Treatment of *n*-hexane in fungal packed bed and stirred tank bioreactors containing two phases

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ABSTRACT. The treatment of hydrophobic volatile pollutants is limited by the poor transfer of the compounds from the gaseous to the liquid biotic phase, where biodegradation occurs. Fungi have shown high Elimination Capacities (EC) with hydrophobic compounds through their increased transfer surface and more favorable partition. This study evaluates silicone oil for enhancing hexane transport and biodegradation by the fungus *Fusarium solani* in various bioreactor configurations. Silicone oil was first selected among various solvents for its biocompatibility, non-biodegradability and good partitioning properties. Biodegradation experiments were conducted in 1.5 L stirred tank or in 2.5 L packed bed biofilters fed with an hexane load of 180 $g.m^{-3}_{reactor}.h^{-1}$ (EBRT: 1 min). Hexane EC of 120 $g.m^{-3}_{reactor}.h^{-1}$ was obtained in the stirred tank bioreactor inoculated with *Fusarium solani*. The addition of 5% of silicone oil to a fungal biofilter improves hexane degradation to 180 $g.m^{-3}_{reactor}.h^{-}$ with a maximum of 360 $g.m^{-3}_{reactor}.h^{-1}$. Control experiments without oil were around 40% lower. These results confirm that the use of an adequate organic phase enhances the transport and subsequently biodegradation of hydrophobic compounds such as hexane.

1 INTRODUCTION

Biological air treatment processes are based on the transfer of the contaminants into an aqueous biotic phase prior to their biodegradation. This transfer is limited for hydrophobic pollutants by their extremely low solubility. Some strategies have been proposed to solve this limitation. Various authors have shown that the use of fungi improved the removal of hydrophobic compounds (García-Peña *et al*., 2001; van Groenestijn and Liu, 2002). For hexane, a highly hydrophobic compound, elimination capacities (EC) around 150 $g.m^{-3}.h^{-1}$ have been obtained (Spigno *et al*., 2003; Arriaga and Revah, 2005) whereas only 35 $g.m^{-3}.h^{-1}$ have been reached with bacteria (Budwill and Coleman, 1999; Kibazohi *et al*., 2004). Other authors have also shown that the addition of a hydrophobic organic phase into two-liquid phase bioreactors (TLPBs) increased the EC of the slightly hydrophobic toluene (Davison and Daugulis, 2003). Van Groenestijn and Lake (1999) reported hexane removal efficiency up to 90 $g.m^{-3}.h^{-1}$ in a biotrickling filter percolated with a mixture of silicone oil and water. Silicone oil

appears to be a common choice in TLPBs but other solvents have used for benzene removal (Yeom and Daugulis, 2000) and in application for liquid effluents (Déziel, 1999).
The objective of this study was to compare the performance of a fungal stirred tank and a packed bed bioreactor supplied with an organic phase.

2 MATERIALS AND METHODS

2.1 Chemicals and microorganism

Hexane with a purity of 95% was obtained from Tecsiquim Chemical Co. (Mexico, City). Silicone oil [Poly(dimethylsiloxane)], hexadecane, tetradecane, undecane, undecanone, diethyl sebacate and 1-decanol were purchased from Sigma-Aldrich with a purity of +99%.
Fusarium solani (CBS 117476) was used for hexane biodegradation. The mineral medium used for fungal growth and the inoculum was reported by Arriaga and Revah, (2005).

2.2 Partition test

Duplicate 55 mL glass tubes were filled with 2 mL of the tested organic solvents and closed with Mininert Teflon Valves (VICI Precision Sampling, Inc., Baton Rouge, LA). Various initial amounts of hexane were added and the tubes were then vigorously shaken for 1 minute and equilibrated for 30 minutes at 30 °C with agitation. Gas samples of 200 µL were then withdrawn from the tubes headspaces to measure the hexane concentration. The hexane partitioning coefficient is the ratio between its concentrations in the gaseous and organic phases.

2.3 Solvent biodegradability and toxicity

Biodegradability tests were conducted in duplicate in 155 mL glass flasks with 19 mL of mineral medium, 2 mL of the tested solvent and 1 mL of fungal inocula. The flasks were closed with butyl septa and incubated on a rotary shaker at 150 rpm and 30 °C. Control flasks were treated identically but no solvent was added. The concentrations of O_2 and CO_2 in the flasks headspace were monitored every 3 days by GC for one month by withdrawing 200 µL gas samples. Solvents were considered biodegradable if the CO_2 production was significantly higher than in the controls.
Toxicity tests were in duplicate as described above but supplied with glucose, yeast extract and peptone at 1, 0.02 and 0.02 $g.L^{-1}$, respectively. Controls contained the nutritive solution without organic phase. The concentrations of O_2 and CO_2 in the flasks headspace were monitored by GC for one month as described above. A solvent was considered toxic if the CO_2 production with the solvent and with the nutritive solution was significantly lower than the control.

2.4 Stirred tank reactor, STR

A 2 L Multigen fermentor (New Brunswick Scientific Co. Inc., New Jersey, USA) equipped with a double Rushton turbine operated at 400 rpm was used for hexane biodegradation at 30°C (Figure 1).The reactor was filled with 1.35 L of mineral medium, 150 mL of silicone oil (organic/aqueous ratio of 1:9) and inoculated with *F. solani* to attain an initial biomass concentration of approximately 220 mg protein.L^{-1}. The reactor was aerated at 1 vvm and fed with a constant hexane load of 180 $g.m^{-3}_{reactor}.h^{-1}$. The pH was controlled at 4.0 with HCl (0.01 M). Similar control experiments

were conducted without silicone. One hundred mL of liquid sample was replaced with fresh fungal medium every two days.

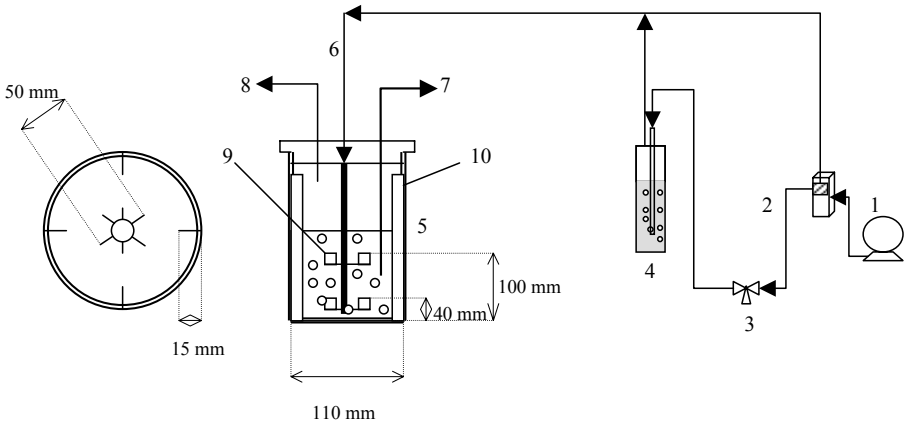

Figure 1. Schema of the stirred tank bioreactor. 1, compressor; 2, mass flow meter; 3, precision valve; 4, hexane evaporator; 5, stirred tank reactor; 6, inlet; 7, sample port; 8, outlet; 9, Rushton turbine; 10, Baffles.

2.5 Packed bed bioreactor

The biofilter consisted of a 1 m cylindrical glass column with an inner diameter of 0.07 m filled with 2.5 L of perlite (average diameter of 4 mm, Hummert, Mexico) (Figure 2). Hexane saturated air was mixed with moist air and fed at the top of the reactor with an Empty Bed Residence Time (EBRT) of 1 min and an hexane inlet load of 180 $g.m^{-3}_{reactor}.h^{-1}$. The reactor was inoculated with an emulsion of silicone oil (5%) and *F. solani* spores ($2x10^7$ $spores.mL^{-1}$) in MSM and containing 5 $g.L^{-1}$ of malt extract. To evaluate the influence of hexane load experiments were carried out with various inlet hexane concentrations at constant EBRT.

2.6 Analytical methods

Hexane gaseous concentration was measured with a FID-GC (García Peña *et al*., 2001). CO_2 and O_2 concentrations were analyzed by TCD (Muñoz *et al*., 2003). The CO_2 concentration in gas samples from the packed bed bioreactor was measured with an infrared analyzer (García Peña *et al*., 2001).

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.

2.7 Calculations

Results from the reactor experiments were expressed in terms of the hexane volumetric elimination capacity (EC in $g.m^{-3}_{reactor}.h^{-1}$) and the hexane gaseous elimination capacity (ECg in $g.m^{-3}_{gas}.h^{-1}$) according the following formulas:

$$EC = \frac{Q_g}{V_{reactor}}(S_{in} - S_{out}) \qquad EC_g(\text{Stirred tank}) = EC\left(\frac{1}{\varepsilon_G}\right)$$

$$EC_g(\text{Packed bed}) = EC\left(\frac{1}{\varepsilon}\right)$$

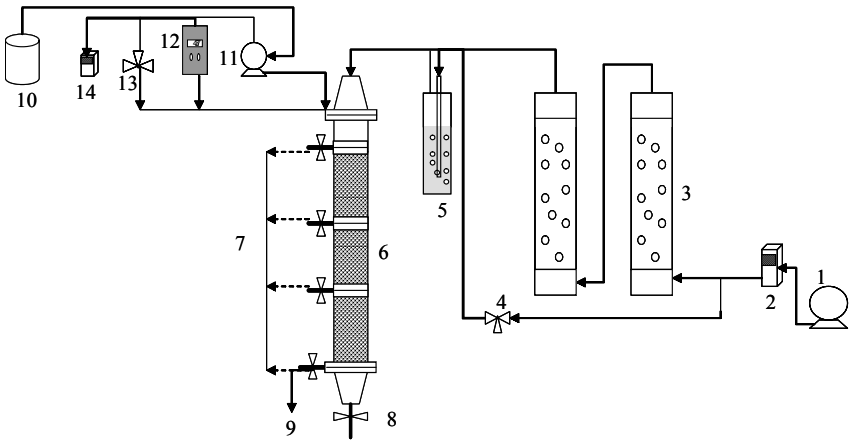

Figure 2. Experimental device of packed bed bioreactor. 1, compressor; 2, mass flow meter; 3, humidifier; 4, precision valve; 5, hexane evaporator; 6, packed column; 7, sampling ports; 8, leachate purge; 9, outlet; 10, nutrient solution; 11, peristaltic pump; 12, spraying system; 13, electric solenoid valve; 14, timer.

where $V_{reactor}$ is the reactor volume ($m^3_{reactor}$); Q_g is the air flow ($m^3_{gas}.h^{-1}$); S_{in} and S_{out} are the inlet and outlet hexane concentrations, ($g.m^{-3}_{gas}$); ε_G ($m^3_{gas}.m^{-3}_{reactor}$) is the gas hold up and was determined experimentally by dividing V_g (gas volume) over the reactor volume ($V_{reactor}$). ε is the bed void fraction for the packed bed bioreactors ($m^3_{gas}.m^{-3}_{reactor}$). For a Q_g of 1.5 $L.min^{-1}$ the gas hold up had a value of 0.133 $m^{-3}_{gas}.m^{-3}_{reactor}$ and the bed void fraction for the reactor packed with Perlite was 0.65 $m^{-3}_{gas}.m^{-3}_{reactor}$.

Table 1. Toxicity and biodegradability tests with *Fusarium solani*.

Solvent	*F. solani*	
	Biodegradable	Toxic
Silicone oil	-	-
Hexadecane, Tetradecane, Undecane	+	-
Diethylsebacate, Undecanone, Decanol	-	+

3 RESULTS AND DISCUSSION

Silicone oil was the only non toxic and non biodegradable organic phase for *F. solani* (Table 1). Partition coefficient of hexane in silicone oil was 0.0034 which is around 9,000 times greater than in the water.

The selection of an appropriate organic phase is an essential parameter for the performance of two phase bioreactors. The relevant characteristics of the organic phase are non biodegradability, biocompatibility, high solubilization for the substrate and non miscibility in water (Déziel *et al*., 1999). Silicone oil was chosen for the two phase bioreactors. The EC expressed in terms of the reactor and gas volumes are shown in

Table 2. In terms of the reactor volume the packed bed bioreactor exhibited an EC 33% greater than the stirred tank bioreactor with silicone oil. The EC obtained with the packed bed bioreactor was greater than the maximum EC of 150 $g.m^{-3}_{reactor}.h^{-1}$ reported by Spigno *et al*., (2003) with *Aspergillus niger*. Hexane uptake mechanisms by fungi in biphasic systems involves besides the uptake from the dissolved hexane in the medium, both the direct assimilation from the gas phase and probably through the contact with the oil. Both uptake mechanisms strongly reduce the mass transfer limitations. Experiments at different loads in the fungal packed bed bioreactor showed a maximum EC of 360 $g.m^{-3}_{reactor}.h^{-1}$ (Data no shown).

Table 2. Maximum hexane elimination capacities, expressed in terms of the reactor and gas volumes for packed bed and stirred tank bioreactors inoculated with *Fusarium solani*.

Elimination Capacity	Packed bed bioreactor*	STR	
		One phase	Two phases
$EC(g.m^{-3}_{reactor}.h^{-1})$	180	50	120
$EC(g.m^{-3}_{gas}.h^{-1})$	270	554	900

STR: Stirred Tank Reactor. * 5% of silicone oil.

When considering the EC referred to the effective gas residence time, the EC_g, in the STR, it was shorter than in the biofilter. The maximum EC_g achieved in the STR was 3 times higher than in the biofilter and almost 2 times greater that the STR without silicone oil (one phase). It can be related with the lack of agitation in packed bed bioreactors which promote coalescence of the oil particles. In contrast, stirring in baffled reactors promotes emulsion dispersion and increased mass transfer rates.

Mass transfer of highly hydrophobic compounds from the gas phase to the biotic aqueous phase usually limits their treatment. Hexane mass transport was increased by the addition of silicone oil. Davison and Daugulis, (2003) reported EC up to 233 $g.m^{-3}_{reactor}.h^{-1}$ for toluene in TLPB which is much higher than those reported for conventional bacterial (Auria *et al*., 2000) or fungal biofilters (García-Peña *et al*., 2001). The lower EC obtained here with STR with hexane could be related to the lower aqueous solubility of hexane (which is approximately 50 times lower than toluene) and to the lower organic/aqueous phase ratio and slower stirring rates used. Stirring rate is a crucial parameter as it promotes increased transfer coefficients and interfacial area of the emulsion and therefore the EC in mass limiting situations.

Addition of silicone oil in a STR and packed bed bioreactors enhanced the mass transfer of hexane and biodisponiblity between the air and *F. solani*. Further work includes the study of less expensive organic phases, the optimization of the solvent phase ratios and the modeling of the two liquid phase bioreactors.

4 ACKNOWLEDGEMENTS

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