Influence of the inlet load, EBRT and mineral medium addition on spore emission by *Fusarium solani* in the fungal biofiltration of hydrophobic VOCs†

Alberto Vergara-Fernández, a,∗ Sergio Hernández, b Raúl Muñoz c and Sergio Revah d

Abstract

BACKGROUND: The effect of the inlet *n*-pentane load (IL), empty bed residence time (EBRT) and mineral medium (MM) addition on spore emission and elimination capacity (EC) was evaluated using the fungus *Fusarium solani* in a vermiculite-based biofilter.

RESULTS: When the process was operated at an EBRT of 3.7 min and an IL of 100 g m⁻³reactor h⁻¹, the weekly addition of MM from 6.8 to 13.6 mLMM L⁻¹vermiculite resulted in fluctuating dynamics of ECs and spore emission.

When varying the *n*-pentane IL from 25 to 875 g m⁻³reactor h⁻¹, a maximum EC of 110 g m⁻³reactor h⁻¹ associated with a spore emission of 1.8 × 10⁴ CFU m⁻³air was recorded. The critical inlet load for 90% *n*-pentane removal was 50 g m⁻³reactor h⁻¹ with spore emission 4.5 × 10³ CFU m⁻³air. Finally, when the EBRT was decreased from 3.7 to 2.1 min at a constant *n*-pentane IL of 50 g m⁻³reactor h⁻¹ the EC decreased by 110%.

CONCLUSIONS: The results show a poor performance of the *n*-pentane biofiltration system at high IL and low EBRT, which was further confirmed by the low final biomass concentrations in the biofilter (62 mg biomass g⁻¹vermiculite)

© 2012 Society of Chemical Industry

Keywords: hydrophobic VOCs; spore emission; *Fusarium solani*; fungal biofiltration

INTRODUCTION

The elimination of volatile organic compounds (VOCs) using fungal biofilters has been studied by several authors1,2,3 because of its advantages over bacterial biofilters.4–6 Fungal biofilters have the ability to degrade a large number of VOCs, resistance to low humidity and pH,7,8 the capacity to colonize empty space with aerial hyphae increasing the transport area and can penetrate the solid support favoring the availability of nutrients.4 Fungal biofilters also have some disadvantages including the potential relevance of spore and bacteria emissions from biofiltration facilities, little research has been devoted to this issue. Therefore, more research is needed to understand and control the mechanisms of spore emission from biofilters.

In this context, the nutrient (mineral medium, MM) content of the packing materials and its bioavailability play an important...
role in the operation of biofilters, both in terms of biodegradation performance and spore emission. Thus, while organic packing materials usually have the necessary nutrients for the maintenance of biological activity, the addition of MM to inorganic materials-based biofilters, especially of a nitrogen source, is essential for efficient biofiltration performance. In addition, operating parameters such as the pollutant inlet load (IL) and the empty bed residence time (EBRT) might play a key role in spore emission.

This paper reports on a study into the effect of IL, EBRT and MM addition on spore emission and gaseous \( n \)-pentane EC in a biofilter inoculated with the fungus *Fusarium solani* and using vermiculite as packing material.

**MATERIALS AND METHODS**

**Microorganisms and inoculum**

*Fusarium solani*, further classified as *Fusarium solani* CBS 117 476 by the Centraalbureau voor Schimmelcultures (The Netherlands) was used in all experiments. Its preservation, cultivation conditions and spore production were carried out as detailed by García-Peña et al.

**Chemicals and mineral medium**

1-pentanol (98.5% ACS reagent) and \( n \)-pentane (99% ACS reagent) were purchased from Merck (KGaA, Darmstadt, Germany). The MM used contained (g L\(^{-1}\)): 18 NaNO\(_3\); 1.3 KH\(_2\)PO\(_4\); 0.38 MgSO\(_4\)·7H\(_2\)O; 0.25 CaSO\(_4\)·2H\(_2\)O; 0.055 CaCl\(_2\); 0.015 FeSO\(_4\)·7H\(_2\)O; 0.012 MnSO\(_4\)·H\(_2\)O; 0.013 ZnSO\(_4\)·7H\(_2\)O; 0.0023 CuSO\(_4\)·7H\(_2\)O; 0.0015 CoCl\(_2\)·6H\(_2\)O; 0.0015 H\(_3\)BO\(_3\). To limit bacterial growth and to support the fungal population, the MM was supplemented with gentamicin (40 mg L\(^{-1}\)) and chloramphenicol (50 mg L\(^{-1}\)) in all experiments.

**Biofilter systems**

The experiments were performed in biofilters consisting of a 0.75 m height cylindrical fiberglass column with an inner diameter of 0.145 m, divided into three equal stages (Fig. 1). The biofilters were packed with 900 g of dry vermiculite (7.4 L, bed void fraction of 60% and particle size of 2–4.5 mm). Each biofilter was inoculated with 3 L of MM containing an active fungal mycelium at 0.35 gbiomass L\(^{-1}\) (equivalent to 1.17 mgbiomass g\(^{-1}\) dry vermiculite) in order to reduce process start-up. Biomass was previously grown for 3 weeks at 25 °C in MM containing potato dextrose agar (PDA, 2.0 g L\(^{-1}\)) and 1-pentanol (1.0 g L\(^{-1}\)).

Air saturated with \( n \)-pentane was mixed with water-saturated air (using a humidification column) and introduced at the top of the biofilter at a flow rate of 2.0 L min\(^{-1}\) (EBRT of 3.7 min) corresponding to a superficial velocity of 7.02 m h\(^{-1}\). The \( n \)-pentane inlet concentration was 5.0 g m\(^{-3}\) during the start-up of the biofilters, corresponding to an IL of 81 g m\(^{-3}\) reactor h\(^{-1}\). All experiments were conducted at 25 °C and a moisture content of the packing material of approximately 45% in wet basis (3.33 g H\(_2\)O g\(^{-1}\) dry vermiculite).

The influence of weekly MM addition (between 6.8 and 13.6 mL-MM L\(^{-1}\)vermiculite) on spore emission and EC was first evaluated in a biofilter operated with 3.7 min EBRT and an \( n \)-pentane IL of 100 g m\(^{-3}\) reactor h\(^{-1}\). The influence of \( n \)-pentane IL on EC and spore formation was also evaluated by stepwise increasing every 3 days...
the n-pentane inlet concentration from 1.0 to 16 g m\(^{-3}\) at an EBRT of 3.7 min. Finally, the influence of the EBRT on EC and spore emission was assessed by varying every 3 days the inlet gas-flow between 2.0 and 3.5 L min\(^{-1}\) (EBRT between 3.7 and 2.1 min) at a constant n-pentane IL of 50 g m\(^{-3}\) reactor h\(^{-1}\).

The results from the biofiltration experiments are expressed in terms of n-pentane inlet load (IL, g m\(^{-3}\) reactor h\(^{-1}\)), biofilter elimination capacity (EC, g m\(^{-3}\) reactor h\(^{-1}\)), spore concentration based on colony forming units (SC, CFU m\(^{-3}\)), and removal efficiency (RE, %) according to:

\[
IL = \frac{Q}{V_r} S_{in} \quad (1)
\]
\[
EC = \frac{Q}{V_r} (S_{in} - S_{out}) \quad (2)
\]
\[
SC = \frac{CFU}{Q \cdot tm} \quad (3)
\]
\[
RE = \left( \frac{S_{in} - S_{out}}{S_{in}} \right) \cdot 100 \quad (4)
\]

where \(S_{in}\) and \(S_{out}\) are inlet and outlet n-pentane concentration, respectively (g m\(^{-3}\)), \(Q\) is the gas-flow (m\(^3\) h\(^{-1}\)), \(V_r\) is the reactor volume (m\(^3\)), \(tm\) is the time elapsed in the spore counting method (h) and CFU is the number of colony forming units.

**Analytical methods**

Gaseous n-pentane and CO\(_2\) concentrations were measured in triplicate in a GC-FID and a GC-TCD, respectively, (Shimadzu 2014, Japan) equipped with a Rx-5 Restex UE capillary column (30 m \(\times\) 0.32 mm \(\times\) 0.25 \(\mu\)m) and a Carbomex 1000 column. The n-pentane concentration was determined at a detector, injector and oven temperatures of 220, 200 and 80 °C, respectively, using nitrogen as carrier gas. The injector, oven and detector temperatures were maintained at 65 °C, 80 °C and 100 °C, respectively, for CO\(_2\) determination.

The moisture content of the packing material was measured online using a ProCheck meter (Decagon Devices, Inc.) equipped with the ‘EC - 5 Soil Moisture’ detector. The temperature in the bioreactor was measured using a thermo-couple system (Cole-Parrmer, USA) connected to an Extech digital meter and controlled with a thermoregulated chamber.

The experimental system for the collection of spores was adapted from Görny et al.\(^{18}\) For the capture of spores (the average spore size is approximately 8 \(\times\) 3 \(\mu\)m\(^{19}\)), the treated gas in the biofilter was passed for a \(tm\) of 5 min through a hydrophilic mixed cellulose esters membrane (MF, Whatman, 0.45 \(\mu\)m, 47 mm diameter) placed on porous glass located between two glass cones of 0.044 m diameter (Fig. 2). Subsequently, the membranes were placed on Petri dishes containing PDA and cultured at 30 °C for five days. The colony count was performed using a Leica lens model 2000 \(\times\) 45. The fungal biomass in the vermiculite was measured as volatile solids with a thermogravimetric analyzer as described by Arriaga and Revah.\(^{17}\) The suspended biomass was determined by dry weight at 105 °C for 24 h. Measurements were done in triplicate. The pressure drop was determined using a differential manometer system with water as the manometric fluid.

**RESULTS AND DISCUSSION**

**Start-up biofilter**

Figure 3 shows the time course of the n-pentane EC, temperature and moisture content of the packing material during the start-up of the fungal biofilter. Process start-up was characterized by an initial adaptation phase of 5 days and a decrease in the moisture content of the packing material, followed by a rapid increase in EC up to an EC\(_{max}\) of 77 g m\(^{-3}\) reactor h\(^{-1}\) concomitant with an increase in the moisture content to over 45% on wet basis, which was controlled by adding water when it decreased below 41%. These results are in agreement with those reported by Ortiz et al.\(^{8}\) during the treatment of n-pentane biofiltration using \(F.\) solani in a perlite-based biofilter. This short start-up period, in both cases, was likely due to the use of an active mycelium previously adapted with 1-pentanol (a common metabolite from n-pentane biodegradation). Deterioration in the EC, probably mediated by depletion of the nitrogen source in the biofilter, was observed by day 11 of operation. Following this start-up period, the effects of the addition of MM, changes in EBRT and n-pentane IL were evaluated. The biofilter temperature remained approximately constant at 25 °C throughout process start-up.

**Effect of mineral medium addition**

Figure 4 shows the effect of the weekly addition of different amounts of MM on the EC, spore emission, temperature and moisture content of the packing material at an EBRT of 3.7 min and an IL of 100 g m\(^{-3}\) reactor h\(^{-1}\). Following the start-up period,
the addition of 13.6 mL MM L⁻¹ vermiculite (100 mL of MM) (time zero) resulted in an increase in the EC of 100% (up to 64 g m⁻³ reactor h⁻¹), which gradually decreased by day 5 to an EC of 20 g m⁻³ reactor h⁻¹. Likewise, the addition of 13.6 mL MM L⁻¹ vermiculite at day 0 mediated a rapid decrease in the spore emission from 1.0 × 10⁴ to 1.0 × 10³ CFU m⁻³ and a subsequent increase to a SC of 2.0 × 10⁴ CFU m⁻³. After 7 days of operation, 13.6 mL MM L⁻¹ vermiculite (100 mL of MM) were added again to the biofilter to reach an EC of 78 g m⁻³ reactor h⁻¹ and a reduction in the spore emission to an average of 5.0 × 10² CFU m⁻³. In summary, an increase in the EC concomitant with a decrease in spore emission was observed when the mineral was added during stage 1. This improvement in biofiltration performance of the biofilter induced by the MM addition was also observed by Arriga and Revah (2017, 2020) during n-hexane biofiltration using both the fungus F. solani and a microbial consortium.

The EC slightly decreased to 50 g m⁻³ reactor h⁻¹ after 14 days of operation and 9.5 mL MM L⁻¹ vermiculite (70 mL of MM) were then added, corresponding to the beginning of stage 2. MM addition increased the EC to 93 g m⁻³ reactor h⁻¹ and decreased the SC from 2.2 × 10⁴ to 5 × 10³ CFU m⁻³. From day 17 to 20, the EC rapidly decreased to 18 g m⁻³ reactor h⁻¹ concomitant with an increase in the spore emission to 4.5 × 10⁴ CFU m⁻³. At the 20th day, 9.5 mL MM L⁻¹ vermiculite (70 mL of MM) were added again to the biofilter, which increased the EC to an average of 71 ± 11 g m⁻³ reactor h⁻¹ for 4 days and decreased the spore emission to 1.0 × 10³ CFU m⁻³.

After depletion of the MM in stage 2, 6.8 mL MM L⁻¹ vermiculite (50 mL of MM) were added by day 27 (beginning of stage 3) with subsequent gradual increase in the EC up to an average values of 65 ± 7 g m⁻³ reactor h⁻¹ and a reduction of spore emissions to negligible values. Finally, 6.8 mL MM L⁻¹ vermiculite (50 mL of MM) were added again by day 35 with an increase in the EC to 70 g m⁻³ reactor h⁻¹ by day 37 and a decrease in the average spore emission from 5.5 × 10³ to 2.0 × 10² CFU m⁻³.

Despite the amount of MM added being higher in stage 1, the highest ECs were reached in stages 2 and 3. This may be related to the gradual biomass development throughout the time course of the experiment. In this context, the increase in the pressure drop by day 14 supported this significant development of biomass in the biofilter (Fig. 7). In addition, the higher performance during stage 2 and 3 might be also related to the higher moisture content of the packing material during stage 1 (49 ± 2.5% vs 45 ± 3.6% in stage 2 and 42 ± 3.10% in stage 3, on wet basis), which might have hindered the solubility of this hydrophobic VOC and therefore its bioavailability for biodegradation. The average n-pentane EC recorded during the operation of the biofilter (60 g m⁻³ reactor h⁻¹) was approx. eight times higher than that obtained by Dupasquier et al. (2017) using Pseudomonas aeruginosa and similar to that reported by Ortiz et al. (2018) using a fungal consortium. The increase in EC recorded immediately after MM addition regardless of the irrigation volume was also reported by Maestre et al. (2016) and Jin et al. (2022) during toluene and α-pinene fungal biofiltration, respectively. On the other hand, the increase in spore emission during periods of low EC was likely due to the low biological activity mediated by the depletion of MM (nitrogen limitation). Indeed, nutrient...
depletion might have created the stress conditions needed to trigger the sporulation of the fungus according to Anderson and Jayaraman.24 Thus, Fig. 3 describes a decrease in EC at a constant moisture content and Fig. 4 shows that the time course of EC was not correlated with the moisture content, which clearly shows that the decrease in EC was not caused by low moisture in the packing material. The results showed average SC during the periods of increased spores emission of 4.5 ± 1.0 × 10^5 CFU m^{-3} (Fig. 4). These values were higher than the concentrations reported for airborne Aspergillus fumigatus emissions (1.2 × 10^3 CFU m^{-3}) from biofilters treating odours in composting facilities14 and higher than the concentrations typically measured in indoor air (1.0 × 10^2 CFU m^{-3}).13

**Influence of the inlet load on reactor performance and spore emission**

Figure 5 shows the influence of n-pentane inlet load on the biodegradation performance and the spore emission of the biofilter. This evaluation was conducted for a period of 57 days at inlet n-pentane concentrations ranging from 1.0 to 16 g m^{-3} and an EBRT of 3.7 min (corresponding to n-pentane inlet loads between 25 and 875 g m^{-3} h^{-1}). Re of 100% were obtained up to a critical n-pentane load of ~40 g m^{-3} h^{-1} while the maximum EC stabilized at 100–110 g m^{-3} h^{-1} at ILs higher than 500 g m^{-3} h^{-1}. The maximum EC and critical load obtained were similar to those reported by Ortiz et al.8 using F. solani in a biofilter packed with perlite. On the other hand, the spore emission also increased when increasing the IL, stabilizing at average SCs of 1.8 × 10^4 CFU m^{-3} for IL greater than 350 g m^{-3} h^{-1}. The lowest spore emissions (6.5 × 10^3 CFU m^{-3}) were obtained at ILs ranging from 50 to 100 g m^{-3} h^{-1} and RE between 90% and 100%, respectively. These SCs were similar to those reported by Zilli et al.13 in laboratory-scale biofilters filled with peat and sieved sugarcane bagasse and treating benzene at ILs between 5 and 40 g m^{-3} h^{-1}. The 4-fold increase in SC and EC recorded when the n-pentane IL was increased from 100 to 350 g m^{-3} h^{-1} suggested poor performance of the system with respect to the spore emission at increasing IL. Based on the absence of nitrogen limitation in this particular study (data not shown), the increase in the spore emission at increasing n-pentane concentration can possibly be explained by the response of the fungus as a defense mechanism to adverse conditions and stress.25,26

**Effects of EBRT**

When the EBRTs was increased from 2.0 to 3.75 min the EC decreased by 110% (from 36 to 17 g m^{-3} h^{-1}) (Fig. 6). These results are in agreement with those reported by Vergara-Fernández et al.27 and were likely due to the lower contact time available for mass transfer between the VOC in the gas phase and the biomass.7 These results confirmed the poor performance of the biofilter when operated at high n-pentane ILs, given the lower final biomass concentration in the biofilter (62 mg biomass g^{-1} m^{-3}pentane) compared with the 130 mg biomass g^{-1} m^{-3}pentane reported by Vergara-Fernández et al.11 during n-hexane fungal biofiltration). On the other hand, the decrease in EBRT resulted in a 4-fold increase in spore emission (from 1.5 × 10^3 CFU m^{-3} to 5.9 × 10^3 CFU m^{-3}) (Fig. 6). However, these SCs were only slightly higher than the concentrations typically encountered outdoors or in indoor air (10^3–10^4 CFU m^{-3}).13 This increase in spore emission was likely associated with the enhanced drag effect at high gas velocity observed by Ottengraf and Konings12 in the emission of microorganisms from biofilters treating VOCs.

**Pressure drop**

Figure 7 clearly shows the steady increase in pressure drop during the time course of biofiltration due to the decreasing void fraction of packing material caused by biomass growth.16 MM addition resulted in an immediate increase in pressure drop as a result of the stagnant liquid retained in the void volume of the reactor. However, this high pressure drop rapidly decreased due to the redistribution and drying of MM added. The maximum pressure drop obtained at the end of the biofilter operation was 66 mmH_2O m^{-3} packing material which was 3 times higher than that reported by Kibazohi et al.28 (12 mmH_2O m^{-3} packing material) using a microbial consortium degrading n-hexane in biofilters packed with perlite and a mixture of peat-perlite, and similar to that reported by Arriaga and Revah17 (50 mmH_2O m^{-3} packing material) or Vergara-Fernández et al.11 using the fungus F. solani in perlite-based biofilters treating n-hexane. These results confirm that the pressure drop in fungal biofiltrers is higher than those observed in bacterial biofilters.29

**CONCLUSIONS**

The biofiltration of gaseous n-pentane using filamentous fungi as a biocatalyst was feasible and highly influenced by the IL, EBRT
and the periodic addition of MM in terms of both EC and fungus spore emission. The periodic addition of MM in biofilters based on inorganic materials promotes rapid recovery of the biodegradation performance and reduces spore emission. However, an excess of irrigation can increase the pressure drop across the biofilter bed and hinder n-pentane transfer from the gas phase, with a subsequent decrease in EC. Interestingly, the increase in n-pentane IL in a non-N-limited scenario triggered spore emission.

Figure 7. Time course of the pressure drop across the biofilter height.

ACKNOWLEDGEMENTS
The present research has been sponsored by CONICYT-Chile (National Commission for Scientific and Technological Research) (FONDECYT Project N°11080036). The Spanish Ministry of Science and Innovation (RYC-2007-01667, CTQ2009-07601 and CONSOLIDER-CSD 2007-00055) is also gratefully acknowledged.

REFERENCES
1 García-Peña EI, Hernández S, Favela-Torres E, Aurià R and Revah S, Toluene biofiltration by the fungus 


