Physical structure determines compression of membrane biofilms during Gravity Driven Membrane (GDM) ultrafiltration

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Abstract

Increasing transmembrane pressure (TMP) can compress and increase the hydraulic resistance of membrane biofilms. The purpose of the present study is to evaluate how compression of membrane biofilms occurs and how structural rearrangement can affect hydraulic resistance. Biofilms with heterogeneous and homogeneous physical structures were grown in membrane fouling simulators (MFS) in dead-end mode for 20 days with either (i) a nutrient enriched condition with a nutrient ratio of 100:30:10 (C: N: P), (ii) a phosphorus limitation (C: N: P ratio: 100:30:0), or (iii) river water (C: N: P ratio: ca. 100:10:1). The structural and hydraulic response of membrane biofilms to (a) changes in transmembrane pressures (0.06 - 0.1 - 0.5 - 0.1 - 0.06 bar) and (b) changes in permeate flux (10 - 15 - 20 - 15 - 10 L/m²/h) were investigated. Optical coherence tomography (OCT) was used to monitor biofilm structural response, and OCT images were processed to quantify changes in the mean biofilm thickness and relative roughness. Nutrient enriched and river water biofilms had heterogeneous physical structures with greater surface roughness ($Ra' > 0.2$) than homogeneous P limiting biofilms ($Ra' < 0.2$). Compression of biofilms with rough heterogeneous structures ($Ra' > 0.2$) was irreversible, indicated by irreversible decrease in surface roughness, partial relaxation in mean biofilm thickness and irreversible increase in hydraulic resistance. Compression of homogeneous biofilm ($Ra' < 0.2$) was on the other hand reversible, indicated by full relaxation of the biofilms structure and restoration of initial hydraulic resistance. Hydraulic response (i.e., change in the specific biofilm resistance) did not correspond with the change in physical structure of heterogeneous biofilms. The presented study provides a fundamental understanding of how biofilm physical structure can affect the biofilm's response to a change in TMP, with practical relevance for the operation of GDM filtration systems.

Keywords: Biofilm physical structure, biofilm compressibility, biofilm hydraulic resistance, membrane filtration.

1. Introduction

Gravity Driven Membrane (GDM) ultrafiltration is a dead-end filtration process operated at constant and low transmembrane pressure (TMP) (0.02 to 0.1 bar). During GDM ultrafiltration, biofilm formation on the membrane surface is tolerated and allows operating the system at stable flux. Permeate flux can intuitively be increased by applying a larger water head, thereby increasing TMP. Increasing TMP by application of a higher water-head can however compress the physical structure of the membrane biofilm, leading to higher hydraulic resistances and thus a limited increase in permeate flux (Valladares Linares et al., 2015, Derlon et al., 2016). It is not known what structural feature of the biofilm rearranges (e.g., surface roughness) during compression, nor how structural rearrangement is linked to the increase in hydraulic resistance. Identifying what factors determine the structural and hydraulic response of membrane biofilms is essential for maintaining an adequate permeate flux. The aim of the present study is to evaluate how compression of membrane biofilms occurs and its implication for hydraulic resistance in GDM filtration systems.

The hydraulic resistance of a filter cake is linked to its compressibility (n). The specific resistance of the cake layers (α , resistance per mass) is dependent on the applied TMP:

$$\alpha = a \cdot TMP^n \quad (1)$$

where a is a constant and n is the compressibility coefficient with 0 (incompressible) $\leq n \leq 1$ (compressible) (Foley, 2013). When compressible cake layers cannot sustain the force exerted by the increased permeate flux, they consolidate to then form a new stable structure (McCarthy et al., 1999, McCarthy et al., 1998). For microbial suspensions, the formation of a consolidated structure is reached by particle rearrangement and/or particle deformation, increasing the packing density of the cake (Foley, 2006). Cake deformation decreases cake porosity and the area of contact between the particles and fluid, consequently increasing hydraulic resistance (Tiller and Kwon, 1998). For microbial suspensions and polymeric gels, compression is

reversible (McCarthy et al., 1999). Therein, initial hydraulic resistances are recovered following restoration of initial TMP, thus indicating recovery of the initial cake porosity. Whether the mechanisms of homogeneous cake layer (e.g., uniform physical structure/hydraulic flow) compression are also valid for membrane biofilms is not known.

Biofilms and cake layers irreversibly compress following application of higher TMPs, as indicated by decreased mean biofilm thickness and an increase in hydraulic resistance upon restoration of initial TMPs (Bugge et al., 2012, Jørgensen et al., 2014, Poorasgari et al., 2015, Valladares Linares et al., 2016, Dreszer et al., 2014). Irreversible compression is against the expectation that fouling layers with high polymeric concentrations (e.g. membranes biofilms) are reversibly compressed (Foley, 2006). It is not known what structural feature of the biofilm becomes irreversibly compressed (e.g. surface roughness/internal pores). Nor is it clear how structural rearrangement is linked to the increase in hydraulic resistance. Valladares Linares et al. (2016) proposed physical compression can cause structural rearrangement on the nano-scale between neighbouring extracellular polymeric substances (EPS) of the biofilms matrix. Valladares Linares et al. (2015) assumed physical deformation on the mesoscale is sufficient to induce short-range intermolecular interactions. However, the basis for said explanation was not experimentally explored, nor is it likely that macro-scale compression can induce micro-scale interaction of EPS functional groups. We suggest physical structure is an important factor determining the biofilms structural response to force. Thus, the role of physical structure (e.g., surface roughness) on the compression and relaxation of membrane biofilms will be investigated.

The specific focus of the current study is to evaluate (a) what structural feature of the biofilm rearranges (e.g., surface roughness) during compression and (b) how structural rearrangement is linked to the increase in hydraulic resistance. Desmond et al. (2018) demonstrated the utility of nutrient conditions in engineering biofilms with different compositions (in terms of EPS and

inorganic particles) and in turn physical structures. Nutrient limitation will be used in the current study to cultivate biofilms with distinct physical structures for the purpose of understanding how physical structure determines biofilm compression. Optical coherence tomography (OCT) will be used to monitor the mesoscale structural response of membrane biofilms with different physical structures to increasing TMP and quantified with respect to mean biofilm thickness and surface roughness. Monitoring biofilm compression while increasing TMP will help determine what structural feature of the biofilm rearranges (e.g., surface roughness) during compression, and whether structural rearrangement is linked to the increase in hydraulic resistance. The composition of the biofilms matrix was analysed in terms of proteins, polysaccharides, and total organic carbon (TOC).

2. Materials and Methods

2.1 Biofilm growth set-up

2.1.1 Biofilm cultivation

Biofilms were cultivated in membrane fouling simulators (MFS) operated as GDM filtration systems in dead-end mode at a constant TMP of 0.06 bar where gravity was the driving force. For each MFS, feed water was pumped into the feed-line and overflow tank with a peristaltic pump (ISM932A, Ismatec, Glattbrugg, Switzerland) at a rate ensuring that permeation was solely gravity driven. The MFS were connected to overhead tanks with overflows through which excess feed water could leave the water head ensuring constant water head of 60 cm could be maintained. Biofilms were grown on ultrafiltration membranes held in transparent membrane fouling simulators (MFS) (Eawag, Dübendorf, Switzerland) with an area of 18.75 cm². Three growth conditions were used: nutrient enriched, a phosphorous limiting and a membrane system fed by river water. Biofilms were grown in duplicate in 3 experimental runs (n = 6). The experiments were undertaken in a 20 °C temperature-controlled room.

2.1.2 Membrane preparation

Ultrafiltration membranes (UP150, MicrodyN Nadir, Wiesbaden, Germany) of polyethersulfone with a nominal cut-off of 150 kDa were used as a substratum for biofilm growth (corresponding to a mean pore size of about 30-40 nm). Membrane' coupons were cut, rinsed and washed in 40% ethanol to disinfect the membranes' surface as described by Heffernan et al. (2013).

2.1.3 Feed-water preparation

Three different growth conditions were used: Nutrient enriched (synthetic), P limiting (synthetic) and river water (surface water). For biofilms grown with synthetic substrate, sterile feed-water was prepared in 20 L glass bottles. Carbon (C), nitrogen (N) and phosphorus (P) were added in the form of acetate (CH_3COO^-), ammonium (NH_4^+) and ortho-phosphate (PO_4^{3-}), respectively, at a ratio of 100 (C):30 (N):10 (P) for the nutrient enriched conditions (Table 1). Use of nutrient limitation was used only as an experimental tool to grow biofilms of different physical structures and EPS compositions as demonstrated by Desmond et al. (2018). Essential trace elements were supplemented to the feed-water along with EDTA to ensure trace elements did not precipitate (Table 2) (Hammes and Egli, 2005).

For biofilms grown under river water conditions, surface water was pumped into a settling tank to allow removal of larger particles and sediments before being fed by gravity into the feed tank of the filtration system. This ensured removal of silt but allowed transfer of colloids/fine particles through the membrane modules. Intake source located in Chriesbach river, Dübendorf, Switzerland (47°24'16. 3"N 8°36'31. 8"E).

Table 1

2.1.4 Bacteria cultivation and membrane MFS inoculation

Bacteria for MFS inoculation were cultivated from the Chriesbach river, Dübendorf, Switzerland (47°24'16. 3"N 8°36'31. 8"E) during spring time. For inoculation of the MFS, microbial suspension was centrifuged for 15 min at 5,000 rpm (UniCen MR, Herolab, Germany). The supernatant was decanted allowing re-suspension of the cell pellet in 30 mL 0.1 M NaCl yielding a final concentration of $1.5 \cdot 10^{10}$ cells/ml as determined by ATP assay assuming $2 \cdot 10^{-18}$ mol of ATP per cell. From the resulting bacterial solution, 2.5 mL was injected into each MFS. The spiked MFS was let settle for 18-24 hours to allow filtration of bacterial solution allowing known cell coverage over the membrane area. Filtration of microbial suspension resulted in a surface coverage in a surface coverage of $2 \cdot 10^{13}$ cells/m² confirmed by ATP assay assuming $2 \cdot 10^{-18}$ mol of ATP per cell.

2.2 Biofilm compression

2.2.1 Biofilm compression setup

Biofilm compression experiments were performed in 3 separate experimental runs to ensure reproducibility. Each run was performed with 2 MFS per condition (total 6 MFS per condition). Variation is due to break in MFS window when critical TMP was breached in certain instances. After 20 days of growth, biofilms were transferred under 0.06 bar pressure to an automated filtration system (Fig. 1). The filtration system was fed with feed waters reflective of the growth conditions of the biofilm being compressed. Biofilm compression was achieved by step-wise increasing and decreasing either the transmembrane pressure or the permeate flux. Biofilms were exposed to increasing and decreasing TMP (0.06-0.1-0.5-0.1-0.06 bar) where exposure was for 15 min or controlled rates of permeation (10-15-20-15-10 L/m²/h) where exposure was for 30 min at each step (Table 3.). Pressure was measured at the feed water and permeate sides

of the MFS to determine the transmembrane pressure across the biofilm-membrane. Return valve was automatically adjusted to match specific TMP or permeate flux.

Figure 1

Table 3

2.3 Hydraulic analysis

2.3.1 Hydraulic parameters

Permeate flux and hydraulic resistances were derived from the mass of collected permeate. The volume of permeate was determined by dividing the permeate mass by the density of water (1000 kg/m³). The following equations were used for the calculations of the permeate flux (J , [L/m²/h])

$$J = \frac{\Delta V}{A \cdot \Delta t} \quad (2)$$

where ΔV of permeate is the change in permeate volume [L], A is the filtration area [m²], Δt is the change time [h]. The total hydraulic resistance [1/m] was calculated as

$$R_{total} = \frac{TMP}{\eta \cdot J} \quad (3)$$

where TMP is the transmembrane pressure [bar], η is the dynamic viscosity of water at a given temperature [Pa s], and R_{total} is the total filtration resistance of the fouled membrane [1/m]. The biofilm hydraulic resistance ($R_{biofilm}$, [1/m]) was calculated according to Eq. (4):

$$R_{biofilm} = R_{total} - R_{membrane} \quad (4)$$

where $R_{membrane}$ is the intrinsic resistance of the clean membrane measured for a period of 24 h with nanopure water prior to bacteria inoculation. Specific resistance (α) was calculated from

the hydraulic resistance of the biofilm ($R_{biofilm}$, Eq. 4) and the accumulated biomass per membrane area (ω) (mg C/m²):

$$\alpha = \frac{R_{biofilm}}{\omega} \quad (5)$$

2.3.2 Determination of the compressibility coefficient n

The effect of transmembrane pressure on the biofilm hydraulic resistance was quantified by plotting its specific resistance vs. TMP as described in Eq. (1). The compressibility coefficient n was estimated from regression using a power function in Excel. The value n is a quantitative measurement of the biofilm compressibility. For the estimation of compressibility, the results from tests (Table 2) varying TMP or flux were pooled.

2.4 Biofilm characterisation

2.4.1 Biofilm structural quantification

The structural response of the membrane biofilm to increasing and decreasing compressive force was resolved on the meso-scale by means of optical coherence tomography (OCT) (model 930 nm Spectral Domain, Thorlabs GmbH, Dachau, Germany) with a central light source wavelength of 930 nm and refractive index of 1.33. For image acquisition, the OCT was coupled to the automated filtration system for in situ analysis (Fig. 1). For the image acquisition OCT images were recorded on line. Three randomized locations were selected from each MFS during each experimental run. The number of locations was restricted due to a time point analysis, which was compensated for by increase number of MFS. Images were analysed on Matlab, therein quantified with respect to:

(a) mean biofilm thickness:

Biofilm thickness was calculated based on the number of pixels found between the top edge of the biofilm and the upper membrane surface of each OCT image, using a pixel scaling factor to obtain the biofilm thickness in microns.

$$\overline{L_F} = \frac{1}{N} \sum_{i=1}^N L_{F,i}$$

where $L_{F,i}$ is the biofilm thickness from a single A-scan (i.e., light reflected from each optical interface) in the corresponding B-scan (i.e., cross-sectional reconstruction of a plane from a series of A-scans across the structure) and N is the total number of A-scans.

(b) relative roughness

The relative roughness coefficient of the biofilm was calculated according to Murga et al., 1995.

$$R_a^* = \frac{1}{N} \sum_{i=1}^N \frac{|L_{F,i} - \overline{L_F}|}{\overline{L_F}}$$

where i represents a A-scan and N the overall number of A-scans. Biofilms with a smooth surface and only a few variations from the mean biofilm thickness have low values close to 0. The higher the roughness coefficient, the more variations are expected from the biofilm surface (Blauert et al., 2015).

(c) porosity

OCT images does provide some indication of how dense or loose a biofilm is structured, and fraction of light/dark pixels or greyscale distribution can be quantified. Wagner et al. (2010) suggest zones of low signal intensity represent areas of absent biomass (i.e., voids, pores). However, the distinction between solid materials and voids relies on the assumption that

absence of OCT signal equals voids. However, such an assumption and the resulting porosity quantification is debatable.

2.4.2 *Biofilm harvesting*

Biofilms were removed from the MFS for chemical analysis at each time point. Biofilms were scraped from the membrane surface via a cell scraper and transferred into sterile glass beakers. A volume of 30 mL 0.1 M NaCl solution was used to rinse the remaining biofilm from the membrane and MFS surface. The collected biomass was re-suspended in the washings of the 30 mL 0.1 M NaCl solution. Residual TOC after scraping is like TOC of a virgin membrane, indicating that no left biofilm remained on the scrapped membrane. Thus, verifying removal of available biomass from the membranes surface.

2.4.3 *Total organic carbon*

The TOC of all biofilms were measured by an automatic total organic carbon analyser (TOC-V, Shimadzu, Japan) from harvested biomass samples re-suspended in 30 mL 0.1 M NaCl solution. Accumulated biomass (ω) was expressed as mg C/m².

2.4.4 *EPS extraction*

EPS and cells were dispersed and separated by sonication and centrifugation respectively. Physical extraction was selected over chemical methods of EPS extraction to limit contamination of sample fractions due to sensitivity of subsequent analytical methods. Different sonication strengths were tested to maximize EPS/cell separation while avoiding cell lysis (detected through soluble ATP measurement). Harvested biofilm suspensions (as described in section 2.4.2) were placed in an ice bath and sonicated for 120 seconds with 10 s rest intervals using a needle sonicator at 65% amplitude (Sonoplus HD 3200, Bandelin, Berlin, Germany). Sonication was repeated three times to ensure full dispersal of the cells and EPS. Thereafter, homogenised samples were centrifuged at 5,000 rpm at 20 °C for 15 min (UniCen MR, Herolab, Germany) to separate the EPS from the cells. Centrifugation was repeated to ensure full

separation of EPS from cells. The supernatant containing the soluble EPS was decanted from the resolved cell pellet and aliquots were obtained from the extracellular fraction for measurement of total organic carbon (TOC) (TOC-L, Shimadzu, Japan), size exclusion chromatography (SEC), and EPS colorimetric assays (Polysaccharide, Protein).

2.4.5 Total polysaccharide quantification

Concentrations of polysaccharides in the EPS fraction were determined by the Anthrone method adapted from Gerhardt et al. (1994). Anthrone was dissolved in absolute ethanol before adding it to 75% H₂SO₄. Absorbance was measured with a UV-Vis spectrophotometer (Infinite M200, TECAN, Männedorf, Switzerland) at a wavelength of 625 nm. Glucose was used as a standard and results were expressed in mg C/m² (conversion factor of 0.4 mg C mg/glucose).

2.4.6 Total protein quantification

Total proteins in the extracted EPS were quantified using a Bicinchoninic Acid (BCA) kit. Kits for lower or higher protein concentrations (Micro BCA Protein Assay Kit 23235/ Pierce BCA Protein Assay Kit 23225, Thermo Fisher Scientific, Waltham, MA, USA). The quantification was carried out according to the instructions of the manufacturer. The sample to reagent ratio was 1:1 for the lower kit and 1:20 for the higher kit. The solutions were incubated at 60 °C for 1 h (lower kit), respectively at 65 °C for 30 min (higher kit). After the samples were cooled down to room temperature, the absorbance was measured with a UV-Vis spectrophotometer (Infinite M200, TECAN, Männedorf, Switzerland) at a wavelength of 562 nm. Bovine serum antigen (BSA) was used as a standard and results were expressed in mg C/m² (conversion factor of 0.83 mg C mg/BSA).

3. Results

3.1 Biofilm characterisation

3.1.1 Total organic carbon

Total biomass concentration was analysed with respect to total organic carbon (TOC) after 20 days of filtration. The nutrient enriched, P limiting, and river water biofilms yielded 916 ± 120 mg C/m² (n = 6 MFS), 716 ± 90 mg C/m² (n = 6 MFS) and 592 ± 60 mg C/m² (n = 3 MFS), respectively (Fig. 2a).

3.1.2 EPS concentration:

Under nutrient enriched conditions EPS concentration yielded 835 ± 50 (n = 6 MFS) compared to 676 ± 100 mg C/m² (n = 6 MFS) and 541 ± 15 mg C/m² (n = 3 MFS) for the P limiting and river water biofilms respectively (Fig. 2b). P limiting biofilms were characterised by a high polysaccharide content while biofilms grown under nutrient-enriched and river water conditions had a high proteins content, in accordance with the findings of Desmond et al., (2018). In the current study, specific composition with respected to protein content yielded 750 ± 20 mg C/m² (n = 2 MFS) for the nutrient enriched condition, compared to 150 ± 10 mg C/m² (n = 2) and 780 ± 20 mg C/m² (n = 2 MFS) for the P limiting and river water conditions respectively (Fig. 2c). Polysaccharide accumulation yielded 180 ± 80 mg C/m² (n = 2 MFS) under nutrient enriched condition compared to 620 ± 10 (n = 2 MFS) and 450 ± 10 mg C/m² (n = 2 MFS) under P limiting and river water conditions respectively (Fig. 2d).

Concentration of polysaccharides and proteins exceeded the total concentration of total organic carbon in river water biofilms but not in biofilms formed with synthetic influents. Similar over reporting of colorimetric assays for EPS characterisation has been reported by Le et al. (2016) who demonstrate false positive results when using colorimetric means of total protein determination.

Figure 2

3.2 Effect of transmembrane pressure and permeate flux on biofilm physical structure

The application of higher TMPs and permeate flux led to a visible compression of the biofilms' structure. Physical compression was defined as the reduction in the average biofilm thickness. For nutrient enriched and river water biofilms, increasing TMPs and filtration rates lead to a reduction in areas of low or absent signal intensity, which are proposed by Wagner et al. (2010) to represent internal pores or voids (Fig. 3). For the P limiting biofilm, lowering of TMPs and filtration rates also relaxed the consolidated structure and allowed restoration in the initial physical structure of the biofilm (Fig. 3).

Figure 3

3.3 Biofilm hydraulic resistance

3.3.1 Transmembrane pressure and biofilm hydraulic resistance

Increasing TMP from 0.06—0.1—0.5 bar led to an increase in hydraulic resistance of the nutrient enriched and P limiting biofilms of 4 times that of the initial hydraulic resistances: $1 \cdot 10^{12} \pm 4.7 \cdot 10^{11}$ 1/m ($n = 6$) and to $2.7 \cdot 10^{12} \pm 4.4 \cdot 10^{11}$ 1/m ($n = 6$) (Fig. 4). Hydraulic resistance of biofilms formed with river water increased only two times that of its initial hydraulic resistance. Increasing in TMP corresponded with an increase in permeate flux (Fig. 5b). After restoring the TMP to an initial value of 0.06 bar, the hydraulic resistance of nutrient enriched and river water biofilms remained elevated at $5 \cdot 10^{11} \pm 3.5 \cdot 10^{09}$ 1/m ($n = 6$ MFS) and $1 \cdot 10^{12} \pm 5.1 \cdot 10^{10}$ 1/m ($n = 2$ MFS) respectively. The irreversible increase in hydraulic resistance contrasted with homogeneous P limiting biofilms, whose hydraulic resistance returned within range of initial values of $5.6 \cdot 10^{11} \pm 1.16 \cdot 10^{11}$ 1/m ($n = 6$ MFS) at 0.06 bar (Fig. 4).

3.3.2 Permeate flux and biofilm hydraulic resistance

A stepwise increase of permeate flux led to an increase in hydraulic resistance (Fig. 5a-c). Increasing permeate flux from 10 L/m²/h to 20 L/m²/h for the nutrient enriched and river water biofilms caused an increase in hydraulic resistance to $1.7 \cdot 10^{12} \pm 2.9 \cdot 10^{10}$ 1/m and $6.2 \cdot 10^{11} \pm 5.8 \cdot 10^{10}$ 1/m (n = 2 MFS), respectively (Fig. 5). Hydraulic resistance was elevated for both nutrient enriched and river water biofilms even upon restoration of the initial permeate flux (Fig. 5).

Hydraulic resistance of P limiting biofilms increased under increasing flux and returned within range of initial values, following restoration of initial permeate flux around $2.5 \cdot 10^{12}$ 1/m $\pm$ $4.9 \cdot 10^{10}$ 1/m (n = 2 MFS) and 0.4 ± 0.04 bar (n = 2 MFS) respectively (Fig. 4b). It should be noted initial hydraulic resistance was higher during controlled flux experiment compared to controlled TMP experiment, due to a greater TMP required to maintain a 10 L/m²/h permeate flux compared to Fig. 5. Restoration of initial hydraulic resistance and TMP after compression is proposed to indicate the presence of a reversibly compressible structure.

3.4 Effect of Transmembrane pressure and permeate flux on biofilm physical structure

3.4.1 Biofilm thickness

For experiments at controlled TMP, compression of nutrient enriched and river water biofilms was irreversible, with only partial relaxation in biofilm thickness. Nutrient enriched biofilms and river water biofilms had an initial thickness of 520 ± 133 μ m (n = 4 MFS) and 126 ± 13 μ m (n = 2 MFS) which recovered to only 205 ± 40 μ m (n = 4 MFS) and 67 ± 3 μ m (n = 2 MFS) after decompression (Fig. 4). Homogeneous P limiting biofilms had full recovery of its initial thickness of 297 ± 161 μ m (n = 3 MFS) to 313 ± 77 μ m after decompression (n = 2 MFS) (Fig. 4).

For experiments at controlled flux, compression of nutrient enriched and river water biofilms was also irreversible, with only limited relaxation in biofilm thickness. Nutrient enriched biofilms and river water biofilms had respective initial thicknesses of $518 \pm 117 \mu\text{m}$ ($n = 2$ MFS) and $112 \pm 3 \mu\text{m}$ ($n = 2$ MFS) which recovered to only $85 \pm 4 \mu\text{m}$ ($n = 2$ MFS) and $52 \pm 2 \mu\text{m}$ ($n = 2$ MFS) after decompression (Fig. 5). Homogeneous P limiting biofilms on the other hand, relaxed within range of its initial thickness of $189 \pm 12 \mu\text{m}$ ($n = 2$ MFS) to 181 ± 9 ($n = 2$ MFS) after decompression (Fig. 5).

3.4.2 Biofilm surface roughness

For experiments at controlled TMP, nutrient enriched and river water biofilms had an irreversible reduction in surface roughness. Nutrient enriched biofilms and river water biofilms respectively initial surface roughness of 0.4 ± 0.1 ($n = 4$ MFS) and 0.33 ± 0.14 which recovered to only 0.3 ($n = 4$ MFS) and 0.15 ± 0.03 after decompression (Fig. 4). Compression and relaxation of P limiting biofilms had no change their initial surface roughness of 0.21 ± 0.11 ($n = 3$ MFS), recovering to 0.21 ± 0.04 ($n = 3$ MFS) after decompression (Fig. 4).

For experiments at controlled flux, nutrient enriched and river water biofilms also had an irreversible reduction in surface roughness. Nutrient enriched biofilms and river water biofilms an initial relative surface roughness of 0.4 ± 0.2 and 0.3 ± 0.1 which recovered to only 0.1 ± 0.07 and 0.1 ± 0.04 ($n = 2$ MFS) after decompression (Fig. 5). Compression and relaxation of P limiting biofilms had no change in initial relative surface roughness of 0.16 ± 0.05 , recovering to 0.1 ± 0.03 ($n = 2$ MFS) after decompression (Fig. 5).

Figure 4

Figure 5

3.5 Compressibility analysis

Eq. (1) defines a material as compressible when the specific resistance increases with TMP (Fig. 6). The specific resistance for nutrient enriched, P limiting, and river water biofilms increased with increasing TMP, with n values of 0.68 (confidence interval for 97.5 % confidence: 0.60 to 0.77), 0.53 (confidence interval: 0.41 to 0.67) and 0.54 (confidence interval: 0.41 to 0.68), respectively (Fig. 6a-c). Monitoring of the biofilms *physical compression*, defined by the change in mean biofilm thickness over increasing TMPs, also revealed a decrease in thickness with increasing TMP – but not consistent with the increase in TMP. For biofilms formed under nutrient enriched and river water conditions most variation in thickness was observed when increasing TMP from 0.06 to 0.1 bar. Conversely above 0.1 bar, biofilm thickness only slightly decreased. In contrast, the specific resistance increased steadily from 0.06 to 1 bar following Eq. (1). Biofilms formed under the P limiting condition, had a lower relative change in mean biofilm thickness agreed with its specific resistance following application of higher TMP.

Figure 6

4. Discussion

4.1 Mechanisms of biofilm physical compression and implication for hydraulic resistance

In the current study, we evaluated to what extent compression of membrane biofilms (defined as the change in the biofilm thickness) influences its hydraulic resistance. Our results indicate that, for all biofilms tested, specific resistance did in fact increased with increasing TMP. An increasing specific resistance indicates biofilms behave as compressible material when compression is hydraulically defined (Eq. 1). The compression of GDM membrane biofilms is thus comparable to the compression of dead-end filtration of microbial suspensions and to cake layers formed in membrane bioreactors in terms of increasing specific resistance over

increasing TMPs (Foley, 2013, Poorasgari et al., 2015, Bugge et al., 2012, Jørgensen et al., 2014). However, membrane biofilms are self-assembled structures, whilst cake layers mainly result from solids accumulation on the membrane surface.

OCT was used to monitor the change in the biofilms thickness/roughness as an indicator of compression. Our results indicate that compression of heterogeneous biofilm structures ($Ra' > 0.2$) differs from the compression of homogeneous biofilms structures ($Ra' < 0.2$). For heterogeneous biofilms, the increase in specific resistance did not correspond to the reduction in its mean thickness and relative roughness. Physical compression of heterogeneous biofilms ($Ra' > 0.2$) was in fact bi-phasic, involving:

(a) a large reduction of the mean biofilm thickness was observed when biofilms were subjected to a slight TMP increase from 0.06 to 0.2 bar. This large reduction in the mean biofilm thickness is linked to the decrease in roughness and results in the formation of a compressed homogeneous biofilm (Fig. 5, 6). The change in the specific biofilm resistance was rather limited despite the large variation of biofilm thickness at low TMPs.

(b) when TMP increases from 0.2 to 0.9 bar; the biofilm was less compressible, as indicated by the minor change in biofilm thickness, which corresponded to the minor increase in specific resistance.

On the other hand, the physical compression of homogeneous biofilm structures ($Ra' \leq 0.2$) corresponded with its increase in specific resistance, similarly to the phase (b) of the compression of heterogeneous biofilms. Homogeneous biofilms formed under P limiting conditions were less compressible than heterogeneous biofilm structures as indicated by the lower change in relative thickness over 0.06-1.2 bar (Fig. 6). We suggest the limited physical compression of homogeneous biofilms is explained by its low roughness and a reduced initial

porosity. Modelling of biofilm compaction indicates that compacted biofilms are characterised by a reduced pore volume due to packing of the particulate materials, which in turn increases biofilm “stiffness” (Laspidou et al., 2014). For river water biofilms however, specific hydraulic resistance increased after 0.1 bar pressure despite no further variations in mean biofilm thickness (based on OCT imaging). We suggest that for the river biofilms, the increase in specific resistance results from the reorganisation of particles at spatial scale lower than the resolution of the OCT. During filtration of particle suspension, the micro-scale reorganisation of particles indeed leads to an increase in hydraulic resistances (McCarthy et al., 2002).

4.2 Reversible vs. irreversible compression—implication for hydraulic resistance

Heterogeneous biofilm structures ($Ra' > 0.2$) were irreversibly compressed, as supported by the partial recovery of initial thickness and hydraulic resistance following decreased TMP. Conversely, compression of homogeneous structures (P limiting biofilms with $Ra' < 0.2$) was fully reversible, as defined by the restoration of initial thickness and hydraulic resistance within range of pre-compressed values. For nutrient enriched heterogeneous biofilms, which were largely constituted by EPS, the irreversible compression of membrane biofilms was against the expectation that polymeric fouling layers are reversibly compressed (Fig. 4, 5).

4.2.1 Effect of EPS chemical composition on compression

Chemical composition of EPS can determine the physical structure and mechanical properties of a biofilm (Peterson et al., 2013, Desmond et al., 2018). During membrane ultrafiltration, Valladares Linares et al. (2016) suggested irreversible biofilm compression is due to chemical consolidation of the EPS. Chemical consolidation was defined as the interaction of neighbouring functional groups to induce bond formation (Valladares Linares et al., 2016). Therein, induction of new bonds, was proposed to cause the EPS to “stick” together, thereby preventing restoration of initial thickness. However, in the current study we demonstrate pre-compacted nutrient enriched biofilms (low polysaccharide concentration, high protein

concentration) had comparable reversible compression than P limiting biofilms (high polysaccharide concentration, low protein concentration). Thus, biofilms with comparable homogeneous structures but different compositions of EPS were reversibly compressible. We thus suggest EPS chemical composition is linked to reversible/irreversible compression of membrane biofilms by the structural function of EPS. Thereof, different compositions of EPS can lead to the formation of distinct physical structures (e.g., surface roughness), which are more susceptible to irreversible deformation.

4.2.2 Effect of physical structure compression

We suggest irreversible compression is due to deformation of surface heterogeneity (e.g., roughness). Supporting this claim, irreversible compression was only observed for biofilms with a large relative roughness ($Ra' > 0.2$). We suggest irreversible compression of heterogeneous biofilms is due to deformation of weak physical structures (e.g., surface filaments). This is supported by the observation that the irreversible decrease in biofilm thickness corresponded to an irreversible reduction in surface roughness (Fig. 4j, 5j). Under tangential flow streamers and surface roughness do in fact have lower structural strength than the homogeneous biofilm bulk (Stoodley et al., 2002). Furthermore, homogeneous biofilms, with low surface roughness ($Ra' \leq 0.2$), did not exhibit a significant change in surface roughness and recovered their thickness and hydraulic resistance following relaxation of TMP within range of initial values.

The irreversible increase in specific resistance suggests deformation of an independent variable of hydraulic resistance such as porosity. Numerical simulation of biofilm compression does indicate that compression can reduce void volume (Laspidou et al., 2014). In turn, an increase in specific resistance has also been numerically linked to reduced porosity following application of higher TMP (Tiller and Kwon, 1998). Valladares Linares et al. (2016) also suggested increase hydraulic resistance of irreversibly compressed biofilms is due to narrowing of

conduits of hydraulic passage (e.g., channels between EPS). Similarly, Fortunato et al. (2017) calculate a decreased porosity from OCT images, which corresponded with decrease permeate flux. In our study, the increase in hydraulic resistance and decrease in thickness following the application of higher TMPs did in fact lead to a reduction of areas of low OCT signal intensity by visual observation, suggested by Wagner et al. (2010) to represent volumetric voids. However, porosity quantification is limited in quantitative surety. Calculated porosity assumes that no signal is an absolute equivalent to void volume. An absence of signal can however result from a lot of other phenomenon: shadowing by particles, decreasing signal intensity with depth, inappropriate thresholding.

We demonstrate irreversible compression of membrane biofilms and increase hydraulic resistance. We suggest this is due to the reduction in the surface roughness of the biofilm and decrease pore volume required for hydraulic transport.

4.3 Practical implications

The “ideal” membrane biofilm formed during GDM filtration should have a heterogeneous physical structure (i.e., low hydraulic resistance) and be incompressible. Biofilms formed under river water or under enriched nutrient conditions had low hydraulic resistances (due to heterogeneous physical structures). But those biofilms were irreversibly compressed. Biofilms formed under P limiting conditions were reversibly compressible, but had a high hydraulic resistance during normal operation, thereby reducing their desirability. Increasing TMP during GDM filtration should thus be avoided due to the low mechanical strength of heterogeneous structures (e.g. roughness). Efforts to increase permeation in GDM systems should focus on increasing membrane area and/or heterogeneity of the biofilm for greater flux production (Klein et al., 2016).

5. Conclusions

- The initial physical structure of membrane biofilms developed during GDM filtration (0.06 bar pressure) governs its compression, relaxation and hydraulic response to increased TMPs.
- When exposed to increased TMPs, the roughness of the biofilms mainly governs its physical compression (change in thickness) while the biofilm's porosity determines its hydraulic responses, i.e., change in specific resistance.
- Compression of heterogeneous biofilm structures ($Ra' > 0.2$) formed during GDM ultrafiltration is phasic, involving:
 - When TMP increases from 0.06 to 0.2 bar: a large decrease in mean biofilm thickness/roughness that results in the formation of homogeneous structure. The reduction of roughness had little to no impact on hydraulic resistance, despite large variation in biofilm thickness, indicating their limited resistance to hydraulic flow
 - When TMP increases from 0.2 bar to 1.2 bar: a lower relative change in mean biofilm thickness, corresponding to the compression of the resulting homogeneous biofilm structure. The compression of heterogeneous biofilms was irreversible once the roughness is collapsed.
- Compression of homogeneous biofilm structures ($Ra' < 0.2$) was reversible and resulted in a corresponding change in the specific resistance over TMP.
- For practice, the application of higher permeate flux by way of increased water head (thereby TMP) during GDM filtration must be avoided due to the negative effect of biofilm compression on its hydraulic resistance.

6. Supplementary information

- Additional images of biofilm compression
- Video of reversible compression.

- 515 • Size exclusion chromatography validating biopolymeric composition of nutrient enriched
516 and P limiting membrane biofilms.

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References:

- BUGGE, T. V., JØRGENSEN, M. K., CHRISTENSEN, M. L. & KEIDING, K. 2012. Modeling cake buildup under TMP-step filtration in a membrane bioreactor: Cake compressibility is significant. *Water Research*, 46, 4330-4338.
- DERLON, N., GRÜTTER, A., BRANDENBERGER, F., SUTTER, A., KUHLLICKE, U., NEU, T. R. & MORGENROTH, E. 2016. The composition and compression of biofilms developed on ultrafiltration membranes determines biofilm hydraulic resistance. *Water Research*.
- DESMOND, P., BEST, J. P., MORGENROTH, E. & DERLON, N. 2018. Linking composition of extracellular polymeric substances (EPS) to the physical structure and hydraulic resistance of membrane biofilms. *Water Research*, 132, 211-221.
- DRESZER, C., FLEMMING, H. C., ZWIJNENBURG, A., KRUIHOF, J. C. & VROUWENVELDER, J. S. 2014. Impact of biofilm accumulation on transmembrane and feed channel pressure drop: effects of crossflow velocity, feed spacer and biodegradable nutrient. *Water Res*, 50, 200-11.
- FOLEY, G. 2006. A review of factors affecting filter cake properties in dead-end microfiltration of microbial suspensions. *Journal of Membrane Science*, 274, 38-46.
- FOLEY, G. 2013. *Membrane Filtration: A Problem Solving Approach with MATLAB*. Cambridge University Press.
- FORTUNATO, L., QAMAR, A., WANG, Y., JEONG, S. & LEIKNES, T. 2017. In-situ assessment of biofilm formation in submerged membrane system using optical coherence tomography and computational fluid dynamics. *Journal of Membrane Science*, 521, 84-94.
- HAMMES, F. A. & EGLI, T. 2005. New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum. *Environmental science & technology*, 39, 3289-3294.
- HEFFERNAN, R., SEMIAO, A. J. C., DESMOND, P., CAO, H., SAFARI, A., HABIMANA, O. & CASEY, E. 2013. Disinfection of a polyamide nanofiltration membrane using ethanol. *Journal of Membrane Science*, 448, 170-179.
- JØRGENSEN, M. K., KEIDING, K. & CHRISTENSEN, M. L. 2014. On the reversibility of cake buildup and compression in a membrane bioreactor. *Journal of Membrane Science*, 455, 152-161.
- KLEIN, T., ZIHLMANN, D., DERLON, N., ISAACSON, C., SZIVAK, I., WEISSBRODT, D. G. & PRONK, W. 2016. Biological control of biofilms on membranes by metazoans. *Water Research*, 88, 20-29.
- LASPIDOU, C. S., SPYROU, L. A., ARAVAS, N. & RITTMANN, B. E. 2014. Material modeling of biofilm mechanical properties. *Math Biosci*, 251, 11-5.
- LE, C., KUNACHEVA, C. & STUCKEY, D. C. 2016. "Protein" Measurement in Biological Wastewater Treatment Systems: A Critical Evaluation. *Environmental science & technology*, 50, 3074-3081.
- McCarthy, A.A., O'Shea, D.G., Murray, N.T., Walsh, P.K., Foley, G., 1998. Effect of cell morphology on dead-end filtration of the dimorphic yeast *Kluyveromyces marxianus* var. *Marxianus* NRRLy2415. *Biotechnol. Prog.* 14, 279e285.
- MCCARTHY, A., GILBOY, P., WALSH, P. & FOLEY, G. 1999. Characterisation of cake compressibility in dead-end microfiltration of microbial suspensions. *Chemical Engineering Communications*, 173, 79-90.
- MCCARTHY, A. A., O'SHEA, D. G., MURRAY, N. T., WALSH, P. K. & FOLEY, G. 1998. Effect of Cell Morphology on Dead-End Filtration of the Dimorphic Yeast *Kluyveromyces marxianus* Var. *marxianus* NRRLy2415. *Biotechnology Progress*, 14, 279-285.
- Murga, R., Stewart, P.S., Daly, D., 1995. Quantitative-analysis of biofilm thickness variability. *Biotechnol. Bioeng.* 45, 503e510.
- PETERSON, B. W., VAN DER MEI, H. C., SJOLLEMA, J., BUSSCHER, H. J. & SHARMA, P. K. 2013. A distinguishable role of eDNA in the viscoelastic relaxation of biofilms. *MBio*, 4, e00497-13.

- POORASGARI, E., VISTISEN BUGGE, T., LYKKEGAARD CHRISTENSEN, M. & KOUSTRUP
JØRGENSEN, M. 2015. Compressibility of fouling layers in membrane bioreactors. *Journal
of Membrane Science*, 475, 65-70.
- STOODLEY, P., CARGO, R., RUPP, C. J., WILSON, S. & KLAPPER, I. 2002. Biofilm material
properties as related to shear-induced deformation and detachment phenomena. *Journal of
Industrial Microbiology & Biotechnology*, 29, 361-367.
- TILLER, F. M. & KWON, J. H. 1998. Role of porosity in filtration: XIII. Behavior of highly
compactible cakes. *AIChE Journal*, 44, 2159-2167.
- VALLADARES LINARES, R., WEXLER, A., BUCS, S. S., DRESZER, C., ZWIJNENBURG, A.,
FLEMMING, H.-C., KRUTHOF, J. & VROUWENVELDER, J. 2015. Compaction and
relaxation of biofilms. *Desalination and Water Treatment*, 1-13.
- VALLADARES LINARES, R., WEXLER, A. D., BUCS, S. S., DRESZER, C., ZWIJNENBURG, A.,
FLEMMING, H. C., KRUTHOF, J. C. & VROUWENVELDER, J. S. 2016. Compaction and
relaxation of biofilms. *Desalination and Water Treatment*, 57, 12902-12914.
- WAGNER, M., TAHERZADEH, D., HAISCH, C. & HORN, H. 2010. Investigation of the mesoscale
structure and volumetric features of biofilms using optical coherence tomography.
Biotechnology and Bioengineering, n/a-n/a.