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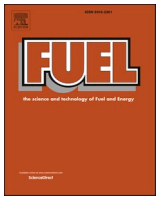

# Assessment of the mass transfer strategy and the role of the active bacterial population on the biological degradation of siloxanes

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ABSTRACT

Upgrading of biogas to remove siloxanes is mandatory to meet the standards required for its use as a substitute of fossil fuels. The biological degradation of these pollutants is a low cost and environmental friendly alternative to conventional techniques, albeit certain limitations, such as the low solubility of siloxanes, still hinder its application. In the present work, two parameters were optimized in aerobic and anoxic two-phase biotrickling filters (TP-BTF): the trickling liquid velocity (TLV) and the internal gas recirculation, with the aim of improving siloxanes biological removal. The results obtained showed that the increase in TLV from 2 to 10 m $h^{-1}$ resulted in higher removal efficiency (RE) under both anoxic and aerobic conditions, reaching maximum values of 55 and 47%, respectively. This effect was more significant for the linear siloxanes. On the contrary, a further increase in the TLV to 20 m $h^{-1}$ together with the implementation of internal gas recirculation caused an excessive turbulence in the liquid side, detaching the biofilm and having a negative effect for the RE. The cyclic siloxanes were more effectively eliminated along the process (maximum REs of 75% were recorded for decamethylcyclopentasiloxane (D5)), but the studied system modifications exerted a minor effect on their RE. The active bacterial population involved in siloxanes degradation (studied throughout RNA extraction and sequencing) was dominated by the clade *Acidithiobacillacea KCM-B-112* and the genus *Parvibaculum* in aerobic conditions, while the members of the family *Phyllobacteriacea* and the genera *Nocardia* and *Baekduia* dominated in anoxic conditions.

## 1. Introduction

Improving energy efficiency and reducing primary energy sources, including nuclear energy, are some of the biggest policy concerns around the world. According to the EU 2030 climate & energy framework, a 32% share of renewable energy is targeted by 2030 [1]. Within this context, biogas constitutes one of the most promising candidates among the different renewable sources [2]. However, the residual gaseous compounds present in biogas should be removed in order to increase its energy efficiency and to avoid operational problems and negative health/environmental impacts. Among them, volatile methyl siloxanes (VMS) are some of the most problematic biogas contaminants, since they damage biogas engines and equipment (abrasion caused by silica particles formed during biogas combustion) raising the operating costs and reducing the efficiency of energy generation facilities. For this reason, there is a concentration limit of siloxanes in raw biogas that depends on its subsequent use. In European legislation, the limit for the injection of biomethane into natural gas grids and for its use as vehicle fuel is set at ~1 mg Si $m^{-3}$ [3].

Commercial physical-chemical technologies mainly based on adsorption, absorption and condensation processes, have demonstrated high VMS removal efficiencies, complying with existing standards. However, their environmental impact together with their excessive operating costs have promoted the development of sustainable alternatives based on biological processes [4]. The most studied bioreactor configurations in the field of VMS abatement, under both aerobic and anoxic conditions, are biotrickling filters (BTFs) [5]. Previous research has identified the poor gas-liquid mass transfer of VMS, associated to their low solubility, as the major limitation during their biological degradation. The optimization of bioreactors to boost mass transfer of

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poorly soluble pollutants has been widely investigated. Mixing the packing material with activated carbon [6] or adding an organic phase to the recycling liquid (in the so called two-phase partitioning BTF, TP-BTF) [7] have been studied as strategies to improve mass transfer of VMS with satisfactory results.

Apart from these solutions, optimization of some operating parameters such as the trickling liquid velocity (TLV) or the internal gas recirculation might lead to an enhanced performance as they determine the mass transfer of pollutants from the gas to the liquid phase. Several studies have assessed the effect of the TLV on the global mass transfer coefficient for the removal of $H_2S$, methane and other VOCs [8–10]. The results concluded that the TLV increases the transport of the target pollutant from the gas phase to the trickling solution, while promoting the growth of biomass in the packing material. On the other hand, the implementation of an internal gas recirculation has been proven as an outstanding mass transfer strategy in submerged culture systems. Bubble column bioreactors with internal gas recirculation operated to remove $CH_4$ or $H_2$, exhibited an increased turbulence and global mass transfer coefficient [11–13].

The present work aimed at testing in a lab-scale TP-BTF the influence of the TLV and the internal gas recirculation in the degradation of VMS under both anoxic and aerobic conditions. The model VMS tested were those commonly found in biogas: hexamethyldisiloxane (L2), octamethyltrisiloxane (L3), octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5). Furthermore, the specialization of the active bacterial population was analyzed throughout RNA sequencing, which allowed to identify the main active bacteria involved in VMS degradation.

## 2. Materials and methods

### 2.1. Mineral salt medium and inocula preparation

The mineral salt medium (MSM) was composed of (g $L^{-1}$): $KH_2PO_4$, 0.7; $K_2HPO_4{\cdot}3H_2O$, 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 micronutrient solution containing (g $L^{-1}$): EDTA, 0.5; $FeSO_4{\cdot}7H_2O$, 0.2; $ZnSO_4{\cdot}7H_2O$, 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; $NiCl_2{\cdot}6H_2O$, 0.002; $NaMoO_4{\cdot}2H_2O$, 0.003. All chemicals used for the preparation of the MSM were purchased from Panreac (Barcelona, Spain). L2 (98.5% purity), L3 (98 % purity), D4 (98 % purity) and D5 (97 % purity) were obtained from Sigma Aldrich (San Luis, USA). The experiments were carried out using consortia enriched from activated sludge of previous TP-BTFs treating siloxanes under anoxic [14] and aerobic [15] conditions. The aerobic consortium was enriched in a TP-BTF fed with a VMS mixture (L2, L3, D4 and D5) as the solely carbon and energy source at concentrations ≈ 800 mg $m^{-3}$ for 124 days, while the anaerobic consortium was enriched in an anoxic TP-BTF fed with a VMS mixture (L2, L3, D4 and D5) as the sole carbon and energy source at a concentration ranging between 100 and 250 mg $m^{-3}$ for 126 days.

### 2.2. Experimental setup and operating procedure

The experimental systems (Fig. 1) consisted of two identical cylindrical PVC columns (8.4 cm diameter, 37.5 cm height) with a working volume of 2 L, packed with Kaldnes K1 Micro rings (Evolution Aqua, UK). The BTFs were inoculated with the anoxic and aerobic enriched cultures obtained as described in previous section 2.1.

The synthetic VMS-loaded inlet stream was prepared by injecting a liquid mixture containing L2, L3, D4 and D5 with a syringe pump (Fusion 100, Chemyx Inc., USA) into an air (aerobic BTF) or a $N_2$ (anoxic BTF) stream of 33 mL $min^{-1}$ controlled by means of a rotameter. The VMS-loaded stream entered a mixing chamber to promote VMS volatilization and homogenization prior feeding at the bottom of the corresponding column. Inlet and outlet VMS and $CO_2$ concentrations in the gas phase were periodically measured with Gastight™ SampleLock™ syringes (Agilent, Santa Clara, California, USA) of 500 and 100 μL, respectively (Fig. 1).

The MSM-silicone oil liquid mixture (70/30 % v/v) was recycled by a peristaltic pump (Watson-Marlow 313D) from an external 1-L holding tank magnetically stirred at 300 rpm. This liquid mixture was continuously recycled to the top of the column countercurrently with the VMS-loaded stream.

An abiotic test was initially performed for 27 days to discard the possibility of siloxanes removal and to ensure that there was no biological activity in the system prior to inoculation. For this purpose, the test was initiated with the empty PVC column, while sterile packing

**Table 1**
Experimental conditions tested in the anoxic TP-BTF.

| | Time course (days) | VMS inlet concentration (mg $m^{-3}$) | TLV (m $h^{-1}$) | Internal gas recirculation (L $min^{-1}$) |
|---|---|---|---|---|
| S1 | 0–26 | 178±20 | 2 | – |
| S2 | 27–42 | 204±25 | 10 | – |
| S3 | 43–70 | 213±10 | 20 | – |
| S4 | 71–96 | 202±18 | 10 | – |
| S5 | 97–116 | 247±14 | 10 | 1 |
| S6 | 117–140 | 243±11 | 10 | 3 |

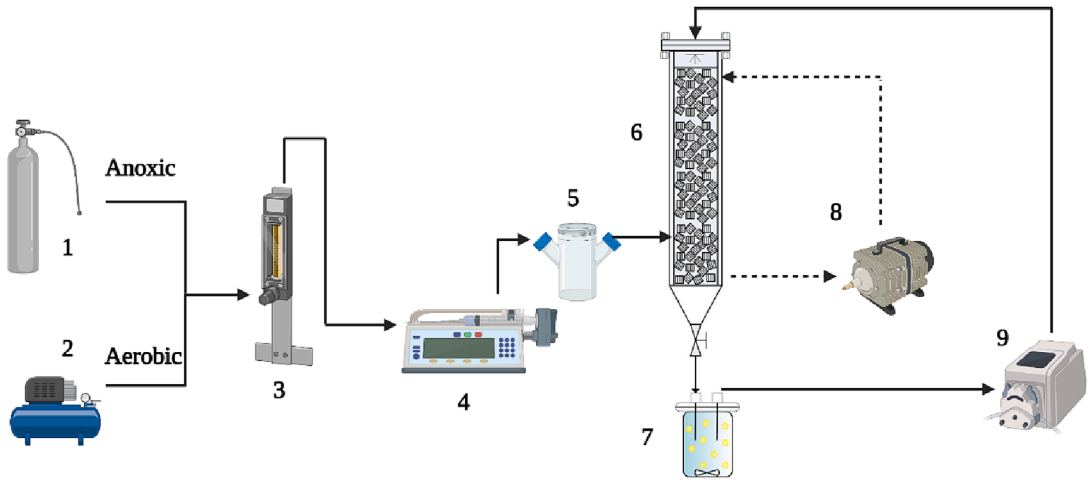


**Fig. 1.** Schematic representation of the experimental set-up. (1) $N_2$ cylinder, (2) air compressor, (3) rotameter, (4) syringe pump, (5) mixing chamber, (6) bio-trickling filter, (7) liquid mixture reservoir, (8) internal gas recirculation pump and (9) liquid recycling pump.

material and MSM were added in subsequent steps.

The anoxic and aerobic systems were operated for 140 (Table 1) and 177 (Table 2) days, respectively, in two different test series devoted to assess (i) the influence of the TLV (S1-S4) and (ii) the effect of the internal gas recirculation (S5 and S6).

The BTFs were operated at an empty bed residence time of 1 h and a periodical replacement of 300 mL of the culture broth with fresh MSM every seven days (equivalent to a hydraulic retention time of 47 days) throughout the entire experiment. The pH and total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), total silicon (Si), nitrite and nitrate concentrations were analyzed in the cultivation broth withdrawn.

The VMS-loaded streams were maintained at concentrations between 200 and 300 mg $m^{-3}$. The trickling liquid solution was progressively increased during the experimental test series from 2 m $h^{-1}$ in S1 to 10 and 20 m $h^{-1}$ in stages S2 and S3, respectively. Finally, the TLV was reduced again to 10 m $h^{-1}$ and maintained at this value during the rest of the experiment. Internal gas recirculation was implemented at 1 and 3 L $min^{-1}$ in stages S5 and S6, respectively.

### 2.3. Analytical procedures

VMS concentration analysis in the gas phase at the inlet and outlet of the BTFs was carried out in an Agilent 8860 gas chromatograph (Santa Clara, California, USA) equipped with a flame ionization detector (FID) and a HP-5 column (15 m × 0.25 mm × 0.25 μm). Both the 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 $min^{-1}$ to 200 °C. Helium was used as the carrier gas at a flow rate of 3.5 mL $min^{-1}$.

$CO_2$ and $O_2$ gas concentrations were daily 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 total organic carbon (TOC), total inorganic carbon (IC), total nitrogen (TN), silicone (Si), nitrite and nitrate concentrations in the cultivation broth were periodically analyzed. Silicon concentration was analyzed by means of an inductively coupled plasma optical atomic emission spectrometer (ICP-OES Radial Simultaneous Varian 725-ES, Agilent). 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 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).

### 2.4. Bacterial community analysis

Samples were taken at the end of operation of the anoxic and aerobic BTFs (BTF-AN and BTF-AE). For this, the biofilm from the Kaldnes K1 Micro rings was resuspended in 40 mL of TE buffer pH 7.4. For RNA preservation, the 40 mL of culture were centrifuged for 10 min at 10,000 *g* at 4 °C and the cell pellet was washed once with 10 mL of TE buffer pH 7.4. After a second centrifugation, the pellet was snap-frozen and stored at −80 °C. RNA 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). RNA extraction was conducted using the RNA PowerSoil® Total RNA Isolation Kit. The RNA extracts were subjected to DNase treatment using the DNase I Kit for Purified RNA in Solution (Mobio Laboratories Inc.) for removal of residual DNA. RNA and DNA concentration was determined using the Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The RNA was subsequently converted to cDNA using the qScriber™ cDNA Synthesis Kit (Mobio Laboratories Inc.). Sequencing was performed by Illumina Miseq platform targeting the 16S V3 and V4 regions (464 bp, *Escherichia coli* based coordinates) with the bacterial primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a- A-21 according to [15]. Archaea communities were not targeted due to the lack of PCR amplification using Archaea specific primers [16]. The 16S rRNA gene sequences were processed and quality filtered using Mothur v1.45.3 following the Mother SOP (https://www.mothur.org/wiki/MiSeq_SOP) [17]. Sequences were then classified and annotated into Operational Taxonomic Units (OTUs) using the Ribosomal Database Project (trainset18) with 100 bootstrap iterations and 80% confidence cutoff [18]. For the accuracy and convenience of taxonomic assignment to species of the most relevant microorganisms, the most abundant sequences were blasted using NCBI. The 16S rRNA gene sequences of the species obtained and their closest relatives were collected from GenBank and updated or supplemented them to the currently RDP 16S rRNA gene database (version 18) used by Mothur (provided as Supplemental Material S6). The nucleotide sequence dataset obtained in this study has been deposited at DDBJ/ENA/GenBank as bioproject: PRJNA895218. After sequence cleaning and taxonomic classification, diversity studies were carried out [19]. The rarefaction with 599 randomizations showed that the smallest representative library was obtained with 41,443 reads. Alpha diversity was calculated with the Inverse Simpson Index resulting from the three biological replicates in each condition using Mothur v1.45.3 [20]. Beta diversity among samples was compared by using the Jaccard Index and AMOVA at $p < 0.05$ of the triplicates [21]. The results obtained of the prokaryotic community structure were plotted using R version 4.1.2 [22] with the package *pheatmap* [23].

**Table 2**
Experimental conditions tested in the aerobic TP-BTF.

| | Time course (days) | VMS inlet concentration (mg $m^{-3}$) | TLV (m $h^{-1}$) | Internal gas recirculation (L $min^{-1}$) |
|---|---|---|---|---|
| S1 | 0–33 | 237±12 | 2 | – |
| S2 | 34–72 | 278±11 | 10 | – |
| S3 | 73–100 | 273±22 | 20 | – |
| S4 | 101–142 | 312±24 | 10 | – |
| S5 | 143–163 | 295±21 | 10 | 1 |
| S6 | 164–177 | 279±4 | 10 | 3 |

## 3. Results and discussion

### 3.1. Optimization of internal liquid and gas recirculation

The abiotic test was performed prior to the inoculation of both BTFs. During stages I, II and III of the abiotic test, no decrease in outlet VMS concentration was observed in the empty PVC column (I), after the addition of the packing material (II) and the startup of the MSM recycling (III), remaining at similar values compared to the inlet concentration (Fig. S1).

Similarly, no significant differences were observed between L2 inlet and outlet concentrations when the silicone oil was added to the recycling liquid during stage IV. However, an instantaneous decrease in the outlet concentration was recorded for the rest of the VMS associated with their absorption in the silicone oil. The outlet concentration tended to increase as the silicone oil saturated with time. No $CO_2$ production was recorded throughout the entire abiotic test.

#### 3.1.1. Influence of the trickling liquid velocity

Both systems were inoculated after the abiotic test. During S1, the anoxic TP-BTF immediately stabilized at an average total VMS removal

efficiency (RE) of 46.7±5.6% by day 9, corresponding to an EC of 83.4 mg $m^{-3}h^{-1}$ (Fig. 2A, Table S1). A higher performance was recorded for D5 (Fig. 3) (66.2±4.6 %) compared to the rest of the VMS (37.5±4.5, 37.6±7.7 % and 47.7±7.7 % for L2, L3 and D4, respectively). In the aerobic TP-BTF, a total VMS removal of 38.8±3.7 % was achieved (Fig. 2B, Table S1), corresponding to an EC of 92.0 mg $m^{-3}h^{-1}$ associated with the slightly higher inlet VMS concentration (237 mg $m^{-3}$ compared to 178 mg $m^{-3}$ in the anoxic BTF). Similar to the anoxic system, the highest performance was recorded for D5 (72.8±2.9%) compared with the rest of VMS (17.0±1.7%, 17.7±1.8% and 45.0±7.9% for L2, L3 and D4, respectively) due to its lower vapor pressure and higher affinity for the organic phase (Fig. 4). This superior performance for D5 compared to D4 and the linear VMS has been consistently reported [15,24].

Previous research has demonstrated that the mass transfer coefficient and the growth of the biomass in a TP-BTF increased at increasing TLVs [8]. In this sense, Caicedo et al. (2018) observed a progressive improvement in the RE and EC of toluene and ethylbenzene by subsequently increasing the TLV from 2 to 4, 15 and 20 m $h^{-1}$ [10]. In our system, the increase in the TLV from 2 to 10 m $h^{-1}$ during S2 resulted in an enhancement in the VMS abatement performance of both anoxic and aerobic BTFs, reaching average RE values of 54.6±3.7 and 46.5±3.2%, respectively. It is worth noting that this enhancement was more remarkable for the linear VMS, with an increase in their removal by up to 1.3 and 2 times in the anoxic and aerobic TP-BTFs, respectively, compared with S1. This superior removal of linear VMS was associated with the increase of the TLV. The TLV leaded to a better distribution of the trickling solution through the packed bed and consequently to an increase in the mass transfer coefficient of the linear VMS. Moreover, the increase of the nutrients availability likely enhanced the biodegradation process. On the contrary, the RE of the cyclic VMS remained at similar values in both systems (Table S1). Fact that was most likely related to the lower vapor pressures of D4-D5. The concentrations of D4 and D5 at the interphase were likely close to the equilibrium concentrations compared to those of linear VMS, thus the mass transfer improvement effect on cyclic VMS was reduced. Thus, the TLV was further increased to 20 m $h^{-1}$ during S3. However, this rise did not trigger a positive effect and lower total VMS REs of 38.0±3.3 and 31.4±4.6% were recorded for both anoxic and aerobic BTFs, corresponding to ECs of 85.2±12.0 and 80.8±6.0, mg $m^{-3}h^{-1}$, respectively. This decrease was especially significant for L2, L3 and D4. In particular, RE values of 19.1±4.9, 33.8 ±4.1 and 37.6±3.1% were obtained in the anoxic TP-BTF, with even lower removal performances in the aerobic BTFs of 13.6±1.8, 13.5±2.1 and 30.7±4.8% for L2, L3, and D4, respectively. Nevertheless, no significant variations were observed for D5 compared to the previous stages, remaining at RE values of 66.0±4.2 and 68.2±8.1% in the anoxic and aerobic TP-BTFs, respectively. According to literature, the negative effect of increasing the TLV can be associated to biofilm detachment, and particularly in BTFs packed with granular activated carbon and plastic rings [8].

The TLV was decreased back to 10 m $h^{-1}$ during S4 in order to restore previous performance of both systems. However, none of the 2P-BTFs recovered the preceding REs, remaining at similar values compared to stage S3 in the anoxic BTF, and even slightly lower values in the aerobic BTF (Table S1).

Overall, a lower abatement performance than that obtained by the authors in previous studies under aerobic conditions was achieved under the optimum conditions tested (i.e. those of Stage 2). In these previous studies, total VMS removals up to 70% were observed working at the same EBRT and silicone oil fraction. Similarly, L2, L3 and cyclic VMS removals were 60, 70, and 90%, respectively, in the mentioned studies. However, it should be noted that the inlet VMS concentration was 2× and 3× higher compared to the present study, which could boost mass transfer associated with an increased concentration gradient [7,15]. However, under anoxic conditions, the BTF performed similarly when working at the same EBRT and inlet VMS concentration with a silicone oil fraction of 45% and a TLV of 2 m $h^{-1}$ [14].

Other authors have tested alternative strategies to improve the removal of these pollutants. For instance, the addition of an adsorbent material (20% of activated carbon) to the packed bed resulted in an increase in the VMS mass transfer in an anoxic BTF and allowed the reduction of the EBRT to 12 min. However, lower REs of 16% and 45 % were recorded for D4 and D5, respectively [6]. Under aerobic conditions, Li et al. achieved a comparable removal of 74 % for D4 at an EBRT of 13.2 min in a BTF inoculated with *Pseudomonas aeruginosa S240*. This good performance was associated to the presence of biosurfactants in the cultivation broth, as the gas/liquid partitioning coefficient is 7 times lower in the aqueous solution containing biosurfactants. However, the performance of this system for linear VMS was not studied [25].

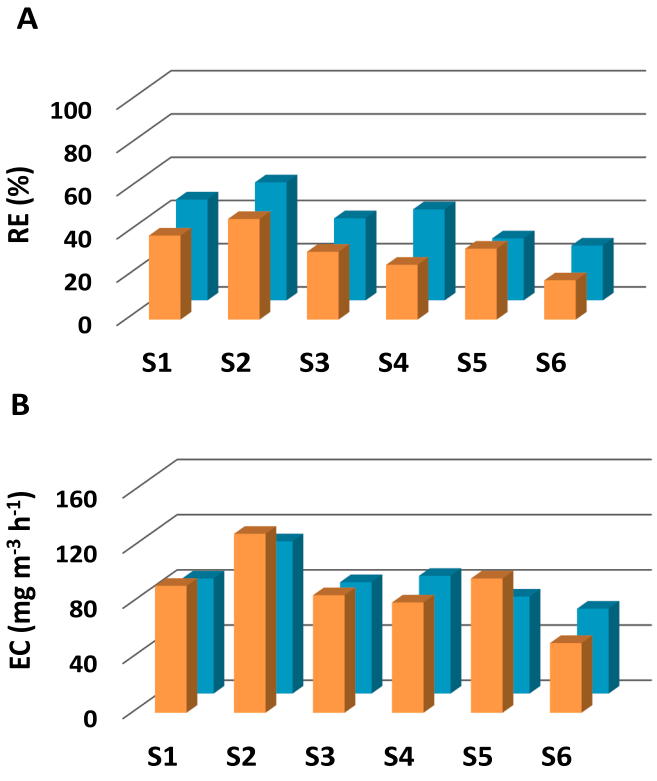


**Fig. 2.** Comparison of the average VMS inlet concentration (A), removal efficiencies (B) and elimination capacities (C) in the aerobic (orange bars) and anoxic (blue bars) TP-BTFs in the different experimental stages: TLV of 2 m $h^{-1}$ (S1), 10 m $h^{-1}$ (S2, S4, S5 and S6), 20 m $h^{-1}$ (S3) and internal gas recirculation of 1 L $min^{-1}$ (S5) and 3 L $min^{-1}$ (S6). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.1.2. *Influence of the internal gas recirculation*

During S5, an internal gas recirculation of 1 L $min^{-1}$ was implemented in both the anoxic and aerobic BTFs in order to increase the mass transfer of the hydrophobic VMS. The total VMS RE in the anoxic TP-BTF initially increased up to 70 %, subsequently decreasing to average steady state values of 28.7±7.8% (Fig. 2). No improvement in the performance of the system was observed for L2 compared to previous stages. However, the RE decreased to 16.1±6.4, 24.5±10.8 and 59.1 ±6.9% for L3, D4 and D5, respectively (compared to those of S4) (Table S1). On the contrary, a notable increase in the RE was observed in the aerobic TP-BTF, stabilizing at average values of 32.8±4.9 %. An increase in the performance of the system was observed for L2, L3 and D5, reaching REs of 25.0±5.7, 22.9±7.0 and 60.9±3.3%, respectively. On the contrary, no improvement was observed for D4 (Table S1).

In order to further studying the effect of the internal gas recirculation, this flow was increased to 3 L $min^{-1}$ during S6. No significant effect

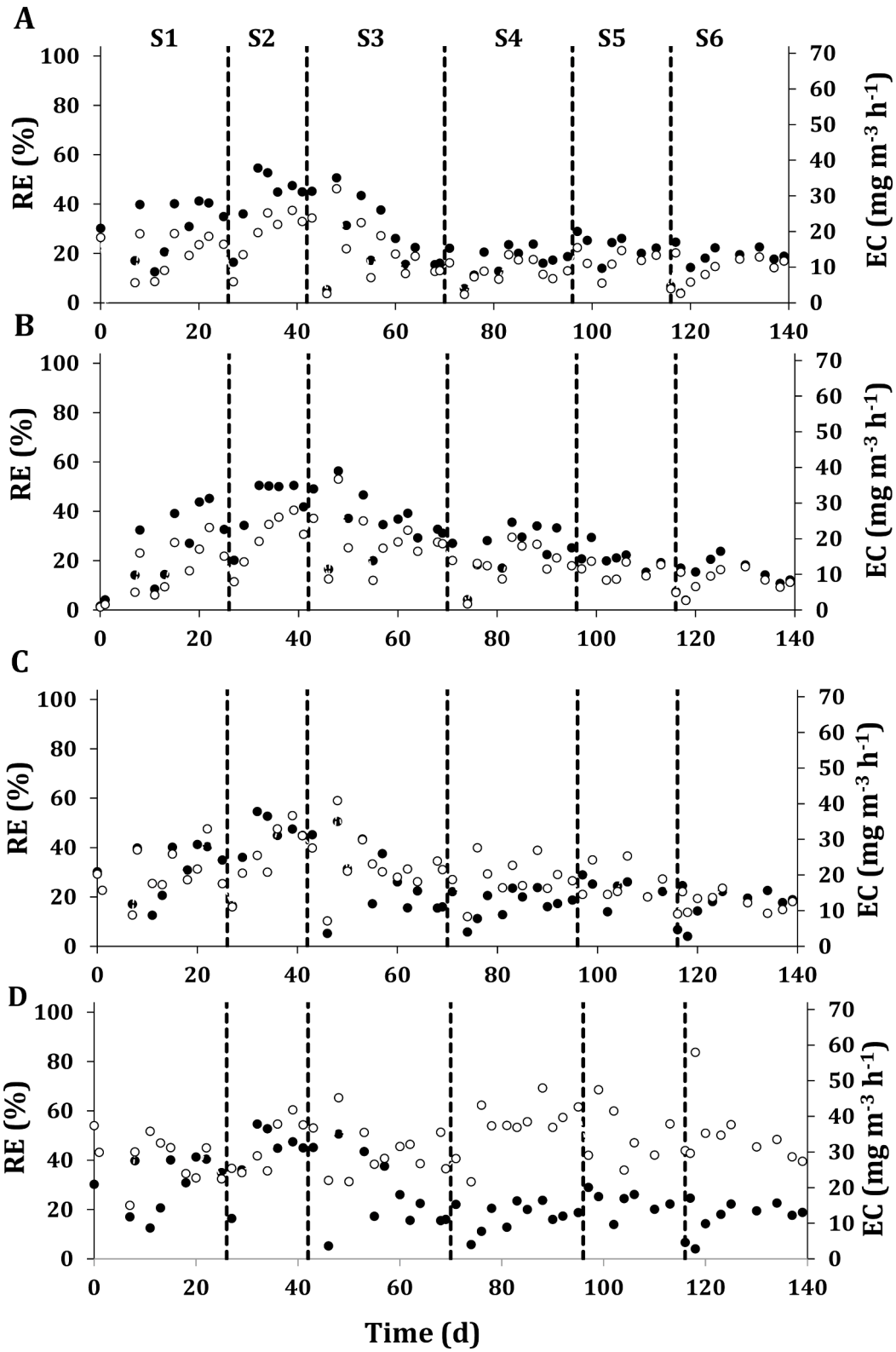


**Fig. 3.** Time course of L2 (A), L3 (B), D4 (C) and D5 (D) removal efficiency (●) and elimination capacity (○) in the anoxic TP-BTF during the different experimental stages: TLV of 2 m $h^{-1}$ (S1), 10 m $h^{-1}$ (S2, S4, S5 and S6), 20 m $h^{-1}$ (S3) and internal gas recirculation of 1 L $min^{-1}$ (S5) and 3 L $min^{-1}$ (S6).

was observed in the anoxic BTF, reaching slightly lower RE values of 25.4±2.2%. This detrimental effect was more significant for D4, whose removal was reduced by 30%. On the contrary, the performance of the aerobic TP-BTF was greatly reduced, with the total VMS RE decreasing by a factor of 1.8.

To the best of the authors' knowledge, internal gas recirculation has not previously been implemented in a BTF intended for siloxanes removal. This configuration has been studied in a BTF for methane removal [9], concluding that the internal gas recirculation improves the performance of the systems by 2.5 times due to an increase in the

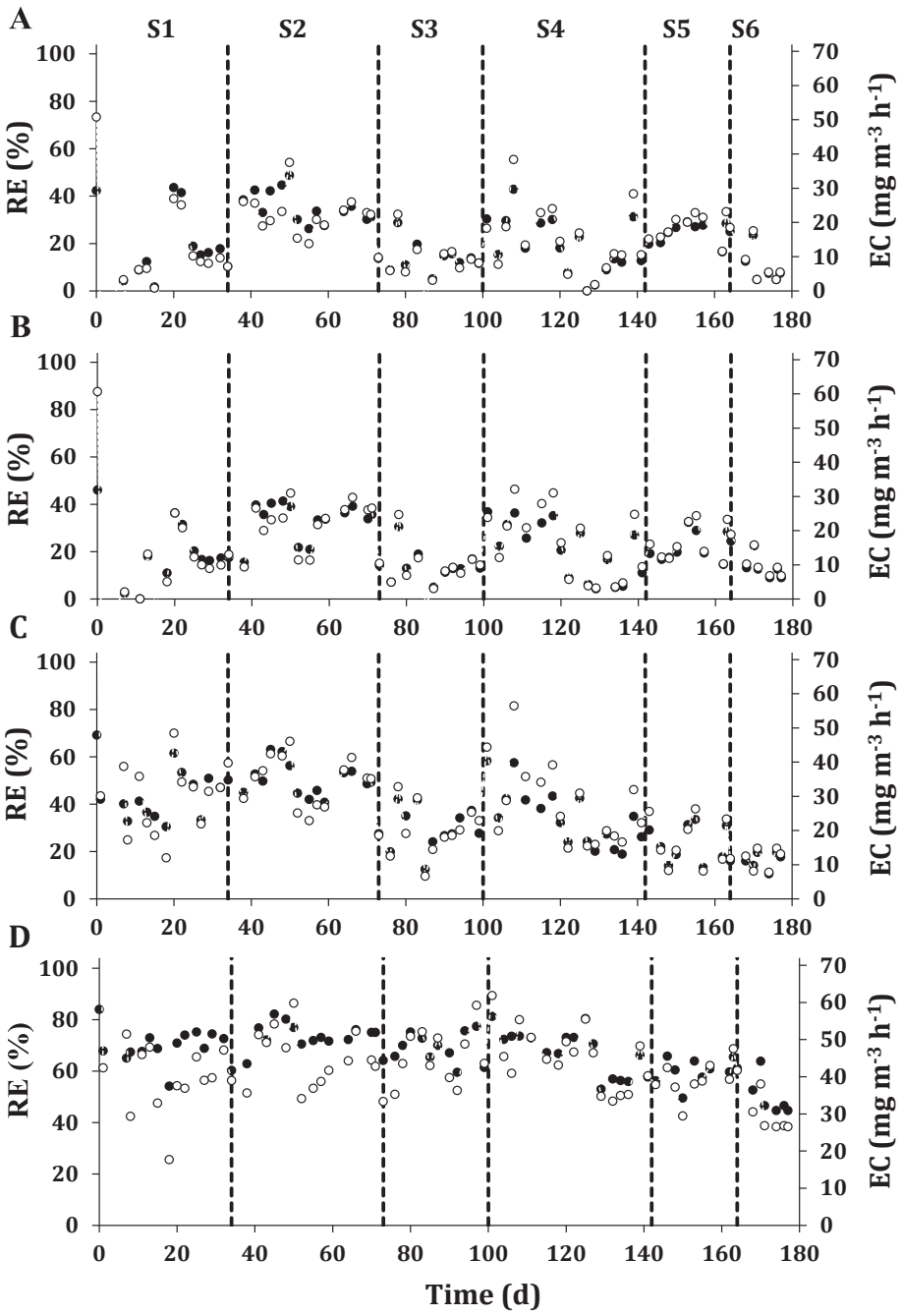


**Fig. 4.** Time course of L2 (A), L3 (B), D4 (C) and D5 (D) removal efficiency (●) and elimination capacity (○) in the aerobic TP-BTF during the different experimental stages: TLV of 2 m $h^{-1}$ (S1), 10 m $h^{-1}$ (S2, S4, S5 and S6), 20 m $h^{-1}$ (S3) and internal gas recirculation of 1 L $min^{-1}$ (S5) and 3 L $min^{-1}$ (S6).

pollutants mass transfer, as it decouples the gas-liquid turbulence inside the reactor from the actual gas residence time.

In the aerobic TP-BTF operated in the present study, this effect was slightly observed when the internal gas recirculation of 1 L $min^{-1}$ was implemented. On the contrary, a further increase to 3 L $min^{-1}$ caused the reduction of the VMS actual concentration entering the TP-BTF due to its dilution effect. This entailed a lower concentration gradient in the biofilm and, consequently, lower mass transfer rates. Moreover, excessive high recirculation flow rates could damage the biofilm in the BTF due to the shear stress resulting from the turbulence, as observed in the present study, or create preferential pathways inside the packed bed. In our particular study, the biofilm, likely affected by the high TLV implemented in the previous stages (20 m $h^{-1}$), did not withstand the turbulence generated in the system. Similarly, Dupnock and Deshusses (2019) observed a decrease in the hydrogen removal by 15% when the internal gas recirculation was implemented in a BTF [26]. The authors concluded that the implementation of the internal gas recirculation in plug flow systems, such as BTFs, decreased the concentration at the inlet of the bioreactor, where the mass transfer is faster due the higher concentration gradient. Hence, this strategy might result more efficient in bioreactors with suspended biomass, such as bubble column or airlift bioreactors, where the implementation of gas recirculation entails the increase of the gas holdup and gas-liquid interfacial area.

### 3.2. *Influence of operating conditions on $CO_2$ production and characterization of the recycling liquid solution*

The $CO_2$ production in the anoxic TP-BTF related to the microbial

activity and the biodegradation of VMS stabilized at average values of 373±41 mg $m^{-3}h^{-1}$ and remained constant throughout the stages S1, S2, S3 and S4 (where the influence of the trickling liquid velocity was tested) (Fig. S2A). At the beginning of stage S5, a gradual increase in the $CO_2$ production to 614 mg $m^{-3}h^{-1}$ was recorded in the anoxic TP-BTF, remaining at 533.2±77.4 mg $m^{-3}h^{-1}$ until the end of the experiment. The inorganic carbon concentration in the recirculating cultivation broth (Fig. S3A), associated with the dissolved $CO_2$, gradually increased to values of 38.6 mg $L^{-1}$ by day 62 and stabilized at average values of 34.4±0.7 mg $L^{-1}$ during stage S4. Then, the IC concentration continuously increased to reach a maximum concentration of 47.6 mg $L^{-1}$ by day 125, slightly decreasing by the end of the experiment.

In the aerobic BTF, the $CO_2$ production remained constant at 1530 ±179 mg $m^{-3}h^{-1}$ until day 48 (Fig. S2B), when a sharp increase to 12215 mg $m^{-3}h^{-1}$ was recorded in the system. This value gradually decreased to 6525 mg $m^{-3}h^{-1}$ by day 69 and increased again at the beginning of S3. The IC concentration, similar to $CO_2$ production, remained at average values of 1.6±0.3 3 mg $L^{-1}$ until day 53, when it increased to a maximum value of 29.5 at the beginning of S3. Then, the IC concentration gradually decreased to 4.8 mg $L^{-1}$ by day 116, fluctuating by the end of the experiment between 6.3 and 19.1 mg $L^{-1}$ (Fig. S3B). These variations were observed throughout the experiment, which were associated with occasional cell debris from biofilm detachment and the subsequent degradation of the organic carbon released to the trickling solution.

The higher $CO_2$ production values achieved in the aerobic TP-BTF compared to the anoxic system were attributed to both the higher TOC concentration and the presence of $O_2$ in the trickling solution, which could have triggered the aerobic degradation of residual organic matter (Fig. S3). The TOC concentration in the anoxic TP-BTF progressively increased to 70.4 mg $L^{-1}$ in stage S3 (day 48), and then decreased to 37.0 mg $L^{-1}$ (day 69). During S4 and S5, the TOC concentration remained at an average value of 51.4±3.0 mg $L^{-1}$, gradually increasing in S6 to average values of 69.4±2.6. However, the TOC concentration in the aerobic TP-BTF sharply increased at the beginning of the experiment to 155.9 mg $L^{-1}$ by day 11, progressively decreasing to values of 72.4 mg $L^{-1}$ by day 46. TOC accumulated again in the system during S2, achieving maximum values of 242.5 mg $L^{-1}$ by day 60. The concentration remained roughly constant until day 88, stablishing afterwards at 156.8±15.9 mg $L^{-1}$.

Similar to the TOC concentration, the Si concentration (Fig. S4A) in the anoxic TP-BTF gradually increased to 13.5 mg $L^{-1}$ by day 48, then decreasing to 5.9 mg $L^{-1}$ at the end of stage S3. The same trend was observed during S4, increasing to 10.2 mg $L^{-1}$ likely due to the removal of siloxanes, and decreasing afterwards to 3.4 mg $L^{-1}$ by day 97. Finally, the Si concentration stabilized at 5.3±0.5 mg $L^{-1}$. In the aerobic BTF, the Si concentration progressively increased to 7.2 mg $L^{-1}$ by day 88, remaining at an average value of 6.8±0.7 mg $L^{-1}$ during stages S3 and S4 (Fig. S4A). Afterwards, the Si concentration slightly decreased to 5.3 mg $L^{-1}$ at the end of the experiment (Fig. S4B). According to the mass flow rate of siloxanes removed in both anoxic and aerobic TP-BTF, and the replacement of MSM, an accumulation of Si up to 208 and 285 mg $L^{-1}$, respectively, was expected in the aqueous phase (assuming that all the Si removed ended up in the aqueous phase). Despite the Si content in the organic fraction was not analyzed, the stabilization of Si concentration in the aqueous phase indicated that part of the Si removed was assimilated by the microbial community.

The TN, nitrate, and nitrite concentrations (Fig. S5) in the anoxic TP-BTF remained at 323.4±15.4 mg $L^{-1}$, 255.8±16.0 mg N-$NO_3^-$ $L^{-1}$ and 15.2±5.7 mg N-$NO_2^-$ $L^{-1}$, respectively. The trace levels of nitrite observed were associated with the incomplete denitrification under anoxic conditions. In the aerobic BTF, TN and nitrate concentrations between 300 and 400 mg N $L^{-1}$ were recorded throughout the entire experiment, while no nitrite was detected regardless of the stage.

### 3.3. Active bacterial community and diversity

The analysis of the active bacterial community resulting from the RNA analysis displayed a total of 388,761 sequences that belonged to 2784 OTUs affiliated with bacterial genera. By the end of the operation, similar number of genera were found in both BTFs (244 and 218 total in the anoxic and aerobic BTFs, respectively). However, the bacterial communities shared <20 % of the identified genera, which was related to the different metabolic conditions (aerobic and anaerobic conditions). In accordance to these results, bacterial alpha and beta diversity was highly dissimilar (AMOVA, $p < 0.05$) between the two BTFs operated (Fig. S6).

The analysis of the bacterial taxonomic classification showed that the different conditions in the BTFs resulted in a highly dissimilar specialized active consortium (Fig. 5). The most abundant active bacteria in the anoxic TP-BTF were uncultured members of the family *Phyllobacteriacea* (21.0±0.9%), followed by the genera *Nocardia* (14.8±0.4) and *Baekduia* (12.7±0.4%), respectively. Other dominant bacteria belonged to the genera *Steroidobacter* (9.3±0.2), *Dokdonella* (7.5±0.3%), *Sphingomonas* (3.7±0.2%), and uncultured Proteobacteria of the clade *Acidithiobacillaceae KCM-B-112* (3.0±0.1%).

In the aerobic BTF, the most representative active bacteria were unclassified members of the order Rhodospirillales (27.2±0.1%), uncultured Proteobacteria of the clade *Acidithiobacillacea* KCM-B-112 (22.6 ±0.1%) and the genera *Parvibaculum* (11.8±0.2%), followed by the genus *Nocardia* (7.1±0.3%), *Aquamicrobium* (5.4±0.5%) and *Pseudoxanthomonas* (5.2±0.4%).

Regarding the anoxic TP-BTF, the bacterial groups found in the RNA study, showed again that KCM-B112 was an active group, although the most dominant bacterial groups were members from the family *Phyllobacteriacea*, followed by the genera *Nocardia* and *Baekduia*. These genera did not represent high abundances in the BTF operated anaerobically for 126 days (Fig. 6). This was associated with: i) the analysis of RNA performed in this study, that shows the active bacteria population; ii) the tension and turbulence applied to the biofilm caused by the increase of the trickling velocity and the internal gas recirculation. This second event, on the one hand weakened the biofilm favoring those bacteria that were more resistant to shear stress, and on the other hand enhanced the mass transfer of siloxanes to the bacterial community prompting the growth of those organisms involved in siloxanes degradation.

In a previous reactor operated under aerobic (124 days) conditions the main bacteria found according to their 16S rRNA gene belonged to the clade *Acidithiobacillaceae_KCMB-112* (65.3±0.2 %), and the genera *Opitutus* (7.6±0.1 %) and *Parvibaculum* (5.8±0.1 %) (Pascual et al., 2021, 2020). This study confirmed that the clade *Acidithiobacillaceae_KCMB-112* and the genera *Parvibaculum* were among the most active bacterial members under aerobic conditions and they most likely have an important role in the degradation of siloxanes. However, the dominant order Rhodospirillales, along with other active representative genera (such as *Nocardia*, *Aquamicrobium* and *Pseudoxanthomonas*) had a relative low abundance according to the DNA analysis, while they appeared as important active bacterial groups after specialization in the aerobic BTF operated according to the RNA analysis (Fig. 6).

Interestingly, some bacteria (members of *Acidithiobacillaceae* and the genus *Nocardia*) were rather active under anaerobic and aerobic conditions. Several *Acidithiobacillaceae* members are facultative anaerobes able to grow as chemolithoautotrophs in complex environments [27]. In previous works, they were recurrently present in reactors treating siloxanes, and it may be possible that they can feed on siloxanes using oxygen and nitrate as electron acceptor. In the case of *Nocardia*, this genus has been classified as a silicon utilizing bacteria [28,29]. However, all the members of *Nocardia* are strictly aerobic. Thus, their high relative abundance under anaerobic conditions is difficult to justify. Nevertheless, bacteria belonging to the family *Phyllobacteriacea,* such as the genus *Nitratireductor* [30], or the genera *Steroidobacter* [31,32],

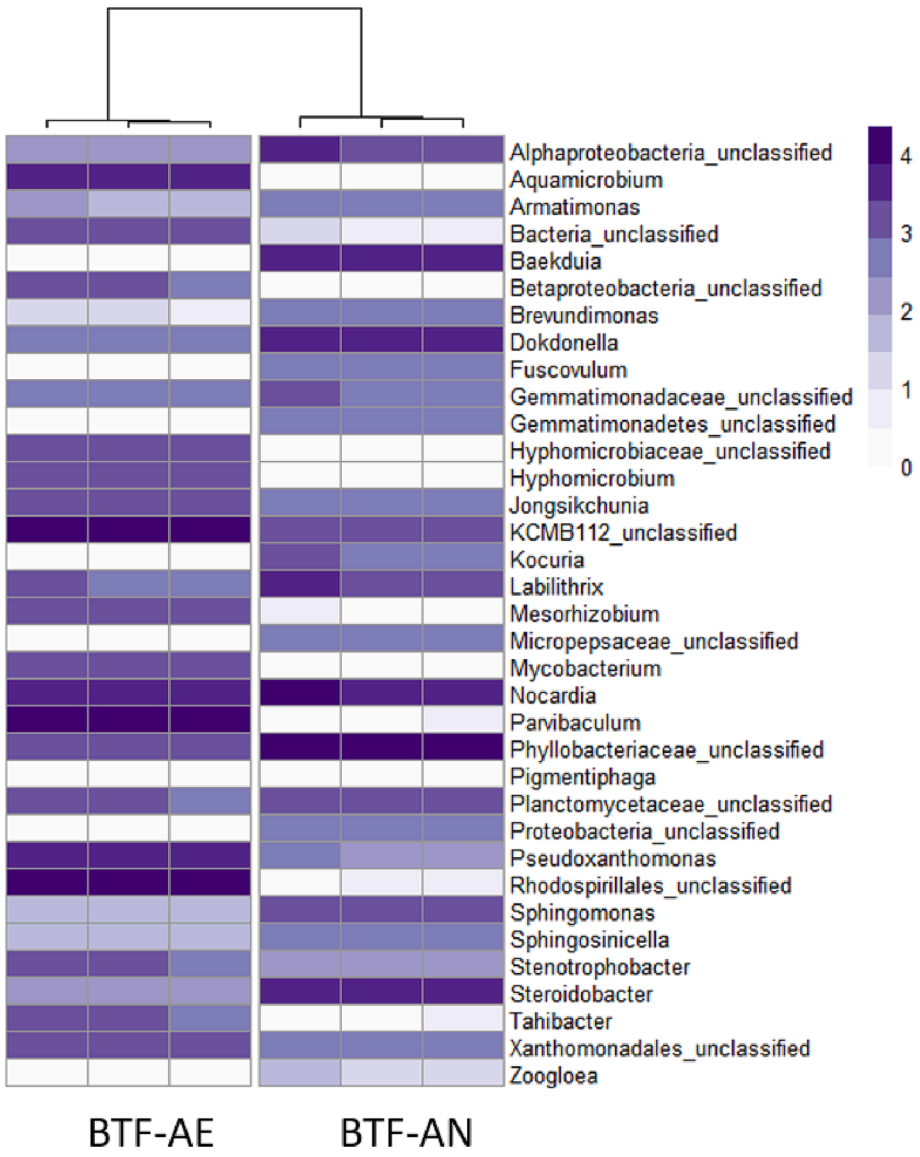


**Fig. 5.** Heat map of the most representative bacterial groups (99 % of the total genera) in the aerobic and anoxic BTFs at the end of the experiment (in triplicate). Data is presented as the log transformation of the relative abundance per sample. The dendrogram on top represents hierarchical clustering of the sample replicates.

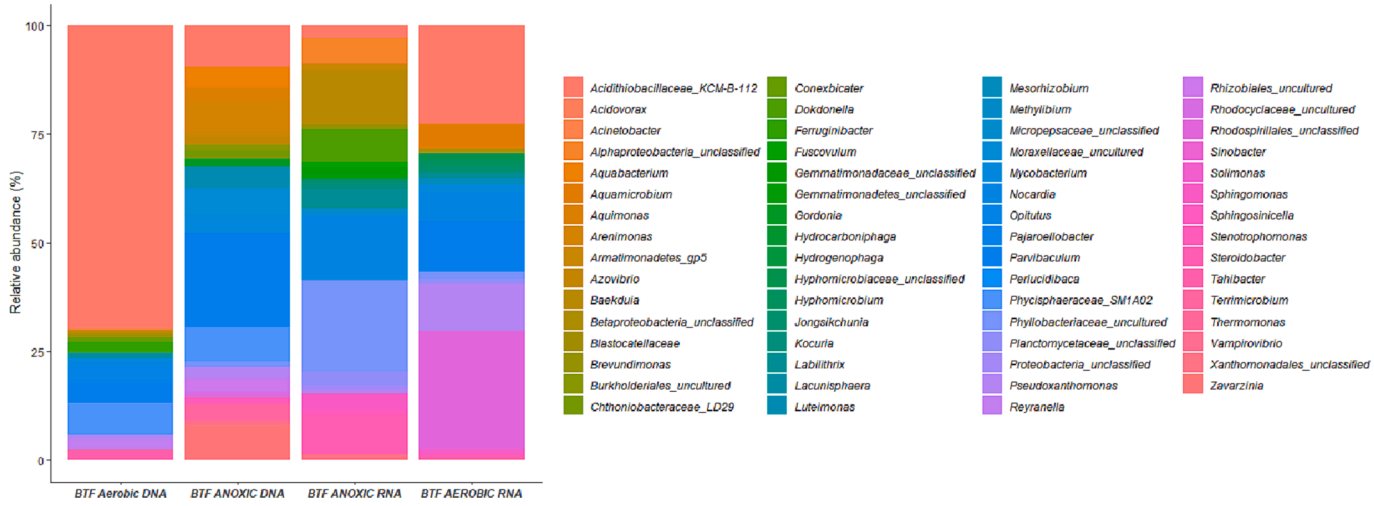


**Fig. 6.** Stacked bar graph of the total bacterial genera found in the aerobic and anaerobic BTFs when there was DNA extraction (Pascual et al. 2022) or RNA extraction (present study).

*Sphingomonas* [33] have been classified as anaerobic bacteria capable to degrade complex organic compounds. Therefore, they could be involved in the oxidation of siloxanes in anoxic conditions using the nitrate present in the culture broth. Moreover, *Aquamicrobium* has been pointed as able to degrade organic pollutants by using both nitrate and oxygen as electron acceptor. Overall, this study revealed the active bacterial population according to the transcriptome when siloxanes were the sole carbon source present.

## 4. Conclusions

The present research brought about important advances in the biodegradation of siloxanes by means of the study of the bacterial community and the implementation of enhanced mass transfer strategies. On the basis of the improvement that TP-BTF entails in the mass transfer of siloxanes, the increase in the TLV from 2 to 10 m $h^{-1}$ enhanced the performance of both anoxic and aerobic TP-BTFs from 47 to 55% and from 39 to 47%, respectively. This effect was more remarkable for the linear VMS, resulting in an increase in the removal performance of 40%. Both L2 and L3 reached similar maximum REs, around 49% and 35% in the anoxic and aerobic TP-BTF, respectively (corresponding to ECs of ~23 and 25 mg $m^{-3}h^{-1}$, respectively). However, a further increase in the TLV to 20 m $h^{-1}$ negatively affected the bacterial community, likely due to biofilm detachment, resulting in a decrease in the total RE in both TP-BTBs. An internal gas recirculation of 1 L $min^{-1}$ did not significantly improve the total VMS removal performances compared to the maximum REs previously obtained, even triggering a system deterioration when the gas recirculation was increased to 3 L $min^{-1}$. This was associated to both a lower VMS inlet concentration entering the TP-BTF and an excessive turbulence promoted in the biofilm. Overall, the highest REs were recorded for D5 under both anoxic and aerobic conditions when operating the system at a TLV of 10 m $h^{-1}$, with values of 75 % corresponding to ECs around 45 mg $m^{-3}h^{-1}$. Moreover, D5 removal was scarcely affected by the modifications implemented in the system. In addition to these findings, this paper substantially contributes to increase the knowledge of the biological degradation of siloxanes, because this is the first time that the active bacterial population of reactors treating VMS has been studied. These results pointed that some bacterial groups are actively involved in the degradation of siloxanes.

## CRediT authorship contribution statement

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

## 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.

## Data availability

No data was used for the research described in the article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2023.128851.

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