Biofiltration of hydrophobic volatile pollutants is intrinsically limited by poor transfer of the pollutants from the gaseous to the liquid biotic phase, where biodegradation occurs. This study was conducted to evaluate the potential of silicone oil for enhancing the transport and subsequent biodegradation of hexane by the fungus *Fusarium solani* in various bioreactor configurations. Silicone oil was first selected among various solvents for its biocompatibility, nonbiodegradability, and good partitioning properties toward hexane. In batch tests, the use of silicone oil improved hexane specific biodegradation by approximately 60%. Subsequent biodegradation experiments were conducted in stirred-tank (1.5 L) and packed-bed (2.5 L) bioreactors fed with a constant gaseous hexane load of 180 g m⁻³ reactor-h⁻¹ and operated for 12 and 40 days, respectively. In the stirred reactors, the maximum hexane elimination capacity (EC) increased from 50 g m⁻³ reactor-h⁻¹ (removal efficiency, RE of 28%) in the control not supplied with silicone oil to 120 g m⁻³ reactor-h⁻¹ in the biphasic system (67% RE). In the packed-bed bioreactors, the maximum EC ranged from 110 (50% RE) to 180 g m⁻³ reactor-h⁻¹ (>90% RE) in the control and two-liquid-phase systems, respectively. These results represent, to the best of our knowledge, the first reported case of fungi use in a two-liquid-phase bioreactor and the highest hexane removal capacities so far reported in biofilters.

Introduction

Air pollution represents a serious health and environmental problem that requires effective control. In particular, many volatile organic compounds (VOCs) can cause odor nuisances and have toxic effects, and must be removed from contaminated streams (I). Compared to classical methods for air treatment, such as absorption, condensation, adsorption, or incineration, biological methods are often considered as most cost-effective for the treatment of large volumes of air contaminated with low concentrations of organic compounds (2).

Biological air treatment processes are based on the transfer of the contaminants into an aqueous phase prior to their biodegradation. Mass transfer is very relevant for the case of hydrophobic pollutants, such as hexane, where this step can limit bioavailability to the microorganisms (3). Hence, new methods must be found to increase the transfer of the pollutants to the microorganisms. In this perspective, it has recently been shown that fungi might improve the removal of hydrophobic compounds because their aerial mycelia, which are in direct contact with the gas, could take up these compounds faster than flat aqueous bacterial biofilms (4–6). As a significant example, hexane EC ranging from 100 to 150 g m⁻³ reactor-h⁻¹ have been obtained with *Aspergillus niger* (7) in comparison to the 10–60 g m⁻³ reactor-h⁻¹, reported with *Pseudomonas* (8, 9) and with activated sludge (10). Fungi are also advantageous in biofilters because of their tolerance to low pH and moisture content that are commonly encountered in such systems (2, 11). On the other hand, fungi generally grow more slowly than bacteria, may induce high-temperature, and in extreme cases, such as severe drying, sporulation may be induced and spores can be liberated to the environment.

Pursuing also the objective to improve mass transfer, various authors (12–14) have shown that the inclusion of a hydrophobic organic phase in the reactors can significantly enhance the transfer of pollutants to the microorganisms and, thereby, their removal. In these systems, commonly known as two-liquid-phase bioreactors (TLPBs), the organic phase is used to rapidly absorb poorly soluble organic pollutants from gaseous streams. The pollutants are then transferred by liquid–liquid partitioning to the aqueous phase at sub-inhibitory levels where they are biodegraded. Hexane EC of up to 80 g m⁻³ reactor-h⁻¹ has been reported in a biotrickling filter percolated with a mixture of silicone oil and water (1:1) (14). Solvent selection is a crucial step to the development of TLPBs, as the organic phase must be nonbiodegradable, nonvolatile, nonmiscible, and biocompatible, and must have good partitioning properties with the target pollutants (15, 16). It can be difficult to combine these properties but silicone oil has often been used to support the biodegradation of hydrophobic pollutants for being biocompatible and highly stable (12–14).

Both the use of fungi and the use of an organic phase have the potential to improve the removal of hydrophobic air pollutants. However, little data is still available to compare these technologies that have never, to the best of our knowledge, been combined together. This study was therefore conducted to systematically compare hexane removal in several bioreactor configurations inoculated with a fungal strain and supplied with silicone oil.

Materials and Methods

**Chemicals.** Hexane (95%) was obtained from Tecsisquim Chemical Co. (Mexico City). Silicone oil (poly(dimethylsiloxane) 200 fluid with a viscosity of 20 cSt and density of 0.95 g mL⁻¹), hexadecane, tetradecone, undecane, 2-undecanone, diethyl sebacate, and 1-decanol were purchased from Sigma-Aldrich (+99%).

**Microorganism and Mineral Salt Medium (MSM).** *Fusarium solani* (CBS 117476) (17) was used for hexane biodegradation. The mineral salt medium (MSM) used for fungal growth contained (g L⁻¹) the following: NaNO₃, 6; KH₂PO₄, 1.3; MgSO₄·7H₂O, 0.38; CaSO₄·2H₂O, 0.25; CaCl₂, 0.055; FeSO₄·7H₂O, 0.015; MnSO₄·H₂O, 0.012; ZnSO₄·7H₂O, 0.013; CuSO₄·7H₂O, 0.0023; CoCl₂·6H₂O, 0.0015; and H₂BO₃, 0.0015. The pH was adjusted to 4 with 0.01 M HCl.
The fungal inoculum contained a spore suspension with an initial concentration of 2 × 10^6 spores mL^-1 of MSM. The spores were obtained by growing the fungus on agar Petri plates with 5 g L^-1 of malt extract and collecting the spores with MSM containing a few drops of Tween 80.

**Partition Test.** Glass tubes of 55 mL capacity were filled with 2 mL of the tested organic solvents and closed with Mininert Teflon Valves (VICI Precision Sampling, Inc., Baton Rouge, LA). For each solvent tested, a duplicate set of tubes was prepared and supplied with 10, 50, 250, 750, or 1250 μL of hexane. The tubes were then vigorously shaken in a vortex for 1 min and allowed to equilibrate during 30 min at 30 °C on a rotary shaker at 150 rpm. Gas samples of 200 μL of each experiment were then withdrawn from the tubes’ headspaces to measure the hexane concentration. The amount of hexane in the silicone oil was calculated by subtracting the amount of hexane in the gas phase at the equilibrium to the initially added hexane. The hexane partition coefficient was then calculated as the ratio between its concentration in the gaseous and organic phases.

**Solvent Biodegradability and Toxicity.** Biodegradability tests were conducted in duplicate in 155 mL glass flasks with 19 mL of MSM, 2 mL of the tested solvent, and 1 mL of fresh fungal inoculum with a concentration of 55 mg protein L^-1. The flasks were closed with butyl septa, sealed with aluminum caps, and incubated on a rotary shaker at 150 rpm and 30 °C. Control flasks were prepared and incubated under similar conditions but were not supplied with the organic solvent. The concentrations of O2 and CO2 in the flasks’ headspaces were monitored every 3 days by GC for one month by withdrawing 200 μL gas samples with a 500 μL syringe. Solvents were considered biodegradable if the CO2 production was greater than 20 g CO2 L^-1 protein h^-1.

Toxicity tests were carried out in duplicate as described above but supplied with glucose, yeast extract, and peptone at 1, 0.02, and 0.02 g L^-1, respectively, as easily available carbon and energy sources. Control flasks were also provided with the nutritive solution without organic phase. The concentrations of O2 and CO2 in the flasks’ headspace were measured with a GC for one month as described above. A solvent was considered toxic if no CO2 was produced.

**Batch Hexane Degradation Experiments.** Glass flasks of 615 mL were supplied with 43 mL of mineral medium, 5 mL of silicone oil, and 2 mL of fungal inoculum to achieve a final concentration of 55 mg protein L^-1. The flasks were closed with butyl septa, sealed with aluminum caps, and incubated on a rotary shaker at 150 rpm and 30 °C. Control flasks were prepared and incubated under similar conditions but were not supplied with the organic solvent. The concentrations of O2 and CO2 in the flasks’ headspaces were monitored every 3 days by GC for one month by withdrawing 200 μL gas samples with a 500 μL syringe. Solvents were considered biodegradable if the CO2 production was greater than 20 g CO2 L^-1 protein h^-1. The protein concentration was determined using the Lowry method by hydrolyzing the sample with a 0.2 M NaOH solution and using bovine serum albumin as standard. The sample was first centrifuged at 8000 rpm for 10 min to separate the aqueous phase from the oil phase.

The biomass content in the carrier was measured as the loss of volatile solids at 550 °C according to Standard Methods (29). Three samples were taken from different levels of the reactor and the measurements were made in duplicate. The reported biomass content is the average of the 3 samples.

**Scanning Electron Microscopy.** Biofilter samples were observed in a digital scanning electron microscope (JSM-5900 LV, JEOL, Japan) using 13 kV accelerating voltage. The
samples were prepared according to the method described by Acuña et al. (18).

Calculations. Results from the reactor experiments were expressed in terms of the hexane volumetric elimination capacity (EC in g·m⁻³reactor·h⁻¹) and the hexane gaseous elimination capacity (ECg in g·m⁻³gas·h⁻¹) according to the following formulas:

\[
EC = \frac{Q_g}{V_{\text{reactor}}} (S_{\text{in}} - S_{\text{out}}) \\
EC_g(\text{Stirred tank}) = EC\left(\frac{1}{G}\right) \\
EC_g(\text{Packed bed}) = EC\left(\frac{1}{\varepsilon}\right)
\]

where \(V_{\text{reactor}}\) is the reactor volume (m³reactor); \(Q_g\) is the air flow (m³gas·h⁻¹); \(S_{\text{in}}\) and \(S_{\text{out}}\) are the inlet and outlet hexane concentrations, respectively (g·m⁻³gas); \(G\) (m³gas·m⁻³reactor) corresponds to the gas hold up and was determined experimentally by dividing \(V_g\) (gas volume) over the reactor volume \(V_{\text{reactor}}\); and \(\varepsilon\) is the bed void fraction of the PBRs (m³gas·m⁻³reactor). For the \(Q_g\) of 1.5 L·min⁻¹, the gas volume was 0.2 L corresponding to a hold up for the STR of 0.133 m³gas·m⁻³reactor. The bed void fraction for the PB reactor was 0.65 m³gas·m⁻³reactor.

Results

Solvent Evaluation. Silicone oil was the only nontoxic and nonbiodegradable organic phase for F. solani (Table 1). The hexane concentration in silicone oil was approximately 300 times greater than that in the gas phase.

Batch Experiments. The kinetic consumption of hexane by F. solani was evaluated in batch experiments (Figure 1).

Stirred-Tank Bioreactors Maximum ECs of 120 and 50 g·m⁻³reactor·h⁻¹ were attained in the biphasic bioreactor and in the control reactor, respectively, after 3 and 4 days of

### TABLE 1. Partition, Toxicity, and Biodegradability Tests with Fusarium solani

<table>
<thead>
<tr>
<th>solvent</th>
<th>log (P_{ow})</th>
<th>aqueous solubility</th>
<th>(K^a)</th>
<th>biodegradable</th>
<th>toxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>silicone oil</td>
<td>NK(^c)</td>
<td>NK</td>
<td>0.0034 ± 0.0003</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>hexadecane</td>
<td>8.25</td>
<td>0.0009</td>
<td>0.0042 ± 0.0004</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>tetradeacne</td>
<td>7.2</td>
<td>0.0022</td>
<td>0.0026 ± 0.0001</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>undecane</td>
<td>6.5</td>
<td>0.0040</td>
<td>0.0038 ± 0.0001</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1-decanol</td>
<td>4.57</td>
<td>37</td>
<td>0.0073 ± 0.0008</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>diethyl sebacate</td>
<td>4.33</td>
<td>80</td>
<td>0.0115 ± 0.0003</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2-undecanone</td>
<td>4.09</td>
<td>19.7</td>
<td>0.0050 ± 0.0009</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\) Logarithm of the octanol–water partitioning ratio and aqueous solubility (mg·L⁻¹) according to the SRC PhysProp Database. \(^b\) \(K^a\): Partitioning coefficient of hexane in organic and gaseous phases calculated as the ratio of the hexane concentrations in gas and organic phases and expressed as the average ± 95% confidence interval calculated from 5 experimental measurements. \(^c\) Not known.
TABLE 2. Maximum Hexane Elimination Capacities Expressed in Terms of the Reactor and Gas Volume, and the Specific Hexane Consumption Rates for Packed-Bed and Stirred-Tank Bioreactors Inoculated with *Fusarium solani*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Load</th>
<th>EC</th>
<th>RE</th>
<th>EC_g</th>
<th>Specific removal rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g·m⁻³_reactor·h⁻¹</td>
<td>g·m⁻³_reactor·h⁻¹</td>
<td>%</td>
<td>g·m⁻³_gas·h⁻¹</td>
<td>mg_hexane·g⁻¹_protein·h⁻¹</td>
</tr>
<tr>
<td>stirred tank bioreactor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>one liquid phase</td>
<td>180</td>
<td>50</td>
<td>28</td>
<td>554</td>
<td>250</td>
</tr>
<tr>
<td>two liquid phases</td>
<td>180</td>
<td>120</td>
<td>67</td>
<td>900</td>
<td>545</td>
</tr>
<tr>
<td>packed-bed bioreactor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>one liquid phase</td>
<td>1575</td>
<td>580</td>
<td>37</td>
<td>4361</td>
<td>2636</td>
</tr>
<tr>
<td>two liquid phases</td>
<td>680</td>
<td>360</td>
<td>54</td>
<td>554</td>
<td>87</td>
</tr>
</tbody>
</table>

* According to the values of Arriaga et al. (6) achieved in a fungal PBR. This study can be used as a control experiment to the fungal PBR tested in this study.

FIGURE 4. Effect of hexane inlet load on elimination capacity in the packed-bed (A) and the stirred-tank (B) bioreactors containing 5% and 10% of silicone oil, respectively, and inoculated with *Fusarium solani*.

The effect of hexane load on EC in the biphasic PBR was evaluated after 45 days of operation and the experiment was carried out during 2 weeks (Figure 4). The critical inlet load was approximately 150 g·m⁻³_reactor·h⁻¹ and a maximum EC of 360 g·m⁻³_reactor·h⁻¹ was attained at a load of 680 g·m⁻³_reactor·h⁻¹ (Table 2).

**Scanning Electron Microscopy.** Samples from the packed-bed bioreactor showed fungal morphologies. It was also possible to observe mycelia adhered to silicone oil and some bacteria on the hyphae (Figure 5).

**Discussion**

The use of silicone oil to increase the mass transport and bioavailability of hexane to *F. solani* was an effective strategy (Table 2). As a significant example, the specific total hexane consumption rate obtained in batch culture with silicone oil was 2.3 times greater than that value without silicone. The use of fungi might improve the removal of hydrophobic compounds because their large aerial mycelia are in direct contact with the gas (4–6). This study shows that fungal activity can be further improved by the addition of a hydrophobic phase to the system. Hence, hexane uptake by fungi in biphasic systems likely undergoes direct assimilation from the gas phase as well as through the observed contact between the fungi and silicone oil. Both uptake mechanisms strongly reduce the mass transfer limitations. In our study, the observed microbial adhesion to silicone oil (Figure 5) was likely favored by the production of surface active molecules, such as the hydrophobins (21), by the fungi, which...
silicone oil (250 mg) transfer to the microorganisms as the solubility of oxygen in improved hexane biodegradation by increasing oxygen hexane mass transfer, the use of silicone oil might also have

2394

inhibitory levels. In fact, 1-decanol was not toxic to times higher than undecane and consequently may attain FIGURE 5. SEM microphotographs for fungal packed bed bioreactors: (a) mycelia over silicone oil particle; (b) hyphae and bacteria. also stabilized the emulsion. This further increased hexane bioavailability and could have allowed direct uptake at the organic aqueous interface (22, 23). Besides increasing the hexane mass transfer, the use of silicone oil might also have improved hexane biodegradation by increasing oxygen transfer to the microorganisms as the solubility of oxygen in silicone oil (250 mg·L⁻¹) is around 30 times higher than in water (24).

The selection of an appropriate and economical organic phase is an essential parameter for the performance of two-phase bioreactors. The most significant characteristics of the organic phase are nonbiodegradability, biocompatibility, high solubilization capacity for the substrate, and nonmiscibility in water (12). Silicone oil was chosen on this basis as it was nonbiodegradable and nontoxic for F. solani and it exhibited an affinity for hexane 10⁴ times greater than water (partition coefficient of hexane in water 30.9) (1). The susceptibility of F. solani to the solvents tested may result from the hydrophobic nature of hyphae, because the toxicity of organic solvents is partially related to their concentration in the cell membrane, which should increase with the hydrophobicity of the membrane in case of hydrophobic solvents (25). However, toxicity also depends on the molecular structure of the solvent used and the partition coefficient of octanol–water (12, 26). Considering that undecane was consumed, it was unexpected to find that 1-decanol inhibited the fungi. However, the solubility of 1-decanol in water is around 10⁶ times higher than undecane and consequently may attain inhibitory levels. In fact, 1-decanol was not toxic to F. solani at a concentration 100 times lower than its solubility in water (37 mg·L⁻¹) (27).

The hexane EC (165 g·m⁻³reactor·h⁻¹) and RE (>90%) obtained with the biphasic PBR were greater than the 10–150 g·m⁻³reactor·h⁻¹ EC and the 40–60% RE previously obtained in bacterial and fungal biofilters (6, 8, 14). The 15% reduction of the EC recorded after day 30 of operation could be related with the instability of the emulsion and the leaching of the oil with the added MSM. The maximum EC of 360 g·m⁻³reactor·h⁻¹ achieved in the PBR was also higher than the maximum EC of 130 g·m⁻³reactor·h⁻¹ previously reported in a similar system not provided with silicone oil (17). The mineralization obtained (45%) was lower than the 79% reported previously (17) but the carbon balance may be influenced by the solubilization of CO₂ (24) and potential hexane oxidation intermediaries (hexanol, hexanal, and hexanoic acid) in the silicone oil phase. This was also observed in the batch tests as CO₂ production was more important in the experiment without silicone oil.

The maximum EC (120 g·m⁻³reactor·h⁻¹) obtained in the biphasic STR at a load of 180 g hexane·m⁻³reactor·h⁻¹ was similar to the values reported in classical biofilters, although the removal efficiencies (67%) and the maximum EC (580 g·m⁻³reactor·h⁻¹) achieved in this study were greater. When considering the short effective gas residence time applied in the STR, the maximum EC achieved in this system was much higher than ECg in the PBs or by other authors such as Kibazohi (10), who reported an ECg of 94 g·m⁻³gas·h⁻¹ for a bacterial biofilter packed with perlite (ε = 0.65). Hence, these results represent, to the best of our knowledge, the highest hexane ECg yet reported in the literature, confirming the potential of two-liquid-phase bioreactors (TLPBs) for the abatement of hydrophobic air pollutants (15, 16). Davison and Daugulis (28) even obtained an EC up to 233 g·m⁻³reactor·h⁻¹ for toluene in TLPB and predicted that a maximum EC of 1290 g·m⁻³reactor·h⁻¹ could be achieved in such systems, which is much higher than the toluene EC of 145 and 350 g·m⁻³reactor·h⁻¹ reported for conventional bacterial (29) or fungal biofilters (5), respectively. The lower EC obtained here with hexane was likely explained by the lower aqueous solubility of hexane (approximately 50 times lower than that of toluene) and to the different operating conditions tested. In our study, the reactor was operated at an organic/aqueous phase rate of 1:9 and an agitation speed of 400 rpm, while Daugulis and co-workers (15, 16) used a ratio of 3.4:6.6 and an agitation speed of 800 rpm. The stirring rate is indeed a crucial parameter in the performance of STRs as it controls the transfer coefficients and the interfacial area of the emulsion. This was illustrated here by the fact that specific consumption rates obtained in batch experiments agitated at 150 rpm were lower than the values achieved in the STR agitated at 400 rpm (Table 2). However, high stirring rates might not be convenient in the case of fungal STRs since shear stress may affect the integrity of the fungal mycelia (30).

The lower ECg recorded in the biphasic PBR than in the biphasic STR can therefore be explained by the lack of agitation in packed bed bioreactors, which would promote the coalescence of the oil particles and a reduction of the aqueous–organic interfacial area. Furthermore, laminar gas flow in biofilters generates reduced transfer coefficients (31). In the long term, macroscopic heterogeneities in biofilters may induce zone clogging or channeling and a further reduction in mass transfer. In contrast, stirring in baffled reactors promotes emulsion dispersion and increased mass transfer rates. These results therefore suggest that two-liquid-phase systems are able to exceed the performance of PBRs when treating low- and high-VOCs gas streams. In addition, the use of TLPBs improves the mass transfer of VOC and oxygen to the microorganism, effectively utilizes the entire reactor volume, and allows a higher ECg (i.e., smaller units to treat the same mass loading). However, this improvement is accompanied by higher energy input that increases the operation costs.

The specific hexane removal rate obtained in the STR with silicone oil was approximately 50% greater than that without oil and can be compared with the value of 339 mg·hexane·g⁻¹proton·h⁻¹ reported by Morales et al. (32) for the
hexane degradation by cometabolism with methyl tert-butyl ether. The lower specific rate recorded in the PBR can be explained by the higher biomass content achieved in this system (approximately 180 mg_organic-mg_1 dry粉末), which could have induced inactive zones (6). This illustrates again the disadvantage of poorly homogenized bioreactors.

Summarizing, the use of silicone oil enhanced hexane removal by _F. solani_ in both stirred-tank and packed-bed bioreactors, which increased the performance of the biphasic systems by approximately 50% compared to their respective controls. This study presents the first reported case of the use of fungi in two-liquid-phase reactors, which broadens the application range of this technology. Solvent selection for the fungal strain was based on the same characteristics and methods proposed for bacteria and the critical log _P_ow below which the solvent was found toxic was between 4.6 and 6.5, which is in the same range of 5–7 reported for gram positive bacteria (33). The combined use of fungi and organic solvent led to a significant process improvement, allowing the highest hexane removal rates reported in the literature hitherto. Further work is needed to select less expensive organic phases, to increase stability, to optimize the solvent phase ratios, to model the process, and to demonstrate the feasibility of the system for other fungal species.

**Acknowledgments**

We thank CONACYT (SEMAR-NAT-120-2001) for financing this work, and also Dr. José Sepulveda for SEM support.

**Nomenclature**

EC: hexane elimination capacity (g m⁻³_3 reactorh⁻¹)
ECₕ: hexane elimination capacity (g m⁻³_gazh⁻¹)
Qₕ: airflow (m³h⁻¹)
Sᵢ: and Sₒ inlet and outlet hexane concentration respectively (g·m⁻³)
Vₕ: reactor volume (m³reactor)
Vgas: gas volume (m³gas)

**Greek Letters**

ε: bed void fraction (m³_gas·m³_3 reactor)
eₒ: gas hold up (m³_gas·m³_3 reactor)

**Literature Cited**

(2) Shareefdeen, Z.; Singh, A. Biotechnology for Odour and Air Pollution; Springer-Verlag: Heidelberg, Germany, 2005.