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THE DEVELOPMENT OF CHARACTERISING AND CLEANING TECHNIQUES TO CLASSIFY FOULANTS AND TO REMOVE THEM FROM ULTRA- AND MICROFILTRATION MEMBRANES BY BIOCHEMICAL MEANS

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by the
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EXECUTIVE SUMMARY

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BACKGROUND AND MOTIVATION

The production of high volumes of effluent with an extremely high chemical oxygen demand (COD) by biologically related industries like abattoirs, woolscouring processes, paper and pulp industries and egg processing plants is a world wide phenomenon. Cowan et al., (1992) reported that in South Africa 300 registered abattoirs use approximately 7 000 000 m3/a of potable quality water and produce about 6 000 000 m3/a of effluent to municipal sewers, an effluent rich in protein and lipid material. The woolscouring process produces 0.5 million m3 of a highly polluting dark slurry with an obnoxious odour and complex composition (Mozes et al., 1981 and Pearson et al., 1976). The content of this effluent includes wool fat (lanolin), suint, protein, sand and clay (Oellermann et al., 1992).

These industries are currently faced with the challenge to substantially reduce the discharge of conventional and toxic pollutants to the receiving environment without reducing economic viability or competitiveness. One method to achieve this goal is to concentrate pollutants and recycle wastewater with the aid of ultrafiltration (UF). UF is a membrane separation process, conservative in energy requirements, and low in operating and maintenance costs (Michaels, 1980 and Rachwal, *et al.*, 1994) which can remove up to 90 % of the (COD) and reduce the potable water demand of an industry, such as an abattoir, by 25% (Cowan *et al.*, 1992).

A major problem is, however, that the materials found in these effluents adsorb onto the membranes which subsequently leads to membrane fouling. Fouling, characterised as an "irreversible flux decline" of water through a membrane, causes an increase in membrane cleaning costs, process down-time, as well as membrane damage due to the effect of the harsh conventional cleaning chemicals often used (Cowan *et al.*,1992). As Cheryan, (1986) concluded: "..fouling and inefficient cleaning are the main reasons why UF has not yet fulfilled its promise as an efficient and economical method for wastewater treatment". Although membrane technology has made great advances recently, the cleaning of fouled membranes have been neglected. Processes which would minimise fouling, like effluent pre-treatment, operational conditions and membrane characteristics, were on the other hand extensively researched and developed (Sedath *et al.*, 1993 and Eckner and Zottola, 1993). This lack of information about the nature and extent of foulants adsorbed to membranes, as well as a lack in eligible cleaning agents, lead to the use of powerful chemical cleaning agents to restore transmembrane flux. These methods, however, caused severe membrane damage (Cheryan, 1986)which influenced membrane characteristics such

as morphological properties (pore shape, pore length, pore density and pore size distribution) as well as the chemical and electrical surface properties (Hanemaaijer *et al.*, 1988). Jacobs (1991) and Coolbear *et al.*, (1992) reinitiated a different approach in membrane cleaning with the introduction of enzymes as cleaning agents. As biocatalysts, enzymes are highly specific for the substrates they react with and in the type of reactions that they catalyse. The group of enzymes called the proteases, for instance, is specific to protein degradation and could, under the right conditions, be used to remove proteins adsorbed onto fouled membranes. Unlike chemical cleaning methods, these enzymes cause no further pollution or membrane damage. Enzymes are, however, considered to be expensive reagents and this fact can impact negatively on their application in cleaning regimes for membranes fouled by biological effluents. Modern molecular biology and genetic engineering, however, enables one to produce many proteins economically and on a large scale in foreign hosts. This technology can also be applied to enzyme production for membrane cleaning once the relevant enzymes have been identified.

This report includes literature surveys on membrane fouling and the consequences of fouling on membrane characteristics, as well as the factors that affect fouling. A review of past and current methods of flux improvement (before and after fouling) as well as some cleaning methods is also given. A broad classification was also made from the available information in the literature to illustrate the origin of different potential foulants derived from biological effluents. The composition of effluents arising from abattoirs and woolscouring processes were analysed and the major foulants in these effluents identified. Polysulphone membranes (PSM) were subsequently statically fouled in these two effluents and the adsorbed foulants were identified and quantified. Biological cleaning mixtures were developed for the removal of adsorbed foulants from statically fouled PSM. Heterologous experession systems for the production of potential enzymes to be used as cleaning agents were also investigated.

OBJECTIVES

The goals set for this project were the following:

1. To establish and develop guidelines and procedures for the classification of organic fouling which may impede the performance of microfiltration membranes. This classification will then be used to develop effective and specific biochemical and physical methods for the removal of foulants.

2. There are a number of commercial enzyme preparations available on the market that are used for cleaning purposes in the food industry. These preparations are not specific and a broad spectrum of biological materials will be removed by them. In membrane installations where fouling can be attributed to one or more main group(s) of biological molecules, the development of specialised enzyme systems, highly specific for particular fouling agents, will be the most cost and time effective method for the removal of foulants from the membrane surface.

In order to achieve these goals:

1. Potential membrane foulants in the effluent of, amongst others, abattoir, maize, egg and woolscouring processing plants will be identified.
2. Methods will be developed to quantitatively bring fouling deposits from these membranes into solution in order to analyse these foulants and to develop methods for the biological removal of these substances from the membranes. Although enzymes can be used effectively to degrade insoluble biopolymers, the effectiveness and rate of the biological cleaning process will be enhanced significantly if foulants could be solubilised.
3. The cloning and large scale preparation of more specialised enzymes for the degradation of specific membrane foulants will be investigated thoroughly.

At the first steering committee meeting it was decided to change the emphasis of the investigation from the egg and maize processing plants to effluent derived from paper and pulp processing. The reason for this shift was that the egg processing plant closed down and the anaerobic digestion-ultrafiltration (ADUF) plant currently in operation in the only maize processing plant in South Africa, is efficient and no real problems were encountered with fouling. A literature study on the effluent from the maize processing is, however, included in this report. In contrast the black liquor from the paper and pulp industry is currently being treated by UF and certain fouling problems do occur on these installations.

RESULTS AND CONCLUSIONS

Abattoir effluent was analysed for potential foulants and it was found that lipids and proteins are quantitatively, the most important foulants. PSM were fouled in this effluent and the fouled membranes were analysed for adsorbed lipid and protein material. With the nature of adsorbed foulants known, specific enzymes were chosen to remove foulants adsorbed onto these fouled membranes. The efficiency of the cleaning experiments were determined by comparing the

potential of each cleaning agent to remove foulants from membranes and to restore the performance and characteristics of the fouled membrane. All the experiments carried out with abattoir effluent fouled membranes, were repeated with wool scouring effluent (WSE). Results obtained in this investigation showed that for industries like the abattoir and wool scouring, the same basic methods could be employed to classify, characterise and remove foulants adsorbed onto PSM membranes. The following conclusions may be drawn from the results obtained in the study of abattoir and WSE effluent:

1. Proteins and lipids are important foulants in abattoir effluent, while protein and wax (lanolin) are the most prominent foulants in WSE. The content of these foulants in the effluent vary significantly from hour to hour and from day to day. This variation in effluent composition renders the use of model foulants and model solutions, to characterise fouling in abattoir and WSE, unserviceable and therefore real effluents were used in fouling experiments;
2. Protein and lipid analysis, developed in this study, proved to be reliable and sufficiently sensitive to determine the nature and amount of these materials adsorbed onto membranes during the fouling period. Results from lipid and protein analysis indicate that multilayer adsorption occurs. The lipids adsorb first and this first layer of lipid material provides an ideal surface for further adsorption of proteins and lipids. In addition to the conventional protein determination methods, a novel rapid staining method was developed to determine protein adsorption onto the membranes. These techniques were also effectively used to determine the efficiency of foulant removal in cleaning experiments.
3. Dynamic contact angle measurements have not previously been used to characterise membrane fouling in abattoir and WSE effluent. It was, however, found that the information obtained from this technique was not only useful in characterising membrane surface characteristics, but also of value in the assessment of cleaning efficiency. Changes in the contact angle correlated well with changes observed on the membrane surface after foulant adsorption.
4. Transmembrane flux measurements of clean water can be seen as the ultimate method to characterise the effect of fouling on membrane permeability. The decline of the clean water flux through statically fouled membranes followed the same pattern as the flux decline observed in an actual UF process. A rapid flux decline was observed within the first hour of fouling, followed by a more steady decline in the following four hours until a steady state was reached. These results indicate that, in future fouling experiments, two to

three hours of fouling will be sufficient and that static adsorption studies could be used as an inexpensive method to characterise foulants and to develop cleaning regimes for real UF processes;

5. Enzymes specific for protein and lipid hydrolysis were used to clean membranes fouled in abattoir effluent. Cleaning experiments, evaluated for their potential to remove lipids and proteins, clearly indicate that specific enzymes and enzyme detergent mixtures can effectively remove foulants adsorbed onto UF membranes. Enzymes alone, and in conjunction with specific detergents, produced good cleaning results. These results could, however, only be obtained with a sound knowledge of the nature of the foulants;
6. The experience gained in effluent and membrane analysis from the abattoir industry was transferred to WSE. In WSE lanolin is the main lipid foulant and the enzymes that could remove lipids from PSM fouled in abattoir effluent, could not remove the lanolin. An esterase, with greater specificity towards ester bonds, was used instead of the lipases which successfully removed lipids from abattoir fouled membranes. The cleaning agent containing an esterase was the most efficient for the removal of foulants and subsequent flux restoration on membranes fouled in WSE;
7. Results obtained during this investigation show that enzymes, as highly specific biocatalysts, are extremely useful and effective components of cleaning mixtures for biologically fouled membranes. In order to use these catalysts effectively it is, however, very important to know the exact composition of the foulants deposited on the membrane.

A novel yeast expression system was developed for the economical and facile expression and purification of proteins in yeast. This system combines the secretory ability of yeast with the use of affinity purification of the expressed protein in a single step. Future studies will concentrate on refining the system and adapting it for the production of specific enzymes that will form part of future cleaning regimes in high yield. Other organisms will also be investigated for enzyme production.

A literature survey of the composition of black liquor derived from the paper and pulp industry, showed that this effluent was considerably more complex and of a total different nature than the abattoir and WSE. Time constraints and the complexity of the paper and pulp effluent lead to the registration of a follow-up study (WRC Project no. 660) in which specific attention will be given to the effluent from the paper and pulp industry and the potential membrane foulants that it might contain.

The promising results obtained in this investigation show that enzyme based, biologically oriented cleaning regimes, hold a great promise for the restoration of UF membranes fouled in biological effluents.

RECOMMENDATIONS FOR FURTHER RESEARCH

This study has opened up new avenues for investigation of:

1. Ideal enzymes, or enzyme detergent mixtures (biological cleaning agents), for the cleaning of UF membranes fouled in the process of recycling abattoir wastewater;
2. The implementation of novel biological cleaning methods on a pilot-scale ultrafiltration plant at an abattoir and the optimisation of cleaning techniques under real UF conditions (once the techniques are optimised it will be introduced to the industry for industrial application);
3. The characterisation of potential foulants present in the wastewater produced in the pulp and paper industry and the identification of enzymes capable of degrading the foulants found in this effluent;
4. Methods for the large scale economical production of enzymes used in cleaning regimes. Expression systems available in bacteria and yeast will be further evaluated in this regard;
5. The implementation of these novel cleaning techniques in field experiments at a pilot plant, followed by optimisation of techniques for actual operating conditions. The successful biological cleaning methods, with all the advantages previously discussed, may then be introduced for industrial application.

ABBREVIATIONS

b	Fouling coefficient
BOD	Biological oxygen demand
BSA	Bovine serum albumin
BSA/FAP	Bovine serum albumin (fatty acid poor)
Cb	Concentration of feed solution
Cg	Concentration of gelation
CIP	Calf intestinal phosphatase
C_m	Concentration of feed solution on the membrane surface
COD	Chemical oxygen demand
CP	Concentration polarisation
CYP17	Gene encoding cytochrome P450c17
Da	Dalton
DNA	Deoxyribonucleic acid
$\Delta\pi$	*Osmotic pressure difference*
ED	Electro dialysis
EDR	Electro dialysis reversal
EDTA	Ethylenediamine tetraacetic acid
FIP	Formed-in-place membranes
GLC-MS	*Gas liquid chromatography-mass spectrometry*
PMSF	Phenyl methyl-sulphonyl fluoride
IPTG	Isopropylthiogalactoside
Jv	Flux
K	Mass transfer coefficient
kPa	Kilo Pascal
L'p	Hydraulic permeability of pure-water
Lp	Hydraulic permeability
MBP	Maltose binding protein
MM	Molecular mass
NMMCO	*Nominal molecular mass cut off*
P450c17	Cytochrome P450-dependent steroid 17α-hydroxylase
PAN	Polyacrylonitrile
PCR	Polymerase chain reaction
pI	Isoelectric point
pMal-c2	*E. coli* expression plasmid encoding maltose binding protein
PSM	Polysulphone membranes
R	Membrane resistance
RO	Reverse Osmosis
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gelelectrophoresis
SE	Standard error
SEM	Scanning electron microscope
SAWTRI	South African Wool and Textile Research Institute
UF	Ultrafiltration
WSE	Wool scouring effluent

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"..THE DEVELOPMENT OF CHARACTERISATION AND CLEANING TECHNIQUES FOR THE CLASSIFICATION OF ORGANIC FOULING OF ULTRA AND MICROFILTRATION MEMBRANES..".

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TABLE OF CONTENTS

CHAPTER 1 1

CHAPTER 2 4

2.1 INTRODUCTION 4

2.2 PRESSURE-DRIVEN MEMBRANE PROCESSES 4

2.2.1 Reverse Osmosis 6

2.2.2 Ultrafiltration 6

2.2.3 Microfiltration 7

2.3 ELECTRICALLY-DRIVEN MEMBRANE PROCESSES 7

2.3.1 Electrodialysis 7

2.4 APPLICATIONS OF MEMBRANES IN INDUSTRIAL BIOTECHNOLOGY 8

CHAPTER 3 9

3.1 INTRODUCTION 9

3.2 CONCENTRATION POLARISATION AND FOULING 11

3.3 EVIDENCE OF FOULING 12

3.3.1 Irreversible Flux Decline 12

3.3.2 Change In Retention Characteristics Of Membranes 12

3.4 POTENTIAL FOULANTS 13

3.5 PROTEIN FOULING 13

3.6 FACTORS THAT AFFECT PROTEIN FOULING 14

3.6.1 Concentration Of Protein Feed Solution 14

3.6.2 Temperature Of The Protein Feed 14

3.6.3 pH And Ionic Environment 15

3.6.4 Associated Molecules 16

3.6.5 The Effect Of Membrane NMMCO And Molecular Mass Distribution Of The Protein Solution 16

3.6.6 Operating Conditions 17

3.6.7 Membrane Character 17

3.7 LIPID FOULING 18

3.8 PHENOLIC FOULING 18

3.9 CARBOHYDRATE FOULING 19

3.10 MATHEMATICAL MODELS OF FOULING 19

3.10.1 Concentration Polarisation Model 20

3.10.2 Gel Polarisation Model 20

3.10.3 Osmotic Pressure Model 20

3.10.4 Adsorption And Osmotic Pressure Model 21

CHAPTER 4 ..... **23**
4.1 INTRODUCTION 23
4.2 FOULING REDUCTION 23
4.2.1 Flow Conditions 23
4.2.2 Pretreatment Of Effluent 24
4.2.3 Membrane Properties And Pretreatment 24
4.2.4 Operational Factors 26
4.2.5 Water Quality 27
4.3 CLEANING AND DISINFECTION OF UF MEMBRANES 27
4.3.1 Mechanical Cleaning 27
4.3.2 Chemical Cleaning 28
4.3.3 Biological Cleaning 29
4.4 CLEANING AGENTS USED ON FOULED UF MEMBRANES 29
4.4.1 Alkalis 29
4.4.2 Acids 30
4.4.3 Enzymes 30
4.4.4 Surface-Active Agents 31
4.4.5 Formulated Agents 32
4.4.6 Disinfectants 32
4.5 CLEANING CONDITIONS FOR FOULED UF MEMBRANES 32
4.5.1 Concentration Of The Cleaning Agent 32
4.5.2 Cleaning Time 33
4.5.3 Temperature 33
4.5.4 pH 33
4.6 FOULING EXTENT AND CLEANING EFFICIENCY 33
CHAPTER 5 ..... **35**
5.1 INTRODUCTION 35
5.2 EXPERIMENTAL 36
5.2.1 Materials 36
5.2.2 Analysis Of Abattoir Effluent 37
5.2.3 Protein Determination Techniques Of Psm Fouled In Abattoir Effluent 38
5.2.4 Determination Of The Lipid Content In The Adsorbed Layer 39
5.2.5 Pure-Water Flux Determination 40
5.3 RESULTS AND DISCUSSION 40
5.3.1 Analysis Of Abattoir Effluent 40
5.3.2 Protein Content Of Fouled Psm 41
5.3.3 Lipid Content Of Fouled Psm 43

5.3.4 Contact Angle Measurements Of Clean And Fouled Psm 44
5.3.5 Pure-Water Flux Measurements Of Fouled Psm 45
5.4 CONCLUSIONS 47
CHAPTER 6 49
6.1 INTRODUCTION 49
6.2 EXPERIMENTAL 50
6.2.1 Materials 50
6.2.2 Fouling Of Membranes 51
6.2.3 Cleaning Methods 51
6.2.4 Determination Of Cleaning Efficiency 52
6.3 RESULTS AND DISCUSSION 52
6.3.1 Optimisation Of Enzyme Concentration And Incubation Time 52
6.3.2 A Comparison Of Various Cleaning Regimes At Optimal Conditions 58
6.4 CONCLUSIONS 63
CHAPTER 7 64
7.1 INTRODUCTION 64
7.2 EXPERIMENTAL 66
7.2.1 Materials 66
7.2.2 Effluent Analysis 67
7.2.3 Fouling 67
7.2.4 Cleaning 68
7.2.5 Determination Of Cleaning Efficiency 68
7.3 RESULTS AND DISCUSSION 68
7.3.1 Effluent Composition 68
7.3.2 Protein Content 68
7.3.3 Lipid Content 69
7.3.4 Contact Angle Measurements 70
7.3.5 Flux Measurements 70
7.4 CONCLUSION 71
CHAPTER 8 73
8.1 INTRODUCTION 73
8.2 EXPERIMENTAL (EXPRESSION OF PROTEINS IN *E.COLI*) 76
8.2.1 Materials 76
8.2.2 Preparation Of The Pmal-C2 Plasmid For CYP17 CDNA Insertion 76
8.2.3 Insertion Of The CYP17 Cdna Fragment Into The PMAL-C2 Vector 76
8.2.4 Expression And The Purification Of The MBP-P450C17 Fusion Protein 77

8.2.5 Isolation Of The MBP-P450C17-Fusion Protein By Affinity Chromatography 78
8.2.6 Separation Of The Two Components Of The Fusion Protein To Obtain Pure Human P450C17 Peptide 78
8.2.7 Results And Discussion 78
8.3 EXPERIMENTAL (EXPRESSION OF PROTEINS IN *S. CEREVISIAE)* 79
8.3.1 Materials 79
8.3.2 Plasmid Construction 79
8.3.3 Bacterial Expression Of pPRL-2Mod17A(His)$_5$ 80
8.3.4 Yeast Transformation And Expression. 80
8.3.5 Purification Of P450C17 (His)$_5$ 81
8.3.6 Results And Discussion 81
CHAPTER 9 83
9.1 INTRODUCTION 84
9.2 EFFLUENT COMPOSITION 84
9.3 TREATMENT OF PULP EFFLUENT 86
9.4 APPLICATION OF MEMBRANE TECHNOLOGY IN PULP EFFLUENT TREATMENT 87
9.5 FLUX DECLINE CAUSED BY FOULING OF MEMBRANES USED IN PULP EFFLUENT TREATMENT 88
9.6 CONCLUSION 88
CHAPTER 10 90
10.1 INTRODUCTION 90
10.2 TREATMENT TECHNIQUES 90
10.3 MEMBRANE FOULING 91
10.4 CONCLUSION 92
CHAPTER 11 93
REFERENCES 99

LIST OF TABLES ANF FIGURES

Fig. 2.1	Schematic presentation of dead-end filtration (Toyomoto and Higuchi, 1992).	5
Fig. 2.2	Schematic presentation of cross-flow filtration. The filtration method used to minimise concentration polarisation (Toyomoto and Higuchi, 1992).	5
Fig. 2.3	Particle range of the different filtration processes. Particle sizes are given in micrometer scale and molecular mass (Croll, 1992).	8
Fig. 3.1	Dependence of flux on operating pressure. C_b is the concentration of solute in the bulk solution (Echner and Zottola, 1993).	9
Fig. 3.2	Schematic presentation of the different stages in flux decline during ultrafiltration. Fouling usually starts with a rapid decline during initial stages and thereafter reaches a plateau (Echner and Zottola, 1993).	10
Fig. 3.3	Factors that could cause flux reduction during the filtration of solutes that are rejected by the membrane (Grund *et al.*, 1992).	10
Fig. 3.4	Diagram of materials that cause problems in membrane filtration. The difficulties caused by these materials are membrane damage, fouling or scaling (Bauser *et al.*, 1982).	13
Fig. 3. 5	General titration curve of a typical protein molecule. The graph reflects the effect of pH on the charge of the molecule. At low pH values all carboxyl and amino groups are protonated, while at a high pH, all carboxyl groups are ionised and amino groups are free (Bohinski, 1987).	15
Fig. 3.6	Diagram of the contact angle between a water drop and the surface of a membrane. The more hydrophilic the surface the smaller the contact angle and water will tend to wet the membrane.	18
Tab. 4.1	Interactive forces between protein molecules, polymer surfaces and membranes and the preventative surface treatment (Bauser *et al.*, 1982)	26
Fig. 5.1	Diagram of the "Perspex" frame membranes were adhered to in static fouling experiments. The 100 x 300 mm strips of PSM were clamped horizontal to the 120 mm posts and submerged into the effluent. Lead weights were also clamped to the frame to keep the frame submerged.	38
Fig. 5.2	Experimental set-up to determine the transmembrane flux of pure-water through unfouled, fouled and cleaned PSM.	40
Tab. 5.1	Major factors in abattoir effluent involved in membrane fouling. Values presented are the mathematical mean ±SD.	41
Fig. 5.3	The colour development of proteins adsorbed onto membranes during fouling. Membranes were treated as follows no's. 1 and 2 fouled followed by staining, 3 and 4 fouled, cleaned and stained, 5 and 6 were unused unstained, 7 fouled and not stained and 8 is an unused reference membrane that was stained.	42
Fig. 5.4	Amount of protein ($\mu g/cm^2$) adsorbed onto PSM vs. time in contact with abattoir effluent. Values presented are the mathematical mean of at least four determinations ±SE.	43

Fig. 5.5	Amount of lipids ($\mu g/cm^2$) adsorbed onto PSM vs. time in contact with abattoir effluent. Values presented are the mathematical mean of at least four determinations ±SE.	44
Fig. 5.6	Contact angle (degrees) of abattoir effluent fouled PSM vs. time of contact with the abattoir effluent. Values presented are the mathematical mean of at least four determinations ±SE.	45
Fig. 5.6	Pure water flux though abattoir effluent fouled PSM vs. time of contact with the effluent. Values presented are the mathematical mean of at least four determinations ±SE.	45
Fig. 5.8	The dynamic flux for a 1% BSA solution as reprinted from Fane and Fell, (1987).	46
Fig. 5.9	The effect of increasing feed pressure on the pure-water flux of an abattoir effluent fouled and an unfouled membrane.	47
Fig. 6.1	Effect of enzyme concentration on protein removal from fouled PSM. Enzymes evaluated were: protease A, lipase A and a lipase A:(0,1%) Triton X100 mixture. Incubation time was 60 minutes, at pH 7,5 and 37 °C.	53
Fig. 6.2	Effect of incubation times on protein removal from fouled PSM. Enzymes evaluated were: protease A, lipase A and a lipase A:(0,1%) Triton X100 mixture. Incubation time was 60 minutes, at pH 7,5 and 37 °C.	53
Fig. 6.3	Effect of enzyme concentration on lipid removal from fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100 mixture. Incubation times were 60 minutes at pH 7,5 and 37 °C.	54
Fig. 6.4	Effect of enzyme incubation time on the lipid removal of fouled PSM. Enzyme evaluated were: protease A, Lipase A and lipase A:(0,1%) Triton X100 mixture. Concentrations used were 1mg/ml at pH 7,5 and 37 °C.	55
Fig. 6.5	Effect of incubation time of a commercial cleaning agent on the lipid and protein removal of fouled PSM. A 3% solution of Alkazyme:Zymex (1:1) was used as prescribed by the manufacturers.	55
Fig. 6.6	Effect of enzyme concentration on the contact angle of fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100, Incubation time was 60 min at pH 7,5 and 37 °C.	56
Fig. 6.7	Effect of enzyme incubation time on the contact angle of fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100, Concentration used was 1mg/ml at pH 7,5 and 37 °C.	57
Fig. 6.8	Effect of enzyme concentration on the pure-water flux of fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100, Concentration used was 1mg/ml at pH 7,5 and 37 °C.	57
Fig. 6.9	Effect of enzyme incubation time on the pure-water flux of fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100, Enzyme concentrations were 1mg/ml at pH 7,5 and 37 °C.	58

Fig. 6.10 Effect of the incubation time of Alkazyme:Zymex (1:1, 3% solution) on the contact angle and pure-water flux of fouled PSM. Conditions were as prescribed by the manufacturers 59

Fig. 6.11 Protein content of abattoir fouled PSM after treatment with different cleaning mixtures. Membranes, fouled for 8 hours, were treated as follows: **[A]** No treatment; **[B]** Buffer, 60 min; **[C]** SDS (0,2%), 60 min; **[D]** Lipase A (3mg/ml), 60 min; **[E]** Protease B (3 mg/ml), 60 min; **[F]** Protease C (3mg/ml), 60 min; **[G]** SDS (0,4%), 60 min; **[H]** Triton X100 (0,1%), 60 min; **[I]** Protease A (3mg/ml), 60 min; **[J]** Lipase A:Triton X100 (1mg/ml:0,1%), 60 min followed by treatment with Protease A (1mg/ml), 60 min; **[K]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[L]** Alkazyme:Zymex (1:1) 3% solution, 60 min; Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD. 59

Fig. 6.12 Lipid content of abattoir fouled PSM after treatment with different cleaning mixtures. Membranes, fouled for 8 hours, were treated as follows: **[A]** No treatment; **[B]** Buffer, 60 min; **[C]** Lipase A (3mg/ml), 60 min; **[D]** Protease B (3 mg/ml), 60 min; **[E]** Triton X100 (0,1%), 60 min; **[F]** Protease C (3mg/ml), 60 min; **[G]** SDS (0,2%), 60 min; **[H]** Protease A (3mg/ml), 60 min; **[I]** SDS (0,4%), 60 min; **[J]** Alkazyme:Zymex (1:1) 3% solution, 60 min; **[K]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[L]** Lipase A:Triton X100 (1mg/ml:0,1%), 60 min followed by treatment with Protease A (1mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD. 60

Fig. 6.13 Contact angle of fouled PSM after treatment with different cleaning mixtures. Membranes fouled for 8 hours, were treated as follows: **[A]** Unfouled membrane; **[B]** Fouled for 8 hours; **[C]** Buffer, 60 min; **[D]** Protease B (3mg/ml), 60 min; **[E]** SDS (0,2%), 60 min; **[F]** SDS(0,4%), 60 min; **[G]** Protease C (3mg/ml), 60 min; **[H]** Lipase A (3mg/ml), 60 min; **[I]** Triton X100 (0,1%), 60 min; **[J]** Protease A (3mg/ml), 60 min; **[K]** Alkazyme:Zymex (1:1) 3% solution, 60 min; **[L]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[M]** Lipase A:Triton X100 (1mg/ml:0,1%), 60 min followed by treatment with Protease A (1mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD. 61

Fig. 6.14 Pure-water flux through fouled PSM after treatment with different cleaning mixtures. Membranes were fouled for 8 hours and treated as follows: **[A]** Fouled for 8 hours; **[B]** Buffer, 60 min; **[C]** SDS (0,2%), 60 min; **[D]** SDS (0,4%), 60 min; **[E]** Protease B (3mg/ml), 60 min; **[F]** Protease C (3mg/ml), 60 min; **[G]** Triton X100 (0,1%), 60 min; **[H]** Lipase A (3mg/ml), 60 min; **[I]** Protease A (3mg/ml), 60 min; **[J]** Unfouled membrane; **[K]** Alkazyme:Zymex (1:1) 3% solution, 60 min; **[L]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[M]** Lipase A:Triton X100 (1mg/ml:0,1%), 60 min followed by treatment with Protease A (1mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD. 62

Tab. 7.1 Characteristics of wool scouring effluent, according to the protein, lipid contents, as well as the temperature and pH. Effluent 1 contains water from the first scouring bowls while effluent 2 contains the later stage scouring water. Effluent 2 is treated with dynamic formed-in-place hydrous zirconium (IV) oxide UF membranes to produce the permeate. Values presented are the mathematical mean ±SD. 68

Fig. 7.1 Protein content of PSM fouled in WSE. **[A]** Fouled for 24 hours; **[B]** Buffer 60 min; **[C]** Protease B (3mg/ml), 60 min; **[D]** Protease A (3mg/ml), 60 min; **[E]** Lipase A (3mg/ml), 60 min ; **[F]** Protease C (3mg/ml), 60 min; **[G]** Esterase A (3mg/ml), 60 min; **[H]** 0,1% Triton X100, 60 min, 37 °C; **[I]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min;**[J]** SDS (0,4%), 37 °C, 60 min; **[K]** Alkazyme:Zymex (1:1), 3%, 60 min; **[L]** Lipase A:Triton X100 (1mg/ml:0,2%), 60 min followed by treatment with Protease A (1mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD. 69

Fig. 7.2 Lipid content of PSM fouled in WSE. **[A]** Fouled for 24 hours; **[B]** Buffer 60 min; **[C]** Protease A (3mg/ml), 60 min; **[D]** Protease C (3mg/ml), 60 min; **[E]** Lipase A (3mg/ml), 60 min; **[F]** 0,1% Triton X100, 60 min, 37 °C; **[G]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[H]** SDS (0,4%), 37 °C, 60 min; **[I]** Protease B (3mg/ml), 60 min; **[J]** Alkazyme:Zymex (1:1), 3%, 60 min; **[K]** Lipase A:Triton X100 (1mg/ml:0.2%), 60 min followed by treatment with Protease A (1mg/ml), 60 min; **[L]** Esterase A (3mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD. 70

Fig. 7.3 Transmembrane flux of clean water through PSM fouled in WSE. **[A]** Fouled for 24 hours; **[B]** Buffer 60 min; **[C]** Protease A (3mg/ml), 60 min; **[D]** Protease C (3mg/ml), 60 min; **[E]** 0,1% Triton X100, 60 min, 37 °C; [F] Lipase A (3mg/ml), 60 min; **[G]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[H]** SDS (0,4%), 37 °C, 60 min; **[I]** Protease B (3mg/ml), 60 min; **[J]** Unfouled membrane; **[K]** Alkazyme:Zymex (1:1), 3%, 60 min; **[L]** Lipase A:Triton X100 (1mg/ml:0.2%), 60 min followed by treatment with Protease A (1mg/ml), 60 min; **[M]** Esterase (A) 3mg/ml, 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD. 71

Fig. 8.1 Plasmid map of pMAL-c2. 77

Fig. 8.2 Plasmid map of pRPL-c2. 80

Tab. 8.1 Summary of the isolation of the modified human P450c17 expressed and exported by *S. cerevisiae*. 82

Fig. 8.3 Elution profile of His-tagged yeast secretion, protein eluted with an imidazole buffer. 82

Fig. 8.4A SDS-page of export protein. Lane 1; Mr-markers, Lane 2-4; 1,2 and 20 microgram protein respectively. 83

Fig. 8.4B Immunoblot of export protein with pig anti-P450c 17 antibody. Lane 1; P450c 17, lane 2; Mr-markers. 83

Fig. 10.1 Schematic diagram of the ADUF process. The anaerobic digester (AD) is coupled to the UF membrane system. 91

Fig. 11.1 Summary of the variables used to characterise fouling of membranes over an eight hour period in abattoir effluent. Protein content and lipid content are in $\mu g/cm^2$ membrane, contact angle in degrees and pure-water flux in $l/m^2 \cdot h$. 94

Fig. 11.2 Comparison of pure-water flux, lipid content, protein content and contact angle measurements for the three cleaning methods that yielded the highest flux recovery of membranes fouled in abattoir effluent. Enzymes were used as follows: 3% of a Alkazyme:Zymex (1:1) solution for 60 minutes, lipase A:Triton X100 (3mg/ml:0,1%) mixture for 60 minutes, lipase A:Triton X100 (1mg/ml:0,1%) mixture for 60 minutes followed by treatment with Protease A (1mg/ml) for an additional 60 min. 95

Fig. 11.3 Comparison of pure-water flux, lipid content and contact angle measurements of membranes fouled in WSE. Enzymes were used as follows: Esterase A (3mg/ml), for 60 min, lipase A:Triton X100 (1mg/ml:0,1%) mixture for 60 min followed by protease treatment with protease A (1mg/ml) for an additional 60 minutes and Alkazyme:Zymex (1:1), 3% solution, for 60 min. 96

Fig. 11.4 The different fouling substances and the different enzymes that could specifically remove specific foulants form UF membranes. 97

CHAPTER 1

INTRODUCTION

Not only in water-stressed South-Africa, but all over the world, industries such as abattoirs and woolscouring processes produce excessive amounts of wastewater. Cowan *et al.*, (1992) reported that in South Africa 300 registered abattoirs use approximately 7 million m^3/a potable quality water and produce about 6 million m^3/a of effluent to municipal sewers. This effluent is rich in protein and lipid material. The woolscouring process produces 0,5 million m^3 of a highly polluting dark slurry with an obnoxious odour and complex composition (Mozes *et al.*, 1981 and Pearson *et al.*, 1976) which contains mainly wool fat (lanolin), suint, protein, sand and clay (Oellermann *et al.*, 1992).

These industries are currently faced with the challenge to substantially reduce the discharge of conventional and toxic pollutants to the receiving environment without endangering their economy or competitiveness. One method to achieve this goal is to concentrate pollutants and recycle wastewater with the aid of ultrafiltration (UF). UF is an energy conserving membrane separation process with low operating and maintenance costs (Michaels, 1980 and Rachwal, *et al.*, 1994). It is also a process that can remove up to 90 % of the chemical oxygen demand (COD) of an effluent and reduce the potable water demand of an industry, such as an abattoir, by 25% (Cowan *et al.*, 1992).

A major problem is, however, that the materials found in these effluents adsorb onto the membranes which subsequently leads to membrane fouling. Fouling, characterised as an "irreversible flux decline" of water through a membrane, causes an increase in membrane cleaning costs, process down-time, as well as membrane damage due to the effect of the harsh conventional cleaning chemicals often used (Cowan *et al.*, 1992). As Cheryan, (1986) concluded: "..fouling and inefficient cleaning are the main reasons why UF has not yet fulfilled its promise as an efficient and economical method for wastewater treatment". Although membrane technology has made great advances recently, the cleaning of fouled membranes has been neglected. Processes which would minimise fouling, such as effluent pretreatment, operational conditions and membrane characteristics, were on the other hand extensively researched and developed (Sedath *et al.*, 1993 and Eckner and Zottola, 1993). This lack of information about the nature and extent of foulants adsorbed to membranes, as well as a lack of eligible cleaning agents, subsequently lead to the use of powerful abrasive chemical cleaning agents to restore transmembrane flux. These methods, however, resulted

in membrane damage (Cheryan, 1986). Damage caused by chemical cleaning agents may influence membrane characteristics such as morphological properties (pore shape, pore length, pore density and pore size distribution) as well as the chemical and electrical surface properties (Hanemaaijer *et al.*, 1988). Jacobs (1991) and Coolbear *et al.*, (1992), however, initiated a different approach to membrane cleaning with the introduction of enzymes as cleaning agents. As biocatalysts, enzymes are highly specific to the substrates they react with and in the type of reactions that they catalyse. The group of enzymes called the proteases, for instance, is specific for protein degradation and could, under the right conditions, be used to remove proteins adsorbed onto fouled membranes. Unlike chemical cleaning methods, these enzymes cause no further pollution or membrane damage. Enzymes are, however, considered to be expensive reagents and this fact can impact negatively on their application in cleaning regimes for membranes fouled by biological effluent. Modern molecular biology and genetic engineering, however, enables one to produce many proteins economically and on a large scale in foreign hosts such as yeast. This technology can also be applied to enzyme production for membrane cleaning once the relevant enzymes have been identified. In this study, using abattoir effluent as a model, the following successes were achieved:

1. identified and quantified the major foulants present in abattoir effluent;
2. characterised fouling agents adsorbed onto membranes under static adsorption conditions in actual effluent. Using variables such as lipid adsorption, protein adsorption, contact angle and pure-water flux, the amount of each foulant adsorbed onto PSM was quantified. In addition the effect of these foulants on the membrane character was studied;
3. developed novel biological cleaning regimes which include enzymes and enzyme/detergent mixtures. These mixtures, containing commercially available enzymes and enzyme detergent mixtures, were evaluated for their cleaning efficiency by using the variables described under point 2; and
4. extended the application of techniques developed for abattoir fouled membranes to WSE, and membranes fouled with this effluent. Enzymes were chosen according to the nature of these foulants.

In this report the basis of membrane filtration, as well as the different types of filtration techniques, are discussed in chapter two. In chapter three the process of membrane fouling and the consequences of fouling on membrane characteristics, as well as the factors that effect fouling, are

described. A review of past and current methods of flux improvement (before and after fouling) as well as some cleaning methods are given in Chapter four.

Chapter five deals with characterising and quantification of foulants present in operational abattoir process effluent. The static fouling of membranes in this effluent and the subsequent investigation of the fouling agents adsorbed onto these membranes are also described in this chapter. Chapter six gives the results obtained with a number of different newly formulated enzymatic cleaning agents, using the variables established in chapter five, to evaluate the efficiency of these newly developed cleaning regimes. The techniques developed to investigate UF membranes fouled in the abattoir industry, were extended to the woolscouring industry as described in chapter seven. Chapter eight describes the development of recombinant DNA techniques to produce enzymes on a large scale and in Chapter nine the conclusions reached in this study are discussed.

CHAPTER 2

MEMBRANE FILTRATION

2.1 INTRODUCTION

Membrane filtration can be defined as the retention of suspended and dissolved particles, of certain size, by a semipermeable membrane. This membrane filtration process may be driven by pressure- or an electrical potential (Croll, 1992). Pressure-driven membrane separation comprises a continuum of processes designed to separate particles of different sizes by the utilisation of membranes containing appropriately sized pores. These processes include microfiltration (MF), ultrafiltration (UF) and Reverse Osmosis (RO) in order of decreasing pore size. The process of electrodialysis (ED) is based on the transport of ions through membranes as a result of an electrical driving force.

2.2 PRESSURE-DRIVEN MEMBRANE PROCESSES

Membrane types, where filtration through the membrane is driven by pressure, may be formed dynamically or statically. Dynamic membranes are formed either *in sito* (i.e.. where they are being used) or by deliberate deposition of the coating material onto the membrane during filtration runs to form a fine pore layer on a coarser substrate. Such membranes can easily be stripped off when fouled and redeposited. Cross-flow techniques, as with other membrane processes, normally are used to improve filtration runs and in many dynamic membrane formation techniques it is an essential part of the membrane depositing process. The main attraction of dynamic membrane systems is the relative ease of membrane replacement when it becomes clogged, thus greatly reducing or eliminating the need for cleaning or backwashing. Dynamic membranes are a special case. More commonly, membranes are produced from a polymer solution by casting a film on an appropriate support. These membranes remain in place and can not be removed. Fouling problems however, occur and cleaning agents must be used to either prevent fouling, or to clean fouled membranes. A number of different organic polymeric materials may be used for the manufacture of these membranes (Croll, 1992).

Pressure-driven membranes may be operated in either dead-end or cross-flow modes of filtration (Fig. 2.1 and 2.2).

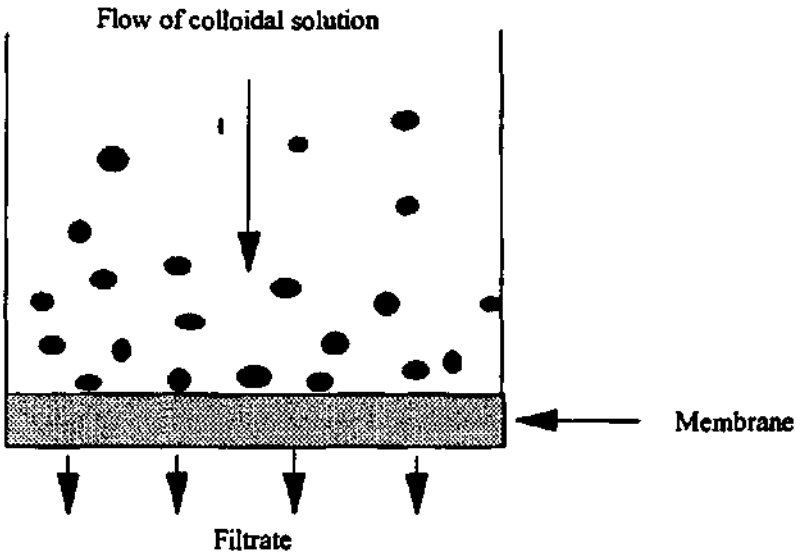

Fig. 2.1 Schematic presentation of dead-end filtration (Toyomoto and Higuchi, 1992).

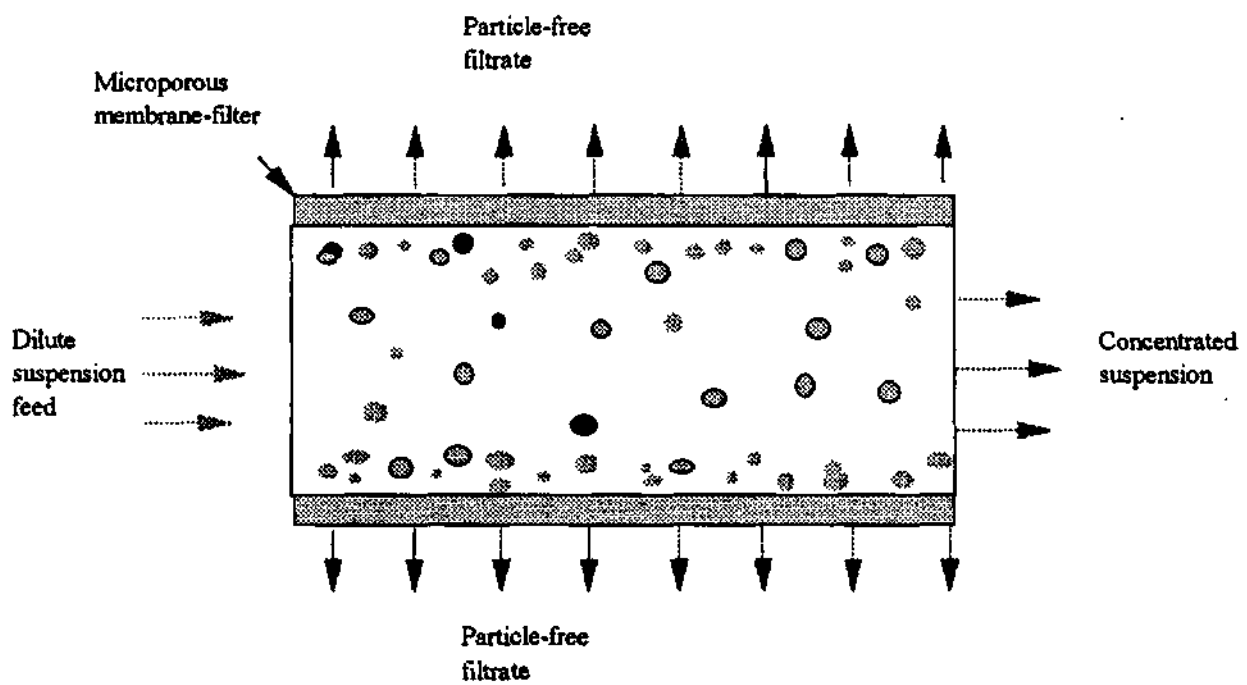

Fig. 2.2 Schematic presentation of cross-flow filtration. The filtration method used to minimise concentration polarisation (Toyomoto and Higuchi, 1992).

Dead-end filtration is suitable for the treatment of clean liquids or for the disposal of solids. Cross-flow filtration on the other hand is suitable for the concentration of particles and the recycling of the liquids and has a more stable filtration rate over time (Toyomoto and Higuchi, 1991). During cross-flow filtration solids accumulate at the surface of the membrane. These solids quickly compact to form a finer filtration surface than the membrane itself, rapidly reducing the rate of filtration to almost zero. Cross-flow filtration reduces this build-up of filtered solids at the membrane surface by keeping solids in suspension. The liquid being filtered is pumped tangentially across the membrane surface at the filtration pressure and at a right angle to the permeate flow direction. The deposited solids thus, are sheared from the membrane surface and carried away with the feed solution. The membrane surface is maintained in a "relatively clean" condition for much longer periods (Croll, 1992).

The following types of pressure-driven processes are used:

2.2.1 Reverse Osmosis

If a solvent and solution (or two solutions of different concentrations) are separated by a semipermeable membrane that permits solvent, but no solute, to diffuse through a flow of solvent through the membrane is commonly observed. If the solvent and solute are under the same pressure, the direction of flow naturally is in the direction of reducing the concentration difference, either from the solvent into the solution or from the more dilute solution into the more concentrated solution. This flow phenomenon is called osmosis. If the pressure on the solution is increased, osmosis is impeded, and at a sufficiently high pressure it is stopped altogether. This pressure is called the osmotic pressure. Still further increase in pressure on the solution reverses the direction of flow and pure solvent can be removed from the solution by passage through the membrane, leaving a more concentrated solution behind. This reverse flow is the basis of reverşe-osmosis as a method of desalting water (Bitter, 1990). The exclusion is not 100% and different membrane materials give different exclusions. Most RO membranes are used for desalination, but can also be used for nitrate removal, water softening and organic material removal (Croll, 1992). The particle range is shown in Fig. 2.3.

2.2.2 Ultrafiltration

The UF process is of importance in this study. The term `ultrafiltration' is applied to membranes with a pore size smaller than MF (bacteriological membrane filters), but larger than reverse-osmosis

membranes. They are generally classified according to the molecular mass (MM) of macromolecules which are excluded by the membrane (NMMCO: nominal molecular mass cut-off values). The molecules retained by membranes may vary from about 500 Da to almost a million Da with operating pressures between 0,5 to 5 bar (Nilsson, 1990). Cross-flow conditions are normally applied.

The advantage of UF over MF is its capacity to exclude viruses and large organic molecules such as humic and fulvic acids (i.e. colour). The disadvantages, however, are increasing operating pressures, the percentage of rejected water and fouling of membranes (Croll, 1992). In addition, UF cannot be applied to the removal of smaller organic materials such as most pesticides, taste and odour-producing compounds (Croll, 1992). Most commercial UF membranes are fabricated from hydrophobic materials which are chemically and physically more resistant than their hydrophilic counterparts. Hydrophobic character, however, often promote fouling (Jacobs, 1991).

2.2.3 Microfiltration

MF membranes are used for the fine filtration of particles including bacteria. This filtration process produces bacterially sterile water. It is possible for some viruses to penetrate MF membranes and extra treatment processes are, therefore, necessary to remove viruses as well as colour compounds, pesticides, tastes, odours, biodegradable organic compounds and possibly nitrates (Croll, 1992).

2.3 ELECTRICALLY-DRIVEN MEMBRANE PROCESSES

2.3.1 Electrodialysis

ED is the transport of ions through membranes as a result of an electrical driving force. Membranes, permeable to ions and electrolytes, can be used to separate these substances from nonelectrolytes. ED can be used for separation processes such as demineralisation, concentration of electrolytes, ion replacement reactions, metathesis reactions, separation of electrolysis products and the fractionation of electrolytes (Bitter, 1990). ED is not effective in the removal of bacteria and viruses. Membrane fouling can be a problem, but has been greatly reduced in recent years by the introduction of 'ED reversal' (EDR), where the polarity of the electrodes is regularly reversed to achieve self-cleaning action (Croll, 1992). In Fig. 2.3 the different membrane types and their respective selectivity ranges are presented.

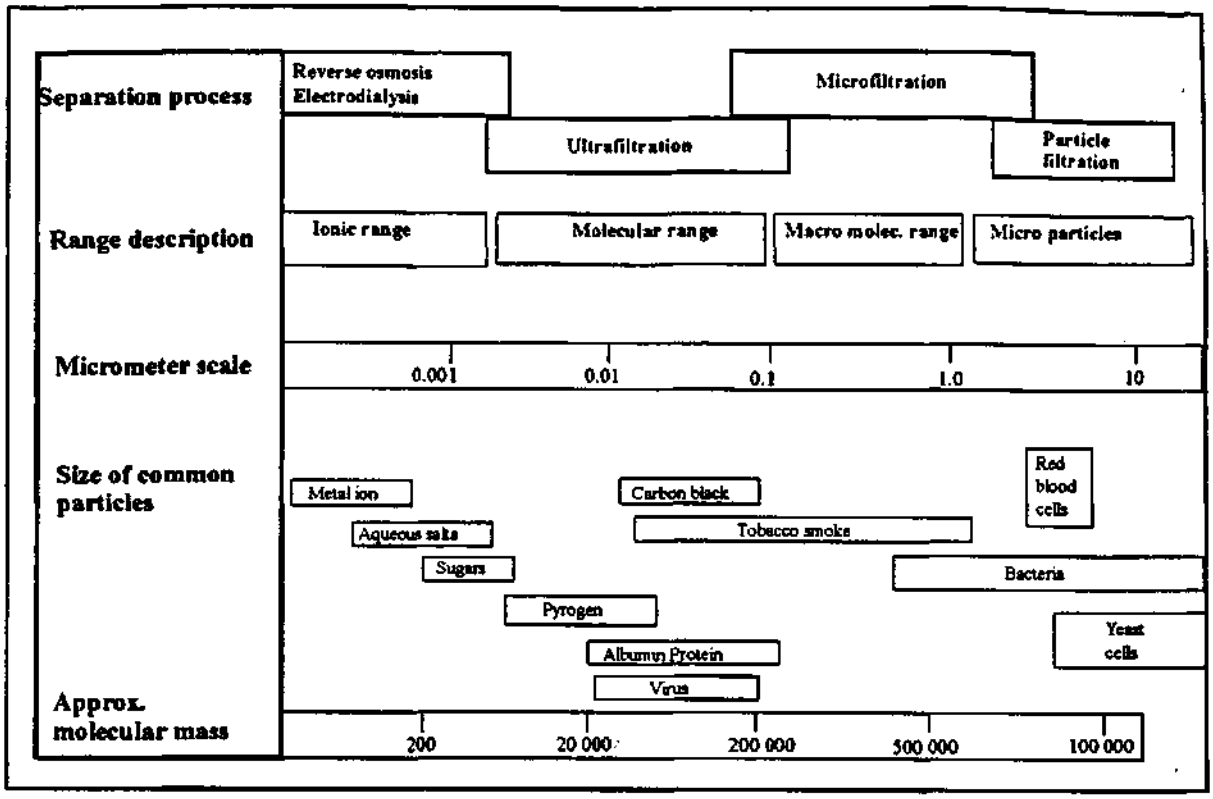

Fig. 2.3 Particle range of the different filtration processes. Particle sizes are given in micrometer scale and molecular mass (Croll, 1992).

2.4 APPLICATIONS OF MEMBRANES IN INDUSTRIAL BIOTECHNOLOGY

Virtually all existing, and most of the proposed, applications of membranes in biotechnological operations call upon the passive permselectivity of membranes as a means of effecting phase or molecular separations. These applications are listed below (Michaels, 1980):

1. separation of microscopic and colloidal particulate solids from aqueous suspensions,
2. concentration of solutes present in dilute solutions (principally by UF and RO),
3. macrosolute/microsolute separations (principally by dialysis, UF, or ED),
4. fractionation of mixtures of macrosolutes in aqueous solution (principally by UF),
5. selective confinement of macrosolutes (e.g., enzymes) or particles (e.g., cells or particles catalysts) and
6. perm-selective barriers for specific-compound-detecting sensors and instruments.

CHAPTER 3

MEMBRANE FOULING

3.1 INTRODUCTION

During the UF of a solution, the transmembrane flux does not increase linearly with an increase in the pressure difference across the membrane. Pressure induced flux increase of a solution subjected to UF is always smaller than the flux increase of a pure solvent under the same conditions. The increase in flux, due to an increase in pressure, reaches a point where it becomes independent of the pressure and no further flux increase can be obtained. This point is known as the J_{vlim} (Fig. 3.1). The J_{vlim} is lowered by an increase in the concentration of solutes in the feed solution (Von Meien, 1994).

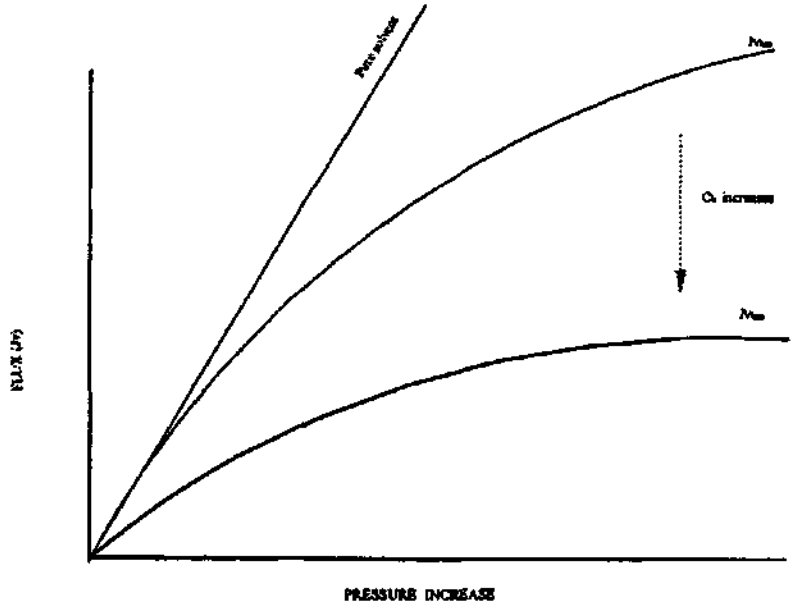

Fig. 3.1 Dependence of flux on operating pressure. Cb is the concentration of solute in the bulk solution (Echner and Zottola, 1993).

During the filtration of a solution under uniform pressure, especially a macromolecular solution, the transmembrane flux usually decreases with time and reaches a steady state Fig. 3.2. The flux of a pure-water solution will stay constant. Several reasons exist why the transmembrane flux of a solution or suspension is usually much lower than the flux observed with pure-water. These reasons are: changes in membrane properties, changes in properties of the feed solution, concentration polarisation (CP) (67 - 95 % flux reduction) and membrane fouling (5 - 30% flux reduction) (see Fig. 3.3)

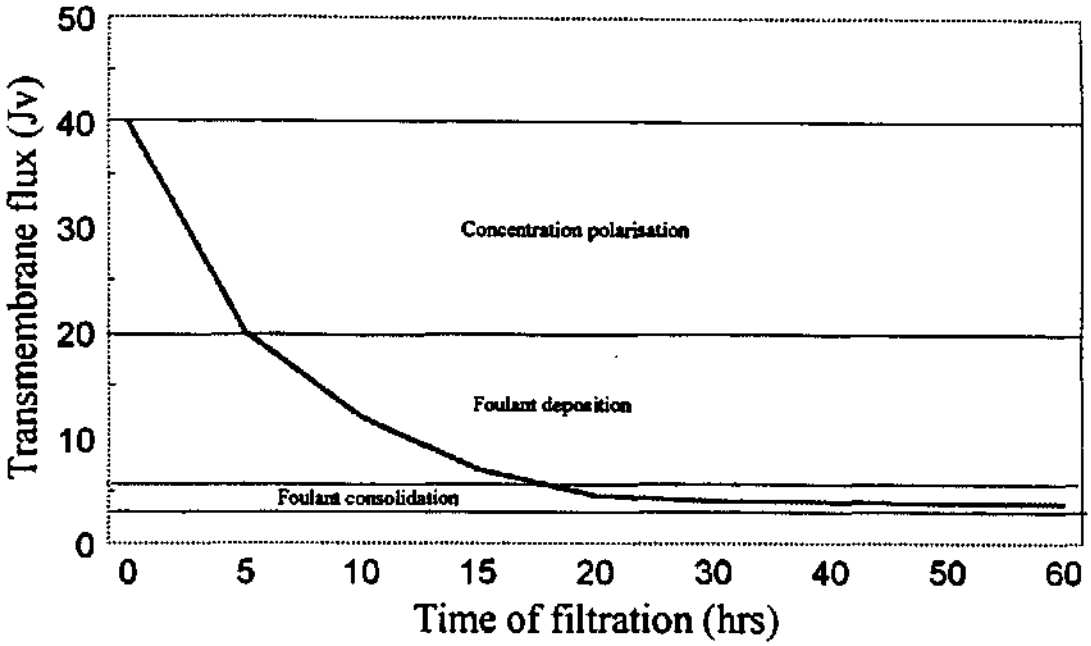

Fig. 3.2 Schematic presentation of the different stages in flux decline during ultrafiltration. Fouling usually starts with a rapid decline during initial stages and thereafter reaches a plateau (Echner and Zottola, 1993).

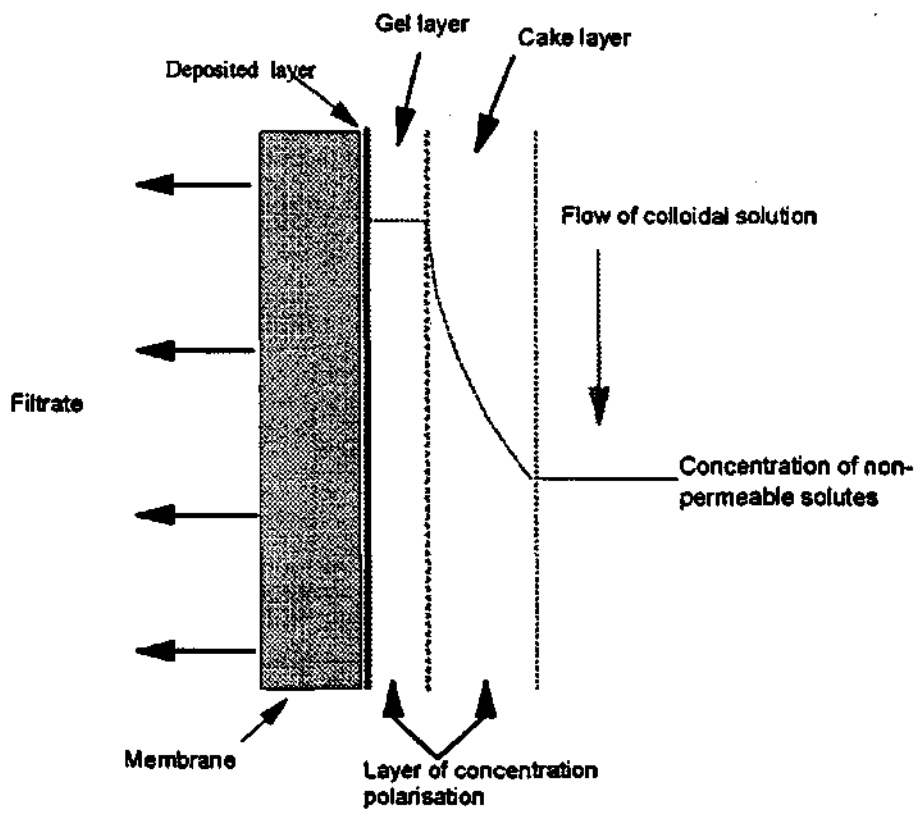

Fig. 3.3 Factors that could cause flux reduction during the filtration of solutes that are rejected by the membrane (Grund *et al.*, 1992).

The same reasons also account for the non-linear flux increase when the pressure of a solution being ultrafiltered is increased. It is evident that CP and fouling are responsible for almost all the flux decline problems and therefore special attention will be given to these two phenomena in the next section (Cheryan, 1986).

3.2 CONCENTRATION POLARISATION AND FOULING

Flux reduction due to fouling and CP in the vicinity of the membrane surface of the membrane are depicted in Fig. 3.3. Due to the retention of soluble substances at the membrane surface, a CP boundary layer forms in the aqueous retentive phase. These solutes form a dense layer with the highest concentration towards the membrane side. If the solute concentration at the membrane surface increases to a point where the viscosity of the solution prevents it from behaving like a Newtonian fluid, this layer starts to function as an elastic or pseudoplastic solid and a gel layer is formed. This gel layer, however, is still part of the CP layer with the only difference being that the macromolecules have reached their critical solubility (Vilker, 1984). Electron-microscopic evidence shows that the lower layers resemble tightly bound aggregates while the upper layers have a more loosely flocculated structure (Suki *et al.*, 1986). This flocculation of solutes can cause up to 67 to 95 % of the flux decline observed when a solute is being filtered. The CP layer provides a "reservoir" of molecules which would gradually become deposited or adsorbed onto the membrane (Suki *et al.*, 1984). The final solute layer to be distinguished is the deposited or adsorbed material, also known as the fouling cake (Toyomoto and Higuchi, 1992). Some of the material adsorbed onto the membrane surface also enters into the membrane pores, providing an additional resistance to the transport of the permeate through the membrane (Grund *et al.*, 1992). Thus the overall resistance a liquid encounters, when permeating through a membrane, is caused by the combination of separate but interactive processes such as CP, protein adsorption on the external surface of the membrane (i.e. protein-protein interaction), an increased adsorption at the membrane surface by soluble protein adsorbed protein interactions, pore plugging by occlusion as well as a gel layer, if it exists (Grund *et al.*, 1992).

Flux reduction, due to CP leads to an increase in the osmotic pressure. The hydrodynamic environment near the membrane surface is of great importance for situations where the osmotic pressure causes the main flux reduction. Many strategies exist for altering the surface hydrodynamics and neutralising the effect of CP. Flux decline caused by membrane fouling, however, is a more complex process, depending on the membrane type as well as the nature and

concentration of the solution (Ko and Pellegrino, 1993). CP and gel layers are removable from the membrane surface by decreasing the feed concentration and/or flushing with pure-water. Fouling on the other hand is more severe and can only be removed by effective cleaning (Echner and Zottola, 1993). At least three kinetic steps are involved in the fouling of UF membranes (Nilsson, 1990). The first kinetic step is the transfer of solute to the surface. This kinetic step is seldom the rate limiting step, as CP and fouling reaches near equilibrium within a few min of operation. The second kinetic step is the transfer of solute into the membrane, to adsorb or pass through after a set of reversible adsorption/desorption steps in the pores. The third kinetic stage reveals the real kinetics of adsorption and, according to Van Dulm and Norde (1983), is a relatively fast process controlled by diffusion.

3.3 EVIDENCE OF FOULING

3.3.1 Irreversible flux decline

Fouling effects are characterised as a process that causes an "irreversible" flux decline. Thus, if flux decline is not reversible by simply altering the operation conditions, it is termed fouling. Depending on the system, the flux may decline in one or more stages, usually rapid in the first few min, followed by a more gradual decline in the later stages (Cheryan, 1986). Fouling of membranes reduces productivity, shortens membrane life (often due to aggressive cleaning regimes) and impairs the fractionation capability of the membrane. If the fouling effect is not properly understood and controlled, the membrane process becomes non-viable (Fane and Fell, 1987). Strong chemical interactions that take place between species in the feed and the membrane surface alter membrane properties dramatically and sometimes irreversibly. The selectivity of the membrane towards the solutes also increases as the amount of adsorbed solutes increases (Bitter, 1990).

3.3.2 Change in retention characteristics of membranes

Mechanical pore blockage takes place if particles of sizes about equal to, or somewhat smaller than the effective pore openings of the membrane are filtered. Blockage also occurs by the precipitation and crystallisation of dissolved species inside membrane pores (Bitter, 1990). It must be noted that fouling under UF conditions with actual effluent can be obscured by effects such as CP, membrane deterioration (hydrolysis, oxidation) and biofouling (growth of living organisms on surfaces) (Flemming, 1990).

3.4 POTENTIAL FOULANTS

Due to the small amount of components deposited, it is often difficult to find analytical methods capable of determining the nature of foulants (Trägårdh, 1989). A number of the problem causing substances documented in membrane filtration are listed in Fig. 3.4. (Bauser *et al.*, 1982).

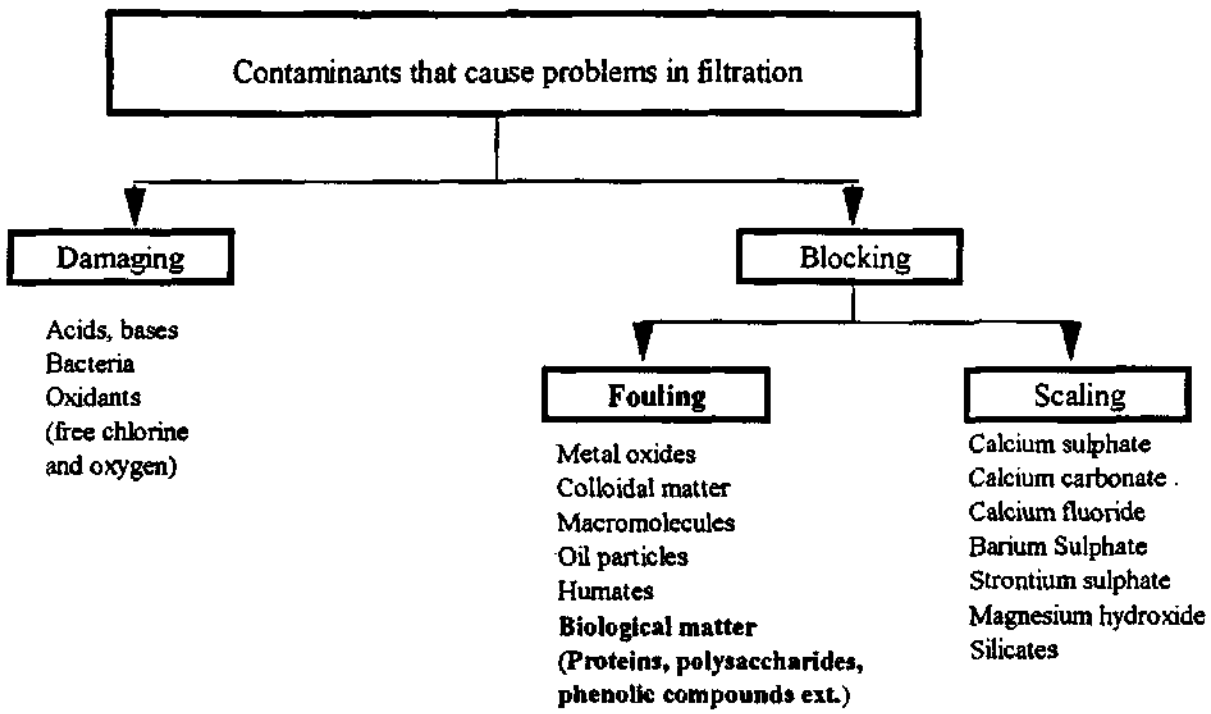

Fig. 3.4 Diagram of materials that cause problems in membrane filtration. The difficulties caused by these materials are membrane damage, fouling or scaling (Bauser *et al.*, 1982).

3.5 PROTEIN FOULING

When proteins are present in UF feed streams, the major cause of flux decline is likely to be the irreversible binding of these proteins onto the membranes. The amphiphilic character of proteins makes this group of molecules highly reactive agents that could adsorb onto almost any interface over a wide range of conditions (Van Dulm and Norde, 1982). The causes and consequences of protein fouling is well documented and will subsequently be discussed in more detail. (Sydney, 1990; Nilsson, 1988; Cheryan, 1986 and Matthiasson, 1983).

3.6 FACTORS THAT AFFECT PROTEIN FOULING

Protein adsorption is influenced by factors, such as solute concentration, pH, ionic strength, temperature, membrane characteristics and operating conditions (Cheryan, 1986).

3.6.1 Concentration of protein feed solution

Using bovine serum albumin (BSA) and bovine serum albumin (fatty acid poor) (BSA/FAP) as fouling proteins, Grund *et al.*, (1992) showed that permeate fluxes decrease when protein concentrations in the feed stream are increased. Fluxes obtained using 6,5% (w/v) protein (BSA/FAP) were up to 43% lower than for a 2% (w/v) solution. The amount of protein adsorbed onto the membranes becomes time independent after 4 h with a bulk concentration lower than 10 g/l. When the bulk concentration is higher than 10 g/l, equilibrium is reached over a longer period. For instance, a concentration of 50 g/l takes more than 14 h to reach equilibrium (Grund *et al.*, 1992). The feed concentration also determines whether monolayer or multilayer adsorption is likely to occur. Nilsson (1990) stated that up to 1 g protein/m^2 membrane area is deposited at higher solute concentrations compared to monolayer adsorption of 5 mg protein/m^2 membrane at low feed concentrations over the same period of time.

3.6.2 Temperature of the protein feed

One advantage of UF is the comparatively mild conditions used. Temperature, however, is a key operational parameter in UF systems, especially when proteins are involved. Temperature is highly influential on the protein conformation. Two physicochemical reasons exist why an UF system should be operated at as high a temperature as possible (Campbell *et al.*, 1993). Firstly, diffusion is increased which subsequently reduces CP at the membrane surface. Secondly, the viscosity of the solution decreases with a concomitant theoretical increase in the flux, resulting in less fouling. When proteins are, however, present in the feed solution an increase in the operational temperature will affect protein conformation. This change in protein conformation can result in the exposure of the inner hydrophobic core of the protein to the environment. Such denatured proteins have the tendency to either aggregate or adsorb onto suitable surfaces. The hydrophobic character of most UF membranes provides such a surface (Campbell *et al.*, 1993).

3.6.3 pH and ionic environment

The pH of the solution affects the charge, conformation (stability), solubility and the tendency of proteins to aggregate (Suki *et al.*, 1984). The influence of pH on protein conformation is, however, different for different proteins due to the variations in their isoelectric points (pI) (Fig. 3.5). The pI of a protein is that pH value where the protein has a net charge of zero (Nilsson, 1990). The variation in charge, conformation and solubility of proteins, at different pH values, will influence adsorption of proteins onto various surfaces. A typical example is the adsorption of BSA to a negatively charged membrane in the absence of salt. At pH values above the pI of BSA (pH 7,4) for instance, the BSA molecule is negatively charged, intramolecular repulsion is high and the protein molecule is in its most expanded conformation. Repulsion forces between the membrane and the protein molecules are also high. If the pH of the solution is decreased to pH 5, the pI of BSA, the negative charge of BSA is decreased, repulsion forces between membrane and protein molecule decrease resulting in higher adsorption (Matthiasson, 1983 and Fane *et al.*, 1983).

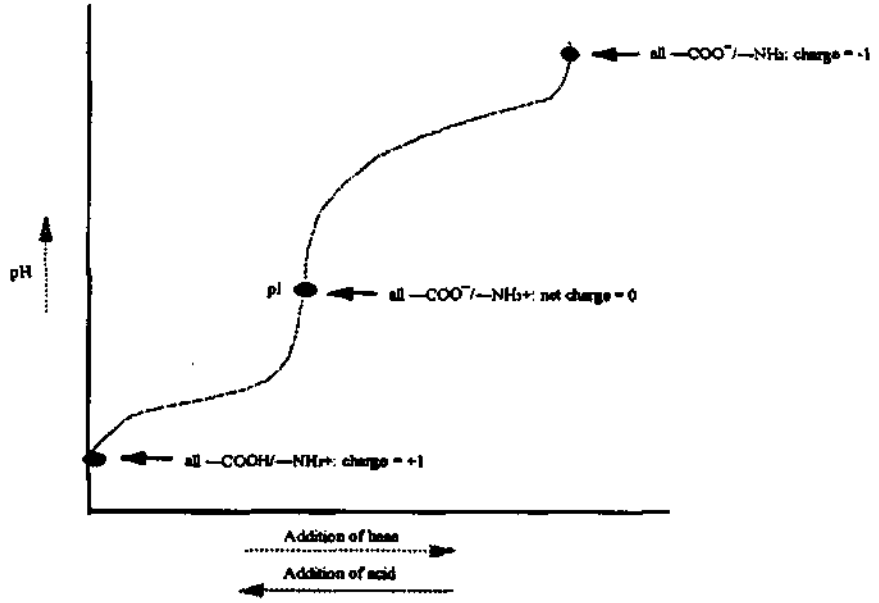

Fig. 3. 5 General titration curve of a typical protein molecule. The graph reflects the effect of pH on the charge of the molecule. At low pH values all carboxyl and amino groups are protonated, while at a high pH, all carboxyl groups are ionised and amino groups are free (Bohinski, 1987).

At pH 5 the BSA molecule is in its most compact conformation (no intramolecular repulsion), carries no net charge and hence the least permeable BSA layers that are adsorbed onto the membrane.

Beyond the pI, the protein becomes positively charged and is more attracted to the negatively charged membrane. This attraction, however, is hampered by the size expansion due to intramolecular repulsion and less adsorption occurs relative to the pI. At pH values well below 5 the protein molecule denatures, causing additional adsorption to the membrane.

The effect of ionic species, such as calcium, on protein fouling, depends on whether the pH is near or far from the pI (Aimar *et al.*, 1986). When the pH = pI, the calcium ions have a great affinity for proteins and increase the polarity, hydrophilicity and adsorption of the macromolecules. At pH values away from the pI, salt ions contribute to neutralise electric charges and subsequently causes less adsorption.

3.6.4 Associated molecules

Grund *et al.*, (1992) used BSA and BSA/FAP to examine the influence of fatty acids on protein fouling. Results suggest that a small amount of fatty acid (about 0,4%), presumably bound to the BSA, has a dramatic influence on the membrane fouling process. Fluxes obtained were significantly lower after fouling with BSA compared to fouling with BSA/FAP. The screening effect of salt ions is another example of the influence of associated molecules.

3.6.5 The effect of membrane NMMCO and molecular mass distribution of the protein solution

According to Nilsson *et al.*, (1990) pores of certain radii are affected more by fouling than others. Using PSM with NMMCO values of 30 000 and 100 000 Da, Grund *et al.*, (1992) observed fluxes 1.5 times higher with the 100 000 NMMCO membrane compared to 30 000 NMMCO after fouling with BSA. The opposite tendency, however, was observed when BSA/FAP was used as the fouling agent. The explanation is that with the 30 000 NMMCO membranes, both proteins cause mostly surface fouling and the additional molecular aggregation caused by fatty acids in the case of BSA, leads to more severe surface fouling. The few proteins that enter into membrane pores are also effected by the additional aggregation of BSA inside pores and cause lower permeate fluxes relative to BSA/FAP. With the 100 000 NMMCO membranes, unaggregated smaller molecules such as the BSA/FAP pass through the membrane, but once inside the membrane pores the molecules adsorb and cause pore plugging, resulting in lower permeate fluxes of the 100 000 NMMCO membranes compared to the 30 000 NMMCO membranes. With the larger and more aggregated molecules of BSA, intrusion of the protein molecules into the pores of the membrane are impeded and only

surface deposition occurs. Interactions between soluble proteins and proteins deposited on the external surface lead to a more open conformation that cause less resistance (Grund *et al.*, 1992).

3.6.6 Operating conditions

High linear flow rates at the membrane surface shear off deposited layers and cause a reduction in the hydraulic resistance of the fouling layer. A critical shear stress, however, exists below which fouling is severe. Pulsatile flow techniques could be used to increase filtration rates without increasing the linear flow rate (Bauser *et al.*, 1982). The other operating parameter of concern is the pressure applied. Fouling is often increased with increasing transmembrane pressure because it leads to temporary flux increases which promotes CP and cause compressing of fouling layers (Aimar *et al.*, 1986). Thus, it is clear that protein fouling is affected by operating conditions and it is therefore necessary to choose the optimal conditions for each feed to be filtered.

3.6.7 Membrane character

The physico-chemical properties of the membrane and the porosity and morphology of the surface are two characteristics of UF membranes that influence fouling (Trägårdh, 1989). Fouling arises when the physico-chemical interactions occur between species in the solution and the membrane material. Proteins interact with polymer surfaces by a variety of mechanisms, including electrostatic interactions, hydrophobic effects, charge transfer (e.g. hydrogen bonding) or through combination of these. These binding mechanisms, however, are profoundly influenced by the membrane physico-chemical character. A correlation between the contact angle and flux lost due to protein binding was observed by Fane and Fell, (1987). The most hydrophilic membrane showed the lowest flux decline. The hydrophobicity of a membrane surface can be determined with contact angle using water as wetting medium. The more hydrophobic the surface the larger the contact angle (see Fig.3.6). Typical UF membranes have a relatively low surface porosity. Low porosity means that solvent flowing towards the membrane does not meet a homogeneous surface, but will have to follow streamlines to the opening of isolated pores. Most solute will pass through the largest pores causing them to plug first, unless the pores are so large that they are unpluggable (Fane and Fell, 1987).

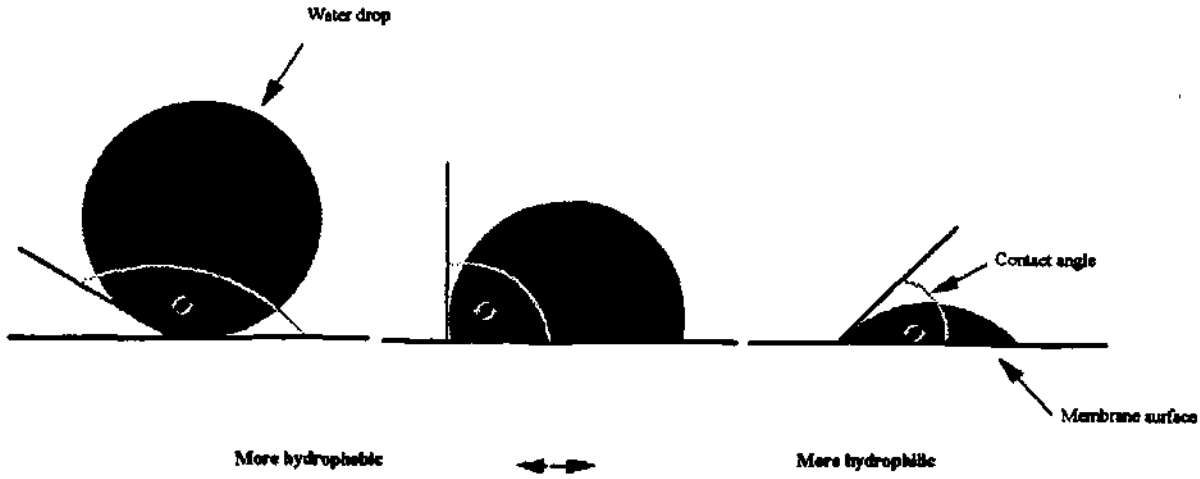

Fig. 3.6 Diagram of the contact angle between a water drop and the surface of a membrane. The more hydrophilic the surface the smaller the contact angle and water will tend to wet the membrane.

3.7 LIPID FOULING

Another important organic foulant is lipid material. Lipids play an important role in the fouling of membranes, however, only a few studies have been done in this area. Early results indicate that the membrane flux of whey improves after lipids have been removed by precentrifugation (Cherayn, 1986). In addition, results from UF experiments with BSA and BSA/FAP indicate that only a small amount of fatty acid bound to the BSA molecule, causes more severe fouling than BSA/FAP (Grund *et al.*, 1992). Animal fat in abattoir effluent is one of the constituents which causes severe fouling of hydrophobic membranes. Fats give rise to particular problems because of their low solubility and extreme hydrophobicity (Jacobs *et al.*, 1991).

3.8 PHENOLIC FOULING

Fouling by phenolic compounds is mostly observed in the UF treatment of pulp and paper effluent. Results obtained by Zaidi *et al.*, (1991) indicated that organic species, such as chlorocatechols, are responsible for the observed flux decline in the UF processing of these effluents. Negligible flux decline was observed during tests with dehydroabietic acid as a model foulant. In contrast, significantly lower fluxes were observed in tests with sulphonated lignin and 4,5-dichlorocatechol as model foulants. Results from experiments with dichloroguaicol and resin acids as model foulants

showed that extensive physico-chemical interactions between the solutes and the membrane exist. Retention of solutes are generally lower for the more hydrophilic membranes. No correlation between fouling by these substances, the retention and the NMMCO of membranes were found (Zaidi *et al.*, 1991). In addition it appears that chlorination of organic compounds increases their affinity for the membrane material. This phenomenon correlates with the Log-P coefficient (octanol/water partition coefficient), which indicates the affinity of a compound for an organic phase. Log-P values of these substances are higher after substitution of hydrogen (H) with chloride (Cl).

3.9 CARBOHYDRATE FOULING

Natural aqueous media contain two main components that have to be removed: solids (siliceous particles, clay, organic residues more or less oxidised) and dissolved matter (organic species including humic substances, biological slimes, macromolecular solutes and large variety of mineral components). Polysaccharides derived from vegetables or bacteria seem to form an important part of dissolved macromolecular solutes which are present in fouling cakes (Baudin *et al.*, 1992). Two polysaccharides (dextran and pullulan) and spherical particles of silica were used to prove that the nature and properties of fouling deposits, accumulated at the membrane surface, depend on the molecular interactions between the suspended matter and the organic solutes. Polysaccharides alone are not a fouling risk, but in conjunction with suspended matter such as silica, adsorption of polysaccharides at the silica water interface occurs and a fouling cake is formed (Baudin *et al.*, 1992). This fouling cake on the membrane surface and inside pores is responsible for the observed flux declines when carbohydrates and silica are present in the mediums being filtered.

3.10 MATHEMATICAL MODELS OF FOULING

Due to the complex nature of UF processes, a number of models based on D'arcy's law have been proposed to predict effects of CP and membrane fouling on transmembrane flux. Two-parameter models are the osmotic pressure model and the gel-layer model. These models lump the effects of CP and solute adsorbed layers on flux into a single-parameter (osmotic pressure, boundary layer resistance or gel layer resistance). Three-parameter models include the boundary layer adsorption model and the osmotic pressure adsorption model, which separately account for the effects of the polarised and adsorbed layers (Ko and Pellegrino, 1993).

3.10.1 Concentration polarisation model

This model explains the flux decrease caused by CP. CP limits flux mostly by decreasing the driving force $\Delta P - \Delta\pi$. This phenomenon causes deviation from the linear relationship between transmembrane flux and pressure driving force (Δ P), with the flux eventually reaching a constant level as illustrated in Fig. 3.1 (Vilker, 1984 and Von Meien, 1994). CP is a phenomenon in which the solute concentration at the membrane surface C_m, is higher than that in the bulk solution C_b. The following equation gives the relationship between flux and CP:

$$C_m - C_p / C_b - C_p = \exp. (J_v/K) \qquad (1)$$

where C_p is the solute concentration in the permeated solution, J_v, is the flux, and **K** is the mass transfer coefficient. The CP model is the basis for the gel-polarisation and the osmotic pressure model, which will be dealt with in the following sections (Toyomoto and Higuchi, 1992).

3.10.2 Gel Polarisation Model

This model explains flux decline due to gel-layer formation. As a consequence of CP, a non-fluid gel-like layer is formed on the surface of the membrane when the solute concentration reaches a limiting value (concentration for gelation C_g). The values of C_m is much higher than that of the bulk solution due to the gel like layer and can therefore be replaced with C_g, which is the concentration in the gel-layer (Wijmans *et al.*, 1984). In general, the retention of the gel-layer forming solute is extremely high and C_p can be ignored. Equation (1) can be rewritten as:

$$J_v = K \ln (C_g - C_p / C_b - C_p) = K \ln C_g/C_b \qquad (2)$$

This model appears valid because the limiting flux is not dependent on the supplied pressure or membrane material, but on the solute concentration in the gel-layer (Toyomoto and Higuchi, 1992).

3.10.3 Osmotic Pressure Model

The osmotic pressure model is of value when the C_m is significantly higher than the bulk concentration and the osmotic pressure of the solution at the membrane surface is no longer negligible (Wijmans *et al.*, 1984). High-concentration macromolecular solutions have a high osmotic pressure, while osmotic pressures of low-concentration macromolecular solutions are negligible. The osmotic pressure model is applied when the gel-layer is not formed, but

high-concentration macromolecular solutes are still concentrated on the surface of the membrane. It explains the experimental results obtained when a solution is filtered, no gel-layer is formed and retention is assumed to be 100%. A typical example would be the filtration of dextran, which does not form a gel-layer. The equations of value are:

$$J_v = L_p(\Delta P - \Delta\pi) \qquad (3)$$

where L_p is the hydraulic permeability for pure-water and $\Delta\pi$ is the osmotic pressure difference. The osmotic pressure of a macromolecular solution can be expressed as follows:

$$\pi(C) = A_1C + A_2C^2 + A_3C^3 \qquad (4)$$

Where C is the concentration of the bulk macromolecular solution and A_1,A_2, and A_3 are constants. The following equation is derived from Eqs. (3) and (4):

$$J_v = L_p\,[\Delta p - (A_1C_m + A_2C^2{}_m + A_3C^3{}_m)] \qquad (5)$$

C_m may be calculating by substituting $C_p = 0$ in Eq. (1)

$$C_m = C_b \exp.(J_v/K) \qquad (6)$$

Thus, when the applied pressure is increased the permeate flux will at first, increase resulting in higher values for C_m and a larger osmotic pressure. In this way the pressure increase is partly cancelled by the osmotic pressure increase, which will lead to a small increase in the permeate flux (Wijmans *et al.*, 1984 and Toyomoto and Higuchi, 1992).

3.10.4 Adsorption and Osmotic Pressure Model

This model explains the decrease in the pure-water flux through membranes due to the adsorption of fouling material. The equation:

$$J_v = L'_p(\Delta P - \Delta\pi)$$

is used instead, where L'_p (hydraulic permeability for pure-water after filtration) is used instead of L_p. The irreversible adsorbed protein molecules form a strongly-bound, immobile layer which grows with filtration. The filtration rate decreases due to two phenomena: a decrease in the hydrodynamic driving force caused by osmotic pressure and fouling resistance (R_f) caused by

surface adsorption and pore-plugging (Ko and Pellegrino, 1993). Through the normalisation of these variables, the relative contribution of osmotic pressure and fouling resistance, their effect on membrane performance can be ascertained and compared for membranes with different material and geometric properties. Results from such an experiment by Ko and Pellegrino, (1993) indicated that the flux reduction of low protein binding membranes (regenerated-cellulose) are due to osmotic pressure and that of protein binding membranes (PVP-coated polycarbonate) are due to protein adsorption. They concluded that transmembrane pressure (kPa), cross-flow velocity and the bulk protein concentration have little influence on the relative flux reduction due to osmotic pressure resistance in severe fouling cases, but has a substantial influence on flux reduction in the osmotic pressure controlling cases. The pH and Na^+ and Cl^- ions have significant effects on flux decline, caused by fouling and osmotic resistance in severe-fouling cases. For osmotic pressure controlled cases, however, the effect of pH and NaCl are negligible.

CHAPTER 4

METHODS USED FOR MEMBRANE CLEANING AND THE PREVENTION OF FOULING

4.1 INTRODUCTION

A major concern when using any UF membrane system, is to accomplish the required separation at an economically acceptable rate of flux. Both of these variables are drastically affected by membrane fouling (Spencer and Thomas, 1991). Although membrane technology has made great advances recently, cleaning has been neglected to some extent despite the difficulties caused by membrane fouling (Echner and Zottola, 1993). A disadvantage of UF membranes, the majority of which are made from artificial polymers, is that they do not have a closed surface but have a porous, sponge-like structure into which product and cleaner constituents can penetrate (Bragulla and Lintner, 1986). Most of these membranes further consist of hydrophobic surfaces that result in much higher adsorption of proteins and lipids compared to hydrophilic surfaces (Toyomoto and Higuchi, 1992). The cleaning of fouled UF membranes has not been investigated extensively in the past. Studies related to fouling (5-30% flux reduction) and CP (67-95% flux reduction), focused primarily on reducing fouling via effluent and membrane pretreatment, processes which would minimise fouling and therefore produce a more economical and energy saving method of membrane separation (Sedath *et al.*, 1993, and Echner and Zottola, 1993). A few of the most prominent examples of treatment methods and operational factors used to reduce fouling will be discussed below.

4.2 FOULING REDUCTION

The most important factors to be considered in fouling reduction are: flow conditions, effluent pretreatment, membrane properties, operational factors and rinse water quality (Spencer and Thomas 1991). Each of these factors will be discussed briefly.

4.2.1 Flow conditions

During production a secondary membrane is formed within the laminar boundary layer of the membrane as a result of CP. The thickness of this secondary membrane is mainly determined by the ratio of flow rate to contact pressure. High flow rates and low production pressures yield thin secondary membranes whilst low flow rates and high pressures produce thick secondary membranes

(Spencer and Thomas, 1991). By varying the hydrodynamics (i.e., increasing the cross-flow velocity), foulant can be swept away from the membrane before adsorption can occur (Sedath *et al.*, 1993), thus preventing the formation of secondary membranes or gel layers. Bauser *et al.*, (1982) used pulsatile flow conditions to increase filtration rate without increasing the linear velocity. An increase in the linear velocity would result in CP and flux decline in the long run.

4.2.2 Pretreatment of effluent

Pretreatment of industrial effluent can be mechanical, thermal or chemical.

1. **Mechanical pretreatment of effluent:** This method includes the use of physical separation techniques such as centrifugation, a method often used to remove fines from whey, for the fractionation or red blood cells from blood serum proteins and for dewatering of effluents (Flemming, 1990).

2. **Thermal pretreatment of effluent:** Pasteurisation is often necessary in order to avoid undesirable changes in the composition of the feed and the products. The pH of whey, for instance, decreases rapidly if it is not heat-treated. Milk and whey are normally heated to 55-60°C and held at this temperature for up to a half an hour before submitting to UF at the same temperature (Trägårdh, 1989).

3. **Chemical pretreatment:** Chemical pretreatment, such as pH adjustment, can in some cases improve the flux due to the fact that proteins are more susceptible to precipitation and adsorption at their pI (Trägårdh, 1989).

4.2.3 Membrane properties and pretreatment

When choosing the most suitable membrane for a liquid feed, it is of course important to choose a membrane which is resistant to the pH, temperature and chemical composition of the feed, as well as one with the desired separation properties. It is also important to consider other membrane properties such as membrane material, electric charge and whether the membrane is hydrophilic or hydrophobic. The ideal UF membrane for most applications would be hydrophilic and homogeneously permeable (Trägårdh, 1989). The only problem with the hydrophilic membranes, such as polyvinyl alcohol and polyacrylonitrile membranes, used for low protein binding is that these membranes do not give thermal stability and are susceptible to chemical degradation. The hydrophobic membranes on the other hand, have thermal stability and some chemical resistance (Toyomoto and Higuchi, 1992).

The red blood cell biomembrane, unlike synthetic polymer membranes, resist protein fouling. This phenomenon is attributed to the unique bilayer structure of predominantly phospholipids which constitute the cell membrane. Orientation of phosphatidylcholine derivatives at the external bilayer surface presents a neutral, zwitterionic interface to the extra cellular environment, which has little or no attraction for protein. These properties of the red blood cell membrane could be mimicked by pretreatment of polyethylene and polyvinylchloride membranes with hydrophilic surfactants and polymers which reduced protein adsorption by 95% (Dudley *et al.*, 1993 and Fane and Fell, 1987). These membranes thus have the required mechanical strength and thermal stability, since only the surface is modified, but the transport characteristics are governed by the hydrophilic skin-layers on the membrane (Toyomoto and Higuchi, 1992). This decrease in physico-chemical interactions between foulant and membrane allows the foulant to diffuse away and subsequently be swept from the surface (Sedath *et al.*, 1993).

Recent results produced by Fane and Fell, (1987) show that similar or better improvements can be achieved by forming a monolayer (so-called Langmuir-Blodgett layer) on the membrane surface. This monolayer produced a higher initial flux for the membrane, possibly due to the effect the precoat has on improving the homogeneity of the membrane surface. A reduction in flux decline was also observed due to the reduction in protein deposition. The precoat presumably lowers the levels of local polarisation and reduces the availability of hydrophobic sites for adhesion (Fane and Fell, 1987).

Sedath *et al.*, (1993) used a fluorination process as a pretreatment step for PSM. Fluorination adds both fluorine and oxygen to the membrane surface which increases the hydrophilic nature of the membranes. An increase in the hydrophilicity of a membrane reduces the adsorption rate of hydrophobic foulants to the surface and thereby decreases the fouling rate. Increased hydrophilicity also decreases the capillary pressure and permits permeate flow through smaller pores.

Spencer and Thomas (1991) used formed-in-place (FIP) or dynamic membranes. These membranes, like cast membranes, are also drastically affected by fouling. However, unlike cast membranes, FIP membranes can be economically removed after extensive fouling and a new membrane formed in place to achieve optimal performance. If this removal-reformation operation has to be done often the process is usually automated (Spencer and Thomas, 1991).

Other mechanisms to enhance the surface hydrophilicity of PSM are through surface sulfonation, generating positively charged PSM with chloromethylation via the Friedel-Crafts reaction, immersion of the polysulphone UF membranes into block copolymer of polyoxyethelene and polyvinyl acetate, polypeptides, polyethylene imine solutions and adsorption of surfactants to membrane surface, to mention but a few of the methods used (Toyomoto and Higuchi, 1992).

The membrane properties are also of importance in the cleaning stage. Smooth, hard surfaces made of glass or polished steel can be cleaned well, in marked contrast to surfaces made of synthetic polymers such as polyethylene, polyvinyl chloride etc. In the latter case, fats penetrate into the plastics and are difficult to remove, as the affinity of fats for plastics is much higher than for inorganic materials (Bragulla and Lintner, 1986).

The various methods that can be used to minimise interactions between membrane and foulant are shown in Table 4.1. Bauser *et al.*, (1982) used different coatings to prevent clogging of membranes. Membranes (0,4 micro metre pore size) were coated with a very thin layer of either polyacrylonitrile (PAN) or carbon. The purpose of the coating was to reduce the protein adsorption. PAN reduces the protein adsorption because it is a hydrogel, and isotropic carbon coatings only interact weakly with proteins if properly deposited. The results obtained showed that the coating alleviates, but does not eliminate, the adsorption problem. During the coating process not all the pores are coated and smaller serum proteins can cause fouling inside the pores (Bauser *et al.*, 1982).

Tab. 4.1 Interactive forces between protein molecules, polymer surfaces and membranes and the preventative surface treatment (Bauser *et al.*, 1982)

Interactive force	Preventative measure
Electrostatic interaction	Co- charged groups
Hydrophobic interaction	Polar groups, hydrogel coatings
Hydrogen bond	No hydrogen-bond forming groups
Dispersion forces	—

4.2.4 Operational factors

Operational factors, such as backwashing, help to control fouling and a particular advantage is gained with air back-flushing for certain types of membranes (MF membranes). The influence of pulsating flow on gel-layer controlled filtration rate and the influence of the tubular pinch effect on

cell distribution at the membrane flood interface were studied. Pulsatile flow was shown to increase the UF rate (Bauser *et al.*, 1982)

4.2.5 Water quality

The chemical and bacteriological quality of the water used for rinsing and cleaning the membrane is important. The presence of iron, silica, manganese, calcium etc. is highly detrimental as they produce precipitates which block the membranes and which are often difficult or impossible to remove (Trägårdh, 1989 and Bragulla and Lintner, 1986). Soft water is more suitable for cleaning membranes than hard water. If hard water has to be used, sufficient amounts of complexing agents must be present (Bragulla and Lintner, 1986). From a microbiological point of view, cleaning water should be as close as possible to drinking water quality (Bragulla and Lintner, 1986).

4.3 *CLEANING AND DISINFECTION OF UF MEMBRANES*

Cleaning can be defined as "a process whereby material which is not an integral part of that substance is relieved from that substance". Disinfection implies the destruction of all pathogenic micro-organisms and the reduction of the number of micro-organisms which degrade the product (Trägårdh, 1989). A surface can be physically clean (free from visible impurities), chemically clean (free from all impurities) or biologically clean (free from living or dead micro-organisms) (Trägårdh, 1989). Great importance is attached to the cleaning and disinfection of UF membrane plants because residues on the membranes can restrict capacity and diminish service life (Bragulla and Lintner, 1986). Membrane equipment has to be cleaned regularly by a combination of the following methods: mechanical, chemical and biological.

4.3.1 Mechanical cleaning

The problem of rapid membrane blockage has been partly overcome through the use of "cross-flow" filtration techniques, gas backwash (where air backwash is applied for a few seconds every few min) and the development of membranes with pore and surface structures which are less liable to clog (Croll, 1992). Periodical backflushing, i.e. applying a pressure on the permeate side thus pushing part of the permeate back through the membrane, is used with some types of membrane modules. The efficiency of cleaning not only depends on the type of suspension to be treated and the type of foulant, but also on the pulse frequency and amplitude as well as the reverse pressure (Trägårdh, 1989). Foam balls are also used as a mechanical cleaning method for the

removal of deposits from tubular membranes (Trägårdh, 1989). During the cleaning phase, flow conditions also play an important role, the contact pressure should be kept as low as possible and the flow rate of the solution as high as possible (Bragulla and Lintner, 1986). Under these conditions the compressible layer is relaxed and less able to withstand shear stresses which will enhance cleaning efforts (Trägårdh, 1989).

4.3.2 Chemical cleaning

A chemical cleaning reaction is a heterogeneous reaction between a detergent solution and the fouling layer. The cleaning reaction can be divided into the following six stages (Trägårdh, 1989):

1. **Bulk reaction of detergents**
2. **Transport of detergents to the fouled surface**
3. **Transport into the fouling layer**. Due to the lower surface tension, surface-active agents have the ability to penetrate through pores and crevices. Their adsorption characteristics allow them to be adsorbed onto the surface of the membrane, thereby weakening the bond between the foulant and the membrane.
4. **Cleaning reactions**. The process which takes place when the cleaning agents come into contact with the fouled layer can be subdivided into physico-chemical transformations and chemical reactions. The physical and physico-chemical transformations include melting, mechanical and thermal stress, wetting, soaking, swelling, shrinking, solvation, emulsification, deflocculation, and adsorption. The chemical reactions involved are hydrolysis, peptisation, saponification, solubilisation, dispersion, chelating, sequestering and suspension. These reactions all contribute to overcome the cohesion forces between foulant particles and adhesion forces between fouling particles and membrane surfaces.
5. **Transport of cleaning reaction products back to the interface**
6. **Transport of products to the bulk solution**

When the mechanical and thermal stress and other physical, physico-chemical and/or chemical processes have weakened the foulant-surface and foulant-foulant bonds, larger sections of the fouling layer can be detached. These six stages do not necessarily always occur. In specific cases some stages will be bypassed. When cleaning equipment, fouled by fatty material for example, it

may be necessary to first melt the fat, or use a detergent or a suitable solvent to dissolve the fat before cleaning can be commenced (Trägårdh, 1989).

4.3.3 Biological cleaning

These methods include the use of biological agents which contain enzymes. A more detailed discussion of this cleaning method will follow in section 4.4.3.

4.4 CLEANING AGENTS USED ON FOULED UF MEMBRANES

When devising an ideal cleaning programme for a membrane system, account must be taken of the type of production residues (fouling) that are to be removed (Bragulla and Lintner, 1986). The cleaning process must remove deposits and restore the capacity and separation characteristics of the equipment. The chemicals used should: (1) loosen and/or dissolve the fouling; (2) keep the foulant in dispersion and solution; (3) avoid new fouling; (4) not degrade the membrane; and (5) disinfect all wetted surfaces. Not only is the cleaning ability of the detergent important, but also such factors as the ease with which it can be dispensed and rinsed away, its chemical stability during use and additional factors such as cost and safety (Trägårdh, 1989). In more recent times the effect of the cleaning agents on the environment has become a most important factor in the choice of chemicals that are potentially useful in cleaning UF membranes.

The following types of cleaning agents are often used for cleaning UF membranes (Trägårdh, 1989): alkalis (hydroxides, carbonates, and phosphates); acids (nitric and phosphoric); enzymes; surface-active agents (anionic, cationic, and nonionic); sequestering agents (EDTA); formulated cleaning agents; combined cleaning and disinfecting agents; and disinfectants (H_2O_2, metabisulphite, hypochlorite, and heat treatment).

4.4.1 Alkalis

Kim *et al.*, (1993) observed that, with the use of basic or acidic cleaners, one of the main mechanisms for protein removal is probably the increased charge on the adsorbed protein molecules packed on the membrane. An increase in the net surface charge of the protein, due to a jump in pH or adsorption of surfactants, enhances electrostatic repulsion between protein molecules and disrupts the protein packing in the cake, allowing it to be gradually swept away. Alkaline cleaners are best suited to remove organic impurities. Sodium and potassium hydroxide saponify fat and solubilise proteins to some extent (Bragulla and Lintner, 1986). Carbonates do not have a very

good cleaning ability but can aid cleaning through their pH-regulating properties while mono-, di- and triphosphates, have limited cleaning effect. Phosphates do, however, act as dispersants, solubilise carbonates, bind ion salts, regulate pH, emulsify fat and peptise proteins. Bohner and Bradley, (1992) prepared a chlorinated alkaline cleaner from sodium hydroxide and household sodium hypochlorite bleach solution. The pH of this mixture was 11 and it contained 200 ppm of active chlorine.

4.4.2 Acids

Acids in general, have a detrimental effect on membrane composition. Acids, such as nitric and phosphoric acid, have, however, been used previously to dissolve precipitations of inorganic salts or oxide films on UF membranes (Bragulla and Lintner, 1986).

4.4.3 Enzymes

Enzymes have three unequalled characteristics. Firstly enzymes are the most efficient catalysts known and most cellular reactions occur about million times faster than they would without enzymes. Secondly, the majority of enzymes are distinguished by their specificity of action. Thirdly, enzymes are regulated, which means that they could be regulated from their most active to their inactive forms. The regulation of enzyme activity may be via internal biological regulation mechanisms or by the creation of optimal pH, temperature and ionic strength conditions for these biocatalysts to operate in. Enzymes and enzyme detergent mixtures are increasingly being incorporated into cleaning mixtures for household uses as well as in industrial applications. In the latter case, for example, the cleaning of equipment involved in food production and processing. Although enzymes are relative expensive components of these cleaning mixtures, their specificity and environmental friendliness more than compensate for the cost factors involved. Detergents, or cleaning mixtures, that contain enzymes have not been used on a large scale in cleaning regimes of biologically fouled UF membranes. Enzymes are, however, ideal cleaning agents for biologically fouled membranes as they are highly specific for the decomposition of biological foulants like proteins and lipids. In addition enzymes act under mild conditions of pH, temperature and ionic strength and will not have a detrimental influence on the membrane surface. Enzymes are, for instance, very feasible cleaning agents for cellulose acetate membranes, as these membranes cannot withstand the high temperature and pH used in most cleaning regimes (Trägårdh, 1989).

A number of factors have, up to now, prohibited the large scale use of enzymes in membrane cleaning regimes. As mentioned earlier, enzymes are costly and formulating them into effective cleaning agents would require relative expensive research and development. There are concerns that residual enzyme activity can affect cultures, such as the starters used in cheese making, which would have an adverse effect on production quality. Enzymes are also perceived to be slow and longer cleaning times are thus needed when they are incorporated into cleaning regimes.

A rapid extension of membrane technology into the treatment of effluent originating from food and other biologically related processes, as well as mounting pressures on the environment, have compelled researchers in the field to re-examine enzymes as possible cleaning agents for fouled UF membranes. Recently proteolytic enzymes from extremely thermophilic eubacteria (65°C) were used to clear UF membranes fouled during the processing of whey (Coolbear *et al.*, 1992). It is important to note that, after restoration of flux with detergents, dilute acid or dilute alkali solutions, the flux decreases rapidly to half of the initial value during the first hour of operation (Chen *et al.*, 1992). To circumvent these problems, a self cleaning membrane was prepared (Chen *et al.*, 1992 and Butterfield *et al.*, 1994). Proteases (pepsin and papain) were adsorbed onto PSM UF membranes via three different adsorbing methods which include physical adsorption (Howell and Velicangil, 1982), cross-linking with gluteraldehyde, and covalent coupling by means of cyanogenbromide activation. Membranes used in abattoir effluent were also successfully cleaned using a commercially available enzyme detergent preparation which had proteolytic activity (Jacobs *et al.*, 1991). Norde (1986) stated that pancreatic lipases, that control the digestion of alimentary fats in the duodenum, show increased activity when adsorbed at the oil-water interface and could well be applied in membrane cleaning regimes.

4.4.4 Surface-active agents

Surface-active agents may be classified as anionic, cationic, or amphoteric electrolytes. Anionic agents are neutral, organic foaming agents, such as soap, alkyl sulphate and alkyl sulphonate. Cationic agents consist of quaternary ammonium compounds and they are less effective detergents than anionic or nonionic agents. Non-ionic surfactants consist of condensation products of compounds such as ethylene oxide, ethoxylated dodecyl alcohol and ethoxylated nonylphenol. Great care has to be taken, however, when introducing surface-active agents into a membrane plant as many cationic agents are adsorbed onto membranes made from aromatic polyamides, for instance, resulting in a decrease in flux (Trägårdh, 1989).

Surfactants help to remove adsorbed proteins by adsorbing onto the protein molecules, thus increasing solubility as well as the charge on the protein (Kim *et al.*, 1993). Unsuitable surfactants defoaming and dedusting agents could, however, cause deposits on the membrane or blockage of the membrane pores, which impairs filtration performance. Many surfactants are also not environmentally friendly, may contribute considerably to pollution and are not biodegradeble.

4.4.5 Formulated agents

Formulated detergents often consist of a mixture of alkali's, phosphates, sequestering agents and wetting agents. The actions of these individual cleaning agents have already been discussed in the preceding sections.

4.4.6 Disinfectants

Disinfectants are used to reduce biofouling, which is the unwanted deposition and growth of living organisms on surfaces (Flemming, 1990). Sanitation programmes usually include physical and chemical methods (Flemming, 1990). Physical methods include flushing, backwashing, air scouring, air bumping, abrasive sponge balls, non-abrasive sponge balls, sand scouring, brushing, hot water, steam, ice nucleating, irradiation and ultrasonic energy. The chemicals used in sterilisation are chlorine, hypochlorite, ClO_2, chloramine, bromine, ozone, H_2O_2, peraceticacid, formaldehyde, gluteraldehyde, isothiazolones and quaternary ammonia compounds.

4.5 CLEANING CONDITIONS FOR FOULED UF MEMBRANES

Cleaning frequency experiments show that both excessive cleaning and infrequent cleaning resulted in lower productivity, indicating that an optimum cleaning frequency exists. It is possible that incomplete cleaning could leave a denatured protein foulant on the membrane surface which is now more strongly bound than the original foulant (Kim *et al.*, 1993). A number of factors are important to consider when designing a cleaning regime for fouled UF membranes.

4.5.1 Concentration of the cleaning agent

Kim *et al.*, (1993) observed that a ten-fold increase in cleaner concentration only marginally improved cleaning effectivity. In addition an indiscriminate increase in cleaning velocity also did not improve cleaning results markedly. It is important that chemicals in powder form should be dissolved in water completely before they are introduced into the system, as undissolved matter

could add to the fouling problem. It is also important to gradually add cleaning chemicals to the balance tank to avoid local over-concentration of chemicals in the system.

4.5.2 Cleaning time

Bragulla and Lintner, (1986) showed that refouling may occur if the preselected cleaning time is too long. A cleaning time of about 30 min has proved ideal for alkaline cleaners. If enzymatic cleaners are employed, cleaning times of 60 to 90 min may be necessary and in some cases several days. (Bragulla and Lintner, 1986).

4.5.3 Temperature

An increase in temperature will normally increase the rate of cleaning. An exception to this rule is where certain substances are unresponsive to the initial temperature increase. Fats, for instance, have a well defined melting point. Raising temperatures under the melting point will not affect the lipid adsorption on the membrane and cleaning temperatures must always exceed the melting point of the fouling lipid (50 to 60 °C). As a rule cleaning should thus always be carried out at the same or slightly higher temperatures than used in the process (Trägårdh, 1989).

4.5.4 pH

Changing the pH of the cleaning solution may often result in desorption of proteins as they form uniformly charged molecules above or below their pI values. These charged forms are more reactive and repel each other, thereby increasing solubility.

4.6 FOULING EXTENT AND CLEANING EFFICIENCY

It is important to develop techniques to characterise membrane fouling and to evaluate the efficiency of any given cleaning method. Analysis of adsorbed foulants, before and after cleaning, is most important for the planning and development of future cleaning strategies. A number of methods have previously been developed to achieve these goals. These methods can be summarised as follows:

Kim *et al.*, (1993) characterised the degree of fouling as the residual resistance (R_{sw}) of the solute due to bound protein using the following equation:

$$R_{sw} = (\Delta P/J_{ww}) - R_m$$

where J_{ww} is water permeability, after the membrane was washed with distilled water to remove unbound proteins, and R_m is the initial membrane resistance. Data for cleaning efficiency are expressed as **flux recovery** and **relative solute** removal. Flux recovery is J_{wc}/J_{wi}, where J_{wc} is the water flux after cleaning and J_{wi} is the initial water flux. The solute resistance removal is given by:

$$(R_{sw} - R_{sc})/R_{sw}$$

where R_{sc} is the residual solute resistance of the cleaned membrane. Flux measurements was the only parameter that was used to determine the efficiency of cleaning experiments.

Echner and Zottola, (1993) determined the **fouling coefficient (b)** as an indicator of long-term fouling, the value of **b** can be calculated from the equation:

$$\ln \Delta V/\Delta t = A - b \ln V$$

where $\Delta V/\Delta t$ is the instantaneous flux rate at time t, A is the initial flux and V is the total volume permeated at time t.

Bragulla and Lintner, (1986) used fouling substances labelled with ^{14}C prior to determine the extent of fouling and the cleaning efficiency after various cleaning strategies were applied. The information obtained with this method was analysed and correlated with the pure-water flux.

Bohner and Bradley, (1992) investigated fouling and cleaning by direct observations of UF membranes using microbiological swabbing and scanning electron microscopy (SEM).

A number of factors that cause ineffective cleaning have been identified. It has been reported by Bragulla and Lintner (1986) that circulating cleaning solution must entrain as little air as possible to prevent oxidation of foulants and to impair bacterial growth in addition to ensuring that maximal contact is established between the cleaning solution and the fouled membrane. Residues on the membrane which are not readily removed during cleaning can be identified with modern chemical and physical methods of analysis (Bragulla and Lintner, 1986). Once identified the cleaning regime may be adjusted to remove these substances. Cleaning agents should always be chosen with a view to obtain optimal cleaning results whilst minimising damage to the membrane (Bragulla and Lintner, 1986).

CHAPTER 5

CHARACTERISATION OF FOULANTS ADSORBED ONTO (UF) MEMBRANES USED IN ABATTOIR EFFLUENT

5.1 INTRODUCTION

Abattoirs are major water users and produce a large volume of effluent rich in proteins and lipids. Discharge of this effluent is of major concern to abattoir management due to its high volume and high COD. It is therefore important to consider treatment of abattoir effluent in order to recycle treated water (i.e. reduce the clean water demand) or produce a smaller volume of effluent. One possible treatment process includes the use of UF membranes. A treatment method that could reduce the COD in the effluent stream by up to 90 % and the potable water demand by 25% (Cowan *et al.*, 1992). The problem is, however, that severe fouling of (UF) membranes occurs in this environment which impairs the acceptance of UF as an economical treatment method of abattoir effluent (Cheryan, 1986). Although chemical cleaning techniques exist for membranes that have been fouled in abattoir effluent treatment operations, these methods pose some problems, such as the reduction of membrane life and added pollution. Biological cleaning techniques (including the use of enzymes to remove specific foulants) on the other hand do not produce additional pollution (biodegradeble) or cause any membrane damage. Enzymes can be recycled and it is also possible to produce enzymes on a large scale at relatively low cost with the aid of modern molecular biology. In order to develop new biological cleaning methods for membranes fouled in abattoir effluent, potential foulants in abattoir effluent streams first needed to be identified.

Real abattoir effluent was chosen as medium for experimentation, as this effluent is complex in nature and may vary from hour to hour and from day to day. Using model foulant mixtures would therefore not give an accurate picture of membrane fouling and would complicate characterisation of foulants. Membranes were fouled in abattoir effluent and the nature and quantity of foulants adsorbed onto membranes were determined. In the present study adsorption was investigated under static conditions, as the hydraulic resistance (R_h) of a membrane may change during an UF experiment due to at least three different phenomena: gelation, mechanical pore blocking and adsorption. During UF, all three phenomena occur, but adsorption occurs even at zero operating pressure. In order to assess the role of adsorption in fouling, it was important to study the process at zero pressure. The contribution of adsorption can then readily be separated from the other phenomena (Aimar *et al.*, 1986). In some cases the dynamic protein loading may even be predicted

from the static kinetics of adsorption (Ko *et al.*, 1993). Fouled membranes were analysed for the major foulant constituents present in abattoir effluent namely proteins and lipids. A rapid colometric detection method for protein fouling was developed to assess and compare protein adsorption onto flatsheet UF membranes fouled in abattoir effluent. In addition the correlation between fouling and changes in the water contact angle and pure-water flux of PSM was also investigated.

5.2 EXPERIMENTAL

5.2.1 Materials

All the glassware used for lipid determination experiments were precleaned in chromic acid, rinsed with distilled water and acetone and dried in an oven. The flasks were subsequently stored in a vacuum desiccator until further use. All chemicals used for analysis of the effluent as well as the fouled membranes were of analytical grade and obtained from BDH Chemicals Ltd., Pool, England unless otherwise mentioned. Bradford reagent was used for the determination of the protein content of the effluent and was prepared as previously published (Bradford, 1976). BSA obtained from Sigma Chemical Co., St. Louis, USA, was used as the protein standard.

All membranes used were flatsheet PSM prepared from Udel P3500 polysulphone and cast from N-methyl 2,piridine 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 (5mg/l) to prevent bacterial growth. A staining solution (62.5 ml of a 1% aqueous Coomassie brilliant blue G250 solution filtered through a Whatman no. 2 filter was combined with 250 ml methanol, 50 ml acetic acid and diluted to 500 ml with distilled water) was used to stain proteins adsorbed onto membranes. Two solutions were used for destaining: the first destaining solution, I, consisted of 125 ml methanol and 25 ml acetic acid diluted to 250 ml with distilled water and the second destaining solution, II, consisted of: 25 ml methanol and 37 ml acetic acid diluted to 500 ml with distilled water.

The following reagents were used to quantify proteins adsorbed onto membranes: solution A (0,2 g sodium tartrate and 10 g Na_2CO_3 dissolved in 55 ml 1M NaOH and diluted to a final volume of 100 ml with distilled water), solution B (2 g sodium tartrate and 1 g $CuSO_4 \cdot 5H_2O$ dissolved in 90 ml of distilled water and 10 ml 1 M NaOH), freshly prepared solution C (1 volume Folin-Ciocalteu reagent (Merck Darmstadt, Germany) diluted with 2 volumes distilled water) and solution D (5% sodium dodecyl sulphate (SDS) from Merck Darmstadt, Germany, dissolved in 0.5 M NaOH). Due

to precipitation at temperatures below 23°C solution D was always heated until all precipitates were dissolved before each experiment.

5.2.2 Analysis of abattoir effluent

All experiments were replicated four times and the values represented is the mathematical mean ±SD and ±SE as indicated. Effluent was collected every hour for a period of four weeks from the Maitland abattoir in Cape Town and preserved at 4°C in 200 ml Shott flasks with sodium azide (1mg/200 ml) until analysis. The effluent used for analysis is the abattoir process effluent stream arriving from the slaughter process areas.

1. Determination of the lipid content

Effluent samples (20 ml) were lyopholized and a total lipid extraction was subsequently achieved as follows: 40 ml hexane:isopropanol (3:2 v/v) was shaken up with the lyopholized residue and the resultant mixture boiled mildly for 15 min. The hot solution was filtered through a Whatman no. 40 filter paper and the filtrate collected. The flask that contained the lyopholized residue was rinsed with another 30 ml of heated organic solution, filtered and recombined with the first extract. The amount of lipid present was determined gravimetrically after the volatile phase of the solution was removed under nitrogen at 40 °C (Radin, 1981).

2. Determination of the protein content

The protein content of abattoir effluent was determined by the Bradford method (Bradford, 1976): Effluent (40 ml) was centrifuged for 10 min at 12000 rpm to remove particulate matter. The supernatant (1 ml) was decanted and diluted with distilled water (5 ml). Of this diluted mixture 1 ml was mixed with the Bradford reagent (4 ml) and the absorbance read under identical conditions as the standards. The protein concentration was subsequently calculated from the standard curve.

3. Static membrane fouling

Membranes were cut into 10 cm x 32 cm rectangulars and transferred to a portable container filled with distilled water. The membrane squares were subsequently adhered to a "Perspex" frame equipped with lead weights and submerged into the abattoir effluent tank (see Fig. 5.1). The effluent used for membrane fouling is the abattoir process effluent stream arriving from the slaughter process areas. Care was taken that the membranes were totally submerged at all times. Two membranes were removed after every hour of contact with the effluent stream, washed thoroughly with tap water to remove all excess adsorbents, placed in distilled water and transported

to the laboratory. In the laboratory the membranes were immediately transferred to freshly distilled water containing sodium azide (5mg/l) and kept at 4°C until analysis.

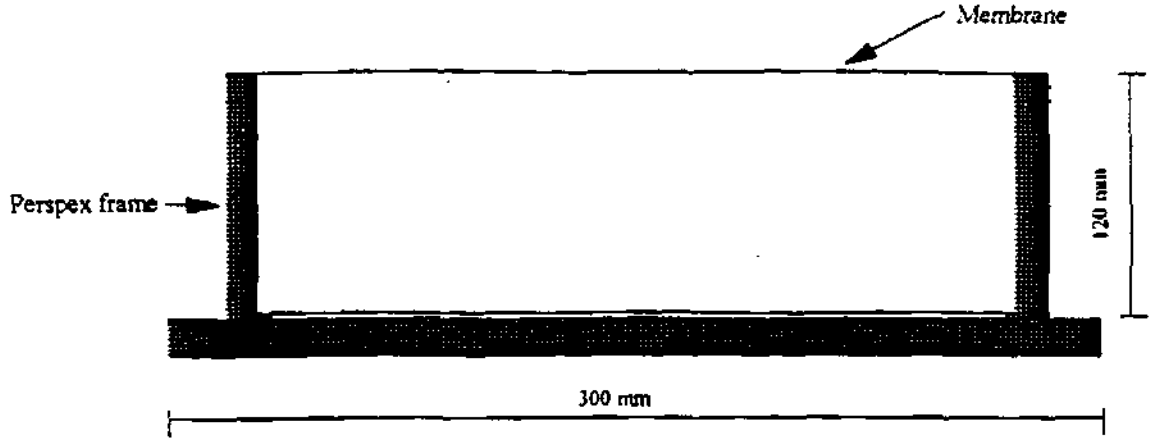

Fig. 5.1 Diagram of the "Perspex" frame membranes were adhered to in static fouling experiments. The 100 x 300 mm strips of PSM were clamped horizontal to the 120 mm posts and submerged into the effluent. Lead weights were also clamped to the frame to keep the frame submerged.

5.2.3 Protein determination techniques of PSM fouled in abattoir effluent

1. Qualitative colorimetric detection of proteins adsorbed onto PSM

Fouled and unused membranes were stained for 30 min with the Coomassie brilliant blue G250 staining solution at room temperature. The membranes were subsequently destained, to remove all excess Coomassie blue not bound to the adsorbed proteins for four 15 minute periods with destain solution I followed by another 2,5 hours of destaining in destain solution II.

2. Quantitative determination of the proteins adsorbed onto membranes

Due to the presence of lipids in the membrane's fouling layer, the protein determination method of Hess *et al.*, (1978) had to modified. Fouled PSM were washed thoroughly with distilled water, cut into squares (25 cm^2) and the proteins subsequently stripped from the membranes by submerging the squares into solution D (5 ml) and distilled water (2,5 ml). This mixture was incubated at 32°C for 2.5 hours and vortexed every 30 min.

The protein content of the resulting stripping solution was determined as follows: To 750 µl of the stripping solution 2M NaOH (500 µl) and solution A (900 µl) were added, the mixture vortexed and left at room temperature for 30 min. Solution B (100 µl) was subsequently added, the mixture vortexed and left at room temperature for 20 minutes. After 20 minutes two 1,5 ml portions of

solution C were added (the mixture was vortexed after each addition) and the resulting solution left at room temperature. After 30 minutes the absorbance was read at 650 nm against a blank solution that was obtained by treating an unused membrane square in the same manner as the fouled membranes. The amount of protein was subsequently determined from the standard curve.

5.2.4 Determination of the lipid content in the adsorbed layer

Fouled membranes were cut into squares (25 cm^2) and left to dry at room temperature for 30 min. The dried membranes were shaken vigorously with hexane:isopropanol (3:2 v/v) (50 ml) for 5 minutes in sealed Erlenmeyer flasks and thereafter left at room temperature for 12 hours. The extraction solution was subsequently boiled mildly for 15 minutes and filtered through a Whatman no. 40 filter paper. The membrane squares, filter paper and flask were subsequently rinsed with another 20 ml of preheated hexane:isopropanol (3:2 v/v) and the filtrate combined with the first. The amount of the lipid residue was determined gravimetrically after the volatile phase of the solution was removed under nitrogen gas at 40°C (Radin, 1981).

1. Contact angle measurements of PSM

Dynamic contact angle analysis, a technique based on the Whilhelmy slide technique, was used to determine the contact angle of water with the membrane (Johnson and Dettre, 1977). The surface under observation, attached to a balance, is positioned in a vertical orientation directly above the liquid. The level is raised gradually until it just touches the membrane suspended from the balance. The increase in mass is then noted and related to the contact angle with the equation (Johnson and Dettre, 1977):

$$y \cos = W/p$$

Where **W** is the change in mass of the membrane sample when it is brought into contact with the liquid, and **p** is the perimeter of the membrane sample. The contact angle of the membranes, statically fouled in the abattoir effluent, were compared with the contact angle of an unused membrane. Membranes were cut into 2 cm wide strips and folded double with the membrane-layer facing outwards. These membrane strips were fitted into the dynamic contact angle analyser coupled to a computer which analysed the results. Fresh deionized analytical grade water was used for each membrane strip.

5.2.5 Pure-water flux determination

Pure-water flux was determined as follows: statically fouled flatsheet membranes were placed in a flat sheet rig and pure-water was pumped through the membranes at 200, 300 and 400 kPa pressures respectively, at a constant flow rate of 1 l/min (see Fig. 5.2). Permeate was collected in 25 ml flasks at 5 min after the settings were adjusted.

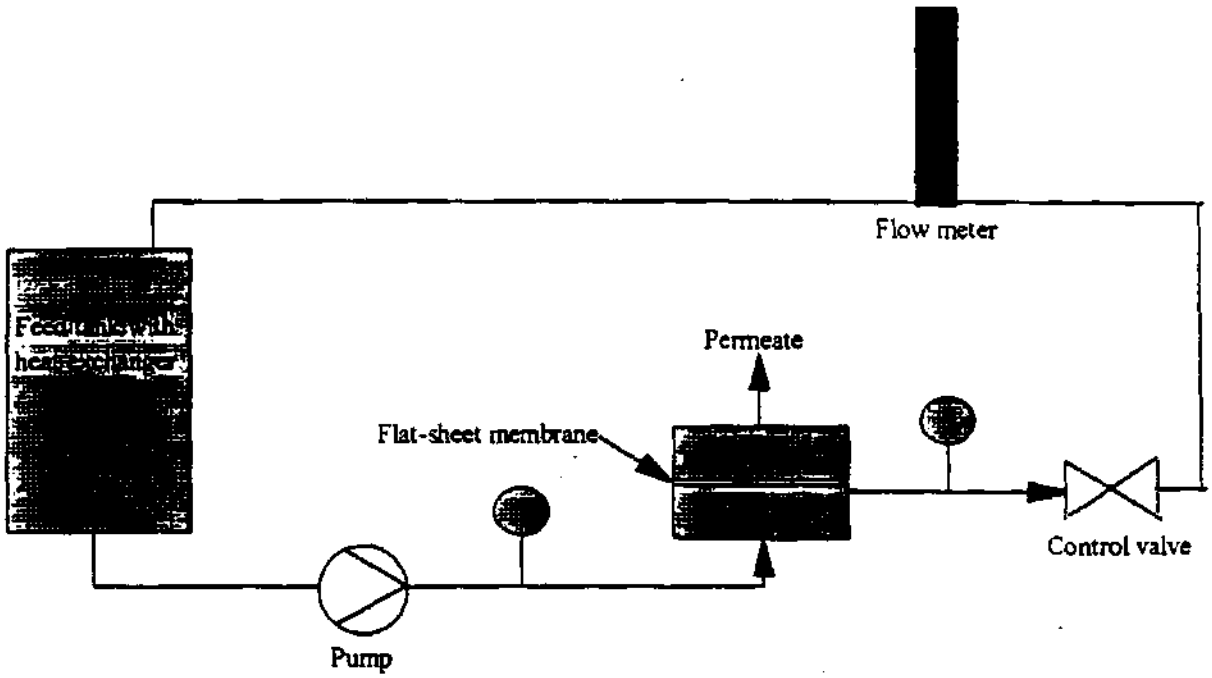

Fig. 5.2 Experimental set-up to determine the transmembrane flux of pure-water through unfouled, fouled and cleaned PSM.

5.3 RESULTS AND DISCUSSION

5.3.1 Analysis of abattoir effluent

To fully utilise the catalytic potential of enzymes in cleaning solutions for the treatment of biologically fouled membranes, the nature and composition of foulants on the membrane must, however, be known. This knowledge is of importance when choosing the correct enzyme(s) or enzyme detergent mixtures. Using abattoir effluent as a case study the main components in the effluent from an operational abattoir process stream was first identified. It was established that proteins and lipids were the only potential foulants present in significant concentrations. Most previous studies on membrane fouling used artificial mixtures of proteins and lipids as model effluents (Grund *et al.*, 1992). This approach, however, oversimplifies the problem and, in our

opinion, will not yield results that can readily be extrapolated to an industrial environment. Our analysis of a typical operational abattoir process effluent over a period of 4 weeks showed that the protein and lipid contents varied significantly. The temperature and pH of the effluent, however, remained remarkably constant. The results obtained from the analysis of abattoir effluent are summarised in Table 5.1. From this data it is clear that abattoir effluent has a high fouling potential due to amount of protein and lipid material present. The concentrations of both organic substances vary considerable. The pH and temperature of the effluent are ideal to support bacterial growth.

Tab. 5.1 Major factors in abattoir effluent involved in membrane fouling. Values presented are the mathematical mean ±SD.

µg Protein/ml effluent	µg Lipid/ml effluent	Temperature of effluent °C	pH
925 ± 445	508 ± 150	29.81 ± 2.75	7,46 ± 0,68

5.3.2 Protein content of fouled PSM

1. Colometric staining of fouled PSM

The results of the membrane staining experiment is presented in Fig. 5.3. There is a marked difference between the stain development of the unused membrane (8) and that of the fouled membranes (1 and 2). The reason why the unfouled membranes also developed some colour, in contrast with clean unstained reference membranes (5 and 6), is that Coomassie blue is a low molecular mass dye. Some of the small dye molecules penetrate the membrane pores and are not washed out during destaining. Unused stained membranes could, however, still be used as a reference to characterise protein fouling on flatsheet PSM as the background staining of unfouled membranes is significantly less than that of fouled membranes.

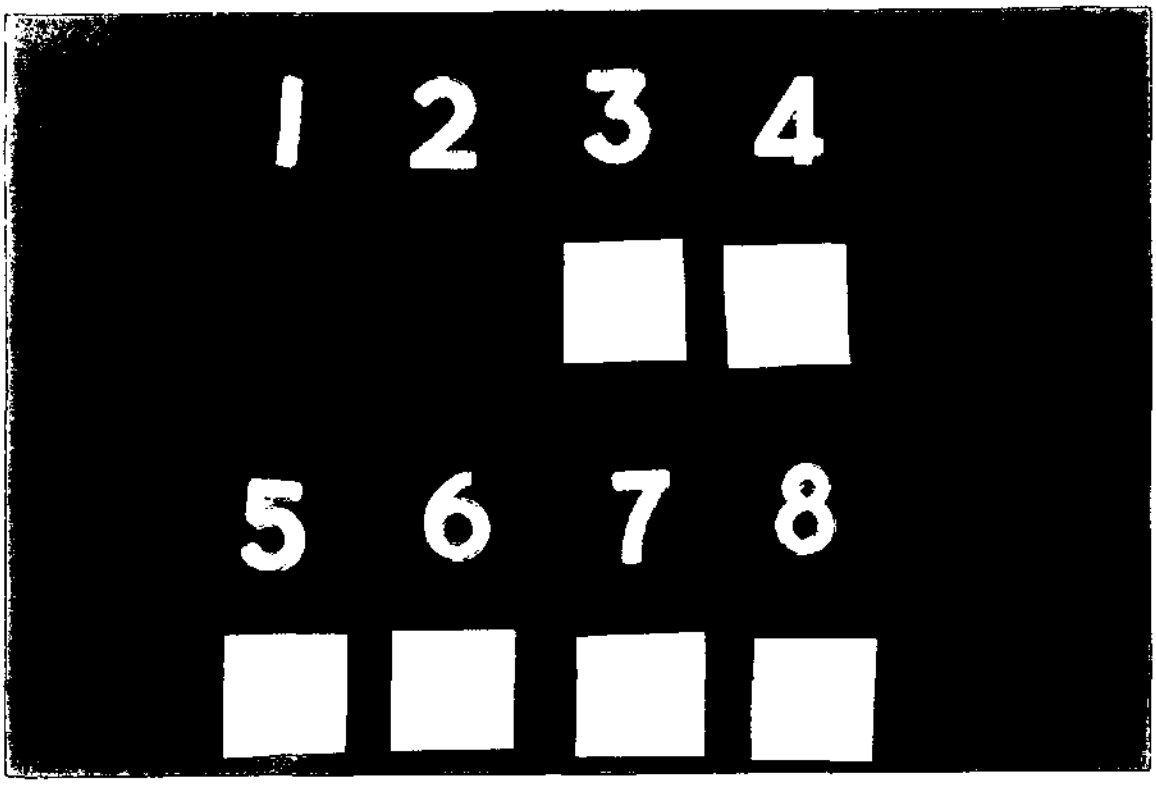

Fig. 5.3 The colour development of proteins adsorbed onto membranes during fouling. Membranes were treated as follows no's. 1 and 2 fouled followed by staining, 3 and 4 fouled, cleaned and stained, 5 and 6 were unused unstained, 7 fouled and not stained and 8 is an unused reference membrane that was stained.

The value of the staining method as a rapid qualitative assessment of membrane fouling can be clearly seen with fouled membranes numbers (3) and (4). Fouled membrane (3) was treated with a biological cleaning agent (Protease type XX111 from *Aspergillus Oryzae* for 30 min) and fouled membrane (4) was stripped with 5% SDS for 2,5 hours. The colour development of these two membranes, when compared to membranes no's 8 and 1, indicates the value of this method as a rapid determination method for the efficiency of protein stripping and cleaning methods. Untreated membranes, fouled in abattoir effluent, have a distinct green-yellow colour as can be seen in 7, Results obtained with the staining experiments clearly indicate that this rapid qualitative method of protein determination can play an important role as a simple approach to assess membrane fouling and determine the efficiency of cleaning and stripping techniques.

2. Protein adsorption to PSM

Three distinct phases of protein adsorption were observed during the 8 hour fouling period. Initially there was a rapid adsorption of proteins in the first hour of fouling, followed by a lower rate of adsorption between 2 and 5 hours. After 5 hours the same adsorption pattern was observed (Fig. 5.4). After 8 hours the protein loading reached a value of 42 $\mu g/cm^2$ membrane. Data from the

literature indicate protein loading ranging from 0,05 μg/cm² , on cellulose acetate membranes, to as high as 150 μg/cm² on polyacrylonitrile membranes (Aimar *et al.*, 1986).

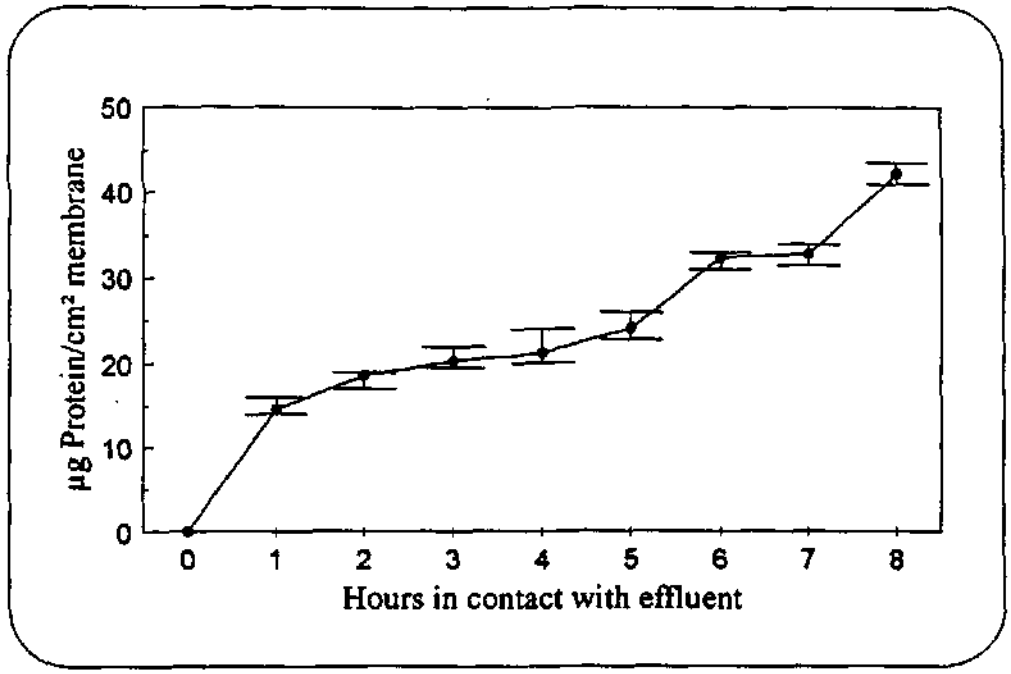

Fig. 5.4 Amount of protein (μg/cm²) adsorbed onto PSM vs. time in contact with abattoir effluent. Values presented are the mathematical mean of at least four determinations ±SE.

5.3.3 Lipid content of fouled PSM

The results summarised in Table 5.1. indicate that, quantitatively, proteins should be the most abundant foulant adsorbed onto PSM exposed to abattoir effluent. Lipid analyses of fouled PSM, however, show that the surface of these membranes adsorb lipids much more effectively than proteins due to the hydrophobic nature of the membrane surface (Fig. 5.5.). This indicates that the surface of PSM is ideal for lipid adsorption. Like protein adsorption, there was a rapid adsorption of lipids in the first hour of fouling followed by a period of slower or almost no adsorption in the next three hours. After 3 hours, a fast adsorption period again occurs, followed by slower or no significant adsorption thereafter. The lipid content reached a maximum value of 330 g/cm² membrane area in 8 hours. Comparing the protein and lipid adsorption it seems that a layer of lipid material initiates membrane fouling. The membrane surface, covered with lipids after adsorption in the first hour then provides an ideal surface for further protein and lipid adsorption.

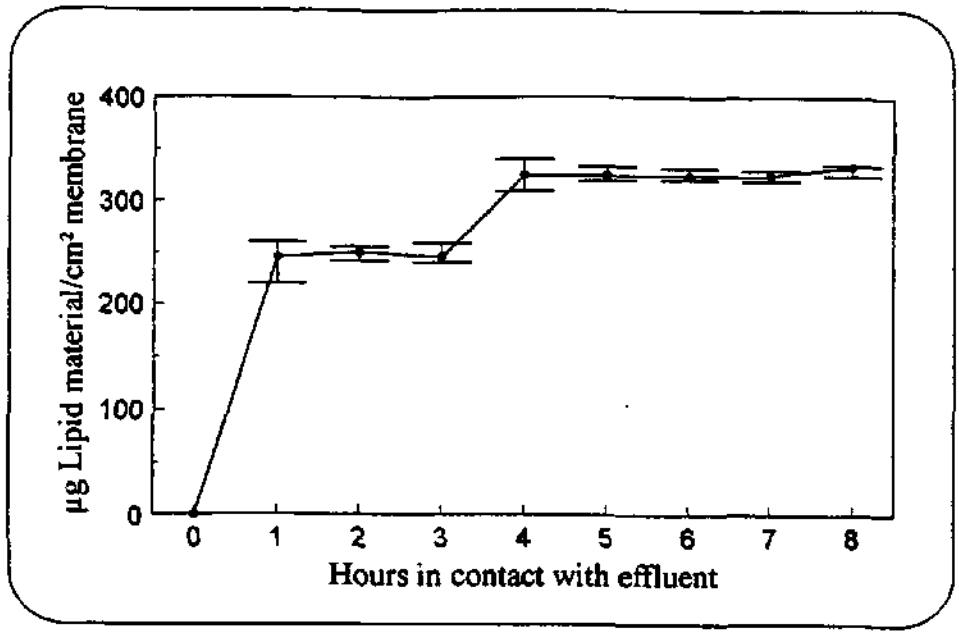

Fig. 5.5 Amount of lipids (µg/cm²) adsorbed onto PSM vs. time in contact with abattoir effluent. Values presented are the mathematical mean of at least four determinations ±SE.

5.3.4 Contact angle measurements of clean and fouled PSM

The results of contact angle measurements are given in Fig. 5.6. An increase in the contact angle, from 63° to 85°, was observed after the first hour of fouling. The contact angle thereafter declined for the next two hours, followed by an increase after 4 hours fouling, declining again and eventially stabilising at approximately 72,5°. The contact angle values represent the characteristics of the membrane surface. If a substance of hydrophobic nature adsorbs onto the membrane, the contact angle will respond by increasing. Within the first hour of fouling the most abundant adsorbing species, in this case lipids, covers the membrane surface. The adsorbed lipids cause an increase in the contact angle of the membrane due to the hydrophobic nature of the new adsorbed surface. Proteins, with both hydrophobic and hydrophilic groups, easily adhere to the lipid-covered membrane surface revealing its hydrophilic part to the outside thereby decreasing the contact angle. Proteins continue to be adsorbed onto the membrane after 2 hours of fouling, covering the lipid surface. A more hydrophilic surface again causes a decrease in the contact angle. Lipid adsorption and contact angle increases are repeated at 4 hours of fouling, followed again with protein adsorption and a decrease in the contact angle.

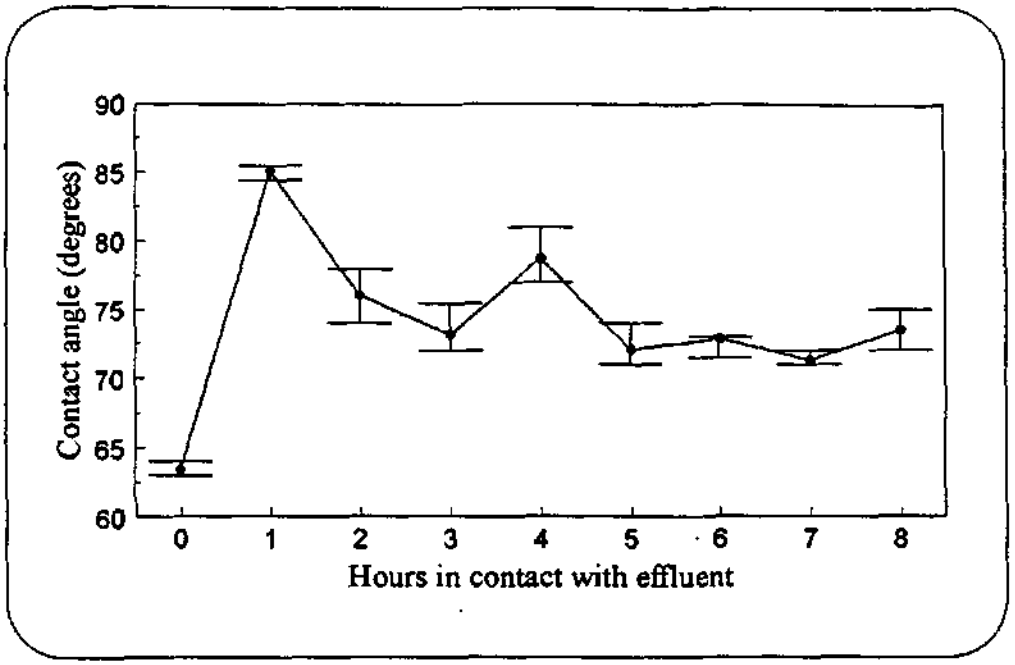

Fig. 5.6 Contact angle (degrees) of abattoir effluent fouled PSM vs. time of contact with the abattoir effluent. Values presented are the mathematical mean of at least four determinations ±SE.

5.3.5 Pure-water flux measurements of fouled PSM

Abattoir effluent is an ideal medium for bacterial growth and the experimental fouling of PSM had therefore to be accomplished on site. In addition, the day to day variations in lipid and protein content of the effluent necessitated the simultaneous fouling of a large number of PSM samples to facilitate comparative studies. Financial as well as logistic constraints forced static fouling instead of dynamic fouling of membranes in this investigation. If one compares the time dependent flux decline in statically fouled membranes, obtained from our investigation in abattoir effluent (Fig. 5.7), to that of dynamically fouled membranes, using a 0,1% BSA solution (Fig. 5.8) (Fane and Fell, 1987), the same flux reduction profile was observed. The decline in the water flux was also matched by a gradual increase in irreversibly deposited material that could not be removed by simple water washing. These results indicate that characterising fouling under static conditions yields the same results as dynamic conditions. Under dynamic conditions the flux decline, however, is the result of a combination between CP, gel-layer formation and fouling. Fouling is therefore often confused with flux depression effects of CP. If the flux decline is reversible, the decline is the result of CP and not fouling. Thus to simplify and appreciate the sole effect of fouling, by adsorption, it is beneficial to employ static fouling experiments. Results of the pure-water permeability experiments are shown in Fig. 5.7.

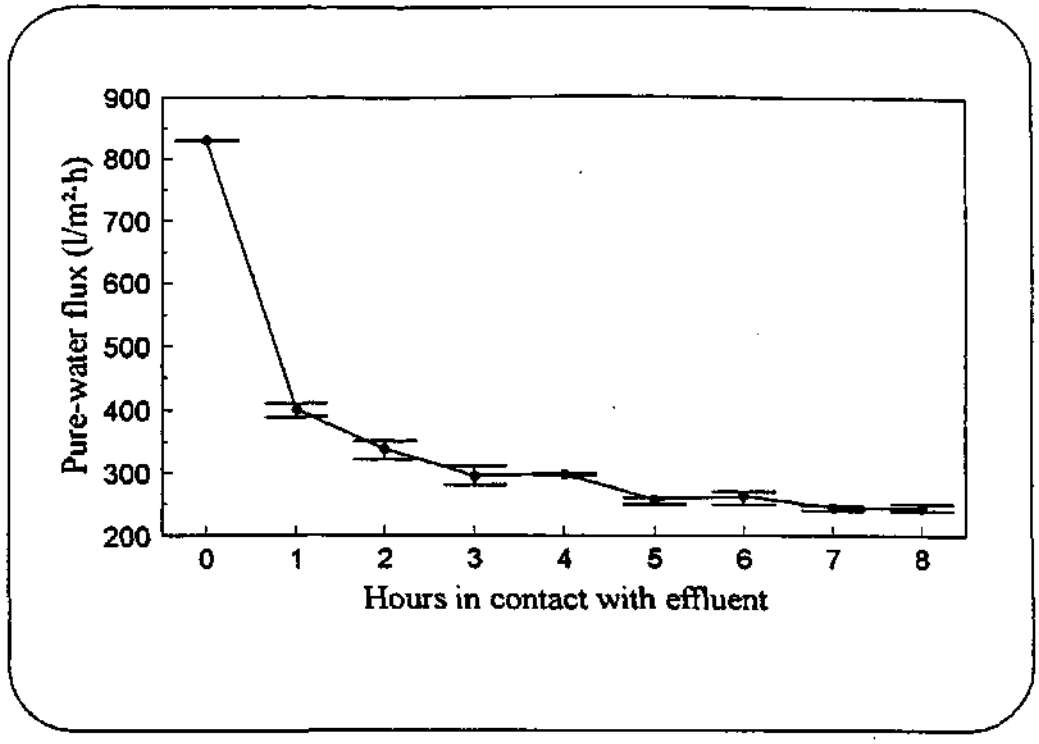

Fig. 5.7 Pure-water flux (l/m²·h) through abattoir effluent fouled PSM vs. contact time with effluent. Values presented are the mathematical mean of at least four determinations ±SE.

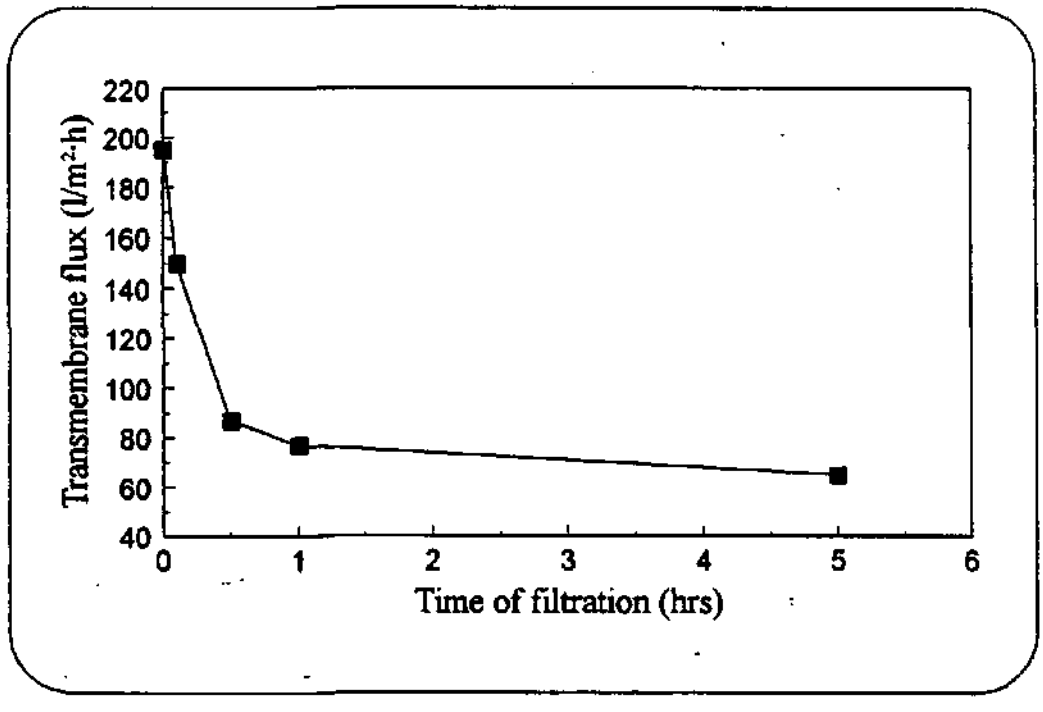

Fig. 5.8 The dynamic flux for a 0,1% BSA solution as reprinted from Fane and a Fell (1987).

The major effect of fouling on the permeability of membranes occurred during the first hour of contact with the effluent resulting in a pure-water flux decline from 832,27 to 400 l/m²·h. The flux decline thereafter is more gradual and stabilised after 5 hours at approximately 242,74 l/m²·h.

The relationship between flux and feed water pressure is shown in Fig. 5.9. For both membranes the flux increases linearly with an increase in pressure. For the fouled membrane, however, the increase in flux with an increase in feed water pressure is significantly less, indicating that pressure increases cannot be used to alleviate fouling.

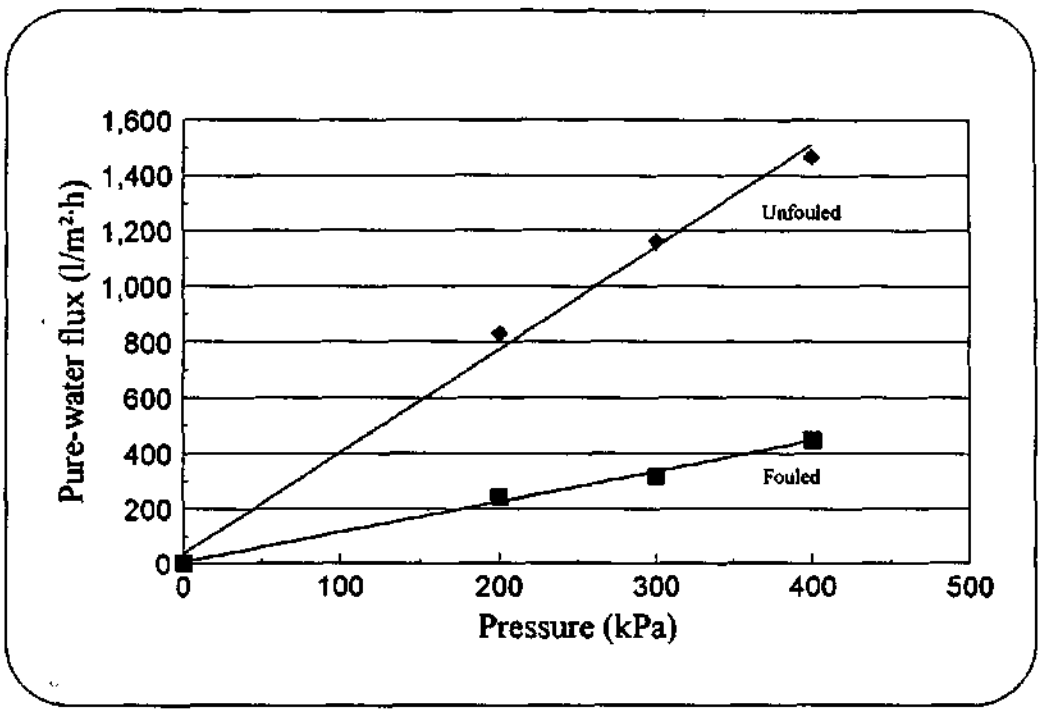

Fig. 5.9 The effect of increasing feed pressure on the pure-water flux of an abattoir effluent fouled and an unfouled membrane.

5.4 CONCLUSIONS

Protein and lipids are the main foulants present in abattoir effluent. These foulants adsorb onto PSM as shown by protein and lipid analysis of fouled membranes. Contact angle and pure-water flux measurements are also affected significantly by these foulants. Protein and lipid content, in conjunction with contact angle measurements and pure-water flux measurements, can therefore be used to characterise the nature and effects of foulants.

To summarise the effect of fouling on each parameter during the 8 hour fouling period:

1. rapid adsorption of lipids (250 g/cm^2) and proteins (15 µg/cm^2) occurred within the first hour of contact;

2. lipids adsorbed onto the membrane during the first hour period change the membrane surface to a more hydrophobic nature resulting in an increase in the contact angle;

3. the greatest pure-water flux decline was also observed within the first hour. Flux decline is caused by the combination of surface and pore adsorption as well as an increase in the hydrophobic nature of the membrane surface;

4. lipid adsorption stabilises between 2 to 4 hours of contact. Protein adsorption, however, still continued during this period and subsequently lead to a decrease in the contact angle;

5. pure-water flux reaches a plateau after 5 hours of fouling and is not affected by additional adsorption;

6. at 4 hours the same pattern of lipid adsorption that initially increases the contact angle occurred, followed by protein adsorption and the resulting decrease in the contact angle. This implies that multilayer adsorption occurs under static adsorption conditions for both the lipid and protein material and the contact angle is influenced by the most abundant foulant specie on the membrane surface at that specific stage;

7. under static adsorption conditions, only the effect of adsorption is present and therefore it can be assumed that the same type of adsorption reactions will occur under dynamic conditions. The amount of adsorbed material, however, will differ due to cross-flow conditions and the effect of CP; and

8. techniques developed and used to characterise the nature of foulants as well as their effects on the membrane performance and character provide some knowledge to aid with the development of new cleaning regimes. Variables described in this chapter will be used in future studies to evaluate the efficiency of cleaning regimes.

CHAPTER 6

BIOLOGICAL REMOVAL OF FOULANTS ADSORBED ONTO PSM DURING THE TREATMENT OF ABATTOIR EFFLUENT

6.1 INTRODUCTION

A major concern when using any UF membrane system, is to accomplish the required separation at an economically and acceptable flux rate. Both of these variables are severely affected by membrane fouling (Spencer and Thomas, 1991). Although membrane technology has recently made great advances, cleaning has been neglected to some extent despite the numerous difficulties that resulted from membrane fouling (Echner and Zottola, 1993). Studies related to fouling (5-30% flux reduction) and concentration polarisation (67-95% flux reduction), focused primarily on processes which would minimise fouling and produce a more economical and energy saving method of membrane separation (Sedath *et al.*, 1993 and Echner and Zottola, 1993). These methods, which concentrate on reducing fouling and not the actual cleaning of fouled membranes, include, optimisation of flow conditions, pretreatment of effluent, acceptable membranes properties (Spencer and Thomas 1991, Flemming, 1990, Fane and Fell, 1987 and Sedath *et al.*, 1993), optimisation of operational factors (Bauser, 1982) and high rinse water quality (Bragulla and Lintner, 1986).

In this chapter we will concentrate on the cleaning of membranes fouled within actual abattoir effluent. Cleaning could be describe as "a process whereby material is relieved of a substance which is not an integral part of that material". Membranes are regularly cleaned by a combination of the following methods: mechanical and/or (Croll, 1992 and Bragulla and Lintner, 1986), chemical (Trägårdh, 1989) and/or biological cleaning. Biological cleaning would include the use of biological agents which contain enzymes. The following types of cleaning agents are often used for cleaning UF membranes: Alkalis (Kim *et al.*, 1993, Bragulla and Lintner, 1986 and Bohner and Bradley, 1992), acids (Bragulla and Lintner, 1986), surface-active agents (Trägårdh, 1989 and Kim *et al.*, 1993), sequestering agents (Flemming, 1990), disinfectants (Flemming, 1990) and enzymes (Trägårdh, 1989, Coolbear *et al.*, 1992, Chen *et al.*, 1992, Howell and Velicangil, 1982 and Jacobs, 1991).

Enzymes, and enzyme detergent mixtures, are increasingly being incorporated into cleaning mixtures for household use as well as in industrial applications. Detergent or cleaning mixtures, that contain enzymes, however, have not been used on a large scale in cleaning regimes of biologically fouled UF membranes before. Enzymes are ideal cleaning agents for biologically fouled membranes as they are highly specific with regard to the reactions they catalyse and the substrates they interact with. Enzymes, that would be specific for the removal of foulants like proteins and lipids from UF membranes, can therefore be used in cleaning regimes for these substances. In addition enzymes act under mild conditions of pH, temperature and ionic strength and will not have a detrimental influence on the membrane surface. A number of factors have, up to now, prohibited the large-scale use of enzymes in membrane cleaning regimes. Enzymes are costly and to formulate them into effective detergents would require relative expensive research and development. Secondly there are concerns that residual enzyme activity can affect cultures such as the starters used in cheese making, which would have an adverse effect on cheese production. Extension of membrane technology into effluent treatment of biologically related processes, as well as mounting pressures on the environment, have compelled researchers to re-examine enzymes as possible cleaning agents for fouled UF membranes (Coolbear *et al.*, 1992, Chen *et al.*, 1992 and Jacobs *et al.*, 1991) or to prevent fouling by adsorption of enzymes onto membranes before fouling occurs (Chen *et al.*, 1992 and Howell and Velicangil, 1982).

In order to use enzymes as cleaning agents one must note that enzymes are highly specific towards substrates and therefore the nature of foulants should be known to make the right choice of enzyme(s). Fouling with actual abattoir process effluent was conducted. This anticipated some problems in choosing the right method to evaluate cleaning efficiency. Four variables were used to evaluate the efficiency of cleaning experiments including the determination of: the concentration of protein and lipid material (potential foulants in effluent) adsorbed onto membranes, the contact angle of the membrane with water and pure-water flux through the membranes. These variables provided us with sufficient information of the exact amount adsorbed foulants onto the membranes as well as the effect of the cleaning agent on membrane character and permeability.

6.2 EXPERIMENTAL

6.2.1 Materials

Enzymes, used in the cleaning experiments, were obtained from Sigma Chemical Co., St. Louis, USA, unless otherwise mentioned and include the following: Protease A (type XX111 from

Aspergillus oryzae, a serine protease with an optimal activity at pH 7,5 at 37 °C), protease B (type II: fungal, crude from *Aspergillus oryzae*, a serine protease with an optimal activity at pH 7,5 at 37 °C), trypsin (Protease C) (a pancreatic protease with an optimal enzymatic activity at pH 7,5 at 35,5 °C obtained from Merck Darmstadt, Germany) and lipase A (a crude porcine type II pancreatic lipase for the hydrolysis of triacylglycerides with an optimal activity at pH 7,5 and 37 °C). Commercial agents (Alkazyme® and Zymex®) were obtained from Syndachem Sales Ltd., Milnerton, South Africa. These are formulated cleaning agents with proteolytic activity. A 3% distilled aqueous solution of Alkazyme:Zymex (1:1) was used at room temperature according to manufacturers specifications. Detergents used were sodium dodecyl sulphate (SDS), 0,2% and 0.4% solutions in distilled water at 37 °C and Triton X100 (0,1% solution in distilled water).

Pancreatic lipases, that control the digestion of alimentary fats in the duodenum, show increased activity when adsorbed at the oil-water interface (Norde, 1986). Therefore, in addition to the abovementioned agents, lipase A (1mg/ml) was dissolved in a 0,1% Triton X100 Clark and Lubs solution (pH 7,5) to create such a oil-water interface. Other combinations between these agents were also evaluated.

Reagents and membranes, used in the determination of the cleaning efficiency, were according to previously described methods (see chapter 5).

6.2.2 Fouling of membranes

Membranes were fouled as previously discused in chapter 5 after which fouled membranes were washed thoroughly with tap water, placed in distilled water and preserved with sodium azide (5mg/l) at 4°C until cleaning experiments and analysis were carried out. All experiments were replicated four times and the values represented is the mathematical mean of the four values obtained ±SD.

6.2.3 Cleaning Methods

Fouled membranes were cut into 30 x 10 cm strips, washed in the Clark and Lubs buffer (pH 7,5), and incubated in a 600 ml solution containing the cleaning agent or mixture of cleaning agents. Cleaning agents were used at optimal conditions for each agent to ensure maximum efficiency. After cleaning the membranes were removed from the cleaning solutions, washed thoroughly with distilled water to remove all excess materials, and stored in distilled water at 4 °C until analysis.

6.2.4 Determination of cleaning efficiency

Cleaned membranes as well as reference membranes were analysed as described previously (see chapter 5). The following reference membranes were used; an 8 hour fouled membrane and a fouled membrane incubated with only the Clark and Lubs (pH 7,5) buffer for one hour.

6.3 RESULTS AND DISCUSSION

6.3.1 Optimisation of enzyme concentration and incubation time

Protease A as well as lipase A, selected for our studies, are enzymes with a relative broad spectrum of activity for proteins and triacylglyceride hydrolysis, respectively. Before these two enzymes could be used in cleaning mixtures, the optimal enzyme concentration and incubation period had to be established for the enzymes on their own, using lipid content, protein content, contact angle measurement and pure-water flux as variables for evaluation (chapter 5). As mentioned previously, lipase activity can be increased with the addition of Triton X100, It was, however, still necessary to determine the optimal enzyme loading and incubation time for this cleaning mixture. In addition, the optimal incubation time of the commercial agent, Alkazyme:Zymex, was also determined.

The effect of enzyme concentration and incubation time on the removal of proteins adsorbed onto PSM are shown in figures 6.1 and 6.2 respectively. The protein content of fouled membranes decreases with an increase in enzyme concentration incubated with protease A for 60 min, however, it was found that beyond a enzyme concentration of 3 mg/ml no further significant protein removal occurred. For the lipase A and the lipase A:Triton X100 mixture no significant protein removal after increasing the enzyme concentration to 2 or 3mg/ml was achieved. As expected, protease A proved to be more effective in protein removal than lipase A, however, lipase A combined with Triton X100 yielded better results. Maximum protein removal was obtained after 60 min incubation with lipase A and protease A. For the lipase A:Triton X100 mixture maximum protein removal occurred after 90 min. The removal with the enzyme detergent mixture was, however, significantly more effective than with the enzyme alone. Protein removal with the lipase A:Triton X100 mixture at 60 min was higher than that achieved with lipase A or protease A. After 90 minutes an increase in protein content was observed for lipase A alone and the protease (results not shown). This increase could be ascribed to re-adsorbtion of the cleaning enzymes onto the membrane surface in the place of the degraded foulants.

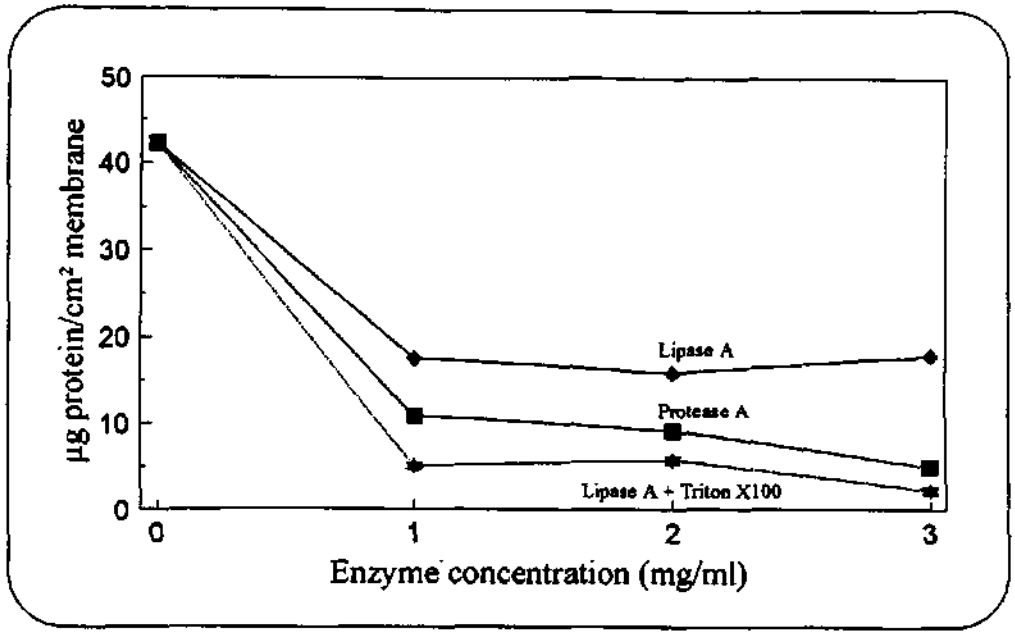

Fig. 6.1 Effect of enzyme concentration on protein removal from fouled PSM. Enzymes evaluated were: protease A, lipase A and a lipase A:(0,1%) Triton X100 mixture. Incubation time was 60 minutes, at pH 7,5 and 37 °C.

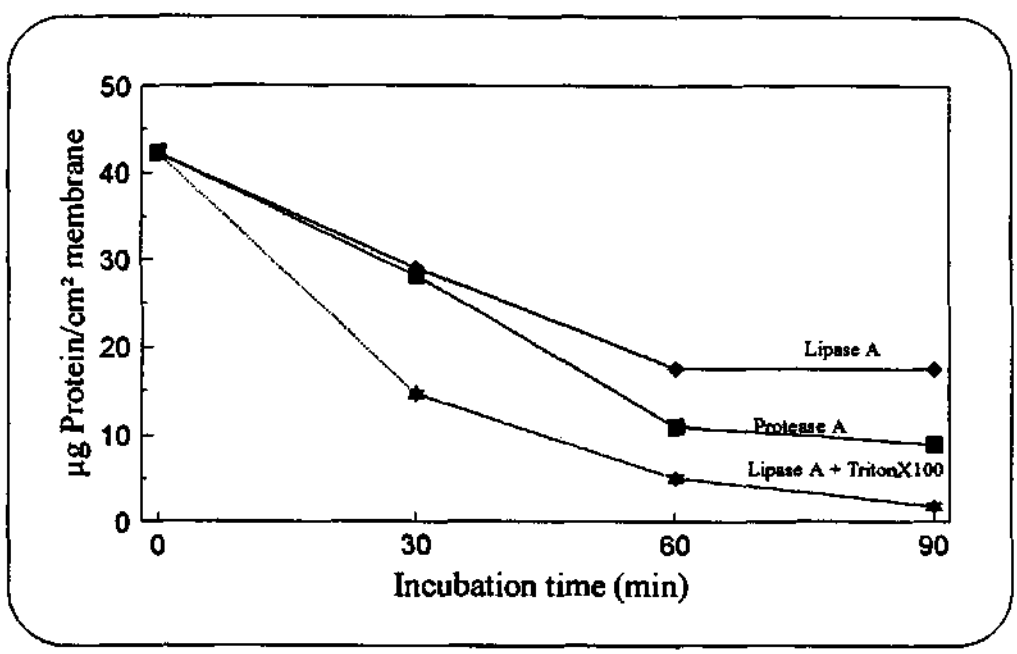

Fig. 6.2 Effect of incubation times on protein removal from fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100 mixture. Concentration used was 1mg/ml at pH 7,5 and 37 °C.

The effect of enzyme concentration and varying incubation times, of the different enzymatic cleaning agents, on the lipid content of fouled PSM are represented in figures 6.3 and 6.4, respectively. A decline in the amount of adsorbed lipid material was observed after an increase in

enzyme concentration for both the protease A and lipase A preparations. The protease A was, most unexpectedly, a better cleaning agent for lipid removal than the lipase A alone. In combination with Triton X100 (0,1%), lipase A, however, yielded optimal lipid removal at a 1mg/ml enzyme concentration. Lipid removal by protease A is most likely due to the disturbance of lipid-protein interactions on the membrane surface when proteins are degraded by this enzyme. After 60 min incubation with protease A the lipid content increased due to readsorption, most probably because the lipids were not degraded by the protease A but only liberated into the solution. Optimal lipid removal by lipase A alone was achieved after 90 minutes and no re-adsorbtion was observed. The lipase A:Triton X100 mixture achieved maximum lipid removal after 60 minutes and 40% of the adsorbed lipid material was removed compared to approximately 25% with the other two solutions. These results prove that lipase A, combined with Triton X100, not only lowers the enzyme concentration required, but also reduces the incubation time needed for more efficient cleaning, a fact that could be of great importance in the practical application of these types of cleaning protocols.

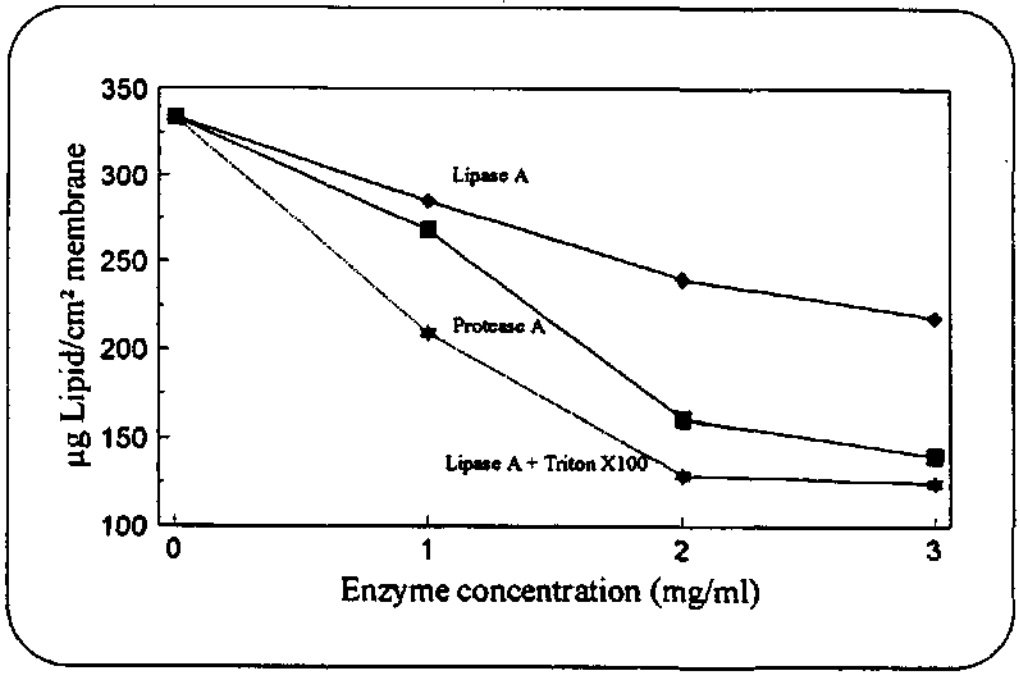

Fig. 6.3 Effect of enzyme concentration on lipid removal from fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100 mixture. Incubation times were 60 minutes at pH 7,5 and 37 °C.

The effect of incubation time, of the commercial cleaning agent (Alkazyme:Zymex), on the protein and lipid removal is presented in Fig. 6.5. Longer incubation periods with the commercial cleaning agents, resulted in more effective lipid and protein removal for fouled membranes. After 60 min

most of the foulants were removed and no significant reduction occurred for periods up tp as long as 8 hours of incubation.

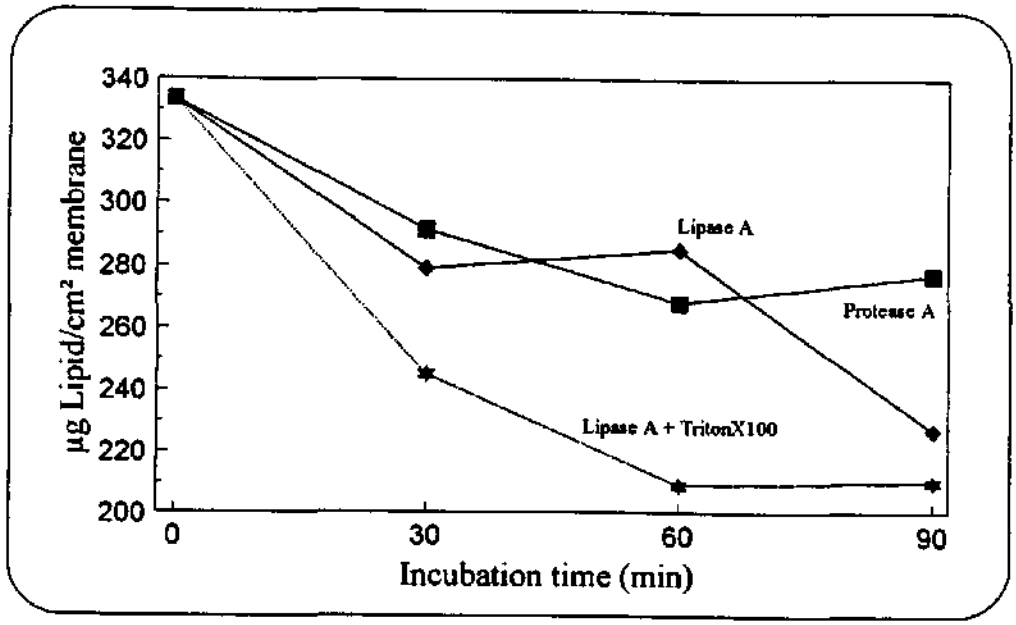

Fig. 6.4 Effect of enzyme incubation time on the lipid removal of fouled PSM. Enzyme evaluated were: protease A, Lipase A and lipase A:(0,1%) Triton X100 mixture. Concentrations used were 1mg/ml at pH 7,5 and 37 °C.

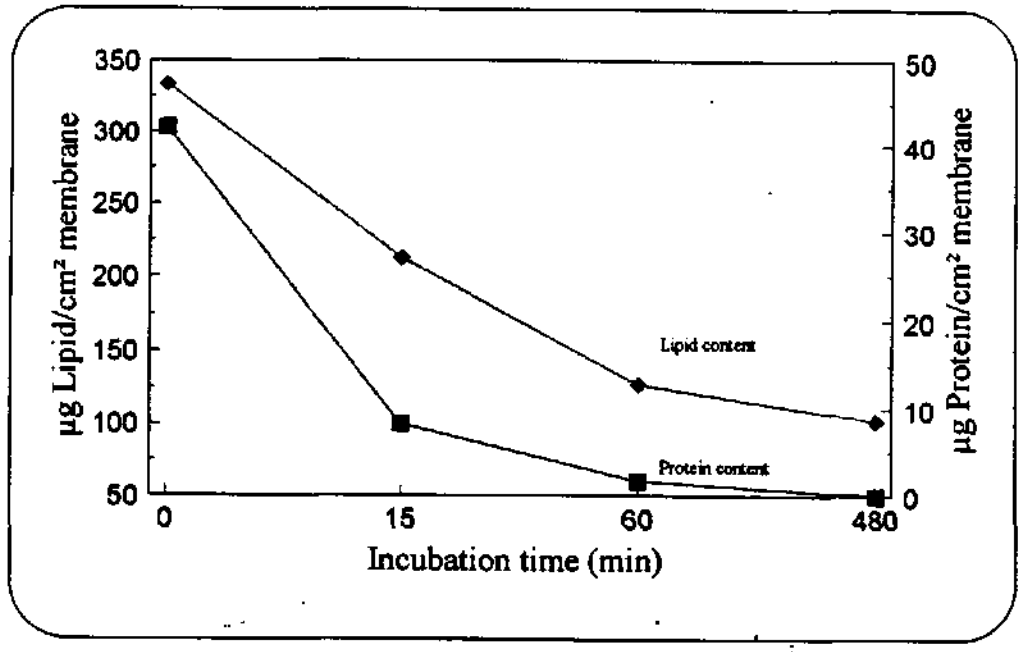

Fig. 6.5 Effect of incubation time of a commercial cleaning agent on the lipid and protein removal of fouled PSM. A 3% solution of Alkazyme:Zymex (1:1) were used as prescribed by the manufacturers.

The effect of enzyme concentration on the contact angle measurements of PSM, fouled in abattoir effluent, is shown in Fig. 6.6 and the effect of incubation time on the same parameter is summarised

in Fig. 6.7, An increase in the lipase A and protease A concentrations produced lower contact angle values which corresponded well with the decrease in lipid content observed for the same treatment (see Fig. 6.3). With the lipase A detergent mixture the contact angle decreased to 38.81 degrees. No further significant decrease in the contact angle was observed with an increase in the enzyme concentration. The effect of incubation times of cleaning agents on the contact angle reveals the same pattern as lipid removal from fouled membranes. With protease A, a significant increase in the contact angle between 60 and 90 min of incubation time was observed, possibly due to the re-adsorption of lipid material to the membrane.

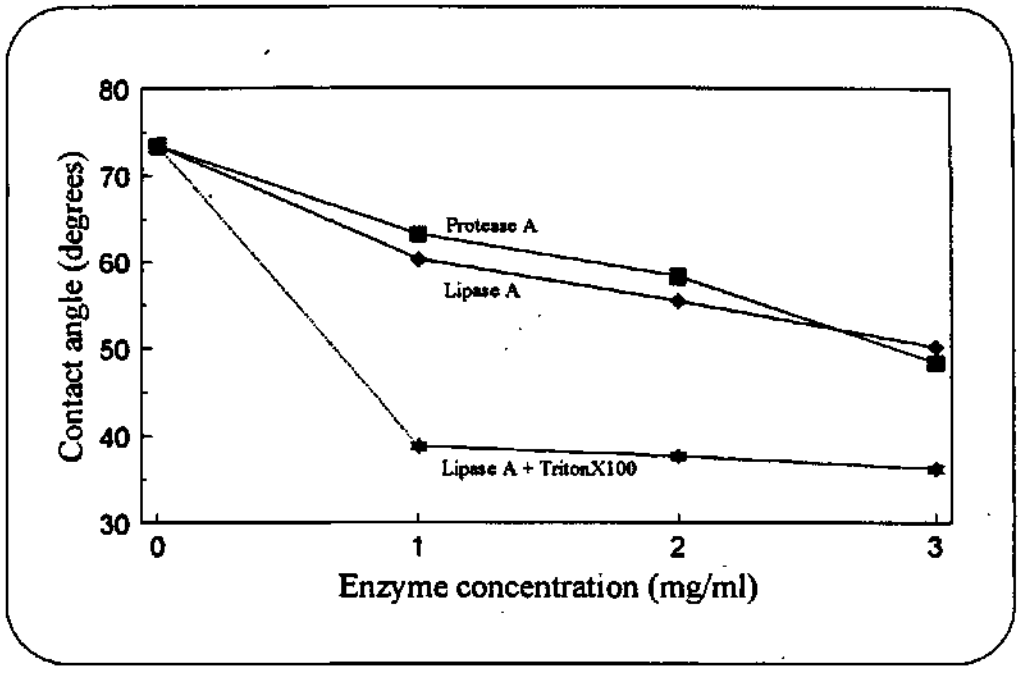

Fig. 6.6 Effect of enzyme concentration on the contact angle of fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100, Incubation time was 60 min at pH 7,5 and 37 °C.

The pure-water flux through fouled PSM, treated with different concentrations of protease A and lipase A, is shown in Fig. 6.8. The pure-water flux showed the expected reverse pattern observed with the lipid removal and contact angle measurements. The Lipase A detergent mixture achieved the best overall flux improvement. Although maximum flux recovery, by all cleaning agents, was achieved at 3mg/ml enzyme concentration, this value was not significantly higher than the increases achieved with a 1mg/ml solution. The cleaning agent that could remove the greatest amount of adsorbed lipid material also yielded the best flux recovery (lipaseA:Triton X100).

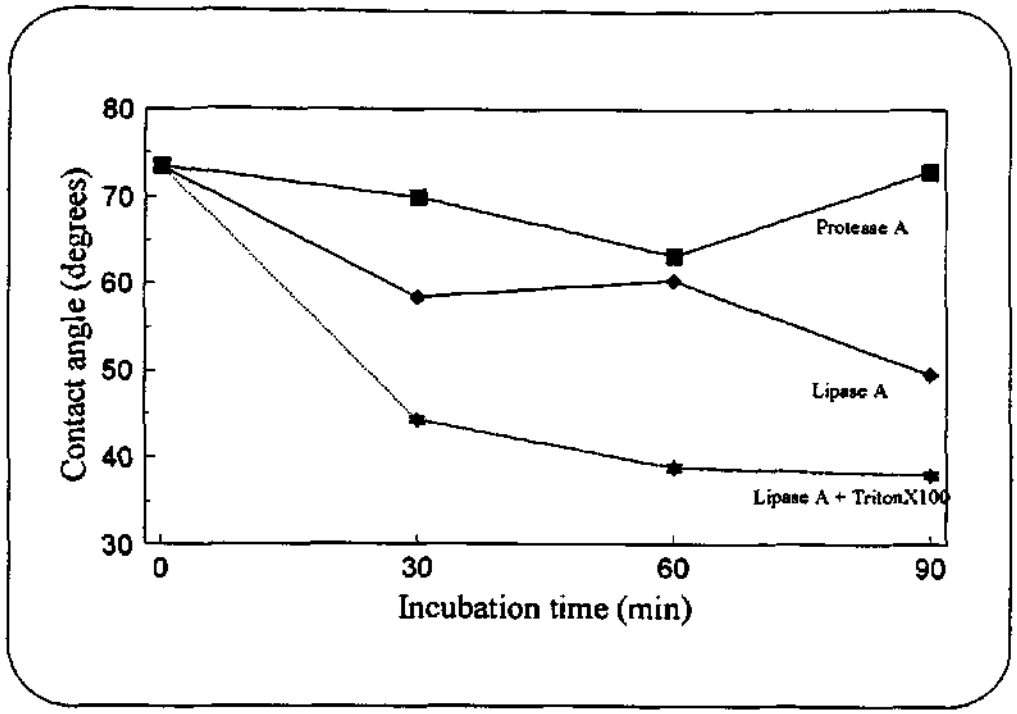

Fig. 6.7 Effect of enzyme incubation time on the contact angle of fouled PSM. Enzymes evaluated were: protease A, lipase A and lipase A:(0,1%) Triton X100, Concentration used was 1mg/ml at pH 7,5 and 37 °C.

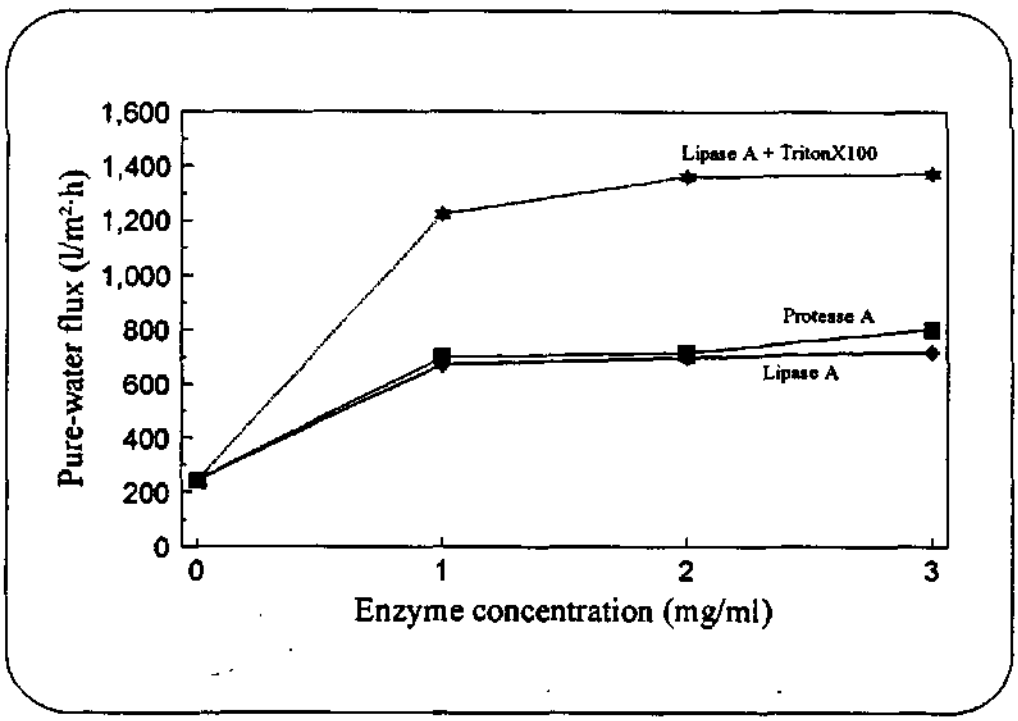

Fig. 6.8 Effect of enzyme concentration on the pure-water flux of fouled PSM. Enzymes evaluated were protease A, lipase A and lipase A:(0,1%) Triton X100, Incubation time was 60 min at pH 7,5 and 37 °C.

The effect of incubation time on the pure-water flux through fouled PSM is shown in Fig. 6.9. A rapid flux increase was obtained after only 30 min of incubation with the different agents after which no significant flux increase could be obtained. For protease A, in fact, the flux decreased slightly between 60 and 90 min incubation corresponding to the possible readsorption of lipids as seen in Fig. 6.4.

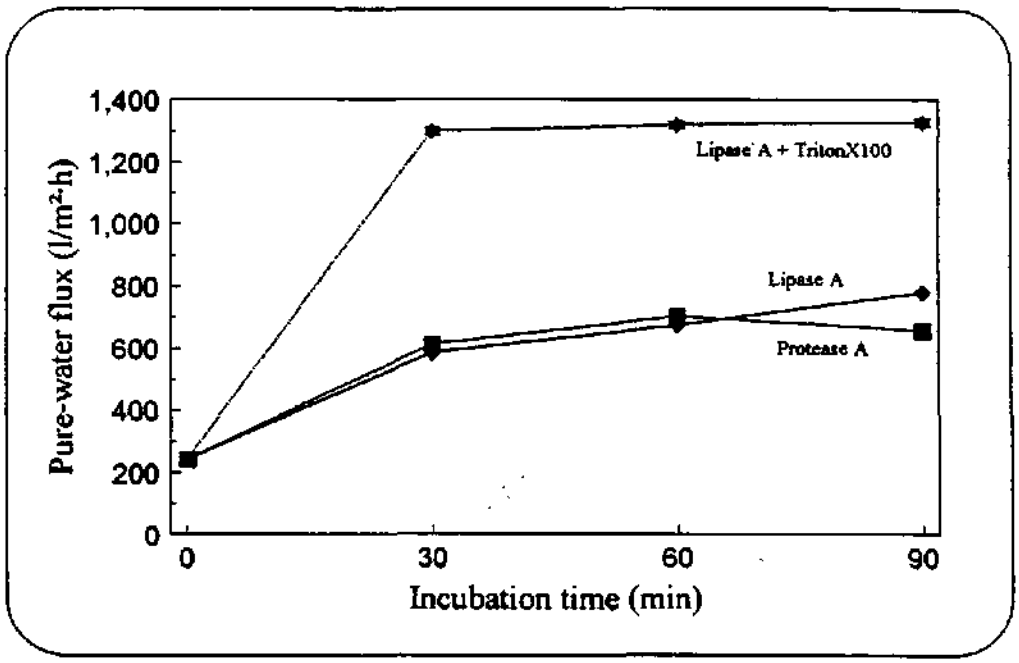

Fig. 6.9 Eeffect of enzyme incubation time on the pure-water flux of fouled PSM. Enzymes evalueted were: protease A, lipase A and lipase A:(0,1%) Triton X100, Enzyme concentrations were 1mg/ml at pH 7,5 and 37 °C.

In Fig. 6.10 the effect of incubation time of the commercial cleaning agent (Alkazyme:Zymex) on the contact angle and pure-water flux is shown. A decrease in the contact angle with a concomitant increase in pure-water flux was observed that corresponded well with the data obtained with the enzyme preparations.

6.3.2 A comparison of various cleaning regimes at optimal conditions

Experiments carried out to optimise the enzyme concentration and incubation time for protease A, lipase A and the lipase A:Triton X100 mixture in membrane-cleaning regimes, showed that an incubation period of 60 min (to prevent readsorption) with an enzyme concentration of 3 mg/ml would be suitable. A number of cleaning mixtures were subsequently evaluated using the variables as described in chapter 5. In Fig. 6.11 the effect of different cleaning agents on the protein content of fouled PSM is shown. The different mixtures used are given in the legend to the figure.

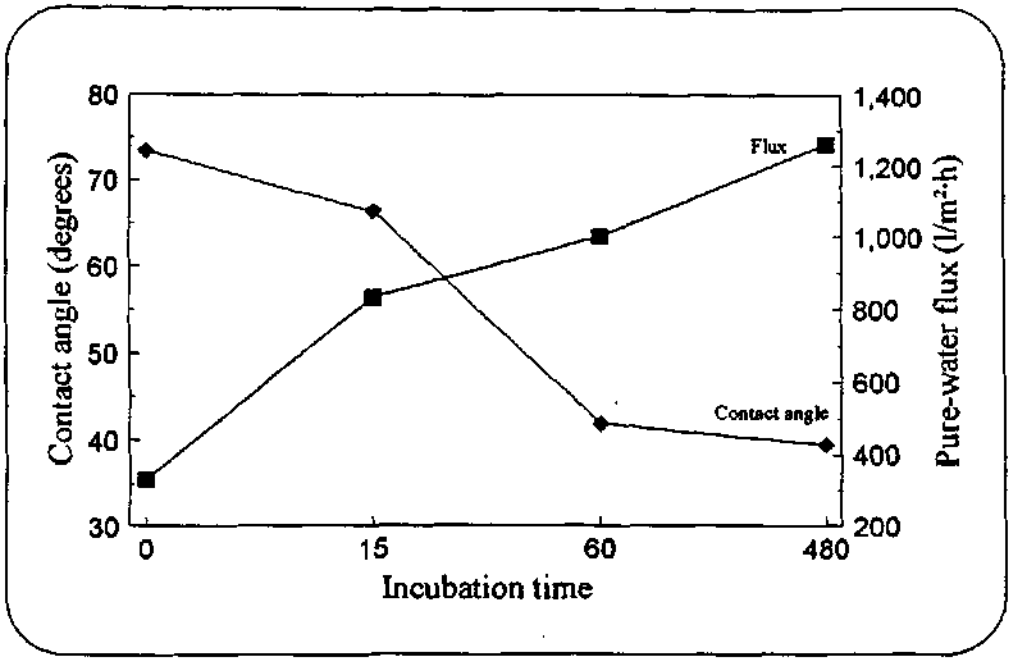

Fig. 6.10 Effect of the incubation time of Alkazyme:Zymex (1:1, 3% solution) on the contact angle and pure-water flux of fouled PSM. Conditions were as prescribed by the manufacturers

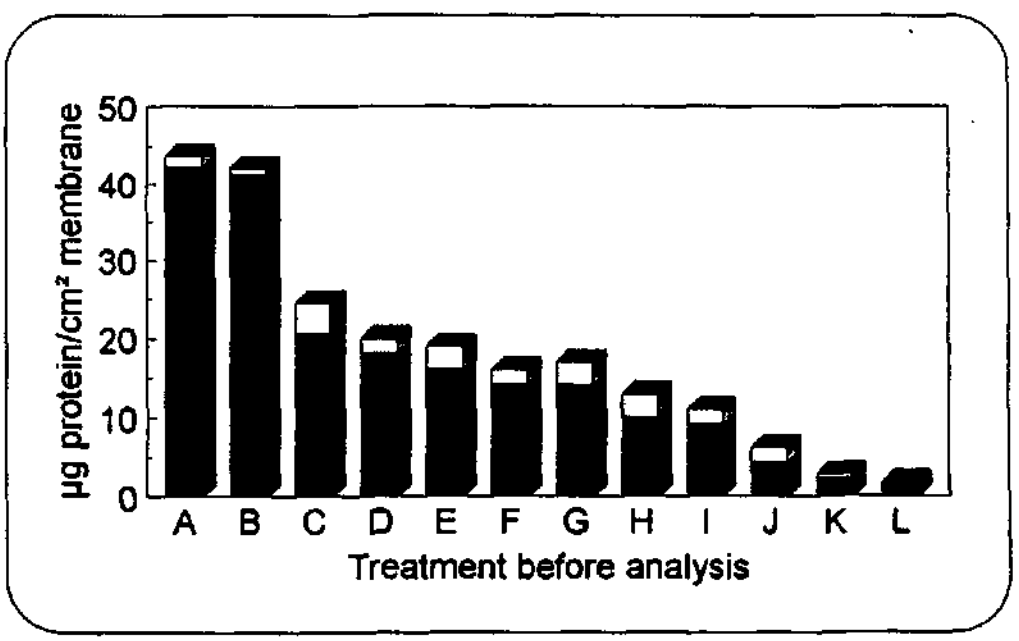

Fig. 6.11 Protein content of abattoir fouled PSM after treatment with different cleaning mixtures. Membranes, fouled for 8 hours, were treated as follows: **[A]** No treatment; **[B]** Buffer, 60 min; **[C]** SDS (0,2%), 60 min; **[D]** Lipase A (3mg/ml), 60 min; **[E]** Protease B (3 mg/ml), 60 min; **[F]** Protease C (3mg/ml), 60 min; **[G]** SDS (0,4%), 60 min; **[H]** Triton X100 (0,1%), 60 min; **[I]** Protease A (3mg/ml), 60 min; **[J]** Lipase A:Triton X100 (1mg/ml:0,1%), 60 min followed by treatment with Protease A (1mg/ml), 60 min; **[K]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[L]** Alkazyme:Zymex (1:1) 3% solution, 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD.

It is interesting to note that treatment with the lipase A:Triton X100 mixture, was also very effective for the removal of proteins. This result indicates that removal of certain lipids enhances the desorption of proteins and that proteins adsorb onto membranes mainly through their interaction with fouling lipids. It was previously shown that, although proteins are more abundant in abattoir effluent, significantly more lipid material adsorb onto statically fouled membranes, due to the extreme hydrophobic character of PSM. Lipid adsorption typically occurred preferentially in the initial stages of the fouling process (250μg/cm²) while protein adsorption was significantly slower (Chapter 5).

Results obtained from lipid analysis of membranes fouled in abattoir effluent before and after treatment with various cleaning agents, are summarised in Fig. 6.12. The different mixtures used are given in the legend to the figure. The values range from 333,75 μg lipid/cm² after 8 hours of fouling to 110 μg lipid/cm² membrane area for membranes treated with a lipase A:Triton X100 1mg/ml:0,1% solution for 60 min followed by treatment with protease A (1mg/ml) for 60 min.

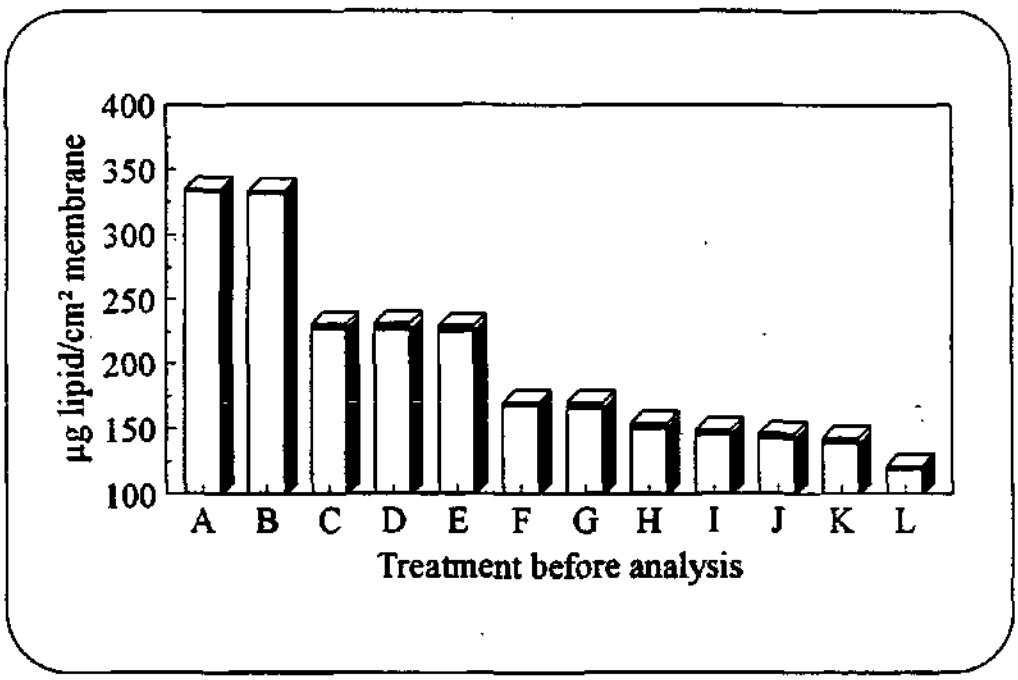

Fig. 6.12 Lipid content of abattoir fouled PSM after treatment with different cleaning mixtures. Membranes, fouled for 8 hours, were treated as follows: **[A]** No treatment; **[B]** Buffer, 60 min; **[C]** Lipase A (3mg/ml), 60 min; **[D]** Protease B (3 mg/ml), 60 min; **[E]** Triton X100 (0,1%), 60 min; **[F]** Protease C (3mg/ml), 60 min; **[G]** SDS (0,2%), 60 min; **[H]** Protease A (3mg/ml), 60 min; **[I]** SDS (0,4%), 60 min; **[J]** Alkazyme:Zymex (1:1) 3% solution, 60 min; **[K]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[L]** Lipase A:Triton X100 (1mg/ml:0,1%), 60 min followed by treatment with Protease A (1mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD.

Not one of the cleaning mixtures could remove more than approximately 60% of the lipid material adsorbed onto the membrane during the eight hour fouling period. It is interesting to note that when lipase A was used as a cleaning agent on its own, it was less effective in removing lipid than after the addition of 0,1% Triton X100. This improvement in lipid removal may be attributed to the enhanced activity of lipases in general, when these enzymes are presented with an organic-aqueous interface. When detergents like SDS or Triton X100 were used alone, less lipid removal was achieved than in conjunction with enzymes, a phenomenon also observed by Coolbear *et al.*, (1992). It must be noted that detergents were not used in conjunction with protease A, as detergents inhibit the activity of these proteolytic enzymes. The effect of different cleaning mixtures on the contact angle of fouled PSM is given in Fig. 6.13. The different mixtures used are given in the legend to the figure. Criteria like lipid and protein content, as well as contact angle measurements, are extremely useful and valuable variables to quantitate fouling, determine the nature of foulants and evaluate cleaning regimes for PSM used in biologically related processes.

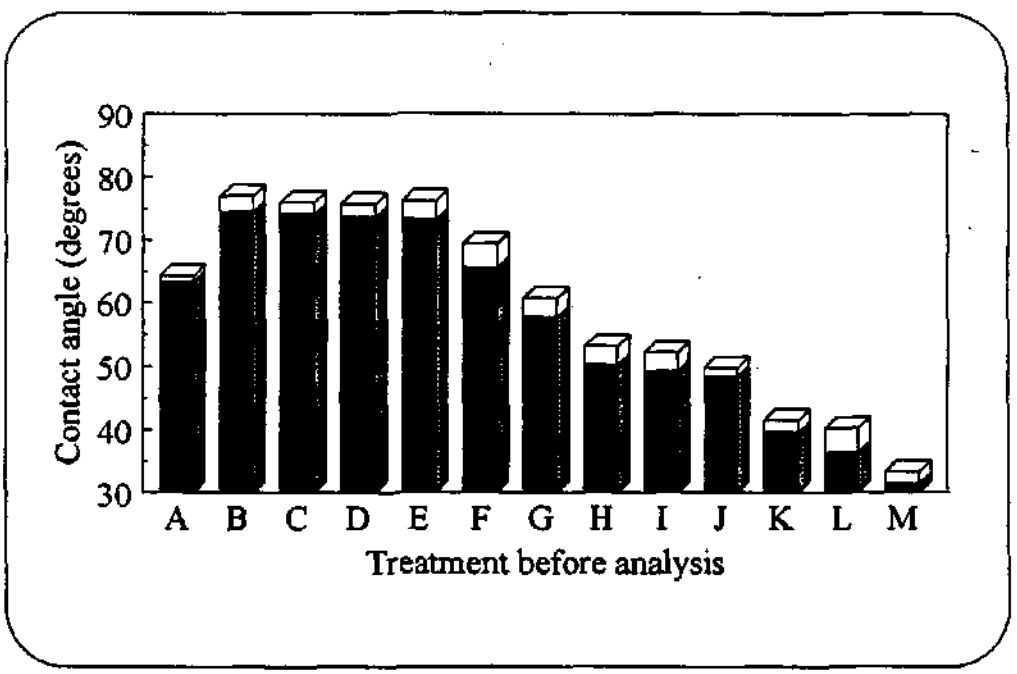

Fig. 6.13 Contact angle of fouled PSM after treatment with different cleaning mixtures. Membranes fouled for 8 hours, were treated as follows: **[A]** Unfouled membrane; **[B]** Fouled for 8 hours; **[C]** Buffer, 60 min; **[D]** Protease B (3mg/ml), 60 min; **[E]** SDS (0,2%), 60 min; **[F]** SDS(0,4%), 60 min; **[G]** Protease C (3mg/ml), 60 min; **[H]** Lipase A (3mg/ml), 60 min; **[I]** Triton X100 (0,1%), 60 min; **[J]** Protease A (3mg/ml), 60 min; **[K]** Alkazyme:Zymex (1:1) 3% solution, 60 min; **[L]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[M]** Lipase A:Triton X100 (1mg/ml:0,1%), 60 min followed by treatment with Protease A (1mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD.

Flux recovery, however, is the ultimate test for the assessment of cleaning techniques. Of the cleaning mixtures used in this study the commercially available preparation (Alkazyme:Zymex) and the lipase A:Triton X100 mixture alone, and in conjunction with protease A, gave the best flux recovery (see Fig. 6.14).

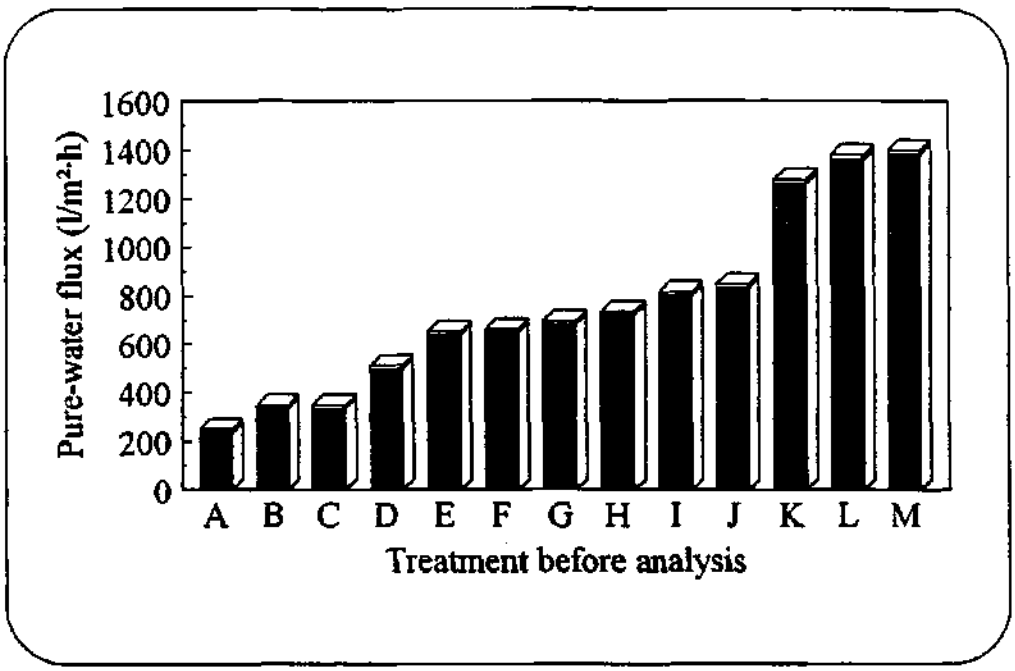

Fig. 6.14 Pure-water flux through fouled PSM after treatment with different cleaning mixtures. Membranes were fouled for 8 hours and treated as follows: **[A]** Fouled for 8 hours; **[B]** Buffer, 60 min; **[C]** SDS (0,2%), 60 min; **[D]** SDS (0,4%), 60 min; **[E]** Protease B (3mg/ml), 60 min; **[F]** Protease C (3mg/ml), 60 min; **[G]** Triton X100 (0,1%), 60 min; **[H]** Lipase A (3mg/ml), 60 min; **[I]** Protease A (3mg/ml), 60 min; **[J]** Unfouled membrane; **[K]** Alkazyme:Zymex (1:1) 3% solution, 60 min; **[L]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[M]** Lipase A:Triton X100 (1mg/ml:0,1%), 60 min followed by treatment with Protease A (1mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD.

The flux recoveries for these three cleaning mixtures were all significantly higher than 100% when compared to the flux through a new unused membrane. This phenomenon could be explained by the fact that all the membranes, where this higher flux was observed, were treated with surface active agents such as Triton X100, The surface active agents cause swelling of the organic material adsorbed onto the membrane as well as the membrane self, thus leading to higher fluxes. To prove this phenomenon, a clean unused membrane was coated with the nonionic surfactant Triton X100 and the pure-water flux and contact angle was subsequently determined. A flux average of 1118,4 l/m^2h and contact angle of 38,88 °, for the unused membrane treated with Triton X100, was obtained. Similar results, of higher initial fluxes, were obtained by Fane *et al.*, (1985) and Chen *et*

al., (1992) after treatment with nonionic surfactants. Chen *et al.*, (1985) explains the higher initial flux as the way in which the surfactant apparently modifies the surface morphology of the membrane. The reason why the flux is not as high as those membranes that were fouled and cleaned, with surfactant containing agents, could be explained by the fact that the contact angles observed in theses cases are lower and membranes are more hydrophilic of nature. The more hydrophilic membranes resulted in lower hydrodynamic resistance and higher fluxes.

It is important to note that, although the best cleaning methods used in this study, did not remove all the lipids, the contact angle for these treated membranes were below that of the unused control. This phenomenon may be attributed to the fact that lipids penetrated the membrane pores as well as the support medium and could not be reached by the higher molecular mass enzyme molecules.

6.4 CONCLUSIONS

For the lipase A:Triton X100 mixture used alone and in conjunction with protease A as well as the Alkazyme:Zymex, the decrease in lipid content correlates well with a decrease in the contact angle. Protein content did not seem to have the same relation to pure-water flux and, as far as abattoir effluent is concerned, it may be deduced that lipid content and contact angle measurements are more reliable variables for the quantification of fouling and the assessment of cleaning regimes. This is understandable if one compares the amount of lipids and proteins adsorbed onto the membranes during the fouling period. Therefore, development of cleaning agents, used for cleaning membranes fouled in abattoir effluent, should concentrate on lipid removal. From the comparison between enzymatic and chemical cleaning agents it is clear that enzymes are the most effective for abattoir effluent fouled membranes. The reason for this is that chemical cleaning agents have a wide range of non-specific applications, while enzymes were chosen specifically for the removal of specific foulants. The combination of noni-onic surfactants could be used to improve initial fluxes and further research is needed to determine the effect, of no-nionic surfactants, on fouling and cleaning..

CHAPTER 7

THE COMPOSITION OF EFFLUENT PRODUCED DURING THE WOOL SCOURING PROCESS AND THE USE OF ENZYME BASED CLEANING REGIMES FOR THE REMOVAL OF FOULANTS FROM POLYSULFONE MEMBRANES FOULED IN WOOL SCOURING EFFLUENT

7.1 INTRODUCTION

Before raw wool can be carded and spun into yarn it has to be cleaned (scoured) from its natural impurities. Scouring is normally carried out by means of an aqueous process using hot water together with small amounts of sodium carbonate and non-ionic detergent (Turpie *et al.*, 1992, Monteverdi *et al.*, 1992). Turpie *et al.*, (1992) reported that, to obtain 1 kg of clean merino wool, 10 litres of water is required. In South Africa, in the order of 50 million kg of wool fibre is scoured per season (Mozes *et al.*, 1981, Pearson *et al.*, 1976). A highly polluting, dark slurry effluent, with an obnoxious odour and complex composition, is produced (Oellermann *et al.*, 1992). The effluent produced by the woolscouring industry comprises approximately 7 million kg grease (melting point about 42 °C), 4,7 million kg suint (the water-soluble secretion of the sudoriferous gland of the sheep), 10,5 million kg sand and clay and 1,2 million kg of other contaminants in a total volume of 0,5 million m^3 of effluent (Mozes *et al.*, 1981 and Pearson *et al.*, 1976). Typically, the effluent with a pH between 7 and 10, contains 10-25 g/l wool grease, 0,1-1 g/l detergent, 7-15 g/l suint and 10-30 g/l sand (Townsend *et al.*, 1992 and Pearson *et al.*, 1976). Further analysis of WSE indicate COD values of between 3 and 150 g/l and biological oxygen demand (BOD) values of between 3 and 25 g/l (Oellermann *et al.*, 1992, Rindone *et al.*, 1991 and Pearson *et al.*, 1976).

WSE contains considerable amounts of complex lipid compounds (10-25 g/l wool grease), composed primarily of long-chain fatty acids, glycerol, glycerides, steroids, wax and steryl esters of often branched and hydroxylated long-chain fatty acids (Monteverdi *et al.*, 1992). The amount of these lipids further vary according to the breed, nutritional status, and origin of the sheep from which the wool was obtained and scoured (Brahimi-horn *et al.*, 1991). Extensive GLC-MS analysis of WSE was conducted by Rindone *et al.*, (1991) and Monteverdi *et al.*, (1992). Results from GLC-MS analysis, after solvent extraction and diazomethane derivatisation, show the presence of several even carbon atom unbranched saturated aliphatic fatty acids containing from 14 to 28 carbons. Several branched chain saturated fatty acids were also found. These include C-15, C-16, C-17 and C-18 carbon chains with a branch at C-(ω-1), and C-14 and C-16 carbon chains with a

branch at C-(ω-2). The C-18 unsaturated acid was also present. Some straight chain even carbon alcohols such as C-22, C-26 and C-27 compounds were also found. The major steroid component in WSE was cholesterol, its methyl ether and two cholestadines. Hexadecanoic acid (C-16), eicosanoic acid (C-20) and tetracosanoic acid (C-24) occur in the highest concentration (Monteverdi *et al.*, 1992).

The conventional disposal of WSE, up to now, has been mainly by solar evaporation or direct discharge. Both these methods, however, have become unacceptable due to environmental considerations. The treatment of fatty wastewaters is a difficult technological and environmental problem, because the concentration of these effluents is fairly high and it contains significant quantities of suspended solids, organic compounds and grease which are difficult to degrade by micro-organisms (Rindone *et al.*, 1991 and Chao and Yang, 1981). The major problems reside with the residual lanolin (grease) and the detergents used in the initial scouring process. This combination produces a relatively stable emulsion (Oellermann *et al.*, 1992) which cannot be treated to the required effluent discharge standards by a single stage biological process and therefore multistage treatments are required (Rindone *et al.*, 1991 and Chao and Yang, 1981). Multistage treatment can, however, often be expensive and difficult to accomplish in practice (Mozes *et al.*, 1981).

There are various possible ways in which WSE can be treated. These methods include: chemical flocculation, evaporation, incineration or biological treatment, aerobic lagooning activated sludge, a combination of anaerobic and aerobic biological treatments and enzymatic pretreatment followed by anaerobic digestion (Mozes *et al.*, 1981, Rindone *et al.*, 1991 and Pearson *et al.*, 1976). In most cases these treatment methods were combined in one way or another to yield the desired result.

An important consideration in the treatment of WSE is the use of UF membranes. The advantages of UF for the treatment of biological effluents have been discussed earlier. Initial research using conventional UF membranes to treat the grease effluent, however, showed that this process was unsuitable for WSE treatment as the characteristics of the effluent is of such a nature that permanent damage and fouling of the membrane occurred within a very short space of time. Membrane performance was reduced due to physical damage and irreversible adsorption of foulants to the surface of the membrane (Townsend *et al.*, 1992). Hogetsu *et al.*, (1992) found that fouling of hollow fibre polyacrylonitrile membranes occurred mainly by the deposition of grease scales on the inner surface of the membranes.

Turpie *et al.*, (1992) treated wastewater from a wool scouring industry by means of dynamic formed-in-place hydrous zirconium (IV) oxide UF membranes in a batch-operated process. WSE from the first scouring bowls were directly filtered through these membranes. The high levels and nature of contaminants, removed from raw wool in the early stages of scouring, made membrane treatment of this early stage effluent difficult and a permeate of poor quality was obtained. A switch to the membrane treatment of a later stage dirty rinse water, proved to be more successful. Most of the colour, all of the dirt and two thirds of the remaining contaminants were removed. A reduction of grease (66%), suint (67%), COD (89%) and detergent (6%) was achieved. Frequent stripping and reforming of the membranes were, however, required (every 10 working days) in order to maintain an acceptable flux. Due to the high frequency of membrane regeneration, the process was automated. Membrane treatment of WSE could, however, not be optimised due to the extensive fouling.

With the experience and information gained from the abattoir study, trials were conducted to obtain information on the nature and composition of WSE using the variables described earlier. The fouling effect of WSE on flatsheet PSM and the use of different cleaning regimes were investigated. The feasibility of cleaning techniques were evaluated according to the methods developed for the abattoir industry.

7.2 EXPERIMENTAL

7.2.1 Materials

The following cleaning agents were used:

Enzymes used are all obtained from Sigma Chemical Co., St. Louise, USA, unless otherwise mentioned and include: protease A (type XX111 from *Aspergillus oryzae*, a serine protease with a optimal activity at pH 7,5 at 37 °C), protease B (type II: fungal, crude from *Aspergillus oryzae*, a serine protease with a optimal activity at pH 7,5 at 37 °C), trypsin (protease C) (pancreatic protease with an optimal enzymatic activity at pH 7,5 at 35,5 °C obtained from Merck Darmstadt, Germany), lipase A (crude porcine pancreatic lipase type II, a lipase for the hydrolysis of triacylglycerol with an optimal activity at pH 7,5 at 37 °C) and esterase A (from hog liver EC 3.1.1.1, a carboxylic-ester hydrolase with optimal activity at pH 7,5 obtained from Boeringer Mannheim Gmbh, Mannheim Germany).

Commercial agents used, were obtained from Syndachem Sales Ltd. Milnerton South Africa (Alkazyme® and Zymex®), are formulated cleaning agents with proteolytic activity. A 3% distilled water solution of Alkazyme:Zymex (1:1) was used at room temperature according to manufacturers specifications.

Detergents include SDS (0,2% and 0,4% solutions in distilled water at 37 °C) and Triton X100 (0,1% solution in distilled water).

Mixtures of enzyme solutions and other agents are: lipase A combined with Triton X100 to create an oil-water interface. Other combinations between these agents were also evaluated as indicated in the subheadings of the figures.

To obtain the adequate pH for each enzyme the enzymes were dissolved in a Clark and Lubs buffer prepared from KH_2PO_4 and NaOH obtained from BHD chemicals Ltd Poole England. (Bower and Bates, 1955). Reagents and membranes used in the determination of the cleaning efficiency are according to the specifications in Chapter 5.

7.2.2 Effluent analysis

Values presented are the mathematical mean of at least four determinations ±SD or ±SE as indicated. Effluent was collected at three different stages in the discharging system and includes: the first stage wash water from the scouring bowls (effluent 1, a high polluted dark slurry), the second stage wash water (effluent 2, water currently treated by dynamic formed-in-place hydrous zirconium (IV) oxide membranes) and the permeate of the filtration plant in operation. Effluent samples were preserved with (5 mg/l) sodium azide at 4 °C.

7.2.3 Fouling

Flatsheet PSM, cast from Udel P3500 polysulphone in N,methyl, 2-piridine, obtained from the Institute for Polymer Science, University of Stellenbosch, were adhered to perspex frames and submerged in the second stage effluent (in the feeding tank of the zirconium (IV) oxide membranes) for a period of 24 hours (see Fig. 5.1). Fouled membranes were removed and preserved in (5 mg/l) sodium azide at 4°C until cleaning experiments and analysis.

7.2.4 Cleaning

Fouled membranes were cut into 30 x 10 cm strips, washed in a Clark and Lubs buffer (pH 7,5) and incubated in a 600 ml solution of the appropriate cleaning agent. After the cleaning, the membranes were removed from the cleaning solutions, washed thoroughly with distilled water to remove all excess agents and stored in distilled water at 4 °C until analysis.

7.2.5 Determination of cleaning efficiency

Treated and reference membranes (which included an unused membrane, a 24 hour fouled membrane and a membrane that was incubated in Clark and Lubs buffer (pH 7,5) for 60 min) were analysed according to specifications in chapter 5.

7.3 RESULTS AND DISCUSSION

7.3.1 Effluent composition

The composition of these effluent samples are presented in Table 7,1. The lipid and protein content and pure-water flux was determined as previously described.

Tab. 7.1 Characteristics of wool scouring effluent, according to the protein, lipid contents, as well as the temperature and pH. Effluent 1 contains water from the first scouring bowls while effluent 2 contains the later stage scouring water. Effluent 2 is treated with dynamic formed-in-place hydrous zirconium (IV) oxide UF membranes to produce the premeate. Values presented are the mathematical mean ±SD.

	Protein µg/ml effluent	Lipid mg/ml effluent	Temperature °C	pH
Effluent 1	356 ± 102	6,23 ± 1,12	60 ± 5,6	10,04 ± 1,09
Effluent 2	140 ± 11	1,18 ± 0,98	40 ± 1,5	7,89 ± 0,75
Permeate	0	0,56 ± 0,12	39 ± 2,5	8 ± 0,82

7.3.2 Protein content

Fig. 7,1 summarises data obtained from protein determinations of PSM fouled in WSE and subsequently subjected to the different cleaning mixtures as described in the legend to the figure. The commercially available product Alkazyme:Zymex, SDS and the combination between lipaseA:Triton followed by protease A removed most of the fouling proteins from the membranes.

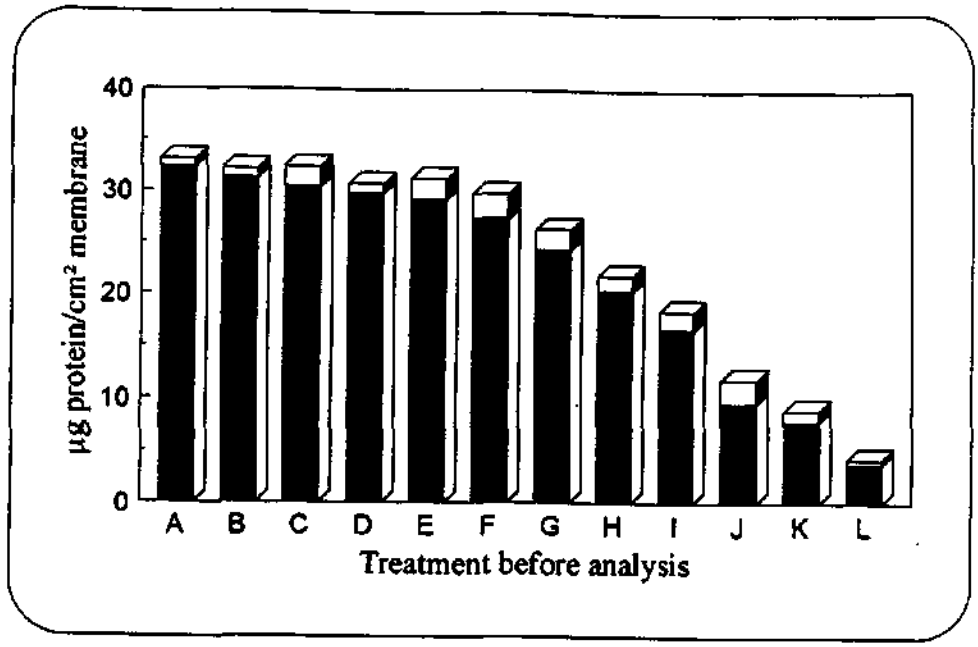

Fig. 7.1 Protein content of PSM fouled in WSE. **[A]** Fouled for 24 hours; **[B]** Buffer 60 min; **[C]** Protease B (3mg/ml), 60 min; **[D]** Protease A (3mg/ml), 60 min; **[E]** Lipase A (3mg/ml), 60 min ; **[F]** Protease C (3mg/ml), 60 min; **[G]** Esterase A (3mg/ml), 60 min; **[H]** 0,1% Triton X100, 60 min, 37 °C; **[I]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min;**[J]** SDS (0,4%), 37 °C, 60 min; **[K]** Alkazyme:Zymex (1:1), 3%, 60 min; **[L]** Lipase A:Triton X100 (1mg/ml:0,2%), 60 min followed by treatment with Protease A (1mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD.

7.3.3 Lipid content

The lipid content of PSM fouled in WSE, after treatment with various cleaning mixtures, is given in Fig. 7,2. The different cleaning regimes used is given in the legend to the figure. It is important to note that, unlike the membranes fouled in abattoir effluent where a 66% reduction in lipid content could be achieved, the lipid content of the WSE fouled membranes could only be reduced by approximately 25% by the same cleaning mixtures. This indicates that the lipids deposited on the WSE fouled membranes are of a different nature than those found in abattoir effluent. Therefore different enzymes are necessary to treat these foulants. The inability of lipase solutions to remove most of the lipids deposited on the membrane surface, can most probably be ascribed to the fact that lanolin is a major component of the lipids in WSE. Lanolin is a wax rather than a triacylglycerol or phospholipid. When esterase A, an enzyme with greater specificity towards ester bonds was used, the adsorbed wax content decreased from 175 to 51 µg/cm² membrane area. This phenomenon emphasised the importance of analysing the nature of foulants adsorbed onto membranes and to subsequently choose an enzyme with specificity towards such a substance. Most

materials of biological origin are biodegradable and specific enzymes that would hydrolyse or breakdown these substances to their simplest form can be found in most cases.

7.3.4 Contact angle measurements

All the contact angle values were above 90 degrees, except for the unused membranes (63°), those treated with esterase A (32.31°) and lipase A:Triton X100 mixture followed by protease A (84°) (data not shown). It seems thus that after fouling, the membrane surface was covered with wax (lanolin) that changes the membrane surface characteristics to a totally hydrophobic nature as revealed by the contact angle measurements. Most of the cleaning experiments, however, did not succeed in removing enough of this wax to produce lower contact angles. Those agents that could remove part of the wax covering the membrane surface, subsequently gave membranes with lower contact angles.

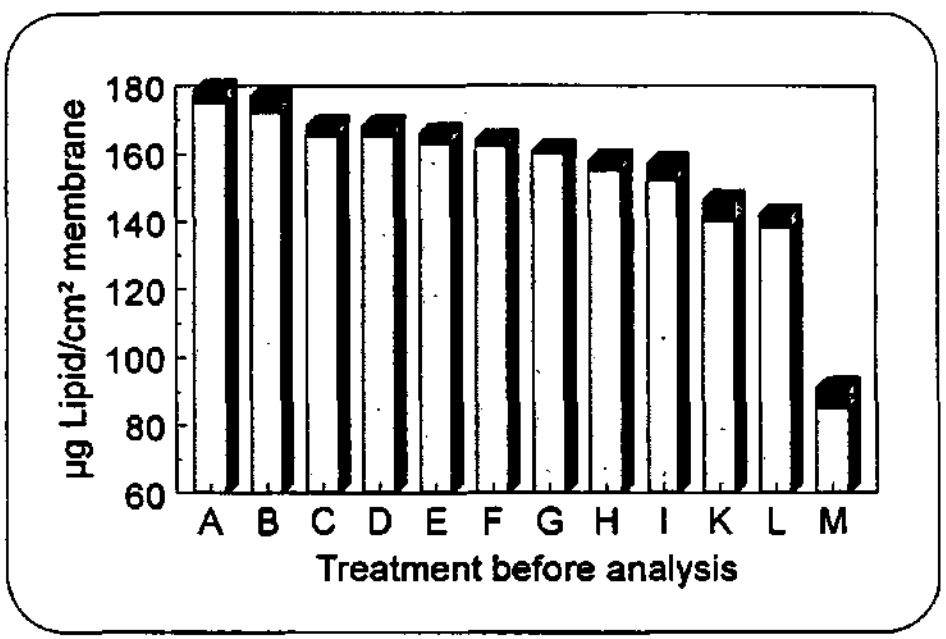

Fig. 7.2 Lipid content of PSM fouled in WSE. **[A]** Fouled for 24 hours; **[B]** Buffer 60 min; **[C]** Protease A (3mg/ml), 60 min; **[D]** Protease C (3mg/ml), 60 min; **[E]** Lipase A (3mg/ml), 60 min; **[F]** 0,1% Triton X100, 60 min, 37 °C; **[G]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[H]** SDS (0,4%), 37 °C, 60 min; **[I]** Protease B (3mg/ml), 60 min; **[J]** Alkazyme:Zymex (1:1), 3%, 60 min; **[K]** Lipase A:Triton X100 (1mg/ml:0.2%), 60 min followed by treatment with Protease A (1mg/ml), 60 min; **[L]** Esterase A (3mg/ml), 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD.

7.3.5 Flux measurements

Flux measurement, as mentioned, is the ultimate test for cleaning efficiency. The effect of cleaning techniques on transmembrane flux is presented in Fig. 7,3. The cleaning regimes followed are given

in the legend to the figure. It was apparent that the enzyme esterase A, lipase A:Triton X100 mixture followed by protease A and Alkazyme:Zymex were the most effective agents to restore flux to above normal values. Interestingly, the same pattern was found between the contact angle, lipid removal and pure-water flux as for membranes fouled in abattoir effluent. Those enzyme or enzyme mixtures that could remove the excessive amount of wax covering the membrane surface and reduce the contact angle below 90° also gave the best transmembrane flux.

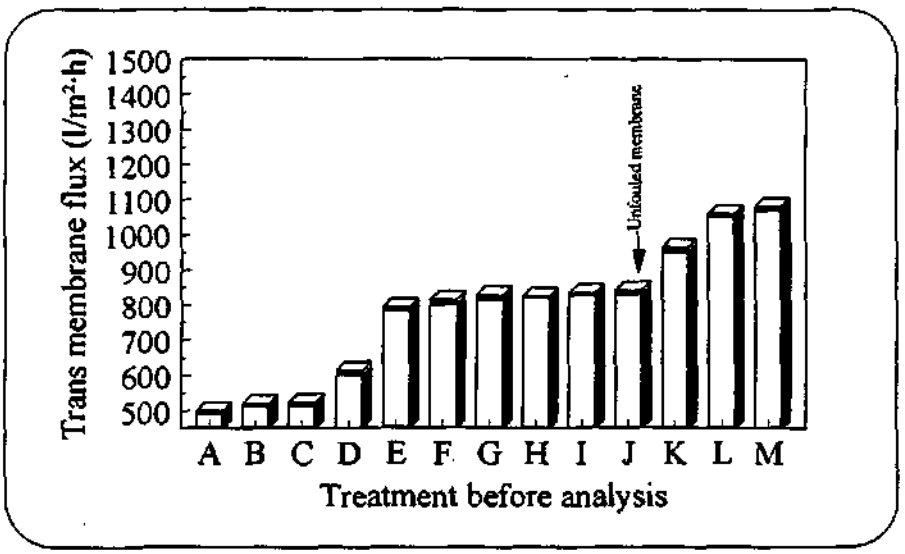

Fig.7.3 Transmembrane flux of clean water through PSM fouled in WSE. **[A]** Fouled for 24 hours; **[B]** Buffer 60 min; **[C]** Protease A (3mg/ml), 60 min; **[D]** Protease C (3mg/ml), 60 min; **[E]** 0,1% Triton X100, 60 min, 37 °C;**[F]** Lipase A (3mg/ml), 60 min; **[G]** Lipase A:Triton X100 (3mg/ml:0,1%), 60 min; **[H]** SDS (0,4%), 37 °C, 60 min; **[I]** Protease B (3mg/ml), 60 min; **[J]** Unfouled membrane; **[K]** Alkazyme:Zymex (1:1), 3%, 60 min; **[L]** Lipase A:Triton X100 (1mg/ml:0.2%), 60 min followed by treatment with Protease A (1mg/ml), 60 min; **[M]** Esterase (A) 3mg/ml, 60 min. Values presented in the stacked bar are the mathematical mean of at least four determinations ±SD.

7.4 CONCLUSION

The techniques developed to clean abattoir effluent fouled membranes, were transferred to the woolscouring industry to characterise and clean foulants adsorbed onto membranes fouled in WSE. Although the abattoir study laid a sound foundation for the investigation of membrane fouling and cleaning in the wool industry, it is clear that considerable differences in effluent composition will necessitate adjustments to the existing techniques and cleaning mixtures. The characterising techniques developed for the abattoir fouled membranes were successfully used to characterise the nature and quantity of adsorbed foulants. In addition, effects of foulants adsorbed onto membranes were effectively characterised with the same methods used in the abattoir industry. Cleaning regimes could, however, not be transferred directly due to the different nature of some of the

adsorbed materials. An esterase, an enzyme specific towards waxes, was chosen with excellent results. The wax content was substantially reduced, the contact angle declined and the flux improved to above expected values. Fluxes above that of the control membrane, as in the abattoir study, were obtained with membranes treated with surface active agents. This phenomenon was discussed in chapter 5.

CHAPTER 8

INVESTIGATION OF EXPRESSION SYSTEMS FOR THE LARGE SCALE PRODUCTION OF ENZYMES USED FOR THE CLEANING OF BIOLOGICALLY FOULED ULTRAFILTRATION MEMBRANES

8.1 INTRODUCTION

Enzymatic cleaning of ultrafiltration membranes, fouled in biologically orientated processes such as abattoirs and the woolscouring industry, is an attractive option because of the specificity of enzymes, the mild conditions under which they are active and their environmental friendliness. The application of these biocatalysts in the cleaning of biologically fouled membranes was well demonstrated in this study and it was clearly shown that enzymes, as major components of cleaning mixtures, can be used to remove specific foulants effectively.

One major draw back in the use of enzymes for the above mentioned purpose, is the fact that enzymes are often expensive and difficult to obtain in large quantities. Previously this factor was a major impediment for the use of specific types of enzymes for the removal of specific foulants. With the development of modern age biotechnology and recombinant DNA-technology, however, it is now possible to obtain the genes (DNA) encoding most proteins, and therefore also enzymes, and to express these genes in organisms such as bacteria (*Eschericia coli*) or yeasts (*Saccharomyces cerevisiae*) to produce large quantities of protein on an economical scale.

The use of transformation systems in recombinant DNA technology has made it possible to express virtually any foreign gene in hosts that range from bacteria to mammals. The procedure for transformation is generally the same for all gene transfer systems. Competent host cells are prepared and induced to take up exogenous DNA. Then selective pressure is applied so that only those cells that have incorporated the DNA are capable of growing. Selection of transformants is achieved by linking the DNA to a vector that contains a selectable marker.

The ability to change the genetic makeup of an organism has led to the design of various heterologous systems for the production of foreign proteins. The choice of a host in a transformation system is often dictated by the desired gene product, and no single system can accommodate every product.

E.coli was the first host for recombinant DNA cloning and *E.coli* plasmids and bacteriophage A were the first cloning vectors (Das, 1990, Holland et al., 1990). Although the proteins expressed in *E.coli* can reach very high levels, a number of problems are encountered in the use of prokaryotic transformation hosts. Many recombinant proteins accumulate intracellularly in an insoluble and inactive form in inclusion bodies. Sophisticated and expensive techniques are subsequently needed to recover soluble and active protein products. Another drawback is that small polypeptides are often rapidly degraded and can only be produced as fusion proteins. After initial purification, fusion proteins must be cleaved to release the peptide and subjected to another round of purification. In addition, prokaryotic cells are unable to perform the post translational modifications needed for eukaryotic protein products. Gram negative bacteria, such as *E.coli*, also produce toxic and pyrogenic cell wall components, which are unsuitable for the production of human pharmaceuticals and fused protein products. Such limitations were the major impetus in the development of eukaryotic hosts for cloned gene expression. There are, however, several successful applications of *E.coli* expressions systems in use today and it was therefore decided to evaluate one such system in this study.

A number of attributes make yeasts attractive transformation hosts (De Baetselier Van Broekhoven, 1994, Gellissen, 1994, Hadfield *et al.*, 1993, Harashima, 1994, Jong and Edwards, 1990 and Jong and Birmingham, 1994). They combine the molecular genetic manipulation and growth characteristics of prokaryotic organisms with the subcellular machinery for performing post translational protein modification of eukaryotes. Moreover, yeasts are free of lipopolysaccharide and associated problems of pyrogenicity. Yeasts can be maintained in either haploid or diploid states, and a combination of classical and modern techniques allows the construction of strains with any desired genetic background. *S. cerevisiae* is the most highly characterized yeast with the most highly developed host vector systems and has been used as a model for other yeasts.

Gene constructs for a yeast host can be created that express a mature heterologous protein rather than a fused protein. The recovery of recombinant proteins or peptides from the growth medium is also easier with yeasts and eliminates the need to disrupt the producing cells. Yeasts do not produce many extracellular proteins; those they do secrete are glycosylated and possess biological activity. The ability of yeasts to grow to high cell densities under controlled fermentation conditions results in correspondingly elevated protein production levels.

The composition of a vector has a profound effect on its stability and function. Vectors for yeast hosts have been developed from bacterial plasmids and possess both prokaryotic and eukaryotic origins of replication. The prokaryotic part enables the vector to be propagated in large amounts in bacteria, while the eukaryotic part allows the plasmid to be replicated in the yeast host cell. In general, vectors comprise *E.coli* plasmid DNA and an appropriate marker for selection in host strains auxotrophic for the marker. Dominant selectable markers, such as resistance to antibiotics or metals, have also been introduced into vectors.

Most *S. cerevisiae* have naturally occurring plasmids, which are stable vectors. For others such as, *Hansenula polymorpha, Candida spp., Schizosaccharomyces pombe* and *Yarrowia lipolytical*, which contain no endogenous plasmids, vectors with autonomously replicating sequences have been constructed. A large assortment of vector types (integrating, multicopy episomal, centromeric, linear, and circular) are now available or can be assembled.

When a yeast host is used in a commercial process for producing a heterologous protein, yield is of paramount importance. Methods for improving yields have often relied on assessing a large number of different host strains. There can be striking differences in expression levels of a given protein using the same vector system in different yeast hosts, presumably a reflection of the varying genetic background of the yeast strains.

Traditional fermentation industries, such as brewing, baking, and distillery spirit production, have attempted to incorporate genes encoding proteins with enzymatic activities to improve process efficiency or capability. Greater progress has been achieved in the area of pharmaceutical biotechnology. Many biomedical products, such as hormones, growth factors, blood proteins, and viral antigens for vaccines, have been produced from yeasts and are in current therapeutic use.

To evaluate heterologous expression systems, it was decided to use the gene encoding for a protein that could be readily detected after expression. It was decided to use a gene encoding for the human 17α-hydroxylase (P450c17), as this DNA was available to us and we could detect the gene product by immunoblot analysis with an antibody that was also available to us. In these studies it is advantageous to first detect protein and not active enzyme, as the protein might be expressed in an inactive form and would therefore not be detected by enzyme assays. Once it has been established that the protein is being produced, however, expression conditions may be altered to yield the active enzyme. If only enzyme activity is used as an assay for successful expression, it would not be

possible to determine where the problem with expression occurred if a negative expression result was obtained.

8.2 EXPERIMENTAL (EXPRESSION OF PROTEINS IN *E.COLI*)

8.2.1 Materials

A commercial expression system from New England Biolabs was used for expression experiments with a TB1 strain of *E.coli.* Experiments were conducted as prescribed in the accompaning literature and according to standard recombinant DNA-technology procedures as described in Sambrook *et al.*, 1989. All other chemicals used were of the highest analytical grade and were used without further purification or modification.

8.2.2 Preparation of the pMAL-c2 plasmid for CYP17 cDNA insertion

Only a small amount of the pMAL-c2 plasmid was supplied with the commercial expression system. To obtain workable quantities the plasmid was multiplied using standard techniques of plasmid preparation and propagation as previously described (Sambrook *et al.*, 1989).

8.2.3 Insertion of the CYP17 cDNA fragment into the pMAL-c2 vector

After treatment with the restriction endonucleases, Eco RI and Hind III, the pMAL-c2 plasmid and the foreign DNA insert both contain non-complimentary protruding termini (a complete plasmid map of the pMAL-c2 vector is given in Fig. 8.1). As the same endonucleases were used in both cases the 5'-end of the foreign DNA, restricted with the Eco RI enzyme, will be complimentary to the 3'-end of the pMAL-c2 vector, restricted with the same enzyme. The 3'-end of the foreign DNA fragment will naturally be complimentary to the 5'-end of the vector as both were restricted with the Hind III enzyme and subsequent directional cloning was achieved. To avoid recircularization of the plasmid without the insertion of foreign DNA, all 5'-phosphate groups of the linear pMAL-c2 plasmids were dephaophorylated with CIP as previously described (Sambrook *et al.*, 1989). To ensure that phosphate groups were available for the ligation reaction, the foreign DNA molecules were subjected to phosphorylation with the enzyme bacteriophage T4 polynucleotide kinase in a separate reaction. The "Magic DNA Clean-Up^{R} " resin (Promega, Madison, USA) was used to remove all traces of the two enzymes from the DNA samples as their presence might adversely affect the ligation reaction (Sambrook *et al.*, 1989). Restriction analyses showed that the

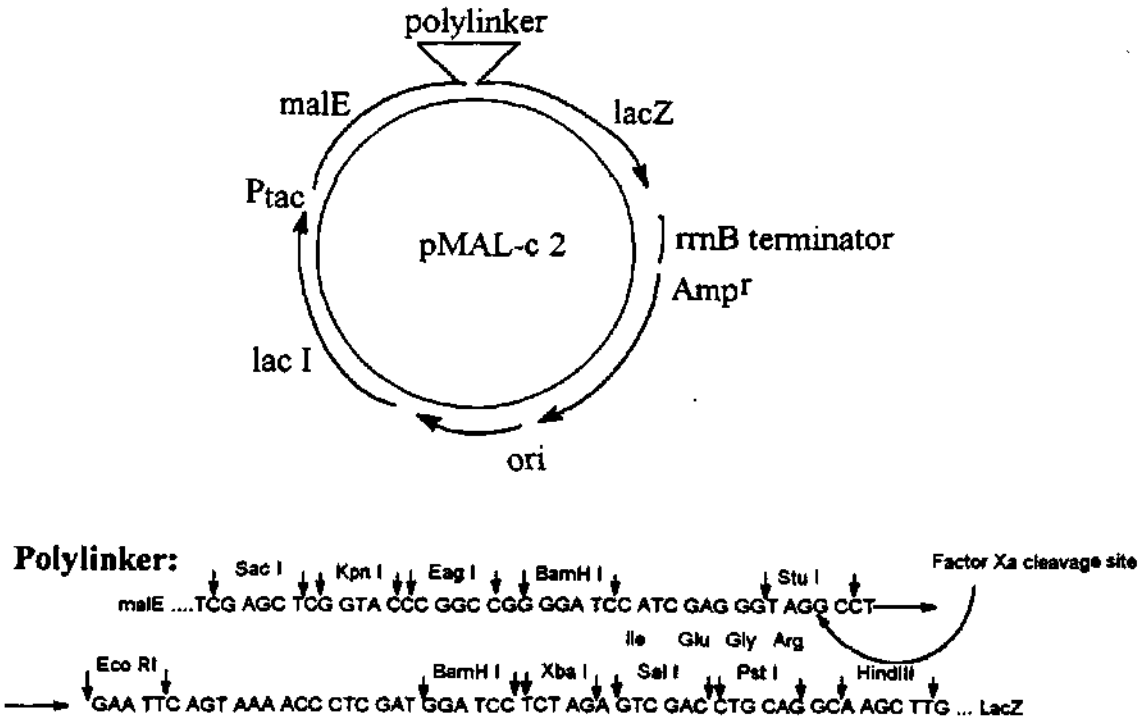

Fig. 8.1. Plasmid map of pMAL-c2

P450c17 was successfully cloned in to the pMAL-c2-plasmid for subsequent expression as a fusion protein.

8.2.4 Expression and the purification of the MBP-P450c17 fusion protein

TB1 bacterial cells containing the recombinant plasmid DNA were used to inoculate an overnight culture containing TB medium with ampicillin and grown overnight at 37°C in a shaking incubator at 225 rpm. Six separate cultures, containing 80 ml each of rich broth and ampicillin, were inoculated with 0,8 ml of the overnight culture and also incubated in a shaking incubator at 37°C until an OD_{600} of 0,4 was reached. IPTG was added to a final concentration of 0,3 mM and the cultures were incubated under the same conditions for an additional 2 hours. Cells were harvested in 12 x 50 ml centrifuge tubes by centrifugation at 4000 x g for 20 min at 4°C and the resultant bacterial pellets were resuspended in a total of 24 ml of column buffer (Phosphate 10 mM, NaCl 0,5 M, EDTA 10 mM, PMSF 1 mM and Emulgen 913 0,5% (v/v), pH 7,4). This was based on the harvesting of 2.4 g (wet mass) of bacterial cells and the use of 10 ml of lysis buffer for each gram of cells harvested. The bacterial suspensions were pooled into a single 24 ml cell suspension, frozen overnight at -20°C and thawed in cold water and sonicated in a ice/water bath for 5 min. NaCl was added to a final concentration of 0,5 M and the entire suspension was centrifuged at 9000 x g for

30 min at 4°C. The supernatant known as the crude extract was kept frozen at -20°C until it had to be applied to the amylose affinity resin.

8.2.5 Isolation of the MBP-P450c17-fusion protein by affinity chromatography

Procedures for the affinity isolation of the fusion protein was followed as given in the instruction accompanying the commercial New England Biolabs kit. Degassed Amylose resin was poured into a 10 cm by 2.5 cm "Perspex" column. Three column volumes of column buffer were used to wash the column before the application of the crude extract. The crude extract, diluted twice with column buffer, was loaded onto the column. The column was subsequently washed with three column volumes of column buffer before the fusion protein fraction was eluted with column buffer containing added maltose, β-mercaptoethanol and EDTA. A total of 21 fractions of 5 ml each were collected and pooled for a total sample of 110 ml. This sample was dialysed, freeze-dried, redissolved in smaller volume of buffer and subsequently analysed for fusion protein content.

8.2.6 Separation of the two components of the fusion protein to obtain pure human P450c17 peptide

The cleavage of the fusion protein was carried out in the same buffer that was used to elute it from the amylose column with the omission of the maltose. The protease Factor Xa was added to each reaction at a 1% (m/m) ratio. In an attempt to relax the fusion protein to make the Factor Xa cleavage site more accessible to the enzyme acetonitrile, SDS and urea were added to the reaction mixtures. The cleavage reaction mixtures were incubated at room temperature for 18 hours and the each reaction mixture was subjected to SDS-PAGE to evaluate the success of the cleavage.

8.2.7 Results and discussion

The sample obtained by affinity chromatography and dialysis was analysed with the aid of SDS-PAGE and Western blot analysis using the anti-MBP antibody. Protein fractions of various molecular masses were recognised by the anti-MBP antibody. This is significant as it indicates that, although the fusion protein was affinity purified, protein degradation remained a problem. Fusion protein was eluted from the column at a concentration of 2 mg/ml. A total of 4 mg of fusion protein was eluted from the amylose resin and recovered after dialysis and freeze-drying. The dialysis of the protein sample was necessary to remove excessive detergent from the elution buffer as its presence led to the formation of large volumes of foam during freeze-drying. Excessive dialysis, however, led to protein aggregation and loss due to precipitation. The fusion protein is recognised by both

anti-MBP antibody and anti-pig P450c17 antibodies and is therefore made up of MBP and human P450c17, A significant amount of fusion protein was, however, present in the insoluble inclusion body fraction. The anti-pig P450c17 antibody did not recognise the smaller proteins present in the sample that was detected by the MBP-antibody. These smaller protein fractions may be due to degradation of the fusion protein and the fact that they are not recognised by the anti-pig P450c17 antibody may indicate that protein degradation starts at the carboxy-terminal of the fusion protein and affects the P450c17 component of the fusion protein first.

The MBP-P450c17-fusion protein could not be successfully cleaved into two separate proteins under any of the different sets of reaction conditions employed. The possible effect of the detergent in the reaction buffer on the activity of the protease is unknown. In addition the tertiary structure of the fusion protein in these reaction mixtures could also be prohibitive to cleavage. Both these factors could therefore be responsible for the failure of the protease to cleave the fusion protein as intended. The results obtained with the pMAL-c2 expression system in *E.coli*, using P450c17 as model, showed that this was not an viable economical route for the production of proteins on a large scale as removal of the MBP, as well as the incorporation of the fusion proteins in inclusion bodies, interfere too much with the production of the heterologously expressed proteins.

8.3 EXPERIMENTAL (EXPRESSION OF PROTEINS IN *S. CEREVISIAE)*

8.3.1 Materials

Microbial Strains and Plasmids: The *E. coli* strain used was DH5α [supE44 Δ lacU169 (ϕ80lacZΔ M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1]. The yeast strain used was YPH 259 [α ura3-52 lys-801^{amber} ade2 101^{ochre} his3-Δ200 leu2-Δ1]. The plasmid pPRL-2, a derivitive of YCplac111 [Amp^r LEU2] was used to express a modified CYP 17 sequence. This plasmid is a yeast-centromeric plasmid and has the $ADC1_P$-$MF\alpha1_S$ expression-secretion cassettes and a yeast terminator sequence $ADC1_T$. inserted in the multi-cloning site (see Fig. 8.2).

8.3.2 Plasmid construction

The modified CYP17 sequence was prepared by polymerase chain reaction (PCR). The template used was a pCD plasmid containing the sequence encoding the human CYP17 which had been linearized by Hind111 digestion. The 5' primer was designed to delete the first 18 amino-terminal residues (Δ1-18) of P450c17, To construct the COOH-terminal modification, the 3' primer

included a sequence encoding five consecutive histidine residues (5 x His tag) and the stop codon was deleted. Both primers contained a Hind111 restriction site. The amplified product was cloned into the Hind111 site of pPRL-2. Standard protocols for DNA and plasmid isolation, purification, manipulation and cloning, transformation of *E.coli*, electrophoreses and western blot hybridization were followed, as previously described (Sambrook *et al.*, 1989).

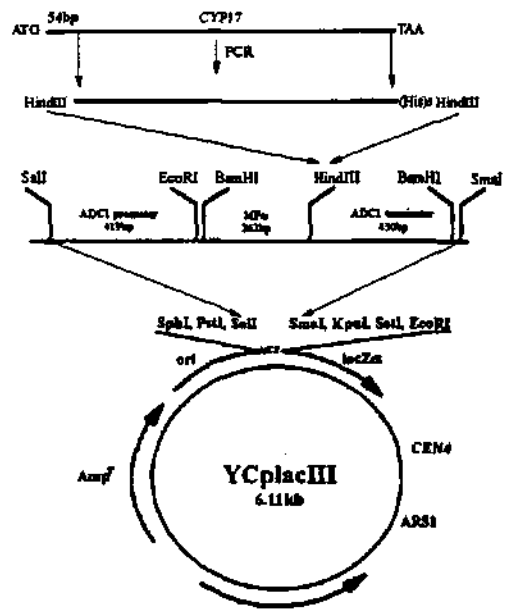

Fig. 8.2. Plasmid map of pPRL-2

8.3.3 Bacterial expression of pPRL-2mod17α(His)$_5$

Single colonies of DH5α cells transformed with the plasmid construct were seeded into Luria-Bertani containing ampicillin (50 µg/ml) and grown overnight at 37°C. Plasmid DNA was isolated and screened for correct insertion and orientation of CYP17 with the appropriate restriction enzymes according to previously published methods (Sambrook *et al.*, 1989).

8.3.4 Yeast transformation and expression.

The *S. cerevisiae* strain, YPH259 was transformed with the recombinant plasmid, pPRL-2mod17α(His)$_5$, by the modified lithium acetate method (Sambrook *et al.*, 1989). Single yeast transformants were inoculated, in triplicate, in 100ml SC^{-Leu} (containing 0,67% yeast nitrogen base without amino acids, 2% glucose and all required growth factors lacking leucine in 0,1 M phosphate buffer pH 6), incubated at 30°C on a rotary platform and grown well into the late stationary phase (96-120h).

8.3.5 Purification of P450c17 $(His)_5$

The culture was centrifuged at 12 000g at 4°C and the supernatant filtered through a Millipore cellulose filter (30 000 NMWL low protein- binding PLTK membrane). The concentrate, 10 ml, was dialyzed overnight at 4°C against 1,5 litres of buffer A containing 50 mM Na-phosphate pH 8,0, 300 mM NaCl. The dialyzed concentrate was subsequently applied to a nickel nitrilotriacetate (Ni^{2+} -NTA) agarose column (1,5 cm in diameter, bed volume 16 ml), previously equilibrated with buffer A. The resin was washed with buffer A until the absorption at 280 nm returned to the base line value. The column was subsequently washed with buffer B containing 50 mM Na-phosphate pH 6.0, 300 mM NaCl, and 10% glycerol. The protein was eluted with a gradient of 0 - 0,5 M imidazole in buffer B and and 0,5 ml fractions having an A_{280} greater than 1,0 were collected. Protein concentrations were determined using the bicinchoninic acid method and bovine serum albumin as the standard. Column samples were fractionated by polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellose membranes. Immunoblot analyses was carried out using rabbit IgG specific for pig testis P450c17,

8.3.6 Results and discussion

As was mentioned earlier, using yeast as an expression host has the advantage of the export of the expressed protein into the medium.To further facilitate isolation and purification of the heterologous gene product, it was decided to clone additional histidine residues to the carboxy terminal end of the P450c17, The five histidine residues would act as an affinity tag and will facilitate the affinity purification of the expressed protein by a nickel nitrilotriacetate (Ni^{2+} -NTA) agarose column.

The purification of the expressed modified P450c17 is summarised in Tab. 8.1. Media from control cultures, transformed with pPRL-2, yielded 5,4 mg of protein per ml while the media of pPRL-2mod17α$(His)_5$ transformed cultures yielded 12,2 mg of protein per ml media. The elution profile of the affinity purification of the expressed P450c17 is shown in Fig. 8.3. Elution with imidazole yielded only one single peak with a significant amount of protein. SDS-PAGE of the pooled protein containing fractions (23-26) showed one major component on SDS-Page (Fig. 8.4A) and the corresponding immunoblot analysis with anti-pig P450c17-IgG indicated that the exported product was a P450-derived peptide (Fig. 8.4B).

Tab. 8.1. Summary of the isolation of the modified human P450c17 expressed and exported by *S. cerevisiae.*

	Volume (ml)	Protein (mg/ml)	Total protein (mg)
Control culture medium	100	-	-
Transformed cell culture medium	300	-	-
Control supernatant concentrate> 30 000	12	5.4	64.8
Transformed supernatant concentrate> 30 000	27.5	12.2	1656
Control imidazole elution	40	-	-
Transformed imidazole solution	13	0.7	9.2

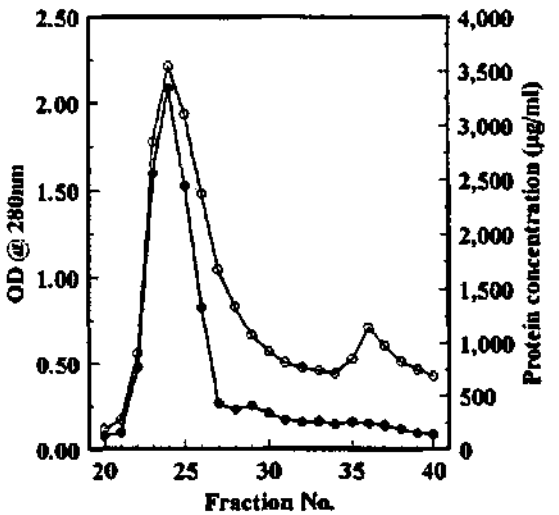

Fig. 8.3. Elution profile of His-tagged yeast secretion protein eluted with an imidazole buffer.

We have demonstrated that a truncated P450c17 with a histidine tag can be expressed and secreted into the medium by *S. cerevisiea.* The secreted peptide could be readily purified from the medium in one step by affinity chromatography. The yeast expression system described here holds a number of distinct advantages over the pMAL-c2 protein fusion and purification system in *E. coli.* The histidine tagged P450-peptide is secreted into the medium in a soluble form while expression in the *E. coli* system yielded most of the P450-peptide in the insoluble inclusion bodies which necessitates further elaborate solubilisation and purification steps. In addition the maltose binding affinity tag, used for the pMAL-c2 protein fusion and purification system in *E. coli,* has to be removed.

The additional histidine tag on the P450-peptide expressed in yeast, on the other hand, should not significantly influence the activity of the secreted protein and no further modification of the product

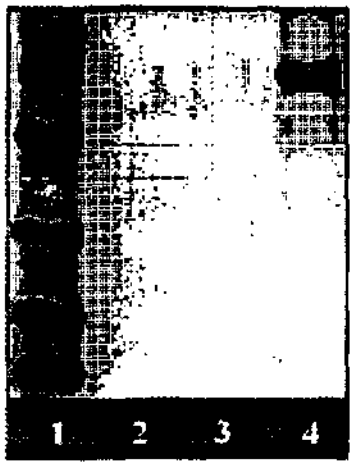

Fig 8.4A: SDS-page of export protein. Lane 1; Mr-markers, Lanes 2-4; 1, 2 and 20 µg protein respectively.

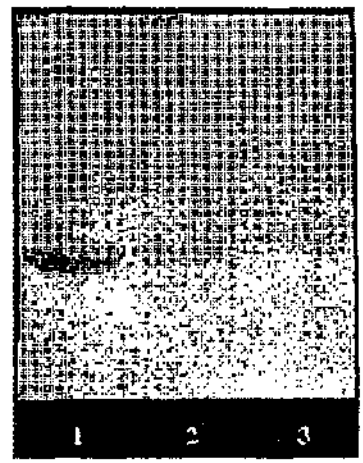

Fig 8.4B: Immunoblot of export protein with pig anti-P450c17antibody. Lane 1; P450c17, lane 3; Mr-markers.

is therefore necessary after purification. Results obtained from SDS-PAGE and immunoblot analysis show that the his-tagged P450-peptide secreted by the transformed yeast has a molecular mass of approximately 64000 which is considerably higher than that expected for the truncated human P450c17 (± 4900) that was originally cloned into the pPRL-2 plasmid. This discrepency can be attributed to the removal of the stop codon of the P450c17 in the modified plasmid and the fact that the electrophoretic mobility of his-tagged proteins is sometimes retarded. It is also possible that extensive glycosalation of the P450c17 peptide could have taken place which increased the molecular mass of the expressed protein. Yeast strains that are deficient in glycosalating enzymes can be used in future if glycosaltion will influence the activity of the expressed enzyme. The yield of protein expressed by the yeast was also satisfactory and although direct comparisons are difficult to make at this stage, it compared well to yields previously obtained in *E. coli* for active P450c17, It is believed that this expression system provides an alternative method for the facile preparation of enzymes on a large scale. Future efforts will concentrate on the investigation of other suitable plasmids and yeast strains. Much refinement will be done once the specific enzymes needed in cleaning mixtures are identified. The use of an Ni^{+}-membrane instead of agarose resin wil also be investigated in future. The experiments conducted in this study showed that yeast expression systems can be used in conjunction with an affinity tag for the large scale production of proteins. The purification of the expressed product is much easier than the *E.coli* system and the same or better yields of active proteins are possible.

CHAPTER 9

A LITERATURE STUDY ON THE FOULING OF UF MEMBRANES BY PULP AND PAPER EFFLUENT

9.1 INTRODUCTION

Most chemical pulping is carried out either according to the Kraft (sulphate) process or the sulphite process. The purpose of these pulping methods is to remove lignin to facilitate fibre separation and to improve paper making properties of fibres. The Kraft process is presently the most popular pulping method in the paper industry. Neither the Kraft nor the sulphite process removes all the lignin. About 5-10% of the original lignin remains in the pulp and this residual lignin calls for a multistage bleaching process. For soft wood Kraft pulps bleaching is normally accomplished by successive treatments with chlorine (C1), alkali (E1), chlorine dioxide (D1), alkali (E2) and chlorine dioxide (D2). Often, a hypochlorite stage (H) may be inserted between the E1 and D1 stages (Kringstad and Lindstrom, 1984). The exact chemical reactions that occur during these steps will not be discussed. It is suffice to say that the effluent discharges from these processes are of complex nature with a high pollution potential. The first caustic extraction filtrate (E-stage effluent) contains the majority of pollutants and 10 m^3 of this stage effluent is produced per ton of pulp. A considerable amount also originates from the first chlorination filtrate (C-stage effluent). Only minor amounts of pollutants originate from the following bleaching stages (Johnson, 1987).

The Kraft pulp and paper industry is therefore currently faced with the challenge of substantially reducing the discharge of conventional and toxic pollutants to the receiving environment without compromising economy or competitiveness.

9.2 EFFLUENT COMPOSITION

The paper industry, which produces bleach chemical pulp consumes considerable quantities of chlorine and discharges large quantities of chlorinated organic matter into rivers, lakes and oceans. Improved knowledge of the chemical composition and biological effects of this effluent would facilitate a meaningful assessment of any risks involved in releasing such material into receiving waters (Kringstad and Lindstrom, 1984).

More than 250 separate chemicals have been identified in bleached Kraft mill effluent, and all but 50 are chlorinated. The compounds include chlorinated resin acids, chlorophenolics, chloroguaiacols,

chlorocatechols, chlorinated dioxins and chlorinated furans (Hodson *et al.*, 1992). The COD and BOD of bleach plant effluents fluctuate, The following values have been reported:

- Dorica *et al.*, (1986) reported COD, BOD, Organics, Cl_2, organic chlorine and Na^+ values of 6.2, 1.3, 3.8, 3.1, 0.4 and 2.98 mg/l respectively for E-stage effluent.

- Zaidi *et al.*, (1992) reported that E-stage effluent has an average COD of 2150 mg/l, BOD of 190 mg/l and an adsorbed organic halogens AOX of 85 mg/l

- Muratore *et al.*, (1983) reported COD values of 2500 mg/l and colour concentrates of 20 000 mg/l.

Kringstad and Lindstrom, (1984) divided pollutants into the following categories:

High-molecular-mass materials: 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). While in the case of the alkali extraction (E-stage) liquor, about 95% of the organically bound chlorine belongs to this class (Kringstad and Lindstrom, 1984). Determination of the elemental composition of these substances yielded the following: $C_9H_9O_4Cl$, $C_{10}H_{14}O_7Cl$ and $C_9H_{10}O_8Cl$ as the representative formula for high molecular mass compounds in spent chlorination liquor. In the alkali extraction the most abundant species were of $C_{14}H_{15}O_8Cl$ and $C_{14}H_{10}O_9Cl$ configurations (Kringstad and Lindstrom, 1984).

Low-molecular-mass materials: About 30% of the organically bound chlorine, in spent chlorination liquor and approximately 5% in alkali extraction liquor, is of low relative molecular mass (Mr < 1000) (Kringstad and Lindstrom, 1984).

These high-molecular-mass and low-molecular-mass materials could further be divided into three main groups: acidic, phenolic, and neutral.

Acidic compounds: Acidic compounds include five categories of acids, including fatty, hydroxy, dibasic, aromatic and resin acids. Formic and acetic acids are qualitatively the most important fatty acids while glyceric acid predominates the hydroxy acids. Dibasic acids, like oxalic, malonic, succinic and malic acids, are present in considerable quantities in both types of spent liquor. Three

kinds of aromatic acids are formed (phenolic, catecholic and guaiacolic) from residual lignin (Kringstad and Lindstrom, 1984).

Phenolic compounds: Chlorinated catechols primarily occur in spent chlorinated liquor, whereas most chlorinated guaiacols and vanillins are found in spent alkali extraction liquor. All of these are formed from residual lignin (Kringstad and Lindstrom, 1984).

Neutral compounds: Methanol and various hemicelluloses are, qualitatively, by far the most dominant neutral compounds in spent liquor (Kringstad and Lindstrom, 1984).

9.3 TREATMENT OF PULP EFFLUENT

Methods for the reduction of pollution that arises from the pulp and paper industry, can be divided into two complementary approaches:

1. Pollution prevention through the implementation of internal measures. The integration of physical/chemical separation processes into the pulp mill with cost effective recycling of process waters (permeate) (Zaidi *et al.*, 1992).
2. Innovative end-of-pipe treatment processes (effluent treatment) (Zaidi *et al.*, 1992). The concentrated stream, which contains most of the toxic compounds, will still need to be disposed of. Its reduced volume, however, would make it easier and cheaper to dispose (Zaidi et al., 1992).

The field of interest in this study include effluent treatment. Existing pulp and paper effluent treatment strategies include: aerobic and anaerobic digestion; lime and alum precipitation, oxidation with hydrogen peroxide; sodium hypochlorite chlorination; adsorption to ion exchange resins; rapid infiltration ultrafiltration and processes based on coagulation (Dorica *et al.*, 1986; Johnson, 1987; Tirsch, 1990 and Dixon *et al.*, 1992).

This overview, however, will concentrate on the use of membrane treatment as the main strategy. In the context of pulp mill effluent treatment and for toxic organic removal, the membrane processes which are of most relevance are: (i) microfiltration (ii) UF (iii) nanofiltration (NF) and (iv) RO (Zaidi *et al.*, 1992).

The available information on the use of UF and NF membranes for the treatment of pulp mill effluents comes from work conducted during the early to mid 1970's to the late 1980's. In the early 70's the focus fell on the removal of colour (not specific toxic organic). High molecular weight chlorolignins were considered to be mainly responsible for this colour and the E-stage effluent was

identified as the main source of chlorolignins. The late 1970's produced chemically and thermally resistant membranes which were made of polymeric products (polysulphone and its derivatives). These membranes could remove 80 to 95% colour and 60 to 85% COD. There was still a lack of interest in the use of membranes as a treatment stage. The reasons being: (a) secondary biological processes were quite adequate for achieving the regulatory requirements (b) expansion of evaporation plants would be necessary and (c) difficulties in controlling the fouling of membranes during the treatment of pulp mill effluents. In the late 1980's up to 1990 there was a revival of interest in membrane processes for the treatment of various effluent streams in the pulp and paper industry. The main reason for this being: (a) increased pressures on the industry to reduce the discharge of toxic organic material and (b) improved membranes and membrane systems that became available (Zaidi *et al.*, 1991).

9.4 APPLICATION OF MEMBRANE TECHNOLOGY IN PULP EFFLUENT TREATMENT

The E-stage effluent is well suited for UF treatment as most of the polluting substances are high molecular weight compounds and easy to remove by UF (Johnson, 1987). C-stage effluent was also treated, but was found to be a more difficult task (Johnson, 1987).

Results from UF treatment of the E-stage indicate a colour removal of 70 to 98%, COD removal of 55-87%, BOD removal of 35 to 44% and AOX rejections from 85-91% (Zaidi *et al.*, (1992), Dorica, (1986), Dorica *et al.*, (1986). Muratore *et al.*, 1983). Ekengren *et al.*, (1991) observed a decrease in toxicity of about 50% and the removal of substances with a bio-accumulation potential was around 90%. A combination between UF and RO increased the removal efficiency.

The composition of the bleach effluents may differ at different mills. It may also differ from time to time at the same mill. This has the consequence that UF results from one mill cannot be directly translated to other mills, but results reported in various experiments indicate that there are good reasons to continue the testing of UF treatments of bleach plant effluents (Johnson, 1987).

9.5 FLUX DECLINE CAUSED BY FOULING OF MEMBRANES USED IN PULP EFFLUENT TREATMENT

A well known phenomenon during membrane filtration is the initial flux decline during the first hours of operation (Johnson, 1987). Only a few studies, however, were conducted to investigate the nature of foulants that caused this decline when treating pulp and paper effluent:

- Dorica, (1986) showed that the flux decline observed in the treatment of first-stage (caustic bleach) plant 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 the initial value could not be attained. Further analysis of the deposits on the fouled membrane indicated that Mg, Ca, and CO_3 were the dominant substances which were easily removed with 5N HCl.

- Results obtained by Zaidi *et al.*, (1991) indicated that organic species, such as chlorocatechols, may also be partly responsible for the observed flux decline. Negligible flux decline was observed during tests in which dehydroabietic acid was used as a model foulant. On the other hand, significant flux decline was observed in UF tests with sulphonated lignin and 4,5-dichlorocatechol.

Additional evidence of extensive physical/chemical interactions between the solutes and the membrane was obtained from experiments with dichloroguaicol and resin acids as model foulants. Rejection of solutes were generally lower for the more hydrophilic membranes. No correlation could be established between the rejection and the molecular eight cut off of these membranes when these compounds were part of the feed solution. (Zaidi *et al.*, 1991). The Log-P (octanol/water partition coefficient), which indicates the affinity of a compound for an organic phase, is higher when substitution of a hydrogen (H) with chloride (Cl) occurred.

9.6 CONCLUSION

From this literature study it is clear that fouling problems exist, however, a more extensive study is needed to find a solution to this fouling problem in the pulp and paper industry. Such a study will include the following:

Implement experimental knowledge, obtained from the abattoir industry, to characterise foulants adsorbed onto membranes during UF treatment of pulp and paper effluent;

identify enzymes/enzyme detergent mixtures capable of degrading and removing foulants characterised in previous paragraph;

imply these new-fashioned cleaning methods to treat membranes fouled within pulp and paper effluent at a pilot plant to optimise the cleaning techniques under actual conditions and this information, of the most successful cleaning method, introduced to the industry.

CHAPTER 10

A LITERATURE STUDY ON THE USE OF UF MEMBRANES IN THE TREATMENT OF MAIZE-PROCESSING EFFLUENT

10.1 INTRODUCTION

A maize-mill processes maize (corn) for the manufacturing of cornflower and a wide range of natural and modified starches, glucose, syrups, dextrins and by-products such as gluten feed (20% protein), maize-gluten (60% Protein) and maize germ (50% oil mass) (Butcher 1989 and Ross *et al.*, 1992). The wastewater resulting from the production of starch and its derivatives from maize grain contain considerable quantities of putrescible material, mostly in solution, and has a high oxygen demand. If adequate treatment and dilution are not available disposal of such effluents can present difficult problems (Hemens, *et al.*, 1962). These effluents are generally biodegradable, but can organically be highly polluted. The composition of the effluent varies according to the processing conditions, but in general consists of soluble inorganic matter, proteins, amino acids and other nitrogenous substances, lactic acid, carbohydrates and also finely divided solids such as, gluten fibre and powdered activated carbon (Ross *et al.*, 1992). A typical nature and variation of maize-processing effluents can be ascertained from results of analyses previously published (Hemens *et al.*, 1962, Butcher 1989 and Ross *et al.*, 1992).

10.2 TREATMENT TECHNIQUES

A number of the techniques that are being used to treat maize-processing effluent were described by the following authors:

Hemens *et al.*, (1962) used a full-scale anaerobic digestion of maize-starch plant effluents in a modified Dorr Oliver Clarigester and reported 88% COD removal. A modification of this process was later used by Faber et al., (1986) and a COD removal of 96% was reported.

Butcher (1989) used an anaerobic digester to treat wheat starch processing waste. A UF plant was connected to the digester every 24 h. The UF process gave very good results, particularly against the colloidally dispersed bacterial solids.

Ross *et al.*, (1992) used the ADUF^{R} (anaerobic digestion ultrafiltration) process that comprises two main unit processes: an anaerobic digester and an external UF unit. The permeate produced by the

UF membranes is the final effluent while the concentrated sludge containing the bacteria, is recycled back to the digester (see Fig. 10.1). Results after 15 months of full-scale operation illustrated the merits of the ADUF^R process producing a colloid-free effluent and COD removal of 97%.

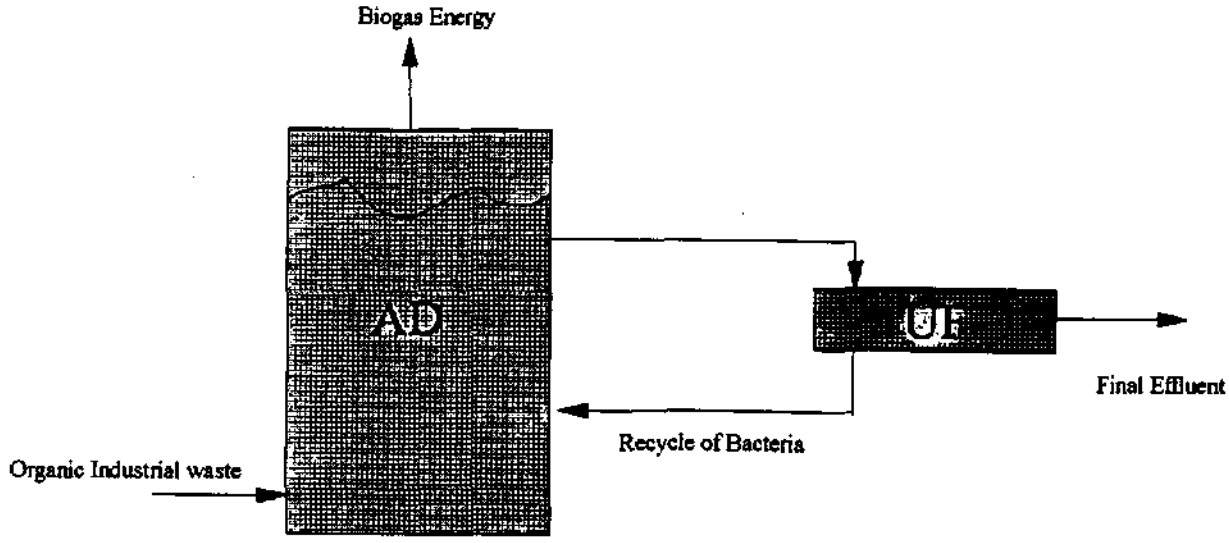

Fig. 10.1 Schematic diagram of the ADUF process. The anaerobic digester (AD) is coupled to the UF membrane system.

10.3 MEMBRANE FOULING

According to the literature membrane fouling does not frequently occur when UF membranes are used in conjunction with an anaerobic digester. Butcher, (1989) concluded that only a monthly wash with caustic hypochlorite was needed to keep up membrane performance. However, it was observed that a rise in either digester solids or volatile acids (VA) could cause a drop in the membrane filtration rate. Ross *et al.*, (1992) reported that membrane cleaning, with EDTA, was necessary after 13 months of operation which indicated that membrane fouling, of this particular system, was minimal. This is explained by the fact that the anaerobic digestion breaks down organic substances that would otherwise foul the membrane. Influent macromolecules and particulate organic substances are rejected at the membrane surface and are selectively retained in the digester by the UF until metabolised to the molecular mass cut-off (MMCO) of the membrane.

10.4 CONCLUSION

From this literature study it is thus clear that no or minimal fouling exists in the processes where anaerobic digestion is combined with an UF membrane system. It was therefore, at present, deemed not necessary to develop cleaning regimes for UF membranes used in these kind of process plants..

CHAPTER 11

CONCLUSIONS

The goals set for this project were the following:

1. identification and characterisation of potential foulants in abattoir and WSE effluents;
2. development of a static fouling method for PSM in these effluents;
3. development of techniques to quantify the amount of major foulants on statically fouled PSM;
4. development of methods to determine the influence of foulants on membrane character;
5. development of enzymatic cleaning agents for abattoir and WSE fouled membranes, using the results obtained from foulant analysis;
6. quantification and comparison of the cleaning efficiency of different enzyme based cleaning regimes of biological as well as chemical origin and
7. investigation of recombinant DNA-methodology for the economical production of enzymes to be used in potential enzyme based cleaning regimes.
8. a literature study of the treatment of maize and pulp and paper industry effluents with UF membranes and the influence of membrane fouling in these processes.

Abattoir effluent was analysed for potential foulants and it was found that lipids and proteins are quantitatively the most important foulants. PSM were fouled in this effluent and the fouled membranes were analysed for adsorbed lipid and protein material. With the nature of adsorbed foulants known, specific enzymes were chosen to remove foulants adsorbed onto these fouled membranes. The efficiency of the cleaning experiments was determined by comparing the potential of each cleaning agent to remove foulants from membranes and to restore the performance and characteristics of the fouled membrane. All the experiments carried out, with abattoir effluent fouled membranes, were repeated with WSE. The following conclusions can be made from the results obtained:

1. Protein and lipids are important foulants in abattoir effluent, while protein and wax (lanolin) are the most prominent foulants in WSE. The content of these foulants in the effluent vary significantly from hour to hour and from day to day. This variation in effluent composition

renders the use of model foulants and model solutions, to characterise fouling in abattoir and WSE, unserviceable and therefore real effluents were used in fouling experiments;

2. Methods used to characterise and quantify membrane fouling in abattoir effluent are summarised in Fig. 11.1. Protein and lipid analysis, developed in this study, proved to be reliable and sensitive to determine the nature and amount of these materials adsorbed onto membranes during the fouling period. Results from lipid and protein analysis indicate that multilayer adsorption occurs with the lipid material adsorbing first. This first layer of lipid material provides an ideal surface for further adsorption. A novel staining method was developed to determine protein adsorption onto the membranes. These techniques were also effectively used to determine the efficiency of foulant removal in cleaning experiments. Dynamic contact angle measurements have not previously been used to characterise membrane fouling in abattoir effluent. It was, however, found the information obtained from this technique not only useful in characterising membrane surface characteristics, but also of value in the assessment of cleaning efficiency;

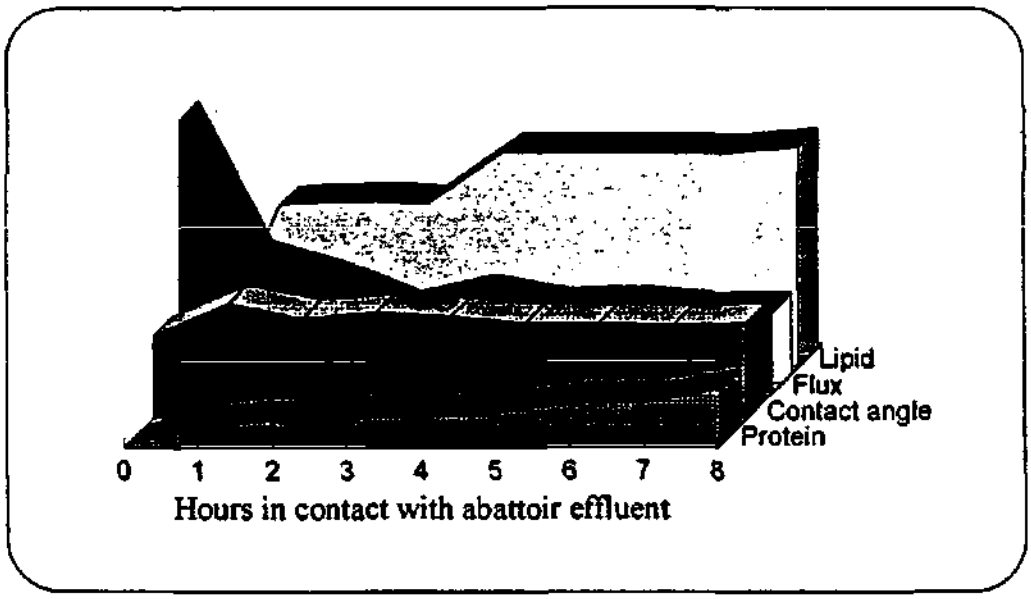

Fig. 11.1 Summary of the variables used to characterise fouling of membranes over an eight hour period in abattoir effluent. Protein content and lipid content are in $\mu g/cm^2$ membrane, contact angle in degrees and pure-water flux in $l/m^2 \cdot h$.

3. The contact angle changes correlate well with changes observed on the membrane surface after foulant adsorption. Lipid adsorption covers the membrane surface with a hydrophobic layer with a concomitant increase in the contact angle. Proteins subsequently adsorb to the hydrophobic lipid surface and expose their hydrophilic parts to the outside, thereby reducing the contact angle of the membrane. Transmembrane flux measurements of clean water can be

seen as the ultimate method to characterise the effect of fouling on membrane permeability. The decline of the clean water flux through membranes follow the same pattern as the flux decline observed in an actual UF process. A rapid flux decline is observed within the first min of fouling followed by a more steady decline. This indicates that, in future fouling experiments, two to three hours of fouling will be sufficient and that static adsorption studies could be used as an inexpensive method to characterise foulants and develop cleaning regimes for real UF processes;

4. Enzymes specific for protein and lipid hydrolysis were used to clean membranes fouled in abattoir effluent. Cleaning experiments, evaluated for their potential to remove lipids and proteins, clearly indicate that specific enzymes and enzyme detergent mixtures can effectively remove specific foulants adsorbed onto UF membranes. In Fig. 11.2 a summary of the cleaning agents that yielded the best flux recovery of membranes, fouled in abattoir process effluent, is given. Enzymes alone, and in conjunction with specific detergents, produced good cleaning results. These results could, however, only be obtained with a sound knowledge of the nature of the foulants;

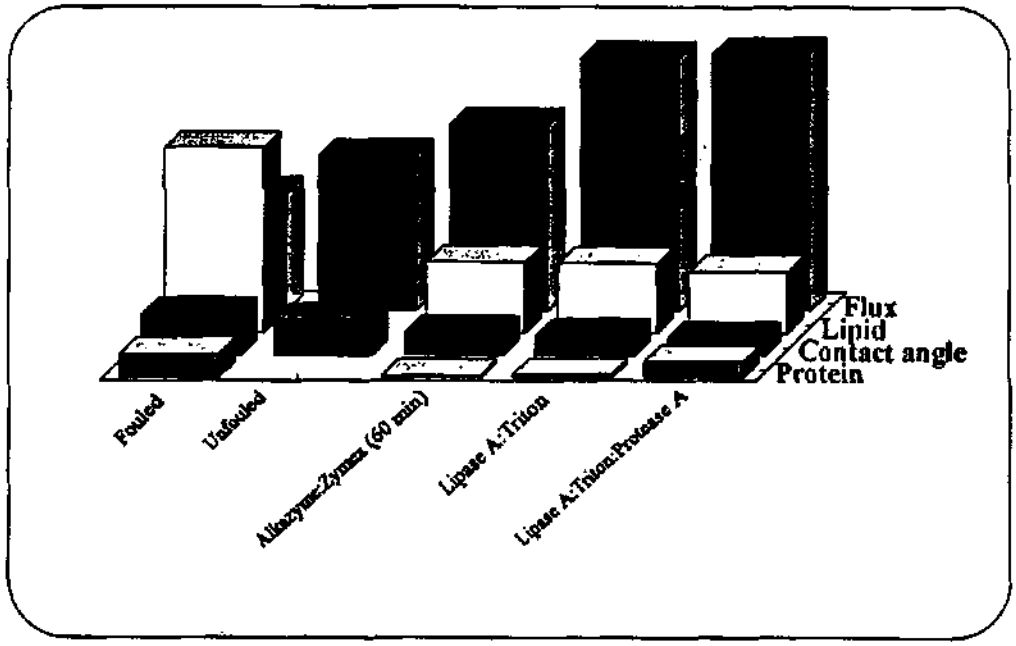

Fig. 11.2 Comparison of pure-water flux, lipid content, protein content and contact angle measurements for the three cleaning methods that yielded the highest flux recovery of membranes fouled in abattoir effluent. Enzymes were used as follows: 3% of a Alkazyme:Zymex (1:1) solution for 60 minutes, lipase A:Triton X100 (3mg/ml:0,1%) mixture for 60 minutes, lipase A:Triton X100 (1mg/ml:0,1%) mixture for 60 minutes followed by treatment with Protease A (1mg/ml) for an additional 60 min.

5. The experience gained in effluent and membrane analysis from the abattoir industry was transferred to the WSE. In WSE lanolin is the main foulant and the enzymes, that could remove lipids from PSM fouled in abattoir effluent, could not remove the lanolin. An esterase, with greater specificity towards ester bonds, was used instead of the lipases which successfully removed lipids from abattoir fouled membranes. In Fig. 11.3 results obtained from cleaning experiments conducted with WSE fouled membranes are presented. The cleaning agent containing an esterase was the most successful in the foulant removal and flux restoration;

6. Results obtained during this investigation show that enzymes, as highly specific biocatalysts, are extremely useful and effective components of cleaning mixtures for biologically fouled membranes. In order to use these catalysts effectively it is, however, very important to know the exact composition of the foulants deposited on the membrane.

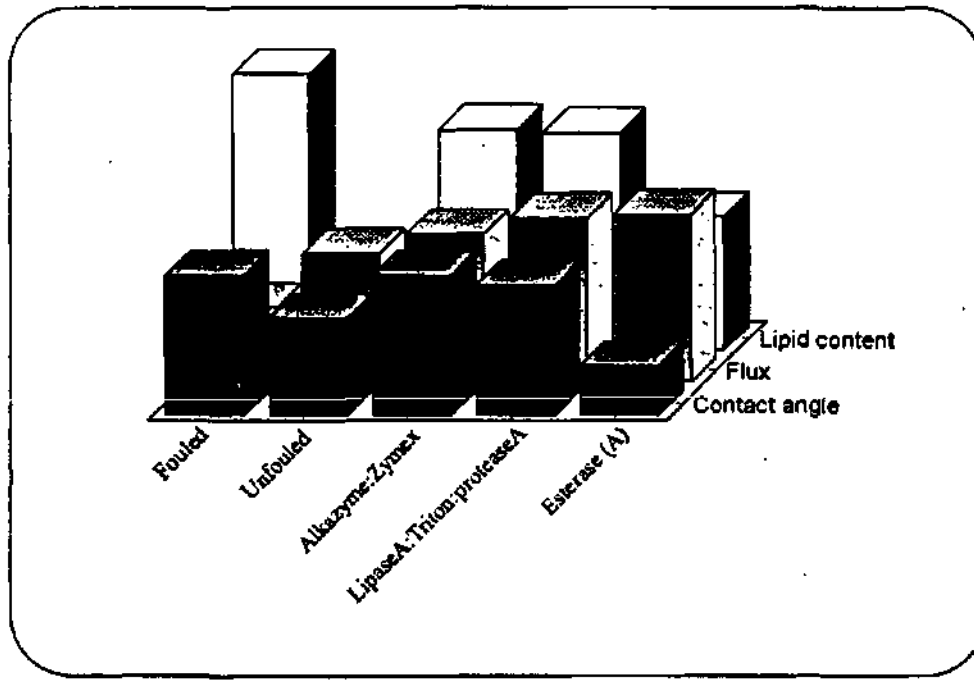

Fig. 11.3 Comparison of pure-water flux, lipid content and contact angle measurementsof membranes fouled in WSE. Enzymes were used as follows: Esterase A (3mg/ml), for 60 min, lipase A:Triton X100 (1mg/ml:0,1%) mixture for 60 min followed by protease treatment with protease A (1mg/ml) for an additional 60 minutes and Alkazyme:Zymex (1:1), 3% solution, for 60 min.

7. A novel yeast expression system was developed for the economical and facile expression and purification of proteins in yeast. This system combines the secretory ability of yeast with the use of affinity purification of the expressed protein in a single step. Future studies will concentrate on refining the system and adapting it for the specific enzymes that will form part of future cleaning regimes.

8. From the literature we concluded the ultrafiltration membranes used in the pulp and paper industry suffer from fouling problems while, the maize industry is almost free from the problem, possibly due to the anaerobic digestion. In Fig. 11.4 the different fouling substances arriving from the processes discussed in this report are summarised as well as the enzymes that could be used for each foulant.

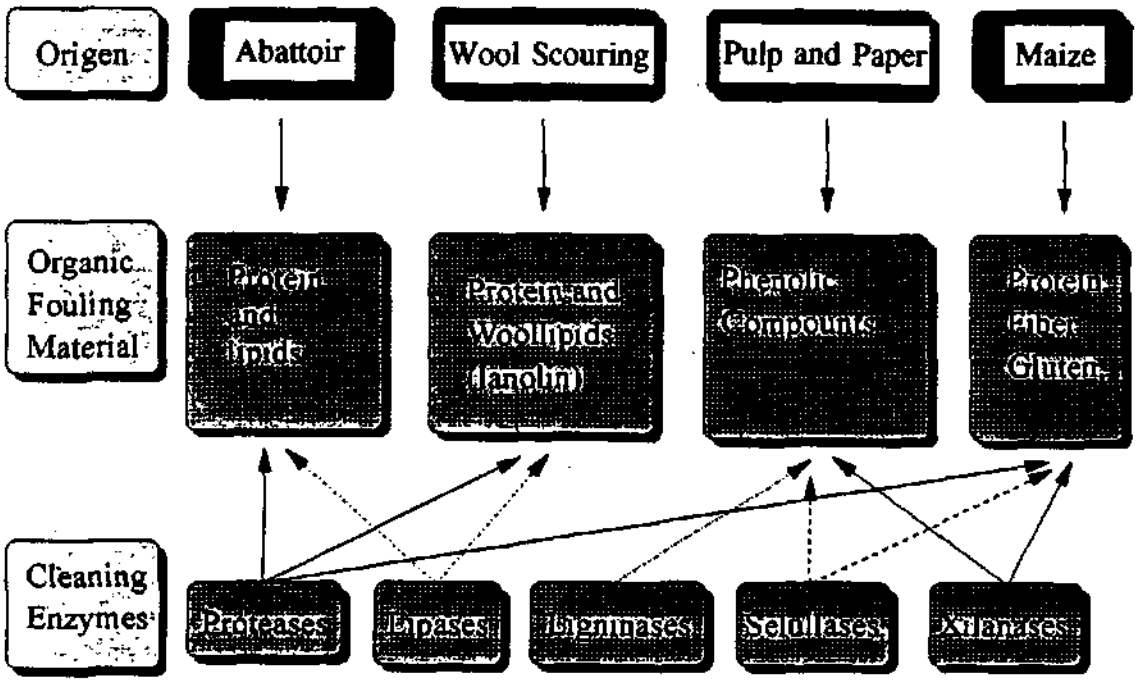

Fig. 11.4 The different fouling substances and the different enzymes that could specifically remove specific foulants form UF membranes.

The promising results obtained in this investigation show that enzyme based, biologically orientated cleaning regimes, hold a lot of promise for the restoration of UF membranes fouled in biological effluents. This study has also opened new avenues for investigation of:

1. Ideal enzymes, or enzyme detergent mixtures (biological cleaning agents), for the cleaning of UF membranes fouled in the recycling process of abattoir wastewater;
2. The implementation of novel biological cleaning methods on a pilot-ultrafiltration plant at an abattoir and the optimisation of cleaning techniques under real UF conditions (once the techniques are optimised it will be introduced to the industry for industrial application);
3. The characterisation of potential foulants present in the wastewater produced in the pulp and paper industry and the identification of enzymes capable of degrading the foulants found in this effluent;

4. Methods for the large scale economical production of enzymes used in cleaning regimes. Expression systems available in bacteria and yeast will be further evaluated in this regard;

5. The implementation of these new-fashioned cleaning methods in field experiments at a pilot plant, followed by optimisation of techniques for actual operating conditions. These successful biological cleaning methods, with all the advantages previously discussed, can then be introduced for industrial application.

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