Performance evaluation of fungal biofilters packed with Pall rings, lava rock, and perlite for α-pinene removal

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ABSTRACT

The most suitable packing material for biofiltration of α-pinene was selected among Pall rings, lava rock, and perlite. In the present study, several biofilters fed α-pinene-polluted air were inoculated with a new fungal isolate of *Ophiostoma stenoceras*. The biofilters were packed either with lava rock or Pall rings alone or with a mixture of perlite and Pall rings. During the approximately 9 months operation, α-pinene's removal efficiency, pressure drops, pH dependence and removal profile were evaluated. α-Pinene removal efficiencies were above 93.8%, 79.4% and 58.6% at an inlet loading rate of 100 $g.m^{-3}.h^{-1}$ in the biofilters packed with Pall rings, lava rock, and a mixture of Pall rings and perlite, respectively. The fungus preferred to grow in lava rock and the mixture of Pall rings and perlite instead of Pall rings alone. Moreover, with sufficient nutrients and buffer solution, the biofilter packed with the mixture of Pall rings and perlite reached the highest elimination capacity compared to the other two packing materials. The pressure drop of the biofilter packed with the mixture of Pall rings and perlite did not exceed 11 mm $H_2O.m^{-1}$. The low-pressure drop reached when using the mixture of Pall rings and perlite as packing material allows to conveniently prevent clogging and channeling problems often associated with conventional biofilter operations.

1 INTRODUCTION

Alpha-pinene is one of the major hydrophobic organic compounds, which is emitted by the forest product industries, pulp and paper industries, and fragrance manufacturers in the form of monoterpene. These emissions into the atmosphere result in the formation

of particulates and free radicals. The former form a blue haze and reduce visibility and the latter deplete the ozone layer. Since the compound is emitted in low concentrations of, usually, less than 300 ppm, treatment of this waste gas by biofiltration is expected to be cost-effective.

Biofiltration is an established technology for air pollution control and the alternative of choice to conventional physico-chemical treatment techniques (Kennes and Veiga, 2001). Biofiltration is a promising technology involving the flow of a polluted air stream through a packed-bed containing microorganisms that are able to degrade pollutants into harmless products. Several studies have been carried out using biofilters based on the action of bacteria. As a result of the low solubility of α-pinene in water (2.5 ppm at 23 °C), that compound is poorly absorbed by the bacterial biofilms. In addition, acidification and drying out of the filter bed often cause biofilter failure. This is why a fungal biofilter was chosen in the present study. For α-pinene abatement, filamentous fungi were isolated from biofilters operated in our laboratory. Fungi develop hyphae which provide a large surface area in contact with the gas phase so that a direct efficient mass transfer from the gas phase to the biological aqueous phase is possible. This allows a faster uptake of hydrophobic compounds than in flat aqueous bacterial biofilms. Furthermore, fungi are generally tolerant to low water activities and a low pH, so that these parameters do not need to be strictly monitored in the biofilters (Kennes and Veiga, 2004).

While biofiltration has emerged as an attractive technique in the treatment of waste gases, it is not completely free of problems and still needs to be further optimized. One potential problem is a high pressure drop which causes high energy consumptions. Conventional biofilters are usually packed with natural carriers, such as compost, peat or soil. They decay over time, causes compaction, clogging, short circuiting and increased headloss across the bed. Therefore, the filter bed usually requires blending with some inert materials to prevent this from happening. Polystyrene, gypsum, perlite, wood chips, and branches have been used as inert materials to be blended into the bed (Kennes and Thalasso, 1998; Devinny *et al*., 1999; Kennes and Veiga, 2001). The oxidation of sulfur, nitrogen, and chlorine-containing compounds produces acidic intermediates or end products, which lowers the bed's pH and subsequently reduces the efficiency of VOCs removal. Calcium carbonate, marl, and oyster shells have been used to neutralize acid products (Ottengraf and van den Oever, 1983). More recently, inert and synthetic filter beds have been used for biofiltration. Inert carriers present several advantages such as a long lifetime and low pressure drop. Besides, they are physically and chemically inert, when compared to conventional natural media (Kennes and Veiga, 2002).

In our previous studies, we have isolated a new strain of *Ophiostoma stenoceras* which proved to be very effective in removing α-pinene. Therefore, *Ophiostoma stenoceras* was used as a biofilter inoculum and different packing materials such as

Pall rings, lava rock, and perlite were used as biofilter bed. The biofilters were operated under similar conditions and their capabilities to degrade α-pinene were measured and compared. In addition, possible operational problems such as a high pressure drop, as well as the optimization of operational parameters were also evaluated.

2 MATERIALS AND METHODS

2.1 Packing material

In choosing packing materials, our concern was to provide a large surface area for microbial adhesion and efficient mass transfer, along with a minimal pressure drop within the column. Microbial compatibility, low cost, and readily availability were additional considerations. The packing materials tested were either Pall rings, lava rock, and perlite, alone or as mixtures. The polypropylene Pall rings were obtained from VFF GmbH & Co (Germany). The lava rock in this study was bought from Burés S.A., (Spain). The macroporous volcanic stone is commonly crushed and sieved for use in decorative landscaping. The perlite was manufactured from Otavi Ibérica S.L.u. (Spain). Perlite is an expanded mineral with high porosity consisting of SiO_2 as the main component. Table 1 summarizes some properties of all three packing materials.

Table 1.
Characteristics of the filter bed materials used in the experiment.

Packing	Density ($kg.m^{-3}$)	Void space (%)	Size (mm)	Specific surface area ($m^2.g^{-1}$)
Pall rings	80	91	15	350 $m^2.m^{-3}$
Lava rock	866.7	50	4-10	0.55
Perlite	94.5	40	4-6	8.75

2.2 Media composition

Batch experiments were undertaken with an aqueous culture medium containing (per liter) (Estévez *et al.*, 2005): 4.5 g KH_2PO_4, 0.5 g K_2HPO_4, 2.0 g NH_4Cl and 0.1 g $MgSO_4.H_2O$. The culture medium was autoclaved at 120 °C for 20 min before adding filter-sterilized solutions of vitamins and trace minerals. The composition of the vitamins solution was (per liter): 0.2 g thiamine·HCl, 0.1 g riboflavin, 1.0 g nicotinic acid, 2.0 g Ca-pantothenate, 0.1 g biotin, 0.1 g thioctic acid, 0.1 g folic acid and 0.25 g pyridoxine HCl. The composition of the trace minerals solution was (per liter): 120 mg $FeCl_3$, 50 mg H_3BO_3, 10 mg $CuSO_4.5H_2O$, 10 mg KI, 45 mg $MnSO_4.H_2O$, 20 mg

$Na_2MoO_4.2H_2O$, 75 mg $ZnSO_4.7H_2O$, 50 mg $CoCl_2.6H_2O$, 20 mg $KAl(SO_4)_2.12H_2O$, 13.25 mg $CaCl_2.2H_2O$ and 10,000 mg NaCl. The original pH of that medium was 5.9.

Stock cultures of the fungus were maintained on petri dishes or on slants using either Potato Dextrose Agar (PDA) or the same mineral medium as described above supplemented with 16 g agar.l^{-1}. When using the mineral medium, the plates were incubated in a tank at 30 °C, in the presence of α-pinene vapors as sole carbon source. Stock cultures on PDA were stored in a refrigerator at 4 °C.

2.3 Enrichment and isolation of the α-pinene degrader

The α-pinene degrading fungus used in this study was obtained from the leachate of a biofilter treating toluene. 10 ml of the liquid was suspended in 90 ml mineral medium as described elsewhere (Estévez *et al.*, 2005). α-Pinene was added as the only source of carbon and energy. Erlenmeyer flasks with a 5:1 headspace/liquid ratio were closed with Teflon wrapped rubber stoppers and were incubated in a rotary shaker (150 rpm) at 35 °C. The flasks were aerated daily, and α-pinene was added as needed. After several serial transfers, a stable microbial consortium developed (Jin *et al.*, 2006). Individual members of the consortium were isolated by streaking on mineral agar medium and incubation under solvent vapor. The isolated strain was identified as *Ophiostoma stenoceras* by the Centraal Bureau voor Schimmelcultures (The Netherlands).

For the preparation of cell suspensions, the fungus was cultured for 10-12 days in 100 mL mineral medium in 500 mL flask at 35 °C with shaking at 150 rpm. The bottle was sealed with a Teflon-lined screw cap, and 15 ìL α-pinene was added to the medium. After the culture had degraded six additions of α-pinene, it was transferred to a 5 L bottle containing 2 L nutrient medium. After the culture had degraded three 0.5 mL additions of α-pinene, it was recirculated through the packed bed bioreactor using a peristaltic pump (model 323E/D, Watson-Marlow Ltd, Falmouth, Cornwall, UK) at a rate of 0.5 L.min^{-1} for 24 hours in order to allow the biomass to attach to the support material.

2.4 Experimental setup

The schematic of the biofilters used in this study is shown in Figure 1. All the bioreactors are cylindrical packed bed reactors made of glass, with different dimensions. Bioreactor 1 and 2 were 75 mm in diameter and 700 mm in height. The active height of packing column, filled with different packing materials were 25cm and 65cm for lava rock (Bioreactor 1) and Pall rings (Bioreactor 2), respectively. The working volumes of Bioreactor 1 and Bioreactor 2 were 1.25 L and 2.78 L. Biofilter 3, packed with the mixture of Pall rings and perlite, consisted of a cylindrical glass column with an inner diameter of 10 cm and a total height of 70 cm. The length of the biofilter bed

was 60 cm, leading to a working volume of approximately 4.71 L. All fittings, connections and tubings were made of teflon. A large stream of compressed air was humidified up to 97% relative humidity by passing it through a tower humidified with water. Another smaller stream of air was bubbled through a vial containing pure α-pinene and was mixed with the larger humidified gas stream. Gas phase α-pinene concentrations ranging from 0 to 460 ppm were obtained by changing the relative flow rates of the gas streams. The resulting synthetic waste gas was introduced through the top of the column (co-current flow). An aqueous mineral medium was recirculated over the packed bed once a week in order to add fresh nutrients and remove the accumulated metabolites. The pH of the leachate was measured on a regular basis.

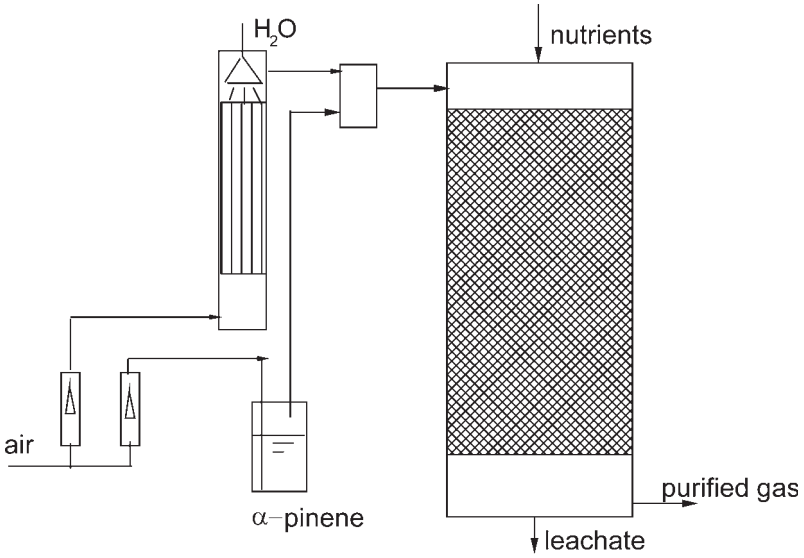

Figure 1. Schematic of the laboratory scale fungal biofilter.

2.5 Analytical methods

Gas phase concentrations of α-pinene in the biofilters were measured by gas chromatography using a Hewlett-Packard 5890 series II chromatograph. The GC was equipped with a flame ionization detector (FID). The flow rates were 30 mL.min^{-1} for H_2 and 300 mL.min^{-1} for air. The inlet and outlet streams were sampled, as well as air aliquots taken at different reactor heights. The GC was equipped with a 50 m TRACER column (TR-WAX, internal diameter 0.32 mm, film thickness 1.2 μm) and Helium was used as the carrier gas (flow rate 2.0 mL.min^{-1}). The α-pinene concentration was determined at an oven temperature of 120 °C and using a FID at 250 °C. Similarly, CO_2 concentrations were measured on another Hewlett-Packard 5890 series II GC

equipped with a thermal conductivity detector (TCD). The CO_2 concentrations were determined at an injection temperature of 90 °C, an oven temperature of 25 °C and using a TCD at 100 °C.

3 RESULTS AND DISCUSSION

3.1 Start-up period

During the start-up period, the inlet loading rate of α-pinene was maintained around 15 $g.m^{-3}.h^{-1}$ in all reactors. It took several weeks before *Ophiostoma stenoceras* had grown and attached enough to the packing material. Almost 28 days were needed before complete removal of α-pinene took place in bioreactors 1 and 3, as shown in Figure 2. However, the removal efficiency of bioreactor 2 only reached 45% in the same period. On day 0, before inoculation, no degradation of α-pinene was observed, indicating the absence of any abiotic removal. During the next 7 days, α-pinene degradation was observed only in the section of the reactor closest to the inlet in both reactors 1 and 3. Subsequently, α-pinene degradation moved progressively toward the outlet section of the biofilter bed. The data indicate that the inoculated *Ophiostoma stenoceras* first grew near the inlet section of the biofilter column and then spread out to the sections of the bed closer to the outlet over a period of 4 weeks. The regular addition of the nutrient solution probably helped fungal spreading. However, the removal of α-pinene in Bioreactor 2 only increased from 0 to 38% in 4 weeks, which is much lower than in the other reactors. It was caused by the limited biomass growth on the surface of the packing. Probably the high void space and plastic surface of Pall rings are unfavorable for a fast and significant biomass growth and adhesion. As can be seen in Figure 2, the performance of Bioreactor 3 improved after mixing perlite with the Pall rings.

3.2 Elimination capacity of á-pinene

Of the three support materials, lava rock performed best, achieving a α-pinene removal rate in excess of 143 $g.m^{-3}.h^{-1}$, followed closely by the mixture of Pall rings and perlite. Pall rings alone gave less favorable results. The relationships between the inlet load and the elimination capacity are shown in Figure 3 for each packing material. The values for the critical load and maximum elimination capacity are summarized in Table 2.

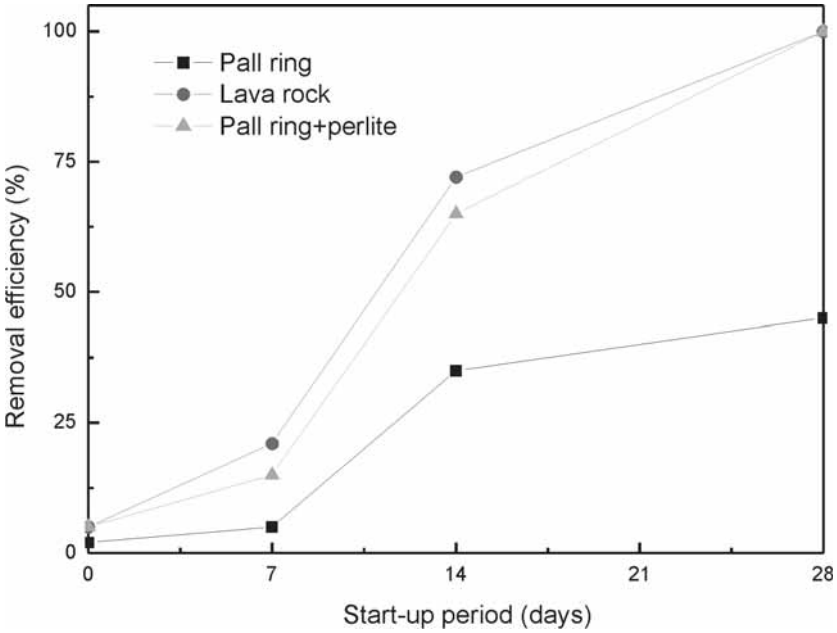

Figure 2. Removal efficiency of α-pinene during the start-up period.

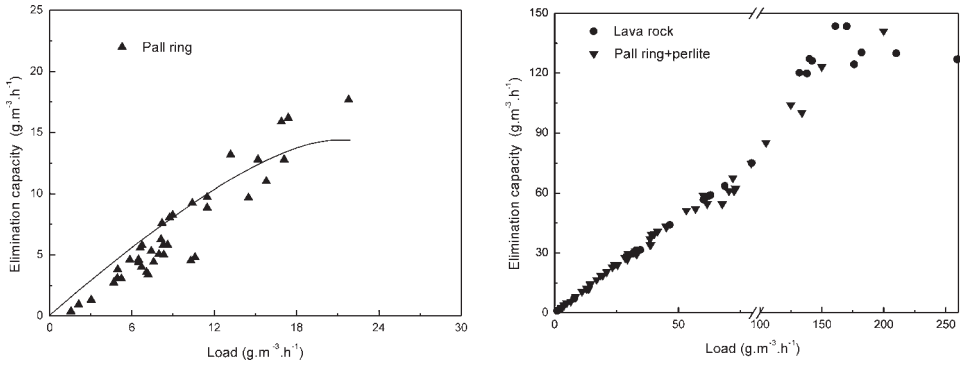

Figure 3. Relationship between load of α-pinene and elimination capacity.
(a) Pall rings. (b) Lava rock, and Pall rings+perlite.

Table 2.
Performance characteristics of the filter bed materials used in the experiment.

Packing	Critical load ($g.m^{-3}.h^{-1}$)	Maximum elimination capacity ($g.m^{-3}.h^{-1}$)
Pall rings	18	17.5
Lava rock	125	143
Perlite+Pall rings	130	135

The poor performance of Bioreactor 2 is due to the low biomass growth on the surface of Pall rings. Although there is no big difference between each other, for Bioreactor 1 and 3, the performance could be improved since most of the pollutant removal took place in only part of the packing. As much as 90% α-pinene removal took place in the inlet section of the reactor, corresponding to the upper one-third filter bed layer. The rest of the packing material of the reactor only played a minor role in the removal. This was mainly due to the non homogeneous distribution of the biomass in the filter bed, as easily confirmed by visual observation and observations under the microscope. Recent studies have shown that biomass distribution and performance could be improved with a directionally switching operation, in which the contaminant inlet feed is periodically reversed between the top and bottom of the bioreactor column or using a split-feed operation mode (Song and Kinney, 2001; Mendoza *et al*., 2003).

3.3 Pressure drop

Pressure drop is a key aspect of biofilter performance. It affects the energy consumption of the blower, which contributes most to the operation costs. In order to reduce such problem the design of high filter beds fed with large air flow rates should be avoided. The pressure drop depends on the nature of the filter bed and its moisture content. Adequate selection and design of the carrier is a means of reducing pressure drop. Compared to bacterial systems, the filamentous fungi may cause some higher head losses due to the fact that fungal biomass fills the pore spaces of the packing media. This may eventually lead to channeling and clogging problems in the biofilter, which ends up in a reduced efficiency. In the fungal biofilters treating α-pinene, no significant pressure drop was detected for any of the three packings even after 12 months operation (Figure 4). The pressure drops remained stable at low values (<2.4 cm $H_2O.m^{-1}$) over all the experimental period. Initially, it increased very slowly in Bioreactors 1 and 3, while the pressure drop of Bioreactor 2 remained at the same value (4 mm $H_2O.m^{-1}$) during that operational period. After 6 months, the pressure drop remained essentially constant in all three reactors. One can speculate that at the end of the experiment, the surface of Pall rings in Bioreactor 2 was covered with the

biomass, but the whole matrix was still empty. The main problem in Biofilter 2 is insufficient biomass. Bioreactor 1 experienced a backwashing for removal of excess biomass on day 424. It was repacked and the pressure drop decreased to 1.4 cm $H_2O.m^{-1}$. Apparently, the porous structure of perlite allowed easy biomass growth and attachment. However, our experience with the perlite packing shows that it may need to be repacked after several months of operation, because heavy overgrowth of biomass causes compaction or channeling phenomena that can adversely affect the performance (Prado *et al*., 2002). It is well known that packing materials with high void fractions can limit pressure drop. In this study, the addition of Pall rings assured minimization of the pressure drop and avoided channeling, resulting in the superior performance of the mixture. This demonstrated that the mixture of Pall rings and perlite is very suitable for use in this fungal biofilter.

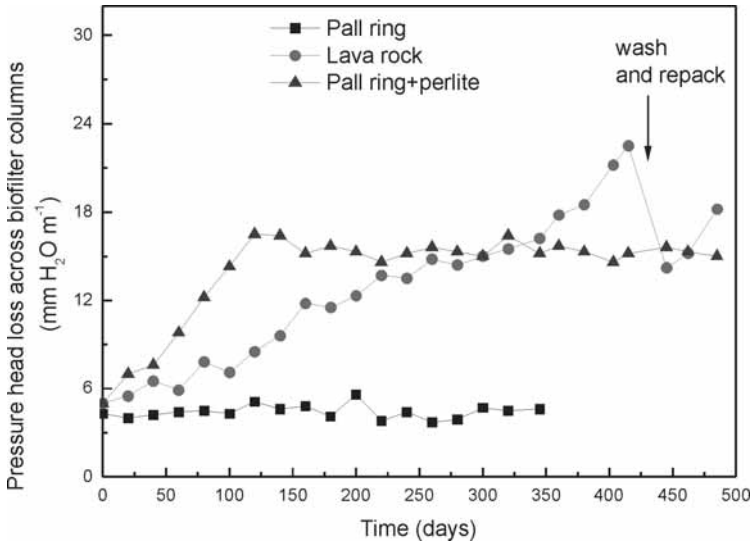

Figure 4. Variations of the pressure drop along the three biofilters.

3.4 SEM PHOTOS

Figure 5 shows all three packing surfaces grown with filamentous fungi. The filamentous fungal structure enhances the mass transfer of hydrophobic pollutants from the gas phase to the biocatalyst, thereby improving the performance of biofilters.

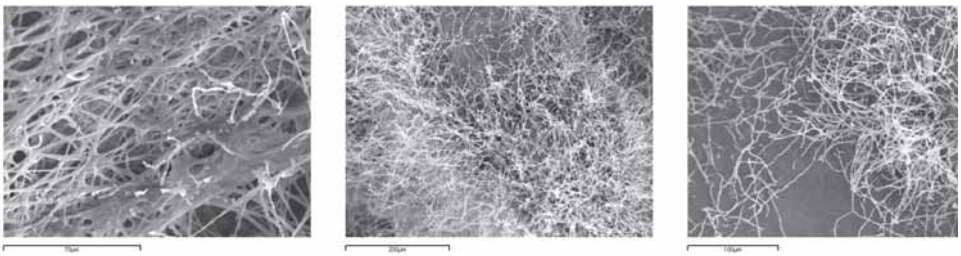

Figure 5. SEM pictures of a filter-bed samples from three bioreactors colonized by a culture of *Ophiostoma stenoceras.* Left: lava rock, middle: Pall rings, right: perlite.

4 CONCLUSIONS

The following conclusions can be drawn from the results presented in this study:

(1) The biofilters packed with lava rock and the mixture of Pall rings and perlite reached a similar maximum elimination capacity around 143 $g.m^{-3}.h^{-1}$. This value is much higher than for the Pall rings-packed biofilter.
(2) Pressure drop of all three bioreactors is below 2.4 cm $H_2O.m^{-1}$ over a one and half year operation. The addition of Pall rings to the perlite packing improved not only the performance of the biofilter, but did also stabilized the pressure drop.
(3) Overall, the mixture of Pall rings and perlite is the best choice for this fungal biofilter treating α-pinene.

5 ACKNOWLEDGEMENTS

The present research was funded by the Spanish Ministry of Education and Science (Project CTM2004-00427/TECNO) and through European FEDER funds. Yaomin Jin was financially supported by the Xunta de Galicia (Project PGIDIT05PCIC10304PN).

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