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Graphical abstract**Biofilm activity and quantity at 40 mbar/cm pressure drop increase**

**Effect of water temperature on biofouling
development in reverse osmosis membrane systems**

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21 Highlights

- 22 • Impact of temperature on biofilm development in RO
23 systems has been evaluated.
- 24 • Biofilm growth and pressure drop increase were faster at
25 higher water temperature.
- 26 • Biofilm thickness decreased at higher feed water
27 temperatures.
- 28 • Biomass accumulation decreased at higher feed water
29 temperatures.
- 30 • Biofilm structure and composition highly affected the
31 pressure drop increase.

Abstract

Understanding the factors that determine the spatial and temporal biofilm development is a key to formulate effective control strategies in reverse osmosis membrane systems for desalination and wastewater reuse. In this study, biofilm development was investigated at different water temperatures (10, 20, and 30 °C) inside a membrane fouling simulator (MFS) flow cell. The MFS studies were done at the same crossflow velocity with the same type of membrane and spacer materials, and the same feed water type and nutrient concentration, differing only in water temperature. Spatially resolved biofilm parameters such as oxygen decrease rate, biovolume, biofilm spatial distribution, thickness and composition were measured using *in-situ* imaging techniques. Pressure drop (PD) increase in time was used as a benchmark as to when to stop the experiments. Biofilm measurements were performed daily, and experiments were stopped once the average PD increased to 40 mbar/cm. The results of the biofouling study showed that with increasing feed water temperature (i) the biofilm activity developed faster, (ii) the pressure drop increased faster, while (iii) the biofilm thickness decreased. At an average pressure drop increase of 40 mbar/cm over the MFS for the different feed water temperatures, different biofilm activities, structures, and quantities were found, indicating

55 that diagnosis of biofouling of membranes operated at different or
56 varying (seasonal) feed water temperatures may be challenging.
57 Membrane installations with a high temperature feed water are
58 more susceptible to biofouling than installations fed with low
59 temperature feed water.

60 **Keywords:** sensor; non-destructive; growth rate; reverse
61 osmosis; seawater desalination; wastewater reuse

1. Introduction

Reverse osmosis (RO) and nanofiltration (NF) membranes are used to produce high-quality drinking water from abundantly available brackish and seawater sources (Elimelech and Phillip 2011). The increase in potable water scarcity in many regions around the world resulted in investments for improvement of this technology, and the share of membrane-based desalination has grown to 80 percent of the total global desalination in the past 40 years (Greenlee et al. 2009). However, biofouling occurrence and its proper control strategies persist as a major concern for the application of membrane desalination processes (Vrouwenvelder et al. 1998, Baker and Dudley 1998). A better understanding of the biofouling process is the key to develop effective control strategies.

Biofouling becomes a problem when biofilm growth in membrane systems results in a performance decline that exceeds a certain threshold. In practice, this threshold is in most cases defined as 15 percent increase in feed channel pressure drop or reduction in permeate flux (Al-Ahmad et al. 2000, Hydranautics 2001, Vrouwenvelder et al. 2008, GE 2009, Bucs et al. 2014, DOW 2016a). Two types of pressure drop can be distinguished in spiral-wound membrane modules: the feed channel pressure drop (PD), differential pressure between feed and concentrate, and the

85 trans-membrane pressure drop (TMP), differential pressure
86 between feed and permeate. Biofouling occurrence results in an
87 increase in PD and flux decline requiring the increase in TMP to
88 maintain the necessary permeate quantity (Bucs et al. 2014). In
89 systems suffering from biofouling cleaning cycles are governed by
90 the pressure drop over the feed channel. The feed channel contains
91 both the feed spacer and membrane on which biofilms develop.
92 Feed spacer presence and biofilm development on this feed spacer
93 results in a much higher PD increase compared to biofouling on
94 membrane surface only; therefore, in both spiral-wound NF and
95 RO systems biofouling is predominantly a feed spacer problem
96 (Vrouwenveder et al. 2009, 2009a).

97 Biofouling development occurs due to the gradual
98 accumulation of biological matter. Planktonic cell attachment is
99 the initial step in the colonization of abiotic and biotic surfaces
100 (Palmer et al. 2007). Cell attachment is a complex process
101 regulated by diverse characteristics of the growth medium,
102 substratum, cell surface and hydrodynamics (Donlan 2002).
103 Following attachment, bacterial growth using available nutrients
104 from the feed water becomes the prevalent process toward the
105 development of the biofilm. As with bacterial cell attachment,
106 bacterial cell growth is affected by the chemical and physical
107 nature of the surrounding. Factors such as water temperature, pH,

108 and nutrient availability have a significant effect on bacterial
109 growth rate.

110 Water temperature can be conceived as a key controlling factor
111 regulating bacterial growth (Madigan 2005) playing a direct and
112 indirect role in affecting the factors that govern bacterial growth
113 (Ratkowsky et al. 1982). Water temperature regulates several
114 environmental genes, sometimes resulting in bacterial cell surface
115 changes that could affect bacterial cell attachment (Giaouris et al.
116 2005). Van Loosdrecht et al. (1990) have shown that water
117 temperature modifies hydrophobicity and microbial cell surface
118 charge thus playing an important role in initial cell attachment.
119 Pompermayer and Gaylarde (2000) showed that some bacteria
120 adhere better at lower temperatures while others adhere better at
121 higher temperatures. Water temperature also has a significant
122 effect on the extracellular polymeric substances (EPS) matrix
123 produced by the bacteria (Lewis et al. 1989) and high temperatures
124 result in a decrease in the EPS matrix viscosity (Morimatsu et al.
125 2012). Temperature might further determine changes in the
126 composition of bacterial populations (Lindstrom et al. 2005).
127 Water temperature also has an effect on water viscosity and
128 nutrient diffusion coefficients. On a larger scale, water
129 temperature influences treatment plant efficiency, disinfection

effectiveness, dissipation of residual disinfectant, corrosion rates,
and distribution system hydraulics.

The impact of water temperature on bacterial growth and metabolism is well documented (Gillooly et al. 2001, Brown et al. 2004, Davidson et al. 2006), and the enzymatic respiratory activities of bacterial cells almost double at a temperature increase of 10 °C (Amthor 1984, Collier and Cummins 1990, Villanueva et al. 2011, Perkins et al. 2012). An increase in bacterial biofilm biomass and activity might be expected under high-temperature conditions. However, this is valid in the exponential bacterial growth phase when there is maximum bacterial growth. At the end of the exponential bacterial growth phase, the growth of biofilms becomes nutrient limited, and the balance between change in nutrient transport and maintenance energy determines the rate of biofilm development in this phase. Biofilm growth rates are expected to increase with temperatures (Ratkowsky et al. 1982), but bacterial growth efficiency (del Giorgio and Cole 1998), the amount of new bacterial biomass produced per unit of organic carbon nutrient assimilated, may decline which may result in less biomass (Sand-Jensen et al. 2007).

The effect of water temperature specifically on biofouling development in RO membrane installations is poorly reported in literature. In this study, transparent luminescent planar O₂ optodes,

153 in combination with an imaging system, were used to map the two-
154 dimensional distribution of O_2 decrease rates inside a membrane
155 fouling simulator (MFS) representative for spiral-wound RO and
156 NF membrane modules (Vrouwenvelder et al. 2006, 2007a,
157 2007b). The imaging system was used together with rhodamine
158 (tracer dye) for a qualitative spatial imaging of flow channels and
159 biofilm. Pressure drop development was recorded throughout the
160 experiments and confocal laser scanning microscopy (CLSM) was
161 done on the membrane sheets at the end of the experiments to
162 distinguish biofilm composition and thickness. The main objective
163 of this study was to evaluate the effect of water temperature on
164 biofilm development, quantity, structure, and how this correlates
165 with pressure drop increase.

2. Materials and methods

2.1. Experimental setup

Biofilms were grown in a membrane fouling simulator (MFS) with a transparent window for visual observations and imaging. The MFS contains a 20 cm $\times$ 4 cm coupon of a membrane and a feed spacer (Vrouwenvelder et al. 2006, 2007b). The inlet is defined as the first 2.5 cm of the MFS. The MFS was operated without permeate production at a pressure of one bar. Earlier studies done with membrane elements in the same position in an NF installation, with and without permeate production, showed the same development of PD increase and biofilm formation (Vrouwenvelder et al. 2007a, 2007b, 2008, 2009c). Mass transfer calculations supported the observations that the permeate flux is not playing a significant role in the nutrient supply to the fouling layer. Hydrodynamic conditions in the MFS were similar to spiral wound membrane modules as applied in practice for water treatment (Vrouwenvelder et al. 2007b). The spacer and membrane sheets were taken from virgin spiral wound membrane elements (4040-TS80-TSF, Trisep Corporation, USA). The feed spacer consisted of a sheet of 31 mil (787 μ m) thick diamond-shaped polypropylene spacer. The feed spacer was placed in the MFS with the same orientation as in spiral wound membrane elements (45° contact angle with feed flow).

189 The system was run as a continuous flow reactor and the setup
190 consisted of a buffer tank (200 L capacity), diaphragm pump,
191 temperature and differential pressure transmitter (Delta bars,
192 Endress+Hauser, PMD75) (Vrouwenvelder et al. 2009b), pressure-
193 reducing valve, nutrient dosing diaphragm metering pump, MFS
194 and a flow sensor (Ultra flow sensor ULF01.H.0, POM/FPM)
195 (Bucs et al. 2015a). Feed water was filtered by a carbon and
196 cartridge filter (filter housing model: UPS BB3 [AWF-UPS-3H-
197 20B] Cartridges model: pore size 4 μm , sediment-carbon [AC-SC-
198 10-NL]) before passing through the MFS (figure 1).

199 FIGURE 1

200 Filtered tap water ($16 \text{ L}\cdot\text{h}^{-1}$) continuously refreshed the water in the
201 buffer tank. Carbon filters were used to remove residual chlorine
202 from tap water. Water from the buffer tank was pumped through
203 the MFS at a flow rate of $16 \text{ L}\cdot\text{h}^{-1}$ equivalent to a linear flow
204 velocity of $0.16 \text{ m}\cdot\text{s}^{-1}$ which is representative for practice
205 (Vrouwenvelder et al. 2009c, Bucs et al. 2015b). The outflow from
206 the MFS was recirculated into the tank. The buffer tank water was
207 aerated to ensure saturated oxygen conditions in the MFS to ensure
208 oxygen decrease detected is due to biofilm formation. A circulating
209 water bath with temperature control (VWR MX07R-20
210 refrigerating / heating water bath) was used to heat and cool the
211 feed water temperature to 10, 20, and 30 °C. The water temperature

212 was kept constant during the whole experiment. A nutrient stock
213 solution containing sodium acetate, sodium nitrate, and sodium
214 phosphate in a mass ratio C:N:P of 100:20:10 was added to the
215 feed water increasing the feed water nutrient concentration by 1
216 $\text{mg-C}\cdot\text{L}^{-1}$ to enhance biofilm formation in the MFS. Acetate and
217 this mass ratio is commonly used (Vrouwenvelder et al. 2009a,
218 Araujo et al. 2012, Creber et al. 2010). All chemicals were
219 purchased in analytical grade from Sigma aldrich. The stock
220 solution was dosed by a diaphragm metering pump (STEPDOS
221 03S, Knf NEUBERGER). All experiments were run in duplicates.

222 **2.2. Imaging system description**

223 Oxygen imaging was done with an Apogee Imaging Systems
224 Ascent A285 CCD Camera equipped with a monochrome Sony
225 ICX-285 Interline CCD Sensor (1392×1040 pixels). A filter
226 wheel (APOGEE CFW31-8R FILTER WHEEL 1.25, 8 POS) was
227 mounted on the camera. A 690-850 nm filter (Astrodon
228 photometrics, sloan photometric filters i'2) was used to take the
229 oxygen images. For the rhodamine images a 560-690 nm filter
230 (Astrodon photometrics, sloan photometric filters r'2) was used.
231 On top of the filter wheel a Nikon F-mount lens adapter was
232 mounted and a Nikon Nikkor 35mm f/1.4 lens was used with
233 Nikon macro extension tube Kit resulting in a focal distance of 15
234 cm, depth of field 1.9 – 2.2 mm, and a pixel size of $45 \mu\text{m} \times 45$

235 μm . Micro-manager 1.4, a freeware microscope-controlling
 236 software program based on ImageJ, was used to control the camera
 237 and filter wheel settings.

238 **2.3. Optode description**

239 The oxygen sensing dye used in the planar optode was based
 240 on the dye PtTPTBPF (excitation 595 nm, emission 775 nm)
 241 immobilized in a polystyrene matrix (4% w/w) (Borisov et al.
 242 2008). The oxygen sensing dye was excited by eight amber light
 243 emitting diodes (1 Watt LEDs, lumiled type, 595 nm) placed
 244 around the camera lens. The power supply for the LEDs was an
 245 AIM-TTI instruments, PL303 power supply, 1CH, 30V, 3A,
 246 providing a stable LED output. The MFS and the camera system
 247 were mounted in a light-tight box to minimize the effect of
 248 external light on the luminescence images.

249 The PtTPTBPF oxygen sensing was calibrated at the three
 250 water temperatures used in the experiments 10, 20, and 30 °C. A
 251 calibration curve can be determined by correlating known oxygen
 252 concentrations with the ratio of the intensity under anoxic
 253 condition to that under different O_2 concentrations according to the
 254 modified stern-volmer equation (Eq.1).

$$255 \quad \frac{I}{I_0} = \frac{1-\alpha}{1+K_{sv}[\text{O}_2]} + \alpha \quad (1)$$

256 I_0 : luminescence intensity in the absence of O_2

257 I : luminescence intensity in the presence of O_2

258 K_{qv} : constant expressing the quenching efficiency of the
259 fluorophore
260 $[O_2]$: oxygen concentration
261 α : non-quenchable fraction of the dye

262 The three calibration curves are shown in figure S1 in
263 supplementary material.

264 **2.4. Oxygen decrease rate**

265 Oxygen distribution was calculated with a luminescence
266 intensity imaging approach (Staal et al. 2011). Details regarding
267 the method and oxygen distribution determination can be found
268 (Farhat et al. 2015). The oxygen decrease rate (ODR) was
269 determined using a stop-flow imaging protocol. The stop-flow
270 imaging protocol consisted of the following sequential steps (i) a
271 dark intensity image, (ii) the stop-flow image series, (iii) and the
272 anaerobic intensity image (Farhat et al. 2015). A dark intensity
273 image was taken and subtracted from all intensity images to
274 eliminate the background effect. Afterwards, a series of 100
275 intensity images was taken during a 10-minute MFS flow
276 interruption at an interval of six seconds. The anaerobic
277 luminescence intensity image was made after pumping of a
278 concentrated solution of sodium hydrosulfite ($20 \text{ g}\cdot\text{L}^{-1}$) into the
279 MFS feed water at a rate of $0.125 \text{ mL}\cdot\text{h}^{-1}$. The anaerobic
280 luminescence intensity image was taken on a daily basis, and
281 sodium hydrosulfite consumed all oxygen present in the monitor in
282 less than one minute. The water flow through the MFS was

diverted to the drain when sodium hydrosulfite was pumped to prevent contamination of the water in the buffer tank. The stop-flow intensity image series was divided by the anaerobic intensity image where after the stack of ratio images is converted into a stack of O_2 concentration image using Stern-Volmer equation (Farhat et al. 2015).

2.5. Rhodamine imaging

Free flowing water was visualized by injecting $20 \text{ mL} \cdot \text{min}^{-1}$ of a diluted (0.5%) rhodamine WT solution (Model # 00298-16, Cole-Parmer, USA) to the MFS feed water using a T-connector placed in the feed water tube before the MFS. Rhodamine WT is an inert, non-adsorbing and stable tracer for flow visualization. Rhodamine imaging is able to differentiate between biofilm filled regions and flow channels when the measurement was carried out immediately after injection of the rhodamine solution. The use of rhodamine dye enables visualization of the spatial distribution of accumulated biomass and biomass quantification by comparing the decrease in intensity signal of rhodamine at the start of the experiment to that at any day as biofilm develops. Rhodamine WT was excited with eight blue light emitting diodes (1 Watt LEDs, lumiled type, 460 nm) and emission was measured at a filter wavelength range of 560-690 nm, which includes rhodamine's emission peak at 585nm.

306 The intensity of fluorescence of rhodamine WT showed an
307 inverse relationship with temperature. Calibration of the rhodamine
308 WT intensity signal was done at the different temperatures.

309 **2.6. Confocal laser scanning microscopy (CLSM)**

310 Confocal Laser Scanning Microscopy (CLSM) (LSM710
311 upright confocal microscope, Zeiss, Germany, equipped with a
312 diode laser (405 nm)) was used to identify and characterize
313 biofouling on the membrane surface after the experiments. Biofilm
314 images were observed with a water immersion lens (60× object and
315 numerical aperture of 1.4) and a series of z-axis images were
316 generated through optical sectioning at a sliced thickness of 1 µm.
317 Membrane samples from the MFS inlet were taken at the end of all
318 the experiments. The samples were stained with the following dyes
319 based on methods described in the literature (Chen et al. 2006, Lee
320 et al. 2010, Valladares Linares et al. 2014): i) 4',6-Diamidino-2-
321 Phenylindole (DAPI) dye (emission wavelength of 461 nm;
322 excitation wavelength of 358 nm) was used to stain DNA to
323 identify total bacterial cells (incubation in dark room for 20 min),
324 ii) Fluorescein Isothiocyanate (FITC) (emission wavelength of
325 500-540 nm; excitation wavelength of 488 nm) was applied to
326 stain the amine-reactive compound-like proteins and amino sugars
327 (incubation time of 60 min); a 0.1 M sodium bicarbonate buffer
328 was used to retain the amine groups so the dying was effective, and

329 iii) Calcofluor White (emission wavelength of 410-480 nm;
330 excitation wavelength of 400 nm) was used to stain β -D-
331 glucopyranose polysaccharides (incubation time of 1 min). After
332 each of these three staining stages, the sample was washed twice
333 by phosphate-buffered saline (PBS) pH 7 to remove excess stain.

334 Comstat software was used to analyze the image stacks of the
335 biofilms determined using CLSM (Heydorn et al. 2000). CLSM
336 was done at five locations from the MFS inlet samples; an average
337 and standard deviation value of all parameters analyzed, was
338 calculated based on the results from these five locations and were
339 presented in the results.

340 **3. Results**

341 In this study, several parameters were used to describe the
342 effect of water temperature on biofouling development in RO
343 membrane systems. All experiments were stopped once an 800
344 mbar increase in overall pressure drop (40 mbar/cm) was observed
345 (figure 2). Oxygen sensing optode imaging was used to evaluate
346 biofilm activity and show the spatial distribution of the developed
347 biofilm (figures 3, 4 and 5). Direct imaging using rhodamine
348 enabled distinguishing areas with biofilm and allowed a qualitative
349 distinction of the level of biofilm present through variations in
350 intensity (figure 6). Confocal laser scanning microscopy (CLSM)
351 was used for biofilm thickness determination and imaging of
352 biofilm composition (figure 7).

353 **3.1. Pressure drop**

354 The pressure drop (PD) development was recorded for all the
355 MFS experiments at the different temperatures. The increase in PD
356 was used as the main criterion for stopping the experiments. All
357 experiments were stopped once an overall PD increase of 800 mbar
358 was observed. The exponential increase in PD was indicative of
359 biofouling formation. A higher water temperature (30 °C) resulted
360 in faster development of biofouling. The PD increase reached 800
361 mbar in approximately two days for 30 °C, five days for 20 °C, and

18 days for 10°C. This indicates that a higher water temperature (30°C) resulted in faster development of biofouling.

FIGURE 2

3.2. Biofilm activity

Oxygen decrease rate (ODR) was used to indicate the activity and extent of biofilm presence. ODR ($\text{mg}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$) is a parameter that measures how fast oxygen decreases at the biofilm attachment surface in the MFS when the water flow is stopped. The ODR measures the net effect of oxygen consumption and diffusion at the biofilm attachment surface. The average ODR increased in time for all the water temperatures (figure 3A). The ODR increase exhibited a similar trend with time as the exponential increase in PD confirming biofouling development, but the ODR increase started one day earlier than the pressure drop increase highlighting the imaging system's sensitivity.

At the end of the experiment at an overall PD increase of 800 mbar (40 mbar/cm), highest biofilm activity (ODR) was observed at the lowest temperature (10°C) (figure 3B). The lowest biofilm activity was found at 20°C. Biofilm activity at 30°C was slightly lower when compared to the highest biofilm activity seen at 10°C. The difference in biofilm activity at an equal PD for all the temperature experiments agree with differences found in biofilm structure (figure 4, 6, &7).

385 FIGURE 3

386 Oxygen sensing optode imaging allowed the observation of
387 the spatial distribution of biofilm activity at the different
388 temperatures. The structure of the developed biofilm, its spatial
389 distribution, and biofilm activity as represented in the ODR are
390 shown in figure 4. Figure 4A shows the ODR at 10°C where most
391 of the biofilm activity (highest ODR) occurred at the feed spacer
392 strand. Lower biofilm activity was observed between the two feed
393 spacer strands. The average biofilm activity at 20°C was not only
394 lower compared to other temperatures but also had different
395 structure (figure 4B). Development of more rounded biofilm
396 structures, as well as formation of preferential flow areas, was
397 clearly visible at 20°C. The growth rate of the biofilm was highest
398 at 30°C, the biofilm showed a moderate average activity that
399 appeared to be more homogenous over the imaged area (figure 4C)
400 compared to the other temperatures. No clear oxygen limitation
401 was observed during or at the end of the experiment.

402 FIGURE 4

403 Figure S2 in supplementary material shows a detailed
404 spatial distribution of the ODR separated into four ranges of low,
405 medium and high ODR. This detailed segregation was made to
406 differentiate low, medium and high oxygen decrease locations.

407 The increase of ODR during biofilm development is shown
408 in figure 5 for the moment when the pressure drop reached 200 and
409 800 mbar in the 20 °C experiment. At the early stage biofilm (figure
410 5A) areas with high and areas with low ODR had developed in a
411 spatially heterogeneous way. The higher biofilm activity areas
412 observed in figures 5A and 5B contain more biofilm volume and
413 will force water to flow through the areas with lower biofilm
414 activity due to a lower water resistance resulting in preferential
415 flow channel formation.

416 FIGURE 5

417 Biofilm activity seemed to be governed by the biofilm's
418 structure and composition with highest biofilm activity observed at
419 10 °C.

420 **3.3. Qualitative biofilm imaging**

421 Rhodamine was used to differentiate between biofilm filled
422 regions and water flow channels. Direct imaging using rhodamine
423 enabled distinguishing areas with biofilm from areas with water
424 and allowed a qualitative spatial estimation of the level of biofilm
425 present through variations in rhodamine fluorescence intensity
426 (figure 6 A, B, and C).

427 FIGURE 6

428 After injection of rhodamine, the regions with no biomass had
429 the highest intensity signal, and the more biomass present, the

430 lower intensity signal was detected. Figure 6 (A, B, and C) shows
431 biofilm distribution based on intensity variation from the
432 rhodamine signal on the last day of the experiments. On average,
433 the areas with high biomass as measured with rhodamine (figure 6)
434 coincide with high activity areas (figure 4). The amount of biofilm
435 present was highest at 10°C (figure 6D), followed by 20°C and was
436 the least at 30°C as confirmed by visual observations after opening
437 the MFS flow cell.

438 **3.4. Confocal laser scanning microscopy (CLSM)**

439 CLSM was used for biofilm thickness determination and
440 imaging of biofilm composition (figure 7) on the membrane
441 surface. Average biofilm thickness was determined by analyzing
442 the image stacks using Comstat software. CLSM imaging was
443 done at five locations from the MFS inlet samples; the average and
444 standard deviation value of biofilm thickness, bacterial cell
445 coverage, and EPS coverage were calculated based on the
446 measurements from these five locations. Figure 7A shows that the
447 thickest biofilm developed at 10°C followed by that at 20°C with
448 the thinnest biofilm developing at 30°C all having same impact on
449 PD. As for biofilm composition the use of comstat software
450 enabled quantification of the coverage of total bacterial cells
451 stained with DAPI, polysaccharides (stained with Calcofluor
452 White) and proteins (stained with FITC). Figure 7B shows the

ratio of bacterial cell coverage to the total EPS matrix (polysaccharides and proteins). The ratio of bacterial cells to total EPS coverage was highest at 30°C followed by 10°C and was lowest at 20°C. The higher the ratio between bacterial cell coverage to total EPS coverage the greater the presence of bacterial cells that can contribute to biofilm activity. Figure 7C shows the bacterial cell coverage per unit area which was determined by multiplying the biofilm thickness with the cell coverage divided by the volume. The bacterial cell coverage per unit area showed a similar trend as the ODR at PD of 800 mbar. In summary, the biofilm became thinner with increasing feed water temperature.

FIGURE 7

465 **4. Discussion**

466 The objective of the study was to assess the effect of water
 467 temperature on the development of biofilm and pressure drop. The
 468 results showed that with increasing feed water temperature (i) the
 469 pressure drop increased faster (figure 2), (ii) the biofilm activity
 470 developed faster (figure 3), while (iii) the biofilm thickness
 471 decreased (figure 7). At an average pressure drop increase of 40
 472 mbar/cm over the MFS, for the different feed water temperatures
 473 different biofilm activities (figures 3B and 4), structures (figure 4)
 474 and quantities (figure 5) were found.

475 **4.1. Different biofilm quantities, activities, and structure** 476 **resulting in equal pressure drop (PD) increase**

477 Feed channel PD measurements are based on the resistance that
 478 water experiences when passing through the feed-concentrate
 479 channel of the membrane module during operation. At constant
 480 cross flow velocity, feed channel PD increases with an increasing
 481 biomass amount, but the location where the biofilm forms will
 482 determine the hydraulic resistance per biofilm volume. At an
 483 average pressure drop increase of 40 mbar/cm over the MFS for
 484 the different feed water temperatures, different biofilm activities,
 485 structures, and quantities were found, indicating that diagnosis of
 486 biofouling of membranes operated at different or varying
 487 (seasonal) feed water temperatures based on PD measurements

488 may be challenging (Vrouwenvelder et al. 2010). It should be
489 mentioned that the MFS studies were performed at a pressure of 1
490 bar. A study from Xie et al. (2015) on organic fouling in forward
491 osmosis (FO) and RO showed differences in fouling layer related
492 to the difference in applied pressure related to process differences.
493 Biofouling studies using NF and RO membranes done with MFS
494 units, single element test rigs, and a full scale installation showed
495 the same performance decline and biofilm development
496 irrespective of the difference in applied feed pressure
497 (Vrouwenvelder et al. 2007a).

498 The oxygen decrease rate (ODR) was used as an indication of
499 biofilm activity. At the end of the experiments, highest ODR was
500 observed for the 10°C experiment. At 10°C, oxygen solubility in
501 water is highest; however, diffusion of nutrients is lowest.
502 Although the biofilm developed much slower with time at 10°C
503 compared to the other temperatures, no nutrient limitation was
504 evident. In other words, both biodegradable carbon (“food for
505 bacteria”) and oxygen were not limiting the biofilm growth at
506 10°C. Biofilm development at 10°C appeared to be growth rate
507 limited only. Biofilm activity was highest at the spacer strand
508 indicating favored attachment and start of biofilm formation at the
509 spacer in agreement with observations in literature
510 (Vrouwenvelder et al. 2009a, Radu et al. 2014). Biofilm activity

(ODR) at 30°C was slightly lower than that at 10°C, however, biofilm quantity was the lowest and biofilm thickness was the thinnest. Higher bacterial cell to EPS ratio at 30°C (either higher cell attachment or lower EPS production) might have resulted in the high ODR value as temperature has a significant effect on bacterial cell attachment and the EPS matrix produced by the bacteria. Most of the studies conducted to evaluate the effect of water temperature specifically on bacterial attachment were performed with single strain bacteria or mixed cultures of two or three bacterial species (Pompermayer and Gaylarde 2000, Abdallah et al. 2015); where some strains adhered less at a higher temperature while others adhered better at a higher temperature (Abdallah et al. 2014, Nguyen et al. 2014). This study uses feed water with a mixed bacterial population, where each temperature may lead to variation in population composition optimized for the prevalent conditions, resulting in this net observed biofilm development. Lewis et al. (1989) showed that some water soluble fractions of the EPS are released at high temperatures. High temperature results in a decrease in the EPS matrix viscosity leading to higher detachment rates (Morimatsu et al. 2012). Van Loosdrecht et al. (1995) reported that faster growing biofilms developing at higher temperatures are more spatially heterogeneous (rough) with a less cohesive structure (weak). The

fact that the thinnest biofilm at 30°C resulted in a similar PD increase as the biofilms that developed at the other temperatures indicates indeed a more spatially heterogeneous (flow distorting) biofilm. Even without biofilm growth, at higher temperatures, the diffusion coefficient of nutrients increases while the oxygen solubility in water decreases later affecting the ODR value when biofilm develops. An increase in diffusion of nutrients as the temperature increases, as well as a thinner biofilm as seen at 30°C; all contribute to a more active biofilm. The activity of the biofilm grown at 20°C was the lowest. This low activity observed is probably the result of the low bacterial cell to EPS ratio calculated from the CLSM images, in agreement with the findings of Ma et al. (2013), indicating a fluffier biofilm, with a relatively low volumetric respiratory potential. Moreover, the contact between a fluffy biofilm and the optode is less tight, resulting in a lower measured activity. The development of preferential flow areas was visible at 20°C, and these heterogonous high and low activity regions were a clearly evident difference between the biofilm that developed at 20°C and the other temperatures. Preferential flow areas are considered to be the result of nutrient starvation (i.e. the nutrient does not reach all bacteria in the biofilm)(Bottero et al. 2013, Hunt et al. 2004); in our study it is most likely that carbon is the limiting nutrient at 20°C. As these preferential flow areas

557 develop and later clear flow channels form, diffusion of the
558 nutrient from the bulk becomes limited, as well as diffusion of
559 metabolic products out of the biofilm (Stewart 2012). As a result,
560 the biofilm's ability to consume oxygen and nutrients after the
561 exponential growth phase will further decrease.

562 The use of oxygen sensing planar optode imaging enabled
563 gaining spatial biofilm development information, *in-situ* non-
564 destructively (Staal et al. 2011, Farhat et al. 2015, Prest et al.
565 2012). Spatial oxygen distribution and oxygen decrease rates were
566 able to give an idea about the complexity of the biofilm structure.
567 The activity of a heterogeneous biofilm depends strongly on
568 characteristics of the bulk liquid-biofilm interface (Debeer et al.
569 1994), thus ODR measurements are highly affected by biofilm
570 thickness, composition, and nutrient limitations. The integration of
571 oxygen sensing optode imaging method with PD measurements
572 and CLSM imaging allowed gaining broader information about
573 biofilm development inside the MFS.

574 **4.2. Practical implications of the effect of temperature on** 575 **biofouling**

576 It is well established that the membrane efficiency (permeate
577 flux and salt retention) is very sensitive to changes in feed water
578 temperature. Water fluxes over the membrane increases almost
579 linearly with increasing temperature (DOW 2016b, Pressdee et al.

2006); primarily due to the higher diffusion rate of water. Increased feed water temperatures also results in lower salt rejection or higher salt passage. Again, this is due to a higher diffusion rate for salt through the membrane (DOW 2016b, Pressdee et al. 2006). The effect of water temperature variations on biofilm formation and thereby biofouling of membrane systems has had very limited attention in literature. This study presents the effect of water temperature on biofilm development however further research has to elucidate the interdependences of e.g. oxygen solubility, transfer of nutrient to the biofilm, conversion of nutrient by biomass, yield of biomass on nutrient, EPS production, and biomass community composition. This study showed that feed water temperature had a strong impact on structure, quantity and thickness of the biofouling layer in RO membrane systems. The presented results can have practical value for diagnosis of biofouling since it shows that at higher water temperatures e.g. a thinner biofilm can cause similar performance decline as a thicker biofilm developed at a low water temperature. This is especially relevant when comparing the extent of fouling of membrane module autopsy studies at a full-scale/pilot plant with other plants, emphasizing the need to look at plants with similar feed water temperatures. Seasonal feed water temperature variations can result in changes in bacterial cell attachment, EPS

603 production, and the whole structure of the developed biofilm,
604 resulting in different effects of accumulated biofilm on membrane
605 performance. Slow growing biofilms, at the lower temperatures,
606 are smooth and strong (Picioreanu et al. 1998). Therefore, the
607 membrane system's cleanability is affected by the influence of
608 temperature on the developed biofilm's structure and composition.
609 Cleanability studies should be done on biofilms developing at
610 different temperatures to test the hypothesis that faster growing,
611 less attached biofilms developing at higher water temperatures can
612 be easier to clean than slow growing, stronger attached biofilms
613 that develop at lower temperatures.

614 An important effect of the growth of a biofilm on the
615 membrane is the formation of an extra diffusion resistance layer
616 limiting the water flux and decreasing the salt rejection (Herzberg
617 and Elimelech 2007). At higher temperatures the biofilm layer
618 formed more rapidly as indicated by the pressure drop. When
619 biofilm layer thicknesses are compared at equal pressure drop
620 increase, results showed that the biofilms developing at lower
621 temperatures were thicker compared to those developing at higher
622 temperatures. Diffusion of oxygen into the biofilm was more
623 retarded at the lower temperatures. This might indicate that despite
624 a lower rate of formation, biofouling at lower temperatures might
625 have a relatively stronger negative impact on the permeate flux and

626 salt retention. Slower mass transfer at the lower temperature means
627 that ions accumulating at the membrane surface (concentration
628 polarization effect) will diffuse slower to the bulk liquid further
629 increasing the concentration polarization effect at low
630 temperatures. Future studies should study the impact of
631 temperature on biofouling development in a system with permeate
632 production to verify the hypothesis “that biofilms developing at a
633 higher water temperature, although causing faster increase in feed
634 channel pressure drop, have a lower impact on the permeate flux
635 and have a lower concentration polarization effect in the biofilm”.

636 5. Conclusion

637 Biofilm development in time was studied *in-situ* using (i) O₂
638 sensing optode imaging, (ii) direct imaging using rhodamine dye,
639 and (iii) feed channel pressure drop in an MFS. CLSM was used
640 for biofilm thickness determination and imaging of biofilm
641 composition. The main objective was to investigate the effect of
642 water temperature on the development of pressure drop and
643 biofilm quantity, structure, composition, and spatial distribution.

644 Based on the results it can be concluded that:

- 645 • Biofilm accumulation was faster at higher water
646 temperatures.
- 647 • At the same pressure drop increase, biofilm thickness
648 decreased with higher feed water temperature.
- 649 • Total biomass accumulation decreased with higher feed
650 water temperatures. Biofilm activity was affected by the
651 biofilm structure, and bacterial cell to extracellular
652 polymeric substances ratio, where the lowest biofilm
653 activity was seen at 20°C with the lowest bacterial cell to
654 extracellular polymeric substances ratio.
- 655 • Feed channel pressure drop results from a complex
656 interaction between biofilm structure, composition, and
657 quantity.

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References

- Abdallah, M., Benoliel, C., Jama, C., Drider, D., Dhulster, P. and Chihib, N.E. (2014) Thermodynamic Prediction of Growth Temperature Dependence in the Adhesion of *Pseudomonas aeruginosa* and *Staphylococcus aureus* to Stainless Steel and Polycarbonate. *Journal of Food Protection* 77(7), 1116-1126.
- Abdallah, M., Khelissa, O., Ibrahim, A., Benoliel, C., Heliot, L., Dhulster, P. and Chihib, N.E. (2015) Impact of growth temperature and surface type on the resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms to disinfectants. *International Journal of Food Microbiology* 214, 38-47.
- Al-Ahmad, M., Aleem, F.A.A., Mutiri, A. and Ubaisy, A. (2000) Biofouling in RO membrane systems Part 1: Fundamentals and control. *Desalination* 132(1-3), 173-179.
- Amthor, J.S. (1984) The Role of Maintenance Respiration in Plant-Growth. *Plant Cell and Environment* 7(8), 561-569.
- Araujo, P.A., Kruithof, J.C., Van Loosdrecht, M.C.M. and Vrouwenvelder, J.S. (2012) The potential of standard and modified feed spacers for biofouling control. *Journal of Membrane Science* 403, 58-70.
- Baker, J.S. and Dudley, L.Y. (1998) Biofouling in membrane systems - A review. *Desalination* 118(1-3), 81-89.
- Borisov, S.M., Nuss, G. and Klimant, I. (2008) Red Light-Excitable Oxygen Sensing Materials Based on Platinum(II) and Palladium(II) Benzoporphyrins. *Analytical Chemistry* 80(24), 9435-9442.
- Bottero, S., Storck, T., Heimovaara, T.J., van Loosdrecht, M.C.M., Enzien, M.V. and Picioreanu, C. (2013) Biofilm development and the dynamics of preferential flow paths in porous media. *Biofouling* 29(9), 1069-1086.
- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. and West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology* 85(7), 1771-1789.
- Bucs, S.S., Farhat, N., Siddiqui, A., Valladares Linares, R., Radu, A., Kruithof, J.C. and Vrouwenvelder, J.S. (2015a) Development

- of a setup to enable stable and accurate flow conditions for membrane biofouling studies. *Desalination and Water Treatment*.
- Bucs, S.S., Linares, R.V., Marston, J.O., Radu, A.I., Vrouwenvelder, J.S. and Picioreanu, C. (2015b) Experimental and numerical characterization of the water flow in spacer-filled channels of spiral-wound membranes. *Water Research* 87, 299-310.
- Bucs, S.S., Linares, R.V., van Loosdrecht, M.C.M., Kruithof, J.C. and Vrouwenvelder, J.S. (2014) Impact of organic nutrient load on biomass accumulation, feed channel pressure drop increase and permeate flux decline in membrane systems. *Water Research* 67, 227-242.
- Chen, M.Y., Lee, D.J., Yang, Z., Peng, X.F. and Lai, J.Y. (2006) Fluorescent staining for study of extracellular polymeric substances in membrane biofouling layers. *Environmental Science & Technology* 40(21), 6642-6646.
- Collier, D.E. and Cummins, W.R. (1990) The Effects of Low Growth and Measurement Temperature on the Respiratory Properties of 5 Temperate Species. *Annals of Botany* 65(5), 533-538.
- Creber, S.A., Vrouwenvelder, J.S., van Loosdrecht, M.C.M. and Johns, M.L. (2010) Chemical cleaning of biofouling in reverse osmosis membranes evaluated using magnetic resonance imaging. *Journal of Membrane Science* 362(1-2), 202-210.
- Davidson, E.A., Janssens, I.A. and Luo, Y.Q. (2006) On the variability of respiration in terrestrial ecosystems: moving beyond Q(10). *Global Change Biology* 12(2), 154-164.
- Debeer, D., Stoodley, P., Roe, F. and Lewandowski, Z. (1994) Effects of Biofilm Structures on Oxygen Distribution and Mass-Transport. *Biotechnology and Bioengineering* 43(11), 1131-1138.
- del Giorgio, P.A. and Cole, J.J. (1998) Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematics* 29, 503-541.
- Donlan, R.M. (2002) Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases* 8(9), 881-890.

- 752 DOW (2016a) DOW FILMTEC™ Membranes, Cleaning
 753 Procedures for DOW FILMTEC FT30 Elements 1-7.
 754 [http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_06](http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_060a/0901b8038060a66f.pdf?filepath=liquidseps/pdfs/noreg/609-23010.pdf&fromPage=GetDoc)
 755 [0a/0901b8038060a66f.pdf?filepath=liquidseps/pdfs/noreg/609-](http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_060a/0901b8038060a66f.pdf?filepath=liquidseps/pdfs/noreg/609-23010.pdf&fromPage=GetDoc)
 756 [23010.pdf&fromPage=GetDoc](http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_060a/0901b8038060a66f.pdf?filepath=liquidseps/pdfs/noreg/609-23010.pdf&fromPage=GetDoc)
 757
- 758 DOW (2016b) FILMTEC Membranes, Factors Affecting RO
 759 Membrane Performance. 1-4.
 760 [http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_00](http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_0030/0901b80380030095.pdf?filepath=liquidseps/pdfs/noreg/609-00055.pdf&fromPage=GetDoc)
 761 [30/0901b80380030095.pdf?filepath=liquidseps/pdfs/noreg/609-](http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_0030/0901b80380030095.pdf?filepath=liquidseps/pdfs/noreg/609-00055.pdf&fromPage=GetDoc)
 762 [00055.pdf&fromPage=GetDoc](http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_0030/0901b80380030095.pdf?filepath=liquidseps/pdfs/noreg/609-00055.pdf&fromPage=GetDoc)
 763
- 764 Elimelech, M. and Phillip, W.A. (2011) The Future of Seawater
 765 Desalination: Energy, Technology, and the Environment. *Science*
 766 333(6043), 712-717.
 767
- 768 Farhat, N.M., Staal, M., Siddiqui, A., Borisov, S.M., Bucs, S.S.
 769 and Vrouwenvelder, J.S. (2015) Early non-destructive biofouling
 770 detection and spatial distribution: Application of oxygen sensing
 771 optodes. *Water Research* 83(0), 10-20.
 772
- 773 GE (2009) Utilization of Chemical Treatments to Maintain and
 774 Restore Membrane Performance. GE Water & Process
 775 Technologies, 1-6.
 776 [https://www.gewater.com/kcpguest/documents/Technical%20Pape](https://www.gewater.com/kcpguest/documents/Technical%20Papers_Cust/Americas/English/tp503.pdf)
 777 [rs_Cust/Americas/English/tp503.pdf](https://www.gewater.com/kcpguest/documents/Technical%20Papers_Cust/Americas/English/tp503.pdf)
 778
- 779 Giaouris, E., Chorianopoulos, N. and Nychas, G.J.E. (2005) Effect
 780 of temperature, pH, and water activity on biofilm formation by
 781 *Salmonella enterica* Enteritidis PT4 on stainless steel surfaces as
 782 indicated by the bead vortexing method and conductance
 783 measurements. *Journal of Food Protection* 68(10), 2149-2154.
 784
- 785 Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. and
 786 Charnov, E.L. (2001) Effects of size and temperature on metabolic
 787 rate. *Science* 293(5538), 2248-2251.
 788
- 789 Greenlee, L.F., Lawler, D.F., Freeman, B.D., Marrot, B. and
 790 Moulin, P. (2009) Reverse osmosis desalination: Water sources,
 791 technology, and today's challenges. *Water Research* 43(9), 2317-
 792 2348.
 793
- 794 Herzberg, M. and Elimelech, M. (2007) Biofouling of reverse
 795 osmosis membranes: Role of biofilm-enhanced osmotic pressure.
 796 *Journal of Membrane Science* 295(1-2), 11-20.

- Heydorn, A., Nielsen, A.T., Hentzer, M., Sternberg, C., Givskov, M., Ersboll, B.K. and Molin, S. (2000) Quantification of biofilm structures by the novel computer program COMSTAT. *Microbiology-Uk* 146, 2395-2407.
- Hunt, S.M., Werner, E.M., Huang, B.C., Hamilton, M.A. and Stewart, P.S. (2004) Hypothesis for the role of nutrient starvation in biofilm detachment. *Applied and Environmental Microbiology* 70(12), 7418-7425.
- Hydranautics (2001) Troubleshooting Your RO - Hydranautics. 1-6.
<http://www.membranes.com/docs/trc/TROUBLES.PDF>
- Lee, W., Ahn, C.H., Hong, S., Kim, S., Lee, S., Baek, Y. and Yoon, J. (2010) Evaluation of surface properties of reverse osmosis membranes on the initial biofouling stages under no filtration condition. *Journal of Membrane Science* 351(1-2), 112-122.
- Lewis, S.J., Gilmour, A. and Johnston, D.E. (1989) Factors Influencing the Detachment of a Polymer-Associated *Acinetobacter* Sp from Stainless-Steel. *International Journal of Food Microbiology* 8(2), 155-164.
- Lindstrom, E.S., Kamst-Van Agterveld, M.P. and Zwart, G. (2005) Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. *Applied and Environmental Microbiology* 71(12), 8201-8206.
- Ma, Z., Wen, X.H., Zhao, F., Xia, Y., Huang, X., Waite, D. and Guan, J. (2013) Effect of temperature variation on membrane fouling and microbial community structure in membrane bioreactor. *Bioresource Technology* 133, 462-468.
- Madigan, M.T. (2005) *Brock Biology of Microorganisms*, 11th edn, SciELO Espana.
- Morimatsu, K., Eguchi, K., Hamanaka, D., Tanaka, F. and Uchino, T. (2012) Effects of Temperature and Nutrient Conditions on Biofilm Formation of *Pseudomonas putida*. *Food Science and Technology Research* 18(6), 879-883.
- Nguyen, H.D.N., Yang, Y.S. and Yuk, H.G. (2014) Biofilm formation of *Salmonella Typhimurium* on stainless steel and

- 842 acrylic surfaces as affected by temperature and pH level. *Lwt-Food*
843 *Science and Technology* 55(1), 383-388.
- 844
- 845 Palmer, J., Flint, S. and Brooks, J. (2007) Bacterial cell attachment,
846 the beginning of a biofilm. *Journal of Industrial Microbiology &*
847 *Biotechnology* 34(9), 577-588.
- 848
- 849 Perkins, D.M., Yvon-Durocher, G., Demars, B.O.L., Reiss, J.,
850 Pichler, D.E., Friberg, N., Trimmer, M. and Woodward, G. (2012)
851 Consistent temperature dependence of respiration across
852 ecosystems contrasting in thermal history. *Global Change Biology*
853 18(4), 1300-1311.
- 854
- 855 Picioreanu, C., van Loosdrecht, M.C.M. and Heijnen, J.J. (1998)
856 Mathematical modeling of biofilm structure with a hybrid
857 differential-discrete cellular automaton approach. *Biotechnology*
858 *and Bioengineering* 58(1), 101-116.
- 859
- 860 Pompermayer, D.M.C. and Gaylarde, C.C. (2000) The influence of
861 temperature on the adhesion of mixed cultures of *Staphylococcus*
862 *aureus* and *Escherichia coli* to polypropylene. *Food Microbiology*
863 17(4), 361-365.
- 864
- 865 Pressdee, J.R., Veerapaneni, S., Shorney-Darby, H.L. and Clement,
866 J.A. (2006) Integration of Membrane Filtration Into Water
867 Treatment Systems, American Water Works Association.
- 868
- 869 Prest, E.I., Staal, M., Kuhl, M., van Loosdrecht, M.C.M. and
870 Vrouwenvelder, J.S. (2012) Quantitative measurement and
871 visualization of biofilm O₂ consumption rates in membrane
872 filtration systems. *Journal of Membrane Science* 392, 66-75.
- 873
- 874 Radu, A.I., van Steen, M.S.H., Vrouwenvelder, J.S., van
875 Loosdrecht, M.C.M. and Picioreanu, C. (2014) Spacer geometry
876 and particle deposition in spiral wound membrane feed channels.
877 *Water Research* 64, 160-176.
- 878
- 879 Ratkowsky, D.A., Olley, J., Mcmeekin, T.A. and Ball, A. (1982)
880 Relationship between Temperature and Growth-Rate of Bacterial
881 Cultures. *Journal of Bacteriology* 149(1), 1-5.
- 882
- 883 Sand-Jensen, K., Pedersen, N.L. and Sondergaard, M. (2007)
884 Bacterial metabolism in small temperate streams under
885 contemporary and future climates. *Freshwater Biology* 52(12),
886 2340-2353.
- 887

- 888 Staal, M., Prest, E.I., Vrouwenvelder, J.S., Rickelt, L.F. and Kuhl,
889 M. (2011) A simple optode based method for imaging O₂
890 distribution and dynamics in tap water biofilms. *Water Res* 45(16),
891 5027-5037.
- 892
893 Stewart, P.S. (2012) Mini-review: Convection around biofilms.
894 *Biofouling* 28(2), 187-198.
- 895
896 Valladares Linares, R., Bucs, S.S., Li, Z., AbuGhdeeb, M., Amy,
897 G. and Vrouwenvelder, J.S. (2014) Impact of spacer thickness on
898 biofouling in forward osmosis. *Water Research* 57, 223-233.
- 899
900 Van Loosdrecht, M.C.M., Norde, W. and Zehnder, A. (1990)
901 Physical chemical description of bacterial adhesion. *Journal of*
902 *biomaterials applications* 5(2), 91-106.
- 903
904 Van Loosdrecht, M.C.M., Eikelboom, D., Gjaltema, A., Mulder,
905 A., Tijhuis, L. and Heijnen, J.J. (1995) Biofilm structures. *Water*
906 *Science and Technology* 32(8), 35-43.
- 907
908 Villanueva, V.D., Font, J., Schwartz, T. and Romani, A.M. (2011)
909 Biofilm formation at warming temperature: acceleration of
910 microbial colonization and microbial interactive effects.
911 *Biofouling* 27(1), 59-71.
- 912
913 Vrouwenvelder, J.S., van Paassen, J.A.M., van Agtmaal, J.M.C.,
914 van Loosdrecht, M.C.M. and Kruithof, J.C. (2009) A critical flux
915 to avoid biofouling of spiral wound nanofiltration and reverse
916 osmosis membranes: Fact or fiction? *Journal of Membrane Science*
917 326(1), 36-44.
- 918
919 Vrouwenvelder, H.S., van Paassen, J.A.M., Folmer, H.C., Hofman,
920 J.A.M.H., Nederlof, M.M. and van der Kooij, D. (1998) Biofouling
921 of membranes for drinking water production. *Desalination* 118(1-
922 3), 157-166.
- 923
924 Vrouwenvelder, J.S., Bakker, S.M., Cauchard, M., Le Grand, R.,
925 Apacandie, M., Idrissi, M., Lagrave, S., Wessels, L.P., van
926 Paassen, J.A.M., Kruithof, J.C. and van Loosdrecht, M.C.M.
927 (2007b) The Membrane Fouling Simulator: a suitable tool for
928 prediction and characterisation of membrane fouling. *Water*
929 *Science and Technology* 55(8-9), 197-205.
- 930
931 Vrouwenvelder, J.S., Bakker, S.M., Wessels, L.P. and van Paassen,
932 J.A.M. (2007a) The Membrane Fouling Simulator as a new tool for
933 biofouling control of spiral-wound membranes. *Desalination*
204(1-3), 170-174.

- 934 Vrouwenvelder, J.S., Buiters, J., Riviere, M., van der Meer, W.G.J.,
 935 van Loosdrecht, M.C.M. and Kruithof, J.C. (2010) Impact of flow
 936 regime on pressure drop increase and biomass accumulation and
 937 morphology in membrane systems. *Water Research* 44(3), 689-
 938 702.
- 939
 940 Vrouwenvelder, J.S., Hinrichs, C., Van der Meer, W.G., Van
 941 Loosdrecht, M.C. and Kruithof, J.C. (2009c) Pressure drop
 942 increase by biofilm accumulation in spiral wound RO and NF
 943 membrane systems: role of substrate concentration, flow velocity,
 944 substrate load and flow direction. *Biofouling* 25(6), 543-555.
- 945
 946 Vrouwenvelder, J.S., Manolarakis, S.A., van der Hoek, J.P., van
 947 Paassen, J.A.M., van der Meer, W.G.J., van Agtmaal, J.M.C.,
 948 Prummel, H.D.M., Kruithof, J.C. and van Loosdrecht, M.C.M.
 949 (2008) Quantitative biofouling diagnosis in full scale nanofiltration
 950 and reverse osmosis installations. *Water Research* 42(19), 4856-
 951 4868.
- 952
 953 Vrouwenvelder, J.S., van Paassen, J.A.M., Kruithof, J.C. and van
 954 Loosdrecht, M.C.M. (2009b) Sensitive pressure drop
 955 measurements of individual lead membrane elements for accurate
 956 early biofouling detection. *Journal of Membrane Science* 338(1-2),
 957 92-99.
- 958
 959 Vrouwenvelder, J.S., van Paassen, J.A.M., Wessels, L.P., van
 960 Dama, A.F. and Bakker, S.M. (2006) The membrane fouling
 961 simulator: A practical tool for fouling prediction and control.
 962 *Journal of Membrane Science* 281(1-2), 316-324.
- 963
 964 Vrouwenvelder, J.S., von der Schulenburg, D.A.G., Kruithof, J.C.,
 965 Johns, M.L. and van Loosdrecht, M.C.M. (2009a) Biofouling of
 966 spiral-wound nanofiltration and reverse osmosis membranes: A
 967 feed spacer problem. *Water Research* 43(3), 583-594.
- 968
 969 Xie, M., Lee, J., Nghiem, L.D. and Elimelech, M. (2015) Role of
 970 pressure in organic fouling in forward osmosis and reverse
 971 osmosis. *Journal of Membrane Science* 493, 748-754.
- 972

Figure 1 – Schematic description of the experimental set-up consisting of the membrane fouling simulator (MFS), a water mixing system, differential pressure transmitter, flow meter and the imaging setup for O₂ detection. The arrows indicate the water flow direction. A refrigerating / heating water bath was used to adjust the water temperature at 10, 20, and 30 °C.

Figure 2 – Pressure drop increase (PD increase) [mbar] with time over the MFS for the three different water temperatures (10, 20, and 30 °C). The MFS feed water was supplemented with 1 mg-C·L⁻¹ biodegradable substrate to increase the rate of biofilm accumulation. All experiments were stopped once a pressure drop increase of 800 mbar was reached. Pressure drop graphs are the average of duplicate experiments.

Figure 3 – (A) Oxygen decrease rate (ODR) as a function of time for three different water temperatures (10, 20, and 30 °C). (B) ODR at the end of the experiments when a PD increase of 800 mbar was reached. Error bar represents the variation between the duplicate experiments.

Figure 4 – Oxygen decrease rate [$\text{mg}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$] images on the last day of the experiments at pressure drop increase of 800 mbar (A, B and C for 10, 20, and 30 °C respectively). The images were taken at the inlet position of the flow cell (first 2.5 cm), ODR calculated based on the decrease rate one minute after stopping the flow. Flow direction is from left to right. Imaged area is 3.8 cm $\times$ 2.5 cm. The net-like structure of the feed spacer, located on the membrane, is visible.

Figure 5 – Oxygen decrease rate [$\text{mg}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$] images (A) on day four at a pressure drop increase of 200 mbar and (B) on the last day of the experiments at pressure drop increase of 800 mbar for the 20 °C experiment. The images were taken at the inlet position of the flow cell (first 2.5 cm), ODR calculated based on the decrease rate two minutes after stopping the flow. Flow direction is from left to right. Imaged area is $3.8\text{ cm} \times 2.5\text{ cm}$. The net-like structure of the feed spacer, located on the membrane, is visible.

Figure 6 – Biomass quantification using Rhodamine imaging; (A,B,C) spatial distribution of accumulated biomass measured as an increase in [%] of the total volume at 10, 20, and 30 °C respectively; (D) Average relative biomass increase [%] in the MFS for the three different water temperatures (10, 20, and 30 °C). All images were taken at the end of the experiments once a pressure drop increase of 800 mbar was observed over the MFS. The arrow indicates the water flow direction.

Figure 7 – (A) Average biofilm thickness (μm) using CLSM, (B) ratio of bacterial cell coverage to the total EPS coverage using CLSM, (C) relative coverage per unit area by bacterial cells.