



Innovative polishing stage in biogas upgrading: Siloxanes abatement in an anoxic two-phase partitioning biotrickling filter

Celia Pascual^{a,b}, Sara Cantera^c, Raúl Muñoz^{a,b}, Raquel Lebrero^{a,b,*}

^a Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina s/n., Valladolid, 47011, Spain

^b Department of Chemical Engineering and Environmental Technology, University of Valladolid, Dr. Mergelina s/n., Valladolid, 47011, Spain

^c Laboratory of Microbiology, Wageningen University and Research Center, the Netherlands

ARTICLE INFO

Handling Editor: Mingzhou Jin

Keywords:

Anoxic biotrickling filter

Biogas upgrading

Silicone oil

Siloxanes

Two-phase partitioning bioreactor

ABSTRACT

The present work investigated for the first time the feasibility of the anoxic biodegradation of siloxanes (hexamethyldisiloxane, L2; octamethyltrisiloxane, L3; octamethylcyclotetrasiloxane, D4; and decamethylcyclopentasiloxane, D5) in a two-phase partitioning biotrickling filter (TP-BTF) using silicone oil as the organic phase. The influence of the silicone oil ratio and the inlet concentration of siloxanes were also evaluated. The highest removals were obtained for D4 (41–54%) and D5 (48–73%). Lower removals were recorded for L2 (12–15%) and L3 (22–38%) as a consequence of their higher volatility. The best performance was obtained at a silicone oil content of 45%. Doubling the inlet load enhanced the elimination capacity of the TP-BTF by 50%, although the removal efficiencies did not vary. A specialized bacteria consortium dominated by the genera *Parvibaculum*, *Zarvaz inia*, *KCM-B-11*, *Arenimonas*, *Moraxellaceae* and *Luteimonas* was related to anoxic siloxanes degradation.

1. Introduction

Volatile methyl siloxanes (VMS) are organosilicon compounds increasingly employed in multiple industrial applications due to their unique and interesting properties, such as their high thermal stability, resistance to oxidation and compressibility or their low surface tension and viscosity (Gaj, 2021). Unfortunately, this increasing widespread utilization results in larger discharges of these compounds into the environment, which accumulate in land and water bodies due to their high mobility/volatility and persistence (Horii and Kannan, 2020). For instance, between 200 and 400 tons of VMS are estimated to be annually discharged to global municipal solid waste landfills, and an increase of 78% is expected within the next 25 years (Tansel and Surita, 2017). The majority of VMS deposited in landfills end up in the biogas produced in such waste treatment facilities, exhibiting a wide concentration range (from 15 to 400 mg m⁻³) (Horii and Kannan, 2020; Kuhn et al., 2017). In particular, hexamethyldisiloxane (L2), octamethyltrisiloxane (L3), octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) are the most abundant VMS. Indeed, D4 contributes to ~60% of the total content of VMS in biogas (Ruiling et al., 2017). During biogas combustion, VMS oxidize forming silica deposits that are responsible for severe damages in energy valorization systems. Thus, VMS

accumulation increases the maintenance costs, decreases the performance of the combustion, and triggers higher emissions of air pollutants in these facilities. Whereas there is yet no legal restriction on the siloxanes concentration released in atmospheric emissions, the maximum concentration required for the injection of biomethane into the natural gas grid as stated by European standards is ~1 mg Si m⁻³ (Standardization, 2016). Hence, VMS abatement is of utmost importance for biogas valorization.

In this context, siloxanes are commonly removed from biogas and biomethane by physical-chemical processes. Adsorption on conventional materials such as activated carbon or silica gel, and chemical scrubbing with organic, acid or basic solutions have demonstrated the best abatement performances (Shen et al., 2018). Notwithstanding their potential, these technologies present high operating costs due to the short lifespan of the packing material, the complex treatment of the adsorbent, and the chemicals employed (Pascual et al., 2021a). In order to overcome these limitations, recent research has focused on improving the adsorption capacity of the sorbent materials (Meng et al., 2020; Tran et al., 2019) or using solid waste for the production of sustainable adsorbents (Papurello et al., 2018). Similarly, an important advance in absorption processes lies in the use of green solvents, such as deep eutectic solvents, characterized by their lower price, higher

* Corresponding author. Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina s/n., Valladolid, 47011, Spain.

E-mail address: raquel.lebrero@iq.uva.es (R. Lebrero).

<https://doi.org/10.1016/j.jclepro.2022.133427>

Received 10 February 2022; Received in revised form 15 June 2022; Accepted 1 August 2022

Available online 8 August 2022

0959-6526/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

biodegradability and lower toxicity (Stupek et al., 2021).

Biotechnologies have recently appeared as an interesting substitute, due to their reduced operating costs and the fact that their environmental impact is up to 10 times lower compared to that of their physical-chemical counterparts (Pascual et al., 2021a). Among them, biotrickling filtration (BTF) represents the most studied biotechnology to treat VMS both aerobic and anaerobically. Overall, the transfer of VMS from the gas phase to the aqueous biofilm, where siloxane-degrading microbial community grows, represents the major limitation in biotechnologies due to the low solubility of these compounds (Pascual et al., 2022). Several strategies have been studied to improve the abatement of hydrophobic volatile organic compounds (VOC) in BTFs. Some of these strategies include the addition of surfactants that reduce surface and interfacial tensions at the liquid-gas or liquid-liquid interfaces; or the implementation of fungal catalyzed BTFs, where the presence of hydrophobins and the mycelial growth increase pollutants mass transfer from the gas phase to the biofilm (Cheng et al., 2016). In the field of siloxanes abatement, Li et al. (2014) observed removals up to 74% in a BTF containing biosurfactants produced by *Pseudomonas aeruginosa* S240, hydrophobic compounds able to increase the availability of hydrophobic contaminants to the degrading bacteria (Li et al., 2014).

A promising bioreactor configuration engineered to boost the mass transfer of hydrophobic pollutant from the gas to the bacterial community are two-phase partitioning bioreactors (TPPBs). TPPBs consist of the addition of an organic phase to the aqueous media of a conventional bioreactor, which traps hydrophobic pollutants due to its higher affinity compared to water and improves their availability for the bacterial community. Thus, the implementation of TPPBs to remove VMS can enhance process performance in conventional BTFs and appears as an interesting and cost-effective alternative to abate these pollutants from biogas. In fact, a recent research demonstrated the superior VMS removal performance of a two-phase partitioning BTF (TP-BTF) compared with that of a conventional BTF under aerobic conditions (total VMS removals of 70% and <30%, respectively) (Pascual et al., 2020). Both systems were operated at an empty bed residence time (EBRT) of 1 h with an organic phase (silicone oil) volume fraction of 30% and an inlet VMS concentration between 600 and 700 mg m⁻³. These promising results encouraged further investigation on the implementation of TP-BTFs for siloxanes abatement under anoxic conditions, thus covering the valorization of biogas into biomethane, where O₂ concentration should not exceed 0.2–0.5% v/v (Muñoz et al., 2015).

This work investigated the effect of different operating parameters on the removal of VMS in an anoxic TP-BTF using silicone oil as organic phase. Thus, the influence of the inlet VMS concentration and the percentage of silicone oil on VMS abatement were evaluated, and the microbial community responsible for VMS biodegradation under anoxic conditions was identified.

2. Materials and methods

2.1. Mineral salt medium

The mineral salt medium (MSM) was prepared according to Pascual et al. (2021b) (g L⁻¹): KNO₃, 3; K₂HPO₄·3H₂O, 0.917; KH₂PO₄, 0.7; MgSO₄·7H₂O, 0.345; NaCl, 0.2; CaCl₂·2H₂O, 0.026; and 2 mL L⁻¹ of a micronutrient solution containing (g L⁻¹): EDTA, 0.5; FeSO₄·7H₂O, 0.2; ZnSO₄·7H₂O, 0.01; MnCl₂·4H₂O, 0.003; H₃BO₃, 0.003; CoCl₂·6H₂O, 0.02; CuCl₂·2H₂O, 0.001; NiCl₂·6H₂O, 0.002; NaMoO₄·2H₂O, 0.003, (Pascual et al., 2021b).

2.2. Experimental setup and operating procedure

The TP-BTF system consisted of a 2 L cylindrical vinyl polychloride (PVC) column (8.4 cm diameter, 37.5 cm height), packed with polymeric rings (Fig. 1) (Pascual et al., 2021b) and installed in a temperature controlled room at 25 °C. The inlet stream was prepared by injecting a

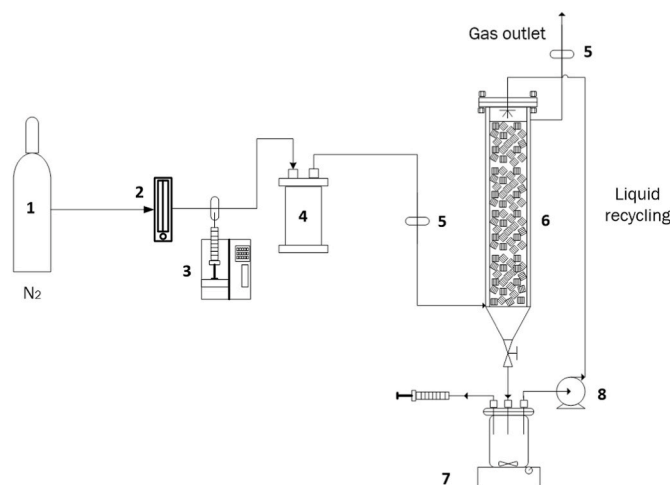


Fig. 1. Schematic representation of the experimental set-up. (1) N₂ cylinder, (2) rotameter, (3) syringe pump, (4) mixing chamber, (5) inlet and (6) outlet gas sampling ports, (7) two-phase partitioning biotrickling filter, (8) nutrient reservoir, and (9) peristaltic pump.

liquid solution with equivalent volumes of L2, L3, D4 and D5 (98.5, 98, 98 and 97% purity, respectively) purchased from (Sigma Aldrich, San Luis, USA) into a 33 ml min⁻¹ N₂ stream by means of a syringe pump (Fusion 100, Chemyx Inc., USA). N₂ was employed to simulate a bio-methane stream in order to simplify the experimental setup. The flow was regulated with a rotameter and was homogenized in a mixing chamber prior entering the column. The siloxanes-loaded inlet stream was fed at the bottom of the TP-BTF while the MSM mixed with the silicone oil and magnetically stirred at 300 rpm was recycled at the top of the TP-BTF from a 1 L glass vessel (nutrient reservoir) by a peristaltic pump (Watson-Marlow 313D) at a linear recycling liquid velocity of 2 m h⁻¹. Silicone oil was selected as the organic phase based on its outstanding properties, as it is non-biodegradable, biocompatible and no toxic effects to the bacterial community have been previously observed (Darracq et al., 2012).

An abiotic test prior TP-BTF start-up was performed in subsequent steps: with the empty PVC column (Stage A1), with the PVC column packed with the sterile polymeric rings (Stage A2), after addition of sterile MSM (Stage A3) and after supplementation of sterile silicone oil at ratio of 15% (Stage A4).

After the abiotic test, 200 ml of fresh activated sludge from wastewater treatment plant (WWTP) of Valladolid (Spain) with a concentration of volatile suspended solids (VSS) of 5.76 g L⁻¹ were employed as inoculum. The pellet obtained after centrifugation at 10000 rpm for 10 min was resuspended in 1 L of a mixture of fresh MSM and silicone oil 20 cts (85:15% v/v). The TP-BTF was operated during 126 days maintaining an empty bed residence time (EBRT) of 1 h. The experiment was divided into four experimental stages (Table 1) of different duration, the corresponding operating parameter being modified upon reaching a steady VMS removal efficiency (standard deviation lower than 10%). 300 mL of culture broth were weekly replaced with fresh MSM, returning to the system the recovered fraction of silicone oil in order to avoid the supply of fresh silicone oil.

Table 1
Experimental stages during the anoxic TP-BTF operation.

Stage	Silicone oil ratio (%)	Time course (days)	Inlet total VMS concentration (mg m ⁻³)
S1	15	0–42	138 ± 14
S2	30	43–56	118 ± 15
S3	30	57–103	239 ± 22
S4	45	104–126	219 ± 18

The inlet VMS-loaded stream was maintained at a total VMS inlet concentration between 110 and 140 mg m⁻³ through stages S1 and S2. The silicon oil ratio was increased from 15% in S1 to 30% in stage S2. By day 56, the VMS inlet concentration was increased to 239 ± 22 mg m⁻³ (stage S3) in order to assess the influence of VMS concentration on the mass transfer of these pollutants from the intel siloxanes-loaded gas stream to the culture broth. Finally, the silicone oil fraction was raised to 45% during S4.

2.3. Analytical procedures

All the parameters analyzed in the present study were carried out according to Pascual et al. (2021a,b). VMS concentration was determined in an Agilent 8860 gas chromatograph (Santa Clara, California, USA) with a HP-5 column (15 m × 0.25 mm × 0.25 μm) and a flame ionization detector (FID). Both detector and injector temperatures were maintained constant at 250 °C. The oven temperature was initially set at 40 °C for 2.0 min, then increased at 30 °C min⁻¹ up to 180 °C, maintained for 1 min and increased again at 30 °C min⁻¹ up to 200 °C. Helium was used as the carrier gas at a flow rate of 3.5 mL min⁻¹.

CO₂ and O₂ concentrations were determined in a Bruker 430 gas chromatograph (Palo Alto, USA) 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 and with a thermal conductivity detector (TCD). 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 total organic carbon (TOC), total inorganic carbon (IC), and total nitrogen (TN) of the cultivation broth were weekly monitored in a TOC-VCSH analyzer coupled with a TNM-1 chemiluminescence module (Shimadzu, Japan). Silicon was analyzed using an inductively coupled plasma optical atomic emission spectrometer (ICP-OES Radial Simultaneous Varian 725-ES, Agilent). Nitrite and nitrate concentrations were monitored by means of HPLC-IC with 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). Finally, the pH of the cultivation broth was measured in a pH meter with a glass membrane electrode PH BASIC 20 (Crison, Barcelona, Spain).

2.4. Bacterial community analysis

Samples of 20 mL of activated sludge from the WWTP (Inoculum) and of the biofilm obtained at the end of the TP-BTF operation (BTF_Final) were preserved at -20 °C for prokaryotic population analysis. DNA extraction in triplicate (biological replicates) and Illumina Miseq amplicon sequencing were carried out in the Foundation for the Promotion of Health and Biomedical Research of the Valencia Region (FISABIO, Spain) according to (González-Martín et al., 2022). Archaea communities were not targeted due to the lack of PCR amplification using Archaea specific primers (Pausan et al., 2019). The 16S rRNA gene sequences were processed and quality filtered using Mothur v1.44.3 according to Pascual et al. (2021a,b) (Pascual et al., 2021b; Schloss, 2020). The gene exangendnce database, SILVA 16S rRNA (Version: 138.1), was used to cluster the obtained sequences into Operational Taxonomic Units (OTUs) at 97% identity. The sequences analyzed in this research can be found as bioproject: PRJNA717278 (<https://trace.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA717278>). After taxonomic classification, diversity studies were developed using Mothur v1.44.3 (Willis, 2019). The smallest representative library had 71377 reads (1000 randomizations) (Phandanouvong-Lozano et al., 2018). The Inverse Simpson Index was used to calculate the alpha diversity among triplicates, while beta diversity was calculated using the Jaccard Index and AMOVA at p < 0.05 (Schloss, 2008). The prokaryotic community structure was analyzed and plotted using R version 1.4.1 (Team, 2016) and the package *phreatmap* (Kolde, 2019).

3. Results and discussion

3.1. Abiotic test

During stages A1 and A2, no significant difference between the inlet and outlet VMS concentrations was observed working with the empty PVC column and the column filled with the packing material (Fig. S1). At the beginning of stage A3, the VMS outlet concentration slightly decreased when the MSM was recirculated through the BTF, which was associated to the initial absorption of the four compounds in the MSM. The outlet concentration rapidly increased to previous values following saturation of the aqueous recycling medium. Finally, a significant decrease in the outlet concentration of the VMS was recorded in stage A4, with the exception of L2, as a result of VMS absorption in the silicone oil. The steady increase in the outlet concentration of L3 and D4 suggested a gradual saturation of the recycling silicone oil. Additionally, no CO₂ production was observed throughout the entire abiotic test, thus ruling out any biotic degradation of the siloxanes.

3.2. Performance of the anoxic TP-BTF

3.2.1. Linear siloxanes abatement

During the start-up of the TP-BTF, large fluctuations in L2 concentrations (Fig. 2) and thus in the removal efficiencies (REs) (Fig. S2) of this siloxane were observed. Inlet and outlet L2 concentrations roughly stabilized by the end of stage S1 at 31.3 ± 6.0 (Fig. 3A) and 29.0 ± 3.9 mg m⁻³, respectively, resulting in a poor removal efficiency (Fig. 3B and C). In comparison, higher removals were recorded for L3. The average inlet and outlet concentrations remained at 34.1 ± 5.5 and 25.4 ± 6.2 mg m⁻³, respectively, resulting in highly fluctuating REs and elimination capacities (ECs) during the entire stage S1 (25.9 ± 20.8% and 9.3 ± 7.8 mg m⁻³ h⁻¹, respectively).

During S2, RE and EC values of 14.7 ± 14.6% and 3.7 ± 4.0 mg m⁻³ h⁻¹, respectively, were achieved for L2. On the other hand, a higher removal was observed for L3 compared to that of the previous stage, with average RE and EC values of 37.7 ± 12.0% and 10.0 ± 3.7 mg m⁻³ h⁻¹, respectively. The higher performance was associated to the increase in the silicone oil share in the recycling liquid (from 15 to 30%), which likely enhanced the mass transport of siloxanes through the interface gas/biofilm and therefore their availability for the VMS degrading microbial community. Similarly, Pascual et al. (2021a,b) observed an improvement of the aerobic L3 removal from 21 to 58% when the silicone oil ratio was increased from 5 to 30% due to the higher mass transport interfacial area. (Pascual et al., 2021b). In this sense, the lower aqueous phase/organic phase ratio likely reduced the surface tension in the gas-liquid interphase, and therefore increased interfacial area available for mass transport of the target pollutants (Quijano et al., 2010).

L2 inlet concentration was increased from 26.5 ± 4.3 to 56.0 ± 8.5 mg m⁻³ in S3 (Fig. 3A). No significant differences were observed in the RE compared to S2, remaining at an average value of 9.5 ± 10.1% from day 47–55, while the average EC increased to 5.1 ± 7.1 mg m⁻³ h⁻¹ (~25% higher compared to S2). The REs of L3 initially ranged between 45 and 55% (Fig. S2) until day 76, when these values steadily decreased and stabilized at an average RE of 22.5 ± 8.0%. This behavior was associated to the saturation of the cultivation broth after the increase in the inlet VMS concentration. In fact, higher inlet pollutant concentrations typically boost the volumetric pollutant mass transfer rate by increasing the gas-liquid pollutant concentration gradient (Muñoz et al., 2014). This effect progressively decreased as the silicone oil and the gas phase reached a thermodynamic equilibrium (Eq. (1)). Despite the lower REs recorded during S3, the higher siloxane load fed into the TP-BTF resulted in an average EC of 14.0 ± 5.9 mg m⁻³ h⁻¹, which represented an increase of 40% compared to that recorded in S2. This fact demonstrated that the performance of the system was hindered by the transport of the gaseous pollutants from the gas phase to the biofilm.

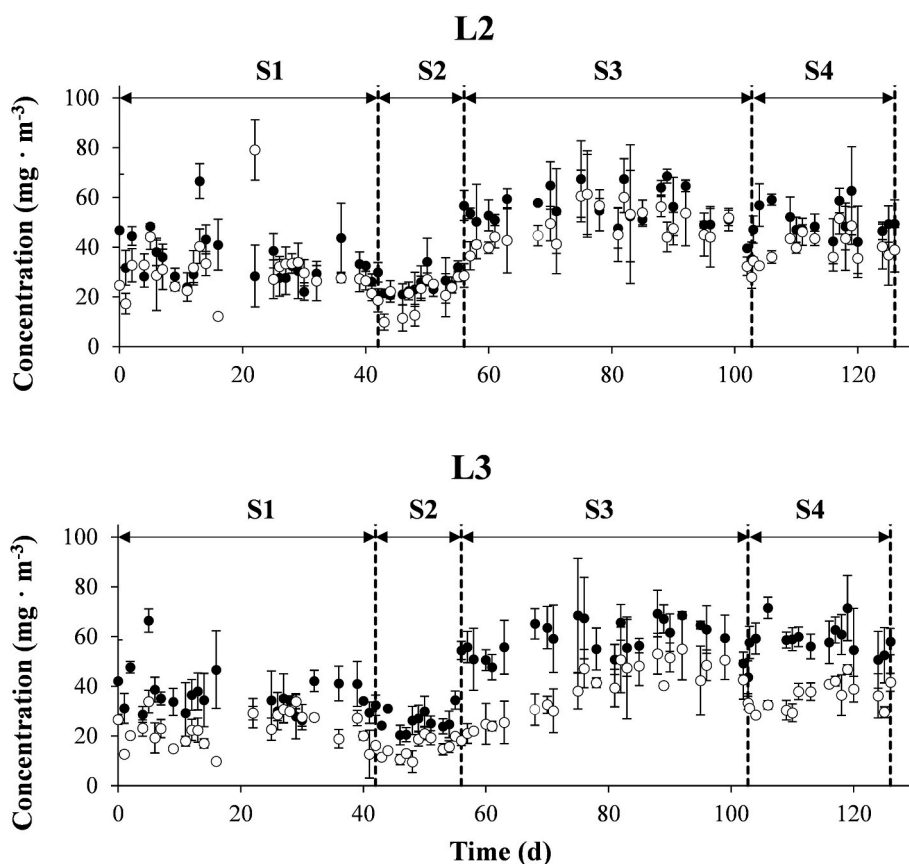


Fig. 2. Time course of inlet (●) and outlet (○) concentrations for L2 and L3 in the TP-BTF during the different experimental stages: silicone oil percentage of 15 (S1), 30 (S2, S3) and 45% (S4). Vertical lines represent standard deviation from triplicate measurements. Vertical dotted lines separate different stages.

At the beginning of S4, a notable increase in L2 removal was observed, with a maximum RE of 43% and an EC of $24 \text{ mg m}^{-3} \text{ h}^{-1}$ achieved by day 104, which was associated with the increase in the silicone oil ratio from 30 to 45%. Subsequently, a sharp decrease in the RE and EC values was recorded, likely related to the saturation of the silicone oil, to finally stabilize at 21.1% and $10.4 \text{ mg m}^{-3} \text{ h}^{-1}$, respectively. Similarly, a maximum RE of 54.6% was achieved for L3 by day 106, corresponding to an EC of $39.1 \text{ mg m}^{-3} \text{ h}^{-1}$. The abatement efficiency progressively decreased to finally obtain average steady values of $34.2 \pm 5.2\%$ and $20.0 \pm 3.8 \text{ mg m}^{-3} \text{ h}^{-1}$ for RE and EC, respectively. The removals achieved in this stage were slightly higher due to the higher silicone oil ratio of 45% that increased the interfacial area involved in the mass transport of the VMS.

Overall, the removals achieved throughout the entire experiment were lower than those previously obtained in aerobic TP-BTFs, especially those reported for L3, with values that amount to 80% (Pascual et al., 2020, 2021b). However, it should be noted that the L3 inlet concentration treated in the aforementioned studies was considerably higher compared to those used in the present experiment ($\sim 200 \text{ mg m}^{-3}$ vs. $30\text{--}60 \text{ mg m}^{-3}$). In this sense, according to Eq. (1), the concentration gradient $\left(\frac{C_G}{H_{G/w}} - C_w\right)$ decreases when decreasing the inlet concentration, reducing the overall mass transfer. Thus, a lower VMS mass transfer rate is expected when treating low pollutant concentrations.

$$F_{G/w} = K_L^{G/w} a \left(\frac{C_G}{H_{G/w}} - C_w \right) \quad (1)$$

where $F_{G/w}$ is the gas-liquid volumetric pollutant mass transfer rate ($\text{g m}^{-3} \text{ h}^{-1}$), $K_L^{G/w} a$ represents the overall volumetric gas-liquid mass transfer coefficient (h^{-1}), C_G and C_w are the pollutant concentrations (g m^{-3}) in the gas and aqueous phase, respectively, and $H_{G/w}$ the

dimensionless Henry's law constant (Muñoz et al., 2014).

3.2.2. Cyclic siloxanes abatement

The TP-BTF supported a higher abatement performance for the cyclic siloxanes compared to that recorded for the linear VMS. However, a similar response of the removal efficiency of the cyclic VMS compared to the linear VMS was observed throughout the different experimental stages (Fig. 4). The average RE values for D4 and D5 during S1 accounted for 47.0 ± 3.4 and $54.1 \pm 8.9\%$, respectively, corresponding to ECs of 14.8 ± 5.2 and $22.0 \pm 6.6 \text{ mg m}^{-3} \text{ h}^{-1}$, respectively (Fig. 3B and C).

During S2, no improvement in the performance of the system was observed when the silicone oil/recycling solution ratio was increased from 15 to 30%. In fact, the REs obtained for D4 and D5 were slightly lower, reaching values of 40.8 ± 8.2 and $48.1 \pm 6.4\%$, corresponding to ECs of 10.3 ± 2.3 and $18.9 \pm 3.4 \text{ mg m}^{-3} \text{ h}^{-1}$, respectively.

The VMS inlet load increase during S3 (corresponding to average inlet concentrations of $53.9 \pm 5.1 \text{ mg D4 m}^{-3}$ and $68.7 \pm 7.7 \text{ mg D5 m}^{-3}$, Fig. 4) supported steady REs of 42.4 ± 4.2 and $54.4 \pm 6.0\%$ for D4 and D5, respectively (Fig. 3). These values were slightly higher compared to those recorded in S3, thus leading to a significant increase in the D4 and D5 ECs up to 23 ± 3.9 and $37.4 \pm 6.2 \text{ mg m}^{-3} \text{ h}^{-1}$, respectively.

The best abatement performance was recorded during S4, which was associated with the increase in the silicone oil fraction (45% silicone oil-55% MSM). D4 removal increased by 26% compared to the previous stage, which entailed an average RE of $53.6 \pm 4.8\%$ and an EC of $27.8 \pm 3.9 \text{ mg m}^{-3} \text{ h}^{-1}$. Similarly, D5 removal increased by 33%, resulting in an average RE of $72.7 \pm 3.8\%$ and an EC of $45.9 \pm 5.9 \text{ mg m}^{-3} \text{ h}^{-1}$. This unprecedented positive effect of the increase in the silicone oil percentage was related to the concomitant increase in VMS concentration

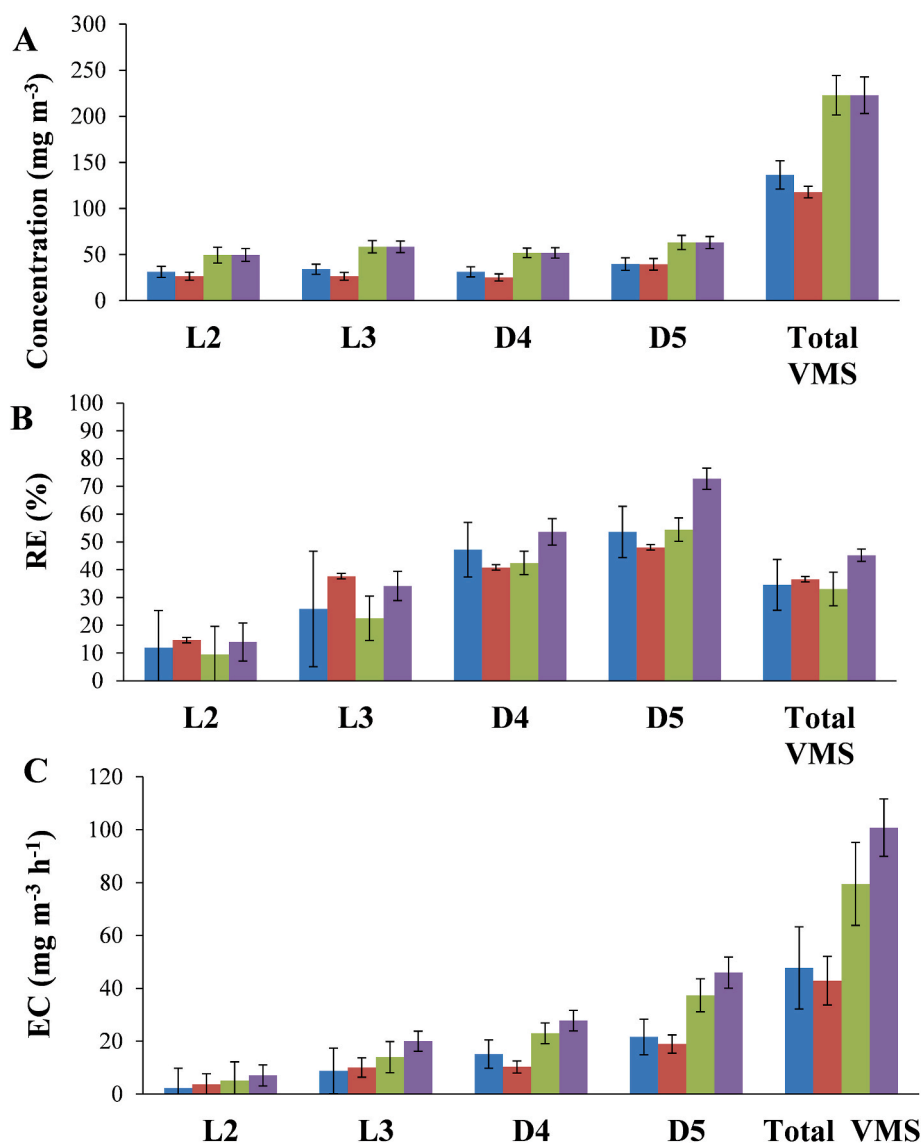


Fig. 3. Average VMS inlet concentration (A), removal efficiencies (B) and elimination capacities (C) in the TP-BTF in the different experimental stages: S1 operated at 15% of silicone oil (blue bars), S2 at 30% of silicone oil (red bars), S3 at 2-fold inlet VMS concentration and 30% of silicone oil (green bars) and S4 at 2-fold inlet VMS concentration and 45% of silicone oil (purple bars). Vertical lines represent standard deviation from measurements under steady state.

implemented during S4. Thus, despite the silicone oil fraction was also increased from S1 to S2, the positive effect of the lower $H_{G/W}$ was counterbalanced by the low concentration gradient, thus reducing the beneficial contribution of the non-aqueous phase (Eq. (1)). Nevertheless, the maximum REs achieved for D4 and D5 were still lower to those previously obtained by Pascual et al. (2020, 2021b), where values up to 80 and 90% for D4 and D5 were recorded. As above explained, this was associated to the significantly lower VMS inlet concentrations tested in this study, thus reducing the overall mass transfer (Pascual et al., 2020, 2021b).

A comparable abatement performance was achieved for D4 (up to 74%) in a conventional BTF operated at 50 mg D4 m^{-3} . In this experiment, the presence of biosurfactants (rhamnolipids) produced by *Pseudomonas aeruginosa* was identified as the critical factor responsible for the enhanced availability of D4 in the biofilm (Li et al., 2014). However, the performance of the system decreased to less than 3% at D4 concentrations of 140 mg m^{-3} likely due to an inhibitory effect of the high D4 load provided to the microorganisms. In this context, a two-phase system usually shows a higher robustness due to its inherent capacity to absorb pollutants surges compared to a conventional BTF.

Despite the total VMS concentration in raw biogas can range between 0 and 400 mg m^{-3} (Nguyen et al., 2021), the typical content emitted in the biogas from both landfills and anaerobic digesters is around 50 mg m^{-3} (Golmakani et al., 2022). Therefore, this study provides a new insight in the removal of siloxanes via TP-BTF, despite better performances have been reported in previous studies working at higher VMS concentration. However, further investigation should be performed in this field. For instance, the supplementation of activated carbon to the BTF packing material has been recently tested by (Santos-Clotas et al., 2019) as a novel strategy to enhance siloxanes abatement. In this case, an increase in the D5 RE from 20 to 45% was achieved working at an inlet concentration around 100 mg m^{-3} . A combined technology using activated carbon as packing material and adding an organic phase to the BTF could significantly increase the abatement of these hydrophobic compounds.

Overall, the total VMS RE remained close to 35% in stages S1, S2 and S3 (34.6 ± 9.1 , 36.6 ± 7.3 , $33.0 \pm 4.3\%$ respectively), increasing to 45.2 ± 2.2 during S4 mediated by the higher silicone oil content in the recycling liquid. Similar to these results, Pascual et al. (2021b) observed a positive response when the silicone oil ratio increased to 45%,

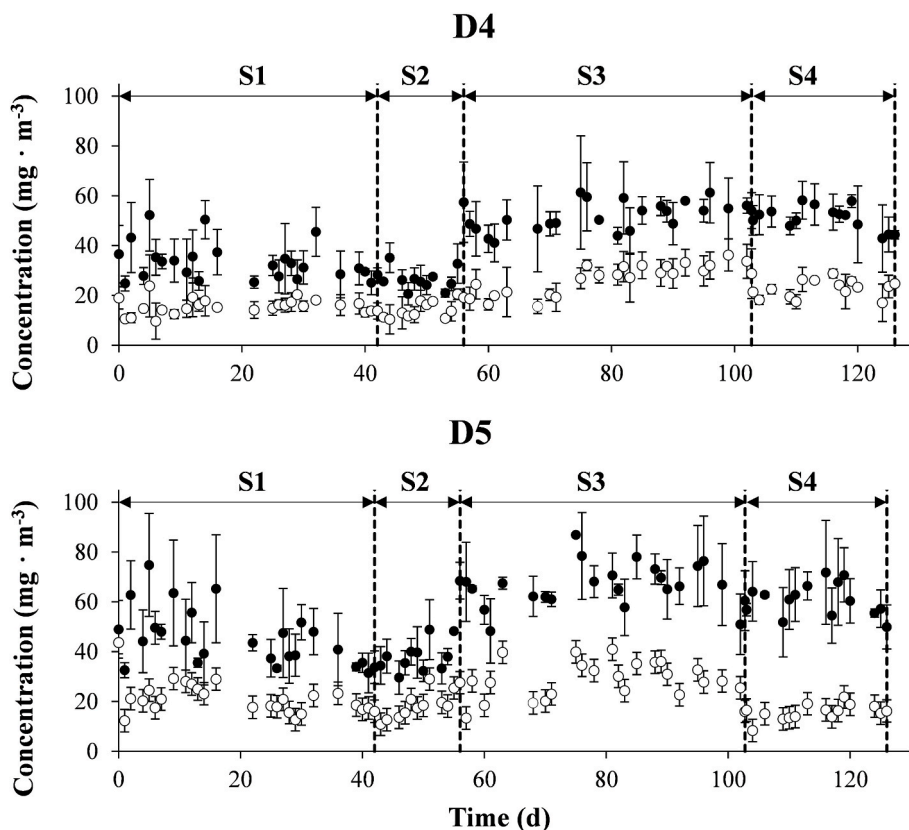


Fig. 4. Time course of inlet (●) and outlet (○) concentrations for D4 and D5 in the TP-BTF during the different experimental stages: silicone oil percentage of 15(S1), 30 (S2, S3) and 45% (S4). Vertical lines represent standard deviation from triplicate measurements. Vertical dotted lines separate different stages.

resulting in the improvement of the total VMS removal from 35 to 52% (Pascual et al., 2021b). ECs remained at 47.7 ± 15.5 and 42.9 ± 9.2 $\text{mg m}^{-3} \text{h}^{-1}$ during S1 and S2, increasing to 79.5 ± 15.7 and 100.8 ± 10.8 $\text{mg m}^{-3} \text{h}^{-1}$ in S3 and S4, respectively, when the TP-BTF was operated at higher inlet concentrations and silicone fractions.

The lower performance observed throughout the entire experiment for the linear VMS, especially for L2, was associated to their high vapor pressure compared to that of the cyclic VMS (L2: 5.626 Pa, L3: 0.52 Pa, D4: 0.132 Pa and D5: 0.023 Pa; at 20 °C), which limited their solubility in the organic phase and also promoted their subsequent desorption to the treated gas stream (Surita and Tansel, 2014). Similar findings have been reported in physical-chemical technologies for VMS abatement, such absorption or cryogenic condensation, where the higher volatility of linear VMS hindered their condensation and promoted their desorption at elevated gas flow rates (Nyamukamba et al., 2020; Ruiling et al., 2017).

3.3. Short-term fate of carbon

The CO_2 production associated to the biodegradation of VMS fluctuated throughout the experiment (Fig. S3). The CO_2 production initially increased to $2618 \text{ mg m}^{-3} \text{h}^{-1}$ by day 36 mainly due to the cell debris and organic carbon degradation from the fresh WWTP inoculum, along with the degradation of VMS. This value gradually decreased and stabilized at $400 \pm 77 \text{ mg m}^{-3} \text{h}^{-1}$ during S2. At the beginning of S3, no variations were recorded until day 71, when the production of CO_2 suddenly increased up to $2967 \text{ mg m}^{-3} \text{h}^{-1}$. This increase was associated to the presence of trace concentrations of O_2 in the new N_2 cylinder used to mimic biogas (Fig. S4), which could have triggered the activation of aerobic heterotrophic metabolisms and the subsequent degradation of recalcitrant dissolved organic compounds (since no increase in VMS degradation was recorded). By day 103, the N_2 cylinder was replaced

again and the CO_2 production decreased to $300 \text{ mg m}^{-3} \text{h}^{-1}$, remaining constant for the rest of the experiment.

During the first 71 days, average TOC, Si and IC concentrations in the trickling solution of 12.6 ± 4.2 , 1.8 ± 0.5 and $4.0 \pm 2.4 \text{ mg L}^{-1}$, respectively, were measured (Figs. S5 and S6). A gradual increase in TOC and Si concentrations was observed by day 77, reaching maximum values of 237.2 and 7.86 mg L^{-1} by day 104, decreasing afterwards to 63.3 and 2.3 mg L^{-1} by the end of the experiment. This behavior was associated with the higher VMS concentration supplemented during S3, which resulted in an enhanced mass transfer and subsequent degradation of these compounds. Likewise, the IC concentration remained at an average value of 5.0 ± 2.4 until day 77, when a gradual increase up to 46.3 mg L^{-1} by day 104 was observed due to the higher CO_2 production.

3.4. Bacterial diversity and community structure

The analysis of the bacterial community in the sample set displayed a total of 508843 sequences that belonged to 6418 OTUs affiliated with bacterial genera. Bacterial richness differed significantly between samples according to the alpha diversity analysis (Fig. S7). The inoculum consisted of 3307 total species, while the richness of the community present in the TP-BTF at the end of the experiment dropped down to 1074 total species (BTF_final, Fig. S8). In fact, both communities shared less than 10% of the species which indicated bacterial specialization towards organisms related to biological siloxane degradation. In accordance to this result, bacterial beta diversity was significantly higher in the inoculum (AMOVA, $p < 0.05$), and highly dissimilar between the two different samples studied.

The analysis of the bacterial taxonomic classification showed that the continuous exposure to siloxanes promoted a specialized consortium highly different from the original inoculum (Figs. S7 and S8). As shown in Fig. 5, in BTF_final the most representative bacteria were the

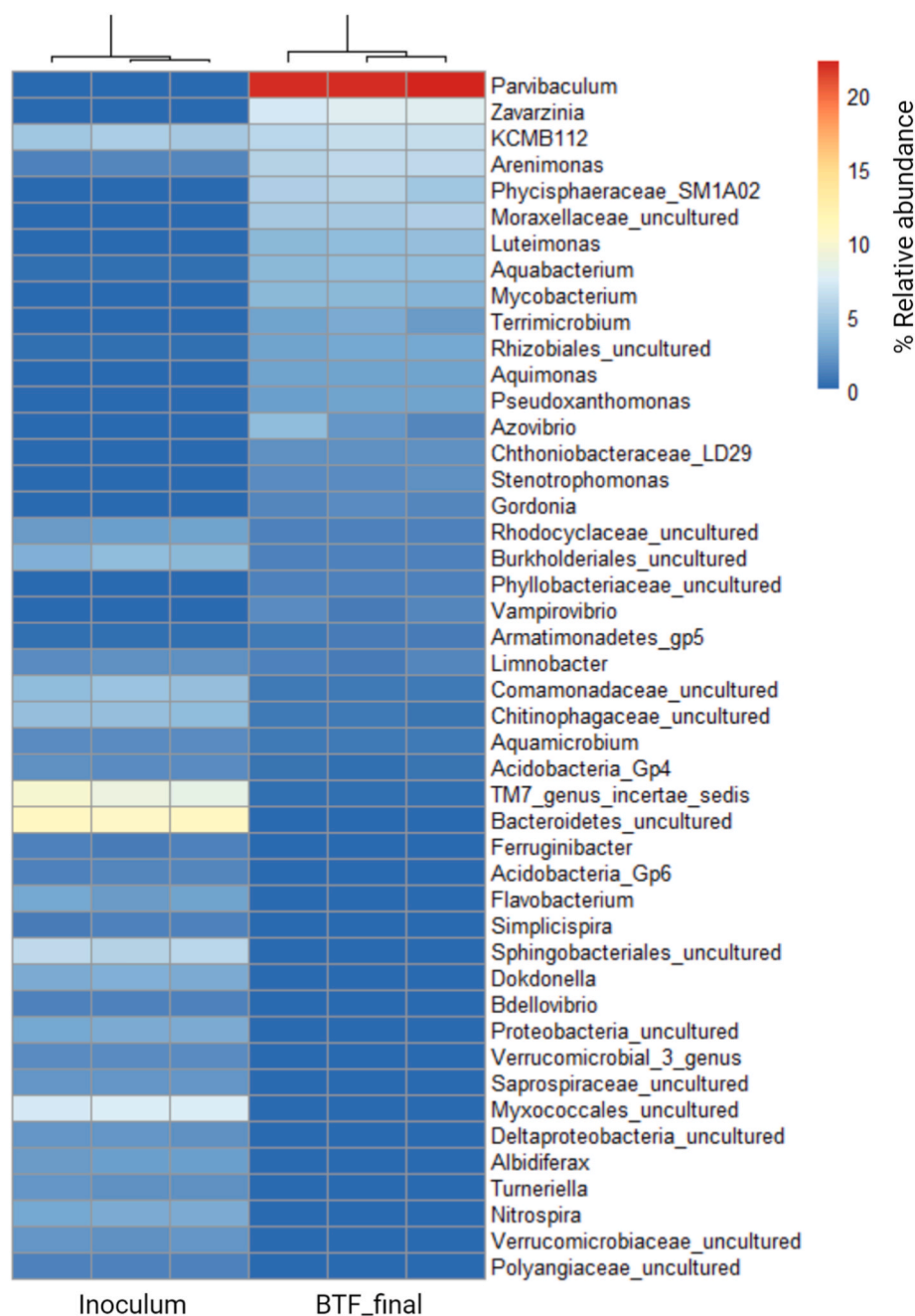


Fig. 5. Heat map of the relative abundance per sample of the most representative bacterial genera (99% of the total genera) in the inoculum and after operation (in triplicate). Hierarchical clustering of the sample replicates is indicated with a dendrogram.

Alphaproteobacteria members of the genus *Parvibaculum* ($22.1 \pm 0.21\%$) and *Zavarzinia* ($7.7 \pm 0.3\%$), followed by Gammaproteobacteria representatives from the genus *KCM-B-11* ($6.2 \pm 0.2\%$), the genus *Arenimonas* ($5.9 \pm 0.3\%$), uncultured members of the family *Moraxellaceae* ($5.1 \pm 0.2\%$) and the genus *Luteimonas* ($4.1 \pm 0.2\%$). Other abundant bacteria belonged to the class Phycisphaerae and Betaproteobacteria, such as the genus *Phycisphaeraceae_SM1A02* ($5.3 \pm 0.4\%$) and the genus *Aquabacterium* ($4.1 \pm 0.2\%$). Recent studies in a BTF supporting siloxanes degradation under aerobic conditions found a clear specialization of the genus *Acidithiobacillaceae_KCMB-112* (20–73% of the population) after 8 months of operation (Pascual et al., 2021b). However, the lack of oxygen in this study promoted the growth of the genus *Parvibaculum* over *KCMB-112*, even if the presence of *Parvibaculum* members was almost negligible in the inoculum. Members of the species *Parvibaculum* have been pointed as able to grow on complex organic compounds as the

only carbon and energy source, such as alkylbenzenesulphonates (Dong et al., 2004; Rosario-Passapera et al., 2012). However, they have not been identified yet as siloxanes degraders. Previous research has detected the enrichment of members of this genus after long exposure to siloxanes under aerobic conditions, but always at abundances lower than 5% (Pascual et al., 2020). Although most members of this genus are considered aerobic heterotrophs, some uncultured species have been found under anoxic conditions. Thus, in this study the operational conditions at very low oxygen levels and in the presence of complex organic compounds may have helped the growth of this genus over others (Blöthe and Roden, 2009). Nevertheless, the most plausible scenario is that a consortium of different organisms was responsible of siloxanes degradation based on the changes on bacterial diversity and the dominance of species that were not detected in the inoculum.

4. Conclusions

The present research evidenced for the first time the technical feasibility of anoxic siloxanes abatement in TP-BTF. While a slight increase in the removal efficiency was recorded for the linear VMS (L2: 12–15% and L3: 26–38%) when the organic phase fraction was raised from 15 to 30%, no significant variations in the abatement of the cyclic VMS (D4: 47–41% and D5: 54–48%) were observed. However, an increase in the EC was recorded for both linear and cyclic VMS (L2: 3.7–5.1 mg m⁻³ h⁻¹, L3: 10–14 mg m⁻³ h⁻¹, D4: 10.3–23 mg m⁻³ h⁻¹ and D5: 18.9–37.4 mg m⁻³ h⁻¹) when their inlet concentration was doubled. These results supported the fact that the performance of the TP-BTF was hindered by the VMS gas phase/biofilm transport. Finally, the highest REs and ECs (54% and 27.8 mg m⁻³ h⁻¹ for D4 and 73% and 45.9 mg m⁻³ h⁻¹ for D5, respectively) were recorded when the silicone oil fraction was increased to 45%. The inferior removal performance recorded for the linear VMS was related to their higher vapor pressure and volatility, that reduces the solubility of these compounds in the organic phase. The organic carbon and silicon concentrations in the aqueous phase along with the CO₂ production evidenced the biological degradation of the VMS. In this sense, a specialized bacteria consortium was identified in the TP-BTF with *Parvibaculum* (22.1 ± 0.21%), *Zarvazinia* (7.7 ± 0.3%), *KCM-B-11* (6.2 ± 0.2%), *Arenimonas* (5.9 ± 0.3%), *Moraxellaceae* (5.1 ± 0.2%) and *Luteimonas* (4.1 ± 0.2%) as the most representative bacteria. The promising results obtained in the present work, along with the reduced operating cost and low environmental impact of this biological alternative compared to the physical-chemical technologies available, could boost the implementation of sustainable biotechnologies for the removal of VMS from biomethane.

CRedit authorship contribution statement

Celia Pascual: Investigation, Formal analysis, Writing – original draft. **Sara Cantera:** Formal analysis, Writing – original draft. **Raúl Muñoz:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Raquel Lebrero:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

Acknowledgment

This work was financed by the URBIOFIN project, receiving funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation program under grant agreement No 745785. The European Commission-H2020-MSCA-IF-2019 is also gratefully acknowledged for the financial support of the project ENHANCEMENT (897284). The support from the regional government of Castilla y León and the EU-FEDER programme (CLU 2017-09, CL-EI-2021-07 and UIC 315) is also acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2022.133427>.

References

Blöthe, M., Roden, E.E., 2009. Microbial iron redox cycling in a circumneutral-pH groundwater seep. *Appl. Environ. Microbiol.* 75, 468–473. <https://doi.org/10.1128/AEM.01817-08>.

- Cheng, Y., He, H., Yang, C., Zeng, G., Li, X., Chen, H., Yu, G., 2016. Challenges and solutions for bio filtration of hydrophobic volatile organic compounds. *Biotechnol. Adv.* 34, 1091–1102. <https://doi.org/10.1016/j.biotechadv.2016.06.007>.
- Darraçq, G., Couvert, A., Couriol, C., Thomas, D., Amrane, A., Dumont, E., Andres, Y., Le Cloirec, P., 2012. Optimization of the volume fraction of the NAPL, silicone oil, and biodegradation kinetics of toluene and DMDS in a TPPB. *Int. Biodeterior. Biodegrad.* 71, 9–14. <https://doi.org/10.1016/J.IBIOD.2012.03.004>.
- Dong, W., Eichhorn, P., Radajewski, S., Schleheck, D., Denger, K., Knepper, T.P., Murrell, J.C., Cook, A.M., 2004. Parvibaculum lavamentivorans converts linear alkylbenzenesulphonate surfactant to sulphophenylcarboxylates, α,β -unsaturated sulphophenylcarboxylates and sulphophenyldicarboxylates, which are degraded in communities. *J. Appl. Microbiol.* <https://doi.org/10.1111/j.1365-2672.2004.02200.x>.
- Gaj, K., 2021. Siloxanes removal from biogas and emerging biological techniques. In: *Emerging Technologies and Biological Systems for Biogas Upgrading*, pp. 255–291. <https://doi.org/10.1016/b978-0-12-822808-1.00010-6>.
- Golmakani, A., Ali Nabavi, S., Wadi, B., Manovic, V., 2022. Advances, challenges, and perspectives of biogas cleaning, upgrading, and utilisation. *Fuel* 317. <https://doi.org/10.1016/J.FUEL.2021.123085>.
- González-Martín, J., Cantera, S., Lebrero, R., Muñoz, R., 2022. Optimization of acrylic-styrene latex-based biofilms as a platform for biological indoor air treatment. *Chemosphere* 287. <https://doi.org/10.1016/j.chemosphere.2021.132182>.
- Horii, Y., Kannan, K., 2020. Main uses and environmental emissions of volatile methylsiloxanes. *Handb. Environ. Chem.* 89, 33–70. https://doi.org/10.1007/698_2019_375.
- Kolde, R., 2019. *Pretty Heatmaps R Package [WWW Document]. Version 1.0.12*.
- Kuhn, J.N., Elwell, A.C., Elsayed, N.H., Joseph, B., 2017. Requirements, techniques, and costs for contaminant removal from landfill gas. *Waste Manag.* 63, 246–256. <https://doi.org/10.1016/j.wasman.2017.02.001>.
- Li, Y., Zhang, W., Xu, J., 2014. Siloxanes removal from biogas by a lab-scale biotrickling filter inoculated with *Pseudomonas aeruginosa* S240. *J. Hazard Mater.* 275, 175–184. <https://doi.org/10.1016/j.jhazmat.2014.05.008>.
- Meng, Z., Liu, Y.H., Ma, Z.C., Hou, X.F., 2020. The regulation of micro/mesoporous silica gel by polyethyleneimine for enhancing the siloxane removal. *Inorg. Chem. Commun.* 112, 107754. <https://doi.org/10.1016/j.inoche.2019.107754>.
- Muñoz, R., Meier, L., Diaz, I., Jeison, D., 2015. A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading. *Rev. Environ. Sci. Biotechnol.* 14, 727–759. <https://doi.org/10.1007/s11157-015-9379-1>.
- Muñoz, R., Quijano, G., Revah Moiseev, S., 2014. Two-phase partitioning bioreactors: towards a new generation of high-performance biological processes for VOC and CH₄ abatement. *Electron. J. Energy Environ.* 2, 34–46. <https://doi.org/10.7770/ejee-V0N0-art661>.
- Nguyen, L.N., Kumar, J., Vu, M.T., Mohammed, J.A.H., Pathak, N., Commault, A.S., Sutherland, D., Zdzarta, J., Tyagi, V.K., Nghiem, L.D., 2021. Biomethane production from anaerobic co-digestion at wastewater treatment plants: a critical review on development and innovations in biogas upgrading techniques. *Sci. Total Environ.* 765, 142753. <https://doi.org/10.1016/J.SCITOTENV.2020.142753>.
- Nyamukamba, P., Mukumba, P., Chikukwa, E.S., Makaka, G., 2020. Biogas upgrading approaches with special focus on siloxane removal—a review. *Energies* 13, 1–17. <https://doi.org/10.3390/en13226088>.
- Paparello, D., Boschetti, A., Silvestri, S., Khomenko, I., Biasioli, F., 2018. Real-time monitoring of removal of trace compounds with PTR-MS: biochar experimental investigation. *Renew. Energy* 125, 344–355. <https://doi.org/10.1016/j.renene.2018.02.122>.
- Pascual, C., Cantera, S., Lebrero, R., 2021a. Volatile siloxanes emissions: impact and perspectives. *Trends Biotechnol.* 39, 1245–1248. <https://doi.org/10.1016/j.tibtech.2021.05.003>.
- Pascual, C., Cantera, S., Muñoz, R., Lebrero, R., 2021b. Siloxanes removal in a two-phase partitioning biotrickling filter: influence of the EBRT and the organic phase. *Renew. Energy* 177. <https://doi.org/10.1016/j.renene.2021.05.144>.
- Pascual, C., Cantera, S., Muñoz, R., Lebrero, R., 2020. Comparative assessment of two biotrickling filters for siloxanes removal: effect of the addition of an organic phase. *Chemosphere* 251, 126359. <https://doi.org/10.1016/j.chemosphere.2020.126359>.
- Pascual, C., Lebrero, R., Cantera, S., 2022. Toward a sustainable and cost-efficient biological-based platform for siloxanes removal. *Crit. Rev. Environ. Sci. Technol.* 1–17. <https://doi.org/10.1080/10643389.2022.2044254>.
- Pausan, M.R., Csorba, C., Singer, G., Till, H., Schöpf, V., Santigli, E., Klug, B., Högenauer, C., Blohs, M., Moissl-Eichinger, C., 2019. Exploring the archaeome: detection of archaeal signatures in the human body. *Front. Microbiol.* <https://doi.org/10.3389/fmicb.2019.02796>.
- Phandanoung-Lozano, V., Sun, W., Sanders, J.M., Hay, A.G., 2018. Biochar does not attenuate triclosan's impact on soil bacterial communities. *Chemosphere.* <https://doi.org/10.1016/j.chemosphere.2018.08.132>.
- Quijano, G., Rocha-Ríos, J., Hernández, M., Villaverde, S., Revah, S., Muñoz, R., Thalasso, F., 2010. Determining the effect of solid and liquid vectors on the gaseous interfacial area and oxygen transfer rates in two-phase partitioning bioreactors. *J. Hazard Mater.* 175, 1085–1089. <https://doi.org/10.1016/j.jhazmat.2009.10.020>.
- Rosario-Passapera, R., Keddis, R., Wong, R., Lutz, R.A., Starovoytov, V., Vetricani, C., 2012. *Parvibaculum hydrocarbonoclasticum* sp. nov., a mesophilic, alkane-oxidizing alphaproteobacterium isolated from a deep-sea hydrothermal vent on the East Pacific Rise. *Int. J. Syst. Evol. Microbiol.* <https://doi.org/10.1099/ijs.0.039594-0>.
- Ruiling, G., Shikun, C., Zifu, L., 2017. Research progress of siloxane removal from biogas. *Int. J. Agric. Biol. Eng.* 10, 30–39. <https://doi.org/10.3965/ij.ajabe.20171001.3043>.
- Santos-Clotas, E., Cabrera-Codony, A., Boada, E., Gich, F., Muñoz, R., Martín, M.J., 2019. Efficient removal of siloxanes and volatile organic compounds from sewage biogas

- by an anoxic biotrickling filter supplemented with activated carbon. *Bioresour. Technol.* 294, 122136 <https://doi.org/10.1016/j.biortech.2019.122136>.
- Schloss, P.D., 2020. Reintroducing mothur: 10 years later. *Appl. Environ. Microbiol.* <https://doi.org/10.1128/AEM.02343-19>.
- Schloss, P.D., 2008. Evaluating different approaches that test whether microbial communities have the same structure. *ISME J.* <https://doi.org/10.1038/ismej.2008.5>.
- Shen, M., Zhang, Y., Hu, D., Fan, J., Zeng, G., 2018. A review on removal of siloxanes from biogas: with a special focus on volatile methylsiloxanes. *Environ. Sci. Pollut. Res.* 25, 30847–30862. <https://doi.org/10.1007/s11356-018-3000-4>.
- Stupek, E., Makoś-Chelstowska, P., Gębicki, J., 2021. Removal of siloxanes from model biogas by means of deep eutectic solvents in absorption process. *Mater* 14, 241. <https://doi.org/10.3390/MA14020241>, 2021, Page 241 14.
- Standardization, E.C. for, 2016. EN 16723-1, *Acta Crystallographica. Section F, Structural Biology and Crystallization Communications*.
- Surita, S.C., Tansel, B., 2014. Emergence and fate of cyclic volatile polydimethylsiloxanes (D4, D5) in municipal waste streams: release mechanisms, partitioning and persistence in air, water, soil and sediments. *Sci. Total Environ.* 468–469, 46–52. <https://doi.org/10.1016/j.scitotenv.2013.08.006>.
- Tansel, B., Surita, S.C., 2017. Historical and projected trends of siloxane use in consumer products, associated impacts on municipal solid waste and landfill gas utilization. *Int. J. Environ. Sci. Technol.* 14, 795–802. <https://doi.org/10.1007/s13762-016-1186-x>.
- Team, R.C., 2016. *R: A Language and Environment for Statistical Computing*. R Found. Stat. Comput.
- Tran, V.T.L., Gélín, P., Ferronato, C., Mascunan, P., Rac, V., Chovelon, J.M., Postole, G., 2019. Siloxane adsorption on activated carbons: role of the surface chemistry on sorption properties in humid atmosphere and regenerability issues. *Chem. Eng. J.* 371, 821–832. <https://doi.org/10.1016/j.cej.2019.04.087>.
- Willis, A.D., 2019. Rarefaction, alpha diversity, and statistics. *Front. Microbiol.* <https://doi.org/10.3389/fmicb.2019.02407>.