HUMIFICATION OF PYRENE IN CONTAMINATED SOIL DURING LAND FARMING

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ABSTRACT

Pyrene, a polycyclic aromatic hydrocarbon (PAH) compound present in contaminated soil at the Champion International Superfund site in Libby, Montana, a site presently undergoing land farming/prepared-bed bioremediation, was evaluated with regard to incorporation into soil components (humification) in biologically active and poisoned treatments under varying environmental conditions including soil moisture, incubation temperature, and electron acceptor concentrations. The methyl isobutyl ketone (MIBK) humic fractionation method was used to separate soil organic matter (SOM) into fulvic acid, humic acid, bound humic acid, bound lipid, and mineral associated fractions. A radiolabeled chemical mass balance approach was used to evaluate the distribution of humified ^{14}C among humic fractions. This is the first application of the MIBK fractionation method to a soil previously contaminated with a complex waste mixture.

Humification, or nonsolvent extractable incorporation, of ^{14}C associated with ^{14}C-pyrene, was significantly higher in biologically active treatments with the humic acid fraction of SOM being the primary sink. The bound lipid SOM fraction was the primary sink for ^{14}C in poisoned microcosms. Treatments incubated at a soil matric potential of -1 bar demonstrated significantly more ^{14}C humification than those incubated at -16 bars. Total humified ^{14}C in treatments incubated at 10° C was equal to that of treatments incubated at 20° and 30° C at the end of 365 days of incubation. Addition of alternate electron acceptors did not significantly affect the extent or distribution of humified ^{14}C compared to the unamended control with only native electron acceptors.

Pyrene or pyrene transformation products associated with SOM fractions are likely to present a low risk to environmental receptors due to their difficulty of extraction. Because of this, humification of pyrene and other hydrophobic organic compounds (HOC) may be an acceptable endpoint in soil systems. Data generated from this project will be applied to the management of the Champion International Superfund site.

Key words: *humification, pyrene, polycyclic aromatic hydrocarbon (PAH), bioremediation*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrophobic organic compounds (HOC) composed of two or more aromatic rings (Menzie et al., 1992). Of the many PAH compounds, 16 have been identified as "priority pollutants" by the United States Environmental Protection Agency (USEPA) and seven of these are considered to be probable carcinogens (USEPA, 1997). PAHs are produced through natural combustion processes causing them to be ubiquitous in the environment, but anthropogenic combustion processes have also caused them to become concentrated at many industrial sites. Many sites where wood preservative application has occurred have been adversely affected because of the historical use of creosote, a combustion residue primarily composed of PAH compounds, as a wood preservative, and present a risk to human health and the environment through contamination of soil and groundwater.

One of these contaminated sites is the Champion International Superfund Site located in Libby, Montana. Wood-treating operations at the site from 1946 to 1969 caused extensive contamination of the soil and groundwater with both PAHs and pentachlorophenol (PCP). The site was listed as a

Superfund site in 1983 and has been undergoing remediation of both soil and groundwater since 1989 to risk-based cleanup levels. Soil is currently being bioremediated in a prepared-bed land treatment unit (LTU) at the Libby site (Piotrowski et al., 1993; USEPA, 1996).

Success of the bioremediation process at the Libby site is evaluated by monitoring contaminant disappearance by chemical analysis of solvent extractable PAHs and PCP. Studies using radiolabeled PAHs have identified mineralization and humification as the two primary loss mechanisms contributing to the disappearance of PAHs in the Libby soil (Hurst et al., 1996) and in other PAH-contaminated soils (Guthrie and Pfaender, 1998; Sims and Abbott, 1993). The term humification in this study is defined as irreversible (nonsolvent extractable) sequestration of contaminant parent compound or transformation products in the soil matrix and encompasses several mechanisms. One such mechanism is the biologically or abiotically mediated oxidative coupling of contaminants to soil organic matter resulting in covalent bonding between the two (Bollag, 1992; Whelan and Sims, 1992). The other sequestration mechanism includes intraorganic matter and intraparticle diffusion of parent compounds and transformation products resulting in a strong resistance to solvent extraction, but may allow for the slow release of these compounds over time (Luthy et al., 1997). There is a current lack of information as to which mechanisms control contaminant humification of PAHs into soil organic matter (SOM) and which environmental variables affect the humification process.

The objective of this study was to characterize the humification process of the four aromatic ring PAH pyrene under environmental conditions which may affect contaminant fate in the Libby soil undergoing bioremediation. These include soil moisture, soil temperature, and concentration of alternate electron acceptor amendments. Poisoned soil samples were evaluated to assess the relative contribution of biological processes to the treatment endpoints measured. The methyl isobutyl ketone (MIBK) humic fractionation method (Rice and MacCarthy, 1989) was employed to evaluate the distribution of nonsolvent extractable ^{14}C (added as ^{14}C-pyrene) among different soil humic fractions. This is the fist use of the MIBK method to evaluate humification of a chemical in a soil previously contaminated with a complex waste.

MATERIALS AND METHODS

Triplicate treatments of contaminated soil from the Libby "waste pit" area, where soil is stored before treatment, were adjusted to matric potentials of either -16 bars (40% of the soil field capacity) or -1 bar (85% field capacity) and were incubated at 10°, 20°, and 30° C. Other treatments of soil from the Libby LTU 2 were amended with two concentrations of three potential alternate electron acceptors: amorphous iron oxyhydroxide (Fe III), manganese oxide (Mn IV), and nitrate (potassium nitrate). The treatments were spiked with ^{14}C-pyrene and incubated in biometer flasks which allowed monitoring of $^{14}CO2$ evolved through mineralization. Head space oxygen concentrations were maintained between 0 and 5%, representative of Libby waste pit soil, by the method of

Hurst et al. (1996, 1997). Replicates of electron acceptor amended treatments were sacrificed at 41 and 294 days. Treatments subjected to different soil moistures and temperatures were sacrificed after 365 days. This range of sampling times is representative of required soil treatment times at the Libby site.

All of the soil in each replicate flask was extracted sequentially and analyzed to provide a radiolabeled ^{14}C chemical mass balance. Water extractable (300 ml of 5mM $CaCl_2$), followed by solvent extractable (16 hour Soxhlet extraction with hexane:acetone (1:1)), and finally non-solvent extractable ^{14}C (by direct combustion) fractions were determined. Following solvent extraction, the soil was divided into humic acid, fulvic acid, mineral matter, and humin components. The humin component was then further fractionated into bound-humic acid and bound-lipid fractions. Separation of these five components was achieved using the methyl isobutyl ketone (MIBK) partitioning procedure (Rice and MacCarthy, 1989). All components were then analyzed for associated ^{14}C by liquid scintillation counting. Poisoned controls of all treatments containing 1000 mg/kg mercuric chloride were also evaluated.

The MIBK Fractionation Method

A modified form of the MIBK fractionation method of Rice and MacCarthy (1989) was used to separate different components of the humic material remaining in the solvent-extracted soil. Ten gram soil samples were stirred in 200 ml of 0.5 N NaOH for 24 hours to extract humic and fulvic acids. The entire mixture was then placed in a 2L separatory funnel and 100 ml of NaOH, used to rinse the extraction flask, were added. Two hundred milliliters of MIBK were then added to the funnel and the entire mixture was acidified with 16 ml concentrated HCl and shaken vigorously for five minutes. The mixture was allowed to separate for 45 minutes and the aqueous phase containing larger soil mineral components and fulvic acid was discharged. Three hundred milliliters of NaOH were then added to the separatory funnel, shaken for five minutes, and allowed to separate for 45 minutes. The aqueous phase containing residual mineral material and humic acid was discharged. Finally, 300 ml of distilled water (DW) were added to the funnel, shaken, and allowed to separate for one hour. The aqueous phase containing residual mineral material and bound-humic acid was discharged leaving the bound-lipid fraction suspended in the MIBK phase.

Residual mineral material in all humic fractions was allowed to settle for six days, after which the aqueous phase of each fraction was sampled for ^{14}C analysis. The bound-lipid extract was mixed vigorously before sub sampling. Residual mineral material from all fractions was combined and combusted to determine ^{14}C associated with the mineral fraction.

Quality Assurance/Quality Control

In accordance with the HSRC project QA/QC plan, the quality of all solvent extractions was evaluated through the use of method blanks and a fluoronaphthalene surrogate spike added to all

samples before extraction. HPLC analysis of all solvent extracts was evaluated through the use of matrix spike duplicates and calibration check analyses as recommended in USEPA (1986).

To assess the quality of data generated from the MIBK fractionation, the sum of all nonsolvent extractable ^{14}C associated with humic fractions isolated by the MIBK fractionation method was compared to nonsolvent, extractable ^{14}C assessed by direct soil combustion in a Harvey Biological Oxidizer (R.J. Harvey Instrument Corporation, Hillsdale, NJ). The MIBK fractionation method was tested for reproducibility, using triplicate spiked samples prior to incubation and repeated analysis of a single sample following incubation, and found to be consistently repeatable.

RESULTS AND DISCUSSION

Biological vs Abiotic Humification

The distribution of humified ^{14}C among humic fractions after 294 days of incubation in biologically active and poisoned microcosms that had been amended with alternate electron acceptors is shown in Figure 1. The percentage of the initial ^{14}C spike, which was nonsolvent extractable at the end of the incubation period, was significantly higher in biologically active microcosms (10.7%) than in poisoned microcosms (3.1%).

While other studies have found increased humification with biological activity (Guthrie and Pfaender, 1998; Hurst et al., 1996; Sims and Abbott, 1993), unique to this study is the finding that the humic-acid fraction of soil organic matter was the primary sink of humified ^{14}C under biologically active conditions. In addition, significant increases in ^{14}C associated with other humic fractions were also observed. Although there was a statistically significant increase in the ^{14}C activity associated with the bound-lipid fraction of biologically active microcosms, this difference was small, indicating that biological activity did not play a major role in humification within this soil humic fraction.

Soil Moisture Effects on Humification

Results of the MIBK fractionation of humified ^{14}C in treatments incubated at matric potentials of -1 bar and -16 bars (85% and 40% field capacity, respectively) are presented in Figure 2. Increased soil moisture resulted in a significant increase in ^{14}C humification. ANOVA analysis of biologically active microcosms showed that the effect of moisture was highly significant ($p<0.0001$) with regard to total ^{14}C humification and to humification within all humic fractions, except for the bound-lipid fraction (Figure 2). In poisoned microcosms, soil moisture status did not effect the extent or distribution of humified ^{14}C.

Effects of Incubation Temperature and Alternate Electron Acceptor Amendments on Humification

Incubation temperature did not have a statistically significant effect on the extent of ^{14}C humification at the end of the 365-day incubation period as shown in Figure 3. This finding is not unexpected since the humification process in the Libby soil appeared to be highly influenced by the

ability of the soil microbial community to mineralize the ^{14}C-pyrene and treatments incubated at all three temperatures had mineralized approximately 25% of the added ^{14}C by the end of the 365-day incubation period. Although temperature did not have an effect on the extent of mineralization, increased temperature did significantly increase the mineralization rates of ^{14}C-pyrene during the first 125 days of the incubation period.

A trend of increased ^{14}C association with increased temperature in the humic acid, bound humic acid, and mineral-associated humic fractions appears to be present, although statistically significant differences between temperature treatments were present only in the fulvic acid and bound-humic acid fractions. A decrease in fulvic acid associated ^{14}C was observed at 20 and 30° C. Similar trends were observed in ^{14}C associated with humic fractions of poisoned treatments.

Percentages of the initial ^{14}C spike sequestered in each AEA-amended treatment evaluated by both MIBK and combustion methods are shown in Figure 4. Some differences in total sequestration between AEA treatments were statistically significant as in the case of the Fe-1x and NO3-2x treatments (Figure 4), but these differences were small. Humification in poisoned treatments, likewise, was not significantly affected by the type or amount of electron acceptor amendment.

AEA amendments had no statistically significant impact on the distribution of ^{14}C among humic fractions isolated by the MIBK fractionation method in biologically active or poisoned treatments. Sequestration of ^{14}C was highest in the humic-acid fraction under biologically active conditions and highest in the bound-lipid fraction in poisoned treatments (Figure 1).

CONCLUSIONS

This is the first study to examine the humification or sequestration of a hydrophobic PAH into SOM fractions which include fractions of soil humin in a soil contaminated with a complex creosote waste. It is also the first application of the MIBK fractionation method to such a complex, waste-contaminated soil.

Results indicate that the mechanism of humification is significantly enhanced under biologically active conditions. The primary sink of humified ^{14}C (added as ^{14}C-pyrene) in biologically active microcosms was the humic-acid fraction of the Libby soil as isolated by the MIBK fractionation method. The primary sink of nonsolvent extractable ^{14}C under poisoned conditions was the bound-lipid fraction of the soil humin. Both humic fractions were resistant to water and solvent extraction, and therefore may be acceptable endpoints for hydrophobic contaminants or transformation products in soil treatment systems.

The humification process was most significantly affected by soil moisture status. Soil incubated at a matric potential of -1 bar (85% field capacity) mineralized approximately 25% and humified approximately 15% of the added ^{14}C-pyrene, while soil incubated at -16 bars (40% field capacity) appeared to be limited in its ability to mineralize and humify the added ^{14}C-pyrene. Incubation

temperatures in the range of 10°-30° C, and alternate electron acceptor amendments above those naturally occurring in the soil did not significantly affect the extent or distribution of humified ^{14}C that was assessed at the end of the incubation period when the extent of mineralized ^{14}C was approximately the same among treatments.

Recommendations for the management of the prepared-bed land treatment facility at the Champion International Superfund site will be made based on data generated from this project. These recommendations include investigation of real-time moisture monitoring in the field based on the significant effect of soil moisture status on both contaminant humification and mineralization. Addition of alternate electron acceptors to the Libby soil is not recommended.

Because the humification mechanism was found to be highly dependent on biological activity, monitoring of soil-gas oxygen concentrations which can limit biological activity (Hurst et al., 1996, 1997) may also be recommended. Results of this study have shown that when sufficient oxygen is present, biologically mediated humification and mineralization will occur even under lower temperatures (10° C). Based on this finding, addition of untreated soil to the land treatment units at the Libby site is recommended even under cold conditions if dewatering can be accomplished to allow for nonlimiting soil-gas oxygen concentrations.

Findings of this study have led to an improved understanding of treatment mechanisms operating in soil at the Libby site and have generated information that can be applied to management of the site. Application of these management techniques has the potential to significantly reduce treatment time and cost at the Champion International Superfund site. The MIBK fractionation method may have application in the characterization of the humic material involved in contaminant humification at other PAH-contaminated sites.

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Figure 1. Average percentage of initial ^{14}C-spike found in each humic fraction in biologically active

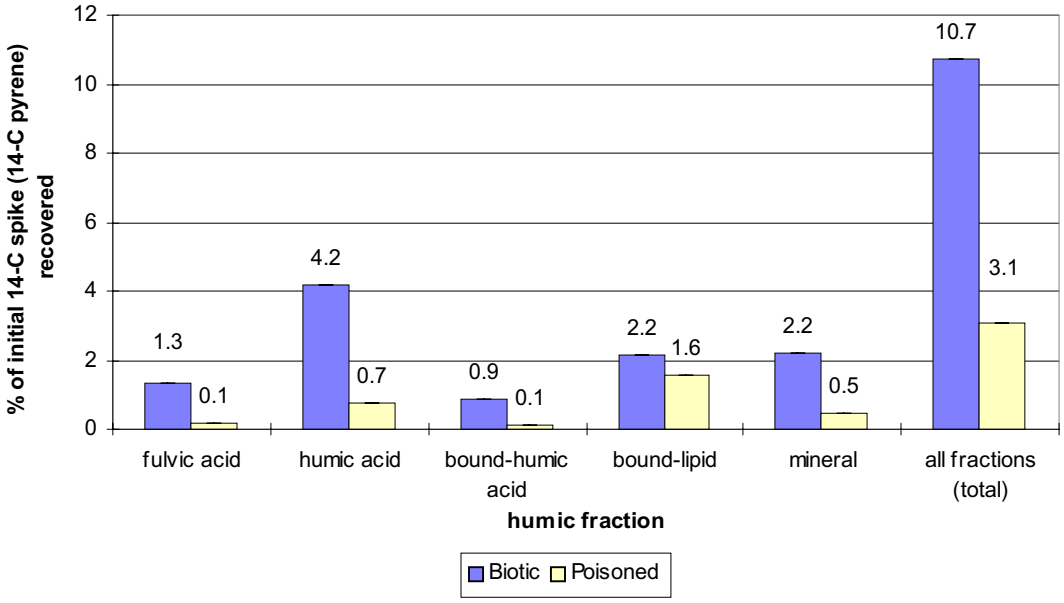

Figure 2. Average percentage of initial 14-C spike found in each humic fraction, isolated by the MIBK fractionation, under biologically active and poisoned conditions at soil matric potentials of -1 bar (85% field capacity (F.C.)) and -16 bars (40% F.C.) after 365 days (Error bars=LSD, n=9).

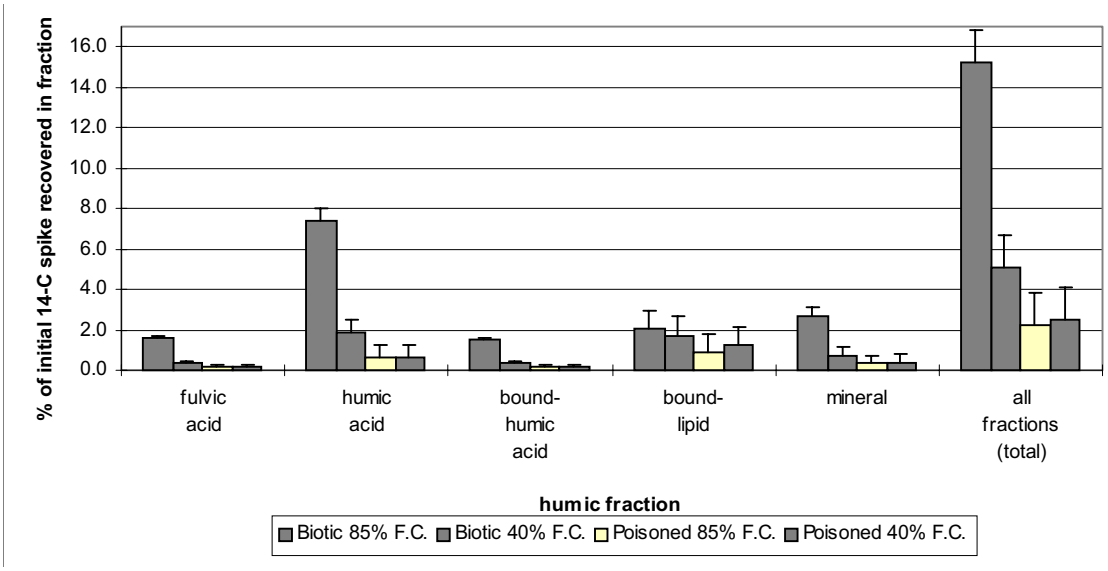

Figure 3. Average percentage of initial 14-C spike found in each humic fraction under biologically active conditions at soil matric potential of -1 bar after 365 days. Error bars are shown for fractions in which statistically significant differences were found (Error bars=LSD, n=3).

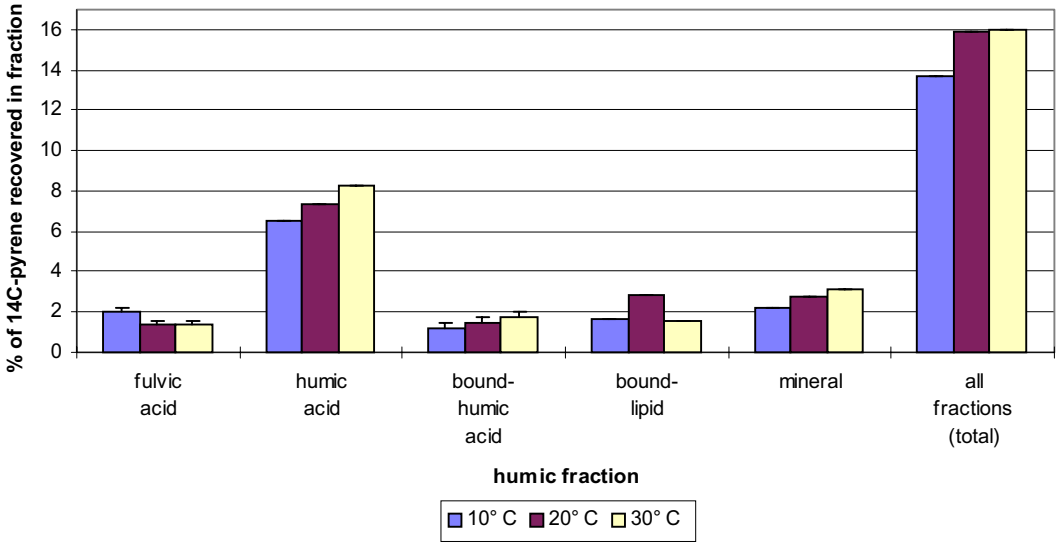

Figure 4. Average percentages of initial 14-C spike sequestered by various treatments as evaluated by combustion and the MIBK fractionation method in biologically active treatments. Error bars indicating LSD are for comparison of sequestration in different treatments within a given evaluation method (combustion or MIBK). Fe=iron, Mn=manganese, NO3= nitrate, O2-Cont=no amendments, 1x= amended with the calculated stoichiometric electron-acceptor requirement, 2x= amended with double the stoichiometric electron-acceptor requirement.