CLEANING OF FOULED MEMBRANES USING ENZYMES FROM A SULPHIDOGENIC BIOREACTOR

A thesis submitted in the fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

of

RHODES UNIVERSITY

in the

Department of Biochemistry, Microbiology and Biotechnology
Faculty of Science

by

Xolisa Melamane

December 2003

TABLE OF CONTENTS

ABSTRACT

Maintenance of membrane performance requires inevitable cleaning or defouling of fouled membranes. Membrane cleaning using enzymes such as proteases, lipases, α-glucosidases from a sulphidogenic bioreactor was investigated. At first, dilute and concentrated enzyme extract were prepared form the sulphidogenic pellet. Enzyme assays on 0.5 % azocaisen, 1 % triacetin and 1 mg/ml ρ-nitrophenyl-α-D-glucopyranoside were performed using the concentrated enzyme extract (0 – 200 mg/ml). For membrane fouling, an abattoir effluent was obtained from Ostritech Pty (Ltd), Grahamstown, South Africa. The effluent was characterised for presence of potential foulants such as lipids, proteins, amino acids and carbohydrates. Static fouling of polysulphone membranes (0.22 μm, 47 mm) was then performed using the abattoir effluent. Cleaning of the fouled membranes was also performed using at first the dilute and then the concentrated form (200 mg/ml) of enzyme extracts. Qualitative and quantitative biochemical analysis for proteins, lipids and carbohydrates was performed to ascertain the presence of foulants on polysulphone membranes and their removal by dilute or concentrated enzyme extracts. The ability of dilute enzyme extracts to remove proteins lipids, and carbohydrates fouling capillary UF membrane module; their ability to restore permeate fluxes and transmembrane pressure after cleaning/defouling was also investigated. Permeate volumes from this UF membrane module were analysed for protein, amino acids, lipids, and carbohydrates concentrations after fouling and defouling. Fouling was further characterized by standard blocking, cake filtration and pore blocking models using stirred UF cell and polyethersulphone membranes with MWCO of 30 000, 100 000 and 300 000. After characterization of fouling, polyethersulphone membranes with MWCO of 30 000 and 300 000 were defouled using the concentrated enzyme extract (100 mg ml).

Enzyme activities at 200 mg/ml of enzyme concentration were 8.071 IU, 86.71 IU and 789.02 IU for proteases, lipases and α-glucosidases. The abattoir effluent contained 553 μg/ml of lipid, 301 μg/ml of protein, 141 μg/ml of total carbohydrate, and 0.63 μg/ml of total reducing sugars.

Proteins, lipids and carbohydrates fouling polysulphone membranes after a day were removed by 23.4 %, when a dilute enzyme was used. A concentrated enzyme extract of 200 mg/ml was able to remove proteins, lipids and carbohydrates up to 5 days of fouling by 100 %, 82 %, 71 %, 68 % and 76 % respectively. Defouling of dynamically fouled capillary ultrafiltration membranes using sulphidogenic proteases was successful at pH 10, 37°C, within 1 hour. Sulphidogenic proteases activity was 2.1 U/ml and flux Recovery (FR %) was 64. Characterization of fouling revealed that proteins and lipids were major foulants while low concentration of carbohydrates fouled polyethersulphone membranes. Fouling followed standard blocking for 10 minutes in all the membranes; afterwards fouling adopted cake filtration model for membranes with 30 000 MWCO and pore blocking model for membranes with 300 000 MWCO. A concentration of 100 mg/ml of enzyme extract was able to remove fouling from membranes with MWCO of 30 000. Defouling membranes that followed pore blocking model i.e. 300 000 MWCO was not successful due to a mass transfer problem. From the results of defouling of 30 000 and 300 000 MWCO it was concluded that defouling of cake layer fouling (30 000 MWCO) was successful while defouling of pore blocking fouling was unsuccessful due to a mass transfer problem. The ratio of enzymes present in the enzyme extract when calculated based on enzymatic activity for proteases, lipases and α-glucosidases was 1.1 %, 11 % and 87.9 %. It was hypothesized that apart from proteases, lipases, α and β-glucosidases; phosphatases, sulphatases, amonipeptidases etc. from a sulphidogenic bioreactor clean or defoul cake layer fouling by organic foulants and pore blocking fouling provided the mass transfer problem is solved. However, concentration of enzymes from a sulphidogenic bioreactor has not been optimized yet. Other methods of concentrating the enzyme extract can be investigated for example use of organic solvents.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AMD	-	ACID MINE DRAINAGE
BSA	-	BOVINE SERUM ALBUMIN
CE	-	CONTROLLED ENVIRONMENT
CFM	-	CAKE FILTRATION MODEL
COD	-	CHEMICAL OXYGEN DEMAND
ED	-	ELECTRODIALYSIS
EDR	-	ELECTRODIALYSIS RERVERSAL
EPS	-	EXTRACELLULAR POLYMERIC SUBSTANCES
FR	-	FLUX RECOVERY
FSBR	-	FALLING SLUDGE BED REACTOR
LSI	-	LANGELVIER SATURATION INDEX
MF	-	MICROFILTRATION
NF	-	NANOFILTRATION
MWCO	-	MOLECULAR WEIGHT CUT-OFF
NOM	-	NATURAL ORGANIC MATTER
PBM	-	PORE BLOCKING MODEL
PSS	-	PRIMARY SEWAGE SLUDGE
PV	-	PERVAPORATION
PWF	-	PURE WATER FLUX
RO	-	REVERSE OSMOSIS
RPM	-	REVOLUTIONS PER MINUTE
RSI	-	RYZNA STABILITY INDEX
SBM		STANDARD BLOCKING MODEL
SDS	-	SODIUM DODECYL SUPHATE
SRB	-	SULPHATE REDUCING BACTERIA
TCA	-	TRICHLOROACETIC
TSS	-	TOTAL SUSPENDED SOLIDS

UF	-	ULTRAFILTRATION
VFA	-	VOLATILE FATTY ACIDS
WSE	-	WOOL SCOURING EFFLUENT

ACKNOWLEDGEMENTS

I extend my sincere gratitude to the following people whom without this thesis would have never materialized.

My supervisor Professor C.G. Whiteley, for his guidance, unwavering support and for the opportunity to be involvedin a challenging research area.

My co-supervisor Dr W.D. Leukes, for his guidance, assistance and motivation.

Dr B.I Pletschke for encouragement, Dr J.A. Burgess for her devotion towards the success of this thesis and members of Lab 309 and 315.

Members of staff in the Department of Biochemistry, Microbiology and Biotechnology for their support and Rhodes Campus Securities for every night I have been escorted home.

National Research Foundation (NRF), Water Research Commision (WRC) and Deutscher Akademischer Austaucherrdienst (DAAD) for financial support.

I thank Grahamstown Sewage Works and Ostritech Pty (Ltd) for their assistance during sampling.

To Tabita, Nosisa, Lungelwa, Mpho D, Loyiso, Mbasa, Vuyo A, Vuyo M, Phehello, Seun, and Tonde.

To my sisters LamaHlubi and Yonela you re the best gifts in the entire universe!

To my other family Granny, Auntie Vuyo, Zowe, Mdala, Sis Lulama, Buti Sivuyile, Azukiwe, Lubabalo, Batandwa, Pumza, Bayanda, Gcobani, Anele, Athi Zenande and Liyavuma thank you for your prayers and support, *niyinto yonke kum*.

This thesis is dedicated to my late Dad Mongezi and Mom Nomawethu Melamane who have realized potential in me before every one could and have believed in me even when I did not believe in myself.

Above all, glory and honour be unto Him my Keeper and Provider, the One who began a good work in me, Jesus Christ.

CHAPTER 5
CHARACTERIZATION AND CLEANING OF FOULING USING STIRRED ULTRAFILTRATION CELL

5.1 Introduction

There is considerable experimental evidence that protein adsorption within the pores of UF membranes can dramatically alter membrane transport properties (Belfort *et al.,* 1994). Protein adsorption is nearly a universal phenomenon, with the extent and strength of the interaction governed by the physical and chemical properties of the protein, membrane surface and of the solvent (e.g. pH and ionic strength) (Chen et al., 1995; Bowen and Gan, 1994; Robertson and Zydney, 1990). In general protein adsorption is entropically driven, but there is little quantitative data on the structural changes in the protein associated with adsorption. Protein adsorption is strongly affected by electrostatic interactions with maximum adsorption typically attained near the protein isoelectric point (Belfort *et al.,* 1994). At the same time proteins can adsorb quite strongly even to surfaces with the same charge as the protein itself. Many investigators have successfully described protein adsorption using a Langmuir-type isotherm, with the plateau values in good agreement with those for a close-packed protein monolayer (Chen et al., 1995; Belfort *et al.,* 1994). Other investigators however, report multilayer adsorption under essentially the same experimental conditions (Chen *et al.,* 1996; Kelly and Zydney, 1995). Understanding protein fouling is fundamental in designing and controlling membrane processes for many biotechnology applications. Direct observations of protein deposits after fouling have revealed that varying types of fouling have been found on both UF and MF membranes. During filtration, significant surface deposits may occur, some in large aggregates (Kelly and Zydney, 1995). However, internal deposition in the form of monolayers or multilayers, is still difficult to determine by direct observation and must still be deduced by analysis of total amount of protein on the membrane using the spectroscopic methods, radio-labeling or other techniques. The basic mechanisms of fouling have also been studied (Chen et al., 1995).

The general consensus appears to be that fouling may be due to one or more of the following mechanisms (1) surface adsorption of solutes (2) gradual, irreversible changes to the polarized membrane such as cake consolidation and (3) adsorption of solutes within the membrane (Czekaj *et al.*, 2001; 2000). When a stream of biological origin is filtered, membrane fouling can be caused by pore size constriction, pore blocking, or the deposition of cells, cell debris, or other particles such as macromolecules or macromolecule aggregates on the top surface of the membrane. These decreases permeate flux and according to Belfort *et al.*, (1994), colloidal fouling occurs in two stages: internal and external fouling. Internal fouling is caused by adsorption or deposition of small particles and macromolecules within the pores. Consequently two models have been proposed to describe internal fouling, namely the standard blocking model (SBM), which assumes that molecules are adsorbed on the pore walls thus reducing their effective diameter [Figure 5.1] and the pore blocking model (PBM), which assumes that molecules or their aggregates completely block some of the pores while leaving the rest unaffected [Figure 5.1]. The two phenomena occur simultaneously during MF (Czekaj *et al.*, 2001; 2000; Mueller and Davis, 1996; Güell and Davis, 1996).

Molecules may reduce the diameter of the larger pores and block smaller pores at the same time. The cake filtration model (CFM) describes external fouling. This model assumes that filtered substances (cells, cell debris and aggregates) build up a cake on the surface of the membrane, and resistance gradually increases [Figure 5.2]. The CFM, PBM and SBM can be distinguished experimentally by plotting resistance versus time [Figure 5.3]. Internal fouling (SBM or PBM) is characterized by a “concave-up” form of the curve, while external fouling (CFM) leads to a “concave-down” form of the curve (Ho and Zydney, 2002; Berg and Smolders, 1989). Proteins, polyphenols and polysaccharides are much smaller than the pore size of typical MF membranes and can cause significant fouling. Some work has been reported on membrane fouling during MF of protein mixtures. It was found that protein fouling does not depend on the size of individual proteins, but on their ability to aggregate (Güell and Davis 1996). Detailed mechanisms of organic membrane fouling caused by polysaccharides and polyphenols are relatively scarce. Vernhet, *et al.*, (1999) evaluated the influence of wine polysaccharides on polyethersulfone membrane fouling and concluded that mannoproteins may cause the strongest decrease in wine filterability, but again this cannot be directly related to their initial concentration.

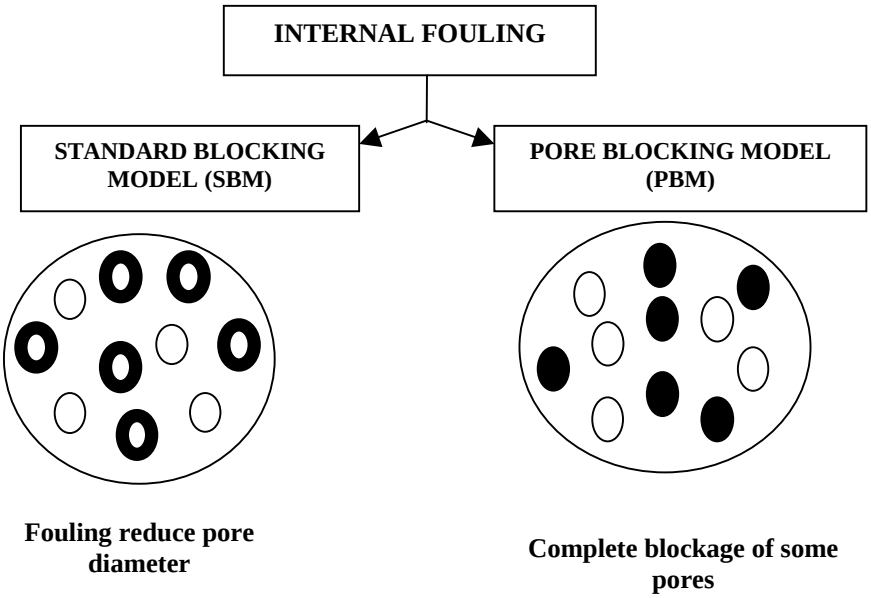


Figure 5.1: Internal fouling occurring as standard blocking and or pore blocking model.

Bellville et al., (1992) studied fouling of an inorganic tubular alumina membrane during red wine MF and mainly attributed fouling to polysaccharides and phenols. Research by Vernhet and Moutounet, (2002) has covered polysaccharide and tannin adsorption on polymeric membranes under static conditions. Their results indicated that the adsorption of polysaccharides and tannins was dependent on membrane polarity. Polyphenol adsorption was stronger on more polar membranes, while the polarity of the membrane surface was a limiting factor in polysaccharide adsorption (Czekaj *et al.*, 2001; 2000). However, it is well known that these results cannot be extrapolated to the dynamic conditions during membrane filtration. Membrane fouling during the filtration of beer with inorganic membranes has also been studied. The flux decreased mainly because of internal fouling caused by carbohydrates. Fouling caused by the mannoprotein was reduced by the pectic polysaccharide which suggests that overall membrane fouling depends on the intermolecular and membrane-foulant interactions (Czekaj *et al.*, 2001; 2000). There is still a large degree of uncertainty regarding a detailed mechanism by which adsorbed foulants alter the membrane as it affects membrane pores differently. Depending on the size of the membrane pores and molecular weight of solutes, pore blockage, pore constriction or cake layer maybe formed.

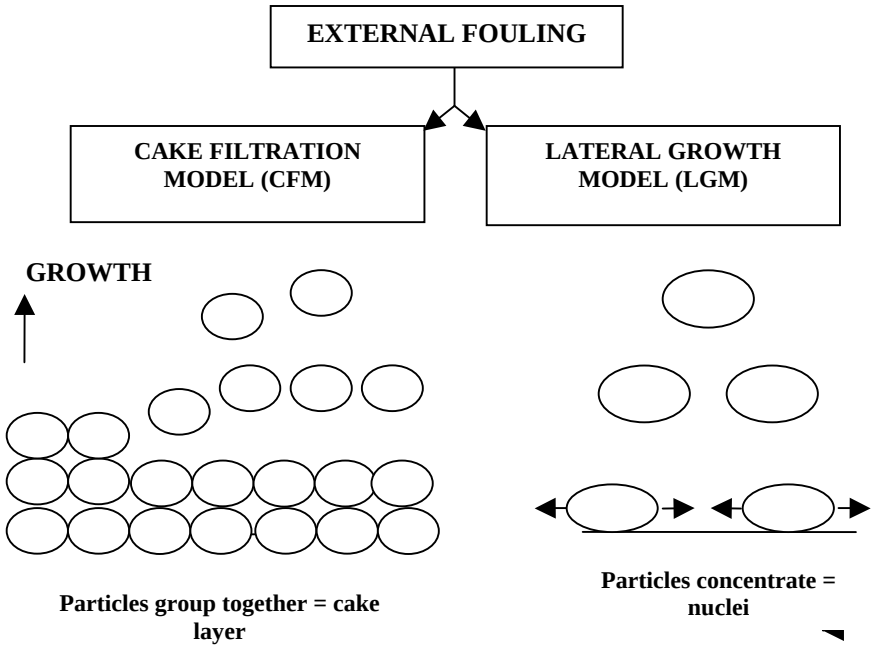


Figure 5.2: External fouling occurring as cake layer or following the lateral growth model.

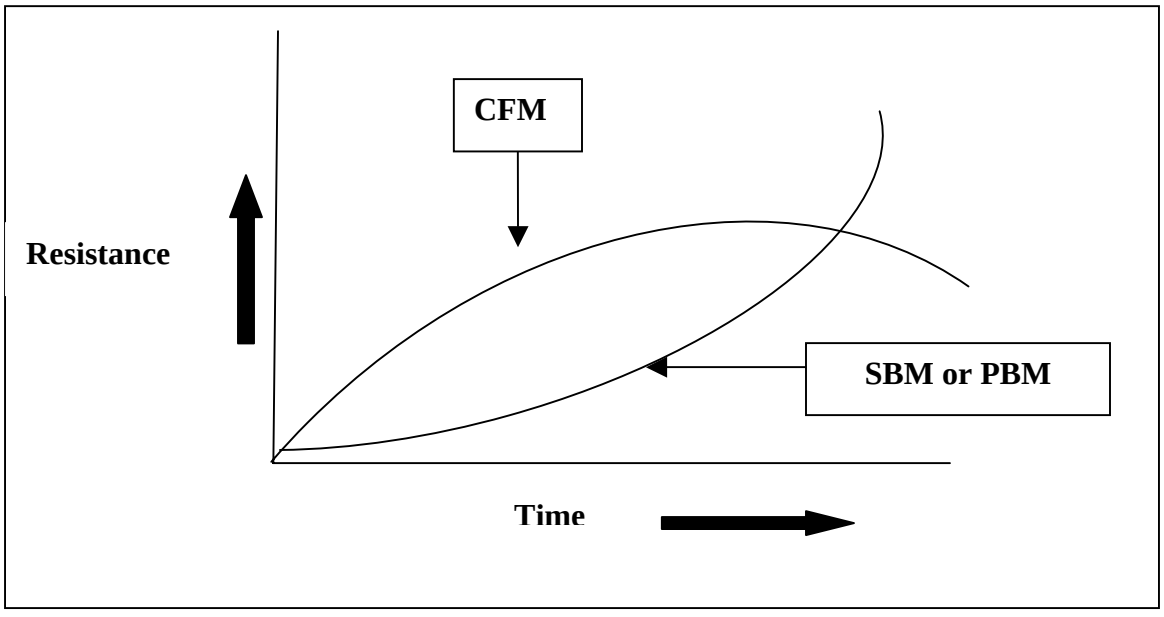


Figure 5.3: Schematic presentation of the mechanisms of internal fouling.

However, researchers still disagree on whether the primary mode of fouling is by surface or pore deposition. As a result it is necessary to understand the mechanism of fouling by abattoir effluent so that effective defouling process can be developed.

5.1.1 Objectives

- Characterization of fouling by abattoir effluent using polyethersulphone membranes with varying pore sizes (30 000, 100 000, and 300 000 MWCO).
- To investigate stirring effect on fouling and fitting the filtration or fouling data into established mathematical models for different fouling mechanisms.
- To clean/defoul fouled polyethersulphone membranes using enzymes from a sulphidogenic bioreactor.

5.2 Materials and methods

5.2.1 Materials

Abattoir effluent used for fouling was obtained from Ostritech (Pty) Ltd, Grahamstown, South Africa. A stirred UF cell used for characterization of fouling was purchased form Microsep (Pty) Ltd, South Africa. Polyethersulphone UF membranes with MWCO of 30 000, 100 000 and 300 000 (Millipore Biomax UF discs) were also purchased from Microsep (Pty) Ltd, South Africa. Nitrogen gas was supplied by Afrox Limited, South Africa. Reagents for protein, glycerol, carbohydrate, reducing sugar assays and for protein staining are as listed in section 3.2.

5.2.2 Stirred ultrafiltration cell set-up

The stirred UF cell was set-up at room temperature (23 ± 2°C) as in figure 5.3. All UF experiments were performed in a batch cell of 180 ml capacity with a membrane area of 3.17 cm^2 . The stirred UF cell consists of a cylindrical vessel containing the test solution, surmounting the porous support on which the membrane is placed. The top end of the cell contains a pressure relief valve and a gas inlet.

The UF cell houses an internal magnetic stirring bar (8 cm long, 63.5 mm diameter) suspended 1 cm above the membrane. Polyethersulphone UF membranes with 30 000, 100 000 and 300 000 MWCO were inserted in the UF cell for each fouling experiment. For UF cell operation, an inlet line was connected to a regulated gas pressure source. Nitrogen gas was used for pressurizing in place of compressed air that can cause large pH shifts, due to dissolution of carbon dioxide and prevents oxidation that would lead to other potential problems.

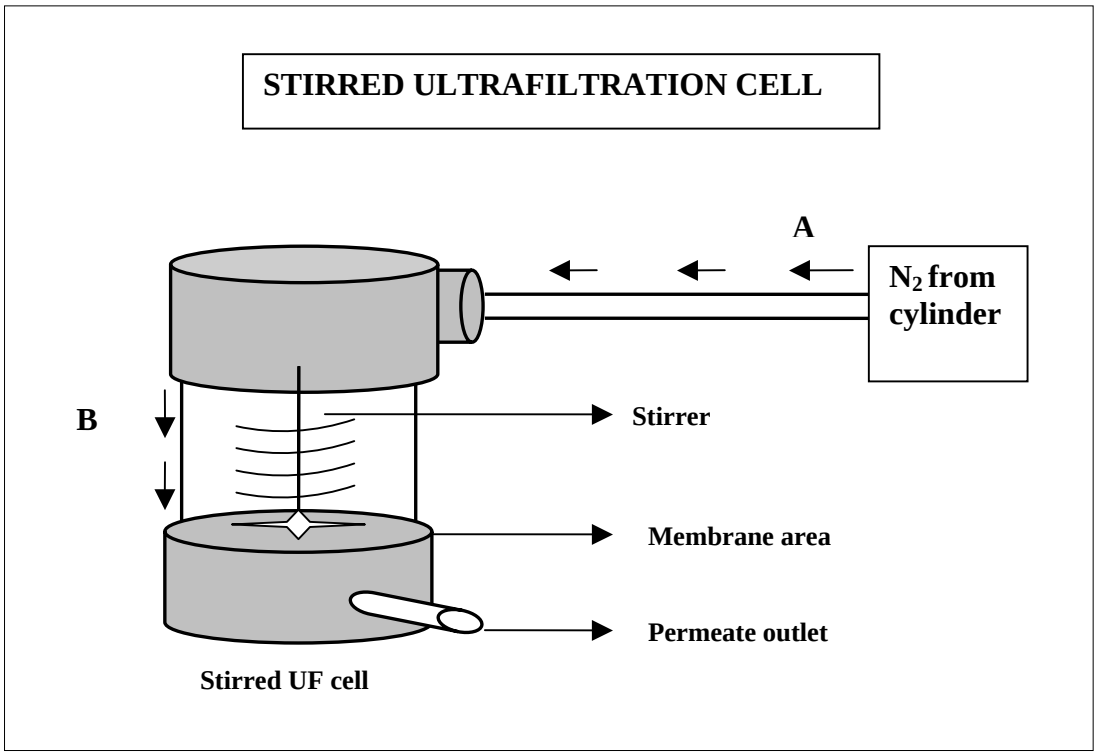


Figure 5.4: Schematic representation of stirred UF cell.

5.2.3 Fouling of polyethersulphone membranes using stirred UF cell

Fouling of polyethersulphone membranes with MWCO of 30 000, 100 000, and 300 000 was performed using the stirred UF cell. The objective was to compare the formation of foulant layer on these membranes and to investigate the effect of stirring (0, 100, and 400 revolutions per minute) on the rate of flux decline. The stirred UF cell was assembled as described in paragraph 5.2.2 and one membrane was inserted for each fouling experiment. The inserted membrane was first rinsed with lukewarm deionized water to remove glycerol preservative.

Pure water flux was measured at 10 minute intervals, 180 ml abattoir effluent was added and ΔP of 50 kPa was used to filter the effluent. Each membrane was fouled for an hour by stirring at 0, 100, and 400 revolutions per minute (RPM). Permeate volumes were collected at 10 minute intervals using a measuring cylinder. After fouling for 1 hour, PWF of the fouled membrane was determined by replacing the remaining effluent with deionised water and collecting permeate volume at 10 minute intervals. Membranes and permeate volumes were preserved by storing them at 0 °C. Permeate flux (J) was calculated as follows:

$$\text{Permeate flux } (L/m^2/hr) = \frac{\text{permeate volume (L)}}{\text{Surface area } (m^2) \text{ X Time (hr)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Abattoir effluent and permeate volumes were analyzed for protein, lipid and carbohydrate concentrations. Protein concentrations from stirred fouling were used to calculate percentage rejection. Percentage rejection was calculated as follows:

$$R\ (\%) = 100[1 - (C_p / C_b)] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

R = rejection
C_p = concentration in the permeate
C_b = concentration in the bulk solution of abattoir

5.2.4 Fitting of filtration data to establish fouling models

A set of equations relating to filtration volume *(V)* and filtration time *(t)* have been developed (Hermia 1982; Sugahara *et al.,* 1979). The equations are known as the constant-pressure blocking filtration laws and are summarized in Table 5.1. The equations were obtained by integration of the general equation:

$$\frac{d^2t}{dV^2} = k\,(dt/dV)^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

In order to establish the mechanisms of fouling the filtration data was plotted as follows:

(i) t/V(min/ml) versus volume (ml) for blocking model,

(ii) Exponential time versus volume (ml) for pore blocking model and

(iii) t/V(min/ml) versus time for cake filtration, standard blocking and pore blocking models.

Table 5.1: Equations for the constant pressure blocking filtration laws (Hermia 1982).

Model	*k*	*n*	Integrated equation	Equation
Complete blocking	$K_b = u_{o\sigma}$	2	$K_b V = Q_o(1 - \exp(-K_b t))$	4
Intermediate blocking	$K_i = \sigma / A$	1	$K_i V = \ln(1 + K_i Q_o t)$	5
Standard blocking	$K_s = (2C/LA_o) Q_o^{0.5}$	1.5	$K_s t = \frac{2t}{V} - \frac{2}{Q_o}$	6
Cake filtration	$K_c = \frac{\alpha\gamma s}{AR_m Q_o(1 - ms)}$	0	$K_c V = \frac{2t}{V} - \frac{2}{Q_o}$	7

Table 5.2: Relationships in Table 5.1.

A	= membrane area	R_m	= membrane resistance
A_o	= initial membrane area	S	= mass fraction of solids in slurry
C	= volume of solid particles	T	= time
L	= membrane thickness	V	= volume
K_b	= complete blocking model	K	= proportionality constant
K_C	= cake filtration model	N	= proportionality constant
K_i	= intermediate blocking model	α	= cake specific resistance
K_S	= standard blocking model	γ	= filtrate density
M	= mass ratio of wet to dry cake	$u_{o\sigma}$	= initial fluid velocity and standard deviation
Q_o	= initial flow rate		

5.2.5 Defouling polyethersulphone membranes characterized by standard blocking and pore blocking models

100 mg/ml enzyme was prepared as in paragraph 2.2.4 in order to defoul polyethersulphone membranes with 30 000 and 300 000 MWCO. A fouled membrane was placed in the stirred UF cell and 10 ml of 100 mg/ml enzyme extract were added. The UF cell was incubated at 37 °C for 1 hour.

After defouling, the enzyme extract was removed and the PWF of each membrane was determined. Pure water fluxes of clean, fouled and defouled membranes were compared to determine the extent of defouling.

5.3 Results and discussion

5.3.1 Fouling of polyethersulphone membranes using abattoir effluent

Reduction in flux over time may be substantial and represents a loss in membrane performance. Figures 5.5, 5.6 and 5.7 show the effect of stirring on the rate of flux decline for membranes with MWCO of 30 000, 100 000 and 300 000 respectively. Figure 5.5 shows a significantly lower flux when the UF cell was operated without stirring. According to Bowen *et al.,* (1991) determination of protein binding at zero applied shear conditions gives incomplete assessment of the effects of protein membrane interactions. As a result, in this study flux decline was also monitored under stirring conditions. Higher fluxes were observed with increased stirring speed with no significant difference in flux decline between stirring at 100 or 400 rpm in all the membranes. Higher fluxes experienced during stirring suggested that stirring moderated the fouling effect by reducing the net transport of the solute to the membranes. These results agreed with Bowen *et al.,* (1991) where stirring of the solution containing foulants caused an increase in membrane permeation. Circular Dichroism and Fourier Transformed Infrared Spectroscopy studies on capillary pore polycarbonate MF membranes by Franken *et al.,* (1990) suggested that small changes occur in the secondary structure of the protein when subjected to shear or stirring. This is the opposite of what occurs during fouling without stirring, where the tendency of proteins to aggregate is very high and causes rapid fouling (Güel and Davis, 1996). Figure 5.5 also shows that rapid fouling occurred within approximately 10 minutes (0.17 h) irrespective of stirring. Rapid fouling can also be attributed to the fact that the abattoir effluent had a high concentration of protein (146.36 μg/ml) as in Table 5.4 and agrees with Bowen *et al.,* (1991) where at higher concentrations of protein rapid flux decline was experienced. Complete fouling was also observed after 25 minutes (0.42 h) for unstirred UF and after approximately 35 minutes (0.58 h) for 100 and 400 rpm.

These results confirmed findings by Chudacek and Fane, (1984) that in unstirred UF, solutes are carried by convective flow to the membrane surface where they accumulate and provide increasing barrier to solvent flow. Rapid fouling can also be attributed to the type of membrane used, in this case polyethersulphone membranes. Hydrophobic membranes such as polyethersulphone and polysulphone have been proven to be very susceptible to fouling (Chaturvedi *et al.*, 2001; Mulder, 1991). Figure 5.6, and 5.7 show the effect of stirring speed when MWCO of the polyethersulphone membranes were increase to a 100 000 and 300 000. The rate of fouling was approximately the same with and without stirring. In both membranes, rapid fouling occurred within approximately 10 minutes (0.17 h) of UF. It can be concluded that flux reduction becomes less pronounced with increasing pore size (from 30 000 to 300 000 MWCO) regardless of whether the system was stirred or not. Comparison of permeate flux decline in all three membranes, higher permeate fluxes were evident with polyethersulphone membranes when MWCO was larger than the particles to be filtered.

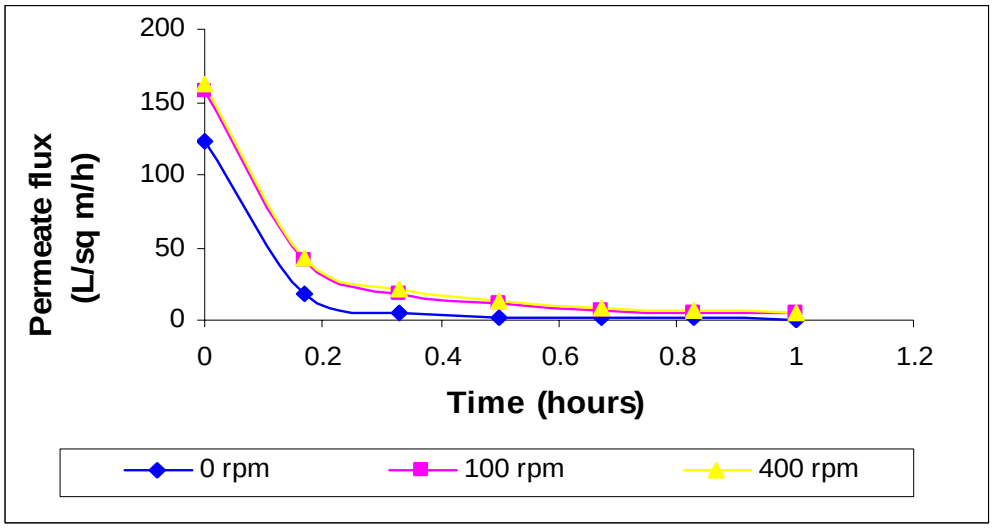


Figure 5.5: Fouling of polyethersulphone membranes with 30 000 MWCO using stirred UF cell.

For unstirred 30 000 MWCO the initial flux was 122.78 L/m^2/h, it was reduced to 17.95 L/m^2/h after 10 minutes; for 100 000 MWCO, initial permeate flux was approximately 406.29 L/m^2/h and was reduced to 24.56 L/m^2/h and for 300 000 MWCO, initial flux was 623.36 L/m^2/h reduced to 90.67 L/m^2/h. These results suggested that cake layer formation occured upon fouling of polyethersulphone membranes with 30 000 MWCO, and that pore constriction was the major cause of fouling in polyethersulphone membranes with 100 000 and 300 000 MWCO.

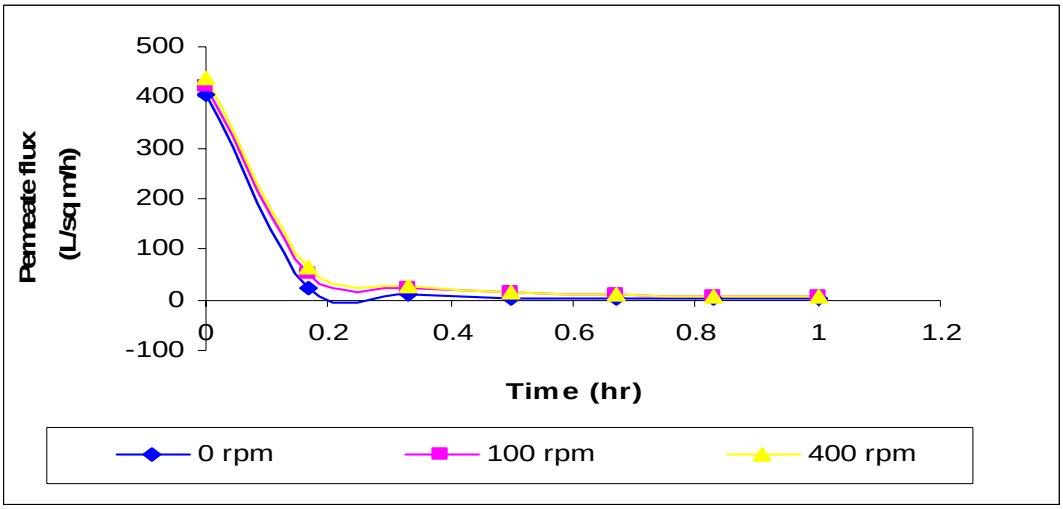


Figure 5.6: Fouling of polyethersulphone membranes with 100 000 MWCO using stirred UF cell.

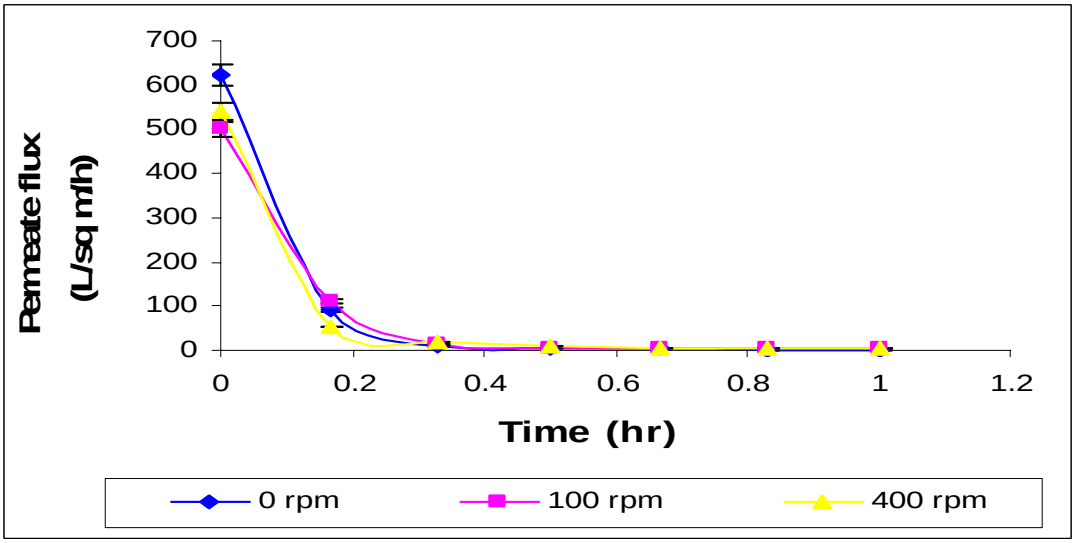


Figure 5.7: Fouling of polyethersulphone membranes with 300 000 MWCO using UF stirred cell.

5.3.2 Characterization of fouling using standard blocking pore blocking and cake filtration models

The unstirred UF data plotted according to the constant pressure blocking laws are shown in Figure 5.8 and 5.9. This was done in order to identify the dominant mechanism of fouling on the polyethersulphone membranes with MWCO of 30 000, 100 000 and 300 000.

A linear relationship for both standard blocking and cake filtration models except for deviations at very early stages for all membranes is shown in Figure 5.8. In the plot of exponential time versus volume [Figure 5.9], early deviations from linearity were also observed. These deviations confirmed that fouling followed the pore blocking model. In the t/V versus volume plot [Figure 5.8], the curves approached a linear relationship after filtration volumes of 14.1 ml, 28.4 ml and 65 ml for 30 000, 100 000 and 300 000 MWCO respectively. On the other hand, exponential time versus volume plots showed linearity from 10 ml, 24 ml and 50 ml which suggested that there was fouling or blockage of a few large pores by particles in the abattoir effluent that had contact first with the membrane surface. These results agreed with Kim *et al.*, (1993) who filtered silver particles on a stirred UF cell. The only difference was that Kim *et al.*, (1993) experienced the same phenomenon with larger filtration volumes. As a result, it is apparent that during filtration and after most of the accessible pores are blocked, the fouling mechanism that prevailed was pore blocking at first followed by the standard blocking model or cake filtration model. There is a high probability that fouling of 30 000 MWCO polyethersulphone membranes followed the cake filtration model, as the protein foulants present in the abattoir effluent, albumin and globulin are larger than the membrane pores. In addition, research on mechanisms for fouling during MF has suggested that large aggregates are formed and serve as attachment (nucleation) sites for deposition by other foulants (Kelly and Zydney, 1995). It is possible that the standard blocking model of fouling occurred simultaneously with the cake filtration model and was possibly contributed to by other foulants such as carbohydrates and lipids present in the abattoir effluent.

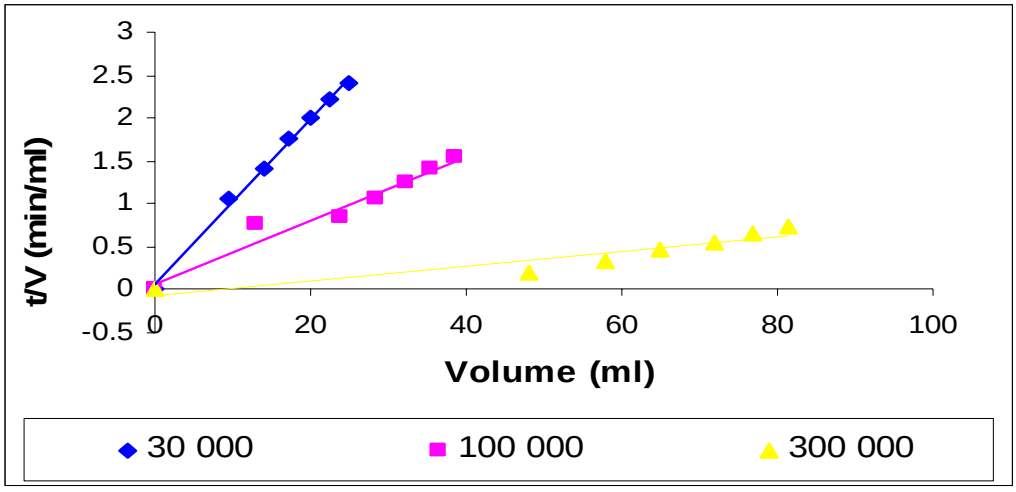


Figure 5.8: Standard blocking and cake filtration models.

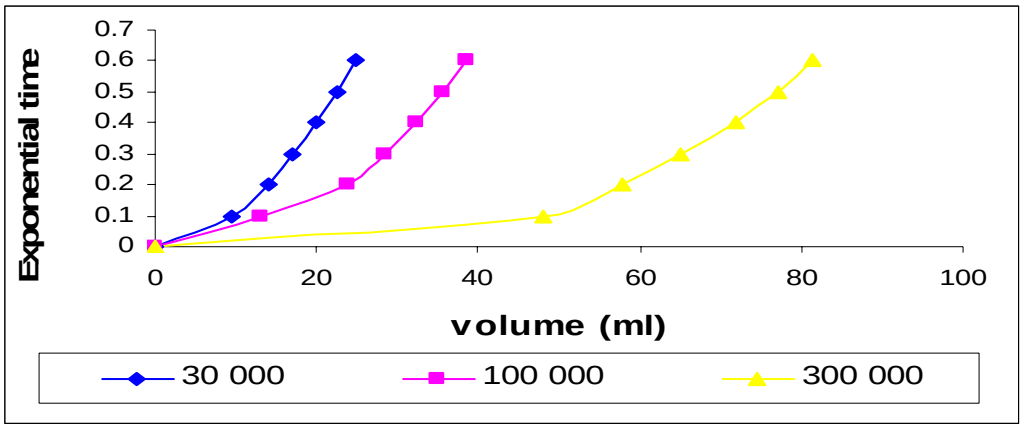


Figure 5.9: Complete blocking model.

The main objective of the research, at this stage was to establish whether fouling occurs, the mechanism and mostly whether fouling can be treated by enzymes from a sulphidogenic bioreactor. At the same time, it is important to note that extensive research on fouling by proteins and polysaccharides has been conducted (Vernhet *et al.*, 1999; Güell and Davis, 1996; Kelly and Zydney, 1995; Cameira-dos-Santos *et al.*, 1994). These researchers have found that standard blocking model, pore blocking model and cake filtration occurs simultaneously during MF.

5.3.3 Defouling polyethersulphone membranes fouled using stirred UF cell

Pure water fluxes of clean, fouled and defouled polyethersulphone membranes with MWCO of 30 000 were 253.15 l/m^2/h, 68.5 l/m^2/h, 94.78 l/m^2/h as shown in Figure 5.10; for 300 000 MWCO PWF were 1833.5 l/m^2/h, 185.5 l/m^2/h and 66.56 l/m^2/h as in Figure 5.11. From Figure 5.10 it is clear that enzymes such as proteases, lipases and α-glucosidases from a sulphidogenic bioreactor were able to defoul polyethersulphone membranes. The defouling efficiency on membranes with MWCO of 30 000 and where the majority of fouling followed the cake filtration model was found to be 27.73 %. This suggested that there was reasonable contact between substrates and enzymes however; defouling is yet to be optimized.

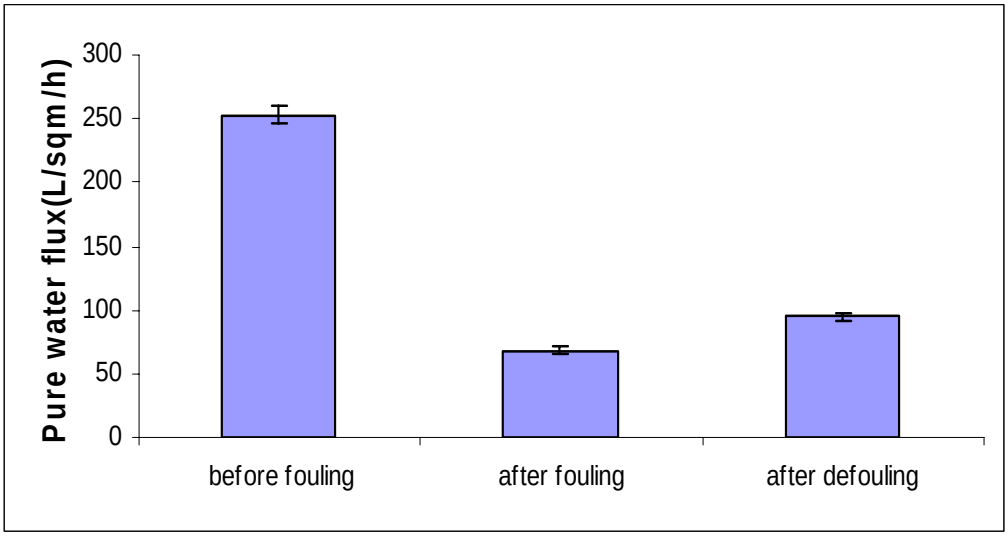


Figure 5.10: Defouling of polyethersulphone membrane with MWCO of 30 000 using enzymes from a sulphidogenic bioreactor.

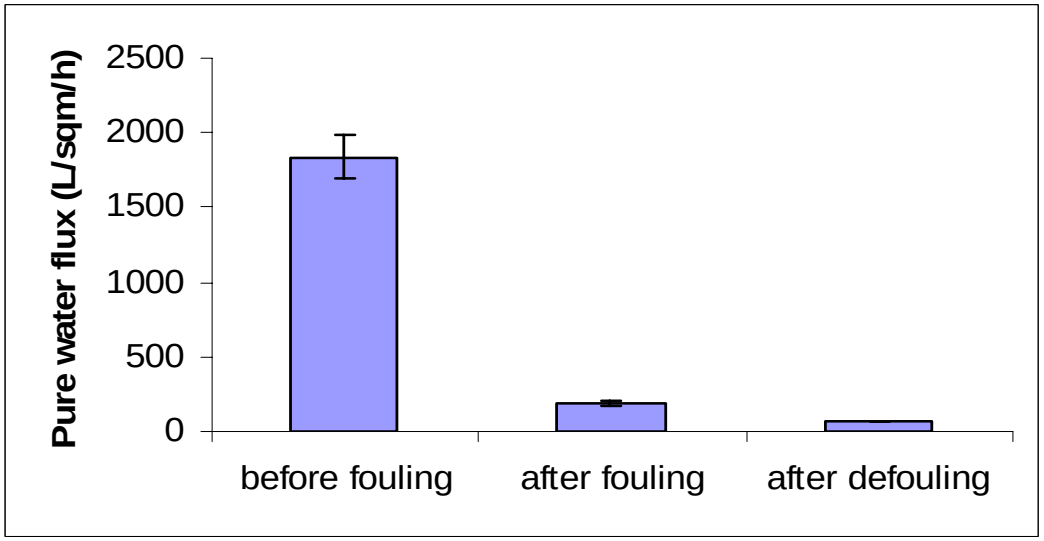


Figure 5.11: Defouling of polyethersulphone membrane with MWCO of 300 000 using enzymes from a sulphidogenic bioreactor.

Successful defouling of polyethersulphone membranes was further confirmed by concentration profiles obtained when membranes were analyzed for protein, glycerol and carbohydrates. Protein concentration after fouling with abattoir effluent was 72.36 μg/ml and 4.72 μg/ml after defouling; the respective glycerol concentrations, an indicator of lipid content was 1.45 μmol/ml and 0.335 μmol/ml and reducing sugar concentration was 4.08 μg/ml after fouling and 6.72 μg/ml after defouling.

There seems to be some refouling by carbohydrates, as reducing sugar concentration increased from 4.08 μg/ml to 6.72 μg/ml. Anthrone assays used to quantify total carbohydrates on fouled and defouled membranes showed that no carbohydrates fouled polyethersulphone membranes. These results suggest that the analyses of carbohydrates within membrane fouling are complicated and can lead to erroneous results. Defouling of polyethersulphone membranes with MWCO of 300 000 was not successful, as the PWF after defouling decreased from 185.5 $l/m^2/h$ to 66.56 $l/m^2/h$.

Table 5.3: Protein, carbohydrate, glycerol concentrations on fouled and defouled polyethersulphone membranes.

MWCO	Samples	Protein concentration (μg/ml)	Glycerol concentration (μmol/ml)	Total carbohydrate concentration (μg/ml)	Reducing sugar concentration (μg/ml)
30 000	Clean membrane	0	0	0	0
	Fouled membrane	72.36 ±2.56	1.450 ±0.078	0	4.67 ±7.170
	Defouled membrane	4.72 ±1.740	0.335 ±0.005	0	6.68 ±0.677
300 000	Clean membrane	0	0	0	0
	Fouled membrane	25.20 ±4.30	0.091 ±0.001	2.48 ±4.49	15.95 ±0.179
	Defouled membrane	5.07 ±0.892	0.116 ±0.001	5.03 ±0.19	13.60 ±1.111

According to Table 5.3 protein and reducing sugar concentrations decreased to 5.07 μg/ml and 13.60 μg/ml, while glycerol and total carbohydrate increased to 0.116 μmol/ml and 5.03μg/ml. This may be due to the fact that the majority of fouling on polyethersulphone membranes with 300 000 MWCO followed standard blocking and pore blocking models which describe internal fouling. At this stage, defouling was possible for membranes that suffered cake filtration model of fouling and was not achievable where blockage occurred within the pores (pore blockage model) Figure 5.11 and Table 5.3. This implies that in an attempt to defoul using enzymes from a sulphidogenic bioreactor a mass transfer problem was experienced which means that there was less or no contact between the enzymes and the foulants.

Therefore, methods need to be explored in order to solve the mass transfer problem, at first to increase filtration pressure during fouling and defouling in order to have the system to produce a laminar flow. Once the laminar flow has been achieved then investigate if defouling efficiency can be improved. Laminar flow also has a potential of not yielding the expected result as enzymes would be exposed to the surface of substrates. Other methods include stirring the enzyme and using a turbulent flow. Turbulent flow has much potential in solving the mass transfer problem as it removes the top layer of foulants with the enzyme thus improving the contact of enzyme with substrate and at the same time exposing a new foulant layer. Turbulent flow in principle allows penetration of the substrate.

5.3.4 Protein, carbohydrate and glycerol concentrations during fouling of polyethersulphone membranes with MWCO of 30 000, 100 000 and 300 000.

Tables 5.4, 5.5 and 5.6 show protein, total carbohydrates, reducing sugars and glycerol concentrations during fouling of polyethersulphone membranes. Protein concentration before fouling (0 minutes) was 146.36 μg/ml, 12.91 μg/ml for total carbohydrates, 16.01 μg/ml for reducing sugars and 0.074 μmol/ml for glycerol. According to Table 5.7 protein concentration remaining after one hour of fouling was 25.8 %, 20.98 %and 21.03 % for 0 rpm,100 rpm and 400 rpm 30 000 MWCO membrane. There was a slight increase on the percentage protein remaining after fouling of 100 000 and 300 000 MWCO membranes. According to Table 5.7 it is clear that proteins were major foulants followed by reducing sugars. There was a high percentage of total carbohydrate and glycerol remaining after one hour of fouling which suggested membranes suffered less fouling by carbohydrates and lipids. Increase in pore size from 30 000 to 100 000 and 300 000 resulted in total rejection of carbohydrates when fouling was performed without stirring as no carbohydrates could be detected on the permeates. Concentration of glycerol decreased on the permeate after defouling from 1.450 to 0.335 μmol/ml. This would be expected as carbohydrates and lipids are far less in molecular weight when compared with pore size of 100 000 and 300 000. The decrease in percentage upon stirring was not expected and seems to contradict the fact that stirring reduces fouling. According to Table 5.6 stirring reduce fouling by proteins only, but encouraged fouling by carbohydrates, reducing sugars and glycerol.

Table 5.4: Protein, carbohydrate, reducing sugars and glycerol concentrations during fouling of polyethersulphone of 30 000 MWCO.

Molecular weight cut-off (MWCO)	Stirring (rpm)	Time (minutes)	Protein concentration (μg/ml)	Total carbohydrate concentration (μg/ml)	Reducing sugar concentration (μg/ml)	Glycerol concentration (μmol/ml)
30 000	0	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	29.730 ±0.413	16.28 ±3.660	7.40 ±0.255	0.033 ±0.015
		20	33.89 ±1.469	9.89 ±1.555	7.44 ±0.605	0.061 ±0.0025
		30	39.69 ±0.643	18.37 ±12.537	8.52 ±0.651	0.062 ±0.002
		40	40.93 ±0.091	6.21 ±0.150	8.87 ±0.348	0.091 ±0.001
		50	40.77 ±0.688	8.19 ±0.150	7.80 ±0.047	0.06 ±0.001
		60	41.84 ±1.010	11.60 ±3.961	6.63 ±0.116	0.12 ±0.001
30 000	100	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	30.21 ±0.000	10.49 ±2.006	5.91 ±0.675	0.084 ±0.002
		20	30.93 ±0.321	8.12 ±0.050	4.87 ±0.186	0.070 ±0.0002
		30	30.41 ±0.367	14.68 ±7.823	4.74 ±0.279	0.071 ±0.0015
		40	30.41 ±0.367	8.014 ±0.200	4.51 ±0.139	0.067 ±0.0009
		50	30.80 ±0.596	7.234 ±0.401	4.69 ±0.255	0.070 ±0.0002
		60	31.45 ±0.413	7.70 ±0.351	6.25 ±0.140	0.078 ±0.0004
30 000	400	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	30.21 ±0.826	6.84 ±0.150	13.21 ±3.884	0.061 ±0.0004
		20	30.86 ±0.046	6.70 ±0.35	5.90 ±0.305	0.096 ±0.001
		30	30.41 ±0.596	7.45 ±0.401	6.94 ±1.163	0.063 ±0.001
		40	30.80 ±0.184	8.33 ±0.451	6.32 ±1.582	0.063 ±0.0001
		50	30.86 ±0.964	7.27 ±0.551	6.5 ±0.768	0.063 ±0.0008
		60	31.51 ±0.229	10.81 ±0.351	1.65 ±0.000	0.061 ±0.001

Table 5.5: Protein, carbohydrate, reducing sugars and glycerol concentrations during fouling of polyethersulphone of 100 000 MWCO.

Molecular weight cut-off (MWCO)	Stirring (rpm)	Time (minutes)	Protein concentration (μg/ml)	Total carbohydrate concentration (μg/ml)	Reducing sugar concentration (μg/ml)	Glycerol concentration (μmol/ml)
100 000	0	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	49.27 ±0.229	12.97 ±3.711	16.99 ±0.675	0.061 ±0.0005
		20	50.53 ±0.55	14.85 ±4.633	51.63 ±0.581	0.096 ±0.0001
		30	51.19 ±0.275	8.79 ±0.902	32.60 ±4.419	0.062 ±0.002
		40	51.38 ±0.091	10.35 ±0.200	30.02 ±1.977	0.062 ±0.0006
		50	51.48 ±0.321	23.30 ±3.159	34.39 ±2.814	0.063 ±0.001
		60	50.60 ±1.286	9.33 ±0.150	6.63 ±0.0116	0.061 ±0.001
100 000	100	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	36.16 ±0.872	8.12 ±0.451	10.83 ±0.558	0.16 ±0.005
		20	26.94 ±0.321	7.55 ±0.150	11.5 ±0.628	0.061 ±0.0006
		30	27.46 ±0.688	9.53 ±0.250	9.56 ±0.488	0.062 ±0.0005
		40	26.48 ±0.596	14.11 ±2.808	10.88 ±4.628	0.064 ±0.0006
		50	27.75 ±0.092	12.51 ±0.150	10.33 ±0.232	0.06 ±0.0007
		60	28.17 ±0.137	13.30 ±0.250	29.87 ±6.094	0.056 ±0.0002
100 000	400	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	35.57 ±0.138	7.98 ±0.651	14.77 ±0.930	0.052 ±0.0005
		20	30.28 ±0.184	9.29 ±0.902	10.43 ±0.465	0.052 ±0.0004
		30	29.11 ±0.642	8.90 ±0.050	11.22 ±1.86	0.05 ±0.0002
		40	30.41 ±0.275	8.19 ±0.250	25.83 ±4.791	0.056 ±0.004
		50	33.72 ±0.091	9.11 ±0.150	15.480 ±5.140	0.051 ±0.003
		60	33.62 ±1.331	10.11 ±0.351	14.61 ±2.512	0.048 ±0.0009

Table 5.6: Protein, carbohydrate, reducing sugars and glycerol concentrations during fouling of polyethersulphone of 300 000 MWCO.

Molecular weight cut-off (MWCO)	Stirring (rpm)	Time (minutes)	Protein concentration (μg/ml)	Total carbohydrate concentration (μg/ml)	Reducing sugar concentration (μg/ml)	Glycerol concentration (μmol/ml)
300 000	0	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	51.81 ±1.239	6.99 ±0.651	2.71 ±0.681	0.31 ±0.005
		20	37.68 ±0.734	8.30 ±0.601	3.33 ±0.764	0.33 ±0.005
		30	45.93 ±6.79	64.36 ±1.253	3.13 ±0.304	0.47 ±0.0362
		40	39.98 ±13.453	39.50 ±1.203	2.97 ±0.144	0.44 ±0.002
		50	42.06 ±14.739	8.51 ±0.100	3.21 ±0.307	0.5 ±0.009
		60	46.06 ±3.765	12.77 ±1.003	3.71 ±0.372	0.41 ±0.005
300 000	100	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	50.11 ±1.515	8.617 ±0.651	39.39 ±3.65	0.4 ±0.0004
		20	35.08 ±1.377	10.43 ±0.501	33.03 ±2.698	0.064 ±0.001
		30	33.10 ±3.351	8.76 ±0.150	16.99 ±1.697	0.055 ±0.0005
		40	27.94 ±1.699	8.26 ±0.050	15.81 ±4.861	0.057 ±0.001
		50	25.41 ±4.040	10.50 ±1.203	14.53 ±3.140	0.045 ±0.0004
		60	26.68 ±0.413	8.54 ±0.351	10.30 ±1.209	0.053 ±0.0009
300 000	400	0	146.36 ±1.648	12.91 ±2.708	16.01 ±0.357	0.074 ±0.002
		10	42.32 ±0.964	13.44 ±0.150	14.77 ±0.930	0.066 ±0.001
		20	31.90 ±1.928	13.33 ±1.303	10.43 ±0.465	0.082 ±0.001
		30	34.01 ±0.229	11.20 ±0.300	11.22 ±1.860	0.073 ±0.0006
		40	32.87 ±2.571	10.63 ±0.501	25.83 ±4.791	0.053 ±0.0001
		50	28.13 ±2.479	9.92 ±0.200	15.48 ±5.140	0.061 ±0.005
		60	22.42 ±0.183	10.85 ±0.601	14.61 ±2.512	0.056 ±0.0006

Table 5.7: Percentage protein, carbohydrates, reducing sugars and glycerol remaining after an hour of fouling of polyethersulphone membranes.

Molecular weight cut-off (MWCO)	Stirring (rpm)	% Protein	% Total carbohydrate	% Reducing sugar	% Glycerol
30 000	0	25.8	91.10	49.60	95.95
	100	20.98	61.11	32.20	98.86
	400	21.03	61.19	42.16	91.89
100 000	0	34.67	102.79	179.33	106.76
	100	19.70	84.04	86.32	104.05
	400	21.95	69.17	96.13	70.27
300 000	0	30.00	181.33	19.86	637.83
	100	22.58	71.14	135.4	151.35
	400	31.94	96.25	96.13	87.87

Thus it can be concluded that organic foulant present in the abattoir effluent fouled polyethersulphone membranes with proteins being major foulants, reducing sugars, carbohydrates and lipids attaching themselves to available sites.

5.4 Conclusions

Characterization of fouling in order to devise the correct cleaning regime and also to test the concentrated enzyme efficiency was performed successfully using the stirred ultrafiltration cell and polyethersulphone membranes of 30 000, 100 000 and 300 000 MWCO. Fouling of polyethersulphone membranes with 30 000 MWCO followed standard blocking model at first and then cake filtration model. Fouling of polyethersulphone membranes with 100 000 MWCO followed all three models namely cake filtration, standard blocking and the pore blocking model. The pore blocking model occurred first on membranes with 300 000 MWCO followed by standard blocking models.

From the results of defouling of 30 000 and 300 000 MWCO it can be concluded that defouling of cake layer fouling (30 000 MWCO) was successful while defouling of pore blocking fouling was unsuccessful due to a mass transfer problem. Further research needs to be conducted to combat the mass transfer problem. Laminar flow in the stirred UF cell has to be established and then ensure that under such conditions defouling is achievable as well. So far turbelant flow has the potential solution to the problem of refouling experienced in polyethersulphone membranes with MWCO of 300 000 [Figure 5.11].

CHAPTER ONE
LITERATURE REVIEW

1.1 Introduction

Membrane processes such as nanofiltration (NF), ultrafiltration (UF), microfiltration (MF), are already very important for water treatment applications as a result of the advances in membrane technology and increasingly stringent requirements for water quality (Tansel *et al.,* 2000). However, membrane fouling which results in loss of productivity is one of the most important operational concerns in membrane technology. For example, membrane fouling severely reduces the economical implementation of ultrafiltration for the purification of biologically related process streams such as abattoir effluent (Jacobs, 1991). A number of theoretical models have been developed to explain membrane fouling mechanisms. These models use system parameters such as viscosity, pore size, membrane thickness, pressure, unsteady-state material and flow balance equations with specific boundary conditions (Chan *et al.,* 2002; Ho *et al.,* 2002; Czekaj *et al.,* 2001; Madaeni *et al.,* 2001; Ho *et al.,* 2000; Chen *et al.,* 1995; Kelly and Zydney, 1995; Kim *et al.,* 1993; Jönsson and Trägårdh, 1989).

Mechanical and chemical removal of foulants usually leads to membrane damage and additional pollution. As a result optimization of membrane defouling/cleaning protocols requires in-depth understanding of complex interactions between the foulant and the membrane (Maartens *et al.,* 1996a). Therefore, it is important to consider the economic impact of cleaning procedures including the costs of the cleaning materials together with the effect of the procedures on membrane lifetime and efficiency (Tansel *et al.,* 2000). For foulants containing proteinaceous components, enzymatic cleaners play a vital role in hydrolysing specific points in the protein strands, while detergent cleaners also interact with protein strands at specific points but in addition rapidly solubilize any small loose protein fragments (Tansel *et al.,* 2000; Maartens *et al.,* 1996a).

Effective cleaning, first with an enzyme and then with a detergent has been shown (Muňoz-Agoudo *et al.,* 1996). Since commercial enzymes are expensive there is a need for a cost effective, readily available and abundant source of enzymes. As a result an investigation has been undertaken to consider enzymes from a sulphidogenic bioreactor for membrane defouling/cleaning as they have proved to be cheap, readily available, and efficient in the solubilization of primary sewage sludge (PSS) under sulphidogenic conditions (Whiteley *et al.,* 2003; 2002a; 2002b; 2002c).

1.2 Membranes in wastewater treatment

Membrane filtration has emerged as a separation technology that is competitive in many ways with conventional techniques, such as centrifugation, distillation, adsorption, absorption, extraction, etc. (Baker, 2000; Fane and Fell, 1987*)*. This technology simultaneously concentrates, fractionates and purifies the products via NF, UF, MF, reverse osmosis (RO), electrodialysis (ED), dialysis and pervaporation (PV) (Beerlange, 2001).

Table 1.1: Membrane processes classified according to the driving force for separation.

DRIVING FORCE	MEMBRANE PROCESS
Pressure gradient (ΔP)	MF, UF, NF, and RO
Electrical potential (ΔE)	ED
Partial pressure	PV
Concentration gradient (ΔC)	Dialysis

A membrane is defined as a selective barrier that permits the separation of certain species in a fluid by a combination of sieving and sorption diffusion mechanisms (Tansel *et al.,* 2000; Mulder, 1991). Membranes can selectively separate components over a wide range of particle sizes and molecular weights, from macromolecular materials such as starch and protein to monovalent ions. In terms of energy, membrane filtration has an important advantage in that unlike evaporation or distillation, no change of phase is involved in the process, thus avoiding the latent heat requirements (Baker, 2000).

Membranes are available in several different configurations such as tubular, hollow-fibre, plate and frame and spiral wound. Some of these designs may work better than others for a particular application, depending on factors such as fluid viscosity, concentration of suspended solids, particle size and temperature (Cheryan, 1986). The degree of filtration or separation of a membrane may be categorised resulting in various membrane processes [Table 1.2] (Cheryan, 1986).

Table 1.2: Characteristics of different membrane processes.

PARAMETERS	MF	UF	NF	RO
Operating pressure (bar)	1 – 4	2 – 7	10 - 40	15 - 100
Pore size (µm)	0.1 – 1.5	0.01 – 0.05	0.001- 0.01	< 0.0002
MWCO range (µm)	> 300 000	300 000 – 100 000	200 000 – 20 000	< 500
Size-cut-off-range (µm)	0.1 - 20	0.005 – 0.1	0.001 – 0.01	< 0.001

These membrane processes are classified according to operating pressure, pore size of the membranes, molecular cut off range and size cut off range [Table 1.2]. Microfiltration is a membrane process that separates micron-size or sub-micron particles from the liquid or gaseous feed stream. The pore sizes of MF membranes are in the range of 0.1 to 1.5 µm. Thus MF typically operates at low transmembrane pressures to minimize build-up of the suspended solids at the membrane surface. Pressures of 0.3 - 3.3 bar and cross flow velocities of up to 3 - 6 m/s in tubular modules are common. On an industrial scale, MF is usually carried out as a multistage (stage in series) operation in a feed and bleed mode of operation. Typical materials removed by MF are starch, bacteria, moulds, yeast and emulsified oils (Kurbekar *et al.,* 1998).

Ultrafiltration is also a low-pressure fractionation process (2 - 7 bar), selecting components by size. It separates dissolved solutes of 0.005 - 0.1 microns. This corresponds to a molecular weight cut-off (MWCO) of about 100,000 to 300,000. Depending on the MWCO selected, the membrane will concentrate high molecular weight species while allowing dissolved salts and lower molecular weight materials to pass through the membrane (Singh[1], 2001; Jönsson and Trägårdh, 1989).

1. Singh, N., Overview of membrane technology: http://www.membraneonline.com/Overview/index.htm

Ultrafiltration membranes are used in numerous industries for concentration and clarification of large process streams (Singh[1], 2001; Jönsson and Trägårdh, 1989). Nanofiltration membranes display excellent rejection of divalent ions while allowing a majority of monovalent ions to pass. Organic molecules in the 200 - 300 molecular weight range are also highly rejected. The unique separation capabilities of NF membranes provide the opportunity to selectively concentrate either valuable or undesirable substances from a process stream with greater effectiveness, consistency, reliability, and economy. Nanofiltration is more cost effective than RO membranes in certain applications. Most water sources contain single charged ions such as Na^+, and Cl^- ions and double charged ions such as Ca^{2+} and SO_4^{2-} ions (Beerlange *et al*., 2001; Kurbekar *et al.,* 1998). Only double charged positive ions in conjunction with certain negatively charged ions can form hard scales within water treatment equipment and piping and are called hardness ions (Beerlange *et al*., 2001; Kurbekar *et al.,* 1998). Depending upon the membrane, water chemistry, and operating conditions, NF membranes can remove more than 90% of feed water's hardness ions. These membranes also remove large colour molecules and many trihalomethane (THM) precursors. The largest users of NF technology are municipal drinking water plants (Beerlange *et al*., 2001; Kurbekar *et al.,* 1998). Reverse osmosis is also growing in the dairy industry for cheese-whey desalting, RO pre-treatment, pharmaceutical concentrations, kidney dialysis units, and maple sugar concentration (Kurbekar *et al.,* 1998). Reverse osmosis is the most complex technique in membrane separation. Its membranes concentrate low molecular weight (MW) organic materials and salts while allowing water and solvents to pass (Girard and Fukomo, 2000). High pressures of about 15 - 100 bar are required in order to overcome high osmotic pressures across the membrane. This permits water to flow from concentrated feed streams to dilute permeates a direction that is just the reverse of what would naturally occur during osmosis. Reverse osmosis has been the most widely used technology for desalinating seawater and reclaiming brackish well water. This membrane process typically rejects all of the organic molecules over 150 MW and a percentage of those between 25 and 150 MW (Girard and Fukomo, 2000; Kurbekar *et al.,* 1998).

1. Singh, N., Overview of membrane technology: http://www.membraneonline.com/Overview/index.htm

Electrodialysis is the transport of ions through the membrane as a result of the application of a direct electric current. If membranes are more permeable to cations than to anions or vice versa, the concentration of ionic solutions increases or decreases, so that concentration or depletion of electrolyte solution is possible. Since in this technique only ionic species are transferred directly, removal of ionic species from non-ionic products can be accomplished, so that purification is possible (Kurbekar *et al.,* 1998). Electrodialysis reversal (EDR) is an ED process in which the polarity of the electrodes is reversed on a prescribed time cycle, thus reversing the direction of ion movement in a membrane stack (Kurbekar *et al.,* 1998).

Pervaporation is the separation of liquid mixtures by partial vaporization through a semi-selective membrane. In this technology, a phase change occurs when the permeate changes from liquid to vapour during its transport through the membrane. Pervaporation is an enrichment technique similar to distillation (Kurbekar *et al.,* 1998). This technique uses a non-porous membrane that either exhibits a high permeation rate for water but does not permeate organics, or permeates organics but not water. The driving force for the process is a gradient in the chemical potential of the substances on the feed side and the permeate side. This force is kept at maximum by applying low pressure to the permeate side of the membrane, combined with immediate condensation of permeated vapours (Beerlange *et al*., 2001; Kurbekar *et al.,* 1998).

The dialysis process relies on concentration-driven transport for separation. It is used for the separation of small solutes from synthetic or biological macromolecules. The membrane material serves to retain molecules larger than the material related cut-off of the membrane by sterically hindering them from entering the membranes. The smaller solutes freely diffuse through the membranes, eventually equilibrating concentration differences. If osmotic pressure is different in the two membrane contacting phases, solvent molecules will also diffuse through the membrane, striving for equilibrium. Dialysis is used in the separation of proteins and other macromolecules from salts in pharmaceutical and biochemical applications (Kurbekar *et al.,* 1998).

One of the most important applications of dialysis is the therapeutic treatment of patients with renal failure. This technique is called haemodialysis and attempts to mimic the action of the nephron of the kidney in the separation of low molecular weight solutes, such as urea and creatinine, from the blood of patients with chronic uraemia (Beerlange *et al.,* 2001; Kurbekar *et al.,* 1998). Dialysis membranes are normally regarded as dense membranes, their porous structure true only in the dry state of the membrane. A dialysis membrane will normally swell in the solvent used, and this introduces a certain separation of the polymer chains in the membrane. This swelling is reversible and introduces a porosity that accounts for the separation of molecules of different sizes (Beerlange *et al.,* 2001; Kurbekar *et al.,* 1998).

1.2.1 Types of membranes

Membranes are classified as symmetric or asymmetric. Asymmetric membranes have tapering pores with larger pore diameter in the top layer as compared with diameter of the pores in bottom layer. In asymmetric membranes the top layer may be non-porous or even made of different material (Mulder, 1991). In symmetric membranes pores can form cylindrical channels; membranes can also have a porous sponge structure (Mulder, 1991). Symmetric membranes can also be non-porous. Most UF membranes have an asymmetric structure with a porous top layer. After preparation of the UF membranes or any other membranes they must be characterized to obtain information about: (i) membrane morphology i.e. pore size and distribution, membrane thickness, and the chemical composition of the membrane surface. and (ii) membrane performance i.e. solvent/gas permeability, separation properties, retention and fouling behavior. Membrane characterization techniques have been classified by Cuperus and Smolders, (1991) as either morphology or performance related [Table 1.1]. Membranes have been further classified by their chemical properties, into hydrophilic; hydrophobic and inorganic membranes. Cellulose acetate, polyacryonitrile, polyvinylchloride, polyimide and polyvinylideneflouride are examples of hydrophilic membranes, while polysulphone, polyethersulphone, and nylon membranes are hydrophobic. Cellulose acetate membranes are easy to prepare, with high flux and high salt retention for RO (Cheryan, 1986).

Chemical stability of cellulose acetate membranes is low; they also have a relatively narrow range of pH tolerance, are highly biodegradable and cannot be used at temperatures above 30 ºC. Polyethersulphone and polysulphone are used successfully in UF as they show a wide range of pH and temperature tolerance. The disadvantage of using this type of membrane is that membrane fouling by adsorption of feed components at the membrane surface may cause a severe flux decline. Polyethersulphone and polysulphone membranes are not very resistant to hydrocarbon media (Chaturvedi *et al.,* 2001; Mulder, 1991). Consequently a number of other polymers have been investigated as UF membrane material. Hydrophilic polymers also known as polymer blends can be used to prevent irreversible fouling (Beelange *et al.,* 2001; Mulder, 1991; Cheryan, 1986). Examples of chemically stable polymers are polyacrylonitrile, polyvinylchloride, etc. Inorganic membranes are gaining importance as UF membranes because they have extremely high chemical and thermal stability. They can be made from glass, metals, or ceramic materials. Disadvantages are that these membranes are brittle and generally more expensive than polymeric membranes. Ceramic membranes are generally based on aluminium oxide (γ-Al_2O_3)/zirconium oxide (ZrO_2). Glass membranes are formed from SiO_2 (Beerlange *et al.,* 2001; Mulder, 1991).

1.2.2 Membrane operation

Membranes operate predominantly in cross-flow filtration mode, where the liquid flow is parallel to the membrane surface. The membrane splits the feed stream into two streams *viz.* permeate and retentate [Figure 1.1] (Tansel *et al.,* 2000). The permeate stream consists of small components that pass through the membrane pores, while the retentate stream consists of components large enough to be retained by the membrane. The retentate stream is usually re-circulated through the membrane module because only one passage through the membrane module may deplete the feed significantly (Girard and Fukumoto, 2000; Tansel *et al.,* 2000). Transmembrane pressure and cross-flow velocity are important parameters that are controlled throughout the membrane modules. Cross-flow velocity is the average rate at which the process fluid flows parallel to the membrane. Velocity has a major effect on the permeate flux, which depends on the applied ΔP for a given surface area up to a threshold ΔP (Tansel *et al.,* 2000).

Above this threshold pressure, which has to be experimentally determined for each application, higher pressures have little or no effect on permeates. In fact, higher pressure may aggravate fouling of the membrane (Tansel *et al.,* 2000).

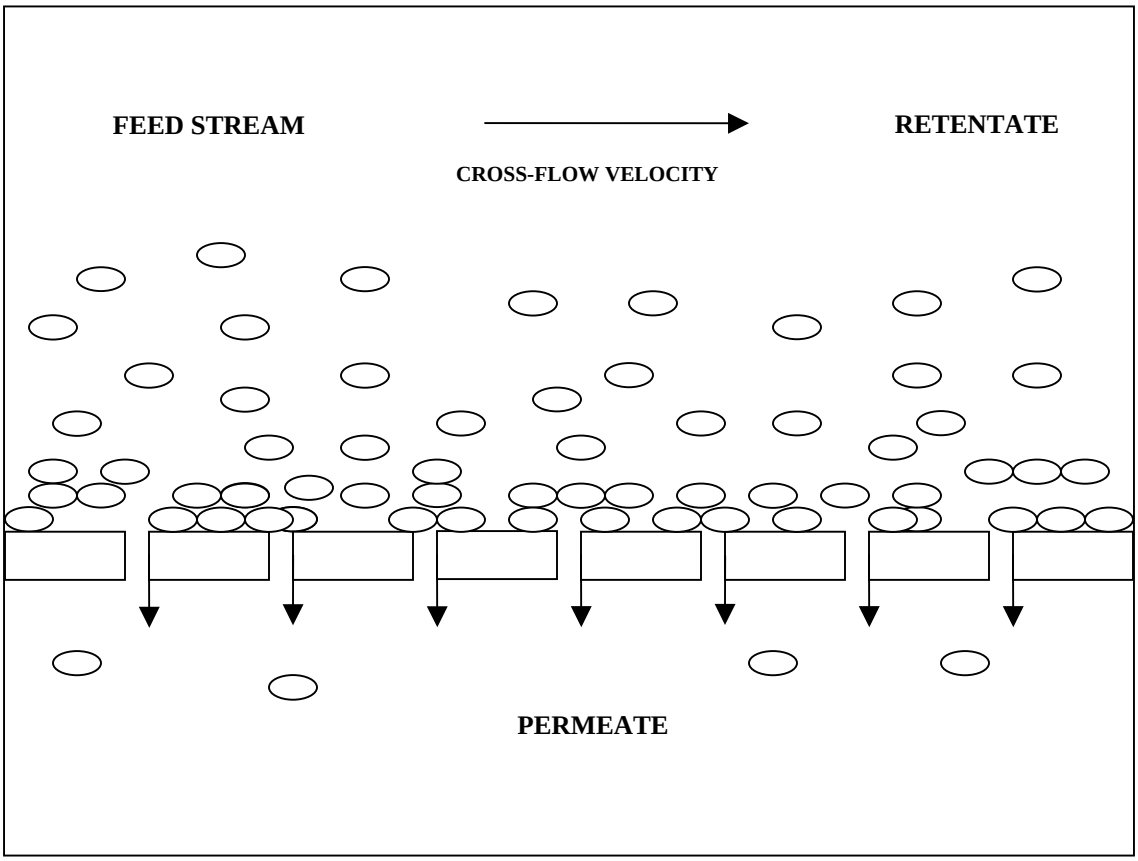


Figure 1.1: Schematic diagram of a feed stream broken into permeate and retentate streams and fouling of the membrane depending on cross-flow velocity.

Membrane processes mentioned in Table 1.1 and 1.2 have been used in water and industrial wastewater treatment applications for volume reduction of aqueous wastes, recovery of chemicals from liquid industrial wastes, desalination, drinking water purification, and removal of oil from oil-water emulsions (Tansel *et al.,* 2000).

1.2.3 Performance of a membrane system

Membrane fouling, which results in loss of productivity, is one of the major operational concerns of membrane processes (Stephenson *et al.,* 2000).

Operational performances in UF systems are often reported in terms of flux through the membrane (Stephenson *et al.,* 2000). When a membrane is clean, the only resistance to flow is the membrane itself. Once the flow starts to permeate through the membrane, fouling begins and flux through the membrane gradually decreases. The overall performance of a membrane system is determined by the following characteristics: (i) membrane selectivity including the characteristics of the membrane material such as its pore size etc. and (ii) permeate flux ($L/m^2/hr$) which is dependent upon the operating pressure, temperature, pH, pore sizes of the membrane, feed composition and flow rate. Typical values may lie within the range of 20 - 2000 $L/m^2/h$ (Beerlange *et al.,* 2000; Stephenson *et al.,* 2000). Particles with effective diameters 2-3 times less than the membrane pore size may be retained, although the efficiency of this sub-pore size rejection depends upon: (i) the loading rate on the membrane and the membrane thickness. (ii) the pore size of the membrane compared to the dimensions of the particles. (iii) the trans-membrane pressure and flux rate, and (iv) the chemical characteristics of the membrane or any charge that is placed on the membrane together with the chemical and physical characteristics of the particles (Stephenson *et al.,* 2000).

1.3 Membrane fouling

Although membrane technology has made great advances recently such as its wide use in wastewater treatment, food and biotechnology industries, fouling still remains a problem. Membrane fouling occurs due to the deposition of suspended or dissolved substances on its external surfaces at or within the pores (Madaeni *et al.,* 2001). Depending on the membrane type, feed composition and process conditions, the membrane performance will decrease due to fouling. Fouling can be quantified by the resistance appearing during the filtration and cleaning can be specified by the removal of this resistance (Güel and Davis, 1996; Kim *et al.,* 1992). Fouling results in (i) loss of membrane performance (ii) lower than expected flux (iii) reduced productivity (iv) need for the use of harsh chemicals as cleaning agents and (v) high cleaning costs. Permanent membrane fouling is caused by the formation of a “gel-layer” which results in severe permanent flux decline and thus a seriously reduced productivity (Güel and Davis, 1996; Kim *et al.,* 1992).

1.3.1 Foulants

Membrane foulants can be classified by their physical type and their location on the membrane *viz.* dissolved solids, suspended solids, biological organisms and non-biological organics. Suspended solids which include colloidal forms of metal oxides such as iron and aluminium/silica, maintain their suspension through a process of repulsion by a double layer of charge. The charge repulsion characteristics of suspended solids also stabilize particulates such as carbon fines which may inadvertently leak from filters (Belfort *et al.,* 1994; Bowen and Gan, 1991).

Biological foulants can be both aerobic and anaerobic living organisms such as bacteria, fungi and algae, and their metabolic waste. Such foulants tend to be present in small concentrations and literally grow into massive quantities that effectively block any flow through the membrane surface (Vernhet *et al.,* 2003).

Organic foulants are substances that contain carbon-based chemical structures like carbohydrates, proteins and lipids (Czekaj *et al.,* 2001; Maartens *et al.,* 1996b; Belfort *et al.,* 1994). Dissolved solids are either cations or anions, such as calcium carbonate, calcium sulphate, barium sulphate and strontium sulphate or scales forming substances which are soluble in feedwaters (Luekes *et al.,* 1999; Bowen and Gan, 1991).

1.3.2 Fouling mechanism and models

Suspended or dissolved solids concentrate or precipitate onto the membrane and when this happens to the point of super-saturation, a nucleus is formed. These nuclei may either precipitate onto the membrane or become attached at a previously fouled area on the membrane surface, known as the site of nucleation (Kelly and Zydney, 1995). In RO higher recovery rates increase the concentration of the salts in the brine. Similarly the calcium and bicarbonate ions or the barium and sulphate ions combine to form a nucleus which precipitates onto the membrane. Calcium carbonate is one of the most common membrane foulants (Kelly and Zydney, 1995).

Its potential for fouling can be measured using the Langelier Saturation Index (LSI), the modified LSI (Stiff and Davis LSI) or the Ryzna Stability Index (RSI) (Beerlage *et al.,* 2000; Bowen and Gan, 1991; Robertson and Zydney, 1990). A positive LSI, which is characteristic of feedwaters with greater hardness, indicates the potential for calcium carbonate scaling. The LSI can be decreased by reducing the pH of the feedwaters using an acid. Lowering the pH decreases the formation of calcium carbonate, but it increases the potential of other foulants such as silica to bind or attach to the membrane (Beerlage *et al.,* 2000). For example, in natural waters silica exists in two physical forms i.e. soluble/reactive silica and colloidal silica. Decreasing the pH to less than 7.0 will decrease the solubility of silica thus encouraging its precipitation onto the membrane. In the presence of metal ions such as magnesium, aluminium, or iron, silica will also form complexes and precipitate onto the membrane. In addition, in the presence of phosphate or phosphonates, silica can form a gel which thickens and retards the flow, eventually blocking the membrane (Belfort *et al.,* 1994).

1.3.3 Amelioration

Reduction of fouling and cleaning of fouled membranes has been approached in a number of ways (Maartens *et al.,* 1998; Eckner *et al.,* 1993; Flemming, 1990), which included optimisation of flow conditions, pre-treatment of the effluent, production of membranes with reduced absorptive conditions by modification of membrane surface, backflushing and harsh chemical cleaning agents which result in high cleaning costs and industrial pollution (Kim *et al.,* 1993a; Trägårdh *et al.,* 1989).

1.3.3.1 Adjustment of operating conditions

A most useful operating condition for fouling control is crossflow velocity. Increasing crossflow velocity decreases the degree of polarization by increasing mass transfer and other back transport mechanisms. Some advantages have been found for the use of pulsating flow and backwashing (Maartens, *et al.,* 1998).

A novel approach to backwashing was developed by Memtec Ltd in Australia who produces UF/UF systems for particulate and colloidal removal, typical applications being fruit juice, beverage and water clarification and effluent treatment. The Memtec system uses hollow fiber membranes with a relatively low bubble-point. The feed suspension is pumped across the outside of the fibers and the filtrate passes out through the lumen (Maartens, *et al.,* 1998; Kuberkar, *et al.,* 1998).

1.3.3.2 Surface modification of membranes

The extent and strength of interaction between foulants and membranes is governed by (i) the physical and chemical properties of the foulants (ii) membrane surface charge and (iii) pH and ionic strength of the solvent being filtered. Inorganic fouling of membranes by Ca^{2+} salts (also called scaling) is controlled by adjusting the pH and addition of antiscalants. Prevention of colloidal and organic fouling is much more difficult. This requires extensive pretreatment steps and surface modification of the membranes (Reimann, 1997; Bowen and Gan, 1991; Robertson and Zydney, 1990).

Membrane surface modification is a method that can reduce fouling and is therefore regarded as a pretreatment step. In UF surface modification of membranes has been performed extensively to prevent fouling. Various techniques have been used: (i) ultraviolet and plasma initiated polymerization, (ii) surfactant adsorption, and (iii) fluorination. In RO membrane systems major commercial manufacturers are currently marketing low fouling membranes which show resistance to organic fouling (Gilron *et al.,* 2001). An *in situ* method for surface modification of commercial membranes is under development (Belfer *et al.,* 1998). Belfer *et al.,* (1998) investigated surface modification of commercial composite polyamide RO membranes, since the thin layer composite polyamide membrane is widely accepted as the optimal system. Taking into account the radical of oxidation, they considered chemical initiation and the use of redox initiators and radical grafting to be a possible way to modify the membrane surface.

Electron spectroscopy for chemical analysis (ESCA) and attenuated total reflection Fourier transform infrared (ATR-FTIR) were used to analyze results after grafting of the membranes. Streaming potential and RO tests were also performed to further characterize the modified membranes (Belfer *et al.,* 1998). It was concluded that it is possible to modify the surface of ready-to-use thin-layer composite polyamide membranes with minimal change of performance. In order to choose the most suitable monomer for grafting onto the surface, long-term fouling studies will have to be carried out (Belfer *et al.,* 1998). The hydrophilicity or hydrophobicity of the membrane surface is the physical parameter that often has been used to indicate susceptibility of the membrane to organic fouling. One measure of surface hydrophibicity is the contact angle which decreases as the membrane becomes more hydrophilic. Clearly if the membranes that are more hydrophilic or more resistant to fouling are chosen, fouling can be reduced. Membrane pretreatment in order to reduce fouling by natural organic matter (NOM) has been investigated (Maartens *et al.,* 2000).

Severe fouling by NOM was experienced due to irreversible adsorption of substances onto the membrane surface, both at the surface of the membrane and within the pores. Chemical cleaning agents could be used to remove that fouling, but as they are expensive, influence retention properties of the membrane, cause severe damage to the membrane and present additional pollution problems, the alternative was to prevent or minimize membrane fouling by changing the membrane surface properties before filtration (Maartens *et al.,* 2000). As membrane surface properties influence foulant adsorption, the most suitable membrane for treating aqueous feed solutions containing NOM would be hydrophilic and possess pores that are homogenously permeable. Most hydrophilic membranes, such as cellulose acetate, are however thermally unstable and susceptible to chemical degradation. As a result membranes used for most UF processes are usually hydrophobic and more susceptible to foulant adsorption (Fane and Fell, 1987). A reduction of fouling in hydrophobic membranes can be achieved by forming a hydrophilic monolayer on the membrane surface (Fane and Fell, 1987). This hydrophilic monolayer (also called the Langmuir Blodgett Layer) gives membranes a hydrophilic character with fewer hydrophobic sites for foulant adsorption (Fane and Fell, 1987).

The effects of membrane pretreatment on the adsorptive behavior of NOM from natural brown water streams and commercial humic acid solutions, under dynamic filtration conditions have been studied (Maartens *et al.,* 2000). Coating substances included commercial nonionic surfactants, Triton X-100 and Pluronic F108. Triton X-100 orients itself with the hydrophobic C_6H_4 group towards the membrane and the hydrophilic CH_2CH_2O group towards the aqueous phase. Pluronic F108 is anchored to the hydrophobic membrane surface by the hydrophobic poly(propylene oxide) group in the middle of the molecule. The two hydrophilic poly(ethylene oxide) buoy groups at both ends of the molecule are facing towards the aqueous phase [Figure 1.2] (Maartens *et al.,* 2000). Capillary membranes coated with 0.5% Triton X-100 increased pure water flux (PWF) from the original of 100% to 137% (Fane and Fane, 1987). These results were confirmed by (Maartens *et al.,* 1998) where cleaning with Triton X-100 also resulted in flux recovery of more than 100 % through polysulphone membranes. While flux through the membrane was increased by coating, the retention of the membrane decreased. Similar results were also obtained on coating with Pluronic F108. Comparable results of increased flux and reduced retention through the membranes were observed by several authors (Fane *et al.,* 1985; Brink and Romijn, 1990; Maartens *et al.,* 1996; Chapman *et al.,* 1998). Other results also confirmed that modified membranes behave better in filtrations as compared to its virgin analogue, and that surface hydrophilization improves membrane performance (Gancarz *et al.,* 2000; Shi-He-Li *et al.,* 2002).

1.3.3.3 *In situ* cleaning

Considerable research has been focused on cleaning of fouled membranes. Milder and more environmentally friendly cleaning regimes such as purified enzymes and detergents have been considered for the removal of biologically derived foulants from polymer membranes (Trägårdh *et al., 1989;* Kim *et al*., 1993). The use of enzymes alone or in combination with biodegradable detergents is an attractive alternative to the classical cleaning regimes (Maartens *et al.,* 1996a; Leukes *et al.,* 1999). Previous work has shown that enzymes, as biocatalysts, can be used effectively in combination with detergents to reduce fouling and restore permeate flux on previously fouled membranes (Maartens *et al.,* 1996a).

Enzymes are also ideal cleaning agents because they are highly specific for the reactions they catalyse and the substrates with which they interact. In addition, enzymes also act under mild conditions of pH, temperature, and ionic strength and will not damage the membrane surface (Maartens *et al.,* 1996a). The consequences of defouling or cleaning fouled membranes using sludge enzymes would be a reduction of high cleaning costs (since large amounts of cleaning agents including purified enzymes are often required to restore permeate fluxes) and increased membrane life (as enzymes are milder and environmental friendly, in comparison with harsh chemical cleaning agents that damage the membrane). Enzymatic cleaning of UF membranes fouled in wool-scouring effluent was performed by Maartens, *et al.,* (1998), who found membranes treated with wool-scouring effluent to be severely contaminated by protein and lipid material.

The effectiveness of enzyme based cleaning agents was determined by comparing their abilities to remove adsorbed protein and lipid material from the fouled membrane, as well as their ability to restore the water contact angle and the pure water flux to the same levels as unfouled membranes. The enzymes used in this study are listed in Table 1.3 (Maartens *et al.,* 1998). After polysulphone membranes were subjected to cleaning by the different cleaning mixtures listed in Table 1.3, changes were observed in membrane contact angle, percentage flux improvement, percentage lipid reduction and percentage protein reduction after cleaning. Adsorbed proteins were successfully removed from fouled membranes by the commercial products called Alkazyme: Zymex, SDS and the combination of lipase A and Triton X100, followed by treatment with protease B (Maartens *et al.,* 1998).

The enzymes used in this study are listed in Table 1.3 (Maartens *et al.,* 1998). After polysulphone membranes were subjected to cleaning by the different cleaning mixtures listed in Table 1.3, changes were observed in membrane contact angle, percentage flux improvement, percentage lipid reduction and percentage protein reduction after cleaning. Adsorbed proteins were successfully removed from fouled membranes by the commercial products called Alkazyme: Zymex, SDS and the combination of lipase A and Triton X100, followed by treatment with protease B (Maartens *et al.,* 1998).

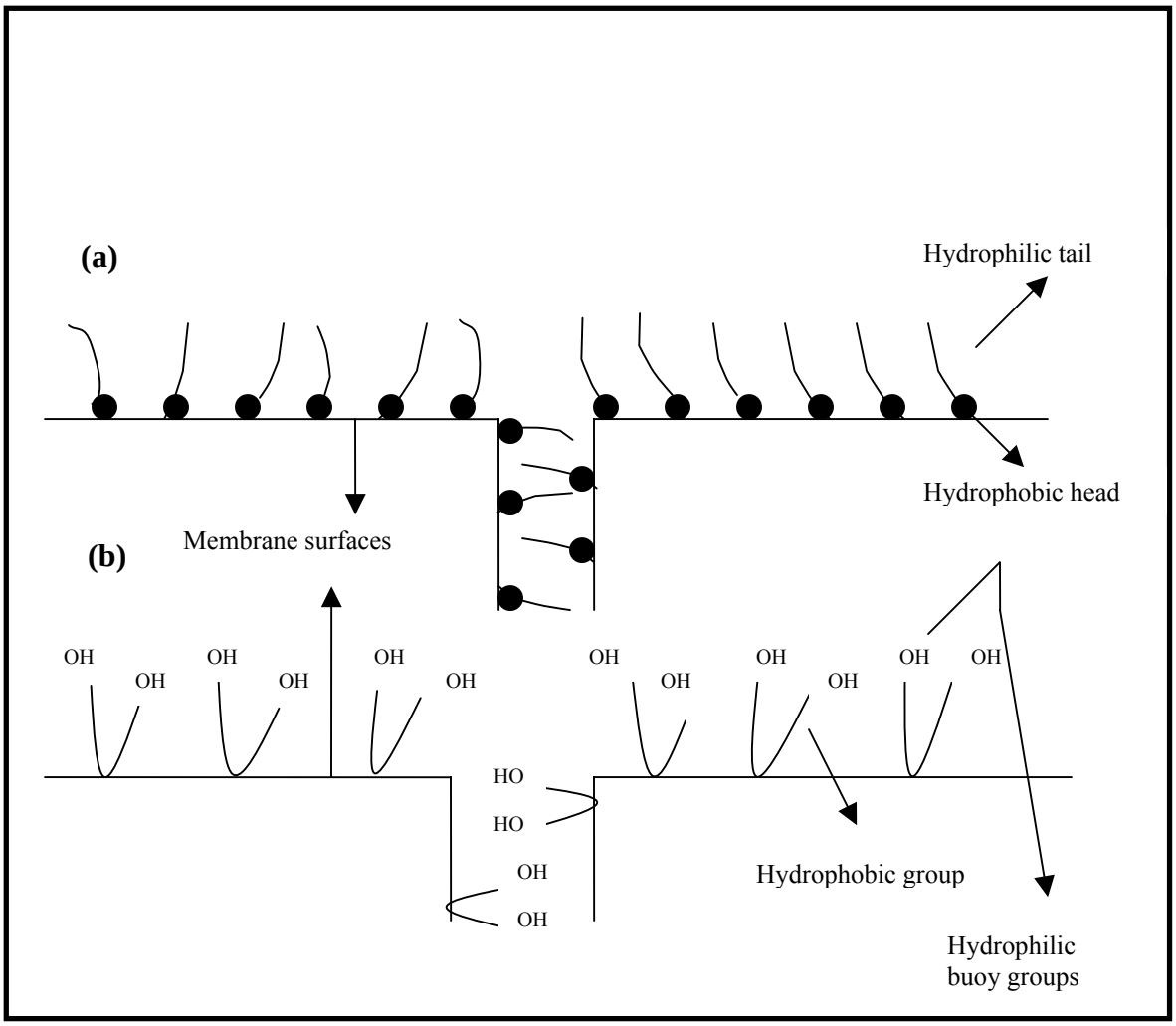


Figure 1.2: Representation of the adsorption of nonionic surfactants onto membranes. (a) Triton X-100 and (b) Pluronic F108.

Results indicated that cleaning regimes identical to those used for membranes fouled by abattoir effluent could not be used with the same efficiency due to differences in the nature of adsorbed lipid material. Enzymatic and detergent cleaning on polysulphone UF membranes fouled using bovine serum albumin (BSA) and whey were also investigated (Muňoz-Aguado *et al.,* 1996). It was found that for foulants containing proteinaceous components, enzymatic cleaners play a vital role in scissioning specific points in the protein strands while detergent cleaners also interact with the protein strands at specific points; in addition there is rapid solubilization of small loose protein fragments (Muňoz-Aguado *et al.,* 1996).

Table 1.3: Enzymes used to clean membranes fouled by wool scouring effluent. These enzymes were used at their optimal pH and temperature recommended by distributors (Maartens *et al.*, 1998).

Types of enzymes	Source of enzyme	Class of enzyme	pH	Temperature optimum
Protease A type XXIII	*Aspergillus oryzae*	Serine proteases	7.5	37 ºC
Protease B type II	*Aspergillus oryzae*	Serine proteases	7.5	37 ºC
Lipase A type II	Porcine pancreas	Triglyceride hydrolase	7.5	37 ºC
Trypsin protease C	Pancreas		7.5	37 ºC
Esterase A	Porcine liver	Carboxyl-ester hydrolase	7.5	35.5

It is also important to consider the economic impact of cleaning procedures, including the cost of the cleaning process itself together with the effect of the procedures on the membrane lifetime and efficiency (Muňoz-Aguado *et al.,* 1996). Muňoz-Aguado *et al.,* (1996) established that it was most effective to defoul/clean first with an enzyme and then with a detergent and recommended that if both the detergent and enzyme are present at the same time, they be formulated in such a way that the action of each does not interfere with the others. Enzymatic cleaners were most effective when operated at concentrations that optimized hydrolysis of protein foulants (Muňoz-Aguado *et al.,* 1996).

The use of higher concentrations did not increase enzymatic cleaning efficiency. The efficiency of detergent cleaners increased with concentration up to a certain point where the detergent itself attacked the membrane. Water rinsing during the defouling/cleaning regime was an effective method of removing loose foulants, but only effective when the rinsing was carried out at the same temperature as the chemical cleaning, otherwise rinsing could cause compaction of the foulant layer (Whiteley *et al.,* 2003; 2002a; 2002b; 2002c).

As commercial enzymes are expensive there is a need for a cost effective, readily available source of enzymes. As a result, hydrolytic enzymes such as phosphatases, sulphatases, proteases, lipases, and glucohydrolases, involved in PSS solubilisation under anaerobic conditions and in the presence of sulphate reducing bacteria (SRB) have been characterized (Whiteley *et al.,* 2003; 2002a; 2002b; 2002c).

1.4 Anaerobic digestion

Anaerobic digestion employs a complex mixture of microorganisms to hydrolyze, ferment, and ultimately reduce organic solids present in sludge in the form of fats, proteins and polysaccharides [Figure 1.3]. Organic solids can be reduced by up to 50% with this process. Hydrolytic enzymes such as lipases, proteases and glucohydrolases released by microorganisms metabolize these organic solids into fatty acids, amino acids and sugars (Tshivhunge, 2001; Henze and Mladenovski, 1991; Sterrit and Lester, 1988; Forday and Greenfield, 1983). Hydrogen-producing acetogenic bacteria will catabolise these monomers to acetate and hydrogen. This is a fermentative process in which amino acids and sugars are readily fermentable substrates. Sugars are usually fermented to alcohols and fermentation has been reported to occur on alkanoic acids, purines and pyrimidines. Pyruvate is the most important intermediate in the fermentation of amino acids, from which lactate, propionate, butyrate, formate and acetate are formed. Acetate, propionate, butyrate, caproate, caprylate, valerate and heptanoate constitute a group of compounds called volatile fatty acids (VFA). When the concentration of VFA becomes sufficiently high, the pH of the anaerobic digester decreases (Tshivhunge, 2001; Henze and Mladenovski, 1991; Sterrit and Lester, 1988). However, acetic acid is a weak acid and this combined with the natural buffering capacity of the system provided by carbon dioxide (in the form of bicarbonate alkalinity) means that a significant increase in the concentration of VFA must occur before any significant decrease in pH is observed. On the other hand, VFA act as an early warning or indicator of impending bioreactor failure. The exact concentration of VFA that induces bioreactor failure depends on the initial pH, the alkalinity and the organic loading (Sterrit and Lester, 1988).

However, metabolism of amino acids results in liberation of ammonia, which in turn neutralizes those acids that have not been converted to acetate, hydrogen and carbon dioxide. In that way the pH rises to a more favorable level for further bacterial growth. The last phase of anaerobic digestion is carried out by methanogenic bacteria (Whiteley *et al.,* 2002a; Sterrit and Lester, 1988). These bacteria metabolize 1-C compounds and acetate to methane and carbon dioxide. Conventional anaerobic digestion is a slow process by comparison with aerobic treatment and is more suitable for wastes with comparatively high biological oxygen demand (BOD). Hatziconstaninou *et al., (*1996) also stated that solubilization of primary sewage sludge (PSS) is slow in conventional anaerobic systems, with maximum soluble product formation reported between 8 and 20 days and at a yield of 10% in the mesophilic temperature range.

1.4.1 Operational parameters in anaerobic digestion

Temperature - The three common temperature ranges at which anaerobic digestion operates are thermophilic (50 - 65°C), mesophilic (20 - 45°C) and psychrophillic (<20°C). In all microbial systems, temperature increase leads to increased microbial activity and thus enzyme activity. However, changes in overall process efficiency due to increased metabolic activity are balanced by a corresponding increase in microbial inactivation, i.e. above the optimum temperature efficiency of the process decreases (Henze and Mladenovski, 1991). The thermophilic digestion process offers a number of advantages, namely, rapid metabolic activity which leads to shorter retention times, higher loading rates and smaller digester volumes. Operation of the bioreactors at thermophilic temperatures prevents accumulation of bacterial pathogens. The disadvantages of thermophilic operations are that they require higher energy inputs for heating and maintenance costs are also high (Banister and Pretorius, 1998; Henze and Mladenovski, 1991).

pH - The optimum pH range is between 6.5 and 8.0. Maintenance of this neutral pH is due to the conversion of acid end-products to methane in the methanogenic anaerobic digestion and H_2S production coupled with precipitation of heavy metals in the sulphate reduction process.

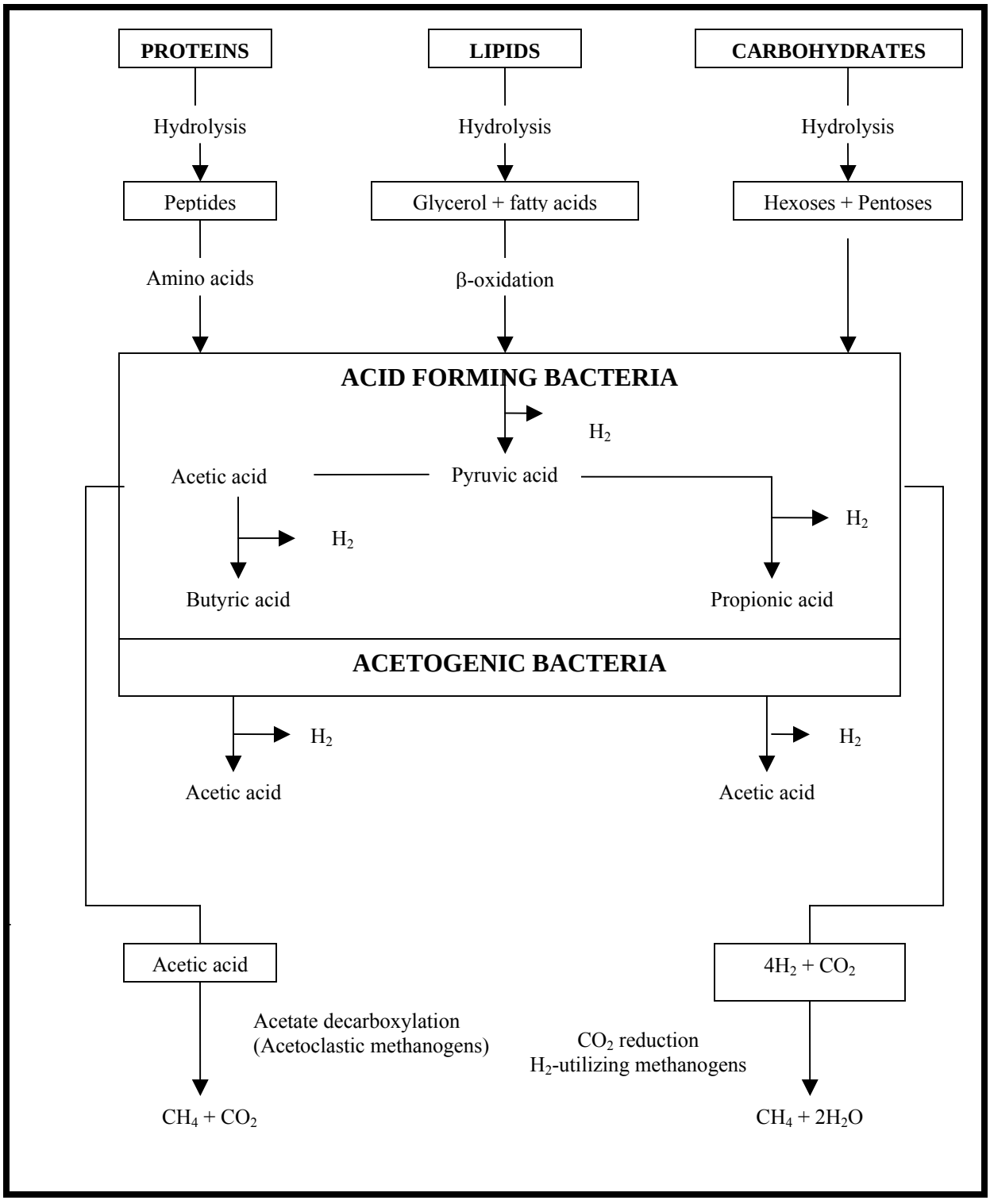


Figure 1.3: Showing an outline of anaerobic digestion at biochemical level. In the absence of Sulphate Reducing Bacteria the major product of anaerobic digestion is methane. The initial attack or hydrolysis of complex substrates is brought about by extracellular enzymes excreted by methanogenic bacteria.

The major controlling buffer is the carbonate-bicarbonate system, with orthophosphoric acid, hydrosulphuric acid, the volatile acids and ammonia contributing to pH stabilization. At lower pH values VFA regulate buffer capacity. Anaerobic digestion is sensitive to pH changes and microbial activities can be altered. Changes in microbial activities imply changes in enzyme activities.

Retention times - Mesophilic and thermophilic digesters can operate at mean sludge retention times typically in the range of 25 - 35 days and sometimes as low as 12 - 15 days (Banister and Pretorius, 1998).

Substrate loading - Chemical oxygen demand (COD) parameters can be used to calculate substrate loading. The COD is a measure of the organic matter content of a sample (sludge/substrate) that is susceptible to oxidation by a strong chemical oxidant. Volatile solids can also be used as a measure of organic content of the sludge and loadings are normally expressed in kg/m^3/day. If a feed containing a lower concentration of biodegradable organics is added at a rate sufficient to maintain the normal organic load, higher volumetric loading is required to reduce the retention times (Banister and Pretorius, 1998; Henze and Mladenovski, 1991).

Volatile acids - Instability in anaerobic digestion occurs when the series of microbiological reactions become uncoupled. Uncoupling may be a result of inhibition of methane-forming organisms or organic overload, which allows faster growing acidogens to outproduce the methanogens. When acid forming bacteria out produce acid consuming bacteria, a sharp rise in volatile acids follows. The presence of a sulphur cycle in sulphidogenic digesters is associated with an effective fracturing of particulates that settle towards a lower level of anaerobic sulphidogenic zone, followed by a subsequent upwelling of dissolved and residual suspended organic matter to the aerobic upper zone. The residual undegraded particulates settle once again to the sulphidogenic zone and undergo a further cycle of degradation, eventually achieving a surprisingly high level of solids removal. Detailed mechanisms of this organic particulate degradation within the sulphidogenic environment were investigated in a model Falling Sludge Bed Reactor (FSBR) (Whittington-Jones *et al.,* 2001).

The simulation of the reciprocating sedimentation and unveiling events observed in the FSBR provided a descriptive model of enhanced solubilisation of PSS under sulphidogenic conditions establishing that there was enhanced hydrolysis of macromolecular polymeric constituents. Microbial aggregates generated in wastewater treatment using activated sludge provide an efficient organization of bacterial communities (Whittington-Jones *et al.*, 2001). The bacteria are embedded in a matrix of extracellular polymeric substances (EPS) which act as a trap for biodegradable colloidal organic matter too large for direct assimilation by SRB (Akhurst et al., 2002). Furthermore, EPS acts as a network confining extracellular enzymes exhibiting hydrolytic activity. Models of anaerobic digestion for complex particulate substrates show that, both the concentration of the hydrolytic enzymes and the contact between these enzymes and their substrates were crucial (Akhurst et al., 2002).

1.5 Biochemistry of organic substrates in wastewaters

1.5.1 Carbohydrates

Carbohydrates are everywhere in nature as they occur in every living organism and are essential to life. The sugar and starch in food and the cellulose in wood, paper, and cotton are nearly pure carbohydrates (Campbell, 1991). Carbohydrates are classified into two groups: (1) Simple sugars or monosaccharides which are carbohydrates like glucose and fructose that cannot be hydrolyzed into smaller molecules. (2) Complex carbohydrates that are made of two or more simple sugars linked together. For example, table sugar is a disaccharide made up of a glucose molecule linked to a fructose molecule. Cellubiose and maltose are also disaccharides, and contain a glycosidic acetal bond between C1 of one sugar and a hydroxyl group at any position on the other sugar (Ngesi, 2001). A glycosidic bond to the anomeric carbon can either be alpha or beta. Maltose, the disaccharide obtained by enzyme-catalyzed hydrolysis of starch, consists of two D-glucopyranoses joined by a 1, 4′-α-glycoside bond. Cellubiose, the disaccharide obtained by partial hydrolysis of cellulose, consists of two D-glucopyranoses joined by a 1, 4′-β-glycoside bond. Both maltose and cellulose are reducing sugars because of the free anomeric carbons have hemi-acetal groups [figure 1.4] (Whiteley *et al.*, 2002c; McMurry, 1992; Campbell, 1991).

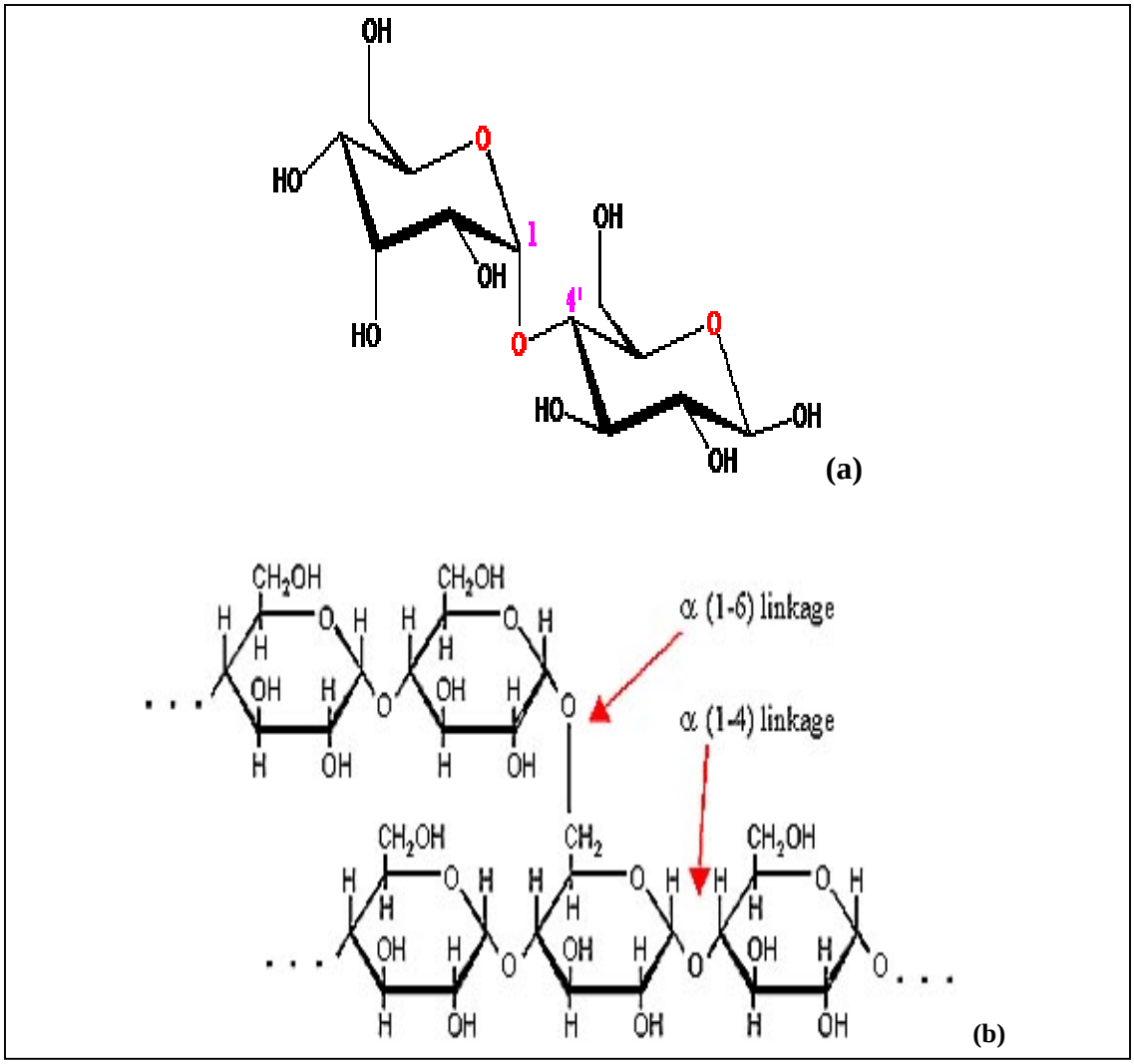


Figure 1.4: Chemical structures of maltose and cellubiose.

Similarly, cellulose is a polysaccharide made up of many D-glucose molecules linked by the 1, 4′-β-glycoside bonds as in cellobiose. The fundamental linkage between monosaccharide units in polysaccharides is an acetal or a ketal, formed by condensation of an alcoholic hydroxyl of one monosaccharide with the hemiacetal (hemiketal) form of carbonyl sugar, accompanied by a loss of water. Glycosidic bonds may occur in a variety of isomeric arrangements. They may be formed between α or β hydroxyls at the anomeric carbon of one sugar and alcoholic hydroxyl groups at carbon 2, 3, 4, or 6 of a second sugar. Nature uses

cellulose primarily as a structural material to impart strength and rigidity to plants [Figure 1.5].

In addition, the polymer may be branched so that the two sugars have glycosidic linkages to a different alcoholic hydroxyl of a third unit, for example a branch point in glycogen (Ngesi, 2001; McMurry, 1992; Campbell, 1991). Each glycosidic linkage employs the reducing carbon of one monosaccharide, thus all oligosaccharides and polysaccharides have only a single reducing end. The α-linked glucose units in glycogen or starch allow the formation of molecules that are accessible to enzymes that can hydrolyze inter-glucose bonds. On the other hand, hydrolytic enzymes do not easily degrade cellulose. Starch or glycogen can be separated into two fractions: a fraction soluble in cold water, called amylopectin and one insoluble in cold water, called amylose. Amylose accounts for about 20% by weight of starch, and consists of several glucose molecules linked together by 1, 4′-α-glycoside bonds [Figure 1.6]. Amylopectin, which accounts for the remaining 80% of starch, is more complex in structure. Unlike amylose and cellulose, which are linear polymers, amylopectin contains 1, 6′-α-glycoside branches approximately every 25 glucose units. As a result, amylopectin has an exceedingly three-dimensional structure [Figure 1.7] (Ngesi, 2001; McMurry, 1992; Campbell, 1991).

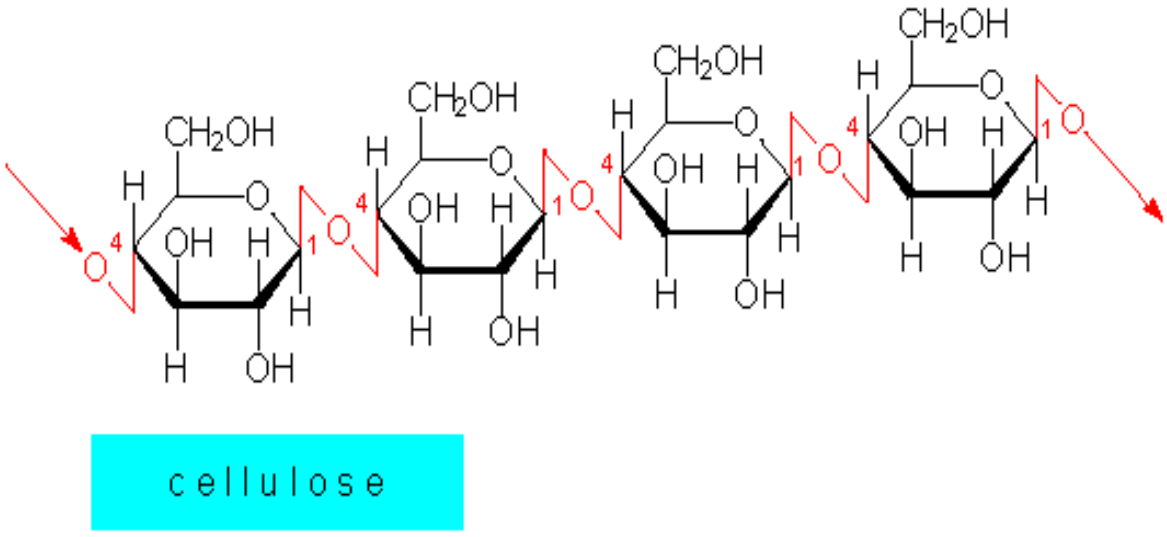


Figure 1.5: Chemical structure of cellulose.

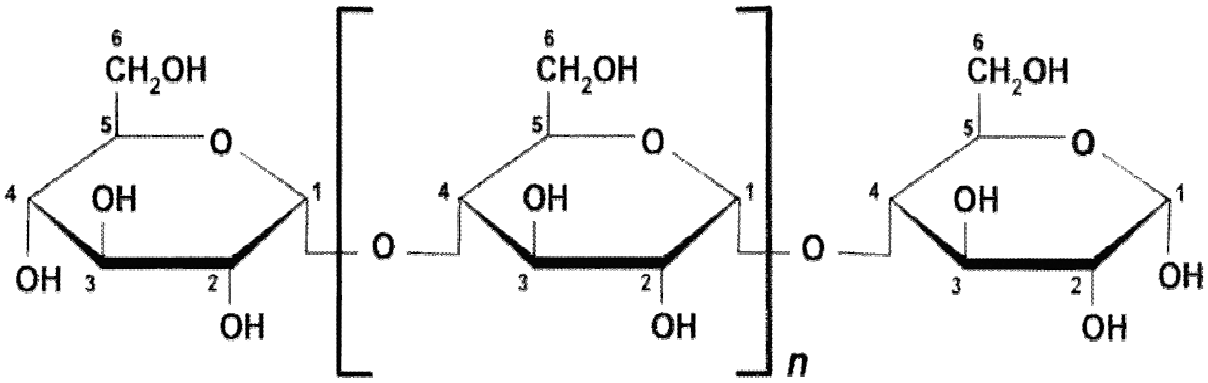


Figure1.6: Chemical structure of starch showing the amylose moiety.

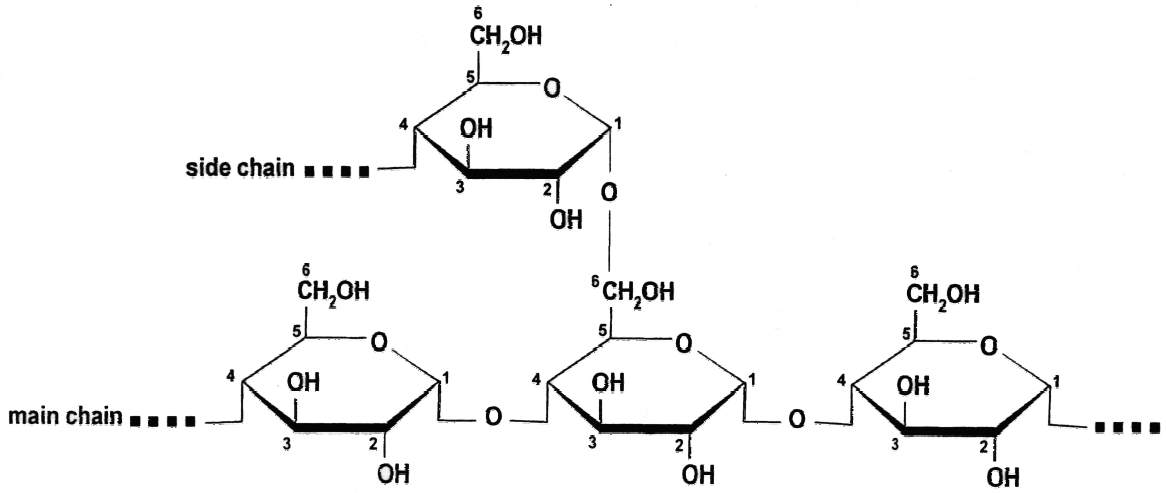


Figure 1.7: Chemical structure of starch showing the amylopectin moiety.

1.5.1.1 Enzymatic hydrolysis of carbohydrates

Cellulases hydrolyze cellulose polymer to smaller oligosaccharides and glucose. They include three major types of enzymes: (i) endoglucanases (EC 3.2.1.4) which randomly attack the cellulose polymer by endoaction; (ii) cellobiohydrolases (EC 3.2.1.91) act as exoenzymes and remove cellubiose or glucose from the non-reducing end of the cellulose chain and (iii) β-glucosidases (EC 3.2.1.21) which hydrolyze cellooligosaccharides and cellobiose into glucose.

These enzymes can either be free, particularly in aerobic microorganisms or grouped in a multicomponent enzyme complex, cellulosome, such as anaerobic cellulolytic bacteria. α-Glucosidase is a membrane boumd enzyme that catalyzes the cleavage of glucose from the disaccharide maltose (Criquet, 2002)

1.5.2 Lipids

Lipids are naturally occurring organic molecules isolated from cells and tissues by extraction with a nonpolar organic solvent. They usually have large hydrocarbon portions in their structure and therefore are insoluble in water but soluble in organic solvents. Lipids can be classified according to their chemical nature into two groups: neutral and polar lipids. Polar lipids contain polar groups such as the phosphate group which is the "base" of phospholipids, the sulphate groups of the sulpholipids and the sugar moiety of glycolipids (McMurry, 1992; Campbell, 1991). Neutral lipids include free fatty acids, simple sterols, sterol esters and triglycerides. The major circulating lipids insoluble or marginally soluble in the blood are transported as lipoprotein particles. Animal fat and vegetable oil are the most widely occurring lipids. Both are tri-esters of glycerol with long-chain saturated fatty acids, in the former and unsaturated fatty acid residues in the latter. Phosphoglycerides such as lecithin and cephalin are closely related to fats. The glycerol backbone in these molecules is esterified to two fatty acids and to one phosphate ester. Sphingolipids are another major class of phospholipids, they have an amino alcohol such as sphingosine for their backbone (Akoh and Min, 1998, McMurry, 1992). These compounds are important constituents of cell membranes. Fatty acids are biosynthesized in nature by a condensation of enzyme bound two-carbon acetate units. Other lipids, like prostagladins, are found in all body tissues and have a wide range of physisological action. Terpenes are often isolated from the essential oils of plants while steroids are plant and animal lipids with a tetracyclic carbon skeleton. Steroids also occur widely in body tissue and have a large variety of physiological activities. The more polar lipids, phospholipids and non-esterified cholesterol reside with certain proteins on the surface of the particles oriented to provide solubility in the aqueous medium. The non-polar lipids, triglycerides and esterified cholesterol are in the core.

Cholesterol and phospholipids are essential constituents of cell membrane whereas the triglycerides are an important fuel source (McMurry, 1992; Campbell, 1991).

1.5.3.1 Enzymatic hydrolysis of lipids

Lipases are lipolytic enzymes that are found in many microorganisms, plants and higher animals. The most unusual feature of all lipases is that although they are water soluble, they catalyze heterogeneous ester hydrolytic processes at the lipid-water interface. The rate of lipolysis is directly determined by the concentration of the substrate molecules at this interface. The initial step in lipase hydrolysis is the splitting of the fatty acids esterified to the primary hydroxyls of glycerol (Akoh and Min, 1998; Campbell, 1991). This reaction is not stereo-specific and the fatty acids in the 1and 3 positions are initially removed at equal rates. Once one fatty acid has been removed, the resulting di-glycerides and subsequently the monoglycerides are more slowly hydrolyzed than the original triglycerides (Akoh and Min, 1998; Campbell, 1991).The preference to remove the 1- and 3- fatty acids together, with the diminished rate of hydrolysis of the subsequent ester linkages, results in the accumulation of monoglycerides as the primary products of lipase hydrolysis. Lipases do not only hydrolyze fatty acids on the outer positions of triglycerides, they also liberate the esterified fatty acid in the 1-position of phospholipids (Akoh and Min, 1998; Campbell, 1991).

1.5.3 Proteins

Proteins are classified into two major types according to their composition. Simple proteins such as blood serum albumin are those that yield only amino acids on hydrolysis. Conjugated proteins are much more common than simple proteins and yield other compounds in addition to amino acids on hydrolysis (Akoh and Min, 1998; Campbell, 1991). Conjugated proteins can be further classified according to the chemical nature of the non-amino acid portion. Glycoproteins contain a carbohydrate part, lipoproteins contain a lipid part and nucleoproteins contain a nucleic acid part. Glycoproteins are widely spread in nature and make up a large part of the membrane coating around living cells. Another way to classify proteins is as fibrous or globular, according to their three-dimensional shape.

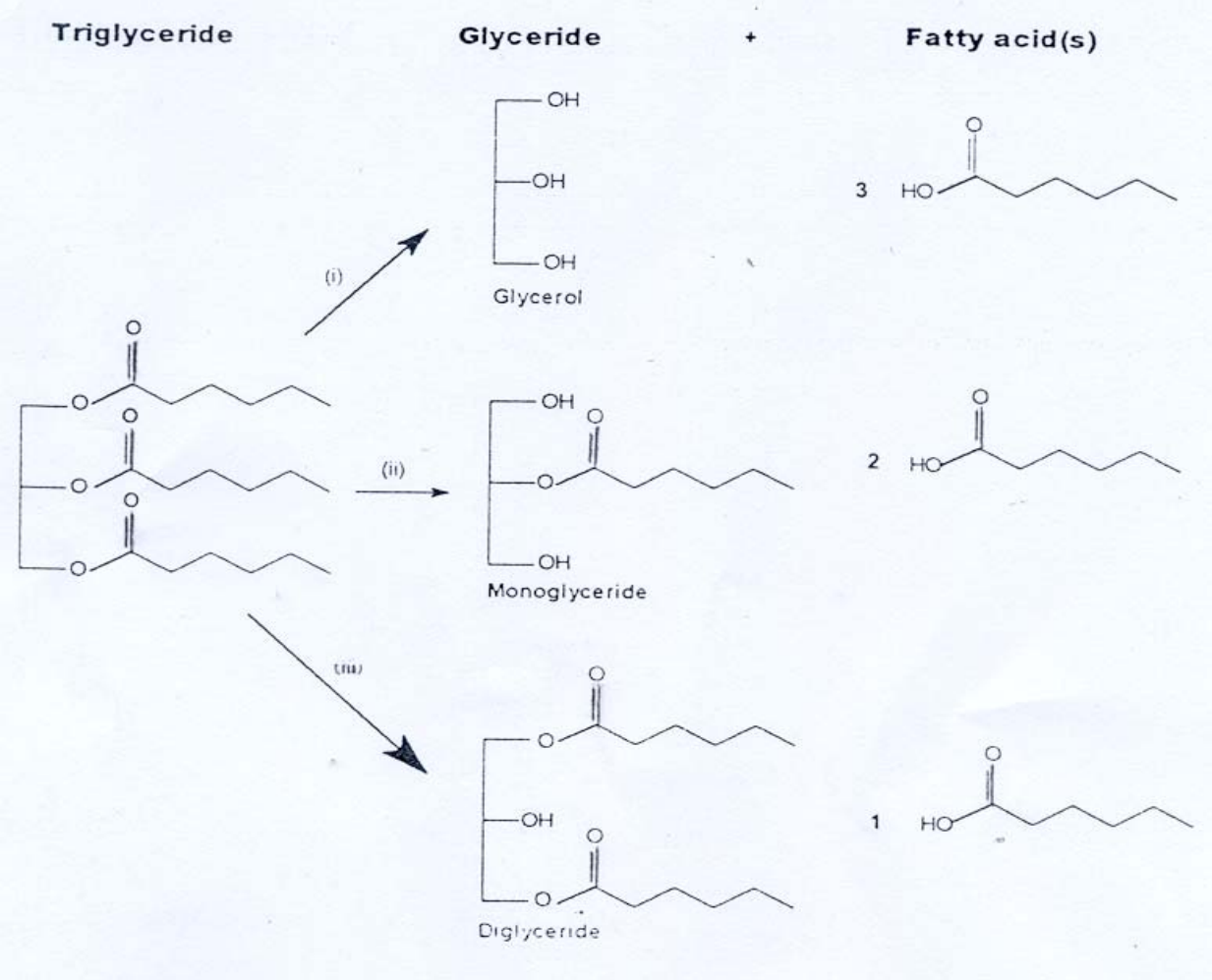


Figure 1.8: Schematic presentation of a triglyceride molecule indicating selectivity of lipases catalyzing the hydrolysis with (i) random lipase yielding glycerol and three fatty acids, (ii) a sn-1,3 specific lipase which on hydrolysis yields a monoglyceride and two fatty acids and (iii) a sn-2 specific lipase liberating a diglyceride and one fatty acid.

Fibrous proteins such as collagen and keratin consist of polypeptide chains arranged side by side in long filaments. Because these proteins are tough and insoluble in water, they are used in nature for structural material such as tendons, hooves, horns and muscles. Globular proteins by contrast, are usually coiled into compact and nearly spherical shapes. These proteins are generally soluble in water and are mobile within cells (Campbell, 1991).

1.5.3.1 Protein structure

Proteins are very large as compared to simple organic molecules. The primary structure of a protein is made up of a sequence of amino acid residues that are bound together by peptide bonds. Secondary structure refers to the way in which segments of the peptide backbone are oriented into a regular pattern. The end result is a right or left - handed helix and or β-sheet. Tertiary structure refers to the way in which the entire protein molecule is coiled into an overall three-dimensional shape. In a quaternary structure several protein molecules come together to yield large aggregate structures (Wilson and Walker, 1994).

1.5.3.2 Protein hydrolysis by proteases

For protein hydrolysis, a number of enzymes are presently available that cleave proteins over a pH and temperature ranges as in Table 1.4.

1.6 Enzymes from sulphidogenic systems

While process development studies have made significant progress the underlying enzymatic mechanisms for the enhanced solubilization in the sulphate reducing system remains obscure. Instead, a lot of research has been performed on enzyme activity in activated sludges (Goel *et al.,* 1998; Frϕlund *et al.,* 1995 and Lötter *et al.,* 1987). Nevertheless, Boczar *et al*., (1992) managed to characterize enzyme activities in activated sludge using a rapid analysis specific for hydrolases. Enzyme activity in activated sludge received particular attention for a number of reasons: (i) Enzymatic activities play a key role in the hydrolysis and mineralization of organic wastes. (ii) Characterization of enzymatic activities can provide insight into the biochemical factors controlling the treatability of xenobiotic chemicals. (iii) Patterns of enzyme activities can be added exogenously to treatment processes to improve overall treatment efficiencies and (iv) There is little published information on the concentrations, activity specificities and cellular location of enzymes in sludge, in general.

Table 1.4: Optimum temperature and pH for various proteases.

ENZYME	pH RANGE	OPTIMUM TEMPERATURE (°C)
Pepsin	2-4	40-45
Acid fungal protease (*A. saitoi*)	2.4-4	45
Papain	4-8	60-65
Neutral fungal protease (A. niger)	5-7.5	50
Neutral bacterial protease	6-8	55-60
Pancreatic protease	6-9	45-50
Alkaline bacterial protease	7.5-9.5	60
High-alkaline bacterial protease	8-12	55

Boczar, *et al.,* (1992) thus managed to localize and compare hydrolytic enzyme profiles in activated sludges, as previous research had focused on enzymatic activity as a microbial population indicator; as a monitor of active biomass; and as an indicator, of specific engineering processes such as COD and phosphorus removal. They also investigated activities of lipases, esterases, proteases, aminopeptidases, phosphatases, and glucosyl hydrolases on mixed liquor, sonicated mixed liquor, freeze - thaw and extracellular functions (Boczar *et al.,* 1996). Their results indicated that API-ZYM and LRA-ZYM esterase systems can be used to characterize the microbial and biodegradation activity of activated sludge. They may also be useful in monitoring physiological changes in the biochemical efficiency of biological treatment processes (Boczar *et al.,* 1996).

1.6.1 Enzymatic activities in sulphidogenic systems

During the sulphate reduction process the pH of the system becomes more alkaline due to increases in the concentrations of HCO_3^-, OH^- and SH^- ions and it is suggested that there is neutralization of the acidic surface of the organic particulate floc. This in turn allows for a disruption of the ionic charges maintaining the integrity of the floc. Consequently, there is an exponential increase in the rate at which polymeric substrate is released for further enzymatic degradation. Process optimization studies are crucially dependent on an understanding of the enzymatic processes and hydrolysis in the sulphur reducing environment (Whiteley *et al*., 2003; 2002a; 2002b; 2002c). Sulphate reduction is a process that has been identified as a method for treating effluents with high concentrations of SO_4^{2-} and heavy metals with the use of obligate anaerobic bacteria called SRB (Du Preez *et al.,* 1992). These microorganisms are known to dissimilate SO_4^{2-} and produce H_2S, and require a constant supply of energy and an electron donor. PSS has been identified as the most abundant and cost effective carbon source, but it is available in a complex form (Bjørn *et al.,* 1996). Solubilization of this complex PSS can be achieved by hydrolysis of polymers, such as cellulose, lipids, proteins and polysaccharides, using enzymes such as cellulases, lipases, proteases and α and β-glucosidases produced in the presence of SRB (Whiteley *et al*., 2003;2002a; 2002b; 2002c; Whittington-Jones, 1999). Whiteley *et al.,* (2003; 2002a; 2002b; 2002c), monitored enzyme activities and mechanisms in the solubilisation of PSS. They stated that the first stage in the degradation and recycling of PSS and particulate organic matter is the solubilisation and enhanced hydrolysis of complex organic carbon structures under anaerobic sulphidogenic conditions. Protease and phosphatases activities were particularly associated with particulate organic matter of sewage sludge.

1.7 Hypothesis

Considering the role that is played by the "sludge enzymes" *(*proteases, lipases, α and β-glucosidases, phosphatases, sulphatases, amonipeptidases etc.) in the solubilization of PSS under sulphidogenic conditions, it can be hypothesized that the key enzymes involved in the first step of solubilization (i.e. hydrolysis) can clean or defoul membranes that have been fouled by abattoir effluent.

1.7.1 Objectives

- To characterize abattoir effluent for potential foulants such as proteins, lipids, polysaccharides and carbohydrates.
- To formulate an enzyme extract from the sulphidogenic bioreactor containing relevant enzymes for cleaning/defouling fouled membranes.
- To foul and defoul UF membranes using abattoir effluent and sulphidogenic sludge enzymes (dynamic fouling and defouling).
- To passively foul and defoul disc polysulphone membranes using the abattoir effluent and sulphidogenic sludge enzymes.
- To ascertain fouling and defouling processes by monitoring protein, lipid and carbohydrate levels on the membranes and enzyme activity assays on organic foulants.

CHAPTER TWO
EXTRACTION AND CONCENTRATION OF ENZYMES FROM A SULPHIDOGENIC BIOREACTOR

2.1 Introduction

Anaerobic digestion is known as the most common process used for sludge minimization and stabilization (Hudson and Lowe, 1996). This process, however, has little application in the treatment of organic industrial wastes due to several limitations, including the low achievable rates of performance; inability to withstand hydraulic and organic shock loads and poor process control. These problems are all inherent to conventional digesters, and are associated with difficulties in retaining biomass within the digesters and very long retention times (Houghton and Stephenson, 2002). However new reactor designs and methods of bacterial bed preparation have allowed retention times to be reduced considerably – sometimes to a few hours. As already mentioned the anaerobic process occurs in discrete stages, the main ones being hydrolysis, acidogenesis and methanogenesis. The first step in anaerobic digestion hydrolysis- is responsible for the degradation of complex organic carbon structures in waste waters and is regarded as the rate limiting step (Eliösor and Argaman, 1995; El-Fadel *et al*., 1996; Vavilin *et al.,* 1996; Fernand *et al.,* 1997). This is illustrated by the fact that high molecular weight compounds are hydrolyzed slowly and moreover, the microbial extracellular polymeric substances (EPS) hinder the diffusion of substrates in sludge flocs and their availability to extracellular enzymes (Aurore *et al.,* 2002). The rate at which hydrolysis proceeds is also described by first order kinetics and may be strongly influenced by both environmental and operational parameters, including the concentration of particulate substrates and soluble products. Particle size has also been shown to have a profound impact on the rate of anaerobic digestion of complex substrates (Chio *et al.,* 1997; Madhakara *et al.,* 1997; Muller *et al.,* 1998; Wentzel *et al.,* 1995).

On the other hand, sewage sludge has received attention as a model system in the degradation of particulate organic matter due to the potential use of solubilized product as an electron donor source in a range of bioprocess applications. These potential bioprocess applications related to waste disposal include biological nutrient removal (BNR) in wastewater treatment (Brinch *et al.,* 1994; Skalsky and Daigger, 1995*;* Hatziconstantinou *et al.,* 1996; Banister and Pretorious, 1998); and biological sulphate reduction (BSR) in acid mine drainage (AMD) (Molipane, 1999; Whittington-Jones, 1999). Several strategies have been employed to enhance enzymatic hydrolysis, to reduce solids and improve settlability and solubilization of primary sewage sludge (PSS) and activated sludge. For example, structural modification of lignocelluloses by pretreatment strategies was investigated in an effort to enhance enzymatic hydrolysis (Gharpuray *et al.,* 1983). The limiting factor in the hydrolysis step in this case was the availability of the substrate, namely lignocelluloses, to the enzyme. Pretreatment strategies employed were physical and chemical, with the former being milling, including ball-milling, Fitz-milling and roller-milling and the latter being peracetic acid and ethylene glycol. These pretreatment strategies decreased the particle size of lignocellulose and increased its available surface area causing an increase in substrate availability to the enzymes and thus enhanced hydrolysis (Gharpuray *et al.,* 1983). Another enzyme based strategy for toxic waste treatment and waste minimization was devised (Smith *et al.,* 1992).

In this case, the enzyme was used for hydrolyzing organophosphates, present in cattle dipping waste. The cattle dipping liquid contained a pesticide called coumaphos, which was used to kill a disease-causing tick. Waste generated form the dipping process had a toxic dechlorination product of coumaphos, called potasan. When potasan accumulated to high concentrations, it became hazardous to cattle. Smith *et al.,* (1992) results showed that hydrolysis of the two organophosphates substrates can be modeled as first order reactions with identical rate constants based upon experimental results describing the system (Smith *et al.,* 1992). Enzyme treatment to reduce solids and improve settling of sewage sludge was also investigated (Parmar *et al.,* 2001). In this study a mixture of commercial cellulase, protease and lipase, in equal proportion by weight, reduced total suspended solids (TSS) by 30-50%. This caused improved settling of solids. Increased solid reduction was also observed when enzyme concentration was increased (Parmar *et al.,* 2001).

Two-enzyme combinations of protease and cellulase produced better solid reduction than individual enzymes and that lipase further augmented this effect (Parmar *et al.,* 2001). Enhanced solubilization accompanied by effective fracturing of settled organic particulate matter was observed in an anaerobic sulphidogenic zone of tannery waste ponding system (Dunn, 1998; Rose *et al.,* 1998). In later work, a technical scale falling sludge bed reactor (FSBR) was developed to provide a descriptive model of enhanced solubilization under sulphate reducing conditions. It was established that there was enhanced hydrolysis of macromolecular polymeric substances (Whittington-Jones, 1999). While process development studies have made significant progress, the underlying enzymatic mechanisms for enhanced hydrolysis and solubilization in sulphate reducing systems are at infant stages (Ngesi, 2001; Tshivhunge, 2001). So far hydrolytic enzymes, namely proteases, cellulases, β-glucosidases, lipases, sulphatases and phosphatases have been characterized (Whiteley *et al*., 2003; 2002a; 2002b; 2002c; Pletschke *et al.,* 2002).

One of the reasons for establishing such fundamentals in accelerated PSS solubilization is that process optimization studies are crucially dependent on understanding these enzymatic mechanisms. Understanding the morphology of sewage sludge, its microorganisms and the enzymes produced is of utmost importance (Muller *et al.,* 1998; Chio *et al.,* 1997; Madhakara *et al.,* 1997; Wentzel *et al.,* 1995). These authors have shown that the rate of hydrolysis of polymers such as polysaccharides, proteases and lipids is directly dependent on particle size. It is well known that before bacteria can assimilate high molecular weight compounds, the compounds are first hydrolyzed by extracellular enzymes (Frϕlund *et al.,* 1995). Extracellular enzymes are either bound to the cell surface (ecto-enzymes) or released by bacteria into the medium in free form (exo-enzymes). Exo-enzymes however, have a high potential of forming complexes with humic substances or other polymers. Extracellular enzymes are therefore responsible for biodegradation of high molecular weight compounds found in wastewaters. Several studies have proved that exo-enzymes are associated with EPS. The architecture of EPS is dependent on the interaction between biopolymers present in that particular sludge, which may be carbohydrates, lipids, proteins, polysulphates and polyphosphates, and various cations and anions. Small changes in ionic strengths and ionic composition can therefore alter the structural properties of the sludge floc (Frϕlund *et al.,* 1995; Cadoret *et al.,* 2002; 2001).

Although bacteria can be incorporated into flocs/EPS, the majority of degradation of EPS is from the outside where the concentration of bacteria is high (Frϕlund *et al.,* 1995; Jinho Jung *et al.,* 2002; Cadoret *et al.,* 2002). Molecules within EPS are protected from enzymatic degradation; therefore disruption of this matrix leads to enhanced solubilization of PSS. Sludge flocs present in the sulphidogenic bioreactor were found to have smaller diameters than their counterparts present in the methanogenic bioreactor (Akhurst, 2002). Addition of hydrolytic enzymes, namely β-glucosidases, proteases, trypsin, chymotrypsin, pronase E and cellulase to methanogenic sludge resulted in increased rate of hydrolysis in the floc matrix, leading to increased deflocculation. Enhanced hydrolysis has been evident also in the study of enzymology of accelerated PSS solubilization (Akhurst, 2002). In this case, primary sewage sludge (PSS) was combined with SRB under anaerobic conditions, and it was established that proteases and phosphatases activities were particularly associated with particulate organic matter of sewage sludge i.e. EPS (Whiteley *et al.,* 2002a; Pletschke *et al.,* 2002).

In an attempt to increase enzyme activity an extraction method called sonication was employed. An increase in enzyme activity was recorded for proteases, lipases, phosphatases cellulases and β-glucosidases upon sonication of the EPS (Whiteley *et al.,* 2002a; 2002b; 2002c). Whiteley *et al., (*2002a; 2002b; 2002c) characterized these enzymes and pH studies showed a broad range of proteolytic activity namely pH 5 - 7 and prominent activity at pH 10, while phosphatases showed optimum activity in the acidic region between pH 4.5 and 5.5. Temperature optimization studies demonstrated neutral proteases surviving temperatures up to 70ºC and those at pH 5 and pH 10 with temperature optima at 50 ºC and 60 ºC respectively. Phosphatase activity was optimum at 60 ºC (Whiteley *et al.,* 2002a; 2002b; 2002c). Lipase activity was also found to be associated with the EPS and also present extracellularly in considerable amounts. Sonication of EPS also led to increased enzyme activity as the enzymes were released into the supernatant. pH and temperature optimization studies showed optima between 6.5 and 8.0 and 50 to 60 ºC, respectively (Whiteley *et al.,* 2003). Cellulase and β-glucosidase activity was also found to be located mainly on the EPS. pH optima for β-glucosidases were between 5.4 and 7.2 and the multiple peaks suggested the presence of different bacteria in the sulphidogenic bioreactor. The optimum temperature was between 50 and 60 ºC (Whiteley *et al.,* 2002c).

In sulphidogenic systems, SRB are known to dissimilate SO_4^{2-} and reduce it under anaerobic conditions to produce H_2S gas and its ionic form, S^{2-}. The pH of the system becomes more alkaline due to an increase in concentration of HCO_3^-, OH^- , and SH^- ions (Whiteley *et al.,* 2002c). As already mentioned, small changes in ionic strengths and ionic composition can therefore alter the structural properties of the sludge floc and result in deflocculation and thus increased enzyme activity. It was also established that proteases, lipases and β-glucosidases activity was elevated in the presence of ≥ 200 mg/L of sulphide and sulphite and their activities decreased by 50% under high sulphate concentrations. Lipase activity was also highly activated at ≥ 200 mg/L of sulphide (1000 fold) and sulphite (15 fold); and inhibited by sulphate. β-Glucosidases were gradually inhibited by sulphate and sulphite while activated by sulphide concentration (Whiteley *et al.,* 2003). Whether sulphide is responsible for the chemical breakdown of EPS, thus increasing the availability of substrates to enzymes, or is the essential component in the formation of a viable enzyme substrate complex, or both processes occur, is yet to be clarified (Whiteley *et al.,* 2002a; 2002b; 2002c; 2003; Pletschke *et al.,* 2002). The sulphidogenic bioreactor has been proven to be a better source of hydrolytic enzymes *viz*. proteases, lipases, β and α-glucosidases, cellulases, sulphatases, ATP-sulphurylases etc. than the methanogenic bioreactor, as enzymatic activity has been proven to be higher. In addition, combinations of enzymes have been employed in enzymatic treatment to reduce solids and successfully reduced solids by 30 - 50 % (Parmar, *et al.,* 2001; Houghton and Stephenson, 2002).

2.1.1 Objectives

- To extract hydrolytic enzymes such as *viz*. proteases, lipases, and α-glucosidases, form the sulphidogenic bioreactor.
- To concentrate these enzymes using freeze-drying method, in order to obtain higher enzyme activity.
- To ascertain increase in enzyme activity of these enzymes by performing assays for proteases, lipases and α-glucosidases on commercial substrates namely azocasein, triacetin and ρ-Nitrophenol-α-D-glucopyranoside ρ-Nitrophenol-α-D-glucopyranoside respectively.

2.2 Materials and methods

2.2.1 Materials

Eppendorf Centrifuge 5810R and Beckman Model J2-21 Centrifuge were used for centrifugation. Spectroquant TR420 was used for heating samples during COD determination. Photometric Spectroquant NOVA 60 was used to read COD concentrations. Ultrasonic apparatus Virtis 60 was used for sonications. Lipase (EC 3.1.1.3) and triacetin were obtained from Sigma-Aldrich (Pty) Ltd., South Africa. Chloroform, chromotropic acid, disodium hydrogen phosphate, glycerol (99 % assay), methanol, potassium chloride, sodium arsenite, sodiumdihydrogen phosphate, sodium periodate, sulphuric acid (98 % assay), azocasein, α-glucosidase, ρ-nitrophenol-α-D-glucopyranoside, sodium hydroxide, solution A, solution B, trichloroacetic acid, Tris-(hydroxylmethyl)-aminomethane GR, were obtained from Merck (Pty) Ltd., South Africa.

2.2.2 Sulphidogenic Bioreactor set-up

Primary sludge was collected from an anaerobic digester at the Grahamstown Municipal Sewage Works, Grahamstown, South Africa. The sulphidogenic bioreactor [Figure 2.1] was setup in glass tank in a fume hood cupboard (23 ± 2°C), as follows:

(i) The sulphidogenic bioreactor (17 L) was seeded with 10% inoculums of SRB, obtained from an anaerobic digester at Grahamstown Municipal Sewage Works; Grahamstown, South Africa.

(ii) The reactor was sealed with a rubber stopper to maintain an anaerobic environment.

(iii) A gas trap containing zinc acetate was used to collect hydrogen sulphide escaping from the sulphidogenic bioreactor.

(iv) The bioreactor was fed with PSS that had been sieved through a 2 mm mesh sieve and diluted to a COD of 2000 mg/L.

(v) Sodium sulphate salt was used as a source of sulphate at a concentration of 2000 mg/L, i.e. a COD:SO_4^{2-} ratio of 1:1 was maintained.

(vi) Hydraulic retention time (HRT) was maintained at 2 days, with the mean organic loading rate of 0.0588 kg COD/L//day.

(vii) The overflow from the sulphidogenic bioreactor was allowed to settle by gravity to yield particulate material.

(viii) The particulate material contained a high number of SRB recycled to the main reactor once a week to minimize washing out and subsequent loss of SRB from the sulphidogenic bioreactor.

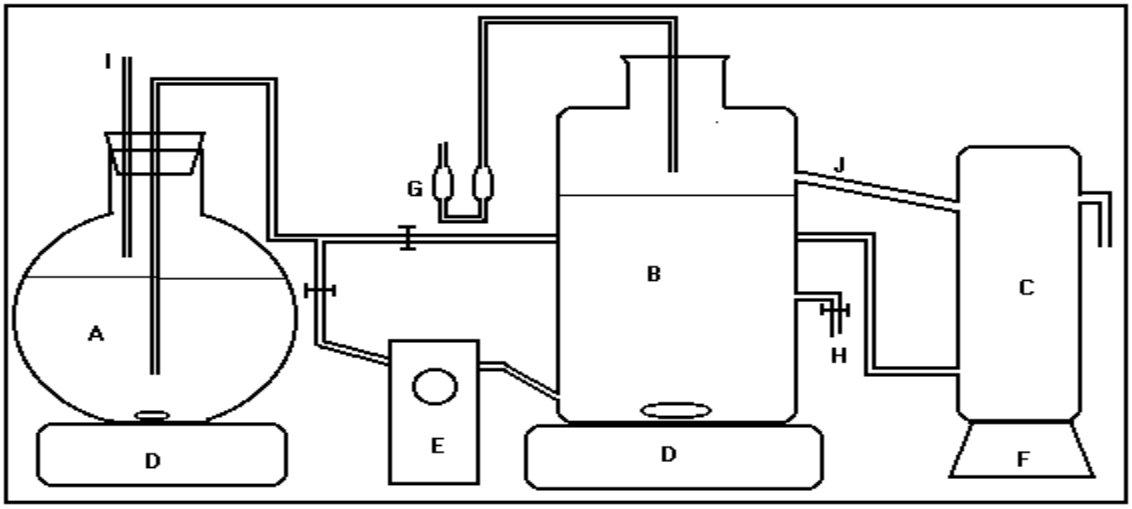


Figure 2.1: Showing the continuous feed sulphidogenic bioreactor under anaerobic conditions. A – Feed vessel, B – Main sulphidogenic bioreactor, C – Overflow vessel, D – Stirrers, E – Peristaltic pump, F- Support base, G – gas trap device, H – Sampling port, I – Influent port, J – Overflow port.

2.2.3 Chemical oxygen demand determination

Principle of the assay

Organic matter is oxidized by boiling the mixed chromic and sulphuric acids. The sample is refluxed in excess potassium dichromate solution that is strongly acidic. After digestion, the remaining potassium dichromate is titrated with ferrous ammonium sulphate. The amount of potassium dichromate consumed is determined and the amount of utilizable organic matter is calculated in terms of oxygen equivalent.

Reagents

1. Solution A: contains ferrous ammonium sulphate (Merck Test Kit).
2. Solution B: contains potassium dichromate and sulphuric acid (Merck Test Kit).

Procedure

Primary sewage sludge was first diluted 100 fold using deionized water. Diluted sludge (3 ml) was measured into a reaction cell. The blank was 3 ml of deionized water. Solution A (0.3 ml) and Solution B (2.3 ml) were added. Samples were vortexed and then incubated in preheated Spectroquant TR420 at (148 °C, 2 h). After cooling to room temperature, COD concentration (mg/L) was measured on a photometer Spectroquant NOVA 60.

2.2.4 Extraction of enzymes from a sulphidogenic biorector

Principle of the experiment

The majority of enzyme activity is located in the solid pellet produced by centrifugation. Sonication disrupts the EPS and bacterial cell walls, thus releasing enzymes into the supernatant.

Reagents

1. Sodium phosphate buffer (0.1 M, pH 7.5): 2.622 g of sodium dihydrogen phosphate monohydrate salt and 11.50 g of disodium hydrogen phosphate salt were dissolved in 1 L of deionised water.

Procedure

The dilute form of the enzyme "cocktail" was prepared by first obtaining large quantities (200 ml) of sulphidogenic sludge. The sludge was centrifuged at 10 000 rpm, at 4°C, for 15 minutes using the Beckman centrifuge Model J2-21. Supernatant and pellet were pooled separately, and this was performed until a suitable wet volume of pellet was obtained. The pellet was resuspended in an equal volume of 0.1 M sodium phosphate buffer at pH 7.5.

Resuspended pellet was sonicated at 2 min/ml, 30 seconds interval using an ultrasonic apparatus Virtis 60, energy setting at 8 W. The sonicated pellet was centrifuged at 10 000 rpm, at 4 °C, for 15 minutes. The blending method was also used to extract enzymes form the sulphidogenic pellet (1sec/ml). Enzyme assays were performed using pooled supernatants and commercial substrates. The supernatants were pooled and assayed for enzyme proteases, lipases and α-glucosidases activity. The pooled supernatants were the dilute enzyme extract. The concentrated enzyme extract was prepared in the same manner, except that the supernatant obtained was freeze-dried using an Edwards freeze dryer Model 25. The freeze-dried enzyme powder was stored at 4 °C. Enzyme assays using the concentrated enzyme "cocktail" and commercial substrates were performed. Enzyme powder was weighed using a Denver APX-200 mass balance and reconstituted with 0.1 M sodium phosphate buffer at pH 7 to make 0 - 200 mg/ml concentrations.

2.2.4.1 Protein assay

Principle of the assay

Coomassie Brilliant Blue G-250 exists in two different color forms, red and blue. The red form is converted to the blue form upon binding of the dye to protein. The protein-dye complex has a high extinction coefficient thus leading to great sensitivity in measurement of the protein. The binding of the dye to protein is very rapid, approximately 2 minutes, and the protein-dye complex remains dispersed in solution for a relatively long time (approximately 1 hour), thus making the procedure very rapid and yet not requiring critical timing for the assay (Bradford, 1976).

Reagents

1. Bradford's reagent: 100 mg of Coomassie Brilliant Blue G-250 dissolved in 50 ml of 95% ethanol, to this solution 100 ml, 85% of phosphoric acid was added. The volume was made up to 1L with deionised water.
2. Bovine serum albumin stock solution (300 μg/ml): 3 mg of BSA were dissolved in 10 ml of deionized water.

3. Sodium chloride (0.15 M): 4.383 g of NaCl was dissolved in 500 ml of deionized water.

Procedure

Bradford's assay (Bradford, 1976) was performed for protein determination on the sonicated and unsonicated or blended and unblended supernatants and pellets. At first a BSA (300 μg/ml) standard curve was prepared (0 – 15 μg/ml) [Table 2.1]. For protein assay, aliquots of supernatant (50 μl) were treated with 0.15 M NaCl (50 μl) and the samples were thoroughly mixed. The blank was prepared by using 50 μl of deionised water in place of abattoir effluent. Bradford's reagent (1 ml) was added and samples were incubated for 2 minutes. Absorbance was determined spectrophotometrically on a Shimadzu UV-160A - UV visible spectrophotometer at 595 nm, using the BSA standard curve [Figure 2.2].

Table 2.1: Preparation of BSA standard curve.

BSA concentration (μg/ml)	0.15 M NaCl (μl)	Bradford's reagent (ml)
0	100	1
3	90	1
6	80	1
9	70	1
12	60	1
15	50	1

2.2.5 Enzyme assays

Enzyme assays were performed on the dilute form of enzyme extract which is the supernatant after extraction and on the concentrated enzyme extract which was prepared by pooling all supernatants and then freeze drying.

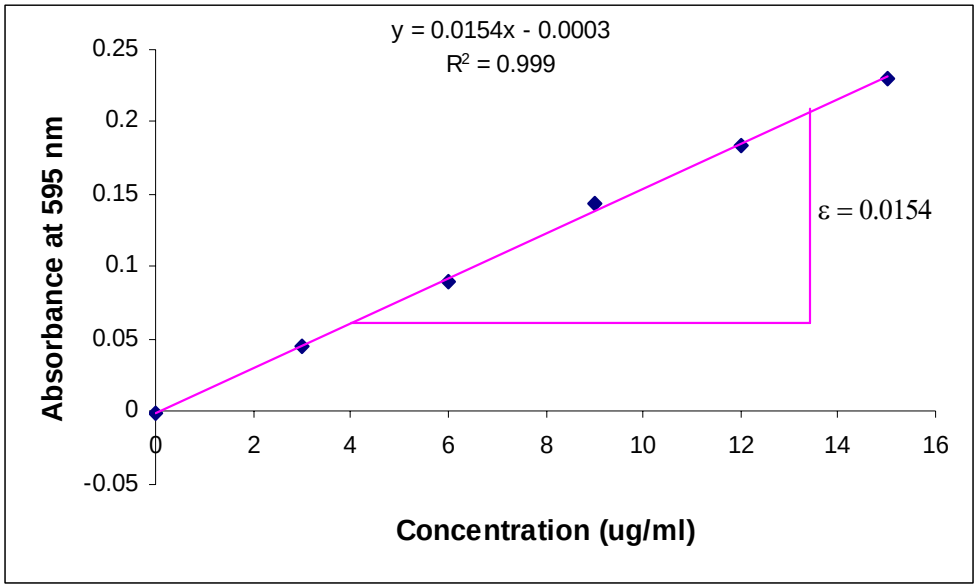


Figure 2.2: BSA standard curve for the determination of protein.

2.2.5.1 Protease assay

Principle of the assay

Azocasein is made up of azo dye bonded to the protein casein. Addition of a protease cleaves this bond releasing the dye and protein, which can be centrifuged down. Addition of hydrochloric acid leads to the development of a color that can be detected colorimetrically at a wavelength of 440 nm. Color intensity is a surrogate measurement of protease activity (Goel *et al.*, 1998).

Reagents

1. Azocasein (0.5 %): 0.5 g of azocasein was dissolved in 100 ml of 0.1 M sodium phosphate buffer at pH 7.

2. Sodium phosphate buffer (0.1 M, pH 7): 5.795 g of sodium dihydrogen phosphate a monohydrate salt and 8.233 g of disodium hydrogen phosphate salt were dissolved in 1 L of deionised water.

3. Sodium hydroxide (2M): 8 g of sodium hydroxide were dissolved in 100 ml deionised water.

4. Trichloroacetic acid (10 %): 10 g of trichloroacetic acid were dissolved in 100 ml deionised water.

Procedure

Azocasein (0.5 % or 5mg/ml) standard curve was prepared [Table 2.2]. For enzyme assays the dilute or concentrated sulphidogenic enzyme (3.0 ml obtained as in paragraph 2.2.4) was added to 0.5 % azocaisen (1.0 ml) and incubated (37°C, 90 mins). The reaction was stopped by adding 10 % trichloroacetic acid (TCA) (2.0 ml). Samples were centrifuged (4000 rpm, 10 mins) and supernatant (2.0 ml) mixed with 2 M NaOH (2.0 ml). Absorbance was determined at 440 nm and the extinction coefficient being the slope of the standard curve [Figure 2.3]. Enzyme activity was calculated as follows:

$$= \frac{V \times (\Delta A/\Delta t)}{\varepsilon.d.v}$$

V = total volume of the assay

ΔA = change of absorbance over time

t = incubation time

ε = extinction co-efficient was the slope of the standard curve

d = path length

v = volume of enzyme

Enzyme activity in International Units (IU) is defined as the amount of enzyme that will catalyze the transformation of 1 μmole of substrate in one minute.

2.2.5.2 α -Glucosidase assay

Principle of the assay

ρ-Nitrophenol-α-D-glucopyranoside is composed of α-D-glucose molecule with a nitrophenol group attached to C 1. α-Glucosidase enzyme hyrolysates this bond and a free glucose molecule and the nitrophenol group are released. The activity of α-glucosidase is determined by first measuring the absorbance at 410 nm (Goel *et al.,* 1998).

Table 2.2: Preparation of azocasein standard curve.

Azocasein concentration (mg/ml)	Azocasein volume (ml)	Deionized water (ml)
0	0	3
1	0.2	2.8
2	0.4	2.6
3	0.6	2.4
4	0.8	2.2
5	1	2

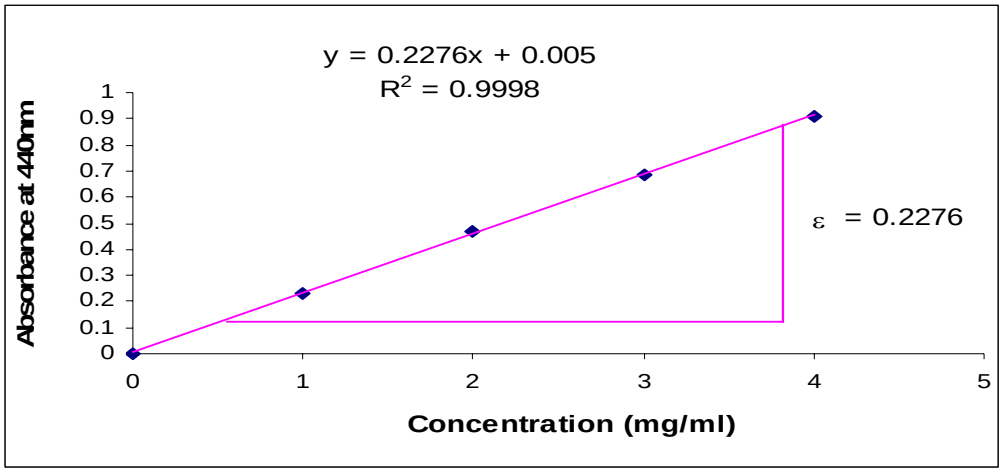


Figure 2.3: Azocasein standard curve for the determination of protease activity.

Reagents

1. ρ-Nitrophenol-α-D-glucopyranoside (1 mg/ml): 0.01 g of the substrate was dissolved in 10 ml of 0.1 M sodium phosphate buffer at pH 7.

2. Sodium phosphate buffer (0.1M, pH7): 5.795 g of sodium dihydrogen phosphate a monohydrate salt and 8.233 g of disodium hydrogen phosphate salt were dissolved in 1 L of deionised water.

3. Tris HCl (0.2M pH 7.6): 2.423 g Tris hydroxymethyl aminomethane was dissolved in 100 ml of deionised water and pH adjusted to 7.6 with 6 M HCl.

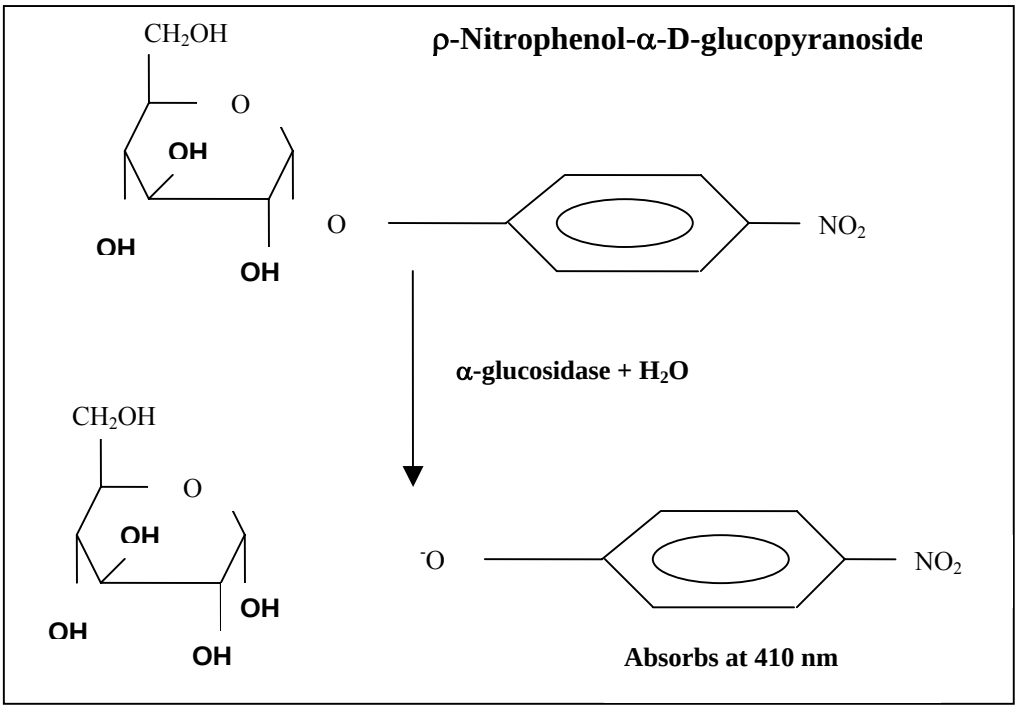


Figure 2.4: Cleavage of ρ-Nitrophenol-α-D-glucopyranoside by α-glucosidase.

Procedure

ρ-Nitrophenol-α-D-glucopyranoside (1 mg/ml; 1 ml) standard curve was prepared [Table 2.3]. ρ-Nitrophenol-α-D-glucopyranoside (1 mg/ml; 1 ml) added to Tris HCl (0.2 M, pH 7.6, 2.0 ml) and sulphidogenic enzyme (1.0 ml) was added and samples incubated (37 ºC, 60 mins). The reaction was stopped by incubating samples in boiling water for 3 minutes and samples were centrifuged (4000 rpm, 4 ºC, 15 mins) using an Eppendorf Centrifuge 5810R. Absorbance was read at 410 nm and enzyme activity was calculated as in section 2.2.5.1[Figure 2.5].

2.2.5.3 Lipase assay

Principle of the assay

Enzymatic hydrolysis of a fatty acid ester releases glycerol which subsequently combines with chromotropic acid to produce a coloured chromotropic acid complex. Enzyme activity is measured by the absorbance of this complex at 570 nm.

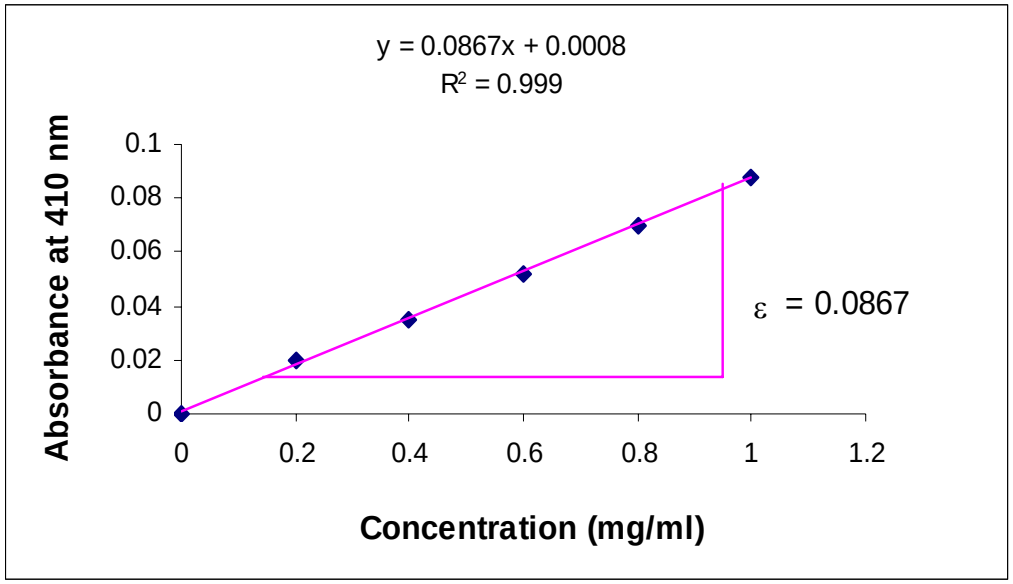


Figure 2.5: ρ-nitrophenol -α-D-glucopyranoside standard curve for the determination of α-glucosidases activity.

Reagents

1. Chromotropic acid (0.18 %): 0.18 g of chromotropic acid was dissolved in 100 ml of deionized water.

2. Chromotropic acid reagent: The ratio was 10 ml of 0.18 % chromotropic acid were always added to 45 ml of 12 M sulphuric acid

3. Glycerol stock solution (250 μM): 99% glycerol (1.85 ml) was added to 298.15 ml deionized water.

4. Sodium arsenite (10 % w/v): Sodium asernite (10.0 g) was dissolved in 100 ml of deionized water.

5. Sodium periodate (0.1 M): Sodium periodate (2.14 g) was dissolved in 100 ml deionized water.

6. Sodium phosphate buffer (0.1M, pH7): 5.795 g of sodium dihydrogen phosphate a monohydrate salt and 8.233 g of disodium hydrogen phosphate salt were dissolved in 1 L of deionised water.

7. Sulphuric acid (12 M): was prepared from concentrated sulphuric acid (98 %).

8. Sulphuric acid (5 M):was also prepared from a concentrated sulphuric acid (98 %).

9. Triacetin (3%): Triacetin (3 ml) was dissolved in 0.1 M Sodium phosphate buffer at pH 7.

Procedure

A glycerol standard curve (0.1 - 0.5 µmol/ml) was prepared from 250 µM glycerol stock solution [Figure 2.4]. For enzyme assay 3 % triacetin (0.5 ml) was added to sulphidogenic enzyme (0.5 ml). H_2SO_4 (5 M, 50 µl) and $NaIO_4$ (0.1 M, 250 µl) were added to the standards and samples. After thorough mixing all samples were incubated at room temperature for 5 minutes and 10 % sodium arsenite (250 µl) was added to each sample. After vortexing, 250 µl were aliquoted out into series of test tubes containing 2.5 ml of chromotropic acid reagent. Samples were then boiled for 60 minutes and cooled. Glycerol levels were obtained by reading absorbance at 570 nm. Lipase activity was calculated as in section 2.2.5.1.

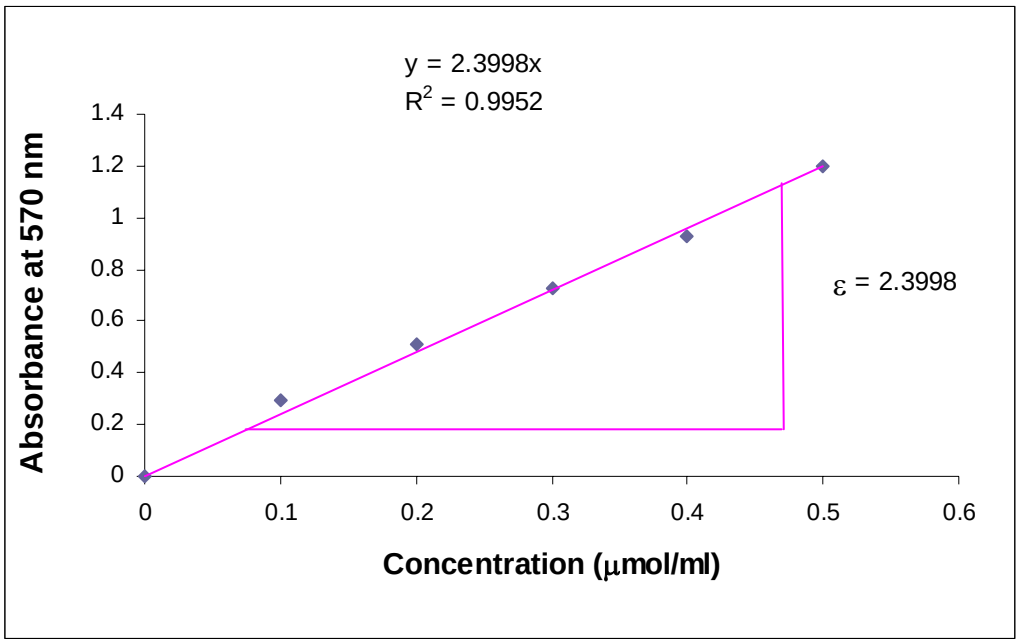


Figure 2.6: Glycerol standard curve for the determination of lipase activity.

2.3 Results and discussion

2.3.1 Localization and extraction of enzymes from a sulphidogenic bioreactor

Localization, extraction and quantification of proteins from a sulphidogenic bioreactor are shown in Figure 2.7.

Sonication studies revealed that the supernatant before extraction, which was obtained by centrifuging the sulphidogenic sludge contained 0.66 % (0.515 μg/ml) of protein while the unsonicated pellet contained 77.49 μg/ml which was taken as 100 % protein. Sonication of the pellet released 11.64 % (9.02 μg/m) of protein into the supernatant and 54.84 % (42.50 μg/ml) remained on the pellet. .It is important to note that 33.51 % (25.97 μg/ml) of protein was lost during sonication. It was concluded that the majority of protein in a sulphidogenic bioreactor is immobilized naturally on the EPS. The majority of protein found in the pellet was also indicative of the location of enzyme activity.

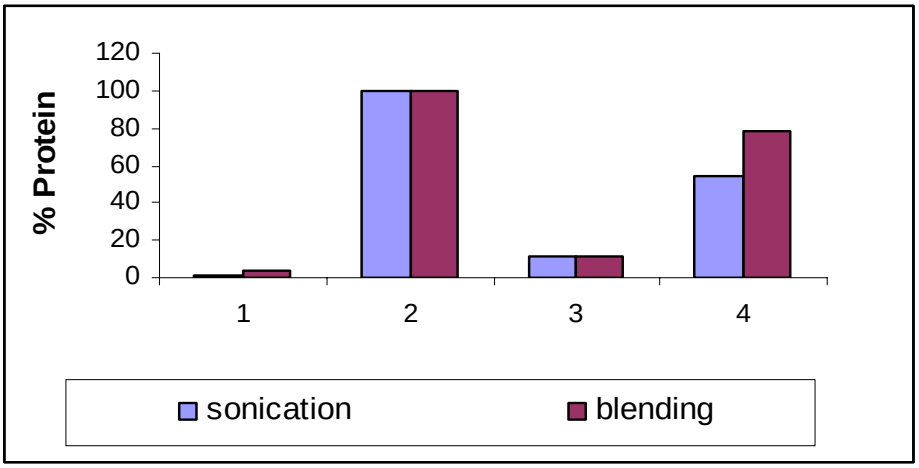


Figure 2.7: Quantitative analysis of protein from a sulphidogenic bioreactor before and after extraction by sonication or blending method. 1 = supernatant before extraction, 2 = pellet before extraction, 3 = supernatant after extraction and 4 = pellet after extraction.

These results supported previous studies which state that the architecture of EPS is dependent on the interaction between biopolymers present in that particular sludge, which may be carbohydrates, lipids, proteins, polysulphates and polyphosphates; and various cations and anions (Frϕlund *et. al.,* 1995; Jinho Jung *et al.,* 2002; Cadoret *et al.,* 2002). These results were further supported by Akhurst (2002) who discovered that the majority of EPS/floc material was carbohydrate, followed by protein. Due to the loss of protein experienced repeatedly upon extraction of enzymes/protein using sonicator at 2 min/ml, it was concluded that sonication was not a viable method.

Other reasons being that sonication was time consuming and there was also a problem of heating-up when large volumes of sulphidogenic sludge was fractionated. Extraction of protein using a blender is a method that was also investigated as various researchers also experienced loss of protein or enzyme activity when extraction was carried out using sonication, ammonium sulphate and detergents such as Triton X-100 and sodium cholate (Rashamuse, 2003; Ngesi, 2001; Tshivhunge, 2001). As a result extraction studies using a blendor were performed. The advantage of using the "blending method" was that, extraction of protein from large volumes (500 ml) of sulphidogenic pellet could be performed at less time (1sec/ml) and in cycles. After each cycle the pellet was stored at 4 °C and the blendor chilled to minimize heat build-up during the process and extraction continued. Results in Figure 2.7 show only the first cycle of protein/enzyme extraction by the blending method. The supernatant before blending contained 4.02 % (1.98 μg/ml), while the unblended pellet contained 100 % (49.31 μg/ml). The supernatant after blending contained 11.63 % (5.73 μg/ml) and blended pellet contained 78.26 % (38.59 μg/ml). From these results, it was clear that loss of protein during blending was less than with the sonication method (10.11 %, compared to 33.53 %). It was concluded that the blending method was more effective in protein/enzyme extraction from a sulphidogenic bioreactor. However, optimization of extraction using blending method that is combining the extract from all cycles and assaying for protein and enzyme activity is yet to be performed.

2.3.2 Enzyme assays

2.3.2.1 Localization and distribution of enzyme activity in the sulphidogenic bioreactor

Enzyme assays (protease, α-glucosidase and lipase) were performed on the enzyme extracts obtained by blending method and their localization and distribution is shown in Figure 2.8, 2.9 and 2.10. Very low protease activity of 3 IU (7.14%) was detected in the unblended supernatant (extracellular fraction) [Figure 2.8]. Lack of protease activity in the extracellular fraction was not surprising as low protein was detected in Figure 2.7.

The unblended pellet (EPS) contained 42 IU (100 %) protease activity and upon first cycle of blending half protease activity of 21.4 IU (51 %) was released into the supernatant and the other half remained. In Figure 2.9 extracellular α-glucosidases activity was detected to be 62.4 IU (10.1 %) and upon blending 38.03 % (235 IU) was released while 58.3 % was immobilized on EPS. Only 3.72 % (23 IU) of α-glucosidases activity was lost during blending. Lipases contained 1270 IU (69.4 %) extracellular activity and 1830 IU (100 %) in the pellet. Blending released 78.5 IU (4.29 %) into the supernatant and 1605 IU (87.7 %) lipases were immobilized on the pellet. Only 8 % (146.5 IU) lipase activity was lost during blending. These results are supported by Whiteley, *et al.,* (2003c) showing that lipases are both extracellular and immobilized on EPS and homogenization or extraction (whether sonication or blending) released 4.29 % of lipases into the supernatant. These findings also suggested that lipases, proteases and β-glucosidases are also immobilized on EPS, as it is where most of the substrates like protein, carbohydrates and lipids are located (Cadoret *et al.,* 2001; 2002; Frϕlund *et al.,* 1995). Results also suggested that proteases and α-glucosidases are not as tightly bound to EPS as lipases, which is to be expected as lipids in EPS are membrane bound in bacterial cell walls. The other reason for highest protein concentrations (enzyme activity) found on the pellet fraction is enzymes are closely associated with substrates (main components of EPS) for enzyme substrate-enzyme complexes to occur easily.

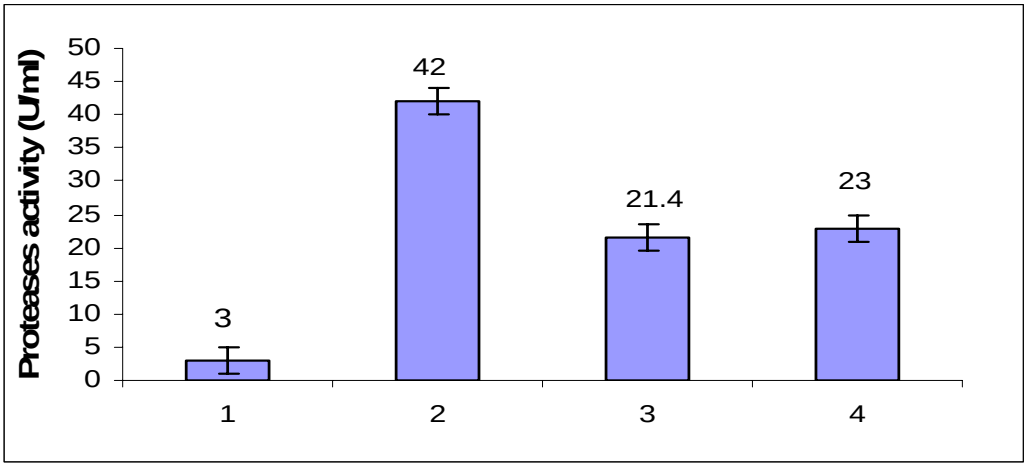


Figure 2.8: Protease activity using 0.5 % azocasein as substrate. 1 = supernatant before blending, 2 = pellet before blending, 3 = supernatant after blending and 4 = pellet after blending**.**

2.3.2.2 Enzyme assays on commercial substrates using the concentrated enzyme extract

As enzymes are catalysts, the initial velocity of the reaction would be expected to be proportional to the concentration of the enzyme. This is indeed the case for most enzyme-catalyzed reactions, where a graph of initial velocity against total enzyme concentration would be a straight line passing through the origin.

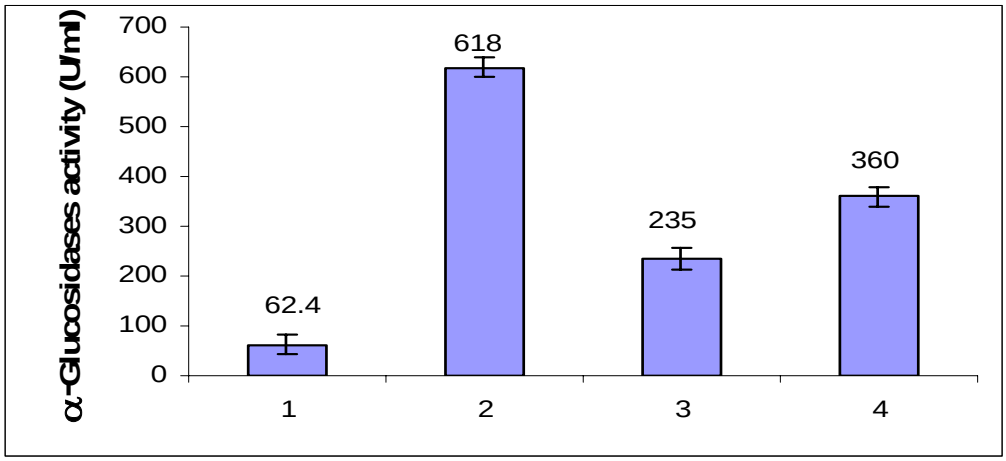


Figure 2.9: α-Glucosidase activity using 1 mg/ml ρ-Nitrophenol-α-D-glucopyranoside as substrate. 1 = supernatant before blending, 2 = pellet before blending, 3 = supernatant after blending and 4 = pellet after blending.

It has been established that there are however some cases where this simple relationship does not appear to hold. It is therefore important to check for linearity in all studies. In some cases departure from linearity maybe because of changes of the pH or ionic strength of the assay mixture as increasing amounts of the enzyme solution are added (Danson, 2002). Therefore, the objective of the study was to determine the highest concentration of the concentrated enzyme extract needed to give highest enzyme activity. Substrate concentrations of 0.5 % azocasein, 3 % triacetin and 1 mg/ml ρ-Nitrophenol-α-D-glucopyranosides were kept constant. Figure 2.8 shows that α-glucosidase activity increased with increased enzyme activity.

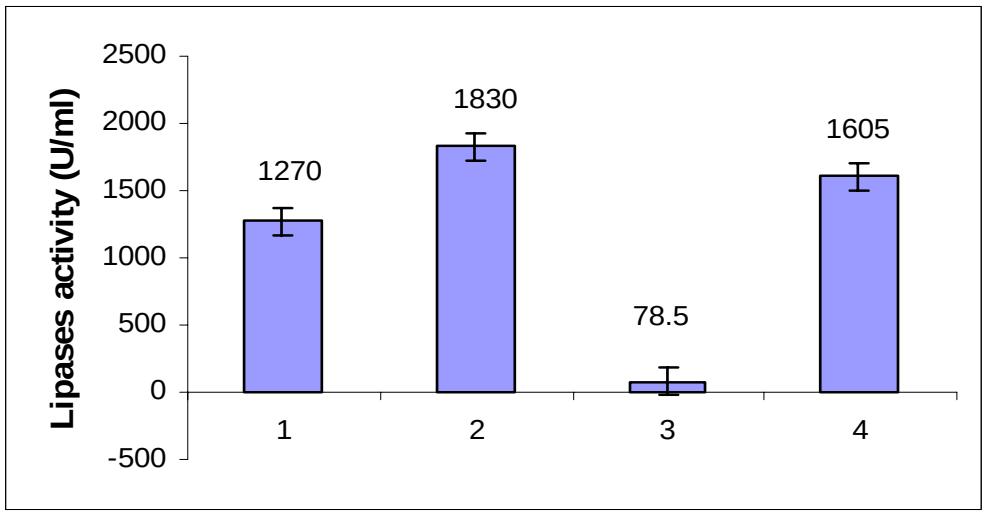


Figure 2.10: Lipase activity using 3 % triacetin as substrate. 1 = supernatant before blending, 2 = pellet before blending, 3 = superanatant after blending and 4 = pellet after blending.

The graph showed linearity between 0 and 200 mg/ml concentration and this was confirmed by 1/[V] versus 1/[E] where the R^2 equaled 0.9541. These results suggested that increased enzyme concentration led to increased enzyme activity. In Figure 2.7 the graph was linear between 0 and about 35 mg/ml. This was proved by the fact that 1/[V] versus 1/[E] plot had R^2 value of 0.7160. In Figure 2.8 the graph was linear between 0 and about 50 mg/ml and R^2 of 0.7999 was obtained when 1/[V] was plotted against 1/[E]. Results from Figure 2.7 and 2.8 also suggest that substrate were the limiting factor as result linearity was reached at low enzyme concentrations. These results also suggest that substrate studies, where the concentration of substrate is varied and enzyme concentration kept constant can be performed at 35 mg/ml, 50 mg/ml and 200 mg/ml for proteases, lipases and α-glucosidases, respectively. Increased enzyme activity with an increase in the concentration of the enzyme was also observed by Parmar *et al.,* (2001) in the enzymatic treatment of sewage sludge, which led to increased solid reduction. It was also established that a mixture of industrial cellulase, protease and lipase, equal in proportion by weight, led to higher solid reduction. The effect of combinations of enzyme treatments was the highest in the two enzyme combinations of protease and cellulase. It was concluded that enzymes function better in combination than individually, which is also true for enzymes from sulphidogenic bioreactors. The other advantage is that the enzymes from a sulphidogenic bioreactor are in combination naturally. This enzyme technology is still at infant stages and has tremendous potential in the treatment of wastewaters.

It must be noted that the "enzyme extract" prepared also contain a number of other enzymes as mentioned in section 1.1.

2.4 Conclusions

It was concluded that in the sulphidogenic bioreactor the majority of protein is localized in the pellet. Extraction of protein/enzymes from a sulphidogenic bioreactor by homogenizing using a blendor yielded higher protein/enzyme activity for proteases, lipases and α-glucosisdases. Localization and distribution of enzyme activity in the sulphidogenic bioreactor followed the same trend as protein concentration for proteases and α-glucosidases except for lipases where it was established that the enzyme activity was also high on the supernatant as well. Extraction and concentration of enzymes from a sulphidogenic bioreactor was successful and the use of commercial substrates on the concentrated enzyme extract proved that enzyme activity was increased upon concentration using the freezedrying method. Objectives set out at the beginning of this chapter were all attempted and achieved, such the concentrated enzyme extract was used to defoul membranes fouled statically (Chapter 3) and fouling in ultrafiltration systems (Chapter 4 and 5).

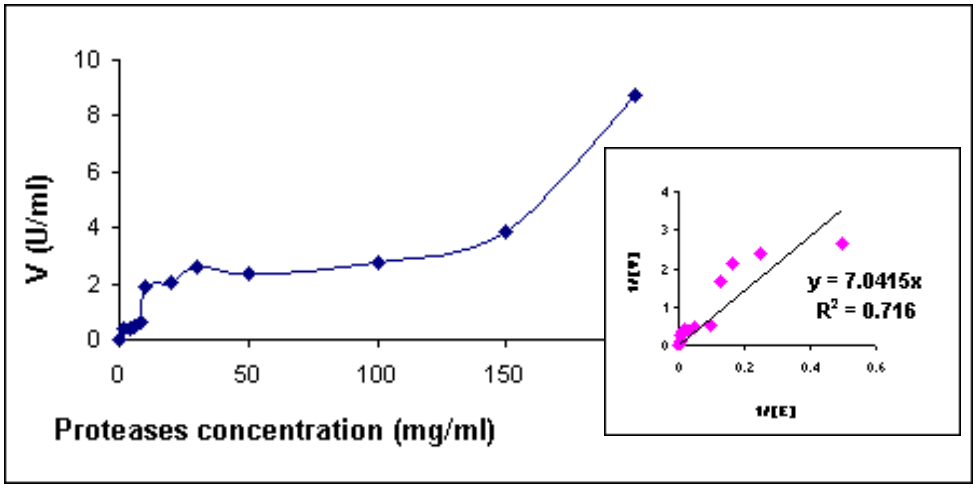


Figure 2.11: Activity of proteases on 0.5 % azocasein over increased enzyme concentration.

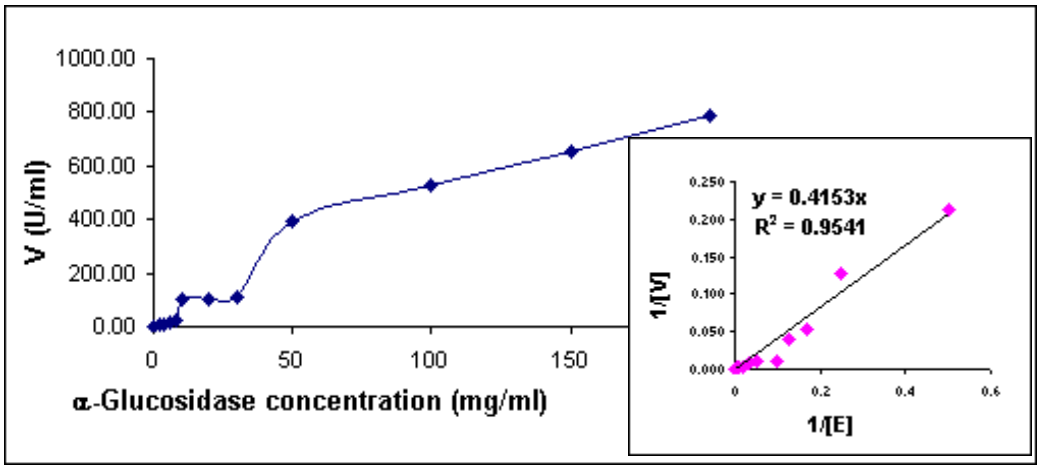


Figure 2.12: Activity of α-glucosidases on 1 mg/ml *ρ*-Nitrophenol-*α*-D-glucopyranoside over increased enzyme concentration.

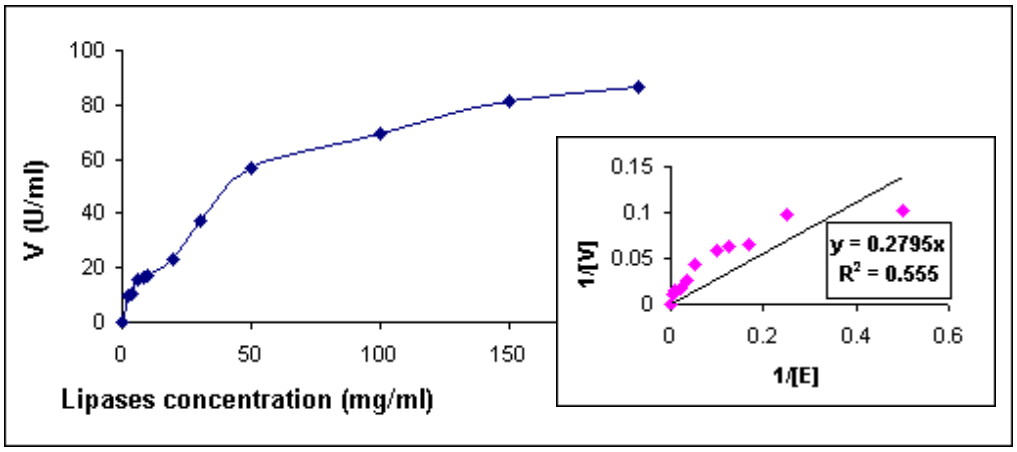


Figure 2.13: Activity of lipases on 3 % triacetin over increased enzyme concentration.

CHAPTER 3
STATIC FOULING AND DEFOULING OF POLYSULPHONE MEMBRANES

3.1 Introduction

Membrane blockage is one of the major factors restricting the effectiveness of membrane filtration in bio-separation and bioengineering. Almost all filtration processes utilizing membranes are characterized by a progressive decline of permeate flux with time as a result of fouling of the membrane (Su *et al.,* 2000; Tracey and Davis, 1994). In general terms, fouling arises from a combination of physicochemical and hydrodynamic conditions. It may occur as a result of the formation of a gel layer of protein on the high-pressure side of the membrane; by deposition of protein inside the membrane pores; or by a combination of both processes (Su *et al.,* 2000). Identification of the exact location of fouling would be beneficial because it might enable better design of the filtration set-up; optimization of the flow conditions; more effective cleaning of fouled membranes; and improvement of surface properties of the membrane pores by surface modification (Su *et al.,* 2000).

3.1.1 Protein fouling mechanisms

The crucial step in understanding the fouling mechanism is then to be able to identify the location of fouling and to follow the development of the foulant layer with time. Unfortunately, almost none of the existing techniques can accomplish this task (Jones and O'Melia, 2001). It is now accepted that in the first stage of the development of fouling, protein molecules form a monolayer through physical adsorption (Ho and Zydney, 2002; Kim *et al.,* 1992; Robertson and Zydney, 1990). This adsorption process is thought to be complete in seconds and is only limited by convective transport of the protein molecules from the bulk to the membrane surface (Ho and Zydney, 2002; Kim *et al.,* 1992; Robertson and Zydney, 1990).

This is followed by a much slower step of further deposition of protein onto the pre-adsorbed monolayer leading to the formation of a densely packed protein layer (Ho and Zydney, 2002; Kim *et al.,* 1992; Robertson and Zydney, 1990). Although it is obvious that this latter step must be a major factor causing the decline in permeates fluxes, the early stage of the development of foulant layer is thought to be crucial to the understanding of fouling (Madaeni *et al.,* 2001). However, in the absence of any *in situ* technique for monitoring the development of fouling, such an assessment remains a hypothesis. Protein adsorption/fouling is hypothesized to proceed in several steps: charge redistribution in overlapping electric double layers of proteins and membrane surfaces, followed by dehydration of these interacting surfaces which is enhanced at higher ionic strengths, and almost irreversible structural rearrangements of the adsorbed protein molecules (Jones and O'Melia, 2001; Fane and Fell, 1987; Norde, 1986). Protein molecules have a tendency of being readily adsorbed on membranes surfaces by a number of interaction mechanisms namely ionic, entropic, hydrophobic, van der Waals, specific/affinity etc., according to their chemical and structural characteristics. This enables adsorption even in the absence of transmembrane pressure difference (Jones and O'Melia, 2001; Roberston and Zydney, 1990; Fane and Fell, 1987; Norde, 1986). Nevertheless, the degree of adsorption depends on the local protein concentration (Bowen and Gan, 1991). The structural changes in protein molecules following adsorption represent a major step of irreversible membrane fouling by proteins. Hydrophilicity of membrane surfaces has been considered necessary to reduce such fouling. In previous studies, the interaction between proteins and microfiltration membranes have been carried out under static conditions, with no pressure applied across the membrane (Tracey and Davis, 1994).

3.1.2 Reversible and irreversible fouling

Fouling on the other can be further classified to reversible and irreversible[1]. Gel layer formation is one form of fouling and is in principle reversible fouling because rinsing with clean water should solve the problem.

1. http://www.ub.rug.nl/eldoc/dis/science/t.r.noordman/c1.pdf

Fouling can become irreversible[1] if the components in the gel layer react with each other and form a dense cross-linked layer on top of the membrane, which is not easy to remove. Another mechanism of fouling is pore blocking which can be highly irreversible. Possibilities for cleaning depend on the fouling type. If fouling is reversible, like gel formation, rinsing with pure water might cure the problem. If fouling is irreversible other solutions have to be sought e.g. rinsing with strongly alkaline or acidic agents at elevated temperature may help in case of adsorption or irreversible fouling[1]. Irreversible protein adsorption is considered as the main fouling mechanism in UF of protein solutions (Madaeni *et al.,* 2001; Su *et al.,* 2000). The adsorbed proteins cannot be easily removed by washing with buffer solutions or water. The morphology of deposited proteins depends on pH and electrolyte concentrations. The specific resistance of adsorbed proteins in the presence of salts is also high. The presence of electrolyte increases flux reduction, because of the conformational changes in the native structure of proteins leading to poor permeability of the adsorbed protein layers. If proteins interact hydrophobically with membrane surfaces then structural changes in the adsorbed protein molecules cannot be ruled out (Robertson and Zydney, 1990; Fane and Fell, 1987; Norde, 1986). The footprint of an adsorbed bovine serum albumin (BSA) molecule on a hydrophobic surface has recently been shown to be five times the globular BSA molecule in the solution (Wertz, 1999).

3.1.3 Existing defouling procedures

Considerable research has been focused on cleaning of fouled membranes. Reduction of fouling and cleaning of fouled membrane have been approached in a number of ways which include optimisation of flow conditions; backflushing; production of membranes with reduced absorptive properties by modification of membrane surface; pre-treatment of the effluent; harsh chemical cleaning agents which results into high cleaning costs and industrial pollution (Czekaj and Davis, 1998; Reis, *et al.,* 1997; Maartens *et al.,* 1996c; Eckner, *et al.,* 1993; Kim *et al.,* 1993; Rodgers and Sparks, 1991; Flemming., 1990; Trägårdh, 1989). Milder and environmentally friendlier cleaning regimes such as purified enzymes and detergents have been considered for the removal of biologically derived foulants from polymer membranes (Maartens, et al., 1998; 1996a; Muňoz-Aguado and Fane, 1996).

1. http://www.ub.rug.nl/eldoc/dis/science/t.r.noordman/c1.pdf

Enzyme immobilized membranes and their self-cleaning and antifouling abilities in protein separations have also been prepared (Chen *et al.,* 1992). Research on complex fouling and cleaning-in-place of reverse osmosis desalination are underway (Luo and Wang, 2001). The use of enzyme alone or in combination with biodegradable detergents is an attractive alternative to the classical cleaning regimes. (Leukes *et al.,* 1999; Maartens *et al.,* 1998; 1996a). Previous studies (Maartens *et al.,* 1996a), showed that enzymes, as biocatalysts, can be used effectively in combination with detergents to reduce fouling and restore permeate flux on previously fouled membranes. Enzymes are also ideal cleaning agents because they are highly specific for the reactions they catalyse and the substrates with which they interact. In addition, enzymes also act under mild conditions of pH, temperature, and ionic strength and will not damage the membrane surface (Maartens *et al.,* 1996a). The consequences of defouling or cleaning fouled membranes using sludge enzymes would be reduction of high cleaning costs, since large amounts of cleaning agents including purified enzymes are often required to restore permeate fluxes; and increased membrane life as enzymes are milder and environmental friendly as compared to harsh chemical cleaning agents that damage the membrane. The source of sludge enzymes would be sulphidogenic bioreactor where sulphate reduction occurs. Robertson and Zydney, (1990) performed static adsorption experiments on ultrafiltration membranes, while Bowen and Gan, (1991) performed static adsorption experiments on microfiltration membranes. These authors concluded that approximately a monolayer of protein adsorbs under static conditions, which causes relatively small increases in the resistances of microfiltration membranes. However, flux decline for microfiltration membranes under dynamic conditions is much greater than can be explained by static protein adsorption (Bowen and Gan, 1991).

3.1.4 Objectives

- To characterize the abattoir effluent for potential foulants such as proteins, lipids and carbohydrates.
- To perform static fouling on polysulphone membranes (PSM) using the abattoir effluent/
- To defoul/clean PSM using at first the dilute and then the concentrated form of the enzyme extract from the sulphidogenic bioreactor.

- Biochemical analysis of fouled and defouled PSM using qualitative and quantitative assays for proteins, lipids and carbohydrates.

3.2 Materials and methods

3.2.1 Materials

Ostrich abattoir effluent used for fouling was obtained from Ostritech (Pty) Ltd, Grahamstown, South Africa. Polysulphone membranes (0.22 micron, 47 mm diameter) were obtained from Osmonic Inc. A Shimadzu UV-160A, UV visible recording spectrophotometer was used for reading absorbance. Eppendorf centrifuge 5810R and Beckman Model J2-21 centrifuge were used for centrifugation. Deluxe vortex mixer MT19 was used to mix contents of samples. Bovine serum albumin (BSA), Bradford reagent, carbofulschin, Coomassie brilliant blue G-250, chromotropic acid, Folin-Cauteleu reagent, glycine, ninhydrin reagent, Schiff reagent, sodium arsenate, sodium arsenite, sodium chloride, sodium dodecyl sulphate, were purchased from Sigma – Aldrich (Pty) Ltd South Africa. Acetic acid, ammonium molydbdate, anthrone, chloroform, copper sulphate, ethanol, glucose, glycerol, methanol, methylene blue, phenol, potassium chloride, potassium sodium tatrate, sulphuric acid, sodium acetate, sodium carbonate, sodium hydroxide, sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium periodate and sodium sulphate were obtained from Merck (Pty) Ltd South Africa.

3.2.2 Characterization of the abattoir effluent

The abattoir effluent was first characterized for presence of organic foulants using assays specific for proteins, amino acids, lipids and carbohydrates.

3.2.2.1 Folin-Lowry assay

Principle of the assay

This assay is the modified version of Lowry *et al.,* (1951).

The original method described by Lowry *et al.,* (1951) combines the use of the biuret reaction of proteins with copper ions in alkali with the reduction of the Folin-Ciocalteu phenol reagent by tyrosine and tryptophan residues. The latter reaction in a complicated way is intensified by the copper-protein complex. The Lowry method is about 10 to 20 times more sensitive than UV absorption measurement and 50 to 100 times more sensitive than the standard biuret assay. A large number of substances interfere with protein quantitation by the Lowry assay. The Lowry assay is subject to interference by compounds potassium ions, magnesium ions, carbohydrates, reductants, EDTA and SDS. As a result the following procedure is a modification of this original method based on the results obtained by Hess *et al.,* (1978) in their investigation for a linear Folin-Lowry assay for both water soluble and SDS solubilized proteins. In order to obtain linearity at higher absorbance and extended the range of protein types that can be assayed without heating in alkali. When insoluble proteins (which often occur in association with lipids) are present in the sample to be assayed, sodium dodecyl sulphate (SDS) dissolves them in a short time and at room temperature. Solubilization of proteins by SDS can occur even in the presence of lipids. High concentrations of Folin-Ciocalteu reagent are also used to achieve linearity.

Reagents

1. Solution A: 0.2 g of sodium tartrate and 10 g of sodium carbonate were dissolved in 55 ml of 1 M sodium hydroxide and diluted to final volume of 100 ml with deionized water.
2. Solution B: 2 g of sodium tartrate and 1 g of copper sulphate were dissolved in 90 ml of deionized water and 10 ml of 1 M sodium hydroxide added.
3. Solution C: 1 part of Folin-Ciocalteu's phenol reagent was added in 2 parts of deionized water.
4. Solution D (5 % SDS): 5 g of sodium dodecyl sulphate were dissolved in 100 ml sodium hydroxide.

Procedure

Bovine serum albumin (300 μg/ml) standard curve was prepared [Table 3.1]. Protein concentration on the abattoir effluent was determined using the Folin-Lowry method (Hess, 1978). The abattoir effluent (2.5 ml) was added to solution D (5 ml of 5 % SDS). After mixing, aliquots of the solution (750 μl) were treated with NaOH (2 M, 500 μl) and solution A (900 μl). After thorough mixing, samples were incubated at room temperature for 30 minutes.

Solution B (100 µl) was added and samples were incubated at room temperature for 20 minutes. Two portions of solution C (1.5 ml) were added. After incubating at room temperature for 30 minutes, absorbance values were determined at 650 nm against a BSA standard curve [Figure 3.1]. Protein concentration was determined using the equation of the standard curve.

Table 3.1: Preparation of BSA standard curve.

BSA concentration (µg/ml)	BSA volume (ml)	Volume of deionized water (ml)
0	0	0.75
12	0.04	0.71
24	0.08	0.67
36	0.12	0.63
48	0.16	0.59
60	0.2	0.55

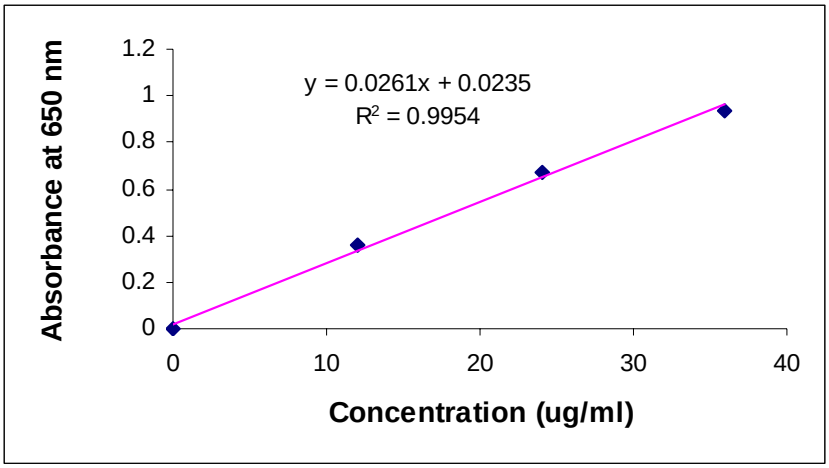


Figure 3.1: Bovine serum albumin standard curve for the determination of proteins.

3.2.2.2 Amino acid assay

Principle of the assay

α-amino acid + ninhydrin ⟶ reduced ninhydrin + α-imino acid + H_2O…….(1)

α-imino acid + H_2O ⟶ α-keto acid + NH_3 … ……(2)

α-keto acid + NH_3 ⟶ aldehyde + CO_2 ..... (3)

Step 1 is an oxidative deamination reaction, removing two hydrogen atoms from α-amino acid to yield α-imino acid.

Ninhydrin is reduced and loses an oxygen atom with the formation of water molecule. In step 2 the NH group in the α-imino acid is rapidly hydrolyzed to form an α-keto acid with the production of ammonia. This α-keto acid under goes decarboxylation (step 3), with heat to produce carbon dioxide and an aldehyde that has one less carbon atom than the original amino acid. These three steps produce reduced ninhydrin and ammonia that is required for color development. The overall reaction is described as:

α-amino acid + ninhydrin $\longrightarrow$ aldehyde + CO_2 + final complex (deep purple) + $3H_2O$

Reagents

1. Ethanol (50 % v/v): 100 ml of 99% ethanol were diluted with 100 ml of deionised water.
2. Glycine stock solution (1 mM): 0.0075g of glycine was dissolved in 0.1 M sodium phosphate buffer.
3. Sodium phosphate buffer (0.1 M, pH 8):0.966 g of sodium dihydrogen phosphate, a monohydrate salt was added to 13.20 g of disodium hydrogen phosphate and dissolved in 1 L of deionised water.
4. Sodium acetate buffer (4 M, pH 5.5): 31.83 g of sodium acetate were dissolved in deionized water and 0.7 µl of acetic acid added. The solution was made up to 100 ml with deionized water.

Procedure

A standard curve was prepared using 1 mM glycine stock solution [Table 3.2]. Abattoir effluent (10 µl) was added to sodium phosphate buffer (40 µl, 0.1 M, pH 8). Sodium acetate buffer (50 µl 4 M, pH 5.5) was then added followed by Ninhydrin reagent (50 µl) and the sample was incubated (60 °C, 20 minutes). After cooling to room temperature, 50 % ethanol (100 µl) was added and absorbance was determined spectrophotometrically at 570 nm. The concentration of amino acids was determined from the standard curve [Figure 3.2].

Table 3.2: Preparation of glycine standard curve.

Glycine concentration (mM)	Volume of glycine (µl)	Sodium phosphate buffer(0.1 M, pH 8) (µl)
0	0	50
0.1	5	45
0.2	10	40
0.3	15	35
0.4	20	30
0.5	25	25

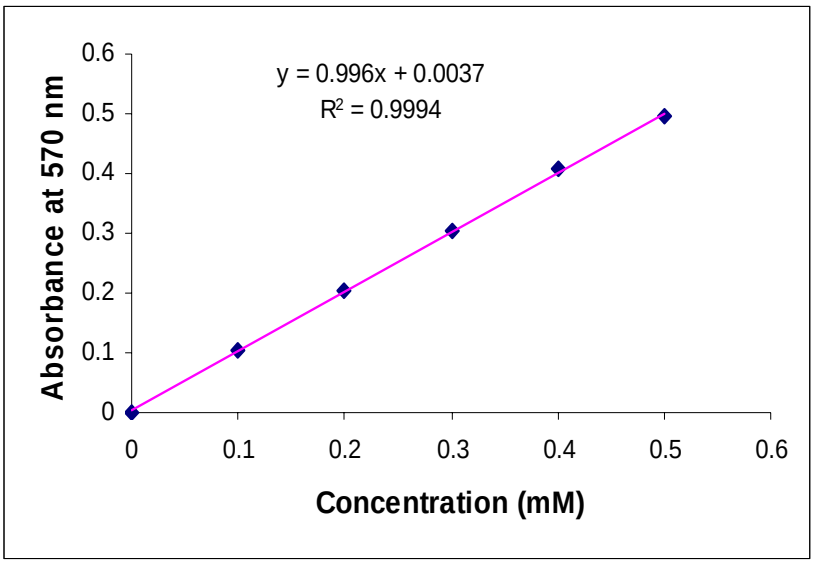


Figure 3.2: Glycine standard curve for the determination of amino acid concentration.

3.2.2.3 Lipid assay

Principle of the assay

Samples are extracted into chloroform-methanol solvent and solubilize the lipid material followed by treatment with potassium chloride (Folch *et al.*, 1957).

Reagents

1. Chloroform- methanol (2:1): 1 L of chloroform was mixed with 500 ml methanol.
2. Potassium chloride (0.88 %): 8.8g of KCl was dissolved in 1 L of deionised water.

Procedure

Abattoir effluent (100 ml) was heated (100 °C, 2 h). After cooling, the sample was homogenized in methanol-chloroform (1:2) (30 ml) and centrifuged (4000 rpm, 10 min, 4 °C). The supernatant was decanted and the pellet resuspended in chloroform-methanol solvent (2:1) (60 ml) and homogenized (2 mins). The sample was then centrifuged (4000 rpm, 10 min, 4°C) and the supernatants pooled and then treated with 0.88 % KCl (25 % v/v). The aqueous layer was removed and the organic layer evaporated to dryness. Lipid content of the abattoir effluent was determined by weight (Folch *et al.,* 1957).

3.2.2.4 Anthrone assay

Principle of the assay

Concentrated sulphuric acid hydrolyses glycosidic bonds to produce monosaccharides which are then dehydrated to furfural and its derivatives. Furfural reacts with anthrone (10-keto-9, 10-dihydroanthracene) to give a blue-green complex (Plummer, 1978).

Reagents

1. Anthrone reagent (2 g/L in concentrated H_2SO_4)
2. 70 % ethanol: 70 ml of 99 % of ethanol were added to 30 ml of deionized water.

Procedure

Glucose (1 mg/ml) standard curve was prepared [Table 3.3]. Total carbohydrate on the abattoir effluent was determined using anthrone assay (Plummer, 1978). Abattoir effluent (1ml) was treated with 70 % ethanol (1 ml) to precipitate protein. After centrifugation (4000 rpm, 5 min, 4 °C) the supernatant (1 ml) was aliqouted. Anthrone reagent (4 ml) was then added. After mixing the samples were boiled for 20 minutes. Absorbance was determined at 620 nm. Total carbohydrate was determined using standard curve.

Table 3.3: Preparation of glucose standard curve.

Glucose concentration (μg/ml)	Volume of glucose stock (ml)	Volume of deionized water (ml)	Anthrone reagent volume (ml)
0	0	1	4
20	0.2	0.8	4
40	0.4	0.6	4
60	0.6	0.4	4
80	0.8	0.2	4
100	1	0	4

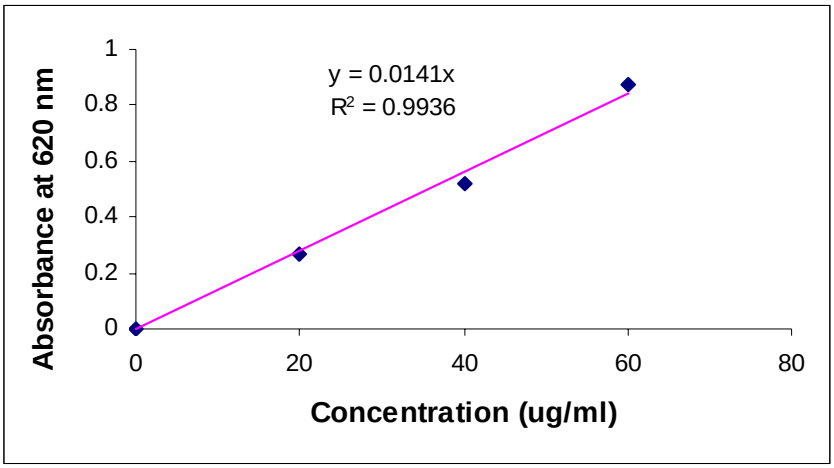


Figure 3.3: Glucose standard curve for the determination of carbohydrate concentration.

3.2.2.5 Reducing sugar assay

Principle of the assay

When a sugar is heated with an alkaline solution of copper tatrate, cuprous oxide is produced. Cuprous oxide reacts with arsenomolybdate and a molybdenum blue colour is formed and this blue colour can be measured spectrophotometrically at 510 nm. Sodium sulphate is included in the reaction mixture to minimize entry of atmospheric oxygen in solution that would cause re-oxidation of the cuprous oxide (Nelson, 1944).

Reagents

1. Arsenomolybdate reagent: Ammonium molydbdate (25 g) was dissolved in 450 ml deionised water and concentrated sulphuric acid (21 ml) was added.

Sodium arsenate (3.0 g) was dissolved in 25 ml deionised water separately, and then added to the first solution. After mixing the reagent was incubated (37 °C, 48 h) in a brown bottle.

2. Copper reagent: Anhydrous sodium sulphate (45 g) was dissolved in 100 ml of boiled deionized water, 6 g of sodium carbonate; 4 g of potassium sodium tatrate and 1 g of copper sulphate were added. Solution was made up to 250 ml with deionized water. The solution was stored at room temperature.

3. Glucose stock solution (1 mg/ml): 10 mg of glucose were dissolved in 10 ml of deionised water.

Procedure

A standard curve was prepared from 1 mg/ml of glucose stock solution [Table 3.4]. Abattoir effluent (1.0 ml) and copper reagent (1.0 ml) were mixed and then incubated (70 °C, 15 min). After cooling to room temperature arsenomolybdate reagent (1.0 ml) was added and the samples were incubated (5 min, ±22 °C). Total reducing sugar was determined by reading absorbance at 510 nm against a glucose standard curve (Plummer, 1978) [Figure 3.4].

Table 3.4: Preparation of glucose standard curve.

Glucose concentration (μg/ml)	Volume of stock glucose (ml)	Volume of deionized water (ml)	Volume of copper reagent (ml)
0	0	6	1
6.25	0.05	5.95	1
12.5	0.1	5.9	1
18.75	0.15	5.85	1
25	0.2	5.8	1
31.25	0.25	5.75	1

3.2.3 Static fouling of polysulphone membranes

In order to properly design experiments around defouling, it was necessary to determine the time required to static foul polysulphone membranes. Maximum fouling time by proteins was determined by first boiling 500 ml of abattoir effluent for 15 minutes and centrifuging (10 000 rpm, 4 °C, 15 min). Polysulphone membranes were then placed in a 1000 ml Erlenmeyer flask with the supernatant of abattoir effluent (1 membrane/100ml).

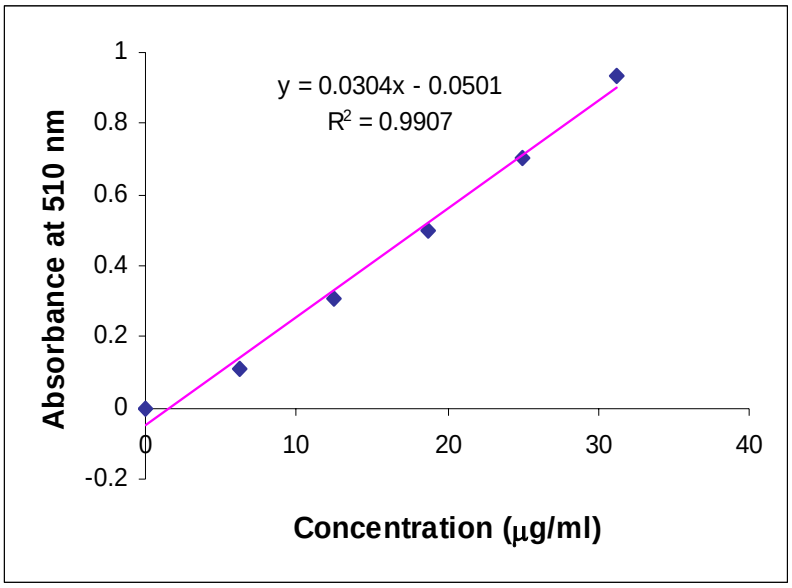


Figure 3.4: Glucose standard curve for the determination of reducing sugar concentration.

The sample was left stationary at room temperature for 48 hours. Every 4 hours a membrane was removed, rinsed with deionised water, let to dry for 5 minutes, cut into 8 equal segments, and assayed for fouling by proteins as in section 3.2.1.1 *(*Maartens *et al.,* 1996). Once maximum time for fouling by proteins was determined polysulphone membranes were fouled by incubating them in the abattoir effluent. Two membranes were removed every 24 hours until the day 10. Each membrane was scoured by soaking in solution D (5 ml of 5 % SDS for proteins and 0.1% for carbohydrates and lipids combined with 2.5 ml deionized water) and incubated (37 °C, 2.5 h, 100 rpm). Protein, carbohydrate and lipid assays were performed in order to determine concentrations fouling the membranes as in [3.2.2.1, 3.2.2.4 and 3.2.3.1].

3.2.3.1 Glycerol assay

Principle of the assay

Hydrolysis of a fatty acid ester by heat releases glycerol which subsequently combines with chromotropic acid to produce a coloured chromotropic acid complex. Enzyme activity is measured by the absorbance of this complex at 570 nm.

Reagents

1. Chromotropic acid (0.18 %): 0.18 g of chromotropic acid was dissolved in 100 ml of deionized water.
2. Chromotropic acid reagent: The ratio was 10 ml of 0.18 % chromotropic acid were always added to 45 ml of 12 M sulphuric acid.
3. Glycerol stock solution (250 μM): 1.85 ml of 99% glycerol was added to 98.15 ml deionized water, afterwards 200 times dilution was prepared from this solution.
4. Sodium arsenite (10 % w/v): 10 g. of sodium arsenite were dissolved in 100 ml of deionized water.
5. Sodium periodate (0.1 M): 2.14g of sodium periodate were in 100 ml deionized water.
6. Sulphuric acid (5 M): 53.33 ml of concentrated sulphuric acid (98 %) was made up to 100 ml with deionized water.
7. Sulphuric acid (12 M): 641.025 ml of concentrated sulphuric acid (98 %) was made up to 1 L with distilled water.

Procedure

A glycerol standard curve (0.1 - 0.5 μmol/ml) was prepared from 250 μM glycerol stock solution [Figure 2.4]. Glycerol-an indicator of lipid fouling was determined by aliqouting (1.0 ml) of solution D (5.0 ml 0.1 % SDS, fouled membrane and 2.5 ml deionized water). H_2SO_4 (5 M, 50 μl) and $NaIO_4$ (0.1 M, 250 μl) were added to the standards and samples. After thorough mixing all samples were incubated at room temperature for 5 minutes and 10 % sodium arsenite (250 μl) was added to each sample. After vortexing, 250 μl were aliquoted out into series of test tubes containing 2.5 ml of chromotropic acid reagent. Samples were then boiled for 60 minutes and cooled. Glycerol levels were obtained by reading absorbance at 570 nm. Lipase activity was calculated as in section 2.2.5.1.

3.2.4 Defouling fouled polysulphone membranes

Dilute and concentrated enzyme extract (200 mg/ml) obtained in 2.2.4 were used to defoul polysulphone membranes fouled by abattoir effluent. Each fouled membrane was incubated with dilute or concentrated enzyme extract (5.0 ml, 37°C, 4 h). Afterwards membranes were rinsed with deionised water.

The remaining foulants were solubilised by incubating membranes in solution D (5.0 ml 5 % SDS for proteins and 0.1 % for carbohydrates and lipids combined with 2.5 ml deionized water) (37 °C, 2.5 h, 100 rpm. Solutions were analyzed for presence or absence of proteins, carbohydrates and glycerol [3.2.2.1, 3.2.2.4 and 3.2.3.1].

3.2.5 Qualitative detection of proteins, carbohydrates and lipids on fouled and defouled membranes

Fouled and defouled polysulphone membranes were stained to ascertain the presence or absence of proteins, lipids and carbohydrates.

3.2.5.1 Protein staining

Principle of the assay

Coomassie Brilliant Blue G-250 exists in two different colour forms, red and blue. The red form is converted to the blue form upon binding of the dye to protein.

Reagents

1. Destaining solution I: a mixture of methanol/ acetic acid/ deionized water (5:1:4).
2. Destaining solution II: a mixture of methanol/ acetic acid/ deionized water (25:37:438).
3. Staining solution I (1 % Coomassie Brilliant Blue G-250):

Procedure

A qualitative colorimetric assay (Dunn *et al.,* 1990; Bradford, 1976) was performed on clean, fouled and defouled membranes to ascertain the presence and or absence of proteins.

Membranes were stained for 15 minutes with 1 % of Coomassie Brilliant Blue G250 staining solution at room temperature. Afterwards they were destained using destaining solution I for 15 minutes at four equal intervals, and then destained for 2.5 hours using destaining solution II.

3.2.5.2 Carbohydrate staining

Principle of the assay

In this staining procedure membranes are first oxidized by periodic acid. The oxidative process results in the formation of aldehyde groups through carbon-to-carbon bond cleavage. Free hydroxyl groups have to be present for oxidation to take place and oxidation is completed when it reaches the aldehyde stage. Aldehyde groupings are detected by the Schiff reagent.

A colourless, unstable di-aldehyde compound is formed and then transformed to the coloured final product by restoration of the quinoid chromotropic grouping.

Reagents

1. Ethanol (50, 70, 80, 95 and 100 %): were prepared using a ratio of ethanol to deionized water 1:1, 7:3, 8:2 and 9.5:1 from 50 % to 95 %.
2. Periodic acid solution (1 %): 1g of Periodic acid was dissolved in 100 ml of deionized water.
3. Schiff reagent: used as purchased from Sigma – Aldrich S.A. (Pty) Ltd, South Africa.

Procedure

Polysulphone membranes that have been fouled and defouled were stained for carbohydrates by first incubating them in 1 % solution of periodic acid for 10 minutes. Membranes were transferred to Schiff reagent for 10 minutes, incubated at room temperature for 1 hour and soaked in ethanol solutions of 50 – 100 % (in series) for 10 minutes. The membranes were dried and stored at 4 °C until they were scanned.

3.2.5.3 Lipid staining

Principle of the assay

Lipids form complexes with basic dye carbofulschin. When exposed to heat, the phenol in the dye strengthens the complexes, even when decolorized with acid-alcohol (95 % ethanol with a lower pH).

Reagents

1. Methylene blue solution
2. Acid-ethanol

Procedure

Fouled or defouled polysulphone membranes were covered with carbolfuschin solution in a petri dish and placed in a steam bath (5 min).

After cooling to room temperature membranes were decolourized with acid ethanol (20 min), counterstained with methylene blue (30 min) and rinsed with deionized water.

3.3 Results and discussion

3.3.1 Static fouling and defouling of polysulphone membranes

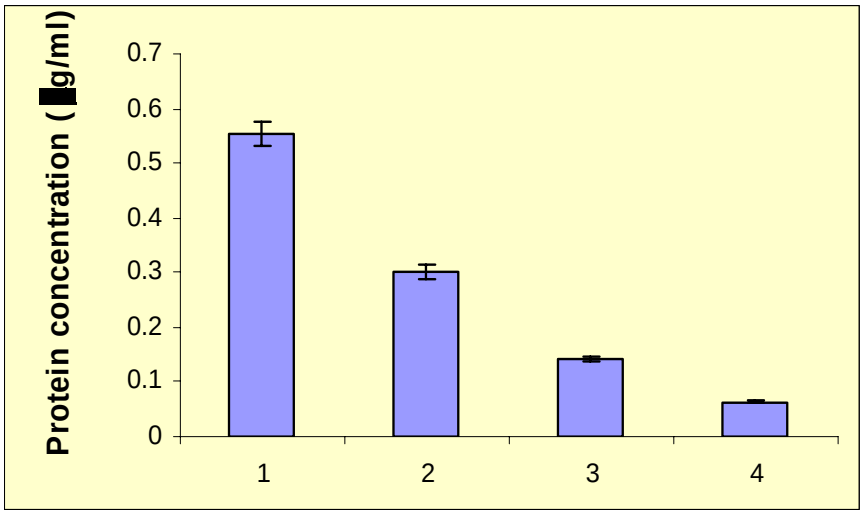


Figure 3.5: Characterization of an ostrich abattoir effluent for lipids (1), proteins (2), total carbohydrate (3), and total reducing sugar (4).

Characterization of ostrich abattoir effluent for lipids, proteins, total carbohydrates, and total reducing sugars is shown in figure 3.5. The abattoir effluent of contained 553 μg/ml of lipid, 301/ml μg of protein, 141 μg/ml of total carbohydrate and 0.63 μg/ml of total reducing sugars. These results suggest that lipids and proteins are major potential foulants in an ostrich abattoir effluent. Similar findings were observed by Maartens *et al.,* (1996). It was also established that amino acid concentration present in the abattoir was 0.153 mM.

The abattoir effluent had a high COD of 31 900 mg/l which also suggest a high concentration of organic foulants. Determination of maximum time for static fouling of polysulphone membranes using the abattoir effluent (Figure 3.6) showed that rapid fouling occurred within 10 hours and at 24 hours fouling had reached maximum. The results agree with Maartens *et al.*, (1996), who found that fouling occurred within eight hours.

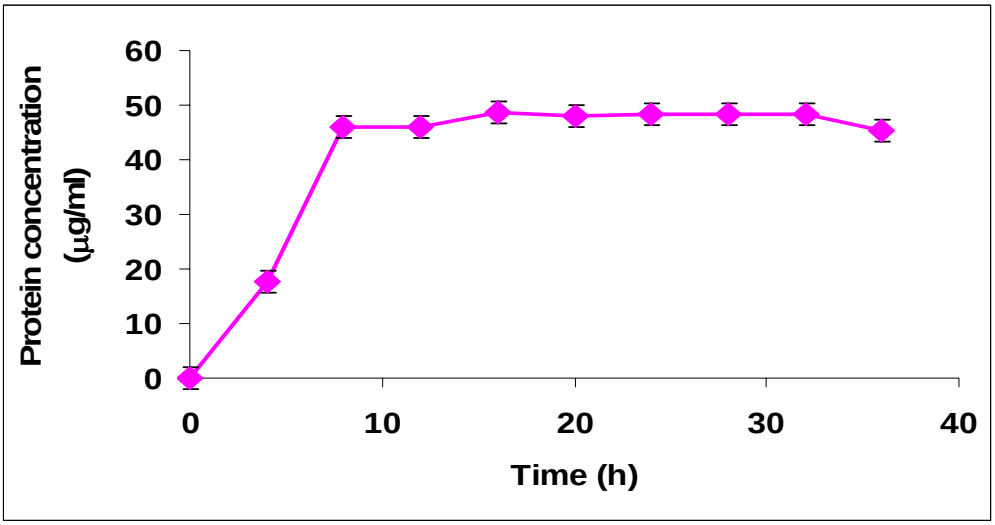


Figure 3.6: Protein concentration on determination of maximum time during static fouling polysulphone membranes, using an ostrich abattoir effluent.

3.3.2 Qualitative assay on statically fouled and defouled membranes

Qualitative assay for proteins adsorbed and desorbed onto PSM during fouling and defouling are shown in Figure 3.7. According to Figure 3.7 defouling of membrane C, previously fouled for 24 hours (membrane B) was successful as it is comparable to membrane A in colour intensity. Membrane D had an increase in colour intensity after fouling for 48 hours as compared to membrane B. Defouling of membrane D using a dilute enzyme extract did not result in a decrease in colour intensity as previously in membrane C. The results suggested that increased colour intensity is directly proportional to protein adsorbed onto the membrane. Colour intensity on membrane E remained the same after defouling and suggested either that fouling for 48 hours resulted in formation of a gel layer as a result of concentration polarization of macromolecules at the membrane/solution interface; or that the enzyme extract used for defouling was too dilute as protein adsorbed onto PSM had increased from membrane B to membrane D.

A concentrated enzyme extract (200 mg/ml) was used to defoul membranes in order to overcome problems of defouling after 48 hours of fouling. Figure 3.8 and 3.9 show results of fouling and defouling using a concentrated enzyme extract. Once the concentrated enzyme extract was obtained, static fouling using abattoir effluent was performed for 120 hours [Figure 3.8]. Defouling using the concentrated enzyme extract from sulphidogenic bioreactor after fouling of 120 hours was then attempted [Figure 3.9]. Results in Figure 3.8 also showed a slight increase in colour intensity occurred starting from membrane C to membrane E which suggested an increase in protein concentration during fouling.

Figure 3.7: A qualitative assay on fouled and defouled polysulphone membranes.
A = clean membrane
B = membrane fouled for 24 hours
C = B defouled for 4 hours
D = membrane fouled for 48 hours
E = D defouled for 4 hours.

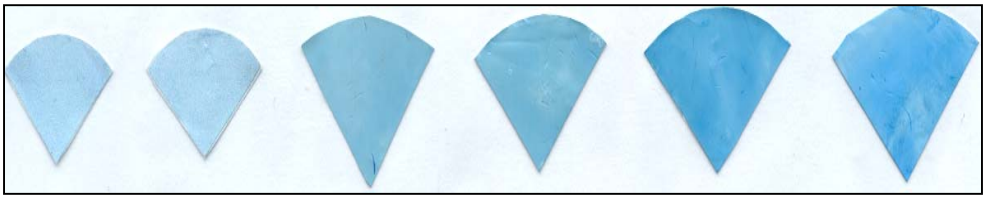
Membrane F had a doubled colour intensity that also suggested increased protein concentration and formation of a second layer of protein foulants. Upon defouling using the concentrated enzyme extract (200 mg/ml) qualitative assay in Figure 3.9 shows a decrease in colour intensity starting from membrane B to F. Membrane B had the same colour intensity as membrane A which suggested complete removal of adsorbed proteins upon defouling after 24 hours.

This result confirmed those in Figure 3.7 and also suggests that a foulant layer that is formed after 24 hours of fouling has weak association between proteins. Figure 3.9 also shows that colour intensity was increasing slightly from membrane C to F as compared membrane B suggesting that the defouling was less effective from membrane B to F and that after 48 hours of fouling stronger bonds were progressively being formed.

A B C D E F

Figure 3.8: A qualitative assay for proteins on fouled polysulphone membranes.
A= clean membrane
B= membrane fouled for 24 hours
C= membrane fouled for 48 hours
D= membrane fouled for 72 hours
E= membrane fouled for 96 hours
F= membrane fouled for 120 hours.

Qualitative assays for carbohydrates and lipids fouling PSM and assays after defouling of the same membranes are shown in Figure 3.10 and 3.11 respectively. In Figure 3.10 colour intensity was used successfully as an indicator for the degree of fouling. According to these results, there was a high concentration of carbohydrates as compared to proteins and lipids fouling the PSM. According to colour intensity carbohydrate fouling was high upon 96 hours of fouling. These results correspond to those in Figure 3.14, where the total carbohydrate concentration peaked after 24 hours, was reduced after 72 hours of fouling and increased again after 96 hours. Results for carbohydrate staining after defouling did not give the pink colour and therefore suggested total removal of carbohydrates when the concentrated enzyme extract was used.



A B C D E F

Figure 3.9: A qualitative assay for proteins on defouled polysulphone membranes.
A = clean membrane,
B = membrane defouled after 24 hours
C = membrane defouled after 48 hours
D = membrane defouled after 72 hours
E = membrane defouled after 96 hours
F = membrane defouled after 120 hours.

Results from the staining procedure for lipids produced membranes of the same colour intensity; as a result it was impossible to differentiate the degree of fouling over time [Figure 3.11]. These results therefore did not shed any light as to whether lipids from the abattoir effluent were able to foul PSM. The only reference for evidence of fouling by lipids is shown in Figure 3.14.

3.3.3 Quantitative assay on statically fouled and defouled membranes

Quantitative assays performed on PSM fouled up to 48 hours and defouled by a dilute form of the enzyme extract is shown in Figure 3.12. These results show that membrane A contained zero protein concentration, while membrane B contained 446.2 μg protein/ml; membrane C contained 104.32 μg protein/ml; membrane D contained 506 μg protein/ml; and membrane E had 483.7 μg protein/ml. Defouling of membrane B showed a decrease in protein concentration, while there was almost negligible decrease in protein concentration upon defouling of membrane D.

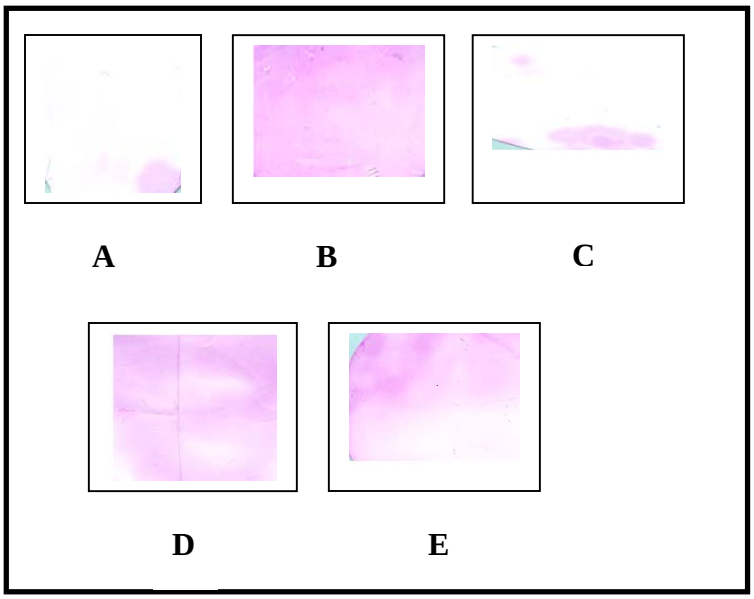


Figure 3.10: Qualitative assay for carbohydrates on fouled polysulphone membranes. A = membrane fouled for 24 hours, B = membrane fouled for 48 hours, C = membrane fouled for 72 hours, D = membrane fouled for 96 hours and E = membrane fouled for 120 hours.

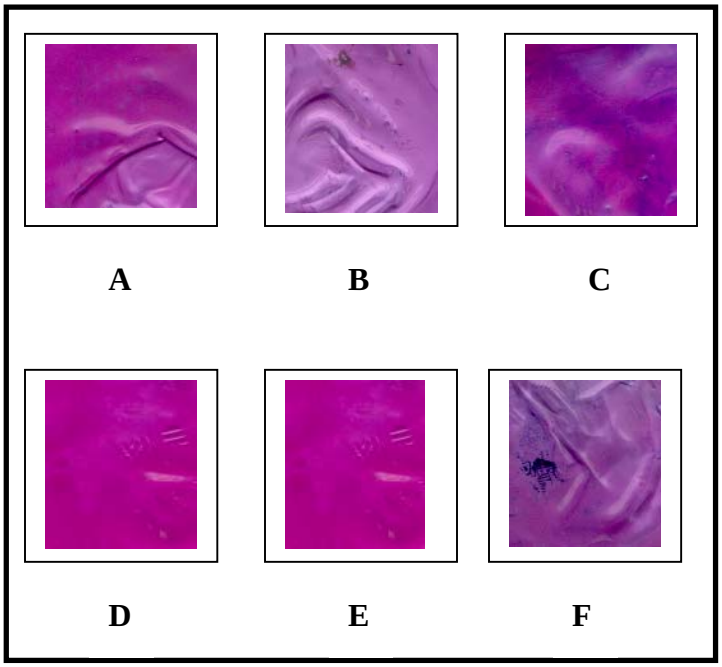


Figure 3.11: A qualitative assay for lipids fouling polysulphone membranes. A = clean membrane, B = membrane fouled for 24 hours, C = membrane fouled for 48 hours, D = membrane fouled for 72 hours, E = membrane fouled for 96 hours and F = membrane fouled for 120hours.

These results confirm observations made in the qualitative assay for proteins and that fouling for 48 hours resulted into increased protein concentration and formation of a gel layer which was hard to remove with the dilute form of the enzyme extract.

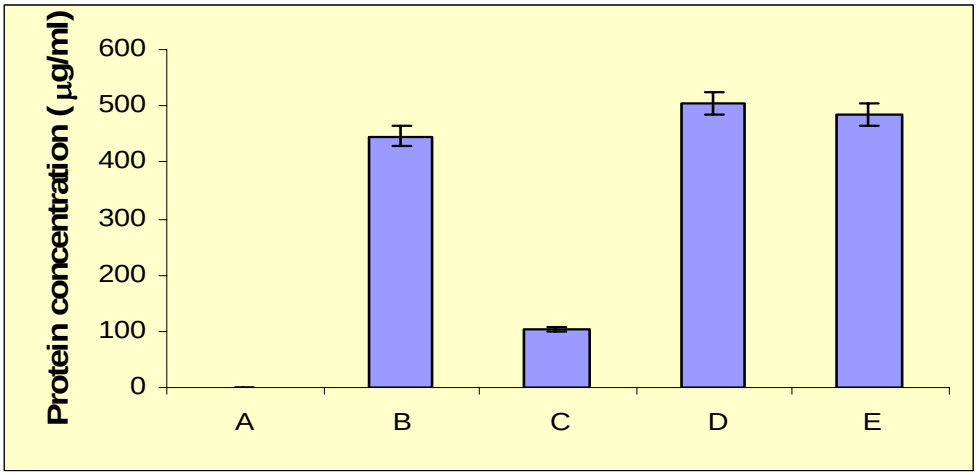


Figure 3.12: Quantitative assay for proteins on clean, fouled, and defouled polysulphone membranes. A = protein on clean membrane, B = protein on membrane fouled for 24 hours, C = B defouled for 4 hours, D = protein on membrane fouled for 48 hours and E = D defouled for 4 hours.

Once the concentrated enzyme extract was obtained, static fouling using abattoir effluent was performed for 120 hours [Figure 3.13]. Defouling using the concentrated enzyme extract from sulphidogenic bioreactor after fouling for 120 hours was then attempted. Protein assays performed on the membranes indicated that upon fouling, protein concentration was 3.43 µg/ml, 7.85 µg/ml, 7.7 µg/ml 9 µg/ml and 13.08 µg/ml from 24 to 120 hours respectively [Figure 3.13]. It is important to bear in mind that low protein concentrations on the fouled membranes are directly proportional to the concentration of proteins present in the abattoir effluent that was used for fouling. These results confirm the observations in Figure 3.10, especially protein concentration on membrane F which is almost double that on membranes C and D. Upon defouling using the concentrated enzyme extract (200 mg/ml) the quantitative assays in Figure 3.13 and qualitative assay in Figure 3.9 indicated 100 % removal of proteins fouling membrane B, while 82 % of the protein fouling membrane C was removed. 71 % of protein on membrane D, 68 % of protein on membrane E and 76 % of protein on membrane F were also removed.

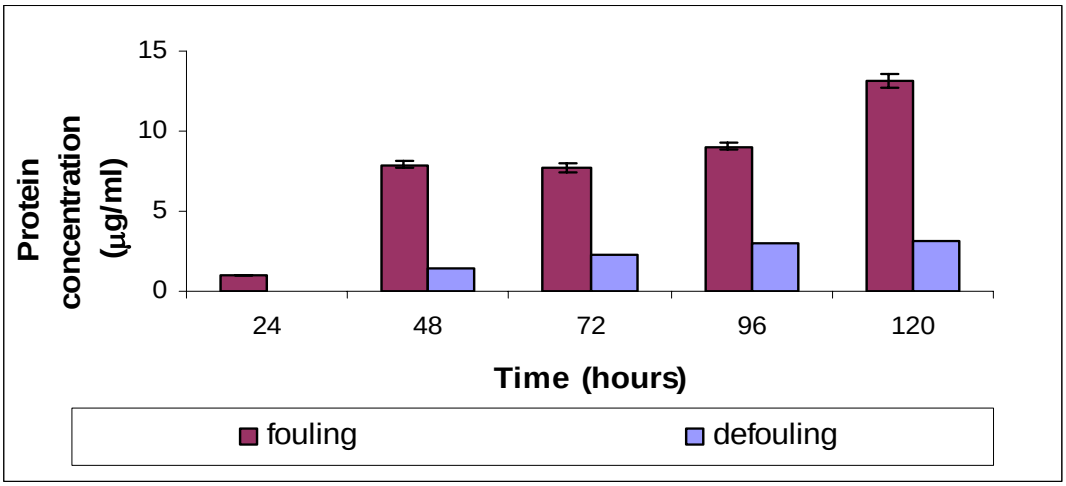


Figure 3.13: A quantitative assay for proteins after fouling and defouling of polysulphone membranes from 24 hours to 120 hours.

These results again suggested that weak associations between proteins and the polysulphone membranes were formed during the first two days of fouling; as a result there was 100 % and 82 % removal upon cleaning/defouling. Defouling efficiency decreased with increased fouling time from 24 to 120 hours. The decrease in efficiency of defouling suggested that stronger bonds were being formed between foulants over increased fouling time. Thus it can be concluded that defouling proteins fouling PSM was successful as the efficiency was above 70 % even, after 120 hours of fouling. At this stage, it is important to note that the abattoir effluent contains a mixture of foulants that have been characterized as lipids, proteins and carbohydrates; therefore fouling of polysulphone membranes by abattoir proteins may occur simultaneously or sequentially with other foulants.

Polysulphone membranes fouled for 120 hours were also assayed for carbohydrate and lipid foulants [Figure 3.14 and 3.15] and defouled using the concentrated enzyme extract [Figure 3.15. Figure 3.14 indicates that there was little carbohydrate and lipid fouling PSM within the first 24 hours, while low levels of reducing sugars fouled the membranes. Total carbohydrate and reducing sugars concentrations on the membranes were constantly low for 96 hours, while lipids were major foulants as compared to carbohydrates. As the concentration of lipid foulants decreased after 96 hours, reducing sugar and carbohydrate concentrations increased.

It can be concluded that proteins from the abattoir effluent were primary foulants, followed by low levels of carbohydrate which appear to be binding sites for lipids and as soon as the lipid layer has been formed or the binding sites are depleted, another protein layer follows and then a carbohydrate layer made up of reducing sugars is formed, which serves as a base for binding for lipid foulants.

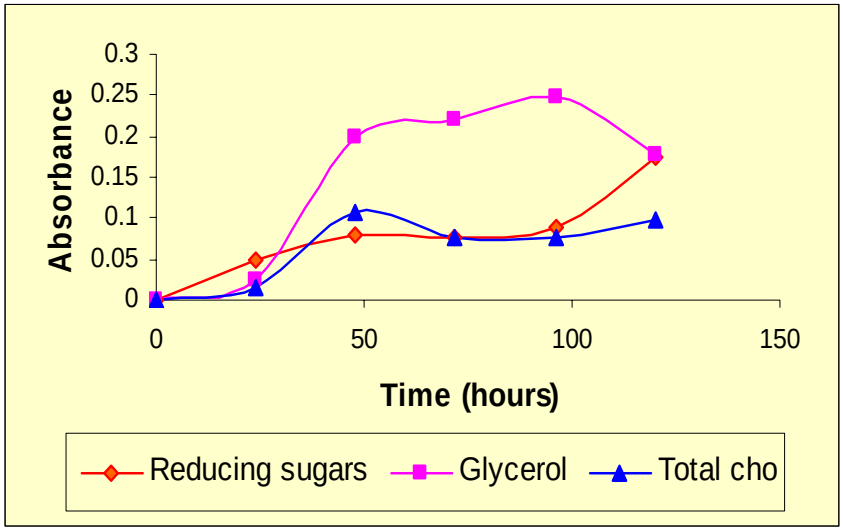


Figure 3.14: Reducing sugars, glycerol, and carbohydrate present levels on fouled membranes, from 24 to 120 hours.

In other results (not shown) defouling membranes that had been fouled for 5 days using the concentrated enzyme extract shows approximately 95 % removal of lipids and carbohydrates and reducing sugars. Defouling of polysulphone membranes fouled for 120 hours showed complete removal of carbohydrates reducing sugars and lipids as a result in order to quantify data defouling using sludge pellet was performed [Figure 3.15]. Upon defouling using sulphidogenic pellet which contains high activity of all enzymes carbohydrates refouled the polysulphone membranes [Figure 3.15]. This occurred because of the high carbohydrate concentration present in the sludge and it was concluded that enzyme extraction from the pellet was necessary, as other foulants form pellet would foul membranes. Improvements on enzyme formulation can increase the ability to remove foulants on membranes fouled for longer than 120 hours. It was difficult to quantify lipid and carbohydrate foulants after 5 days of fouling.

Lipids and proteins are the major foulants present in an ostrich abattoir effluent. Static fouling using an abattoir effluent occurred maximally at 24 hours of incubation, while fouling occurred rapidly and maximally within 1 hour on dynamic of capillary ultrafiltration membranes. Quantitative and qualitative assays indicated that defouling of statically fouled membrane using sulphidogenic proteases was achieved but after fouling for 24 hours, and not after 48 hours

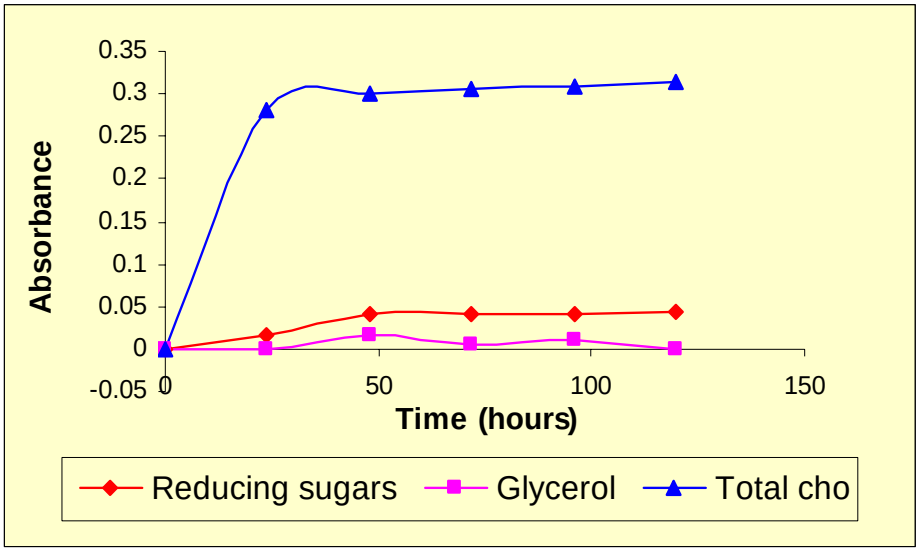


Figure 3.15: Reducing sugars, glycerol, and carbohydrate present levels on defouled membranes, from 24 hours to 120 hours.

3.4 Conclusions

Defouling of PSM that have been fouled statically was possible only on 24 hour fouling. The concentrated enzyme extract was able to remove fouling of up to 120 hours with efficiency of about 70 % on average. It was therefore concluded that defouling using the concentrated enzyme extract from a sulphidogenic bioreactor was successful. Only low concentrations of carbohydrates and lipids from the abattoir effluent were able to foul PSM. Removal of carbohydrates and lipids using the concentrated enzyme extract was also achieved. The primary aim of this study which was to clean or defoul PSM tha have been fouled statically using the abattoir effluent was achieved successfully. On the other more sensitive methods like high performance liquid chromatography need to be employed especially for quantifying carbohydrates and lipids as this would shed more light to the mechanisms of fouling and in turn lead to better formulation of cleaning agents.

In industry, most of the membrane modules used employ filtration, there the success of the concentrated enzyme extract is not complete until proven to work in these filtration systems. Thus, the next two chapters' *viz*. Chapter 4 and 5 deal with cleaning of fouling in the capillary ultrafiltration (UF) membrane module and in the stirred UF cell.

CHAPTER 4
DYNAMIC FOULING AND DEFOULING OF CAPILLARY ULTRAFILTRATION MEMBRANES

4.1 Introduction

Ultrafiltration is a separation process that allows a solution to be fractionated into two streams using a selective membrane and a pressure gradient. Owing to its mechanical and physiochemical properties, the membrane allows the solvent and the low molecular weight solutes through while retaining the large solutes or solid particles. (Aimar *et al*., 1986). Ultrafiltration can be a cost effective method for the treatment of wool scouring effluent to prepare it for recycling (Maartens *et al.,* 1998). However, fouling is a major obstacle in the successful implementation of UF membranes for treatment of high organic content effluents like the abattoir effluent and like the wool-scouring effluent (WSE). Maartens *et al.,* (1996a) stated that when those UF membrane systems are used for the treatment of biological process effluents, the main aim is to obtain the required water purification as economically as possible and at acceptable flux rates. The realization of this goal in the direct treatment of effluents is severely hampered by membrane fouling. The fouling problem has been previously approached from a number of angles which include the optimization of flow conditions, pretreatment of the effluent, the production of membranes with reduced absorptive properties, the optimization of operational factors and the use of high quality water for membrane rinsing (Maartens *et al.,* 1996a; Eckner *et al.,* 1993; Flemming, 1990). All these methods yielded moderately satisfactory results but at a relatively high cost. It was suggested that an alternative approach to the fouling problem would be to reduce pre-treatment to minimum acceptable levels and to introduce extensive but simple membrane-cleaning protocols (Maartens e*t al.,* 1996a). Membranes are normally cleaned by a combination of mechanical and chemical and biological methods using alkalis, surface active agents, sequestering agents, disinfectants and enzymes (Kim *e .al.,* 1993; Trägårdh *et al.,* 1989). Biological cleaning can be described as the use of cleaning mixtures that contain bioactive agents to enhance the removal of foulants.

Enzymes are ideal biological agents for this purpose as they are specific for the reactions they catalyze and substrates with which they interact. In addition, enzymes act under mild conditions of pH, temperature and ionic strength and will not damage the membrane surface (Leukes *et al.,* 1999). A number of factors have prohibited the large-scale use of enzymes for cleaning membranes. Extension of membrane technology to effluent treatment of biological related processes, as well as the increasing pressure on the environment has, however, compelled researchers to re-examine enzymes for membrane cleaning. The performance of UF processes is affected by the feed water matrix, operating conditions, the physical and chemical characteristics of the membrane used. Since 1997 progress has been made in minimizing fouling by increasing membrane permeability (through surface porosity), and the chemical resistance of polymeric materials to fouling. Chemical resistance of the membrane to fouling has already been explained in section 1.3.3.2, where surface modification is a method that when employed can reduce fouling and is also known as the pretreatment step. Surface modification is normally carried out on hydrophobic membranes to make them hydrophilic. Clearly if membranes are hydrophilic they become more resistant to fouling.

Ultrafiltration and MF involve the separation of macromolecules, as already mentioned in Table 1.2 (Kim *et al.,* 1993b). The mechanism of transport is mainly a sieving process. The differences are in the operating pressures, pore sizes, molecular cut-off (MWCO) range and size cut off range. Operating pressures for UF are between 2 to 7 bars and 1 to 4 bars for MF. Pressures needed for UF and MF are therefore lower than for RO and NF and so higher fluxes can be reached. The major causes of flux decline in UF are concentration polarization and fouling (Chan *et al.,* 2002). Due to the larger pore size and structure, these membranes can also be affected by phenomena such as pore blocking and standard blocking. Typical effects of operating parameters on the flux for UF processes are illustrated in Figure 4.1. At low transmembrane pressure (ΔP), flux increase is directly proportional to ΔP and is also governed by the rate at which solvent passes a porous material. This region is called pressure controlled region. This relationship continues until a stage where further increase in ΔP does not lead to increase in flux. At point the flux becomes independent of pressure due to concentration polarization and this is called the mass transfer region.

For optimal flux, ΔP at the point that flux levels off is usually chosen (Matzinos and Álvarez, 2002; Aimar *et al*., 1986). The flux in the mass-transfer controlled region can be described by film model theory, which gives an interpretation of the steady-state mass transfer in the boundary layer:

$$J = k \ln [C_G/C_B] \quad (1)$$

k = mass transfer coefficient is equal to D (diffusion coefficient)/ δ (thickness of the boundary layer)

C_G = gel concentration at the boundary layer

C_B = bulk concentration of the rejected solute

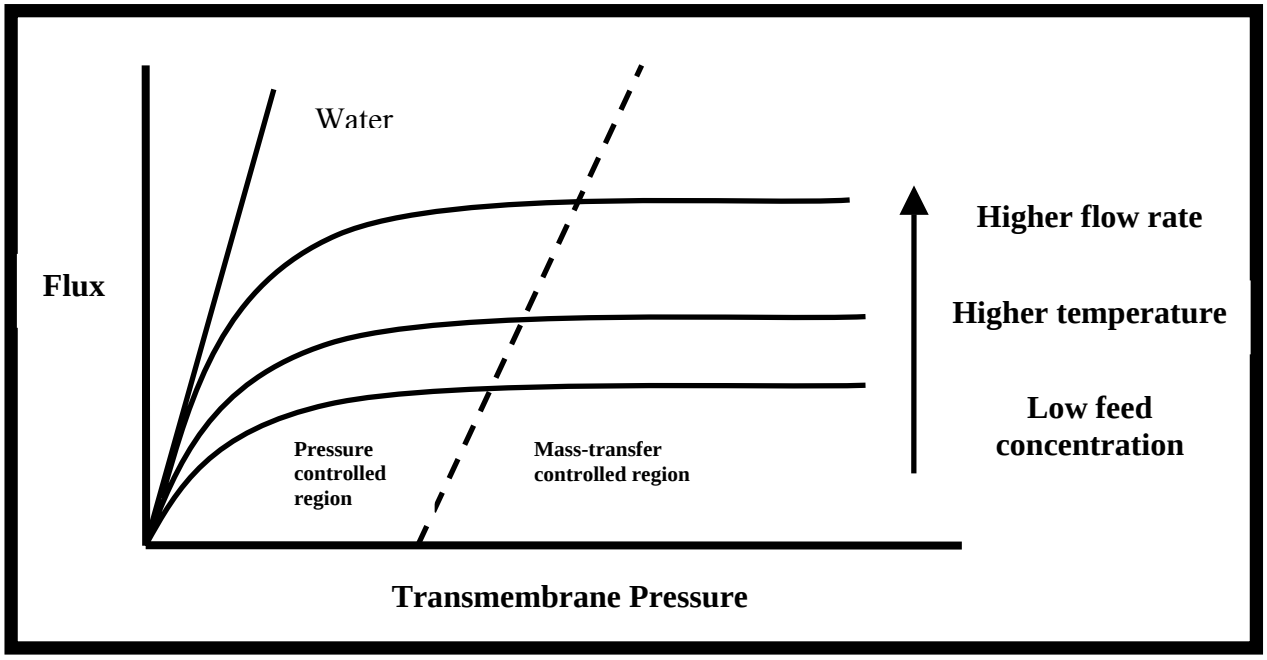


Figure 4.1: Profile of permeates flux and transmembrane pressure influenced by pressure, temperature, concentration of feed and mass transfer.

The semi-empirical Sherwood correlations can be useful to calculate a mass transfer coefficient for the boundary layer. Several factors such as crossflow velocity, flow regime, geometrical profile of the membrane module, viscosity of the fluid and diffusivity of the components, affect this mass transfer. Because of the importance of the mass-transfer step, conventional dead-end operation of ultrafilters is very rare.

There are many ways to depolarize a membrane, but crossflow is by far the most widespread. Turbulent flow is more common than laminar flow. To find optimum operating parameters for a particular system one parameter e.g. ΔP, crossflow velocity and concentration of the feed; is varied while keeping the others constant. For example, if the effect of ΔP is to be examined, temperature, crossflow velocity and concentration of the feed are kept constant while pressure is increased and allowed to stabilize before measuring the flux and curves such as those illustrated in Figure 4.1 can be derived (Chan *et al.,* 2002; Matzinos and Álvarez, 2002; Aimar *et al*., 1986). Before testing the next operating parameter, the system should be cleaned with agents for fouled UF membranes and for prevention of fouling by the adsorption of enzymes onto the membranes.

4.1.1 Objectives

- To dynamically foul the capillary ultrafiltration membrane module using the abattoir effluent.
- To defoul or clean the UF membrane module with the dilute enzyme extract first and then the concentrated enzyme extract.
- To evaluate the effectiveness of rinsing procedures employed after foul when rinsing is performed with distilled water, at buffering pH of 5.0, 7.0 and 10.0.
- Permeates and retentates collected during fouling and defouling of the capillary membrane module will be biochemical analyzed for the presence or absence of proteins, lipids and carbohydrates, in order to confirm the occurrence of fouling and defouling.

4.2 Materials and methods

4.2.1 Materials

Abattoir effluent was obtained form Ostritech (Pty) Ltd, Grahamstown, South Africa. Double skinned capillary polysulphone membranes were obtained from University of Stellenbosch, South Africa. Manometers were constructed by a glassblower, Department of Chemistry, Rhodes University, Grahamstown, South Africa. A Beckman Model J2-21 Centrifuge was used to centrifuge the abattoir effluent.

Enzymes for defouling fouled capillary polysulphone membranes were obtained from a sulphidogenic bioreactor. A Virsonic 100 sonicator was used to extract enzymes from sulphidogenic sludge. Sodium dihydrogen phosphate, sodium carbonate, sodium hydrogen carbonate, glycine, Ninhydrin reagent, sodium acetate, ethanol were purchased from Merck (Pty) Ltd, South Africa.

4.2.2 Ultrafiltration membrane module set-up

The capillary ultrafiltration membrane module was set-up at room temperature (23 ± 2°C), with a pore size of 0.2 μm and membrane surface area was calculated to be 1.76 x 10^{-3}. The capillary UF membrane module is presented in Figure 4.2. The abattoir effluent was pumped into the membrane using a Watson Marlow 504S peristaltic pump and transmembrane pressure was measured using manometers. The capillary membrane was first rinsed free of the preservative glycerol with warm deionized water for 2 hours. Rinsing was performed by connecting tubing from (c) to the abattoir reservoir and retentate tubing to the permeate outlet [Figure 4.2]. After membrane rinsing the permeability of the membrane was determined by first measuring permeate flux and ΔP at different pump speeds and permeability was then calculated using these parameters. Membrane surface area was also calculated. Pure water fluxes (PWF) were determined and fouling experiments performed as below. During water flux and fouling experiments, the effluent was pumped from (a) following route indicated by arrows, permeate volumes were collected at point (f) and retentate was recycled to the reservoir.

4.2.3 Fouling of capillary ultrafiltration membrane using ostrich abattoir effluent

Permeability of UF capillary membranes was determined by measuring PWF at different pump speed and using a Watson Marlow 504S pump at 15 %, 25 % and 40 % pump speed. Permeability was calculated as follows:

$$k = \frac{J}{\Delta P} \qquad (2)$$

k = permeability of the membrane

J = permeate flux

ΔP = transmembrane pressure

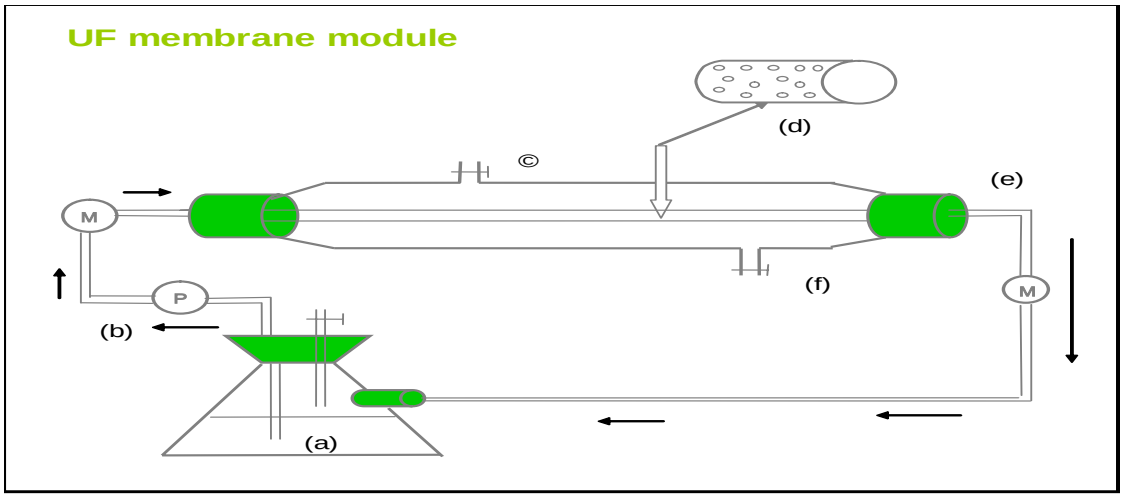


Figure 4.2: Ultrafiltration membrane module. (a) = abattoir reservoir (b) = route for pumping abattoir effluent, (c) = closed outlet, (d) = membranes pores around capillary polysulphone membrane, (e) = outlet for retentate, (f) = outlet for permeate, P = pump and M = manometer.

Table 4.1: Permeability of capillary UF membranes at different pump speeds

Pump speed (%)	**Permeability (L/m^2/kPa)**
15	6.51
25	6.4
40	6.58

Abattoir effluent (500 ml) was centrifuged (10 000 rpm, 15 min, 4 °C) and the supernatant used for dynamic fouling of the capillary UF membrane module. Parameters such as pressure; permeate flux, inlet and outlet flow rates were measured at 30 minute intervals for 2.5 hours. Simultaneously samples were collected at the retentate and permeate outlets for biochemical analysis. Calculations for permeate fluxes were as follows:

$$\text{Membrane surface area } (m^2) = \pi\ d\ l \qquad (3)$$

d = membrane pore diameter

l = length of capillary membrane

$$\text{Transmembrane pressure } (\Delta kPa) = \frac{\rho\, g\, m^2}{1000}$$

ρ = density of mercury

g = gravitational acceleration

m^2 = membrane surface area

$$\text{Permeate flux } (J) = \frac{V}{t\, m^2}$$

V = permeate volume

T = fouling time

m^2 = membrane surface area

4.2.4 Defouling of the capillary ultrafiltration membrane module

Reagents

1. Carbonate-bicarbonate buffer (0.1 M, pH 10): 5.242 g of sodium hydrogen carbonate and 3.985 g of sodium carbonate were dissolved in 1 L of deionised water.

Procedure

The dilute form of the enzyme was extracted as in 2.2.4 from a sulphidogenic bioreactor. The sludge was centrifuged (12 000 rpm, 25 min, 4 °C) and the pellets were collected to make a final volume (100 ml). The pellet was resuspended in 400 ml 0.1 M sodium phosphate buffer at pH 5.0 and 7.0. 0.1 M carbonate-bicarbonate buffer was used for pH 10. The resuspended pellet was sonicated for 2 ml/min, 8 W using the Virsonic 100 sonicator. The sludge was then centrifuged (4 °C, 10 000 rpm, 25 mins) and the supernatant was placed in a reservoir and pumped through the fouled membrane. Defouling was performed at 37 ºC. Permeate flux, inlet flow rate; outlet flow rate and ΔP were measured as in the fouling experiments. Samples were also collected for biochemical analysis at points (e) and (f) [Figure 4.2].

4.2.5 Analysis of samples collected during fouling and defouling

Samples were assayed for protein content using Bradford's methods as in section 2.2.4.1. Amino acids were assayed using a micro-titre Ninhydrin assay as follows: 50 µl of each sample was measured and of sodium acetate buffer (4 M, pH 5.5). Ninhydrin reagent (50 µl) was added and the samples were incubated at 60 °C for 20 minutes. After cooling at room temperature 100 µl of 50 % ethanol was added to each sample. Amino acid concentrations were determined by reading samples at 570 nm against a 1 mM glycine standard curve (Plummer, 1978). Carbohydrate and reducing sugar assays were performed as described in 3.2.2.4 and 3.2.2.5.

4.3 Results and discussion

4.3.1 Fouling of the capillary ultrafiltration membrane module using an abattoir effluent

Results on dynamic fouling of the capillary UF membrane module using abattoir effluent are shown [Figure 4.3 (a), (b)]. These graphs show that major fouling occurred within the first 60 minutes as there was a severe decrease in specific flux from 1.06 L/m^2/h/kPa to 0.22 L/m^2/h/kPa (Figure 4.3(a)) and from 0.648 L/m^2/h/kPa to 0.26 L/m^2/h/kPa (Figure 4.3(b)). Rapid fouling by abattoir effluent can be attributed to the fact that polysulphone membranes, which are highly hydrophobic, have been characterized to be more susceptible to fouling compared to hydrophilic membranes (Chaturvedi *et al.,* 2001; Mulder, 1991). The other reason for rapid fouling could be, the fact that albumin (69 000 Da) and globulin (159 000 Da) proteins, found in blood serum form protein aggregates, on the surface of PSM and within the pores due to concentration polarization occurring during filtration. During fouling increased transmembrane pressure (ΔP) was observed to be occurring with a decrease in specific flux [Figure 4.4] and this further confirmed that fouling was achieved. Transmembrane pressure increased from 11.163 kPa to 25.5 kPa and specific flux decreased from 0.64 L/h/m^2/kPa to 0.21 L/h/m^2/kPa over the period of fouling.

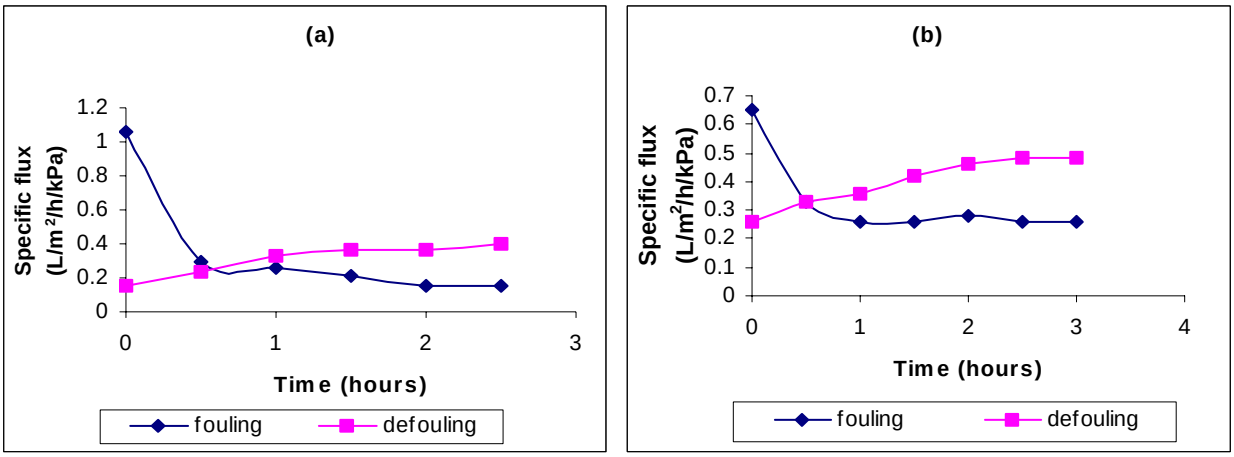


Figure 4.3: Dynamic fouling and defouling of the capillary UF membrane module. (a) Fouling and defouling using sulphidogenic proteases in water. (b) Fouling and defouling using sulphidogenic proteases in 0.1M carbonate-bicarbonate buffer at pH 10.

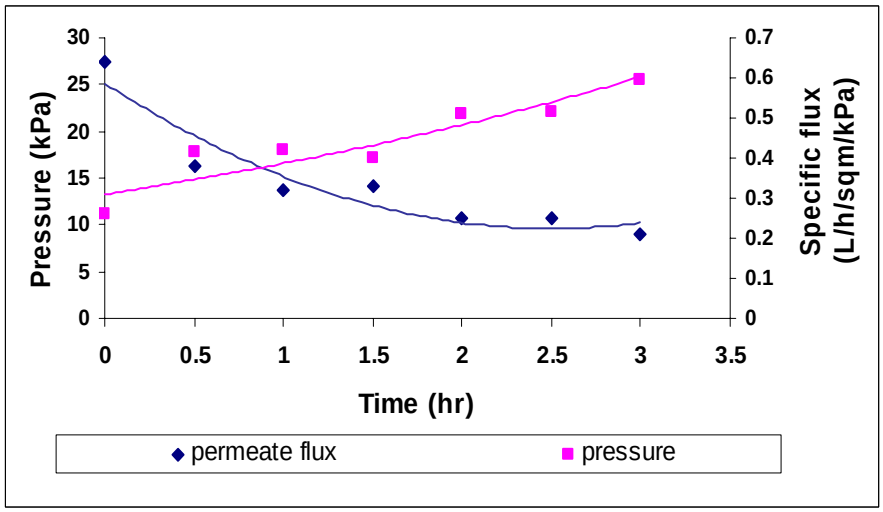


Figure 4.4: Transmembrane pressure and specific flux profile during fouling of the capillary UF membrane module.

Increased ΔP suggested that it was harder to pump the fluid through the constricted pores and led to a formation of a cake layer during fouling. This was proven true by biochemical analysis of permeate and retentate volumes collected during fouling and defouling [Table 4.3 and 4.4]. Rinsing with deionised water after fouling did not restore permeate flux. This suggested that foulants had formed strong associations with each other and the membrane surface.

Fouled membranes were also rinsed with 0.1 M sodium phosphate buffer at pH 5.0 and 7.0 which led to further fouling by sodium and phosphate salts, as the permeate fluxes decreased even further [Figure 4.5]. Similar findings were observed by Matzinos et al., (2002) where membranes were rinsed with increased concentration of sodium chloride and sodium ions replaced fouling calcium ions, thus emphasized the importance of the rinsing step before chemical cleaning of the membrane. Rinsing of fouled membranes was also performed using 0.1 M carbonate bicarbonate buffer at pH 10 [Figure 4.5]. After rinsing with this buffer a significant increase in permeate flux and decrease in ΔP was observed, indicating that some defouling had taken place. The increase led to a slight flux recovery (FR) upon rinsing with carbonate bicarbonate buffer at pH 10. This is to be expected since the carbonate bicarbonate buffer is not unlike the normal chemical cleaning solutions of sodium hydroxide.

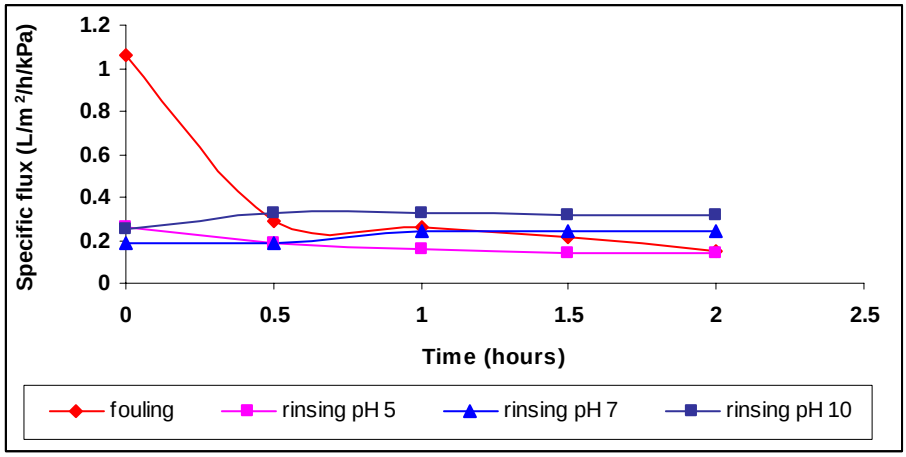


Figure 4.5: Specific fluxes profile during fouling and rinsing cycles.

4.3.2 Defouling of capillary ultrafiltration membranes using enzymes from a sulphidogenic bioreactor

Dynamic defouling of capillary ultrafiltration membrane module [Figure 4.3] using at first dilute enzyme extract from the sulphidogenic bioreactor led to restoration of permeate fluxes from 0.19 L/m^2/h/kPa to 0.4 L/m^2/h/kPa in Figure 4.3(a) and 0.26 L/m^2/h/kPa to 0.48 L/m^2/h/kPa in Figure 4.3 (b). Flux recovery (FR %) was 39 % for Figure 4.3(a) and 64% for Figure 4.3(b).

It was observed that when the dilute enzyme extract was prepared in 0.1 M carbonate bicarbonate buffer at pH 10.0 led to increased flux recovery as compared to the enzyme extract prepared in water. Transmembrane pressure and specific flux during defouling of capillary UF membrane module are shown in Figure 4.6. Upon defouling permeate flux increased from 3.4 L/h/m^2 to 5.82 L/h/ m^2 while transmembrane pressure decreased from 4.23kPa to 2.9 kPa which proved that defouling was successful. After defouling amino acid assay indicated that proteins, previously adsorbed on the membranes were hydrolysed by sulphidogenic proteases maximally in a carbonate–bicarbonate buffer system. Biochemical analysis (Ninhydrin assay) on the permeate volumes revealed that, a low concentration of amino acids was detected. During defouling, the concentration of amino acids on the permeate volumes increased and levelled off after an hour. As this defouling was performed using a 0.1 M carbonate-bicarbonate buffer at pH 10, the activity of sulphidogenic proteases was 0.0021mM/ml/min, calculated per amino acid concentration. These results agreed with (Kubekar *et.al.,* 1998; Baker, 2000) that fouling decreases permeate/water fluxes and increases transmembrane pressure and cleaning or defouling restores them.

4.3.3 Analysis of samples after fouling and defouling

Protein concentration in the permeate volumes decreased from 151.2 μg/ml to 1.207 μg/ml after 2.5 hours of fouling with the abattoir effluent [Table 4.2]. Average protein concentration that was present on permeates after fouling for 2.5 hours was 0.8%. At the same time, amino acid concentration decreased from 0.0906 mM to 0.0436 mM leaving 48.12 % after the same period. Reducing sugar concentration decreased from 6.38 μg/ml to 1.64 μg/ml with 25.7 % remaining. Protein concentration of a dilute enzymes extract from a sulphidogenic bioreactor was 3.715 μg/ml [Table 4.2].

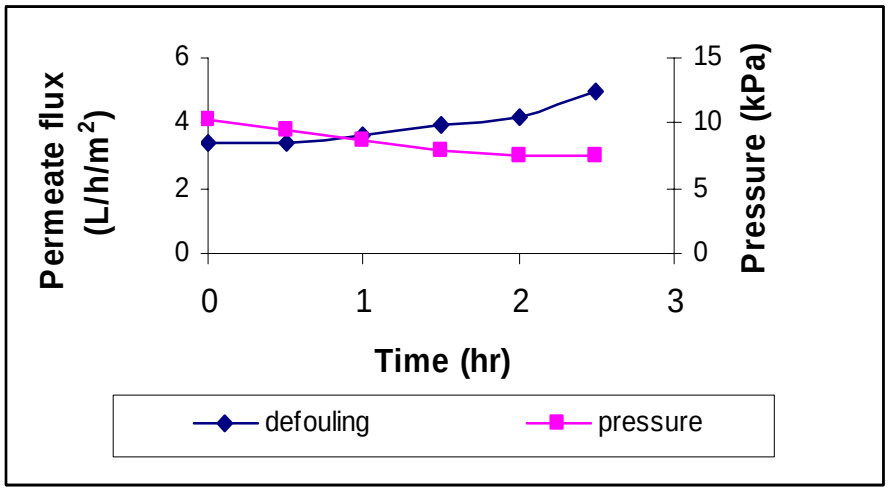


Figure 4.6: Transmembrane pressure and permeate flux profile during defouling of the capillary UF membranemodule.

According to Table 4.3 protein and amino acid concentration of permeate volumes decreased and reducing sugar concentrations also decreased except at one hour where it was 18.26 µg/ml as compared to 15.00 µg/ml before fouling (time 0). These results suggested that defouling occurred within one hour as 3.26 µg/ml (18.26 – 15.00 µg/ml) of reducing sugars permeated through the membranes pores. Biochemical analysis of retentate volumes revealed that most of the foulant that formed a cake layer were removed as protein and amino acid concentrations increase [Table 4.3]. However, there was no increase in the concentration of reducing sugars which suggested that, reducing sugars fouled the pores of capillary UF membranes. It can be concluded that fouling of capillary UF polysulphone membranes was successful as in Figure 4.3 and 4.4 however; defouling of these membranes using sulphidogenic enzymes could not be confirmed by biochemical analyses. This study has confirmed than the fouling phenomenon is far to complex especially when there is more than foulant, normaly true for natural occurring systems. It was further complicated by using sulphidogenic enzymes which exist in a microcosm however; further research like use of more sensitive biochemical assays (HPLC) would shed more light on this black box. It must be noted that the development of an efficient enzyme extract is at infant stages and has been proven to be effective in static fouling [chapter 3 and 5].

Table 4.2: Protein, amino acid and reducing sugars concentrations on permeate and retentate during fouling.

FOULING	Time (h)	Protein concentration (μg/ml)	Amino acid concentration (mM)	Reducing sugars (μg/ml)
Permeates	0	151.2 ±8.273	0.0906 ±0.001	6.38 ±0.000
	0.5	4.255 ±1.704	0.0361 ±0.001	1.64 ±0.000
	1	0.441 ±0.595	0.0407 ±0.001	1.71 ±0.000
	1.5	0.461 ±0.621	0.0518 ±0.001	1.64 ±0.000
	2	0.000 ±0.000	0.0444 ±0.000	1.64 ±0.000
	2.5	1.207 ±0.597	0.0436 ±0.000	1.64 ±0.000
Retentate	0	151.2 ±8.273	0.0906 ±0.001	6.38 ±0.000
	0.5	16.625 ±0.742	0.1035 ±0.0007	5.036 ±0.000
	1	16.245 ±1.322	0.0926 ±0.006	5.036 ±0.000
	1.5	17.695 ±1.421	0.095 ±0.000	5.95 ±0.000
	2	14.81 ±1.301	0.105 ±0.000	4.84 ±0.000
	2.5	15.4 6.750	0.099 ±0.001	4.12 ±0.000

Table 4.3: Protein, amino acid and reducing sugars concentrations on permeate and retentate during defouling**.**

DEFOULING	Time (h)	Protein concentration (μg/ml)	Amino acid concentration (mM)	Reducing sugars (μg/ml)
Permeates	0	3.715 ±0.841	0.0445 ±0.001	15.00 ±0.000
	0.5	1.705 ±0.756	0.0353 ±0.000	14.05 ±0.000
	1	3.260 ±0.000	0.0362 ±0.000	18.26 ±0.000
	1.5	1.990 ±1.141	0.0403 ±0.000	13.88 ±0.000
	2	1.991 ±1.596	0.0384 ±0.0001	12.08 ±0.000
	2.5	1.991 ±1.596	0.053 ±0.002	11.02 ±0.000
Retentate	0	3.715 ±0.841	0.0445 ±0.001	15.00 ±0.000
	0.5	6.280 ±0.028	0.0444 ±0.000	4.38 ±0.000
	1	8.870 ±0.212	0.0432 ±0.001	16.45 ±0.000
	1.5	8.865 ±0.219	0.0434 ±0.002	5.33 ±0.000
	2	9.90 ±0.057	0.0779 ±0.002	5.00 ±0.000
	2.5	8.638 ±0.809	0.058 ±0.000	4.48 ±0.000

4.4 Conclusions

Fouling of the capillary UF membrane module was achieved mostly within an hour. Defouling using the dilute enzyme extract was successful when the enzyme was prepared in water; where a flux recovery of 39 % was obtained. There was greater success on defouling when the dilute enzyme extract was prepared in the carbonate bicarbonate buffer at pH 10 where a flux recovery of 64 % was obtained. The increase in pressure accompanied by decrease in the specific flux obtained during fouling further confirmed fouling to be occurring in the capillary UF membrane module. Similarly a decrease in transmembrane pressure was obtained with and increase in specific flux when defouling was performed, this result as well confirmed defouling to be taking place. However, defouling using the concentrated enzyme extract still has to be investigated. Overall little success was attained in an attempt to defoul the capillary UF membrane module and more research has to be done on the parameters like laminar and turbelant flow in trying to improve the cleaning process. So far a combination of procedures like backpulsing, backflushing, pretreatment of the abattoir effluent would have to be used together with the enzyme extract in order to obtain better results.

CHAPTER 6
GENERAL CONCLUSIONS

6.1 Introduction

Membrane fouling is a universal problem in membrane technology. It severely curtails performance as filtration has important applications in the environment, for example, in wastewater treatments, abattoir effluents, and in food and biotechnology industries (Cheryan, 1986; Kulkarni *et al.*, 1992). A number of theoretical models have been developed to explain membrane-fouling mechanisms. These models use system parameters such as viscosity, pore size, membrane thickness, pressure, unsteady-state material and flow balance equations with specific boundary conditions (Chan *et al.*, 2002; Ho *et al.*, 2002; Czekaj *et al.*, 2001; Madaeni *et al.*, 2001; Ho *et al.*, 2000; Chen *et al.*, 1995; Kelly and Zydney, 1995; Kim *et al.*, 1993; Jönsson and Trägårdh, 1990). As a result fouling can be defined as deposition of suspended or dissolved substances on the membrane's external surfaces, at its pore, or within the pores (Madaeni *et al.*, 2001). Fouling results in loss of membrane performance such as lower than expected flux, reduced productivity, high cleaning costs, use of harsh chemical cleaning etc (Leukes, 1999). Permanent membrane fouling caused by the formation of "gel-layer" results in severe flux decline and thus reduced productivity (Howell *et al.*, 1982). Extensive research on the problem of fouling has focused mainly on protein and little is known about other foulants such as polysaccharides, lipids, microorganisms and salts such as sulphates, nitrates and phosphates. It has been established that the rate of fouling depends on the concentration of foulants present in the solution to be filtered, pH of the fouling solution and the physical and chemical structure of the membrane. Reduction of fouling and the cleaning of fouled membrane have been approached in a number of ways (Flemming, 1990; Eckner *et al.*, 1993; Maartens *e .al.*, 1996), which included optimisation of flow conditions, pre-treatment of the effluent, production of membranes with reduced absorptive conditions, backflushing, harsh chemical cleaning agents which results in high cleaning costs and industrial pollution (Trägårdh *et al.*, 1989; Kim *et al.*, 1993). Chemical cleaning agents do not only add to the pollution problem, they also shorten membrane life which is a problem as membranes are very expensive.

Another problem with cleaning regimes employed so far is that pure water flux at the beginning of the filtration process is never restored. Considerable research has also been focused on cleaning of fouled membranes using milder and environmentally friendlier regimes. Commercial enzymes and detergents have been considered for the removal of biologically derived foulants from polymer membranes (Trägårdh *et al.,* 1989; Kim *et al.,* 1993). The use of enzymes alone or in combination with biodegradable detergents is an attractive alternative to the classical cleaning regimes (Maartens *et al.,* 1996; Leukes *et al.,* 1999). Previous studies (Maartens *et al.,* 1996), show that enzymes, as biocatalysts, can be used effectively in combination with detergents to reduce fouling and restore permeate flux on previously fouled membranes. Enzymes are considered ideal cleaning agents because they are highly specific for the reactions they catalyse and the substrates with which they interact. In addition, enzymes also act under mild conditions of pH, temperature, and ionic strength and will not damage the membrane surface (Maartens *et al.,* 1996).

6.2 Enzymes from a sulphidogenic bioreactor as cleaning agents of fouling

Challenges of membrane fouling and cleaning regimes experienced in membrane technology have led to a need for an environmental friendly, abundant and cost effective source of enzymes. As a result enzymes from a sulphidogenic bioreactor, which have been proven to accelerate solubilization of PSS, treatment of AMD and tannery effluents were extracted and concentrated. (Whiteley *et al.,* 2003; 2002a; 2002b; 2002c). Considering the role that is played by the "sludge enzymes" *(*proteases, lipases, α and β-glucosidases, phosphatases, sulphatases, aminopeptidases etc.) in the solubilization of PSS under sulphidogenic conditions, it was hypothesized that the key enzymes involved in the first step of solubilization (i.e. hydrolysis) can clean or defoul membranes that have been fouled by abattoir effluent. From the extraction processes such as sonication, or fractionation using a blender, it can be concluded that the blending method was far better as loss in enzyme activity was less. The dilute form of the enzyme was able to hydrolyse organic foulants such as proteins, carbohydrates and lipids upon its incubation with the abattoir effluent. Enzyme activity was successfully increased when a concentrated enzyme as extract was formulated by freeze-drying after extraction (blending) and reconstituting the enzyme powder in less volume.

Enzyme activities like proteases increased from 0.488 IU to 8.071 IU, lipases from 9.24 IU to 86.71 IU and α-glucosidases 70.01IU to 789.02 when the concentrated enzyme extract (200 mg/ml) was used on commercial substrates. It can be concluded that enzymes from a sulphidogenic bioreactor can be used for pretreatment of effluents containing organic substrates in this case the abattoir effluent. More research has to be done so that the enzyme extract is formulated in such a way that ratios of enzymes present are known and can be manipulated to suite any treatment, depending on the effluent constituents.

6.3 Fouling and defouling of polysulphone membranes

The abattoir effluent was characterized for the presence of organic foulants and it was confirmed that lipids and proteins were major potential foulants while carbohydrates were present at lower levels. Static fouling of polysulphone membranes using the abattoir effluent was successful within 24 hours and in terms of degree of fouling the major foulants were proteins, followed by lipids and then reducing sugars. These results confirmed first the presence of organic foulants in the abattoir effluent and that polysulphone membranes which are hydrophobic are susceptible to fouling as major fouling occurred within 24 hours. Defouling of polysulphone membranes was successful up to 5 days of fouling with decreasing efficiency which confirmed the fact that stronger bonds were formed between foulants and membranes (gel layer). Another area of research would be to foul for longer to allow gel layer formation and then defoul with the concentrated enzyme extract, thus testing the cleaning efficiency of these enzymes.

6.4 Dynamic fouling and defouling in ultrafiltration membrane modules

Some work has been reported on membrane fouling during MF of protein mixtures. It was found that protein fouling does not depend on the size of individual proteins, but on their ability to aggregate (Güell and Davis 1996). Detailed mechanisms of organic membrane fouling caused by polysaccharides and polyphenols are relatively scarce. Vernhet, *et al.,* (1999) evaluated the influence of wine polysaccharides on polyethersulfone membrane fouling and concluded that mannoproteins may cause the strongest decrease in wine filterability, but again this cannot be directly related to their initial concentration.

Thus in the fouling of capillary UF membrane module using the abattoir effluent it can be concluded that protein aggregation occurs as the membrane pore of about 0.2 micron retained globulins and albumins of 159 000 and 69 000 daltons. Low concentration of protein was present on the permeate volumes after fouling and this also confirmed the mechanism of fouling. Low molecular weight foulants such as reducing sugars and amino acids were also able to foul capillary membranes and thus contribute to the gel layer formation. Defouling with the concentrated enzyme extract was unsuccessful as the enzymes fouled the membranes further. Defouling with a dilute enzyme extract in 0.1 M carbonate-bicarbonate buffer at pH 10 was successful within 30 minutes. It can be concluded that enzymes in the dilute enzyme extract lost activity and freeze drying was a viable method for concentrating and stabilizing the enzymes in powder form. Characterization of fouling in order to devise the correct cleaning regime and also to test the concentrated enzyme efficiency was performed successfully using the stirred ultrafiltration cell and polyethersulphone membranes of 30 000, 100 000 and 300 000 MWCO. It was established that fouling of polyethersulphone membranes with 30 000 MWCO followed standard blocking model at first and then cake filtration model. The pore blocking model occurred first on membranes with 300 000 MWCO followed by standard blocking models. Polyethersulphone membranes with 100 000 MWCO followed all three models.

From the results of defouling of 30 000 and 300 000 MWCO it can be concluded that defouling of cake layer fouling (30 000 MWCO) was successful while defouling of pore blocking fouling was unsuccessful due to a mass transfer problem. Therefore apart from proteases, lipases, α and β-glucosidases it can be hypothesized that phosphatases, sulphatases, aminopeptidases etc. from a sulphidogenic bioreactor clean or defoul cake layer fouling by organic foulants and pore blocking fouling provided the mass transfer problem is solved. However, concentration of enzymes from a sulphidogenic bioreactor has not been optimized yet. Other methods of concentrating the enzyme extract can be investigated for example use of organic solvents. The ratio of enzymes present in the enzyme extract when calculated based on enzymatic activity for proteases, lipases and α-glucosidases is 1.1 %, 11 % and 87.9 %.

References

Akhurst, T., Watson, S., Rose, P.D., Whiteley, C.G. and Pletschke, B.I., (2002) Accelerated sludge solubilisation under sulphate reducing conditions: the effect of hydrolytic enzymes on sludge floc size distribution and EPS composition. In: *Abstracts from Biennial WISA Conference and Exhibition, ICC, Durban, South Africa 19-23 May 2002*, p32.

Babu, P.R., and Gaikar, V.G., (2001) Membrane characteristics as determinants in fouling of ultrafiltration membranes. *Separation and Purification Technology,* 24: 23 - 34.

Baker, R.W., (2000) Membrane technology and applications. The McGraw-Hill Companies, USA.Chapter 1,2,6 and 8.

Banister, S.S. and Pretorius, W.A., (1998) Optimisation of sludge acidogenic fermentation for biological nutrient removal. *Water SA*, 24: 35 – 41.

Beerlange, M.A.M., Mulder, M.H.V., Smoulders, C.A. and Strathmann, H., (2001) Ultrafiltration membranes for non-aqueous systems. *Literature Review,* chapter one.

Belfer, S., Purinson, Y., Fainshtein, R., Radchenko, Y. and Kedem, O., (1998a) Surface modification of commercial composite polyamide reverse osmosis membranes. *Journal of Membrane Science,* 139: 175 – 181.

Belfort, G., Davis, R.H. and Zydney, A.L., (1994) The behaviour of suspensions and macromolecular solutions in crossflow microfiltration. *Journal of Membrane Science*, 96: 1 - 8.

Bjørn, C., Morteen, L. and Torleiv, L., (1996) Treatment of acid mine water by sulphate-reducing bacteria; results from a bench scale experiment. *Water Research*, 30: 1617 - 1624.

Boczar, B.A., Forney, L.J., Begley, W.M., Larson, R.J. and Federle, T.W., (1996) Characterisation and distribution of esterase activity in activated sludges. *Water Research,* 35(17): 4208 – 4216.

Boczar, B.A., Begley, W.M. and Larson, R.J., (1992) Characterization of enzyme activity in activated sludge using rapid analyses for specific hydrolases. *Water Environment Research,* 64: 792 - 797.

Bouhabila, E.H., Aïm, R.B. and Buisson, H., (2001) Fouling characterisation in membrane bioreactors. *Separation and Purification Technology,* 22-23: 123 - 132.

Bowen, W.R. and Gan, Q., (1991) Properties of microfiltartion membranes: Flux loss during constant pressure permeation of bovine serum albumin. *Biotechnology and Bioengineering,* 38: 688 – 696.

Bradford, M.M., (1976) A rapid sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72: 248 - 254.

Busto, M.D., Ortega, N. and Perez-Mateos, M., (1997) Effect of immobilization on the stability of bacterial and fungal β-D-glucosidase. *Process Biochemistry*, 32: 441 - 449.

Cadoret, A., Conrad, A. and Block, (2002) Availability of low and high molecular weight substrates to extracellular enzymes in whole and dispersed activated sludge. *Enzyme and Microbial Technology,* 6085: 1 - 8.

Campbell, M.K., (1991), Biochemistry. Saunders College Publishing, Florida USA. p 126 – 133

Canziani, R., Pollice, A. and Ragazzi, M., (1996) Design considerations on primary sludge hydrolysis under psychrophillic conditions. *Environmental Technology,* 17: 747 - 754.

Chan, R., Chen, V. and Bucknall, M.P., (2002) Ultrafiltration of protein mixtures: Measurement of apparent critical flux, rejection performance, and identification of protein deposition. *Desalination,* 146: 83 - 90.

Chaturvedi, B.K., Ghosh, A.K.,, Ramachandhran, V., Trivedi, M.K., Hanra, M.S. and Misra, B.M., (2001) Preparation, characterization and performance of polyethersulfone ultarfiltration membranes. *Desalination,* 133: 31 - 40.

Chen, V., Kim K.J. and Fane, A.G., (1995) Effect of membrane morphology and operation on protein deposition in ultrafiltration membranes. *Biotechnology and Bioengineering,* 47: 174 - 180.

Chen, J., Wang, L. and Zhu, Z., (1992) Preparation of enzyme immobilized membranes and their self-cleaning and anti-fouling abilities in protein separations. *Desalination,* 86: 301 - 315.

Cheryan, M., (1986), Ultrafiltration handbook, *Technomic,* Lancaster.

Choi, H.B., Hwang, K.Y. and Shin, E.B., (1997) Effects of anaerobic digestion of sewage sludge pre-treatment. *Water Science and Technology,* 35: 207 - 211.

Criquet, S., (2002) Measurement and characterization of cellulose activity in sclerophyllous forest litter. *Journal of Microbiological Methods*, 50: 165 - 173.

Cuperus, F.P. and Smolders, C.A., (1991) Characterization of ultrafiltration membranes: Membrane characteristics and characterization techniques. *Advanced Colloid Interface Science,* 34: 135 - 137.

Cyster, H., (1999) The characterization and fractionation of lipid foulants adsorbed onto ultrafiltration membranes during contact with abattoir effluent. MSc Thesis, University of Stellenbosch, South Africa.

Czekaj, P., López, F. and Güell, C., (2001) Membrane fouling by turbidity constituents of beer and wine: Characterization and prevention by means of infrasonic pulsing. *Journal of Food Engineering*, 49: 25 - 35.

Czekaj, P., López, F., and Güell, C., (2000) Membrane fouling during microfiltration of fermented beverages, *Journal of Membrane Science,* 166: 199 - 212.

Danson, M.J. and Eisenthal, R., (2002) Enzyme assays, 2nd edition, Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY UK.

De Villiers, G., (2000) Abattoir effluent treatment and production: Full-scale application. *Water SA,* 26: 599 - 562.

Dunn, M.J. and Angel, S., (1990) Protein Purification Methods: A practical approach, Oxford University Press, New York, p 10-16.

Du Preez, L.A., Odendaal, J.P. and Ponsonby, M., (1992) Biological removal of sulphate from industrial effluents using producer gas as energy source. *Environmental Technology* 13: 875 - 882.

Eliösor, B. and Argaman, Y., (1995) Hydrolysis of particulate organics in activated sludge systems. *Water Research*, 29: 155 - 163.

Euromemebrane, (2000) Highlights of membrane based water treatment technologies, *Membrane Technology,* 130: 4 - 8.

El-Fadel, M., Findikakis, A.N. and Leckie, J.O., (1996) Temperature in modelling solid waste biodegradation. *Environmental Technology*, 17: 915 - 935.

Elefsiniotis, P. and Oldham, W.K., (1994) Anaerobic acidogenesis of primary sludge: The role of solids retention time. *Biotechnology and Bioengineering*, 44: 7 - 13.

Fane, A.G. and Fell, C.J.D., (1987) A review of fouling and fouling control in ultrafiltration. *Desalination,* 62: 117 - 136.

Flemming, H.C., (1990) Biofouling in water treatment. *Proc. Int. Workshop Biofouling Biocorrosion*, Stuttgart, Germany, 13-14.

Folch, J., Lees, M. and Sloane-Stanley, G.H., (1957) *Journal of Biological Chemistry*, 49: 7.

Forday, W. and Greenfield, P.F., (1983) Anaerobic digestion. *Effluent and Water Treatment Journal.*

Frϕlund, B., Griebe, T. and Nielsen, P.H., (1995) Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology Biotechnology,* 43: 755 - 761.

Gilron, J., Belfer, S., Väisänen, P. and Nyström, (2001) Effects of surface modification on antifouling and performance properties of reverse osmosis. *Desalination,* 140: 167 - 179.

Girard, B. and Fukumoto, L.R., (2000) Membrane processing of fruit juices and beverages: A review. *Critical Reviews in Food Science and Nutrition,* 40(2): 91 - 157.

Goel, R., Mino, T., Satoh, H. and Matsou, T., (1998) Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. *Water Research,* 32: 2081 - 2088.

Grund, G., Robinson, C.W. and Glick, B.R., (1992) Protein type effects on steady-state crossflow membrane ultrafiltration fluxes and protein transmission. *Journal of Membrane Science,* 70: 177 - 192.

Güell, C. and Davis, (1996) Membrane fouling during microfiltration of protein mixtures. *Journal of Membrane Science*, 119: 269 - 284.

Hatziconstantinou, G.J., Yannakopoulos, P. and Andreadakis, A., (1996) Primary sludge hydrolysis for nutrient removal. *Water Science Technology,* 34: 417 - 423.

Henze, M. and Mladenoski, C., (1991) Hydrolysis of particulate substrate by actvated sludge under aerobic, anoxic, and anaerobic conditions. *Water Research,* 61 - 64.

Hess, H.H., Lees, M.B. and Derr, J.E., (1978) A linear Lowry-Folin assay for both water-soluble and sodium dodecyl sulphate-solubilized proteins, *Analytical Biochemistry,* 85: 295 - - 300.

Ho, C.C. and Zydney, A.L., (2002) Transmembrane pressure profiles during constant flux microfiltration of bovine serum albumin, *Journal of Membrane Science,* 232: 389 - 399.

Ho, C.C. and Zydney, A.L., (2000) A combined pore blockage and cake filtration model for protein fouling during microfiltration. *Journal of Colloid and Interface Science,* 232: 389 - 399.

Houghton, J.I. and Stephenson, T., (2002) Effect of influent organic content on digested sludge extracellular polymer content and dewaterability. *Water Research,* 36: 3620 - 3628.

Howell, J.A. and Velicangil, O., (1982) Theoretical considerations of membrane fouling and its treatment with immobilized enzymes for protein ultrafiltration. *Journal of Applied Polymer Science, 27: 21 - 32.*

Hudson, J.A. and Lowe, P., (1996) Current technologies for sludge treatment and disposal. *J Chart Inst Water Environ Management,* 10: 436 - 441.

Jacobs, E.P., (1991) Cleaning of polyethersulphone tubular UF membranes: An abattoir case-study, *WRC Report No.K5/362,* Pretoria, South Africa.

Jones, K.L. and O'Melia, C.R., (2001) Ultrafiltration of protein and humic substances: effect of solution chemistry on fouling and flux decline. 193: 163 - 173.

Jönsson, A. and Trägårdh, G., (1990) Ultrafiltration applications. *Desalination,* 77: 135 - 179.

Kelly, S.T. and Zydney, A.L., (1995) Mechanisms for BSA fouling during microfiltartion. *Journal of Membrane Science* 107: 115 - 127.

Kelly, S.T. and Zydney, A.L., (1994) Effects of intermolecular thiol-disulfide interchange reactions on BSA fouling during microfiltration. *Biotechnology and Bioengineering,* 44: 972 - 982.

Kim, K., Sun, V., Chen, D.E. and Fane, A.G., (1993a) The cleaning of UF membranes fouled by protein. *Journal of Membrane Science, 80: 241 - 249.*

Kim, K.J., Chen, V. and Fane, A.G., (1993b) Ultrafiltration of colloidal silver particles: Flux, rejection and fouling. *Journal of Colloid and Interface Science,* 155: 347 - 359.

Kim, K., Fane, A.G., Fell, C.J.D. and Joy D.C., (1992) Fouling mechanisms of membranes during protein ultrafiltration. *Journal of Membrane Science,* 68: 79 - 91.

Kuberkar, V., Czekaj, P. and Davis, R., (1998) Flux enhancement for membrane filtration of bacterial suspensions using high-frequency backpulsing. *Biotechnology and Bioengineering*, *60: 77 - 87.*

Kulkarni, S.S., Funk, E.W. and Li, N.N., (1992) Ultrafiltration: Applications and economics, chapter 30, page 446, Van Nostrand Reinhold, New York.

Lettinga, G. and Hulshoff, P., (1991) UASB-process design for various types of wastewaters. *Water Science and Technology,* 24: 87 - 107.

Leukes, W., Buchanan, K. and Rose, P.D., (1999) Defouling of ultrafiltration membranes by linkage of defouling enzymes to membranes for the purpose of low cost, maintenance ultrafiltration of river water. *WRC Report 791/1/99.*

Lötter, L.H. and Van der Merve, H.M., (1987) The activities of some fermentation enzymes in activated sludge and their relationship to enhanced phosphorus removal. *Water Research,* 21: 1307 - 1310.

Luo, M. and Wang, Z., (2001) Complex fouling and cleaning-in-place of a reverse osmosis desalination system. *Desalination,* 141: 15 - 22.

Lowry, O. H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., (1951) Protein measurement with the Folin phenol reagent, *Journal of Biological Chemistry,* 193: 265 – 275.

Maartens, A., Swart, P. and Jacobs, E.P., (2002) Ultrafiltration of pulp and paper effluent: membrane fouling-prevention and cleaning. *Journal of Membrane Science,* 209: 81 - 92.

Maartens, A., Swart, P. and Jacobs, E.P., (2000) Membrane Pretreatment: A method for reducing fouling by Natural Organic Matter. *Journal of Colloid and Interface Science,* 22: 137 - 142.

Maartens, A., Swart, P. and Jacobs, E.P., (1998) Enzymatic cleaning of ultrafiltration membranes fouled in wool-scouring effluent. *Water SA,* 24: 71 - 76.

Maartens, A., Swart, P. and Jacobs, E.P., (1996a) An enzymatic approach to the cleaning of ultrafiltration membranes fouled in abattoir effluent. *Journal of Membrane Science,* 119: 9 - 16.

Maartens, A., Swart, P. and Jacobs, E.P., (1996b) Characterisation techniques for organic foulants adsorbed onto flat-sheet UF membranes used in abattoir effluent. *Journal of Membrane Science,* 119: 1 - 8.

Maartens, A., Swart, P. and Jacobs, E.P., (1996c) Membrane pre-treatment: A method for reducing fouling by natural organic matter. *Journal of Collodial Interface Science*, 221:137 – 142.

Madaeni, S.S., Mohamadi, T. and Moghadam, M.K., (2001) Chemical cleaning of reverse osmosis membranes. *Desalination,* 134: 77 - 82.

Madhukara, K., Srilatha, H.R., Srinath, K., Bharathi, K. and Nand, K., (1997) Production of methane from green pea shells in floating dome digesters. *Process Biochemistry*, 32: 509 - 513.

Martzinos, P. and Álvarez, R., (2002) Effect of ionic strength on rinsing and alkaline cleaning of ultrafiltration inorganic membranes fouled with whey proteins. *Journal of Membrane Science*, 208: 23 - 30.

McMurry, J., (1992) Organic Chemistry, 3rd edition, Brooks/Cole Publishing Company, California, USA.

Mathews, C.K. and Van Holde, K.E., (1996) Biochemistry, 2nd edition, The Benjamin/Cumming Publishing Company Inc. USA, pp142.

Mulder, M.H.V., (1991), Basic principles of membrane technonolgy, Kluwer, Dordrecht.

Müller, J., Lehne, G., Schwedes, J., Battenberg, S., Näveke, R., Kopp, J., Dichtl, N., Scheminski, A., Krull, R. and Hempel, D.C., (1998) Disintegration of sewage sludges and influence on anaerobic digestion. *Water Science and Technology*, 38: 425 - 433.

Muňoz-Aguado, M.J., Wiley, D.E. and Fane, A.G., (1996) Enzymatic and detergent cleaning of a polysulphone ultrafiltration membrane fouled with BSA and whey. *Journal of Membrane Science,* 117: 175 - 187.

Nelson, N., (1944), A photometric adaptation of the Somogyi method for the determination of glucose *Journal of Biological Chemistry*, 153:375.

Norde, W., (1986) Adsorption of proteins from solution at the solid-liquid interface. *Advances in Colloid and Interface Science,* 25: 267 - 340.

Ngesi, N., (2001) The role of celluloses and glucohydrolases in the solubilization of Primary Sewage Sludge, MSc thesis, Rhodes University, Grahamstown, South Africa.

Panapanit, S. and Visvanathan, C., (2001) The role of bentonite addition in flux enhancement mechanisms for oil/water emulsion. *Journal of Membrane Science,* 184: 59 - 68.

Parmar, N., Singh, A. and Ward, O.P., (2001) Enzyme treatment to reduce solids and improve settling of sewage sludge, *Journal of Industrial Microbiology and Biotechnology,* 26: 383 - 386.

Pletschke, B.I., Rose, P.D. and Whiteley, C.G., (2002) The enzymology of sludge solubilisation utilising sulphate reducing systems: Identification and properties of ATP-sulphurylases. *Enzyme and Microbial Technology*, 31: 329 - 336.

Robertson, B.C. and Zydney, A.L., (1990) Protein adsorption in asymmetric ultrafiltration membranes with highly constricted pores. *Journal of Colloid and Interface Science,* 134: 563 - 575.

Rose, P.D., Boshoff, G.A., Van Hille, R.P., Wallace L.C.M., Dunn, K.M. and Duncan, J.R., (1998) An integrated algal sulphate reducing high rate ponding process for the treatment of acid mine drainage wastewaters. *Biodegradation,* 9: 247 - 257.

Shimizu, T., Kudo, K. and Nasu, Y., (1993) Anaerobic waste activated sludge digestion – a bioconversion mechanism and kinetic model. *Biotechnology Bioengineering,* 41: 1082 - 1091.

Smith, J.M., Payne, G.F. and Lumpkin, J.A., (1992) Enzyme-based strategy for toxic waste treatment and waste minimization. *Biotechnology and Bioengineering,* 39: 741 - 752.

Sosnowiski, P., Wieczorek, A. and Ledakowicz, S., (2002) Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. *Advances in Environmental Research,* 1 - 8.

Spencer, G. and Thomas, R., (1991) Fouling, cleaning, and rejuvenation of formed-in-place membranes. *Food Technology,* 12: 98 - 99.

Sterritt, R.M. and Lester, J.N., 1988, Microbiology for Environmental and Public Health Engineers, p 179 - 189, E & F N Spin 4th Edition, London.

Stevens, P.V., Nyström, M. and Ehsani, N., (1998), Modification of Ultrafiltration membrane with gelatin protein. *Biotechnology and Bioengineering,* 57: 26 - 34.

Su, T.J., Lu, J.R., Cui, Z.F. and Thomas, R.K., (2000), Fouling of ceramic membranes by albumins under dynamic filtration conditions. *Journal of Membrane Science,* 173:167-178.

Swart, P., Maartens, A., Engelbrecht, J., Allie, Z. and Jacobs, E.P., 1999, The development and implementation of biological cleaning techniques for ultrafiltration and reverse osmosis membranes fouled by organic substances, *WRC report, 660/1/99.*

Tansel, B., Bao. W.Y. and Tansel I.N., (2000), Characterization of fouling kinetics in ultrafiltration systems by resistances in series model. *Desalination,* 129:7-14.

Tracey, E.M. and Davis, R.H., (1994), Protein fouling of track-etched polycarbonate microfiltration membranes, *Journal of Colloid and Interface Science,* 167: 104 - 116.

Trägårdh, G., (1989), Membrane cleaning, *Desalination, 71: 325 - 335.*

Tshivhunge, A.S., (2001), Enzymology of activated sewage sludge during anaerobic treatmentof wastewaters: Identification, characterization, isolation and partial purification of proteases, MSc Thesis, Rhodes University, Grahamstown, South Africa.

Van De Berg, G.B., Smoulders, (1989) The boundary-layer resistance model for unstirred ultrafiltration: A new approach. *Journal of Membrane Science,* 40: 149 - 172.

Van Reis, R., Gadam S., Frautschy L.N., Orlando, S., Goodrich, E.M., Saksena, S., Kuriyel, R., Simpson, C.M., Pearl, S. and Zydney, A., (1997) High performance tangential flow filtration. *Biotechnology and Bioengineering,* 56: 71 - 82.

Vavilin, V.A., Rytov, S.V. and Lokshina, L.Y., (1996) A desciprion of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *BioResources Technology,* 56: 229 - 237.

Vernhet, A., Cartalade, D. and Moutounet, M., (2003) Contribution to the understanding of fouling build-up during microfiltration of wines. *Journal of Membrane Science,* 211: 357 - 370.

Wenten, I.G., (1995) Mechanisms and control of fouling in crossflow microfiltration. *Filtration and Separation,* 3: 252 - 253.

Wentzel, M.C., Mbewe, A. and Ekama, G.A., (1995) Batch test for measurement of readily biodegradable COD and active organism concentrations in municipal wastewaters. *Water SA,* 21: 117 - 124.

Whiteley, C.G., Burgess J.E., Melamane, X., Pletschke, B.I. and Rose P.D., (2003) The enzymology of sludge solubilisation utilising sulphate-reducing systems: The properties of lipases. *Water Research*, 37: 419 – 424.

Whiteley, C.G., Heron, P., Pletschke, B.I., Rose, P.D., Tshivunge, S., Van Jaarsveld, F.P., and Whittington-Jones, K., (2002a) Enzymology of sludge solubilisation utilising sulphate reducing systems: properties of proteases and phosphatases. *Enzyme and Microbial Technology,* 6088: 1 - 6.

Whiteley, C.G., Rose, P.D. and Pletschke, B., (2002b) Environmental Enzymology:Enzymology of accerelated sewage solubilization: Role of ATP sulphurylases. *Enzyme Microbial Technology,* 31(3): 329 - 336.

Whiteley, C.G., Pletschke, B., Rose, P. and Ngesi, N., (2002c) Specific sulphur metabolites stimulate β-glucosidase activity in an anaerobic sulphidogenic bioreactor. *Biotechnology Letters,* 24(18): 1509 - 1513.

Whittington-Jones, K., (1999), Sulphide-enhanced hydrolysis of Primary Sewage Sludge: Implications for the bioremediation of sulphate-enriched wastewaters, PhD Thesis, Rhodes University, Grahamstown.