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Removal of *n*-hexane by *Fusarium solani* with a gas-phase biofilter

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Abstract A gas-phase biofilter inoculated with the fungus *Fusarium solani*, isolated from a consortium grown on hexane vapors, was used to degrade this compound. The biofilter, packed with perlite and operated with an empty bed residence time of 60 s, was supplied with hexane concentrations between 0.5 gm^{-3} and 11 gm^{-3}. Biofilter performance was evaluated over 100 days of operation. Several strategies for supplying the nutritive mineral medium were assayed to maintain favorable conditions for the fungal growth and activity. The *Fusarium* system was able to sustain an average elimination capacity of 90 $gm^{-3}_{reactor}\ h^{-1}$ with a maximum of 130 $gm^{-3}_{reactor}\ h^{-1}$. The mass transfer limitations due to high biomass development in the biofilter were confirmed in batch experiments. Bacterial contamination was observed, but experiments in the biofilter and in batch reactors using selective inhibitors and controlled pH confirmed the predominant role of the fungus. Results indicate that fungal biofilters can be an effective alternative to conventional abatement technologies for treating hydrophobic compounds.

Keywords Fungal biofilter · Hexane · *Fusarium solani* · Biodegradation · Mass transfer · Hydrophobic substrate

Introduction

The emission of volatile organic compounds (VOCs) has a strong impact on air quality and human health. Traditionally, the emissions of VOCs have been treated by physicochemical process such as thermal and catalytic oxidation and carbon adsorption. VOCs emission treatment by these technologies is energy-intensive and may produce secondary air pollution (i.e., NO_x [7]). Biological processes, such as biofilters, trickling beds and bioscrubbers, have been successfully applied to treat waste gases containing VOCs and toxics emitted from industrial facilities such as foundries, chemical plants, oil-extraction industries and print shops. They are suitable for treating large volumes of air containing low concentrations of pollutants. Biofiltration has been employed widely due to its low cost, simple process control and low energy requirements.

Biofiltration is a multi-step process involving the partition of the gaseous VOCs and oxygen to the liquid phase containing the microorganisms, followed by transport through the biofilm to the cells. Bacteria, yeast and fungi in the biofilm metabolize the VOCs to CO_2 and H_2O [4]. When hydrophobic compounds are degraded in biofilters, the process is generally limited by the mass transport of the sparingly soluble compounds [9].

Alkanes are emitted by various industries, e.g., the petrochemical industry, edible oil procedures (*n*-hexane) and polystyrene foam producers (pentane). Vegetable oil production involves the cleaning and crushing of the seeds and then solvent oil extraction. While most of the solvent is recovered, a fraction is usually lost. Average emission values of 3.3 L of hexane per tonne of soybean oil have been reported.

Filamentous fungi have shown promising results for the degradation of hydrophobic pollutants [5, 15]. Fungi are microorganisms that are able to colonize and degrade a great number of substrates and are employed in a large variety of biotechnological processes. For slightly hydrophobic compounds, such as toluene, fungal biofilters show very high elimination capacities, (ECs; $>200\ gm^{-3}_{reactor}\ h^{-1}$) and support higher loads and fewer transfer limitations than bacterial biofilms [8]. Recently, a study carried out by Spigno et al. [12] reported greater degradation rates of hexane with the fungus

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Aspergillus niger than those obtained with bacterial systems [8]. Fungal mycelial growth favors the transport of hydrophobic compounds and oxygen from the gas to the biological phase by its enhanced surface/biofilm thickness and hydrophobic cell membrane [5, 16]. Probably a direct transport of hydrophobic pollutants from the gas phase to the biofilm phase is involved [14].

The present study describes the potential of using fungal biofiltration for the treatment of hexane as a model hydrophobic compound. A biofilter inoculated with *Fusarium solani*, isolated from a consortium [2], was characterized. Mineral medium addition was optimized to improve the reactor performance and selected environmental conditions (pH control, intermittent addition of bacterial inhibitor) were studied to limit bacterial growth.

Materials and methods

Organism and inoculum

Fusarium sp. was isolated as described by Arriaga and Revah [2] and was identified as *Fusarium solani* CBS 117476 by the Centraalbureau voor Schimmelcultures, The Neatherlands. Spores for inoculation were produced on mineral medium containing 15 gL^{-1} of agar in 250-mL Erlenmeyer flasks, incubated in a desiccator at 30°C for 7 days with hexane vapors. The gas phase biofilter was inoculated with a mineral medium solution containing 2×10^7 spores mL^{-1} and 5 gL^{-1} of malt extract.

Mineral medium

The medium used for the biofilter contained (per liter): 18 g $NaNO_3$, 1.3 g KH_2PO_4, 0.38 g $MgSO_4{\cdot}7H_2O$, 0.25 g $CaSO_4{\cdot}2H_2O$, 0.055 g $CaCl_2$, 0.015 g $FeSO_4{\cdot}7H_2O$, 0.012 g $MnSO_4{\cdot}H_2O$, 0.013 g $ZnSO_4{\cdot}7H_2O$, 0.0023 g $CuSO_4{\cdot}7H_2O$, 0.0015 g $CoCl_2{\cdot}6H_2O$, 0.0015 g H_3BO_3. The pH of the medium was adjusted to 4.0 with 1.0 N hydrochloric acid or 1.0 N sodium hydroxide.

Reactor

The gas-phase biofilter consisted of a 1-m cylindrical glass column with inner diameter of 0.07 m (Fig. 1). The biofilter was packed with 2.5 L of perlite and the inoculum (described above) was recirculated through the bed at the beginning of the experiment. Hexane-saturated air was mixed with moistened air and introduced at the top of the reactor with a flow rate of 2.5 L min^{-1} (Mass-flow controller 60061; Cole Parmer, Vernon Hills, Ill., USA). Mineral medium was recirculated for 30 min at the top of the reactor through a spray nozzle to maintain the pH at 4.0, to improve moisture content and to supply nutrients. In some experiments, chloramphenicol (20 gm^{-3}) and gentamycin sulfate (50 gm^{-3}) were added to the mineral medium to limit bacterial growth. To evaluate the influence of hexane load, different concentrations of hexane between 0.5 gm^{-3} and 11 gm^{-3} were tested, maintaining the gas flow rate constant.

Results from the biofiltration experiments are expressed in terms of hexane inlet load (L), biofilter elimination capacity (EC) and removal efficiency (%RE) according to:

$$L = \frac{Q}{V_r} S_{in} \tag{1}$$

$$EC = \frac{Q}{V_r}(S_{in} - S_{out}) \tag{2}$$

$$\%RE = \frac{S_{in} - S_{out}}{S_{in}} \times 100 \tag{3}$$

where S_{in} and S_{out} are inlet and outlet hexane concentration, respectively (gm^{-3}), Q is air flow ($m^3\ h^{-1}$), V_r is

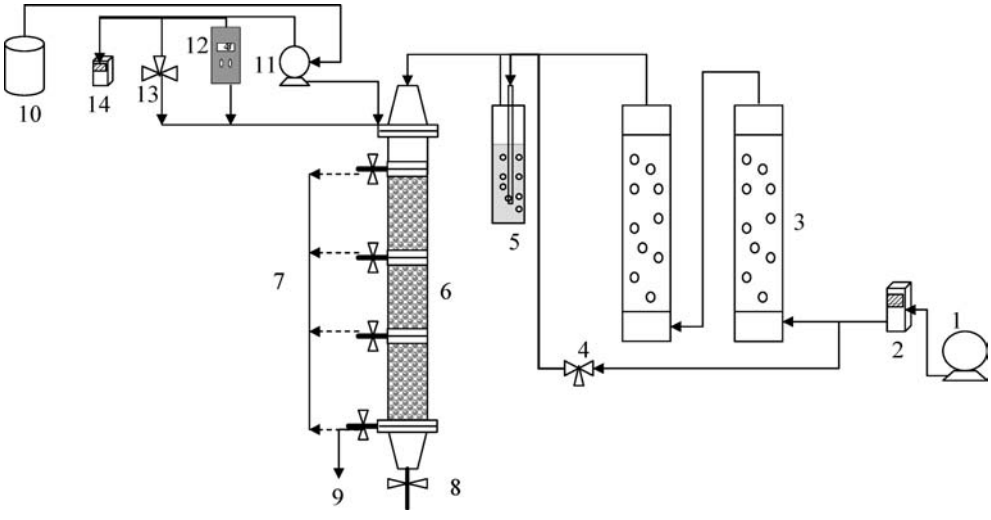

Fig. 1 Experimental device: *1* compressor, *2* mass flow meter, *3* humidifier, *4* precision valve, *5* hexane evaporator, *6* packed column, *7* sampling ports, *8* leachate purge, *9* outlet, *10* nutrient solution, *11* peristaltic pump, *12* spraying system, *13* electric solenoid valve, *14* timer

reactor volume ($m^3_{reactor}$), EC is $gm^{-3}_{reactor}\ h^{-1}$, RE is % and L is inlet load ($gm^{-3}_{reactor}\ h^{-1}$).

Batch experiments

Glass bottles of 125 mL, stoppered with Mininert teflon valves (VICI Precision Sampling, , Baton Rouge, La., USA), were used to determine the consumption rate of hexane. One gram of biofilter medium was added to the bottles and then 20 mL of mineral medium and 3 μL of liquid hexane were injected to obtain an initial headspace concentration of approximately 14 gm^{-3}. In some experiments, bacterial (20 mgL^{-1} chloramphenicol, 50 mgL^{-1} gentamycin sulfate) or fungal (50 mgL^{-1} nystatin) inhibitors were included in the mineral medium. Also, some experiments without medium addition were performed.

Hexane consumption was followed until exhaustion. The integrated Gompertz model was used to obtain the maximum volumetric hexane consumption rate (V_{max} [1]).

Analytical methods

The hexane concentration in the gas stream of the biofilter and from the batch experiments was determined by injecting 200-μL gas samples into a GC with a flame ionization detector (FID; GowMac FID-GC series 580; Bridgewater, N.J., USA), equipped with a 1.83 m long, 3.2 mm diam., 0.085 μm film SS column (2% Silar 10C, Graphpac GC 80/100; Alltech, Deerfield, Ill., USA). Nitrogen was used as a carrier gas at a flow rate of 20 mL min^{-1}. The FID was supplied with hydrogen (30 mL min^{-1}) and reconstituted air (300 mL min^{-1}). The column was operated at 180°C and the injector and detector were maintained at 190°C and 210°C, respectively.

The CO_2 concentration at the outlet of the biofilter was measured with an infrared analyzer (3400 Gas analyzer; Analytical Instruments, Calif., USA); and the reported values correspond to the difference between the outlet and the ambient CO_2 concentrations.

In batch experiments, the CO_2 was quantified from the headspace with 100-μL samples using a gas-tight syringe. The samples were analyzed in a TCD-GC with thermal conductivity detector (TCD; GowMac TCD-GC series 550) equipped with a concentric column (CTR-1; Alltech). Operating conditions were: injector 70°C, oven 30°C, detector 60°C; and the carrier gas was helium at 60 mL min^{-1}.

Biomass in the support was measured as volatile solids with a thermogravimetric analyzer (2950 TGA; TA Instruments, New Castle, Del., USA). Approximately 30 mg of sample were heated from room temperature to 550°C at a heating rate of 10°C min^{-1}, using a nitrogen flow rate of 100 ml min^{-1} as purge gas. Measurements were made in duplicate. The biomass content was expressed in milligrams of biomass per gram of dry perlite.

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. [1].

Results and discussion

Effect of nutrients addition on biofilter performance

The hexane biofiltration experiment was initiated with a hexane inlet load of $160 \pm 12\ gm^{-3}_{reactor}\ h^{-1}$. In the first 10 days of operation, the inoculum was recirculated to the reactor to favor fungal colonization and development. The activity of *F. solani* from the consumption of malt extract was registered by the CO_2 production (Fig. 2). As fungi have slower growth rates than bacteria, the use of an alternative carbon source to favor fungal biomass development is a very important parameter for the rapid start-up of a fungal biofilter. Spigno et al. [12] reported that the use of malt extract as a concurrent carbon source for the growth of *A. niger* maintained a higher hexane EC than when the fungus was grown with glucose, which acted as catabolic repressor. After 7 days, the hexane began to be slowly degraded, with EC values around 30 $gm^{-3}_{reactor}\ h^{-1}$ and CO_2 production of 60 $gm^{-3}_{reactor}\ h^{-1}$. In terms of carbon balance, the conversion yield of hexane to CO_2 was 0.65 g CO_2 g^{-1} hexane and represented only 21% mineralization.

Subsequently, over days 24–45, mineral medium was added to the reactor every 2 days to improve biofilter performance. The EC and CO_2 production increased rapidly, favored by the nutrients added (Fig. 2). During

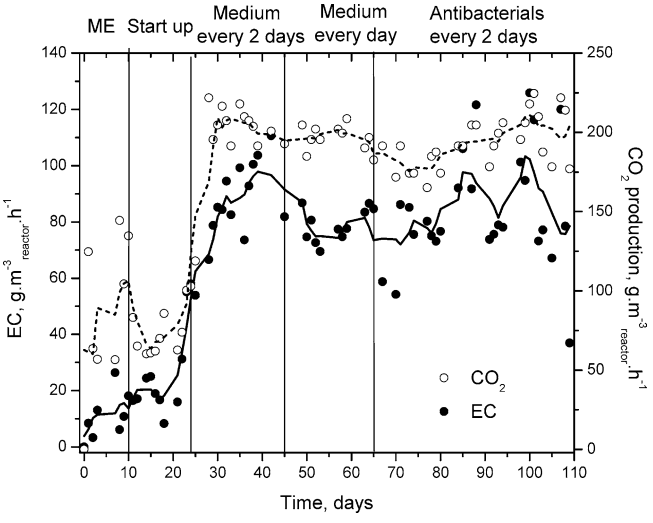

Fig. 2 Evolution of the EC (*filled circles*) and CO_2 production (*open circles*) over time in a biofilter grown with *Fusarium solani* Hexane inlet load was $160 \pm 12\ gm^{-3}_{reactor}\ h^{-1}$. *ME* Malt extract

the consumption of malt extract in the start-up period, the nutrients were probably exhausted and the system was limited by nutrients [6]. During this period, EC values around 100 $gm^{-3}_{reactor}\ h^{-1}$ and CO_2 production of 200 $gm^{-3}_{reactor}\ h^{-1}$ were obtained, which are greater than those obtained with bacterial systems [8]. In this work, the continuous addition of nutrients improved the EC, but also increased the biomass content in the biofilter. A sample from the reactor which contained 28 $mg_{biomass}\ g^{-1}_{dry\ perlite}$ was taken at day 24 and used for the batch experiments.

After day 45, an increment in the pH in the leachate was registered and a daily mineral medium addition was established for days 45–65, to maintain an acid pH at 4.0 and to ensure moisture content. The EC and CO_2 production began to decrease to values of approximately 70 $gm^{-3}_{reactor}\ h^{-1}$ and 170 $gm^{-3}_{reactor}\ h^{-1}$, respectively (Fig. 2). This decrease can probably be related to conditions not being favorable to fungi because of higher moisture conditions. In these conditions, mass transfer limitations could be augmented by the presence of water; and possibly a higher water content in the biofilm may limit the development of the aerial mycelium by increasing the water surface tension [16]. The results obtained in the first 65 days of operation showed the performance-dependence of the fungal biofilter on water content control. As with bacterial biofilters, low water content promotes slow growth rates and activity. However, fungal biofiltration of hydrophobic substrates seems to be sensible to excess water, by promoting further mass-transfer resistance and favoring bacterial growth. During this period, a pressure drop of 50 mm $H_2O\ m^{-1}_{reactor}$ was attained. This value was higher than the value of 12 mm $H_2O\ m^{-1}_{reactor}$ reported by Kibazohi et al. [8] for the same superficial velocity (40 m h^{-1}). Jorio et al. [6] reported the same tendency in the periodicity of nitrogen supply to the biofilter, in their case it enhanced styrene EC but also caused excess biomass and clogging.

Biofilters, being open systems, are subject to microbial contamination and bacteria were found in the leachate and in samples from the support. To evaluate the effect of bacteria in the hexane biodegradation, over days 65–109 mineral medium with antibiotics was added to the reactor every 2 days to inhibit bacterial activity. The EC began to increase to values of approximately 85 $gm^{-3}_{reactor}\ h^{-1}$ with a CO_2 production of 220 $gm^{-3}_{reactor}\ h^{-1}$. These results corroborated the predominant role of the fungi in the process and showed that the decrease in EC during days 45–65 was principally due to unfavorable humidity. The fact that there was a contribution of bacteria (around 10%) to the overall hexane removal can be shown by comparing the EC obtained during days 65–109 (with antibiotics) with the EC obtained during days 24–45 (without bacterial antibiotics). Microscopic observations (Fig. 4) confirmed that *F. solani* was the predominant species in the biofilter and was able to maintain the stability of the system during 85 days.

The biomass at the final day of operation was approximately 253 mg biomass g^{-1} dry perlite; and from this data and the integration of the CO_2 and EC values from Fig. 2, it was found that 9% of the consumed hexane carbon was incorporated into biomass and 79% was mineralized to CO_2. The remaining carbon (12%) can be accounted as biomass in the leachate, metabolic intermediates and carbonates [10].

Influence of the inlet load on reactor performance

The influence of the inlet hexane concentration was tested in the biofilter experiments: values between 0.5 gm^{-3} and 11 gm^{-3} were applied, maintaining the air flow constant (Fig. 3). The elimination of hexane was complete below the critical inlet load of around 70 $gm^{-3}_{reactor}\ h^{-1}$ with a

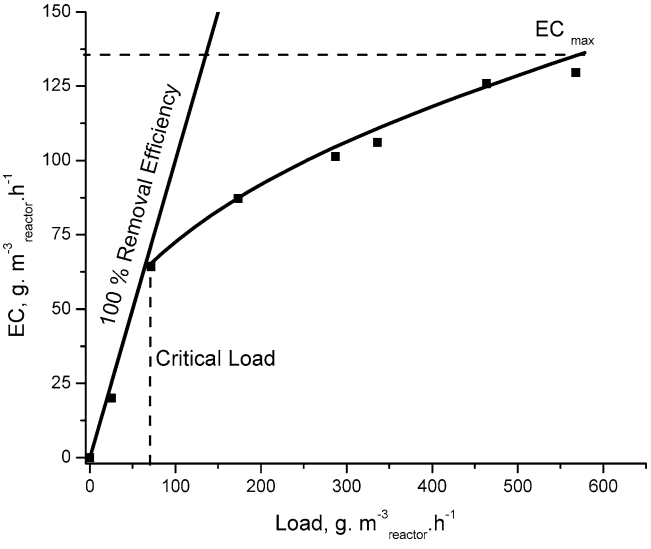

Fig. 3 Load effect on the hexane elimination capacity of a *Fusarium solani* biofilter

Table 1 Hexane consumption rate and CO_2 production (batch experiments) for reactor sample (RS) from the biofilter, with different treatments. RS were obtained at the end of a 109-day experiment and contained 253 mg biomass g^{-1} dry perlite. RS-24 was obtained at day 24 and contained 28 mg biomass g^{-1} dry perlite

Sample	Specific V_{max} ($mg_{hexane}\ g^{-1}_{biomass}\ h^{-1}$)	Mineralization (% hexane to CO_2)
RS	3.24	57
RS with mineral medium	0.84	93
RS with mineral medium and bacterial inhibitor	0.75	66
RS with mineral medium and fungal inhibitor	0.13	100
RS-24	6.00	76

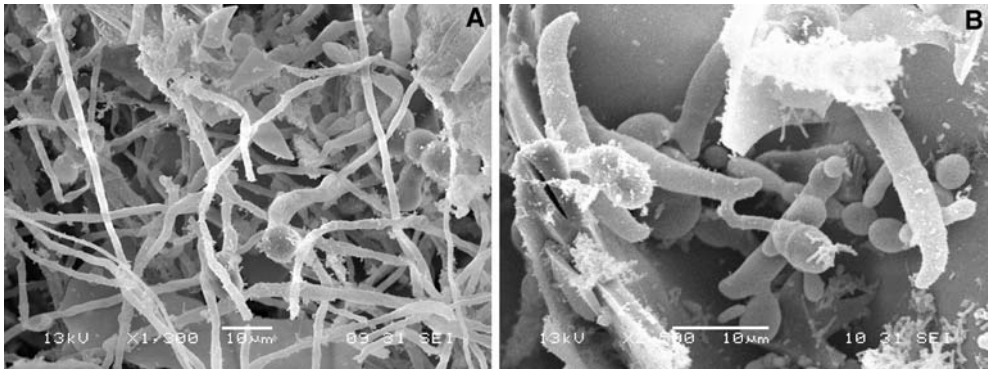

Fig. 4 SEM photomicrographs of *Fusarium solani* in the biofilter, samples taken at final day of operation. **a** Hyphae growth. **b** Typical spores for *Fusarium solani*

maximum EC of 130 $gm^{-3}_{reactor}\ h^{-1}$. The maximum EC obtained with *F. solani* was at least two times greater than the values obtained using bacterial systems [8], but was lower than the value obtained by Spigno et al. [12], who found that a biofilter containing the fungus *A. niger* was able to support average ECs of 150 $gm^{-3}_{reactor}\ h^{-1}$ with concurrent utilization of malt extract.

Batch experiments

Reactor samples (RS) from the end of the experiment were analyzed in closed batch reactors to evaluate the relative contribution of fungi and bacteria in the hexane degradation and also to demonstrate the mass transport limitations due to water and high biomass development in the biofilter. As seen in Table 1, the rate and mineralization data for batch experiments indicate that 95% of the specific consumption velocity was related to fungal activity and 15% was linked to the bacterial activity, confirming the results obtained in the biofilter. As the total percentage found was greater than 100%, probably a synergistic effect between fungi and bacteria was involved, as reported by Prenafeta-Boldú et al. [11], although the unspecific action of the inhibitors cannot be discarded. SEM photomicrographs (Fig. 4) show the presence of bacteria and the interaction with fungi (bacteria on the hyphae). Since biofilters are open systems, it was not possible to sustain an axenic fungal culture during all the operation, despite pH control and the addition of antibiotics. Nevertheless, the fungal population was predominant. The difference in the mineralization rate reflects the diversity of the metabolic activities of the bacteria and fungi.

The specific rate obtained with the biofilter solid sample was around four times greater than those obtained with liquid medium, indicating the existence of a liquid phase limitation to the transfer of hexane and oxygen (hydrophobic compounds) to the submerged fungi. This result also suggests that the treatment of hydrophobic compounds with biofilters may be more favorable than other technologies with extensive water use, such as biotrickling filters, bioscrubbers and fermentors.

The specific rate obtained with the reactor solid sample at day 24 (28 mg biomass g^{-1} dry perlite) was 1.8 times greater than the sample from day 109 (253 mg biomass g^{-1} dry perlite). The volumetric hexane consumption rates were, for these experiments, 1.12 $g_{hexane}\ m^{-3}\ h^{-1}$ and 1.94 $g_{hexane}\ m^{-3}\ h^{-1}$, respectively. These results confirm that, while increased biomass results in higher EC, in the long run the performance was diminished. Fungal overgrowth induces inactive zones (high biomass density), channeling the diminution in the effective residence time and increased pressure drop and mass transfer limitations [3, 13].

Conclusions

This work confirms previous results showing that fungal biofiltration improves the EC of hexane. Kinetic studies with this biomass under biofilter conditions show the importance of the biomass and water content control in the fungal reactor, since this can cause reduced activity, inactive zones and mass transfer limitations. Furthermore, this work shows that, since it is very difficult to maintain an axenic culture in biofilters, the study of fungal and bacterial synergism may improve both their performance and the practical feasibility of such processes.

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