Biomass Control in Waste Air Biotrickling Filters by Protozoan Predation

Huub H. J. Cox, Marc A. Deshusses

Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521; telephone: 909-787-2477; fax: 909-787-3188; email: mdeshuss@engr.ucr.edu

Abstract: Two protozoan species as well as an uncharacterized protozoan consortium were added to a toluene-degrading biotrickling filter to investigate protozoan predation as a means of biomass control. Wet biomass formation in 23.6-L reactors over a 77-day period was reduced from 13.875 kg in a control biotrickling filter to 11.795 kg in a biotrickling filter enriched with protozoa. The average toluene vapor elimination capacity at 1 g/m^3 toluene and 64 m^3/(m^3 · h) was 31.1 g/(m^3 · h) in the control and 32.2 g/(m^3 · h) in the biotrickling filter enriched with protozoa. At higher toluene inlet concentrations, toluene degradation rates increased and were slightly higher in the biotrickling filter enriched with protozoa. The lower rate of biomass accumulation after the addition of protozoa was due to an increase of carbon mineralization (68% as compared to 61% in the control). Apparent biomass yield coefficients in the control and enriched trickling filter were 0.72 and 0.59 g dry biomass/g toluene, respectively. The results show that protozoan predation may be a useful tool to control biomass in biotrickling filters, however, further stimulation of predation of the biomass immobilized in the reactor is required to ensure long-term stability of biotrickling filters. © 1999 John Wiley & Sons, Inc. *Biotechnol Bioeng* **62**: 216–224, 1999.
Keywords: waste air treatment; biotrickling filter; biomass control; protozoa

INTRODUCTION

Biotrickling filters for waste air treatment have been extensively studied in the laboratory and efficient removal of a wide variety of organic and inorganic compounds from polluted air streams has been reported. However, deployment of biotrickling filters in an industrial environment is still limited (Hugler et al., 1997; Loy et al., 1997; Webster and Deshusses, 1997), although waste air treatment in biotrickling filters is a relatively cheap alternative as compared to physical and chemical techniques (Van Groenestijn and Hesselink, 1993; Zuber et al., 1997).

A major obstacle to implementation of biotrickling filters in industry appears to be the instability of these reactors over the long term. Due to rapid biomass accumulation in the packed bed, the pressure drop, i.e., the operational costs will increase. At the same time, pollutant removal will decline mostly because of the decrease in interfacial area for mass transfer (Alonso et al., 1997; Cox et al., 1998; Deshusses et al., 1998). If no remedial action is taken, the reactor will ultimately clog. In the case of a toluene-degrading biotrickling filter with an elimination capacity of 50 g toluene/(m^3 filter bed · h) with 50% conversion of carbon into biomass and the production of a biofilm with 0.1 kg/L dry matter, 45% carbon on dry weight basis, and a density of 1 kg/L, it would take 82 days of continuous operation to produce a biomass volume as large as the reactor volume. Indeed, rapid clogging of biotrickling filters has been observed (Diks, 1992; Smith et al., 1996; Sorial et al., 1995; Weber and Hartmans, 1996). Interestingly, the minimum amount of biomass in the reactor to obtain maximal pollutant-degrading activity is only 16 L/m^3 reactor, if one assumes a specific surface area of 200 m^2/m^3 (Van Groenestijn and Hesselink, 1993) and an effective biofilm thickness of about 80 μm as estimated in biofilters for waste air treatment (Cox et al., 1997; Shareefdeen et al., 1993). This implies that prolonged biotrickling filter operation primarily results in formation of biomass not actively involved in pollutant degradation. This was confirmed by Zuber (1995), who demonstrated that no improvement of pollutant removal occurred as biomass accumulated over time in methylene chloride-degrading biotrickling filters.

Several options to either prevent excessive biomass production or to remove excess of biomass in biotrickling filters and biofilters have been investigated, including nutrient limitation (Hekmat et al., 1997; Morgenroth et al., 1996; Schönduve et al., 1996; Weber and Hartmans, 1996), addition of growth-limiting concentrations of NaCl (Schönduve et al., 1996; Van Lith et al., 1994), discontinuous operation with starvation periods (Farmer et al., 1995), biomass removal by chemical washing (Weber and Hartmans, 1996) or by backwashing (Smith et al., 1996; Sorial et al., 1995) or mechanical removal by stirring of the trickling filter bed (Wübker et al., 1997). Unfortunately, these methods suffer from various drawbacks. In the case of limitation of bacterial growth, decrease of bacterial activity and hence, pollutant elimination capacity is generally observed. Chemical biomass removal suffers from considerable down-time after treatment, while mechanical biomass removal requires com-

plex reactor design. Finally, full media fluidization (back-washing) requires expensive equipment and generates large amounts of high BOD wastewater.

We recently reported on several approaches to control the rate of biomass formation in biotrickling filters (Cox and Deshusses, 1997a). One innovative strategy that was investigated is the use of protozoa that prey upon bacteria (Cox and Deshusses, 1997b). Because protozoan predation is coupled with CO_2 and heat generation, overall biomass production per amount of pollutant degraded should be less in the presence of protozoa. Ideally, protozoan predation should balance bacterial production to obtain a stable biotrickling filter with a constant amount of biomass. Such an equilibrium has been shown to exist in natural aquatic ecosystems (Bloem et al., 1989; Nagata, 1988; Sanders et al., 1989), although bacterial production rates in those systems is much lower than in biotrickling filters. However, protozoan predation increases carbon mineralization in systems with higher biomass production rates such as wastewater treatment plants, and protozoa has been suggested as a means to decrease sludge production (Ratsak et al., 1994; 1996). Protozoa have been observed in biofilters and biotrickling filters for waste air treatment (Diks, 1992; Ottengraf, 1986), but there have been no study reports on the role of protozoa in these reactors, nor has there been any attempt prior to this study to stimulate protozoa to control the rate of biomass accumulation in biotrickling filters for air pollution control. In the present article, we investigated a laboratory-scale toluene-degrading biotrickling filter to which protozoa were added and compared its performance to that of a control trickling filter to which no protozoa were added.

MATERIALS AND METHODS

Organisms and Culture Media

A toluene-degrading bacterium, enriched from activated sludge and tentatively identified as *Pseudomonas corrugata* using the Biolog test (GN Microplate™ from Biolog, Inc., Hayward, CA), was cultured in a mineral medium containing per L: 1 g KH_2PO_4, 1 g K_2HPO_4, 1 g $KNO_3 \cdot 1$ g NaCl, 0.2 g $MgSO_4$, 26 mg $CaCl_2 \cdot 2H_2O$, 5.2 mg EDTA $Na_4(H_2O)_2$, 1.5 mg $FeCl_2 \cdot 4H_2O$, 0.12 mg $CoCl_2 \cdot 6H_2O$, 0.1 mg $MnCl_2 \cdot 2H_2O$, 0.07 mg $ZnCl_2$, 0.06 mg H_3BO_3, 0.025 mg $NiCl_2 \cdot 6H_2O$, 0.025 mg $NaMoO_4 \cdot 2H_2O$, 0.015 mg $CuCl_2 \cdot 2H_2O$. For inoculation of the biotrickling filters, *P. corrugata* was cultured under continuous aeration of 10-L mineral medium in an open container with repeated addition of 40 μL/L toluene until a culture of appropriate density was obtained. *Tetrahymena pyriformis* (ATCC 3005) and *Vorticella microstoma* (ATCC 30897) were selected as protozoan predators, and cultured in ATTC medium 357 and a culture of *Klebsiella pneumoniae* (ATTC 27889, grown at 37°C in ATCC medium 802), respectively. *T. pyriformis* is a free-swimming, fast-growing ciliate and an effective predator of bacteria (Curds and Cockburn,

1968; Ratsak et al., 1994), while *V. microstoma* is a sessile protozoa frequently associated with activated sludge in wastewater treatment plants (Al-Shahwani and Horan, 1991). A mixed protozoan consortium originating from activated sludge was grown using a culture of toluene-grown *P. corrugata* as substrate. Protozoa cultures were performed in unstirred Erlenmeyer flasks with foam stoppers at room temperature. Bacterial cell counts were done on Plate Count Agar (PCA) (Difco, Detroit, MI) and on solid mineral medium (MMAT) containing 8 g/L agarose with toluene vapors as the carbon source.

Biotrickling Filter Equipment and Inoculation Procedure

Two identical biotrickling filters were operated in parallel. Protozoa were added to one of the reactors while the other reactor, without added protozoa, served as control. The reactors were made from clear PVC pipe with an internal diameter of 0.152 m and filled with 2.46 kg polypropylene Pall rings with a diameter of 2.5 cm (Flexirings, Koch Engineering, Wichita, KS). The resulting reactor had a packed bed height of 1.3 m and a bed volume of 23.6 L. The reactors were operated in a cocurrent mode. The air flow rate was regulated using a Series 200F mass flow controller (Porter Instrument Company, Inc., Hatfield, PA). Toluene inlet concentrations were varied by pumping liquid toluene at different flow rates into the air stream with a metering pump (model QVG50-RH00STY, Fluid Metering, Inc., Oyster Bay, NY). The trickling liquid was recycled over the filter bed with a centrifugal pump (model 1P677A, Dayton Electric Mfg. Co., Chicago, IL) at a superficial velocity of 7.9 m/h. Fresh mineral medium was continuously fed at an average rate of 238 mL/h to a vessel at the bottom of the reactor to collect the recycle liquid. Excess liquid was drained via an overflow outlet to maintain a constant liquid volume in the vessel of 1.6 L. A schematic of the experimental apparatus is shown in Figure 1.

The procedure of inoculation of the biotrickling filters was as follows. 2.5 L culture of toluene-grown *P. corrugata* was recirculated over the filter bed for 3 h. After removing the bacterial liquid, one of the reactors was inoculated with protozoa by overnight circulation of a mixture containing 1.5 L of a *T. pyriformis* culture, 0.93 L of a *V. microstoma* culture, and 0.86 L of a culture containing the mixed-protozoan consortium. In the control reactor, mineral medium without protozoa was circulated. Standard operation of the biotrickling filters began the next day.

Biotrickling Filter Experiments

Apart from the inoculation, the operation of the two biotrickling filters was identical, and their performances were compared over a 77-d period. During this period, the biotrickling filters were usually operated at standard conditions, i.e., a volumetric load of 64 $m^3/(m^3 \cdot h)$, an average toluene inlet concentration of 1.025 g/m^3, a superficial liq-
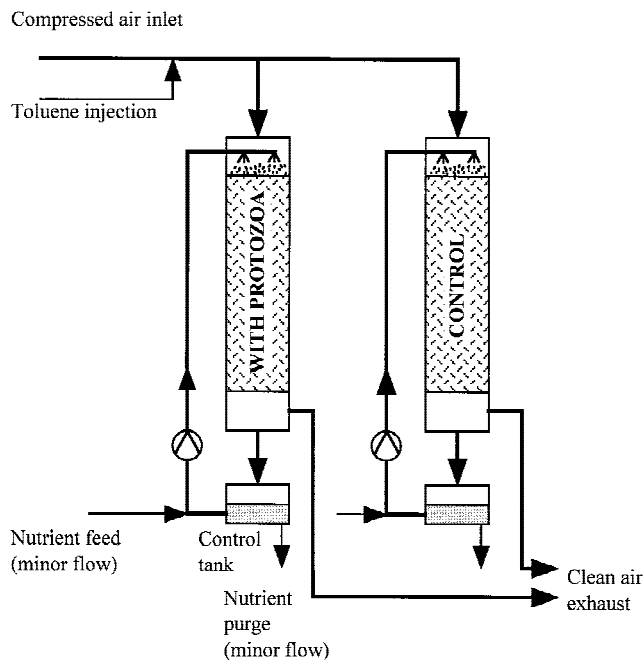
Figure 1. Schematic of the experimental set-up.

uid velocity of 7.9 m/h, and an average mineral medium feed rate of 238 mL/h. Toluene and CO_2 inlet and outlet concentrations, pressure drop, and wet weight of biomass accumulating in the reactors were determined on a daily basis. On a weekly or biweekly basis, the liquid phase was analyzed for pH, total carbon, total number of heterotrophs and toluene-degrading microorganisms, and for the presence of protozoa.

The performance of the two systems as a function of the toluene-loading rate was investigated between days 37–42. During these experiments, the toluene inlet concentration was varied between 0 and 3.5 g/m^3, while the gas-flow rate was kept constant at a volumetric load of 64 $m^3/(m^3 \cdot h)$. After a change in the toluene inlet concentration, the toluene and CO_2 outlet concentrations were continuously measured until constant values were obtained.

On day 55, the trickling filter enriched with protozoa was reinoculated with the same protozoa as on day 0. 2.5-L *T. pyriformis* culture, 2.0-L *V. microstoma* culture, and 1.5-L culture of the mixed consortium were centrifuged, and the pellets were combined and suspended in 2.63-L mineral medium. The resulting protozoa concentration was approximately 10^4 to 10^5 protozoa per mL of solution. The liquid phase of the biotrickling filter enriched with protozoa was removed and substituted by the protozoa culture suspended in mineral medium. The control received the same volume of pure mineral medium. Subsequently, for a period of 4 d (days 55–59), the toluene-inlet concentration was decreased to 0 and the gas-flow rate was decreased to a volumetric load of 16 $m^3/(m^3 \cdot h)$. CO_2 in the inlet and outlet gases were regularly analyzed, as well as the number of protozoa in the liquid phase.

On days 65–66, mass-balance studies were performed for

both biotrickling filters, which included continuous determination of toluene and CO_2 in the gas phase, biomass in the reactor, and suspended solids, bacteria, protozoa, and total carbon in the liquid phase. Standard operation of the biotrickling filters was maintained during these experiments.

Analytical Methods

Samples from inlet and outlet streams were automatically withdrawn via heated 1/8″ stainless steel tubing sampling lines connected to a 16-port valve (Valco, Houston, TX) and injected into a HP 5890 Series II gas chromatograph (Hewlett-Packard, Wilmington, DE) via a 10-port valve. Simultaneous analysis of CO_2 and volatile organic compounds was achieved with a packed column and a TCD detector, and with a capillary column and a FID detector, respectively. At standard operating conditions, influent and effluent streams were analyzed in duplicate twice a day. For determination of the amount of wet biomass in the reactor, the liquid circulation was stopped, and the liquid was allowed to drain from the reactor for 10 min. The reactor was weighed with a precision of 5 g using a model 7300 scale from Pennsylvania Scale Company (Leola, PA). The amount of wet biomass in the reactor was calculated as the increase of reactor weight as compared to the weight of the dry and clean reactor including the packing on day 0. Pressure drop measurements over 1.3-m filter bed was determined with a water U-tube during standard operation. Dry-matter content of immobilized biomass was determined in quadruplicate by overnight drying at 95°C of known amounts of wet biofilm sampled from the biotrickling filters after drainage of the dynamic hold-up as for the reactor weight determination. Analysis of C, H, N, and O in the dried biomass was done by Desert Analytics Laboratory (Tucson, AZ). Total carbon in the liquid phase was determined in duplicate using a model 5050 Total Carbon Analyzer (Shimadzu, Kyoto, Japan). Suspended solids in the liquid was determined by centrifugation of a known volume and overnight drying at 95°C of the pellet. Microbial counts in the liquid were performed by duplicate 10-fold dilution series of samples in 8.5 g/L NaCl, followed by duplicate plating of dilution series on PCA for the count of total heterotrophs and plating on MMAT for the count of toluene-degrading microorganisms. Incubation was at room temperature and counting was done after 4 to 7 d. MMAT-plates were placed in a closed box, and toluene was supplied to the atmosphere by diffusion via a syringe needle through the septum of a vial containing liquid toluene. Protozoa in the liquid samples were counted in nine fields of $0.1 \times 1 \times 1$ mm in a hemacytometer after fixation in 10% (v/v) Lugol solution (Sigma, St. Louis, MO).

RESULTS AND DISCUSSION

Toluene Elimination Capacity

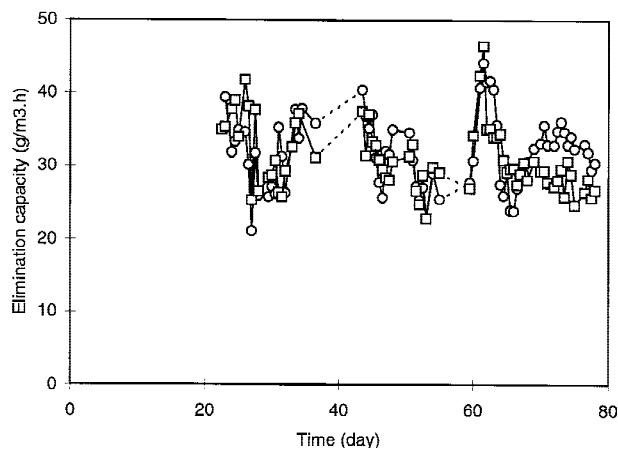Figure 2 shows the toluene elimination capacity at standard operating conditions. GC analyses started on day 22, at

Figure 2. Toluene degradation rate of the control (□) and enriched (○) filter at standard operation (64 m³/m³ · h, 1 g/m³ toluene).

which time both trickling filters had reached a steady state with respect to the toluene-degradation rate. In previous studies (Cox and Deshusses, unpublished results), start-up times of less than 10 d were observed. Between day 22 and 78, the average toluene elimination capacity of the control was 31.1 g/(m³ · h) whereas the elimination capacity of the biotrickling filter enriched with protozoa was 32.2 g/(m³ · h) at a volumetric load of 64 m³/(m³ · h) and an average toluene inlet concentration of 1.025 g/m³. Performance vs. load curves (Fig. 3) were determined between days 37–42, when the amount of wet biomass was about the same in both reactors (Fig. 4). At low loadings, both performance curves showed a non-zero-order behavior with increasing toluene elimination capacities at higher toluene inlet concentrations. At loadings above 150 g/(m³ · h), a constant elimination capacity was observed. We did not perform further investigations to discern the reaction- controlled regime from the diffusion-controlled regime. Mea-
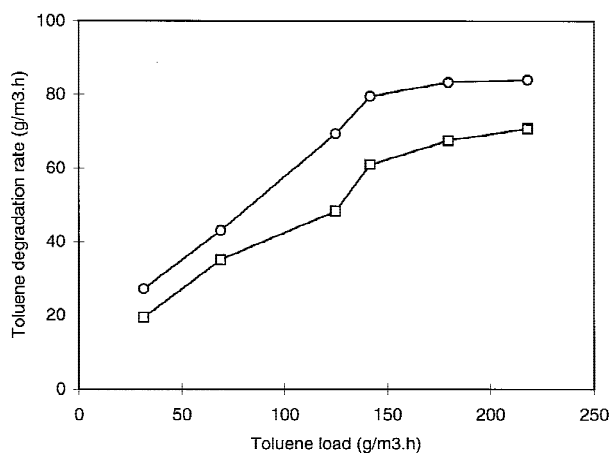

Figure 3. Performance curves of the control (□) and enriched trickling filter (○) at various toluene inlet concentrations and a constant volumetric load of 64 m³/m³ · h.

surement of dissolved oxygen in the liquid at the outlet of the reactor indicated that oxygen in the liquid was not limiting at standard operating conditions. This may not have been the case at higher inlet concentrations, and further, dissolved oxygen in the liquid may not reflect the true dissolved oxygen concentration in the biofilm. Toluene degradation reached a maximum at inlet concentrations of 2–2.5 g/m³ with maximal toluene elimination capacities of 71 and 83 g/(m³ · h) for the control and enriched trickling filter, respectively (Fig. 3). At all toluene concentrations tested, the biotrickling filter enriched with protozoa degraded toluene at a slightly higher rate than the control. The increased bacterial activity may be due to protozoan excretion of growth factors and/or an increased turnover of nutrients (Ratsak et al., 1996; Wright et al., 1995). The elimination capacities obtained herein are at the higher end of values usually reported for toluene. Pederson and Arvin (1997) compared various studies and found that toluene elimination capacities ranged from 12 to 84 g/(m³ · h) at loadings ranging from 12 to 220 g/(m³ · h) and residence times between 1.2 sec to 4 min.

Ecological Aspects

The liquid phase of both reactors was regularly investigated for the presence of bacteria and protozoa to obtain a general description of the species developing inside the reactor. Because the population in the liquid phase does not necessarily reflect the population immobilized on the packing in the reactor, it should be noted that this approach may result in an underestimation of sessile protozoa inside the reactor. Total heterotrophic bacteria counts in the liquid of both reactors were relatively similar and declined from initially 10^8/mL to 10^6–$3 \cdot 10^6$/mL at the end of the investigation. The decrease in bacterial counts in the liquid did not correlate with a decrease in reactor activity, which indicates that most of the pollutant elimination was performed by immobilized microorganisms. Counts of toluene-degrading bacteria in the liquid phase were of the same order as total heterotrophs, indicating that most of the bacterial population consisted of toluene-degraders. This is in contrast to the results of Møller et al. (1996), who determined that *Pseudomonas putida* constituted only 4% of the total biofilm population, but was responsible for about 65% of the degradation of the toluene vapors in their biotrickling filter.

At least six different bacterial species were observed on both the general plate-count medium and the selective medium with toluene. Specific precautions were taken to prevent cross-contamination between the biotrickling filters, but otherwise the reactors could be considered as open systems. Hence, the different bacterial species observed may have originated from the air, the liquid feed, and from the mixed protozoa inoculum in the case of the enriched biotrickling filter. *T. pyriformis* disappeared from the liquid within a few days, whereas *V. microstoma* was observed up to 8 d after inoculation. Possible explanations are discussed

below. Over the duration of the experiment, the liquid in both biotrickling filters showed a succession of different protozoan species. Both flagellates and ciliates were observed, most of them smaller than 50 μm and motile. These may have originated from the mixed protozoan population added to the protozoan inoculum in case of the enriched biotrickling filter. In addition, both reactors may have acquired protozoa from the laboratory environment because the biotrickling filters were open systems. Protozoan cell concentrations in the liquid of the enriched biotrickling filter fluctuated with time, and they were regularly too low for accurate counting, i.e., less than one cell per counting field of $1 \times 1 \times 0.1$ mm. In the liquid of the control reactor, protozoa counts fluctuated as well, but appeared, in general, to be about one order of magnitude lower than in the reactor enriched with protozoa. No attempts were made to limit protozoa in the control biotrickling filter with antibiotics specific for eukaryotic organisms such as cycloheximide because it would have altered yeast and fungal populations. Also, in related predator-prey experiments performed with rotating disk contactors in our laboratories, protozoan resistance to 100 mg/L cycloheximide was observed after 6 d (Cox and Deshusses, unpublished results).

Biomass Accumulation and Carbon Mineralization

An accurate determination of wet biomass in the reactor was possible by daily comparing the reactor wet weight to the weight of the dry reactor without biomass. An average volume of about 2.1 L drained from the reactor in 10 min, which constituted more than 90% of the dynamic hold-up. Biomass accumulation shown in Fig. 4 was more rapid in the enriched biotrickling filter up to day 34. This was not initially expected. A plausible explanation is that inoculation of the biotrickling filter enriched with protozoa resulted in the addition of extra nutrients, i.e., protozoan biomass and residual nutrients from the protozoan cultures. Also, it has been suggested (Ratsak et al., 1996) that protozoa produce bacterial growth factors or that protozoa induce bacterial flocculation (Bossier and Verstraete, 1996), which may have accelerated the initial start-up of the biotrickling filter enriched with protozoa. Another possible explanation is that polymers secreted by protozoa may have affected the wetting of the support, creating a more favorable environment for rapid bacteria attachment. From day 34 on, the trend reversed with biomass accumulation being more rapid in the control biotrickling filter. At the end of the experiment, the wet weight of immobilized biomass was 13.875 kg in the control trickling filter and 11.795 kg in the trickling filter enriched with protozoa. This had a marked effect on the pressure drop as shown in Fig. 4. Initially, the pressure drop increased very slowly in both reactors. After 60 days, a rapid increase was observed in the control filter only, whereas pressure drop remained essentially constant in the reactor inoculated with protozoa. One can speculate
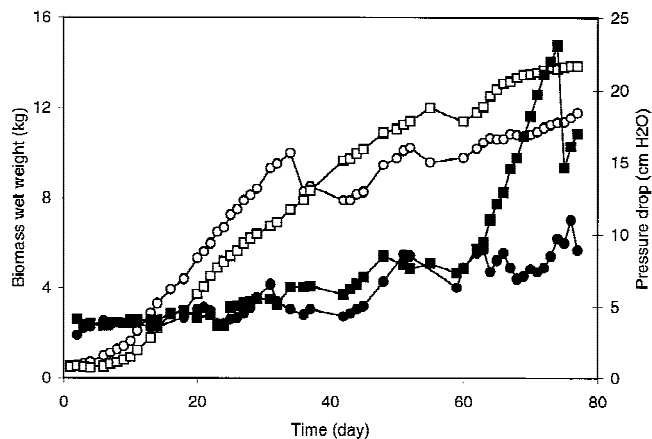
Figure 4. Biomass accumulation (open symbols) and pressure drop (closed symbols) over 1.3-m filter bed height of the control (□,■) and enriched (○,●) biotrickling filter at standard operation.

that at the end of the experiment, with more than 60% of the bed volume occupied by biomass, flooding would have occurred if the control reactor had been operated in a countercurrent mode.

Because both biotrickling filters were identical and operated in an identical way except for the inoculation of the enriched biotrickling filter with protozoa, we conclude that the lower rate of biomass accumulation in the enriched biotrickling filter was caused by the addition of protozoa. This is further supported by the examination of carbon mineralization values, i.e., C-CO$_2$ produced / C-toluene degraded reported in Figure 5. Between day 22–34, carbon mineralization was approximately the same for the control and enriched biotrickling filter, with average values of 50 and 52%, respectively. However, carbon mineralization in the enriched biotrickling filter increased thereafter, to an average of 68% while only 61% of the toluene was mineralized in the control reactor. This confirms that addition of protozoa indeed resulted in an increase of carbon mineralization as expected when the food chain is extended to include
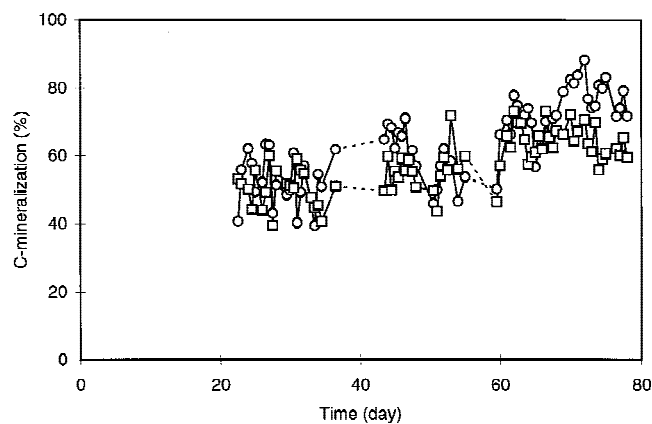
Figure 5. Degree of carbon mineralization of the control (□) and enriched (○) biotrickling filter at standard operation.

higher organisms preying on lower organisms. Closure of the carbon balance is discussed in the next section.

Carbon Balances

In Table I, carbon balances for the control and enriched biotrickling filter are compared for the entire period of investigation (day 22 to 77). The carbon balance includes average C-pollutant elimination, C-CO$_2$ production, carbon washed out via the purge and C-biomass increases in the reactor.

Biofilm samples from the inlet and outlet of both reactors had the same dry mass and carbon content of 0.046 g/g wet weight, and 0.44 g C/g dry matter, respectively. These values are comparable to others reported for various types of biofilms (Christensen and Characklis, 1990). Total carbon concentrations in the liquid phase were relatively constant during the entire experiment with average concentrations of 0.28 g C/L and 0.24 g C/L in the control and enriched biotrickling filter, respectively. Removal of carbon via the liquid discharge was slightly higher in the control reactor (Table I), indicating that wash-out of carbon-biomass (protozoa or bacteria) could not explain the observed lower rate of biomass accumulation in the enriched reactor. Table I clearly illustrates that although more toluene was degraded in the enriched biotrickling filter, biomass accumulation was lower in this reactor because of the increased rate of carbon mineralization due to protozoan predation. Interestingly, we believe predation must have occurred preferentially on the biofilm rather than in the liquid, based on three observations: (1) bacterial cell densities in the liquid were very similar for both biotrickling filters; (2) bacterial cell densities in the liquid were close to levels known to persist in the presence of protozoa (Alexander, 1981); and (3) protozoan concentrations in the liquid were, in general, lower

than as found in activated sludge (Al-Shahwani and Horan, 1991; Ratsak et al., 1996). The values presented in Table I permit calculation of a biomass yield coefficient for both trickling filters. In most studies on biotrickling filters, biomass yield coefficients are indirectly determined by either estimation of model equations or experimentally using batch- or continuous-liquid cultures. This leads to large uncertainties, because flask and bioreactor systems can generally not be directly compared. Here, assuming that total carbon in the liquid is biomass, various yield coefficients can be calculated as defined in Equations (1) and (2):

$$Y_{\text{C-biomass/C-substrate}} = \frac{\text{C-biomass formed}}{\text{C-toluene degraded}}$$

$$= \frac{\text{C-biofilm formed + C-biomass washed out}}{\text{C-toluene degraded}} \quad (1)$$

$$Y_{X/S} = \frac{\text{Dry biomass formed}}{\text{Toluene degraded}} \quad (2)$$

Using data from Table I, average values over the experiment and over the entire reactor can be calculated. A C-biomass to C-substrate yield coefficient of 0.31 g C-biomass/g C-toluene in the control biotrickling filter and 0.21 g C-biomass/g C-toluene in the enriched filter was obtained, which corresponded to apparent biomass yield coefficients $Y_{X/S}$ of 0.65 and 0.43 g dry biomass/g toluene, respectively. Those values fall within the range of yield coefficients reported for *Pseudomonas* species growing on toluene (Mirpuri et al., 1997). The lower apparent yield coefficient in the enriched bioreactor confirms that addition of protozoa has a positive effect in reducing the rate of biomass accumulation in biotrickling filters.

Reinoculation with Protozoa

Carbon mineralization approaching 100% is required to prevent clogging of biotrickling filters. However, this was not observed in the bioreactor enriched with protozoa, and consequently, a tendency for clogging and decreasing performance over the long-term operation was also observed with this reactor. In an attempt to increase protozoa density, the enriched filter was reinoculated with protozoa on day 55. During this phase of the experiment (5 d), the volumetric load was decreased to 16 m^3/(m$^3 \cdot$ h) and the toluene supply was stopped in both reactors. Protozoan cell cultures, harvested by centrifugation and suspended in mineral medium (to minimize introduction of dissolved organic nutrients), were then introduced in the enriched biotrickling filter. The production of carbon dioxide was monitored as an indication of the overall endogenous respiration and of protozoan activity. Values for CO$_2$ concentrations in the outlet gases are compared in Figure 6. Four hours after the addition of protozoa, CO$_2$ production in the enriched biotrickling filter was twice as high as in the control biotrickling filter. However, CO$_2$ production rates declined in both biotrickling

Table I. Overall carbon balances over days 22–27 for the control and the biotrickling filter enriched with protozoa.

Parameter	Control filter	Enriched filter
C-toluene degraded (g)[a]	898	930
C-biomass formed (g)[b]	190	118
C washed out (g)[c]	88	75
C-mineralization (%)		
Average[d], days 22–78	66	72
Determined[e], days 22–34	50	52
Determined[e], days 35–78	61	68

[a]Average toluene elimination capacity 31.1 and 32.2 g/m$^3 \cdot$ h for the control and enriched filter, respectively (except for days 55–59, during which no toluene was supplied).

[b]Amount of wet biomass on days 77–day 22, multiplied by dry matter (4.62%) and biomass carbon (44.04%) content.

[c]Average total carbon concentration in liquid multiplied by the medium purge rate.

[d](C-toluene degraded – C-biomass formed – C-washed out)/C-toluene degraded) × 100%.

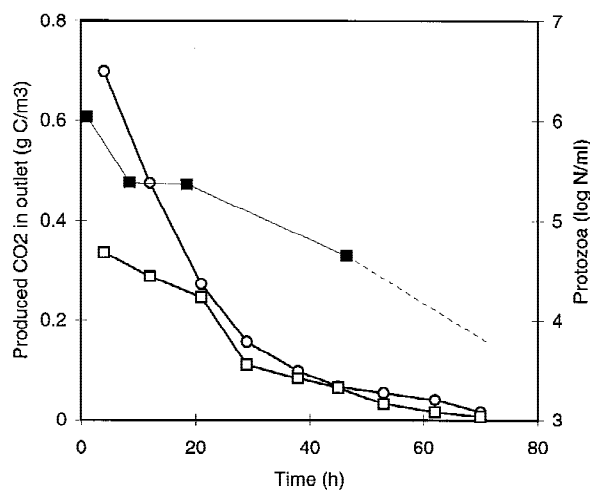[e](C-CO$_2$ produced/C-toluene degraded) × 100%.

Figure 6. Effect of reinoculation at time 0 of the enriched bioreactor with protozoa on CO_2 production ($\square$, control filter; $\bigcirc$, enriched filter) and protozoan cell concentration in the liquid phase of the enriched biotrickling filter ($\blacksquare$).

filters and were comparable only 1 d after reinoculation with protozoa. The protozoan cell concentration in the liquid of the enriched biotrickling filter coincidentally decreased from 1.1×10^6/mL to 4×10^4/mL within 2 d, while protozoa in the liquid of the control biotrickling filter was less than 10^4/mL during this experiment (Fig. 6). Small flagellates predominated in the liquid of the enriched filter, while *T. pyriformis* was observed for 2 d at concentrations of 6×10^3 to 10^4/mL. *V. microstoma* disappeared from the liquid within 1 d. Because *V. microstoma* is a sessile protozoa frequently associated with activated sludge in wastewater treatment plants (Al-Shahwani and Horan, 1991), its disappearance from the liquid may be explained by attachment to immobilized biomass, although this would require confirmation by sampling biofilm inside the reactor. Disappearance of *T. pyriformis* was unexpected, because it is a free-swimming ciliate and an effective predator of bacteria in batch (Curds and Cockburn, 1968) and continuous cultures (Ratsak et al., 1994). In our laboratory, we also found that *T. pyriformis* rapidly grew and effectively reduced bacterial concentrations in toluene-degrading suspended cultures with continuous aeration of 1 g/m^3 toluene in air (results not shown). Conditions in the trickling filter may not have been optimal for the growth of *T. pyriformis* because it was washed out at a dilution rate of 0.06 h^{-1}, which is lower than reported previously. Ratsak et al. (1994) reported stable protozoa populations at dilution rates of 0.06 up to 0.18 h^{-1} and Curds and Cockburn (1971) at dilution rates ranging from 0.1 to 0.4 h^{-1}. Possible explanations could be that bacterial concentrations in the liquid were too low to support growth of *T. pyriformis* and that *T. pyriformis* could not effectively prey upon biofilms. Alternatively, repeated passage through the centrifugal pump may have had a detrimental effect on *T. pyriformis*. As judged from CO_2 production (Fig. 6), addition of large amounts of various protozoa to the recirculating liquid of biotrickling filters did not

result in an immediate decrease of biomass by protozoan predation. Instead, the addition of protozoa that specifically prey upon immobilized biomass should be considered.

Carbon Balance Studies over Days 65–66

Between days 64 and 70, the weight of the enriched reactor remained almost constant (see Fig. 4). Optical density measurements of the recycling liquid indicated an unusually high turbidity, with maximal densities observed at days 65–66. Details of carbon balance studies for these days are presented in Table II. At this time, toluene degradation was slightly higher in the control bioreactor, while carbon mineralization was the same in both biotrickling filters and comparable to the average degree of carbon mineralization (Table II). However, the liquid in the reactors showed marked differences. While bacterial concentrations were about the same in both biotrickling filters, the liquid in the enriched trickling filter contained high concentrations of two types of protozoa. These protozoa were not identified but were probably ciliates with average diameters of 10 and 30 μm. Suspended solids and total carbon were significantly higher in the liquid of the enriched trickling filter, which is likely to be the result of unusually high protozoan and bacterial cell concentrations. The carbon balance of the enriched biotrickling filter (Table II) showed that at this time 22% of C-toluene degraded was being removed (as total carbon) via the liquid discharge whereas this was only 4% for the control. The reason for this is unclear. The observation that significant amounts of biomass flocs were found in the liquid of the enriched biotrickling filter and that the reactor weight remained constant seems to indicate that biofilm detachment was an important factor. This is further supported by separate experiments with rotating disk contactors (Cox and Deshusses, unpublished results), where it was found that biofilm detachment was more likely to occur when protozoa were added compared to controls without protozoa. On the other hand, during the trickling filter experiments, the toluene-induced oxygen uptake rate for the liquid of the enriched biotrickling filter was much higher than usual (results not shown). This indicates that significant toluene-removal activity occurred in the liquid. Hence, it is likely that the unusually high carbon content in the liquid was a combination of biomass growth in the liquid and biofilm detachment from the packing, probably triggered by protozoa. Unfortunately, because of the extreme complexity of the phenomena involved during these experiments and because of the lack of fundamental understanding in heterogeneous predator-prey systems, it was not possible to firmly attribute a cause to these unusual observations. Neither was it possible to maintain the conditions observed during these 6 d over an extended period of time, which would have guaranteed a stable and 0-growth situation. At this time, one can only speculate that a cause-effect relationship exists between the reinoculation of the reactor with protozoa on day 55 and the sudden protozoan bloom 10 d

Table II. Analyses if the gas phase, biomass, and the liquid phase, and calculation of carbon-mass balances over days 65–66.

Parameter	Control filter		Enriched filter	
	Day 65	Day 66	Day 65	Day 66
Gas phase				
g C-toluene degraded/m^3	0.423	0.410	0.380	0.364
g C-CO_2 produced/m^3	0.267	0.276	0.224	0.242
Reactor				
Biomass wet weight (kg)	12.830	13.100	10.635	10.640
Liquid phase				
Total heterotrophs (log N/mL)	6.0	6.5	6.2	6.6
Total toluene-degraders (log N/mL)	6.2	6.3	6.9	6.6
Small protozoa, 10 μm (log N/mL)		<4		5.3
Large protozoa, 30 μm (log N/mL)		<4		4.3
Suspended solids (g/L)		0.016		0.361
Total carbon (g/L)	0.116	0.095	0.571	0.445
Carbon balance[a]				
C-toluene degraded (g)		14.994		13.392
C-CO_2 produced (g)		9.774		8.388
C-biomass formed (g)		5.494		0.102
C washed out (g)		0.603		2.902

[a]Carbon balances are calculated over a 24 h period, using the following parameters/conversion factors: gas flow rate = 1.5 m^3/h, medium feed and purge rate = 238 mL/h, 0.0462 g dry biomass/g wet biomass, 0.4404 g C/g dry biomass. When available, average values for days 65 and 66 were used.

later. If such a cause-effect relationship does indeed exist, it is possible that repeated or continuous inoculation of protozoa would enable one to achieve a stable biomass content in the reactor. Further research to explain and exploit these phenomena is warranted.

CONCLUSION

Stimulation of protozoan predation appears to be a new and promising strategy to control biomass accumulation in biotrickling filters. Using a black-box approach, it was possible to show that addition of protozoa improved the performance and stability of toluene-degrading biotrickling filters in such a way that, without any loss of toluene-degradation activity, a decreased rate of biomass accumulation was obtained. This was mainly caused by an increase of pollutant mineralization. However, further stimulation of protozoa preying on biofilms is still required to obtain a no-growth situation where near-complete carbon mineralization is achieved. This should ensure long-term stability of biotrickling filters for waste air treatment with little reactor maintenance, while sustaining high pollutant elimination rates. Future research should focus on the selection of appropriate protozoa specialized in grazing of biofilms and the stimulation of protozoan activity inside complex bioreactor systems.

References

Alexander M. 1981. Why microbial predators and parasites do not eliminate their prey and hosts. Ann Rev Microbiol 35:113–133.

Alonso C, Suidan MT, Sorial GA, Smith FL, Biswas P, Smith PJ, Brenner RC. 1997. Gas treatment in trickle-bed biofilters: Biomass, how much is enough? Biotechnol Bioeng 54:583–594.

Al-Shahwani SM, Horan NJ. 1991. The use of protozoa to indicate changes in the performance of activated sludge plants. Wat Res 25:633–638.

Bloem J, Ellenbroek FM, Bär-Gilissen M-J, Cappenberg TE. 1989. Protozoan grazing and bacterial production in stratified Lake Vechten estimated with fluorescently labeled bacteria and by thymidine incorporation. Appl Environ Microbiol 55:1787–1795.

Bossier P, Verstraete W. 1996. Triggers for microbial aggregation in activated sludge. Appl Microbiol Biotechnol 45:1–6.

Christensen BE, Characklis WG. 1990. Physical and chemical properties of biofilms. In: Characklis WG and Marshall KC, editors. Biofilms. New York: Wiley. p 93–120.

Cox HHJ, Moerman RE, Van Baalen S, Van Heiningen WNM, Doddema HJ, Harder W. 1997. Performance of a styrene-degrading biofilter containing the yeast Exophiala jeanselmei. Biotechnol Bioeng 53:259–266.

Cox HHJ, Deshusses MA. 1997a. Evaluation of different technologies to control biomass growth in biotrickling filters for waste air treatment. In: Proceedings of Emerging Technologies in Hazardous Waste Management IX, September 15–17, 1997, Pittsburgh, PA. p 5–8.

Cox HHJ, Deshusses MA 1997b. The use of protozoa to control biomass growth in biological trickling filters for waste air treatment, Paper 97–RA71C.05. In: Proceedings of the Annual Meeting and Exhibition of the Air and Waste Management Association, June 9–13, 1997, Nashville, TN.

Cox HHJ, Nguyen TT, Deshusses MA. 1998. Elimination of toluene vapors in biotrickling filters: Performance and carbon balances, Paper 98–WAA.04P. In: Proceedings of the Annual Meeting and Exhibition of the Air and Waste Management Association, June 15–17, 1998, San Diego, CA.

Curds CR, Cockburn A. 1968. Studies on the growth and feeding of Tetrahymena pyriformis in axenic and monoxenic culture. J Gen Microbiol 54:343–358.

Curds CR, Cockburn A. 1971. Continuous monoxenic culture of Tetrahymena pyriformis. J Gen Microbiol 66:95–108.

Deshusses MA, Cox HHJ, Miller DW. 1998. The use of CAT scanning to characterize bioreactors for waste air treatment, Paper 98–TA20B.04. In: Proceedings of the Annual Meeting and Exhibition of the Air and Waste Management Association, June 15–17, 1998, San Diego, CA.

Diks RMM. 1992. The removal of dicloromethane from waste gases in a biological trickling filter. Ph.D. thesis, Technical University Eindhoven, The Netherlands.

Farmer RW, Chen J-S, Kopchynski DM, Maier WJ. 1995. Reactor switching: Proposed biomass control strategy for the biofiltration process, In: Hinchee RE, Sayles GD, and Skeen RS, editors. Biological unit processes for hazardous waste treatment. Columbus, OH: Batelle Press. p 243–248.

Hekmat D, Linn A, Stephan M, Vortmeyer D. 1997. Biodegradation dynamics of aromatic compounds from waste air in a trickle-bed reactor. Appl Microbiol Biotechnol 48:129–134.

Hugler W, Benavente JL, Acosta CM. 1997. BIOCYDR technology demonstration project for carbon disulfide bioremediation in waste gas. In: Proceedings of Emerging Technologies in Hazardous Waste Management IX, September 15–17, 1997, Pittsburgh, PA.

Loy J, Heinrich K, Egerer B. 1997. Influence of filter material on the elimination rate in a biotrickling filter, Paper 97–RA71C.01. In: Proceedings of the 1997 Annual Meeting and Exhibition Air Waste Management Association, June 9–13, 1997, Nashville, TN.

Mirpuri R, Jones W, Bryers JD. 1997. Toluene degradation kinetics for planktonic and biofilm-grown cells of Pseudomonas putida 54G. Biotechnol Bioeng 53:535–546.

Møller S, Pedersen AR, Poulsen LK, Arvin E, Molin S. 1996. Activity and three-dimensional distribution of toluene-degrading Pseudomonas putida in a multispecies biofilm assessed by quantitative in situ hybridization and scanning confocal laser microscopy. Appl Environ Microbiol 12:4632–4640.

Morgenroth E, Schroeder ED, Chang DPY, Scow KM. 1996. Nutrient limitation in a compost biofilter degrading hexane. J Air Waste Manage Assoc 46:300–308.

Nagata T. 1988. The microflagellate-picoplankton food linkage in the water column of Lake Biwa. Limnol Oceanogr 33:504–517.

Ottengraf SPP. 1986. Exhaust gas purification. In: Rehm HJ and Reed G, editors. Biotechnology, volume 8. Weinheim, Germany: VCH. p 426–452.

Pedersen AR, Arvin E. 1997. Toluene removal in a biofilm reactor for waste gas treatment. Wat Sci Tech 36:69–76.

Ratsak CH, Kooi BW, Van Verseveld HW. 1994. Biomass reduction and mineralization increase due to the ciliate Tetrahymena pyriformis grazing on the bacterium Pseudomonas fluorescens. Wat Sci Tech 29:119–128.

Ratsak CH, Maarsen KA, Kooijman SALM. 1996. Effects of protozoa on carbon mineralization in activated sludge. Wat Res 30:1–12.

Sanders RW, Porter KG, Bennett SJ, DeBiase AE. 1989. Seasonal patterns of bactivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol Oceanogr 34:673–687.

Schönduve P, Sára M, Friedl A. 1996. Influence of physiologically relevant parameters on biomass formation in a trickle-bed bioreactor used for waste gas cleaning. Appl Microbiol Biotechnol 45:286–292.

Shareefdeen Z, Baltzis BC, Oh Y-S, Bartha R. 1993. Biofiltration of methanol vapor. Biotechnol Bioeng 41:512–524.

Smith FL, Sorial GA, Suidan MT, Breen AW, Biswas P, Brenner RC. 1996. Development of two biomass control strategies for extended, stable operation of highly efficient biofilters with high toluene loadings. Environ Sci Technol 30:1744–1751.

Sorial GA, Smith FL, Suidan MT, Biswas P, Brenner RC. 1995. Evaluation of trickle bed biofilter media for toluene removal. J Air Waste Manage Assoc 45:801–810.

Van Groenestijn JW, Hesselink PGM. 1993. Biotechniques for air pollution control. Biodegradation 4:283–301.

Van Lith CPM, Ottengraf SPP, Diks RMM. 1994. The control of a biotrickling filter. VDI Berichte 1104:169–180.

Weber FJ, Hartmans S. 1996. Prevention of clogging in a biological trickle-bed reactor removing toluene from contaminated air. Biotechnol Bioeng 50:91–97.

Webster TS, Deshusses MA. 1997. Optimization of a full-scale biotrickling filter reactor in the treatment of industrial styrene off-gas emissions. Presented at the 1997 Annual Meeting of the American Institute of Chemical Engineers, November 18, 1997, Los Angeles, CA.

Wright DA, Killham K, Glover LA, Prosser JI. 1995. Role of pore-size location in determining bacterial activity during predation by protozoa in soil. Appl Environ Microbiol 61:3537–3543.

Wübker S-M, Laurenzis A, Werner U, Friedrich C. 1997. Controlled biomass formation and kinetics of toluene degradation in a bioscrubber and in a reactor with a periodically moved trickle-bed. Biotechnol Bioeng 55:686–692.

Zuber L. 1995. Trickling filter and three-phase airlift bioreactor for the removal of dichloromethane from air. Ph.D. thesis, Swiss Federal Institute of Technology Zurich, Switzerland.

Zuber L, Dunn IJ, Deshusses MA. 1997. Comparative scale-up and cost estimation of a biological trickling filter and a three-phase airlift bioreactor for the removal of methylene chloride from polluted air. J Air Waste Manage Assoc 47:969–975.