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1 Early non-destructive biofouling detection and
2 spatial distribution: application of oxygen sensing
3 optodes

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22

23 Highlights

- 24 • Imaging allowed direct non-destructive biofouling
25 detection and quantification
- 26 • Spatially resolved information on biofilm growth
- 27 • Biofilm started to develop mainly at the feed spacer
28 crossings
- 29 • Early detection of O₂ consumption under cross-flow
30 operation conditions
- 31 • Stop-flow images provided information on O₂
32 consumption rates

33

Abstract

Biofouling is a serious problem in reverse osmosis/nanofiltration (RO/NF) applications, reducing membrane performance. Early detection of biofouling plays an essential role in an adequate anti-biofouling strategy. Presently, fouling of membrane filtration systems is mainly determined by measuring changes in pressure drop, which is not exclusively linked to biofouling. Non-destructive imaging of oxygen concentrations (i) is specific for biological activity of biofilms and (ii) may enable earlier detection of biofilm accumulation than pressure drop.

The objective of this study was to test whether transparent luminescent planar O₂ optodes, in combination with a simple imaging system, can be used for early non-destructive biofouling detection.

This biofouling detection is done by mapping the two-dimensional distribution of O₂ concentrations and O₂ decrease rates inside a membrane fouling simulator (MFS).

Results show that at an early stage, biofouling development was detected by the oxygen sensing optodes while no significant increase in pressure drop was yet observed. Additionally, optodes could detect spatial heterogeneities in biofouling distribution at a micro scale. Biofilm development started mainly at the feed spacer crossings.

58 The spatial and quantitative information on biological
59 activity will lead to better understanding of the biofouling
60 processes, contributing to the development of more effective
61 biofouling control strategies.

62

63 **Keywords:** early warning, sensor, imaging, water treatment,
64 desalination.

65

66 **Nomenclature**67 **Acronyms**

68 RO: Reverse osmosis

69 NF: Nanofiltration

70 MFS: Membrane fouling simulator

71 PD: Pressure drop

72 LED: Light emitting diode

73 PtTPTBPF: platinum (II) meso-tetra (4-fluorophenyl)

74 tetrabenzoporphyrins

75 AOD : Average oxygen concentration decrease

76 : Average oxygen concentration on day 0- Average

77 oxygen concentration on day x

78 (x= 1, 2, 3, 4, 5)

79 Stop-flow OCR: Stop-flow oxygen consumption rate

80

81 **Main equation**

$$O_2 = \frac{(1 - \frac{I}{I_0})}{((\frac{I}{I_0} - \alpha) * K_{sv})}$$

82 α : Non-quenchable fraction of the dye83 K_{sv} : quenching efficiency coefficient of the dye84 I_0 : luminescence intensity in the absence of O_2 85 I : luminescence intensity in the presence of O_2

86

87 **1. Introduction**

88 **1.1. Biofouling in NF/RO membranes**

89 Many areas in the world are experiencing water shortage
90 problems as a result of the increase in human population and
91 water demand (Viljoen 2010). Reverse osmosis (RO) and
92 nanofiltration (NF) systems are pressure driven, passing water
93 through a semipermeable membrane while retaining particles,
94 ions, and microorganisms (Fritzmann et al. 2007). Production
95 of high quality drinking water depends increasingly on NF and
96 RO membrane processes (Shaban 1990). A potential drawback
97 of NF and RO is that membranes foul with time, resulting in an
98 increase in pressure drop (PD) and/or a decrease of permeate
99 flux and/or increase in salt passage (Vrouwenvelder et al.
100 2009a). Major fouling types are scaling, particulate and organic
101 fouling, and biofouling. These different types of fouling may
102 occur simultaneously and can influence each other (Flemming
103 2003). Scaling and particulate fouling can be relatively easy to
104 control by physical and chemical pretreatment, but biofouling
105 and organic fouling seem to be less controllable
106 (Vrouwenvelder et al. 2008).

107 Biofouling is referred to as the unwanted accumulation of
108 biofilm by attachment and growth (Flemming 2002).
109 Biofouling can be operationally defined as the formation of
110 biofilm that results in an unacceptable degree of system
111 performance loss in industrial systems (Roe et al. 1994).

112 Biofilm formation, which is considered a prerequisite for the
113 occurrence of biofouling, may not always be detected by the
114 present techniques. Detection of fouling is typically done
115 indirectly through measurements of membrane performance
116 decline (Vrouwenvelder et al. 2011). Existing methods to detect
117 biofouling are mostly based on autopsy and require the
118 destructive opening of a membrane module from the system
119 (Tamachkiarow and Flemming 2003, Vrouwenvelder et al.
120 2003, Vrouwenvelder and Van der Kooij 2003). The ideal
121 method for biofilm detection should be able to quantify
122 biofouling, and spatial variability *in-situ*, in real time, non-
123 destructively and at an early stage. Determination of the
124 relevant spatial parameters will result in the formulation of
125 effective biofouling control strategies (Flemming 2003,
126 Vrouwenvelder et al. 2006, Vrouwenvelder et al. 2007a).

127 Earlier detection of biofouling enables corrective actions at
128 an earlier stage which may be more effective than cleaning
129 measures at a later stage (Vrouwenvelder et al. 2008,
130 Vrouwenvelder et al. 2011, Creber et al. 2010). In this study,
131 transparent luminescent planar O₂ optodes, in combination with
132 an imaging system, were used to map the two-dimensional
133 distribution of O₂ concentrations and O₂ decrease rates inside a
134 membrane fouling simulator (MFS)(Vrouwenvelder et al. 2006,
135 Vrouwenvelder et al. 2007a, Vrouwenvelder et al. 2007b).
136 Planar optode measurements offer a unique opportunity to

137 resolve both spatial and temporal variations on a sub-millimeter
138 scale for a range of analytes (Larsen et al. 2011). So far, the
139 majority of planar optode studies have been focused on O₂
140 dynamics in marine sediments (Glud et al. 1999, Glud et al.
141 1998, Behrens et al. 2007, Jensen et al. 2005). This new
142 quantitative and non-destructive method allows direct
143 quantitative assessment of biological activity of fouling in
144 membrane filtration systems, thereby quantifying biofouling.

145 The primary objective of this study was to evaluate whether
146 biofouling could be detected and quantified with the optode at
147 an early stage, before a significant pressure drop increase is
148 observed (Vrouwenvelder et al. 2009b), providing non-
149 destructive early biofouling detection and diagnosis. This study
150 gives insight into the behavior of biofilms in membrane
151 systems during their initial developmental phase.

152 **1.2. Planar optodes**

153 Oxygen plays a vital role in microbial ecology, and flux of
154 O₂ has been used to quantify primary biomass production, total
155 bacterial community respiration, and mineralization rates
156 (Fenchel and Finlay 2008). Oxygen concentrations can vary
157 sharply at μm to mm scale, and precise quantification of the O₂
158 distribution and dynamics is a crucial requirement in
159 understanding spatial and temporal biofilm processes. Planar
160 optodes are a tool that can map spatial distribution of O₂ (Glud
161 et al. 1996). Planar optodes use luminescent O₂ indicators

(dyes) immobilized in an oxygen permeable polymeric matrix and can be fixed on foils or glass surfaces. The measuring principle is based on the dynamic collisional quenching of the indicator luminescence by O₂ (De Graff and Demas 2005). The dye is excited by an excitation light where after the excited dye can release absorbed energy either by luminescence or collisional quenching by molecular oxygen. The ideal response of optical O₂ sensors is described by Stern–Volmer relation (Bacon and Demas 1987).

171

$$\frac{I}{I_0} = \frac{1}{1+K_{sv}[O_2]} \leftrightarrow \frac{I_0}{I} = 1 + K_{sv}[O_2] \quad (1)$$

173 I₀: luminescence intensity in the absence of O₂

174 I: luminescence intensity in the presence of O₂

175 K_{sv}: constant expressing the quenching efficiency of the
176 fluorophore

177 [O₂]: oxygen concentration

178

179 In planar optodes, only a certain fraction of the O₂ indicator dye
180 remains quenchable upon immobilization thus exhibiting a non-
181 ideal Stern-Volmer response (Carraway et al. 1991). The
182 oxygen dependent quenching can be quantified by a modified
183 Stern-Volmer equation. The relationship can be described by
184 the equation:

$$\frac{I}{I_0} = \frac{1-\alpha}{1+K_{sv}[O_2]} + \alpha \quad (2)$$

186 Where α is the non-quenchable fraction

187 For many applications, the most common and simplest form of

188 oxygen sensing involves excitation of a supported dye using an

189 intensity modulated light emitting diode (LED). The excitation

190 light is turned on until the luminescence level of the dye

191 reaches a steady state (Prest et al. 2012). The luminescence

192 intensity is dependent on the O₂ concentration with maximal

193 luminescence intensity reached under anoxic conditions.

194 Recently ratiometric approaches have been published that allow

195 spatial oxygen measurements with low cost, commercially

196 available cameras. The ratiometric method is based on

197 simultaneous measurement of luminescence intensities of the

198 oxygen indicator dye and of the reference (oxygen-insensitive)

199 dye. If the two emissions are spectrally separable, a color

200 camera can be used for imaging (Larsen et al. 2011). The

201 ratiometric approach allows compensation for the variation in

202 the intensity of the excitation light, sensitivity of the detector

203 and dye density. However, if these parameters are kept

204 constant, reliable measurements are also possible by simply

205 measuring luminescence intensity at anoxic conditions and

206 under different oxygen concentrations (Staal et al. 2011a). Due

207 to its simplicity, this approach was used in our study. Using this

208 method, a calibration curve can be determined by correlating

209 known oxygen concentrations with the ratio of the intensity

210 under anoxic condition to that under different O₂

- 211 concentrations. From the calibration curve, any luminescence
212 intensity image can be converted to an O₂ concentration image.

213 2. Materials and methods

214 2.1. Experimental setup description

215 Biofilm was grown in a membrane fouling simulator (MFS)
216 containing a 20 cm × 4 cm coupon of a membrane and a spacer
217 and a transparent window for visual observations
218 (Vrouwenvelder et al. 2006, Vrouwenvelder et al. 2007b). The
219 MFS flow cell was operated without permeate production
220 (Vrouwenvelder et al. 2009) at a pressure < 4bar.
221 Hydrodynamic conditions (e.g. flow field distribution and the
222 relationship between cross flow and feed channel pressure
223 drop) in the monitor are similar to spiral wound membrane
224 modules as applied in practice for water treatment. The spacer
225 and membrane coupons were taken from virgin spiral wound
226 membrane elements (4040-TS80-TSF, Trisep Corporation,
227 USA). The feed spacer consisted of a sheet of 31 mil (787 µm)
228 thick diamond-shaped polypropylene spacer (porosity 0.85).
229 The feed spacer was placed in the MFS with the same
230 orientation as in spiral membrane elements (45° contact angle
231 with feed flow). The system was run as a continuous flow
232 reactor and the setup consisted of a large buffer tank (200 L
233 capacity), diaphragm pump, temperature and differential
234 pressure transmitter (Delta bars, Endress+Hauser, PMD75),
235 pressure-reducing valve, substrate dosing diaphragm metering
236 pump, MFS and a flow sensor (Ultra flow sensor ULF01.H.0,

POM/FPM) (Figure 1). Feed water is filtered by a carbon and cartridge filter (4 μm pore size).

FIGURE 1

2.2. Operating condition

Filtered (carbon and cartridge filter, filter housing model: UPS BB3 [AWF-UPS-3H-20B] Cartridges model: sediment-carbon [AC-SC-10-NL]) tap water ($16 \text{ L}\cdot\text{h}^{-1}$) continuously refreshed the water in a buffer tank. The tap water is produced from chlorinated RO effluent so in order to have biofilm growth carbon filters were used to remove residual chlorine. Water from the buffer tank was pumped through the MFS at a flow rate of $12 \text{ L}\cdot\text{h}^{-1}$ equivalent to a linear flow velocity of $0.12 \text{ m}\cdot\text{s}^{-1}$, which is representative for practice (Vrouwenvelder et al. 2009c). The outflow from the MFS was recirculated into the tank. Water recirculation was required to increase bacterial cell numbers in the feed water of the monitor. The buffer tank water was aerated to ensure saturated oxygen conditions in the MFS. Water temperature was maintained at 24°C during the whole experiment. A nutrient stock solution containing sodium acetate, sodium nitrate, and sodium phosphate in a mass ratio C: N: P of 100:20:10 was added to the feed water up to $1 \text{ mg}\cdot\text{C}\cdot\text{L}^{-1}$ to enhance biofilm formation in the MFS. The stock solution was dosed by a diaphragm metering pump (STEPDOS 03S, Knf NEUBERGER) at a flow rate of $0.05 \text{ L}\cdot\text{h}^{-1}$. To restrict bacterial growth in the nutrient dosage vessel, the pH was set at

11 by adding sodium hydroxide at a concentration of $1 \text{ mol}\cdot\text{L}^{-1}$. The nutrient dosage flow rate ($0.05 \text{ L}\cdot\text{h}^{-1}$) was low compared to the feed water flow rate ($12 \text{ L}\cdot\text{h}^{-1}$). Thus, the effect of the nutrient dosage on the pH of the feed water was insignificant.

Pressure drop was measured over the MFS as an indicator of fouling development (Vrouwenvelder et al. 2009b, Vrouwenvelder et al. 2009c). The development of pressure drop (PD) with time over the monitor length was measured and recorded by a differential pressure transmitter (Delta bars, Endress+Hauser, PMD75). The pressure drop development was normalized by subtracting the pressure drop value at time 0.

2.3. Dye and optode description

The planar optode was coated on a Mylar (thin polyethylene terephthalate, thickness equals 0.13 mm) sheet, which was placed into the flow cell under the transparent cover window. The optode sheet was in direct contact with the feed spacer and the water. Biofilm formation occurs on the membrane, the spacer and the optode sheet. The planar optode used in this study was based on the dye PtTPTBPF (excitation 595 nm , emission 775 nm) immobilized in a polystyrene matrix (4% w/w PE/chloroform) (Borisov et al. 2008). Coating the dye directly on the transparent window of the flow cell was not possible since the solvent of the dye also dissolves the MFS window material, making the sight window turbid.

2.4. Imaging system

Oxygen imaging was done with an Apogee Imaging Systems Ascent A285 CCD Camera equipped with a monochrome Sony ICX-285 Interline CCD Sensor (1392×1040 pixels). A filter wheel (APOGEE CFW31-8R FILTER WHEEL 1.25, 8 POS) was mounted on the camera. A filter (690-850 nm, Astrodon photometrics, sloan photometric filters i'2) was used to take the oxygen images. On top of the filter wheel a Nikon F-mount lens adapter was mounted and a Nikon Nikkor 35mm f/1.4 lens was used with Nikon macro extension tube Kit resulting in a focal distance of 15 cm, depth of field 1.9 – 2.2 mm, and a pixel size of $45\ \mu\text{m} \times 45\ \mu\text{m}$. Micro-manager 1.4, a freeware microscope-controlling software program based on ImageJ, was used to control the camera and filter wheel settings. The oxygen sensing dye was excited by eight amber LEDs (lumiled type, 595 nm) placed around the lens. The power supply for the LEDs was an AIM-TTI instruments, PL303 power supply, 1CH, 30V, 3A, providing a stable LED output. The MFS and the camera system were mounted in a light-tight box to minimize the effect of external light on the luminescence images.

2.4.1. Image calculation and analysis

Oxygen distribution was calculated with a luminescence intensity imaging approach (Staal et al. 2011a). In this approach, the spatial O_2 distribution was calculated directly

from the ratio of a luminescence image obtained under anoxic conditions (I_0) and a luminescence image (I) at the actual O_2 concentration. A schematic description of the calculation of O_2 concentration from aerobic and anaerobic luminescence intensity images is shown in Figure 2. For the conversion from ratio to O_2 concentration a Stern-Volmer equation determined from the calibration of the optode was used.

$$O_2 = \frac{(1 - \frac{I}{I_0})}{((\frac{I}{I_0} - \alpha) * K_{sv})} \quad (3)$$

α is the non-quenchable fraction of the dye and K_{sv} is the quenching efficiency coefficient of the dye.

Calibration of the planar optode was done by circulating water with different O_2 concentrations through the MFS at 24°C. Different O_2 concentrations were generated using a gas mixing system based on electronic mass flow controllers (Sensor Sense, Nijmegen). Intensity images were taken for each defined O_2 concentration, where after a calibration curve was obtained. K_{sv} and α were determined by fitting the adapted Stern-Volmer equation (eq. 2) through the calibration curve.

All post-acquisition image processing was done using the freeware ImageJ version 1.49g (<http://rsbweb.nih.gov/ij/>). After the import to ImageJ, all 16 bit images were converted to a 32 bit floating point format. I/I_0 ratio intensity images were then calculated using the “process > image calculator” option in ImageJ. Conversion of ratio I/I_0 intensity images to O_2

concentration distribution images was done in the process >
math > macro menu based on calibration equation linking the
intensity ratio values to defined O₂ concentrations. False
coloring was applied to visualize the differences in O₂
concentration.

2.4.2. Analysis of spatial indicators

Analysis of spatial indicators of images taken under both
cross-flow and stop-flow operation of the MFS was done using
the ISA software (Beyenal et al. 2004a). Quantitative
parameters describing biofilm physical structure such as
homogeneity and contrast to monitor biofilm development were
analyzed (Yang et al. 2000). Biofilm homogeneity is a textural
parameter that measures the similarity of the spatially close
image structures: a higher homogeneity indicates a more
homogeneous image structure, the homogeneity decreases with
increasing numbers of biofilm clusters (Beyenal et al. 2004b).
Contrast is determined by the difference in the color and
brightness of an object and other objects within the same field
of view.

2.5. Cross-flow versus stop-flow imaging

Two different types of oxygen measuring protocols (cross-
flow and stop-flow) were tested for their potential to measure
biofouling in an MFS. The cross-flow imaging protocol
consisted of sequential imaging of luminescence intensity

359 images under (i) dark, (ii) aerobic and (iii) anaerobic conditions
360 for the inlet of the MFS. The dark intensity image was
361 subtracted from all intensity images to eliminate the
362 background effect. The aerobic intensity image was determined
363 under standard cross-flow operation, while the anaerobic
364 luminescence intensity image was made after dosing a
365 concentrated solution of sodium hydrosulfite ($20 \text{ g}\cdot\text{L}^{-1}$) into the
366 feed water at a rate of $0.125 \text{ mL}\cdot\text{h}^{-1}$. The anaerobic
367 luminescence intensity image was taken once a day and sodium
368 hydrosulfite consumed all oxygen present in the monitor. The
369 water flow through the MFS was directly diverted to the drain
370 when sodium hydrosulfite was pumped to prevent
371 contamination of the buffer tank water.

372 In the stop-flow imaging protocol, a series of 100 intensity
373 images was taken during a 10-minute flow interruption at an
374 interval of 6 seconds. The stop-flow intensity image series was
375 divided by the anaerobic intensity image where after the stack
376 of ratio images is converted into a stack of O_2 concentration
377 image. The stop-flow series gave spatially resolved information
378 about O_2 concentration decrease rates and biofilm development.

379 FIGURE 2

380 3. Results

381 In this study, three methods were compared to evaluate
382 their potential for early biofouling detection and diagnosis; (i)
383 pressure drop development with time, (ii) O_2 decrease under

384 MFS cross-flow conditions, and (iii) O₂ decrease under MFS
 385 stop-flow conditions. No pressure drop increase was observed
 386 during the first 1.5 days of the experiment (Figure 3). Both O₂
 387 measuring techniques already showed a decrease in O₂
 388 concentration at day one. Spatially resolved information about
 389 oxygen depletion locations was identified from images taken
 390 during cross-flow operation (Figure 4). These areas indicate
 391 local biofilm development. In addition to average O₂
 392 concentration decrease with time, spatial indicators such as
 393 image homogeneity and contrast were used as an indicator of
 394 biofilm presence (Figure 5). Earliest biofilm development
 395 detection was based on stop-flow O₂ decrease rates (Figures 6 -
 396 8). Furthermore, Figures 6-8 confirm the oxygen depletion
 397 locations identified during cross-flow operation (Figure 4) as
 398 these locations have highest depletion rate during stop-flow.
 399 Comparison between different indicators for biofouling
 400 presence with time is given in Figure 9.

401 **3.1. Pressure drop**

402 No increase in pressure drop (PD) over the MFS was
 403 observed during the first 1.5 days of the experiments (Figure 3).
 404 After 1.5 days of MFS operation, an exponential increase in PD
 405 started as a result of biofilm accumulation in the flow cell. A
 406 small effect of flow disruption (stop-flow) on PD can be seen at
 407 day three and five (Figure 3). Nonetheless, the increasing PD
 408 trend recovered within one hour after the original flow (0.12

409 $\text{m}\cdot\text{s}^{-1}$) was restored. The PD increased to 250 mbar within the
410 five day experiment.

411 FIGURE 3

412 **3.2. Oxygen concentration during cross-flow MFS** 413 **operation**

414 The spatial distribution of oxygen concentrations ($\text{mg}\cdot\text{L}^{-1}$)
415 were measured daily under cross-flow conditions for a period
416 of five days at the inlet of the MFS (first 2.5 cm) (Figure 4A).
417 Regions with lower oxygen concentrations appeared at the feed
418 spacer crossing on day one, indicating that these were the areas
419 where respiratory activity started and showing initial biofilm
420 development. The regions of lower oxygen concentration
421 become more pronounced with time (Figure 4A). In the
422 supplementary material information (Figure S4), the contrast of
423 the O_2 concentration images was increased to emphasize the
424 heterogeneity of oxygen distribution. The differential image
425 day 0 – day 5 (Figure 4B) shows the variability in change in
426 oxygen concentration within the imaged area. Almost every
427 pixel shows a decrease in oxygen concentration during the
428 experiment. Compared to day 0, approximately 73% of the area
429 had an O_2 decrease of $\sim 1\text{-}1.5 \text{ mg}\cdot\text{L}^{-1}$ while nearly 16% showed
430 an O_2 decrease of $1.5\text{-}2.5 \text{ mg}\cdot\text{L}^{-1}$. Some small areas ($\approx 1\%$ of the
431 total area) showed even a stronger decrease in oxygen

432 concentration ($4\text{--}5\text{ mg}\cdot\text{L}^{-1}$), occurring predominantly at feed
433 spacer crossings.

434 FIGURE 4

435 The average O_2 concentrations were determined from the
436 images. The highest decrease in average O_2 concentration was
437 found on the first day of the experiment: A reduction of 6%
438 was observed. The O_2 concentration decreased further during
439 the rest of the experiment but at lower daily rates (Figure 5A).
440 A suite of parameters were evaluated using ISA software
441 (Beyenal et al. 2004a). Here only image homogeneity and
442 contrast are shown (Figure 5B and 5C). Both homogeneity and
443 contrast decreased with time. The largest decline in image
444 homogeneity was found at day three ($\approx 13\%$); (day one $\approx 2\%$
445 decline and day two $\approx 11\%$ decline).

446 It was also tested whether the size of the sampled area
447 within the O_2 image affected the results. The O_2 concentration
448 plotted in Figure 5A is an average value of the whole inlet area
449 of the MFS ($2.5\text{ cm} \times 4.0\text{ cm}$). The smallest sampling area
450 giving the same average oxygen concentration values was
451 found to be as small as $0.30\text{ cm} \times 0.30\text{ cm}$. This area is almost
452 as small as one feed spacer frame. Table 1 summarizes the
453 minimum surface area needed for representative detection of
454 change in oxygen concentration, where an area as small as 0.30
455 $\text{cm} \times 0.30\text{ cm}$ can be used to detect O_2 concentration decrease
456 representatively. Table 1 shows an increase in the standard

deviation of the oxygen values obtained from the different sampled areas as these areas become smaller. The O_2 concentration determined from a smaller area deviated more from the value obtained over a larger area as biofilm spatial distribution becomes more heterogeneous with time.

FIGURE 5

TABLE 1

3.3. Oxygen concentration during stop-flow MFS operation

During a stop-flow event, the flow was interrupted for a period of 10 minutes. During this period, a series of 100 intensity images was taken at 6 seconds intervals. In addition to biofilm development locations, average oxygen decrease with time (10 minutes) can be determined from the stop-flow image series. Figure 6 shows the spatial distribution of oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$) assessed after the water flow interruption (0 and 2 minutes) and a difference image (t_0 - t_2) derived by subtracting the image at t_2 from the image at t_0 to show locations with the highest oxygen concentration decrease. Figure 7 shows the same differential images, but the scale in every individual image is changed so that O_2 decrease values and locations become more pronounced. Figures 6 and 7 show that the highest oxygen concentration decrease, and thus largest quantity of biofouling present can be seen on and near the feed spacer crossing as early as day two.

On day one only a minor decrease in O_2 concentration was found two minutes after stopping the water-flow through the MFS. The O_2 concentration at t_2 compared to t_0 had an average O_2 decrease of $0.005 \text{ mg}\cdot\text{L}^{-1}$ and a maximum decrease of $3 \text{ mg}\cdot\text{L}^{-1}$. The decrease in O_2 concentration between t_0 and t_2 on day one was relatively homogenous distributed. On day two the O_2 decrease (t_0-t_2) became more evident and more heterogeneous with an average of $0.56 \text{ mg}\cdot\text{L}^{-1}$ and a maximum of $4 \text{ mg}\cdot\text{L}^{-1}$. The first 1 cm of the flow cell inlet had an O_2 decrease average of $0.65 \text{ mg}\cdot\text{L}^{-1}$; the remaining 1.5 cm of the inlet of the flow cell had an O_2 decrease average of $0.50 \text{ mg}\cdot\text{L}^{-1}$. More biofilm is present at the first 1 cm (inlet side) of the flow cell. On day three a higher average O_2 decrease ($1.25 \text{ mg}\cdot\text{L}^{-1}$) over the whole inlet area was seen compared to day two. The largest O_2 decrease was still seen at the first 1 cm of the inlet on day three with a maximum O_2 decrease (t_0-t_2) of $5 \text{ mg}\cdot\text{L}^{-1}$. On day four, the first 1 cm of the inlet with the most biofilm growth had a lower O_2 concentration decrease. Overall the biofilm continued to grow with time; the O_2 consumption rate (OCR) during stop-flow increased rapidly until the MFS became completely anoxic within five minutes of stop-flow on day four and within three minutes of stop-flow on day five (Figure 6).

Figure 8 shows the average oxygen decrease with time after stopping the water flow on days one to five. On day one the

507 OCR was $0.2 \mu\text{g}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$ while on day two it increased to 7.65
 508 $\mu\text{g}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$; illustrating that respiration increased in the flow cell.
 509 Stop-flow images clearly showed regions with lower oxygen
 510 concentrations mainly at the feed spacer crossing indicating the
 511 initial location of biofilm development and activity (Figure 7,
 512 Day 1(t_2), Day 2 (t_2)).

513 FIGURE 6

514 FIGURE 7

515 FIGURE 8

516 **3.4. Comparison of the three methods**

517 Three methods were compared in terms of their early
 518 biofouling detection potential: pressure drop development with
 519 time, O_2 decrease under cross-flow conditions, and O_2 decrease
 520 under stop-flow conditions. Figure 9 (A) shows the normalized
 521 feed channel pressure drop (mbar) development in time, (B) the
 522 average oxygen concentration decrease (AOD) ($\text{mg}\cdot\text{L}^{-1}$) in time
 523 under cross-flow conditions; AOD is calculated by subtracting
 524 the average oxygen concentration on any day from the average
 525 oxygen concentration on day 0, and (C) the stop-flow OCR
 526 ($\text{mg}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$) with time. Normalized pressure drop development,
 527 AOD, and stop-flow OCR all showed an increase with time.
 528 The normalized feed channel pressure drop showed an
 529 exponential increasing trend. Compared to feed channel
 530 pressure drop development, the AOD showed a different trend.

531 The first two days had the highest decrease in O₂ concentration.
532 This was attributed to large clusters of bacterial cells attaching
533 in the MFS and resulting in hotspots of low O₂ concentration.
534 These clusters will already experience diffusion limitation,
535 reducing their O₂ consumption (OCR) locally. Another factor
536 that explains uncoupling of oxygen concentration distribution
537 from OCR is the fact that the oxygen concentration at the base
538 of a biofilm depends on a combination of oxygen consumption
539 and the diffusive boundary layer (DBL) thickness. The DBL
540 thickness is dependent on flow rate and surface roughness.
541 Biofilm development will increase surface roughness and
542 reduce water flow. Both will increase the DBL and therefore
543 biofilms can reduce oxygen concentrations with relative low
544 respiration rates. The stop-flow OCR exhibited an
545 exponentially increasing trend similar to the PD development
546 trend. A decrease in O₂ concentration and an increase in stop-
547 flow OCR were observed within one day after the start of the
548 experiment.
549 Control experiments conducted with no biodegradable substrate
550 dosing and no biofilm development showed that pressure drop
551 measurements can fluctuate up to ± 5 mbar. Thus, the 3 mbar
552 increase observed on day one is still within the sensitivity error
553 of the differential pressure drop transmitter and cannot be used
554 to indicate biofilm development. As for the oxygen
555 concentration, a variation up to $\pm 0.184 \text{ mg}\cdot\text{L}^{-1}$ was observed

when no biodegradable substrate was dosed. The average oxygen concentration was $7.488 \text{ mg}\cdot\text{L}^{-1}$; resulting in an error of 2.8% of the measured value. A decrease in average oxygen concentration of $0.184 \text{ mg}\cdot\text{L}^{-1}$ was considered as the sensitivity error of the imaging methods and any decrease higher than $0.184 \text{ mg}\cdot\text{L}^{-1}$ was attributed to biofilm growth. Table 2 summarizes average time (days) required to detect and quantify the biofouling accumulation inside the MFS. Earliest biofilm development detection was based on average O_2 decrease and stop-flow OCR as these methods showed a higher signal when compared to pressure drop.

FIGURE 9

TABLE 2

4. Discussion

4.1. Early detection of biofouling development

The recent recognition that membrane biofouling during nanofiltration (NF) and reverse osmosis (RO) processes is inevitable, has led to a paradigm shift in which biofouling management rather than prevention is now becoming a practice being considered (Habimana et al. 2014, Bucs et al. 2014a). Earlier, the key focus of biofouling studies was on developing control strategies that mainly relied on the reduction in the feed water of biodegradable substrates, and the reduction of microorganism's numbers (Vrouwenvelder and van der Kooij

2001, Kim et al. 2009, Rand et al. 2007) to control biofouling. This approach was not successful in controlling biofouling in all cases. Early detection plays an essential role in an adequate biofouling management strategy (Vrouwenvelder et al. 2011, Creber et al. 2010). In this study, a sensitive differential pressure drop transmitter was used to monitor the feed channel pressure drop over the MFS length. No increase in feed channel pressure drop was observed during the first 1.5 days of the experiment (Figure 3).

Monitoring O₂ concentration changes in time during MFS cross-flow operation was possible using planar optodes. Average O₂ concentration in the MFS decreased with time as a result of biofilm development. This O₂ decrease was seen already one day after starting the study while no feed channel pressure drop increase was recorded. Observing this O₂ concentration decrease under cross-flow operation conditions is considered crucial emphasizing the imaging setup's capability of being an *in-situ*, non-destructive tool to detect biofouling. Stop-flow oxygen imaging was used to acquire more information on oxygen decrease rates (OCR) and biofouling locations. Usually, stop-flow oxygen imaging can detect biofouling earlier than imaging during cross-flow operation. An oxygen concentration decrease during this 10-minute flow interruption, no matter how small it is, was detected even when no significant decline in O₂ concentration under cross-flow

operation was observed (data not shown). In this study, the decrease in O_2 concentration during stop-flow between t_0 and t_2 on day one was relatively homogenous signaling already the start of formation of a thin layer of biofilm (Figures 6 - 8). The first 1 cm of the flow cell inlet had the most biofilm development with highest O_2 decrease range. The OCR at the first 1 cm of the flow cell inlet was higher than the remaining 1.5 cm up until day four. On day four, the first 1 cm of the inlet that had the most biofilm growth had a lower OCR and a lower O_2 difference (t_0-t_2) (Table S3, supplementary material). One reason for the reduction in the OCR can be that O_2 respiration rates can become carbon limited in the thicker biofilm due to a lower diffusive transport of carbon into the biofilm compared to O_2 diffusive transport.

Cross-flow and stop-flow imaging of oxygen concentration, as methods to detect biofouling, require imaging skills and are more labor intensive compared to pressure drop measurements. Nevertheless, the superiority of these two O_2 imaging methods is in the detailed information received on biofouling development.

4.2. Spatially resolved biofouling development information

In full-scale installations, membrane performance assessment is done according to three operational parameters:

629 feed channel pressure drop, permeate flux, and salt retention.

630 An increase in feed channel pressure drop, decline in flux or

631 increase in salt retention would indicate the existence of

632 fouling. The membrane performance sensors employed are

633 pressure transmitter, flow meter, and conductivity meter. These

634 measured parameters are general and not specific to biofouling

635 (Vrouwenvelder and Van der Kooij 2003), they only signal the

636 existence of a problem. Increase in feed channel pressure drop

637 is considered an indirect method to detect biofouling as it can

638 occur due to the accumulation of any fouling type on the

639 membrane and feed spacer. Feed channel pressure drop

640 measurements are based on the resistance that water

641 experiences when passing through the membrane module

642 during operation. Under constant cross flow velocity, feed

643 channel pressure drop increases with an increasing biomass

644 amount, but the location where the biofilm forms will

645 determine the resistance per biofilm volume. The primary roles

646 of feed spacers are to promote water mixing and to provide an

647 open flow channel with little resistance (Tran et al. 2007, Bucs

648 et al. 2014b, Araujo et al. 2012); however the largest pressure

649 drop along the feed channel occurs in the spacer region, with

650 the narrowest open flow area (Radu et al. 2010). Therefore,

651 biomass present on the spacer will have higher effect on feed

652 channel pressure drop signal than biomass present on the

653 membrane area inside a spacer frame. Measuring the increase

654 in feed channel pressure drop does not provide information
655 about fouling location and distribution.

656 Planar optode imaging specifically detects biofouling
657 through assessment of oxygen concentration as a direct
658 correlation to biofilm activity (Staal et al. 2011b, Debeer et al.
659 1994). Monitoring the change in O₂ concentration under cross-
660 flow operation conditions is considered very important,
661 emphasizing the imaging setup's capability of being an *in-situ*,
662 non-destructive tool to detect biofouling. Spatial distribution of
663 oxygen concentration over the imaged area is the most
664 appealing feature of such a system, enabling identification of
665 the regions where the biofilm starts to grow and how it
666 develops with time. In contrast with differential pressure drop
667 measurements, biomass present on the membrane area inside
668 the feed spacer frame has a higher impact on oxygen
669 consumption signal than biomass present on the spacer since
670 the optode material is directly present under the transparent
671 MFS window. This information about biomass location is
672 mentioned to compare the sensitivity of the measuring methods
673 (pressure drop versus optodes) to show that each method has a
674 different measuring principle, thus illustrating the difference in
675 response with time.

676 In terms of membrane performance, biomass accumulation
677 on the feed spacer affects the water flow distribution in
678 membrane modules. This flow channeling has a substantial

679 impact on feed channel pressure drop, but also on the permeate
680 flux and salt passage. Flow channeling results in a reduction of
681 the (i) residence time of water in the membrane system and the
682 (ii) effective membrane area. Areas with reduced fresh water
683 flux (stagnant water) experience increased concentration
684 polarization, reducing locally the permeate flux and increasing
685 salt passage. Therefore, the direct consequence of biofilm
686 presence on the membrane and the indirect consequence of
687 biofilm presence on the spacer have a huge effect on membrane
688 performance.

689 Both cross-flow and stop-flow imaging methods were able
690 to provide spatially resolved information about oxygen
691 depletion locations. The cross-flow method showed local
692 depletion of oxygen concentration mainly starting near the feed
693 spacer crossings. Stop-flow imaging revealed that these same
694 locations (near the feed spacer crossing) had the highest OCR
695 thus confirming the observations seen using cross-flow. Direct
696 biofilm imaging using rhodamine dye (data not shown here)
697 substantiated that the locations with highest O₂ depletion had
698 the highest quantity of biofilm, confirmed also by results seen
699 in (Staal et al. 2011b).

700 Analysis of the images in terms of spatial parameters such
701 as homogeneity and contrast was also used to determine how
702 the present biofilm is distributed. Thus regardless of the actual
703 pixel values of the image in terms of oxygen, the change in the

704 distribution of these pixels was used to describe biofilm
705 development. Image homogeneity and contrast decreased over
706 time as a result of biofilm development.

707 Spatially resolved oxygen concentration can be used to
708 determine the minimum surface area required to detect a
709 representative measurement of the change in O₂ concentration.
710 Information on the minimum sample area to get a
711 representative sample of the monitor can be used to implement
712 less labor-intensive non-spatial resolved lifetime oxygen
713 sensing with photodiodes. This minimum sample area
714 information is relevant when spatial distribution is not as
715 important as measuring a decrease in O₂ for biofouling
716 detection. It is essential however for the biofilm bacteria to
717 actively respire O₂; otherwise this method cannot be used.

718 **4.3. Effect of stop-flow imaging on biofouling** 719 **development**

720 Stop-flow imaging of oxygen consumption rate enabled a
721 more evident and earlier decrease in average oxygen
722 concentration, as well as indicating the (the locations of)
723 initialization of biofouling development. Stop-flow images
724 clearly showed regions with lower oxygen concentrations
725 mainly at the feed spacer crossings, showing the initial location
726 of biofilm development and activity at these spots. This
727 observation on the start of biofouling at the spacer is consistent

728 with results obtained by others (Creber et al. 2010, Bucs et al.
729 2014b, Baker et al. 1995, van Paassen et al. 1998)

730 However, stop-flow imaging, although superior to cross-
731 flow imaging in terms of earlier biofouling detection, resulted
732 in local biofilm detachment. This biofilm detachment drawback
733 was more evident at higher cross-flow velocities (data not
734 shown here) where more biofilm detachment occurred resulting
735 in a reduced biomass amount and lower pressure drop
736 development with time.

737 **4.4. Practical applications and future studies**

738 A unique feature of O₂ sensing optode imaging is the
739 quantitative and detailed information this method generates,
740 especially in terms of the spatial distribution of the biofilm.
741 Therefore, the optode method can be used to study the
742 fundamentals of development of biofilms under different
743 conditions such as the effect of different spacer geometries,
744 configurations, materials, and operational aspects on biofouling
745 development. Various cleaning chemicals and protocols can
746 also be evaluated as long as the chemicals do not affect the
747 performance of the optode. O₂ sensing optodes are only
748 relevant for biologically active biofilms that consume O₂. For
749 biofilms under anaerobic conditions, for example, other types
750 of detection dyes can be used such as ones that detect changes

751 in pH or CO₂. In practice, most biofilms in spiral-wound NF
752 and RO membrane systems consume oxygen.

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754 5. Conclusion

755 Biofilm development with time was studied using O₂ optode
756 luminescence imaging and compared with normalized feed
757 channel pressure drop. Surface O₂ imaging was done during
758 cross-flow and during stop-flow operation of membrane fouling
759 simulators. The main objective of these experiments was to
760 evaluate whether O₂ optode imaging can detect biofouling
761 presence at an early stage providing a non-destructive early
762 biofouling diagnosis. Based on the results it can be concluded
763 that:

- 764 1. An increase in normalized feed channel pressure drop
765 started to occur after 1.5-2 days from the beginning of
766 the experiment signalling the fouling development.
- 767 2. A decrease in the average oxygen concentration during
768 cross-flow operation, and an increase in the stop-flow
769 OCR were observed as early as day one from the
770 experimental start indicating earlier detection of fouling
771 and diagnosis of biofilm activity.
- 772 3. O₂ optode imaging, in addition to earlier biofouling
773 detection, provided quantitatively the spatial
774 distribution of oxygen concentration over the imaged
775 surface area, identifying with high resolution the
776 regions where the biofilm starts to grow and how it
777 develops with time.

778 4. O₂ optode imaging provided detailed quantitative
779 information on biofouling development therefore it can
780 be successfully implemented in evaluating control
781 strategies or/and practical applications.

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785

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Table 1 – Minimum surface area needed for representative detection of change in oxygen concentration.

Biofilm development at an initial stage (Day 1)		
Number of Pixels	Area (cm × cm)	Average oxygen concentration (mg L ⁻¹) ± SD
247,390	2.5 × 4.0	6.354
32,900 *	1.2 × 1.2	6.246 ± 0.069
10,800 **	0.60 × 0.60	6.236 ± 0.086
1,080 **	0.30 × 0.30	6.230 ± 0.144
Biofilm development at a late stage (Day 5)		
Number of Pixels	Area (cm × cm)	Average oxygen concentration (mg L ⁻¹) ± SD
247,390	2.5 × 4.0	5.695
32,900 *	1.2 × 1.2	5.575 ± 0.057
10,800 **	0.60 × 0.60	5.556 ± 0.062
1,080 **	0.30 × 0.30	5.555 ± 0.091

SD: Standard deviation of the values from the different sampled areas

(*): Measurements were done at six different locations of the same surface area

(**): Measurements were done at twelve different locations of the same surface area

Table 2 – Average time (days) required to detect biofouling accumulation inside the MFS

Method	Detection period (Days)
Normalized pressure drop (NPD)	1.5-2
Cross flow	
Oxygen concentration	1
Spatial indicators (Homogeneity, Contrast)	1
Stop flow	≤ 1

Figure 1–Schematic description of the membrane fouling simulator (MFS) including a water mixing system, differential pressure transmitter, flow meter and the imaging setup for O₂ detection. The arrows indicate the water flow direction. The filter wheel gives the possibility to shift between different filters.

Figure 2–Schematic description of the calculation of the spatial oxygen concentration derived from aerobic and anaerobic luminescence intensity images. (A) Scale bar represents intensity values; (B) scale bar represents oxygen concentration (mg·L⁻¹).

Figure 3—Pressure drop increase (PD) with time over the MFS. The MFS feed water was supplemented with 1 mg- $\text{C}\cdot\text{L}^{-1}$ biodegradable substrate to increase the rate of biofilm accumulation. Arrows indicate the time of O_2 imaging during a short flow interruption (stop-flow) in the MFS.

Figure 4–(A) Spatial distribution of oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$) assessed during cross-flow operation at the inlet side of the MFS (first 2.5 cm) with time (Day 0 to Day 5) at constant cross-flow velocity. (B) Differential image showing oxygen image Day 0 – oxygen image Day 5, indicating the locations where the biofilm is present. The arrow indicates the water flow direction. Scale bar represents oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$).

Figure 5—(A) Biofilm average oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$), and image spatial indicators: (B) homogeneity and (C) contrast change with time determined from images taken at constant cross-flow velocity.

Figure 6–Spatial distribution of oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$) assessed during water flow interruption (0 and 2 minutes) at the MFS inlet (first 2.5 cm) on day 1–5. The locations with the highest oxygen concentration decrease (t_0-t_2) were derived by subtracting the image after 2 minutes (t_2) from the initial image (t_0). The arrow indicates the water flow direction. Scale bar in first and second column represents oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$), Scale bar in third column represents change in oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$).

Figure 7–Spatial distribution of difference (t_0-t_2) in oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$) assessed after water flow interruption (0 and 2 minutes) at the MFS inlet (first 2.5 cm) on day 1–5. Range of O_2 concentration decrease is narrowed to emphasize the oxygen decrease values and locations.

Figure 8–Stop-flow plot: Average oxygen concentration (mg·L⁻¹) decrease with time on day 1 to 5 due to a 10 minute water flow interruption of the MFS.

Figure 9—(A) pressure drop increase (NPD), (B) average oxygen concentration decrease (AOD), and (C) stop-flow oxygen consumption rate (OCR) with time at constant cross-flow operation of the MFS.

Figure S4– Spatial distribution of oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$) assessed during cross-flow operation at the inlet side of the MFS (first 2.5 cm) with time (Day 0 to Day 5) at constant cross-flow velocity. The arrow indicates the water flow direction. Scale bar represents oxygen concentration ($\text{mg}\cdot\text{L}^{-1}$).

Table S3 – Comparison between the first 1 cm and the remaining 1.5 cm of position 1

Day	Average O ₂ concentration (mg·L ⁻¹) first 1 cm during cross-flow	Average O ₂ concentration (mg·L ⁻¹) remaining 1.5 cm of position 1 during cross-flow	Average O ₂ concentration difference t ₀ -t ₂ (mg·L ⁻¹) first 1 cm	Average O ₂ concentration difference t ₀ -t ₂ (mg·L ⁻¹) remaining 1.5 cm of position 1
1	6.3	6.4	0.018	-0.007
2	6.0	6.1	0.643	0.503
3	5.9	6.1	1.408	1.188
4	5.9	6.0	3.276	3.652
5	5.5	5.8	4.932	5.134