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Active compost biofiltration of toluene

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Abstract

Composting of leaves and alfalfa (i.e. active compost) was used for the biofiltration of toluene-contaminated air in a 6-L biofilter (initial bed height: 180 mm). During the thermophilic phase (45 to 55 °C), toluene biodegradation rates reached 110 g toluene.m^{-3}.h^{-1} at an inlet concentration of about 5 g.m^{-3} and a gas residence time of 90 seconds. The highest rates were obtained late in the thermophilic phase suggesting a microbial adaptation was occurring. Biodegradation rates decreased rapidly (50% in 48h) in the cooling stage. Under mesophilic conditions, the maximum biodegradation rates that could be obtained by increasing the inlet toluene concentration were near 89 g toluene·m^{-3}·h^{-1} which is similar to that reported in the literature for mature compost biofilters. No volatile by-product was detected by gas chromatography. Mineralization of ^{14}C-toluene and benzene showed that they were completely degraded into CO_2 and H_2O under both thermophilic and mesophilic conditions. Bacteria isolated from late mesophilic stage had the capacity to degrade all BTEX compounds but were not able to transform chlorinated compounds. No organisms were isolated which could use toluene as their sole source of carbon and energy at 50 °C. Active compost biofiltration should be an excellent process for the treatment of gaseous BTEX by biofiltration. This is the first report of thermophilic biofiltration of toluene.

Introduction

Toluene and other monoaromatic compounds such benzene, ethylbenzene and xylenes (BTEX) are commonly found in gasoline-contaminated sites (EPA 1995). The presence of BTEX compounds in the environment is a major concern because of their toxicity. This, combined with their high aqueous solubility which allows them to migrate through soil and groundwater, has made them priority pollutants for the US Environmental Protection Agency. BTEX-contaminated soils are generally excavated and treated by bioventilation (Saberiyan et al. 1994). Biofiltration is often used to treat the exhaust air from this process. Similarly, air sparging followed by biofiltration can be used for *in situ* treatment if soil structure permits. Biofiltration is also widely used for treatment of low concentration process off-gases. Pollutants are transformed into carbon dioxide and water.

Biofiltration costs are generally lower than those of other technologies such as incineration or adsorption (Leson & Winer 1991; Bohn 1992) but due to relatively low specific biodegradation rates, biofilters must be very large. Biofilter beds usually consist of soil, compost, wood bark or peat moss (Leson & Winer 1991). These materials provide a suitable environment for biofilm formation. Biodegradation rates in the biofilm are limited either by microbial activity or contaminant diffusion through the biofilm (Ottengraf & Van den Oever 1983). Thus, in cases where diffusion is not limiting, biofilters with increased microbial activity should have a superior performance. The first stage of composting is thermophilic due to extremely active microbial metabolism (Fogarty & Tuovinen 1991). Temperatures up to 65 °C can be reached during this stage but the optimal temperature for degradation of the organic phase is below 60 °C (Nakasaki et al. 1985b). Aeration is necessary to maintain aerobic conditions and can increase the degradation rate up to 25% (Miller

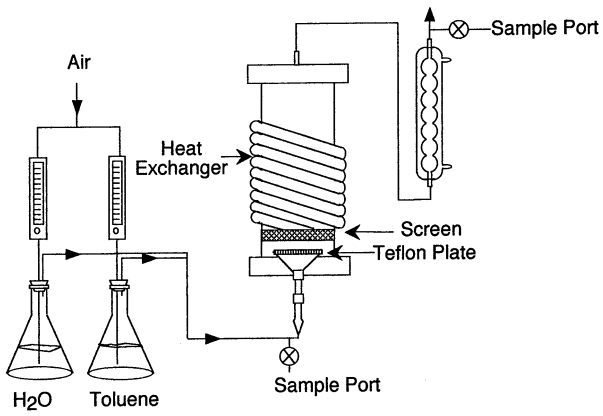

Figure 1. Experimental biofilter. All materials in contact with toluene were Teflon, glass or stainless steel.

1984). Intense microbial activity in the thermophilic stage may increase the possibility of co-oxidation of added contaminants. *Bacillus stearothermophilus,* one of the most numerous micro-organisms in the thermophilic stage of composting (Strom 1985) has been reported to metabolize benzene and toluene (Natarajan et al. 1994). Studies have demonstrated that recalcitrant pollutants such as polycyclic aromatic hydrocarbons nitro-and chloro-aromatics can be transformed during composting (Crawford et al. 1993; Breitung et al. 1996; Laine & Jorgensen 1996). Co-composting of hydrocarbon-contaminated soils with leaves and alfalfa has increased the extent of weathered hydrocarbon degradation by at least 40% (Beaudin et al. 1996). In this work, we report on the use of an active compost of leaves and alfalfa for the biofiltration of toluene vapors. The term active compost biofiltration is used to differentiate the process from those using mature compost.

Materials and methods

Reactor description

A 6-liter reactor (Figure 1) was used to perform composting and biofiltration. The reactor consisted of a glass column with an inner diameter of 152 mm and a height of 300 mm. Initial compost bed height was 180 mm (volume: 4L). Simulated off-gases were generated by bubbling a portion of the air in toluene. The remaining air was sparged through water for humidification. Toluene was chosen as the model contaminant because its characteristics are representative of monoaromatic compounds (Allen-King et al. 1994). Toluene concentration and flowrate were controlled with rotameters. Inlet gases were passed through a perforated Teflon plate (perforation diameter: 0,5 mm). A stainless-steel screen supported the compost 8 cm above the plate. An external heat exchanger consisting of plastic tubing connected to a recirculating bath was used to limit heat loss during composting. The temperature of the recirculating water bath was adjusted to maintain the compost temperature just below 55 °C in thermophilic stage. It was maintained at 2–3 °C below the interior compost temperature during the cooling and mesophilic stages. Two sample ports allowed the measurement of inlet and outlet toluene concentrations. Outlet gasflow was cooled by a condenser.

Compost material

A blend of maple leaves and Rabbit Chow (#5315, Ralston Purina, Mississauga, Canada) served as the composting substrates. The initial C/N ratio was adjusted to 14 (w/w). Calcium carbonate (70 g.kg of dry substrate^{-1}) was added as a pH buffer. Water was added to keep the moisture content between 55 and 60% (w/w). Pressure drop during the composting did not exceed 2,5 cm of water. The inoculum (85 g·kg of dry substrate^{-1}) was compost that had been previously used for biofiltration. The compost was mixed manually every 48–72 h to prevent channeling and drying. Samples for pH, moisture and ash content analyses were taken after mixing. Water content was measured in duplicate samples by drying 10 to 15 g of compost overnight at 105 °C. The dried solids were then weighed and heated at 550 °C for 90 min to determine ash content. A slurry of 10 g of compost in 90 ml of distilled water was used to monitor pH.

Mineralization experiments

Toluene mineralization was determined in microcosms. Compost samples (5 g wet, although all calculations were based on dry weight) were placed in 120-ml serological bottles sealed with Teflon Mininert valves (Supelco, Bellafonte, PA, USA). Uniformly ^{14}C-ring-labelled toluene (9,7 mCi·mmol^{-1} Sigma Chemicals, St-Louis, MI, USA) or benzene (19.3mCi.mmol^{-1}, Sigma Chemicals) was added at 100 000 dpm per microcosm. Unlabeled toluene or benzene was added to obtain the desired vapor phase concentrations. A CO_2 trap (1N KOH) was placed inside each microcosm. Microcosms were incubated in the dark at room

temperature or 50 °C as specified. Abiotic controls were performed by adding sodium azide to samples (0,2% w/w). The recovered $^{14}CO_2$ was quantified using a liquid scintillation counter (Wallac LSC 1409, Turku, Finland). The $^{14}CO_2$ measurement was converted to mg of toluene mineralized based on its specific activity (17331 dpm/mg).

Microbiological studies

Cultivable heterotrophic bacteria and specific counts were performed by the poured plate method. Nutrient agar (with one-tenth the normal concentration), Sabouraud Agar (Difco, Detroit, MI, USA) and minimal salts agar (Greer et al. 1990) were used. Ten g of compost was suspended in 90 ml of sterile NaCl solution (0,85% w/v) and shaken 45 min with a wrist action shaker (Burrell Scientific, Pittsburg PA, USA). Tenfold dilutions were made and 100 μL was mixed with molten agar at 45 °C before pouring. Plates were placed in a 2-L jar with a Teflon-lined lid. When indicated, BTEX, 1,2-dichloroenzene or trichloroethylene were added to obtain a vapor phase concentration of 10 g·m^{-3}. Plates were incubated at 30 °C and 50 °C. Toluene-degrading bacteria were isolated on nutrient agar, Gram stained and identified using the GN microplate system (Biolog, Hayward, CA, USA).

Analytical methods

Triplicate samples of toluene were measured by a HP 5890 gas chromatograph equipped with a 25 m HP-5 capillary column and a flame ionization detector (FID). The temperature profile began at 60 °C, with heating at a rate of 5 °C·min^{-1} to 90 °C, then heating at 10 °C·min^{-1} to a final temperature of 120 °C. Injector and detector temperatures were maintained at 90 °C and 220 °C respectively. Helium was used as the carrier gas. For sampling, 25 to 100 μL of gas was taken by a gastight syringe (Hamilton, Reno, NE, USA) with a valve (Supelco) and injected manually. Standards were made by injecting a known volume of toluene into a closed bottle of known volume. Calibration curves were linear in a range from 0 to 30 g·m^{-3}. Biodegradation rates in g of carbon· m^{-3} of biofilter bed·h^{-1} were calculated as:

$$\text{rate} = \frac{C_o - C}{V} \times \nu$$

where

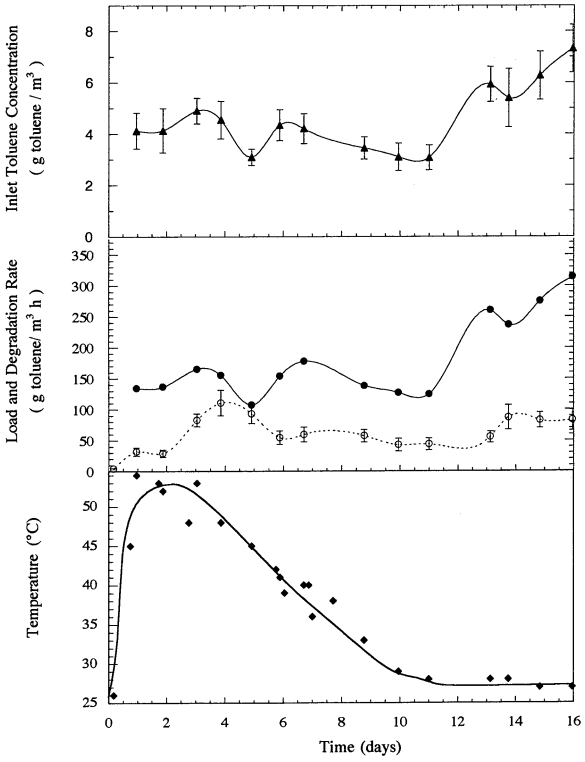

Figure 2. Batch operation of an active compost biofilter treating toluene vapors. Inlet toluene concentration (▲); toluene degradation rate (○); organic load (●); and temperature (♦).

C_o = inlet toluene concentration(g·m^{-3});
C = outlet toluene concentration (g·m^{-3});
ν = gas flowrate (m^3/h);
V = volume of biofilter bed (m^3).

Oxygen and carbon dioxide in the gas phase were monitored via a mass spectrometer (VG Quadropoles, Middlewich, England).

Results

Toluene biodegradation during composting

An aerated composting process was studied for its capacity to purify toluene-contaminated air. The data presented (Figure 2) for the thermophilic, cooling and early maturation stages are typical of 3 experiments conducted under very similar conditions. The air flowrate and residence time were adjusted to avoid oxygen limitation of composting activity at least at a macroscopic level. The total compost dry weight (cal-

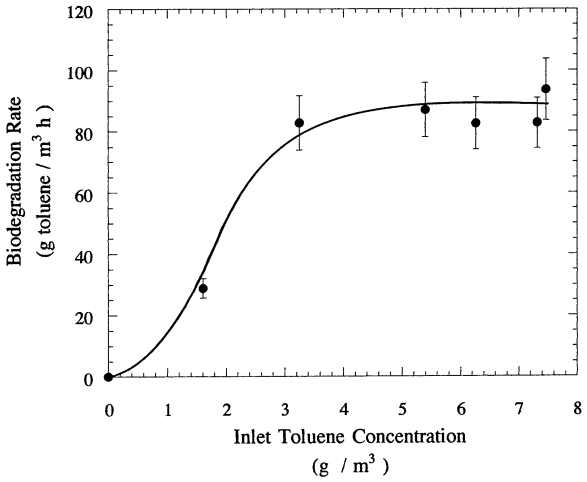

Figure 3. Biofilter response to inlet concentration under mesophilic conditions. Samples were taken 45 minutes after the stepchange.

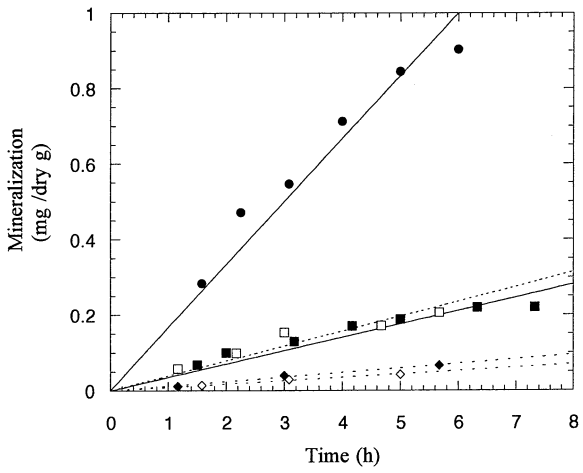

Figure 4. Mineralization of toluene and benzene by active compost under mesophilic (squares) and thermophilic (circles) conditions. Toluene (● and ■); benzene (□); benzene abiotic control(◇); toluene abiotic control (◆). The initial concentration of toluene and benzene was 6 $g \cdot m^{-3}$.

culations based on ash content) decreased by almost 45% due to utilization of organic matter during the 17-day period. Water content remained stable at 50 to 60% due to humidification of inlet air and to metabolic production of water. The gas residence time in the solid (compost) phase was measured frequently and gradually decreased from 100 to 90 seconds as the height of the compost bed, and thus its volume, decreased. Maximum toluene biodegradation (110 g toluene·m-^{3}·h^{-1}) was obtained several days into the thermophilic stage. Apparently thermophilic toluene degraders required this time to increase their numbers and to acclimatize to toluene. Temperatures remained high for 3 days. The biodegradation rate decreased at least 50% during the cooling phase (up to day 8) probably due to a shift from a thermophilic to a mesophilic microbial population and perhaps to a decrease in cometabolism related to an overall decrease in metabolic activity. A brief increase in the degradation rate was observed in several experiments just as the compost temperature decreased to 37 °C (data not shown). As yet, no explanation has been found for this phenomenon.

During the mesophilic stage, the degradation rate increased after 14 days to 89 g toluene·m-^{3}·h^{-1} due to an increase in the inlet toluene concentration. Effects of inlet toluene concentration were studied by stepwise increases in the inlet concentration from 1 to 8 $g \cdot m^{-3}$ (Figure 3). The maximum biodegradation rate in mesophilic systems reached a plateau of 80-90 g toluene·m^{-3}·h^{-1} at a concentration of about 4 $g \cdot m^{-3}$.

Toluene mineralization

Gas chromatography of the outlet gases showed no detectable peaks other than residual toluene. In order to prove, complete mineralization of toluene to CO_2 and H_2O, studies with radioactive toluene were conducted (Figure 4). Regardless of whether the compost was taken from thermophilic or mesophilic stages, degradation began without a lag phase. After 5 h, more than 50% of labelled toluene was recovered in both cases. However, as in all composting processes, oxygen limitation due to diffusion into the compost particles may have occurred from the beginning. Nevertheless, the degradation rates were constant during the first 5 h indicating the absence of oxygen limitation on a macroscopic level in the microcosms. On the basis of the calculated O_2 uptake rate from mass spectrometry data from the laboratory-scale composting unit, the oxygen concentration could have become limiting after about 6 h. At a concentration of 6 $g \cdot m^{-3}$, benzene was mineralized at a rate equal to that of toluene mineralization under both mesophilic (Figure 4) and thermophilic conditions (different experiment, data not shown).

Metabolic capacity of microorganisms

Microbial counts were made on the inoculum (compost from previous toluene active compost biofiltration) and on the compost substrates (leaves and alfalfa) to determine the source of toluene-degrading microor-

Table 1. Microbiological characterization of substrates used for active compost toluene biofiltration

Substrate	Bacterial concentration (CFUs/dry g)			
	Heterotrophs		Toluene-degraders	
	30 °C	50 °C	30 °C	50 °C
Leaves	$6{,}51 \pm 0{,}53 \times 10^4$	$< 10^3$	n.g.[a]	n.g.[a]
Alfalfa	$6{,}97 \pm 0{,}58 \times 10^5$	n.g.[a]	n.g.[a]	n.g.[a]
Inoculum	$4{,}39 \pm 0{,}41 \times 10^9$	$6{,}17 \pm 0{,}82 \times 10^8$	$2{,}20 \pm 0{,}23 \times 10^5$	n.g.[a]

[a] n.g.: no growth was detected.

ganisms. The majority of the initial microbial population came from the inoculum (Table 1). The low water content of leaves (12,35%) and alfalfa (11,8%) limits their microbial population. Less than 0,01% of the cultivable mesophilic, heterotroph population in the compost mixture showed the capacity to utilize toluene as sole carbon source. Three different bacterial strains which use toluene as sole carbon and energy source were obtained from mesophilic stage. These strains were also able to grow on benzene, ethylbenzene or a mixture of o-, m- and p-xylene isomers supplied in the vapor phase. No growth was observed with 1,2-dichlorobenzene and trichloroethylene vapors. All three were Gram-negative rods identified as *Pseudomonas* sp. Thermophilic microorganisms which use toluene as sole carbon and energy source were not isolated under the culture conditions tested, although thermophilic microflora were found to grow in the presence of an aqueous toluene concentration of up to 250 $mg{\cdot}L^{-1}$ if yeast extract (100 $mg{\cdot}L^{-1}$) was supplied. No fungi were isolated that were able to metabolize toluene as sole carbon source under the conditions tested.

Discussion

In the first stages of active composting, the toluene biodegradation rate increased constantly until the temperature began to descend (Figure 2). The highest toluene biodegradation rate (110 g toluene$\cdot m^{-3}\cdot h^{-1}$) was achieved when the temperature was near 50 °C. A similar effect has been observed in studies on hydrocarbon co-composting were two optimal temperatures were found (25 and 50 °C) (Beaudin et al. 1995). Lengthening the thermophilic phase greatly increased the extent of hydrocarbon degradation (Beaudin et al. 1995). In our experiments, the thermophilic phase lasted only 3 days since the high aeration rate allowed rapid utilization of the most easily degradable growth substrates. A longer thermophilic period would allow for more growth and acclimatization which could result in an even higher thermophilic degradation rate. The use of thermophilic biofiltration for treatment of waste has been reported. Phenol and formaldehyde vapors at a concentration of 0,1 $g{\cdot}m^{-3}$ were degraded in a wood chip bed with a biodegradation rate of 9 g $C{\cdot}m^{-3}{\cdot}h^{-1}$ at temperatures between 40 and 55 °C (Knauf & Zimmer 1994). In comparison, the thermophilic rates obtained with active compost in this present study were 12 times higher than those measured by Knauf and Zimmer.

Toluene biodegradation rates in the mesophilic stage of active compost biofiltration were similar to those obtained with mature compost biofilters (Rho et al. 1994). The solubility of toluene in an aqueous film decreases with temperature. The toluene biodegradation rate did not increase above a certain value (about 80 g toluene$\cdot m^{-3}\cdot h^{-1}$) during mesophilic biofiltration but was sometimes much higher (up to 110 g toluene$\cdot m^{-3}\cdot h^{-1}$during the thermophilic operation). This shows that biological reactions rather than mass transfer (i.e. diffusion) must have limited the reaction rate during the mesophilic active compost biofiltration.

Some of the toluene-degraders isolated from the maturation (later mesophilic) stage were Pseudomonas sp. which tend to have optimal growth temperatures around 30 °C. Nevertheless, the high temperature before maturation stage did not appear to decrease the activity of toluene degrading bacteria in the mesophilic stage. It has been reported that mesophilic bacteria are able to survive above 60 °C but do not contribute to composting activity at that temperature (Nakasaki et al. 1985a).

Toluene biodegradation under thermophilic and mesophilic conditions is quite different. Our attempts to isolate thermophilic microorganisms which use toluene as sole carbon source failed, suggesting that toluene-degrading microorganisms from active com-

post need other organic compounds for growth. As opposed to mesophilic toluene-degraders which are able to use toluene as the sole carbon source, it has been demonstrated that both a *Thermus* sp (Chen & Taylor 1995) and *Bacillus stearothermophilus* (Natarajan et al. 1994) need another nutrient source such as yeast extract for toluene degradation under thermophilic conditions. Presence of a large amount of available nutrients during the thermophilic stage of composting could be one of the reasons for the high toluene biodegradation rate in this stage. Nutrient limitation during the late thermophilic stage lowers the overall microbial activity and the temperature (Fogarty & Tuovinen 1991). In the present study, this corresponded to a decrease in the biodegradation rate although it is difficult to separate the effect of carbon limitation from that of temperature.

Mineralization studies in microcosms and the absence of by-products in GC analyses confirmed that toluene and benzene were completely degraded to CO_2 and H_2O. Microcosm data may also be useful in biofilter characterization and in the design of pilot-scale biofilters. In our case, thermophilic conditions showed a higher degradation rate both in the microcosms (Figure 4) and in the bioreactor (Figure 2). Nevertheless a direct relation between these systems cannot be made. After conversion of the reactor degradation rates to mg toluene.g of dry compost^{-1}.h^{-1}, the microcosm rates were 2 to 20 times lower than reactor rates. Mass transfer rates in the microcosm are much lower than those in a reactor. No airflow is provided in microcosms so that anaerobic microzones can be formed and toluene and/or oxygen may not reach all parts of the biofilm. In addition, carbon used for biosynthesis is not measured in microcosms. This can lead to an underestimation of the biodegradation rate. It has been reported that 15 to 40% of toluene transformed in groundwater microcosms was incorporated into biomass (Bianchi-Mosquera et al. 1994). Another study has shown that only 50% of initial radioactive 2,4-dichlorophenoxyacetic acid was recovered as ^{14}C-CO_2 when all of the contaminant was degraded (Comeau et al. 1993). Humic substances may also bind toluene by physico-chemical mechanisms such as is the case for 3-4 dichloroaniline which polymerize with humic macromolecules during its degradation in organic soil (Völkel et al. 1994). Nevertheless, microcosm data can give a rqelative idea of toluene degradation under different physiological conditions.

Mature compost is widely used as a biofilter bed (Leson & Winer 1991). It provides good physiological conditions for microbial colonization. On the other hand, this paper shows that immature compost (i.e. active compost) could also be used as a filter bed. Instead of purchasing mature compost, inexpensive local waste materials could be used for biofiltration processes. The composting process seems not to be affected by toluene biodegradation activities and the compost produced could be sold at a profit. During the bioventilation of BTEX-contaminated soil, the highest concentration of BTEX is found during the first days of operation (Donaldson et al. 1992). This peak coincides with the maximum rate of the toluene degradation in active compost biofiltration so these processes could be linked. In the case of mixed volatile and non-volatile contamination , compost produced could be also added to the soil as a rich microbial inoculum before treatment of non-volatile contaminants. Finally, in contrast to conventional systems, higher rates are achieved above 45 °C during active compost biofiltration. A variation of this process could be used in an industrial off-gas treatment where expensive gas cooling is normally required.

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