temperature of the effluent in contact with the membranes, a perspex framework with perspex membrane holders in which membranes could be clamped at fixed angles to the effluent steam and a stainless steel industrial pump (Ebara CD 70/5). Due to the very corrosive nature of the effluent, only stainless steel and perspex components were used in this experimental setup.

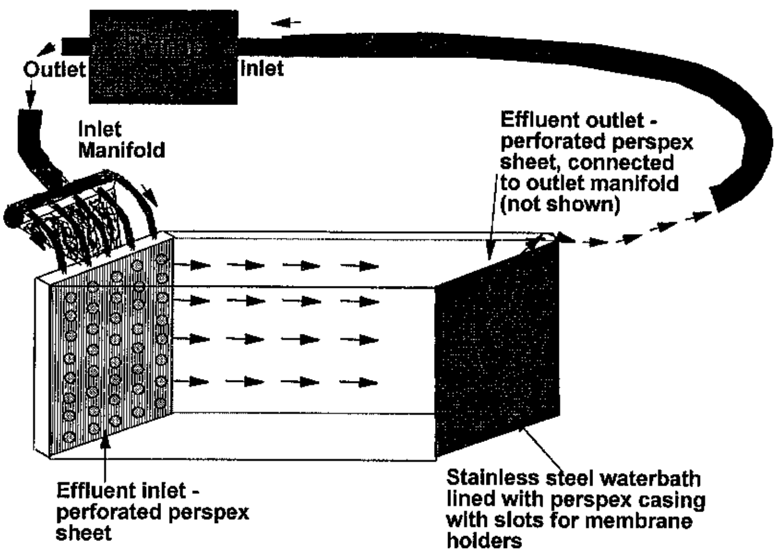

Figure 2.5 Flat sheet membrane fouling system consisting of a stainless steel water bath, Perspex framework with perspex membrane holders and a stainless steel pump.

For this reason, even the screws used to clamp the membranes in the membrane holders were made of Teflon to prevent damage to the apparatus used and to prevent leakage of any metal ions into the effluent. A detailed diagram of the perspex membrane holders is shown in Figure 2.6.

Membrane strips (four per holder) were clamped in the holders by perspex bars (1 cm across) that were secured onto the perspex frame with Teflon screws. Flat sheet membranes were clamped with their cotton backing facing to the inside and the membrane surfaces facing towards the effluent. This precaution was taken to minimise adsorption onto the cotton backing.

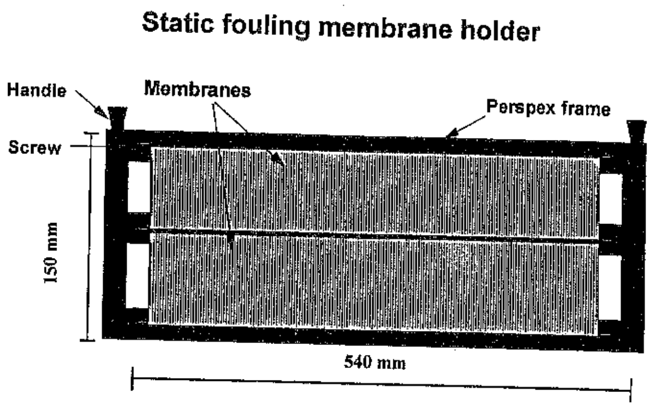

Figure 2.6 Perpex flat sheet membrane holders for static fouling apparatus.

2.2.5 STATIC FOULING OF FLAT SHEET MEMBRANES

Flat sheet polysulphone membranes were cast on 25 cm wide polyester backing to a thickness of 200 μm. The casting solution was obtained from Membratek and the casting apparatus was supplied by the Institute of Polymer Science, University of Stellenbosch. The membranes were clamped in the membrane holders as described above and placed in the stainless steel water bath, filled with effluent. The effluent was subsequently circulated through the bath at 40 °C. Fouled membranes were removed after 24, 36, 48, 60 and 72 h of effluent circulation and washed thoroughly in distilled water. Each fouled membrane strip was cut into four evenly sized pieces for the measurement of pure water flux in the flat sheet system previously described by Maartens *et al.* (1995). After this initial experiment to determine the optimal fouling time, all subsequent cleaning experiments were performed on membranes fouled in this manner for 48 h.

Results: The static fouling of flat sheet membranes in black liquor is summarised in Figure 2.7. The flux decline stabilised after fouling for 48 h at approximately 60 % of the original flux value. For this reason all subsequent cleaning experiments were performed on membranes fouled for 48 h.

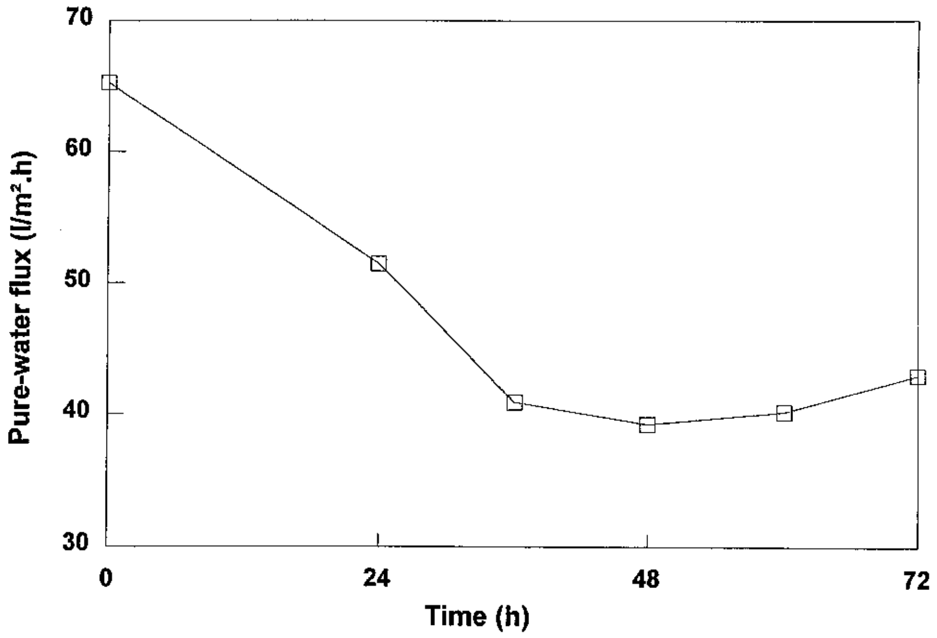

Figure 2.7 Fresh water flux decrease as a result of static fouling of flat sheet membranes in paper mill effluent. Flat sheet polysulphone membrane strips (20 x 25 cm) were fouled in real paper mill effluent. Membrane strips were removed at the times indicated and wash in distilled water, before the fresh water flux was measured as described by Maartens *et al.* (1995).

2.2.6 DYNAMIC FOULING OF TUBULAR MEMBRANES

Tubular polysulphone membranes were subjected to fouling by effluent arriving from the pulp and paper plant at Piet Retief. Membranes were fouled for a period of time to determine the tempo of membrane fouling and the subsequent flux decline caused by fouling. The following steps were followed:

Polysulphone membranes were pre-compacted with clean reverse osmosis water at 800 kPa, 25°C and a linear flow rate of 500 l/min.

After precompacting of the membranes the pure-water flux was determined at 200 kPa, 25°C at a flow rate of 500 l/min and used as a reference.

Precompacted membranes were then subjected to real effluent at 500 kPa, 30°C and a flow speed of 500 l/min which resembles the real ultrafiltration conditions used in practice where operational conditions usually are: 400-600 kPa pressure, temperatures between 30-55°C and a linear flow rate of 500 l/min. The product flux of the effluent was determined after 5 min of contact with the effluent and again at regular intervals through the experiment.

After fouling for a certain period of time the membranes were washed thoroughly with reverse osmosis water and the pure-water flux measured at 200 kPa, 25°C with a flow rate of 500 l/min. The difference between this flux and the initial pure-water flux were used to characterise the extent of irreversible foulant adsorption.

Steps 3 and 4 were repeated until the decline in the pure-water flux stabilised.

The effect of membrane pre-coating on fouling: The hydrophobic charater of the foulants in paper and pulp effluent prompted an investigation into the possibility of non-covalently coating the polysulphone membranes with a hydrophilic agent to reduce hydrophobic adsorption. Triton X100® and Pluronic® F108 were used in the coating trials. To investigate the effect of non-covalent membrane coating on membrane fouling, membranes were pre-treated with the above mentioned agents and steps 1-5 were repeated. In step 1 membranes were, however, pre compacted with either a 1% solution of Triton X100® or a 1.2% solution of Pluronic® F108.

Results. The pure water flux decline profiles, of coated and uncoated polysulphone membranes fouled by paper and pulp effluent over a period of time are shown in Figure 2.8. The pure-water flux through the untreated polysulphone membranes was reduced by approximately 47% after 21 h of contact with the effluent. Prolonged contact with the effluent, up to 70 h, resulted in almost no further flux decline an indication that, for fouling and cleaning studies, a fouling time of 15-20 h would be sufficient. The initial pure-water flux through membranes pre-treated with the detergent Triton X100® was 55% higher than that through an untreated membrane. This increase in the water flux was, however, markedly decreased after contact with effluent. The pure-water flux decreased by 89 % after 21 h of filtration. Again the flux decline stabilised at this point and with prolonged filtration only a slight decrease occurred.

The pure water flux for polysulphone membranes pre coated with Pluronic® F108, on the other hand, showed a significantly different pattern. The pure water flux after precompaction with the Pluronic® F108 was approximately 60% lower than that of the untreated membrane, indicating that extensive adsorption of the Pluronic® F108 had occurred. After extended contact with the effluent (70 h), however, there was no meaningful further decline in the pure-water flux. The product flux resulting from effluent filtration through untreated and treated polysulphone membranes are shown in Figure 2.9. Over the first 20 h of contact with the effluent the pattern was the same for the permeate flux for both the untreated membranes and pre coated membranes. This pattern included a gradual decline in the permeate flux until the membranes were washed with water prior to the pure-water flux measurements. After the pure-water flux was measured ultrafiltration of the effluent was continued. The product flux measured directly after the pure-water flux measurement was as high as the initial product flux for coated as well as uncoated membranes. Again the product flux followed a gradual decline towards the end of the filtration run. The increase of the product flux after flushing with pure water indicated the effect of concentration polarisation and no permanent foulant adsorption was observed, but according to the pure-water flux measurements irreversible foulant adsorption had occurred with both the untreated and the Triton X100® pre-treated membranes. This emphasises the importance of measuring the degree of membrane fouling with pure-water flux to eliminate flux decline caused by concentration polarisation and to detect foulant adsorption at an early stage.

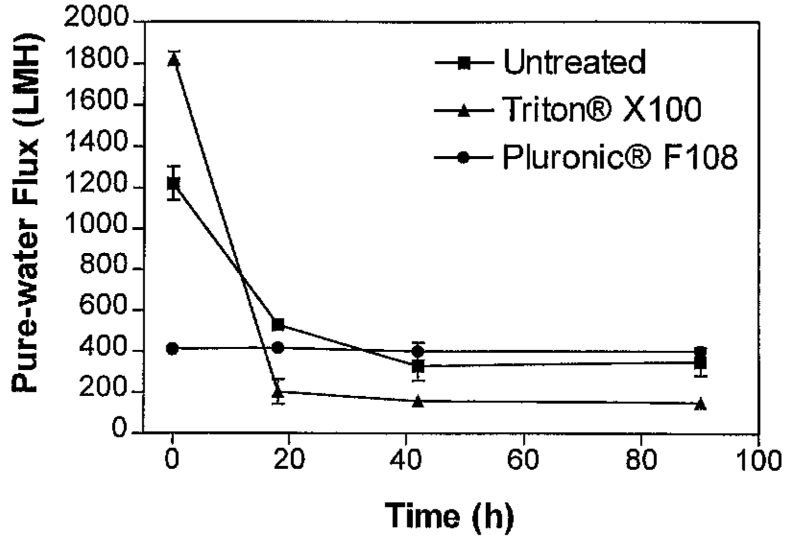

Figure 2.8 Pure-water flux decline profiles of untreated, Triton X100® and Pluronic® F108 treated membranes during contact with pulp and paper effluent. Values presented are the mathematical means of at least three determinations ± SD.

2.2.7 ENZYMATIC CLEANING OF FLAT SHEET MEMBRANES FOULED IN PAPER AND PULP EFFLUENT

The following enzymes were investigated for the removal of adsorbents from flat sheet polysulphone membranes fouled in black liquor: ligninases, cellulases, cellulase and xylanase mixtures, pectinases and a horse radish peroxidase preparation.

Ligninases: A ligninase preparation was obtained from Prof. P. Rose and Mr. W. Leukus from the Biotechnology Department, Rhodes University. This ligninase was prepared using an immobilised strain of *Phanerocheate chrysosporium* (Leukus and Rose (1995) (SA patent, USA and UK patent pending). The lyophilised preparation was dissolved in 0.1 mM sodium tartrate buffer pH 2.5 for membrane cleaning studies.

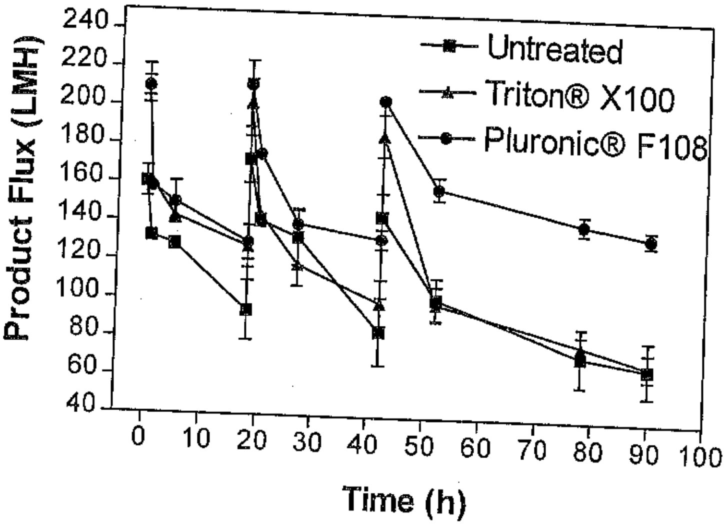

Figure 2.9 Product flux decline profiles of untreated, Triton X100® and Pluronic® F108 treated membranes during contact with pulp and paper effluent. Values presented are the mathematical means of at least three determinations ± SD.

Cellulases: Zumizyme C (Seravac Fine Chemicals Company, Epping Industria, Cape Town) is a cellulase isolated from *Trichoderma viridae*. The activity, as determined by the manufacturer, was 20.925 units per mg solid. This assay was performed as previously described by Almin and Erikson (1967). One unit of enzyme was defined as the amount of enzyme which produced a relative fluidity change of 1.0 over a five minute period in a defined carboxymethylcellulose substrate. Cellulase CPT (a liquid form) and Cellulase CPD (a powder form) (ENZYMES SA, Pretoria) have combined cellulase and xylanase activity. Both preparations were prepared from the fungus *Penicillium funiculosum*. The cellulase activity of these mixtures were confirmed using the Remazol Brilliant Blue-CMCellulose assay (Wirth and Wolf, 1992; Appel *et al.*, 1995). Zumizyme C was used as reference cellulase. The cellulase activity of Cellulase CPT was found to be 60.528 Units per mg solid, while the activity of Cellulase CPD was found to be 650.29 Units per mg solid.

Pectinases: Two pectinase preparations were used in membrane cleaning experiments: Pectinase CPT (ENZYMES SA, Pretoria) and Zumizyme AP (Seravac Fine Chemicals Company, Epping Industria, Cape Town). The activity of Zumizyme AP was given by the manufacturer as 600 apple juice depectinising units per mg solid, as determined by a viscometric assay described previously by Almin and Erikson (1967). The activity of Pectinase CPT was determined by the same assay by Seravac to be 700 000 000 apple juice depectinising units per ml.

Peroxidases: A horse radish peroxidase preparation was obtained from Seravac Fine Chemicals Company, Epping Industria, Cape Town. The peroxidase activity of this preparation was given by the manufacturer as 276 units per mg solid. The activity was determined by a guiacol-based assay described previously by Bergmeyer (1974). The activity of Pectinase CPT was determined by the same assay to be 700 000 000 units per ml or 2 333 333 units per mg solid.

The protein content of all the above enzyme solutions were determined as described previously by Bradford (1976), as modified by Appel (1996).

The results of the protein content determinations of enzyme solutions are summarised in Table 2.3. For each enzyme, the measured or supplied enzyme activities are also given.

Table 2.3 Protein concentration and enzyme activities of enzyme solutions used in membrane cleaning experiments.

Enzyme solution	Protein content (mg/mg solid)	Type of enzyme	Enzyme activity Units/mg solid
Ligninase	0.050	Lignin peroxidase	430.000
Zumizyme C	0.320	cellulase	20.925
Cellulase CPT	0.635	cellulase/ xylanase	60.528
Cellulase CPD	0.580	cellulase/ xylanase	650.290
Zumizyme AP	0.160	pectinase	600.142
Pectinase CPT	0.386	pectinase	2 333 333
Horse radish peroxidase	0.440	peroxidase	275.800

2.2.8 CLEANING EXPERIMENTS WITH FLAT SHEET MEMBRANES

The buffers and incubation temperatures used with different enzyme preparations are summarised in Table 2.4.

Table 2.4 Conditions used with various enzymes in the treatment of flat sheet membranes fouled in paper mill effluent.

Enzymes	Buffer	pH	Temperature
Zumizyme C	0.1 M Sodium acetate	4.80	28°C
Cellulase CPD	0.1 M Sodium acetate	4.80	28°C
Cellulase CPT	0.1 M Sodium acetate	4.80	28°C
Pectinase CPT	0.1 M Sodium acetate	4.80	28°C
Zumizyme AP	0.1 M Sodium acetate	4.80	28°C
Lignin peroxidase	0.1 mM Sodium tartrate	2.50	37°C
Horse radish peroxidase	0.1 M Potassium phosphate	7.00	28°C

Membranes fouled in the flat sheet fouling chamber for 48 h were cut into 5 x 10 cm strips. The means of the fresh water flux of four control (unfouled) and four fouled pieces were determined as reference fluxes. These reference membrane strips, as well as four control and four fouled pieces cut from the same membrane strips, were treated with 100 mg/ 100 ml solutions of the enzymes in a shaking incubator (speed 80 rpm, Innova 4000 incubator).

Results: Figure 2.10 shows the cleaning results obtained with various enzyme and buffer treatments. Results are presented as the mean of four fresh water flux determinations.

The initial fouling is expressed as a percentage of the pure-water flux of the unfouled membrane pieces and is shown as a negative value. This method of presentation allows the increase in flux after enzyme treatments to be shown more clearly.

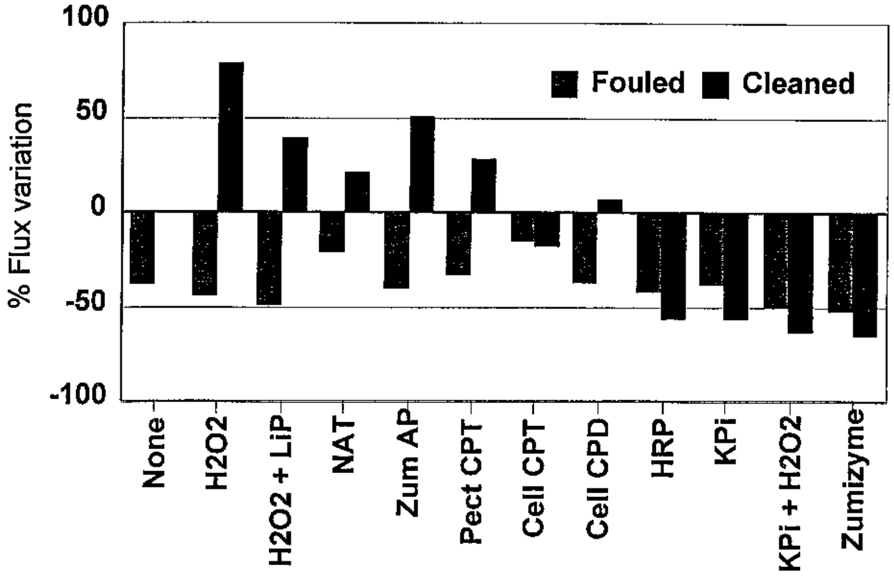

Figure 2.10 Fouling of flat sheet membranes after 48 hour contact with pulp and paper effluent (red bars) are shown as negative values in order to indicate more clearly the restoration of flux achieved by various membrane treatments. The percentage increase in fresh water flux after various treatments are shown as green bars.

Increase in flux is indicated as a percentage of the flux of the fouled control membrane. In Figure 2.11, results are shown to demonstrate the effectiveness of different enzyme treatments.

A selection of the enzyme treatments resulting in the highest flux recovery was made and these treatments were evaluated by plotting the increase in flux obtained per enzyme unit.

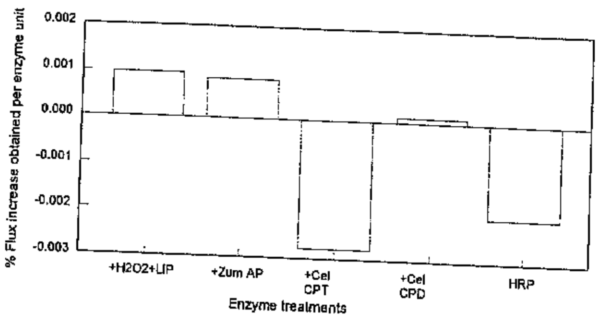

Figure 2.11 Flux restoration achieved by different enzyme treatments expressed as percentage flux increase per enzyme unit used.

Discussion: Figure 2.10 shows clearly that the best results were obtained by treating fouled flat sheet membranes with hydrogen peroxide in buffer, followed by pectinase and ligninase treatment. Hydrogen peroxide is an essential co-factor for ligninases and needed to be evaluated alone for the determination of its role in the flux increase after ligninase treatment. Hydrogen peroxide had no effect on the selectivity of native polysulphone membranes.

During the treatment of fouled membranes with lignin peroxidase and hydrogen peroxide in buffer, the hydrogen peroxide donates a single electron to the lignin peroxidase to form the active enzyme. As a result, the hydrogen peroxide levels decreased no more substrate was available for the enzyme or the hydrogen peroxide was depleted. The cleaning effect of ligninases can therefore not be described as the cumulative effect of the enzyme and hydrogen peroxide, but only as the effect of the active enzyme complex.

The second best treatment at the concentrations used and in the prevalent experimental conditions, were treatment with the pectinase preparation Zumizyme AP (Seravac Fine Chemicals Company, Epping Industria, Cape Town). The other pectinase preparation Pectinase CPT (ENZYMES SA, Pretoria) also yielded good results. It is, however, clear from Figure 2.11 that better cleaning efficiency was obtained with Zumizyme AP than with Pectinase CPT. From Figure 2.11 it can also be concluded that the ligninase preparation used yielded approximately the same cleaning efficiency value as the pectinase preparation Zumizyme AP.

2.2.9 MEMBRANE CLEANING

Tubular membranes were cleaned with different chemical protocols to investigate the extent to which the flux could be restored. The type of cleaning solution or cleaning combination and the concentrations used in cleaning trials are summarised in Table 2.5. After fouling in effluent for a period of 17 h, membranes were flushed thoroughly with pure-water to remove all loosely adsorbed foulants. Membranes were then subjected to cleaning by the respective cleaning solutions and combinations. From data discussed in previous sections and the literature it was decided that the a number of enzymes should be evaluated in addition to chemical cleaning trials. The type of enzyme, the optimal operational conditions and concentrations used in enzymatic cleaning trials are summarised in Table 2.5. Pluronic® F108 coated and uncoated membranes, fouled for 17 h in effluent, were dynamically cleaned by recycling the enzyme mixtures for a period of 2 h at the optimal temperature and pH of each enzyme. Cleaning conditions included an operating pressure of 200 kPa and flow speed 500 l/min. After being cleaned the membranes were flushed thoroughly with pure-water to remove all loosely adsorbed foulants, cleaning chemicals and enzymes. The rinsing solution was then replaced with pure-water and the flux measured. The increase in the pure-water flux was again used to determine the effectiveness of cleaning solutions.

Table 2.5 Chemical and enzymatic cleaning of tubular membranes fouled in paper and pulp effluent.

Cleaning Solution	**pH**	**Concentration**	**Temperature (°C)**	**Cleaning Time (h)**
Triton X100®	**N/D**	**0.2 %**	**30**	**2**
NaOH	**>11**	**0.01 M**	**30**	**2**
Sodium dodecyl sulphate (SDS) + NaOH	**11.7**	**0.1% SDS dissolved in 0.01 M NaOH**	**30**	**2**
H_2O_2	**N/D**	**2 %**	**28**	**2**
CellulaseCPT	**4.8**	**0.5 %**	**28**	**2**
0.1% Triton X100® + CellulaseCPT	**4.8**	**0.5 %**	**28**	**2**
Horse radish peroxidase	**7**	**0.5 %**	**28**	**2**
Zumizyme AP	**4.8**	**0.5 %**	**28**	**2**

Results: The cleaning efficiency of all cleaning solutions was evaluated by calculating the flux improvement after cleaning. Untreated and Pluronic® F108 treated membrane were fouled for a period of 17 h followed by cleaning with the different cleaning solutions. During fouling the pure-water flux of the untreated and the Pluronic® F108 treated membranes decreased from 980 and 400 LMH to 498 and 382 LMH, respectively. After cleaning with the soduim dodecyl sulphate (SDS)/NaOH solution flux of pure-water through the uncoated polysulphone membranes showed a slight increase. The Pluronic® F108 coated membranes on the other hand did not respond to the sodium dodecyl sulphate (SDS)/NaOH wash. The Triton X100® wash proved to be more effective, the pure-water flux for the uncoated membrane increased by 53%, whereas the pure-water flux for the Pluronic® F108 coated membrane increased by 288%. The pure-water flux reached after cleaning of the Pluronic® F108 coated membranes was equal to that of an unfouled untreated membrane. This implied that the fouling material as well as the Pluronic® F108 coating material were removed by Triton X100®. Enzymatic cleaning under dynamic conditions did not increase the flux significantly. In some cases it appeared as if the enzyme itself adsorbed onto the membrane causing additional fouling. In one of the cleaning trials mechanical cleaning was combined with the Triton X100® chemical cleaning. As mentioned before, Triton X100® alone increased the pure-water flux of a fouled untreated membrane by only 53%. When this cleaning agent was combined with sponge ball treatment (two sponge balls were flushed through the system under pressure) the flux could be restored to values higher than the original flux, of an unfouled membrane. Repeating the cleaning experiment with the Pluronic® F108 treated membrane showed no additional flux improvement. These results indicated that foulants from the pulp and paper effluent adsorbed to a much greater extent onto untreated membranes than to membranes coated with Pluronic® F108. During cleaning with Triton X100® some of the foulants adsorbed onto the untreated membranes could be removed while most of the foulants adsorbed onto the Pluronic® F108 treated membranes were removed. Triton X100® removed most of the coating material as well but these membranes could be recoated and the same performance could be obtained.

Table 2.6 Effect of cleaning on the water flux through membranes. Untreated and Pluronic® F108 treated membranes were fouled for 17 h followed by chemical or enzymatic cleaning. Values presented are the mathematical means of at least three determinations ± SD.

Cleaning Solution	Pure -water flux (LMH) (uncoated)	Percentage of original flux (LMH) (uncoated)	Pure-water flux (LMH) (Pluronic)	Percentage of Original Flux (LMH) (Pluronic)
Unfouled membrane	980	100	498	100
None	400	41	382	77
Triton X100®	766 ± 24	78	1152 ± 27	288
Triton X100® + Sponge ball	1012 ± 13	103	1167 ± 18	290
NaOH	512 ± 10	25	394 ± 12	98.5
SDS + NaOH	597 ± 13	61	389 ± 10	97
H_2O_2	507 ± 9	52	402 ± 11	100
CellulaseCPT	523 ± 11	53	400 ± 3	100
0.1% Triton X100® + CellulaseCPT	770 ± 13	78.5	979 ± 20	244
Horse radish peroxidase	432 ± 25	44	366 ± 13	92
Zumizyme AP	446 ± 23	45.5	392 ± 15	98

Foulants remaining adsorbed onto the untreated membranes after Triton X100® treatment, were easily removed mechanically. It was clear that fouling by substances from pulp and paper effluent can be reduced by membrane pre-treatment and that the efficiency of chemical cleaning can be increased by the addition of mechanical cleaning. To determine whether Pluronic® F108 treated membranes should be recoated after each chemical wash, Triton X100® cleaned membranes were refouled by pulp effluent. The initial flux of these membranes was almost as high as that of an untreated, unfouled membrane. During contact with the effluent the pure-water flux declined with the same profile as observed with an uncoated membrane. These results, therefore, confirmed that the Pluronic® F108 pre-adsorbed onto the polysulphone membranes, was removed by the Triton X100® wash and that, unless membranes were re-coated, refouling would occur.

2.3 CONCLUSIONS

1. Under dynamic fouling conditions the pure-water flux was reduced by 53 % over a 20 h period, indicating irreversible fouling of polysulphone membranes by pulp and paper effluent.
2. Pretreatment of the membranes with Pluronic® F108 decreased the flux of pure-water through the membrane, but significantly reduced the magnitude of foulant adsorption onto the membranes. The product flux was, however, unaffected by pre-coating
3. Chemical cleaning, in combination with mechanical cleaning, restored the flux to values above those of unfouled membranes.
4. Pluronic® F108 coated membranes were more easily cleaned with Triton X100®, but the coating material was also removed during the cleaning trials.
5. Fouling can be reduced by membrane pre-treatment with Pluronic® F108 without any loss in product volume.
6. None of the enzymes evaluated in flat sheet static fouling trials could increase the pure-water flux more efficiently than Triton X100® in dynamic studies. This phenomenon will need further investigation.

2.4 RECOMMENDATIONS

1. Additional characterisation of the pulp and paper effluent in order to develop more specialised anti-fouling strategies.
2. An investigation into more suitable commercially available membrane pre-coating agents for fouling prevention.
3. An investigation into the transfer of enzymatic cleaning techniques, used on statically fouled membranes, to dynamic systems.
4. Development of a pilot scale cleaning regime based on the results obtained in the present study with the objective being implemented in industry.

ABATTOIR

3.1 INTRODUCTION

Water effluent streams originating from abattoirs contain several proteinaceous and other constituents (Jacobs *et al.*, 1992; [a]Maartens *et al.*, 1995). Treatment of such an effluent with hydrophobic ultrafiltration membranes leads to membrane fouling. We have previously shown that proteins and lipids, the major foulants that adsorb onto ultrafiltration membranes, can be effectively removed with enzymes ([b]Maartens *et al.* 1996). The same studies also showed that although proteins were the most abundant fouling agents in abattoir effluent, lipids absorbed more strongly onto the membranes and lipid removal not only gave the largest flux improvement, but also facilitated protein removal.

The initial success achieved with the removal of proteins and lipids from membranes fouled in abattoir effluent prompted a further investigation into the use of enzymes in cleaning regimes. The aim of this study was to find the most suitable commercially available lipases (enzymes used to degrade lipids) and proteases (enzymes used to degrade proteins) for the formulation of membrane-cleaning agents. Emphasis was, however, placed on lipases as lipid removal is the key to effective flux recovery for membranes fouled in abattoir effluent. In addition the use of lipases is potentially advantageous in cleaning solutions because they are:

1. commercially available in large quantities (economically more attractive);
2. very selective towards their substrates (substrate specificity of lipases offers an advantage over purely chemical modifications);
3. effective catalysts which are effective under mild operating conditions (the industrial potential of lipases are broadened by their ability to remain active in organic solvents);
4. efficient and selective catalysts at ambient temperatures (increased stability, especially to high temperatures, of lipases in the absence of appreciable amounts of water, also contribute to their industrial potential) ([b]Maartens *et al.*, 1996; Björkling, *et al.*, 1991; Harwood, 1989).

The mechanism and conditions of lipase action will be discussed later.

3.1.1 LIPASES

Lipases (triacylglycerol hydrolases; EC 3.1.1.3) are widely distributed among animals, plants and micro organisms that hydrolyse ester bonds in neutral lipids (Figure 3.1). Lipases differ from classic esterases in that their natural substrates (long-chain triacylglycerols) are insoluble in water and they are activated by interfaces.

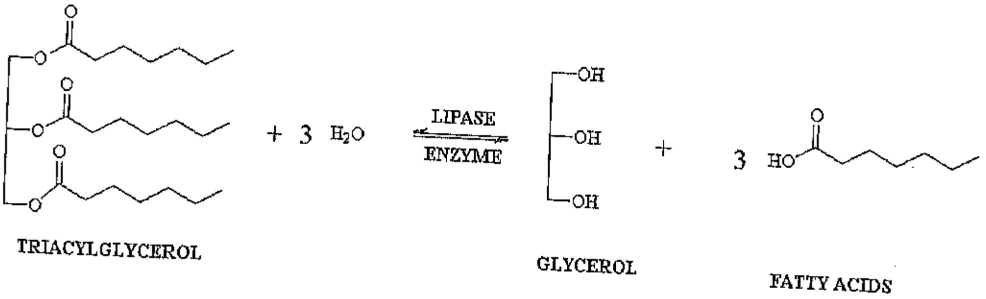

Figure 3.1 Enzymatic reaction of a lipase catalysing the hydrolysis or synthesis of a triacylglycerol substrate.

Microbial lipases are attractive components in industrial cleaning solutions due to their vast diversified enzymatic properties and substrate specificities. Hydrolysis, ester synthesis and interesterifications are some of the reactions carried out during the chemical modification of lipids in the presence of lipases.

Hydrolysis reactions involve an attack on the ester bond of glycerides in the presence of water molecules to produce both an alcohol functionality and a carboxylic acid. Hydrolysis of fats and oils is an equilibrium reaction. It is therefore possible to change the direction of the reaction to ester synthesis by modifying the reaction conditions. The equilibrium between forward and reverse reactions is controlled by the water content of the reaction mixture and in a non-aqueous environment lipases will catalyse ester synthesis reactions.

Decrease of the water content in the reaction mixture allows lipases to carry out interesterification of fats and oils as well as esterification of alcohols with fatty acids. Interesterification is the exchange of acyl radicals between an ester and an acid (acidolysis), an ester and an alcohol (alcoholysis) or an ester and another ester (transesterification) (Figure 3.2). These reactions require a small amount of water, in addition to the amount needed for the enzyme to maintain an active hydrated state.

The presence of too much water will decrease the amount of ester synthesis products. The transesterification reaction would not be possible in water because of the dominance of the hydrolysis reaction (Stead, 1986; Jaeger *et al.*, 1994; Malcata *et al.*,1992).

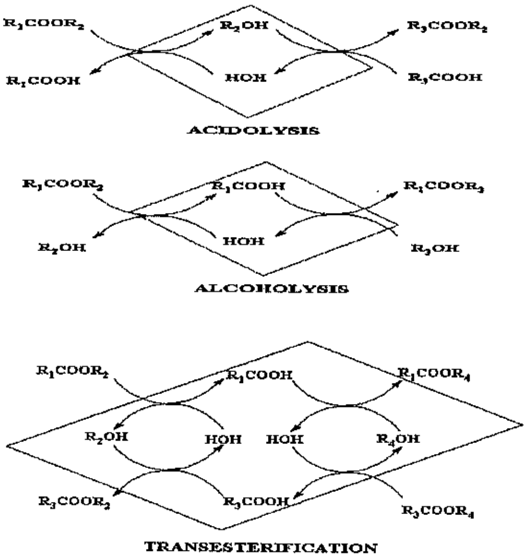

Figure 3.2 Industrially important reactions catalysed by lipases (Malcata *et al.*,1992).

Three-dimensional structure of lipases: Understanding of the structural and mechanistic properties of lipases will contribute to their development as industrial enzymes. The elucidation of the crystal structure of three non-homologous lipases from the fungi, *Rhizomucor miehei* and *Geotrichum candidum* and from the human pancreas revealed essential information concerning the structure-function relationships of lipases. The only X-ray structure of a bacterial lipase published thus far, however, is of the lipase from *Pseudomonas glumae*. A better understanding of the catalytic mechanism and structure of the active site of lipases will facilitate selections of specific modifications for protein engineering of lipases to enhance stability and activity (Stead, 1986; Taipa *et al.*, 1992; Derewenda and Sharp, 1993; Derewenda, 1994).

The catalytic triad: The catalytic centre of lipases contains a Ser-protease-like catalytic triad consisting of Ser-His-Asp residues. A serine and a carboxylate residue are hydrogen (H)-bonded to the same His-residue. The carboxylate may originate either from an Asp or a Glu residue. The well-conserved "lipase box" with a consensus sequence of Gly-X-Ser-X-Gly, differs in lipases from *Bacillus* strains where the first Gly residue is replaced by an Ala.

The active site of lipases is completely buried under a lid-like structure composed of one or two α-helices. This characteristic feature leads to the hypothesis that TAG lipases undergo a conformational change in response to adsorption at the oil-water interface. The lid moves, thereby allowing the active centre to become more accessible for the substrate. This conformational change also leads to a significant increase in the hydrophobic surface of the enzyme which is involved in the lipid-surface recognition.

All *Pseudomonas* species contain a well-conserved calcium binding site, but in contrast to other lipases, *P. glumae* contains a bound calcium ion. Although the latter is located close to the active site, its seems not to be catalytic, but to stabilise the local structure adjacent to the active centre (Harwood, 1989; Derewenda and Sharp, 1993).

***Reaction mechanism of a lipase ester bond hydrolysis*:** During these reactions (Figure 3.3), a tetrahedral intermediate is formed which degenerates into an acyl-enzyme complex. Regeneration of the free lipase is then achieved by a hydrolytic reaction mediated by a water molecule. First, the oxygen of the Ser side chain carries out a nucleophilic attack on the carbonyl C-atom of the ester (Figure 3.3, reaction 1) which leads to the formation of a tetrahedral intermediate. The histidine enhances the nucleophilicity of the Ser hydroxyl group. The histidine imidazole ring becomes protonated and positively charged and is then stabilised by the negative charge of the acid residue (Figure 3.3, reaction 2). The tetrahedral intermediate is stabilised by two H-bonds formed with amide bonds of residues belonging to the oxyanion hole. The alcohol is finally released leaving behind the acyl-enzyme complex (Figure 3.3, reaction 3). The fatty acid is released by the nucleophilic attack of a hydroxyl ion and the enzyme is thus regenerated (Figure 3.3, reaction 4) (Jaeger *et al.*, 1994).

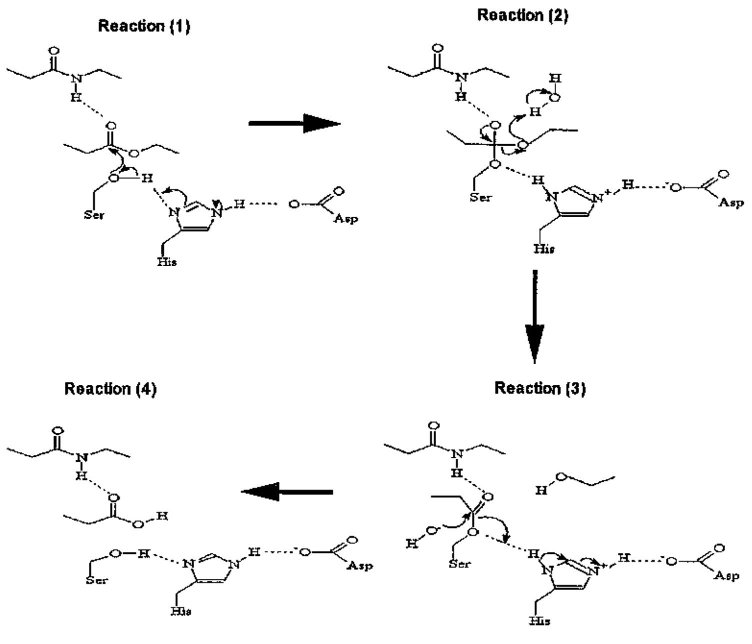

Figure 3.3 Reaction mechanism of a lipase hydrolysing an ester bond (see text for explanation) (Jaeger *et al.*, 1994).

Mechanism of lipolysis: Reactions catalysed by lipases are energy-saving processes which can be carried out at normal temperature and normal pressure. The reactions occur exclusively at the lipid-water interface generated by the hydrophobic lipid in a hydrophilic aqueous medium (Figure 3.4). Thus, the rate of lipolysis is directly determined by the concentration of the substrate molecules at this interface (Gilbert, 1993; Kosugi and Suzuki, 1988).

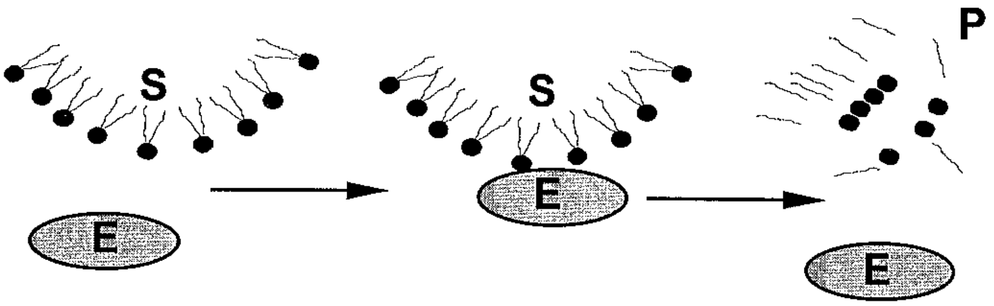

Figure 3.4 Schematic illustration of the mechanism of lipolysis catalysed by lipases [Verger & de Haas, 1976]. S = substrate; E = enzyme; P = products.

Esterase and lipase activities differ in their ability to be activated by interfaces. Esterase activity is a function of substrate concentration as described by Michealis-Menten kinetics where the maximal reaction rate is reached long before the solution becomes substrate-saturated. Thus, the formation of a substrate-water emulsion does not change the reaction rate. In contrast, lipases show almost no activity with the same substrate as long it is in its monomeric state. When the solubility limit of the substrate is exceeded (substrate-saturation) there is a sharp increase in enzyme activity as the substrate forms an emulsion. There is a dramatic increase in lipase activity when the substrate is presented in an insoluble phase and is referred to as interfacial activation. This phenomenon known as interfacial activation, is one of the most characteristic properties of lipase-catalysed reactions. Interfacial lipase-catalysed reactions involve binding of the enzyme from the bulk phase to a surface phase followed by catalysis at that interface. Lipases also differ from classic esterases in that their natural substrates (long-chain TAG's) are insoluble in water (Stead, 1986; Verger and de Haas, 1976). This fact is of particular importance when these enzymes are applied to the removal of lipids adsorbed onto a polysulphone ultrafiltration membrane.

Kinetics and interfacial activation: Previous studies showed that the overall catalytic behaviour of lipases are regulated by the physicochemical as well as the organisation and dynamics of the interface, since it is this interfacial binding step that regulates the lipase concentration at the interface. Thus, interfacial binding is the key regulatory factor that determines the catalytic properties of lipases (although this may not be a true affinity of the enzyme for a substrate) (Verger and de Haas, 1976; Maragoni, 1994).

The observed burst in enzyme activity upon substrate aggregation can be explained by any of the following three hypotheses, but it is more likely that a combination of all three theories is needed to explain lipase activity. The hypotheses are:

- the **enzyme theory** assumes a conformational change in the interface-adsorbed enzyme that is controlled by the micro-environment of the lipid-water interface. This results in an optimisation of the active site of the enzyme;
- the **substrate theory** assumes a higher susceptibility of substrate molecules towards the enzyme in the lipid-water interface;
- the **product theory** assumes that the rate-limiting step of product release (being very slow in water) is noticeably increased in the hydrophobic lipid-water interface.

With the elucidation of the three-dimensional structure of lipases, the enzyme theory was postulated. These studies showed that the active site of lipases is covered by a lid-like polypeptide chain which blocks substrate molecules from entering the active centre. This leads to the inactivity of the lipase on monomeric substrate molecules. When a lipase is bound to a lipid interface, a conformational change takes place. which causes the lid to move away so that the active centre of the lipase becomes fully accessible to the substrate. Thus, hydrophobic interactions are promoted between the enzyme and lipid surface due to the exposure of the hydrophobic side of the lid to the lipid phase. This mechanism explains the enzyme theory of the interfacial activation phenomenon where the lipase undergoes a conformational change upon association with an interface which makes the enzyme more active towards the substrate.

The substrate theory, on the other hand, confers interfacial activation to the changes in the concentration and organisation of substrate molecules at the interface upon lipase binding. Previous work showed that interfacial activation could be induced by increasing the proportion of monolayer substrate lipid (to which the lipase would bind) versus monolayer non-substrate lipid (to which the lipase would not bind). A refined theory (referred to as a "percolation theory") was used to explain these results. The binding of lipases at the interface is proportional to the concentration of substrate lipid. Hence, an increase in concentration of substrate lipid will lead to more lipases binding to the interface. A change is observed in monolayer composition from a non-substrate lipid-continuous to a substrate-lipid continuous phase at a specific mole percentage substrate lipid. Lipase activity was found to depend on the specific size of the lipid cluster (cluster size, number and shape) and therefore lipase activity also depends on lipid-lipid interactions at the interface (Verger and de Haas, 1976; Maragoni, 1994).

Another kinetic model for lipolysis was proposed by Maragoni (1994). It concludes that the rates of catalysis are controlled by the binding of lipases to the substrate interface, which is a co-operative process. This involves a conformational change in the enzyme which includes a rigid body movement of an α-helical lid which covers the active site and controls solvent and substrate accessibility to the active site.

Also, a structural change takes place in the substrate interface and a penetration of the lipase into that substrate interface. Lipolytic catalysis was shown to obey the Hill model since binding of a lipase molecule to an interface facilitates the binding of a second lipase molecule to the interface and so forth, showing that binding of lipases is in itself a co-operative process (Verger and de Haas, 1976).

However, the current working theory to describe kinetically the action of a lipolytic enzyme at an interface, is proposed by Verger *et al.* (1976). Their simplified model can theoretically describe the action of any soluble enzyme on an insoluble substrate (Figure 3.5). The water-soluble enzyme penetrates reversibly into the interface (E <==> E*) in the first step. This penetration is followed by the binding of the enzyme to a substrate molecule (S) to give the interfacial lipase-substrate complex (E*S). The catalytic steps now take place, regenerating the enzyme with the liberation of the product (P). It is presumed that the product is soluble in the water phase, diffuses away rapidly and does not accumulate at the interface and that no change is induced in the physicochemical properties of the interface during the reaction. This model proposes that the reversible penetration of the enzyme into the interface confers on the enzyme a new conformation (E*) which has more efficient catalytic properties than the soluble enzyme (E). In addition, it has been shown that a proportion of the interface lipases will become irreversibly inactivated on time ($E^* \rightarrow E^*_i$) where E^*_i is an irreversible inactivated interface lipase (Kosugi and Suzuki, 1988; Verger and de Haas, 1976).

According to all the theories mentioned here, lipolytic enzymes displays a two-step kinetic mechanism: the physical adsorption of the enzyme at the lipid interface may include an activation of the enzyme when the lid opens, and the formation of the enzyme/substrate complex which can be hydrolysed to give the product and regenerate the enzyme.

Substrate specificity of lipases: The investigation of biocatalysts for industrial transesterification was a fruitful area of research compared with chemical methods due to the specificity of enzyme-catalysed reactions and the regioselectively of lipases. Enzyme products developed for transesterification processes were based on their high regioselectively and good thermostability. Thus, an enzyme must meet the rigorous requirements without loss of productivity or selectivity.

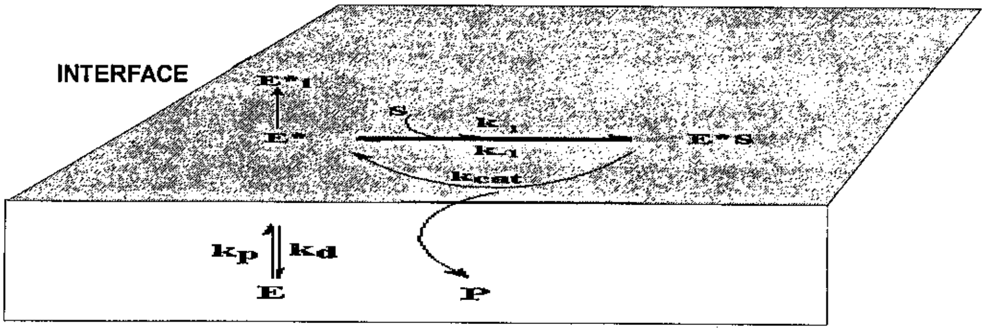

Figure 3.5 Model for describing interfacial kinetics of a water-soluble lipase enzyme acting on a insoluble substrate (Verger & deHaas, 1976) (see text for detail).

The interest in lipases arises primarily from their ability to hydrolyse long/short or saturated/unsaturated fatty acyl residues preferentially. The glycerol molecule, as the basic building block of the lipase substrate TAG, contains two primary and one secondary OH-group. According to lipase substrate specificity, these groups can be classified as follows:

1. the first group (random lipases) shows no positional and no specificity with respect to the chemical structure of the fatty acid (i.e. *Chromobacterium viscosum*);
2. the second group (*sn*-1,3 specific lipases) hydrolyses only primary ester bonds (i.e. ester bonds at atoms C1 and C3 of the glycerol molecule) i.e. *Pseudomonas fragi* and *Pseudomonas fluorescens*. Most bacterial lipases have a certain preference for the primary ester bonds but are also capable of hydrolysing the secondary ester bond i.e. *P. flourescens*;
3. the third group (*sn*-2 specific lipases) displays a clear fatty acid preference i.e. the fungal lipase of *Geotrichum candidum* (lipase B) which is specific for fatty acids with a double bond between C9 and C10. As yet no bacterial lipase is known to belong to this group (Harwood, 1989; Stead, 1986).

Lipase assays: In view of the increasing interest in biotechnology, the enzymatic hydrolysis or synthesis of lipids has become of more than academic interest. A reliable method of measurement of lipase activity is needed to evaluate the efficiency of enzymatic hydrolysis or synthesis.

Lipolytic activity has been determined by an assortment of techniques which include:

- titrimetric and indicator dye methods for detecting free fatty acids (FFA's) released from the hydrolysis of triglycerides;
- changes in the appearance of the agar surrounding colonies of lipolytic bacteria either as a result of the hydrolysis of opaque lipid emulsion or production of insoluble FFA's from solubilised lipids;
- enzyme immunoadsorbant assays of lipases;
- the use of chromogenic and fluorogenic substrates (Harwood, 1989; Stead, 1986).

Chromogenic substrates are water-soluble and are, therefore, not considered to be true lipase substrates. These substrates may also be hydrolysed by esterases which posed a specificity problem which had to be overcome. With the use of a range of 5-(4-hydroxy-3,5-dimethoxyphenylmethylene)-2-thiooxothiazoline-3-acetic acid (SRA) derivatives (Gilbert, 1993) it was shown that the short-chain water-soluble esters of SRA function as substrates for esterases while the longer-chain derivatives could act as lipase substrates (Maragoni, 1994; Hawthorne and Ansell, 1982).

In this study, the artificial substrate, para-nitrophenyl palmitate (*p*NPP) (Figure 3.6), was used to assay lipase activity. The release of para-nitrophenol (*p*NP) from *p*NPP was measured spectrophotometrically at a wavelength of 410 nm and a temperature of 37°C. The lipase activity in U/ml (µmole FA's released per minute per ml enzyme) was calculated from the following equation:

$$\mathbf{U/ml} = \frac{\mathbf{V}}{\mathbf{v \times \varepsilon \times d \times \Delta A/min},}$$

Where: V = volume of substrate solution(ml),

v = volume of enzyme solution (ml),

ε = extinction coefficient of *p*NP at 410 nm

= 15 (l x mmole^{-} x cm^{-} = ml x µmole^{-} x cm^{-}),

d = light path of cuvette (cm) and

ΔA/min = change of absorbance per minute.

One unit (U) of lipase activity is defined as the amount of enzyme that releases 1 μmole of FA per minute.

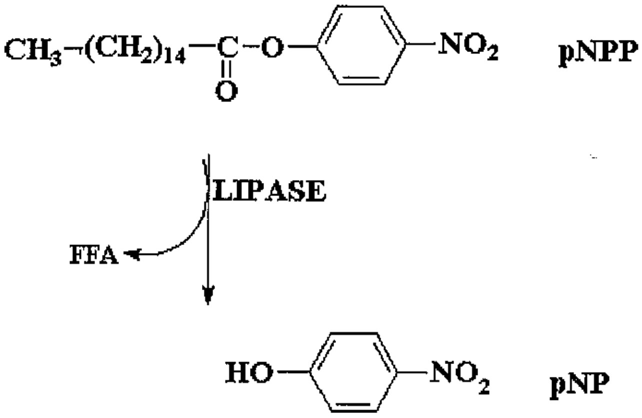

Figure 3.6 Enzymatic action of a lipolytic enzyme on a artificial substrate, *p*NPP.

In this study proteins and lipids adsorbed onto flat-sheet polysulphone membranes from abattoir wastewater streams, were removed by means of proteases and lipases. Enzymatic cleaning of polysulphone membranes included the use of lipases alone and lipases in combination with proteases. Optimal cleaning conditions for each cleaning agent were determined by measuring the reduction in adsorbed lipid and protein substances as well as the pure-water flux recovery through cleaned membranes.

3.2 EXPERIMENTAL

Materials: All chemicals used were of analytical grade and were obtained from BDH Chemicals Ltd., Poole, UK, unless otherwise mentioned. All membranes used were flat-sheet polysulphone membranes prepared from Udel P 3500 polysulphone cast from N'-methyl 2'-pyridine solutions, obtained from the Institute for Polymer Science, University of Stellenbosch, South Africa. Membranes were stored in distilled water at 4°C with sodium azide (5 mg/ml) to prevent bacterial growth. The enzymes used in this study is given in Table 3.1. In addition to their origin and supplier's reported activities, optimal pH and temperatures for each enzyme are also listed. The artificial lipase substrate paranitrophenol palmitate (*p*NPP) was obtained from Sigma Chemical Co., St. Louis, USA.

3.2.1 ENZYME ASSAYS

Lipase assay: The activity of the different lipases was determined by measuring the release of paranitrophenol (*p*NP) from *p*NPP spectrophotometrically at 410 nm and 37°C as previously published (Vorderwülbecke *et al.*, 1992; Fujii *et al.*,1986; Fu *et al.*, 1995).

Protease assay: The activity of the different proteases was assayed using a method based on the Coomassie Blue G-250 protein assay with casein as a soluble substrate (Bradford, 1976; Bickerstaff and Zhou,1993). Proteolysis was monitored by the decrease in absorbance at 595 nm.

3.2.2 MEMBRANE FOULING

Flat sheet polysulphone membranes (10 cm x 30 cm) were statically fouled in the abattoir effluent stream originating from the slaughter process areas as previously described [9]. After a 4 hr fouling, membranes were rinsed with tap water, placed in distilled water and transported to the laboratory where they were preserved in distilled water containing sodium azide (5 mg/ml) and kept at 4°C until analysis.

3.2.3 MEMBRANE CLEANING

Membranes fouled in abattoir effluent were washed in Clarks and Lubs Buffer (pH 7.5). The membranes (unfouled and fouled) were incubated for 1 hr in a 600 ml solution of cleaning agent or cleaning mixture at optimum pH and temperature for each agent to ensure maximum efficiency. The non-ionic detergent, Triton X-100 (0.1%), was also included in the enzymatic cleaning solutions. During enzyme cleaning trials, lipases were used alone and in conjunction with proteases. When lipases were used in conjunction with proteases, the lipases were applied to the fouled polysulphone membranes first. The lipases were then removed by thorough flushing with distilled water and the proteases were subsequently applied to the same membranes. This procedure was followed to prevent the degradation of the lipases by the proteases. After they have been cleaned, membranes were removed, washed thoroughly with distilled water to remove excess material, and lipid content, protein content and pure water flux were determined as previously described ([b]Maartens *et al.*, 1996).

The lipid content of fouled and cleaned membranes was determined gravimetrically after extraction with a hexane/isopropanol mixture (3:2, v/v) ([a]Maartens *et al.*, 1996).

The protein content of fouled and cleaned membranes was determined by a modified technique of Hess *et al.* (1978) ([a]Maartens *et al.*, 1996).

The pure-water flux determinations were done on a flat-sheet rig at an operational pressure of 300 kPa, a constant flow rate of 500 l/hr and 20°C ([a]Maartens *et al.*, 1996).

Table 3.1 List of enzymes and suppliers used in this investigation to clean flat sheet polysulphone membranes fouled in abattoir effluent.

Enzyme source	**Product name**	**Company (Location)**	**Enzyme activity (suppliers units)**	**Optimal pH and temperature**
***Candida cylindracea* lipase**	**n.a.**	Sigma Chemical Co., St. Louis, USA	**943 units/mg solids; 1.31 units/mg protein (Biuret)**	pH 7.2 & 37°C
*******Pseudomonas mendocina* lipase**	**Lumafast ™**	Genencor, Deflt, The Netherlands	**Minimum 20 000 *GLU/kg**	pH 7-10 & 20-50°C
*******Aspergillus oryzea* lipase**	**Lipolase ™**	NOVO-Nordisk, Denmark	**100 *KLU/g**	pH 7-11 & 30-55°C
***Bacillus licheniformis* protease	Optimase ™	Solvay Enzymes, Hannover, Germany	660 000 *DU/ml ±5%	pH 9-10 & 60-70°C
**Protease A (a protein engineered protease)	Properase ™	Genencor, Deflt, The Netherlands	Minimum 300 *PU/g	pH 8-11 & 20-30°C
Aspergillus oryzea protease	n.a.	Sigma Chemical Co., St. Louis, USA	0.13 units/mg solid; one unit will hydrolyze casein to produce color equivalent to 1 μmole (181 μg) of tyrosine per minute (Folin-Ciocalteu)	pH 7.5 & 37°C

* Enzyme activity as defined by the specific company: GLU = Genencor Lipase Units; KLU = Kilo Lipase Units; DU = Deflt Unit; PU = Properase Units

** These enzymes are distributed from Enzyme SA, Johannesburg, South Africa

3.3 RESULTS

All experiments were done in triplicate and the values presented are the mathematical means of all the experimental values (the standard deviation, SD, values are indicated).

3.3.1 ENZYME ASSAYS

It is evident from the data presented in Table 3.1 that suppliers of lipases quoted activities in different units. In order to make a comparison between the activities of these enzymes and to be able to estimate the amounts of lipases needed for a successful cleaning protocol, a standard lipase assay was performed on all the lipases. The specific lipolytic activities of the three different lipases are shown in Table 3.2. The *Candida* lipase showed the highest specific activity and only 1 mg/ml of this enzyme was needed to give an activity value of 0.78 U/ml. This may be explained by the broad substrate specifities that is common for lipases. The *Candida* lipase is a random lipase and is able to hydrolyze the triglyceride molecule at all three ester bonds. Thus, the hydrolysis reactions are rapid and complete. The *Pseudomonas* and *Aspergillus* lipases, however, are 1,3-specific lipases which hydrolyze the glycerol molecule primarily at the C1 and C3 atoms (Malcata, *et al.*, 1992; McNeill, 1991; Muñoz-Aguado *et al.*,1996; Sedmak and Grossberg,1977; Verger and de Haas,1976).

The specific activities of the different proteases were quantitatively calculated as previously described and the results are presented in Table 3.2 (Bickerstaff, 1993).

For all protease enzymes a decrease in absorbance at 595 nm was immediately observed after the incubation of the enzyme with the Coomassie-casein complex. This decrease in the absorbance is proportional to the degradation of casein by proteases. Therefore, the specific protease activity was determined by using the change in absorbance at 595 nm. This assay is an extention of the Bradford protein assay (Bradford, 1976; Bickerstaff and Zhou, 1993) where the shift in absorbance from 465 nm (orange-coloured unbound dye) to 595 nm (blue dye-protein complex) is used to determine protease activity.

Table 3.2 The amount of each enzyme used for the enzymatic cleaning trials performed on membranes fouled in abattoir effluent.

Enzyme source	[Enzyme] (mg/ml)	Lipase activity (U/ml)	Protease activity (U)	[Enzyme] (g/600ml)
***Candida* lipase**	1.00	**0.78 (± 0.28)**	**	0.39
***Pseudomonas* lipase**	10.00	**0.59 (± 0.02)**	**	5.05
***Aspergillus* lipase**	10.00	**0.43 (± 0.04)**	**	6.91
***Bacillus* protease**	0.0005	**	2.46 (0.09)	0.31
Protease A	0.005	**	0.28 (0.02)	0.11
***Aspergillus* protease**	0.005	**	0.22 (0.01)	0.13

* One unit (U) of lipase activity is defined as the amount of enzyme that releases 1 µmole of fatty acid/min. Protease activity is defined as the amount of casein (mg) hydrolysed per 1 mg of protease under the specified assay conditions (0.5 U were used for all lipases and 10.0 U for all proteases except for the *Bacillus* protease where 2500 U was used)

**These enzyme activities are insignificantly low

3.3.2 MEMBRANE CLEANING

Enzymatic membrane cleaning was carried out with lipases alone and lipases in combination with proteases. The enzyme combinations used in cleaning regimes are listed in Table 3.3 together with the parameters used to determine their cleaning efficiencies.

With lipases used alone, the lipid content on the membranes after enzymatic cleaning were reduced by 46%, 55% and 33% for the *Candida, Pseudomonas* and *Aspergillus* lipase, respectively. A higher percentage lipid reduction was observed in both the *Pseudomonas* and *Candida* lipases compared with the *Aspergillus* lipase. The highest lipid reduction was obtained with the *Pseudomonas* lipase which, in contrast to the *Aspergillus* lipases, has the lower enzyme activity towards the artificial substrate. Although the *Aspergillus* and the *Pseudomonas* lipases are both *sn*-1,3 specific lipases, the latter enzyme gave a higher percentage lipid reduction. The *Candida* lipase which is a random lipase, gave the lowest percentage lipid reduction.

Table 3.3 Cleaning efficiencies of the enzymes used in different combinations of enzyme-based cleaning solutions for polysulphone membranes fouled in abattoir effluent. All cleaning solutions also contained 0.1% of the non-ionic detergent, Triton X-100.

Enzymatic cleaning action	**% LIPID REDUCTION**	**% PROTEIN REDUCTION**	**** PURE-WATER FLUX (l/m^2.hr)**
***Candida* lipase alone**	**45.77 (± 3.09)**	**58.01 (± 2.30)**	127.42 (± 10.50)
***Candida* lipase + *Bacillus* protease**	47.74 (± 0.81)	84.91 (± 6.94)	155.05 (± 5.56)
***Candida* lipase + Protease A**	46.69 (± 10.24)	83.35 (± 8.73)	147.92 (± 21.29)
***Candida* lipase + *Aspergillus* protease**	47.77 (± 2.38)	82.63 (± 2.68)	142.20 (± 18.02)
***Pseudomonas* lipase alone**	**55.32 (± 10.40)**	**74.49 (± 0.98)**	163.93 (± 15.21)
***Pseudomonas* lipase + *Bacillus* protease**	66.52 (± 3.39)	93.43 (± 4.92)	207.57 (± 78.97)
***Pseudomonas* lipase + Protease A**	68.83 (± 3.75)	87.26 (± 3.26)	216.28 (± 23.60)
***Pseudomonas* lipase + *Aspergillus* protease**	67.09 (± 11.09)	86.67 (± 1.57)	205.19 (± 17.13)
***Aspergillus* lipase alone**	**33.09 (± 4.17)**	**29.30 (± 4.46)**	101.33 (± 3.32)
***Aspergillus* lipase + *Bacillus* protease**	43.53 (± 1.10)	35.58 (± 1.10)	106.79 (± 9.49)
***Aspergillus* lipase + Protease A**	44.23 (± 1.40)	49.33 (± 8.38)	101.73 (± 18.87)
***Aspergillus* lipase + *Aspergillus* protease**	43.39 (± 5.70)	61.06 (± 1.89)	135.47 (± 9.48)

*Enzymatic membrane cleaning trials were performed as previously described

**The flux of the control membrane (unfouled membrane treated with 0,1% Triton X-100) was 219.84 l/m^2.hr and was used as the reference value for the calculation of cleaning performance.

When lipases were used in combination with proteases, lipase cleaning was done first, followed by the addition of the proteases. This protected the lipase enzymes from degradation by proteases. A significantly greater lipid reduction (Table 3.3) was observed after cleaning of the membranes with lipases (*Candida*, *Pseudomonas* and

Aspergillus) in conjunction with proteases (*Bacillus*, Protease A and *Aspergillus*), compared with lipases alone. The *Pseudomonas* lipase showed a lipid reduction of aproximately 70% when used in conjunction with the proteases.

The protein reduction, after membrane cleaning with lipases alone were, 58%, 75% and 29% for the *Candida*, *Pseudomonas* and *Aspergillus* lipases, respectively. Although lipases showed only low protease activity, proteins were removed from fouled membranes by lipase treatment alone. This can be explained by the association of several proteins with lipids which are adsorbed onto the membranes. During lipolytic cleaning, lipids are hydrolysed and the proteins adsorbed onto the lipids are therefore also solubilised into the cleaning solution. The presence of detergents in the lipolytic cleaning solutions further prevented proteins from readsorbing onto the membranes.

A general increase in percentage protein reduction was found after cleaning of polysulphone membranes with lipases in conjunction with proteases when compared to lipases alone. Protein reductions of 80-90% were obtained when the *Pseudomonas* and *Candida* lipases were used in combination with all the identified proteases.

The determination of the pure-water flux through polysulphone membranes is the fundamental method for showing the enzymatic cleaning efficiency of the identified lipases and proteases.

After cleaning of fouled polysulphone membranes with lipases alone, the highest flux improvement was obtained with the *Pseudomonas* lipase in comparison with the *Candida* and *Aspergillus* lipases, as shown in Table 3.3. These results were determined where flux through the unfouled membrane (219 l/m^2.h) treated with Triton X-100 was used as the reference value.

Pure-water flux improvements with lipases in conjunction with proteases as cleaning agents, were moderately higher in comparison with those calculated for lipases alone. The percentage increase in pure-water flux is shown in Table 3.3. Notably, the *Pseudomonas* lipase in conjunction with all the identified proteases showed a flux improvement of nearly 200%.

Statistical analysis according to Pearson's method was subsequently done to determine the correlation between the pure-water flux and lipid and protein reduction after enzymatic cleaning. Results from this statistical analysis are shown in Table 3.4. There was a linear relationship between flux improvement and lipid and protein reduction so that a reduction in the adsorbed lipid and protein material is associated with an increase in the water flux through the membrane.

Table 3.4 Statistical analysis, according to Pearson's method, of the correlation between lipid and protein reduction with pure-water flux of polysulphone membranes after cleaning with lipases alone.

Parameter	% Lipid reduction	% Protein reduction
Number of XY Pairs	4	4
Pearson r	0.95	0.99
99% confidence interval	-0.6195 to 0.9997	-0.1156 to 0.9999
P value (two-tailed)	0.04	0.01
P value summary	*	*
Is the correlation significant? (alpha=0.05)	Yes	Yes
R squared	0.91	0.97

For membrane cleaning with lipases in combination with the proteases, the same linear relationship was obtained for the statistical analysis of the correlation of flux improvement and lipid and protein reduction (Table 3.5). The correlation of percentage lipid reduction was, however, higher than the percentage protein reduction when lipases were used in combination with proteases as when lipases were used alone as cleaning agent.

Table 3.5 Statistical analysis, according to the Pearson's method, of the correlation between lipid and protein reduction with pure-water flux of polysulphone membranes after cleaning with lipases in combination with proteases.

Parameter	% Lipid reduction	% Protein reduction
Number of XY Pairs	9	9
Pearson r	0.95	0.72
99% confidence interval	0.6402 to 0.9935	-0.1454 to 0.9609
P value (two-tailed)	0.00	0.02
P value summary	***	*
Is the correlation significant? (alpha=0.05)	Yes	Yes
R squared	0.90	0.52

3.4 DISCUSSION

In this study a number of commercially available lipases and proteases were evaluated as possible candidates in cleaning regimes for polysulphone ultrafiltration membranes fouled in abattoir effluent. Most attention was given to the lipases as it was previously shown that the performance of fouled membranes was significantly enhanced after lipid reduction. In order to assess and compare the activities of commercially available enzymes, assay systems were set up for lipases as well as proteases. For lipases the soluble artificial substrate, *p*NPP was used the most active lipases were identified. It is interesting to note that the *Candida* lipase was the most active when using the artificial substrate. In cleaning trials, however, the *Pseudomonas* lipase gave the most efficient lipid removal results. This result can be explained by the fact that the *Pseudomonas* enzyme is a protein engineered lipase specifically for the laundry industry. The lipids in abattoir effluent are a heterogenous population of molecules and a somewhat less specific enzyme would therefore be able to hydrolyse a wider range of esters than a more substrate specific enzyme. It must, however, be noted that the use of the artificial substrate is still important in order to establish activity in commercial lipase preparations and that enzymes which had very low activity towards the soluble substrate, could not significantly reduce the lipid content of fouled membranes.

Although it was found that the use of lipases alone could reduce the amount of adsorbed foulants on membranes fouled in abattoir effluent, the combination of proteases with lipases (in a suitable detergent) significantly enhanced the efficiency of the enzymatic cleaning regime. Initial treatment of the fouled membranes with lipases in Triton X100®, not only removed lipids, but also enhanced protein removal by subsequent protease treatment.

The results obtained in this study allowed for the formulation of an effective enzyme-based cleaning regime for membranes fouled in abattoir effluent. This cleaning regime can now be implemented in a pilot study and combined with mechanical and pulsatile techniques as well as membrane pretreatment to yield a practical and economical membrane cleaning system for industrial application.

3.5 CONCLUSIONS

1. Proteins and lipids are the major foulants that adsorb onto polysulphone membranes fouled during treatment of abattoir effluent by ultrafiltration. These foulants severely limit the effective application of ultrafiltration in abattoir effluent purification and therefore necessitated the development of effective enzyme-based cleaning protocols.
2. Cleaning with lipases alone effectively reduced the quantity of membrane foulants (proteins and lipids) adsorbed onto the membranes. The combination of lipases with proteases, however, was more effective than lipases alone in cleaning trials.
3. An increase in pure-water flux values after enzymatic cleaning correlated well with a reduction in the quantity adsorbed protein and lipid substances.
4. Lipase and proteases are effective biological cleaning agents for flat sheet polysulphone membranes fouled in abattoir effluent.

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