

# Extra-Cellular Polysaccharides, Soluble Microbial Products, and Natural Organic Matter Impact on Nanofiltration Membranes Flux Decline

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Extra-cellular polysaccharides (EPS), soluble microbiological products (SMP), dispersed bacterial cells, and a well-characterized natural organic matter (NOM) isolate were observed to determine their influence on the flux decline of model nanofiltration membrane systems. Biofouling tests were conducted using bench-scale, flat-sheet membrane modules, fed with particle-free (laboratory) waters and natural waters, some of which were augmented with readily biodegradable organic carbon. The modules were operated  $6.7 \times 10^5$  Pa, and  $21 \pm 2^\circ$  C. Membrane flux-decline was associated with increases in surface EPS mass: between 30 and 80% of normalized flux decline occurred when membrane-associated EPS content increased from 5 to 50  $\mu\text{g}/\text{cm}^2$ . As judged by standard culturing, heterotrophic cell densities recovered from membrane biofilm samples showed no significant correlations with the different carbon sources present in the feedwaters, or flux decline rates. Results suggested that, in the absence of microbiological activity, SMP and NOM have intrinsic membrane fouling properties at levels that are operationally significant to commercial-scale membrane treatment practices. Results also suggested that SMP may have a biofouling potential significantly greater than some types of NOM. Trends obtained relating these compounds with flux decline were successfully described by expanding existing resistance-in-series models.

## 1. Introduction

Nanofiltration (NF) has demonstrated the capacity to control disinfection byproducts precursors, hardness, some pesti-

cides, and other emerging micropollutants (1–2). However, wider application of this technology in the drinking water field is in part hindered by loss of production due to flux decline, caused by membrane fouling. Flux decline associated with the activity of microorganisms on membrane surfaces (i.e., biofouling) is one of the most commonly reported problems among membrane facilities (1, 3–5).

A few studies have focused on describing the classes of polymers and microorganisms that are associated with membrane (bio)fouling (1,6). However, there still remains a basic lack of understanding as to which foulants, i.e., microorganism cells and their fragments, natural organic matter (NOM), extra-cellular polysaccharides (EPS), and byproducts of microbial metabolism (i.e., soluble microbial products (SMP)), govern membrane (bio)fouling, or how their interactions with membrane surfaces affect membrane separation processes. EPS, SMP, and NOM have all been identified as part of a “conditioning layer” that may help retain other fouling materials, as well as directly cause membrane fouling (1,6). The sorption of microbiological cells, EPS, SMP, and NOM onto membrane surfaces may affect local charge density and other critical separation characteristics. Active microorganisms also affect local nutrient availability, redox gradients, and pH gradients, which has implications for interactions of membrane surfaces with other fouling agents (7). Foulant interactions with membranes have been shown to be dependent on the membrane physical/chemical properties, the water quality parameters (e.g., pH, hardness, and ionic strength), and local system hydrodynamics (8).

Limited studies have focused on how SMP may influence the performance of membrane separation processes (9–10), and affect biofouling. SMP is operationally defined as any soluble microorganism components, including those excreted as byproducts of microbial metabolism. The degree to which these compounds affect flux decline is difficult to determine, because of their chemical complexity, biodegradation potential and dependence on the water (or wastewater) source (11, 12). There is a growing body of evidence suggesting that SMP derived from local microbiological cells may have a negative impact on membrane separation processes (9, 10, 14, 16). These findings bear increased importance with respect to some current biofouling control strategies, since many utilities disinfect prior to membrane process for the express purpose of minimizing biofouling. Estimating the fouling potential of SMP using available data sets has been difficult, and strategies to minimize biofouling from this perspective have yet to be developed.

The main objective of this study was to better understand how EPS, SMP, and NOM may influence NF membranes flux and performance. Surrogate compounds were used to represent each of these pools. Biofouling (BF) tests, in which biofilm growth was promoted, were run with waters containing different biodegradable carbon sources (i.e., acetate, glucose, and NOM) and a pure culture inoculum. Fouling potential (FP) tests, without a pure culture inoculum, were run with isolated NOM, SMP, and a natural water (Barker Lake, CO). Since many common foulants can be negatively charged at neutral pH, the impact of the membrane surface charge-density on biofouling potential was isolated as a process variable by examining two different membranes. The flux decline of bench-scale NF membranes was monitored at different stages of the (bio)fouling process. A better understanding of biofilm precursors, biofilm constituents, and their interactions with surfaces, may provide for improved pretreatments, as well as operation and membrane selection strategies.

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## 2. Experimental Apparatus and Design

**2.1. Sterile Rapid Bench-Scale Membrane Test System.** All membrane tests were performed with a rapid bench-scale membrane test (RBSMT) system, adapted to operate under sterile conditions (17). A diaphragm pump was used to minimize impact on microbial cells. Through the use of concentrate recycle, the RBSMT is able to simulate full-scale membrane performance, using smaller water volumes than pilot-scale systems, with reliable control of system recovery, flow regime, and operating pressure (and consequently permeate flux). The sterile RBSMT was composed of a flat sheet membrane module placed downstream of a chemostat, which was seeded with a pure culture of *Pseudomonas aeruginosa* FDR1 (mucA22), and maintained using aseptic techniques. This strain (supplied by the Center for Biofilm Engineering, Bozeman, MT) was isolated from a cystic fibrosis patient, and is known to excrete relatively large masses of alginate with respect to its size. The median areas of single *Pseudomonas aeruginosa* cells associated with biofilms on membrane filters have been reported between  $0.71 \mu\text{m}^2 \pm 0.03 \mu\text{m}^2$  and  $0.97 \mu\text{m}^2 \pm 0.06 \mu\text{m}^2$  for wild type and genetically engineered strains respectively (31). This apparatus was developed to maximize microbial growth on membrane surfaces, while maintaining sterility, and was used during all biofouling tests. During fouling potential tests, the same system was used without the inoculation step. All solution components were either autoclaved (all media with the exception of NOM) and/or filter-sterilized (NOM) through  $0.22 \mu\text{m}$  polycarbonate membrane filters (Millipore, Bedford, MA).

**2.2. System Operations and Inoculation Procedures.** Membrane samples ( $14.5 \text{ cm} \times 9.5 \text{ cm}$  rectangular permeation area) were mounted in a flat sheet, cross-flow cell (Osmonics, Minnetonka, MN) operated at a constant operating pressure of  $6.7 \times 10^5 \text{ Pa}$ , 70% recovery, temperature between  $21 \pm 2 \text{ }^\circ\text{C}$ , pH between 6 and 7, and a flow rate of 250 mL/min, yielding a cross-flow velocity of 0.05 m/s. Velocity scalars between 0.05 and 1.5 m/s are commonly observed in spiral-wound elements with mesh spacers (3, 17) and cross-flow velocity of 0.05 m/s was used to maximize biofouling potential in this study. Dissolved oxygen was not measured in any of the fluid components associated with this study. Given the configuration and size of the feed stock and reactor, the relatively small amounts of sessile biomass present, and the time frame which the feed stock was spent (and changed), it is likely that the bulk fluid dissolved oxygen content was near saturation during the entire course of these experiments.

At the beginning of each test, laboratory grade deionized (DI) water was permeated through the membrane under the operating conditions cited above, to allow for membrane saturation and compaction (i.e., membrane setting). The system was designated to be at steady-state when the pure water flux varied less than 4% over a period of 12 h (17), after which different feed solutions were introduced in accordance with each of the respective experiments. This procedure was performed in order to differentiate between the flux loss caused by membrane compaction (usually observed during the first hours of operation) and subsequent membrane (bio)-fouling.

As determined by direct microscopy, the system was rapidly inoculated with 3 L of planktonic *Pseudomonas aeruginosa* cells following membrane setting. This inoculum was washed and suspended in a dilute phosphate buffer (c.a.  $10^5 \text{ cell/mL}$ ), where >90% of the cells could be recovered by standard culturing techniques (CFU). In all cases, a sterile feed solution was immediately introduced following the inoculation, and the flux decline was monitored. The carbon source in the sterile feed was isolated as a process variable

in otherwise identical dilutions of Stanier's minimal medium (18) adapted for the express purpose of providing adequate nutrients for microbial growth, while minimizing concentration polarization effects.

**2.3. Feed Preparation and Key Analytes.** **2.3.1. Readily Degradable Carbon Sources.** Sodium acetate and glucose were used as sole carbon sources during the biofouling studies. For baseline experiments, media contents were adjusted to a total carbon organic concentration (TOC) of 3 mg C/L, including 1.15 mg/L  $\text{NH}_4\text{Cl}$ , 7.5 mg/L  $\text{K}_2\text{HPO}_4$ , 17.5 mg/L  $\text{KH}_2\text{PO}_4$ , and 1:100 dilution of the standard Stanier's mineral base additives. Acetate was also tested at 10 mg C/L (where all media contents adjusted accordingly).

**2.3.2. Soluble Microbial Products.** SMP was defined for this study as membrane-free cytoplasmic extracts of metabolically active bacteria. To harvest SMP, activated sludge was collected and washed with laboratory grade phosphate buffered saline (PBS), centrifuged at  $1500 \times g$  for 30 min (centrifuge: BECKMAN CS6, rotor G.H. 3.8, Beckman Coulter, Palo Alto, CA), after which the supernatant was discarded. This cycle was repeated three times. Washed cells were subsequently lysed in a french-press (American Instrument Company, Urbana, IL) operated at  $1.1 \times 10^7 \text{ Pa}$  and the cytoplasmic extract released into solution; pressure-lysing was repeated three times to increase recovery. The cytoplasmic extract was separated from all other compounds (cell wall debris and remaining particulate matter) by ultra centrifugation at  $17\,000 \times g$  for 20 min (centrifuge: Sorval RC 5B Plus, rotor SS-34, Sorval Haraeus, Kendro Laboratory Products, Newton, CT), after which the supernatant was collected in autoclaved, acid-washed bottles, and stored at  $4 \text{ }^\circ\text{C}$ . The carbon content of SMP stocks were characterized by their dissolved organic carbon (DOC). Due to their poor stability, i.e., SMP are readily biodegradable, a stock solution was produced immediately before each flux decline test. SMP solutions were prepared for membrane tests by diluting their stocks to one of three DOC levels: 0.6 mg C/L, 1.6 mg C/L, and 2.5 mg C/L, in a buffer containing 7.5 mg/L  $\text{K}_2\text{HPO}_4$ , 17.5 mg/L  $\text{KH}_2\text{PO}_4$ , and 0.5 g/L of NaCl, which provided similar ionic strengths to that in all other tests.

**2.3.3. Natural Organic Matter.** A 170 mg C/L solution of a NF concentrate from Manatee Lake, FL, was used in this study as the NOM source. NOM feed solutions were prepared using protocols identical to those detailed in section 2.2 except that NOM was added as a sole carbon source.

**2.3.4. Natural Water.** Barker Lake water (BLW) was collected from the source water of Betasso Water Treatment Plant, Boulder, CO and filtered through a  $0.22 \mu\text{m}$  filter (PTFE, Cole-Parmer, Vernon Hills, IL). Two parallel fouling potential tests were executed, under treatment conditions similar to those of SMP and NOM solutions. One test was executed with BLW, and a subsequent test with BLW supplemented with model compounds normally detected in ozonated waters: formaldehyde (140  $\mu\text{g/L}$ ), glyoxal (42  $\mu\text{g/L}$ ), oxalic acid (420  $\mu\text{g/L}$ ), and sodium acetate trihydrate (952  $\mu\text{g/L}$ ).

**2.3.5. Organic Carbon Concentration.** Organic carbon concentration (i.e., DOC and TOC) was measured by Persulfate-Ultraviolet Oxidation Method (APHA, 1992) with a total carbon analyzer (SIEVERS 800, Boulder, CO). DOC samples were filtered through a  $0.45 \mu\text{m}$  pore size membrane (HVLP, Millipore, Bedford, MA), after which the sample was acidified to pH of 2 and stored at  $4 \text{ }^\circ\text{C}$  for no longer than 7 days. The minimum detection limit (MDL) for TOC (0.08 mg C/L) and all other critical analytes were calculated based on the standard deviation of the lower measured concentration (19).

**2.3.6. Proteins and Polysaccharides.** A UV-VIS spectrophotometer (DR/4000U, HACH Company, Loveland, CO) was used for colorimetric determination of the protein and polysaccharide content of membrane-associated biofilms.

Proteins were measured using the conventional Bradford assay modified for small sample volumes (BioRad Laboratories, Hercules, CA) using Bovine Serum Albumen as a standard; the MDL for the method was 15  $\mu\text{g/L}$ . Polysaccharides were measured using a Phenol-Sulfuric acid digestion method modified for small sample volumes (20). Polysaccharides were used as a surrogate parameter for EPS (the major fraction of the biofilm mass). This absorbance method was standardized to a calibration curve developed with glucose solutions (Sigma Chemical, St. Louis, MO). The MDL was 10  $\mu\text{g/mL}$ .

**2.3.7. Colony Forming Units.** CFUs were used to quantify the number of culturable microorganisms that could be harvested from membrane surfaces. Samples were diluted appropriately for statistical counting constraints (i.e., between 30 and 300 colonies per plate), and triplicate samples were plated in agar media containing 3 g/L soy agar, using a semi-automated spiral plating machine (Spiral Biotech, Inc, Bethesda, MD). Colonies were counted after 36 h of incubation at 37 °C.

**2.4. Nanofiltration Membranes.** Two thin-film composite NF membranes, NF70 (Dow/FilmTec, Minneapolis, MN) and ESPA3 (Hydranautics, Oceanside, CA), were selected because they are commonly used in the water treatment industry and had different zeta potential (NF70 varied between  $-28$  and  $-20$  mV, and ESPA3 varied between  $-40$  and  $-37$  mV, where  $6 < \text{pH} < 7$ ), while all other parameters describing their performance were relatively similar. Both membranes have a polyamide base, with salt rejection performance higher than 95%; the average molecular weight cutoff (MWCO) was 200 Da for ESPA3, and varied between 300 and 500 Da for NF70.

**2.5. Membrane Sampling.** At the end of each experiment, membranes were sacrificed under sterile conditions, and biofilm composition was evaluated. Coupons were cut from the membrane and biofilm was scraped with sterile razors and eluted with low-energy sonication. From each membrane, a minimum of nine 2.5 cm  $\times$  2.5 cm coupons were eluted into sterile centrifuge tubes, containing 3 mL of cold 8.5% NaCl solution (21), centrifuged for 30 min at  $9000 \times g$ , and filtered through a low protein binding 0.22  $\mu\text{m}$  filter (PSU, Whatman Inc., Clifton, NJ). The resulting solutions were placed in acid washed test tubes and triplicate observations for proteins and polysaccharides were recorded. For bacterial assays, nine 1.5 cm  $\times$  1.0 cm coupons were cut from the membrane. Samples were sacrificed and eluted into sterile test tubes containing 9 mL of phosphate buffer saline.

### 3. Resistance-in-Series Model

The flux decline behavior of the NF membranes was modeled using a resistance-in-series (R-I-S) model (8,13–16). This model describes flux loss as a function of four resistance parameters associated with the membrane materials ( $R_m$ ), buffer solution ( $R_{\text{buffer}}$ ), microorganisms cells ( $R_{\text{cells}}$ ), and polysaccharides ( $R_{\text{EPS}}$ ).

$$J = \frac{P - \Delta\pi}{\mu(R_m + R_{\text{buffer}} + R_{\text{cells}} + R_{\text{EPS}})} \quad (1)$$

Here  $J$  ( $\text{m}^3 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) represents the permeate flux,  $P$  represents the applied pressure (Pa),  $\Delta\pi$  represents the osmotic pressure (Pa),  $\mu$  represents viscosity ( $\text{Pa} \cdot \text{s}$ ), and  $R_i$  represents the respective resistances ( $\text{m}^{-1}$ ).

### 4. Results

A normalized flux—the flux at any given time normalized by the flux obtained at the end of the membrane setting phase—was used to compare the performance of different membrane tests. This provided for a relative analysis of flux decline,

**TABLE 1. Relationship between Polysaccharides and Flux Decline during Biofouling and Fouling Potential Tests Performed with Different Membranes, Carbon Sources, and Feed Solution Concentrations**

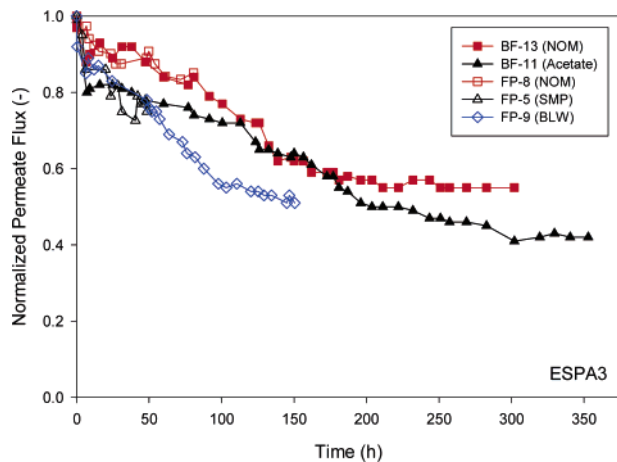
test number	membrane	carbon source	feed DOC (mg/L)	run time (h)	polysaccharides ( $\mu\text{g}/\text{cm}^2$ )	flux decline (%)
BF-1	NF70	acetate	10 $\pm$ 1.8	154	60 $\pm$ 20	79
BF-2			10 $\pm$ 1.8	96	51 $\pm$ 9.3	80
BF-3			3.4 $\pm$ 0.3	275	66 $\pm$ 33	65
BF-4			3.4 $\pm$ 0.9	244	73 $\pm$ 15	68
BF-5			3.5 $\pm$ 0.8	60	5 $\pm$ 0.7	35
BF-6			3.0 $\pm$ 0.2	39	<DL	13
BF-7			3.1 $\pm$ 0.1	52	<DL	20
BF-8		glucose	3.5 $\pm$ 0.3	127	41 $\pm$ 6.5	65
BF-9		NOM	3.0 $\pm$ 0.0	270	50 $\pm$ 11	69
BF-10		NOM	3.2 $\pm$ 0.4	270	75 $\pm$ 19	78
BF-11	ESPA3	acetate	3.2 $\pm$ 0.3	353	23 $\pm$ 3.1	58
BF-12			3.2 $\pm$ 0.3	230	16 $\pm$ 1.7	47
BF-13		NOM	3.1 $\pm$ 0.2	302	15 $\pm$ 2.0	45
FP-1		SMP	0.6 $\pm$ 0.15	70	6 $\pm$ 1.5	13
FP-2		NOM	0.6 $\pm$ 0.12	56	<DL	5
FP-3		SMP	1.6 $\pm$ 0.28	74		17
FP-4		NOM	2.3 $\pm$ 0.24	72		12
FP-5		SMP	2.2 $\pm$ 0.35	49	5 $\pm$ 1.9	25
FP-6		NOM	2.5 $\pm$ 0.13	49	<DL	15
FP-7		SMP	2.5 $\pm$ 0.35	84		40
FP-8		NOM	2.5 $\pm$ 0.12	84		13
FP-9		BLW	2.0 $\pm$ 0.10	150	33 $\pm$ 8.6	49
FP-10		BLW-S	2.4 $\pm$ 0.30	169	40 $\pm$ 6.9	55

regardless of intrinsic microscale membrane variability. Average steady-state fluxes at the end of the membrane setting phase were  $43.6 \pm 7.4 \text{ L}/\text{m}^2 \cdot \text{h}$  and  $34.8 \pm 2.6 \text{ L}/\text{m}^2 \cdot \text{h}$  for NF70 and ESPA3, respectively.

Thirteen BF tests, where microbial activity was purposely promoted, were performed to assess the impact of three carbon sources, two carbon concentrations, and two NF membranes on membrane associated biological growth and flux decline. The test matrix and results are summarized in Table 1. The MDL of the polysaccharide test, on an area basis, was 4.8  $\mu\text{g}/\text{cm}^2$ . DOC rejection varied between 90 and 97%, and conductivity rejections ranged between 91 and 96%, agreeing with values previously reported for “tight” NF membranes (17,22).

Ten FP tests, where microbial activity was minimized to the extent possible, were performed with the ESPA3 membrane (Table 1). The impact of three different carbon sources and three carbon concentrations was assessed. DOC and conductivity rejections higher than 90% was measured for all runs.

Typical permeate flux responses obtained for the BF and FP tests with acetate, NOM and BLW (with ESPA3 membranes) are shown in Figure 1. For all BF and FP tests, after membrane setting, the flux rapidly declined until it reached an initial plateau, which was likely due to concentration polarization effects and nonspecific adsorption of organic and inorganic molecules (1,3). The duration of this initial plateau, typically ranged between 25 and 50 h. For the BF and BLW tests, the development of microbial biomass on membrane surfaces resulted in additional flux decline until a second plateau was reached, usually after 200–250 h of operation (FP tests were shorter to avoid biofilm formation). Irreversible fouling was observed during this phase. This phenomenon was operationally isolated and defined by decreasing system recovery (generally maintained at 70%), where concomitant decreases in concentrate conductivity did not result in any increases in permeate flux. As with all tests in this study, steady-state was defined as less than 4 percent flow change over a period of 12 h. This steady-state behavior, previously described in ref 23, was observed for all



**FIGURE 1.** Flux decline response of ESPA3 membranes for different carbon sources.

tests and is likely dynamic equilibrium involving biological growth, sloughing, and rejection induced by cross-flow velocity and its associated shear.

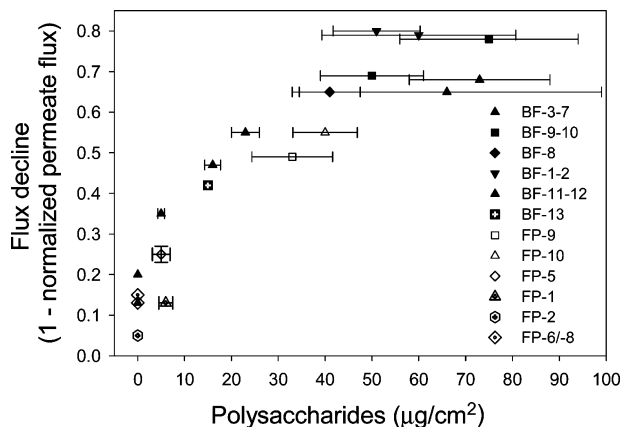
The trends obtained for acetate and NOM did not vary significantly between BF and FP tests. Conversely, BLW showed a faster flux decline than acetate and the synthetic solution of NOM.

The DOC of the SMP stock extract solutions contained, on average,  $48 \pm 9\%$  and  $35 \pm 4\%$  of proteins and polysaccharides, respectively. The NOM solutions contained  $35 \pm 1\%$  and  $28 \pm 9\%$  of proteins and polysaccharides, respectively.

## 5. Discussion

**5.1. Impact of Biofouling on Membrane Flux Decline.** The data summarized in Table 1 show that for acetate and NOM, membrane-associated polysaccharides, EPS, and the concurrently observed flux decline, was higher for the NF70 (BF-3, 4, 9 and 10) than for the ESPA3 (BF-11 to BF-13) after similar solution volumes (95 L) and carbon mass (314 mg) had been processed. The higher negative zeta potential of the ESPA3 membrane may, in part, have been responsible for the lower biofouling responses observed. Membrane surface charge has been reported to affect microbial sorption potential on polymeric membrane surfaces. Maximum adsorption potential has been observed on uncharged membrane surfaces (7). Since acetate moieties and many NOM- and SMP-associated molecules have negative charge at neutral pH, the lower membrane zeta potential of the NF70 would tend to decrease the electrostatic repulsion potential between the substrate and the membrane surface and, therefore, increase their adsorption and fouling potential.

Figure 2 shows the relationship between membrane-associated polysaccharides, here also a surrogate for EPS, and flux decline for the biofouling and fouling potential tests. The results suggest an operational relationship between membrane-associated EPS and flux decline up to a polysaccharide concentration of c.a.  $50 \mu\text{g}/\text{cm}^2$ . Above this polysaccharide level, higher surface-associated polysaccharides did not result in a significant increase in flux decline, most likely because of a surface saturation effect with respect to hydrodynamics through a mature biofilm. In three of the BF tests, 5, 6, and 7, the systems were terminated after short run times, 39–60 h, with the flux decline between 13 and 35%, to evaluate the EPS impact on early stages of biofilm development. The polysaccharides measured are within ranges previously reported. Ridgway and co-workers (24) reported levels of membrane-associated polysaccharides near



**FIGURE 2.** Membrane-associated polysaccharide density and corresponding flux decline at different feed solution concentrations (Horizontal error bars represent variation of triplicate measurements).

$75 \mu\text{g}/\text{cm}^2$  after 200 days of RO membrane operations in a pilot system treating secondary wastewater effluent. Speth et al. (25) reported levels of membrane-associated EPS between 47 and  $99 \mu\text{g}/\text{cm}^2$  on NF pilot membranes system treating Ohio River water, which was present when flux declines ranged between 25 and 35%.

The surface cell densities calculated from cultured samples of homogenized membrane biofilms were also within ranges previously reported (4, 6, 26, 27). Regardless of the surface-averaged polysaccharide densities observed, culturable cell densities ( $10^7$ – $5 \times 10^8$  CFU/ $\text{cm}^2$ ) recovered from final membrane autopsies were statistically similar ( $P < 0.05$ ), suggesting that the flux declines observed were associated with the polymeric substances excreted, rather than bacterial cells alone. Cell densities recovered from membrane surfaces were confirmed with epifluorescent microscopy, using fluorescent stains (21). As suggested by these results, it seems, there was a spatial (aerial) saturation of biofilm on the membrane. Ridgway (27) estimated numbers of finite adhesion sites on cellulose acetate membranes near  $3 \times 10^6/\text{cm}^2$ , 1 order of magnitude lower than the theoretical binding capacity of the membrane, based upon spatial and geometric considerations. A similar saturation condition is likely to occur in membrane-associated biofilm “layers”.

The results obtained during this study also suggest that carbon sources, otherwise considered relatively refractory in the environment (such as NOM), can support extensive biofouling when adequate micronutrients are present and/or are concentrated by a membrane separation system. Nutrients that may otherwise limit bacterial growth can occur naturally in source waters, or be introduced in the system. These may be contaminants present in other common operating chemicals, such as mineral acids and anti-scalants (3,28) and/or liberated during oxidation disinfection processes common to water treatment practices. Thus, minimizing nutrient availability during water treatment may be an effective biofouling reduction strategy achieved through the following practices: (i) biological filtration, which allows for assimilable carbon, nitrogen, and phosphorus to be removed prior to downstream membrane process, (ii) relocating disinfection/oxidation to avoid oxidation before membrane elements (Speth et al. (25) reported higher flux declines in membrane systems treating chloraminated than non-chloraminated waters), and (iii) assuring the purity of all process chemicals, such that growth-limiting macro- and trace nutrients are not introduced to membrane feedwaters.

**5.2. Fouling Potential of SMP, NOM, and BLW.** Similar to the results obtained for the BF tests, the accumulation of membrane-associated polysaccharides, from the SMP, NOM solutions, and the BLW in the fouling potential tests with the

**TABLE 2. Parameters Used to Calibrate and Validate the Resistance-in-series Model (model Simulations and Predictions Are Shown in the Last Column).**

test number	carbon source	$M_{EPS}$ ( $\times 10^{-4}$ ) (Kg/m <sup>2</sup> )	$J_{MO}$ ( $\times 10^{-7}$ ) (m <sup>3</sup> /m <sup>2</sup> /s)	$J_{SS}$ ( $\times 10^{-7}$ ) (m <sup>3</sup> /m <sup>2</sup> /s)	$R_m$ ( $\times 10^{14}$ ) (m <sup>-1</sup> )	$R_{EPS}$ ( $\times 10^{14}$ ) (m <sup>-1</sup> )	$R_{TOTAL}$ ( $\times 10^{14}$ ) (m <sup>-1</sup> )	flux loss (%)	
								measured $1 - J_{SS}^d/J_{MO}^c$	calculated $1 - J_{CALC}/J_{MO}^c$
<b>Simulations<sup>a</sup></b>									
BF-6	acetate	0	7.3	6.3	9.2	0	10.6	13	12
BF-7		0	9.2	7.9	7.3	0	8.5	20	15
BF-5		0.5	14	9.1	4.8	1.2	7.3	35	34
BF-12		1.6	11	5.9	6.2	3.8	11.3	47	48
BF-11		2.3	9.3	3.9	7.2	8.8	17.3	58	47
BF-1		6.0	15	5.4	4.4	6.6	12.3	79	76
BF-2		6.0	14	4.6	4.2	8.3	13.8	80	75
<b>Predictions<sup>b</sup></b>									
BF-8	glucose	4.2	12	4.4	5.4	7.1	8.4	65	61
BF-13	NOM	1.5	9.7	5.3	6.9	4.1	5.4	45	44
BF-9		5.0	14	4.5	4.7	7.6	8.9	69	65
BF-10		7.5	14	3.1	4.7	8.6	9.9	78	67
FP-9	BLW	3.3	9.9	5.1	6.8	6.4	7.7	49	66
FP-10	BLW-S	4.0	11	5.0	6.2	6.9	8.2	55	70
FP-5	SMP	5.0	7.6	3.9	8.8	7.6	8.9	25	20
FP-6	NOM	0	8.3	4.6	8.0	0	1.3	15	10

<sup>a</sup> Model calibration performed with using 3 and 10 mg C/l acetate solutions. <sup>b</sup> Model validation performed with 3 mg C/L glucose, NOM, Barker Lake water, and 2.5 mg C/L SMP and NOM solutions. <sup>c</sup>  $J_{MO}$  are flux observations during membrane setting with particle free deionized water. <sup>d</sup>  $J_{SS}$  are flux observations during steady-state operations with the noted feed stock.

sterilized membrane system, correlated well with the extent of membrane flux decline (Figure 2). However, initial DOC concentration emerged as a factor, as higher feed concentrations resulted in higher flux declines, when membrane-associated EPS levels were at or near the detection limit, 5  $\mu\text{g}/\text{cm}^2$  (FP-1, -3, and -5 for SMP and FP-2, -4, and -6 for NOM).

Sterilized membrane systems fed SMP solutions experienced higher flux losses and polysaccharide densities on the membrane than those fed NOM, under otherwise identical conditions (Table 1 and Figure 2).

Proteins could not be detected on any of the membranes tested, although they were present in higher concentrations than the polysaccharides in both the SMP and NOM feed solutions, suggesting that polysaccharides were preferentially adsorbed onto the membrane, and/or that protein sorption was sufficiently strong that it could not be eluted from the membrane surfaces for assay. Evidence for preferential sorption of polysaccharides to NF membrane surfaces has been previously reported (25). However, other studies have also recovered significant concentrations of proteins from biofouling layers (24–25).

It appears the degree of fouling depended on the extent to which the different groups of molecules interacted with the membrane surfaces, rather than their rejection efficiency. More than 95% of DOC rejection was obtained with the ESPA3 membranes, regardless of the feed solution. The impact of polymeric molecules on flux decline has been previously addressed in the literature (29,30), suggesting that fouling potential depends on molecules' charge and conformation. These properties are themselves dependent on the water quality parameters such as pH and ionic strength. The results obtained in this study support these observations. Similar flux decline (13–15%) was observed with the SMP solution at 0.6 mg C/L and the NOM solution at 2.5 mg C/L. Despite the lower carbon concentration of the SMP feed solution and associated lower polysaccharide and protein concentrations, higher membrane-associated polysaccharides were measured, suggesting a higher fouling potential.

The BF potentials of SMP and NOM were also compared by challenging the membrane with solutions of similar

concentration (2.5 mg C/L) for different periods (FP-5–FP-8). An additional 30 h of testing (i.e., 80 h total) did not result in additional flux loss for the membranes challenged with NOM solutions; the flux decline was maintained at 15%. However, an additional 15% flux decline was observed on the membrane challenged with SMP during the latter 30 h of these tests, adding up to a total flux loss of 40%. These observations again suggest that SMP had a higher biofouling potential than did the NOM solution.

The natural water, BLW, and supplemented natural water, BLW-S, displayed very fast flux decline rates (Figure 1 and Table 1). BLW-S had the fastest and most extensive flux decline of any of the runs with the ESPA3 membrane, including the inoculated BF tests, in both the short term (<50 h), when bioactivity was not likely significant and in the long-term (>100 h) when it was. The rate of polysaccharide development (Table 1) at the end of the run was also the highest. The BLW results followed the general behavior shown in Figure 2, indicating the robustness of the relationship.

**5.3. Resistance-in-Series Model: Simulation and Predictions.** The R-I-S model was calibrated with results from the BF tests with acetate at a DOC concentration between 3.0 and 3.5 mg/L and inoculated with active *Pseudomonas aeruginosa* cultures (BF-3–BF-7, BF-11, BF-12). Validation was done with the glucose and NOM results from the other BF tests (BF-8–BF-10, and BF-13) and SMP, NOM, and BLWs results from the FP (FP-5, -6, -9, and -10) with DOC concentrations ranging between 2.0 and 3.5 mg C/L. Values for several measured parameters were needed: flow rates (using DI water, buffer and media solutions), cells, and EPS associated with the membrane.

The resistance offered by the membrane,  $R_m$ , was calculated based on  $J_{DI}$ , steady-state flux during membrane challenges using DI water, which was obtained at the end of the membrane setting phase, using eq 2.

$$J_{DI} = \frac{P - \Delta\pi}{\mu R_m} \quad (2)$$

A similar strategy was used to calculate  $R_{buffer}$ ,  $R_{cells}$ , and  $R_{EPS}$ .

$R_{\text{buffer}}$  (only considered for SMP and NOM buffer solutions) was calculated with eq 1 using the  $R_m$  value and the flux with the buffer solution and found to be  $4.2 \times 10^{14} \text{ m}^{-1}$ . The osmotic pressure ( $\Delta\pi$ ) was considered to be negligible at the recovery levels and concentrations used in this study. The maximum  $\Delta\pi$  calculated for the system was 2% (21).

$R_{\text{cells}}$  was derived from biofouling flux decline tests where no EPS (biofilm carbohydrate) could be detected (BF-6 and -7), but bacterial cells were associating with the membrane surface in numbers similar to those entrained in biofilms. This parameter represents the resistance associated with cells, concentration polarization, and primary microbiological compounds that were present below the detection limit or not measured at all (such as lipids and other cellular byproducts). Therefore,  $R_{\text{cells}}$  accounted for the impact of cells and associated biopolymers which could impact flux, but could not be isolated and identified.  $R_{\text{cells}}$  was found to range from  $1.2 \times 10^{14}$  to  $1.4 \times 10^{14} \text{ m}^{-1}$  with an average of  $1.33 \times 10^{14} \text{ m}^{-1}$ .

After determining all other parameters in eq 1, the resistance associated with polysaccharides present in (bio)-fouling layers,  $R_{\text{EPS}}$ , was calculated using flux at the end of the run,  $J_{\text{ss}}$ . After calculating the  $R_{\text{EPS}}$  values for the acetate runs, they were related to the mass of membrane-associated polysaccharides,  $M_{\text{EPS}}$ , using eq 3. Parameters  $a$  and  $b$  were determined to be  $1.2 \times 10^{15}$  and  $2.9 \times 10^{-4} \text{ m}^{-1}$ , respectively.

$$R_{\text{EPS}} = \frac{aM_{\text{EPS}}}{b + M_{\text{EPS}}} \quad (3)$$

Using these values of  $a$  and  $b$  and eqs 1 and 3, permeate fluxes were calculated ( $J_{\text{CALC}}$ ) for the acetate runs and the flux losses compared to the measured values (Table 2). The model simulations were within 4 and 18% of the experimental values with an  $R^2$  of 0.979.

The R-I-S model was then used to predict the non-acetate permeate fluxes ( $J_{\text{PRED}}$ ) by using the measured  $M_{\text{EPS}}$  values, and eqs 1 and 3. The comparison to measured values is shown in Table 2. Predicted flux declines for glucose, NOM, SMP, and BLW were within 3–35% of the experimental values, with a  $R^2$  of 0.813, reflecting the relationship between membrane-associated EPS and flux loss.

Flux decline tests in sterile systems using sterile feed stock, suggested that in the absence of significant microbial activity and biofilm formation, SMP and NOM themselves may have intrinsic membrane fouling properties at levels that are operationally significant to the membrane treatment processes. These results are consistent with the fact that many SMP components are readily biodegradable, compared to the more refractory NOM molecules. The natural water results also displayed significant biofouling potential. These findings bear increased importance with respect to some membrane biofouling control strategies, since many utilities use strong chemical oxidants as a pretreatment to membrane processing for the express purpose of minimizing biofouling. Regardless of the polysaccharide source (extra- or intracellular), the trend obtained relating these compounds with flux decline was verified for different carbon sources and membrane types, and was successfully described by expanding the R-I-S model. Improvement in our understanding and could be achieved by further researching the nature of the microbial and molecules-membrane interactions, as well as the impact of inorganic and organic molecules and colloids on the development of a (bio)fouling layer.

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