Comparative assessment of two biotrickling filters for siloxanes removal: effect of the addition of an organic phase

Celia Pascual[a,b], Sara Cantera[c], Raúl Muñoz[a, b], Raquel Lebrero[a, b]*

[a] Department of Chemical Engineering and Environmental Technology, University of Valladolid, Dr. Mergelina s/n., Valladolid 47011, Spain.

[b] Institute of sustainable processes, University of Valladolid, Dr. Mergelina s/n., Valladolid 47011, Spain

[c] Laboratory of Microbiology, Wageningen University and Research Center, The Netherlands

*Author for correspondence: raquel.lebrero@iq.uva.es

Keywords: Biogas upgrading, Biotrickling filter, Silicone oil, Siloxanes, Two-phase partitioning bioreactor.

Abstract

Biogas produced at wastewater treatment plants and landfills contains trace levels of volatile methyl siloxanes (VMS) that are responsible for abrasion, corrosion and erosion of equipment during biogas storage and combustion. This research comparatively evaluated the removal of the most common VMS (L2, L3, D4, and D5) under aerobic conditions in a conventional biotrickling filter (BTF) and a two-phase partitioning BTF (TP-BTF) with silicone oil (at 30 %) as organic phase. The TP-BTF showed a superior performance compared to the conventional BTF, increasing the total VMS removal from < 30 % in the BTF up to ~70 % in the TP-BTF. The highest REs in the TP-BTF were recorded for D4 and D5, reaching values of 80-90 %, corresponding to ECs between 0.12 and 0.17 $g.m^{-3}.h^{-1}$. Slightly lower values were obtained for L3 (70-80 %), and the lowest performance was recorded for L2 (20-60%) due to the high vapor pressure of this siloxane and therefore its lower affinity by the organic phase. Surprisingly, despite the different inocula used, a similar microbial community was found by the end of operation of both BTFs, with *KMBC-112*, *Reynarella* and *Chitinophaga* as the dominant genera.

1. Introduction

Biogas produced at wastewater treatment plants (WWTPs) and landfills contains trace amounts of undesirable contaminants such as hydrogen sulfide, NH_3, halogenated hydrocarbons, volatile organic compounds and siloxanes (Muñoz et al., 2015; Ryckebosch et al., 2011). Of them, siloxanes lead to severe detrimental effects when using biogas as an energy vector in turbines, microturbines or fuel cells (Ajhar et al., 2010). Polydimethylsiloxanes (PDMS) are organosilicon compounds widely used in household and industrial products, such us cosmetics and personal care products, detergents, building materials and textiles (Li et al., 2014; Soreanu et al., 2011). When these products reach WWTPs, organosilicon hydrolyzation results in the formation of volatile methyl siloxanes (VMS), a group of low molecular weight compounds that are eventually volatilized to biogas (Soreanu et al., 2011). These VMS are further oxidized to crystalline deposits of silicon dioxide (SiO_2) during biogas combustion (Eq. 1), which cause abrasion, corrosion and erosion of turbine parts. Furthermore, SiO_2 can deactivate catalytic converts resulting in undesirable gas emissions of CO and SO_x (Läntelä et al., 2012; Li et al., 2014).

$$((CH_3)_2SiO)_n + 4nO_2 \rightarrow n\,SiO_2 + 2nCO_2 + 3nH_2O$$

Eq. 1. VMS oxidation reaction during combustion process, where *n* indicates the numbers of Si atoms (n = 3 –6) (Ruiling et al., 2017).

The main VMS present in biogas are hexamethyldisiloxane (L2), octamethyltrisiloxane (L3), hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), D4 and D5 being the most abundant in biogas from landfills and anaerobic digesters (Shen et al., 2018). VMS concentration in raw biogas typically ranges from 16 up to 400 $mg.m^{-3}$ (Dewil et al., 2006). These values far exceed

55 the maximum VMS concentration allowed for biomethane injection into natural gas grid,
56 which according to the most recent European standard 16723 must be lower than 1 mg
57 $Si.m^{-3}$ (Standardization, 2016).

58 Therefore, the removal of siloxanes prior biogas usage is of utmost importance in any
59 biogas-to-energy application, and mandatory when the upgraded biogas is envisaged as
60 vehicle fuel or as a substitute of natural gas. In this context, the main VMS abatement
61 technologies commercially available are based on physical-chemical processes, such as
62 adsorption, absorption and cryogenic condensation. Despite the successful removal
63 efficiencies achieved, these technologies only transfer the undesired compounds from one
64 phase to another, resulting in hazardous wastes that require further treatment, which
65 entails high investment and operating costs (Gaj, 2017; Muñoz et al., 2015).
66 On the contrary, biotechnologies have arisen as a cost-effective and environmentally
67 friendly alternative to these physical-chemical processes. Whereas little is known on the
68 microbiology underlying the biodegradation of VMS, there are several studies focused
69 on the implementation of biotechnologies for continuous VMS removal. For instance,
70 Accettola et al (2008) and Popat and Deshusses (2008) operated aerobic biotrickling
71 filters (BTF) inoculated with isolated D4-degrading bacteria, reaching removals
72 efficiencies of this VMS of 20-43 % at empty bed residence times (EBRTs) ranging from
73 2.2 up to 19 min. Li et al (2014) significantly improved these preliminary results
74 achieving REs over 74 % for D4 at an EBRT of 13.2 min in an aerobic BTF inoculated
75 with *Pseudomonas aeruginosa* S240. This superior process performance was attributed
76 to the presence of rhamnolipids, biosurfactants produced by *P. aeruginosa* that could have
77 increased the mass transfer of D4 from the gas phase to the aqueous phase. Overall, these
78 investigations suggested that the main bottleneck during biological VMS removal is the
79 low solubility of these compounds in the aqueous phase, and hence their poor availability

to the microbial community. Since bioreactors operation at high EBRTs results in prohibitive reactor volumes, the addition of a non-aqueous (organic) phase with a high affinity for the VMS represents as a potential solution to overcome mass transfer limitation. In this context, the superior performance of two-phase partitioning bioreactors (TPPB) for the removal of hydrophobic volatile organic compounds such as hexane has been consistently demonstrated during the past decade. TPPBs are based on boosting the mass transfer of the target gas compound by adding an organic water-immiscible and non-volatile phase with a high affinity of the gas pollutant (Muñoz et al., 2007). Organic solvents such as hexadecane and silicone oil have been employed as organic phase in TPPBs. A recent research demonstrated the enhancement of chlorobenzene biodegradation by using a water-silicone oil biphasic system (Ye et al., 2019). Nevertheless, TPPBs have not been yet applied for the removal of VMS from biogas.

This study aimed at comparatively evaluating the removal of a mixture of VMS (L2, L3, D4, and D5) by using both a conventional BTF (without organic phase) and a two-phase BTF (TP-BTF). The organic phase selected in the TP-BTF was silicone oil, due to its high affinity for VMS. In addition, the bacterial community structure of both bioreactors was analyzed with the aim of identifying the most representative microorganisms involved in siloxanes degradation. This research constitutes an important contribution to the field of biogas upgrading since it explores and demonstrates the viability of siloxanes biodegradation in two-phase BTFs, a promising, sustainable and innovative technology scarcely studied so far

2. Materials and Methods

105 **2.1. Mineral Salt Medium**

106 The mineral salt medium (MSM) was composed of ($g.L^{-1}$): KH_2PO_4, 0.7; $K_2HPO_4 \cdot 3H_2O$,
107 0.917; KNO_3, 3; NaCl, 0.2; $MgSO_4 \cdot 7H_2O$, 0.345; $CaCl_2 \cdot 2H_2O$, 0.026; and 2 $mL.L^{-1}$ of a
108 micronutrients solution containing ($g.L^{-1}$): EDTA, 0.5; $FeSO_4 \cdot 7H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$,
109 0.01; $MnCl_2 \cdot 4H_2O$, 0.003; H_3BO_3, 0.003; $CoCl_2 \cdot 6H_2O$, 0.02; $CuCl_2 \cdot 2H_2O$, 0.001; $NiCl_2$
110 $\cdot 6H_2O$, 0.002; $NaMoO_4 \cdot 2H_2O$, 0.003. All the chemicals used for the preparation of the
111 MSM were purchased from Panreac (Barcelona, Spain). L2 (98.5% purity), L3 (98 %
112 purity), D4 (98 % purity) and D5 (97 % purity) were obtained from Sigma Aldrich (San
113 Luis, EEUU).

114

115 **2.2 Culture enrichment conditions**

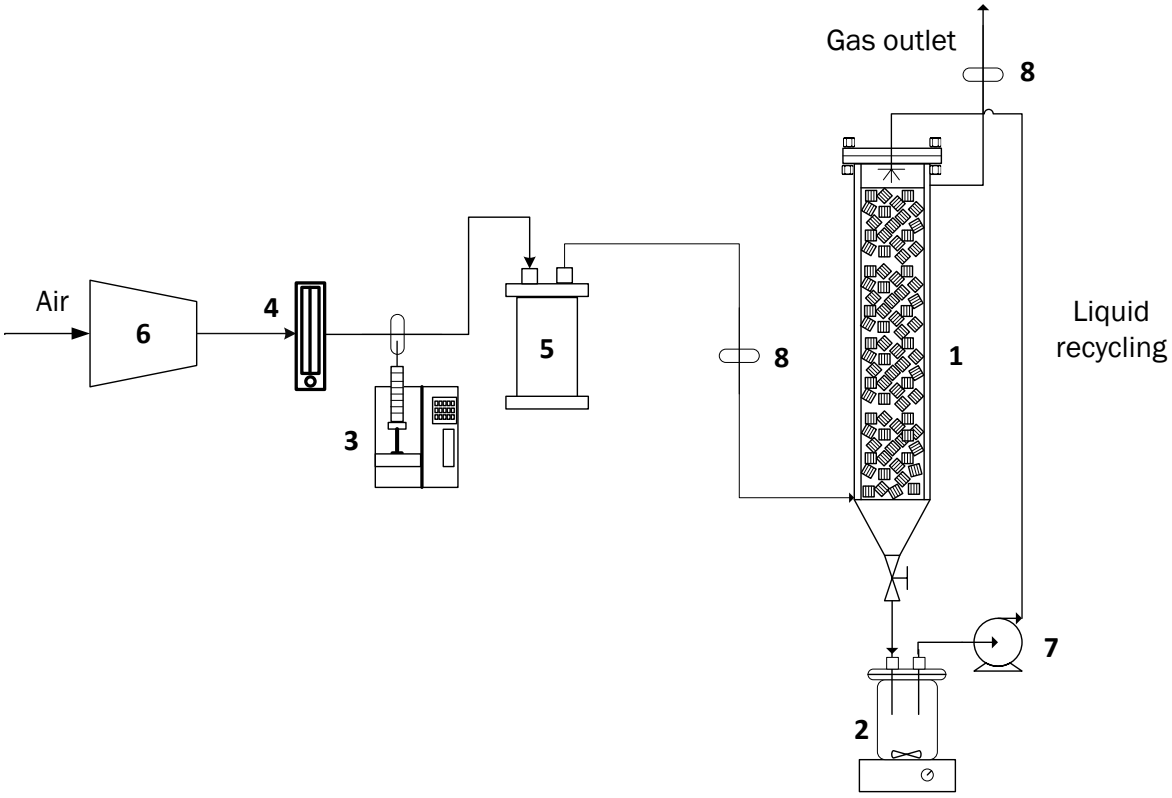
116 Three aerobic batch tests were operated to enrich a VMS degrading culture using
117 activated sludge as inoculum (Valladolid WWTP, Spain). For this purpose, 1.2 L bottles
118 were filled with 0.2 L of mineral salt medium at a pH ~ 7 and inoculated with 100 mL of
119 activated sludge. A 20 mL gas mixture of VMS (containing trimethylsilanol (TMSOL),
120 L2, L3, L4, L5, D4 and D5 at individual concentrations of ~50 $mg.m^{-3}$) was added to the
121 headspace as the only carbon and energy source at an initial total concentration of ~350
122 mg $VMS.m^{-3}$. The enrichment was conducted for 360 days. Both gas and liquid phases
123 were replaced by day 39, 174 and 230. For this purpose, the cultivation broth was
124 centrifuged for 10 min at 10000 rpm and the pellet was resuspended in 0.3 L of fresh
125 mineral medium. The bottles headspace was flushed with fresh air prior supplementation
126 of 20 mL of VMS gas mixture. By days 95 and 304, 20 mL of VMS gas mixture were
127 added to the headspace without replacing neither the headspace nor the liquid phase.
128 VMS, CO_2, O_2 and N_2 concentrations were periodically analyzed in the headspace of the

129 bottles, and samples of the cultivation broth were periodically withdrawn to determine
130 pH, NO_3^-, NO_2^-, TN, TOC and IC concentrations.
131

132 **2.3. Experimental setup and operating procedure.**

133 The experimental systems (Fig. 1) consisted of a cylindrical PVC column of 2 L of
134 working volume (8.4 cm diameter, 37.5 cm height). A 1.2 L holding tank magnetically
135 stirred at 100 rpm was used as MSM reservoir. The MSM in the BTF, and the mixture
136 MSM + silicone oil in the TP-BTF, were continuously recycled to the top of the column
137 by a peristaltic pump at a linear velocity of 2 $m.h^{-1}$. The VMS-loaded inlet air stream was
138 prepared by injecting a liquid mixture containing L2, L3, D4 and D5 with a syringe pump
139 (Fusion 100, Chemyx Inc. USA) into a 33 $mL.min^{-1}$ air stream controlled by means of a
140 rotameter. The VMS-loaded stream entered a mixing chamber and was subsequently fed
141 at the bottom of the column countercurrently with the trickling liquid flow.

142



143

144 Fig. 1. Schematic representation of the experimental set-up. (1) Biotrickling filter, (2)
145 nutrient reservoir, (3) syringe pump, (4) rotameter, (5) mixing chamber, (6) air
146 compressor, (7) peristaltic pump, (8) gas sampling ports.

147

148 An abiotic test was initially performed to discard the possibility of siloxanes removal by
149 photolysis or adsorption, and to ensure that there was no biological activity in the system
150 prior to inoculation. For this purpose, the test was initiated with the empty PVC column,
151 while sterile packing material and MSM were added in subsequent steps.

152 The BTF was inoculated with the previously enriched siloxane-degrading culture. The
153 system was operated during 160 days in five different stages (Table 1) at an EBRT of 1 h
154 and a periodical replacement of the culture broth with fresh MSM of 200 mL every three
155 days (equivalent to a hydraulic retention time of 18 days). During stages S1, S3 and S5,

156 the system was fed with a VMS-loaded stream with a total VMS concentration between
157 500 and 700 mg.m^{-3}. By day 81, the original enriched culture was used to re-inoculate the
158 system. During stage S2, VMS feeding was stopped in order to evaluate potential
159 emissions of VMS adsorbed in the packing material or biofilm, and CO_2 production.
160 During this period, no MSM exchange was performed. Finally, a two-fold increase in the
161 VMS concentration up to ~1300 mg.m^{-3} was implemented during stage S4.

162 Table. 1. Experimental conditions tested in BTF.

	Feed Stream	Time course (days)	VMS Concentration (mg.m^{-3})
S1	VMS loaded air stream	0 - 46	515 ± 126
S2	VMS free air stream	47 - 76	-
S3	VMS loaded air stream	77 - 124	719 ± 203
S4	VMS loaded air stream	125 -137	1288 ± 217
S5	VMS loaded air stream	138 - 160	651 ± 127

163

164 In the particular case of the TP-BTF, constructed with 30 % of silicone oil 20 cts,
165 siloxanes removal and CO_2 production were observed by day 10. Therefore, no additional
166 inoculum was added and the TP-BTF was operated in three different stages at an EBRT
167 of 1 h (Table 2). From day 84 onwards, 200 mL of the culture broth were withdrawn
168 every three days (equivalent to a hydraulic retention time of 18 days). The silicone oil
169 was recovered by settling, supplemented with fresh MSM up to 200 mL and returned to
170 the system. Thus, the silicone oil initially added to the TP-BTF was reused during the
171 entire experiment without necessity of replacement or further supply. During stages S1
172 and S3, the system was fed with a VMS-loaded stream at a concentration of ~650 mg.m-
173 ^{3}, while during S2 both VMS feeding and culture broth replacement were stopped.

174

175 **Table. 2**. Experimental conditions in TP-BTF.

	Feed Stream	Time course	VMS Concentration

		(days)	($mg.m^{-3}$)
S1	VMS loaded air stream	0 - 21	651 ± 181
S2	Clean air stream	22 - 70	-
S3	VMS loaded air stream	70 - 127	625 ± 137

Inlet and outlet VMS and CO_2 gas concentrations were daily analyzed. The samples were taken directly from the VMS-loaded air stream by means of a gas sampling port located at both inlet and outlet gas streams of the BTFs The TOC, TN, nitrite and nitrate concentrations and the pH of the cultivation broth were periodically analyzed every three days.

2.4. Analytical procedure

VMS gas concentration was analyzed in a Bruker 3900 gas chromatograph (Palo Alto, USA) equipped with a flame ionization detector and a HP-5-MS (30 m × 0.25 mm × 0.25 µm) column. Both the detector and injector temperatures were maintained constant at 250 ºC. The oven temperature was initially set at 40 ºC for 2.0 min, then increased at 20 ºC min^{-1} up to 180 ºC, maintained for 1 min and increased again at 20 ºC min^{-1} up to 200 ºC. Finally, this temperature was maintained for 0.5 min. N_2 was used as the carrier gas at a flow rate of 1 $mL.min^{-1}$. CO_2 and O_2 gas concentrations were determined in a Bruker 430 gas chromatograph (Palo Alto, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m × 0.53 mm × 15 µm) and a P-PoraBOND Q (25 m × 0.53 mm × 10 µm) columns. Oven, detector and injector temperatures were maintained constant at 45, 200 and 150 °C for 5 min, respectively. Helium was used as the carrier gas at a flow of 13.7 $mL.min^{-1}$.

The pH of the cultivation broth was analyzed using a glass membrane electrode PH BASIC 20 (Crison, Barcelona, Spain). TOC, IC and TN concentrations were measured

using a TOC-VCSH analyzer coupled with a TNM-1 chemiluminescence module (Shimadzu, Japan). Finally, nitrite and nitrate were determined in a HPLC-IC by using a Waters 515 HPLC pump coupled with a conductivity detector (Waters 432) and equipped with an IC-PAK Anion HC column (4.6 × 150 mm) and an IC-Pak Anion Guard-Pak (Waters). Samples were eluted isocratically at 2 $mL.min^{-1}$ (at room temperature) with a solution of distilled water/acetonitrile/n-butanol/buffer at 84/12/2/2% v/v (Muñoz et al., 2013).

2.5 Bacterial community analysis

Aliquots of 40 mL of fresh activated sludge (AS), the two inocula of the BTF (BTF-InA – day 0, BTF-InB – day 81), the end of the experimental period of the BTF (BTF-END – day 160), stage S1 of the TP-BTF (TP-S1 – day 127) and the end of the experimental period of the TP-BTF (TP-END – day 21) were sent for DNA extraction and Illumina Miseq amplicon sequencing to the Foundation for the Promotion of Health and Biomedical Research of Valencia Region (FISABIO, Spain). Amplicon sequencing was developed targeting the 16S V3 and V4 regions (464bp, *Escherichia coli* based coordinates) with the bacterial primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a- A-21, forward and reverse. Illumina adapter overhang nucleotide sequences were added to the gene-specific sequences according to Perez et al., 2019 (Pérez et al., 2019). Library construction was carried out using the Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, CA). Libraries were then normalized and pooled prior to sequencing. Non-indexed PhiX library (Illumina, San Diego, CA) was used as performance control. Samples containing indexed amplicons were loaded onto the MiSeq reagent cartridge for automated cluster generation paired-end sequencing with a 2×300pb paired-end run (MiSeq Reagent kit v3 (MS-102-3001)) according to manufacturer's

instructions (Illumina). The 16S rRNA gene sequences were processed using Mothur v1.40.5 following the Mother SOP (https://www.mothur.org/wiki/MiSeq_SOP (Schloss et al., 2009)). Quality filtered sequences were aligned to the Silva 16S rRNA gene reference database (Quast et al., 2013; Yilmaz et al., 2014), preclustered by abundance and chimeric sequences were removed according to Phandanouvong-Lozano et al., 2018 (Phandanouvong-Lozano et al., 2018). Sequences were then classified and annotated into Operational Taxonomic Units (OTUs) using the Ribosomal Database Project, RDP with 100 bootstrap iterations and 80% confidence cutoff (Cole et al., 2009). The plots were generated with R version 3.5.1 and RStudio 1.1.442, and the gplot package 3.0.1 for R was used to build the heatmap (Team, 2016).

3. Results

3.1 Abiotic test

During the abiotic test, no siloxanes removal was observed in the empty PVC column or after the addition of the packing material (Fig. S1). A slight decrease in the outlet concentration of the four compounds was observed when the mineral medium was recirculated through the bed. This removal was associated to a partial absorption of siloxanes in the liquid media rather than to biological removal, since no CO_2 production was detected (Fig. S2). As expected, a higher absorption was recorded after the addition of silicone oil due to the higher affinity of this organic phase for siloxanes, with no CO_2 production observed during the first 3 days.

3.2 Performance of the BTF

246 No significant differences were observed in terms of VMS removal efficiency (RE)
247 through the different stages in BTF, with values lower than 30 % for the different VMS
248 throughout the entire experiment (Fig. 2.A). D5 reached the highest RE of 26.6 ± 15.3 %
249 during S1, while the total VMS RE and elimination capacity (EC) accounted for 19.1 ±
250 13.3 % and 0.10 ± 0.08 $g.m^{-3}.h^{-1}$, respectively. During S2 (without VMS feeding to the
251 system), no significant VMS emission was observed, which confirms the low absorption
252 capacity of the mineral medium due to the low aqueous solubility of siloxanes (Fig. S3)

253 A slight decrease in RE was observed for the 4 compounds in the subsequent feeding
254 stages. In particular, the total VMS RE and EC averaged 12.9 ± 13.1 % and 0.14 ± 0.15
255 $g.m^{-3}.h^{-1}$, respectively, in S3. The increase in VMS inlet concentrations during S4 did not
256 significantly affect VMS removal, which remained at 10.1 ± 14.7 %, but increased the
257 EC to 0.16 ± 0.16 $g.m^{-3}.h^{-1}$ due to the higher VMS inlet concentration in S4 compared to
258 S1 and S3. Finally, values of 7.9 ± 11.5 % and 0.06 ± 0.09 $g.m^{-3}.h^{-1}$ were obtained for the
259 total VMS RE and EC, respectively, during S5.

260

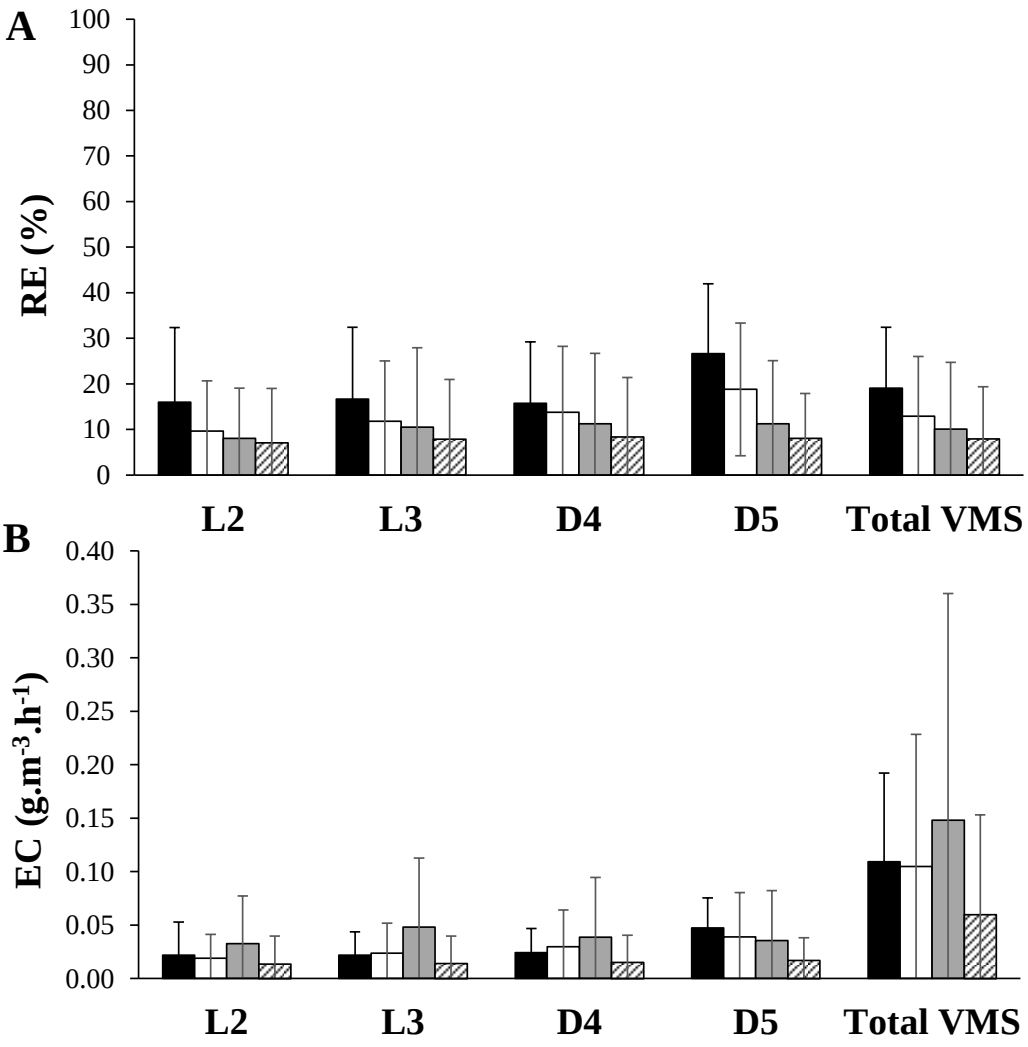


Fig. 2. Average VMS removal efficiencies (A) and elimination capacities (B) in the BTF during the four feeding stages: S1 (black bars), S3 (white bars), S4 (grey bars) and S5 (striped bars). Vertical lines represent standard deviation from measurements under steady state.

The biodegradation of VMS resulted in an average CO_2 production of 0.43 ± 0.11 $g.m^{-3}.h^{-1}$ during the entire experiment. Likewise, the pH of the cultivation broth remained constant at 6.9 ± 0.1. The TN concentration decreased from 402.7 to 274.3 $mg.L^{-1}$ by day 80, gradually increasing afterwards to reach a steady value of 341.2 ± 21.3 $mg.L^{-1}$ from day 97 onwards. Similarly, $N\text{-}NO_3^-$ concentration initially decreased from ~400 to 301.9 $mg.L^{-1}$ by day 80, steadily increasing afterwards to an average value of 342.9 ± 7.5 $mg.L^{-}$

$^{-1}$ from day 97 onwards. No $N\text{-}NO_2^-$ production was observed throughout the entire experiment.

The TOC concentration in the cultivation broth rapidly increased from 3.3 to 15.0 $mg.L^{-1}$ by day 6, progressively decreasing down to 5.1 $mg.L^{-1}$ by day 80. From day 80 onwards, TOC concentration remained constant at an average value of 3.9 ± 1.0 $mg.L^{-1}$. Conversely, IC concentration remained stable at 1.6 ± 0.5 $mg.L^{-1}$ throughout the entire experiment.

3.3 Performance of the TP-BTF

A total VMS RE of ~ 70 % was achieved during both S1 and S3 in the TP-BTF (Fig. 3). Initially, higher REs were recorded due to absorption of the VMS in the fresh silicone oil. These values gradually decreased and stabilized by day 6. The highest REs were obtained for D4 and D5. The average REs for D4 were 91.1 ± 2.1 % during S1 and 81.0 ± 6.6 % during S3, corresponding to ECs of 0.17 ± 0.05 $g.m^{-3}.h^{-1}$ and 0.14 ± 0.04 $g.m^{-3}.h^{-1}$, respectively. Similarly, REs for D5 remained at 87.8 ± 4.3 % during S1 and 80.3 ± 6.0 % during S2, corresponding to ECs of 0.17 ± 0.05 $g.m^{-3}.h^{-1}$ and 0.12 ± 0.05 $g.m^{-3}.h^{-1}$, respectively. Slightly lower REs and ECs were obtained for L3, remaining at 78.4 ± 6.2 % and 0.11 ± 0.03 $g.m^{-3}.h^{-1}$ during S1 and 70.6 ± 12.3 % and 0.12 ± 0.04 $g.m^{-3}.h^{-1}$ during S3, respectively. In contrast, L2 abatement performance in TP-BTF was considerably lower and fluctuating compared to the rest of the compounds, with values ranging between 20 and 60 %, corresponding to ECs between 0.02 and 0.15 $g.m^{-3}.h^{-1}$.

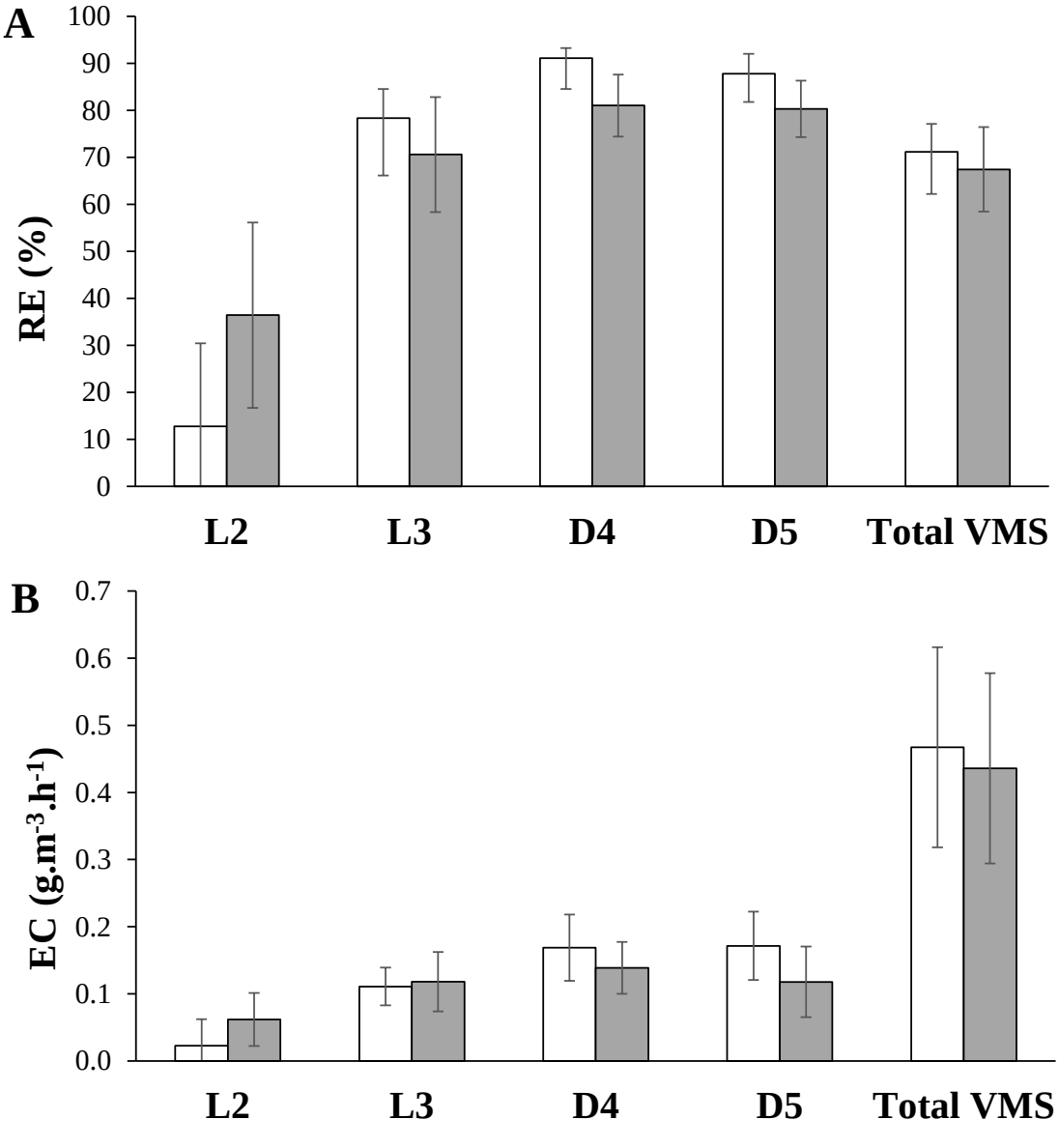
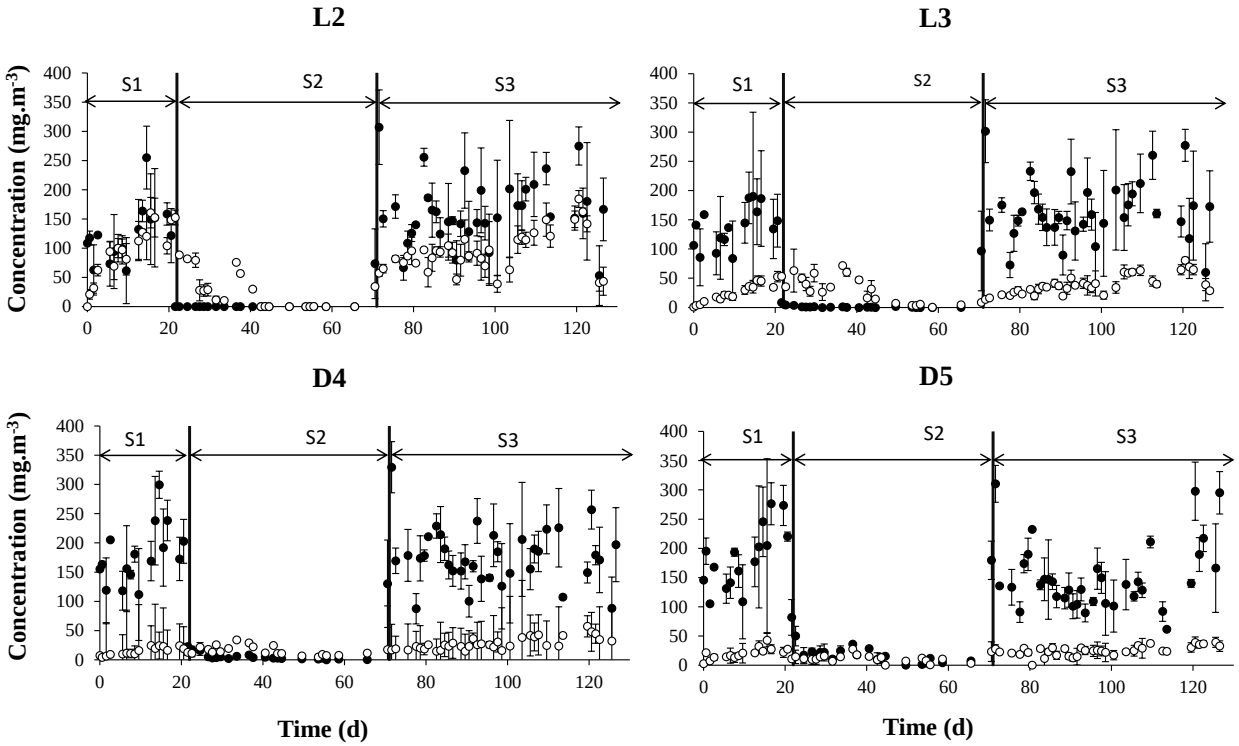


Fig. 3. Average VMS removal efficiencies (A) and elimination capacities (B) in the TP-BTF during the two feeding stages: S1 (white bars) and S3 (grey bars). Vertical lines represent standard deviation from measurements under steady state.

The total VMS outlet concentration remained at average values of 184.7 ± 54.1 and 204.0 ± 66.0 mg.m^{-3} during S1 and S3, respectively. The lowest values were obtained for D4 and D5, with outlet concentrations of 16.5 ± 6.2 and 23.0 ± 8.5 mg.m^{-3} during stage S1 and 27.9 ± 8.1 and 24.0 ± 6.6 mg.m^{-3} during S3, respectively. Finally, a slight VMS

303 emission was observed during S2, probably due to the desorption of the VMS from the
304 silicone oil (Fig. 4).

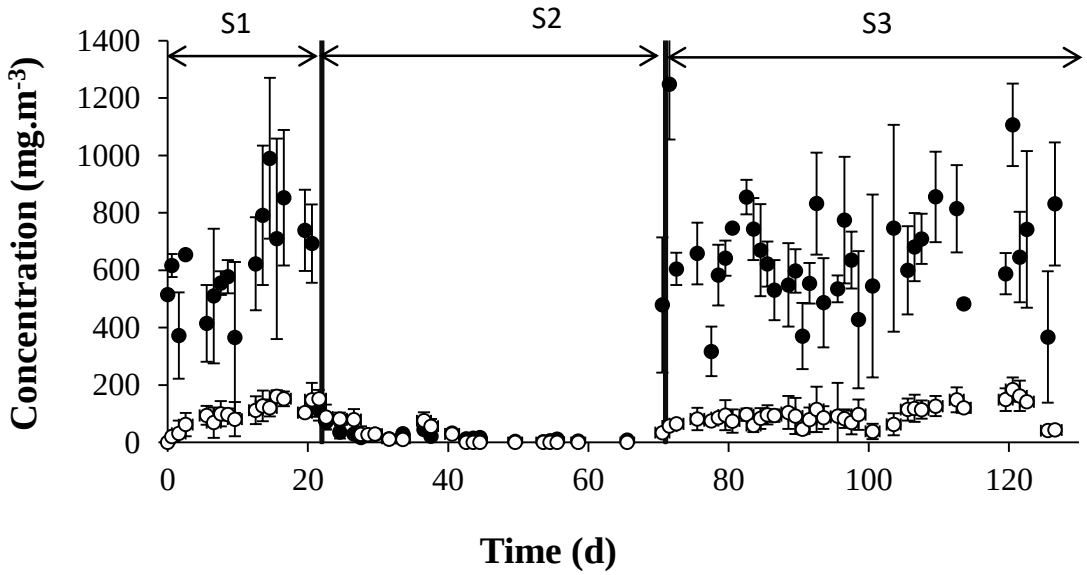
305



306

307 **Fig. 4.** Time course of L2, L3, D4 and D5 inlet (●) and outlet (○) concentration in the
308 TP-BTF. Vertical lines represent standard deviation from triplicate measurements.

309



310

311 **Fig. 5.** Time course of total VMS inlet (●) and outlet (○) concentration in the TP-BTF.
312 Vertical lines represent standard deviation from triplicate measurements.

313

314 The biodegradation of VMS resulted in a CO_2 production of 2.24 ± 0.29 and 2.26 ± 0.96
315 $g.m^{-3}.h^{-1}$ during S1 and S3, respectively. Similarly, pH remained constant at 7.1 ± 0.3
316 during the entire experiment. TN concentration in the cultivation broth decreased from
317 402.7 to 214.3 $mg.L^{-1}$ by day 84. Afterwards, a gradual increase was recorded up to a
318 maximum concentration of 376.9 $mg.L^{-1}$ by day 127 due to the mineral medium
319 exchange. Similarly, $N\text{-}NO_3^-$ concentrations of 403.3, 229.8 and 343.2 $mg.L^{-1}$ were
320 recorded by days 0, 84 and 127, respectively. No NO_2^- production was observed.

321 Finally, TOC concentration in the cultivation broth increased initially from 4.3 to 219.5
322 $mg.L^{-1}$ by day 84, gradually decreasing afterwards to 100.7 $mg.L^{-1}$ by day 127. IC
323 concentration remained constant at 6.5 ± 4.2 $mg.L^{-1}$ throughout the entire experiment.

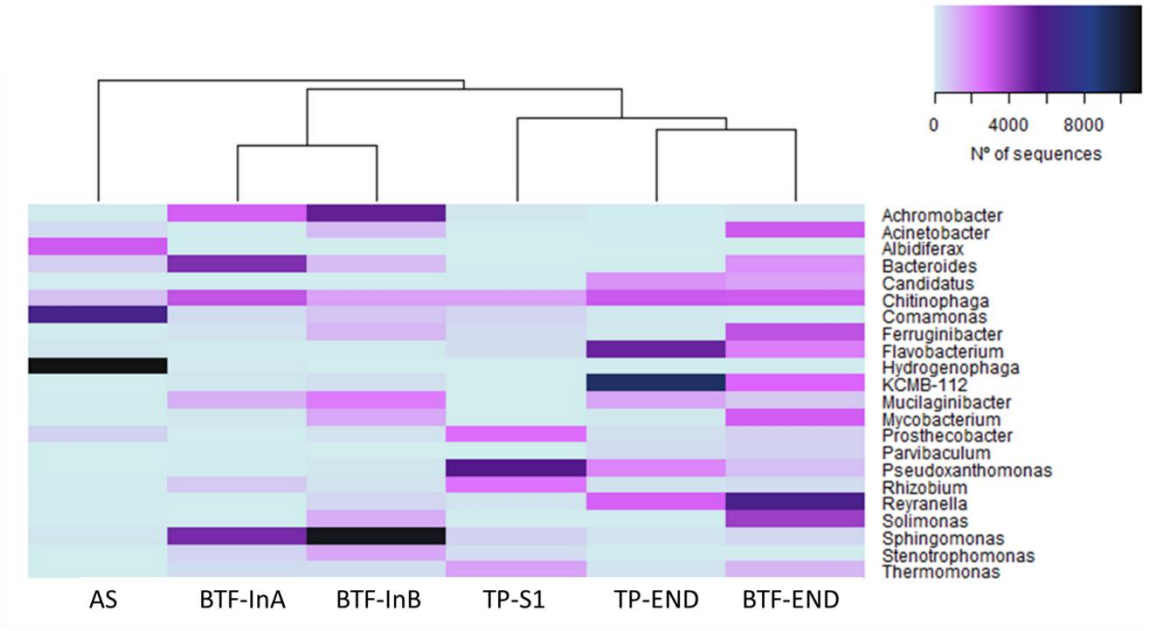
324

325 **3.4 Analysis of the microbial community**

Activated sludge (AS) was used as inoculum for the enrichment of a siloxane degrading consortium. The dominant genera in the AS were *Hydrogenophaga*, *Comamonas*, *Albidiferax* and *Chitinophaga*, with abundances of 27.7, 15.9, 7.8 and 2.3 %, respectively (Fig.6). However, during the enrichment of BTF-InA (day 278) and BTF-InB (day 360) a significant shift in the microbial population was observed. In these samples, the genera *Hydrogenophaga*, *Comamonas* and *Albidiferax* represented less than 1 % of the total population, while bacteria from the genus *Chitinophaga* increased their abundance up to 11.8 % in BTF-InA and to 4.7 % in BTF-InB. Regardless of the enrichment duration, both samples presented a similar population structure, which consisted mainly of 5 genera (data shown as relative abundance in BTF-InA and BTF-InB, respectively): *Sphingomonas* (17.1 and 29.9 %), *Achromobacter* (10.5 and 14.4 %), *Bacteroides* (16.4 and 3 %) and *Mucilaginibacter* (4.4 and 6.3 %), as well as the above mentioned *Chitinophaga* (Fig. 5). Interestingly, by the end of the experimental period (stage S5), the diversity and richness of the bacterial population in BTF increased in terms of absolute abundance. The continuous exposure to siloxanes supported the growth of genera with negligible abundances in the inocula, such as *Reyranella* (10.1 %), *Solimonas* (6.8 %), *Ferruginibacter* (5.6 %), *Mycobacterium* (5.0 %) and an uncultured genus from the family *Acidithiobacillaceae*, *KCMB-112* (4.9 %) (SILVA database Accession Nr: FJ914601). Moreover, the genus *Chitinophaga* (5.1 %) still represented an important share of the population in the sample BTF-END.

Interestingly, biomass grew in the TP-BTF without previous inoculation using siloxanes as the only carbon and energy source. The main genera retrieved in stage S1 (TP-S1) were *Pseudoxanthomonas* (21.2 %), *Proteobacter* (9.9 %), *Rhizobium* (9.4 %) and the genus *Chitinophaga* (5.9 %). Nevertheless, after operation of the TP-BTF (TP-END) the bacterial population shifted towards a more specialized community that was similar to the

351 bacterial population independently reached during the operation of BTF. The main genera
352 were *KCMB-112* (20 %), *Flavobacterium* (11.2 %) *Reyranella* (7.0 %) and *Chitinophaga*
353 (6.9 %).
354



355

356 **Fig. 6**. Heatmap showing the differential number of sequences of the most significant
357 22 bacterial genera in the activated sludge (AS), the enriched culture used as inoculum
358 of the BTF (BTF-InA, BTF-InB), the end of operation of the BTF (BTF-END) and the
359 TP-BTF operation (TP-S1, TP-END). OTUs with absolute abundances < 1 were not
360 included in the data analysis.
361

362 **4. Discussion**

363 A clear improvement in the siloxanes abatement performance of the BTF was observed
364 with the addition of silicone oil at 30 %. In this sense, the presence of the organic phase
365 resulted in an increase in the total VMS RE from 20 % in the BTF to 70% in the TP-BTF,
366 which resulted in an EC 5 fold higher. The re-inoculation of the BTF by day 81 had no
367 effect on performance of the system, the poor VMS REs recorded being associated to the

low solubility of the VMS in the aqueous phase and hence to their reduced mass transfer. Several studies have confirmed that the presence of an organic phase in a TPPB increases the elimination performance of poorly soluble contaminants such as CH_4, toluene and styrene (Cantera et al., 2016; San-Valero et al., 2017; Nourmohammadi et al., 2018). To the best of our knowledge, this is the first study validating the potential of a silicon oil-based BTF for VMS removal. Moreover, the negligible VMS desorption observed during S2 (no VMS feeding) supported the high affinity of silicone oil for siloxanes. This result, along with the significantly higher CO_2 production recorded in the TP-BTF compared to the BTF, confirmed biodegradation as the main mechanism of VMS removal. In addition, the higher TOC concentration in the trickling solution of the TP-BTF (~ 150 $mg.L^{-1}$ vs. ~ 4 $mg.L^{-1}$ in BTF) further evidenced the biological activity. Based on the results obtained by Accettola et al (2008) and Li et al (2014), the increase in TOC concentration was attributed to Si-containing metabolites such as silicic acid. At this point is should be stressed that the aqueous solubility of silicone oil is negligible.

When analyzing the individual removal performance of the different VMS, the lowest enhancement was observed for L2, increasing from an overall RE of ~10 % in BTF to 27 % in TP-BTF. This improvement in RE was significantly higher for L3, reaching an overall RE of ~ 76 % in the TP-BTF compared to the average RE of 12 % recorded in the BTF. Nevertheless, the most remarkable enhancement was obtained for D4 and D5, with REs between 85 and 90 % in the TP-BTF *vs* 13 and 18 % in the BTF, respectively. These cyclic siloxanes are typically the most abundant VMS in biogas, and therefore the biological removal of lineal VMS has not been reported to date. For instance, Popat and Deshusses (2008) obtained D4 removals of 43 % at an EBRT of 19.5 min under aerobic conditions, while Li et al (2014) significantly improved previous results reaching D4 REs > 74 % in an aerobic BTF operating at an EBRT of 13.2 min. This enhanced performance

393 was attributed to the presence of rhamnolipids in the trickling solution, which likely
394 fostered the mass transfer of D4 from the gas to the aqueous phase. The effect of the gas
395 residence time and the packing material in the removal of both D4 and D5 was studied
396 by Santos-Clotas et al (2019) in an anoxic BTF using nitrate as electron acceptor. The
397 highest REs obtained were 13 % and 37 % for D4 and D5, respectively, at the highest
398 EBRT (14.5 min). Moreover, the addition of activated carbon to the packing material
399 resulted in an increased mass transfer of D4 and D5, which supported REs of 16 % and
400 45 %, respectively. The low L2 removal was attributed to its high vapor pressure, which
401 hindered the solubility of this compound in the organic phase (Table 3). As demonstrated
402 by Rojas Devia and Subrenat (2013), the lower the vapor pressure of siloxanes the easier
403 their removal from the gas phase by absorption into different oils (L2>L3>D4>D5).
404 These researchers also reported a significant effect of the temperature on VMS mass
405 transfer, with a significant increase in absorption efficiency when operating at the lowest
406 temperature, which provided removals of 80 % and 60 % for D4 and L2, respectively.

407

408 **Table 3.** Physical properties of volatile methyl siloxanes present in biogas.

Compound	Formula	Boiling point (ºC)[a]	Molar Mass ($g.mol^{-1}$)	Saturated vapor pressure at 25°C (Pa)[a]	Water solubility at 25 ºC ($mg.L^{-1}$)[a]
Hexamethyldisiloxane (L2)	$C_6H_{18}OSi_2$	106.9	162.4	5626.2	0.93
Octamethyltrisiloxane (L3)	$C_8H_{24}O_2Si_3$	153.0	236.5	445.0	0.034
Decamethyltetrasiloxane (L4)	$C_{10}H_{30}O_3Si_4$	194.0	310.7	50.0	0.00674
Dodecamethylpentasiloxane (L5)	$C_{12}H_{36}O_4Si_5$	232.0	384.8	9.0	0.000309
Hexamethylcyclotrisiloxane (D3)	$C_6H_{18}O_3Si_3$	135.2	222.5	471.0	1.56
Octamethylcyclotetrasiloxane (D4)	$C_8H_{24}O_4Si_4$	175.7	296.6	132.0	0.056
Decamethylcyclopentasiloxane (D5)	$C_{10}H_{30}O_5Si_5$	211.2	370.8	23.2	0.017
Dodecamethylcyclohexasiloxane (D6)	$C_{12}H_{36}O_6Si_6$	245.1	444.9	4.0	0.005

409 [a] Adapted from Ruiling et al (2017).

410

411 Finally, the microbial analysis showed significant differences between the bacterial
412 community of the enriched inocula and those retrieved by the end of operation of the
413 bioreactors. This could be attributed to the different VMS mixture employed: while the
414 enriched culture was fed with a wide range of VMS (TMSOL, L2, L3, L4, L5, D4 and
415 D5), only L2, L3, D4 and D5 were fed to the BTF and TP-BTF. In this sense, the
416 enrichment test promoted the growth of the members from the genus *Sphingomonas* and
417 *Chitinophaga* in BTF-InA and BTF-InB, which represented 40 % of the total population.
418 In the case of BTF and TP-BTF, the continuous exposition to siloxanes shifted the initial
419 microbial population to a similar bacterial community regardless of the inoculum used.
420 *KMBC-112*, *Reyranella* and *Chitinophaga* were the main genera favored by VMS
421 exposure (L2, L3, D4 and D5), representing 30 % of the population. Although no previous
422 studies have pointed out any of the genera found in this study as VMS degraders, the
423 genus *Chitinophaga*, *Sphingomonas* and *Reyranella* consist of highly versatile
424 microorganisms capable of growing in a wide range of environments and have been found
425 before in bioreactors devoted to the removal of volatile organic compounds such as
426 toluene or dichloromethane (Cheng et al., 2018; Xu et al., 2019). In the case of *KMBC-*
427 112, it has been retrieved from urban deposits and contaminated soils (Marti et al., 2017).
428 In this regard, siloxanes can be efficiently eliminated by a bespoke consortium that will
429 vary based on the target VMS.

430

431 **5. Conclusions**

432 This study demonstrated the superior siloxanes abatement performance of a two-phase
433 partitioning BTF compared to a conventional BTF. While the BTF achieved a total VMS
434 removal lower than 30 %, this value increased up to ~ 70 % due to the addition of a non-
435 aqueous phase (i.e. silicon oil), corresponding to an EC 5× higher than that of the BTF.

436 The highest REs were recorded for D4 and D5, reaching values between 80-90 %
437 throughout the entire experiment (corresponding to ECs between 0.12-0.17 $g.m^{-3}.h^{-1}$).
438 The RE of L3 was slightly lower (70-80 %), while only 20-60 % of L2 was removed
439 depending on the operating conditions. This outstanding performance of the TP-BTF was
440 associated to the presence of silicone oil that boosted the mass transfer of VMS from the
441 gas phase to the liquid phase. The removal of L2 was hindered by its higher vapor pressure
442 compared to L3, D4 and D5, decreasing the solubility of this compound in the organic
443 phase. Finally, bacterial genera *KMBC-112*, *Reynarella* and *Chitinophaga* represented
444 more than 30 % of the total population retrieved by the end of operation of both BTF
445 despite the different inocula and operating conditions in both bioreactors. Up to date, no
446 evidence of siloxanes biodegradation capacity of these genera has been reported.

447

448 **Acknowledgements**

449 This work was supported by the regional government of Castilla y León and the EU-
450 FEDER programme and by the URBIOFIN project. The project has received funding
451 from the Bio Based Industries Joint Undertaking under the European Union's Horizon
452 2020 research and innovation program under grant agreement No 745785. The authors
453 also thank Ramon Areces Foundation for the financial support of this work via sponsoring
454 of Sara Cantera Postdoctoral grant.

455

456

457 **References**

458 Accettola, F., Guebitz, G.M., Schoeftner, R., 2008. Siloxane removal from biogas by

biofiltration: Biodegradation studies. Clean Technol. Environ. Policy 10, 211–218. doi:10.1007/s10098-007-0141-4

Ajhar, M., Travesset, M., Yüce, S., Melin, T., 2010. Siloxane removal from landfill and digester gas - A technology overview. Bioresour. Technol. 101, 2913–2923. doi:10.1016/j.biortech.2009.12.018

Cantera, S., Estrada, J.M., Lebrero, R., García-Encina, P. a., Muñoz, R., 2016. Comparative performance evaluation of conventional and two-phase hydrophobic stirred tank reactors for methane abatement: Mass transfer and biological considerations. Biotechnol. Bioeng. 113, 1203–1212. doi:10.1002/bit.25897

Cheng, C., Zhou, Z., Pang, H., Zheng, Y., Chen, L., Jiang, L.M., Zhao, X., 2018. Correlation of microbial community structure with pollutants removal, sludge reduction and sludge characteristics in micro-aerobic side-stream reactor coupled membrane bioreactors under different hydraulic retention times. Bioresour. Technol. 260, 177–185. doi:10.1016/j.biortech.2018.03.088

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., Tiedje, J.M., 2009. The Ribosomal Database Project: Improved alignments and new tools for rRNA analysis. Nucleic Acids Res. doi:10.1093/nar/gkn879

Dewil, R., Appels, L., Baeyens, J., 2006. Energy use of biogas hampered by the presence of siloxanes. Energy Convers. Manag. 47, 1711–1722. doi:10.1016/j.enconman.2005.10.016

Gaj, K., 2017. Applicability of selected methods and sorbents to simultaneous removal of siloxanes and other impurities from biogas. Clean Technol. Environ. Policy 19, 2181–2189. doi:10.1007/s10098-017-1422-1

Läntelä, J., Rasi, S., Lehtinen, J., Rintala, J., 2012. Landfill gas upgrading with pilot-

484 scale water scrubber: Performance assessment with absorption water recycling.
485 Appl. Energy 92, 307–314. doi:10.1016/j.apenergy.2011.10.011
486 Li, Y., Zhang, W., Xu, J., 2014. Siloxanes removal from biogas by a lab-scale
487 biotrickling filter inoculated with Pseudomonas aeruginosa S240. J. Hazard. Mater.
488 275, 175–184. doi:10.1016/j.jhazmat.2014.05.008
489 Marti, R., Bécouze-Lareure, C., Ribun, S., Marjolet, L., Bernardin Souibgui, C., Aubin,
490 J.B., Lipeme Kouyi, G., Wiest, L., Blaha, D., Cournoyer, B., 2017. Bacteriome
491 genetic structures of urban deposits are indicative of their origin and impacted by
492 chemical pollutants. Sci. Rep. 7, 1–14. doi:10.1038/s41598-017-13594-8
493 Muñoz, R., Meier, L., Diaz, I., Jeison, D., 2015. A review on the state-of-the-art of
494 physical/chemical and biological technologies for biogas upgrading. Rev. Environ.
495 Sci. Biotechnol. 14, 727–759. doi:10.1007/s11157-015-9379-1
496 Muñoz, R., Souza, T.S.O., Glittmann, L., Pérez, R., Quijano, G., 2013. Biological
497 anoxic treatment of O2-free VOC emissions from the petrochemical industry: A
498 proof of concept study. J. Hazard. Mater. 260, 442–450.
499 doi:10.1016/j.jhazmat.2013.05.051
500 Muñoz, R., Villaverde, S., Guieysse, B., Revah, S., 2007. Two-phase partitioning
501 bioreactors for treatment of volatile organic compounds. Biotechnol. Adv. 25,
502 410–422. doi:10.1016/j.biotechadv.2007.03.005
503 Pérez, R., Cantera, S., Bordel, S., García-Encina, P.A., Muñoz, R., 2019. The effect of
504 temperature during culture enrichment on methanotrophic polyhydroxyalkanoate
505 production. Int. Biodeterior. Biodegrad. doi:10.1016/j.ibiod.2019.04.004
506 Phandanouvong-Lozano, V., Sun, W., Sanders, J.M., Hay, A.G., 2018. Biochar does not
507 attenuate triclosan's impact on soil bacterial communities. Chemosphere.
508 doi:10.1016/j.chemosphere.2018.08.132

509 Popat, S.C., Deshusses, M.A., 2008. Biological removal of siloxanes from landfill and
510 digester gases: Opportunities and challenges. Environ. Sci. Technol. 42, 8510–
511 8515. doi:10.1021/es801320w
512 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J.,
513 Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project:
514 Improved data processing and web-based tools. Nucleic Acids Res.
515 doi:10.1093/nar/gks1219
516 Rojas Devia, C., Subrenat, A., 2013. Absorption of a linear (l2) and a cyclic (d4)
517 siloxane using different oils: Application to biogas treatment. Environ. Technol.
518 (United Kingdom) 34, 3117–3127. doi:10.1080/09593330.2013.804588
519 Ruiling, G., Shikun, C., Zifu, L., 2017. Research progress of siloxane removal from
520 biogas. Int. J. Agric. Biol. Eng. 10, 30–39. doi:10.3965/j.ijabe.20171001.3043
521 Ryckebosch, E., Drouillon, M., Vervaeren, H., 2011. Techniques for transformation of
522 biogas to biomethane. Biomass and Bioenergy 35, 1633–1645.
523 doi:10.1016/j.biombioe.2011.02.033
524 San-Valero, P., Gabaldón, C., Penya-roja, J.M., Quijano, G., 2017. Enhanced styrene
525 removal in a two-phase partitioning bioreactor operated as a biotrickling filter:
526 Towards full-scale applications. Chem. Eng. J. 309, 588–595.
527 doi:10.1016/j.cej.2016.10.054
528 Santos-Clotas, E., Cabrera-Codony, A., Boada, E., Gich, F., Muñoz, R., Martín, M.J.,
529 2019. Efficient removal of siloxanes and volatile organic compounds from sewage
530 biogas by an anoxic biotrickling filter supplemented with activated carbon.
531 Bioresour. Technol. 294, 122136. doi:10.1016/j.biortech.2019.122136
532 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B.,
533 Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B.,

534 Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-
535 source, platform-independent, community-supported software for describing and
536 comparing microbial communities. Appl. Environ. Microbiol.
537 doi:10.1128/AEM.01541-09
538 Scope, H., Press, I., Press, I., Nourmohammadi, M., Golbabaei, F., Karimi, A.,
539 Pourmand, M.R., Poor, A.N., Foroushani, A.R., Nourmohammadi, E., 2018.
540 Absorption and Biodegradation of Toluene in a Two-Phase Low-Pressure
541 Bioscrubber Using Cutting Oil as the Organic Phase. Heal. Scope 1–8.
542 doi:10.5812/jhealthscope.65219.Research
543 Shen, M., Zhang, Y., Hu, D., Fan, J., Zeng, G., 2018. A review on removal of siloxanes
544 from biogas: with a special focus on volatile methylsiloxanes. Environ. Sci. Pollut.
545 Res. 25, 30847–30862. doi:10.1007/s11356-018-3000-4
546 Soreanu, G., Béland, M., Falletta, P., Edmonson, K., Svoboda, L., Al-Jamal, M., Seto,
547 P., 2011. Approaches concerning siloxane removal from biogas - A review. Can.
548 Biosyst. Eng. / Le Genie des Biosyst. au Canada 53.
549 Standardization, E.C. for, 2016. EN 16723-1, Acta crystallographica. Section F,
550 Structural biology and crystallization communications.
551 Team, R.C., 2016. R: A Language and Environment for Statistical Computing. R
552 Found. Stat. Comput.
553 Ye, J.X., Lin, T.H., Hu, J.T., Poudel, R., Cheng, Z.W., Zhang, S.H., Chen, J.M., Chen,
554 D.Z., 2019. Enhancing chlorobenzene biodegradation by delftia tsuruhatensis using
555 awater-silicone oil biphasic system. Int. J. Environ. Res. Public Health 16.
556 doi:10.3390/ijerph16091629
557 Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T.,
558 Peplies, J., Ludwig, W., Glöckner, F.O., 2014. The SILVA and "all-species Living

559 Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res.
560 doi:10.1093/nar/gkt1209
561