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## Dynamic bioreactor operation: effects of packing material and mite predation on toluene removal from off-gas

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**Abstract** Recent studies have focused on using vapor-phase bioreactors for the treatment of volatile organic compounds from contaminated air streams. Although high removal capacities have been achieved in many studies, long-term operation is often unstable at high pollutant loadings due to biomass accumulation and drying of the packing medium. In this study, three bench-scale bioreactors were operated to determine the effect of packing material and fungal predation on toluene removal efficiency and pressure drop. Toluene elimination capacities (mass toluene removed per unit packing per unit time) above  $100 \text{ g m}^{-3} \text{ h}^{-1}$  were obtained in the fungal bioreactors packed with light-weight, artificial medium, and submersion of the packing in mineral medium once per week was found to provide sufficient moisture and nutrients to the biofilm. The use of mites as fungal predators improved performance by increasing the overall mineralization of toluene to  $\text{CO}_2$ , and by dislodging biomass along the bioreactor.

### Introduction

Much research is currently being carried out in the area of biofiltration of hazardous air pollutants. The motivation for this research is the economic advantage that biological treatment may provide compared to physical-chemical treatment systems, as well as the fact that biological treatment is a final treatment step, reducing pollutants to compounds such as  $\text{CO}_2$  and  $\text{H}_2\text{O}$  (Leson and Winer 1991; Ottengraf and Diks 1991; van Groenestijn

and Hesselink 1993; van Lith et al. 1997). Although many researchers have demonstrated that biofiltration systems can achieve high pollutant removal capacities and degrade a variety of pollutants, several problems with biofiltration systems continue to hinder their wide-scale use. Two common problems are their inability to sustain optimal moisture conditions and a tendency to clog with excess biomass during extended use (Soriat et al. 1995; Weber and Hartmans, 1996; van Lith et al. 1997; Morales et al. 1998; Cox and Deshusses, 1999a). When the moisture content in the packing medium is insufficient, a viable biofilm is unable to form. Excessive moisture, on the other hand, will reduce mass transfer and clog pore space. Both conditions will reduce the potential elimination capacity of the biofilter (van Lith et al. 1997). Humidification of the off-gas stream and the use of sprayer systems are commonly employed to maintain the proper moisture content (Groenestijn and Hesselink 1993). These methods, however, are not always sufficient or feasible with certain packing materials (Ortiz et al. 1998; García et al. 2000). Clogging, like moisture control, is also problematic in biofiltration systems subjected to high pollutant loadings, leading to increased pressure drop and channeling of the gas flow through the filter bed. To avoid clogging, many different approaches to biofilter operation have been proposed, including chemical rinsing, backwashing, nutrient limitation, and directionally switching operation (Cox and Deshusses 1999b; Schönduve et al. 1996; Smith et al. 1996; Wübker and Friedrich 1996; Song and Kinney 2000). The use of various packing media and the predation of bacterial biofilms by protozoa also have been suggested for controlling clogging problems in highly loaded biofilters (Ortiz et al. 1998; Yamashita and Kitagawa 1998; Cox and Deshusses 1999a).

To overcome some of the problems associated with biofiltration systems, the use of fungal-based systems has been investigated. Fungal systems have been shown to maintain high degradation capacities of volatile organic contaminants even under adverse operating conditions, such as low water activity and acidic pH (Cox et al. 1993;

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Weber and Hartmans 1996; Woertz et al. 2001). Although fungal systems are resilient, they, too, are prone to clogging problems over time (Woertz et al. 2001). Preliminary studies have suggested that mites may be used to control fungal biomass (van Groenestijn et al. 2001; Kraakman et al. 2000); however, little work has been done to characterize the impact of mite grazing on column performance.

This report focuses on comparing different modes of operation in order to determine the strategy that enhances long-term performance of toluene-treating, fungal biofiltration systems. Only a small fraction of the biofiltration studies to date have focused on using fungi for the removal of volatile organic compounds from waste gas streams, and little work has been carried out to optimize these systems. Therefore, in this study, packing material type and predation by mites were investigated for their effect on toluene removal capacities and column pressure drop in fungal bioreactors.

## Materials and methods

### Cultures and medium

Perlite packing medium covered with a biofilm consisting primarily of *Cladophialophora* sp. was obtained from a toluene-degrading bioreactor at the TNO Department of Environmental Biotechnology (Apeldoorn, the Netherlands). The *Cladophialophora* sp. was aseptically removed from the packing and resuspended in 2 l of sterile mineral medium. The culture was maintained by bubbling toluene-laden air through mineral medium (Cox et al. 1993). The pH of the medium was adjusted to 4 using 85% H<sub>2</sub>PO<sub>4</sub> (Acros, Geel, Belgium).

Mites were obtained from the leachate of a second toluene-degrading bioreactor (TNO, Apeldoorn, the Netherlands), which had been inoculated with *Tyrophagus putrescentiae* (Koppert Biological Supplies, Berkel en Rodenrijs, the Netherlands).

### Experimental set-up and reactor inoculation

Three reactors were operated in parallel in a 25 °C temperature-controlled room. Each reactor was constructed from glass tubing (7 cm ID) and had a total volume of 3 l. Each reactor was filled with 2 l of packing material and supplied with a toluene-contaminated air stream at superficial gas velocities between 150 and 200 m<sup>3</sup>gas m<sup>-3</sup>packing h<sup>-1</sup> in downflow operation. The gas velocities were varied to produce dynamic loading conditions that mimic those encountered in field applications such as paint-spray booths.

The pollutant stream was produced by passing a portion of the total gas stream through a gas-washing bottle filled with liquid toluene (Lab-Scan Analytical Sciences, HPLC grade, Dublin, Ireland) and then mixing this stream with the remainder of the total air stream. The air stream was prehumidified by bubbling it through a series of water baths prior to toluene addition.

The operating conditions for each of the three reactors are given in Table 1. Two reactors were packed with perlite (sieved fraction 3.45–4.75 mm average diameter, Pull BV, Rhenen, the Netherlands) and one reactor was packed with a hydrophobic, polyurethane foam (1.4 cm average length of cubic side, Fa. Linde AG, Munich, Germany). The reactors were wetted by submerging the entire packed bed in nutrient medium for 15 min. The wetting frequency for all reactors was between 7 and 10 days. Each reactor was inoculated with a 1-l suspension of *Cladophialophora* sp. in mineral medium on day 7 of operation. The inoculum was

**Table 1** Operating parameters of bioreactors

Reactor	Packing	Mite addition
P1	Perlite	No
P2	Perlite	Yes
F1	Polyurethane foam	No

poured over the top of the reactor and allowed to drain through the bottom. The inoculum was then collected and poured over the top of the reactor a second time. The inoculum was left in each reactor for 1 h before it was drained out of the reactor again. Mites were added to one of the perlite-packed reactors on day 32 of operation by pouring 500 ml of leachate from a mite-infested bioreactor through the reactor.

The reactor packed with perlite and not amended with mites (P1) was considered the control for all experiments. The effect of mites was determined by comparing reactors P1 and P2. Reactors P1 and F1 were compared to determine the effect of packing medium.

### Analytical procedures

The off-gas from each reactor was sampled automatically every 2 h, and the toluene concentration was analyzed using a total-carbon analyzer (Thermo Environmental Instruments, model 51, Franklin, Mass.). A Sixnet 60-PIB/IO data acquisition system (Digitronics Sixnet, Clifton Park, Mass.) was used to collect the data. The analyzer was calibrated with a calibration gas containing 300 ppm propane in air (Aga gas BV, Amsterdam, The Netherlands).

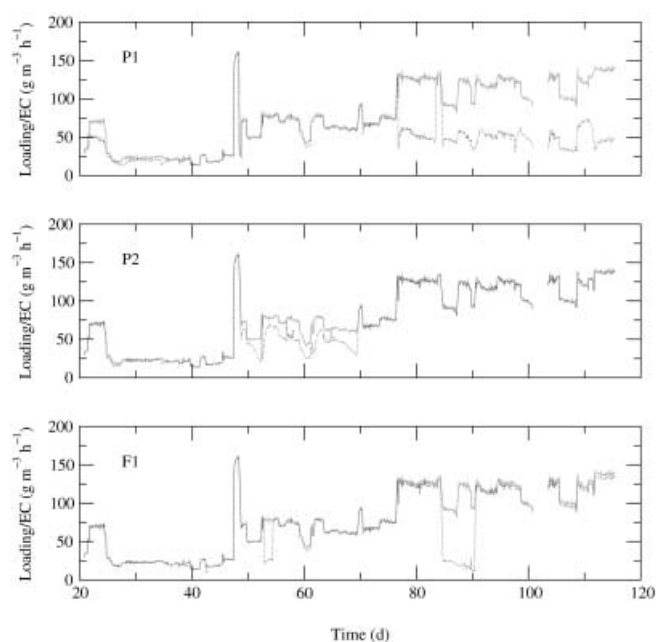
Samples to measure CO<sub>2</sub> production in the reactors were collected in 50-ml gas sampling tubes by connecting tubing directly from the inlet and outlet of the reactors to the sampling tubes. The gas was passed through the tubes for 2 min, and the tubes were closed with stopcocks on either end. A 100-μl sample of the gas was then withdrawn and injected onto a Varion Model 3700 gas chromatograph (Varion Benelux BV, Amsterdam, The Netherlands) equipped with Chrompack Carboplot P7 column (Chrompack/Varian, The Netherlands), a flame ionization detector (FID), and a methanizer (self-made, copper tube filled with 10% nickel nitrate on Chromosorb G AW 100/120, heated at 380 °C). The oven, detector, and injector were maintained at 80 °C (isothermal), 275 °C, and 80 °C, respectively. Data were collected using a Chrompack Maestro acquisition system (Varian Chrompack BV, The Netherlands). The analyzer was calibrated using gas standards made by adding known volumes of CO<sub>2</sub> gas to a 1-l Duran bottle that had been flushed with nitrogen gas. All samples and standards were prepared at 25 °C.

The pressure drop across the entire reactor length was measured by connecting a U-tube manometer to the inlet and outlet of the reactor. The protein content of the drain-water was determined using Coomassie assay reagent (Pierce, Rockford, Ill.) after boiling the samples in 1 N NaOH for 10 min (Cox et al. 1996). The protein content was measured in triplicate for each drain-water sample. An eight-point calibration curve was prepared prior to each test using bovine serum albumin standards.

## Results

### Packing material

The differences in toluene removal in the bioreactor packed with perlite (P1) and the bioreactor packed with polyurethane foam (F1) are compared in Fig. 1. Both reactors performed well at toluene loadings below



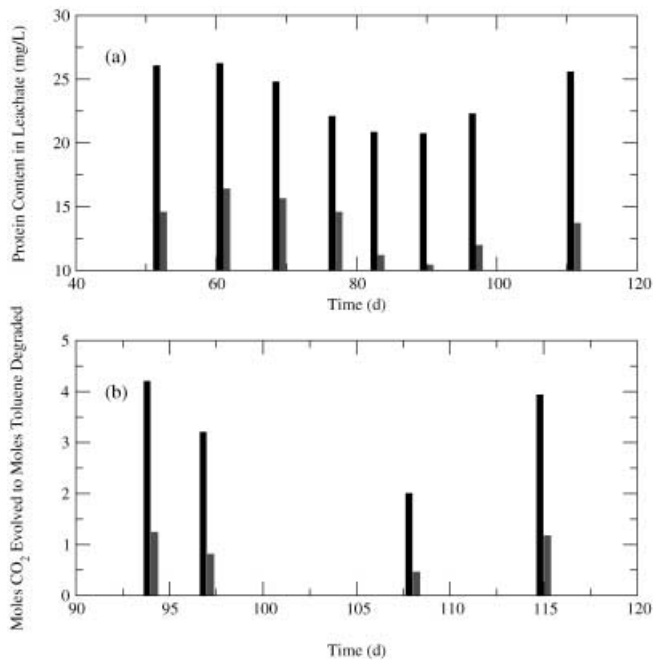
**Fig. 1** Toluene loading (solid line) and toluene elimination capacity (hatched line) for each of the for reactors: P1, P2, and F1. The air stream was shut off between days 101 and 104

$75 \text{ g m}^{-3} \text{ h}^{-1}$ ; however, the performance of P1 declined after the toluene loading increased above  $120 \text{ g m}^{-3} \text{ h}^{-1}$ . The poor performance of F1 on days 85–91 was a result of the packing medium floating to the top of the reactor during wetting on day 83, leaving much of the packing unsubmerged. This resulted in insufficient wetting of the packing and a drop in performance. Once the system was wetted again on day 90, the performance increased and a removal efficiency >99% was observed.

The pressure drop in P1 fluctuated between 0.5 and 1.5 cm  $\text{H}_2\text{O}$  over the course of the experiment. The pressure drop in F1, on the other hand, remained consistently below 0.5 cm  $\text{H}_2\text{O}$  throughout the entire experiment.

### Fungal predation

The use of mites grazing on the fungi as a biomass removal mechanism was evaluated in reactor P2. Figure 1 compares the performance of this reactor to a reactor that was operated in an identical manner but contained no mites (P1). The performance of both reactors was consistent until day 76, although bioreactor P2 had slightly poorer removal during days 50–70. This was presumed to be due to excessive drying, since the wetting frequency was decreased to once every 10 days during this period. After again wetting the packing on a weekly basis, the performance of bioreactor P2 improved. The performance of P1, however, was much poorer after day 76, when the average toluene loading to the reactors was increased.



**Fig. 2a, b** Effect of mites on bioreactor performance. **a** Protein content of the reactor leachate after rinsing in P2 (black bars) and P1 (gray bars); **b**  $\text{CO}_2$  production relative to toluene degradation in reactors P2 (black bars) and F1 (gray bars)

Since the mites were mobile and sensitive to changes in the packing medium, biomass samples were not collected from the column. Instead, the leachate was analyzed for protein content and the outlet gas was analyzed for  $\text{CO}_2$  concentrations as surrogate measures of biomass accumulation in the reactors. Since loading of all the reactors was the same, if the protein content in the leachate and the  $\text{CO}_2$  concentrations in the off-gas were higher in P1 than in the control, less biomass would have been accumulated.

The protein content of the drain-water from reactors P1 and P2 was measured from days 51 to 112. It was found that the protein content in the drain-water from P2 was much higher than in the drain water from P1 (Fig. 2a), indicating that improved washout of the biomass occurred during wetting.

$\text{CO}_2$  production was measured in all reactors to compare the mineralization of toluene between the systems after toluene loading was increased above  $120 \text{ g m}^{-3} \text{ h}^{-1}$  (days 94–115). The molar ratio of  $\text{CO}_2$  evolved to toluene degraded in reactor P2 was approximately four times that in reactor F1 over the 4-week sampling period (Fig. 2b). In reactor P2, 2–4 mol  $\text{CO}_2$  were evolved for every mol toluene degraded. In reactor, F1, however, only 0.5–1 mol  $\text{CO}_2$  were evolved for every mol toluene degraded even though both reactors had almost identical removal capacities during this period of operation. Only reactors P2 and F1 were compared, since they had similar elimination capacities during the time frame when the  $\text{CO}_2$  measurements were collected and were representative of efficient operation. Removal in reactor P1 was

poor (<50%) after the toluene loading was increased. The CO<sub>2</sub> production in this reactor was unstable, with ratios of moles CO<sub>2</sub> produced to moles toluene degraded ranging between 0.1 and 5.7 (data not shown). The higher ratios sometimes observed in this reactor were most likely due to endogenous respiration caused by the decay of the existing biomass.

## Discussion

### Effect of packing material

The choice of packing material greatly influenced reactor performance. Although perlite and polyurethane foam are both synthetic media, the structure of the two materials differs greatly. Polyurethane foam has a very open network of pores and, therefore, a large surface area. The fungus was found to grow in these internal pores rather than in the pores between the foam cubes. With the perlite, however, the fungus had a tendency to grow in the pores between the packing granules. This would account for the difference in pressure drop between the two systems, with a lower pressure drop being observed across the foam packing material. Yamashita and Kitagawa (1998) also reported that the pressure drop across a toluene-treating column packed with urethane foam was minimized. In our system, the higher biofilm surface area on the polyurethane foam may have allowed for more efficient mass transfer of the pollutant, thereby improving toluene uptake and degradation. This resulted in higher elimination capacities in the polyurethane foam reactor (F1) than in the perlite control (P1) when toluene loading to the systems was increased. Since the performance of the foam reactor was better than that of the control, it is unlikely that there were gas distribution problems in F1 even though the ratio of column diameter to packing medium size was only 5:1.

### Effect of mite predation

A large difference in performance was observed between the two reactors packed with perlite (P1 and P2). After the mites were added to the filter bed of P2 on day 34, the pressure drop declined from 3.9 to 1.3 cm H<sub>2</sub>O after 5 days of operation. Also, when toluene loading of the reactors was increased on day 76, P2 had nearly complete toluene removal, whereas a decline in performance was observed in P1. Two mechanisms were found to be responsible for the superior performance observed in the reactor containing the mites (P2). First, the mites in reactor P2 fed on the fungus, increasing the overall mineralization of toluene to CO<sub>2</sub>. This is evident from the CO<sub>2</sub> evolution ratios obtained between days 94 and 115 of operation (Fig. 2b). Although reactors P2 and F1 were degrading the same quantity of toluene, reactor P2 had a much higher CO<sub>2</sub> mineralization rate. Im-



**Fig. 3** Scanning electron micrograph of *Tyrophagus putrescentiae* grazing on *Cladophialophora* sp. attached to perlite packing. *A* The mite is transporting pieces of the packing and fungus. *B* The burrowing capabilities of the mites

proved mineralization of toluene to CO<sub>2</sub> reduced the biomass accumulation rate in the system and led to the steady pressure drop observed across the system after the mites were added ( $\Delta P < 0.5$  cm H<sub>2</sub>O). Cox and Deshusses (1999a) observed a similar increase in CO<sub>2</sub> production when protozoa were used to control the growth of bacterial cultures in biotrickling filters. The overall mineralization of toluene to CO<sub>2</sub> in both reactors, however, was lower than anticipated. The low conversion rate may have been due to the accumulation of reserve materials (Griffin 1994), particularly in reactor F1, since there had been a period of poor performance prior to when CO<sub>2</sub> sampling began.

The mites, when grazing on the fungus, also burrowed into the fungal mycelium, causing some of the biofilm to become dislodged from the packing material (Fig. 3). Cox and Deshusses (1999a) also speculated that grazing by protozoa caused detachment of a bacterial biofilm from the packing material. During wetting, this dislodged material was washed out of the system, along with some of the mites, decreasing the overall biomass retained in the system and increasing the protein content of the leachate (see Fig. 2a).

The results of this study show that prolonged operation of highly loaded bioreactors treating hazardous air pollutants is possible when proper design parameters are chosen. The use of a light-weight, open-networked packing allows for minimal pressure drop at high superficial gas velocities, and submersion of this material in nutrient medium allows for even distribution of moisture and nutrients to the hydrophobic packing. Addition of mites, which prey on the fungal biomass, may promote more complete mineralization of the substrate as well as improved removal of excess biomass.

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