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Use of solvents for PAHs extraction and enhancement of the PAHs bioremediation in coal-tar-contaminated soils

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Use of solvents for PAHs extraction and enhancement of the PAHs bioremediation in coal-tar-contaminated soils

by

Pak-Hing Lee

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ABSTRACT

Bioremediation of coal-tar-contaminated soils is a challenging task because of the low solubility of the polycyclic aromatic hydrocarbons (PAHs) and their high partitioning with soils. As such, not all the PAHs present in the soil will be available for biodegradation. Enhancing the availability of PAHs will make bioremediation attractive as a treatment technology. Because of the PAH low solubility and high partitioning, the analyses of PAHs in coal-tar-contaminated soils using standard protocols are usually time-consuming and expensive.

In the first part of the study, a direct solvent extraction method was evaluated to assess its application for the extraction and analysis of PAHs in coal-tar-contaminated soils. The direct solvent extraction method using acetone as an extraction solvent was shown to be equivalent to the Soxhlet extraction method (EPA Method 3540). In this study, five coal-tar-contaminated soils from four manufactured gas plant (MGP) sites in Iowa were used.

In the second part of the study, a mild extraction method was developed as an assessment tool to estimate the extent of PAH degradation or availability in contaminated soils. The percent of PAHs biodegraded after 35 days for 16 individual PAHs using soil slurry reactors were found to correlate well with the percent of PAHs extracted with an acetone-water mixture of 0.6. Two correlations relating the percent biodegraded and the percent of PAHs extracted with 0.6 acetone-water mixture along with the soil properties and PAH properties were developed. This assessment tool using acetone-water mixture of 0.6 may be used to estimate the availability of PAHs in soils.

In the third part of the study, two miscible solvents - acetone and ethanol - were used to enhance the biodegradation of PAHs using soil slurry reactors. The degradation rates of PAHs were shown to be significantly enhanced for soils pretreated with solvents. Four- and 5-ring PAHs in pretreated soils were found to degrade by at least 2 to 6 times faster than soils that were not pretreated. The results show that solvents such as acetone and ethanol may enhance the availability of PAHs.

Using mixed cultures from a soil-slurry reactor, the degradation of [^{14}C]benzo(a)pyrene (BaP) in various aqueous phase and solid phase treatments was studied.

The purpose of these studies was to investigate the effects of low molecular weight PAHs on the degradation of BaP. In liquid phase treatments with and without 2- and 3-ring PAHs, no significant mineralization was found for all treatments for 14 weeks even though there was significantly microbial respiration. This indicates that BaP may not be used as a microbial source of carbon and energy and 2- and 3-ring PAHs, most probably, were not used for the cometabolization of BaP. In solid phase treatments, 18.6% of initial ^{14}C were mineralized to CO_2 for the land-farming soil from Vandalia road site after 25 weeks incubation. Results of the solid phase treatment showed that the degradation of PAHs was sequential in that the lower molecular weight PAHs were degraded first followed by the higher molecular weight PAHs such as BaP.

CHAPTER 1. INTRODUCTION AND OBJECTIVES

Introduction

In the United States, manufactured gas plants (MGPs) were used for over a century to generate gaseous fuel, called town gas, for cooking, heating, and lighting. In the early 1950s, all of the MGPs were abandoned when a cheaper gas, natural gas, was available. Coal, coke, and crude oil were used in the gasification processes to produce town gas. Several by-products were also generated. Although some by-products were reused for other purposes, the less valuable by-products or wastes, were usually disposed of on site. Among the wastes, coal tar containing polycyclic aromatic hydrocarbons (PAHs) was the most common. A number of the PAHs in the coal tar are carcinogens presenting a serious risk to humans and the environment. The U.S. Environmental Protection Agency (USEPA) has selected 16 PAHs to be on the list of priority pollutants. At many former MGP sites, the surrounding soils and groundwater are contaminated with PAHs. There are more than 5,000 former MGP sites located throughout the United States that require some form of remedial action. In Iowa alone, there are about 100 sites.

Bioremediation is a destructive technology that converts PAHs to carbon dioxide, water, and biomass and is a relatively inexpensive technology. However, bioremediation is relatively slow and may not remove the PAHs to the regulatory clean-up levels or to the background concentration levels. Factors affecting the biodegradation of PAHs include the chemical and physical properties of the PAHs and soils, availability of nutrient, temperature, and the types of microorganisms present. Although much are known about the conditions for the biological degradation of PAHs, these conditions tend to be very site specific.

In general, 2- and 3-ring PAH compounds are readily degraded by soil bacteria and fungi under aerobic conditions. Aromatic compounds of 4- and more rings are significantly more difficult to degrade. The low solubilities and the highly hydrophobic properties of these compounds have been cited as some of the reasons for their lack of biodegradability. The bioavailability of PAHs varies for different soils and is dependent on the contact time between the PAHs and the soil. For these reasons, meeting the regulatory clean-up levels for 4- and more ring PAHs in coal-tar-contaminated soils or PAH-contaminated soils may be

difficult. Much efforts have been expended investigating the issues of PAH availability for biodegradation and developing methods to enhance biodegradation. Several researchers have used surfactants and solvents to enhance the availability of PAHs. Results of these studies were mixed with some studies showing enhanced biodegradation while some showing negative effects. To estimate the extent of PAHs availability, several simple assessment tools have been proposed. However, there is no one tool that can estimate the extent of PAH availability.

Dissertation Objectives

The dissertation has four separate objectives as listed below. The unifying theme of the objectives is the availability of PAHs in various soils and enhancing their availability for bioremediation by using solvents. This research is not to investigate the fundamentals of bioavailability but rather explore the use of various solvents to enhance bioavailability and therefore the degradation of PAHs. The objectives of the dissertation are as follows:

1. To develop a simple extraction method to analyze PAH-contaminated samples. The conventional Soxhlet method for extracting PAHs from soils requires intensive handling and attention and use of high volumes of solvent. Development of a simple extraction method that is comparable to USEPA-approved analytical method will provide an inexpensive method for determining PAHs in contaminated soils.
2. To assess the use of solvent-water mixture as an assessment tool for the estimation of the amounts of PAH present in contaminated soils that may be biologically available. The assessment approach used acetone and ethanol at various solvent-water mixture ratios. Acetone and ethanol were chosen because they are relatively less toxic in comparison to other solvents.
3. To investigate the application of solvents to enhance the bioavailability of PAHs and the bioremediation of PAH-contaminated soils. PAH-contaminated soils were pretreated with acetone or ethanol to enhance the availability and degradation of PAHs.
4. To asses the impact of 2- and 3-ring PAHs and different soil types on the biodegradation of a 5-ring PAH, benzo(a)pyrene.

Dissertation Organization

This dissertation is organized into seven chapters with the Introduction and Objectives and the Literature Review in Chapter 1 and 2, respectively. The Literature Review is followed by four chapters that contained four manuscripts prepared for publication in various journals. Each chapter addresses the specific objectives listed above. The first manuscript has been published in the American Society of Civil Engineers (ASCE) Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management. The general conclusions for this dissertation are drawn in the last chapter, Chapter 7.

CHAPTER 2. LITERATURE REVIEW

2.1 Background

During the mid-1800s to the early 1950s, manufactured gas plants (MGPs) were a major supplier of gaseous fuel in the United States (US) for heating, lighting, and cooking (Hatheway, 1997). Coke, coal, and fossil oil were gasified to produce gaseous fuel known as town gas. This process of incomplete combustion in the absence of air at high temperatures but without burning, drives off volatile gases from the coal or coke. Coal tar was a byproduct of the gasification process. It has been estimated that during the era of MGPs, 11 billion gallons of coal tar were produced. A large portion of which was discarded as a waste product at more than 5,000 MGP sites located throughout the US (Larsen, 1997; Findlay et al., 1995). The Iowa Department of Natural Resources estimates that there are over 100 sites in Iowa alone (Golchin et al., 1997).

Most of the MGPs were operated at a time when the state of environmental awareness in the US was not strong and environmental regulations were not stringent or in existence. Coal tar was disposed of in tar wells, sewers, landfills and nearby pits or streams. Most of the disposal facilities were unlined. As a result of these practices, coal tar contaminants from these disposal sites have contaminated soils and have leached into aquifers - becoming a serious environmental problem (Iowa Department of Natural Resource, 1995). Coal tar contains polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), phenolics, inorganic compounds of sulfur, cyanide, nitrogen, and metals (Luthy et al., 1994). The compounds of concern in the coal tar are PAHs. Sixteen PAHs have been placed on the United States Environmental Protection Agency (USEPA) list of priority pollutants (Keith and Telliard, 1979). These PAHs have been found to have toxic, mutagenic, and carcinogenic properties (International Agency for Research on Cancer, 1983; World Health Organization, 1998). Sites where refining and distillation of crude oil have occurred are frequently contaminated with PAHs and other aromatic and aliphatic hydrocarbons. PAH contamination at industrial sites is commonly associated with spills and leaks from storage tanks and with the conveyance, processing, use, and disposal of these fuel oil products. Wood-treatment activities are also linked with PAH contamination (Mueller et al., 1989a) as

PAHs are the major constituents in wood treatment chemicals such as creosote (approximately 85% PAHs by weight) and anthracene oil.

Several technologies have the potential to remediate soils contaminated with PAHs. These technologies include solvent extraction, thermal desorption, wet air oxidation, supercritical extraction, and bioremediation. Solvent extraction is the transfer of organic contaminants from a solid or liquid matrix into a solvent or solvents (Helsel, 1991; Jones, 1992; Anderson, 1995). Thermal desorption is the application of heat, either directly or indirectly, to volatilize and remove organic contaminants present in the solid matrix (American Academy of Environmental Engineers, 1998; Stryker, 1999). Wet air oxidation uses elevated temperatures and pressures to oxidize the organic constituents of a liquid or slurry waste stream from PAH-contaminated soils or sediments (Timberlake and Garbaciak, 1995). Supercritical extraction is an advanced technique of separation based on enhancing the solvating power of water at elevated temperatures and pressures to achieve a complete and unhindered extraction of contaminated soils (Kothandaraman et al., 1992; Kocher et al., 1995). Bioremediation is the use of microorganisms to break down hazardous organic materials to harmless compounds (Cacciatore and McNeil, 1995; Rogers et al., 1993; Wilson and Jones, 1993). Bioremediation has several advantages over thermal and physical-chemical treatment techniques. For example, bioremediation is relatively inexpensive in comparison to thermal and physical-chemical treatment techniques (see Table 2-1) and the properties of the soil are generally not altered. Furthermore, bioremediation is a destructive

Table 2-1. Comparison of treatment costs for contaminated soils (Helsel, 1991; Cacciatore and McNeil, 1995)

Treatment Process	Cost ($/ton)
Solvent extraction	90-300
Thermal desorption	100-300
Wet air oxidation	40-500
Supercritical extraction	250-450
Bioremedaition	
In-situ	15-70
Bioslurry	75-180
Landfarming	20-75

technology that converts organic contaminants to carbon dioxide, water, and biomass. Other technologies such as solvent extraction transfer contaminants from one phase to another.

2.2 Polycyclic Aromatic Hydrocarbons

PAHs consist of two or more fused benzene rings in linear, angular, or cluster arrangements. By definition, they contain only carbon and hydrogen atoms, although nitrogen, sulfate, and oxygen atoms may readily substitute in the benzene ring to form heterocyclic aromatic compounds, which are commonly grouped with PAHs. Polycyclic aromatic molecular structures are formed whenever organic substances are exposed to high temperatures and rich carbon environment. Over hundreds tons of PAHs are generated through natural and human activities annually. The major natural source is forest fire. Active human sources include automobile exhaust systems, industrial stacks, household cooking, and fire places. In addition, PAHs are one of the many constituents in fossil fuel.

At MGPs, PAHs were generated under similar conditions. Several types of manufactured gas processes were employed in the US and they are described in various specialized technical literatures (Lowry, 1945; Wilson and Wells, 1950; Gas Engineers Handbook, 1966; Rhodes, 1966). Primarily, three processes were used to manufacture town gas. The processes were coal carbonization process, carburetted water gas process, and oil gas process. The quantities and characteristics of the by-products and residuals, including PAHs in the coal tar, greatly varied with the process. Even for the same type of process, the yield and quality of gas and by-products were different, depending on the temperature of the gas generator, the class of feedstock, the period of distillation and other factors (Royle, 1907). In general, the gasification process consists of heating the coal, coke, and crude oil to produce combustible gases such as methane, hydrogen, carbon dioxide or carbon monoxide. The weaker aliphatic bonds are broken and aromatic rings are fused during the combustion and pyrolysis process. Since aromatic products are more stable than their precursors, high temperatures are needed for the formation of PAHs (Dias, 1987). As some of these PAHs are probable human carcinogens, their distribution in the environment and possible exposure to human beings have been the focus of much research. The total potential dose of carcinogenic

PAHs for humans from water, air, sediment, soil, and food was estimated by Menzie et al. (1992). The estimated intake of PAHs by nonsmokers ranged from 3 to 15 μg/day.

2.2.1 The chemical structure of PAHs

The chemical structures of 16 USEPA priority PAHs are shown in Figure 2-1. The stability of PAHs is indicated by the ring arrangement, linear being the most unstable and angular (rings in step) the most stable (Blumer, 1976). However, the stability of the PAHs based on the position of the benzene rings is not always true for the biodegradation of PAHs. For example, the angular ring arrangement of phenanthrene, a 3-ring compound, is considered thermodynamically more stable than the linear arrangement of anthracene which is also a 3-ring compound. However, phenanthrene is found to be more rapidly degraded than anthracene in soil (Mahmood and Rama Rao, 1993; Park, 1990). The major reason is that phenanthrene (1 mg/L) is more soluble in water than anthracene (0.045 mg/L).

The 16 USEPA priority PAHs may be classified into two major groups: non-carcinogenic and carcinogenic PAHs. Each group includes eight compounds. Non-carcinogenic PAHs with 2- to 4- benzene rings include: naphthalene, acenaphthene, anthracene, phenanthrene, acenaphthylene, fluorene, fluoranthene, and pyrene. Carcinogenic PAHs with 4- to 6- benzene rings include: benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3,c-d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene.

2.2.2 Physical-chemical characteristics of PAHs

Although PAHs as a whole may behave similarly in the environment, each PAH compound has a unique set of physical and chemical properties (see Table 2-2). At ambient temperatures, pure PAH compounds are solids. The general characteristics of PAHs are high melting and boiling points, low vapor pressures, Henry's Law constants, and very low water solubilities. Because of their hydrophobicities, PAHs tend to be asorbed to the organic matter in soil. Based on the PAH physical-chemical properties, Smith et al. (1999) illustrated the correlation between environmental fate mechanisms of PAHs and their molecular weight or number of benzene rings (Figure 2-2). For example, PAH compounds with higher number

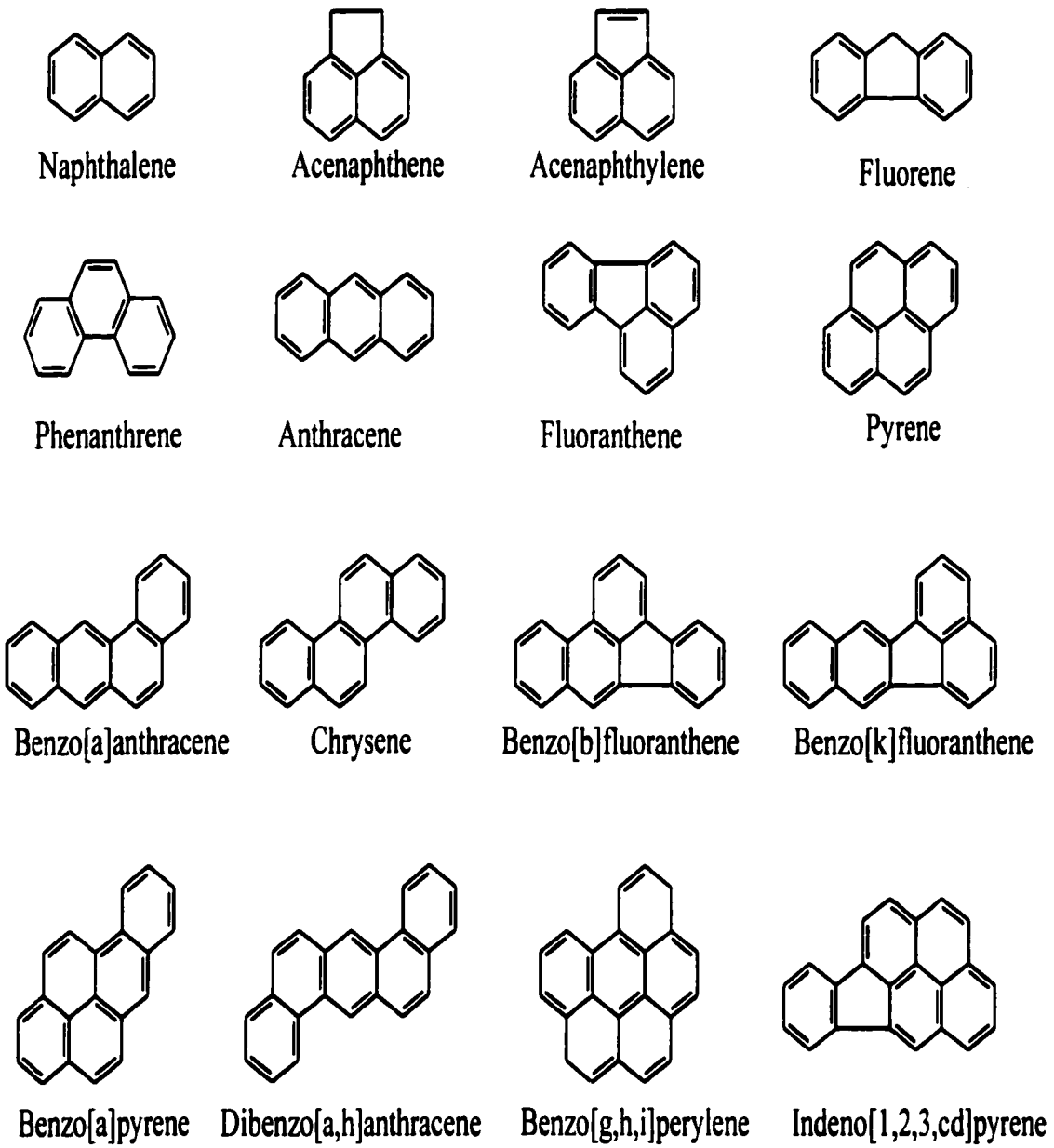

Figure 2-1. Chemical structures of 16 polycyclic aromatic hydrocarbons

Table 2-2. Physical-chemical properties of polycyclic aromatic hydrocarbons (LaGrega et al., 1994; World Health Organization, 1998)

Compounds	No. of rings	Chemical formula	Melting point (°C)	Molecular weight (g/mole)	Solubility in water at 25°C (μg/l)	Vapor pressure at 25°C (Pa)	Henry's Law constant at 25°C (KPa m^3 $mole^{-1}$)	n-octanol water partition coefficient (log K_{ow})	Partition coefficient in organic carbon (log K_{oc})
Naphthalene	2	$C_{10}H_8$	81.0	128.18	31690	10.4	4.89E-2	3.37	3.11
Acenaphthene	3	$C_{12}H_{10}$	95.0	154.21	3420	2.9E-1	1.48E-2	4.00	3.65
Acenaphthylene	3	$C_{12}H_{10}$	93.0	152.20	3930	8.9E-1	1.14E-3	3.70	3.40
Anthracene	3	$C_{14}H_{10}$	216.4	178.24	45	8.0E-4	7.30E-2	4.45	4.15
Fluorene	3	$C_{13}H_{10}$	116.0	166.22	1690	8.0E-2	1.01E-2	4.18	3.86
Phenanthrene	3	$C_{14}H_{10}$	100.5	178.24	1000	1.6E2	3.98E-3	4.46	4.15
Fluoranthene	4	$C_{16}H_{10}$	108.8	202.26	206	1.2E-3	6.5E-4 (20°C)	4.90	4.58
Pyrene	4	$C_{16}H_{10}$	150.4	202.26	130	6.0E4	1.1E-3	4.88	4.58
Chrysene*	4	$C_{18}H_{12}$	253.8	228.30	1.8	8.4E-5 (20 °C)	NA	5.61	5.30
Benzo(a)anthracene*	4	$C_{18}H_{12}$	160.7	228.30	5.7	2.8E-5	NA	5.60	6.14
Benzo(b)fluoranthene*	5	$C_{20}H_{12}$	168.3	252.32	14	6.7E-5 (20 °C)	5.1E-5	6.06	5.74
Benzo(k)fluoranthene*	5	$C_{20}H_{12}$	215.7	252.32	4.3	1.3E-8 (20 °C)	4.4E-5(20°C)	6.06	5.74
Benzo(a)pyrene*	5	$C_{20}H_{12}$	178.1	252.32	3.8	7.3E-7	3.4E-5(20°C)	6.06	6.74
Dibenzo(a,h)anthracene*	5	$C_{22}H_{14}$	266.6	278.36	0.5	1.3E-8 (20 °C)	7.0E-6	6.80	6.52
Indeno(1,2,3-cd)pyrene*	6	$C_{22}H_{12}$	163.6	276.34	0.53	1.3E8 (20 °C)	2.9E-5(20°C)	6.50	6.20
Benzo(g,h,i)perylene*	6	$C_{22}H_{12}$	278.3	276.34	0.26	1.4E-8	2.7E-5(20°C)	6.51	6.20

*Carcinogenic PAHs
NA: data not available

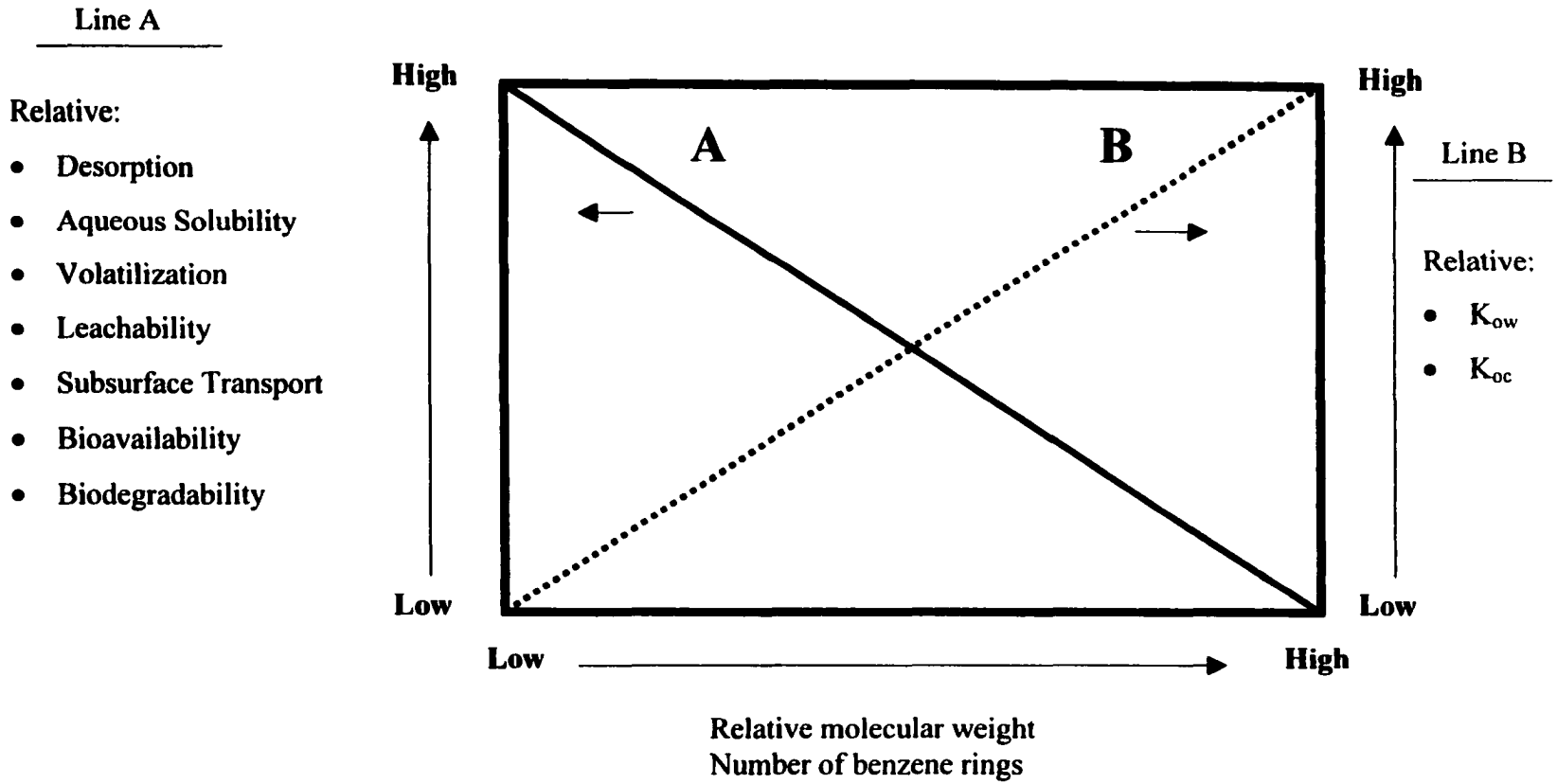

Figure 2- 2. Influence of PAH physical and chemical characteristics on environmental fate mechanisms (Smith et al., 1999).

of benzene rings would exhibit high values of octanol-water partition coefficients (K_{ow}) and soil organic carbon partition coefficients (K_{oc}), and would tend to be highly adsorbed to the organic matter of soils or sediments. In contrast, lower molecular weight compounds tend to be more volatile, soluble, and bioavailable. Therefore, they tend to have a higher rate of biodegradation. The solubilities of the 16 PAHs are inversely proportional to the number of fused benzene rings. For example, the solubility of naphthalene in distilled water at 25°C is approximately 31.69 mg/L while that of benzo(g,h,i)perylene is approximately 0.00026 mg/L.

2.2.3 Abiotic removal of PAHs

The volatility of PAH is inversely proportional to the number of fused benzene rings. Thus, volatilization is expected to be significant for 2- and 3-ring PAHs. Park et al. (1990) found that the abiotic losses of 2- and 3-ring PAH compounds from soil samples were approximately 2% to 18% over 48 hours. The mean volatilization loss of naphthalene was measured at 31%. However, no significant volatilization losses were found for PAH compounds containing more than three benzene rings. Lauch et al. (1992) also found that only small amounts of 2- and 3-ring PAHs such as naphthalene, fluorene, and phenanthrene were detected in the air samples for the first few days of operation of a bio-slurry reactor for the treatment of creosote-contaminated soils. Wild and Jones (1993) found that loss of PAHs from sewage sludge amended soils through abiotic processes has a significant effect only on PAHs with less than four benzene rings. Bossert and Bartha (1986) also reported that abiotic losses of 3-ring PAHs may be significant and primarily through volatilization. An inverse correlation between the number of rings in PAHs and their volatilization losses from soil is generally assumed (see Figure 2-2).

PAHs are chemically stable with no functional groups that can result in hydrolysis under environmental conditions. Therefore, hydrolysis does not contribute to the abotic degradation of PAHs (Radding et al., 1976; Howard et al., 1991). PAHs can be expected to be photodegraded in air and water, but to a very low extent in soils and sediments (Sims and Overcash, 1983).

2.3 Bioremediation

Bioremediation involves the use of microorganisms to degrade hazardous organic constituents to harmless substances such as carbon dioxide and water. If bioremediation is to be considered as a treatment technology the following questions must be asked before proceeding (Crawford and Crawford, 1996; King et al., 1998; Adriano et al., 1999): (1) can the target contaminants be biologically degraded? (2) are there specific microorganisms present in the matrix? and (3) what are the environmental conditions needed to sustain biodegradation? Similarly, Rogers et al. (1992) summarized the primary factors that must be considered to control PAHs biodegradation in soil when bioremediation is applied:

1. Types of microorganisms present
2. Accessibility of the target compound
3. Nutrients present (usually nitrogen and phosphorous)
4. Electron acceptors present (usually oxygen)
5. Soil chemical conditions (pH, redox, acidity/alkalinity, and organic matter)
6. Soil physical conditions (texture, moisture content, temperature)
7. Supplemental organic compounds present.

Depending on the above factors, organic compounds may be completely biodegraded in a matter of days or weeks or may persist unchanged for a long period of time. Changing the chemical or physical conditions of the soil such as soil pH, moisture, aeration, and nutrient levels may enhance the degradation process. Bioagumentation, adding specifically adapted microorganisms, may also enhance the process. More details are described below.

2.4 Environmental Factors Affecting Bioremediation

2.4.1 Soil Moisture

Soil moisture is a measure of the amount of water present in the soil. Water is essential for not only meeting the physiological requirements of microorganisms but also for the transport of nutrients and metabolic by-products in and out of the cell. Water also affects the pH, aeration, and osmotic pressure in soil. In addition, soil bacteria tend to live in soil

water at all times (Killham, 1994). Thus, soil biological activity is dependent on an adequate supply of water within the soil environment. In general, aerobic biodegradation of hydrocarbons in soil systems requires soil moisture of between 50% and 70% of the soil field capacity (Riser-Roberts, 1992). The field capacity is a rough estimate of the amount of water retained within the soil micropores under gravity flow and is highly dependent on the texture and porosity of the soil. As shown by Sims et al. (1990), the control of soil moisture resulted in enhanced biodegradation of PAHs with the shorter half-lives of the PAH compounds for higher soil moisture contents (Table 2-3).

Table 2-3. Effects of soil moisture on PAH degradation (Sims et al., 1990).

Moisture Content	Half-life in waste soil mixture (days)		
	Anthracene	Phenanthrene	Fluoranthene
20-40% field capacity	43	61	559
60-80% field capacity	37	54	231

2.4.2 Soil pH

The soil pH, a measure of the hydrogen ion (H^+) activity in soil water, is an important parameter for microbial growth. The pH of the soil water affects the microbial enzymes directly and the availability of required nutrients and trace heavy metals that indirectly influence the growth of microorganisms. In general, fungi can tolerate a pH as low as 5, but most bacteria and actinomycetes prefer normal to slightly alkaline pH conditions. Joshi and Lee (1996b) reported that most bacteria in PAH-contaminated soils from MGP sites require a pH range between 6.5 and 7.5. Walter et al. (1991) showed a maximum degradation rate of pyrene at pH 7 for *Rhodococcus* sp. UW1. For a compost system, Kastner and Mahro (1996) observed that there were no significant differences in the degradation rates of phenanthrene, anthracene, fluoranthene, and pyrene for an artificially contaminated soil with pH 5.2 and pH 7. Although the dioxygenase activity from specific microorganisms showed a high tolerance between pH 5.5 and 9.0 for the degradation of PAHs (Walter et al., 1991), researchers recommended neutral pH conditions for the treatment of PAH-contaminated soils. Mueller et al. (1991b) found that higher degradation of PAHs was obtained in a slurry phase reactor when the soil pH was adjusted to 7.1 from 10. Sims et al. (1990) had similar results in that

the half-lives of PAH compounds were lower when the soil pH was adjusted from 6.1 to 7.5 for a solid phase treatment system.

2.4.3 Electron acceptors

In order to achieve biodegradation of PAH compounds, electron donors (carbon sources) must be brought into contact with suitable electron acceptors and with metabolically active microorganisms. Most studies, showing the degradation of PAH, are under aerobic conditions (Hurst et al., 1996; Prince and Drake, 1999). Bauer and Capone (1985) have shown that the initial attack on the PAH molecule is an aerobic process involving oxygen. The eukaryotic and prokaryotic PAH degradation pathways are well characterized whereby bimolecular oxygen is incorporated into the PAH ring during the initial enzymatic attack (Pothuluri and Cerniglia, 1994). The pathway of degradation is provided in a later section (see Section 2.5). Hurst and coworkers (1996) reported that the degradation rates of ^{14}C-pyrene and the 16 priority PAH compounds were enhanced under soil gas oxygen concentrations of between 2% and 21% while statistically insignificant mineralization was found to occur at 0% oxygen concentration. Joshi and Lee (1996b) indicated that oxygen amendment via hydrogen peroxide solution improved remediation in a packed bed with the degradation rates of the packed bed being comparable with that of a completely mixed system.

Several studies have reported that low molecular weight PAHs can be degraded under anaerobic conditions. For example, Mihelicic and Luthy (1991) demonstrated that degradation of naphthalene could occur under denitrifying conditions but no degradation of naphthalene or anthracene was found under strictly anaerobic conditions. Coates et al. (1996) showed that anaerobic mineralization of naphthalene and phenanthrene in contaminated sediment under sulfate-reducing conditions can occur. Similarly, Zhang and Young (1997) evaluated the biodegradation of seven PAHs under denitrifying, Fe-reducing, sulfate-reducing, and methanogenic conditions and found that PAHs degradation was noted only under sulfate-reducing conditions after 5 months of incubation. Recently, McNally et al. (1998) reported that pyrene (4-ring compound) could be degraded under denitrifying conditions. However, pyrene was completely degraded within 50 hours under aerobic

Table 2-4. Half-lives of 16 PAHs in soil under aerobic and anaerobic conditions (Howard et al., 1991)

PAH compounds	Aerobic conditions (days)	Anaerobic conditions (days)
Naphthalene	0.5-20	25-258
Acenaphthene	42.5-60	170-240
Acenaphthylene	12.3-102	49.2-408
Anthracene	32-60	128-240
Fluorene	16-200	64-800
Phenanthrene	50-460	200-1,840
Fluoranthene	140-440	560-1,760
Pyrene	210-1,900	840-7,600
Chrysene	102-680	408-2,720
Benzo(a)anthracene	371-1,000	1,484-4,000
Benzo(b)fluoranthene	360-610	1,440-2,440
Benzo(k)fluoranthene	910-2,140	3,640-8,560
Benzo(a)pyrene	57-530	228-2,120
Dibenzo(a,h)anthracene	600-720	2,400-2,920
Indeno(1,2,3-cd)pyrene	361-940	1,444-3,760
Benzo(g,h,i)perylene	590-650	2,360-2,600

conditions but 72 hours was needed for complete degradation of pyrene under anaerobic conditions. The half-lives of 16 PAHs under aerobic and anaerobic conditions are presented in Table 2-4.

2.4.4 Nutrients

For a typical bioremediation system, microorganisms must obtain their nutrients for growth from the soil environment or from nutrients that are artificially added to the system. Design of a successful bioremediation project considers the availability of carbon, nitrogen, and phosphorus for microbial growth. Carbon is usually provided for by the substrate. The nitrogen and phosphorus needed at a site may be estimated by assuming the microbial requirements are approximately similar to the composition of their cells (Alexander, 1977; Zitrides, 1983). Other elements such as potassium, manganese, calcium, iron, cobalt, copper, and zinc are generally available in adequate concentrations in most soils and usually there is no need to add these nutrients for a bioremediation process.

In general, PAH-contaminated soils from MGP sites do not have enough nitrogen and phosphorus to support microbial growth (Ghiorse et al., 1989; Erickson et al., 1993). Russell

(1992) proposed that the carbon: nitrogen: phosphorus (C:N:P) ratio of 100:5:1 to 100:20:0.5 should be adequate to support biological growth. The C:N:P ratio based on the composition of biomass is 100:10:1. However, there is no general consensus on how to estimate the amount of nitrogen and phosphate needed. In addition, the amount of carbon present may be equal to the total organic carbon present which includes organic matter and the target contaminants. If the amount of carbon present is based on the total organic carbon in the soil, the amount of nutrient application may be overestimated since some of the organic matter are not biodegradable. If the amount of carbon is based on the target contaminants, the amount of nutrient applied may be underestimated since some of the organic matter in the soil may be biodegradable. In addition, the nutrient cycle in the soil will also affect the nutrient consumption. Brook et al. (1997) found that nitrate may be inhibitory at concentrations equal to 500 mg N/kg. As such the nitrogen nutrient needed may have to be based on the total contaminant concentration.

The various forms of nitrogen applied for bioremediation have their own unique advantages and disadvantages. Nitrate and ammonium are the readily available forms of nitrogen responsible for microbial nutrition whereas urea must be transformed to release the ammonium ion. Nitrate is highly soluble and can be lost by leaching while ammonium can be readily volatilized depending on the soil pH. For now, the form of nitrogen that works best for microbial degradation of PAH-contaminated soils is still unclear (Walworth et al., 1997). Brook et al. (1997) reported that hydrocarbon degradation rates of diesel fuel in nutrient-limited soil were highest for urea than ammonium nitrate. However, their studies were done with one type of soil and a single concentration of nitrogen. The nitrogen level used may be too high and may have inhibited microorganism growth since no significant degradation of total petroleum hydrocarbon from ammonium nitrate-amended soil was observed.

Laboratory and field data on the importance of adding nutrients for the successful implementation of bioremediation are inconclusive. Many laboratory studies (Demque et al., 1997; Weir et al., 1995) showed significant improvement on the rate of degradation with nutrient supplements but field data often demonstrated little effect (Lehtomaki and Niemela, 1975; Morgan and Watkinson, 1992; Morgan et al., 1993; Venosa et al., 1996; Braddock et

al., 1997). Inadequate delivery and distribution of nutrients may be a problem for field studies while other environmental parameters may be limiting the degradation of organic compounds.

2.5 Microbial Degradation of PAHs

Two- and 3-ring PAHs have been shown to be easily biodegradable whereas 4-, 5-, and 6-ring PAHs tend to be recalcitrant. In laboratory studies, Sims et al. (1990) showed that the degradation of 2-ring PAHs in sandy soils was extensive with half-lives of approximately two days. In comparison, the half-lives for the 3-ring PAHs such as anthracene and phenanthrene were 16 and 134 days, respectively. The 4-, 5-, and 6-ring PAHs generally exhibited half-lives that were over 200 days. The work of Heitkamp and Cerniglia (1987) showed similar results for PAH degradation in sediment/water microcosms. McGinnis et al. (1988) performed laboratory treatability studies on creosote-contaminated soils from wood treatment sites and found that PAHs with two rings generally exhibited half-lives of less than 10 days and those with three rings had half-lives of less than 100 days. However, most of the 4- and 5-ring PAHs exhibited half-lives that were over 100 days. Studies on the persistency of PAHs in sewage-sludge-amended soil have shown that half-lives estimated for soils under artificial laboratory conditions may be considerably shorter than those actually observed in the field (Wild et al., 1990).

Complete mineralization of lower molecular weight (LMW) PAHs, 2- to 3-ring compounds has been demonstrated by a number of researchers (Cerniglia, 1984; Heitkamp and Cerniglia 1989; Mueller et al., 1989b; Weissenfels et al., 1990). PAHs with four or more rings are less soluble, more stable, and therefore more recalcitrant. Only recently, in laboratory studies, have a number of researchers isolated microorganisms from contaminated soils that demonstrated the ability to mineralize 4-ring PAHs as their sole carbon and energy sources (Mueller et al., 1990; Walter et al., 1991; Weissennfels et al., 1991). However, microbial mineralization of PAHs with four or more rings has generally been reported to occur via cometabolism (Bouchez et al., 1995; Ye et al., 1996; Aitken 1998). A partial list of microorganisms that have been isolated from contaminated soils is presented on Table 2-5.

Table 2-5. Representative PAHs metabolized by different microorganisms

Compounds	Rings	Microorganism(s)	Com.[1]	References
Naphthalene	2	*Rhodococcus* sp.	S	Bouchez et al., 1996
		Pseudomonas sp.	S	Bouchez et al., 1996
		Pseudomonas sp.	S	Aitken et al., 1998
Acenaphthene	3	*Pseudomonas* sp.	S	Komatsu et al., 1993
		N. *naphthovorans*	C	Hedlund et al., 1999
Acenaphthylene	3	*Pseudomonas* sp.	S	Komatsu et al., 1993
Anthracene	3	*Rhodococcus* sp.	S	Bouchez et al., 1996
		Pseudomonas sp.	S	Bouchez et al., 1996
		Bjerkandera sp.	S	Field et al., 1995
Fluorene	3	*Pseudomonas* sp.	S	Foght and Westlake, 1988
		Rhodococcus sp.	S	Bouchez et al., 1996
		P. *saccharophila*	C	Stringfellow and Aitken, 1995
		Mycobacterium sp.	C	Boldrin et al., 1993
Phenanthrene	3	*Rhodococcus* sp.	S	Bouchez et al., 1996
		Pseudomonas sp.	S	Bouchez et al., 1996
		M. flavescens	S	Dean-Ross and Cerniglia, 1996
		Mycobacterium sp.	S	Boldrin et al., 1993
		Flavobacterium sp.	S	Stucki and Alexander, 1991
		Beijerinckia sp.	S	Stucki and Alexander, 1991
Fluoranthene	4	*Rhodococcus* sp.	S	Bouchez et al., 1996
		Pseudomonas sp.	S	Bouchez et al., 1996
		M. *flavescens*	S	Dean-Ross and Cerniglia, 1996
		Mycobacterium sp.	S	Boldrin et al., 1993
		P. *paucimobilis*	S	Mueller et al., 1990
Pyrene	4	*Rhodococcus* sp.	S	Bouchez et al., 1996
		Pseudomonas sp.	S	Bouchez et al., 1996
		M. *flavescens*	S	Dean-Ross and Cerniglia,1996
		Mycobacterium sp.	C	Ye et al., 1996
		Mycobacterium sp.	S	Boldrin et al., 1993
		Rhodococcus sp.	S	Walter et al., 1991
		Xanthamonas sp.	S	Grosser et al., 1991
		Mycobacterium sp.	S	Heitkamp et al., 1988
		Mycobacterium sp.	S	Heitkamp and Cerniglia, 1989
Chrysene	4	S. *paucimobilis*	C	Ye et al., 1996
		P. *fluorescens*	S	Caldini et al., 1995
		Achromobacter sp.	S	Cutright and Lee, 1994
Benzo(a)anthracene	4	S. *paucimobilis*	C	Ye et al., 1996
		P. *fluorescens*	C	Caldini et al., 1995
		Pleurotus sp. Florida	C	Wolter et al., 1997
Benzo(b)fluoranthene	5	S. *paucimobilis*	C	Ye et al., 1996
Benzo(k)fluoranthene	5	*Achromobacter* sp.	C	Cutright and Lee, 1994
Benzo(a)pyrene	5	P. *saccharophila* P15	C	Chen and Aitken, 1999
		Bjerkandera sp. BOS55	C	Kotterman et al., 1998
		S. *paucimobilis*	C	Ye et al., 1996
		Xanthamonas sp.	C	Grosser et al., 1991
		Mycobacterium sp.	C	Heitkamp and Cerniglia, 1989
Dibenzo(a,h)anthracene	5	S. *paucimobilis*	C	Ye et al., 1996
		Achromobacter sp.	C	Cutright and Lee, 1994

[1]Comments indicate whether the microorganism utilized the PAH compound as sole carbon (S) source or require co-substrate through cometabolism (C) mechanism.

In natural environments, mixed cultures play an important role in the degradation of xenobiotic compounds. Thus the biotransformation of PAHs by the natural microflora of contaminated soils has been the subject of numerous investigations (Bauer and Capone, 1988; Mueller et al., 1989b; 1990; Shiaris, 1989). These cultures often involve significant degradation capabilities because the various microbial strains can complement one another due to their physiological properties. Therefore, some members of the culture might be able to provide important degradation enzymes whereas others may supply bio-surfactants or growth factors.

2.6 Acclimatization, Adaptation, and Bioaugmentation

Studies have shown that prior exposure and acclimatization of subsurface and surface microbes to PAHs or other hydrocarbons in soil or sediment tend to enhance degradation rates. Bauer and Capone (1985, 1988) found that maximum mineralization of PAHs occurred after intermediate periods of pre-exposure of the microbes to alternative aromatic hydrocarbons. Herbes and Schwall (1978) found that the degradation rates of PAHs were 3,000 to 725,000 times faster than in unacclimatized soil. Although the factors limiting acclimatization are not well understood, it is apparent that a threshold PAH concentration or a specific exposure time is necessary. Hence, in order to reduce the acclimatization period and increase the rate of PAH biodegradation, microbes used in remediation projects are frequently isolated from the contaminated site itself. Those that show the fastest rates of PAH degradation are cultured and reapplied to the soil for bioremediation. This approach is generally called bioaugmentation. Although this is a logical approach, application of microorganisms into contaminated soils is a challenge. Many microorganisms introduced from one soil to another may have difficulty in adapting to the new ecological conditions of the soil requiring remediation (King et al., 1998; Edgehill, 1999). The specific degraders have to compete with indigenous microbes which are already acclimatized to the existing environment. Grosser et al. (1991) reported enhanced mineralization of pyrene (55% enhancement in two days) after bacteria isolated and cultured from the contaminated soil were reintroduced at 10^6-10^8 cells per gram of soil. In some studies, specifically adapted strains from one site were cultured in the laboratory and applied to a second site

contaminated by similar compounds. Vecchioli et al. (1990) reported a 22% increase in hydrocarbon removal when cultured bacteria were added to petroleum-contaminated soil. Heikamp et al. (1988) demonstrated that *Mycobacterium* sp. mineralized multiple doses of pyrene. Competition with indigenous microorganisms did not adversely affect survival of the bacteria or pyrene degradation. However, many researchers have reported that bioaugmentation technology showed no benefit for the biodegradation of target compounds (Moller et al., 1995; Margesin and Schinner, 1997)

2.7 Biochemical Pathways

The ultimate goal of bioremediation is to degrade organic contaminants completely to harmless constituents, such as carbon dioxide and water. However, intermediate metabolites such as dihydrodiols, phenolic compounds, and arena oxides may result which may be carcinogenic, mutagenic, and teratogenic. The intermediate compounds may be more soluble than the parent compounds (Park et al., 1988). Therefore, the degradation pathways should be investigated in order to identify potential problems.

Degradation of the saturated aromatic-ring structure of PAHs is dependent on the ability of enzymes produced by microorganisms to incorporate oxygen into the ring structure. The degradation pathway for a 2-ring compound is show in Figure 2-3. Gibson and Subramanian (1984) reported that catechol is a principal intermediate product from the degradation of naphthalene by bacteria. The enzymes are generally compound-specific and many different microbes may be required to degrade the PAH compounds at a contaminated site. Aerobic biodegradation is currently the most common form of bioremediation practiced for soils contaminated with PAHs.

The initial activation and oxidation of PAHs involve enzymes called oxygenases produced by the microorganisms that catalyze oxygen-fixing reactions. The two groups of oxygenases are monooxygenases and dioxygenases. Fungi generally produce monooxygenases, which incorporate one oxygen atom into the substrate to form arena oxides. This is followed by the enzymatic addition of water to yield trans-dihydrodiols and phenols. Bacteria characteristically produce dioxyenases which incorporate two oxygen atoms into the substrate to form dioxethanes. These are further oxidized to cis-dihydrodiols

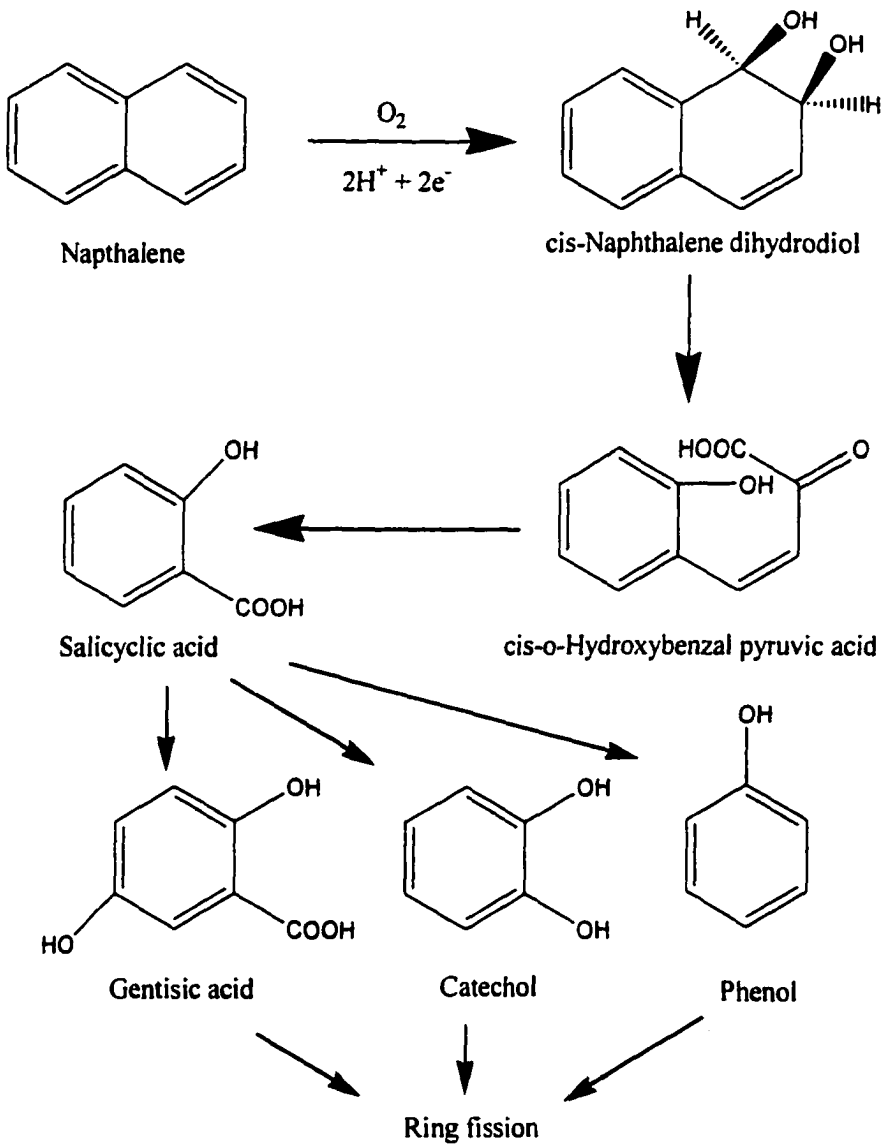

Figure 2-3. Proposed pathway for the degradation of naphthalene by bacteria (Gibson and Subramanian, 1984)

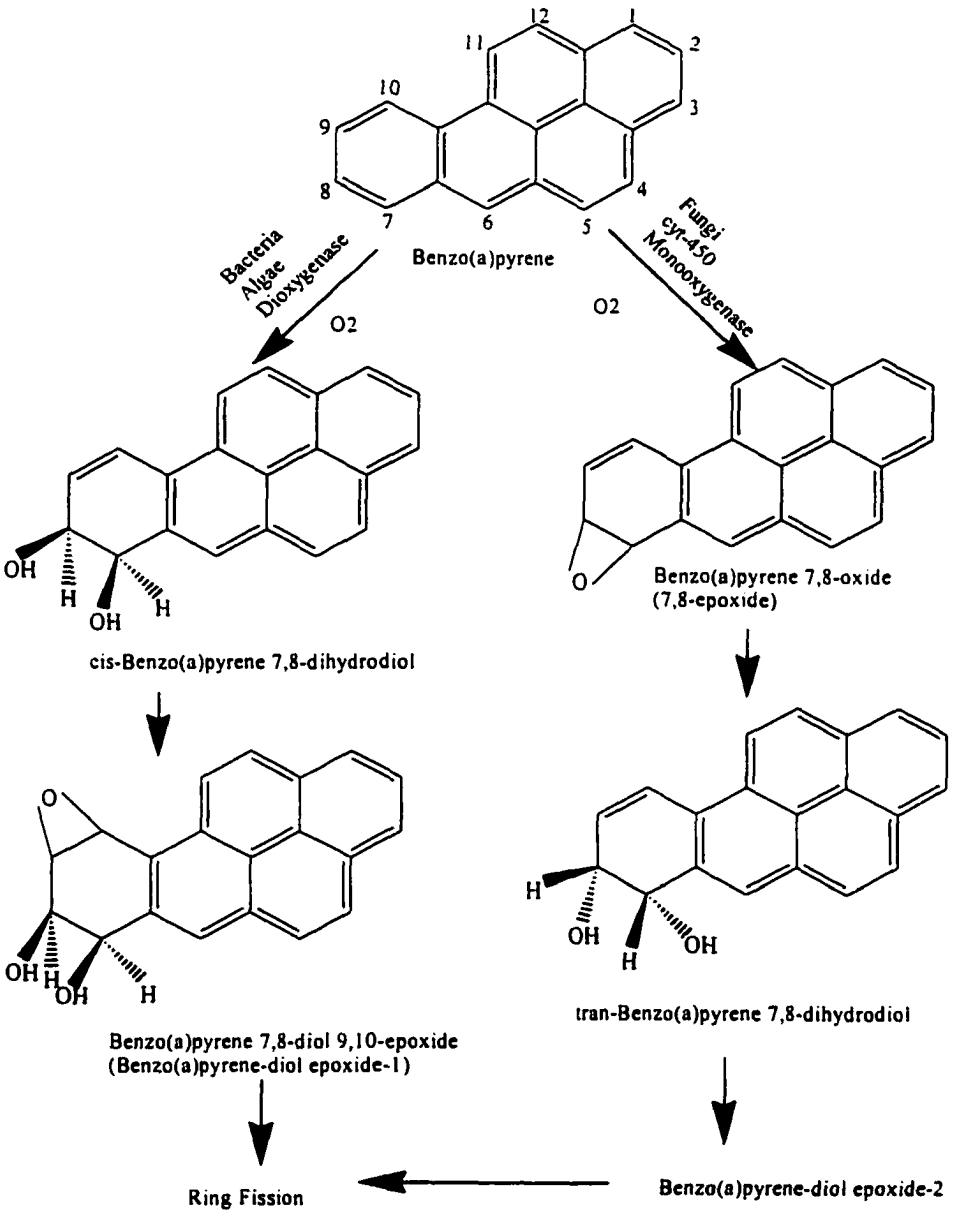

Figure 2-4. Fungal transformation of benzo(a)pyrene. Pathway adapted from Cerniglia and Heitkamp (1989)

and then to dihydroxy products. As an example, the degradation pathways of benzo(a)pyrene are presented in Figure 2-4. The initial ring oxidation is the rate-limiting step in the biodegradation reaction of PAHs (Cerniglia, 1984) after which degradation proceeds relatively quickly with little or no accumulation of intermediates (Herbes and Schwall, 1978).

Although the initial reaction of specific PAH degradation differ to yield different initial oxidation products, only a few common intermediate metabolites, namely catechol, protocatechuic, and gentisic acids, are produced. Catechol is the most frequently formed compound. The specific compound produced depends on whether the hydroxyl groups in the dihydrodiols are ortho or para to each other. These metabolites are degraded by five similar pathways which include ring cleavage to produce succinic, fumaric, pyruvic, and acetic acids and acetaldehyde, all of which are utilized for cell-protein synthesis and energy by microorganisms with the production of carbon dioxide and water (Sims and Overcash, 1983).

There has been much interest recently in the ability of the white-rot fungus, *Phanerochaete chrysosporium,* to degrade a number of xenobiotic compounds including PAHs (Brodkorb and Legge, 1992; McFarland and Qiu, 1995). The white-rot fungus produces an extracellular lignin-degrading enzyme (ligninase) which has the ability to degrade high molecular weight (HMW) including 4- and more rings, compounds that will not pass through bacteria cell walls (Hammel et al., 1986; Sanglard et al., 1986). The ligninases rely on a supplemental enzyme system to supply the necessary hydrogen peroxide to start the oxidation of lignin. Lignin has a random composition and a high-polymer structure. The ligninases, therefore, have a wide substrate range and low specificity and thus have the potential to degrade many different organic pollutants.

2.8 Cometabolic Biodegradation

The definition of cometabolism is that the cometabolized compound does not serve as the carbon and energy source for synthesis of cell biomass but a separate compound is required as the primary substrate. Enzymes produced by the microorganism to degrade the primary substrate are fortuitously used to degrade the target compound. Bouchez et al. (1995) isolated six bacterial strains from MGP soils capable of using PAHs (naphthalene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene) as sole carbon and energy

sources. All individual strains were found to be capable of cometabolic degradation of PAHs. Boldrin et al. (1993) used a pure culture of *Mycobacterium* sp. to confirm that fluorene was degraded through cometabolic process. Keck et al. (1989) used complex wastes (oil refinery wastes) to amend the soil and found that 4- and 5-ring PAHs disappeared more rapidly than unamended soils while degradation rates for 3-ring compounds in all matrices were similar. Complex wastes contain a mixture of compounds of which many are readily oxidizable by bacteria and some are capable of being utilized as substrates in the cooxidization of recalcitrant compounds. Kanaly et al. (1997) reported that benzo(a)pyrene could be completely degraded when soil contained suitable cosubstrates such as crude oil, even for soils which have no contamination history. Morgan et al. (1993) found that addition of organic substrates such as glucose, hay, wood chips, pine bark, loam and peat promoted growth and degradation of benzo(a)pyrene. Cooney and Shiaris (1982) reported that growth substrates could support cometabolism of phenanthrene - their effective order was: yeast extract plus peptone > glucose > benzoate > oil plus kerosene.

As in the cometabolism of PAHs, an inducer may be used to stimulate the degradation of PAHs. An inducer is usually an intermediate compound, downstream of the degradation pathway. Since induction of enzyme synthesis may be important for the degradation of the compounds that do not serve as growth substrates for the degrading microorganisms, inducers may stimulate the production of broad-specificity enzymes for PAH metabolism. Chen and Aitken (1999) found that salicylate may be used as an inducer to enhance the degradation of HMW PAHs such as benzo(a)anthracene, chrysene, and benzo(a)pyrene.

On the other hand, Wiesel et al. (1993) found that 4-ring PAHs were only metabolized significantly when phenol, naphthalene, and most of the three rings PAHs were already degraded. Similarly, other authors have reported the same degradation sequence pattern (Bossert and Bartha, 1986; Mueller et al., 1991a). Zappi et al. (1996) reported that addition of rapidly metabolizing substrates such as sodium acetate and/or phenanthrene did not enhance the degradation of PAHs containing more than three aromatic rings. However, the augmented phenanthrene was rapidly metabolized.

The contradictory results of cometabolism from different research groups indicate that degradation of HMW PAH through cometabolism processes is still unknown. The primary substrates needed to enhance the degradation of HMW PAHs are not definite at this time. However, most people believe that the degradation of HMW PAHs is limited by their bioavailability rather than by the conditions for cometabolism (Erickson et al., 1993; Zappi et al., 1996). Erickson and coworkers (1993) added naphthalene and phenanthrene in contaminated soil in order to enhance the degradation of PAHs. However, the added naphthalene and phenanthrene were degraded rapidly with no change in the concentrations of indigenous PAHs. They concluded that the PAHs were bound to the soil in a way that made them unavailable for degradation. Zappi et al. (1996) reported similar results and found that bioavailability may be the reason for limiting the degradation of PAHs.

2.9 Bioavailability

2.9.1 Bioavailability of PAHs

Bioavailability is defined as the availability or accessibility of organic compounds for the utilization of microorganisms. Bioavailability represents one of the most important factors controlling PAH biodegradability in the environment (Alexander, 1994; Mueller et al., 1996). Since PAHs are hydrophobic compounds, they tend to partition onto soil organic matter and to a lesser extent, sorb to soil mineral surfaces. In addition, PAHs have very low solubility in water. Dissolved organic compounds are more available to the microorganisms than insoluble compounds (Wodzinski and Coyle, 1974; Volkering, 1993). Therefore, sorbed PAHs may be physically unavailable to microorganisms and may be protected from microbial degradation. Weissenfels et al. (1992) evaluated the influence of sorption on microbial PAH degradation by adding anthracene oil to different sorbents: sand, soil, and amberlite resin. The results showed that the percentages of biodegradation of sorbed PAHs were 100%, 77%, and 0% for sand, soil, and resin, respectively, after 28 days of incubation. This implied that sorption limit the bioavailability of PAHs. Similar conclusions were found by many researchers (Guerin and Boyd, 1992; White and Alexander, 1996; Carmichael et al., 1997; Bosma et al., 1997). Sorption may involve physical adsorption on a surface or by partitioning into a phase such as natural organic matter (Pignatello and Xing, 1996). The

intermolecular interactions such as van der Waals forces, hydrogen bonding, ion exchange, or chemisorption are common in adsorption. Besides adsorption, PAHs may be deposited and entrapped in a micropore through diffusion processes. The size of a micropore may be physically unavailable to any living organisms since even the smallest bacterium may have a larger dimension than the micropores (Nam and Alexander, 1998). Several factors affect the sorption of organic compounds including the concentration and type of solutes in the surrounding solution, the type of clay minerals, the amount of organic matter in the soil or sediment, pH, temperature, and the characteristics of the specific compound involved (Means et al., 1980; Kan et al., 1994; Luthy et al., 1997).

Additionally, several different studies have shown that contaminants become increasingly more resistant with time to extraction and biodegradation (Alexander, 1995; Hatzinger and Alexander, 1995). The "aging" process results in a significant portion of the PAHs to be adsorbed on soil particles and possibly entrapped in intraparticle micropores. The microbial utilization of hydrophobic compounds requires solubilization or emulsification prior to uptake and metabolism (Wodzinski and Coyle, 1974). In soil slurry studies, Zhang (1995) showed that the degradation rates of PAHs are dependent on the soil:water ratio and the partition coefficient. For strongly sorbed compounds, the degradation rates may be controlled by the sorption-desorption kinetics even for soils that may contain a large and active community of PAH-degrading microorganisms (Carmichael et al., 1997).

Thomas et al. (1986) and Volkering et al. (1992) found that mass transfer from the solid phase to the liquid phase was the rate-limiting step for microorganism growth based on PAH. Microbial limitations can be overcome by either improving environmental conditions, such as nutrients or electron acceptors, or in some instances, augmentation of the microbial population with desired microbes. However, a critical analysis of bioremediation data reveals that the intrinsic microbial activities limit bioremediation only in a few cases. In most cases, mass transfer limitation prevented the full exploitation of the microbial degradative potential (Bosma et al., 1997). To enhance the PAH bioavailability, many researchers in recent years have investigated the use of surfactants or co-solvents to enhance biodegradation (Luthy et al., 1994; Mueller et al., 1996; Zhang et al., 1998).

2.9.2 Measurement of bioavailability

Since organic compounds adsorbed to the soil matrix are not available to microorganisms, many researchers have attempted to correlate desorption rates and biodegradability of PAHs in order to understand the issue of bioavailability (Scow and Hutson, 1992; Bosma et al., 1997; Zhang et al., 1998). However, desorption studies cannot completely explain the bioavailability of PAHs in soil. For example, Zhang (1995) reported that the in-situ degradation rates of PAHs at a former MGP site were several times slower than the estimated degradation rates using mass transfer (desorption) principles. Cornelissen et al. (1998) studied the desorption kinetics of 15 PAHs from sediments. They found that the initial rapidly desorbing fraction could be roughly used to predict the extent of possible PAH degradation from PAH-contaminated sediments. However, the desorption rate constants for the rapidly desorbing fraction of the PAHs were found to be much higher than their degradation rate constants of each PAH.

Extraction techniques have recently been used to predict the bioavailability or toxicity of PAHs from the coal-tar-contaminated soils. Nakles and Harju (1998) used a mild solvent extraction method to predict the availability of PAHs to earthworms and plants. They used 1-butanol, 1-propanol, methanol, and ethyl acetate as extraction solvents to correlate the uptake of PAH compounds such as anthracene, pyrene and fluoranthene by earthworms, barley, and wheat. The correlation coefficients were greater than 0.86 for all of the comparisons. Similarly, Kelsey et al. (1997) found that mild extraction with n-butanol showed the best prediction of phenanthrene mineralization. Loehr et al. (1998) used a resin to quantify the total fraction of PAHs that may be released into water. In their studies, the amounts of PAHs released into the aqueous phase were much less than the amounts that were degraded.

2.10 Enhancement of Bioavailability

2.10.1 Surfactant addition

Many PAHs sorb strongly onto soils which limit their availability for microbial degradation. Indeed, low aqueous solubility may be the rate-limiting factor controlling the degradation rates of the HMW PAHs in soils. Surfactants have been shown to enhance

desorption and solubilization of PAHs (Edwards et al., 1991; Jafvert, 1991; Joshi and Lee, 1996a; Guha and Jaffe, 1996; Grimberg et al., 1996), with appreciable desorption when the surfactant concentration exceeds the critical micelle concentration (CMC) of the surfactant.

Nonionic surfactants are often used because unlike ionic surfactants, they adsorb less extensively onto mineral regions of the soil particles. In addition, they display lower sensitivity to water hardness, are less toxic, and are more susceptible to removal from the environment by biodegradation. Nonionic octyl and nonyl phenylethoxylates with nine to twelve ethoxylate units have been shown to be most effective in enhancing the degradation of PAHs such as anthracene, phenanthrene, and pyrene in soil-water suspensions (Tiehm, 1994).

Different types of bacteria, yeast, and fungi produce metabolic products or extracellular polymers that behave like surfactants. These metabolic products or polymers are known as biological-produced surfactants or biosurfactants. Microorganisms growing on insoluble substrates usually produce biosurfactants (Gerson and Zajic, 1979). Kanga et al. (1997) reported that naphthalene showed a substantial increase in their apparent solubilities in the presence of surfactants. The increase in solubilities was significantly greater for the biosurfactant than a synthetic surfactant. Willumsen and Karlson (1997) found significant surfactant and emulsifier production in the microbiota of the PAH-contaminated soils. However, the degradation of PAHs did not correlate with the production of surfactants and emulsifiers by the isolated microorganisms.

Synthetic surfactants can be effectively employed to increase bioavailability especially for water-soil system. However, several studies have shown that the presence of surfactant micelles inhibited degradation (Deschenes et al., 1995; Auger et al., 1995a, b). Liu et al. (1991) showed that nonionic synthetic surfactants might inhibit the biodegradation process when used at a concentration exceeding the CMC. The positive, negative, and no effects of surfactants on microbial degradation of hydrocarbons were summarized by Liu et al. (1995). Biosurfactants have several potential advantages over synthetic surfactants. They are more acceptable since they are natural and indigenous and are readily degraded.

2.10.2 Co-solvents

Addition of an organic solvent can increase the solubility of PAHs which may result in an enhancement of the degradation of PAHs. Because of the hydrophobic nature of PAH, a high solvent:water ratio would enhance the solubility of PAHs (Morris et al., 1988). Peters and Luthy (1993) found that coal tar partitions as a pseudocomponent in systems with a solvent present, but not in systems with only coal tar and water. Field et al. (1995) reported that acetone and ethanol at a concentration of 5% were toxic to fungi when added at the time of inoculation. However, with addition of 11% - 21% (v/v) acetone or ethanol to 9-day old cultures, the rate of anthracene bioconversion to anthraquinone in liquid medium increased by a factor of two to three in comparison to fungal cultures receiving 1% - 3% of solvent. Caldini et al. (1995) reported that the number of microbial cells and degradation rate increased when HMW PAHs dissolved in a water miscible solvent such as acetonitrile was added to the mineral solution as compared to a system without a water miscible solvent. Jimenez and Bartha (1996) found that by adding a hydrophobic solvent such as paraffin oil (0.8% v/v) into a medium with PAHs, the mineralization rate of pyrene by the *Mycobacterium* sp. doubled. Birman and Alexander (1996) reported that phenanthrene degradation was enhanced in the presence of a model nonaqueous-phase liquid (NAPL). Dibutyl phthalate was selected as the model NAPL in their experiments. When grown in a medium with phenanthrene in dibutyl phthalate as the carbon source, the bacteria were shown to use the hydrocarbon that was initially present in the NAPL phase. Such microorganisms presumably were more capable of using the hydrocarbon in the nonaqueous liquid than in the media with hydrocarbon alone. The organisms in the NAPL might be more active since they may produce an extracellular surfactant that promoted pseudosolubilization of the substrate or adhered to the NAPL and used the substrate at an enhanced rate without excreting a water-soluble surfactant (Efroymson and Alexander, 1991).

Kilbane (1997) showed that ethanol extraction combined with biological techniques may enhance the degradation of PAHs from contaminated soils. The results showed that less than 10% of PAHs were removed in 14 days for a weathered MGP soil in a bio-slurry reactor. For the same period, about 95% biodegradation was achieved for PAHs extracted from this soil by ethanol and subsequently degraded by aqueous bacterial suspensions.

However, the aqueous phase bio-reactor cannot be loaded with more than 1% of ethanol as ethanol itself may be toxic to the bacterial suspensions.

Recently, a number of research groups investigated the effectiveness of using solvents for the removal of PAHs from contaminated soils and sediments (Augustijn et al., 1994; Errett et al., 1996). Since the rate of PAH desorption from the solid phase is dependent on the composition of the solvent-water mixture, it is expected that the mobility of PAHs would be significantly enhanced with increasing amounts of solvent in the solution phase. MacDonald and Rao (1998) reported that a pilot-scale field study was conducted to demonstrate enhanced contaminant solubilization by in situ co-solvent flushing with conjunction with "pump and treat" technology. Over a 10-day period, 40,000 L of a co-solvent mixture (70% ethanol, 12% pentanol, and 18% water) were injected through four injection wells. The results showed 85% removal of nonaqueous-phase liquid mass. The extracted co-solvent-water mixture which contained high concentration of PAHs must be treated either by re-using the mixture or treated using above ground treatment technologies.

Solvents and surfactants may enhance the availability of PAHs for biodegradation from the contaminated soils, but at the time they may cause an expansion of the contaminated area. Generally, solvents are better than synthetic surfactants as solvents tend to have higher extractability of PAHs from aged contaminated soil and are more easily biodegraded (Kilbane, 1997; Zhang et al., 1998).

2.11 Field-Scale Bioremediation Technologies for PAH-Contaminated Soils

2.11.1 In situ bioremediation

In situ treatment is the enhancement of the native microbes to biodegrade target organic compounds through the passive management of environmental parameters. Most in-situ treatments involve the biodegradation of contaminants within the saturated zone of the contaminated soil. This involves the addition of nutrients, electron acceptors, and sometimes specifically adapted microorganisms to enhance degradation. Mueller et al. (1995) showed that introduction of oxygen and nitrogen in an in situ bioremediation system may be used to stimulate the indigenous microflora to degrade PAH in the soil. After 16 weeks of incubation period, they found that 62% of PAHs were degraded by adding air and liquid

nutrients but only 19.3% of PAHs were removed for a system without any amendment. Flyvberg et al. (1991) reported that nitrate may be used to enhance the in situ biodegradation of PAHs from creosote and oil but the reported removal was limited to LMW PAHs. Ellis (1994) reported a full-scale in-situ remedial system at Blekholmstorget, Sweden for the treatment of 20,000 yd^3 of undisturbed creosote-contaminated soil. About 10 to 13 ft depth of the contaminated soil was saturated soil and the groundwater was withdrawn with pumps. The groundwater was transferred to two 1,300-gallon steel reactors by perforated pipes laid at the base of the contaminated soil. The effluent was aerated along with the addition of hydrogen peroxide (30 to 100 mg/L using a 35% peroxide solution). Nutrients, microorganisms, and other additives were added in sequence to the reactor tanks. After 4 months of the first phase treatment, approximately 60% of the total creosote were removed from the soil. A large fraction of the removal was the degradation of 2-ring PAH compounds such as naphthalene (approximately 100% removal) while 4-ring PAH compounds such as chrysene were only degraded by 11%.

Mueller et al. (1996) reported that there are only a few in situ applications for the treatment of PAH-contaminated soils. Most in situ applications are limited by the physical-chemical properties of the soil and the PAH which in turn limit the biodegradation of PAHs (i.e., bioavailability) while certain factors such as electron acceptors and nutrients can be easily added. In general, sites with hydraulic conductivity greater than 10^{-4} cm/s, in a fairly homogeneous stratigraphy, are good candidates for in-situ bioremediation (Rogers et al., 1993). If the contamination is shallow and can be excavated or the site physical and/or chemical characteristics prohibit in-situ bioremediation, an ex-situ system may be applied. The favorable and unfavorable factors affecting the feasibility of in-situ bioremediation are summarized in Table 2-6.

2.11.2 On-site/Prepared bed systems

Land-farming treatment of contaminated soils is one of the many on-site treatment techniques available. It has been widely used in the oil industry for the disposal of oily sludge. Prepared beds, which require a greater degree of engineering and containment, have been used to minimize contaminant movement from the land-farming treatment area. The prepared area is usually lined with a low permeability material such as high-density

Table 2-6. Favorable and unfavorable factors affecting bioremediation (Rogers et al., 1993).

Favorable chemical, biological, and hydrogeologic factors	Unfavorable chemical, biological, and hydrogeologic factors
Small number of organic contaminants	Numerous contaminants/complex mixture of inorganic and organic compounds
Non-toxic concentrations	Toxic concentrations
Diverse microbial populations	Sparse microbial activity
Suitable electron acceptor conditions	Absence of appropriate electron acceptors
pH 6 to 8	Extreme pH
Granular porous media	Fractured rock
High permeability ($>10^{-4}$ cm/s)	Low permeability
Uniform mineralogy	Complex mineralogy/high organic carbon content
Homogeneous media	Heterogeneous media
Saturated conditions	Unsaturated state, or intermittently saturated conditions

polyethylene (HDPE) or clay. A leachate collection system and, sometimes, an emission control system are included. Prepared beds are usually situated close to the area where the contaminated material is removed. Degradation of the contaminants in the bed is optimized by fertilization, irrigation, pH control, and sometimes microbial and surfactant additions.

Warith et al. (1992) presented the results of a pilot program to bioremediate about 5,000 m^3 of PAH-contaminated soil at a former oil gasification plant. The contaminated soil was placed in a clay-lined bed of 120 m long and 60 m wide. After 3 months of treatment, volatile organic compounds such as benzene, toluene and xylene decreased by 73%, oil and grease by 36%, and PAH compounds by 86%. For this treatment, the 4- and 5-ring PAH compounds such as pyrene and benzo(a)pyrene were only reduced by 60%. Similar results were obtained by several researches (Ellis et al., 1991; Huling et al., 1995; Guerin, 1999) using land-farming techniques for PAH-contaminated soils. Haught et al. (1995) combined nutrient and fungi addition to the wood preserving-contaminated soil in laboratory bench-scale experiments and reported similar degradation results for four and five ring PAHs.

Mueller et al. (1991a) reported that the pattern of degradation with creosote-contaminated sediment using land-farming technique was as follows: phenolics > heterocycles > LMW PAHs > HMW PAHs > pentachlorophenol. When they also classified the PAHs in three groups with group 1 - 2-ring PAHs, group 2 - 3-ring PAHs, and group 3 - 4- to 6-ring PAHs, the PAH biodegradation pattern was as follows: group 1 > group 2 >

group 3. This trend is most probably related to the differences in aqueous solubilities of the PAH compounds of these groups. In general, as the size of a compound increased with additional rings, its aqueous solubility decreased logarithmically (see Table 2-2). This decreased solubility negatively impacts the bioavailability with a concomitant effect on biodegradability. Therefore, the addition of inorganic nutrients to surface soils may enhance the biodegradation of certain creosote constituents but land-farming bioremediation may not be effective in destroying HMW PAHs and other more recalcitrant pollutants (Mueller et al., 1991a).

2.11.3 Bioreactors

Excavated contaminated soils may be treated in a bioreactor. Usually, the soil is slurried with water and then treated in the reactor where conditions for bioremediation are enhanced. There is considerable control over the operating conditions that often result in relatively faster and effective treatment than land-farming techniques. An acclimatized microbial population from a previously treated batch of soil is usually used as seed to start the degradation. After treatment, the material is passed through a water-separation system and the water recycled.

Recently, a semi-solid phase bioreactor was investigated to reduce water usage in the reactor. Pinelli et al. (1997) compared three different reactors (solid phase, semi-solid phase, and slurry phase) for the treatment of PAH-contaminated soils. As expected, the slurry-phase system provided the most effective and fastest removal of the PAHs. About 73% of the total PAHs originally present in the soil were removed in slurry system after 17 days, whereas only 60% and 65% were removed in the semi-solid phase treatments over the same period. The advantages of slurry-phase reactors are as follows: greater and more uniform process control, enhanced solubilization of organic chemicals, physical breaking of soil-sludge particles, increased contact between microorganisms and contaminants, ability to enhance solubility of contaminants with surfactant applications, and improved distribution of nutrients, electron acceptors, or primary substrates (Cookson, 1995)

Lewis (1993) and Lauch et al. (1992) reported 96-97.4% degradation of the 2- and 3-rings PAHs and 83-90% degradation of the 4- to 6-ring PAHs in soil slurry reactor for

creosote-contaminated soils. Jerger et al. (1994) reported similar results for PAH degradation in a 180,000 gallon soil slurry reactor. The overall treatment efficiencies for non-carcinogenic PAHs ranged from 85% to 95% for the first two weeks. Treatment of the carcinogenic PAHs ranged from 55% to 85% for same period. Since the initial concentrations of PAHs in soils for the above researches were over 5,000 mg/kg of Total PAH and 1,000 mg/kg of 8 carcinogenic PAHs, the removal percentages of PAHs from the soil were high. For both studies above, the final concentrations of PAHs remaining in the soil were over 500 mg/kg of Total PAH and 200 mg/kg of 8 carcinogenic PAHs.

2.12 Summary

Bioremediation is a cost-effective technology for the removal of PAHs in contaminated soils from MGP sites. LMW PAHs have been shown to be degraded by indigenous microorganisms under appropriate environmental conditions. However, the bioavailability of PAHs has been shown to be a limiting factor in the degradation of PAHs, especially for HMW PAHs. Understanding the bioavailability mechanism and how to enhance PAH availability is an essential step in accelerating the degradation rates of PAHs. Bioavailability not only limits the degradation rate of PAHs, it also limits the remediation technology in achieving lower concentrations of PAHs to meet regulatory clean-up levels. The regulatory cleanup levels are generally set at less than 500 mg/kg for Total PAHs and 100 mg/kg for Total carcinogenic PAHs.

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CHAPTER 3. EXTRACTION METHOD FOR ANALYSIS OF PAHS IN COAL-TAR-CONTAMINATED SOILS

A paper published in the ASCE Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management

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ABSTRACT

A direct solvent extraction method was evaluated to assess its application for the extraction and analysis of polycyclic aromatic hydrocarbons (PAHs) in coal tar-contaminated soils. Eight individual solvents and two combined solvents were used in a screening test to determine the suitability of different solvents for the direct solvent extraction method. Based on the extracted concentrations of the Total PAH, non-carcinogenic PAH (N-PAH), and carcinogenic PAH (C-PAH), three different solvents: acetone, ethanol, and methylene chloride were selected for further investigation by testing these solvents on four coal tar-contaminated soils and a certified PAH-contaminated soil. The extraction results for the Total PAH and N-PAH concentrations showed that there were no statistical differences for the three solvents at a confidence interval of 95%. However, ethanol gave lower concentrations of C-PAH than acetone and methylene chloride. Fifteen coal tar-contaminated soil samples from a land farming treatment unit were used to compare the direct solvent extraction method using acetone as the extraction solvent with the conventional Soxhlet extraction method (EPA Method 3540) and ultrasonic extraction method (EPA Method 3550). The results showed that the direct solvent extraction method gave higher

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mean PAH concentrations than the Soxhlet and ultrasonic extraction method and that the direct extraction method was equivalent to the Soxhlet method at a confidence interval of 99%. Due to its simplicity in use and its equivalent extraction capability with the Soxhlet extraction method, the direct solvent extraction method may be used as a technique for the extraction and analysis of PAHs in contaminated soil.

Keywords: solvent extraction, PAHs, coal tar, acetone, Soxhlet

INTRODUCTION

The most widely used extraction method for the extraction and analysis of polycyclic aromatic hydrocarbons (PAHs) in contaminated soils and sediments is the Soxhlet extraction method (EPA Method 3540) (US EPA, 1996). Even though this method is relatively straightforward in its application, it still requires continuous observation, i.e., a time consuming procedure, and a relatively large volume of solvent. In the Soxhlet extraction method, 10 g of soil is extracted with 300 mL of solvent under reflux conditions in a Soxhlet extractor for 16 - 24 hours. The solvent is usually not recovered and is disposed of as a waste. Recently, several extraction methods have been developed to reduce the operator's time and the volume of solvents used. These methods include the automated Soxhlet extraction method (EPA Method 3541) where a shorter extraction time and a smaller volume of solvent are used as compared to the conventional Soxhlet extraction method (US EPA, 1996; Lopez-Avila et al., 1993). In this method, the soil sample is immersed in the boiling solvent during the extraction procedure instead of reflux conditions as in the conventional Soxhlet method. In the accelerated solvent extraction method (EPA Method 3545), high temperature (up to 200° C) and high pressure (up to 20 MPa) are used to improve the solvent penetration into the soil particles and to enhance the desorption of the organic compounds (Fisher et al., 1997; Heemken et al., 1997; Richter et al., 1996; and Saim et al., 1998). Other enhancement techniques used include the application of ultrasound (EPA Method 3550) and microwave energy to enhance desorption of organic compounds from soil matrices (US EPA, 1996; Golden and Sawicki, 1978; Oostdyk et al., 1993; Eiceman et al., 1980; Dean et al., 1995; Lopez-Avila and Young, 1994; Lopez-Avila et al., 1995; Noordkamp et al., 1997).

The supercritical fluid extraction method (EPA Method 3561) uses carbon dioxide and a small volume of organic solvent for the extraction of organic compounds at a temperature of 80 – 120° C and 33 MPa (Paschke et al., 1992; Chester et al., 1994; Barnabas et al., 1994; Dean et al., 1995; Schantz et al., 1997). In the methanolic saponification extraction method, the availability of contaminants is enhanced by using solvents (methanol or ethanol) and bases (potassium hydroxide or ammonium hydroxide) to hydrolyze and solvate the soil organic matter to release the organic contaminants (Heemken et al., 1997; Eschenbach et al., 1994). A summary of the extraction conditions for the different methods is presented in Table 3-1.

Table 3-1. Summary of extraction conditions for different extraction techniques.

Extraction method	Soil sample (g)	Solvent volume (mL)	Recommended Solvents (vol:vol ratio)	Extraction time	Temperature or/and pressure
Soxhlet extraction (EPA Method 3540)	10	300	• Acetone/Hexane (1:1) • Methylene Chloride/Acetone (1:1) • Methylene Chloride • Toluene/Methanol (10:1)	16 – 24 hours	Boiling point of the solvent
Automated Soxhlet extraction (EPA Method 3541)	10	50	• Acetone/Hexane (1:1) • Methylene Chloride/Acetone (1:1)	2 hours	Boiling point of the solvent
Ultrasonic extraction (EPA Method 3550A)	2 - 30	300	• Methylene Chloride/Acetone (1:1) • Methylene Chloride • Hexane	3 minutes for each cycle, require 3 cycles	
Accelerated solvent extraction (EPA Method 3545)	10 - 30	3.5 - 32	• Hexane/Acetone (1:1) • Methylene Chloride/Acetone (1:1)	10 minutes	100° C 13.5 MPa
Supercritical fluid extraction (EPA Method 3561)	2 - 3	180 (CO_2)	• Carbon dioxide and mixture of Carbon dioxide/methanol/ water at ratio (95/1/4)	90 minutes	80 - 120° C 33.2 MPa
Microwave assisted extraction	5	30	• Acetone/Hexane (1:1)	20 minutes	80 - 145° C

Although the extraction methods described above may require less of the operator's time than the Soxhlet extraction method, these methods, however, require special and expensive equipment and careful sample handling. For example, Dean et al. (1995) reported that the equipment costs for the microwave-assisted extraction and the supercritical fluid extraction method were 20 to 40 times higher than that of the Soxhlet extraction method. The skills level needed for conducting the Soxhlet extraction, the microwave-assisted extraction, and the supercritical fluid extraction may be classified as low, medium, and high, respectively (Dean et al., 1995). The more expensive equipment and the higher skills level needed with the improved extraction methods have caused several researchers to evaluate a simpler and more direct extraction method for the extraction of organic compounds from contaminated soils and sediments (Noordkamp et al., 1997; Fowlie and Bulman, 1986; Coover et al., 1987; Chen et al., 1996). Chen et al. (1996) reported that direct extraction with a 1:1 (v/v) methanol-methylene chloride mixture may be a suitable extraction method for determining the concentrations of PAH in coal tar-contaminated soil. The direct extraction method used by Chen et al. was relatively simple and cost-effective but the extraction time used was 5 days. In their method, water present in the soil sample was removed by adding sodium sulfate before the extraction since methylene chloride is an immiscible solvent. Using methylene chloride as the extraction solvent, Coover et al. (1987) found that the Soxhlet extraction method yielded slightly better recoveries of PAHs than the direct extraction method for two PAH-spiked soils. Fowlie and Bulman (1986) found that the Soxhlet extraction method with 1:1 hexane:acetone gave higher recoveries of ^{14}C labeled benzo(a)pyrene and anthracene than a direct extraction method with a high-velocity homogenizer or mixer/shearer. The extraction time used was only 2 minutes, which may result in the lower recovery for the direct extraction method. Extraction efficiencies of PAHs using the direct solvent extraction method have been shown to vary with the initial concentrations of PAHs in soil, the extraction procedure, soil textures, and solvents used (Coover et al., 1987; Chen et al., 1996). Further investigation is needed to assess the usefulness of applying the direct solvent extraction method for the extraction and analysis of coal tar-contaminated soil. For an extraction method to be useful, the solvent must be used in

small quantities and must not be toxic, and the extraction should be conducted within an acceptable time period with minimum attention.

The objective of this study was to assess the applicability of a direct solvent extraction method for the extraction and analysis of PAHs in coal tar-contaminated soils. Sixteen US EPA priority PAH compounds grouped as Total PAH, noncarcinogenic PAH (N-PAH), and carcinogenic PAH (C-PAH) were examined in this study. The direct solvent extraction method allowed the extraction to be performed directly in a 10-mL glass tube thus minimizing the solvent used and sample handling. Because a smaller volume of organic solvent is used, significant savings on solvent costs and disposal costs as compared to a conventional Soxhlet extraction method may be realized.

MATERIALS AND METHODS

The approach used in evaluating the direct solvent extraction method for coal tar-contaminated soils was as follows. Eight individual solvents and two combined solvents were initially screened to assess their extraction abilities on a coal tar-contaminated soil. Based on the initial screening test, three solvents were selected for further investigation to evaluate the extraction method. The evaluation test was conducted on four coal tar-contaminated soils and a certified coal tar-contaminated soil. A solvent was then selected and the direct extraction method was compared with the Soxhlet extraction method (EPA Method 3540) and the ultrasonic extraction method (Method 3550) by using 15 coal tar-contaminated soil samples from a land farming treatment unit.

Direct Extraction Method

Two g of soil was placed with 5 mL of solvent (soil:solvent ratio of 1:2.5 (w/v)) in a 10-mL glass tube with Teflon-lined screw cap. All assays were conducted in triplicates. The contents in the tube were mixed for 24 hours with a wrist action shaker (Model 75, Burrell Scientific, Pittsburgh, PA) at a room temperature of $22 \pm 2^{\circ}$C. The tubes were then centrifuged at 3,000 rpm for 40 minutes. A 5 μL aliquot of the supernatant was analyzed with a gas chromatograph (GC) (Model HP5890 A, Hewlett-Packard, Palo Alto, CA) equipped with a HP-5 capillary column and a flame ionization detector (FID). The initial

oven temperature was 50° C followed by a temperature ramp rate at 8° C/min to a final temperature of 302° C for 5 minutes. The injector temperature was set at 240° C and the detector temperature was 320° C. The dry mass of the soil was determined by decanting the supernatant and drying the soil in the tube in an oven at 105° C for 24 hours. PAH concentrations in soil were reported on a dry weight basis.

Screening of Different Solvents

For the initial screening test, eight solvents and two combined solvents were used with the direct extraction method. The solvents or combined solvents used were acetone, hexane, acetone/hexane, methylene chloride, methylene chloride/acetone, ethanol, toluene, 2-propanol, methanol, and acetonitrile. The combined solvents used had a volume ratio of 1:1. All solvents except for ethanol were purchased from Fisher Scientific Co., Chicago, IL and were pesticide or HPLC grade. Ethanol was purchased from McCormick Distilling Co., Weston, MO.

Coal-Tar-Contaminated Soils

The soil used for the initial screening test was collected from the Vandalia Road site near Pleasant Hill, Iowa. The soil, identified as Vandalia (EXC), was obtained from an excavation pit where manufactured gas plant (MGP) residuals were landfilled in the 1940s. For the evaluation tests, the Vandalia (EXC) soil and three other coal tar-contaminated soils and one certified soil were used. One of the three soils, called Vandalia (LTU), was collected from a land farming treatment unit where the Vandalia (EXC) soil was thoroughly mixed with lesser contaminated soil from the site at a ratio of 1:1. The other two soils were collected from former MGP sites at Charles City, Iowa and Hampton, Iowa. Both MGP sites started operations in 1915 and were closed around the mid-1940s. All four soils were placed in airtight aluminum containers and stored at 4° C. Before the soil was used, the soils were homogenized by sieving through a 2-mm sieve. The physical-chemical properties of the four soils are presented in Table 3-2.

Table 3-2. Physical-chemical properties of four different MGP soils

Properties	Hampton Soil	Vandalia (EXC) Soil	Charles City Soil	Vandalia (LTU) Soil
Soil Texture	Loam soil	Sandy loam	Sandy loam	Sandy clay loam
Sand (%)	41	60	64	54
Silt (%)	35	26	18	24
Clay (%)	24	14	18	22
Organic carbon (%)	3.5	4.0	2.3	3.0
Initial soil moisture (%)	12.97	8.72	8.40	3.70

The certified soil, a coal tar-contaminated soil from a Superfund site located in the Western United States, was purchased from Resource Technology Corporation, Laramie, WY. The reported PAH concentrations for the certified soil were based on a round-robin study involving 20 laboratories. The extraction method used in the round-robin study was Soxhlet extraction (EPA Method 3540) with the PAHs analyzed using gas chromatography/mass spectroscopy (GC/MS).

For the final evaluation, soil samples were obtained from the Vandalia Road land farming treatment unit. The land farming treatment unit has an area of 100 ft x 300 ft and is divided into 16 subplots. Composite samples were collected from all subplots except for one subplot. The soil samples were mixed thoroughly and three split soil samples of 150 gms each from each subplot were obtained. One split sample was sent to the University of Iowa Hygiene Laboratory (UHL), Iowa City, Iowa where the soil samples were analyzed using EPA Method 3550. Another split sample was sent to the National Environmental Testing Laboratory (NET), Cedar Falls, Iowa where EPA Method 3540 was used for the analysis of the coal tar-contaminated soils. The third split sample was extracted and analyzed using the direct extraction method as described above. The extraction conditions for the three methods are summarized in Table 3-3.

Table 3-3. Analytical conditions for different extraction methods

Extraction Conditions	Extraction Method		
	Soxhlet Extraction (EPA Method 3540)	Ultrasonic Extraction (EPA Method 3550)	Direct Extraction Method
Soil sample	15 g	30 g	2 g
Solvent	Methylene Chloride	Acetone/Methylene Chloride (1:1 v/v)	Acetone
Solvent volume	200 mL	100 mL	5 mL
Extraction time	16-24 hours	3 minutes	24 hours
Extraction apparatus	Soxhlet extractor	Ultrasonic system	Shaker
Quantification method	GC/MS	GC/MS	GC/FID

RESULTS AND DISCUSSION

Solvent Screening Test

The results of the screening test using the direct extraction method for the different solvents and combined solvents are presented in Table 3-4. The concentrations of the 16 individual PAHs are reported along with the Total PAH, N-PAH and C-PAH concentrations. The Total PAH concentration was estimated by summing the 16 individual PAH concentrations. The N-PAH compounds include naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluroanthene, and pyrene while the C-PAH compounds were benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, Indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene.

As shown in Table 3-4, hexane and acetone/hexane gave the lowest mean Total PAH concentrations and the largest 95% confidence interval variation while acetone and methylene chloride gave one of the highest mean Total PAH concentrations and the smallest 95% confidence interval variation. Toluene gave the highest mean PAH concentration but had one of the largest 95% confidence interval variations. Statistically, the Total PAH concentrations measured by the different solvents were not different at a confidence interval of 95%. The N-PAH concentrations showed similar trends as the Total PAH concentrations for the different solvents used and were not statistically different at 95% confidence interval. This generally indicates that low molecular weight PAHs, 2- and 3-ring compounds, were

Table 3-4. Screening Test - Mean PAH concentrations (mg/kg ± 95% confidence interval) for Vandalia (EXC) soil using individual and combined solvents

Compound	Acetone	Acetone/ MC[a]	Acetone/ Hexane	Acetonitrile	Ethanol	Hexane	Methanol	MC[a]	2-propanol	Toluene
Naphthalene*	207±11	196±9	176±43	217±11	256±79	213±17	230±76	190±33	225±62	233±35
Acenaphthylene*	236±2	243±4	215±40	238±23	263±51	219±14	238±54	243±22	238±43	260±34
Acenaphthene*	139±2	130±4	118±18	140±4	154±27	138±10	143±29	132±19	145±24	146±22
Fluorene*	226±6	212±7	193±40	259±108	246±38	223±26	227±44	216±30	231±36	236±31
Phenanthrene*	703±53	678±36	595±160	688±74	709±72	676±124	667±64	690±70	683±82	708±103
Anthracene*	214±13	208±27	183±52	207±20	204±20	191±46	202±16	210±29	202±15	207±41
Fluroanthene*	274±18	269±16	224±50	261±20	245±18	244±51	236±12	271±7	239±6	252±36
Pyrene*	398±29	394±14	328±60	378±26	359±19	362±65	341±20	398±25	351±10	406±46
Benzo(a)anthracene+	145±5	144±9	119±12	137±2	124±2	119±19	119±3	146±16	116±0**	141±13
Chrysene+	153±9	154±10	125±10	145±2	129±4	116±23	122±3	157±16	121±2	150±16
Benzo(b)fluoranthene+	63±12	66±4	54±0**	59±6	52±8	43±7	47±4	69±8	44±11	64±6
Benzo(k)fluoranthene+	67±15	71±12	54±1	62±6	52±3	42±6	48±2	72±9	46±6	67±5
Benzo(a)pyrene+	104±3	109±16	90±1	97±5	82±12	64±13	71±3	116±4	68±4	105±7
Indeno(1,2,3-cd)pyrene+	38±4	44±4	36±5	37±0	29±1	20±6	24±2	47±6	25±1	44±2
Dibenzo(a,h)anthracene+	14±1	14±1	12±1	13±1	11±0**	8±0**	10±0**	15±1	9±0**	14±2
Benzo(g,h,i)perylene+	42±2	45±3	36±3	37±5	28±1	16±2	24±2	45±5	25±3	51±14
Totai PAH[b]	3023±61	2977±158	2557±490	2974±45	2942±354	2694±395	2749±237	3017±106	2770±269	3084±379
N-PAH[c]	2396±109	2330±99	2032±462	2387±63	2434±324	2266±319	2284±252	2349±171	2315±280	2448±349
C-PAH[d]	626±48	648±59	525±28	586±19	508±30	429±77	465±14	667±65	455±11	636±30

[a] MC = methylene chloride
[b] Total PAH = sum of 16 individual PAHs
[c] N-PAH = sum of 8 non-carcinogenic PAHs (indicated by *)
[d] C-PAH = sum of 8 carcinogenic PAHs (indicated by +)
** Confidence interval less than 0.5, rounded up as zero

easily extracted. Typically, N-PAH may make up about 80 to 90 % of the total PAHs in the contaminated soil. The measured C-PAH concentrations for the different solvents used were found to be statistically different at 95% confidence interval. Of all the solvents used, ethanol, hexane, methanol, 2-propanol, and a combined solvent of acetone/hexane gave the lowest mean concentrations of C-PAH. However, these solvents generally gave the highest mean concentrations of two-ring compounds such as naphthalene. The results of the screening test showed that solvent extractability was not only dependent on the properties of the solvent but was also dependent on the properties of each PAH. Alcohols such as ethanol and methanol are suitable for extracting two-ring PAHs such as naphthalene but are not as suitable for C-PAH such as benzo(a)pyrene (see Table 3-4). Noordkamp et al. (1997) reported that the extraction efficiency of PAHs from contaminated soils with ethanol was less than acetone at room temperature. Therefore, a solvent that may be appropriate for Total PAH measurement may not be suitable for the extraction and measurement of individual PAH.

Based on the above screening test and the properties of the solvent used, three solvents: acetone, ethanol, and methylene chloride were selected and tested against four different coal tar-contaminated soils. Although methylene chloride is more toxic than the other solvents, this solvent has been widely used for the extraction of PAHs from contaminated soil and was shown to have high extraction efficiencies. Coover et al. (1987) found that the direct extraction method with methylene chloride gave high percent recoveries (62% to 113%) for each PAH. Acetone in the preliminary test gave similar results to that of methylene chloride. Even though extraction of C-PAH by ethanol was slightly less than the other solvents, ethanol was selected for further evaluation as it is an environmentally benign solvent.

Comparison of Extracted Concentrations for Four Coal-Tar-Contaminated Soils

The extraction results for the four different soils using acetone, ethanol, and methylene chloride are shown in Table 3-5. At a confidence interval of 95%, the mean Total PAH concentrations for all three solvents were statistically similar. Further examination of the mean Total PAH concentrations showed that both Vandalia (LTU) soil and Vandalia

Table 3-5. Evaluation Test - Mean PAH concentrations (mg/kg + 90% confidence interval) for four different soils using three different solvents

	Hampton Soil			Vandalia (EXC) soil			Charles City soil			Vandalia (LTU) soil		
Compounds	Acetone	Ethanol	MC[d]	Acetone	Ethanol	MC	Acetone	Ethanol	MC	Acetone	Ethanol	MC
Naphthalene*	48±24	52±25	49±21	31±5	23±1	32±4	986±165	1081±103	786±219	62±3	64±7	52±19
Acenaphthylene*	192±18	241±13	188±42	403±21	403±16	439±25	325±66	343±38	284±53	61±3	51±2	58±14
Acenaphthene*	38±3	51±4	33±5	198±10	210±7	181±13	80±19	84±2	70±14	34±1	34±3	29±12
Fluorene*	141±12	178±27	131±31	297±20	300±13	302±18	181±42	194±13	203±114	62±3	61±4	51±20
Phenanthrene*	353±50	456±47	356±79	947±113	926±49	869±7	408±127	432±17	376±70	128±14	121±14	108±45
Anthracene*	115±13	135±11	116±17	296±36	250±10	251±1	141±35	146±10	134±18	54±3	50±3	50±8
Fluroanthene*	130±15	145±12	140±11	289±39	256±9	277±21	126±34	119±9	116±15	98±10	93±1	94±12
Pyrene*	176±19	196±17	191±13	418±58	382±12	405±30	171±44	156±11	153±20	146±17	139±1	142±16
Benzo(a)anthracene*	68±9	70±3	75±3	144±22	111±3	137±12	66±12	56±8	61±7	52±7	48±3	52±1
Chrysene*	66±10	68±2	74±3	155±23	117±4	146±13	66±12	54±3	59±6	53±13	48±7	53±2
Benzo(b)fluoranthene*	37±35	36±2	43±3	63±9	46±6	64±7	25±3	19±5	26±3	30±3	25±2	31±1
Benzo(k)fluoranthene*	37±3	36±2	41±2	65±11	49±2	64±6	30±7	21±5	25±3	25±4	20±1	25±1
Benzo(a)pyrene*	61±7	57±3	72±4	110±17	77±3	112±12	48±10	32±1	43±7	36±3	28±2	38±2
Indeno(1,2,3-cd)pyrene*	29±3	26±2	33±3	41±4	25±2	42±4	18±3	11±2	15±2	18±3	14±1	20±1
Dibenzo(a,h)anthracene*	9±1	9±2	10±1	14±4	10±1	15±2	7±1	5±1	7±0	7±2	6±1	8±1
Benzo(g,h,i)perylene*	24±4	20±2	30±3	44±7	26±4	42±4	17±4	8±4	11±3	18±3	10±1	20±1
Total PAH[a]	1522±176	1777±140	1583±225	3516±344	3211±121	3378±116	2694±572	2762±202	2368±546	884±84	814±10	831±147
N-PAH[b]	1191±145	1455±123	1205±213	2880±248	2749±115	2756±77	2417±523	2556±180	2122±518	645±50	614±20	585±144
C-PAH[c]	331±38	322±17	378±21	636±96	462±16	622±61	276±51	206±29	246±30	239±35	199±14	246±4

[a] Total PAH = sum of 16 individual PAHs

[b] N-PAH = sum of 8 non-carcinogenic PAHs (indicated by *)

[c] C-PAH = sum of 8 carcinogenic PAHs (indicated by *)

[d] MC = methylene chloride

(EXC) soil gave higher mean concentrations with acetone than methylene chloride and ethanol. For Charles City and Hampton soils, the mean Total PAH concentrations using ethanol as the extraction solvent were found to be higher than that of the other two solvents. The higher mean Total PAH concentrations were due to the higher extractability of the two- and three-ring PAHs by ethanol (see Table 3-5).

As expected, all four soils gave lower mean C-PAH concentrations with ethanol than with the other two solvents. Statistically at a confidence interval of 95%, the mean C-PAH concentrations using ethanol were different from that of methylene chloride for all soils except Charles City soil. In comparison with acetone, only the Vandalia (EXC) soil was found to have a mean C-PAH concentration that was statistically different from that of ethanol as an extraction solvent. For all soils, there were no statistical differences in the C-PAH concentrations for acetone and methylene chloride as the extracting solvents. Generally, the evaluation test results agreed with the screening test results.

Comparison of Different Solvents Using a Certified Soil

Three different solvents: acetone, ethanol, and methylene chloride and a certified soil were used to further evaluate the direct extraction method. The analytical results are presented in Table 3-6. The reported PAH concentrations for the certified soil are shown in the last column of Table 3-6. The Total PAH, N-PAH and C-PAH concentrations for acetone and methylene chloride were found to be statistically similar to the reported concentrations of the certified soil at a 95% confidence interval. With ethanol as the extraction solvent, the measured N-PAH concentration was found to be lower than the reported concentration for the certified soil. Unlike the results of the screening test, ethanol for this evaluation test gave lower N-PAH concentrations than the other two solvents. Examination of the concentrations of each individual PAH concentration shows that acetone and methylene chloride as the extraction solvents gave similar concentrations as the reported concentrations of the certified soil. The results indicated that the direct extraction method may be used for the extraction and analysis of PAHs from contaminated soils. Of the three solvents used, both acetone and methylene chloride may be used as a solvent for the direct extraction method. Acetone is preferred since acetone is more biodegradable and less toxic

Table 3-6. Comparison of mean PAH concentrations (mg/kg±95% CI) of certified soil using three different solvents

Compounds	Acetone	Ethanol	Methylene Chloride	Certified Soil
Naphthalene*	43±13	40±1	40±21	35±4.4
Acenaphthylene*	61±2	46±8	61±16	17
Acenaphthene*	492±6	459±60	492±74	627±168
Fluorene*	412±7	363±47	412±46	443±45
Phenanthrene*	1823±50	1511±179	1800±155	1925±209
Anthracene*	450±9	342±38	448±32	431±217
Fluroanthene*	1298±41	1014±105	1287±91	1426±166
Pyrene*	1036±34	804±84	1031±69	1075±141
Benzo(a)anthracene+	240±45	192±35	269±25	264±24
Chrysene+	355±29	272±23	369±32	316±30
Benzo(b)fluoranthene+	154±33	80±4	138±5	115
Benzo(k)fluoranthene+	84±37	69±18	119±53	64
Benzo(a)pyrene+	96±32	86±43	110±6	97±12
Indeno(1,2,3-cd)pyrene+	56±7	41±1	36±6	32±8
Dibenzo(a,h)anthracene+	14±2	13±1	20±6	14
Benzo(g,h,i)perylene+	29±3	27±5	28±11	26
Total PAH[a]	6644±246	5359±613	6660±588	6905
N-PAH[b]	5616±117	4579±522	5571±481	5978
C-PAH[c]	1028±172	779±92	1089±121	927

[a]Total PAH = sum of 16 individual PAHs
[b]N-PAH = sum of 8 non-carcinogenic PAHs (indicated by *)
[c]C-PAH = sum of 8 carcinogenic PAHs (indicated by +)

than methylene chloride.

Comparison of the Direct Extraction Method with Other Extraction Methods

In this experiment, 15 samples from the Vandalia Road land farming treatment unit were analyzed using the direct extraction method and the Soxhlet extraction and Ultrasonic extraction method. Two approaches were used to statistically compare the results of the direct extraction method and the other extraction methods. In the first approach, the 15 samples from the land farming treatment unit were assumed to be from the same sample set. With this approach, all the analytical data for the 15 samples were pooled and evaluated together statistically. In the second approach, the 15 samples were assumed to be

independent, i.e., not from the same data set. This assumption may be justified since each subplot within the land farming treatment unit may have different rates of treatment even though the soil may come from the same source. Based on this assumption, the Student paired t-test was conducted on the 15 samples for each extraction method.

The results of the first approach for the 15 soil samples using the direct extraction method and the Soxhlet and ultrasonic extraction methods are shown in Table 3-7. The Total PAH and N-PAH concentrations measured using the ultrasonic extraction method (Method 3550) were lower and statistically different from the Soxhlet extraction method (Method 3540) and the direct extraction method. However, the C-PAH concentrations measured using the ultrasonic extraction method were not statistically different from the Soxhlet extraction method, but were significantly different from the direct extraction method. There were no significant statistical differences in the total PAH, N-PAH, and C-PAH concentrations measured by the Soxhlet extraction method and the direct extraction method. Note that the direct extraction method consistently gave higher mean concentrations of each individual PAH as compared to the other two methods.

Instead of pooling the results of all 15 samples together, the concentrations of each sample from a given subplot and for each extraction method were compared using the Student paired t-test. The analytical results for Total PAH, N-PAH and C-PAH for all 15 samples using the three different extraction methods are presented in Table 3-8. The Student paired t-test results are presented in Table 3-9. For an extraction method to be significantly different from another method at a 95% confidence interval, the p-value for the paired test should be less than 0.05 or the t value should be larger than 2.145 for 14 degrees of freedom. For all three methods, the Total PAH concentrations measured were found to be significantly different. For the N-PAH concentrations, there was no statistical difference between the Soxhlet extraction method and the direct extraction method but these two methods were found to be statistically different from ultrasonic extraction method. This result was similar to that reported by Brilis and Marsden (1990) where the sonication extraction method was found to be less efficient than the Soxhlet extraction method for coal tar-contaminated soil. For C-PAH concentrations, all three methods were statistically different at 95% confidence interval. Even though the direct extraction method was statistically different from the

Table 3-7. Average PAH concentrations (mg/kg ± 95% CI) of 15 samples using different extraction methods

Compounds	Soxhlet extraction method (EPA 3540)	Ultrasonic extraction method (EPA 3550)	Direct extraction method
Naphthalene*	143±47	63±19	224±63
Acenaphthylene*	97±22	55±12	118±27
Acenaphthene*	35±9	23±4	63±14
Fluorene*	92±24	37±7	88±19
Phenanthrene*	176±41	107±23	183±37
Anthracene*	60±14	40±8	76±15
Fluroanthene*	69±13	51±7	85±12
Pyrene*	108±21	79±12	128±17
Benzo(a)anthracene+	40±8	30±4	45±5
Chrysene+	39±7	31±4	48±6
Benzo(b)fluoranthene+	18±3	20±12	26±2
Benzo(k)fluoranthene+	22±4	15±2	26±3
Benzo(a)pyrene+	35±6	27±3	40±4
Indeno(1,2,3-cd)pyrene+	11±2	10±1	20±2
Dibenzo(a,h)anthracene+	4±1	1±0	7±0
Benzo(g,h,i)perylene+	16±2	15±2	21±1
Total PAH[a]	963±206	605±89	1199±203
N-PAH[b]	779±179	456±78	965±188
C-PAH[c]	184±31	148±15	234±23

[a].Total PAH = sum of 16 individual PAHs

[b]. N-PAH = sum of 8 non-carcinogenic PAHs (indicated by *)

[c]. C-PAH = sum of 8 carcinogenic PAHs (indicated by +)

Table 3-8. Mean Total PAH, N-PAH and C-PAH concentrations (mg/kg) of 15 samples using different extraction methods

	Soxhlet extraction method (EPA 3540)			Ultrasonic extraction method (EPA 3550)			Direct extraction method		
Samples	Total PAH[a]	N-PAH[b]	C-PAH[c]	Total PAH[a]	N-PAH[b]	C-PAH[c]	Total PAH[a]	N-PAH[b]	C-PAH[c]
1	1269	1083	186	979	782	197	1055	898	157
2	787	642	146	530	405	125	1604	1317	287
3	1297	1071	226	516	332	184	1153	932	221
4	601	492	109	599	468	131	1377	1139	237
5	874	699	175	521	397	124	828	631	197
6	1443	1180	263	765	592	173	1539	1266	273
7	938	755	184	661	489	172	798	549	250
8	926	754	172	524	394	130	1362	1098	263
9	923	734	190	729	578	151	1155	941	214
10	1760	1483	277	678	513	165	1984	1665	319
11	1297	1019	278	759	584	175	1092	909	183
12	532	362	170	348	235	113	680	437	242
13	642	493	149	444	313	131	750	542	207
14	615	510	106	593	467	126	1074	861	213
15	546	413	134	428	298	130	1529	1287	242
Average (w')	963	779	184	605	456	148	1199	965	234

[a]Total PAH = sum of 16 individual PAHs
[b]N-PAH = sum of 8 non-carcinogenic PAHs
[c]C-PAH = sum of 8 carcinogenic PAHs

Table 3-9. Summary of Student paired t-test results for three extraction methods at 95% and 99% confidence interval (C.I.)

Paired t-test Comparisons	Degrees of freedom	t*	p-value	Statistically Different at	
				95% C.I.	99% C.I.
For Total PAH					
Direct extraction method and Soxhlet extraction method	14	2.36	0.03332	Yes	No
Direct extraction method and Ultrasonic extraction method	14	6.16	0.00002	Yes	Yes
Soxhlet extraction method and Ultrasonic extraction method	14	4.66	0.00036	Yes	Yes
For Noncarcinogenic PAH (N-PAH)					
Direct extraction method and Soxhlet extraction method	14	2.14	0.05	No	No
Direct extraction method and Ultrasonic extraction method	14	5.86	0.00004	Yes	Yes
Soxhlet extraction method and Ultrasonic extraction method	14	4.71	0.00032	Yes	Yes
For Carcinogenic PAH (C-PAH)					
Direct extraction method and Soxhlet extraction method	14	2.99	0.0096	Yes	No
Direct extraction method and Ultrasonic extraction method	14	6.10	0.000027	Yes	Yes
Soxhlet extraction method and Ultrasonic extraction method	14	3.30	0.00552	Yes	Yes

* t is given by the equation below

$$t = \sqrt{\frac{1}{n-1}\sum_{1}^{n}(w_i - w')} \qquad \textit{where } w_i \textit{ and } w' \textit{ are given in Table } 3-8$$

Soxhlet extraction method for Total PAH and C-PAH concentrations, it should be noted that the direct extraction method consistently extracted more PAHs than Soxhlet extraction method and ultrasonic extraction method (see Tables 3-7 and 3-8). At a confidence interval of 99% (t value of 2.98), the direct extraction method was statistically similar to the Soxhlet method for the Total PAH, N-PAH and C-PAH concentrations.

The above experiments indicate that the direct extraction method may be used for the extraction of PAHs from the coal-tar-contaminated soil for chemical analysis. Acetone was shown to be a suitable solvent for the direct solvent extraction method. The soils used in the study had a Total PAH concentration in the range of between 800 and 7,000 mg/kg. If the soil has low PAH concentrations, the extract from the direct extraction method probably need to be concentrated before the PAHs are analyzed with a GC. The above experiments also showed that the direct extraction method could give comparable analytical results with that of the Soxhlet extraction method for soils with organic carbon content as high as 4%.

CONCLUSION

The direct extraction method using various solvents was evaluated to assess it suitability for extracting PAHs from coal tar-contaminated soils. Based on the screening and evaluation tests, acetone was found to be a suitable solvent for the extraction of PAHs from coal tar-contaminated soil. Acetone has similar extraction properties as methylene chloride but is less toxic than methylene chloride. Ethanol was also evaluated and was found to be a suitable extractant for N-PAHs but consistently gave lower concentrations of C-PAHs as compared to acetone and methylene chloride. The direct extraction method using acetone as the solvent was found to extract a higher mass of PAHs than the Soxhlet and ultrasonic extraction methods (Method 3540 and Method 3550, respectively). At a confidence interval of 99%, the Total PAH, N-PAH and C-PAH concentrations for the direct extraction method and Soxhlet method were statistically similar. In conclusion, the direct extraction method is an easy method requiring minimum sample handling and operator's attention. The extraction can be performed directly in a 10-mL glass tube with a small volume of solvent.

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CHAPTER 4. ESTIMATION OF POTENTIAL PAHS BIODEGRADATION FOR COAL-TAR-CONTAMINATED SOILS

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ABSTRACT

The availability of PAHs in contaminated soils has an impact on the extent of PAH degradation in the soil. In this study, a simple solvent-water extraction method is proposed as an assessment tool to estimate the percent of PAHs in the soil that can be degraded, i.e., that will be available for biodegradation. The approach taken is to measure the percent of PAHs that can be extracted by a solvent-water mixture and comparing the results with the percent of PAHs degraded in a soil slurry reactor. Five soil samples from three former manufactured gas plant (MGP) sites and a coal tar disposal site were used in this study. Extraction experiments were conducted using solvent volume fraction in solvent-water mixtures of 1.0 to 0.4 (vol/vol). The solvents used were acetone and ethanol. The extractability of PAHs from the various soils was shown to decrease when the solvent fraction of solvent-water mixture was reduced. PAH biodegradation experiments were conducted using a soil slurry reactor system. The percent of PAHs degraded over 35 days and the first order biodegradation rate constants in the soil slurry reactor were determined for 16 individual PAHs. The percentage of PAHs degraded was found to strongly correlate with the extent of PAHs extracted for a acetone-water mixture of 0.6 for all four soils. Two correlations to predict the percent of PAHs that will degrade in the soils were developed. In the first correlation, a linear correlation was developed between the percent of PAHs extracted, percent of PAHs degraded and the molecular weight and the K_{ow} of the PAHs. In the second correlation, the percent of PAHs degraded was related to the percent of PAHs extracted using the acetone-water mixture and percent organic matter and percent clay and silt in the soil. The second correlation was tested against a fifth soil from another site and was found to predict well the percent of PAHs degraded or available for biodegradation. Although the experiments were conducted for a limited number of soils, the proposed

assessment tool using acetone-water mixture appeared to be a good starting point in estimating the availability of PAHs for biodegradation in contaminated soils from MGP sites.

INTRODUCTION

Manufactured gas or town gas was used in the mid-1800s to the early 1950s for heating, cooking, and lighting in the United States. Town gas was produced by manufactured gas plants (MGPs) with coal, coke, and oil as raw materials. One of the primary byproducts from MGPs was coal tar. Improper disposal of coal tar has contaminated valuable land and aquifers, and poses a serious threat to the environment. With more than 5,000 former MGP sites in United States requiring some forms of remediation (Hatheway, 1997; Luthy et al., 1994), several treatment technologies have been developed in the last decade to address this problem. These technologies include: solvent extraction, thermal desorption, wet air oxidation, supercritical extraction, and bioremediation (Helsel, 1991). Of the above remediation technologies, bioremediation is probably the most cost-effective technology (Crawford, 1996; Cacciatore and McNeil, 1995).

The primary constituents in coal tar wastes from former MGPs are polycyclic aromatic hydrocarbons (PAHs). Information on the biodegradation of PAHs is well documented in the literature. For example, Pothuluri and Cerniglia (1994) reported that PAHs were metabolized to ring fission products via dioxygenase-, monooxygenase-, or peroxidase-catalyzed reactions. A summary of the enzymatic mechanisms used by microorganisms to metabolize and detoxify various PAHs may be found in Sutherland et al. (1995). A majority of the biodegradation studies reported in the literature tend to use artificially contaminated soils with pure microbial culture (Wiesche et al., 1996; Wiesel et al., 1993), while data on the mineralization of PAHs in contaminated soils under field conditions are limited, especially for high molecular weight (HMW) PAHs (MacGillivray and Shiaris, 1994; Cookson, 1995). HMW PAHs are defined as compounds containing four or more fused benzene rings. In general, HMW PAHs have very low aqueous solubilities and very high partition coefficients as compared to low molecular weight (LMW) PAHs. LMW PAHs which contain two or three fused benzene rings are readily degraded under aerobic conditions in the presence of appropriate organisms (Wilson and Jones, 1993) while

HMW PAHs degraded slowly and, in some situations, may be completely recalcitrant (Mueller et al., 1991a, 1991b).

A pressing issue with regard to the bioremediation of PAHs in soil is their availability for biodegradation. PAHs may be adsorbed or bound to humic material making them unavailable for microbial degradation (Manilal and Alexander, 1991; Haider, 1992). In addition, PAHs may reside in the micropores or nanopores of the soil particles that are smaller than 100 nm making them unavailable to the smallest bacterium (Nam and Alexander, 1998; Hatzinger and Alexander, 1997). Several researchers have speculated that bioremediation of PAHs in soil may be visualized as a sequential process in which sorbed PAHs must desorb first into the aqueous phase before the PAHs become available to the microorganisms. Some researches have shown that the overall biodegradation rate of PAHs is controlled by the desorption and diffusion of the PAHs through the intraparticles and not on the activity of the degrading microorganisms in the aqueous phase (Zhang et al., 1998; Pigntello and Xing, 1996; Scheunert and Mansour, 1992; Volkering et al., 1992). Many efforts have gone towards understanding the relationship between desorption and biodegradability (Scow and Hutson, 1992; Bosma et al., 1997; Zhang et al., 1998). However, desorption studies cannot completely explain the lack of bioavailability of PAHs in soils. For example, Zhang (1995) reported that the in-situ degradation rates of PAHs at a former MGP site were several times slower than the estimated degradation rates using mass transfer or desorption principles. Cornelissen et al. (1998) studied the desorption kinetics of 15 PAHs from sediments by using a Tenax solid-phase extraction method. They found that the initial rapidly desorbing fraction could be roughly used to predict the extent of possible PAH degradation in PAH-contaminated sediments and soils. In their work, the percent of the rapidly desorbing fraction was much lower than the percent of degradation for each PAH.

Information on the mechanism and the extent of PAH "bioavailability" or the extent at which the PAHs will degrade within a time period are limited. Development of a simple tool that predicts the extent of PAH degradation potential for a given soil or the limit at which the soil may be cleaned within a reasonable time period will be highly useful (Loehr and Webster, 1997; Kelsey et al., 1997).

Extraction techniques may be used to predict the extent of PAH bioavailability from the coal-tar-contaminated soils. Nakles and Harju (1998) reported that mild solvent extraction might predict the availability of PAHs to earthworms and plants. They used 1-butanol, 1-propanol, methanol, and ethyl acetate as extraction solvent to correlate the uptake by earthworms, barley and wheat plants for PAH compounds such as anthracene, pyrene and fluoranthene. Correlation coefficients were greater than 0.86 for all of the combinations of solvents and test organisms. Similarly, Kelsey et al. (1997) found that mild extraction with n-butanol predicted phenanthrene mineralization in PAH-contaminated soil. In addition, Cuypers et al. (1998) found that solvent extraction (1:1 acetone-water mixture) of the sediments of different size fractions gave a qualitative indication of the availability of PAHs from PAH-contaminated sediment. However, the use of mild solvent extraction to estimate the availability of PAHs in the contaminated soils is still in the development stage. Additional data on the use of different mild organic solvents for different kinds of PAH-contaminated soils is needed to evaluate this approach.

The objective of this study was to assess the feasibility of using miscible solvent extractants for PAH-contaminated soils to estimate the PAH fraction in the coal-tar-contaminated soils that may be biologically available. Solvent-water mixtures of two water miscible solvents, acetone and ethanol, were used as extraction solutions. Slurry phase reactors were used to determine the extent of PAH biodegradation in five coal-tar-contaminated soils. The extraction of sixteen U.S. EPA priority PAHs by the solvent-water mixtures were correlated with the extent at which each of the 16 PAHs were degraded in the soil slurry phase reactors.

MATERIALS AND METHODS

Soil Characteristics

Five different soil samples from four different coal-tar-contaminated sites were used for the experiments. The first two soil samples were collected from the Vandalia road site near Pleasant Hill, Iowa. The site was operated between 1945 and 1950 when MGP residuals were landfilled into a former creek channel. The first soil sample was taken directly from the former creek channel and is designated as Vandalia (EXC). The second soil sample, referred

to as Vandalia (LTU), was taken from a land-farming treatment unit located near the former creek channel. The soil was made up of a mixture of lesser contaminated soil and coal tar-contaminated soil from the former creek channel. The third soil sample was collected from a former MGP site at Charles City, Iowa. This site was operated between 1915 and 1937. The fourth soil sample was collected from Hampton, Iowa where a former MGP site operated between 1915 and 1947. The fifth soil sample was collected from Independence, Iowa where a former MGP site operated between 1880 and 1947. All soils were placed into airtight aluminum containers and kept in a refrigerator at 4 °C. Before each soil was used in the soil slurry and extraction studies, the soil was homogenized by sieving through a 2-mm opening mesh. Measured PAH concentrations and physical-chemical properties of the five soils are listed in Tables 4-1 and 4-2. PAH concentrations were measured using a solvent extraction method and gas chromatography (GC) with a flame ionization detector (see details in later section). Soil texture was determined based on soil particle distribution test and USDA modified soil texture triangle (Boulding, 1994). Organic carbon contents in the soils were determined by the rapid dichromate oxidation method (Nelson and Sommers, 1982).

Solvent Extraction Study

The two solvent-water mixtures used were either acetone-water or ethanol-water. The volume fraction of the solvents used in the solvent-water mixtures ranged from 0.4 to 1.0. Deionized water was used for all dilutions. Two grams of soil in 5 mL of solvent-water mixture were placed in a 10-mL glass tube with Teflon-lined screw cap. The tubes were shaken for 24 hours using a wrist action shaker (Model 75, Burrell Scientific, Pittsburgh, PA) at room temperature of 22 ± 2 °C. The tubes were then centrifuged at 3,000 rpm for 30 minutes. A 5 μL aliquot of the supernatant was analyzed with GC (5890 series II, Hewlett-Packard, Palo Alto, CA). The GC was equipped with a HP-5 capillary column and a flame ionization detector (FID). The initial oven temperature of the GC was set at 50 °C followed by a temperature ramp rate of 8 °C /min to a final temperature of 302 °C for 5 minutes. The injector temperature was 240 °C and the detector temperature was 320 °C. To obtain the dry mass of the soil, the supernatant was decanted and the mass of soil was determined after the

Table 4-1. PAH concentrations on dry-weight basis (mg/kg) for five different coal-tar-contaminated soils

Compound	Hampton, IA		Vandalia (EXC), IA		Charles City, IA		Vandalia (LTU), IA		Independence, IA	
	Conc.	Std. Dev.	Conc.	Std. Dev.	Conc.	Std. Dev.	Conc.	Std. Dev.	Conc.	Std. Dev
Naphthalene	48	24	35	3	986	54	62	1	379	12
Acenaphthylene	190	5	485	19	325	22	61	1	272	6
Acenaphthene	37	1	239	9	80	6	34	0	103	3
Fluorene	139	3	358	13	181	14	62	1	197	3
Phenanthrene	347	11	1,238	40	408	42	128	4	602	6
Anthracene	113	3	386	12	141	12	54	1	177	2
Fluroanthene	128	2	378	9	126	11	98	3	209	3
Pyrene	173	3	545	12	171	14	146	5	287	3
Benzo(a)anthracene	67	1	188	3	66	4	52	2	92	1
Chrysene	65	2	202	3	66	4	53	4	92	1
Benzo(b)fluoranthene	36	1	82	2	25	1	30	1	53	1
Benzo(k)fluoranthene	36	0	84	1	30	2	25	1	54	1
Benzo(a)pyrene	60	1	143	3	48	3	36	1	90	2
Indeno(1,2,3-cd)pyrene	28	1	54	2	18	1	18	1	51	4
Dibenzo(a,h)anthracene	9	0	18	1	7	0	7	0	15	1
Benzo(g,h,i)perylene	24	1	57	2	17	1	18	1	51	3
Total PAH	1,500	34	4,494	129	2,694	188	884	28	2,724	10
N-PAH*	1,174	32	3,664	112	2,417	172	645	16	2,227	15
C-PAH**	326	6	830	18	276	17	239	11	498	14
N-PAH (%)	78		82		90		73		82	
C-PAH (%)	22		18		10		27		18	

*N-PAH: non-carcinogenic PAHs (first eight compounds)

**C-PAH: carcinogenic PAHs (second eight compounds)

Table 4-2. Physical-chemical properties of five different coal-tar-contaminated soils

Properties	Hampton, IA	Vandalia (EXC), IA	Charles City, IA	Vandalia (LTU), IA	Independence, IA
Soil Texture*	Loam soil	Sandy loam	Sandy loam	Sandy clay loam	Sandy loam
Sand (%)	41	60	64	54	64
Silt (%)	35	26	18	24	25
Clay (%)	24	14	18	22	11
Organic carbon (%)	3.5	4.0	2.3	3.0	2.5
Soil moisture (%)	12.97	8.72	8.40	3.70	4.00
Soil pH	7.22	7.65	6.52	7.10	7.80

*Based on USDA modified soil texture classification

soil in the tube was dried in an oven at 105 °C for 24 hours. The PAH concentrations in the soil were reported on a dry weight basis.

Soil Slurry Biodegradation Studies

Soil slurry biodegradation studies were conducted in a batch mode in a 1-L glass reactor. The constant contact of the soil with water coupled with the mixing in soil slurry reactors generally provides an environment that maximizes availability of PAH. Therefore, the amount of PAH remaining, after steady state conditions are reached, will reflect the amount of PAH that are not available. Approximately 150 g (dry weight basis) of contaminated soil were loaded into the reactor along with 750 mL of distilled water. Each reactor was seeded with 20 mL of supernatant from a mother reactor which has been treating PAH-contaminated soil for several months. The soil slurry was supplemented with ammonium nitrate as a nitrogen source and KH_2PO_4 as a phosphate source. The amounts of nutrient added were based on a C:N:P ratio of 100:10:2 where the carbon fraction was assumed to be the total carbon content of the 16 PAHs present in the soil. Each reactor was mixed with a mixer (Model PM6015, EMI Incorporated, Clinton, CT) and aerated using an air stone diffuser at an airflow rate of 200 mL/min. The dissolved oxygen concentration was maintained at above 3.0 mg/L and the pH of the slurry was kept between 6.5 and 8. Slurry samples were taken at various time periods and the concentration of each PAH in the soil was measured according to the method described by Pinelli et al. (1997) and Lee et al. (1999). A 10-mL slurry sample was placed in a 10-mL glass tube and centrifuged at 3,000 rpm for 30 minutes. The supernatant was discarded. Preliminary tests have shown that the supernatant contained negligible amount of PAHs. Five mL of pure acetone were then added to the soil residue and the tube was shaken for 24 hours. The suspension was centrifuged at 3,000 rpm for 30 minutes and 5 μL of the supernatant was analyzed by GC. The dry weight of soil was then determined by drying the soil sample at 105 °C for 24 hours.

RESULTS AND DISCUSSION

Solvent-water Extraction Experiments

Instead of plotting the amount of PAH extracted for every 16 PAHs of each soil, the percent extracted for the low molecular weight (LMW) and high molecular weight (HMW) PAHs for the five soils with different acetone-water and ethanol-water mixtures are presented in Figures 4-1 and 4-2. The eight PAHs that make up the LMW PAHs and the HMW PAHs are listed in Table 4-1. The trends seen in Figures 4-1 and 4-2 are reflective of the individual PAH within its broad grouping of LMW and HMW PAHs. The PAH extracted are expressed as a percentage of the PAH extracted using 100% acetone. Lee et al. (1999) have shown that pure acetone is an effective solvent for the extraction and measurement of the PAHs present in the soil.

Except for the Vandalia (EXC) soil, the percentages of PAH extracted were all greater than 90% for acetone-water mixtures of 0.8 or more. The percent extracted declined precipitously when an acetone-water mixture of 0.7 or less was used. Less than 30% of the PAH were extracted with an acetone-water mixture of 0.4. As seen in Figure 4-1 (and also in Figure 4-2), the LMW PAHs were more easily extracted than the HMW PAHs for different solvent-water mixtures. Less PAH were extracted for the Vandalia (EXC) soil than from the other four soils for all acetone-water mixtures. A possible reason for the lower extraction percentages may be the higher organic carbon content of the soil in comparison to the other four soils.

As expected, PAH extraction by the ethanol-water mixtures were all less than that of the acetone-water mixtures for all five soils. In comparison with 100% acetone, the percent of HMW PAH extracted with pure ethanol were 79%, 79%, 79%, 73% and 70% for Hampton, Charles City, Vandalia (LTU), Vandalia (EXC), and Independence soils, respectively. Pure ethanol extracted similar amounts of LMW PAHs as pure acetone except for Hampton soil where a higher percentage of LMW PAH was extracted. With a lower fraction of ethanol in the ethanol-water mixture, the percent of PAH extracted declined. The percent extracted for various ethanol-water mixtures were always less than that of the corresponding volume fraction of acetone-water mixtures. For example, for an ethanol-water mixture of 0.7, the percent of HMW PAH extracted were 55%, 63%, 56%, 38%, and 39% for

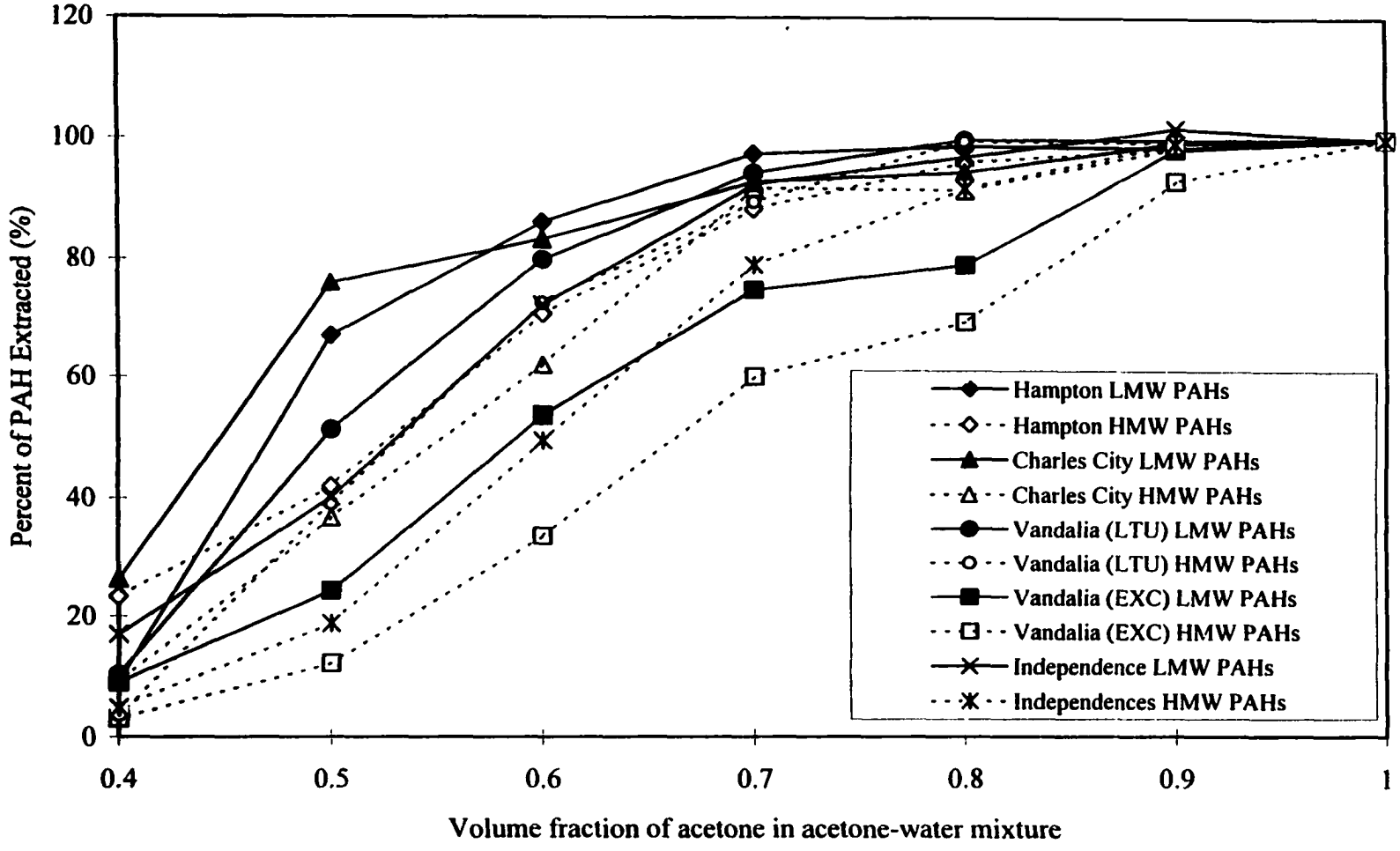

Figure 4-1. Extraction of PAHs using different acetone-water mixtures for five different soils

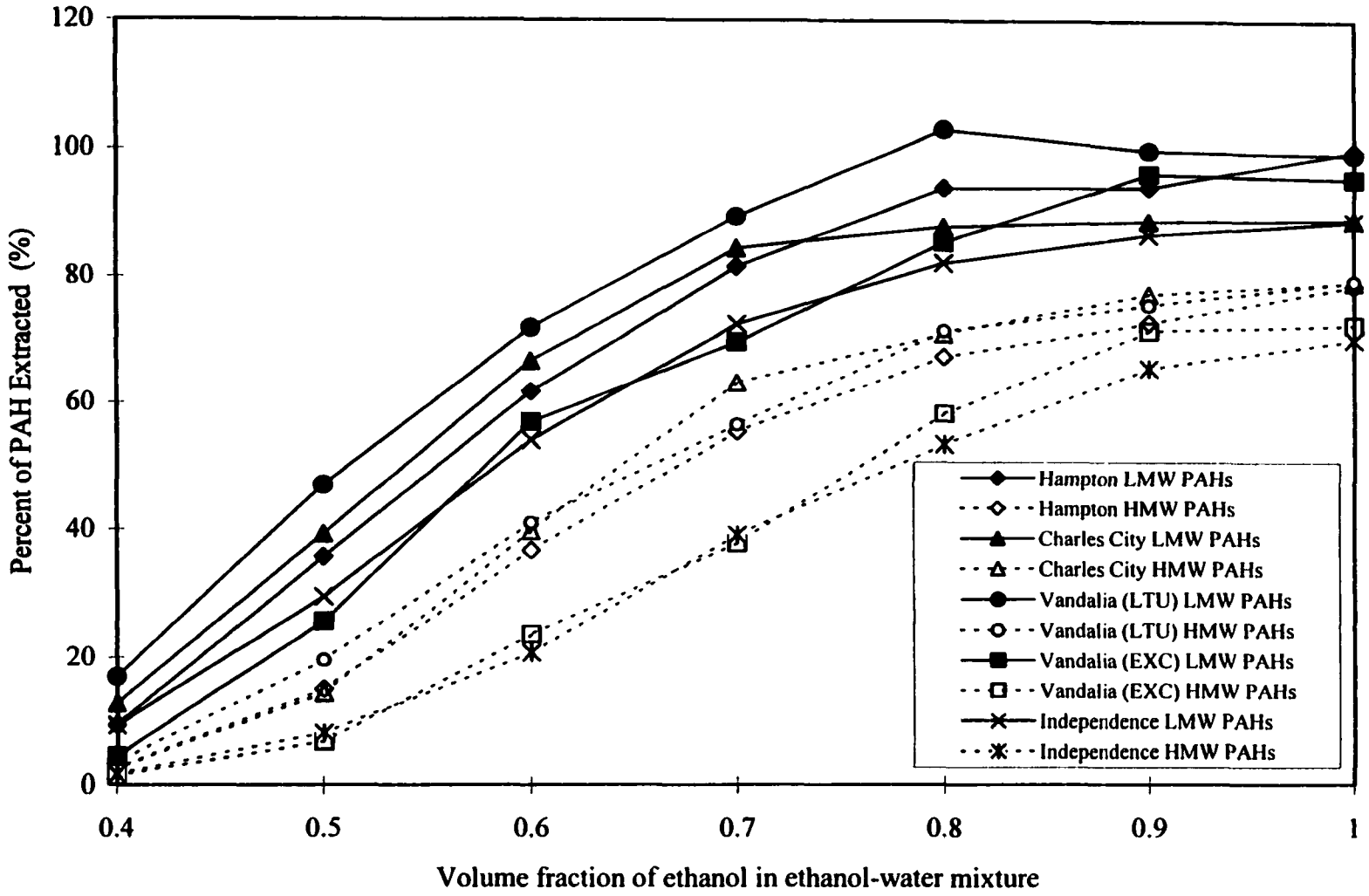

Figure 4-2. Extraction of PAHs using different ethanol-water mixtures for five different soils

Hampton, Charles City, Vandalia (LTU), Vandalia (EXC), and Independence soils, respectively while the percent extracted were 88%, 92%, 90%, 60% and 79% for the same soils. Just as in the acetone-water mixture experiments, the percents of LMW and HMW PAHs extracted from the Vandalia (EXC) soil by ethanol-water mixture were lower than that of the other four soils.

The above results showed that the pattern of decreasing extractability of PAHs with a lower volume fraction of solvent in the solvent-water mixture may be used to estimate the extent of PAH availability for biodegradation in the soil. As seen in Figures 4-1 and 4-2, the lower extraction potential of HMW PAHs as compared to LMW PAHs may be similar, to a certain extent, to the biodegradation potential of PAHs in the soil where LMW PAHs are more easily degraded than HMW PAHs (Cookson, 1995).

Extent of PAH Biodegradation in Different Soils

Figures 4-3 – 4-7 present the biodegradation results of sixteen PAHs using the soil slurry reactors for all five soils. Except for one or two PAHs (for example, benzo(k)fluoranthene in Charles City soil), most of the PAHs in the five soils have reached steady state concentrations. Biodegradation of PAHs for all five soils followed a similar degradation pattern. A large fraction of the 2- and 3-ring PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene) degraded within 14 days while the 4-ring PAHs (fluroanthene, pyrene, benzo(a)anthracene, and chrysene) only started to degrade after most of the 2- and 3-ring PAHs were removed. The 5- and 6-ring PAHs that included benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(g.h.i)perylene were degraded slowly with less than 20% degraded after 35 days. For all soils, a residual amount of PAHs remained after 35 days and these were generally 4-, 5- and 6-ring PAHs.

Of the five soils tested, degradation of 2- and 3-ring PAHs was the fastest for Vandalia (LTU) soil (see Figure 4-3) which may be related to its initial concentration (Loehr and Webster, 1997). Naphthalene was completely removed from Charles City soil within 4 days but for Vandalia (LTU) and Vandalia (EXC) soils, naphthalene continued to persist even up to 28 days. In the case of Hampton soil, low concentrations of naphthalene

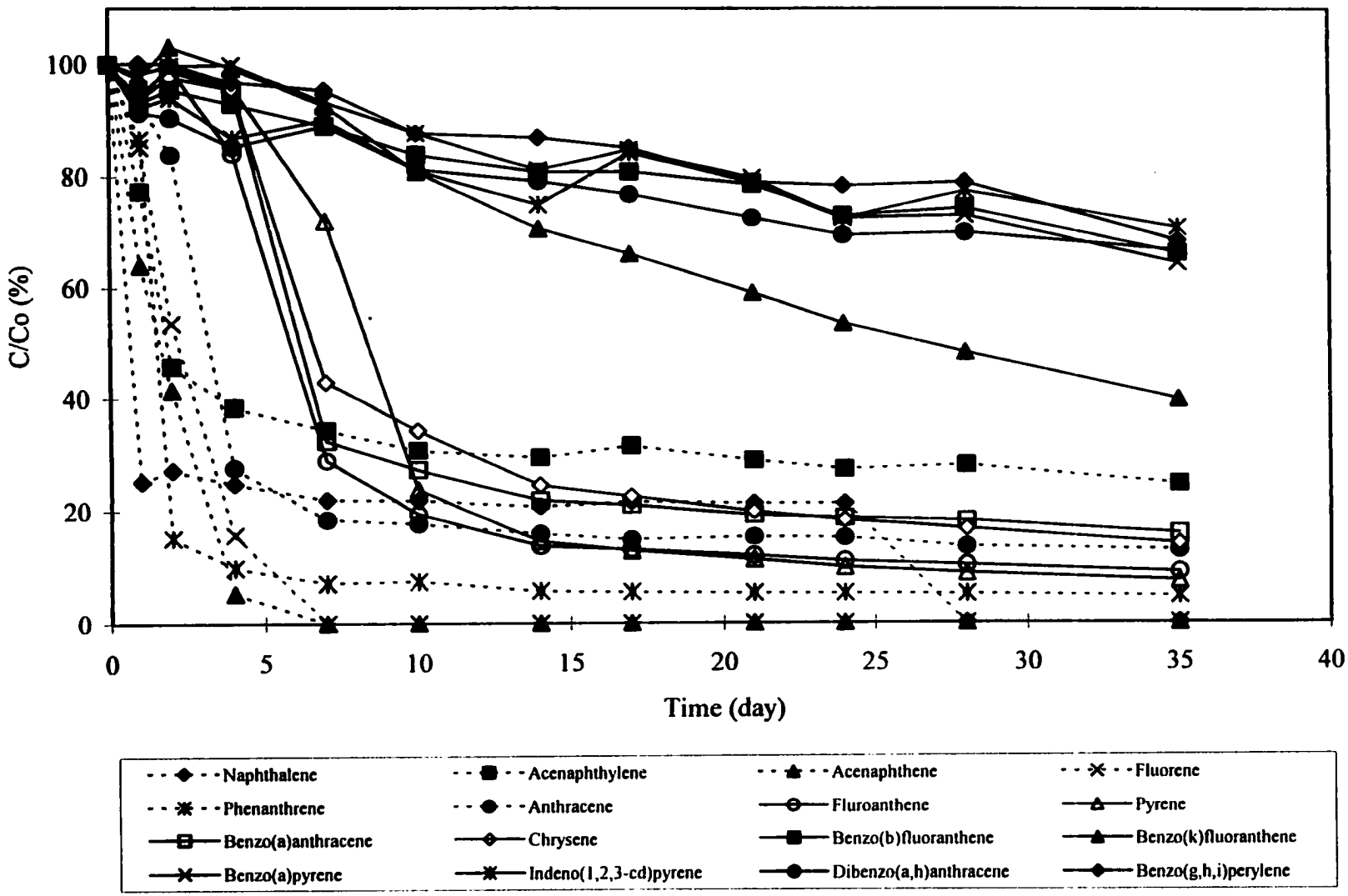

Figure 4-3. Biodegradation of PAHs for Vandalia (LTU) soil in soil slurry reactor

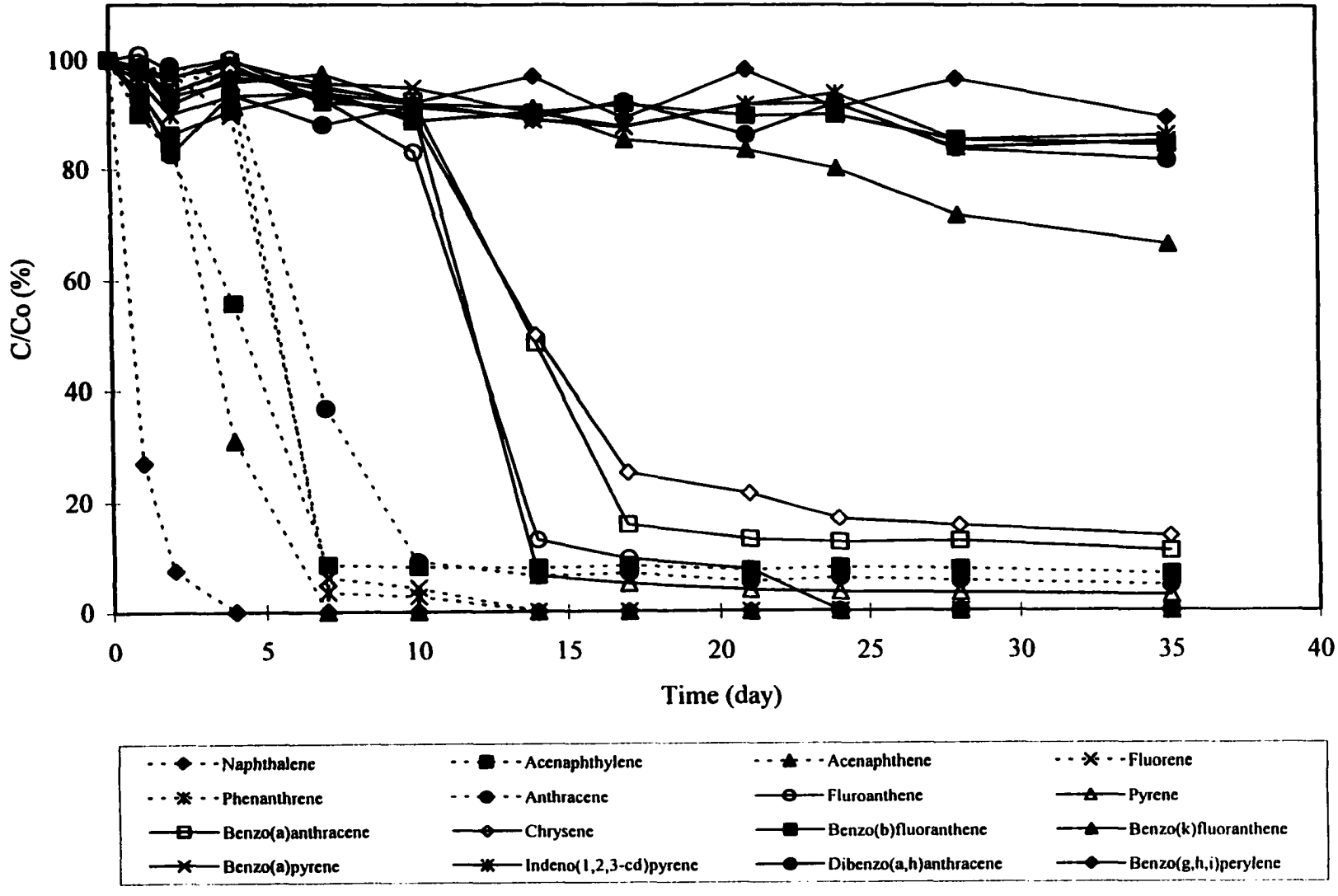

Figure 4-4. Biodegradation of PAHs for Charles City soil in soil slurry reactor

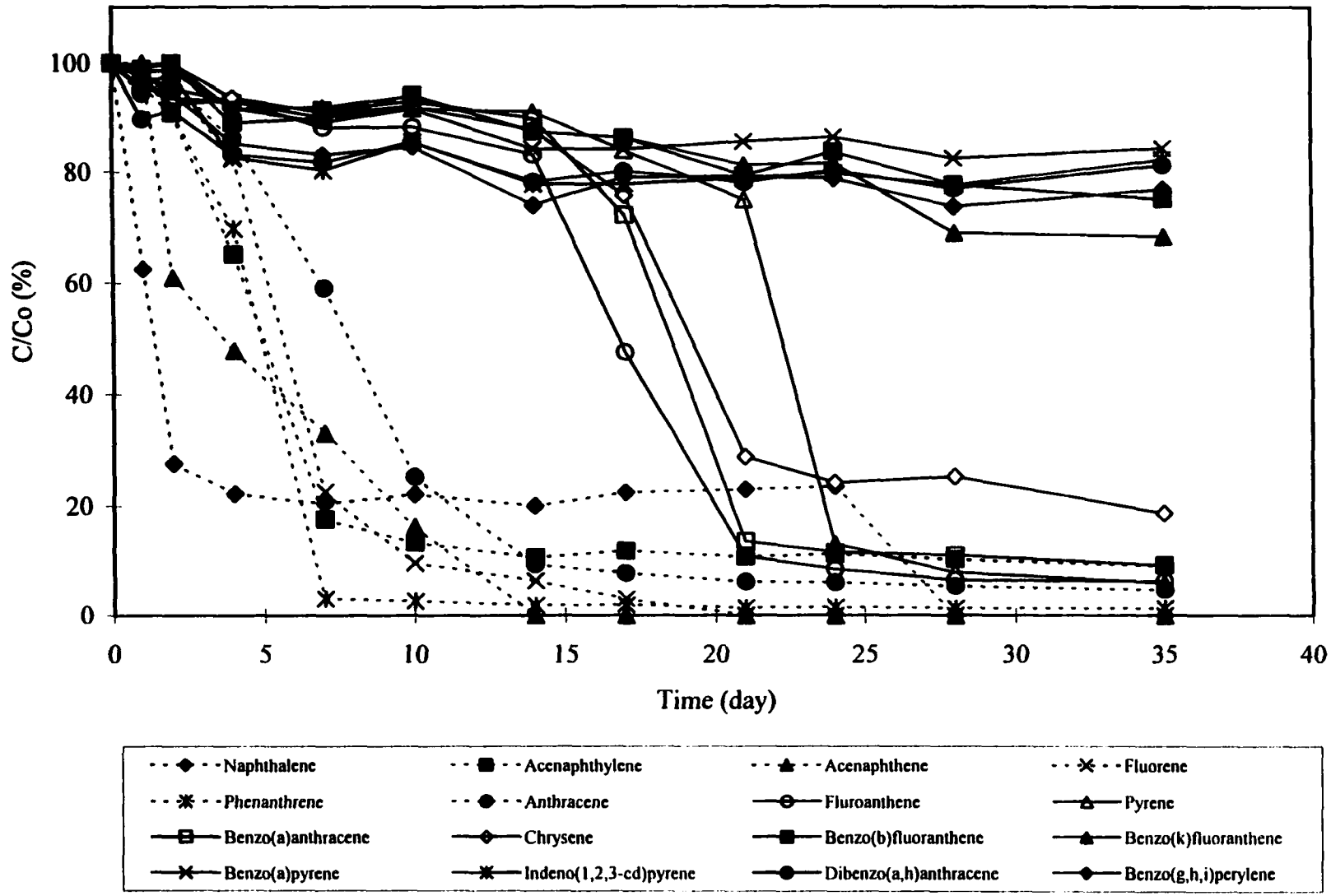

Figure 4-5. Biodegradation of PAHs for Vandalia (EXC) soil in soil slurry reactor

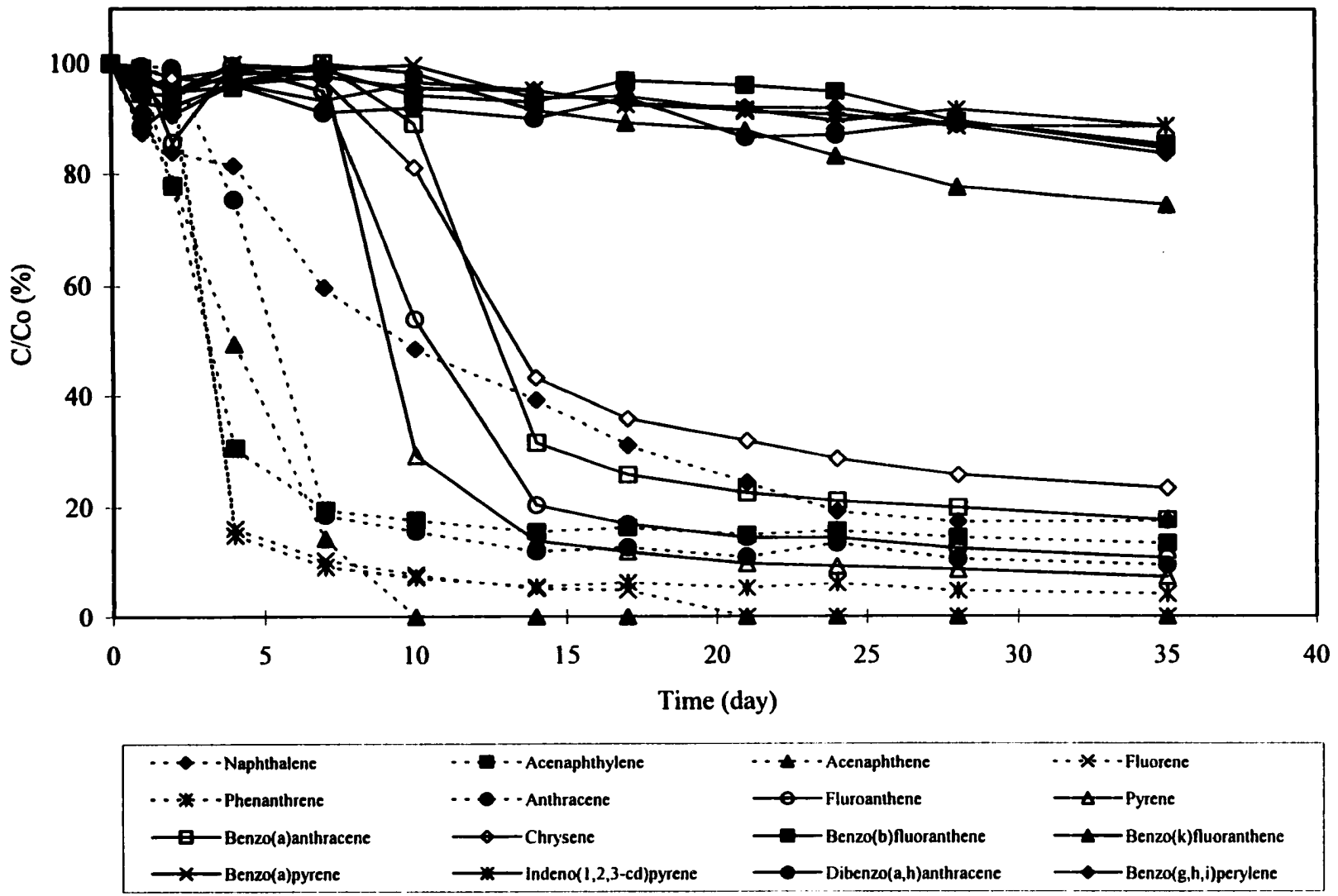

Figure 4-6. Biodegradation of PAHs for Hampton soil in soil slurry reactor

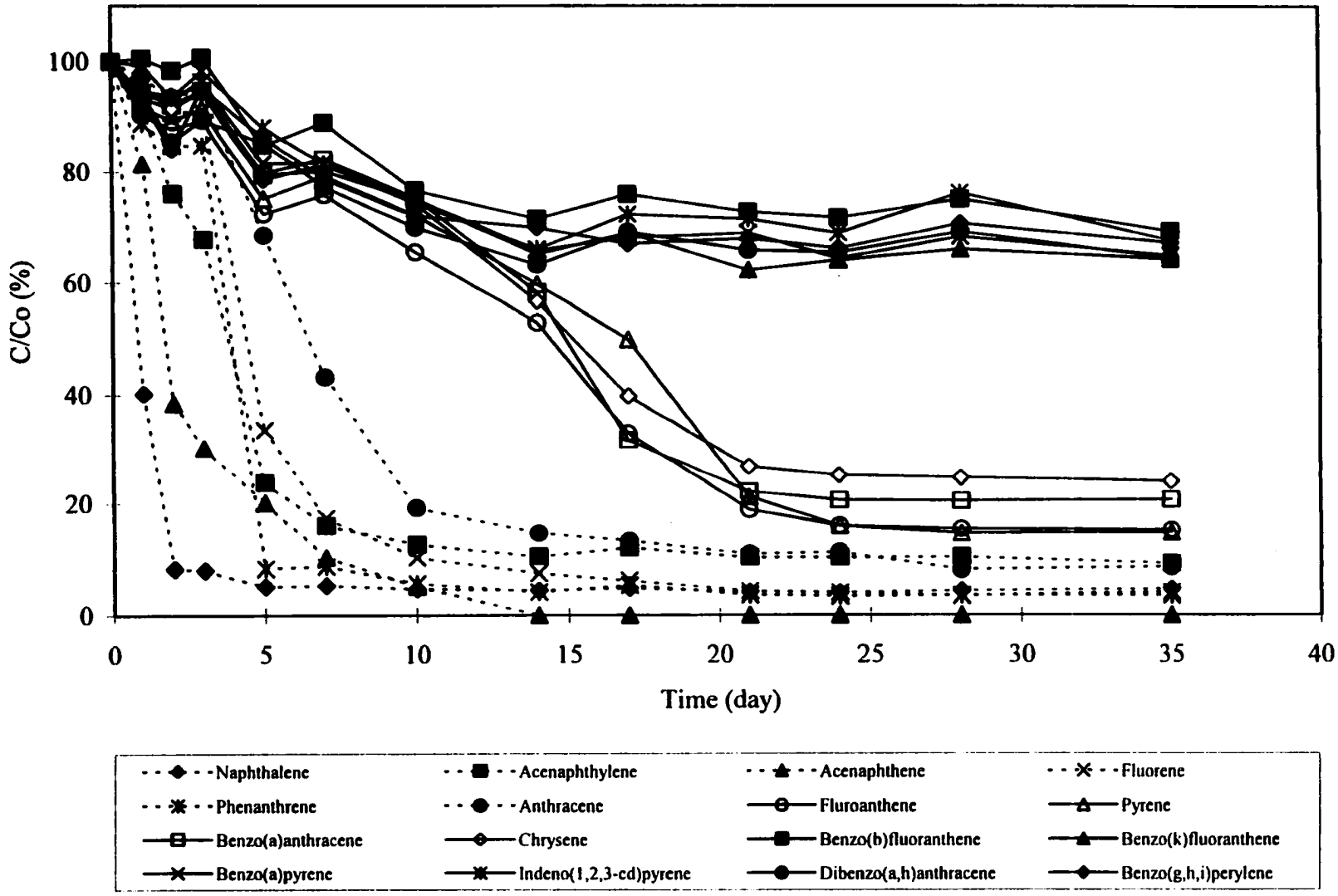

Figure 4-7. Biodegradation of PAHs for Independence soil in soil slurry reactor

(6 mg/kg) remained even after 35 days. Besides naphthalene, other PAHs compounds that were completely removed were fluorene and acenaphthene. Benzo(k)fluoranthene was the only 5- and 6-ring PAH that was degraded significantly (about 50%) for Vandalia (LTU) soil within 35 days (see Figure 4-3) while for all other soils approximately 20% were degraded. The residual amounts of 2- and 4-ring PAHs remaining in the soil after 35 days clearly point towards the slowly desorbing or unavailable fraction present in the soil. For the 5- and 6-ring PAHs, the slow degradation observed may be due to their lack of availability and/or the lack or absence of microbial activity essential for their degradation (Suntherland et al., 1995). The above data can be viewed differently by using the degradation of Total PAH as shown in Figure 4-8 instead of all 16 individual PAHs. There was a lag phase for both Hampton soil and Vandalia (EXC) soil. Degradation of PAHs in Charles City soil appeared to be the fastest of the five soils tested. For all soil samples, the Total PAH concentration reached steady state concentrations with a residual amount of PAHs (between 150 to 300 mg/kg) remaining after 35 days. These PAHs were generally 4-, 5-, and 6- rings PAHs.

To correlate the biodegradation of the PAHs for the five soils with the percent extracted using solvent-water mixtures, the extent of PAH degraded (expressed as a percentage) after 35 days were determined along with the first order degradation rate constants for the 16 PAHs and Total PAHs. The extent of degradation and the degradation rate constants are presented in Table 4-3.

Correlation between Percent Extracted and Biodegradation

Since the extent of PAH degradation in the soil is dependent on its availability, the percent of PAH extracted for various solvent-water mixtures were plotted against the percent of PAH degraded or the first order degradation rate constants to elucidate any trends. The percent of PAH degraded reflected the total amount of each individual PAH in the soil that will be eventually degraded. The first order degradation rate constant was also used since it reflected how easily the PAH is degraded and, indirectly, the availability of the PAH for degradation. Figure 4-9 shows a typical plot of the percent biodegraded for all five soils and the percent extracted while Figure 4-10 shows a similar plot but with the first order degradation rate constants. The figures were plotted using Hampton soil and each data point

Table 4-3. First order degradation rate constants of PAHs after 35 days for five soils in slurry reactor

Compound	Hampton		Vandalia (EXC)		Charles City		Vandalia (LTU)		Independence	
	k (1/day)*	R^2	k (1/day)	R^2	k (1/day)	R^2	k (1/day)	R^2	k (1/day)	R^2
Naphthalene	0.038±0.014	0.910	0.083±0.050	0.903	1.296±0.100	0.999	0.651±0.534	0.706	0.644±0.838	0.779
Acenaphthylene	0.199±0.092	0.900	0.188±0.065	0.917	0.347±0.233	0.882	0.107±0.084	0.759	0.146±0.037	0.938
Acenaphthene	0.281±0.121	0.948	0.178±0.042	0.972	0.300±0.276	0.856	0.741±0.499	0.953	0.203±0.045	0.965
Fluorene	0.237±0.110	0.860	0.222±0.041	0.967	0.366±0.202	0.863	0.481±0.309	0.957	0.145±0.023	0.96
Phenanthrene	0.231±0.160	0.734	0.347±0.160	0.862	0.424±0.254	0.843	0.394±0.393	0.772	0.212±0.085	0.872
Anthracene	0.183±0.071	0.897	0.154±0.030	0.955	0.224±0.077	0.918	0.273±0.145	0.923	0.085±0.018	0.926
Fluroanthene	0.106±0.031	0.903	0.087±0.024	0.869	0.139±0.052	0.849	0.163±0.047	0.941	0.061±0.014	0.923
Pyrene	0.132±0.101	0.906	0.074±0.038	0.758	0.132±0.162	0.836	0.146±0.185	0.884	0.059±0.023	0.878
Benzo(a)anthracene	0.061±0.017	0.864	0.071±0.032	0.770	0.080±0.022	0.866	0.128±0.051	0.892	0.055±0.013	0.905
Chrysene	0.050±0.011	0.911	0.049±0.013	0.876	0.069±0.015	0.917	0.112±0.035	0.932	0.047±0.009	0.925
[illegible]	0.602±0.001	0.426	0.007±0.003	0.788	0.003±0.002	0.401	0.010±0.001	0.961	0.011±0.004	0.792
Benzo(k)fluoranthene	0.007±0.002	0.899	0.009±0.003	0.845	0.011±0.002	0.926	0.028±0.002	0.988	0.015±0.004	0.885
Benzo(a)pyrene	0.003±0.001	0.625	0.005±0.002	0.725	0.004±0.002	0.809	0.012±0.002	0.966	0.014±0.004	0.876
Indeno(1,2,3-cd)pyrene	0.001±0.002	0.165	0.006±0.004	0.531	0.003±0.002	0.481	0.008±0.003	0.783	0.012±0.005	0.767
Dibenzo(a,h)anthracene	0.002±0.002	0.239	0.005±0.003	0.624	0.003±0.003	0.301	0.011±0.002	0.825	0.013±0.004	0.807
Benzo(g,h,i)perylene	0.002±0.002	0.547	0.007±0.003	0.678	0.001±0.002	0.186	0.010±0.001	0.968	0.012±0.004	0.835
Total PAHs	0.080±0.018	0.940	0.078±0.013	0.960	0.144±0.025	0.970	0.092±0.025	0.930	0.080±0.014	0.956

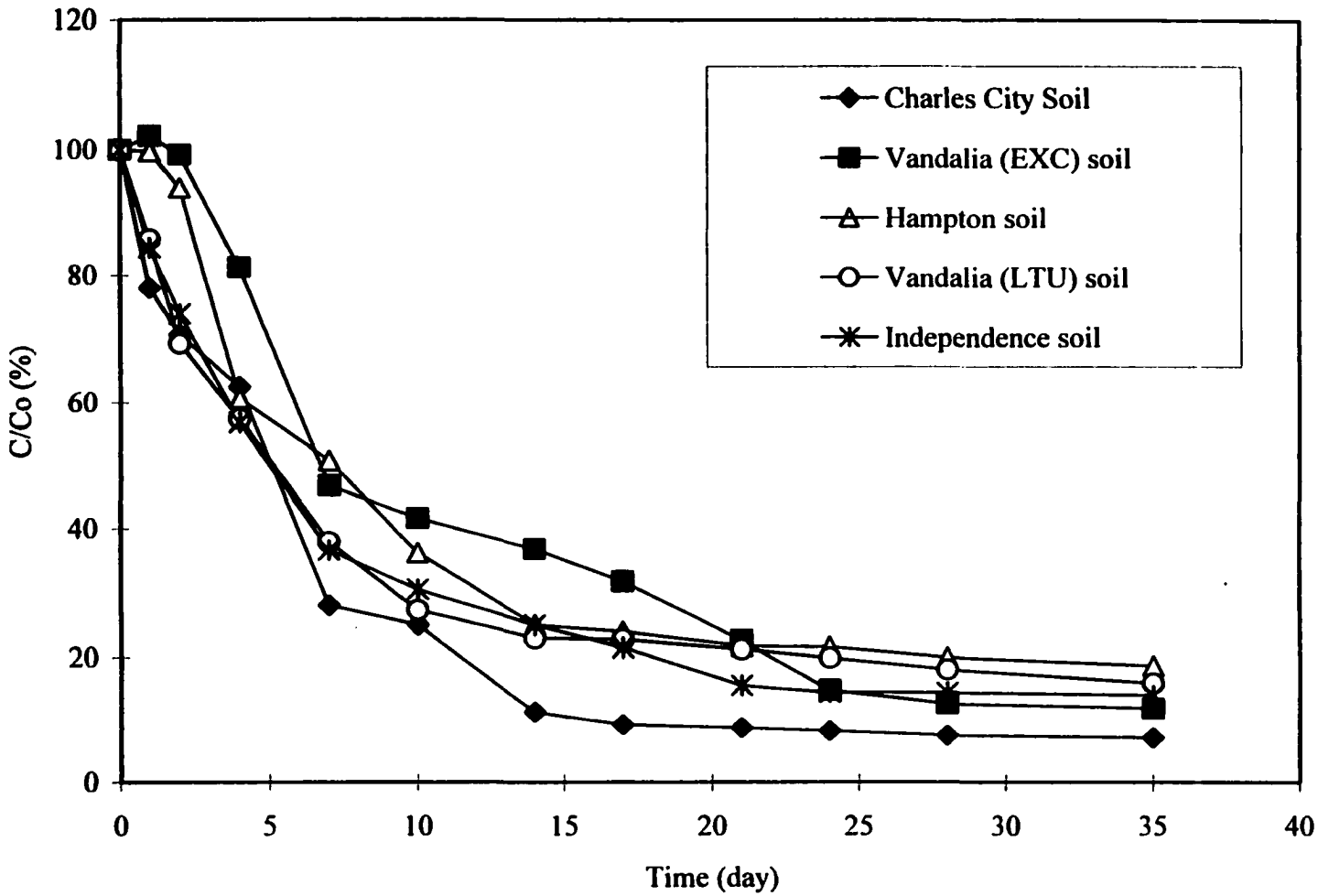

Figure 4-8. Degradation of Total PAHs for five different soils in soil slurry reactor

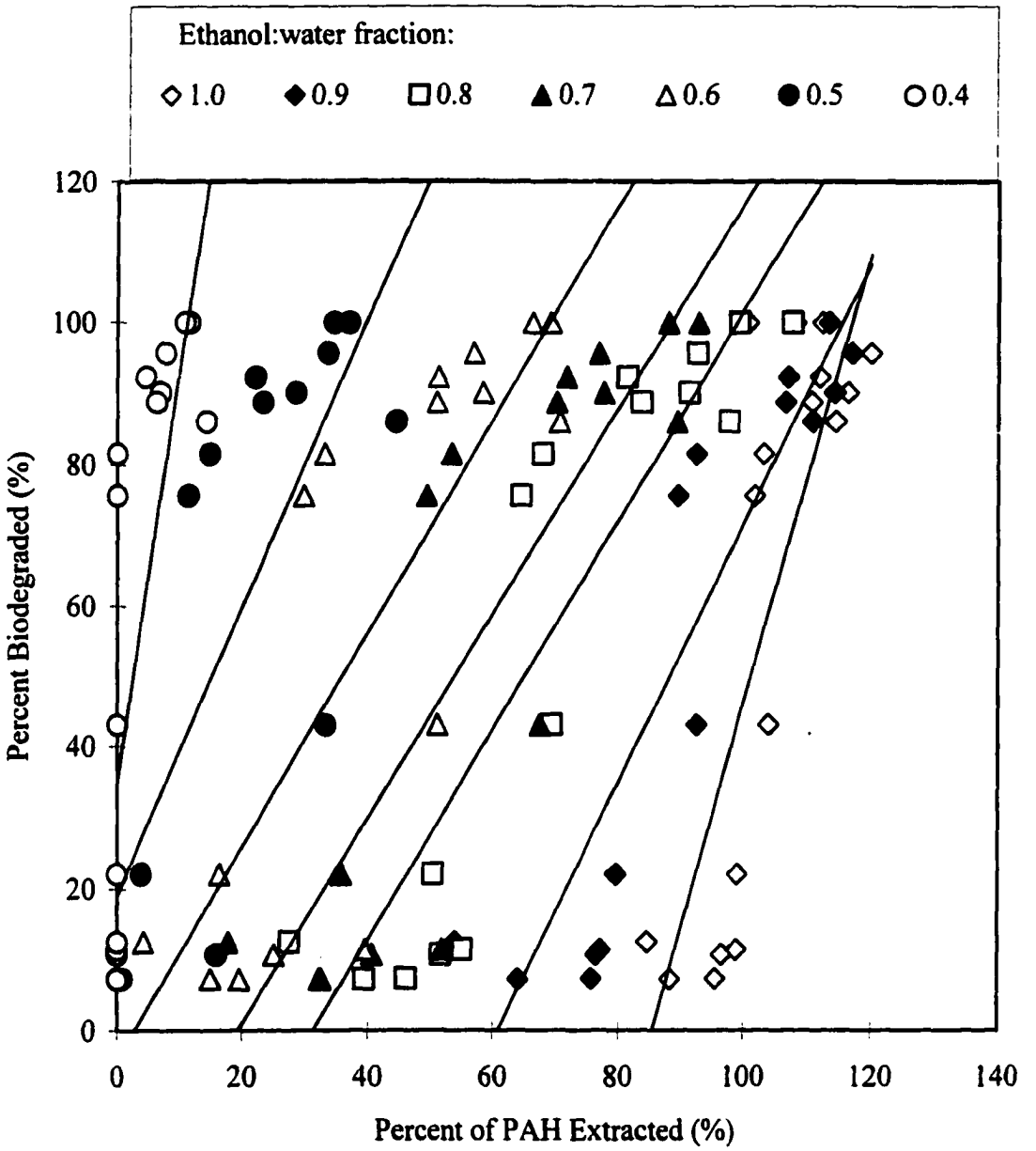

Figure 4-9. Plot of percent PAH extracted and percent biodegraded for Hampton soil using different ethanol-water mixtures

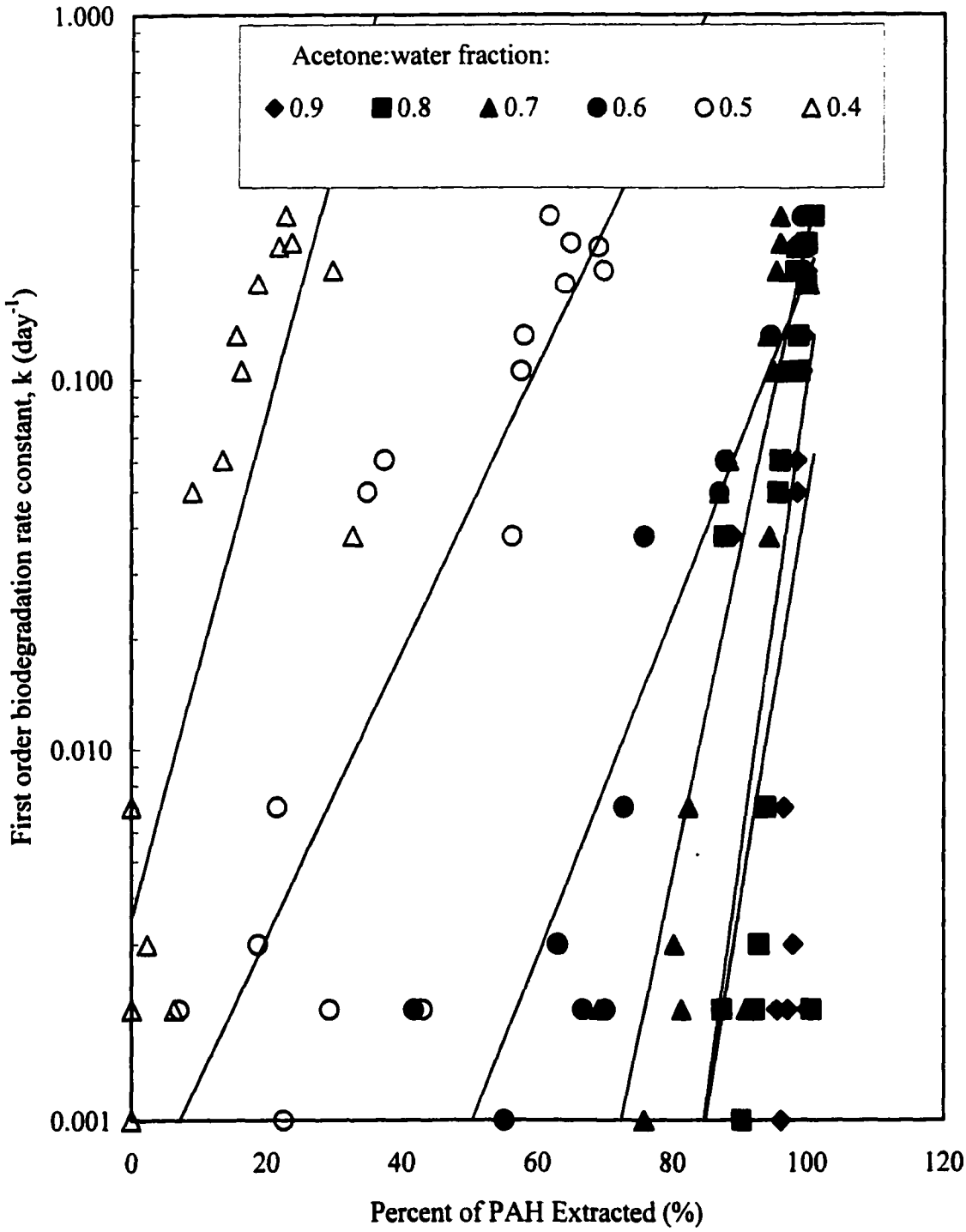

Figure 4-10. Plot of percent PAH extracted and biodegradation rate constant (k) for Hampton soil using different acetone-water mixtures

represent one of the 16 PAHs. Both figures showed that there were positive trends between the percent PAH degraded or the PAH degradation rate constant for all solvent-water mixtures and the percent PAH extracted except for 0.9 acetone-water mixture where the trend seemed to flatten out. Similar plots were generated for the other four soils but are not presented here. For each of the soil and each solvent-water mixtures used, the correlation coefficients (R^2) for the plot of the percent biodegraded or the first order degradation rate constants and the percent of PAH extracted were determined. The correlation coefficients (R^2) of the linear regression for each soil and each solvent-water mixture are presented in Table 4-4. The R^2 values provide an indirect measure of the strength of the correlation between the extent of PAH biodegraded in the soil after 35 days or the first order degradation rate constants and the percent PAH extracted.

Several observations can be made. For ethanol as the extracting solvent, the R^2 values for the percent biodegraded and the percent extracted ranged from 0.75 to 0.85 for ethanol-water mixtures of 0.6 to 0.9. When the degradation rate constants were correlated with the percent extracted, the R^2 values were slight greater (from 0.85 and 0.90) than that of the percent degraded. For acetone, the highest R^2 values for all five soils were obtained when an acetone-water mixture of 0.6 was used. This can be seen more clearly in Figures 4-11 and 4-12 where the R^2's were the maximum for an acetone-water mixture of 0.6. In contrast, the R^2 values (see Table 4-4) for various ethanol-water mixtures did not change dramatically compared to that of the acetone-water mixture experiments. The above indicates that acetone-water mixtures may provide the necessary differences in PAH extractability to clearly delineate the extent of PAH availability for the prediction of PAH degradation in a PAH-contaminated soil. The volume fraction of acetone-water mixture of 0.6 seemed to be an appropriate solvent fraction for predicting the extent of PAH degradation. This approach, however, seemed to be rather simplistic and must be further expanded since the extent of PAH degradation for a given soil is also very much dependent on the PAH properties, soil type and properties and the aging process.

Figure 4-13 presents a plot of the percent biodegraded and the percent extracted while Figure 4-14 presents a plot of the logarithm of the first order biodegradation rate constants

Table 4-4. Summary of R^2 values of linear regression between percent biodegradated/first order degradation rate constants and percent extracted with two solvents for five soils

Solvent volume fraction	Percent biodegraded vs. percent extracted										First order degradation rate constant vs. percent extracted									
	Ethanol					Acetone					Ethanol					Acetone				
	S#1	S#2	S#3	S#4	S#5	S#1	S#2	S#3	S#4	S#5	S#1	S#2	S#3	S#4	S#5	S#1	S#2	S#3	S#4	S#5
1.0	0.34	0.59	0.70	0.74	0.80						0.39	0.72	0.73	0.80	0.75					
0.9	0.63	0.72	0.77	0.84	0.87	0.22	0.17	0.01	0.20	0.40	0.71	0.83	0.84	0.89	0.80	0.16	0.23	0.01	0.01	0.46
0.8	0.73	0.75	0.76	0.83	0.89	0.57	0.34	0.03	0.49	0.71	0.80	0.85	0.86	0.88	0.83	0.52	0.48	0.05	0.05	0.67
0.7	0.75	0.74	0.71	0.74	0.86	0.70	0.59	0.39	0.65	0.80	0.81	0.84	0.81	0.81	0.82	0.62	0.71	0.49	0.49	0.77
0.6	0.85	0.72	0.64	0.67	0.75	0.83	0.74	0.87	0.87	0.88	0.90	0.81	0.73	0.73	0.87	0.88	0.84	0.83	0.83	0.89
0.5	0.70	0.35	0.54	0.63	0.60	0.74	0.61	0.58	0.69	0.69	0.82	0.51	0.64	0.75	0.85	0.83	0.64	0.64	0.64	0.82
0.4	0.43	0.41	0.41	0.57	0.43	0.58	0.44	0.46	0.55	0.57	0.63	0.55	0.54	0.60	0.78	0.73	0.60	0.55	0.55	0.80

Note: S#1 = Charles City soil, S#2 = Vandalia (EXC) soil, S#3 = Vandalia (LTU) soil, S#4 = Hampton soil, S#5 = Independence soil.

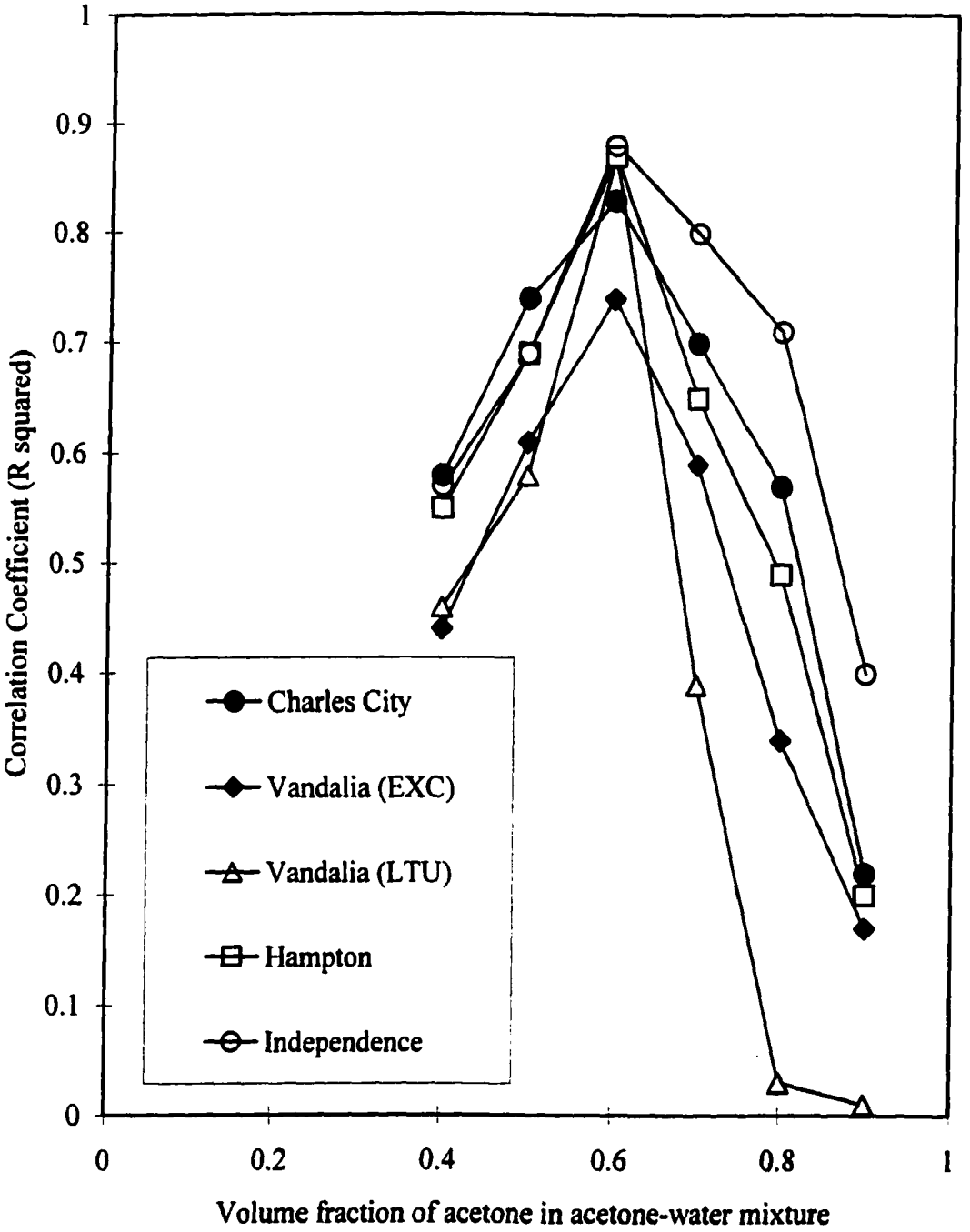

Figure 4-11. Plot of R^2 vs. acetone-water volume fraction for five different soils. R^2 is for percent PAH extracted and percent biodegraded

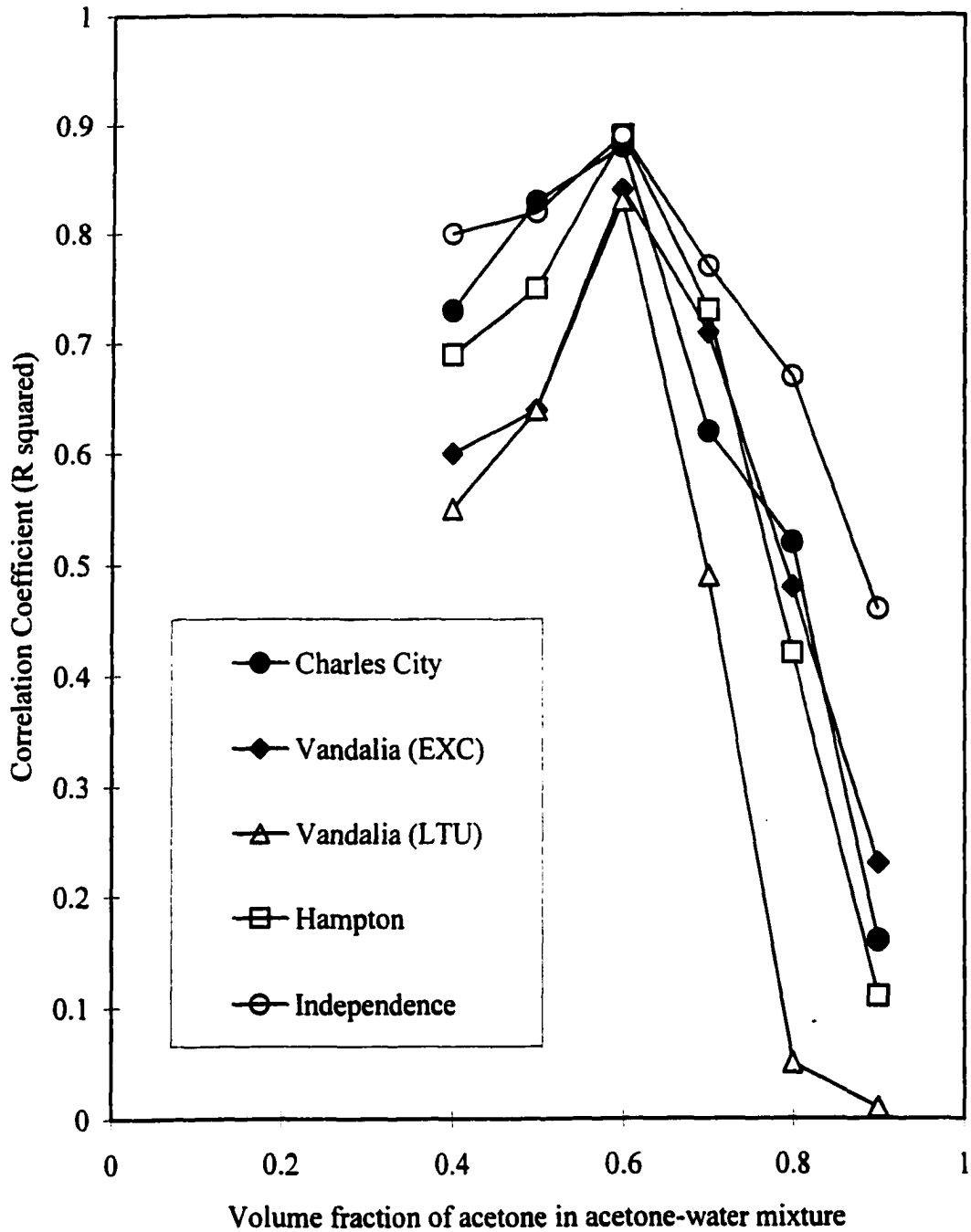

Figure 4-12. Plot of R^2 vs. acetone-water volume fraction for five different soils. R^2 is for percent extracted and first order degradation rate constants

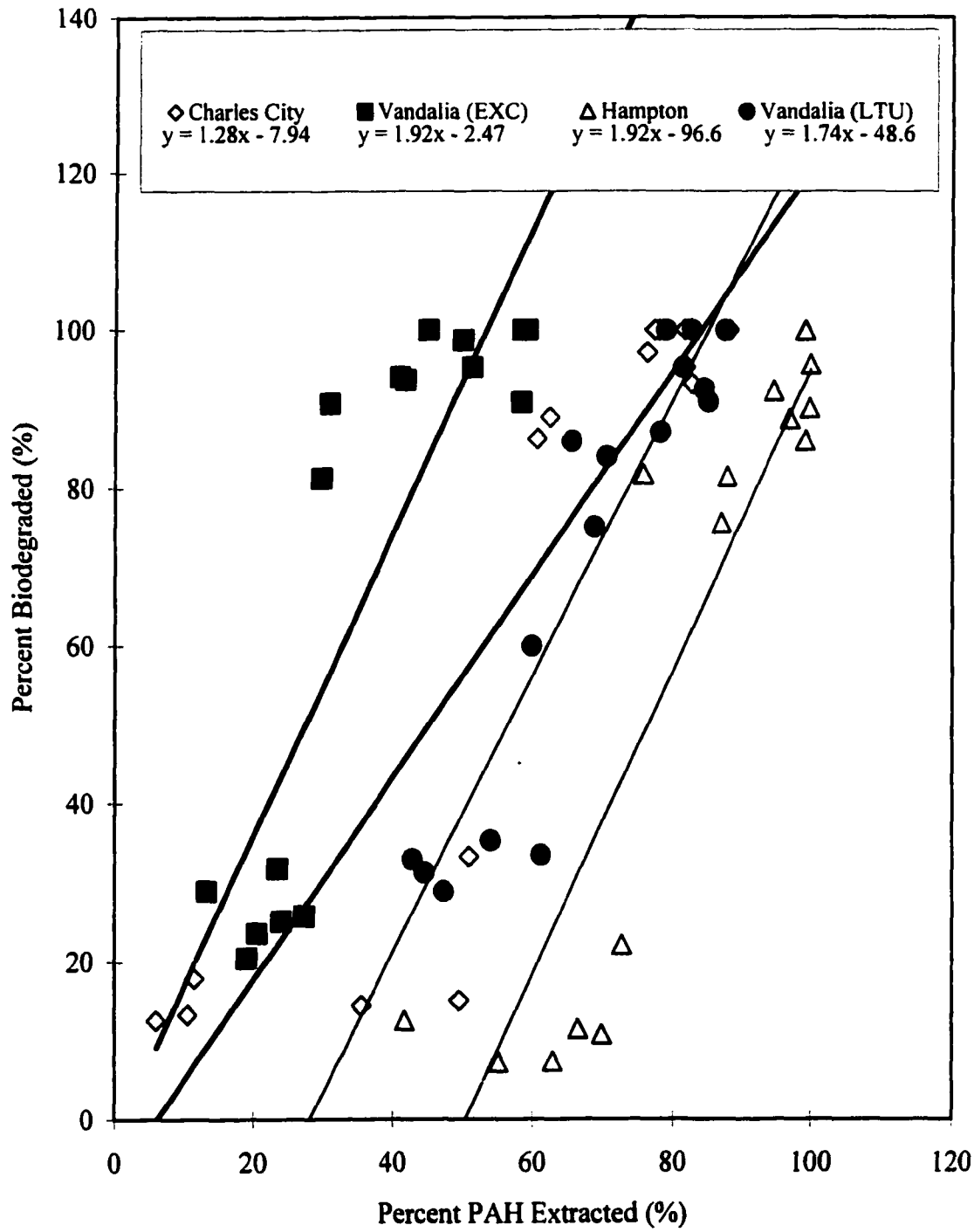

Figure 4-13. Plot of percent PAH extracted and percent biodegraded for four different soils using 0.6 volume fraction of acetone in acetone-water mixture

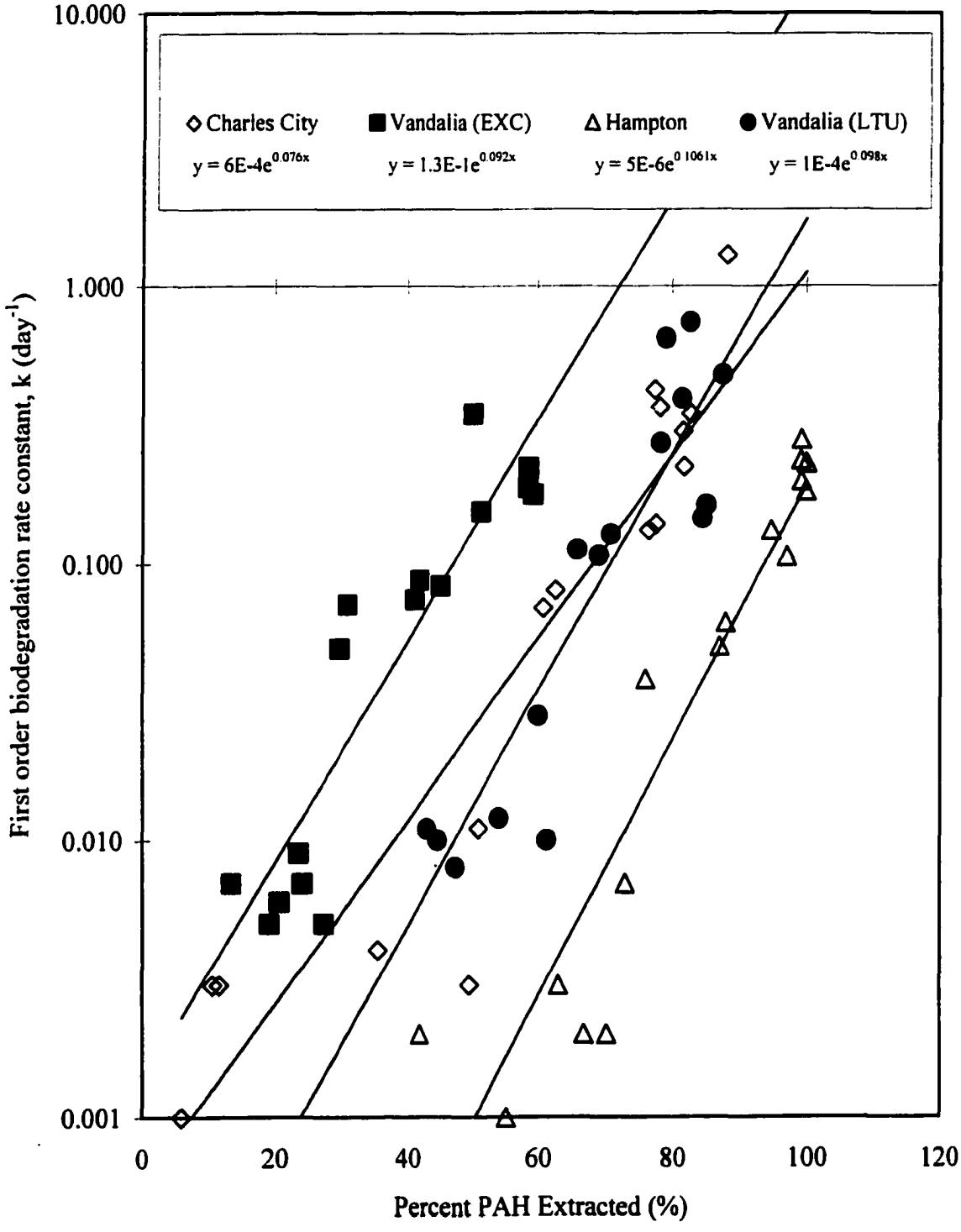

Figure 4-14. Plot of percent PAH extracted and biodegradation rate constant (k) for four different soils using 0.6 volume fraction of acetone in acetone-water mixture

and the percent extracted. Both plots are for all five soils using an acetone-water fraction of 0.6. As seen in Figures 4-13 and 4-14, each soil with the 16 PAHs plotted gave a different regression line. If an ideal bioavailability test method is available, then the percent biodegraded or the first order rate constants and the percent extracted would be plotted with a slope of one and an intercept of zero. This ideal line assumes that any available (or extracted) PAH will be degraded completely and that the ideal test method measures the available PAH. Since it is not possible to have an ideal test method that can be used universally for all soils, adjustments must be made on any other tests to account for the properties of the soil and the PAHs, and the aging process. As seen in Figure 4-13, the slopes of the correlations were larger than one. This means that the acetone-water mixture of 0.6 extracted less of the more biodegradable PAHs (usually the LMW PAHs) but extracted more of the less biodegradable PAHs (usually the HMW PAHs). To provide a more generalized approach that includes the properties of the soils and the PAHs, the following two approaches were taken. Only the percent biodegraded with respect to the percent extracted (as Figure 4-13) will be discussed.

In the first approach, the percent biodegraded and the percent extracted were modified by various ratios. The ratios were the ratio of the molecular weight of naphthalene and the molecular weight of the particular PAH and the ratio of the log K_{ow} of naphthalene and the log K_{ow} of the particular PAH, and the ratio of the log K_{oc} of naphthalene and the log K_{oc} of the particular PAH. Molecular weight and log K_{ow} were used since they are generally correlated with the solubility of the PAH in water while log K_{oc} reflects the extent of adsorption to the soil. Attempts were made to modify the percent degraded and the percent extracted with the above ratios. Of all the attempts, the greatest correlation obtained with the percent extracted using an acetone-water mixture of 0.6 were that with the percent biodegraded modified by the square of the ratio of the molecular weight of naphthalene and the molecular weight of the particular PAH and the percent extracted was modified by the square of the ratio of the log K_{ow} of naphthalene and the K_{ow} of the particular PAH. This is plotted as shown in Figure 4-15. The correlation coefficient for this plot was 0.94. Note that most of the soils fall within the slope of one and within the 95% prediction interval of the regression line.

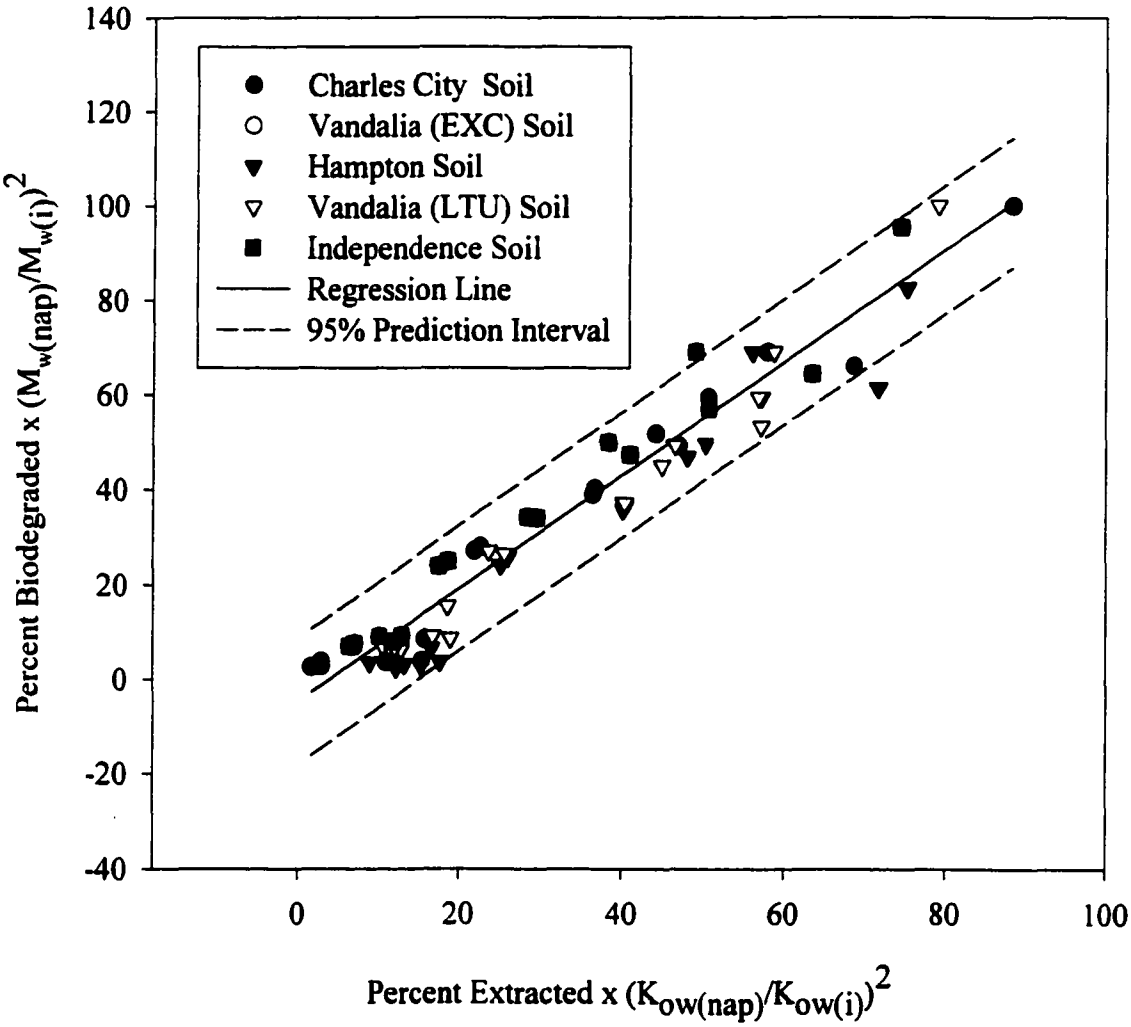

Figure 4-15. Plot of percent PAH extracted (modified with K_{ow}) and percent biodegraded (modified with M_w) for all five soils using 0.6 volume fraction of acetone. (M_w = molecular weight, nap = naphthalene, i = particular PAH)

In the second approach, the slopes and the intercepts of the regressions as shown in Figure 4-13 were correlated with the various soil fractions. In this approach, only four soils will be used while the fifth soil, i.e., the Independence soil, will be used as the test soil for the correlation. Independence soil was chosen as it was received about four months after the other four soils were tested. As seen in Figure 4-13 and pointed earlier, the slopes of the correlation of the percent degraded and the percent extracted as in Figure 4-13 were all greater than one. Using the slopes of four soils, the slopes were regressed against the various soil fractions. Of all the different soil fractions, organic carbon content was found to correlate well with the slopes ($R^2 = 0.87$, see Table 4-5 and Figure 4-16). As seen in Figure 4-16, the larger the organic carbon content, the larger is the slope. This means that for a given PAH in a soil with high organic carbon content, less is extracted by the acetone-water mixture than what is actually degraded in a soil slurry reactor. The relationship between the organic carbon content and the slope is given by:

$$\text{Slope} = 0.39\ (\%\ \text{OC}) + 0.48 \qquad \text{.....(1)}$$

On the other hand, the intercepts were also regressed against the different soil fractions. The percent clay plus silt fraction was found to correlate well with the intercepts with an R^2 of 0.95 as presented in Table 4-5 and shown in Figure 4-17. The correlation shows that with more clay and silt present, the percent biodegraded for a given PAH will be lower for a given percent extraction. The relationship between the clay plus silt fraction and the intercept is given by:

$$\text{Intercept} = -4.22\ (\%\ \text{Clay + silt}) + 152.1 \qquad \text{.....(2)}$$

Using the above two relationships, the following equation relating the percent biodegraded and the percent extracted using acetone-water mixture of 0.6 was obtained:

$$\%\ \text{Biodegraded} = [0.384\ (\%\ \text{OC}) + 0.485]\ (\%\ \text{Extracted}) + [-4.22\ (\%\ \text{Clay + silt}) + 152.1] \qquad \text{.....(3)}$$

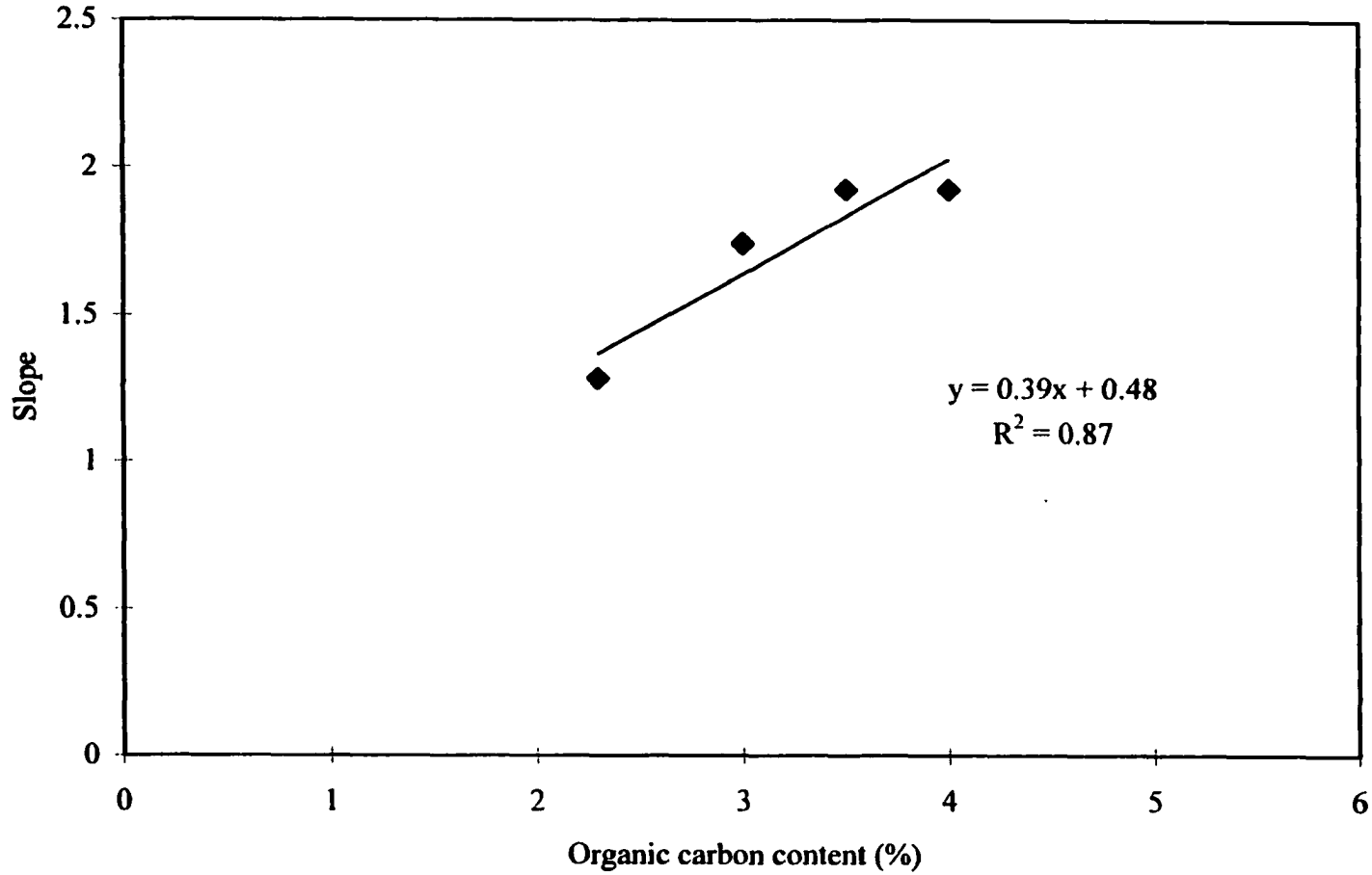

Figure 4-16. Plot of the slopes of regression line (percent biodegraded vs. percent extracted using 0.6 acetone-water mixture) and organic carbon content for four different soils

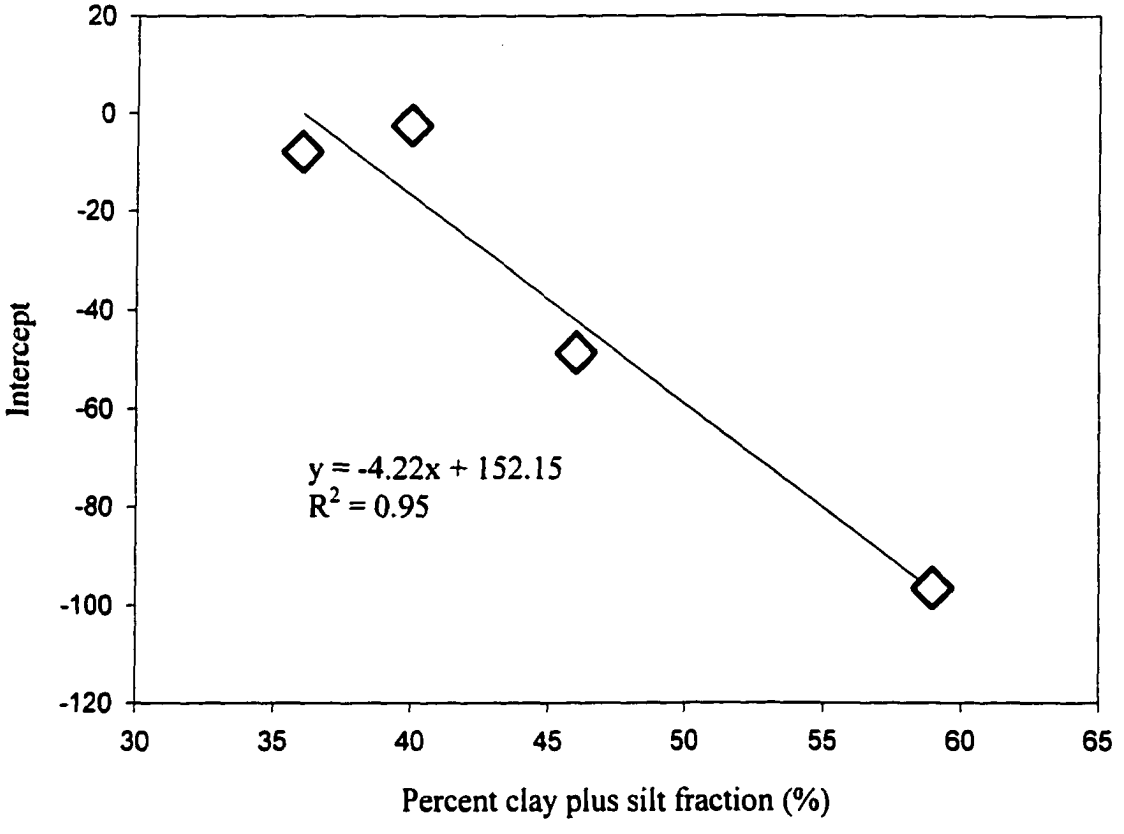

Figure 4-17. Plot of intercepts of regression line (percent biodegraded vs. percent extracted using 0.6 acetone-water mixture) and percent clay plus silt fractions for four different soils

Table 4-5. Regression coefficients for slopes and intercepts versus soil fractions of four soils

	Soil Fractions				
Parameters	O.C. (%)	Clay (%)	Silt (%)	Sand (%)	Clay + Silt (%)
Slope	0.87	0.02	0.68	0.41	0.41
Intercept	0.02	0.84	0.66	0.95	0.95

Table 4-6. Biodegradation of PAHs in the Independence soil estimated by acetone-water mixture extraction and soil properties

Compounds	Extracted by acetone:water mixture (%)	Measured biodegraded (%)	Estimated-biodegraded* (%)	Difference between measured and calculated (%)
Naphthalene	74	95	108	+12
Acenaphthylene	77	91	112	+21
Acenaphthene	69	100	101	+1
Fluorene	78	96	114	+18
Phenanthrene	67	97	98	+1
Anthracene	71	91	104	+13
Fluroanthene	63	85	91	+6
Pyrene	60	85	87	+2
Benzo(a)anthracene	52	79	75	-4
Chrysene	48	76	71	-5
Benzo(b)fluoranthene	38	31	55	+24
Benzo(k)fluoranthene	41	36	60	+24
Benzo(a)pyrene	33	35	47	+12
Indeno(1,2,3-cd)pyrene	25	32	36	+4
Dibenzo(a,h)anthracene	28	36	41	+5
Benzo(g,h,i)perylene	24	33	35	+2

*Estimated by Equation (3)

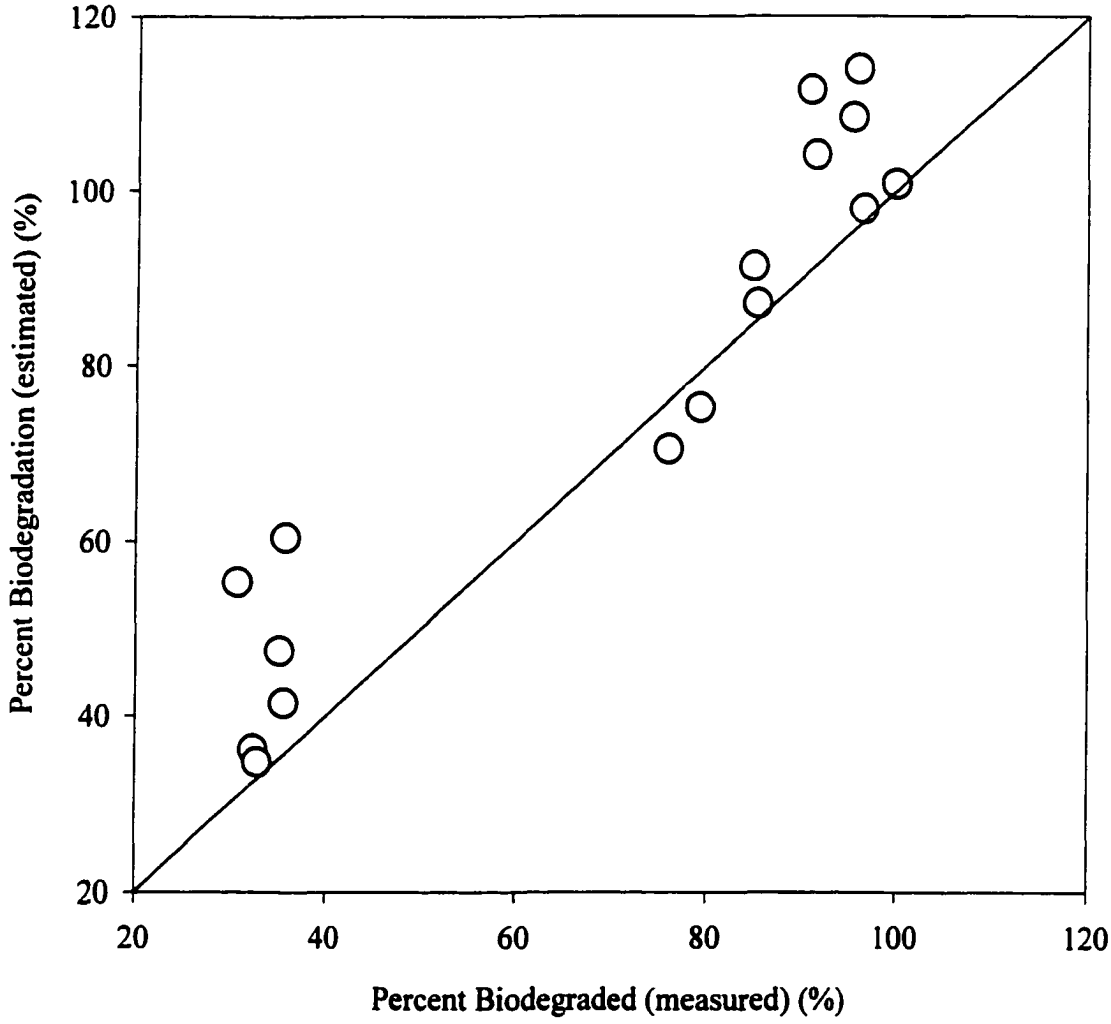

Figure 4-18. Comparison of experimental and calculated percent of biodegraded of 16 PAHs by using acetone-water mixture (0.6 volume fraction) for Independence soil.

Equation 3 was tested against the fifth soil, Independence soil, to evaluate the appropriateness of the equation for the estimation of the percent of PAH degraded. The estimated results are presented in Table 4-6 and in Figure 4-18. The estimated percent of each PAH degraded varied from about 1% to 24% of the actual measured percent biodegraded. The equation overestimated the percent biodegraded for LMW PAHs such as naphthalene, acenaphthylene, acenaphthene, anthracene, and fluorene. The estimations were shown to be over 100%. Although overall estimation of percent biodegraded PAH was higher than the experimental results in this case, most of the differences between the estimation and experiment were less than 13%. The above experimental results show that the mild solvent extraction method can be used as an assessment tool to estimate the extent of PAH degradation in soil. Although the number of soils tested was small, this assessment tool is a first step towards developing a method that estimates the availability of PAHs in soils. This tool may be used to estimate the feasibility of a bioremediation technology in treating a given soil to regulatory clean-up levels.

CONCLUSIONS

A simple solvent-water extraction method was proposed and tested as an assessment tool to estimate the percent of PAHs in soils that may be available for biodegradation. The percent of PAHs extracted using an acetone-water mixture of 0.6 for four different soils were found to strongly correlate with the percent of PAHs degraded using soil slurry reactors. The percent of PAHs extracted and PAHs degraded were for 16 different PAHs.

Two correlations relating the percent PAHs degraded, percent of PAHs extracted using 0.6 acetone-water mixture, soil and PAH properties were developed. One of the correlations was used to estimate the percent of PAHs degraded for a test soil. The correlation was found to predict well the biodegradation potential of 16 PAHs for the test soil with an average error of approximately $\pm$ 12 percent. The mild solvent extraction method appeared to be simple and quick assessment tool that can be used for the estimation of the extent of PAH degradation and their bioavailability in the soil.

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CHAPTER 5. USE OF SOLVENT TO ENHANCE PAHS BIODEGRADATION OF COAL-TAR-CONTAMINATED SOILS

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ABSTRACT

Bioremediation of coal tar-contaminated soils containing polycyclic aromatic hydrocarbons (PAHs) is highly challenging because of the low solubility and strong sorption properties of PAHs. Five coal tar-contaminated soils from former manufactured gas plant (MGP) sites were pretreated with two solvents, acetone and ethanol to enhance the bioavailability of the PAH compounds. The biodegradation of various PAHs in the pretreated soils were assessed using soil slurry reactor studies. The Total PAH degradation rates for soils pretreated with solvents were found to be two times faster than soils that were not pretreated with solvents. For example, the Total PAH first order degradation rate constants were 0.165 ± 0.032 day^{-1}, 0.147 ± 0.020 day^{-1}, and 0.076 ± 0.009 day^{-1} for Vandalia (EXC) soil that were pretreated with acetone, ethanol, and with no solvent, respectively. A distinctive advantage for soil pretreated with solvents was the enhanced removal of 4- and 5-ring PAH compounds such as chrysene and benzo(a)pyrene. Even for soils with over 3.5% organic carbon content, the degradation rates of chrysene were found to be two times faster than soils without solvent pretreatment. The degradation rates of benzo(a)pyrene were enhanced between 2 and 6 times for all five contaminated soils that were pretreated with solvents. To further elucidate trends that control the solvent treatment, the percent improvement (rate constants for pretreated soils divided by rate constants for non-treated soils) were found to correlate with the PAH partition coefficients but not with the properties of the soils. This implies that the availability of PAHs using solvent treatment is driven by the distribution of the PAHs between the solvent and the adsorbed PAHs.

Key words: PAHs, MGP, solvent, biodegradation.

INTRODUCTION

In the 1940s and 1950s, many manufactured gas plants (MGPs) in the United States were closed as a result of competition and availability of a cheaper source of energy, natural gas. These MGPs left behind an environmental legacy of contaminated sites containing a major waste product, coal tar (Luthy et al., 1994). Polycyclic aromatic hydrocarbons (PAHs) are the primary contaminants in coal tar. An estimate by Larsen (1997) indicated that there may be more than 5,000 former MGP sites in United States, with many requiring some form of remedial action. At many of the sites, the waste products may be in the form of non-aqueous phase liquids providing a constant source of contamination of groundwater (Hatheway, 1997). Several microorganisms have been isolated that are capable of degrading PAHs (Ye et al., 1996; Dean-Ross and Cerniglia, 1996; Aitken et al., 1998). However, PAHs are relatively persistent and recalcitrant in soils and are more difficult to degrade than many other organic contaminants under natural conditions (Wilson and Jones, 1993; MacGillivray and Shiaris, 1994).

Since PAHs are hydrophobic compounds with low solubility in water, they have a greater tendency to bind with organic matter or soil, limiting their availability to microorganisms (Volkering et al., 1992; Pignatello and Xing, 1996; Zhang et al., 1998). Another effect that has an impact on PAH bioavailability is the aging effect whereby the PAHs diffused into the micropores of the soil making it unavailable for biodegradation. Hatzinger and Alexander (1997) demonstrated that the extent of mineralization and the rate of phenanthrene biodegradation declined with increasing contact time between phenanthrene and the soil. Tang et al. (1998) also reported that aging decreased the amount of phenanthrene, anthracene, fluoranthene, and pyrene available to microorganism.

To enhance biodegradation, researchers have used surfactants (Aronstein and Alexander, 1991; Grimberg et al., 1996; Yeom et al., 1996) and organic solvents (Weissenfels et al., 1992; Field et al., 1995; Jimenez and Bartha, 1996; Kilbane, 1997) to improve the availability of PAHs. Addition of surfactants to contaminated soils may result in positive, negative, or even no effects on the degradation of PAHs (Liu et al., 1995). In addition, nonionic surfactant may adsorb on the soil particles (Adeel and Luthy, 1995) which may then require a long time to be completely flushed out of the subsurface. Using paraffin

oil and squalene at 0.8% by volume, Jimenez and Bartha (1996) found that mineralization of pyrene at the organic solvent interface was 8.5 times faster than in the aqueous medium. Similar results were obtained by Field et al. (1995) where addition of acetone or ethanol appeared to increase the degradation rate of anthracene in aqueous phase medium.

The objective of this study was to assess the application of solvents to enhance the bioavailability of PAH compounds for the bioremediation of PAH-contaminated soils. Acetone and ethanol were selected as they are relatively safe chemicals, fairly inexpensive, and are easily available. Extraction studies conducted by Lee et al. (1999) on PAH-contaminated soils have shown that acetone and ethanol were fairly good solvents for extracting PAHs from contaminated soils. Most microorganisms including fungi are inhibited by 1 to 15% of ethanol (Ingram and Buttke, 1984). Work done by Field et al. (1995), however, indicated that a fungi, *Bjerkandera* sp., can survive in 21% (v/v) of acetone and ethanol. In this study, the soil was pretreated with acetone and ethanol to maximize the extraction of PAHs. The solvent was then evaporated and recovered for reuse before the contaminated soil was biologically treated in a soil slurry reactor to assess the effectiveness of the solvent in enhancing bioavailability.

MATERIALS AND METHODS

Coal-Tar-Contaminated Soils

Five different soils from several coal-tar-contaminated sites were used for the experiments. Two contaminated soils were collected from the Vandalia road site near Pleasant Hill, Iowa. The site was created between 1945 and 1950 when MGP residuals were landfilled in a former creek channel. The first soil was taken directly from the former creek channel and is designated as Vandalia (EXC). The second soil, called Vandalia (LTU), was taken from a land-farming treatment unit containing a mixture of lesser contaminated soil and coal-tar-contaminated soil from the former creek channel. The third soil was collected from a former MGP site at Charles City, Iowa. This site was operated between 1915 and 1937. The fourth and fifth soils were collected from Hampton, Iowa and Independence, Iowa where a former MGP site operated between 1915 and 1947 and between 1880 and 1947, respectively. All soils were placed in airtight aluminum containers and kept in a refrigerator

at 4 °C. Before each soil was used in the soil slurry and extraction studies, the soil was homogenized by sieving through a 2-mm mesh sieve. The physical-chemical properties of the five soils are listed in Table 5-1. The PAH concentrations were measured using a solvent extraction method and a gas chromatograph (GC) with a flame ionization detector (see details in later section). Soil texture was determined based on the soil particle distribution test and USDA modified soil texture triangle (Cookson, 1995). Organic carbon in soil was determined by the rapid dichromate oxidation procedure (Nelson and Sommers, 1982).

Solvent Pretreatment

Two hundred grams of soil were mixed with 100 mL of ethanol or acetone in a 500 mL glass bottle with Teflon-lined screw cap for about 24 hours. Two hundred mL of deionized water were added to the soil-solvent mixture to cause the extracted PAHs to precipitate. The solvent-water mixture was then allowed to evaporate. The total PAH concentration of the pretreated soil was measured to monitor possible lost of PAHs through volatilization. The concentrations of each PAH in the five soils before and after solvent pretreatment are presented in Table 5-2. Most of the PAHs that were lost during this process were 2- and 3-ring compounds especially naphthalene (2-ring PAH) which tend to have high vapor pressure properties. The PAHs lost were less than ten percent of the initial Total PAH with naphthalene being the majority of the PAHs lost.

Soil Slurry Biodegradation Studies

Soil slurry biodegradation studies were conducted in a batch mode in a 1-L glass reactor. Approximately 150 g (dry weight basis) of the solvent-pretreated or non-pretreated contaminated soil were loaded into the reactor along with 750 mL of distilled water. Each reactor was seeded with 20 mL of supernatant from a soil slurry reactor which has been treating PAH-contaminated soil for several months. The soil slurry was supplemented with

Table 5-1. Physical-chemical properties of five different MGP soils

Parameters	Hampton, IA	Vandalia (EXC), IA	Charles City, IA	Vandalia (LTU), IA	Independence, IA
Soil Texture[1]	Loam soil	Sandy loam	Sandy loam	Sandy clay loam	Sandy loam
Sand (%)	41	60	64	54	64
Silt (%)	35	26	18	24	25
Clay (%)	24	14	18	22	11
Organic carbon (%)	3.5	4.0	2.3	3.0	2.5
Soil moisture (%)	12.97	8.72	8.40	3.70	4.00
Soil pH	7.22	7.65	6.52	7.10	7.80

[1]Based on USDA modified soil texture classification

Table 5-2. PAH concentrations on dry-weight basis (mg/kg) for five different MGP soils before and after solvent pretreatment

	Hampton, IA			Vandalia (EXC), IA			Charles City, IA			Vandalia (LTU), IA			Independence, IA		
Compound	Initial	ACE[1]	ETH[2]	Initial	ACE	ETH	Initial	ACE	ETH	Initial	ACE	ETH	Initial	ACE	ETH
Naphthalene	48	25	28	35	27	30	986	245	275	62	22	18	379	164	152
Acenaphthylene	190	177	186	485	421	463	325	275	298	61	51	40	272	232	234
Acenaphthene	37	34	38	239	223	245	80	75	83	34	28	21	103	97	100
Fluorene	139	122	160	358	362	386	181	181	173	62	63	52	197	205	214
Phenanthrene	347	373	398	1,238	1,154	1,171	408	416	405	128	122	143	602	587	560
Anthracene	113	108	122	386	330	370	141	135	148	54	48	49	177	165	152
Fluroanthene	128	128	137	378	311	357	126	119	143	98	95	98	209	203	192
Pyrene	173	172	183	545	472	540	171	176	188	146	140	145	287	277	261
Benzo(a)anthracene	67	68	69	188	162	182	66	69	69	52	51	53	92	88	93
Chrysene	65	65	67	202	170	192	66	66	66	53	49	53	92	87	92
Benzo(b)fluoranthene	36	39	37	82	79	86	25	30	30	30	31	30	53	56	58
Benzo(k)fluoranthene	36	36	36	84	74	83	30	28	33	25	23	24	54	58	59
Benzo(a)pyrene	60	59	59	143	127	140	48	47	52	36	35	40	90	92	93
Indeno(1,2,3-cd)pyrene	28	28	29	54	50	56	18	20	23	18	20	19	51	57	43
Dibenzo(a,h)anthracene	9	9	10	18	19	21	7	9	11	7	8	8	15	16	13
Benzo(g,h,i)perylene	24	22	25	57	50	55	17	16	17	18	17	17	51	54	42
Total PAH	1,500	1,464	1,584	4,494	4,030	4,379	2,694	1,907	2,013	884	803	809	2,724	2,437	2,358
N-PAH[3]	1,174	1,138	1,253	3,664	3,300	3,562	2,417	1,623	1,712	645	569	566	2,227	1,931	1,864
C-PAH[4]	326	326	331	830	730	817	276	284	300	239	234	243	498	508	494
N-PAH (%)	78	78	79	82	82	81	90	85	85	73	71	70	82	79	79
C-PAH (%)	22	22	21	18	18	19	10	15	15	27	29	30	18	21	21

[1]ACE: concentration of PAHs in soil after pretreatment with acetone
[2]ETH: concentration of PAHs (mg/kg) in soil after pretreatment with ethanol
[3]N-PAH: non-carcinogenic PAHs (first eight compounds)
[4]C-PAH: carcinogenic PAHs (second eight compounds)

ammonium nitrate as a nitrogen source and KH_2PO_4 as a phosphate source. The amounts of nutrient added were based on a C:N:P ratio of 100:10:2 where the carbon fraction was assumed to be the total carbon content of the 16 PAHs present in the soil. Each reactor was mixed with a mixer (Model PM6015, EMI Incorporated, Clinton, CT) and aerated using an air stone diffuser at an airflow rate of 200 mL/min. The dissolved oxygen concentration was maintained at above 3.0 mg/L and the pH of the slurry was between 6.5 and 8. A killed control with Vandalia (EXC) soil was also set up with 2% (w/w) sodium azide to inhibit microorganisms growth and to monitor the loss of PAHs through abiotic processes such as volatilization.

Analysis of Soil PAH Concentrations

Soil suspensions were regularly removed from the soil slurry reactor and analyzed for PAHs according to the method described by Lee et al. (1999). A 10-mL slurry sample was placed in a 10-mL glass tube and centrifuged at 3,000 rpm for 30 minutes. The supernatant was discarded since preliminary tests have shown that the aqueous phase contained negligible amount of PAHs. Five mL of acetone were then added to the soil residue and the tube was shaken for 24 hours. The suspension was centrifuged at 3,000 rpm for 30 minutes and 5 μL of the supernatant was analyzed by GC for the 16 US EPA priority PAHs. The dry weight of the soil in the glass tube was then determined by drying the soil sample at 105 °C for 24 hours.

RESULTS AND DISCUSSION

Total PAH Degradation

An example of the Total PAH degradation result for the soil slurry studies is presented in Figure 5-1. The soil presented in Figure 5-1 is Vandalia (EXC) soil. As seen in Figure 1, PAHs in soil pretreated with solvents degraded faster than the soil that was not pretreated. Approximately 90% of the Total PAH in the acetone- and ethanol-pretreated soil were removed within 15 days while 35 days were required to achieve the same removal

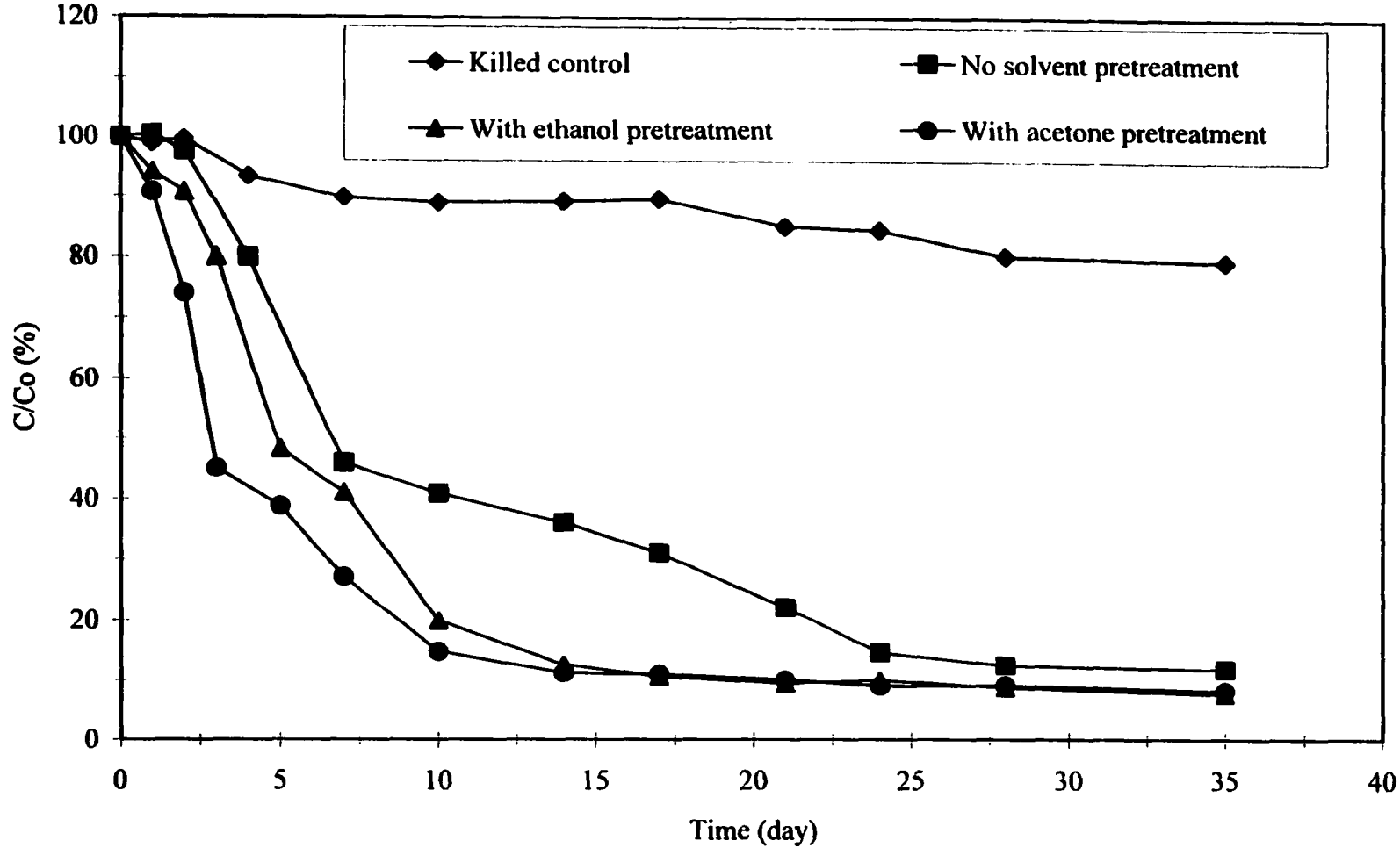

Figure 5-1. Comparison of Total PAHs removal for Vandalia (EXC) soil with and without solvent pretreatment

percentage for non-pretreated soil. First order degradation rate constants for the Total PAH were estimated to be 0.076 ± 0.009 day^{-1}, 0.147 ± 0.020 day^{-1}, and 0.165 ± 0.032 day^{-1} for non-pretreated, ethanol-pretreated, and acetone-pretreated soils, respectively. Statistically at 95% confidence interval, the rate constants for the solvent-pretreated soils were significantly different from that of the non-pretreated soil. The PAHs in the soil that was pretreated with acetone were found to degrade faster than the soil that was pretreated with ethanol but statistically there was no difference in the degradation rate constants at 95% confidence interval (see Figure 5-1). The above results showed that acetone or ethanol might be used as a solvent to increase the bioavailability of PAH resulting in faster degradation of Total PAH.

For the killed control, about 80% of the Total PAH remained after 35 days. Since it was not possible to completely inhibit microbial degradation with sodium azide, some of the losses may be microbial while abiotic losses were the primary reason. Two- and 3-ring PAH compounds such as naphthalene, acenaphthylene, acenaphthene, and fluorene may be lost through volatilization (Lewis, 1993). After 35 days, the percentage of naphthalene, acenaphthylene, acenaphthene, and fluorene remaining in the killed control were 64%, 31%, 34%, and 71%, respectively. Concentrations of the other PAHs remained the same as their initial concentration.

Degradation of 3-ring PAH

Further information on the impact of solvent pretreatment can be obtained by examining the degradation of individual PAHs in the five soils. Figure 5-2 shows the removal of phenanthrene, a 3-ring compound, for all five soils. Biodegradation of phenanthrene was by no means complete with a residual fraction of about 1-7% remaining after 35 days. Ninety percent of phenanthrene were removed within 7 days for all five soils with different pretreatments. Except for Vandalia (LTU) soil, removal of phenanthrene was fastest for soils pretreated with acetone. For Vandalia (EXC) soil and Charles City soil which have the highest initial concentration of non-carcinogenic PAHs, over 90% of phenanthrene were removed within 3 days, 5 days, and 7 days for soils pretreated with acetone, ethanol, and no solvent, respectively. Statistically, the first order degradation rate

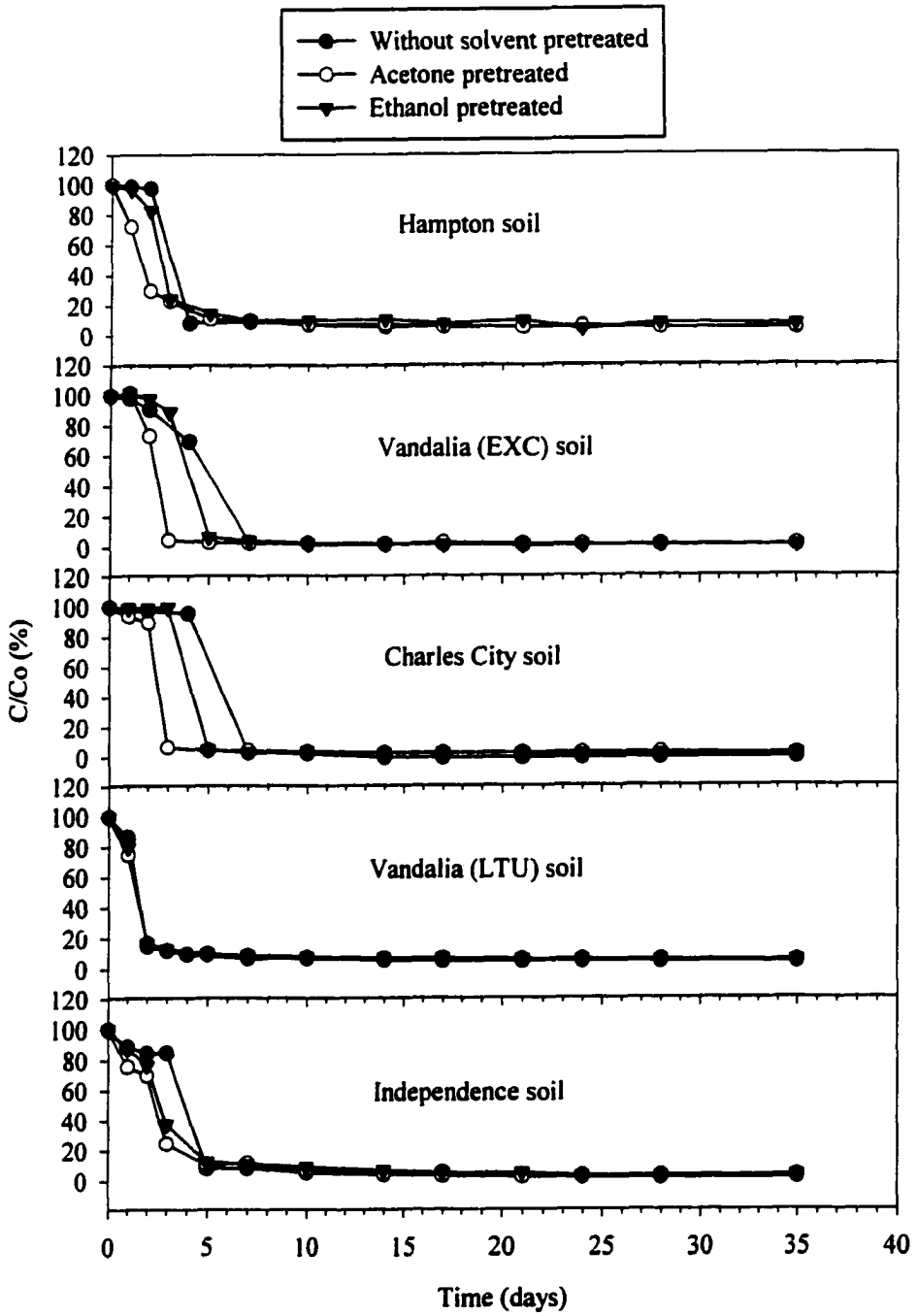

Figure 5-2. Phenanthrene degradation for different solvent pretreatments and different MGP soils

constants of phenanthrene for the five soils using the three treatments were not different at 95% confidence interval (see Table 5-3). This indicates that solvent pretreatment has only a slight advantage in enhancing the biodegradation of phenanthrene in contaminated soil as phenanthrene is relatively available for the microorganisms. Similar results by White and Alexander (1996) showed that 4% methanol increased the rate and extent of mineralization of phenanthrene in loamy soil.

Degradation of 4-ring PAH

Chrysene removal for the five contaminated soils with and without solvent pretreatment are presented in Figure 5-3. Since 4-ring compounds have higher partition coefficients and lower water solubilities than 3-ring compounds, bioavailability may be a major factor that may limit their degradation. As seen in Figure 5-3, removal of chrysene was slightly faster for acetone-pretreated soils than ethanol-pretreated soils for all soils, except for Charles City soil. There were differences in the extent of removal and the time needed to achieve similar removal percentages of chrysene for the solvent-pretreated and the non-pretreated soils. For example, approximately 70% of chrysene were removed within 10 days for Hampton soil that was pretreated with solvents but 24 days were needed to achieve similar removal percentage when the soil was not pretreated. Similar results were found in Vandalia (EXC) soil, Charles City soil, and Vandalia (LTU) soil, but not in Independence soil. Based on the experimental data for this study, it is difficult to speculate a probable reason. Furthermore, the Independence soil has lower organic carbon content and clay fraction than other soils which under current understanding may make the PAHs more readily available.

First order degradation rate constants of chrysene for different soils and pretreatments are presented in Table 5-3. Based on the first order degradation rate constants, there were no statistical differences between acetone-pretreated soils and ethanol-pretreated soils using 95% confidence interval. With respect to solvent-pretreated and non-pretreated soil, there were statistical differences in the rate constants for Hampton soil and Vandalia (EXC) soil. Both Hampton soil and Vandalia (EXC) soil have higher organic carbon content (over 3.5%)

Table 5-3. First order degradation rate constants of three PAHs for different soils and pretreatments

Compound	Soils	Without solvent pretreatment		With acetone pretreatment		With ethanol pretreatment	
		Rate constant[a] (day^{-1})	R^2	Rate constant[a] (day^{-1})	R^2	Rate constant[a] (day^{-1})	R^2
Phenanthrene	Hampton soil	0.309±0.158	0.86	0.310±0.164	0.93	0.258±0.098	0.93
	Vandalia (EXC) soil	0.347±0.160	0.86	0.572±0.424	0.86	0.363±0.193	0.87
	Charles City soil	0.424±0.202	0.86	0.509±0.395	0.85	0.385±0.225	0.85
	Vandalia (LTU) soil	0.639±0.845	0.84	0.570±0.735	0.85	0.556±0.780	0.83
	Independence soil	0.212±0.081	0.87	0.307±0.093	0.97	0.333±0.094	0.98
Chrysene	Hampton soil	0.050±0.011	0.91	0.094±0.020	0.89	0.084±0.017	0.95
	Vandalia(EXC) soil	0.049±0.013	0.88	0.103±0.028	0.92	0.098±0.034	0.87
	Charles City soil	0.069±0.015	0.92	0.114±0.038	0.88	0.105±0.027	0.91
	Vandalia(LTU) soil	0.101±0.026	0.94	0.127±0.039	0.93	0.111±0.025	0.96
	Independence soil	0.046±0.010	0.92	0.059±0.010	0.89	0.054±0.014	0.95
Benzo(a)pyrene	Hampton soil	0.003±0.001	0.63	0.022±0.005	0.89	0.017±0.005	0.83
	Vandalia(EXC) soil	0.005±0.002	0.73	0.015±0.003	0.92	0.018±0.002	0.82
	Charles City soil	0.004±0.002	0.81	0.019±0.002	0.97	0.014±0.002	0.96
	Vandalia(LTU) soil	0.012±0.002	0.97	0.024±0.005	0.92	0.017±0.005	0.85
	Independence soil	0.013±0.004	0.82	0.025±0.002	0.98	0.022±0.004	0.94

[a]with 95% confidence interval.

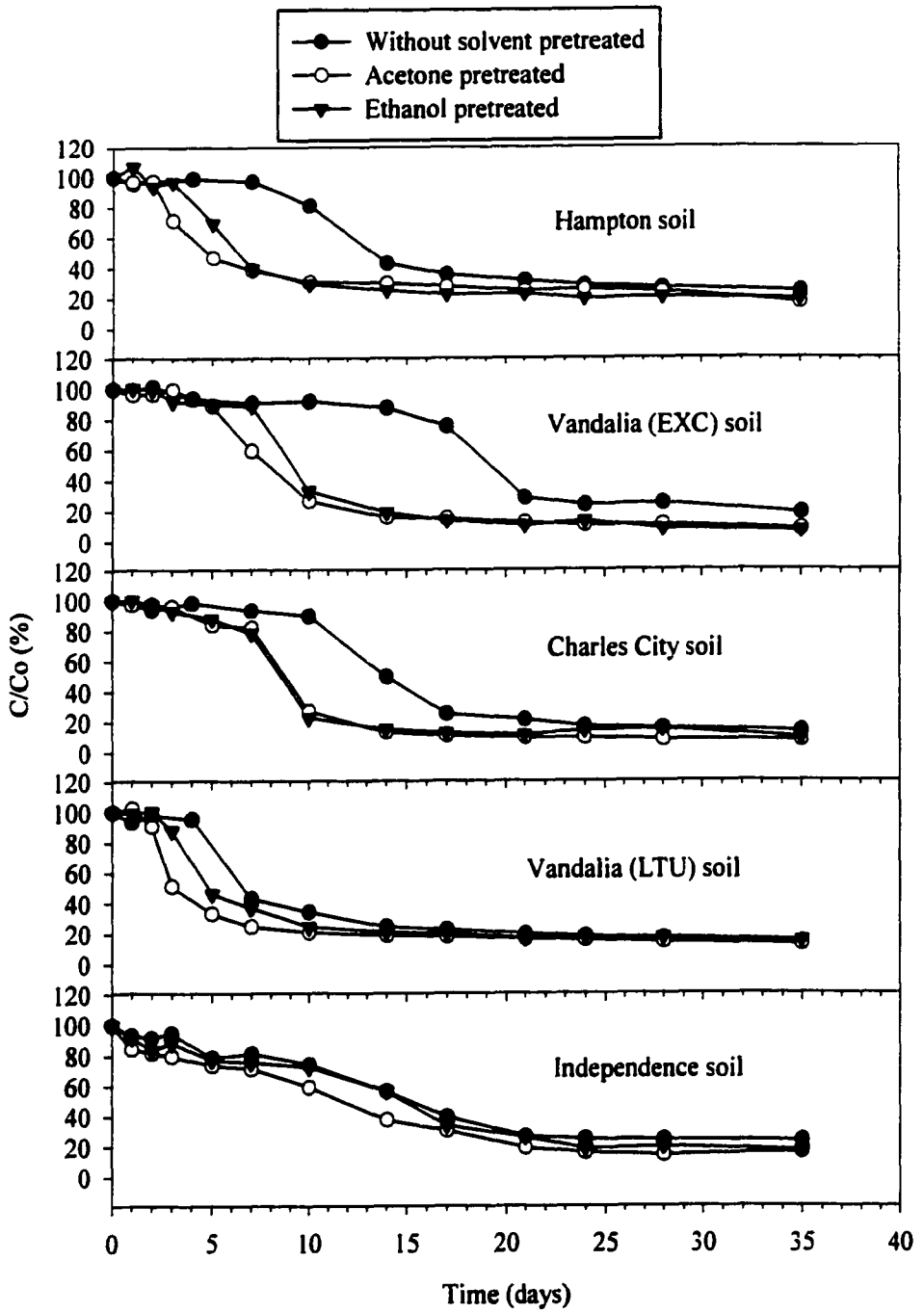

Figure 5-3. Chrysene degradation for different solvent pretreatments and different MGP soils

than the other three soils and that may have limited the availability of chrysene. The degradation rates of chrysene for both soils were found to be two times faster for solvent pretreatment than without solvent treatment. Caldini et al. (1995) reported that the degradation rate of 4-ring PAH compounds increased by adding acetonitrile to enhance their bioavailability.

Degradation of 5-ring PAH

Figure 5-4 illustrates the removal of benzo(a)pyrene, a 5-ring compound, in all five soils. The percents of benzo(a)pyrene remaining after 35 days in soils treated with acetone were approximately 54%, 50%, 51%, 48%, and 45% for Hampton soil, Vandalia (EXC) soil, Charles City soil, Vandalia (LTU) soil, and Independence soil, respectively. In contrast, the percents of benzo(a)pyrene remaining were higher for the soils without solvent pretreatment, i.e., approximately 89%, 80%, 85%, 65%, and 65% for Hampton soil, Vandalia (EXC) soil, Charles City soil, Vandalia (LTU) soil, and Independence soil, respectively. In addition, the first order degradation rate constants of benzo(a)pyrene were statistically different for pretreated soils and non-pretreated soils for all five soils (see Table 5-3). The degradation rates of benzo(a)pyrene were enhanced between 2 and 6 times for all five contaminated soil that were pretreated with solvents. This results indicate that use of solvents may enhance the bioavailability of benzo(a)pyrene. This result may be compared to that of May et al. (1997) where a higher biodegradation of benzo(a)pyrene was obtained when benzo(a)pyrene became more bioavailable to the fungus by using an extraction technique with 0.1% (v/v) Tween 80 surfactant.

Effects of Properties of Soil and PAHs on Solvent Enhancement

The above results clearly showed that the use of organic solvent is a promising approach to increase the availability of PAHs in contaminated soils. This approach is advantageous for 4- and 5-ring PAH compounds which have low solubilities and high partition coefficients. Although the enhancement mechanism was not studied, the organic solvent most probably resulted in the dissolution of PAHs by creating a thermodynamically suitable environment. This may include the transfer of PAHs from the inner parts to the

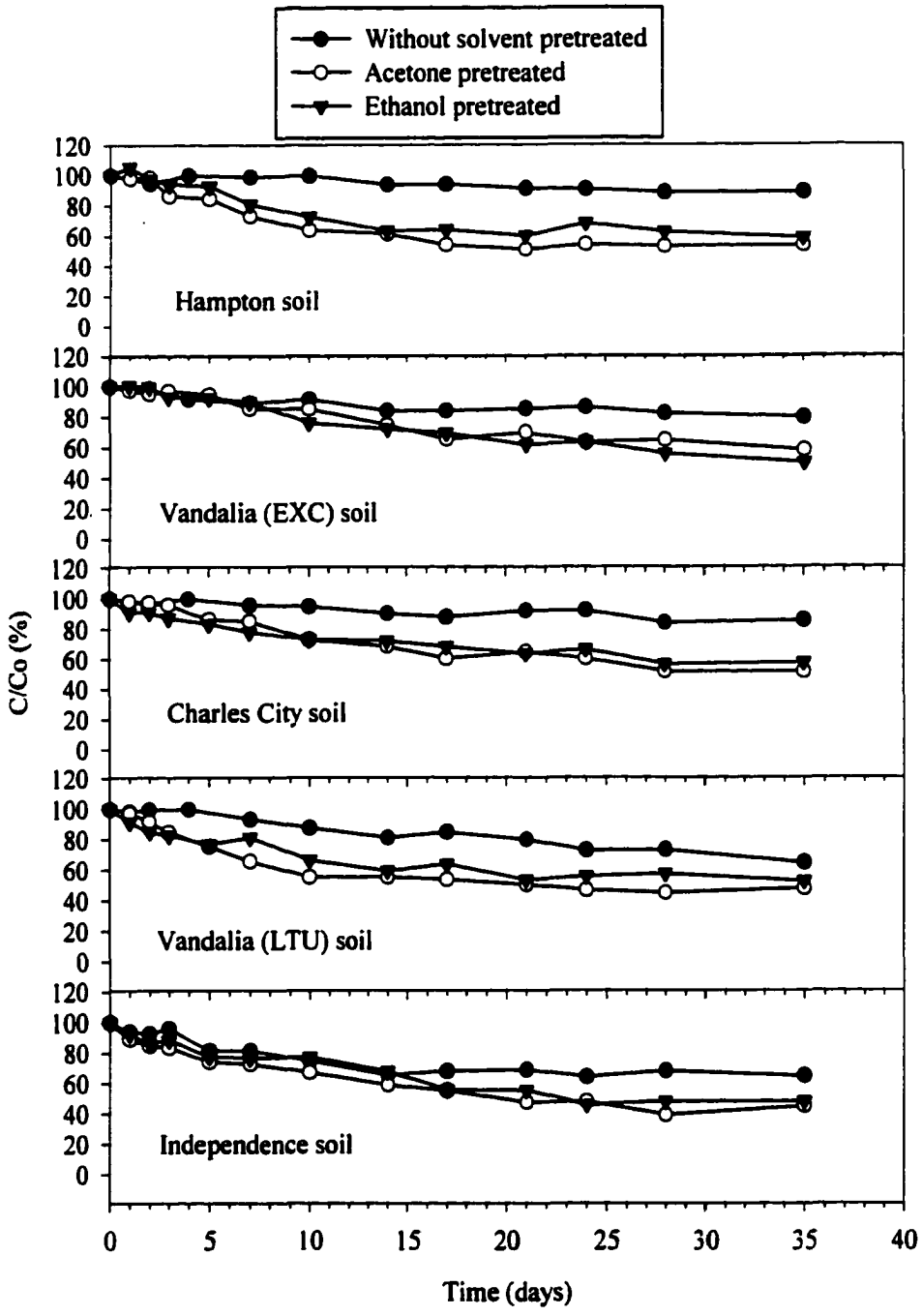

Figure 5-4. Benzo(a)pyrene degradation for different solvent pretreatments and different MGP soils

outer parts of the soil particles or to the surface of the soil (Brusseau et al., 1991; Pignatello and Xing, 1996). Several researchers have shown the importance of using water-miscible solvent for the solubilization and enhancement of PAH availability for degradation (White and Alexander, 1996; Caldini et al., 1995). To further elucidate trends from the above experimental results, the percent improvement in the degradation rates (i.e., rate constants for pretreated soils divided by rate constants for non-pretreated soils) for the 16 PAHs was plotted and regressed against some of the physical-chemical properties of the PAHs as shown in Figure 5-5 and Table 5-4. For each soil, the percent improvement in degradation rate was shown to be strongly dependent on the octanol-water partition coefficients of PAH compounds. Figure 5-5 presents a plot of the percent improvement in degradation rates for all five soils versus the log K_{ow}. Although the positive correlation is obvious, the R^2 is 0.26. This is expected since the enhancement effect of the solvent will be most effective for the more hydrophobic compounds. A similar conclusion was found by Fu and Luthy (1986) in their study on the effect of organic solvents on the sorption of aromatic hydrocarbon onto soil. They suggested that the organic solvent may cause the organic carbon associated with the soil to swell and thereby increasing PAH accessibility. The percent improvement also showed positive correlation with the molecular weights of the PAHs but was not correlated with the logarithm of the solubility of PAHs in water. The results indicate that the water solubility of PAHs may not be a limiting factor on the degradation rates of PAH. For example, the solubility of chrysene (1.8 μg/L) is lower than that of benzo(a)pyrene (3.8 μg/L) but the degradation rate constants of chrysene were higher than that of benoz(a)pyrene (see Table 5-3).

The percent improvement in degradation rate of phenanthrene, chrysene, and benzo(a)pyrene for all five soils were correlated with the physical-chemical properties of soils. Table 5-5 showed high correlation for acetone as a solvent and the initial Total PAH concentrations for phenanthrene but not for chrysene and benzo(a)pyrene. In contrast, there were no correlations between the percent improvement in degradation rate of all three PAHs and the initial Total PAH concentration when ethanol was used as a solvent. This result

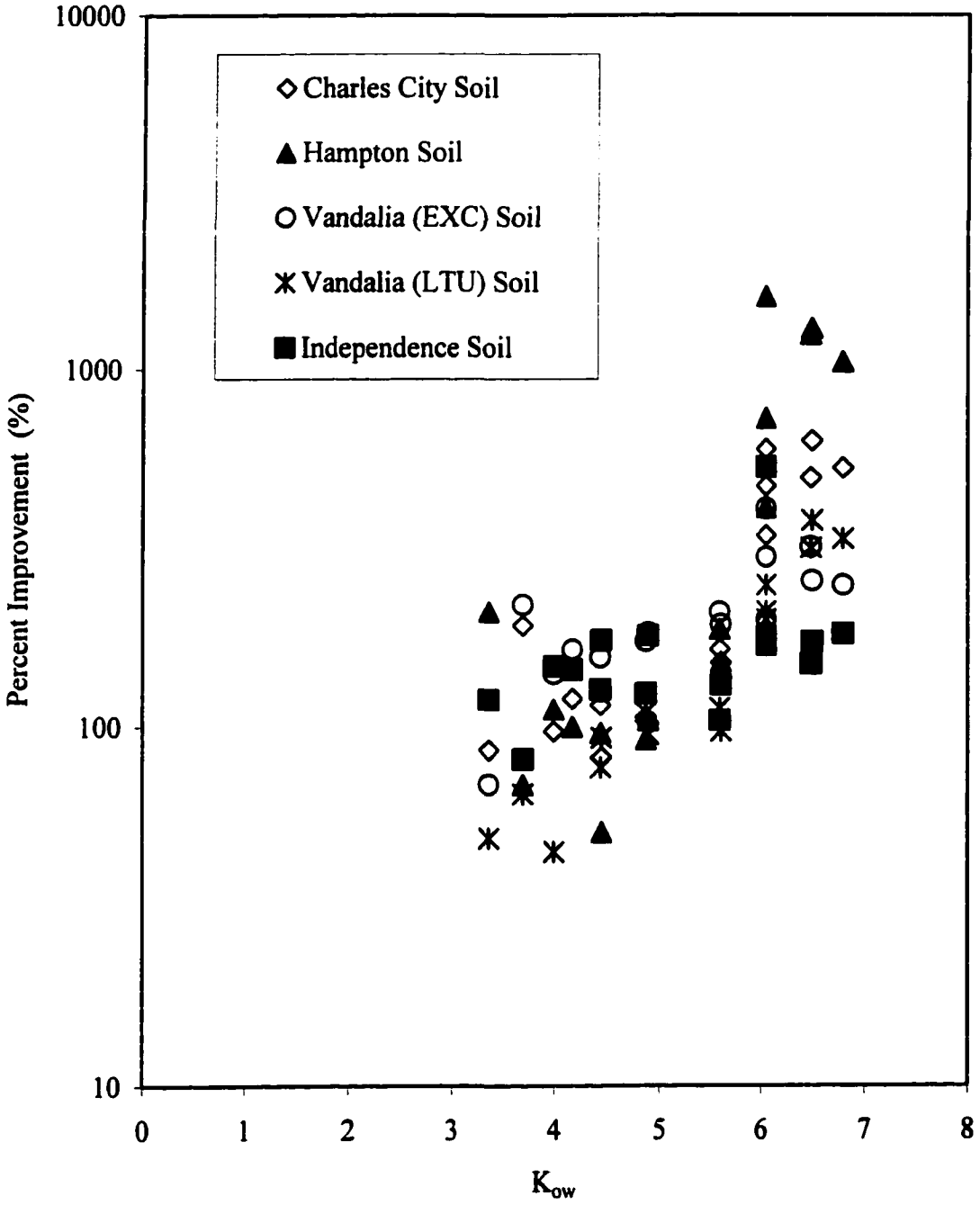

Figure 5-5. Correlation between K_{ow} and percent improvement in degradation rates for five different soils by using acetone pretreatment

Table 5-4. Correlation coefficients (R^2) between percent improvement in degradation rates and properties of 16 PAHs for different soils

Properties of PAHs	Solvents	Hampton Soil	Vandalia (EXC) Soil	Charles City Soil	Vandalia (LTU) Soil	Independence Soil
Log K_{oc}	Ethanol	0.92	0.99	0.95	1.00	0.08
	Acetone	0.90	0.98	0.89	0.98	0.57
Log K_{ow}	Ethanol	0.68	0.85	0.73	0.89	0.00
	Acetone	0.64	0.82	0.63	0.82	0.26
Molecular Weight	Ethanol	0.72	0.88	0.77	0.92	0.00
	Acetone	0.69	0.86	0.67	0.86	0.31
Log (solubility in water)	Ethanol	0.30	0.50	0.35	0.55	0.02
	Acetone	0.26	0.46	0.25	0.46	0.16

Table 5-5. Correlation coefficients (R^2) of percent improvement in degradation rates and properties of soil for different PAH compounds

PAH compounds	Solvents	Organic carbon content	Clay fraction	Initial total PAH concentration
Phenanthrene	Ethanol	0.10	0.73	0.12
	Acetone	0.06	0.77	0.92
Chrysene	Ethanol	0.50	0.00	0.46
	Acetone	0.53	0.00	0.36
Benzo(a)pyrene	Ethanol	0.21	0.20	0.01
	Acetone	0.10	0.38	0.04

indicates that improved biodegradation rates of phenanthrene may be obtained for soils with high initial total PAH concentration when acetone pretreatment is used.

For all three PAHs, only the percent improvement in degradation rate for phenanthrene showed positive correlation with the clay fractions of the soils while the percent improvement of chrysene showed a weak correlation with the organic carbon contents. Crocker et al. (1995) studied the effects of clay fraction and bioavailability of naphthalene (2-ring PAH) and found that clay-sorbed naphthalene was essentially unavailable to microorganisms. Solvents may enhance the desorption of phenanthrene from the clay fraction and promote the availability of phenanthrene. Carmichael et al. (1997) suggested that the desorption of aged phenanthrene and chrysene may control their degradation and may explain the persistence of PAHs even in soils containing a large and active community of PAH-degrading microorganisms. However, the overall correlation between the soil properties and percent improvement in degradation rate was not significant. This generally implies that solvent pretreatment is not affected by the soil properties but by the physical properties of the PAHs.

CONCLUSION

Pretreatment of contaminated soil from MGP sites with solvents was shown to enhance the extent and the biodegradation rates of PAHs. Acetone was found to be slightly more effective as a pretreatment solvent than ethanol. Solvent pretreatment was found to half the time needed to degrade chrysene by 90% as compared to non-pretreated soil. Similarly, the percent removed for benzo(a)pyrene was almost doubled for pretreated soil over the same period of treatment. Solvent pretreatment appeared to be effective for 4- and 5-ring PAH compounds that have high partition coefficients. The percent improvement in the biodegradation rates for both solvents were shown to be highly correlated with the octanol-water partition coefficients of PAHs and partition coefficients of PAHs in organic carbon. This may imply that the availability of PAHs using solvent treatment is driven by the distribution of the PAHs between the solvent and the adsorbed PAHs. Although soil properties such as organic carbon and clay fraction may affect the desorption and degradation of PAHs, no significant correlations were found between several physical properties of the

soils such as percent organic carbon and clay and the percent improvements in biodegradation rates.

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CHAPTER 6. EFFECTS OF LOW MOLECULAR WEIGHT PAHS ON THE BIODEGRADATION OF BENZO(A)PYRENE IN WATER AND PAH-CONTAMINATED SOILS

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ABSTRACT

The degradation of [7,10-^{14}C]benzo(a)pyrene (BaP) was investigated in liquid phase and solid phase treatment studies. The liquid phase treatment was used to evaluate the effect of co-substrates on the mineralization of BaP. For the solid phase treatment studies, different soil types with varying amounts of PAHs were used to study the mineralization of BaP. In the liquid phase treatment studies, only treatments with 2- and 3-ring PAH compounds added as co-substrates or with an extracted solution of PAH-contaminated soil, showed high bioactivity but mineralization of the [^{14}C]BaP was less than 1% for both treatments over 25 weeks. The liquid phase treatments showed that about 20 to 40% of the ^{14}C were non-extractable and were bound to biomass. Naphthalene added in the 9th week of the liquid phase treatments promoted the bioactivity but did not result in further mineralization of BaP. In the solid phase treatment studies, four different PAH-contaminated soils were used. After 25 weeks of incubation, 18.6% of the initial [^{14}C]BaP in one of the soils (with the lowest initial Total PAH concentration) was mineralized to $^{14}CO_2$ while mineralization of [^{14}C]BaP was not exhibited in the other soil treatments. As in the liquid phase treatments, about 10 to 20% of the ^{14}C were non-extractable. For the extractable fraction of the ^{14}C, no radioactive metabolites other than [^{14}C]BaP were detected for both treatments. The liquid phase treatment showed that BaP was not used as a source of microbial carbon and energy and mineralization of BaP was not promoted by 2- and 3-ring PAHs. The solid phase treatment showed that degradation of PAHs in soil was sequential with the utilization of the more easily degradable organic compounds first followed by the more difficult BaP compound.

Keywords: Benzo(a)pyrene, PAHs, mineralization, MGP, coal-tar

INTRODUCTION

Polycyclic aromatic hydrocarbon (PAH) are recalcitrant compounds commonly found in coal-tar-contaminated soils at former manufactured gas plant (MGP) sites. These compounds are regulated by the U.S. Environmental Protection Agency (EPA), as some of them are mutagenic and/or carcinogenic (Keith and Telliard, 1979). Bioremediation has been applied at the MGP sites to remediate and remove PAHs from contaminated soils but successful removal of higher molecular weight (HMW) PAHs (containing 4 benzene rings or more) to the regulatory cleanup levels is limited (Mueller et al., 1991; Warith et al., 1992; Erickson et al., 1993; Zappi et al., 1996).

Many microorganisms with an ability to utilize PAHs have been identified, but only microorganisms utilizing 4-ring PAH compounds or less as a sole source of carbon and energy have been reported (Walter et al., 1991; Komatsu et al., 1993; Bouchez et al., 1996; Dean-Ross and Cerniglia, 1996; Aitken et al., 1998; Chen and Aitken, 1999). This means that PAH compounds containing more than 4 benzene rings may not support microbial growth and may require the presence of other organic substrates for biodegradation (Keck et al., 1989; Morgan et al., 1993; Ye et al., 1996). Chen and Aitken (1999) reported that less than 1% of benzo(a)pyrene (BaP) was mineralized when BaP was the only carbon source, but approximately 30% and 20% were mineralized when phenanthrene and salicylate, respectively, were added as co-substrates. In addition, Keck et al. (1989) observed that the degradation rates of 5-ring PAHs were higher in soils amended with high amounts of carbon in the form of oil and grease than in soils amended with a single PAH constituent or a mixture of PAHs. Kanaly et al. (1997) reported that BaP can be completely degraded when other hydrocarbon substrates such as crude oil are present. In the case of contaminated soils, the presence of other organic carbons may provide the necessary co-substrates for the degradation of HMW PAHs. For example, in soils contaminated with coal tar, there may be as many as 3,000 separate organic compounds present (Hatheway, 1997; Mueller et al., 1989a). Although there are many studies investigating the degradation of HMW PAHs in "clean" systems with isolated microorganisms, the role of lower molecular weight (LMW)

PAHs on the degradation of HMW PAHs in actual contaminated soils has not been fully established.

In a contaminated soil environment, the persistence of HMW PAHs may be due to their strong partitioning with organic matter in soil and their low water solubilities. Many researchers have shown that PAHs bound to clay or humic materials are less available and biodegradable than the free dissolved compounds (Landrum, 1989; Scheunert and Mansour, 1992; Guthrie and Pfaender, 1998). In general, sorbed organic chemicals in labile sites may be available for microbial transformation. Labile sites are defined as sites where diffusion is not limiting the desorption of chemicals at these sites (Wauchope and Meyers, 1985). Chemicals sorbed in more restrictive and bound sites such as micropores may be out of reach of the mircoorganisms (Novak et al., 1995) and degradation may be controlled by the desorption of the PAH from the micropores (Carmichael et al., 1997). Therefore, different soils with different physical-chemical properties will have an effect on the fate of HMW PAHs. However, Guerin and Boyd (1992) pointed out that attributing the lack of biodegradation to low bioavailability may not be appropriate since specific microorganisms for the degradation of the compounds may not be present. Therefore, introducing isolated PAH-degrading bacteria into the soil may significantly enhance the mineralization of PAHs (Grosser et al., 1991). Many of the studies reported in the literature on the biodegradation of HMW PAHs are for pure cultures in artificial media while data on the degradation of PAHs in actual contaminated soils and indigenous mixed cultures are limited.

The objective of this study was to investigate the degradation of a representative HMW PAH compound, BaP (5-ring compound), in aqueous phase with LMW PAHs and in PAH-contaminated soils with varying amounts of PAHs. BaP is highly recalcitrant under natural environmental conditions. Two sets of experiments were conducted: degradation of BaP in aquous phase and solid phase. Both treatments were seeded with a mixed culture from a soil slurry reactor. For the solid phase treatments, different contaminated soils were used to assess the degradation of BaP in these soils and in the presence of various carbon sources.

MATERIALS AND METHODS

Chemicals

[7,10-^{14}C]benzo(a)pyrene (54 mCi/mmol, 50 μCi/ml in toluene; 97.2% purity) was obtained from Amersham Pharmacia Biotech Inc. (Piscataway, NJ). Unlabeled BaP was purchased from Sigma Chemical Co. (St. Louis, MO), at a purity of 98%. Naphthalene, anthracene, and phenanthrene were obtained from Aldrich Chemical Co. (Milwaukee, WI) with a purity of more than 99%. HPLC grade solvents used for extraction were methylene chloride, acetone, and acetonitrile and were purchased from Fisher Scientific (Fair Lawn, NJ).

BaP Degradation in Liquid Phase Treatment

A mineral salt medium containing 1 g of $(NH_4)_2SO_4$, 0.8 g of K_2HPO_4, 0.2 g of KH_2PO_4, 0.25 g of Na_2HPO_4, 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of $MgSO_4 \cdot 7H_2O$, and 10 ml of trace metals solution in 1 liter of Milli-Q water (Millipore Corporation, Bedford, MA) was used (Mueller et al., 1989b). The trace metal solution contained 25 mg of EDTA, 111 mg of $ZnSO_4$, 50 mg of $FeSO_4 \cdot 7H_2O$, 15.2 mg of $MnSO_4 \cdot H_2O$, 4 mg of $CuSO_4 \cdot 5H_2O$, 4 mg of $Co(NO_3)_2 \cdot 6H_2O$, and 4 mg of $NaB_4O_7 \cdot 10H_2O$ in 1 liter of water (Protzman et al., 1999). The mineral medium was autoclaved before use.

For the liquid phase treatment, four different treatments identified as Treatment A, B, C, and D were conducted. Each Treatment consisted of duplicate biometer flasks (Belco, Vineland, NJ). Each flask contained 30 ml of mineral medium with 5 mg of [^{14}C]BaP with a specific activity of 0.149 μCi/mg BaP. In addition, the following were added for each treatment. Treatment A was the control flask with 20 ml of sterilized water added. Treatment B was inoculated with 10 ml of supernatant from a soil slurry reactor and 10 ml of sterilized water. The soil slurry reactor has been treating PAH-contaminated soils for several months. Degradation of 2- to 3-ring PAH compounds in the slurry reactor was rapid with more than 90% removal within 14 days, but degradation of 4-ring compounds was slower with over 90% removal in 35 days. For 5- and 6-ring compounds, less than 25% were removed in 35 days. The soil slurry was maintained by adding approximately 300 g of PAH-contaminated soil from Vandalia road site, Des Moines, Iowa, with 1.5 liter water every 5 to

6 weeks to the 2-liter soil slurry reactor. Nutrients used were ammonium nitrate as nitrogen source and potassium dihydrogen phosphate as a phosphate source. The amounts of nutrient added were based on a C:N:P ratio of 100:10:2 where the carbon fraction was assumed to be the total carbon content of the 16 PAHs present in the soil. For Treatment C, the same volumes as in Treatment B were added but with 10 mg each of naphthalene, anthracene, and phenanthrene (2-3 ring compounds). Treatment D was similar to that of Treatment C except that 10 ml of dissolved soil extract were added instead of sterilized water. The soil extract was obtained by extracting 50 g of land-farming soil from Vandalia road site at 100 °C for one hour with 100 ml of tap water, filtered through a 1.2 μm glass fiber filter (Whatman) and autoclaved before used. The soil extract contained approximately 100 mg C/L of dissolved organic carbon with non-detectable amounts of PAH, which may support the degradation of BaP. All treatments were incubated at 25 °C. All the 2- and 3-ring PAH compounds, non-labeled BaP and [^{14}C]BaP were dissolved in acetone, transferred into the flask, and the solvent was evaporated before the various solutions were added. The side arm reservoirs contained 10 ml of 0.5 M NaOH. Each week, the entire volume of NaOH was removed and replaced with fresh NaOH. A three-ml aliquot of the NaOH was transferred into a scintillation vial with 12 ml of Ultima Gold™ scintillation cocktail (Packard Instrument Co., Meriden, CT) and analyzed by a Packard 1600 liquid scintillation counter (LSC) (Packard Instrument Co., Meriden, CT). The radioactivity counted by the LSC was corrected for quenching and background radioactivity.

At the end of the 9th week of treatment, 5 mg of naphthalene was added to Treatments C and D to determine the potential for enhancing bioactivity and mineralization of BaP. After 14 weeks of treatment, the solution in the flask was filtered with a 0.2 mm aluminum oxide filter (Whatman Anodisc™ Membrane filter) to separate the biomass and the solution. No significant radioactivity (<1%) was found in the filtered solution. The filter with the biomass was extracted three times with 50 ml of methylene chloride. Water in the methylene chloride was removed by passing over 10 g of sodium sulfate. The methylene chloride was evaporated with a rotary evaporator and approximately 7 ml of acetonitrile was added. The radioactivity in the acetonitrile was determined by LSC. Initial and final concentrations of BaP in each treatment were determined by a Waters high pressure liquid chromatography

(HPLC) system with a Model 470 scanning fluorescence detector. Separation was achieved with a C18 reverse-phase column (25 cm x 4.6 mm, 5-mm-particle size, Supelco, Bellefonte, PA). The mobile phase was 50% acetonitrile in water at a flow rate of 1.2 ml/min for 26 minutes. Excitation wavelength was 406 nm. The radioactivity of [^{14}C]BaP and metabolites were quantified by using a Radiomatic radioactive detector (Packard Instrument Co., Meriden, CT) which was connected to the HPLC.

BaP Degradation in Solid Phase Treatment

Five different treatments for the solid phase treatment experiments were used. Treatments 1 and 2 contained soil from a coal tar disposal site at Vandalia road site. Treatment 1 was a killed control with the soil autoclaved twice for 25 minutes before it was placed into a biometer flask. This treatment was used to quantify any abiotic loss of BaP. Treatment 2 was used to assess the degradation of BaP in the Vandalia road soil. The reason for using Vandalia road soil is that the soil was used as a feed for the soil slurry reactor mentioned earlier. Treatment 3 contained treated soil from the soil slurry reactor which was fed with soil from the Vandalia road site. The treated soil was separated from the aqueous phase by centrifuging at 1,000 rpm for 15 minutes. This slurry treated soil contains a higher clay fraction but lower Total PAH concentration than the untreated Vandalia road soil. Treatment 4 contained a mixture of four different soils of equal proportion from four different MGP sites. The Total PAH concentration of mixed soil was between that of Vandalia road soil and the treated soil. Treatment 5 contained soils from a land-farming treatment unit that had been treating a mixture of Vandalia road site soil and a lesser contaminated soil for four months (Kayser et al., 1998). The land-farming soil contained the same soil texture as the Vandalia road soil but with low Total PAH concentration. Information on the soils used is presented in Table 6-1. The initial concentrations of 16 PAHs for the control and the four different soils are presented in Table 6-2.

Table 6-1. Physical-chemical properties of four different MGP soils

Properties	Vandalia Road Soil	Treated Soil	Mixed Soil	Land-farming Soil
Soil Texture	Sandy loam	Clay loam	Sandy loam	Loam
Sand (%)	60	42	59	50
Silt (%)	26	28	24	30
Clay (%)	14	30	18	20
Organic carbon (%)	4.0	3.8	3.1	3.0
Initial soil moisture (%)	15	15	15	15
Initial soil moisture (%)	15	15	15	15
Soil pH	7.65	7.38	7.26	7.55

Each treatment was performed in duplicate biometer flasks and each biometer flask contained 50 g (dry weight basis) of soil. Five ml of supernatant from the soil slurry reactor were added to each flask as an inoculum except for the control flasks. The soil moisture content for each flask was adjusted to 15% with Milli-Q water. One ml of acetone containing 5 mg of [^{14}C]BaP with a specific activity of 0.149 μCi/mg BaP was added to each biometer. The soil was mixed thoroughly to evenly incorporate the BaP. Ten ml of 0.5 M sodium hydroxide was added to the biometer flask side-arm to collect $^{14}CO_2$. All treatments were incubated in the dark at 28 °C. The sodium hydroxide traps were changed weekly to monitor BaP mineralization and CO_2 production. Midway through the experiments (at week 12), 50 g (dry weight basis) of land-farming soil was added to the flasks of Treatments 2, 3, and 4. The reason for doing this was that significantly greater BaP mineralization was obtained with the land-farming soil and that addition of the land-farming soil may improve the PAH degradation of the other treatments. After 25 weeks, soils from each biometer were transferred to a 250 ml Teflon bottle. The flask was rinsed thoroughly three times with 5 ml of acetonitrile and placed in the Teflon bottle. The soil was then extracted with 100 ml of acetonitrile for 24 hours using a horizontal vibration shaker. The slurry was centrifuged at room temperature (25±3°C) and 4,000 rpm for 30 minutes and the supernatant was collected. The extraction procedure was repeated three times. The supernatant was then concentrated to approximately 50 ml with a rotary evaporator. Three ml of the final supernatant were

Table 6-2. Degradation of 16 PAH compounds in five different soil treatments

Compounds	Treatment 1* (Control: sterilized soil)			Treatment 2* (Vandalia road soil)			Treatment 3* (Treated soil/slurry)			Treatment 4* (Mixed soil)			Treatment 5* (land-farming soil)		
	Initial[1] Conc.	Final[2] Conc.	R.P.[3] (%)	Initial Conc.	Final Conc.	R.P. (%)	Initial Conc.	Final Conc.	R.P. (%)	Initial Conc.	Final Conc.	R.P. (%)	Initial Conc.	Final Conc.	R.P. (%)
Naphthalene	31	0	100	31	0	100	0	0	-	44	29	34	0	0	-
Acenaphthylene	487	394	19	487	312	36	35	29	17	206	197	4	17	10	41
Acenaphthene	247	183	26	247	132	47	0	0	-	124	81	35	0	0	-
Fluorene	360	354	2	360	330	8	0	0	-	208	197	5	0	0	-
Phenanthrene	1,273	1,216	4	1,273	1,007	21	44	32	27	646	394	39	15	12	23
Anthracene	335	307	8	335	310	8	12	11	5	150	132	12	10	7	35
Fluroanthene	381	377	1	381	370	3	52	45	13	202	172	15	24	15	40
Pyrene	565	556	2	565	551	2	74	65	13	297	252	15	37	18	51
Benzo(a)anthracene	196	187	5	196	181	8	35	33	7	95	85	10	23	15	37
Chrysene	209	203	3	209	194	7	39	36	8	101	88	13	23	13	43
Benzo(b)fluoranthene	89	86	3	89	83	7	41	39	5	49	38	22	15	14	7
Benzo(k)fluoranthene	85	79	7	85	78	8	40	36	9	46	44	3	14	10	29
Benzo(a)pyrene	241	218	10	241	207	14	216	179	17	171	142	17	123	49	61
Indeno(1,2,3-cd)pyrene	58	55	5	58	54	7	21	20	6	27	26	5	12	12	4
Dibenzo(a,h)anthracene	21	20	5	21	19	12	10	9	7	10	9	7	5	5	0
Benzo(g,h,i)perylene	56	53	5	56	55	1	21	19	9	39	37	6	13	9	31
Total PAH	4,634	4,286	8	4,634	3,893	16	640	557	13	2,415	1,932	20	332	186	44
N-PAH[4]	3,679	3,385	8	3,679	3,017	18	217	182	16	1,877	1,464	22	103	61	41
C-PAH[5]	955	901	6	955	869	9	423	372	12	538	468	13	229	126	45

*All treatments received 5 mg of [^{14}C]BaP with a specific activity of 0.149 μCi/mg BaP

[1]Initial concentration (mg/kg) (dry weight basis) was measured before incubation

[2]Final concentration (mg/kg) (dry weight basis) was measured after incubation for 25 weeks

[3]Removal percentage

[4]N-PAH: non-carcinogenic PAHs (first eight compounds)

[5]C-PAH: carcinogenic PAHs (second eight compounds)

filtered through a 0.22 μm nylon syringe filter into a 4-ml HPLC vial. A 0.2 ml aliquot of the supernatant was transferred into a scintillation vial with 7 ml of Ultima Gold™ scintillation and measured with the LSC. HPLC and Radiomatic detector were used to quantify the [^{14}C]BaP and its metabolites. The presence of other aromatic organic compounds in the contaminated soils had a quenching effect, making the determination of radioactive compounds by Radiomatic detector difficult. Instead, a fractional collection technique (Ye et al., 1996) was used to affirm the presence of radioactive compounds in the soils. The supernatant was passed through a HPLC separation system and collected in 8-ml scintillation vials every 30 seconds. Seven ml of Ultima Gold™ scintillation cocktail was added to each vial and analyzed by the LSC. PAH compounds in the extract were analyzed by injecting 5 μl aliquot of the supernatant in a HP 5890A gas chromatograph (GC) (Hewlett Packard, Palo Alto, CA). The GC was equipped with a HP-5 capillary column and FID. The initial oven temperature was 50° C followed by a temperature ramp rate at 8° C/min to a final temperature of 302° C for 5 minutes. The injector temperature was set at 240° C and the detector temperature was 320° C.

The extracted soil was air dried, powderized, and triplicate samples of one gram were oxidized in a OX500 biological oxidizer (R. J. Harvey Instrument Corporation, Hillsdale, NJ). The carbon dioxide was trapped and the radioactivity measured.

RESULTS

BaP Degradation in Liquid Phase Treatment

The mineralization results of BaP in different liquid phase treatments are shown in Figure 6-1. Small amounts of $^{14}CO_2$ were produced by mixed culture treated with 2- and 3-ring PAHs (Treatment C, 0.6% of applied ^{14}C) and PAHs with soil extract and 2- and 3-ring PAHs (Treatment D, 1.1%), but not for Treatments A and B. Carbon dioxide released from each treatment is presented in Figure 6-2. As expected, the highest amount of CO_2 produced was obtained for Treatment D, followed by Treatment C and Treatment B. No $^{14}CO_2$ was measured for Treatment B but small amounts of CO_2 were measured, indicating that BaP alone in the medium as a carbon and an energy source could not support the growth of the

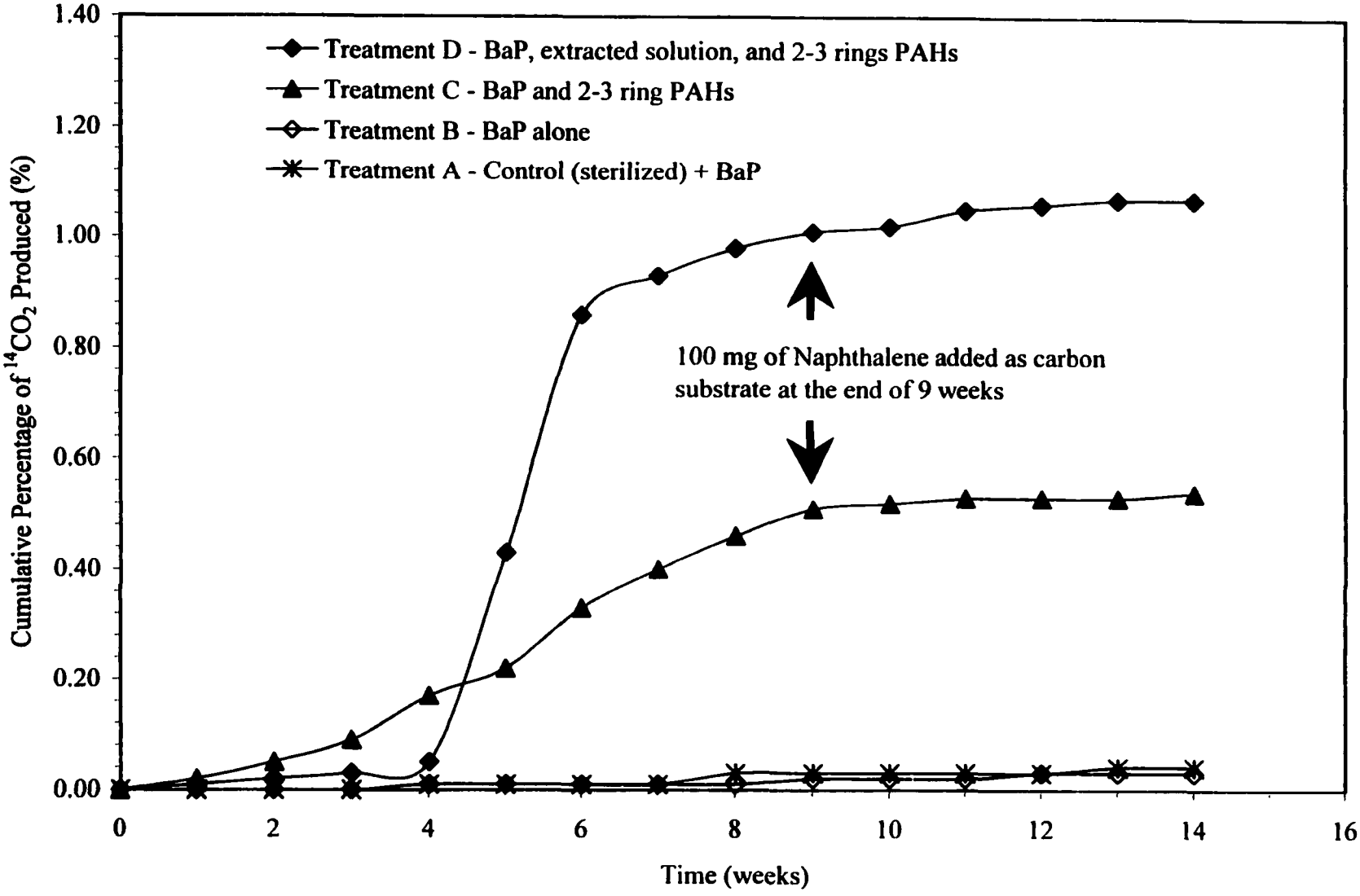

Figure 6-1. Mineralization of benzo(a)pyrene for different liquid treatments

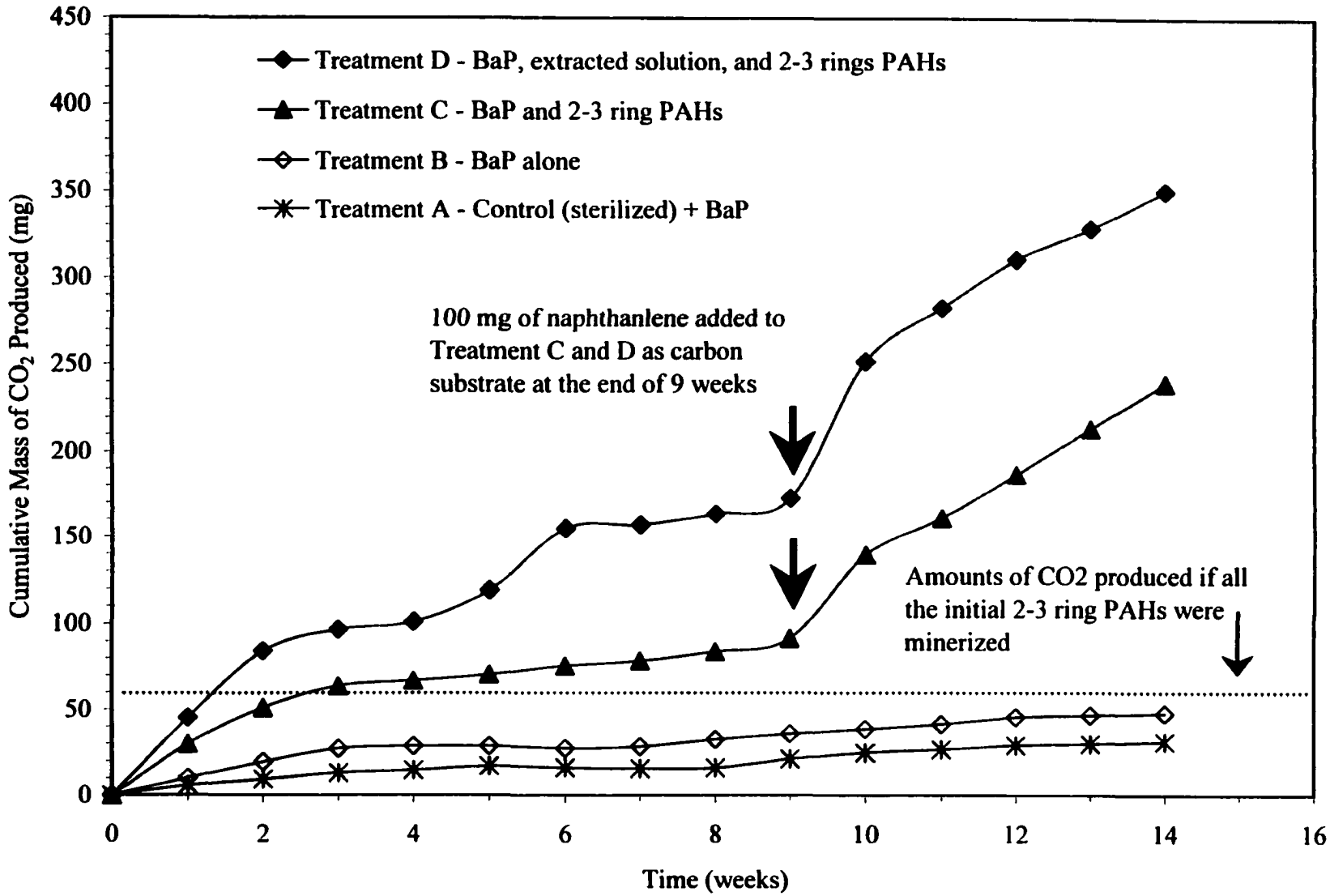

Figure 6-2. Carbon dioxide released for different liquid phase treatment

mixed culture. When naphthalene was added after 9 weeks to Treatments C and D, large amounts of CO_2 were produced (see Figure 6-2) but the amounts of $^{14}CO_2$ produced remained similar to those in the previous weeks. Even though the bioactivity was promoted by adding a carbon source such as naphthalene, naphthalene did not promote the mineralization of BaP. This is contrary to other studies where naphthalene has been shown to increase cometabolism of HMW PAHs (Bouchez et al., 1995; Aitken et al., 1998). Note that the measured CO_2 produced corresponded with the theoretical CO_2 produced if all the 2- and 3-ring compounds were mineralized (50% of carbon for respiration) (see Figure 6-2). This result implies that the mixed culture utilized 2- and 3-ring compounds as carbon and energy sources, but this process did not affect the mineralization of BaP.

The mass balance results of [^{14}C]BaP in liquid phase treatment are shown in Table 6-3. Over 93% of [^{14}C]BaP were recovered for all four treatments. Most of the [^{14}C]BaP were extracted by methylene chloride, but for Treatments C and D approximately 22% and 40% of ^{14}C remained with the biomass as non-extractable ^{14}C, respectively. According to the results of the HPLC, the percentages of unlabeled BaP that were not recovered in the liquid phase treatments were similar to that of the labeled [^{14}C]BaP. The percentages of unlabelled BaP not recovered were 0.4%, 1.4%, 21.1% and 41.1% for Treatments A, B, C, and D, respectively. In addition, no radioactive metabolites were found for these different treatments.

BaP Degradation in Solid Phase Treatment

The mineralization results of BaP in different solid treatments are shown in Figure 6-3. Only the land-farming soil (Treatment 5) produced significant amounts of $^{14}CO_2$ production. The average accumulated amount of $^{14}CO_2$ from Treatment 5 after 25 weeks was 18.6% - with each flask producing 21.3% and 15.9% of $^{14}CO_2$. Although the land-farming soil for the duplicate experiments of Treatment 5 was collected from the same place, a lag time of 5 weeks was observed for the first flask and 10 weeks for the second flask. After the lag period, the mineralization rates of [^{14}C]BaP for both flasks were similar at 1.1% and 1.0% of $^{14}CO_2$ per week, respectively.

Table 6-3. Mass balance for [^{14}C]BaP in liquid and solid treatments

Phase	Treatment[1]	Description	$^{14}CO_2$ (%)	Extracted ^{14}C (%)[2]	Non-extracted ^{14}C (%)	Total recovery of ^{14}C (%)
Liquid (50 ml)	A	Control: sterilized solution + 5 mg of BaP + no inoculation	0.04	95.79	0.56	96.39
	B	5 mg of BaP + mixed culture[3]	0.03	94.23	0.48	94.74
	C	5 mg of BaP + 5 mg of each naphthalene, phenanthrene, and anthracene + mixed culture	0.54	74.14	21.92	96.59
	D	5 mg of BaP + 5 mg of each naphthalene, phenanthrene, and anthracene + 10 ml of soil extract + mixed culture	1.07	51.75	40.46	93.28
Solid (50 g)	1	Control: sterilized soil + 5 mg of BaP + no inoculation	0.03	85.49	10.19	95.71
	2	Vandalia road soil + 5 mg of BaP + mixed culture	0.04	75.46	19.10	94.59
	3	Treated soil from slurry reactor + 5 mg of BaP + mixed culture	0.24	67.40	23.38	91.01
	4	Mixed soil + 5 mg of BaP + mixed culture	0.07	77.96	15.64	93.67
	5	Land-farming soil + 5 mg of BaP + mixed culture	18.56	62.00	12.69	93.20

[1]Each Treatment was added 5 mg of [^{14}C]BaP with a specific activity of 0.149 μCi/mg BaP.
[2]Extracted with methylene chloride for liquid treatment and with acetonitrile for solid phase treatment.
[3]Mixed culture was obtained from a soil slurry reactor, 10 ml of supernatant was added in each liquid treatment and 5 ml of supernatant was added in each solid treatment.

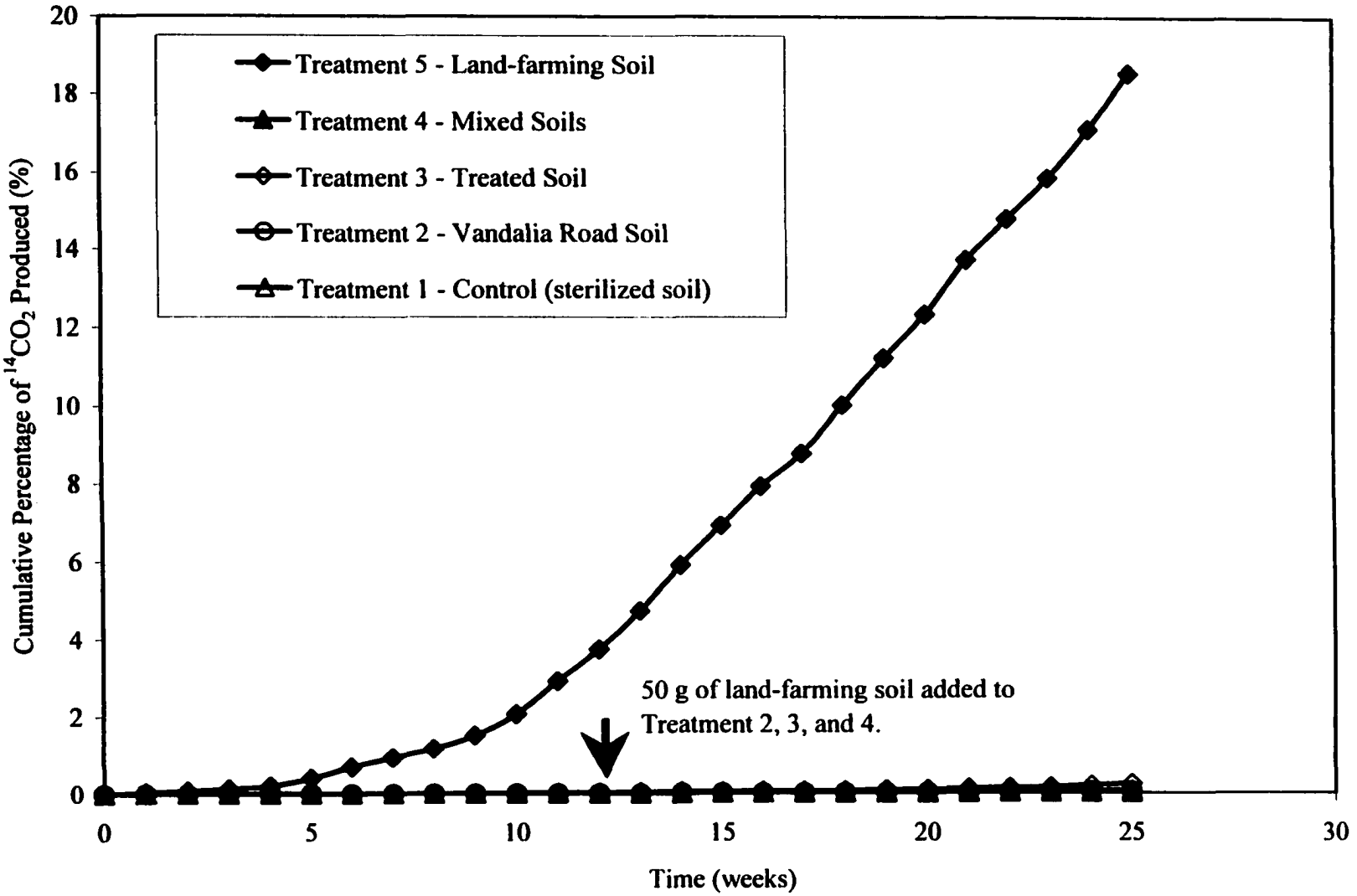

Figure 6-3. Mineralization of benzo(a)pyrene for different solid phase treatments

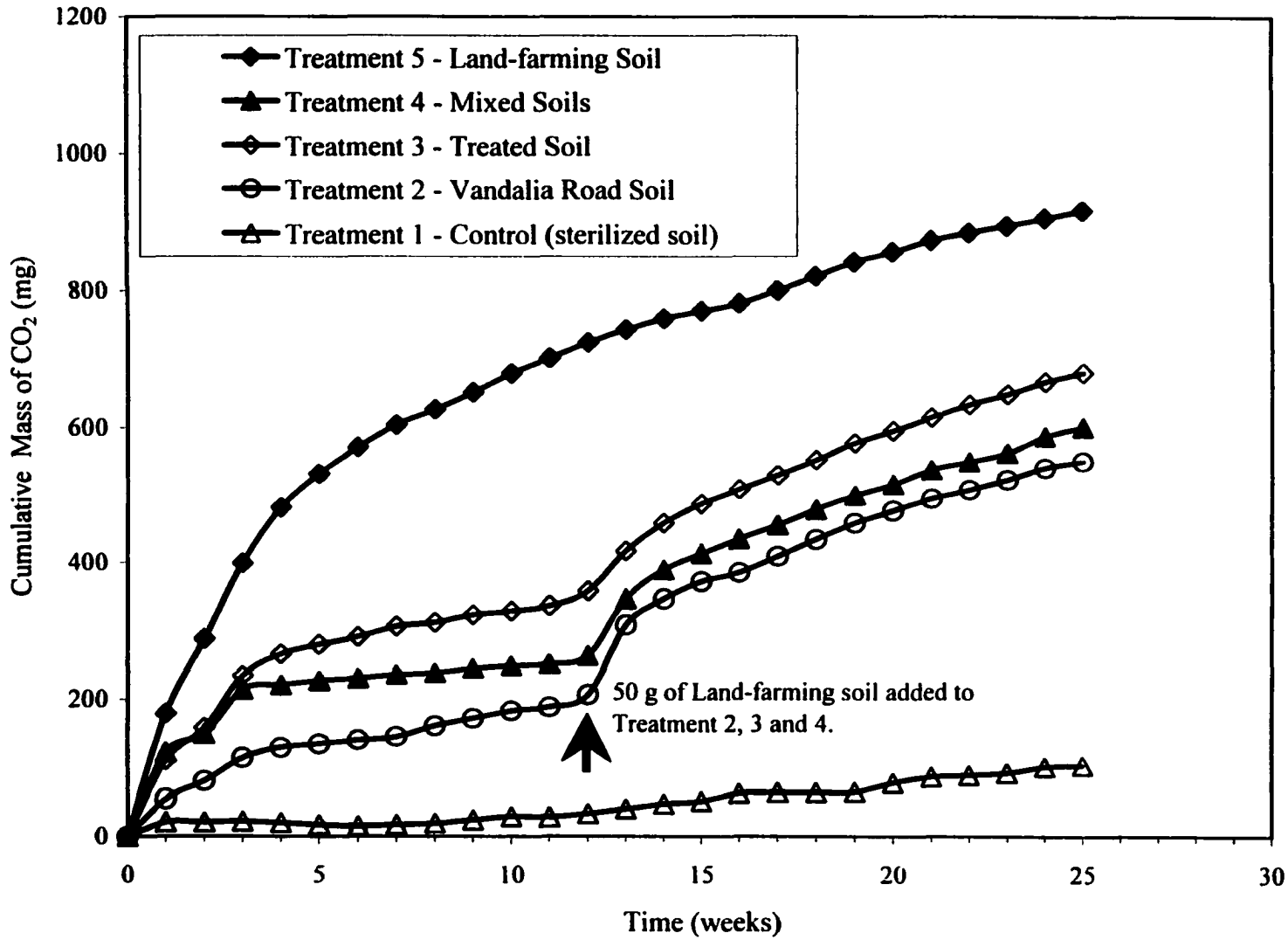

Figure 6-4. Carbon dioxide released from different solid phase treatments

After monitoring Treatments 1 - 4 for 12 weeks without $^{14}CO_2$ production, 50 g (dry weight basis) of land-farming soil was added to Treatments 2 - 4. This was taken to assess whether microorganisms in the land-farming soil were controlling the mineralization of [^{14}C]BaP. There was an improvement in the bioactivity of Treatments 2 – 4 with the added land-farming soil as shown by the CO_2 produced. However, no increase in $^{14}CO_2$ production was obtained for Treatments 2 - 4 even after 25 weeks. The accumulated CO_2 produced for each treatment (Figure 6-4) showed that Treatment 5 had higher production of CO_2 even though the concentrations of PAH present were significantly lower than that of Treatments 2 - 4 (see Table 6-2).

The concentrations of each PAH for all treatments are shown in Table 6-2. There were no significant changes in PAH concentrations for Treatments 2 - 4 over the 25 weeks of treatment. Even the more easily degradable 3-ring compounds such as acenaphthene, fluorene, and phenanthrene continued to remain in the soil. In contrast, lower concentrations of these PAH compounds were found for Treatment 5 after 25 weeks. The removal percentages of each PAH for the different soil treatments are shown in Table 6-2. The soil moisture contents for the various treatments were kept at 15% throughout the study. BaP metabolism measured as $^{14}CO_2$ production increased only when the amount of CO_2 began to decrease, after about 7 weeks incubation (Figure 6-5).

No solvent-extractable metabolites of BaP were found in any treatments. Even for Treatment 5 with a large production of $^{14}CO_2$, no radioactive compounds other than [^{14}C]BaP were identified by the fraction-collection technique that combined both HPLC separation and LSC determination. This implies that mineralization of [^{14}C]BaP without production of solvent-extractable intermediates was obtained.

Mass balances of [^{14}C]BaP in the solid phase treatments are shown in Table 6-3. The distribution of ^{14}C in the solid phase treatments were similar to that of liquid phase treatments with about 10% to 25% non-extractable ^{14}C remaining in all treatments.

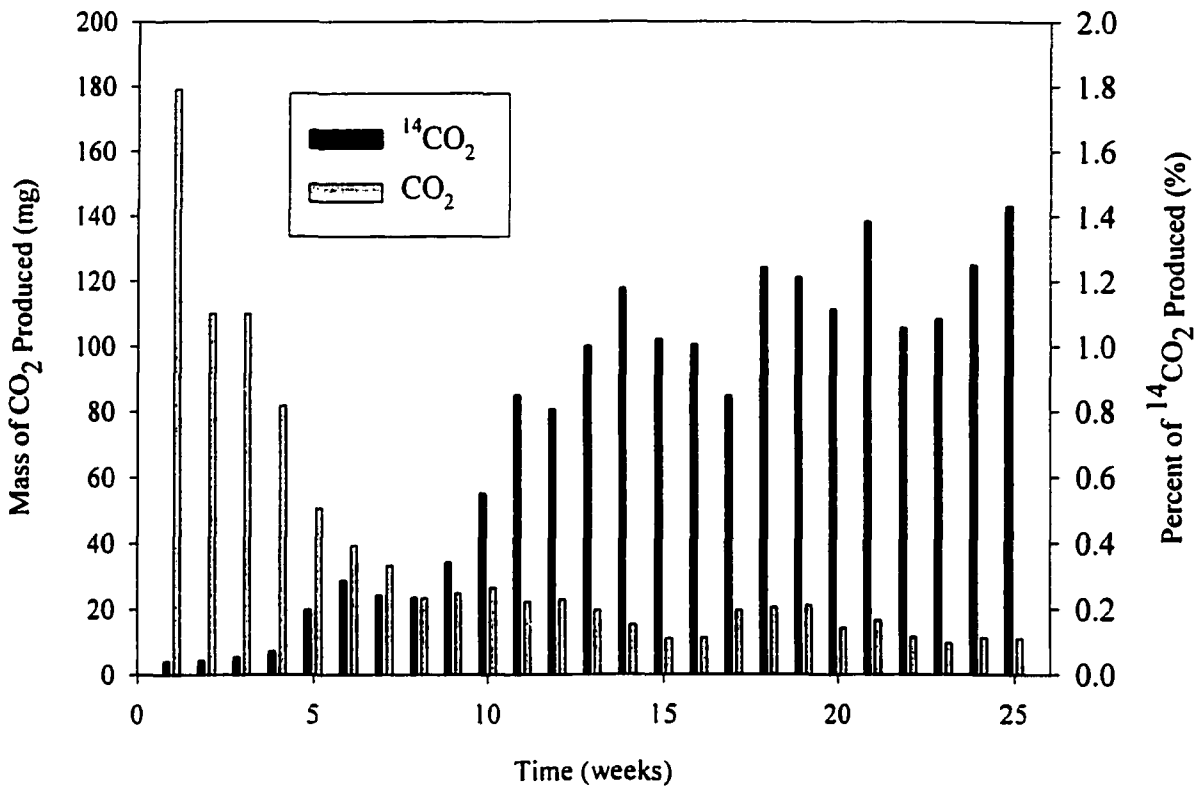

Figure 6-5. Comparison of $^{14}CO_2$ and CO_2 produced by land-farming soil for Treatment 5

DISCUSSION

Microorganisms without additional carbon sources (Treatment B) did not metabolize BaP as a carbon and energy source. This is similar to the results of other researchers since no single organism has been isolated and reported that can use BaP or other 5-ring PAH compounds as carbon and energy source at this time (Pothuluri and Cerniglia, 1994; Aitken et al., 1998; Smith et al., 1999). To degrade HMW PAHs such as BaP, several researchers have proposed using metabolites as inducers to stimulate the degradation (Chen and Aitken, 1999) or use crude oil as co-substrate to enhance the degradation of BaP from soil (Kanaly et al., 1997). However, in our experiments, when 2- and 3-ring PAH compounds were added as possible inducers or co-substrates, microbial respiration was promoted, but not the mineralization of BaP. High microbial activity does not necessarily mean that BaP will be degraded, since microorganisms need specific catabolic enzymes to break the aromatic rings of BaP (Sanglard et al., 1986). Although 2- and 3-ring PAH compounds may serve as co-substrate for the cometabolization of HMW PAH (Bouchez et al., 1995; Aitken et al. 1998), this was not exhibited in these experiments. Erickson et al. (1991) reported that no improvement of HMW PAHs degradation when naphthalene and phenanthrene were added to contaminated soil. In their experiments, the added naphthalene and phenanthrene were readily degraded. Erickson and co-workers reasoned that HMW PAHs in the soils were not available to the microorganisms.

With 20% and 40% of the ^{14}C incorporated in the biomass as non-extractables for Treatments C and D, binding of BaP with the microbial biomass may be an important mechanism. Stringfellow and Alvarez-Cohen (1999) examined different groups of bacteria and found that PAH sorption capacity varied with bacterial species and strain. Note that similar amounts of unlabeled BaP were also incorporated in the biomass for the liquid phase treatments. Kastner et al. (1999) reported that most of the non-extractable residues were incorporated during microbial degradation of PAHs. Guthrie and Pfaender (1998) reported that the highest amounts of non-extractable pyrene degradation products were found in the most biologically active soil samples. They speculated that the pyrene metabolites were bound to soil organic matter and therefore were resistant to solvent extraction. In our study, the highest non-extractable ^{14}C was not found in the land-farming soil (Treatment 5) which

had the highest bioactivity and mineralization but were in the treated soil (Treatment 3). One possible reason for the high non-extractable ^{14}C for Treatment 3 was that it contained more clay than the soils of the other treatments (see Table 6-1). Kastner et al. (1999) found that the extent of non-extractable residue formation depended on the metabolic capacity of the soil microflora and the characteristics of the soil. Identification of the metabolites in the non-extractable fraction was not possible.

Other than the non-extractable ^{14}C compounds which were bound in the biomass or soil, no other ^{14}C products were identified in the extractable portion except for the parent compound [^{14}C]BaP in solid phase treatment. This is contrary to work done by others where water-soluble and ethyl acetate-soluble compounds and oxidized compounds such as phenols, epoxides, or quinones were detected in the degradation of BaP by white rot fungus *Phanerochaete chrysosporium* (Sanglard et al., 1986). Gibson et al. (1975) reported that bacteria *Beijerinckia* sp. oxidized BaP to the cis-7,8- and cis-9,10-dihydrodiols. Shiaris (1989) found that 57% of [^{14}C]BaP was transformed to polar metabolites by three estuarine sediments from the Boston Harbor. Herbes and Schwall (1978) suggested that experiments with microorganisms that were isolated on the basis of their ability to grow on specific PAH compounds may result in the accumulation of oxidized intermediates, since the initial oxidation step by the microbes may proceed more rapidly than the later ring cleavage metabolic steps. Similar results were observed by Heitkamp et al., (1988) for the degradation of PAHs containing three or more rings in sediment-water microcosms. However, Weissenfels et al. (1990) found that no metabolites were detected in the aqueous phase or in the organic extracts for degradation of phenanthrene, fluorene and fluoranthene by pure bacterial cultures. In addition, intermediates or metabolites are rarely found from mixed cultures where metabolites are rapidly utilized by the complex biological communities (Heitkamp et al., 1988; Ellis et al., 1991).

Many researchers have observed that significant degradation of 4-ring compounds were only observed after most of 1-, 2-, and 3-ring compounds were removed (Mueller et al., 1989; Wiesel et al., 1993). This degradation pattern implies a sequential utilization of easily biodegradable compounds to the most difficult. In contrast, cometabolism has been proposed as a mechanism for the degradation of HMW PAHs whereby LMW PAHs are used as co-

substrate (Bouchez et al., 1995; Atiken et al., 1998). Since different PAHs are degraded by the same enzymes from some microorganisms, sequential metabolic degradation may be more likely to occur among PAH-degrading organisms than cometabolism (Stringfellow and Aitken, 1995). Based on this, the high concentrations of LMW PAHs in the contaminated soils may limit the degradation of HMW PAHs. Therefore, the higher mineralization of BaP in Treatment 5 may be due to lower concentrations of LMW PAHs in the soil and not because of specific microorganisms present in the land-farming soil. Since all other treatments eventrally had land-farming soil. In the case of the treated soil (Treatment 3), the higher clay fraction may have limited the availability of BaP for biodegradation. Mineralization of BaP may occur in contaminated soil when specific microorganisms and growth factors are present. In this study, the concentrations of LMW PAH and probably sorption to clay may have limited the degradation of BaP.

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CHAPTER 7. GENERAL CONCLUSIONS AND FUTURE STUDIES

The direct extraction method using various solvents was evaluated to assess its suitability for extracting PAHs from coal-tar-contaminated soils. Based on the screening and evaluation tests, acetone was found to be a suitable solvent for the extraction of PAHs from coal-tar-contaminated soils. The direct extraction method using acetone as the solvent was compared with the Soxhlet and ultrasonic extraction methods (Method 3540 and Method 3550, respectively) and was found to be equivalent to the Soxhlet method at a confidence interval of 99%. The direct extraction method is an easy method requiring minimum sample handling and operator's attention.

A simple solvent-water extraction method was proposed and tested as an assessment tool to estimate the percent of PAHs in soils that may be available for biodegradation. The percent of PAHs extracted using an acetone-water mixture of 0.6 were found to strongly correlate with the percent of PAHs degraded using soil slurry reactors. Two correlations were developed relating the percent degraded, percent extracted using 0.6 acetone-water mixture, soil and PAH properties. This mild extraction method may be used as a simple and quick method to evaluate the biodegradation potential of coal-tar-contaminated soils.

Pretreatment of contaminated soil with solvents showed that solvents can be used to enhance the degradation of PAHs in coal-tar-contaminated soils. Acetone may be a slightly better pretreatment solvent than ethanol but no statistical differences in their degradation rates were found between the two solvents. Solvent pretreatment was found to be especially good for 4- and 5-ring PAH compounds by enhancing the degradation rates by 2 to 6 times.

Mineralization studies of BaP in liquid and solid phase treatments indicate that BaP was not used as a microbial source of carbon and energy and that the presence of 2- and 3-ring PAHs did not enhance the degradation of PAH. The solid phase treatment studies indicate that the low molecular weight PAHs were degraded first followed by the high molecular weight PAHs such as BaP.

Future studies to extend this research include:

1. Investigating the correlation between the bioavailability and the soil properties using the mild solvent extraction method with acetone for other PAH-contaminated soils. The approach used should include artifically contaminated and aged soils plus a variety of contaminated soils with different soil properties. The correlations may be used as an assessment tool to estimate the amount of PAH that is available for biodegradation.
2. Investigating the optimum conditions for using solvent to enhance the degradation of PAHs from the contaminated soils and its practical application for contaminated sites. These optimum conditions can be used to scale up this process and become a practical and applicable technology.
3. Investigating the effects of the interactions between each PAH during biodegradation processes and the removal mechanisms of PAHs when other carbon sources are present. This information may be used to enhance the biodegradation of PAHs with adjustment of carbon sources in the soil environment.

APPENDIX

RAW DATA

Figure 4-1

Hampton soil	Extraction study with acetone-water mixture (volume fraction)					
PAH	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	89.17	87.79	94.60	75.18	56.42	32.82
Acenaphthylene	100.02	98.35	95.61	86.37	69.78	29.79
Acenaphthene	100.00	101.05	96.12	79.11	61.77	22.82
Fluorene	99.51	99.97	96.13	88.08	64.93	23.71
Phenanthrene	97.96	99.29	98.42	88.23	69.03	21.79
Anthracene	100.00	99.88	100.44	83.75	64.11	18.69
Fluroanthene	99.39	98.99	95.25	84.83	57.71	16.23
Pyrene	99.60	98.81	94.33	84.17	58.15	15.50
Benzo(a)anthracene	98.61	96.19	88.64	72.00	37.47	13.53
Chrysene	98.70	95.83	87.12	69.61	34.93	9.05
Benzo(b)fluoranthene	97.19	92.40	81.35	57.03	29.35	6.32
Benzo(k)fluoranthene	96.66	94.00	82.45	53.52	21.58	0.00
Benzo(a)pyrene	97.95	93.02	80.25	49.59	18.72	2.31
Indeno(1,2,3-cd)pyrene	96.19	90.45	75.80	45.08	22.58	0.00
Dibenzo(a,h)anthracene	100.00	100.65	91.11	53.60	43.11	0.00
Benzo(g,h,i)perylene	95.59	87.48	69.10	33.01	7.15	0.00
Total 16 PAHs	99.80	98.76	93.99	83.97	57.44	26.04
LMW PAHs	98.50	98.73	97.42	86.14	66.92	23.33
HMW PAHs	98.43	96.10	88.36	70.55	41.97	9.02

Charles City soil	Extraction study with acetone-water mixture (volume fraction)					
PAH	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	96.37	90.90	88.49	88.28	88.58	31.90
Acenaphthylene	98.49	93.19	90.39	82.79	77.19	31.93
Acenaphthene	98.91	93.14	92.53	81.73	60.55	17.49
Fluorene	101.19	98.02	95.08	78.12	65.42	20.73
Phenanthrene	103.26	99.57	99.41	77.39	62.47	19.27
Anthracene	103.62	97.78	98.22	81.85	65.52	17.30
Fluroanthene	102.06	95.72	97.60	77.52	57.49	14.02
Pyrene	98.84	94.68	97.34	76.43	56.81	12.19
Benzo(a)anthracene	97.78	91.94	90.54	62.60	26.51	11.26
Chrysene	96.57	90.71	89.67	60.76	26.53	6.61
Benzo(b)fluoranthene	93.45	87.78	87.13	49.54	34.89	0.00
Benzo(k)fluoranthene	93.14	87.39	89.67	50.93	1.05	0.00
Benzo(a)pyrene	99.96	81.12	79.24	35.34	0.00	0.00
Indeno(1,2,3-cd)pyrene	97.93	89.41	80.83	10.66	0.00	12.61
Dibenzo(a,h)anthracene	98.35	93.61	84.26	11.66	0.00	0.00
Benzo(g,h,i)perylene	98.30	82.83	76.45	6.03	0.00	0.00
Total 16 PAHs	99.18	93.81	92.56	78.24	66.56	22.19
LMW PAHs	99.35	94.48	92.76	83.27	75.85	26.30
HMW PAHs	98.48	91.53	91.78	61.98	36.70	9.01

Vandalia (EXC) soil	Extraction study with acetone-water mixture (volume fraction)					
PAH	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	96.62	67.97	59.49	56.48	35.11	0.00
Acenaphthylene	100.21	81.39	76.45	58.51	35.32	16.88
Acenaphthene	100.97	85.18	80.38	59.29	22.74	8.33
Fluorene	99.55	83.40	79.82	58.63	28.31	9.98
Phenanthrene	97.04	76.38	72.40	50.13	18.91	6.56
Anthracene	96.99	78.13	73.04	51.40	23.59	6.97
Fluroanthene	95.65	71.81	64.91	41.85	16.45	4.78
Pyrene	95.61	72.29	65.89	41.06	16.23	4.27
Benzo(a)anthracene	89.95	67.64	58.53	30.81	10.37	4.06
Chrysene	89.08	66.25	56.79	29.60	9.52	2.32
Benzo(b)fluoranthene	95.11	70.83	56.38	23.99	9.15	0.00
Benzo(k)fluoranthene	94.11	69.91	55.56	23.44	4.82	0.00
Benzo(a)pyrene	86.45	62.42	48.68	19.12	3.84	0.00
Indeno(1,2,3-cd)pyrene	91.31	68.40	50.09	20.57	8.41	0.00
Dibenzo(a,h)anthracene	101.36	72.46	60.36	27.18	5.44	0.00
Benzo(g,h,i)perylene	90.13	60.44	42.01	13.43	1.45	0.00
Total 16 PAHs	96.23	75.27	68.92	45.72	19.45	6.64
LMW PAHs	98.28	79.11	74.74	53.68	24.25	9.00
HMW PAHs	93.06	69.40	60.05	33.56	12.07	3.04

Vandalia (LTU) soil	Extraction study with acetone-water mixture (volume fraction)					
PAH	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	100.00	100.00	94.81	79.08	67.22	41.89
Acenaphthylene	100.00	97.05	85.56	69.01	48.35	25.60
Acenaphthene	98.71	101.61	97.64	82.82	57.40	24.09
Fluorene	99.59	99.59	97.98	87.62	61.11	29.11
Phenanthrene	100.00	100.00	96.21	81.47	39.19	17.54
Anthracene	100.00	101.85	92.43	78.28	51.43	22.48
Fluroanthene	100.22	102.26	95.48	85.19	51.43	19.20
Pyrene	100.04	99.31	91.61	84.56	50.90	17.60
Benzo(a)anthracene	98.18	98.18	88.85	70.79	36.48	14.55
Chrysene	98.28	98.27	85.61	65.79	31.57	9.37
Benzo(b)fluoranthene	96.77	97.24	85.45	61.26	29.09	16.06
Benzo(k)fluoranthene	100.00	99.30	82.63	59.98	21.04	3.71
Benzo(a)pyrene	100.00	100.00	84.33	54.05	17.84	1.06
Indeno(1,2,3-cd)pyrene	99.27	97.08	84.48	47.37	25.31	18.05
Dibenzo(a,h)anthracene	100.00	100.00	95.52	42.86	45.52	0.00
Benzo(g,h,i)perylene	100.35	103.22	85.90	44.60	6.50	0.11
Total 16 PAHs	99.75	99.45	91.40	75.39	44.21	18.84
LMW PAHs	99.83	99.87	94.27	79.84	51.39	10.24
HMW PAHs	99.43	99.63	89.53	72.43	39.03	3.62

Independence soil	Extraction study with acetone-water mixture (volume fraction)					
PAH	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	103.20	96.39	91.11	74.40	53.51	29.17
Acenaphthylene	101.81	97.77	92.91	76.57	53.55	21.32
Acenaphthene	103.65	99.63	95.87	69.16	32.45	10.82
Fluorene	101.86	100.02	96.42	78.19	46.05	16.38
Phenanthrene	100.66	95.22	91.14	67.18	26.75	10.39
Anthracene	101.39	97.98	92.18	71.44	34.06	10.59
Fluroanthene	100.40	94.52	86.42	62.63	27.10	8.12
Pyrene	100.28	94.39	86.20	59.71	25.32	6.82
Benzo(a)anthracene	99.45	94.04	83.12	51.62	18.82	7.41
Chrysene	98.79	92.99	81.11	48.36	16.23	4.35
Benzo(b)fluoranthene	97.40	91.92	76.35	37.87	14.70	0.00
Benzo(k)fluoranthene	96.29	91.33	76.46	41.43	10.54	0.00
Benzo(a)pyrene	95.00	87.62	68.91	32.51	6.65	0.00
Indeno(1,2,3-cd)pyrene	99.17	81.13	55.82	24.72	3.89	0.00
Dibenzo(a,h)anthracene	98.99	77.96	58.37	28.39	7.16	0.00
Benzo(g,h,i)perylene	103.26	83.69	51.77	19.70	5.86	0.00
Total 16 PAHs	100.89	95.11	87.56	63.86	32.36	12.51
LMW PAHs	101.79	96.96	92.40	72.05	40.12	16.95
HMW PAHs	99.22	91.78	79.06	49.55	18.81	4.76

Figure 4-2

Hampton soil	Extraction study with ethanol-water mixture (volume fraction)						
PAH	1.0	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	104.07	92.46	69.47	67.54	51.06	33.37	0.00
Acenaphthylene	114.59	111.00	97.69	89.36	70.69	44.54	14.37
Acenaphthene	130.74	128.24	107.81	92.74	69.25	37.12	11.66
Fluorene	112.49	113.59	99.39	87.93	66.42	34.75	10.93
Phenanthrene	120.13	117.12	92.63	77.03	56.99	33.71	7.88
Anthracene	116.52	114.46	91.28	77.82	58.48	28.65	7.11
Fluroanthene	110.90	106.73	83.68	70.20	50.93	23.42	6.41
Pyrene	112.11	107.12	81.50	71.77	51.20	22.19	4.64
Benzo(a)anthracene	103.29	92.45	67.96	53.34	33.22	14.89	0.00
Chrysene	101.83	89.49	64.53	49.33	29.88	11.45	0.00
Benzo(b)fluoranthene	96.51	76.57	51.56	40.66	25.12	15.95	0.00
Benzo(k)fluoranthene	99.02	79.70	50.33	35.83	16.40	3.95	0.00
Benzo(a)pyrene	95.49	75.78	46.00	32.34	15.00	0.80	0.00
Indeno(1,2,3-cd)pyrene	88.21	64.02	39.47	32.61	19.60	0.00	0.00
Dibenzo(a,h)anthracene	98.90	77.22	55.09	51.78	39.65	0.00	0.00
Benzo(g,h,i)perylene	84.51	53.90	27.54	17.78	4.31	0.00	0.00
Total 16 PAHs	112.92	106.57	84.11	71.70	52.09	27.51	6.68
LMW PAHs	119.87	114.09	94.00	81.60	61.74	35.61	9.36
HMW PAHs	98.74	92.82	67.43	55.31	36.59	14.90	2.58

Charles City soil	Extraction study with ethanol-water mixture (volume fraction)						
PAH	1.0	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	88.03	88.94	87.60	80.49	66.23	46.89	18.55
Acenaphthylene	86.47	86.83	87.60	86.47	71.98	51.68	13.99
Acenaphthene	90.24	90.48	90.31	86.03	64.56	32.44	6.70
Fluorene	99.27	91.09	92.28	92.29	75.23	35.89	8.50
Phenanthrene	89.00	88.13	85.94	85.46	61.45	23.28	6.27
Anthracene	92.79	90.72	89.70	88.63	64.77	28.20	6.45
Fluroanthene	89.77	86.73	83.40	79.96	56.55	23.52	6.58
Pyrene	83.76	81.78	80.34	71.83	53.81	20.99	3.78
Benzo(a)anthracene	85.14	79.33	72.20	63.81	37.67	15.01	0.00
Chrysene	82.12	75.46	66.98	58.18	33.24	11.53	0.00
Benzo(b)fluoranthene	92.35	82.09	70.88	57.40	22.83	0.00	0.00
Benzo(k)fluoranthene	74.18	63.98	53.50	42.82	18.33	0.00	0.00
Benzo(a)pyrene	72.93	66.03	50.40	40.03	17.43	0.00	0.00
Indeno(1,2,3-cd)pyrene	77.85	59.59	49.54	39.93	0.00	0.00	0.00
Dibenzo(a,h)anthracene	89.10	70.02	61.31	56.35	0.00	0.00	0.00
Benzo(g,h,i)perylene	51.16	37.30	26.68	17.86	0.00	0.00	0.00
Total 16 PAHs	87.93	86.10	83.96	79.44	60.28	33.33	10.31
LMW PAHs	89.08	88.84	87.94	84.48	66.70	39.26	12.73
HMW PAHs	79.26	77.32	71.18	63.24	39.66	14.28	2.52

Vandalia (EXC) soil	Extraction study with ethanol-water mixture (volume fraction)						
PAH	1.0	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	72.67	67.83	63.71	56.17	51.55	0.00	0.00
Acenaphthylene	100.01	99.55	90.87	79.07	73.62	48.55	8.53
Acenaphthene	105.88	107.40	97.32	82.57	71.89	31.55	5.71
Fluorene	101.02	100.92	91.05	75.64	62.30	29.24	4.89
Phenanthrene	97.74	95.85	83.54	65.43	48.96	16.10	2.87
Anthracene	84.55	83.57	73.15	57.66	44.00	19.50	2.92
Fluroanthene	88.72	83.89	69.68	48.25	35.38	12.83	2.48
Pyrene	91.28	85.96	72.51	51.66	34.70	12.51	1.58
Benzo(a)anthracene	77.43	66.23	53.30	32.76	18.01	0.00	3.65
Chrysene	75.81	64.09	51.08	31.57	17.66	0.00	0.00
Benzo(b)fluoranthene	72.43	55.56	40.41	20.44	8.73	0.00	0.00
Benzo(k)fluoranthene	76.12	58.10	43.93	21.28	7.70	0.00	0.00
Benzo(a)pyrene	69.63	46.98	35.96	16.70	4.66	0.00	0.00
Indeno(1,2,3-cd)pyrene	61.45	42.72	31.30	12.71	0.00	0.00	0.00
Dibenzo(a,h)anthracene	70.88	54.33	40.01	22.62	0.00	0.00	0.00
Benzo(g,h,i)perylene	58.56	35.53	23.04	4.21	0.00	0.00	0.00
Total 16 PAHs	91.32	86.78	75.06	57.49	44.09	18.33	3.27
LMW PAHs	95.45	96.21	85.48	69.73	56.81	25.56	4.42
HMW PAHs	72.58	71.61	58.27	37.74	23.55	6.65	1.42

Vandalia (LTU) soil	Extraction study with ethanol-water mixture (volume fraction)						
PAH	1.0	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	102.25	99.07	99.91	89.95	77.07	61.90	38.67
Acenaphthylene	82.26	78.28	78.32	68.35	54.35	37.62	13.41
Acenaphthene	99.74	102.31	105.78	91.80	69.62	45.59	14.93
Fluorene	98.53	103.16	113.80	97.37	78.46	49.20	13.84
Phenanthrene	114.53	111.34	114.10	99.54	80.70	18.82	13.11
Anthracene	93.31	90.48	92.76	75.95	57.25	33.39	10.50
Fluroanthene	94.88	87.62	86.77	72.19	55.69	28.72	8.30
Pyrene	95.81	87.15	85.97	71.16	54.13	26.97	5.96
Benzo(a)anthracene	86.76	74.57	70.21	53.91	38.28	18.34	0.00
Chrysene	83.43	69.17	64.34	47.61	32.39	13.40	0.00
Benzo(b)fluoranthene	79.41	65.53	55.12	41.79	30.09	17.97	0.00
Benzo(k)fluoranthene	78.70	62.24	52.80	36.43	19.90	4.59	0.00
Benzo(a)pyrene	76.28	58.63	49.62	31.40	16.58	2.88	0.00
Indeno(1,2,3-cd)pyrene	74.06	54.88	46.47	38.63	26.03	17.77	0.00
Dibenzo(a,h)anthracene	80.65	65.89	60.76	52.61	46.87	0.00	0.00
Benzo(g,h,i)perylene	56.95	38.98	31.00	14.98	2.49	0.00	0.00
Total 16 PAHs	93.95	86.77	86.05	71.59	55.17	32.07	9.60
LMW PAHs	99.32	99.94	103.18	89.47	71.98	46.93	16.93
HMW PAHs	79.43	75.63	71.55	56.47	40.94	19.50	3.40

Independence soil	Extraction study with ethanol-water mixture (volume fraction)						
PAH	1.0	0.9	0.8	0.7	0.6	0.5	0.4
Naphthalene	98.16	93.87	89.79	81.51	75.21	49.06	18.86
Acenaphthylene	90.55	87.31	84.72	76.32	63.23	36.93	11.32
Acenaphthene	91.27	91.02	87.35	75.71	52.15	23.04	5.64
Fluorene	92.06	89.68	87.09	81.69	57.93	25.82	7.26
Phenanthrene	84.87	80.60	75.34	62.72	38.02	18.96	4.73
Anthracene	88.00	84.66	77.63	69.04	44.94	19.30	5.05
Fluroanthene	84.17	75.89	66.35	55.08	32.00	13.20	4.12
Pyrene	84.57	76.94	67.31	52.01	30.85	11.87	2.63
Benzo(a)anthracene	81.57	69.91	56.67	39.37	19.12	9.32	0.00
Chrysene	81.03	66.73	53.13	36.26	16.75	6.18	0.00
Benzo(b)fluoranthene	72.80	55.98	40.43	23.70	11.85	7.92	0.00
Benzo(k)fluoranthene	74.21	58.07	43.05	26.78	10.52	0.00	0.00
Benzo(a)pyrene	66.50	49.10	33.04	18.77	4.60	0.00	0.00
Indeno(1,2,3-cd)pyrene	55.01	38.23	21.72	16.68	0.00	0.00	0.00
Dibenzo(a,h)anthracene	58.49	44.93	26.89	0.00	0.00	0.00	0.00
Benzo(g,h,i)perylene	49.24	30.72	16.87	7.07	1.56	0.00	0.00
Total 16 PAHs	85.50	78.96	71.74	60.34	41.80	21.64	6.46
LMW PAHs	88.86	86.63	82.27	72.56	53.96	29.44	9.24
HMW PAHs	70.29	65.62	53.42	39.08	20.65	8.06	1.63

Figure 4-3

PAHs	Percent removal of each PAH for Vandalia (LTU) soil during 0-35 days											
	0	1	2	4	7	10	14	17	21	24	28	35
Nap	100.0	25.2	27.2	24.8	21.9	21.8	20.7	21.6	21.3	21.3	0.0	0.0
Acy	100.0	77.4	45.8	38.4	34.4	30.8	29.6	31.7	29.1	27 5	28.2	24.9
Acn	100.0	64.0	41.5	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Flu	100.0	85.3	53.6	15.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phe	100.0	86.7	15.1	9.8	7.1	7.4	5.8	5.5	5.4	5.3	5.1	4.8
Ant	100.0	93.6	83.9	27.7	18.4	17.6	15.9	14.8	15.3	15.1	13.5	12.9
Fla	100.0	93.9	99.4	84.1	29.0	19.4	13.6	13.0	11.9	11.0	10.3	9.0
Pyr	100.0	93.9	99.3	96.0	72.1	23.8	14.4	12.8	11.3	9.9	8.8	7.5
BaA	100.0	94.3	98.9	95.4	32.5	27.3	21.8	21.0	19.2	18.7	18.2	16.0
Chy	100.0	93.7	97.7	95.5	42.9	34.3	24.6	22.5	19.9	18.3	16.8	14.0
BbF	100.0	93.2	95.4	92.9	89.0	83.9	80.8	81.0	78.7	73.1	74.6	66.5
BkF	100.0	97.5	103.1	99.3	92.7	80.9	70.7	66.2	59.2	53.7	48.5	40.0
BaP	100.0	98.2	99.7	99.9	93.3	87.8	81.2	84.9	79.9	72.8	73.3	64.7
InP	100.0	92.3	94.0	86.9	90.1	81.1	75.0	84.4	79.0	72.7	77.7	71.1
DbA	100.0	91.4	90.5	85.3	89.1	81.3	79.3	76.9	72.7	69.6	70.1	67.0
BgP	100.0	100.2	99.9	96.8	95.4	87.8	87.0	85.2	79.2	78 5	79.2	68.7

Where Nap = naphthalene, Acy = acenaphthylene, Acn = acenaphthene, Flu = fluorene, Phe = phenanthrene, Ant = anthracene, Fla = fluroanthene, Pyr = pyrene, BaA = benzo(a)anthracene, Chy = chrysene, BbF = benzo(b)fluoranthene, BkF = benzo(k)fluranthene, BaP = benzo(a)pyrene, InP = Indeno(1,2,3-cd)pyrene, DbA = Dibenzo(a,h)anthracene, BgP = benzo(g,h,i)perylene

Figure 4-4

PAHs	Percent removal of each PAH for Charles City soil during 0-35 days											
	0	1	2	4	7	10	14	17	21	24	28	35
Nap	100.0	27.0	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acy	100.0	90.0	83.3	55.6	8.6	8.2	8.0	8.4	7.6	8.1	7.7	6.8
Acn	100.0	93.5	86.2	31.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Flu	100.0	98.4	97.9	89.8	6.1	4.4	0.0	0.0	0.0	0.0	0.0	0.0
Phe	100.0	97.4	97.6	95.8	3.6	2.8	0.0	0.0	0.0	0.0	0.0	0.0
Ant	100.0	98.0	99.0	97.0	36.9	9.2	6.5	7.1	5.4	6.1	5.5	4.7
Fla	100.0	101.0	98.2	100.2	92.8	83.2	13.1	9.7	7.7	0.0	0.0	0.0
Pyr	100.0	98.9	96.8	99.2	92.3	91.3	6.6	5.0	3.9	3.5	3.3	2.9
BaA	100.0	98.7	94.0	97.9	94.8	92.6	48.5	15.9	13.2	12.7	12.8	11.0
Chy	100.0	98.1	93.8	98.3	93.4	89.8	50.0	25.3	21.6	17.0	15.7	13.7
BbF	100.0	94.0	86.2	90.6	94.4	88.8	90.4	92.0	89.9	90.1	85.7	84.9
BkF	100.0	98.5	93.3	95.8	97.5	91.9	91.4	85.5	83.7	80.4	71.9	66.8
BaP	100.0	97.7	96.7	99.8	95.7	95.0	90.4	87.9	92.0	92.3	84.1	85.6
InP	100.0	96.2	90.0	93.4	93.9	92.0	89.1	87.8	92.0	94.1	85.6	86.7
DbA	100.0	92.9	82.7	93.6	88.2	91.4	89.4	92.6	86.4	92.3	84.0	82.1
BgP	100.0	98.8	91.7	96.8	95.1	92.2	97.1	89.3	98.4	91.4	96.7	89.8

Figure 4-5

PAHs	Percent removal of each PAH for Vandalia (EXC) soil during 0-35 days											
	0	1	2	4	7	10	14	17	21	24	28	35
Nap	100.0	62.5	27.6	22.1	20.4	22.0	20.0	22.4	22.9	23.4	0.0	0.0
Acy	100.0	95.9	90.8	65.1	17.6	13.3	10.7	11.9	10.8	11.2	10.3	9.1
Acn	100.0	100.0	61.0	47.8	33.0	16.3	0.0	0.0	0.0	0.0	0.0	0.0
Flu	100.0	97.4	96.7	82.4	22.5	9.6	6.4	3.0	0.0	0.0	0.0	0.0
Phe	100.0	98.2	90.9	69.7	3.1	2.6	1.9	1.9	1.5	1.5	1.4	1.3
Ant	100.0	94.4	96.7	86.0	59.0	25.2	9.4	7.8	6.3	6.1	5.4	4.8
Fla	100.0	97.5	92.4	93.3	88.1	88.3	83.1	47.5	11.0	8.5	6.5	6.3
Pyr	100.0	97.9	95.4	93.0	89.9	91.6	91.0	83.8	74.9	13.2	7.9	6.0
BaA	100.0	98.8	93.6	92.8	91.4	93.0	89.8	72.1	13.6	11.6	11.1	9.3
Chy	100.0	99.5	99.9	93.7	90.9	92.0	87.7	75.6	28.7	24.2	25.2	18.7
BbF	100.0	99.0	99.9	89.0	89.8	94.2	87.3	86.2	79.2	83.6	77.5	74.9
BkF	100.0	99.7	99.9	91.6	91.9	93.9	87.4	86.2	81.2	81.5	68.9	68.2
BaP	100.0	98.5	98.6	91.8	89.0	91.6	84.1	84.1	85.5	86.3	82.4	84.2
InP	100.0	95.7	98.2	82.6	80.2	85.5	77.7	77.7	78.7	79.6	77.4	82.2
DbA	100.0	89.7	91.2	83.1	81.6	85.5	78.1	80.0	78.0	80.1	76.9	81.1
BgP	100.0	95.3	94.7	85.2	83.1	84.6	73.9	78.9	79.0	78.7	73.6	76.8

Figure 4-6

PAHs	Percent removal of each PAH for Hampton soil during 0-35 days											
	0	1	2	4	7	10	14	17	21	24	28	35
Nap	100.0	87.4	83.9	81.4	59.6	48.4	39.1	31.0	24.3	19.2	17.3	17.4
Acy	100.0	97.6	77.9	30.6	19.4	17.6	15.5	16.3	15.0	15.7	14.4	13.3
Acn	100.0	91.5	78.2	49.4	14.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Flu	100.0	98.4	94.3	16.1	10.4	7.7	5.2	4.9	0.0	0.0	0.0	0.0
Phe	100.0	99.0	97.9	14.9	9.2	7.2	5.6	6.3	5.3	6.1	4.7	4.1
Ant	100.0	99.6	99.2	75.4	18.5	15.6	11.9	12.7	10.9	13.4	10.5	9.4
Fla	100.0	99.4	85.7	99.6	94.9	53.9	20.4	16.9	14.4	14.4	12.4	10.7
Pyr	100.0	98.2	94.6	99.3	99.0	29.2	13.9	11.9	9.7	9.2	8.6	7.3
BaA	100.0	99.1	97.4	96.6	99.9	88.9	31.5	25.7	22.5	21.0	19.8	17.6
Chy	100.0	95.7	97.3	98.8	97.0	81.0	43.2	35.8	31.8	28.6	25.7	23.3
BbF	100.0	94.5	94.3	98.0	98.8	94.1	92.9	96.9	96.0	94.8	89.3	85.4
BkF	100.0	97.2	95.0	95.6	99.9	98.2	91.3	89.1	87.8	83.1	77.5	74.5
BaP	100.0	98.3	94.7	99.8	98.9	99.6	93.7	94.0	91.1	90.6	88.5	88.6
InP	100.0	92.8	92.1	96.4	93.2	96.5	95.1	92.4	91.5	89.4	91.5	88.7
DbA	100.0	88.6	92.1	96.3	90.9	91.8	89.9	93.7	86.5	86.9	89.5	84.7
BgP	100.0	96.1	90.6	96.2	97.6	95.5	95.1	92.6	91.9	91.8	88.7	83.6

Figure 4-7

PAHs	Percent removal of each PAH for Independence soil during 0-35 days												
	0	1	2	3	5	7	10	14	17	21	24	28	35
Nap	100.0	40.2	8.2	8.0	5.1	5.3	4.6	4.4	4.8	4.1	4.0	4.3	4.6
Acy	100.0	91.5	76.0	67.8	23.8	16.0	12.6	10.5	12.0	10.3	10.3	10.4	9.1
Acn	100.0	81.4	38.5	30.3	20.2	10.3	5.1	0.0	0.0	0.0	0.0	0.0	0.0
Flu	100.0	95.5	90.8	90.9	33.6	17.4	10.3	7.4	6.1	4.3	4.0	3.4	4.1
Phe	100.0	88.9	84.8	84.8	8.3	8.7	5.6	4.1	5.3	3.6	3.3	3.5	3.5
Ant	100.0	97.1	93.6	94.7	68.5	43.3	19.2	14.7	13.3	10.9	11.2	8.1	8.6
Fla	100.0	90.5	87.7	89.4	72.4	75.8	65.5	52.9	32.9	18.9	16.0	15.4	15.2
Pyr	100.0	91.7	89.4	91.4	75.1	79.2	72.2	59.7	49.9	21.2	15.9	14.6	14.8
BaA	100.0	94.3	92.1	94.7	79.6	82.4	75.3	58.3	31.7	22.1	20.6	20.4	20.7
Chy	100.0	93.3	91.5	94.5	78.5	81.3	74.3	56.6	39.8	26.8	25.1	24.7	24.0
BbF	100.0	100.6	98.4	100.7	84.7	89.0	76.7	71.5	75.9	72.8	71.7	75.1	69.3
BkF	100.0	94.6	93.7	95.6	79.4	80.1	75.0	65.1	69.0	62.3	64.1	66.0	64.3
BaP	100.0	94.1	93.0	96.3	81.5	81.6	75.1	65.8	68.1	69.0	64.4	68.3	64.9
InP	100.0	99.3	93.4	98.3	88.2	81.5	74.3	66.1	72.3	71.5	69.0	76.2	67.6
DbA	100.0	94.1	85.5	89.5	85.3	77.3	69.9	63.2	69.1	65.8	65.5	69.2	64.4
BgP	100.0	93.3	84.1	95.2	86.4	78.8	72.0	69.9	67.0	67.9	66.2	70.7	67.2

Figure 4-8

Soils	Percent removal of Total PAH for five different soils during 0-35 days											
	0	1	2	4	7	10	14	17	21	24	28	35
CC	100	78	70.6	62.4	27.9	24.9	11.4	9.3	8.8	8.3	7.6	7.2
EXC	100	102.2	99.2	81.3	46.8	41.6	36.8	31.7	22.5	14.9	12.7	12
Ham	100	99.7	93.9	60.6	50.8	36.2	24.9	23.9	21.7	21.6	20	18.7
LTU	100	85.8	69.2	57.4	37.9	27.2	22.9	22.7	21.2	19.9	18.1	16
Ind	100	84.4	73.9	56.8	36.6	30.4	24.9	21.5	15.6	14.5	14.5	14.1

Where CC = Charles City soil, EXC = Vandalia (EXC) soil, Ham = Hampton soil, LTU = Vandalia (LTU) soil, Ind = Independence soil

Figure 5-1

Time (days)	Killed Control (% removal)	No solvent pretreatment (% removal)	Time (days)	Ethanol pretreatment (% removal)	Acetone pretreatment (% removal)
0	100.00	100.00	0	100.00	100.00
1	98.87	100.48	1	94.07	90.64
2	99.69	97.51	2	90.70	74.07
4	93.27	79.92	3	80.01	45.16
7	89.75	45.99	5	48.35	38.90
10	88.98	40.94	7	41.13	27.09
14	89.20	36.18	10	19.89	14.75
17	89.63	31.13	14	12.63	11.28
21	85.20	22.13	17	10.68	11.05
24	84.58	14.65	21	9.57	10.12
28	80.34	12.49	24	10.01	9.08
35	79.52	11.82	28	8.86	9.18
			35	7.68	8.24

Figure 5-2

Time (days)	Phenanthrene removal (%) for five different soils without solvent pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100.00	100.00	100.00	100.00
1	99.01	98.23	97.42	86.72	88.85
2	97.86	90.89	97.60	15.09	84.77
4	8.38	69.70	95.82	9.81	46.56
7	9.17	3.05	3.55	7.09	8.68
10	7.22	2.61	2.78	7.43	5.64
14	5.55	1.86	0.00	5.75	4.10
17	6.29	1.89	0.00	5.52	5.28
21	5.31	1.45	0.00	5.36	3.60
24	6.09	1.54	0.00	5.32	3.34
28	4.93	1.36	0.00	5.14	3.48
35	4.30	1.34	0.00	4.77	3.48

Time (days)	Phenanthrene removal (%) for five different soils with acetone pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100.00	100.00	100.00	100.00
1	72.11	101.48	93.92	74.87	75.76
2	29.80	73.28	89.78	16.82	69.76
3	23.11	4.83	6.87	12.08	24.68
5	10.83	3.39	4.89	9.95	10.77
7	9.90	2.92	4.55	8.64	11.73
10	7.72	2.22	3.82	7.08	6.95
14	7.29	1.85	2.96	6.73	4.54
17	5.71	3.06	2.92	6.61	3.41
21	5.27	1.98	2.95	6.25	2.79
24	5.47	1.67	2.96	6.27	2.48
28	4.88	1.60	3.16	5.72	2.18
35	4.70	1.52	2.10	5.22	2.47

Time (days)	Phenanthrene removal (%) for five different soils with ethanol pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100.00	100.00	100.00	100.00
1	96.89	101.50	99.53	100.74	87.62
2	83.11	97.82	99.18	97.39	77.10
3	24.50	89.17	99.69	13.97	37.64
5	14.65	6.69	5.34	11.41	12.56
7	9.99	4.37	3.72	9.27	10.99
10	10.10	2.81	4.28	8.55	9.05
14	10.38	2.28	2.77	8.16	6.83
17	7.63	1.86	2.95	7.34	5.18
21	9.67	1.56	2.75	6.73	5.48
24	3.89	1.39	2.43	7.21	3.03
28	7.87	1.66	2.43	5.85	2.83
35	6.93	1.31	2.42	6.50	4.07

Figure 5-3

Time (days)	Chrysene removal (%) for five different soils without solvent pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100.00	100.00	100	100
1	95.67	99.52	98.06	93.69	91.93
2	97.29	101.44	93.84	97.71	83.55
4	98.81	93.67	98.30	95.49	82.77
7	97.04	90.86	93.42	42.91	75.59
10	81.01	92.01	89.79	34.33	72.69
14	43.23	87.65	49.96	24.57	56.80
17	35.75	75.61	25.30	22.53	33.89
21	31.78	28.71	21.57	19.87	26.44
24	28.60	24.16	16.97	18.3	18.64
28	26.78	25.20	15.67	16.75	19.93
35	24.36	18.68	13.73	14.01	17.48

Time (days)	Chrysene removal (%) for five different soils with acetone pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100.00	100.00	100.00	100.00
1	97.04	96.55	97.43	102.42	84.49
2	97.12	96.72	97.41	90.84	81.80
3	71.38	99.52	96.05	51.18	79.56
5	46.89	89.07	84.03	33.08	73.61
7	38.64	59.56	82.24	24.75	71.52
10	30.65	26.67	27.03	21.06	59.28
14	29.94	16.10	13.39	18.97	37.73
17	27.87	15.21	11.10	18.40	30.78
21	24.98	12.59	9.79	16.73	19.36
24	26.06	10.96	9.60	16.03	16.35
28	24.30	11.07	8.33	14.80	14.49
35	17.12	8.26	7.87	13.28	16.75

Time (days)	Chrysene removal (%) for five different soils with ethanol pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100.00	100.00	100.00	100.00
1	106.99	100.71	100.44	99.26	91.93
2	93.91	99.66	97.29	100.52	83.55
3	96.94	92.25	92.98	88.04	88.31
5	69.59	90.27	88.05	46.25	82.77
7	39.54	89.08	78.62	36.85	75.59
10	29.61	33.20	22.81	24.48	72.69
14	25.29	19.15	15.39	21.12	56.80
17	22.63	14.08	12.93	20.12	33.89
21	23.18	10.91	11.49	16.98	26.44
24	19.59	13.43	14.37	17.67	18.64
28	20.79	8.55	15.31	17.36	19.93
35	19.20	6.95	9.60	15.51	17.48

Figure 5-4

Time (days)	Benzo(a)pyrene removal (%) for five different soils without solvent pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100	100	100.00	100.00
1	98.28	98.52	97.65	98.19	94.08
2	94.68	98.58	96.69	99.66	92.99
4	99.84	91.76	99.76	99.85	88.94
7	98.93	89.04	95.65	93.31	81.59
10	99.63	91.61	94.97	87.80	75.13
14	93.66	84.09	90.37	81.21	65.79
17	93.96	84.09	87.94	84.92	68.08
21	91.12	85.46	92.03	79.85	68.96
24	90.58	86.32	92.27	72.75	64.36
28	88.52	82.4	84.14	73.25	68.28
35	88.60	79.59	85.58	64.71	64.91

Time (days)	Benzo(a)pyrene removal (%) for five different soils with acetone pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100.00	100.00	100.00	100.00
1	97.94	97.56	98.01	97.46	89.37
2	98.53	95.26	97.30	91.95	85.01
3	86.33	96.93	95.76	84.72	83.67
5	84.58	94.46	86.24	75.42	74.24
7	73.02	85.67	85.25	65.76	72.78
10	63.88	85.33	73.16	55.50	67.50
14	61.28	74.66	68.71	55.35	59.06
17	53.99	65.58	60.48	53.71	55.43
21	50.94	69.75	64.87	50.31	47.58
24	54.16	63.36	60.34	46.79	48.25
28	52.97	64.91	51.69	44.99	39.32
35	53.51	58.14	51.47	47.90	45.09

Time (days)	Benzo(a)pyrene removal (%) for five different soils with ethanol pretreatment				
	Hampton soil	Vandalia (EXC) soil	Charles City soil	Vandalia (LTU) soil	Independence soil
0	100.00	100.00	100.00	100.00	100.00
1	105.49	100.60	91.15	91.54	92.70
2	97.37	99.74	91.07	85.05	86.56
3	94.07	93.16	87.22	82.93	88.68
5	92.89	92.52	83.44	76.74	77.58
7	80.93	89.82	78.05	81.31	76.68
10	72.71	76.23	73.23	66.59	77.24
14	63.25	72.14	72.13	59.48	67.94
17	64.06	69.45	68.51	63.93	55.80
21	60.02	61.84	63.65	53.31	55.59
24	68.26	63.79	66.64	55.93	46.00
28	62.55	55.98	56.65	57.46	48.32
35	58.54	50.03	57.59	52.59	48.20

Figure 6-1

^{14}C (dpm) in CO_2 trapped solution* (initial 1,650,000 dpm) during degradation of benzo(a)pyrene in liquid phase treatment

Time	Treatment A		Treatment B		Treatment C		Treatment D	
(weeks)	#1	#2	#1	#2	#1	#2	#1	#2
1	11	12	2	10	119	115	61	72
2	0	0	2	5	127	115	23	46
3	7	3	6	8	223	166	38	80
4	18	12	2	20	556	261	27	129
5	0	8	5	4	316	178	838	2977
6	0	0	0	10	974	129	2630	1574
7	51	2	0	6	504	202	446	281
8	131	3	0	0	396	146	284	163
9	38	0	125	2	392	174	200	142
10	25	1	15	10	50	15	46	60
11	0	2	7	2	22	83	207	113
12	15	2	0	21	3	3	72	23
13	16	3	3	5	5	3	13	6
14	23	9	10	15	26	2	22	15

*Data was obtained from 3 mL of 10 mL CO_2 trapped solution (0.5 N NaOH)

Figure 6-2

CO_2 (mg) produced during degradation of benzo(a)pyrene in liquid phase treatment

Time	Treatment A		Treatment B		Treatment C		Treatment D	
(weeks)	#1	#2	#1	#2	#1	#2	#1	#2
1	6.6	5.0	11	9	19.2	40.4	45.6	44.6
2	3.3	3.9	9.2	9.5	26.0	16.2	38.5	39.2
3	3.7	3.8	8.5	7.0	10.4	14.7	9.4	15.4
4	3.3	0	2.2	0.6	3.6	2.8	4.3	5.0
5	0.0	4.5	0.7	0.0	4.1	3.3	14.6	21.3
6	0.0	0.0	0.0	0.0	7.7	2.2	36.3	34.5
7	0.0	0.0	1.7	0.2	2.7	2.9	1.5	2.8
8	0.0	1.1	2.4	6.1	3.9	6.9	6.0	6.6
9	4.4	5.6	4.0	2.8	5.9	9.5	10.3	8.2
10	2.8	4.5	3.2	2.2	64.8	30.9	8.4	74.3
11	2.8	1.0	4.0	1.5	33.6	9.5	49.8	29.9
12	2.2	2.6	3.7	4.4	28.4	22.6	33.5	22.0
13	1.0	0.6	1.1	1.1	11.7	42.4	17.6	18.0
14	1.9	1.1	5.0	0.0	18.5	33.1	18.1	25.8

Figure 6-3

Time (weeks)	^{14}C (dpm) in CO_2 trapped solution* (initial 1,650,000 dpm) during degradation of benzo(a)pyrene in solid phase treatment								
	Treatment 1	Treatment 2		Treatment 3		Treatment 4		Treatment 5	
		#1	#2	#1	#2	#1	#2	#1	#2
1	10	19	11	26	15	49	24	111	339
2	0	6	5	9	2	3	13	41	439
3	6	7	9	7	1	4	11	30	561
4	1	3	4	6	5	7	5	342	454
5	6	5	3	4	2	1	10	1508	705
6	3	28	5	7	2	1	3	2470	716
7	1	4	0	37	1	3	8	2303	381
8	0	2	1	10	1	3	1	2297	310
9	0	0	2	7	3	0	5	3486	307
10	14	7	4	10	5	8	9	4768	1350
11	11	15	6	14	7	9	0	7124	2295
12	2	0	0	58	97	0	6	6285	2656
13	1	7	7	15	103	43	3	7329	3792
14	2	5	20	12	44	64	6	7576	5495
15	58	6	25	30	22	19	8	7261	4095
16	4	10	0	40	14	30	1	7865	3294
17	5	6	5	31	2	5	8	5864	3535
18	0	3	36	114	7	32	0	7368	6430
19	6	8	7	182	8	19	4	8084	5370
20	0	3	7	150	0	8	0	6136	6232
21	0	2	7	215	7	29	2	7442	7924
22	16	19	18	218	12	35	7	5329	6414
23	5	9	14	259	0	17	6	5496	6531
24	11	17	20	350	13	10	134	5852	8829
25	11	40	27	461	19	107	4	5865	10032

*Data was obtained from 3 mL of 10 mL CO_2 trapped solution (0.5 N NaOH)

Figure 6-4

Time (weeks)	CO_2 (mg) produced during degradation of benzo(a)pyrene in solid phase treatment								
	Treatment 1	Treatment 2		Treatment 3		Treatment 4		Treatment 5	
		#1	#2	#1	#2	#1	#2	#1	#2
1	20.7	44.8	62.7	73.6	146.9	115.7	127.6	198.4	160.0
2	0.0	20.4	32.5	27.0	69.3	30.0	24.6	110.0	110.0
3	1.5	34.6	33.0	110.0	44	20.4	110.0	110.0	110.0
4	0.0	11.0	19.3	50.6	13.8	13.8	0.0	110.0	53.4
5	0.0	7.5	2.8	20.1	7.0	9.4	2.2	66.8	34.7
6	0.0	9.5	2.8	15.4	7.15	7.0	2.5	51.8	26.8
7	1.6	8.4	1.1	24.2	5.0	7.2	1.8	46.8	19.8
8	1.9	5.5	27.0	4.8	6.6	3.3	2.2	31.4	15.2
9	5.5	14.7	5.7	14.3	9.6	8.0	5.8	30.4	19.1
10	4.3	10.0	13.3	3.3	5.6	5.5	3.5	28.3	24.8
11	0.0	8.14	2.6	11.7	4.0	4.9	0.0	23.8	14.8
12	5.0	14.3	23.1	30.0	13.0	17.0	7.9	20.1	25.7
13	7.5	100.0	103.4	56.1	61.1	81.4	82.5	18.2	21.2
14	7.3	41.3	35.2	43.5	39.6	42.4	41.8	15.2	15.1
15	3.7	25.3	24.9	29.4	26.8	25.4	22.0	11.0	10.9
16	12.3	0.7	24.5	21.5	21.3	25.3	18.7	13.1	9.4
17	0.4	21.3	26.6	20.5	21.1	21.7	19.4	15.6	23.6
18	0.0	26.0	25.3	27.2	19.7	19.6	26.5	20.7	20.3
19	1.6	24.1	22.9	21.9	28.1	23.7	17.5	25.3	17.2
20	12.1	16.7	18.4	18.0	18.4	14.2	17.9	17.7	11.0
21	9.5	17.1	18.9	18.9	21.2	24.5	19.3	15.6	24.5
22	1.7	14.2	13.2	12.7	24.8	14.3	10.3	12.1	10.8
23	3.8	14.2	14.3	13.5	15.5	13.0	13.2	12.2	6.9
24	8.8	17.9	18.2	16.0	20.9	18.5	30.2	9.4	18.5
25	14.5	32.3	33.7	32.8	36.2	40.0	32.4	24.0	20.4

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