IDENTIFICATION OF TRANSPARENT EXOPOLYMER PARTICLES (TEP) ON RO MEMBRANES

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Abstract

The relevance of studying transparent exopolymer particles (TEP) in surface water has been increasing in recent years in the field of membrane water treatment. TEP are natural organic substances seasonally abundant in most freshwater sources and often in higher concentrations in seawater. Some TEP may pass through some pre-treatments and may potentially accumulate in RO systems. Their sticky surfaces make them a favourable platform for bacteria and colloidal materials to attach to as well as facilitate their adhesion to membrane surfaces.

In this research, autopsies performed on reverse osmosis (RO) membranes revealed the presence of TEP on membrane surfaces. The main objective of this research was to develop a technique to visualize TEP on membrane surfaces.

Fouled membranes from full scale reverse osmosis (RO) plants treating fresh water and coastal seawater were analyzed in this study. Membrane and feed spacer samples were stained with alcian blue - a dye specific for acidic polysaccharides. The stained TEP were visualized using an optical microscope. Additionally, TEP and biopolymer concentrations were measured using spectrophotometric technique and liquid chromatography organic carbon detection (LC-OCD), respectively. Some membrane samples were also analyzed using scanning electron microscopy (SEM) to verify the foulant accumulation.

Based on microscopy and spectrophotometric analyses, TEP was found present in all the membrane samples. Not only TEP could be visualized on the membranes but other gel-like substances such as proteins and possibly other polysaccharides and colloidal materials were also present. Some particles and inorganic flocs were found embedded on some TEP gels. TEP and other foulant were also found in the feed spacer. Some suspected bacterial colonies were also found within the vicinity of TEP gels on the spacer mesh. Accumulation of organic and inorganic foulants and the presence of bacteria were also found in SEM images.

I. INTRODUCTION

The importance of studying transparent exopolymer particles (TEP) has been increasing in recent years in the field of membrane water treatment [1, 2 and 3]. TEPs are natural organic substances (negatively charged acidic polysaccharides) seasonally abundant in most freshwater sources and often in higher concentrations in seawater [4]. TEP are mostly produced by algae (e.g. diatoms, dinoflagellates, etc.) and bacteria in surface water environments [5]. Considering that their sizes may vary between 3 nm to 200 μm, some TEP may pass through some pre-treatments and reach the RO membrane system downstream. Their sticky surface makes them a favorable platform for bacteria and colloidal material to attach to and facilitates their attachment to membrane surface as well [6].

Several research have been using alcian blue, a dye specific for acidic polysaccharides, for staining TEP to visualize them under the microscope [6, 7]. The staining mechanism is based on the ionic reaction between TEP (negatively charged polysaccharides) and the cationic dye alcian blue [8]. The result of the reaction is non-ionic blue precipitate, revealing the structure of the transparent TEP gels.

The main purpose of performing this study is to apply the alcian blue staining technique in visualizing TEP substances on RO membrane surfaces. This new application was done together with known parameters, such as organic and inorganic composition of the foulants, to quantitatively support the qualitative microscopy analysis.

II. MATERIALS AND METHODS

2.1 RO Membrane Samples

The reverse osmosis membrane samples analyzed in this study were from 2 full scale reverse osmosis plants treating coastal seawater and surface (river) water, respectively. The characteristics of the water source and treatment process of the plants are described in Table 1:

Table 1. Data of sampled reverse osmosis treatment plants.

Plant	Water source	TOC (mg C/L)	TEP >0.4um (abs/cm/L)	Pretreatment	Chemical addition (RO feedwater)	NPD increase (%)	Element analyzed
1	Seawater	1.0 - 2.1	0-8	– 50μm strainer – Coagulation (Fe or Al) – UF	acid	~ 5	1st and 6th element; 1st stage
2	River water	1.9 - 4.5	0-3.5	– Holding basin – 100μm strainer – Coagulation (Fe) – UF	anti-scalant/acid	>25	1st element, 1st stage

2.2 Membrane Autopsy

The spiral wound RO membranes were taken out by the plant operators and sent to UNESCO-IHE laboratory for autopsy. The membranes were delivered wrapped in plastic bags and consisted in three leaves of the spiral wound element containing each feed spacer, membrane and permeate spacer. The membranes were kept at 4 °C until the moment of analysis.

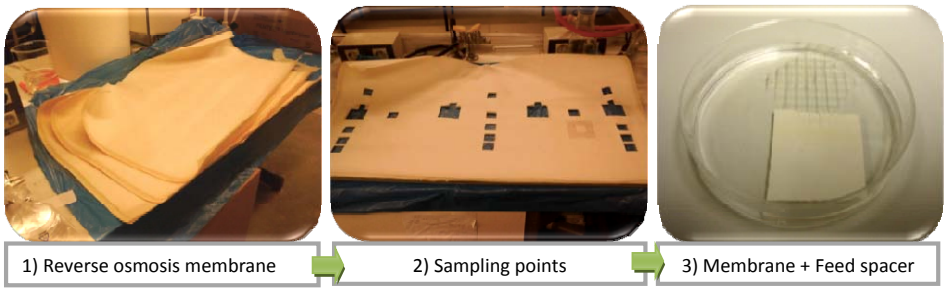

Figure 1**.** Reverse membrane autopsy: From the middle leaf of each membrane, 3 samples were cut (feed end, middle and concentrate end). The samples were then placed on petri-dishes and stored at 4 °C until analysis.

2.2 Alcian Blue Staining and Microscopy

Alcian blue 0.025% solution was prepared by mixing for 18 hrs at 1000 rpm 300 mL of ultrapure water with 0.075 g of Alcian blue 8GX powder (Sigma-Aldrich®). Before adding the alcian blue powder, the pH of the ultrapure water was lowered to 2.5 by adding some drops of acetic acid. A small volume of the standard solution was filtered twice at vacuum pressure 0.2 bar through 0.05 μm polycarbonate filters to eliminate flocks.

The staining procedure is the following: 1) Place a piece of the sample of about 3 cm x 3 cm on a petri-dish; 2) Soak the sample in ultrapure water for about 1 minute and discard the water; 3) Add the filtered alcian blue solution until it totally submerged the membrane samples for 10 minutes and then discard the liquid solution; 4) Rinse the sample again with ultra pure water for 1 minute and discard the water. The sample is then placed on glass slides for microscopy analysis. Figure 2 illustrates the above-mentioned procedure.

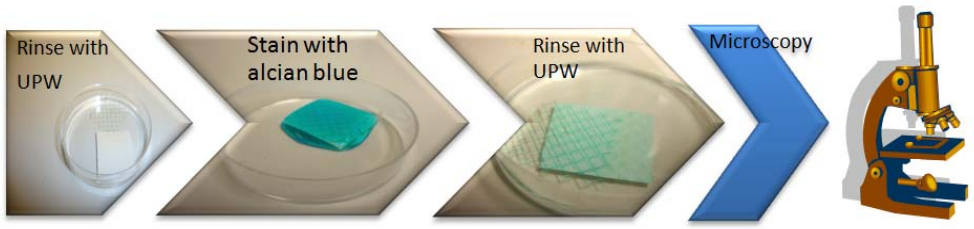

Figure 2. Staining procedure of RO membrane samples for TEP visualization.

The microscope used was an Olympus BX51 optical microscope set at 200x magnification. Since reverse osmosis membranes are rather thick, it was necessary to use external lights for microscopy. A microscope illuminator was used to light the upper surface of the membrane. In this way, it was also possible to visualize gel-like substances. In the case of the feed spacer there was no need to use external lights.

This procedure can be applied to other types of flat-sheet membranes (e.g. UF/MF). When the TEP are not firmly attached to the membrane surface, it was unavoidable not to see TEP particles floating during rinsing. In this case, it may be advisable to skip the rinsing steps and the alcian blue solution must be applied very carefully. Floating particles can be captured with a small volume pipette and placed on a glass slide and viewed under the microscope.

2.3 Supplementary Analyses

A series of analyses were performed on the membrane foulant to study the presence of TEP, organic and inorganic materials using different techniques (Table 2).

Table 2. Tests performed on the reverse osmosis membranes sampled.

	Microscopy for TEP	TEP concentration	Microscopy for protein	LC-OCD	SEM	Aluminum	Iron
Plant #1	X	X	X	X	X	X	X
Plant #2	X	X	X				

To analyze the foulant composition, membrane samples were submerged in ultra-pure water and ultrasonicated. The membrane and feed spacer samples were placed on containers filled with a known volume of ultrapure water and ultrasonicated until the membrane and feed spacer appeared clean. The procedure is illustrated in Figure 3.

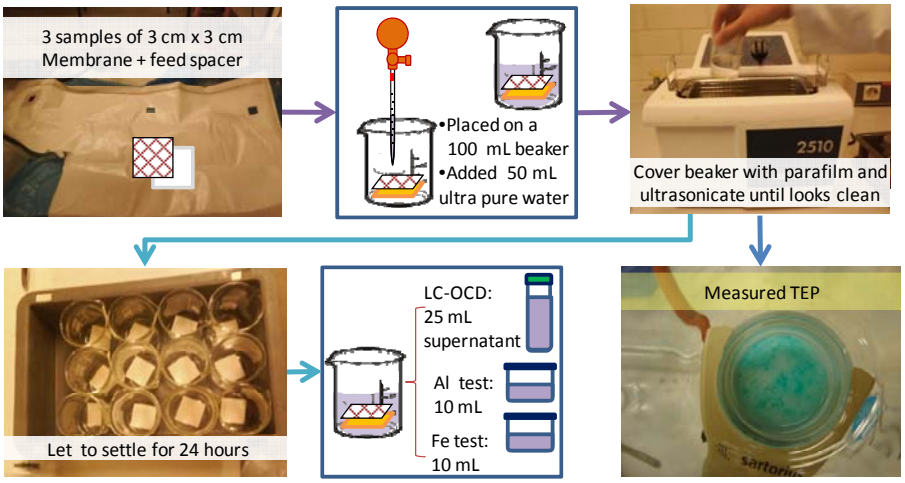

Figure 3. Extraction of foulants using ultrasonication. The foulants extracted from the membrane and feed spacers were re-suspended in ultra pure water.

2.3.1 - TEP Concentration Test – For both plants samples, TEP concentrations of extracted foulants were measured using the modified TEP method proposed by Villacorte et al. [2]. This method is still under development and is based on the method developed by Passow and Alldredge [9]. Results were calculated as abs/cm/m^2 of membrane and spacer area.

2.3.2 - Microscopy for Protein Visualization – For visualizing proteins, staining with Coomassie Brilliant Blue was performed in the samples. The Coomassie Brilliant Blue dye has been used for many years for staining proteins in analytical biochemistry and later in aquatic chemistry [10]. Protein substances react with dye and turn normally transparent protein gels to bluish colour. The staining procedure is similar to the one used for TEP staining with alcian blue (Figure 2).

2.3.3 - Liquid Chromatography Organic Carbon Detection (LC-OCD) Test – Samples for LC-OCD were prepared in UNESCO-IHE laboratory (Figure 3) and sent for analysis to a commercial Het

Waterlaboratorium in Haarlem, The Netherlands. Integration of chromatogram results was performed in UNESCO-IHE to calculate the carbon concentration of the biopolymer fraction of NOM.

2.3.4 - Scanning Electron Microscopy (SEM) – Scanning electron microscopy was performed at Wetsus laboratory in Leeuwarden, The Netherlands. The samples were submerged in artificial sea water and stored at 4°C until the day of the test.

2.3.5 - Aluminum and Iron Concentrations Tests – The eriochrome cyanine R method was used to measure aluminum. Iron was measured using atomic absorption spectrometry (AAS). Results were converted from mg/L to mg/m^2 of membrane and spacer area.

III. RESULTS AND DISCUSSION

3.1 Plant #1 (SWRO) Results

For this plant, the front (1st element, 1st stage) and rear (6th element, 1st stage) elements were tested. In this paper, the front element is designated as membrane #1 and the rear element as membrane #2.

3.1.1 - TEP Microscopy (Plant #1) – The pictures taken from microscopy analysis showed the presence of TEP in the membrane surface and the feed spacer (Figure 4). Stained membrane samples showed significant concentrations of TEP materials deposited on Membrane #1. In contrast, the presence of TEP on Membrane #2 was very minimal. More TEP were also detected around the spacer mesh fibers of Membrane #1 while few small TEPs were found in the spacer mesh of Membrane #2. In terms of coverage, TEP has been heterogeneously spread all over the membrane area of Membrane #1. Gel thickness can vary significantly from different parts of the membrane samples. This can be observed based on the shades of blue color of the stained gels. The bluer is the layer, the thicker is the gel.

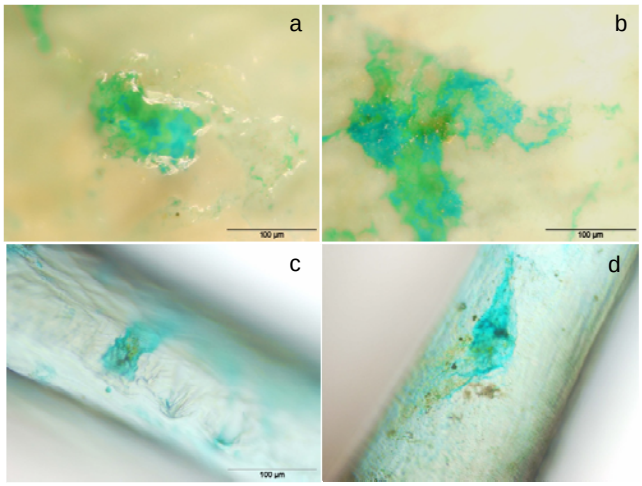

Figure 4. Microscope pictures of reverse osmosis membrane Plant#1, membrane #1: a) Membrane middle sample; b) Membrane concentrate end sample; c) Feed spacer middle sample, and d) Feed spacer concentrate end sample. All pictures 200x magnification.

Aside from alcian blue-stainable substances (TEP), several other forms of foulants were also observed on the membranes. Some dark particles (dark brown to dark gray) were found on the membrane surface,

mostly embedded within a layer of gel-like materials. This type of material was also found around the fibers of the spacer mesh (mostly on the rough side) and sometimes resembling a colony of bacteria (cluster of several small dots). The most abundant among non-TEP foulants were the slimy brown-orange substances. Based on visual observation and elemental measurement, these materials may have consisted of inorganic materials. Several transparent slimy gels that were not stained by Alcian Blue were also observed on the membrane surface. These can be other forms of biopolymers such as neutral polysaccharides and proteins. The dye can only react with acidic polysaccharides and not with neutral species nor with proteins other than glycoprotein. However, it is possible that some of the non-stainable materials were originally acidic polysaccharides but eventually neutralized by iron compounds present in membrane surface.

3.1.2 - TEP Concentration (Plant #1) – TEP measurements showed the relative difference in total TEP (> 0.1um) concentration between Membrane #1 and Membrane #2 was about a factor of 5 (Figure 5).

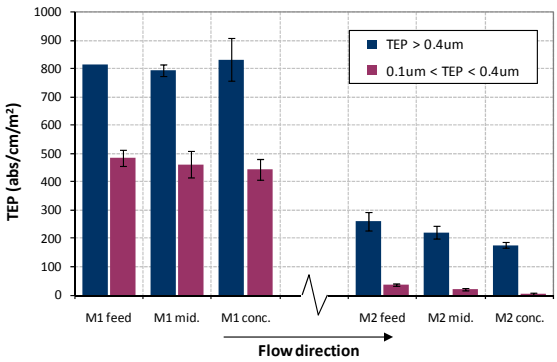

Figure 5. Relative TEP concentration of extracted foulants from membrane samples following the flow direction of feedwater.

3.1.3 - Microscopy for Protein Visualization (Plant #1) – In Membrane #1, most of the protein materials were found along the section where part of the spacer mesh touches the membrane surface (Figure 6). In Membrane #2, few clusters of protein materials were found on some of the membrane samples (Figure 6).

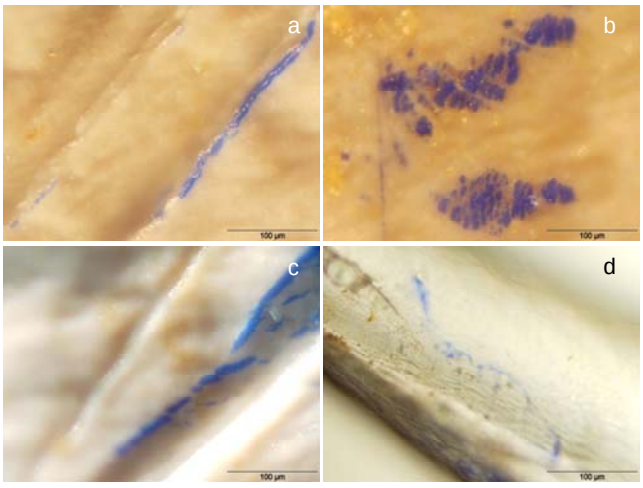

Figure 6. Coomassie blue stained proteins (CSP) found on the RO membrane and spacer samples: a) and c) Membrane #1 and, b) and d) Membrane #2.

3.1.4 - LC-OCD Results (Plant #1) – Since TEP was found to be abundant on membranes #1 and #2, specifically in Membrane #1, it was expected that polysaccharides were the dominant component of biopolymers. The protein concentration of the foulant materials extracted from membrane samples was found to range from 1.5 to 24.2 mg-C per square meter of membrane and feed spacer area. This was about 35% of the total biopolymer (BP) concentration of Membrane #1 and about 60% of BP in Membrane #2 (Figure 7). Polysaccharides were a dominant component of BP in Membrane 1 (lead element) but not in Membrane #2 (rear element) probably because most of the TEP materials were already deposited in the first 5 elements of the SWRO train. Proteinic substances usually originate from bacteria. Their presence on the RO membranes suggests that biofilm were already building up on the membrane surface, especially in the lead elements. This is not unusual since the plant has been running for almost two years with few chemical cleanings.

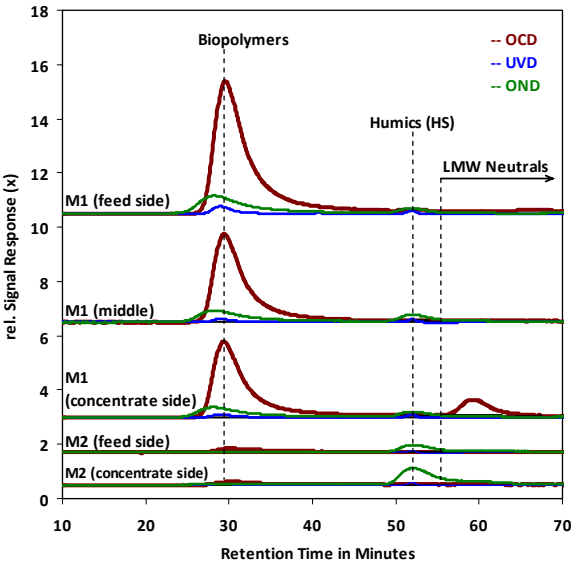

Figure 7. LC-OCD chromatograms of foulant materials extracted from membrane samples.

3.1.5 - Scanning Electron Microscopy (SEM) Analysis (Plant #1) – The SEM pictures showed the presence of bacteria on the membrane surface. Membrane #1 appeared to be more fouled than Membrane #2 (Figure 8). This was consistent with the results of the examination using the optical microscope. Membrane #1 was totally covered with foulants, which was relatively homogenous over the membrane samples. Several bacterial cells were also found (white spheres, Figure 8) mostly embedded in the foulant layer. In several parts of the sample, the RO membrane surface is still visible. Only few and isolated bacterial cells were found on Membrane #2.

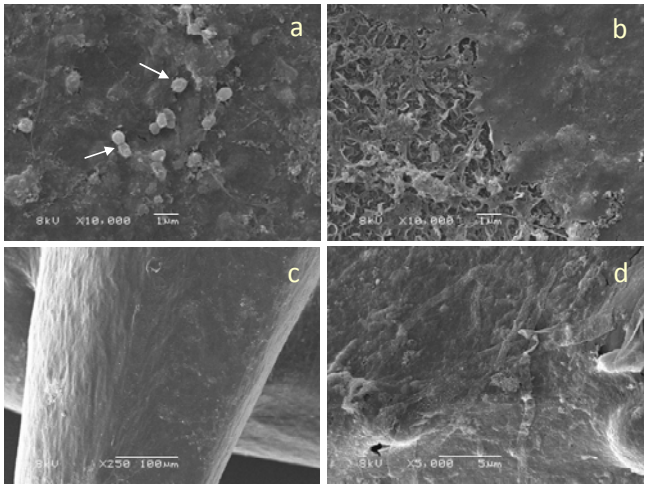

Figure 8. SEM pictures of RO membranes samples: a) Membrane # 1 with bacterial cells visible on top of the foulant layer; b) Membrane # 2 partially covered with foulants; c) Membrane # 1 spacer fiber with white small dots identified as bacterial cells, and d) high magnification photo at the crosslink of the membrane spacer showing accumulation of long fibrous organic materials.

3.1.6 - Aluminum and Iron (Plant #1) – Results showed that there was more iron than aluminum compounds in the accumulated foulants on the RO membrane (Figure 9). The origin of these iron compounds is likely from the iron-based coagulant applied prior to the UF system. In the case of the aluminum the values obtained were below the detection limit.

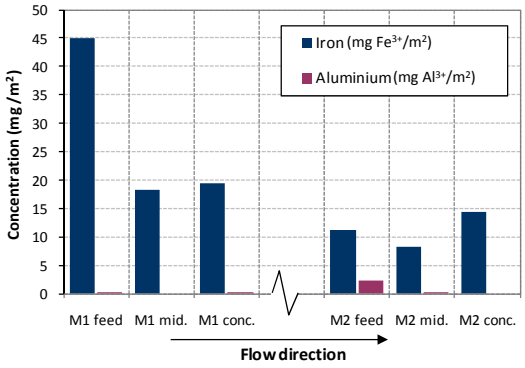

Figure 9. Concentration of iron and aluminum accumulated on RO membrane samples.

3.2 Plant #2 (River water) Results

3.2.1 - TEP Microscopy (Plant #2) – Microscopy analysis of Alcian blue stained membranes showed abundant presence of TEP. Several parts of the membrane were covered with thin layer of TEP dyed in light blue color (Figure 10). Spacer samples also showed deposition of TEP-like materials. TEP accumulation was considerably high near the feed side of the membrane. The accumulation appears to decrease along the feed channel to the direction of the concentrate (Figure 10).

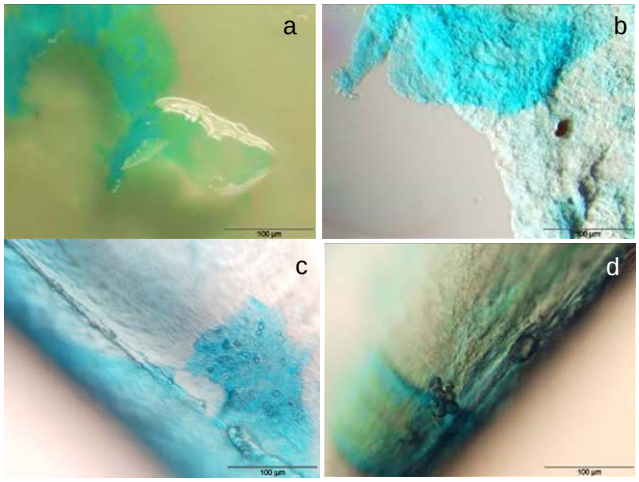

Figure 10. Microscope pictures of reverse osmosis membrane Plant #2: a) Membrane feed end sample; b) Material attached to feed spacer, feed end sample; c) Feed spacer, feed end sample; d) Feed spacer , concentrate end sample. All pictures 200x magnification.

3.2.2 - TEP Concentration (Plant #2) – TEP measurement of extracted membrane foulant materials showed highest TEP concentration near the feed end of the membrane and lowest concentration near the concentrate end (Figure 11). Total TEP near the feed was 928 abs/cm/m^2 while it was 447 abs/cm/m^2 near the concentrate end. Majority of the TEP are larger than 0.4 um, comprising of about 57 to 88% of total TEP (>0.1 um).

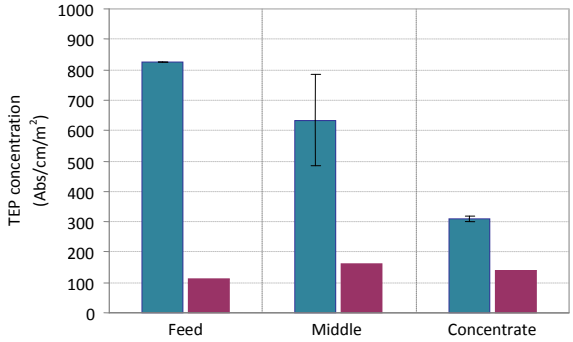

Figure 11. TEP levels of extracted foulants of different parts of the membrane, plant #2.

3.2.3 - Microscopy for Protein Visualization (Plant #2) – Coomassie blue stained protein particles (CPS) were also present on the membrane surface. Photographs from optical microscopy showed accumulation of these materials on the membrane but it was much less than the TEP accumulation. Near the feed end of the membrane, mostly fibrous CSP's were found while it was mostly beads/spots of CSP's found near the concentrate end (Figure 12). These are suspected protein substances possibly from bacterial colonies growing on the membrane.

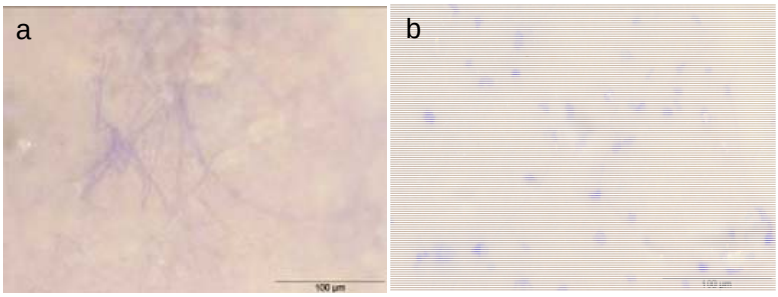

Figure 12. Protein materials (stained blue) found on RO membrane samples: a) Feed end and, b) Concentrate end.

During microscopy analysis, several unidentified foulant materials were found. Some dark brown colored materials were found on the membrane samples resembling that of dirt and/or bacterial colonies. Some transparent gel-like materials that were not stained by Alcian blue or Coomassie blue were also found. These may be neutral polysaccharides which do not react with either of the two stains used. Some orange materials associated with the coagulant used in the treatment process were also observed. Few large crystalline-like particles (~500 μm) were also found on the membrane samples. The feed spacer has some attached materials like dirt and some particles as well.

IV. CONCLUSIONS AND RECOMMENDATIONS

- By staining with alcian blue it was possible to visualize TEP attached to the surfaces of reverse osmosis membranes and spacers under the microscope. The procedure is simple and the chemical needed is available in the market.
- It is important to consider the staining mechanism of alcian blue. If there are metal ions on the membrane that can neutralize the negatively charged TEP, this may result in under-staining.
- TEP microscopy analyses of fouled elements are important but should be always accompanied by other tests like TEP concentration measurements and others (e.g. ATP, TOC, inorganic ions, etc.). Information of the feed water and treatment process is very useful for a more complete analysis.

V. REFERENCES

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