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Performances of protease and amylase cleaning for microporous membranes used in wastewater applications

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ABSTRACT

Enzymes have been successfully applied to clean fouled membranes, especially those affected by the deposition of protein- and lipid-based compounds. In the water and wastewater industries, enzymatic cleaners are increasingly considered as potential alternatives from conventional chemical agents. Their very specific targeted actions and environmental impact are indeed some of the drivers for using enzymes in microporous membrane processes. This paper aims to assess the cleaning performance of two types of enzyme (protease and amylase) to remove typical fouling materials found in wastewater treatment (protein and carbohydrate modeled in this study by bovine serum albumin and sodium alginate, respectively). Under the experimental conditions used in this study, results showed relatively low cleaning efficiencies, with optimum efficiencies of 68% and 73% for protease and amylase, respectively. Although enzymes are not known to cause membrane ageing/deterioration, this type of cleaners was expected to potentially foul the membrane, as residual enzyme may attach to the membrane during the cleaning process. To assess this potential, cyclical cleanings were performed in addition to single cycle cleaning. Results showed that fouling occurred four times faster after membrane was re-used for 16 cycles. The sequential use of the two enzymatic cleaners in series did not provide any improvement in efficiency compared to the use of single enzyme. However, analysis of residual foulants indicated the lower amount of material found on the membrane.

Keywords: Enzyme; Protease; Amylase; Cleaning; Membrane bioreactor

1. Introduction

Membrane technology has become increasingly popular for its application in water and wastewater treatment. Despite its wider usage, the main drawback of membrane usage still exists, namely membrane fouling. In membrane bioreactor (MBR), protein and polysaccharides are the two main foulants which appear in the forms of extracellular polymeric substances (EPS) and soluble microbial products (SMP). EPS and SMP are the results of bacterial process occurring in the activated sludge. EPS are polymeric materials which encapsulate bacterial cells in a microbial biofilm while SMP are cellular components that are soluble and released in cell lysis [1]. SMP and EPS are able to form strong cake layer in membrane surface and even penetrate to membrane pores due to its small size which then create severe fouling. To overcome this problem, numerous studies have been conducted on suitable operating parameters and membrane cleaning.

Cleaning agents are commonly used to eliminate foulants from membrane surface and pores. Acids and

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sodium hypochlorite (NaOCl) are the popular cleaning agents in wastewater applications as they are aimed to remove inorganic and organic foulants [2]. Nevertheless, some drawbacks arise from chemical usage. Increasingly strict environmental rules has made it a problem to dispose the chemical solutions, not to mentioned the possibility of contamination of the chemicals towards the permeate. In addition, as these chemicals possess harsh characteristics (high pH, oxidizing capability), they may cause membrane ageing and pose a threat of membrane lifetime reduction. In the end, this means undesired extra cost. Alternative cleaning agent is then sought to provide cleaning solution without costing membrane lifetime.

Enzyme has been used as membrane cleaning agent in food industry. Earlier studies have found that protease provides good efficiency for membrane cleaning. For membrane fouled with abbatoir effluent, protease A from *Aspergillus oryzae* (Sigma) was used as cleaning agent with concentration between 1 and 3 mg/ml, pH 7.5 and temperature 37 °C. These high concentrations resulted in 80% protein removal and flux recovery of more than 90% [3]. Another study reported the use of 0.005 g/L protease A for membrane fouled with whey, resulting in 65% flux recovery [4]. Enzymatic cleaning of membrane used for abbatoir effluent provided convincing outcomes [5]. Enzymes used in that study were different types of lipases and proteases. Lipase was applied first to avoid lipase degradation by protease. Lipase alone, besides reducing lipid in the system is also able to reduce the amount of protein. Cleaning by *Pseudomonas* lipase and protease showed flux recovery of almost 100% [5]. For foulants consisting of whey proteins, enzyme mixture (protease and detergent) yield membrane resistance recovery of almost 100% [6]. Another study showed that enzymatic cleaning was effective for membrane fouled with effluent from wastewater treatment plant [7]. Protease recovered 100% of the flux, which was higher than the results obtained with alkali and acid cleanings.

Cleaning with enzymes is performed under mild condition, that is moderate temperatures, as high temperatures can denature the enzyme [6]. This is economically beneficial because high temperatures means high energy requirement which eventually leads to higher cost. Mild conditions also mean that enzymes do not work in extreme pH, resulting in good compatibility with membrane materials as some of the materials have narrow pH range [6]. Another advantage is their nature as biological substance which makes them easier to be disposed through biodegradation [6]. Therefore, enzymes may provide alternative to chemical cleaning agents which potentially causes ageing on membrane material. Enzymatic cleaning also presents challenges to the membrane user: maintaining the enzymatic activity when enzymes are mixed with protein solutions has to be particularly considered.

As mentioned previously, proteins and polysaccharides are major components of EPS, the main MBR foulant. While proteases has been investigated as a common cleaning agents to overcome protein foulants [4,7], no previous study has assessed the performances of amylase (capable of degrading polysaccharides) in membrane cleaning. This study will therefore attempt to investigate the performances of protease- and amylase-type-enzymes to clean the membrane fouled with MBR model solutions. The effect of repetitive enzyme usage on membrane hydraulic resistances and foulants density on membrane surface was also examined.

So far, most of the cleaning studies published were not greatly emphasized on experiments reproducibility. It is necessary for the initial and the fouling state of the membrane to be similar between different samples. These ensure the same baseline of the cleaning experiments, which then produce experiments with good repeatability. Rigorous method was applied in this study to have the same experiments parameters and provide reproducible results.

2. Methods and materials

The membrane used in this study was Durapore®, a flat sheet polyvinylidene fluoride (PVDF) from Millipore™. This membrane is hydrophilic with pore size 0.22 μm. A cross flow cell was used for filtration with active membrane area of 27.5 cm^2. Model solution throughout the whole study was consisted of BSA (Moregate Biotech) and sodium alginate (Ajax Chemicals), both used at 1 g/L and dissolved in MilliQ water.

Filtration was conducted under constant flux of 30 L m^{-2} h^{-1} and performed until the TMP reached 50 kPa. After fouling, rinsing with MilliQ water for 2 min was performed followed by enzymatic cleaning for 10 min. Another rinsing for 4 min was performed to remove the residual enzymes. Clean water tests were performed to obtain membrane resistance in the beginning of the experiments (R_m), after fouling (R_{fouled}), after rinsing (R_{rinsed}) and after cleaning process ($R_{cleaned}$). Average values of the resistances are presented in Table 1.

Cleaning efficiency was assessed by calculating the enzymatic cleaning efficiency (ECE) and the overall cleaning efficiency (OCE; Eqs. (1) and (2)). The OCE value takes into account the entire resistance removed during the rinsing and enzymatic, while ECE does not encompass the resistance removal obtained by rinsing process towards enzyme performance.

Table 1
Average R_m, R_{fouled} and $R_{cleaned}$ value throughout the study

Parameter	Average	Standard deviation	% error
R_m ($\times 10^{10}$ m^{-1})	5.21	0.48	9.2
R_{fouled} ($\times 10^{10}$ m^{-1})	60.08	5.88	9.8
R_{rinsed} ($\times 10^{10}$ m^{-1})	46.89	6.43	13.7
Fouling duration (min)	52.9	7.5	14

$$ECE = \frac{R_{rinsed} - R_{cleaned}}{R_{rinsed} - R_m} \times 100\% \quad (1)$$

$$OCE = \frac{(R_{fouled} - R_{cleaned})}{(R_{fouled} - R_m)} \times 100\% \quad (2)$$

Cleaning was based on the use of two different types of enzymes; protease and amylase. Protease was protease M amano from *Aspergillus melleus* (P4032), obtained from Sigma Aldrich and amylase was Purastar® ST L, a liquid α-amylase for laundry detergents from Genencor International which contains 1–5% active protein as alpha amylase. Concentration, temperature, and pH of the experiments were determined through preliminary experiments and supplier suggestions. Protease was used at concentration 0.1%, temperature 37 °C and unadjusted pH (6.5 ± 0.3), while amylase study was using 0.05%, temperature 50 °C, and pH adjusted to 6.5 using sodium hydroxide (NaOH). In sequential cleaning, both enzymes were used one after another in two different sequences, and another involves a rinsing step in between the two enzyme application.

The modified Lowry method was used to determine the amount of protein left on the membrane [8]. This method was performed by dissolving the attached proteins into sodium dodecyl sulphate (SDS) and NaOH. Solution was further reacted with sodium tartrate ($Na_2C_4H_4O_6$), sodium carbonate (Na_2CO_3), copper sulphate pentahydrate ($CuSO_4.5H_2O$), NaOH, Folinciocalteau reagent, SDS, and NaOH. Solutions obtained were then analyzed using Cary UV Spectrophotometer. Measurement was carried out in 750 nm. From the absorbance obtained, protein mass on the membrane was determined. The amount of polysaccharides attached on the membrane was assessed using the Dubois method [9]. In this paper, NaOCl was added to extract the alginate from the membrane. Solution was then reacted with phenol and sulphuric acid (H_2SO_4) solution. Measurement performed in 480 nm using Cary UV Spectrophotometer. Field Emission Scanning Electron Microscope (FESEM) was used for membrane imaging (Hitachi S900). Membrane samples were vacuum dried overnight and chromium coated prior to imaging process.

3. Results and discussion

3.1 Protease cleaning

3.1.1. Single cleaning

The optimum concentration of protease was assessed during single cleaning. Three different concentrations (ranging from 0.01 to 0.3%) were investigated under the same experimental conditions. The OCE values ranged from 48 to 68% for protease concentration increasing from 0.01 to 0.3% (the 0.1% protease concentration led to 61% of OCE). These relatively low cleaning efficiency results might be caused by the inability of the protease to remove sodium alginate from the membrane surface. This result was expected as protease aims to degrade protein rather than polysaccharides.

Due to the time constraints of this study, a relatively short cleaning duration of 10 min was applied. This relatively low cleaning time may also contribute to the small OCE obtained. Previous publications on enzymatic cleaning reported longer cleaning durations. Full recovery of initial water flux was reported in the case of protease cleaning (total cleaning time of 2 h) membrane fouled by effluent from wastewater treatment plant. [7]. The enzymatic agent (Maxatase XL) reached optimum cleaning for whey proteins fouling after 20 min [10]. Another publication reported maximum protease cleaning within 1 h [11], although, residual enzyme was found on membrane surface after that time. Protease detergent for cleaning of membrane fouled with aqueous extract of soy flour only needed 15 min to reach maximum cleaning capacity (a poor flux recovery of only 42%) [12].

Given that 0.3% of enzymatic cleaner is considered a relatively high concentration, greater level of protease was not tested. Above this concentration, enzymes were expected to attach and foul the membrane significantly (see following Section). For cyclical cleaning, concentration of 0.1% was chosen as it provided a reasonable efficiency.

3.1.2 Fouling potential of protease

The main concern in using enzyme as cleaning agent is its potential to foul the membrane. Previous research already reported that the usage of enzymatic cleaning agent can result in foulant and attachment of

Table 2
Resistance increase for virgin membrane cleaned by protease

Protease concentration	$R_m \times 10^{-10}$ (m^{-1})	$R_{cleaned} \times 10^{-10}$ (m^{-1})	R increase (%)
0.005%	5.42	5.44	0.3
0.1%	3.62	4.79	32.3

enzyme on the membrane [6]. Due to its proteinaceous nature, enzyme can deposit on the membrane surface and cause fouling. To assess this potential in this study, an experiment was conducted by performing the cleaning step on a virgin membrane. Different concentrations of protease were tested with temperature 50 °C and pH 8.5. Results reported in Table 2 indicate that protease can interact with the membrane directly by increasing the membrane resistance. While low concentration of protease has little influence on R_m, 0.1% of protease caused resistance to increase by more than 30%. Resistance increase on virgin membrane indicates that some of the protease deposited on the membrane surface, and proved that protease potentially participates in membrane fouling.

To avoid this effect, inactivation step of the enzyme might be beneficial, but was not studied in details within the scope of this work. Previous publication mentioned that, for commercial protease Alcalase, water rinsing was able to remove the residual activity present on post cleaning surface [13]. In this study, a rinsing step was also incorporated to remove the remaining residual enzyme.

3.1.3. Cyclical cleaning

To further assess the fouling potential of the enzyme, cyclical foulings and cleanings using protease 0.1% were performed. The ageing of foulant layer with cyclical fouling/cleaning is also an important factor and will be under investigation in this section. Experimental steps for each cycle were the same than single fouling/cleaning.

Fig. 1 shows the $R_{cleaned}$ values of the membrane which was fouled and cleaned with protease and amylase for 16 cycles. The R_m value (5.5×10^{10} m^{-1}) was indicated as cycle number 0 as initial references. The $R_{cleaned}$ after the first cycle of protease cleaning (26×10^{10} m^{-1}) was four times higher than R_m and this value increased then regularly from the second to the sixteenth cycle. After 16 cycles, membrane resistance (46.3×10^{10} m^{-1}) was eight times higher than R_m which implies that protease was ineffective in cleaning the membrane entirely. It is interesting to report that most of the increase in $R_{cleaned}$ occurred within the first

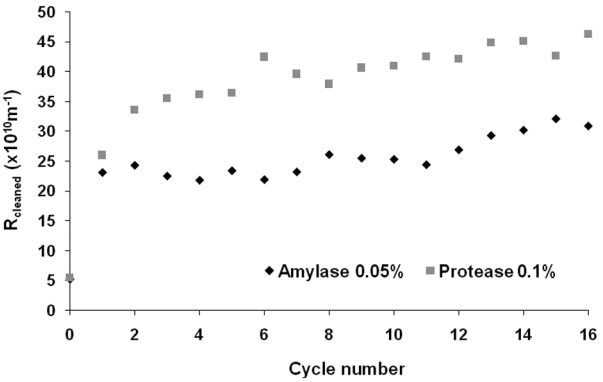

Fig. 1. $R_{cleaned}$ values for 16 cleaning cycles using protease 0.1% and amylase 0.05%.

fouling/cleaning cycle, revealing the importance of the first deposited fouling layer on the long-term performances of the membrane. This was also revealed in Fig. 2, which reports the time necessary for the membrane system to foul up to 50 kPa in consecutive fouling episodes. Fouling duration was found to significantly decrease with the number of cycles (from 58 down to around 15 min). The time plateau reached after 5 cycles suggests the formation of a steady fouling layer which was not removed by the enzymatic cleaning. However, the resistance could also originate from the accumulation of polysaccharides which were not degraded or removed by protease. A fraction of protease may also be left on the membrane, and became foulant itself due to its proteinaceous nature and its absorbance affinity with the membrane.

Compared to the cleaning efficiencies reported in previous studies [3,4], the protease appears to be less efficient in removing membrane foulants in these series of experiment. The feed mixture used here could be

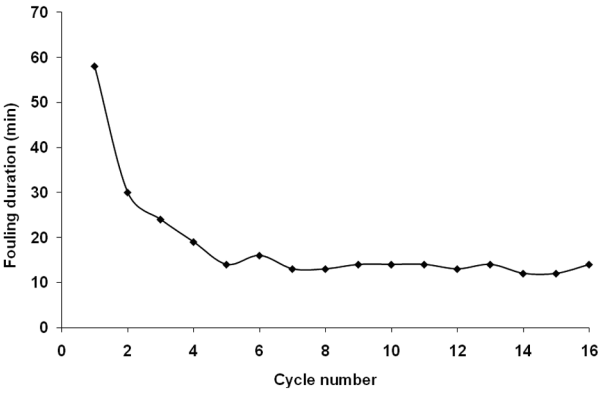

Fig. 2. Fouling duration for 16 cyclical filtrations using protease 1%.

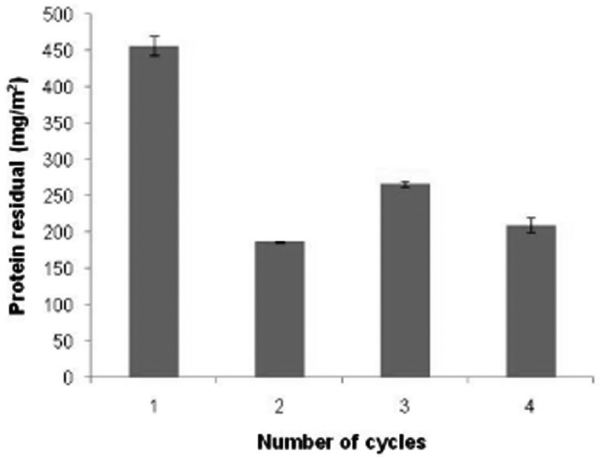

Fig. 3. Protein residual on the membrane cleaned by protease 0.1%.

partially responsible for the relatively poor enzymatic performances observed in the experiments, indicating the limitation of enzymes in cleaning membrane used for complex solutions. In addition, different types of membrane and operating parameters are expected to affect the cleaning efficiency of the enzyme.

Fouled membranes were analysed in terms of their relative protein loading. While 457 mg of protein was measured per m^2 of fouled membrane, this value decreased to 187 mg/m^2 after the first cleaning cycle (Fig. 3). For three cleaning cycles, more protein accumulated on the membrane (267 mg/m^2). This originated from both the BSA and the residual protease. With 16 fouling/cleaning cycles, protein level on membrane surface stabilized, although the $R_{cleaned}$ value was higher, revealing the increasingly higher contribution of alginate in fouling, while the ratio of protein in the fouling layer generally decreased due to the protease effect.

3.2. Amylase cleaning

3.2.1. Repeatability of cleaning experiments

This section assesses the repeatability of amylase cleaning. Membrane was fouled and cleaned with 0.05% of amylase under pH 6.5 and temperature 50 °C. The experiment was repeated three times and the results obtained (Table 3) show relatively low standard deviation with maximum error percentage of around 14% for RE and fouling duration. Therefore, it can be concluded that this set of experiments has good repeatability. All parameters and methods were kept the same throughout the experiments to ensure reproducible results.

Table 3
Average values of three single cleaning cycles with amylase

Parameter	Average	Standard deviation	% error
R_m ($\times 10^{10}$ m^{-1})	5.3	0.5	8.8
R_{fouled} ($\times 10^{10}$ m^{-1})	64.9	3.9	6.1
R_{rinsed} ($\times 10^{10}$ m^{-1})	54.4	4.5	8.3
Fouling duration (min)	52.9	7.5	14
ECE (%)	66.2	3.4	5.1
OCE (%)	71.3	4.6	6.5

3.2.2. Determination of optimum concentration

In these series of experiments, the fouled membranes were cleaned with different concentrations of amylase and the resulting cleaning efficiencies were reported in Fig. 4. For amylase concentrations of 0.006–0.075%, the OCE were not significantly different, averaging 70%, while concentration higher than 0.1% showed decreasing trend of cleaning efficiency.

Within the concentration range examined in this study, amylase resulted in higher cleaning efficiency compared to protease. A previous study focusing on the filtration of BSA and alginate investigated their fouling potential and revealed that alginate presented the highest fouling potential [14]. In addition, the membrane fouled with alginate also showed the lowest resistance recovery after backwashing. Hence, membrane fouling was expected to originate more significantly from alginate rather than BSA. It was therefore expected to obtain better performance recovery from the amylase compared to the protease cleaner.

The density of polysaccharide on the fouled and cleaned membranes was measured and results indicated that the membranes which were cleaned with amylase experienced decrease in polysaccharides density compared to the fouled membrane (Fig. 5). While

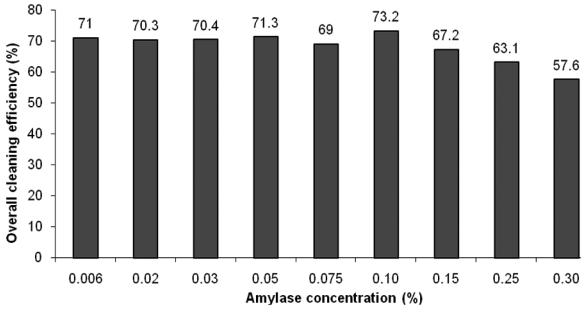

Fig. 4. Overall cleaning efficiencies with different concentrations of amylase.

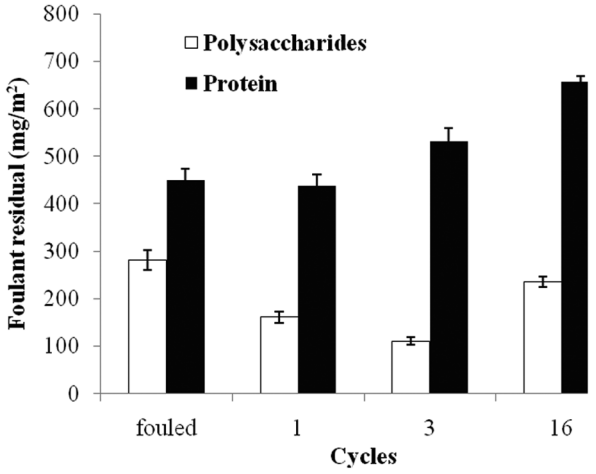

Fig. 5. Protein and polysaccharides residuals for membrane cleaned with amylase.

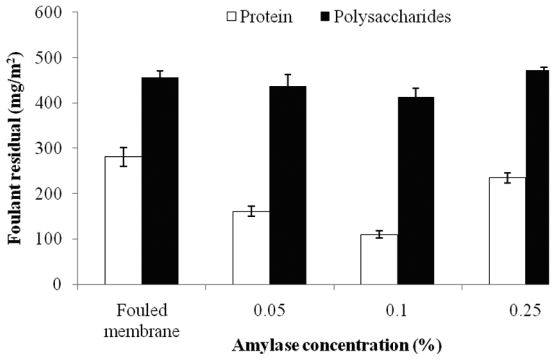

Fig. 6. Protein and polysaccharides density on membrane cleaned with amylase 0.05% with different numbers of cycles.

fouled membrane was found to contain 281 $\pm$ 21 mg/m^2 of polysaccharides, cleaning with 0.05% of amylase decreased this amount to 161 $\pm$ 11 mg/m^2 (43% removal) and with 0.1% of amylase, the polysaccharide density on the membrane found was 110 $\pm$ 8 mg/m^2 (61% removal). Amylase concentration of 0.25% was found to be ineffective compared to the other concentrations analyzed, given that polysaccharides density found on the membrane was 235 $\pm$ 11 mg/m^2 (i.e. only 17% removal). These results showed that amylase reduced the polysaccharides foulant on the membrane, with concentration 0.1% being the most effective.

Protein density for fouled membrane was observed to be 457 $\pm$ 14 mg/m^2. Cleaning with 0.05 and 0.1% of amylase decreased the protein density to 437 $\pm$ 25 and 413 $\pm$ 19 mg/m^2 respectively (Fig. 5). Cleaning with 0.25% amylase was found to slightly increase the protein level on the membrane to 472 $\pm$ 7 mg/m^2. These results suggest that amylase was not effective to eliminate the protein foulant on the membrane. This was expected as amylase works by eliminating polysaccharides. It appeared that residual amylase was attached to the membrane and analyzed by the Lowry method as the total protein. Despite its incapability to clean membrane from protein foulant, amylase was able to provide better cleaning efficiency compared to protease.

3.2.3. Cyclical cleaning

Cyclical cleaning involved the same protocol as the previous enzymatic cleaning, repeated 16 times. Amylase concentration was 0.05%, pH adjusted to 6.5 and temperature was 50 °C. After the first fouling/cleaning cycle, membrane resistance increased to more than 20×10^{10} m^{-1} (5 times the R_m value; Fig. 1). Membrane resistance kept increasing until it reached approximately 30×10^{10} m^{-1} after 16 cycles. This increase was less significant compared to rise of $R_{cleaned}$ for 16 cycles of protease cleaning, which indicates that amylase provided better (but still not complete) cleaning performance compared to protease. Residual foulants were left on the membrane, some of them potentially being residual enzyme. Similar trend of fouling duration for 16 cycles of protease cleaning was found with 16 cycles of amylase cleaning (results not shown). First filtration took 46 min to reach 50 kPa, while for the second filtration, the time decreased to almost half of the initial value with 26 min. The duration kept decreasing until it reached a rather stable value on the ninth cycle with 15 min. Fouling was found to occur faster for cleaned membrane on consequent filtrations. Residual foulant which was left on the membrane was suspected as the cause of decreasing time of fouling.

Previous study has found that BSA was easier to be removed by backwashing compared to alginate [14]. This means that it was likely for BSA to constitute the foulant layer in the membrane surface while alginate caused internal pore fouling. Internal fouling causes membrane performances to decrease more severely. Another study also mentioned that alginate caused more rapid flux decline compared to BSA [15]. Amylase was expected to eliminate alginate instead of BSA. Therefore, when alginate was eliminated, higher removal of resistance was expected and the results reported in this present work are in accordance with the above observations.

Fig. 6 shows the protein and polysaccharides density on the membranes which have been cleaned with amylase 0.05%. Single cycle cleaned membrane shows slight removal of protein from 457 $\pm$ 14 to

437 ± 25 mg/m^2 (4.4% removal), while membrane cleaned with 3 cycles had 531 ± 27 mg/m^2 protein which was more than fouled membrane (16% increase). At the end of 16 cycles of fouling and cleaning, the protein density was 656 ± 13 mg/m^2 which equals to 43% increase. This increase of protein level was assumed to originate from BSA which was not removed by amylase, combined with residual amylase attaching gradually on the membrane. Therefore, these results confirmed that amylase was not able to clean protein foulant and that there was a possibility that the residual enzyme participates in membrane fouling. Fouled membrane was found to contain 281 ± 21 mg/m^2 of polysaccharides and membrane cleaned with a single cycle showed 43% polysaccharides removal to 161 ± 11 mg/m^2. More removal was achieved after three fouling and amylase cleaning cycles, where membrane featured 110 ± 8 mg/m^2 of polysaccharides. However, after 16 cycles, polysaccharides density increased to 235 ± 110 mg/m^2. This increase was suspected as an accumulation of alginate from the previous cycles which was not been able to be removed from the membrane.

SEM pictures of new, fouled and cleaned membranes were taken to examine the amylase cleaning performance physically (Fig. 7). Fig. 7b showed fouled membrane which has been through rinsing step and revealed the foulant layer covering the membrane surface. Following one and three cycles of fouling and cleaning, membrane appears to be relatively clean and similar to the image of virgin membrane (Fig. 7a), although $R_{cleaned}$ value was four and five times R_m respectively. After 16 cycles of cleaning (Fig. 7e), foulant flocs were observed on the membrane surface. This picture supports the $R_{cleaned}$ data which was much higher than membrane with 1 and 3 cleaning cycles. Fig. 7e also supports the finding of residual foulant analysis, where polysaccharides was still found on the sample and protein amount was the highest compared to fouled membrane and membrane with one and three cleaning cycles.

Amylase did not seem to be an effective cleaning agent, since, with repeated usage, fouling occurred faster, membrane resistance increased high and more protein and polysaccharides was deposited compared to single cleaning cycle. Enzyme works by cutting the linkage of a specific bond. Sodium alginate is build with 1,4-α linkage while the amylase in this study is targeted for starch based polysaccharides which posses 1,4-β linkage. This difference may cause the enzyme not to work optimally [16] and the use of alginate lysase is recommended for future study. However, in real applications, the type of polysaccharides found on membrane surface is expected to cover a wide range of characteristics and a mixture of enzymatic cleaners would be recommended for optimum performances.

3.3. Sequential cleaning

As the model solution consists of two main substance, protein and polysaccharides, while enzyme works specifically, sequential cleaning was investigated. Sequential cleaning used both types of enzymes sequentially. Three combinations were examined; amylase/protease, protease/amylase and amylase/rinsing/protease. In this section, four fouling/cleaning cycles were imposed on each membrane. Sequential cleanings were expected to provide better cleaning results compared to single enzyme.

3.3.1. Cleaning performance

Fig. 8 shows the R_m and $R_{cleaned}$ values after each cleaning cycle for four cycles. Following the first cycle, hydraulic resistance increased approximately 5–6 times, before stabilizing during the remaining three cycles. From the three cleaning modes, the amylase/protease provided the best result. Final $R_{cleaned}$ after four cycles of fouling and cleaning was 32.8×10^{10} m^{-1} compared to $R_{cleaned}$ of 37.2×10^{10} m^{-1} for protease followed by amylase and 32.2×10^{10} m^{-1} for amylase followed by rinsing and protease.

Comparing the final $R_{cleaned}$ of the sequential cleaning and single enzyme, single enzyme was found to provide better result. With four cycles of cleaning by protease only, final $R_{cleaned}$ value was 36.3×10^{10} m^{-1}. This value is lower than the protease/amylase option, although higher than the other two sequential cleaning modes. Furthermore, four cycles of fouling and cleaning with amylase resulted in $R_{cleaned}$ of 21.8×10^{10} m^{-1} which was lower than all the sequential cleaning modes. This implies that amylase provided better cleaning performance without being combined with protease and rinsing process. Although resistance removal with amylase/rinsing/protease was slightly lower than amylase/protease, it was concluded that the rinsing step did not assist the cleaning process as expected.

As mentioned earlier, fouled membrane was found to contain 457 ± 14 mg/m^2 of protein and 281 ± 21 mg/m^2 of polysaccharides (Fig. 9). Cleaning with amylase/protease was able to removed 64% of the protein and 70% of the polysaccharides. When protease was applied first, protein removal was slightly higher (65%), but less polysaccharide were removed (62% removal). With amylase/rinsing/protease, 68% of protein and 68% of polysaccharides were removed. This slight difference in removal (along with the hydraulic results) was not enough to recommend a specific sequential cleaning protocol for this feed mixture.

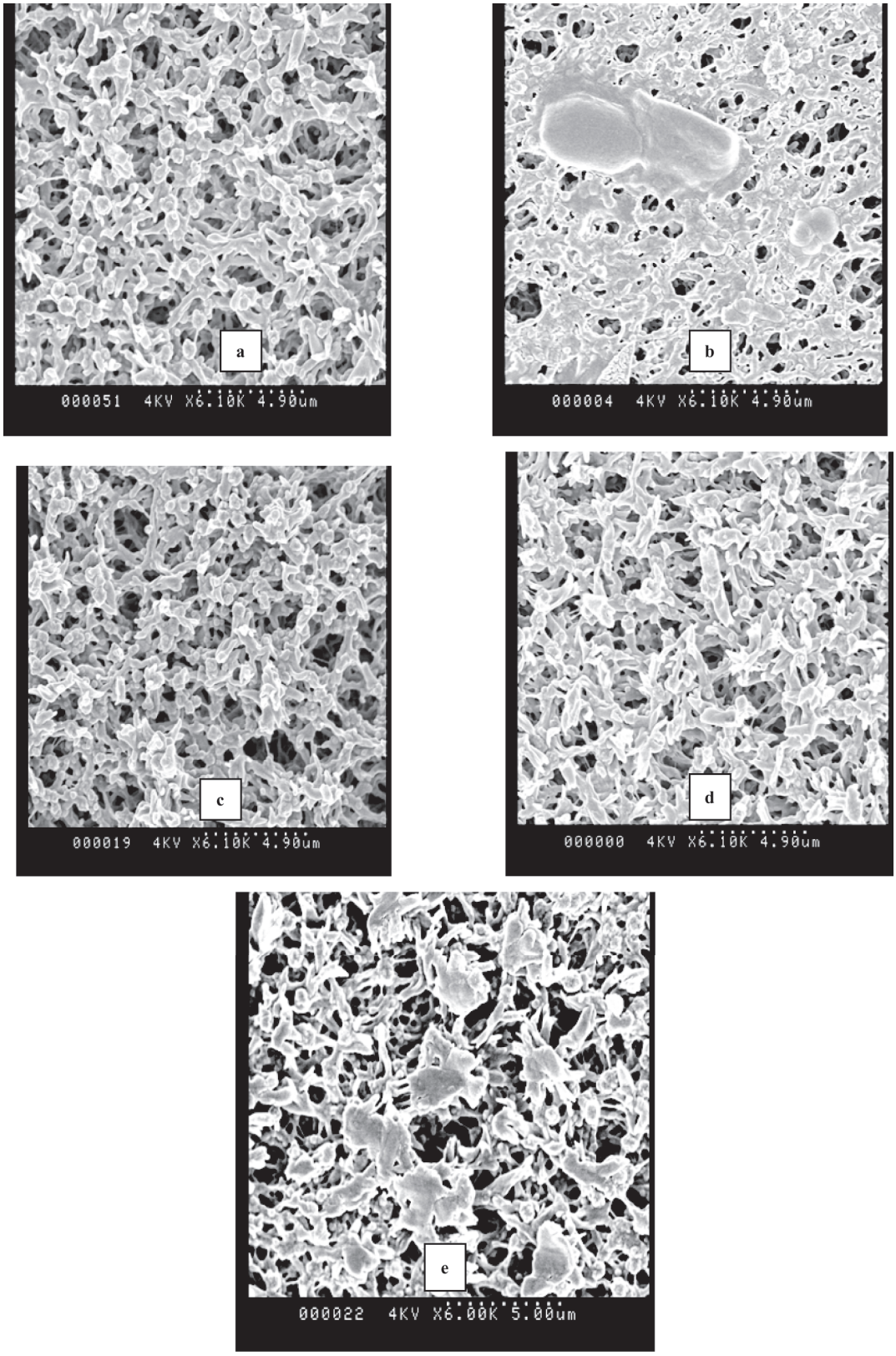

Fig. 7. SEM pictures of membranes (6000× enlargement): (a) virgin, (b) fouled and rinsed, and membranes with (c) 1 cycle, (d) 3 cycles, (e) 16 cycles of fouling/cleaning using amylase 0.05%.

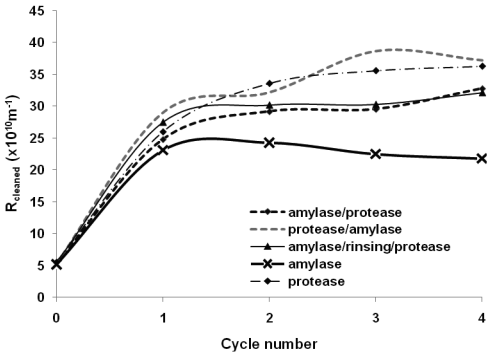

Fig. 8. Resistance evolution for membrane with various enzymatic sequential cleanings.

4. Conclusions

Within the operating conditions used in this study, the protease and amylase tested to clean the fouled membranes resulted in maximum cleaning efficiencies of 68 and 73% respectively, during optimized single cycles. The cleaning performances of the enzymes were further studied with repeated fouling/cleaning cycles (up to 16), at the end of which, fouling was found to occur four times faster than with single cycle. This rapid fouling was due to the residual foulant still attached on the membrane. This was supported by the SEM pictures which revealed large foulant flocs left on the membrane after few cycles, inducing faster fouling process on the consequent filtration runs. Analysis of the remaining foulant indicate that cleaning by protease was able to remove up to 60% of the protein deposited on the membrane during the first fouling/cleaning cycle. However, the relative concentration of protein on the membrane surface was found to increase in subsequent cycles. Similar observations were reported for cleaning by amylase, revealing the attachment of the enzymatic cleaners on the membrane. Water rinsing did not seem to be sufficient to remove all residual enzymes from the membrane surface after cleaning. The use of different sequential cleaning protocols, aimed to eliminate both foulants, did not produce the expected higher removal efficiencies.

Based on the results presented in this paper, amylase and protease did not provide overall, satisfying performances. With their environmental advantages over conventional chemical cleaners, further research is sought in order to better assess the performance of enzymatic compounds (in particular more specifically targeted enzymes) for the cleaning of microporous membranes in wastewater treatment.

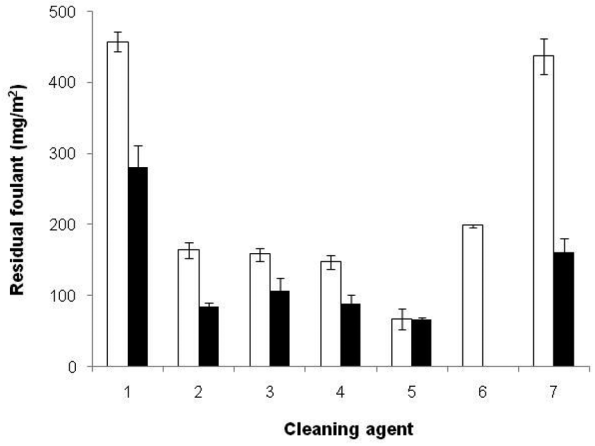

Fig. 9. Residual foulants on membrane with sequential cleaning (1. fouled membrane, 2. Amylase/protease, 3. protease/ amylase, 4. Amylase/rinsing/protease, 5. NaOCl 1%, 6. protease 0.1%, 7. amylase 0.05%).

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