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Title: A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations

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Abstract: The performance of a flat-membrane biofilm reactor (MBR) for the removal of acetone, toluene, limonene and hexane at concentrations ranging from 1.3 to 3.2 mg m-3 was investigated at different gas residence times (GRT): 60, 30, 15 and 7 s. A preliminary abiotic test was conducted to assess the mass transport of the selected volatile organic compounds (VOCs) through the membrane. A reduced transport of limonene and hexane was observed with water present over the dense side of the membrane. The presence of a biofilm attached on the dense side of the membrane following bioreactor inoculation significantly increased VOC transport. High acetone and toluene removals (>93%) were recorded in the MBR regardless of the GRT. To remediate the low hexane removal performance (RE< 24 %) recorded at the initial stages of the process, a re-inoculation of the membrane with a hexane-degrading consortium embedded in silicon oil was performed. Although hexane removal did not exceed 27%, this re-inoculation increased limonene removals up to 90% at a GRT of 7s. The absence of inhibition of hexane biodegradation by substrate competition confirmed that hexane removal in the MBR was indeed limited by the mass transfer though the membrane. Despite the low carbon source spectrum and load, the microbiological analysis of the communities present in the MBR showed high species richness (Shannon-Wiener indices of 3.2-3.5) and a high pair-wise similarity (84-97 %) between the suspended and the attached biomass.

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Cover Letter, For Editor only

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Dear Editor,

Please find enclosed our manuscript "**A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations**" co-authored by Raquel Lebrero, Diëgo Volckaert, Rebeca Pérez, Raúl Muñoz and Herman Van Langenhove. The paper is submitted for publication in **Water Research**.

Conventional biological techniques for odour abatement face severe mass transfer limitations when treating hydrophobic odorants, which directly impacts the footprint of these biotechnologies. Membrane bioreactors (MBRs) may overcome this mass transfer limitation due to the high permeability and affinity of some particular membranes for hydrophobic pollutants. However, little is known about the mass transfer and the removal performance of MBRs when treating mixtures of volatile organic compounds (VOCs), especially at the low concentrations typically found in odorous emissions. The study herein submitted constitutes, to the best of our knowledge, the first evaluation of MBR performance for the treatment of mixtures of VOCs at trace level concentrations. Besides, the inoculation of the membrane with a hydrophobic microbial inoculum embedded in a non-aqueous phase was tested for the first time. The results showed that MBRs are a reliable technology for treating acetone, toluene and limonene at low gas residence times, while the selection of an adequate membrane material is mandatory for an efficient hexane removal. The experimental findings herein obtained were also supported by an abiotic mass transfer characterization of the membrane, biodegradation tests and molecular biology techniques (DGGE).

We look forward to your evaluation.
Best regards,

Valladolid, 29 September 2012

Herman Van Langenhove

Raquel Lebrero

Research Highlights

- Membrane bioreactors are an efficient technology for the treatment of soluble VOCs
- High removals were obtained for acetone, toluene and limonene at GRTs of 7 s
- Microbial activity mediated higher concentration gradients over the membrane
- Selection of membrane material is a key design criterion to achieve high removals
- A high microbial biodiversity was observed despite the limited carbon source

A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations

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Abstract

The performance of a flat-membrane biofilm reactor (MBR) for the removal of acetone, toluene, limonene and hexane at concentrations ranging from 1.3 to 3.2 mg m^{-3} was investigated at different gas residence times (GRT): 60, 30, 15 and 7 s. A preliminary abiotic test was conducted to assess the mass transport of the selected volatile organic compounds (VOCs) through the membrane. A reduced transport of limonene and hexane was observed with water present over the dense side of the membrane. The presence of a biofilm attached on the dense side of the membrane following bioreactor inoculation significantly increased VOC transport. High acetone and toluene removals (>93%) were recorded in the MBR regardless of the GRT. To remediate the low hexane removal performance (RE< 24 %) recorded at the initial stages of the process, a re-inoculation of the membrane with a hexane-degrading consortium embedded in silicon oil was performed. Although hexane removal did not exceed 27%, this re-inoculation increased limonene removals up to 90% at a GRT of 7s. The absence of inhibition of hexane biodegradation by substrate competition confirmed that hexane removal in the MBR was indeed limited by the mass transfer though the membrane. Despite the low carbon source spectrum and load, the microbiological analysis of the communities present in the MBR showed high species richness (Shannon-Wiener indices of 3.2-3.5) and a high pair-wise similarity (84-97 %) between the suspended and the attached biomass.

Keywords: membrane bioreactor, odorous VOCs, waste gas treatment, trace level concentrations

Introduction

Biological technologies such as biofilters, biotrickling filters and bioscrubbers are nowadays the best available techniques for odour abatement from both an economical and environmental perspective (Estrada et al., 2011). In addition to their lower operating costs, biotechnologies exhibit lower energy/chemical consumptions and CO_2 emissions than their physical-chemical counterparts (e.g. activated carbon adsorption, chemical scrubbing, incineration, etc.). However, these biotechnologies face severe mass transfer limitations when treating hydrophobic odorants. In bioscrubbers, the gaseous pollutants are absorbed in a water recycling phase prior to its biodegradation and thus only odorants with a low Henry constant ($H = C_g\ C_{aq}^{-1} < 0.01$, where C_g and C_{aq} are the pollutant concentrations in the gas and aqueous phases, respectively) are efficiently treated. Similarly, the presence of a trickling and a stagnant water layer over the packing bed of biotrickling filters and biofilters, respectively, also limits the odorant mass transfer, although in a lesser extent (odorant with $H < 0.1$ for biotrickling filters and $H < 10$ for biofilters; Mudliar et al. 2010). Hence, biotechnologies for odour treatment usually present low removal efficiencies (RE) for the less water soluble odorants (i.e. terpenes, volatile organic sulfides, alkanes, hydrocarbons, etc.) (Iranpour et al. 2005). This mass transfer limitation directly impacts on the footprint of biotechnologies: lower mass transfer rates entail higher gas residence times and therefore higher bioreactor volumes.

Membrane bioreactors for waste gas treatment (MBR) can overcome these mass transfer limitations due to the high permeability and affinity of some particular membranes for hydrophobic pollutants (Kumar et al. 2008). In MBRs, the membrane also serves as a support for the growth of the microbial population responsible for pollutant biodegradation (although biomass might be also suspended in the aqueous phase), which significantly increases the pollutant concentration gradients available for mass transport (Kumar et al. 2008). In a typical membrane bioreactor configuration, the volatile organic compound (VOC) and O_2 laden gas stream circulates through one side of the membrane, while on the other side, an attached biofilm is submerged into a mineral salt solution that provides the water and nutrients required for microbial growth. This mineral salt solution is usually recycled, buffered to maintain a suitable pH and replaced periodically with fresh solution to replenish nutrients and avoid toxic by-products accumulation. The performance of MBRs is determined by the membrane material (polydimethylsiloxane (PDMS), polypropylene (PP), polyethylene (PE), polyvinylidene difluoride (PVDF), etc., Kumar et al. 2008) and the type of membrane configuration (plate and frame, spiral wounded, tubular, capillary or hollow fiber modules) (Mulder, 1997). To date, most of the studies on MBRs focused on the removal of individual compounds such as toluene, propene, benzene, etc. at high concentrations (g m^{-3}) (Kumar et al., 2008), while research on the performance of MBRs for the removal of mixtures of VOCs is scarce. In this context, since odorous emissions are complex mixtures of sulphur/nitrogen derived compounds and VOCs at concentrations in the order of mg m^{-3}- μg m^{-3}, the results reported in literature studies for MBRs cannot be directly applied to odour abatement. Besides, it has been hypothesized that the low

82 substrate concentrations is one of the main limitations of MBRs since they might not
83 sustain an active microbial population (Kumar et al. 2008).

84

85 The present study aims at investigating the performance of a flat MBR for the treatment
86 of a mixture of VOCs (acetone, toluene, limonene and hexane) at trace level
87 concentrations in order to evaluate: i) the influence of VOC nature on the transport
88 through the membrane and on the biodegradation, ii) the performance of MBRs at the
89 low VOC loads typically found in WWTP odorous emissions (mg m^{-3}), iii) the
90 dynamics of microbial biodiversity linked to the MBR performance.

91

92 **Materials and Methods**

93 **Chemicals and reagents**

94 Acetone was purchased from Chem-Lab (+99%), toluene and limonene from Sigma
95 Aldrich with a purity >99% and >97%, respectively, and hexane (purity +99%) from
96 Acros Organics (USA). All chemicals for mineral salt medium (MSM) preparation were
97 purchased from Acros Organics (USA) with a purity of at least 98%, and vitamins were
98 obtained from Laboratories Vitarmonyl (France).

99

100 **Bioreactor set-up**

101 A laboratory-scale flat membrane bioreactor made of Perpex was used (Fig. 1).
102 Although hollow fibre MBRs offer higher specific gas-liquid surface areas, a flat-sheet
103 configuration is preferred due to its easier operation (cleaning and membrane

replacement) (Ergas and McGrath, 1997). A commercially available composite flat membrane was provided by GKSS Forschungszentrum Geesthacht (Germany). The hydrophobic dense top layer material was polydimethylsiloxane (PDMS) with an average thickness of 0.3 μm while the porous hydrophobic support layer was polyacrylonitrile (PAN) with a thickness of 50 μm. The membrane was clamped between the two identical compartments of the reactor and placed in an isothermal chamber at 23ºC. The total volume of the reactor was 16 mL (8 mL of gas volume and 8 mL of liquid volume) and the contact area of the membrane was 40 cm^2.

The MSM solution was continuously recycled along the dense side (liquid side) of the membrane at a velocity of 30 mL min^{-1} by a peristaltic pump (Masterflex, Cole Parmer, USA). The necessary macro and micronutrients were supplied via a buffered nutrients solution containing KNO_3 53.6 g L^{-1}, KH_2PO_4 3.0 g L^{-1}, K_2HPO_4 3.0 g L^{-1}, $MgSO_4$ 2.5 g L^{-1}, micronutrients (P, Ca, Fe, Zn, Co, Mn, Mo, Ni, B) and vitamins at trace concentrations (Álvarez-Hornos et al. 2011). The fresh nutrients solution was periodically supplied to the MBR to maintain nitrogen concentration above 20 mg L^{-1} in the recycling solution. The total liquid volume in the reservoir, maintained under continuous agitation in a thermostatic bath at 23ºC, was 800 mL. The contaminated air stream was obtained by evaporating a mixture of the target VOCs. The liquid VOC mixture was loaded in a syringe (Hamilton Gastight, Switzerland) and dosed into the air stream by means of a syringe pump (model NE 1000, Qis, USA). The pumping velocity was controlled to maintain the inlet concentrations of acetone, toluene, limonene and hexane at 2.5±0.1, 2.4±0.1, 3.2±0.1 and 1.3±0.0 mg m^{-3}, respectively. The volumetric loading rates (based on gas side reactor volume) fed to the MBR ranged between 0.1-10.2 g m^{-3} h^{-1} for acetone, 0.2-13.4 g m^{-3} h^{-1} for toluene, 0.1-5.6 g m^{-3} h^{-1} for limonene

and 0.1-9.6 g m^{-3} h^{-1} for hexane. The gas flow rates (in a counter current configuration) through the porous side (gas side) of the MBR were accurately controlled by a mass flow controller (Brooks, Holland).

Abiotic mass transfer characterization

The mass transport of the four VOCs through the membrane was determined according to Kumar et al. (2009) under two different scenarios in the dense/porous sides of the membrane: air/air and air/water. In the air/air scenario, the polluted air containing the target VOCs was introduced through the porous side at three different gas residence times (GRTs, defined as the volume of the gas chamber divided by the gas flowrate): 30, 16 and 7 s, while clean air passed through the dense side at a constant velocity of 30 mL min^{-1}. The inlet and outlet concentrations of the VOCs in the polluted stream and the outlet concentration of the clean air were periodically measured. Each experimental condition was maintained until the standard deviation of three consecutive measurements was lower than 10% and the mass balance over the reactor was evaluated to ensure the accuracy of the results obtained. In the air/water scenario, MSM instead of clean air was continuously fed to the dense side of the membrane at 30 mL min^{-1}. In this case, the transport of the VOCs was determined at 4 different GRTs: 60, 30, 16 and 7 s.

Inoculation and bioreactor operation

The bioreactor was inoculated with aerobic activated sludge from the Ossemeersen WWTP (Ghent, Belgium) previously stored at 4ºC for one month. The initial biomass concentration in the recycling nutrients solution was 0.6 g of total suspended solids

(TSS) L^{-1}. The reactor was operated at 60 s of GRT for the first 23 days. At day 24, the MBR was re-inoculated with fresh activated sludge from Valladolid WWTP (Spain) (TSS in the recycling liquid = 3.4 g L^{-1}) and operated under similar conditions until day 42. At day 42, the MBR was stopped and re-inoculated with a hydrophobic microbial consortium due to the low limonene and hexane removal efficiencies. This hydrophobic bacterial consortium consisted of hexane-degrading bacteria immersed in silicon oil (Hernández et al., 2012). The silicone oil containing the hydrophobic bacteria was spread on the membrane surface of the liquid side (dense layer). The reactor was operated for 2 days with no liquid recycling to allow bacteria to grow on the membrane surface and avoid their removal by liquid shearing. At day 44 the liquid recycling was restarted and the performance of the MBR was then evaluated at GRTs of 60, 30, 15 and 7 s. Each steady state was maintained for at least 8 days. Finally, in order to assess any potential inhibition of hexane biodegradation in the MBR by the presence of the other VOCs, hexane (1.6±0.3 mg m^{-3}) was directly fed to the MBR, which contained a new membrane impregnated with the hydrophobic microbial consortia. This MBR was operated under these conditions at a GRT of 7 s for 21 days. The inlet and outlet gas concentrations were daily measured by SPME-GC-FID.

Biodegradation tests

At days 18 and 33, two sets of VOC biodegradation tests were performed to assess the catabolic potential of the biomass present in the MBR. In both tests, 5 mL of bacterial suspension from the recycling liquid were added to 12 serological bottles of 120 mL. The bottles were maintained under continuous magnetic agitation (100 min^{-1}) at 22ºC. In 2 serological bottles, 5 mL of distilled water instead of bacterial culture were added

to serve as control. The bottles were sealed with mininert valves (Sigma-Aldrich, USA) and the VOCs were added to the headspace at initial concentrations of 0.05 and 0.4 mg m^{-3} of acetone, 2.3 and 2.3 mg m^{-3} of toluene, 2.5 and 3.1 mg m^{-3} of limonene and 1.7 and 1.8 mg m^{-3} of hexane in the first and second tests, respectively. The concentration of the VOCs was periodically measured for 9 hours by SPME-GC-FID by removing a test bottle each time due to the destructive nature of the analysis.

Analytical Methods

Gas samples from the inlet and outlet sampling ports of the experimental setup were periodically collected in 125 mL glass bulbs (Alltech, USA) and pre-concentrated for 15 min by SPME using a 75 μm PDMS-Carboxen fiber (Supelco, USA). The VOC concentrations were then determined in a GC-FID (Agilent 4890, USA) equipped with a HP-1 column (30 m × 0.53 mm × 5 μm). The injector and detector temperatures were 300ºC and 250 ºC, respectively. The oven temperature was maintained at 35ºC for 2 min, then increased at 10ºC min^{-1} up to a temperature of 75ºC, at 20 ºC min^{-1} up to 220 ºC and finally hold at this temperature for 1 min. The He flow was 5.2 mL min^{-1}.

Liquid samples of 20 mL were periodically collected from the nutrients storage bottle to analyze the concentration of phosphate, nitrate, total nitrogen and COD by Nanocolor Test Tubes (Macherey- Nagel, Germany). The pH was analyzed by a pHmeter (Jenway, UK; electrode from Hamilton, Switzerland).

Microbiological procedures

198 To evaluate the richness and composition of the bacterial communities, biomass
199 samples of the three inocula (activated sludge from Ossemeersen WWTP -sample A-,
200 activated sludge from Valladolid WWTP -sample B-, and the hydrophobic microbial
201 consortium -sample F-), of the liquid recycling media at day 28 (sample C), 42 (sample
202 D) and 80 (sample G) and of the membrane biofilm at day 42 (sample E) and 80
203 (sample H) were collected and stored immediately at – 20ºC. The biofilm samples were
204 retrieved by removing the membrane from the reactor and scrapping part of the biofilm
205 from the membrane surface.

206

207 The genomic DNA was extracted according to Lebrero et al. (2012b). The PCR mixture
208 (50 µL) was composed of 25 µL of BIOMIX ready-to-use 2× reaction mix (Bioline,
209 Ecogen), containing reaction buffer, magnesium, deoxynucleotide triphosphates
210 (dNTPs), Taq polymerase and additives, l or 2 µL of the extracted DNA, PCR primers
211 968-F-GC and 1401-R (10µM) (Sigma- Aldrich, St. Louis,MO, USA) for bacterial 16S
212 rRNA gene amplification, and Milli-Q water up to a final volume of 50 µL. The PCR
213 thermo-cycling program used was previously described in Lebrero et al. (2012b).

214

215 DGGE analysis of the amplicons was performed with a D-Code Universal Mutation
216 Detection System (Bio Rad Laboratories) using 8% (w/v) polyacrylamide gels with a
217 urea/formamide denaturing gradient of 45 to 65%. The DGGE running conditions were
218 applied according to Roest et al. (2005). The gels were stained with SYBR Green I
219 nucleic acid gel stain (Sigma Aldrich, St.Louis, MO, USA) for 1 h. The obtained DGGE
220 patterns were processed using the GelCompar IITM software (Applied Maths BVBA,
221 Sint-Martens-Latem, Belgium). After image normalization, bands were defined for each
222 sample using the bands search algorithm within the program. Similarity indices of the

compared profiles were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient (Häne et al. 1993). The peak heights in the densitometric curves were also used to determine the Shannon–Wiener diversity index (H), which considered both the relative number of the DGGE bands (richness) and their relative intensities (evenness):

$$H = -\sum \left[P_i \ln(P_i) \right]$$

where P_i is the importance probability of the bands in a lane ($P_i = n_i/n$, n_i is the height of an individual peak and n is the sum of all peak heights in the densitometric curves).

Sequencing and DNA sequence analysis

Some bands were excised from the DGGE gel in order to identify the microorganisms present both in the inocula and in the MBR. The procedure was previously described in Lebrero et al. (2011). The taxonomic position of the sequenced DGGE bands was obtained using the RDP classifier tool (50% confidence level) (Wang et al. 2007). The closest matches to each band were obtained using the BLAST search tool at the NCBI (National Centre for Biotechnology Information) (McGinnis and Madden, 2004). Sequences were deposited in GenBank Data Libray under accession numbers JX627815-JX627846.

Results and Discussion

Abiotic mass transfer characterization

Acetone was completely transferred at a GRT higher than 16 s when air was present at both sides of the membrane. The transfer efficiency decreased to about 60 % at a GRT

246 of 7 s. When water was flowing at the dense membrane side RE decreased to 89% at
247 GRT of 16 and 30 s. However, at 7 s of GRT, the acetone transport through the
248 membrane with water was superior than with air flowing through the dense side (Fig.
249 2a). The transport of toluene in the air/air experiments was similar to that of acetone,
250 but decreased noticeably in the air/water scenario: 64%, 52% and 13% at GRTs of 30,
251 16 and 7 s, respectively (Fig. 2b). These results were in agreement to those obtained by
252 Kumar et al. (2009) with a similar composite membrane (PDMS 0.3µm/PAN 185µm)
253 and toluene as the only pollutant in the gas phase. Hexane and limonene presented the
254 lowest percentages of mass transfer through the membrane (Fig. 2c and 2d). The mass
255 transfer percentages of hexane (53%, 68% and 94% in an air/air scenario at GRTs of 7,
256 16 and 30 s, respectively) decreased noticeably with water in the dense side (4% at a
257 GRT of 7 s and <35% at GRTs <60 s). Similarly, 50%, 90% and 98% of the limonene
258 was transferred at GRTs of 7, 16 and 30 s in an air/air scenario, respectively, while its
259 transport severely decreased when water was recycled through the dense side of the
260 membrane (<10% at GRTs <30 s and 40% at a GRT = 40 s). The presence of a biofilm
261 significantly increased the transport of acetone, toluene and limonene at 7 s of GRT
262 compared to the gas/gas scenario, whereas no improvement was recorded for hexane.

263

264 A summary of the gas-water ($K_{g/w}$), octanol-water ($K_{o/w}$) and octanol-gas ($K_{o/g}$,
265 calculated from $K_{g/w}$ and $K_{o/w}$) partition coefficients of the target compounds is shown
266 in Table 1 (data collected from Sander 1999, Schwarzenbach et al. 2002, Copolovici
267 and Niinemets, 2005). Mass transfer in the system can be conceptually described by a
268 number of transfer resistances in series. Moving from the air towards the biofilm there
269 are: a stagnant laminar boundary layer at the bulk air/porous membrane interphase,
270 diffusion trough the stagnant air in the pores; air-membrane transfer, diffusion across

271 the membrane, membrane-air transfer and a laminar boundary layer at the dense
272 membrane-bulk air output. Since the interphase at both sides of the membrane is the
273 same in the air/air situation, a compound with greater affinity for PDMS will benefit at
274 the input side but not at the output side. Besides, whereas in the output side the
275 resistance is constant because the velocity, and hence the thickness of the stagnant layer
276 are constant, at the input side the lower GRTs (higher air velocity) reduce the thickness
277 of the layer and subsequently the resistance to mass transfer. Therefore, it is difficult to
278 predict whether the behaviour of the components is dominated either by equilibrium
279 constant or by the flow dynamics, since the mass transfer is a combination of the
280 different resistances at the different GRTs.

281

282 In the air/water scenario, the driving force will be determined by $K_{o/g}$ at the input side,
283 which is highest for limonene and lowest for acetone. However, at the output side, the
284 VOC transfer will be determined by the partition coefficient between the membrane and
285 water (estimated by $K_{o/w}$), improving acetone transport and hindering that of hexane.
286 Therefore, there are two driving forces with opposed effects on the transfer of the
287 VOCs, and hence the limiting step cannot be directly elucidated from the experimental
288 data. Nevertheless, the substitution of the air phase by an aqueous phase in the dense
289 side mediated an enhancement in the transport of acetone, followed by toluene,
290 limonene and hexane, which corresponds to the relative order of $K_{g/w}$.

291

292 When biofilm was present on the dense side of the membrane, the experimental data
293 suggested that the transport depended on the existing concentration gradients and was
294 not likely correlated to $K_{o/w}$ or $K_{g/w}$, due to the addition of a biodegradation step (VOC

295 sink) to the physical transport. In this case, physical and biological processes cannot be
296 separately considered.

297

298 *Membrane bioreactor performance*

299 The formation of a thin biofilm over the dense side of the membrane was visually
300 observed four days after the inoculation of the MBR. REs higher than 99% were
301 recorded for acetone already one day after the inoculation of the membrane. It can be
302 hypothesized that pollutant biodegradation in MBRs is not only due to the
303 microorganisms present in the biofilm but also to the suspended biomass, especially for
304 highly water soluble VOCs. A high acetone removal performance was observed during
305 the entire experimentation period, regardless of the inoculation strategies and the GRTs
306 tested, probably due to its high biodegradability (Fig. 3a).

307

308 Four days were necessary to achieve toluene REs higher than 99% following the MBR
309 start-up (Fig. 3b). Toluene removals higher than 99% were maintained during operation
310 at GRTs of 60 s and 30 s. When the GRT was decreased to 15 and 7 s, the REs slightly
311 declined to 97 ± 1% and 93 ± 0%, respectively. Steady REs (considering a steady state
312 as the operation period with a STD<5% in the average removal efficiency) were always
313 achieved immediately after each change in the operating conditions, except for the last
314 decrease in GRT when the MBR required 1 day to achieve steady state performance.
315 Several studies on the performance of membrane bioreactors for the treatment of VOCs-
316 contaminated gas streams used toluene as the model compound. An efficient removal of
317 toluene as single pollutant in MBRs has been consistently shown in these laboratory
318 studies at high inlet concentrations (30-4650 mg m^{-3}) and GRTs as low as 1.4 s (Ergas

and McGrath 1997, Ergas et al. 1999, Jacobs et al. 2003, Kumar et al. 2008b). Toluene is relatively easy to degrade and elimination capacities (ECs) up to 2520 g m^{-3} h^{-1} have been obtained in a hollow fiber membrane reactor configuration at a GRT of 1.8 s (Ergas et al. 1999). However, at such high ECs, the corresponding REs were much lower than those observed in the present study (RE = 35%, Ergas et al. 1999). To the author's knowledge, the only study testing low toluene inlet concentrations (4 mg m^{-3}) was performed by Jacobs et al. (2003) in a flat MBR with a composite membrane inoculated with *Pseudomonas putida* TVA8. These authors recorded REs of ~75%, ~55% and 53% at GRTs of 8, 4 and 2 s, respectively, which were lower than those here achieved at a GRT of 7 s (RE = 93%).

Hexane was the VOC with the lowest REs at all GRTs evaluated. Steady REs of 15±4% were achieved six days after the inoculation of the membrane. However, the biodegradation tests showed that none of the microbial communities present in the MBR (the bacterial community in the recycling liquid presented the same structure than in the biofilm) were able to degrade hexane (Fig. 4.1c and 4.2c). At day 42, the MBR was inoculated with a hydrophobic hexane-degrading consortium growing immersed in silicon oil by spreading it on the dense side of the membrane surface (Hernández et al. 2012). The addition of the silicon oil consortium slightly improved the removal performance to 24±3%, the highest values observed throughout the experimentation period. When the GRT was further decreased to 30, 15 and 7 s, the RE remained at 14 ± 3% regardless of the GRT tested. Based on the proven hexane degrading capacity of this microbial consortium (Hernández et al. 2012), it could be hypothesizes that the low hexane removal performance recorded in the MBR could be caused by either a substrate competition between the different microorganisms present in the biofilm and/or in the

liquid suspension or by a mass transport competition of the 4 VOCs in the membrane. In this regard, Zhao et al. (2011) found interactions during the simultaneous biotreatment of two volatile pollutants, toluene and hexane, in a hollow fiber MBR, although at higher inlet concentrations (30-1100 mg m^{-3}). To clarify the reasons underlying this consistently low performance, the membrane was operated with the silicon-oil inoculum and fed only with hexane for 30 days. However, the recorded REs were always lower than 27%, which suggests that hexane mass transport through the membrane was the limiting factor during hexane biodegradation.

Limonene removal efficiency increased gradually from the start-up of the bioreactor to finally achieve a maximum RE of 93% at day 14 but, surprisingly, the limonene removal performance decreased progressively to values of 52 ± 5% by day 23 (Fig. 3d). This deterioration in the removal performance of MBRs during the start-up period has been already observed by other authors in fixed-film bioreactors degrading VOCs (Arcangeli and Arvin, 1992; Reij et al. 1998) and also in hollow fiber MBRs (Ergas et al. 1999). This drop in the RE was attributed to the starvation of the suspended microbial community in the aqueous phase following biofilm formation in the membrane. A change in the hydrophobicity of the membrane was also pointed out as a probable reason underlying this behavior (Ergas et al. 1999). This decrease in the membrane hydrophobicity is often due to the coating of the membrane pores by polysaccharide materials excreted by the biomass, although this mechanism was unlikely in our particular case since the biofilm was formed on the dense side of the composite membrane. The re-inoculation of the MBR with fresh activated sludge did not improve the limonene removal performance, and indeed, a minimum RE of 4% was recorded by day 35. This deterioration was attributed to the formation of a thick biofilm

on the membrane surface (which was visually noticeable and likely induced mass transfer limitations in the process). At day 35 the membrane was partially cleaned by increasing the velocity of the recycling pump for a few seconds to promote biofilm sloughing due to the increased shear forces, and the removal performance immediately increased to steady REs of 35±5%. The biodegradation tests performed at days 18 and 33 with the suspended culture demonstrated the capacity of the existing microorganisms to degrade limonene (Fig. 4.1d and 4.2d). The degradation curves are notably different in both tests, which can be attributed to a change in the structure or concentration of the microbial community. However, the limonene degradation line is clearly below the control, which suggested that the MBR was indeed mass transfer limited for limonene in the absence of a biofilm. The high similarity coefficient (83.8%, further discussed in the *Internal structure and molecular composition of the bacterial communities* section) between the microbial population in the suspended culture and in the biofilm validated this assumption.

The re-inoculation of the membrane by day 42 with the hydrophobic microbial consortium increased the limonene RE up to 98 ± 1% in the following 3 days. Similar REs were recorded only 1 day after decreasing the GRT to 30 s, while the removal performance slightly decreased to 95 ± 1% and 90 ± 1% when the gas flow rate was further increased to GRTs of 15 and 7 s, respectively. These high elimination efficiencies (much higher than the mass transfer rates recorded under abiotic conditions) were likely related to the presence of a hydrophobic silicone-oil layer on the membrane surface, which eventually improved the mass transfer of limonene (a low water soluble compound with high affinity for silicone oil, Table 1) to the degrading microorganisms present in the biofilm (the inoculum was spread on the membrane surface, without

replacing the existent microbial suspension). It can be also hypothesized that a highly active limonene-degrading bacterial population was present in the inoculated silicon-oil layer. This, together with the improved mass transport of limonene, could explain the high limonene removal observed in our MBR. To the authors knowledge, this is the first study where a non-aqueous phase (here silicone oil) was combined with a biological membrane bioreactor. Considering the promising results obtained for limonene, this two-phase system deserves further investigation.

The REs here obtained for the less hydrophobic compounds (acetone, toluene) and for the moderately hydrophobic limonene were comparable to those observed in previous studies in activated sludge systems, biofilters and biotrickling filters under similar inlet concentrations and gas residence times (Lebrero et al. 2011, 2012a, Prenafeta et al. 2012). For instance, Prenafeta et al. (2012) recorded high REs for limonene (RE > 99%) during the biofiltration of a real odorous emission from a composting plant. However, hexane REs up to 70% were recorded under comparable conditions in a biotrickling filter at GRTs as low as 11 s, in contrast with the low efficiencies obtained in this study.

Finally, it was observed that microbial activity (either in the form of biofilm or suspended culture) mediated a higher concentration gradient for acetone, toluene and limonene over the membrane (thus increasing the driving force), as shown by the increased mass transport efficiencies of these VOCs through the membrane compared to those measured under abiotic conditions (Fig. 2a, b and d). However, in the particular case of hexane, the transport was only slightly increased at the lowest GRT (7 s) and it was hypothesized that the biofilm established over the membrane could create an

418 additional resistance to hexane transport at the highest GRTs. The selection of a
419 membrane material with a higher affinity for hexane is therefore mandatory to achieve
420 higher eliminations for this particular VOC.

421

422 *Internal structure and molecular composition of the bacterial communities*

423 The activated sludge inoculum from Valladolid WWTP showed the highest species
424 evenness and richness among the inocula evaluated as demonstrated by its high
425 Shannon-Wiener diversity index (3.3, with values usually ranging from 1.5 to 3.5,
426 McDonald 2003). The biodiversity of the inoculum from Ossemeersen WWTP (A) was
427 slightly lower (2.9), while the microbial inoculum contained in the silicone oil (F)
428 presented the lowest diversity (H = 2.2). The samples retrieved from the liquid phase
429 recirculation (C, D and G) and the biofilm (E, H) on the membrane surface also
430 presented a high species evenness and richness during the whole experimentation period
431 (H varying from 3.2 to 3.5) despite the low carbon source spectrum. This high
432 biodiversity and richness in bioreactors fed with low VOCs concentrations has been
433 previously reported (Estrada et al. 2012, Lebrero et al. 2012a, 2012b). In this context,
434 Estrada et al. (2012) observed that low toluene concentrations mediated a higher
435 biodiversity in a suspended bioreactor, while the biodiversity was significantly reduced
436 at high toluene loadings.

437

438 The pair-wise similarity indices showed a high correspondence between the community
439 profiles of the samples from the recirculation liquid and the biofilm (83.8% between
440 samples D and E and 97.5% between samples G and H), which confirmed that most
441 microorganisms developed in both the suspended culture and the biofilm. After the

second re-inoculation of the membrane, similarity coefficients of 66.3% and 51.7% were observed between the inoculum (sample B) and the suspended cultures on days 28 and 42 days, respectively (samples C and D). These empirical findings demonstrated the progressive acclimation of the microorganisms to the operating conditions. Finally, the highest similarity in the phylogenetic composition of the communities (~95%) was obtained between the silicon oil inoculum (sample F) and the biofilm sample retrieved from the membrane at the end of the experiment (sample H).

From the DGGE gel, 32 bands were sequenced (Fig. 5). Seven different phyla were retrieved according to the RDP classifier tool (bootstrap value of 50 %) in the RDP database: *Actinobacteria* (10 bands), *Proteobacteria* (10 bands), *Chlamydiae* (5 bands), *Acidobacteria* (2 bands), *Chlorobi* (2 bands), *Firmicutes* (2 bands), and *Chloroflexi* (1 band). The closest matches for each band (BLASTN) using the NCBI database are shown in Table 2, together with the similarity percentages and the sources of origin.

Most of these phyla have been previously found in biological odour abatement systems (Lebrero et al., 2011, 2012a, 2012b), exhibiting a demonstrated VOC biodegradation ability. Microorganisms in the *Actinobacteria* phylum (DGGE fragments 1-10), which includes aromatic and aliphatic hydrocarbon-degrading bacteria, were detected with high intensity in samples A, F, G y H. The DGGE fragments 3 and 4 showed a 99% similarity with *Rhodococcus phenolicus* (NR042950), a species capable of degrading aromatic compounds (Rehfuss and Urban, 2005). Similarly, microorganisms belonging to the *Mycobacterium* genus (fragment 5), known as slow-growing bacteria, are able to degrade toluene at low concentrations (Juteau et al., 1999). Previous literature studies have detected members of the *Proteobacteria* phylum in biological gas treatment

systems (Bayle et al., 2008). In our particular study, the *Gammaproteobacteria* class is significantly present in all samples analyzed. Fragments 18 and 19 were affiliated to the *Dokdonella* genus, which has been previously detected in bioreactors treating sulfurous compounds, ammonia and VOCs (Maestre et al. 2010, Lebrero et al. 2012a, 2012b). Member of the *Chlamydiae* phylum (DGGE bands 21- 25) were also found in a biorreactor treating gaseous toluene (Estrada et al. 2012). Despite present in our MBR, the ability of members of the phyla *Acidobacteria* (DGGE bands 26 and 27), *Chlorobi* (DGGE bands 28 and 29) and *Firmicutes* (DGGE bands 30 and 31) to degrade VOCs has not been reported yet. This fact clearly confirms the scarce knowledge available on the microbiology of off-gas treatment biotechnologies. Microorganisms classified into the *Chloroflexi* phylum (DGGE band 32) have been commonly retrieved from a wide variety of biological systems, but information about their functional role is scarce. Finally, it must be stressed that the biodiversity of the microbial community present in the bioreactor remained constant over time for the phyla *Actinobacteria, Proteobacteria* and *Chlamydiae,* while *Acidobacteria, Chlorobi, Firmicutes* and *Chloroflexi* were no longer found after day 42 of operation (Fig. 5 and Table 2).

Conclusions

This work confirmed the efficiency of MBRs for the treatment of water soluble and moderately soluble VOCs. Whereas the abiotic test showed that the presence of an aqueous phase over the dense side of the membrane induced a higher overall mass transfer resistance, biofilm activity mediated higher concentration gradients over the membrane and therefore a more efficient VOC transport. Thus, REs higher than 93% were always obtained in the MBR for acetone and toluene at GRTs as low as 7 s. In the particular case of limonene, the inoculation of the membrane with an inoculum

embedded in silicon-oil increased its removal performance up to 90% at 7 s of GRT. Nevertheless, hexane biodegradation was limited by its mass transfer over the membrane regardless of the GRT (RE < 24 %), which pointed out towards the selection of the optimum membrane material as a key design criterion determining the performance of MBRs for the treatment of highly hydrophobic VOCs. Finally, the microbiological analysis of the communities present in the MBR showed a high species richness despite the limited C source spectrum, and a high structural similarity between the microbial populations present in the suspended culture and in the biofilm.

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595

596

Fig 1. Schematic representation of the experimental setup

Fig 2. Influence of the gas residence time on the transport efficiency of acetone (a), toluene (b), hexane (c) and limonene (d) through the membrane reactor in the air/air (■), air/liquid (□) and air/biofilm (●) scenarios.

Fig 3. Time course of the inlet (Δ) and outlet (♦) concentrations, and removal efficiency (▬) of acetone (a), toluene (b), hexane (c) and limonene (d) in the MBR. The re-inoculation of the MBR is represented by continuous vertical lines, while vertical dashed lines correspond to the changes in GRT. Membrane cleaning is represented by a vertical grey arrow.

Fig 4. Time course of the acetone (a), toluene (b), hexane (c) and limonene (d) headspace concentration (○) in the batch biodegradation tests conducted with MBR biomass at days 18 (Fig. 4.1) and 33 (Fig. 4.2). The dashed lines represent the VOC concentrations in the control bottles.

Fig 5. Bacterial DGGE profiles in the MBR. The samples names and the Shannon-Weiner diversity indices are shown in the upper part of the gel.

Table 1. Partition coefficients for the target VOCs (C_g: concentration in the gas phase, C_{aq}: concentration in the aqueous phase, C_{oct}: concentration in an octanol phase)

Compound	$\log (K_{g/w} (C_g C_{aq}^{-1}))$	$\log (K_{o/w} (C_{oct} C_{aq}^{-1}))$	$\log (K_{o/g} (C_{oct} C_g^{-1}))$
Acetone	-2.82	-0.24	2.58
Toluene	-0.59	2.69	3.29
Limonene	0.06	4.23	4.17
Hexane	1.77	4.11	2.33

Table 2
Click here to download Table: Table 2.docx

Table 2. RDP classification of the DGGE bands sequenced and corresponding matches (BLASTN) using the NCBI database with indication of the similarity percentages of sources of origin. The presence (x) / absence of each band in each sample tested is also shown.

Taxonomic placement (50% confidence level)	Band n°	A	B	C	D	E	F	G	H	Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
Phylum *Actinobacteria*												
Class *Actinobacteria*												
Subclass *actinobacteridae*												
Order *actinomycetales*												
Suborder *Actinomycineae*												
Family *Actinomycetaceae*												
Genus *Actinomyces*												
	1	**X**								Uncultured bacterium (AY953348)	100	Anaerobic sludge
Suborder *Corynebacterineae*												
Family *Nocardiaceae*												
	2						**X**	**X**	**X**	*Rhodococcus phenolicus* (NR_042950)	97	Culture collection
Genus *Rhodococcus*												
	3					X	X	**X**	X	*Rhodococcus phenolicus* (NR_042950)	99	Culture collection
										Rhodococcus sp.(EU0174049)	99	Soil
	4								**X**	*Rhodococcus phenolicus* (NR_042950)	99	Culture collection
										Rhodococcus phenolicus (JN180180)	99	Soil
Family *Mycobacteriaceae*												
Genus *Mycobacterium*												
	5	X		X	X		**X**	**X**	**X**	*Mycobacterium fortuitum* (JF734327)	99	Soil
Suborder *Propionibacterineae*												
Family *Propionibacteriaceae*												
Genus *Propionibacterium*												
	6	X								*Propionibacterium sp.*(AB540663)	95	Ditch sludge
	7	**X**								Uncultured bacterium (EU186882)	97	Cachaca yeast
	8	**X**				X				*Propionibacterium jensenii* (AY883044)	100	Culture collection
										Propionibacterium sp. (AB540663)	98	Ditch sludge
Order *Bifidobacteriales*												
Family *Bifidobacteriaceae*												
Genus *Bifidobacterium*												
	9	**X**	X	X	X	X		X	X	Uncultured bacterium (JN620462)	97	Activated sludge from a bioreactor treating synthetic wastewater
	10	**X**								Uncultured bacterium (JN620462)	97	Activated sludge from a bioreactor treating synthetic wastewater
Phylum *Proteobacteria*												
	11		X	X	X	X		X	X	Uncultured bacterium (JQ426388)	96	Soil
Class *Alphaproteobacteria*												
	12		X	X	X	X				Uncultured bacterium (CT574092)	99	Evry municipal wastewater treatment plant
Order *Rhodobacterales*												
Family *Rhodobacteraceae*												
Genus *Rhodobacter*												
	13	X						X	X	Uncultured bacterium (AB286495)	98	Activated sludge
	14	**X**		X			X			Uncultured bacterium (AB286495)	97	Activated sludge
Order *Rhodospirillales*												
	15	X				X				*Acetobacteraceae* bacterium (HQ687487)	97	Culture collection
										Uncultured bacterium (FN667149)	97	Full scale municipal waste compost
Class *Gammaproteobacteria*												

Order *Xanthomonadales*											
Family *Xanthomondaceae*											
	16	**X**		X	X	X		X	X	Uncultured *Xanthomonadales* bacterium (AM936405)	95	Bioremediation process of a hydrocarbon-contaminated soil
Genus *Pseudoxanthomonas*											
	17	**X**								Uncultured *gamma proteobacterium* (AB669240)	99	Anaerobic digester sludge
Genus *Dokdonella*											
	18	**X**	X	**X**	**X**	**X**		**X**	**X**	Uncultured bacterium (JQ038783)	100	Biotrickling filter (BTF) treating low concentrations of VOCs
										Uncultured *Dokdonella sp.*(JN679149)	99	Membrane bioreactor
										Uncultured bacterium (FM213064)	99	Biotrickling filter removing H2S from water treatment sludge
	19		X	**X**	X	**X**		**X**	**X**	Uncultured bacterium (JQ038783)	100	Biotrickling filter (BTF) treating low concentrations of VOCs
										Uncultured bacterium (FJ660574)	99	Activated sludge
										Uncultured *Dokdonella sp.*(JN679149)	99	Membrane bioreactor
										Uncultured bacterium (FM213064)	99	Biotrickling filter removing H2S from water treatment sludge
Family *Sinobacteraceae*											
Genus *Steroidobacter*											
	20		X				**X**	**X**	**X**	Uncultured *Pseudomonadales* bacterium (EU193058)	92	Agricultural soil
Phylum *Chlamydiae*											
Class *Chlamydiae*											
Order *Chlamydiales*											
Family *Parachlamydiaceae*											
	21				X	X		X	X	Uncultured bacterium (JQ056534)	95	Soil
Genus *Parachlamydia*											
	22		X		X	X				Uncultured bacterium (JQ050078)	92	Soil
	23		X	X	X	X	X	X	X	*Criblamydiaceae* bacterium (JF706725)	94	Culture collection
	24		X	X	X	X		X	X	Uncultured bacterium (JN606107)	99	Reactors treating toluene at different concentrations
	25		X	X	X	X		X	X	Uncultured bacterium (JQ053179)	100	Soil
Phylum *Acidobacteria*											
	26		X	X	X	X				Uncultured bacterium (FQ659784)	100	PAH-contaminated soil; retention systems which treat road runoffs
Class *Acidobacteria_Gp4*											
Genus Gp4											
	27			X	X	X				Uncultured bacterium (FN827223)	99	Activated sludge from a membrane bioreactor
Phylum *Chlorobi*											
Class *Ignavibacteria*											
Order *Ignavibacteriales*											
Family *Ignavibacteriaceae*											
Genus *Ignavibacterium*											
	28		X	X						Uncultured bacterium (GQ397077)	98	Soil
	29			**X**	X	X				Uncultured bacterium (FN824912)	98	Biofilm sampled in a treatment system for groundwater contaminated with BTEX, MTBE and ammonium
Phylum *Firmicutes*											
Class *Clostridia*											
Order *Clostridiales*											
Family *Lachnospiraceae*											
Genus *Clostridium XlVa*											
	30		**X**	X						*Clostridiaceae* bacterium (AB298726)	100	Rice straw residue in a methanogenic reactor of cattle farm waste
										Uncultured bacterium (CR933122)	99	Evry municipal wastewater treatment plant
	31		X	X	X					Uncultured bacterium (CR933122)	95	Evry municipal wastewater treatment plant
Phylum *Chloroflexi*											
	32	X	X	X	**X**	**X**				Uncultured bacterium (AB630830)	98	Aquatic moss pillars
										Uncultured bacterium (JQ800911)	96	Soil

Figure 1

Fig. 1.

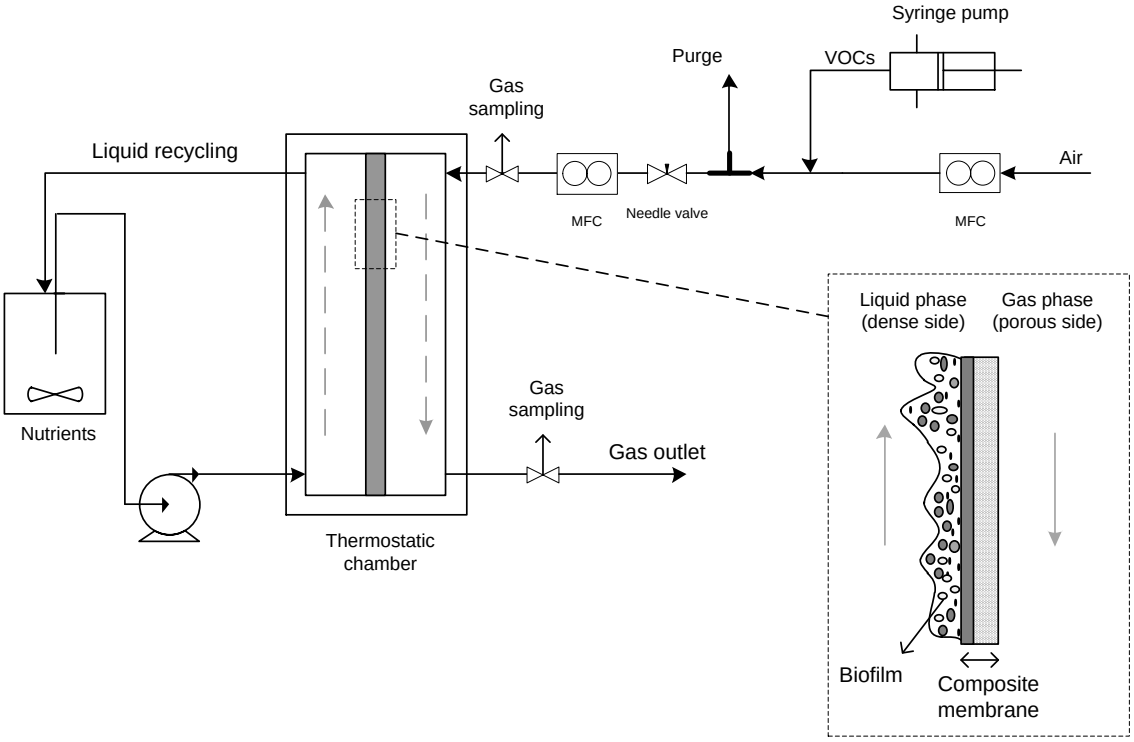


Figure 2

Fig. 2.

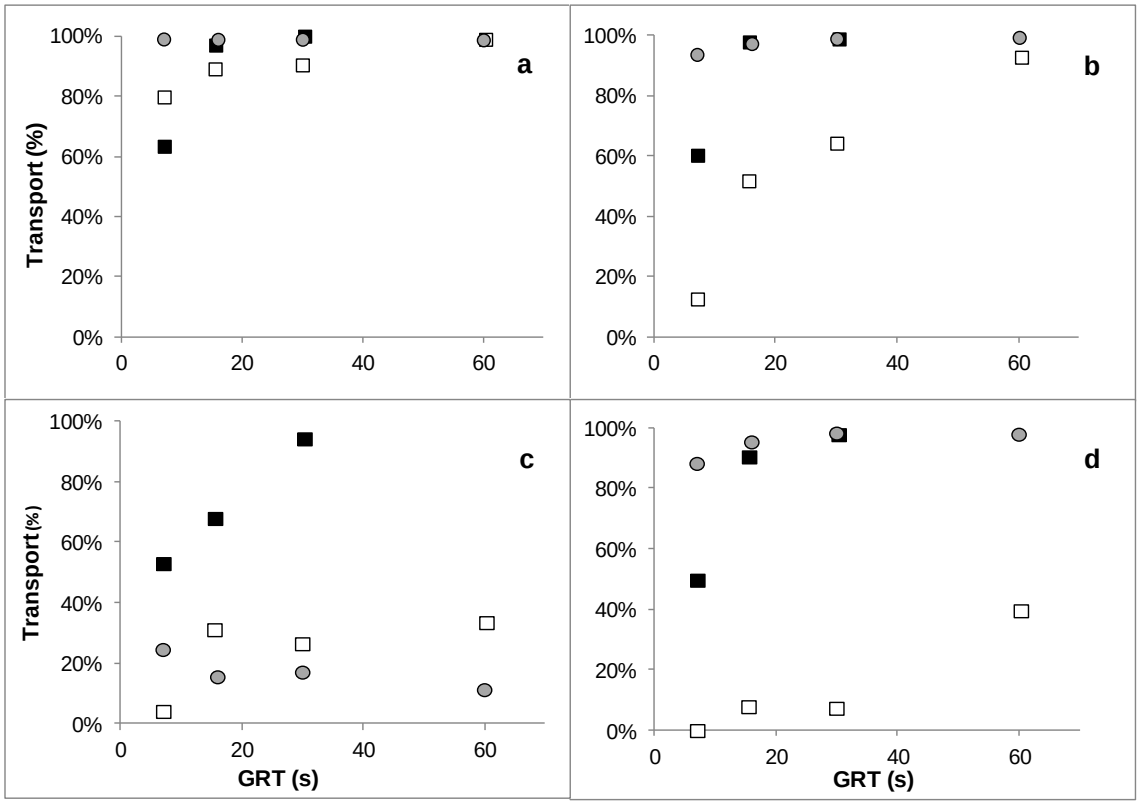


Figure 3

Fig. 3.

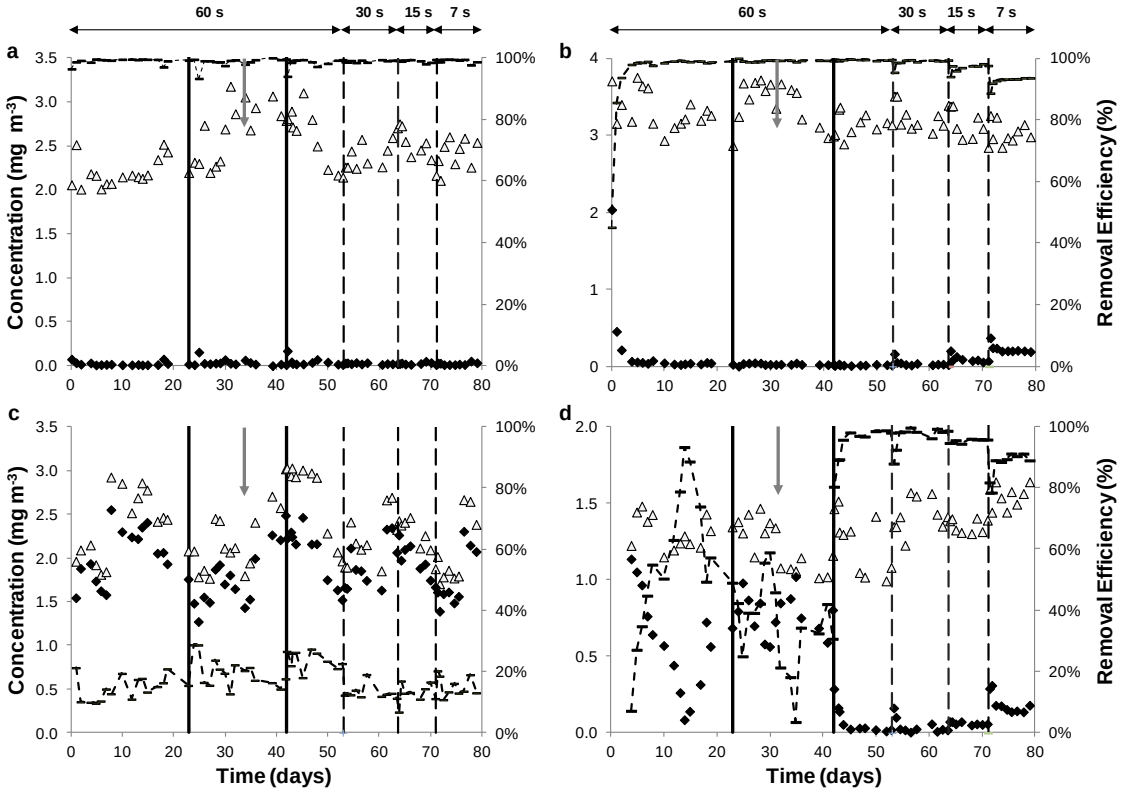
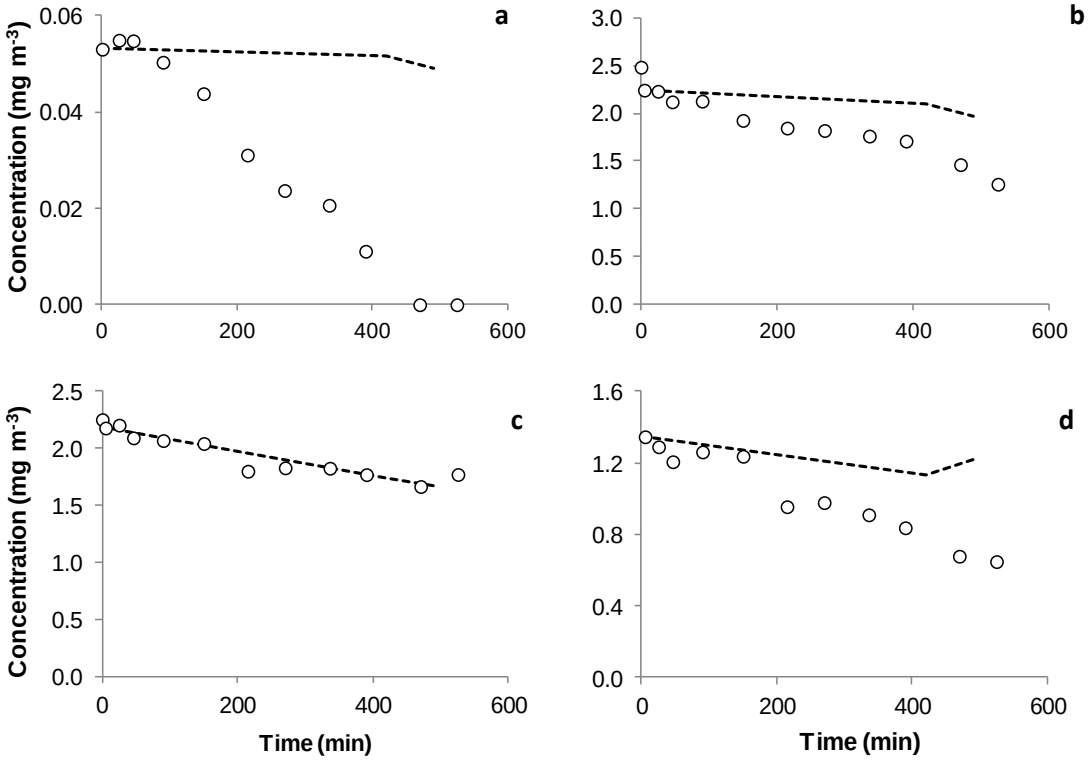


Figure 4

Fig. 4

4.1



4.2

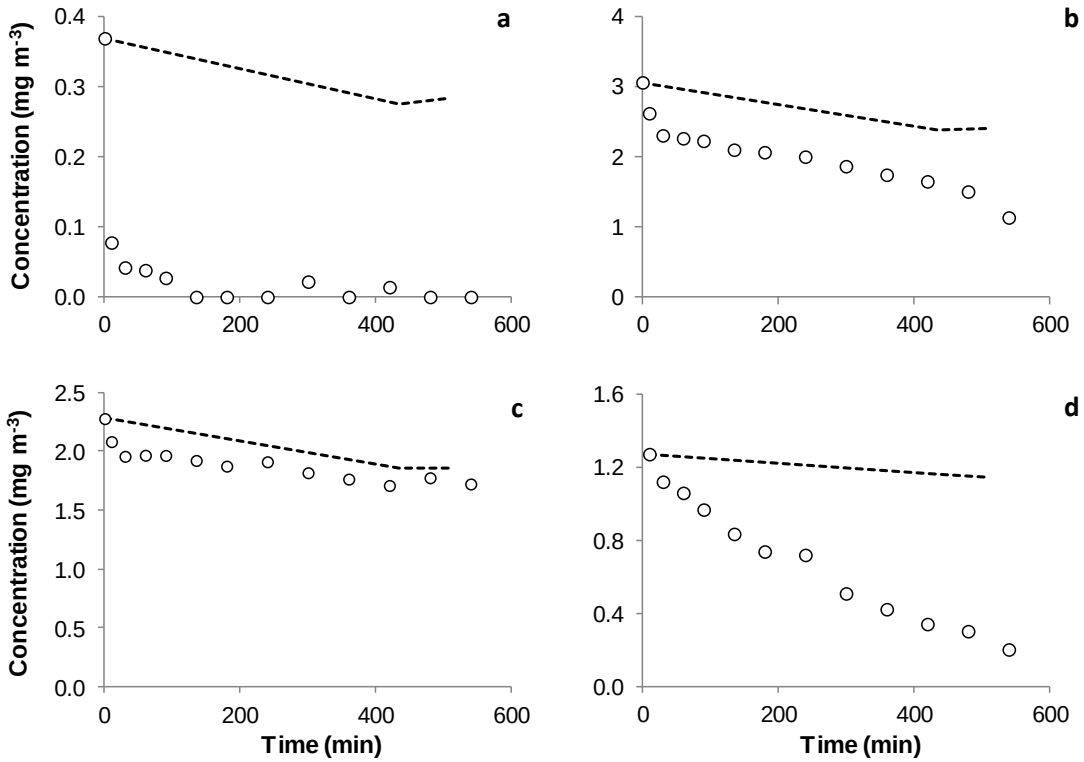
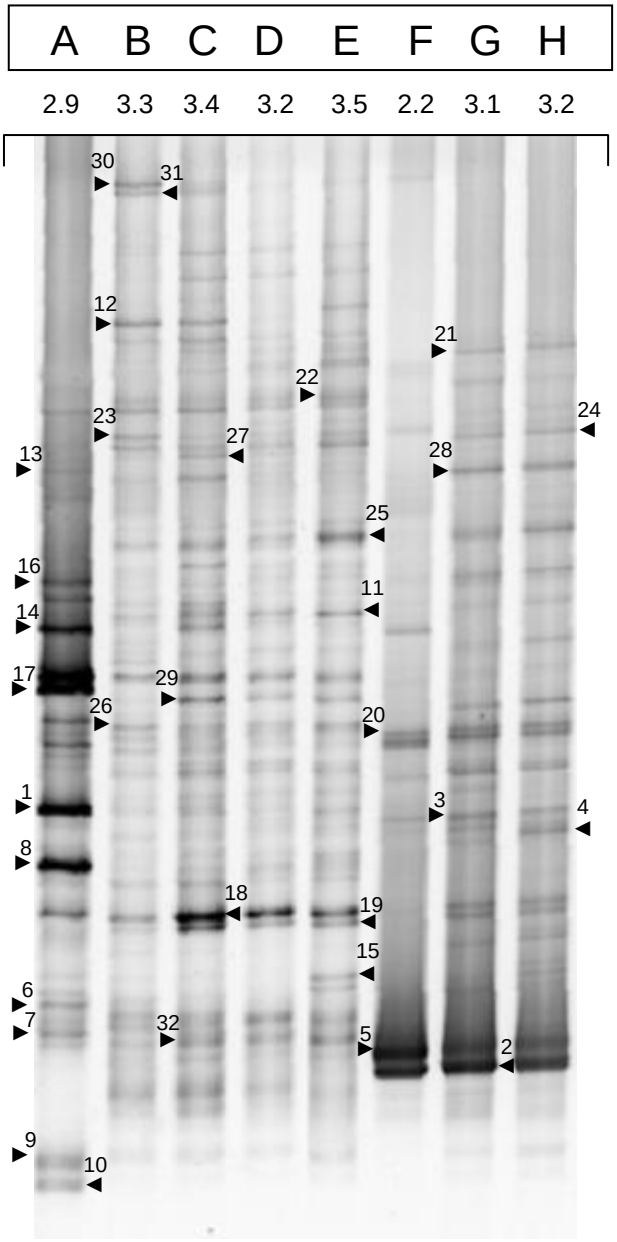


Fig. 5.


A B C D E F G H
2.9 3.3 3.4 3.2 3.5 2.2 3.1 3.2
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