Appl Microbiol Biotechnol (2008) 79:319–327
DOI 10.1007/s00253-008-1433-2

ENVIRONMENTAL BIOTECHNOLOGY

Influence of synthetic packing materials on the gas dispersion and biodegradation kinetics in fungal air biofilters

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Received: 4 January 2008 / Revised: 22 February 2008 / Accepted: 24 February 2008 / Published online: 11 April 2008

Abstract The biodegradation of toluene was studied in two lab-scale air biofilters operated in parallel, packed respectively with perlite granules (PEG) and polyurethane foam cubes (PUC) and inoculated with the same toluene-degrading fungus. Differences on the material pore size, from micrometres in PEG to millimetres in PUC, were responsible for distinct biomass growth patterns. A compact biofilm was formed around PEG, being the interstitial spaces progressively filled with biomass. Microbial growth concentrated at the core of PUC and the excess of biomass was washed-off, remaining the gas pressure drop comparatively low. Air dispersion in the bed was characterised by tracer studies and modelled as a series of completely stirred tanks (CSTR). The obtained number of CSTR (n) in the PEG packing increased from 33 to 86 along with the applied gas flow (equivalent to empty bed retention times from 48 to 12 s) and with operation time (up to 6 months). In the PUC bed, n varied between 9 and 13, indicating that a stronger and steadier gas dispersion was achieved. Michaelis–Menten half saturation constant (k_m) estimates ranged 71–113 mg m^{-3}, depending on the experimental conditions, but such differences were not significant at a 95% confidence interval. The maximum volumetric elimination rate (r_m) varied from 23 to 50 g m^{-3} h^{-1}. Comparison between volumetric and biomass specific biodegradation activities indicated that toluene mass transfer was slower with PEG than with PUC as a consequence of a smaller biofilm surface and to the presence of larger zones of stagnant air.

Keywords Air biofiltration · Packing materials · Biodegradation kinetics · Dispersion dynamics · Fungi · Toluene abatement

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Introduction

Biofiltration is gaining acceptance as an economical and reliable air pollution control technology for treating gases contaminated with relatively low concentrations of biodegradable volatile organic compounds (Kennes and Veiga 2001; van Groenestijn and Kraakman 2005). In air biofilters, waste gases are forced through a filter bed consisting of a porous particulate carrier material that serves as support for microorganisms that metabolise volatile compounds contained in the gas phase. To maintain biological activity, the filter bed is humidified, but conversely to other biological waste-gas treatment technologies (i.e., bioscrubbers and biotrickling filters), gas biofilters are characterised by the absence of a continuous and mobile aqueous phase. This lack of free flowing water makes control of parameters, which influence microbial activity, such us water activity, pH, and nutrient supply more difficult.

Conventional biofilters employ organic packing media like compost, peat, wood chips, and sawdust, alone or in combinations with other materials. Although these biofilters have been used successfully in many applications, several common problems, such as medium clogging and channelling, excessive pressure loss of the gas flow, and the occurrence of secondary and co-metabolic reactions, appear over the operation time as a result of packing deterioration and biomass accumulation due to an uncontrolled delivery of nutrients (Kennes and Veiga 2001). Hence, there is a current tendency to investigate the use of biologically inert materials as support media, which are physically and chemically more stable. Material porosity is a key feature of the biofilter bed, and the presence of a compact network of micropores was initially thought to be indispensable for a proper water holding capacity (Sakuma et al. 2006). Yet, small porosity promote biomass growth around the packing, which results in an increasing pressure drop and even in the clogging of the filter bed after long operational periods. These problems have prompted the experimentation with new synthetic foam-like materials that are characterised by the structural predominance of macropores (Moe and Irvine 2000, 2001; Woertz et al. 2002).

In combination with the use of synthetic packing materials, which are usually devoid of indigenous microflora, recent advances in air biofiltration have been focussed on the inoculation or enrichment of specialised microbial populations. In particular, research on the biofiltration of volatile aromatic hydrocarbons is being directed towards the use of fungi growing on inert packing material (Kennes and Veiga 2004; van Groenestijn and Kraakman 2005). Fungi are generally more resistant than bacteria to the relatively acidic and dry conditions commonly present in the biofilter bed. Moreover, it has also been speculated that the fluffy aerial mycelia might take up hydrophobic compounds faster than flat and aqueous bacterial biofilm surfaces (Dorado et al. 2008; van Groenestijn et al. 2001). This phenomenon is thought to be the result of the relatively large surface area of fungal filaments, in combination with the enhanced diffusivity of hydrophobic compounds due to the minimal presence of a water film between the gas phase and the biomass.

A third important trend in biofiltration progress refers to the development of numerical models for the simulation of relevant biochemical and physical processes to improve the understanding on biofiltration and to optimise the design of biological gas treatment systems (Devinny and Ramesh 2005). In this context, the overall elimination efficiency of a biofilter is commonly limited either by the pollutant diffusion transfer from the gas phase to the biofilm or by the biodegradation reaction rate. Most biofilter models presume that the air flow within the reactor packing can be modelled as a plug flow (Devinny and Ramesh 2005). Biodegradation kinetics have generally been assumed to follow the Michaelis–Menten model for enzyme activity. The determination of biodegradation kinetic parameters in air biofilters is not an easy task, and such data are frequently taken from the literature. However, the experimental conditions in which biodegradation parameters have been obtained may be considerably different from those found in real biofilters. Depending on the culture conditions, the half saturation constant (k_m) measured for the same strain and substrate combination has been found to differ up to one order of magnitude (Kennes and Veiga 2001). Such differences might be attributed to diffusion rate limiting phenomena or to biomass physiological changes that are embedded in the observed kinetic parameters and cannot be neglected in the development of accurate biofiltration models.

The goal of this work was to study the influence of two selected synthetic packing materials, polyurethane foam cubes (PUC) and perlite granules (PEG), on the dispersion of the air flow and the biodegradation kinetics in continuous lab-scale biofiltration experiments. The two packed beds had a comparable total porosity, were devoid of chemicals that might interfere with the biological activity, and had no significant adsorption capacity, but the average pore size of polyurethane foam was much larger than that of perlite. Toluene and the fungus *Cladophialophora* sp. strain CBS 110553 were used as model contaminant and biocatalyst, respectively. The effect of biomass accumulation and gas flow rate on the air mixing regime and biodegradation kinetic parameters was also assayed.

Materials and methods

Microbial strain

Biofilters were inoculated with the fungus *Cladophialophora* sp. strain CBS 110553 (ATCC MYA-2335) by submerging the reactor bed with a spore suspension (approximately 1.5 dm^3) obtained by washing fully grown oatmeal agar plate cultures with a phosphate buffer solution (50 mM). This strain is able to grow on toluene as the sole source of carbon and energy (Prenafeta-Boldú et al. 2001). Despite its close phylogenetic relationship to pathogenic black yeasts from the *Herpotrichiellaceae* family (Prenafeta-Boldú et al. 2006), recent experimental evidence has shown that this fungus is non-virulent (de Hoog GS, unpublished results).

Biofilter setup and operation

Experiments were carried out in two glass columns (6 cm internal diameter, 2 dm^3 empty volume, 1.7 dm^3 of reactor bed volume) packed respectively with PEG and PUC to a

height of 60 cm. The physicochemical characteristics of these materials are given in Table 1. Each reactor was supplied with a continuous toluene-contaminated air stream at an approximate flow rate of 120 $m^3\ m^{-3}\ h^{-1}$ (relative to the reactor bed volume) in downflow operation mode, and the toluene concentration was set up to 500 mg m^{-3}. The polluted air flow was produced by passing a known portion of the total incoming air, as determined with a mass flow meter Model 5850TR (Brooks Instruments BV, Veenendaal, The Netherlands), through a gas-washing bottle filled with liquid toluene (Lab-Scan Analytical Sciences, HPLC grade, Dublin, Ireland). This toluene saturated stream was subsequently mixed with the main air stream that had previously been humidified by bubbling through a series of water baths. Biofilters were continuously operated during 6 months in a temperature controlled room at 25°C. Operation was stopped once a week to supply a mineral medium to the microorganisms (Cox et al. 1993); the filter bed was then fully submerged in approximately 1.5 dm^3 of mineral medium for about 30 min, and the excess of medium was drained off for an additional 30 min before resuming the process.

Residence time distribution tests

The gas mixing regime in the packing was characterised by continuously recording the methane concentration (M) at the biofilter outlet after injecting a pulse of methane (1 cm^3) at the flow inlet. These experiments were performed at volumetric air flow rates (Q) that ranged from 50 to 350 $m^3\ m^{-3}\ h^{-1}$, equivalent to an EBRT range of 72 to 10 s and at different stages of biomass development: before biofilter inoculation and after 1 and 6 months of operation. The recorded pulse residence time distribution (RTD) curves were modelled as being equivalent to a series of n completely stirred tank reactors (CSTR; Eq. 2). Two additional CSTR compartments of constant volume were added to account for the void spaces present at the top (V_{in}=274 cm^3; Eq. 1) and at the bottom (V_{out}=56 cm^3; Eq. 3) of the packing. The mass balance of methane in each of the modelled biofilter tank series produces the following governing differential equations set:

$$\frac{dM_0}{dt} = -\frac{Q}{V_{in}} M_0 \tag{1}$$

$$\frac{dM_i}{dt} = \frac{n}{\tau}(M_{i-1} - M_i), \quad i = 1, \ldots, n. \text{ with } M_{i-1} = 0 \text{ if } i = 1 \tag{2}$$

$$\frac{dM_{n+1}}{dt} = \frac{Q}{V_{out}}(M_n - M_{n+1}) \tag{3}$$

where M_i is the methane gas concentration in tank i and τ is the mean residence time of the methane pulse in the packing. The initial conditions were defined as $M_0(0)=m/V_{in}$ and $M_i(0)=0$, $i=1,\ldots,n+1$, where M_0 is the methane concentration in the first tank due to the pulse injection of a mass m. The three parameters of the model (n, τ and m) were determined by numerically fitting modelled data against experimental RTD curves minimising the sum of quadratic errors. Assuming that the obtained residence time τ of the tracer gas is equivalent to that of the flowing air, the effective bed void volume was calculated as:

$$V_e = \tau \times Q \tag{4}$$

Biodegradation kinetics

The biodegradation of toluene was characterised by applying increasing step changes at the influent toluene concentration (C_0), from about 20 up to 200 mg m^{-3} at a constant flow (Q), and by measuring the effluent concentration (C_e) once steady operation was reached (standard

Table 1 Characteristics of the assayed biofilter packing materials

Bed characteristics	Biofilter A	Biofilter B
Material	Perlite	Polyurethane foam
Supplier	Puil B.V. (Rhenen, The Netherlands)	Betapol B.V. (Beesd, The Netherlands)
Approximate particle geometry	Spherical	Cubical
Average particle size	5 mm diameter	13 mm edge
Relative density[a]	100 g dm^{-3}	28 g dm^{-3}
Median pore size[a]	<16 μm	<3,000 μm
Material porosity[a]	94%	97%
Interstitial space[b]	44%	56%
Specific particle surface area[b]	5.3 $cm^2\ cm^{-3}$	1.7 $cm^2\ cm^{-3}$

[a] According to the information provided by the supplier
[b] Estimated from particle counts on given packing volumes

deviation of at least three C_e measurements after more than 1 h of operation ≤5%). These experiments were performed at two different flows (approximately 150 and 300 $m^3\ m^{-3}\ h^{-1}$) and at two stages of biomass accumulation (once biomass had just covered the whole packing material and with an aged biofilm after 6 months of operation). Biodegradation was modelled on the basis of the following assumptions: (1) steady-state conditions prevailed during each of the constant organic load step measurements, (2) biomass activity was evenly distributed throughout the biofilter bed, and (3) the toluene depletion rate r_x inside the biofilter followed the Michaelis–Menten kinetics (Eq. 6), with k_m and r_m as constant parameters. Experimental C_0 and C_e values were used to fit two air mixing regime models (M1 and M2). In model M1, the bed was discretised into n tanks as determined previously according to the series of CSTR air dispersion model. The toluene stationary mass balance in tank i (i=1,...,n) was then quantified by Eq. 5,

$$Q(C_{i-1} - C_i) + r_i \frac{V}{n} = 0 \tag{5}$$

where C_i is the in air concentration at the ith tank and r_i is the corresponding volumetric degradation rate, which was assumed to be depended on the toluene concentration in the air (Eq. 6).

$$r = -r_m \frac{C}{k_m + C} \tag{6}$$

The substitution of r_i in Eq. 5 by the degradation rate given by Eq. 6 produced the set of second order Eq. 7, which were solved successively to obtain the effluent concentration (C_e=C_n).

$$C_i^2 + \left(k_m + \frac{V \times r_m}{n \times Q} - C_{i-1}\right) C_i - k_m \times C_{i-1} = 0, \quad i = 1, \ldots, n \tag{7}$$

In model M2, biofilters were considered as a steady plug flow reactor with the air toluene concentration (C) profile given by Eq. 8,

$$\frac{dC}{dx} = \frac{A}{Q} r_x \tag{8}$$

where A was the section of the packed column, Q the air flow and r_x the volumetric biodegradation rate (Eq. 6). The substitution of Eq. 6 in Eq. 8 and subsequent integration along the height of the column, with bed volume (V), results in expression 9.

$$C_0 - C_e - k_m \ln \frac{C_e}{C_0} - r_m \frac{V}{Q} = 0 \tag{9}$$

In both models, the resulting toluene concentration in the effluent is a function of the form C_e=$f(C_0, k_m, r_m)$. The kinetic parameters k_m and r_m were obtained by minimising the sum of quadratic errors when fitting model predicted effluent concentrations to experimental data. Confidence intervals on k_m and r_m estimates were obtained from contour plots of the objective function at critical confidence values (Beale 1960). The sets of ordinary differential equations were numerically solved by the *ode15s* routine from MATLAB software package (Mathworks, USA). Simulations were performed on a Pentium IV computer.

Analytical methods

For tracer studies, the methane concentration at the biofilter outlet was measured each second with a Foxboro TVA-1000 portable FID detector (Foxboro, USA). The concentration of toluene at the biofilter inlet and outlet was measured by taking gas samples (100 μl) with a valve syringe and injecting them in a Varion 3800 gas chromatograph equipped with a FID (Varian Chromatography Systems, Walnut Creek, CA). Gas valve-rotameter assemblies were used to adjust the flow passing through the biofilters. Precise flow determination in fluidodynamic and kinetic experiments was performed with a wet gas flow meter (Actaris Meterfabriek B.V., Dordrecht, The Netherlands). The pressure drop across the entire reactor length was measured by connecting a water filled U-tube manometer to the inlet and outlet of the reactor. For protein determination, the biofilter bed was suspended in 2 dm^3 of a NaOH solution (1 N) and boiled for 20 min, before colorimetric analysis with the Coomassie-dye method using bovine serum protein as reference (Pierce Biotechnology).

Results

Biomass accumulation and gas pressure drop

After 1 month start up of the biofilters operation, the filter media in the columns packed with PEG and PUC appeared to be fully covered by a brownish and velvety biofilm. Microscopic observations of samples were taken regularly from the bed (or the leachate) and showed that the predominant biomass morphology was concordant to the inoculated fungus throughout the biofiltration experiments. Closer observations on the biofilm/support structure revealed that the mycelium was unable to penetrate the perlite micropores and grew only over the surface of the particles. Conversely, the larger pore size of polyurethane foam allowed the fungus to develop in the interior of the cubes. As biofilter operation time progressed, the appearance of the fungal biofilm developing around the PEG became progressively thicker compared to that of the PUC,

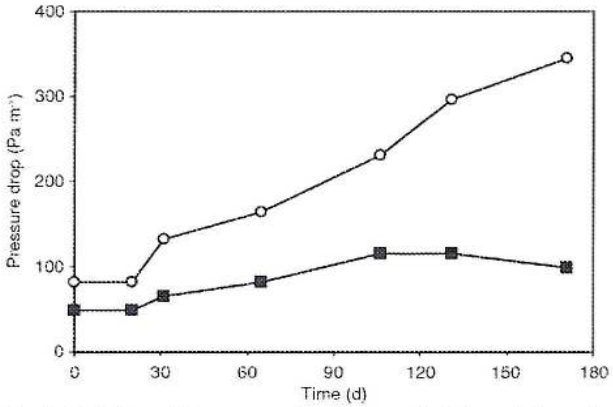

Fig. 1 Evolution of the pressure drop between the inlet and the outlet of two air biofilters packed with perlite granules (*circle*) and polyurethane foam cubes (*square*) measured at a volumetric loading rate of 150 $m^3\ m^{-3}\ h^{-1}$

in which the interstitial particle space remained appreciably free of biomass. The gain in time of pressure drop between inlet and outlet airflows was considerably higher in the PEG biofilter (Fig. 1). Measurements on the total amount of protein contained in both biofilter columns after 6 months of operation, yielded 428 and 255 g m^{-3} for PEG and PUC, respectively. These values were used for the calculation of specific toluene biodegradation rates, relative to the amount of biomass contained in the biofilter bed.

Gas residence time distribution

The series of CSTR air dispersion model provided good adjustments to experimental residence time distribution (RTD) curves of a methane pulse measured under the tested conditions (Fig. 2). The air mixing regime determined in this way with the two tested packing materials under analogous flow and runtime conditions was found to be significantly different (Table 2). Considerably higher n values were determined in PEG biofilter, indicating that air dispersion within this material was lower than in the PUC column. With the uninoculated PEG biofilter, n increased from 33 to 70 along with an air flow range of about 50 to 350 $m^3\ m^{-3}\ h^{-1}$. The progressive accumulation of biomass also increased the plug-flow behaviour of the perlite packing as the n values obtained with similar air flows after 6 months of operation ranged from 52 to 86. Model fittings in this latter case were slightly worse, as shown by the standard error (Table 2), indicating that biomass accumulation caused a greater deviation from the modelled ideal air dispersion. When the RTD curves were fitted directly to the series of CSTR model, without the inclusion of the biofilter void plenum compartments, the n values obtained for perlite were a 39–65% lower (results not shown). Model fitting in this case was generally worse, compared to the model where plenum spaces were taken into account. Interestingly, under analogous test conditions but using PUC as packing, n remained comparatively invariable at the range of 9 to 13, irrespective of the air flow, biomass growth and of the consideration of the unpacked biofilter compartments into the air dispersion modelling.

The effective gas bed volume (V_e) occupied by the passing air flow was calculated from Eq. 4 and compared against the interstitial particle space and the total bed porosity (Fig. 3). The results showed that at a volumetric flow rate in the range of 50 to 100 $m^3\ m^{-3}\ h^{-1}$, air stagnant pockets of similar magnitude were formed in the two biofilters and V_e was very similar to the particle interstitial space of the bed. Higher air flows increased V_e, particularly in the polyurethane foam bed, where it reached values that approached the total bed porosity (particle interstitial space + material porosity). The specific biomass accumulation pattern of each support material had a differential effect on V_e. After 6 months of operation, biomass growth on the PEG reduced significantly V_e, while this effect was hardly noticeable in PUC (Fig. 3).

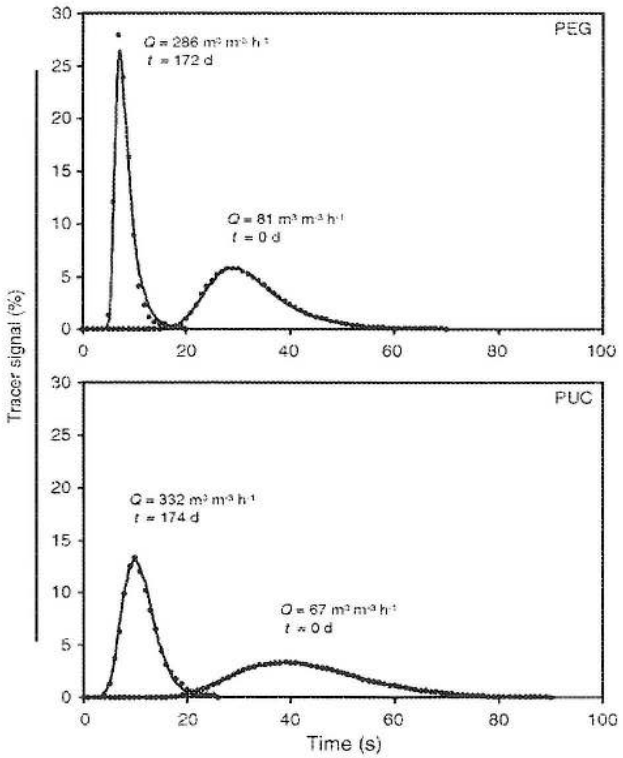

Fig. 2 Examples on the fitting of the series of CSTR model (*solid lines*) to the residence time distribution curves of a pulse of methane measured at the outlet (*dots*) in biofilters packed with perlite granules (*PEG*) and polyurethane foam cubes (*PUC*). The shown figures correspond to the longest and the shortest assayed pulse retention time

Table 2 Adjusted parameter values corresponding to the number of CSTR connected in series (n), the mean residence time of the gas tracer pulse (τ) in the packing and the mass (m) of the gas tracer pulse

Packing material	Runtime (d)	Flow ($m^3\ m^{-3}\ h^{-1}$)	n	τ (s)	m^a (%)	S_E (% m)
Perlite granules	0	81	33	24	112	0.68
		161	57	12	94	0.79
		357	70	8	97	0.76
	31	98	33	20	119	1.29
		159	40	13	110	1.29
		182	50	11	99	0.87
		299	63	8	95	1.01
	172	87	52	15	120	3.59
		99	58	12	65	2.11
		165	60	9	95	1.19
		286	86	6	104	1.51
Polyurethane foam cubes	0	67	12	33	90	0.29
		165	11	16	98	0.68
		224	12	13	98	0.68
		376	13	8	84	0.78
	33	82	10	30	97	0.45
		146	10	20	106	0.76
		204	10	16	112	0.79
		299	13	10	84	0.95
	174	76	11	32	93	0.29
		89	12	28	107	0.29
		213	9	14	125	0.41
		332	11	9	97	0.49

The standard error (S_E) of the modelled tracer residence time distribution (RTD) curve in relation to the experimental data

[a] Adjusted mass of the gas tracer pulse of the modeled RTD curve, which corresponded to the injection of 1 cm^3 of methane, in relation to the average value obtained with all the different treatments.

Degradation macrokinetics

Biodegradation kinetic parameters (k_m, r_m) and confidence intervals obtained with the air dispersion model M1 are presented in Table 3. These parameter values were somewhat lower than those obtained with the model M2, but these differences were generally below 5% (Fig. 4). The exception were the k_m values from the bed packed with PUC, in which the discrepancy between the two models exceeded the 5% level in most of cases and reached a maximum of 12%. Contour plots of the sum of squared errors at the 95% confidence region with respect to the adjusted kinetic parameters displayed a elliptic region indicating that, to some extent, k_m and r_m were correlated (Fig. 4). The degree of this correlation appears to be related to the magnitude of the standard error on the measured values in relation to the simulated data. The significant overlapping of the individual 95% confidence interval corresponding to the k_m estimates indicates that this parameter is not very sensitive to the tested conditions of different packing materials, gas flow, and biomass content. The apparent maximum substrate conversion rate (r_m), on the other hand, increased with the volumetric loading rate, particularly in the perlite packing and upon biomass accumulation (Fig. 5; Table 3). The r_m values were also higher with the perlite packing than with polyurethane foam, but conversely, the specific maximum biodegradation rate, in relation to the protein content measured after 6 months of operation, was higher in the biofilter packed with PUC.

Discussion

Materials with a predominant pore size range above 100 μm, such as polyurethane foam, have been cited as a very suitable biofilter packing (Moe and Irvine 2001). The relative wide pores of this material constrained biomass growth inside the particle core, resulting in low pressure loss levels as the interstitial spaces remained available for the flowing air. However, good biodegradation rates with polyurethane foam appeared to be linked to fungal growth

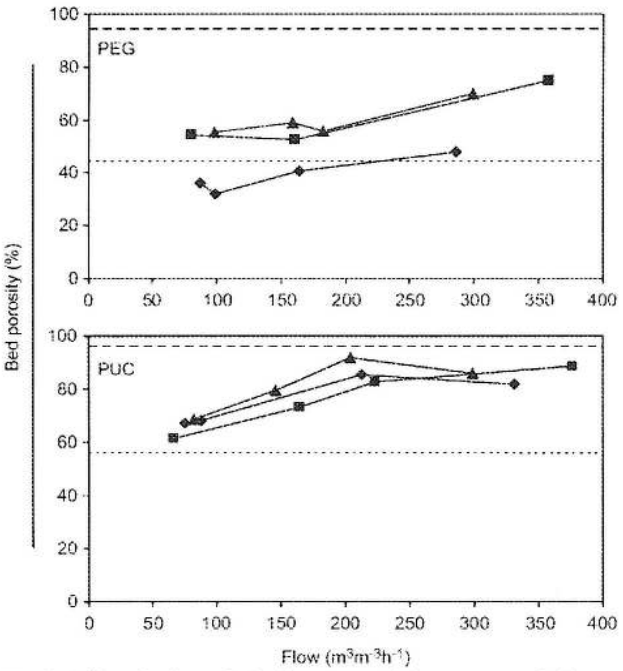

Fig. 3 Effect of volumetric loading rate and biomass accumulation on the gas phase volume of the packing that is occupied by a pulse of methane in two air biofilters filled respectively with perlite granules (*PEG*) and polyurethane foam cubes (*PUC*). Measurements were taken at time zero (*square*), after 1 month of operation (*triangle*) and at the end of experiments after 6 months (*diamond*). *Dotted lines* correspond to calculated particle interstitial space and the *dashed lines* to the total bed porosity

Table 3 Estimated Michaelis–Menten kinetic parameters (for model M1) corresponding to the biodegradation of toluene (at 25°C) in biofilters inoculated with the fungus *Cladophialophora* sp. (CBS 110553) under different conditions of packing material, runtime and flow

Packing material	Runtime	Flow	n^a	k_m	$r_m{}^b$		S_E
	(d)	($m^3\ m^{-3}\ h^{-1}$)		($mg\ m^{-3}$)	($g\ m^{-3}\ h^{-1}$)	($mg\ g^{-1}\ h^{-1}$)	($mg\ m^{-3}$)
Perlite	34	154	33	74 (65–89)	34 (31–36)	–	6.15
		297	63	79 (62–101)	40 (36–45)	–	8.14
	178	144	52	71 (64–81)	31 (29–32)	72	4.80
		341	86	98 (76–126)	50 (44–56)	116	10.29
Polyurethane foam	35	154	10	113 (93–150)	23 (20–26)	–	9.33
		297	13	99 (83–122)	30 (27–33)	–	5.31
	179	144	11	72 (71–82)	28 (27–29)	111	3.06
		341	11	90 (72–115)	33 (29–36)	130	6.99

Individual 95% confidence intervals on kinetic parameters are shown between brackets

[a] The number of CSTR compartments considered in the model at the experimental flow values were obtained by linear extrapolation of the previously calculated data (Table 2).

[b] Maximum toluene biodegradation rate units are presented in relation to the reactor bed volume and to the total protein content.

(van Groenestijn and Liu 2002; Woertz et al. 2002), and the use of such material has been reported as inadequate when the predominant biofilm populations were formed by bacteria (Sakuma et al. 2006). This difference can be attributed to the poor water holding capacity of polyurethane foam, in combination with the higher sensitivity of bacteria to dry conditions. Moreover, the highly branched filamentous hyphae are better retained inside the particle macropore network than the typical clumped bacterial biofilms. This relationship between pore size, biomass distribution in the packing and higher pressure drop has been confirmed in our study by comparing perlite and polyurethane foam as support media for air biofiltration.

Besides, and because of the effect of the carrier structure on biomass retention and distribution, the selection of the packing material also had important implications on the biofilter gas mixing regime. Air biofilters are often considered to behave as a plug-flow reactor regardless of the packing material (Devinny and Ramesh 2005). However, the limited number of literature references on air biofilter tracer studies provides conflicting evidence as the reported n values ranged from 2 to 100 (Devinny et al. 1999; Kraakman et al. 1997; Lee et al. 2001; Mendoza et al. 2004; Morgan-Sagastume et al. 2001), well above and below the threshold of 10 over which ideal plug flow conditions are generally considered (Levenspiel 1972).

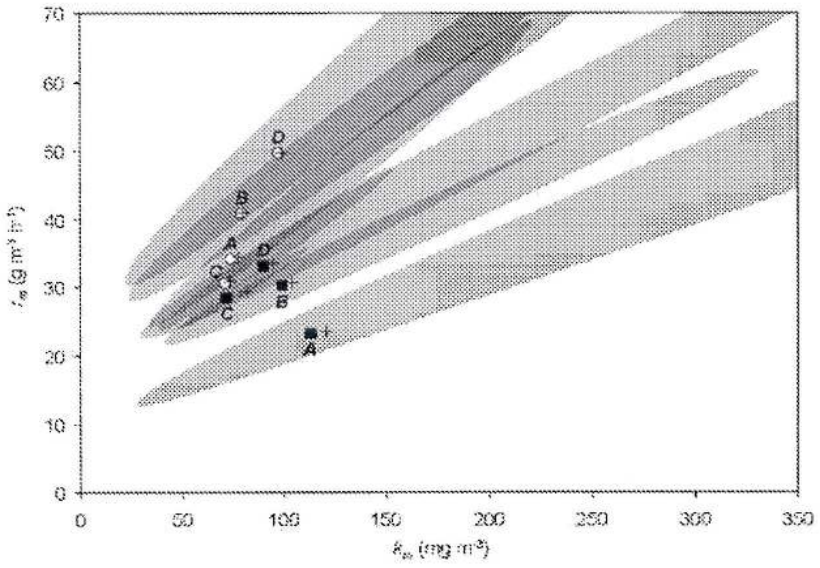

Fig. 4 Contour plots on the 95% confidence regions of the Michaelis–Menten parameters estimated by model M1 for the biodegradation of toluene measured in biofilters packed with perlite granules (*circle*) and polyurethane foam cubes (*square*), and measured at different gas flow and operation time combinations of approximately 150 $m^3\ m^{-3}\ h^{-1}$ after 30 days (*A*) and after 180 days (*B*); and 300 $m^3\ m^{-3}\ h^{-1}$ after 30 days (*C*) and after 180 days (*D*). Kinetic parameters obtained with model M2 are also displayed (*plus symbol*)

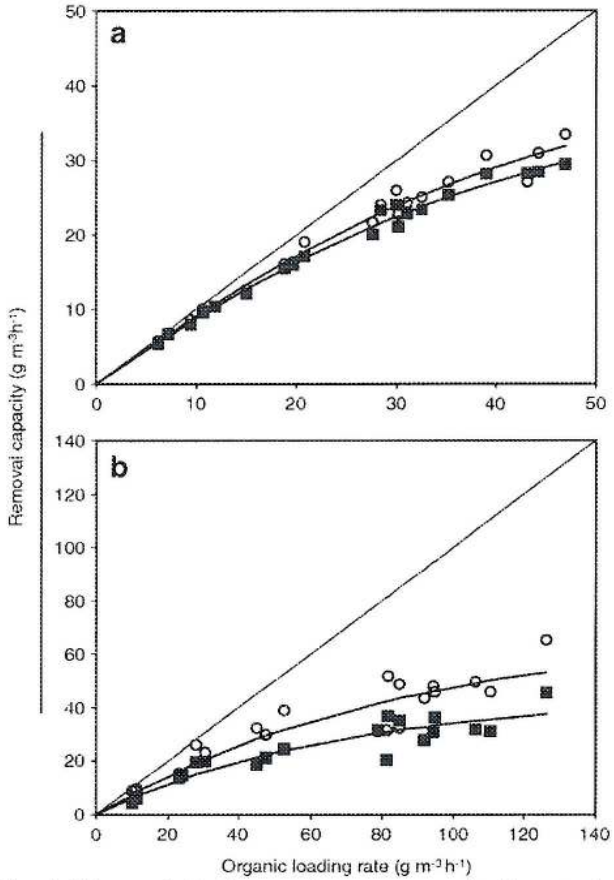

Fig. 5 Toluene elimination capacity versus mass loading rate in biofilters packed with perlite granules (*circle*) and polyurethane foam cubes (*square*) after 6 months of operation, measured at two volumetric loading rates: 150 m^3 m^{-3} h^{-1} (**a**) and 300 m^3 m^{-3} h^{-1} (**b**). *Dots* correspond to measured values and *solid lines* to simulated; *dashed lines* represent the 100% removal efficiency

Such diversity of values is excessive to be attributed to differences on experimental conditions (biofilter configuration, bed composition and applied gas flow), as well as to methodological bias (inappropriate time sensor responses and adsorption/absorption phenomena of the tracer).

The clue for understanding this disparity on the biofiltration n parameter is provided by comparing the present work against a similar study published earlier (Mendoza et al. 2004). In that report, air dispersion was characterised in a lab-scale biofilter packed with perlite, used for the biodegradation of styrene, by measuring RTD curves of methane pulses. The reported n values at an applied gas flow of about 60 m^3 m^{-3} h^{-1} before biomass development was only 9, which is much lower than the n value of 33 obtained in the present work under similar flow conditions and identical packing material and tracer gas (Table 2). Interestingly, the column used in that study had a large empty section at the top of the packing (the exact volume was not reported) to allow fluidisation of the perlite during moistening. In the present work, when model calculations were repeated without considering the relatively small void spaces present at the top and the bottom of the packing, n decreased down to 20 (results not shown). Hence, a reasonable explanation concerning the diversity of n found in the literature arises from the fact that, to our knowledge, tracer studies have not considered the effect of the biofilter plenum. The common presence of void air distribution and collection chambers, placed before and after the packed bed where biodegradation takes place, will inevitably have a diluting effect on the applied tracer pulses. Consequently, if not corrected conveniently, the resulting n values will be lower than those that strictly correspond to the gas dispersion into the bed. The magnitude of this bias will depend on the size of the plenum in relation to the volume of the modelled CSTR compartments, and in fact, n values as low as 2 have been registered in full-scale biofiltration units (Kraakman et al. 1997), which are likely to have a substantially larger plenum to bed volume ratio when compared to lab-scale columns. On the other hand, this effect might be negligible with support materials that already cause a strong air dispersion, like PUC. The tracer studies also indicated that an important fraction of the air contained in the biofilter bed (interstitial space plus material porosity) was not mixed with the passing polluted gas stream (Fig. 3). The volume of this stagnant air was inversely correlated to the pore size of the packing material and the applied air flow. Therefore, the smallest volumes of stationary air were measured in the polyurethane foam packing and at the higher tested air volumetric loading rates. The greater air dispersion into the larger pores of polyurethane foam also resulted in a more effective mixing (lower n values) of the flowing air when compared to perlite.

As biodegradation kinetics was studied at bioreactor level, the resulting parameter values do not account for concentration gradients within the biofilm. Thus, the measured maximum biodegradation rate (r_m) encompasses both external (through the air boundary layer) and internal (through the biofilm) substrate diffusion. The observed dependency of r_m on the volumetric gas loading rate (Table 3), especially in the PEG packing after long runs, could be interpreted as a diffusion limitation through the air boundary layer. In this sense, a higher air flow enhanced the convective transport of the substrate from bulk gas turbulent phase to the biofilm surface, resulting in a higher r_m. The PEG biofilter had superior r_m values, but the biomass specific biodegradation rate was lower when compared to the PUC biofilter. This phenomenon could be attributed to internal transport rate-limiting phenomena due to the presumably smaller biofilm air contact area to biomass ratio. The elimination capacities obtained in the present study where lower than those reported earlier under

very similar operational conditions and a highly related fungal species (Woertz et al. 2002), which indicates that the selection of the inoculum has important consequences on biofilter performance. The half saturation constant (k_m) was less affected by the carrier materials and the operational conditions than r_m, indicating that the assayed conditions did not significantly influence the toluene degradation at a metabolic level. The k_m range for the biodegradation of toluene obtained in this study (71–113 mg m^{-3}) were rather similar to the value of 66 mg m^{-3} measured previously in batch liquid suspensions of harvested cells grown on toluene of the same strain (Prenafeta-Boldú et al. 2002). Such coincidence is remarkable considering the complex physiology and morphology of fungi from the *Herpotrichiellaceae* family, which grow as budding cells in liquid cultures and as filaments in solid media. On the other hand, these values are slightly lower than the k_m of 210 mg m^{-3} that has recently been determined for the biodegradation of toluene in an undefined fungal biofilter packed with coconut fibre (Dorado et al. 2008).

In summary, the characterisation of the air mixing by means of trace gases has been proposed as a precise quantitative method for the diagnostic of the packing condition. In such case, it is very important that the void chambers present at the packing inlet and outlet are conveniently included in the air dispersion modelling. Yet, in practical applications and under relatively short retention times, the air mixing regime in biofilters packed with synthetic materials can acceptably be assimilated to an ideal plug flow. Biofiltration is affected by the presence of stagnant air zones and compact biofilm formation. Diffusion limitation in such case can be reduced by increasing the volumetric loading rate and by increasing the biofilm contact area with support materials that favour the formation of loose biomass. Hence, for each packing, a compromise has to be found between an air flow that optimises the substrate mass transfer and an organic load rate that does not saturate the biological metabolic capacity of the bed.

Acknowledgements This work has partly been funded by grants BIO4 CT 972295 from the European Commission and CTM2006-07976 from the Spanish Ministry of Science and Education. We acknowledge Dr. Guillermo Baquerizo Araya for his critical reading of the manuscript.

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