Enzymatic cleaning of ultrafiltration membranes fouled in wool-scouring effluent

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Abstract

Polysulphone membranes used for the ultrafiltration of raw biological streams, like abattoir and wool-scouring effluents, are subjected to severe fouling. In a previous study the authors developed a static membrane fouling system to investigate the nature of foulants from abattoir effluent and the effects of fouling on membrane performance and character Enzymes specific for the degradation of proteins and lipids were subsequently successfully used in cleaning mixtures to remove foulants from membranes fouled in abattoir effluent. Membranes fouled by wool-scouring effluent were also found to be severely contaminated by protein and lipid material. In this study the same approach, followed for the enzymatic removal of proteins and lipids from abattoir-effluent-fouled membranes, was applied to membranes that were statically fouled in wool-scouring effluent. The effectiveness of a number of enzyme-based cleaning agents was determined by comparing their ability 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 of unfouled membranes. This study showed that the enzymatic approach to biological foulant removal could be successfully applied to polysulphone ultrafiltration membranes used in the wool-scouring industry.

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Introduction

The wool-scouring process uses hot water, sodium carbonate and non-ionic detergents to produce a highly polluting, malodorous, effluent with a complex composition (Pearson et al., 1976; Mozes et al., 1981 and Monteverdi et al., 1992). This effluent, rich in grease, suint, clay and proteins, causes major pollution problems (Rindone et al., 1991 and Oellermann et al., 1992). Ultrafiltration (UF) can be a cost-effective method for the treatment of this effluent to prepare it for recycling and the advantages of this method for the treatment of biological effluents are well documented (Cheryan, 1986; Jacobs, 1991; Cowan et al., 1992; and Echner and Zottola, 1993). Fouling, however, is the major obstacle in the successful implementation of UF membranes for the treatment of high organic content effluents like wool-scouring effluent (WSE). Permanent membrane fouling, caused by the formation of grease scales on the inner membrane surface, results in severe flux decline (Turpie et al., 1992 and Hogetsu et al., 1992). Previously the fouling problem was approached in a number of ways which included optimisation of flow conditions, pretreatment of the effluent, the production of membranes with reduced adsorptive properties (Fane et al., 1983; Flemming, 1990; Spencer and Thomas, 1991; Echner and Zottola, 1993 and Sedath et al., 1993), the optimisation of operational factors (Bauser et al., 1982) and the use of high-quality rinse-water (Bragulla and Lintner, 1986). All these methods have yielded moderately satisfactory results but at a relatively high cost. 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. The success achieved with the use of enzyme-based cleaning regimes for membranes fouled by abattoir effluent, opened new avenues for the use of this method in other biological process streams, such as WSE (Maartens et al., 1996). To fully realise the catalytic potential of enzymes in cleaning mixtures it is, however, important that the nature and composition of foulants adsorbed onto membranes, are known.

In this study real WSE was used as the fouling medium as artificially prepared foulant mixtures yield results that cannot be readily extrapolated to an industrial process (Grund et al., 1992). Trials were conducted to obtain information on the nature and composition of foulants in WSE and to determine which of the foulants in the effluent adsorbed onto UF flat-sheet polysulphone membranes (PSM). The effects of the statically adsorbed foulants on membrane surface and permeability characteristics were also determined. With the nature of the foulants known a number of different cleaning regimes and cleaning agents were evaluated according to methods previously used in trials for cleaning abattoir-effluent-fouled membranes (Maartens et al., 1996). The cleaning methods and agents tested included specific enzymes and enzyme/detergent mixtures as well as commercial and conventional cleaning agents.

Experimental

Materials

The enzymes used in this study are listed in Table 1. Commercial detergents, Alkazyme and Zymex (formulated cleaning agents with proteolytic activity), were obtained from Syndachem Sales Ltd., Milnerton, South Africa. A 3% aqueous solution of Alkazyme:Zymex (1:1) was used at room temperature according to the manufacturer's specifications. Detergents used were sodium dodecyl sulphate (SDS) (2% and 4 % solutions (m/v) in distilled water at 37°C) and Triton X100 (0.1% solution (m/v) in distilled water at 37°C). To create an artificial oil-water interface for lipase action, lipase A (1 mg/ml) was dissolved in an 0.1%

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TABLE 1
ENZYMES USED TO CLEAN MEMBRANES FOULED BY WSE. ENZYMES WERE USED AT OPTIMAL TEMPERATURE AND PH AS RECOMMENDED BY THE DISTRIBUTORS

Enzyme	Origin	Type	Optimum pH	Optimum temperature °C
Protease A type XXIII	*Aspergillus oryzae*	serine protease	7.5	37
Protease B type II	*Aspergillus oryzae*	serine protease	7.5	37
Lipase A type II	porcine pancreas	triglyceride hydrolase	7.5	37
Trypsin protease C	pancreas		7.5	35.5
Esterase A	hog liver	carboxylic-ester hydrolase	7.5	375

TABLE 2
FOULANT CONTENT, TEMPERATURE AND PH OF TYPICAL WSE. VALUES PRESENTED ARE THE MATHEMATICAL MEANS OF AT LEAST TEN DETERMINATIONS ±SD

Protein content (mg/l)	Lipid content (mg/l)	Temperature °C	pH
140 ± 11	1.18 ±1	40 ± 1.5	7.89 ± 0.75

Triton X100 Clark and Lubs solution (pH 7.5). The PSM used in this study were of flat-sheet conformation, cast from a solution of Udel P3500 polysulphone in N-methyl-2-pyrrolidone and stored at 4°C in sodium azide (5 mg/l). All other chemicals were of the highest analytical grade and were used without further purification.

Effluent analysis

WSE was collected from the second-stage wash water of an active wool-scouring plant and preserved with (5 mg/l) sodium azide at 4°C. Effluent samples were analysed for proteins and lipids as previously described by Maartens et al. (1996).

Membrane fouling

Membranes were statically fouled in the second-stage WSE for a period of 24 h. During this process PSM rectangles (10 x 32 cm) were affixed to a Perspex frame containing two 120 mm posts equipped with lead weights to keep the frame submerged in the WSE for the entire fouling period (Maartens et al.,1996). After static fouling the membranes were removed, washed thoroughly with tap water, transferred to distilled water containing sodium azide (5 mg/l) and stored at 4°C. To monitor membrane fouling the lipid and protein material adsorbed onto the fouled membranes were determined by modified methods of Radin (1981) and Hess et al. (1978) respectively. The dynamic water contact angle changes of the fouled membranes were determined by a technique based on the Whilhelmy slide technique (Johnson and Dettre, 1977). Membranes were cut into 2 cm wide strips and folded double with the membrane layer facing outwards. The membranes under observation were attached to a balance and positioned vertically above freshly deionised analytical grade water. The level of the liquid was raised gradually until it just touched the membrane suspended from the balance. The increase in mass was recorded by the dynamic contact angle analyser coupled to a computer that related the mass increase to the contact angle. The pure-water flux changes were determined as follows: the statically fouled flat-sheet membranes were placed in a flat-sheet rig and pure water was pumped through the membranes at pressures of 200, 300 and 400 kPa with a constant flow rate of 1 l/min. By measuring the volume and rate of water permeation through the membrane the pure-water flux in $l/m^3 \cdot h$ was calculated (Maartens et al., 1996).

Membrane cleaning

Fouled membranes were cut into 30 x 10 cm strips, washed in a Clark and Lubs buffer (pH 7.5) and incubated in a 600 ml solution of the appropriate cleaning agent (Bower and Bates, 1955). Cleaning agents were used at optimal pH and temperature for each enzyme to ensure maximum efficiency. After incubation, the membranes were removed from the cleaning solutions, washed thoroughly with distilled water to remove all excess materials, and stored in distilled water at 4°C until analysed.

Determination of cleaning efficiency

To determine the efficiency of the different cleaning agents, membranes cleaned with the various cleaning protocols, unused membranes, 24 h-fouled membranes and membranes incubated in only the Clark and Lubs buffer (pH 7.5 for 60 min) were analysed as previously described (Maartens et al., 1996).

TABLE 3
CHARACTERISTICS OF UNUSED AND FOULED PSM. MEMBRANES WERE FOULED FOR A PERIOD OF 24 H IN WSE CONTAINING THE LATER STAGE-SCOURING WATER. VALUES PRESENTED ARE THE MATHEMATICAL MEAN OF AT LEAST FOUR DETERMINATIONS ± SD

Membrane treatment	Adsorbed protein µg/cm²	Adsorbed lipids µg/cm²	Pure water flux l/m²·h	Contact angle °C
Unused	0	0	829.2 ± 13	63.4 ± 1.1
Fouled	32.3 ± 0.8	175.5 ± 4	433.9 ± 4.6	above 90

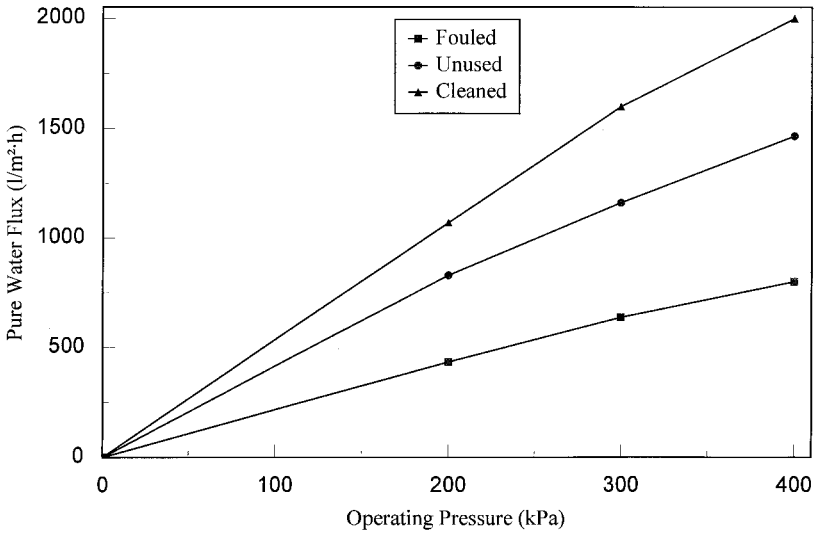

Figure 1
Correlation between the pure-water flux and the operating pressure as an indication of fouling and cleaning efficiency

Results and discussion

Effluent composition

The composition of the different WSE samples is presented in Table 2. The results indicate that quantitatively, proteins can be considered the major foulant. The effluent composition, pH and temperature of the WSE will support bacterial growth and membrane fouling should therefore be conducted on site. In addition, fouled membranes should be treated to prevent bacterial growth before membranes are analysed.

Membrane fouling

The four variables used to characterise fouling are summarised in Table 3. Relatively high amounts of lipid and protein material were adsorbed onto the membrane during the 24 h fouling period causing a flux decline in the order of 52%. The relatively large standard deviations observed in the effluent composition are due to the fact that alterations to the effluent cannot be controlled in the plant. Comparative fouling studies should therefore always be conducted in batches. The water contact angle values above 90 indicated that the membrane surface characteristics were drastically changed from slightly to totally hydrophobic indicating lipid fouling rather than protein fouling. The correlation between the pure-water flux and the operating pressure, at a constant linear flow speed, for fouled and cleaned membranes, is indicated in Fig. 1. By comparing the slopes of the graph for the unfouled membrane with those of the fouled membranes, the extent of fouling could be determined. Cleaning efficiency could also be estimated using these graphs as a steeper slope would indicate a better cleaning performance.

Membrane cleaning

PSM fouled in WSE for 24 h were subjected to cleaning by different cleaning mixtures. The changes observed in membrane contact angle, percentage flux improvement, percentage lipid reduction and percentage protein reduction, after cleaning are summarised in Table 4. Adsorbed proteins could be most successfully removed from the fouled membranes by the commercial product Alkazyme:Zymex, SDS and the combination between lipase A and Triton X100 followed by protease B. These cleaning regimes were, however, not very successful and only 20% of the adsorbed lipid material could be removed. These results clearly indicated that cleaning regimes, identical to those used for membranes fouled in the abattoir effluent, could not be used with the same efficiency in WSE membranes due to differences in the nature of adsorbed lipid material.

A literature study revealed that lanolin, which is classified as a wax rather than a tri-acylglycerol or phospholipid, is the major constituent of lipids in WSE. An enzyme, esterase A, with greater

TABLE 4
CLEANING EFFICIENCY OF THE DIFFERENT CLEANING AGENTS AND COMBINATIONS USED TO CLEAN UF MEMBRANES FOULED IN WSE. VALUES PRESENTED ARE THE MATHEMATICAL MEAN OF A MINIMUM OF FOUR DETERMINATIONS ± SD

Cleaning technique	% Flux improvement	Contact angle °C	% Lipid reduction	% Protein reduction
Fouled for 24 h	0	> 90	0	0
Buffer 60 min	17.5 ± 2	> 90	1.7 ± 3	3.53 ± 0.1
Protease A (3 mg/ml) 60 min	18 ± 2.8	> 90	5.55 ± 1.8	8.1 ± 0.3
Protease C (3 mg/ml) 60 min	38.3 ± 6.5	> 90	5.8 ± 1.8	14.4 ± 0.7
0.1% Triton X100, 60 min, 37°C	80 ± 4.1	> 90	7.43 ± 1.4	37.3 ± 4
Lipase A (3 mg/ml) 60 min	84.4 ± 4.5	> 90	6.9 ± 1.6	9.8 ± 0.62
Lipase A:Triton X100 (3 mg/ml:0.1%), 60 min	87.8 ± 4.1	> 90	8.6 ± 1	48.6 ± 4.2
SDS(0.4%), 60 min, 37°C	89 ± 3.1	> 90	11.4 ± 1.8	70.6 ± 6.2
Protease B (3 mg/ml), 60 min	90.8 ± 4.5	> 90	13.1 ± 3	6 ± 0.6
Alkazyme:Zymex (1:1), 3% 60 min	119 ± 9.1	86 ± 3.9	20 ± 3.6	90.2 ± 5
Lipase A:Triton X100 (1 mg/ml:0.1%) 60 min, followed by Protease B (1 mg/ml) 60 min	141.9 ± 8	84 ± 2.2	21.14 ± 2.8	65.4 ± 0.25
Esterase A (3 mg/ml), 60 min	146 ± 9.1	62.31 ± 6.5	49.3 ± 3.6	25 ± 0.6

TABLE 5
STATISTICAL ANALYSIS OF THE CORRELATION BETWEEN LIPID REMOVAL AND FLUX IMPROVEMENT AND PROTEIN REMOVAL AND FLUX IMPROVEMENT OF PSM MEMBRANES FOULED IN ABATTOIR EFFLUENT. VALUES PRESENTED IN TABLE 4 (THE MATHEMATICAL MEAN OF AT LEAST FOUR DETERMINATIONS ± SD) WERE ANALYSED USING THE PEARSON r TEST

Parameter	Protein removal	Lipid removal
Number of XY Pairs	10	10
Pearson r	0.52	0.76
99% confidence interval	-0.3769 to 0.9140	0.02957 to 0.9624
P value (two-tailed)	0.12	0.01
Is the correlation significant?(α=0.05)	No	Yes
R squared	0.27	0.58

specificity towards ester bonds was subsequently used and the adsorbed wax material could be decreased by 50%. The inability of the lipase A solution to remove the lipids deposited on the membrane surface can be ascribed to the fact that this enzyme is specific for tri-acylglycerols. This result emphasised the importance of the analysis of foulants adsorbed onto membranes before choosing a cleaning enzyme. Most materials of biological origin are biodegradable and specific enzymes that would hydrolyse or break down these substances to their simplest form can usually be found.

After fouling for 24 h in WSE the membrane surfaces were totally hydrophobic as shown by the values of the contact angle measurements. The results obtained from contact angle measurements show that only three cleaning regimes, esterase A (62.31), lipase A:Triton X100 mixture followed by protease B (84) and Alkazyme:Zymex (86) could significantly reduce the contact angle with a concomitant increase in pure-water flux. As expected, these three regimes were also the most successful in lipid reduction. These results demonstrate the important supporting role physical techniques like contact-angle measurements can play in the assessment of cleaning regimes for membranes fouled in biological effluents.

A statistical analysis of the correlation between lipid removal and PWF and protein removal and PWF improvement is summarised in Table 5. The analysis shows a correlation only between lipid removal and flux increase, a clear indication that the adsorption of lipophilic substances to the hydrophobic PSM surface is the most important cause for flux decline during the ultrafiltration of WSE and that future cleaning regimes should concentrate more on lipid removal for the regeneration of WSE-fouled membranes.

Flux improvements greater than 100% were achieved by some of the cleaning agents, a phenomenon that can be explained by the effect of detergents in the cleaning protocols on the membrane surface and foulant layer. The surfactants and buffers included in these cleaning solutions changed the membrane surface morphology to a more homogeneously permeable surface (Fane et al.,1985). This effect, however, is only noticeable when excessive membrane foulants are removed by means of effective cleaning agents such as enzymes. This phenomenon is also noticeable in Fig.1, where the slope of the pressure vs. pure-water flux graph for the best cleaning agent, was higher than that of an unused membrane. In addition this figure shows that by increasing the operating pressure on a system the effect of fouling cannot be reduced. From the results obtained in this study it was apparent that the enzyme esterase A, the lipase A:Triton X100 mixture followed by protease B and Alkazyme:Zymex were the most effective agents to restore the pure-water flux.

Conclusion

- Potential foulants in WSE are proteins and lipids, the same type of foulants that adsorbed onto PSM fouled in abattoir effluent. The main lipid component in WSE was lanolin while tri-acylglycerols were the major lipid foulants in abattoir effluent.
- Lipids adsorbed to a much larger extent than proteins.
- The techniques developed to characterise membrane fouling by abattoir effluent were successfully transferred to characterise membrane fouling by WSE.
- The enzyme-based cleaning methods used on membranes fouled in abattoir effluent, could be applied to PSM fouled in WSE.
- Due to the difference in lipid composition, lipids adsorbed onto WSE membranes could most effectively be removed with an esterase in contrast with the lipases which were most effective on abattoir effluent membranes.

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