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Polycyclic aromatic hydrocarbon biodegradation as a function of oxygen tension in contaminated soil

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Abstract

Laboratory tests were conducted to determine the effect of soil gas oxygen concentration on the degradation and mineralization of spiked ^{14}C-pyrene and nonspiked 16 priority pollutant polycyclic aromatic hydrocarbons (PAH) present in the soil. The soil used for the evaluation was taken from a prepared-bed land treatment unit at the Champion International Superfund Site in Libby, Montana. This soil was contaminated with wood preserving wastes including creosote (composed primarily of polycyclic aromatic hydrocarbons and pentachlorophenol). Degradation rates of ^{14}C-pyrene and PAH compounds were found to be enhanced under soil gas oxygen concentrations between 2% and 21% in the contaminated soil. Between 45% and 55% of ^{14}C-pyrene spiked onto the soil was mineralized after 70 days at soil gas oxygen levels between 2% and 21%. No statistically significant mineralization was found to occur at 0% oxygen concentrations. Mineralization of ^{14}C-pyrene in contaminated soil poisoned with mercuric chloride was determined to be less than 0.5%. Degradation of indigenous nonradiolabeled PAH in non-poisoned soil was statistically significantly greater than in poisoned soil. These results indicated that the degradation of ^{14}C-pyrene and PAH compounds was biological and would occur under low oxygen concentrations. For example, the use of soil aeration technology in order to achieve continued treatment for buried lifts of soil while new lifts are added will decrease the total time for soil remediation of the prepared-bed.

Keywords: Polycyclic aromatic hydrocarbon; Biodegradation; Oxygen tension; Contaminated soil

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1. Introduction

Polycyclic aromatic hydrocarbons (PAH) include a group of organic priority pollutants of critical environmental and public health concern due to the following characteristics: (1) chronic health effects (carcinogenicity); (2) microbial recalcitrance; (3) high bioaccumulation potential; and (4) low removal efficiencies in traditional treatment processes [1]. PAH are classified as both carcinogenic and noncarcinogenic compounds. Carcinogenic PAH refer to fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)pyrene. Non-carcinogenic PAH refer to naphthalene, acenaphthylene, acenapthene, fluorene, anthracene, and phenanthrene. These compounds are produced by high-temperature industrial processes such as petroleum refining, coke production, wood preservation, and synthetic oil and gas production. PAH are associated with a wide range of hazardous waste sites (e.g., Superfund sites, uncontrolled hazardous waste sites not listed as Superfund sites, and hazardous waste land treatment sites) [2,3].

Though PAH are considered recalcitrant, losses do occur over time. These losses may occur through biotic processes and a variety of abiotic processes including leaching, photodegradation, and volatilization [4]. Several environmental factors are known to influence the capacity of indigenous microbial populations to degrade PAH. The interactions among environmental factors such as temperature, pH, soil gas oxygen concentrations, oxidation-reduction potential, and the presence of other substrates often influence feasibility of biodegradation [3,5,6]. However, the soil gas oxygen concentration is generally thought to be the limiting constituent in the biodegradation of PAH when nutrients are not limiting [7].

In order to achieve biodegradation of PAH compounds, these electron donors must be brought into contact with electron acceptors and with metabolically active microorganisms. It has been shown that the initial attack on the reduced hydrocarbon PAH molecule is an aerobic one and involves oxygen [8–10]. Dihydroxylation of the aromatic nucleus is a prerequisite for enzymatic cleavage of the benzene ring by oxygenases that incorporates molecular oxygen into the ring [11]. The incorporation of molecular oxygen into the PAH molecule is an activation step that makes the molecule biologically active and also a substrate for ring fission. A demonstration that molecular oxygen is involved in hydrocarbon utilization by microorganisms was presented by Hansen and Kallio [12]. Consequently, molecular oxygen is required for the insertion of oxygen into the hydrocarbon molecule, but not for the subsequent utilization of the oxygenated intermediates. Park and Sims [7] demonstrated that the concentration of oxygen was strongly correlated with the rate of apparent disappearance of 7,12-dimethylbenzanthracene spiked onto unsaturated soil in laboratory flask reactors.

In the presence of the appropriate organisms, biodegradation of lower molecular weight PAHs such as naphthalene occurs rapidly. Biodegradation of 3-ring PAH compounds such as acenaphthene, anthracene, and phenanthrene is much slower. PAH compounds with four or more fused benzene rings are believed to be biodegraded through the process of cooxidation whereby these non-growth PAH are oxidized when present as cosubstrates in a medium where one or more hydrocarbons are available for

growth [13]. Low concentrations of oxygen may limit the cooxidative process as discussed by Sims and Overcash [3]. The initial attack through cooxidation on a recalcitrant molecule such as a PAH is a coincidental attack on that compound that is probably of little consequence to the microorganism involved in the reaction. Neither energy nor carbon for biosynthesis results from this oxidation, and the reaction probably results in a loss of energy for the organism involved [14,15].

Bioremediation of PAH at field scale is generally accomplished using three types of systems: (1) In situ; (2) Prepared-bed; or (3) Bioreactor (e.g. slurry reactors) systems. This discussion will focus on the biodegradation of PAH in prepared-bed systems. This type of contaminated soil treatment generally consists of applying soil layers or lifts of about 6–12 inches into a prepared-bed bioreactor (also called a Land Treatment Unit (LTU)) [16]. The LTU is lined with clay and/or plastic liners. A leachate collection system, rainfall runoff/leachate storage, and a passive moisture control system are all components that may be used in LTU design. When the contaminated soil lift reaches the cleanup levels prescribed for the site, a new lift is applied and the remediation process is continued until all the soil reaches cleanup levels. The LTU is then capped. For microorganisms to get both air and water, the soil moisture content in the LTU is maintained between 70%–80% field capacity. Nutrients are also added as needed to the LTU either as solids or dissolved in the irrigation water. Tilling of the top lift is typically conducted on a weekly basis when weather permits [17,18].

Subsurface unsaturated soil in prepared-bed systems (buried lifts) is expected to be lower in oxygen concentration with increasing depth through the prepared-bed. This is expected to be caused by a rate of oxygen utilization by microorganisms that is higher than the rate of supply of oxygen through the prepared-bed caused by diffusion from the atmosphere. Diffusion has been considered the primary mechanism of oxygen transport in soils [19], and it is known that the oxygen diffusion rate decreases with an increase in soil depth [20].

As aerobic biological oxidation was found by Lin [21] to be the prime mechanism for the biodegradation of selected compounds of wood-preserving wastes, and because the rate of oxygen diffusion is known to decrease with an increase in soil depth, it is likely that the rate of biodegradation is buried lifts of a prepared-bed system is limited by the anticipated low concentrations of molecular oxygen.

There is currently a lack of information concerning the fate of PAHs in soil at field scale, most importantly those that are considered hazardous to human health and the environment. A small number of cases of land treatment of hazardous wastes have been documented with respect to predicting degradation, immobilization, and detoxification of hazardous wastes when mixed with soil and subjected to engineering management [16–18,21–23].

An important problem related to prepared-bed bioremediation of wood preservative contaminated unsaturated soil is the length of time required for bioremediation. Treatment time may be shortened by placing a new contaminated soil lift on top of a lower lift before the soil comprising the lower lift reaches target remediation levels. If critical oxygen levels necessary for biodegradation of PAH are present, then continued biodegradation of target chemicals in the lower lift may allow the lower lift to reach target remediation levels as the upper lift begins the bioremediation process.

The soil at the Champion International Superfund Site in Libby, Montana was contaminated as a result of wood preserving operations between 1946–1969. The contaminants consisted mainly of residuals from creosote, primarily PAH, and pentachlorophenol (PCP) wood preservatives. The prepared-bed system at this site consists of two 1 acre LTUs lined with a 60 mil high density polyethylene liner, compacted clay, and geotextile filter fabric. Nutrients, including ammonium sulfate and ammonium phosphate, are added periodically to the prepared-bed to prevent nutrient limitations to biodegradation. Design criteria for this site were total containment of contaminated soils, surface runoff, and leachate, with treatment and disposal of all contaminated soils within the LTU [17,23].

Oxygen concentrations previously measured in the prepared-bed at the site ranged from 0% to 21% in the soil gas phase. Results of sampling at greater depths than the top lift (nine-inch layer) of soil indicated a decrease in oxygen concentration with depth. This decrease is most likely the result of the oxygen at the greater depths being consumed rapidly relative to the rate of diffusion from the soil surface [24,25].

The objective of this study was to evaluate the effect of oxygen concentration in the soil gas phase on the rate and extent of biodegradation of PAH that occur in wood preservative contaminated soil undergoing bioremediation within the prepared-bed treatment system at the Champion International Superfund Site in Libby, Montana. Results from this research will be used to determine the potential for decreasing the required time for treatment of contaminated soil in prepared-bed systems by maintaining the necessary concentration of oxygen in the soil gas phase for active aerobic biodegradation of PAH in buried lifts within the prepared-bed.

2. Materials and methods

To determine the effect of oxygen concentration in the soil gas phase on biodegradation in the contaminated soil, the rates of biodegradation of radiolabeled pyrene and the 16 priority pollutant PAH compounds were measured in contaminated soil taken from the prepared-bed bioreactor at the Libby site.

2.1. Soil characterization

The contaminated soil evaluated in this study was taken from the Prepared-Bed LTU Number One at the Champion International Superfund Site in Libby, Montana [26]. A soil characterization was conducted by the Utah State University Soils Analysis Laboratory. Results are presented in Table 1. No additional nitrogen or phosphorous was added. Soil was taken from the top 6 inches of the LTU on August 24, 1994, that had been treated for two months but had not reached the cleanup level of 88 mg kg^{-1} total carcinogenic PAHs (including fluoranthene and pyrene, which are included in the list of total carcinogenic PAHs for this site [26]) and PCP (37 mg kg^{-1}).

2.2. Mass balance studies of ^{14}C-pyrene degradation in laboratory microcosms

Radiolabeled pyrene was chosen because it was identified as the rate limiting constituent at the atmospheric oxygen level in soil bioremediation at the site [23]. In

Table 1
Soil physical and chemical characteristics

Soil characteristics	Values
Physical	
% sand	48
% silt	39
% clay	13
Texture	Loam
Moisture retention(% water)	
1/3 Bar (Referred to as Field Capacity)	12.9
15 Bar	5.5
pH	6.6
EC ($mhos\,cm^{-1}$)	4.5
Organic Carbon (%)	1.88
CEC ($meq\,100g^{-1}$)	6.1
NO_3-N ($mg\,kg^{-1}$)	6.8
Total N (%)	0.10
P ($NaHCO_3$ extractable) ($mg\,kg^{-1}$)	20.8
Total Fe ($mg\,kg^{-1}$)	16216
Total Mn ($mg\,kg^{-1}$)	273.6

addition, the concentrations of unlabeled carcinogenic PAH compounds present in the contaminated soil were measured. A schematic of the glass laboratory biometer flask test reactors that were used for evaluation of the effect of oxygen concentration on PAH biodegradation is shown in Fig. 1.

The experiment consisted of evaluating the soil in laboratory biometer flask tests to determine the biodegradation response at five soil gas oxygen concentrations: (1) 0% (anaerobic), (2) 2% O_2, (3) 5% O_2, (4) 10% O_2, and (5) 21% O_2 (atmospheric). Laboratory tests were conducted by adding 50 grams of contaminated soil into each reactor. The soil moisture was maintained at 70% to 80% of field capacity (9–10% soil

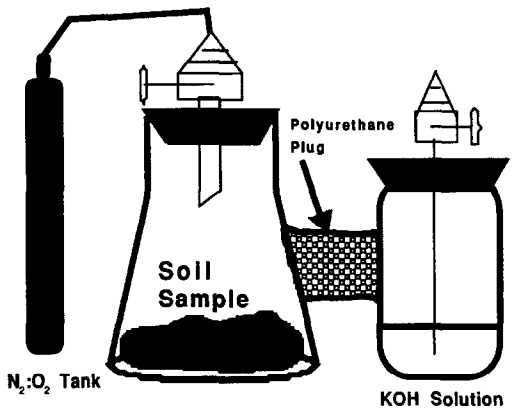

Fig. 1. Biometer flask laboratory test reactor for determining the effect of oxygen concentration in the headspace gas phase on PAH (^{14}C-pyrene) degradation.

moisture on dry weight basis) for the duration of the experiment by weighing each microcosm weekly to check for moisture loss. If the weight of the microcosm had decreased, a fine mist of water was sprayed onto the soil to bring it back to the initial weight recorded. 1.2 μCi of ^{14}C-pyrene with a specific activity of 55 mCi mmol^{-1}, and labeled on the 4, 5, 9, and 10 positions on the molecule, was spiked into triplicate sets of microcosms to determine the rate of mineralization to $^{14}CO_2$ as well as transformation of the parent compound as a function of oxygen concentration within the headspace of the microcosms. The use of radiolabeled chemicals allowed a chemical mass balance to be calculated that included fractions of radiolabeled carbon that were mineralized, volatilized, solvent extractable, and soil bound. For each biometer flask, a carbon dioxide trap (10 ml of 0.1 N KOH) was used to collect $^{14}CO_2$ to determine the amount of mineralized ^{14}C-pyrene. A polyurethane plug, located in the side of each flask, was used for collection of volatile parent compounds and intermediates.

The desired O_2 concentrations were maintained in the reactors using premixed ratios of N_2:O_2 in the feed stock gases. Each reactor was flushed with the appropriate O_2 concentration three times per week. Flushing was accomplished using a vacuum system where 0.5 atmospheres was drawn out of the reactor and replaced with the premixed gas. This was repeated seven times. Measurements of the headspace oxygen concentrations in the reactors were done repeatedly using gas chromatography. Leaks were not found in the reactors and oxygen concentrations were maintained at ±1% of the desired concentrations.

2.3. Experimental design

Triplicate reactors for each oxygen concentration were used for a total of 60 reactors, i.e., 3 replicates × 5 oxygen concentrations × 2 modes (non-poisoned and poisoned) × 2 times (day 0 and day 70) = 60 reactors. The reactors were incubated at the different oxygen concentrations for two to three weeks before the ^{14}C-pyrene was added in order to ensure steady state with regard to oxygen concentration. Immediately before the radiolabeled pyrene was added, a set of reactors was extracted to obtain concentrations at the beginning of the experiment. Radiolabeled pyrene was added as well as the 10 mL of KOH and reactors were monitored at seven day intervals for $^{14}CO_2$. The soil gas phase and KOH solution within the biometer flasks were accessible through two hypodermic needles with two-way Luer-Lock stop cocks attached. After 70 days (day 91), each reactor was destructively analyzed using the entire soil contents (50 g).

For evaluation of the biological component of degradation, a triplicate set of reactors at each oxygen concentration was poisoned with mercuric chloride ($HgCl_2$) that served as controls to evaluate abiotic loss within the soil. The poisoning procedure consisted of first air drying the soil to be poisoned, then mixing a solution of 10 000 mg l^{-1} $HgCl_2$ and water with the soil to be poisoned and bringing it to a concentration of 1 000 mg $HgCl_2$ kg^{-1} dry weight of soil at a water content of 70% to 80% of field capacity.

2.4. Quality assurance / Quality control

As part of the QA/QC plan, method blanks were extracted and analyzed as well as matrix spike duplicates following the guidelines given in Test Methods for Evaluating

Solid Wastes, U.S. EPA SW-846 [26]. Method blank recoveries were less than the method quantitation limits for the GC/MS. Matrix spike duplicate recoveries for quadruplicate spikes for phenanthrene (74.8% to 101.9%) and for pyrene (69.3% to 95.4%) were within QC limits set by U.S. EPA SW-846 [26]. Method detection and quantitation limits were determined for the PAH using gas chromatography/mass spectrometry (GC/MS) analysis. The method quantitation limits for each PAH are as follows: naphthalene, 1.4 mg kg^{-1}; acenaphthylene, 1.1 mg kg^{-1}; acenapthene, 1.3 mg kg^{-1}; fluorene, 1.5 mg kg^{-1}; phenanthrene, 2.1 mg kg^{-1}; anthracene, 1.7 mg kg^{-1}; fluoranthene, 2.8 mg kg^{-1}; pyrene, 2.8 mg kg^{-1}; benzo(a)anthracene, 1.3 mg kg^{-1}; chrysene, 1.7 mg kg^{-1}; benzo(b)fluoranthene, 2.4 mg kg^{-1}; benzo(k)fluoranthene, 1.9 mg kg^{-1}; benzo(a)pyrene 1.6 mg kg^{-1}; dibenzo(a,h)anthracene, 2.2 mg kg^{-1}; benzo(g,h,i)perylene, 1.6 mg kg^{-1}; and indeno(1,2,3-cd)pyrene, 1.7 mg kg^{-1}. Internal standards and surrogate standards for GC/MS were used and analyzed within the guidelines found in SW-846 [26].

2.5. Chemical analyses

The soil samples from the microcosms were Soxhlet extracted with methylene chloride using U.S. EPA Method 3540 [26]. Silica gel cleanup, U.S. EPA Method 3630 [26], was used to separate the PAH compounds from the heavy oils and greases. The instrumental analysis used to measure non-radiolabeled PAH in the soil was gas chromatography/mass spectrometry (GC/MS), U.S. EPA Method 8270 [26]. A 1 mil aliquote of the extract was analyzed for ^{14}C.

After the soil in the microcosms was extracted using the Soxhlet procedure, the soil was allowed to air dry. After the soil was air dried it was mixed thoroughly, triplicate subsamples of soil from each sample were weighed out and combusted for determination of soil bound ^{14}C (unextracted residues). The polyurethane plug was extracted using the Soxhlet extraction procedure, and the extract also was analyzed for ^{14}C. All ^{14}C analysis was performed on a Beckman LS 6000 Liquid Scintillation Counter.

3. Results and discussion

Results of the mineralization study for ^{14}C-pyrene are plotted in Fig. 2 and Fig. 3 for the non-poisoned and poisoned microcosms, respectively. Each data point plotted represents the mean of triplicate measurements. Results of $^{14}CO_2$ measurements for ^{14}C-pyrene in poisoned reactors (Fig. 3) show less than 0.5% mineralization at any soil gas oxygen concentration. This trend is consistent throughout the 70 day incubation period, indicating that biodegradation was responsible for the mineralization of ^{14}C-pyrene.

Results shown in Fig. 2 indicate that mineralization increased significantly as oxygen concentrations in the soil gas phase were increased above zero percent in the non-poisoned microcosms. Both rates and extents of mineralization were similar for all non-zero oxygen concentrations over the 70 day incubation. Ranges for cumulative mineralization of added ^{14}C-pyrene after 70 days of incubation were 45% to 55% within the oxygen concentration range of 2% to 21%. Although mineralization was measured as 13% after

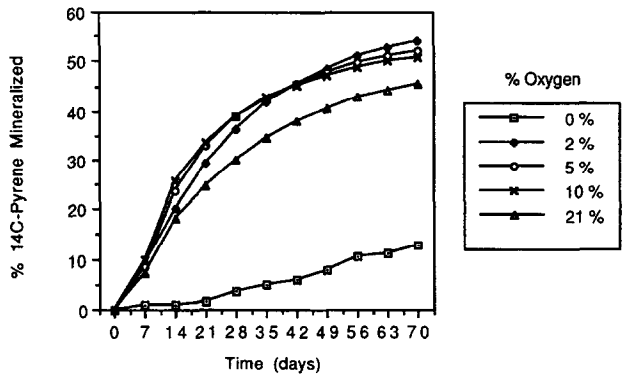

Fig. 2. Mineralization of ^{14}C-pyrene in non-poisoned soil microcosms as a function of oxygen concentration. Values are the means for triplicate reactors.

70 days incubation at 0% oxygen, the standard deviation was 13%. Mineralization in the microcosms incubated at 2%, 5%, 10%, and 21% oxygen was not significantly different until day 56. At day 56, the mineralization in 2% oxygen samples became significantly larger than the mineralization in the 21% oxygen samples. Significant differences were determined using the least significant difference (LSD) obtained from the two-way analysis of variance (ANOVA) method. Mineralization in the 5% and 10% microcosms was not significantly different from either the 2% or 21% samples throughout the duration of the experiment. At 0% oxygen concentration in the soil gas phase, mineralization of ^{14}C-pyrene was found to be statistically insignificant.

Fig. 4 shows results of a two-way analysis of variance for oxygen concentrations and percent mineralization for non-poisoned reactors averaged over the 70 day incubation period. Fig. 4 indicates that average mineralization was similar at 35% to 40% for all non-zero oxygen concentrations in the soil gas phase and significantly less for reactors incubated at zero oxygen concentration. The least significant difference was ± 1.20.

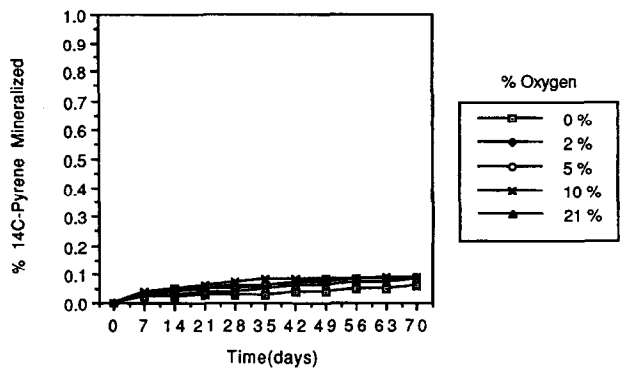

Fig. 3. Mineralization of ^{14}C-pyrene in poisoned soil microcosms as a function of oxygen concentration. Values are the means for triplicate reactors.

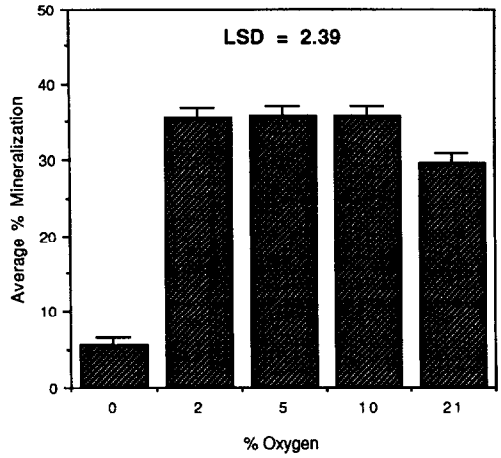

Fig. 4. Two-way analysis of variance for oxygen concentrations and percent mineralization of ^{14}C-pyrene in non-poisoned soil microcosm reactors with a least significant difference (LSD) of 2.39% mineralization.

Using this LSD, average mineralization at the atmospheric oxygen level is significantly lower than at the 2%, 5%, and 10% levels. This analysis was not appropriate for poisoned reactors because there was no significant mineralization observed at any soil gas oxygen concentration.

Results of the mineralization evaluation indicate that even small soil gas oxygen concentrations substantially below atmospheric oxygen levels are sufficient to allow mineralization of pyrene in the contaminated soil evaluated. One reason for production of $^{14}CO_2$ in the 0% microcosms would be from trace impurities in radiochemicals that may lead to formation of small quantities of $^{14}CO_2$ that does not originate from the substrate of interest [27].

Results of the mineralization evaluation indicate that even low soil gas oxygen concentrations, substantially below atmospheric oxygen levels, are sufficient to allow mineralization of pyrene in the contaminated soil evaluated. Results of the soil analysis shown in Table 1 indicate iron and manganese were present in the contaminated soil. These metals may have been used as alternate electron acceptors by microorganisms in mineralizing the ^{14}C-pyrene [28]. Under aerobic conditions iron and manganese have low water solubilities and exist in oxidized states [28]. However, as the soil system becomes more reduced and the iron and manganese are biologically reduced, the metals become water soluble. If the system becomes aerobic again, these metals will become reoxidized and precipitate out of solution. Under these conditions the iron and manganese may become more available to microorganisms as alternate electron acceptors. If aerobic conditions are prevalent, these metals may be found in a crystalline form that is not readily available for use by microorganisms. However, if they become solubilized and then reoxidized and precipitated, they are in an amorphous state and are readily available to microorganisms [29]. Mineralization of pyrene that occurred under low oxygen concentrations in the soil atmosphere might be supported by this phenomenon. The lower oxygen concentrations in the soil atmosphere provides more anoxic sites in

Table 2
Average distribution of ^{14}C in non-poisoned microcosms spiked with ^{14}C-pyrene ± standard deviation

Oxygen concentration	% Mineralized	% Volatilized	% Soil extractable	% Soilbound	% ^{14}C Mass recovered
0%	12.96 ± 12.86	0.27 ± 0.03	69.68 ± 14.03	8.47 ± 0.8	91.39 ± 19.05
2%	53.95 ± 4.76	0.10 ± 0.08	22.32 ± 4.59	15.08 ± 2.40	91.46 ± 7.03
5%	51.99 ± 1.21	0.06 ± 0.02	20.74 ± 1.21	15.66 ± 1.11	88.45 ± 2.04
10%	50.92 ± 1.45	0.14 ± 0.14	20.47 ± 0.69	14.20 ± 1.29	85.73 ± 2.06
21%	45.51 ± 1.02	0.08 ± 0.01	24.66 ± 1.54	15.41 ± 0.77	85.66 ± 2.00

the soil system and therefore more possibilities for microbial use of alternate electron acceptors.

It is also plausible that mineralization was less at 21% oxygen than at 2%, 5%, and 10% oxygen concentrations due to the involvement of microaerophilic soil microorganisms in the degradation process. In water-saturated marine environments and in uncontaminated saturated soil it has been shown that pyrene and other PAHs biodegraded at low oxygen concentrations [7,30,31].

Results for the chemical mass balance on ^{14}C-pyrene after 70 days of incubation are presented in Tables 2 and 3 for the non-poisoned and poisoned reactors, respectively. Results presented in these tables indicate that in every case the total mean recovery of ^{14}C from the microcosms was above 85% for all mass balances conducted. With regard to mineralization, values at the termination of the incubation period are provided, and indicate that mineralization was the most significant pathway for pyrene transformation, with the exception of microcosms incubated at 0% oxygen concentration, where mineralization was statistically equivalent to zero. Soil extractable ^{14}C was the second most significant fraction with a range of 20% to 25% recovery for non-zero oxygen concentrations. For the soil incubated at 0% oxygen concentration, solvent extractable ^{14}C accounted for the most significant fraction of the ^{14}C. Soil extractable ^{14}C could include any ^{14}C that partitions into the solvent phase and may include the parent compound as well as solvent soluble intermediates. The solvent extractable phase was not evaluated further.

The third most significant fraction of ^{14}C in the non-poisoned reactors was the soil bound (non-solvent extractable or humified) fraction. Relative to the 0% O_2 micro-

Table 3
Average distribution of ^{14}C in poisoned microcosms spiked with ^{14}C-pyrene ± standard deviation

Oxygen concentration	%Mineralized	%Volatilized	% Soil extractable	% Soil bound	% ^{14}C Mass recovered
0%	0.06 ± 0.01	0.35 ± 0.04	86.01 ± 0.90	8.56 ± 0.64	94.98 ± 1.12
2%	0.09 ± 0.01	0.22 ± 0.05	82.01 ± 0.59	8.77 ± 0.52	91.09 ± 0.79
5%	0.09 ± 0.01	0.29 ± 0.06	77.78 ± 0.73	10.97 ± 1.71	89.13 ± 1.86
10%	0.09 ± 0.01	0.29 ± 0.08	76.79 ± 1.46	12.46 ± 1.40	89.63 ± 2.02
21%	0.08 ± 0.02	0.36 ± 0.02	87.93 ± 10.31	8.34 ± 0.37	96.71 ± 10.32

cosms, a greater amount of soil bound ^{14}C-pyrene occurred in the non-zero O_2 microcosms where biological mineralization was the dominant fate mechanism. Additionally, except for the 10% O_2 microcosms, the amount of soil bound ^{14}C-pyrene in the non-zero O_2 microcosms was also greater than the poisoned controls. This may be due to chemical binding of intermediates to soil organic matter generated in the biodegradation of pyrene in a process referred to as humification [23].

Regarding volatilization of ^{14}C, results in Table 2 indicate that less than 0.3% ^{14}C was volatilized over the 70 day incubation period.

Results presented in Table 3 for poisoned microcosms indicate insignificant mineralization and volatilization, with the major fraction of ^{14}C measured as soil extractable ^{14}C within the range of 77% to 88%. This high value for solvent recovery is likely due to the absence of biotic transformations of the ^{14}C-pyrene in the soil. Soil bound ^{14}C was observed to be within the range of 8% to 12%. There were no differences between observations at non-zero oxygen concentrations and 0% oxygen concentrations. Total mean ^{14}C mass balances for the poisoned microcosms were all above 85% as observed with the non-poisoned microcosms.

Initial and final PAH concentrations were determined for all 16 priority PAH compounds in both the poisoned and non-poisoned microcosms. Eight of the 16 PAH compounds were found to be below the method quantitation limit (MQL) discussed in Section 2.3. As the concentrations of these compounds were below the MQL, statistical analysis could not be conducted with good reliability, therefore, results for the degradation of these compounds are not reported. Eight PAH compounds were found in concentrations above the method quantitation limit for both poisoned and non-poisoned reactors at day 0 and day 70. These eight compounds were: naphthalene, anthracene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, and benzo(a)pyrene.

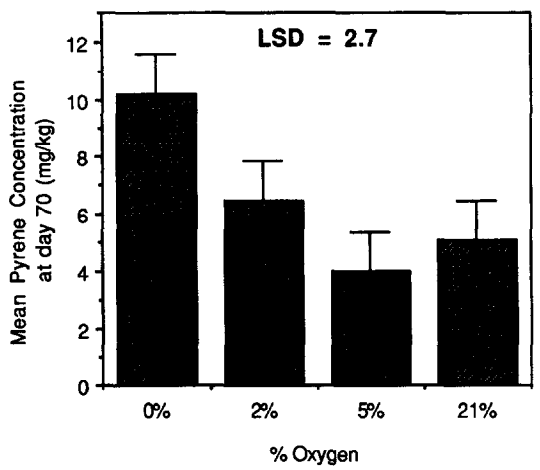

Fig. 5. Non-radiolabeled mean pyrene concentrations in non-poisoned reactors after 70 days as a function of soil gas oxygen concentration with an LSD of 2.7 mg kg^{-1}.

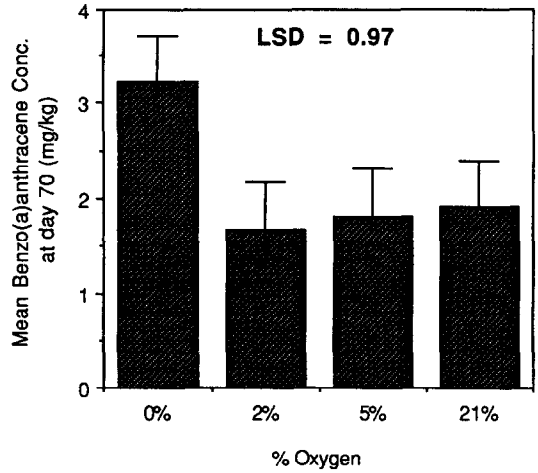

Fig. 6. Mean benzo(a)anthracene concentrations in non-poisoned reactors after 70 days as a function of soil gas oxygen concentration with an LSD of 0.97 mg kg^{-1}.

An analysis of variance (ANOVA) was used to determine whether oxygen concentration had a significant effect on PAH loss in the non-poisoned reactors. Three 4-ring compounds (pyrene, benzo(a)anthracene, and chrysene) were selected for the evaluation with regard to the effect of soil gas oxygen tension. For all three compounds (Figs. 5–7), there were significant differences after 70 days between the 0% oxygen level and the 2%, 5%, and 21% oxygen levels. Results indicate a larger degree of biodegradation when oxygen is present compared with when it is not present. Results shown in these

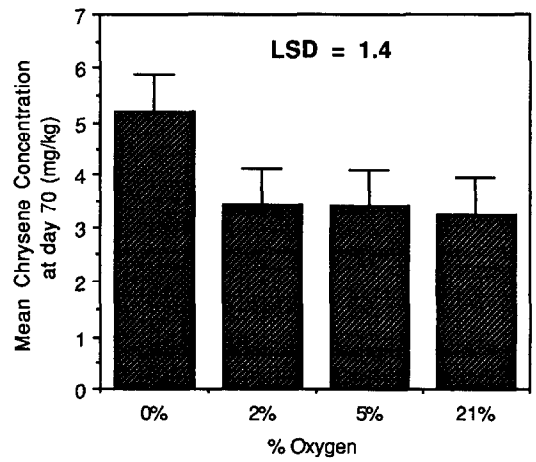

Fig. 7. Mean chrysene concentrations in non-poisoned reactors after 70 days as a function of soil gas oxygen concentration with an LSD of 1.4 mg kg^{-1}.

Table 4
Poisoned and non-poisoned microcosms PAH concentrations ($mg\,kg^{-1}$), percent difference, and least significant difference averaged over time and non-zero oxygen levels [a]

PAH compounds	Poisoned	Non-poisoned	% Loss	LSD
Naphthalene	7.3	6.85	6.2	0.37
Anthracene	23.13	11.96	48.3	3.28
Pyrene	15.73	6.72	57.3	1.73
Benzo(a)anthracene	4.88	2.66	45.5	0.45
Chrysene	8.06	4.73	41.3	0.76
Benzo(b)fluoranthene	8.9	7.31	17.9	0.92
Benzo(k)fluoranthene	8.39	6.74	19.7	0.88
Benzo(a)pyrene	11.06	9.31	15.8	1.21

[a] Eight PAH compounds were found below the MQL and therefore were not quantified.

figures indicate that the addition of oxygen to the soil gas phase caused a statistically significantly greater loss of pyrene, benzo(a)anthracene, and chrysene after 70 days when compared to concentrations of these compounds after 70 days at zero oxygen levels. For all non-zero oxygen levels, results indicate no significant difference between concentrations of these three 4-ringed compounds after 70 days. This means that the amount of the compound degraded in microcosms incubated at 21% oxygen is not significantly different from the amount of compound degraded in microcosms incubated at the 2% oxygen level.

There were also significant differences between PAH concentrations in the poisoned and non-poisoned microcosms for all eight compounds at all non-zero oxygen concentrations. These results are given in Table 4. From this Table, it is shown that when the soil was not poisoned there was a significant loss of PAH compared to the poisoned soil when oxygen was present. Statistically, there were not significant differences in PAH concentration among the 2%, 5%, 10%, and 20% oxygen levels within either non-poisoned or poisoned micrososms. Results for non-radiolabeled PAH agree with ^{14}C-pyrene results with regard to greater reduction in PAH concentration measured in non-poisoned non-zero oxygen microcosms compared with poisoned micrososms.

4. Conclusions

Results using ^{14}C-pyrene as an indicator of the amount of degradation that occurs under different oxygen concentrations demonstrated that oxygen tension does have a significant effect on rate and amount of degradation of ^{14}C-pyrene. At oxygen concentrations of 2% to 21%, mineralization accounted for between 45% to 55% of the ^{14}C-pyrene spiked onto the soil after 70 days. At these non-zero soil gas oxygen concentrations, mineralization of ^{14}C-pyrene was statistically significantly higher than at the 0% oxygen concentration while mineralization of ^{14}C-pyrene was not significant at the 0% oxygen level. Based on the negligible amount of mineralization measured in

poisoned microcosms, mineralization that occurred in the non-poisoned microcosms was caused by biological processes.

Based on a chemical mass balance and analysis for ^{14}C-pyrene, biological mineralization was the dominant fate mechanism at all non-zero oxygen concentrations. Soil extractable ^{14}C was the second largest fraction of recovered ^{14}C from microcosms incubated at non-zero oxygen concentrations, and was the dominant fate of ^{14}C-pyrene incubated at 0% oxygen concentrations in non-poisoned microcosms and in all poisoned microcosms. Soil bound (non-solvent extractable) ^{14}C was the third largest fate mechanism at all non-zero oxygen concentrations. Volatilization was minimal for all oxygen concentrations evaluated.

A recommendation for soil gas oxygen would be a minimum of 2% to 5% in buried lifts of a prepared-bed bioreactor in order to maintain conditions conducive for continued biodegradation of pyrene and other PAH. Low oxygen concentration requirements indicate minimal pumping of oxygen through the buried lifts would be required and then only when the soil gas oxygen concentration decreases to below 2% to 5%. If these critical oxygen levels necessary for biodegradation of PAH are present, then continued biodegradation of target chemicals in the lower lifts may allow the lower lift to reach target remediation levels after an upper lift has been applied. Treatment time may be shortened by placing a new contaminated soil lift on top of a lower lift before the soil comprising the lower lift reaches target remediation levels. Results from this research will be used to determine the potential for decreasing the required time for treatment of contaminated soil in prepared-bed systems by maintaining the necessary concentration of oxygen in the soil gas phase for active aerobic biodegradation of PAH in buried lifts within the prepared-bed.

5. Disclaimer

Although the research described in this article has been funded by the U.S. EPA, it has not been subject to internal Agency peer review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

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