Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*

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**HIGHLIGHTS**
- A fungal BF and BTF treating hydrophobic VOCs were systematically compared.  
- *subhashii* supported an effective removal of VOCs at short EBRT of 30 s.  
- Fungal BTF supported a slightly higher VOC abatement performance than fungal BF.  
- The ability of *C. subhashii* to remove VOCs was also confirmed in batch assays.  
- A consistent biodegradation pattern was recorded: toluene $\approx$ \textit{n}-hexane $>$ \textit{α}-pinene $>$ TCE.

**ABSTRACT**

This work systematically compared the potential of a conventional fungal biofilter (BF) and a fungal biotrickling filter (BTF) for the abatement of a mixture of hydrophobic volatile organic compounds (VOCs). *Candida subhashii* was herein used for the first time, to the best of the author’s knowledge, to remove \textit{n}-hexane, trichloroethylene, toluene and \textit{α}-pinene under aerobic conditions. *C. subhashii* immobilized on polyurethane foam supported steady state removal efficiencies of \textit{n}-hexane, trichloroethylene, toluene and \textit{α}-pinene of 25.4 $\pm$ 0.9\%, 20.5 $\pm$ 1.0\%, 19.6 $\pm$ 1.5\% and 25.6 $\pm$ 2.8\% in the BF, and 35.7 $\pm$ 0.9\%, 24.0 $\pm$ 1.6\%, 44.0 $\pm$ 1.7\% and 26.2 $\pm$ 1.8\% in the BTF, respectively, at relatively short gas residence times (30 s). The ability of *C. subhashii* to biodegrade \textit{n}-hexane, TCE, toluene and \textit{α}-pinene was confirmed in a batch test conducted in serum bottles, where a biodegradation pattern (toluene $\approx$ \textit{n}-hexane $>$ \textit{α}-pinene $>$ trichloroethylene) comparable to that recorded in the BF and BTF was recorded.

1. Introduction

The removal of hazardous gas pollutants prior discharge into the environment entails many technical problems and is often costly. When selecting the best technology to remove hazardous gas pollutants, parameters such as a high pollutant removal efficiency, low capital and operational costs, environmentally friendliness and low environmental impacts are typically considered (Kosmider et al., 2012; Revah et al., 2011). In recent years, biological waste gas and odor treatment methods have become increasingly popular (Gospodarek et al., 2019; Szulczyński...
et al., 2019; Abraham et al., 2015). One of the most common biological gas purification techniques is biofiltration, which is a relatively cheap and ecologically safe process based on packed bed systems. Biofiltration consists of circulating the contaminated gas stream through a filter bed inhabited by pollutant degrading microorganisms (Rybackzyk et al., 2019a; Lebrero et al., 2016; Guieysse et al., 2008). Pollutants diffuse from the gas phase to the so-called biofilm formed on the surface of the packing material. The compounds adsorbed on the surface or absorbed into the biofilm undergo biodegradation in the presence of nutrients (N, P, etc) and electron acceptors (typically O2), and the purified and odorless gas stream leaves the biofiltration unit (Rybackzyk et al., 2019b; Raboni et al., 2017). The biofiltration process can be engineered as a biofilter or biotrickling filter, the latter involving a continuous recirculation of a nutritive aqueous solution that helps controlling the pH, supplying nutrients and improving gas-biofilm pollutant mass transfer (Mudliar et al., 2016; Estrada et al., 2019).

Biofiltration is cost effective for waste gases with a low concentration (<3 g m⁻³) of volatile organic compounds (VOCs) (van Groenestijn and Hesselink, 1993; OJ Velga and Kennes, 2006). However, conventional biofilters, based on the action of microorganisms typically growing on organic packing materials such as compost, and biotrickling filters constructed with inorganic packing materials, still face problems during the abatement of hydrophobic VOCs such as aromatics, alkenes and alkanes. Indeed, these gas pollutants are poorly absorbed in bacterial biofilms or in the recirculating aqueous solution due to the low aqueous solubility of hydrophobic VOCs. In addition, the operational stability of conventional biofilters is often hampered by acidification and drying of the filter bed. In this context, fungal colonization of inert packing material in biofilters or biotrickling filters has been proposed in order to overcome these operational problems (Cox, 1995; Groenestijn et al., 1995).

Fungi are more resistant to acidic and dry conditions than bacteria, and their cell wall contains hydrophobic proteins called hydrophobins that can enhance the abatement of hydrophobic VOCs (Cox, 1995; Jorio et al., 2009; Marycz et al., 2022). In recent years, the potential of fungi in biofiltration applications has been revisited, since these microorganisms have been shown to be able to biodegrade many complex organic compounds. For example, Fusarium solani supported an effective removal of hydrophobic VOCs in biofilters (Arriaga and Revah, 2005; Arriaga et al., 2006; Vergara-Fernández et al., 2016; Rybackzyk et al., 2021). Similarly, Cladosporium sphaerospermum was able to remove BTEX, methyl propyl ketone, MEK, toluene and n-butyl acetate in a biotrickling filter (Raboni et al., 2017; Qi et al., 2005). Two types of fungi are typically used in biofiltration: molds and yeasts. Mold fungi form a mycelium composed of loosely collected hyphae, the so-called air mycelium. This aerial mycelium of mold fungi, which is in direct contact with the gas phase, can adsorb and biodegrade hydrophobic VOCs. On the other hand, yeasts are single-celled fungi that reproduce by budding. The species Candida subhashii, which was pioneering in the removal of mixtures of hydrophobic and hydrophilic VOCs, belongs to the group of yeasts. Indeed, C. subhashii was successfully used in a biotrickling filter for the continuous cyclohexane removal with ethanol as a co-substrate (Rybackzyk et al., 2021) based on its effective immobilization on biofilter packing materials (Marycz et al., 2020). Applications of bacterial biofiltration as an alternative for odor abatement has been extensively investigated and reviewed in the past 35 years (Gospodarek et al., 2019; Marycz et al., 2022). However, the potential of yeasts for air biofiltration has not been used in biofiltration to remove hydrophobic VOCs, and their biodegradation potential was just stated. Vergara-Fernández et al. (2018) stated that the removal efficiency of hydrophobic compounds is lower in bacterial biofilters than in fungal biofilters (Vergara-Fernández et al., 2018a). Moreover, Prenaeta-Boldú et al. (2018) (Prenaeta-Boldú et al., 2018) indicated that special attention should be paid to elucidate the phenomena underlying fungal and yeast based biofiltration process such as heat, momentum and mass transport, as well as microbial growth and biodegradation kinetics. Indeed, systematic studies comparing the ability of fungi to abate hydrophobic VOCs in different bioreactor configurations have not been reported to date. Moreover, the performance of C. subhashii for the biofiltration of a hydrophobic VOC mixture has never been assessed.

VOCs are emitted in industrial activities (e.g. chemical, printing, petrochemical industries). VOCs are generally classified as aliphatic hydrocarbons (e.g., n-hexane), halogenated hydrocarbons (e.g., TCE), aromatic hydrocarbons (e.g., toluene), and terpenes (e.g., α-pinene) (Yang et al., 2009). n-hexane, TCE, toluene and α-pinene are representative VOCs from each class and are considered to be relevant air pollutants as a result of their toxicity (Yang et al., 2009; Liu et al., 2007). In addition, these VOCs exhibit a moderate to high hydrophobicity, which is typically considered the main technical limitation of biofilters and biotrickling filters.

The present work aims at systematically comparing a conventional biofilter and a biotrickling filter colonized by C. subhashii in terms of their ability to remove a hydrophobic VOC mixture composed of hexane, pinene, trichloroethylene (TCE) and toluene. Additionally, batch biodegradation tests were carried out in order to confirm the metabolic capacity of C. subhashii to biodegrade the target mixture of hydrophobic VOCs.

2. Materials and methods

2.1. Microorganisms and inoculum

C. subhashii was used in this work as a biocatalyst (Marycz et al., 2020). Aliquots of 333 mL of sterile liquid Sabouraud medium (BTL, Poland) (containing the carbon and energy source) in 500 mL Erlenmeyer flasks (E-flasks) were inoculated with C. subhashii agar using an inoculation loop under sterile conditions. The E-flasks were incubated at 25 °C for 9 days in a rotary shaker (Thermo Fisher Scientific, U.S.) at 200 rpm. Then, 1000 mL of inoculum were centrifuged under sterile conditions at 24 °C for 5 min at 3000 rpm (Sorvall Legend RT Plus Centrifuge, U.S.). After centrefugation, the fungal pellet was re-suspended and washed with 200 mL of minimal nutrient medium (MSM), and centrifuged again under similar conditions. The fungal pellet was re-suspended in 200 mL of MSM and 90-mL cell culture aliquots were used to inoculate the polyurethane foam used as a packing material in the fungal biofilter and biotrickling filter.

2.2. VOCs and minimal nutrient medium

n-hexane (Sigma-Aldrich, South Korea), trichloroethylene (Panreac AppliChem, Spain), toluene (Sigma-Aldrich, USA) and α-pinene (Sigma-Aldrich, USA) were used as model hydrophobic indoor air pollutants. The MSM used for fungal growth in the batch liquid cultures and biofiltration columns was previously reported by Marycz (Marycz et al., 2020). This medium was composed of: Na₂HPO₄ 2H₂O (Panreac AppliChem, Spain) (15.2 g L⁻¹), KH₂PO₄ (Panreac AppliChem, Spain) (3 g L⁻¹), NaCl (Panreac AppliChem, Spain) (0.5 g L⁻¹) and NH₄Cl (Sigma-Aldrich, USA) (1 g L⁻¹). After mixing all components, the pH of the medium was ~7. The composition of the Sabouraud mineral salt medium was as follows: casein hydrolyzate (Panreac AppliChem, Spain) (5 g L⁻¹), meat extract (Panreac AppliChem, Spain) (5 g L⁻¹), glucose (Panreac AppliChem, Spain) (40 g L⁻¹). The pH of the Sabouraud mineral salt medium was 5.6 ± 0.2.

2.3. Batch VOC biodegradation assay

Batch biodegradation tests were carried out in 1.2-L gas-tight glass
bottles (closed with butyl septa and sealed with plastic caps) containing 200 mL of MSM inoculated with a loop inoculum of *C. subhashii* taken from the solid medium. *n*-hexane, TCE, toluene and α-pinene were injected into the headspace of the bottles to achieve an initial concentration of 300, 175, 150 and 145 mg m$^{-3}$, respectively. Positive and negative controls were also prepared with *C. subhashii* (without VOCs) and with VOCs (without *C. subhashii*), respectively. The bottles were magnetically agitated at 300 rpm and incubated for 28 days at 25 °C. All tests were conducted in duplicate. The headspace concentrations of the target VOCs were daily determined by GC-FID using a 100 μL gastight syringe (Hamilton, Australia).

2.4. Continuous VOC abatement in a fungal biofilter and a fungal biotrickling filter

A conventional biofilter (BF) and a biotrickling filter (BTF) (Fig. 1) made of clear PVC columns (10 cm internal diameter × 100 cm height) were set up. Each column was filled with 34 cm of polyurethane foam (PUF), reaching a total packed bed volume ($V_p$) of 2.5 L. The PUF exhibited a density of 0.01 g mL$^{-1}$, a specific surface area of 1000 m$^2$ m$^{-3}$, a porosity of 96% and a water retention capacity of 0.12 L$_{water}$ L$_{PUF}^{-1}$. Air was initially humidified in a clear PVC column (0.1 m internal diameter × 1.6 m height, filled with 1.2 m of water) and mixed with a liquid mixture of *n*-hexane, TCE, toluene and α-pinene injected at 0.3 mL h$^{-1}$ using a syringe pump (KDS100 Legacy, Fisherbrand, USA), resulting in average concentrations of 206.6 ± 6.9, 237.3 ± 7.6, 310.3 ± 10.7 and 393.5 ± 21.2 mg m$^{-3}$, respectively. Both the fungal biofilter and fungal biotrickling filter were fed with the polluted air from the bottom at 5 L min$^{-1}$, which resulted in an empty bed residence time of 0.5 min. The air flowrates were controlled using rotameters (Aalborg, USA). The BF was periodically irrigated at 17.8 mL MSM h$^{-1}$ picking d$^{-1}$. In the BTF, a recycling nutritive solution (MSM) was continuously agitated in an external 1-L tank and recycled at a rate of 2 mL h$^{-1}$ using a peristaltic pump (Watson Marlow, USA). In addition, a MSM renewal rate of 40 mL d$^{-1}$ was implemented in the BTF. Gas samples were periodically collected from each module at the gas inlet and outlet using a 100-μL gastight syringe (Hamilton, Australia) in order to determine the concentration of the target VOCs and CO$_2$.

The pH and culture absorbance at 600 nm (OD$_{600}$) for the monitoring of the suspended biomass concentration were daily measured in the leachate of the BF (from aliquots of 40 mL) and in the liquid effluent of the BTF by withdrawing a 40-mL aliquot from the external 1-L mineral medium tank during the MSM renewal. The experiment lasted 48 days.

2.5. Analytical methods

CO$_2$ and O$_2$ gas concentrations were quantified using a Bruker 430 gas chromatograph (Bruker Corporation, Palo Alto, USA) equipped with a CP-Molsieve 5A and a CP-PoraBOND Q columns and a thermal conductivity detector. Oven, injector and detector temperatures were kept at 45, 150 and 200 °C, respectively, while helium was employed as a carrier gas at 13.7 mL min$^{-1}$. This method is described elsewhere (Estrada et al., 2014). The concentrations of VOCs were measured in a GC-FID (Varian 3900) equipped with an Agilent HP-5MSI capillary column (30 m × 0.25 mm × 0.25 μm) as described by González-Martin and co-workers (González-Martin et al., 2022). Measurements of the OD$_{600}$ were performed in a SPECTROstar Nano spectrophotometer (BMG LABTECH, Germany). pH was determined using a pH-meter Basic 20 (Crison, Spain).

2.6. Calculations

Results from the biofiltration experiments were herein expressed in terms of VOC removal efficiency (RE, %), which was calculated according to Equation (1):

$$RE = 100 \times \frac{C_{in} - C_{out}}{C_{in}}$$

where $C_{in}$ and $C_{out}$ stand for the inlet and outlet VOCs concentrations. The average values of RE along with its standard deviation were calculated for each VOC under steady state.

The volumetric CO$_2$ production (g m$^{-3}$ h$^{-1}$) is defined as:
$CO_2\ production = \frac{Q (CO_2^{\text{out}} - CO_2^{\text{in}})}{V_{\text{bed}}}$ \hspace{1cm} (2)

where $CO_2^{\text{out}}$ and $CO_2^{\text{in}}$ are the CO$_2$ concentrations at the gas outlet and inlet of the reactor, Q is the gas volumetric flow rate and $V_{\text{bed}}$ is the packed bed volume.

The pollutant elimination capacity (EC) is defined according to Equation (3):

$$EC = \frac{Q (C^{\text{in}} - C^{\text{out}})}{V_{\text{bed}}}$$ \hspace{1cm} (3)

as a function of the air flow rate (Q), the inlet and outlet gas concentrations ($C^{\text{in}}$ and $C^{\text{out}}$), and the packed bed volume ($V_{\text{bed}}$).

3. Results and discussion

3.1. Batch VOC biodegradation assay

The concentrations of the target VOCs in the headspace of the bottles started to decrease by day 6. The highest removal at the end of the experiment was recorded for n-hexane (~38%), followed by toluene (~33%), α-pinene (~26%) and TCE (~22%) (Fig. 2). An unexpected deterioration in the biodegradation capacity of $C.\ subshashii$ was observed for n-hexane by day 18, and for α-pinene by day 23, which might have been due to the accumulation of inhibitory metabolites in the cultivation medium. Interestingly, the biodegradation of TCE and toluene continued until the end of the experiment. A gradual decrease in the concentration of the target VOCs in the abiotic controls was observed as a result of pollutant adsorption onto the glass wall or butyl septum. This phenomenon occurred to a greatest extent for TCE and α-pinene compared to n-hexane and toluene, but a more rapid decrease in pollutant concentration was recorded in the assays inoculated with $C.\ subshashii$.

Fig. 2. Time course of the headspace concentration of (a) n-hexane, (b) TCE, (c) toluene, (d) α-pinene in the abiotic control (◆) and biodegradation assays (○).

Fig. 3. Time course of (a) pH and (b) OD$_{600}$ in the BTF trickling solution (■) and BF leachate (▲).
3.2. Continuous VOC abatement in a fungal biofilter and a fungal biotrickling filter

A start-up period of 20 days, characterized by low VOC removal efficiencies, was observed in the BF and BTF inoculated with *C. subhashii* (Fig. 3). The gradual formation of the fungal biofilm in the packing material of both BF and BTF resulted in a steady increase in biofiltration performance. Rybarczyk et al. (2021) (Rybarczyk et al., 2021) presented investigations on the removal of cyclohexane from air in a BTF inoculated with *C. subhashii*, where the start-up period lasted 30–35 days. On the other hand, Vergara-Fernández et al. (2018a, 2018b) observed a start-up period of 18 days for toluene during the simultaneous abatement of formaldehyde, toluene and benz[a]pyrene in a biofiltration reactor inoculated with *Fusarium solani* fungi and *Rhodococcus erythropolis* bacteria. In this context, the initiation of the process of biotransformation of a compound by a given species of fungus is directly related to the type of compound to be removed, the operational conditions in the bioreactor and the physiological state of the inoculum.

A slight decrease in the pH of both the BF leachate and BTF trickling solution was observed, probably due to the release of acidic fungal biodegradation metabolites (Fig. 3a). Interestingly, this drop in pH occurred in a larger extent in the BTF, with pHs fluctuating between 6.7 and 7, as a result of the higher VOC removals supported by this bioreactor configuration. Typically, pH fluctuations of ±1 do not alter Candida metabolism as previously reported by (Rane et al., 2019). Biomass concentration in the trickling solution of the BTF, estimated as OD$_{600}$, gradually increased from day 10 (OD$_{600}$ = 0.10) to 31 (OD$_{600}$ = 0.73) (Fig. 3b). This phenomenon was likely due to biofilm detachment from the packaging material and its subsequent washout by the trickling solution. On the other hand, biomass concentration in the leachate of BF decreased significantly to finally stabilize by day 20. From this day onwards, the OD$_{600}$ remained constant in the BF at 0.055 ± 0.015, which suggest that cells did not detach from the biofilm formed in the polyurethane foam. The low OD$_{600}$ and strong biofilm attachment in the BF may be likely due to the absence of trickling solution and the associated shear stress in the biofilm. In the BTF, the higher VOC removal efficiencies recorded entailed a higher biomass growth. It should be noted that the measurement of optical density also takes into account dead cells with preserved integrity of cytoplasmic membranes. Therefore, a high OD$_{600}$ value does not fully reflect the physiological state of the culture and their potential for biofilm formation.

Maximum and stable n-hexane removal efficiencies of 34–37% in BTF and 25–27% in BF were recorded under steady state at 30 s of EBRT, indicating that *C. subhashii* did not only promote the mass transfer of n-hexane to the biofilm but contributed to n-hexane biodegradation (Fig. 4a). Interestingly, a similar start-up period of 20 days was observed in both biofiltration configurations. In this context, n-hexane has been effectively removed using *Fusarium solani* in a 2.5 L BF packed with perlite operated at EBRT of 60 s with elimination capacities of 90–130 g m$^{-3}$ h$^{-1}$ and a maximum RE of 100% below inlet concentrations of 1.8 g m$^{-3}$ (which corresponded to a critical inlet load of around 70 g m$^{-3}$ h$^{-1}$) (Arriaga and Revah, 2005). The discrepancy in the results was likely due to the fact that Arriaga and Revah (2005) operated a BF with a twice higher EBRT and pure n-hexane instead of a mixture of VOCs. In addition, process operation at hexane concentrations 10 folds higher than in the study herein presented promoted an active fungal growth and therefore an effective hexane capture and biodegradation.

The start-up period to achieve a significant TCE removal in both biofiltration units was approximately 21 days. The steady state REs of TCE in BTF fluctuated between 22 and 26% from days 38–48. Similarly, steady state REs of TCE in BF oscillated between 21 and 23% from days 33–48 (Fig. 4b). Apart from carbon and hydrogen, TCE also contains chlorine atoms, which hinders its enzymatic degradation by microorganisms. TCE abatement in the presence of methanol has been conducted with a consortium of *Fusarium verticillioides* and *Fusarium solani* in a BTF operated at an EBRT of 9 s, with eliminations capacities of 3.2–12.9 g m$^{-3}$ h$^{-1}$ and maximum REs of TCE of 87.1% (Chheda and Sorial, 2017). Similarly, TCE elimination capacities of 4.9–3.6 g m$^{-3}$ h$^{-1}$ and maximum REs of 52.9% were recorded in a BTF inoculated with an *Ascomycota* strain and operated at an EBRT 405 s (Quan et al., 2018).

Fig. 4. Time course of the inlet (●) and outlet concentration of (a) n-hexane, (b) TCE, (c) toluene, (d) α-pinene in the biofilter inoculated with *C. subhashii* (■) and the biotrickling filter inoculated with *C. subhashii* (▲) and operated at a gas residence time of 30 s.
Interestingly, higher toluene REs were achieved under steady state in BTF than in BF likely due to the moderate aqueous solubility of this aromatic VOC. Thus, steady state toluene REs fluctuated in the range of 41–46% (day 40–50) with a reduced start-up period of 10 days. Toluene REs of 18–21% were reached in BF during steady state (Fig. 4c). Toluene removal has been conducted with a non-virulent consortium of black yeast Cladothialaphora sp. in a BF operated at an EBRT of 12–48 s, with elimination capacities of 5.0–30.0 g m⁻³ h⁻¹ and maximum REs of toluene of 100% (Prenafeta-Boldú et al., 2008).

Finally, steady state α-pinene REs of 25–29% and 20–27% were recorded in BTF and BF, respectively, with a start-up period of ~17 days (Fig. 4d). Preliminary studies carried out α-pinene removal with Ophiostoma sp. in a BF operated at EBRTs of 26–72 s, with elimination capacities of 143.0 g m⁻³ h⁻¹ and REs of α-pinene of up to 95% (Gonzalez-Martín et al., 2022). Similarly α-pinene REs of 89% were achieved in a BF operated at an EBRT of 31 s with Ophiostoma sp. (Jin et al., 2007). Finally, the operation of a BTF at an EBRT of 26–38 s with Candida boidinii and Ophiostoma stenoceras consortium resulted in pinene elimination capacities of 175.0 g m⁻³ h⁻¹ and REs of 67% (López et al., 2013).

Fungal cells colonizing the packing material in the BTF produced more CO₂ than those in BF mediated by the higher efficiency in the removal of the hydrophobic VOCs in the former biofiltration unit (Fig. 5). At this point it should be highlighted that since the emission treated was a diluted VOC air stream, O₂ never limited the biodegradation process. The VOCs mineralization ratio (CO₂ production/VOCs-EC) averaged 0.12 ± 0.01 and 0.07 ± 0.02% in the BTF and the BF, respectively.

### 3.2.1. Henry’s law constants and Hansen solubility parameters

The ability of a compound to be dissolved in a given solvent can be predicted through Hansen’s solubility parameters. The principle of the Hansen three dimensional solubility parameters (the Hildebrand parameter) can be expressed according to Equation (4):

$$
\delta_i^2 = \delta_D^2 + \delta_H^2 + \delta_P^2
$$  \( (4) \)

where \( \delta_i \) is the total solubility parameter (the so-called Hildebrand solubility parameter) and \( \delta_D, \delta_H \), and \( \delta_P \) are the components of the Hansen parameters of the compound due to dispersion, polar and hydrogen bonding, respectively. The \( \delta_D, \delta_H \), and \( \delta_P \) components of the Hildebrand parameter for each individual compound were listed in Table 1.

The solubility distance (\( R_s \)) is the distance between the solvent (water) and the solute (compound) in Hansen (Hildebrand) solubility parameters and can be defined according to Equation (5) (Li et al., 2016):

$$
R_s^2 = 4(\delta_H - \delta_D)^2 + (\delta_P - \delta_D)^2 + (\delta_H - \delta_P)^2
$$  \( (5) \)

The closer the solubility parameters of the compound and solvent are, the more likely the compound is to dissolve in a given solvent. The values of the solubility distance (\( R_s \)) showed in Table 1 are consistent with the biodegradation pattern herein obtained: toluene \( \approx n\)-hexane > α-pinene > TCE. Thus, the higher the \( R_s \) value calculated for a given compound and the solvent used, the better removal through biofiltration.

Hydropobicity patterns differed from those theoretically expected according to Henry’s law constants (in order of decreasing solubility: \( n\)-hexane > α-pinene > TCE > toluene). To date, the mechanisms of degradation of these VOCs individually by C. subhashii have not been investigated. In this context, it is not possible to lay the foundation of possible inhibition mechanisms using this VOC mixture. Interestingly, the presence of trickling liquid phase did not alter the patterns of degradation observed.

### 4. Conclusion

C. subhashii immobilized in PUF supported an effective abatement of hydrophobic VOCs at a relatively short EBRT regardless of the gas-phase bioreactor configuration evaluation. Both the batch and continuous VOC biodegradation assays showed a consistent biodegradation pattern: toluene \( \approx n\)-hexane > α-pinene > TCE. The biotrickling filter supported a slightly higher VOC abatement performance, as confirmed by the higher CO₂ concentrations recorded in the BTF off-gas. The decrease in the pH of the cultivation broth and the unexpected deterioration in VOC biodegradation in the batch assays suggested the release of inhibitory metabolites from fungal metabolism during VOC mineralization. Despite the promising results herein obtained, further research on the optimization of process parameters such as EBRT, inlet load and pH, and on the elucidation of potential inhibition mechanisms in the VOC mixture, is required to enhance the efficiency of the biofiltration process for the abatement of hydrophobic compounds by C. subhashii.

### CRediT authorship contribution statement

Milena Marycz: Conceptualization, Methodology, Investigation, Formal analysis, Roles./. Yadira Rodríguez: Writing – review & editing, Methodology. Jacek Gębićki: Supervision, Writing – review & editing. Raúl Muñoz: Supervision, Conceptualization, Methodology, Project administration, Writing – review & editing, Funding acquisition, Resources.

### Table 1

Henry’s constant and Hansen parameters for water and some VOCs.

| Compounds | Henry’s constant | Hansen solubility parameters [MPa] | \( R_s \) (relative for a water) | Reference |
|-----------|-----------------|----------------------------------|---------------------------------|-----------|
| n-hexane  | \( 1.0\times10^{-3} \) | 15.2 | 0.8 | 2.0 | 43.08 | Filly et al. (2014) |
| TCE       | \( 9.9\times10^{-2} \) | 18.0 | 3.1 | 5.3 | 39.48 | Hansen (2007) |
| Toluene   | \( 1.6\times10^{-1} \) | 18.0 | 1.4 | 2.0 | 43.13 | Hansen (2007) |
| α-pinene  | \( 4.9\times10^{-2} \) | 17.0 | 1.3 | 2.0 | 42.99 | Filly et al. (2014) |
| Water     | – | 15.6 | 16.0 | 42.3 | – | Subrahmanyam et al. (2015) |

* Henry’s law constants at 298.15 K [M/atm] (Henry’s law constants \( n \), 2021).*
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.

Acknowledgments

The present work has been sponsored by Gdańsk University of Technology with a project co-financed from European Funds ‘Integrated Programme of Development of Gdańsk University of Technology’ (the project no POW.03.05.00–00–Z44/17). The Regional Government of Catalonia y León and the EU-FEDER programme [grant number CLU 2017-09 and UIC 315] are also gratefully acknowledged.

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