Cross flow frequency determines the physical structure and cohesion of membrane biofilms developed during gravity-driven membrane ultrafiltration of river water: Implication for hydraulic resistance

Nicolas Derlon a,*,1, Peter Desmond a,b, Patrick A. Rühs d, Eberhard Morgenroth a,c

a Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600, Dübendorf, Switzerland
b Institute of Environmental Engineering, RWTH Aachen University, D-52074, Aachen, Germany
c ETH Zürich, Institute of Environmental Engineering, 8093, Zürich, Switzerland
d Complex Materials, Department of Material Science, ETH Zürich, 8093, Zürich, Switzerland

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ABSTRACT

We evaluated how intermittent shear influences the physical structure, material properties and hydraulic resistance of membrane biofilms developed during gravity-driven ultrafiltration of river water, with the ultimate goal of increasing the filtration performances. Our results indicate intermittent shear helps slowing-down the flux decline but does not help to increase the level of stabilisation of the permeate flux. After several weeks, the biofilms exposed to different shear regimes were indeed characterised by similar hydraulic resistance. But the characteristic time to achieve a stable flux increased from 7 d to 25d when increasing the shear frequency. Also, most of the hydraulic resistance (up to 95%) was governed by the base layer that remained attached after erosion tests. With increasing exposure to shear conditions, the biofilms became more cohesive and more elastic, thus resisting better to cross flow conditions. Overall, our results demonstrate that engineering membrane biofilms with a desired permeability is not feasible using intermittent shear due to significant adaptability of the biofilms to their hydraulic environment.

1. Introduction

Gravity-driven membrane (GDM) ultrafiltration is a relevant alternative to conventional ultrafiltration for small-scale and decentralized applications of wastewater treatment and drinking water production systems, due to its low energy- and chemical-demand [33]. GDM ultrafiltration thus received more and more attention during the past few years, and various GDM-based technologies were developed: decentralized production of drinking water in developing countries [31], recycling of greywater within toilet or building [26], pre-treatment of seawater before reverse-osmosis [36] or treatment of rainwaters collected from roofs [15]. Whatever the type of applications, the design of GDM filters remains however hindered by the low values of permeate flux achieved in those systems (< 20 L m⁻² h⁻¹) [33]. Currently, a main challenge is therefore to increase the permeate flux, but with a minimal amount of maintenance.

GDM filters are operated in dead-end mode, usually without backwashing, chemical cleaning, or cross-flow. The development of a biofilm on membrane surfaces is tolerated – in fact it is desirable. Tolerating biofilm formation on membrane surfaces allows operating the GDM filters at a low but stable permeate flux for several months, thus significantly reducing the maintenance needs [33]. Sustained filtration conditions are achieved without backwashing, air sparging or chemical cleaning, indicating that these fouling control measures can be eliminated [30]. However, the stable flux values reported in literature are usually rather low: 4–20 L m⁻² h⁻¹ during river water treatment [10,34], <5 L m⁻² h⁻¹ during rain water treatment [15], 3.6–7.3 L m⁻² h⁻¹ during sea water treatment [1], 1–4.5 L m⁻² h⁻¹ during treatment of municipal wastewater [20,28], or as low as 0.5–3 L m⁻² h⁻¹ during grey-water treatment [34]. Few recent studies however indicated that the stable flux can be increased with some control strategies: permeate flux was for example more than doubled by application of air scouring/relaxation during treatment of municipal WW [20] or surface water [30]. However [20], also noticed the specific biofilm resistance (m⁻¹/μm biofilm thickness)

a Corresponding author.
E-mail address: nicolas.derlon@eawag.ch (N. Derlon).
1 Nicolas Derlon and Peter Desmond contributed equally to this study. See CRediT author statement for more details.

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increased with air scouring due to compaction of the biofilm. A main question is therefore whether long-term operation (several months/years) at a higher stable flux can be sustained with a minimal control of the biofilm formation (e.g., with an intermittent cross-low)? A main challenge is in engineering a biofilm with an increased permeability maintained over long-term (several weeks).

Biofilms developed during GDM ultrafiltration are characterised by the stratification of their cohesion and in turn of their hydraulic resistance [11]. The cohesion of the biofilms increases towards their base, so does their hydraulic resistance [11]. As demonstrated over short-term erosion tests, the removal of the loose top layers of the biofilms help reducing the hydraulic resistance, and thus increasing the permeate flux [11]. However, it is unclear how a regular removal of those top layers would impact the biofilm permeability over long-term. In the case of biofilms developed on solid substrata, higher shear conditions may for example result in the formation of more resistant biofilms [4]. Based on this observation, we hypothesize that membrane biofilms may adapt to intermittent shear conditions and get more resistant over time. Also, the permeate flux does not improve proportionally to the amount of biofilm actually detached [11]. A cohesive base layer resists detachment and dominates the overall hydraulic resistance [11,23]. If the base layer of the biofilm is very cohesive and represents most of the hydraulic resistance of the biofilms, we then hypothesize that the removal of the top layer might only allow operation at a slightly higher permeate flux. Such shearing of the biofilm could be performed intermittently (e.g., weekly) and with a minimal shear force, due to the very low cohesiveness of the top layers [11], indeed reported top layers of membrane biofilms can be efficiently removed at a shear stress as low as 1 Pa. A main objective of the current study was thus to better understand the link between the frequency of hydraulic shearing and the level of flux stabilisation.

If shear conditions are intermittently applied during GDM filtration, another relevant question is how the long-term exposure of the membrane biofilms to intermittent shear conditions influence their formation and permeability? Dead-end operation may, for example, limit the availability of nutrient at the base of the biofilm, thus promoting the secretion of extracellular polymeric substances (EPS) and especially of polysaccharides [11]. The higher the density of polysaccharide within the biofilm, the lower its permeability [11]. If so, intermittent shear could help increasing the availability of nutrient at the biofilm base, thus improving its permeability. But intermittent shear could also compress the residual biofilms, and in turn promote the growth of homogeneous and cohesive biofilms with a reduced permeability. During GDM filtration of synthetic grey-water, high aeration shear led to a thinner but denser fouling layer with a higher EPS content, ultimately leading to an increase of its hydraulic resistance from 10-10 to 30-10-12 m1 [12] A second objective of the present study will be to evaluate the response of GDM biofilms intermittently exposed to shear conditions, in terms of physical structure, mechanical properties and permeability.

The main purpose of this study was to evaluate how the application of intermittent shear conditions influence the physical structure and mechanical properties of membrane biofilms and in turn their hydraulic resistance, during GDM filtration of river water. Biofilms were grown in parallel Membrane Fouling Simulators (MFS) operated with different frequencies of hydraulic shear: (a) Dead-end mode (no cross-flow), (b) weekly cross-flow (c), daily cross-flow, and (d) 30 min cross-flow. The weekly structures of the different biofilms were assessed using optical coherence tomography. The cohesion of the different biofilms was assessed by submitting the biofilms to erosion tests. Oscillatory rheology was used to assess the biofilm’s material characteristics (e.g., yield stress, elasticity).

2. Materials and methods

2.1. Experimental set-up

2.1.1. Membrane filtration system

A programmable automated membrane filtration system (MMS AG, Switzerland) was used for the online analysis of biofilm development and hydraulic resistance. The membrane filtration system consisted of 4 independent lines, each equipped with a membrane fouling simulator (MFS) placed in parallel (STT products, The Netherlands), gear pump, pressure sensors and regulators to allow setting of a constant TMP (Fig. 1). This system allows operating the biofilm-membrane composite systems under dead-end conditions only, or with intermittent or continuous cross-flow. Under dead-end conditions only, the permeate valve (Fig. 1e) is open while the retentate solenoid valve (Fig. 1g) is closed. The gear pump is controlled to apply a given trans-membrane pressure set by the user. Intermittent cross-flow is triggered by closing the permeate valve (Fig. 1e) while opening the retentate solenoid valve (Fig. 1g). The gear pump is in this case controlled to apply a given flow velocity at the surface of membrane, to achieve a targeted shear stress.

The membrane fouling simulators had length x width dimensions of 250 × 80 mm, and a channel height of 1.5 mm. Such channel height is sufficient to allow biofilm formation in the range of thicknesses usually reported in literature during GDM filtration of river waters, i.e., below 500 μm [8-10]. The inlet was branched into 5 distribution channels to ensure even flow distribution over the width of the module. Due to the very permeate flux, the water velocity under dead-end condition is very slow. Therefore, feed-channel pressure drop can be neglected. During dead-end filtration, a trans-membrane pressure of 0.1 bar was maintained.

Membrane biofilms were grown under 4 distinct growth-conditions: (1) dead-end only (label: “dead-end”), (2) dead-end with weekly hydraulic shear (label: “weekly shear”), (3) dead-end with daily shear (label: “daily shear”) and (4) dead-end with hydraulic shear every 30 min (label: 30 min shear). Three different runs were performed to assess the reproducibility of our observations. Shear conditions were always applied for 5 min at a shear stress value of 1 Pa (calculated based on the dimensions of empty flow chambers). Our previous investigation on the permeability of three different types of membrane biofilms had evidence stratification of their hydraulic resistance [12]. Our results had consistently shown the top biofilm layer can be removed at 1 Pa and resulted in a partial recovery of permeate flux. We therefore selected a shear stress of 1 Pa to try engineering biofilm permeability over long term (several weeks).

2.1.2. Membrane preparation

Ultrafiltration membranes made of polyethersulfone with a nominal cut-off of 150 kDa were used in this study (UP150, Microdyn Nadir, Wiesbaden, Germany). Membrane coupons of 0.02 m2 were cut, washed in 40% ethanol for 1 h and then rinsed in nanopore water, as described by Ref. [21].

2.1.3. Feed water composition

Water from Chriesbach river (Dübendorf, Switzerland (47 24′16.3″ N 8′36′31.8″)E)was used as feed water. The composition of the Chriesbach river water is typically as follow: Total organic carbon: 2.5-3 mg C L-1, particulate organic carbon: 0.1-1.5 mg C L-1, assimilable organic carbon: 0.1-1 mg C L-1, biopolymers (measured with SEC-OCD): 30-100 μg C L-1 [9].

2.2. Permeate flux and hydraulic resistances

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 m-3). The following equations were used for the calculations of the permeate flux

\[
\text{Flux} = \frac{\text{Volume}}{\text{Area} \times \text{Time}}
\]

\[
\text{Resistance} = \frac{\text{Pressure}}{\text{Flux}}
\]
(J, L m$^{-2}$ h$^{-1}$):

\[ J = \frac{\delta V}{A \delta t} \quad (1) \]

where $\Delta V$ is the change in permeate volume (L), $A$ is the filtration area (m$^2$), $\Delta t$ is the duration of permeate collection (hrs). The characteristic time to achieve “flux stabilisation” was calculated for each experimental run and then correlated to the different shear conditions. The characteristic time to achieve flux stabilisation was defined as the time to reach (1) a permeate flux value equal to the average flux $\pm$ 10% and (2) a flux variation of less than 0.5 L m$^{-2}$ h$^{-1}$ between two successive days. The average flux values were calculated over the last 7 days of filtration for run #2 and #3. For run #1, the average flux was calculated between day #23 and #29 due to operational problems on the system starting from day #29 that ultimately deteriorated the flux values.

The total hydraulic resistance of the fouled membrane ($R_{\text{total}}$ in m$^{-1}$) was calculated as:

\[ R_{\text{total}} = \frac{\text{TMP}}{\eta J} \quad (2) \]

where TMP is the transmembrane pressure (Pa) and $\eta$ is the dynamic viscosity of the permeate at a given temperature (Pa s) and $J$ is the permeate flux. The biofilm hydraulic resistance ($R_{\text{biofilm}}$) was calculated according to eq. (3):

\[ R_{\text{biofilm}} = R_{\text{total}} - R_{\text{membrane}} \quad (3) \]

where $R_{\text{membrane}}$ is the intrinsic resistance of the clean membrane measured for a period of 24 h with nanopure water prior to bacteria inoculation. All data accessible here: data.eawag.ch/dataset/publication_derlonetal_jms_2022.

2.3. Biofilm characterization

2.3.1. Biofilm physical structure

The change in the physical structure of the biofilms subjected to different shear conditions was evaluated using optical coherence tomography (OCT). An OCT system with a central light source wavelength of 930 nm was used (Spectral Domain, Thorlabs GmbH, Dachau, Germany). The movable scanning lens of the OCT was fixed on a rack to allow easy in-situ scanning of the different biofilms developed in the parallel MFS. A refractive index of 1.33 was set during image acquisition. The OCT system was coupled to the automated filtration system for in situ monitoring of the biofilms physical structures (Fig. 1). 10–12 images at viewports located at the front and back of the membrane fouling simulators were recorded. Images were analysed using a specific Matlab script [10], therein quantified with respect to the biofilm thickness and relative roughness.

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. The average thickness was calculated as the arithmetic mean of the A-scan thicknesses:

\[ L_F = \frac{1}{N} \sum_{i=1}^{N} L_{F,i} \quad (4) \]

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.

The relative roughness of the biofilm was calculated using the following equation:

\[ R_\star = \frac{1}{L_F} \sum_{i=1}^{N} \left| L_{F,i} - L_F \right| \quad (5) \]
films with a smooth surface and only a few variations from the mean where...

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biofilm thickness are characterised with $R^2$ values close to 0. The higher the roughness coefficient, the larger the heterogeneities of the biofilm’s surface [10]. All data accessible here: data.eawag.ch/dataset/publication_derlonetal_jms_2022.

2.3.2. Stratification of the biofilm cohesion

The different membrane biofilms were subjected to erosion tests to determine to what extent exposure to different shear frequencies during the growth period influences their cohesion and in turn of their hydraulic resistance. Erosion tests were conducted at the end of run #2 and #3. Erosion tests consisted in gradually increasing the hydraulic shear stress from 0 to 2.8 Pa with an exposure time of 5 min. During those erosion tests, the permeate valve (Fig. 1e) was closed to prevent permeation and consequent compaction of the biofilm. The retentate solenoid valve (Fig. 1g) is open and the frequency of the gear pump is modulated to vary the cross-flow rate. The hydraulic resistance of the residual biofilm was measured after each step-wise increase of the shear stress, by closing the cross-flow valve and opening the permeation valve (TMP controlled at 0.1 Bar, similar to the TMP applied during the growth period). The permeate flux after each erosion step was measured for 10 min. All data accessible under data.eawag.ch/dataset/publication_derlonetal_jms_2022.

2.3.3. Biofilm harvesting

Membrane biofilms were scraped from the membrane surface using a cell scraper and then 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 surface. The collected biomass was re-suspended in the washings of the 30 mL 0.1 M NaCl solution.

2.3.4. Total and residual biofilm mass

Total and residual biomass was measured as total organic carbon (TOC) and refers to the total mass or the mass of biofilm remaining on the surface of the membrane following exposure to hydraulic shear stress. Biofilm mass is expressed as mgC m$^{-2}$. For measurement of their Total TOC, biofilms were first scraped from the membrane surface using a cell scraper and collected into sterile glass beakers. A volume of 30 mL 0.1 M NaCl solution was used to rinse the remaining biofilm from the membrane surface. The collected biomass was then re-suspended in the washings of the 30 mL 0.1 M NaCl solution. This solution was then homogenized using an ultra-turrax treatment (1 min at 10 000 rpm) prior TOC measurement. Detached biomass was also collected during erosion experiments and analysed with respect to TOC according to the same procedure. The residual biofilms that remained attached onto the membrane surface after erosion at 2.8 Pa was harvested using a cell scraper. The TOC of the detached biomass was summed to the TOC of the residual biofilm after erosion at 2.8 Pa to determine total biofilm mass.

The TOC of the membrane biofilms was measured using an automatic total organic carbon analyser (TOC-V, Shimadzu, Japan). The suspensions of collected biofilms were mixed during the injection into the TOC analyser using a magnetic stirrer (Polytron PT 3100, Kinematica, Bohemia (NY), USA) (2 min with 15,000 rpm), to avoid sedimentation and ensure the quality of the measurement. Inorganic carbon from the river water can contribute to a large fraction of the biofilm mass due to its continuous accumulation on the membrane surface [8]. However, the inorganic carbon fraction of the GDM biofilms represents only a very small volume of biofilm structure, and does not increase its hydraulic resistance [8]. Engineering the biofilm permeability can be successfully achieved only at high dosage of inorganics [6,24]. Therefore, the inorganic carbon content of the biofilms was not measured in this study. All data accessible here: data.eawag.ch/dataset/publication_derlonetal_jms_2022.

2.3.5. Extraction of the extracellular-polymeric substances (EPS)

Extracellular-polymeric Substances (EPS) were extracted from the resuspended biofilm by sonication. Sonication was selected over existing chemical methods to limit contamination of sample fractions due to sensitivity of subsequent analytical methods. Three sequential sonication were performed to maximize EPS extraction while avoiding cell lysis. Lysis of the cells was evaluated for each extraction through quantification of the soluble ATP release. The harvested biofilm suspensions were placed in an ice bath and sonicated for 120 s with 10 s rest intervals using a needle sonicator (Sonopuls HD 3200, Bandelin, Berlin, Germany). This was repeated three times to maximize the EPS extraction. Thereafter, homogenized samples were centrifuged at 5000 rpm at 20 °C for 15 min (Unicen MR, Herolab, Germany) to separate the EPS from the cells. Centrifugation step was repeated to ensure full separation. 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), EPS analysis (Polysaccharide/Protein). No cell lysis was detected through the measurement of the soluble ATP during this extraction procedure.

2.3.6. Total polysaccharide quantification

The concentrations of extracted polysaccharides in the EPS extract were determined by the Anthrone method. Anthrone was dissolved with a UV–Vis spectrophotometer (Infinite M200, TECAN, Männedorf, Switzerland) at a wavelength of 625 nm. Glucose was used as a standard. All data accessible under ***final link to be inserted here prior publication***. No detailed characterisation of the different polysaccharides (e.g., functional group, molecular size, etc.) was performed.

2.3.7. Total protein quantification

The concentrations of total proteins in the EPS extract 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) or for higher concentrations were used for the characterization of the different biofilms. 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 albumin (BSA) was used as a standard. All data accessible here: data.eawag.ch/dataset/publication_derlonetal_jms_2022.

2.3.8. Rheological properties of membrane biofilms

Plate rheology was used to identify how exposure to different shear frequencies influence the rheological properties of the membrane biofilms. The different biofilms were first scraped off the membrane and placed on the lower plate. All measurements were performed at 20 °C in triplicate using a MCR 501 rheometer (Anton Paar, Austria) equipped with a 25-mm diameter plate-plate configuration set to a gap size of 0.5 mm. The upper and lower plates were both stainless steel. The upper plate was slowly lowered onto the biofilm. A rest period of 5 min was then applied prior to measurements. To minimize evaporation a solvent trap containing moist sponges was placed over the entire plate-plate flow geometry. The yield stress values were determined graphically from the flow curves. In simple shear mode, the yield stress was extracted from the intersection of the shear stress curve with the y-axis at low shear rates. To determine the viscoelastic properties, the biofilms were subjected to oscillatory flow at a constant frequency. All data accessible here: data.eawag.ch/dataset/publication_derlonetal_jms_2022.
3. Results

3.1. How does the shear frequency influence filtration performance?

The change in the permeate flux was monitored over 50 days for each experimental condition (Fig. 2). The frequency of shear stress influenced the rate at which the flux declined (i.e., the time requested to reach a stable flux), but not the level of flux stabilisation. The more frequent the shear events, the slower the decline of the flux and thus the longer the time needed to reach a stable flux. MFS operated under dead-end conditions were associated with the quickest flux decline and flux stabilisation was thus observed after 6.6 ± 1.8 d of operation (Fig. 3). Increasing the frequency of shear stress slowed down the flux decline. MFS operated under 30 min frequency of hydraulic shear stress exhibited flux stabilisation after 26 ± 6 d of operation (Fig. 3). But over long-term (few days to weeks depending on the system), flux stabilisation within a comparable range of 12–15 L m⁻² h⁻¹ was observed for all experimental lines. The weekly and daily shear were on the other hand associated with characteristics times of 7 ± 0.8 d and 11 ± 2 d to reach flux stabilisation (Fig. 3). Thus, efficiently controlling the biofilm formation by mean of intermittent shear is time-dependant, as increasing the exposure to shear conditions successfully reduces biofilms formation during the first days of filtration but not over long-term. Despite exposure to very different shear frequencies, all the biofilms thus had similar hydraulic resistances (Fig. 2, e-h). Average hydraulic resistances of 2.1e⁻¹² to 2.4e⁻¹² m⁻¹ were measured for the different biofilms.

3.2. Does the shear frequency influence the biofilm concentration and composition?

The influence of the shear frequency on the biofilm concentration and composition (in terms of polysaccharides and proteins) was evaluated at the end of each experiment (Fig. 4a). The shear frequency did not influence the biofilm accumulation over long-term (several weeks) (Fig. 4a). Similar biofilm concentrations of 1425 ± 25 mg C m⁻², 1225 ± 31 mg C m⁻², 1437 ± 150 mg C m⁻² and 1462 ± 100 mg C m⁻² (n = 2) were measured for the biofilms grown under dead-end conditions, weekly, daily and 30 min exposures to hydraulic shear stress, respectively (Fig. 3a).

Also, no effect of the shear frequency on the protein content of the biofilms was noticed (Fig. 4c). The concentrations of extracted proteins represented around 250 mg BSA m⁻² for all the different biofilms. However, a noticeable effect of the shear conditions on the concentrations of extracted polysaccharides was observed (Fig. 4b). The larger the shear frequency, the larger the concentration of extracted polysaccharides from the biofilms. Biofilms grown under dead-end conditions for example yielded 516 ± 48 mgGlucose m⁻², while biofilm exposed to shear conditions every 30 min yielded up to 1048 ± 133 mgGlucose m⁻². Biofilms exposed to weekly and daily shear conditions were, on the other hand, characterised by intermediary polysaccharides concentrations of 381 ± 48 mgGlucose m⁻², 792 ± 47 mgGlucose m⁻².

3.3. How does the shear frequency influence the morphology and physical structure of the membrane biofilms?

Quantification of the mean biofilm thickness and relative roughness confirmed the link between the biofilm structure and the exposure to different frequencies of hydraulic shear (Fig. 5). Overall, all biofilms had a similar thickness (no statistical difference) but were associated with different roughness coefficients. At day 30, all biofilms were associated with an average thickness of around 200 μm, irrespective of the shear frequency.
conditions applied during their growth. The average thickness of the different biofilms measured after 30d were however not statistically different, i.e., the dead-end biofilms were not thicker than the biofilms exposed to intermittent shear conditions. Only the dead-end biofilms of run #3 got thicker than the other biofilms between 30d and 40d of filtration.

Quantification of relative surface roughness revealed on the other hand that biofilms grown under frequent shear conditions (daily and 30 min) had a lower surface roughness than the biofilms formed under less frequent hydraulic shear (dead-end, weekly) (Fig. 5). Biofilms grown under dead-end conditions were characterised by a very large relative roughness, ranging from 0.2 to 0.8 (Fig. 5e). On the contrary, biofilms

Fig. 4. Influent of shear frequency on composition of membrane biofilms with respect to (a) the overall total organic carbon (mg C m⁻²), (b) the extracted total polysaccharides (mg Glucose m⁻²) and (c) extracted total proteins (mg BSA m⁻²).

Fig. 5. Change in the physical structure of membrane biofilms exposed to different frequencies of hydraulic shear.

Fig. 6. Rheology of membrane biofilm: Comparison of yield stress and viscoelasticity of biofilm formed under dead-end operation to weekly, daily and 30 min applications of hydraulic shear stress.
exposed to very frequent shear conditions were associated with much lower relative roughness values of 0.2–0.3 (Fig. 5h). Also, it can be noticed that the relative roughness coefficient tends to decrease over a longer period of exposure to shear conditions.

3.4. How does the shear frequency influence the mechanical properties of the biofilm?

3.4.1. Biofilm rheological properties

The yield stress and elastic modulus of the different biofilms were measured using simple shear and oscillatory rheology (Fig. 6). Tests performed in simple shear mode allow determining the yield stress (Fig. 6, a-d), whereas oscillatory measurements allow determining the yield point and the viscoelasticity (G′, G″) of the biofilms.

Overall, the more frequent the shear conditions, the larger the value of yield stress of the biofilms. Values of yield stress increased with more frequent exposure to shear conditions: weekly shear: 262 Pa, daily shear: 974 Pa and 30 min shear: 2605 Pa. Dead-end operation resulted in the formation of membrane biofilms with a yield stress of 411 Pa, thus slightly larger than of weekly sheared biofilm but lower than of daily and 30 min sheared biofilms. The observed trend in yield stress of the different biofilms is also reflected by their viscoelastic properties (Fig. 6, e-h). The dynamic yield point, defined as the crossover between G′ and G″, also increased with an increasing exposure to hydraulic shear conditions. Yield points lower than 1000 Pa were measured for biofilms grown under dead-end or weekly hydraulic shear conditions. Much higher values, of around 2000 Pa and 30'000 Pa, were measured for biofilms frequently exposed to shear conditions (daily and 30 min shear).

3.4.2. Biofilm cohesion and link with hydraulic resistance

At the end of the filtration experiments, the membrane biofilms were then submitted to erosion tests to evaluate how exposure to different frequencies of hydraulic shear influence their cohesion and hydraulic resistance (Fig. 7). Results of the erosion tests confirmed that regular exposure to shear conditions reinforces the cohesion of the biofilm. The more frequent the shear conditions during the formation of the biofilms, the more cohesive the biofilms (Fig. 7). During the erosion tests, the biofilms grown under dead-end only or weekly shear conditions were almost or fully detached. The fraction of the “dead-end” biofilms that resisted to erosion at 2.8 Pa represented only 38 ± 9% of its total mass (Fig. 7e). But biofilms that were frequently exposed to shear conditions during their growth were only partially detached during the erosion tests: the fraction of the “daily sheared” and “30 min sheared” biofilms that resisted erosion at 2.8Pa represented 63 ± 11 and 65 ± 7%, respectively (Fig. 7f and h).

The hydraulic resistance of the residual biofilms did not increase proportionally to the reduction in the residual biofilm mass observed during the erosion tests (Fig. 7 a to d). The hydraulic resistance of the biofilms grown under dead-end, daily shear and 30 min shear exposure remained stable during the erosion tests, despite a significant (-70%) or partial (-50%) biofilm removal. Only the biofilms exposed to weekly shear conditions, that were fully detached, exhibited a significant reduction of their hydraulic resistance following the erosion tests: from around 2.0·10^{12} m^{-1} to 1.0·10^{12} m^{-1}.

4. Discussion

4.1. Intermittent exposure to shear conditions increases the cohesion of membrane biofilms

A main result of our study is that increasing exposure to shear conditions, even intermittently, results in a significant change in the biofilms’ material properties. This change in the biofilms’ material properties is indicated by both their long-term accumulated mass, their rheological properties and the results of the erosion tests. When exposed more frequently to shear conditions, the biofilms become more elastic and more cohesive. The yield stress values of the biofilms increased from <500 Pa to more than 2'500 Pa, indicating these biofilms can sustain higher shear stress before being irreversibly deformed (Fig. 6). Also, the biofilms yield point increased from around 1’000 to 30’000 Pa, indicating an increase in their elasticity (Fig. 6). As a result of the change in their mechanical properties, biofilms that were frequently exposed to shear conditions were only partially detached during erosion tests (Fig. 7g–h). On the contrary, the biofilms that were not or weekly exposed to shear conditions were almost fully detached during the erosion tests (Fig. 7e–f). The more frequent the exposure to shear conditions, the larger the fraction of the biofilms that resisted to erosion at high shear. Laboratory observations demonstrated the ability of biofilms to adapt to changing shear stress environments and their versatility [35]. In the case of biofilms developed on solid substrata, continuous exposure to higher shear conditions may result in the formation of mechanically more stable biofilm [5]. Reinforcement of the mechanical stability of biofilm exposed to intermittent starvation/relaxation/erosion periods was recently reported for membrane biofilms [7]. The same phenomenon was also observed on full-scale plant (nanofiltration

![Fig. 7. Change in the hydraulic resistance (first row) and residual biofilm mass (in mgC.m^{-2}) of the different biofilms submitted to two distinct erosion tests (shear stress gradually increased from 0 to 2.8 Pa).](image-url)
systems) due to combined biofouling and scaling [3]. In our study, such response was observed for biofilms exposed to intermittent shear conditions only. Even a weekly exposure to shear for several minutes triggered such a reinforcement of the membrane biofilm. A main question is what mechanisms govern the mechanical response of membrane biofilms, when intermittently exposed to shear conditions?

Both biotic and abiotic mechanisms can result in such change in the biofilms material properties when exposed to intermittent shear conditions. A first explanation is a metabolic response of the bacteria. Biofilms can modulate their strength in response to their physical environment. Such adaptation can occur either via an increased production of EPS [2] or via a change in the type of EPS that are secreted [19]. In our study, an increased polysaccharide concentration was measured via colorimetric assays in response to an increasing exposure to shear conditions (Fig. 4). Despite colorimetric assays provide semi-quantitative measurements of polysaccharides only [27], those assays offer relevant insights about the composition of biofilms. Our results thus suggest that increasing exposure to intermittent shear conditions results in an increased secretion of polysaccharides (Fig. 3b). The polysaccharide concentration of “30 min shear” biofilms was for example two times larger than the one of the “dead-end” biofilms (1048 ± 133 vs. 516 ± 48 mg glucose m⁻², respectively). Similar results were reported in literature [14, 37]. Higher polysaccharide content was observed in the EPS extracts of biofilms grown at high shear rate condition, while a lower protein content was observed [37, 14] also reported higher polysaccharides contents in the case of biofilms grown on microfiltration membrane when increasing the shear rate. In addition to the overall amounts of polysaccharides produced, the type of EPS that are secreted might also influence the biofilm cohesion. Higher shear forces selects for the EPS with higher cohesion forces, while those that cannot stick to the matrix were washed away [18]. However, in our study, no characterisation of the different polysaccharides was performed. No effect on the production of proteins was on the other hand quantified (Fig. 3c). Many mechanisms influence the protein content found in the EPS matrix of the biofilms: decay of bacterial cells, enzyme production, presence of Fimbria, etc. It is not entirely clear why we did not observe any effect of intermittent shear on the protein content. One hypothesis is that biofilms were exposed to a similar organic substrate loading (as a result of similar flux values), resulting in similar amounts of bacterial cells, and ultimately in similar protein production rates among the different biofilms.

Another mechanism that may explain the biofilm’s consolidation is the reorganisation of the polymer network. Biofilms exposed to shear conditions experience significant compression by the fluid [32]. As demonstrated for drinking water biofilms, the compactness of the EPS matrix increases with hydrodynamic stress [29]. When the EPS matrix is compressed, the mesh size of the polymeric network decreases, thus increasing the number of contact points between the polymers and ultimately the biofilm’s cohesiveness [17, 29]. The entanglement of the biopolymer contributes to the matrix’s stability [25]. Extrusion of the water from the biofilms due to compression thus makes the biofilms’ structures more rigid [32]. Such rearrangement of the EPS matrix during intermittent shear conditions might also contribute to the consolidation of the biofilms observed in our study.

4.2. Biofilm consolidation reduced biofilm hydraulic permeability

Another main finding of our study is that intermittent exposure to shear condition influences the rate of flux decline, but not the final level of flux stabilisation. After few weeks, all biofilms had a similar hydraulic resistance despite very different exposures to shear conditions during their growth (Fig. 2). But the characteristic time required to reach this stable flux value increased with an increasing frequency of exposure to shear conditions, e.g., from around 1 week to 1 month for biofilms exposed to weekly and 30 min shear conditions, respectively (Fig. 3). A main question is why different shear conditions resulted in the formation of biofilms with similar hydraulic resistance?

A first factor influencing the hydraulic resistance of membrane biofilms is their meso-scale physical structure. In our study, increasing exposure of the biofilms to shear conditions did not result in the formation of thinner biofilms, despite intuitive (Fig. 5). Overall, all biofilms had a similar average thickness after 30d of operation (no statistical difference) (Fig. 5), and also a similar accumulated mass (Fig. 4). This suggests that all biofilms had, in average, a similar density. An increasing density reduces the porosity of the biofilms, and eventually their permeability [23]. In our study, the different biofilms had similar density and were ultimately associated with similar hydraulic resistances and flux values.

We also noticed a large contribution of the biofilm’s base layer to the overall hydraulic resistance (Fig. 7). Biofilms that grew under “dead-end” or “weekly shear conditions” were largely (60%) or fully detached during the erosion tests, respectively. On the contrary, biofilms exposed to frequent shear conditions during their growth were only partially detached during the erosion tests (30–40%) (Fig. 7, e-h). However, the recovery of permeate flux following erosion tests was limited for all biofilms except the “weekly sheared” ones (Fig. 7, a-d). Removing 30, 40 or even 60% of the biofilm mass through erosion at 2.8 Pa did not help to increase the permeate flux. Only the full removal of the “weekly shear” biofilms help to recover flux. In our study, the very cohesive base layers of membrane biofilms that resisted to erosion at 2.8Pa represented up to 65 ± 9% of the biofilm total mass, but more than 95% of their hydraulic resistance [11]. already observed that for membrane biofilms grown under dead-end conditions, erosion of the biofilms top layer has negligible impact on its hydraulic resistance. In their numerical study [22], further confirmed the major contribution of the base layer on the overall permeability of membrane biofilms. If the base layers of biofilms are associated with a very high cohesiveness and hydraulic resistance, measures that aim at increasing permeate flux through biofilm engineering should thus directly target the engineering of biofilm’s base layers.

4.3. Intermittent shear conditions do not represent a relevant approach to engineer biofilms with increased permeability during GDM filtration

The initial goal of our study was to engineer biofilms with a reduced hydraulic resistance by applying intermittent shear conditions. Our goal was not to fully remove the biofilms, as GDM filtration takes advantage of their presence (operation at stable flux and improved permeate quality). Applying a low shear stress value of 1 Pa was sufficient to detach the surface layer of the biofilms, despite the biofilms were overall characterised by much larger yield stress value (minimum 260 Pa). Our results however indicate that intermittent shear conditions do not help reducing the biofilm’s hydraulic resistance and in turn increasing the system performances. Over long-term, the hydraulic resistance of the base layer (layer remaining attached to the membrane after erosion at 1Pa) increases, to ultimately reach a similar value than the one of dead-end biofilms. Measures to engineer biofilms with an increased permeability should thus target engineering their base layer. If increasing the permeability of membrane biofilms is not achievable during GDM filtration, increasing the production of treated water then requires using a larger surface of membrane. However, increasing the membrane surface to produce more water is only achievable at the expense of an increasing investment cost as a result of the increasing membrane area.

But intermittent shear conditions might actually help reducing the amount of organic detritus originating from the influent and accumulating in the system. The river water used in our study contain up to 1400 μgC/L of particulate organic substrate, that continuously accumulate on the membrane surface during filtration [9]. The long-term accumulation of organic detritus in GDM filters results in a deterioration of the permeate quality due to the increasing release of assimilable organic carbon, as a result of their hydrolysis [9]. Therefore, we hypothesize that an intermittent removal of the organic detritus helps controlling the accumulation of organic detritus in the systems and...
could help improving the permeate quality. Thus, the benefit of intermittent shear might not be the recovery of flux, but rather the long-term improvement of the permeate quality.

5. Conclusions

- Membrane biofilms developed during gravity-driven ultrafiltration of river water and exposed to intermittent shear conditions significantly adapted to their hydraulic environment. Intermittent exposure to shear conditions resulted in an increasing resistance to detachment and, over long-term, in a similar hydraulic resistance.
- Intermittent shear conditions triggered a major change in the mechanical properties of the membrane biofilms developed during GDM filtration of river water. The more frequent the exposure to shear conditions, the more elastic and cohesive the biofilms. Ultimately, the biofilms that were the most frequently exposed to shear conditions were also the most resistant to erosion.
- The more frequent the exposure to shear conditions, the slower the flux decline during the first week of gravity-driven ultrafiltration. However over longer-term, all biofilms developed during GDM filtration of river water had a similar hydraulic resistance despite very different exposures to shear conditions. Frequent shear conditions resulted in the formation of thinner and denser biofilms, especially in terms of polysaccharide density.
- Over long-term, intermittent shear at value of 1 Pa is not a useful strategy to engineer highly permeable biofilms and does not help increasing permeate flux during gravity-driven membrane ultrafiltration of river water.

CRediT authorship contribution statement

Nicholas Derlon: Conceptualization, data interpretation, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition. Peter Desmond: Conceptualization, data interpretation, Investigation, Validation, Writing – original draft, Writing – review & editing, Visualization. Patrick A. Rihs: Investigation. Eberhard Morgenroth: Conceptualization, data, interpretation, Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eberhard Morgenroth reports financial support was provided by Swiss National Science Foundation.

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