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Luca Fortunato, TorOve Leiknes

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***In-situ* biofouling assessment in spacer filled channels using optical coherence tomography (OCT): 3D biofilm thickness mapping**

Luca Fortunato*, TorOve Leiknes

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

*Corresponding author: Tel. +966 12 808 22 89; Email: Luca.Fortunato@kaust.edu.sa

Abstract

Membrane systems for water purification can be seriously hampered by biofouling. The use of optical coherence tomography (OCT) to investigate biofilms in membrane systems has recently increased due to the ability to do the characterization *in-situ* and non-destructively. The OCT biofilm thickness map is presented for the first time as a tool to assess biofilm spatial distribution on a surface. The map allows the visualization and evaluation of the biofilm formation and growth in membrane filtration systems through the use of a false color scale. The biofilm development was monitored with OCT to evaluate the suitability of the proposed approach. A 3D time series analysis of biofilm development in a spacer filled channel representative of a spiral-wound membrane element was performed. The biofilm thickness map enables the time-resolved and spatial-resolved evaluation and visualization of the biofilm deposition pattern *in-situ* non-destructively.

1. Introduction

Membrane filtration systems have increasingly been used in the last decades to produce high-quality water. Biofilm formation is inevitable in all water treatment systems and is a key challenge in all membrane filtration systems applied to both saline and freshwater environments. The development of a biofilm on the membrane surface causing an unacceptable decline in membrane performance is defined as biofouling. Performance losses are observed by an increase in feed channel pressure drop, permeate flux reduction, and/or salt passage increase in desalination systems (Characklis and Marshall, 1990). To maintain water production, fouled membrane elements require periodic cleaning and eventually replacement when cleaning is no longer effective. Biofouling has been identified as one of the major operational problems in spiral-wound nanofiltration and reverse osmosis membrane operation (Vrouwenvelder et al., 2008; Winters and Isquith, 1979).

A key aspect of biofilm studies involves the analysis of the biofilm structure (Halan et al., 2012), which can predict the biofilm behavior, and thus, the impact on membrane filtration performance. Several approaches are reported in the literature to study biofilm formation in membrane systems, most of them requiring destructive procedures. Optical coherence tomography (OCT) has gained popularity in the study of biofilm formation and evolution on surfaces in aqueous environments. OCT is capable of evaluating the internal structure of a biological tissue, performing high resolution cross sectional images by measuring the backscattered light. This technology was first applied in ophthalmology for imaging the retina in real time (Huang et al., 1991). Due to its ability to investigate biofilm formation *in-situ*, non-destructively and without additional preparation of the biofilm (e.g. staining procedures), OCT has recently been used to visualize/monitor biofilm growth in membrane filtration systems (Dreszer et al., 2014; Valladares Linares et al., 2016).

So far, the OCT has mainly been used for two-dimensional (2D) studies in which the structural data are gained by analyzing each single slice of a scan, and calculating the biofilm thickness from the 2D scans (Derlon et al., 2012; Fortunato et al., 2016). OCT scans were also used to evaluate the efficiency of two-phase cleaning flow in a spiral-wound nanofiltration element (Wibisono et al., 2015). In spacer filled the feed channel pressure drop was correlated with the amount of biofilm (Fortunato et al., 2017a; West et al.,

2015). Gao et al. (2014) used the OCT to characterize the dynamic process of the fouling during membrane filtration. In another study, the OCT was used to study *in-situ* the evolution of the fouling layer morphology in a gravity driven membrane bioreactor (Fortunato et al., 2017b).

In this study, 3D OCT image analysis is used to visualize and evaluate the biofilm spatial distribution *in-situ* under continuous operation in a spacer filled channel representative of spiral wound element. A novel approach is presented using image analysis to provide biofilm thickness maps as a tool to assess biofouling formation and distribution in membrane filtration systems. The maps allow visualization and evaluation of the biofilm formation and growth through the use of a false color scale.

2. Material and methods

2.1. Spacer filled channel – spiral-wound module

A modified membrane fouling simulator (MFS) was used to grow a biofilm on the surface of a membrane and feed spacer, and the glass lid of the feed channel, simulating spiral-wound membrane modules. Details of the initial MFS system can be found elsewhere (Vrouwenvelder et al., 2007). The modified MFS was operated with a linear crossflow velocity of 0.05 m/s, without permeate production, for 40 h. A 5 mm thick glass cover on the MFS allowed *in-situ* non-destructive observation of the biofouling formation and evolution, monitored through a series of OCT scans. The flow channel height was 787 μm to accommodate a standard 31 mil thick feed spacer (TriSep) commonly used in commercial spiral-wound modules. A TriSep TS80 polyamide-based nanofiltration (NF) membrane, molecular weight cut-off (MWCO) 100-200 Daltons, was used. The system was operated under gravity driven conditions, adjusting the flow rate by setting the height of the air bubble trap on the feed prior to the MFS (fig S1). A feed solution of 0.3 g·L⁻¹ of yeast extract was used and re-circulated in the system. The feed solution was pumped through a bubble trap to avoid the presence of bubbles in the system. Feed solution was refreshed every 10 h.

2.2. OCT and data processing approach

An Optical Coherence Tomography (OCT), Ganymede 930 Spectral Domain, Thorlabs, GmbH, fitted with a 5X telecentric scan lens (Thorlabs LSM03BB) was used to investigate the biofouling evolution. A video camera included in the OCT device enables focusing and selection of the area of interest (referred to as “camera” in this paper). The MFS was fixed under the OCT stage in order to monitor the same area for the duration of the experiments. An area representing one square of the feed spacer was chosen, resulting in a scanned space of 6 mm × 6 mm × 0.96 mm. The corresponding pixel size for the scanned image is 600 × 600 × 482, giving an axial resolution of 2.7 μm and a lateral resolution of 10 μm .

The OCT images were processed using the open source software, ImageJ (Version 1.48). A multi-step sequence was applied, consisting of: (1) the image at time zero subtracted from all the subsequent images, (2) the contrast and brightness adjusted, (3) the image cropped, (4) the image filtered, and (5) the image binarized. The subtraction operation allows eliminating external elements from the OCT stack (e.g.

82 membrane, glass and feed spacer) in order to assess only the biofilm data. The resulting stack was then
83 processed with a customized MATLAB code to obtain the thickness map.

3. Results and discussion

3.1. Fouling thickness map

In biofouling monitoring, there is a need to quickly assess and evaluate the spatial development of the biofilm on the membrane over time. Biofilm thickness maps are presented in this study as a new tool to assess the biofilm spatial distribution on a surface. It is a similar approach as the classical distribution map that depicts the distribution of a phenomenon on a surface, where different colors are used to show and evaluate the distribution in a physical map.

In figure 1 the biofilm thickness maps are shown for a spacer filled channel representative of a spiral-wound membrane. Compared to the method used for direct observation through the membrane (DOTM) (Chen et al., 2004), 3D visualization and thickness maps allow a better understanding of the biofilm deposition, providing a depth-resolved biofilm structure. As matter of fact, cross-sectional analysis is necessary to enable the distinction of biofilm accumulation in different elements (i.e. membrane, feed spacer and glass for the spacer filled channel) and for quantifying the thickness. Applying 3D OCT image analysis to a specific area enables the analysis of information related to the spatial distribution and to the homogeneity of the biofilm. In Figure 1, areas shown in red represent higher amounts of biofilm, and are more easily and rapidly detected. With respect to the 3D volume rendering image analysis (Fortunato et al., 2017a), thickness mapping images require less imaging skills, less computational resources and automated data handling is therefore more feasible. Furthermore, interpretation of fouled systems applying 3D rendered volume images is more difficult compared to the thickness maps. In the 3D rendering images, biofilm deposited on the glass may obscure visualization of lower areas and thus impact the image results. Thickness maps can be obtained directly from raw images without any correction (e.g. time zero as baseline) or data segmentation, where the image data is not only due to the biofilm but also the other elements (e.g. feed spacer, glass and membrane).

Figure 1. Biofilm development in a spacer filled channel (flow direction left to right) b) flat sheet membrane. A camera mounted on the OCT probe (left), 3D biofilm rendered volume (center) and biofilm thickness map (right column).

Figure 2. b) Biofilm thickness map in a spacer filled channel. The calibration bar allows estimating the biofilm thickness deposited on the flat sheet membrane.

Another advantage of mapping images is the calibration of the color scales, making it possible to relate each color with a corresponding thickness value. In this way, quantifying the amount of biofilm deposited over a specific area can be done while evaluating the spatial distribution. An example of calibrated maps for the system studied in this paper is shown in Figure 2.

The approach proposed can easily be applied to any membrane configuration. It is possible to evaluate the biofilm thickness distribution on the membrane surface and distinguish between different fouled areas. The colored scale allows identifying zones with lower biofilm deposition, which can further be related to the water flow (i.e. lower hydraulic resistance). In this study, the approach was used to assess the biomass thickness and the spatial distribution over a single frame (image 2), to evaluate the biofouling development over the time (figure 3) and to assess the pattern distribution on each elements of the flow cell (figure 4). The same approach can be extended to the evaluation of the biofilm distribution over any surfaces.

3.2. Applicability of OCT imaging and analysis

In this study, the 3D OCT image analysis was tested on a spiral-wound membrane module mimicked in a modified MFS. Imaging the spacer filled channel with OCT is complex due to the presence of the feed spacer. The region monitored in this study corresponds to an area of $6 \text{ mm} \times 6 \text{ mm} \times 0.96 \text{ mm}$. The possibility to observe an area in the mm range (meso-scale) is a beneficial feature of the OCT, and particularly important to better understand biofouling in a spiral-wound element where a complete section of the feed spacer can be studied. Although other imaging techniques (i.e. CLSM and SEM) can provide images with a higher resolution, it is a destructive method and the area observed is too small and includes only a small part of the membrane or the feed spacer. However, the common practice consists in the investigation of just membrane without the feed spacer. It is therefore also not suited to fully investigate and understand biofouling behavior and responses over time and how this may impact the membrane filtration processes in spacer filled channel. In fact, the structural information at micro-scale and nano-scale level might be of minor relevance to characterize the behavior of macro-scale biofilm processes (Wagner et al., 2010). The capacity of OCT to capture images at the meso-scale is an important feature in the analysis of biofouling behavior and response in different membrane module configurations under realistic operating conditions, however, obtaining images with detailed information at this scale is a challenge. Applying the biofilm thickness mapping approach as proposed in this study appears to be a useful tool.

3.3. Time Series

One of the main benefits of the *in-situ* non-invasive 3D image analysis using OCT is that biofilm development, evolution and change can be monitored over a given time frame. The mapping approach is well suited for monitoring biofouling development over time as image acquisition and analysis is relatively quick and gives detailed analysis for a predefined specific area. An example of such an analysis is shown in Figure 3. Results show that after circulating the feed through the flow cell for 20 h, the initial biofilm growth could be observed on the feed spacer filaments through 3D OCT image analysis. The biofilm thickness map shown in Figure 3 corresponds only to the biofilm. Image signals of the glass, feed spacer and membrane (i.e. MFS setup) are removed using the time zero image as baseline. Therefore, the 3D image analysis make it possible to quickly and selectively assess the evolution of the biofilm distribution in a specific area. In the spacer filled channel (Figure 3), most of the biofilm development was found on the feed spacer filaments at the early stages. After 40 h operation, the biofilm covered most of the flow channel.

Figure 3. Biofilm development in spacer filled channel (20 hours, 30 hours, 40 hours). Biofilm thickness map. Flow direction is from left to right.

3.4. Deposition Pattern

Moreover, starting from the acquired 3D OCT scans (stack) it is possible to select the corresponding area occupied specifically by each element: glass, feed spacer and membrane. Hence, the resulting thickness map can be used to analyze the biofilm development patterns on each element. As shown in figure 4, it is possible identifying different deposition patterns for each element. As reported by Radu et al. the deposition patterns in spiral wound NF/RO membranes are a function of the feed spacer orientation (Radu et al.,

2014). After 40 hours of operation for the given experimental conditions of this study (fig 4), the most fouled area was observed to be on the thinner part of the upper filament of the feed spacer, upstream the bottom filaments on the glass, and downstream the upper filament on the membrane.

Figure 4. Biofilm thickness map for each element of the flow cell after 40 h of operation (the flow is from left to right). The map can be used to evaluate the biofilm deposition pattern in spacer filled channel on feed spacer and membrane.

3.5. Feed Spacer

Feed spacers are essential for spiral-wound NF and RO membrane modules, with the dual functions of keeping the membranes apart to form a flow channel and positively affecting channel hydraulics by generating flow turbulence for improved performance. Various models based computational fluid dynamics CFD and biofilm formation have been proposed to describe biofouling behavior in spiral wound modules. The proposed approach could be used as an experimental tool to validate such theoretical models, considering that the area examined in CFD models corresponds to a single feed spacer frame, the same area covered in a single scan in this study. Vrouwenvelder et al. (2009) reported that biofouling is mainly a feed spacer problem, being more significant than fouling of the membrane surface, causing increase in feed channel pressure drop and subsequently performance decline. Recently, Fortunato et al. (2017a) observed that during the early stage of biofouling the biomass accumulated mainly on the feed spacer, impacting the feed channel pressure drop. At the reported experimental conditions, biofilm is mainly observed to accumulate on the feed spacer at an early stage, as shown in Figure 3. This may be an indication of either a higher affinity of biofilm to attach to the feed spacer material (e.g. polypropylene) or preferential deposition on the feed spacer due to the hydrodynamics of the system.

4. Conclusion

In summary, 3D OCT image analysis has been demonstrated to be an effective tool for the evaluation of biofilm development over the time in a membrane filtration system. The biofilm thickness mapping is an approach and method that can be used as a tool to rapidly analyze a predefined fouled area, providing time series to assess the evolution. The approach can be applied to characterize the biofouling and can also be beneficial in assessing the effectiveness of biofouling mitigation and antifouling strategies.

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Supporting information

Figure S1. Schematic representation of the experimental setup consisting of a tank containing feed solution, pump, air bubble trap, membrane fouling simulator (MFS), and optical coherence tomography (OCT) unit, used to study biofilm development in time in 3D.

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270 **Highlights**

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- A novel method to assess the biofilm formation is presented for the first time.

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- Tool for *in-situ* biofouling evaluation in membrane filtration systems.

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- The method allows a quick evaluation of fouling spatial distribution.

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- 3D biofilm mapping is suitable to assess the biofilm development over the time.

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In-situ biofouling OCT thickness map in spacer filled channel

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