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# Enhanced biofilm solubilization by urea in reverse osmosis membrane systems

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ABSTRACT

Chemical cleaning is routinely performed in reverse osmosis (RO) plants for the regeneration of RO membranes that suffer from biofouling problems. The potential of urea as a chaotropic agent to enhance the solubilization of biofilm proteins has been reported briefly in the literature. In this paper the efficiency of urea cleaning for RO membrane systems has been compared to conventionally applied acid/alkali treatment. Preliminary assessment confirmed that urea did not damage the RO polyamide membranes and that the membrane cleaning efficiency increased with increasing concentrations of urea and temperature. Accelerated biofilm formation was carried out in membrane fouling simulators which were subsequently cleaned with (i) 0.01M sodium hydroxide (NaOH) and 0.1M hydrochloric acid (HCl) (typically applied in industry), (ii) urea ($CO(NH_2)_2$) and hydrochloric acid, or (iii) urea only (1340 g/$L_{water}$). The pressure drop over the flow channel was used to evaluate the efficiency of the applied chemical cleanings. Biomass removal was evaluated by measuring chemical oxygen demand (COD), adenosine triphosphate (ATP), protein, and carbohydrate content from the membrane and spacer surfaces after cleaning. In addition to protein and carbohydrate quantification of the extracellular polymeric substances (EPS), fluorescence excitation–emission matrix (FEEM) spectroscopy was used to distinguish the difference in organic matter of the remaining biomass to assess biofilm solubilization efficacy of the different cleaning agents. Results indicated that two-stage $CO(NH_2)_2$/HCl cleaning was as effective as cleaning with NaOH/HCl in terms of restoring the feed channel pressure drop (>70% pressure drop decrease). One-stage cleaning with urea only was not as effective indicating the importance of the second-stage low pH acid cleaning in weakening the biofilm matrix. All three chemical cleaning protocols were equally effective in reducing the concentration of predominant EPS components protein and carbohydrate (>50% reduction in concentrations). However, urea-based cleaning strategies were more effective in solubilizing protein-like matter and tyrosine-containing proteins. Furthermore, ATP measurements showed that biomass inactivation was up to two-fold greater after treatment with urea-based chemical cleanings compared to the conventional acid/alkali treatment. The applicability of urea as an alternative, economical, eco-friendly and effective chemical cleaning agent for the control of biological fouling was successfully demonstrated.



## 1. Introduction

Biofouling is an unavoidable problem in RO membrane treatment processes. Biofilm formation is the result of unwanted growth and adhesion of micro-organisms on a membrane and feed spacer surface that excrete extracellular polymeric substances (EPS) (Flemming, 1997). Biofouling can be defined as the formation of biofilm that results in operational problems (Characklis and Marshall, 1990) such as an increase in the normalized pressure drop by 15% or a decrease in the normalized flux by 10% of the start-up values (DOW, 2014; Hydranautics, 2001; SUEZ, 2009). Biofouling

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imposes a large economic burden on RO plants (>30% of total operating costs), due to membrane cleaning, labor and pretreatment costs, increased energy demand, and early membrane replacement (Flemming, 2011). Effective control of biofouling is important to maintain membrane performance, extend membrane life span, and reduce operating costs.

The periodic application of chemical cleaning agents is routinely carried out to control biofouling in RO membranes. Chemical cleaning using combinations of acidic and alkaline chemicals is commonly applied for the regeneration of membranes (Madaeni and Samieirad, 2010; Yu et al., 2017). The cleaning chemicals disrupt the biofilm structure through several mechanisms, by denaturing the macromolecules such as polypeptides and polysaccharides. Alkaline solutions such as sodium hydroxide (NaOH) remove organic fouling and biofouling by hydrolysis and solubilization; whereas acidic solutions such as hydrochloric acid (HCl) dissolve scaling, disrupt the bacterial cell wall structure and precipitate proteins (Beyer et al., 2017; Jiang et al., 2017). Cleaning protocols for spiral wound RO membranes consist of several phases of high flow recirculation and soaking, lasting anywhere between 6.5 and 24 h in duration at a typically applied temperature of 35 °C. (Beyer et al., 2017).

Conventionally applied acid/alkali treatments often fail to fully restore membrane performance (Beyer et al., 2014; Huiting et al., 2001; Vrouwenvelder et al., 1998). A recent study examining chemical cleaning in three full-scale RO plants in the Netherlands underlined the need for novel cleaning approaches as none of the standard cleaning chemicals resulted in highly effective membrane regeneration (Beyer et al., 2017). Moreover, rapid biofilm regrowth is observed, inducing more frequent and rigorous cleaning, resulting in increased costs and chemical waste (Vrouwenvelder et al., 1998). There is an apparent need for novel chemical cleaning strategies to enhance biofilm solubillization and removal from membrane modules, achieve higher recoveries of membrane performance parameters, and decrease the required cleaning frequency.

Chaotropic agents such as urea ($CO(NH_2)_2$) and guanidine hydrochloride ($CH_6ClN_3$) have been reported in literature as possible chemical cleaning agents. Whittaker et al. (1984) studied a variety of chemical cleaning strategies, of which urea (2M) in combination with a detergent (SDS) was amongst the most successful treatments in terms of biofilm removal. Applying 6–8 M urea demonstrated excellent biofilm removal (Whittaker et al., 1984). Another study confirmed the effectiveness of 2 M urea in achieving >70% biofilm protein removal (Chen and Stewart, 2000). A recent study examined the effect of 5 M urea on oral biofilms derived from clinical isolates, and showed that urea was much more efficient at removing biofilm coverage than chlorhexidine, a traditionally used antimicrobial agent (Rasmussen et al., 2016).

Though the exact action mechanism of urea cleaning is not fully understood, its ability to disrupt the hydrogen bonds involved in stabilizing the structure of proteins and other biopolymers has been reported (Mallevialle et al., 1996). Urea may enhance biofilm solubilization by extracting non-covalently bound extracellular matrix and cellular proteins (Ashraf Kharaz et al., 2017). Urea is also efficient for cell and tissue lysis (Rasmussen et al., 2016; Su and Dias, 2017), resulting in higher biomass inactivation.

Previously, urea cleaning has been performed on small strips of membranes (Whittaker et al., 1984) or on biofilm covered slides (Chen and Stewart, 2000). The novelty of this study lies in; (i) the use of membrane fouling simulators (MFSs) to mimic the conditions of spiral-wound RO membrane elements used in practice (Vrouwenvelder et al., 2006, 2007), (ii) the application of a more concentrated urea solution to disrupt the structure of EPS components, and (iii) the detailed analysis of the effect of urea on biomass removal.

This study aimed to comprehensively investigate the potential to use saturated urea solution as a cleaning agent for fouled membrane systems, compared to the conventional acid/alkali cleaning protocol commonly applied in practice.

## 2. Materials and methods

### 2.1. Experimental set-up

The laboratory set-up consisted of a granular activated carbon filter followed by two cartridge filters in series (10 μm pore size), mass-flow controllers, nutrient dosage pump, MFSs and back-pressure valves (Fig. 1) (Bucs et al., 2016). The MFS flow channel dimensions were 200 mm × 40 mm × 0.863 mm. Membrane and feed spacer coupons (200 mm × 40 mm) were placed inside each MFS to mimic the structure of spiral-wound membrane elements. The feed spacer consisted of a sheet of 34 mil (0.863 mm) thick diamond-shaped polypropylene spacer. The feed spacer and membrane sheets were taken from virgin spiral-wound membrane elements (BW30-400/34i, DOW FILMTEC, USA). The carbon filter was installed to remove residual chlorine from the incoming tap (feed) water to protect RO membrane from chlorine damage and enable microbial growth. Cartridge filters were used to retain any particles emerging from the carbon filter. The MFS was operated in crossflow mode without permeate production at a pressure of 2 bar. Feed water entered the MFS from the inlet side and exited from the outlet after flowing through the spacer-filled channel. Four MFSs were operated in parallel and a flow controller was installed for each MFS. A differential pressure transmitter measured the pressure drop over each MFS. A back-pressure valve was installed at the outflow to avoid degassing by pressurizing the set-up. Nutrients and cleaning solutions were dosed to the MFSs using separate flow controllers and dosage pumps.

Feed water was pumped to the MFSs at a flow rate of 17.0 L/h equivalent to a linear flow velocity of 0.16 m/s, representative of practice (Vrouwenvelder et al., 2009a). Feed water used for the experiments was tap water from the KAUST desalination treatment plant (Table 1).

The product water from the desalination plant (Belila et al., 2016) is chlorinated ensuring a concentration of 0.5 mg/L before entering the distribution network. The residence time from the desalination plant to the laboratory set-up is between 4 and 6 h. The residual chlorine concentration in the tap (feed) water is $0.3 \pm 0.1$ mg/L.

Biofilm development in the MFSs was accelerated by dosing a biodegradable nutrient solution containing acetate, nitrate and phosphate in a mass ratio C:N:P of 100:20:10 to the feed water (Miller et al., 2012). The concentration of acetate carbon added to the MFS feed water was 250 μg/L. During operation, the MFS window was covered with a light-tight lid to prevent growth of phototrophic organisms. The development of fouling was monitored by measuring the pressure drop increase over the feed spacer channel of the MFS, using a pressure difference transmitter (Endress & Hauser, type Deltabar S, PMD70-AAA7FKYAAA) (Vrouwenvelder et al., 2009b). The active biomass accumulated on the feed spacer and membranes in each MFS was quantified and characterized at the end of operation.

### 2.2. Preliminary assessment of urea cleaning

Studies were carried out to investigate (i) whether RO polyamide membranes can withstand high urea concentrations, and (ii) the effect of urea concentration and temperature on biofilm solubilization. For all preliminary tests, membrane and spacer sheets

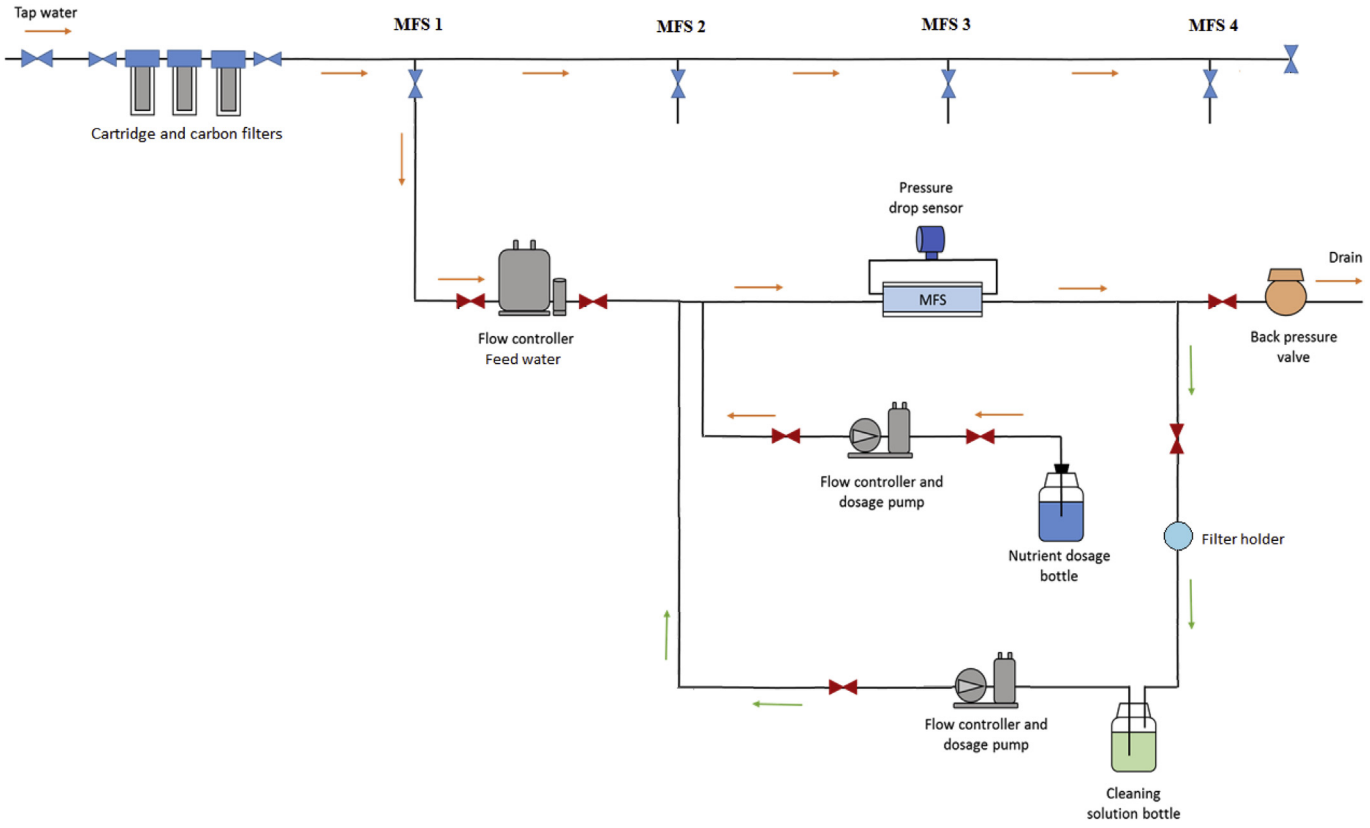


**Fig. 1.** Schematic diagram of the automated system for lab scale membrane fouling simulation. Arrow indicates water flow direction.

**Table 1**
Feed (tap) water quality parameters.

| Parameter | Unit | Value |
|---|---|---|
| Specific conductivity at 25 °C | µS/cm | 345 ± 15 |
| Total hardness | mg/L | 42 |
| pH | – | 7.9 ± 0.2 |
| Temperature | °C | 23 ± 1 |
| Bacterial load | Cells/mL | $10^4$ ± 100 |
| Total organic carbon | mg/L | <0.2 |
| Residual chlorine | mg/L | 0.3 ± 0.1 |

with pre-grown biofilm (obtained using the MFS set-up described above) were used.

First, the compatibility of urea with RO polyamide membranes was evaluated using the Congo red dye test (U.S.D.I., 1996). This test can detect membrane damage as the Congo red dye does not attach to the intact polyamide layer of the membrane but is absorbed by the porous supporting layer of the membrane resulting in an intense color on the membrane surface where the membrane is damaged. Fouled membrane coupons were submerged in urea solutions at concentrations ranging from 294 to 756 g/$L_{water}$ for two weeks. After urea treatment, membrane coupons were rinsed with MilliQ water and air dried. A solution of Congo red dye (1% w/w) was swabbed on the membrane surface and damages were visually assessed. The membrane coupons were compared to a damaged membrane (positive control) and to an intact virgin membrane (negative control). The damaged membrane was prepared by 24 h of continuous stirring of the membrane coupon with a magnetic stirrer.

The influence of urea solution concentration and temperature on biofilm solubilization was examined. The fouled membranes and spacers were cut into 10 pieces of 8 $cm^2$ each and incubated for 24 h in centrifuge tubes containing 20 mL of urea solution at varying concentrations (294–1147 g/$L_{water}$) and at a constant temperature of 23 °C to eliminate the effect of heat. Similarly, membrane and spacer coupons were incubated for 24 h at varying temperatures (23–80 °C) at a constant urea concentration of 1147 g/$L_{water}$. Incubation was performed in a thermostatic bath without stirring. As negative controls, fouled membrane and spacer coupons were immersed in MilliQ water. After 24 h, membrane and spacer coupons were taken out of the solution and rinsed by successive immersions in beakers of MilliQ water to remove the residual urea. The remaining biomass on the membrane and spacer coupons was measured using the chemical oxygen demand (COD) kits from HACH (LCK314 and LCK114), with a Lange HT200S thermostat and Lange DR3900 spectrophotometer.

### 2.3. MFS chemical cleaning studies

Laboratory-scale studies were carried out in order to evaluate the efficiency of urea as a chemical cleaning agent for biofilm solubilization compared to the conventional acid/alkali treatment. Accelerated biofilm development was carried out in membrane fouling simulators using the experimental set-up and operating conditions described in Section 2.1. Four MFSs containing the same brackish water reverse osmosis (BWRO) membrane and feed spacers (34 mil) were operated simultaneously until the pressure drop reached a threshold value of 200 mbar (in 5–6 days, which is the average duration of accelerated biofouling studies with MFSs). Subsequently, the flow cells were cleaned according to a predetermined cleaning protocol (Table 2). The temperature of the cleaning solutions (NaOH and $CO(NH_2)_2$) was kept at 35 °C as this temperature is applied for enhanced flux recovery in practice

**Table 2**
Cleaning protocols applied to each MFS.

| MFS | Code | Cleaning protocol | Comment |
|---|---|---|---|
| 1 | C | None | Positive control |
| 2 | R | • NaOH, pH 12, 0.01M, 35 °C, 1 h<br>• HCl, pH 1, 0.1M, room temp., 1 h | Reference MFS. Conventional cleaning protocol. |
| 3 | U + A | • Saturated $CO(NH_2)_2$ solution (1340 g/$L_{water}$), pH 9.6, 35 °C, 1 h<br>• HCl, pH 1, 0.1M, room temp., 1 h | NaOH replaced by $CO(NH_2)_2$. Two-stage cleaning using $CO(NH_2)_2$ + HCl |
| 4 | U | Saturated $CO(NH_2)_2$ solution (1340 g/$L_{water}$), pH 9.6, 35 °C, 1 h | One-stage cleaning using $CO(NH_2)_2$ only. |

(Madaeni and Samieirad, 2010). The applied concentration of urea (1340 g/$L_{water}$) is the saturated concentration at 30 °C to ensure that urea remains in solution at 35 °C. This concentration of urea also did not damage the polyamide membrane as it was tested on membrane modules in pilot-scale and no increase in salt passage was observed. The pH of the cleaning solutions was also within the operating range (pH 1–12) of polyamide membranes (Kucera, 2011). BioReagent grade (≥98%) urea, ACS reagent (37%) HCl, and reagent grade (≥98%) NaOH were purchased from Sigma Aldrich.

Each chemical cleaning agent (1L) was recirculated at the feed side of the MFS for 1 h at the same flow rate as the feed water (17.0 L/h). A membrane filter (10 μm pore size) was placed between the outlet of the MFS and the dosing pump to avoid the recirculation of detached biofilm particles back to the MFS. In between and after each cleaning, the MFSs were rinsed with feed water for 5 min to displace the previous cleaning solution. Feed channel pressure drop development was monitored throughout the duration of the experiment, including the chemical cleaning phases. Membrane autopsies were subsequently carried out on retrieved membrane and feed spacer coupons for the qualitative and quantitative analysis of the fouling deposits. Membrane and spacer coupons were cut from the inlet side of the MFS using sterile scissors and tweezers. The coupon dimensions were measured with calipers so that the results can be reported per area of the combined membrane and spacer surface. The extent and composition of the fouling layer was assessed using various analytical procedures described below. All experiments were run in duplicates and the figures show the average and standard deviation from the two runs.

### 2.4. Biomass analysis

Adenosine triphosphate (ATP) analysis was used for biomass quantification. ATP is present in all metabolically active microorganisms, thus ATP analysis can be used to measure the viable biomass content. It is a generally accepted parameter for diagnosis of biofouling (Hijnen et al., 2011; Vrouwenvelder et al., 2008). The biomass detachment and quantification method has been described previously (Sanawar et al., 2017).

A well-established EPS extraction procedure was adapted (Liu and Fang, 2002). Biomass removed from membrane and feed spacer coupons was suspended in 10 mL of phosphate-buffered saline (PBS) solution. The samples were treated with 0.06 mL formaldehyde at 4 °C for 1 h and incubated with 4 mL 1M NaOH at 4 °C for 3 h. After centrifuging the samples for 20 min at 20000×g, the supernatant was filtered through a 0.2 μm pore size membrane filter and purified using a 3500 Da dialysis membrane for 24 h. The dialyzed samples were lyophilized (−50 °C, 48 h). Fluorescence excitation-emission matrix (FEEM) was used to identify the predominant organics in the extracted EPS such as protein-like substances, humic/fulvic-like substances and others (Chen et al., 2003b; Valladares Linares et al., 2012). FEEM was measured using a Fluoromax-4 spectrofluorometer (Horiba Scientific, Japan) under excitation of 240–450 nm and emission of 290–600 nm at a speed of 1500 nm.min$^{-1}$, a voltage of 700 V, and a response time of 2 s.

Carbohydrate and protein components of the extracted EPS were quantified. Carbohydrates were quantified using the standard sulfuric acid-phenol method (Masuko et al., 2005) which involves mixing 200 μL of the extracted EPS with 600 μL sulfuric acid and 120 μL of 5% phenol in a centrifuge tube. The samples were incubated for 5 min at 90 °C in a dry block heater and cooled down to room temperature for 5 min. An aliquot of 200 μL of the sample was then transferred to a microplate and the absorbance at 490 nm was measured using a Spectra A max 340pc microplate reader (Molecular devices, USA).

Proteins were quantified using the BCA assay kit (Thermo Scientific, USA) according to manufacturer's guidelines.

### 2.5. Statistical evaluation

Averages and standard deviations were calculated for each of the duplicate sets of experiment, and a two-tailed Student's t-test was used for statistical comparisons. The standard deviation between duplicate experiments is represented by error bars in the graphs. The variance in the sample means is denoted by a single asterisk * if there is a significant difference ($p = <0.05$), and by "ns" if there is no significant difference ($p = >0.05$).

## 3. Results

### 3.1. Membrane resistance to urea

In order to determine whether urea has a damaging effect on RO polyamide membranes, Congo red dye was applied to membrane coupons which had been submerged in 294–756 g/$L_{water}$ urea solutions for 2 weeks. No absorption of the dye was visible on the treated membrane surface contrary to the degraded membrane control, confirming that urea did not damage the polyamide layer of the membrane. In a separate study, no increase in salt passage was observed when full-scale RO membrane modules were cleaned with saturated urea solution (1340 g/$L_{water}$), reiterating the compatibility of urea with the membrane.

### 3.2. Impact of urea concentration and temperature on biofilm solubilization

COD analysis revealed that urea cleaning efficiency was enhanced with increasing urea concentration and temperature. More than 80% reduction in COD was achieved with urea treatment at concentrations above 700 g/$L_{water}$ (Fig. 2A), whereas above 30 °C urea solutions (1147 g/$L_{water}$) resulted in >90% reduction in COD (Fig. 2B). In accordance with industrial cleaning practice, 35 °C was set as the cleaning temperature for RO membranes, and 1340 g/$L_{water}$ urea was the corresponding concentration just below the saturation point at 30 °C.

### 3.3. Efficiency of chemical cleanings

Four MFSs containing the same BWRO membrane and feed

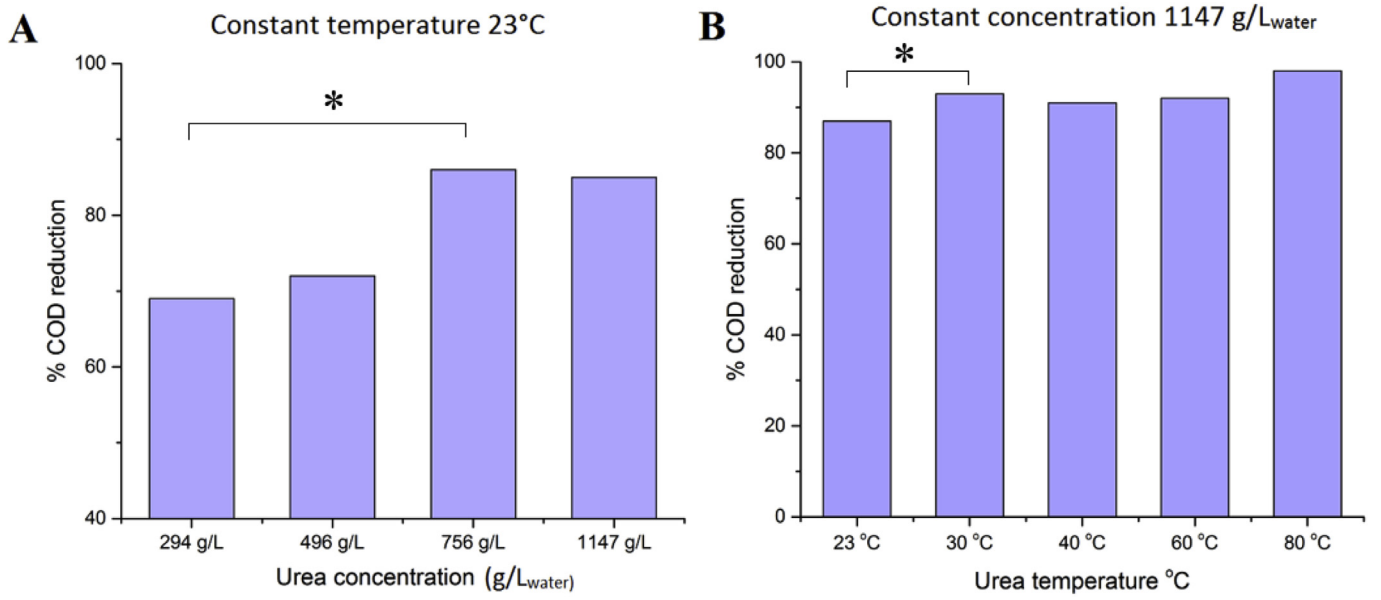


**Fig. 2.** Impact of urea concentration (A) and temperature (B) on biomass removal measured indirectly as percent chemical oxygen demand (COD) reduction. Results are based on single experiments.

spacer (34 mil) were operated in parallel with a constant supply of nutrients to the feed water to accelerate biofilm formation. Biofilm development was monitored by means of feed channel pressure drop increase in the MFSs until it reached a threshold value of 200 mbar in each MFS. Different chemical cleaning protocols consisting of the standard acid/alkali treatment and two alternative urea treatments were applied (Table 2). Pressure drop reduction was used as a measure of membrane performance restoration after each of the applied chemical cleanings (Section 3.3.1), active biomass removal post chemical cleanings was determined by means of ATP analysis (Section 3.3.2), and biofilm solubilization efficiency of the chemical cleaning agents was studied by quantifying the predominant EPS components as well as FEEM analysis (Section 3.3.3).

### 3.3.1. *Performance restoration*

The increase in pressure drop (ΔP) was 160 mbar in the control MFS, and 180 mbar in all other MFSs (Fig. 3A), starting with an average initial pressure drop in all MFSs of 20 mbar and increasing to about 200 mbar (over the 0.20 m long MFS) over a period of 5–6 days.

Membrane performance was restored equally well in the MFS cleaned with the conventional reference (R) chemicals (NaOH + HCl) and the MFS cleaned with $CO(NH_2)_2$ + HCl (U + A). More than 70% reduction in pressure drop was achieved with both the chemical treatments (Fig. 3B) and a *t*-test revealed no significant difference (p = >0.05) between the performance of the R and U + A cleaning protocols. However, one-stage cleaning with urea only (U) resulted in a 50% decrease in pressure drop only,

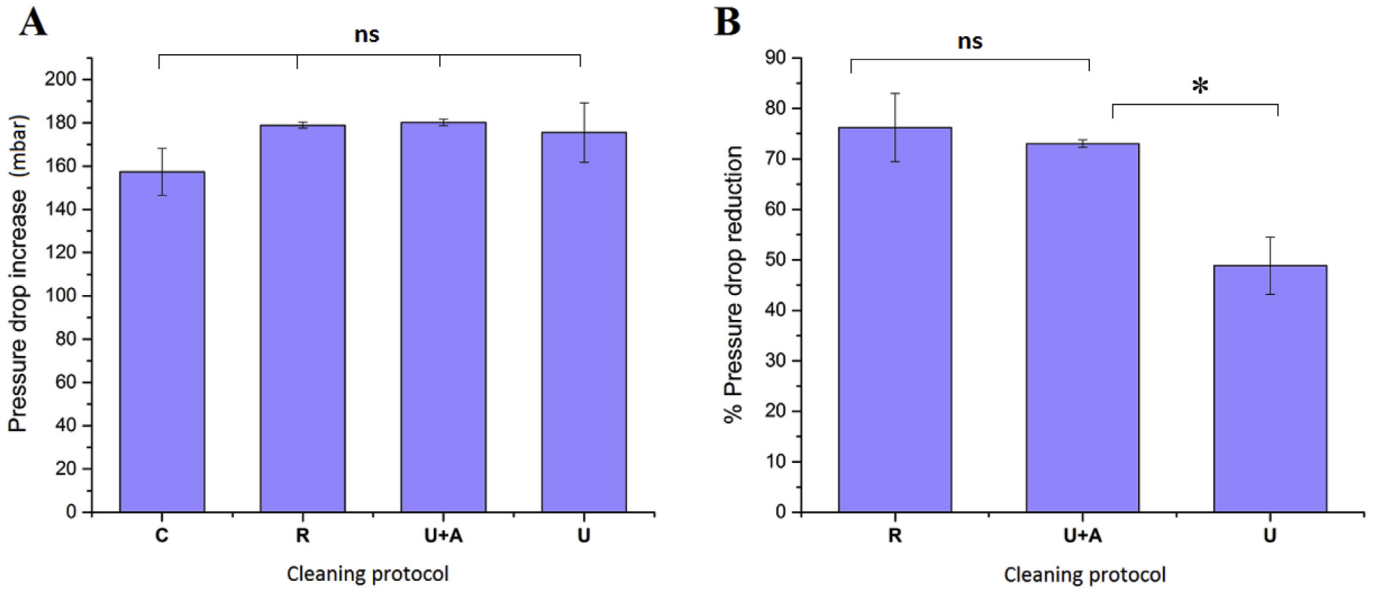


**Fig. 3.** (A) Pressure drop increase (mbar) in each MFS, and (B) percent reduction in pressure drop after chemical cleaning. Error bars represent standard deviation of duplicate experiments. C = Control (no cleaning); R = Reference (NaOH + HCl); U + A = ($CO(NH_2)_2$ + HCl); U = $CO(NH_2)_2$ only.

suggesting that the acid recirculation phase plays an important role in the effectiveness of the cleaning cycles.

### 3.3.2. Biomass inactivation and removal

The concentration of viable biomass remaining in each MFS after cleaning was quantified using ATP analysis (Fig. 4). Aside from the uncleaned control MFS which contained $8.85 \times 10^4$ pg ATP/cm$^2$, the reference (R) MFS had the highest concentration of active biomass (662 pg ATP/cm$^2$). After cleaning by protocol U and U + A, the remaining active biomass content in the MFSs was 446 and 318 pg ATP/cm$^2$, respectively. Urea-based cleaning protocols resulted in significantly higher biomass inactivation ($p = <0.05$) than the reference protocol R.

### 3.3.3. Biofilm solubilization

The two predominant components of EPS - total proteins and total carbohydrates were quantified. Fig. 5 presents the concentration of proteins (Fig. 5A) and carbohydrates (Fig. 5B) remaining after the different cleaning strategies. Compared to the uncleaned control (C), chemical cleaning resulted in >50% reduction in protein and carbohydrate content. Solubilization of proteins was slightly enhanced by urea-based cleaning protocols, however, compared to the reference acid/alkali treatment the difference was not significant ($p = >0.05$). Likewise, the same applies to the reduction of the carbohydrate content for which all three cleaning protocols produced similar results ($p = >0.05$).

Additionally, FEEM analysis was used to examine the extracted EPS. Fig. 6 shows the FEEM plots divided into four regions – I (humic-like matter; $E_x = 320$ nm, $E_m = 425$ nm), II (protein-like matter; $E_x = 275$ nm, $E_m = 330$ nm), III (fulvic acid-like substances; $E_x = 260$ nm, $E_m = 475$ nm), and IV (tyrosine-containing proteins; $E_x = 235$ nm, $E_m = 330$ nm).

The FEEM plot of the uncleaned control membrane shows a distinct peak for protein-like substances and a strong peak for tyrosine-containing substances which are typically proteinaceous and associated with amino acids (Fig. 6A). Both peaks (II and IV) are present at a much lower intensity on the membranes treated by chemical cleaning. After cleaning with R, U and U + A, the average peak intensity for protein-like matter was reduced by 19%, 27% and 40%; while the average peak intensity for tyrosine-containing proteins was reduced by 24%, 19%, and 52% respectively, compared to the uncleaned control. In other words, urea-based cleaning protocols were able to solubilize protein-like substances and tyrosine-containing proteins more effectively than the reference acid/alkali cleaning treatment, indicating that urea cleaning has advantages over the acid/alkali cleaning.

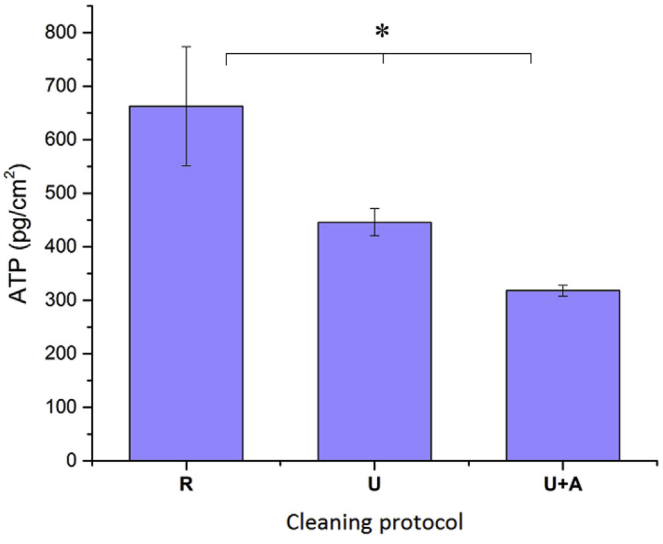


**Fig. 4.** Active biomass content measured as ATP (pg/cm$^2$) remaining in each MFS after cleaning. Error bars represent standard deviation of duplicate experiments.

## 4. Discussion

### 4.1. Selection of chemical cleaning protocol

Periodic membrane cleaning is unavoidable to maintain the performance of membrane-based water treatment processes and the selection of a chemical cleaning protocol is usually governed by the fouling types deposited on the membranes (Jiang et al., 2017). In this study, the effectiveness of urea chemical cleaning was evaluated by performing short-term accelerated biofouling experiments (Sanawar et al., 2017) in which a biofilm was grown in membrane fouling simulators with the supplementation of biodegradable nutrients. Biofilm accumulation was monitored in real time by measuring the feed channel pressure drop development. The choice of cleaning chemicals in this study was based on the predominant nature of the fouling layer i.e. biological fouling. Hence, cleaning with NaOH and $CO(NH_2)_2$ is an appropriate strategy since both cleaning agents initiate chemical reactions involved in the removal of biological fouling (Beyer et al., 2017; Mallevialle et al., 1996). Typically in practice, a high pH cleaning solution (such as NaOH) is used first to remove biological matter, followed by a low pH cleaning chemical (such as HCl) to further weaken the bonds between the foulants and the membrane surface, and destabilize the chemical bonds within the EPS matrix itself (Hydranautics, 2014). This study investigated the effect of substituting the standard alkaline chemical (NaOH) with $CO(NH_2)_2$ which acts as a chaotropic (hydrogen bond disrupting) agent and enhances the solubility of organic foulants by denaturing proteins.

### 4.2. Optimization of the urea cleaning protocol

Standard RO membranes used in water practice are thin-film composite polyamide membranes. When selecting a chemical cleaning agent, it is important to assess whether or not the chemical can cause damage to the membrane material. The compatibility of $CO(NH_2)_2$ with RO polyamide membranes was confirmed during the initial evaluation of urea as a cleaning agent. A recent patent also confirms the suitability of urea and its derivatives with RO polyamide membranes (Kawakatsu and Ishii, 2017). Furthermore, the effectiveness of chemical cleaning depends on the type, circulation time, pH and temperature of the cleaning solutions (Al-Amoudi and Lovitt, 2007; Madaeni and Samieirad, 2010). Two of the main chemical aspects of cleaning – urea concentration and temperature – were investigated to determine the optimal cleaning conditions. Using COD as an indicator of biomass removal, it was shown that the urea cleaning efficiency increases at higher concentration (756 g/L$_{water}$ > 294 g/L$_{water}$) and temperature (30 °C > 23 °C). Urea concentration higher than 756 g/L$_{water}$ and temperatures higher than 30 °C did not increase the cleaning efficiency. The results are in agreement with literature which have shown that chemical cleaning efficiency increases at higher concentration and at elevated temperatures (50–60 °C) (Bartlett et al., 1995; Madaeni et al., 2001; Shorrock and Bird, 1998; Sohrabi et al., 2011). This is because higher temperatures accelerate the chemical reactions involved in the breakdown of deposits at the membrane surface (Chen et al., 2003a). However, with repeated exposure to temperatures higher than 45 °C the polyester support and polyamide layer of the membrane can be damaged (Kucera, 2011). Therefore, 35 °C was set as the optimal

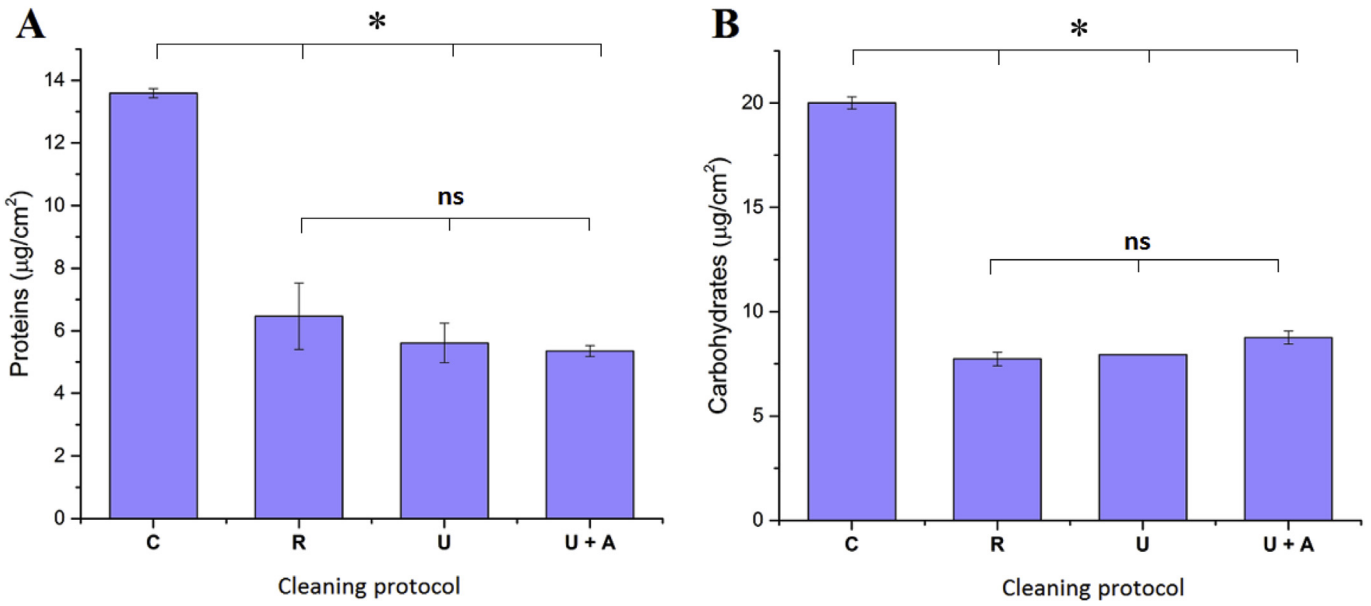


**Fig. 5.** (A) Concentration of proteins (μg/cm$^2$) and (B) concentration of carbohydrates (μg/cm$^2$) in each MFS after cleaning compared to the uncleaned control C MFS. Error bars represent standard deviation of duplicate experiments.

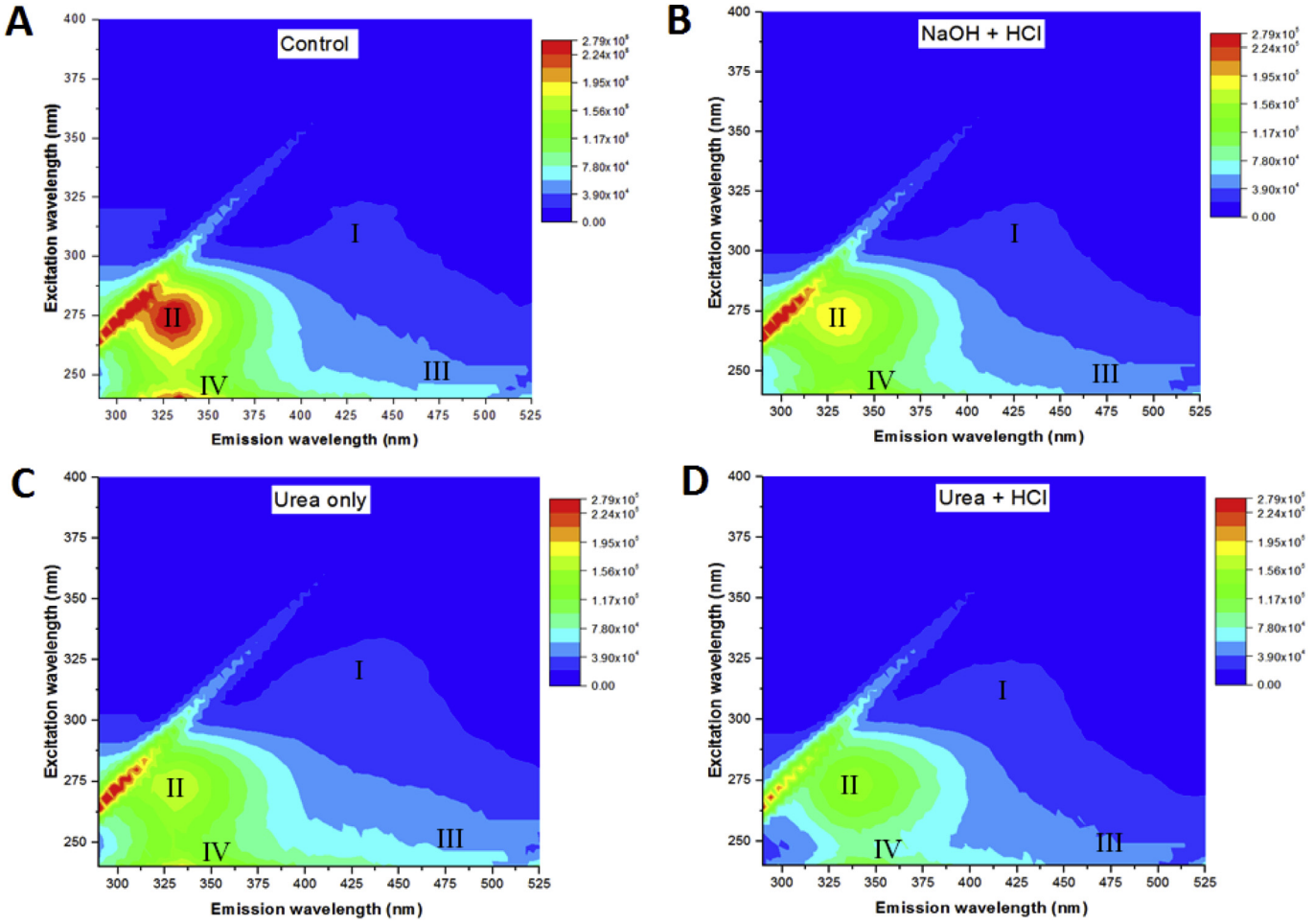


**Fig. 6.** FEEM plots of EPS extracted from membrane/spacer coupons of each of the cleaned MFSs (B, C, D) compared to the uncleaned control MFS (A). The plots show the presence of (I) humic-like matter, (II) protein-like matter, (III) fulvic acid-like substances, and (IV) tyrosine-containing proteins.

temperature for NaOH and $CO(NH_2)_2$ cleaning solutions in accordance with practice and guidelines for polyamide membranes (Hydranautics, 2014; Madaeni and Samieirad, 2010).

### 4.3. Efficiency of chemical cleaning

#### 4.3.1. Performance restoration

After determining the optimal cleaning conditions, fouled MFSs were subjected to the reference cleaning protocol (R = NaOH + HCl) and to alternative urea-based chemical cleanings (U = Urea only, U + A = Urea + HCl). Feed channel pressure drop increase is a result of biomass accumulation on the membrane/spacer surface (Vrouwenvelder et al., 2009a, 2009c). Restoration of membrane performance after chemical cleaning was therefore monitored by means of feed channel pressure drop reduction. Cleaning with R and U + A yielded >70% reduction in pressure drop, with no significant difference between the efficiency of both chemical cleaning strategies in terms of membrane performance restoration. However, one-stage cleaning with urea only was not as effective indicating the importance of applying a second-stage low pH acid cleaning. The possible physico-chemical interactions between acidic cleaners such as HCl and foulants include hydrolysis and solubilization (Porcelli and Judd, 2010; Zondervan and Roffel, 2007). Therefore, these mechanisms may be responsible for enhanced biofilm solubilization during the secondary acid cleaning stage.

#### 4.3.2. Biomass parameters

The performance of urea-based chemical cleanings is superior to that of the reference cleaning in terms of biomass inactivation. In order of biomass inactivation efficiency, the cleaning protocols can be ranked as U + A > U > R. In a recent study, urea was shown to cause significant internal stresses within the biofilm within 15 min of exposure, likely due to osmotic forces (Rasmussen et al., 2016). The extracellular and intracellular swelling of the biofilm caused by the diffusion of urea molecules into the biofilm space and bacterial cells eventually leads to osmotic lysis (bursting of cell membrane due to osmotic imbalance). This may be the reason why in this study biomass inactivation was up to two-fold greater after treatment with urea-based chemical cleanings compared to the conventional acid/alkali treatment (Fig. 4).

Urea as a chemical cleaning agent proved to be more efficient in terms of biofilm protein solubilization. FEEM plots (Fig. 6) illustrate that urea acts as a protein denaturant and results in significantly lower intensities of protein-like matter and tyrosine-containing proteins when compared with the membrane cleaned with the conventional cleaning chemicals. This may be attributed to the ability of urea to: (i) cause unfolding of proteins by interrupting hydrogen bonding between amide and carbonyl groups of proteins; and (ii) inhibit protein aggregation. The deposition of proteins on the membrane surface has been shown to accelerate fouling by serving as a nucleation site for the formation of larger protein aggregates (Kelly et al., 1993, Kelly and Zydney, 1994, 1995). Aggregation of proteins occurs predominantly through intermolecular disulfide bonds (Kelly and Zydney, 1994; Steinhauer et al., 2015). The availability of free sulfhydryl groups is essential to the aggregation reactions (Huggins et al., 1951). Urea eliminates the formation of protein aggregates by blocking the free sulfhydryl group (Kelly and Zydney, 1994; Mo and Ng, 2010), thereby minimizing the accumulation of the foulant on the membrane surface.

Several studies have demonstrated that by strategically pairing chemical agents that have complementary cleaning mechanisms, foulants can be removed from the membrane surface more efficiently (Ang et al., 2011; Bartlett et al., 1995; Madaeni and Samieirad, 2010; Shi et al., 2014). Pairing of urea and HCl in this study yielded higher cleaning efficiencies. It is plausible that (i) the first stage urea cleaning disrupts the biofilm stability by interfering with the hydrogen-bond network of biofilm and (ii) the second stage acid cleaning minimizes the ligand binding of divalent atoms between negatively charged groups on the EPS due to lower pH. In other words, urea creates a loose fouling layer that allows for deeper penetration of HCl into the biofilm, consequently enhancing the solubilization of deposits. These results (Fig. 6) are based on the analysis of total EPS extracted from the biofilm present on membrane/spacer coupons using the formaldehyde/NaOH method. The total EPS extract was then subjected to subsequent extraction methods for individual EPS components i.e. proteins and carbohydrates. Analysis of the individual EPS components resulted in similar concentrations of total proteins and carbohydrates after cleaning with R, U and U + A (Fig. 5) suggesting possible limitations of the extraction methods. All three cleaning protocols yielded >50% reduction in total proteins and carbohydrates.

### 4.4. Future research

The potential of urea as an alternative cleaning agent should also be evaluated in full-scale spiral-wound RO membrane modules when several fouling types might exist simultaneously. The possibility of reclaiming urea from the waste solution post-cleaning should be explored. All the used urea can potentially be recovered by eutectic freeze crystallization as its eutectic point lies around −12 °C (and 33 wt.%), which temperature is high enough to allow for energy-efficient operation (Van der Ham, 1999). This would allow for urea to be reused after the removal of impurities, in an effort to reduce the volume of chemical waste and the costs associated with cleaning, and treating chemical waste. Appropriate urea waste disposal methods must be implemented leading to eco-friendly and preferential use of urea over the conventional cleaning agents. Bacterial cells in biofilms are known to develop resistance to antimicrobial agents and cleaning chemicals (Bridier et al., 2011). Several studies have reported that repeated cleaning cycles select for microbial groups that strongly attach to the RO membrane surface by producing rigid and adhesive EPS (Al Ashhab et al., 2017) and that the type and amount of deposits remaining after cleaning will have an effect on the rate and extent of biofilm regrowth (Bereschenko et al., 2011). Therefore, investigating the changes in biofilm structure and composition after repetitive urea cleaning cycles and the impact of urea on membrane performance restoration during long-term membrane operation should be pursued. Chemical cleaning combined with physical cleaning methods such as increase in shear (Vrouwenvelder et al., 2010) or the use of air bubble scouring (Cornelissen et al., 2007) may enhance biofilm removal since urea weakens the fouling layer. Furthermore, studies have shown that the composition of the EPS matrix can influence the hydraulic biofilm resistance (Desmond et al., 2018; Dreszer et al., 2013). It may be addressed in future studies how urea cleaning affects the hydraulic resistance of biofilms and possibly leads to enhanced permeate flux.

## 5. Conclusions

The suitability of alternative chemical cleaning strategies, employing concentrated urea for biofilm solubilization, was studied in comparison with the conventional cleaning protocol as applied in practice to reverse osmosis membranes. Based on the results of the lab-scale membrane fouling simulator studies, it can be concluded that;

- Urea is a compatible chemical cleaning agent for reverse osmosis polyamide membranes.

- Enhanced biomass removal is achieved with increasing urea concentration and temperature, until the effect begins to plateau.
- Performance of urea as a chemical cleaning agent is superior to conventional cleaning in terms of biomass inactivation and biofilm protein solubilization (based on FEEM analysis of total extracted EPS).
- Chemical cleaning with urea is as effective as the standard acid/alkali cleaning in terms of membrane performance restoration and extracellular polymeric substances reduction.
- Alternative urea-based cleaning strategy is an inexpensive, simple and successful approach to control organic and biological fouling.

Research is underway to recover and reuse urea from the waste solution.

## Conflict of interest

The authors declare no conflict of interest.

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