Performance optimization of the fungal biodegradation of α-pinene in gas-phase biofilter

Yaomin Jin, María C. Veiga, Christian Kennes

Process Biochemistry, Volume 41, Issue 8, August 2006, Pages 1722–1728
DOI: 10.1016/j.procbio.2006.03.020

Abstract

A *Ophiostoma* species using α-pinene as the sole source of carbon and energy converted it into biomass, carbon dioxide and water. The strain was inoculated in a biofilter fed α-pinene polluted air, and it remained the dominant biocatalyst throughout the study. Near complete removal was observed up to a load of 100 g m^{-3} h^{-1}, while 89% removal efficiency was reached at an elimination capacity of 143 g m^{-3} h^{-1}. The influence of gas flow rate, pollutant concentration and load was evaluated. For a similar load, the highest removal efficiency and corresponding elimination capacity were reached at the lowest flow rate and at an inlet concentration of 2.47 g m^{-3}. Oxygen had a significant effect on the removal efficiency since the maximum α-pinene degradation rate exceeded 187 g m^{-3} h^{-1} at 45% oxygen in the inlet air suggesting that at high loads oxygen transfer becomes limiting. Biokinetic studies led to a good fit between experimental data and mathematical equations, yielding a calculated maximum α-pinene degradation rate of 198.42 g m^{-3} h^{-1} with a half-velocity constant, K_S, of 0.76 g m^{-3}.

Keywords

Biokinetics; α-Pinene; Filamentous fungi; Biofiltration; Lava rock

1. Introduction

Emissions of volatile organic compounds (VOCs) have recently become of increasing regulatory concern. Off-gases can be treated by means of various technologies such as absorption, adsorption, scrubbing, and thermal or catalytic oxidation [1]. Over the past decades much effort has been made to develop and improve biological treatment technologies due to their low cost, operational simplicity, and minimum secondary pollution. Biofiltration is currently an accepted and mature technique to treat large volumes of waste gases with low pollutant concentrations [2].

Wood industries represent an important industrial sector in regions of the Northwest of Spain, Scandinavia, South America, and Canada, among others. α-Pinene ($C_{10}H_{16}$) is a hydrophobic and recalcitrant volatile organic compound emitted from the forest products industry (e.g., wood products, pulp and paper industries) with a maximum water solubility of 5–10 mg l^{-1}. It contributes to the formation of photochemical smog and tropospheric ozone [3]. Because of its low solubility in water, the 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 [4]. 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 [5].

Some researchers have isolated microorganisms that grow with α-pinene as sole carbon source. Most of them are bacteria such as *Pseudomonas* strain PL, *Pseudomonas fluorescens* NCIMB 11671, *Pseudomonas* PX1 (NCIMB 10684), *Pseudomonas putida* PIN11, *Nocardia* P18.3, *Pseudomonas* PL and PIN 18 (NCIMB 10687) [6]. Most of the published research data focused on the degradation pathway of α-pinene. Recently, Savithiry et al. [7] reported that a thermophilic *Bacillus* strain, *Bacillus pallidus* BR425 was isolated from an α-pinene enrichment culture. Farooq et al. [8] isolated a plant pathogenic fungus, *Botrytis cinerea* that could biotransform the α-pinene into 3β-hydroxy-(−)-β-pinene, 9-hydroxy-(−)-α-pinene, 4β-hydroxy-(−)-α-pinene-6-one, and verbenone. Agrawal and Joseph [9] also isolated an *Aspergillus niger* strain that converts α-pinene into verbenone.

Until now there is no published study on the inoculation of fungi as pure biocatalyst in a bioreactor treating waste gases polluted with α-pinene (Table 1) nor on the complete mineralization of that pollutant by fungal strains. Therefore, the goal of the present work was to obtain fungi capable to metabolize it and verify their efficiency in purifying waste gases in a biofilter. Several experimental runs were carried out in order to investigate the best operational conditions for reaching a high removal efficiency, in terms of pollutant concentration, nutrients addition, gas flow rate, load, and oxygen concentration. Besides, relevant biokinetic parameters were calculated.

Table 1.

Performance of biofilters treating α-pinene

Media	Inlet (ppm)	EBRT (s)	Performance ($g\ m^{-3}\ h^{-1}$)	Ref.
Perlite; expanded clay granules; polyurethane foam cubes; compost	71	18–36	24; 33; 38; 24	[10]
Proprietary wood waste	1–100	120	6	[11]
BIOSCRUB RBC	25–35	13–24	2.7–4.2	[12]
Aspen wood chips	6–451	1248–1812	3.9	[13]
Celite R-635	15	660; 1080	3.5	[14]
Wood, compost, and perlite mixture	30–35	50	10–12	[15]
Wood chips	38–109	20; 60	14.6–44.6	[16]
Lava rock	2–739	26; 38; 72	143	Present study

2. Material and methods

2.1. Media composition

Batch experiments were undertaken with an aqueous culture medium containing (per liter) [17]: 4.5 g KH_2PO_4, 0.5 g K_2HPO_4, 2.0 g NH_4Cl and 0.1 g $MgSO_4 \cdot 7H_2O$. 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 vitamin 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 $CuSO_4 \cdot 5H_2O$, 10 mg KI, 45 mg $MnSO_4 \cdot H_2O$, 20 mg $Na_2MoO_4 \cdot 2H_2O$, 75 mg $ZnSO_4 \cdot 7H_2O$, 50 mg $CoCl_2 \cdot 6H_2O$, 20 mg $KAl(SO_4)_2 \cdot 12H_2O$, 13.25 mg $CaCl_2 \cdot 2H_2O$ and 10,000 mg NaCl. The original pH of that medium was 5.9.

Stock cultures of the fungi 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 or a desiccator 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.

2.2. Enrichment and isolation of α-pinene degrader

Biomass from a biofilter treating toluene was mixed with sludge and inoculated in a biofilter fed α-pinene-polluted air. The α-pinene degrading fungus used in this study was obtained from the leachate of the biofilter treating α-pinene. Ten milliliters of the

liquid were suspended in 90 ml mineral medium as described elsewhere [17]. 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, stable microbial consortia developed. Individual members of the consortia were isolated by streaking on mineral agar medium and incubation under solvent vapor. The isolated strain was sent to the Central Bureau voor Schimmelcultures (The Netherlands) for identification.

2.3. α-Pinene biodegradation in batch assays

Batch assays were performed at least in duplicate, with the corresponding controls. Both uninoculated media (called "blanks") and inoculated autoclaved vials (called "controls") were used. The mineral medium described above (200 ml) was introduced into 500 ml bottles closed with Viton septa and screw caps. The pollutant was added, to reach a gas-phase concentration of about 28 mg l^{-1} air. Although data are reported as gas-phase concentrations, the substrate is a volatile compound and is distributed between the gas and liquid phases. As described below, biodegradation was followed by sampling the gas phase. The concentration in the liquid phase can easily be calculated based on Henry's partition coefficient. A stock culture of the fungus was used as inoculum. That stock culture was grown in the mineral medium with α-pinene until reaching an optical density of 0.2 at 660 nm. After homogenization, 10 ml of that stock culture was inoculated into the bottles for the biodegradation assays, allowing all experiments to start with identical biomass concentrations. The bottles were maintained in a thermostatic shaker at 30 °C with constant shaking at 200 rpm.

2.4. Biofilter operation

The schematic of the biofilter used in this study is shown in Fig. 1. It is a cylindrical packed-bed reactor made of glass, 75 mm in diameter and 700 mm in height. The active height of packing column, filled with lava rock, was 250 mm. The cylindrical glass column contained four equidistant sampling ports. 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 packing tower humidified with water. A small 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 739 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 in a co-current flow. An aqueous mineral medium was added over the packed bed once a week in order to add fresh nutrients and remove any accumulated metabolites. The pH of the leachate was measured. For inoculation, the fungus was first grown in liquid medium with α-pinene as sole carbon and energy source. Then, the biofilter was filled with that solution and the liquid phase was drained off after 1 h. Afterwards, α-pinene was fed to the biofilter.

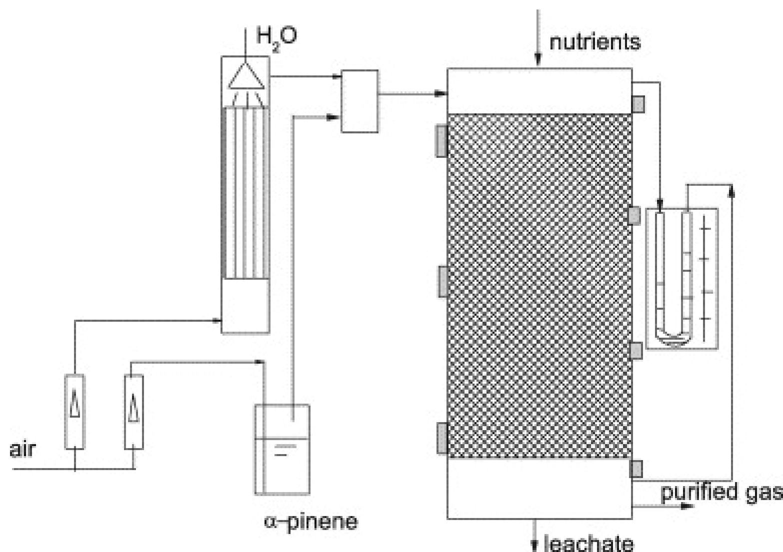

Fig. 1.

Schematic of the laboratory scale biofilter.

During the experimental study on the effect of O_2, pure oxygen from a gas cylinder flew first through a pressure gauge (RBDE-30/PS-3.5, Carburos Metalicos S.A., La Coruña, Spain) and then through a flowmeter (Model VCD 1000 flow controller, Porter Instrument Co. Inc., Hatfield, PA, USA) in order to reach 45% O_2 concentration in the inlet gas.

2.5. Analytical methods

Gas-phase concentrations of α-pinene in the biofilters were measured using a Hewlett-Packard 5890 series II gas chromatograph (GC). The GC was equipped with a flame ionization detector (FID) maintained at 250 °C. The flow rates were 30 ml min^{-1} for H_2 and 300 ml min^{-1} for air. The inlet and outlet streams of the biofilter 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 at a flow rate of 2.0 ml min^{-1}. The α-pinene concentration was determined at the oven temperature of 120 °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. The head loss across the biofilter bed was measured by a U-tube manometer filled with water (Fig. 1).

3. Results and discussion

3.1. Microorganism identification

The recently isolated organism used for the present studies was identified as *Ophiostoma* species. This organism formed a filamentous network when grown in packed-bed reactors such as the gas-phase biofilter used in this work (Fig. 2). This is an interesting characteristic, since it has been suggested that the growth of filamentous

organisms enhances the mass transfer of hydrophobic pollutants from the gas to the biocatalyst, thereby improving the performance of biofilters.

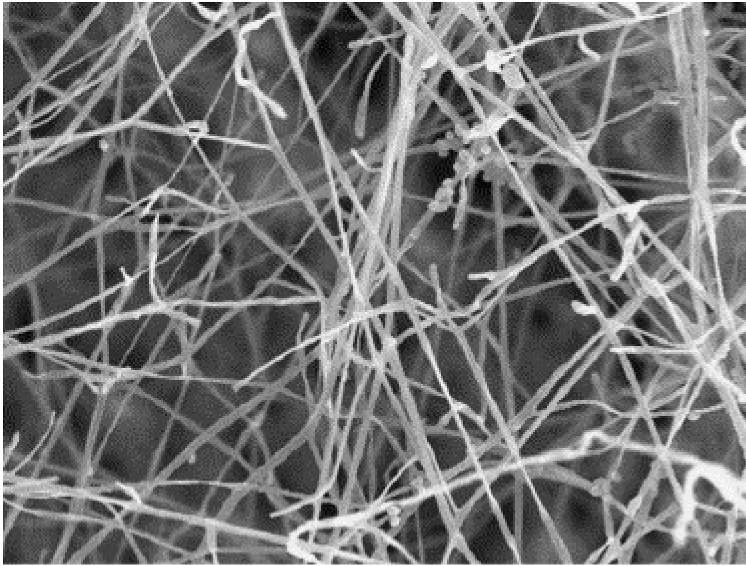

Fig. 2.

SEM picture of a filter-bed sample from a gas-phase packed-bed bioreactor colonized by a culture of *Ophiostoma* species.

3.2. Batch assays and biomass yield

The *Ophiostoma* species was transferred to the aqueous nutritive solution at pH 5.9 with α-pinene as the sole carbon source, demonstrating its ability to use that compound as single carbon and energy source (Fig. 3). Carbon dioxide was identified as end-product, the rest of the substrate being converted into biomass (Fig. 4). To the best of our knowledge, this is the first report on the complete biodegradation of α-pinene by a pure fungal culture.

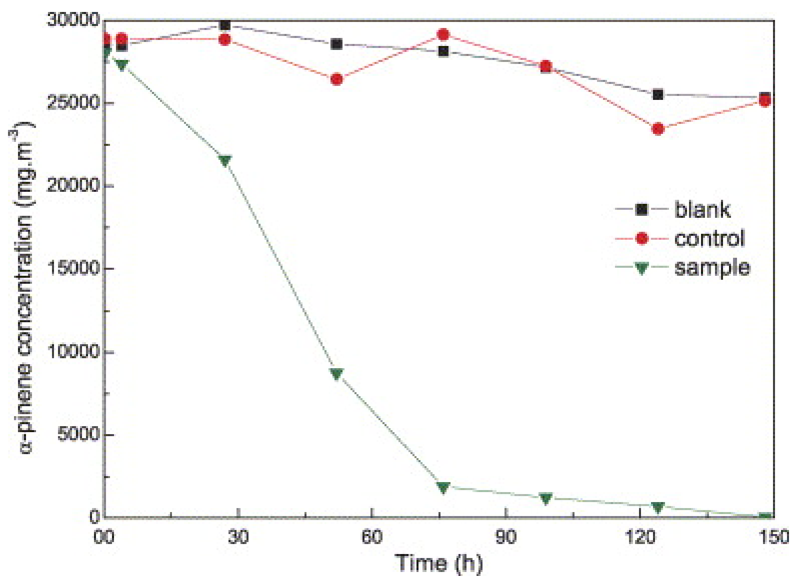

Fig. 3.

Biodegradation of α-pinene by *Ophiostoma* species.

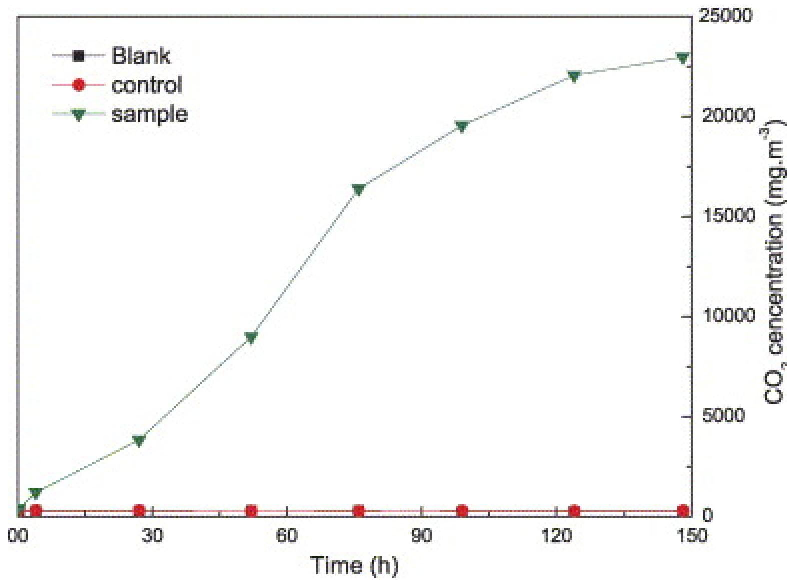

Fig. 4.

CO_2 production during the biodegradation of α-pinene by *Ophiostoma* species.

The yield coefficient of biomass on α-pinene has been determined from the shake flask experiments as 1.25 kg dry biomass/kg α-pinene consumed. A typical cellular composition can be represented by the formula $C_5H_7NO_2$[1]. Taking into account the fact that NH_4Cl was the nitrogen source and considering the yield coefficient, one can deduce the following stoichiometric equation:

$$C_{10}H_{16} + 6.5O_2 + 1.5NH_4Cl \rightarrow 1.5C_5H_7NO_2 + 5H_2O + 2.5CO_2 + 1.5HCl$$

3.3. Influence of the inlet α-pinene concentration and gas flow rate

Fig. 5 shows the removal efficiencies as a function of the α-pinene concentration at different volumetric flow rates of the gas stream during biofilter operation. A gradual decrease in efficiency was observed as the pollutant concentration increased, showing a higher elimination efficiency under conditions of low flow rate. The removal efficiency of the biofilter remained higher than 90–95% up to an inlet concentration of about 400 ppm, at an air flow rate of 0.055 $m^3 h^{-1}$. At such flow rate, a maximum elimination capacity of 143 g $m^{-3} h^{-1}$ was reached when feeding 444 ppm α-pinene, corresponding to 2.47 g m^{-3} (Fig. 6). When using higher flow rates, the elimination capacities reached 65 and 57 g $m^{-3} h^{-1}$, at 0.105 and 0.155 $m^3 h^{-1}$, respectively. For similar loads, the optimum inlet concentration corresponding to the highest elimination capacity ranged from 120 to 400 ppm depending on the gas flow rate. Within this range, the elimination capacity increased with increasing inlet concentrations for the low gas flow rate (0.055 $m^3 h^{-1}$), but it decreased at the highest gas flow rate (0.155 $m^3 h^{-1}$). Thus, as shown in Fig. 6, the optimum inlet concentration and the corresponding elimination capacity are decreasing functions of the gas flow rate. These data suggest that fungal

biofilters would be highly efficient at relatively high inlet concentrations combined with moderate flow rates. High flow rates, i.e. short residence times, result in reduced efficiencies.

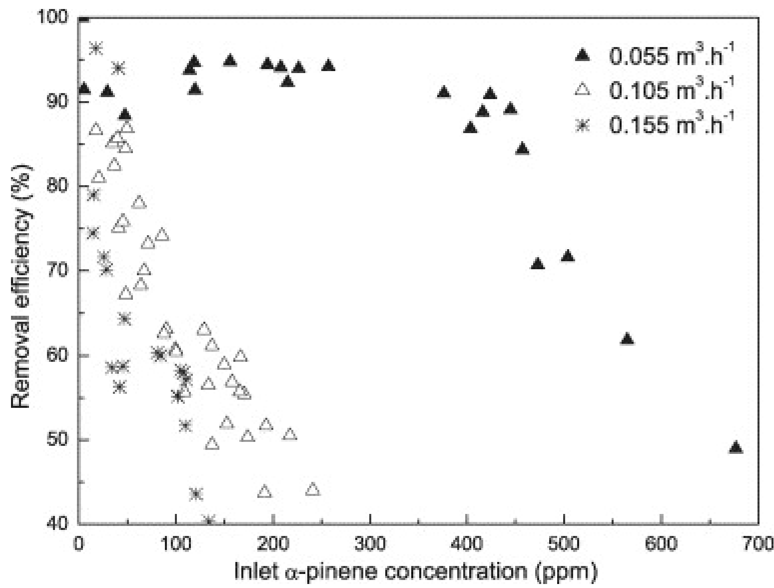

Fig. 5.

Removal efficiency of the biofilter as a function of the inlet concentration of α-pinene, at different flow rates of the gas stream.

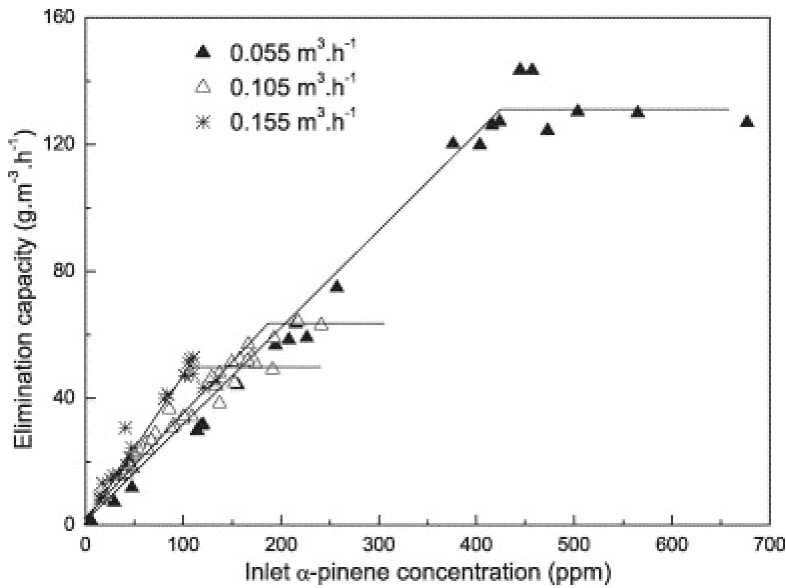

Fig. 6.

α-Pinene elimination capacity vs. the inlet load for various gas flow rates obtained in the biofilter.

3.4. Load effect on the elimination capacity

The effect of the α-pinene load on the biofilter's elimination capacity was evaluated at a gas flow rate of 0.055 m^3 h^{-1} (Fig. 7). The relationship was linear up to a critical value, after which the elimination capacity approached a maximum value asymptotically. The

removal efficiency was close to 100% below the critical inlet load of 100 g m^{-3} h^{-1} and a maximum elimination capacity of 143 g m^{-3} h^{-1} was obtained at an inlet load of 161 g m^{-3} h^{-1} or higher, still yielding a high removal efficiency of 89%. The maximum elimination capacity obtained with this *Ophiostoma* species was 3–50 times higher than the values obtained using bacteria-dominant systems (Table 1). Although studies on fungal biofilters started only a few years ago, several researchers have reported and shown experimentally that fungal biofilters may offer contaminant removal rates equal or greater than those observed in bacterial systems [4] and [5].

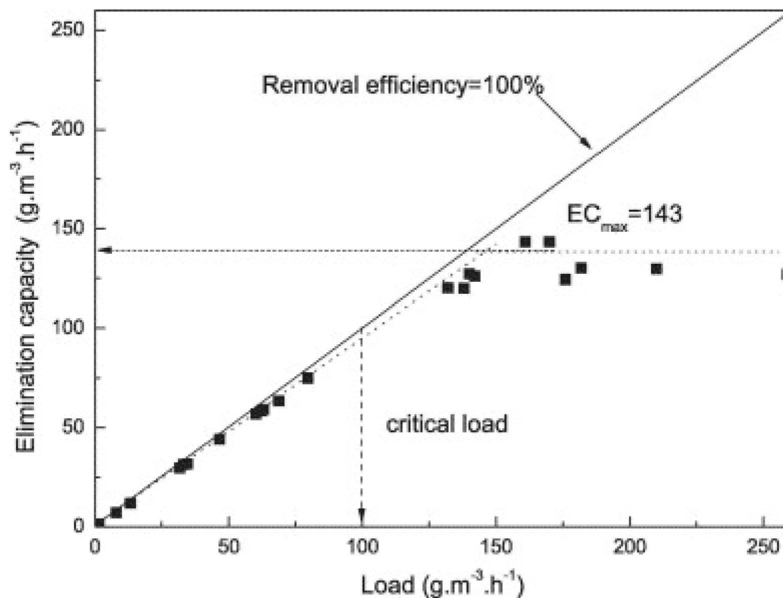

Fig. 7.

Load effect on α-pinene elimination capacity.

3.5. pH evolution and pressure drop in the biofilter

The assimilation of ammonium nitrogen (NH_4^+–N) results in a decrease of the pH of the medium. If the medium can neutralize the protons released from the assimilation of the nitrogen source, the pH will be relatively constant. Otherwise, acidification of the filter bed will occur.

The pH of the aqueous solution drained from the biofilter following the regular nutrient addition procedure was around 5.0. However, inside the biofilter, the fungi still grew, even at this low pH value, during the periods between the weekly nutrient additions. For the fungal biofilter used in this research, the pH drop did never exhibit any apparent adverse impact on the reactor's performance.

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 biofilter treating α-pinene, no significant pressure drop was detected, even after 6 months operation. This demonstrated that lava rock is a very suitable packing material for use in this fungal biofilter.

3.6. Carbon dioxide balance

In the biofiltration process, α-pinene is converted under aerobic conditions to carbon dioxide, water, and biomass. Hence, monitoring the carbon dioxide concentration in the gas phase provides valuable information on the operation of the biofilter and on the extent of mineralization of the pollutant. The ratio of daily measurements of the carbon dioxide production and removal of α-pinene are summarized in Fig. 8. The measurements in Fig. 8 show that this ratio was rather constant. Experimental data reveal that the variation of RCO_2RCO2 versus RC10H16RC10H16 is sensibly linear. The equation of the line shown in Fig. 8 is $y = 11x$. Thus, the slope of this line is 11 indicating that the average ratio of the measured RCO_2RCO2 to RC10H16RC10H16 was equal to 11 with a correlation coefficient of 0.99. The theoretical number of moles of carbon dioxide that should be produced per mole of α-pinene eliminated should not exceed 10, even when neglecting biomass growth, since 1 mole of α-pinene with 10 carbon atoms ($C_{10}H_{16}$) would yield 10 moles of carbon dioxide. If biomass growth is accounted for, this value should even be lower, as indicated earlier. However, the experimental value was somewhat higher than 10. The discrepancy between these two ratios may result from the following events: (1) even when no α-pinene was added to the reactor, there was still some CO_2 generated by endogenous respiration, (2) there is some carbonate of the lava rock packing dissolved by the acid released from NH_4^+ assimilation, this reaction may cause some additional CO_2 emission too.

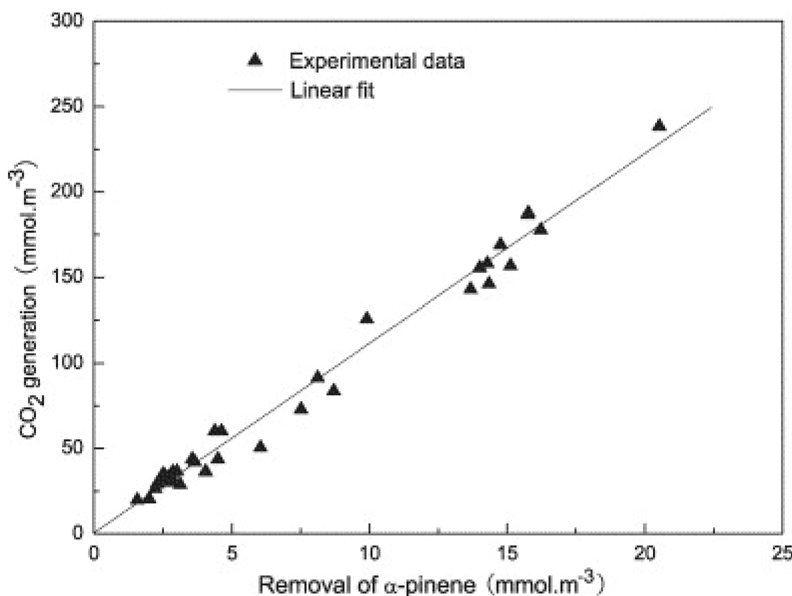

Fig. 8.

Carbon dioxide produced vs. the elimination capacity of α-pinene.

Other authors also observed that the CO_2 recovery in gas-phase biofilters may sometimes exceed 100% as a result of high rates of endogenous respiration [18]. It was shown that after long-term operation this may be the dominant CO_2 generation process. This would also explain the negligible pressure drop observed in our system and described above, due to limited biomass accumulation and significant endogenous respiration.

In any case, the high CO_2 recovery confirms the data of batch assays and indicates that the removed α-pinene is eliminated by biodegradation rather than by any other physical or chemical process such as adsorption. It can be concluded that α-pinene was basically completely transformed into CO_2.

3.7. Influence of O_2 on performance

In this experiment the influence of the oxygen concentration was investigated by comparison of α-pinene degradation and CO_2 production rates at 21% (air) and 45% oxygen (air enriched with pure oxygen) in the inlet gas.

According to the data shown in Fig. 9, the degradation rate of α-pinene shows a linear relationship with the inlet concentration. However, the removal efficiency of α-pinene decreased when increasing the inlet concentration. The maximum α-pinene degradation rate increased from 143 to 187 g m^{-3} h^{-1} with 45% oxygen in the gas while no effect on the recovery of removed α-pinene as CO_2 was observed. This maximum elimination capacity was reached at an inlet pollutant concentration of 739 ppm, which is as high as 4.12 g m^{-3}. Apparently, the maximum degradation rate at high α-pinene concentrations is limited by the availability of oxygen. Contrary to what happened with normal air (i.e., 21% O_2) where the elimination capacity leveled off when reaching 125–130 g m^{-3} h^{-1} (Fig. 6), such a plateau was not found in presence of 45% O_2 (Fig. 9) suggesting that elimination capacities exceeding 190–200 g m^{-3} h^{-1} could reasonably haven been reached if higher loads had been applied.

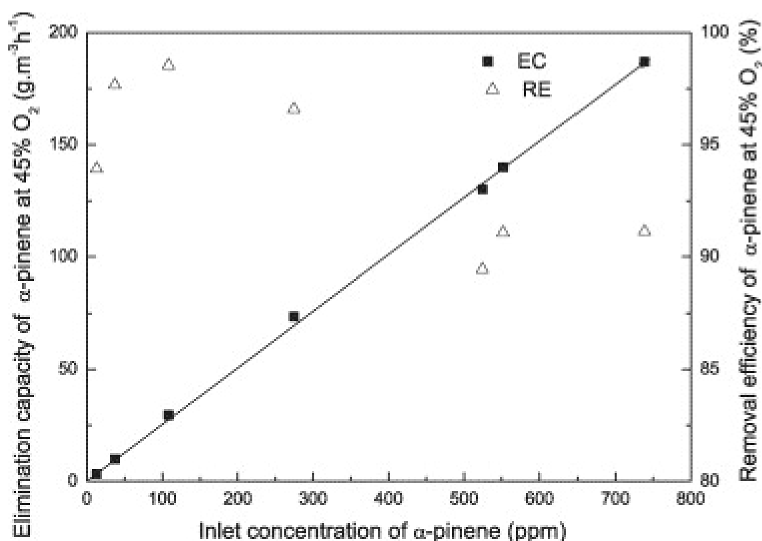

Fig. 9.

Effect of O_2 concentration on α-pinene removal.

3.8. Biokinetic parameters calculation from bioreactor data

Different methods can be used to size biofilters. If a modeling approach is chosen, the knowledge of specific biokinetic parameters is necessary. In the present case, biokinetic parameters were calculated in biofiltration studies undertaken at the optimal air flow rate of 0.055 m^3 h^{-1}. The kinetics for cellular systems can be described by the Monod Equation [1] when no inhibitory effects are observed:

$$\mu = \frac{\mu_m S}{K_S + S}$$

The constant K_S is known as the saturation constant or half-velocity constant and is equal to the concentration of the rate-limiting substrate when the specific growth rate is equal to one-half of its maximum value, that is, $K_S = S$ when $\mu = (1/2)\mu_m$. In general, $\mu = \mu_m$ for $S \gg K_S$ and $\mu = (\mu_m/K_S)S$ for $S \ll K_S$.

There is a relationship between the substrate consumption rate and the specific microbial growth rate, that can be described as follows:

$$r_S = -\frac{dS}{dt} = \frac{B}{Y_{X/S}}\mu = \frac{B\mu_m}{Y_{X/S}}\frac{S}{K_S + S} = r_{S,max}\frac{S}{K_S + S}$$

$r_{S,max}$, the maximum substrate degradation rate, corresponds to $B\mu_m/Y_{X/S}$.

Considering the mass balance over a small segment of biofilter volume as shown in Fig. 10, one can derive the following equation:

$$-\int_{C_i}^{C_o} \frac{K_S + S}{S} dS = r_{S,max}\frac{A}{Q}\int_0^H dz$$

After integration over the full height of the biofilter, we obtain:

$$(C_i - C_o) + K_S \ln\left(\frac{C_i}{C_o}\right) = r_{S,max}\frac{V}{Q}$$
(Ci−Co)+KS lnCiCo=rS,maxVQ

The left side of Eq. (4) can be simplified as:

$$\text{left side} = (C_i - C_o)\left[1 + \frac{K_S}{(C_i - C_o)/\ln(C_i/C_o)}\right]$$

After substituting V/Q by $C_i - C_o/r_S$, the right side of Eq. (4) becomes:

$$\text{right side} = r_{S,max}\frac{C_i - C_o}{r_S} = (C_i - C_o)\left(1 + \frac{K_S}{\bar{C}}\right)$$

where

$$\bar{C} = \frac{C_i - C_o}{\ln(C_i/C_o)}$$

Comparing Eqs. (5) and (6), it appears that there is a relationship between the substrate consumption rate and the mean log of the inlet and outlet concentrations. r_S can be expressed as a function of the log mean of the concentrations $\bar{C}$, i.e., $r_S = f(\bar{C})$.

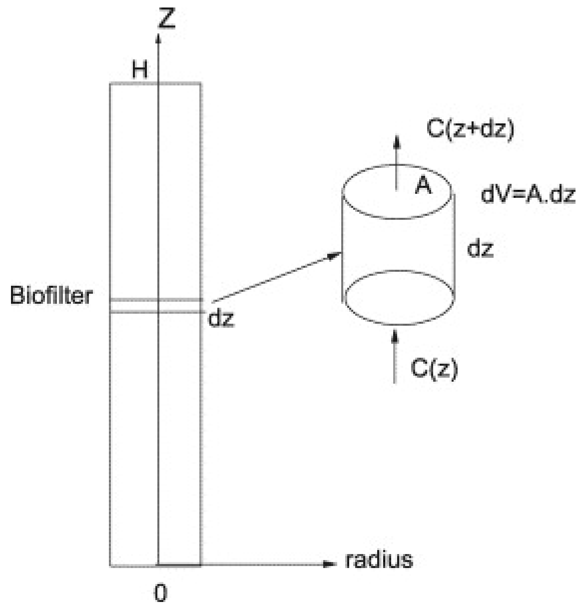

Fig. 10.

Mass balance in biofilter.

Based on the relationship between r_S and $\bar{C}C^{-}$, the data can be plotted in order to determine $r_{S,max}$ and K_S by non-linear regression of the experimental data at the gas flow rate of 0.055 $m^3\ h^{-1}$ (Fig. 11). This shows that the data can be fitted satisfactorily by linear regression in the low inlet concentration range, meaning that the bioreaction follows first-order kinetics. However, the degradation rate approaches a constant value at higher concentrations, indicating a shift from first to zero-order kinetics. Under such circumstances, the kinetic parameters for the biofiltration of α-pinene were $r_{S,max}$ = 198.42 $g\ m^{-3}\ h^{-1}$ and K_S = 0.76 $g\ m^{-3}$, with a regression coefficient R^2 = 0.902.

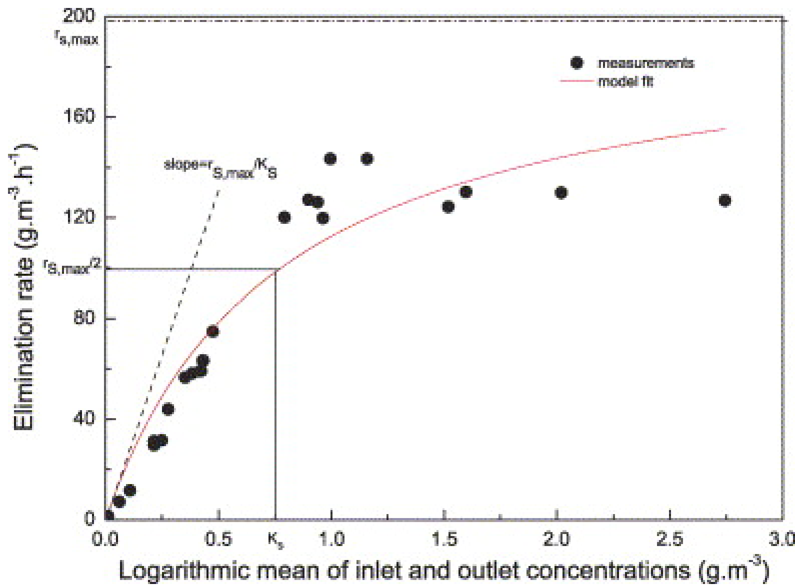

Fig. 11.

Evaluation of the elimination capacity in the biofiltration of α-pinene at the logarithmic mean of inlet and outlet concentrations.

In real full-scale application, these biokinetic parameters can be used for biofilter design. Unlike the rule of thumb method, these parameters come from experimental data and can better reflect the real situation. The volume of the biofilter can be calculated from the waste gas concentration (C_i), the gas flow rate (Q) and the required outlet concentration after biotreatment (C_o), by rearranging Eq. (4):

$$V = \frac{Q}{r_{S,max}} \left[(C_i - C_o) + K_S \ln\left(\frac{C_i}{C_o} \right) \right]$$

4. Conclusions

Batch studies and laboratory scale biofilter experiments conducted with the strain *Ophiostoma* sp. confirmed that the fungus is able to use α-pinene as a sole carbon and energy source and completely degrade it in both liquid and vapor phase. The fungal biofilter inoculated with *Ophiostoma* species was found to be more efficient than bacterial bioreactors treating α-pinene contaminated air streams, achieving a maximum elimination capacity of 143 g m^{-3} h^{-1} with 89% removal efficiency, while complete pollutant degradation was reached up to a load of 100 g m^{-3} h^{-1}. Optimal performance and maximum elimination capacity were observed when simultaneously increasing the pollutant concentration while decreasing the air flow rate, i.e. increasing the residence time. Oxygen was shown to significantly enhance the α-pinene's removal efficiency. The kinetic parameters for the biofiltration of α-pinene were $r_{S,max}$ = 198.42 g m^{-3} h^{-1} and K_S = 0.76 g m^{-3} at the optimal air flow rate of 0.055 m^3 h^{-1}. These kinetic parameters can be used for the design of full-scale biofilters.

Acknowledgements

The present research was financed by the Spanish Ministry of Education and Science (Project CTM2004-00427). Yaomin Jin was financially supported through a fellowship of the Agencia Española de Cooperación Internacional (AECI) and the Spanish Ministry of Foreign Affairs.

Appendix A. Nomenclature

A	cross area of the biofilter (m^2)
B	biofilm density (g-cell m^{-3})
C_i	α-pinene inlet concentration (g m^{-3})
C_o	α-pinene outlet concentration (g m^{-3})
$\bar{C}C^{-}$	the mean log of the inlet and outlet concentrations (g m^{-3})
H	height of the packed bed (m)
K_S	saturation constant or half-velocity constant (g m^{-3})
Q	waste gas flow rate (m^3 h^{-1})
r_S	substrate degradation rate (g m^{-3} h^{-1})
$r_{S,max}$	maximum substrate degradation rate (g m^{-3} h^{-1})
RCO_2RCO2	molar carbon dioxide production (mmol m^{-3})
RC10H16RC10H16	molar of α-pinene removal (mmol m^{-3})
S	concentration of substrate (g m^{-3})
t	residence time (h)
V	volume of the biofilter bed (m^3)
$Y_{X/S}$	biomass yield coefficient based on the substrate (g-cell g-substrate^{-1})
z	height of the biofilter (m)

Greek letters

μ	specific microbial growth rate
μ_m	maximum growth rate

References

C. Kennes, M.C. Veiga
Bioreactors for waste gas treatment
Kluwer Academic Publishers, Dordrecht, The Netherlands (2001)

C. Kennes, F. Thalasso
Waste gas biotreatment technology
J Chem Technol Biotechnol, 72 (1998), pp. 303–319

C. Dirk-Faitakis, D.G. Allen
Development and simulation studies of an unsteady state biofilter model for the treatment of cyclic air emissions of an alpha-pinene gas stream
J Chem Technol Biotechnol, 80 (2005), pp. 737–745

J.W. van Groenestijn
Biotechniques for air pollution control: past, present and future trends
Proceedings of the international congress biotechniques for air pollution control, La Coruña, Spain, October 5–7 (2005), pp. 3–12

C. Kennes, M.C. Veiga
Fungal biocatalysts in the biofiltration of VOC-polluted air
J Biotechnol, 113 (2004), pp. 305–319

P.W. Trudgill
Microbial metabolism of monoterpenes—recent developments
Biodegradation, 1 (1990), pp. 93–105

N. Savithiry, D. Gage, W. Fu, P. Oriel
Degradation of pinene by *Bacillus pallidus* BR425
Biodegradation, 9 (1998), pp. 337–341

A. Farooq, S. Tahara, M.I. Choudhary, Atta-ur-Rahman, Z. Ahmed, K.H.C. Başer, *et al.*
Biotransformation of (−)-α-pinene by *Botrytis cinerea*
Zeitschrift für Naturforschung C, 57c (2002), pp. 303–306

R. Agrawal, R. Joseph
Bioconversion of alpha pinene to verbenone by resting cells of *Aspergillus niger*
Appl Microbiol Biotechnol, 53 (2000), pp. 335–337

J.W. van Groenestijn, J.X. Liu
Removal of alpha-pinene from gases using biofilters containing fungi
Atm Environ, 36 (2002), pp. 5501–5508

B.D. Lee, W.A. Apel
Effect of bed medium moisture on α-pinene removal by biofilters
Proceedings of the USC-TRG 1996 conference on biofiltration, Los Angeles, California, October 24–25 (1996), pp. 214–222

M. Mohseni, D.G. Allen, K.M. Nichols
Biofiltration of alpha-pinene and its application to the treatment of pulp and paper air emissions
TAPPI J, 81 (1998), pp. 205–211

G.T. Kleinheinz, S.T. Bagley
A filter-plate method for the recovery and cultivation of microorganisms utilizing volatile organic compounds
J Microbiol Methods, 29 (1997), pp. 139–144

G.T. Kleinheinz, S.T. Bagley, W.P. St John, J.R. Rughani, G.D. McGinnis
Characterization of alpha-pinene-degrading microorganisms and application to a bench-scale biofiltration system for VOC degradation
Arch Environ Contam Toxicol, 37 (1999), pp. 151–157

M. Mohseni, D.G. Allen
Transient performance of biofilters treating mixtures of hydrophilic and hydrophobic volatile organic compounds
J Air Waste Manage Assoc, 49 (1999), pp. 1434–1441

M. Mohseni, D.G. Allen
Biofiltration of mixtures of hydrophilic and hydrophobic volatile organic compounds
Chem Eng Sci, 55 (2000), pp. 1545–1558

E. Estévez, M.C. Veiga, C. Kennes
Biofiltration of waste gases with the fungi *Exophiala oligosperma* and *Paecilomyces variotii*
Appl Microbiol Biotechnol, 67 (2005), pp. 563–568

R.M.M. Diks, S.P.P. Ottengraf, S. Vrijland
The existence of a biological equilibrium in a trickling filter for waste gas purification
Biotechnol Bioeng, 44 (1994), pp. 1279–1287