Biodegradation (2006) 17: 293–302
DOI 10.1007/s10532-005-4217-8

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Pyrene mineralization capacity increases with compost maturity

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Accepted 22 March 2005

Key words: compost, composting, PAH, polyaromatic hydrocarbons, pyrene

Abstract

Experiments were conducted to determine the effects of composting or simple addition of compost to the mineralization of *n*-hexadecane, pyrene and benzo(a)pyrene in soil. Soil (contaminated or clean) was composted with maple leaves and alfalfa. Samples from different composting phases were spiked with radiolabeled and cold *n*-hexadecane, pyrene or benzo(a)pyrene, placed in aerated microcosms at different temperatures, and monitored for mineralization. It was determined that neither composting nor the addition of compost had any effect on *n*-alkane or benzo(a)pyrene mineralization. In contrast, the pyrene mineralization rate increased dramatically with the amount of time that soil had been composted. Highest pyrene mineralization rates and extents (more than 60% after 20 days) were obtained when pyrene was in contact with composted soil from the curing stage. Neither thermophiles (55 °C) nor fungi were responsible for pyrene mineralization.

Introduction

Composting has been shown to be effective in the decontamination of soils containing nitroaromatic compounds (Daun et al. 1998; Lenke et al. 1998; Williams & Mayler 1990), aliphatic compounds (Beaudin et al. 1996), chlorophenols (Semple & Fermor 1995; Valo & Salkinoja-Salonen 1986), herbicides (Dooley et al. 1995), pharmaceutical compounds (Rhodes & Peck 1995) and polycyclic aromatic hydrocarbons (PAH) (Joyce et al. 1998; McFarland et al. 1992; Potter et al. 1999). Composting appears to be particularly useful in the bioremediation of petroleum hydrocarbons, especially the PAH fraction.

The mechanism by which composting or addition of compost aids in PAH bioremediation has not been completely elucidated. Fragments of PAH may be incorporated into the organic matrix (Berry & Boyd 1985). Fungal (*Phanerochaete chrysosporium*) composting of soil contaminated with benzo(a)pyrene has been shown to increase formation of bound residues of this PAH, thus decreasing its detectability (Qiu & McFarland 1991). Addition of mature compost to soil has been found to render significant quantities of anthracene and *n*-hexadecane unextractable by conventional solvent extraction techniques (Kästner et al. 1995). Although immobilization of hydrocarbons may reduce their effective toxicity, incomplete degradation sometimes produces intermediates that are more toxic than the parent compound (Belkin et al. 1994; Cerniglia 1984). Processes which lead to complete mineralization are desirable since they assure detoxification.

Composting is typically used for soil decontamination when other methods are too expensive or ineffective. For example, composting has been found to be highly effective in the bioremediation of a highly weathered petroleum-contaminated soil when another bioremediation process (landfarming) was ineffective (Beaudin et al. 1996). We have proposed the addition of compost to contaminated soil as an alternative to actually composting the soil. However, there may be significant differences between the mechanisms at work in the two bioremediation processes.

Possible mechanisms for enhanced hydrocarbon mineralization during composting include increased microbial activity due to the addition of easily metabolized organics, increased microbial diversity resulting from the constantly changing environment during batch composting (Crawford et al. 1993; Williams & Mayler 1990), increased desorption due to thermophilic temperatures, and co-metabolism due to the addition of substances with structures similar to PAH (i.e. lignins). Both soil composting and/or simply adding compost to the soil may increase bioavailability due to hydrocarbon mobilization by humic or other materials.

Since it is very difficult to determine the quantitative impact of each of these factors on a particular process, we set out to answer the following practical questions. Do composting or compost addition increase the mineralization of both *n*-alkanes and PAH? Do the thermophilic conditions typical of composting need to be incorporated into a simple compost addition process? Which composting stage should be used to obtain the most rapid mineralization when adding compost to contaminated soil? Should that compost be produced in the presence of the contaminants to acclimatize the microbial population? Are fungi important in the mineralization process? This paper presents and discusses results of research designed to answer these questions using pyrene and *n*-hexadecane as model compounds. It was hoped that answering these questions might also shed some light on the mechanisms leading to increased mineralization of contaminants during composting and/or compost addition processes.

Materials and methods

Chemicals

[1-^{14}C] *n*-Hexadecane (2.6 mCi per mmol), unlabeled *n*-hexadecane, [4, 5, 9, 10-^{14}C] pyrene (32.3 mCi per mmol), and [7-^{14}C] benzo(a)pyrene (26.6 mCi per mmol) were all purchased from Sigma-Aldrich Ltd., Oakville, Ont., Canada and were at least 95% pure. Unlabeled pyrene was purchased from Supelco Inc., Bellefonte, PA, USA. 2.5 M KOH (Anachemia Inc., Ville St-Pierre, Canada) was used to trap CO_2. The scintillation cocktail was OptiPhase 'HiSafe' 3 (Fisher Scientific Ltd., Nepean, Ont.). 35% HCl (Anachemia) was used for acidification. As a drying agent, prior to solvent extraction, anhydrous sodium sulfate (Anachemia) was used. Methylene dichloride (pesticide grade, Fisher) was employed to extract the remaining radioactivity. Cycloheximide (Aldrich) was used to suppress the fungal population. The ingredients for the mineral salts solution (Greer et al. 1990) as well as all other chemicals were purchased from Anachemia, Montreal, PQ.

Soil and amended organic material

Two soils were used. One had been contaminated with petroleum hydrocarbons while the other was not. The contaminated soil (TPH = 40,000 mg/kg and PAH = 630 mg/kg) was obtained from the Thouin sand quarry near Montreal, Quebec, which had served as an illegal dumping site for hazardous waste during the late 1960s. The soil was sieved to less than 2 mm in particle diameter. The clean soil (Bomix Golden Sand, Daubois Inc., Montreal, Quebec) consisted of 97.1% (wt/wt) sand ($75\ \mu m < d_p < 2$ mm), 0.3% stones ($d_p > 2$ mm) and 2.6% fines ($d_p < 75\ \mu m$). The clean soil TPH content was below the detectable level. A mixture of dry maple leaves and alfalfa (Rabbit Chow, Ralston Purina Canada Inc.) with a molar C/N ratio of 17 was used as the source of carbon and nitrogen for composting. Further information on the clean and contaminated soils is found in tables in Beaudin et al. (1999) and Haderlein et al. (2001), respectively.

Soil composting

An 11-l aerated stainless steel batch reactor equipped with heating jackets was used to prepare composted soil samples for mineralization experiments. Thermocouples were installed at different points inside the reactor, at the inner wall, and between the heating jackets and the outer wall. During the mesophilic stage, the difference between these two temperatures was used to calculate heat loss through the wall. The heating jackets were automatically activated to compensate for lost heat. The maximum compost temperature was limited to 55 °C by increased aeration. The compost cooled at the end of the thermophilic phase due to decreased heat generation although the heating jackets continued to compensate for heat loss (curing stage).

The initial composition of all composted soils was 640 g of soil (either contaminated or clean), 250 g leaves, 720 g alfalfa and 82 g $CaCO_3$. The water content was adjusted to 50% (w/w). Composted soil from the mesophilic stage was obtained after 23 h by following an autoregulated temperature profile as in Figure 1. The relatively short thermophilic phase (when compared to windrow composting) is typical of reactor-based composting. The temperature and CO_2 production rate at sampling were about 30 °C and 60 mmol kg^{-1} h^{-1}, respectively. The composted soil from the thermophilic stage was obtained after 49 h (temperature and CO_2 production rate at sampling, 55 °C and 75 mmol kg^{-1} h^{-1}, respectively). Composted soil from the curing stage was obtained after 1 month of composting and 3 months of maturation at ambient temperature. The composted clean soil from the curing stage was produced in the same manner as with contaminated soil, except that uncontaminated sand was used to prepare the mixture and the thermophilic phase was extended to 1 month by holding the temperature at 50 °C. It was used after subsequent maturation at ambient temperature during 11 months. Aliphatic hydrocarbons were measured by gas chromatography according to Ministry of the Environment, Quebec (MA410-HYD1.0). Soil samples were analyzed by Maxxam Analytique Inc, Lachine PQ by HPLC after extraction.

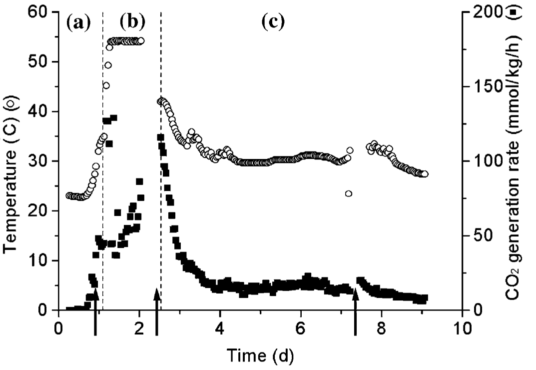

Figure 1. CO2 generation rate and temperature profile during composting of contaminated soil (a) mesophilic stage; (b) thermophilic stage (temperature was limited at 55 °C by increasing the aeration rate); and (c) curing stage. Arrows indicate when the reactor contents were mixed.

Mineralization experiments

All mineralization experiments employed a continuously aerated 125 ml microcosm connected to a series of two KOH traps which were designed for complete capture of all evolved CO_2 (Haderlein et al. 1999). The first CO_2 trap contained 14 ml KOH solution designed to capture more than 99% of generated CO_2. The second trap contained 4 ml KOH solution to capture the majority of the remaining CO_2.

Depending on the objectives of the experiment each microcosm contained:

1. Ten grams of moist composted clean or contaminated soil (moisture content 50% (w/w)) from either the mesophilic, thermophilic or curing stage (Figure 2a) or,
2. Five grams of a mixture of 3 parts (by weight) contaminated soil and 1 part mature composted clean soil plus 3.7 ml distilled water to adjust moisture content to 57% (Figure 2b) or,
3. Five grams contaminated soil plus 5 ml mineral salts medium (Figure 2c).

The above mixtures were spiked with either *n*-hexadecane (0.046 μCi of ^{14}C-hexadecane and 2 mg non-radiolabeled hexadecane per microcosm) or pyrene (0.046 μCi of ^{14}C-pyrene and 1 mg non-radiolabeled pyrene dissolved in 100 μl of toluene per microcosm) or benzo(a)pyrene (0.046 μCi of ^{14}C-benzo(a)pyrene). Microcosms (triplicates) were aerated continuously at ambient temperature, except for composted soil from the thermophilic stage which was continuously aerated at either ambient temperature or 55 °C. Abiotic controls (duplicates) contained 0.4% (w/DW) of NaN_3 applied as an aqueous solution (40 mg in 0.5 ml per microcosm).

Quantification of mineralization

The KOH solution in the traps was periodically replaced with fresh KOH solution. The KOH solution from both traps was combined, adjusted to a uniform volume and mixed vigorously. Ten ml of scintillation cocktail was added to 3 ml of the sampled KOH solution and mixed vigorously.

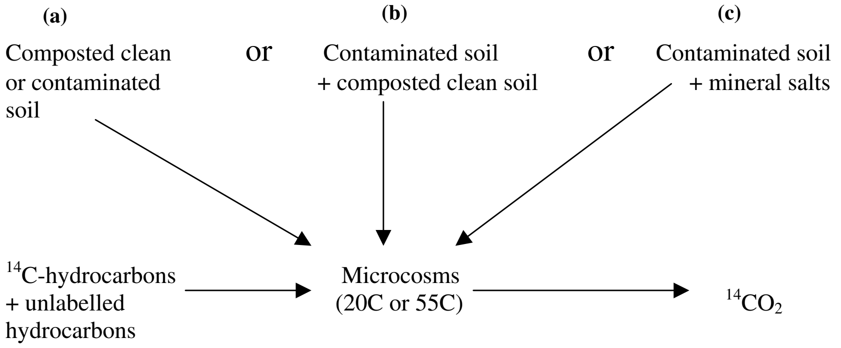

Figure 2. Unlabeled and ^{14}C-hydrocarbons were added to: (a) moist composted clean or contaminated soil from either the mesophilic, thermophilic or curing stage or, (b) a mixture of 1 part (by weight) mature composted clean soil and 3 parts contaminated soil or, (c) five (5) g contaminated soil plus 5 ml mineral salts medium. The microcosms were then placed at either 55 °C or ambient temperature and continuously aerated. $^{14}CO_2$ evolution was collected in KOH traps was monitored over time.

Radioactivity levels were determined with a liquid scintillation counter (model LSC 1409, Wallac Scintillation Products).

Abiotic controls never showed mineralization values greater than 1% of the initially added radioactivity, except when composted soil from the thermophilic stage was incubated at 55 °C where radioactive material collected in the CO_2 trap originating from abiotic transformations still accounted for less than 2.6% initially added radioactivity.

Water (50 ml) was added to the microcosms after each sampling. The pH was measured after vigorous mixing for 5 min. The pH was then lowered to 2.0 to release any $^{14}CO_2$ bound as carbonate. In order to verify the mass balance of radioactive carbon, solvent extraction was carried out on composted soil from the mesophilic stage after the acidification step. After the aqueous phase had been filtered off (Whatman (1 qualitative), about 10 g of anhydrous sodium sulfate was added to the microcosms, which were then shaken vigorously for about 5 min. Methylene dichloride (50 ml) was added. The microcosms were then shaken at 110 rpm (Lab-Line® Environ-Shaker) overnight. The extraction was repeated once. Ten ml scintillation cocktail was added to 4 ml of each extract, was mixed vigorously and analyzed for radioactivity.

Calculation of lag phase and maximum mineralization rate

The lag phase was calculated by plotting the logarithm of the percentage of remaining radioactivity versus time and determining the intercept of the steepest tangent with the $y = \ln(100)$ line. The maximum mineralization rate was calculated using the steepest tangent on the % mineralization versus time graph. Both tangents were calculated by linear regression on three data points using error bar weighing where possible.

Results and discussion

Effect of composting on extractable hydrocarbon concentration

Composting the contaminated soil caused a decrease in the concentration of C_{10} to C_{16} aliphatic hydrocarbons as well as certain extractable PAH (Figure 3). The rate of decrease in aliphatics concentration showed a concentration dependence (first-order kinetics) typical of biochemical reactions without mass transfer limitations. In contrast, the rate of pyrene decrease was linear (zero-order kinetics) indicating a dependence on mass transfer rather than on the extractable concentration. Benzo(a)pyrene was not degraded, either because it was not bioavailable, because there were no microorganisms present that could degrade it, or because it was below the threshold concentration necessary to support growth (Roch & Alexander 1997). Much of the decrease in hydrocarbon concentration occurred well into the curing stage.

Effect of the presence of compost on n-hexadecane mineralization

While the concentration of extractable C_{10} to C_{16} aliphatics had decreased during composting, it was not known if composting or the presence of compost affects their degradation. Composted contaminated soil from different stages of the composting process was spiked with cold and ^{14}C-labeled *n*-hexadecane and incubated at a desired temperature. Composted soil from all three composting stages was able to mineralize hexadecane readily when incubated at ambient temperature. A lag phase (3.0 days) was originally observed with composted soil from the mesophilic stage (Figure 4, open circles). This was probably a result of low reproducibility caused by the high heterogeneity of the mesophilic stage (i.e. distinct pieces of alfalfa pellets and leaves) and the rapidly changing physico-chemical conditions of this early stage of composting. Repetition of this experiment produced similar kinetics but no lag phase (Figure 4, closed circles).

A lower final *n*-hexadecane mineralization extent was observed for composted soil from the thermophilic stage when incubated at 55 °C (Figure 4). Although efforts were made to avoid this problem, this was likely due to drying of the aerated materials at 55 °C.

Although the initial mineralization rates were slightly lower, no lag phase was observed nor was there a significant difference in the mineralization potential of the resulting mature composted soils whether the soil was clean or contaminated with petroleum hydrocarbons (Figure 5). Thus, previous exposure to petroleum hydrocarbons and hence adaptation to aliphatics, does not greatly increase the ability of the population present in mature composted soil to mineralize *n*-hexadecane. This may be attributed to the fact that *n*-hexadecane is easily metabolized by many common mesophiles (Chaîneau et al. 1995; Oudot et al. 1987) which may be present in soil or mature compost. Adding composted clean soil to contaminated soil did not significantly increase *n*-hexadecane mineralization.

Amending contaminated soil with composted clean soil did not increase the mineralization rate. Similar results were obtained simply by adding mineral salts to contaminated soil (Figure 5) Therefore, for unbranched aliphatics similar in size to *n*-hexadecane, there is little or no benefit to composting nor of compost addition other than bulking to increase oxygen transfer when composting on a large-scale or (in the case of composting) to generate heat to obtain mesophilic temperatures in cold climates.

Pyrene mineralization

Mineralization rates and extents were highest in composted petroleum-contaminated soil from the curing stage (Figure 6). A similar effect has been reported in a study of the composting of simulated municipal waste where PAH mineralization extents

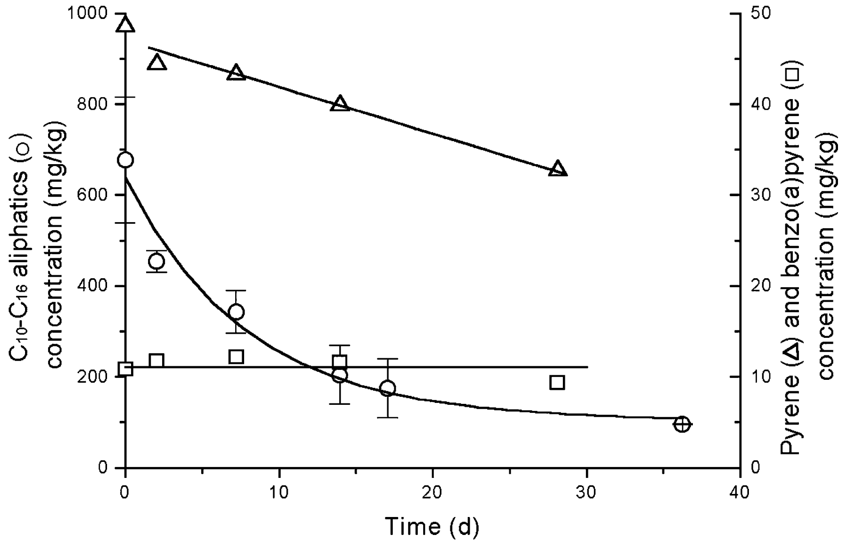

Figure 3. Concentrations of C_{10}–C_{16} aliphatics, pyrene, and benzo(a)pyrene during active composting of contaminated soil. Concentrations were standardized based on ash content. Error bars indicate standard errors (*n* = 2).

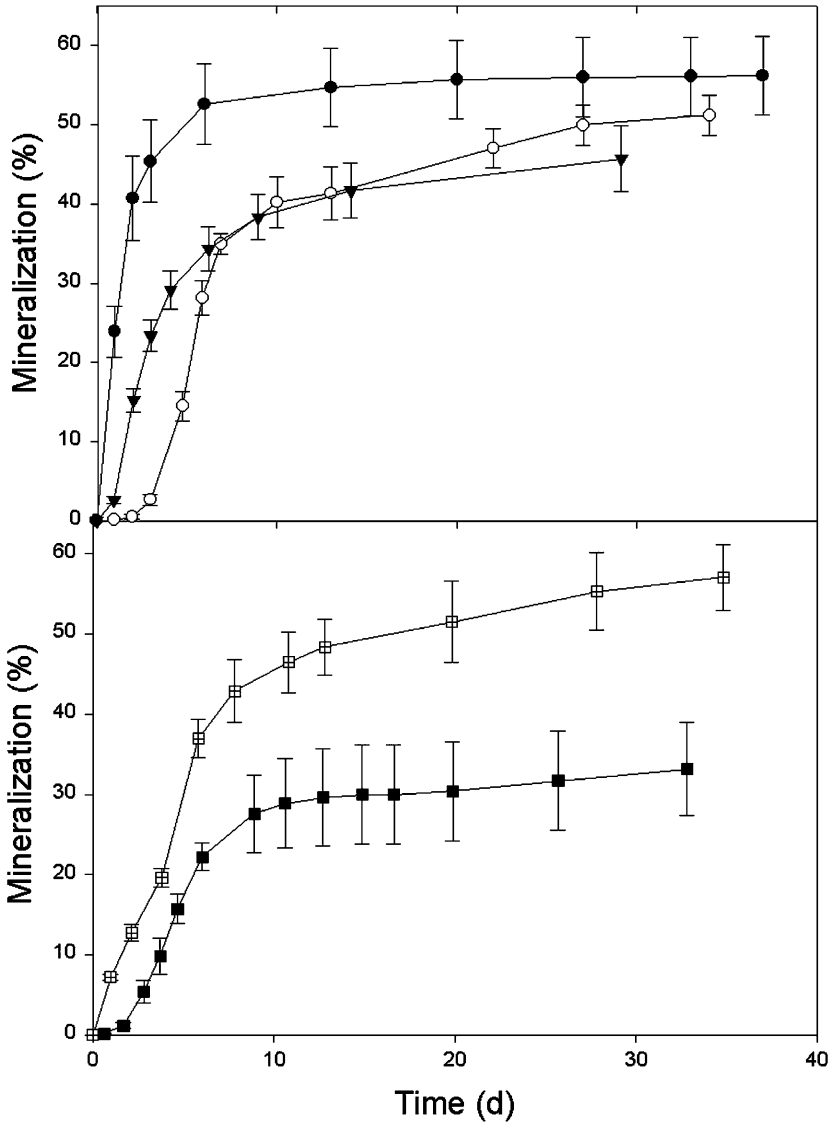

Figure 4. Mineralization of *n*-hexadecane in materials taken from different stages of composting contaminated soil. Mesophilic stage (○). Mesophilic stage again (sample from a different composting run) (●). Thermophilic stage incubated at ambient temperature (□). Thermophilic stage incubated at 55 °C (■). Curing stage (▼). Error bars indicate the standard error (*n* = 3 microcosms).

were higher with mature compost than with fresh compost (Martens 1982). When composted petroleum-contaminated soil from the mesophilic stage was incubated at ambient temperature, significant mineralization of pyrene occurred only after a lag phase of 18 days (Figure 6). A similar mineralization curve (but with an even more extended lag phase) was obtained when composted petroleum-contaminated soil from the thermophilic stage was incubated at ambient temperature. Very little mineralization was observed when composted soil from the thermophilic stage was incubated at 55 °C demonstrating that thermophilic microflora are not responsible for pyrene mineralization during composting. This conclusion is supported by the findings of Potter et al. (1999) who reported a disappearance of phenanthrene-degrading microorganisms at temperatures exceeding 60 °C and suggested that thermophilic temperatures destroy PAH degraders. Addition of cycloheximide to mature composted soil incubated under mesophilic conditions had little effect on pyrene mineralization

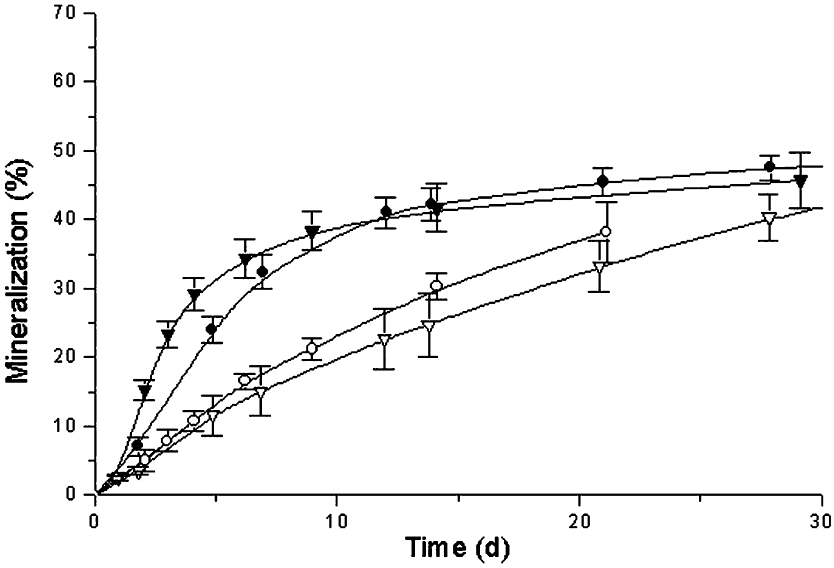

Figure 5. Mineralization of *n*-hexadecane in materials taken from the curing stage of composting contaminated soil (▼), the curing stage of composting clean soil (●), contaminated soil amended with composted clean soil from the curing stage (▽) and contaminated soil amended with mineral salts (○). Error bars indicate standard error ($n = 3$ microcosms).

(data not shown) demonstrating that prokaryotes are primarily responsible for PAH mineralization under these conditions.

In order to determine whether previous exposure to pyrene is required, composted clean soil from the curing stage was spiked with pyrene. After a 2-week lag phase, pyrene was mineralized relatively quickly even though it had not previously been exposed to PAHs (Figure 7). Kästner and Mahro (1996) have reported a similar finding. The increased lag phase suggests that although pyrene degraders were present, their enrichment resulted in

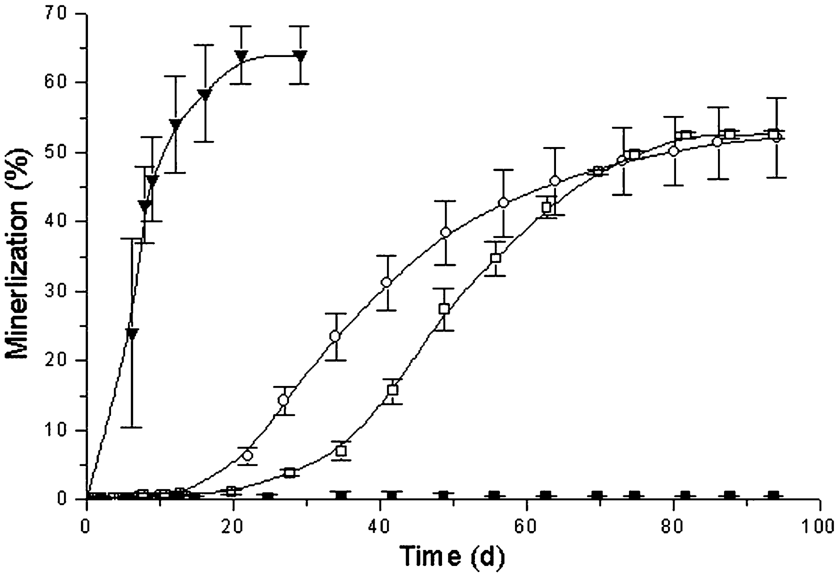

Figure 6. Mineralization of pyrene in materials taken from different stages of composting contaminated soil. Mesophilic stage (○). Thermophilic stage incubated at ambient temperature (□). Thermophilic stage incubated at 55 °C (■) Curing stage (▼). Error bars indicate standard error ($n = 3$ microcosms).

increased mineralization rates. However, even with time for enrichment, composted clean soil was able to mineralize only 50% of the radiolabeled pyrene while composted contaminated soil mineralized 65%. This may be due to sequestration of pyrene in the organic matrix during the lag phase, a process that has been demonstrated previously to render pyrene inaccessible to bacterial degradation (Bogan & Sullivan 2003).

PAH degradation rates during composting can best be modeled by first- or zero-order kinetics, reaching a plateau where no more degradation occurs (Joyce et al. 1998; McFarland et al. 1992; Potter et al. 1999). During experiments using the 11-l batch composting reactor, a constant pyrene removal rate of 0.31 mg kg^{-1} day^{-1} was observed during the first month (Figure 3). A pyrene concentration of 10 mg/kg was reached after 3 months of subsequent curing (data not shown). The initial apparent degradation was probably not due to mineralization but to sequestration. While Figure 3 shows an immediate decline in extracted pyrene, Figure 6 shows that mineralization could not begin until almost 3 weeks into the composting process.

The presence of mineral-associated humic acids can increase the CO_2 generation rate during pyrene degradation (Seibel et al. 1996). Furthermore, it has been suggested that sorption of both the microorganisms and the PAHs to the colloidal surfaces of humic matter stimulates their biodegradation (Laor et al. 1999). The amount of humic acids was much higher in the curing stage than in the earlier stages (Haderlein et al 2001) and it is well known that the degree of humification typically increases with compost age (Ciavatta et al. 1990). In this light, the likely explanation for increased pyrene mineralization in mature composted soil is the presence of humic matter, since it could play a major role in increasing the bioavailability of pyrene. Addition of mature composted clean soil to contaminated soil increased the amount of pyrene mineralization (Figure 7, open triangles) over that which could be attained by simply adding mineral salts (Figure 7, open circles). However, it resulted in much less mineralization than in composted clean soil alone (Figure 7, closed circles) likely due to a much lower compost to soil ratio. This indicates that if simple addition of compost to contaminated soil is to be an effective remediation method, either large amounts of compost must be used or the compost must have a very high humic content.

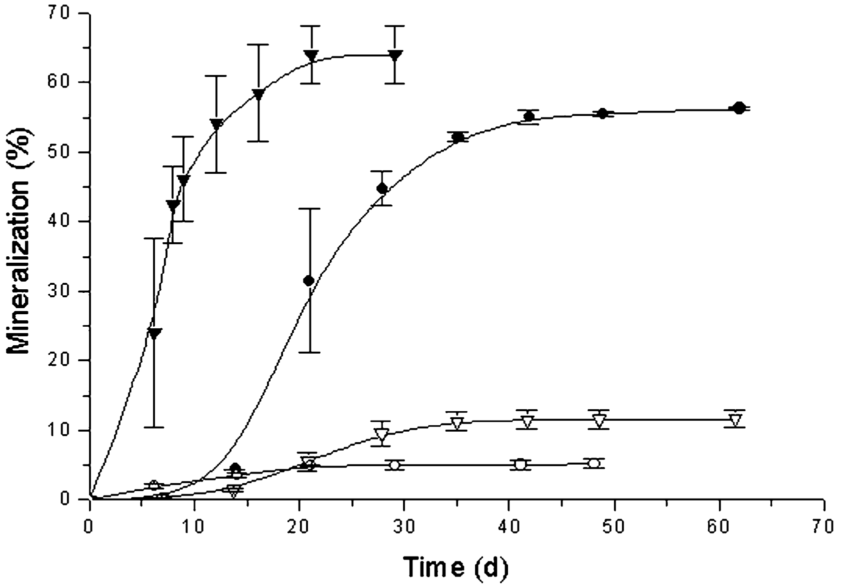

Figure 7. Mineralization of pyrene in materials taken from the curing stage of composting contaminated soil (▼), the curing stage of composting clean soil (●), contaminated soil amended with material from the curing stage of composting clean soil (▽), and contaminated soil amended with mineral salts (○). Error bars indicate standard errors ($n = 3$ microcosms, except for (●) where $n = 2$).

Benzo(a)pyrene mineralization

Composted contaminated soil from the curing stage that had been shown to mineralize pyrene readily was used to assess the benzo(a)pyrene mineralization potential. No significant mineralization was observed after 109 days (data not shown). This is in accordance with the data for extractable benzo(a)pyrene during active composting (Figure 1) where no decrease in benzo(a)pyrene concentration occurred. The concentration measured after 3 months of subsequent composting (11 mg kg^{-1}) did not differ significantly from the initial value and is consistent with reports that 5 and 6 ring PAHs are poorly degraded during composting (Potter et al. 1999). Although it has been demonstrated that bacteria can grow on the surfaces of PAH crystals (Tiehm & Fritsche 1995) other research shows a direct correlation between the rate of degradation and the aqueous phase concentration (Ramsay et al. 2003). Benzo(a)pyrene is much less soluble in water than pyrene (Sverdrup et al. 2002). Nevertheless, it is not known whether the difficulty in degrading benzo(a)pyrene is due to poor solubility or lack of enzymatic capacity in the system studied. Further investigation is required to extend the mineralization potential of mature compost to PAHs with more than 4 rings.

Radiolabeled-carbon balance

The ^{14}C balance conducted on material from mineralization studies of composted soil from the mesophilic stage (open circles in Figures 4 and 6) recovered only 58.9% ± 0.8 of initially added hexadecane radioactivity and 51.1% ± 5.6 of initially added pyrene radioactivity in the form of CO_2, carbonate or methylene dichloride extractable residues. Less than 2% of the initially added amount was bound in the form of carbonates. Thus, significant amounts of the initially added hydrocarbons had been rendered unextractable by conventional solvent extraction techniques. Such low recovery yields are common for PAHs and are observed even when the organic solvent extraction is followed by an alkaline saponification step (Eschenbach et al. 1998; Kästner et al. 1995). Although it has been shown that the addition of compost to contaminated soil allows a higher amount of PAHs to be recovered by extraction than in unamended soil (Kästner et al. 1995), there was still a considerable amount of hydrocarbon apparently unavailable for mineralization.

Conclusions

This study clearly shows that the thermophilic temperatures attained during a typical composting process do not directly aid in the mineralization of pyrene. It also shows that the high degree of microbial activity associated with the early stages of composting does not directly increase the rate of pyrene mineralization. It was also demonstrated that the rate of pyrene mineralization increases with the age of the compost. Although, addition of compost to contaminated soil was less effective than composting the soil, this was likely due to the much lower compost to soil ratio. Both composting soil and simply adding compost to the soil have the significant drawback of diluting the contaminants, thus increasing the overall volume. A preferred method would be to use an extract of humic materials. A challenge to the production of such extracts is that they typically are either acidic or contain potentially inhibitory amounts of salts. Bioaugmentation would benefit such a process. It would reduce the lag time, thus decreasing the amount of potentially irreversible PAH sequestration to humic materials.

Acknowledgements

This project was supported by funds contributed by the Environmental Science and Technology Alliance of Canada (ESTAC) and the Natural Science and Engineering Research Council of Canada (NSERC). The authors would like to thank Dr. Barth Smets for his helpful advice. Special thanks go to Dr. Marie-Claire Bonin Aly-Hassan for her assistance in microcosm preparation and sampling.

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