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An in-situ technique for the direct structural characterization of biofouling in membrane filtration

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Abstract

In the present work, a convenient and direct technique which enables to characterize the intrinsic structure and the mechanical properties of the biofilm without altering its chemical and physical properties is proposed. By utilizing the Optical Coherence Tomography (OCT) as a structural imaging tool coupled with an advance mathematical framework, thickness, micro-porosity, normal stress-strain curve, bulk modulus and total permeability of the biofilm structures are determined. The accuracy of this mathematical technique for the *in situ* characterization is validated by analyzing two different membrane structures for porosity and permeability values against the mercury intrusion porosimetry method. Three-dimensional images of biofouling were obtained with high resolution aided to numerically analyze the intrinsic biofilm structure at microscale. Growth of biofilm in a dead-end filtration experimental setup was investigated by varying the feed flow rate which allowed uniform compression and decompression to compute normal stress-strain relation of the evolving biofilm structure. At early development of biofilm (day 3), the thickness and normal stress/strain curve showed that the biofilm structure behave similar to elastic material. However, hysteresis-like trend starts to appear with the growth of biofilm suggesting the deviation of biofilm properties to viscoelastic nature at day 8. The microstructure porosity increased from 0.214 (day 3) to 0.482 (day 8) at a feed flow rate of 15 mL/min. The total membrane/biofilm permeability decreased with biofilm age to reach $5.19 \times 10^{-15} \text{ m}^2$ at day 8 at the same flow rate, leading to a reduction of permeate flux over time. All the structural properties were found to be time dependent as the biofilm continuously evolved.

Keywords: Biofilm, mechanical and structural characterization, OCT, membranes, fluid shear.

1. Introduction

Permeate flux recovery is commonly impended by bacterial and biomass accumulation on membrane surface during the filtration process. Similarly to other types of membrane fouling, the biofouling causes unacceptable decline in membrane performance in terms of the flux decline and associated pressure drop [1-4]. The biofilm is as an aggregation of microbial communities protected by an adhesive matrix, the extracellular polymeric slime (EPS), which otherwise provides an intrinsic structural arrangement and mechanical stability to the bacterial cells [5-8].

The growth of biofilms on membranes and its intrinsic architecture is related to biological, chemical and physical factors such as, the bacteria type [9, 10], hydrophobicity and the repulsive forces of membrane/bacterial cells [11-14], heterogeneity of the membrane surface [15, 16], chemical characteristics of the feed solutions [5, 17, 18] and the operating hydrodynamic conditions [5, 15, 19, 20]. The shear force created by the fluid flowing over the biofilm surface affects structure of the cell colonies and influences mechanical and structural properties of biofilm [21, 22]. Along with the deep understanding of the biofilm behavior in response to the fluid hydrodynamics stress, there is a need for the characterization and evaluation of the intrinsic structural and mechanical variation acting on the biofilm properties.

Several characterization techniques aimed at investigating the biofilm structure which is developed on the membrane surface as a result of membrane filtration process have been reported. Prest et al. [23] suggested the oxygen optode imaging based on measuring the oxygen distribution and consumption rates within the biofilms in order to elucidate the heterogeneity and the biological dynamics in biofouling [23, 24]. The microscopic analysis tools are widely known in the investigation of biofilm development. Yun et al. [25] assessed the variation of textural and volumetric parameters and their effect on a permeability of membrane in a bioreactor as a function of biofilm growth by using confocal laser scanning microscopy (CLSM) [25, 26]. Other studies evaluated scanning electron microscopy (SEM) as a tool for monitoring and visualization of biofouling layers deposited on the membrane surfaces [27, 28]. The magnetic resonance imaging (MRI) was also recognized in providing spatial insights on fluid flow progress in filtration systems and quantifying biomass accumulation [29, 30]. Although the available techniques allow to monitor biofouling growth as well as characterize its structural parameters, they suffer from several drawbacks such as limited image resolution, complex sample

preparation procedure, limitation of depth measurement and need for a membrane autopsy. All these factors eventually result in losses of the biofilm integrity and/or its destruction [31].

In the evolved biofilm, the fluid force (normal or shear) created by incoming fluid manipulates its structure. From a biological perspective, it is important to comprehend, predict and stipulate how a biofilm will behave in response to varying fluid flow conditions. Also, knowing the mechanical strength of the biofilm structure is critical in augmenting physical techniques for removing biofilms from membrane surfaces or material property inputs to various modeling techniques [25, 27]. Evaluation of the effect of biofilm's environment on its material properties is challenging as biofilms are very difficult to test mechanically. Thus, the biofilm cannot be stretched or expanded like conventional solids to determine biomass properties and associated changes when forces are applied. The biofilms are generally difficult to handle as they are deformable, have a thickness of only few microns, and are attached to surfaces. The moment the biofilm is released from the attached surface for analysis, its structure will be inevitably damaged. Therefore, in order to accurately determine material properties of the biofilm, it is critical to use a technique that is relevant to the physical environment to which the biofilm is exposed. While existing techniques have provided useful fundamental data (e.g. microbial activity, biofilm composition and structure, etc.) [23, 26, 28, 29], determination of the biofilm's material properties in filtration systems where the external factors should not alter their characteristics is still a challenge.

Recently, the optical coherence tomography (OCT) has evolved as a non-invasive, real time *in situ* tool which enables three-dimensional imaging of biofilm structure on the membrane surface with the micron-range resolution without any stain integration [32-35]. Having the ability to penetrate in the biofilm depth, OCT can provide detailed structural biofilm parameters [31, 36]. Akhondi et al. [37] applied this technique to study the effect of biofilm structure and thickness on permeate flux in the gravity-driven membrane ultrafiltration process [9]. The OCT was further used by West et al. [38] to quantify the biofouling which developed in the feed spacer channel of a spiral wound reverse osmosis (RO) membrane. The autopsy showed that the spacer geometry and the feed water composition affected biofilm distribution and structure. By compressing/decompressing grown biofilm, Dreszer et al. [39] confirmed that the loss of membrane's performance was not only related to the biofilm thickness but also to its internal structure. They further investigated the suitability of OCT technique to visualize the various

stages of biofilm development in nanofiltration (NF) and RO membranes (biofouling formation, development and detachment). As of now, OCT is currently being utilized to access macroscopic properties (heterogeneous biofilm thickness and macro-porosity) [40]. However, details of biofilm microscopic structural properties have not been realized owing to large scale data analytics of 3-D structures and lack of mathematical tools for analysis.

In present study, an *in situ* technique using OCT as a structure imaging tool along with advance micro-scale image analysis algorithms is reported for the first time to determine the material characteristics of the biofilm at various stages of its growth. In the proposed technique, the biofilm which is formed on a membrane surface is not compromised by staining with dyes or by addition of other chemicals which can alter its structure. These factors make the proposed technique unique by eliminating all the native drawbacks associated with common biofilm characterization techniques (e.g. CLSM, SEM, and MRI). By utilizing OCT scans and mathematical tools, a wide range of biofilm internal parameters such as thickness, micro-porosity, micro-permeability, strain rates as well as bulk modulus are determined. The proposed approach is universal and can easily be adapted to any biological, environmental or physiological systems. Furthermore, the technique is validated by comprehensive evaluating properties of a biofilm which was grown on a typical UF membrane [9] in a custom-made UF cell. The UF system was operated in the dead-end filtration mode to produce a denser biofilm on the membrane surface in order to demonstrate the efficiency of this technique with respect to more rigid, and adherent biofilm (that has dense micro-structures), where both measurement and analysis of biofilm properties are more challenging.

2. Materials and methods

2.1. Experimental setup

Figure 1 illustrates the schematic of the experimental setup. The synthetic feed solution was pumped to the UF flow cell by a gear pump (Model 75211-70, Cole-Parmer). The flow cell had a filtration area of 6 cm x 1.5 cm and a filtration channel with a height of 1.2 mm on the feed side. The UF flat sheet polyethersulfone (PES) membranes (Synder Filtration, Vacaville, CA, USA) with a molecular weight cut-off of 100 kDa were used in the entire series of experiments. The standard feed spacer was prototyped by 3-D printer technology according to the dimensions mentioned in Kerdi et al. [41] and was placed on the feed side of the flow cell. The filtration was

conducted in the dead-end batch mode at a constant feed flow rate of 15 mL/min at first three days. When the biofilm developed, the feed flow rates was varied between 15 mL/min to 150 mL/min with a step of 15 mL/min to conduct uniform biofilm's compression and decompression. Two sets of data, one for biofilm compression experiments and other one for decompression experiments were acquired after 3, 6, 7 and 8 days of biofilm growth. The transmembrane pressure differential was monitored by two gauge pressure sensors P_A and P_B (OMEGA, Model PX309-050G) placed at the feed and permeate sides, respectively. Permeate flux was collected in the permeate tank and its weight was recorded by a digital balance (MS3002S, Mettler Toledo). Permeate flux and pressures (P_A and P_B) produced at different feed flow rates were automatically recorded by using the data acquisition system (National Instruments, LabVIEW, 2016) every 15 seconds. At each feed flow rate, the 3-dimensional scans of biofilm were taken *in situ* by the OCT (Thorlabs, Hyperion). The structure of the biofilm obtained from each dataset (compression and decompression) was taken at a fixed location in the flow cell. The structure analysis of the acquired biofilm images was conducted by the AVIZO software (Field Electron and Ion Company, Hillsboro, OR, USA).

Fig. 1. Schematic of the experimental setup. The flow cell was operated in dead-end mode at $Q = 15$ mL/min.

2.2. Feed bacterial suspension

A synthetic feed solution was used to expedite the biofilm growth. It allowed the growth of bacteria on the membrane surface while minimizing the development of other types of fouling.

The synthetic feed solution was prepared by dissolving 0.5 g of the Bacto™ Yeast Extract (Extract of Autolyzed yeast cells, Becton Dickinson and Company) in 0.5 L of tap water (dominantly containing *Proteobacteria* [42]) followed by incubation (Isotemp incubator, Fisher Scientific) at 30°C for 24 hours. The incubated solution was further diluted with another 1.5 L of tap water to make up the final volume of 2 L and the yeast extract concentration of 1 g/L in each batch. During filtration experiments, the feed solution was continuously stirred with a magnetic stirrer at 300 rpm. The feed concentration factor in each batch was 1.16.

2.3. Data processing

2.3.1. Three-Dimensional (3-D) visualization of biofilm

OCT scans were performed at a fixed location on the membrane by using the same scan parameters (Mode: 3-dimensional, Model: Hyperion Thorlabs, A-Scan rate: 127 Hz). The scan area was 1 mm x 1 mm with a scan depth of 1.198 mm (x-z plane) such that it covered both biofilm and membrane thickness with a resolution of 800 x 795 x 439 pixels. Keeping the same raw data features, the 3-D scans obtained by OCT technique were exported to *AVIZO* software and processed with multi-sequences such as volume rendering, fix colormap intensity adjustment for all 3D images and color system selection (physics.icol).

Physical dimensions using OCT are always challenging especially in multiple material layers or configurations (such as biofilm growing on membrane submerged under feed and encased inside an acrylic flow module) as group refractive index is usually hard to determine [43]. The refractive index determines the direct relation between the optical length and the physical length [44]. Therefore, to measure the physical length accurately using OCT, the thickness of new membrane was physically measured before the start of the experiment. The same membrane was then mounted inside the flow cell under experimental conditions and the group refractive index of the OCT was adjusted until the physical thickness and optical thickness match. Refractive index of 1.35 was chosen at central wavelength of 930 nm that accurately determines the physical length in OCT scanning. However, slight changes in refractive index might come up as biofilm grows which are small and can be neglected [45].

2.3.2. Biofilm thickness

The thickness of biofilm was estimated by digitally isolating the membrane from the biofilm in each OCT scans. To achieve this, multi-AVIZO sequences were applied, which include, binarization, thresholding and segmentation of the image in order to assess the biomass. For accuracy, instead of direct thickness measurement of 3-D biofilm, a slice-based technique was utilized. Two-dimensional (2-D) slices in x-z plane were extracted from the 3-D raw dataset at each pixel location in y-direction. The area of biomass (including pores) was computed for each 2-D slice. The average thickness was then calculated:

$$H_{av} = \sum_{j=0}^{y_n} A_j / W \quad (1)$$

where H_{av} is the average biofilm thickness in μm , A_j is the 2-D slice area of each slice in μm^2 , y_n is the number of 2-D slices ($y_n = 799$ slices) in y-direction and W corresponds to the width of biofilm attached to the membrane ($W = 1000 \mu\text{m}$ for all images and dataset).

2.3.3. Normal strain and bulk modulus

The normal stress corresponded to uniformly applied flow pressure (recorded by the pressure gauge A, Figure 1) to growing biofilm. The axial strain (ϵ) was calculated by;

$$\epsilon = \frac{(H_{av(t)} - H_{av(o)})}{H_{av(o)}} \quad (2)$$

where $H_{av(o)}$ is the biofilm original thickness (μm) measured before the application of pressure on the biofilm and $H_{av(t)}$ is the final thickness (μm) obtained after application of either compression or decompression. The values of all thicknesses were acquired from AVIZO software using 3-D OCT scans as mentioned in Section 2.3.2.

The bulk modulus (G) of biofilm were computed under compression and decompression processes according to the following equation [46]:

$$G = -V_o \frac{dP}{dV} \quad (3)$$

where V_o is the initial volume (μm^3) and dP and dV represent the change in pressure and volume, respectively. As the cross-section area (length x width) of the biofilm taken in the analysis is kept same for all cases, the bulk modulus definition can be approximated as follows:

$$\frac{dV}{V_o} = -\frac{1}{G} dP \quad (4)$$

$$\frac{dV}{V_o} = \frac{(H_{av(t)} - H_{av(o)})}{H_{av(o)}} = \frac{\Delta H_{av}}{H_{av(o)}} = -\frac{1}{G} (P_t - P_o) \quad (5)$$

where P_t (kPa) represents P_A (kPa) at each Q and P_o (kPa) is the initial pressure applied.

2.3.4. Biomass porosity

The biofilm's porosity was also estimated by *AVIZO*. The multi-sequences steps were used for each dataset acquired by 3-D OCT scans. These multi-sequences steps involved: (1) binarization of 3-D image by interactive thresholding, (2) 3-D image volume rendering for membrane subtraction, (3) segmentation of image and selection of pixel zones corresponding to void zone (pores) and biomass, and (4) calculation of void space (V_p , μm^3) and biomass (V_M , μm^3) volumes through material statistics of image segmentation. The porosity (P) was then calculated by using the following equation [47]:

$$P = \frac{V_p}{V_p + V_M} \quad (6)$$

2.3.5. Absolute permeability by numerical tensor computations

The absolute permeability is defined as the influence of solid biomass on the liquid flowing through the pores of the biofilm. To determine this, a change of scale is realized to obtain equations valid for the entire volume of biofilm matrix (i.e including biomass and pore region where fluid can flow). A well established volume averaging technique is used to achieve change of scale by spatially smoothing the equations on a volume of scanned biofilm structure. The mathematical averaging technique leads to closure problem which transforms the Stokes equations [48] into a high order tensorial problem. The detailed mathematical derivation of closure problem and averaging [49] yields:

$$\begin{aligned} \nabla \cdot \bar{\bar{D}} &= 0 \\ \nabla^2 \bar{\bar{D}} - \nabla \bar{d} &= \bar{\bar{I}} \end{aligned} \quad (7)$$

where $\overline{\overline{D}}$ represents the tensor of the spatial deviation of the velocity field (velocity perturbation), $\overline{\overline{d}}$ represents the vector of spatial deviation of the pressure field (pressure perturbation) and $\overline{\overline{I}}$ is the unit tensor representing arbitrary force function to drive the system of equation.

The above system of equations are solved using periodic boundary conditions along all the faces of the scanned biofilm structure, given by;

$$\overline{\overline{D}}(r_i + l_i) = \overline{\overline{D}}(r) \quad \overline{\overline{d}}(r_i + l_i) = \overline{\overline{d}}(r) \quad i = x, y, z \quad (8)$$

where r_i is the spatial edge coordinate in respective direction, while l_i is the total length of the biofilm sample in that particular direction. At solid-liquid interface (inside the biofilm structure)

$$\overline{\overline{D}} = 0 \quad (9)$$

boundary condition is enforced representing no-slip condition.

The above system of equations along with boundary conditions were solved into *Avizo* software using acquired 3D biofilm structures through OCT. Finally the permeability tensor ($\overline{\overline{k}}$) was obtained by the mean value of $\overline{\overline{D}}$ over the volume V represented by the OCT scan.

$$\overline{\overline{k}} = \frac{1}{V} \int_V \overline{\overline{D}} dV \quad (10)$$

Finally, the total permeability was determined by the magnitude of $\overline{\overline{k}}$ tensor in direction perpendicular (fluid-flow direction) to the biofilm.

2.3.6. Total permeability from experimental measurements

The total permeability comprised biofilm's permeability and membrane's permeability was calculated at different biofilm growth days under compression/decompression processes by using Darcy's law [50]:

$$K = -Q\mu \frac{L}{A(P_B - P_A)} \quad (11)$$

where K is the total permeability (m^2), Q is the flux (m^3/s) recorded in the experiments, μ is the viscosity of the feed solution ($\mu = 0.00089$ Pa.s), L is the total biofilm and membrane thicknesses

at different flow rates obtained through OCT scans, A is the scan area ($A = 10^{-6} \text{ m}^2$), and $(P_B - P_A)$ is the pressure differential (ΔP , kPa) across the membrane.

2.4. Procedure

The filtration experiments started at the initial feed flow rate of 15 mL/min and after 3 days of filtration, a cake type biofilm structure appeared on the membrane surface. Once the stable biofilm thickness was formed, the feed flow rate was gradually increased from 15 mL/min to 150 mL/min at a step of 15 mL/min for 3 min to minimize possible permanent biofilm structural damages associated with pressure increase. As the flow rate increased, the applied pressure which acted on biofilm also increased to result in the uniform compression of the biofilm structure. After the flow rate reached its highest value of 150 mL/min, it was gradually reduced in the same sequence which leading to biofilm decompression. For each flow rate at both compression/decompression processes, 3-dimensional OCT scans were acquired generating series of structural scans corresponding to a specific pressure and flow rates. The procedure was repeated for other days as well (days 6, 7 and 8) to investigate the evolution of the biofilm structure.

3. Validation of technique

The accuracy of the proposed technique was first validated by comparison of permeability and porosity of two virgin polymeric membranes made of PES with the molecular weight cut-offs of 100 kDa (Synder Filtration, Vacaville, CA, USA) and 150 kDa (Microdyn Nadir, Wiesbaden, Germany) obtained under OCT technique with those obtained by standard mercury intrusion porosimetry method (Micromeritics Autopore IV 9510 Instrument, Norcross, GA, USA). Figure 2 shows computed and measured porosity and permeability values (Figures 2A and 2B, respectively) and 3-D structures of two virgin UF membranes (Figure 2C). As seen in Figure 2 (A and B), porosity and permeability values produced by OCT technique and common mercury intrusion porosimetry method were close to each other.

Fig. 2. Porosity (A) and permeability (B) of virgin membranes (100 kDa and 150 kDa) by using mercury intrusion porosimetry method and our current technique; and 3-D OCT images of membrane structure (C). In these images, blue and black colors correspond to solid and free space, respectively.

As shown in Figures 2A and B, porosity of 150 kDa membrane was found to be larger than that of 100 kDa membrane by both tested techniques. The OCT images (Figure 2C) revealed that both membranes contained macro-voids which are typically formed in membrane matrix during the phase-inversion casting process [51]. Moreover, more macro-voids were present in the structure of 100 kDa membrane as compared to that of 150 kDa membrane (inset of Figure 2C). As a result, 100 kDa membrane was characterized by significantly higher permeability because increase in the free space due to macro-voids formation reduced its resistance to the flow so that more fluid could penetrate this membrane from the feed to permeate side.

Based on this data, it can be concluded that present technique could attain an accurate estimation of physicochemical parameters of membranes such as porosity and permeability. Therefore, a simple, *in situ* and direct internal analysis of materials can be conducted by using this proposed approach without any requirement to cease the operating conditions.

4. Results and discussion

A range of previous studies attempted to evaluate the biofouling development by visualization of a biofilm grown on spacer or membrane surface by applying 2-D visualization techniques [4, 19, 52, 53]. In our study, we applied OCT technique to investigate mesoscale structural and mechanical properties of biofilm deposited on the membrane surface. OCT allowed capturing biofilm structure in its native environment and digital analysis of biofilm structure aided in characterization of biomass thickness, porosity, permeability and mechanical properties. The 3-D biofilm images obtained in our study during the dead-end filtration at various stages of biofilm development revealed formation of uniform cake-like biofilm structure. For dead-end filtration, it is expected to have cake-like biofilm structure as the evolving biofilm is always under constant applied hydrodynamic feed pressure in the normal direction of the biofilm.

4.1. Flux variation under compression and decompression processes

The permeate flux production in response to biofilm compression and decompression was investigated. Figure 3 (A and B) shows the evolution of permeate flux as a function of the feed flow rate at various days of biofilm development with respect to compression and decompression processes. At each testing day, permeate flux first increased due to increase in the feed flow rate from 15 mL/min to 150 mL/min and then declined due to decompression process. As filtration evolved, a gradual decrease in permeate fluxes at each feed flow rate was observed due to biofilm development and increase in biomass thickness. As seen in Figure 3C, at the highest hydraulic force applied to the biofilm/membrane ($Q = 150$ mL/min), the permeate flux declined by almost four times, from 33.60 LMH at day 3 to 8.16 LMH at day 8. The reduction in permeate flux was attributed to development and accumulation of biofilm which fully covered the surface of the membrane.

Fig. 3. Permeate flux produced at various feed flow rate applied on the biofilm/filtration system at different growth time under compression (solid line)/decompression (dotted line) processes (A, B) and highest flux produced over time at $Q = 150$ mL/min (C).

4.2. Biomass thickness and 3-D biofilm visualization

The resulted biofilm thicknesses (H_{av}) were plotted as a function of the feed flow rate at different days when compression and decompression processes were applied on growing biofilm (Figure 4A). At each applied feed flow rate, an increase in biomass thickness was observed with an increase in filtration time from day 3 to day 8. However, when comparing the absolute values of biofilm thickness during compression process, we found that biofilm thickness increased to a higher extent from day 6 to day 7 as compared to its increase from day 7 to 8.

On the other side, a decrease in biofilm thickness was observed with the increasing feed flow rate during the compression procedure at any testing day (solid lines in Figure 4A). As the feed

flow increases from 15 mL/min to 150 mL/min, a highest reduction in biomass thickness was observed at day 8 from 330 μm to 293 μm , indicating that biofilm became more fragile as it evolves over the filtration time. When decompression process was applied, different trends in biofilm behavior were observed at different testing days (dotted lines in Figure 4A). While no changes in the biofilm thickness was detected in case of sequential compression/decompression at day 3, a thickness of biofilm measured at days 6, 7 and 8 was always higher after decompression process comparing to that of compression at any given feed flow rate.

The evolution of biofilm thickness over filtration time was previously studied by Fortunato et al. [40] in a gravity driven submerged membrane bioreactor. The authors found that the highest increase in the biofilm thickness occurred between days 3 and 7 with its further stabilization until day 9. Our data exhibited similar trend where a significant increase in biofilm thickness was also observed until day 7 (Figure 4A). However, the increase in the biomass thicknesses slowed down between days 7 and 8 comparing to days 6 and 7 suggesting the beginning of the stability phase.

Biofilms are mainly composed of bacterial cells embedded in EPS matrix which is described as a highly hydrated gel [5, 54]. Presence of EPS in biofilm matrix causes its compression and expansion under increased or decreased pressure (or flow rate), respectively and modifies biofilm's thickness [55]. At day 3, the biomass thicknesses showed the same values in response to compression and decompression processes (Figure 4A). However, as the biofilm was further developing (days 6, 7 and 8), its response to stress became different. The changes in biofilm reaction to stress clearly indicated that at early biofilm growth stage, the biofilm structure was more resilient and tougher as compared to later growth stages exhibiting characteristic properties of elastic material. As the biofilm structure evolved, the changes in biofilm thickness followed the hysteresis-like trend under compression/decompression processes, thus suggesting that the structural properties of biofilm deviated from the elastic-type behavior towards the viscoelastic-type behavior. This finding is well-correlated with the results of other studies where the viscoelastic nature of the biofilm material was often assumed [21, 56].

Fig. 4. Variation of biomass thickness as a function of the feed flow rate during compression (solid line, red error bars) and decompression (dotted line, black error bars) processes at different days of filtration (A), and deviation of thickness between the compression and decompression of biofilm over time at $Q = 15, 60$ and 105 mL/min (B).

We further calculated the deviation in biofilm thickness at three different feed flow rates (15 mL/min, 60 mL/min and 150 mL/min) for compression and decompression processes and plotted it as a function of filtration time (Figure 4B). As seen in Figure 4B, the deviation of thicknesses followed the same trend with its initial increase with increasing filtration time from day 3 to day 6 at feed flow rates of 60 mL/min and 105 mL/min. Whereas, for the feed flow rate of 15 mL/min initial increase of thickness deviation was found from day 3 to day 7. In addition, reductions in thickness deviation are observed for all feed flow rates toward the end of the experiments (day 8).

The lowest deviations of compression/decompression processes (Figure 4B) were observed at day 3, followed by their increase at days 6 and 7 when the biofilm structure exhibited elastic-plastic type of behavior [57, 58]. The deviations in thickness tended to decrease towards day 8 suggesting transformation of biofilm structure from the elastic to the viscoelastic type. The appearance of hysteresis-like trend with respect to compression/decompression processes suggested that porosity of biofilm was changed after compression, so its further response to decompression was different which resulted in increasing biofilm thickness. A similar phenomenon was observed by Dreszer et al. [39] who studied the biofilm growth and

development on NF membrane. The authors attributed this effect to changes in the internal voids (i.e., porosity) of biofilm community.

The high resolution 3-D imaging of both biofilm and membrane was achieved by OCT technique allowing to estimate their different intrinsic properties. The biofilm development was evaluated with respect to increase in biofilm thickness over filtration time (days 3, 6, 7 and 8) with respect to compression process at 15 mL/min and 150 mL/min, as shown in Figure 5. The processed scan elucidates a dense smooth layer of biofilm with the uniform morphology. Three main elements can be distinguished from the images in Figure 5: (i) the active layer of membrane (orange middle line), (ii) the porous structure of membrane (bottom side of the active layer), and (iii) the biomass cake (top side from the active layer). At day 3, a hydraulic force exerted on the biofilm through the compression process resulted in a decrease in biofilm thickness from 113 μm at a feed flow rate of 15 mL/min to 85.4 μm at a feed flow rate of 150 mL/min. The visualized orange color in Figure 5 indicated fluid travelling through the biofilm and membrane structures. As seen in Figure 5A, more orange color, i.e. more fluid intensity appeared in the biomass structure at day 3 corresponding to early stage of biofilm growth when the biofilm thickness was lowest. The intensity of orange color further increased during biofilm compression (Figure 5B). As filtration time increased, the biofilm evolved and thickens. As such, the intensity of orange color appeared in biofilm and membrane structures progressively decreased from day 6 to day 8 pointing out on the reduction in the filtration processes efficiency. On the other hand, some defects were observed on the biofouling cake surface at high flow rate of 150 mL/min (Figures 5D, 5F and 5H) caused by the fluid shear exerted through compression process at various days of biofilm growth.

It is also important to mention that OCT technology is based on measuring low-coherence light reflection from imaging samples (in this case biofilm and membrane) through interference pattern. Also, the scan of depth is fixed by OCT hardware. At day 3, when the scan of biofilm and membrane are taken, the OCT light source can penetrate until the depth of the membrane. Therefore, we see well-defined structure of both membrane and biofilm. Since the reflection of light from the material of biofilm and membrane's support layer is very similar, the displayed color spectrum for both materials is same (in shades of green). Thus, the green color does not mean that biofilm has penetrated inside the membrane matrix. It is indicative that some material exists, either biomass or polymeric matrix. As the filtration progressed on days 6, 7 and 8, the

biofilm on membrane surface grew thicker. The light reflection from the membrane support layer reaching the OCT probe changes significantly due to thicker biofilm. As a result, the color intensity changed from green to shades of blue.

Fig. 5. 3-D visualization of biomass grown on UF membrane at a feed flow rate of 15 mL/min and 150 mL/min during the compression process exerted at days 3, 6, 7 and 8.

4.3. Normal strain and bulk modulus

The mechanical properties of biofilm were investigated by studying the normal strain developed during compressing and decompressing the biofilm. The results are shown in Figure 6, which represents axial strain as a function of applied pressure (normal stress). As seen in Figure 6A, at day 3, the applied normal stress was roughly proportional to the strain and is slightly non-linear while at days 6 and 7 the strain response became non-linear. Additionally, hysteresis in the stress-strain curves under compression and decompression processes was also clearly visible. The observed results suggests the elastic nature of biofilm in the early days of filtration (day 3). By comparing images of Figures 6B-D, it can be concluded that the strain hysteresis difference between the compression and decompression processes was highest at day 6, and then gradually decreased with an increase in the filtration time (days 7 and 8). A non-linear response of the stress/strain curve to the applied force predominated (Figures 6B-D) suggested biofilm's deviation to a non-elastic structure. Moreover, after day 6 of biofilm development, a hysteresis-like shape of compression/decompression stress/strain curves suggested an elastic-plastic behavior of the biofilm [58]. It is clearly seen that the areas under the hysteresis-like curves corresponding to days 6, 7 and 8 progressively decreased indicating a shift towards the viscoelastic structure of biofilm.

The mechanical properties of biofilm are mainly related to EPS that provides the structural stability to the bacterial matrix [60]. Increasing the normal forces could initiate various mechanical responses such as deformation, mixing, movement and removal of bacterial cells [22, 61, 62]. The deformation nature (reversible or irreversible) is a function of the intermolecular bonds that dominate the intrinsic structure of biopolymers [57]. The agreement of biofilm thickness values under compression and decompression processes observed on day 3 of filtration suggested that the biofilm exhibited elastic properties at the early stage of its development (Figure 4A) so that the deformation of biofilm under compression and decompression processes was reversible (Figure 6A). This result is consistent with the findings reported by Stoodely et al. [22] who investigated the elastic behavior of biofilm grown under laminar flow in response to the increase and the decrease of fluid shear stress over a short time scales. However, as time of filtration increased, the biofilm started acquiring non-elastic material properties (irreversible deformation) (Figures 6B-D) resulting in the formation of hysteresis-like trend that implied a breakage of bonds under exerted fluid force [57].

Fig. 6. Normal Stress/strain curves obtained at different times of biofilm development during compression (solid line) and decompression (dotted line) (A, B, C, D) and variation of bulk modulus (G) of biofilm over time under compression/decompression processes (E).

Figure 6E shows the value of bulk modulus (G) calculated on the basis of compression/decompression of biofilm as the initial slope of the normal stress-strain curve corresponded to the bulk modulus of the biofilm. The bulk modulus of biofilm gradually increased in the compression process with increasing filtration time and reached maximum of 666 kPa at day 7 and then decreased to 522.4 kPa at day 8. The bulk modules obtained during decompression process deviated from the bulk modulus achieved during the compression

processes and the extent of deviation varied from 6% at day 6 to 26% at day 7. Further, the bulk modulus which is also defined as inverse of the compressibility of the material also suggests similar trend. For day 3, lower values (both compression and decompression cases) of bulk modulus clearly indicate that structure is least compressible compared to other days. As the filtration progresses the structure became more compressible and the highest compressibility is observed for day 7. In addition, the highest difference in bulk modulus under compression and decompression is also found in day 7 and day 8, suggesting irreversible deformation in the internal structure of biofilm owing to high compressibility of the structure.

4.4. Porosity of biofilm

The impact of compression/decompression on biomass porosity was investigated at various steps of biofouling growth. Figure 7 shows the micro-structure porosity variation of the biofilm evolving on membrane. At day 3 (Figure 7A), the variation of porosity at each feed flow rate during compression/decompression was negligible under applied normal stress. A non-linear response of the biomass porosity to stress over applied feed flow rates was observed upon biofilm compression and decompression processes conducted on days 6, 7 and 8. Thus, the biomass porosity gradually decreased with initial increase in the feed flow rate, followed by a slight increase in the middle range of the applied flow rates and then decreased again with further flow rate increase to 150 mL/min.

Fig. 7. Evolution of biomass porosity over feed flow rate at different times of biofilm growth obtained after application of compression (solid line, red error bars) and decompression (dotted line, black error bars) on the filtration system.

In the case of decompression process when the feed flow rate was gradually reduced from 150 mL/min to 15 mL/min, the biofilm porosity responded to the releasing stress with a significant variation which depended on biofilm age. While steady increase in biofilm porosity was observed at days 6 and 7, the biofilm behavior during decompression at day 8 was more complicated with the initial steep increase in the biomass porosity upon reducing feed flow rate from 150 mL/min to 135 mL/min followed by its steady decline during the remaining decompression time. It was hypothesized that at this stage of development, the internal porosity of biofouling was greatly modified upon restoring the applied fluid stress. Since initial biofouling layer developed on the membrane surface by day 3 was less porous comparing to that grown on membrane surface after 6 days of filtration (Figure 7 (A and B)), the compressibility would significantly influence the mature biofilm leading to a hysteresis-like response to the applied stress.

We further compared porosity values obtained at compression and decompression tests at the same applied feed flow rate. As seen in Figure 7, while only minimal difference in biomass porosities was detected at day 3 and the variation in biomass porosities increased with increasing filtration time. The variation in biomass porosities reached its maximum at day 8 where the porosity values observed during the compression and decompression processes were not maintained and a significant variation was detected at low and high feed flow rates. Thus, the porosity under compression at day 8, estimated at the feed flow rate of 15 mL/min ($P = 0.482$) was higher than that under decompression ($P = 0.332$), an opposite trend was detected at the feed flow rate of 135 mL/min ($P = 0.320$ and $P = 0.411$, respectively).

In order to explain the differences in biomass porosity response to the applied stress at day 8, the orthoslices from the 3-D biofilm structure were extracted and plotted, as shown in Figure 8. The structure analysis of biofilm at the feed flow rate of 15 mL/min revealed the presence of a larger voided space (biofilm detachment from membrane) in the vicinity of the membrane surface which was expected to contribute to the increase in porosity under biofilm compression (Figure 8A) as compared to decompression process (Figure 8B) where less voids were detected. On the contrarily, at the feed flow rate of 135 mL/min, this high void space was found under decompression process (Figure 8D) explaining the high value of biofilm porosity observed at day 8 (Figure 7B).

Fig. 8. AVIZO orthoslices extracting from 2-D OCT imaging of biofilm/membrane at a feed flow rate of 15 mL/min (A and B) and 135 mL/min (C and D) achieved at day 8 of biofilm development under compression and decompression processes. The biofilm detachment area was shown inside red rectangles in Figures 8A and 8D.

The results of this study suggested an increase in porosity with increasing the biofilm age. As discussed above, the porosity of biofilm was affected differently by its compression at low and high thicknesses. In general, the development of biofilm is arbitrary and its porosity is influenced by several factors including type of bacterial species and EPS which are present within the biofouling matrix, the age of biofilm and the fluid flow stress [28, 39, 59, 60]. Ica et al. [59] found that the porosity of biofilm evolved differently when the bacterial species in the substrate were changed. Other studies confirmed that the distribution of porosity itself depends on the depth of biofilm [61, 62]. Zhang and Bishop [62] suggested that the porosity varied in the range of 84%-93% in the top layers of biofilm to 58%-67% in the bottom layers of biofilm. Herzberg and Elimelech [28] explained the mechanism of biofouling development on the membrane

surface in presence of *P. aeruginosa*. The authors distinguished three stages of this process including accumulation of micro-colonies, maturation of biofilm followed by the detachment of bacterial cells in the membrane vicinity. The bacterial detachment, which depends on the hydrodynamic shear and microbial growth rates [60] caused the creation of crevices and holes in the mature biofilm and increased porosity [39]. The latter is well-correlated with our data where higher biofilm porosity was observed at the membrane surface after 8 days of biofilm growth at a feed flow rate of 15 mL/min after compression process (Figures 5G and 8A).

4.5. Total membrane/biofilm permeability

The evolution of the total biofilm/membrane permeability as a function of applied flow rate is presented in Figure 9. A decrease in the total biofilm/membrane permeability with increasing filtration time was observed. Thus, during the compression process, at the feed flow rate of 15 mL/min, the initial permeability was estimated to be $4.54 \times 10^{-14} \text{ m}^2$ after 3 days of biomass growth, it further decreased to reach $5.19 \times 10^{-15} \text{ m}^2$ after 8 days of filtration. While the total biofilm/membrane permeability underwent a monotonic decrease with the increase in the feed flow rate at day 3, an initial increase of total biofilm/membrane permeability followed by its almost linear decrease with an increase of the feed flow rate was observed on days 6, 7 and 8. Further decompression process caused a significant decrease in total membrane/biofilm permeability as comparing to compression process. It was hypothesized that the compression process caused collapse of the biofilm voids which were not immediately recovered leading to a decline of the total biofilm/membrane permeability values. In addition to the total permeability measured by the experiments (Figure 9A-B), the biofilm/membrane structures were also analyzed by numerical technique as described in Section 2.3.5. Figure 9C shows the comparison of total permeability values of the numerical technique with the experimental values for day 3 and day 7 at flow rates of 45 mL/min, 90 mL/min, and 120 mL/min, respectively. Total permeability values compare quite well with the experimental values.

In agreement with previous studies [63, 64], changes in membrane's permeability and biofilm nature over the time of filtration caused decrease in the total biofilm/membrane permeability. With the evolvement of bacterial population, the porous structure of membrane is mitigated due to the membrane pore bio-clogging [65]. On the other side, biofilm became stiffer with the increase in its thickness making the EPS and the biofilm pores less accessible during

filtration. Therefore, the accumulation of EPS as the filtration time is expected to hinder permeation of water due to increase of hydraulic resistance [66-68]. As mentioned in Section 4.6.3, biofilm porosity varied as a function of biofilm depth. The effect of biofilm depth on other internal structural properties of biofilm such as density, ratio of living/dead cells and the mean pore radius of biofilm has been shown [61, 62]. Thus, Zhang and Bishop [62] demonstrated that the bottom layers in the vicinity of membrane surface are denser, have less living cells and lower mean pore radius than the top layers. These factors could also hamper water diffusion through the membrane and reduce permeate flux.

Fig. 9. Variation of the total permeability biofilm/membrane as a function of feed flow rate calculated corresponding to Darcy's Law at different days of biofilm growth under compression/decompression processes (A and B) and comparison of numerical and experimental values of the total permeability at day 3 and day 7 (C).

5. Conclusions

An *in situ* technique for the intrinsic structural characterization of biofilm grown on membrane is successfully developed in this study. This technique which is based on OCT as a structural imaging tool, is able to overcome the limitations associated with currently used biofilm characterization techniques. It is important to emphasize here that structural properties of biofilm are quite sensitive to the applied stress. As such, the present *in situ* biofilm analysis technique is not invasive and is not able to damage or disturb the biofilm structure at any stage of sample preparation or analysis. Present work, therefore, holds the advantage over existing methods as it does not require biofilm removal from its natural growing environment, neither addition of chemicals or reagents that might potentially alter its structure.

In this work, the microstructural morphology of biofilm grown on UF membrane was visualized by using 3-D imaging resolution. The mechanical and structural properties of biofilm against the shear fluid flowing through its structure at various feed flow rates ($Q = 15 \text{ mL/min} - 150 \text{ mL/min}$) and at different stages of biofouling development were comprehensively investigated. Under compression/decompression processes, the changes in biofilm thickness and strain rates values confirmed that the biofilm acquired an elastic properties at the initial growth time (day 3) followed by its transition to viscoelastic type as the biofilm evolved and matured (day 8). Moreover, the internal biomass porosity increased with biofilm development to reach a maximum of 0.482 after 8 days of filtration at the feed flow rate of 15 mL/min. The evolution of the biofilm porosity as a function of a feed flow rate confirmed the elasticity nature of initial biofilm formed after 3 days of filtration. Furthermore, the total permeability of biofilm/membrane decreased over course of filtration reaching $5.19 \times 10^{-15} \text{ m}^2$ on day 8 at an applied feed flow rate of 15 mL/min. Therefore, the decrease of permeability hindered the diffusion of the feed solution thus explaining the reduction of permeate flux produced with biofilm development.

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Highlights

- An in-situ technique to characterize biofilms in membrane filtration is proposed
- It enables direct structural characterization and mechanical properties of biofilms
- It is based on 3-D OCT imaging coupled with an advanced mathematical framework
- Biofilm thickness, micro-porosity, normal stress-strain, bulk modulus and permeability are determined
- Structural properties were found to be time dependent as the biofilm continuously evolved