

# Biodegradation in Biofilters: Did the Microbe Inhale the VOC?

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## ABSTRACT

Biofiltration is a promising technique for air pollution control. Unfortunately, very little is known about the physiology of the biofilter microflora and about the metabolism of the pollutant undergoing treatment. A question of interest for process optimization and basic understanding of the biofiltration is whether the pollutants are only biocatalyzed to carbon dioxide, or whether the pollutants are incorporated into new cellular material. In the experiments reported herein,  $^{14}\text{C}$ -toluene was used as a tracer, and the dynamic pattern of the  $^{14}\text{CO}_2$  release after injection of labeled toluene to a bench-scale biofilter was studied. The release of labeled carbon dioxide was very rapid (most within 5 hours after labeled toluene injection) and depended on the age of the packing, the oldest packing exhibited the slowest release. For experiments with packing that was more than two months old, a radioactivity recovery of about 70% of the value expected was obtained. The recovery dropped to 30% for packing that was three weeks old, suggesting an incorporation of C-toluene into the cells during the early phase of the biofilter life. The rate of  $^{14}\text{CO}_2$  release could be linearly correlated to the overall performances of the biofilter, providing a biological explanation for the decrease observed during the operation of a biofilter.

**KEYWORDS:** Biofilter, VOC, toluene, air toxics, biodegradation, microbiology

## INTRODUCTION

As the number of full-scale biofilters for air pollution control is increasing, one is forced to admit that very little is known on the basic biological mechanisms essential to biofiltration. This is unfortunate since in most cases, biofilters are limited by the biodegradation step rather than by mass transfer. Hence, rational troubleshooting and process optimization are limited. A key question for understanding biofiltration pertains to the physiology of the process culture and to the metabolism of pollutant degrading cultures in biofilters. Is the culture growing or is it essentially resting? Are the pollutants simply biocatalyzed to carbon dioxide, for example by resting cells satisfying their maintenance requirements? Or are the pollutants incorporated in the cell metabolism so that new cellular material is synthesized?

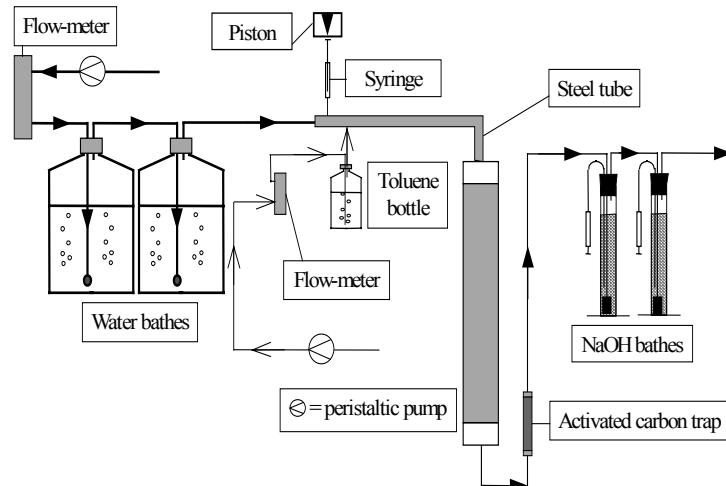
In order to better understand the fate of pollutants undergoing treatment, experiments were setup where a discontinuous feed of a  $^{14}\text{C}$  radiolabeled toluene was injected in a laboratory scale compost biofilter and the dynamic of  $^{14}\text{CO}_2$  production was monitored over time. Such experiments were performed at various stages in the lifetime of the biofilter and attempts were made to correlate variable describing the release of  $^{14}\text{CO}_2$  from the biofilter to the actual performance of the biofilter.

## MATERIALS AND METHODS

### Biofilter Setup and Operation

A bench-scale biofilter (Figure 1) was used to perform all experiments with radiolabeled toluene ( $^{14}\text{C}$ -toluene). The reactor was made of clear PVC pipe (4 cm ID and bed height of 40 cm). A high humidity (>95%) air stream was produced by sparging compressed air into two 500 mL wash-bottles filled with deionised water. A smaller metered stream of air was sparged in pure toluene and toluene vapors were injected into the main air stream to achieve the desired inlet toluene concentration ( $0.7\text{-}0.8\text{ g m}^{-3}$ ). Under standard operating conditions, the bench-scale biofilter was operated at an empty bed retention time (EBRT) of 32 s, and an inlet concentration of toluene of  $0.8\text{ g m}^{-3}$ . When the biofilter was loaded with used packing from the lab-scale biofilter (see below), it was allowed to reach a steady-state (usually 2-4 days) prior to performing significant experiments.

Parallel studies were performed with a lab-scale biofilter (15.2 cm ID and total bed height of 105 cm, bed volume 19 L). Operation of the bench-scale and the lab-scale biofilter was similar. Details of the experimental setup have been published elsewhere.<sup>1</sup> The larger biofilter also served as a source of packing material for the bench-scale biofilter for the labeled pulses. For this operation, the lab-scale biofilter was opened and a 500-800 mL representative sample of packing was taken usually about 5-10 cm below the top of the bed surface. It was then carefully transferred to the bench-scale biofilter. Standard operating conditions for the lab-scale biofilter were an inlet toluene concentration of  $\sim 0.8\text{ g m}^{-3}$  and an EBRT of 1 minute.



**Figure 1.** Schematic of the experimental setup.

### Biofilter Packing Material

The biofilter medium was prepared by mixing approximately 12.5 L of wet bark chips (3.11 kg, size 0.5-2 cm) with approx. 12.5 L of compost (6.56 kg). 425 g of limestone (calcium carbonate: final concentration of  $17\text{ kg}\cdot\text{m}^{-3}$ ) and 625 g of a natural compost activator (WhitneyFarms<sup>TM</sup>: 4 % of nitrogen, 4 % of  $\text{P}_2\text{O}_5$  and 2 % of  $\text{K}_2\text{O}$  in mass) were added and the mixture was carefully mixed. The medium was inoculated by spray addition of approximately 2 L of the recycle liquid

from a biotrickling filter degrading toluene. The initial moisture content of the medium was about 65% on a wet weight basis and it remained relatively unchanged (55-70%) during the experiments.

## Analytical Methods

Gaseous samples were automatically injected in a HP 5890 gas chromatograph using a 10 port injection valve. The GC was fitted with a 30 m Supelcowax 10 (Supelco, Bellefonte, PA) capillary column and a flame ionization detector (FID) for quantification of the volatiles, and with a 2.4 m 80/100 Chemosorb 1/4" packed column (Supelco, Bellefonte, PA) and a thermal conductivity detector (TCD) for carbon dioxide. Helium was used as the carrier gas. In selected cases, CO<sub>2</sub> was measured using a portable non-dispersive infrared analyzer (Q-Check Model 8730, TSI Inc., St. Paul, MN).

The elimination capacity (EC) of toluene in the biofilter was calculated as:

$$EC = \frac{C_{in} - C_{out}}{V_{bed}} \cdot G \quad (1)$$

where  $C_{in}$  is inlet toluene concentration (in  $\text{g}\cdot\text{m}^{-3}$ ),  $C_{out}$  the outlet toluene concentration,  $V_{bed}$  the volume of biofilter bed ( $\text{m}^3$ ), and  $G$  is the air flowrate ( $\text{m}^3\cdot\text{h}^{-1}$ ).

## Radiolabeled Toluene Pulses

5  $\mu\text{L}$  of a solution of <sup>14</sup>C-ring-labelled toluene (Sigma Chemicals, St-Louis, MI) diluted with non labeled toluene were used per experiment. This corresponded to about 100,000 counts per minute (CPM) on our analyzer. The labeled solution was introduced directly into the main air stream using a custom made syringe pump while the normal toluene feed was by-passed to an activated carbon trap. Injection usually lasted for 5.5 minutes. At the end of the labeled toluene injection, the trap was by-passed and the cold toluene injection supply to the biofilter was resumed. Dry run experiments with cold toluene injections via the syringe pump coupled with inlet concentration monitoring by GC revealed that this was the best setup to ensure a constant toluene inlet concentration. This was important because the biofilter needed to remain at steady-state during the pulse of labeled contaminant to avoid transient artifacts. In doing so, one enables use of the previously determined removal efficiency and apply it to the labeled toluene injected to calculate the amount of <sup>14</sup>CO<sub>2</sub> expected. During the experiments with labeled toluene, an activated carbon trap was placed at the outlet port of the biofilter to capture the remaining toluene. The carbon dioxide in the outlet air stream was absorbed in two small wash-bottles in series filled with 60 mL of 4M sodium hydroxide. The capture efficiency of the carbon trap and the NaOH baths was confirmed by gas chromatography. As the experiment proceeded, 1 mL samples were taken from the NaOH and baths and mixed with 5 mL of scintillation cocktail (Scintiverse II, Pittsburgh, PA). <sup>14</sup>C was quantified using a Hidex Triathler liquid scintillation counter (Wallac, Gaithersburg, MD) and the measurements were corrected for the samples taken previously (Equation 2). After a sample was taken, each NaOH bath was replenished with an equivalent volume of fresh NaOH 8M; the higher molarity was to compensate for the consumption of alkalinity by the absorbed CO<sub>2</sub>.

$$CPM_{total,n} = \frac{cpm_{sample,n}}{V_{sample,n}} \cdot V_{bath,n} + \sum_{i=0}^{n-1} cpm_{sample,i} \quad (2)$$

where  $CPM_{total,n}$  is the corrected activity of the sample in question (CPM stands for counts per minute),  $cpm_{sample,n}$  is the measured activity for the sample,  $V_{sample,n}$  the volume of the sample,  $V_{bath,n}$  the volume of the NaOH bath at the time of sampling, and  $cpm_{sample,i}$  any sample taken prior to the sample in question.

As the volume of the baths varied during the experiment but could only be measured accurately at the beginning and at the end of the experiment, it was interpolated from the initial value and the final value (Equation 3). In doing so, the uncertainty on the volume of the NaOH baths was about 0.2%.

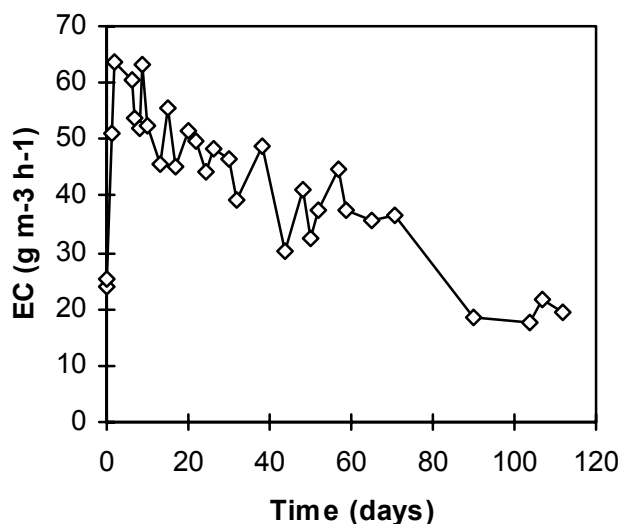
$$V_{bath,n} = \frac{V_{bath,tf} - V_{bath,to}}{t_f - t_0} \cdot t_n + V_{bath,to} \quad (3)$$

where  $V_{bath,n}$  is the corrected volume of the NaOH bath at the time of sampling,  $V_{bath,tf}$  is the final (measured) volume of the bath,  $V_{bath,to}$  is the initial (measured) volume of the bath,  $t_f$  and  $t_0$  are the final and initial time, and  $t_n$  the time of sampling.

## RESULTS

### Biofilter Performance Over Time

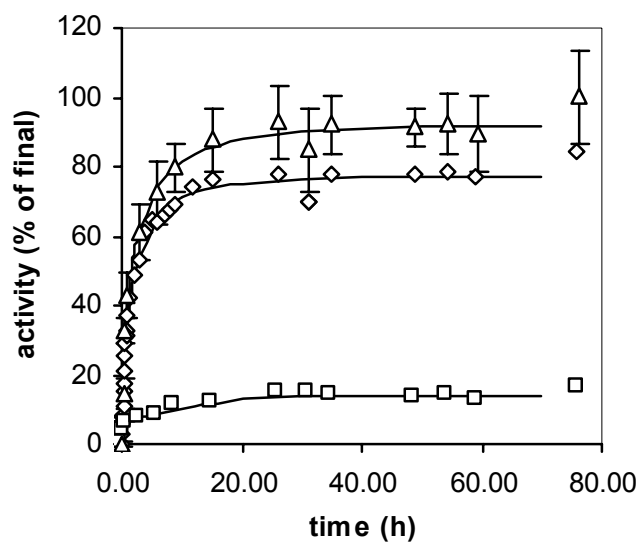
The source biofilter (lab-scale) was monitored during four months following the startup and its performance is reported in Figure 2. A very rapid startup was observed (2 days to reach pseudo steady-state) which was most probably the result of the effective inoculation of the packing. After startup, the biofilter exhibited relatively high performance (overall removal of 92 to 100%) for about 2 weeks. In particular the first segment of the biofilter which was exposed to a higher loading reached elimination capacities of over  $100 \text{ g m}^{-3} \text{ h}^{-1}$ . After this initial period, a continuous decrease in the removal performance was observed, and after about 80 days, the toluene elimination capacity stabilized around  $20 \text{ g m}^{-3} \text{ h}^{-1}$ , which is a value comparable to this reported in other works on toluene degradation in biofilters.<sup>1-5</sup> This slow decrease in performance has been attributed to various causes by different authors. According to Cherry and Thompson,<sup>6</sup> the decrease is due to a shift from a growing culture to a resting culture satisfying its maintenance requirements. Others reasons that have been proposed include nutrient limitations,<sup>7,8</sup> reduced air-biofilm interphase area caused by biomass growth,<sup>9</sup> or air short-circuiting.<sup>10,11</sup> It is likely that in most cases, a combination of the above is the reason for such a decrease in performance. Further research was warranted. The changes in the metabolism of the process culture over time were further investigated by injecting radiolabeled toluene to the biofilter and monitoring the release of labeled carbon dioxide.



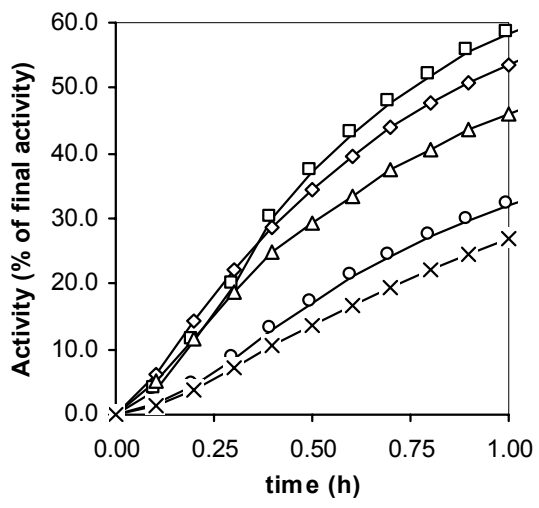
**Figure 2.** Evolution of the toluene elimination capacity of the source biofilter over time at standard operating conditions (EBRT: 1 minute; inlet concentration:  $0.8\text{-}1.1\text{ g m}^{-3}$ , i.e., the toluene loading:  $48\text{-}66\text{ g m}^{-3}\text{ h}^{-1}$ ).

### Radiolabeled Toluene Pulses

Figure 3 shows a typical curve for the  $^{14}\text{CO}_2$  recovered after a pulse of labeled toluene in the bench-scale biofilter. To allow for a better comparison between all the experiments, the recovery in Figure 3 is expressed as the percentage of the total activity recovered at the end of the experiment, i.e., when no noticeable increase in labeled  $\text{CO}_2$  absorbed occurred. This was usually 60 to 80 hours after the injection of  $^{14}\text{C}$ -toluene. The experiment was repeated at various times in the lifetime of the biofilter. The results are reported in Figure 4 where only the first hour of  $^{14}\text{CO}_2$  accumulation for packings of 3, 22, 29, 71 and 112 days old is shown. The derivatives of the  $^{14}\text{CO}_2$  release with respect to time of Figure 4 are plotted in Figure 5. They represent the instantaneous rate of release of  $^{14}\text{CO}_2$  from the biofilter after the injection of labeled toluene.

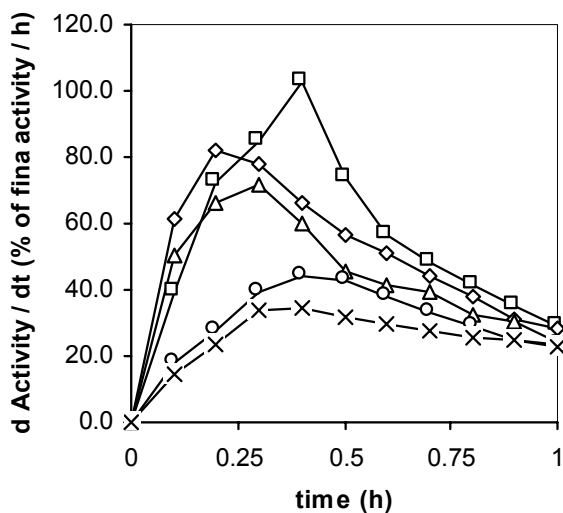


**Figure 3.** Radioactivity recovered as  $^{14}\text{CO}_2$  in the outlet air following a pulse of radiolabeled toluene to the bench-scale biofilter operated under standard conditions. First NaOH bath ( $\diamond$ ), second bath ( $\square$ ), total ( $\Delta$ ). The biofilter packing was 29 days old, error bars are shown for the total activity only. The percentages reported are based on the total radioactivity recovered at the end of the experiment.



**Figure 4.** Radioactivity recovered as  $^{14}\text{CO}_2$  in the outlet air following pulses of radioactive toluene to toluene packed with media of different ages. 3 days ( $\square$ ); 22 days ( $\diamond$ ); 29 days ( $\Delta$ ); 71 days ( $\circ$ ); 112 days ( $\times$ ). Note that the recovery is the percentage of the total radioactivity recovered during the experiment.

The first observation is that toluene is rapidly converted to CO<sub>2</sub>: within 1 hour about 25 to 60% of the total labeled carbon dioxide is emitted (Figure 4). This should be compared to the various time constants of the biofilter. The empty bed retention time of the gas in the biofilter during the experiment was 32 s and with an approximate bed porosity of 50%, the true retention time of the gas is about 16 s in the biofilter. Other experiments (not shown) revealed that a spike signal of toluene in the inlet air stream takes 4 to 7 minutes to pass through the biofilter under conditions similar to those of Figure 4. The relatively low retardation of toluene in biofilters is clearly linked to its hydrophobicity. More hydrophilic compounds are much more absorbed into the damp material and much greater pollutant retardations are expected.<sup>12</sup> Finally, the largest time constant of the system is that of the specific growth rate of toluene degraders in the biofilter. It is unknown but a conservative estimate can be done looking at systems such as shake flasks, aerated flasks or flat-plate biofilm. In those reactors, the specific growth rates of toluene degraders ranged from 0.13 to 0.86 h<sup>-1</sup>, which corresponds to doubling times of 5.3 to 0.8 hours.<sup>13-15</sup> Since these growth rates were measured under conditions more favorable for growth than the conditions in biofilters, one should be careful to extrapolate these values to biofilters. Even so, they express the very slow growth rate of toluene degraders when compared to the time scale of Figures 4 and 5.



**Figure 5.** Instantaneous <sup>14</sup>CO<sub>2</sub> production over time. Symbols as in Figure 4.

The rapid release of carbon dioxide from the biofilter is further illustrated by the graph in Figure 5. <sup>14</sup>CO<sub>2</sub> release peaked only 12 to 40 minutes after starting the injection of labeled toluene. The shape of all the release curves is typical for the combination of a Gaussian distribution to which a first order exponential decrease is added to obtain the significant tailing. Thus, one can hypothesize that conversion of labeled toluene to carbon dioxide is by two different but simultaneous mechanisms. The first mechanisms resulting in a Gaussian distribution is supposedly the rapid biocatalytic conversion of toluene to carbon dioxide. This includes gas-biofilm interphase mass transfer of toluene, diffusion to the cell, transport through the cell membrane and to key oxidase(s), catabolic reactions, and subsequent carbon dioxide release and

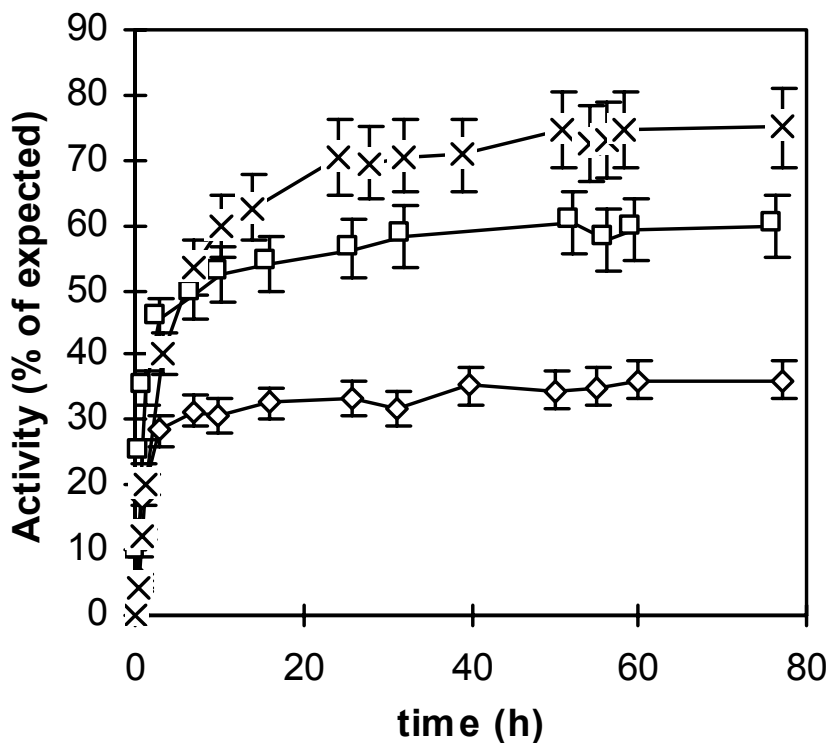
transfer back to the gas phase. The second mechanism which is responsible for the tailing effect, is much slower and is expected to follow a first order decrease, similar to the wash-out of a tracer from a stirred tank reactor. It includes the initial incorporation of the carbon-toluene into cellular material or storage compounds until the subsequent release of  $^{14}\text{CO}_2$  upon either normal turnover of cellular material or following death, lysis and reuse by secondary degraders or by predation by higher organisms. The above two mechanisms are consistent with the assimilation of a growth and energy substrate by a bacterial culture in a complex environment.

Further examination of Figures 4 and 5 reveals that the older biofilter packing, the lower the maximum rate of  $^{14}\text{CO}_2$  release. The opposite was expected based on reasoning including only cell growth and maintenance energy.<sup>6</sup> After biofilter startup, significant cell growth is expected and toluene consumption for maintenance (i.e., the fast release mechanism) is expected to be limited. Over time the cell metabolism switches to maintenance and fast release is expected based on this reasoning.<sup>6</sup> The results of Figures 4 and 5 are not necessarily contradicting this reasoning because the performance of the biofilter significantly changed over time (Figure 2). Hence the decrease of the  $^{14}\text{CO}_2$  release rate with the aging of the biofilter should rather be viewed as the result of the decrease of the overall metabolic activity of the process culture over time.

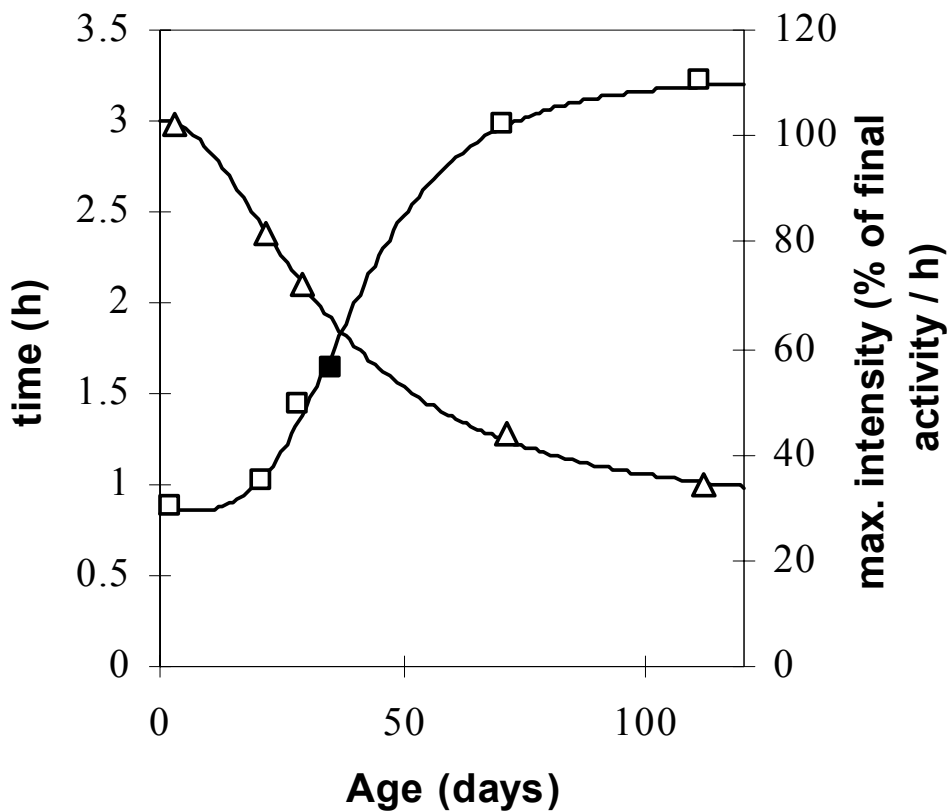
In Figure 6, the same data as in Figure 3 are reported, but as a percentage of the total expected  $^{14}\text{CO}_2$  released calculated using the amount of labeled toluene injected and the toluene removal at the time of the experiment. In all cases, the recovery of radioactivity was below 100% suggesting some incorporation of labeled toluene into biomass. As discussed above, this would have resulted in a slow release, but it was not experimentally observed (Figure 6 at 40-80 hours). It was then hypothesized that the  $^{14}\text{C}$  could have been sequestered in the cells as storage compounds. To test whether this was true, the toluene supply was stopped, inducing a starvation which would have accelerated the use of the storage compounds and released the labeled  $\text{CO}_2$ . But this was not experimentally observed. It was subsequently attempted to locate the missing radioactivity in the packing and in the microflora of the biofilter. To test for labeled carbonate, the packing was acidified and the resulting carbon dioxide was absorbed in NaOH and analyzed by liquid scintillation. To test for labeled cellular materials, the packing was extracted using a modified Blight and Dyer extraction method<sup>16</sup> and the three fractions (hydrophobic, amphiphilic and hydrophilic) of the cellular material were tested for radioactivity. None of these attempts was successful in finding any significant portion of the missing radioactivity. Further research is yet to be conducted to determine the fate of the missing  $^{14}\text{C}$ .

Even so, the results of Figure 6 show clearly that there were important changes in the biology during the first 30 days. On the other hand, after about two months, all experiments showed a very similar pattern (see also Figure 4) suggesting that a quasi-steady state with respect to the process culture metabolism had been obtained. This is consistent with the performance data of Figure 2. The final values of  $^{14}\text{CO}_2$  released reported in Figure 6 support the fact that the metabolism of toluene changed with the age of the biofilter. It appears that during the early phases of the biofilter life (22 days), more toluene was incorporated into biomass, i.e., more growth occurred, while over time (112 days) a major fraction of the toluene was simply biocatalyzed to  $\text{CO}_2$  without really entering the anabolism of the cell. The relatively high recovery for the experiment conducted after 3 days compared to the this performed after 22 days

is probably a transient effect. It can be explained by the fact that immediately after startup, the biofilter is extremely active. Some composting probably still occurs and although it was not measured, one can speculate that predators such as protozoa and rotifers are present in very high density, which may decrease over time because of the toxicity of high concentrations of toluene.<sup>15</sup> Predators certainly play an important role in releasing the <sup>14</sup>C incorporated into the biomass of the primary degraders.

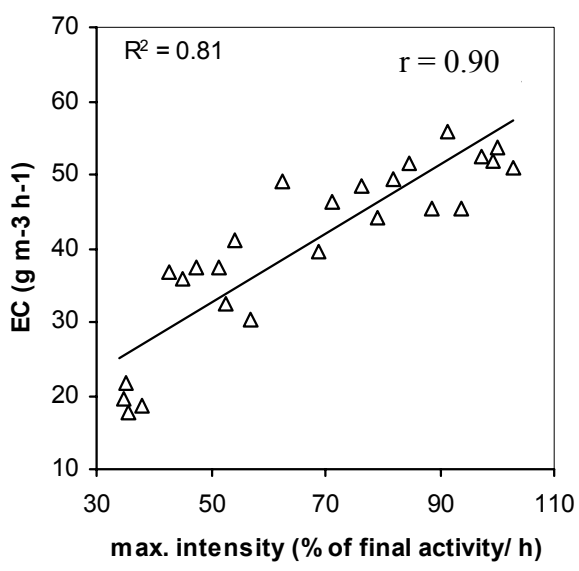


**Figure 6.** Recovery of the <sup>14</sup>CO<sub>2</sub> as percentage of the expected value based on steady-state toluene removal prior to the labeled pulse. Biofilter age: 3 days (□); 22 days (◇); 112 days (×).

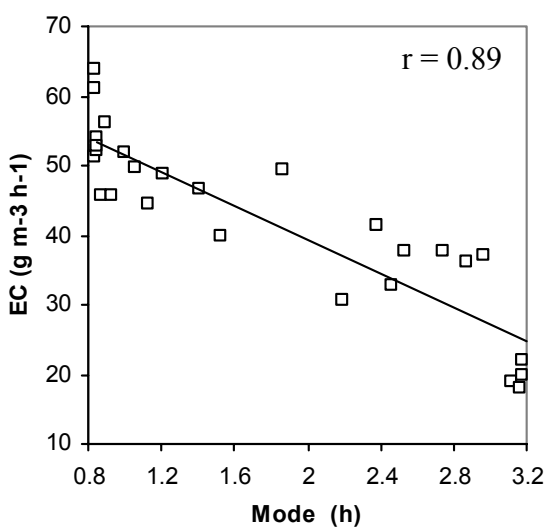


**Figure 7.** Evolution of the maximum intensity of radioactivity production (in % of the total radioactivity recovered) and its mode with the age of the packing. Mode (□); maximum intensity (Δ); data of a sixth pulse for comparison (■); manual fittings (—).

In Figure 7, the location of the mode and the maximum rate of  $^{14}\text{CO}_2$  release from the biofilter are plotted against the age of the biofilter. The data show that as the biofilter is aging, the absolute rate of carbon turn-over in the biofilter is decreasing (i.e., the mode of the release appears later and the intensity of  $^{14}\text{CO}_2$  release decreases). Interestingly, the mode and the maximum rate of  $^{14}\text{CO}_2$  release are linearly correlated with the performance of the biofilter, as described in Figures 8 and 9. High maximum intensity of  $^{14}\text{CO}_2$  releases and short modes of  $^{14}\text{CO}_2$  release correspond to high rates of toluene elimination.



**Figure 8.** Toluene elimination capacities (from the lab-scale biofilter) reported vs. the maximum intensity of the <sup>14</sup>CO<sub>2</sub> release from the bench-scale biofilter.



**Figure 9.** Elimination capacities (from the lab-scale biofilter) reported vs. the mode of the <sup>14</sup>CO<sub>2</sub> release from the bench-scale biofilter.

## CONCLUSIONS

Overall, the findings of this study should be placed in a general perspective for biofilter design and operation. As discussed in the first section of the results, as biofilters age, it is common to find a decrease of pollutant removal. The results discussed herein provide a biological explanation for this phenomenon. Still, a number of unanswered question remains on the principle of biofiltration. The challenge will be first to understand the causes for the changes in the metabolism of process culture, and second to engineer reactors or controls that allow for sustained operation at the rates observed shortly after reactor startup.

## REFERENCES

1. Deshusses, M.A., Johnson, C.T., Leson, G. Biofiltration of high loads of ethyl acetate in the presence of toluene. *J. Air Waste Manage. Assoc.* 1999, 49, 973-979.
2. Acuña E. et al. Studies on the microbiology and kinetics of a biofilter used to control toluene emissions, In *Proceedings of the 89th Annual Meeting & Exhibition of Air & Waste Management*, Nashville, Tennessee, June 23-28, 1996. The Air & Waste Management, Pittsburgh, PA.
3. Sabo, F. Behandlung von Deponiegas im Biofilter. Ph.D. Thesis, University of Stuttgart, Germany, 1991
4. Ottengraf, S.P.P., Van Den Oever, A.H.C. Kinetics of organic compound removal from waste gases with a biological filter. *Biotechnol. Bioeng.* 1983, 25, 3089-3102
5. Morales, M., Perez, F., Auria, R., Revah, S. In *Advances in Bioprocess Engineering*, E. Galindo and O. T. Ramirez, Eds. Kluwer Academic, Dordrecht, The Netherlands, 1994: pp. 405-411
6. Cherry, R.S., Thompson, D.N. The shift from growth to nutrient-limited maintenance kinetics during acclimation of a biofilter. *Biotechnol. Bioeng.* 1997, 56, 330-339
7. Morgenroth, E., Schroeder, E.D., Chang, D.P.Y., Scow, K.M. Nutrient limitation in a compost biofilter degrading hexane. *J Air Waste Manage Assoc.* 1996, 46, 300-308
8. Weckhuysen, B., Vriens, L., Verachtert, H. The effect of nutrient supplementation on the biofiltration removal of butanal in contaminated air. *Appl. Microbiol. Biotechnol.* 1993, 39, 395-399
9. Alonso, C., Suidan, M.T., Sorial, G.A. Smith, F.L., Biswas, P., Smith, P.L., Brenner, R.C. Gas treatment in trickle-bed biofilters: biomass, how much is enough? *Biotechnol. Bioeng.* 1997, 54, 583-594
10. Auria, R., Frere, G., Morales, M., Acuña, M.E., Revah, S. Influence of mixing and water addition on the removal rate of toluene vapors in a biofilter *Biotechnol. Bioeng.* (in press)

11. Morales, M., Revah, S., Auria R. Start up and gaseous ammonia addition on biofilter for elimination of toluene vapors. *Biotechnol. Bioeng.* 1998, *60*, 483-491
12. Deshusses, M.A. Transient behavior of biofilters: Startup, carbon balances, and interactions between pollutants. *J. Environ. Engrng.* 1997, *123*, 563-568
13. Pedersen, A.R., Arvin, E. Activity of toluene-degrading *Pseudomonas putida* in the early growth phase of a biofilm for waste gas treatment. *Biotechnol. Bioeng.* 1997, *54*, 131-141
14. Mirpuri, R., Jones, W., Bryers, J.D. Toluene degradation kinetics for planktonic and biofilm-grown cells of *Pseudomonas putida* 54G. *Biotechnol. Bioeng.* 1997, *53*, 535-546
15. Cox, H.H.J., Nguyen, T.T., Deshusses, M.A. Predation of bacteria by the protozoa *Tetrahymena pyriformis* in toluene-degrading cultures. *Biotechnol. Lett.* 1999, *21*, 235-239
16. White, D.C. et al. Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia*, 1979, *40*, 51-62