Water Research 198 (2021) 117163

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Biofouling control: the impact of biofilm dispersal and membrane flushing

Hendrik J. de Vries[a,b,1], Eva Kleibusch[b], Gerben D.A. Hermes[a], Paula van den Brink[b,2], Caroline M. Plugge[a,b,*]

[a] Laboratory of Microbiology, Wageningen University & Research, Stippeneng 4, 6708 WE Wageningen, The Netherlands
[b] Wetsus, European Centre of Excellence for Sustainable Water Technology, Oostergoweg 9, 8911 MA Leeuwarden, The Netherlands

ARTICLE INFO

Article history:
Received 25 September 2020
Revised 12 April 2021
Accepted 13 April 2021
Available online 18 April 2021

Keywords:
Biofilm dispersal
Bacterial community
Flow velocity
Membrane biofouling
Membrane fouling simulator
Nutrient limitation

ABSTRACT

Pure culture studies have shown that biofilm dispersal can be triggered if the nutrient supply is discontinued by stopping the flow. Stimulating biofilm dispersal in this manner would provide a sustainable manner to control unwanted biofilm growth in industrial settings, for instance on synthetic membranes used to purify water. The response of multispecies biofilms to nutrient limitation has not been thoroughly studied. To assess biomass dispersal during nutrient limitation it is common practise to flush the biofilm after a stop-period. Hence, flow-stop-induced biomass removal could occur as a response to nutrient limitation followed by mechanical removal due to biofilm flushing (e.g. biofilm detachment). Here, we investigated the feasibility to reduce membrane biofouling by stopping the flow and flushing the membrane. Using a membrane fouling simulator, biomass removal from synthetic membranes after different stop-periods was determined, as well as biomass removal at different cross flow velocities. Biomass removal from membrane surfaces depended on the nutrient limiting period and on the flow velocity during the biofilm flush. When flushed at a low flow velocity (0.1 $m.s^{-1}$), the duration of the stop-period had a large effect on the biomass removal rate, but when the flow velocity was increased to 0.2 $m.s^{-1}$, the length of the stop period became less considerable. The flow velocity during membrane flushing has an effect on the bacterial community that colonized the membranes afterwards. Repetition of the stop-period and biofilm flushing after three repetitive biofouling cycles led to a stable bacterial community. The increase in bacterial community stability coincided with a decrease in cleaning effectivity to restore membrane performance. This shows that membrane cleaning comes at the costs of a more stable bacterial community that is increasingly difficult to remove.

1. Introduction

Membrane filtration is the leading technology for seawater purification (Gude 2016). One disadvantage is the inevitable accumulation of material at the membrane surface, causing membrane fouling. Biofouling is the fouling type that most frequently diminishes membrane performance and leads to operational problems, such as an increase in pressure drop, decrease in normalized flux and altered membrane selectivity (Nguyen et al. 2012). For reverse osmosis (RO) membranes, fouling removal is limited to chemical based cleaning in place. Microfiltration (MF) and ultrafiltration (UF) membranes are also cleaned physically via forward flush, backflush, pneumatic cleaning by gases or by applying ultrasound or electrical fields (Regula et al. 2014). Despite these alternatives, cleaning in place is the only option for MF and UF membranes to remove recalcitrant fouling components such as proteins (Maskooki et al. 2010).

Biofilms are multicellular microbial aggregates surrounded by extracellular polymeric substances (EPS) (Donlan and Costerton 2002). When biofilms develop on synthetic membranes, the EPS layers add to the hydraulic resistance of the membrane and provides the embedded cells protection against chemical cleaning and nutrients after the chemical treatment (Flemming et al. 1997, Hijnen et al. 2012). Hence, membrane biofouling is a biofilm problem (Flemming et al. 1997). Three biofilm removal mechanisms can be distinguished: desorption, detachment and dispersal (Petrova and Sauer 2016). Desorption is the reverse of bacterial

* Correspondence: Caroline M. Plugge, Stippeneng 4, 6708 WE Wageningen, The Netherlands.
E-mail address: caroline.plugge@wur.nl (C.M. Plugge).
[1] Present address: WLN, Rijksstraatweg 85, 9756 AD, Glimmen, the Netherlands.
[2] Present address: Evides Industriewater, Schaardijk 150, 3063 NH Rotterdam, the Netherlands.

https://doi.org/10.1016/j.watres.2021.117163

attachment, e.g. surface-attached cells leave the substratum to enter the bulk liquid. Detachment describes the process in which biofilm embedded cells dislocate passively from the biofilm. This occurs when external forces disrupt the biofilm structure. Two examples are sloughing, via frictional fluid forces, and grazing by eukaryotic consumption (Klein et al. 2016, Petrova and Sauer 2016). Biofilm embedded cells can also disseminate actively from the biofilm in response to changes in the environment, which is referred to as biofilm dispersion (Guilhen et al. 2017). Biofilm dispersion is always performed to stimulate surface colonization, but its inducers can be either beneficial or harmful (Petrova and Sauer 2016). In Pseudomonas aeruginosa, biofilm dispersion can be induced by a sudden increase in glucose availability but also by glucose limitation; hence biofilm dispersion is a complex process (Sauer et al. 2004, Schleheck et al. 2009). To disperse from the biofilm, bacteria increase their motility or secrete proteins that disrupt the integrity of the EPS layer (Guilhen et al. 2017). Another way to disperse from the biofilm is via regulated cell lysis of a part of the community (Webb et al. 2003). External factors that can trigger biofilm dispersal include, amongst others, nutrient limitation, oxygen depletion, hydrogen peroxide stress and concentration fluctuations of certain carbon sources (Guilhen et al. 2017). Recent studies have proposed that also signal molecules trigger biomass release from biofilms (Nagaraja et al. 2017, Xie et al. 2019). Nitric oxide is suggested to induce biofilm dispersal by modulating intracellular signal molecules, but as free radical, nitric oxide also causes oxidative damage to DNA, proteins and polysaccharides (Nagaraja et al. 2017, Xie et al. 2019). These compounds therefore not only induce biofilm dispersal but also physically disassemble the EPS layer.

Dispersal of multispecies biofilms is understudied and it is unknown how these biofilms react to stimulants that disperse single species biofilms (Petrova and Sauer 2016). The aim of this study was to examine the feasibility to remove membrane fouling, caused by a multi-species biofilm, using nutrient limitation as a stimulant for biofilm dispersal. Nutrient depletion was achieved by stopping the flow of the feed water. To untangle the biological (e.g. dispersal) from the mechanical (e.g. flush) impact, we determined and compared the efficiency of biomass removal after different stop-periods (4 min, 24h and 4 weeks) and with different flow velocities.

2. Material and Methods

2.1. Experimental setup

Three or four identical lab scale cross flow filtration cells with hydrodynamics similar to spiral wound nanofiltration (NF) and RO membrane elements were used to investigate membrane fouling. For each flow cell, an identical experimental design was applied to investigate membrane fouling under equivalent conditions (Figure S1). A detailed overview of the experimental setup is presented in the supplementary information.

2.2. Fouling simulation experiments

Three or four flow cells, supplied with a polyethersulfone microfiltration membrane (nominal pore size 0.05 µm; Nadir MP 005, Microdyn-Nadir GmbH Wiesbaden, Germany), were operated simultaneously to investigate membrane biofouling control by stimulation of biofilm dispersal via nutrient limitation. The flow rate of the retentate stream was measured by a calorimetric flowmeter (omni-FIN, Honsberg, Remscheid, Germany) and controlled using custom-made flow controllers at a linear flow velocity of 0.1 m s^{-1}. Nutrients were dosed ahead of the filtration cells by a peristaltic pump (Masterflex L/S pumps, Cole-Palmer Instrument Company, Vernon Hills, Illinois, USA) (Figure S1). Carbon, nitrogen and phosphorus were dosed to a final concentration of 1 mg L^{-1}, 0.2 mg L^{-1} and 0.1 mg L^{-1}, respectively. A 10 L nutrient solution, containing sodium acetate (VWR Chemicals, Amsterdam, The Netherlands), $NaNO_3$ (VWR Chemicals, Amsterdam, The Netherlands) and NaH_2PO_4 (VWR Chemicals, Amsterdam, the Netherlands) was autoclaved. After autoclaving the nutrient bottles were connected to the setup using a stainless steel connector (Swagelok, Waddinxveen, The Netherlands) that was flamed before use to prevent microbial contamination of the nutrients.

Table 1
Experimental design and analyses per experiment

Experiment	Flow velocity	Stop-periods	TOC	ATP	NGS
I	0.1 m s-1	4 min, 24h	+	+	-
II	0.1 m s-1	4 min, 4 weeks	+	+	-
III	0.2 m s-1	4 min, 24h	+	+	-
IV	0.2 m s-1	4 min, 4 weeks	+	+	-
V	0.1 m s-1	4 min	+	+	+
VI	0.2 m s-1	4 min	+	+	+

After a minimum feed channel pressure drop (FCP) increase of 125 mbar, the flow was stopped for either 4 min, 24 h or 4 weeks The reasons for selecting these stop periods have been described in detail in the supplementary information. After the stop period, the membrane was flushed at a linear flow velocity of either 0.1 or 0.2 m s^{-1} (Table 1). As a reference, we selected the flow cell with the lowest increase in FCP. The FCP at the start of the experiment ranged between 80 and 110 mbar. At initiation of the membrane flush, a total of 250 mL of the retentate stream was collected in aliquots of 50 mL. From each 50 mL aliquot, 5 mL was used for total organic carbon (TOC) measurement, 10 mL for adenosine triphosphate (ATP) content, and the remaining 35 mL was used for DNA extraction. Reduction in FCP, hereafter referred to as pressure drop reduction (PDR) was used as a measure to determine membrane fouling control via biofilm dispersal.

2.3. Repeated membrane flushing

We investigated how the efficiency to remove biomass after flow interruption periods and membrane flushing events was affected by repetition of these steps. For all membranes used in the repeated flushing experiments, duration of the stop-period was 4 min and the linear flow velocity during flushing was either 0.1 m s^{-1} or 0.2 m s^{-1} (Table 1). For the experiments investigating the effect of repeated membrane flushes, the experimental setup was operated as described in section 2.2, with the exception that for the repeated fouling and cleaning cycles, the complete setup was cleaned before and after the experiment.

2.4. Total organic carbon (TOC) and adenosine triphosphate (ATP) determination

A membrane autopsy was performed after membrane flushing. For three times intermittingly fouled and cleaned membranes, one autopsy was performed after the last cleaning step. TOC and ATP measurements were performed as described before (Beyer et al, 2014).

2.5. Membrane bacterial community composition

Bacterial genomic DNA was extracted from fouled membrane biofilms and from membrane released biomass to perform 16S rRNA gene amplicon sequencing. Membranes were cut in pieces of

8 cm^2 and placed in a 7 mL tube containing 6 mL of sterile milli-Q water. These samples were bead-beaten for 1 min at 50.000 rpm to dissolve the biomass and subsequently frozen using liquid nitrogen and stored at -80°C. After thawing, DNA was isolated using the Power Water DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA) following manufacturer's instructions, and visualised on a 1% agarose.

Amplification of the V4 region of the 16S rRNA gene was performed using double barcoded primers covering the variable region V4 of the 16S rRNA gene: 515F (5'-GTGCCAGCMGCCGCGGTAA) and 806R (5'-GGACTACHVGGGTWTCTAAT) (Caporaso et al. 2011, Ramiro-Garcia et al. 2018). PCR amplification and amplicon quality check was performed as described before (Müller et al. 2020). The resulting library was sent to GATC Biotech AG (now part of Eurofins Genomics Germany GmbH, Konstanz, Germany) for 2×150nt sequencing on an Illumina Hiseq2500 instrument. Sequence analysis was performed in NG-Tax 2.0 using default settings (Poncheewin et al. 2019). In short: Paired-end libraries were demultiplexed using read pairs with valid and perfectly matching barcodes. Amplicon sequence variants (ASV) were picked as following: sequences were ordered by abundance per sample and reads were considered valid when their cumulative abundance was ≥ 0.1%. Taxonomy was assigned using the SILVA reference database version 128 (Quast et al., 2013). ASVs are defined as individual sequence variants rather than a cluster of sequence variants with a shared similarity above a specified threshold (generally 97%) such as operational taxonomic units (OTU). Nucleotide sequences are available in the European Nucleotide Archive under accession number PRJEB38042.

2.6. Statistical analysis

For the bacterial communities, all analyses were performed in R version 3.4.0. ASV richness was calculated to define microbial alpha-diversity (within sample diversity) for each sample as implemented in the vegan package (Oksanen et al. 2018). Beta diversity (between sample diversity) was calculated with Bray-Curtis dissimilarity the ASV level and Principle Coordinate analysis (PCoA) was used to visualize the resulting pairwise dissimilarity matrix (Bray and Curtis 1957). Data were normalized for the bar graphs into relative abundance for each sample, by dividing the reads for each taxon by the total number of valid reads per sample without rarefaction, because alpha diversity is independent of sequencing depth with NG Tax 2.0 (Müller et al. 2020). All plots were visualized using the ggplot2 package (Wickham 2016).

3. Results

It is common practise to assess the effectivity of biofilm dispersal stimulants by determining the amount of released biomass after the liberated components have been removed by a flush. To untangle the biological (e.g. dispersal) from the mechanical (e.g. flush) impact of this procedure, we compared the biomass removal efficiency after different stop-periods (4 min, 24h and 4 weeks) at the same flushing flow velocity, and we assessed the biomass removal efficiency after the same stop-periods at two different flow velocities. In experiment I and II the membranes were flushed at 0.1 m s^{-1} and in experiment III and IV the membranes were flushed at 0.2 m s^{-1} (Table 1). In experiment V and VI the membranes were fouled and cleaned for three successive times to simulate the effect of repeated membrane cleaning. An FCP increase of 125 mbar was used as criterion to start the stop-period, but due to the logarithmic biomass increase, this criterion was frequently exceeded (Figure S2, S3 and S4).

3.1. Effect of different stop-periods and flushing at 0.1 m s^{-1}

3.1.1. Biomass indicators

ATP measurements generally confirmed that elongation of the stop-period led, independent of the flow velocity, to a decrease in microbial activity within the biofilm (Figure S5). During the membrane flush, a total of 250 mL of the retentate stream was collected in aliquots of 50 mL. TOC was determined in each of the 5 aliquots and ATP was measured in the first 50 mL aliquot. When the membrane was flushed after a 4 min stop-period, the TOC increased from 6.8 mg L^{-1} in the feed stream to 18.8 mg L^{-1} in the concentrate stream, suggesting that biomass was released from the membrane (Fig. 1A). For the second, third, fourth and fifth 50 mL aliquots of the retentate stream, the TOC was, for each flow cell in each experiment, comparable to the feed stream. This suggests that most of the released biofilm is collected in the first 50 mL retentate stream (Fig. 1A, 1D, 2A, 2D 3A and 3B).

When the duration of the stop-period was increased to 24h, the amount of membrane released biomass (feed: 6.8 mg L^{-1} and concentrate: 120.6 mg L^{-1}) was substantially higher compared to the 4 min stop-period. These differences cannot be explained by the amount of biomass accumulated at the membrane surface, which were comparable for the 4 min (158.3 ng cm^{-2}) and 24 h (224.3 ng cm^{-2}) stop-period (Fig. 1B). The effect of a 4 min stop period was investigated as biological replicate, while the stop-period of 24h (experiment I) was exchanged for a 4 week stop-period (experiment II; Fig. 1D, E and F). Although the amount of membrane accumulated TOC was substantially higher for the 4 min stop period (Fig. 1B; TOC of 158.2 ng cm^{-2}) compared to its replicate (Fig. 1E; TOC of 38.2 ng cm^{-2}), the increase in FCP was slightly lower for the 4 min stop period (Figure S2; FCP of 168 mbar) compared to its biological replicate (FCP of 244 mbar).

For the 4 week stop-period, the difference between the biomass concentration in the feed (5.7 mg L^{-1}) and retentate (30.6 mg L^{-1}; Fig. 1D) was lower compared to the 24h stop-period (Fig. 1A), and indicated that not much more biomass was released from the membrane compared to the 4 min stop period (feed: 7.8 mg L^{-1} and retentate: 19.6 mg L^{-1}; Fig. 1D). However, the amount of biomass accumulated at the membrane surface was a factor 4 lower for the 4 week stop-period (2.68 ng cm^{-2}; Fig. 1E) relative to the 4 min stop period (8.56 ng cm^2). The TOC and ATP concentrations at the reference membrane were always the lowest because the flow cell with the lowest increase in FCP was selected as reference due to the chosen selection criteria.

3.1.2. Membrane performance

FCP measurements showed that membrane performance was comparable for the flow cells per experiment but not between different experiments (Figure S2, S3 and S4). Reduction in PDR was used as a measure to determine the membrane cleaning effect of the inflow-stop and membrane-flushing on membrane performance.

In experiment I, the increase in membrane performance after the stop and flush event was considerably higher for the 4 min stop-period (Fig. 1C; PDR of 22.5%) relative to the 24h stop period (Fig. 1C; PDR of 6.5%). But for membranes that were flushed at 0.1 m s^{-1} the stop-period of 4 weeks (Fig. 1F; PDR of 32.7%) gave the highest pressure drop reduction in experiment II. The 4 min stop-period (Fig. 1C; PDR of 22.5%) gave a higher increase in membrane performance compared to its biological replicate (Fig. 1F; PDR of 12.6%), illustrating large differences between biological replicate experiments, which agrees with the differences in the amount of accumulated TOC between biological replicates (Fig. 1B and 1E).

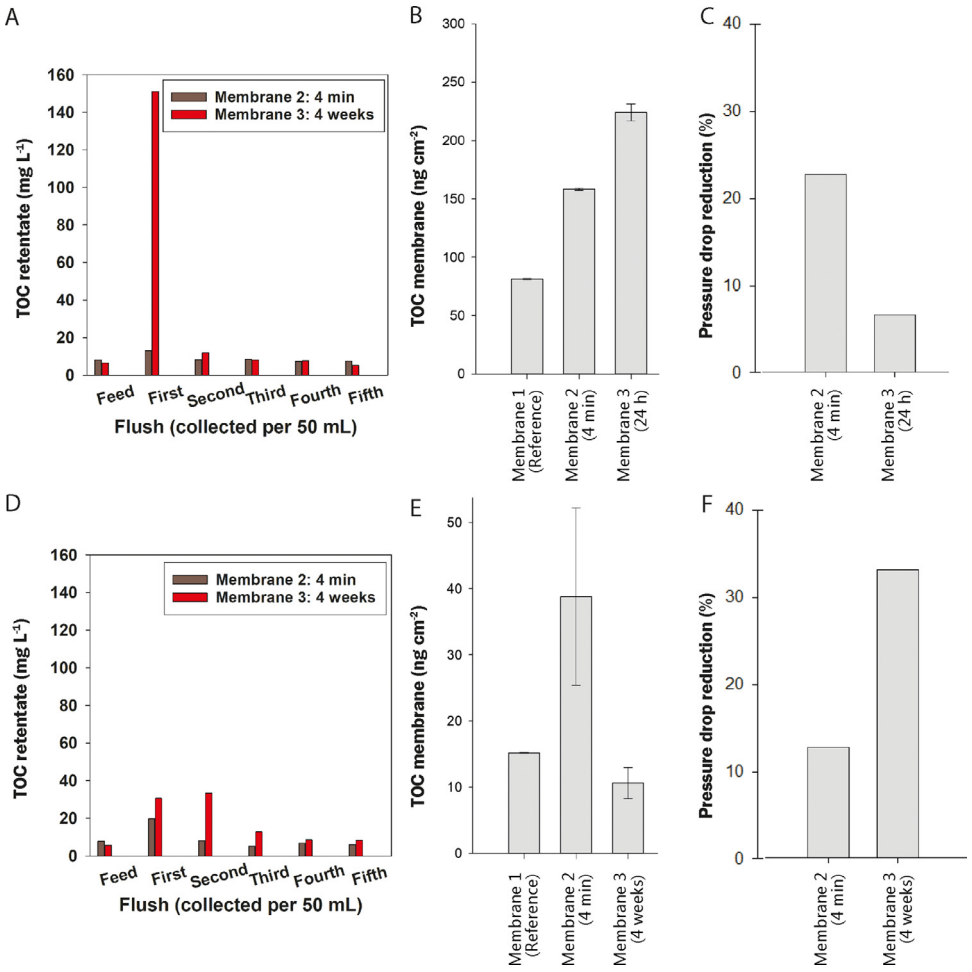

Fig. 1. Biomass indicators and membrane performance for the reference membrane and the membranes flushed at 0.1 m s^{-1} after stop-periods of 4 min, 24 h and 4 weeks (Experiment I and II). TOC of the retentate stream (A, D) and at the membrane (B, E) during or after membrane flushing, respectively, and pressure drop reduction (C, F). After the flush, five aliquots of 50 mL of retentate were collected to determine the amount of released biomass (A, D). Error bars give standard deviation between TOC concentrations at the inlet and outlet side of the flow cell (C, D).

3.2. Effect of stop-period and flushing at 0.2 m s^{-1}

To determine the effect of biofilm sloughing (e.g. mechanical biofilm removal) on biomass removal and membrane performance recovery, we repeated the fouling simulation experiments but increased the flow velocity during the membrane flush from 0.1 m s^{-1} to 0.2 m s^{-1}.

3.2.1. Biomass indicators

In experiment III, the TOC concentration increased after the 4 min stop-period from 4.8 mg L^{-1} in the feed to 22.6 mg L^{-} in the retentate (Fig. 2A). Despite the fact that the amount of biomass accumulated at the membrane surface was slightly higher in experiment IV relative to experiment III (Fig. 2B and Fig. 2E) the same stop-period of 4 min gave a lower TOC increase, from 8.12 mg L^{-1} in the feed to 12.90 mg L^{-1} in the retentate (Fig. 2D). The TOC increase after the 24h stop-period was, with 42.8 mg L^{-1} (feed: 6.2 mg L^{-1} and retentate: 49.0 mg L^{-1}, Fig. 2A) higher compared to both 4 min stop-periods, but the 4 weeks stop-period gave the highest biomass release (144.5 mg L^{-1}; feed: 6.4 mg L^{-1} and retentate 151.0 mg L^{-1}, Fig. 2D).

3.2.2. Membrane performance

Irrespective of the stop-period, the recoveries in membrane performance were very comparable for the membranes flushed at 0.2 m s^{-1}. In experiment III, the 4 min stop-period (PDR of 50.4%) gave a higher PDR compared to the 24h stop-period (PDR of 37.3%; Fig. 2C). Similarly, a stop-period of 4 weeks (PDR of 51.7%) in experiment IV led to a comparable recovery of membrane performance relative to the biological replicate of the 4 min stop-period (PDR of 51.7%; Fig. 2F) .

3.3. Effect of repeated cleaning

For membranes used in practise it becomes difficult over time to efficiently clean the membrane and restore their performance to the starting value (Beyer et al. 2014). To investigate whether repeated cleaning by a stop and flush procedure leads to a more stable bacterial community, membrane fouling and cleaning was performed for three consecutive times.

3.3.1. Biomass indicators

After flushing at 0.1 m s^{-1}, the TOC concentrations of the first 50 mL of retentate increased for three distinct membranes to 17.7 ng L^{-1}, 33.4 ng L^{-1} and 13.20 ng L^{-1} (Fig. 3A). Hence the amount of TOC was slightly higher compared to the feed stream. For two membranes (i.e. 3 flushes and its technical duplicate: 3 flushes duplicate; membrane 3 and 4), the biofouling and cleaning steps were performed for three consecutive times. During the second consecutive flush, the TOC concentrations of the first 50 mL aliquot ranged, between 18.3 ng L^{-1} and 14.5 ng L^{-1}, and for the third consecutive flush between 16.6 ng L^{-1} and 16.5 ng L^{-1} (Fig. 3A).

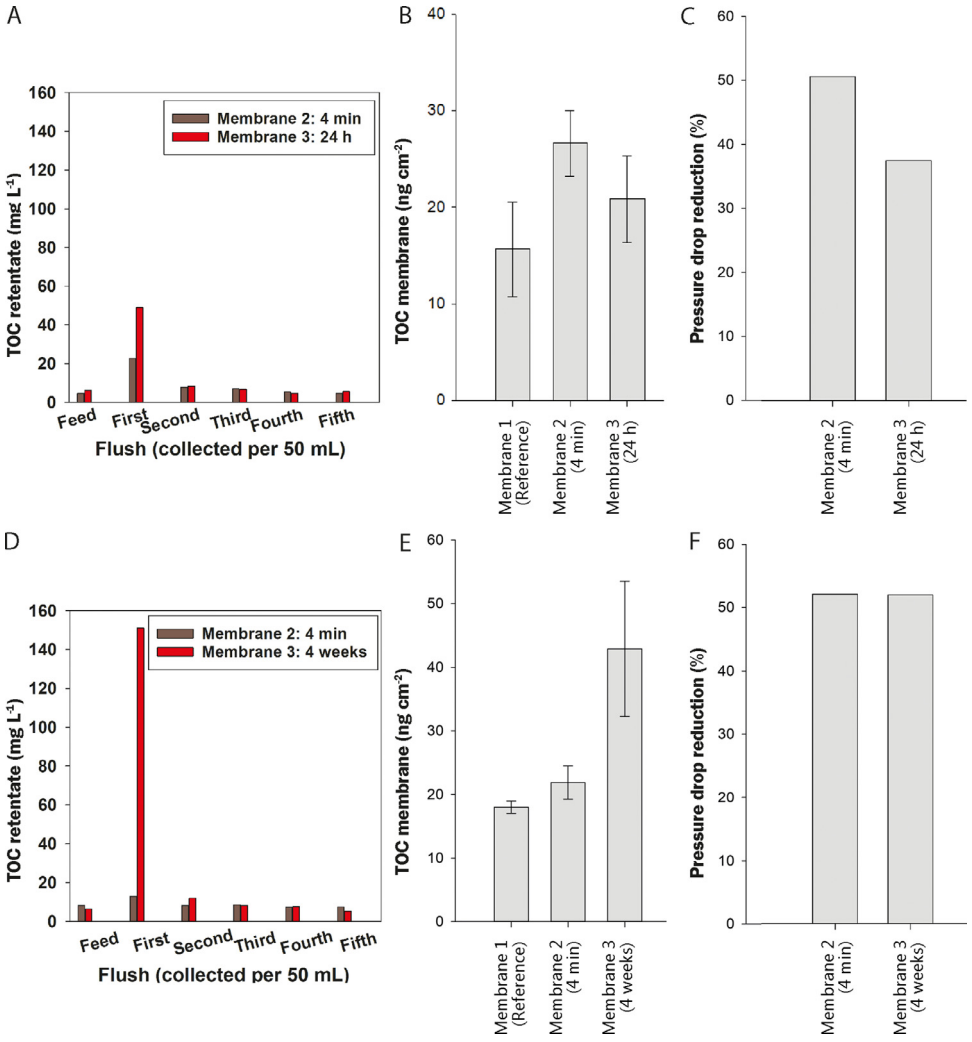

Fig. 2. Biomass indicators and membrane performance for the reference membrane and the membranes flushed at 0.2 m s^{-1} after stop-periods of 4 min, 24 h and 4 weeks (Experiment III and IV). TOC of the retentate stream (A, D) and at the membrane (B, E) during or after membrane flushing, respectively, and pressure drop reduction (C, F). After the flush, five aliquots of 50 mL of retentate were collected to determine the amount of released biomass (A, D). Error bars give standard deviation between TOC concentrations at the inlet and outlet side of the flow cell (C, D).

The comparable amounts of TOC released during the first, second and third cleaning step suggests that the susceptibility of the membrane biofilm did not change throughout successive membrane fouling cycles.

Generally more biomass was released from the membranes flushed at 0.2 m s^{-1} (Fig. 3B; experiment VI), indicating that flow-velocity has a strong effect on the amount of released biomass. During the first flush at 0.2 m s^{-1}, the TOC concentrations increased in the first 50 mL of retentate for the three membranes to 65.8 ng L^{-1}, 165.0 ng L^{-1} and 126.6 ng L^{-1} (Fig. 3B). During the second consecutive flush a comparable amount of biomass was released; as the TOC concentrations in the retentate stream increased to 139.4 ng L^{-1} and 218.0 ng L^{-1} (Fig. 3B). But during the third cleaning step the TOC in the retentate stream was for both membranes substantially lower (TOC concentrations of 40.4 ng L^{-1} and 65.8 ng L^{-1}). The amount of membrane attached biomass was comparable between experiment V and VI (Fig. 3C, 3D).

3.3.2. Membrane performance

Membrane performance was in line with the biomass measurements. For the membranes flushed at 0.1 m s^{-1} the PDR values varied between 3.5% and 30.2% (Fig. 4A). The membranes flushed at 0.2 m s^{-1} showed higher PDR values, ranging from 40.7% to 73.9% (Fig. 4B). These performance measurements confirm that flushing at 0.2 m s^{-1} instead of 0.1 m s^{-1} has a vast influence on the biomass removal efficiency. The efficiency of the stop-period and membrane flush to recover membrane performance was comparable for the first two cycles, but the PDR of the third cycle was generally much lower (Fig. 4). For the membranes flushed at 0.1 m s^{-1}, this trend is less apparent but for the membranes flushed at 0.2 m s^{-1} a clear trend can be distinguished. For the latter, the PDR values for the first and second cycle ranged from 71.6% to 73.0% but dropped to 43.5% for the third cycle (Fig. 4B, membrane 3). Comparably, for the second membrane repeatedly flushed at 0.2 m s^{-1} (membrane 4), the PDR values for the first and second cycle changed from 74.0% to 70.4% and dropped to 40.7% for the third cycle.

3.3.3. Effect on the bacterial community composition

For experiment V and VI 16S rRNA gene amplicon sequencing was used to identify the bacterial community composition present on the membrane surface and in the retentate stream directly after each flush. ASV picking resulted in yielded 154 166 ± 70 887 high quality reads per sample and an average of 37 ASVs per sample (Table S1). For three randomly chosen samples replicate PCRs and sequencing was performed. The high uniformity between each

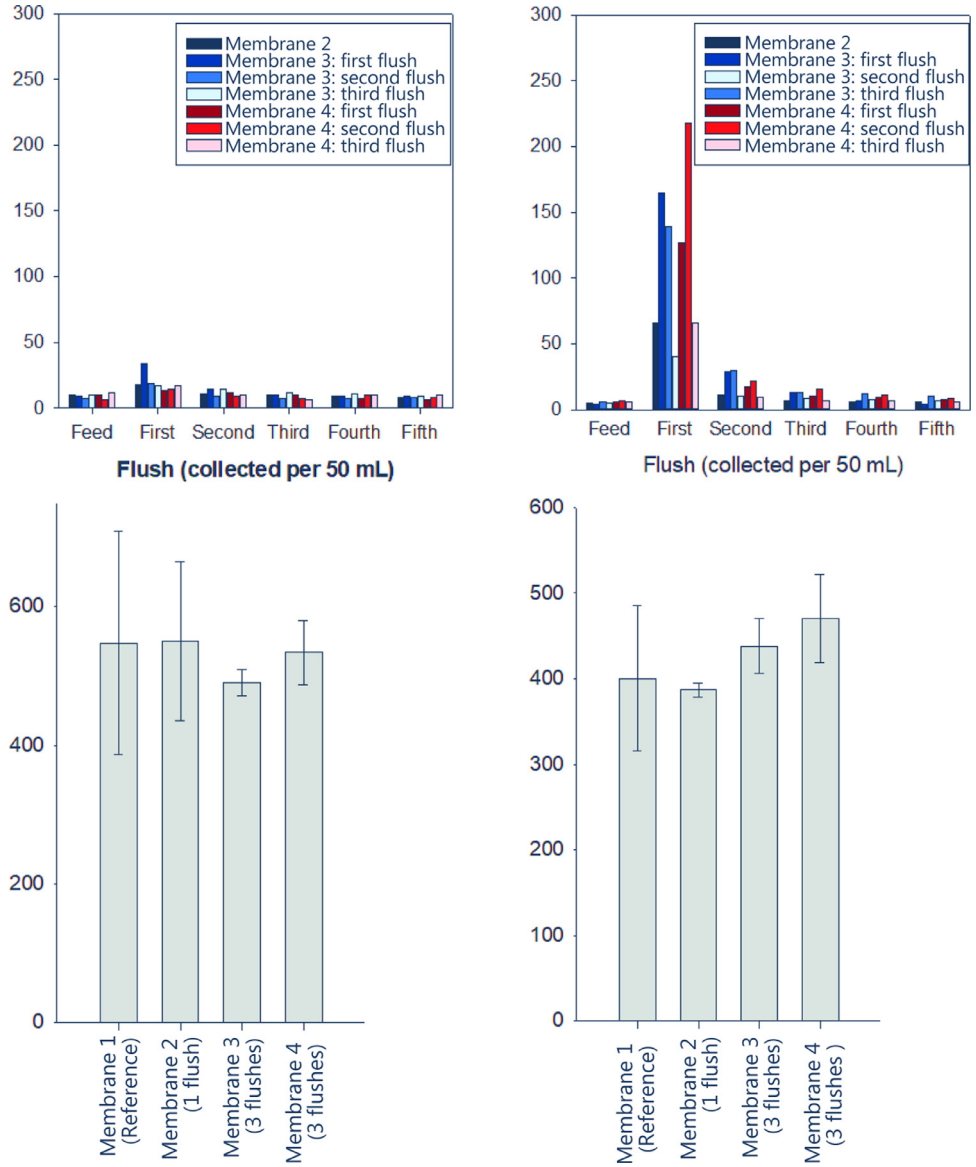

Fig. 3. TOC of the retentate stream directly after the flush (A, B) and at the membrane surface (C, D). In experiment V and VI, membrane 1 (reference) was fouled but not cleaned. Membrane 2 was fouled and cleaned once by stopping the flow for 4 minutes and flushing at either 0.1 m s^{-1} (A, C) or 0.2 m s^{-1} (B, D). For membrane 3 and 4, the cycle of membrane fouling and cleaning, as described for membrane 2, was repeated three consecutive times, i.e. the first, second and third flush. During each flush a total of 250 mL of the retentate stream was collected in aliquots of 50 mL and analysed for TOC. Error bars give standard deviation between TOC concentrations at the inlet and outlet side of the flow cell.

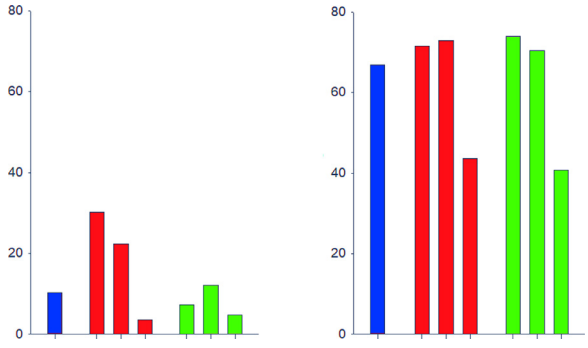

Fig. 4. Pressure drop reduction (%) for (A) the membranes that were flushed at 0.1 m s^{-1} and (B) the membranes that were flushed at 0.2 m s^{-1} (Experiment V and VI). In each experiment, one membrane was flushed once (membrane 2) and two membranes were three times intermittingly fouled and cleaned (membrane 3; 3 flushes and membrane 4; 3 flushes (duplicate)).

of the technical replicates illustrates that the NGS data is highly consistent (Figure S6).

The most abundant taxa in all samples (feed stream, membrane attached biomass and retentate stream during flushing) were mainly ASV belonging to the Family Comamonadaceae and the genera Acinetobacter, Ferribacterium, Flavobacterium and Zoogloea (Fig. 5 and 6). This close resemblance in bacterial community composition between feed and membrane suggests that the membrane surface does not select for specific bacterial taxa. Although the biofilms developing on the membranes that were flushed at a flow velocity of 0.1 m s^{-1} and 0.2 m s^{-1} were dominated by the same taxa, Acinetobacter appears to be generally more dominant in experiment V (flushing at 0.1 m s^{-1}, Fig. 5) while members from the Comamonadaceae Family appear to be generally more dominant in experiment VI (flushing at 0.2 m s^{-1}, Fig. 6).

Microbial alpha diversity, defined as ASV richness varied in experiment V for the detached biomass present in the retentate after flushing the membrane (Fig. 7A). For the membranes that were

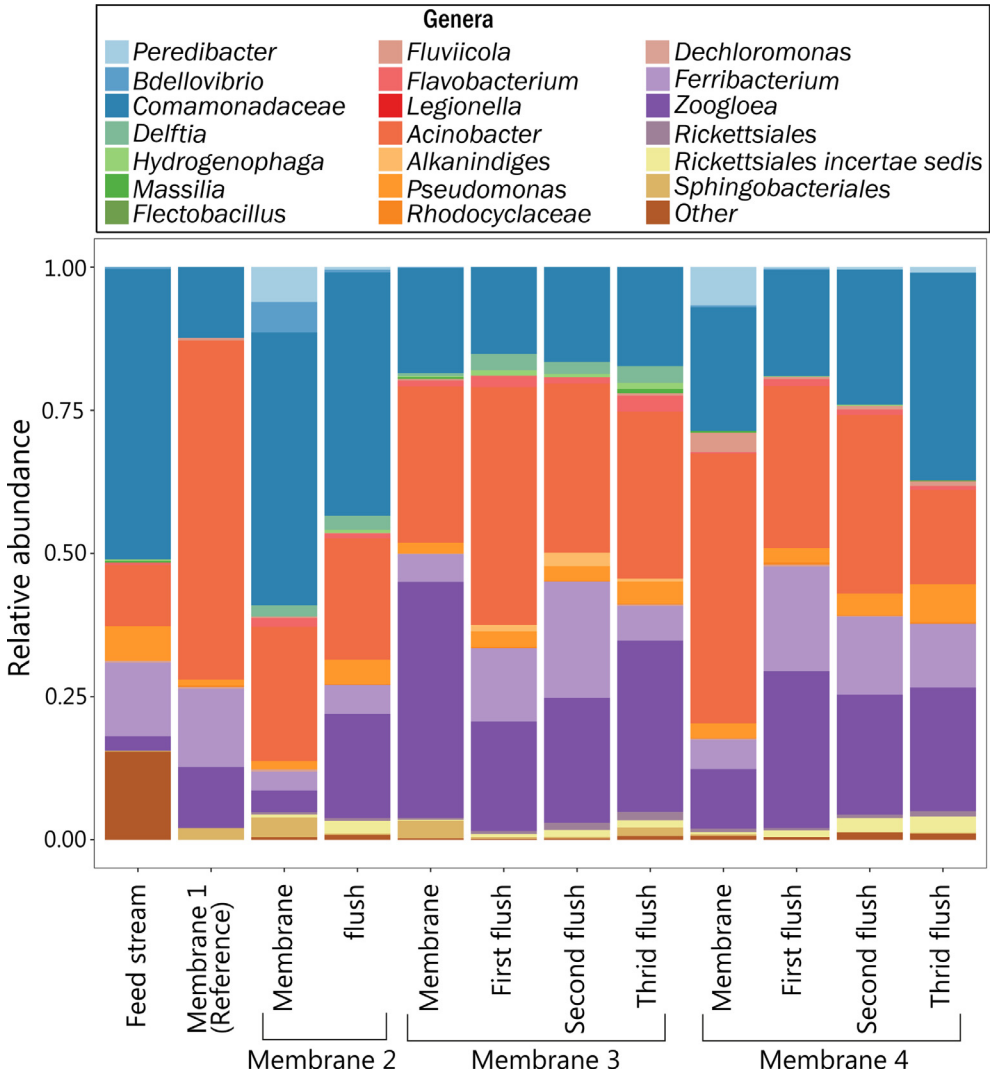

Fig. 5. Relative abundance of the twenty most dominant bacterial genera in the feed, on the membrane and in the retentate after the membranes were flushed at 0.1 m s^{-1} identified using the 16S rRNA marker gene (Experiment V). The abundance is presented in terms of percentage in total effective bacterial sequences in a sample.

flushed three consecutive times at 0.1 m s^{-1}, richness of the detached biomass decreased between the first and second flush, but increased between the second and third flush. For the membranes that were flushed three consecutive times at 0.2 m s^{-1}, detached biomass richness increased most substantially between the first and second flush after which the number of ASVs decreased. Beta diversity (between sample diversity) based PCoA was used to visualise pairwise dissimilarity between samples, where distance between samples is a proxy for community dissimilarity (Fig. 8). Bacterial communities of the detached biomass in experiment V clustered by membrane and were thus similar. Bacterial communities on membrane 3 and 4 were different from the detached biomass of the first, second and third flush, because these samples clustered together and were distant from the membrane attached communities (Fig. 8A). For experiment VI detached biomass communities clustered together with the membrane it originated from (Fig. 8B). In both experiment V and VI, bacterial communities of membrane 1, which was used as reference and underwent no flushing event, was most closely related to that of the feed water, indicating that membrane flushing has an effect on bacterial community composition.

4. Discussion

Cleaning in place remains the only universal applicable method to control membrane (bio)fouling but it has major drawbacks, such as loss of efficiency over time, potential membrane damage, the production of a waste stream and the CO_2 footprint of the applied chemicals. Application of biofilm dispersal for cleaning purposes in full-scale membrane installations would most likely entail an increase in membrane modules to balance the likely gain in membrane downtime. The stop period required to disperse a multispecies biofilm is therefore critical in determining the benefit of this cleaning method.

4.1. Effect of biofilm dispersal and membrane flushing at 0.1 m s^{-1}

Literature reports on different stop periods, ranging from 5 minutes to several days to stimulate biofilm dispersal (Petrova and Sauer 2016). As the EPS layer serves as nutrient, we postulate that nutrient limitation is enhanced by increasing the stop period duration. This was supported by the ATP concentrations of the membrane biomass, which in general decreased with increas-

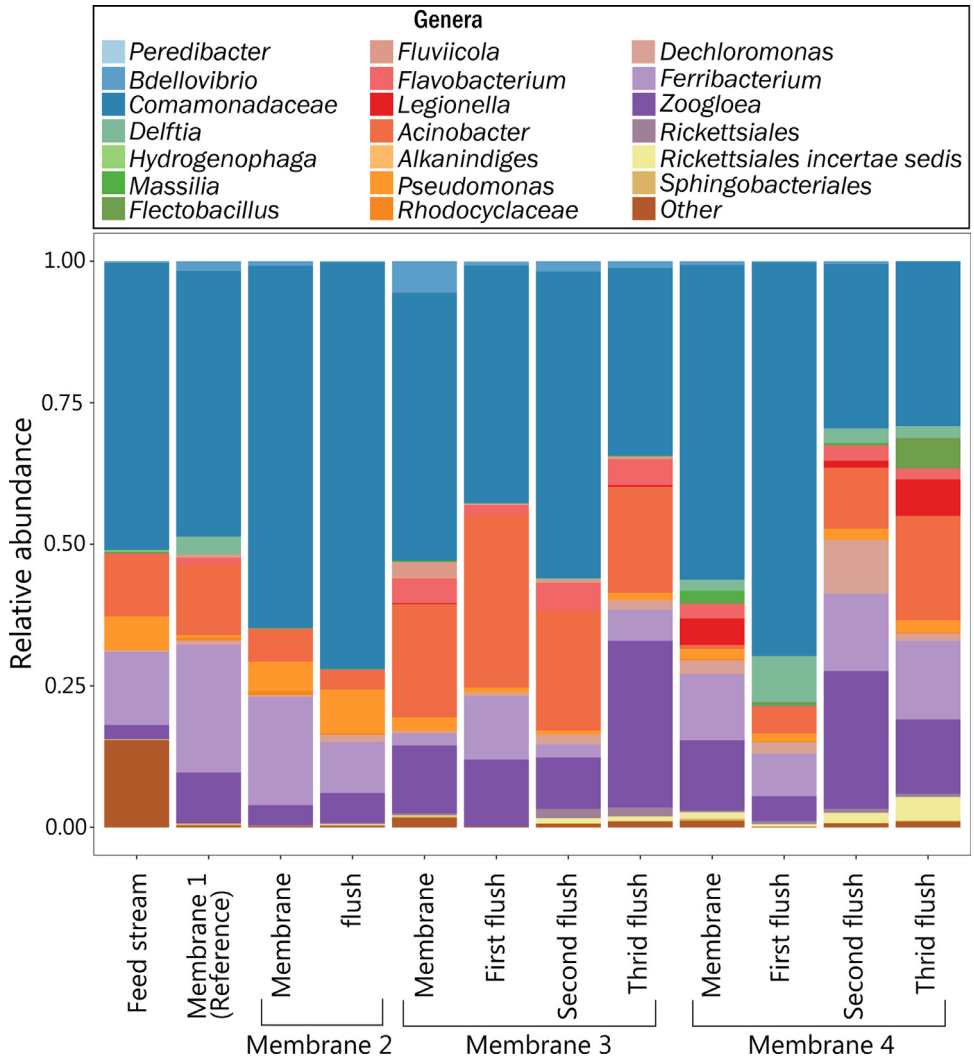

Fig. 6. Relative abundance of the twenty most dominant bacterial genera in the feed, on the membrane and in the retentate after the membranes were flushed at 0.2 m s^{-1} identified using the 16S rRNA marker gene (Experiment VI). The abundance is presented in terms of percentage in total effective bacterial sequences in a sample.

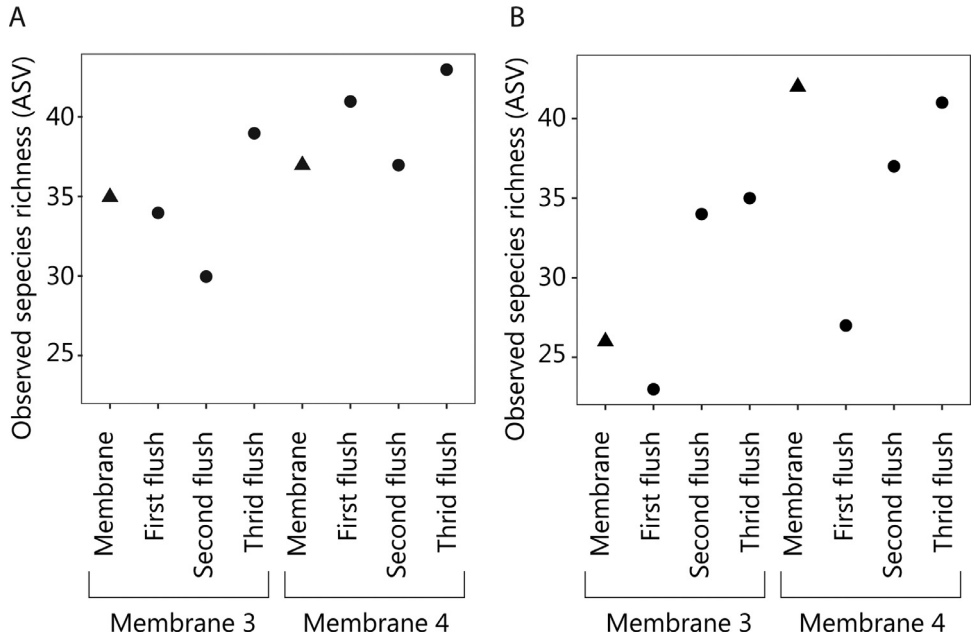

Fig. 7. Observed species richness (i.e. alpha diversity) of the membrane (triangle) and for retentate stream during the first (circle), second (circle) and third (circle) flush at (A) 0.1 m s^{-1} and (B) at 0.2 m s^{-1} (Experiment V and VI).

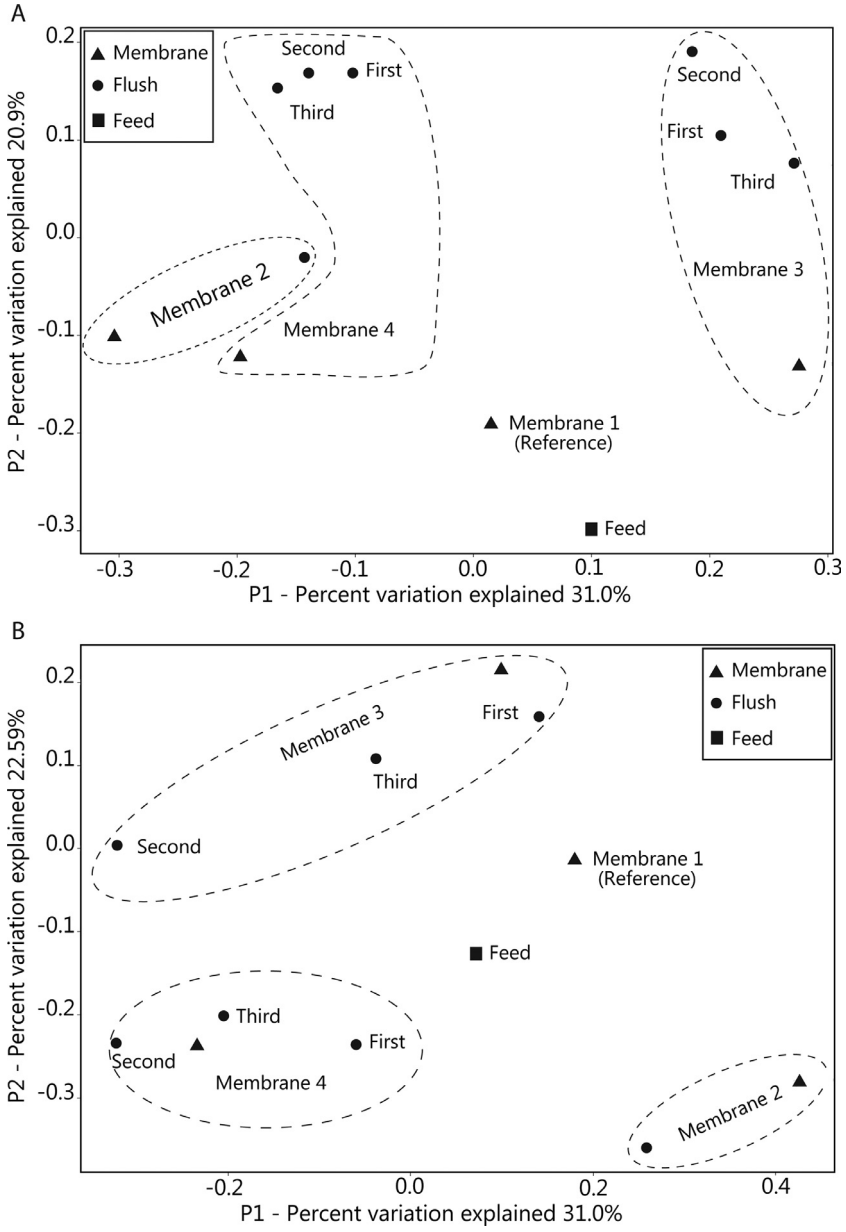

Fig. 8. Bray-Curtis dissimilarity based Principal coordinate analysis of the bacterial community composition of the feed (square), on the membrane (triangle) and in the retentate (circle) after the membranes were flushed (A) at 0.1 m s^{-1} and (B) at 0.2 m s^{-1} (Experiment V and VI).

ing stop-periods (Figure S5). For the membranes that were flushed at 0.1 m s^{-1}, it was shown that longer stop periods lead to more biomass removal (Fig. 1A and Fig. 1D). This indicates that, like single-species biofilms, nutrient limitation is a stimulant for multispecies biofilm dispersal.

No correlation of biomass removal to membrane performance was found; the large amount of TOC that was removed after a 24h stop period was associated with a low PDR (Fig. 1A and Fig. 1C). For the stop period of 4 min and its biological replicate, the TOC present in the retentate after the flush were very similar (respectively: 18.8 mg L^{-1} and 19.6 mg L^{-1}; Fig. 1A and 1D) but resulted in deviating PDRs (respectively: 22.5% and 12.6%; Fig. 1C and 1F). This difference between biomass and membrane performance can be explained by development of flow channels on the membrane surface that form a passage for the feed between the accumulated biomass (Bucs et al. 2014, Radu et al. 2014). No recovery in FCP is observed when biomass removal from the membrane does not widen the flow channels. Therefore biomass accumulation does not necessarily relates to membrane performance (Bucs et al., 2014).

Another explanation that fits our observations is that the TOC differences between the biological replicates was caused by the lack of a setup cleaning step between each of the first four experiments (experiments I, II, III and IV). Because the setup was not cleaned, bacteria that attach during the first experiment will colonize the setup during the experiment that follows. While the entire setup will be colonized, bacteria at the inlet side of setup will grow faster at the expense of the dosed nutrients. In respect to the differential pressure meter, when bacteria exclusively colonize the tubing at the inlet side, this will lead to an increase in the feed channel pressure drop that erroneously may be explained as membrane fouling. Hence, although the same performance criterion (FCP increase of 125 mbar) was used throughout the experiments, the TOC values declined from experiment I to IV because fouling of the setup contributed to the FCP increase. This corroborates with the observations during the repeated cleaning experi-

ments in §3.3; whereby the setup was cleaned between each experiment. In these experiments, the biomass indicator corresponds well with the membrane performance indicator

4.2. Biofilm dispersal at different flow velocities

To untangle the influence of mechanical biofilm detachment from biofilm dispersal, we repeated the experiments but altered the flow velocity of the flush. Overall, the increase in membrane performance was very similar for the membranes flushed at 0.2 m s^{-1} (Fig. 2C and 2F) and consistently higher compared to the membranes flushed at 0.1 m s^{-1} (Fig. 1C and 1F).

For the membranes flushed after a 4 min stop period, the amount of membrane accumulated biomass was comparable for three membranes (Fig. 1E, 2B and 2E) but was substantially higher for one membrane (Fig. 1B). In terms of biomass removal, the differences between the 4 min stop period were comparable (Fig. 1A, 1D, 2A and 2D) and appeared therefore not to be affected by the flush flow velocity, nor by the amount of membrane accumulated biomass. During repeated fouling and cleaning cycles, the stop period was the same, but in contrast to the membranes that were cleaned once, the different flow velocity led to large deviations; both in terms of amount of removed biomass (Fig. 3A and 3B) as well as in membrane performance (Fig. 4A and 4B). These apparent discrepancies can be explained by differences in the amount of membrane accumulated biomass, which was substantially lower for the membranes cleaned once (Fig. 1B, 1E, 2B and 2E; experiment I, II, III, IV) relative to the membranes cleaned three times (Fig. 3C and 3D; experiment V, VI). This low amount of membrane accumulated biomass, might provide an alternative explanation why the biomass parameters could not be correlated to membrane performance for the membranes that were cleaned once (Fig. 1 and 2).

For the 24h stop period, the amount of membrane accumulated biomass was a factor ten higher for the membrane flushed at 0.1 m s^{-1} (Fig. 1B) compared to the membrane flushed at 0.2 m s^{-1} (Fig. 2B). The amount of removed biomass for these membranes was around a factor two higher for the membrane flushed at 0.1 m s^{-1} (Fig. 1A) than those flushed at 0.2 m s^{-1} (Fig. 2A). Hence, although flushing at 0.1 m s^{-1} removed in absolute sense a higher amount of biomass, when the amount of released biomass is compared to the amount of accumulated biomass, it seems that flushing at 0.2 m s^{-1} is more effective in removing microbial aggregates. These observations indicate that the flush flow velocity rather than the duration of the stop period has a substantial effect on the cleaning efficiency.

For the membranes flushed after a 4 week stop period, the amount of accumulated biomass (Fig. 1E and 2E) was a factor 4 lower for the membrane flushed at 0.1 m s^{-1} relative to the membrane flushed at 0.2 m s^{-1}. In comparison, the amount of removed biomass was only a factor two lower for the membrane flushed at 0.1 m s^{-1} relative to the membrane flushed at 0.2 m s^{-1}. This indicates that, in contrast to the membranes stopped for 4 minutes and 24h, for the 4 week stop period the stop period seems to strongly influence the amount of released biomass.

4.3. Biofilm dispersal to control membrane biofouling

Based on single-species biofilm studies, biofilm dispersal appears an attractive membrane cleaning strategy because no chemicals are required (Delaquis et al. 1989, Gjermansen et al. 2005, Guilhen et al. 2017, Petrova and Sauer 2016, Sauer et al. 2004). The disadvantage of using stop-periods is the demand for increased membrane surface area to counteract membrane downtime. The results presented here indicate that multispecies biofilms can partly be removed using biofilm dispersal. When flushed at 0.1 m s^{-1}, the highest membrane cleaning efficiency of 32.7% was obtained after the longest stop period (Fig. 1F). In literature it has been reported that chemical cleaning with urea, or a combination of urea with sodium hydroxide and hydrochloric acid, leads to PDRs of 50% and >70%, respectively (Sanawar et al. 2018). This shows that membrane flushing at 0.1 m s^{-1} leads to small performance recoveries. One factor to improve the cleaning efficiency is by increasing the flush flow velocity. The highest PDR of 79.0% for the 0.2 m s^{-1} flush was obtained during the repeated fouling and cleaning cycle (Fig. 4B). These cleaning efficiencies exceed those of two-stage cleaning using sodium hydroxide and hydrochloric acid, but have to be considered within their experimental context. As was illustrated here (Fig. 2F and Fig. 4B), the efficiency of membrane cleaning depends on the degree of membrane fouling: higher PDRs are obtained at higher FCP increases (Figure S2, S3 and S4).

There has not been much research performed on repeating membrane cleaning and whether this changes the membrane's efficiency. The membrane cleaning efficiency at 0.2 m s^{-1} was very comparable between the first (72 and 74%) and second (73 and 70%) cleaning step but then declined with 30% to 44 and 41% (Fig. 4B). For the same membranes, the relatively low amount of TOC present in the retentate stream (Fig. 3A, 3B) combined with the high amount of membrane attached biomass (Fig. 3C and 3D) suggests that cleaning repetition reduces cleaning efficiency.

4.4. Bacterial communities of fouled membranes

The observed trend in ASV richness of the released biomass (a small decrease followed by a small increase) shows that richness remained relatively stable for the membranes flushed at 0.1 m s^{-1} (Fig. 7). This is in line with biomass indicators (Fig. 3A) and membrane performance (Fig. 4A), which showed that the removal efficiency was independent on the number of repetitions. For the membranes flushed at 0.2 m s^{-1}, the small increase in ASV richness (Fig. 7) between the second and third flush corroborated with a low amount of released biomass (Fig. 3B) and low regain of membrane performance (Fig. 4B). Hence, for the membranes flushed at 0.2 m s^{-1}, the bacterial community became more stable during the consecutive cleaning and fouling cycles.

It is usually considered that biofilms growing under the same conditions show a high resemblance in bacterial community composition (Khan et al. 2013, Kim et al. 2014, Tan et al. 2017). The flow cells used in this study were fed with the same feed water, were dosed the same nutrients at the same flow velocity and had the same dimensions; hence they were operated under identical conditions. However, 16S rRNA amplicon sequencing showed that the bacterial assemblages differed substantially in composition (Fig. 5 and 6). This lack of resemblance between biological replicates might be driven by the fact that phylogenetic diverse bacteria do share multiple functional traits, such as being oligotrophic, producing pili, flagella and EPS, which equally aid their change of growth and survival on the membrane. Distinct bacterial groups may therefore prevail in the same environment (Barberán et al. 2014).

Members from the Orders Burkholderiales, Pseudomonadales, Rhizobiales, Sphingomonadales and Xanthomonadales are frequently detected on fouled membranes (de Vries et al. 2020). In the present study, the Family Comamonadaceae and the genera Acinetobacter, Ferribacterium, Flavobacterium and Zoogloea were dominant taxa (Fig. 5 and 6). This shows that, from the frequently detected Orders on fouled membranes, members of the Rhizobiales and Sphingomonadales were below detection limit, although they were abundant in the feed water. The membranes used in present study deviate from membranes used in practise due to the addition of nutrients to the feed stream and the lack mem-

brane cleaning events. A recent study has shown that membrane cleaning efficiencies of lab-scale and full-scale experiments deviate substantially due to the differences in the chemical composition of the biofilms EPS layer (Jafari et al. 2020). At least for members of the Sphingomonas genus it has been shown that their dominance on fouled membranes is due to membrane cleaning (Bereschenko et al. 2010). For the other frequently detected orders, i.e. Burkholderiales, Pseudomonadales, Rhizobiales and Xanthomonadales the same relation between abundance and being recalcitrant to membrane cleaning has been suggested (Al Ashhab et al. 2017, Hong et al. 2016). Our results show, that besides the influence of the chemical composition of the cleaning agents, also the mechanical force of the membrane flush plays a pivotal role in selecting which bacterial taxa remain attached after the cleaning cycle. This might provide an explanation for the results obtained by Jafari et al (2020).

The few studies that used cultivation dependent methods to characterize membrane isolated bacteria suggest that bacterial appendages (flagella and pili), EPS composition, metabolic plasticity and (dissimilatory) nitrate reduction are important physiological traits for membrane colonization (de Vries et al. 2019, Nagaraj et al. 2019, Pang et al. 2005, Pang and Liu 2007). In line with these studies, members of the Comamonadaceae, Acinetobacter, Ferribacterium, Flavobacterium and Zoogloea have shown to be flagellated and produce pili, and many are able to reduce nitrogen or nitrate (Bernardet and Bowman 2006, Clemmer et al. 2011, Cummings et al. 1999, Shao et al. 2009).

4.5. Biofilm dispersal of multispecies communities

Microscopic imaging has shown that dispersal of single-species biofilms leads to almost complete removal of all attached biomass (Delaquis et al. 1989, Gjermansen et al. 2005, Schleheck et al. 2009, Thormann et al. 2005). The high TOC concentrations on the membrane surface after the membrane flush indicates that biomass removal of the multispecies biofilms was incomplete (Fig. 1B, 1E, 2B, 2E, 3C, 3D). Besides the technological approach used to illustrate biofilm dispersal, there might be several other reasons to explain this apparent contradiction between pure-culture studies and the results presented here on multispecies biofilms. Different species use different molecular mechanisms to disrupt a biofilm and have different abilities to remain surface attached under high hydrodynamic forces (Guilhen et al. 2017, Petrova and Sauer 2016).

Another cause for the disagreement between single-species biofilm dispersal and the results presented here might be the (mechanical) conditions on synthetic membranes. The feed spacer is located on the membrane surface to separate two membrane sheets and stimulate mixing, but its geometry creates positions with low shear forces where biofilm formation commonly starts. Circumstantially the feed spacer thus provides a physical barrier that renders membrane flushing less effective for biomass removal. Other studies have revealed that biofilms contain a basal layer that is more cohesive and denser than the outer biofilm layers (Abe et al. 2012, Paul et al. 2012, Stoodley et al. 1998). This heterogeneity in cohesiveness within a biofilm might, besides biofilm detachment, also affect biofilm dispersal. Particularly for biofilms that develop under shear stress, such as those on membranes, the biofilms cohesion strength increases as it becomes compressed by the flow (Paul et al. 2012).

5. Conclusions

In full scale membrane filtration installations, biofilm dispersal would, at the costs of additional membranes and membrane downtime, provide an economic and eco-friendly alternative for cleaning in place. This study shows that biomass removal after a stop period has a biological and mechanical cause, and uncovers that more biomass can be removed by increasing the flow velocity of the flush, but after repeated cleaning-cycles this leads to a stable and resilient bacterial community on the membrane.

7. Author contributions section

H.J.d.V., P.v.d.B. and C.M.P. designed the study. E.K. performed the experiments. H.J.d.V., P.v.d.B., G.D.A.H. and E.K. analyzed the data. H.J.d.V., P.v.d.B., G.D.A.H and C.M.P put the analyses in perspective. H.J.d.V. drafted the manuscript. All authors contributed to obtain the final version manuscript revision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was performed in the cooperation framework of Wetsus, European Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is cofounded by the Dutch Ministry of Economic Affairs and Ministry of Infrastructure and Environment, the European Union Regional Development Fund, the Province of Fryslân and the Northern Netherlands Provinces. Gerben D. A. Hermes was supported by the project MASTER (Microbiome Applications for Sustainable food systems through Technologies and Enterprise) funded by the European Union's H2020 research and innovation program under grant agreement No. 818368.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117163.

References

Abe, Y., Skali-Lami, S., Block, J.C., Francius, G., 2012. Cohesiveness and hydrodynamic properties of young drinking water biofilms. Water Res 46 (4), 1155–1166.
Al Ashhab, A, Sweity, A., Bayramoglu, B., Herzberg, M., Gillor, O., 2017. Biofouling of reverse osmosis membranes: effects of cleaning on biofilm microbial communities, membrane performance, and adherence of extracellular polymeric substances. Biofouling 33 (5), 397–409.
Barberán, A., Ramirez, K.S., Leff, J.W., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. Ecol. Lett. 17 (7), 794–802.
Bereschenko, L.A., Stams, A.J.M., Euverink, G.J.W., Van Loosdrecht, M.C.M., 2010. Biofilm formation on reverse osmosis membranes is initiated and dominated by Sphingomonas spp. Appl. Environ. Microbiol. 76 (8), 2623–2632.
Bernardet, J.F., Bowman, J.P., 2006. The genus Flavobacterium. The Prokaryotes 7, 481-531. DOI: 10.1007/0-387-30747-8_17
Beyer, F., Rietman, B.M., Zwijnenburg, A., van den Brink, P., Vrouwenvelder, J.S., Jarzembowska, M., Laurinonyte, J., Stams, A.J.M., Plugge, C.M., 2014. Long-term performance and fouling analysis of full-scale direct nanofiltration (NF) installations treating anoxic groundwater. J. Membr. Sci. 468, 339–348.
Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27 (4), 325–349.
Bucs, S.S., Radu, A.I., Lavric, V., Vrouwenvelder, J.S., Picioreanu, C., 2014. Effect of different commercial feed spacers on biofouling of reverse osmosis membrane systems: a numerical study. Desalination 343, 26–37.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U.S.A. 108 (Supplement 1), 4516–4522.
Clemmer, K.M., Bonomo, R.A., Rather, P.N., 2011. Genetic analysis of surface motility in Acinetobacter baumannii. Microbiology 157 (Pt 9), 2534–2544.
Cummings, D.E., Caccavo Jr,, F., Spring, S., Rosenzweig, R.F., 1999. Ferribacterium limneticum, gen. nov., sp. nov., an Fe (III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. Arch. Microbiol. 171 (3), 183–188.
de Vries, H.J., Beyer, F., Jarzembowska, M., Lipińska, J., van den Brink, P., Zwijnenburg, A., Timmers, P.H.A., Stams, A.J.M., Plugge, C.M., 2019. Isolation and characterization of Sphingomonadaceae from fouled membranes. NPJ Biofilms Microbiomes 5 (1), 6.

de Vries, H.J., Stams, A.J.M., Plugge, C.M., 2020. Biodiversity and ecology of microorganisms in high pressure membrane filtration systems. Water Res 172, 115511.
Delaquis, P.J., Caldwell, D.E., Lawrence, J.R., McCurdy, A.R., 1989. Detachment of Pseudomonas fluorescens from biofilms on glass surfaces in response to nutrient stress. Microb. Ecol. 18 (3), 199–210.
Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. 15 (2), 167–193.
Flemming, H.-C., Schaule, G., Griebe, T., Schmitt, J., Tamachkiarowa, A., 1997. Biofouling—the Achilles heel of membrane processes. Desalination 113 (2), 215–225.
Gjermansen, M., Ragas, P., Sternberg, C., Molin, S., Tolker-Nielsen, T., 2005. Characterization of starvation-induced dispersion in Pseudomonas putida biofilms. Environ. Microbiol. 7 (6), 894–904.
Gude, V.G., 2016. Desalination and sustainability–an appraisal and current perspective. Water Res 89, 87–106.
Guilhen, C., Forestier, C., Balestrino, D., 2017. Biofilm dispersal: multiple elaborate strategies for dissemination of bacteria with unique properties. Mol. Microbiol. 105 (2), 188–210.
Hijnen, W., Castillo, C., Brouwer-Hanzens, A., Harmsen, D., Cornelissen, E., van der Kooij, D., 2012. Quantitative assessment of the efficacy of spiral-wound membrane cleaning procedures to remove biofilms. Water Res 46 (19), 6369–6381.
Hong, P.Y., Moosa, N., Mink, J., 2016. Dynamics of microbial communities in an integrated ultrafiltration–reverse osmosis desalination pilot plant located at the Arabian Gulf. Desalination Water Treat 57 (35), 16310–16323.
Jafari, M., D'haese, A., Zlopasa, J., Cornelissen, E., Vrouwenvelder, J.S., Verbeken, K., Verliefde, A., van Loosdrecht, M.C.M., Picioreanu, C., 2020. A comparison between chemical cleaning efficiency in lab-scale and full-scale reverse osmosis membranes: Role of extracellular polymeric substances (EPS). J. Membr. Sci. 609, 118189.
Khan, M.T., Manes, C.-L.d.O., Aubry, C., Croué, J.-P., 2013. Source water quality shaping different fouling scenarios in a full-scale desalination plant at the Red Sea. Water Res 47 (2), 558–568.
Kim, I.S., Lee, J., Kim, S.-J., Yu, H.-W., Jang, A., 2014. Comparative pyrosequencing analysis of bacterial community change in biofilm formed on seawater reverse osmosis membrane. Environ. Technol. 35 (2), 125–136.
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 Res 88, 20–29.
Maskooki, A., Mortazavi, S.A., Maskooki, A., 2010. Cleaning of spiral wound ultrafiltration membranes using ultrasound and alkaline solution of EDTA. Desalination 264 (1-2), 63–69.
Müller, M., Hermes, G.D.A., Canfora, E.E., Smidt, H., Masclee, A.A., Zoetendal, E.G., Blaak, E.E., 2020. Distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans with slow colonic transit. Am. J. Physiol. Gastrointest. Liver Physiol. 318 (2) G361-G369.
Nagaraj, V., Skillman, L., Li, D., Xie, Z., Ho, G., 2019. Culturable bacteria from a full-scale desalination plant: Identification methods, bacterial diversity and selection of models based on membrane-biofilm community. Desalination 457, 103–114.
Nagaraja, N., Skillman, L., Xie, Z., Jiang, S., Ho, G., Li, D., 2017. Investigation of compounds that degrade biofilm polysaccharides on reverse osmosis membranes from a full scale desalination plant to alleviate biofouling. Desalination 403, 88–96.
Nguyen, T., Roddick, F.A., Fan, L., 2012. Biofouling of water treatment membranes: a review of the underlying causes, monitoring techniques and control measures. Membranes 2 (4), 804–840.
Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., 2018. vegan: Community Ecology Package. R package version 2.5-2. 2018.
Pang, C.M., Hong, P., Guo, H., Liu, W.-T., 2005. Biofilm formation characteristics of bacterial isolates retrieved from a reverse osmosis membrane. Environ. Sci. Technol. 39 (19), 7541–7550.
Pang, C.M., Liu, W.T., 2007. Community structure analysis of reverse osmosis membrane biofilms and the significance of Rhizobiales bacteria in biofouling. Environ. Sci. Technol. 41 (13), 4728–4734.
Paul, E., Ochoa, J.C., Pechaud, Y., Liu, Y., Liné, A., 2012. Effect of shear stress and growth conditions on detachment and physical properties of biofilms. Water Res 46 (17), 5499–5508.
Petrova, O.E., Sauer, K., 2016. Escaping the biofilm in more than one way: desorption, detachment or dispersion. Curr. Opin. Microbiol. 30, 67–78.
Poncheewin, W., Hermes, G.D.A., Van Dam, J.C., Koehorst, J.J., Smidt, H., Schaap, P.J., 2019. NG-Tax 2.0: A semantic framework for high-throughput amplicon analysis. Front. Genet. 10, 1366.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41 D590-D596.
Radu, A., van Steen, M.S.H., Vrouwenvelder, J.S., Van Loosdrecht, M.C.M., Picioreanu, C., 2014. Spacer geometry and particle deposition in spiral wound membrane feed channels. Water Res 64, 160–176.
Ramiro-Garcia, J., Hermes, G.D.A., Giatsis, C., Sipkema, D., Zoetendal, E.G., Schaap, P.J., Smidt, H., 2018. NG-Tax, a highly accurate and validated pipeline for analysis of 16S rRNA amplicons from complex biomes [version 2; peer review: 2 approved, 1 approved with reservations, 1 not approved]. F1000Research 5, 1791 2018.
Regula, C., Carretier, E., Wyart, Y., Gésan-Guiziou, G., Vincent, A., Boudot, D., Moulin, P., 2014. Chemical cleaning/disinfection and ageing of organic UF membranes: a review. Water Res 56, 325–365.
Sanawar, H., Pinel, I., Farhat, N., Bucs, S.S., Zlopasa, J., Kruithof, J., Witkamp, G., van Loosdrecht, M.C.M., Vrouwenvelder, J.S., 2018. Enhanced biofilm solubilization by urea in reverse osmosis membrane systems. Water Res. X 1, 100004.
Sauer, K., Cullen, M., Rickard, A., Zeef, L., Davies, D., Gilbert, P., 2004. Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. J. Bacteriol. 186 (21), 7312–7326.
Schleheck, D., Barraud, N., Klebensberger, J., Webb, J.S., McDougald, D., Rice, S.A., Kjelleberg, S., 2009. Pseudomonas aeruginosa PAO1 preferentially grows as aggregates in liquid batch cultures and disperses upon starvation. PloS one 4 (5), e5513.
Shao, Y., Chung, B.S., Lee, S.S., Park, W., Lee, S.-S., Jeon, C.O., 2009. Zoogloea caeni sp. nov., a floc-forming bacterium isolated from activated sludge. Int. J. Syst. Evol. Microbiol. 59 (3), 526–530.
Stoodley, P., Dodds, I., Boyle, J.D., Lappin-Scott, H., 1998. Influence of hydrodynamics and nutrients on biofilm structure. J. Appl. Microbiol. 85 (S1) 19S-28S.
Tan, Y.-J., Sun, L.-J., Li, B.-T., Zhao, X.-H., Yu, T., Ikuno, N., Ishii, K., Hu, H.-Y., 2017. Fouling characteristics and fouling control of reverse osmosis membranes for desalination of dyeing wastewater with high chemical oxygen demand. Desalination 419, 1–7.
Thormann, K.M., Saville, R.M., Shukla, S., Spormann, A.M., 2005. Induction of rapid detachment in Shewanella oneidensis MR-1 biofilms. J. Bacteriol. 187 (3), 1014–1021.
Webb, J.S., Thompson, L.S., James, S., Charlton, T., Tolker-Nielsen, T., Koch, B., Givskov, M., Kjelleberg, S., 2003. Cell death in Pseudomonas aeruginosa biofilm development. J. Bacteriol. 185 (15), 4585–4592.
Wickham, H., 2016. ggplot2: elegant graphics for data analysis. Springer International Publishing doi:10.1007/978-3-319-24277-4.
Xie, Z., Skillman, L., Nagaraj, V., Li, D., Ho, G., 2019. Experimental investigation into the use of sodium nitroprusside for controlling polysaccharide fouling in membrane separation. J. Water Process. Eng. 27, 171–176.