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Research article

Enhancing the biodegradation of hydrophobic volatile organic compounds: A study on microbial consortia adaptation and the role of surfactants

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ABSTRACT

The emission of hydrophobic Volatile Organic Compounds (VOCs) is a serious environmental issue. Typically, biofilters (BFs) are employed for their treatment, with the potential enhancement of mass transfer through the addition of surfactants. However, disparate results in previous studies have been observed, attributed to uncontrolled conditions during the introduction of surfactants to BFs. Additionally, there has been limited exploration of microbial consortium adaptation to surfactants. To address these gaps, this study followed two approaches. First, the long-term (247 days) removal of cyclohexane was studied in a stirred tank bioreactor (STBR) inoculated with *Rhodococcus erythropolys* E1 and using Tween 80 at three times the critical micelle concentration (CMC). Second, the short-term (9 days) impact of two (bio)surfactants [Tween 80 (1 × CMC) and Quillaja Saponin (QS, 1 × CMC)] on the removal of cyclohexane, hexane and toluene was investigated in batch tests using three types of inocula: a pure culture of *Rhodococcus erythropolys* E1 (X_0), a microbial consortium adapted to cyclohexane (X_1), and a microbial consortium adapted to cyclohexane with Tween 80 (X_2). For long-term operation, the addition of Tween 80 at 3 × CMC improved cyclohexane removal efficiency (RE) to 87 ± 1% (elimination capacity, EC = 145 ± 25 mg m^{-3} h^{-1}, gas residence time, GRT = 20 min, inlet concentration, C_{in} = 14.9 ± 2.5 ppm_v), compared to a RE of 32 ± 9% (EC = 44 ± 8 mg m^{-3} h^{-1}, GRT = 20 min, C_{in} = 15.1 ± 0.7 ppm_v) under similar conditions without surfactants. For short-term operation, the addition of QS at 1 × CMC significantly increased biomass growth, resulting in lower maximum specific consumption rates for X_1 and X_2 compared to scenarios without surfactants or 1 × CMC Tween 80. The most abundant genera in X_1 and X_2 were *Paludisphaera* (26-23%), *67-14* genus (17–23%), and *Rhodococcus* (9–18%), respectively.

1. Introduction

Volatile organic compounds (VOCs) are ubiquitous pollutants emitted by various industrial processes, making their efficient and cost-effective treatment a matter of paramount importance because of their detrimental environmental and health effects (Han et al., 2018). VOCs play a significant role in the formation of ground-level ozone and secondary organic aerosols, thereby contributing to air quality degradation and climate change (David and Niculescu, 2021). Moreover, exposure to VOCs has been linked to a range of health issues, including respiratory disorders, neurological abnormalities, and immunological responses (Dörter et al., 2020; Sarkar et al., 2022). The hazards associated with VOCs extend beyond human health, affecting ecosystems and contributing to the formation of photochemical smog (Wu et al., 2023). Therefore, the development of effective strategies for VOCs removal is essential for both environmental protection and public health.

Among the various technologies available for VOC removal, biological methods have gained considerable attention owing to their advantages over conventional physical-chemical techniques (e.g., absorption, chemical oxidation, and adsorption). The removal of VOCs by biofiltration is appealing because of its low operating costs, minimal energy consumption, and reduced secondary pollution compared with other methods (Sheoran et al., 2022).

However, efficient removal of hydrophobic VOCs remains a challenge in biofiltration systems. The low solubility of hydrophobic VOCs translates into mass transfer limitations from the gas to the aqueous

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biofilm phase, leading to reduced bioavailability and lower removal efficiency when compared to their hydrophilic counterparts (Lamprea Pineda et al., 2021). This challenge is particularly pronounced in industrial settings where complex mixtures of VOCs are often encountered (Wu et al., 2024). To address this issue, researchers have explored various strategies for enhancing the solubility and bioavailability of hydrophobic VOCs, with the addition of surfactants emerging as a promising approach (Wu et al., 2022). Surfactants are amphiphilic compounds with hydrophilic and hydrophobic regions. They reduce the surface and interfacial tension between two immiscible phases, such as gas and liquid, and significantly increase the solubility of a compound in a medium. The increase in solubility is attributed to the formation of surfactant aggregates known as micelles, which occurs at the critical micelle concentration (CMC). These micelles, created by surfactants, effectively encapsulate VOC molecules in their hydrophobic cores, facilitating their transport to microorganisms (Percival et al., 2019). Nevertheless, the effects of surfactant addition to biological systems are complex and context-dependent, as evidenced by the divergent results in the literature. For instance, Woertz and Kinney (2004) observed negative effects with a reduction in elimination capacity (EC) from 63–156 g m^{-3} h^{-1} to 38–148 g m^{-3} h^{-1} when adding 50 mg L^{-1} (3.1 × CMC) of Tween 20 to a BF treating toluene. In contrast, Wang et al. (2018) reported the highest maximum elimination capacity (EC_{max}) of a biotrickling filter (BTF) treating m-xylene in the presence of 100 mg L^{-1} (6.3 × CMC) Tween 80 (EC_{max}: 159 g m^{-3} h^{-1}, removal efficiency (RE) ≈ 80%) compared to the BTF without surfactant (EC_{max}: 128 g m^{-3} h^{-1}, RE ≈ 59%). The disparate outcomes can be attributed to a number of variables. These include the suboptimal distribution of surfactants, which is typically achieved by pouring the surfactant solution on top of the BFs. This results in areas with high concentrations of surfactants and areas with surfactant concentrations below the CMC. Furthermore, interactions with the support media, the chemical properties of the specific VOCs being targeted, and the varying microbial communities must be considered (Dewidar and Sorial, 2021; Lamprea Pineda et al., 2021). Therefore, for systematic research on surfactant-enhanced biodegradation, the use of a stirred tank bioreactor (STBR) was proposed. By avoiding the use of packing materials, this configuration ensures a uniform distribution of surfactants, effectively addressing the limitations encountered in studying surfactant addition within BFs and BTFs.

Furthermore, the long-term impact of the addition of these surfactants onto the microbial consortia remains underexplored. Despite the proven enhancement of mass transfer (Lebrun et al., 2022; Sardeing et al., 2006), limited knowledge exists regarding the long-term effects of surfactant addition on the biodegradation capabilities of microorganisms. Existing literature provides minimal insights into the potential phenomena that may occur after biomass adaptation to prolonged surfactant exposure. For instance, little is known about the potential utilization of surfactants as a preferred carbon source over different hydrophobic VOCs, or the possible differing effects of surfactant introduction to consortia with no prior exposure to surfactants compared to those that have adapted to their presence.

To address these gaps, in this study, a STBR was employed to cultivate different microbial consortia specialized in removing VOCs. The STBR operation generated two distinct microbial consortia: (i) consortia unadapted to surfactants, and (ii) consortia adapted to the presence of surfactants. The ability of these microbial consortia to remove three model VOCs covering a broad range of Henry's coefficients [hexane (with a dimensionless Henry's coefficient at 25 °C, H (25 °C) = 73.3), cyclohexane (H (25 °C) = 7.6), and toluene (H (25 °C) = 0.269] was examined through batch tests, a proven technique for assessing short-term effects in biological systems (Valenzuela-Reyes et al., 2014; Yang et al., 2013). These batch tests were performed in both the presence and absence of (bio-)surfactants to discern the impact of their addition on different types of microbial consortia. Two distinct types of (bio)surfactants were selected. Tween 80, a synthetic surfactant, was chosen for its commercial availability, low concentration requirement to reach the CMC value (0.016 g L^{-1}), and prior successful application in biofiltration systems (Cheng et al., 2016; Hajizadeh et al., 2018; Liu et al., 2007). Next to that, saponin, a biological surfactant, was chosen for its lower toxicity compared to synthetic ones, affordability, environmental friendliness and limited previous research regarding its utilization in biofiltration systems (Lamprea Pineda et al., 2021).

This study aims to address key gaps in our understanding of surfactant-enhanced VOC removal in biofiltration systems, focusing on both short- and long-term impacts of (bio)surfactants on the performance and dynamics of microbial consortia. The innovative aspects include the use of a stirred tank bioreactor (STBR) to ensure uniform surfactant distribution, comparative analysis of adapted and unadapted microbial consortia, and evaluation of both synthetic (Tween 80) and biological (saponin) surfactants on a range of hydrophobic VOCs. By conducting STBR operations and batch tests, we provide insights into the effects of surfactant addition on microbial consortia and their VOC removal capabilities. Specifically, we evaluated the removal rates of a pure culture of *Rhodococcus erythropolys* and two microbial consortia adapted to hydrophobic VOC removal, with and without Tween 80. The impact of (bio)surfactants on microbial community dynamics was further investigated through 16S metagenomic analysis. This comprehensive approach offers valuable information on microbial adaptation to surfactants and the subsequent impact on biodegradation performance, potentially guiding the optimization of biofiltration systems for improved efficiency of hydrophobic VOC removal in various industrial applications.

2. Materials and methods

2.1. Chemicals

The structure and physical-chemical properties of the VOCs [cyclohexane (99.9% assay, VWR, Belgium), hexane (technical grade, VWR, Belgium), and toluene (100% assay, VWR, Belgium)] as well as the surfactants [Tween 80 (oleic acid, ≥58.0% (balance primarily linoleic, palmitic, and stearic acids), Sigma-Aldrich, Belgium) and Quillaja saponin (Sapogenin content 20–35%, Sigma-Aldrich, Belgium)] are listed in Table 1.

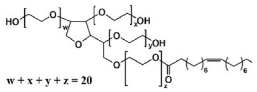
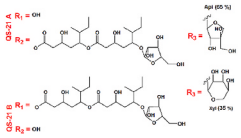
2.2. Inoculum preparation

Rhodococcus erythropolys E1 (strain number LMG 21994) was selected as inoculum because it is a bacterium that is known for its ability to degrade various hydrocarbons (Cappelletti et al., 2015; Thi Mo et al., 2022; Vergara-Fernández et al., 2018). The strain was acquired from the Belgian Coordinated Collection of Microorganisms (BCCM) and was incubated at 30 °C and 180 rpm for approximately 12 days in Bushnell Hass (BH) medium supplemented with 5 g L^{-1} of glucose. The BH medium contained 0.2 g L^{-1} $MgSO_4$ (assay: 99–101%, Chemlab-Analytical bvba, Zedelgem, Belgium), 0.02 g L^{-1} $CaCl_2$ (assay: >95%, Chemlab-Analytical bvba, Zedelgem, Belgium), 1 g L^{-1} KH_2PO_4 (assay: ≥99.0%, Sigma Aldrich, Hoeilaart, Belgium), 1 g L^{-1} K_2HPO_4 (assay: ≥98.0%, Sigma Aldrich, Hoeilaart, Belgium), 1 g L^{-1} NH_4NO_3 (assay:99%, VWR International, Leuven, Belgium) and 0.05 g L^{-1} $FeCl_3$ (assay: 97.0%, Sigma Aldrich, Hoeilaart, Belgium). The pH of the medium was adjusted to 7. After the strain was grown, a 400 mL culture of the mature strain was used to inoculate the STBR. The inoculum was then mixed with 1.6 L of fresh BH medium (excluding glucose) to achieve a total working volume of 2 L. Before use, both the BH medium and all instruments that came into contact with the microbial culture were autoclaved at 121 °C and 1 bar for 15 min.

2.3. Experimental setup

The experiments were conducted using an STBR with a working volume of 1 L (Fig. 1). The bioreactor was magnetically stirred at 150

Table 1
Physical-chemical properties at 25 °C of the VOCs of interest in this study (Dean, 1999; Howard and Meylan, 1997; Max-Planck Institute, 2023) and of the (bio-) surfactants tested (Jarzębski et al., 2020; Mitra and Dungan, 1997; Pedebos et al., 2014; Sigma-Aldrich, 2024).

Parameters	Unit	Cyclohexane	Toluene	Hexane	Tween 80	Quillaja saponin
Chemical structure	-					
CAS	–	110-82-7	108-88-3	110-54-3	9005-65-6	8047-15-2
Molecular Formula	–	C_6H_{12}	C_7H_8	C_6H_{14}	$C_{64}H_{124}O_{26}$	$C_{92}H_{148}O_{46}$
Molecular weight	g mol^{-1}	84.2	92.1	86.2	1310	1990
Vapor pressure	kPa	12.92	3.79	20.13	–	–
Solubility in H_2O	mg L^{-1}	5.50×10^1	5.26×10^2	9.50×10^0	–	–
Henry's law constant	- (gas/aq)	7.611	0.269	73.345	–	–
Critical micelle concentration (CMC)	g L^{-1}	–	–		0.016	0.510
γ_{CMC}[a]	mN m^{-1}	–	–		46.2	40.2[b]
Purity, supplier's name, internal reference, and country of origin	–	≥99%, VWR, 23223, Belgium	≥99%, VWR, 28675, Belgium	Tech., VWR, 24574, Belgium	Oleic acid, ≥58.0% (balance primarily linoleic, palmitic, and stearic acids), P4780, Belgium	Sapogenin content 20–35%, Sigma-Aldrich, S4521, Belgium

[a] γ_{CMC} indicates the surface tension (γ) value at the CMC.
[b] at 0.5 g L^{-1}

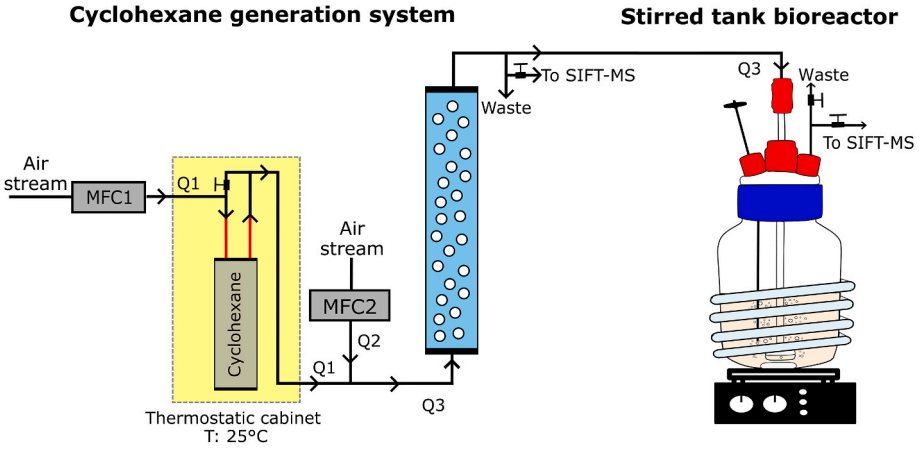


Fig. 1. Experimental setup of both the gas generation system and the stirred-tank bioreactor. RM represents the rotameter, MFC represents the mass flow controller, Q represents the different gas flows and SIFT-MS represents the selected-ion flow-tube mass spectrometry equipment used to analyze VOC concentrations.

rpm and maintained at 30 °C in a thermostatic bath (1136D, VWR, Belgium).

It is imperative to clarify that the primary objectives of this study were not to achieve large ECs using this bioreactor. Instead, the overarching goal was to investigate the influence of Tween 80 to a liquid bioreactor operated at varying conditions of inlet loads (IL) and gas residence time (GRT). The deliberate choice of a liquid-phase bioreactor was made to ensure the even distribution of Tween 80 throughout the system. Furthermore, it must be remarked that the bioreactor was not optimized from a mass transfer perspective.

Cyclohexane was selected as the sole VOC for bioreactor feeding because of its intermediate level of hydrophobicity when compared to the other two VOCs under investigation (hexane and toluene), its widespread global use (Dahiru et al., 2021), and the limited existing research on its bioremediation. A cyclohexane-polluted air stream was produced using a custom-made generation system (Fig. 1). The configuration was based on passing a small air stream (Q1, 1–30 mL min^{-1}) through a stainless-steel vessel containing pure cyclohexane liquid. The gas flow was controlled using a mass flow controller (MFC; Brooks Instruments, USA). The polluted gas stream (Q1) was then diluted with an

air stream (Q2) to a final total flow of 12.5–100 mL min^{-1}. To maintain a constant headspace concentration of cyclohexane, the setup was temperature controlled in a thermostatic cabinet (25 °C). The contaminated gas was passed through a humidification system to maintain a relative humidity above 95%. The contaminated gas stream was fed into the bioreactor.

2.4. Long-term effect of inlet load (IL) and Tween 80 addition on cyclohexane removal

To obtain a microbial consortium that is adapted to remove cyclohexane and to assess the long-term impact on bioreactor performance and microbial consortium composition, the addition of Tween 80 (3 × CMC) was evaluated in a STBR. This concentration was chosen based on conclusions from previous studies, which reported its ability to improve cyclohexane mass transfer (Lamprea Pineda et al., 2023b) without promoting excessive microbial growth that could lead to preferential degradation of the surfactant over cyclohexane (Lamprea-Pineda et al., 2024).

The bioreactor was operated under various cyclohexane ILs ranging from 80 ± 15 to 376 ± 83 mg m^{-3} h^{-1}, by adjusting the GRT (10–40 min). The parameters employed for assessing the performance of the bioreactor were determined using Eqs. (1)–(4).

$$IL = \frac{C_{in}}{V} \bullet Q \quad \text{(Eq. 1)}$$

$$RE = \frac{C_{in} - C_{out}}{C_{in}} \bullet 100 \quad \text{(Eq. 2)}$$

$$EC = \frac{C_{in} - C_{out}}{V} \bullet Q = IL \bullet \frac{RE}{100} \quad \text{(Eq. 3)}$$

$$GRT = \frac{V}{Q} \quad \text{(Eq.4)}$$

where IL stands for inlet load (mg m^{-3} h^{-1}), EC for elimination capacity (mg m^{-3} h^{-1}), RE for removal efficiency (%), C_{in} and C_{out} for inlet and outlet concentration (mg m^{-3}), respectively, V for bioreactor volume (m^3), Q for gas flow rate (m^3 h^{-1}) and GRT for gas residence time (s).

The operation of the bioreactor spanned 247 days and was divided into seven stages, each conducted under different conditions as outlined in Table 2.

The STBR was fed with an air stream polluted with cyclohexane to maintain a constant cyclohexane concentration throughout the operation (≈16 ppm_v). The bioreactor was started-up for 78 days to acclimatize the inoculum to the carbon source (Stage 0). Aiming to improve the cyclohexane removal efficiency (RE), during Stages I, II, and III, the GRT was progressively increased (from 10 to 40 min), which resulted in a decrease in IL from 244 ± 73 to 80 ± 15 mg m^{-3} h^{-1}. At the beginning of Stage IV (day 147), 3 × CMC Tween 80 (0.048 g L^{-1}) was added to the bioreactor to achieve a concentration of 3 × CMC. Then, ILs and GRTs comparable to those in the previous stages without surfactants (Stages I-III) were applied to the STBR during Stages IV and V. Once the STBR reached a stable RE (standard deviation < 5%), the effect of short-term starvation on BF performance was studied by stopping the air feed for seven days (Stage VI). Feeding was then restored, and recovery was studied for 30 days. Finally, the GRT was reduced to 10 min to test the system limits in the presence of 3 × CMC Tween 80 (Stage VII).

The biomass concentration in the bioreactor was determined by the spread plate method using tryptone soya agar (TSA) solid plates (Oxoid, Basingstoke, England). All plates were incubated at 30 °C for 2 days, after which bacterial colonies were counted.

2.5. Short-term effect of (bio-)surfactants on cyclohexane, hexane and toluene removal

The short-term effect of adding (bio-)surfactants to the removal of cyclohexane, hexane, and toluene at three different initial biomass compositions (X_0, X_1, and X_2) was studied in 118 mL amber glass penicillin bottles. X_0 was the pure culture (*R. erythropolis*) used in the inoculation of the STBR. X_1 and X_2 were sampled and selected from the STBR considering the operational aspects detailed in the preceding section (section 2.4). The STBR underwent three distinct phases where biomass was different throughout its operational timeline. After 146 days of continuous cyclohexane feeding as the exclusive carbon source, an improvement in the bioreactor's removal performance was noted, suggesting a shift in the microbial composition, denoted as X_1. Subsequently, 3 × CMC Tween 80 was added and another enhancement in the bioreactor's removal performance was observed after an additional 101 days. To discern the impact of introducing this surfactant, a new biomass sample was collected, referred to as X_2. Microbial analysis of X_2 and X_3 biomass was performed by 16S metagenomic analysis (see section 2.7).

BH medium with the corresponding amount of (bio)surfactant (1 × CMC) was added to the penicillin bottles to obtain a total volume of 20

Table 2
Summary of operating conditions during biomass adaptation to the removal of cyclohexane in presence and absence of Tween 80 for all experiments. Stage 0 corresponds to the start-up phase.

Stage	Time (days)	Action	GRT[a] (min)	IL[b] (mg m^{-3} h^{-1})	C_{in}[c] (ppm_v)
0	0–78	Start-up	10	253 ± 28	12.1 ± 1.4
I	79–100	IL effect (I)	10	244 ± 73	11.9 ± 3.6
II	101–115	IL effect (II)	20	154 ± 8	15.1 ± 0.7
III	116–146	IL effect (III)	40	80 ± 15	15.8 ± 2.9
IV	147–174	IL effect (III) + 3 × CMC Tween 80	40	85 ± 14	16.7 ± 2.9
V	175–183	IL effect (II) + 3 × CMC Tween 80	20	202 ± 5	19.0 ± 0.6
VI	184–223	Short-term starvation (7 days) and recovery	20[d]	155 ± 26[a]	14.9 ± 2.5
VII	224–247	IL effect (I) + 3 × CMC Tween 80	10	376 ± 83	17.9 ± 3.9

[a] Gas residence time.
[b] Inlet Load.
[c] Inlet concentration.
[d] Values once feeding was restored.

mL, with an initial concentration of approximately 1×10^5 cells mL^{-1}. The bottles were sealed with Mininert valves (BGB Analytik, Harderwijk, The Netherlands), and saturated headspaces of cyclohexane, hexane, and toluene were separately injected into the bottles to obtain an initial headspace VOC concentration of 1200 ppm_v. Thus, each bottle always had only one VOC in the headspace. The bottles were incubated at 180 rpm for nine days. In all cases, the experiments were carried out in triplicate, and control experiments were considered (i) without (bio) surfactants but maintaining the same amount of medium and bacteria, and (ii) without (bio)surfactants and bacteria but maintaining the same amount of medium. The specific substrate consumption rates (qS) were determined using Eq. (5).

$$qS = \left(\frac{C_{ti} - C_{ti+1}}{\overline{X}}\right) \quad \text{(Eq. 5)}$$

where C_{ti} was the VOC concentration on day i, C_{ti+1} was the VOC concentration in the headspace at the day after day i, and $\overline{X}$ the average biomass concentration present in each bottle. In the batch tests, biomass quantification was performed by cell counting using an improved Neubauer chamber (Hecht Assistent, Germany).

2.6. Analysis of the gas stream

In this study, real-time monitoring of the STBR performance was carried out using Selected-Ion Flow Tube Mass Spectrometry (SIFT-MS). SIFT-MS is an analytical technique that relies on the chemical ionization of gaseous organic compounds through precursor ions, including NO^+, H_3O^+, and O_2^+, which generate product ions (PIs). For this investigation, $C_6H_{11}^+$ [83]/NO^+ PI was used to measure the concentration of cyclohexane. Measurements were taken daily at both the inlet and outlet streams of the STBR, with each measurement lasting for at least 3 min. Consequently, the reported concentrations represent the average derived from over 150 data points.

To measure cyclohexane, hexane, and toluene concentrations during the batch tests, gas samples (0.5 mL) collected from the different penicillin bottles were injected using a 1 mL gas syringe (Hamilton, USA) into a gas chromatograph connected to a flame ionization detector (Agilent 6890, HP, USA) and equipped with a CP-Sil 8 CB 25 m × 0.32 mm capillary column. The oven temperature was maintained at 110 °C, and the detector, supplied with hydrogen (40 mL min^{-1}) and air (400 mL min^{-1}), was maintained at 200 °C. Helium was used as the carrier gas at a flow rate of 5 mL min^{-1}.

To measure the CO_2 production in the STBR, a CO_2 probe (MI70, Vaisala, Finland) was employed. A thermo-hygrometer (Testo 625, Testo, Germany) was used to gauge the temperature and relative humidity at both the inlet and outlet points of the STBRs. All figures in this study were generated using SigmaPlot 15.0 (Grafiti LLC, CA, USA) and Inkscape 1.2.

2.7. Microbial analysis by 16S metagenomic analysis

The microbial community shifts that occurred after acclimatization of the biomass to cyclohexane feeding for 146 days without Tween 80 (X_1) and after acclimatization of the biomass to cyclohexane feeding in the presence of 3 × CMC of Tween 80 for another 101 days (day 247) (X_2) were determined using 16S metagenomic analysis. The biomass present in 100 mL of the bioreactor was harvested by centrifugation (10,000×*g*, 10 min). Genomic DNA extraction was performed using the DNeasy PowerSoil Pro Kit (Qiagen, Germany), which served as the input material for STAB VIDA (Inv. e Serv. Ciências Biológicas, Lda., Portugal). A library was constructed following the Illumina 16S Metagenomic Sequencing Library preparation protocol employing a specific set of primers (341F and 785R). Subsequently, the DNA fragments were subjected to sequencing using the MiSeq Reagent Kit v3 on the Illumina MiSeq platform, which involved 300 bp paired-end sequencing reads (DNA libraries). For subsequent bioinformatic analysis, the data were processed using QIIME2 v2021.4 (Caporaso et al., 2010). Reads were denoised using the DADA2 plugin (Callahan et al., 2016). These reads were then organized into operational taxonomic units (OTUs) and classified by taxon using a clustering criterion of 99% similarity, employing a fitted classifier trained on the SILVA database (version 138 QIIME). Only OTUs containing ≥10 sequence reads were deemed significant, with the relative abundance of reads for each OTU being represented.

3. Results and discussion

3.1. Long-term effect of IL and Tween 80 addition on cyclohexane removal

To gain insights into the long-term effects of adding Tween 80 to a bioreactor treating cyclohexane, the STBR (see section 2.3) was operated for 247 days (Fig. 2).

After 79 days of acclimatization (Stage 0, data not shown), the bioreactor operation was monitored. During Stage I, the cyclohexane RE remained very low, staying below 14% (EC = 44 ± 3 mg m^{-3} h^{-1}). These results could be mainly attributed to the poor mass transfer of

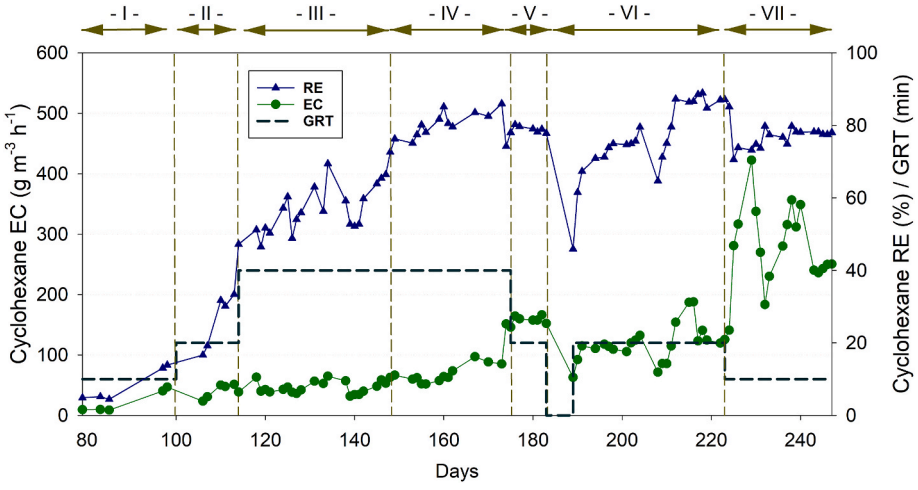


Fig. 2. Elimination capacity (EC), removal efficiency (RE) and gas residence time (GRT) of cyclohexane in the stirred tank bioreactor. Roman numerals represent the operational stages as outlined in Table 2. Stage 0 is excluded to emphasize the key stages. The bioreactor was operated at 30 °C and a pH of 7.0 ± 0.2, a GRT ranging from 10 to 40 min, a cyclohexane inlet load (IL) between 80 and 376 mg cyclohexane m^{-3} h^{-1}, and an inlet concentration ranging from 11.9 to 19.0 ppm_v.

cyclohexane from the gas to the liquid. Therefore, to enhance the EC, the GRT was increased from 10 min (Stage I) to 20 (at day 99) and 40 min (at day 119) in Stages II and III, respectively. This GRT increase resulted in an increase in EC in both stages. While in Stage II the EC remained constant to an average 44 ± 8 mg m^{-3} h^{-1} (RE = 32 ± 9%), the increase of the GRT to 40 min (Stage III) increased the RE to 55 ± 4% (EC = 49 ± 8 mg m^{-3} h^{-1}). As previously mentioned, the bioreactor operated in batch mode, meaning that the bioreactor medium was never discharged or replenished with fresh medium. Under these conditions, the biomass concentration remained remarkably stable, averaging around (9.7 ± 2.9) × 10^6 CFU mL^{-1} throughout the entire operation. Therefore, the increase in RE and EC could not be attributed to a higher biomass concentration in the bioreactor.

The CO_2 production of the STBR obtained during the operation was represented in Fig. S1. Notably, the similarity between the measured CO_2 production and the theoretical net CO_2 production expected, assuming complete chemical oxidation of the removed cyclohexane, indicated that almost all the carbon supplied to the STBR was converted into CO_2. This observation indicates that the biomass was in the stationary phase, implying no assimilation of carbon by the biomass (Bordoloi and Gostomski, 2019). This consistency strongly implies that the increase in mass transfer and adaptation of the microbial consortium are the primary factors accounting for this observed increase in RE and EC.

After 30 days of constant conditions in Stage III, Tween 80 was added at a concentration of 3 × CMC. Although not immediate, the EC gradually increased and stabilized at 90 ± 5 mg m^{-3} h^{-1} (RE = 84 ± 1%). Under these conditions, the addition of Tween 80 resulted in an increase by a factor of 1.8 compared to the EC obtained in Stage III under the same IL and GRT, without the addition of surfactant. To continue comparing these data with the earlier stages, the GRT was reduced to 20 min (which resulted in an IL increase from 85 to 202 mg m^{-3} h^{-1}) as tested in Stage II, but now in the presence of Tween 80. The STBR maintained a nearly constant RE (78 ± 1%), indicating an increase in EC to an average of 159 ± 6 mg m^{-3} h^{-1}. On day 184, the supply of contaminated air was stopped, leaving the bioreactor without oxygen for 5 days. On day 189, the air supply was resumed. This interruption in air supply led to a decrease in RE from 78% (EC = 152 mg m^{-3} h^{-1}) to 46% (EC = 63 mg m^{-3} h^{-1}), presumably due to oxygen depletion in the reactor. This outcome was expected given the comparatively less resilient nature of suspended growth bioreactors compared to three-phase bioreactors such as BFs and BTFs. In these latter systems, the attachment of biomass to the packing material enhances their resilience against brief shutdowns of the polluted stream (Almenglo et al., 2023). Despite this, the STBR was able to recover, surpassing the EC just prior to the interruption after 33 days of recovery, and achieved the highest average RE in the study from day 212–224 (87 ± 1%, EC = 145 ± 25 mg m^{-3} h^{-1}). The recovery observed in this study aligns with findings from systems considered to be more robust such as BTFs and BFs. For instance, in BTFs treating H_2S, starvation periods can lead to a significant decrease in viable bacterial numbers, particularly when the airflow is maintained without liquid spraying, as reported by Zhang et al. (2009). However, BTFs were able to recover almost completely, achieving REs near 98% even after starvation periods, due to the ability of the remaining viable bacteria to re-establish activity under normal operating conditions. A similar pattern is observed in BFs treating toluene vapors, where prolonged interruptions in air and pollutant supply led to a significant drop in performance, with REs dropping from 97.7 to 45.4% after starvation periods ranging from 12 to 96 h (Jiménez et al., 2017). Notably, BFs that maintained an airflow during starvation periods also experienced greater challenges in recovery, possibly due to the accumulation of inactive biomass and inhibitory compounds.

Finally, on day 226, the GRT was reduced to 10 min to compare it with Stage I. During this stage, the EC increased to an average of 248 ± 3 mg m^{-3} h^{-1} (RE = 78 ± 0.2%) resulting in an increase by a factor of 5.6 compared to the EC obtained exactly under the same conditions during Stage I, without the addition of surfactants. To compare the results obtained under the different operating conditions, Fig. S2 shows a compilation of the results obtained in terms of EC versus IL. As previously mentioned, the experimental setup is not optimized from a mass transfer point of view, since the objective was not to obtain large ECs but to evaluate the effect of the use of surfactants in liquid media. Therefore, in bioreactors with optimized mass transfer such as BTFs, much higher ECs have been observed. For example, in BTFs, REs of 80%–99% and ECs in the range of 6300 to 44,400 mg cyclohexane m^{-3} h^{-1} have been reported (Salamanca et al., 2017). Similarly, in BFs fed with a VOC mixture composed of toluene, cyclohexane and hexane, a maximum average EC of 11,000 ± 2 mg cyclohexane m^{-3} h^{-1} (RE = 86 ± 6 %; empty bed residence time, EBRT = 163 s) was reported (Lamprea-Pineda et al., 2024).

The performance improvement found in the bioreactor in Stages IV, VI and VII must be related mainly with the addition of Tween 80, a non-ionic surfactant that has been recognized in recent studies as an effective agent for enhancing the solubility of hydrophobic VOCs in aqueous solutions (Lamprea Pineda et al., 2023b). This solubility improvement is achieved through the formation of micelles, aggregations of surfactant molecules capable of solubilizing hydrophobic substances (Miller et al., 2020). Thus, an improved solubilization of cyclohexane in the aqueous phase has the potential to render it more accessible to microorganisms, consequently leading to a better removal of the VOC within the bioreactor. Given the scarcity of research on the use of surfactants for the biological removal of hydrophobic VOCs, it is difficult to compare the results of the present study. Most studies studying the addition of Tween 80 in bioreactors have dealt with relatively high soluble VOCs (H (25 °C) below 5 × 10^{-4}) what rendered the use of surfactants relatively unnecessary. For example, Wang et al. (2018) explored the role of Tween 80 in facilitating the removal of m-xylene (H (25 °C) = 9.8 × 10^{-8}) in a BTF, concluding that concentrations ranging from 50 to 100 mg L^{-1} (approximately 3–6 × CMC) could enhance the bioavailability of m-xylene and improve its degradation efficiency. Hajizadeh et al. (2018) investigated the influence of adding Tween 80 (approximately 2.8 mg L^{-1}, approximately 0.18 × CMC) to a BF packed with scoria-bagasse and compost for the treatment of 1,1-dimethylhydrazine. Notably, the addition of Tween 80 did not significantly affect the BF's EC (approximately 0.7 g m^{-3} h^{-1}). This observation can be attributed to the high solubility of the compound (H (25 °C) = 5.3 × 10^{-4}) and the relatively low concentration of Tween 80 applied. Other studies have also documented the favorable impact of introducing Tween 20 into bioreactors but again for the treatment of highly soluble VOCs. Wang et al. (2014) explored the effects of adding Tween 20 (11.8 mg L^{-1}, approximately 0.74 × CMC) and Zn (II) (1 mg L^{-1}) to a BTF containing polyurethane foam for the treatment of ethylbenzene (H (25 °C) = 8.9 × 10^{-8}) Their findings indicated that the RE and EC of the supplemented BTF (EC = 61–181 g m^{-3} h^{-1}, RE = 61–94%) exceeded those of the control BTF (EC = 48–132 g m^{-3} h^{-1}, RE = 37–74%). Lamprea Pineda et al. reported the effects of Tween 80 and saponin on the removal of cyclohexane and hexane in biofilters (BFs) treating polluted air streams. Three biofilters were tested: BF1 and BF2 filled with a compost and wood chip mixture, and BF3 filled with expanded perlite. Tween 80 and saponin were selected from five surfactants based on their ability to enhance VOC removal by decreasing gas-liquid partitioning and supporting microbial growth. Lamprea-Pineda et al. (2024) recently evaluated the addition of (bio)surfactants in BFs packed with a mixture of compost and wood chips as well as expanded perlite to enhance the removal of cyclohexane and hexane from a polluted gas stream. Results showed that adding Tween 80 at 1 × CMC slightly improved cyclohexane removal in the BF packed with wood chips (EC = 7.0 ± 0.6 g cyclohexane m^{-3} h^{-1}; RE = 85 ± 2% at 163 s), compared to no surfactant (EC = 6.7 ± 0.4 g m^{-3} h^{-1}; RE = 76 ± 2%). However, Tween 80 had a negative effect in the BF packed with perlite, reducing cyclohexane removal at 1 × CMC (EC = 2.4 ± 0.4 g cyclohexane m^{-3} h^{-1}; RE = 30 ± 4%) compared to no surfactant (EC = 4.6 ± 1.0 g cyclohexane m^{-3} h^{-1}; RE = 43 ± 8%). In

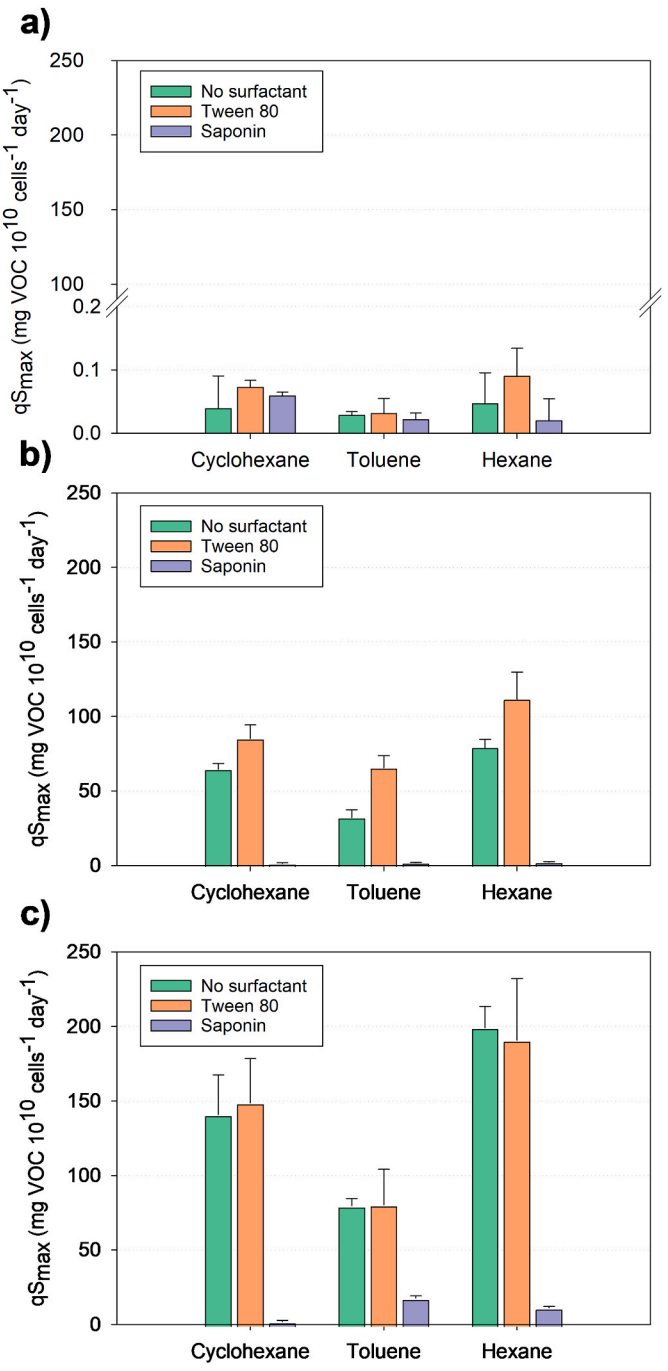


Fig. 3. Maximum specific substrate consumption rates (qS_{max}) for cyclohexane, toluene, and hexane without surfactant, with 1 × CMC Tween 80, and with 1 × CMC saponin by a) pure culture of *R. erythropolis* (X_0), b) a microbial consortium adapted to cyclohexane removal (X_1), and c) a microbial consortium adapted to cyclohexane removal in the presence of Tween 80 (X_2).

contrast, saponin at 3 × CMC significantly enhanced removal in all BFs, especially in the one packed with perlite where cyclohexane removal doubled (EC = 9.1 ± 0.6 g cyclohexane m^{-3} h^{-1}; RE = 49 ± 3% vs. EC = 4.5 ± 0.4 g cyclohexane m^{-3} h^{-1}; RE = 23 ± 3% without surfactant).

3.2. Short-term effect of (bio)surfactants on cyclohexane, hexane and toluene removal

To study the impact of (bio)surfactants on the removal of three model VOCs by different biomasses, batch tests were conducted using *R. erythropolys* (X_0) and the microbial consortia harvested from the STBR after adapting to cyclohexane removal in either the absence (X_1) and presence (X_2) of Tween 80. Additionally, this experiment aimed to provide new insights into the long-term adaptation of the biomass to Tween 80 in the STBR towards the removal of other VOCs with and without (bio)surfactants. To consider the influence of surfactants on biomass concentration, the removal of these VOCs was examined using specific substrate consumption rates (qS) calculated by Eq. (5). Fig. 3 presents a compilation of the maximum qS values (qS_{max}) for each VOC, obtained from the experiments conducted with and without Tween 80 and saponin.

In view of Fig. 3, it is clear that the pure culture of *R. erythropolys* (Fig. 3a) exhibited significantly lower qS_{max} values compared to the microbial consortia that were adapted by cyclohexane treatment for removing hydrophobic VOCs, both with and without surfactants (Fig. 3b and c). The reason for these low qS_{max} values in the pure culture of *R. erythropolys* (X_0) is that none of the VOCs studied (cyclohexane, toluene, and hexane) were significantly eliminated, regardless of the presence or absence of surfactants. Fig. S3 indeed shows that only toluene exhibited degradation in the presence of saponin, after 7 days. This poor performance contrasts with the literature, where this species is reported to be capable of degrading a wide variety of VOCs, including n-alkanes, such as hexane, and aromatics such as toluene or benzo[α] pyrene (Cappelletti et al., 2015; Thi Mo et al., 2022; Vergara-Fernández et al., 2018). This may be attributed to the necessary adjustment time required for the strain's metabolism. Like *R. erythropolys*, many microorganisms are able to grow on various substrates, and typically, the change of substrate requires an intermediate lag phase, the duration of which depends on the pre-culture conditions and the type and concentration of the substrates (Bate et al., 2023; Nicola and Bååth, 2019). It should be noted that this strain was initially grown on glucose and, after a period of starvation (4 days), was directly exposed to the VOCs, which may have rendered it unable to degrade these compounds during the studied period (9 days).

Afterwards, the same experiments were repeated using the biomass sampled from the STBR on day 146 and adapted to remove cyclohexane (X_1). Unlike X_0, this consortium was able to remove cyclohexane, toluene, and hexane within the 9-day experiment, both in the presence and absence of surfactants (Fig. S4). Consequently, this removal caused the qS_{max} to be much higher than those obtained by the pure *R. erythropolys* culture. The best results were obtained using Tween 80 with qS_{max} ranging from 66 ± 8 to 112 ± 18 mg VOC 10^{-10} cells day^{-1}, and without surfactants with qS_{max} ranging from 32 ± 5 to 79 ± 5 mg VOC 10^{-10} cells day^{-1} (Fig. 3b). Using saponin, qS_{max} ranged from 1 to 2 mg VOC 10^{-10} cells day^{-1}. Although the VOCs were also consumed using saponin to a similar degree or even faster than without surfactants or with 1 × CMC Tween 80 (Fig. S4), the bacterial growth-promoting effect of this surfactant (final biomass without surfactants or with 1 × CMC Tween 80 was (9.0 ± 2.6) x 10^5 cells mL^{-1}, while with saponin it grew to (340 ± 41) x 10^5 cells mL^{-1}) resulted in a much lower qS_{max}. In contrast, as shown in Fig. 3b, the addition of Tween 80 to X_1 improved the qS_{max} of all tested VOCs compared to the experiments without surfactant. Thus, it improved the removal of cyclohexane (65 ± 4 mg VOC 10^{-10} cells day^{-1} without surfactants versus 85 ± 9 mg VOC 10^{-10} cells day^{-1} with Tween 80), toluene (32 ± 5 mg VOC 10^{-10} cells day^{-1} without surfactants versus 66 ± 8 mg VOC 10^{-10} cells day^{-1} with Tween 80) and hexane (79 ± 5 mg VOC 10^{-10} cells day^{-1} without surfactants versus 112 ± 18 mg VOC 10^{-10} cells day^{-1} with Tween 80). Furthermore, the inclusion of Tween 80 resulted in a faster removal of various VOCs in all tests, occurring 24–48 h earlier compared to experiments without surfactants (Fig. S4).

Finally, the same experiments were carried out using biomass from the STBR (sampled at day 247) that had been adapted to cyclohexane removal for 101 days with 3 × CMC Tween 80 (X_2). The qS_{max} obtained by this biomass were the highest obtained in the study. Despite the low concentration of biomass present especially in the tests without

surfactant and Tween 80, i.e. (5.1 ± 1.6)x10^5 cells mL^{-1}, the different VOCs were removed at the same or even higher rate than with biomasses X_0 and X_1 (Fig. S5). However, unlike previous findings, the addition of Tween 80 did not result in a significant enhancement on the VOCs removal by this biomass. Overall, the addition of Tween 80 had a neutral effect, with no greater than 6% variation in qS_{max}. These results suggest that the biomass may have developed the ability to degrade Tween 80 as an alternative carbon source, indicating an adaptive metabolic response within the microbial communities. The utilization of Tween 80 as a competitive substrate may reduce the consumption of VOCs during biodegradation (Cheng et al., 2018b). This observation could explain the reduced mass transfer enhancement typically expected when incorporating surfactants into microbial consortia. For example, the study of Zhao et al. (2015) demonstrated that the degradation of beta-cypermethrin by *Bacillus licheniformis* was influenced by the presence of Tween 80. While Tween 80 increased the solubility of the contaminant, the bacteria preferentially utilized it as a substrate, thereby decreasing the degradation of the target compound. Similarly, other authors reported that although the addition of Tween 80 facilitated the adsorption of contaminants such as organochlorines, this did not lead to improved removal by microbial consortia (Betancur-Corredor et al., 2015). In conclusion, these findings align with the main conclusion of our study, which demonstrated that the effect of surfactants is highly dependent on the type of VOC and the specific microbial consortium involved.

The highest qS_{max} values obtained in this study were 149 ± 27 mg cyclohexane 10^{-10} cells day^{-1}, 80 ± 5 mg toluene 10^{-10} cells day^{-1}, and 199 ± 14 mg hexane 10^{-10} cells day^{-1} when using biomass X_2 adapted to Tween 80. These values align well with those reported by Lee and Cho (2008) who characterized the degradation of cyclohexane and hexane by *Rhodococcus* sp. *EC1*. In their study, they found maximum specific VOC degradation rates of 246 μmol cyclohexane g-dry cell $weight^{-1}$ h^{-1} and 361 μmol hexane g-dry cell $weight^{-1}$ h^{-1}. According to biomass conversion factors reported by Myers et al. (2013), this translates to values of 146 mg cyclohexane 10^{-10} cells day^{-1} and 102 mg hexane 10^{-10} cells day^{-1} which are of similar magnitude to the values observed in our study. Wei et al. (2016) investigated the impact of various surfactants (Brij 30, Brij 35, Triton X-100, and Tween 80) on hexane biodegradation by bacteria and fungi using batch tests. While they reported the removal of hexane per unit biomass (up to ≈55 mg hexane g^{-1} biomass), they did not provide details regarding the time required for this removal or the methodology used to determine the biomass, making it challenging to compare their findings to our study.

3.3. Microbial analysis

Since the results obtained in the present study suggest that the effect of surfactant addition is highly dependent on the microbial consortium

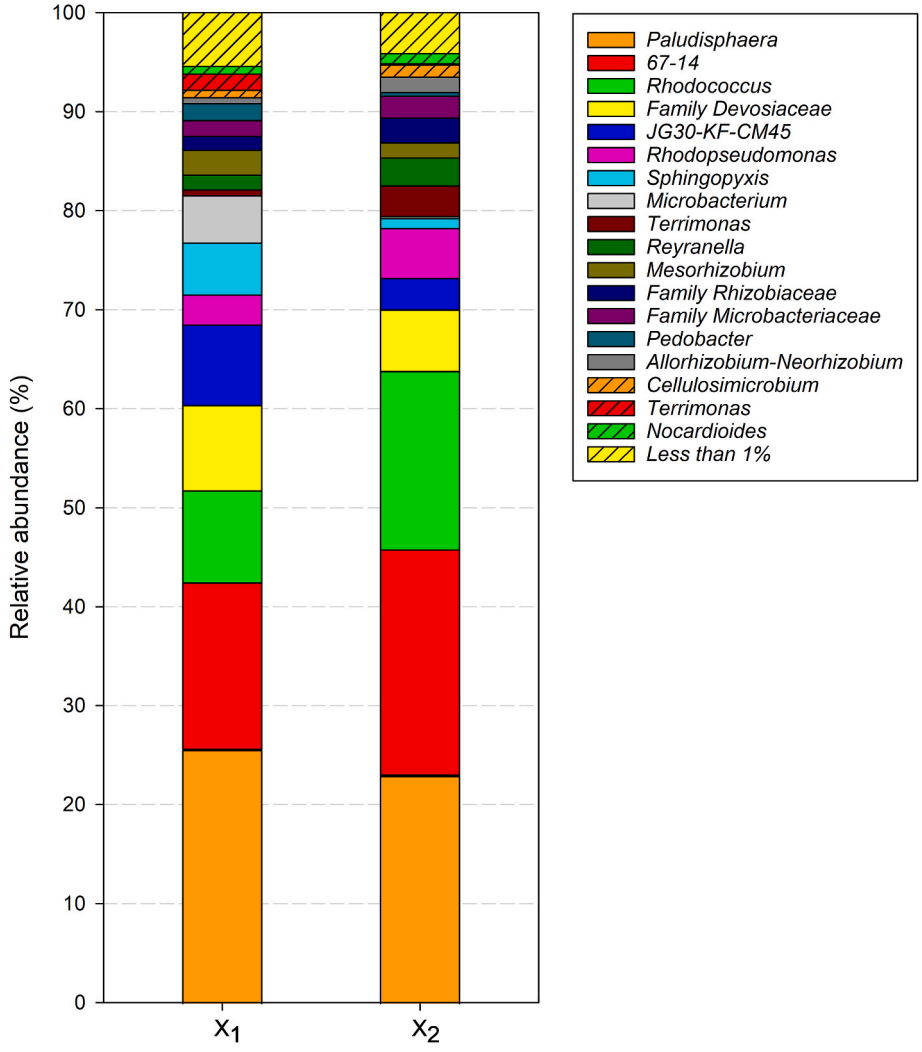


Fig. 4. Relative abundance of operational taxonomic units (OTUs) at the genus level with a relative abundance exceeding 1% in the microbial consortium adapted to cyclohexane removal without Tween 80 (X_1) and with Tween 80 (X_2).

present, the composition of the microbial consortia adapted to cyclohexane removal in the absence (X_1) and presence (X_2) of Tween 80 was analyzed by sequencing the 16s ribosomal gene. After denoising, 55 distinct operational taxonomic units (OTUs) were identified. Simpson ($X_1 = 0.8774$, and $X_2 = 0.8522$) and Shannon ($X_1 = 2.606$, and $X_2 = 2.387$) diversity indices showed that the samples lacked a single dominant microorganism. However, these values do not show as much diversity as in bioreactors with immobilized biomass such as BTF or BF, where the Simpson and Shannon indices easily exceed 0.9 and 2.5, respectively (Lamprea Pineda et al., 2023a). This is because of the selective nature of the environment where the biomass is located, which, being liquid, provides more restrictive conditions for growth.

The dominant phyla were *Actinobacteriota* (35% in X_1 and 47% in X_2), *Proteobacteria* (26% in X_1 and 23% in X_2), and *Planctomycetota* (26% in X_1 and 23% in X_2), and – to a lesser extent – the phyla *Chloroflexi* (8% in X_1 and 3% in X_2) and *Bacteroidota* (5% in X_1 and 4% in X_2).

The distribution of the OTUs at the genus level is shown in Fig. 4. More than 65% of both consortia were formed by 5 OTUs (genera *Paludisphaera, 67-14, Rhodococcus* and *JG30*-KF-*CM45,* and family *Devosiaceae*).

The genus *Paludisphaera* was the majority in both samples (23–26%). Although the phylum to which they belong (*Planctomycetes*) is widely distributed across various environments, little is known about their potential functions in the environment. These chemo-organotrophic bacteria have mainly been attributed to be slow-acting decomposers of plant-derived organic matter (Hao et al., 2023; Ivanova et al., 2017). However, the genomes of various *Planctomycetes* have been reported to encompass metabolic pathways responsible for breaking down a variety of VOCs, such as ethylbenzene, aminobenzoate, chloroalkanes, polycyclic aromatic hydrocarbons (PAHs), toluene, benzoic acid, chlorocyclohexanes, and chlorobenzenes (Lage et al., 2019). Given their high long-term relative abundance and metabolic capabilities of the order in which this genus is classified, these results suggest that this genus possesses the metabolic tools necessary to degrade cyclohexane.

The second most abundant OTU was the genus 67-14 (17–23%) belonging to the order *Solirubrobacterales*. This genus has been reported as a potent degrader of a variety of hydrocarbons, including polycyclic aromatic hydrocarbons (Lin et al., 2023) and xenobiotic substances such as (polychlorinated-) biphenyls (Pagé et al., 2015), suggesting that it plays a relevant role in the removal of cyclohexane and VOCs in the STBR and batch tests.

The genus *Rhodococcus* was found to have a relative abundance between 9.3% (X_1) and 18% (X_2). This genus has been widely reported in bioreactors carrying out the removal of a large number of hydrocarbons (Cappelletti et al., 2015; Thi Mo et al., 2022; Zhao et al., 2022). Although the nature of the analysis employed does not allow us to know the species to which this OTU belongs, given that the bioreactor was inoculated with *R. erythropolys* and the operating conditions were optimal for its development, it is very likely that it belongs to this species. In the probable case that this OTU belongs to the species *R. erythropolys*, the question remains why this species in pure culture was not able to degrade VOCs in the batch tests, while it prevailed in a bioreactor only fed with cyclohexane for 146 and 247 days, respectively. The most plausible explanation for this phenomenon is the adaptation of the species or the existence of complementary metabolic pathways between the different members of the microbial community (Cao et al., 2022). In microbial consortia, different species often possess diverse metabolic pathways. While *R. erythropolys* alone may lack certain enzymes or pathways required for VOC degradation, other microorganisms in the consortium could fill these metabolic gaps, allowing for a more comprehensive degradation of VOCs (Cao et al., 2022). In this context, other consortium members may produce intermediate metabolites during cyclohexane biodegradation, which are subsequently used by *R. erythropolys* to complete VOC degradation, enhancing the overall efficiency of VOC removal. These facts can also be explained by other phenomena observed in microbial consortia, such as the stress response where some microorganisms release stressors or signaling molecules (Lories et al., 2020). These molecules may have induced stress responses in *R. erythropolys*, leading to the activation of VOC degradation pathways that might not occur when *R. erythropolys* is in isolation. Additionally, cooperative gene expression could be at play, where the presence of specific species triggers the expression of particular genes in others, further contributing to the degrade certain substrates (Duncker et al., 2021).

A better performance of *R. erythropolys* forming part of a microbial consortium has been reported by other authors, such as Morales et al. (2017), using serum bottles with vermiculite as a solid support to mimic a biofilter. The authors reported that the use of a consortium of *Fusarium solani* and *R. erythropolys* shows improved performance in terms of mineralization rate towards the degradation of formaldehyde, toluene, and benzo[a]pyrene compared with a pure culture of *R. erythropolys*. The authors attributed the observed improvement to two key factors. First, the complementary metabolic pathways and capabilities of the two microorganisms facilitated the more efficient degradation of pollutants. Second, the higher surface hydrophobicity and lower partitioning coefficient of the mixed-species biofilm in comparison to the monospecific biofilm played a crucial role. The partitioning coefficient represents the ratio of the concentration of a substance in two different phases, indicating the equilibrium distribution of the substance between them. In this case, the lower partitioning coefficient of the mixed-species biofilm suggests that the pollutants are more readily distributed in the biofilm, contributing to improved pollutant transport and enhanced biodegradation.

In relation to the differences found between both samples, the negative effect of the addition of Tween 80 on *Microbacterium* (4.7% in X_1 versus 0.2% in X_2), *Sphingopyxis* (5.3% in X_1 versus 1.0% in X_2) and *JG30*-KF-*CM45* (6.6% in X_1 versus 1.5% in X_2), members with a high capacity to eliminate a wide variety of hydrocarbons (Brzeszcz and Kaszycki, 2018; Sharma et al., 2021; Speirs et al., 2019), were the most important.

On the contrary, there were also OTUs that were promoted by the addition of Tween 80 such as *Rhodopseudomonas* (3.1% in X_1 versus 5.1% in X_2), *Terrimonas* (0.6% in X_1 versus 3.1% in X_2) or *Reynarella* (1.5% in X_1 versus 2.8% in X_2). *Rhodopseudomonas* could degrade and employ Tween 80 as a carbon source given its extraordinary metabolic versatility, carbon source diversity and metabolite diversity. (Brown et al., 2022; Li et al., 2022). *Terrimonas* is a bacterium known to degrade VOCs such as benzo[a]pyrene (Song et al., 2015) and methyl tert-butyl ether (Liu et al., 2009). In several independent studies, an evident surge in its relative abundance was noted following the introduction of surfactants, including polyoxyethylene sorbitan (Tween) (Adrion et al., 2016; Singleton et al., 2016). This trend suggests that *Terrimonas* is resilient to environmental shifts induced by surfactant addition, possibly indicating its ability to utilize these compounds as a source of carbon and energy. *Reyranella* comprises exceptionally adaptable microorganisms that thrive across a diverse array of environments. They have previously been identified in bioreactors dedicated to eliminating VOCs such as toluene and dichloromethane (Cheng et al., 2018a; Xu et al., 2019).

In summary, the microbial samples revealed an intricate web of potential VOC degraders that play a crucial role in the effective operation of the system. The abundance of the genus *Rhodococcus* remained below 20%, indicating that if bioaugmentation strategies are to be applied to improve removal, it will be necessary to pre-adapt the species comprising the inoculum with the VOCs to be removed.

4. Conclusions

This study elucidates the impact of (bio-)surfactants on microbial consortium adaptation within bioreactors, providing insights into the mechanisms underlying the enhanced biodegradation process. The significant influence of Tween 80 on the biodegradation of cyclohexane in a

suspended growth bioreactor has been demonstrated. The addition of 3 × CMC Tween 80 led to a substantial increase in the cyclohexane EC and RE from 32 ± 1 to 145 ± 25 mg m^{-3} h^{-1} and from 29 ± 6 to 87 ± 1%, respectively, highlighting the potential of surfactant-mediated biofiltration as an effective strategy for improving hydrophobic VOC removal. A pure culture of *Rhodococcus erythropolys EC1* grown on synthetic medium enriched with glucose exhibited very limited VOC removal. Concentrations between 1 × and 3 × CMC of Tween 80 improved removal using biomass that was not previously exposed to the surfactant (X_1) in the short- and long-term, respectively. This enhancement effect of Tween 80 addition was reduced after the microbial consortium was adapted to Tween 80 for 101 days (X_2). The addition of Quillaja saponin at 1 × CMC significantly increased biomass growth, highlighting its potential as an alternative carbon source for microorganisms and thereby limiting its use in biofiltration. The predominant genera in X_1 and X_2 were *Paludisphaera* (23–26%), *67-14* (17–23%), and *Rhodococcus* (9.3–18%). The insights gained from this study have the potential to be applied not only to cyclohexane, but also to other hydrophobic VOCs, expanding its practical relevance in environmental and industrial settings.

CRediT authorship contribution statement

J.J. González-Cortés: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **P.A. Lamprea-Pineda:** Writing – review & editing, Methodology, Investigation, Formal analysis. **M. Ramírez:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **H. Van Langenhove:** Writing – review & editing, Supervision, Conceptualization. **K. Demeestere:** Writing – review & editing, Supervision, Conceptualization. **C. Walgraeve:** Writing – review & editing, Supervision, Project administration, 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.jenvman.2024.122874.

Data availability

Data will be made available on request.

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