Biofiltration of waste gases with the fungi Exophiala oligosperma and Paecilomyces variotii

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Abstract

Two biofilters fed toluene-polluted air were inoculated with new fungal isolates of either Exophiala oligosperma or Paecilomyces variotii, while a third bioreactor was inoculated with a defined consortium composed of both fungi and a co-culture of a Pseudomonas strain and a Bacillus strain. Elimination capacities of 77 g m^{-3} h^{-1} and 55 g m^{-3} h^{-1} were reached in the fungal biofilters (with removal efficiencies exceeding 99%) in the case of, respectively, E. oligosperma and Paecilomyces variotii when feeding air with a relative humidity (RH) of 85%. The inoculated fungal strains remained the single dominant populations throughout the experiment. Conversely, in the biofilter inoculated with the bacterial–fungal consortium, the bacteria were gradually overgrown by the fungi, reaching a maximum elimination capacity around 77 g m^{-3} h^{-1}. Determination of carbon dioxide concentrations both in batch assays and in biofiltration studies suggested the near complete mineralization of toluene. The non-linear toluene removal along the height of the biofilters resulted in local elimination capacities of up to 170 g m^{-3} h^{-1} and 94 g m^{-3} h^{-1} in the reactors inoculated, respectively, with E. oligosperma and P. variotii. Further studies with the most efficient strain, E. oligosperma, showed that the performance was highly dependent on the RH of the air and the pH of the nutrient solution. At a constant 85% RH, the maximum elimination capacity either dropped to 48.7 g m^{-3} h^{-1} or increased to 95.6 g m^{-3} h^{-1}, respectively, when modifying the pH of the nutrient solution from 5.9 to either 4.5 or 7.5. The optimal conditions were 100% RH and pH 7.5, which allowed a maximum elimination capacity of 164.4 g m^{-3} h^{-1} under steady-state conditions, with near-complete toluene degradation.

Introduction

Conventional waste gas treatment technologies are based on mass transfer processes in adsorption and absorption towers, or oxidation reactions in incinerators. Recently, bioprocesses became widely accepted as a cost-effective alternative to conventional treatment technologies (Kennes and Veiga *2001*). Biofilters packed with natural filter beds in which bacterial populations are dominant have been used for several decades, mainly at wastewater treatment plants and composting facilities, for the removal of low concentrations of odors. More recently, efforts have been made to extend the use of bioreactors to new sources, with the need to develop systems able to efficiently treat higher loads. Therefore, several new reactor designs have been developed and tested at either the laboratory- or industrial-scale. Some examples are the biotrickling filter, the membrane bioreactor, the rotating biological contactor, and systems based on combined oxidation and biodegradation processes (Kennes and Veiga *2001*).

Besides the search for new types of bioreactors, efforts are also being made to find high-performance biocatalysts. Inoculating fungi rather than bacteria in biofilters or simply favoring their growth seems to represent an interesting alternative to improve the

performance of biofilters treating air contaminated with hydrophobic compounds (Kennes and Veiga *2004*). Toluene is one of the most frequently encountered alkylbenzenes in waste gases. The biodegradation of toluene by bacteria was proven several decades ago; and a large number of papers have been published on bacterial biofilters. However, fungi in biofilters present the advantage of being more resistant than bacteria to extreme conditions, mainly at reduced moisture content and low pH (Kennes and Veiga *2001*; van Groenestijn and Hesselink *1993*). Moreover, it is hypothesized that the aerial hyphae of filamentous fungi could enhance the rate of transfer of pollutants from the gas phase to the biocatalyst. Nevertheless, clear evidence on the use of pollutants as substituted benzenes as the sole source of carbon and energy in fungi was only published recently and, so far, only very few fungal strains have been isolated on such pollutants (Kennes and Veiga *2004*).

In the present study, two new fungal strains were obtained and inoculated individually in biofilters for the removal of toluene from waste gases in order to evaluate the behavior and efficiency of biofilters in which fungi, rather than bacteria, are dominant. A defined bacterial–fungal culture was also used in a third biofilter, in order to compare its efficiency with the fungal systems. Performance data, mass balance calculations, and studies of biomass accumulation and distribution are reported and discussed. The stability of the inoculated biocatalysts was checked in all three biofiltration experiments. Biofilter performance was evaluated and optimized through modification of the relative humidity (RH) of the waste air and the pH of the nutrient solution.

Materials and methods

Inocula

The fungi Exophiala oligosperma (CBS-113408) and Paecilomyces variotii (CBS-113409) were isolated on toluene from a biofilter originally inoculated with a defined bacterial–fungal consortium in which a high elimination capacity of 120 g m^{-3} h^{-1} with 100% removal efficiency had been reached (Veiga and Kennes *2001*). Both the inoculated strains and new fungal populations were present in that biofilter after long-term operation. In the biofiltration experiments with bacteria, a Pseudomonas sp. and a Bacillus sp. were used (Veiga et al. *1999*).

Biofilter operation

All biofilters were made of glass, using either Viton or Teflon tubing for feeding the pollutant. The biofilters were packed with Perlite, with a size of 5±1 mm. The packed volume was 3.5 l and the empty bed residence time (EBRT) was 80 s. Operation took place at room temperature, which was maintained at 20°C during the day. Six sampling ports were located along the biofilter for the analysis of air samples and the removal of filter bed samples for biomass analysis. The biofilters were operated in a downflow mode as described by Mendoza et al. (*2004*). Polluted air was fed to the reactors by mixing a humidified air flow with a smaller air stream containing toluene. The flow rates of each stream were modified during the experiment in order to maintain a constant EBRT while varying the inlet toluene concentration, i.e. toluene load. A sterilized nutrient solution was added once a week to the biofilters and drained off after a few minutes, as described by Prado et al. (*2002*). Oxytetracycline was added to the mineral medium during the first month of operation of the biofilters inoculated with pure fungal strains. It was not used in the biofilter inoculated with the defined bacterial–fungal consortium. The RH of the waste air was adjusted by modifying the flow rates of both a dry and a humidified air stream. Biofilter performance was estimated by

calculating the elimination capacity and removal efficiency at different toluene loads, according to equations defined in the literature (Kennes and Veiga *2001*).

Analytical methods

Inlet and outlet toluene concentrations were measured daily on a HP-5890 series II gas chromatograph (GC) equipped with a flame ionization detector and a 30-m HP5 column (Veiga and Kennes *2001*). Carbon dioxide was measured either with a CO_2 gas sensor or by gas chromatography on a HP-6890 GC equipped with a thermal conductivity detector and a Porapak Q-column W80/100. The protein concentrations were measured using Lowry's method (Lowry et al. *1951*). Bovine serum albumin was used as a standard. Samples of colonized packing material were removed from the biofilters and treated for protein analysis as described by Veiga and Kennes (*2001*). Volatile suspended solids (VSS) were determined as described by Mendoza et al. (*2004*). A Crison model 507 pH meter, connected to an Ingold electrode was used for measuring the pH.

Results and discussion

Inoculation and start-up of fungal biofilters

E. oligosperma is a new species belonging to the group of so-called black yeasts. The recently isolated strains of E. oligosperma and Paecilomyces variotii were shown to grow on toluene as sole source of carbon and energy (Estévez et al. *2004*). Both strains grow optimally in a slightly acidic medium at 30°C. No previous information has been published on the growth of E. oligosperma nor any Paecilomyces strain on toluene. In shake-flasks, P. variotii on toluene grows mainly in the form of pellets. A similar phenomenon has also been reported for P. japonica grown on sucrose (Sinha et al. *2001*). The formation of pellets was not observed in E. oligosperma grown in liquid phase; and the strain produced long filaments. This characteristic allowed a more homogenous inoculation of the packing material in case of the black yeast than with the Paecilomyces strain. The formation of pellets resulted in a relatively uneven initial colonization of the packing material in the biofilter inoculated with P. variotii.

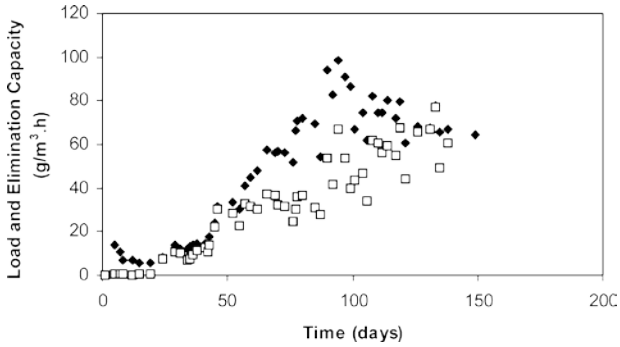
In both reactors, fungal growth started much faster in the lower part of the filter beds. After the second week of operation, heavy colonization was observed in that zone, while little biomass was present in the upper region of the packing material. This phenomenon resulted probably from the regular supply of the nutrient solution displacing part of the biomass from the top to the bottom of the reactors, mainly during liquid drainage. The addition of an antibiotic to the solution used for inoculation and during nutrient supply avoided bacterial invasion of the biofilters. The presence of the inoculated strains as single dominant populations in the filter beds was checked and confirmed 1 month after inoculation, using plating techniques and observations under the microscope, thus confirming that biodegradation was due to fungal activity only. The formation of filamentous structures was clear with both strains growing in the filter bed, according to observations with a scanning electron microscope (SEM). No pellets were visible after a few weeks in the reactor inoculated with the Paecilomyces strain.

Long-term operation of the fungal biofilters

In a first set of experiments, the RH of the waste air was maintained at 85% and the pH of the nutrient solution at pH 5.9. Two biofilters operated under similar conditions were inoculated, each with one of the pure fungal cultures; and the toluene load was increased stepwise from about 10 g m^{-3} h^{-1} to more than 100 g m^{-3} h^{-1}. The biofilter inoculated with E. oligosperma appeared to perform better than the other one. While

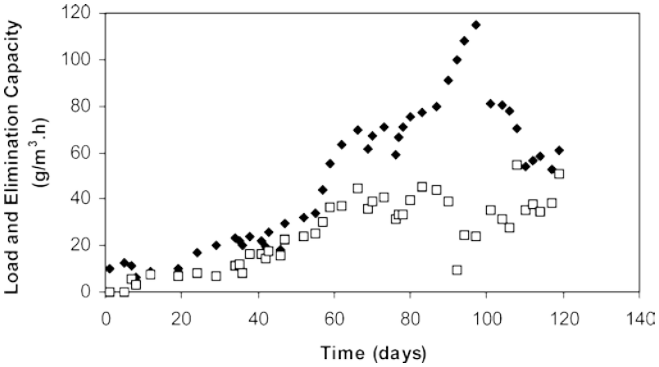
removal efficiencies of more than 70% could be maintained in the case of E. oligosperma, even at toluene loads around 100 g m^{-3} h^{-1} (Fig. 1), the removal efficiency dropped in the case of P. variotii from more than 95% at a load of 40 g m^{-3} h^{-1} to about 40% at a load of 100 g m^{-3} h^{-1} (Fig. 2). It is worth observing that, at loads of 100 g m^{-3} h^{-1} or higher, the biofilter performance dropped with P. variotii, resulting in a transient decrease in the elimination capacity when increasing the load, while in case of the black yeast the removal efficiency increased continuously when increasing the toluene load (Fig. 1). The presence of the inoculated fungi as single dominant organisms was confirmed approximately once a month by serially diluting filter bed samples and checking the organisms present at the three highest positive dilutions. Samples were also regularly observed under the microscope in order to confirm the absence of bacteria as dominant populations. It is worth mentioning that in the few other studies undertaken with Exophiala spp, a mixed bacterial–fungal community was present in the reactors (Cox et al. *1997*; Woertz et al. *2001*). During the fourth month of operation, the toluene concentration in the feed was decreased in both reactors, in order to evaluate the maximum load allowing complete toluene removal (>99%). Elimination capacities of 55 g m^{-3} h^{-1} and 77 g m^{-3} h^{-1} were reached with P. variotii and E. oligosperma, respectively. Similar results were obtained, under the same conditions, in the studies on the influence of RH and pH performed later and described below.

Fig. 1



Toluene load (▪) and corresponding elimination capacity (□) versus time in the biofilter inoculated with the black yeast E. oligosperma (85% RH, pH 5.9)

Fig. 2



Toluene load (▪) and corresponding elimination capacity (□) versus time in the biofilter inoculated with the fungus P. variotii (85% RH, pH 5.9)

Mineralization of toluene

Different methods were used to confirm that the fungi mineralized toluene. First, batch assays undertaken simultaneously to the biofiltration studies indicated that at least two-thirds of the toluene added to the vials was recovered as carbon dioxide. Similar values have been reported for other fungi grown on toluene (Prenafeta-Boldu et al. *2001*). Carbon dioxide production was also measured approximately twice a week throughout the biofiltration experiments. Neglecting biomass formation, the theoretical CO_2 production in the biofilters corresponds to 7× (inlet minus outlet toluene concentration), taking into account that (by volume) the mineralization of 1 ppm toluene yields 7 ppm CO_2, according to the following stoichiometric reaction:

$$C_7H_8+9O_2\rightarrow 7CO_2+4H_2O$$

Nevertheless, when biomass growth is taken into account, 6.35 moles CO_2 are generated for 1 mol toluene degraded, according to the following reaction:

$$1.55C_7H_8 + 12.0O2 + 0.2NH_4Cl\rightarrow 1CH_{1:8}N_{0.2}O_{0.5} + 9.85CO_2 + 5.6H_2O\ 0.2HCl$$

Here, $CH_{1.8}N_{0.2}O_{0.5}$ represents a typical average composition for biomass (Atkinson and Mavituna *1991*). Carbon dioxide recoveries reported in the literature normally neglect biomass growth.

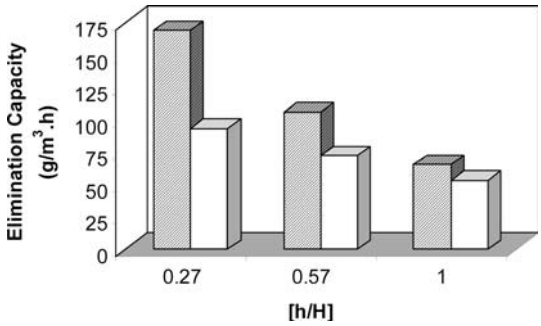
In order to determine the actual CO_2 production in the biofilters, the difference between the inlet and outlet CO_2 concentrations were calculated. The CO_2 recovery, neglecting biomass growth, was typically 70–80%, although it occasionally exceeded 100% in both reactors, above all near the end of the experiment. On average, somewhat lower CO_2 recoveries were found during the start-up phase, compared with long-term operation, but the difference was not really significant. Carbon dioxide production exceeding 100% is not unusual in biofilters when endogenous respiration becomes a relevant process. Cox et al. (*1997*) reported CO_2 recoveries ranging over 40–100% over a 300-day biofiltration period for E. jeanselmei growing on styrene, while Woertz et al. (*2001*) reported recoveries around 57% (4 moles CO_2 from 1 mol substrate) for E. lecanii-corni grown on toluene in a biofilter. Between 2.0 moles and 5.7 moles CO_2 from 1 mol toluene degraded were found in a biofilter inoculated with a Cladophialophora sp. (Woertz et al. *2002*). The absence of any soluble intermediate product, according to HPLC and GC analysis, also suggests that complete mineralization can reasonably be admitted in our systems.

Biofilm characterization

Uneven biomass distribution is not unusual during long-term biofilter operation (Kennes and Veiga *2002*). Therefore, biomass concentrations were checked in both reactors at the end of the experiment at two different ports located at (h/H)=0.12 and (h/H)=0.72, considering that the feed port and the outlet port correspond, respectively, to (h/H)=0 and (h/H)=1. Contrary to what might have been expected, both VSS and protein concentrations were, on average, 20% higher near the outlet than near the inlet of the reactors. Usually, the opposite is observed in this type of system (Veiga and Kennes *2001;* Mendoza et al. *2003*). This trend was also confirmed by SEM observations, indicating that the thickest biolayers on average were found closest to the outlet of the biofilters. For E. oligosperma the biofilm depth varied between 101 μm and 613 μm after 4 months of operation, while it varied between 70 μm and 379 μm for P. variotii, with the lowest values corresponding in both cases to samples closest to the inlet of the biofilters.

Some authors have observed that a large fraction of the biomass may be inactive in biofilters and does not degrade the pollutant fed to the reactor (Arcangeli and Arvin *1992*; Juteau et al. *1999*). The inactive biomass may reach as much as 50% of the total biomass. In the present case, it is highly probable that, although the total VSS and protein concentrations were higher near the outlet of the biofilter, that fraction of the biomass was less active or was involved to a lower extent in toluene removal. Data published in the literature indicate that the active biofilm layer, into which oxygen and VOCs can diffuse, does not usually exceed 100–200 μm (Kennes and Veiga *2001*). Since the substrates do not reach deeper zones, part of the deep biofilm at the bottom of the filter bed must have been inactive. Also, the large amount of biomass accumulated in the lower zones of the reactor promotes channeling phenomena, leading to a reduced overall removal efficiency. Local elimination capacities were calculated in both the upper and lower zones of the biofilters. These calculations indicated that local elimination capacities as high as 170 g m^{-3} h^{-1} and 94 g m^{-3} h^{-1}, respectively, were found in the upper zone of the biofilters near the end of the experiment for E. oligosperma and P. variotii, while lower values were found near the outlet of the reactor (Fig. 3). Some other authors also observed higher rates of pollutant removal near the inlet of biofilters, both with bacteria (Lu et al. *2001*) and with fungi (Woertz et al. *2001*). The larger accumulation of biomass at the bottom of the system, observed in the present study, must be due to faster and heavier biomass growth and accumulation in that zone, above all during the start-up phase and the first month of operation, as described in the previous section.

Fig. 3



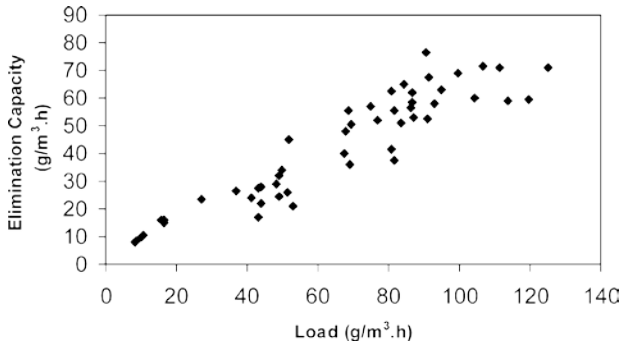
Local elimination capacities in different zones of the biofilters around the end of the experiments, after 4 months operation, for E. oligosperma (left column) and Paecilomyces variotii (right column), showing that toluene removal was more significant near the inlet (h/H=0) than near the outlet (h/H=1) of the reactor

Bacterial–fungal biofilter

In a parallel experiment, a biofilter was inoculated with a defined mixed culture containing both fungi (E. oligosperma, P. variotii) and two bacterial cultures (Pseudomonas sp., Bacillus sp.) known to actively grow on alkylbenzenes under acid conditions, such as those prevailing in the biofilter (Veiga et al. *1999*). The biofilter was run for a period of 4 months and was operated in the same way as the fungal biofilters. The load was increased from an initial value of 10 g m^{-3} h^{-1} to a maximum value of 125 g m^{-3} h^{-1}. Basically identical results were obtained as with the pure culture of E. oligosperma, reaching a maximum elimination capacity of 77 g m^{-3} h^{-1} (Fig. 4a). Serial dilutions done with filter bed samples taken near the inlet zone, middle zone, and outlet zone of the biofilter at the end of the experiment indicated that only E. oligosperma and P. variotii were present in samples from the different zones at the two highest positive

dilutions. Both organisms were detected at similar concentrations, but hardly any bacteria were found. Overgrowth of bacterial populations during the early stages of the study must have been due to the low pH, around pH 3.0–3.5, of the filter bed. Although both inoculated bacterial strains are known to degrade toluene under acid conditions (Veiga et al. *1999*), their activity is notably lower than that of the fungal strains below pH 3.5. The amount of carbon dioxide produced was similar to that in the previous experiments.

Fig. 4



Toluene elimination capacity versus load in the biofilter inoculated with the bacterial–fungal consortium

Influence of RH and pH on biofiltration with E. oligosperma

Since better results were obtained with E. oligosperma than with P. variotii, further studies were undertaken with the black yeast to evaluate the effect of RH and pH on biofilter performance. The same experimental procedure was followed as during the previous experiments. The load was continuously increased until maximum performance was reached. In a first set of experiments, the RH was 85%, as in the previous studies, and the pH of the nutritive solution was adjusted to either pH 4.5, 5.9, or 7.5. As shown in Fig. 5, the highest pH of 7.5 allowed the highest elimination capacity, namely 95.6 g m^{-3} h^{-1}. With the nutrient solution adjusted to pH 4.5 or 5.9, the pH dropped to values around pH 2.5–3.5 in the aqueous medium and the drain water of the biofilters. Conversely, with a nutrient solution originally adjusted to pH 7.5, the pH of the reactor's water phase was pH 5.9±0.5, which is known to be near optimal for the black yeast (unpublished data). Since the best results were obtained at pH 7.5, the effect of increasing the RH was evaluated at this pH. The RH was increased from 85% to 100%, which produced a high maximum elimination capacity of 164.4 g m^{-3} h^{-1}, with near complete toluene removal. Elimination capacities around 60–125 g m^{-3} h^{-1} have been reported by different authors using fungal biofilters (Table 1). Although fungi are more active than bacteria at low pH and reduced water content, the present data clearly show that the reactor performance drops under such conditions and that further studies aimed at optimizing operation parameters will allow us to improve and optimize the performance. Although some authors have occasionally reported elimination capacities exceeding 100 g m^{-3} h^{-1} in bacterial biofilters (Jorio et al. *1998*; Zilli et al. *2000*), such results are the exception rather than the rule, compared with the very high number of papers published on bacterial biofilters.

Table 1

Maximum elimination capacities (EC) reached with fungal biofilters under stable conditions

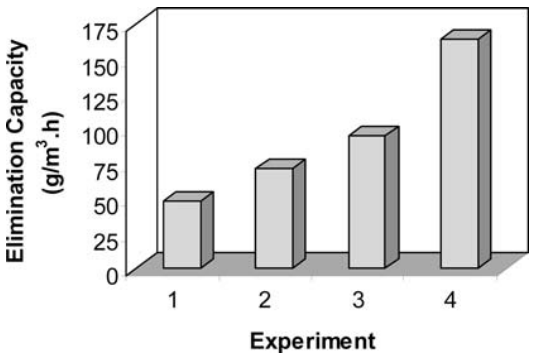
Inoculum	**Dominant strain**	**Pollutant**	**RH (%)**	**Maximum EC (g $m^{-3}.h^{-11}$)**	**Reference**
Mixed culture	E. jeanselmei	Styrene	90–95	62(91)[a]	Cox et al. (*1997*)
Mixed culture: primarily Pseudomonas putida	E. lecanii-corni	Toluene	–	≈80–90(270)[b]	Woertz et al. (*2001*)
E. oligosperma	E. oligosperma	Toluene	85	77–96	Present study
			100	164	
Scedosporium apiospermum	S. apiospermum	Toluene	>95	≈100 (220–361)[b]	García-Peña et al. (*2001*)
Cladophialophora sp.	Cladophialophora sp.	Toluene	–	125[c]	Woertz et al. (*2002*)
Paecilomyces variotii	P. variotii	Toluene	85	55	Present study

[a]Air enriched with 40% O_2

[b]Short-term experiment or unstable conditions

[c]In the presence of mites

Fig. 5



Maximum toluene elimination capacities under different conditions of RH and pH: (1) 85 % RH, pH 4.5, (2) 85 % RH, pH 5.9, (3) 85 % RH, pH 7.5, (4) 100 % RH, pH 7.5

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