Bioactive Coating Bioreactors for Indoor Air Treatment

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In this work, bioactive coating-based bioreactors have been applied to the treatment of three odorous indoor air pollutants (i.e., toluene, α-pinene, n-hexane), which are volatile organic compounds (VOC) of different hydrophobicities. The removal efficiency (RE) was evaluated at different empty bed residence times (EBRT) and inlet concentrations. The setup consisted of three bioreactors (namely BR 1, BR 2 and BR 3), packed with porous expanded clay. An enriched consortia of microorganisms was inoculated in BR 1 and BR 2, while fresh activated sludge was used in BR 3. A styrene-acrylate copolymer was employed for bioactive coating formulations in BR 2 and BR 3, while BR 1 performed as a conventional bioreactor.
BR 1 and BR 2 achieved REs over 90 % for toluene and α-pinene at an EBRT as low as 30 s. On the contrary, BR 3 only achieved REs >90 % when operated at an EBRT of 60 s, toluene and α-pinene removals decreasing to 67.0 % and 49.8 % at 30 s, respectively. The poorest performance was recorded at 15 s, with REs of toluene and α-pinene of 41.5% and 55.7% in BR 1, 44.3 % and 10.5 % in BR 2, and 22.2 % and 8.0 % in BR 3. n-hexane removal was poor and non-consistent. When decreasing inlet concentrations at EBRT 15 s, toluene and α-pinene REs progressively increased to reach 87.1% and 90.9 % in BR 1, 86.5 % and 76.6 % in BR 2, and 64.2 % and 70.6 % in BR 3. Overall n-hexane removal slightly increased but was still poorly reproducible.
Generally, no big differences were observed between the control and the bioactive coating bioreactor, suggesting that the bioactive coating can perform as good as conventional biofilm reactors. On the other hand, BR 3 performance was always inferior due to the lack of an adapted microbial community.

1. Introduction

Indoor air pollution has been proved as a serious health problem by several agencies (European Environmental Agency, 2020; USEPA, 2021; World Health Organization, 2018). Pollution levels are often higher indoors, where people spend as much as 90% of their time. This situation might worsen as new building regulations leading to energy efficiency, and thus air tightness, could increase indoor air pollution levels (González-Martín et al., 2021b). Among indoor air pollutants, volatile organic compounds (VOCs) represent one of the most relevant groups that includes a great number of odorous compounds. Some studies have demonstrated that, even in the absence of physical symptoms, odors have a strong psychological effect, influencing mood and cognitive performance (Nordin et al., 2013). Apart from odor annoyance, the so called sick building syndrome has been associated to these contaminants, inducing some non-specific symptoms like membrane and skin irritation, mental fatigue and headaches (Ghaffarianhoseini et al., 2018). Even below irritation thresholds, indoor odorous VOCs can also trigger asthma episodes or irritation in sensitive persons (Wolkoff and Nielsen, 2017). Additionally, severe long-term health problems, such as pulmonary and heart diseases, lung cancer or strokes, can be caused by these pollutants (Lee et al., 2020; Van Tran et al., 2020). All the above points out the need to develop efficient technologies for indoor air treatment.

Biotechnologies have the potential to be applied for the abatement of odorous VOCs at different concentrations. However, a good performance of these technologies is hampered by the variable concentrations and the poor mass transfer of some hydrophobic VOCs (Kraakman et al., 2021). Bioactive coatings, which have recently emerged as a potential alternative, consist of a porous polymer matrix in which microorganisms are embedded in a restricted growing state.

This configuration avoids the formation of natural biofilms, which represents the main impediment for the assimilation of hydrophobic compounds due to their high-water content. Therefore, mass transfer of hydrophobic VOCs from gas phase to microorganisms would be greatly enhanced (Cortez et al., 2017).

This concept has been already developed in some small-scale studies. Estrada et al. (2015) used a *Pseudomonas putida* F1 based bioactive coating to degrade toluene in batch conditions, achieving degradation rates 10 times higher than those achieved by agarose biofilms in the same conditions. Piskorska et al. (2013) immobilized *Rhodopseudomonas palustris* CGA009 for H_2 bio-generation, obtaining good bacterial activity even after 2-week dry storage. Although promising, these studies tested experimental conditions still far from a real, long-term application to indoor air purification. In a previous lab-scale study developed by our research group, several parameters related with bioactive coating formulation were optimized, and the concept of bioactive coatings was proven at a higher scale (González-Martín et al., 2021a). In this study, bioactive coatings were applied to conventional bioreactors for the treatment of toluene, α-pinene and n-hexane as examples of odorous indoor air VOCs. The removal efficiency (RE) of each compound was evaluated at different empty bed residence times (EBRTs) and inlet concentrations.

2. Materials and Methods

The experimental setup consisted of three PVC bioreactors (BR 1, BR 2 and BR 3) (Ø 10 cm) packed to a height of 32 cm (V = 2.5 L) with expanded clay beads (Arlite Light Plus, LECA Portugal S.A., Portugal). Plastic Kaldness K1 Micro rings (Evolution Aqua, UK) of Ø 1 cm were placed over the packing (5 cm layer) to ensure a homogeneous irrigation. A timer and a peristaltic pump (model 205S; Watson-Marlow Limited, Falmouth, UK) were used to regulate the irrigation flow. A syringe pump (Fusion 100, Chemyx Inc., USA) and glass syringes (Hamilton, USA) were used to feed the odorous VOCs into the system. The air flow was provided by an air compressor (ABAC B2500-50 2, Italy), humidified in a 1 m height water column and regulated by air flow meters (Aalborg, New York, USA) at the inlet of each reactor. Glass bulbs (Sigma-Aldrich, Madrid, Spain) of 250 mL were placed at the inlet and outlet of each bioreactor to measure VOC concentration by solid-phase microextraction (SPME) followed by GC-FID analysis. The bioreactors were maintained at 22 ± 2 ºC.

2.1 Chemical products

A mixture of odorous compounds composed by 22 % of α-pinene (CAS 80-56-8), 34 % of toluene (CAS 108-88-3) and 44 % of n-hexane (CAS 110-54-3) (%V/V) was used as feed for the bioreactors. α-pinene was purchased from Sigma-Aldrich (Madrid, Spain); n-hexane and toluene were purchased from Panreac® (Barcelona, Spain). The nutrient solution composition was described in González-Martín et al. (2021a). For the formulation of bioactive coatings, PRIMAL™ SF-208 ER (acrylic-styrene copolymer, solids content 48.05 %; pH 8.0 - 9.5; Dow Chemical, Germany) was used. Additionally, D (+)-saccharose and glycerol were used as osmoprotectants. Salts for nutrient solution, glycerol, n-hexane and toluene were supplied by Panreac® (Barcelona, Spain), α-pinene was supplied by Sigma-Aldrich (Madrid, Spain) and D (+)-saccharose was supplied by Labkem (Barcelona Spain).

2.2 Microorganisms and biocoating preparation

An enriched bacterial culture was used for BR 1 and BR 2. For the enrichment, activated sludge was fed for 3 months with toluene, α-pinene and n-hexane in a 3-liter stirred tank bioreactor. On the other hand, BR 3 was inoculated with fresh activated sludge obtained from a local wastewater treatment plant.

Bioactive coatings were used in BR 2 and BR 3, while BR 1 was operated as a conventional biofilm bioreactor to compare the performance. The formulation of bioactive coatings consisted of 115 mL of raw polymer suspension mixed with 30 mL of a saccharose solution (0.72 g L^{-1}) and 16 mL of glycerol (100 % V/V), as described in Gosse and Flickinger (2011). For BR 1, the polymer was replaced with distilled water. The microorganisms were obtained by centrifugation of an aliquot of enriched culture/activated sludge (10000 rpm; 10 min; 4 ºC) to obtain ≈ 1.4 g (dry weight) for each bioreactor. The cell pellet was then mixed homogenously with the formulation and applied to the previously washed and dried expanded clay beads. The coated packing was left to dry overnight before the start of the experiment.

2.3 Experimental conditions

The 218-day experiment was divided in 8 stages, ended when a steady state of VOC removal was achieved. Inlet air was humidified by using a 1 m water column prior to VOC addition. Stages 1 to 4 consisted of progressive EBRT decreases (120, 60, 30 and 15 s) by increasing air flow, while maintaining the same inlet concentrations (average 15.2 ± 2.3, 15.7 ± 3.2 and 9.1 ± 3.4 mg m^{-3} for toluene, α-pinene and n-hexane, respectively). At Stage 5, the irrigation frequency was changed from an intermittent mode of 100 mL day^{-1}, divided in 16 intervals, to a continuous recirculation of 8 L day^{-1}, maintaining EBRT and inlet concentration.

From Stages 5 to 8, the inlet concentrations (Table 1) were reduced by decreasing the inlet VOC flow rate at a constant EBRT of 15 s, to achieve toluene, α-pinene and n-hexane concentrations of 14.4 ± 1.5, 15.9 ± 2.6, and 14.2 ± 1.9 mg m^{-3} in Stage 5; 7.7 ± 0.9, 8.4 ± 1.1 and 5.8 ± 0.7 mg m^{-3} in Stage 6; 4.2 ± 0.6, 4.0 ± 0.8 and 2.9 ± 0.4, mg m^{-3} in Stage 7; and 2.0 ± 0.2, 1.9 ± 0.1 and 1.5 ± 0.2 mg m^{-3} in Stage 8.

2.4 Analytical

For VOCs analysis, a preliminary preconcentration step was performed using a SPME fiber (85 µm CAR/PDMS; Supelco, Bellefonte, USA) which was exposed for 10 min to the contaminated air contained in 250 mL glass bulbs. After that, the thermally desorbed VOCs were analyzed in a GC-FID (Varian 3900; Agilent HP-5MSI capillary column of 30 m × 0.25 mm × 0.25 µm). GC analysis parameters are described in González-Martín (2021a). Odorous VOCs were measured every day at the inlet and outlet of the reactors. A blank injection was performed prior analysis to ensure a clean SPME fiber. External standards of each compound were used for SPME calibration.

2.5 Data processing

Removal efficiency percentages were calculated as shown in Equation 1 using daily inlet (C_{in}) and outlet (C_{out}) concentration values of each VOC. Data are reported as averaged REs when a steady state was achieved.

$$\%RE = 100 * \frac{C_{in} - C_{out}}{C_{in}} \tag{1}$$

Table 1: Duration and average inlet concentration (with standard deviation) of the odorous VOCs at the different experimental stages

Stage	1	2	3	4	5	6	7	8
Duration (days)	41	24	36	38	31	19	14	15
C_{Hex} (mg m^{-3})	7.8 (1.3)	8.2 (0.8)	10.8 (2.5)	14.3 (3.1)	14.2 (1.9)	5.8 (0.7)	2.9 (0.4)	1.5 (0.2)
C_{Tol} (mg m^{-3})	14.6 (2.2)	14.9 (1.1)	16.7 (2.7)	14.6 (1.6)	14.4 (1.5)	7.7 (0.9)	4.2 (0.6)	2.0 (0.2)
C_{Pin} (mg m^{-3})	13.4 (3.2)	18.3 (1.5)	17.5 (2.8)	15.1 (1.7)	15.9 (2.6)	8.4 (1.1)	4.0 (0.8)	1.9 (0.1)

3. Results and discussion

The %REs achieved in each bioreactor throughout the experiment are represented in Figure 1 (A-C).

3.1 Effect of the EBRT

The EBRT was modified from 120 s in Stage 1, to 60 s in Stage 2, 30 s in Stage 3 and 15 s in Stage 4. An acclimation phase was observed in the three bioreactors at the beginning of the experiment, with initially high REs of toluene and α-pinene that rapidly decreased afterwards. After a 3 to 4-weeks period of gradual recovery, depending on bioreactor and VOC, a steady removal performance was reached. The highest REs, 92.1 ± 3.8 % and 92.8 ± 7.4 % for toluene and α-pinene, respectively, were observed in BR 1, which also experienced the shortest acclimation phase. BR 2 reached REs of 90.1 ± 4.6 % and 87.8 ± 9.6 % for toluene and α-pinene, while BR 3 achieved REs of 90.9 ± 5.1 % and 90.0 ± 8.4 % for toluene and α-pinene despite exhibiting the longest acclimation period. No n-hexane removal (< 5 %) was observed in any of the bioreactors. The longest acclimation period of BR 3 was attributed to the lack of adaption of the microorganism to the selected VOCs. The reduction in the EBRT to 60 s had little effect on VOC removal in BR 1 and BR 2. REs of toluene and α-pinene remained over 90 %, while no increase in n-hexane removal was observed. A slight deterioration of BR 3 performance was recorded after the EBRT reduction, rapidly recovering average REs of 89.9 ± 1.6 % for toluene and 87.3 ± 4.1 % for α-pinene. No n-hexane abatement was observed. A further decrease in the EBRT to 30 s in Stage 3 resulted in an initial decrease in toluene removals in BR 1 and BR 2, with a more severe effect on α-pinene removal (≈ 30 % decrease). Removals of toluene and α-pinene recovered within a week to achieve steady state values of 91.3 ± 3.0 % and 97.9 ± 2.3 % in BR 1, and 92.6 ± 1.7 % and 95.2 ± 6.4 in BR 2. On the contrary, BR 3 could not recover previous REs, and after the initial decrease, steady state REs for toluene and α-pinene remained at 67.0 ± 7.8 % and 49.8 ± 12.0 %. A slight increase in n-hexane degradation was observed in the three bioreactors, although outlet concentrations showed a high variability. As previously stated, the lack of specialization of the fresh activated sludge to the VOCs was the most likely cause of the performance deterioration in BR 3. The reduction in the EBRT to 15 s entailed an overall decline in the removal values.

Toluene REs decreased by ≈ 50 % in the three bioreactors. While a similar deterioration in toluene REs was observed in BR 1 and BR 2 (to 41.5 ± 11.2 % and 44.3 ± 5.4 %, respectively), a much faster deterioration was recorded in BR 3, reaching values of 22.2 ± 16.1 %. The reduction in α-pinene RE was moderated in BR 1, decreasing to 41.5 ± 11.2 %. On the other hand, BR 2 and BR 3 experienced steeper reductions to REs of 10.5 ± 10.2 % and 8.2 ± 9.3 %, respectively. No variation on n-hexane removal was observed in any of the bioreactors. This general drop in VOCs removals could be attributed to a deterioration of the bacterial activity, especially in the bioactive coatings, caused by the loss of moisture at higher inlet gas flowrates. Additionally, a higher gas flow implies a shorter contact time that might hinder VOCs mass transfer.
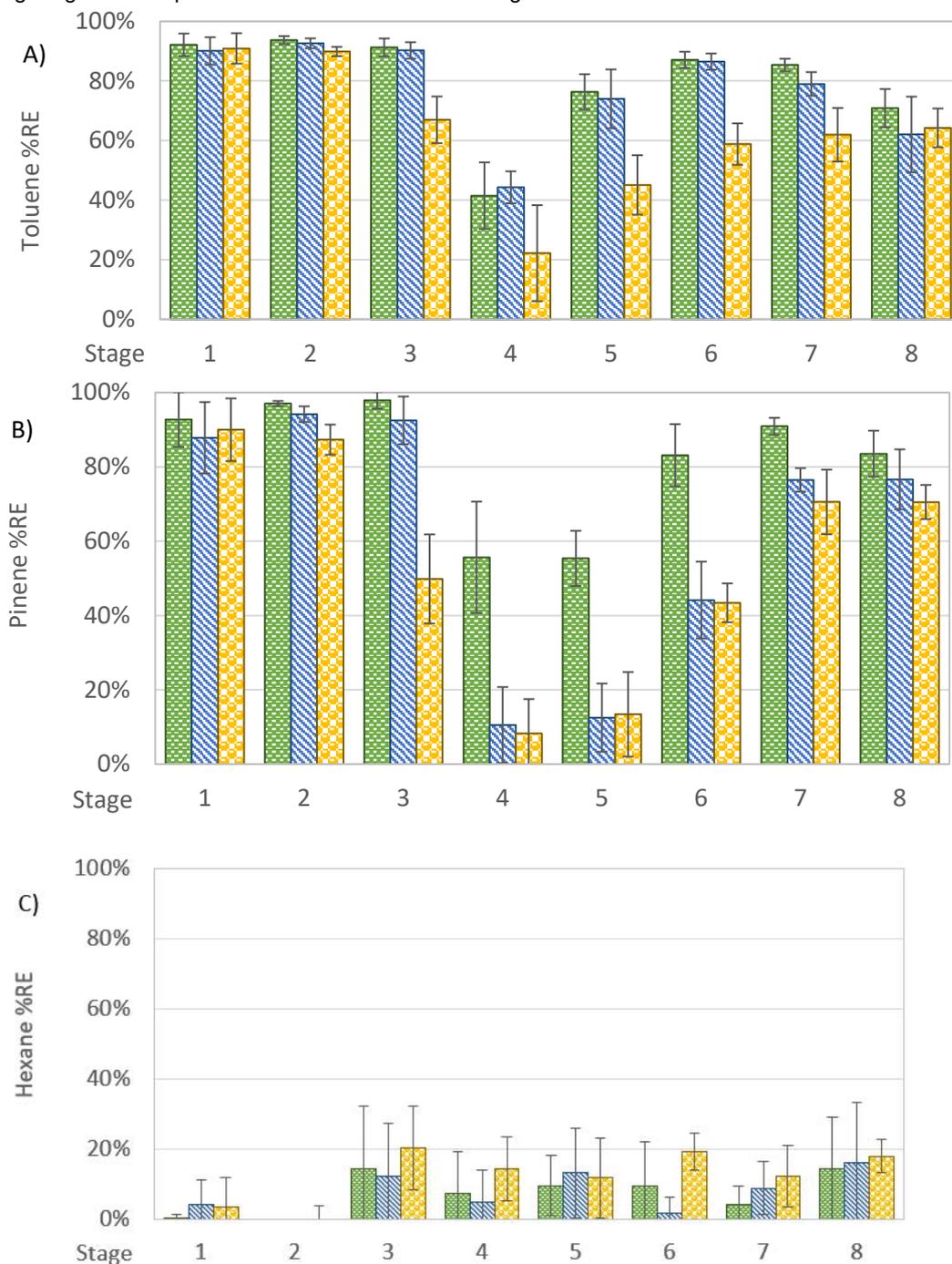

Figure 1: Steady-state average REs and standard deviation of A) toluene, B) α-pinene and C) n-hexane recorded in BR 1 (■), BR 2 (▨) and BR 3 (▨) at stages 1-8.

3.2 Effect of the irrigation frequency

At Stage 5, EBRT and inlet VOC concentrations remained the same as those of Stage 4, but the system configuration was shifted from a biofilter (intermittent irrigation) to a biotrickling filter (continuous liquid recirculation). The irrigation was increased from intermittent intervals of 6.25 mL, 16 times a day (100 mL day^{-1}) to a continuous recirculation at a liquid velocity of 8 L day^{-1}. In this stage, a ≈ 30 % toluene removal recovery was observed, reaching 76.3 ± 5.9 % in BR 1, 74.0 ± 9.9 % in BR 2, and 45.1 ± 10.0 % in BR 3. However, both α-pinene and n-hexane abatement performance remained similar to that recorded in Stage 4. Despite the higher irrigation rate contributed to the reactivation of bacterial activity, the different behavior could be explained by the higher hydrophobicity of α-pinene and n-hexane compared to that of toluene.

3.3 Effect of the inlet VOCs concentration

In Stage 6, the inlet VOC concentration was reduced to test the performance of the biotrickling filters. An increase of ≈ 11 and 30 % was observed for toluene and α-pinene removals, respectively, regardless of the bioreactor. n-hexane removal seemed slightly higher in BR 2 and BR 3, but no conclusive findings could be obtained due to the high variability of the results. In Stage 7, toluene RE marginally decreased in BR 1 and BR 2 (85.4 ± 2.1 % and 79.0 ± 4.0 %, respectively), slightly increasing in BR 3 to 61.9 ± 9.0 %. An improvement in α-pinene removal was recorded in this stage, however, whereas in BR 1 α-pinene RE only increased to 90.9 ± 2.3 % (< 10 %), a significant enhancement was observed in BR 2 and BR 3 (up to 30 %), to reach values of 76.5 ± 3.2 % and 70.6 ± 8.7 %, respectively. The final decrease in VOCs inlet concentration below 2 mg m^{-3} in Stage 8 had a different impact on the bioreactors: in BR 1, toluene and α-pinene REs decreased to 70.8 ± 6.4 % and 83.5 ± 6.2 %, respectively; in BR 2, toluene RE decreased to 62.0 ± 12.7 % but no effect was recorded on α-pinene removal; and in BR 3, an enhanced toluene RE (64.2 ± 6.5 %) was obtained, with similar α-pinene abatement performance. Regardless of the inlet concentration, the removal of n-hexane was very inconsistent, thus no reliable conclusions can be drawn. Overall, the decrease in inlet concentrations, which corresponds to lower inlet loading rates, promoted the progressive recovery of toluene and α-pinene REs. This could be also supported by the higher moisture inside the reactors. Nevertheless, REs over 90 % could not be recovered, probably due to the low EBRT and the permanent damage to bacterial activity during Stage 4. In this sense, high removals of toluene and α-pinene at the trace level concentrations typically recorded in odorous emissions have been reported at EBRTs of 8 and 4 s (Lebrero et al., 2014). The poor removal of n-hexane was attributed to the high hydrophobicity of this compound, which highly limits its bioavailability. n-hexane removal in bacterial bioreactors has been reported both at high (Amin et al., 2017; Cheng et al., 2020) and low concentrations (Lebrero et al., 2014, 2013), thus further optimization of the bioactive coating bioreactors here tested is necessary to achieve significant and stable n-hexane removals.

4. Conclusions

In this work, bioactive coatings were implemented in conventional bioreactor configurations to assess their performance on odorous indoor air purification. Overall, the acclimation of the bacterial community to the odorous VOCs was decisive in bioreactor performance, as inoculation with fresh activated sludge (BR 3) always resulted in lower REs and inferior robustness towards changes in operating parameters compared to BR 1 and BR 2, in which an enriched consortium was used as inoculum. The bioactive coating-based reactor and the conventional biofilm reactor exhibited similar results. Removal efficiencies for toluene and α-pinene of ≈ 90 % were achieved by both reactors at EBRTs as low as 30 s. At 15 s, the removal performance clearly decreased in the three bioreactors probably caused by the combination of a progressive desiccation and the reduced contact time, which limited VOCs mass transport to the degrading community. Toluene removal started to recover after shifting the system configuration from a biofilter to a biotrickling filter, while α-pinene abatement was restored only after the reduction in inlet VOCs concentration. Nevertheless, the permanent damage to the microbial activity associated to the successive reductions in EBRT prevented from recovering initial removal performances. n-hexane abatement was poor throughout the experiment, which was attributed to the high hydrophobicity of this pollutant. Optimization of the biocoating formulation and the operating parameters is still necessary to ensure the removal of the most hydrophobic odorants commonly present in polluted indoor air.

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