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Gary S. Sayler, Major Professor

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Date 5/24/91

EFFECT OF SOIL COMPOSITION AND CONTAMINATION ON NAPHTHALENE MINERALIZATION

A Thesis

Presented for the

Master of Science

Degree

University of Tennessee, Knoxville

Mary L. Korde

August 1991

DEDICATION

This thesis is dedicated to my husband, Uday, whose support and encouragement helped to make it possible.

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Many people deserve thanks for their help during my thesis research. I am grateful to Dr. Gary S. Sayler, my advisor, for his guidance and support for my research. I thank Dr. Greg Reed for his advice, enthusiasm for this research, and support. Dr. David C. White's comments have been very helpful. I would like to thank Rebecca Pullen for her crucial contribution to this research. I am indebted to Dr. John Sanseverino for his patient guidance and being my mentor through all stages of this research. I would also like to thank Bruce Applegate, Claudia Werner, Dr. Henry King, and others in the laboratory for their help. Dr. John Graveel has been helpful with soil collection and characterization. I would like to thank the University of Tennessee's Waste Management Research and Education Institute, a state funded center of excellence, for funding this research.

ABSTRACT

The extent of biodegradation in soil may be determined by factors such as sorption of the compound to soil and different soil fractions. It is anticipated that knowledge gained in laboratory studies can eventually be applied to field conditions (i.e. bioremediation).

In this study, naphthalene (a nonpolar hydrocarbon) biodegradation was examined in batch cultures under aqueous conditions and in soil slurries. Four soils with different characteristics (varying amounts of organic matter, clay, silt, and sand) were used to make the soil slurries. Biodegradation assays were performed with and without Strain 5R, a known naphthalene degrader. The soils used to make the soil slurries were contaminated with naphthalene using either a dry or wet contamination procedure.

This study resulted in a number of findings: 1) Colony hybridizations determined that the frequency of the NahA (a naphthalene degrading) genotype increased with exposure to naphthalene. 2) Changes in the soil pH can affect the total amount of mineralization. 3) The method of contamination (wet or dry) may have an effect on the amount of mineralization. 4) Dry contaminated soil slurries with Strain 5R had faster mineralization rates than Strain 5R by itself in water. 5) Mercuric chloride is not the best method to sterilize soil.

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LIST OF SYMBOLS AND ABBREVIATIONS

Adsorpt.	adsorption
AVER.	average
°C	degrees in Celsius
^{14}C	a radioactive form of carbon
C.E.C.	cation exchange capacity
cfu	colony forming unit(s)
cmol	centimole
CO_2	carbon dioxide
dATP	deoxyribose adenine triphosphate
dCTP	deoxyribose cytosine triphosphate
Desorpt.	desorption
dGTP	deoxyribose guanine triphosphate
dpm	disintegrations per minute (radioactive)
dTTP	deoxyribose thymine triphosphate
Exper.	experimental
g	gram(s) or gravitational acceleration (9.81 m/s^2)
hrs	hours
K_{ads}	the adsorption capacity
K_{des}	the desorption capacity
kg	kilogram
K_{om}	the number of sites on organic matter
l	liter
m	meter(s)
M	molar
Max.	maximum
mCi	milliCurie
mg	milligram
Miner.	mineralization
ml	milliliter
mm	millimeter
mM	millimolar
mmol	millimole
N	Normal
ng	nanogram
pg	picogram
PAH	polycyclic aromatic hydrocarbon
ppm	parts per million
r	correlation coefficient
rpm	rotations per minute
s	second(s)
S.D.	standard deviation
Taq	Thermus aquaticus
Tot. Pop.	Total population
μl	microliter
μm	micrometer

CHAPTER I

INTRODUCTION

Naphthalene is considered to be a priority pollutant by the U.S. Environmental Protection Agency and has been selected as one of many organic compounds for further study by the U.S. Department of Energy (Zachara et al., 1984). Naphthalene is a possible carcinogen (Pitts et al., 1977). Naphthalene is a polycyclic aromatic hydrocarbon (PAH) consisting of two unsubstituted aromatic rings. Polycyclic aromatic hydrocarbons are components of fossil fuels and are found ubiquitously in the environment. Both natural and anthropogenic processes lead to the occurrence of PAHs in the environment (Blumer, 1976). Some of the major sources of PAHs are forest and prairie grass fires, volcanic ash, heat and power generation, refuse burning, motor vehicle emissions, industrial processes, petroleum leakage and spills, fallout from urban air pollution, coal liquefaction and gasification processes, and cigarette smoke (Blumer, 1976; Cerniglia, 1984). Due to atmospheric fallout, naphthalene in the atmosphere ends up on the earth's surface. Naphthalene, like other polycyclic aromatic compounds, is a hydrophobic compound, and tends to adsorb onto surfaces such as soil particles and sediments rather than remain in the aqueous phase (Stumm and Morgan, 1970; Kipling, 1965). Even compounds considered to have a weak

affinity for soils and sediments can exist predominantly in the sorbed state (Pignatello, 1989). Sediments tend to act as temporary or long-term sinks for many organic compounds (Wakeham and Farrington, 1980). Chemicals that become associated with sediments persist for a longer period of time and are released at a slower rate to the moving water than chemicals that are readily soluble and dissolve completely (Thibodeaux et al., 1980).

Biodegradation of a compound in soils and sediments is determined, in part, by the bioavailability of the compound. A compound is considered to be bioavailable if it is physically accessible to living organisms. Generally, it is believed that if a compound is tightly adsorbed to soil or sediment particles, it is not bioavailable, and therefore not able to be biodegraded. The compound that is found in the aqueous phase is considered to be bioavailable and should be able to be biodegraded more easily. Diffusion, along with adsorption, is thought to control the availability of many organic substrates to microorganisms and influence the rate of degradation of these chemicals (Alexander and Scow, 1989). The diffusion of organic compounds into and out of micropores in soil, which are inaccessible to microorganisms because of their size, may be an important factor controlling the rate of mineralization of the compounds.

In many cases, sorption of a chemical has been found to limit its bioconversion. A reduction in the observed second-order microbial rate constant (k_{obs}) for chlorpropham and di-n-butyl phthalate biodegradation was seen as the sediment:water ratio was increased (Steen et al., 1980). Ogram et al., 1985, found that 2,4-D sorbed onto soil particles was completely protected from biological degradation. In this experiment, biodegradation was measured by CO_2 evolution and sorption was determined by a batch slurry method as described by Green et al., 1980. Ogram et al., however, did not compare desorption rates and biodegradation rates. Other researchers compared these two rates. Wszolek and Alexander, 1979, compared biodegradation of an amine (decylamine) complexed to clay (bentonite or montmorillonite) and in solution. They found that the clay-bound n-[1-^{14}C]decylamine was converted to $^{14}CO_2$ much more slowly than the labeled amine in the clay-free solution when starting at the same initial cell density (2×10^8 cells/ml). They also attempted to examine the relationship between desorption and biodegradation. To estimate the desorption rate, a purely physicochemical technique, continuous dialysis at a high flow rate, was used to simulate microbial removal of a chemical from the suspension. It appeared that the biodegradation rate at the two highest cell densities (2×10^{10} cells/ml and 6×10^9 cells/ml) exceeded the highest rate of amine removal by

continuous dialysis by factors of more than 5 and 10. Rijnaarts et al., 1990, using alpha-hexachlorocyclohexane, also examined biodegradation and desorption in contaminated soil. Desorption was measured at time intervals by taking samples, centrifuging them, and analyzing the supernatants with GC and HPLC. Bioconversion was monitored by measuring the release of chloride (non-radioactive). One fault with this experiment was that the soil was contaminated with other chlorinated compounds. However, these other compounds were present at a relatively low concentration. The initial rates of alpha-hexachlorocyclohexane biodegradation were consistently higher than the initial rates of desorption for all concentrations except the highest one (200 mg/kg) in a stirred system. Subba-Rao and Alexander (1982) used benzylamine-clay complexes to study biodegradation and desorption. Increases in the clay concentration decreased the percentage of the organic compound that was mineralized at amine levels of 20 pg to 200 ng, but not at 20 μg/ml. They found that, except at high amine and clay concentrations, amine mineralization was more rapid than desorption during the early periods of decomposition when the amine concentration in solution was relatively high. Although desorption in some studies (Wsolzck and Alexander, 1979; Rijnaarts et al., 1990; Subba-Rao and Alexander, 1982) appeared to be rate limiting for biodegradation, the biodegradation rate was greater than the rate of desorption

in sterile systems. The desorption rates in non-sterile systems may differ from the rates in sterile systems as a result of microbial activity (van Loosdrecht et al., 1990). In some cases, sorption of a compound has been found to stimulate its bioconversion. This happens when the compound or its metabolites are toxic to the microorganisms (Rijnaarts et al., 1990).

There is controversy whether the organic fraction or the mineral fraction (sand, silt, and clay) is more important in sorption (MacIntyre and Smith, 1984; Chiou et al., 1984) In many cases, the sorption of nonpolar organic compounds by soil has been shown to be correlated with organic carbon or soil organic matter (humus) content (Chiou et al, 1979; Hassett et al., 1980; Karickhoff et al., 1979; McCall et al., 1980; Means et al., 1980). Soil organic matter is thought to have great sorption properties due to its large surface area. However, recently, Chiou (1990), by using a different technique for analyzing surface area, found that the surface area of organic matter was much lower than shown previously. Soil properties such as the type and amount of clay, soil pH, hydrous oxide content were considered to have little effect on the sorption process except in low organic matter systems (Hassett and Banwart, 1989). However, there are cases where clay has been found to be important in the sorption process. That estimates of the clay fraction of soils are used to determine application

rates of some pesticides and herbicides is testament to the role clay surface chemistry plays in the transport and immobilization of organics in soils (Zielke et al., 1989). In low organic matter soils, the hydrophobic sites associated with mineral surfaces are not covered by natural humus materials and therefore contribute to the sorption of nonpolar compounds (Stevenson, 1976; Walker and Crawford, 1968). Different clay minerals have different sorptive capacities (Bailey and White, 1964; Zierath et al., 1980). Montmorillonite (bentonite) has greater adsorption capacity than kaolinite because it has more surface area and a higher cation exchange capacity (C.E.C.).

Naphthalene is able to be degraded by a number of organisms. Different species of bacteria can metabolize naphthalene, including Pseudomonas spp. (Jeffrey et al., 1975; Stanier et al., 1966; Treccani et al., 1954; Kiyohara and Nagao, 1978), Nocardia sp. (Treccani et al., 1954), Aeromonas sp. (Kiyohara and Nagao, 1978), and Corynebacterium renale (Dua and Meera, 1981). Cyanobacteria, such as Oscillatoria sp., Coccochloris elabens, Agmenellum quadruplicatum (Cerniglia and Gibson, 1979; Cerniglia et al., 1980a), Microcoleus chthonoplastes, Anabaena sp., Nostoc sp., and Aphanocapsa sp. (Cerniglia et al., 1980b) have been found to partially degrade naphthalene. Green algae, such as Chlorella sp., Dunaliella tertiolecta, Chlamydomonas angulosa, and Ulva fasciata

metabolize naphthalene (Cerniglia et al., 1980b). A red alga, Porphyridium cruentum, and a brown alga, Petalonia fascia, also have been found to break down naphthalene (Cerniglia et al., 1980b). Diatoms, such as Cylindrotheca sp., Amphora sp. (Cerniglia et al., 1980b), Navicula sp., Nitschia sp., and Synedra sp. (Cerniglia et al., 1982), metabolize naphthalene. Fungi, such as Cunninghamella elegans (Cerniglia and Gibson, 1977), Aspergillus spp., Cunninghamella spp., Streptomyces spp., Rhizopus stolonifer, and Helicostylum periforme (Smith and Rosazza, 1974) also can metabolize naphthalene.

In bacterial aerobic metabolism of naphthalene (see Figure 1), both atoms of a molecule of oxygen are incorporated into one of the aromatic rings by a dioxygenase enzyme, naphthalene oxygenase, to form cis-1,2-dihydro-1,2-dihydroxynaphthalene (Davies and Evans, 1964; Jerina et al., 1971)(step A in Figure 1). This compound is then converted to 1,2-dihydroxynaphthalene (step B) by another enzyme, cis-naphthalenedihydrodiol dehydrogenase (Jeffrey et al., 1975). The 1,2-dihydroxynaphthalene can be degraded further (steps C,D, and E) to pyruvic acid and salicylaldehyde. Salicylaldehyde can be degraded (step F) to salicylic acid, which can be degraded (step G) to catechol (Davies and Evans, 1964; Rochkind et al., 1986). Ring fission of catechol, which is a dihydroxy aromatic compound, can occur by either ortho or meta cleavage (Davies and Evans, 1964;

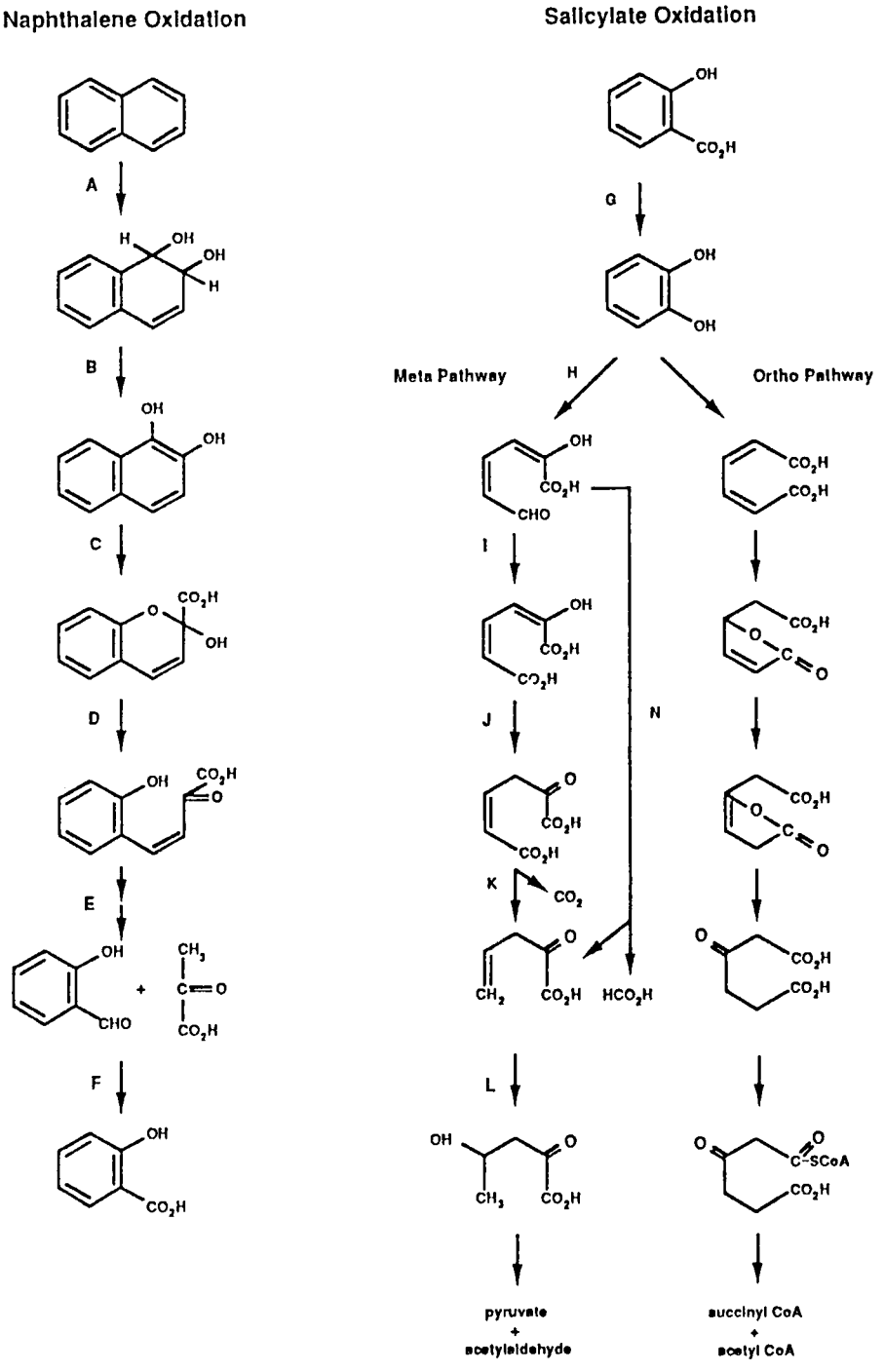

Figure 1. Bacterial aerobic metabolism of naphthalene. courtesy of Bruce Applegate of the Microbiology Department of The University of Tennessee, Knoxville.

Jeffrey et al., 1975; Kiyohara and Nagao, 1978; Lal and Saxena, 1982). The type of cleavage induced depends partly on the substrate and partly on the genetics of the particular bacterial species (Nakazawa and Yokota, 1973). Ortho cleavage of catechol and breakdown eventually leads to the products of acetyl-CoA and succinic acid (Ornston and Stanier, 1966; Stanier and Ornston, 1973). With meta cleavage of catechol, the endproducts are acetaldehyde and pyruvic acid (Bayly and Dagley, 1969; Dagley and Gibson, 1965; Sala-Trepat and Evans, 1971; Sala-Trepat et al., 1971; Sala-Trepat et al., 1972; Williams et al., 1971).

Naphthalene was found to be degraded under anaerobic conditions in the presence of excess nitrate (denitrification conditions) (Mihelcic and Luthy, 1988a and b). Apparently, under these conditions, nitrate is used as an electron acceptor instead of oxygen. They found that under anaerobic conditions without the addition of nitrate, no bacterial biodegradation of naphthalene occurred. However, as they stated, the soil used in the slurries was obtained from an upper soil horizon and may not be truly representative of the activity of microbial populations from anaerobic soils.

Fungal metabolism of aromatic structures is different from that of bacteria. Instead of a dioxygenase enzyme, which bacteria utilize, fungi initially attack the aromatic structure with a monooxygenase. With the monooxygenase, one

atom of molecular oxygen is incorporated into the benzene ring, while the other oxygen atom is converted to water. The resulting intermediate is an epoxide. The epoxide, in the case of naphthalene, can either be isomerized to form 1- or 2-naphthol, a phenol (Gibson, 1978), or with hydration, form a trans-1,2-dihydro-1,2-dihydroxynaphthalene, which then converts to trans-1,2-dihydroxynaphthalene (Gibson, 1976). Further breakdown of 1,2-dihydroxynaphthalene leads to 1,2-naphthoquinone and breakdown of 1-naphthol leads to 1,4-naphthoquinone (Cerniglia and Gibson, 1977; Cerniglia and Gibson, 1978; Cerniglia et al., 1978). Breakdown of aromatic compounds by mammals is similar to that by fungi and due to cytochrome P-450 (Cerniglia and Gibson, 1978).

A number of factors can influence the fate of PAHs in the environment. Such factors include the physicochemical properties of the PAH, such as molecular size, water solubility, lipophilicity, volatility, concentration, and the presence of various substituents, functional groups, and many types of chemical bonds. Environmental factors include temperature, pH, oxygen concentration, salinity, water, pressure, season, light intensity, sediment type, presence of cosubstrates (for cometabolism), and nutrient status. Microbial factors include the genetics, enzymes, enzyme accessibility to the substrate, types, population, and distribution of microorganisms present as well as their

predators and other microbiota and the bioavailability of the PAH (Cerniglia, 1984; Alexander, 1979).

The objectives of this thesis were: 1) to compare biodegradation of naphthalene in slurries of different soil types with and without a known naphthalene-degrader (strain 5R, apparently Pseudomonas fluorescens) (King et al., 1990). Soils having different physical properties were chosen so that their properties could be examined for their role in affecting biodegradation. 2) to compare biodegradation of naphthalene in water using a known naphthalene-degrader and soil microorganisms from each soil type, separately. 3) to determine if there are differences in biodegradation due to the type of contamination (wet or dry). 4) to enumerate the total heterotrophic microorganisms and organisms with the naphthalene-degrading genotype (possessing the NahA gene) in each soil type to compare with biodegradation and the presence of a NAH 7 (naphthalene-degrading) genotype and to determine if the frequency of the genotype changes over time with exposure to naphthalene, and 5) to determine if there is a correlation between desorption and biodegradation of naphthalene.

CHAPTER II

MATERIALS AND METHODS

Soils

Soils were chosen to give a wide variety of physical and chemical characteristics. After air-drying and being passed through # 100 mesh sieves, the soils were characterized by Jennifer Panter and Dr. John Graveel of the Plant and Soil Sciences Department of The University of Tennessee. Sieving through # 100 mesh sieves gave soil particles a maximum diameter of 149 μm. The particle size analysis was done by using the hydrometer method (Gee and Bauder, 1986). The % organic matter was calculated by obtaining the % organic carbon by combustion and multiplying it by a constant factor (1.724)(Buol et al., 1980). The cation exchange capacity (C.E.C.) was obtained using the method described by Rhodes, 1982. Soil characteristics are described in Table 1.

Table 1. Soils Used

Soil Name	Date Collected	% Organic Matter	% Sand	% Silt	% Clay	C.E.C.	Other Comments
Sequatchie	Feb/1990	1.09	59.0	25.5	15.5	7.9	A loam. Clay consists of kaolinite and hydroxy inter-layered vermiculite & clay-sized mica.
Etowah	March/1990	2.9	26.5	40.5	33.0	5.7	Clayish soil which is clayey, kaolinitic, or thermic typic hapludult. Clay consists of kaolinite & hydroxy inter-layered vermiculite.
"S. Carolina" (Pamlico)	July/1987	13.91	45.0	48.5	6.5	73.3	Sandy loam which is a salicious, dysic, firmic, terric metasaparist. Clay consists of kaolinite & hydroxy inter-layered vermiculite.
"Indiana"	June/1989	36.05	34.5	35.5	30.0	34.4	Sandy loam. Clay consists of montmorillonite. <u>Heavy metals analysis</u>[a] arsenic: 13.6 ppm (4 ppm[b]) barium: 47.2 ppm (0.2 ppm[b]) cadmium: <0.5 ppm (0.5 ppm[b]) chromium: 28 ppm (1 ppm[b]) lead: 129 ppm (3 ppm[b]) selenium: <6 ppm (6 ppm[b]) silver: <0.5 ppm (0.5 ppmb) mercury: <0.1 ppm (0.1 ppm[b])

[a]analysis performed by International Technology Corporation, Knoville, TN.
[b]detection limit for the heavy metal analysis.

Media and Reagents

Yeast extract peptone salicylate succinate (YEPSS) broth consisted of the following: one liter of distilled water, 0.2 g yeast extract, 1 g polypeptone peptone, 0.5 g sodium salicylate, 2.7 g sodium succinate, and 0.2 g ammonium nitrate.

Yeast extract peptone glucose (YEPG), 1/4 strength, broth consisted of the following: one liter of distilled water, 0.25 g dextrose, 0.5 g polypeptone, 0.05 g yeast extract, and 0.05 g ammonium nitrate, pH 7.0.

Radiolabeled naphthalene-1-^{14}C (8.0 mCi/mmol) was obtained from Sigma Chemical Co., St. Louis, MO.

Microbial Cultivation

To cultivate indigenous soil microorganisms, 0.1 g of each soil was inoculated into 50 ml of 1/4 strength YEPG broth and incubated at 25°C with shaking at 100 rpm for 48 hours. After incubation, each culture was allowed to settle for 2 minutes after which 25 ml of the broth was transferred into 50 ml of fresh 1/4 strength YEPG broth. The new culture was incubated at 25°C with shaking at 100 rpm for 24 hours. The culture was centrifuged in a Beckman model J2-21 centrifuge at 15,300 x g for 10 minutes. The supernatant

was decanted and the cell pellet was resuspended in 10 ml sterile water to make up the cell suspension used in the soil microorganisms aqueous mineralization assay. Microbial cell densities were determined as described below.

Strain 5R is a known naphthalene degrader and has tentatively been identified as Pseudomonas fluorescens (King et al., 1990). The following procedure was used to obtain the 5R cell suspension used in the mineralization assays: strain 5R from a slant of YEPG agar was used to inoculate 50 ml of YEPSS broth. It was incubated at 25°C with shaking at 100 rpm until turbid, 24 to 48 hours. One-half ml of this culture was inoculated into 50 ml of fresh YEPSS broth. When this culture became fairly turbid, about 24 hours, it was centrifuged at 15,300 x g for 10 minutes. The cell pellet was resuspended in 25 ml sterile distilled water. This cell suspension was used for the mineralization assay.

Enumeration of Cells

Strain 5R and soil microorganism cell suspensions were enumerated by serial dilutions in sterile distilled water and spread-plating on 1/4 strength YEPG agar. The agar plates were incubated at 27°C. Colonies were counted after 72 hours.

To enumerate microorganisms in soil, the soil was blended with 0.1% sodium pyrophosphate to remove the soil microorganisms from the soil particles (Balkwill and Ghiorse, 1985) and serially diluted. The dilutions were spread-plated on 1/4 strength YEPG agar. The agar plates were incubated at 27°C. Colonies, which included both bacteria and fungi, were allowed to grow for 14 days and counted.

Experimental Naphthalene Contamination of Soil

For "dry" contamination of soil, air-dried, # 100 mesh-sieved soil was placed in 110 x 285 mm borosilicate Wheaton tissue culture bottles, which had 4 equidistant lengthwise baffles. The baffles were to facilitate soil mixing. The soil was contaminated with a mixture of unlabeled (10 g/100 ml of naphthalene in acetone) and radiolabeled naphthalene-1-^{14}C (49,000 dpm/μl) to give approximately 150,000 dpm and 300 ppm of naphthalene per gram of soil. The bottles, which had teflon-lined lids, were sealed and covered with Parafilm. The tissue culture bottles were then covered with aluminum foil to prevent photodegradation, and placed onto a Wheaton Modular Cell Production Roller Apparatus (Model 3) at 80% motor speed in a 4°C coldroom for 20 days.

The wet contamination of soil was done in collaboration with Rebecca Pullen, of the Civil Engineering Dept. at the University of Tennessee, Knoxville. The contamination was done by the following procedure: An empty 25 ml Screw Cap Septum Vial (Pierce, Rockford, IL) with a teflon-lined septum and screw cap was weighed. One gram of air-dried, # 100 mesh-sieved soil was weighed out into each vial. One-hundred μl of mercuric chloride solution (49.955 g/l water) were added to those vials that were used as bacteriostatic controls. The proper amount of water and 25 μg/ml naphthalene solution needed to achieve approximately 50 ppm of naphthalene adsorbed onto each soil (known from previous adsorption experiments, in Table B-1 in Appendix B) was calculated. The distilled, deionized water was added to the vial. The unlabeled 25 μg/ml naphthalene solution was added to the vial. To each vial, naphthalene-1-^{14}C was added as a tracer. The vials were vortexed vigorously. The vials were shaken at 100 rpm in closed boxes at 4°C to help prevent photodegradation and biodegradation. After the naphthalene in solution had reached equilibrium, as determined by previous adsorption experiments, the vials were centrifuged in a swinging bucket rotor at 3000 rpm for 15 minutes. An aliquot was taken from the supernatant and placed in 10 mls of aqueous scintillation fluor (Packard Ultima Gold) and counted in a scintillation counter. The supernatant was then decanted into a waste jar. The vial with the wet soil

solids was then weighed to determine how much liquid was remaining in the vial. These vials were kept in a 4°C refrigerator until the following day when they were used for the wet contaminated soil mineralization assay. Control and experimental vials were set up in triplicate. Vials were set up at the same time, which contained CO_2 traps in them, to determine if any mineralization occurred during the contamination process.

Procedure for Dry Contaminated Soil Mineralization Experiments

One gram of dry soil (previously contaminated by the preceding procedure) was placed into a sterile 25 ml Screw Cap Septum Vial (Pierce, Rockford,IL). If strain 5R (presumably Pseudomonas fluorescens), was used, 100 μl of approximately 10^7 cells/ml were added to each vial. To those vials that were used for controls, mercuric chloride solution (Fletcher and Kaufman, 1980; Wolf et. al., 1989) was added to reach a final concentration of 830 ppm. Five ml of sterile distilled water were added. A sterile 8 ml S/P Screw Cap Vial (Baxter Healthcare Co., McGaw Park, IL) was inserted to be used as a CO_2 trap. One-half ml of 0.8 N NaOH (freshly made) was added to the CO_2 trap vial. The vials were sealed with teflon-lined septums (Pierce) and screw caps. The vials were incubated in closed boxes with

shaking (100 rpm) at 27°C. Control and experimental vials were set up in triplicate. At different time points, the reaction was stopped by injecting 0.5 ml of 2 N H_2SO_4 through the septum and the vials were shaken for at least 1 hour. The extraction procedure, described below, was followed.

Mineralization Assay of Naphthalene "Wet" Contaminated Soil

The following procedure was used for the mineralization of naphthalene "wet" contaminated soil: Five ml of sterile, distilled water were added to each vial for the Indiana, Sequatchie, and unbuffered South Carolina soil experiments. Five ml of 5 mM K_2HPO_4 was added as a buffer to all Etowah soil vials. Five ml of 50 mM K_2HPO_4 was added as a buffer to some South Carolina soil vials. An 8 ml vial was added to each vial and 1/2 ml of 0.8 N NaOH (freshly made) was put into this vial to trap CO_2. One hundred μl of 5R cell suspension was added to each vial. The vials were sealed and incubated at 27°C in closed boxes with shaking at 100 rpm. At different time points, the reaction was stopped by injecting 0.5 ml of 2 N H_2SO_4 through the septum and the vials were shaken for at least 1 hour. The extraction procedure, described below, was followed.

Procedure for Aqueous Mineralization Experiments

The following procedure was followed for the aqueous mineralization experiments (naphthalene mineralization by strain 5R or soil microorganisms): Five ml of distilled water was put into a 25 ml Screw Cap Septum Vial (Pierce, Rockford, IL). A Baxter S/P Micro Samp Vial (8 ml volume) was inserted. The vials were sealed loosely with teflon-lined septums (Pierce) and screw caps. They were autoclaved and allowed to cool. To each vial, 100 μl of cell suspension (strain 5R or soil microorganisms which had been cultured by the previous procedure) was added. The cell suspension was enumerated as described earlier. To those vials that were used for controls, mercuric chloride solution was added to each vial to reach a final concentration of 1000 ppm. Each vial received approximately 100,000 dpm per vial and 250 μg of naphthalene to give a final concentration of 50 ppm in each vial. One-half ml of 0.8 N NaOH (freshly made) was put in all of the 8 ml inner vials. The 25 ml vials were sealed with teflon-lined septums (Pierce) and screw caps. The vials were incubated at 27°C in closed boxes with shaking at 100 rpm. Control and experimental vials were set up in triplicate. At different time points, the reaction was stopped by injecting 0.5 ml of 2 N H_2SO_4 through the septum and the vials were

shaken for at least 1 hour. The extraction procedure, described below, was followed.

Extraction Procedure

After mineralization assays were stopped by the injection of acid, the following procedure was performed: Five ml of hexane:isopropanol (4:1) were injected into each vial and they were shaken at 100 rpm for at least 0.5 hour. One-half ml of distilled water was added to a scintillation vial, the NaOH from the CO_2 trap was added to it, it was swirled, and then 10 ml of Beckman Ready Safe scintillation fluor was added to it. After 24 hours, this vial was counted in a Beckman scintillation counter (model LS 3801, Beckman, Fullerton, CA). The CO_2 trap vial was removed and discarded into radioactive waste. The mineralization vial was resealed, vortexed briefly, and shaken for at least one hour. The soil was allowed to settle and the upper hexane phase was transferred to another vial. Another 5 ml of hexane:isopropanol (4:1) were added to the mineralization vial. The vial was vortexed, shaken for at least one hour, and then the upper hexane phase was transferred to the vial with the first hexane phase. One ml of the combined hexane extract was added to 10 ml of Dupont Econofluor. This was counted in the scintillation counter. The soil was allowed

to settle and 1 ml of the polar phase was added to 10 ml of Beckman Ready Safe scintillation fluor. This was counted in the scintillation counter after 24 hours. If the following steps could not be completed immediately, the soil slurries were frozen at -85°C. The soil was allowed to settle and the polar phase removed by vacuum. The soil was allowed to air dry in a fume hood. The soil was removed from each mineralization vial and placed into porcelain boats for oxidation. The sample was combusted in a Harvey Biological Material Oxidizer (model OX-500, R.J. Harvey Instrument Corporation, Hillsdale, N.J.) for 4 minutes. The remaining residue was discarded into radioactive waste. The radioactivity in the scintillation fluor obtained from the Biological Material Oxidizer was then counted in the scintillation counter.

Adsorption and Desorption

Rebecca Pullen from the Department of Civil Engineering obtained the adsorption and desorption data for each soil used. The methods and data are in Appendix B.

Colony Hybridizations

Colony hybridizations were done on air-dried, #100 mesh-sieved soil before "dry" contamination of the soil, after "dry" contamination and mixing of the soil, and after the mineralization experiments. Colonies from soil microorganism enumerations, which included both bacteria and fungi, were allowed to grow for 14 days and counted. After incubation of the agar plates, the following procedure for colony hybridizations (Sayler et. al., 1985; Sayler and Layton, 1990) was performed:

A Biotrans nylon membrane was placed on the agar surface for 5 minutes. Two ml of Denaturing Solution (1.5 M NaCl, 0.5 M NaOH) were placed on plastic wrap and the membranes (colony-side up) were set on the solution for 5 minutes. Two ml of Neutralizing Solution (3 M Sodium Acetate, pH 5.5) were placed on plastic wrap and the membranes (colony-side up) were set on the solution for 5 minutes. The membranes were dried on blotter paper for 30 minutes. The membranes were wrapped in aluminum foil and baked at 80° C for 1 hour. The filters were stored at room temperature until needed.

The gene probe (a single-stranded probe made from the Iron Sulfur Protein of NahA, Naphthalene dioxygenase) for the colony hybridizations was made by the following procedure: The first amplification used PCR (Polymerase

Chain Reaction) (Sambrook et al., 1989) to amplify double-stranded ISP_{α} (Iron Sulfur Protein). Into an Eppendorf tube, the following was pipetted:

2 μl dATP

2 μl dTTP

2 μl dCTP

2 μl dGTP

10 μl Reaction buffer 10x

1 μl Taq polymerase

2.5 μl of pUC primer # 1201

2.5 μl of pUC primer # 1212

1 μl of pUC - ISP (clone obtained from Burt Ensley, Envirogen Co., NJ)

75 μl of sterile, distilled water

The pUC primers were obtained from BRL, Maryland. The other reagents were obtained from Perkin Elmer.

The above reaction mixture was incubated at 94° C for 1 minute to melt the DNA, then incubated at 37° C for 2 minutes to anneal the DNA, and finally incubated at 72° C for 3 minutes to allow for the extension of strands. These incubations were repeated 38 times. A DNA Thermal Cycler (from Perkin Elmer Cetus Co.) carried out these incubations.

A second amplification was performed to generate single-stranded probe, which was done by the following procedure:

Into an Eppendorf tube, the following was pipetted:

10 μl of template from the first amplification

2 μl dATP

2 μl dTTP

2 μl dGTP

10 μl ^{32}P-dCTP

10 μl Reaction buffer 10x

1 μl Taq polymerase

2.5 μl primer # 3 (a NahA primer)

60.5 μl sterile, distilled water

This reaction mixture was incubated the same as for the first amplification. After incubation, the whole volume from the second amplification was put through a 110 x 285 mm NucTrap push column (Stratagene Co.). To whatever volume (approximately 70 μl) that came out of the push column, 930 μl of TE buffer (1 mM Tris and 1 mM EDTA, pH 7.5) was added. Five μl of this diluted solution was put into Beckman Ready Safe scintillation fluor. The counts were multiplied by 200 to get the total number of counts. The probe was put in the freezer until use.

Into each plastic bag, six filters and 100 μl of the gene probe were added and allowed to hybridize overnight at 65° C. The filters were removed from the bags and washed 4 times for 15 minutes at 65° C in 50 ml of high stringency wash (1.17 g NaCl, 4.84 g Tris, 0.74 g EDTA, 10 g SDS, 2 l distilled water, pH 7-8). The filters were placed on blotter paper and allowed to dry at room temperature. The

filters were exposed to Kodak Diagnostic X-ray film (X-Omat AR, "gray", or X-Omat RP XRP-5 "blue") between 2 light intensifying screens (Dupont), placed between 2 aluminum plates and placed in an 80° C freezer overnight or longer. The X-ray film was developed with an automatic film developer (AFP Imaging Corp., Elmsford, N.Y.).

Correlation Analysis

Correlation analysis has been done between variables for the following mineralization experiments: dry contaminated soil slurry with and without strain 5R, and wet contaminated soil slurry with strain 5R. The variables used in the correlation analysis included the following: % sand, % silt, % clay, % organic matter, cation exchange capacity, % naphthalene desorbed, initial number of naphthalene degraders, final number of naphthalene degraders, the change in the number of degraders from the beginning to the end of the mineralization experiment, initial number of total heterotrophs, the final number of total heterotrophs, the change in the number of total heterotrophs during the mineralization experiment, the adsorption isotherm slope, the desorption isotherm slope, K_{ads} (the adsorption capacity, which is a measure of the number of adsorption sites available), K_{om} of adsorption (the number of adsorption

sites on organic matter), K_{des} (the desorption capacity, which is a measure of reversible sites), K_{om} of desorption (the number of reversible sites on organic matter), and maximum percentage of mineralization. Not all of these variables were available for each of these experiments. When available, each of these variables were correlated with the other variables.

Analysis for Metals in Soil

The Indiana soil was found to have a population of mercury-resistant organisms. Therefore, it was analyzed for heavy metals by International Technology Corporation, Knoxville, TN. The cold vapor atomic absorption was used to check for the presence of mercury. An Inductively Coupled Plasma (ICP) Spectroscopy scan was used to check for the presence of arsenic, barium, cadmium, chromium, lead, selenium, and silver.

CHAPTER III

RESULTS

Colony Hybridizations

Colony hybridizations were performed to determine the frequency of the NahA genotype in the soil microorganisms, and to see if it changed during the "dry" contamination procedure and mineralization experiment. The results are shown in Table 2.

The total population counts (which include both bacterial and fungal heterotrophs) for all soils decreased (approximately one order of magnitude) with the dry contamination procedure. This could be due to the effects of handling or storage (Castle and Hulm, 1989). Population counts for all soils except S. Carolina, which had the longest storage time, increased (approximately one to two orders of magnitude) from before contamination to after the mineralization assay. This could be due to the addition of water (Hickman and Novak, 1989) and/or the naphthalene, a possible carbon and energy source.

The range in the last column of Table 2 (results for colony hybridization) is due to the intensity of the spots

Table 2. Colony Hybridizations

Soil	Time Probed	Total Population (cfu/g soil)	Colony Hybridization (# NahA$^+$/g soil)
Sequatchie	before contamination	1.6×10^7 ($\pm 3.3 \times 10^6$)[a]	0.0 ($\pm$0.0)[a] - 3.3×10^4 ($\pm 4.7 \times 10^4$)[a]
Sequatchie	after contamination	4.8×10^6 ($\pm 7.6 \times 10^5$)	0.0 ($\pm$0.0) - 3.3×10^4 ($\pm 4.7 \times 10^4$)
Sequatchie	after mineralization	4.7×10^7 ($\pm 1.1 \times 10^7$)	3.8×10^6 ($\pm 3.1 \times 10^6$) - 1.5×10^7 ($\pm 1.4 \times 10^7$)
Etowah	before contamination	1.8×10^7 ($\pm 1.4 \times 10^6$)	0.0 ($\pm$0.0) - 0.0 ($\pm$0.0)
Etowah	after contamination	7.4×10^6 ($\pm 6.2 \times 105$)	2.5×10^4 ($\pm 4.3 \times 10^4$) - 3.3×10^4 ($\pm 4.7 \times 10^4$)
Etowah	after mineralization	2.1×10^8 ($\pm 2.4 \times 10^7$)	5.3×10^5 ($\pm 2.9 \times 10^5$) - 6.3×10^5 ($\pm 2.7 \times 10^5$)
"S. Carolina" (Pamlico)	before contamination	7.1×10^6 ($\pm 1.5 \times 10^6$)	7.5×10^4 ($\pm 8.3 \times 10^4$) - 3.0×10^5 ($\pm 3.3 \times 10^5$)
"S. Carolina" (Pamlico)	after contamination	3.3×10^6 ($\pm 9.4 \times 10^5$)	3.3×10^4 ($\pm 4.7 \times 10^4$) - 1.0×10^5 ($\pm 1.4 \times 10^5$)
"S. Carolina" (Pamlico)	after mineralization	5.3×10^6 ($\pm 1.5 \times 10^6$)	1.8×10^5 ($\pm 1.1 \times 10^5$) - 3.3×10^5 ($\pm 2.0 \times 10^5$)
Indiana	before contamination	1.6×10^7 ($\pm 1.3 \times 10^6$)	0.0 ($\pm$0.0) - 3.3×10^4 ($\pm 4.7 \times 10^4$)
Indiana	after contamination	2.9×10^6 ($\pm 5.2 \times 10^5$)	0.0 ($\pm$0.0) - 3.3×10^4 ($\pm 4.7 \times 10^4$)
Indiana	after mineralization	9.1×10^7 ($\pm 2.0 \times 10^7$)	7.6×10^6 ($\pm 6.0 \times 10^6$) - 3.9×10^7 ($\pm 2.1 \times 10^7$)

[a]the standard deviation.

probing positive. The lower numbers include only the very dark spots. The higher numbers include both the very dark spots and lighter colored spots. The control filter showed very dark spots for the positive control, and nothing for the negative control. The degree of intensity may be due to the cell lysis efficiency or the degree of homology with the gene probe.

Generally, the number of NahA-probing colonies did not increase greatly in any soil from before dry contamination to after dry contamination. This may be due to lack of exposure of the naphthalene to all of the soil microorganisms because of mass transfer difficulties from lack of water. Also, the water activity (a_w) of the environment is important. A certain water activity value is required for microorganisms for enzymatic activity, metabolism, and growth. Some organisms require a higher water activity than others (Rose, 1981). All the soils used were air-dried before storage. The S. Carolina soil had the longest storage time. Also, the S. Carolina soil had the lowest clay content of the soils used. Clay is important in the retention of water in the soil. It may be that because of these two factors that the S. Carolina soil naphthalene degraders had lost much of their metabolic activity and were not able to reproduce.

For all the soils, there was an increase in the number of NahA-probing colonies from before contamination to after

mineralization. Of all the soils, the South Carolina soil had the smallest increase in the frequency of the NahA genotype. The increase for this soil was slight (less than one order of magnitude). This may be due to the long storage time (approximately 35 months) of the South Carolina soil before the experiment.

Naphthalene Mineralization by Strain 5R in Water

In the experiment for naphthalene mineralization by strain 5R, 7.7×10^7 cells were added to each vial. The initial concentration of naphthalene in each vial was 50 μg/ml (50 ppm). During the experiment, there was a net increase of radioactivity in the CO_2 traps, indicating mineralization. Upon termination, 29.1% of the naphthalene had been mineralized. There was a linear rate of naphthalene mineralization for 12 hours (Figure 2), at which time the experiment was terminated. The rate of naphthalene mineralization was determined to be 1.16 ppm/hour for this time period. In the experimental vials, the radioactive counts in the aqueous phase increased (from 1.2% to 7.3%) and decreased in the hexane phase (from 104.2% to 69.5%) from the beginning to the end of the experiment. In the controls, the radioactivity remained fairly constant in both the aqueous (0.5 - 0.8%) and hexane phases (90.5 - 104.7%).

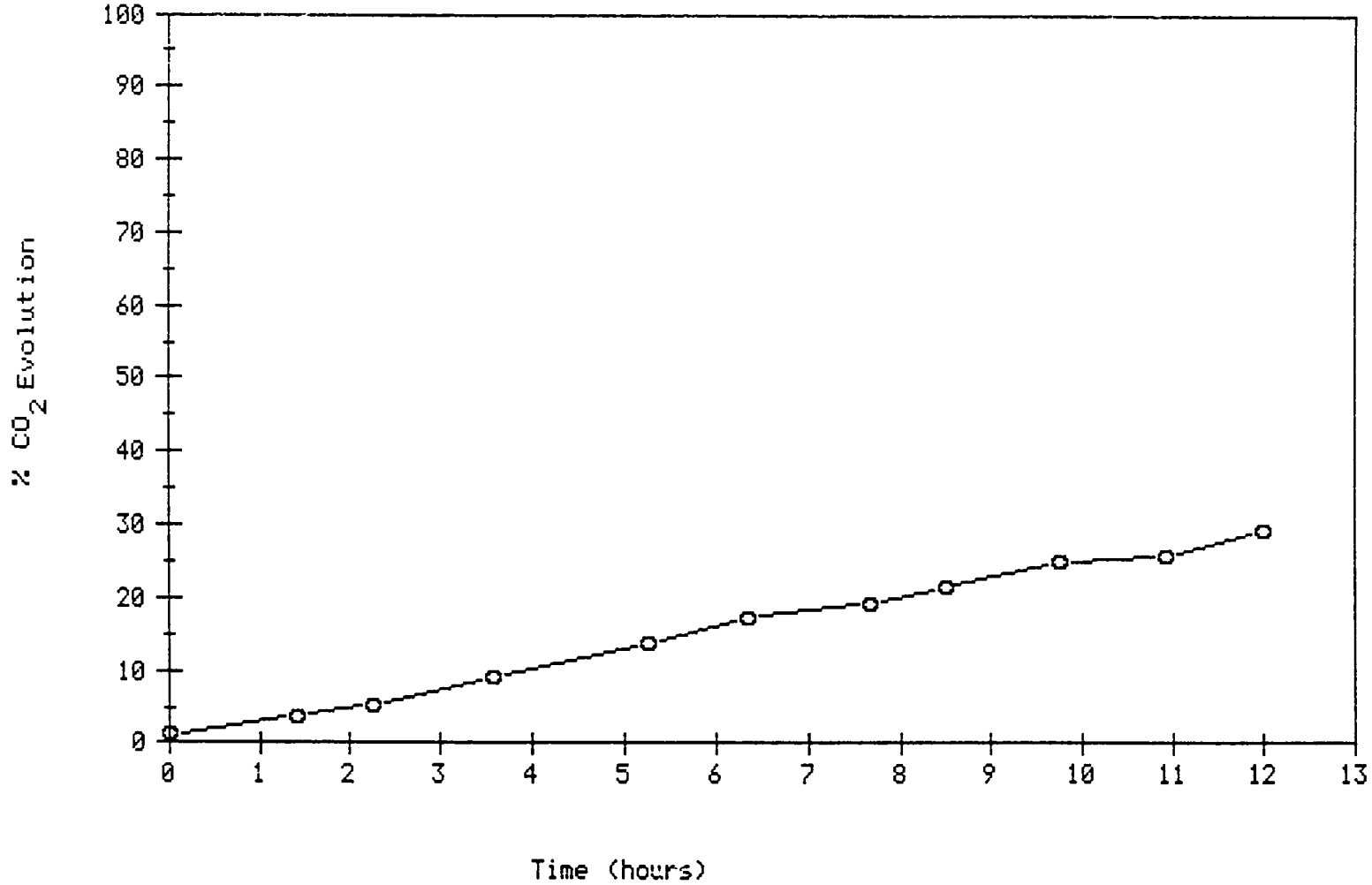

Figure 2. Naphthalene Mineralization by Strain 5R in Water

These results are expected during biodegradation and transformation. The mass balance recovery for the experimental vials ranged from 104% to 130%. Table A-1 in Appendix A contains the data spreadsheet.

Naphthalene Mineralization by Soil Microorganisms in Water

In the experiment for naphthalene mineralization by soil microorganisms in water, 1.3×10^8 cells were added to the Sequatchie vials, 2.6×10^7 cells were added to the Etowah vials, 4.7×10^7 cells were added to the South Carolina vials, and 2.4×10^7 cells were added to the Indiana vials. None of these aqueous assays showed any significant mineralization (less than 1.0%) even after 90 days. See Figure 3. Table A-2 in Appendix A contains the data spreadsheet. In the Etowah, South Carolina, and Indiana soil slurries, there were decreases of radioactivity in the solvent phases (32.3%, 8.1%, and 18.3%, respectively) and slight increases in the aqueous phases (0.05%, 0.14%, and 0.08%, respectively), indicating some transformation may have occurred. No apparent transformation occurred in the Sequatchie soil slurries. It is possible that the media used to cultivate the soil microorganisms (YEPG broth, 1/4 strength) allowed non-naphthalene degraders to outcompete

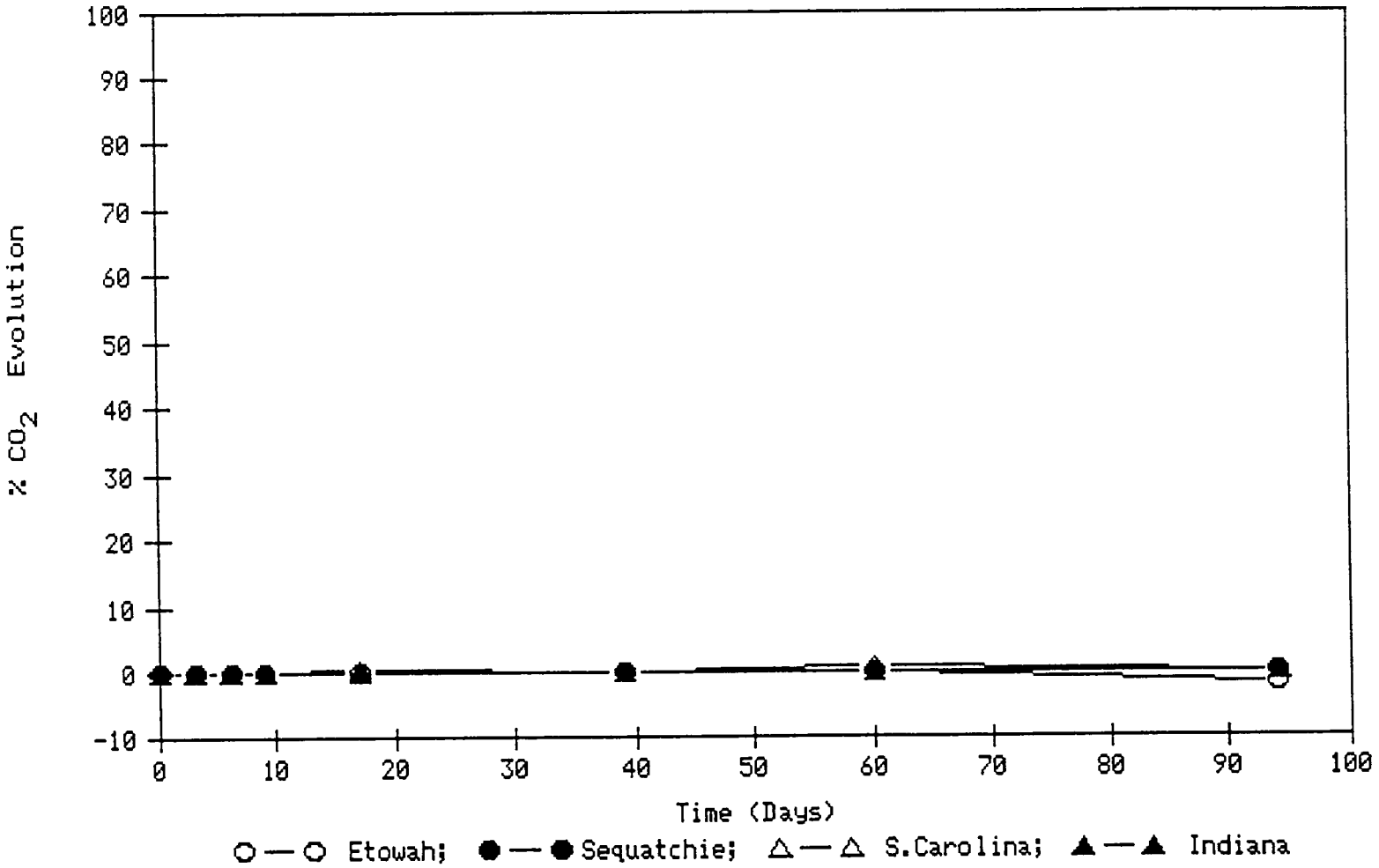

Figure 3. Naphthalene Mineralization by Soil Microorganisms in Water

the degraders or was not the appropriate media for culturing the naphthalene degraders. It is also possible that these naphthalene degraders have higher metabolic rates on solid substrates (van Loosdrecht et al., 1990; ZoBell, 1943) than in aqueous media.

Naphthalene Mineralization by Soil Microorganisms in "Dry" Contaminated Soil Slurry

Soil microbial population densities at the beginning of these experiments (before contamination) are given in Table 2 in the column for Total Population. Table A-3 in Appendix A contains the data spreadsheet. The percentage of naphthalene mineralized for the four soils ranged from 53.6 to 75.3% at the termination of the experiment (see Table 3).

The reason for the time range in the last column is that the incubator had mechanical problems and all of the vials for each soil type were frozen for up to 22 days during the incubation period. After the vials were found frozen, they were allowed to thaw and then were reincubated. Only the Indiana soil slurry was able to mineralize a significant amount (66.3%) by the 3 day timepoint. The other soil slurries mineralized less than 1% in 3 days. All of the soil slurries by 38 to 60 days were able to mineralize naphthalene (ranging from 53.6% to 75.3%). As

Table 3. Naphthalene Mineralization by Soil Microorganisms in Dry Contaminated Soil Slurry.

Soil	% Mineralization at 0 days	% Mineralization at 3 days	% Mineralization at 38 - 60 days
Etowah	0.0 (±0.0[a])	-0.2 (±0.3[a])	53.6 (±1.3[a])
Sequatchie	0.0 (±0.0)	0.2 (±0.2)	70.8 (±0.6)
S. Carolina	0.0 (±0.0)	0.0 (±0.0)	75.3 (±1.2)
Indiana	0.1 (±0.1)	66.3 (±2.7)	73.5[b] (±3.3)

[a] standard deviation.
[b] The control vial was not subtracted from the experimental vial because it appeared that biodegradation had occurred in the control vials. Mineralization percentages were higher in the control vials than in the experimental vials.

radioactivity increased in the CO_2 traps in the experimental vials, radioactivity in the solvent phase decreased (from greater than 95% to less than 15%) and increased slightly (up to 3%) in the aqueous phase. In the different soil slurries, there were increases (increases of 3.7% to 9.5%) in the radioactivity associated with the soils. This may be due to partitioning onto biological membranes and settling of the cells with the soil particles during extraction. There was a difference in the amount mineralized at 38-60 days by each of the different soil slurries.

Since no time points were taken after the 38-60 days timepoint, it is not known whether or not the maximum amounts of mineralization had occurred by this time. It is possible that the maximum percentage of mineralization had

not been reached at the termination of this experiment. For correlation analysis, though, it was assumed that it had.

The number of organisms possessing the NahA genotype increased (Table 2) from before the "dry" soil contamination (exposure) to after the completion of the mineralization experiment. This was discussed earlier (see Colony Hybridizations, this chapter). However, with correlation analysis (Figure 4) the maximum percentage of mineralization was not correlated highly with the initial number of degraders (r=0.54), the final number of degraders (r=0.44), or the change in the number of degraders (r=0.43) throughout the experiment. Other researchers have also noted increases in the numbers of degraders of a specific substrate (para-nitrophenol) upon exposure and adaptation of the substrate (Spain et al., 1984; Aelion et al., 1987; Wiggins et al., 1987).

The maximum percentage of mineralization was correlated inversely (Figure 4), but highly, with the final total population (r=-0.93) and the change in the total population (r=-0.93) during the experiment. There were no high correlations ($r>|0.80|$) for other variables (% sand, % silt, % clay, % organic matter, % desorbed, the cation exchange capacity, the number of degraders, or the initial heterotrophic population) with the maximum percentage of mineralization. There were some high ($r>|0.80|$) correlations between other variables: the number of initial

	Total % Miner.	% Sand	% Silt	% Clay	% Organic Matter	% Desorbed	% C.E.C.	# Initial Degraders	# Final Degraders	Change in # Degraders
Total % Miner.	* 1.00000	0.60717	-0.04273	-0.65201	0.50775	-0.54666	0.65414	0.54080	0.43819	0.43259
	* 0.00000	0.39280	0.95730	0.34800	0.49230	0.45330	0.34590	0.45920	0.56180	0.56740
% Sand	0.60717	1.00000	-0.49906	-0.74249	-0.31675	0.31898	0.07448	0.24531	-0.00660	-0.00847
	0.39280	0.00000	0.50090	0.25750	0.68320	0.68100	0.92550	0.75470	0.99340	0.99150
% Silt	-0.04273	-0.49906	1.00000	-0.20993	0.16487	-0.60362	0.72579	0.70872	-0.48094	-0.48483
	0.95730	0.50090	0.00000	0.79010	0.83510	0.39640	0.27420	0.29130	0.51910	0.51520
% Clay	-0.65201	-0.74249	-0.20993	1.00000	0.22993	0.10671	-0.64507	-0.82462	0.37921	0.38433
	0.34800	0.25750	0.79010	0.00000	0.77010	0.89330	0.35490	0.17540	0.62080	0.61570
% Organic	0.50775	-0.31675	0.16487	0.22993	1.00000	-0.86100	0.42746	0.06362	0.78426	0.78121
	0.49230	0.68320	0.83510	0.77010	0.00000	0.13900	0.57250	0.93640	0.21570	0.21880
% Desorbed	-0.54666	0.31898	-0.60362	0.10671	-0.86100	1.00000	-0.80101	-0.52609	-0.37646	-0.37117
	0.45330	0.68100	0.39640	0.89330	0.13900	0.00000	0.19900	0.47390	0.62350	0.62880
C.E.C.	0.65414	0.07448	0.72579	-0.64507	0.42746	-0.80101	1.00000	0.92878	-0.10414	-0.11097
	0.34590	0.92550	0.27420	0.35490	0.57250	0.19900	0.00000	0.07120	0.89590	0.88900
# Initial Degraders	0.54080	0.24531	0.70872	-0.82462	0.06362	-0.52609	0.92878	1.00000	-0.42058	-0.42692
	0.45920	0.75470	0.29130	0.17540	0.93640	0.47390	0.07120	0.00000	0.57940	0.57310
# Final Degraders	0.43819	-0.00660	-0.48094	0.37921	0.78426	-0.37646	-0.10414	-0.42058	1.00000	0.99998
	0.56180	0.99340	0.51910	0.62080	0.21570	0.62350	0.89590	0.57940	0.00000	0.00010
Change # Degraders	0.43259	-0.00847	-0.48483	0.38433	0.78121	-0.37117	-0.11097	-0.42692	0.99998	1.00000
	0.56740	0.99150	0.51520	0.61570	0.21880	0.62880	0.88900	0.57310	0.00010	0.00000
Initial Total Pop.	-0.6167									
	0.3833									
Final Total Pop.	-0.92887									
	0.07113									
Change in Tot. Pop.	-0.93337									
	0.06663									

* Top number = Correlation Coefficient, r. Bottom number = probability.

Figure 4. Correlation Analysis for Naphthalene Mineralization in Dry Contaminated Soil Slurry

degraders with the cation exchange capacity, and the number of final degraders with the change in the number of degraders (r=0.99). There were also some high inverse correlations: the number of initial degraders with the % clay (r=-0.82), the % desorbed with the % organic matter (r=-0.86), and the % desorbed with the cation exchange capacity (r=-0.80).

Naphthalene Mineralization by Strain 5R and Soil Microorganisms in "Dry" Contaminated Soil Slurry

In this experiment, 2.0 X 10^6 cells of strain 5R were added to each vial. Population counts for the soils at the beginning of the experiment can be seen in Table 2. The results of the experiment are shown in Figure 5. Table A-4 in Appendix A contains the data spreadsheet. With time, there were increases of radioactivity in the experimental CO_2 traps for each slurry. All of the different soil slurries with strain 5R were able to mineralize naphthalene (67% to 82%). The fastest rates of mineralization for the Etowah, Sequatchie, Indiana, and South Carolina soil slurries were determined to be 6.0, 4.2, 3.3, and 2.4 ppm/hour, respectively. During the experiment, there were corresponding decreases of radioactivity in the solvent phases (from greater than 95% to less than 5%) and initial increases in the aqueous phases (increases of 3.9% to 7.8%),

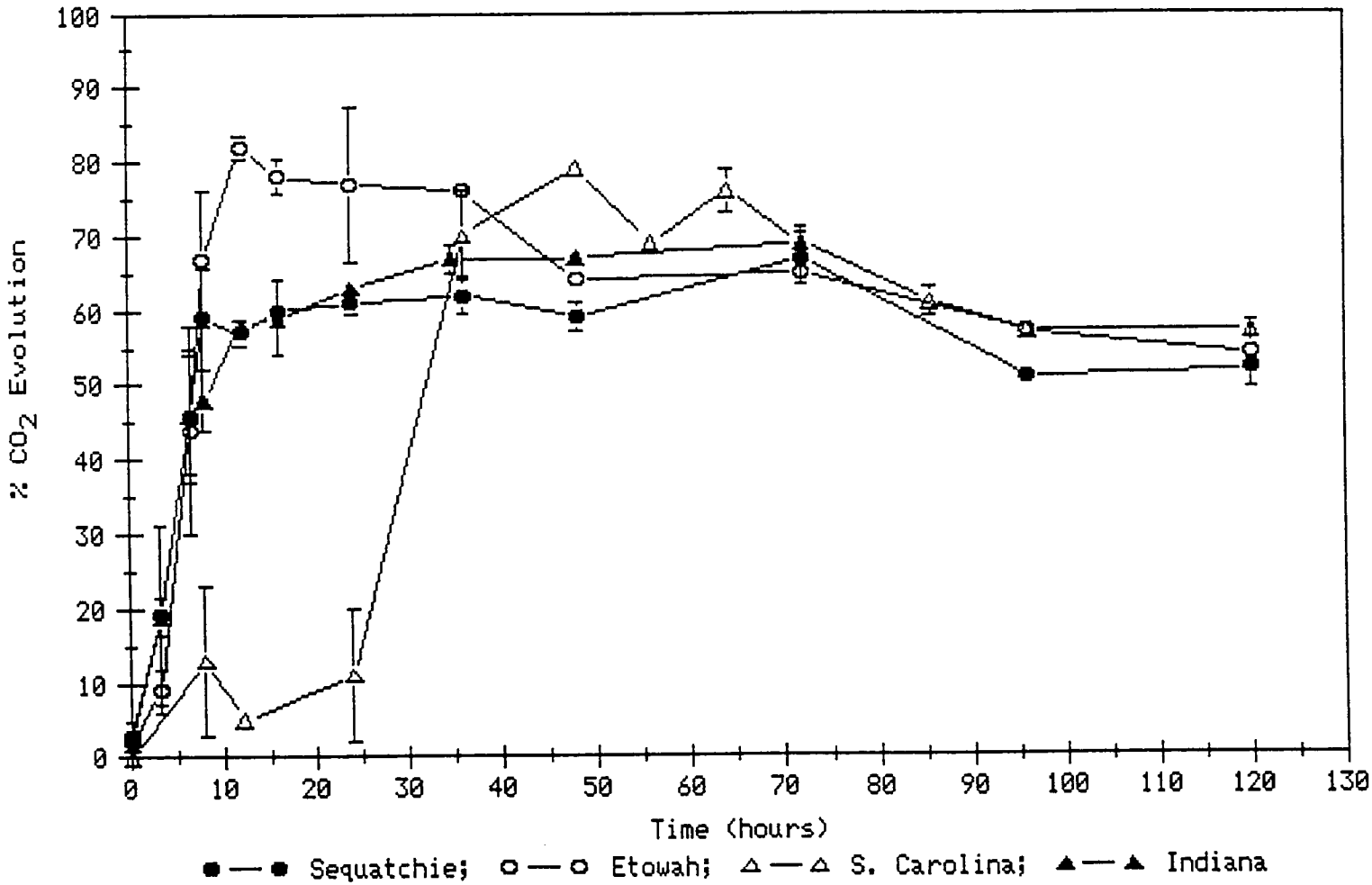

Figure 5. Naphthalene Mineralization by Strain 5R and Soil Microorganisms in Dry Contaminated Soil Slurry

indicating a change from the nonpolar parent compound (naphthalene) and nonpolar metabolites to polar metabolites. With time, the radioactivity associated with the soil increased (increases of 3.7% to 5.8%) in the experimental vials, probably due to bioconcentration.

Unlike the other soil slurries, the S. Carolina soil slurry had a lag time (about 24 hours) before reaching the same amount of mineralization. This may be due to the South Carolina soil having had the longest storage time (Castle and Hulm, 1989).

All of the soil slurries had approximately the same maximum percentage of mineralization (Figure 5). Therefore, differences between the soils were not accountable for the level of the maximum percentage of mineralization. It is likely that some other variable (i.e. oxygen concentration) limited the amount of mineralization.

Because it appeared that the maximum percentage of mineralization had been limited by something other than soil characteristics, correlation analyses would not be appropriate between the "maximum" percentage of mineralization and soil characteristics. However, with correlation analysis (Figure 6), there were some high, but inverse, correlations between other variables. The % desorbed had a high inverse correlation with the % organic matter ($r=-0.86$) and with the cation exchange capacity ($r=-0.80$).

	% Sand	% Silt	% Clay	% Organic Matter	% Desorbed	C.E.C.	Max. % Miner.
% Sand	* 1.00000	-0.49906	-0.74249	-0.31675	0.31898	0.07448	-0.61118
	* 0.00000	0.50090	0.25750	0.68320	0.68100	0.92550	0.38880
% Silt	-0.49906	1.00000	-0.20993	0.16487	-0.60362	0.72579	0.81332
	0.50090	0.00000	0.79010	0.83510	0.39640	0.27420	0.18670
% Clay	-0.74249	-0.20993	1.00000	0.22993	0.10671	-0.64507	0.06087
	0.25750	0.79010	0.00000	0.77010	0.89330	0.35490	0.93910
% Organic Matter	-0.31675	0.16487	0.22993	1.00000	-0.86100	0.42746	-0.30583
	0.68320	0.83510	0.77010	0.00000	0.13900	0.57250	0.69420
% Desorbed	0.31898	-0.60362	0.10671	-0.86100	1.00000	-0.80101	-0.07226
	0.68100	0.39640	0.89330	0.13900	0.00000	0.19900	0.92770
C.E.C.	0.07448	0.72579	-0.64507	0.42746	-0.80101	1.00000	0.22209
	0.92550	0.27420	0.35490	0.57250	0.19900	0.00000	0.77790
Max. % Miner.	-0.61118	0.81332	0.06087	-0.30583	-0.07226	0.22209	1.00000
	0.38880	0.18670	0.93910	0.69420	0.92770	0.77790	0.00000

* Top number = Correlation Coefficient, r. Bottom number = Probability.

Figure 6. Correlation Analysis for Naphthalene Mineralization in Dry Contaminated Soil Slurry + 5R

In the control Indiana soil slurry (Table A-4), there was also an indication of mineralization. There was a sudden increase in radioactivity in the CO_2 traps at the 96 hours time point. In the control vials, 1.7%, 22.1%, and 50.9% of the naphthalene was mineralized at 72, 96, and 120 hours, respectively. From the 72 hours to the 120 hours timepoint, there was a decrease in the radioactivity in the solvent phase (down to 5.1%) and an increase in the aqueous phase (from 1.0% to 7.1%). There was also an increase in the radioactivity associated with the soil (from 2.4% to 10.3%). Apparently, the Indiana soil microorganisms required a lag time (4 days) to adapt resistance to mercuric chloride and then were also able to biodegrade naphthalene.

Naphthalene Mineralization by Strain 5R and Soil Microorganisms in "Wet" Contaminated Soil Slurry

In this experiment, 2.4×10^8 cells of strain 5R were added to each vial in this assay. Soil microorganism enumerations were not done for this experiment. The South Carolina soil slurry was set up with and without buffer. Without the buffer, the slurry pH was 5.19. With the buffer (50 mM K_2HPO_4), the slurry pH was 6.8. The Etowah soil slurries were set up only with buffer (5 mM K_2HPO_4) to adjust the pH to 7.1. The results are shown in Figure 7. Table A-5 in Appendix A contains the data spreadsheet. With

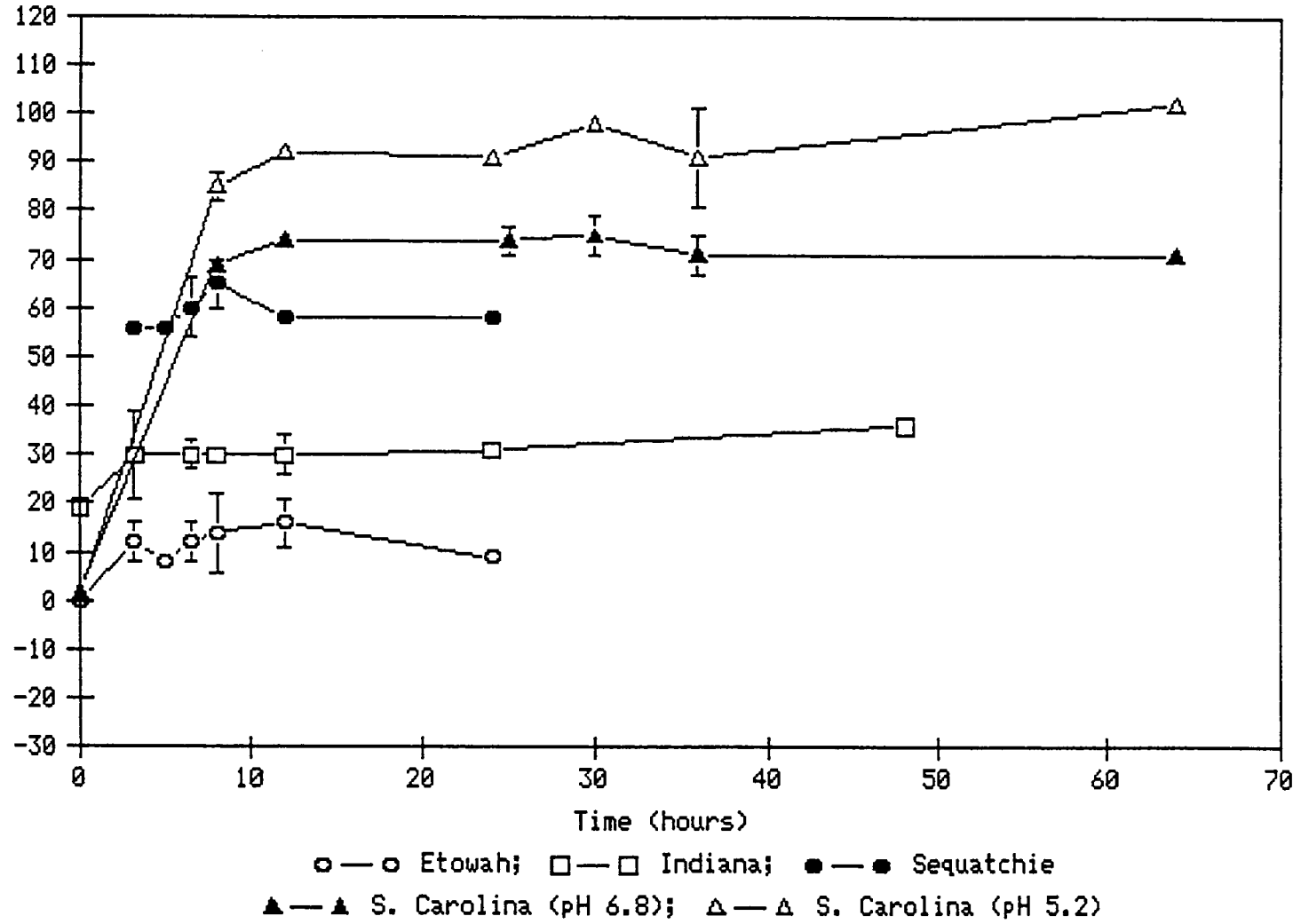

Figure 7. Naphthalene Mineralization by Strain 5R and Soil Microorganisms in Wet Contaminated Soil Slurry

the Etowah, South Carolina with buffer, South Carolina without Buffer, and Indiana soil slurries, there were increases in the radioactivity in the CO_2 traps, with decreases (decreases ranging from 17% to 82%) in the solvent phases and initial increases in the aqueous phases (increases of 1% to 4%) with time.

The first time point (time 0) was lost for the Sequatchie experimental vials. Most mineralization occurred (56% mineralized) before the next time point (3.17 hours). The maximum percentage of mineralization reached in the experiment was 65%. From 3.17 hours, there was a decrease in radioactivity in the solvent phase (10% to 4%) and in the aqueous phase (6% to 3%) for the Sequatchie vials. It would be expected that the radioactivity in the aqueous phase would have increased initially, indicating an increase in polar metabolites. This might have been observed if it hadn't been for the missing first time point (time 0).

All of the soil slurries had a different amount of maximum mineralization (ranging from 16% to 102%), apparently due to some difference(s) between the soils. The amount mineralized by the Etowah, Indiana, Sequatchie, buffered and unbuffered S. Carolina soil slurries was 16%, 36%, 65%, 75%, and 102%, respectively. The highest rates of mineralization for the Etowah, Indiana, unbuffered South Carolina, and buffered South Carolina soil slurries were 0.30, 0.26, 0.82, and 0.66 ppm/hour, respectively. No rate

could be obtained for the Sequatchie soil slurry because the zero timepoint was missing. It is interesting to note that there was an obvious difference between the buffered and unbuffered South Carolina soil slurries, with the unbuffered South Carolina soil slurry having a higher total amount of mineralization (102%) and rate of mineralization (0.82 ppm/hour) than the buffered S. Carolina (75% total amount of mineralization and 0.66 ppm/hour for the rate of mineralization). It is possible that a change in soil pH causes some nutrients to become unavailable or causes the levels of other elements, that may be toxic, to change (Brady, 1984), adding buffer causes hypertonic osmotic pressures (Stotzky and Rem, 1966), or that soil microorganisms are adapted to the original pH of the soil.

There were increases in the radioactivity associated with the soil in the Etowah (8% increase) and buffered South Carolina (1% increase) soil slurries. It remained fairly constant for the other soil slurries.

Correlation analyses (Figure 8) found that the maximum percentage of mineralization was found to correlate inversely ($r=-0.98$) with the clay fraction. This inverse correlation was high, despite different clay minerals present in the four soil slurries. This meant that the clay fraction did not enhance the maximum percentage of mineralization. The maximum percentage of mineralization did not correlate highly ($r>|0.80|$) with any other known

	% Sand	% Silt	% Clay	% Organic Matter	Adsorpt. Slope	Desorpt. Slope	Kads	Kom of Adsorpt.	Kdes	Kom of Desorpt.	C.E.C.	Max. % Miner.
% Sand	* 1.00000	-0.49906	-0.74249	-0.31675	-0.33276	0.75545	-0.26510	0.34129	-0.06876	0.51166	0.07448	0.66889
	* 0.00000	0.50090	0.25750	0.68320	0.66720	0.24450	0.73490	0.65870	0.93120	0.48830	0.92550	0.33110
% Silt	-0.49906	1.00000	-0.20993	0.16487	-0.55534	-0.45724	-0.23234	-0.67273	-0.40111	-0.75839	0.72579	0.29676
	0.50090	0.00000	0.79010	0.83510	0.44470	0.54280	0.76770	0.32730	0.59890	0.24160	0.27420	0.70320
% Clay	-0.74249	-0.20993	1.00000	0.22993	0.80472	-0.49890	0.47870	0.13496	0.38764	0.00895	-0.64507	-0.98408
	0.25750	0.79010	0.00000	0.77010	0.19530	0.50110	0.52130	0.86500	0.61240	0.99100	0.35490	0.01590
% Organic Matter	-0.31675	0.16487	0.22993	1.00000	0.51903	0.37519	-0.74219	-0.81439	-0.79961	-0.76740	0.42746	-0.06204
	0.68320	0.83510	0.77010	0.00000	0.48100	0.62480	0.25780	0.18560	0.20040	0.23260	0.57250	0.93800
Adsorpt. Slope	-0.33276	-0.55534	0.80472	0.51903	1.00000	0.11302	0.05421	0.04134	0.05939	0.03241	-0.55030	-0.76104
	0.66720	0.44470	0.19530	0.48100	0.00000	0.88700	0.94580	0.95870	0.94060	0.96760	0.44970	0.23900
Desorpt. Slope	0.75545	-0.45724	-0.49890	0.37519	0.11302	1.00000	-0.72404	-0.16840	-0.56446	0.02942	0.27849	0.53650
	0.24450	0.54280	0.50110	0.62480	0.88700	0.00000	0.27600	0.83160	0.43550	0.97060	0.72150	0.46350
Kads	-0.26510	-0.23234	0.47870	-0.74219	0.05421	-0.72404	1.00000	0.79536	0.97737	0.65816	-0.79648	-0.61534
	0.73490	0.76770	0.52130	0.25780	0.94580	0.27600	0.00000	0.20460	0.02260	0.34180	0.20350	0.38470
Kom of Adsorpt.	0.34129	-0.67273	0.13496	-0.81439	0.04134	-0.16840	0.79536	1.00000	0.90550	0.97982	-0.83149	-0.30602
	0.65870	0.32730	0.86500	0.18560	0.95870	0.83160	0.20460	0.00000	0.09450	0.02020	0.16850	0.69400
Kdes	-0.06876	-0.40111	0.38764	-0.79961	0.05939	-0.56446	0.97737	0.90550	1.00000	0.80242	-0.85110	-0.54258
	0.93120	0.59890	0.61240	0.20040	0.94060	0.43550	0.02260	0.09450	0.00000	0.19760	0.14890	0.45740
Kom of Desorpt.	0.51166	-0.75839	0.00895	-0.76740	0.03241	0.02942	0.65816	0.97982	0.80242	1.00000	-0.76960	-0.17642
	0.48830	0.24160	0.99100	0.23260	0.96760	0.97060	0.34180	0.02020	0.19760	0.00000	0.23040	0.82360
C.E.C.	0.07448	0.72579	-0.64507	0.42746	-0.55030	0.27849	-0.79648	-0.83149	-0.85110	-0.76960	1.00000	0.76164
	0.92550	0.27420	0.35490	0.57250	0.44970	0.72150	0.20350	0.16850	0.14890	0.23040	0.00000	0.23840
Max. % Miner.	0.66889	0.29676	-0.98408	-0.06204	-0.76104	0.53650	-0.61534	-0.30602	-0.54258	-0.17642	0.76164	1.00000
	0.33110	0.70320	0.01590	0.93800	0.23900	0.46350	0.38470	0.69400	0.45740	0.82360	0.23840	0.00000

* Top number - correlation coefficient, r. Bottom number = probability.

Figure 8. Correlation Analysis for Naphthalene Mineralization in Wet Contaminated Soil Slurry + 5R

soil variable. There were some inverse, but high correlations between some variables: K_{om} of adsorption with % organic matter (r=-0.81), K_{om} of adsorption with the cation exchange capacity (r=-0.83), and K_{des} with the cation exchange capacity (r=-0.85). There were some high correlations ($r>|0.80|$) found between variables. These were: % clay with the slope of adsorption (r=0.80), K_{des} with the K_{om} of adsorption (r=0.91), K_{des} with the K_{om} of desorption, K_{des} with K_{ads} (r=0.98), and K_{om} of adsorption with K_{om} of desorption (r=0.98). It appears that adsorption and desorption are very closely related. In the correlation analyses, the value for the maximum percentage of mineralization for the South Carolina soil slurry was taken from the unbuffered soil slurry.

Mass Balances

In some of the mineralization experiments, the mass balances varied quite greatly from 100%. Naphthalene and some other polycyclic aromatic compounds naturally produce chemiluminescence. Unless precautions are taken (i.e. storage of scintillation vials in the dark before placement in the scintillation counter for counting), the chemiluminescence may produce exaggerated counts, and lead to high mass balances. For all experiments, scintillation

vials were stored in the dark at least 24 hours prior to placement in the scintillation counter to minimize this problem. Low mass balances were also seen. Generally, the mass balances decreased in the soil slurry experiments as the time increased before extraction. This may be due to incomplete extraction. Karickhoff and Morris, 1985, observed that there can be a rather dramatic reduction in extractability of sorbed chemical with increased incubation time.

Mass balances varied considerably for the dry contaminated soil slurries with Strain 5R. Therefore, correlation analysis was done again for some variables with the mass balances normalized. This analysis determined the following correlation coefficients between the maximum percentage of mineralization and other variables: % sand ($r=0.09$), % silt ($r=0.11$), % clay ($r=-0.19$), % organic matter ($r=0.90$), % desorbed ($r=-0.84$), and the cation exchange capacity ($r=0.62$). However, as noted previously (see "Naphthalene Mineralization by Strain 5R and Soil Microorganisms in "Dry" Contaminated Soil Slurry", this chapter), correlation analyses would not be appropriate in this experiment between the "maximum" percentage of mineralization and other soil variables.

Mass balances varied considerably for the wet contaminated soil slurries with Strain 5R. Therefore, correlation analysis was done again for some variables with

the mass balances normalized. Correlation analysis found that the maximum percentage of mineralization had a high inverse correlation with the clay fraction (r=-0.91). The maximum percentage of mineralization did not correlate highly with the organic matter fraction (r=-0.20), the sand fraction (r=0.65), or the silt fraction (r=0.22). As noted before in previous analyses, the highest correlation for the maximum percentage of mineralization with any soil fraction was with the clay fraction. The maximum percentage of mineralization had a high correlation with the cation exchange capacity (r=0.80) and a high inverse correlation with k_{ads} (r=-0.80). The maximum percentage of mineralization did not correlate highly with the adsorption slope (r=-0.55), desorption slope (r=0.71), or K_{des} (r=-0.73).

Analysis for Metals in Soil

The results of the metals analysis for the Indiana soil are in the last column (Other Comments) in Table 1. As can be seen in Table 1, the levels of arsenic, barium, chromium, and lead were higher than detection levels. Although it appeared that Indiana soil microorganisms were resistant to mercury, mercury was not detected.

CHAPTER IV

DISCUSSION

The Relationship Between Total Heterotrophs, Degraders and Mineralization

The total heterotrophic population increased up to 2 orders of magnitude (Table 2) for all soil slurries during the mineralization assay for dry contaminated soil slurries without Strain 5R (compare total populations from "after contamination" with "after mineralization"). The frequency of the NahA genotype, also, increased for all soils from before contamination to after mineralization. Apparently, mineralization was affected by these population increases.

Correlation analysis was done to determine the relationship between the maximum percentage of mineralization, and the initial total heterotrophic microbial population, the final heterotrophic population and the difference between the initial and final populations. The correlation coefficients (r) were found to be -0.62, -0.93, and -0.93, respectively. It is surprising that these correlation coefficients were negative, meaning that a greater number of total heterotrophs did not enhance mineralization. It is generally expected that the

population density would increase with mineralization. One possible explanation for these results is that the non-degraders may be outcompeting the degraders. Another possible explanation is that with a higher population density and biomass, more of the substrate may have partitioned onto cell walls or incorporated into cell constituents by metabolism, rather than released as $^{14}CO_2$ (mineralization). When large fractions of ^{14}C were incorporated into microbial cellular material, measurement of rates of $^{14}CO_2$ alone would seriously underestimate transformation rates (Herbes and Schwall, 1978). Other researchers have examined the relationship between biodegradation and microbial density. Total heterotrophic microbial populations were not good indicators of PAH mineralization rates (Herbes, 1981; Spain and Van Veld, 1983; Heitkamp and Cerniglia, 1987). Hickman and Novak (1989) found that biodegradation and microbial density were directly related, as determined by linear regression, but the correlation coefficients were generally low. Coefficients of determination indicated that, at best, 87%, 92%, and 37% of the variability in subsurface biodegradation rates of methanol, phenol, and tert-butyl alcohol, respectively, could be attributed to differences in the measures of total bacterial density. When measuring microbial density to relate it to biodegradation, it has been assumed, although not necessarily correctly, that all

biomass is active in the substrate degradation and that specific utilization rates (rate of substrate removal per unit biomass) are fairly constant, even between different mixed cultures (Hickman and Novak, 1989). Spain et al. (1984) found that the total number of heterotrophs remained about the same during biodegradation of para-nitrophenol (PNP), while PNP degraders increased 3-fold.

Correlation analysis was performed to determine the relationship between the maximum percentage of mineralization, and the initial number of NahA positive colonies, the final number of NahA positive colonies, and the change in the number of the NahA frequency through the experiment. Correlation coefficients were found to be 0.54, 0.44, and 0.43, respectively. These correlation coefficients were positive, but low. It is unknown why these correlation coefficients were so low.

Lag Period

In the naphthalene mineralization experiment with dry contaminated soil slurry and strain 5R, it was seen that there was a lag, or adaptation period, of about 24 hours for the South Carolina soil slurry (see Figure 5) before the rate of mineralization increased. The South Carolina soil had been collected earlier than the other soils and,

therefore, been in storage longer than the other soils prior to starting the experiments. Mihelcic and Luthy (1988b) found in their experiments that the acclimation period associated with the onset of naphthalene degradation increased with the age of the soil from the time of collection. Adaptation can be defined functionally as an increase in the rate of degradation with exposure to a compound (Aelion et al., 1987). It is thought that the adaptation process may involve one or a combination of 1) induction or derepression of enzymes specific for degradation pathways of a particular compound, 2) a random mutation in which new metabolic capabilities are produced which allow degradation that was not previously possible, or 3) an increase in the number of organisms in the degrading population (Spain et al., 1980). Other factors, such as diauxie, nutrient limitation, time needed for the mineralizing species to acclimate to toxins or for inhibitors that are present in the environment to be destroyed, and predation of degraders by protozoa, may influence the length of the adaptation period (Wiggins et al., 1987). Lewis et al., 1986, found that lag periods decreased in samples amended with nutrients (nitrogen or phosphorus) when concentrations of these nutrients may have been limiting. Some researchers (Spain et al., 1984; Wiggins et al., 1987) concluded that adaptation was probably due to increases in the number of specific degraders.

Mineralization by Soil Microorganisms in Water

In the experiment in which naphthalene mineralization by soil microorganisms was studied, a slight amount of transformation may have occurred. However, no significant mineralization was seen to occur even after 90 days. It is possible that the media used to cultivate the soil microorganisms (YEPG broth, 1/4 strength) allowed non-naphthalene degraders to outcompete the degraders, or was not the appropriate media for culturing the naphthalene degraders. It is also possible that these naphthalene degraders have a higher metabolic rate on solid substrates (van Loosdrecht et al., 1990; ZoBell, 1943). Solid surfaces may help to concentrate nutrients and microorganisms. In this case, microorganisms may be more able to acquire the naphthalene (a nutrient) from surfaces than in the water column.

Dry vs. Wet Contamination

There was an observed difference in mineralization due to the type of contamination (wet or dry). There was a large range for the maximum percentage of mineralization among the wet contaminated soil slurries with Strain 5R (16% to 102%) (Figure 7), while the dry contaminated soil

slurries with Strain 5R (Figure 5) all had approximately the same amount of mineralization. Because the maximum percentage of mineralization was similar among the different dry soil slurries, the amount of mineralization was apparently not due to differences between soil types, and may have been due to some unknown variable(s). Because mineralization in the dry contaminated soil slurries appeared to be limited, the "maximum" percentage of mineralization was not appropriate for correlation analysis. There were some high inverse ($r<-0.80$) correlations between other variables: % desorbed with % organic matter, and % desorbed with the cation exchange capacity.

The maximum percentages of mineralization varied greatly between the different wet contaminated soil slurries. Apparently, some characteristic(s) in the soil caused these differences. The maximum percentage of mineralization was found to correlate highly, but inversely, with the % clay. This means that increasing the clay fraction does not enhance mineralization. Clay was the only known soil variable that correlated highly (positively or inversely), with the maximum percentage of mineralization in the wet contaminated soil slurries. Zielke et al., 1989, stated that it is generally agreed that the clay fraction is most important in influencing the reactivity of organic molecules, and that this is supported in part by commonly encountered correlations between the clay content of the

soil and the behavior of organic molecules applied to the soil.

Correlation analysis for the wet contaminated soil slurries with Strain 5R (Figure 8) found some high inverse correlations ($r<-0.80$) between other variables: K_{om} of adsorption with % organic matter ($r=-0.81$), K_{om} of adsorption with the cation exchange capacity ($r=-0.83$), and K_{des} with the cation exchange capacity ($r=-0.85$). High positive correlations ($r>|0.80|$) were: % clay with the slope of adsorption ($r=0.80$), K_{des} with the K_{om} of adsorption ($r=0.91$), K_{des} with the K_{om} of desorption, K_{des} with K_{ads} ($r=0.98$), and K_{om} of adsorption with K_{om} of desorption ($r=0.98$).

Researchers have found that soil appears to behave differently in dry and wet states in which the soil mineral and organic constituents show different effects on non-ionic compound uptake by soil (Chiou, 1989). In many previous studies, researchers found that sorption of non-ionic organic compounds by soil in aqueous systems is controlled mainly by the organic matter content of the soil, while sorption of nonpolar organic solvents by dry and subsaturated soils is determined mainly by the mineral type and content (Chiou, 1989). Hance (1965) found this to be the case with diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a hydrophobic herbicide. In experiments done for this thesis, it appears that with the different types of

contamination (dry or wet), the organic matter and clay fractions played different roles. However, the results were different than those observed by other researchers. For the wet contaminated soils, adsorption was correlated highly with the clay fraction (r=0.80), but not with the organic matter fraction (r=0.52). Also, mineralization in the wet contaminated soil slurries was highly correlated, but inversely, with the clay fraction (r=-0.98), but was not correlated with the organic matter fraction (r=-0.06). In the dry contaminated soil slurries, though, desorption inversely correlated with the organic matter fraction (r=-0.86) but wasn't correlated with the clay fraction (r=0.11). It is not known why there is a discrepancy between the results here in this thesis and those of other researchers.

Part of the reason for the differences observed between the dry and wet contaminated soil slurries may be due to the concentration of substrate used. The concentrations of naphthalene used in the "dry" contaminated soil slurries and in the "wet" contaminated soil slurries were different. In the "dry" contaminated soil slurries and the "wet" contaminated soil slurries, the concentration of naphthalene in the slurries was 50 ppm and approximately 8.3 ppm (see Table B-1 in Appendix B), respectively. Each slurry for the biodegradation study was composed of 1 gram of soil and 5 ml of water. The same ratio (2 g soil:10 ml water) was used for the sorption isotherms performed by Rebecca Pullen. It

has been seen that the concentration of a compound can affect the extent of its biodegradation. Little mineralization of some organic compounds occurred when these compounds were present at initial concentrations of 2 to 3 ng/ml or less (Boethling and Alexander, 1979), although at higher concentrations, 60% or more of the chemical initially present was converted to $^{14}CO_2$. There may be a threshold concentration below which no significant mineralization occurs. A possible explanation for this is that energy is obtained too slowly from oxidation of the substrate at low concentrations to allow the initially small population active on the compound to proliferate to reach cell densities sufficient to cause appreciable chemical loss.

A difference in the concentration of substrate added would result in different substrate:soil solids ratios. This ratio has been found to be important in determining amounts of mineralization or microbial growth. Protein utilization by soil microorganism isolates in the presence of clay minerals was studied by Marshman and Marshall (1981). Protein-clay complexes were formed by adding sterile solutions of minerals salts medium, clay minerals, and protein, in that order, to a sterile flask, by centrifugation and resuspension. They found that growth of the microorganisms was diauxic and that protein hydrolysis took place in the second phase of growth. The second phase of growth could be divided into three types which was

dependent on the protein-to-clay ratio. At high protein-to-clay ratios, growth was not affected by the clay. At intermediate protein-to-clay ratios, the growth but not the final yield was reduced. With low protein-to-clay ratios, the protein was unavailable for hydrolysis. The growth rate was dependent on the protein (gelatin)-to clay ratio and was independent on the absolute concentrations of gelatin or clay minerals in the medium. Subba-Rao and Alexander (1982) also studied the effects of clay on mineralization. Using benzylamine-clay complexes formed with different concentrations of clay and amines, they found that the extent of amine mineralization at the lower amine levels (20 pg/ml to 200 ng/ml) declined with increased clay concentration. However, at a higher amine concentration of 20 μg/ml, total mineralization was not affected greatly by different concentrations of clay. At a low amine-clay (montmorillonite) ratio, the extent of mineralization was affected, apparently because desorption was affected by the clay.

Another possible explanation for the differences observed between the dry and wet contaminated soil slurries might be due in part to the contamination process. In the wet contaminated soil, water may have carried naphthalene into micropores in the soil where the naphthalene would be physically inaccessible to microorganisms. During dry contamination of soil, the naphthalene would adsorb to the

outer surfaces of the soil particles. In this case, naphthalene would be more accessible to the microorganisms.

Comparison of Mineralization Rates

In the naphthalene mineralization assay with Strain 5R in water, 7.7×10^7 cells of Strain 5R were added to each vial containing 5 ml of water with 50 ppm of naphthalene. There was a constant linear rate (1.16 ppm/hour) of naphthalene mineralization until the experiment was terminated (a 12 hour period). Another researcher, J. Sanseverino (Applegate et al., 1990) found a comparable rate (1.7 ppm/hr) for Strain 5R mineralization of naphthalene. However, he used more cells (approximately 5×10^8 cells/vial), a different concentration of naphthalene (100 ppm) and mineral salts buffer.

In the naphthalene mineralization assays using dry contaminated soil slurries with Strain 5R, the initial concentration of naphthalene was 50 ppm and 2.0×10^6 cells of Strain 5R were added to each vial. Each of the slurries did not have a constant rate of mineralization, so the fastest rates of naphthalene mineralization were compared. The fastest rates for the Etowah, Sequatchie, Indiana, and South Carolina soil slurries were 6.0, 4.2, 3.3, and 2.4 ppm/hour, respectively. It is interesting to note that with

each of these slurries, the rate was higher than with Strain 5R alone, even though fewer cells of Strain 5R were added to these slurry vials. Apparently, the presence of soil helped to increase the rate of naphthalene mineralization. Surfaces, such as soil surfaces, can help to concentrate nutrients (i.e. naphthalene) and microorganisms from the bulk aqueous phase. This could allow for faster metabolic and mineralization rates.

In the naphthalene mineralization assays using wet contaminated soil slurries with Strain 5R, the initial concentration of naphthalene was 7.8 to 7.9 ppm and 2.4 x 10^8 cells of Strain 5R were added to each vial. Each of the slurries did not have a constant rate of mineralization, so the fastest rates of naphthalene mineralization were compared. The fastest rates for the Etowah, Indiana, buffered and unbuffered South Carolina soil slurries were 0.30, 0.26, 0.66, and 0.82 ppm/hour, respectively. Although more cells of Strain 5R were added in the wet contaminated soil slurries than in the dry contaminated soil slurries with Strain 5R or with Strain 5R in water, the highest rates of naphthalene mineralization were lower. This may be partially accounted for because the naphthalene concentrations used in the wet contaminated soil slurry assays was lower.

The dry contaminated soil slurries without Strain 5R, except for the Indiana soil slurry, did not have any

significant mineralization within 3 days. In comparison, all of the dry contaminated soil slurries with Strain 5R had reach a mineralization plateau by this time. Apparently, most mineralization in the dry contaminated soil slurries with Strain 5R could be attributed to Strain 5R and not to the indigenous soil microorganisms, except for the Indiana soil slurry.

Effect of Soil Organic Matter On Mineralization

Although the maximum percentage of mineralization in the dry contaminated soil slurry without Strain 5R or in the wet contaminated soil slurry with Strain 5R was found not to be correlated with the total organic matter content of the soils, it may be correlated with a particular fraction(s) of organic matter. This was not tested. Soils can vary greatly in their proportions of the fractions (nonhumic substances and humic substances such as humic acids, fulvic acids, etc.) that make up organic matter (Stevenson, 1972). Humic and fulvic acids are known to be compositionally different based on elemental analysis (Chiou et al., 1986). Humic substances may be important in the movement of organic compounds because soluble humic acids may carry sorbed compounds into solution (Ballard, 1971). Dissolved organic matter of soil and aquatic origins may increase the

solubility of some relatively water-insoluble solutes (Gschwend and Wu, 1985; Chiou et al., 1986). Gschwend and Wu found that even stringent centrifugal conditions do not completely eliminate non-settling particles. Therefore, during the wet contamination of soil, when the supernatant was discarded, any non-settling organic matter which may have adsorbed naphthalene would have likewise been discarded. This may have allowed for erroneous observations (i.e. organic matter is less important than clay in influencing mineralization). However, a study of partitioning of hydrophobic organics between dissolved organic matter, particulates, and Great Lakes water found that the amount of hydrophobic organic contaminant associated with the dissolved organic matter averaged less than 5%, and did not exceed 10% of the total (Eadie et al., 1990).

Bioaccumulation

In many of the experimental soil slurry vials, it was seen that, with time, there was an increase in the radioactivity associated with the soil. This may be due to bioconcentration. If this increase is due to bioaccumulation, a biologically mediated process, then live organisms should accumulate greater amounts of residues than

dead organisms (Swindoll, 1986). Bioconcentration is defined as an increase in residue concentration in an organism by direct uptake from water (Brungs and Mount, 1978). Bioconcentration has been found to be increased with the hydrophobicity of the chemical (Neely et al., 1974; Chiou et al., 1977; Veith et al., 1979; Oliver and Niimi, 1983). Swindoll, 1986, found that increasing the bacterial biomass exposed to HCB contaminated sediment resulted in a linear increase in HCB sorbed to bacteria. Initially, the bioaccumulation of hydrophobic (and therefore, lipophilic) chemical residues is mainly due to the passive sorption of the residues onto the surface of the organism (Steen and Karickhoff, 1981; Spacie and Hamelink, 1985). The second, slower phase of bioaccumulation could be due to the sorbed residues moving across the surface membranes into the organisms (Sugiura et al., 1975). Tulp and Hutzinger (1978) stated that the aqueous solubility, steric factors, and metabolism were important factors influencing bioaccumulation of the compound.

Effect of Buffer

In the naphthalene mineralization experiment that was performed with wet contaminated soil slurry and strain 5R, the South Carolina soil slurry was used with (pH 6.8) and

without (pH 5.2) buffer. It was seen (see Figure 7) that the South Carolina soil slurry had a higher maximum percentage of mineralization without the buffer than with it. It is possible that a change in soil pH causes some nutrients to become unavailable or causes the levels of other elements, that may be toxic, to change (Brady, 1984), adding buffer causes hypertonic osmotic pressures (Stotzky and Rem, 1966), or that soil microorganisms are adapted to the original pH of the soil. Another possibility is that adding a buffer (salt) may have reduced the solubility of the naphthalene. Generally, the presence of electrolytes reduces the solubility of non-polar compounds: the "salting out" effect (Long and McDevit, 1952; McDevit and Long, 1952; Paul, 1952). Decreasing the solubility of naphthalene may have made it more likely to sorb, and therefore less bioavailable.

Mercury Resistance

It appeared that naphthalene mineralization occurred in the control vials for the Indiana soil slurries in experiments that were run for 4 days or longer. Five mg of mercuric chloride were added to the vials that were used for controls. With soil slurries composed of 1 g soil and 5 ml of water, the final concentration of mercuric chloride in

the vial was 833 ppm. To check for mercury resistance, Indiana soil, water, and mercuric chloride (in the same ratios), and an unmeasured amount of naphthalene were put into an Erlenmeyer flask and shaken at room temperature. After 39 days, this soil slurry was serially diluted and spread-plated onto YEPG agar with 10 μg/ml of Hg^{2+}. The plates were incubated for 10 days at 27° C. Colonies with at least 3 different phenotypes grew on the mercury plates. As a control, Indiana soil unexposed to mercury was serially diluted and plated out on the same mercury plates. No colonies from the unacclimated soil grew on the mercury plates. This confirmed that mercury resistance was present in the Indiana soil microorganisms, and that an acclimation period was necessary to allow the mercury-resistant genotype to increase to a noticeable frequency. Mercury resistance is not uncommon among soil microorganisms (Kelly and Reanney, 1984). Fungicides and atmospheric contamination from evaporation of metallic mercury are the major sources of soil contamination by mercury (Bowen, 1966). Generally, the proportion of heavy metal-resistant microorganisms is higher at heavy metal contaminated sites than at uncontaminated sites (Jordan and Lechevalier, 1975; Timoney et al., 1978), although mercury- resistant bacteria have been isolated from soils which had no known history of exposure to mercury (Radford et al., 1981; Kelly and Reanney, 1984). However, neither Radford et al nor Kelly

and Reanney did any analyses to confirm whether or not there was mercury present in the soil. When heavy metal analysis was done on the Indiana soil, mercury was not detected. Reasons for this may include: 1) the microorganisms may have genetically encoded resistance to other heavy metals that also confer resistance to mercury (R factors that work in a similar manner against more than one chemical), 2) the microorganisms already volatilized the mercury from formerly higher levels to undetectable levels, 3) because the soil was analyzed a long time after collection (17 months), the level of the metals detected may be lower than originally present in the soil due to physical oxidation by the atmosphere, or 4) the microorganisms may contain a plasmid or chromosome that contains genes for resistance against more than one heavy metal. Of the microorganisms that are tolerant to a heavy metal, many are tolerant to more than one (Silver, 1981; Duxbury and Bicknell, 1983; Mergeay et al., 1985).

CHAPTER V

CONCLUSIONS

Colony hybridizations were done to determine if the frequency of the NahA genotype (a naphthalene degrading genotype) increased with exposure to naphthalene. From before naphthalene contamination of soil to after the mineralization assays, there were increases in the NahA genotype for each soil type. The South Carolina soil had the smallest increase (less than one order of magnitude) in the frequency of the NahA genotype. The other soils had increases of up to 6 orders of magnitude in the frequency of the NahA genotype.

The method of contamination (wet or dry) may have an effect on the amount of mineralization. There was a large range in the maximum percentages of mineralization among the wet contaminated soil slurries with Strain 5R (16% to 102%), while the dry contaminated soil slurries with Strain 5R all had approximately the same amount of mineralization.

The maximum % of mineralization was found to be affected by different soil characteristics in the different types of soil slurries. Mineralization in the dry contaminated soil slurries without Strain 5R was found to

correlate inversely with the final total heterotrophic population (r=-0.93) and with the change in the total heterotrophic population during the experiment (r=-0.93). For the wet contaminated soil slurries with Strain 5R, the maximum % of mineralization correlated inversely with the clay fraction (r=-0.98). It was not correlated with any other known variables.

The dry contaminated soil slurries with Strain 5R had faster mineralization rates than Strain 5R by itself in water. In the naphthalene mineralization assay with Strain 5R in water, 7.7×10^7 cells of Strain 5R were added to each vial containing 5 ml of water with 50 ppm of naphthalene. There was a constant linear rate (1.16 ppm/hour) of naphthalene mineralization until the experiment was terminated (a 12 hour period). In the naphthalene mineralization assays using dry contaminated soil slurries with Strain 5R, the initial concentration of naphthalene was 50 ppm and 2.0×10^6 cells of Strain 5R were added to each vial. Each of these slurries did not have a constant rate of mineralization, so the fastest rates of naphthalene mineralization were compared. The fastest rates for the Etowah, Sequatchie, Indiana, and South Carolina soil slurries were 6.0, 4.2, 3.3, and 2.4 ppm/hour, respectively. It is interesting to note that with each of these slurries, the rate was higher than with Strain 5R alone, even though fewer cells of Strain 5R were added to these slurry vials.

Apparently, the presence of soil and its characteristics helped to increase the rate of naphthalene mineralization.

Changes in the soil pH can affect the total amount of mineralization. Mineralization assays were performed using wet contaminated South Carolina soil slurry with and without buffer. Without the buffer, the slurry pH was 5.19. With the buffer, the slurry pH was 6.8. The unbuffered slurry had a higher maximum amount of mineralization (102%) than the buffered slurry (75%).

Mercuric chloride is not the preferable method to prepare soil for controls, because mercury-resistant soil microorganisms do exist. Mercury-resistant microorganisms were found in the Indiana soil even though it did not have any detectable levels of mercury. However, the soil did have detectable levels of other heavy metals (arsenic, barium, chromium, and lead).

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APPENDIXES

APPENDIX A

DATA FOR NAPHTHALENE MINERALIZATION EXPERIMENTS

Table A-1: Data for Naphthalene Mineralization by Strain 5R in Water

		CO2 Trap Results				Aqueous Phase						Solvent Phase						Mass Balances			
		Control	Exper.	Net	% Miner	Control	Control *15	% in Control	Exper.	Exper. *15	% in Exper.	Control	Control *8	% in Control	Exper.	Exper. *8	% in Exper.	Control Total dpm	Exper. Total dpm	Control	Exper.
Time 0	AVER	510	2267	1757	1	63	950	1	110	1650	1	15737	125893	90	18123	144987	104	127353	148903	92	107
	S.D.	57	393	363	0	9	141	0	14	212	0	540	4322	3	827	6616	5	4445	7221	3	4
1.42 hrs	AVER	560	5660	5100	4	63	950	1	187	2800	2	16263	130107	93	19437	155493	112	131617	163953	95	118
	S.D.	290	818	1096	1	5	71	0	34	510	0	101	806	1	449	3593	3	844	4921	1	7
2.25 hrs	AVER	343	7830	7487	5	57	850	1	227	3400	2	16995	135960	98	19930	159440	115	137155	179870	99	129
	S.D.	41	178	215	0	5	71	0	12	187	0	695	5560	4	830	6640	5	5505	7005	4	5
3.58 hrs	AVER	363	12877	12513	9	67	1000	1	5	71	3	17143	137147	99	19482	155853	112	138510	168301	100	124
	S.D.	53	1187	1225	1	9	141	0	5	71	0	88	705	1	4406	35249	25	524	36507	0	25
5.25 hrs	AVER	590	19640	19050	14	60	750	1	367	5500	4	16723	133787	96	14983	119867	96	135127	145007	97	104
	S.D.	78	951	1003	1	8	122	0	24	354	0	365	2917	2	500	4001	3	3065	5306	2	2
6.34 hrs	AVER	1077	25207	24130	17	57	850	1	417	6250	4	17247	137973	99	14813	118507	85	139900	149963	101	108
	S.D.	559	29	534	0	9	141	0	12	187	0	189	1510	1	474	3793	3	1752	4009	1	3
7.67 hrs	AVER	530	27293	26763	19	67	1000	1	470	7050	5	17053	136427	98	15757	126053	91	137957	160397	99	115
	S.D.	404	664	434	0	5	71	0	14	212	0	349	2791	2	501	4006	3	2979	4882	2	3
8.50 hrs	AVER	223	30323	30100	22	60	900	1	537	8050	6	18213	145707	105	15843	126747	91	146830	165120	106	119
	S.D.	31	1307	1296	1	8	122	0	24	354	0	176	1411	1	616	4932	4	1471	6592	1	2
9.77 hrs	AVER	343	34850	34507	25	67	1000	1	623	9350	7	17627	141013	101	17097	136773	98	142357	180973	102	130
	S.D.	41	832	844	1	5	71	0	26	394	0	926	7410	5	1178	9426	7	7364	10702	5	6
10.92 hrs	AVER	430	36363	35933	26	63	950	1	663	9950	7	18037	144293	104	16133	129067	93	145673	175380	105	126
	S.D.	243	1688	1447	1	5	71	0	21	308	0	725	5797	4	3026	24211	17	5913	26207	4	16
12.0 hrs	AVER	357	40910	40553	29	77	1150	1	680	10200	7	17310	138480	100	12093	96747	70	139987	147857	101	106
	S.D.	12	1635	1632	1	5	71	0	64	957	1	489	3913	3	110	882	1	3989	3473	3	2

Table A-2: Data for Naphthalene Mineralization by Soil Microorganisms in Water

		South Carolina soil microorganisms CO2 Trap Results				Solvent Phase				Aqueous P hase				Total dpm		Mass Balances (%)	
		Control	Exper.	Net	% Miner.	Control *8	% in Control	Exper. *8	% in Exper.	Control *15	% in Control	Exper. *15	% in Exper.	Control	Exper.	Control	Exper.
Time 0	AVER	70	63	-7	-0	88667	66	102427	76	700	1	750	1	89437	103240	66	76
	S.D.	14	5	9	0	5110	4	2862	2	71	0	0	0	5061	2864	4	2
3 days	AVER	67	63	-3	-0	88267	65	97520	72	800	1	800	1	89133	98383	66	73
	S.D.	9	5	5	0	686	1	4252	3	71	0	71	0	758	4221	1	3
6 days	AVER	70	80	10	0	93707	69	104640	77	750	1	825	1	94527	105560	70	78
	S.D.	14	29	36	0	1681	1	5280	4	122	0	75	0	1785	5380	1	4
9 days	AVER	113	107	-7	-0	92400	68	107520	79	750	1	750	1	93263	108377	69	80
	S.D.	47	26	73	0	2743	2	3173	2	0	0	122	0	2726	3031	2	2
17 days	AVER	120	127	7	0	85787	63	100160	74	800	1	1000	1	86667	101287	64	75
	S.D.	0	0	10	0	4790	4	4413	3	141	0	71	0	4888	4478	4	3
39 days	AVER	733	793	60	0	69947	52	93707	69	750	1	1100	1	71430	95600	53	71
	S.D.	76	60	131	0	11497	9	1411	1	0	0	394	0	11566	1435	9	1
60 days	AVER	677	1983	1307	1	67733	50	96853	72	850	1	950	1	69260	99787	51	74
	S.D.	388	2423	2590	2	12047	9	322	0	71	0	71	0	12215	2601	9	2
94 days	AVER	690	763	73	0	72293	53	91387	68	750	1	950	1	73733	93100	55	69
	S.D.	119	110	144	0	5478	4	17642	13	212	0	71	0	5717	17745	4	13
		Indiana soil microorganisms															
Time 0	AVER	103	87	-17	0	75147	60	100960	81	1000	1	750	1	76250	101797	61	82
	S.D.	34	12	37	0	11129	9	1359	1	354	0	0	0	11287	1350	9	1
3 days	AVER	70	70	0	0	88560	71	100507	81	800	1	850	1	89430	101427	72	81
	S.D.	0	0	0	0	1941	2	3407	3	71	0	71	0	1915	3477	2	3
6 days	AVER	80	200	120	0	72480	58	105040	84	700	1	800	1	73260	106040	59	85
	S.D.	8	170	170	0	16840	14	1210	1	71	0	71	0	16917	1316	14	1
9 days	AVER	80	83	3	0	91387	73	102347	82	700	1	750	1	92167	103180	74	83
	S.D.	8	9	5	0	5110	4	3216	3	71	0	0	0	5128	3207	4	3
17 days	AVER	123	693	570	0	81733	66	79280	64	800	1	750	1	82657	80723	66	65
	S.D.	9	769	779	1	5545	4	22861	18	71	0	0	0	5572	22094	4	18
39 days	AVER	723	837	113	0	63547	51	82027	66	850	1	850	1	65120	83713	52	67
	S.D.	74	37	102	0	3344	3	5550	4	71	0	71	0	3280	5644	3	5
60 days	AVER	823	700	-123	-0	64373	52	82667	66	750	1	850	1	65947	84217	53	68
	S.D.	533	581	1096	1	3282	3	10342	8	122	0	71	0	3905	9940	3	8
94 days	AVER	647	773	127	0	65520	53	78187	63	850	1	850	1	67017	79810	54	64
	S.D.	123	176	160	0	11532	9	3950	3	71	0	141	0	11579	4225	9	3

Etowah soil microorganisms
CO_2 Trap Results

						Solvent Phase				Aqueous Phase				Total dpm		Mass Balances	
		Control	Exper.	Net	% Miner.	Control *8	% in Control	Exper. *8	% in Exper.	Control *15	% in Control	Exper. *15	% in Exper.	Control	Exper	Control	Exper.
Time 0	AVER	60	63	3	0	84373	62	106213	79	700	1	750	1	85133	107027	63	79
	S.D.	8	5	12	0	8669	6	1984	1	71	0	122	0	8613	2106	6	2
3 days	AVER	90	73	-17	-0	87840	65	106053	79	700	1	900	1	88630	107027	66	79
	S.D.	22	5	17	0	6214	5	1875	1	71	0	212	0	6166	1670	5	1
6 days	AVER	73	63	-10	-0	94480	70	110027	81	800	1	800	1	95353	110890	71	82
	S.D.	5	12	14	0	3943	3	2274	2	71	0	71	0	4011	2205	3	2
9 days	AVER	557	397	-160	-0	91947	68	104293	77	800	1	750	1	93303	105440	69	78
	S.D.	24	164	185	0	3588	3	2852	2	71	0	0	0	3655	2899	3	2
17 days	AVER	100	127	93	0	102480	76	108747	81			750	1	102580	109623	76	81
	S.D.	0	24	62	0	0	0	3639	3			0	0	0	3618	0	3
39 days	AVER	857	573	-283	-0	87200	65	89280	66	650	0	800	1	88707	90653	66	67
	S.D.	111	26	90	0	1977	1	10272	8	141	0	71	0	1775	10179	1	8
60 days	AVER	517	467	-50	-0	55893	41	62613	46	650	0	825	1	57060	63630	42	47
	S.D.	117	53	86	0	2506	2	16755	12	71	0	75	0	2693	16504	2	12
94 days	AVER	1210	1117	-93	-0												
	S.D.	337	280	547	0												

Sequatchie soil microorganisms

Time 0	AVER	83	73	-10	-0	70987	60	100853	85	800	0	750	1	71870	101677	61	86
	S.D.	5	9	8	0	24179	20	5231	4	71	0	122	0	24218	5128	20	4
3 days	AVER	60	70	10	0	87360	74	102213	86	750	0	800	1	88170	103083	75	87
	S.D.	8	8	8	0	623	1	2324	2	122	0	71	0	699	2252	1	2
6 days	AVER	70	73	3	0	81733	69	102320	87	750	0	800	1	82553	103193	70	87
	S.D.	8	5	12	0	1622	1	7360	6	0	0	71	0	1624	7410	1	6
9 days	AVER	67	60	-7	-0	88453	75	108933	92	750	0	850	1	89270	109843	75	93
	S.D.	5	8	9	0	2341	2	5215	4	0	0	71	0	2342	5284	2	4
17 days	AVER	120				76000	64			850	0			76970		65	
	S.D.	0				960	1			71	0			1035		1	
39 days	AVER	767	785	18	0	69627	59	95200	80	850	0	750	1	71243	96473	60	82
	S.D.	143	95	185	0	9751	8	7676	6	141	0	122	0	9798	7488	8	6
60 days	AVER	4040	1370	-2670	-2	82160	69	87520	74	850	0	750	1	87050	89640	74	76
	S.D.	2208	0	0	0	10609	9	0	0	71	0	0	0	8949	0	8	0
94 days	AVER	1080	910	-170	-0	98507	83	117973	100	1050	0	1050	1	100637	119933	85	101
	S.D.	99	136	212	0	7263	6	840	1	324	0	122	0	7330	702	6	1

Table A-3: Data for Naphthalene Mineralization in Dry Contaminated Soil Slurry

		Etowan soil CO2 Trap Results			Solvent Phase				Aqueous Phase				Oxidizer				Total dpm		Mass Balances (%)	
		Control	Exper.	Net	% Min	Control #8	% in Control	Exper. #8	% in Exper.	Control #15	% in Control	Exper. #15	% in Exper.	Control	% in Control	Exper.	% in Exper.	Control	Exper.	Control	Exper.
Time 0	AVER	103	67	-37	-0	129893	83	132853	85	1650	1	1725	1	2660	2	1985	1	134307	136630	86	87
	S.D.	68	5	66	0	7222	5	2100	1	0	0	75	0	813	1	35	0	6752	1715	4	1
3 days	AVER	513	163	-350	-0	137840	88	136800	87	1600	1	2150	1	1727	1	2230	1	141680	141343	90	90
	S.D.	488	9	497	0	2692	2	5050	3	71	0	187	0	125	0	209	0	3012	5395	2	3
60 days	AVER	248	84250	84003	54	109940	70	2800	2	1800	1	5438	3	1228	1	10958	7	113215	103445	72	66
	S.D.	23	1955	1970	1	6238	4	113	0	150	0	373	0	113	0	567	0	6409	1199	4	1
		Sequatchie soil																			
Time 0	AVER	63	77	13	0	121067	108	128507	114	2450	2	2450	0	2080	2	1640	1	125660	132673	112	118
	S.D.	5	12	12	0	3432	3	5056	5	71	0	141	0	110	0	119	0	3279	5234	3	5
3 days	AVER	70	307	237	0	119787	107	124133	111	2150	2	2250	0	1417	1	1580	1	123423	128270	110	114
	S.D.	8	271	278	0	2382	2	3328	7	71	0	122	0	104	0	50	0	2299	8595	2	8
60 days	AVER	285	79835	79550	71	92133	82	2400	2	2175	2	5625	0	1288	1	7053	6	95831	94913	85	85
	S.D.	72	651	662	1	2198	2	113	0	75	0	708	0	38	0	1656	1	6052	2334	5	2
		South Carolina soil																			
Time 0	AVER	53	47	-7	-0	139253	121	143813	125	800	1	850	1	2657	2	3240	3	142763	147950	124	129
	S.D.	5	12	9	0	3262	3	2496	2	71	0	71	0	496	0	100	0	3735	3066	3	3
3 days	AVER	57	93	37	0	141413	123	145787	127	800	1	1050	1	1890	2	2237	2	144160	149167	126	130
	S.D.	5	5	5	0	3704	3	4392	4	71	0	324	0	354	0	54	0	4053	4446	4	4
60 days	AVER	63	86525	86462	75	126107	110	16360	14	1150	1	3225	3	2547	2	7443	6	129867	113553	113	99
	S.D.	5	1443	1423	1	811	1	5903	5	71	0	605	1	123	0	3608	3	850	3074	1	3
		Indiana soil																			
Time 0	AVER	123	197	73	0	143733	137	153640	143	950	1	950	1	2433	2	2290	2	147140	154077	140	147
	S.D.	5	97	96	0	8348	8	1643	2	71	0	71	0	95	0	377	0	8326	1404	8	1
3 days	AVER	363	70073	69710	66	150187	143	8773	8	800	1	3900	4	2000	2	12287	12	153350	95033	146	90
	S.D.	281	2764	2796	3	3854	4	2188	2	71	0	442	0	276	0	394	0	3313	461	3	0
60 days	AVER	90128	77235	-12893	-12	5067	5	6000	6	4313	4	4050	4	6268	6	7488	7	105774	94773	101	90
	S.D.	12090	3446	11743	11	317	1	4709	4	927	1	541	1	2036	2	573	1	3622	7344	3	7

Table A-4: Data for Naphthalene Mineralization in Dry Contaminated Soil Slurry & Strain 5R

		SOUTH CAROLINA SOIL CO2 Trap Results				Solvent Phase			Aqueous Phase				Oxidizer				Total dpm		Mass Balances (%)	
		Control	Exper.	Net	% Miner.	Control #8	% in Control	Exper. #8	% in Exper.	Control #15	% in Control	Exper. #15	% in Exper.	Control	% in Control	Exper.	% in Exper.	Control	Exper.	Control	Exper.
Time 0	AVER	150	520	370	0			158267	125	1200	1	1100	1	2177	2	2173	2		162060		128
	S.D.	22	192	205	0			3086	2	122	0	141	0	141	0	380	0		3420		3
8 hrs	AVER	170	16470	16300	13	158187	125	134827	107	1050	1	3300	3	2227	2	3493	3	161633	158090	128	125
	S.D.	37	12574	12581	10	1376	1	18875	15	127	0	957	1	336	0	1009	1	1058	4517	1	4
12 hrs	AVER	167	6213	6047	5	168613	134	151170	120	1150	1	2700	2	2083	2	2743	2	172013	162217	136	129
	S.D.	29	1674	1646	1	5689	5	4373	3	187	0	71	0	133	0	144	0	5992	3875	5	3
24 hrs	AVER	157	14447	14290	11	155120	123	132133	105	1200	1	2400	2	2050	2	3323	3	158527	152303	126	121
	S.D.	17	11225	11223	9	3679	3	18262	14	0	0	803	1	217	0	1002	1	3875	7648	3	6
36 hrs	AVER	157	88477	88320	70	160213	127	22800	18	1250	1	6000	5	1837	1	9633	8	163457	176910	129	101
	S.D.	17	7603	7620	6	3631	3	11846	9	71	0	324	0	164	0	292	0	3762	4943	3	4
48 hrs	AVER	130	99990	99860	79	160800	127	9120	7	1125	1	5700	5	1870	1	9090	7	163925	123900	130	98
	S.D.	20	570	625	0	1760	1	480	0	75	0	0	0	10	0	250	0	1825	160	1	0
56 hrs	AVER	177	87360	87183	69	159680	126	16320	13	1250	1	6050	5	1807	1	9550	8	162913	119280	129	94
	S.D.	17	7732	7747	6	1585	1	6619	5	141	0	71	0	131	0	199	0	1677	7376	1	2
64 hrs	AVER	150	95897	95747	76	160400	127	8133	6	1350	1	5000	4	1875	1	8430	7	163723	117460	130	93
	S.D.	24	61	66	0	5741	5	1802	1	122	0	495	0	121	0	556	0	5872	2763	5	2
72 hrs	AVER	150	87147	86997	69	161920	128	8107	6	1150	1	4950	4	1820	1	9903	8	165040	110107	131	87
	S.D.	8	3573	3563	3	7248	6	1513	1	71	0	367	0	74	0	1696	1	7281	1418	6	1
85.5 hrs	AVER	127	76843	76717	61	159570	122	7013	6	1600	1	4000	3	3120	2	9570	8	158167	97427	125	77
	S.D.	24	1321	1299	1	5410	4	1740	1	71	0	464	0	136	0	452	0	5773	2759	4	2
96 hrs	AVER	203	71797	71593	57	151547	120	8160	6	1350	1	3800	3	2217	2	10943	9	155817	94700	123	75
	S.D.	147	2347	2313	2	5361	4	988	1	122	0	141	0	642	1	1825	1	5916	3241	5	3
120 hrs	AVER	135	72520	72385	57	155480	123	5760	5	1575	1	3300	3	2340	2	9370	7	159530	90950	126	72
	S.D.	25	1900	1875	1	4360	3	640	1	75	0	0	0	400	0	190	0	4060	2730	3	2
		INDIANA SOIL																			
Time 0	AVER	1460	4730	3270	2			151387	110	1700	1	2300	2	3927	3	3670	3		162087		118
	S.D.	449	850	1201	1			754	1	187	0	141	0	1188	1	767	1		1883		1
3.17 hrs	AVER	7020	28553	26533	19	160080	117	85187	62	1950	1	5550	4	4297	3	8681	6	168147	128173	123	94
	S.D.	636	17610	17005	12	3815	3	14596	11	324	0	745	1	521	0	878	1	7410	18598	2	14
6.5 hrs	AVER	2303	64823	62520	46	163947	120	47307	34	2050	1	8400	6	3777	3	9260	7	177077	129790	125	94
	S.D.	859	9947	10661	8	1243	1	9812	7	374	0	1181	1	381	0	979	1	365	2524	0	14
8 hrs	AVER	1613	68037	66423	48	164373	120	31013	23	1800	1	9250	7	3420	2	9110	7	171207	117410	125	86
	S.D.	274	5305	5343	4	1136	1	13827	10	122	0	1393	1	300	0	906	1	598	6907	0	5
12 hrs	AVER	1403	80570	79117	58	166800	122	17307	13	1650	1	10050	7	3057	2	10260	7	177910	119465	126	87
	S.D.	384	1455	1658	1	4550	3	1033	1	122	0	122	0	411	0	2480	2	4187	1515	3	1
16 hrs	AVER	1743	82190	80447	59	158773	116	10853	8	1800	1	7300	5	3320	2	11757	9	165637	112100	121	82
	S.D.	416	7420	7390	5	2643	2	698	1	122	0	552	0	339	0	1394	1	1975	6667	1	5
24 hrs	AVER	7063	88300	86237	63	156107	114	6987	5	1950	1	5150	4	3360	2	13073	10	163480	113510	119	83
	S.D.	413	1562	1841	1	4688	3	481	0	122	0	1042	1	778	0	483	0	4378	1379	3	1
34.67 hrs	AVER	1600	94113	92513	67	159967	116	5573	4	1800	1	4300	3	2940	2	12790	9	165807	117177	121	85
	S.D.	353	2648	2940	2	3715	3	1046	1	122	0	675	0	628	0	594	0	3495	2410	3	2
48 hrs	AVER	1540	97817	97177	67	160053	117	3947	3	1350	1	3150	3	2970	2	9973	7	165913	110487	121	81
	S.D.	324	1995	1706	1	5493	4	38	0	0	0	0	0	233	0	2606	2	5384	4428	4	3
72 hrs	AVER	2400	96737	94337	69	161733	118	3653	3	1400	1	4500	3	3263	2	7328	5	168797	113283	123	83
	S.D.	467	2007	2442	2	2112	2	189	0	141	0	0	0	360	0	1050	1	2044	1662	1	1
96 hrs	AVER	30317	92615	62298	36	113760	83	3240	2	2550	2	3900	3	6933	5	11225	8	153560	110980	112	81
	S.D.	38385	9595	40075	23	59692	44	120	0	1592	1	450	0	5243	4	335	0	14489	9590	11	7
120 hrs	AVER	69830	82517	12747	14	7013	5	3067	2	9750	7	3350	2	14193	10	11647	8	100787	100640	73	73
	S.D.	6678	3859	9865	7	1815	1	136	0	4060	3	552	0	3848	3	959	1	6558	5476	5	4

ETOWAH SOIL

		CO2 Trap Results				Solvent Phase				Aqueous Phase				Oxidizer				Total dpm		Mass Balances (%)	
		Control	Exper.	Net	% Miner.	Control *8	% in Control	Exper. *8	% in Exper.	Control *15	% in Control	Exper. *15	% in Exper.	Control	% in Control	Exper.	% in Exper.	Control	Exper.	Control	Exper.
Time 0	AVER	540	3073	2533	2	118853	102	141947	122	3400	3	5300	5	2323	2	4747	4	125117	155067	108	133
	S.D.	166	132	273	0	27543	24	5620	5	495	0	464	0	461	0	475	0	27052	4922	23	4
3.17 hrs	AVER	560	10873	10313	9	144107	124	118240	102	2900	2	6350	5	2193	2	4987	4	149760	140450	129	121
	S.D.	45	3493	3529	3	6794	6	8552	7	187	0	255	0	41	0	405	0	6719	6450	6	6
6.5 hrs	AVER	575	51980	51405	44	157227	135	68512	59	3000	3	9700	8	1623	1	8493	7	162233	139685	139	119
	S.D.	65	16185	16224	14	15616	13	27275	23	122	0	2420	2	675	1	1311	1	15702	7107	13	7
8.0 hrs	AVER	660	78553	77893	67	154187	132	23413	20	3750	3	13200	11	2320	2	10977	9	160917	126143	138	108
	S.D.	102	10626	10699	9	9320	8	6202	5	324	0	1901	2	222	0	192	0	8962	7904	8	7
12.0 hrs	AVER	627	96363	95737	82	150213	129	7013	6	3900	3	9850	8	2120	2	10587	9	156860	122813	135	106
	S.D.	69	2009	2056	2	2446	2	528	0	122	0	561	0	22	0	253	0	2533	1614	2	1
16.0 hrs	AVER	923	91660	90737	78	156187	134	6320	5	3950	3	8300	7	2520	2	12677	11	163580	118957	141	102
	S.D.	429	2630	2566	2	4006	3	428	0	354	0	187	0	404	0	2279	2	3423	4447	3	4
24.0 hrs	AVER	510	90297	89787	77	137680	118	4667	4	3350	3	7150	6	1543	1	10343	9	143083	112457	123	97
	S.D.	24	12090	12092	10	13718	12	200	0	187	0	187	0	845	1	373	0	14575	11438	13	10
36.0 hrs	AVER	490	89367	88877	76	132613	114	3893	3	3450	3	6050	5	2243	2	11360	10	138797	110670	119	95
	S.D.	50	1691	1723	1	19998	17	100	0	648	1	283	0	139	0	403	0	20482	1895	18	2
48.0 hrs	AVER	1280	76013	74733	64	142213	122	3813	3	4850	4	5600	5	3020	3	10980	9	151363	96407	130	83
	S.D.	422	1589	1309	1	10494	9	100	0	903	1	374	0	714	1	1339	1	10848	884	9	1
72.0 hrs	AVER	573	76510	75937	65	150693	129	3333	3	3700	3	5100	4	2010	2	9507	8	156977	94450	135	81
	S.D.	34	1616	1628	1	11336	10	247	0	71	0	0	0	45	0	284	0	11397	1465	10	1
96.0 hrs	AVER	600	66920	66320	57	146773	126	3280	3	3900	3	5050	4	2447	2	9910	9	153720	85160	132	73
	S.D.	127	467	490	0	6273	5	113	0	245	0	71	0	535	0	1014	1	5590	610	5	1
120.0 hrs	AVER	477	63193	62717	54	146027	125	3253	3	3750	3	4850	4	1990	2	9120	8	154715	80417	133	69
	S.D.	83	5187	5105	4	6214	5	322	0	122	0	71	0	30	0	354	0	6555	4561	6	4

SEQUATCHIE SOIL

Time 0	AVER	1623	5273	4650	3	132133	116	107733	94	4000	4	5600	5	2300	2	4097	4	140057	122703	123	107
	S.D.	650	176	870	1	2930	3	5057	4	255	0	255	0	495	0	205	0	2603	4903	2	4
3.17 hrs	AVER	1260	72530	71270	59	132987	116	85547	75	3750	3	7900	7	2410	2	6803	6	140467	122780	123	107
	S.D.	67	3053	3097	3	1243	1	5927	5	122	0	71	0	442	0	257	0	1472	3506	1	3
6.5 hrs	AVER	7037	54270	52733	46	130933	115	17573	15	4250	4	14500	13	2640	2	11680	10	139860	98023	122	86
	S.D.	430	10251	10079	9	4070	4	7088	6	834	1	1020	1	850	1	375	0	2203	6615	2	6
8.0 hrs	AVER	2260	69883	67623	59	128427	112	10187	9	4350	4	13000	11	2517	2	11880	10	137553	104950	120	92
	S.D.	941	7847	7533	7	5586	5	3149	3	490	0	1202	1	417	0	249	0	3866	4131	3	4
12.0 hrs	AVER	1853	67163	65310	57	125387	110	7947	7	3850	3	11100	10	2083	2	12203	11	133173	98413	117	86
	S.D.	844	2033	1857	2	7806	7	189	0	141	0	122	0	236	0	314	0	6623	2170	6	2
16.0 hrs	AVER	1297	69480	68183	60	130640	114	6187	5	3750	3	9950	9	2093	2	11207	10	137780	96823	121	85
	S.D.	414	497	350	0	7174	6	628	1	122	0	675	1	105	0	310	0	6572	1262	6	1
24.0 hrs	AVER	1053	71033	69950	61	134240	117	3520	3	3750	3	7000	6	2013	2	12263	11	141057	93787	123	82
	S.D.	127	1935	1948	2	4298	4	236	0	122	0	71	0	58	0	1068	1	4185	2885	4	3
36.0 hrs	AVER	2523	72850	70327	62	127493	112	3573	3	4150	4	6800	6	2297	2	11407	10	136463	94630	119	83
	S.D.	1552	2791	2693	2	8638	8	136	0	579	1	187	0	728	1	728	1	5976	3009	5	3
48.0 hrs	AVER	2190	69133	66943	59	123920	108	3093	3	3600	3	6150	5	2327	2	11307	10	132037	89683	116	78
	S.D.	805	2136	1816	2	1156	1	151	0	122	0	245	0	196	0	642	1	405	1744	0	2
72.0 hrs	AVER	950	77467	76617	67	130400	114	2907	3	3450	3	5850	5	1877	2	10387	9	136577	96610	120	85
	S.D.	102	4015	4099	4	3488	3	38	0	122	0	0	0	91	0	1136	1	3424	4168	3	4
96.0 hrs	AVER	1927	60373	58447	51	123280	108	2693	2	3900	3	5900	5	2303	2	9090	8	131410	78057	115	68
	S.D.	1666	1315	403	0	7190	6	100	0	957	1	464	0	819	1	16	0	3786	1636	3	1
120.0 hrs	AVER	1717	61140	59423	51	129013	113	2640	2	3800	3	6000	5	2637	2	9460	8	137167	79240	120	69
	S.D.	1497	10	1520	1	7723	6	80	0	834	1	150	0	1137	1	740	1	3871	660	3	1

Table A-5: Data for Naphthalene Mineralization in Wet Contaminated Soil Slurry + Strain 5R.

		Sequatchie soil CO2 Trap Results			Hexane Phase		Aqueous Phase		Oxidizer		Mass Balance (%)	
Time		% in Control	% in Exper.	Net % Miner.	% in Control	% in Exper.	% in Control	% in Exper.	% in Control	% in Exper.	Control	Exper.
0 Hrs	AVER	0			78		2		3		83	
	S.D.	0			7		0		0		7	
3.17 Hrs	AVER	0	56	56	77	10	1	6	3	8	81	81
	S.D.	0	2	2	6	1	0	0	0	1	6	2
5.0 Hrs	AVER	0	56	56	87	9	2	5	3	8	92	78
	S.D.	0	2	2	8	0	0	0	0	0	9	2
6.5 Hrs	AVER	0	60	60	82	7	2	5	3	8	87	81
	S.D.	0	6	6	6	0	0	0	0	1	7	7
8.0 Hrs	AVER	0	65	65	114	7	2	5	4	8	121	85
	S.D.	0	5	5	4	0	0	0	0	1	5	6
12.0 Hrs	AVER	0	58	58	76	5	1	4	3	9	81	76
	S.D.	0	0	0	7	1	0	0	1	1	8	2
24.0 Hrs	AVER	0	58	58	78	4	1	3	3	8	83	73
	S.D.	0	2	2	15	0	0	0	1	0	16	3

South Carolina with buffer
CO2 Trap Results

Time		% in Control	% in Exper.	Net % Miner.	Hexane Phase % in Control	Hexane Phase % in Exper.	Aqueous Phase % in Control	Aqueous Phase % in Exper.	Oxidizer % in Control	Oxidizer % in Exper.	Mass Balance (%) Control	Mass Balance (%) Exper.
0 Hrs	AVER	0	2	2	86	85	1	1	6	6	94	94
	S.D.	0	0	0	1	4	0	0	0	1	1	3
8.0 Hrs	AVER	0	69	69	87	12	1	5	6	9	93	95
	S.D.	0	0	0	3	1	0	0	1	0	2	1
12.0 Hrs	AVER	0	74	74	90	9	1	5	6	9	97	97
	S.D.	0	2	2	0	1	0	0	1	0	1	3
25.0 Hrs	AVER	0	75	74	91	7	1	5	6	8	98	95
	S.D.	0	3	3	2	1	0	0	1	0	1	2
30.0 Hrs	AVER	0	75	75	97	5	1	5	5	8	104	93
	S.D.	0	4	4	1	1	0	1	0	1	1	4
36.0 Hrs	AVER	0	71	71	95	4	1	4	6	8	103	88
	S.D.	0	4	4	0	1	0	0	0	0	1	4
64.0 Hrs	AVER	0	71	71	97	3	1	2	5	7	104	84
	S.D.	0	2	2	3	0	0	0	0	1	3	2

South Carolina without buffer

Time		CO2 Trap Results % in Control	% in Exper.	Net % Miner.	Hexane Phase % in Control	% in Exper.	Aqueous Phase % in Control	% in Exper.	Oxidizer % in Control	% in Exper.	Mass Balance (%) Control	Exper.
0 Hrs	AVER	0	2	2	93	84	1	2	7	8	101	96
	S.D.	0	0	0	4	4	0	0	1	1	3	4
8 Hrs	AVER	0	85	85	94	10	1	6	9	5	105	106
	S.D.	0	3	3	2	1	1	0	2	0	2	3
12 Hrs	AVER	0	92	92	98	8	1	6	8	6	107	113
	S.D.	0	2	2	5	0	0	0	1		3	
24 Hrs	AVER	0	91	91	92	5	1	5	8		101	
	S.D.	0	0	0	4	1	0	0	1		3	
30 Hrs	AVER	0	98	98	98	4	1	6	8	7	106	114
	S.D.	0	1	1	3	0	0	0	1	0	3	1
36 Hrs	AVER	0	91	91	96	7	1	6	8	6	105	109
	S.D.	0	10	10	2	4	0	1	1	0	2	5
64 Hrs	AVER	0	102	102	92	2	1	2	8	6	101	112
	S.D.	0	1	1	6	0	0	0	1	0	5	1

Indiana soil
CO_2 Trap Results

Time		% in Control	% in Exper.	Net % Miner.	Hexane Phase % in Control	Hexane Phase % in Exper.	Aqueous Phase % in Control	Aqueous Phase % in Exper.	Oxidizer % in Control	Oxidizer % in Exper.	Mass Balance (%) Control	Mass Balance (%) Exper.
0 Hrs	AVER	1	20	19	90	18	1	3	3	9	94	50
	S.D.	0	1	1	2	9	0	1	0	1	2	6
3.17 Hrs	AVER	1	31	30	85	4	1	4	4	7	90	47
	S.D.	1	8	9	2	1	0	0	1	1	1	7
6.5 Hrs	AVER	1	31	30	89	2	1	4	5	8	95	44
	S.D.	0	3	3	1	0	0	1	1	2	1	5
8.0 Hrs	AVER	1	30	30	78	2	1	3	3	9	82	44
	S.D.	0	1	1	10	0	0	0	0	1	10	1
12.0 Hrs	AVER	1	30	30	82	2	1	3	3	8	86	44
	S.D.	0	4	4	2	0	0	1	0	1	1	4
24.0 Hrs	AVER	1	33	31	78	1	1	3	3	9	83	45
	S.D.	1			5	0	0	0	1	0	4	
48.0 Hrs	AVER	1	37	36	81	1	1	2	3	9	89	50
	S.D.	0	1	1	4	0	0	0		1		1

Etowah soil

Time		CO2 Trap Results % in Control	% in Exper.	Net % Miner.	Hexane Phase % in Control	% in Exper.	Aqueous Phase % in Control	% in Exper.	Oxidizer % in Control	% in Exper.	Mass Balance (%) Control	Exper.
0 Hrs	AVER	0	0	0	78	56	1	1	2	1	81	59
	S.D.	0	0	0	3	20	0	0	0	0	3	20
3.17 Hrs	AVER	0	12	12	72	11	1	5	2	9	75	37
	S.D.	0	4	4	5	2	0	0	0	0	5	3
5.0 Hrs	AVER	0	8	8	77	3	1	4	2	8	80	24
	S.D.	0	0	0	5	1	0	0	0	1	5	2
6.5 Hrs	AVER	0	12	12	79	5	1	5	2	9	81	30
	S.D.	0	4	4	2	2	0	0	0	0	2	6
8.0 Hrs	AVER	0	14	14	77	5	1	4	2	9	79	32
	S.D.	0	8	8	4	2	0	0	0	0	4	6
12.0 Hrs	AVER	0	16	16	79	3	1	4	2	8	81	30
	S.D.	0	5	5	2	0	0	0	0	1	2	5
24.0 Hrs	AVER	0	9	9	76	3	1	3	2	8	78	22
	S.D.	0	2	2	5	0	0	0	0	0	5	1

APPENDIX B

ADSORPTION/DESORPTION DATA BY REBECCA PULLEN

ADSORPTION/DESORPTION OF NAPHTHALENE ON WET CONTAMINATED SOIL METHODS AND MATERIALS

Adsorption and desorption isotherms were run for all four soils (Etowah, Sequatchie, South Carolina, and Indiana). Air-dried soils were sieved with a # 100 mesh brass screen before testing. Triplicate samples with a 1:5 adsorbent:solution ratio were used (10 ml solution with 2 g air-dried soil).

Distilled-deionized H_2O was used as the solvent. Containers were glass test tubes with teflon-lined, screw caps. An empty weight was recorded for each tube. 100 microliters of HgCl (49.955 g/l solution) were added to each tube to sterilize the soil. Six initial concentrations of naphthalene, 0.0, 5.0, 10.0, 15.0, 20.0, and 25.0 micrograms/ml were equilibrated with each adsorbent. 2 μl/ml radiolabeled (C-14) naphthalene were added to each tube as a tracer. One set of concentrations was run without soil in order to determine adsorption of naphthalene to the container walls.

Immediately after addition of the solution, the tubes were vigorously agitated with a vortex mixer. The containers were then placed in a closed styrofoam test tube container to prevent exposure to light. The test tube container was placed on a reciprocating shaker and shaken at 150 cycles per second to keep the adsorbent in suspension

during the shaking period. After 24 hours, the suspensions were centrifuged at 3,000 rpm for 15 minutes. The aqueous solution was immediately sampled by removing 0.5 ml of solution which was placed in 10 mls of aqueous scintillation cocktail (Packard Ultima Gold) and counted on a Packard scintillation counter using the standard C-14 protocol. The tubes were then placed back on the shaker and sampled again after 24 hours to verify equilibrium conditions. Sampling was repeated until repeatable aqueous solution concentrations showed that equilibrium had been achieved.

After reaching equilibrium, the tubes were centrifuged at 3,000 rpm for 15 minutes, the supernatant was decanted and the tubes were weighed to determine the volume of solution remaining with the soil. 10 ml distilled-deionized H_2O were then added to each tube. The tubes were again agitated with a vortex mixer, placed in the styrofoam container, and returned to the shaker for desorption. The sampling procedure given above for adsorption was repeated for desorption until the tubes had again reached equilibrium conditions.

After completing desorption sampling, the supernatant was again decanted and an extraction was performed on the soil using 4:1 hexane:isopropanol as a solvent. 5 ml of solvent were added to each tube. The tubes were vortexed and then placed on the shaker for one hour. After shaking, 5 ml of distilled-deionized H_2O were added to each tube, the

tubes were vortexed, then centrifuged at 3,000 rpm for 15 minutes. 1 ml of the solvent layer was subsequently removed and placed in 10 mls of organic scintillation cocktail (Packard Optifluor O) and counted. The solvent layer was decanted, and 0.5 ml of the water phase was added to 10 ml of aqueous scintillation cocktail and counted.

To complete a mass balance, the soil and solution remaining in each tube were vortexed and emptied into aluminum weigh pans, and dried under a hood. The emptied tubes were also left under the hood with caps off to dry any remaining material. The dried soil in the weigh pans was oxidized with a Harvey Biological Material Oxidizer, and the samples were counted to determine the amount of naphthalene remaining on the soil after desorption and extraction. Finally, the dried tubes were weighed to determine the mass of soil which had adhered to the sides during emptying.

Table B-1: Wet Contamination of Soil

Soil Type	Water (ml)	Naphthalene solution[a] (ml)	% Naphthalene Adsorbed	Concentration on Soil (μg/g)	Concentration in Slurry (ppm)
S. Carolina	3	2	95.0	47.5	7.9
Etowah	2	3	63.6	47.7	7.95
Indiana	3	2	93.7	46.8	7.8
Sequatchie	0	5	42.9	53.6	8.9

[a]concentration of naphthalene in solution is 25 μg/ml.

Table B-2: Adsorption/Desorption Isotherm Data for Naphthalene in Wet Contaminated Soil

	Adsorption			Desorption		
Soil Type	Slope	K (μg/g)	K_{om} (μg/g)	Slope	K (μg/g)	K_{om} (μg/g)
Etowah	0.8507	30.39	1048	0.9742	6.165	212.6
Sequatchie	0.8448	16.10	1477	1.0104	4.606	422.6
Indiana	0.9215	0.147	0.360	1.0067	0.005	0.0134
South Carolina	0.7620	0.476	3.422	1.000	0.005	0.0360

VITA

Mary Louise Korde was born in St. Paul, Minnesota on November 19, 1961. She attended elementary schools in that city and graduated from St. Paul Central High School in June, 1979. The following August she entered The University of Miami and studied Marine Science and Biology for two years. In the autumn of 1981, she transferred to San Diego State University, and in December 1983, she received a Bachelor of Science degree in Biology.

After a two-year agroforestry position with the United States Peace Corps in Kenya and other technical positions, she accepted a teaching assistantship at The University of Tennessee, Knoxville and started study toward a Master's of Science degree in Microbiology in the Fall of 1988. This degree was awarded in August 1991.