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Development and application of an accelerated biofouling test in flat cell

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ABSTRACT

This paper discusses the steps followed to establish an accelerated reverse osmosis membrane biofouling test protocol. Easily bioassimilable nutrients were dosed into water from the Ebro River which was fed into a membrane flat cell pilot plant. The membrane biofouling results were determined by measuring adenosine triphosphate and total organic carbon extracted from the exposed membrane and feed spacer. This strategy was chosen because the equipment does not have pressure drop measurement capability and the observed change in flux or salt rejection was not significant under the testing conditions. The duration of the experiment was set to 3 d to enable rapid screening of different membrane types, yet enable a smooth evolution of biofilm. This study determined that achieving a similar amount of biofouling in each of the three cells was very sensitive to the level of bioassimilable nutrients were dosed to the water. For the Ebro River water, 0.2 mg/L of carbon, 0.04 mg/L of nitrogen, and 0.02 mg/L of phosphorous was determined to provide the best result.

Keywords: Biofouling; Reverse osmosis; Membranes; Flat cell; Biofilm; Feed spacer

1. Introduction

Reverse osmosis membranes are prone to suffer from fouling due to the trace contaminants found in natural feedwater [1]. The term fouling in reverse osmosis refers to the accumulation of material on the membrane surface and/or within the feed channel of the spiral wound element. If this phenomenon is not addressed, the element could suffer from a severe loss of performance [2]. There are four main types of fouling in the reverse osmosis membranes including colloidal/particle, biological, organic, and scaling (precipitated inorganic salts). Biological fouling is characterized to be especially challenging to prevent and control [3].

Laboratory experimental methods are needed to more rapidly and systematically optimize the reverse osmosis membrane chemistry to have a higher

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biofouling resistance. Current published methods for reproducing biofilm on reverse osmosis membranes are based on bacteria attachment determination [4,5]. The main protocols applied are the immersion test using the Center for Disease Control biofilm reactor [6] and filtration with a high concentration of bacterial solutions [7–9].

Although these methods are commonly used, they are not realistic when simulating reverse osmosis operating conditions. Variables such as feed pressure, cross-flow velocity, feed spacer hydrodynamics and/or feedwater composition are not measured or controlled. Therefore, the challenge remains to correlate the data obtained using these methods with observations in the field [3]. Moreover, these methods require sophisticated laboratory equipment and safety standards as they involve bacteria culturing.

The Tarragona Global Water Technology Center has access to natural water sources including the Ebro River water, wastewater, and seawater. These waters have natural sources of bacteria which can form biofilms.

The goal of this project is to utilize the continuous supply of the Ebro River water and a membrane flat cell unit to create an accelerated biofouling test protocol to study membrane biofouling. This membrane flat sheet testing capability can provide an efficient fouling performance screening without the need of extra investment to build an entire reverse osmosis module, yet allow parameters such as membrane flux, feed velocity, and feed pressure to be adjusted for each test.

2. Experimental procedures

2.1. Flat cell description

The flat cell pilot unit used has three side-by-side flat cells. The system was manually operated in once-through mode without temperature control (Fig. 1). Feed spacer and membrane coupons were cut to fit the cell using a template. The O-rings on the top and bottom flat cell plates ensure that the system is water tight when bolted together, as observed in Fig. 2. Each cell provides an active membrane area of 84 cm^2. In the assembly, the membrane feed side has a void space which is 31 mil (31 thousandth of an inch) deep. A 28 mil feed spacer coupon is placed in this space to provide a representative shear environment at the membrane surface. There is a 3 mil difference between the void space and the feed spacer thickness which can create a potential for by-pass in the feed channel. However, feed spacer imprinting is observed on the membrane after each experiments, which indicates that the spacer is in contact with the membrane when mounted. Additionally, the feed spacer did not appear to deform during the experiment (i.e. shift to one end), suggesting that the fit in the channel is secure enough to maintain its position on the membrane surface when mounted. Since the focus for this test method was to study the impact of membrane chemistry on biofouling and not the impact of the feed spacer, further measures to eliminate the hypothetical by-pass were not implemented at this time.

A concentrated nutrient solution (labeled "Acetate" in Fig. 1) was dosed to the Ebro River water in order to accelerate the fouling process. Feed pressure and feed flow were adjusted using a by-pass needle valve. Individual cell feed water flows and recoveries were set by adjusting the feed and concentrate valves. Feed flows were measured using individual flow-meters with interval range of 1–4 L/min.

2.2. Operating conditions

The system was operated with three flat cells in parallel in once-through mode (vs. recirculating permeate and concentrate back to the feed and reusing). The same operational conditions were set for all three flat cells used in each experiment. The aim was to provide triplicate data points and measure the reproducibility in terms of the amount of accumulated

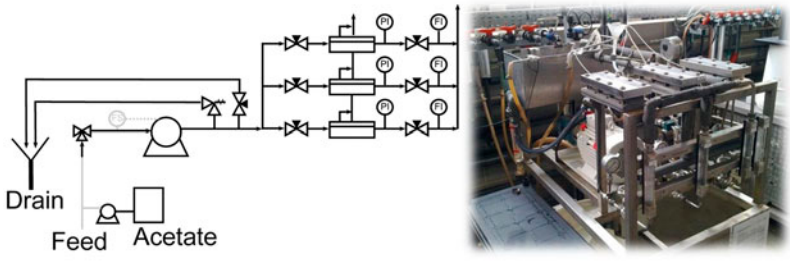

Fig. 1. Pilot unit for flat cell testing.

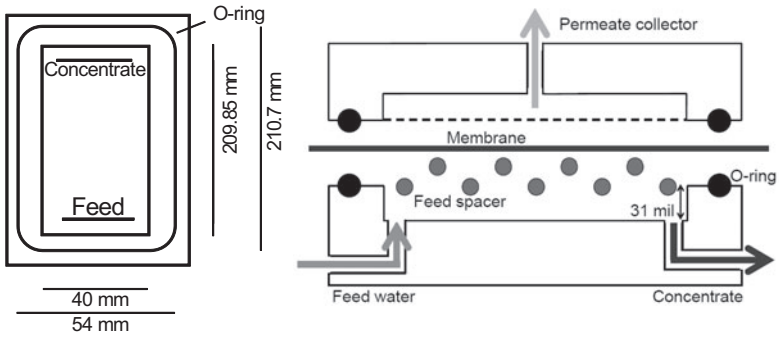

Fig. 2. Flat cell sketch and cross section configuration.

biomass on each flat cell operated in parallel under the same experimental conditions.

The permeate flow and feed flow were controlled within a range to most closely mimic typical commercial element operation (Table 1). However, due to equipment limitations, the lowest cross-flow velocity achievable for the flat cell unit is 0.6 m/s which is higher than a commercial element [10]. The concentrate pressure of each cell was adjusted individually to ensure they were operating at the same flux.

The feed flow was controlled to provide a set cross-flow velocity over the membrane surface. The cross-flow velocities were calculated by Eq. (1):

$$v = \frac{Q}{w \cdot h - \frac{m}{L \cdot \rho}} \quad (1)$$

where v is the cross-flow velocity (m/s), Q is the average feed flow (m/s), m is the mass of the spacer (g) and ρ is the density of the water (g/m). The dimensions L, w, and h (meter) correspond to the length of the channel, width, and thickness of the feed spacer sample that is cut for weighing.

2.3. Flat sheet membranes

DOW FILTMTEC™ BW30 membrane sheet was selected as the reference membrane chemistry for the development of the biofouling test method. This membrane is well-known and extensively used across many different applications. With a stabilized salt rejection of 99.5% and a pH resistance from 1 to 13, this membrane offers reliable performance and robustness across a wide range of feed conditions.

2.4. Feed spacers

The 28 mil polypropylene feed spacers used are defined by their strands per inch, thickness, and angle (Table 2). Feed spacer coupons were oriented in the flat cell so the feed flow intersects the angle formed by the strands knot. For example, in the picture in Table 2, the flow would be from right to left.

2.5. Brackish water characterization

The feedwater was taken from the Ebro River in L'Ampolla (Tarragona, Spain) after pretreatment with coagulation, flocculation, sand filtration, and chlorination. Water is distributed through AITASA (Aguas Industrials de Tarragona SA) which applies a dechlorination step before supplying it to the Water Center. Table 3 provides a general summary of the water composition, highlighting especially those most commonly linked to biofouling, including phosphate (PO_4), nitrate (NO_3), total organic carbon (TOC), and adenosine triphosphate (ATP). The concentration of each of these contaminants in addition to total dissolved solids was monitored over the course of the flat cell

Table 1
Quick biofouling method for flat cell

Parameter	Flat cell operating range evaluated	Commercial element typical operating range
Flux (L/m^2 h)	20–58	20–27
Flow velocity (m/s)	0.7–1.3	0.1–0.3
Recovery (%)	0.2–0.3	10–15

Table 2
Details of the spacers used

Spacer	Thickness (mil)	Strands/in	Angle (°)	Overview
28 mil	28	9	90	

Table 3
Feedwater composition

Feedwater characteristics	Average ± σ (n = 13)
Total dissolved solids (mg/L)	800 ± 190
Conductivity (μS/cm)	1,028 ± 210
Total organic carbon (mg/L)	1.3 ± 0.2
Adenosine triphosphate (ng/L)	11 ± 5
Nitrate (mg/L)	8 ± 2
Phosphate (μg/L)	25 ± 22
Chlorine (mg/L)	<0.02

trials (July–November 2013). Sodium metabisulfite was injected prior to use in the flat cell unit as an additional measure to ensure that chlorine concentration is lower than 0.02 mg/L.

The water composition remains reasonably constant during the year and shows a relatively low biofouling potential, compared to the ATP concentration found in tap water ranging from 5 to 20 ng/L [11].

2.6. Nutrients dosing strategy

Carbon, nitrogen, and phosphate are needed by bacteria to grow, reproduce, and eventually build a biofilm [12]. Since these values are relatively low in the Ebro River water supply, in order to promote biological fouling and reduce the duration of each experiment, readily bioassimilable nutrients were continuously dosed to the feedwater of the flat cell unit. A nutrient stock solution was prepared in an external tank using sodium acetate (VWR, USA), sodium nitrate (Sigma-Aldrich, USA), and sodium dihydrogen orthophosphate hydrate (Sigma-Aldrich, USA) to achieve a C:N:P ratio of 100:20:10. Sodium hydroxide (VWR, USA) was used to adjust the tank solution to pH 12 to avoid contamination, before being injected to the feedwater using a peristaltic pump.

The nutrient ratio was chosen based on typical C, N, P compositions found in biomass and ensured that there was enough of each constituent to avoid limiting biofouling development [13]. Since these inorganic salts can be directly used by bacteria, the concentration needed for accelerating biofouling development is very low, less than 1 mg/L. The calculation to determine the injection rate was based on the feedwater flow rate, the dosing pump frequency, and the nutrient stock tank concentration. An example calculation for an experiment needing 0.2 mg/L of carbon in the feedwater is provided in Table 4. Nutrient dosing rate is typically expressed as the concentration of carbon (mg/L of C) required in the feed water, assuming C:N:P ratio to be always constant at 100:20:10.

2.7. ATP analysis

ATP is the nucleoside triphosphate found in all living cells, including bacteria. This molecule is involved as a quick energy transfer unit in many endothermic biochemical reactions. This characteristic is the reason for its correlation with active biomass [14]. ATP acts as a phosphate group donor, releasing energy when the phosphodiester bond is hydrolyzed to adenosine diphosphate or adenosine monophosphate [15].

ATP content in liquid samples was measured using a Celsis Advance Luminometer, with luciferin as a reagent. This equipment has a detection limit of 2 ng/L, and the sampling volume is 100 μL. Biofouled samples of the membrane and feed spacer (4 × 4 cm) were submerged in 20 mL of ultrapure water to extract and dissolve the biofilm. A physical removal of the attached biofilm was achieved by applying a 6 min sonication in an ultrasonic bath (Fisher Scientific FB15061) at room temperature. The liquid sample was

Table 4
Example for a nutrient loading of 0.2 mg/L carbon in feed water

Parameter	Value
Feed flow (L/h)	700
Nutrient pump stroke (%)	80
Nutrients dosing pump (L/h)	0.8
Dosing tank volume (L)	60
CH_3COONa in tank (g)	36.3
$NaNO_3$ in tank (g)	12.9
$NaH_2PO_4{\cdot}H_2O$ in tank (g)	5.3

transferred into a sterile Eppendorf, where it was immediately analyzed or stored at –20℃ for no longer than 7 d to avoid any potential degradation. The samples were analyzed by adding luciferin reagent, which reacts with ATP emitting light that is detected by the instrument and converted to an ATP concentration using a calibration curve (bioluminescence). ATP results are expressed as ng/cm^2, based on the sample area used for extraction.

2.8. TOC analysis

TOC content in liquid samples was measured using TOC-L Shimadzu using UNE EN-1484:1998 method. The sample is oxidized via high temperature catalytic combustion and quantified using an infrared detector. The equipment has a detection limit of 0.01 mg/L and the sampling volume is 50 μL.

Fouled (4 × 4 cm) membrane and feed spacer samples were extracted using the same procedure described for the ATP analysis (Section 2.7). The liquid extraction sample was either analyzed immediately or was stored at 5℃ after sample acidification, for no longer than 7 d. This is done to prevent degradation of the organic compounds present in the sample.

Samples were measured and expressed as TOC concentration using the equipment internal calibration curve. Taking into account the size of the surface and the extraction volume, TOC results are expressed as mg/m^2.

3. Results and discussion

In general, it is recognized that biofilms are formed as a defense mechanism by bacteria to protect from their surroundings [5]. The biofilm also serves as a mechanism to capture nutrients from the water and provide an environment to colonize and thrive [16,17]. If the nutrients in the feedwater are reduced, bacteria will survive by switching to a dormant state or consume the polysaccharides in their biofilm.

With this basic understanding, the development of an accelerated flat cell biofouling screening test was focused on three main variables shear forces, membrane flux, and nutrient concentration. Shear force is expected to impose stress on the bacteria and promote biofilm formation. The shear is related to the velocity of the water flowing through the feed channel and the resistance generated by the feed spacer. Membrane flux provides a means to draw organic contaminants to the membrane surface to develop a conditioning layer for biofilm initiation. It also provides a high concentration of nutrients to feed the bacteria at the surface of the membrane. The higher the membrane flux is, the greater the concentration polarization and the accumulation of nutrients on the membranes surface. This in combination with the concentration of nutrients in the feedwater and water temperature are expected to affect the rate of biofilm formation [17].

3.1. Initial biofouling protocol set-up

Initial probing experiments identified the following conditions for obtaining a thick biofilm within 3 d: cross-flow velocity of 1.2 m/s, operating flux of 34 L/m^2 h, and nutrient addition to provide 0.4 mg/L carbon. The flux or salt rejection of the membranes showed little change over the course of the experiment (Fig. 3). The ATP and TOC values from the extracted membrane and spacer collected at the end of the experiment were 205 ± 166 ng/m^2 and 122 ± 16 mg/m^2, respectively. These values are well above the detection threshold of the measurement methods and appear to correlate well with the visual observation of high levels of biofilm growth (Table 5, 1–1). Thus, further optimization using these measurements was conducted to develop the method. The ATP and TOC values are the average for the three individual cells operated in parallel. To achieve good reproducibility, the objective is to reduce the variation of the results to less than 20% relative standard deviation.

The same operating conditions were repeated in a second experiment (Table 5, 1–2). However, the standard deviations of the ATP and TOC measurements between each flat cell within an experiment were still unacceptably high. In addition, poor reproducibility between experiments was observed. Further optimization to adjust the cross flow velocity, flux, and nutrient loading was pursued to reduce the standard deviation.

Table 5
Initial probing experiment results

Experiment	1–1	1–2
C (mg/L)	0.4	0.4
N (mg/L)	0.08	0.08
P (mg/L)	0.04	0.04
Flux (L/m^2 h)	34	34
Velocity (m/s)	1.2	1.2
ATP (ng/cm^2 ± 1σ)	205 ± 166	132 ± 51
TOC (mg/m^2 ± 1σ)	122 ± 15	233 ± 112
Flux loss (% ± 1σ)	3 ± 15	15 ± 4

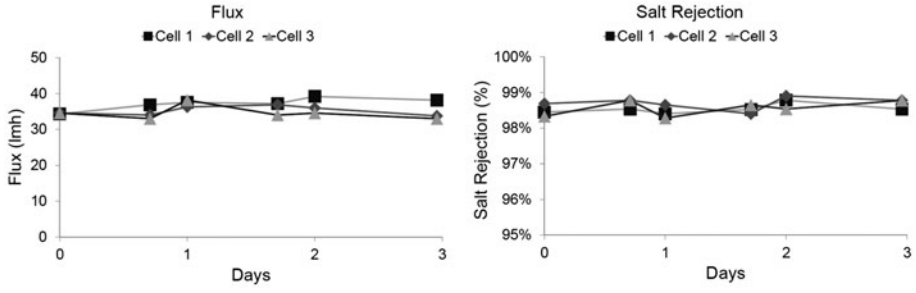

Fig. 3. Operating results example for flux and salt rejection.

3.2. Cross-flow velocity optimization

Since the cross-flow velocity in the first set of experiments was much higher than the one typically observed in an element, the effect of reducing the feed flow was explored. High cross-flow velocity is expected to impose a high shear stress on the bacteria, but it may also be very disruptive to the growing biofilm and cause sloughing, which may create measurement variability. Keeping all other conditions the same, but reducing the cross-flow velocity of 1.2–0.6 m/s, provided a reduction in the variability. The results of two experiments are summarized in Table 6. Further optimization of the nutrient loading was pursued in an attempt to reduce the measurement variability.

3.3. Nutrient loading optimization

Since mature biofilms slough over time, the formation of a less mature biofilm during the 3 d test was targeted by lower nutrient concentrations. The operating flux was also lowered to further slow the rate of the biofilm. Both were expected to reduce the differences in the ATP and TOC measurements between cells at the end of the test. The results are summarized in Table 7. When comparing the ATP and TOC results of experiment 3–1 (Table 7) to 2–1 and 2–2 (Table 6), the reduced flux did not appear to significantly lower the amount of biofilm formed. Lowering the nutrient level, however, lowered the ATP and TOC values, but they were still well above the detection limit of the methods. The relative standard deviations were similar to those in Table 6; however, test conditions with lower levels of nutrients were chosen for the final test validation since there is less risk of reaching a too mature biofilm, prone to sloughing.

Table 7
Nutrient loading effect on lower flux experiments

Experiment	3–1	3–2	3–3
C (mg/L)	0.4	0.2	0.1
N (mg/L)	0.08	0.04	0.02
P (mg/L)	0.04	0.02	0.01
Flux (L/m^2 h)	20	20	20
Velocity (m/s)	0.6	0.6	0.6
ATP (ng/cm^2 ± 1σ)	280 ± 50	76 ± 26	21 ± 5
TOC (mg/m^2 ± 1σ)	91 ± 11	59 ± 3	17 ± 5
Flux loss (% ± 1σ)	3 ± 3	3 ± 2	3 ± 2

3.4. Testing method validation

Operating at lower flux and nutrient loading levels of 0.1–0.2 mg/L carbon provided acceptable results on

Table 6
Results at lower cross-flow velocity

Experiment	2–1	2–2
C (mg/L)	0.4	0.4
N (mg/L)	0.08	0.08
P (mg/L)	0.04	0.04
Flux (L/m^2 h)	33	32
Velocity (m/s)	0.6	0.6
ATP (ng/cm^2 ± 1σ)	139 ± 3	200 ± 38
TOC (mg/m^2 ± 1σ)	132 ± 8	105 ± 15
Flux loss (% ± 1σ)	5 ± 4	6 ± 6

Table 8
Validation of the quick biofouling test at 0.2 ppm C

Experiment	4–1	4–2	4–3
C (mg/L)	0.2	0.2	0.2
N (mg/L)	0.04	0.04	0.04
P (mg/L)	0.02	0.02	0.02
Flux (L/m^2 h)	20	20	20
Velocity (m/s)	0.6	0.6	0.6
ATP (ng/cm^2 ± 1σ)	59 ± 8	98 ± 9	52 ± 3
TOC (mg/m^2 ± 1σ)	28 ± 2	47 ± 2	56 ± 4
Flux loss (% ± 1σ)	0 ± 1	5 ± 3	2 ± 1

Table 9
Validation of the quick biofouling test at 0.1 ppm C

Experiment	4–4	4–5	4–6
C (mg/L)	0.1	0.1	0.1
N (mg/L)	0.02	0.02	0.02
P (mg/L)	0.01	0.01	0.01
Flux (L/m^2 h)	20	20	20
Velocity (m/s)	0.6	0.6	0.6
ATP (ng/cm^2 ± 1σ)	34 ± 11	18 ± 7	8 ± 4
TOC (mg/m^2 ± 1σ)	96 ± 35	15 ± 2	14 ± 3
Flux loss (% ± 1σ)	2 ± 1	2 ± 2	0.7 ± 0.5

the three flat cells operated in parallel. Final validation of each of these conditions was completed by conducting three replicate experiments (Tables 8 and 9).

Good reproducibility and acceptable standard deviations were observed within each run. The standard deviation and amount of fouling are in accordance with other biological fouling test found in the literature [18–20]. Nevertheless, variability between runs still exists, which may be due to uncontrolled changes in the natural water composition [17]. The results are summarized in Table 8. With these optimized conditions, only a very small change in flux was noticed, but the standard deviation of the measurement was low enough to be used to compare biofouling formation.

Using only 0.1 ppm C as nutrients, in general, provided less biofouling than 0.2 ppm C, as can be observed in Table 9. Experiment 4–6 had especially low levels of biofouling, which is closer to the detection limit of the measurements, so the relative error in the measurement of ATP and TOC is more pronounced. Because of this, the nutrient loading of 0.2 ppm C was chosen in the final test protocol.

4. Conclusions

An accelerated, 3 d biofouling test protocol to compare biofilm formation on reverse osmosis membranes coupons has been developed using a membrane flat cell unit. Measuring the ATP and TOC extracted from coupons of membrane and feed spacer after the prescribed method presented in this report was an effective means to quantify biofouling. Good reproducibility between the three parallel operated flat cells is obtained. The feed velocity, permeate flux, and nutrient dosing levels were each evaluated and optimized. Of these three variables, nutrient dosing level had the biggest impact on improving the measurement variability within a test. The method development work completed in this study provides the foundation to enable rapid screening of the biofouling resistance of new reverse osmosis membranes.

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