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Fouling development on Hydrophobic/Hydrophilic membranes

Day 1

No selection of specific foulants

Day 30

Similar composition of foulants layer

Temporal Changes in Extracellular Polymeric Substances on Hydrophobic and Hydrophilic Membrane Surfaces in a Submerged Membrane Bioreactor

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Abstract

Membrane surface hydrophilic modification has always been considered to mitigating biofouling in membrane bioreactors (MBRs). Four hollow-fiber ultrafiltration membranes (pore sizes $\sim 0.1 \mu\text{m}$) differing only in hydrophobic or hydrophilic surface characteristics were operated at a permeate flux of $10 \text{ L/m}^2\cdot\text{h}$ in the same lab-scale MBR fed with synthetic wastewater. In addition, identical membrane modules without permeate production ($0 \text{ L/m}^2\cdot\text{h}$) were operated in the same lab-scale MBR. Membrane modules were autopsied after 1, 10, 20 and 30 days of MBR operation, and total extracellular polymeric substances (EPS) accumulated on the membranes were extracted and characterized in detail using several analytical tools, including conventional colorimetric tests (Lowry and Dubois), liquid chromatography with organic carbon detection (LC-OCD), fluorescence excitation - emission matrices (FEEM), fourier transform infrared (FTIR) and confocal laser scanning microscope (CLSM). The transmembrane pressure (TMP) quickly stabilized with higher values for the hydrophobic membranes than hydrophilic ones. The sulfonated polysulfone (SPSU) membrane had the highest negatively charged membrane surface, accumulated the least amount of foulants and displayed the lowest TMP. The same type of organic foulants developed with time on the four membranes and the composition of biopolymers shifted from protein dominance at early stages of filtration (day 1) towards polysaccharides dominance during later stages of MBR filtration. Nonmetric multidimensional scaling of LC-OCD data showed that biofilm samples clustered according to the sampling event (time) regardless of the membrane surface chemistry (hydrophobic or hydrophilic) or operating mode (with or without permeate flux). These results suggest that EPS composition may not be the

dominant parameter for evaluating membrane performance and possibly other parameters such as biofilm thickness, porosity, compactness and structure should be considered in future studies for evaluating the development and impact of biofouling on membrane performance.

Keywords: wastewater treatment; water reuse, hydrophobicity, hydrophilicity, membrane bioreactor, membrane biofouling.

53 Nomenclature

- 54 EPS: Extracellular polymeric substances
- 55 FEEM: Fluorescence excitation - emission matrices
- 56 FTIR: Fourier transform infrared
- 57 HRT: Hydraulic retention time
- 58 LC-OCD: Liquid chromatography with organic carbon detection
- 59 MBR: Membrane bioreactor
- 60 MLSS: Mixed liquor suspended solids
- 61 nAg: Silver nano-particles
- 62 NF: Nanofiltration
- 63 NMDS: Non-metric multidimensional scaling
- 64 PDA: Polydopamine
- 65 PE: Polyethylene
- 66 POX: Polyoxadiazole
- 67 PS: Polysulfone
- 68 PTA: Polytriazole
- 69 PVDF: Polyvinylidene fluoride
- 70 SMP: Soluble microbial products
- 71 SPSU: Sulfonated polysulfone
- 72 SPTA: Sulfonated polytriazole
- 73 SRT: Sludge retention time
- 74 TMP: Trans-membrane pressure
- 75 UF: Ultra-filtration

1. Introduction

Membrane bioreactors (MBRs) are gaining worldwide merit as a promising solution for wastewater treatment and reuse, offering several advantages over conventional activated sludge systems, especially a smaller footprint, a better effluent quality (Santos et al., 2011), and an improved disinfection capability (Vanysacker et al., 2014). However, membrane biofouling in MBRs remains one of the most problematic challenges that result into a dramatic decrease in the quantity and quality of permeate flux production, and a sharp increase in the energy demand and trans-membrane pressure (TMP) (Meng et al., 2009; Gao et al., 2014). In addition, when severe and irreversible biofouling occurs, the premature replacement of membrane modules becomes inevitable, escalating the overall MBR operation costs (Verrecht et al., 2010).

Biofouling has been considered as the “Achilles heel” of membrane systems (Flemming et al., 1997), and several control strategies have been proposed to reduce biofouling in MBRs including physical and chemical cleaning, membrane surface modification (e.g. charge, hydrophobicity, roughness), and biological-based antifouling strategies (e.g. quorum quenching, enzymatic disruption, energy uncoupling) (Malaeb et al., 2013). Among these strategies, membrane surface modification to reduce biofouling has attracted a lot of attention, not only in MBRs, but in reverse osmosis (RO) systems and membrane spacers as well (Yang et al., 2009; Araújo et al., 2012a; Nikolaeva et al., 2015). In particular, increasing the membrane surface hydrophilicity allows for an efficient initial surface wetting and a higher initial permeate flux; however, the long-term effectiveness of hydrophilic surfaces in reducing biofouling is still debatable. For instance, ultrafiltration (UF) and nanofiltration (NF) membranes coated with

polydopamine (PDA) increased the hydrophilicity of the membrane surface and reduced bacterial attachment during short-term filtration (one hour). However, PDA lost its hydrophilic character after ten days of filtration and became ineffective in reducing bacterial adhesion (Miller et al., 2012). Moreover, polysulfone (PS) membranes with silver nano-particles (nAg) reduced bacterial attachment on membrane surfaces and demonstrated a potential antimicrobial activity due to the toxicity of the silver nano-particles (Zodrow et al., 2009; Tang et al., 2015). However, the effectiveness of nAg were not tested for extended filtration and these studies (Zodrow et al., 2009; Tang et al., 2015) were performed in flow-cell filtration systems and used commercial organic foulants or model microorganisms, which are not representative for the membrane biofouling phenomenon in MBRs.

Extracellular polymeric substances (EPS) have been recognized as the main fouling components in MBRs and contribute to membrane biofouling phenomenon, which leads to a fast and sharp increase in TMP (Kimura et al., 2012; Gao et al., 2013). The complexity of the EPS matrix, composed of polysaccharides, proteins, humic acids and metal ions (Gao et al., 2013), increases the difficulty of understanding the interactions between different EPS components and the membrane surface. Previously, the increase in the membrane fouling resistance was correlated to higher protein concentrations on the membrane and resulted in a dramatic rise in the TMP (Ng and Ng, 2010). On the other hand, another study reported that the polysaccharide fraction of the EPS and soluble microbial products (SMP) was responsible for membrane biofouling and created sharper TMP increase when compared with the protein fraction (Kimura et al., 2004; Jarusutthirak and Amy, 2006). Although several studies characterized the EPS

components responsible for biofouling on different membrane surfaces (Ng and Ng, 2010; Kimura et al., 2012; Gao et al., 2013), a fundamental understanding on the temporal dynamics of EPS components according to modified membrane surfaces is still lacking. Modified membranes correspond to membranes that are subjected to specific surface modifications including but not limited to; changes in the hydrophobic or hydrophilic surface character, increase or decrease in the surface charge, or modifications of membrane surface roughness.

Therefore, the objective of this study was to assess the role of membrane hydrophilicity and surface chemical composition on biofouling and EPS composition and temporal dynamics in long-term experiments using a lab-scale MBR. Membrane modules corresponding to different membrane types (hydrophobic or hydrophilic) were operated in parallel in the same MBR tank, and membrane samples were collected following 1, 10, 20 and 30 days of continuous filtration. In parallel, additional modules were inserted without permeate production, to compare the effect of membrane surface selection against the permeate drag force. To the best of our knowledge, this is the first study that monitored the temporal dynamics of the EPS biofouling layer that developed on different membrane types, and extensively characterized the EPS fractions using several analytical and microscopic tools.

2. Materials and methods

2.1. Membranes manufacture and characteristics

Four UF hollow-fiber membranes were synthesized in the lab, composed of fluorinated polyoxadiazole (POX), fluorinated polytriazole (PTA), sulfonated polytriazole (SPTA) and sulfonated polysulfone (SPSU) (Table 1). The POX and PTA polymers were synthesized and manufactured into hollow fiber membranes, following a previously reported procedure (Maab et al., 2012, 2013). SPTA was synthesized using a procedure previously reported by Ponce et al. (Ponce et al., 2008) and manufactured into hollow fibers. Polysulfone was sulfonated by treatment with sulfuric acid before the SPSU hollow fiber manufacture. POX and PTA hollow fiber membranes are highly hydrophobic due to fluorinated groups. SPTA and SPSU are hydrophilic due to the functionalization with sulfonic groups.

2.2. Lab-scale MBR construction and operation

A lab-scale MBR with a working volume of 20 L was constructed and operated under intermittent aeration (30 min aerobic; 30 min anoxic) (Fig. 1), to achieve simultaneous carbon and nitrogen removal (Wang et al., 2013). The aeration flowrate was maintained at 2 L/min to achieve a dissolved oxygen (DO) of 8 ± 1 mg/L during the aerobic phase. The main objective for aeration was to provide oxygen for chemical oxygen demand (COD) removal and nitrification and not for air scouring the membranes for fouling reduction. The air diffusers were placed in the corner of the reactor away from the membrane surface. The MBR was operated at a solid retention time (SRT) of 15 d and a hydraulic retention time (HRT) of 12 h. The SRT of 15 d was maintained by intentionally

wasting 1.3 L of mixed liquor from the MBR tank every day. The lab-scale MBR was fed with synthetic wastewater (Table S1, Supporting information) and was inoculated with activated sludge collected from a local wastewater treatment plant (Al Ruwais district, Jeddah, KSA). Synthetic medium was used in this study to obtain a soluble COD (SCOD = 404 ± 9 mg/L) and ammonia nitrogen ($\text{NH}_3\text{-N} = 46 \pm 2$ mg/L) level typical of domestic wastewater since local wastewater at the KAUST site has a much lower COD than that typical of a wastewater treatment plant, and it was quite variable. The activated sludge was acclimated to the synthetic wastewater for 45 days under continuous filtration mode using commercial ultrafiltration (UF) hollow-fiber membranes (Pall) before experiments were initiated with the four different UF hollow-fiber membranes synthesized in the lab. During the acclimation period, the MBR was maintained at a constant permeate flux of $10 \text{ L/m}^2\cdot\text{h}$, which is close to the design flux ($15\text{-}25 \text{ L/m}^2\cdot\text{h}$) for municipal wastewater treatment (Judd, 2015), with a filtration cycle of 9 min followed by 1 min relaxation (no filtration).

Four membrane modules corresponding to the four different membranes were constructed following identical procedure, and 12 hollow-fibers were used to achieve a total membrane surface area of 56.5 cm^2 per module. A membrane cassette that holds the four modules was inserted in the MBR tank, and the four modules were run in parallel using the same permeate flux of $10 \text{ L/m}^2\cdot\text{h}$, with cycles of 9 min filtration followed by 1 min relaxation. This low flux was chosen because the lab-scale MBR was operated for the whole duration of the experiment without any cleaning procedure (e.g. air scouring, backwashing or chemical treatment) of the membranes. In parallel, a similar membrane cassette that holds identical hollow-fiber modules were inserted in the MBR tank without

permeate production ($0 \text{ L/m}^2 \cdot \text{h}$), to compare the effect of the membrane surface chemistry with the permeate drag force. All membrane modules (flux and no flux) were run for 1, 10, 20 and 30 d, and membrane modules were sacrificed completely for membrane autopsy and replaced with virgin modules after each run. Lastly, 15 mL of mixed liquor suspended solids (MLSS) was collected from the MBR tank at each sampling event. The MBR was kept at a constant temperature of $21 \pm 1^\circ\text{C}$. The TMP for all the membranes operated at $10 \text{ L/m}^2 \cdot \text{h}$ was measured using a pressure transducer (68075-32, Cole-Parmer Instrument Company), and recorded using a data acquisition system (LabVIEW, National Instruments) connected to a computer.

The intrinsic membrane resistance of the virgin membranes was measured in a separate reactor using deionized water (DI) according to Vrijenhoek et al. (Vrijenhoek et al., 2001). Briefly, the virgin membranes were pre-conditioned by running them with DI water where 2 L of permeate was produced and consequently, the membranes were run with DI water for a period of 24 h, which allowed the membranes to reach steady-state performance.

Samples for chemical analysis were collected from the feed and effluent and analyzed for SCOD, $\text{NH}_3\text{-N}$, nitrite nitrogen ($\text{NO}_2^- \text{-N}$), and nitrate nitrogen ($\text{NO}_3^- \text{-N}$) according to the Standard Methods for the Examination of Water and Wastewater (APHA/ AWWA/ WEF, 2005). SCOD was analyzed using the HACH DR/2010 Portable Datalogging Spectrophotometer (Hach Co., Loveland, CO, USA) and $\text{NH}_3\text{-N}$, $\text{NO}_3^- \text{-N}$, and $\text{NO}_2^- \text{-N}$ were analyzed using the HACH DR/4000U Spectrophotometer (Hach Co., Loveland, CO, USA).

2.3. EPS extraction

In this study, total EPS solutions refer to the soluble microbial products (SMPs) or soluble EPS and the non-soluble fraction of the EPS. For each extraction, two fibers (10 cm length each) from the same module were sacrificed, and total EPS were extracted according to Gonzalez-Gil et al., with minor modifications (Gonzalez-Gil et al., 2015). Briefly, membrane fibers were cut into small pieces of 1 cm length and placed in a falcon tube. Then, 15 mL of 0.1 M NaCl (prepared in milliQ water) were added and samples were vortexed at high speed for 45 min, to achieve complete biofilm detachment. Next, 6 mL of 0.4 M NaOH was added to the EPS solution, followed by a heating phase at 60°C for 30 min. Finally, the EPS solutions were centrifuged at 12,000g for 20 min at 4°C, to remove remaining bacterial cells.

2.4. Analysis of the EPS solution

2.4.1. Liquid chromatography with organic carbon detection

A Shimadzu TOC-Vcsh Analyzer was used to measure the total dissolved organic carbon (TOC) of EPS solutions, then a liquid chromatography with organic carbon detection model 8 (LC-OCD) (DOC-LABOR, Germany) (Huber et al, 2011) equipped with a size exclusion chromatography was used to separate the EPS fractions according to their molecular weight. The ChromCALC® software, compatible with the LC-OCD was used to integrate the resulting peak areas of different EPS fractions and convert them into carbon concentrations (mg/L) (Huber et al, 2011). For each sample, the injection volume was 1000 µL and the analysis time was 130 min (Wang et al., 2014).

2.4.2. Fluorescence excitation - emission matrices

A Fluoro Max-4 spectrofluorometer (Horiba Scientific, Japan) was used to measure the fluorescence excitation - emission matrices (FEEM) of the EPS solutions. EEM matrixes were collected with excitation and emission wavelengths that ranged from 200 to 600 nm and from 200 to 400 nm, respectively. The emission integration time was fixed at 1 s and both excitation and emission bandwidths were adjusted to 5 nm (Wang et al., 2014), and the EEM signals were corrected using blank subtraction process (Murphy et al., 2010).

2.4.3. Fourier transform infrared spectroscopy

Membrane dialysis was performed according to (Khan et al., 2014) to remove remaining salts and NaOH from the EPS solutions, then the EPS solutions were lyophilized to obtain dried foulant material, and 100 mg of dried foulant material was mixed with 100 mg of potassium bromide salt (KBr) and pressed into KBr pellets (Khan et al., 2014). Finally, fourier transform infrared (FTIR) spectroscopy with an attenuated total reflection (PerkinElmer, USA) was used to obtain the FTIR spectra of the lyophilized EPS material.

2.4.4. Proteins and polysaccharides quantification

Proteins and polysaccharides in the EPS samples were quantified using the Lowry method (Lowry et al., 1951) with the bovine serum albumin as standards, and the phenol-sulphuric method (Dubois et al., 1956) with glucose as standards, respectively.

2.5. Confocal laser scanning microscopy

Membrane fibers were cut into small pieces of 1 cm length and the biofilms were stained with SYTO 9 for 30 min in the dark, to visualize the spatial distribution of the bacterial cells. Then, membrane samples were rinsed with 1× phosphate buffer saline (PBS)

solution to remove excess dye, and were incubated for 30 min in the dark, with a mixture of Sypro Orange, Con A Alexa and WGA Alexa, to target the total proteins, α -Man polysaccharides and β -GlcNAc polysaccharides, respectively (Table S2, Supporting information). Finally, membrane fibers were rinsed with 1 $\times$ PBS to remove excess dye. The membrane samples were then embedded with Jung Tissue Freezing medium. Then, membrane pieces were finely cut in transverse direction, into 20 μ m thick slices using a Cryostat CM 3050 E (Leica Biosystems), at -20°C. Triplicate slices were performed for each membrane sample.

Cryosection slices were mounted on microscope glass slides and covered with a cover slip, and examined immediately using LSM710 confocal laser scanning microscope (Zeiss, Germany). On average, triplicate images were taken for each sample using a 20 $\times$ -magnification lens.

2.6. Statistical analysis

Multivariate statistical analysis was used to identify the agglomerative trends of different membranes with regards to EPS fractions (Varmuza and Filzmoser, 2009). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances was created to visualize differences in the EPS samples extracted from the different membrane surfaces. EPS fractions were characterized using LC-OCD and then normalized and Hellinger transformed as recommended for Bray-Curtis distances.

A principal component analysis (PCA) biplot was generated to visualize ordination space distances, between different membrane surfaces and the abundances of EPS fractions measured by LC-OCD. EPS fractions were normalized and Hellinger transformed as recommended for Euclidian distance based ordinations such as PCA

273 (Legendre and Gallagher, 2001). All analyses were conducted using RStudio (Rstudio,
274 2012) with the package Vegan (Oksanen et al., 2013), respectively.

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3. Results

3.1. Reactor performance and TMP measurements

The concentrations of COD, $\text{NH}_3\text{-N}$, $\text{NO}_3^- \text{-N}$ and $\text{NO}_2^- \text{-N}$ were measured continuously during both acclimatization and experimental phase. The reactor achieved 92 ± 3 % COD removal and 86 ± 6 % $\text{NH}_3\text{-N}$ removal. The concentrations of $\text{NO}_3^- \text{-N}$ and $\text{NO}_2^- \text{-N}$ in the permeate were 0.17 ± 0.09 and 0.12 ± 0.10 mg/L, respectively. The TMP was monitored for the four different membranes during the experiment (Fig. 2). Although all membranes featured similar pore size (Table 1), the TMP of the hydrophobic membranes (POX and PTA) after one day of filtration became much higher (reached 80 kPa) than the TMP of hydrophilic membranes (SPTA and SPSU). This could be a consequence of the high hydrophobicity, which favors the adhesion of hydrophobic solutes, leading to a lower effective permeation (higher TMP value). In addition, the SPSU membrane exhibited lower TMP compared with the SPTA membrane (Fig. 2).

The SPSU membrane surface was characterized with the lowest contact angle (higher hydrophilicity) and most negative charge (-106 mV) (Table 1), and displayed the lowest intrinsic membrane resistance (R_m) measured with deionized water compared with the remaining three other membranes. R_m values are summarized in the following order: POX ($17 \times 10^{12} \text{ m}^{-1}$) > PTA ($16.5 \times 10^{12} \text{ m}^{-1}$) > SPTA ($12.2 \times 10^{12} \text{ m}^{-1}$) > SPSU ($9.1 \times 10^{12} \text{ m}^{-1}$) (Table 1). It should be noted that membrane thickness, pore size and porosity, which could also affect the intrinsic membrane resistance, were similar in the four membrane types.

The TMP was quickly established (after 2 hr for hydrophilic and 5 hr for hydrophobic membranes), and the stabilized TMP values can be summarized in the following series:

POX $\approx$ PTA $\gg$ SPTA>SPSU, with the four membranes showing an increasing trend in contact angle measurements: POX (96.4°) > PTA (85.2°) > SPTA (65.4°) > SPSU (54.5°) (Table 1). The quick TMP increase could be due to the initial adsorption of foulants on membrane surfaces, not necessarily removed for analysis.

3.2. Characterisation of the initial fouling layer

The initial fouling layer that developed on the membrane surfaces after 1 day of filtration was analyzed using FTIR for the membranes operated with permeate flux (Fig. 3). The results revealed that all membranes accumulated an identical fingerprint profile, composed mainly of protein-like substances. Peaks related to amide (1640 cm⁻¹, 1560 cm⁻¹, and 1414 cm⁻¹) were detected on the surfaces of the four different membrane (Fig. 3), and contributed along with other accumulated organics to masking the specific functional groups of the virgin membrane surfaces (Fig. S1, Supporting information). Similar results were observed with the membranes operated with no flux (Fig. S2, Supporting information). In addition, the proteins and polysaccharides concentrations in the EPS samples were quantified using standard colorimetric methods, and results showed that proteins were more abundant than polysaccharides at day 1 for the four membranes operated with 10 L/m².h (Fig. 4A), suggesting that the initial conditioning fouling layer was composed predominantly of proteins. The SPSU membrane accumulated significantly lower amounts of organic foulants ($p < 0.05$), compared to the remaining three other membranes (Fig. 4B).

3.3. Temporal changes in fouling characteristics

3.3.1. LC-OCD

The LC-OCD chromatograms of the four membranes operated with 10 L/m².h permeate flux evolved similarly with time (Fig. S3, Supporting information), and the difference in composition of organic foulants in the four membranes was not large, but it can be seen for instance that in days 1 and 10 the accumulation of neutral biopolymers, humic substances and other medium molecular weight building blocks was lower in SPSU membranes compared to the other membranes (POX, PTA and SPTA) (Fig. 5), which might explain the lower TMP values for SPSU. Finally, the amount of humic substances that accumulated on the membranes almost doubled between day 20 and 30 for all the membranes, and when comparing the four membranes at the final stage of filtration, PTA and SPTA membranes slightly accumulated more humics than POX and SPSU respectively (Fig. 5 and Fig. S3). The membranes operated without permeate flux behaved similarly (Fig. S4, Supporting information).

NMDS analysis showed that EPS samples clustered together according to the day of sampling, regardless of the membrane surface characteristic (hydrophobic or hydrophilic), and no clear differences were observed between membranes that undergo filtration and no filtration (Fig. 6). In addition, samples from days 1 and 10 were more distant from each other, while samples from days 20 and 30 clustered closely together. It should be noted that on days 20 and 30 the EPS samples from the mixed liquor were distant from the EPS samples extracted from the four membranes.

A biplot was generated based on the LC-OCD data with the different samples represented by blue circles (day 1) and red circles (day 30) and the gray arrows correspond to the variables (i.e. the different EPS components) (Fig. 7). The EPS samples extracted from the membrane surfaces (combining flux and no flux data) at day 1

occupied the two left quadrants (blue circles), while the majority of the samples extracted from day 30 were located in the two right quadrants (red circles) (Fig. 7). The EPS structure shifted from proteins-like substances, low molecular weight acids and building blocs after day 1 of filtration, towards polysaccharides-like substances, humics and low molecular weight neutrals after 30 days of filtration (Fig. 7). In addition, the proteins and polysaccharides quantification showed that, the protein production continued to increase in the EPS samples (Fig. 4A), however the polysaccharides fraction became more abundant after 30 days of filtration.

3.3.2. FEEM

The excitation and emission profiles were measured for the EPS samples of the different membranes operated with 10 L/m².h permeate flux using a spectrofluorometer, and their dynamics was monitored over time (Fig. 8). The EPS from the four membranes evolved similarly in composition and exhibited a reproducible pattern. Initially at day 1, low intensity peaks related to aromatic proteins were detected in regions I (Ex = 250 nm and Em = 330 nm) and II (Ex = 250 nm and Em = 330 - 380 nm), and peaks related to fulvic acid-like material were detected in region III (Ex = 250 nm and Em = 380 - 550 nm) on all membrane surfaces, with SPTA membrane displaying the highest signal. High intensity peaks corresponding to humic acid-like material were detected in region V (Ex = 250 - 330 nm and Em = 380 - 500 nm) for all membranes samples at day 30 (Fig. 8) (Murphy et al., 2010). Even though the intensities of the detected peaks corresponding to various organics were different, the same signals were identified on all the membranes. This could suggest that following the development of a biofouling layer on the

membranes, the hydrophobic or hydrophilic membrane surface loses its characteristic, and the different membranes accumulated identical EPS fractions.

3.4. Visualisation of the biofilm architecture

The buildup of the protein and polysaccharide fractions of the EPS on the four different membrane surfaces operated with 10 L/m².h permeate flux was monitored with CLSM imaging (Fig. 9). Imaging results confirmed LC-OCD and FEEM results, where the four different membranes behaved similarly (Fig. 9). Also, the conditioning layer after day 1 was composed mainly of proteins, which shifted towards polysaccharides-like substances after 30 days of continuous filtration. In addition, the thickness of the biofouling layer that developed on the four membranes continued to increase with time; whereas initially a thin layer covered the membranes at day 1, and at the end of the experiment, a thick and uniform biofouling layer was formed (100 µm) (Fig. 9).

4. Discussion

4.1. Effect of surface hydrophilicity and charge on biofouling

In this study, we measured the contact angle for the four ultrafiltration membranes as an estimate of hydrophilicity (Table 1). Despite the fact that contact angle measurement remains an approximate test method to determine surface hydrophilicity (Subhi et al., 2012), results showed differences among the four tested membranes, with SPSU membrane having the lowest contact angle value (54.5°) and the highest hydrophilicity. Highly hydrophobic POX and PTA hollow-fiber membranes distinguished themselves from hydrophilic SPTA and SPSU membranes as far as TMP is concerned. Hydrophobic membranes had higher TMP values than hydrophilic ones throughout the experiment. Due to the hydrophobicity of the membrane surface, more energy would be required to push the same volume of water through the hydrophobic membranes than hydrophilic membranes, which lead to differences in the TMP profile.

The influence of hydrophilicity and surface chemistry on biofouling has been demonstrated by many research groups, justifying for instance with the fact that a layer of absorbed water molecules on hydrophilic surfaces, reduces the adhesion of foulants on membrane surfaces (Elimelech and Phillip, 2011). However, fouling behavior in long-term filtration of membranes in MBRs, as reported in this work, are much less dependent on hydrophilicity. This supports previous observations by other groups (Miller et al., 2012) who coated UF and NF membranes and feed spacers with polydopamine to increase their hydrophilic surface character, and their fouling tendency were tested under continuous filtration mode during 10 days (Miller et al., 2012). Their results also showed that biofilm developed on the modified membranes independently of the hydrophilic

surface character. In this study, the hydrophilic SPSU membranes accumulated lower amounts of EPS compared with other more hydrophobic membranes (Fig. 4B) potentially due to the highly negative surface charge (Table 1). The influence of the surface zeta potential on interactions with foulants particles for other membrane surfaces has been reported by Zhang et al. as being even more important than hydrophilicity (Zhang et al., 2015). Similarly, Qu et al. showed that membrane's negative surface charge increased the electrostatic repulsion with the organic matter and reduced their adherence on membrane surfaces (Qu et al., 2012). Therefore, designing membranes with high zeta potential could alleviate membrane fouling in MBRs, but the long-term effectiveness of high zeta potential membrane surfaces in reducing biofouling still requires further investigations.

It should be noted that a number of factors that were not evaluated as part of this study, such as membrane surface roughness, could potentially affect the development of membrane fouling. For example, UF membranes in anaerobic MBR with the largest initial water flux and the roughest surface exhibited the most severe flux decline (He et al., 2006). Kang et al. showed that the initial adhesion rate of *Saccharomyces cerevisiae* was significantly lower on the membrane which had more negative, hydrophilic, and smooth surfaces (Kang et al., 2006). Jin et al. showed that ceramic membranes submerged in a lab-scale MBR with the roughest surface and biggest pore size had the highest fouling with respect to TMP profile, whereas ceramic membranes with a smoother surface and relatively uniform smaller pore openings experienced least membrane fouling with respect to TMP increase (Jin et al., 2010). Furthermore, Vrijenhoek et al. used atomic force microscopy analysis to demonstrate that colloidal fouling of RO and NF membranes was correlated with membrane surface roughness

where more colloidal particles deposited on rough membranes than on smooth RO/NF membranes (Vrijenhoek et al., 2001).

4.2. Dynamics of the EPS deposition on different membrane surfaces

Studying the temporal dynamics of EPS and how their components evolve with time when the biofilm matures according to different membrane surfaces could be essential to establish a better understanding of the membrane biofouling phenomenon in submerged MBRs, especially that these studies are still lacking in MBRs compared with RO and NF membrane systems (Ivnitsky et al., 2007; Dreszer et al., 2014a; Farias et al., 2014). The development of the fouling layer on RO membranes was investigated in a bench-scale RO system over two weeks (Farias et al., 2014), and their results showed that carbohydrate materials increased with time after 14 d, but the amounts of proteins remained stable. Dreszer et al found during 4 d biofilm development studies that the biofilm contained more proteins than polysaccharides (Dreszer et al., 2014a), which was in agreement with our findings following similar short membrane operation time. The evolution of microbial communities and their EPS products on NF membranes were monitored over 24 d, and findings revealed that the accumulation of EPS on NF membranes was initiated by polysaccharides (after 8 hours), and contributed to permeate flux decline (Ivnitsky et al., 2007).

All the aforementioned studies used colorimetric and/or microscopic techniques to characterize EPS that developed on the membrane surface. Conventional colorimetric analytical tests, such as Lowry (Lowry et al., 1951) and Dubois (Dubois et al., 1956) are applied to quantify proteins and polysaccharides fractions in the MBR fouling layer, respectively. However, these colorimetric tests remain non-exclusive since nitrate or

nitrites can potentially affect the Dubois results, and since the Lowry test can respond to humic substances (Drews, 2010). Instead, several other chemical analytical techniques such as LC-OCD, FEEM and FTIR are currently being used to characterize EPS on membrane surfaces (Meng et al., 2010; Malaeb et al., 2013). LC-OCD is based on size exclusion chromatography and is applied to characterize biopolymers (proteins and carbohydrates), humics and low molecular weight molecules (acids and neutrals) in MBR fouling layer, membrane permeate and sludge supernatant (Meng et al., 2010). On the other hand, FEEM allows detection of aromatic proteins, humic acid-like substances, fulvic acid-like substances and SMP (Murphy et al., 2010; Bridgeman et al., 2011). FTIR allows detecting the functional groups of membrane foulants, such as polysaccharides, proteins, amino acids and humic-like substances (Meng et al., 2010).

In the current study, conventional colorimetric tests (Lowry and Dubois), LC-OCD, FEEM, FTIR, and CLSM were applied to monitor the temporal dynamics of EPS on four different membranes. The initial fouling layer was predominantly composed of proteins based on the Lowry method (Fig. 4A) and CLSM images (Fig. 9). At later stages polysaccharides became dominant based on Dubois method (Fig. 4A) and CLSM (Fig. 9). FTIR results showed a very low peak at 1070 cm^{-1} corresponding to sugars at day 1 (Fig. 3 and Fig. S2), and that confirmed the low concentration of polysaccharides obtained using colorimetric methods. On the other hand, LC-OCD analysis showed low molecular weight neutrals were dominant at day 1 and their relative abundance decreased with time with the concomitant increase in the relative abundance of humic substances and biopolymers (Fig. 5; Fig. 7 and Fig. S4). The biopolymer fraction (proteins and polysaccharides are major components of biopolymers) identified with LC-OCD was low

despite the fact that the concentrations of proteins and polysaccharides measured using conventional colorimetric tests was high. This is not surprising as the LC-OCD method provides information on the different EPS fractions including biopolymers, humic substances, low molecular weight neutrals, low molecular weight acids and building blocks, whereas the colorimetric tests (i.e. Lowry and Dubois) are used to specifically quantify the concentrations (expressed in this study as mg/cm^2 of membrane surface) of proteins and polysaccharides in the EPS fractions. Also, the two analytical tools work completely differently. For example, the biopolymers identified using LC-OCD consist of large and intact proteins and polysaccharides, whereas the colorimetric tests will react with functional groups of potentially breakdown protein products and polysaccharide monomers.

FEEM diagrams at day 30 showed the dominance of humic substances (Fig. 8) and are in coherence with the results obtained by LC-OCD. The intensity of peaks corresponding to proteins in FEEM were not consistent with the concentration of proteins detected by Lowry method. This is due to the fact that the 3D FEEM results correspond to the intensities of peaks detected for EPS fractions mainly proteins and humics, whereas the Lowry method specifically quantifies the concentrations of proteins in the EPS fractions. Also, the relative abundance of humics increased with time and therefore, the peaks that correspond to the humic substances became more intense when compared to the peaks that belonged to the proteins fractions. It should be noted that the 3D FEEM results are presented using the same intensity scale (Fig. 8). It is clear from these results that a combination of complementary analytical tools is required to get a better insight on the composition and type of foulants selected on membrane surfaces.

Although the aforementioned analytical tools are powerful tools for investigating the footprint of organic matter, the detailed information on their protein or polysaccharide species would not be obtained by the analytical techniques listed above. Alternatively, state-of-the-art analytical tools such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and mass spectrometry-based proteomics could help in elucidating the detailed chemical composition of EPS. Gonzalez-Gil et al. reported the presence of alginate-like exopolysaccharides in the EPS matrix of anaerobic granular sludge using MALDI-TOF MS (Gonzalez-Gil et al., 2015). Also, mass spectrometry-based proteomics can be applied to identify and precisely quantify protein components (Aebersold and Mann, 2003). Given the complex nature of the foulant mixture obtained in MBRs and characterizing EPS from mixed microbial systems is not straightforward, it is proposed that the use of LC-OCD, combined with NMDS could potentially be used as a method for monitoring or comparing profiles of EPS from various modified membrane surfaces.

Irrespective of the analytical technique used to characterize foulants, the same organic foulants were observed on the surfaces of the four different membranes regardless of membrane surface characteristic (Fig.3; Fig. 5 and Fig. 8) or mode of operation (10 vs. 0 L/m².h) (Fig. S2 and Fig. S4). Also, the same temporal dynamics in EPS composition was observed on the four different membranes (Fig. 6). The only remarkable difference between the four membranes was the TOC concentration (Fig. 4B). The amount of foulants that amassed on the SPSU membrane at the initial (day 1) and final stages of filtration (day 30), were significantly lower than the three other membranes (Fig. 4B), possibly due to the very high negatively charged membrane surface (Table 1).

Collectively, these results suggest that other parameters such as biofilm thickness, porosity, compactness and structure should be considered in future studies for evaluating membrane performance and effectiveness of membrane surface modifications. For example, recent studies showed that membrane operational conditions such as the imposed permeate flux caused variations in the compactness, morphology and thickness of the biofilm on the membrane, which lead to losses in the membrane system's performance and an increase in the TMP (Dreszer et al., 2014b; Valladares Linares et al., 2015).

Despite the fact that membrane surface chemistry might contribute to selecting the conditioning fouling layer during early stages of filtration, this fouling selection becomes of reduced importance since the membrane loses its intrinsic affinity following the accumulation and development of a mature fouling layer. The results presented in this study suggest that the conditioning fouling layer might have developed quickly on the membrane surface and masked its intrinsic characteristic after one day of filtration, where the four membranes showed similar composition of EPS.

A recent study investigated the effect of organic nutrient load on biofouling of spiral wound NF, RO and forward osmosis membrane systems (Bucs et al., 2014). Their results showed that the organic nutrient load dictated the accumulation of biomass on membrane surfaces and feed spacers, and the impact of accumulated biomass on membrane performance was reduced when adopting lower crossflow velocity combined with a modified geometry feed spacer (Bucs et al., 2014). Future studies that focus on controlling membrane biofouling should include strategies to reduce the impact of accumulated biofilm on membrane performance and potentially applying advanced

cleaning strategies (Cornelissen et al., 2007, Vrouwenvelder et al. 2010, Van Loosdrecht et al., 2012) that are simple and cost-effective, to reduce the overall membrane operating costs. For such studies, a suite of tools for non-destructive in-situ analysis of fouling is available, such as optical coherence tomography (Derlon et al., 2012; Dresser et al., 2014, Valladares Linares et al., 2015; West et al., 2016) and nuclear magnetic resonance imaging (Graf von der Schulenberg, 2008; Creber et al., 2010a,b).

4.3. Effect of permeate production on membrane biofouling

Operating membranes systems at sub-critical permeate flux values has been considered as an alternative approach to reduce membrane biofouling, and several studies confirmed that biofilm formation occurs even at low permeate flux. Despite operating the MBR at sub-critical flux values defined between 30 and 40 L/m².h, membrane fouling occurred progressively and contributed to the gradual increase in transmembrane pressure (Ognier et al., 2004). Similar results were observed in an MBR operated at much lower flux ranging between 10 and 18 L/m².h, and concluded that membrane fouling in an MBR is unavoidable even at low flux rates, yet it changes considerably when the critical flux is reached (Le-Clech et al., 2003). Likewise, the imposed permeate flux (10 L/m².h) did not affect the pressure drop increase in NF or RO membranes; neither did it impact the membrane biofouling rate, consequently the critical flux concept was not applicable (Vrouwenvelder et al., 2009a; 2009b).

Despite the very low flux of 10 L/m².h, membrane biofouling was unavoidable, and the accumulation of microorganisms and their EPS products increased with time, on the surfaces of the four hollow-fiber membranes (Fig. 9). EPS attachment onto the four

different membranes occurred under passive adsorption (i.e. no flux) and active (i.e. filtration mode) conditions, as described previously (Drioli and Giorno, 2009). The rate of passive bacterial adsorption on the membrane surface is expected to be different from those obtained during filtration. During initial membrane contact with the mixed liquor components (filtration or not), SMP interact with the membrane surface on which specific foulants adsorb depending on its hydrophobicity. Once the membrane is covered, additional SMP and EPS products interact with the organic-covered membrane and its intrinsic characteristics do not affect the fouling rate any further. Recently, hydrophilic fractions of natural organic matter composed of biopolymers (proteins and polysaccharides), resulted into irreversible fouling of different membranes after only 13 h of filtration (Yamamura et al., 2014). Our results showed that identical relative abundances of EPS fractions accumulated on the four different membranes using LC-OCD analysis (Fig. 5), and similar accumulation of EPS fractions occurred on the membranes without flux ($0 \text{ L/m}^2 \cdot \text{h}$) (Fig. S4, Supporting information). However, subsequent biofouling steps that include attachment of pioneer microorganisms and their growth into a mature biofilm happened eventually on the four membranes under flux and no flux conditions. Studying these modified membranes under sub-critical flux was not the main objective of this work, yet biofilm formation happened on all the membranes, regardless of the chemical characteristics of the membrane surface or the imposed permeate flux.

5. Conclusion

This is the first study that adopted several analytical tools to characterize the temporal dynamics of EPS, on four polymeric ultrafiltration membranes differing in hydrophobic and hydrophilic character, tested in parallel in the same membrane bioreactor tank.

The main outcomes can be summarized as follows:

(1) The intrinsic membrane characteristics does not seem to impact long term membrane fouling, yet it might contribute to the initial transmembrane pressure value that was established within couple of hours after filtration.

(2) Despite clear differences in the transmembrane pressure measurements, the different surface characteristics of the membranes did not affect the selection of specific foulants at the initial stages of filtration (day 1).

(3) Hydrophilic sulfonated polysulfone membrane accumulated the lowest amounts of foulants and developed the lowest transmembrane pressure compared with the other four membranes, potentially due to the high negative surface charge.

(4) The same temporal changes in EPS were observed on the surfaces of all the membranes tested.

(5) The combination of analytical tools provided a comprehensive analysis of the accumulated foulants selected on membrane surfaces in the lab-scale MBR.

A potential alternative explanation for the impact of accumulated biomass on transmembrane pressure was discussed, involving the thickness, biofilm compactness (density) and spatial structure including *in-situ* non-destructive methods for characterization such as optical coherence tomography and NMR.

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Figure Captions

- Fig. 1 – Schematic of the lab-scale membrane bioreactor (MBR).**
- Fig. 2 - Transmembrane pressure (TMP) profiles for the four membranes during filtration (with permeate production).**
- Fig. 3 - Fourier transform infrared (FTIR) spectroscopic analysis of the foulant material extracted from the four different membrane surfaces during early stages of filtration, operated with 10 L/m².h permeate production (1 day).**
- Fig. 4 – A) Concentrations of proteins and polysaccharides in the extracellular polymeric substances (EPS) samples extracted from the four membrane surfaces on day 1 and 30, and quantified using colorimetric methods (Lowry et al., 1951; Dubois et al., 1956). B) TOC concentrations (mg/cm²) accumulated on the four different membranes operated in continuous mode for 1 and 30 days.**
- Fig. 5 - Temporal dynamics of organic foulants extracted from the surfaces of four different membranes operated with 10 L/m².h permeate flux, and analyzed using liquid chromatography with organic carbon detection (LC-OCD). The black arrows correspond to the chronological order of the membrane sample collection.**
- Fig. 6 - Non-metric multidimensional scaling (NMDS) results of the EPS solutions extracted from four different membranes with (10 L/m².h) and without permeate production (0 L/m².h). The red circles correspond to the EPS samples that clustered together based on their percent similarity. POX (Polyoxadiazole), PTA (Polytriazole), SPTA (Sulfonated Polytriazole) and SPSU (Sulfonated Polysulfone) and MLSS (mixed liquor suspended solids) correspond to the membrane and activated sludge EPS samples collected according to the sampling day. NF correspond to the EPS samples extracted from the membranes operated without permeate production.**
- Fig. 7 - Biplot of the EPS solution, extracted from the different membrane surfaces. Blue circles and red circles correspond to samples from day 1 and day 30, respectively. The gray arrows point towards different EPS fractions, and PC1 (component 1) and PC2 (component 2) explain 77.4% and 19.5% of the variance between samples, respectively.**
- Fig. 8 - Evolution of 3D Fluorescence Excitation Emission (FEEM) diagrams for four different membranes operated with permeate production for 1, 10, 20 and 30 days. The color scale corresponds to the intensity of the peaks, which correlates to their concentration, where red correspond to the highest intensity (22,000) and blue corresponds to the lowest intensity. Fluorescence emission intensity in arbitrary units.**
- Fig. 9 - Confocal laser scanning microscopy (CLSM) images showing the evolution of different EPS fractions accumulated on the four membrane surfaces. Total cells,**

843 proteins and α - and β -Polysaccharides were stained with their corresponding dyes
844 (Table S2, Supporting information). The scale bar length corresponds to 50 μm .

Table 1 – Membrane properties and polymer composition

Membrane type	Acronym	Membrane properties				Polymer composition and characteristics
		Pore size (μm)	Contact Angle (°)	Zeta potential (mV)*	R_m (m ⁻¹)**	
Polyoxadiazole	POX	0.1	96.4±3.2	-28±1	17×10 ¹²	Very hydrophobic (fluorinated)
Polytriazole	PTA	0.1	85.2±12.0	-31±1	16.5×10 ¹²	Hydrophobic (fluorinated)
Sulfonated Polytriazole	SPTA	0.1	65.4±7.5	-23±1	12.2×10 ¹²	Hydrophilic (sulfonic group)
Sulfonated Polysulfone	SPSU	0.1	54.5±3.9	-106±1	9.1×10 ¹²	Hydrophilic (sulfonic group)

*Zeta potential measured using 10 mM NaCl as ionic solution, and presented for pH 7.5

**Intrinsic membrane resistance measure with deionized water

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Fig. 2 - Transmembrane pressure (TMP) profiles for the four membranes during filtration (with permeate production).

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Day 1

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Fig. 3 - Fourier transform infrared (FTIR) spectroscopic analysis of the foulant material extracted from the four different membrane surfaces during early stages of filtration, operated with 10 L/m².h permeate production (1 day).

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Fig. 4 – A) Concentrations of proteins and polysaccharides in the extracellular polymeric substances (EPS) samples extracted from the four membrane surfaces on day 1 and 30, and quantified using colorimetric methods (Lowry et al., 1951; Dubois et al., 1956). B) TOC concentrations (mg/cm²) accumulated on the four different membranes operated in continuous mode for 1 and 30 days.

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2 Low MW acids Low MW neutrals Building blocs Humics Substances Biopolymers

3 **Fig. 5 - Temporal dynamics of organic foulants extracted from the surfaces of four**
 4 **different membranes operated with 10 L/m².h permeate flux, and analyzed using**
 5 **liquid chromatography with organic carbon detection (LC-OCD). The black arrows**
 6 **correspond to the chronological order of the membrane sample collection.**

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Fig. 6 - Non-metric multidimensional scaling (NMDS) results of the EPS solutions extracted from four different membranes with (10 L/m².h) and without permeate production (0 L/m².h). The red circles correspond to the EPS samples that clustered together based on their percent similarity. POX (Polyoxadiazole), PTA (Polytriazole), SPTA (Sulfonated Polytriazole) and SPSU (Sulfonated Polysulfone) and MLSS (mixed liquor suspended solids) correspond to the membrane and activated sludge EPS samples collected according to the sampling day. NF correspond to the EPS samples extracted from the membranes operated without permeate production.

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Fig. 7 - Biplot of the EPS solution, extracted from the different membrane surfaces. Blue circles and red circles correspond to samples from day 1 and day 30, respectively. The gray arrows point towards different EPS fractions, and PC1 (component 1) and PC2 (component 2) explain 77.4% and 19.5% of the variance between samples, respectively.

Fig. 8 - Evolution of 3D Fluorescence Excitation Emission (FEEM) diagrams for four different membranes operated with permeate production for 1, 10, 20 and 30 days. The color scale corresponds to the intensity of the peaks, which correlates to their concentration, where red correspond to the highest intensity (22,000) and blue corresponds to the lowest intensity. Fluorescence emission intensity in arbitrary units.

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Fig. 9 - Confocal laser scanning microscopy (CLSM) images showing the evolution of different EPS fractions accumulated on the four membrane surfaces. Total cells, proteins and α - and β -Polysaccharides were stained with their corresponding dyes (Table S2, Supporting information). The scale bar length corresponds to 50 μ m.

1 **Highlights**

- 2 • Four membranes differing in hydrophobic and hydrophilic character
- 3 • All membranes showed similar temporal changes of extracellular polymeric
- 4 substances
- 5 • Membranes with modified surfaces did not affect the EPS composition
- 6 • Sulfonated polysulfone had the lowest zeta potential, TMP and EPS
- 7 • Membranes with modified surfaces should be tested for extended durations

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