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Using Free Nitrous Acid for Biofouling Removal and Control of Reverse Osmosis Membranes

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Abstract: The effects of FREE NITROUS ACID (FNA) with or without H_2O_2 on biofouling of REVERSE OSMOSIS (RO) membranes were investigated, five RO membranes with different degree of biofouling were cleaned using FNA solutions (10, 35 and 47 mg HNO2-N/L) at pH 2.0, 3.0 and 4.0 under cross-flowconditions for 24 hours.The cleaning tests demonstrated that FNA cleaning solutions were efficient at biomass removal and inactivation. At the optimum cleaning conditions (35 mg HNO2-N/L at pH 3.0), FNA has achieved higher biomass removal for both heavily fouled (86-96% versus 41-83%) and moderately fouled (92-95% against 89-92%) membranes, respectively. In accordance to the biomass removal, 6-32% of viable cells remained on the moderately fouled RO membranes under the impact of FNA cleaning (pH 3), whereas 38-58% of viable cells stayed on the heavily fouled RO membranes. Preservation trials were conducted with FNA concentrations of 0.1, 1, 3 and 10 mg HNO_2-N/L at pH 5.0 (adjusted with hydrochloric acid).The pH of the FNA-based preservation solution increased by 31% after storage, while nitrite concentration decreased by 20%. This indicates that denitrification occurred. After 6-month storage, FNA residual of 0.06 mg HNO_2-N/L remains in the solution.

Key words: FNA · Biofouling · Scaling · Biomass removal · Denitrification

INTRODUCTION

Due to freshwater scarcity across Egypt and the world, membrane technologies have gained enormous attention for water purification applications such as seawater desalination and wastewater recycling. Compare to micro, ultra and nano-filtration, reverse osmosis (RO) filtrations can achieve high rate of contaminant removal using low energy consumption [1].

Due to this reason, RO membrane filtration has been widely applied for water purification in recent years [2]. However, membrane fouling and specially biofouling are the major obstacles hindering the full potential of RO purification processes [3]. Biofouling is defined as the undesired development of microbial layers on RO membranes [4] and well known by its adverse effects to the membranes. Studies have reported biofouling is likely to cause the increase of energy and chemical costs, loss of water production and quality and eventually membrane deterioration [5, 6].

To restore the performances of RO membranes, chemical cleaning is regularly required.

Chemical cleanings involve alkali cleaning (i.e. sodium hydroxide) for organics and biofilmremoval and acid cleaning (i.e. hydrochloric acid, citric acid) for scaling removal. However, many studies have reported that biofouling cannot be removed effectively using the standard chemical cleaning method [7-9]. The application of chemical cleaning agents in large quantities has also caused significant operational costs and environmental issues for their disposal [10-12]

Free nitrous acid (FNA) has been reported to have a strong biocidal effect on sewer biofilm and waste activated sludge [13-17]. Studies have reported FNA potentially induce cell death and biofilm detachment at parts per million levels (0.2 mg HNO_2-N/L) and the biocidal effect of FNA was increased by 43–51% when FNA is combined with hydrogen peroxide (H_2O_2) [13, 14]. Based on these studies, it is anticipated that FNA not only can damage the structure of biofouling layers but also can inactivate the microbes in the biofilm formed on RO membranes. FNA as acid can hydrolyze organic constituents of biofouling layers such as proteins and polysaccharides [18], resulting a loose biofilm that may be

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susceptible to biocidal attracts. As a biocidal agent, inactivation of bacteria induced by FNA can inhibit the development or the regrowth of biofilm. Moreover, the synergistic biocidal effect of FNA and H_2O_2 has been well demonstrated on sewer biofilms and waste activated sludge [13-17], as afore mentioned, H_2O_2 is a strong oxidant agent that can also cause the death of bacteria.

Therefore, an alternative anti-fouling strategy using FNA with or without H_2O_2 for RO membranes was studied. Additionally, it is expected FNA as an acid could potentially removescaling from RO membranes. The descaling efficiency of FNA was also investigated.

The potential inactivation and cleaning effects of FNA on RO membrane biofilms and the application of FNA to replace the conventional two-stage cleaning strategy under cross-flow conditions have formed the motivation for the work in this research.

MATERIALS AND METHODS

The main objective of this study is to use FNA with or without H2O2 for RO membrane biofouling and scaling removal. The specific goals are:

- To design a cleaning protocol under cross-flow conditions at lab-scale.
- To determine the cleaning effects of FNA with or without H2O2 on different biofouling matrix.
- To reveal the optimal cleaning conditions for RO biofouling removal by studying the impacts of FNA concentration, pH and H_2O_2 concentration.
- To determine the descaling efficiency of FNA in comparison with standard acid cleaning solutions (i.e. hydrochloric acid and citric acid).
- To investigate the ability of FNA to prevent the accumulation of fouling on RO membranes at the bench-scale.

Membranes: Membrane autopsies have been done on five fouled RO membranes. All RO membranes are commercial thin-film composite polyamide membranes, which were collected from full-scaleplants (Table 1) and stored in the cold room at 4? until membrane autopsy took place. In membrane autopsy, biological fouled RO modules (RO1-5).

Sample Preparation: Foulant samples were collected in two ways: (1) the *in situ* method, which membrane coupons with foulant attachments were cut directly from RO modules, (2) the destructive extraction methods, which foulant was physically scraped or brushed off the membranes [20].

Size of membrane coupons and extra preparation procedures are varying depending on the limit of detection of each analysis. For microscopy based analyses, *in situ* biofilm samples on RO membrane coupons were prepared.

In order to conduct comprehensive membrane autopsy, five biomass samples were collected from different location of RO modules for each analysis.

Cleaning Set-Up and Operation: Cleaning trials were carried out at lab-scale under dynamic (with cross-flow recirculation) conditions. Five cleaning cells made of Perspex were operated in parallel with cross-flow, without permeate production, as they were designed to simulate the configuration of RO filtration system only (Figure 1). In a test, membrane coupons (150 cm^2 of membrane active surface) cut out from a fouled membrane and the respective feed spacers were placed in the cleaning cells. All cleaning cells were connected to the same pump (Cole Parmer, Master flex L/S economy drive pump), this pump was assembled with five pump heads allowing cleaning cells to run in parallel with similar flows. In order to simulate industry cleaning practice, a cross flow velocity of 0.1 m/s was applied for the cleaning trials [21]. To conduct a cleaning test, the following operational procedure was applied:

- Tap water was pumped through the cell for 2 hours to remove the loose, external biofilm layer that is removable using shear force. The external layer of the marine biofilm on RO5 could further be affected by tap water due to osmotic shock.

Table 1: RO membranes used in this study.

Membrane #	Source	Fouling Type	Membrane 1Autopsy Date
RO1	Industrial wastewater recycling plant	Biofouling	2013/10/16
RO2		Biofouling	
RO3	Water reclamation plant	Biofouling	
RO4	Water reclamation plant	Biofouling	
RO5	Seawater desalination plant	Biofouling	

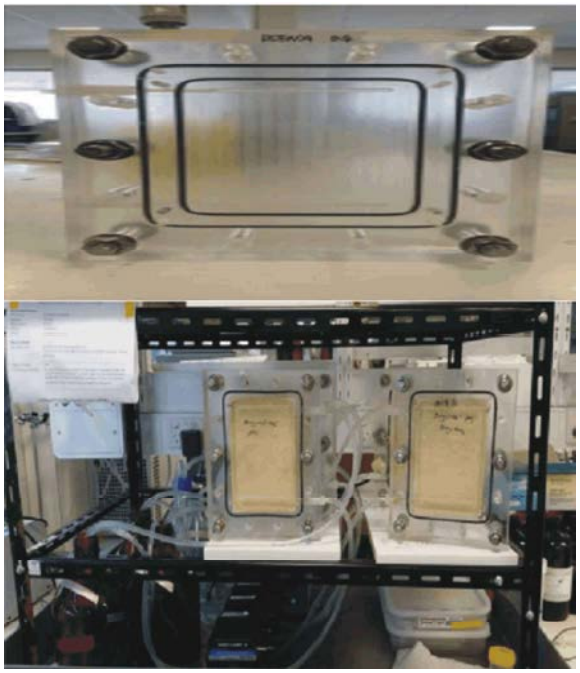

Fig. 1: The cleaning cell (up) and the entire cleaning system with five cleaning cells in parallel, fed with a single peristaltic pump with five pump heads.

- A cleaning solution was pumped through the cell for 22 hours;
- Tap water was pumped through for 15 minutes to remove chemicals.

Design of Cleaning Tests: Based on the preliminary results of static cleaning tests (data not shown), the nitrite concentration used in the dynamic tests was 50 mgNO2 --N/L. Sodium nitrite (≤99%, Sigma Aldrich) and hydrochloric acid, HCl (32%, Univar) were used to prepare the FNA solution at different pH level. The concentration of FNA is related to the total nitrite concentration, the pH and the temperature and is calculated based on the following equation which is extracted from [**22**]:

$$FNA = NO2 --N / (Ka \times 10pH),$$

where Ka is the ionization constant of the nitrous acid (Ka = e-2300/(T+273)) and T is the temperature (°C). In this study, the FNA concentration was varied by adjusting the pH level of cleaning solutions. FNA cleaning solutions at concentrations of 47, 35 and 10 mg HNO2-N/L were prepared by acidifying the nitrite solution (50 mg NO_2 --N/L) to pH 2, 3 and 4, respectively (T=20°C). The cleaning efficiency of FNA was compared with that of sodium hydroxide solution (NaOH, Univar) at pH 11 and deionised (DI) water, as controls. Although DOW and LENNTECH (RO membrane manufacturers) indicate that caustic solutions at pH level higher than pH 11 are more effective at biofouling cleaning, it is generally not recommended to use such harsh cleaning solutions since NaOH at pH 11.5 can shorten the membrane life due to hydrolysis [23, 24]. Hence, NaOH at pH 11 was selected to be the control solution in this paper.

Post-Cleaning Analyses

Post-Cleaning Analysis for Biofouling Cleaning Tests: The characteristics of the biofouling layer after different cleaning tests were determined using following methods.

- Biomass (ATP) measurement.
- Live/dead cells staining method.
- FISH analysis.
- Protein and polysaccharides measurement
- Hydraulic performances of RO membranes.

Based on the biofouling conditions before cleaning, the percentage of biomass residuals and removal percentage of protein and polysaccharides under the impact of different cleaning agents were obtained based on following equations:

Biomass residuals (%) = ATP (pg/[cm²]) of biofilm after cleaning / ATP (pg/cm²) of biofilm before cleaning×100

Protein removal (%) = (1-BSA/m2 of biofilm after cleaning / BSA/m2 of biofilm before cleaning) ×100

Polysaccharides removal (%) = (1- Glucose/m2 of biofilm after cleaning / Glucose/m^2 of biofilm before cleaning) ×100

From the FISH analysis, the distribution and relative abundance of major groups of proteo-bacteria (*Alpha-* and *Beta-proteobacteria*) and archaea were determined and compared with the biofilm communities before cleaning. The detail procedure and oligonucleotide probes which were applied for FISH analysis. The percentage of each microbe was estimated using relative abundance of the microbe (i.e. *Alphaproteobacteria*) over that of total targeted microbes (bacteria + archaea).

Live/dead Cell Staining: The microbial viability of biofilm was determined using the LIVE/DEAD® BacLight$_{TM}$

Bacterial Viability Kits (Molecular Probes, L-7012). The viability kits contains with two nucleic acid stains, the green-fluorescent SYTO-9 labels viable cells, whereas

red-fluorescent Propidium iodide (PI) stains dead cells [15]. 2×2 cm^2 membrane coupons (n=5) with *in situ* biofilm attachment were cut and then submerged into 1 ml MilliQ water in the centrifuge tubes (1 membrane coupon per tube). After mixing 1 uL of each SYTO-9 and PI stains in the tubes, samples were incubated for 25 mins under dark condition. The stained membranes were air dried first, then mounted onto a glass slide and observed under the Zeiss 510 Confocal Laser Scanning Microscopy (CLSM) (School of Chemistry and Molecular Biosciences at UQ). Two excitation/emission wavelengths were used for the two fluorescent stains: 488 nm/500 nm for SYTO-9 and 510 nm/635 nm for PI. CLSM images (n=15-60) were randomly taken from each sample. CLSM images were used to demonstrate the distribution and abundance of live and dead cells in the biofilm before and after the cleaning tests. DAIME (Center for Organismal Systems Biology, Austria) was applied to estimate the relative abundance of live and dead cells by counting green and red pixels respectively. The proportion of viable cell was obtained using relative abundance of viable cells (green pixels) over that of total cells (red + green pixels).

Post-cleaning Analysis for Descaling Tests: From membrane autopsy, the concentration (21.7±3.8 g Ca/m^2) of calcium carbonate scale was determined. Given the size of membrane used in the cleaning tests, the concentration of calcium carbonate scale that was removed can be estimated by measuring the calcium contents in the cleaning solutions via ICP-OES. The descaling efficiency of different cleaning solutions can be determined by comparing the calcium contents in the cleaning solution with membrane autopsy result. SEM-EDS analysis was also performed on the membrane coupons and its results can be used to justify the ICP-OES results. The characteristics of the biofouling layer after different cleaning tests were determined using following methods.

Table 2: Design of the biofouling control tests.

		Cell #1	Cell #2	Cell #3	Cell #4	Cell #5
RO1	Test 1	47 mg FNA-N/L, pH 2.0	35 mg FNA- /L, pH 3.0	10 mg FNA- /L, pH 4.0	NaOH, pH 11.0	DI water
	Test 2					
	Test 3					
RO2	Test 4		35 mg FNA- /L, pH 3.0		NaOH, pH 11.0	DI water
	Test 5	35 mg FNA-N/L, pH 3.0	35 mg FNA- /L, pH 3.0; 50 mg/L H_2O_2	35 mgFNA- N/L, pH 3.0; 150 mg/L H_2O_2		
RO3	Test 6	35 mg FNA-N/L, pH 3.0	50 mg/L H2O2	35 mgFNA-N/L, pH 3.0; 50 mg/L H_2O_2	NaOH, pH 11.0	DI water
	Test 7					
RO4	Test 8	47 mg FNA-N/L, pH 2.0	35 mg FNA-N/L, pH 3.0	10 mg FNA-N/L, pH 4.0	NaOH, pH 11.0	DI water
	Test 9		35 mg FNA-N/L, pH 3.0			
	Test 10	35 mg FNA-N/L, pH 3.0	35 mg FNA-N/L, pH 3.0	35 mgFNA-N/L, pH 3.0; 50 mg/L H_2O_2	NaOH, pH 11.0	
RO5	Test 11	47 mg FNA-N/L, pH 2.0	35 mg FNA- N/L, pH 3.0	10 mg FNA-N/L, pH 4.0	NaOH, pH 11.0	DI water
	Test 12					

The replication of the cleaning tests (cleaning solutions) was summarized in the following table.

Table 3: The repetition of the cleaning tests

Cleaning Solutions	47 mg FNA N/L, pH 2.0	35 mg FNA N/L, pH 3.0	10mg FNA-N/L, pH 4.0	35mg FNA -N/L, pH 3.0; 50 mg/L H_2O_2	35 mg FNA N/L, pH 3.0; 150 mg/L H_2O_2	50 mg/L H_2O_2	NaOH, pH 11.0	DI water
RO1	n=3	n=3	n=3				n=3	n=3
RO2		n=2		n=1	n=1		n=2	n=2
RO3		n=2		n=2		n=2	n=2	n=2
RO4	n=1	n=3	n=1	n=1		n=1	n=3	n=3
RO5	n=2	n=2	n=2				n=2	n=2

RESULTS AND DISCUSSION

Effects of FNA Cleaning on RO Biofouling under Cross-Flow Conditions

Effects of FNA Cleaning on Active Biomass (ATP): ATP was used to quantify the biomass contents within biofouling layers after cleaning tests. Figure 2 shows the biomass residual in percentage of the pre-cleaning level for five different fouled RO membranes after the cleaning tests. The percentage of biomass residual was estimated using ATP measurements after cleaning tests over initial ATP contents of biofilm, which quantitatively demonstrates biomass residual under impacts of different cleaning solutions.

For all RO membranes tested in the cleaning tests, the FNA cleaning solutions were more efficient than conventional cleaning solution, namely NaOH (pH11), for removing biomass.

Acidified nitrite cleaning solutions removed 7-45% more biomass than NaOH for heavily fouled membranes, RO1-3. For moderately fouled membranes, RO4 and RO5, 3-5% and 2-3% more biomass were removed by nitrite

cleaning solution at low pH level for RO4 and RO5 respectively. Although the RO2 biofilm contained the highest biomass load (4234±15423 pgATP/cm2), FNA solution at pH 3 has achieved the best cleaning efficiency for RO2 among heavily fouled membranes. Hijnen *et al*. [25] reported the resistance of biofilm against cleaning varies based on the structure of biofilm. It is likely that the natural structure of biofilm formed on RO2 has created less resistance to FNA cleaning solution. [25] also applies to cleaning tests for moderately fouled membranes. The biomass residual results have shown FNA cleaning was more efficient for moderately fouled membranes, implying that cleaning efficiency of cleaning agents is influenced by the degree of fouling.

The results showed all cleaning solutions were more efficient for cleaning moderately fouled membranes than heavily fouled membranes, suggesting cross-flow conditions have created great effects on the loose biofilm layers. It is likely that cross-flow conditions helped to facilitate the diffusion of cleaning solutions into biofouling layers, detach bacterial cells and flushed them away [26]. However, it is evident that biomass removal was not mainly caused by hydrodynamic shear, since removal caused by FNA cleaning solutions is significantly higher compared to removal with DI water only. The results have suggested that applying cross-flow enhances the cleaning efficiency of FNA.

Figure 2 (a) also demonstrated the effect of pH level on the cleaning efficiency of nitrite cleaning solutions. For heavily fouled membrane RO1, the best cleaning efficiency of nitrite cleaning solutions is observed at pH 3. Based on the

three cleaning tests, more than 86% of biomass was removed by nitrite cleaning solutions at pH 3. The concentration of FNA is inversely correlated to the pH level of nitrite solutions. The results have proved higher FNA concentration at pH 3 has resulted in better cleaning efficiency. However, there was no obvious further improvement to biomass removal for nitrite solutions at pH 2, likely due to the loss of nitrite concentration induced by the conversion of nitrite to nitrate [27]. Hence, 50 mg NO_2-N/L at pH 3.0 as optimum conditions were used to clean RO2 and RO3.

For moderately fouled membranes, there was no obvious difference between the cleaning efficiency of nitrite cleaning solutions at different pH levels. The results have suggested that lower concentration of nitrite can be applied to clean moderately fouled membranes.

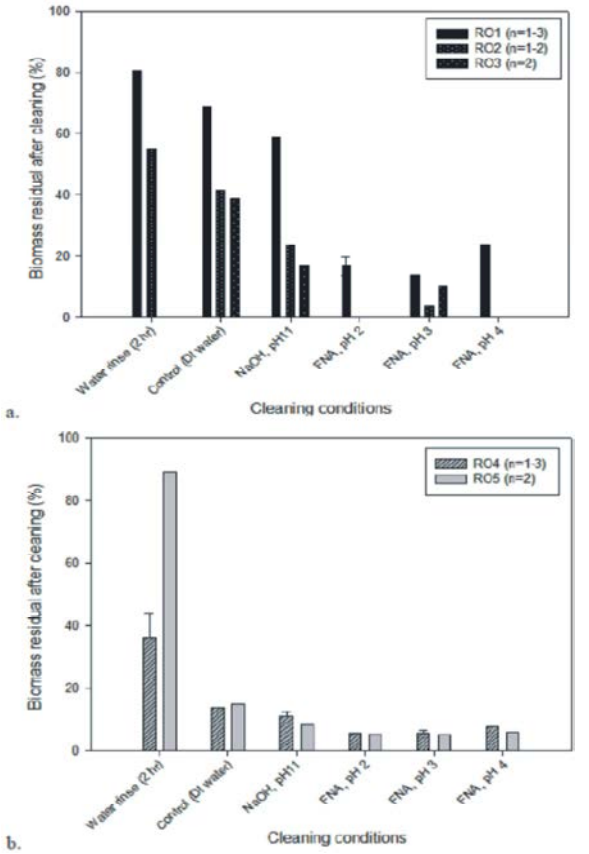

Fig. 2: Biomass residual after 24 hours cleaning tests performed in cross-flow conditions with the membranes (a) heavily fouled RO1, RO2 and RO3 and (b) moderately fouled RO4 and RO5. The cross-flow velocity applied was 0.1 m/s. The error bars show the standard errors of three replicate experiments. The results without error bars are based on three measurements from each experiment.

Influence of FNA on Viability of Biomass: The biocidal effect of FNA has already been demonstrated on anaerobic sewer biofilm and waste activated sludge applications [15, 28]. Thus, it is anticipated FNA would affect biofilm formed on RO membranes in a similar way. The potential inhibition of FNA was assessed by measuring live and dead bacterial cells after cleaning tests. The CLSM image analysis was carried out directly on biofilm on the RO membranes. Due to the density and thickness of the biofilm on the heavily fouled membranes (RO1 to RO3), it was difficult to reveal the accurate quantification of live and dead cells. CLSM imaging could not give quantitative results due to the density of biofilm

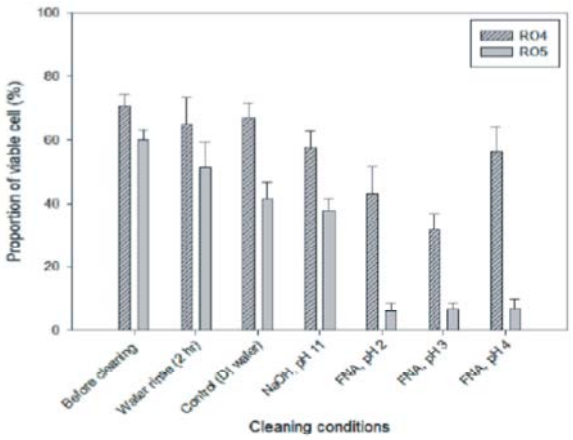

Fig. 3: Proportion of viable cells in membrane biofilm before and after 24 hours cleaning tests for membranes RO4 and RO5. A cross-flow velocity of 0.1 m/s was applied. The error bars show the standard errors of 15 to 60 CLSM images.

changing from location to location on the membrane [29]. Therefore, live and dead analysis did not appear to be applicable to heavily fouled biofilm.

In situ CLSM observation and image analysis could be performed on the moderately fouled membranes (RO4&5) due to lower biofilm density. The CLSM images of RO4&5 before and after cleaning are given in Appendix Figures C-1 and C-2. Comparing to the biomass before cleaning, 13-39% and 22-54% of viable cells were inactivated and removed by chemical cleaning solutions for RO4 and RO5, respectively (Figure 3). The trends of viable cell revealed the best inactivation effect of nitrite cleaning solutions is at pH 3 for RO4 and there was no distinct difference between the inactivation effects of nitrite cleaning solutions at different pH levels on RO5 biofilm.

In accordance with the biomass residual measured as ATP, the inactivation efficiency of FNA is better than NaOH (pH 11). 32±5% of total cells remained activated in the biofilm on RO4 under the impact of FNA at pH 3, which is 26% less than that under impact of NaOH. For RO5, the biofilm contained only 6-7% of viable cells after cleaning with nitrite solutions, whereas 38±4% of viable cells remained in the biofilm which was cleaned by NaOH. CLSM images (n=15-45) also explain the more superior inactivation effect of FNA solutions on RO5 biofilms in comparison to that on RO4. CLSM images revealed that the biofilm formed on RO4 was denser than biofilm on RO5. It appears the denser biofilm on RO4 creates resistance to the hydrodynamic flow, which then reduces the interaction between cleaning chemicals and biofilm, hence hinders the cleaning efficiency. The results of live/dead cells staining confirm that the bactericidal efficiency of FNA on bacteria formed on RO membranes. The results additionallydemonstrated FNA is more efficient than NaOH in killing bacteria.

Biofilm Community Structure Changes after FNA Cleaning: The selected FISH probes have revealed that more than 60% of biofilm communities on RO1 and RO5 were *Alphaproteobacteria,Betaproteobacteria, Gammaproteobacteria* and *archaea*, so the impacts of FNA on these microbes were further investigated using the same probes. The relative quantity of each detected bacterium was determined by DAIME based on FISH images.

FISH results showed that the total amount of detected bacteria has been dropped after the cleaning tests. Figure 4 (a) and (b) show targeted bacteria in the biofilm of RO1 decreased 70% after FNA cleaning at its optimal condition (pH 3). Figure 4 (b) and (d) indicate that 38% of total detected bacteria of RO5 biofilm were removed under the same treatment. This result also revealed that the microbe communities of RO1 were more susceptible to the impact of FNAcleaning than that of RO5. The rise of *Alphaproteobacteria* in the population of RO5 biofilm is likely due to the drastic removal of *Betaproteobacteria* and archaea, the relative quantity of all bacteria detected and *Alphaproteobacteria* has been reduced under the impact of FNA cleaning. FISH analysis results show that *Betaproteobacteria* and archaea were greatly affected by FNA cleaning for both membranes. However, the FNA effects were less consistent on *Alphaproteobacteria*. Similar phenomenon has been reported by Bereschenko *et al.* [30], the weekly chemical cleaning (30% sodium bisulfite solutions and mixed acid detergent descaler) has more impact on *Beta-* and *Gamma-proteobacteria* than *Alpha-proteobacteria* on the membranes after 3-6 month operation. Due to limited availability of biofilm residues after cleaning, limited oligonucleotide probes were chosen for FISH analysis. Large proportion of bacteria was not detected by the selected probes, especially for RO5, suggested that the results of FISH analysis are not comprehensive for the purpose of this paper.

Effect of FNA on Protein and Polysaccharide Removal: In addition to biomass analyses, the impact of FNA and other cleaning solutions on

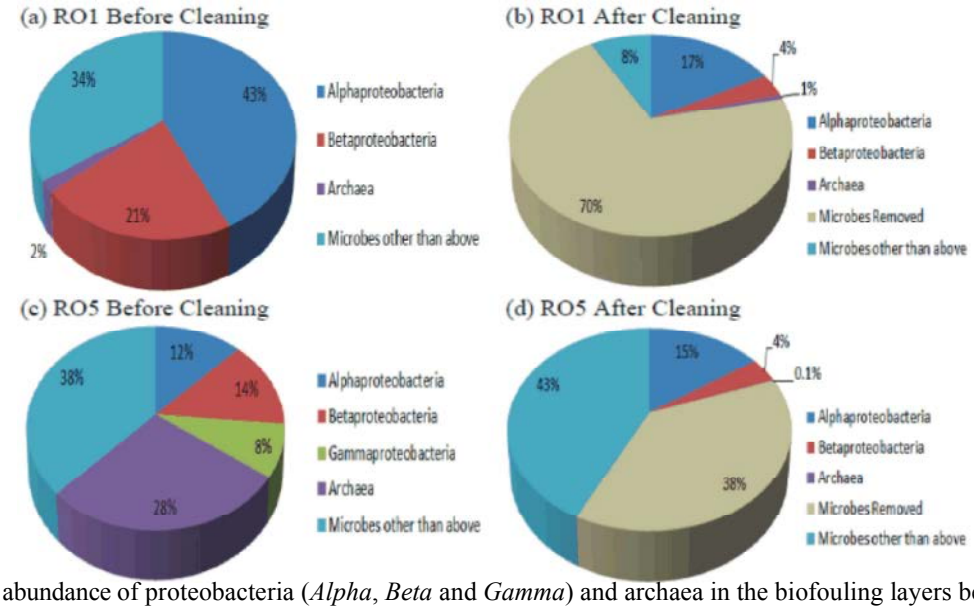

Fig. 4: The abundance of proteobacteria (*Alpha*, *Beta* and *Gamma*) and archaea in the biofouling layers before and after 24 hours cleaning tests for the membranes RO1 and RO5, respectively. Standard test conditions: FNA (50 mgN-NO2/L), pH 3, cross-flow velocity 0.1 m/s.
The abundances of each microbe were calculated based on the FISH images (n=20±5).

Table 4: Comparison of FNA and NaOH cleaning effects on proteins and polysaccharides.

Cleaning Agents	FNA		NaOH	
Membran #	RO4	RO5	RO4	RO5
Protein removal rate (%)	93.1±3.4	67.1±2.5	60.3±14.9	59.16
Polysaccharides removal rate (%)	72.9±2.0	86.5±1.8	61.7±7.5	79.36

The deviation ranges show the standard errors of the FNA cleaning solutions (at pH 2.0, 3.0 and 4.0)

the removal of the organic constituents of biofilm was studied for moderately fouled membranes (RO4&5).

This impact was reflected by evaluating the removal of proteins and polysaccharides by the cleaning. By comparing the average removal efficiency of proteins and polysaccharides achieved by the FNA cleaning solutions with that by NaOH (Table 4), FNA cleaning solutions have shown similar or better efficiency for proteins and polysaccharides removal. The removal of proteins and polysaccharides was likely due to the interaction between FNA or its reactive nitrogen species (RNS) and biofilm, as FNA is able to damage chemical bond and structure of EPS in waste activated sludge [19]. Based on results obtained from all analyses, FNA can be a substitute cleaning chemical for NaOH.

The comparison of the effects of all cleaning solutions on biomass, protein and polysaccharides is given in Figure 4-5. Figure 5 (b) shows the removal rate for biomass and polysaccharides and proteins presented a similar trend. However, FNA cleaning solution was still more efficient on biomass removal (94-95%) than on protein and polysaccharides removal, respectively (Table 4). Many studies have reported that bacteria are more susceptible than EPS to the cleaning chemicals, in which polysaccharides and proteins have been used as proxy of EPS [25, 29, 30]. [25, 30] revealed that the biofilm matrix consisting of polysaccharides and proteins creates greater resistance to the cleaning attack, though the bacteria cells are removed during the cleaning processes.

Synergistic cleaning effects of FNA and H_2O_2 on RO Membrane Biofouling: [17] reported adding H_2O_2 can enhance the biocidal efficiency of FNA by 43-51%compared with FNA alone. Hence, the synergistic cleaning effects of FNA and H_2O_2 on RO biofouling were studied and compared with effects of FNA and H2O2 individually, as shown in Figure 6. For both heavily fouled RO2 and moderately fouled RO4, minimal enhancement(less than 1%) was obtained after adding H2O2 to FNA. Increasing the concentration of H_2O_2 to150 mg/L showed no improvement in cleaning for RO2. However, FNA and FNA/H_2O_2 are stillmore efficient than NaOH on biomass (ATP) removal for all three RO membranes. Given thatthe cleaning efficiency of H2O2 alone is lower than FNA or the combination of FNA and H2O2,this implies that FNA is still the main cleaning agent for the biofouling removal.

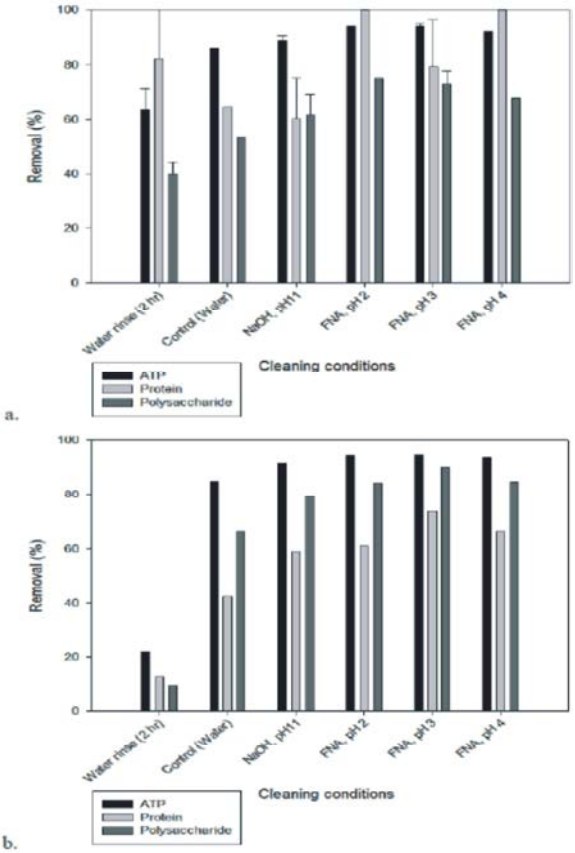

Fig. 5: Biomass removal (%, based on ATP values), protein and polysaccharide removal (%) after 24 hours cleaning tests for the membranes (a) RO4 and (b) RO5. A cross-flow velocity 0.1 m/s was applied. The error bars in the plot show the standard errors of 2-3 replicate experiments.

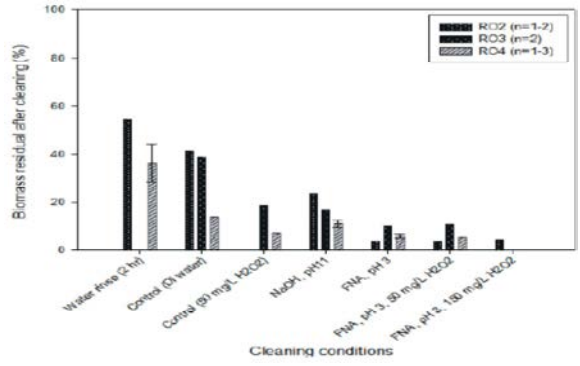

Fig. 6: Biomass removal after 24 hours cleaning tests for the membranes heavily fouled RO2 and RO3 and moderately fouled RO4. A cross-flow velocity 0.1 m/s was applied. The error bars show the standard errors of three replicate experiments. The results without error bars are based on three measurements from each experiment.

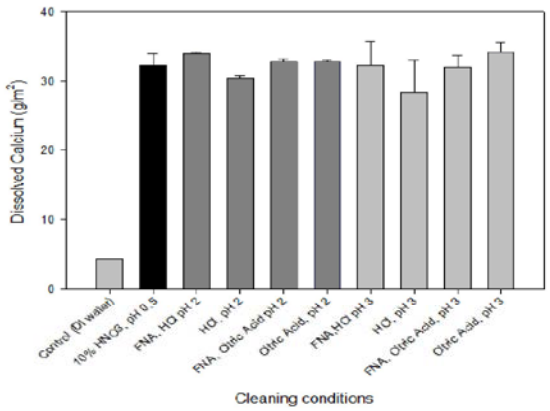

Fig. 7: Dissolved calcium content removed from the membrane surface after 24 hours cleaning tests with membrane RO6. A cross-flow velocity 0.1 m/s was applied in all tests. The error bars show the standard errors of four measurements from two replicate experiments.

Descaling Efficiency of FNA: Since the FNA cleaning solutions are formed by combining nitrite with acid, it is expected that FNA cleaning solutions would act like acid to remove scaling from membrane surfaces. The same cleaning procedures used for biofouling were carried out to remove scaling from RO membrane (RO6). After 24 hour cleaning tests, the descaling efficacy of FNA and the other cleaning solutions (as controls) were assessed by evaluating the dissolved inorganic elements in the cleaning solutions. Fig 7 presents the dissolved calcium content based on ICP-OES results. 28.5±4.6 to 34.3±1.4 g/cm^2 calcium was removed by all acidic solutions, which suggests that all cleaning solutions are efficient at calcium scaling removal. The results suggested that nitrite has no influence on the descaling efficiency of acidic solutions, since the descaling efficient of FNA at pH 2 and 3 (either adjusted by HCl or citric acid) was similar to conventional descaling agents (HCl and citric acid). In accordance to the cleaning tests for biofouling removal, it was proven again that hydrodynamic shear is beneficial for the cleaning tests as it improves the solubility of the fouling layer. HNO_3 (10 v/v % at pH 0.5) the control cleaning solution successfully removed 32.4±1.7 g/cm2 versus the autopsy results 21.7±3.8 g/cm^2.

The calcium removal rate is higher in cross-flow conditions. However, scaling removal was not mainly caused by hydrodynamic shear, since only 4.4±0.1 g/cm^{2}of calcium was removed from the membrane surface by water washing.

Table 5: Hydraulic performances of membranes after cleaning tests.

	Membrane autopsy	Cleaning Tests					
Membrane #	Permeability (L/m2.h.bar, 25°C)	Water Rinse (2hr)	Water	NaOH pH 11	FNA pH 2	FNA pH 3	FNA pH 4
RO1	4.1			1.3		3.7	
			4.7	2.9		4.8	4.6
RO2	4.3	4.0	3.8	4.7		4.3	
		4.0	4.2	4.2		3.9	
RO3	2.3	2.2	1.9	2.2		2.2	
			3.6	3.6	3.4	3.7	3.2
RO4	3.6			4		2.7	
				2.9		3.7	
RO5	2.5			2.7	2.7	2.8	2.8
						2.6	2.6
	Rejection (%)	Water Rinse (2hr)	Water	NaOH pH 11	FNA pH 2	FNA pH 3	FNA pH 4
RO1	95.1	95.4		95.9		95.1	
		92	95.4	96.5		96.1	95.6
RO2		98.6	95.5	96.4		97.5	
			96.3	95.9		97.6	
RO3	98.1		98.5	98.7		98.2	
			97.3	98.8	99.4	96.3	99.1
RO4	98.4			99.0		98.0	
			98.1	98.5		98.3	
RO5	99.0		99.2	98.9	99.3	99.4	98.8
						98.1	98.7

In addition, SEM-EDS analyses were conducted on the membrane after cleaning for nitric acid and all cleaning solutions at pH 3, which is also the optimal pH level selected for biofouling removal. SEM images and element distribution (wt %) are available.

In agreement with the ICP-OES results (Fig 7), the SEM-EDS results revealed that the calcium contents of scaling layers reduced from 24 to 1% under impacts of all cleaning solutions adjusting pH to 3, implying that calcium carbonate scaling has been effectively removed. This result is supported by SEM images of calcium carbonate scaled RO membrane before and after cleaning. SEM images revealed that the crystal structure of calcium carbonate scaling was removed by all cleaning solutions at pH 3. Overall, the results of the cleaning tests for biofouling and scaling removal suggested that FNA can be used as a single cleaning agent for both biofouling and scaling removal.

Hydraulic Performances of RO Membranes after Cleaning: As result of effective cleaning for RO membrane, it is anticipated that permeability would be improved and salt rejection would be increased. However, based on the filtration results, there was no significant difference in permeability and salt rejection for all tested RO membranes after cleaning. According to RO membrane manufacturers, the permeability of RO module is normally ±15–20% of its nominal rate due to membrane manufacturing and experimental error [31]. Since all permeability changes remained in the range of this deviation (Table 5), no conclusive statement could be made based on the hydraulic results for this study. In addition, fouling is likely non-uniformly spread out on membranes surface. The small coupons (active area 42 cm^2) used in the filtration trials do

CONCLUSION

Five biofouled membranes (RO1-5) were used in 12 dynamic cleaning tests in which 0.1 m/L cross-flow was applied. The cleaning tests have shown that FNA solutions are more efficient at biofouling cleaning than NaOH (pH 11) and 35 mgFNA-N/L at pH 3.0 is the optimum cleaning conditions in this study, based on the results of biomass, protein and polysaccharides analysis and live/dead cells staining.

The ATP results showed that all nitrite cleaning solutions (pH 2.0, 3.0 and 4.0) removed 7-45% more biomass than NaOH for heavily fouled membranes, RO1-3.

Although the superior cleaning efficiency of nitrite cleaning solutions was not obvious for moderately fouled membranes, there was still 3-5 and 2-3% more biomass removed by nitrite cleaning solutions than NaOH for RO4 and RO5, respectively. This result suggested that all chemical cleaning solutions are more efficient for cleaning moderately fouled membranes than heavily fouled membranes, under cross-flow cleaning conditions.

Live/dead cells, protein and polysaccharides measurements were able to be performed on moderately fouled membranes (RO4&5), due to their less dense biofilm structure. In accordance to ATP results, live/dead cells staining has revealed that less viable cells remain in biofouling layers after FNA cleaning at pH 3.0 (32±5% for RO4 and 7±2% for RO5) than NaOH (57±5% for RO4 and 38±4% for RO5). The removal rate (%) of protein and polysaccharides showed a similar trend as the results of ATP and CLSM analysis. However, FNA cleaning appeared more efficient for biomass removal.

FISH analysis has demonstrated that the overall abundances of targeted bacteria on RO1 and RO5 have been reduced under the impact of FNA cleaning at pH 3.0. The results of FISH analysis also revealed that *Betaproteobacteria* and archaea were greatly affected by FNA cleaning than *Alphaproteobacteria*.

Although applying FNA alone, or combine FNA and H_2O_2 have shown better efficient at biofouling removal than NaOH, the percentage of biomass residual showed combining FNA with

H_2O_2 was not able to improve the cleaning/removal efficiency of FNA significantly (less than 1% of enhancement).

The scaling cleaning tests revealed that FNA solutions at pH 2.0 and 3.0 were as efficient as conventional descaling acids based on elemental analyses (ICP-OES and SEM-EDS). The results of both analyses showed that the scaling layers were effectively cleaned by all acidic cleaning solutions. Based on the outcomes of biofouling and scaling cleaning tests, FNA has showed its better at both biofouling and scaling cleaning. Hence, FNA can be a promising cleaning agent to achieve biofouling and scaling removal at a single stage.

For all cleaning tests, performances of tested membrane coupons were examined in terms of permeability and salt rejection. No significant difference was observed for all membranes, which was likely due to the small size of filtration cells used in this research.

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