BIOAVAILABILITY OF SORBED ORGANIC CONTAMINANTS

By

Jeong-Hun Park

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Civil and Environmental Engineering

2000

ABSTRACT

BIOAVAILABILITY OF SORBED ORGANIC CONTAMINANTS

By

Jeong-Hun Park

The bioavailability of sorbed contaminants was investigated because it has been an important issue in applying bioremediation technology to the restoration of contaminated sites. Experimental and mathematical approaches were developed to evaluate the bioavailability of contaminants sorbed by silica particles and natural soils. Biokinetic parameters in column and batch assays were compared in order to evaluate the role of cell attachment and flow on biodegradation rates.

Commercially available silica, natural soils, and Ottawa sand were selected as sorbents and 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthalene were used as sorbates/substrates. Four organisms capable of naphthalene degradation, and one for 2,4-D degradation were studied. Sorption/desorption isotherms, biodegradation rate studies, and bioavailability assays were conducted in batch systems. Bio-kinetic parameters were also evaluated in both of batch and Ottawa-sand packed columns. A three-site desorption model was developed to depict the desorption process of contaminants sorbed in natural soils. A bioavailability model, which is counted for sorption, desorption, and biodegradation of contaminants in both solid and liquid phases, was developed to evaluate the bioavailability of sorbed contaminants.

This study demonstrates the existence of enhanced bioavailability for some sorbed contaminants, which cannot be explained by desorption and liquid phase degradation alone. Enhanced bioavailability of 2.4-D was observed in silica-slurry systems and an enhanced transformation factor was introduced to express the increased biodegradation rate over that expected from the liquid phase only. However, the bioavailability of sorbed naphthalene in soils varied with the distribution coefficient and biokinetics. For the less sorptive soil, the results could be explained by sequential desorption and degradation processes. For the other soil, enhanced degradation was clearly observed for the organisms with first-order and Michaelis-Menten rates. No enhancement was found for the organism with zero-order kinetics. In all soils, degradation of non-desorbable naphthalene, which could not be removed by successive water extractions, was observed. Several explanations are explored including the development of elevated substrate concentrations at the organism/sorbent interface. Enhanced bioavailability could be described using a model formulation that included sorbed-phase degradation.

Liquid-phase degradation rates using attached organisms in column experiments were found to be lower than with suspended cells in batch experiments. It is believed that this results from at least two factors – exposed reduction of exposed cell surface area and heterogeneity of cell distribution.

DEDICATION

To my wife, Hae-Sook Kim

ACKNOWLEDGMENTS

This achievement could not have been completed without the special assistance of following people. My great thanks go to my major professor, Dr. Thomas C. Voice who gave me guidance, encouragement and advice through the research. I appreciate my committee's professional help with expert knowledge: Dr. Stephen A. Boyd of the Department of Crop and Soil Science for his introduction to soil organic chemistry; Dr. Roger B. Wallace for helping me to understand groundwater systems; Dr. Syed Hashsham for helping me to understand biological treatment processes; and Dr. Xianda Zhao for introducing laboratory and experimental skills, and guiding direction through the research (also my tennis partner).

I would also like to thank other people for their special help. Dr. Denise Kay did the experiments for chapter 3 and helped me analyze the data. Dr. Paul Loconto and Mr. Yanlyang Pan taught me the operation of the analytical instruments and helped with method development, and secretarial personnel, Linda Steinman and Lori Hesse, assisted me in many administrative aspects.

I cannot forget the initial laboratory help of Mr. Dave Berends and John Zimmerman. I enjoyed the valuable information exchange on my research with my fellow graduate student Stephen Callister (also my tennis partner), my office mates Chris Saffron (also my racquet ball partner), Kyung-Hyuk Lee, and Dr. Jerry Wu. I appreciate the encouragement and friendship of Dr. Jae-Woo Park in Anadigics Co. and his family.

I am grateful to all my family for their encouragement and support for my study abroad. Especially, my heartfelt thanks go to my parents and my parents-in-law for their unwavering love and support that encouraged me to complete this goal.

I owe a great dept to my lovely wife, Hae-Sook Kim. She has supported my study by dint of rare diligence and perseverance, and managed our home hopefully and enjoyably. I appreciate my lovely daughter, So-Young, and son, Ki-Lyang, for their healthy and happy faces.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION AND OBJECTIVES

INTRODUCTION

Organic contaminants in soils and sediments originate from a variety of anthropogenic activities. Major contaminants such as benzene, toluene, ethylbenzene, and xylene (BTEX), poly-aromatic hydrocarbons (PAHs), poly-chlorinated biphenyls (PCBs), pesticides and herbicides are of environmental concern because of their documented or suspected mutagenic and carcinogenic effects. Therefore, the fate of these compounds at contaminated sites and the restoration of the sites are of high public concern. Over 50,000 organic contaminated sites and several hundred thousand leaking underground storage tanks were reported in United States (Singleton, 1994). The US Environmental Protection Agency (EPA) has spent over a billion dollars each year from 1990 to clean up hazardous waste sites; a large portion of which are contaminated with organic chemicals (Hileman, 1999).

Bioremediation is an emerging technology, which is attractive for cleaning up these contaminants because it can treat them in-situ with little disturbance to the contaminated matrix and because the contaminants can often be completely mineralized to inorganic materials (Head, 1998). Also, bioremediation is relatively inexpensive compared to incineration, soil washing, and pump and treat (Singleton, 1994; Hughes et al., 1997). For example, Hughes et al. (1999) reported that bioremediation was used successfully and economically (compared to incineration: $55 million vs. $120 million) to restore lagoon sediments containing mixture organic contaminants including PCBs, PAHs, bezene, toluene, ethylbenzene, xylenes (BTEX), chlorinated solvents, and pesticides. However, this technology is not widely adopted because of the uncertainty of

the success. PAHs and PCBs are composed of aromatic rings and are hydrophobic, resulting in low aqueous solubility and a strong tendency to sorb to the matrix of organic material in soil or to partition into oily phases at contaminated sites (Harayama, 1997). Due to these chemical characteristics, the bioavailability of sorbed contaminants in natural sorbents has been an important issue in applying bioremediation technology to remediation of contaminated sites (Willumsem and Arvin, 1999). Bioavailability of sorbed contaminants in soils plays a significant role in successful bioremediation and it is an important factor in determination of environmentally acceptable end-points (ES&T, 1998). Bioavailability is defined as the extent of contaminant that is externally available for utilization by organisms (Hamelink et al., 1994).

OBJECTIVES

This study was designed to investigate bioavailability of contaminants sorbed by solids/materials. In order to investigate this phenomenon systematically, four main objectives were developed. Each of the following chapters is dedicated to a single objective.

The first objective (chapter 3) was designed to develop experimental and mathematical approaches to evaluate bioavailability of contaminants sorbed by an artificial sorbent. Desorption rates were measured in the absence of organisms and biodegradation rates were measured in the absence of solids. Combined desorption/biodegradation experiments were then performed, and the results were analyzed using a simple mathematical model incorporating three processes: desorption, liquid-phase degradation and sorbed-phase degradation. This approach allowed us to

determine whether a sorbed-phase degradation mechanism was consistent with the bioavailability data. 2,4-dichlorophenoxyacetic acid (2,4-D) was used as the sorbate and substrate, silica as the sorbent, and *Flavobacterium* sp. FB4 as 2,4-D degrading organism.

The second objective (chapter 4) was designed to evaluate the effects of different biokinetic processes on the bioavailability of sorbed naphthalene in natural soils. In this study, four organisms (ATCC 17484, NP-Alk, *Pseudomonas putida* G7, and NCIB 9816-4) haveing different degradation kinetics (zero-order, first-order, and Michaelis-Menten) were selected. Two of the organisms (ATCC 17484, NP-Alk) have been previously used to study bioavailability of sorbed naphthalene based on mineralization. *Pseudomonas putida* G7 and NCIB 9816-4 were selected because it was recently reported that they were chemotactic to solid naphthalene.

The third objective (chapter 5) was designed to develop a mathematical model to quantitatively describe the bioavailability. In this study, degradation assays were used instead of mineralization assays, and two organisms (which were chemotactic to solid naphthalene) and four soils (which have different organic carbon contents and different distribution coefficients of naphthalene) were selected. The developed model described the relationship among sorption/desorption, and biodegradation of chemicals in soil slurries. Model parameters for sorption, desorption and biodegradation of a dissolved chemical were evaluated independently. Sorbed-phase degradation rate coefficients, which were an indicator to explicitly describe the enhanced bioavailability of the sorbed chemical, were enumerated by non-linear regression fitting of the model to experimental data.

The fourth objective (chapter 6) was designed to evaluate the relationship between kinetic parameters in both batch and column systems. This was accomplished by selecting a sand/contaminant/organism combination that resulted in significant biomass attachment but only minimal contaminant sorption to the sand in the columns. In addition, relatively high flow rates and low total biomass levels were used to minimize external and internal mass-transfer resistances, respectively. The effect of residence time could be evaluated independent of flow rate by using columns of different lengths, and it became possible to simultaneously evaluate all of the degradation parameters.

REFERENCES

Hamelink, J. L., P. F. Landrum, H. L. Bergman and W. H. Benson. 1994. "Bioavailability: Physical, chemical, and biological interactions." *Chelsea, Michigan: Lewis Publishers.*

Harayama, S. 1997. "Polycyclic aromatic hydrocarbon bioremediation design." *Current Opinion in Biotechnology* 8: 268-273.

Head, I. M. 1998. "Bioremediation: towards a credible technology." *Microbiology-UK* 144: 599-608.

Hileman, B. 1999. "Research targets hazardous waste." *Chem. Eng. News* 77: 24-25.

Hughes, J. B., D. M. Beckles, S. D. Chandra and C. H. Ward. 1997. "Utilization of bioremediation processes for the treatment of PAH-contaminated sediments." *Journal of Industrial Microbiology & Biotechnology* 18: 152-160.

Singleton, I. 1994. "Microbial Metabolism of Xenobiotics: Fundamental and Applied Research." *J. Chem. Tech. Biotechnol.* 59: 9-23.

Willumsem, P. A. and E. Arvin. 1999. "Kinetics of Degradation of Surfactant-Solubilized Fluoranthene by a *Sphingomonas paucimobilis.*" *Environ. Sci. Technol.* 33: 2571-2578.

CHAPER 2. LITERATURE REVIEW

The purpose of this chapter is to review the factors affecting sorption of chemicals to natural sorbents and bioavailability of sorbed contaminants.

FACTORS AFFECTING SORPTION OF CHEMICALS

Sorption of organic chemicals by soil decreases the liquid-phase concentration in the system. The decreased liquid concentration decreases the available amount of contaminants to the organisms that can utilize only dissolved chemicals. Therefore, it is generally known that sorption decreases bioavailability of contaminants, at least in comparison to systems with the some contaminant mass and no soil. Factors affecting sorption of organic chemicals are discussed below.

Sorbent

Mineral surfaces can be charged positively or negatively depending on the pH and solution composition (Stumm and Morgan, 1995). The surface charge affects the sorption of polar and ionic organic chemicals, such as pyridine and triazines (Laird and Fleming, 1999). Surface charge is established by exchanging of protons or metal ions that exist in the solution. For example, the negatively charged surface of montmorillonite is converted into a positive surface in the presence of hydroxy aluminium ($Al(OH)_x$) (Sannino et al., 1997). The modified surface promotes the adsorption of the negatively charged 2,4-Dichlorophenoxiacetic acid (2,4-D) (Sannino et al., 1997). Sannino et al. also reported that there was adsorption of 2,4-D into montmorillonite in phosphate buffer solution (Sannino et al., 1997). They explained that the adsorption could not occur explicitly because both 2,4-D and montmorillonite are negatively charged in the solution and both

of them repel each other preventing adsorption. However, they concluded that some impurities of magnesium or calcium ions, which came from magnesium or calcium phosphate, collect on montmorillonite surfaces. Therefore, the positive sites created by magnesium and calcium ions are used as adsorption sites for 2,4-D anions.

The sorption of non-ionic organic compounds (NOCs) to soils strongly correlates with soil organic matter (SOM) content of the soils (Novak, 1999) and the contaminant's relative hydrophobicity, which is described by its octanol-water partition coefficient (Voice and Weber, 1983). This is because SOM fractions as a separate sorbing phase, with a capacity that normally overshadows that of mineral soil core. Sorption to the mineral surface is typically thought of a surface phenomenon, and is non-linear and competitive, while the sorption of chemicals into organic matter is known to follow a partitioning that is a linear and a noncompetitive sorption process (Chiou et al., 1979). The distribution coefficient of a nonpolar organic compound is proportional to the amount of SOM in the soil, because the amount of adsorption on mineral surface is negligible compared to the amount of partitioning into SOM.

Aggregated clay has different sorption characteristics from the mineral surface and SOM. Geerdink et al (1996) evaluated sorption onto three soil fractions: sand (massive silicate particles), organic matter, and clay (agglomerates of clay platelets). Sorption and desorption of the contaminants was fast in sand. Sorption into organic matter was faster than into clay. The clay fraction exhibited extremely low desorption rate for nonpolar chemicals; about 1000 times lower than the organic matter fraction.

Dissolved organic matter (DOM)

DOM enhances water solubility of organic pollutants and decreases the distribution coefficient of organic contaminants in soils and sediments (Chiou et al., 1986). The effect of DOM on solubility and sorption of organic chemicals varies with the properties of the chemicals. Plaehn et al.(1999) reported that the effect of DOM on sorption and desorption of naphthalene to soil was not significant. They concluded that the negligible impact on the distribution coefficient of naphthalene resulted from being only moderately hydrophobic. However the sorption of phenanthrene to humic acid, which is composed of hydrophilic and hydrophobic moieties, was observed to increase the solubility in a study by Ortega-Calvo and Saiz-Jimenez (1998). In Chiou et al study, DOM strongly enhanced the solubility of DDT and 2,4,5,2',5'-PCB which have low water solubility (Chiou et al., 1986), while the solubility enhancement of lindane and 1,2,3-trichlorobenzene was negligible due to their highly water soluble properties. The enhanced solubility of hydrophobic chemicals stems from partitioning of hydrophobic chemicals into an intramolecular nonpolar organic environment provided by DOM (Chiou et al., 1986). The main forces for binding of zenobiotic chemicals into DOM are nonbonded forces (e.g., van del Waals) and hydrogen bonds (Schulten, 1999).

Other factors

Aging, temperature and pH are also important factors on sorption of organic compounds. Aging increases the sorption of organic chemicals, allowing more time to partition into the hard organic polymeric matrix and to sorb into micro-voids or microporous minerals (e.g., zeolites) (Luthy et al., 1997). The distribution coefficient of a hydrophobic organic contaminant typically decreases as temperature increases, because

the heat of sorption for many hydrophobic organic contaminants is negative, i.e sorption is an exothermic reaction (Zhang et al., 1998). The enthalpy values (ΔHs) of sorption become more negative as the size of the organic chemical increases. Piatt et al (1996) have shown that ΔHs of pyrene was more negative than that of naphthalene, and the distribution coefficient of pyrene decreased more sensitively as temperature increased. pH can increase or decrease sorption of ionic organic compounds due to changing the charge of the mineral surface and ionic organic compounds (Laird and Fleming, 1999).

FACTORS AFFECTING BIOAVAILABILITY OF SORBED CHEMICALS

There has been a debate as to the occurrence of direct bioavailablility, which means that organisms can utilize directly the sorbed chemicals (Marshman and Marshall, 1981; Subba-Rao and Alecander, 1982; Amador and Alexander, 1988; Griffith and Fletcher, 1991; Guerin and Boyd, 1992; Guerin and Boyd, 1993; Crocker et al., 1995; Calvillo and Alexander, 1996; Guerin and Boyd, 1997; Ortega-Calvo and Saiz-Jimenez, 1998; Tang et al., 1998), and indirect bioavailability, where the sorbed chemicals can only be utilized after desorption (Weber and Cole, 1968; Moyer et al., 1972; Steen et al., 1980; Ogram et al., 1985; Shimp and Young, 1988; Smith et al., 1992; Weissenfels et al., 1992; Shelton and Doherty, 1997). The bioavailability of sorbed chemicals appears to depend on the properties of organisms used as well as the characteristics the sorbent and experimental methodology.

Sorbent

The bioavailability of organic compounds sorbed to solids is dependent on physical-chemical properties of the sorbents such as pore size distribution, aggregation,

hydrophobicity, and organic matter content. An inverse relationship has been suggested between bioavailability and sediment organic carbon content, resulting from an increased distribution coefficient of organic chemical and a decrease in liquid-phase concentration. Also, direct relationship between particle size and chemical availability has been reported since fine sediments increase the surface area available for adsorption (Knezovich et al., 1987).

The effect of surface hydrophobicity on bioavailability was studied by Nam and Alexander (Nam and Alexander, 1998). The mineralization of phenanthrene was rapid and extensive in the presence of beads with hydrophilic surface such as glass and silica. The rate of biodegradation in a bead slurry system was the same as a solids-free system, suggesting that the bacterium readily degraded the sorbed phenanthrene to hydrophilic surface. However, bioavailability of phenanthrene (through mineralization of phenanthrene) was reduced by sequestration and sorption into hydrophobic sites inside nanopores.

A few researchers have studied the relationship between organic matter content in soils and bioavailability of partitioned contaminants. Guerin and Boyd (1997) reported that no correlation existed between initial mineralization rate (IMR) or extent of mineralization and soil organic matter content, while Ortega-Calvo et al (1997) reported that the mineralization rate of phenanthrene was related to the content of organic matter in soils; that is, the rates and extents of mineralization decreased with organic matter in soils.

The physical characteristics of sorption sites have also been reported to affect bioavailability of sorbed chemicals. Knaebel et al.(Knaebel et al., 1994) used dodecyl

linear alkylbenzene sulfonate (LAS), dodecyl linear alcohol ethoxylate(LAE), sodium stearate and stearyl trimethylammonium chloride (STAC) as organic compounds, and sand, montmorillonite, kaolinite, illite, and humics as sorbents in mineralization assays to study bioavailability of sorbed organic compounds. The extent of bioavailability of organic compounds sorbed on sand, kaolonite and illite was higher than that on montmorillonite and humics. It is reported that this is because sand has exclusively external binding sites, and kaolinite and illite are nonswelling clays whose binding surface are primarily external. In contrast, montmorillonite is an expandable clay and 80% of the binding surfaces are interstitial. Externally sorbed organic compounds were reported to be more accessible to organisms. In addition, nonporous sorbent provides more enhanced bioavailability than sorbent with microporous.

The effects of humic acid (HA), "bare minerals (sematite and montmorillonite)" (mineral with no HA coating) and mineral-HA complex on bioavailability of phenanthrene were addressed by Laor et al (Laor et al., 1999). They used mixed culture (dominated by *Pseudomonas* sp.) that was enriched from a coal tar contaminated soil. HA did not enhance nor inhibit initial phenanthrene mineralization; that is, the ratios of initial mineralization rate (IMR) in the presence of dissolved HA to that of the control were close to one. While the mineral-HA complexes always stimulated the initial mineralization of phenanthrene, resulting in a higher value than one for the ratios of IMR in the presence of the mineral-HA complexes to that of the control. However, in the presence of bare materials, IMR was either stimulated or inhibited. In conclusion, their study suggested some important information on bioavailability. There was no evidence that HA stimulated the mineralization by providing an addition carbon source to bacteria.

IMR value increased with sorbed phenanthrene to bare mineral and mineral-HA complex, suggesting sorption of bacteria to phenanthrene-enriched surfaces might enhance IMR.

DOM

The DOM effect on bioavailability is more complex, depending on substrate, characteristic and concentration of the DOM. The effect of DOM or HA on the mineralization rate of naphthalene has been reported to be negligible (Meredith and Radosevich, 1998; Plaehn et al., 1999), while humic acid significantly increased the mineralization rate of benzoic acid and phenylactic acid at low concentration (Amador and Alexander, 1988). Ortega-Calvo and Saiz-Jimenez (Ortega-Calvo and Saiz-Jimenez, 1998) insisted that phenanthrene mineralization is enhanced, the extent of mineralization is increased, and the acclimation period is shortened by the presence of dissolved humic acids and humic acid-clay complex. Hatzinger and Alexander (Hatzinger and Alexander, 1995) also showed that the mineralization rate of phenanthrene was rapid in presence of DOM. On the other hand, Laor et al (1999) reported that the IMR of phenanthrene was not affected by the presence of HA. Haitzer et al (Haitzer et al., 1998) claimed that DOM can decrease the bioavailability of organic chemicals in aquatic systems due to binding of organic chemicals into DOM. Knaebel et al. reported that the initial mineralization of the chemicals presorbed to humics much lower than those of clay complexes, because binding between organic chemicals and humics is hydrophobic, and includes some covalent interactions, while binding with clays involves ionic or hydrogen bonding (Knaebel et al., 1994). The strong binding between organic chemicals and organic matter would result in longer persistence of organic chemicals preventing utilization by organisms.

The enhanced bioavailability of organic chemicals in the presence of DOM can be explained by the sorption of chemicals into DOM; that is, the sorption into DOM increases the chemicals' concentration in the vicinity of the bacterial cells that can physically attach to the DOM (Ortega-Calvo and Saiz-Jimenez, 1998). Inhibition in presence of DOM results from decreased concentration of organic chemicals in aquatic system due to binding of chemicals into DOM (Haitzer et al., 1998). Further research is needed to clarify the relationship between DOM and bioavailability of organic chemicals resulting from sorption, aging, DOM structure, organism characteristic, and organic chemical property effects.

Substrate

The enhanced bioavailability of sorbed chemicals mainly happens in high molecular substrate (Knezovich et al., 1987). For example, Griffith and Fletcher reported that strongly sorbed bovine serum albumin (BSA; molecular weight, ca. 68,000) was rarely available to suspended cells, but was rapidly hydrolyzed by attached cells due to excreted enzymes. And non-sorbed methyl-coumarinyl-amide-leucine (MCA-leucine; molecular weight, ca. 385) was hydrolyzed faster by suspended than attached cells(Griffith and Fletcher, 1991). They suggested that when the sorption extent of a substrate was low, it was easily accessible to suspended bacteria, in contrast, when the sorption extent of a substrate was high it was more available to surface-attached bacteria (Knezovich et al., 1987). The limit of molecular weight was not clearly defined.

Aging

The aging effect on bioavailability of sorbed contaminant in soil, sediment and aquifer materials is poorly understood and published articles on it are rare. It is reported

that the bioavailability of organic chemicals decreases with aging, the contact time between organic chemicals and solid. For example, the bioavailability of chemicals such as phenanthrene, atrazine (Chung and Alexander, 1998), 4-nitrophenol (Hatzinger and Alexander, 1995), 1,2-dibromoethane (Steinberg et al., 1987), simazine (Scribner et al., 1992) and native herbicide (Pignatello and Huang, 1991) declined over aging times.

If bioavailability can be predicted by physicochemical properties of sorbed chemicals, the reasonable endpoint of bioremediation would be estimated by physicochemical properties of sorbed chemicals. A clear correlation has not been reported between bioavailability and physicochemical properties of sorbed chemicals. Pignatello and Huang (Pignatello and Huang, 1991) found that the nonequilibrium site sorption fraction of atrazine and metolachor increased and the bioavailability decreased with aging time in field. Hatzinger and Alexander reported that both bioavailability and extractability of phenanthrene and 4-nitrophenol added to soil decreased with aging time (Hatzinger and Alexander, 1995). However, Chung and Alexander found it was difficult to generalize the relationship between the bioavailability and the extent of extraction of aged phenanthrene and atrazine sorbed to soil(Chung and Alexander, 1998). Carmichael et al. reported that desorption rates of phenanthrene and chrysene freshly sorbed to soil were much faster than the measured mineralization rates; however the desorption rates of aged phenanthrene and chrysene in contaminated soil were equal to or slower than mineralization rates (Carmichael et al., 1997). More research is required to evaluate the relationship between bioavailability and physicochemical properties of sorbed organic chemicals with and without aging.

Contaminant aging effects with organic matter and humic materials have not been extensively studied. One published study claimed that organic matter and humin aging effect on mineralization of phenanthrene was not significant compared to the soil aging effect (Hatzinger and Alexander, 1995).

Organisms

Characteristics of organisms are an important factor on determining bioavailability of sorbed chemicals. It is known that organisms, which secret extracellular enzymes, can utilize the sorbed chemicals (Griffith and Fletcher, 1991). It is also reported that some sorbed bacteria without extracellular enzymes have access to the sorbed chemicals and are able to utilize sorbed chemicals (Guerin and Boyd, 1992; Harms and Zehnder, 1995).

Kefford et al. (1982) used three bacteria; *Leptospira biflexa patoc* 1, which adheres reversibly, pigmented *Serratia marcescens* EF190, which adheres irreversibly, and a non-pigmented hydrophilic mutant of EF190, to study the effect of bacterial adhesion on utilization of stearic acid coated on a glass surface (Kefford et al., 1982). The hydrophobic pigmented *Serratia* strain had greater scavenging ability in removing sorbed fatty acid than the hydrophilic non-pigmented mutant. They suggested that the strong association of the hydrophobic strain with the solid allowed closer interaction to sorbed stearic acid. *Serratia* initially showed a faster rate of removal, but the overall rate was considerably slower than that of the *Leptospira*. It was suggested that *Leptospira* could readily move to other surface areas as substrate was depleted in its vicinity, while Serratia could not move to other surface site after depleting substrate near its locality due to its strong attachment.

Guerin and Boyd found that sorbed naphthalene was directly available to *Pseudomonas putida* strain 17484, while it was not directly available to *Alcaligenes* sp. Strain NP-Alk, suggesting bioavailability of sorbed contaminants is organism-specific (Guerin and Boyd, 1992). They also suggested that an organism attached to the soil more efficiently utilized sorbed naphthalene than a nonattaching strain (Guerin and Boyd, 1992). Tang et al (1998) reported that bacteria cultured on sorbed phenanthrene on a solid easily mineralized the compound sorbed to polyacrylic beads or sediments, while bacteria cultured on non-sorbed phenanthrene were not able to utilize sorbed phenanthrene to the beads (Tang et al., 1998). They suggested that the microorganisms grown on nonsorbed compounds might be inappropriate for evaluation of biodegradation and bioremediation of sorbed contaminants. Calvillo and Alexander (1996) reported that a microbial consortium mineralized biphenyl sorbed to polyacrylic beads, but pure cultures of bacteria isolated from the consortium were not able to mineralize the sorbed compounds, suggesting that cells from consortium were able to attach to the beads and the attachment of cells was an important factor in mineralization of sorbed biphenyl (Calvillo and Alexander, 1996). Harms and Zehnder showed that the rate of utilization by *Sphingomonas* sp. of 3-dichlorodibenzofuran sorbed to porous Teflon was influenced by the tendency of the bacterium to adhere to the sorbent, indicating bacteria adhesion is an important factor in degradation of sorbed chemicals(Harms and Zehnder, 1995). In aquatic environment, epibenthic and infaunal organisms may more accumulate sorbed pollutants by direct contact than pelagic and epifaunal organisms (Knezovich et al., 1987). The attachment of bacteria to sorbent appears to play a significant role on the bioavailability of sorbed chemicals.

However, no mechanisms on the direct uptake have been suggested and the specific characteristics of the organisms such as cell surface structure, biodegradation kinetics, morphology, and mobility at surface have not been explicitly studied.

CONCLUSION

The amount of sorption of organic chemicals into soil is dependent on properties of the sorbent, characteristics of dissolved organic matter, aging, temperature, pH, and the properties of sorbate. Generally, sorption decreases the bioavailability of organic chemicals due to decreased liquid phase concentration of the chemicals. However, the enhanced bioavailability of organic chemicals in the presence of the sorbent has been reported. The bioavailability of sorbed chemicals is complex, depending on properties of sorbent, DOM, and organism, and aging time. Surface sorption of organic chemicals and the attachment of organisms have a positive correlation with the bioavailability of sorbed chemicals, but mechanisms have not been clearly delaminated. More investigation is needed to evaluate the relationship between bioavailability, the physicochemical properties of sorbents and sorbed chemicals, and the characteristics of organisms.

REFERENCES

Amador, J. A. and M. Alexander. 1988. "Effect of Humic Acids on the Mineralization of Low Concentrations of Organic Compounds." *Soil Biol. Biochem.* 20: 185-191.

Calvillo, Y. M. and M. Alexander. 1996. "Mechanism of microbial utilization of biphenyl sorbed to polyacrylic beads." *Appl. Microbiol. Biotechnol* 45: 383-390.

Carmichael, L. M., R. F. Christman and F. K. Pfaender. 1997. "Desorption and Mineralization Kinetics of Phenanthrene and Chrysene in Contaminated Soils." *Environ. Sci. Technol.* 31: 126-132.

Chiou, C. T., R. L. Malcolm, T. I. Brinton and D. E. Kile. 1986. "Water Solubility Enhancement of Some Organic Pollutants and Pesticides by Dissolved Humic and Fulvic Acids." *Environ. Sci. Technol.* 20: 502-508.

Chiou, C. T., L. J. Peters and V. H. Freed. 1979. "Physical Concept of Soil-Water Equilibria for Nonionic Organic Compounds." *Science* 206: 831-832.

Chung, N. and M. Alexander. 1998. "Differences in Sequestration and Bioavailability of Organic Compounds Aged in Dissimilar Soils." *Environ. Sci. Technol* 32: 855-860.

Crocker, F. H., W. F. Guerin and S. A. Boyd. 1995. "Bioavailability of Naphthalene Sorbed to Cationic Surfactant-Modified Smectite Clay." *Environ. Sci. Technol* 29: 2953-2958.

Geerdink, M. J., M. C. M. van Loosdercht and K. C. A. M. Luyben. 1996. "Model for Microbial Degradation of Nonpolar Organic Contaminants in a Soil Slurry Resctor." *Environ. Sci. Technol* 30: 779-786.

Griffith, P. C. and M. Fletcher. 1991. "Hydrolysis of Protein and Model Dipeptide Substrates by Attached and Nonattached Marine Pseudomonas sp. Strain NCIMB 2021." *Appl. Environ. Microbiol* 57: 2186-2191.

Guerin, W. F. and S. A. Boyd. 1992. "Differential Bioavailability of Soil-Sorbed Naphthalene to Two Bacterial Species." *Appl. Environ. Microbiol* 58: 1142-1152.

Guerin, W. F. and S. A. Boyd. 1993. "Bioavailability of Sorbed Naphthalene to Bacteria: Influence of Contaminant Aging and Soil Organic Carbon Content." *Sorption and Degradation of Pesticides and Organic Chemicals in Soil. SSSA Special Publication no* 32: 197-208.

Guerin, W. F. and S. A. Boyd. 1997. "Bioavailability of naphthalene associated with natural and synthetic sorbents." *Water Res.* 32: 1504-1512.

Haitzer, M., S. Hoss and W. Traunspurger. 1998. "Effects of Dissolved Organic Matter (DOM) on the Bioconcentration of Organic Chemicals in Aquatic Organisms-a Review." *Chemosphere* 37: 1335-1362.

Harms, H. and A. J. B. Zehnder. 1995. "Bioavailability of Sorbed 3-Chlorodibenzofuran." *Appl. Environ. Microbiol* 61: 27-33.

Hatzinger, P. B. and M. Alexander. 1995. "Effect of aging of chemicals in soil on their biodegradability and extractability." *Environ. Sci. Technol* 29: 537-546.

Hughes, J. B., D. M. Beckles, S. D. Chandra and C. H. Ward. 1997. "Utilization of bioremediation processes for the treatment of PAH-contaminated sediments." *Journal of Industrial Microbiology & Biotechnology* 18: 152-160.

Kefford, B., S. Kjelleberg and K. C. Marshall. 1982. "Bacterial Scavenging: Utilization of Fatty Acids Localized at a Solid-Liquid Interface." *Arch Microbiol* 133: 257-260.

Knaebel, D. B., T. W. Federle, D. C. Mcavoy and J. R. Vestal. 1994. "Effect of Mineral and Organic Soil Constituents on Microbial Mineralization of Organic Compounds in a Natural Soil." *Appl. Environ. Microbiol.* 60: 4500-4508.

Knezovich, J. P., F. L. Harrison and R. G. Wilhelm. 1987. "The Bioavailability of Sediment-Sorbed Organic Chemicals: A Review." *Water, Air, and Soil Pollution* 32: 233-245.

Laird, D. A. and P. D. Fleming. 1999. "Mechanisms for adsorption of organic bases on hydrated smectite surfaces." *Environ. Toxicol. Chem.* 18: 1668-1672.

Laor, Y., P. F. Strom and W. J. Farmer. 1999. "Bioavailability of Phenanthrene Sorbed to Mineral-Associated Humic Acid." *Wat. Res.* 33: 1719-1729.

Luthy, R. G., G. R. Aiken, M. L. Brusseau, S. D. Cunningham, P. M. Gschwend, J. J. Pignatello, S. M. Reinhard, J. Traina, J. R. W.J. Weber and J. C. Westall. 1997. "Sequestration of Hydrophobic Organic Contaminants by Geosorbents." *Environ. Sci. Technol* 31: 3341-3347.

Marshman, N. A. and K. C. Marshall. 1981. "Bacterial Growth on Proteins in the Presence of Clay Minerals." *Soil Biol. Biochem.* 13: 127-134.

Meredith, C. E. and M. Radosevich. 1998. "Bacterial degradation of homo- and heterocyclic aromatic compounds in the presence of soluble/colloidal humic acid." *J. Environ. Sci. Health Part B Pestic. Contam. Agric. Wastes* 33: 17-36.

Moyer, J. R., R. J. Hance and C. E. McKone. 1972. "The Effect of Adsorbents on the Rate of Degradation of Herbicides Incubated with Soil." *Soil Biol. Biochem.* 4: 307-311.

Nam, K. and M. Alexander. 1998. "Role of Nanoporosity and Hydrophobicity in Sequestration and Bioavailability: Tests with Model Solids." *Environ. Sci. Technol.* 32: 71-74.

Novak, J. M. 1999. "Soil factors influencing atrazine sorption: Implications on fate." *Environ. Toxicol. Chem.* 18: 1663-1667.

Ogram, A. V., R. E. Jessup, L. T. Ou and P. S. C. Rao. 1985. "Effects of Sorption n Biological Degradation Rates of (2,4-Dichlorophenoxy)acetic Acid in Soils." *Appl. Environ. Microbiol* 49: 582-587.

Ortega-Calvo, J. J., M.Lahlow and C. Saiz-Jimenez. 1997. "Effect of Organic Matter and Clays on the Biodegradation of Phenanthrene in Soils." *Int. Biodeterior. Biodegrad* 40: 101-106.

Ortega-Calvo, J. J. and C. Saiz-Jimenez. 1998. "Effect of humic fractions and clay on biodegradation of phenanthrene by a Pseudomonas fluorescens strain isolated from soil." *Appl. Environ. Microbiol.* 64: 3123-3126.

Park, J. H., X. Zhao and T. C. Voice. 1999. "Comparison of Kinetic Parametes in Batch and Column Systemes." *Environmental Progress* in process: .

Pignatello, J. J. and L. Q. Huang. 1991. "Sorptive Reversibility of Atrazine and Metolachor Residues in Field Soil Samples." *J. Environ. Qual.* 20: 222-228.

Plaehn, W. A., X. Zhao, B. E. Dale and T. C. Voice. 1999. "Dissloved Organic Material Impact on the Desorption Rate and Mineralization Rate of Naphthalene." *J. Soil Contam.* 8: 491-507.

Sannino, F., A. Violante and L. Gianfreda. 1997. "Adsorption-Desorption of 2,4-D by Hydroxy Aluminium Montmorillonite Complexes." *Pestic. Sci* 51: 429-435.

Schulten, H. R. 1999. "Interactions of dissolved organic matter with xenobiotic compounds: Molecular modeling in water." *Environ. Toxicol. Chem.* 18: 1643-1655.

Scribner, S. L., T. R. Benzing, S. Sun and S. A. Boyd. 1992. "Desorption and Bioavailability of Aged Simazine Residues in Soil from a Continuous Corn Field." *J. Environ. Qual.* 21: 115-120.

Shelton, D. R. and M. A. Doherty. 1997. "A model describing pesticide bioavailability and biodegradation in soil." *Soil Sci. Soc. Am. J* 61: 1078 – 1084.

Shimp, R. J. and R. L. Young. 1988. "Availability of organic chemicals for biodegradation in settled bottom sediments." *Ecotoxicol Environ Saf* 15: 31-45.

Smith, S. C., C. C. Ainsworth, S. J. Traina and R. J. Hicks. 1992. "Effect of sorption on the biodegradation of quinoline." *Soil Sci. Soc. Am. J.* 56: 737-746.

Steen, W. C., D. F. Paris and G. L. Baughman. 1980. "Effects of Sediment Sorption on Microbial Degradation of Toxic Substances." *Contaminants and Sediments. Vol. 1. Ann Arbor Sci. Publ., Ann Arbor, MI.* 1: 477-482.

Steinberg, S. M., J. J. Pignatello and B. L. Sawhney. 1987. "Persistence of 1,2-dibromoethane in soils: Entrapment in intra-particle micropores." *Enviorn. Sci. Technol* 21: 1201-1208.

Stumm, W. and J. J. Morgan. 1995. Aquatic Chemistry (Chemical Equilibria and Rates in Natural Waters). New York, John Wiley and Sons, Inc.

Subba-Rao, R. V. and M. Alecander. 1982. "Effect of Sorption on Mineralization of Low Concentrations of Aromatic Compounds in Lake Water Samples." *Appl. Environ. Microbiol.* 44: 659-668.

Tang, W. C., J. C. White and M. Alexander. 1998. "Utilization of sorbed compounds by microorganisms specifically isolated for that purpose." *Appl. Microbiol. Biotechnol.* 49: 117-121.

Voice, T.C. and W.J. Weber, jr. 1983. " Sorption of hydrophobic compounds by sediments, soils and suspended solids-I; Theory and Background". *Water Res.* 17:1433-1441.

Weber, J. B. and H. D. Cole. 1968. "Microbial Decomposition of Diquat Adsorbed on Montmorillonite and Kaolinite Clays." *J. Agric. Food Chem.* 16: 475.

Weissenfels, W. D., H. J. Klewer and J. Langhoff. 1992. "Adsorption of Polycycleic Aromatic-Hydrocarbons (PAHS) by soil particles- Influence ofn Biodegradatility and Biotoxicity." *Appl. Microbiol. Biotechnol.* 36: 689-696.

Zhang, W. X., E. J. Bouwer and W. P. Ball. 1998. "Bioavailability of hydrophobic organic contaminants-effects and implications of sorption-related mass-transfer on bioremediation." *Ground Water Monit. R.* 18: 126-138.

CHAPTER 3. KINETIC MODELING OF BIOAVAILABILITY FOR SORBED-PHASE 2,4-DICHLOROPHENOXYACETIC ACID

ABSTRACT

The degradation rate of 2,4-dichlorophenoxyacetic acid (2,4-D) was studied in silica-slurry systems to evaluate the bioavailability sorbed-phase contaminant. After the silica particles were saturated with 2,4-D, the system was inoculated with the 2,4-D degrading microorganism *Flavorbacterium* sp. strain FB4. The disappearance rate of 2,4-D was measured and found to be greater than the rate predicted based upon liquid-phase 2,4-D concentrations. A kinetic formulation, termed the enhanced bioavailability model, was developed to describe the desorption and biodegradation processes in this batch system. The approach assumes that 2,4-D resides in both the liquid and solid phases and degradation occurs via both suspended and attached biomass. All biomass can degrade liquid-phase 2,4-D at one rate, while only attached biomass can degrade sorbed 2,4-D at another rate. An enhanced transformation factor (E_f) was introduced to express the increased biodegradation rate over that expected from the liquid phase only. This approach was able to account for the increased degradation rates observed experimentally. The results provide evidence that desorption to the bulk solution is not prerequisite to degradation, and that sorbed substrate may be available for degradation.

INTRODUCTION

Bioavailability of sorbed contaminants in soils and sediments affects the clean-up time, cost, and end-point of bioremediation processes. Published results suggest that bioavailability varies with the sorbent (Geerdink et al., 1996), substrate (Griffith and Fletcher, 1991) and organisms (Guerin and Boyd, 1992). Rigorous interpretation of such results requires formulation of a conceptual model describing the component processes, conducting experiments to allow quantification of individual process rates, and mathematical analysis of bioavailability data to evaluate the validity of the model formulation. Potential interactions between processes must also be assessed. One critical issue that arises in this approach is whether desorption is prerequisite to biodegradation, or expressed alternatively, whether direct sorbed-phase degradation can occur. Some researchers have reported that sorbed contaminant is not directly available to attached or suspended cells (Steen et al., 1980; Ogram et al., 1985; Shimp and Young, 1988; Smith et al., 1992; Weissenfels et al., 1992; Shelton and Doherty, 1997), while others concluded that sorbed substrate can be directly utilized by attached cells (Guerin and Boyd, 1992; Guerin and Boyd, 1993; Crocker et al., 1995; Calvillo and Alexander, 1996; Guerin and Boyd, 1997; Ortega-Calvo and Saiz-Jimenez, 1998; Tang et al., 1998; Lahlou and J.J., 1999; Laor et al., 1999; Feng et al., 2000). While these conflicting interpretations may reflect differences related to the characteristics of the sorbents, substrates and organisms employed, they may also result from inadequate experimental and mathematical resolution of individual processes and the factors affecting them. For example, none of the above studies reporting direct sorbed-phase degradation fully described desorption kinetics, resulting in uncertainty in the substrate concentration driving degradation.

The purpose of this study was to develop an experimental and mathematical approach to evaluate bioavailability, and specifically whether direct sorbed-phase degradation occurs and at what rate if it does. To do this, desorption rates were measured in the absence of organisms and biodegradation rates were measured in the absence of solids. Combined desorption/biodegradation experiments were then performed, and the results were analyzed using a mathematical model incorporating three processes: desorption, liquid-phase degradation and sorbed-phase degradation. Using this approach, we were able to determine whether a sorbed-phase degradation mechanism is consistent with the bioavailability data and the rate of this process. Experimentally, we used 2,4-dichlorophenoxyacetic acid (2,4-D) as the sorbate and substrate, silica as the sorbent, and a 2,4-D degrading organism, *Flavobacterium* sp. FB4. The aquatic and systematic herbicide 2,4-D is widely used on wheat, corn and sorghum to control broad-leaf weeds and is a potential pollutant of groundwater (Sannino et al., 1997).

MATERIALS AND METHODS

The *Flavobacterium* sp. strain FB4 used in this study is a proteobacterium that is able to internally metabolize 2,4-D as a sole carbon and energy source (Ogram et al., 1985). Cells were grown until early stationary phase in minimal salts media ([MSM] 1419.6 mg Na_2HPO_4, 1360.9 mg KH_2PO_4, 0.3 mg $(NH_4)_2SO_4$, 50 mg $MgSO_4$ $7H_2O$, 5.88 mg $CaCl_2$ $2H_2O$, 3.2 mg EDTA disodium salt, 2.78 mg $FeSO_4$ 7H2O, 1.15 mg $ZnSO_4$ $7H_2O$, 1.69 mg $MnSO_4$ H_2O, 0.375 mg $CuSO_4$ $5H_2O$, 0.233 mg $Co(NO_3)_2$ $6H_2O$ and 0.1236 mg $(NH_4)_6Mo_7O_{24}$ $4H_2O$ per liter of distilled water) that contained 400 ppm 2,4-

D. Cells to be used as inoculum were rinsed once and resuspended in phosphate buffered saline (PBS).

Sterile stock solutions of radio-labeled 2,4-D (Sigma, ≥96 pure, 12.8 mCi $mmol^{-1}$) were prepared in PBS. The solutions contained approximately 200,000 dpm ml^{-1} ring-U-[^{14}C] 2,4-D with a final concentration of 10 mg L^{-1}. The solutions were filter sterilized and stored in five 100 ml portions in light shielded bottles at 4°C.

Uncoated silica was obtained from the J.T. Baker company. The irregular-shaped particles have a size of 40 μm with 60-angstrom pores. To create the silica slurries, 2.5 g silica was weighed into sterile a 10 ml Falcon® tube and 9 ml PBS were carefully layered on top of the silica. The tubes were placed in a Speed Vac® for one minute. A disposable sterile loop was used to re-suspend the silica. The silica was rinsed with 200 ml of PBS in 10 ml aliquots in a 25 ml glass column with a glass frit. After rinsing, the silica was transferred to a 20 ml serum vial. The solution from the last rinse was used as the supernatant control.

Sorption kinetics were evaluated to determine the silica-liquid contact time necessary to reach apparent steady state in batch experiments. PBS and stock solutions containing ^{14}C-labeled 2,4-D were added to serum vials containing silica to achieve the appropriate slurry density and contaminant concentration. The vials were placed on a platform rocker and mixed well enough to prevent the silica from settling. The slurries were sampled at prearranged time intervals and the samples were immediately centrifuged at 11750 relative centrifuge force in a centrifuge filter vial to separate the silica from the solution. After centrifugation the filter cup was separated from the vial and the cap was cut off. The filter cup and vial were dropped into separate scintillation

vials containing 10 ml of scintillation cocktail fluid. Activity was counted on a liquid scintillation analyzer.

Desorption kinetics were measured by first equilibrating silica and 2,4-D, diluting the supernatant with 2,4-D-free PBS, and resuspending the particles. Liquid-phase and sorbed-phase concentrations were measured over time as described above.

Biodegradation in the presence or absence of the sorbent was measured in similar batch systems. After the system reached sorption steady state, it was sampled as previously described to determine the sorbed and liquid-phase contaminant concentrations. The slurry was then inoculated with a pure bacterial culture (FB4) at a cell density of approximately 1×10^{8} CFU ml^{-1}. Depletion of liquid phase 2,4-D was monitored over time. Cell-free control vials were also prepared and monitored to examine abiotic losses in the batch system. The liquid-phase degradation rate for 2,4-D was also determined in the supernatant control solutions.

In the proposed model, shown schematically in Figure 3-1, the contaminant can reside in two phases, solid and liquid, and degradation occurs via both suspended and attached biomass. Distribution of 2,4-D between the two phases is described by an equilibrium distribution coefficient, as the kinetic experiments indicated that this process is rapid and reversible. All of the biomass in the system can degrade liquid-phase contaminant at one rate, while only an attached fraction degrades sorbed-phase contaminant at another rate.

The linear sorption distribution coefficient (K_d) decribes the relationship between the sorbed-phase (S_e) and liquid-phase (C_e) concentrations at equilibrium.

$$K_d = \frac{S_e}{C_e} \qquad \text{Eq. 1}$$

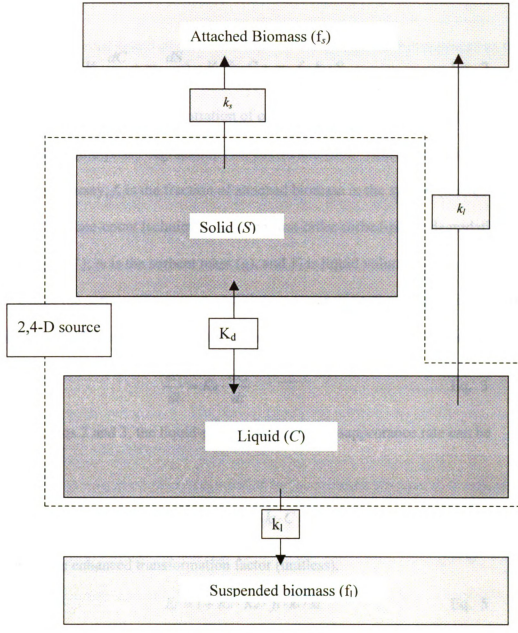

Figure 3- 1. Depicted model for disappearance of 2,4-D in slurry systems. Where, C and S are sorbed and liquid phase concentration, respectively. fs is attached cell fraction. Kd is distribution coefficient. kl and ks are liquid and sorbed phase degradation rate coefficients, respectively.

The overall mass balance equation of contaminant in the batch system is expressed as:

$$-(V_l \cdot \frac{dC}{dt} + m \cdot \frac{dS}{dt}) = V_l \cdot k_l \cdot C + m \cdot f_s \cdot k_s \cdot S \qquad \text{Eq. 2}$$

where C is the liquid-phase concentration of contaminant ($\mu g\ L^{-1}$); t is time (min); k_l is the first-order liquid-phase degradation rate coefficient (min^{-1}) determined from the biodegradation assay; f_s is the fraction of attached biomass in the system (unitless) determined by plate-count techniques; k_s is the first-order sorbed-phase degradation rate coefficient (min^{-1}); m is the sorbent mass (g), and V_l is liquid volume (ml).

Under the sorption/desorption equilibrium assumption, the rate of change in the sorbed phase can be calculated from the change in liquid phase concentration:

$$\frac{dS}{dt} = K_d \cdot \frac{dC}{dt} \qquad \text{Eq. 3}$$

Rearranging Eqs.2 and 3, the liquid-phase contaminant disappearance rate can be expressed as:

$$-\frac{dC}{dt} = E_f \cdot B_f \cdot k_l \cdot C \qquad \text{Eq. 4}$$

where, E_f is the enhanced transformation factor (unitless),

$$E_f = 1 + R_{sl} \cdot K_d \cdot f_s \cdot k_s / k_l \qquad \text{Eq. 5}$$

B_f is the bioavailability factor (Zhang et al., 1998),

$$B_f = \frac{1}{1 + R_{sl} \cdot K_d} \qquad \text{Eq. 6}$$

and R_{sl} is solid to liquid ratio ($g\ ml^{-1}$).

$$R_{sl} = m / V_l \qquad \text{Eq. 7}$$

Integrating Eq. 4, the liquid-phase contaminant concentration is expressed as

$$C = C_o \cdot e^{-E_f \cdot B_f \cdot k_l \cdot t} \qquad \text{Eq. 8}$$

where C_o is the initial liquid-phase concentration. We designated this approach as the Enhanced Bioavailability (EB) model.

The solid/liquid distribution coefficient, K_d, was determined from the sorption isotherm experiments. The liquid-phase degradation rate coefficient, k_l, was determined from the silica-free biodegradation experiment using Eq. 4 when E_f and B_f are set to one. The fraction of attached biomass, f_s, was determined by comparison of the cell plate counts from the initial inoculant and the liquid phase of the silica slurry. The ratio of solid to liquid, R_{sl}, was calculated from the silica weight and liquid volume. The bioavailability factor, B_f, was calculated from Eq. 6. The enhanced transformation factor, E_f, was determined from the change in liquid-phase concentration of contaminant in the bioavailability assay using Eq. 8. The sorbed-phase degradation rate coefficient, k_s, was calculated after E_f was determined using Eq. 5.

If there is no sorbed-phase degradation by the attached biomass, k_s is zero and E_f is one. The equation reduces to the bioavailability equation (B_f model), that assumes sorption/desorption equilibrium and only liquid-phase degradation (Zhang et al., 1998). B_f varies between zero and one, with a value of one corresponding to a system with no sorbent. If E_f is equal to one, the microorganisms are only able to degrade contaminant in liquid phase. Values of E_f greater than one indicate a 2,4-D biodegradation rate faster than that expected based on liquid-phase concentrations, whereas values less than one indicate slower rates. In the later case, the value of k_s will be negative.

RESULTS

The 2,4-D sorption isotherm was linear over the concentration range employed in this study. Steady-state conditions were reached within 5 minutes and the sorption distribution coefficient (K_d) was 2.5 (± 1.0) ml g^{-1}. Desorption was also rapid, with the system reaching the value predicted by the sorption K_d by the first time point at 30 seconds (Figure 3-2). Three consecutive dilution desorption experiments were performed and no desorption hysteresis was observed. It was therefore concluded that sorption/ desorption could be described as completely reversible and instantaneous for this study.

Two sets of experiments were completed to evaluate the bioavailability of sorbed 2,4-D in slurry systems. In the first set, the ratio of silica to liquid, R_{sl}, was varied while the amounts of 2,4-D were held constant. In the second set of experiments the initial 2,4-D amount was varied while R_{sl} was held constant. Since the experiments were performed using cells harvested from independent cultures, cell activity varied slightly. Bio-kinetic parameters were evaluated from silica-free controls and normalized for each set of experiments.

Four silica to liquid ratios, 0.12, 0.19, 0.29, and 0.43 g ml^{-1}, were used in the first experiment (Table 3-1). The amount of 2,4-D and volume of solution remained constant. As a result, the initial liquid-phase 2,4-D concentrations were between 750 and 360 μg L^{-1} for the four ratios. An example of the concentration data and prediction by the B_f model are shown in Figure 3-3. The B_f model consistently over-predicted liquid-phase concentrations (i.e. under-predicted 2,4-D depletion), suggesting the need for an additional or enhanced rate process. The enhanced transformation factor (E_f) was

determined from the 2,4-D concentration-time profile using Eq. 8. It can be seen that the added degradation described by this term results in a fit consistent with the data. The enhanced transformation factors (E_f) were greater than 1 for all silica to liquid ratios and increased linearly with this ratio (Figure 3-4).

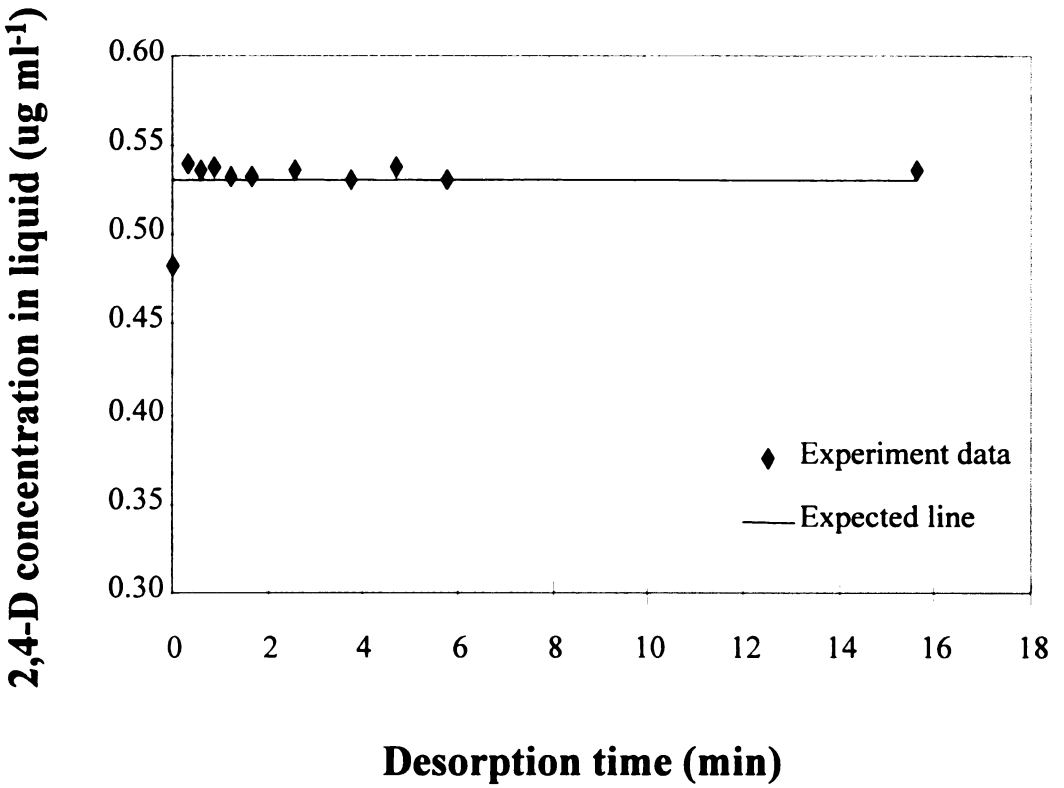

Figure 3- 2. 2,4-D desorption from silica following a single dilution of the solution phase. Initial liquid concentration was 483 μg L^{-1}. The distribution coefficient (K_d) was 2.5(±1.0). Time points represent single measurements.

Table 3- 1. Analysis of experimental results for a range of R_{sl} (0.12 - 0.43) at 1.2 ×107 CFU ml^{-1}

R_{sl}	C_i[a]	C_o[b]	k_l[c]	B_f	k_s	E_f
g mL^{-1}	μg mL^{-1}	μg mL^{-1}	min^{-1}	-	min^{-1}	-
0	0.92	0.92	0.0060			
0.12	1.00	0.75		0.75	0.030	1.51
0.19	1.00	0.63		0.63	0.032	1.89
0.29	1.00	0.49		0.49	0.024	2.10
0.43	1.00	0.36		0.36	0.023	2.68

[a] Initial solution concentration before adding silica
[b] initial concentration in liquid phase for degradation reaction.
[c] measured first order degradation coefficient in liquid phase

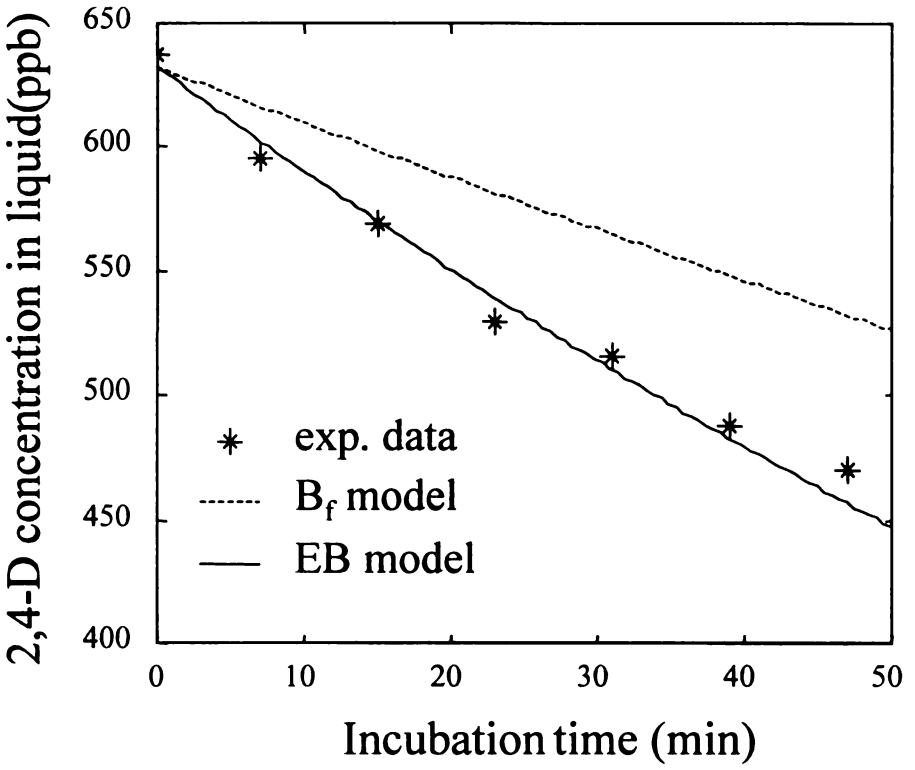

Figure 3- 3. 2,4-D liquid-phase concentration versus incubation time. The two depicted lines were plotted using following values of R_{sl} (0.19), k_l (0.0058), k_s (0.032), and f_s (0.28). Inoculum was 1.2×10^7 CFU ml^{-1}.

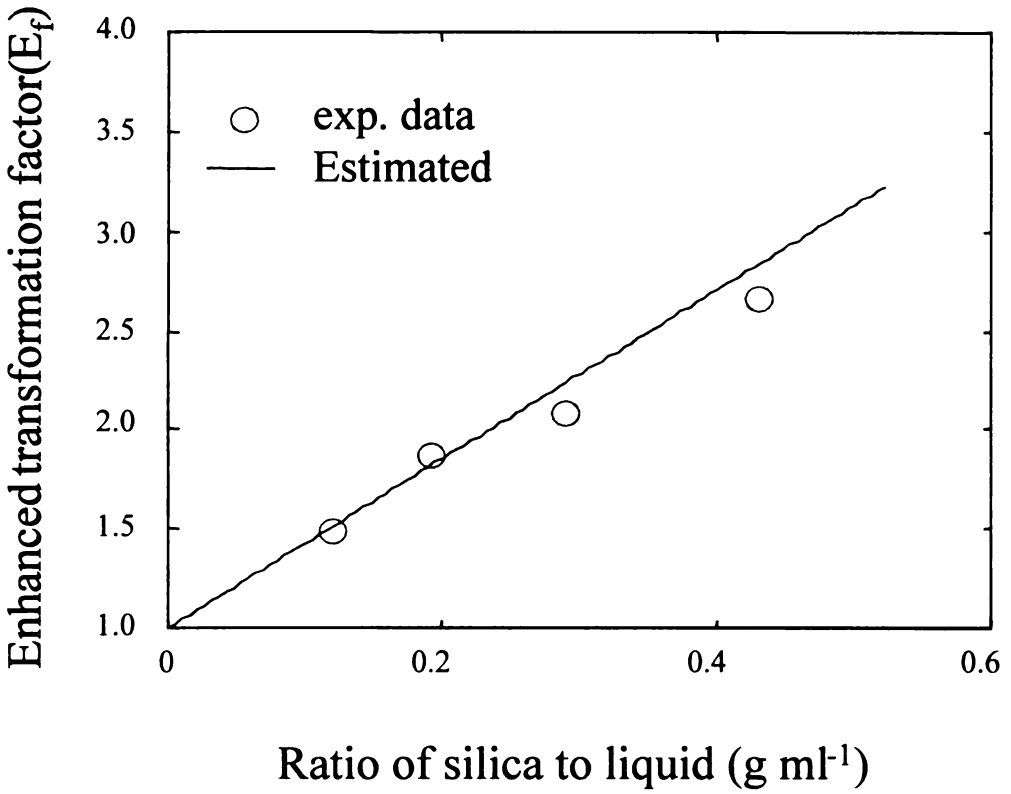

Figure 3- 4. Enhanced transformation factor versus the ratio of silica to liquid. Estimated line was plotted using average values of k_l(0.0060), k_s(0.027), and f_s(0.28). Inoculum was 1.2×10^7 CFU ml^{-1}.

In the second experiment, four different 2,4-D amounts were used with a constant silica to liquid ratio (R_{sl} = 0.25) (Table3-2). This resulted in initial concentrations between 70 to 550 μg L^{-1}. Again, the B_f model significantly underestimated initial 2,4-D depletion rates while the EB model consistently fit the results (Figure 3-5). The average value of the enhanced transformation factor (E_f) was 1.70 ± 0.15.

Table 3- 2. Analysis of experimental results for different initial concentrations and same R_{sl} (0.25) at 5.6×10^6 CFU ml^{-1}

R_{sl}	C_i [a]	C_o [b]	k_l [c]	B_f	k_s	E_f
g mL^{-1}	μg mL^{-1}	μg mL^{-1}	min^{-1}	-	min^{-1}	-
0	0.93	0.93	0.0045			
0.25	0.13	0.07		0.53	0.010	1.54
0.25	0.38	0.21		0.57	0.013	1.60
0.25	0.63	0.35		0.56	0.017	1.86
0.25	1.00	0.55		0.55	0.016	1.79

[a] Initial solution concentration before adding silica
[b] initial concentration in liquid phase for degradation reaction.
[c] measured first order degradation coefficient in liquid phase

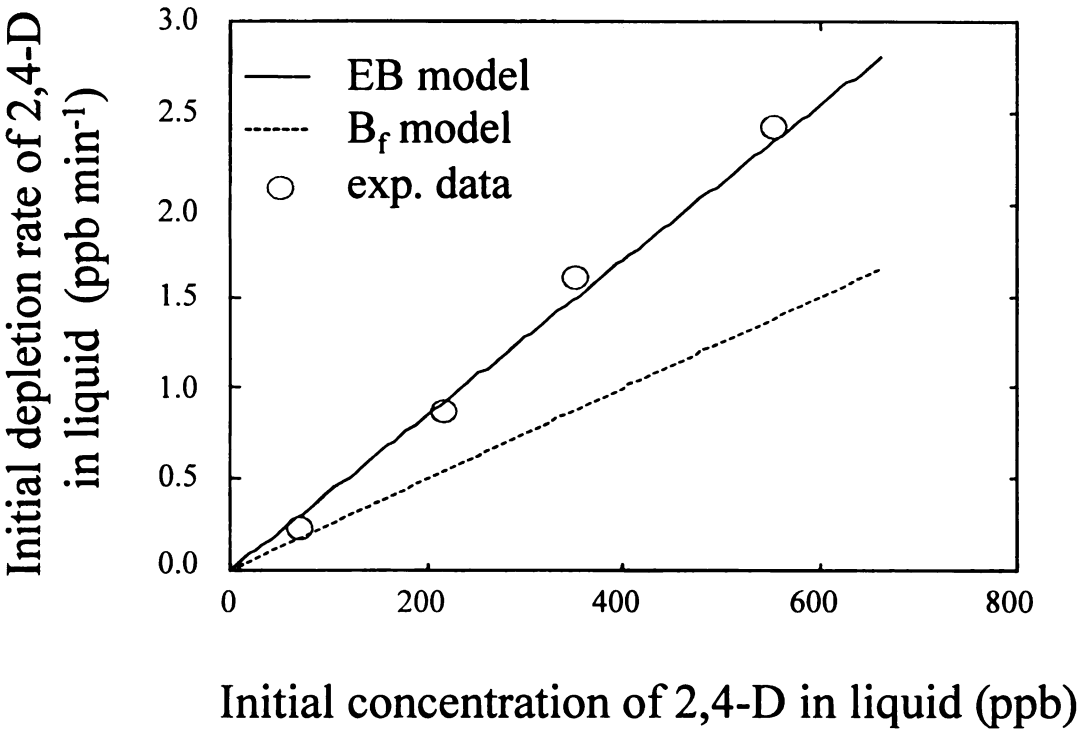

Figure 3- 5. Initial depletion rate versus initial equilibrium concentration at a constant silica to liquid ratio. Inoculum was 5.5×10^6 CFU ml^{-1}. Values used for model; k_l (0.0045); k_s (0.014); f_s (0.28).

DISCUSSION

The proposed approach for assessing bioavailability defines a combination of experimental and mathematical tools that can be used to describe biodegradation in the presence of a sorbent. We have accounted for the dependence and the effect of both desorption and biodegradation on liquid-phase concentration. By doing this we are able to evaluate whether desorption and degradation are sequential processes, with degradation dependent only on liquid-phase concentration. In our experimental system, this was not the case. In all experiments degradation proceeded at a rate faster than would be expected based on the liquid concentration alone.

The EB model presented in this study was developed using formulations for reversible and instantaneous sorption/desorption processes and first-order biodegradation reactions in both liquid and solid phases, and the assumption that the liquid-phase degradation rate coefficient is not affected by the presence of solids. Since desorption can never be truly instantaneous, the validity of this formulation rests on whether it is significantly faster than degradation. As shown in Figure 3-6, if the ratio of the desorption rate coefficient to the degradation rate coefficient is larger than 10, the concentration error would be less than 2 %. A desorption rate coefficient, α (min^{-1}), was estimated using the following equation

$$\frac{dS}{dt} = -\alpha \cdot (S - K_d \cdot C) \qquad \text{Eq. 9}$$

and found to be at least 30 min^{-1} for the data shown in Figure 3-2. Because the liquid-phase degradation rate coefficients were less than 0.006 min^{-1}, the rate ratio was greater than 1000. Thus, the error is expected to be insignificant.

In order to describe the enhanced 2,4-D disappearance rate in the silica slurry system, the enhanced transformation factor (E_f) was introduced to the previously published B_f model. When the E_f value is higher than 1, as in all cases from this study, degradation is faster than expected based on liquid-phase concentrations. One possible mechanistic interpretation of E_f is an enhanced system degradation rate resulting from attached biomass accessing adjacent elevated concentrations of contaminant prior to complete dilution in the liquid phase. This has been previously termed "direct sorbed-phase utilization" (Guerin and Boyd, 1992; Guerin and Boyd, 1993). Another possibility is an increase in bacterial metabolic rates resulting from the presence of the sorbent. When considering the practical implication, the important conclusion is that bacterial degradation in the presence of a sorbent may be faster than described by the independent processes of desorption and liquid-phase degradation.

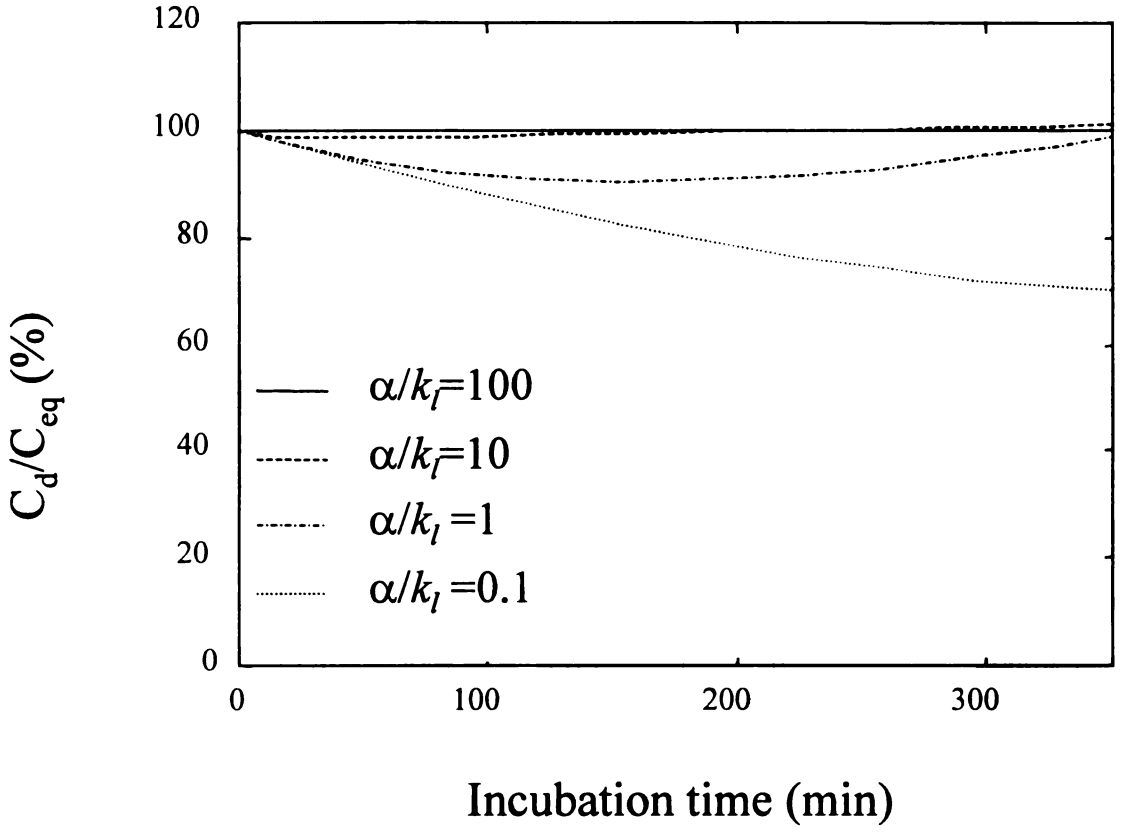

Figure 3- 6. The ratio effect of desorption rate coefficient to degradation rate coefficient on liquid-phase concentration over incubation time. C_{eq} is calculated using EB model with E_f value of one. C_d is calculated using Eqs. 2 (overall mass balance equation with k_s value of zero) and 9 (first-order desorption-rate equation). K_d value is assumed 2.5. R_{sl} is 0.1. k_l is 0.006 min^{-1}.

ACKNOWLEDGEMENTS

This work was supported, in part, by the Center for Microbial Ecology (National Science Foundation, Grant DEB9120006), and by the Great Lakes and Mid-Atlantic Center for Hazardous Substance Research (Environmental Protection Agency, Michigan Department of Environmental Quality).

REFERENCES

Calvillo, Y. M. and M. Alexander. 1996. "Mechanism of microbial utilization of biphenyl sorbed to polyacrylic beads." *Appl. Microbiol. Biotechnol* 45: 383-390.

Crocker, F. H., W. F. Guerin and S. A. Boyd. 1995. "Bioavailability of Naphthalene Sorbed to Cationic Surfactant-Modified Smectite Clay." *Environ. Sci. Technol* 29: 2953-2958.

Feng, Y., J. H. Park, T. C. Voice and S. A. Boyd. 2000. "Bioavailability of Soil-Sorbed Biphenyl to Bacteria." *Environ. Sci. Technol.* Accepted.

Geerdink, M. J., M. C. M. van Loosdercht and K. C. A. M. Luyben. 1996. "Model for Microbial Degradation of Nonpolar Organic Contaminants in a Soil Slurry Resctor." *Environ. Sci. Technol* 30: 779-786.

Griffith, P. C. and M. Fletcher. 1991. "Hydrolysis of Protein and Model Dipeptide Substrates by Attached and Nonattached Marine Pseudomonas sp. Strain NCIMB 2021." *Appl. Environ. Microbiol* 57: 2186-2191.

Guerin, W. F. and S. A. Boyd. 1992. "Differential Bioavailability of Soil-Sorbed Naphthalene to Two Bacterial Species." *Appl. Environ. Microbiol* 58: 1142-1152.

Guerin, W. F. and S. A. Boyd. 1993. "Bioavailability of Sorbed Naphthalene to Bacteria: Influence of Contaminant Aging and Soil Organic Carbon Content." *Sorption and Degradation of Pesticides and Organic Chemicals in Soil. SSSA Special Publication no* 32: 197-208.

Guerin, W. F. and S. A. Boyd. 1997. "Bioavailability of naphthalene associated with natural and synthetic sorbents." *Water Res.* 32: 1504-1512.

Lahlou, M. and O.-C. J.J. 1999. "Bioavailability of labile and desorption-resistant phenanthrene sorbed to montmorillonite clay containing humic fractions." *Environ. Toxicol. Chem.* 18: 2729-2735.

Laor, Y., P. F. Strom and W. J. Farmer. 1999. "Bioavailability of Phenanthrene Sorbed to Mineral-Associated Humic Acid." *Wat. Res.* 33: 1719-1729.

Ogram, A. V., R. E. Jessup, L. T. Ou and P. S. C. Rao. 1985. "Effects of Sorption n Biological Degradation Rates of (2,4-Dichlorophenoxy)acetic Acid in Soils." *Appl. Environ. Microbiol* 49: 582-587.

Ortega-Calvo, J. J. and C. Saiz-Jimenez. 1998. "Effect of humic fractions and clay on biodegradation of phenanthrene by a Pseudomonas fluorescens strain isolated from soil." *Appl. Environ. Microbiol.* 64: 3123-3126.

Sannino, F., A. Violante and L. Gianfreda. 1997. "Adsorption-Desorption of 2,4-D by Hydroxy Aluminium Montmorillonite Complexes." *Pestic. Sci* 51: 429-435.

Shelton, D. R. and M. A. Doherty. 1997. "Estimating Losses of Efficacy Due to Pesticide Biodegradation in Soil: Model Simulations." *Soil Sci. Soc. Am. J* 61: 1085–1090.

Shimp, R. J. and R. L. Young. 1988. "Availability of organic chemicals for biodegradation in settled bottom sediments." *Ecotoxicol Environ Saf* 15: 31-45.

Smith, S. C., C. C. Ainsworth, S. J. Traina and R. J. Hicks. 1992. "Effect of sorption on the biodegradation of quinoline." *Soil Sci. Soc. Am. J.* 56: 737-746.

Steen, W. C., D. F. Paris and G. L. Baughman. 1980. "Effects of Sediment Sorption on Microbial Degradation of Toxic Substances." *Contaminants and Sediments. Vol. 1. Ann Arbor Sci. Publ., Ann Arbor, MI.* 1: 477-482.

Tang, W. C., J. C. White and M. Alexander. 1998. "Utilization of sorbed compounds by microorganisms specifically isolated for that purpose." *Appl. Microbiol. Biotechnol.* 49: 117-121.

Weissenfels, W. D., H. J. Klewer and J. Langhoff. 1992. "Adsorption of Polycycleic Aromatic-Hydrocarbons (PAHS) by soil particles- Influence ofn Biodegradatility and Biotoxicity." *Appl. Microbiol. Biotechnol.* 36: 689-696.

Zhang, W. X., E. J. Bouwer and W. P. Ball. 1998. "Bioavailability of hydrophobic organic contaminants-effects and implications of sorption-related mass-transfer on bioremediation." *Ground Water Monit. R.* 18: 126-138.

CHAPTER 4. DEVELOPMENT OF A KINETIC BASIS FOR BIOAVAILABILITY OF SORBED NAPHTHALENE IN SOIL SLURRIES

ABSTRACT

The degradation of naphthalene in soil-slurry systems was studied using four different organisms and two soils. Organims with zero-order, first-order, and Michaelis-Menten rates were selected. The soils had substantially different sorption distribution coefficients. Sorption and desorption was evaluated in abiotic soil-slurry systems. The desorption process was described by a model that accounts for equilibrium, rate-limited and non-desorbing sites. Biodegradation parametes were measured in soil-extract solutions. Bioavailability assays, inoculated soil-slurries, were conducted and both liquid- and solid-phase naphthalene concentrations were measured over time. For the less sorptive soil, the results could be explained by sequential desorption and degradation processes. For the other soil, enhanced degradation was clearly observed for the organisms with first-order and Michaelis-Menten rates. Several explanations are explored to explain these observations including the development of elevated substrate concentrations at the organism/sorbent interface. No enhancement was found for the organism with zero-order kinetics.

Key words- naphthalene, sorption, desorption, bioavailability, soil, model, biodegradation, kinetics.

INTRODUCTION

Bioavailability of organic contaminants has been identified as a major limitation to complete bioremediation of contaminated soils affecting clean-up time, cost, and the end-point of the process (Head, 1998). The importance of bioavailability is evidenced by numerous recent studies on this topic (Guerin and Boyd, 1992; Guerin and Boyd, 1993; Crocker et al., 1995; Calvillo and Alexander, 1996; Guerin and Boyd, 1997; Ortega-Calvo and Saiz-Jimenez, 1998; Tang et al., 1998; Laor et al., 1999; Park et al., 1999; Feng et al., 2000; Park et al., 2000; Park et al., 2000). Most of this work has focused on understanding the affects of soil sorption on first-order mineralization. As a result of this perspective, these studies have typically utilized very low contaminant concentrations (e.g. less than 100 μg/L), only monitored CO_2 production in the presence and absence of soil, and did not independently assess desorption processes.

In attempting to understand how desorption and biodegradation processes interactively control bioavailability, it must be considered that biodegradation of sorbed materials involves at least two rate processes: desorption and biodegradation. It is understood that desorption is controlled by the concentration gradient across the solid/liquid interface. The simplest view of biodegradation involves a rate driven by liquid-phase contaminant, but different organisms and concentration regions result in different rate formulations. Thus, the liquid-phase concentration of substrate is both a result of, and the driving force for, both rate processes. The situation is further complicated by the possibility of process interactions, for example if the presence of organisms serves to either accelerate or retard desorption rates.

It has not been possible in previous studies to fully investigate these potential process interactions for several reasons. First, desorption has not generally been separately quantified. Second, when this has been attempted using mineralization data, an assumption that CO_2.

production equates with substrate disappearance is required. There is considerable evidence in the literature, however, that contradicts this assumption for many of the organisms and substrates that were used in bioavailability experiments (Whitman et al., 1998; Willumsem and Arvin, 1999; Park et al., 2000). For example, Whiteman et al. (1998) and Park et al. (2000) found that substrate disappearance was rapid, while CO_2 production lagged. As a result, the desorption driving force would be greater than that expected by inferring liquid-phase substrate concentrations from CO_2 measurements. Third, while first-order kinetics have almost always been used to describe mineralization rates, this may not be applicable to degradation rates.

In this study, a mathematical representation of bioavailability was developed by measuring both rate processes independently, and investigating rate interactions in systems with both processes active. Two soils, with different sorption capacities, and four organisms having three different biokinetic formulations, were studied. Experimental data were evaluated using coupled kinetic models. The overall purpose was to understand the kinetic basis of bioavailability, including exploration of rate interactions. Naphthalene was selected as a test contaminant, since it has often been used as a model compound for PAHs, and this class of contaminant is a critical bioavailability issue at numerous petroleum contamination sites.

MATERIALS AND METHODS

Soil

Two sandy loam soils were collected from a forest environment in Michigan: SPCF (Spinks loamy sand) and Kalkaska-A. The soil samples were air-dried and sieved through a U.S. Sieve Series #20 sieve (> 650 μm) to remove larger components. Prior to use, the soils were sterilized by gamma irradiation (^{60}Co source) at a dosage of 2 Mrad. Following sterilization, sealed containers were maintained at room temperature. Before the soils were used, 0.1g of each soil was placed on a nutrient agar plate and incubated at 30 °C for 3 days to verify sterility. No colony forming units were observed. Soil characteristics are summarized in Table 4-1.

Organism and Growth conditions

Four naphthalene-degrading strains, *Pseudomonas putida* ATCC 17484, *Alcaligenes* sp. NP-Alk, *Pseudomonas putida* G7 and *Pseudomonas* sp. NCIB 9816-4, were used. ATCC 17484 and NP-Alk were selected because they were previously used in mineralization assays to study bioavailability of sorbed naphthalene (Guerin and Boyd, 1992). G7 and NCIB 9816-4 were selected because it was reported recently that these organisms are chemotactic to naphthalene (Grimm and Harwood, 1997). ATCC 17484 was obtained from the American Type Culture Collection, NP-Alk from S. A. Boyd at Michigan State University, and G7 and NCIB9816-4 from C. S. Harwood at the University of Iowa.

Table 4- 1 Soil characteristics[†]

Soils	Organic content (%)	Mechanical characteristics (%)		
	Organic matter	Sand	Silt	Clay
SPCF	1.9	78	17	5
Kalkaska-A	3.9	90.6	7.7	1.7

[†] Analysis completed by Plant and Soil Science Laboratory in Michigan State University.
SPCF: This was collected between 15 and 31 cm depth of forest in Michigan State University, East Lansing, Michigan.
Kalkaska-A: This was collected at surface (0-6 cm depth) of forest in the northern half of Michigan's lower peninsula (The more detailed location was documented by Vance (Vance, 1984))

ATCC 17484 and NP-Alk were grown in 500 ml high-buffer broths (HBB) composed of 2.0 g of NaCl, 3.0 g of $(NH_4)_2HPO_4$, 1.2 g of KH_2PO_4, 3 mg of $MgSO_4$, 1 ml of ferric quinate (Guerin and Boyd, 1992), 1 ml of vitamin solution (Wolin et al., 1963), per liter of distilled water at pH 7.0. Naphthalene was added to the sterilized liquid medium as a concentrated stock solution in acetone (200 g/L) to a final concentration of 200 mg/L. The liquid medium was inoculated by adding 5 ml of the starved liquid culture (cell density of ~10^7 CFU/mL) to 500 mL medium, and stirred. Growth was monitored by absorbance at 600 nm, and cells were harvested at a point determined to correspond to early stationary phase based on a full growth curve. Cells were separated by centrifugation (1900 × g, 20 min) and resuspended in a phosphate buffer saline (PBS) solution before use. The PBS contained 8.5 g of NaCl, 0.6 g of Na_2HPO_4, and 0.3 g of KH_2PO_4 per liter. This procedure was repeated three times to ensure the removal of remaining naphthalene from the cell-growth medium. G7 and NCIB 9816-4 were grown in 100 ml minimal mineral salt media (Harwood et al., 1994). Solid naphthalene was added to the sterilized liquid at a final concentration of 200 mg/L. The liquid medium was inoculated by adding one ml of the starved liquid culture (cell density of ~10^7 CFU/mL) to 100 mL medium, and stirred. Growth was monitored by absorbance of 600 nm, and cells were harvested at a point determined to correspond to early stationary phase. Cells were separated by centrifugation (1900 × g, 20 min) and resuspended in a chemotaxis buffer (CB) (Harwood et al., 1994) before use. The CB contained 13.6 g of potassium phosphate and 7.4 mg of EDTA per liter of distilled water, and it was adjusted by 10 N NaOH solution to be pH 7. This procedure was repeated

three times to ensure the removal of remaining naphthalene from the cell growth medium. All the cell suspensions were kept at room temperature and used within 3 hours.

Sorption, desorption and extraction

Naphthalene soil/water distribution coefficients were measured experimentally. An aliquot of each sterile soil (0.68 g of SPCF and 0.18 g of Kal-A) and 4.2 ml of CB containing ^{14}C-naphthalene stock (in methanol) were prepared in 5 mL screw cap vials with Teflon-lined septa. The soil/water ratios were carefully selected to achieve approximately equal masses of naphthalene in both liquid and solid phases at end of sorption period. The headspace was less than 1mL in volume. Initial liquid-phase concentrations ranged from 0 to 3,400 μg/L. Control vials without soil were also prepared in triplicate. Vials were tumbled at 6 rpm for 2 days in the dark. After mixing, each vial was centrifuged for 5 min at 1,200 × g to separate soil, and the supernatant was sampled. The concentration of naphthalene in the supernatant was determined by liquid scintillation counting (LSC) and high pressure liquid chromatography (HPLC). The naphthalene concentration on soils were calculated by the difference between the initial and final liquid phase concentrations.

Desorption rates were measured in batch soil slurries with initial naphthalene concentrations of 800 μg/L. The vials were tumbled at 6 rpm for 2 days in the dark. The final concentration of naphthalene in the liquid phase was determined by LSC, and the amount of sorbed naphthalene calculated by difference. The supernatant was then decanted to the extent possible, the residual water determined by weight, and naphthalene-free CB was added to the original volume. The vials were then tumbled again at 6 rpm, with periodic liquid phase sampling and analysis.

Recovery studies were performed to determine extraction efficiencies. Triplicate soil slurries were performed in the same manner as the desorption rate studies except methanol was used as the desorption solution and the concentration was only measured after 2 days of tumbling in the extraction solvent. Concentrations were measured by HPLC, and efficiency calculated by mass balance.

Cell attachment assays

Cell attachment was measured in duplicate soil slurries with initial liquid-phase naphthalene concentrations of 800 μg/L. The vials, and controls were tumbled at 6 rpm for 2 days in the dark. The slurries and soil-free controls were inoculated to a density of ~10^7 CFU/mL

from an inoculum harvested during the early stationary phase. The inoculated vials were tumbled at 6 rpm for 1 hr to facilitate cell attachment. The soil was then allowed to settle quiescently for 2 hr and 0.1 mL of supernatant was removed for plate counting. Cell attachment efficiency was calculated by difference between soil-slurries and soil-free controls.

Bioavailability assays

Bioavailability assays were performed using soil slurries in 5 mL serum vials with Teflon-lined septa. Using the same soil:water ratio as in the desorption assays, naphthalene was added to an initial liquid-phase concentration of approximately 800 μg/L, and the vials tumbled for 2 days in the dark. Sterility of the soil slurries was checked by plating out the suspension on nutrient agar plates. The vials were then inoculated with 0.05 mL of cell suspension harvested during the early stationary phase to

initiate biodegradation of naphthalene. Cell density in the serum vials was approximately 10^7 CFU/mL. Inoculated vials were tumbled at 6 rpm. At predetermined time intervals, vials were removed and centrifuged at 1200 × g for 5 min to separate soil. One ml of supernatant was transferred a vial containing 0.01 ml 10N NaOH to stop naphthalene degradation, and analyzed by HPLC. The remaining supernatant was decanted and extracted with methanol as described above to determine solid-phase concentrations by HPLC.

Biodegradation rates were measured in soil-extract solutions. These were prepared by tumbling soil slurries for 2 days at the same soil to water ratio as in the desorption and bioavailability experiments. Kinetic data were obtained using the same bioavailability assay procedure, except no soil was added to the vials. Both sterile-CB/PBS and soil-extract controls were also used to monitor abiotic losses of naphthalene during the sorption and degradation periods. All experiments were performed at room temperature (24 ± 1 °C) unless otherwise mentioned.

Chemical analysis

Naphthalene was analyzed by HPLC using a C_{18} reverse-phase column, an 80% acetonitrile/20% water mobile phase, and fluorescence detection (280 nm excitation, 340 nm emission). The analytical detection limit was 0.2 μg/L. The detection limit for LSC was less than 1 μg/L for the naphthalene solution used in this study.

THEORY

Three-site desorption model

Sorption and desorption processes have commonly been described using a model with both equilibrium and kinetically-limited sites in the soil phase (van Genuchten and Wagenet, 1989; Shelton and Doherty, 1997; Shelton and Doherty, 1997). There is considerable recent evidence, however, that some fraction of the sorbed material either does not desorb at all, or only desorbs very slowly (Ahn, 1998; Deitsch and Smith, 1999; Tomson and Pignatello, 1999). In this study, a three-site model was developed by assuming the solid is composed of equilibrium, non-equilibrium and non-desorption sites. The non-desorption sites are defined in this work as those containing sorbate that can not be released to aqueous solutions during the experimental desorption period (3 days). However, the non-desorbed naphthalene is extractable with methanol.

In the three-site model, equilibrium and non-desorption partitioning are described by:

$$S_{eq} = f_{eq} \cdot K_d \cdot C_{des} \qquad \text{Eq. 9}$$

$$S_{nd} = f_{nd} \cdot K_d \cdot C_{e(sorp)} \qquad \text{Eq. 10}$$

while the release from non-equilibrium sites follows the first-order expression

$$\frac{dS_{neq}}{dt} = \alpha \cdot [f_{neq} \cdot K_d \cdot C_{des} - S_{neq}] \qquad \text{Eq. 11}$$

where, S_{eq}, S_{neq} and S_{nd} (μg/kg) are the sorbed concentrations in solid, equilibrium site, non-equilibrium site, and non-desorption site, respectively. K_d, (L/kg) is the sorption distribution coefficient, C_{des} (μg/L) is liquid phase concentration in a desorption assay, $C_{e(sorp)}$ is liquid phase concentration in sorption equilibrium. f_{eq} is the equilibrium

site fraction, f_{neq} is non-equilibrium site fraction, f_{nd} is non-desorption site fraction, t is desorption time, and α (min^{-1}) is the first order desorption rate coefficient for non-equilibrium sites. $K_{d,}$ was calculated from the sorption isotherm. f_{eq}, f_{neq}, f_{nd} and α were estimated by non-linear regression analysis of desorption data.

Bioavailability model

The bioavailability models add biodegradation to the three-site desorption model. In model I, biomass can utilize only liquid-phase contaminant. Degradation may be described by zero-order, first-order, or Michaelis-Menten kinetic expressions. In model II, an additional degradation rate based on solid-phase concentrations is added. This addresses what some investigators have described as direct solid-phase degradation (Guerin and Boyd, 1992; Guerin and Boyd, 1993; Crocker et al., 1995; Calvillo and Alexander, 1996; Tang et al., 1998), but might also represent any rate enhancement, perhaps resulting from elevated concentrations at the cell/sorbent interface (Harms and Zehnder, 1995; Ortega-Calvo and Saiz-Jimenez, 1998; Feng et al., 2000). A first-order expression based on the concentration in the equilibrium sites is used for this rate.

Total disappearance rate in slurry systems for model II can be expressed

$$-(V_l \cdot \frac{dC}{dt} + m \cdot \frac{dS}{dt}) = V_l \cdot R_{bio} + m \cdot k_{eq} \cdot S_{eq} \qquad \text{Eq. 12}$$

where S (μg/kg) is the sorbed concentrations in solid. C (μg/L) is the liquid phase concentration in a slurry system. m (kg) is the soil amount. V_l (L) is the liquid total volume, k_{eq} is the first order biodegradation coefficients of sorbed contaminant. R_{bio} is the liquid-phase biodegradation rate expression, which can be formulated as:

$R_{bio} = k_0 \cdot C$ for zero-order kinetics, Eq. 13

$$R_{bio} = k_1 \cdot C \quad \text{for first-order kinetics, and} \qquad \text{Eq. 14}$$

$$R_{bio} = \frac{k_m \cdot C}{K_s + C} \quad \text{for Michaelis-Menten kinetics,} \qquad \text{Eq. 15}$$

where k_o, k_1 and k_m are the zero-order, first-order and maximum degradation rate coefficient for dissolved contaminant, respectively, and K_s is the half saturation coefficient.

In the system of equations above, k_o, k_1, k_m, and Ks were estimated by regression of the soil-extract biodegration experiments, and k_{eq} by non-linear regression analysis of the bioavailability data. For Model I a value of zero is used for k_{eq}..

RESULTS

Sorption of naphthalene was found to be linear for both soils (Figure 4-1). The distribution coefficients (K_d) were calculated by least squares regression and found to 4.3 L/kg for SPCF and 26 L/kg for Kal-A. Desorption was essentially complete within a few hours, with the release of naphthalene from SPCF occurring somewhat faster than from Kal-A. Coefficients in the three-site model were determined by non-linear regression (Table 4-2). It can be seen that the higher organic carbon soil (Kal-A) was found to have a substantially larger proportion of equilibrium sites, but desorption from the non-equilibrium sites was slower. The fraction of non desorption sites was essentially the same in both soils, at approximately 0.13. Overall naphthalene recovery in the sorption-desorption experiments was found to 99.0 (± 1.8) % for Kal-A and 99.0 (± 3.5) for SPCF. No evidence of naphthalene degradation was observed.

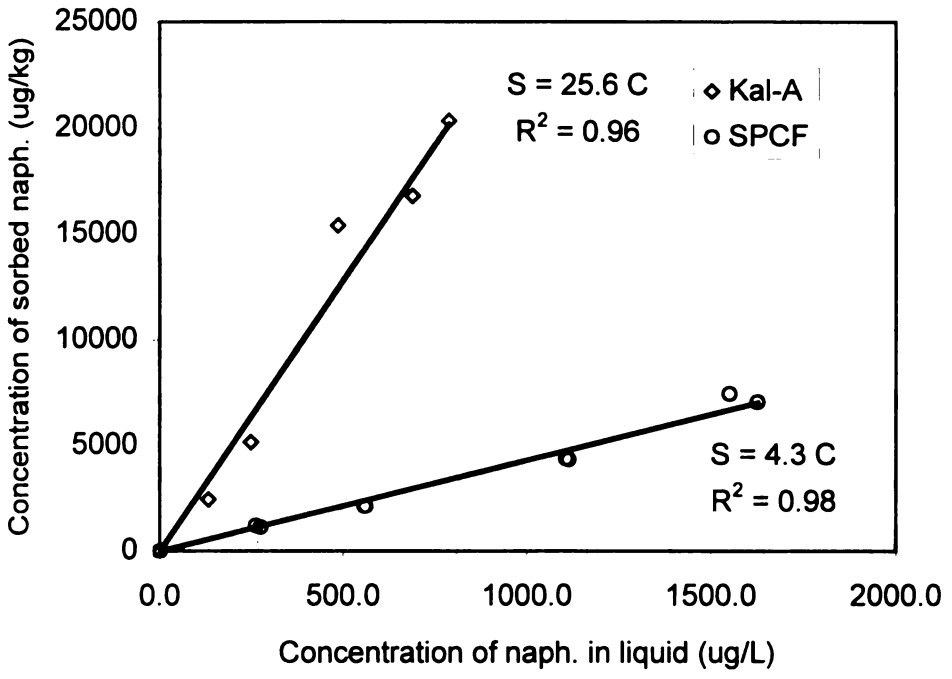

Figure 4- 1. Sorption isotherm of naphthalene in two-soil slurries.

Table 4- 2. Desorption site fraction and desorption rate coefficient estimated by three-site desorption model.

Soil	Site fraction			Desorption rate coefficient	R^2
	Desorbable		Non-desorption		
	f_{eq}	f_{neq}	f_{nd}	α (min^{-1})	
SPCF	0.37	0.50	0.13	0.019	0.95
Kalkaska-A	0.70	0.16	0.14	0.0045	0.96

The attachment of the four bacterial strains to the two soils ranged from 13 % to 43 % (Table 4-3). No clear pattern of dependence on either soil or organism alone was observed – attachment appeared to depend on both factors. From the soil extract control rate data, it was found that naphthalene degradation followed a zero-order relationship for ATCC 17484, and first-order expression for NP-Alk and NCIB, and Michaelis-Menten kinetics for G7. The appropriate rate parameters were determined from these experiments.

The disappearance of naphthalene in the bioavailability assaies was the predicted using these degradation rate parameters, and the distribution coefficient from the sorption isotherms, using model I with the appropriate biokinetic expression. Correlation coefficients describing the goodness of fit for the predictions are shown in Table 4-4, and plots comparing the predictions to the experimental data are shown in Figures 4-2 ~ 4-6.

To assess whether degradation related to the solid-phase concentration occurs, model II was used to the fit the experimental data by non-linear regression with k_{eq} as a fit parameter. The improvements in correlation coefficients were recorded, and the F-test used to determine whether the use of model II results in a significantly better description of the data at the 95% confidence level. Model II fits are also shown in Figures 4-3 ~4-6 and the model coefficients in Table 4-4. In the figures presented, only the total amount of naphthalene is shown, however separate plots of liquid and solid-phase concentrations shown similar results.

Table 4- 3. Each cell attachment percent in both soil slurries.

	Cell attachment %			
cell	SPCF		Kal-A	
	average	stdev	average	stdev
ATCC	25	12.5	13.4	6.3
NP-Alk	37.4	10.7	43.4	9.8
NCIB	14.0	16.8	16.9	15.2
G7	14.8	9.7	39.0	8.5

Table 4- 4. Kinetic parameters estimated in control and soil-slurry systems.

Bacteria	Soil		Degradation rate parameters†		Solid-phase degradation rate††		F-test result of 95% confidence level†††
			k_o		k_{eq}	R^2	
ATCC 17484	SPCF	Prediction	3.62		0	0.99	F
		Regression	3.62		0.00061	0.99	
	Kal-A	Prediction	2.01		0	0.97	F
		Regression	2.01		-0.00034	0.97	
			k_1		k_{eq}	R^2	
NP-Alk	Kal-A	Prediction	0.00417		0	0.76	T
		Regression	0.00417		0.00252	0.98	
NCIB	SPCF	Prediction	0.0183		0	0.99	F
		Regression	0.0183		0	0.99	
	Kal-A	Prediction	0.0144		0	0.69	T
		Regression	0.0144		0.0103	0.99	
			k_m	Ks	k_{eq}	R^2	
P. putida G_7	SPCF	Prediction	4.94	36.4	0	0.96	F
		Regression	4.94	36.4	0.00441	0.99	
	Kal-A	Prediction	6.83	36.4	0	0.90	T
		Regression	6.83	36.4	0.0068	0.99	

†Degradation rate parameters from soil-extract controls

††Solid-phase degradation rate from regression

††† F test results (α=0.05)

F: The difference of k_{eq} in between regression and prediction was not significant at a confidence level of 95%.

T: The difference of k_{eq} in between regression and prediction was significant at a confidence level of 95%.

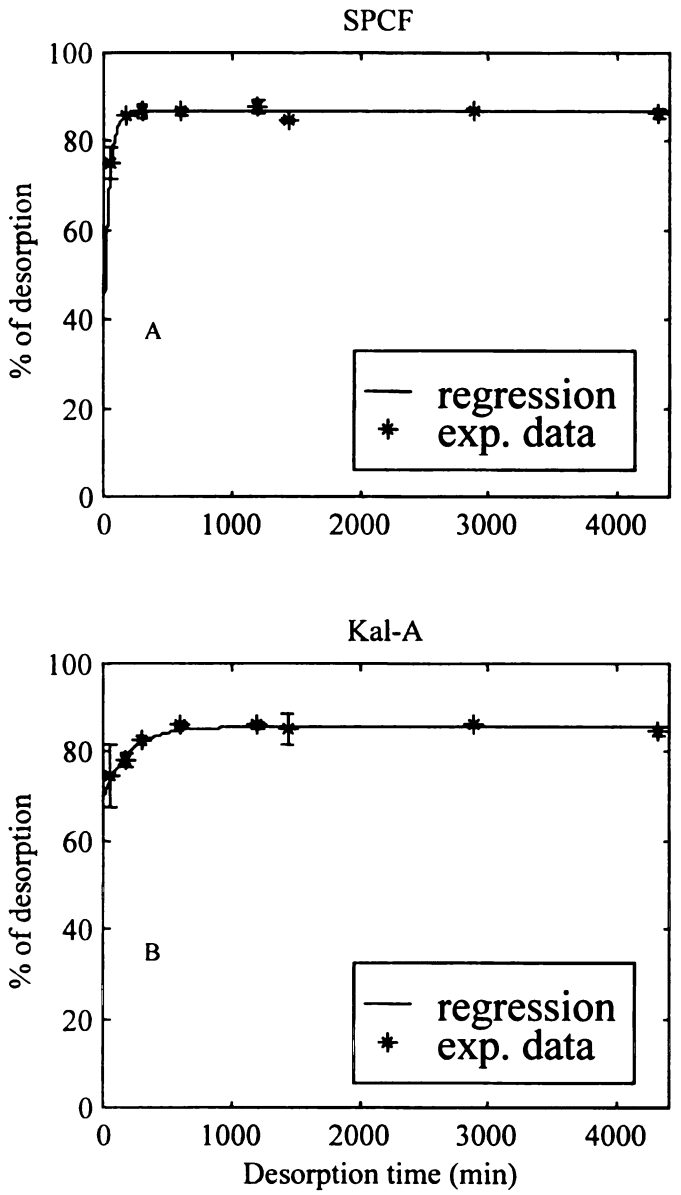

Figure 4- 2. Percent of desorption vs. desorption time. Three-site model was used for the regression.

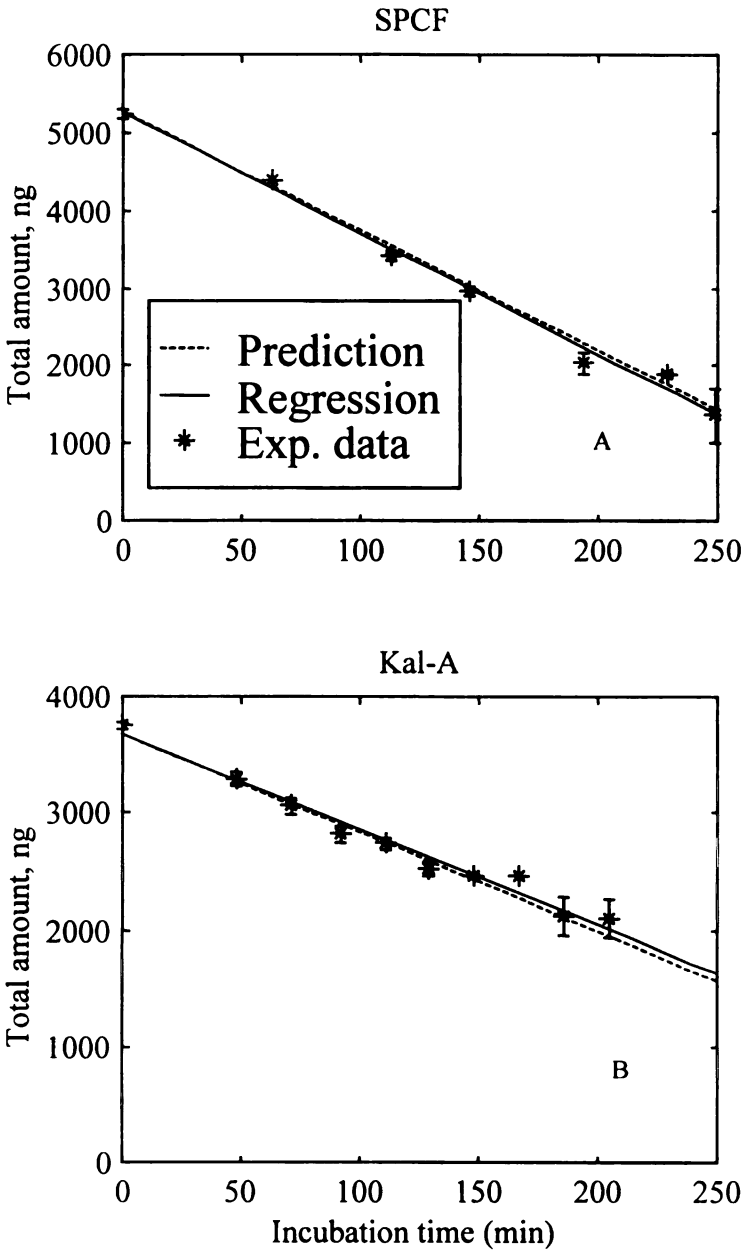

Figure 4- 3. Total amount of naphthalene in SPCF and Kal-A soil slurries as incubation time of strain ATCC 17484

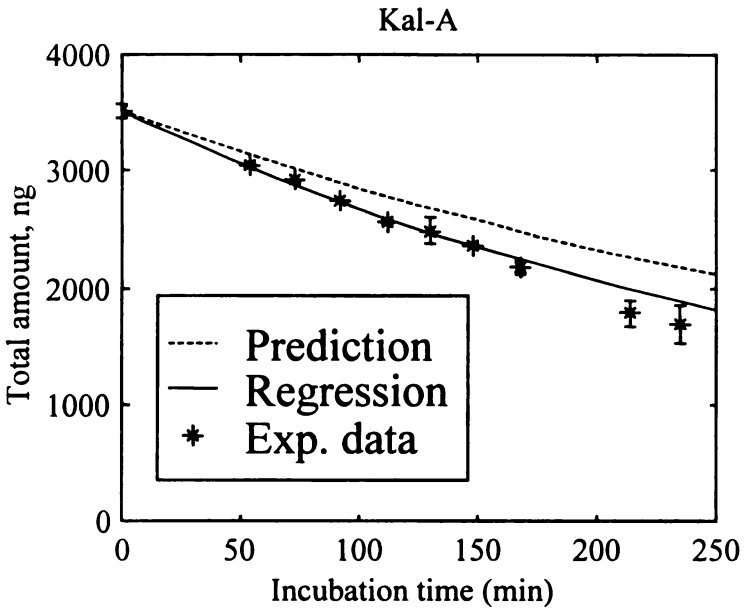

Figure 4- 4. Total amount of naphthalene in Kal-A soil slurry as incubation time of strain NP-Alk

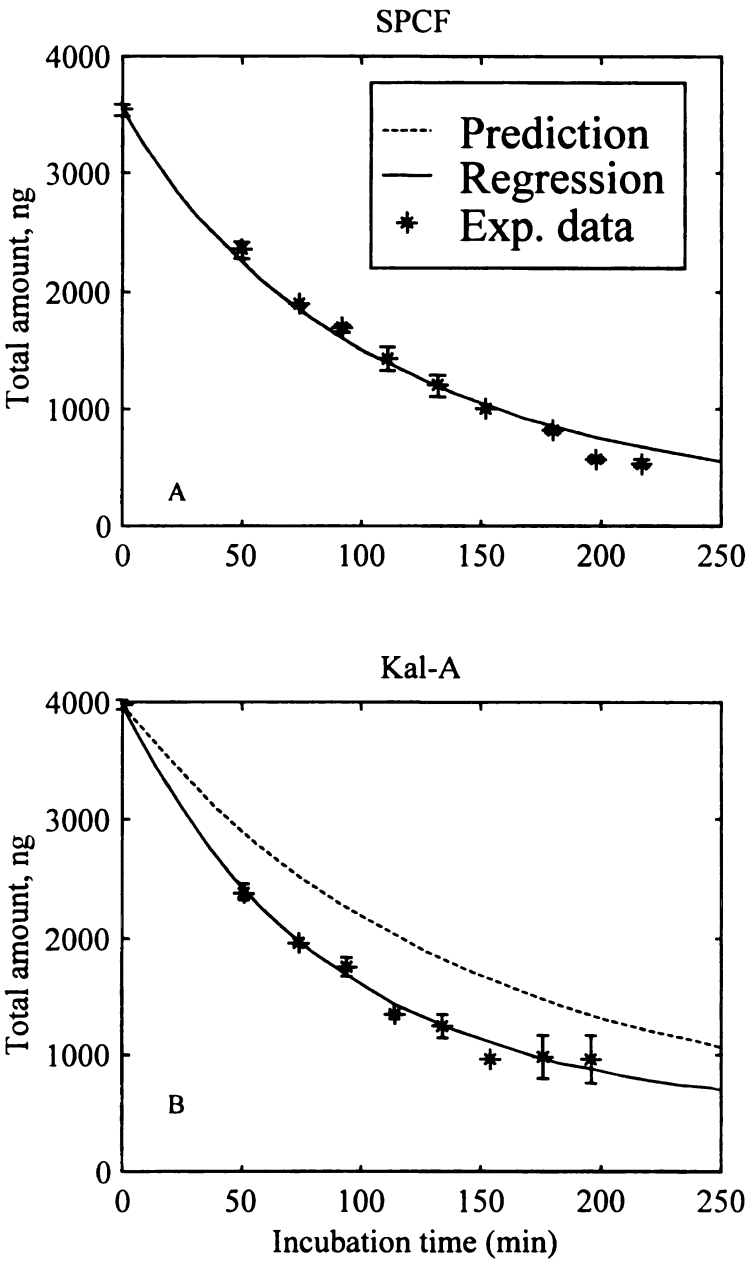

Figure 4- 5. Total amount of naphthalene in SPCF and Kal-A soil slurries as incubation time of strain NCIB.

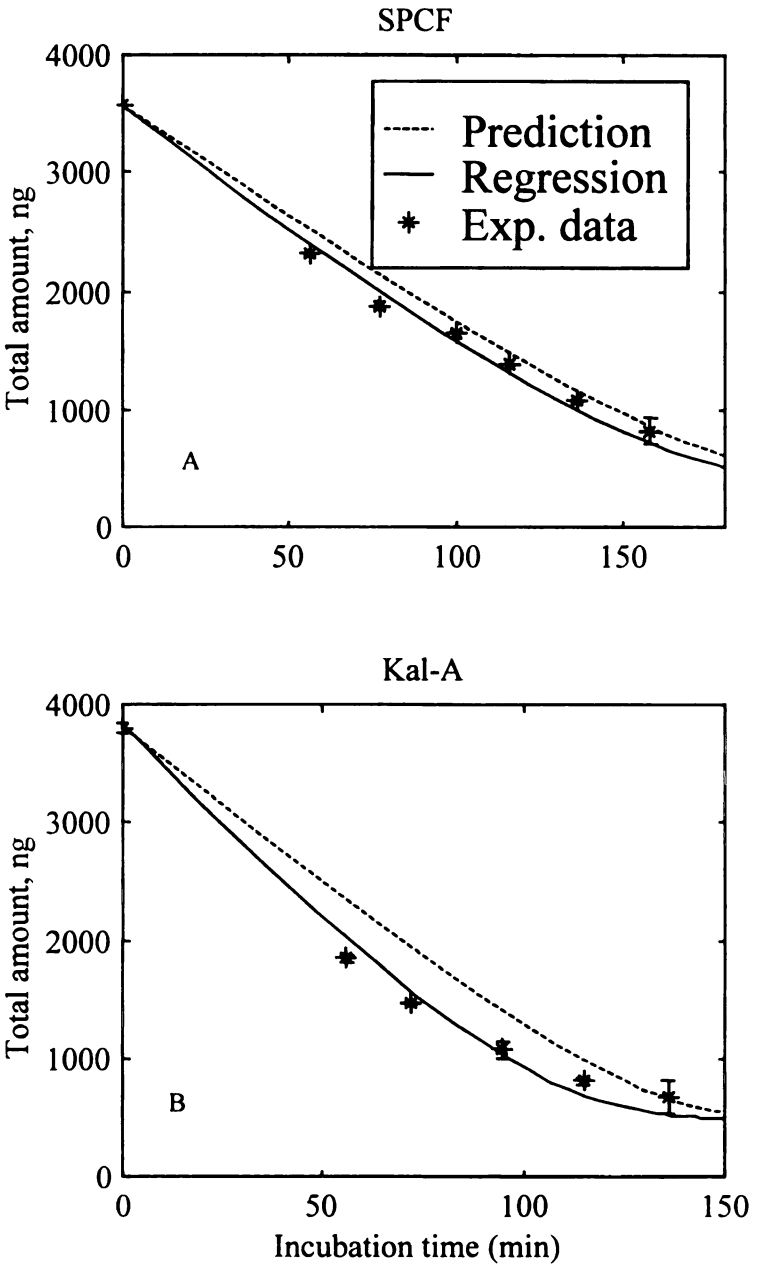

Figure 4- 6. Total amount of naphthalene in SPCF and Kal-A soil slurries as incubation time of strain *P.p.*G7.

For strain ATCC 17484, the disappearance of dissolved and sorbed naphthalene was well predicted by Model I and the use of Model II was not justified statistically (Figure 4-3). For strain NP-Alk (only Kal-A soil was studied), Model I predicted higher than observed concentrations. A good fit to the data could be obtained with Model II, indicating the need for additional or enhanced degradation (Figure 4-4). This was supported by the F-test results (Table 4-4). The two soils performed differently with strain NCIB. The soil with the lower K_d value (SPCF) was well described by Model I, while that with the higher sorption capacity was fit better by Model II (Figure 4-5). Similar results were obtained from strain G7 (Figure 4-6). Note that even though the line predicted from model I was slightly above the experimental data, the estimated k_{eq} (0.00441 min^{-1}) value was not statistically significant from zero at a confidence level of 95 % (Table 4-4, Figure 4-6a).

DISCUSSION

The assumptions used in Model I (which was used for the predictions) were that a) desorption could be described by a model incorporating equilibrium, rate-limited and non-desorption sites, b) desorption parameters developed in abiotic experiments were valid in the bioavailability assays, c) biodegradation is dependent upon only liquid-phase concentrations, and d) degradation rates in developed in soil-extract solutions were applicable to the bioavailability assays. This model predicted the behavoir in all of the experiments using the lower organic content soil, SPCF. However, it underestimated the disappearance rate of naphthalene for three organisms (NP-Alk, NCIB, and G7) with the higher organic-content Kal-A soil. This provides clear evidence that the simple sequential desorption-degradation model using independent process parameters is not universally applicable to systems in which both processes occur.

In this study, the observed enhancement was described using a second degradation process dependent upon solid-phase substrate concentrations. There are several possible mechanistic explanations for this result. The first is that degradation by some portion of the biomass is linked to solid-phase substrate concentrations. This might result from what has been termed "direct solid phase degradation", or equivalently, from zones of concentration higher than in the mixed-liquid-phase, such as might occur at the interface between attached organisms and the soil. The second is that the liquid phase degradation process is somehow enhanced by the presence of soil. We have attempted to account for other chemical species that might desorb and stimulate degradation by measuring the biokinetic parameters in soil extracts. However, if these species are

depleted by degradation, further release might occur in the soil slurries, thereby further stimulating degradation. A third possibility is that the biomass serves to increase the desorption rate coefficient. It is difficult to postulate a mechanism by which this occurs. We therefore conclude that degradation rate enhancement occurs with some soil-organism combinations, but the precise cause of this remains elusive.

In an attempt to further distinguish between these explanations, predictions were made assuming the same fraction of non-desorption sites, but the balance of sorbed material was in equilibrium with the liquid. This represents the limiting case for enhanced desorption rates. A slight change in the predictions was observed, but degradation rate enhancement was still clearly evident. Thus the explanation that biomass increases desorption can not alone explain the enhancement in degradation. This result also indicates that it may not be possible to distinguish between the first two explanations. This is because there is no mathematical difference between an increase in the liquid-phase degradation rate and formulation of a second rate process based on the solid-phase concentration, when the two concentrations are in equilibrium.

Degradation rate enhancement was only observed with the more sorptive soil. In this soil, the solid-phase concentrations are approximately six times higher for a given liquid-phase concentration. As a result, experiments with the less sorptive soil are approximately six times less sensitive to rate enhancement. It is entirely possible, therefore, that rate enhancement occurs with this soil, but is not observed because this process represents a negligible contribution to the overall rate. This is perhaps substantiated by the result with G7 and SPCF, where a slight enhancement can be seen in the plot (Figure 6), but this was not found to be statistically significant (Table 4-4).

It was also observed that degradation rate enhancement did not occur even with the more sorptive soil for the ATCC 17484 organism. The kinetics of this reaction are described by a zero-order formulation, indicating that the degradation rate is limited by a factor other than substrate concentration. Thus, we probably should not expect any enhancement due to the presence of a sorptive solid phase because if concentration is not controlling the rate direct access to the solid or locally higher concentrations are not likely to produce an effect.

Two of the bacterial strains used in this effort, ATCC 17484 and NP-Alk, have been used in previous bioavailability studies (Guerin and Boyd, 1992; Guerin and Boyd, 1997). Guerin and Boyd reported that the bioavailability of sorbed naphthalene was different for two organisms, that is, sorbed naphthalene was directly available to ATCC 17484 but not available to NP-Alk. In the present study, the opposite result was found.

We did not attempt to directly address the causes for this apparent contradiction, but conclude that differences in the two approaches could cause this. In the previous work, only CO_2 production was measured as the focus was on changes in mineralization rates. In this study, desorption and biodegradation were independently quantified and both dissolved and sorbed naphthalene were directly measured over the incubation period because the objective was on mechanistic interactions. In addition, experimental conditions such as the soil used and substrate concentrations, were substantially different.

ACKNOWLEDGEMENTS

This research was supported, in part, by the Center for Microbial Ecology (National Science Foundation, Grant DEB9120006) and by the Great Lakes and Mid-Atlantic Center for Hazardous Substance Research (Environmental Protection Agency, Michigan Department of Environmental Quality).

REFERENCES

Ahn, I. S. 1998. Mathematical Modeling and Experimental Verification of Naphthalene Transport and Biodegradation in Soil. Environmental Engineering and Biotechnology. Ithaca, Cornell University: 176.

Calvillo, Y. M. and M. Alexander. 1996. "Mechanism of microbial utilization of biphenyl sorbed to polyacrylic beads." *Appl. Microbiol. Biotechnol* 45: 383-390.

Crocker, F. H., W. F. Guerin and S. A. Boyd. 1995. "Bioavailability of Naphthalene Sorbed to Cationic Surfactant-Modified Smectite Clay." *Environ. Sci. Technol* 29: 2953-2958.

Deitsch, J. J. and J. A. Smith. 1999. "Sorption and desorption rate comparisons for 1,2-dichlorobenzene to a peat soil." *Environ. Toxicol. Chem.* 18: 1701-1709.

Feng, Y., J. H. Park, T. C. Voice and S. A. Boyd. 2000. "Bioavailability of Soil-Sorbed Biphenyl to Bacteria." *Environ. Sci. Technol.* 34: 1977-1984.

Grimm, A. C. and C. S. Harwood. 1997. "Chemotaxis of Pseudomonas spp. to the Polyaromatic Hydrocarbon Naphthalene." *Appl. Environ. Miceobiol.* 63: 4111-4115.

Guerin, W. F. and S. A. Boyd. 1992. "Differential Bioavailability of Soil-Sorbed Naphthalene to Two Bacterial Species." *Appl. Environ. Microbiol* 58: 1142-1152.

Guerin, W. F. and S. A. Boyd. 1993. "Bioavailability of Sorbed Naphthalene to Bacteria: Influence of Contaminant Aging and Soil Organic Carbon Content." *Sorption and Degradation of Pesticides and Organic Chemicals in Soil. SSSA Special Publication no* 32: 197-208.

Guerin, W. F. and S. A. Boyd. 1997. "Bioavailability of naphthalene associated with natural and synthetic sorbents." *Water Res.* 32: 1504-1512.

Harms, H. and A. J. B. Zehnder. 1995. "Bioavailability of Sorbed 3-Chlorodibenzofuran." *Appl. Environ. Microbiol* 61: 27-33.

Harwood, C. S., N. N. Nichols, M. K. Kim, J. L. Kitty and R. E. Parales. 1994. "Identification of the pcaRKF Gene Cluster from Pseudomonas putida: Involvement in Chemotaxis, Biodegradation, and Transport of 4-Hydroxybenzoate." *J. Bacteriol.* 176: 6479-6488.

Head, I. M. 1998. "Bioremediation: towards a credible technology." *Microbiology-UK* 144: 599-608.

Laor, Y., P. F. Strom and W. J. Farmer. 1999. "Bioavailability of Phenanthrene Sorbed to Mineral-Associated Humic Acid." *Wat. Res.* 33: 1719-1729.

Ortega-Calvo, J. J. and C. Saiz-Jimenez. 1998. "Effect of humic fractions and clay on biodegradation of phenanthrene by a Pseudomonas fluorescens strain isolated from soil." *Appl. Environ. Microbiol.* 64: 3123-3126.

Park, J. H., D. Kay, X. Zhao, S. A. Boyd and T. C. Voice. 1999. Bioavailability of 2,4-dichlorophenolxyacetic acid in silica-slurry systems. ACS 218th National Meeting, New Orleans, LA, American Chemical Society, Washington D.C.

Park, J. H., D. Kay, X. Zhao, S. A. Boyd and T. C. Voice. 2000. "Bioavailability Model of 2,4-dichlorophenolxyacetic acid in silica-slurry systems." in preparation: .

Park, J. H., X. Zhao and T. C. Voice. 2000. "Comparison of Kinetic Parametes in Batch and Column Systemes." *Environmental Progress* in press (expected May 2001): .

Shelton, D. R. and M. A. Doherty. 1997. "Estimating Losses of Efficacy Due to Pesticide Biodegradation in Soil: Model Simulations." *Soil Sci. Soc. Am. J* 61: 1085–1090.

Shelton, D. R. and M. A. Doherty. 1997. "A model describing pesticide bioavailability and biodegradation in soil." *Soil Sci. Soc. Am. J* 61: 1078 – 1084.

Tang, W. C., J. C. White and M. Alexander. 1998. "Utilization of sorbed compounds by microorganisms specifically isolated for that purpose." *Appl. Microbiol. Biotechnol.* 49: 117-121.

Tomson, M. B. and J. J. Pignatello. 1999. "Causes and effects of resistant sorption in natural particles." *Environ. Toxicol. Chem.* 18: 1609.

van Genuchten, M. T. and R. J. Wagenet. 1989. ""Two-site/two-region models for pesticide transport and degradation: theoretical development and analytical solutions"." *Soil Science Society of America Journal* 53: 1303-1310.

Whitman, B. E., D. R. Lueking and J. R. Mihelcic. 1998. "Naphthalene uptake by a Pseudomonas fluorescens isolate." *Can. J. Microbiol.* 44: 1086-1093.

Willumsem, P. A. and E. Arvin. 1999. "Kinetics of Degradation of Surfactant-Solubilized Fluoranthene by a *Sphingomonas paucimobilis*." *Environ. Sci. Technol.* 33: 2571-2578.

Wolin, E. A., M. J. Wolin and R. S. Wolfe. 1963. "Formation of Methane by Bacterial Extracts." *J. Biol. Chem.* 238: 2882-2886.

Zhao, X., and Voice, T. (2000). "Assessment of bioavailaibility using a multicolumn system." *Environmental Science & Technology*, 34(8), 1506-1512.

CHAPTER 5 BIOAVAILABILITY OF NON-DESORBABLE NAPHTHALENE IN SOIL SLURRIES

ABSTRACT

The degradation of naphthalene was studied in soil-slurry systems, and a quantitative model was developed to evaluate the bioavailability of sorbed-phase contaminant. Four soils with different organic matter contents were used as sorbents. Two naphthalene degrading organisms, *Pseudomonas putida* G7 and NCIB 9816-4, were also selected. Sorption isotherms, single and series-dilution desorption studeis were conducted to evaluate distribution coefficients, to estimate desorption parameters, and to measure the non-desorbed amount of naphthalene. Biodegradation kinetics were measured in soil extract solutions and rate parameters developed. For the bioavailability assays, sorption equilibrium was established, and the systems were inoculated. Naphthalene concentrations in both sorbed and dissolved phases were measured over time. Enhanced bioavailability, as evidenced by faster than expected degradation rates, was observed in soils with the higher sorption distribution coefficients. These observations could be desrcribed by using a model formulations that included solid-phase degradation. In all soils, degradation of non-desorbable naphthalene was observed.

INTRODUCTION

Numerous researchers have made the assumption, either explicitly or implicitly, that sorbed contaminants are not directly available for biodegradation (Ogram et al., 1985; Scow et al., 1986; Shimp and Young, 1988; Scow et al., 1989; Scow and Hutson, 1992; Estrella et al., 1993; Tabak et al., 1994; Shelton and Doherty, 1997; Zhang et al., 1998). The most commonly accepted conceptual model is that sorbed contaminant first desorbs, and then degrades. Recently, other studies have suggested that adsorbed substrate may be directly available for degradation by attached cells (Guerin and Boyd, 1992, 1993,1997; Crocker et al., 1995; Calvillo and Alexander, 1996; Tang et al., 1998; Ortega-Calvo and Saiz-Jimenez, 1998). It should be noted that all of the evidence for direct availability is derived from mineralization studies. This approach, while useful for demonstrating the effects of sorbing materials on complete degradation, is somewhat limited in its potential to elucidate the underlying mechanims. Amongst these limitations are that CO_2 measurements do not indicate whether the mineralized material originates in the sorbed or dissolved phases, may not directly reflect substrate disappearance, and provide only an indicator of the combined effects of desorption and degradation, rather than of the individual processes. Central to this issue is that to independently evaluate a system controlled by multiple rate processes, multiple time-concentration data sets are required. Finally, the availability of irreversbly sorbed contaminant to biodegradation has not been explicitly studied.

In the present study, substrate depletion, instead of mineralization, was used to investigate the bioavailability of sorbed naphthalene. Sorption and degradation could be tracked independently by measuring naphthalene concentrations in both the sorbed and

dissolved phases. Models were developed to quantitatively analyze the data. Naphthalene was selected as a test contaminant, since it has often been used as a model compound for PAHs, and this class of contaminant is a critical bioavailability issue at numerous petroleum contamination sites.

MATERIALS AND METHODS

Experimental

Four sandy-loam soils with organic matter contents from 1 to 4% were collected from forest environments in Michigan. Soil samples were air-dried and sieved through a U.S. Sieve Series a #20 sieve (> 650 μm) to remove larger components. Prior to use, the soils were sterilized by gamma irradiation (^{60}Co source) at a dosage of 2 Mrad. Following sterilization, sealed containers were maintained at room temperature. Before the soils were used, 0.1g of each soil was placed on a nutrient agar plate and incubated at 30 °C for 3 days to verify sterility. No colony forming units were observed. Soil characteristics are summarized in Table 5-1.

Table 5- 1. Soil characteristics*

Soils	Organic matter content (%)	Mechanical characteristics (%)			Distribution coefficient
		Sand	Silt	Clay	K_d
SPCF	1.9	78	17	5	4.3
Rubicon-BS1	1.4	87	8.2	4.7	8.8
Rubicon-A	2.0	89	8.4	2.7	11.0
Kalkaska-A	3.9	91	7.7	1.7	25.6

* Analysis completed by Plant and Soil Science Laboratory in Michigan State University.
SPCF. This was collected in 15-31 cm depth of forest in Michigan State University, East Lansing, Michigan.
Rubicon-BS1 (subsurface in 15-31 cm depth), Rubicon-A (surface in 0-6 cm depth), and Kalkaska-A (surface in 0-6 cm depth). These were collected at uncultivated forest in the northern half of Michigan's lower peninsula (The detail location was documented by Vance (Vance, 1984)).

Two naphthalene-degrading strains, *Pseudomonas putida* G7 and *Pseudomonas* sp. strain NCIB 9816-4, were selected because these organisms have been reported to be motile and chemotactic to naphthalene (Grimm and Harwood, 1997; Grimm and Harwood, 1999). G7 and NCIB 9816-4 were grown in 100 mL minimal mineral salt media (Harwood et al., 1994). Solid naphthalene was added to the sterilized liquid at a final concentration of 200 mg/L. The liquid medium was inoculated by adding one mL of starved liquid culture (cell density of ~10^7 CFU/mL) to 100 mL medium, and stirred. Growth was monitored by absorbance at 600 nm, and cells were harvested at a point determined to correspond to early stationary phase based on a full growth curve. Cells were separated by centrifugation (1900 × g, 20 min) and resuspended in a chemotaxis buffer (CB) (Harwood et al., 1994) before use. The CB contained 13.6 g/L of potassium phosphate and 7.4 mg/L of EDTA, and was adjusted by 10N NaOH to pH 7. This procedure was repeated three times to ensure the removal of remaining naphthalene from the cell growth medium. All the cell suspensions were kept at room temperature and used within 3 hours.

Naphthalene soil/water distribution coefficients were measured experimentally. An aliquot of each sterile soil (0.68g of SPCF, 0.57g of Rub-BS1, 0.39g of Rub-A, and 0.18g of Kal-A) and 4.2 ml of CB containing ^{14}C-naphthalene stock (in methanol) were prepared in 5 mL screw-cap vials with Teflon-lined septa. The soil/water ratios were carefully selected to achieve approximately equal masses of naphthalene in both liquid and solid phases at end of the sorption period. The headspace was less than 1mL in volume. Initial liquid-phase concentrations ranged from 0 to 3,400 μg/L. Control vials

without soil were prepared in triplicate. Vials were tumbled at 6 rpm for 2 days in the dark. After mixing, each vial was centrifuged for 5 min at 1200 × g to separate soil, and the supernatant was sampled. The concentration of naphthalene in the supernatant was determined by liquid scintillation counting (LSC) and high pressure liquid chromatography (HPLC). The naphthalene concentrations on soils were calculated by the difference between the initial and final liquid-phase concentrations.

Desorption rates were measured in batch soil slurries with initial naphthalene concentrations of 800 μg/L. The vials were tumbled at 6 rpm for 2 days in the dark. The final concentration of naphthalene in the liquid phase was determined by LSC, and the amount of sorbed naphthalene calculated by difference. The supernatant was then decanted to the extent possible, the residual water determined by weight, and naphthalene-free CB was added to the original volume. The vials were then tumbled again at 6 rpm, with periodic liquid-phase sampling and analysis.

Series-dilution desorption assays were conducted to measure the amount of non-desorbable naphthalene. Six replicate soil slurries were prepared for each soil using the desorption procedure described above. The slurries were tumbled for 24 hours, the supernatant decanted, sampled and analyzed for naphthalene, and the bottles refilled with naphthalne-free CB to the original volume. This procedure was repeated for a total of 6 successive desorption periods. The non-desorbable naphthalene was determined using a methanol extraction of the soil following this series dilution procedure. Recovery studies were performed to determine extraction efficiencies. Triplicate soil slurries were prepared in the same manner as the desorption rate studies except methanol was used as

the desorption solution. The concentration was measured after 2 days of tumbling by HPLC, and the efficiency calculated by mass balance.

Cell attachment was measured in duplicate soil slurries with initial liquid-phase naphthalene concentrations of 800 μg/L. The vials, and controls were tumbled at 6 rpm for 2 days in the dark. The slurries and soil-free controls were inoculated to a density of ~10^7 CFU/mL

from an inoculum harvested during the early stationary phase. The inoculated vials were tumbled at 6 rpm for 1 hr to facilitate cell attachment. The soil was then allowed to settle quiescently for 2 hr and 0.1 mL of supernatant was removed for plate counting. Cell attachment efficiency was calculated by difference.

Bioavailability assays were performed using soil slurries in 5 mL screw-cap vials with Teflon-lined septa. Using the same soil to water ratio as in the desorption assays, naphthalene was added to an initial liquid-phase concentration of approximately 800 μg/L, and the vials tumbled for 2 days in the dark. Sterility of the soil slurries was checked by plating out the suspension on nutrient agar plates. The vials were then inoculated with 0.05 mL of cell suspension harvested during the early stationary phase to initiate biodegradation of naphthalene. Cell density in the serum vials was approximately 10^7 CFU/mL. Inoculated vials were tumbled at 6 rpm. At predetermined time intervals, vials were removed and centrifuged at 1200 × g for 5 min to separate soil. One ml of supernatant was transferred a vial containing 0.01 ml 10N NaOH to stop naphthalene degradation, and analyzed by HPLC. The remaining supernatant was decanted and extracted with methanol as described above to determine solid-phase concentrations by HPLC.

Biodegradation rates were measured in soil-extract solutions. These were prepared by tumbling soil slurries for 2 days at the same soil to water ratio as in the desorption and bioavailability experiments. Kinetic data were obtained using the same bioavailability assay procedure, except no soil was added to the vials. Both sterile-CB and soil-extract controls were also used to monitor abiotic losses of naphthalene during the sorption and degradation periods. All experiments were performed at room temperature (24 ± 1 °C) unless otherwise mentioned.

Naphthalene was analyzed by HPLC using a C_{18} reverse-phase column, an 80% acetonitrile/20% water mobile phase, and fluorescence detection (280nm excitation, 340nm emission). The analytical detection limit was 0.2μg/L. The detection limit for LSC was less than 1 μg/L for the naphthalene solution used in this study.

Mathematical

Four related kinetic models employing different assumptions for the biodegradability of different contaminant pools were used to evaluate the experimental results. The liquid phase concentration is increased by desorption of sorbed contaminant and decreased by biodegradation. All models assume that soils have three types of desorption sites: equilibrium, non-equilibrium and non-desorption sites (Park et al., 2000).

$$S = S_{eq} + S_{neq} + S_{nd} \qquad \text{Eq. 16}$$

where S (μg/kg) is the total sorbed contaminant concentration, which is subcripted for the different solid phase compartments (eq for equilibrium, neq for non-

equilibrium, and nd for non-desorption). Desorption from equilibrium sites is described by a linear partitioining model

$$S_{eq} = f_{eq} \cdot K_d \cdot C \qquad \text{Eq. 17}$$

where C (μg/L) is the liquid-phase concentration, f_{eq} is the equilibrium site fraction and K_d (L/kg) is the sorption distribution coefficient. A first-order linear driving force formulatio is used to describe desorption from non-equilibrium sites:

$$J_n = -\alpha \cdot (S_{neq} - f_{neq} \cdot K_d \cdot C) \qquad \text{Eq. 18}$$

where J_n is the desorption flux, f_{neq} is non-equilibrium site fraction in desorbable site, and α (min^{-1}) is first-order desorption rate coefficient.

In model I, biomass can utilize only liquid-phase contaminant. Model II allows biomass to also utilize contaminant in equilibrium sites on the solid phase. Non-equilibrium, or rate limited sites, are available in model III , and all three sites, including the non-desorption sites, can be accessed in model IV (Figure 5-1). The biodegradation rate coefficients for the various contaminant pools are independent. The mathematical basis for model IV is developed below. Models I, II and III are simplifications of this model, achieved mathematically by setting the appropriate degradation rate coefficients to zero. The total disappearance rate in slurry systems for model IV can be expressed as

$$-(V_l \cdot \frac{dC}{dt} + m \cdot \frac{dS}{dt}) = V_l \cdot R_{bio} + m \cdot k_{eq} \cdot S_{eq} + m \cdot k_{neq} \cdot S_{neq} + m \cdot k_{nd} \cdot S_{nd} \qquad \text{Eq. 19}$$

where m (kg) is the mass of soil, V_l (L) is the liquid volume, t is time and k_{eq}, kneq, and knd are the first order biodegradation coefficients of sorbed contaminants in equilibrium, non-equilibrium and non-desorption sites respectively. R_{bio} is the liquid-phase biodegradation rate expression, which can be formulated as:

$$R_{bio} = k_1 \cdot C \quad \text{for first-order kinetics, and} \qquad \text{Eq. 20}$$

$$R_{bio} = \frac{k_m \cdot C}{K_s + C} \quad \text{for Michaelis-Menten kinetics,} \qquad \text{Eq. 21}$$

where k_1 and k_m are the first-order and maximum degradation rate coefficient for dissolved contaminant, respectively, and K_s is the half saturation coefficient.

Mass balances on the non-equilibrium and non-desorption sites, produces

$$\frac{dS_{neq}}{dt} = J_n - k_{neq} \cdot S_{neq} \qquad \text{Eq. 22}$$

$$\frac{dS_{nd}}{dt} = -k_{nd} \cdot S_{nd} \qquad \text{Eq. 23}$$

In the system of equations above, K_d was calculated from sorption isotherm data, f_{eq}, f_{neq}, f_{nd} and α were estimated by non-linear regression analysis of the desorption rate data, k_1, k_m, and Ks by regression of the soil-extract biodegradation experiments, and k_{eq}, k_{neq} and k_{nd} by non-linear regression analysis of the bioavailability data.

For model I, k_{eq}, k_{neq} and k_{nd} are set to zero, for model II k_{neq} and k_{nd} are set to zero, and for model III, k_{nd} is set to zero.

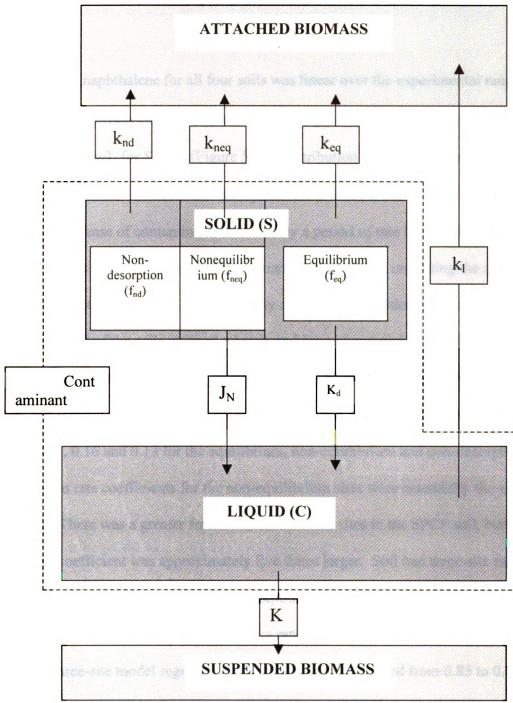

Figure 5- 1. Model IV description. k_{eq}, k_{neq} and k_{nd} are deleted in Model I. k_{neq} and k_{nd} are deleted in Model II. k_{nd} is deleted in Model III.

RESULTS

Sorption of naphthalene for all four soils was linear over the experimental range of liquid-phase equilibrium concentrations (0 to 1000 μg/L for Kal-A, Rub-A and Rub-BS1, and 0 to 1700 μg/L for SPCF) (Figure 5-2). Distribution coefficients ranged from 4.3 to 25.6 mL/g (Table 5-1). From the desorption rate profiles it can be seen that there is an instantaneous release of contaminant, followed by a period of rate limited desorption (Figure 5-3). Also shown on these plots is a horizontal dashed line indicating the amount that would be expected in solution for completely reversible desorption of all of the sorbed material. All of the soils stopped releasing additional material well before reaching this level. The distribution of desorption sites obtained from the three-site model was remarkably similar for three soils (Kal-A, Rub-A and Rub-BS1) with average fractions of 0.71, 0.16 and 0.13 for the equilibrium, non-equilibrium and non-desorption sites. Desorption rate coefficients for the non-equilibrium sites were essentially the same for these soils. There was a greater fraction of rate-limited sites in the SPCF soil, but the desorption rate coefficient was approximately five times larger. Soil had three-site model (Park et al., 2000) was used to estimate the desorption rate coefficients and each site fraction for all four soils. The correlation factors ranged from 0.91 to 0.97 (Figure 5-3). Analyzing the three-site model regression, desorbable fraction ranged from 0.85 to 0.89 (Table 5-2). The non-desorption site ranged from 0.11 to 0.15. The desorption rate coefficient on non-equilibrium site ranged from 0.00011 to 0.019 min^{-1}. Overall naphthalene recoveries in the sorption/desorption experiments were 99.0 (± 1.8) % for

Kal-A, 98.0 (± 1.7) % for Rub-A, 99.2 (± 5.2) % for Rub-BS1, and 99.0 (± 3.5) for SPCF.

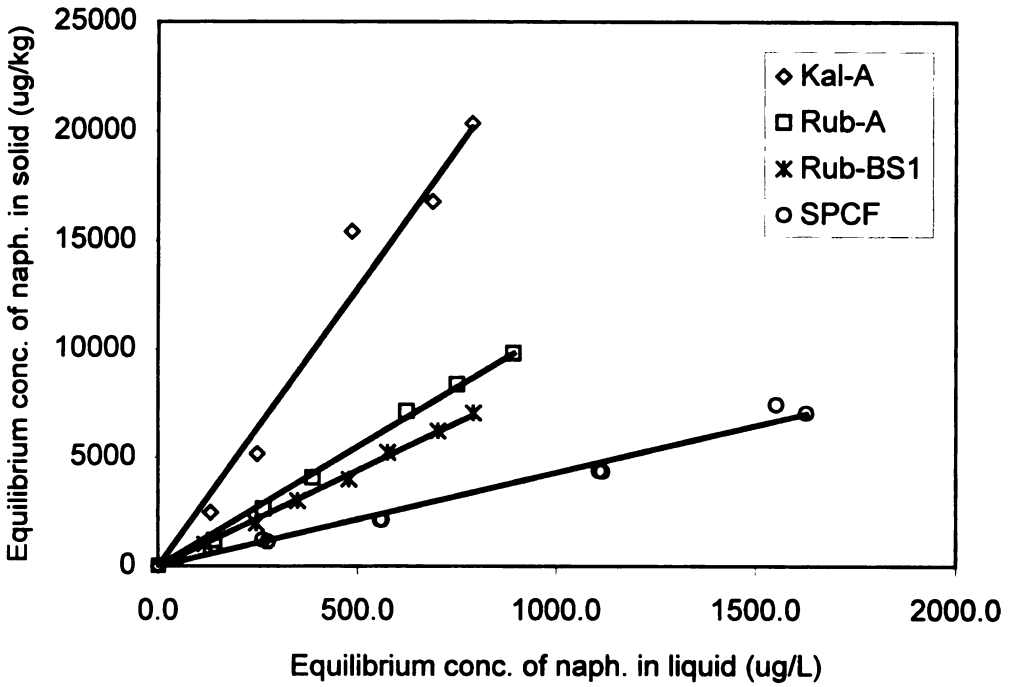

Figure 5- 2. Sorption isotherm of naphthalene in four-soil slurries.

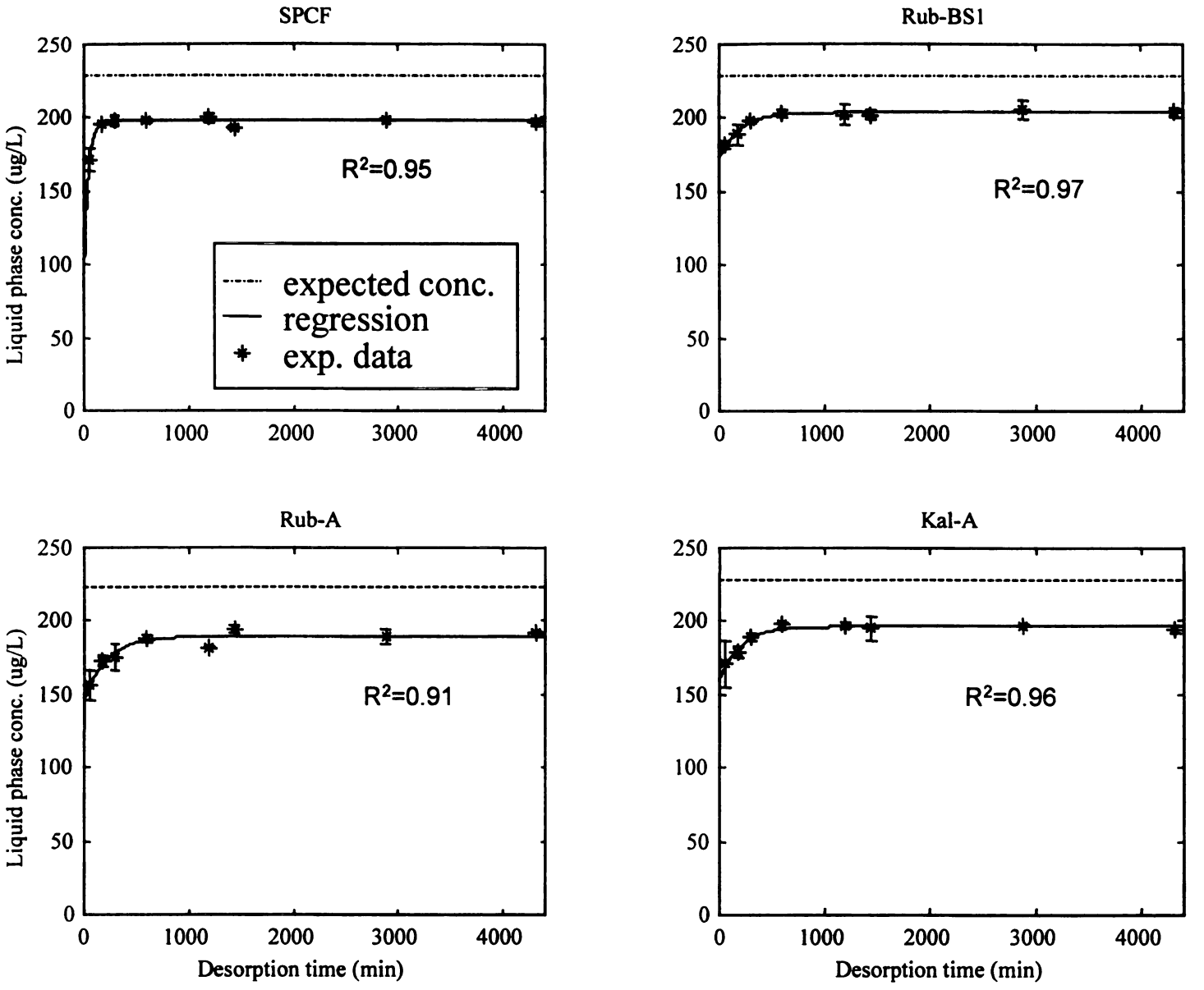

Figure 5- 3. Liquid-phase concentration over desorption time. Three-site model was used for the regression.

Table 5- 2. Desorption site fraction and desorption rate coefficient obtained from desorption, and non-desorption site fraction from series-dilution desorption experiment. Each site fraction and desorption rate coefficient were estimated from non-linear regression analysis.

Soil	Site fraction			Series-dilution desorption	
	Desorbable		Non-desorbable	Non-desorbable	Desorption rate coefficient
	f_{eq}	f_{neq}	f_{nd}	f_{nd}	α (min^{-1})
SPCF	0.37	0.50	0.13	0.11	0.019
Rubicon-BS1	0.76	0.13	0.11	0.12	0.0045
Rubicon-A	0.66	0.19	0.15	0.17	0.0039
Kalkaska-A	0.70	0.16	0.14	0.18	0.0045

The series dilution experiments show clear evidence of the existence of a non-desorption fraction, as the desorption isotherms all lie significantly above the sorption data (Figure 5-4). This fraction can be calculated from the intercept of the desorption isotherm and the initial solid-phase concentration (Table 5-2). The calculated value was remarkably close to that found by regression analysis. This result was further confirmed by extraction of the soil after the last dilution. It was also found that the slope of the desorption isotherm was essentially the same as the sorption isotherm, providing experimental validation of the assumption of this equivalence used in the regression analysis.

The degradation kinetics in the soil extract solutions followed by first order kinetic for NCIB and the Michaelis-Menten equation for G7. Rate parameters are shown in Tables 5-3 and 5-4.

Data from the bioavailability assays for both organisms and all four soils are shown in Figures 5-5 ~ 5-12. The depletion of liquid-phase substrate can be seen to occur over the first 400 minutes for all combinations. Solid-phase concentrations continue to decrease after this time however. In all cases total solid-phase contaminant concentrations dropped to levels below the non-desorbable amount, shown by the dashed horizontal line. Thus it appears that more contaminant was removed from the solids in these inoculated systems than could be accomplished in abotic systems.

Also shown in these figures are predictions of both the liquid- and solid-phase data using model I with liquid-phase degradation parameters from the biodegradation assays. Liquid-phase data are reasonably well predicted by model I for

the two soils with the lower distribution coefficients (SPCF and Rub-BS1), but not for the other two. Model I could not predict the solid-phase data well for any of the combinations, however, especially at later times. These predicted curves reached a plateau at or near the measured non-desorbable amount. Fitting the data using models II and III provided a better fit of the liquid-phase data, but were not able to describe solid-phase data below the non-desorbable amount. Regression using model IV was able to provide a reasonable description of both liquid- and solid-phase data for all combinations over the entire time period. Model parameters are shown in Tables 5-3 and 5-4.

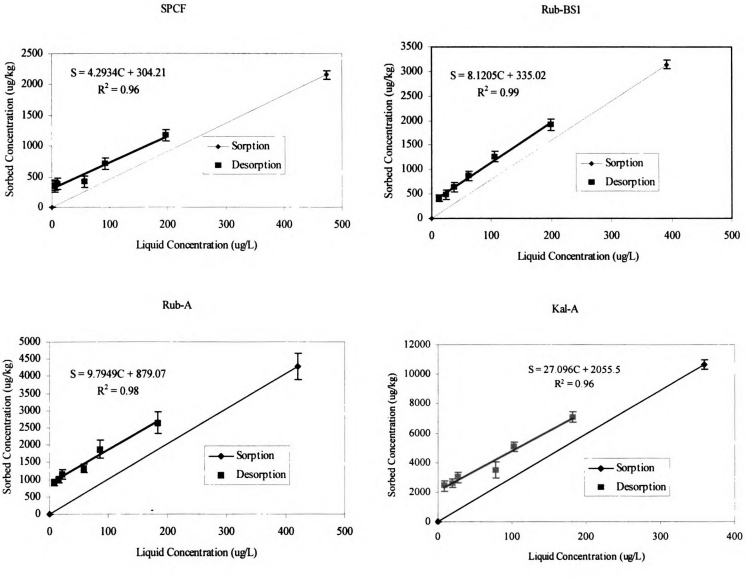

Figure 5- 4.. Series dilution desorption in four soils.

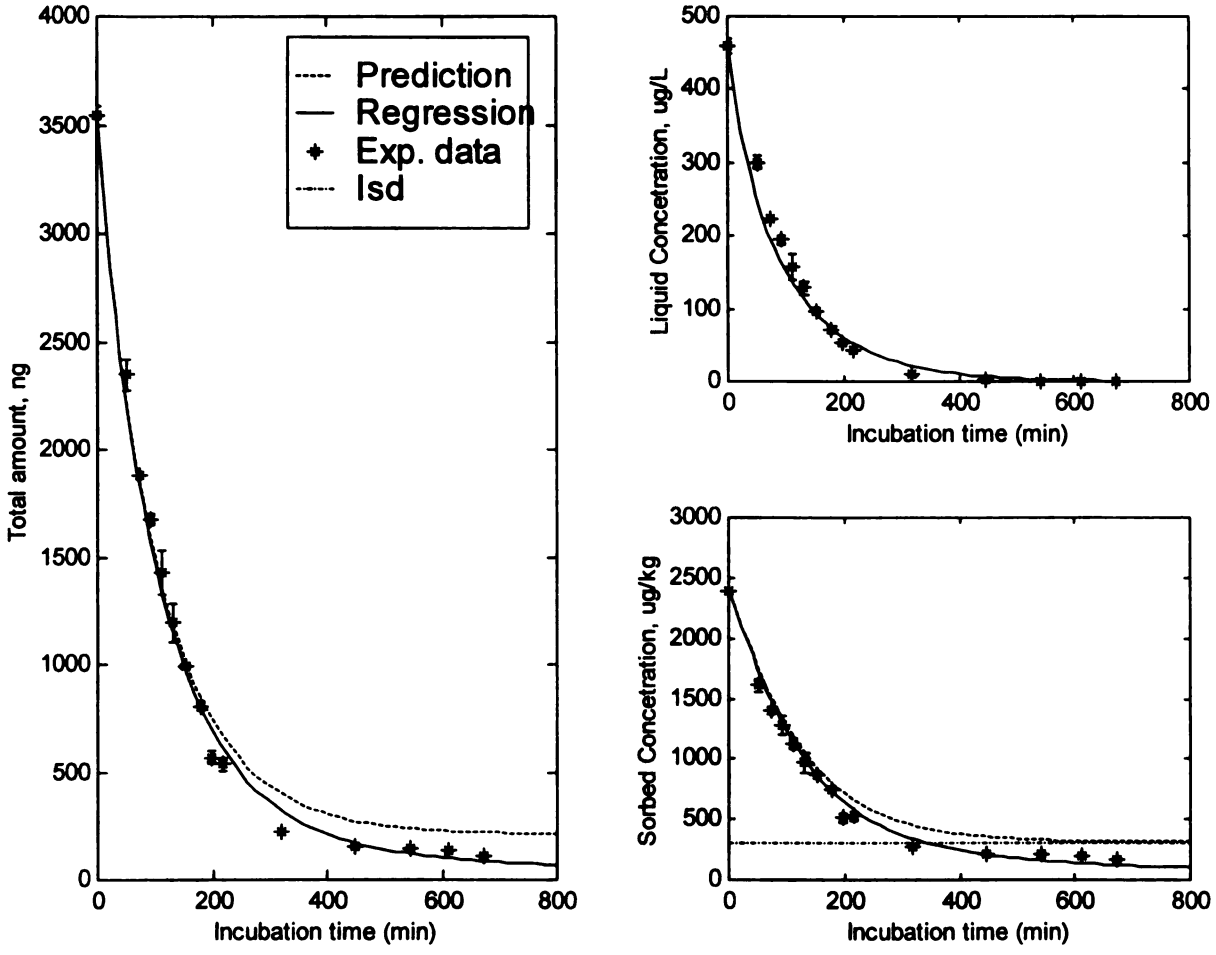

Figure 5- 5. Naphthalene disappearance under strain NCIB in SPCF soil slurry over incubation time.

Prediction. The line predicted by Model I
Regression. The line used to estimate the degradation kinetic parameters of sorbed phase with Model IV.
Exp. data. Measured data.
Isd. The intercept calculated in the series dilution desorption (Figure 5-4-SPCF)

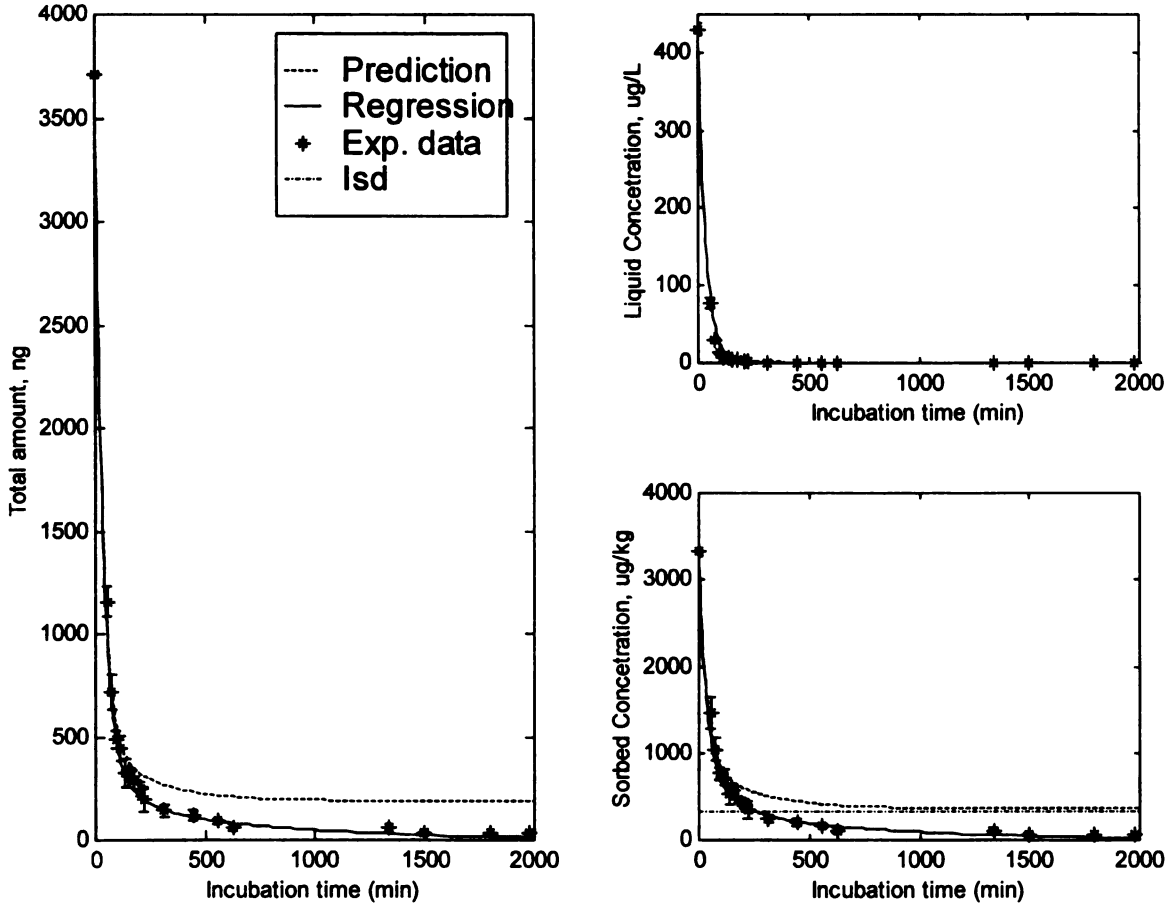

Figure 5- 6. Naphthalene disappearance under strain NCIB in Rub-BS1 soil slurry over incubation time.

Prediction. The line predicted by Model I
Regression. The line used to estimate the degradation kinetic parameters of sorbed phase with Model IV.
Exp. data. Measured data.
Isd. The intercept calculated in the series dilution desorption (Figure 5-4-Rub-BS1)

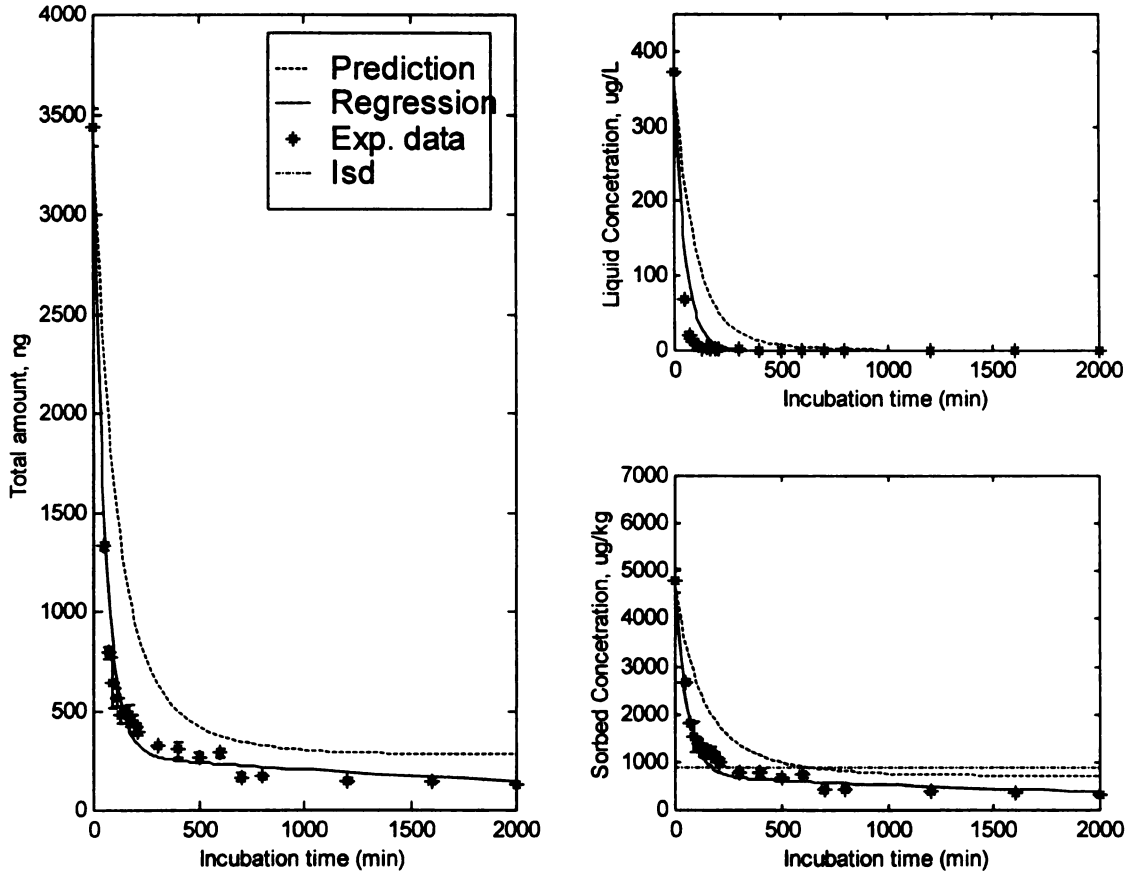

Figure 5- 7. Naphthalene disappearance under strain NCIB in Rub-A soil slurry over incubation time.

Prediction. The line predicted by Model I
Regression. The line used to estimate the degradation kinetic parameters of sorbed phase with Model IV.
Exp. data. Measured data.
Isd. The intercept calculated in the series dilution desorption (Figure 5-4-Rub-A)

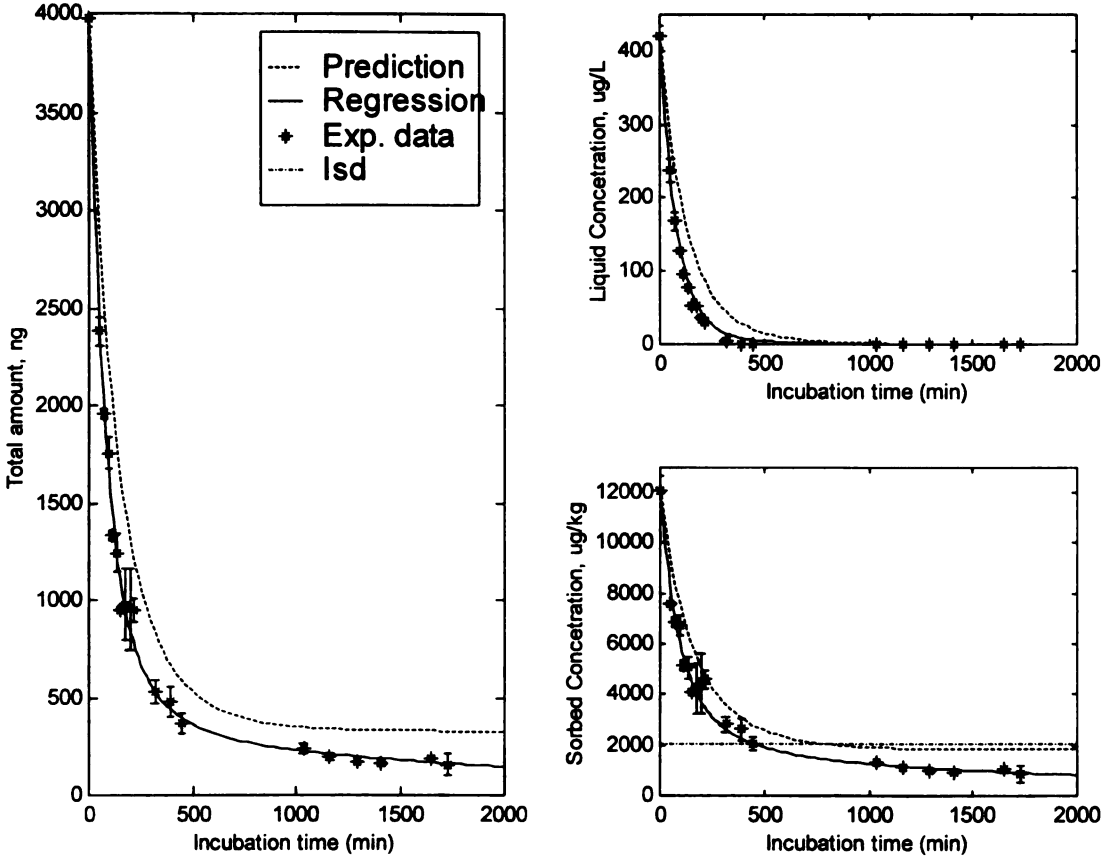

Figure 5- 8. Naphthalene disappearance under strain NCIB in Kal-A soil slurry over incubation time.

Prediction. The line predicted by Model I
Regression. The line used to estimate the degradation kinetic parameters of sorbed phase with Model IV.
Exp. data. Measured data.
Isd. The intercept calculated in the series dilution desorption (Figure 5-4-Kal-A)

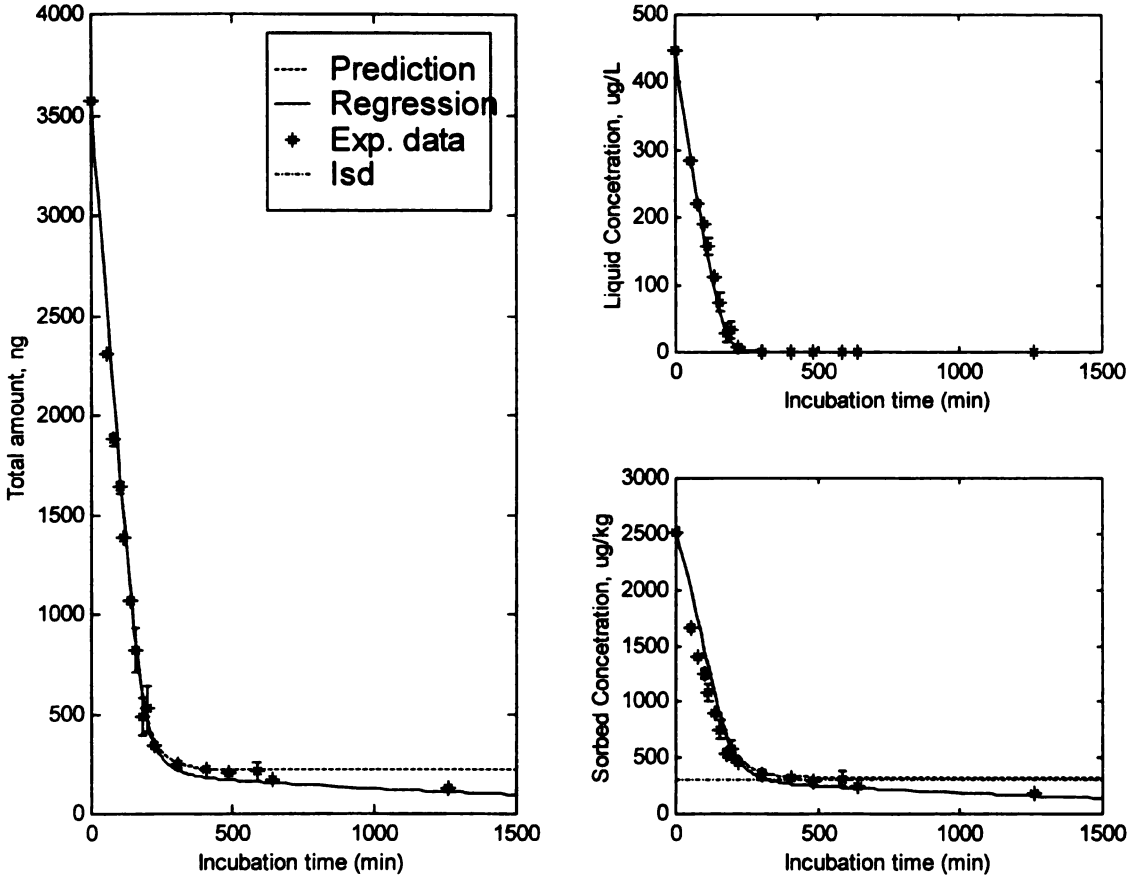

Figure 5- 9. Naphthalene disappearance under strain G7 in SPCF soil slurry over incubation time.

Prediction. The line predicted by Model I
Regression. The line used to estimate the degradation kinetic parameters of sorbed phase with Model IV.
Exp. data. Measured data.
Isd. The intercept calculated in the series dilution desorption (Figure 5-4-SPCF)

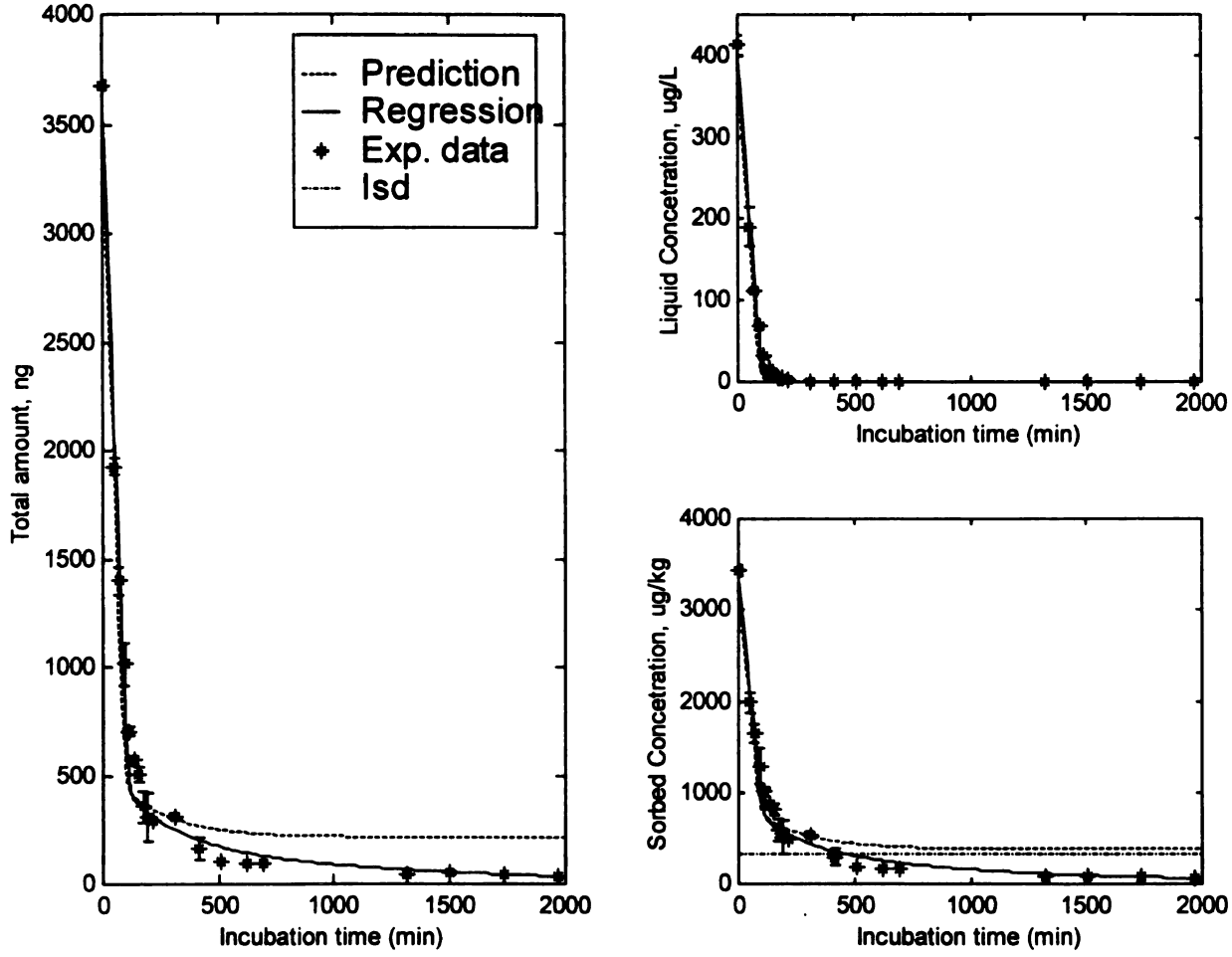

Figure 5- 10. Naphthalene disappearance under strain G7 in Rub-BS1 soil slurry over incubation time.

Prediction. The line predicted by Model I
Regression. The line used to estimate the degradation kinetic parameters of sorbed phase with Model IV.
Exp. data. Measured data.
lsd. The intercept calculated in the series dilution desorption (Figure 5-4-Rub-BS1)

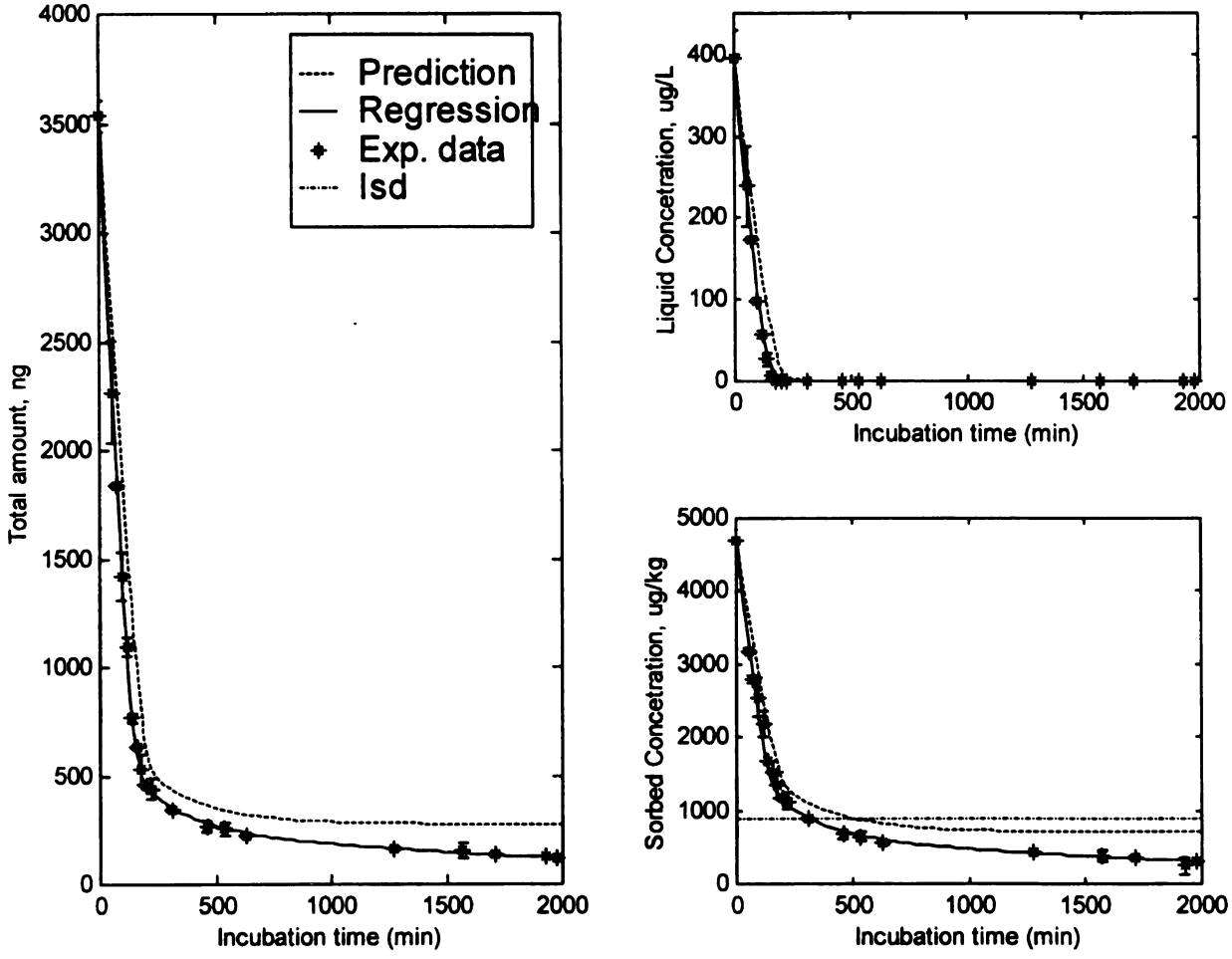

Figure 5- 11. Naphthalene disappearance under strain G7 in Rub-A soil slurry over incubation time.

Prediction. The line predicted by Model I
Regression. The line used to estimate the degradation kinetic parameters of sorbed phase with Model IV.
Exp. data. Measured data.
Isd. The intercept calculated in the series dilution desorption (Figure 5-4-Rub-A)

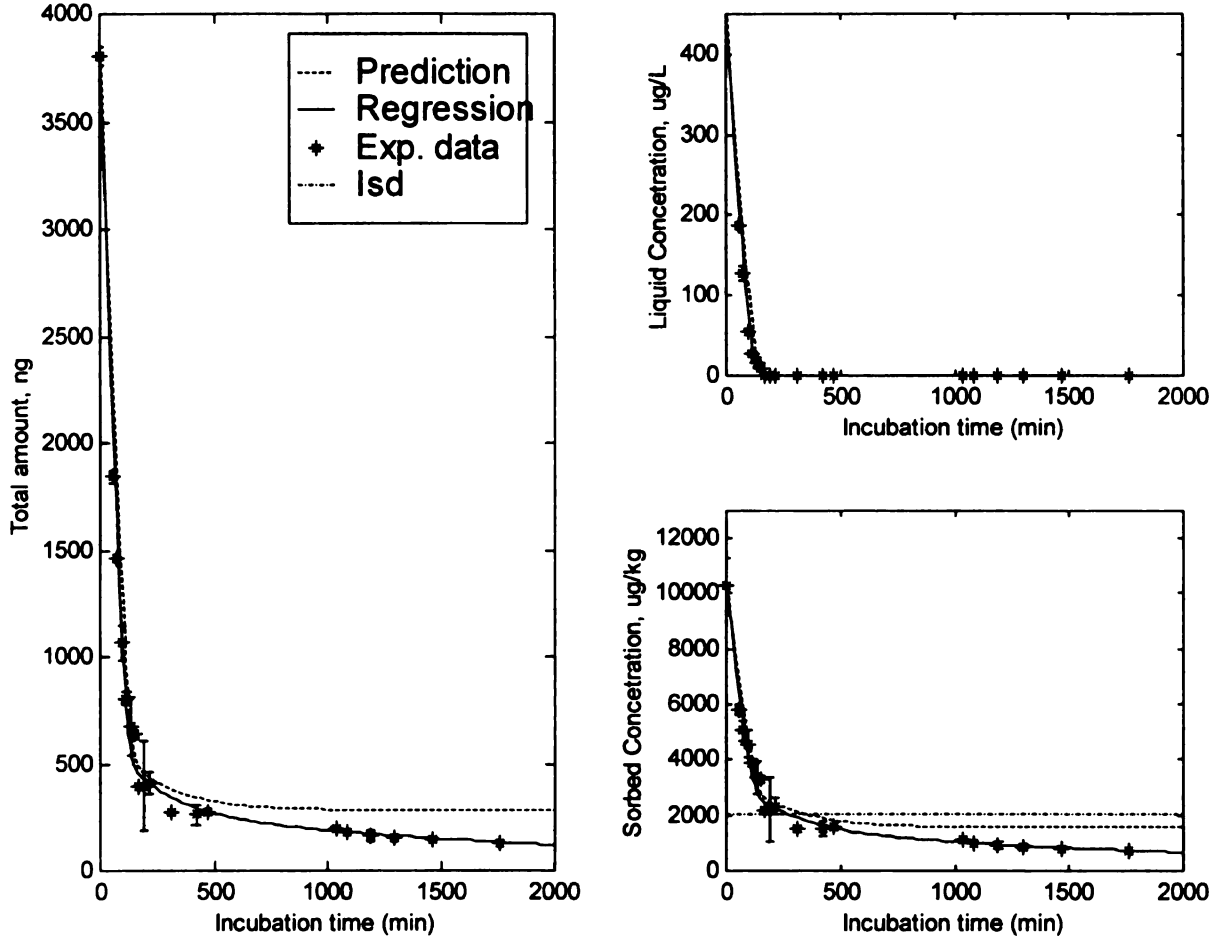

Figure 5- 12. Naphthalene disappearance under strain G7 in Kal-A soil slurry over incubation time.

Prediction. The line predicted by Model I
Regression. The line used to estimate the degradation kinetic parameters of sorbed phase with Model IV.
Exp. data. Measured data.
Isd. The intercept calculated in the series dilution desorption (Figure 5-4-Kal-A)

Table 5- 3. Estimated each model parameters for NCIB.

Soil	Model	Degradation rate parameters[†]	Solid-phase degradation rate[††]			R^2
		k_1 (min^{-1})	k_{eq} (min^{-1})	k_{neq} (min^{-1})	k_{nd} (min^{-1})	
SPCF	Model I	0.01831	N/A	N/A	N/A	0.989
	Model II		0	N/A	N/A	0.989
	Model III		0	0	N/A	0.990
	Model IV		0	0	0.0015	0.996
Rub-B	Model I	0.05206	N/A	N/A	N/A	0.975
	Model II		0.0083	N/A	N/A	0.977
	Model III		0	0.0074	N/A	0.986
	Model IV		0	0.0039	0.0014	0.999
Rub-A	Model I	0.01842	N/A	N/A	N/A	0.321
	Model II		0.052	N/A	N/A	0.980
	Model III		0.023	0.027	N/A	0.985
	Model IV		0.023	0.012	0.00032	0.990
Kal-A	Model I	0.0144	N/A	N/A	N/A	0.836
	Model II		0.0103	N/A	N/A	0.988
	Model III		0.0103	0	N/A	0.988
	Model IV		0.0098	0	0.00040	0.995

[†]Degradation rate parameters from soil-extract controls
[††]Solid-phase degradation rate from regression

Table 5- 4. Estimated each model parameters for *P. putida* G7.

Soil	Model	Degradation rate parameters[†]		Solid-phase degradation rate[††]			R^2
		k_m	Ks	k_{eq}	k_{neq}	k_{nd}	
SPCF	Model I	4.938	36.37	N/A	N/A	N/A	0.987
	Model II			0	N/A	N/A	0.987
	Model III			0	0	N/A	0.987
	Model IV			0	0	0.00056	0.990
Rub-B	Model I	9.958	36.37	N/A	N/A	N/A	0.972
	Model II			0	N/A	N/A	0.977
	Model III			0	0	N/A	0.977
	Model IV			0	0	0.00091	0.988
Rub-A	Model I	4.490	36.37	N/A	N/A	N/A	0.899
	Model II			0.0078	N/A	N/A	0.991
	Model III			0.0066	0.0020	N/A	0.993
	Model IV			0.0070	0.00087	0.00042	0.999
Kal-A	Model I	6.826	36.37	N/A	N/A	N/A	0.946
	Model II			0.0068	N/A	N/A	0.985
	Model III			0.0068	0	N/A	0.985
	Model IV			0.0067	0	0.00043	0.991

[†]Degradation rate parameters from soil-extract controls
[††]Solid-phase degradation rate from regression

DISCUSSION

The desorption profiles show clearly that three types of desorption occur: equilibrium release as evidenced by the elevated solution concentrations at the first time point, rate limited release shown by the increases over the next several time points, and a non-desorbable fraction indicated by failure to reach the expected equilibrium levels. The presence of the non-desorbable fraction is supported by the series dilutions experiments, indicating that this material could not be removed by six successive water extractions. Confirmation that this material was actually sorbed, rather than resulting from a loss, is provided by the methanol extractions and the essentially complete closure of the sorption/desorption mass balance. Thus it appears that not only is the use of the three-site model consistent with the desorption rate data, but the data provide information sufficient to estimate the rate parameters.

The ability of model I to fit the liquid-phase concentration data for the SPCF and Rub-BS1 soils suggests that most of the degradation occurs in the liquid-phase, as assumed in the model (Figures 5-5, 5-6, 5-9 and 5-10). This result does not eliminate the possibility of degradation based on the solid phase contaminant, but if this occurs it is apparently not significant. Because these soils have relatively low sorption capacities, solid-phase concentrations are low, and rates based on these compartments would also be expected to be low. It is seen that model I does not predict the liquid-phase rate profiles very well for the higher capacity soils (Figures 5-7, 5-8, 5-11 and 5-12). Models II and III, which incorporate degradation of the reversibly-sorbed material, provide successively better fits of the liquid-phase rate profiles for these soils (Table 5-3 and 5-4). Apparently,

the higher concentrations of sorbed substrate result in significant increases in degradation rates. This extends recent published evidence of enhanced mineralization rates (Guerin and Boyd, 1992; Guerin and Boyd, 1993; Guerin and Boyd, 1995; Guerin and Boyd, 1997; Ortega-Calvo and Saiz-Jimenez, 1998; Laor et al., 1999), suggesting that substrate degradation can also be enhanced over that expected on the basis of liquid-phase concentration alone.

This result is consistent with what has been labeled "direct solid-phase degradation" by some researchers, and other explanations such as elevated concentrations at the sorbent/organism interface and stimulation of liquid-phase rates by the presence of solids (Guerin and Boyd, 1992; Guerin and Boyd, 1993; Guerin and Boyd, 1995; Harms and Zehnder, 1995; Guerin and Boyd, 1997; Ortega-Calvo and Saiz-Jimenez, 1998; Laor et al., 1999; Feng et al., 2000). The results using models I, II and III do not address these alternative explanations. They simply indicate that rate enhancement occurs and that the behavior of systems involving both desorption and degradation can not necessarily be predicted based on independent assessments of these processes. All three models provide the same prediction of the long-term behavior of liquid-phase concentrations: that substrate levels drop below the detection limit within 400 minutes.

The solid-phase concentration data provide some additional insights, however. The total amount of contaminant in the solid phase continued to decrease after liquid phase was depleted, dropping below the amount known to be held in the non-desorption sites. Thus it appears that the naphthalene that could not be removed by exhaustive aqueous extraction, was removed in the presence of bacteria. This addressed mathematically by model IV, which incorporates degradation of the non-desorption

material. It can be seen that this formulation provides a good description of the experimental data.

We offer two possible explanations for this unexpected result. First, the presence of bacteria may serve to extract the non-desorbable fraction, much as an organic solvent would. Living bacterial cultures continually produce a wide array of soluble organic materials, and it is possible that some of these can facilitate desorption. Second, the bacteria may able to degrade this material directly, with desorption. This could occur through direct partitioning to the cell membrane, or via degradation by extracellular enzymes. The present study was not designed to discriminate between these explanations, and further research in this area may be warranted. Nonetheless, it can be concluded that material that was non-desorbable in these experimental systems was bioavailable.

ACKNOWLEDGEMENTS

We thank Dr. Harwood in University of Iowa for providing the bacteria.

REFFERENCES

Estrella, M. R., M. L. Brusseau, R. S. Maier, I. L. Pepper, P. J. Wierenga and R. M. Miller. 1993. "Biodegradation, Sorption, and Transport of 2,4-Dichlorophenoxyacetic Acid in Saturated and Unsaturated Soils." *Appl. Environ. Microbiol* 59: 4266-4273.

Feng, Y., J. H. Park, T. C. Voice and S. A. Boyd. 2000. "Bioavailability of Soil-Sorbed Biphenyl to Bacteria." *Environ. Sci. Technol.* 34: 1977-1984.

Grimm, A. C. and C. S. Harwood. 1997. "Chemotaxis of Pseudomonas spp. to the Polyaromatic Hydrocarbon Naphthalene." *Appl. Environ. Miceobiol.* 63: 4111-4115.

Grimm, A. C. and C. S. Harwood. 1999. "NahY, a Catabolic Plasmid-Encoded Receptor Required for Chemotaxis of Pseudomonas putida to the Aromatic Hydrocarbon Naphthalene." *J. Bacteriol.* 181: 3310-3316.

Guerin, W. F. and S. A. Boyd. 1992. "Differential Bioavailability of Soil-Sorbed Naphthalene to Two Bacterial Species." *Appl. Environ. Microbiol* 58: 1142-1152.

Guerin, W. F. and S. A. Boyd. 1993. "Bioavailability of Sorbed Naphthalene to Bacteria: Influence of Contaminant Aging and Soil Organic Carbon Content." *Sorption and Degradation of Pesticides and Organic Chemicals in Soil. SSSA Special Publication no* 32: 197-208.

Guerin, W. F. and S. A. Boyd. 1995. "Maintenance and Induction of Naphthalene Degradation Activity in Pseudomonas putida and an Alcaligenes sp. under Different Culture Conditions." *Appl. Environ. Microbiol* 61: 4061-4068.

Guerin, W. F. and S. A. Boyd. 1997. "Bioavailability of naphthalene associated with natural and synthetic sorbents." *Water Res.* 32: 1504-1512.

Harms, H. and A. J. B. Zehnder. 1995. "Bioavailability of Sorbed 3-Chlorodibenzofuran." *Appl. Environ. Microbiol* 61: 27-33.

Laor, Y., P. F. Strom and W. J. Farmer. 1999. "Bioavailability of Phenanthrene Sorbed to Mineral-Associated Humic Acid." *Wat. Res.* 33: 1719-1729.

Ogram, A. V., R. E. Jessup, L. T. Ou and P. S. C. Rao. 1985. "Effects of Sorption n Biological Degradation Rates of (2,4-Dichlorophenoxy)acetic Acid in Soils." *Appl. Environ. Microbiol* 49: 582-587.

Ortega-Calvo, J. J. and C. Saiz-Jimenez. 1998. "Effect of humic fractions and clay on biodegradation of phenanthrene by a Pseudomonas fluorescens strain isolated from soil." *Appl. Environ. Microbiol.* 64: 3123-3126.

Park, J. H., X. Zhao and T. C. Voice. 2000. "Development of a kinetic basis for bioavailability of naphthalene in soil slurries." *Water Res.* Submitted: .

Scow, K. M. and J. Hutson. 1992. "Effect of Diffusion and Sorption on the Kinetics of Biodegradation Theoretical Considerations." *Soil Sci. Soc. Ame. J* 56: 119.

Scow, K. M., S. K. Schmidt and M. Alexander. 1989. "Kinetics Of Biodegradation Of Mixtures Of Substrates In Soil." *Soil Biol. Biochem.* 21: 703.

Scow, K. M., S. Simkins and M. Alexander. 1986. "Kinetics of Mineralization of Organic Compounds at Low Concentration in Soil." *Appl. Environ. Microbiol* 51: 1028.

Shelton, D. R. and M. A. Doherty. 1997. "A model describing pesticide bioavailability and biodegradation in soil." *Soil Sci. Soc. Am. J* 61: 1078 – 1084.

Shimp, R. J. and R. L. Young. 1988. "Availability of organic chemicals for biodegradation in settled bottom sediments." *Ecotoxicol Environ Saf* 15: 31-45.

Tabak, H. H., C. Gao, L. Lai, X. S. Yan, S. Pfanstiel, L. S. Kim and R. Govind. 1994. "Determination Of Bioavailability and Biodegradation Kinetics Of Phenol and Alkylphenols In Soil." 554: 51.

Zhang, W. X., E. J. Bouwer and W. P. Ball. 1998. "Bioavailability of hydrophobic organic contaminants-effects and implications of sorption-related mass-transfer on bioremediation." *Ground Water Monit. R.* 18: 126-138.

CHAPTER 6. COMPARISON OF BIODEGRADATION KINETIC PARAMETERS FOR NAPHTHALENE IN BATCH AND SAND COLUMN SYSTEMS BY *PSEUDOMONAS PUTIDA*

ABSTRACT

Kinetic parameters for the degradation of naphthalene by *Pseudomonas putida* (ATCC 17484) were estimated in both batch and column assays, in order to evaluate the role of cell attachment and flow on biodegradation rates. Suspended cells and cells attached to Ottawa sand were used under a variety of biomass levels, column flow-rates, and substrate concentrations. The maximum specific utilization coefficient (k_m), the zero-order reaction coefficient (k_o) for degradation, and zero-order reaction coefficient (k_{co2}) for mineralization differed by factors similar to the calculated reduction in exposed cell surface area. In batch systems, degradation followed zero-order kinetics across the entire concentration range, while the columns exhibited decreased rates at concentrations less than 100 (μg/L), describable by Michaelis-Menten kinetics. This is reflected in elevated values of the half-saturation constant, K_s, in columns. We offer the explanation that this may result from reactive-heterogeneity within the porous media, imposing a distribution of length-scales for transfer of substrate to the cell surfaces. Well-mixed batch systems are expected to have both shorter and more uniform transfer distances. This explanation was also shown to be consistent with observations of the effects of flow rate and biomass levels in column experiments. When kinetic parameters obtained in batch system are used for prediction of degradation in columns, at least two factors – exposed reduction of exposed cell surface area and heterogeneity of cell distribution – will likely reduce overall column degradation rates.

INTRODUCTION

Biokinetic parameters are commonly measured in batch systems using suspended cells in liquid medium. One of the advantages of this approach is that mass-transfer limitations are minimized and the resultant kinetic parameters are thought to reflect the "intrinsic" biodegradation capability of the organisms. It is further assumed that the more complicated soil environments in which biodegradation processes may occur can be described by incorporating appropriate formulations for flow through the porous media, mixing, cell attachment, mass-transfer to and from cell surfaces, and sorption/desorption. It is commonly assumed that the basic biokinetic processes are unaltered by system configuration and the parameters measured in soil-free suspended-cell assays can be applied directly in these dissimilar systems. Finding little evidence in the literature to support this assumption, this effort was designed to evaluate whether there are differences between biodegradation rates by suspended-cells in well-mixed batch systems without solids and attached-cells in sand columns.

Bacterial adhesion effects on biodegradation or cell activity have been studied in batch systems by van Loosderacht *et al.* (van Loosdrecht et al., 1990) These investigators classified the potential influences of interfaces into two categories: direct and indirect. Direct influences include changes in the structure and permeability of the cell membranes as the result of adhesion to solid surfaces. Indirect influences involve alteration of cell activity because of changes in the composition of the medium resulting from sorption and desorption phenomena at the interfaces, and the physical geometric structure around an attached organism. van Loosderacht *et al.* did not find conclusive evidence that the adhesion of cells to solid surfaces directly influences bacterial

metabolism. They concluded that differences are largely indirect, resulting from decreases in the "apparent" substrate affinity because of a reduction in the cell surface exposed to the bulk solution and an increased mass-transfer resistance to the non-exposed surface (Ngian et al., 1977; McLaren, 1978; Ellwood et al., 1982; Firestone, 1982; Bright and Fletcher, 1983; Dolfing, 1985; Myrold and Tiedje, 1985; Caldwell and Lawrence, 1986; Jeffrey and Paul, 1986; Focht and Shelton, 1987). It should be noted that all of these studies were conducted in well-mixed systems and may not be applicable to degradation in flow through porous media.

A few recent investigations have attempted to explore the differences between batch (free cells) and column (adhered cells) experiments. Harms and Zehnder (Harms and Zehnder, 1994) studied the effect of substrate diffusion on the biodegradation rate of dibenzofuran and 3-chlorodibenzofuran by attached and suspended *Sphingomonas* sp. strain HH19K. A batch system was used to obtain kinetic parameters including maximum specific growth rate (μ_m) and the half-saturation coefficient (K_s) for suspended cells, and columns packed with glass beads were used to obtain K_s for the attached cells. They found that K_s increased as surface coverage of cells increased and as bulk flow rates decreased, while the maximum specific activity was assumed to be the same in the both systems. Kelly *et al.* conducted both suspended culture batch and quartz-sand-packed column experiments to measure biodegradation kinetic parameters for benzene, toluene and xylene, using a mixed culture of organisms derived from creosote-contaminated soil (Kelly et al., 1996). They found that the maximum specific growth rate was similar for both systems but it was not possible to estimate half-saturation coefficients for the columns. Mass-transfer limitations in the columns were not explicitly addressed.

Estrella *et al.* studied the biodegradation rate parameters for 2,4-dichlorophenoxyacetic acid by indigenous soil microorganisms in batch systems and soil-packed columns under different flow and biomass conditions (Estrella et al., 1993). They reported that the maximum specific growth rate coefficients were greater in the column systems. However, because of limitations in their mathematical formulation, it was necessary for them to assume the same yield coefficient and half-saturation constant for both batch and column systems.

The objective of the present study was to evaluate the relationship between kinetic parameters in both batch and column systems without the assumptions that have been necessary in previous column studies. This was accomplished by selecting a sand/contaminant/organism combination that resulted in significant biomass attachment but only minimal contaminant sorption to the sand in the columns. In addition, relatively high flow rates and low total biomass levels were used to minimize external and internal mass-transfer resistances, respectively. Finally, by using columns of different lengths, the effect of residence time could be evaluated independent of flow rate, and it became possible to simultaneously evaluate all of the degradation parameters.

Naphthalene was selected as the substrate for this study. As one of the components in petroleum products such as gasoline and diesel fuel, naphthalene contamination of soil and groundwater is not uncommon. Because naphthalene sorbs significantly to soils with relative low organic content, bioavailability of this compound has been studied by several researchers (Guerin and Boyd, 1992; Guerin and Boyd, 1995; Zhao and Voice, 2000). However, in order to fully understand the interactions between sorption and biodegradation processes, each processes must be evaluated independently.

This study was designed specifically to understand naphthalene degradation rates *per se*, so that interactions between biodegradation and sorption/desorption could be addressed in a subsequent study, with an overall goal of understanding the bioavailability of naphthalene in the soil environment (Zhao and Voice, 2000).

MATERIALS AND METHODS

Materials

Pseudomonas putida (ATCC 17484), which has been established to grow on naphthalene as a sole source of carbon and energy was obtained from the American Type Culture Collection (Guerin and Boyd, 1995). Bacteria were grown in 500 mL high-buffer broth (HBB) composed of 2.0 g of NaCl, 3.0 g of $(NH_4)_2HPO_4$, 1.2 g of KH_2PO_4, 3 mg of $MgSO_4$, 1 mL of ferric quinate (Blakemore et al., 1979), 1 mL of vitamin solution (Wolin et al., 1963), per liter of distilled water at pH 7.0. Naphthalene was added to the sterilized liquid medium as a concentrated stock solution in acetone (200 g/L) to a final concentration of 200 mg/L. The liquid medium was inoculated by adding 5 mL of starved liquid culture to 500 mL medium, and stirred. Growth was monitored by absorbance at 600 nm, and cells were harvested at a point determined to correspond to early stationary phase based on a full growth curve. Cells were separated by centrifugation (1900 × g, 20 min) and resuspended in phosphate buffer saline (PBS) solution before use. The PBS contained 8.5 g of NaCl, 0.6 g of Na_2HPO_4, and 0.3 g of KH_2PO_4 per liter and had an ionic strength of 0.16 M. This procedure was repeated three times to ensure the removal of remaining naphthalene in the cell growth medium. The cell suspension was stored at room temperature and used within 2 hours.

To produce the best estimates of the biodegradation rates in column experiments, the effects sorption to the solids were minimized. Ottawa sand was selected as the solid medium because sorption of naphthalene to the sand was negligible, having a measured distribution coefficient (K_d) of 0.089 mL/g. The following characteristics of the sand were measured: bulk density of 1.64 g/mL; particle size distribution, 26.3% coarse sand (0.5-0.7 mm), 73.2% medium sand (0.25-0.5 mm), 0.5% fine sand (0.18-0.25 mm); organic matter content below the detection limit of 0.03%; and pH of 6.5. The sand was placed in sealed container and was sterilized by gamma irradiation at a dosage of 2 Mrad in a cobalt-60 irradiator (Phoenix Memorial Laboratory, University of Michigan, Ann Arbor, Michigan). The sealed sand was stored at 25 °C. Before the sand was used, microbial growth was examined by plating 0.1 g sand on a nutrient agar plate. There was no colony formation after 4 days of incubation at 30 °C.

Analytical Methodology

Concentrations of naphthalene, $^{14}CO_2$ and intermediate degradation products were determined at each time point in the batch and column assays. One-mL samples were placed into 10 mL of liquid scintillation cocktail for determination of total ^{14}C activity by liquid scintillation counting. A second 1-mL sample was centrifuged (1200 × g, 10 min.) to separate sand and biomass and the supernatant analyzed for naphthalene by high pressure liquid chromatograph (HPLC) or gas chromatography (GC). In the HPLC method, separation was accomplished using a C_{18} reverse-phase column and an 80% acetonitrile in water mobile phase under isocratic conditions with a flow rate of 1 mL/min. Naphthalene was detected by ultraviolet absorption at 225 nm. Using an injection volume of 50 μL the detection limit was 2 μg/L. The GC methodology utilized

the headspace technique reported by Voice and Kolb (Voice and Kolb, 1993). Separation was accomplished using a PE-624 capillary column followed by detection using a photoionization detector (PID). The detection limit was 17 μg/L.

Mineralization rates for naphthalene were evaluated by measuring the production of $^{14}CO_2$. To accomplish this, naphthalene was first removed from the sample by stirring with a magnetic stirring bar for 2 hours at 700 rpm after adjusting pH to above 12 to minimize the volatilization of CO_2. This procedure was found to remove greater than 95% of the naphthalene from prepared standards. After the sample was acidified to less than pH 2.0 with 0.2 mL of 6N HCl, $^{14}CO_2$ was trapped within 300 μL of base (0.1 M KOH) suspended from a tube stopper inside a plastic cap. After overnight degassing, 280 μL of the KOH was transferred to scintillation vials with 10 mL of liquid scintillation cocktail. The radioactivity was measured by liquid scintillation counting. The efficiency of trapping $^{14}CO_2$ was found to be greater than 90% using $NaH^{14}CO_3$ standards (Plaehn et al., 1999). Subsequent to CO_2 release, a one-mL aliquot of the remaining solution was transferred to a vial which contained 10 mL scintillation cocktail to measure the amount of ^{14}C contained as intermediate degradation products.

Batch experiments

Degradation parameters were determined under two batch experimental conditions: sand-free solutions of phosphate buffer saline (PBS), and sand-free solutions with the supernatant from the sand-PBS mixture. In both types of experiments, ten mL of re-suspended cells (10^7-10^8 CFUs/mL) were placed into 20 mL serum vials. A stirring bar was added to the vial to provide mixing. Biodegradation was initiated by spiking an

aliquot of ^{14}C-labeled naphthalene stock (in methanol) into the vials at an initial concentration of 400-700 μg/L. Two types of control vials, prepared by adding NaOH to prevent degradation, were used as a check for losses. One contained only PBS and the other contained PBS with dispersed cells. The naphthalene-spiked samples were mixed at 800 rpm on a magnetic stirrer. Each bottle was removed at predetermined time intervals and biodegradation was stopped by increasing pH to greater than 12 with injection 0.1 mL of 10 N NaOH. Naphthalene concentrations were measured by GC or HPLC and the activity of $^{14}CO_2$ and intermediate products were monitored by liquid scintillation counting. All experiments were performed at 24 ± 1 °C.

To test the effect of soil organic matter that might be released from the sand to the aqueous solution, a degradation-rate experiment was conducted using PBS that had been in contact with the sand. 200 g of sterilized sand was mixed with 200 mL PBS and tumbled for 2 hours at 6 rpm. The mixture was centrifuged at 2100 × g for 20 min to separate the sand and supernatant. The supernatant was then used in the degradation experiment, as described above, and compared to the results from PBS that had not been in contact with the sand.

Column experiments

Sand columns were constructed from stainless steel tubing (I.D. 1.1 cm) and Swagelok™ fittings in lengths of 5, 10, 15, and 20 cm. Porous stainless-steel frits (thickness, 3 mm: pore size, 0.02 mm) were placed at each column end to prevent loss of sand. All column parts were sterilized by autoclaving prior to packing, and the sterilized sand was added to the columns under a laminar flow hood. Sand columns were saturated

and washed with 5 pore volumes of PBS solution at a flow rate of 10 mL/min. The average porosity of the packed column was 0.38 determined by a weight measurement before and after water saturation of the columns.

The columns were inoculated by pumping 2.67 pore volumes of cell suspension at a flow rate of 5 mL/min using a syringe pump. Flow was stopped for at least 15 minutes to facilitate attachment. Nonattached cells were then washed out by flushing the columns with 3 pore volumes of PBS solution at a flow rate of 3 mL/min. ^{14}C-labeled naphthalene solution (68,000 DPM/mL) was pumped through the inoculated sand columns.

The experimental matrix included four column lengths, four flow rates, and four cell densities (Table 6-1). Four column lengths and three influent concentrations at a constant cell density were designed for kinetic parameter estimation. Four different-flow rates at one column length, one cell-density level and one influent concentration were used to evaluate the effect of velocity on degradation rate. Four different cell densities at one column length and one flow rate were used to evaluate of the effect of biomass level on degradation rate. In all cases, the naphthalene concentrations in the effluent were stable after 3-pore volumes of solution were fed.

Effluent samples were collected directly into 20-mL headspace vials, which contained NaOH to stop biodegradation. Naphthalene losses resulting from sorption or bacterial contamination were evaluated using non-inoculated packed columns, which were prepared in the same way as the inoculated columns, at two different flow rates. After three pore volumes, influent and effluent concentrations were equal, indicating that sorption to the system was complete and there were no fortuitous losses.

The amount of attached biomass was determined by the difference between protein measurements of the inoculum and the effluent during the wash step, using bovine serum albumin as a standard protein (Pierce, 1989). 33±3% of the inoculum cells were attached to the sand for all column lengths. To study the distribution of biomass in the sand column, sand samples were removed from three 10-cm columns after washing, divided into three segments, digested in 4.5 mL of 0.1 N NaOH solution for 2 hours, and the protein content measured. No significant differences were found in the distribution of cells in the inlet, middle and outlet one-third portions of the three columns.

Table 6- 1. Experimental matrix for the column assays

Experiments	Column length (cm)	Flow rate (mL/min)	Protein concentration (μg/L in pore volume)	Cell Density (CFU/g sand)	Influent concentration (μg/L)
Kinetic parameter estimation	5	3	2100	1.36×10^{7}	506 368 221
	10	3	2100	1.36×10^{7}	506 368 221
	15	3	2100	1.36×10^{7}	506 368 221
	20	3	2100	1.36×10^{7}	506 368 221
Flow rate effect	5	0.5 1 2 3	2490	1.62×10^{7}	498
Cell density effect	10	3	6353	4.13×10^{7}	699
			3071	1.99×10^{7}	613
			2065	1.34×10^{7}	613
			1006	6.53×10^{6}	613

To estimate the dispersion coefficient and effective porosity in the columns, a step-increase of tritiated water (^3H_2O) (81,000 DPM/mL) was pumped through a control sand column at several flow rates ranging from 0.5 to 3 mL/min. The effluent was sampled every 10 sec until the concentration was same as the influent as measured by liquid-scintillation counting. The dispersivity and effective porosity were found to be 0.11 cm and 0.38 respectively, using the solution to the equation for one-dimensional saturated homogeneous flow in porous media (Freeze and Cherry, 1979).

Calculation of kinetic parameters

The non-growth Michaelis-Menten equation was used to calculate the half-saturation coefficient (K_s, μg/L) and maximum specific utilization coefficient (k_m, min^{-1}) in both batch and column assays. The non-growth assumption was based upon several experimental conditions designed to minimize the potential for growth: nitrogen was not included in the PBS solution, the amount of inoculation was large when compared with the naphthalene concentration, and the experimental time was relatively short. The validity of this assumption was also examined by measuring the cell concentrations between before and after one experiment; no significant differences were found. The non-growth equation can be used directly for batch experiments:

$$-\frac{dC}{dt} = \frac{k_m X_o C}{K_s + C} \qquad \text{(Eq. 1)}$$

where X_o is initial protein content (μg/L), C is naphthalene concentration in liquid (μg/L), and t is time (min). In columns, the advection/dispersion equation was used to formulate the system mass balance, with a Michaelis-Menten reaction term:

$$R\frac{dC}{dt} = D\frac{dC^2}{dx^2} - V\frac{dC}{dx} - \frac{k_m X_o C}{K_s + C} \qquad \text{(Eq. 2)}$$

where R is the retardation coefficient, D is the dispersion coefficient (cm^2/sec) and V is average pore velocity (cm/sec). At steady state, Eq. 2 can be simplified to:

$$D\frac{dC^2}{dx^2} - V\frac{dC}{dx} = \frac{k_m X_o C}{K_s + C} \qquad \text{(Eq. 3)}$$

Because dispersion was small when compared with advection in these systems (D = 0.01 cm^2/sec), it can be ignored, and Eq. 3 can be further simplified:

$$-V\frac{dC}{dx} = \frac{k_m X_o C}{K_s + C} \qquad \text{(Eq. 4)}$$

Converting the independent variables length (x) and pore velocity (V) to residence time (t_r), Eq. 4 becomes:

$$\frac{dC}{dt_r} = -\frac{k_m X_o C}{K_s + C} \qquad \text{(Eq. 5)}$$

In batch systems, the terms k_m and K_s were estimated simultaneously for each batch experiment using concentration-time data and Eq. 1. In the column experiments, this was not possible because each column only produces one steady-state concentration, so the parameters were estimated from multiple experimental conditions (initial naphthalene concentration, flow rate and column length). In both cases, estimations were accomplished using the nonlinear regression algorithm in the ModelMaker software package (Cherwell Scientific Publishing, Oxford, United Kingdom).

If the value of K_s is significantly smaller than the value of C, then Eqs. 1 & 5 can be simplified to a zero-order relationship and the zero-order reaction coefficient, k_o (μg/L-min) can be determined from:

$$-\frac{dC}{dt} = k_o X_o \quad \text{(for batch)} \qquad \text{(Eq. 6)}$$

$$-\frac{dC}{dt_r} = k_a X_o \quad \text{(for column)} \qquad \text{(Eq. 7)}$$

The initial production of CO_2 was found to follow a zero-order relationship in both systems (see Figures 6-3 and 6-6). The parameter k_{CO2} (μg/L-min) was estimated from:

$$\frac{dC_{co2}}{dt} = k_{co2} X_o \text{ (for batch)} \qquad \text{(Eq. 8)}$$

$$\frac{dC_{co2}}{dt_r} = k_{co2} X_o \text{ (for column)} \qquad \text{(Eq. 9)}$$

where C_{co2} is the concentration of produced CO_2, (μg/L as naphthalene).

RESULTS AND DISCUSSION

Degradation and mineralization of naphthalene by suspended cells in batch systems

It can be seen in Figure 6-1 that naphthalene was rapidly removed within the first five minutes in the batch experiments. An increase in measurable intermediates mirrored naphthalene disappearance until the point of naphthalene depletion. CO_2 production was much more gradual, and continued to increase after the concentration of intermediates peaked. The recovery of total ^{14}C-activity was greater than 90% throughout the experiment.

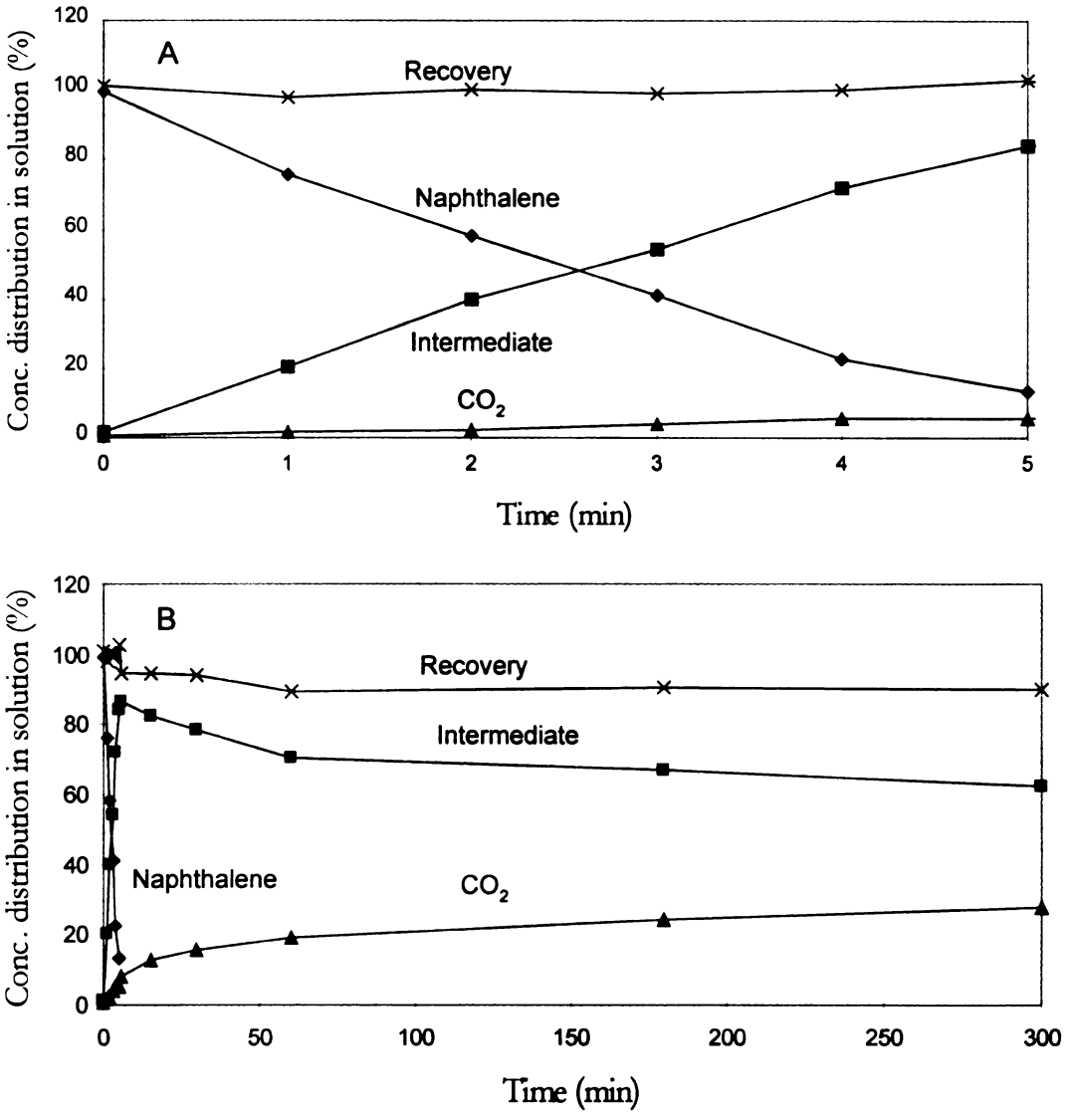

Figure 6- 1. Degradation of naphthalene, production of intermediates and carbon dioxide in a batch experiment with free suspended cells. Protein content; 780 μg/L (2.15×10^7 CFU/mL), Initial substrate concentration; 520 μg/L of naphthalene.

A: Initial incubation time within 5 min
B: Extended incubation time up to 300 min

This sequence indicated that the first step in the process, removal of naphthalene, was not rate-limiting with respect to the overall mineralization process. Several possible intermediates can be formed during the degradation of the naphthalene. Barnsley reported that ATCC 17484 is able to transform naphthalene to 1,2-dihydroxy-naphthalene, which is then converted to catechol. Catechol can be further utilized following an *ortho*-cleavage pathway (Barnsley, 1976, Appendix A). Because this study focused on the degradation of the naphthalene, identification of the intermediates was not attempted. However, it was noted that the intermediates were found to be hydrophilic and nonvolatile, were not retained (i.e same retention time with water) on a C_{18} reverse-phase HPLC column in this separation condition, and absorbed light in the 220 nm UV.

The maximum specific utilization rate coefficient, k_m, and the half-saturation constant, K_s, were estimated as 0.115 (±0.004) min^{-1} and 5.5 (±0.26) μg/L , respectively (Figure 6-2). Because of the extremely low value of K_s, the disappearance of the naphthalene could be adequately described as a zero-order reaction and the rate coefficient, k_o, was estimated to be 0.110 (±0.003) min^{-1}. The production of CO_2 also followed a zero-order relationship and the coefficient, k_{CO_2}, was 0.0068 (±0.00007) min^{-1} (Figure 6-3). Correlation coefficients (r^2) were 0.99 for the Michaelis-Menten equation, 0.99 for the zero-order equation, and 0.98 for the mineralization of naphthalene. Guerin and Boyd studied the naphthalene utilization activity of ATCC 17484 and estimated the utilization activity to be in the range 0.0025 – 0.015 min^{-1}, which is one magnitude less than our value (k_o=0.11 min^{-1}) (Guerin and Boyd, 1995). This may be explained by experimental differences between the two studies. Guerin and Boyd utililized much greater initial concentrations (25,600 vs. 450 μg/L) and performed the degradation

experiments in quartz cuvettes without mixing, possibly resulting in oxygen transfer limitations. We utilized serum bottles and continuous stirring. In addition, we analyzed naphthalene chromatographically (HPLC and GC), whereas the earlier study monitored UV absorption at 277 nm, which may be biased by other light absorbing species.

To determine whether leachable material from the sand could have affected degradation rates, batch studies using PBS and sand supernatant were compared. As can be seen in Figure 6-4, there was no apparent difference in disappearance of naphthalene between two solutions.

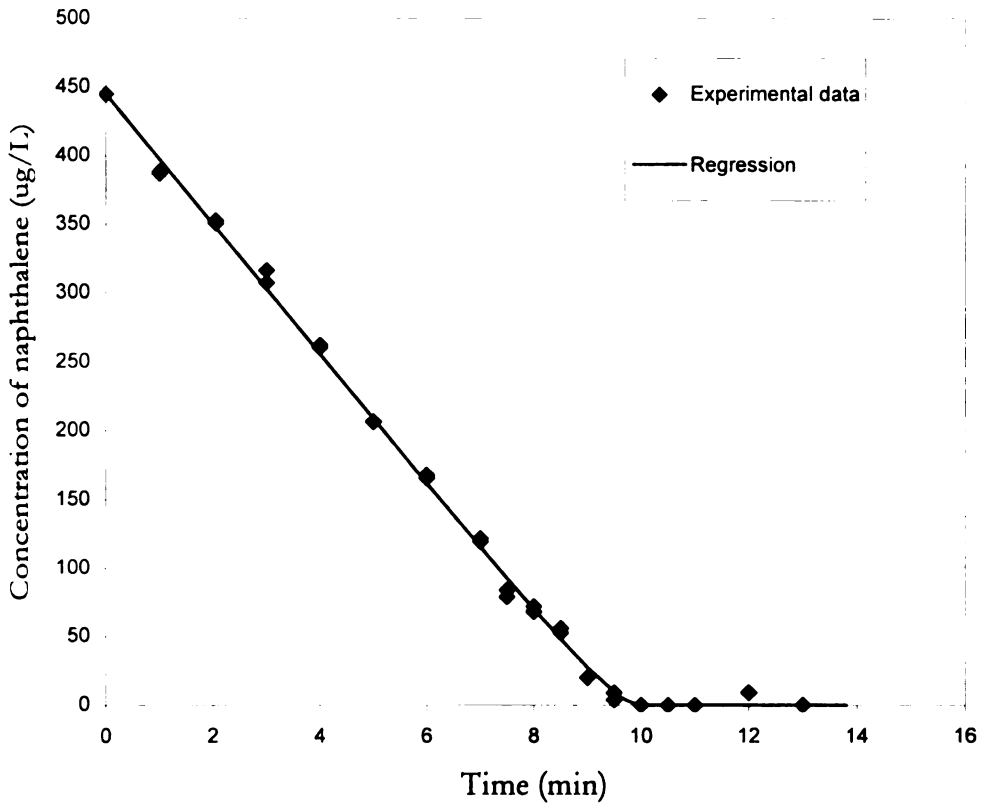

Figure 6- 2. Estimation of degradation parameters of naphthalene in a batch experiment with free suspended cells. Protein content; 420 μg/L (1.16×10^7CFU/mL), Initial concentration of naphthalene; 445 μg/L, K_s; 5.5 (±0.26) μg/L, k_m; 0.115 (±0.0004) min^{-1}, k_o; 0.110(±0.003) min^{-1}. The values in the parentheses are the standard deviations obtained by nonlinear regression.

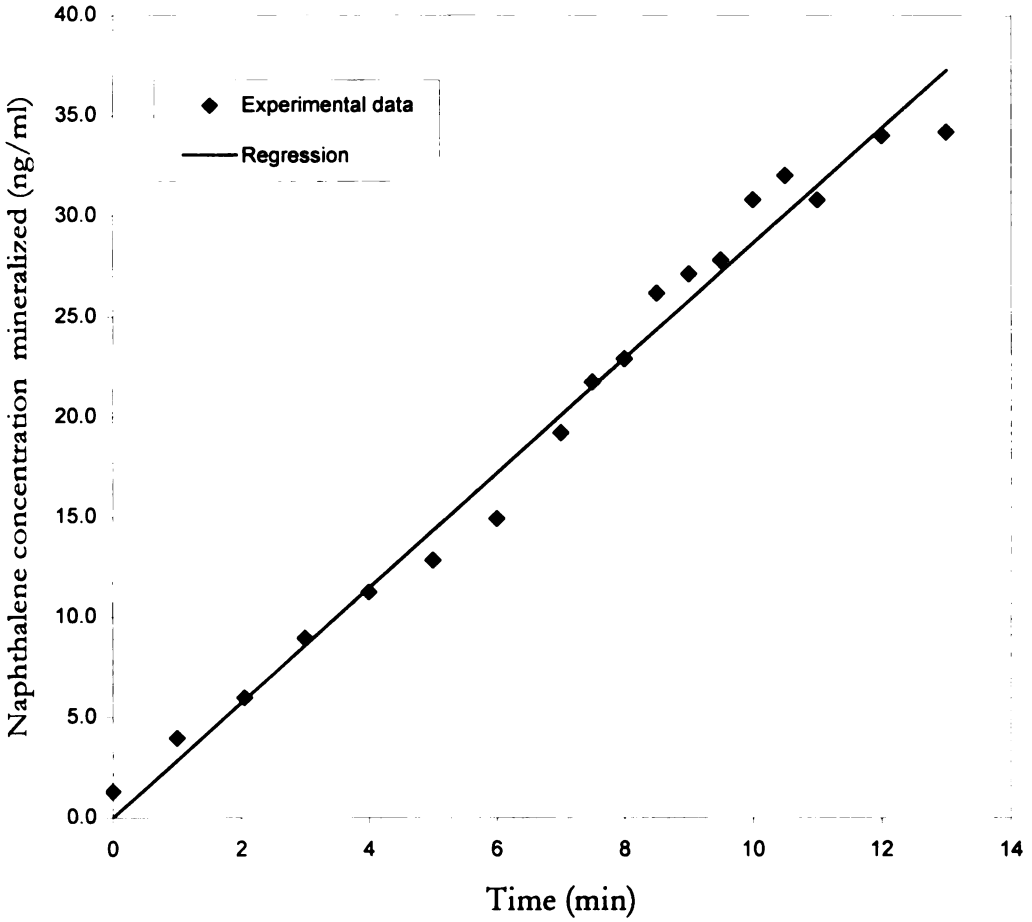

Figure 6- 3. Estimation the initial mineralization rate of naphthalene in a batch experiment with free suspended cells. Protein content; 420 μg/L (1.16×10^{7}CFU/mL), Initial concentration of naphthalene; 445 μg/L, k_{co2}; 0.0068 (±0.00007) min^{-1}. The value in the parentheses is the standard deviation.

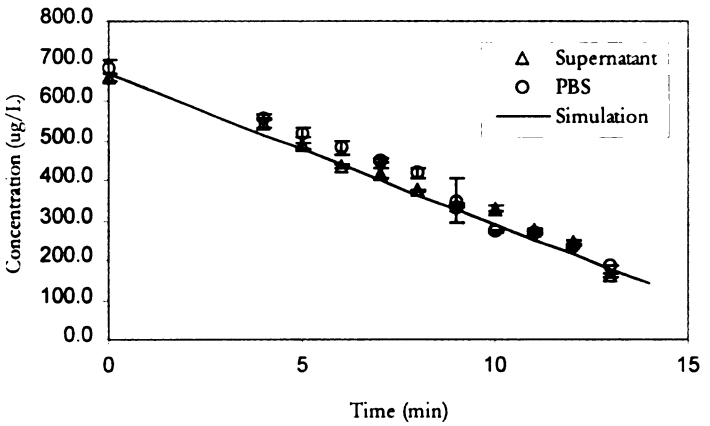

Figure 6- 4. Comparison of degradation rates of naphthalene in PBS and supernatant in batch experiments. Protein content; 337 μg/L ($9.3x10^6$ CFU/mL), initial concentration; 667 μg/L. Error bars indicate standard deviation.

Degradation and mineralization of naphthalene by attached cells in sand columns

A series of column assays were conducted to evaluate naphthalene degradation and mineralization rates by attached cells in sand columns (Table 6-1). The residence time was calculated based upon the flow rate and pore volume of each column. Using Eq. 5, the half-saturation constant, K_s, and maximum specific utilization rate, k_m, were estimated to be 49.6 (±1.4) μg/L and 0.0965 (±0.0005) min^{-1}, respectively (Figure 6-5). The zero-order reaction coefficient for degradation, k_o, was calculated to be 0.080 (±0.010) min^{-1} using the data points when the effluent naphthalene concentration was greater than 100 μg/L (Figure 6-5) and the zero-order reaction coefficient of mineralization, k_{co2}, was 0.0062 (±0.00013) min^{-1} (Figure 6-6). Correlation coefficients (r^2) were 1.00 for the Michaelis-Menten equation, 0.97 for zero-order degradation, and 0.94 for mineralization.

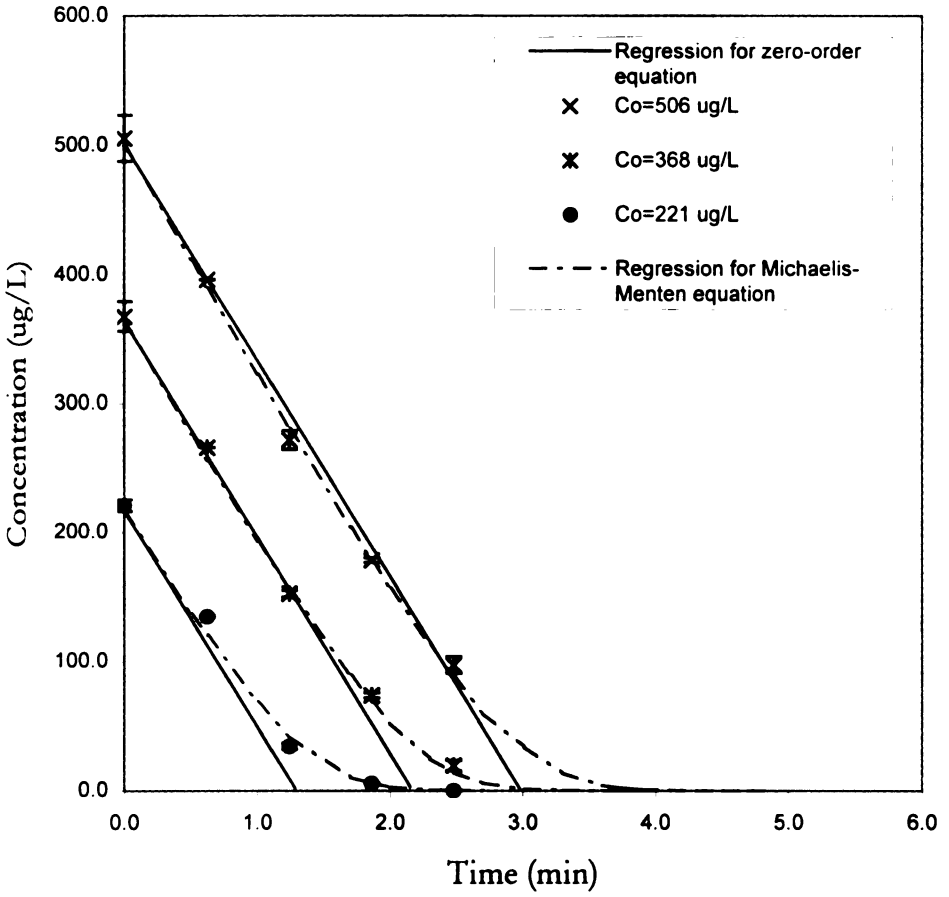

Figure 6- 5. Estimation of degradation parameters of naphthalene in column experiments with attached cells. Protein content; 2100 μg/L (1.36×10^7 CFU/g sand), flow rate; 3ml/min, influent concentrations; 506, 368, 221 μg/L, k_o; 0.080 (±0.010) min^{-1}, k_m; 0.0965 (±0.0005) min^{-1},K_s; 49.6 (±1.4) μg/L. Error bars indicate standard deviations. The values in the parentheses are the standard deviations obtained by nonlinear regression.

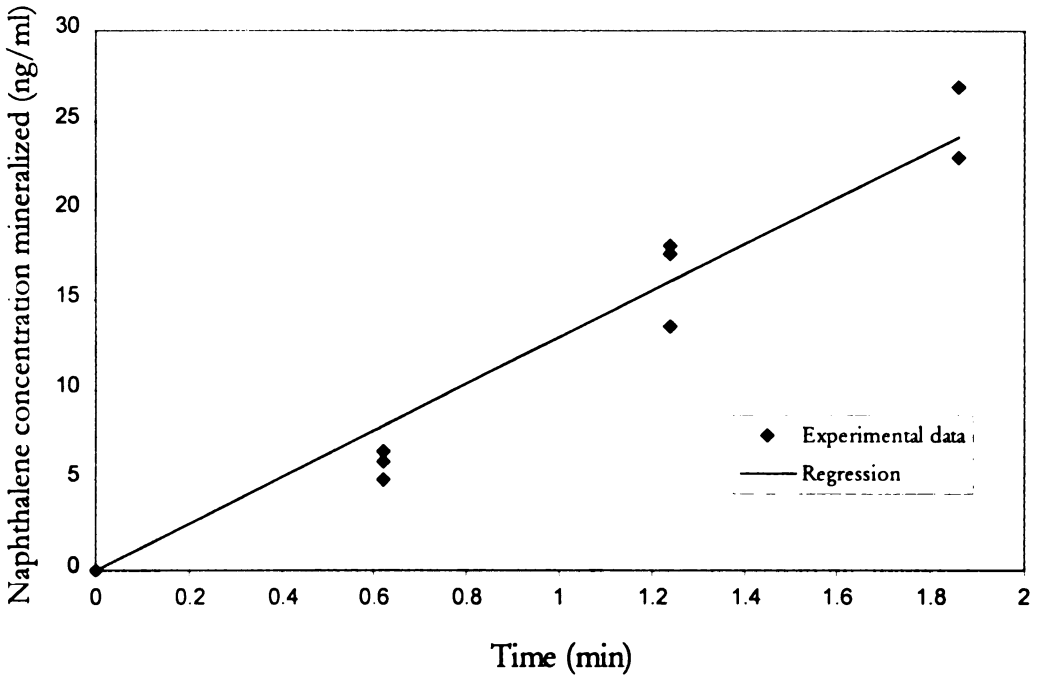

Figure 6- 6. Estimation of initial mineralization of naphthalene in column experiments with attached cells. Protein content; 2100 μg/L (1.36×10^7 CFU/g sand), flow rate; 3ml/min, influent concentrations of naphthalene; 506, 368, 221 μg/L, k_{co2}; 0.0062 (±0.00013) min^{-1}. The value in the parentheses is the standard deviation.

Comparing rate coefficients in both batch and column systems, it could be seen that k_o was reduced by a factor of 27% and k_{co2} by 8% in columns when compared to batch systems (Table 6-2). The decreases in k_o and k_{co2} were found to be significant at the 98% and 56% confidence levels, respectively. It has been suggested that the exposed surface area of attached cells is less than suspended cells, which can reduce degradation rates (Harms and Zehnder, 1994). To estimate the effect of this surface area reduction, we measured the dimensions for the rod-shaped cells (0.5 μm diameter x 1.2 μm length). Assuming monolayer-cell coverage (the portion of the sand surface covered by cells is less than 0.5 %), the attached cell presented 17 % less area to bulk solution. This value must be treated as only approximate, because we were not able to account for cell clustering or irregularities in the sand surface. It was noted that this value was similar to the reductions in k_o and k_m, which were reduced by 27% and 16% respectively. Because the k_o, k_m, and k_{co2} values represent a series of mass-transfer and reaction processes occurring within the cell membrane, the dependence on surface area likely has a mechanistic basis.

Activity decreases with attached bacteria have also been observed in batch assays by other workers (Gordon et al., 1983; Jeffrey and Paul, 1986). Jeffery and Paul (Jeffrey and Paul, 1986) found that the thymidine incorporation rate and the ATP/DNA ratio of free suspended cells were greater than those of attached cells. They ascribed the reduced rates to physiological differences between the two systems, primarily a reduction in the amount of cell surface available for nutrient uptake. Gordon *et al.* (Gordon et al., 1983) reported that attached cells had a decreased metabolic efficiency and that the rate of respiratory metabolism was slower than that of suspended cells in their assays. They

suggested several possibilities including reduced availability of inorganic nutrients or oxygen, pH effects, and reduction of exposed cell surface area.

In the present study, we had no reason to believe that the decrease in cell activity was caused by limitations in the availability of the electron acceptor, substrate, or other nutrients in the bulk solution of the column systems. Air-saturated PBS was used in both systems. Because the maximum initial concentration of the naphthalene was less than 700 μg/L, dissolved oxygen was sufficient, remaining more than 7.0 mg/L during the entire course of the experiment. Sorption of naphthalene to the sand and column components was negligible after 3 pore volumes passed the columns (verified in non-inoculated packed columns). pH effects on sand surface were eliminated because the sand was thoroughly washed and equilibrated with PBS before inoculation, and ATCC 17484 was found to have same activity at both the original sand pH of 6.5 and the buffer pH of 7.0. The degradation rate of naphthalene was also tested in the supernatant, and was found to be the same as that in PBS (Figure 6-4). The reduction of exposed cell surface was therefore considered to be the primary factor in the decrease in cell activity as reflected by the values of k_o and k_m.

The differences between batch and column experiments became more problematic at low concentrations (i.e., substrate concentrations less than 100 μg/L), corresponding to large levels of removal. While the batch systems appeared to follow zero-order kinetics across the entire concentration range (Figure 6-2), the columns deviated from this model below approximately 100 μg/L (Figure 6-5). The behavior at low concentrations is described by the half-saturation coefficient, K_s. These values were significantly greater in the columns than those in batch systems (Table 6-2). Harms and Zehnder also found

greater K_s values for degradation of dibenzofuran and 3-chlorodibenzofuran by attached cells of *Sphingomonas* sp. strain HH19k. They attempted to model this considering the change in diffusive flux resulting from the smaller surface area of attached cells. However, their experimental K_s values were much greater than theoretical predictions. They concluded that the discrepancy could have resulted from the assumptions used in their calculations, non-uniform distribution of cells within the column, variations in the boundary layer across which diffusive transport occurs, and the presence of preferential flow paths.

The dispersion coefficient, which reflects *hydraulic heterogeneity*, was evaluated in the tritium tracer study in the non-inoculated packed columns. As noted previous, the dispersion term in Eq. 3 was negligible when compared with the advection term. However, it is possible that *reactive heterogeneity* might occur in the inoculated columns as a result of the differences between the distribution of cells and the paths of water flow. Reactive-heterogeneity implies that all flow paths do not necessarily contain the same biomass and therefore do not offer the same reactivity. This effect is not captured by the dispersion coefficient, and the advection-dispersion equation assume that all paths are similarly reactive.

Table 6- 2. Comparison of kinetic parameters between batch and column experiments

	k_o (min^{-1})	k_m (min^{-1})	K_s (μg/L)	k_{co2} (min^{-1})
Batch	0.110 (±0.003)*	0.115 (±0.0004)*	5.5 (±0.26)*	0.0068 (±0.00007)*
Column	0.080 (±0.01)*	0.0965 (±0.0005)*	49.6 (±1.4)*	0.0062 (±0.00013)*
Comments (comparing column to batch values)	27 % reduction	16 % reduction	9-fold increase	8 % reduction

*: Standard deviation given by ModelMaker software during nonlinear regression.

The sand pore structure can be viewed as a "bundle of capillary tubes" of different sizes and shapes (Tindall and Kunkel, 1999). Cells will colonize the most favorable locations, which may be far from the primary flow paths, so as to avoid shearing effects. Thus, the solution to cell transfer distance for the substrate in packed columns may be on the order of several sand particle diameters. In a suspended-cell system, the distance is the aqueous boundary layer surrounding the cell, which will likely be much smaller. Furthermore, the variation in transfer distance will be much greater in columns, as cells may reside both close to and far from the various flow paths. The result is essentially increased dispersion of substrate in inoculated columns, where substrate in some paths encounters fewer cells than in others. Thus, reactive-heterogeneity decreases reaction efficiency and increases the relative substrate concentration in the effluent over that which would be expected from the assumption of uniform reactivity implicit in the advection-dispersion equation. The result is greater measured/fit K_s values. Thus K_s is not an intrinsic parameter. Rather, it is a lumped mass-transfer parameter that describes the overall process of transport from the bulk fluid to the cell surface, and is always dependent upon the nature of the system in which degradation occurs.

External mass-transfer effects

The naphthalene concentrations in the effluents at different retention times were measured to evaluate the effect of external mass transfer in the pore space on degradation rates (Figure 6-7). Also shown is the predicted curve using the degradation parameters obtained from the previous column experiments with the assumption that overall degradation rates are not dependent on the liquid flow rate. It appeared that this

assumption was true for flows of 1 mL/min or greater, based on the coincidence of the measured values and the simulation for all but the lowest flow rate. At 0.5 mL/min, effluent concentrations were greater than predicted. Harms and Zehnder reported that the half-saturation coefficient (K_s) increased as flow rate decreased, producing greater-than-expected effluent concentrations (Harms and Zehnder, 1994). Tros et al. suggested that this was the result of channeling and immobile water, because channeling reduces residence time and mass-transfer resistance increases in immobilized water regions (Tros et al., 1998). In the present study, however, the immobilized-water region was less than 5% of the pore volume for all pore-water velocities used, as determined by the tritium tracer study. Furthermore, Li *et al.* has shown that the immobile water fraction was determined by the sand structure but was independent of the velocity (Li et al., 1994), while Griffioen *et al.* reported mobile water fraction was constant or decreased with increasing pore water velocity in their literature review (Griffioen et al., 1998). We therefore conclude that the effects observed in our data did not result from immobile water.

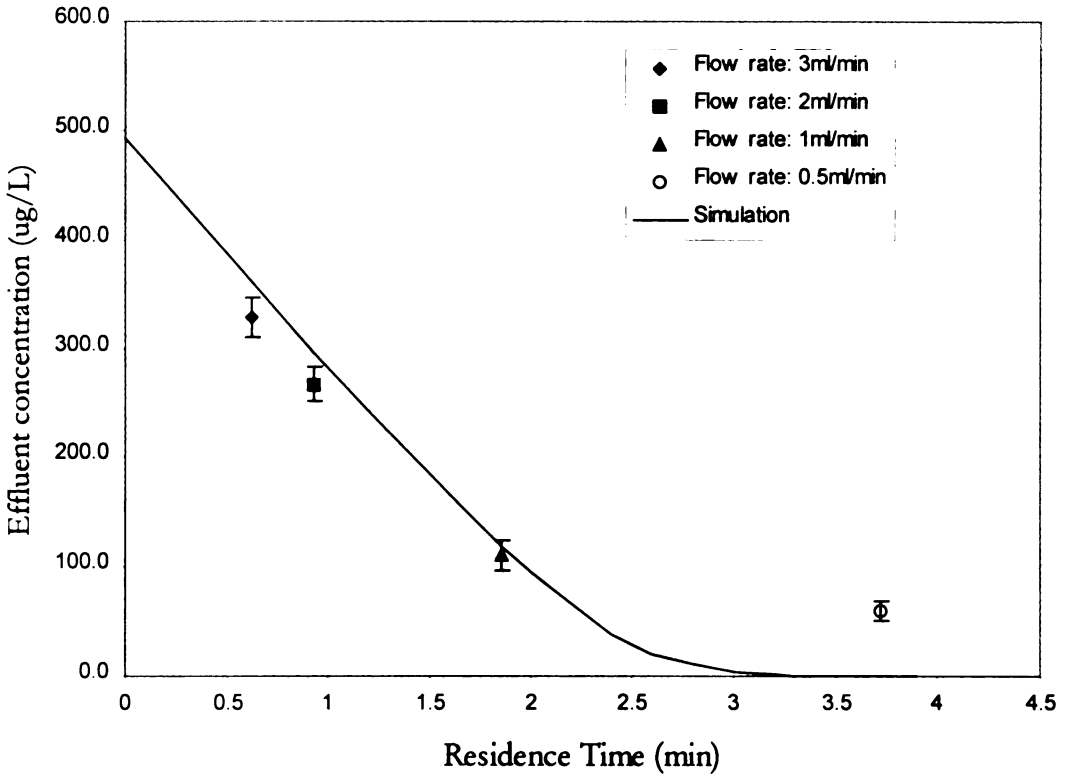

Figure 6- 7. Flow rate effect on degradation of naphthalene in column experiments. Column length; 5 cm, Protein content; 2490 μg/L (1.62×10^7 CFU/g sand), initial naphthalene concentration; 498 μg/L, flow rates; 3 mL/min, 2 mL/min, 1ml/min and 0.5 mL/min, K_s; 49.6 μg/L, k_m; 0.0965 min^{-1}. Error bars indicate standard deviations.

As discussed previously, K_s is a lumped mass-transfer parameter describing transport to the cell surface. It is commonly observed that the external mass-transfer resistance increases with decreases in pore-velocity, as evidence by correlation between mass-transfer coefficients and Reynolds numbers (Weber and DiGiano, 1996). Although these results do not provide a mechanistic basis for our observations, the dependence of K_s on velocity is not unexpected. We suggest that this dependence may be another manifestation of reactive-heterogeneity of bioactivity. As velocity decreases, the residence time in all of the capillary pathways will increase proportionally. The result should be increased removal of substrate. However, in highly reactive pathways (i.e pathways with more cells), this increase will result in depletion of the substrate before the flow reaches the column exit. Any bio-reactivity in the pathway after the point of depletion is effectively wasted. This wasted activity does not contribute to removal in the column system, and thus the integrated removal rate on a biomass basis is reduced. The result is that column effluent concentrations will be greater than would be expected by the implicit assumption that all of the biomass is active.

Internal mass-transfer effects

Internal mass-transfer limitations (within the biological matrix) might also affect degradation rates because cells interior to the matrix may see a reduced substrate flux. Such a limitation would result in rates that are not strictly proportional to biomass levels, but drop below expected rates. This possibility was investigated by inoculating the sand columns with four different cell densities (Table 6-1). The predicted values in Figure 6-3 were calculated using the degradation parameters estimated from previous sand column

experiments in Figure 6-5. There were no significant differences between the measured and predicted values for the low-cell-density cases (1006 and 2065 μg/L). As the cell density increased, the measured values were greater than the predicted values (Table 6-3) indicating some internal mass-transfer resistance. Even at the highest inoculation levels, the cells theoretically covered less than 1% of the sand surface. Harms and Zehnder also reported a similar result: half-saturation coefficients (K_s) for DF and 3CDF degradation by strain HH19k increased as cell density increased in their columns (Harms and Zehnder, 1994).

Table 6- 3. The predicted and measured values of effluent naphthalene concentrations at different cell densities

Protein density (μg/L)	Cell Density (CFU/g sand)	Predicted value (μg/L)	Measured value (μg/L)	Difference (μg/L)
6353	4.13×10^{7}	70	192 (±5.09)*	122
3071	1.99×10^{7}	294	370 (±9.9)*	76
2065	1.34×10^{7}	397	419 (±12.7)*	22
1006	6.53×10^{6}	503	510 (±9.4)*	7

* Standard deviation of three measurements.

There is another factor that could cause the higher naphthalene concentrations in the effluent at greater inoculation densities: increased cell washout. However, the optical density of the effluent did not increase and the effluent cell density was below the detection limit (5×10^5 CFU/mL) after inoculation in all of the columns, and the degradation rate was stable after the acclimation period. These observations suggest that cell detachment was negligible.

It should be noted that this result might also derive from reactive-heterogeneity. At increased biomass levels substrate depletion will occur earlier in the most reactive flow paths. Thus, even more of the bioactivity would be wasted, as compared to smaller biomass levels, and the overall inefficiency would be greater. The result, as discussed above, would be greater than expected K_s values and effluent naphthalene concentrations.

REFERENCE

Barnsley, E. A. 1976. "Role and Regulation of the ortho and meta Pathways of Catechol Metabolism in Pseudomonads Metabolizing Naphthalene and Salicylate." *J. Bacteriol* 125: 404-408.

Blakemore, R. P., D. Maratea and R. S. Wolfe. 1979. "Isolation and pure culture of a freshwater magnetotactic spirillum in chemically defined medium." *J. Bacteriol.* 140: 720-729.

Bright, J. J. and M. Fletcher. 1983. "Amino Acid Assimilation and Respiration by Attached and Free-living Population of a Marine Pseudomonas sp." *Microb. Ecol* 9: 215-226.

Caldwell, D. E. and J. R. Lawrence. 1986. "Growth kinetics of Pseudomonas fluorescens microcolonies within the hydrodynamic boundary layers of surface microenvironments." *Microb. Ecol.* 12: 299-312.

Dolfing, J. 1985. "Kinetics of methane formation by granular sludge at low substrate concentrations." *Appl. Environ. Microbiol.* 22: 77-81.

Ellwood, D. C., C. W. Keevil, P. D. Marsh, C. M. Brown and J. N. Wardell. 1982. "Surface-associated growth." *Pjilos. Trans. R. Soc. London Ser. B.* 297: 517-532.

Estrella, M. R., M. L. Brusseau, R. S. Maier, I. L. Pepper, P. J. Wierenga and R. M. Miller. 1993. "Biodegradation, Sorption, and Transport of 2,4-Dichlorophenoxyacetic Acid in Saturated and Unsaturated Soils." *Appl. Environ. Microbiol* 59: 4266-4273.

Firestone, M. K. 1982. Biological denitrification. Madison, WI., ASA, CSSA, and SSSA.

Focht, D. D. and D. Shelton. 1987. "Growth kinetics of Pseudomonas alcaligenes C-O relative to inoculation and 3-chlorobenzoate metabolism in soil." *Appl. Environ. Microbiol* 53: 1846-1849.

Freeze, R. A. and J. A. Cherry. 1979. Groundwater. Englewood Cliffs, New Jersey., Prentice-Hall. Inc.

Gordon, A. S., S. M. Gerchakov and F. J. Millero. 1983. "Effects of Inorganic Particles on Metabolism by a Periphytic Marine Bacterium." *Appl. Environ. Microbiol* 45: 411-417.

Griffioen, J. W., D. A. Barry and J. Y. Parlange. 1998. "Interpretation of two-region model parameters." *Water Resour. Res.* 34: 373-384.

Guerin, W. F. and S. A. Boyd. 1992. "Differential Bioavailability of Soil-Sorbed Naphthalene to Two Bacterial Species." *Appl. Environ. Microbiol* 58: 1142-1152.

Guerin, W. F. and S. A. Boyd. 1995. "Maintenance and Induction of Naphthalene Degradation Activity in Pseudomonas putida and an Alcaligenes sp. under Different Culture Conditions." *Appl. Environ. Microbiol* 61: 4061-4068.

Harms, H. and A. J. B. Zehnder. 1994. "Influence of Substrate Diffusion on Degradation of Dibenzofuran and 3-Chlorodibenzofuran by Attached and Suspended Bacteria." *Appl. Environ. Microbiol* 60: 2736-2745.

Jeffrey, W. H. and J. H. Paul. 1986. "Activity of an Attached and Free-Living Vibrio sp. as Measured by Thymidine Incorporation, p-Iodonitrotetrazolium Reduction, and ATP/DNA Ratios." *Appl. Environ. Microbiol* 51: 150-156.

Kelly, W. R., G. M. Hornberger, J. S. Herman and A. L. Mills. 1996. "Kinetics of BTX biodegradation and mineralization in batch and column systems." *J. Contam. Hydrol.* 23: 113-132.

Li, L., D. A. Barry, P. J. Culligan-Hensley and K. Bajracharya. 1994. "Mass transfer in soils with local stratification of hydraulic conductivity." *Water Resour. Res.* 30: 2891-1900.

McLaren, A. D. 1978. Kinetics and consecutive reactions of soil enzymes. New York, Academic Press.

Myrold, D. D. and J. M. Tiedje. 1985. "Diffusional constraints on denitrification in soil." *Soil Sci. Soc. Am. J.* 49: 651-657.

Ngian, K. F., S. H. Lin and W. R. B. Martin. 1977. "Effect of mass transfer resistance on the Linweaver-Burk plots for flocculating microorganisms." *Biotechnol. Bioeng.* 19: 1773-1784.

Pierce. 1989. "Coomassie Protein Assay Reagent." *Pierce Chemical Company Caltaloge 23200* -: -.

Plaehn, W. A., X. Zhao, B. E. Dale and T. C. Voice. 1999. "Dissloved Organic Material Impact on the Desorption Rate and Mineralization Rate of Naphthalene." *J. Soil Contam.* 8: 491-507.

Tindall, J. A. and J. R. Kunkel. 1999. Unsaturated Zone Hydrology for Scientists and Engineers. New Jersey, Prentice-Hall, Inc.

Tros, M. E., G. Schraa, A. J. B. Zehnder and N. P. Bosma. 1998. "Anomalies in the Rransformation of 3-Chlorobenzoate in Percolation Columns with Pseudomonas sp. Strain B13." *Wat. Sci. Tech.* 37: 89-96.

van Loosdrecht, M. C. M., J. Lyklema, W. Norde and A. J. B. Zehnder. 1990. "Influence of Interfaces on Microbial Activity." *Microbiol. Rev.* 54: 75-87.

Voice, T. C. and B. Kolb. 1993. "Static and Dynamic Headspace Analysis of Volatile Organic Compounds in Soils." *Environ. Sci. Technol* 27: 709-713.

Weber, W. J., Jr. and F. A. DiGiano. 1996. "Process Dynamics in Environmental Systems." *John Wiley & Sons, Inc.* -: -.

Wolin, E. A., M. J. Wolin and R. S. Wolfe. 1963. "Formation of Methane by Bacterial Extracts." *J. Biol. Chem.* 238: 2882-2886.

Zhao, X. and T. C. Voice. 2000. "Assessment of Bioavailability Using a Multi-Column System." *Environ. Sci. Technol.* 34:1506-1512.

CHAPTER 7 SUMMARY AND CONCLUSIONS

SUMMARY

The objective of this study was to investigate the bioavailability of sorbed contaminants in solids, which has been an important issue in applying bioremediation technology to the restoration of contaminated sites. In order to accomplish this, four main objectives were pursued in sequence.

The first objective was to develop an experimental and mathematical approach to evaluate bioavailability of contaminants sorbed in artificial solids (silica particles). Desorption rates of 2,4-dichlorophenoxyacetic acid (2,4-D) were measured in the absence of organisms and the biodegradation rates were measured in the absence of the solids. After the silica particles were saturated with 2,4-D, the system was inoculated with the 2,4-D degrading microorganism *Flavorbacterium* sp. strain FB4. The disappearance rate of 2,4-D was measured and found to be greater than the rate predicted based upon liquid-phase 2,4-D concentrations. A kinetic formulation, termed the enhanced bioavailability model, was developed to describe the desorption and biodegradation processes in this batch system. The approach assumes that 2,4-D resides in both the liquid and solid phases and degradation occurs via both suspended and attached biomass. All biomass can degrade liquid-phase 2,4-D at one rate, while only attached biomass can degrade sorbed 2,4-D at another rate. An enhanced transformation factor (E_f) was introduced to express the increased biodegradation rate over that expected from the liquid phase only. This approach was able to account for the increased degradation rates observed experimentally. The results provide evidence that desorption to the bulk solution is not prerequisite to degradation, and that sorbed substrate may be available for degradation.

The second objective was to evaluate the effects of biodegradation kinetics on the bioavailability of sorbed naphthalene. Degradation assays were used instead of mineralization assays. Four different organisms with zero-order, first-order, and Michaelis-Menten rates, and two soils with different sorption distribution coefficients were selected. Sorption and desorption was evaluated in abiotic soil-slurry systems. The desorption process was described by a model that accounts for equilibrium, rate-limited and non-desorbing sites. Biodegradation parametes were measured in soil-extract solutions. Bioavailability assays, inoculated soil-slurries, were conducted and both liquid- and solid-phase naphthalene concentrations were measured over time. For the less sorptive soil, the results could be explained by sequential desorption and degradation processes. For the other soil, enhanced degradation was clearly observed for the organisms with first-order and Michaelis-Menten rates. No enhancement was found for the organism with zero-order kinetics.

The third objective was to develop a quantitative mathematical model to describe the bioavailability of chemicals sorbed by natural soils. Two naphthalene degrading organisms (which were chemotactic to solid naphthalene) and four soils with different distribution coefficients were selected. Sorption isotherms and single/series dilution desorption experiments were conducted to evaluate distribution coefficients, to estimate desorption parameters, and to measure the non-desorbable amount of naphthalene. Biodegradation kinetics were measured in soil extract solutions and rate parameters developed. For the bioavailability assays, sorption equilibrium was established, and the systems were inoculated. The naphthalene concentrations in both sorbed and dissolved phases were measured over time. Enhanced bioavailability, as evidenced by faster than

expected degradation rates, was observed in soils with the higher sorption distribution coefficients. These observations could be described by using a model formulation that included solid-phase degradation. A portion of the sorbed naphthalene could not be desorbed by successive water extractions, but was available for biodegradation.

The fourth objective was to evaluate the relationship between kinetic parameters in batch and column systems, in order to assess the role of cell attachment and flow on biodegradation rates. Under this objective, Ottawa sand-packed-columns were used to evaluate the effects of fluid flow, soil to water ratio, cell attachment/detachment, and the mixing of contaminants, on the degradation of naphthalene by *Pseudomonas putida* (ATCC 17484). Column results were compared to degradation rates found in well-mixed batch systems with suspended cells. The maximum specific utilization coefficient (k_m), the zero-order reaction coefficient (k_o) for degradation, and zero-order reaction coefficient (k_{co2}) for mineralization differed by factors similar to the calculated reduction in exposed cell surface area. In batch systems, degradation followed zero-order kinetics across the entire concentration range, while the columns exhibited decreased rates at concentrations less than 100 (μg/L), describable by Michaelis-Menten kinetics. This is reflected in elevated values of the half-saturation constant, K_s, in columns. This may result from reactive-heterogeneity within the porous media, imposing a distribution of length-scales for transfer of substrate to the cell surfaces. Well-mixed batch systems are expected to have both shorter and more uniform transfer distances. This explanation was also shown to be consistent with observations of the effects of flow rate and biomass levels in column experiments. When kinetic parameters obtained in batch system are used for prediction of degradation in columns, at least two factors – exposed reduction of

exposed cell surface area and heterogeneity of cell distribution – will likely reduce overall column degradation rates.

CONCLUSIONS

1. There is enhanced bioavailability of sorbed contaminants, which cannot be explained by desorption and liquid-phase degradation alone.
2. The bioavailability of sorbed contaminants in natural soils is dependent on biokinetic characteristics as well as the sorption distribution coefficient.
3. Contaminants held in non-desorption sites, may be available for biodegradation.
4. Biodgradation rate may be reduced in columns due to factors including the reduction of exposed cell surface area and heterogeneity of cell distribution.

APPENDICES

APPENDIX A

Biodegradation pathway of naphthalene

Naphthalene properties

PAHs' solubility

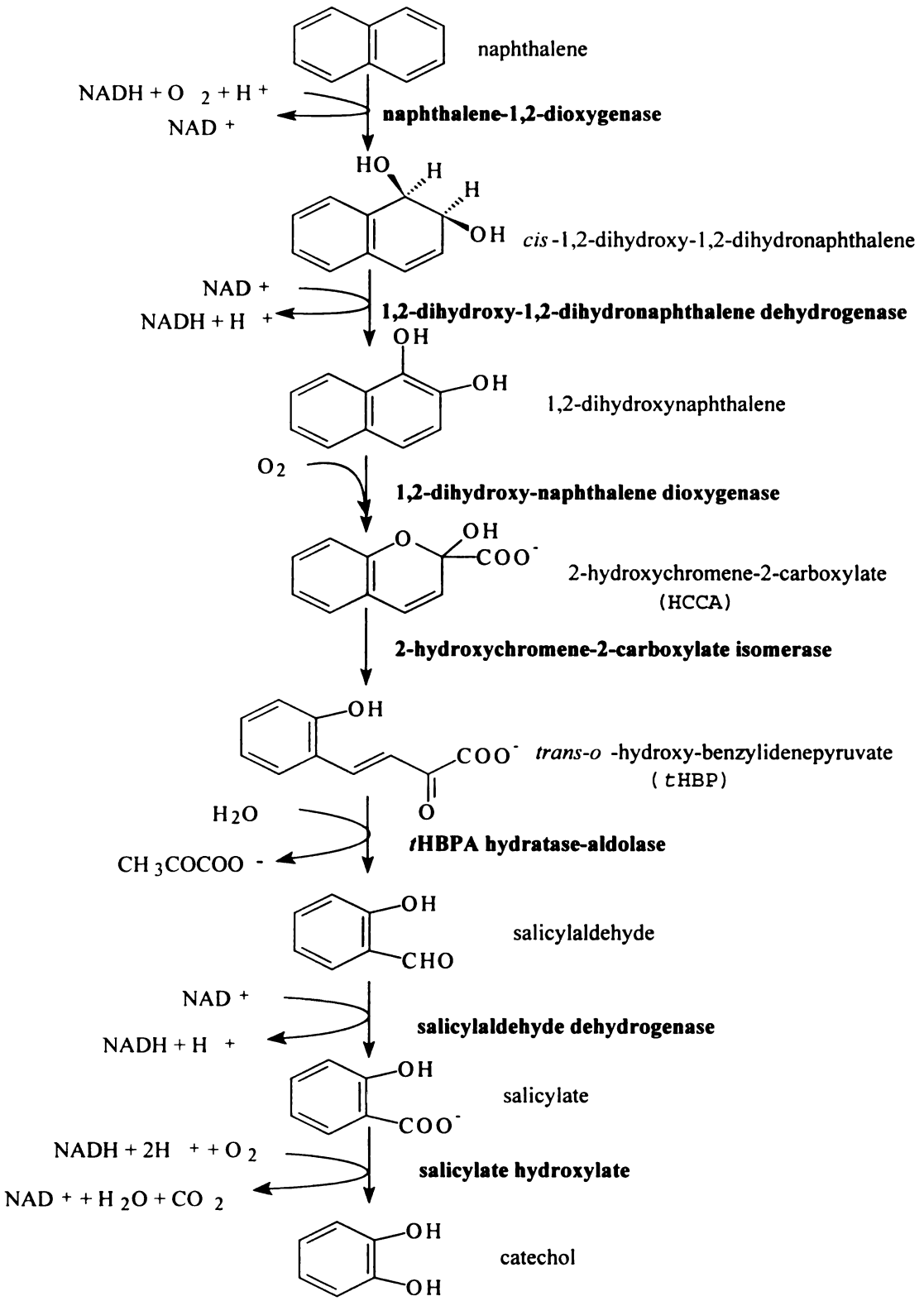

Figure A- 1. Degradation pathway from naphthalene to catechol (after Wackett, 1997)

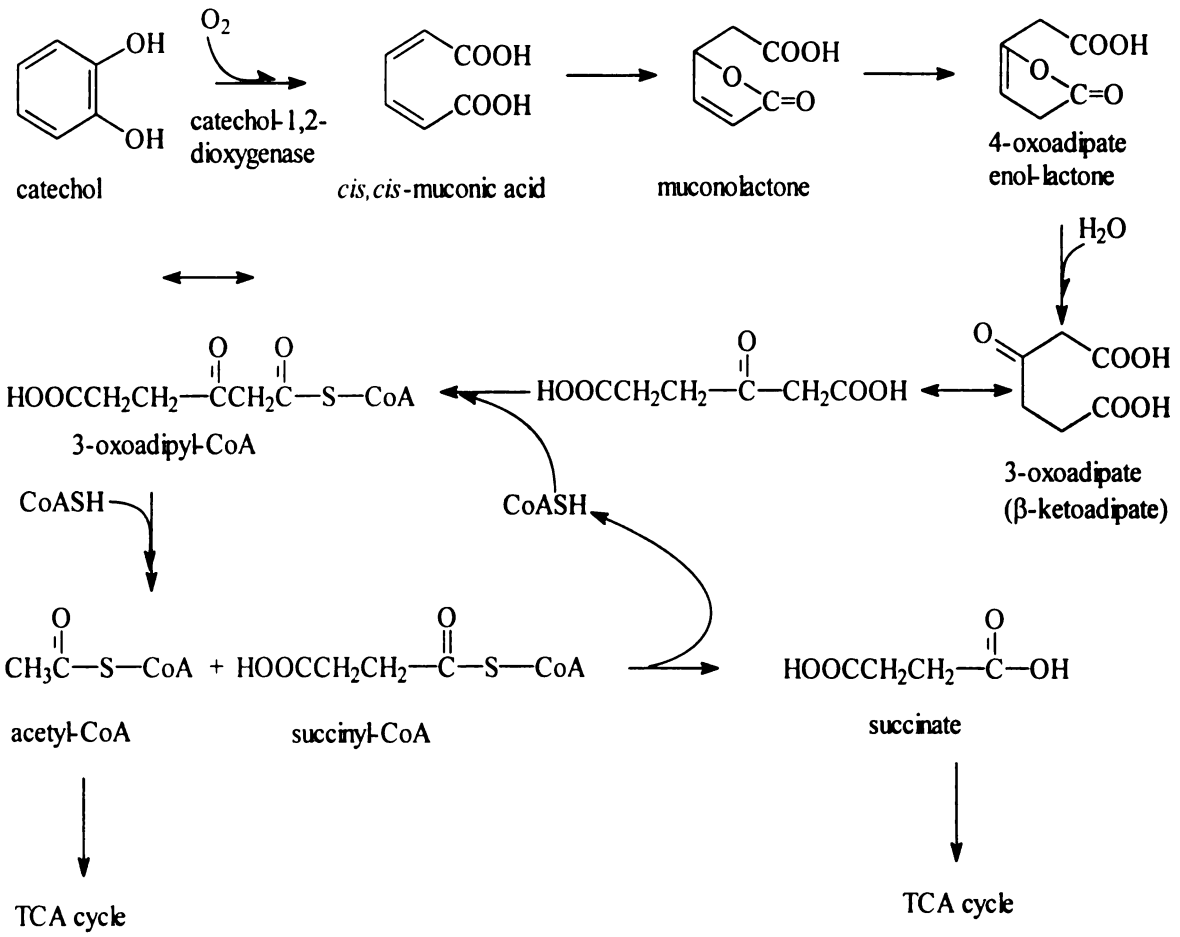

Figure A- 2. Ortho-cleavage pathway for catechol catabolism (after Rittman et al., 1994)

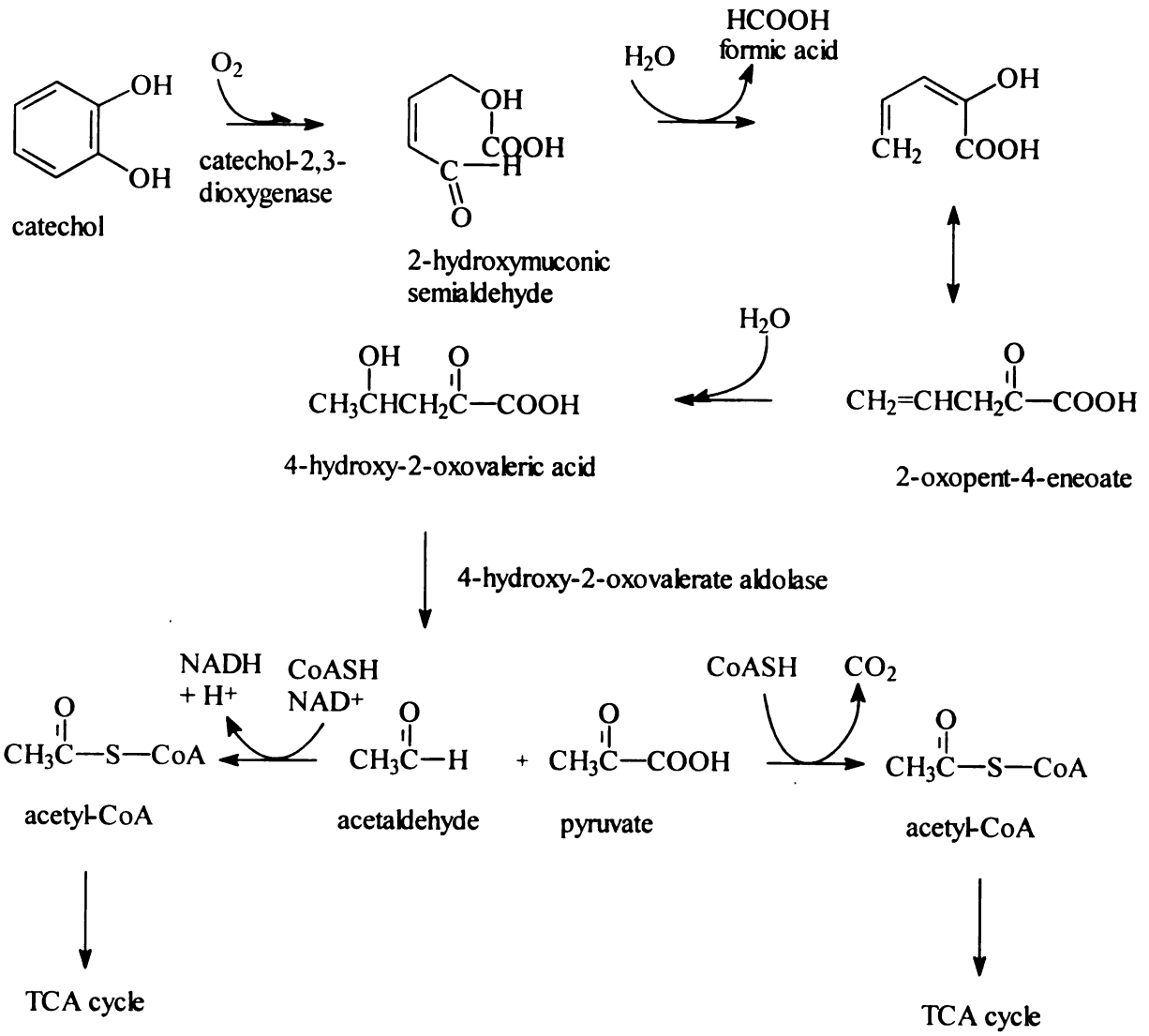

Figure A- 3. Meta-cleavage pathway for catechol catabolism (after Rittman et al., 1994)

Table A- 1. Naphthalene Properties

Parameters	Value (unit)	Description	reference
M.W	128 (g/mol)	Molecular weight	
T_m	80.6 (^oC)	Melting point	Schwarzenbach et al., 1992
K'_H	0.0197	Dimensionless Henry's law constant (mol L_{atm}^{-1})/(mol L_w^{-1})	
C_w^{sat} (s)	2.5×10^{-4} (mol L_{water}^{-1}) or 32 (mg L_{water}^{-1})	Solid naphthalene solubility At 25 ^oC and 1 atm	
C_w^{sat} (l)	8.7×10^{-4} (mol L_{water}^{-1}) or 111 (mg L_{water}^{-1})	Subcooled liquid naphthalene solubility At 25 ^oC and 1 atm	
K_{ow}	2300 ($L_{water}/L_{octanol}$)	Octanol-water distribution coefficient Log K_{ow} = -0.87 log C_w^{sat} (l) + 0.68	Chiou, 1998
K_{om}	182 (L_{water}/kg_{om})	Organic matter-water distribution coefficient Log K_{om} = 0.904 log K_{ow} – 0.779 Log K_{om} = -0.729 log C_w^{sat} (l) + 0.001	
K_{oc}	313 (L_{water}/kg_{oc})	Organic carbon-water distribution coefficient 1.72 K_{om} (Using organic carbon is 58% of organic matter, OM=OC × 1.74)	

Table A- 2. Aqueous solubility of PAH compounds (Linz and Nakles, 1997)

	PAHs	Maximum Aqueous Solubility* (mg/L)
2-Ring	Naphthalene	30.0
3-Ring	Acenaphthene	3.42
	Acenaphthylene	3.93
	Anthracene	1.29
	Fluorene	1.90
	Phenanthrene at 21 °C	0.816
4-Ring	Benzo(a)anthracene at 24 °C	0.0100
	Chrysene	0.00600
	Fluoranthene	0.265
	Pyrene at 26 °C	0.160
5-Ring	Benzo(a)pyrene	0.00380
	Benzo(b)fluoranthene	0.0140
	Benxo(k)fluoranthene	0.00430
	Dibenzo(a,h)anthrancene	0.000500
6-Ring	Benzo(g,h,I)perylene	0.000260
	Indeno(1,2,3-cd)pyrene	0.000530

* Pure single compound solubility in water; at 25 °C unless otherwise mentioned.

APPENDIX B

F-test in statistics

F test[1] definition

Comparison of two variances; A variance ratio test used to decide whether two independent estimates of variance can reasonably be accepted as being two estimates of the variance of a single normally distributed universe.

$F= S_a^2/S_b^2$

Where S_a^2= the larger variance estimate

S_b^2= the smaller variance estimate

F test for a group of sets[2].

$$F = \frac{(RSS_{12} - RSS_1 - RSS_2)/(df_{12} - df_1 - df_2)}{RSS_{12}/df_{12}}$$

Where

RSS(or SSE)=$\sum(y_i-\hat{y}_i)^2$; Residual sum of square.

SST=$\sum(y_i-\bar{y}_i)^2$; Total sum of square

yi=datum point.

$\hat{y}_i$=predicted point by equation.

$\bar{y}_i$=mean of data.

RSS_1= Residual sum of square at first set

RSS_2= Residual sum of square at second set

RSS_{12}= Residual sum of square at combined set

[1] Anderson, R.L. 1987. Practical Statistics for Analytical Chemists. Van Nostrand Reinhold, New York, New York.

df_1= degree of freedom at first set (n_1-p_1)

df_2= degree of freedom at second set (n_2-p_2)

df_{12}= degree of freedom at combined set (n_1+n_2-p_{12})

p=parameter used in the equation.

n=number of data

F test for equations[3]

$$F = \frac{(RSS_f - RSS_r)/(df_f - df_r)}{RSS_f / df_f}$$

RSS_f= Residual sum of square (or Error sum of squares) at full equation

RSS_r= Residual sum of square (or Error sum of squares) at reduced equation

df_f= degree of freedom at full equation (n-p_f)

df_r= degree of freedom at reduced equation (n-p_r)

(ex. See the book: it says SSE (sum of square of error) instead RSS)

p=parameter used in the equation.

n=number of data

---

[2] Communication with Denise P. Kay

[3] Neter, J., W. Wasserman, G.A. Wlitmor. (1993) . Applied Statistics. 4th ed. Boston ; Allyn and Bacon. Or Jay L. Devore. (1995) Probability and Statistics for Engineering and the Sciences. 4th ed. Duxbury Press. p561.