Cleaning fouled membranes using sludge enzymes

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Abstract

Maintenance of membrane performance requires inevitable cleaning or "defouling" of fouled membranes. Membrane cleaning using sludge enzymes, was investigated by first characterising ostrich abattoir effluent for potential foulants, such as lipids, proteins and polysaccharides. Static fouling of polysulphone membranes using abattoir effluent was also performed. Biochemical analysis was performed using quantitative and qualitative methods for detection of proteins on fouled and defouled membranes. The ability of sulphidogenic proteases to remove proteins adsorbed on polysulphone membranes and capillary ultrafiltration membranes after static fouling, and ability to restore permeate fluxes and transmembrane pressure after dynamic fouling was also investigated. Permeate volumes were analysed for protein and amino acids concentrations. 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. Static fouled membranes removed 23.4% of proteins. Defouling of dynamically fouled capillary ultrafiltration membranes using sulphidogenic proteases was successful at pH 10, 37°C, within 1 h. Sulphidogenic protease activity was 2.1 U/ml and Flux Recovery (FR%) was 64%.

Introduction

Membrane filtration has important applications in the environment, for example, in wastewater treatments, abattoir effluents, and in food and biotechnology industries (Cheryan, 1986, and Kulkarni et al., 1992). It is a separation technology that is competitive with conventional separation techniques such as centrifugation, distillation, adsorption, absorption, extraction, etc. (Kuberkar et al., 1998). This technology selectively separates components over a wide range of particle size. It also concentrates, fractionates and purifies the product (Baker, 2000). Although membrane technology has made great advances recently, membrane fouling is a problem. Fouling occurs due to the deposition of suspended or dissolved substances on its external surfaces, at its pore, or within the pores (Madaeni et al., 2001). It is a process resulting in loss of membrane performance such as lower than expected flux, reduced productivity, high cleaning costs, use of harsh chemical cleaning etc (Leukes et al., 1999). Permanent membrane fouling caused by the formation of "gel-layer" results in severe flux decline and thus reduced productivity (Howell and Velicangil, 1982).

Reduction of fouling and cleaning of fouled membrane have been approached in a number of ways (Flemming, 1990; Maartens et al., 1996b), which included optimisation of flow conditions, pretreatment of the effluent, production of membranes with reduced absorptive conditions, backflushing, harsh chemical cleaning agents which results into high cleaning costs and industrial pollution (Trågard et al., 1989; and Kim et al., 1993).

Considerable research has been focused on cleaning of fouled membranes. Milder and environmentally more friendly cleaning regimes such as purified enzymes and detergents have been considered for the removal of biologically derived foulants from polymer membranes (Trågard et al., 1989; Kim et al., 1993). The use of enzyme alone or in combination with biodegradable detergents is an attractive alternative to the classical cleaning regimes (Maartens et al., 1996b; and Leukes et al., 1999). Previous studies (Maartens et al., 1996b), 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 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., 1996b). 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 compared to harsh chemical cleaning agents that damage the membrane. The source of sludge enzymes would be sulphidogenic bioreactors where sulphate reduction occurs.

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 sulphate-reducing bacteria (SRB) (Du Preez et al., 1992). These micro-organisms are known to dissimilate SO_4^{2-} and produce H_2S, however they require a constant supply of energy and an electron donor. Primary sewage sludge (PSS) has been identified as the most abundant and cost-effective carbon source, but it is available in a complex form (Bjorn et al., 1996). Solubilisation of this complex PSS can be achieved by hydrolysis of polymers, such as cellulose, lipids, proteins, polysaccharides, using enzymes such as cellulases, lipases, proteases, α and β-glucosidases; produced in the presence of sulphate reducing bacteria (SRB) (Whittington-Jones, 1999).

In a study of Enzymology of Accelerated Sewage Solubilisation these enzymes were characterised (Whiteley et al., 2000). Solubilisation and hydrolysis of polymers in PSS by lipases, proteases, and β-glucosidases was enhanced in the presence of sulphate reducing bacteria (Whiteley et al., 2000). These enzymes had high optimum temperature between 50°C and 60°C. pH optima for proteases, lipases, and β-glucosidases were 5, 7 and 10; 6 to 8; and

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5.4 to 6.8 respectively. Protease activity was highly activated at > 200 mg/l of sulphide and sulphite while inhibited by 50% by sulphate. Lipase activity was also highly activated at > 200 mg/l of sulphide (1 000-fold) and sulphite (15-fold); and inhibited by sulphate. β-Glucosidases were gradually inhibited by sulphate and sulphite while activated by sulphide (Whiteley et al., 2000b). The aim of this study therefore is to clean or defoul membranes that have been fouled by abattoir effluent using sludge enzymes. Sludge enzymes for the defouling process will be obtained from a sulphidogenic bioreactor. It also investigates whether these enzymes can be used to produce self-cleaning membranes.

Materials and methods

Ostrich abattoir effluent used for fouling was obtained from Ostritech (Pty) Ltd, Grahamstown, South Africa. Enzymes used for defouling experiments were obtained from a sulphidogenic bioreactor at the laboratory. Polysulphone disc membranes (MSI Micron-PES – Polysulphone disc filters, 0.22 μ, 47 mm) were purchased from Osmonics Inc, Minnesota, USA. Ninhydrin reagent solution and Bradford reagent were purchased from SIGMA Inc, Germany. All other reagents of analytical grade were purchased from Saarchem Inc, South Africa. Shimadzu-Spectrophotometer (UV-160A UV visible recording spectrophotometer) was used for reading absorbance values. Eppendorf Centrifuge 5810R and Beckman Model J2-21 Centrifuge were used for centrifugation.

Characterisation of the abattoir effluent

The abattoir effluent was characterised for proteins, lipids, and carbohydrates.

Protein assay

Bradford's method (Bradford, 1976) was used for protein determination on the ostrich abattoir effluent. 50μl were aliquoted from a 15 min boiled abattoir effluent, 50 μl of 0.15 M NaCl was added, and the samples were vortexed. The blank was prepared by using 50 μl of distilled water. 1 ml of Bradford's reagent was then added; the samples were incubated for 2 min. Absorbance was read at 595 nm against a BSA standard curve.

Lipid assay

100 ml of abattoir effluent was heated in an oven at 100°C for 2 h. After cooling the sample was homogenised in methanol-chloroform (1:2) 30 ml. The sample was then centrifuged (4 000 r/min, 10 min, 4°C). The pellet was resuspended and homogenised for 2 min in 60 ml of chloroform-methanol (2:1) solvent. The sample was centrifuged (4 000 r/min, 10 min, 4°C). The supernatants were pooled and 25 ml of 0.88 % KCl was added. The aqueous layer was drawn out and the remaining layer evaporated to dryness in the laminar hood flow. Lipid content of the abattoir was determined by weight (Folch et al., 1957).

Total carbohydrate assay

Total carbohydrate concentration on the ostrich abattoir effluent was determined by a modification of the phenol-sulphuric acid method (Taylor, 1995). 1 ml of abattoir effluent was measured in triplicate and 1 ml of 5% phenol-sulphuric reagent was added. 5 ml of concentrated H_2SO_4 were also added and the samples were cooled at room temperature. Total carbohydrate was determined by reading the absorbance at 488 nm against a glucose standard curve.

Total reducing sugar assay [Nelson-Somogyi]

1ml of abattoir was measured in triplicate and 1 ml of copper reagent was added. Samples were then incubated in a waterbath at 70°C for 15 min. After cooling 1 ml of arsenomolybdate reagent was added and the samples were incubated for 5 min at room temperature. Total reducing sugar was determined by reading absorbance at 510 nm against a glucose standard curve.

Determination of maximum fouling time for polysulphone disc membranes

In order to properly design experiments around defouling it was necessary to determine the time required to statically foul the membranes. Maximum fouling time was determined by first boiling 500 ml of abattoir effluent for 15 min. Polysulphone membranes were then placed in a 1 000 ml Erlenmeyer flask with the boiled abattoir effluent (1 disc membrane/100 ml of abattoir). The sample was incubated stationary at room temperature for 48 h. Every 4 h a membrane was removed, let to dry for 5 min, cut into 8 equal halves, and assayed for protein fouling using the Bradford method (Maartens et al., 1996a).

Static fouling and defouling of polysulphone membranes

Polysulphone membranes (1 disc membrane/100 ml) were fouled for 48 h using abattoir effluent that had been boiled for 15 min. After fouling each disc membrane was rinsed with distilled water and allowed to dry for 5 min then cut into 4 equal halves. Sulphidogenic proteases were obtained from sulphidogenic bioreactor and sonicated for 2 min/1 ml using a Virsonic 100 sonicator. The sludge was then centrifuged (4 000 r/min, 15 min, 4°C). Fouled membranes were incubated in 5 ml of supernatant at 37°C for 4 h.

Qualitative colorimetric detection of proteins on fouled and defouled membranes

A qualitative colorimetric assay (Bradford, 1976; and Dunn and Angel, 1990) was performed on clean, fouled and defouled membranes to ascertain the presence and/ absence of proteins. Membranes were stained for 15 min with 1% of Coomassie Brilliant Blue G250 staining solution at room temperature. Afterwards they were destained using Destaining Solution I (methanol/acetic acid/water – 5:1:4) for 15 min in four equal intervals, and then destained for 2.5 h using Destaining Solution II (methanol/acetic acid/water – 25:37:438).

Quantitative method for detection of proteins on fouled and defouled membranes

A fouled membrane disc was cut into 4 equal halves and incubated in a mixture of 5 ml of 5% SDS and 2.5 ml of distilled water. The samples were then incubated at 37°C for 2,5 h. The protein content was determined by measuring out 750 μl of the stripping solution and then adding 500 μl of 2M NaOH and 900 μl Solution A. The samples were vortexed and incubated for 30 min at room temperature. Then 100 μl of Solution B were added; the samples were vortexed and incubated for 20 min at room temperature; 2 portions (1.5 ml) of Solution C were added and the samples were incubated at room temperature for 30 min. Protein was determined by measuring absorbance at 650 nm against the BSA standard curve. Bradford's assay was also performed on the same set of membranes (Bradford, 1976; Dunn and Angel, 1990).

Dynamic fouling and defouling of capillary ultrafiltration membranes

Fouling using abattoir effluent

500 ml of diluted abattoir effluent was centrifuged (12 000 g, 25 min, 4°C). The supernatant was used for dynamic fouling of the capillary ultrafiltration membrane module. Water flux experiments were conducted with pressure, permeate flux, inlet and outlet flow rates measured every 30 min intervals for 2.5 h.

On fouling using the prepared abattoir effluent, inlet and outlet pressure, permeate flux; inlet and outlet flow rates were recorded every 30 min. At the same time samples were collected at the retentate and permeate outlets for biochemical analysis.

Defouling using sulphidogenic proteases

Sulphidogenic proteases were from the obtained sulphidogenic bioreactor. The sludge was centrifuged (12 000 g, 25 min, 4°C) and the pellets were collected to make 100 ml. The pellet was resuspended in 400 ml of carbonate-bicarbonate buffer (0.1M, pH 10). The resuspended pellet was sonicated for 2 min/1 ml, 8W using Virsonic 100 sonicator.

The sludge was then centrifuged at 4°C, 12 000g for 25 min, and the supernatant was placed in a reservoir and pumped through the fouled membrane. Defouling was performed at 37ºC. Parameters were recorded as in fouling experiments and samples were also collected for biochemical analysis.

Biochemical analysis of samples collected during fouling and defouling

Samples were assayed for protein content using Bradford's and Folin-Lowry methods. Amino acids were assayed using micro-titre Ninhydrin assay. 50 µl of each sample was measured and 50 µl of sodium acetate buffer (4M, pH 5.5) was added. Ninhydrin reagent (50 µl) was added and the samples were incubated at 60°C for 20 min. After cooling off, 100 µl of 50% ethanol was added. Amino acid concentration was determined reading samples at 570 nm against 1 mM glycine standard curve.

Results and discussion

Characterisation of abattoir effluent

Characterisation of an ostrich abattoir effluent for lipids, proteins, total carbohydrates, and total reducing sugars is shown in Fig. 1. 1 ml of an abattoir effluent contained 553 µg of lipid (100%), 301 µg of protein (54.4%), 141 µg of total carbohydrate (25.5%), and 0.63 µg of total reducing sugars (0.11%). These results suggest that lipids and proteins are major potential foulants in an ostrich abattoir effluent (Cyster, 1999 and Maartens et al., 1996a). The abattoir effluent had a high chemical oxygen demand (COD) of 31 900 mg/l which also suggests a high concentration of foulants.

Maximum time for static fouling of polysulphone membranes

Determination of maximum time for static fouling of polysulphone membranes using the abattoir effluent (Fig. 2) showed that rapid fouling occurred within 10 h and at 24 h fouling had reached maximum. The results agree with the findings of Maartens et al., 1996a where fouling occurred within 8 h.

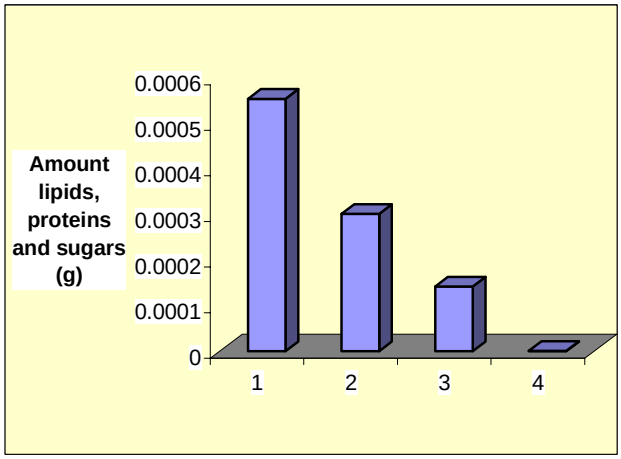

Figure 1
Characterisation of an ostrich abattoir effluent for lipids (1), proteins (2), total carbohydrate (3), and total reducing sugar (4).

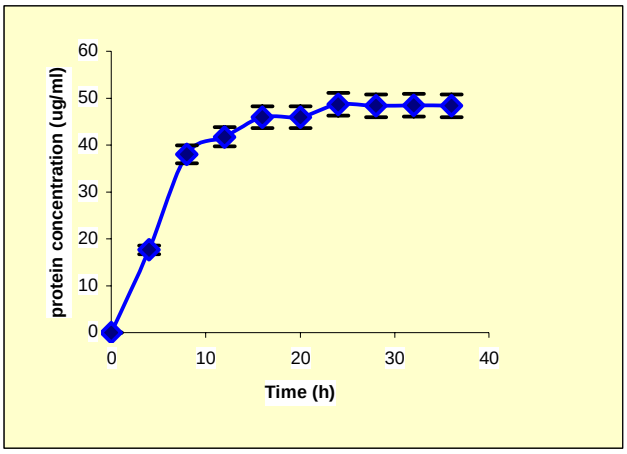

Figure 2
Concentration of proteins on determination of maximum time for static fouling on disc-shaped polysulphone membranes, using an ostrich abattoir effluent

Static fouling and defouling of polysulphone membranes

Colour development and quantitation of proteins adsorbed onto polysulphone membranes during static fouling using abattoir effluent is shown in Figs. 3 and 4. According to Fig. 3 defouling of Membrane C previously fouled for 24 h (Membrane B) was successful, as it is comparable to Membrane A. Membrane D had a slight increase of colour intensity when compared to Membrane B that suggested a slight increase in protein concentration.

Defouling of Membrane D, using sulphidogenic proteases did not remove adsorbed proteins as shown in membrane E. Fouling and defouling of Membrane D confirm (Howell and Velicangil, 1986) that fouling results in the formation of a cake layer (irreversible), formed as a result of concentration polarisation of macromolecules at the membrane/solution interface. Figure 4 shows protein concentrations obtained on membranes that had been fouled and defouled as in Fig. 3. Membrane 2 had 446.2 µg protein/ml, membrane 3 had 104.32 µg protein/ml and Membrane 4 had 506 µg protein/ml.

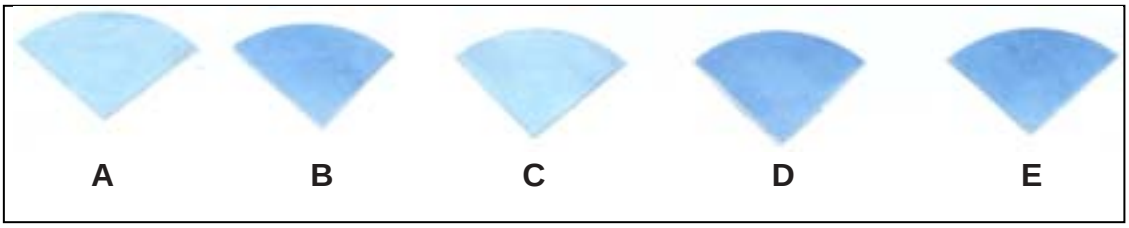

Figure 3

A qualitative colorimetric assay on fouled and defouled membranes. A = clean membrane, B = membrane that had been fouled for 24 h, C = same as B except it had been defouled for 4 h, D = membrane fouled for 48 h, and E = same as D except that it had been defouled for 4 h.

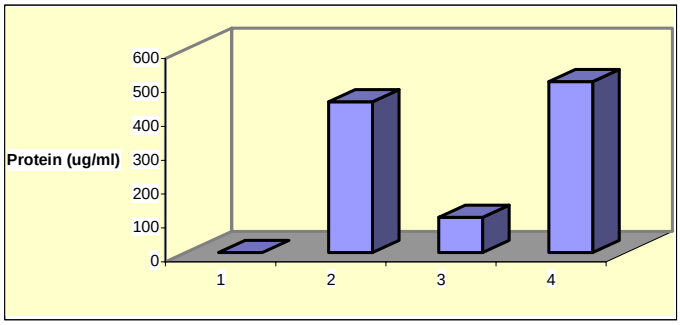

Figure 4

Quantitative assay for proteins on clean, fouled, and defouled polysulphone membranes. 1 = protein on clean membrane, 2 = protein on membrane that had been fouled for 24 h, 3 = protein on membrane same as 2 but defouled for 4 h, and 4 = protein on membrane that had been fouled for 48 h and defouled for 4 h.

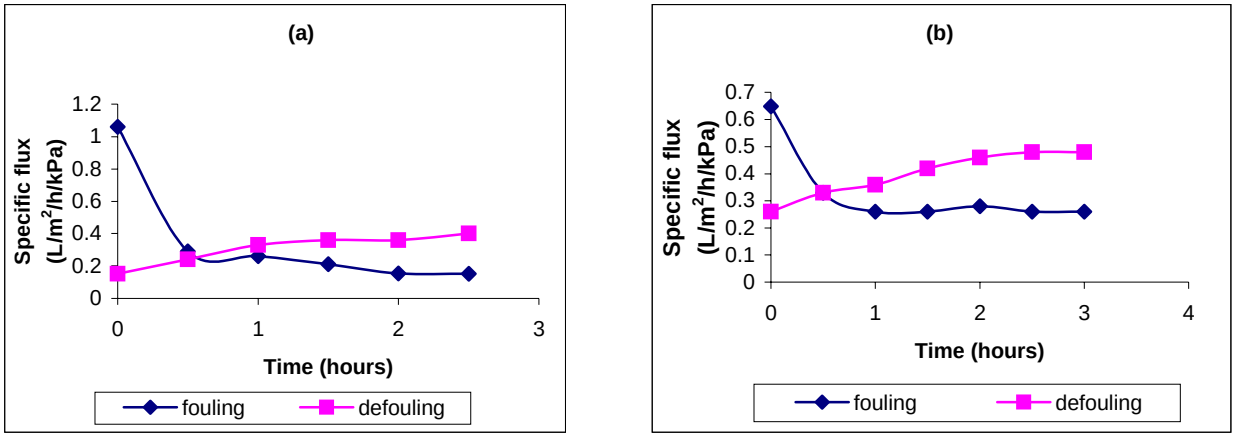

Figure 5

Dynamic fouling and defouling of capillary ultrafiltration membranes. (a) fouling and defouling using sulphidogenic proteases in water, (b) fouling and defouling using sulphidogenic proteases in 0.1 M carbonate-bicarbonate buffer at pH 10.

These results confirmed those in Fig. 3 that fouling for 48 h resulted in increased protein concentration and formation of a thicker cake layer. However activity of sulphidogenic proteases had not been optimised.

Dynamic fouling and defouling of the capillary ultrafiltration membrane module is shown in Fig. 5. These results indicate that major fouling occurred within 1 h, as there was a severe decrease in specific fluxes from 1.06 l/m²·h·kPa to 0.26 l/m²·h·kPa in Fig. 5(a) and 0.648 l/m²·h·kPa to 0.26 l/m²·h·kPa in Fig. 5(b). Defouling using sulphidogenic enzymes led to restoration of permeate fluxes from 0.26 l/m²·h·kPa to 0.4 l/m²·h·kPa in Fig. 5(a) and 0.26 l/m²·h·kPa to 0.28 l/m²·h·kPa in Fig. 5(b). Flux recovery (FR%) was 39 % for Fig. 5(a) and 64% for Fig. 5(b).

No protein was detected on permeate volumes after fouling, and upon defouling amino acid assay indicated that proteins previously adsorbed on the membranes were hydrolysed by sulphidogenic proteases maximally using a carbonate-bicarbonate buffer system. Biochemical analysis (Bradford and Ninhydrin assays) of the permeate volumes revealed that, on fouling no proteins were detected while a low concentration of amino acids was detected. During defouling, the concentration of amino acids on the permeate volumes increased and levelled after an hour. The activity of

sulphidogenic proteases was 0.0021 mM/ml·min, calculated per amino acid concentration. These results agreed with (Kuberkar et al., 1998, and Baker, 2000) that fouling decreases permeate/water fluxes and increases transmembrane pressure and cleaning or defouling restores them.

Conclusions

Lipids and proteins are the major foulants present in an ostrich abattoir effluent. Static fouling using an abattoir effluent occurred maximally at 24 h of incubation, while fouling occurred rapidly and maximally within 1 h on dynamic capillary ultrafiltration membranes.

Quantitative and qualitative assays indicated that defouling of statically fouled membrane using sulphidogenic proteases was achieved only for fouling after 24 h, and not after 48 h. Dynamic fouling caused a decrease in specific fluxes and an increase in transmembrane pressure. The activity of sulphidogenic proteases was 0.0021mM/ml·min after dynamic defouling. Flux recovery (FR%) was 39% when enzymes were suspended in distilled water and 64% when sulphidogenic proteases were in carbonate-bicarbonate buffer system at 0.1M, pH10, 37°C. However, defouling using sulphidogenic proteases has not been optimised. More experiments have to be performed, such as SDS-PAGE to ascertain protein hydrolysis during passive and active defouling, addition of sulphate, sulphite and sulphide to sulphidogenic enzymes and investigating whether they will enhance or inhibit enzyme activity, for optimisation.

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