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Microalgal-bacterial consortium for the removal of volatile methylsiloxanes from biogas in a multi-channel capillary photobioreactor

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ABSTRACT

Volatile methylsiloxanes (VMS) are widely occurring biogas contaminants that hinder the performance and lifetime of energy recovery systems. Despite the feasibility of biological VMS removal, important limitations remain, and the use of microalgal-bacterial consortia has not yet been investigated. The present study presents the first assessment of the removal of seven VMS from simulated biogas using a multi-channel capillary photobioreactor (PBR). *Chlorella vulgaris* was initially used as the sole inoculum, followed by the addition of mixed recirculation sludge, VMS-enriched sludge, and the surfactant Tween 80. *C. vulgaris* provided CO_2 fixation rates up to 302 mg_{CO2} L^{-1} d^{-1} and average total VMS removal and elimination capacity of 36 ± 10% and 1142 ± 436 μg L^{-1} h^{-1}, respectively. PBR re-inoculation with sludge did not significantly improve the average VMS removal due to gas-liquid mass transfer limitations. The addition of Tween 80 increased the total VMS removal efficiency and elimination capacity to 60 ± 4% and 2136 ± 195 μg L^{-1} h^{-1}, respectively. This improvement was attributed to enhanced mass transfer of VMS such as D5 (decamethylcyclopentasiloxane) from the simulated biogas to the culture, along with a substantial increase in siloxane adsorption and accumulation in the biomass. The combination of a microalgal-bacterial consortium with a capillary reactor was proved effective for VMS removal, namely in the presence of a surfactant. These findings open new perspectives for the integration of microalgae-based systems and advances PBR designs into biogas upgrading technologies, which are essential to enable the reliable large-scale use of biogas as a renewable energy source.

1. Introduction

Population growth has led to a substantial increase in waste generation, which calls for the development of sustainable technologies capable of managing waste while producing renewable energy [1]. Biogas production through the anaerobic digestion of solid waste and sewage sludge is an interesting valorisation pathway. During anaerobic digestion, organic carbon is converted into biogas, which is mainly composed of CO_2 (15–60%) and CH_4 (40–75%). Additionally, it contains trace-level impurities such as H_2S (0.005–2%), O_2 (0–1%), N_2 (0–2%), NH_3 (<1%) and siloxanes (0–0.02%) [2,3]. These compounds can reduce the heating value of biogas (i.e. CO_2) and accumulate in gas engines, leading to mechanical damage (particularly volatile methylsiloxanes, VMS) [4]. VMS are hydrophobic organosilicon compounds with alternating silicon and oxygen atoms and methyl side chains forming either linear or cyclic structures. They are used globally in a broad spectrum of applications and pose risks to the environment and human health due to their toxic and bioaccumulative properties [5–8]. Many of them reach wastewater treatment plants (WWTPs) or landfills, where they are volatilised into biogas during anaerobic digestion. Octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) represent >90% of the total siloxanes in biogas [9]. Upon combustion, VMS are oxidised to SiO_2, which deposits on engine components and energy-generation systems, causing severe and often irreversible damage [10]. Several physical/chemical technologies are currently commercially available for biogas upgrading, but their application is often limited by high energy and chemical

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Nomenclature

$(A_{VMS}/A_{M4Q})_c$	Ratio between peak VMS and M4Q areas for the diluted culture extract, –
$(A_{VMS}/A_{M4Q})_{LB}$	Ratio between peak VMS and M4Q areas for the laboratory blank, –
a	General designation for the calibration curve slopes, –
C_c	Mass-based VMS concentration in culture samples, mg g_{VSS}^{-1}
CC_b	Carbon content in the biomass, mg_C mg^{-1}
C_{in}	VMS concentration in the inlet gas, μg L^{-1}
C_{out}	VMS concentration in the outlet gas, μg L^{-1}
DF	Dilution factor, –
EC	VMS elimination capacity from the gaseous phase, μg L^{-1} h^{-1}
IL	VMS inlet loading in the gaseous phase, μg L^{-1} h^{-1}
M_C	Carbon molecular weight, g mol^{-1}
M_{CO2}	CO_2 molecular weight, g mol^{-1}
p	p-value, –
P	Biomass productivity, mg_{VSS} L^{-1} d^{-1}
P_{avg}	Average biomass productivity, mg_{VSS} L^{-1} d^{-1}
P_d	Daily biomass productivity, mg_{VSS} L^{-1} d^{-1}
Q_g	Inlet gas flow rate, L min^{-1}
r_{CO2}	CO_2 fixation rate, mg_{CO2} L^{-1} d^{-1}
RE	VMS removal efficiency from the gaseous phase, %
t_0	Beginning of the exponential microalgal growth phase, d
t_7	End of the exponential microalgal growth phase, d
v	Ratio between the culture volume periodically exchanged with culture medium and the total culture volume, –
VSS_{t0}	Volatile suspended solids at the beginning of the exponential growth phase, g_{VSS} L^{-1}
VSS_{t7}	Volatile suspended solids at the end of the exponential growth phase, g_{VSS} L^{-1}
VSS_y	Volatile suspended solids at time y, g_{VSS} L^{-1}
VSS_z	Volatile suspended solids at time z, g_{VSS} L^{-1}
V_l	Total working volume of the photobioreactor, L
y	Time point y, d
z	Time point z, consecutive to y, d
μ	Microalgal specific growth rate, d^{-1}

Acronyms

ANOVA	Analysis of variance
ASVs	Amplicon Sequence Variants
BF	Biofilter
BTF	Biotrickling filter
C_{18}	Octadecylsilane
C-0	Negative control from the preliminary surfactant experiments
C-m	Positive control from the preliminary surfactant experiments
CMC	Critical micelle concentration
D3	Hexamethylcyclotrisiloxane
D4	Octamethylcyclotetrasiloxane
D5	Decamethylcyclopentasiloxane
DIC	Dissolved inorganic carbon
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EC	Elimination capacity
FID	Flame ionisation detector
GC–MS	Gas chromatography-mass spectrometry
$H_{G/W}$	Dimensionless Henry's law constant
IL	Inlet loading
IS	Internal standard
k_{ow}	Octanol-water partition coefficient
L2	Hexamethyldisiloxane
L3	Octamethyltrisiloxane
L4	Decamethyltetrasiloxane
L5	Dodecamethylpentasiloxane
LB	Laboratory blank
LED	Light-emitting diode
LOD	Limit of detection
M4Q	Tetrakis (trimethylsilyloxy)silane
NH_4-N	Ammonium-nitrogen
NO_3-N	Nitrate-nitrogen
PBR	Photobioreactor
PO_4-P	Phosphate-phosphorus
PSA	Primary and secondary amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RE	Removal efficiency
rpm	Rotations per minute
TCD	Thermal conductivity detector
TDC	Total dissolved carbon
TIN	Total inorganic nitrogen
TSS	Total suspended solids
VOCs	Volatile organic compounds
VMS	Volatile methylsiloxanes
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

consumption [11,12]. In this context, biological processes may represent a low-cost and environmentally friendly alternative [13].

Several studies have evaluated the removal of cyclic and linear VMS from simulated or real biogas in bioreactors such as biofilters (BFs) and biotrickling filters (BTFs). Accettola et al. [14] employed bacteria isolated from activated sludge, predominantly *Pseudomonas* spp., in an anaerobic BTF to treat an inlet air stream with hexamethylcyclotrisiloxane (D3) at 46–77 μg L^{-1}. However, the low removal efficiencies (REs, 10–20%) led the authors to highlight the need for further optimisation, particularly through strategies such as the use of surfactants and/or structured packing materials to overcome mass transfer limitations. These constraints are widely recognised as a major bottleneck in biological siloxane removal systems, and recent research has focused on addressing them by adding organic phases or surfactants. Surfactants decrease the surface tension between liquid and gaseous phases and enhance the solubility of hydrophobic volatile organic compounds (VOCs) in the liquid, improving their bioavailability [15,16]. Li et al. [17] reported enhanced REs (up to 74%) in a laboratory-scale BTF inoculated with *Pseudomonas aeruginosa* for D4 removal from an inlet air stream (20–140 μg L^{-1}). The authors attributed this improvement to the microbial production of biosurfactants detected in the liquid phase. Similarly, González-Cortés et al. [18] found that the addition of surfactants Tween 80 and QS significantly improved L3 and D4 removal in aerobic BFs inoculated with activated sludge from a municipal WWTP. For inlet concentrations of 500–1361 μg L^{-1}, the total VMS elimination capacity (EC) increased from 0.07 mg L^{-1} h^{-1} to 0.24 mg L^{-1} h^{-1} with Tween 80 and 0.43 mg L^{-1} h^{-1} with QS. These values reflected L3 and D4 REs up to 31.2% and 32.8%, respectively.

Mass transfer can be further enhanced using advanced reactors with high gas-liquid mass-transfer efficiency. In particular, capillary reactors operated under a Taylor flow regime are specifically designed for this purpose through the co-current upward flow of alternating liquid slugs and gas bubbles. They combine relatively low pressure drops with mass transfer rates per unit gas volume that are one order of magnitude higher than those achieved in traditional gas treatment reactors (e.g., BTFs and bubble columns) [19,20]. Recently, a multi-channel capillary configuration was successfully applied for the bacterial remediation of hydrophobic VOCs from contaminated air [20]. A synergistic microalgal-bacterial treatment was also explored, in which bacteria mineralise VOCs and produce CO_2, while microalgae photosynthetically fix CO_2 and provide the O_2 required for bacterial metabolism. Microalgal-bacterial co-cultures have been described as effective mitigation strategies for VOCs and CO_2 in contaminated gas streams or real biogas [21–24]. Thus, these systems may represent a promising solution for the removal of VOCs such as VMS. In this context, microalgae such as *Chlorella vulgaris* and native wastewater bacteria have been associated with the bioadsorption and bioaccumulation of siloxanes in urban effluents [25]. However, additional research is required to fully exploit the potential of microalgal-bacterial symbioses, particularly under conditions relevant to complex gas mixtures such as biogas.

The present work aimed to address the challenges associated with siloxanes in WWTPs and landfills through the use of a microalgal-bacterial consortium. The effects of microalgae, activated sludge, VMS-enriched sludge, and surfactant Tween 80 on VMS removal from simulated biogas were assessed in a multi-channel capillary photobioreactor (PBR). Although previous studies on biological VMS removal have focused solely on L2 (hexamethyldisiloxane), L3 (octamethyltrisiloxane), D3, D4, or D5, the current research also investigated L4 (decamethyltetrasiloxane) and L5 (dodecamethylpentasiloxane) due to their increasing environmental relevance [26,27]. Another novel aspect of this study was the simultaneous assessment of nutrient removal, CO_2 fixation, and quantification of siloxanes in the liquid culture to evaluate bioadsorption and bioaccumulation mechanisms. In addition, dynamic loading tests were conducted to evaluate the system's response to increased VMS inlet loads and to identify potential mass transfer limitations. Parallel experiments were also conducted to select an appropriate surfactant to enhance mass transfer. To the best of the authors' knowledge, this study represents the first evaluation of microalgae for VMS removal from a gaseous stream and the first application of a capillary reactor for siloxane abatement.

2. Materials and methods

2.1. Chemicals

All chemicals used for the preparation of culture media were acquired from Panreac (Barcelona, Spain). For VMS analysis in the liquid phase (matrix-matched calibration curves), a VMS mixture containing L2, L3, L4, L5, D3, D4, and D5 at individual concentrations of 0.5 g L^{-1} was prepared in *n*-hexane. Tetrakis(trimethylsilyloxy)silane (M4Q) was used as an internal standard (IS), and a 1 g L^{-1} solution was prepared in *n*-hexane. Stock VMS and M4Q solutions were obtained from Sigma-Aldrich (San Luis, USA) with >97% purity. Analytical grade *n*-hexane was purchased from VWR (Fontenay-sous-Bois, France). Primary and secondary amine (PSA) and octadecylsilane (C_{18}) were acquired from Supelco (Bellefonte, PA, USA), anhydrous $MgSO_4$ and the surfactant Tween 80 from Panreac (Barcelona, Spain) and the surfactant Brij 58 from Sigma-Aldrich (Dorset, UK). Synthetic biogas was simulated using a gas mixture of N_2 (70% v/v) and CO_2 (30% *v*/v) supplied by Carburos Metalicos (Sant Esteve Sesrovires, Spain). This gas composition was selected to represent the CO_2 fraction typically found in raw biogas, while avoiding methane use and ensuring safe and controlled operation. To simulate a contaminated air stream, a VMS injection mixture was prepared with 73 g L^{-1} of L2, L3, and D3, 145 g L^{-1} of D4 and L4, and 182 g L^{-1} of D5 and L5, using the stock solutions.

2.2. Inocula and culture media

Detailed cultivation conditions are described in Section I.A of the Supplementary Material for all inocula. For SI, the microalgal strain *Chlorella vulgaris* SAG 211-11b was acquired from the Culture Collection of Algae at the University of Göttingen (SAG, Germany) and cultured in a modified BG-11 medium. In parallel, mixed recirculation sludge was collected from Valladolid WWTP (Spain) and enriched with the seven target VMS. After the enrichment period, the VMS-adapted sludge was also used as inoculum for SIII.

2.3. Experimental setup and operating procedure

The capillary PBR consisted of two reservoirs connected by 25 glass capillary tubes with inner and external diameters of 2.4 and 4.4 mm, respectively, and a length of 1.5 m, corresponding to a total working volume (V_l) of 7.1 L (Fig. 1). These specifications provided a volumetric ratio between mixing and capillary zones of approximately 14. Two LED (light-emitting diode) panels (Roof 30 W CCT, Led Hispania, Madrid, Spain) were used to continuously illuminate the top and bottom reservoirs at artificial photosynthetic active radiation of 145 and 74 μmol m^{-2} s^{-1}, respectively. The capillary tubes were exposed only to indirect light to minimise biofilm growth and clogging. The liquid was continuously recirculated from the top to the bottom of the reactor at a flow rate of 2.3 L min^{-1} using a D25V plus peristaltic pump (Dinko Instruments, Barcelona, Spain). A mixture of N_2 (70% *v*/v) and CO_2 (30% v/v) was supplied to the reactor at 0.240 L min^{-1} (Q_g) using a mass flow controller (GFC17, Aalborg Instruments, Orangeburg, USA). A Legato 100 syringe pump (KD Scientific, Holliston, USA) coupled with a 5 mL 1005 RN liquid syringe (Hamilton, Nevada, USA) was used to inject the liquid mixture of seven VMS into the N_2:CO_2 gas, simulating a siloxane-contaminated biogas stream. Glass wool (Panreac, Barcelona, Spain) was placed at the injection port to stabilise VMS volatilisation and ensure uniform evaporation of the different compounds. The syringe pump was set at a velocity of 0.33 μL min^{-1} to provide theoretical concentrations of 100 μg L^{-1} of L2, L3, and D3, 200 μg L^{-1} of D4 and L4, and 250 μg L^{-1} of D5 and L5 in the inlet gas stream. The total VMS concentration (1200 μg L^{-1}) falls within the range typically reported in other studies [18,28,29], and the individual concentrations were selected based on compound volatility and chromatographic response. This stream was directed to a first mixing chamber (1 L) to promote VMS volatilisation and ensure homogenization of the inlet gas.

A compressor (H5P3 P 1, EAD pumps, Barcelona, Spain) was used to recirculate a fraction of the outlet gas at a flow rate of 6.2 L min^{-1}, regulated by a rotameter (102–16-N, Aalborg Instruments, Orangeburg, USA), which was mixed with the inlet stream in a 2-L second mixing chamber. This resulted in a total inlet gas flow rate to the PBR of 6.44 L min^{-1} and a gas residence time of approximately 30 min. Preliminary tests were performed to confirm that the selected gas and liquid flow rates provided a segmented flow within the capillary tubes, alternating gas bubbles and liquid slugs. A water condenser was installed in the recirculation line to avoid operational issues due to water condensation. The water was maintained at 14 °C using a Haake A 10 refrigerated circulator (Thermo Scientific, Waltham, USA). An Ifm Electronic PN709s7 pressure sensor (Essen, Germany) was used to monitor the pressure in the inlet, recirculating, and outlet gas streams. To enhance gas-liquid contact, scrubber packing (6 mm Kaldness K1 rings, Evolution Aqua, Wigan, UK) was placed in the bottom reservoir. The experiment was conducted at 25 °C in a temperature-controlled room.

Prior to biological operation, an abiotic test was conducted for 3 days under these conditions using only the modified alkaline BG-11 culture medium. The medium was prepared as described in Section 2.2. but with Na_2CO_3 and $NaHCO_3$ concentrations of 3.9 and 8 g L^{-1}, respectively, corresponding to a theoretical inorganic carbon concentration of 1.6 g L^{-1}. This composition promotes CO_2 mass transfer while providing significant buffering capacity [30]. Subsequently, the reactor was operated in four experimental stages: (i) SI, with *C. vulgaris* as the sole inoculum; (ii) SII, in which this microalga was cultivated in consortium with mixed recirculation sludge from a municipal WWTP; (iii) SIII, where VMS-enriched sludge was added to the PBR; and (iv) SIV, in which the effect of surfactant addition was evaluated.

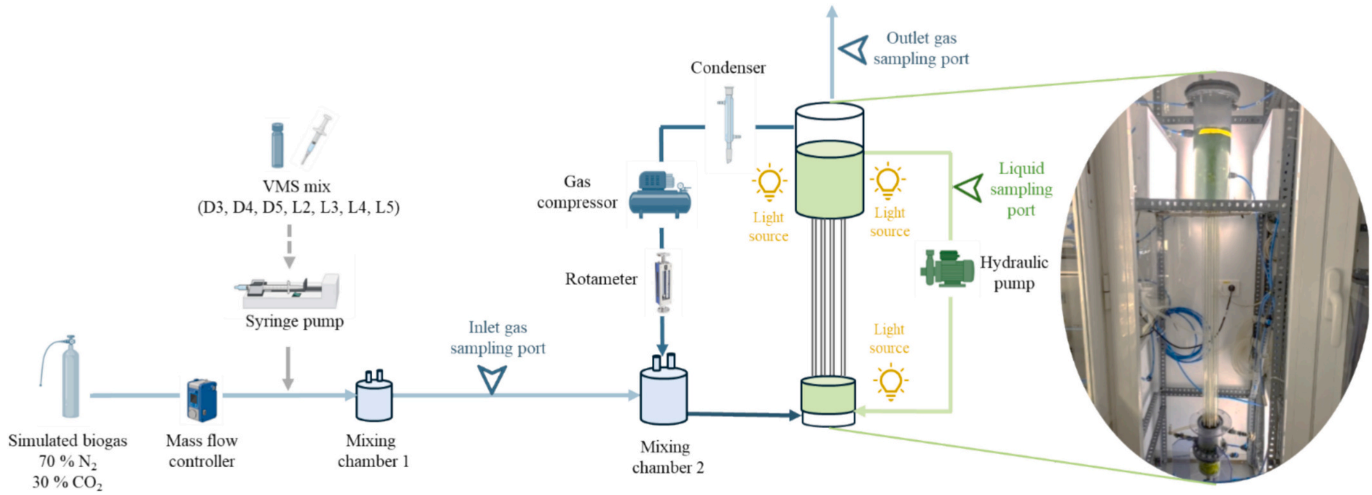

Fig. 1. Schematic representation of the experimental setup of the capillary photobioreactor system.

2.3.1. SI: Inoculation with Chlorella vulgaris

Before inoculation, the *C. vulgaris* culture was centrifuged at 4200 rpm (4122 ×*g*) for 10 min using a Sorvall X4R Pro Series centrifuge (Thermo Scientific, Waltham, USA) to obtain a biomass pellet. The pellet was resuspended in the PBR culture medium and returned to the system, thereby initiating SI with a biomass concentration of 89 mg_{VSS} L^{-1}. After 7 days of operation, aliquots of alkaline modified BG-11 medium with adjusted NH_4Cl (0.37 g L^{-1}) and K_2HPO_4 (0.056 g L^{-1}) concentrations were exchanged at a rate of 0.25 L d^{-1} until the end of the experiment. These conditions allowed maintaining the biomass concentration at approximately 1 g L^{-1}, with a dilution rate of 0.035 d^{-1} and a hydraulic retention time of 27 days. This target biomass concentration was selected to prevent clogging of the capillary tubes. The reactor was operated for a total of 25 days with *C. vulgaris* as the sole inoculum.

2.3.2. SII: Addition of mixed recirculation sludge

A volume of 100 mL of mixed recirculation sludge was centrifuged at 7000 rpm (7500 ×*g*) for 10 min (Sorvall X4R Pro Series centrifuge). The pellet was washed twice with distilled water and centrifuged under the same conditions to decrease the soluble organic carbon content. After discarding the supernatant, the pellet was resuspended in culture medium and inoculated into the reactor on day 25, increasing the biomass concentration by 115 mg L^{-1}. The reactor was operated under these conditions until day 52.

2.3.3. SIII: Addition of VMS-enriched sludge

Prior to inoculation, 100 mL of VMS-enriched sludge (Section 2.2) was centrifuged under the same conditions as in SII. The supernatant was discarded, and the pellet was resuspended in alkaline culture medium and inoculated into the reactor on day 52, increasing the biomass concentration by 220 mg L^{-1}. The reactor was subsequently operated under these conditions until day 76.

2.3.4. SIV: Addition of surfactant

Two biodegradable non-ionic surfactants, Tween 80 and Brij 58, were evaluated. Four preliminary VMS partition experiments were conducted (Fig. SM1): (i) two abiotic experiments using only modified alkaline BG-11 culture medium, one supplied with different concentrations of Tween 80 and the other with Brij 58; and (ii) two biological experiments using liquid culture withdrawn from the PBR on day 67, likewise supplied with different concentrations of each surfactant. All assays were conducted for 8 h in 250 mL sealed flasks at 25 °C under continuous agitation at 250 rpm. At the start of each experiment, the flask headspaces were flushed with gas from the PBR inlet. For each assay, two control flasks were prepared: an empty negative control (C-0) and a positive control with culture medium without surfactant (C-m). Three surfactant concentrations were tested in duplicate, based on the critical micelle concentration (CMC) of the surfactants in water: 0.5CMC, 1CMC and 1.5CMC, which corresponds to 8, 16 and 24 mg L^{-1} for Tween 80, and 45, 90 and 135 mg L^{-1} for Brij 58. VMS concentration in the headspace was analysed at the beginning and end of the experiments, as described in Section 2.4.1. Tween 80 at a concentration of 16 mg L^{-1} (1CMC) was selected for SIV and was added to the PBR on day 76. From this day onward, culture medium exchanges were performed with alkaline medium containing Tween 80 at this concentration until the end of the experiment (day 94).

2.4. Analytical procedures and sampling

2.4.1. Gas phase analysis

VMS concentrations were periodically analysed in the inlet and outlet gas streams using an Agilent 8860 gas chromatograph (Santa Clara, USA) equipped with a flame ionisation detector (FID) and an HP-5 capillary column (15 m × 0.25 mm × 0.25 μm). Detector and injector temperatures were maintained at 250 °C. Helium was used as the carrier gas at a flow rate of 3.5 mL min^{-1}, and gas samples were manually injected in splitless mode using a 500 μL Hamilton gas syringe (Reno, USA). The oven temperature was set at 40 °C for 1.5 min, then increased at 30 °C min^{-1} until 180 °C, held for 1 min and increased again at 30 °C min^{-1} until 200 °C and held for 0.5 min, resulting in a total run time of 8.33 min (see calibration curves in Table SM1). Daily inlet loading (*IL*, μg L^{-1} h^{-1}), removal efficiency (*RE*, %) and elimination capacity (*EC*, μg L^{-1} h^{-1}) were calculated for individual and total VMS according to Eqs. (1)–(3), where C_{in} and C_{out} correspond to the inlet and outlet VMS concentrations (μg L^{-1}), respectively. The total working volume of the PBR (V_l) was considered for these calculations, assuming that biological VMS removal occurred not only in the capillary tubes, but also in the upper and bottom reservoirs. The average values were determined for each operational stage as the mean of positive daily parameters.

$$IL\ (\mu g\ L^{-1}h^{-1}) = \frac{C_{in} \times Q_g \times 60}{V_l} \quad (1)$$

$$RE\ (\%) = \frac{(C_{in} - C_{out})}{C_{in}} \times 100 \quad (2)$$

$$EC\ (\mu g\ L^{-1}h^{-1}) = \frac{(C_{in} - C_{out}) \times Q_g \times 60}{V_l} \quad (3)$$

Inlet and outlet gas samples were collected using a 250 μL Hamilton gas syringe to monitor N_2, CO_2 and O_2 concentrations through manual injection into an Agilent 8860 gas chromatograph (Santa Clara, USA), coupled with a thermal conductivity detector (TCD), with the specifications described by Regueira-Marcos et al. [31]. The daily and average CO_2 REs were determined as described for VMS but based on inlet and outlet CO_2 concentrations (%*v*/v).

2.4.2. Liquid phase analysis

Culture samples were periodically collected to monitor temperature, pH, dissolved oxygen (DO), total suspended solids (TSS), volatile suspended solids (VSS), total inorganic nitrogen (TIN), total dissolved carbon (TDC), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), and phosphate-phosphorus (PO_4–P) concentrations (details provided in Section I·B of the Supplementary Material). During SI, the specific growth rate of *C. vulgaris* (μ, d^{-1}) and nutrient removal rates and efficiencies were calculated as described by Salgado et al. [32], using VSS as a proxy for biomass concentration. The average volumetric biomass productivity during the exponential growth phase, P_{avg} (mg L^{-1} d^{-1}), was determined according to Eq. (4), where VSS_{t0} and VSS_{t7} represent the VSS content (g_{VSS} L^{-1}) at the beginning (t_0, d) and at the end of the exponential growth phase (t_7, d), respectively. During semi-continuous operation in SI, daily microalgal productivities (P_d, mg_{VSS} L^{-1} d^{-1}) were determined between consecutive experimental points (*z* and *y*) as shown in Eq. (5), based on the corresponding VSS values (VSS_z and VSS_y) and the dilution factor (v), defined as the ratio between the exchanged culture volume and the total culture volume. Average and daily CO_2 fixation rates (r_{CO2}, mg_{CO2} L^{-1} d^{-1}) were calculated using Eq. (6), where *P* represents the average/daily biomass productivity (mg_{VSS} L^{-1} d^{-1}); CC_b is the carbon content in microalgal biomass (assumed to be 0.5 mg_C mg^{-1}); and M_{CO2} and M_c are the molecular weight of CO_2 and carbon, respectively (g mol^{-1}).

$$P_{avg} = \frac{(VSS_{t7} - VSS_{t0}) \times 10^3}{t_7 - t_0} \tag{4}$$

$$P_d = \frac{(VSS_z - (1 - v)VSS_y) \times 10^3}{z - y} \tag{5}$$

$$r_{CO2} = P \times CC_b \times \frac{M_{CO_2}}{M_C} \tag{6}$$

Samples were also collected to evaluate the presence of target VMS in intact and disrupted culture samples from each stage (days 23, 49, 73, and 94) through a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS)-based extraction method combined with gas chromatography–mass spectrometry (GC–MS) analysis (details provided in Section I.B of the Supplementary Material). The calibration curves were determined through a matrix-matched method for intact and disrupted culture samples (Table SM2). Limit of detection (LOD) ranges were 0.003–0.211 μg L^{-1} and 0.006–771 μg L^{-1} for intact and disrupted culture methods, respectively. These analyses were performed in a Maxisafe 2020 biosafety cabinet (Thermo Scientific, Langenselbold, Germany) and under strict practices to prevent sample contamination, as described by Ferreira et al. [33]. The siloxane mass-based concentration in culture samples, C_c (mg g_{VSS}^{-1}), was determined according to Eq. (7), where: $(A_{VMS}/A_{M4Q})_c$ is the ratio between peak VMS and M4Q areas for the diluted extract; $(A_{VMS}/A_{M4Q})_{LB}$ is the ratio between peak VMS and IS areas for the laboratory blank (LB); *VSS* is the volatile suspend solids content in the culture; *a* is the slope of the respective matrix-matched calibration curve in L μg^{-1}; and *DF* is the dilution factor (10 fold).

$$C_c = \frac{(A_{VMS}/A_{M4Q})_c - (A_{VMS}/A_{M4Q})_{LB}}{VSS \times a} \times DF \times 10^{-3} \tag{7}$$

2.5. Dynamic loading test

Dynamic loading tests were conducted in SI, SII, and SIII to evaluate the system's response to a 3-fold increase in VMS inlet loading (IL) and to assess potential mass transfer limitations. Therefore, on days 23, 49, and 73, the syringe pump velocity was increased to 0.99 μL min^{-1} for approximately 12 h and subsequently returned to the original operating conditions. During these tests, VMS concentrations at the reactor inlet and outlet were regularly monitored, and liquid samples were collected at the beginning (0 h) and end (12h) of each test to determine VMS concentrations in both intact and disrupted cultures.

2.6. Microbial community analysis

Samples were collected from the enriched sludge prior inoculation and from the PBR during SII (day 52, before inoculation) and SIV (day 94) to characterise the microbial community composition. Total genomic DNA was extracted, the 16S rRNA was amplified, and metataxonomic analysis was conducted by Novogene (Cambridge, UK) using an Illumina NovaSeq 6000 instrument (details provided in Rodríguez-Gallego et al. [34]). Raw demultiplexed sequence data and associated metadata can be found in the Sequence Read Archives (SRA) under BioProject accession number PRJNA1358825.

2.7. Statistical analysis

Average and standard deviation values were determined for each parameter. Significant differences between the parameters were assessed through one-way or two-way analysis of variance (ANOVA) with Tukey's multiple-comparison tests at a significance level of 0.05, using GraphPad Prism V. 8.0. Shapiro-Wilk tests were performed to ensure normal distribution of the data.

3. Results and discussion

3.1. Abiotic test in the photobioreactor system

Upon system start-up, the supplied CO_2 dissolved into the culture medium and was converted to HCO_3^-, releasing protons and causing a rapid decrease in pH. Thus, the initial pH of 9.9 decreased to 7.7 within the first day of operation and remained stable until the end of the abiotic test, indicating that gas-liquid equilibrium had been reached. After 2 days of operation, inlet and outlet siloxane concentrations stabilised for nearly all VMS. No removal was observed for L2, L3, D4, L4, and D5. In contrast, low REs of ~13% and 14% were detected for D3 and L5, corresponding to ECs of 27 μg_{D3} L^{-1} h^{-1} and 104 μ_{L5} L^{-1} h^{-1}. This removal can be attributed to the partial absorption of these siloxanes into the culture medium, as previously reported by Pascual et al. [29]. In addition, the delayed stabilisation of L5 concentrations may be related to its lower volatility and higher boiling point compared to other VMS. After 2–3 days of operation, no significant CO_2 removal was observed in the outlet gas, confirming the absence of biological activity.

3.2. Surfactant selection

The ratios between final (8 h) and initial VMS concentrations in the headspace were calculated for the preliminary experiments with Tween 80 and Brij 58 (Fig. SM2). A decrease in VMS concentration was observed in most experiments after 8 h, as evidenced by average ratios below 1. Higher values were generally observed for L2, D3, L3, and D4 compared to L4, L5, and D5. While the former compounds exhibit a higher tendency to remain in the gas phase, the latter were more likely to partition into the mineral medium and/or liquid culture. The VMS concentration also decreased in the control experiments, indicating their adsorption to the flasks/septa in the empty controls (C-0) and to the mineral medium or liquid culture in the absence of surfactant (C-m). The headspace concentration ratios in C-0 controls presented great variability among replicas, varying between 0.74 and 0.96, 0.77–0.97, 0.61–0.82, 0.55–0.97, 0.25–0.88, 0.21–0.90, 0.11–0.68 for L2, D3, L3, D4, L4, D5, and L5, respectively. Similarly, high variability was recorded in the C-m conditions. In the experiments with Tween 80, L2, D3, L3, D4, L4, D5, and L5 ratios ranged between 0.80 and 0.98, 0.32–0.80, 0.63–0.91, 0.56–1.1, 0.41–0.76, 0.30–0.82, and 0.12–0.46, respectively. In addition, ranges of 0.77–0.92, 0.66–0.90, 0.70–1.01, 0.72–1.29, 0.37–0.81, 0.27–0.76, and 0.04–0.45 were observed with Brij 58. The relatively high variability among duplicates under surfactant-supplemented conditions hindered the identification of statistically significant differences compared to the controls and between the two surfactants. Even though Brij 58 has previously been tested in capillary reactors and shown to promote dilute methane removal [35], Tween 80 has been more extensively studied in relation to microalgal growth and siloxane or hydrophobic compounds removal. Qin et al. [36] reported that Tween 80 concentrations between 0.125 and 1.2% (*v*/v) promoted both microalgal growth and nitrogen removal in *Chlorella pyrenoidosa* cultures. Yuan et al. [37] observed adverse effects on the growth and survival of *Scenedesmus quadricauda* and *C. vulgaris* only at concentrations exceeding 100 mg L^{-1} (over 6 times the CMC). Subashchandrabose et al. [38] demonstrated that Tween 80 at 0.001–0.005% (v/v) enhanced pyrene degradation by *Chlorella* ssp. MM3 in aqueous medium and soil slurry. In addition, González-Cortés et al. [18] reported that Tween 80 at 1 CMC improved VMS EC in a BF, while higher dosages up to 3 CMC did not further improve the removal performance. Considering the results from the preliminary experiments and the available literature, Tween 80 at a concentration of 1 CMC was selected for use in the capillary PBR (SIV).

3.3. VMS removal performance

Removal performance and fate of VMS in biological systems must be interpreted in relation to their physicochemical properties (Table 1). Volatility-related parameters, such as boiling point, saturated vapour pressure, and dimensionless Henry's law constant ($H_{G/W}$) govern the partitioning of VMS between gas and liquid phases, whereas the octanol-water coefficient (k_{OW}) reflects their lipophilicity and tendency to adsorb onto microbial biomass [39,40]. Accordingly, higher variability was observed during system operation for L4, D5, and L5 inlet concentrations compared to the remaining siloxanes (Figs. 2 and 3). Given the different volatilisation rates of the studied VMS, these less volatile compounds are more prone to remain in the liquid phase within the syringe or inlet tubing before entering the reactor, leading to fluctuations in inlet concentrations over time.

During SI, a total VMS RE of 36 ± 10% was achieved, corresponding to an EC of 1142 ± 436 μg L^{-1} h^{-1} (Table 2). Average RE and EC values followed the trend L2 < D3/L3 < D4 < L4 < D5 < L5, ranging from 10 ± 6% and 21 ± 14 μg_{L2} L^{-1} d^{-1} for L2 to 60 ± 13% and 422 ± 192 μg_{L5} L^{-1} d^{-1} for L5. The observed VMS removal trend is consistent with the combined influence of volatility and lipophilicity. Higher vapour pressure and lower k_{ow} values indicate limited gas-liquid mass transfer, reduced biomass adsorption and subsequent accumulation and degradation, respectively (Table 1). Thus, the poorer removal of compounds such as L2, D3, and L3 may be attributed to these properties, which reflect a decreased solubility in the liquid culture compared to the remaining VMS. In addition, their relatively lower inlet concentrations may further limit mass transfer, as reduced concentration gradients can hinder VMS transport between the gas and liquid phases [41]. Nonetheless, the highest RE and EC for L3 were recorded during this stage on day 14, reaching 33% and 110 μg_{L3} L^{-1} h^{-1}, respectively. The maximum EC for D4 was also recorded during SI (243 μg_{D4} L^{-1} h^{-1}), driven by a punctual elevated inlet concentration on the second day of operation.

The removals achieved during SI were in accordance with those reported in previous studies without the addition of mass transfer-enhancing compounds (e.g., organic phases or surfactants), despite the higher overall IL due to the simultaneous feed of seven VMS at comparable individual concentrations. For instance, Pascual et al. [29] operated a BTF inoculated with enriched activated sludge for the removal of L2, L3, D4, and D5 from a contaminated air stream. For an average total VMS IL of 0.51 mg L^{-1} h^{-1} and inlet concentration of 515 μg L^{-1}, the authors reported total siloxane RE and EC of 19.1 ± 13.3% and 0.10 ± 0.08 mg L^{-1} h^{-1}, respectively. Increasing the total VMS inlet concentration to values comparable to the present study (1288 ± 217 μg L^{-1}) did not significantly improve the RE but increased the EC to 0.16 ± 0.16 mg L^{-1} h^{-1}. Individual L2, D3, D4, and D5 REs remained below 20% under these conditions. Identically to the present study, L2 showed the lowest removal parameters, primarily attributed to its low vapour pressure. In contrast, a higher average D5 RE is reported here (43 ± 11%).

Results from the dynamic loading tests in SI (section 3.5) showed compound-dependent limitations, and the removal of some VMS was not

Table 1
Summary of the physical and chemical properties of linear and cyclic VMS.

Compound Cas no./Chemical formula	Water solubility at 25 °C (mg L^{-1})[a]	Molecular weight (g mol^{-1})[b]	Boiling point (°C)[b]	Saturated vapour pressure at 25 °C (Pa)[b]	Log k_{OW} at 25 °C[c,d]	$H_{G/W}$[e,f]
Hexamethyldisiloxane (L2) 107-46-0/$C_6H_{18}OSi_2$	0.93	162.4	106.9	5626.2	4.2	397
Octamethyltrisiloxane (L3) 107-51-7/$C_8H_{24}O_2Si_3$	0.034	236.5	153.0	445.0	4.8	1465
Decamethyltetrasiloxane (L4) 141-62-8/$C_{10}H_{30}O_3Si_4$	0.00674	310.7	194.0	50.0	5.4	943
Dodecamethylpentasiloxane (L5) 141-63-9/$C_{12}H_{36}O_4Si_5$	0.000309	384.8	232.0	9.0	6.0	nd
Hexamethylcyclotrisiloxane (D3) 541-05-9/$C_6H_{18}O_3Si_3$	1.560	222.5	135.2	471.0	3.85	72
Octamethylcyclotetrasiloxane (D4) 556-67-2/$C_8H_{24}O_4Si_4$	0.056	296.6	175.7	132.0	4.45	259
Decamethylcyclopentasiloxane (D5) 541-02-6/$C_{10}H_{30}O_5Si_5$	0.017	370.8	211.2	23.2	5.2	185

$H_{G/W}$: dimensionless Henry's law constant (gas-water partition coefficient); k_{OW}: octanol-water partition coefficient; nd: not determined.
[a] de Arespacochaga et al. [58].
[b] Pascual et al. [29].
[c] Meyers et al. [59].
[d] Oshita et al. [60].
[e] Kochetkov et al. [61].
[f] Pascual et al. [44].

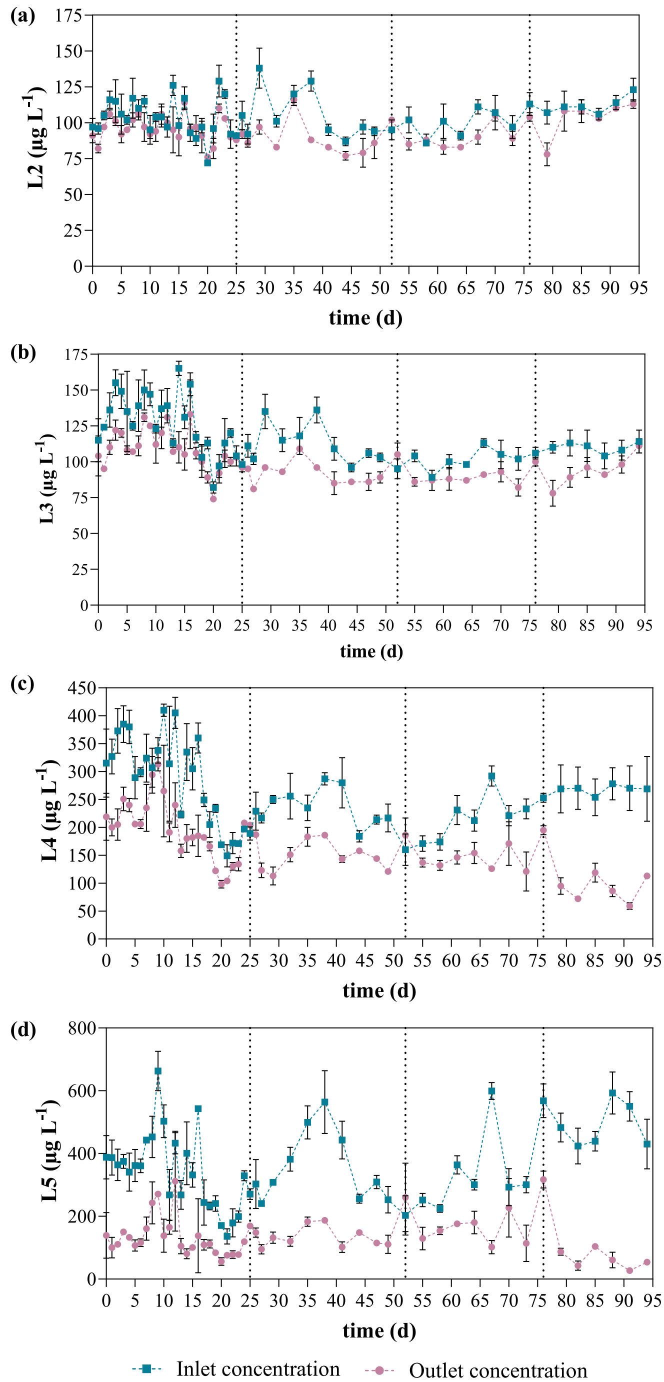

Fig. 2. Time-course evolution of linear siloxanes L2 (a), L3 (b), L4 (c), and L5 (d) concentrations in the inlet and outlet gas from the capillary photobioreactor in SI (days 0–25), SII (days 25–52), SIII (days 52–76), and SIV (days 76–94).

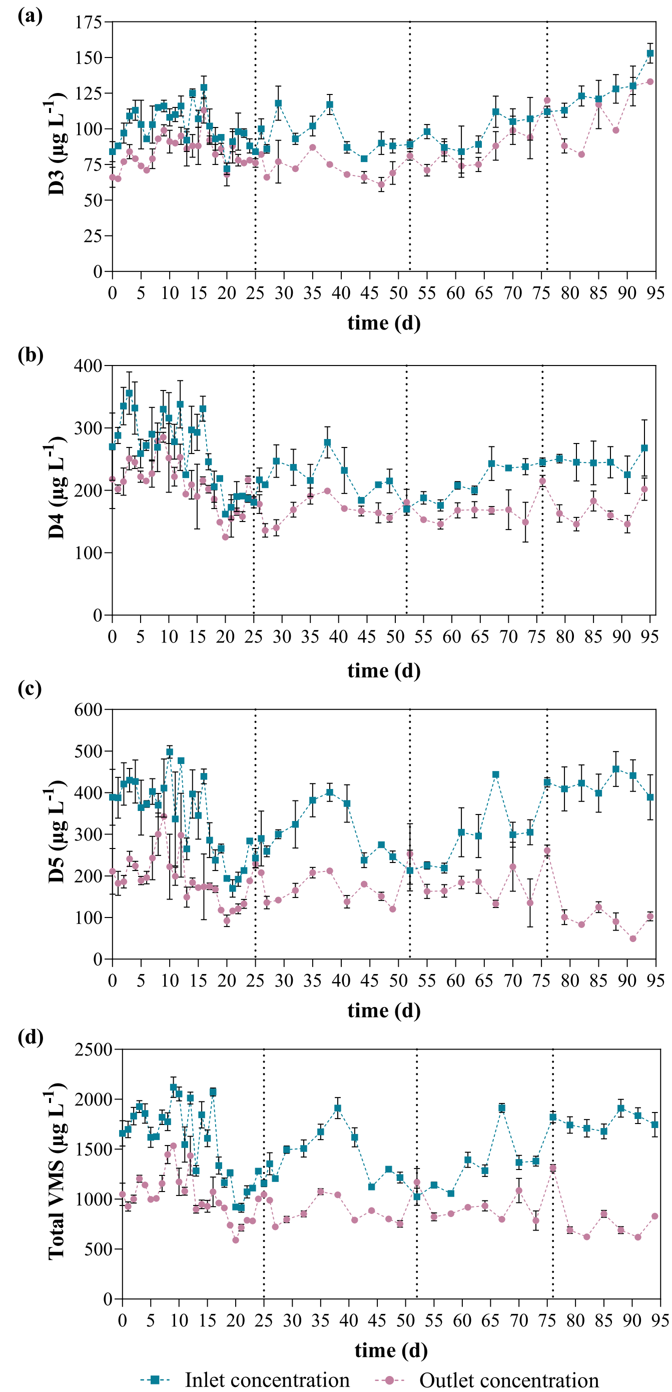

Fig. 3. Time-course evolution of cyclic siloxanes D3 (a), D4 (b), and D5 (c), and total VMS (d) concentrations in the inlet and outlet gas from the capillary photobioreactor in SI (days 0–25), SII (days 25–52), SIII (days 52–76), and SIV (days 76–94).

purely mass-transfer driven. Therefore, re-inoculation of the PBR was conducted in SII and SIII to increase microbial diversity and broaden the metabolic potential for siloxane transformation and degradation. Incorporating mixed recirculation sludge into the PBR did not significantly enhance the average siloxane removal performance in SII compared to SI ($p > 0.05$) and previous biological removal studies. During this stage, total VMS RE and EC were 39 ± 7% and 1148 ± 299 μg L^{-1} h^{-1}, respectively. These parameters ranged between 15 ± 9% and 35 ± 27 μg_{L2} L^{-1} h^{1} for L2 and 61 ± 10% and 447 ± 192 μg_{L5} L^{-1} h^{-1} for L5 (Table 2). Nevertheless, the maximum RE values for L2, D3, and D4 were registered during this stage, reaching 32% (day 38), 36% (day 38), and 43% (day 29), respectively. Similarly, the maximum EC values for L2 and D3 were observed on day 38, reaching 83 μg_{L2} L^{-1} h^{-1} and 84 μg_{D3} L^{-1} h^{-1}, respectively.

The subsequent addition of enriched sludge in SIII also did not significantly improve average gas-phase VMS removal compared to the earlier stages ($p > 0.05$) or results from previous studies. Total VMS RE and EC during SIII were 34 ± 10% and 980 ± 392 μg L^{-1} h^{-1}, respectively. As in earlier stages, the lowest RE and EC values were observed for L2 (11 ± 6% and 24 ± 14 μg_{L2} L^{-1} h^{-1}), whereas the highest values corresponded to L5 (48 ± 19% and 380 ± 282 μg_{L5} L^{-1} h^{-1}).

Table 2
Average VMS inlet concentration ($C_{in,avg}$), inlet loading (IL_{avg}), elimination capacity (EC_{avg}), and removal efficiency (RE_{avg}) at each stage in the capillary photobioreactor. Statistical differences were assessed across stages using one-way ANOVA followed by Tukey's multiple-comparison test. For each VMS and parameter ($C_{in,avg}$, IL_{avg}, EC_{avg}, and RE_{avg}), values sharing at least one common letter (a, b) are not statistically different across stages ($p > 0.05$).

VMS	Stage	$C_{in,avg}$ (μg L^{-1})	IL_{avg} (μg L^{-1} h^{-1})	EC_{avg} (μg L^{-1} h^{-1})	RE_{avg} (%)
L2	I	107 ± 11^{a}	216 ± 23^{a}	21 ± 14^{a}	10 ± 6^{a}
	II	106 ± 17^{a}	214 ± 35^{a}	35 ± 27^{a}	15 ± 9^{a}
	III	103 ± 8^{a}	209 ± 16^{a}	24 ± 14^{a}	11 ± 6^{a}
	IV	112 ± 6^{a}	226 ± 12^{a}	18 ± 21^{a}	8 ± 10^{a}
D3	I	101 ± 13^{a}	204 ± 27^{a}	36 ± 17^{a}	17 ± 8^{a}
	II	95 ± 13^{a}	192 ± 25^{a}	44 ± 22^{a}	22 ± 9^{a}
	III	97 ± 11^{a}	196 ± 22^{a}	28 ± 18^{a}	14 ± 8^{a}
	IV	127 ± 15^{b}	258 ± 31^{b}	48 ± 28^{a}	19 ± 11^{a}
L3	I	126 ± 11^{a}	255 ± 42^{a}	36 ± 24^{a}	13 ± 8^{a}
	II	$113 \pm 14^{a,b}$	$228 \pm 28^{a,b}$	43 ± 22^{a}	18 ± 7^{a}
	III	102 ± 7^{b}	206 ± 14^{b}	25 ± 14^{a}	12 ± 6^{a}
	IV	$110 \pm 4^{a,b}$	$222 \pm 7^{a,b}$	32 ± 21^{a}	15 ± 9^{a}
D4	I	270 ± 58^{a}	544 ± 118^{a}	127 ± 63^{a}	22 ± 8^{a}
	II	224 ± 25^{b}	453 ± 51^{b}	116 ± 54^{a}	$25 \pm 10^{a,b}$
	III	217 ± 27^{b}	438 ± 55^{b}	100 ± 48^{a}	$22 \pm 9^{a,b}$
	IV	$246 \pm 14^{a,b}$	$498 \pm 28^{a,b}$	161 ± 29^{a}	33 ± 6^{b}
L4	I	293 ± 79^{a}	592 ± 160^{a}	196 ± 99^{a}	32 ± 12^{a}
	II	$237 \pm 32^{a,b}$	$479 \pm 64^{a,b}$	174 ± 77^{a}	35 ± 14^{a}
	III	223 ± 40^{b}	451 ± 80^{b}	153 ± 90^{a}	32 ± 14^{a}
	IV	$268 \pm 8^{a,b}$	$542 \pm 16^{a,b}$	359 ± 57^{b}	66 ± 9^{b}
D5	I	$343 \pm 94^{a,b}$	$693 \pm 189^{a,b}$	303 ± 130^{a}	43 ± 11^{a}
	II	309 ± 59^{a}	624 ± 119^{a}	289 ± 105^{a}	45 ± 11^{a}
	III	$315 \pm 82^{a,b}$	$636 \pm 165^{a,b}$	270 ± 169^{a}	40 ± 16^{a}
	IV	420 ± 26^{b}	848 ± 52^{b}	662 ± 94^{b}	78 ± 7^{b}
L5	I	342 ± 123^{a}	691 ± 248^{a}	422 ± 192^{a}	60 ± 13^{a}
	II	$356 \pm 112^{a,b}$	$718 \pm 226^{a,b}$	447 ± 192^{a}	61 ± 10^{a}
	III	$363 \pm 142^{a,b}$	$733 \pm 287^{a,b}$	380 ± 282^{a}	48 ± 19^{a}
	IV	487 ± 70^{b}	983 ± 142^{b}	856 ± 167^{b}	87 ± 7^{b}
Total	I	$1582 \pm 185^{a,b}$	$3194 \pm 374^{a,b}$	1142 ± 436^{a}	36 ± 10^{a}
	II	1440 ± 135^{a}	2909 ± 273^{a}	1148 ± 299^{a}	39 ± 7^{a}
	III	1420 ± 172^{a}	2868 ± 347^{a}	980 ± 392^{a}	34 ± 10^{a}
	IV	1770 ± 78^{b}	3575 ± 158^{b}	2136 ± 195^{b}	60 ± 4^{b}

D3: hexamethylcyclotrisiloxane; D4: octamethylcyclotetrasiloxane; D5: decamethylcyclopentasiloxane; L2: Hexamethyldisiloxane; L3: octamethyltrisiloxane; L4: decamethyltetrasiloxane; L5: dodecamethylpentasiloxane; VMS: volatile methylsiloxanes.

Although re-inoculation in SII and SIII was intended to enhance biodegradation capacity, results from the dynamic loading test performed in SIII indicated that gas-liquid mass transfer remained a limiting factor for specific VMS, particularly the less volatile compounds (section 3.5). The competition between dominant bacterial genera involved in symbiotic interactions with microalgae and commonly reported VMS-degraders could be another factor influencing these results (Section 3.7). Based on these observations, the addition of a surfactant was proposed as a strategy to mitigate mass transfer limitations and enhance VMS availability to the microalgal-bacterial culture. Accordingly, the introduction of Tween 80 in SIV resulted in a marked improvement in removal performance, with an average total VMS RE of 60 ± 4% (1.8-fold increase compared to SIII), which corresponds to an EC of 2136 ± 195 μg L^{-1} h^{-1} (2.1-fold increase). Average RE values for L4, D5, and L5 increased to 66 ± 9%, 78 ± 7%, and 87 ± 7%, respectively, while EC values reached 359 ± 57 μg_{L4} L^{-1} h^{-1}, 662 ± 94 μg_{D5} L^{-1} h^{-1}, and 856 ± 167 μg_{L5} L^{-1} h^{-1} ($p < 0.05$). Peak removal values for these compounds were also recorded during SIV, with maximum RE and EC values of 78% and 426 μg_{L4} L^{-1} h^{-1} (day 91) for L4, 89% and 790 μg_{D5} L^{-1} h^{-1} (day 91) for D5, and 95% (day 91) and 1074 μg_{L5} L^{-1} h^{-1} (day 88) for L5. Given that D5 is frequently reported as one of the dominant siloxanes in biogas, its removal up to nearly 90% is particularly relevant and indicates that the proposed approach may contribute to mitigate the broader challenge of VMS contamination.

The findings from SIV are consistent with those reported by González-Cortés et al. [18], who observed a significant increase in siloxane removal following the addition of Tween 80 (1 CMC) to an aerobic BF packed with woodchips and compost and inoculated with activated sludge. At ILs of 0.63–0.67 mg_{L3} L^{-1} h^{-1} and 0.82–0.89 mg_{D4} L^{-1} h^{-1}, the EC increased from 0.04 ± 0.01 mg_{L3} L^{-1} h^{-1} (6.2 ± 1.9%) and 0.07 ± 0.02 mg_{D4} L^{-1} h^{-1} (9.1 ± 2.2%) to 0.13 ± 0.00 mg_{L3} L^{-1} h^{-1} (19.5 ± 1.1%) and 0.19 ± 0.02 mg_{D4} L^{-1} h^{-1} (21.0 ± 0.3%). Moreover, the total VMS EC increased more than threefold, from 0.07 ± 0.04 mg L^{-1} h^{-1} to 0.24 ± 0.08 mg L^{-1} h^{-1}. Thus, the present study achieved comparable L3 and D4 removal parameters for lower ILs. Similarly, Pascual et al. [29] proposed an innovative two-phase biotrickling filter (TP-BTF) with silicon oil as the organic phase for the removal of L2, L3, D4, and D5 from a contaminated atmospheric air stream with a total VMS concentration of 625–651 μg L^{-1} (IL of 0.62–0.64 mg L^{-1} h^{-1}). Consistent with the present study, the total VMS RE increased from approximately 20% in the conventional BTF to 70% in the presence of a mass transfer-enhancing phase. Individual L2, L3, D4, and D5 REs ranged 20–60%, 71–78%, 82–91%, and 80–88%, corresponding to ECs of 0.02–0.15, 0.11–0.12, 0.14–0.17, and 0.12–0.17 g m^{-3} h^{-1}, respectively. The higher L2 and L3 removal values in this bacterial TP-BFT indicate a greater capacity to remove highly volatile compounds compared with the microalgal-bacterial capillary system.

While the present work achieved total VMS REs comparable to those reported in other biological systems (usually up to ~70%), conventional technologies such as adsorption using activated carbon can achieve significantly higher values (90–99%). Although adsorption is the most well-established alternative, it presents some drawbacks, such as pore blockage caused by siloxane polymerisation, which hinders effective adsorbent regeneration and increases operational costs. Another disadvantage is the non-selective and competing adsorption between different VMS and other gas impurities [42,43]. Considering the limitations of both approaches, recent studies suggested combining biological and adsorption technologies, using activated carbon as a packing material for BFs or BTFs, which allowed L2, L3, D3, D4, and D5 removals ranging from 80 to 100% [28,44]. Nonetheless, these reactors rely on packed beds, which have some limitations such as mass-transfer constraints and increased pressure drop after long-term operation due to biofilm overgrowth and pore size reduction [42,45]. Conversely, multi-channel capillary reactors are specifically designed to increase mass-transfer at a relatively low pressure drop. Thus, under optimal conditions and with a specialised microalgal-bacterial consortium, this system could address the limitations of current biological methods and provide superior removal performance.

3.4. VMS fate within the photobioreactor system

Steady-state VMS concentrations in intact and disrupted culture samples generally followed the same trend observed for gas-phase removal (Table 3), consistent with both the compounds' inlet concentrations and their physicochemical properties (Table 1). Thus, the highest overall concentrations were obtained for the compounds with the lowest vapour pressure and highest k_{ow}, namely D5 and L5, and vice-versa. Most of the target VMS were detected in intact culture samples across the four stages, confirming siloxane adsorption to the culture medium and/or cells (bioadsorption). The latter mechanism is a passive process in which compounds are adsorbed to the cell wall components or to organic substances like extracellular polymeric substances [46]. Higher VMS concentrations were detected in disrupted biomass compared to intact culture samples, indicating intracellular uptake (bioaccumulation) without necessarily fast conversion. Bioaccumulation can be defined as the active transport of a pollutant through the cell wall into the cell and consequent binding to intracellular proteins [39,46]. Statistically significant differences were compound- and stage-dependent. Across all stages, concentrations of L2, D3, and D5 were significantly higher in disrupted samples ($p < 0.05$), whereas no significant differences were found for L3 ($p > 0.05$).

Table 3
Steady-state concentrations of L2, D3, L3, D4, L4, D5, L5, and total VMSs in intact ($C_{c,int}$) and disrupted ($C_{c,drp}$) culture samples from Stages I, II, III, and IV. Statistical differences were assessed across stages and culture type using a two-way ANOVA followed by Tukey's multiple-comparison test. For each VMS, values sharing at least one common letter (a-g) are not statistically different ($p >$ 0.05).

VMS	Stage	Day	$C_{c,int}$ (mg g_{VSS}^{-1})	$C_{c,drp}$ (mg g_{VSS}^{-1})
L2	I	23	0.0004*,a	0.058 ± 0.008b
	II	49	0.0002*,a	0.025 ± 0.002c
	III	73	0.0005*,a	0.024 ± 0.005 c
	IV	94	0.009 ± 0.001a	0.028 ± 0.007c
D3	I	23	0.03 ± 0.01a	0.19 ± 0.02b
	II	49	0.07 ± 0.03a,c	0.19 ± 0.03b
	III	73	0.10 ± 0.01c	0.20 ± 0.01b
	IV	94	0.21 ± 0.01b	0.29 ± 0.01d
L3	I	23	0.02 ± 0.01a	0.03 ± 0.001a
	II	49	0.02 ± 0.01a	0.04 ± 0.01a,b
	III	73	0.09 ± 0.01b,c	0.13 ± 0.01c
	IV	94	0.72 ± 0.01d	0.71 ± 0.05d
D4	I	23	0.43 ± 0.01a	0.68 ± 0.03a
	II	49	0.38 ± 0.03a	0.63 ± 0.02a
	III	73	1.1 ± 0.1b	1.61 ± 0.07c
	IV	94	6.48 ± 0.03d	6.7 ± 0.3d
L4	I	23	0.51 ± 0.01a	0.96 ± 0.05b,c
	II	49	0.65 ± 0.03a,b	1.04 ± 0.01c
	III	73	2.4 ± 0.3d	3.09 ± 0.09e
	IV	94	17.4 ± 0.1f	17.4 ± 0.2f
D5	I	23	1.37 ± 0.01a	2.50 ± 0.07b,c
	II	49	2.0 ± 0.2a,b	3.15 ± 0.04c
	III	73	6.5 ± 0.8d	8.39 ± 0.07e
	IV	94	36.6 ± 0.1f	37.9 ± 0.2g
L5	I	23	3.09 ± 0.01a	3.64 ± 0.06a,b
	II	49	5.2 ± 0.3b,c	6.06 ± 0.01c
	III	73	15 ± 2d	17.0 ± 0.3d
	IV	94	61.6 ± 0.4e	64.5 ± 0.3f

* Values corresponding to the limit of detection. D3: hexamethylcyclotrisiloxane; D4: octamethylcyclotetrasiloxane; D5: decamethylcyclopentasiloxane; L2: Hexamethyldisiloxane; L3: octamethyltrisiloxane; L4: decamethyltetrasiloxane; L5: dodecamethylpentasiloxane; VMS: volatile methylsiloxanes.

During SI, L2 was not detected in intact samples, and measured concentrations ranged from 0.02 ± 0.01 mg_{L3} g_{VSS}^{-1} to 3.09 ± 0.01 mg_{L5} g_{VSS}^{-1}. In disrupted biomass, concentrations ranged from 0.058 ± 0.008 mg_{L2} g_{VSS}^{-1} to 3.64 ± 0.06 mg_{L5} g_{VSS}^{-1}. In addition to L2, D3, and D5, L4 levels were significantly higher in disrupted samples during SI (p = 0.005). In contrast, no significant differences were observed for L3, D4 and L5 between intact and disrupted samples ($p = 0.167$ and $p = 0.961$, respectively). Culture samples from SII followed a similar trend. The detected values in intact samples varied between 0.02 ± 0.01 mg_{L3} g_{VSS}^{-1} and 5.2 ± 0.3 mg_{L5} g_{VSS}^{-1}, with L2 below the LOD. Disrupted samples values fell between 0.025 ± 0.002 mg_{L2} g_{VSS}^{-1} and 6.06 ± 0.01 mg_{L5} g_{VSS}^{-1}. The key difference from the SI was the higher L5 concentrations in both intact and disrupted samples ($p = 0.024$ and $p = 0.009$, respectively). These results suggest improved L5 adsorption associated with sludge addition, despite no significant enhancement in gas-phase siloxane removal. In SIII, L2 was again not detected in intact samples, and the measured values ranged from 0.09 ± 0.01 mg_{L3} g_{VSS}^{-1} to 15 ± 2 mg_{L5} g_{VSS}^{-1}. Disrupted samples exhibited concentrations between 0.024 ± 0.005 mg_{L2} g_{VSS}^{-1} and 17.0 ± 0.3 mg_{L5} g_{VSS}^{-1}. The most notable differences from previous stages were the bioaccumulation of D4 (higher concentration in the disrupted culture, $p = 0.0006$) and the increased L3, D4, L4, D5, and L5 levels ($p < 0.05$) in both types of samples. Although part of this increase could be attributed to VMS carried over with the inoculum, the observed concentrations exceeded theoretical estimates based solely on inoculum addition (maximum increase in VMS concentration of 0.28 mg_{L2} g_{VSS}^{-1}, 1.85 mg_{D3} g_{VSS}^{-1}, 0.28 mg_{L3} g_{VSS}^{-1}, 3.70 mg_{D4} g_{VSS}^{-1}, 0.57 mg_{L4} g_{VSS}^{-1}, 0.71 mg_{D5} g_{VSS}^{-1}, and 0.71 mg_{L5} g_{VSS}^{-1}), indicating enhanced adsorption/accumulation of these compounds in the PBR.

In SIV, a wider range of VMS concentrations was observed in intact and disrupted samples, ranging from 0.009 ± 0.001 and 0.028 ± 0.007 mg_{L2} g_{VSS}^{-1} to 61.6 ± 0.4 and 64.5 ± 0.3 mg_{L5} g_{VSS}^{-1}, respectively. Most VMS were present at significantly higher concentrations than in previous stages ($p < 0.05$), indicating a substantial improvement in VMS adsorption/accumulation with the addition of Tween 80. Notably, SIV was the only stage in which significant L5 bioaccumulation was detected (higher concentration in disrupted samples, $p = 0021$). Together, these findings confirm that Tween 80 promoted siloxanes mass transfer from the simulated biogas to the microalgal-bacterial culture, leading to the higher removal performance observed for L4, L5, D5, and total VMS. Conversely, removal of L2, D3, L3, and D4 during SIV may have been biologically limited, with mass transfer no longer representing the primary bottleneck.

3.5. Dynamic loading tests

Dynamic loading tests are useful for elucidating between mass transfer and biological limitations during VMS removal. When removal is biologically limited, a step increase in inlet concentration results in a stable EC, whereas under mass transfer limitation, an increase in the concentration gradient leads to a proportional increase in EC [47]. During the dynamic loading test in SI (Fig. 4), the increase in the syringe pump flow rate resulted in average fold increases in the IL of approximately 2.4–2.6 for L2, D3, L3, D4 and L4, 2.1 for D5, and 1.5 for L5 once steady-state was reached. Due to their lower volatility, stabilisation of inlet concentrations was slower for D5 and L5 (~10 h) compared to the remaining compounds (between 3.5 and 5 h). The results indicated that L2 removal was limited by biological activity, as the EC did not increase proportionally to the IL. Similarly, the EC of L3 increased only 1.5-fold after 12 h, despite a larger IL increase, suggesting a combined mass transfer with biological limitation. In contrast, the EC of the remaining VMS increased proportionally with IL, achieving values of ×2.2 for D3, ×4.3 for D4, ×5.6 for L4, ×3.3 for D5 and 1.6 for L5 after 12 h, indicating mass transfer limitation. Following restoration of the original inlet conditions, IL and EC values decreased to previous steady-state values within 48 h.

Similar conclusions were drawn from the tests in SII and SIII (Figs. SM3 and SM4, respectively). In SII, steady-state fold IL increases ranged from approximately 1.1 for L5 to 3.0 for L2. After 12 h, the EC increased by a factor of 1.9 for L2, 3.4 for D3, 2.9 for L3, 3.0 for D4, 2.4 for L4, 2.2 for D5 and 1.2 for L5, indicating that L2 was also affected by a combination of mass transfer and biological limitations during this stage. In SIII, IL exhibited greater variability after 8–12 h, with fold increases between 1.3 for L5 and 3.2 for L2. Nonetheless, a clear proportional increase in EC was observed for all VMS, confirming persistent mass transfer limitation. The EC increased by factors of 5.1 for L2, 7.0 for D3, 2.3 for L3, 2.7 for D4, 3.5 for L4, 2.8 for D5, and 2.2 for L5 after 10.5–12 h.

VMS measurements in intact and disrupted culture samples (Fig. 5) provided further insight into the mechanisms underlying the gas-phase observations, namely bioadsorption and bioaccumulation. Biodegradation was inferred as a VMS removal pathway when the EC increased proportionally to the IL, but no corresponding increase in liquid-phase concentrations was detected. This pathway can be defined as the metabolic breakdown of compounds into simpler molecules, either by catabolic enzymes or by their usage as a carbon source [48].

L2 concentrations were below the LOD before and after the step increase in intact cultures across all stages, likely due to its low tendency to adsorb to biomass compared to the remaining siloxanes. However, in SI and SII, L2 levels in disrupted samples increased significantly ($p <$ 0.05) after 12 h. These results support the biological limitation inferred from the gas phase data and suggest bioaccumulation as a mechanism to cope with the increased IL. In contrast, L2 levels remained stable in disrupted biomass after the step increase in SIII, consistent with the

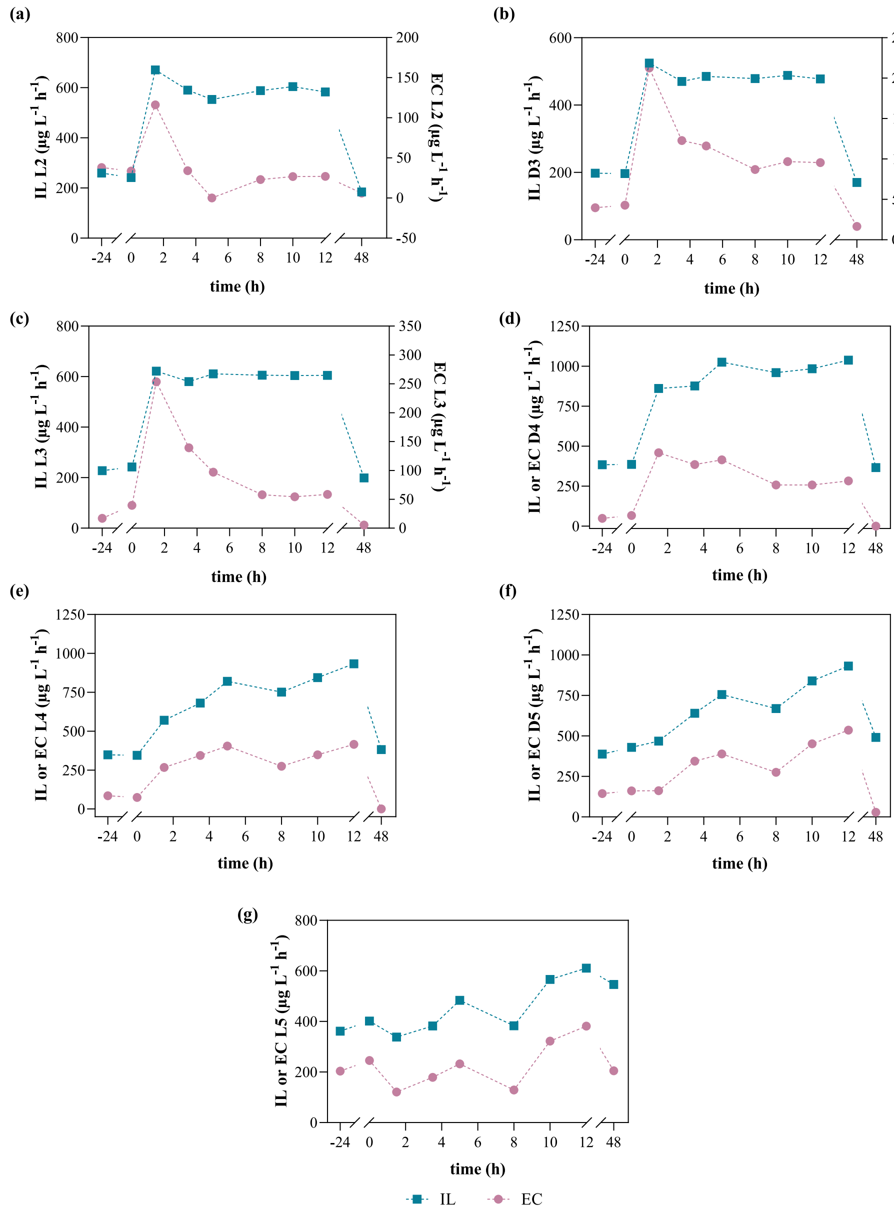

Fig. 4. Inlet loading (*IL*) and elimination capacity (*EC*) for L2 (a), D3 (b), L3 (c), D4 (d), L4 (e), D5 (f), and L5 (g), in the dynamic loading test performed during stage I (day 23).

proportional EC increase and indicating L2 biodegradation limited by mass transfer.

For the remaining VMS, D3, L4, and D5 levels were consistently higher ($p < 0.05$) in all disrupted samples than in intact cultures, both before and after the step increase, indicating significant VMS bioaccumulation. This behaviour was only observed for L3 and D4 after the step increase in SII and SIII, and for L5 after 12 h in SIII. During SI, no significant differences were detected between VMS levels at beginning and end of the test ($p > 0.05$), indicating that the observed increase in EC was primarily associated with degradation rather than additional accumulation in the culture. In SII, this behaviour was maintained for D4, L4, D5, and L5, whereas L3 concentrations increased by factors of 2.8 and 2.7 in intact and disrupted cultures, respectively. In SIII, D3, L3, and D4 concentrations increased 1.4, 2.3, and 1.5-fold, respectively, in the intact culture. In the disrupted biomass, L3, D4, and L4 concentrations increased by a factor of 2.3, 1.4, and 1.2, respectively. In contrast, D5 and L5 concentrations remained stable before and after the step increase, as observed in the previous stages. In combination with the observed increases in EC and RE, these results suggest that D5 and L5 were likely degraded during all three dynamic loading tests.

Even though a dynamic loading test was not performed in SIV, the addition of Tween 80 appeared to mitigate mass transfer limitations previously identified for L4, D5, and L5, as evidenced by their higher EC, RE, and *Cc*.

3.6. Biomass growth and nutrients dynamics

Temperature, pH, TDC, and DIC remained constant throughout all stages, with average values of 24 ± 1 °C, 8.0 ± 0.3, 1.9 ± 0.1 g_C L^{-1}, and 1.9 ± 0.1 g_C L^{-1}, respectively. The stable pH evidences the strong buffering capacity of the culture medium, which compensates for the regeneration of carbonate species during microalgal growth and supports a high CO_2 mass transfer [49]. Constant DIC values were observed because the high CO_2 inlet load rapidly replenished the inorganic carbon consumed by microalgae. DOC values remained considerably lower and highly variable, with an average of 0.08 ± 0.08 mg_C L^{-1} (Fig. SM5 a). DO varied between 0.66 and 3.71 mg_{O2} L^{-1} throughout the experiment.

C. vulgaris exhibited exponential growth until day 7 of SI (Fig. SM5 b)

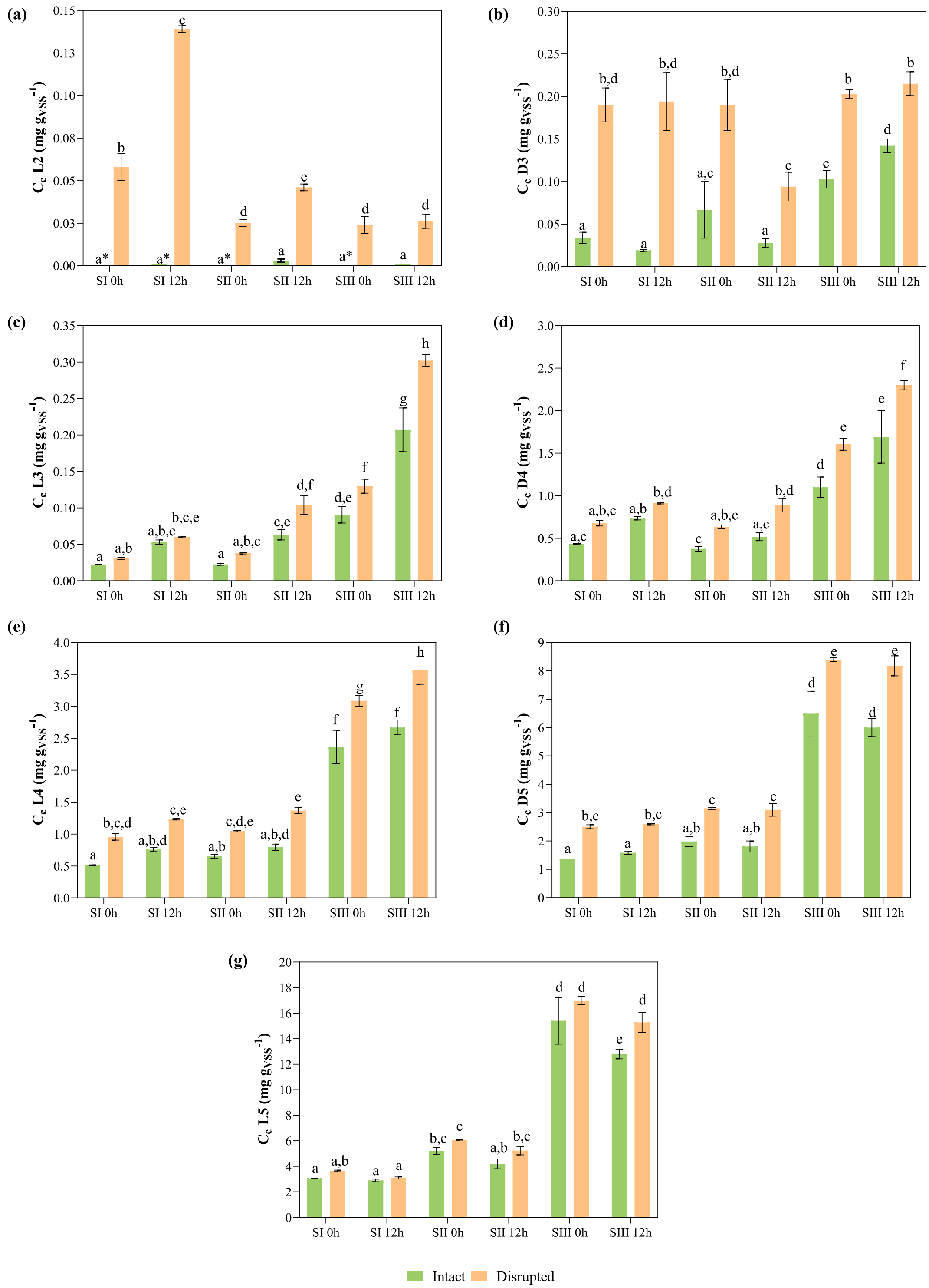

Fig. 5. Concentrations (C_c) of L2 (a), D3 (b), L3 (c), D4 (d), L4 (e), D5 (f), and L5 (g) in intact and disrupted cultures at the beginning (0 h) and end (12 h) of the dynamic loading tests in SI (day 23), SII (day 49), and SIII (day 73). Statistical differences were assessed using a two-way ANOVA followed by Tukey's multiple-comparison test. For each VMS, values sharing at least one common letter (a-h) are not statistically different ($p > 0.05$). *Value corresponding to the limit of detection.

at a specific growth rate of 0.293 d^{-1}, corresponding to an average biomass productivity and CO_2 fixation rate of 85 mg_{VSS} L^{-1} d^{-1} and 155 mg_{CO2} L^{-1} d^{-1}, respectively. Fixation rates between 140 and 480 mg_{CO2} L^{-1} d^{-1} have been reported for this microalga under inlet volumetric CO_2 concentrations of 25–40% (*v*/v) [50,51]. The exponential growth was accompanied by average TIN and PO_4-P removal rates of 5.95 mg_N L^{-1} d^{-1} and 0.71 mg_P L^{-1} d^{-1}, and efficiencies of 95% and 61%, respectively (Fig. SM5 c). During the remainder of SI, periodic culture medium exchanges were implemented to ensure nutrient availability and stabilise average volumetric and areal microalgal biomass productivities at theoretical values of 35 mg $L^{-1}d^{-1}$ and 2.15 g d^{-1} m^{-2}, respectively. Nutrient concentrations remained stable and the maximum VSS content (1.29 g_{VSS} L^{-1}), daily biomass productivity (165 mg_{VSS} L^{-1} d^{-1}), and corresponding CO_2 fixation rate (302 mg_{CO2} L^{-1} d^{-1}) were observed on the last day of this stage. Inlet and outlet O_2 and CO_2 volumetric concentrations varied considerably throughout the stage (Fig. SM6). The average CO_2 removal from the gaseous phase during this period was 8 ± 5% (v/v), with a maximum value of 16.6% (v/v) recorded on day 9. These relatively low REs are attributed to the high CO_2 IL relative to the microalgal fixation capacity.

The addition of sludge to the PBR in SII led to an increase in TIN and PO_4-P concentrations, possibly due to the presence of nitrogen and phosphorus in the inoculum. Nonetheless, nutrient concentrations returned to stable levels by day 44. The VSS content exhibited moderate fluctuations, with an average of 1.4 ± 0.1 g_{VSS} L^{-1} and a maximum of 1.72 g_{VSS} L^{-1}. The maximum CO_2 removal during this stage was higher compared to SI (19.3% on day 41), but no significant differences were observed in the average value (10 ± 7%, $p = 0.362$). The incorporation of enriched sludge and Tween 80 in SII and SIV did not impact nitrogen and phosphorus concentrations, which remained stable until the end of the experiment. Average VSS contents of 1.5 ± 0.2 g_{VSS} L^{-1} and 1.8 ± 0.1 g L^{-1} were achieved in SIII and SIV, respectively, with maximum values of approximately 1.8 g_{VSS} L^{-1}. Average CO_2 removals of 9 ± 3% in SIII and 10 ± 5% in SIV were comparable to those of earlier stages ($p > 0.05$). Conversely, differences were observed in the maximum CO_2 RE, which reached 13.2% and 17.2% on days 55 and 91, respectively.

3.7. Characterisation of the microbial community

The microbial community was analysed in enriched sludge and culture samples from the PBR in SII and SIV. A larger number of shared Amplicon Sequence Variants (ASVs) was observed between SII and SIV (51) than between the enriched inoculum and the reactor samples (7 with SII and 10 with SIV, Fig. SM7). Enriched sludge presented the highest taxonomic diversity, with 9 phyla identified at relative abundances above 0.5% (Fig. 6). *Actinomycetota* was the dominant phylum (27%), however, most of the corresponding genera belonged to the low-abundance or unclassified fraction in Fig. 6 (b). Consequently, the most abundant bacterial taxa identified in the enriched sludge belonged to members of the *Macellibacteroide* (9.9%), *Acinetobacter* (3.5%), and *Romboutsia* (2.2%) genera from the *Bacteroidota*, *Pseudomonadota*, and *Bacillota* phyla, respectively. In contrast, the PBR microbial community was dominated by members of the *Bacteroidota* phylum accounting for 67% of the community in SII and 70% in SIV. Within this phylum, members of the *Saprospiraceae* family prevailed, with members of the *OLB8* (19%) and *Phaeodactylibacter* (29%) genera dominating in SII and members of the *Phaeodactylibacter* (66%) genus in SIV. These genera were not identified in the enriched sludge, indicating that their presence in the PBR likely resulted from the proliferation of bacteria from the sludge inoculum in SII. This was likely facilitated by a symbiotic interaction with *C. vulgaris*, as members of the *Saprospiraceae* family have been positively correlated with microalgal growth [52]. Additionally, Kondrotaite et al. [53] described the *Saprospiraceae* family as frequently detected in WWTPs and capable of degrading proteins, polysaccharides, and other complex compounds. To the authors' best knowledge, the genera from this family have not yet been identified as VMS degraders. Nonetheless, Kraakman et al. [20] reported *OLB8* as the dominant genus (42%) in a capillary bioreactor treating ambient air contaminated with a mixture of VOCs (α-pinene, hexane, toluene). The introduction of a microalgal inoculum composed of *Pseudianabaena* sp. and *C. vulgaris* led to a marked decline in the relative abundance of this genus, in contrast to the sustained dominance observed in the present work. Other relatively abundant genera in SII included *Sphingopyxis* (7.9%) and *Mesorhizobium* (5.0%), from the *Pseudomonadota* phylum, and *Fluviicola* (7.8%), which belongs to the *Bacteroidota* phylum. In SIII, the abundance of members of the *Mesorhizobium* genus increased to 6.6%, but *Fluviicola* and *Sphingopyxis* members decreased to 0.74% and 1.5%, respectively. From the same phylum, members of the genus *Stappia* also dominated (5.3%) at this stage.

In addition to dominant taxa, several low-abundance genera of potential relevance were consistently detected. These included: (i) *Microbacterium* (*Actynomycetota*) in enriched sludge (0.18%), SII (0.91%), and SIV (0.55%); (ii) *Mycobacterium* (*Actynomycetota*) in enriched sludge (1.3%) and SII (0.07%); (iii) *Chitinophaga*, (*Bacteroidota*) in SII (1.3%) and SIV (0.15%); and (iv) *Terrimonas*, from the same family as *Chitinophaga* (*Chitinophagaceae*), in enriched sludge (0.16%), SII (1.5%) and SIV (0.04%). These genera, together with *Mesorhizobium* and *Sphingopyxis*, have been repeatedly reported in biological VMS degradation systems. González-Cortés et al. [18] found that *Mycobacterium* was the dominant genus in an aerobic BF operated with Tween 80 at 1 CMC to remove L3 and D4, at a relative abundance of 7.5%. Similarly, Pascual et al. [29] reported an enhanced growth of this genus during continuous exposure to L2, D3, D4, and D5 in an aerobic BTF, where *Chitinophaga* members also became one of the dominant genus. Members of the *Terrimonas* genus were reported at 6.8% abundance in a sludge enriched for 176 days in an aerobic TP-BTF with silicone oil treating the same VMS [54]. Other studies have identified *Mycobacterium* and *Mesorhizobium* in aerobic TP-BTF supplemented with the previously mentioned siloxanes [55]. In addition, several of these genera have been identified in anoxic bioreactors. Boada et al. [56] isolated D4-degrading bacteria from an anoxic BTF and classified 2 of the 17 recovered isolates as members of the genera *Sphingopyxis* and *Microbacterium*. Subsequently, Boada et al. [57] detected *Chitinophaga*, *Terrimonas*, *Mesorhizobium*, and *Mycobacterium* at relative abundances up to 0.41%, 0.14%, 0.41%, and 9.99%, respectively, in an anoxic BTF treating D4, D5, toluene, limonene, and hexane, both in the absence and presence of activated carbon. More recently, *Mycobacterium* was again identified in an anoxic BTF supplemented with L2, L3, D4, and D5, reaching a relative abundance of 5.5% by the end of the experiment [44].

There is a significant knowledge gap regarding specific VMS degradation pathways and microorganisms directly involved in the process. Degradation studies focus mostly on members of the *Pseudomonas* genus, commonly isolated from WWTP sludge [8,14,17]. However, members of this genus were not found in the communities of the present study, suggesting that operational conditions or competition with established microorganisms might have prevented their growth. Although no single genus could be unequivocally identified as a key VMS degrader in the present study, the reactor selected for a specialised microbial community enriched in taxa previously associated with microalgal growth or the degradation of VMS and hydrophobic VOCs, supporting the observed VMS removal performance.

4. Conclusions

The present work demonstrates for the first time the feasibility of combining a microalgal-bacterial consortium with a capillary reactor configuration for biological VMS removal. *C. vulgaris* grew efficiently in this PBR, with average total VMS RE and EC of 36 ± 10% and 1142 ± 436 μg L^{-1} h^{-1}. The addition of sludge did not improve siloxane removal, as mass transfer limitations were identified for most VMS. A significant improvement was observed when the surfactant Tween 80 was added to the PBR, resulting in a total VMS RE of 60 ± 4% (1.8-fold increase) and an EC of 2136 ± 195 μg L^{-1} h^{-1} (2.1-fold increase). Bioadsorption, bioaccumulation, and biodegradation were identified as possible removal pathways, but additional research is required to elucidate the specific metabolic pathways and microorganisms involved in the process. Although biological approaches could be environmentally and economically advantageous, further optimisation is required to achieve removals comparable to conventional processes and ensure their large-scale applicability. In summary, the current work opens opportunities for biological VMS removal not only from biogas, but also from related matrices where these compounds may pose an environmental threat, which is crucial to mitigate their persistence and toxicity. It also opens new perspectives for the integration of microalgae-based systems and advances PBR designs into biogas upgrading technologies, essential for its reliable large-scale use as a renewable energy source.

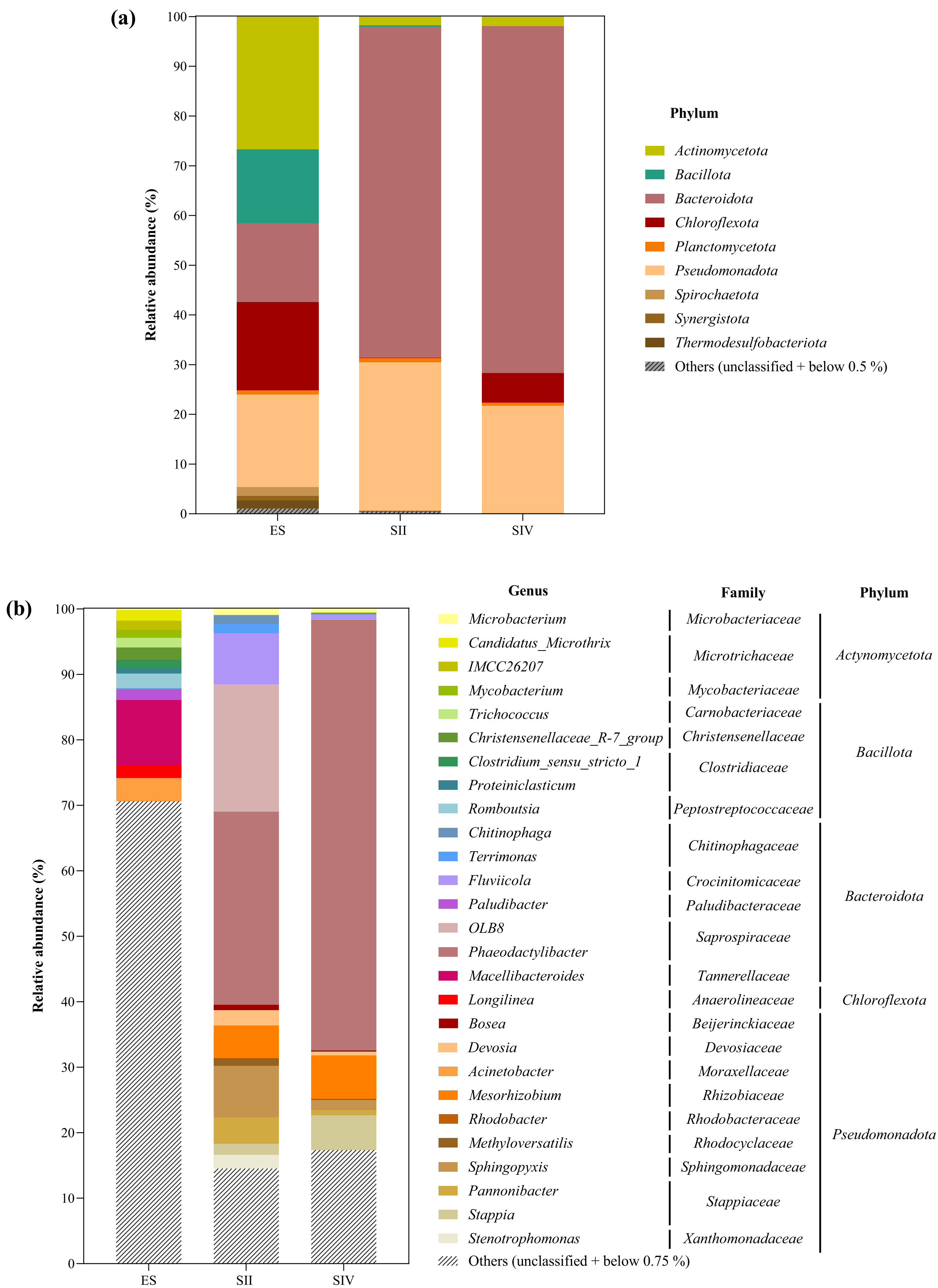

Fig. 6. Relative abundance (%) of bacterial phyla (a) and genera (b) in the enriched sludge inoculum (ES) and in the capillary photobioreactor in SII (day 52) and SIV (day 94).

CRediT authorship contribution statement

Eva M. Salgado: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Nerea Rodríguez:** Writing – review & editing, Investigation. **Roxana Ángeles-Torres:** Writing – review & editing, Investigation. **Ana L. Gonçalves:** Writing – review & editing, Supervision. **Nuno Ratola:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition. **José C.M. Pires:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition. **Sara Cantera:** Writing – review & editing, Methodology. **Raquel Lebrero:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2026.175747.

Data availability

Data will be made available on request.
BioProject: PRJNA1358825 (Original data) (NCBI)

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