Effects of Inoculum Type, Packing Material and Operating Conditions on Pentane Biofiltration

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Original scientific paper
Received: February 9, 2007
Accepted: September 4, 2007

Biofilters are an interesting alternative to treat airstreams polluted with gaseous alkanes from industrial activities. These hydrophobic compounds are difficult to treat by bacterial communities which are generally used in biofiltration. In this work, four fungal populations (3 consortia and *Fusarium solani*) were used as inocula in biofilters for treating pentane and hexane. The biofilters were packed with inorganic and organic materials (perlite and peat) and operated with the periodic addition of mineral medium at pH 4 supplemented with antibacterial agents to favor the development of fungi. To reduce the lag phase, the biofilters were inoculated with active mycelia. Lower performance was obtained with the peat biofilters. Sustained 100 % removal efficiencies were obtained with biofilters at an operation pentane load of $\Gamma = 32.9 \pm 8.1$ g m^{-3} h^{-1}. Maximum elimination capacity of $C_{max} = 100$ g m^{-3} h^{-1} was obtained with one of the fungal consortia; this value is higher than those usually reported for pentane degrading bacterial biofilters.

Key words:
Fungal biofiltration, alkane, pentane, hexane, air pollution

Introduction

Medium chain alkanes (C_5 to C_8) are used in many industrial processes. Among these, pentane is used in polystyrene production, whereas hexane is used as solvent in oil extraction and in the printing and painting industries. As a consequence, air polluted with these volatile compounds is commonly found. The hydrophobic nature of these compounds reduces the bioavailability and makes their biological treatment difficult.

In biofiltration, either non-defined mixed or pure bacterial cultures have been commonly used.[1] For pentane, an elimination capacity (EC) of $C = 20$ g m^{-3} h^{-1} in a trickle-bed air biofilter inoculated with activated sludge was reported[2] and an EC of $C = 12$ g m^{-3} h^{-1} with *Pseudomonas aeruginosa*.[3] For hexane biofiltration, an EC of $C = 95$ g m^{-3} h^{-1} was attained using perlite as packing material and activated sludge as inoculum.[4] The use of fungi for air pollution control in recent years has received the attention of research groups. For hexane biofiltration, an average elimination capacity (EC) of $C = 150$ g m^{-3} h^{-1} was reported with *Aspergillus niger*.[5] In our previous report, a maximum EC of $C = 150$ g m^{-3} h^{-1} hexane was obtained using predominantly fungal populations.[6] Despite its industrial relevance, less work has been performed with pentane.

The higher EC obtained with fungi has been related to the increase in mass transfer favored by the aerial mycelial growth (increase in exchange surface) and with better partition coefficient between the hydrophobic gaseous pollutant and the fungal biomass.[7] Furthermore, fungi are capable of degrading numerous organic substrates under a wide range of environmental conditions regarding pH, low water content and limited nutrient conditions[1] being an interesting option in air pollution control treatments. On the other hand, fungal biofilters may be faced with slower growth rates and clogging problems.

As biofilters are open systems operated for long periods, it is difficult to maintain a pure microbial population all along experiment. However, the operating conditions can be settled to favor the development of a defined population; in this case, fungi inoculated at the start-up of the biofiltration experiment. Thus, the objective of this work was to compare different start-up strategies, packing materials and operating conditions to favor predominant fungal population activity and to evaluate their potential for biofiltration of pentane.

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Materials and methods

Microbial populations

From preliminary studies, four degrading populations were selected as inocula and adapted for pentane consumption in liquid cultures with mineral weekly pentane addition and monthly medium replacement.

Consortium A (CA) was obtained from a leachate of a compost/peat biofilter used to eliminate gasoline vapors.

Consortium (E2) was obtained from a biofilter for hexane vapors treatment.[6]

Consortium (CM) was prepared with different fungal species that proved to degrade pentane and were isolated from polluted soil and biofilter samples.

Fusarium solani (F) strain was isolated previously and reported for hexane degradation.[6]

Observations of the colonies formed by CA, E2 and CM on Petri dishes and with the optical microscope showed a predominant fungal population with varied morphology and with the presence of bacteria.

Packing material

Perlite (4 mm > particle diameter > 2.36 mm) and peat (imported from Canada by Peat Moss of Mexico) were used. Perlite has been previously reported[6] and peat was selected to evaluate the contributions that natural organic and inorganic compounds can have on fungal growth and activity. The water retention capacity was 68 % and 78 % for perlite and peat, respectively.[6,8] The low pH of peat (3.2) was an important characteristic in the selection of this packing material to favor fungal growth. The peat composition was w = 49.5 % carbon, 5.11 % hydrogen, 1.26 % nitrogen and 0.52 % sulfur measured by elemental analysis (CHNS analyzer 2400 series II, Perkin Elmer).

The pre-grown biomass in liquid medium was decanted and mixed with perlite in a closed system where the fungal population continued to spread in the support during seven weeks with periodical supplements of pentane and mineral medium. The pre-grown biomass on support (10 %) was mixed with fresh perlite and then the sterile liquid medium was added to attain the maximum water retention capacity (68 %). For the experiments with peat, the pre-grown culture (10 %) was mixed with mineral medium and added to the dry support to attain the maximum water retention capacity (78 %).

Mineral medium

It was prepared in a buffered phosphate solution (pH 4) and contained γ/g L^{-1}: 18 $NaNO_3$; 1.3 KH_2PO_4; 0.38 $MgSO_4 \cdot 7H_2O$; 0.25 $CaSO_4 \cdot 2H_2O$; 0.055 $CaCl_2$; 0.015 $FeSO_4 \cdot 7H_2O$; 0.012 $MnSO_4 \cdot H_2O$; 0.013 $ZnSO_4 \cdot 7H_2O$; 0.0023 $CuSO_4 \cdot 7H_2O$; 0.0015 $CoCl_2 \cdot 6H_2O$; 0.0015 H_3BO_3. To prevent bacterial growth and to favor the fungal population, the mineral medium was supplemented in all experiments with gentamicin (γ = 40 mg L^{-1}) and chloramphenicol (γ = 50 mg L^{-1}) except in some of the microcosm experiments where it is explicitly mentioned.

Chemicals

Pentane was fed from gas cylinders (Praxair, Mexico); the mole fraction was χ = 0.35 % which was below the lower explosive limit (φ = 1.65 %). Hexane (Tecsiquim, Mexico) was fed as reported previously.[9]

Biofiltration experiments

The experimental system has been described previously.[9] The air that feeds the columns was provided by a compressor and dosed by a mass flow meter. This air stream was bubbled through a prehumidifier that contained a dilute NaOH solution to eliminate the inlet CO_2 in the air, and passed then to a humidifying system where the air was saturated with water. The humid air was mixed with the pentane coming from the tank and this stream was delivered to the reactors through a manifold provided with valves. For the experiments with hexane, the solvent was evaporated with a small air stream and then mixed with the humid air stream before being conducted to the manifold. The volumetric flows of pentane, hexane and humid air were set to obtain the required concentration. The 0.5 L glass columns have hermetic Teflon caps with ports for gaseous sampling.

Operation conditions

The columns were maintained in a chamber at θ = 30 °C ± 2. Alkane mass concentration was γ = 2.6 ± 0.4 g m^{-3} for pentane and γ = 4.1 ± 1.1 g m^{-3} for hexane. The empty bed gas residence time was τ = 5 min. For biofilters packed with perlite (packing density ρ = 377 g L^{-1}) 10 mL of fresh mineral medium was added every 2 days to maintain the humidity of the support and to maintain the pH around 4. No extra medium was added to the peat biofilters (packing density ρ = 260 g L^{-1}) to avoid compaction. The initial and final pH values were evaluated in 1 g of support with 5 mL of distilled water. Duplicated biofilters were evaluated for each fungal

population. A peat biofilter without inoculation was assayed as a control.

As the original fungal consortia were enriched with hexane, the experimental protocol included biofiltration periods with both hexane and pentane. The experiments were divided in three operation periods. In period I a pentane load of $\Gamma = 32.9 \pm 8.1$ g m^{-3} h^{-1} was fed for the first 14 days of operation. In period II a hexane load of $\Gamma = 38.9 \pm 5.2$ g m^{-3} h^{-1} was fed from day 14th and maintained for 24 days. In period III the pentane feed was reestablished at the same load from day 38th until the end of experiments (t = 49 days).

Evaluation of the maximum experimental EC was made at the end of period III by increasing the pentane loads up to $\Gamma = 290$ g m^{-3} h^{-1} and evaluating the experimental EC. Although the response of the systems to increments in load was about two hours final EC was evaluated after 1 day. This variable load experiments were performed in biofilters CA-Perlite, E2-Perlite, F-Perlite, CM-peat where 100 % of removal efficiency was obtained.

Microcosm experiments

Microcosms experiments to determine bacterial and fungal activity were conducted by triplicate using 125 mL hermetic flask capped with Mininert valves (VICI precision sampling Inc., USA). In these experiments, pentane consumption was evaluated in 5 g of samples taken at the conclusion of the biofiltration experiments. To the microcosms, 4 μL of pentane (around $\gamma = 20$ g m^{-3}) and 5 mL of fresh mineral medium (pH 4) were added. The pentane consumption was evaluated in the presence and absence of antibacterial agents ($\gamma = 40$ mg L^{-1} gentamicin and $\gamma = 50$ mg L^{-1} chloramphenicol). The mineralization (*i.e.* production of CO_2) was also measured.

Analyses

The gaseous pentane concentration was measured by gas chromatography with ionization flame detector (HP 5890 Series II, USA) equipped with a column HP624 (Agilent, USA) the temperatures of the detector, injector and oven were of $\theta = 200$, 220 and 100 °C, respectively. For hexane quantification the oven temperature was $\theta = 80$ °C. In both cases nitrogen was used as carrier gas. The concentration of n-pentane and n-hexane was measured in 100 μL gaseous samples at the inlet and outlet for each biofilter.

The CO_2 concentration was measured in 250 μL gaseous phase samples by CG-TCD (GOW MAC Series 550, USA) and equipped with a concentric column CTR1 (Alltech, USA). The oven, detector and injector temperatures were $\theta = 40$ °C, 100 °C and 30 °C, respectively. Helium at a volumetric flow rate of $Q = 65$ mL min^{-1} was used as carrier gas.

Biomass estimation

Biomass was estimated by thermal gravimetric method using $m = 60 – 100$ mg of humid duplicated samples. This analysis was performed with a Thermo Gravimetric and Differential Thermal Analyzer (TG-DTA) (STA 409 EP, Netzsch, Germany) in a range of $\theta = 20$ to 600 °C and $U = 0$ to 1000 mV. This analysis allowed quantifying the mass losses and associated them with the processes of the water and carbon combustions. Biomass was considered to contain 50 % carbon.

Results and discussion

Elimination capacity

Fig. 1 shows the adaptation of microorganisms and the elimination capacity (EC) obtained with the two supports. Start-up was faster in the biofilter inoculated with pre-grown mycelia E2 which had been previously adapted in perlite. In this period, CA showed a similar lag phase while very low elimination was observed for the perlite biofilter inoculated with *Fusarium solani* (F). In period II a hexane load of $\Gamma = 38.9 \pm 5.2$ g m^{-3} h^{-1} was fed from day 14th and maintained until day 38th.

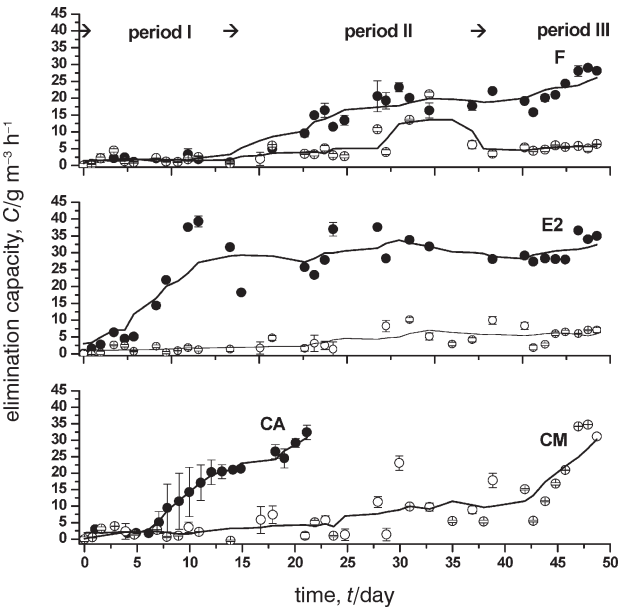

Fig. 1 – *Elimination capacities for (●) Perlite biofilters and (○) Peat biofilters. CA-consortium A. CM- consortium M. E2-consortium E2. F-Fusarium solani. Period I: pentane operation for the first 14 days. Period II: hexane operation from day 14th to day 38th. Period III: pentane operation from day 38th to 49th. Error bars mean standard deviation of 4 samples.*

Under these conditions (F) started to grow and degrade hexane on perlite. It would seem that hexane was a better substrate for (F) as it allowed initial growth but once the population was established, it was capable of attaining up to 100 % of elimination efficiency that was sustained when pentane feeding was reestablished in period III. In CA-Perlite and E2-Perlite biofilters, 100 % efficiency was obtained in period II. The CA-Perlite adaptation period was 6 days, which was shorter than the 15 days reported for *Aspegillus niger* for hexane biofiltration.[5] In this biofilter, a progressive increase of the EC was observed after the lag phase attaining complete alkane consumption that was maintained throughout the experiment. For E2-Perlite, there was no adaptation phase and after 8 days an elimination efficiency of 100 % was obtained and maintained for 42 days. At this operation condition, 100 % elimination efficiency corresponded to an EC around C = 35 g m^{-3} h^{-1}, which was higher than that of C = 20 g m^{-3} h^{-1} reported for a trickle-bed air biofilter.[2] In general, the perlite systems adapted well to the transition between period II and period III.

Contrary to the perlite biofilters, where growth and elimination were found in all the assays, the biofilters packed with peat showed less satisfactory performance. Only the CM-peat biofilter attained a similar EC than the perlite reactors but only after an adaptation period longer than 40 days. In the case of F-peat, after a period of 14 days with hexane, the degradation activity started but it was sustained only for a short period and then the EC decreased, the low EC shown (around C = 5 g m^{-3} h^{-1}), was comparable to the uninoculated control. The less favorable results obtained with peat may be a consequence of the difficulties to maintain moisture and possibly to the existence of competitive interactions between the inocula and the peat native population.

Maximum experimental EC

It was evaluated in the biofilters with CA-Perlite, E2-Perlite, CM-peat and F-Perlite, where 100 % removal efficiencies were sustained. The highest EC, Fig. 2, was obtained with CA-Perlite where EC reached C = 100 g m^{-3} h^{-1}. Both biofilters E2-Perlite and CM-peat reached similar EC, around C = 70 g m^{-3} h^{-1} while an EC of C = 56 g m^{-3} h^{-1} was obtained for F-Perlite. These values were higher than those reported previously with bacterial populations. Pentane EC maximum reported ranged between C = 8 and 20 g m^{-3} h^{-1} in trickle-bed air biofilter and packed biofilters.[2,3,10]

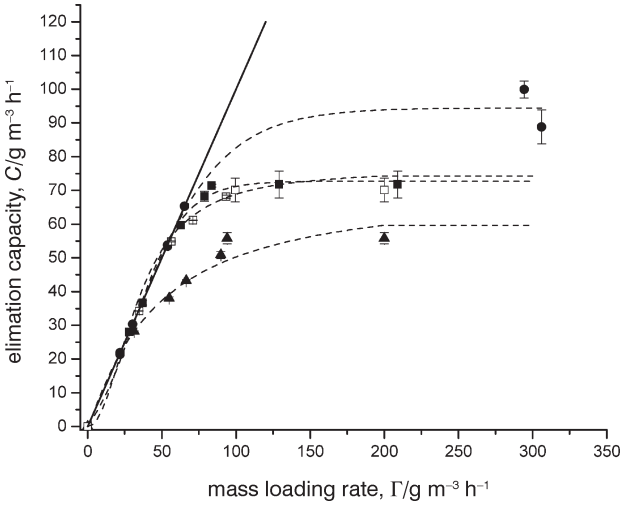

Fig. 2 – *Maximum experimental EC. (●) CA-Perlite. (■) E2-Perlite. (▲) F-Perlite. (□) CM-peat. (——) 100 % removal efficiency. Error bars mean standard deviation of 4 samples.*

Carbon balances

Carbon balances, Table 1, were carried out by integrating the total amount of consumed alkanes and the total CO_2 and biomass produced. The biofilters were operated 21 days and 28 days with pentane and n-hexane, respectively. The highest mineralization was obtained for biofilter E2-Perlite close to 60 % and in this case also the balance was close to 100 %. The balance carbon for the F-peat biofilter was comparable with the values obtained in the peat control biofilter, which was in agreement with the poor performance of this biofilter. In E2-peat and CM-peat biofilters, the percentages of biomass quantified were close to twice to that obtained in the peat control biofilter. However, this

Table 1 – *Carbon balances and pH values*

Population/ Support	Carbon fractions, w_c/%			pH	
	mineralization	biomass	balance	initial	final
CA/Perlite	47.46	18.68	33.86	4.89	5.45
E2/Perlite	58.69	32.34	8.97	4.72	7.59
F/Perlite	30.04[a]	22.34	47.62	4.27	6.96
E2/peat	19.44[b]	58.18	22.38	4.2	4.14
CF/peat	10.45[b]	26.78	62.77	4.2	3.96
CM/peat	30.91[b]	60.00	9.09	3.89	5.39
PA/peat (Control)	6.27[b]	30.94	58.61	3.27	3.86

a. From pentane consumed in 14 days
b. Including CO_2 produced by pentane and n-hexane consumption

higher amount of biomass did not increase of pentane mineralization. The unaccounted carbon was possibly adsorbed in the packing material or transformed to other organic compounds that might have accumulated or emitted in the outlet gas stream. The significant carbon amounts not quantified suggest a biotransformation production of metabolites that are not susceptible for further degradation by the present microorganisms.

Table 1 also presents the initial and final pH values for each biofilter. In perlite biofilters mineral medium was added as a strategy of to maintain humidity and reduce extreme variations in pH. The higher final pH values in Table 1 (CA, E2 and F with Perlite) correspond to the experiments with higher EC as shown in Fig. 1. The initial acidity of peat and its natural buffering capacity allowed maintaining the pH values around 4 except for CM where pH increased probably was coupled to a better performance as shown in Fig. 1.

Microcosm experiments

These experiments were conducted with final samples of CA biofilter to establish the activity attributable to fungal populations. Pentane, fresh mineral medium and in some cases, antibacterial agents were added to the samples. As seen in Fig. 3, pentane consumption with the samples of the biofilters was not significantly different to the results obtained with the antibacterial additions, suggesting that fungi were mainly responsible of pentane degradation. In this work, the operational conditions were selected to favor the preferential establishment of fungal populations. However, it is well documented that fungi-bacterial association can result in increased degradation rates. In a recent report, it was shown that fungal hyphae act as vectors for bacterial transport and it was suggested that stimulation of fungi might be a strategy to mobilize pollutant-degrading bacteria and consequently improve the degradation of pollutants.[11]

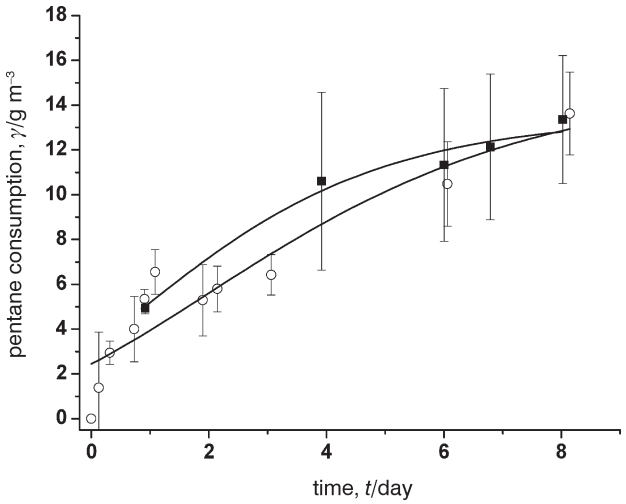

Fig. 3 – *Pentane consumption in microcosms experiments final samples from CA-Perlite biofilter, added with 5 mL of mineral medium. (■) without antibacterial agents (○) with antibacterial agents (40 mg L^{-1} gentamicin and 50 mg L^{-1} chloramphenicol). Error bars mean standard deviation of 6 samples.*

Conclusions

The microbial activity of fungi was shown to be induced in the pentane and hexane vapors biofiltration and although the start-up periods were in general long, the different consortia were then able to sustain 100 % of elimination efficiency corresponding to an EC around $C = 35$ g m^{-3} h^{-1} which was higher than those reported in the literature.

The maximum EC was $C = 100$ g m^{-3} h^{-1}, obtained with the consortium extracted from a gasoline vapors biofilter using perlite as packing material. Also good results were obtained for the biofilters operated with the consortium E2 (from a hexane biofilter) and that inoculated with a mixture of fungi able to degrade pentane. The maximum EC obtained were of $C = 70$ g m^{-3} h^{-1} and $C = 55$ g m^{-3} h^{-1}, respectively. *Fusarium solani* showed to prefer hexane to pentane in the initial colonization period, but was able to consume it efficiently once the population was established.

Although peat was expected to favor fungal establishment on biofilters due to low pH and its nutrient content, better results were observed with perlite as packing material. These results may be attributed to the pre-grown inoculum used for perlite biofilter and the possibility of controlling pH and water content.

Although some improvements in reducing the start-up time were made by adapting the inoculum, this remains an important issue when considering the industrial application of fungal biofilters.

ACKNOWLEDGEMENTS

This research was partially sponsored by Conacyt (Mexican Council for Science and Technology) project SEMARNAT-00120.

List of symbols

Γ – mass loading rate, $m_{substance}\, V_{reactor}^{-1}\, t^{-1}$, g m^{-3} h^{-1}

C – elimination capacity, $m_{substance}\, V_{reactor}^{-1}\, t^{-1}$, g m^{-3} h^{-1}

m – mass, g

Q – volumetric flow rate, mL min^{-1}

t – time, day

τ – empty bed gas residence time, min
V – volume, L, m^3
w – mass fraction, %
χ – mole fraction, %
γ – mass concentration, g L^{-1}, g m^{-3}
θ – temperature, ° C
ρ – packing density, $m_{wet}\ V_{reactor}^{-1}$, g L^{-1}
φ – volume fraction, %
U – electric potential, mV

List of abbreviations

CA – consortium A
E2 – consortium E2
CM – consortium M
F – *Fusarium solani*
EC – elimination capacity

References

1. *Kennes, C., Veiga, M. C.,* J. Biotechnol. **113** (2004) 305.
2. *Chungsying, L., Min-Ray, L., Innho, W.,* J. Chem. Technol. Biotechnol. **76** (2001) 820.
3. *Dupasquier, D., Revah, S., Auria, R.,* Environ. Sci. Technol. **36** (2002) 247.
4. *Kibazohi, O., Yun, S.-I., Anderson, W. A.,* W. J. Microbiol. Biotechnol. **20** (2004) 337.
5. *Spigno, G., Pagella, C., Fumi, M. D., Molteni, R., De Faveri, D. M.,* Chem. Eng. Sci. **58** (2003) 739.
6. *Arriaga, S., Revah, S.,* Biotechnol. Bioeng. **90** (2005) 107.
7. *Vergara-Fernandez, A., Van Haaren, B., Revah, S.,* Biotechnol. Lett. **28** (2006) 2011.
8. *Ortiz, I., Revah, S., Auria, R.,* Environ. Technol. **24** (2003) 265.
9. *Ortiz, I., Auria, R., Sigoillot, J.-C., Revah, S.,* Environ. Sci. Technol. **37** (2003) 805.
10. *Barton, J. W., Klasson, K. T., Koran, L. J., Davison, B. H.,* Biotechnol. Prog. **13** (1997) 814.
11. *Kohlmeier, S., Smits, T. H. M., Ford, R. M., Keel, C., Harms, H., Wick, L.Y.,* Environ. Sci. Technol. **39** (2005) 4640.