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Comparative evaluation of bacterial and fungal removal of indoor and industrial polluted air using suspended and packed bed bioreactors.

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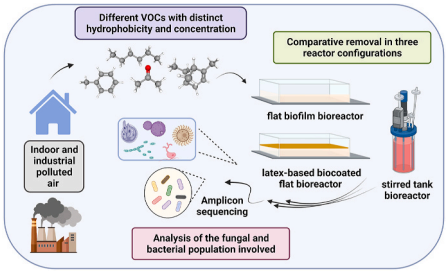
HIGHLIGHTS

- Comparison of different reactor configurations for the removal of indoor air VOCs.
- The stirred tank reactor showed the best VOC removals at high inlet concentrations.
- VOC removals>97% achieved at low inlet concentration in all reactor configurations.
- *n*-hexane was more efficiently removed in a flat biofilm reactor without latex.
- The specific operation mode affected the bacterial and fungal population.

GRAPHICAL ABSTRACT



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ABSTRACT

The abatement of indoor volatile organic compounds (VOCs) represents a major challenge due to their environmental risk, wide nature and concentration variability. Biotechnologies represent a cost-effective, robust and sustainable platform for the treatment of hazardous VOCs at low and fluctuating concentrations. However, they have been scarcely implemented for indoor air purification. Thus, little is known about the influence of the reactor configuration or the VOC nature and concentration variability on the removal, resilience and the microbial population of bioreactor configurations susceptible to be implemented, both in indoors and industrial environments. The present study aims at comparing the removal performance of four VOCs with different hydrophobicity and molecular structure -acetone, *n*-hexane, α-pinene and toluene-at two inlet concentrations (5 and 400 mg m^{-3}), which mimics the concentrations of contaminated indoor and industrial air. To this aim a stirred tank, flat biofilm and latex-based biocoated flat bioreactor were comparatively evaluated. The results demonstrated the superior performance of the stirred tank reactor for the removal of hydrophilic VOCs at high inlet concentrations, which achieved removals >99% for acetone and toluene. At low concentrations, the removal efficiencies of acetone, toluene and α-pinene were >97% regardless of the bioreactor configuration tested. The most hydrophobic gas, *n*-hexane, was more efficiently removed in the flat biofilm reactor without latex. The microbial community analyses showed that the presence of VOCs as the only carbon and energy source didn't promote the growth of dominant bacterial members and the populations independently evolved in each reactor configuration and operation mode. The fungal population was more diverse in the biofilm-based bioreactors, although, it was mainly dominated by uncultured fungi from the phylum Cryptomycota.

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1. Introduction

Air pollution from both outdoor and indoor sources constitutes one of the most critical environmental risks to public health globally (World Health Organization, 2017). There is a clear evidence of the detrimental effects of poor indoor air quality (IAQ) on human health: respiratory illnesses, allergies and even cancerous and neurodegenerative diseases (Tan et al., 2022; Tran et al., 2019). In fact, according to the statistics of the World Health Organization (WHO), it has been estimated that around 5.5 million people die prematurely every year due to indoor air pollution (World Health Organization, 2017; Yue et al., 2021). These values are expected to increase in the current scenario of rising economic activity and the increasing human exposition to indoor air as a result of the construction of energetically efficient residential and public utility buildings (Śmiełowska et al., 2017).

Indoor air contains hundreds of different pollutants including chemicals, particulate matter, biological pollutants (virus, allergens, bacteria, mold, fungi, spores, etc.) and physical agents (temperature, electromagnetic fields). Chemical pollutants are divided into inorganic compounds (CO, NO_x, O_3) and volatile organic compounds (aromatic hydrocarbons, aldehydes and terpenes) (González-Martín et al., 2021; Yue et al., 2021). Due to the increasing exposure to indoor air pollutants, their severe impact on human health, and the enforcement of IAQ standards, the development and optimization of versatile technologies for *in-situ* indoor air purification is increasingly demanded. For instance, particle removal is performed by well-established and highly efficient technologies based on mechanical and/or electronic filtration (Cheek et al., 2021). Moreover, technologies to treat inorganic chemical pollutants are actively being developed due to the hazardous nature of CO, NO_x and O_3. However, effective preventive measures or abatement technologies for VOCs are very unusual and rarely implemented in indoor air purification systems due to the large number of VOCs found in indoor air (over 400) and their concentration variability (González-Martín et al., 2021).

The few technologies commercially available for the abatement of indoor air VOCs are based on physical-chemical methods such as adsorption or catalytic oxidation (Yue et al., 2021). These technologies exhibit a limited performance at typical indoor environmental conditions, such as high humidity, fluctuating pollutant loads or complex VOCs mixtures. Besides, pollutants are either retained or transformed into secondary hazardous by-products (González-Martín et al., 2021). Biotechnologies have emerged as a promising alternative for VOC removal, the most common conventional bioreactors implemented for VOCs removal being bioscrubbers, biofilters and biotrickling filters (Barbusiński et al., 2020). They are based on the transformation of pollutants throughout the biocatalytic action of specific organisms in safely-closed bioreactors (Biswas et al., 2015; Estrada et al., 2013). Microorganisms can cope with low pollutant concentrations, and can simultaneously remove a wider range of VOCs. Thus, biotechnologies based on the use of microorganisms, if well implemented, can be more cost-effective, sustainable and robust than physical-chemical methods (Lamprea Pineda et al., 2021). Despite their potential, biotechnologies for VOCs treatment are still limited by the low mass transfer of the highly hydrophobic VOCs, which hampers their availability to the bacterial community and increases bioreactor footprint, and by the small portfolio of organisms capable of biodegrading these hazardous and poorly biodegradable compounds.

To increase the mass transfer of VOCs to the microbial community, innovative high-mass transfer bioreactor configurations can be implemented. A novel bioreactor configuration that has been proven to enhance the treatment performance of VOCs are latex-based biocoating bioreactors (Estrada et al., 2015; González-Martín et al., 2022). They consist of packed bed bioreactors where a bioactive polymeric coating (such as latex), with a high affinity for the target pollutant, is added to the packing material. According to previous macroscopic studies, cells grow confined within the polymeric coating forming a biofilm, thus preventing an undesired microbial release and decreasing potential pathogenic risk (Estrada and Quijano, 2020). The latex-based biocoating allows for a direct contact between the gas pollutants and the attached cells, improving the bioavailability of hydrophobic VOCs for the microorganisms responsible for their degradation (Estrada and Quijano, 2020). As a result, a lower gas residence time will be required to achieve good removal performances, boosting the compactness of the bioreactor. Nevertheless, little is still known about the effect of this type of bioreactors on biological activity in terms of the availability of nutrients and water in the biocoating, and about the effect of latex on the structure of the microbial communities (Cantera et al., 2015).

The best known microorganisms able to metabolize VOCs are pure bacterial strains with large-genomes and metabolic versatility, such as the genera *Pseudomonas*, *Burkholderia* and *Rhodoccocus* (He et al., 2019; Kumar et al., 2008; Zampolli et al., 2021). However, recent metagenomic and amplicon sequencing analyses in VOC rich environments have shown that there is a wide spectrum of bacterial species (several of them with very small genomes) that could be applied in the degradation of persistent VOC compounds (Chu et al., 2021; González-Martín et al., 2021; Toth et al., 2021). Besides bacteria, several fungi have shown to play a key role in the degradation of aromatic hydrocarbons and terpenes (Marycz et al., 2022a,b). Nevertheless, there is a limited understanding of the potential and role of fungi in VOCs catabolism in gas-phase bioreactors (Prenafeta-Boldú et al., 2019; Zhang et al., 2020).

This research assessed the elimination performance, resilience, and microbiology of three types of bioreactors (with and without latex-based immobilization) when they were exposed to different model VOCs with distinct hydrophobicity and concentrations (mimicking indoor and industrial pollution). To this aim, we evaluated the VOCs elimination performance of a suspended-growth stirred tank reactor, a flat biofilm bioreactor packed with a microfiber fabric, and a flat biofilm bioreactor packed with a microfiber fabric and a latex-based biocoating. Acetone, *n*-hexane, α-pinene and toluene were selected as model VOCs with different hydrophobicity and molecular structure, and tested at two different pollutant loads (5 and 400 mg m^{-3}).

2. Materials and methods

2.1. Reagents and mineral salt medium

The model indoor air pollutants used were *n*-hexane (CAS-110-54-3), α-pinene (CAS-80-56-8), toluene (CAS-108-88-3) and acetone (CAS-67-64-1). *n*-hexane, acetone and toluene were supplied by Panreac® (Barcelona, Spain), while α-pinene was supplied by Sigma-Aldrich (Madrid, Spain). The mineral salt medium (MSM) was prepared according to González-Martín et al. (2022). The reagents were purchased from Panreac® (Barcelona, Spain) with a purity >99%. PRIMAL™ SF-208 ER (Dow Chemical, Germany) supplied by Brenntag Química (Barcelona, Spain) was used as latex for biocoatings formulations (alkylphenol ethoxylates and biocide free, acrylic-styrene copolymer; solids content 48.05%; pH 8.0–9.5).

2.2. Experimental set-up and operational procedure

The experimental setup consisted of two parallel lines, designated as high concentration (HC) (inlet VOC concentration of ~400 mg m^{-3}) and low concentration (LC) (inlet VOC concentration of ~5 mg m^{-3}). Three different reactor configurations were tested under each concentration (Fig. 1): two stirred tank reactors (STR_HC and STR_LC) as model suspended growth bioreactors, two flat bed bioreactors (FB_HC and FB_LC) as model biofilm reactors, and two flat bed bioreactors with latex as biocoating (FBL_HC and FBL_LC). The STRs had a volume of 0.5 L (working volume of 0.4 L) and were magnetically stirred at 200 rpm. The four flat bed bioreactors consisted of transparent PVC chambers with internal dimensions of 70 × 10 × 2 cm, resulting in a working volume of 1.4 L. The chambers were packed with microfiber fabric (a

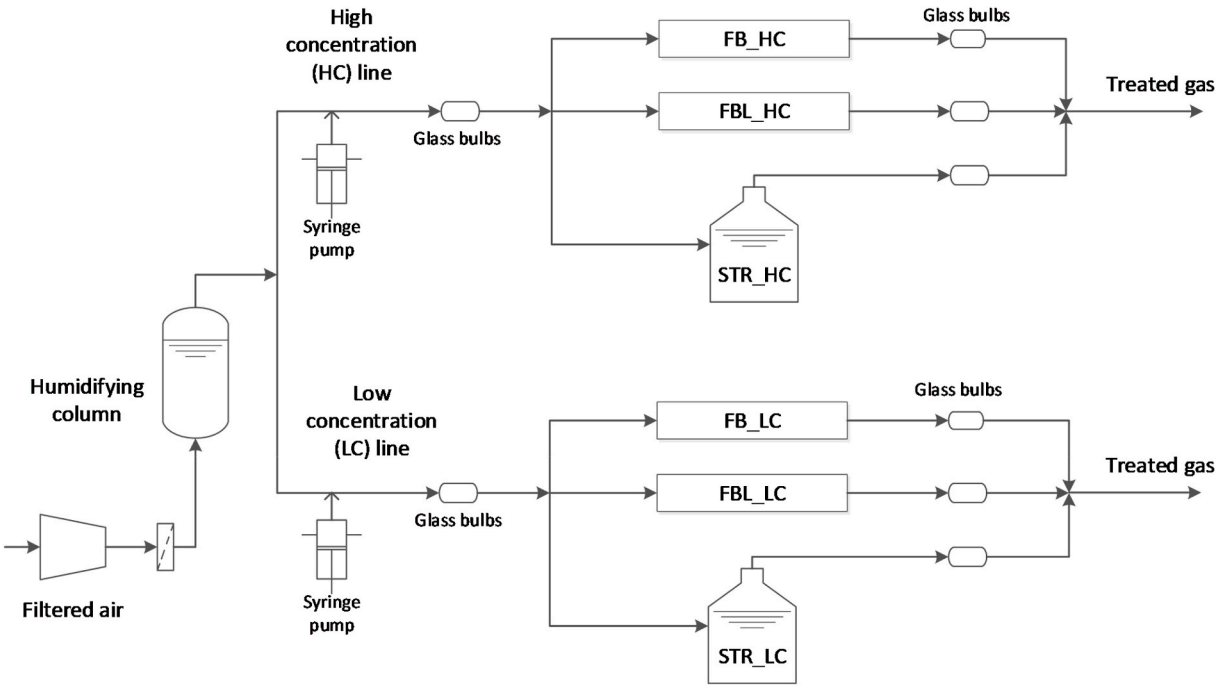

Fig. 1. Schematic representation of the experimental set-up. STR: Stirred tank reactor; FB: flat bed bioreactors; FBL: flat bed bioreactors with latex as biocoating.

non-woven material composed of 80% polyester and 20% polyamide) with a diameter between fibers of approximately 10 μm.

Air was compressed (ABAC B2500-50 2, Italy), filtered through a PTFE filter (0.22 μm pore size) and humidified in a 1 m water bubble column to prevent the desiccation of the biocoating. The humidified and filtered air stream was divided into two lines prior injection of the liquid model VOCs. The VOC mixture (30% hexane, 23% toluene, 23% pinene and 24% acetone w/w) was injected in the airstreams using a syringe pump (Chemyx Fusion 100, USA) and liquid syringes of different volumes (Hamilton, Australia). The final inlet concentrations fed to all the reactor configurations tested were 390.8 ± 26.9 and 5.0 ± 0.7 mg *n*-hexane m^{-3}; 455.8 ± 23.9 and 6.0 ± 0.6 mg toluene m^{-3}; 481.2 ± 46.4 and 7.2 ± 0.7 mg acetone m^{-3}; and 363.7 ± 38.9 and 4.0 ± 0.5 mg α-pinene m^{-3} in the HC and LC lines, respectively. The corresponding polluted air stream was fed into the reactors at different flow rates (i.e. empty bed residence time, EBRT) and inlet loads (Table 1). To carry out a systematic comparative evaluation, the reactors with different volumes were exposed to the same inlet loads at the two concentrations tested by applying a gaseous flow that was proportional to the reactor volume. The VOC laden streams were sparged into the STRs through 10 μm porous stainless steel diffusers located at the bottom of the reactors. Similarly, the VOC laden stream was injected through one side of the chamber and flowed over the biofilm surface.

Prior inoculation, a 2-days abiotic test was performed feeding the corresponding VOC mixture to the empty bioreactors in order to rule out any removal not related to microbiological activity. Inlet and outlet VOCs concentrations were analyzed every 10 h to ensure the stability of the system (Fig. S1, Supplementary materials). Fresh aerobic settled activated sludge (6.9 g dry weight L^{-1}) from the denitrification-nitrification wastewater treatment plant (WWTP) of Valladolid (Spain) was used as inoculum. Before inoculation, the sludge was centrifuged at 10,000 rpm for 10 min to remove external carbon sources. The pellet was then resuspended in the same volume of MSM. Each bioreactor was inoculated with ~80 mg of biomass (as volatile suspended solids). In the case of the STRs, 11.6 mL of the inoculum were added to the reactor and then filled up to 0.4 L with MSM. The same volume of inoculum was mixed with 28.5 mL of MSM and added to the flat bed bioreactors. In addition, either 20 mL of water or 40 mL of latex dilution (containing approximately 50% water) were added on the microfiber support.

The systems were operated at a constant temperature of 25 °C. Distilled water was added weekly to compensate water losses by evaporation in the STRs. In the FB and FBL, 15 mL of water were added daily during operation to ensure high humidity in the microfiber. Four different sets of operational conditions were tested at the two concentrations (Table 1). From day 42, biomass growth was likely hindered by the accumulation of metabolites in the STR operated at HC. Thus, from this day on, 200 mL of the bioreactor cultivation broth were replaced every week with biomass retention (the aqueous cultivation broth drawn was centrifuged at 10,000 rpm for 5 min, the biomass pellet resuspended in fresh MSM and returned to the bioreactor). By day 62, the flow was doubled in the reactors working at low VOC concentrations, and by day 92 the flow was halved in the reactors operated at high

Table 1
Operational conditions during VOC treatment in the different reactor configurations.

Line	Reactor	Stage (days)	Inlet load (g m^{-3} h^{-1})	Flow rate (mL min^{-1})	EBRT (min)
HC	STR_HC	I (1–91)	6	125	4
		II (92–157)	3	62.5	8
	FB/FBL_HC	I (1–91)	6	350	4
		II (92–157)	3	175	8
LC	STR_LC	I (1–61)	0.075	125	4
		II (62–157)	0.15	250	2
	FB/FBL_LC	I (1–61)	0.075	350	4
		II (62–157)	0.15	700	2

VOC concentration. Biological samples to study the microbial population structure were taken at the end of each operational stage in all bioreactors and preserved at −20 °C. To determine the removal efficiency (RE), VOC concentrations were periodically measured in 250 mL glass bulbs (Sigma-Aldrich, Madrid, Spain) at the inlet and outlet of the bioreactors as described in section 2.3. Total organic carbon (TOC), inorganic carbon (IC), pH and total suspended solids (TSS) were measured in the liquid samples withdrawn from the STR_HC from day 42 of experimentation.

2.3. Analytical procedures

VOCs were measured by gas chromatography in a GC-FID (Varian 3900) equipped with an Agilent HP-5MSI capillary column (30 m × 0.25 mm × 0.25 μm). Injector and detector temperatures were set at 150 and 200 °C, respectively. The oven temperature was set at 40 °C for 1.5 min, increased at 10 °C min^{-1} to 50 °C (held for 1 min), and finally increased at 40 °C min^{-1} to 250 °C (held for 1 min). Nitrogen was used as carrier gas (2.5 mL min^{-1}) and as make-up gas (25 mL min^{-1}). Hydrogen and air flowrates were set at 30 and 300 mL min^{-1}. In the experiments at low VOC concentration sampling was carried out by 10 min preconcentration of the pollutants in 250 mL glass bulbs using 85 μm CAR/PDMS SPME fibers (Supelco, Bellefonte, USA). The fibers were initially conditioned at 300 °C for 1 h prior to calibration, and a cleaning run was performed before sampling with the above-described GC-FID method. External standards of the VOCs, prepared in 250 mL glass bulbs, were used for quantification using the preconcentration conditions above-mentioned.

TOC and IC were determined using a TOC-VC_{SH} analyzer (Shimadzu, Japan). The pH was analyzed using a pH/mV/°C meter (CRISON pH meter BASIC 20+, Spain), and TSS were quantified using Standard Method 2540 D (APHA-AWWA-WPCF).

VOC removal efficiency (%RE), inlet load (IL) and empty bed residence time (EBRT) were calculated as follows:

$$\%RE = (1 - \frac{C_{out}}{C_{in}}) \times 100$$

$$IL = Q \times \frac{C_{in}}{V}$$

$$EBRT = \frac{V}{Q}$$

where C_{out} and C_{in} are the outlet and inlet pollutant concentrations (g m^{-3}), respectively, Q is the air flow rate (m^3 h^{-1}), and V the total volume of the reactor (m^3). The use of the total reactor volume allowed us to compare the actual space occupied by the different reactor configurations, an important fact when implemented for indoor air treatment.

2.4. Bacterial and fungal community analysis

Samples of 20 mL of the sludge from the WWTP of Valladolid (Inoculum) and of the cultivation broth and biofilm of each bioreactor were taken on day 62, 92 and at the end of operation (day 157) and preserved at −20 °C for bacterial and fungi population analysis. DNA extraction and Illumina Miseq amplicon sequencing were carried out by the Foundation for the Promotion of Health and Biomedical Research of the Valencia Region (FISABIO, Spain). Amplicon sequencing to analyze the bacterial population was developed targeting the 16 S V3 and V4 regions (464bp, *Escherichia coli* based coordinates) with the bacterial primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a- A-21. Amplicon sequencing was also developed targeting the 18 S V4 (position 571–980) in order to analyze the fungal population. The following forward and reverse tailed primers were used EUKA-F: 5′-GCCGCGGTAATTC-CAGCTC-3′ and EUKA-21 R: 5′- GCYTTCGYYCTTGATTRA-3′ following Illumina protocols. Libraries were prepared and sequenced on a Miseq-Illumina platform using nucleotide paired-end reads at FISABIO (Spain). The 16 S and 18 S rRNA gene sequences were processed and quality filtered using Mothur v1.44.3 following the Mother SOP (https://www.mothur.org/wiki/MiSeq_SOP) (Schloss, 2020). Bacterial sequences were then clustered at 97% identity threshold into Operational Taxonomic Units (OTUs) using the SILVA 16 S rRNA gene reference database (Version: 138.1). Fungal sequences were cluster at 90% identity using the population reference database UNITE (V. 8.3). The results obtained were doubled checked using SILVA 18 S rRNA gene reference database (Version: 138.1) through the silva next generation sequencing (Silva.ngs) platform (Quast et al., 2013). The nucleotide sequence dataset obtained in this study has been deposited at DDBJ/ENA/GenBank as bioproject: PRJNA843378. The rarefaction with 1000 randomizations showed that the smallest representative library for bacteria was obtained with 40,493 reads and for fungi with 57,834. Both community structures were then analyzed using R version 1.4.1 (Team, 2016). The main genera are shown in heatmaps plotted using the package *pheatmap* (Kolde, 2019).

3. Results and discussion

3.1. Influence of the bioreactor configuration, gas concentration and inlet load on the removal of VOCs

The abatement performance of the target VOCs was clearly impacted by the bioreactor configuration. In all the reactors tested, an initial removal of acetone, toluene and α-pinene during the first days of operation was observed (Fig. 2), likely due to the adsorption of the contaminants to the active sludge. After saturation, biotic removal slowly increased up to steady state due to microbial specialization and growth. At high VOCs concentrations, the suspended-growth STR showed the best removal efficiencies (REs) for acetone (99.5 ± 2%) and toluene (90.6 ± 2.1%), with a slight removal of α-pinene (14.7 ± 7.0%) and a negligible degradation of hexane during stage I. The STR_HC performance was not significantly influenced by MSM exchange from day 42 onwards. The TOC periodically measured in the liquid medium of STR_HC confirmed that there was biological degradation of the pollutants, since the VOCs concentration decreased in the liquid phase along time and the biomass dry weight increased along operation (Fig. S2 and Table S1). In the case of the flat bed bioreactor (FB), acetone removal remained roughly constant during the first 44 days at ~20%, progressively increasing up to average removals of 78.2 ± 9.9% by the end of stage I. Neither toluene, α-pinene nor hexane were degraded in FB. The higher outlet concentrations recorded for alpha-pinene in FB and FBL could be due to the random desorption kinetics of this VOC or the coelution in the GC column of any desorbed compound from the microfiber fabric. The presence of the latex biocoating in the flat bed bioreactor (FBL) improved the acetone RE recorded in stage I, with average values of 99.4 ± 0.3% from day 73 onward. The degradation of toluene was also slightly increased in comparison to FB, stabilizing by day 63 at ~18%. However, the degradation of α-pinene and *n*-hexane in FBL was negligible (Fig. 2). The lack of degradation of toluene, α-pinene and hexane in the FB and FBL during stage I was most likely related to the slow adaptation of the non-previously enriched population to the new culture conditions. The restrictive conditions that were harsher in FB and FBL (growth on a fixed support, restrictive carbon and energy sources and low water content, as well as the toxic concentrations of VOCs (higher mass transfer in the flat bed reactors)) most likely hindered the development of the microbial population, which required of longer time to acclimate to VOC degradation.

The gas residence time in the bioreactors operated at high inlet VOC concentrations was doubled by day 92 in order to decrease the inlet VOCs load. This change did not affect the removal efficiency of acetone in STR and FBL (>99%), while acetone removal immediately increased in FB to average values of 99.4 ± 0.2%. Similarly, toluene abatement

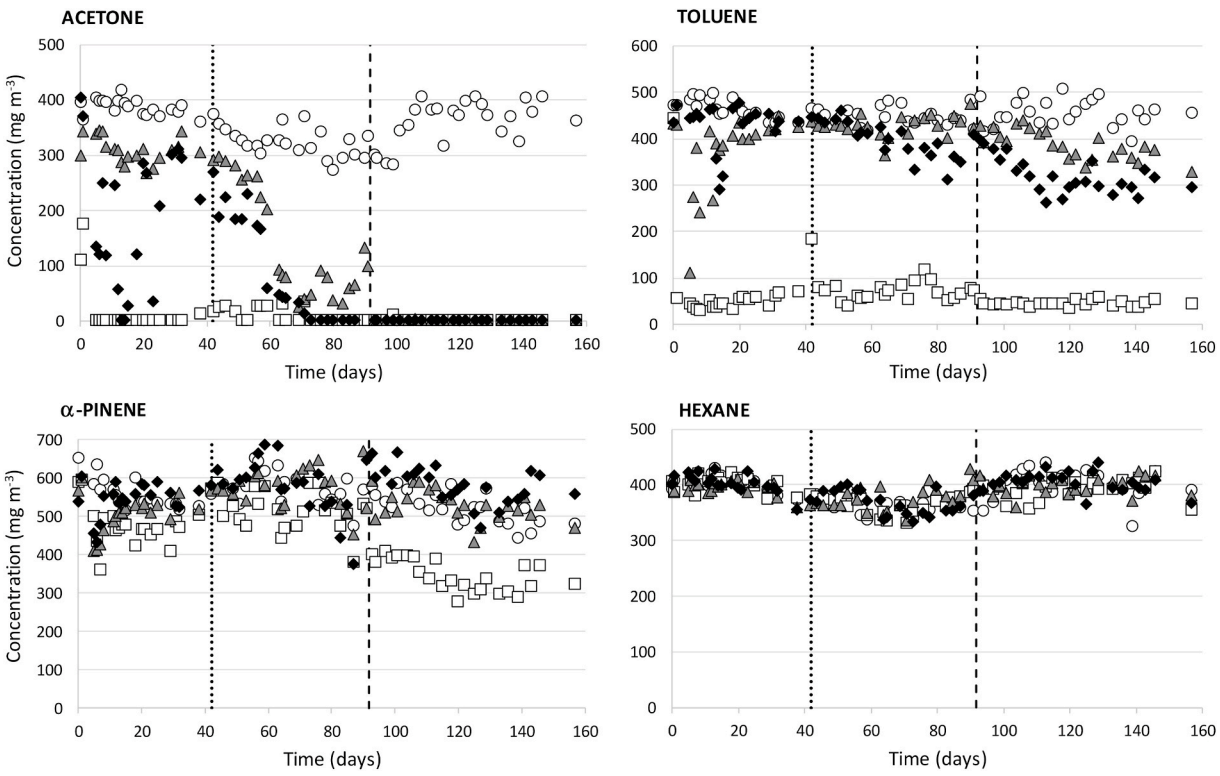

Fig. 2. Time course of acetone, toluene, α-pinene and hexane concentrations at the inlet (circle, ○) and outlet of the HC bioreactors: STR (square, □), FB (triangle, ▲) and FBL (diamond, ◆). Dotted line indicates the start of MSM exchange in the STR_HC and the dashed line represents the decrease in the VOCs inlet load.

also improved when increasing the gas residence time, with average values of 20 ± 6.1% and 33.5 ± 6.2% in FB and FBL, respectively. The higher gas residence time ~~likely~~ improved the availability of this pollutant to the microbial community present in the flat bed bioreactors, increasing its degradation, as it has been observed in previous research devoted to VOC treatment in stirred tank bioreactors (Estrada et al., 2015). However, no enhancement in the removal of α-pinene or hexane was recorded regardless of the bioreactor configuration. This could be attributed to the lower biodegradability of α-pinene compared to that of toluene and acetone, as previously reported by other authors (Marycz et al., 2022a,b), and to a lower mass transfer in the particular case of hexane, based on its higher hydrophobicity. Overall, acetone and toluene were effectively degraded in the stirred tank bioreactors regardless of the inlet load tested. Acetone has low hydrophobicity, while toluene and α-pinene are moderately hydrophobic VOCs ($H = 2.7 \times 10^{-1}$, $H = 1.5 \times 10^{-4}$, 2.1×10^{-4} mol m^{-3} Pa^{-1}, respectively (Sander, 2015)). On the other hand, *n*-hexane is more hydrophobic ($H = 6.0 \times 10^{-6}$ mol m^{-3} Pa^{-1}). Hence, its reduced bioavailability for the microbial community likely hindered the degradation of this compound.

At low VOC concentrations, the REs of acetone (>97%), toluene (97.5 ± 1.2%, 98.6 ± 1.8% and 98.8 ± 1.4% in STR, FB and FBL, respectively) and α-pinene (97.7 ± 1.1%, 99.1 ± 0.3% and 99.4 ± 0.3% in STR, FB and FBL, respectively) recorded during stage I were similar between the different configurations tested (Fig. 3). While a short acclimation period was necessary in FB and FBL to achieve a steady toluene abatement performance (5 and 10 days, respectively), a longer adaptation period was required in STR prior stabilization of α-pinene removal (20 days) compared to that of FB (7 days) and FBL (11 days). In the particular case of hexane, the highest removals were obtained in the FB_LC, achieving average values of 70.6 ± 9.2% from day 13–53, and increasing by the end of this stage I to ~90%. On the contrary, average hexane removals of 56.4 ± 10.5% from day 13 onward and of 45.9 ± 6.2% from day 32 onward were recorded in STR_LC and FBL_LC, respectively (Fig. 3).

The increase in the VOCs inlet load during stage II resulted in a slight increase in acetone removal regardless of the bioreactor configuration to values of ~99%, the STR requiring 20 days to recover previous steady state performance. Similar toluene removals were also recorded in the STR and FB compared to previous stage, the latter after a 17-day adaptation period. On the contrary, a slight decrease in toluene degradation in FBL to 90.8 ± 4.7% was observed from day 62–112, increasing to ~97% by the end of stage II. An effective removal was recorded for α-pinene and hexane in the STR at the beginning of stage II, achieving average values of 95.2 ± 2.7% and 86.1 ± 3.0%, respectively. However, by day 87, a failure in the air compressor for 24 h resulted in the injection of high VOC concentrations to the system, stabilizing two days after at 5.3 ± 0.4, 5.7 ± 0.4, 7.5 ± 0.6, 5.4 ± 0.5 mg m^{-3} of hexane, toluene, α-pinene and acetone, respectively, until the end of operation. This operational up-set triggered an initial deterioration of the removal of α-pinene and hexane in the STR. Nevertheless, after a stabilization period, the RE stabilized at ~80% of α-pinene and 37% of hexane, demonstrating the robustness of the process. The increase in the inlet load at the expenses of a lower gas residence time also resulted in lower α-pinene and hexane removals in the flat bed bioreactors. In FB_LC, α-pinene removals stabilized by day 93 at 73.6 ± 9.4% after an initial period of fluctuations. The detrimental impact of the air failure was also observed in the α-pinene abatement in FBL, finally stabilizing at 85.0 ± 4.4%. Finally, poor and unstable hexane removals were recorded in both flat bed bioreactors, ranging from ~3 to 40% throughout stage II. Similar results for hexane elimination were observed in a previous study

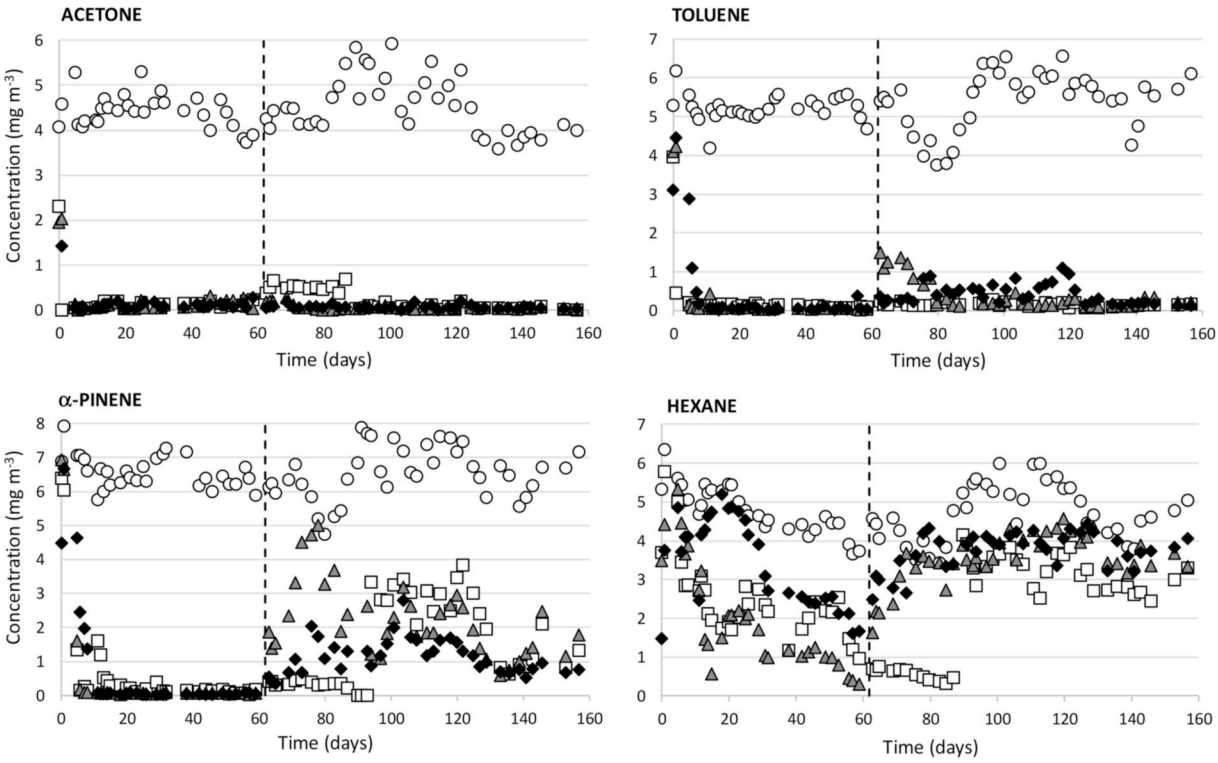

Fig. 3. Time course of acetone, toluene, α-pinene and hexane concentrations at the inlet (circle, ○) and outlet of the LC bioreactors: STR (square, □), FB (triangle, ▲) and FBL (diamond, ◆). Dashed line represents the increase in the VOCs inlet load.

where the operation of latex biocoating bed reactors packed with polyurethane foam achieved very low removals of this scarcely soluble pollutant (0–30%) (González-Martín et al., 2022).

The increase in the inlet load by increasing the air flowrate exerted a dual effect on the VOCs biodegradation performance. On the one hand, the higher load provided additional carbon to support the growth of the bacteria/fungi growing either in suspension, attached to the microfiber fabric packing material or embedded in the latex biocoating. This could support a better removal if the system is limited by microbial activity. On the other hand, the increase in the air flowrate resulted in a concomitant decrease in the gas contact time, which could hinder pollutants mass transport to the microbial community, thus decreasing substrate availability. In the event that the bioreactor is mass transfer limited, a detrimental impact on the VOC removal performance would be observed. Overall, a decrease in the abatement of the most hydrophobic indoor pollutants, α-pinene and toluene, was recorded regardless of the bioreactor configuration, suggesting a reduction in the gas-liquid mass transport to the degrading community.

It is also worth noting that the flat bed bioreactors did not outperform the conventional stirred tank reactor either at high or low concentrations, despite being designed to enhance the mass transfer of hydrophobic pollutants. Additionally, while α-pinene removal in FBL at low concentrations was higher than that supported by FB during stage II, FB provided a better hexane removal performance compared to the FBL during stage I. This fact could have been related to the different water absorption capacity of the microfibers when latex is present, which can modify the transport of the different VOCs. Nevertheless, a different specialization of the bacterial community could also explain the different removal performance of both packed bed reactors. In this context, a complete degradation of toluene and α-pinene has been successfully achieved in flat bed bioreactors, including latex biocoating flat bed configurations operated with pure and mixed cultures (González-Martín et al., 2022; López de León et al., 2019; Sun et al., 2013). However, the concentrations tested in these previous investigations were 10–50 times lower than the ones used in this study (~500 mg m^{-3}). In fact, when low concentrations were used in this experiment (~5 mg m^{-3}) a complete removal of toluene was obtained and α-pinene was almost completely abated.

3.2. *Influence of the bioreactor configuration, gas concentration and inlet load on the microbial population*

3.2.1. *Fungal community structure*

The fungal analysis displayed a total of 985,898 sequences that belonged to one uncultured fungi member and 4 fungal phyla Ascomycota, Basidiomycota, Cryptomycota and Chytridiomycota (Fig. S3). Overall, the population was dominated in all the reactors and conditions tested by members of the phylum Cryptomycota and the uncultured Fungi_LKM15, the most predominant fungal members in the inoculum. Only in the flat bed reactors operated at low concentrations (FB/FB_LC) the phylum Ascomycota became an important representative of the fungal community structure.

The fungal population in the inoculum presented a low richness (Fig. 4) and was dominated by uncultured members of the phylum Cryptomycota (74.0%) and members of the uncultured fungi_LKM15 (19.3%). In the STRs operated at high and low concentrations these fungal phyla were also the most dominant. At low concentrations, uncultured members from the phylum Cryptomycota represented 61.8 and 48.0% in STR during stage I and II, respectively, and the uncultured fungi LKM15 represented 23.5 and 41.0%. However, the majority of Cryptomycota members in STR operated at high concentrations belonged to the genus *Paramicrosporidium*. These fungi represented 61.3% in STR_HC in stage I and 56.0% by the end of stage II. Cryptomycota is a newly discovered phylum of early-diverging fungi that is estimated to comprise a massive amount of unstudied biodiversity and inhabit a wide variety of environments (Lazarus and James, 2015). Members of this phylum, such as *Paramicrosporidium,* have been previously related to the degradation of toluene (Marycz et al., 2022a,b; Zhang et al., 2020).

In the flat bed bioreactors operated at high concentrations

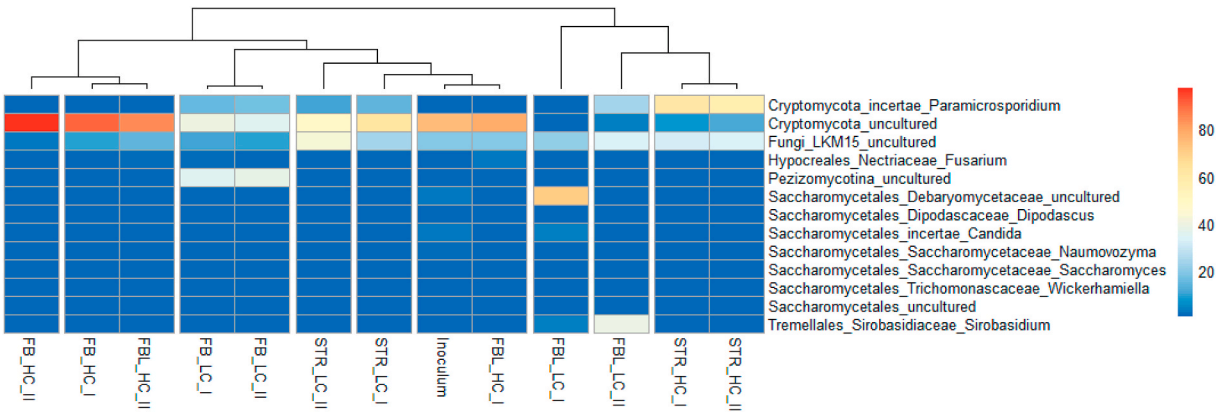

Fig. 4. Heat map of the fungal population in the different bioreactor configurations and conditions tested. The dendrogram on top represents hierarchical clustering of the sample. Data is presented as the logarithm of the total sequences found per sample.

uncultured members of Cryptomycota were the most dominant fungi, regardless of the presence of latex as biocoating. These uncultured fungi represented 97% and 85% of the total population by the end of operation of FB and FBL_HC. Interestingly, the genus *Paramicrosporidium* was almost negligible compared to the fungal population structure of STR_HC.

At low VOC concentrations the fungal populations diverged between the different flat bed reactors. Hence, the fungal population in FB_LC was dominated by members of uncultured Cryptomycota (~35%), and of the subphylum Pezizomycotina (phylum Ascomycota) (~35%) independenly of the inlet load. Members of Pezizomycotina, such as *Cladosporium* and *Fusarium*, represent some of the most common fungi identified during the biodegradation of n-alkanes (Marycz et al., 2022a, b; Zhang et al., 2019) and have been used before for indoor air purification (Prenafeta-Boldú et al., 2019). The addition of latex as biocoating in the flat biroeactor affected the fungal community structure in the FBL operated at low concentrations. The main fungi in FBL during stage I were uncultured members from the family *Debaryomycetaceae* (Ascomycota), which represented 70% of the population. However, the increase in the inlet load in FBL_LC during stage II promoted the growth of the fungal genus *Paramicrosporidium* (Cryptomycota), which represented 24% of the population and *Sirobasidium* (Basidiomycota), which outcompeted the representative members of *Debaryomycetaceae* by the end of stage II in FBL_LC (~40%). To the best of our knowledge, there are not reports associating these fungi with the degradation of VOCs. However, they possess the cytochrome P450s from the CYP52 family, which has been demonstrated to participate in the assimilation of alkanes and fatty acids in fungi (Ortiz-Álvarez et al., 2020).

Lower VOC concentrations promoted the growth of a more diverse and distinct fungal population in the flat bed bioreactors. Nevertheless, the fungal population did not significanthy diverge from the one found in the inoculum, with uncultured members of the phylum Cryptomycota and the uncultured Fungi_LKM15 as the most detected fungi.

3.2.2. Bacterial diversity and community structure

The analysis of the bacterial community in the sample set displayed a total of 872,303 sequences that belonged to 7287 OTUs affiliated with bacterial genera. The most diverse sample was the inoculum, which indicated that in each reactor and condition there was an specialization of the bacterial community, although, maximum dissimilarities were observed between the STRs and the FBs (FB and FBL) (Fig. S4).

Fig. 5 shows the most representative bacteria observed in the inoculum and during operation in the different reactor configurations. The main bacteria found in the inoculum corresponded to members of uncultured Candidatus Saccharibacteria (27.4%), Bacteroidetes_SJA_28 (12.9%), *Planctomycetaceae* (7.4%), *Rhodobacteraceae* (6.8%) and the genus *Methylibium* from the order Burkholderiales (6.3%). In the STR operated at high VOC concentrations, the most representative bacterial members during stage I belonged to uncultured candidates of the family *Anaerolineaceae* (34.0%) and Bacteroidetes_SJA_28 (14.8%). Doubling the EBRT time in STR_HC during stage II favoured the growth of *Stenotrophobacter* (11.2%) by the end of operation, while the uncultured members from the family *Anaerolineaceae* (35.5%) were still the most dominant bacteria. Uncultured members from this family have been already related with the degradation of long and short chain *n*-alkanes, complex naphtha hydrocarbons and benzene (Liang et al., 2016; Mohamad Shahimin et al., 2016). On the other hand, the presence of *Anaerolineaceae* members in the STR operated at low VOC concentrations was almost negligible, which can indicate that members of this family dominate when they are exposed to high concentrations of the hazardous VOCs studied here. In fact, the most dominant bacterial genus in the STR working at the lowest VOC concentration and inlet load (STR_LC in stage I) belonged to the genus *Stenotrophobacter* (14.7%). This genus was not representative in the inoculum and specialized and dominated both STRs when VOCs were the only carbon source. The genus *Stenotrophobacter* has been recently described and therefore its potential metabolic capabilities still have to be investigated (Pascual et al., 2015). Increasing the inlet load in STR_LC during stage II promoted the growth of members from the family *Planctomycetaceae* and *Gemmataceae* (26.7 and 15.6%, respectively). This increase of uncultured members of the class Planctomycetia (family *Planctomycetaceae* and *Gemmataceae*) could be correlated to the degradation of VOCs. Several genera from these two families have been linked to the metabolism of n-alkanes, pinene or acetone (Boada et al., 2021). Interestingly, by the end of operation of STR_LC, members of the phylum Cyanobacteria were also present in this bioreactor (12.3%). This indicates that the bacterial population was not only feeding from the gaseous VOCs, but also it was obtaining the energy from light and the carbon from the surrounding CO_2.

The bacterial community structure was also influenced by the different VOCs concentrations tested and the operational regime in the flat bed reactors. In FB_HC the most dominant genera during stage I were *Azospirillum* (30.7%), and members from the candidatus Saccharibacteria (12.1%) and the family *Phyllobacteriaceae* (11.5%). The increase in EBRT resulted in bacterial population in FB_HC radically changed in comparison to the previous stage and dominated by *Reyranella* (16.2%), uncultured *Blastocatellaceae* (12.8%) and uncultured *Myxococcales* (6.9%). *Azospirillum* is a well described alkane degrader (Roy et al., 1988), while *Reyranella* has been previously found in soil contaminated with polycyclic hydrocarbons and in bioreactors treating persistent cyclic compounds such as siloxanes (Pascual et al., 2020; Wang et al., 2018).

At low VOCs concentrations, the bacterial population in FB_LC exhibited the highest alpha diversity. FB_LC I was dominated by

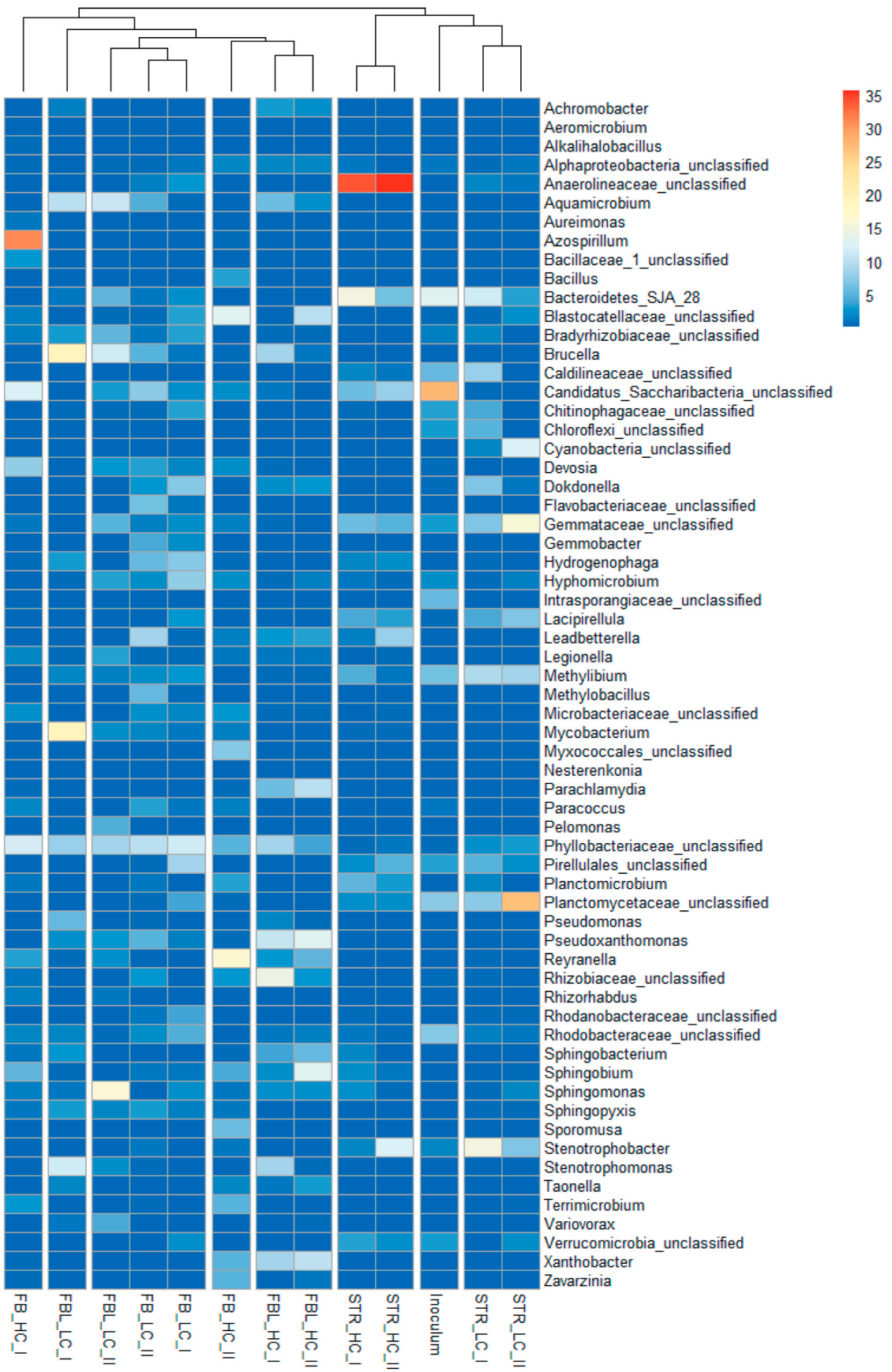

Fig. 5. Heat map of the most representative bacterial genera (95% of the total genera) in the inoculum and after operation. The dendrogram on top represents hierarchical clustering of the samples. Data is presented as the relative abundance (%).

uncultured members of the family *Phyllobacteriaceae* (9.8%) and the genera *Leadbetterella* (8.4%), *Hydrogenophaga* (5.6%) and *Methylobacillus* (5.6%). The modification in the inlet load (FB_LC II) affected the bacterial population that was still dominated by *Phyllobacteriaceae* members (9.8%) and *Hydrogenophaga* members (7.0%), although members of the order Pirellulales (8.3%) and the genera *Hyphomicrobium* (7.8%) and *Dokdonella* (6.9%) increased their abundance by 8–6%. *Hydrogenophaga, Hyphomicrobium* and *Dokdonella* have been linked before to the treatment of alkanes and also of complex hydrocarbons (Pascual et al., 2020; Rojas-Gätjens et al., 2020). Moreover, it has been recently discovered that members of the family *Phyllobacteriaceae* have the necessary genes for the metabolism of complex alkanes (Wang et al., 2021).

The addition of a latex biocoating also had an effect on the bacterial dynamics. In FBL_HC I the population was dominated by members of the family *Rhizobiaceae* (14.1%) and the genera *Pseudoxanthomonas* (10.5%), *Xanthobacter* (8.7%) and *Phyllobacteriaceae* (8.5%). After doubling the EBRT (FBL_HC II), the population shifted again, however the most abundant bacteria were similar to the previous stage. *Pseudoxanthomonas* (12.8%), *Sphingobium* (12.6%), *Xanthobacter* (10.1%), *Parachlamydia* (9.8%) and uncultured members of *Blastocatellaceae* (9.6%) were the most representative. All these genera have the metabolic potential to degrade alkanes and aromatic hydrocarbons (Brzeszcz and Kaszycki, 2018; TerryMcGenity et al., 2019). In FBL_LC I the dominant organisms detected belonged to the genera *Brucella* (19.0%), *Mycobacterium* (18.7%), *Stenotrophomonas* (11.1%) and *Aquamicrobium* (9.7%) and to uncultured members from the family *Phyllobacteriaceae* (8.0%). When the EBRT was halved (FBL_LC II), members of the genera *Sphingomonas* became the most dominant bacteria (15.9%) and the number of *Mycobacterium* and *Stenotrophomonas* was significantly reduced. *Brucella* (11.3%), *Aquamicrobium* (10.9%) and members of the family *Phyllobacteriaceae* (8.4%) persisted in similar abundances until the end of operation of FBL_LC. Several species from the genus *Sphingomonas* possess a unique group of genes for aromatic degradation which makes them very good candidates for the elimination of these compounds even at low concenrtations (Pinyakong et al., 2003). Members of *Brucella* and, as abovementioned, the family *Phyllobacteriaceae*, including *Aquamicrobium*, have been related to the degradation of complex aromatic hydrocarbons and terpenes and have been found as one of the most dominant bacteria in petroleum contaminated soil and contaminated river and sea sediments (Acer et al., 2021; Lebrero et al., 2013; Wang et al., 2021).

Interestingly, the typical bacterial genera related to toxic VOCs metabolism, such as *Pseudomonas*, *Burkholderia* or *Rhodococcus* were not identified in any of the experiments (He et al., 2019; Kumar et al., 2008; Zampolli et al., 2021). Overall, the presence of VOCs as the only carbon and energy source supplemented to the reactors didn't promote the growth of a dominant bacterial member and the populations independently evolved in each reactor configuration and operation mode being different from the populations found in the inoculum. In general, there were some bacteria members that were always abundant in the presence of VOCs, which suggests their possible enrolment in the degradation of these pollutants. Members of the family *Phyllobacteriaceae* and *Pseudoxanthomonas* were detected in all the reactor configurations. However, they were more dominant in flat bed bioreactors. Members of the family *Gemmatacea* and the genus *Methylibium* were also observed in all the configurations tested, although they were more prominent in suspended growth bioreactors.

4. Conclusions

Overall, the different reactor configurations and pollutants inlet loads entailed changes on the removal efficiencies of the different VOCs treated. At high concentrations (400 mg m^{-3}) the best performance was obtained in the STR at an EBRT of 8 min and an inlet load of 3 g m^{-3} h^{-1}, where acetone and toluene were completely removed. Low α-pinene removals (15%) and negligible hexane degradation were recorded in all configurations tested. At low inlet VOCs concentrations (5 mg m^{-3}), the REs of acetone, toluene and α-pinene were >97% independently of the configuration tested. In this case, degradation of hexane was also observed, the flat bed bioreactor without latex biocoating exhibiting the best degradation performance (~90%). Unfortunately, no enhancement on the VOC elimination was achieved in this study with the addition of a non-aqueous latex biocoating compared to traditional STRs. In this sense the latex biocoating could have a negative effect on the growth of microorganisms, reducing their biocatalytic activity due to the limitation of nutrients or water. Further optimization of the biocoating is necessary to overcome these drawbacks.

The different operational conditions had a major effect on the bacterial communities performing VOC degradation than on the fungal community. The fungal community found along operation changed in the different configurations, but still was very similar to the one found in the inoculum. This could have been due to the broader panoply of metabolic activities characteristic of fungi, meaning that the majority of fungi from the inoculum were able to feed on the treated VOCs. Moreover, it could also mean that the fungal population enhanced the degradation of VOCs through the creation of an environmental niche for bacteria that increased the mass transfer of these hydrophobic pollutants, but was not directly implied in the metabolism of the tested VOCs. Bacteria are rather inefficient in the complete degradation of VOCs, generating several intermediary metabolites that accumulate in the culture broth and that simultaneously could be used as carbon source for the fungi. This would also help reducing the accumulation of toxic metabolites.

In the case of the bacterial population, there was a greater evolution of the community during operation due to stricter microbial selection based on the available substrates. Nevertheless, the diverse community enriched in all the configurations tested was resilient to modifications in the pollutant load and retention times. Specific conditions benefited some bacterial members over others, likely due to their affinity for the hydrophobic VOCs and the mechanisms that the different bacterial members developed to resist the toxicity of these hazardous VOCs.

Credit author statement

Sara Cantera: Conceptualization; Methodology; Writing - Original Draft; Writing - Review & Editing. **Martino López**: Methodology. **Raúl Muñoz**: Conceptualization; Writing - Review & Editing; Funding acquisition; Supervision. **Raquel Lebrero**: Conceptualization; Methodology; Writing - Original Draft; Writing - Review & Editing; Funding acquisition; Supervision; Project administration.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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