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Method for distinguishing between abiotic organic and biological fouling of reverse osmosis elements used to treat wastewater

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ABSTRACT

Fouling is one of the issues that is hindering the long-term performance of reverse osmosis systems. The aim of this study is to selectively quantify the extracellular polymeric substances (EPS) from the organic substances present in samples of autopsied elements. Samples were collected from elements used to treat wastewater, operated under two different fouling scenarios. In the first, fouling was mainly organic while in the second, biofouling was promoted by dosing nutrients. The overall percentage of carbohydrates and proteins as organic foulants was quantified. In the first test, the percentage of the foulant consisting of EPS was only 19–34% vs. approximately 100% in the biofouling scenario. At the same time, the increase in the feed-concentrate pressure for the elements after the first test was only 50%, but more than 500% after the second test. This confirms that quantifying the EPS percentage on the foulant can be useful for determining the extent of biofouling versus abiotic organic fouling.

Keywords: Biofouling; Organic fouling; EPS; Wastewater; Reverse osmosis; Pressure drop; Protein; Carbohydrate

1. Introduction

1.1. Fouling in reverse osmosis

Reverse osmosis (RO) is often used as one of the most cost-effective strategies for producing high quality water for a variety of applications. However, fouling is still one of the major hurdles in membrane technology and especially in RO systems, because it increases the energy needed and requires frequent shutdowns for cleanings [1]. When designing a new system, it is very complex to predict the severity of fouling, which might result in a higher frequency of cleaning and reduced productivity. Therefore, improving the ability to predict, troubleshoot and reduce the fouling of reverse osmosis systems continues to be a topic of great interest.

There are different types of membrane fouling. The two most problematic types are biological and organic [2]. Biological fouling is usually associated with an increase in differential pressure in the first stage pressure vessel [3–5]. Organic fouling usually causes an increase in resistance to transport water through the membrane,which reduces permeate flow [6]. Both types of fouling usually occur together so it is difficult to optimize systems because steps to improve biological fouling may worsen organic fouling and vice versa. The characteristics and distribution of each type of fouling must be studied if membrane performance is to be more sustainable [7]. Determining whether biological or organic fouling is dominant is important for designing improvements in pretreatment. If biopolymers such as proteins or carbohydrates are identified as the primary type of fouling, biocide dosing or nutrients limitation using biological pretreatment would likely improve RO fouling problems [8]. However, if the source of the foulants is not determined and fouling was actually caused by abiotic organic compounds, these

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same pretreatments solutions might result in inefficient fouling control. Unfortunately, commonly used fouling quantification techniques do not reveal the source of the organic compounds present in fouling samples. Information about the type of RO contamination is also needed so that cleaning recommendations can be adapted to the pilot unit systems [9].

1.2. Fouling characterization

System fouling is assessed by monitoring performance and analytically characterizing the foulants. Changes in permeate flow rate, feed pressure, salt passage and pressure drop over time are symptoms of the onset and severity of fouling. After operation elements are usually autopsied. ATP (adenosine triphosphate), TOC (total organic carbon) and TN (total nitrogen) are the most common analytical characterization techniques used to study both organic and biological fouling [10]. In most cases, biological and organic fouling are found together [11,12]. Only system differential pressure and foulant ATP concentration can be correlated with biological fouling [13].

1.3. Biofouling vs. organic fouling: characteristics and composition

The main component of biofouling is a polymer matrix excreted by bacteria [14]. This matrix is a strongly hydrated mixture of polysaccharides, proteins, nucleic acids and lipids known as extracellular polymeric substances (EPS) [5,15]. Polysaccharides and proteins are, on a mass basis, the main components of the biofilm matrix [16]. The amount and type of proteins and carbohydrates found in EPS depends on the bacteria strain, environmental conditions and stress events [17,18]. Carbohydrates and proteins are rich in organic carbon, and peptides are also rich in nitrogen, so TOC and TN concentrations are high when biofilms are analyzed [19].

Organic fouling occurs when organic compounds found in the feed water are deposited on the membrane surface. These compounds usually contain carbon and nitrogen and, therefore, can be detected by a positive response for TOC and TN.

It has been suggested that comparing the ATP to TOC levels can be a way of determining whether organic foulants come from biofilm formation or abiotic compounds [1]. However, ATP degrades quickly and is highly sensitive to external factors [20] that can influence the bacterial metabolic state (chemical cleaning, biocides, etc.), so ATP levels can be unreliable. The ATP concentration does not correlate with the presence of EPS under certain conditions [21,22]. The presence of biomass rather than its metabolic activity is more directly correlated with the fouling problems in RO systems [23]. Nonetheless, TOC and TN levels alone are not enough to differentiate the source of the foulants quantified (biological or abiotic).

Liquid chromatography-organic carbon and nitrogen detection (LC-OCD-OND) has emerged as a useful technique for identifying and quantifying the various fractions of the natural organic matter pool (protein and polysaccharide, humics, fulvics, building blocks and low-molecular-weight organics) [24]. This technique has been successfully used in some studies to calculate the fraction of organic carbon associated to biopolymer (proteins and polysaccharides) [25,26]. However, the results can be complex to interpret and the technique is generally not available for routine membrane fouling samples [27]. Other studies suggest that the proportion of biopolymer on a membrane foulant sample can be calculated using the area of the pyrochromatograms, obtained using pyrolysis gas chromatography–mass spectrometry [28]. However and like with the LC-OCD-OND method, analyzing samples is time consuming and technically challenging.

This paper reports a simplified approach for determining the relative extent of biofouling over organic fouling in RO samples. Carbohydrates and proteins are the main constituents of the biofilm matrix and whether they are present or not, can discern between biological and organic foulants [29]. The proportion of carbohydrates and proteins in the TOC and TN pool will be calculated to determine the fraction of biological carbon and nitrogen, respectively. To validate the viability of the approach, the protocol will be applied to determine the EPS fraction of various RO samples operated under two different conditions. The protocol uses techniques that are available at most analytical laboratories. The proportions intend to provide information about the source and proportion of these compounds in complex fouling samples.

2. Materials and methods

Reverse osmosis elements were exposed to two different fouling conditions and autopsied to provide samples for analysis. The concentrations of the parameters quantified were compared to correlate the biopolymer levels with to the testing conditions.

2.1. Fouling field testing

Two separate RO element exposure tests were conducted, one with a high and one with a low biofouling tendency. Both tests used water from the secondary effluents collected from a municipal wastewater treatment plant (Vilaseca WWTP, Spain). The typical composition of the feed water is summarized in Table 1. The high chemical oxygen demand (COD) and TOC concentrations present an inherent organic fouling potential for the RO elements. Foulants were mainly non-biodegradable organic compounds, according to the ratio of COD to the biological oxygen demand (BOD5) [1]. To promote high biofouling

Table 1
Characterization of feed wastewater

Feed water	Concentration
Total dissolved solids (mg/L)	1,880
Total suspended solids (mg/L)	0.14
COD (mg/L O_2)	21.2
BOD5 (mg/L O_2)	2.1
TOC (mg/L)	6.0
ATP (ng/L)	38

levels, an external dosing pump was used to dose nutrients in the feed stream and to stimulate bacteria present in the feed water (*trial with nutrients added*) [30]. In the experiment aiming for a low biofouling tendency, no dosing was used (*trial with no nutrients added*).

In each trial, either six (*trial with nutrients added*) or eight (*trial with no nutrients added*) 1.8-inch-diameter by 12-inch-long reverse osmosis elements were operated in parallel and allowed to treat the wastewater without any recycling. Similar conditions were used in both trials (10 bar, 4.5% recovery and 25 L/m² h) for approximately one week (temperature from 17–26ºC). To promote biofouling nutrients were dosed in the feed water (*trial with nutrients added*). These include a source of carbon (0.1 mg/L C as acetate), nitrogen (0.02 mg/L N as nitrate) and phosphorous (0.01 mg/L P as phosphate). These compounds are readily bioavailable and promote rapid biofilm growth. After each test, exposed elements were autopsied and samples taken for analysis.

2.2. Membrane foulant extraction

After opening the elements lengthwise, a 4×4 cm (16 cm²) sample from the middle region of the membrane and spacer was placed in a glass vial. A 20 mL phosphate-buffered saline (PBS, VWR) solution was added to dissolve the foulant present [31]. Strong acid cation resin (DOWEX-®MARATHON™C Na^+, Dow Chemical) was used (1 g) to improve EPS solubility [32,33]. The vial was sonicated using an ultrasonic cleaning bath (FB15061, Fisher Scientific) at room temperature for 2 min in triplicate [34,35]. After this treatment, all the foulant was fully dissolved. Samples were stored at –21ºC until analysis.

2.3. Adenosine triphosphate quantification

Adenosine triphosphate (ATP) concentration was used to estimate the amount of viable biomass present [17]. ATP content in the fouling extract was measured using a luminometer (Celsis Advance). The amount of light produced was converted to ATP concentration using the equipment calibration curve.

2.4. Total organic carbon and total nitrogen quantification

Both biological and organic foulants are rich in organic carbon and nitrogen. Thus, total organic carbon (TOC) and total nitrogen (TN) are good methods for capturing both types of fouling. TOC and TN were determined by catalytic combustion using a TOC/TN analyzer (TOC-L Shimadzu), calibrated using potassium hydrogen phthalate and urea-BSA (1:1), respectively.

TN is the sum of total inorganic nitrogen (TIN) and total organic nitrogen (TON) in a sample. However, fouling analysis from previous studies, using the same wastewater, has shown that the nitrogen present was over 92% organic [11]. Consequently, in this study it was assumed that the inorganic nitrogen portion (NH_4, NO_3 and NO_2), was negligible in comparison to the organic nitrogen. The TN analysis of the autopsied elements was considered equal to TON.

2.5. Carbohydrate quantification

The polysaccharides from EPS were measured using the Dubois method, also known as the phenol-sulfuric acid method [36]. The Dubois method has been widely reported for EPS polysaccharide quantification as a simple colorimetric method [37–40]. The carbohydrate concentration of the fouling extract was detected colorimetrically using the Hach DR 5000 spectrophotometer (λ = 490 nm).

Glucose (Sigma Aldrich) was used to calibrate the method. Glucose (180 g/mol) contains a significant amount of carbon (40 wt% C) [25]. This factor was used to convert glucose concentration to carbohydrate carbon units ($C_{carb.}$), so that it could be compared with the TOC measurements.

2.6. Protein quantification

The Bicinchoninic acid method (BCA) [41,42] was selected to quantify proteins in the membrane fouling extract. The BCA method can be readily used as a fast and simple colorimetric kit (Micro BCA™ Protein Assay Kit, Thermo Fisher). Absorbance was measured using the Hach DR 5000 spectrophotometer (λ = 562 nm).

Bovine serum albumin (BSA, Sigma Aldrich) was used to calibrate the method. Albumin (66,463 g/mol) contains a significant amount of nitrogen (16 wt% N) [43]. This factor was used to convert the BSA concentration to protein nitrogen units ($N_{proteins}$), so that it could be compared with the TN measurements.

2.7. EPS fraction quantification

To compare the carbohydrate and protein results with the TOC and TN concentrations, the theoretical correlations for the calibration compounds used were checked.

For organic carbon, glucose TOC results were the same as the theoretical carbon percentage (40 wt% C). The TOC results for BSA showed that it contained a 2.5 wt% C, a factor that was later used to calculate the protein carbon ($C_{protein}$). Although this percentage was lower than the reported BSA elemental composition [43], it might be due to a low oxidation yield of the BSA carbon.

The sum of the protein and carbohydrate carbon divided by the TOC result gave the theoretical fraction of organic carbon associated with EPS, according to Eq. (1).

$$EPS\ in\ TOC(\%) = \frac{C_{carb.} + C_{protein}}{TOC} \cdot 100 \quad (1)$$

Likewise, the proportions of nitrogen in BSA and glucose solutions were determined using the TN method. As expected, glucose showed no nitrogen is present. The nitrogen proportion measured for BSA was in agreement with its nitrogen composition (16 wt% N).

The percentage of protein nitrogen divided by the TN result, expressed the fraction of organic nitrogen associated with EPS, according to Eq. (2).

$$EPS\ in\ TN(\%) = \frac{N_{protein}}{TN} \cdot 100 \quad (2)$$

Potential interferences of BCA and Dubois method measurements were also discarded. The presence of glucose and BSA in the sample did not affect the quantification of proteins and carbohydrates, respectively.

3. Results and discussion

ATP, TOC, TN, $C_{carb.}$, $C_{protein}$ and $N_{protein}$ were measured in samples from the elements exposed to the *nutrients added* conditions or the *no nutrients added* environment.

3.1. Wastewater trial with nutrients added

The measured analytical parameters and the corresponding calculations of the composition of the foulants from the elements of the *trial with nutrients added* are summarized in Fig. 1 and Fig. 2. The biological fraction (C-carb., C-protein and N-protein) almost matches the overall organic carbon and nitrogen measured. The material balance was not perfect because of accumulative errors of the various quantification methods involved. However, the results leave little doubt that when nutrients were dosed, most of the foulants present on the membrane were biopolymers (carbohydrates and proteins).

3.2. Wastewater trial with no nutrients added

In the first trial, the carbohydrate and protein concentrations accounted for approximately all the TOC and TN present. However, when no nutrients were dosed, carbohydrates and proteins only account for approximately one fourth of the TOC or TN of the foulants (see Fig. 3 and Fig. 4). These differences could be explained by the high concentration of abiotic organic material present in the feed water, as its high COD/BOD5 ratio (>10) suggests [1].

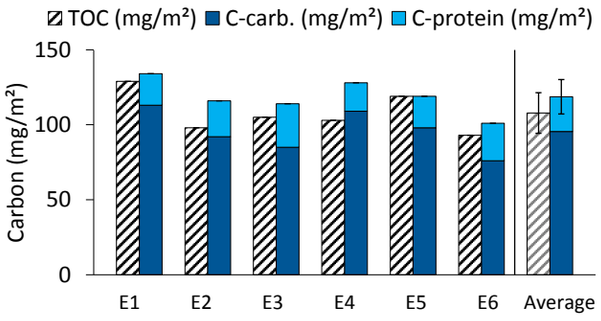

Fig. 1. TOC and carbon from carbohydrates and proteins measurements from elements, E1–E6, operated with nutrients added to the feed water.

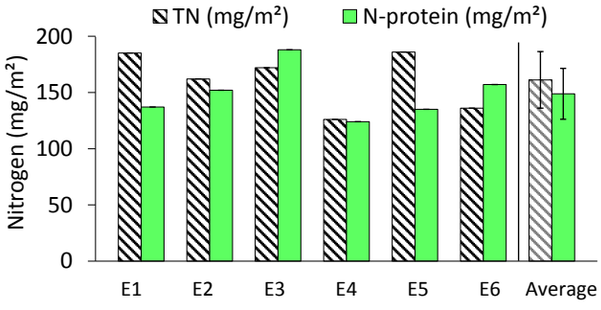

Fig. 2. TN and nitrogen from proteins measurements from elements, E1–E6, operated with nutrients added to the feed water.

3.3. Correlation between EPS fraction and membrane performance

Using the equations described in section 2.7, the EPS fraction was calculated for the elements from the test with and without nutrients (section 3.1 and 3.2, respectively).

The different EPS fractions (based on organic carbon and nitrogen distribution) are plotted in Fig. 5. The same graph also shows the percent increase in the measured feed-concentrate pressure drop for each element at the end of the test. As expected, a clear correlation between the calculated EPS fraction and the dP increase can be observed. When nutrients were dosed (biofouling promoted), the pressure drop increased considerably and biopolymers accounted for almost all the organic nitrogen and carbon measured. However, in the samples from the *trial with no nutrients added*, the differential pressure increases and the EPS fractions were much lower. The method provided similar conclusions as other publications using LC-OCD-OND, where the biopolymer peak for samples containing biofouling was significantly larger than for samples containing organic fouling [44]. However a clear correlation between carbohydrates and proteins, detected by photometric meth-

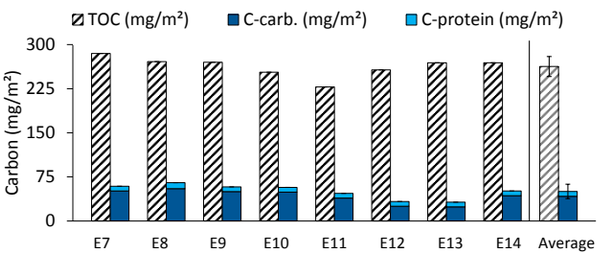

Fig. 3. TOC and carbon from carbohydrates and proteins measurements from samples of elements, E7–E14, operated without nutrients added to the feed water.

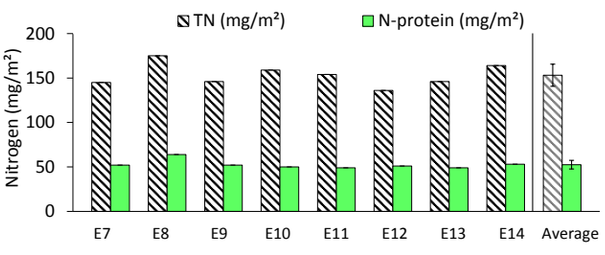

Fig. 4. TN and nitrogen from proteins measurements from elements, E7–E14, operated without nutrients added to the feed water.

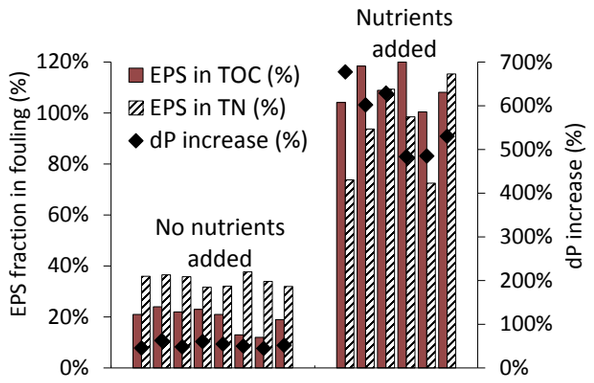

Fig. 5. Correlation of membrane performance and EPS fraction for the two testing conditions.

Table 2
Average ATP, dP and EPS fraction summary for the two testing conditions

Parameter	*No nutrients added test*	*Nutrients added test*
EPS in TOC (%)	19±4	111±9
EPS in TN (%)	34±2	94±18
ATP (ng/cm²)	1±0.1	40±10
dP increase (%)	51±5	568±80

ods, and performance decline, caused by biofouling, was not observed in other publications [45].

A comparison of ATP results revealed good correlation with EPS percentages obtained in the two sets of samples (Table 2). The operational and analytical results both showed that when nutrients were used, fouling observed was mainly attributed to biofilm growth. Foulants found on the samples had higher concentrations of ATP as compared to foulants found on the elements operated without nutrients added. This highlights the importance of readily bioavailable nutrients to enhance bacteria colonization, reproduction and biofilm formation.

4. Conclusions

The fouling found in RO elements that treatwastewater is usually a complex mixture of biological and abiotic organic compounds. The methods commonly used for membrane fouling quantification are non-specific and measure all organic compounds present as either total organic carbon or total nitrogen. The analysis of the contribution of carbohydrates and proteins in the measured TOC and TN values, can be linked with the proportion of EPS in the fouling. This allows a more clear understanding of whether the main source of the organic contaminants is biologic or abiotic. Samples from elements taken from two testing conditions were used to validate that the new proposed method can distinguish between the foulants accumulated in a high biofouling environment (nutrients dosed) or a low biofouling environment (no nutrients).

When nutrients were added, the percentage of TOC and TN accounting for carbohydrates and proteins was nearly 100%. However, the percentages were much lower when no nutrients were dosed, indicating that a greater fraction of the fouling was caused by abiotic organic compounds. Additionally, the concentration of bacteria measured as ATP and the pressure drop increase was found to be much higher than when the feed water was dosed with nutrients.

The ability to determine the proportion of EPS in the TOC and TN results has shown to be useful to determine the source of the compounds present as membrane foulants. The method described in this paper, will enable industrial water treatment plants to easily quantify the proportion of biological fouling present versus the proportion of non-biological organic fouling. Once assessed, pretreatments, operating conditions and cleaning protocols can be adjusted to tackle the primarily type of fouling occurring. Pretreatment optimization strategies, such as biocide dosage or nutrient limitation, could be implemented when biofouling is determined to be the main type fouling. Additionally, chemical cleaning protocols can be adapted for the predominant type of foulant present, such as the use of sanitizers or protease-based enzymatic cleaners for biofouling. Pre-concentration protocols for water samples will be explored in the future, to adapt the method to characterize the feed water foulant composition. This will provide a method to monitor the removal of each particular foulant type after specific pretreatments steps or linked the compounds found on the feed water with the compounds causing RO fouling.

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