Accepted Manuscript

Title: Real-time monitoring of membrane fouling development during early stages of activated sludge membrane bioreactor operation

Authors: Luca Fortunato, Nirenkumar Pathak, Zahid Ur Rehman, Hokyong Shon, TorOve Leiknes

PII: S0957-5820(18)30546-9
DOI: <https://doi.org/10.1016/j.psep.2018.09.022>
Reference: PSEP 1527

To appear in: *Process Safety and Environment Protection*

Received date: 22-7-2018
Revised date: 10-9-2018
Accepted date: 24-9-2018

Please cite this article as: Fortunato L, Pathak N, Ur Rehman Z, Shon H, Leiknes T, Real-time monitoring of membrane fouling development during early stages of activated sludge membrane bioreactor operation, *Process Safety and Environmental Protection* (2018), <https://doi.org/10.1016/j.psep.2018.09.022>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Real-time monitoring of membrane fouling development during early stages of activated sludge membrane bioreactor operation

Luca Fortunato^{1*}, Nirenkumar Pathak², Zahid Ur Rehman¹, Hokyong Shon², TorOve

Leiknes¹

¹ Water Desalination and Reuse Center (WDRC), Division of Biological & Environmental Science & Engineering (BESE), 4700 King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia.

² School of Civil and Environmental Engineering, University of Technology, Sydney, Post Box 129, Broadway, NSW 2007, Australia.

*Corresponding author: Tel. +966 12 808 2289; Email: Luca.Fortunato@kaust.edu.sa

Graphical Abstract

Highlights

- OCT was employed to monitor the fouling formation and development in AS-MBR.
- The increase in TMP was related to the biomass thickness and to the SMP concentration.
- The membrane autopsy values were normalized for the volume of biomass.
- The abundance of *Proteobacteria* was observed in in sludge and fouling layer.

Abstract

Non-invasive analysis and a final destructive analysis were employed to study the fouling formation during the initial days of AS-MBR operation. The fouling layer development was quantified *in-situ* non-invasively with Optical Coherence Tomography (OCT). The increase in biomass thickness was related to the transmembrane pressure (TMP) and to the increase in concentration of soluble microbial products (SMP) in the reactor. The OCT non-destructive analysis allowed normalizing the final autopsy values for the amount of biomass deposited on the membrane. After 8 days of operation, the cake layer presented a biomass activity of 400 pg/mm³ of intra-ATP and EPS concentration of 9.8 mg/ mm³. The microbial community analysis of sludge and biofouling on the membrane surface revealed the abundance of *Proteobacteria*.

Keywords: Cake layer; Membrane fouling; Optical coherence tomography (OCT); Membrane autopsy; Microbial community

1. Introduction

Over the past three decades, membrane bioreactor (MBR) technology that combines the biological activated sludge process with membrane filtration has emerged as an innovative and alternative technology for tertiary wastewater treatment and reuse. Compared to the conventional activated sludge (CAS) process, which has been the most common wastewater treatment process to date, in MBRs the secondary clarifiers are replaced by membrane filtration using polymeric membranes (Ivanovic and Leiknes, 2012). The advantages of MBR technology compared to conventional systems are the high effluent quality achieved, a lower plant footprint, and efficient nutrient removal. Most commercial MBR systems are based on the activated sludge (AS) process coupled with membrane filtration. The membrane units are typically submerged in the AS, either directly in the aeration tank or in a separate membrane-holding tank.

Although the application of MBR has grown worldwide, membrane fouling still represents one of the main drawbacks and challenges. Membrane fouling leads to an increase in TMP under constant flux operation and decrease in flux under constant pressure operation. In MBR, the fouling layer is typically composed of microorganisms encased in self-produced extracellular polymeric substances (EPS), commonly referred to as biofouling. The composition of EPS varies with microbial community, which in turn depends on wastewater characteristics and process configuration (Ziegler et al., 2016).

Membrane fouling starts with adhesion of fouling particle to the membrane. The adhesion on the membrane surface depends on interfacial interaction energy between the fouling particle and membrane, that is influenced by size of the fouling particle and the surface topography of membrane and fouling particle (Cai et al., 2017; Chen et al., 2017). Small size flocs easily adhere to membranes due to high specific interaction energy (Shen et al., 2015), those together with biopolymers greatly increase the hydraulic resistance of fouling layer. Biopolymers and foulants that forms a gel layer on the membrane surface that leads to the increase of the filtration resistance by a chemical potential mechanism (Chen et al., 2016; Zhang et al., 2017).

Commercial MBR systems are typically designed for operation under constant flux mode, which is achieved by permeate extraction by vacuum. Under constant flux operation, the feed water flowing through the membrane is more constant compared to systems operated under fixed TMP (Li et al., 2013). A common practice to reduce CAPEX (*i.e.* investment in number of membrane modules) has been to increase the operating flux thereby reducing the required membrane area and number of membrane modules. However, high fluxes lead to an overall increase of OPEX as membrane fouling increases requiring more fouling mitigation efforts such as increased air scouring and more frequent membrane cleaning procedures. Hence, a sustainable option is to operate the system below a critical flux reducing the rate of fouling deposition on the membrane (Kwon et al., 2000).

Many efforts have been made in the last years to reduce membrane fouling, as well as proposing different approaches to monitor fouling in MBR processes. Most of the methods are based on characterization of the sludge properties (*i.e.* filterability) and defining a matrix for fouling potential (Sabia et al., 2014). The most common method consists in monitoring the change in TMP over time during operation and interpreting fouling development as a function of TMP change. However, the TMP represents an indirect way to assess the fouling since it doesn't provide direct information about the fouling layer deposited on the membrane surface.

Image analysis of the fouling layer offers a lot of insight to the structure, morphology and nature of the fouling layer formed, however, most imaging techniques require a membrane sample to be removed from the membrane module and a varying degree of sample preparation depending on the imaging technique (e.g. microscopy, confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), etc.). Optical coherence tomography (OCT) is an imaging characterization technique that offers the advantage of acquiring scans in the system without any need of extracting samples or sample preparation using staining agents (Valladares Linares et al., 2016). Recently the use of OCT in membrane filtration system has been evaluated and tested in different membrane configurations and processes (Wagner and Horn, 2017). In spacer filled channels, such as spiral wound membranes, OCT imaging enabled the assessment of biomass accumulation over time in different elements in a flow cell simulating spiral wound

membranes (L. Fortunato et al., 2017b; Fortunato and Leiknes, 2017). In another study, OCT was employed to understand the fouling mechanism in gravity driven membrane filtration processes (L. Fortunato et al., 2017a).

Considering the key role of different microorganisms in biofouling (Jeong et al., 2016a), more detailed characterization of the microbial community involved has gained attention in recent years (Inaba et al., 2018; Wu et al., 2017; Zhu et al., 2017). A better understanding of the microbial community composition will enhance our knowledge of biofouling in MBRs and ultimately help develop rational biofouling control strategies (Meng et al., 2017). In this study, the microbial community of the suspended activated sludge and the biofilm formed on the membrane surface during the early stages in AS-MBR operation was characterized.

The objective of this study was to investigate the membrane fouling formation in a submerged AS-MBR during the initial days of operation (*i.e.* first week of operation). *In-situ* and non-destructive observations were performed directly in a bench-scale MBR reactor treating primary treated wastewater effluent collected from a full-scale wastewater treatment plant. Membrane performance was monitored using conventional parameters (e.g. TMP), which is an indirect measurement of membrane fouling, as well as water quality parameters associated with membrane fouling (e.g. changes in SMP and EPS concentrations in the reactor). On completion of the experimental trial a membrane autopsy was performed to relate the final fouling characteristics with the *in-situ* online observations. By using *in-situ* OCT

imaging combined with other destructive fouling characterization techniques, a better understanding of the membrane fouling formed in the early stages of a membrane filtration processes can be achieved.

2. Material & Methods

2.1 Baffled membrane bioreactor set-up and operation

A bench-scale baffled membrane bioreactor (BMBR) was used in this study and a schematic of the system is shown in Fig. 1. The BMBR consisted of a feed solution reservoir, a bioreactor with a submerged flat-sheet UF membrane, and a permeate collection tank. The reactor was constructed with PMMA (Polymethylmethacrylate) to enable direct observation and imaging of the membrane module inside the reactor. A polysulfone (PS) ultrafiltration (UF) membrane (PHILOS, Korea), with a 20 KDa molecular weight cut-off (MWCO), flat-sheet membrane module was used, manufactured by MemSis Turkey. The effective membrane surface area was 0.04 m². The bioreactor tank had an effective volume of 10.5 L (L=24.5 cm, W=15.5 cm, H=40 cm).

Fig. 1. Lab-scale baffled ultrafiltration (UF) membrane bioreactor system

The concept and operating details of the baffled reactor are discussed elsewhere (Kimura et al., 2008; Pathak et al., 2017). The seed sludge was collected from the wastewater treatment plant (WWTP) at the King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia, which is a conventional AS-MBR plant. The sludge was acclimatized for two weeks before adding it to the baffled MBR system. The feed to the hybrid MBR system was primary treated wastewater collected from an equalization tank at the KAUST wastewater treatment plant. The primary treated wastewater was collected every 3 days and stored at 4° C in a cold storage room while being fed to the bench-scale system. The characteristics of the feed are shown in Table 1.

The bench-scale MBR system was operated continuously for 8 days under a constant flux of 9.5 LMH. The mixed liquor suspended solids (MLSS) was initially adjusted to

3.5 g/L. Throughout the operation, the sludge retention time (SRT) was controlled at 70 d by daily wasting 150 mL of mixed liquor from the bioreactor. The HRT of the baffled MBR system was set at 27 h. The pH and total dissolved solids (TDS) of the mixed liquor were measured at regular intervals (HACH, Germany). Transmembrane pressure (TMP) was measured employing pressure gauge (Keller, Reinacherstrasse, Basel, Switzerland).

Table 1: Characteristics of wastewater collected from the KAUST WWTP

Parameters	Value*
pH	7.45±0.2
TSS	65±24
TDS	645±32
FOG**	9.8±2.1
COD	180±29.5
TN	14.7±3.4
NH ₄ -N	12.6±0.5
PO ₄	5.1±0.3

*All values are in mg/L except pH.

** Fat, oil and grease (FOG)

2.2 Optical Coherence Tomography

An optical coherence tomography (OCT) camera was employed for *in-situ* membrane fouling monitoring in the experimental configuration as shown in Fig. 1. This allows online monitoring of the membrane fouling behavior development over

time without removing the membrane module from the reactor. The OCT probe was mounted on a motorized system (Velmex) that enables the movement of the probe in three dimensions (Luca Fortunato et al., 2017b). This enables taking images in various positions on the membrane module. 2D-scans (2333×1024 pixel) corresponding to $7.0 \text{ mm} \times 2.00 \text{ mm}$ (width $\times$ depth) were periodically acquired during the whole experiment. After 8 d of operation, a three-dimensional (3D) analysis was performed on ten different positions of the fouled membrane in the tank. The 3D scans ($666 \times 666 \times 1024$ pixel), acquired on the last day of operation, corresponded each to an area of $4.00 \times 4.00 \times 2.77 \text{ mm}$. Hence, it was possible to gain structural information of the fouling layer formed on the membrane under operating conditions prior to performing the membrane autopsy.

2.3 Analytical Methods

Total organic carbon (TOC) concentration in the reactor was measured with a TOC analyzer (TOC-V-CSH, Shimadzu, Japan). The mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) in the bioreactor were measured according to APHA, AWWA, WEF (1998) standards. The concentration of dissolved oxygen (DO) was measured by using a DO meter (Vernier, USA). Chemical oxygen demand (COD) of the wastewater was analyzed according to standard methods (APHA, 1998). $\text{NH}_4\text{-N}$, and $\text{PO}_4\text{-P}$ were measured using Hach TNTplus™ reagent vials and HACH® Spectrophotometer DR5000. The mean particle size of the

sludge flocs was measured by using a particle size analyzer (Malvern Mastersizer, UK).

2.4 Fouling Characterization

2.4.1 Membrane biomass extraction

Coupons with an area of 25 cm² were collected from the flat sheet membrane and placed in tubes containing 50 mL of Milli-Q water. The tubes were then placed in an ultrasonic bath where sonication was carried out three times for 5 min followed by 10 s of vortex mixing. The aliquots were filtered through a 0.45 µm pore size syringe filter as described by Jeong et al., (2016b).

2.4.2 EPS extraction and quantification

The formaldehyde-NaOH method was used to extract the extracellular polymeric substances (EPS) from the biomass. The extracted samples were analyzed for protein (PN) and polysaccharide (PS) concentrations, following the modified Lowry method (Sigma, Australia) and Anthrone-sulfuric acid method respectively. Standard curves of PN and PS were developed using various concentrations of Bovine Serum Albumin (BSA) and glucose, respectively.

2.4.3 Liquid Chromatography Analysis

The dissolved organic carbon (DOC) concentration of the biomass deposited on the membrane was characterized using liquid chromatography with organic carbon detection (LC-OCD, DOC-Labor, Germany). A mobile phase of phosphate buffer, 2.5 g/L KH₂PO₄ (Fluka, USA) + 1.5 g/L Na₂HPO₄·2H₂O (Fluka, USA) at a flow rate of

1.5 mL/min was employed to elute the sample. The main fractions identified by the detector are biopolymers (BP), humic substances (HS), building blocks (BB), low molecular weight (LMW) acids and neutrals.

2.4.4 Adenosine triphosphate (ATP) analysis

The active biomass in the fouling layer on the membrane was determined by measuring the adenosine triphosphate (ATP) concentration in extracted samples. ATP quantification was performed using the ATP Analyzer (Celsis, USA) employing the luciferin–luciferase bioluminescence. By filtering the sample through a 0.22 μm filter using a sterile syringe, the ATP was divided into intracellular and extracellular ATP. The 0.22 μm filter retains the microbes, hence, it is possible to determine the intracellular ATP by subtracting extracellular ATP from the total ATP (Fortunato et al., 2016).

2.4.5 Microbial community analysis

The DNA extraction was performed on 1 gram of sludge sample from the bulk phase and biomass extracted from a 0.5 cm^2 area of the membrane surface by using QIAamp® DNA mini kit (Qiagen, Germany) according to the manufacturer instructions. The quantity and quality of extracted DNA were measured using Nanodrop and Qubit BR DNA assay kits (Invitrogen, USA). Library for shot-gun metagenome sequencing was prepared by using TruSeq® Nano DNA LT kit according to instruction given by the manufacturer (illumine, USA). The resulting library was sequenced by HiSeq™ 4000 high throughput (2x150bp paired-end)

platform at the KAUST biosciences core labs.

Reads obtained in the FASTQ format were processed for quality filtering using Cutadapt package (Martin, 2011). Taxonomic classification of the sequencing reads was done using KAIJU (Menzel et al., 2016). Classification by KAIJU is done at the protein level by translating reads into amino acids.

3. Results and Discussion

3.1 Relate the process performance to the *in-situ* monitoring

Fouling in MBR is one of the factors that contributes considerably to the operational costs. As fouling builds up on the membrane surface it leads to an increase of the hydraulic resistance across the membrane and subsequent increase in TMP to keep a constant flux. The TMP increase is necessary to compensate for the loss in permeability and causes an increase in energy consumption (Fane, 2007).

In this study, the evolution of the fouling deposition on the membrane surface was monitored *in-situ* non-destructively using Optical Coherence Tomography imaging. Compared to the other imaging techniques employed to characterize the fouling, OCT allows studying the cake layer formation and development in real time. Hence, monitoring biomass formation with OCT images provides an on-line window on the system, which enables relating fouling properties observed on the membrane surface (i.e., thickness) directly to operational data such as changes in TMP profile commonly used as an indicator of the overall performance decrease. Visualizing and characterizing the cake layer properties and morphology directly online while in

operation in the reactor in ASMBR represents a novelty as current studies reported in literature are based on membrane autopsies (*i.e.* destructively, *ex-situ*, at the end of an operating period). The *in-situ* observation with OCT was employed for the first to monitor the fouling in a reactor employing activated sludge to treat a primary effluent collected from a full-scale wastewater treatment plant. So far the use of the OCT in submerged system was tested only with synthetic secondary effluent with low turbidity and low content of TSS and TOC (L. Fortunato et al., 2017b; Luca Fortunato et al., 2017a). Fig. 2 shows that is possible to evaluate the cake layer development on the membrane during operation over 8 days. A significant increase in biomass thickness was observed during the first 2 days (0-40 μm) compared to a moderate increase during the following 6 days (40-63 μm). The TMP profile showed a similar trend, increasing rapidly in the first 2 days then moderately from day 2 to 8 (e.g. increase from 2.18 to 3.00 Kpa (Fig. 3)). As biomass deposits and increases on the membrane surface an increase in TMP can be measured. The accumulation of biomass on the membrane surface and TMP increase correlate with the resistance in series model.

The approach proposed in this study enabled simultaneous monitoring of the TMP change and observation of the fouling deposited on the membrane. In this study, the MBR reactor was operated under sub-critical operational conditions (Le Clech et al., 2003). A limited rise in TMP (e.g. from 0.5 kPa to 3 kPa (Fig. 3)) was observed as the system was operated at a subcritical flux of 9.5 LMH. Low TMP values and moderate increase are characteristic of low flux operation, a preferred mode of operation to maintain a sustainable permeability over time to increase the membrane lifetime

and decrease energy consumption. Similar values have been reported in other studies, e.g. Le Clech et al. (Le Clech et al., 2003) registered an increase in TMP up to 2.2 kPa after 10 days by operating a reactor at 9 LMH with a MLSS of 3 g/L operated and an HRT in the range of 16-24 h. Kraume et al. (Kraume et al., 2009) reported a pilot plant using flat sheet membranes operated at 16 LMH, where a TMP of 2.5 kPa was observed after 20 d of operation reaching 20 kPa after 70 d. Hwang et al. (Hwang et al., 2008) reported a TMP lower than 5 kPa for the first 3 weeks when operating a MBR at a subcritical flux of 13 LMH.

This study focused on the evaluation of fouling development during the early stage of operation. Although the time is not illustrative for the long term operation, initial results demonstrate the efficiency of OCT for real-time monitoring of cake layer deposition and changes in submerged AS-MBR. As the approach is *in-situ* and noninvasive it can be extended to longer operational times to study the variations and impact of the biomass accumulation on the membranes during different phases under full-scale MBR operation.

Fig.2. Evolution of the biomass morphology over the time in ASMBR during 8 days

of operation.

Fig.3. (a) Biomass thickness calculated through the OCT scans. (b) TMP profile over the time.

3.2 Monitoring the SMP and EPS in the reactor over the time

Although monitoring TMP changes has been the common approach to assess MBR performance, other methods have been used to assess fouling in MBR studies. Measuring the concentration of specific compounds that have been identified as foulants or fouling indicators have been used. It is commonly agreed that SMP and EPS play important roles in membrane fouling, they are both heterogeneous and generally include a range of organics mainly polysaccharides, proteins, humic acid, glycolipids and deoxyribonucleic acid (DNA) (Wang et al., 2014). In this study the concentration and composition of SMP and EPS in the MBR reactor was monitored during the whole experiment.

The concentration of SMP in the reactor increased over the time, showing a similar trend to the TMP measured and fouling layer thickness determined by OCT (Fig. 4 and in Table S1 and S2). The trend comprises two phases with two different rates of concentration/growth. A steep rise was observed in the first two days, 69% of total increase in the SMP concentration observed. From day 2 to 8, a stable trend was observed with a modest increase, representing 21% of increase in SMP values measured in the reactor.

The SMP concentration in activated sludge is considered as a major contributor to initial membrane fouling due to adsorption on the membrane surfaces and within membrane pores (Shen et al., 2012). The SMP are in direct contact with membrane surface, thus the protein and polysaccharide fractions of SMP have a pronounced impact on flux profile as compared to EPS components (Johir et al., 2013). Over time the SMP are retained and rejected by UF membranes and start to accumulate in the reactor. The increase in SMP is mainly due to plasmolysis and release of intracellular constituents as well as accumulation of intermediate and unmetabolized fractions produced by biomass (Johir et al., 2013).

Fig.4. Increase of (a) SMP and (b) EPS concentration in the reactor over the time

In contrast, the EPS content in the reactor mixed liquor showed a constant and less enhanced increase over the time respect to TMP, biomass thickness and SMP concentration (Fig. 4b). The EPS concentration was normalized as the sum of protein and carbohydrate concentrations to the volatile mixed liquor concentration in the reactor (Table S2). The protein represented a higher percentage than carbohydrates in the EPS formed. At higher SRTs of 70 d and lower food to microorganism (F/M) ratios ($< 0.1 \text{ d}^{-1}$), the carbohydrate in microbial flocs diminishes, affecting the available carbon. The amount of protein on the cell surface increased probably due to the excretion of intracellular polymers or cell lysis (Lee et al., 2003). Moreover, due to the hydrophobic nature of protein, it has been suggested to interact more with membrane surface, attaching easily and leading to biofouling. Over time the EPS accumulate in the reactor contributing also to the increase in the fouling layer and flocs size.

The EPS content in the reactor can also be related to flocs size. The particle volume distribution of sludge flocs in the ASMBR was measured at the beginning and the end of the experiment. The sludge floc size distribution was found to increase with average volume sizes from 92 μm to 112 μm during the 8 days of peration (data not shown). The increase in floc size of 21% is comparable to the increase in EPS concentration in the reactor (16%). Indeed, the flocs are formed by physicochemical interactions between microorganisms and EPS. In this study, the EPS concentration in the reactor appears to correlate to the flocs size confirming previous findings. Wilen et al. (Wilén et al., 2003) studied the relation between flocculation and EPS composition, showing that the amount of EPS is related to the flocs proprieties. Guo et al. (Guo et al., 2016) studied the effect of the EPS in the microbial aggregation observing a significant increase in flocs size with the increase in EPS.

3.3 Destructive biomass characterization

Besides providing the possibility of on-line monitoring of a system, OCT can be used as an additional tool for a membrane autopsy (Fortunato et al., 2018). By using a motorized frame connected to the OCT probe, it is possible to scan and characterize different areas of the fouled membrane directly in the reactor immediately before the final autopsy. A similar approach was previously used to characterize 75% of a membrane surface (Fortunato et al., 2016). In this study, ten different areas were scanned non-destructively, where Fig. 5 shows the biomass thickness deposited on the flat sheet membrane in the AS-MBR after 8 days of operation for the ten

locations. The values of biomass thickness, except for area 6, show little variation among the different locations. After 8 days of operation, the biomass thickness and distribution was found to be quite even.

Fig.5. Cake layer thickness in different location of the membrane performed in the reactor before the membrane autopsy. The biomass structure was analyzed after 8 days of operation non-destructively inside the reactor.

To get a better understanding of the biomass formed in the MBR after 8 days of operation, the fouling deposited on the membrane surface was characterized according to the procedure reported in section 2.4. Scanning non-destructively the membrane surface with OCT afore the membrane autopsy allowed to normalize the analyzed valued in the final destructive characterization for the amount of biomass deposited on the membrane surface (Fortunato et al., 2016). Table 2 report the value of autopsy normalized for the volume of biomass formed after 8 days of operation. In membrane filtration processes (i.e., MBR) the biomass formed in the fouling layer mainly consist of adsorbed EPS and microorganisms (Flemming and Wingender, 2010). After 8 days of operation, the autopsy analysis showed an EPS value of 157 mg/m² (value expressed for membrane area), corresponding to 9.8

mg/mm³ (value expressed for biomass volume) (Table 2). In this study, the EPS values are expressed in function of the biomass giving an estimate of the “density” of the matrix. This approach could be then extended to different works to relate the hydraulic resistance and the EPS content for mm³ of fouling deposited on the membrane area. The accumulation of EPS over the time is due to the pore size of the membrane employed in this study (20 kDa), leading to the rejection of protein and carbohydrate due to their hydrophilic nature. The foulant layer consisted of 98 mg/m² (6.1 mg/mm³) protein and 59 mg/m² (3.7 mg/mm³) carbohydrate respectively 62% and 38%. The liquid chromatography analysis organic carbon detection (LC-OCD) analysis performed on the biomass confirmed the same ratio between protein and carbohydrate of 63% and 37% (E-supplementary data of this work can be found in online version of the paper).

The amount of protein present in the fouling layer was higher than in the mixed liquor (ratio of 1.7 vs. 1.15), implying that the proteins in the activated sludge contribute more to the fouling, and due to the higher hydrophobicity of the proteins lead to a stronger adhesion onto the membrane surface, contributing more to the membrane fouling (Qiu and Ting, 2014).

This can be correlated with increasing fouling layer thickness during UF-MBR operation. Preferential accumulation of EPS in the membrane fouling layer has also been reported by Zhang and co-workers who confirmed that EPS could be an important factor governing membrane fouling (Zhang et al., 2012). Hwang et al. also reported that proteins were the main contributors to biofouling layer at higher SRT

and corresponding EPS in biocake and biofilm thickness was around 95 mg/m² and 18 µm respectively (Hwang et al., 2008).

Another analytical parameter employed in biofouling autopsy is the ATP, which is often used to quantify the biomass activity. The Table 2 shows the values of intracellular and extracellular ATP in the biomass, where the extracellular ATP excreted by the cell lysis is determined by filtering the sample with a 0.22 µm filter. By expressing the value for the membrane area, 2928 pg/cm² of intracellular ATP was measured in the fouling formed after 8 days in an ASMBR reactor treating primary effluent. In a previous study, a similar value of 2130 pg/cm² intracellular ATP was observed after 6 weeks in a GDMBR treating secondary effluent. However, the OCT enables expressing the autopsy value, in this case the extra-ATP, normalized by the volume of biomass. By normalizing the values for the amount of deposited biomass it is possible to compare the amount of ATP of the two different conditions. Although the fouling deposited on the two different studies have a similar value of extra-ATP/cm² (for membrane area), there was a significant difference in biomass thickness (80 µm vs 170 µm) and biovolume. Therefore, after expressing the values as function of the biomass volume, the intra-ATP in the ASMBR had a value of 400 pg/mm³ compared to 60 pg/mm³ for the GDMBR. The significant difference between the two values is due to the different nature of the two systems, where the ASMBR is characterized by a certain biomass activity.

Table 2. Destructive characterization performed by membrane autopsy after 8 days

of operation. The values are expressed as function of membrane area and Biomass Volume deposited on the membrane surface (calculated non destructively with OCT).

	For Membrane Area (X/cm ²)	For deposited Biomass (X/mm ³)
Total ATP	4573 (pg/cm ²)	284 (pg/mm ³)
Extracellular ATP	1245 (pg/cm ²)	77 (pg/mm ³)
Intracellular ATP	3328 (pg/cm ²)	207 (pg/mm ³)
EPS	157 (mg/m ²)	9.8 (mg/mm ³)
PN	98 (mg/m ²)	6.1 (mg/mm ³)
PC	59 (mg/m ²)	3.7 (mg/mm ³)

3.4 Microbial community

Fouling development in ASMBR is mainly due to growth of microorganisms on the membrane surface, that over the time form a biofilm, leading to pore clogging and cake layer formation. In this study the metagenomic shotgun sequencing approach was employed to study the composition of bulk sludge and biomass formed on the membrane microbial communities. For taxonomic classification of sequencing reads the Kaiju was used, which uses protein level classification being more accurate compared to traditional nucleotide-level alignments (Harb et al., 2016; Menzel et al., 2016). The *Proteobacteria* were found as the most abundant species, representing 45% of the total community, in both sludge and biomass (Fig. 6). Although the biomass and sludge microbial communities contain the same relative abundance of *Proteobacteria*, there were differences in relative abundance of different classes of

Proteobacteria (Fig. 6B). *Alphaproteobacteria* and *Gammaproteobacteria* increased to 29% and 32% in the biomass compared to 24% and 18% in the sludge, respectively. These two Proteobacterial classes were recently shown to play an important role in initial colonization of membranes (Nagaraj et al., 2017). *Deltaproteobacteria* were slightly increased (10%) and *Betaproteobacteria* were decreased (26%) in the biomass compared to the sludge, 7% and 49% respectively. A similar distribution at early stage was previously observed by Miura et al. (2007) after 9 days on a full-scale membrane bioreactor treating municipal wastewater. In our study, *Caulobacteraceae* were more than double in the biomass samples (2%) compared to the sludge (0.8%) (E-supplementary data of this work can be found in online version of the paper). Members of this family are known for their ability to irreversibly attach to surfaces using polysaccharides with high adhesion strength. Also, they have the ability to spread rapidly to form monolayers of cells on the surfaces, which can develop into multicellular mushroom shaped structures (Tsang et al., 2006). Furthermore, *Flavobacteriia* that contribute in flocs and biofilm formation were double in the biofilm community as compared to the sludge (Jo et al., 2016) (E-supplementary data of this work can be found in online version of the paper). *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Planctomycetes* and *Chloroflexi* were found to be the dominant bacterial phyla in both sludge and membrane biofilm communities (Jo et al., 2016; Lim et al., 2012; Ziegler et al., 2016). *Chloroflexi* was more abundant in sludge (14%) compared to the biofilm (10%) (Fig. 6). It is possible that the *Chloroflexi* filaments have better access to nutrients in the bulk phase compared to the biofilm (Ziegler et al., 2016).

Either none or very small differences were detected in the relative abundance of *Proteobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Acidobacteria* between the sludge and membrane biomass bacterial communities. *Bacterioidetes* and *Nitrospirae* were slightly higher in the sludge compared to the biofilm. While, *Actinobacteria*, *Firmicutes* *Cyanobacteria* and unclassified were slightly higher in the biofilm. The higher abundance of non-classified bacteria in the biomass deposited on the membrane suggests that some differentiation of the microbial community has occurred (Fig. 6).

Overall, our results are in good agreement with a previous study, wherein the microbial communities of sludge and membrane biofilm of 10 full scale MBRs were compared (Jo et al., 2016). Although, there are differences in the relative abundance of bacterial groups in the sludge and the biofilm, the microbial community is largely similar in both samples. Therefore, based on our results it can be suggested that at the early stage, the microbial community in the sludge determines the microbial community composition of biofilm (Ziegler et al., 2016).

Fig. 6. Relative abundance of bacterial phyla in sludge and membrane biofilm community after 8 days.

4. Conclusions

The approach presented in this work was used to investigate the fouling development in an AS-MBR during early stage operation. The proposed approach helps to evaluate the impact of the fouling deposition on the system performance.

The approach is based on the simultaneous quantification of the (i) fouling thickness, (ii) TMP increase and (iii) reactor feed characteristics (SMP and EPS).

Besides monitoring the fouling growth under continuous operation, the *in-situ* monitoring was used to express the values of the final autopsy normalized for the amount of biomass, enabling assess the density and activity of the biomass . The presented approach provides additional information for a better understanding of fouling mechanism. Furthermore, this work opens the way to study in real-time the impact of different operating conditions, on the fouling development in AS-MBR.

Acknowledgement

This study was supported by funding from King Abdullah University of Science and Technology (KAUST).

References

- Cai, X., Zhang, M., Yang, L., Lin, H., Wu, X., He, Y., Shen, L., 2017. Quantification of interfacial interactions between a rough sludge floc and membrane surface in a membrane bioreactor. *J. Colloid Interface Sci.* 490, 710–718.
doi:10.1016/J.JCIS.2016.12.005
- Chen, J., Lin, H., Shen, L., He, Y., Zhang, M., Liao, B.-Q., 2017. Realization of quantifying interfacial interactions between a randomly rough membrane surface and a foulant particle. *Bioresour. Technol.* 226, 220–228.
doi:10.1016/J.BIORTECH.2016.12.025
- Chen, J., Zhang, M., Li, F., Qian, L., Lin, H., Yang, L., Wu, X., Zhou, X., He, Y., Liao, B.-Q., 2016. Membrane fouling in a membrane bioreactor: High filtration resistance of gel layer and its underlying mechanism. *Water Res.* 102, 82–89.
doi:10.1016/J.WATRES.2016.06.028
- Fane, A.G., 2007. Sustainability and membrane processing of wastewater for reuse. *Desalination* 202, 53–58. doi:10.1016/j.desal.2005.12.038
- Flemming, H.-C., Wingender, J., 2010. The biofilm matrix. *Nat Rev Micro* 8, 623–633.
- Fortunato, L., Jang, Y., Lee, J.-G., Jeong, S., Lee, S., Leiknes, T., Ghaffour, N., 2018. Fouling development in direct contact membrane distillation: Non-invasive monitoring and destructive analysis. *Water Res.* 132.
doi:10.1016/j.watres.2017.12.059
- Fortunato, L., Jeong, S., Leiknes, T., 2017a. Time-resolved monitoring of biofouling development on a flat sheet membrane using optical coherence tomography. *Sci. Rep.* 7. doi:10.1038/s41598-017-00051-9

- Fortunato, L., Jeong, S., Leiknes, T., 2017a. Time-resolved monitoring of biofouling development on a flat sheet membrane using optical coherence tomography. *Sci. Rep.* 7, 15. doi:10.1038/s41598-017-00051-9
- Fortunato, L., Jeong, S., Wang, Y., Behzad, A.R., Leiknes, T., 2016. Integrated approach to characterize fouling on a flat sheet membrane gravity driven submerged membrane bioreactor. *Bioresour. Technol.* 222, 335–343. doi:10.1016/j.biortech.2016.09.127
- Fortunato, L., Leiknes, T., 2017. In-situ biofouling assessment in spacer filled channels using optical coherence tomography (OCT): 3D biofilm thickness mapping. *Bioresour. Technol.* 229, 231–235. doi:10.1016/j.biortech.2017.01.021
- Fortunato, L., Qamar, A., Wang, Y., Jeong, S., Leiknes, T., 2017b. In-situ assessment of biofilm formation in submerged membrane system using optical coherence tomography and computational fluid dynamics. *J. Memb. Sci.* 521. doi:10.1016/j.memsci.2016.09.004
- Fortunato, L., Qamar, A., Wang, Y., Jeong, S., Leiknes, T., 2017b. In-situ assessment of biofilm formation in submerged membrane system using optical coherence tomography and computational fluid dynamics. *J. Memb. Sci.* 521, 84–94. doi:10.1016/j.memsci.2016.09.004
- Guo, X., Wang, X., Liu, J., 2016. Composition analysis of fractions of extracellular polymeric substances from an activated sludge culture and identification of dominant forces affecting microbial aggregation. *Sci. Rep.* 6, 28391. doi:10.1038/srep28391

- Harb, M., Wei, C.-H., Wang, N., Amy, G., Hong, P.-Y., 2016. Organic micropollutants in aerobic and anaerobic membrane bioreactors: Changes in microbial communities and gene expression. *Bioresour. Technol.* 218, 882–891. doi:10.1016/J.BIORTECH.2016.07.036
- Hwang, B.-K., Lee, W.-N., Yeon, K.-M., Park, P.-K., Lee, C.-H., Chang, in-S., Drews, A., Kraume, M., 2008. Correlating TMP Increases with Microbial Characteristics in the Bio-Cake on the Membrane Surface in a Membrane Bioreactor. *Environ. Sci. Technol.* 42, 3963–3968. doi:10.1021/es7029784
- Inaba, T., Hori, T., Navarro, R.R., Ogata, A., Hanajima, D., Habe, H., 2018. Revealing sludge and biofilm microbiomes in membrane bioreactor treating piggery wastewater by non-destructive microscopy and 16S rRNA gene sequencing. *Chem. Eng. J.* 331, 75–83. doi:10.1016/J.CEJ.2017.08.095
- Ivanovic, I., Leiknes, T.O., 2012. The biofilm membrane bioreactor (BF-MBR)-a review. *Desalin. Water Treat.* doi:10/5004/dwt.2012.2769
- Jeong, S., Cho, K., Bae, H., Keshvardoust, P., Rice, S.A., Vigneswaran, S., Lee, S., Leiknes, T., 2016a. Effect of microbial community structure on organic removal and biofouling in membrane adsorption bioreactor used in seawater pretreatment. *Chem. Eng. J.* 294, 30–39. doi:10.1016/J.CEJ.2016.02.108
- Jeong, S., Naidu, G., Vollprecht, R., Leiknes, T., Vigneswaran, S., 2016b. In-depth analyses of organic matters in a full-scale seawater desalination plant and an autopsy of reverse osmosis membrane. *Sep. Purif. Technol.* 162, 171–179. doi:10.1016/j.seppur.2016.02.029
- Jo, S.J., Kwon, H., Jeong, S.-Y., Lee, C.-H., Kim, T.G., 2016. Comparison of microbial

communities of activated sludge and membrane biofilm in 10 full-scale membrane bioreactors. *Water Res.* 101, 214–225.

doi:10.1016/J.WATRES.2016.05.042

Johir, M.A.H., Vigneswaran, S., Kandasamy, J., BenAim, R., Grasmick, A., 2013. Effect of salt concentration on membrane bioreactor (MBR) performances: Detailed organic characterization. *Desalination* 322, 13–20.

doi:10.1016/J.DESAL.2013.04.025

Kimura, K., Nishisako, R., Miyoshi, T., Shimada, R., Watanabe, Y., 2008. Baffled membrane bioreactor (BMBR) for efficient nutrient removal from municipal wastewater. *Water Res.* 42, 625–632. doi:10.1016/J.WATRES.2007.08.012

Kraume, M., Wedi, D., Schaller, J., Iversen, V., Drews, A., 2009. Fouling in MBR: What use are lab investigations for full scale operation? *Desalination* 236, 94–103.

doi:10.1016/J.DESAL.2007.10.055

Kwon, D.Y., Vigneswaran, S., Fane, A.G., Aim, R.B., 2000. Experimental determination of critical flux in cross-flow microfiltration. *Sep. Purif. Technol.* 19, 169–181.

doi:10.1016/S1383-5866(99)00088-X

Le Clech, P., Jefferson, B., Chang, I.S., Judd, S.J., 2003. Critical flux determination by the flux-step method in a submerged membrane bioreactor. *J. Memb. Sci.* 227, 81–93. doi:10.1016/J.MEMSCI.2003.07.021

Lee, W., Kang, S., Shin, H., 2003. Sludge characteristics and their contribution to microfiltration in submerged membrane bioreactors. *J. Memb. Sci.* 216, 217–

227. doi:10.1016/S0376-7388(03)00073-5

Li, J., Zhang, X., Cheng, F., Liu, Y., 2013. New insights into membrane fouling in

submerged MBR under sub-critical flux condition. *Bioresour. Technol.* 137, 404–408. doi:10.1016/j.biortech.2013.03.158

Lim, S., Kim, S., Yeon, K.-M., Sang, B.-I., Chun, J., Lee, C.-H., 2012. Correlation between microbial community structure and biofouling in a laboratory scale membrane bioreactor with synthetic wastewater. *Desalination* 287, 209–215. doi:10.1016/J.DESAL.2011.09.030

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17, 10. doi:10.14806/ej.17.1.200

Meng, F., Zhang, S., Oh, Y., Zhou, Z., Shin, H.-S., Chae, S.-R., 2017. Fouling in membrane bioreactors: An updated review. *Water Res.* 114, 151–180. doi:10.1016/J.WATRES.2017.02.006

Menzel, P., Ng, K.L., Krogh, A., 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nat. Commun.* 7, 11257. doi:10.1038/ncomms11257

Miura, Y., Watanabe, Y., Okabe, S., 2007. Membrane biofouling in pilot-scale membrane bioreactors (MBRs) treating municipal wastewater: Impact of biofilm formation. *Environ. Sci. Technol.* 41, 632–638. doi:10.1021/es0615371

Nagaraj, V., Skillman, L., Ho, G., Li, D., Gofton, A., 2017. Characterisation and comparison of bacterial communities on reverse osmosis membranes of a full-scale desalination plant by bacterial 16S rRNA gene metabarcoding. *npj Biofilms Microbiomes* 3. doi:10.1038/s41522-017-0021-6

Pathak, N., Chekli, L., Wang, J., Kim, Y., Phuntsho, S., Li, S., Ghaffour, N., Leiknes, T., Shon, H., 2017. Performance of a novel baffled osmotic membrane bioreactor-microfiltration hybrid system under continuous operation for simultaneous

- nutrient removal and mitigation of brine discharge. *Bioresour. Technol.* 240, 50–58. doi:10.1016/j.biortech.2017.03.069
- Qiu, G., Ting, Y.-P., 2014. Short-term fouling propensity and flux behavior in an osmotic membrane bioreactor for wastewater treatment. *Desalination* 332, 91–99. doi:10.1016/J.DESAL.2013.11.010
- Sabia, G., Ferraris, M., Spagni, A., 2014. Online monitoring of MBR fouling by transmembrane pressure and permeability over a long-term experiment. *Sep. Purif. Technol.* 122, 297–305. doi:10.1016/J.SEPPUR.2013.11.022
- Shen, L., Lei, Q., Chen, J.-R., Hong, H.-C., He, Y.-M., Lin, H.-J., 2015. Membrane fouling in a submerged membrane bioreactor: Impacts of floc size. *Chem. Eng. J.* 269, 328–334. doi:10.1016/J.CEJ.2015.02.002
- Shen, Y., Xiao, K., Liang, P., Sun, J., Sai, S., Huang, X., 2012. Characterization of soluble microbial products in 10 large-scale membrane bioreactors for municipal wastewater treatment in China. *J. Memb. Sci.* 415–416, 336–345. doi:10.1016/J.MEMSCI.2012.05.017
- Tsang, P.H., Li, G., Brun, Y.V., Freund, L.B., Tang, J.X., 2006. Adhesion of single bacterial cells in the micronewton range. *Proc. Natl. Acad. Sci. U. S. A.* 103, 5764–5768. doi:10.1073/pnas.0601705103
- Valladares Linares, R., Fortunato, L., Farhat, N.M., Bucs, S.S., Staal, M., Fridjonsson, E.O., Johns, M.L., Vrouwenvelder, J.S., Leiknes, T., 2016. Mini-review: novel non-destructive in situ biofilm characterization techniques in membrane systems. *Desalin. Water Treat.* 1–8. doi:10.1080/19443994.2016.1180483
- Wagner, M., Horn, H., 2017. Optical coherence tomography in biofilm research: A

comprehensive review. *Biotechnol. Bioeng.* 114, 1386–1402.

doi:10.1002/bit.26283

Wang, X., Chen, Y., Yuan, B., Li, X., Ren, Y., 2014. Impacts of sludge retention time on sludge characteristics and membrane fouling in a submerged osmotic membrane bioreactor. *Bioresour. Technol.* 161, 340–347.

doi:10.1016/J.BIORTECH.2014.03.058

Wilén, B.M., Jin, B., Lant, P., 2003. Relationship between flocculation of activated sludge and composition of extracellular polymeric substances. *Water Sci. Technol.* 47, 95–103.

Wu, L., Tang, B., Chen, G., Bin, L., Zhang, W., Huang, S., Fu, F., 2017. Co-existence of diverse sludge granules in a single membrane bioreactor. *Chem. Eng. J.* 326, 849–852. doi:10.1016/J.CEJ.2017.06.036

Zhang, J., Loong, W.L.C., Chou, S., Tang, C., Wang, R., Fane, A.G., 2012. Membrane biofouling and scaling in forward osmosis membrane bioreactor. *J. Memb. Sci.* 403–404, 8–14. doi:10.1016/J.MEMSCI.2012.01.032

Zhang, M., Lin, H., Shen, L., Liao, B.-Q., Wu, X., Li, R., 2017. Effect of calcium ions on fouling properties of alginate solution and its mechanisms. *J. Memb. Sci.* 525, 320–329. doi:10.1016/J.MEMSCI.2016.12.006

Zhu, Y., Wang, Y., Jiang, X., Zhou, S., Wu, M., Pan, M., Chen, H., 2017. Microbial community compositional analysis for membrane bioreactor treating antibiotics containing wastewater. *Chem. Eng. J.* 325, 300–309.

doi:10.1016/J.CEJ.2017.05.073

Ziegler, A.S., McIlroy, S.J., Larsen, P., Albertsen, M., Hansen, A.A., Heinen, N., Nielsen,

P.H., 2016. Dynamics of the Fouling Layer Microbial Community in a Membrane Bioreactor. PLoS One 11, e0158811. doi:10.1371/journal.pone.0158811