BIODEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOIL/WATER SYSTEMS

by

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February 1991

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Engineering

ABSTRACT

This study is concerned with the biodegradation of polycyclic aromatic hydrocarbons (PAHs), particularly the utilization of biological processes in the bioremediation of PAH-contaminated soils. In this regard, the effects of the contaminant bioavailability and the redox environment on the biodegradation process have been investigated.

First, the mineralization of naphthalene in soil/water systems under denitrifying conditions has been studied. The effect of the compound's initial concentration and oil-contamination on the mineralization kinetics of naphthalene was investigated. Results showed that naphthalene mineralization is influenced by its availability to the microbial population, which in turn is a function of the compound initial concentration, the sorption/desorption characteristics of the soil/contaminant complex and the organic content of the soil. Mineralization exhibited zero order kinetics with respect to total naphthalene concentration when the aqueous concentrations of naphthalene were maintained close to the solubility limit. However, when the aqueous concentration of naphthalene dropped appreciably below its saturation limit, mineralization showed higher order kinetics and became substrate dependent.

Second, the biodegradation of four PAH compounds, acenaphthene, acenaphthylene, fluorene and anthracene, in a soil/water system under four redox environments has been studied. Both aerobic and denitrifying environments supported appreciable PAH biodegradation rates. In

comparison to the denitrifying conditions, the aerobic environment exhibited slightly higher PAH biotic losses, but on the other hand, it resulted in substantial abiotic losses, mainly volatilisation. Sulfate-reducing and methanogenic conditions did not result in any significant biodegradation of the PAH compounds. For the above mentioned reasons, the denitrifying environment was chosen for a further experiment to investigate the performance of a bioreactor system in treating PAH-contaminated soils. A bench-scale bioreactor with a total capacity of four litres was used and the PAH compounds tested were: acenaphthene, acenaphthylene, fluorene and anthracene. Results showed that by enlarging the scale of the reactor by approximately eight times and simultaneously reducing the mixing intensity of the soil slurry, the biodegradation rates of the PAH compounds remained virtually unchanged.

RÉSUMÉ

La présente étude porte sur l'utilisation de procédés biologiques pour restaurer des sites contaminés par des hydrocarbures aromatiques polycycliques (HAP). À ce propos, l'effet de la disponibilité des contaminants et du potentiel rédox sur la biodégradation a été examiné.

La première partie de l'expérience consistait à étudier la minéralisation du naphtalène dans des conditions favorisant la dénitrification dans un mélange sol/eau. L'effet de la concentration initiale du naphtalène ainsi que l'effet de l'utilisation d'un sol contaminé par des huiles sur la cinétique de minéralisation du naphtalène ont été étudiés. Les résultats obtenus montrent que la minéralisation du naphtalène par les populations microbiennes dépend de la disponibilité de celui-ci, qui elle, dépend de la concentration initiale du naphtalène, des caractéristiques d'adsorption et de désorption du complexe sol/polluant ainsi que du contenu organique du sol. La minéralisation a présenté une cinétique d'ordre zéro par rapport à la concentration totale du naphtalène lorsque celle-ci était suffisamment élevée pour maintenir une concentration aqueuse proche de la limite de solubilité. Au contraire, lorsque la concentration aqueuse du naphtalène a diminuée de façon appréciable sous la limite de saturation, la minéralisation a présenté une cinétique d'ordre plus élevé jusqu'à devenir dépendante de la concentration du substrat.

La deuxième partie de l'expérience consistait à étudier la biodégradation de quatre HAP, soit l'acénaphthène, l'acénaphthylène, le

fluorène et l'anthracène, sous différentes conditions de potentiel redox dans un mélange sol/eau. Les taux de biodégradation les plus élevés ont été obtenus en condition d'aérobiose et en condition de dénitrification. Parmi ces deux environnements, celui contenant de l'oxygène a démontré un meilleur potentiel de biodégradation quoique des pertes de HAP dues à la volatisation ont été notées, un problème qui n'existe pas dans les conditions de dénitrification. Les deux autres conditions étudiées, soit en sulfato-réduction et méthanogénique n'ont pas démontrées de résultats significatifs de biodégradation des composés HAP. De plus, la présente étude traite de la faisabilité du développement d'un bioréacteur dédié au traitement des sols contaminés sous des conditions de dénitrification, à partir d'un modèle réduit de bioréacteur ayant une capacité totale de quatre litres. Les résultats ont montré qu'un agrandissement de l'ordre de huit fois de l'échelle du bioréacteur, tout en réduisant de manière substantielle la puissance de mélange dans la suspension de sol ont produit peu d'effet sur les taux de biodégradation des isomères de HAP étudiés.

ACKNOWLEDGEMENTS

The author is most and foremost indebted to Professor Roland Leduc for his invaluable guidance and support throughout the study and without whose help the author would not have had the opportunity to conduct this research.

Also, the author acknowledges greatly the constant help provided to him by Dr. Tibor Cseh, at the Biotechnology Research Institute, in analytical chemistry and research techniques.

Furthermore, the author's deepest appreciation goes to the Environmental Engineering Group at the Biotechnology Research Institute particularly Dr. Réjean Samson, the Group Leader, for giving the author the opportunity to conduct this research at their facility and Dr. Jalal Hawari for his frequent valuable consultations.

Finally, the author is grateful to Ms. Chantale Beaulieu for technical assistance and Ms. Sylvie Sanschagrin for technical assistance and for translating the abstract.

The author was supported by an NSERC (Natioal Sciences and Engineering Research Council of Canada) operating grant.

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CHAPTER 1

1.1 FOREWORD

This report provides an account on the research project carried out to investigate the biodegradation of polycyclic aromatic hydrocarbons in soil slurries. Prior to the writing of this report, the material appearing in chapters 3 and 4, has been already written and prepared in the form of two manuscripts to be submitted for publication in scientific journals. So far, one of them found its way to the pages of a journal (Applied Microbiology and Biotechnology; see Appendix E) and the other one is awaiting to follow suit. However, the fact that this research work could be divided into two self-contained manuscripts favoured incorporating them as such in this report to emphasize the central issue studied in each of them and to make use of the fact that they could be read independently of each other.

Having explained the reasons behind the overall format of this report, now a summary of each of the chapters to follow and their order is given.

Chapter 2: The introduction, literature review and objectives of study are included here. The literature review provides a background to the problem of soil contamination with PAHs, the role of biological processes in determining their fate and in the development of a treatment technology. Special attention is given to three factors affecting the biodegradation of PAHs: the redox environment, PAH bioavailability and

the co-presence of other contaminants. A review of the important studies dealing with these issues is presented in this section. Also, the literature review establishes the grounds for the objectives of the study.

Chapter 3: This chapter consists of a study which investigates the biodegradation and mineralization of ^{14}C-naphthalene in a soil slurry and under denitrifying conditions in relation to naphthalene initial concentration and the adsorptive-desorptive properties of the soil-contaminant complex. This study has been published (Al-Bashir et al., 1990a) and some of its results were presented elsewhere (Al-Bashir et al., 1990b, Leduc et al., 1990).

Chapter 4: Here, a biodegradation study is provided which investigates the role and significance of four redox environments (aerobic, denitrifying, sulfate-reducing and methanogenic) in the biodegradation of four PAH compounds: acenaphthene, acenaphthylene, fluorene and anthracene. Also, in this study, the performance of a bench-scale bioreactor is evaluated. Some of the results obtained from this study were presented at a conference (Leduc et al. 1990).

Chapter 5: The important findings and the main conclusions of this study are stated here. Also, comments are made on the analytical techniques adopted in performing the experiments and some suggestions are put forward for further research in the field.

Also, four appendices are attached to this study and these are:

Appendix A presents the tables of results obtained in chapter 3 in their numerical form;

Appendix B is constituted of three figures, which show the Langmuir, Freundlich and B.E.T. adsorption functions fitted to naphthalene

adsorption data in chapter 3;

Appendix C presents tables of the results appearing in Fig.4.2, in chapter 4;

Appendix D outlines the procedure utilized to perform the statistical analysis that appears in chapter 4.

Appendix E contains a reprint of the published manuscript.

Finally, it should be noted that, because of the special format of the thesis, references cited in a given chapter appear listed at the end of that chapter.

1.2. STATEMENT REGARDING AUTHORSHIP

The original proposal for this research work was agreed upon by Dr. Réjean Samson (Biotechnology Research Institute, BRI) and Dr. Roland Leduc (McGill University). The objectives of the study were stated as follows:

1. Compare the performance of a solid-state bioreactor to the standard erlenmeyer techniques for the detoxification of PAHs. Naphthalene, acenaphthene and a sample from a contaminated site will be used as substrates;

2. Evaluate the rate of biodegradation as a function of major operational variables such as water content and redox conditions;

3. Evaluate some kinetic parameters based on the reduction in PAH concentration, production of metabolites and increase in biomass.

The research work was carried out at the BRI and using the technical facilities available there. Also, the prototype of the bioreactor was

developed by the BRI research staff. The installation of the bioreactor and management of troubleshootings were undertaken by Dr. Tibor Cseh who also assisted the author in developing the method of analysis and in deciding on the analytical techniques to be used.

Drs. Roland Leduc and Réjean Samson contributed to the writing of and revised the final manuscript in preparation for it to be published.

1.3. REFERENCES

1. **Al-Bashir, B., T. Cseh, R. Leduc and R. Samson.** 1990a. Effect of soil/contaminant interactions on the biodegradation of naphthalene in flooded soil under denitrifying conditions. Appl. Microbiol. Biotechnol., 34: 414-419.

2. **Al-Bashir, B., T. Cseh, R. Leduc and R. Samson.** 1990b. Biodegradation of [^{14}C]naphthalene in flooded soil under denitrification conditions. Paper presented at the 6th Eastern Region Conference, ACRPEM/CAWPRC, 1st November, Montreal.

3. **Leduc, R., B. Al-Bashir, T. Cseh and R. Samson.** 1990. Biodegradation of polycyclic aromatic hydrocarbons in soil/water systems. Poster presented at the 15th Biennial Conference of IAWPRC, Kyoto: 187-189.

CHAPTER 2

2.1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants that occur in soil, water bodies and air. Due to their genotoxic effects and high bioaccumulation potential, PAHs are receiving considerable attention in the field of environmental research.

A major part of PAH input into the environment ultimately reaches the soil where their fate and persistence are largely determined by biological activities. The rate of biodegradation of PAHs in soils is a function of several physico-chemical factors of the soil-contaminant complex. Under naturally occurring conditions, only a few of these factors constitute the rate limiting step of the reaction. Hence it is of vital importance for research in the field to identify these limiting factors as well as to investigate the possibility of manipulating them as a means to explore new and innovative treatment techniques.

This study investigates the biodegradation of PAHs in soil/water systems with respect to two important factors: redox environment and substrate bioavailability. Both of these factors are believed to have a significant effect on the rate of PAH disappearance from the natural soil environment. Also, this study explores the feasibility of bioremediating PAH-contaminated soil slurries in a bioreactor system.

2.2. LITERATURE REVIEW

2.2.1. SOIL CONTAMINATION

Prior to the 1980s, soil contamination was generally viewed as part of surface and ground water contamination problems and, as a result, its study has lagged considerably behind that of water. This is partly due to the fact that, unlike water or air, soil is relatively immobile and is not commonly ingested by human beings. Furthermore, only simple and low cost measures were initially taken, such as restricting access to contaminated sites or covering soil, in order to reduce the risk of an accidental direct exposure to soil contaminants (Bauman 1989).

However, concerns over soil contamination have significantly increased in the last two decades following the discovery of a large number of industrial sites all over the world, with high contamination levels and serious environmental and health risks. Haines (1988) reported that as much as 0.2% of the total land area of five countries (Denmark, Netherlands, Germany, UK and France) is contaminated industrial land which requires immediate treatment either because it presents a health risk or because it cannot be put to human use without prior decontamination.

Also, the fact that soil could act as a source of contaminant release into other media (water or air; see Fig. 2.1), has prompted governments, environmental bodies and researchers to address the soil environment separately in order to seek new techniques for treating contaminated soils as a further solution to water contamination problems (Bauman

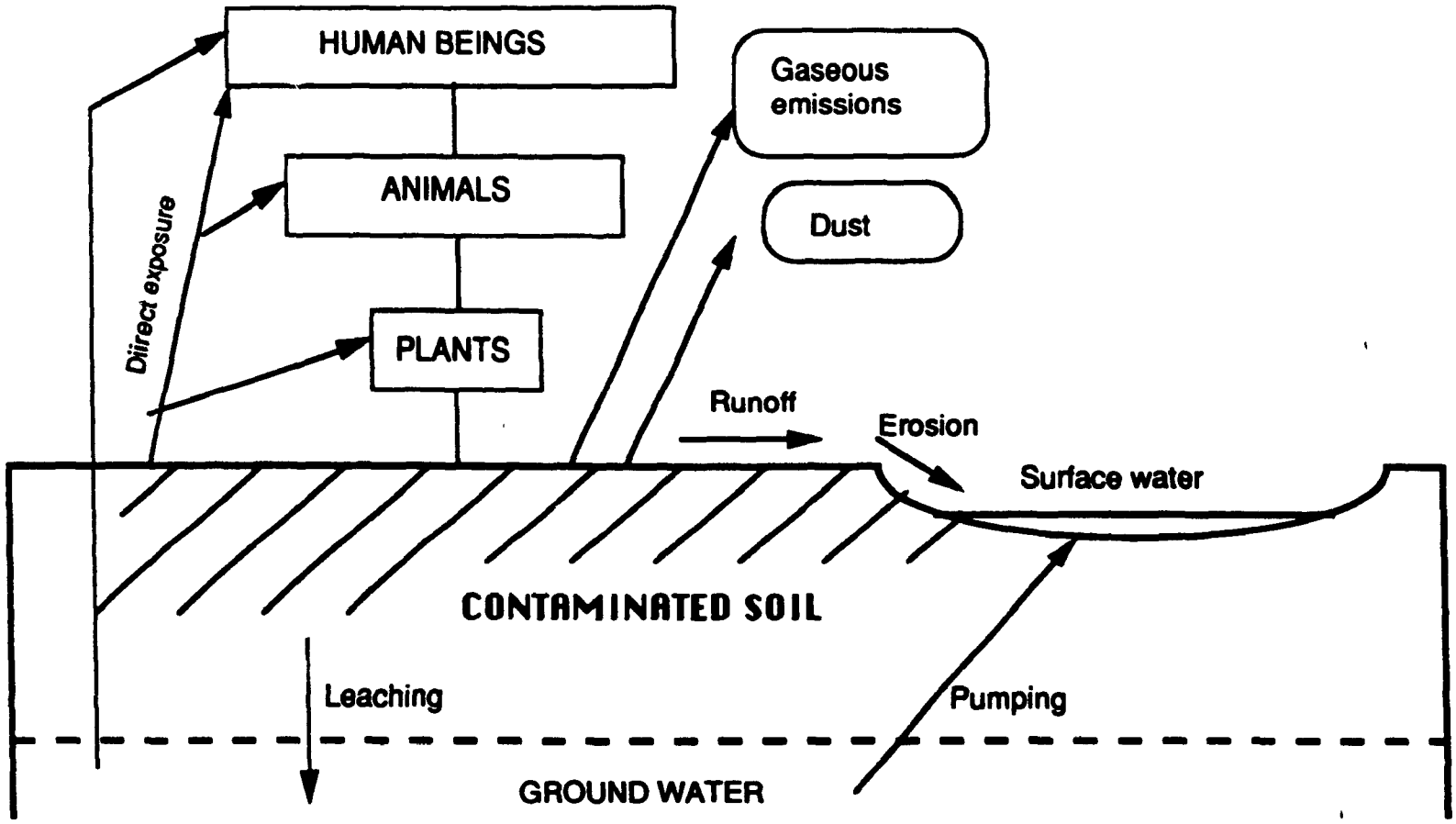


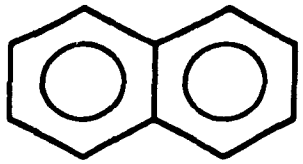
Figure 2.1. Possible contamination paths from a contaminated soil (adapted fromGoetz 1988).

1989, Higgins and Byers 1989).

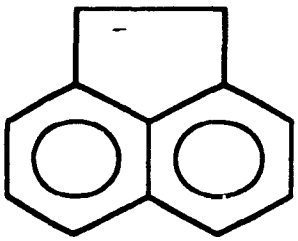
2.2.2. POLYCYCLIC AROMATIC HYDROCARBONS AS SOIL CONTAMINANTS

2.2.2.1. Structure and properties

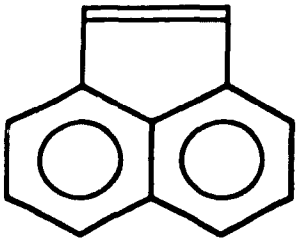
PAHs are ubiquitous environmental contaminants that exist in air, water bodies and soils. These are a class of organic compounds that consist of two or more fuzed benzene rings, arranged in linear, angular and cluster arrangements (see Fig. 2.2). They could occur as unsubstituted or substituted compounds thus forming a larger group termed polynuclear aromatic hydrocarbons. In a substituted PAH compound, a carbon atom in the benzene ring is replaced by nitrogen, sulfur or oxygen to form a heterocyclic aromatic compound (Bulman et al. 1985). PAHs have been found to exhibit mutagenic and/or carcinogenic effects and they form the largest class of chemical carcinogens known today (Heitkamp et al. 1987, Lee et al. 1981, Withey 1986). As a result, several PAHs have been listed by the US Environmental Protection Agency as priority pollutants to be monitored in the environment (Richards and Shieh 1986). Also, PAHs possess hydrophobic and lipophilic properties which accounts for their bioaccumulation potential in soils and living matter (Bulman et al. 1985). In general, as the molecular weight increases, solubility decreases and the K_{ow} (octanol-water coefficient) increases, thus increasing the bioaccumulation potential of the PAH compound (see Table 2.1).



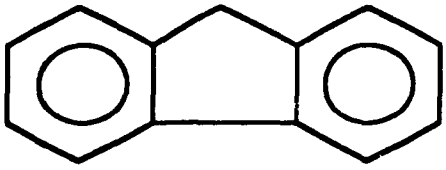
NAPHTHALENE



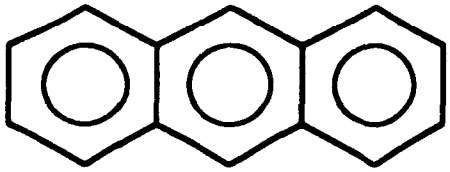
ACENAPHTHENE



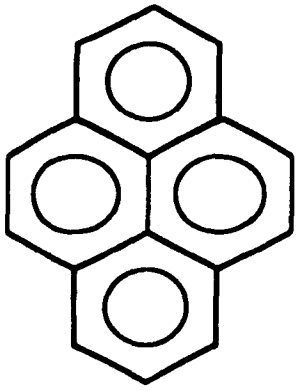
ACENAPHTHYLENE



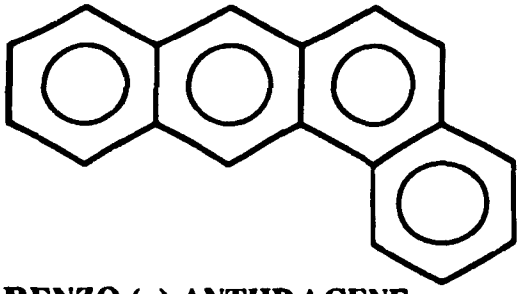
FLUORENE



ANTHRACENE



PYRENE



BENZO (a) ANTHRACENE

Figure 2.2. Chemical structure of some PAH compounds.

Table 2.1. PAHs listed as priority pollutants by the U. S. Environmental Protection Agency: their structure and physical-chemical properties.

Compound[1]	Number of benzene rings[2]	Molecular formula (weight)	Solubility[2] (mg/l)	log K[2,3]
Acenaphthene	3	$C_{12}H_{10}$ (154)	3.47	4.33
Acenaphthylene	3	$C_{12}H_8$ (152)	3.93	4.07
Anthracene	3	$C_{14}H_{10}$ (178)	0.07	4.45
Benzo(a)anthracene	4	$C_{17}H_{12}$ (216)	0.014	5.61
Benzo(b)fluoranthene	5	$C_{20}H_{12}$ (252)	0.0012	6.57
Benzo(k)fluoranthene	5	$C_{20}H_{12}$ (252)	0.00055	6.84
Benzo(g,h,i)perylene	6	$C_{22}H_{12}$ (276)	0.00026	7.23
Benzo(a)pyrene	5	$C_{20}H_{12}$ (252)	0.0038	6.04
Chrysene	4	$C_{18}H_{12}$ (228)	0.002	5.61
Dibenzo(a,h)anthracene	5	$C_{22}H_{14}$ (278)	0.0005	5.97
Fluoranthene	4	$C_{16}H_{10}$ (202)	0.26	5.33
Fluorene	3	$C_{13}H_{10}$ (166)	1.98	4.18
Indeno(1,2,3,c,d)pyrene	6	$C_{22}H_{12}$ (276)	0.062	7.66
Naphthalene	2	$C_{10}H_8$ (128)	30	3.37
Phenanthrene	3	$C_{14}H_{10}$ (178)	1.29	4.46
Pyrene	4	$C_{16}H_{10}$ (202)	0.14	7.66

1 Dzombak and Luthy (1984)
2 Sims and Overcash (1983)
3 Log K = logarithm of octanol:water partition coefficient

2.2.2.2. Sources and sinks

Production of PAHs is a result of natural and anthropogenic processes. They are produced naturally through forest and grass lot fires and biochemical synthesis in bacteria. However, man-made activities are by far the major source of PAH production. This includes fossil fuel combustion such as coal, wood and oil, petroleum refining and coke production. Soil is contaminated with PAHs directly through oil spillage and industrial waste discharge or indirectly through particle deposition and precipitation of PAH atmospheric emissions (Lee et al. 1981, Sims and Overcash 1983).

However, a natural balance was maintained between PAH production and destruction prior to the twentieth century. The degradation processes managed to keep PAH concentrations in soils at low levels, considered now as background levels. However, as a result of the increased industrial activity, PAH inputs into the terrestrial environment have increased dramatically and subsequently the natural degradation processes have lagged behind production (Jones 1988).

2.2.3. BIODEGRADATION PROCESSES

The disappearance of PAHs from soils is governed by several biotic and abiotic processes. The major processes through which PAHs disappear are volatilisation, photodecomposition, microbial biodegradation and leaching (Jones et al. 1989).

While photodecomposition, leaching and volatilisation are not considered as significant pathways for removal of sorbed PAHs from the

soil environment, biological transformation is believed to be the principal process affecting the PAH fraction of hazardous wastes in soil systems (Sims and Overcash 1983).

Several PAH compounds are amenable to microbial degradation, especially those with two or three benzene rings. A wide variety of microorganisms including bacteria (Heitkamp and Cerniglia 1988, Kiyohara and Nagao 1978, Schocken and Gibson 1984), fungi (Bumpus et al. 1985, Bumpus 1989, Cerniglia et al. 1989) and algae (Cerniglia and Heitkamp 1989) have been identified and isolated as being capable of bioconverting PAHs. Also, some possible metabolic pathways have been proposed (Gibson 1971, Rogoff 1967). However, PAHs with a higher number of benzene rings have been found to be recalcitrant. A recalcitrant molecule was defined by Perry (1979) "as one that is resistant to biological degradation but that does disappear from the environment at a slow rate, this persistance is due to the inability of microorganisms to manage such molecules readily, or in many cases virtually non-biodegradable".

Several factors influence the rate of PAH biodegradation or, for that matter, their degree of recalcitrance and these have been summarized by Cerniglia (1984) as follows:

1. PAH-related: type, concentration, ring number, solubility, lipophility;
2. Environment-related: sediment type, organic content, nutrient status, salinity, soil-to-water ratio, temperature, pH, redox potential;
3. Bacteria-related: types, population, distribution, previous exposure.

A considerable amount of research has been conducted on the

significance of these factors and their impact on the degradation process and substantial scientific knowledge has been acquired in this field. However, their is a gap existing between our knowledge on PAH biodegradation and translating it into a practical application in the form of an innovative treatment method (Atlas 1984). Very few studies have aimed at bridging such a gap and the field requires further research into this subject.

Among the several possible techniques that have been suggested for treating contaminated soils is the use of bioreactor systems in which biological processes act as the degradation mechanism (see Table 2.2). The several advantages that are normally associated with biological activity, such as effective detoxification, high waste volume reduction, low operation costs, versatility, wide applicability and safe end products or residues make the development of bioreactors a favorable alternative to physical-chemical treatment methods (De Kreuk 1986).

Pertinent to the development of bioreactors is the investigation into two important mass-transfer mechanisms which are bound to exhibit substantial influence on the treatment process (Bachmann and Zehnder 1988, De Kreuk and Annokkee 1988). As indicated by Fig. 2.3, the two mechanisms are the transfer of oxygen from the gaseous to the liquid phase and the transfer of substrates from the solid to the liquid phase of the soil system. The first of these mechanisms determines the oxygen availability to the biological reaction and hence the redox environment, and the second mechanism controls the availability of the contaminant to the microbial population.

For the remainder of this chapter, the major studies dealing with these two issues will be reviewed. Also, since the co-presence of other

TABLE 2.2. REVIEW OF REMEDIAL TECHNIQUES (Hoogendoorn 1986)

TREATMENT AFTER EXCAVATION	
THERMAL TREATMENT	steam stripping evaporation by thermal treatment (T = ± 300 °C) evaporation by thermal treatment (T = ± 700 °C) incineration (T > 800 °C)
EXTRACTION	aqueous solution organic solvent flotation
MICROBIAL TREATMENT	landfarming composting bio-reactor system
IN SITU TREATMENT	
THERMAL TREATMENT	steam stripping
EXTRACTION	aqueous solution
MICROBIAL TREATMENT	landfarming bio-extraction
ISOLATION AFTER EXCAVATION	
Packing in drums of containers (Temporary) storage Physical-chemical treatment (immobilization)	
IN SITU ISOLATION	
Capping and/or vertical barriers Horizontal barriers in the subsoil Combination of capping, barriers and geohydrological methods	

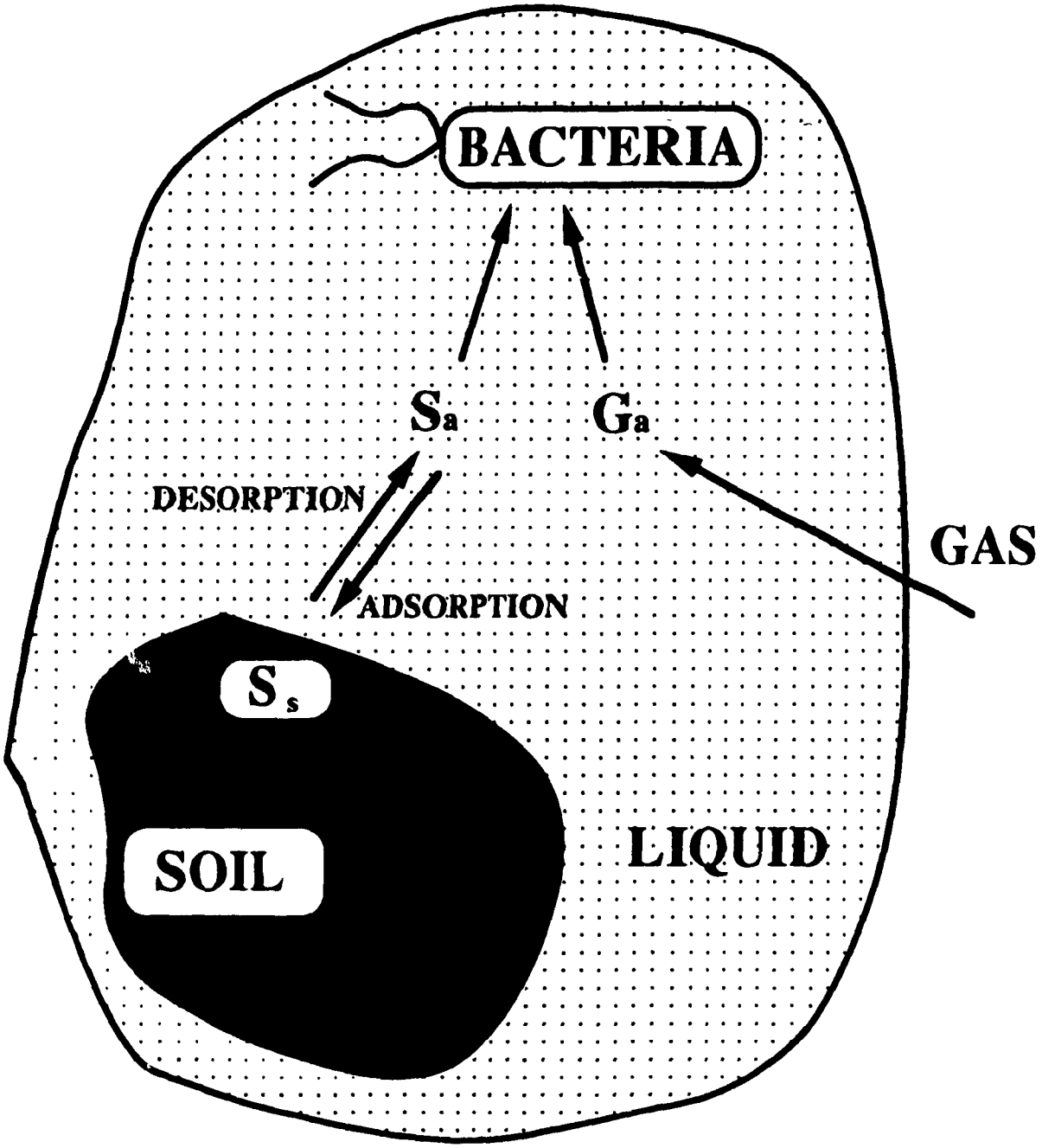


Figure 2.3. Mass transfer considerations in the biodegradation of soil contaminants (adapted from McCarty 1988).

$S_{s,a}$: substrate in solid and aqueous phase, respectively;
G_a : gas in aqueous phase.

organic contaminants have profound effects on PAH biodegradation, therefore it becomes necessary to provide an account of these effects.

2.2.4. THE REDOX ENVIRONMENT

The influence of oxygen and, in the case of its absence, other electron acceptors on the biodegradation of PAH compounds has been the concern of several researchers.

2.2.4.1. Aerobic environment

Bauer and Capone (1985) investigated the effect of oxygen on the biodegradation of naphthalene and anthracene by bacteria in intertidal marine sediments. A variation in the oxygen concentration in the head space of the incubated samples resulted in significant changes in the mineralization rates of the PAH compounds. For naphthalene, a maximum mineralization rate of 3.8%/day was noted at 40% oxygen concentration and a maximum mineralization of 33.4%, over a period of 14 days, was noted at 10% concentration. Anthracene maximum mineralization rate and percent recovered occurred at 40% oxygen concentration. In all the cases where acclimated, unacclimated or anoxic sediments had been used, no naphthalene or anthracene was mineralized at 0% oxygen concentration.

Also, the effect of oxygen bioavailability on the biodegradation of two hydrocarbons in salt marsh sediments was studied by Hambrick et al. (1980). Here, the oxidation-reduction potential was used as a measure of oxygen availability. Both compounds, octadecane and naphthalene, exhibited significantly higher mineralization rates under oxidizing conditions than under reducing ones. The total amounts of naphthalene

recovered as CO_2 increased consistently from near zero at -250 mV up to 63.3% at +250 mV. However, the consistent increase in the hydrocarbon mineralization rates with the increase in redox potential did not hold at relatively higher redox conditions. A drop in the mineralization rate was observed as a result of a further increase in the redox potential from 250 to 480 mV.

These above studies and others (Bouwer and McCarty, 1983) have suggested that molecular oxygen is a basic requirement for the cleavage of the aromatic ring of PAH compounds. Perry (1979) reported that initial oxidative microbial attack on a PAH compound is an aerobic one where the aerobic environment is only required for the insertion of oxygen into the hydrocarbon molecule but not for subsequent utilization of the oxygenated intermediates. Cerniglia (1984) proposed a general model for the metabolic pathway along which PAHs are degraded aerobically. This involves an initial hydroxylation reaction in which both atoms of the molecular oxygen are incorporated into a benzene ring to create dihydrodiols with a cis configuration. The enzyme catalyzing the oxygen fixing reaction is identified as dioxygenase. In turn, the cis-dihydrodiols undergo a further enzymatic reaction to produce catechols. The latter could act as substrates for ring cleavage enzymes (see Fig. 2.4).

2.2.4.2. Denitrifying environment

Under denitrifying conditions, Bouwer and McCarty (1983) reported that naphthalene, along with other aromatic compounds, were not utilized by the microbial population. Their biodegradation study was conducted in a general growth liquid medium inoculated with primary sewage influent. These researchers concluded that, under anoxic conditions, the basic

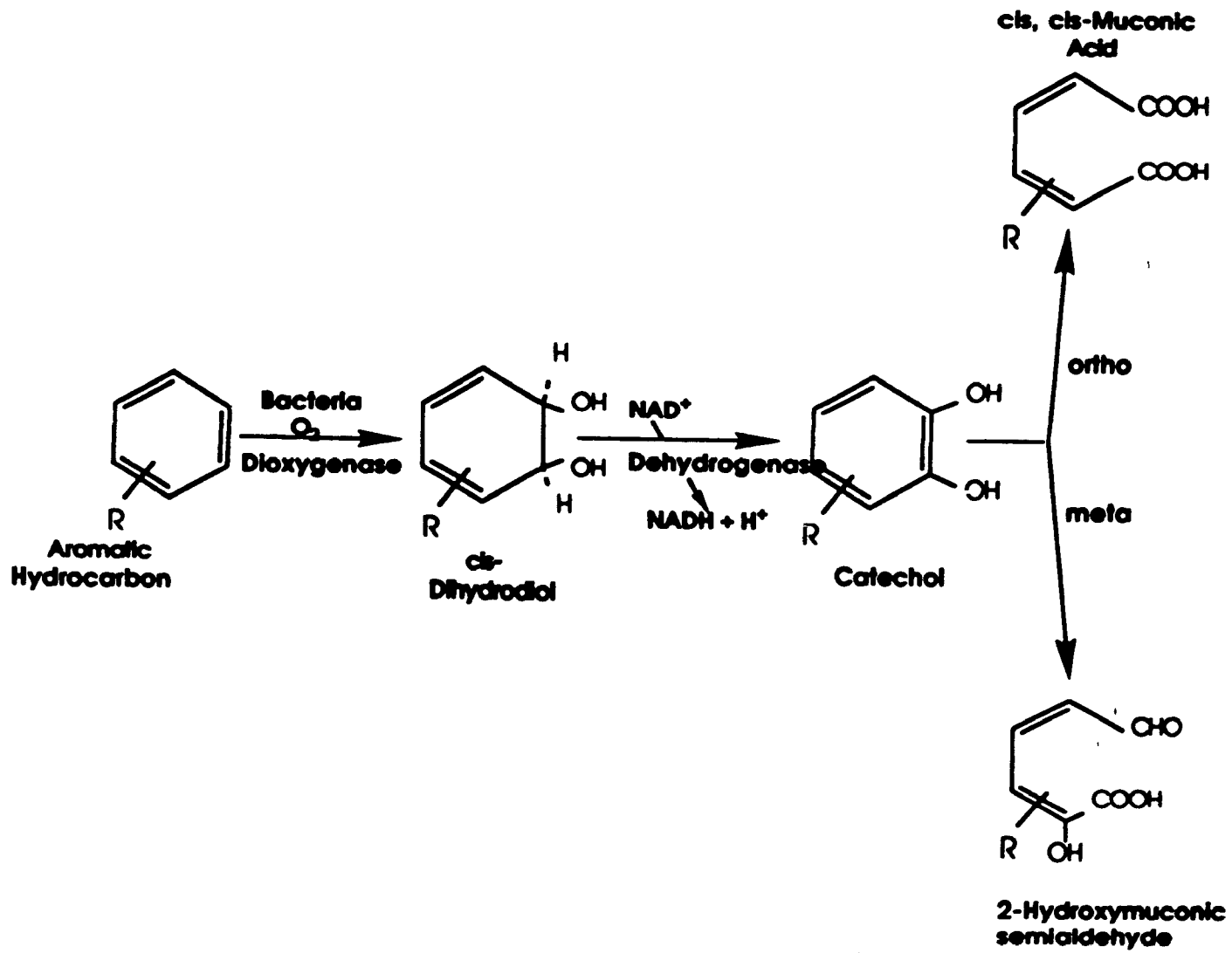


Figure 2.4. Metabolic pathway for bacterial oxic degradation of PAHs (Cerniglia 1984).

requirement for the aromatic ring cleavage is the presence of oxygen on the ring or ring substituent.

Mihelcic and Luthy (1988a), using a soil/water system as the growth medium, studied the biodegradation of naphthol, naphthalene and acenaphthene under various redox conditions. Their results showed that all compounds biodegraded under denitrifying conditions from initial concentrations of 8, 7 and 0.4 mg/l to non detectable levels in 16, 45 and 40 days respectively. Under an anaerobic environment, only naphthol was degraded to non detectable levels, while naphthalene and acenaphthene were recalcitrant over 50 and 70-day periods, respectively.

The effect of nitrate availability on PAH degradation rates in a soil slurry was further investigated by Mihelcic and Luthy (1988b). Under nitrate excess conditions, both naphthalene and acenaphthene were biodegraded to non-detectable levels from initial aqueous phase concentrations of 0.6 mg/l and 4.4 mg/l respectively in less than 9 weeks. However, under nitrate-limiting conditions, both compounds were resistant to microbial biodegradation.

In the two studies by Mihelcic and Luthy (1988a and b), the initial PAH concentration and the soil-to-water ratio were relatively low, with the later being 2-4%. In this case, the soil particles acted more as an inoculum rather than as the contaminated medium.

2.2.4.3. Sulfate-reducing and methanogenic environments

So far, no study has reported biodegradation of PAHs under sulfate-reducing nor methanogenic conditions. However, this does not mean that the benzene ring is not degradable in those two environments. On the contrary, there is strong evidence in the literature to show otherwise.

Grbic-Galic and Vogel (1987) reported that toluene and benzene were biodegraded by a mixed methanogenic culture obtained from an enriched ferulic acid-degrading sewage sludge, with CO_2 acting as the only electron acceptor. Also, ZoBell and Prokop (1966) found that oil was biodegraded in anaerobic sediments when sulfate was present as the principal electron acceptor. Moreover, several studies have shown the ability of microorganisms from marine sediments and soils to biodegrade homocyclic (e.g. benzoate) and heterocyclic (e.g. nicotinate) aromatic compounds under denitrifying, sulfate-reducing and methanogenic conditions. In the case of benzoid, a common reductive pathway has been suggested for all anaerobic microorganisms including denitrifiers, sulfate-reducers and fermenters. This metabolic pathway consists of an initial ring hydrogenation step followed by a reaction sequence of ring hydration and ring cleavage (Berry et al. 1987).

2.2.5. BIOAVAILABILITY OF PAHs

The factors governing the bioavailability of the PAH compounds to the microbial population and the possible effects they may have on the biodegradation rate of PAHs have also received the attention of a number of researchers. Among the several factors considered are: physical state, dissolution rate, solubility, molecular weight, adsorption, the effect of surfactants and initial concentration.

2.2.5.1. Physical state

The effect of the physical state of the organic compound on its uptake rate by bacteria was investigated by Wodzinski and Bertolini

(1972). A comparison was sought between the degradation rate of naphthalene by bacteria in a mineral medium containing solely dissolved naphthalene and its degradation rate in a medium containing a mixture of dissolved and solid naphthalene. The results showed that bacterial growth rate was independent of the amount of solid naphthalene present and this rate was the same as that obtained in the medium containing only dissolved naphthalene. Hence, it was concluded that bacteria utilize naphthalene in the dissolved form and cannot do so by extracting naphthalene directly from solid particles.

To generalize this finding and to investigate if a less soluble compound could induce bacteria to utilize it directly from the solid phase, Wodzinski and Coyle (1974) conducted a similar experiment, but with phenanthrene substituting naphthalene as the carbon source for bacterial growth. The results were similar to those obtained from naphthalene degradation and confirmed the proposition that these compounds are only available for bacterial uptake when present in the dissolved state.

2.2.5.2. Rate of dissolution

Since PAHs are slightly water soluble and they are utilized only when present in the dissolved state, the rate of dissolution from the solid phase is expected to affect PAH biodegradation rates. Thomas et al. (1986) addressed this issue and found that in an uninoculated salt solution the rates of dissolution of naphthalene and chlorobiphenyl were inversely proportional to the solid particle size, i.e. the rate of dissolution increases with the increase in the surface area of the compound. Also, these researchers reported that in the inoculated sample the maximum concentration of the compounds attained as well as the final cell count

were inversely proportional to the particles' size.

2.2.5.3. Solubility

Solubility is another factor that affects the bioavailability of PAHs. Wodzinski and Johnson (1968) noted that the growth rate of cultures growing on aromatic hydrocarbons varied proportionally with solubility. More importantly, none of the cultures grew on hydrocarbons that are more insoluble than the one in which the cultures were isolated. The solubility and the generation time for each of the PAHs studied were as follows:

naphthalene: 98 μM, 2.0 hr;

phenanthrene: 9.0 μM, 19 hr;

anthracene 0.45 μM, 29 hrs;

naphthalene: 0.0066 μM, no growth.

Herbes and Schwall (1978) studied the transformation of naphthalene (2 rings), anthracene (3 rings), benz(a)pyrene (5 rings) and benz(a)anthracene (6 rings) in stream sediment slurries. The transformation rates for these compounds were determined and their order was as follows:

naphthalene > anthracene > benz(a)pyrene > benz(a)anthracene.

The authors concluded that the initial degradation rate is inversely related to the number of benzene rings present.

Bossert and Bartha (1986) studied the biodegradation of 10 PAH compounds in soil in the presence of 1-phenyldecane as a primary substrate. They observed that the transformation of PAHs was inversely affected by the number of aromatic rings and correlated positively with

water solubility.

The solubilities of the PAH compounds have been found to correlate with the number of benzene rings or the molecular weight. However the cluster arrangement of the rings could also affect solubility (Lee et al 1981). Therefore, in the several studies which have reported that the biodegradation rates of PAHs decrease with an increase in the number of benzene rings or the molecular weight, it is not possible to say if this decrease is attributable solely to solubility considerations or if some other chemical properties come into effect.

2.2.5.4. Adsorption by solid matter

Studies published so far have shown that PAH bioavailability to a microbial population grown in a liquid media could constitute a limiting factor to the biodegradation process. Also, in a soil/water system, substrate bioavailability can restrict the biodegradation process even to a greater extent. This is so, because centrifugable solids in natural waters accumulate PAHs several thousand-fold over water levels (Herbes 1976).

To investigate how adsorption restricts the availability of contaminants to bacteria, Ogram et al. (1985), working with 2,4-Dichlorophenox acetic acid (2,4-D), suggested three models to describe the reaction process mechanistically:

Model 1: Only 2,4-D in solution is available for biodegradation and only by bacteria in solution. Sorbed 2,4-D is completely protected from biodegradation and sorbed bacteria are incapable of degrading sorbed or desorbed 2,4-D;

Model 2: Bacteria in solution utilize only 2,4-D in solution and sorbed bacteria utilize only sorbed 2,4-D;

Model 3: 2,4-D in solution is utilized by both bacteria in solution and sorbed bacteria. Sorbed 2,4-D is completely unavailable for bacterial uptake.

The data obtained from the 2,4-D mineralization and sorption experiments agreed fairly well with model 3 while models 1 and 2 did not describe the results accurately. The researchers proposed two possible reasons for the unavailability of the organic compound to bacterial activity: either 2,4-D is adsorbed deeply in the soil particles such that it is inaccessible to bacteria or simply bacteria are unable to uptake 2,4-D in the sorbed form.

The work by Ogram et al. (1985) suggests that the partitioning of highly insoluble hydrocarbon compounds between the solid and liquid phases of the soil/water system affects the biotic reaction rate and its extent. Also, the reversibility of the sorption process and the rate at which desorption takes place are expected to have a significant influence on the biodegradation process (see sections 3.3.4 and 3.4).

The partitioning of PAHs between the solid and liquid phases of a soil/water system has been studied by a number of researchers with the objective of determining its effect on the migration of these contaminants through the vadoze zone and the potential threat it constitutes to groundwaters. However, so far there is no study which investigates the effect of partitioning on PAH biodegradation.

Dzombak and Luthy (1984) reported that the Freundlich adsorption coefficient (K) is directly proportional to the organic content of soil and that its normalized value with respect to percent organic content (K_{oc}) is independent of other soil properties. Also, for a particular PAH compound, the K_{oc} value has been found to correlate highly with the compound K_{ow}

(octanol-water partition coefficient). The following expression has been derived to relate K_{oc} to K_{ow} for PAH compounds (Means et al. 1980):

$$\log K_{oc} = \log K_{ow} - 0.317$$

2.2.5.5. Effect of surface active agents

Researchers have investigated the ability of surfactants to increase the solubility of PAHs in soil/water systems. Due to the presence of polar and non-polar regions on the same molecule, surfactants may reduce the work of adhesion between the hydrophobic compound and soil, thus resulting in the increased desorption of contaminants (Liu et al. 1991, Brickell and Keinath 1991). However, the various studies that have investigated the effect of dispersants on the biodegradation of hydrocarbons have shown increases, decreases and transitory or slight increases in the compounds' biodegradation rates (Leahy and Colwell 1990).

Foght et al. (1989) reported that the use of the disperser heteropolysaccharide (produced from *Acinetobacter calcoaceticus* RAG-1) resulted in a 90% decrease in the biodegradation of the aromatic fraction of crude oil by a mixed population. However, aromatic biodegradation by pure culture was either unaffected or slightly stimulated by the emulsifier.

Mulkins-Phillips and Stewart (1974) investigated the effect of four dispersants on the biodegradation of the n-alkane fraction of crude oil: Corexit 8666, GmlenSea Clean, G. H. Woods Degreaser-Formula 11470 and Sugee 2. Results showed that only Sugee 2, which had the poorest

emulsifying capacity, enhanced n-alkane degradation while the other three retarded biodegradation.

2.2.5.6. Initial concentration

The effect of the initial compound concentration on PAH degradation in stream sediments was examined by Herbes and Schwall (1978) with anthracene acting as a model PAH compound. The results showed that the transformation rate of anthracene is linearly related to its initial concentration when below 1 g/l. Exceeding this concentration resulted in a gradual decrease in the microbial transformation rate. Three reasons were proposed to account for the decrease in the transformation rate:

- saturation of interstitial water with the PAH;
- saturation of the microbial enzymatic pathways;
- toxic inhibition of microbial activity by the PAH compound.

Sims and Overcash (1983) summarized the general trend in PAH degradation as indicated by several studies as follows:

(1) for a given PAH compound the initial rate of transformation increases with increasing initial concentration in soil;

(2) within the class of PAHs, the initial rate of transformation decreases with increasing number of fuzed benzene rings or molecular size (see section 2.5.3).

2.2.5.7. Membrane permeability

Cell membrane permeability has been reported to affect the bioavailability of PAHs to biodegradation. McKenna (1976) compared the oxidation of several PAHs by whole-cell suspensions of *Pseudomonas putida* with that by cell-free extracts. With naphthalene oxidation set at

100%, 9.8% anthracene was oxidized by whole-cell suspension compared to 61.9% oxidized by cell-free preparations. Also, for pyrene, 1.1% was oxidized by whole cell suspension and 81.9% was oxidized by cell-free extracts. It was concluded that cell membrane permeability is a factor in determining the degree of recalcitrance of PAHs. Furthermore, McKenna noted that the initial PAH oxidation rates in cell-free extracts were proportional to their water solubilities.

2.2.6. PRESENCE OF OTHER CONTAMINANTS

PAHs do not exist in soils as single constituents but rather as a complex mixture of a number of PAHs, other organic contaminants and naturally-occurring organic material. The presence of these compounds has a considerable effect on PAH bioavailability and their susceptibility to biodegradation.

The co-occurrence of PAHs and other contaminants in a soil matrix could give rise to one or more of the following mechanisms: co-oxidation and adaptation.

2.2.6.1. Co-oxidation

Co-oxidation is an important mechanism that could account for the loss of recalcitrant PAHs from soil (Heitkamp et al. 1988). Foster (1962) defined co-oxidation in the following manner: "non-growth hydrocarbons are oxidized when present as co-substrates in a medium in which one or more different hydrocarbons are furnished for growth". Also, co-oxidation has been used to describe the process in which microorganisms oxidize the co-substrate without being able to utilize the energy resulting from

the oxidation reaction. Foster (1962) wrote that "the inability to grow at the expense of a particular hydrocarbon is not a consequence only of an organism's inability to attack the substrate; obviously, failure to grow may be due then to its inability to assimilate the oxidation products".

Keck et al. (1989) studied the degradation of 13 PAHs in a fine sandy soil. Four different soil matrix conditions were created such that the PAH compounds were present as:

1. single constituents, incubated singly;
2. a synthetic mixture, applied and incubated together;
3. a mixture of oil refinery waste;
4. a creosote wood preserving waste.

Keck et al. (1989) found that for three-ring compounds the degradation rates were similar in all four matrices. However the degradation rates of four and five ring compounds were significantly higher when present as a complex waste than as single constituents.

2.2.6.2. Adaptation

Adaptation is defined as a change in the microbial community that increases the rate of biodegradation of a test compound as a result of a prior exposure to the test compound (Spain and Van Veld 1983). Evidence for adaptation includes shortening of lag period, changes in biodegradation kinetics or increase in biodegradation rate (Moorman 1990).

Upon exposure to a new substrate, the microbial population can adapt following three mechanisms (Spain et al. 1980):

(i) induction or depression of a specific enzyme not present in the population prior to exposure;

(ii) selection of new metabolic capabilities produced by genetic

changes;

(iii) increase in the number of organisms capable of catalyzing a particular transformation.

Bauer and Capone (1988) investigated the effect of pre-exposure on the degradation of some PAH compounds. They reported that prior exposure to anthracene, phenanthrene, naphthalene and benzene enhanced naphthalene mineralization in marine sediments. Anthracene mineralization was only enhanced by anthracene and benzene pre-exposure. The study concluded that the enhanced degradation of a PAH compound after sediments have been exposed to the same compound is a result of selection and proliferation of a specific microbial population capable of degrading it. However, the enhanced degradation of a PAH compound as a result of sediment pre-exposure to a completely different PAH compound indicates that the proliferated population has either broad specificity for PAHs, common pathways of PAH degradation, or both. In the latter case, the more the compounds are structurally similar, the more similar their oxygenase or metabolic pathways.

2.3. OBJECTIVES OF THE STUDY

Redox conditions and PAH availability to degrading microorganisms are two important factors of significant influence on the degradation process of PAHs in soil/water systems. Besides helping to understand the fate of PAHs in the environment, a better understanding of their effects is necessary for the development of an innovative technology for treating soils contaminated with PAHs.

This study therefore has the following objectives:

1. To examine the effect of PAH (naphthalene) bioavailability on its degradation rate in the soil/water system under a denitrifying environment.

2. To investigate the effect of the redox environment on the biodegradation of four PAH compounds (acenaphthene, acenaphthylene, fluorene, anthracene) in a soil/water system with a relatively high solid content (slurry).

3. To investigate the performance of a bench-scale bioreactor system when treating PAH-contaminated soil slurries.

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CHAPTER 3

Effect of soil/contaminant interactions on the biodegradation of naphthalene in flooded soil under denitrifying conditions

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3.1. SUMMARY

The mineralization of [^{14}C]-labelled naphthalene was studied in pristine and oil-contaminated soil slurry (30% solids) under denitrifying conditions using a range of concentrations from below to above the aqueous phase saturation level. Results from sorption-desorption experiments indicated that naphthalene desorption was highly irreversible and decreased with an increase in the soil organic content, thus influencing the availability for microbial consumption. Under denitrifying conditions, the mineralization of naphthalene to CO_2 occurred in parallel

with the consumption of nitrate and an increase in pH from 7.0 to 8.6. When the initial substrate concentration was 50 ppm (i.e. close to the aqueous phase saturation level), about 90% of the total naphthalene was mineralized within 50 days, and a maximum mineralization rate of 1.3 ppm/day was achieved after a lag period of approx. 18 days. When added at concentrations higher than the aqueous phase saturation level (200 and 500 ppm), similar mineralization rates (1.8 ppm/day) occurred until about 50 ppm of the naphthalene was mineralized. After that the mineralization rates decreased logarithmically to a minimum of 0.24 ppm/day for the rest of the 160 days of the experiments. For both of these higher concentrations, the reaction kinetics were independent of the initial concentration. However, as the concentration of naphthalene in the aqueous phase dropped below the solubility limit, as in the case of the 50 ppm total naphthalene concentration, the mineralization rate became substrate dependent and exhibited higher order kinetics, indicating that desorption of the substrate governs the mineralization rate. Other results showed that pre-exposure of soil to oil contamination did not improve the degradation rates nor reduce the lag periods. This study clearly shows the potential of denitrifying conditions for the biodegradation of low molecular weight PAHs.

3.2. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds that are composed of two or more fused benzene rings, and have been found to exhibit mutagenic and carcinogenic effects (Heitkamp

and Cerniglia 1988; Withey 1986). They are introduced into the environment through natural and anthropogenic processes, such as the incomplete combustion of fossil fuel and organic matter in coke production and petroleum refining (Lee 1981; Sims and Overcash 1983). In the last few decades, contamination with PAHs has received increased attention as a result of the unprecedented levels they have reached in the environment, particularly in soils and sediments (Jones 1988; Yland 1986). Due to their lipophilic properties, PAHs tend to accumulate and persist in soils for a relatively long time (Bulman et al. 1988). One of the PAH's that has been extensively studied is naphthalene, being with its methylated derivatives among the most toxic compounds found in the water soluble fraction of petroleum (Heitkamp et al. 1987). Much work in this field has focused on the mechanisms and the effects of various environmental factors on the biodegradation of naphthalene. Most of these studies related to naphthalene biodegradation under aerobic conditions in which free oxygen acts as the final electron acceptor (Bauer and Capone 1985; Hosler et al. 1988).

Only a few authors have investigated naphthalene biodegradation in a soil/water system under denitrifying conditions. Furthermore, only few research workers have considered the sorption-desorption pattern of a pollutant to the soil matrix as a variable controlling the kinetics of biodegradation. The information available indicates that the dissolution rate of some PAHs, including naphthalene, is an important factor in controlling the rate of biodegradation (Thomas et al. 1986; Stucki and Alexander 1987). Mihelcic and Luthy (1988a and b) showed that the soil desorption kinetics and the reversibility of desorption influences the microbial biodegradation of naphthalene. However these studies were

conducted with concentrations much below the aqueous phase saturation level (reported as 31.7 mg/l at 25 °C, MacKay and Shul 1977. This is of particular importance because at concentrations higher than the aqueous phase saturation level the sorption-desorption pattern of the contaminant is expected to be somewhat different. Furthermore, sites where soil is contaminated with very high concentrations of PAHs are common. Such information can help design better processes for remediation of subsurface soils and groundwaters where denitrifying conditions are present. One study that has investigated naphthalene biodegradation in primary sewage effluent under denitrifying conditions reported no significant biodegradation rates (Bouwer and McCarty 1983). In another study, naphthalene was mineralized to a very low concentration (7 mg/l), in a soil-to-water ratio of 1:50 (Mihelcic and Luthy 1988b).

The objective of this work was to pursue the subject further by studying the biodegradation of a wide range of naphthalene concentrations (50 to 500 ppm) under denitrifying conditions in a flooded system having a high soil-to-water ratio (1:3). Furthermore the study took into consideration the naphthalene sorption-desorption pattern as one of the parameters that possibly influence the biodegradation rate.

3.3. MATERIALS AND METHODS

3.3.1. Soil. Two bulk soil samples were used in this study. First, a pristine soil obtained from the Biotechnology Research Institute land premises, Montreal, Quebec, and second, a weathered oil-contaminated soil sample obtained from the vicinity of a petroleum oil refinery in

Montreal. In both cases, the top 2-5 cm of soil was scraped off and the soil samples were collected at 5-10 cm depth. The latter site has been contaminated for a long period of time (approximately forty years). Both soils were dried at 35°C for 24 hours and large particles removed with a 500 micron mesh sieve. The sieved soils were analysed for total organic content and particle size distribution using the U.S. Department of Agriculture Scheme (Gee and Bauder 1986). These soils were further analysed for extractable oil content by extracting 5 g of soil with benzene in a Soxhlet extractor. The solvent was then evaporated (Büchi Rotavapor RE 120, Laboratoriums-Technik AG, Flawil, Switzerland) until the oil part was brought to dryness. The weight of the oil remaining was then measured. The characteristics of the two soils were as follows: for the pristine soil the content of sand, silt and clay expressed as a percentage of the dry weight was 74, 21 and 5%, respectively, and the organic content was 2.1%. No extractible oil was measured. For the oil-contaminated soil, the sand, silt and clay contents were 78, 20 and 2%, respectively and the organic content 4.3%. In this soil the extractible oil content represented 1.2% of the dry weight.

The two soils tested in both the sorption-desorption and biodegradation experiments were: (1) a soil mixture consisting of 90% pristine soil and 10% weathered oil-contaminated soil (Soil 1) and (2) a 100% weathered oil-contaminated soil (Soil 2).

3.3.2. Mineral medium. A mineral medium was used to flood the soil and to provide an excess of nutrients. This medium was prepared by dissolving the following salts in tap water: KH_2PO_4, 0.170 g/l; K_2HPO_4, 0.430 g/l; $Na_2HPO_4 \cdot 7H_2O$, 0.660 g /l; $FeCl_3 \cdot 6H_2O$, 0.003 g /l; NH_4Cl, 0.034

g/l; $MgSO_4 \cdot 7H_2O$, 0.450g /l; $NaNO_3$, 20 g /l. The final pH of the medium was adjusted to 7.0 (Mihelcic and Luthy 1988a).

3.3.3. Naphthalene solution. Naphthalene was obtained from Aldrich Chemical Inc. (Milwaukee, Wisc., USA) as a scintillation-grade crystalline powder with 99%+ purity. The uniformly radio labelled [^{14}C]naphthalene was obtained from Sigma (St-Louis, Mo., USA) with 98%+ purity and 4.7 mCi/mmol specific activity. A solution with a specific activity of 10000 dpm/mg of naphthalene was prepared by adding the radio labelled [^{14}C]naphthalene to a stock solution. This solution contained 66.7 g /l naphthalene dissolved in HPLC-grade methanol (Caledon Lab. Ltd., Georgetown, Ont. Canada).

3.3.4. Sorption-desorption experiment. This experiment was conducted according to the procedure described by DiToro and Horzempa (1982). Centrifugation vials (16 ml) with Teflon lined screw caps were used for this experiment. They were filled with a slurry made from a mixture of Soil 1 and mineral medium in a 30% (w/v) ratio. The naphthalene concentration in these vials was adjusted to 10, 25, 35, 50, 100, 200 and 500 ppm. Another vial containing 200 ppm of naphthalene was prepared as above, but in this case Soil 2 was used. To verify the soil sorption capacity a control experiment, using the same procedure but without adding soil, was made. All vials were placed on a Wrist-Action® Shaker, Model 75 (Burrell Co., Pittsburgh, Pa., USA) and incubated at 35°C. After 24 hrs of shaking, the vials were centrifuged for 10 min at 2000 g (Centra 4, International Equipment Co, Needham Heights, Mass., USA). To determine the desorbed naphthalene concentration 1 ml of the supernatant

was added to 15 ml scintillation cocktail (ACS, Amersham, Arlington Heights, Ill., USA) and tested in a scintillation counter (Packard Tri-Carb model 4530, Downers Grove, ILL., USA). The rest of the supernatant was discharged and replaced with fresh mineral medium. The vials were returned to the shaker for a further 24 hrs incubation. The procedure of introducing fresh amounts of mineral medium was repeated twice more, such that each time a new equilibrium state was determined.

3.3.5. Biodegradation experiments. The soil samples were incubated in 50-ml glass vials containing 9 g of soil and 21 g of mineral medium. Each vial was equipped with a CO_2 trap containing 0.7 ml of 5 N NaOH in a 1.2 ml glass cup. Each glass cup was supported by a steel rod suspending the cup above the slurry level in the head space of the vial. The soil sample and mineral medium were added to the vial, the NaOH cup was put in place and a known amount of [^{14}C]-labelled naphthalene solution was injected into the soil slurry to obtain the required initial naphthalene concentration in terms of ppm total slurry weight. Each vial was then sealed with a teflon-lined stopper, wrapped in aluminum foil to prevent photodegradation of naphthalene, and then placed in a shaking water bath set at 100 rpm and kept at 35°C. To avoid oxygen contamination, the above procedure was conducted in an anaerobic chamber filled with nitrogen gas. A total of 100 vials was prepared in this manner, covering the four different series of experimental conditions. For each series, 20 vials were prepared using the procedure described above and 5 vials received 500 ppm of $HgCl_2$ to halt bacterial activity (Wolf et al. 1989). These controls were also used to verify the results obtained

from the other experiments. The incubation conditions, in terms of naphthalene initial total concentration and soil type, were, (A) 50 ppm in Soil 1, (B) 200 ppm in Soil 1, (C) 500 ppm in Soil 1 and (D) 200 ppm in Soil 2.

In the case of the 500 ppm initial concentration, spiking was performed in two steps. First, a known amount of non-labelled naphthalene was dissolved in ether in a boiling flask and 10% of the soil sample designated for this experiment was added to the prepared solution. After mixing the contents of the flask vigorously, the organic solvent was evaporated using a Büchi Rotavapor (RE 120, Laboratoriums-Technik AG, Flawil, Switzerland) until the soil fraction was dry. The spiked soil was then mixed thoroughly with the rest of the soil sample to produce a homogeneous mixture. One gram of the resulting soil was extracted with benzene in a Soxhlet apparatus and the naphthalene concentration was determined by HPLC, using a 25 cm long reverse phase column (model RP8, Brownlee Co., Santa Clara, Calif., USA) and a fluorescence detector (model Spectroflow 980, Kratos Co., Ramsey, N.J., USA). The measured concentration of naphthalene was approximately 300 ppm, [^{14}C]naphthalene was then added to reach a final concentration of 500 ppm. The above procedure was followed to avoid the addition of large amounts of methanol. If large amounts were added, higher denitrifying activity would have probably occured and this in turn would have lead to an undesirable increase in the pH of the slurry (see Fig 3.2 in section 3.4).

3.3.6. Sampling and analysis. At given time intervals, two vials (duplicates) were terminated by the addition of 2 ml of concentrated phosphoric acid, by syringe, to halt bacterial activity as well as to

liberate any CO_2 trapped in the soil slurry. The punctured hole in the stopper was sealed with silicon and the vials were returned to the shaker for another 24 hrs. The vials were then connected through a silicon tube to an external CO_2 trap containing a solution of 10N NaOH. The gas in the head space of each vial was bubbled through the solution to recover all CO_2. This procedure was continued until no radioactivity was detected in the trap. To remove any traces of [^{14}C]naphthalene, the NaOH solution in the glass cup was extracted with hexane. The residual solution was then weighed and 0.1 ml was mixed with 15 ml of scintillant. The radioactivity of the trapped CO_2 was quantified using a liquid scintillation counter. Correction for color quenching was applied using an external standard. The nitrate concentrations in the test samples were measured using a HPLC (model SP8100 with integrator model SP4200, Spectra-Physics, San Jose, Calif., USA) equipped with conductivity type detector (model 430, Waters Chromatography Division, Milford, Mass., USA) and anion exchange column (model PRP-X100, Hamilton Co., Reno, Nev., USA).

3.4. RESULTS

Results from the sorption-desorption experiment are shown in Figure 3.1. The saturation concentration of naphthalene in the soil slurry (liquid fraction) was about 50 ppm. When naphthalene was added at concentrations higher than the aqueous phase saturation level the sorption isotherm was bent upwards indicating a substantial reduction in the solubility and a significant naphthalene increase in the soil fraction (Fig. 3.1A). For these high concentrations, naphthalene in the solid phase would

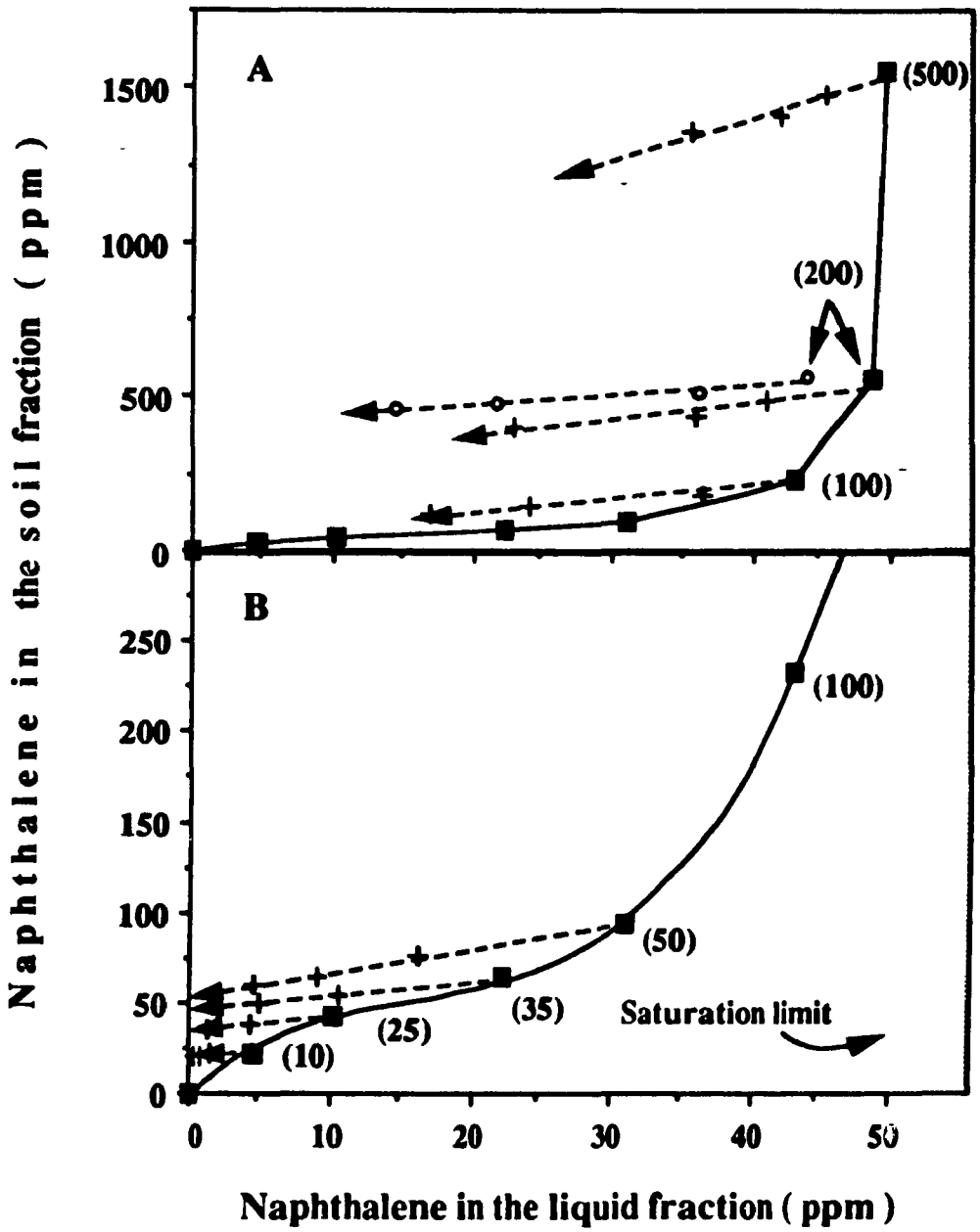

Figure 3.1A, B. Partition of naphthalene between the liquid and the solid phases after addition of a known amount. Numbers in parentheses indicate the concentration of naphthalene injected (ppm). For the desorption experiment, each data point represents the new equilibrium obtained after 24 h of mixing with fresh medium. A: full-scale presentation; B: enlarged area for lower concentrations.

—■— adsorption isotherm for pristine soil slurry (soil 1)

- - +- - desorption isotherm for pristine soil slurry (soil 1)

- - o- - desorption isotherm for oil-contaminated slurry (soil 2)

probably be found both sorbed to the soil and precipitated. At concentrations lower than the saturation level, the sorption isotherm is best described by the trinomial function (r^2 = 0.99).

$$y = -1.68 + 7.15x - 0.35x^2 + 7.33 \cdot 10^{-3}x^3,$$

where x represents the fraction of naphthalene dissolved in the aqueous phase and y the fraction sorbed in the soil (see Fig. 3.1B). The inflexion point for the above function corresponds to an aqueous phase concentration of 16 mg/l. This function provided a better fit than the Langmuir (r^2 = 0.94), Freundlich (r^2 = 0.92) or B.E.T. (r^2 = 0.98) isotherm functions (Green 1984; see Appendix B).

Desorption isotherms (Soils 1 and 2) were established from the seven data points used to construct the sorption isotherm. For each of these points a desorption curve was obtained, suggesting that the desorption is partly irreversible and dependent on the amount of naphthalene previously adsorbed (Fig. 3.1). The control desorption experiment, realized without soil, showed that the equilibrium concentration in the aqueous phase after each of the four desorption sequences always remained close to the saturation limit (50 ppm). This phenomenon illustrates the strong sorptive capacity of soil for naphthalene. It also shows that the solubilisation of naphthalene in solid form (precipitate) never reached the saturation limit when the soil was present. The desorption behavior of the weathered oil-contaminated soil (Soil 2) was studied only for the 200 ppm naphthalene concentration, which was the concentration used later in the mineralization experiments. Comparison between the desorption isotherm for Soils 1 and 2 indicated that the naphthalene partition of the weathered oil-contaminated soil shifted more towards the solid phase and that the concentration found in

the aqueous phase decreased significantly below the saturation concentration. The latter soil exhibited a greater degree of sorption (smaller slope) suggesting that the desorption is influenced by the soil organic content. In fact, the organic content of the weathered oil-contaminated soil was approximately twice the concentration of the pristine soil. The confidence level for the difference in the slopes of the desorption isotherms corresponding to soil 1 and 2 exceeded 99% (Walpole 1982). The results also showed that the methanol introduced in different amounts in the system did not influence the solubility of naphthalene. In a separate experiment, it was found that when the total naphthalene concentration was increased to 500 ppm, the solubility of naphthalene in the aqueous phase increased only by 2 ppm. As a result, the impact of the variations in methanol concentration on the sorption-desorption process was neglected.

Biodegradation of naphthalene under denitrifying conditions was monitored for 160 days (Fig. 3.2). The results obtained from the analysis of trapped $^{14}CO_2$ corresponded to the amounts of naphthalene mineralized and represented the average of two readings (see Appendix A). Analysis of the traps from the control samples showed radioactivity levels similar to those of the background, indicating that mineralization due to abiotic processes is negligible.

Mineralization occurred under all experimental conditions. An adaptation period of approximately 18 days was observed in all cases. When the initial naphthalene concentration was 50 ppm (Fig. 3.2A) the rate of mineralization reached a maximum of 1.3 ppm/day after 18 days. The same rate was observed until about 80% of the naphthalene was mineralized. Following this period, the mineralization rate decreased

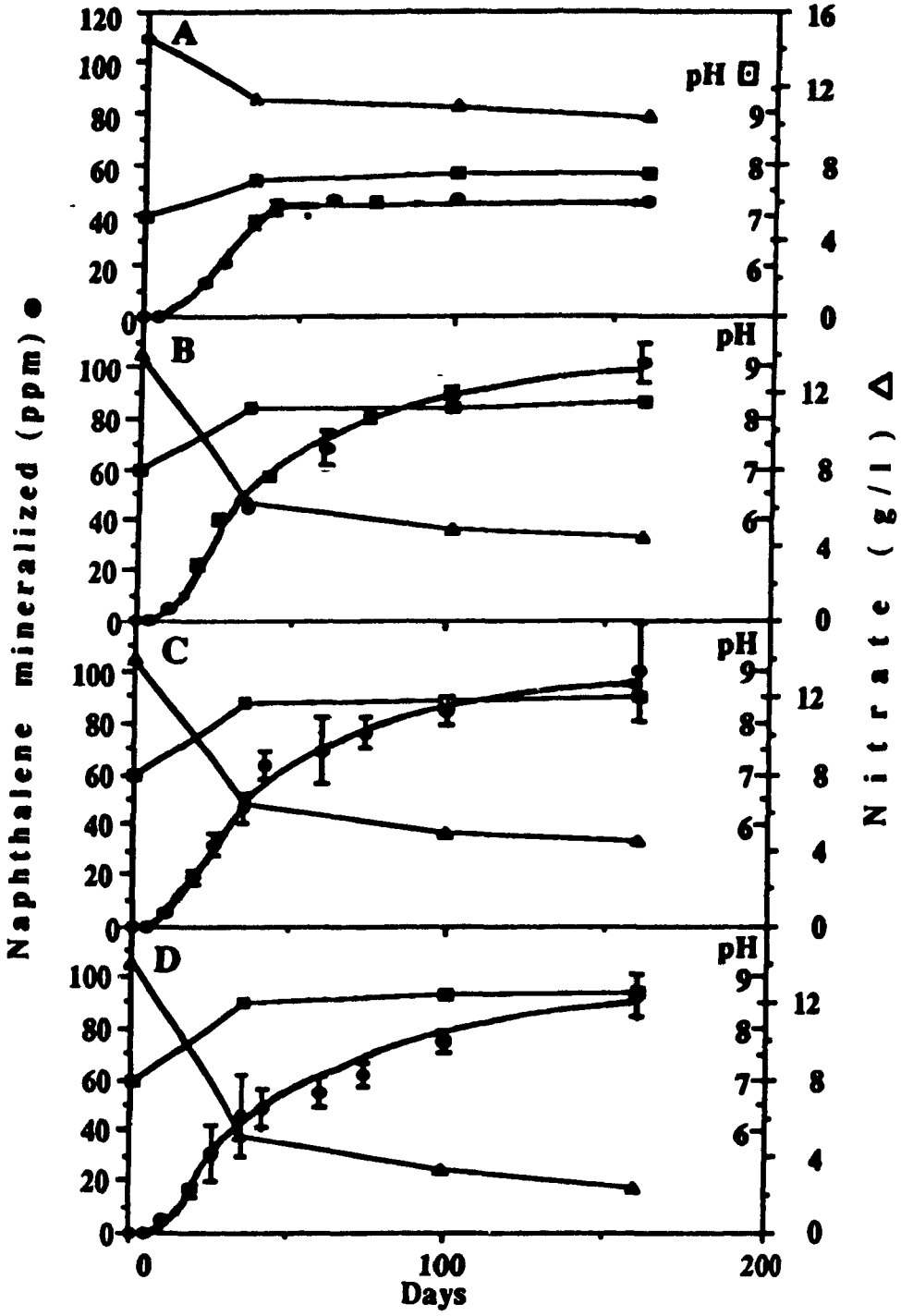

Figure 3.2A-D. Mineralization of [$_{14}$C]naphthalene in soil slurries. A 50 ppm in Soil 1. B 200 ppm in Soil 1. C 300 ppm total naphthalene concentration in Soil 1. D 200 ppm total naphthalene concentration using oil-contaminated soil (Soil 2).

rapidly. The total amount of naphthalene recovered in the form of $^{14}CO_2$ attained 90% after a 50-day period.

In the experiments where naphthalene was added at concentrations higher than the aqueous phase saturation level (200 and 500 ppm, Soil 1 and 2) mineralization resulted in similar curve profiles (see Figs. 3.2B, C and D). After the adaptation period, all curves showed an initial region (almost linear) followed by a gradual logarithmic decrease in the mineralization rate. This indicated that the factors controlling the mineralization process were similar for these three different experiments. The first region lasted until approximately 50 ppm was mineralized. The highest mineralization rates, corresponding to these sections, were 1.8 ppm day^{-1}. These values decreased to 0.24 ppm/day on the 160th day. The mineralization rate curves indicated that pre-exposure of the soil to hydrocarbon contamination did not effect the initial rate of biodegradation, but the subsequent biodegradation was less rapid (see Fig. 3.2D), with a minimum rate of 0.15 ppm/day observed on the 160th day.

Nitrate consumption and pH increase were associated with the denitrifying process (Figs. 3.2A-D). In all cases, nitrate was in excess of the biological demand and did not act as a limiting factor to the mineralization process. The lowest nitrate consumption was observed in the case of the 50 ppm naphthalene concentration and the highest with the oil-contaminated soil. Moreover, there was no detectable nitrate consumption in the control experiment indicating that denitrification was the predominant biological process. This was in agreement with the nitrate demand to be expected as a result of the biodegradation of methanol and naturally occurring organic carbon in the soil samples. Also, the nitrate consumption rate was highest in the initial stages of the

experiment, when the easily biodegradable compounds (such as the introduced methanol) were metabolized. In all experiments, the pH increased in parallel with the utilization of nitrate. The highest pH value (8.6) was recorded for the experiment with the weathered oil-contaminated soil at 200 ppm naphthalene, while the lowest pH (8.0) was observed for Soil 1 with 50 ppm.

3.5. DISCUSSION

The main objective of this work was to study the biodegradation of naphthalene in a soil-slurry system under denitrifying conditions. More specifically, the objective was to investigate the mineralization of ^{14}C-labelled naphthalene when it was present at concentrations close to or above the aqueous phase saturation levels, and the effect of the naphthalene sorption-desorption pattern on the biodegradation rate.

The results obtained in this study illustrate that naphthalene mineralization under denitrifying conditions has occurred in all experimental set-ups. These results also show that different mineralization rates were obtained in response to the induced changes in naphthalene concentration and soil type (Soil 1 vs Soil 2). In order to understand these differences it was necessary to study the naphthalene sorption-desorption pattern for the two soil compositions. The sorption isotherm presented in Fig. 3.1 show that addition of various amounts of naphthalene to the soil slurry result in different concentrations in the aqueous phase. Hence, when the initial naphthalene concentrations were 200 and 500 ppm, the corresponding concentration in the aqueous phase

never exceeded 50 ppm. Also, it was noted that the biodegradation rates obtained for these two different concentrations were similar. Therefore, it is concluded that when naphthalene concentration in a soil slurry is maintained close to, or higher than the aqueous phase saturation level, the kinetics of mineralization are zero order with respect to the total substrate concentration. However, in the experiment where the initial concentration was equal to the aqueous phase saturation level (50 ppm) the actual concentration available in the aqueous phase was significantly less (about 32 ppm) and the mineralization rate represented about 60% of that obtained for the 200 and 500 ppm experiments. This decrease in the mineralization rate indicates that the availability of naphthalene as a carbon source for the microorganisms was an important factor controlling the reaction speed. This also suggests that once the concentration of naphthalene in the aqueous phase decreases below the saturation level, the mineralization rate becomes substrate dependent and exhibits higher order kinetics. In this case, the desorption of naphthalene could control the rate of biodegradation provided that the desorption rate is smaller than that of biodegradation. However, to confirm this, a further study has to be conducted to measure the rate of desorption.

The low biodegradation rate (0.24 ppm/day) as observed after the first 50 ppm were mineralized, for the 200 and 500 ppm naphthalene total concentration experiments (Figs. 3.2B, C and D) can be attributed to the following reasons. Firstly, it could be due to sorption of naphthalene on the relatively high specific surface area of the solid material. For instance, Fig. 3.1 shows that the sorption of naphthalene on soil was very strong, thus limiting the amount available in the liquid phase for the microbial attack. Therefore, in spite of the high amounts of naphthalene

added, the aqueous concentration dropped quickly below the saturation level. In the experiment with the weathered oil-contaminated soil (Soil 2), the decrease in the reaction rate was found to be higher than with Soil 1, which was mostly pristine soil. However, for the same time period, the naphthalene mineralized in Soil 2 was 13% less than in Soil 1 (Figs. 3.2B and D). This can probably be attributed to increased sorption due to the higher organic content of the contaminated soil. Secondly, increase in pH probably inhibited the microbial activity.

As expected, when compared with pristine soil, the weathered oil-contaminated soil did not support higher naphthalene-degrading activity nor a shorter lag period. This lack of difference cannot be associated with a possible lack of denitrifying activity because denitrifiers are ubiquitous in soil. On the other hand the higher concentration of organic matter (4.3% versus 2.1%) in the oil-contaminated soil lead to a quick pH rise (up to 8.6, see Fig. 3.2D). This increased pH could have reduced the activity of the denitrifiers.

Comparisons between the results of this study and studies conducted by other researchers are difficult to evaluate partly because the soils used were different and did not exhibit identical sorption-desorption patterns (Table 3.1). From that table, it appears that naphthalene biodegradation rates obtained under denitrifying conditions were in the same range as those obtained under aerobic conditions. It also indicates that denitrifying conditions could play an important role in the decomposition of low molecular PAHs in nature. From an engineering perspective, it would be easier and probably more economical to supply the needed amounts of electron acceptor for the decontamination reaction to take place in the soil/water system in the form of nitrate, rather than

Table 3.1. Biodegradation rates of naphthalene under aerobic and denitrifying conditions.

Type of ecosystem	Initial naphthalene concentration [ppm]	Tempe-rature [°C]	Redox condition	Maximum bio-degradation rate [ppm/day]	Reference
marine sediment slurry	100	23	aerobic	1.87 [a]	(Bauer and Capone 1988)
marine sediment slurry	100	23	aerobic	11.70 [a,c]	(Bauer and Capone 1988)
soil-water slurry	7	room temp.	aerobic	appr. 1.5 [b]	(Mihelcic and Luthy 1988)
soil-water slurry	7	room temp.	denitrifying	appr. 0.25 [b]	(Mihelcic and Luthy 1988)
soil-water slurry	500	35	denitrifying	1.7-1.8 a	this study
soil-water slurry	200	35	denitrifying	1.7-1.8 a	this study
soil-water slurry	50	35	denitrifying	1.3 [a]	this study

a: mineralized fraction; b: measured upon disappearence from sample; c: previous pre-exposure to naphthalene.

as molecular oxygen. These results show also that, in order to reach a better understanding of the biodegradation process of an organic pollutant in a soil/water system, a certain amount of knowledge is needed about the interaction of the contaminant with the solid phase. Work is being pursued to assess the best operating conditions for using denitrification as a means to treat PAH-contaminated soils and to better understand the influence of PAH sorption-desorption patterns on the biodegradation kinetics using soils of various compositions.

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CHAPTER 4

BIODEGRADATION OF FOUR PAH COMPOUNDS IN A SOIL/WATER SYSTEM UNDER VARIOUS REDOX ENVIRONMENTS

4.1. SUMMARY

Biodegradation of acenaphthene, acenaphthylene, fluorene and anthracene was studied in soil/water slurries under four redox conditions. Under aerobic and denitrifying conditions, biodegradation of all four compounds occurred. The degradation rates observed were 0.4 ppm•day^{-1} for the aerobic environment and 0.2-0.4 ppm•day^{-1} for the denitrifying one. However, no significant biodegradation occurred under sulfate-reducing nor methanogenic conditions. The aerobic environment exhibited much higher volatilisation rates of the studied compounds than the denitrifying one. The denitrifying environment was then chosen to conduct a further study to investigate the performance of a bench-scale bioreactor. The results showed that scaling up the size of the bioreactor while simultaneously reducing the mixing intensity did not result in any significant change in the degradation rates of the PAH compounds. This study suggests that the denitrifying environment could play an important role in the development of an effective, economical and environmentally safe detoxification technology for treating PAH-contaminated soils.

4.2. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread organic contaminants that are produced from natural and man-made processes, mainly petroleum refining and coke production (Bulman et al. 1985). Because of their toxicity and/or mutagenicity, the U.S. Environmental Protection Agency has listed 16 PAH compounds as priority pollutants (Richards and Shieh 1986, Heitkamp et al. 1987). Large amounts of PAHs find their way to the terrestrial environment through atmospheric deposition, agricultural and industrial waste discharges and chemical spills (Jones 1988). As a result, several sites are being discovered annually, all over the industrialized world, with high contamination levels and serious environmental health risks such that they require immediate remedial action (Haines 1988).

PAHs are amenable to microbial biodegradation and their persistence in ground waters, soils and sediments is largely determined by biological processes (Herbes and Schwall 1978). Several studies, mainly concerned with the fate of these contaminants in various environments, have investigated the effect of the different factors on the biodegradation rates of PAHs. These factors have been summarized as follows (Cerniglia 1984):

- compound-related: type, concentration, ring number, solubility;
- microbial-related: types, population, distribution, previous exposure;
- environment-related: sediment type, organic content, nutrient

status, salinity, soil-to-water ratio, salinity, temperature, pH, redox potential.

Currently, a gap exists between the scientific knowledge on PAH degradation and putting this knowledge into practical use in the form of an innovative and economically feasible treatment technology (Atlas 1988). Bridging the existing gap could lead to the development of a highly efficient soil bioremediation technology which, in comparison to other physico-chemical treatment techniques, would hopefully possess several advantages such as low cost, low energy consumption, high waste reduction and detoxification efficiencies, minimal air pollution problems and a biologically healthy decontaminated soil (De Kreuk 1986).

Two biotic treatment techniques of contaminated soils have been proposed: in-situ biological treatment and use of a bioreactor system. The latter technique is still in the stage of development. In comparison with in-situ treatment, bioreactors are thought to have the following advantages (Soczo and Staps 1988):

- better control of several process parameters;
- lower chance of dissipating pollutants to uncontaminated areas;
- option for use of indigenous microbial population or a specifically developed microbial population.

However, for a bioreactor system to bioremediate contaminated soil effectively and within a reasonable time frame, it is necessary to optimise the set of factors that influence the reaction rate. One of the most important factors that have to be investigated is the redox environment (De Kreuk and Annokkee 1988).

In general, PAHs are readily biodegradable under oxic conditions, particularly those containing two or three aromatic rings (Bauer and

Capone 1985, Heitkamp et al. 1988). However, there is a paucity of information in the literature regarding the amenability of these compounds to biodegradation under anoxic conditions.

Bauer and Capone (1985), investigating the degradation of a number of PAH compounds in marine sediments, reported significant mineralization of naphthalene and anthracene under oxic conditions, while both compounds were resistant to biodegradation under anoxic ones. Similarly, Bouwer and McCarty (1983) showed that, under the denitrifying environment, naphthalene along with other aromatic compounds were not utilized in a mineral growth medium inoculated with primary sewage influent.

On the other hand, Mihelcic and Luthy (1988), using a soil/water system as the growth medium, were able to obtain biodegradation of naphthol, naphthalene and acenaphthene under the denitrifying environment at rates of 0.5, 0.16 and 0.01 mg/l-day respectively. In their study, Mihelcic and Luthy used a low soil-to-water ratio (1:25 and 1:50) and relatively low initial compound concentrations (8, 7, 0.4 mg/l, respectively).

Al-Bashir et al. (1990) investigating the effect of sorption-desorption equilibria on the mineralization of naphthalene under the denitrifying environment, obtained a mineralization rate of 1.7-1.8 ppm/day. The initial naphthalene concentration used was 200 mg/g (soil slurry) and the soil-to-water ratio was 30%.

The literature reports no study on the degradation of PAHs under sulfate-reducing nor under methanogenic conditions. However, the benzene ring has been shown to be amenable to biodegradation under these two environments. For example, benzene and toluene were biodegraded by

mixed methanogenic cultures obtained from an enriched ferulic acid-degrading sewage sludge, with CO_2 acting as the only electron acceptor (Grbic-Galic and Vogel 1987). Also, benzoate was mineralized to CO_2 by a sulfate-reducing organism in the presence of reducible sulfur compounds (Berry et al 1987).

From the above it is seen that there is a need to examine the role and significance of redox environments in the development of a remediation technique that utilizes biological processes to treat PAH-contaminated soil slurries. Therefore the purpose of this study is to investigate the following:

1. the biotic and abiotic losses of four PAH compounds from the soil slurry treated under various redox conditions (aerobic, denitrifying, sulfate-reducing and methanogenic). The compounds investigated are acenaphthylene, acenaphthene, fluorene and anthracene.
2. the performance of a bench-scale bioreactor under the optimum redox environment, based on results of part 1.

4.3. MATERIALS AND METHODS

4.3.1. Soil. A bulk soil sample was obtained from the Biotechnology Research Institute land premises, Montreal, Quebec. After scraping off the top 2-5 cm layer, soil was sampled at a depth of 3-10 cm from the surface. The sample was dried at 35 °C for 24 hours, sieved through a 500 micron mesh sieve, and analysed for particle size distribution and total organic content (Gee and Bauder 1986). The soil composition was as follows: sand 74%, silt 21 %, clay 5% and the organic

content 2.1%.

4.3.2. Mineral medium. The mineral medium used as the aqueous phase of the soil slurry was prepared by dissolving the following salts (all analytical grade) in tap water: KH_2PO_4, 0.170 g/l; K_2HPO_4, 0.430 g/l; $Na_2HPO_4 \cdot 7H_2O$, 0.660 g/l; $FeCl_3 \cdot 6H_2O$, 0.003 g/l; NH_4Cl, 0.034 g/l; $MgSO_4 \cdot 7H_2O$, 0.450 g/l. The final pH of the medium was adjusted to 7.0 (Mihelcic and Luthy 1988).

4.3.3. PAH chemicals. The PAH compounds, acenaphthylene, acenaphthene, fluorene and anthracene were obtained as crystalline powder from Aldrich Chemical Company Inc. (Milwaukee, Wis., USA) with a corresponding purity of 95, 99, 98 and 99.9% respectively. A stock solution of the four PAHs was prepared by dissolving a known amount of each compound in HPLC-grade ether.

4.3.4. Soil spiking with PAHs. 10% of the bulk soil sample was isolated and placed in a 250-ml boiling flask which also received the PAH stock solution. The contents of the flask were mixed thoroughly prior to evaporating the organic solvent (Büchi Rotavapor RE 120, Laboratoriums-Technik AG, Flawil, Switzerland) under vacuum and at room temperature. The spiked soil was then added to the bulk soil sample and both were mixed thoroughly to obtain a uniform distribution of the PAHs in the soil matrix.

4.3.5. Experimental setup

i) Redox conditions experiment. Four redox conditions were tested **a**: aerobic, **b**: denitrifying, **c**: sulfate-reducing and **d**: methanogenic.

For each redox environment two 500-ml erlenmeyer flasks were used, of which one acted as a control by receiving a dose of mercury chloride at a concentration of 5 g/kg soil to halt bacterial activity (Wolf et al. 1989). Each flask contained 187 g of soil and 200 ml of mineral stock solution. The initial concentration of each individual PAH compound in the slurry samples was 100 ppm (dry soil weight). The aerobic flasks were plugged with a polyurethane stopper to allow for gas exchange with the atmosphere. The anoxic flasks were purged with nitrogen gas, received 0.5 g glucose and sealed with air-tight teflon-lined screw caps. Glucose was added to the latter flasks, as an easily available organic compound for microbial uptake in order to consume any free oxygen present and to lower the oxidation-reduction potential to that corresponding to the denitrifying, sulfate and methanogenic environments. The flasks used for both of the denitrifying and sulfate-reducing environments received respectively 3.6 g/l of nitrate (as sodium nitrate) and 4.0 g/l sulfate (as sodium sulfate). Finally, all flasks were wrapped in aluminum foil and placed in an incubator shaker (model G25, New Brunswick Scientific Co., Inc., Edison, N.J., USA) and shaken continuously at 250 rpm and incubated at 25°C.

ii) The bench-scale bioreactor experiment. Two bioreactors were used here, of which one acted as a control by adding 5 g/kg mercury chloride. The bioreactors were constructed from a multi-function mixer (model K5SS, KitchenAid, Inc., St. Joseph, Michigan, USA). As shown in Fig. 4.1, the stainless steel bowl (4 liters total capacity) was covered with a steel lid equipped with rubber bands around the rotor and bowl edges to insure a closed and air-free operation mode. A number of screw-type ports were fitted to the covering lid to facilitate the sampling procedure. The

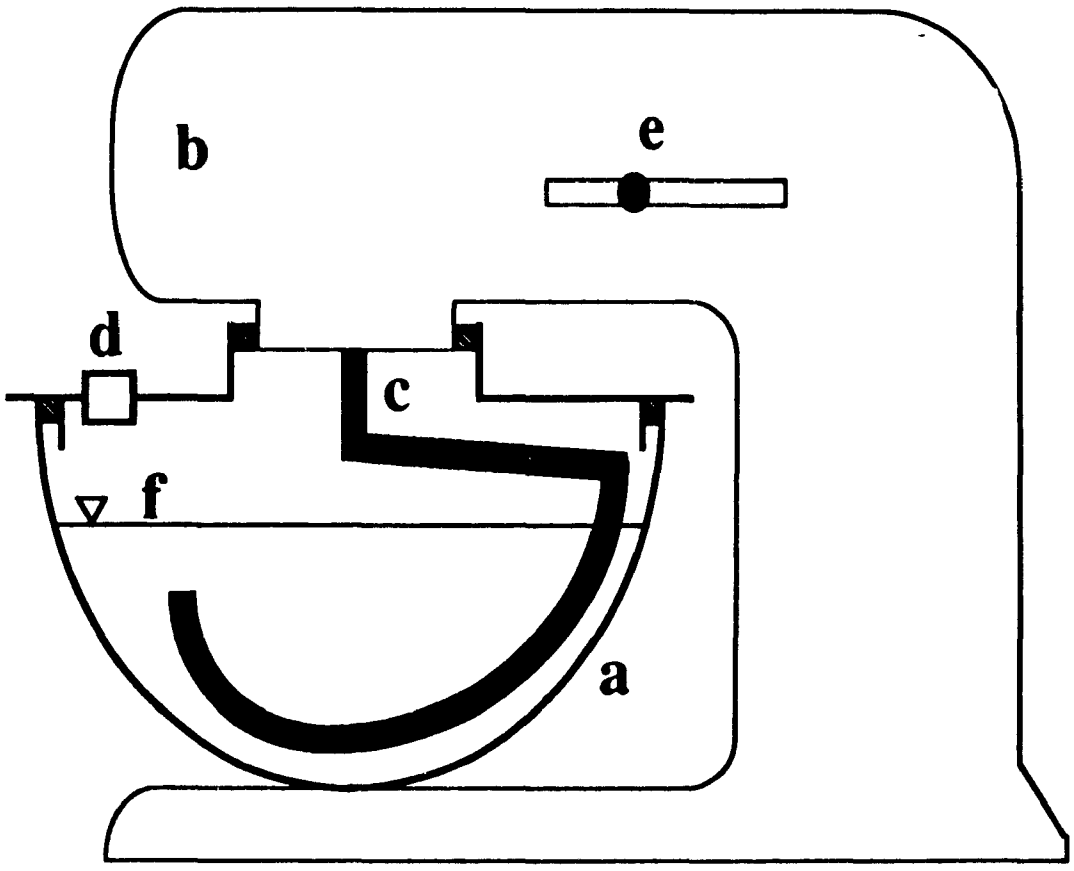

Figure 4.1. A schematic representation of the bench-scale bioreactor. a: stainless steel bowl, b: motor, c: mixing blade, d: sampling port, e: speed regulator, f: soil slurry.

bioreactor received 1.5 kg of soil and 1.5 kg of mineral solution. The initial individual PAH concentration was 120 ppm (dry soil weight). Sodium nitrate was added to the slurry, bringing the final nitrate concentration to 8 g/l. The mixing frequency and duration of the bioreactors were controlled using an electronic timer (model CD-4, ChronTrol, Linburg Enterprises, Inc., San Diego, Calif., USA). Mixing was performed three times a day for 20 seconds at a speed of 120 rpm. Temperature was maintained at 20±1°C.

4.3.6. Sampling and analysis.

i) Sampling. Sampling from the erlenmeyer flasks was conducted in an anaerobic chamber that was charged with oxygen-free nitrogen gas. Prior to sampling from the bench-scale bioreactors, their contents were mixed thoroughly in order to maintain a good sampling consistency throughout the duration of the experiment. Samples were collected for analysing PAH concentration, pH, sulfate and nitrate ions and methane gas.

ii) PAH analysis. Each soil slurry sample was first mixed with 5 g of anhydrous Na_2SO_4 and then extracted with benzene in a Soxhlet extractor for five hours. The solvent was then concentrated on the Rotavapor at 30°C under vacuum and analysed on a capillary gas chromatograph (model Sigma 200, Perkin-Elmer, Norwalk, Conn., USA) equipped with a flame ionizing detector and a 50-m column (Ultra-1, Hewlett-Packard, North Hollywood, Calif., USA). The PAHs recovery efficiencies for the extraction and concentration were 86% for acenaphthylene, 87% for acenaphthene and 85% for fluorene and anthracene.

iii) Anion analysis. One gram of sampled slurry was centrifuged

(model 5415, Eppendorf Centrifuge, Brinkmann, N.Y., USA) at 11000 g for 5 minutes. The supernatant was then analysed for nitrate and sulfate using an HPLC (model SP800 with integrator model SP4200, Spectra-Physics, San Jose, Calif., USA) equipped with conductivity type detector (model 430, Waters Chromatography Division, Milford, Mass., USA) and anion exchange column (model PRP-X100, Hamilton Co., Reno, Nev., USA).

iv) pH measurement. The pH values of slurry samples were measured using a combined electrode and pH meter (Accumet 925, Fisher Scientific, Montreal, Canada).

v) Methane gas. The methanogenic conditions were confirmed by detecting methane gas formation in a sample collected from the head space of the flask. The sample was analysed using a gas chromatograph (Perkin Elmer, Sigma 2000GC, Norwalk, Conn., USA) with a Perkin Elmer LC-100 integrator and a thermal conductivity detector. The GC column (Chromosorb 102, Bellefonte, Pa., USA) was 12 ft long.

4.4. RESULTS and DISCUSSION

4.4.1. Redox conditions. The results obtained from the redox-condition experiment are shown in Figs. 4.2 and 4.3. Although all four PAH compounds were present simultaneously in any flask, Fig. 4.2 presents the disappearance of each compound under the four redox environments separately for ease of comparison.

The loss of the PAH compounds from the oxic and anoxic control samples, as shown in Fig. 4.2, is attributed to abiotic processes, mainly volatilisation, since no biological activity was detected in these samples

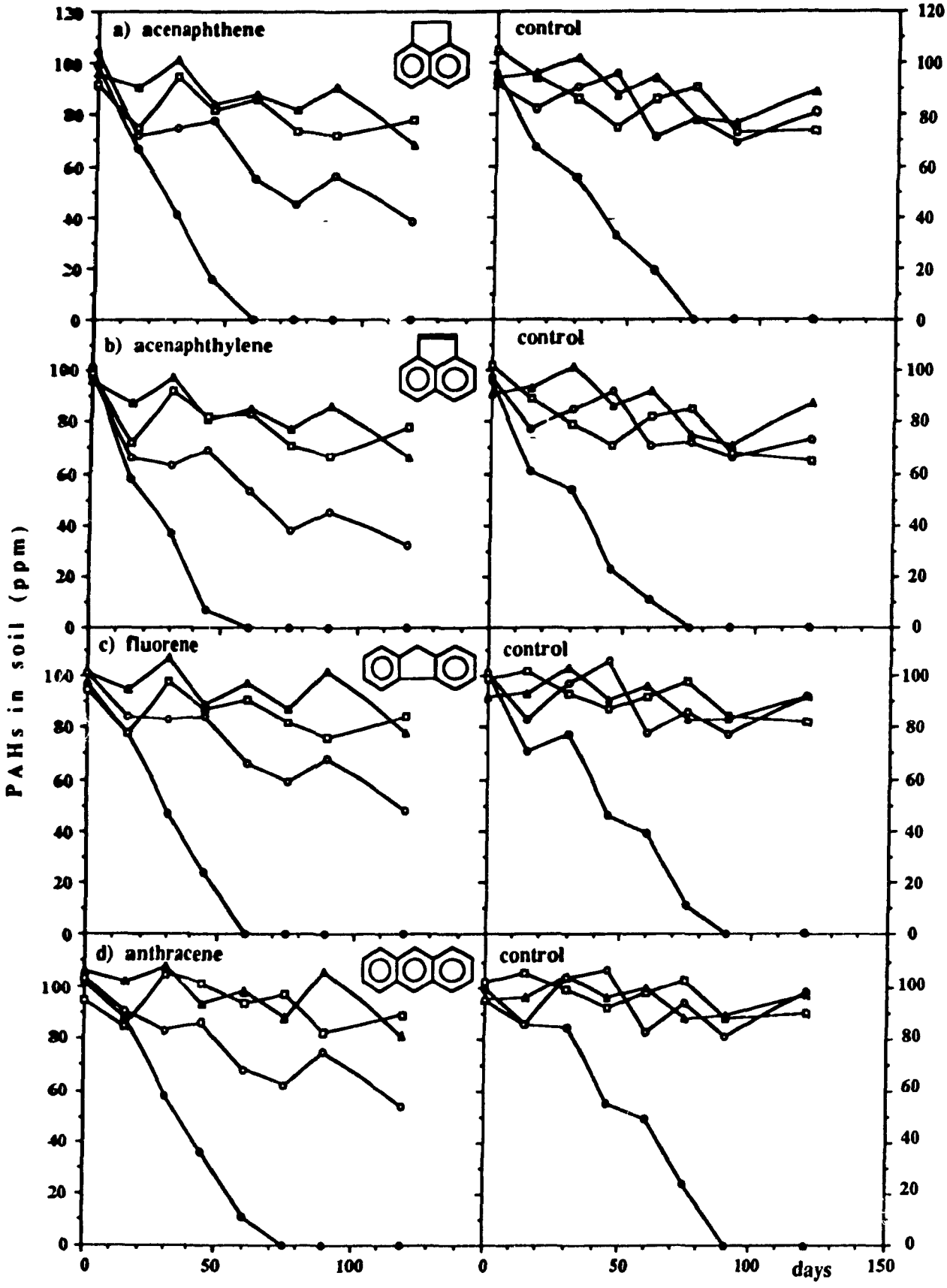

Figure 4.2. Disappearance of PAHs under various redox conditions.
a: acenaphthene, b: acenaphthylene, c: fluorene, d: anthracene.

— methanogenic — denitrifying
— sulfate-reduction — aerobic

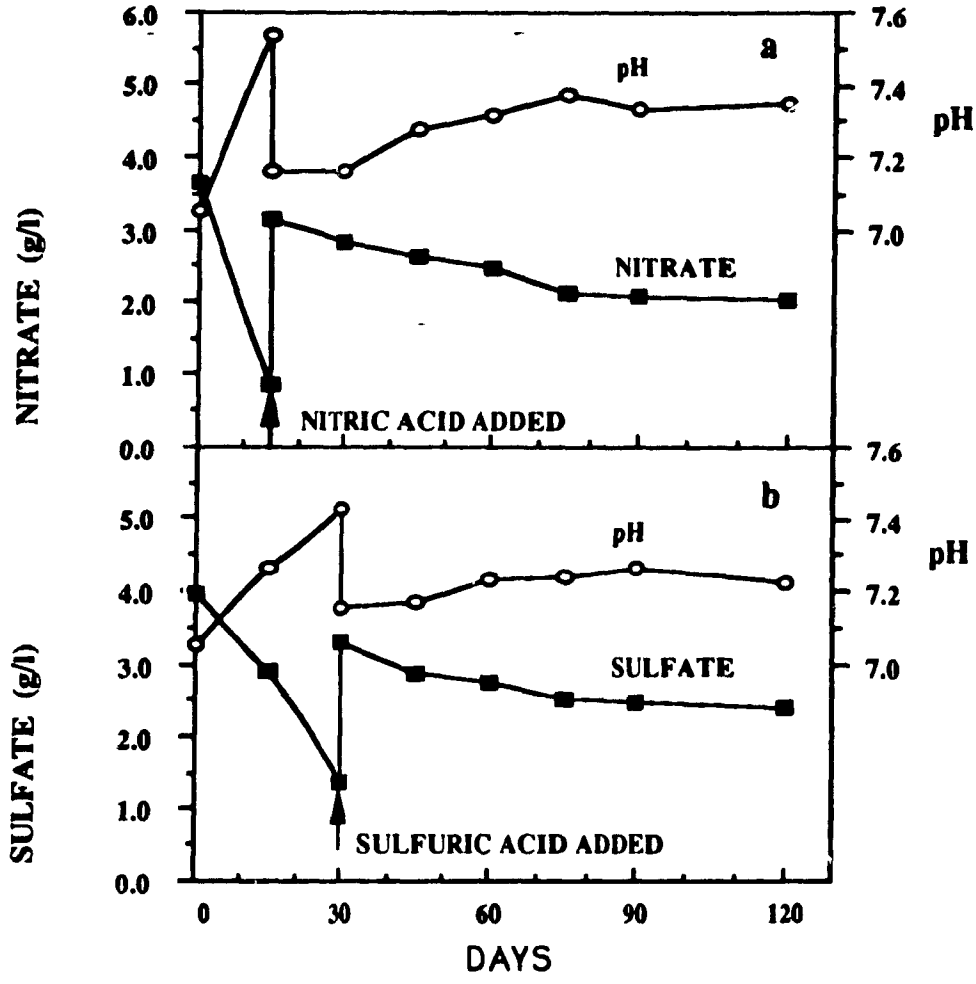

Figure 4.3. Anion and pH measurements for the denitrifying and sulfate-reducing environments.
a. Nitrate and pH measurements under denitrifying environment.
b. Sulfate and pH measurements under sulfate-reducing conditions.

using the plate counting technique. Under the aerobic environment, intensive volatilisation of the PAH compounds from the erlenmeyer flasks occurred. A large percentage of the volatilised compounds was recovered by extracting the polyurethane stopper. Other abiotic processes could have contributed to the loss of PAHs from the controls. Park et al. (1990) suggested that sorption, artifact of the assay, hydrolysis, Hg-catalyzed reactions and autoxidation are possible mechanisms for PAH losses from bioinhibited soil samples.

The continuous and intensive mixing of the slurry, in order to maintain the soil particles in suspension and to ensure adequate oxygen diffusion, may have contributed to the fairly rapid volatilisation of the compounds in the aerobic flasks. However, volatilisation also occurred, though to a much lesser extent, under anoxic conditions. This is probably due to the escape of certain amounts of the PAH compounds from the flasks, especially those saturating the head space, during sampling in the anaerobic chamber.

In the biologically active flasks, the loss of PAHs is attributed to both biotic and abiotic processes. Under the denitrifying and sulfate-reducing environments, the microbial population exerted a significant demand for nitrate and sulfate respectively (see Fig. 4.3). The nitrate and sulfate consumption at the early stage of the experiment was higher than that at subsequent stages. The rapid initial rates of nitrate and sulfate reduction are probably due to the biodegradation of easily degradable carbon (the added glucose and the naturally occurring organic matter in the soil).

Also, Fig. 4.3 indicates that nitrate and sulfate microbial uptake was associated with a slight increase in the slurry pH. To avoid further

increase in the pH of the media, the depleted nitrate and sulfate ions were replenished by adding fresh amounts of nitric and sulfuric acids, respectively. Besides acting as sources for nitrate and sulfate, these acids provided the hydrogen ions needed to neutralize the hydroxyl group resulting from the denitrifying and sulfate-reducing bacterial activities. The nitric and sulfuric acids were added at 10% aqueous concentration to minimize local pH fluctuations.

A statistical analysis was performed on the set of results obtained from the redox conditions experiment (see Appendix D). To determine the biodegradation rates of the PAH compounds under the various redox environments, the linear least-squares method was used to calculate the two rates of disappearance of each PAH compound from any flask and its control. The difference between these two rates represents the rate of disappearance due to biological activity. The confidence limit corresponding to the difference was also determined (Walpole 1982).

Results from the statistical analysis are shown in Table 4.1. It is seen that both the aerobic and denitrifying environments exhibited significant PAH biodegradation rates. The confidence levels for the aerobic and the denitrifying environments exceeded 95%. However, all four compounds were recalcitrant under the sulfate-reducing and methanogenic environments and their confidence limits were significantly low. Therefore, it was not possible to confirm whether the compounds were biodegradable or completely recalcitrant under these two redox environments. Also, the gas chromatograph did not detect any accumulation of PAH metabolites, thus indicating that the reaction limiting step lies in the initial microbial attack on the aromatic ring.

Furthermore, Table 4.1 shows that the PAH biotransformation rates

Table 4.1. Volatilisation and biodegradation rates of PAH compounds (ppm/day).

PAH	Process	Aerobic	Denitrifying		Sulfate-reducing	Methanogenic
			a	b		
Acenaphthylene	Volatilisation	1.60±0.06	0.32±0.02	0.36±0.03		
	Biodegradation	0.53±0.06	0.35±0.03	0.37±0.04	*	*
Acenaphthene	Volatilisation	1.39±0.01	0.26±0.02	0.34±0.03		
	Biodegradation	0.39±0.04	0.32±0.03	0.30±0.03	*	*
Fluorene	Volatilisation	1.18±0.03	0.16±0.03	0.15±0.03		
	Biodegradation	0.50±0.03	0.29±0.03	0.33±0.02	*	*
Anthracene	Volatilisation	1.01±0.02	0.10±0.03	1.11±0.02		
	Biodegradation	0.38±0.03	0.29±0.03	0.28±0.02	*	*

a: erlenmeyer flasks. b: bench-scale bioreactor . *: below 80% confidence limit.

under the denitrifying environment compare favorably with those under the aerobic one, with the former being on average 15% lower. Also, a rough negative correlation exists between the volatilisation rates of the four PAH compounds and their molecular weight (the molecular weights for the PAH-compounds are: acenaphthylene : 152, acenaphthene : 154, fluorene : 166 and anthracene : 178).

In this study, the method of assay resulted in a significant scatter of the results. This is evident in Fig 4.3 and Appendix C where some of the measured PAH concentrations rise above their corresponding initial values. The fluctuation in the concentration of PAHs was as high as 15%, determined by analysing a triplicate sample of the soil slurry. This is largely due to the inconsistency in soil sampling in spite of all precautions taken to standardise its procedure. A slight change in the distribution of the soil particles in the sample inherently gave rise to a significant change in its organic content and consequently a change in the concentration of the PAH compounds.

The scatter of the results hindered any attempt to make a valuable assessment of the effect of the PAH compound's physico-chemical properties, such as aqueous solubility, octanol:water partition coefficient and molecular weight, on their biodegradation rates. Also, this scatter has made it, in certain cases, difficult to decide on the kinetics of the biotic and abiotic processes.

However, in determining the biodegradation and volatilisation rates reported in Table 4.1, zero order kinetics were assumed. Besides giving a better description of the results, other reasons favour making such an assumption. In the case of bioinhibited samples, volatilisation from the soil slurry departed from simple first order kinetics observed in water

bodies due to the fact that the process is complicated with the sorption of the PAHs on soil particles, and that the amount of PAHs in soluble form constituted only a small fraction of the total amount present (Bulman et al. 1985). Also, for the biologically active samples that were kept under anoxic conditions, the initial and final concentrations of the compounds at the end of the experimental run were relatively high. Hence, it is unlikely that the depletion of the soluble PAHs have imposed limitations on the reaction rates (Al-Bashir et al. 1990, Herbes and Schwall 1978).

A comparison between the biodegradation results obtained in this study and those reported in the literature needs to take into consideration the limitations of such a comparison arising from the variations in the biodegradation studies in terms of the nature of the soil matrix, experimental conditions and the assay artifacts. For example, in this study, the biodegradation experiment lasted 120 days, during which a microbial population shift had probably occurred thus questioning the value of extrapolating the data to their initial conditions and comparing them to other studies with a different time frame (Herbes and Schwall 1978).

Under an aerobic environment, Bauer and Capone (1988) obtained a biodegradation rate of 1.6 ppm/day for anthracene biodegradation in marine sediment slurries that had been previously acclimated to anthracene contamination, and a biodegradation rate of 0.07 ppm/day for unacclimated sediments. The initial concentration of anthracene was 100 ppm and the incubation time was 50 hrs. The biodegradation rate of anthracene under aerobic conditions was found to be 0.38 ppm/day. In this study, the long exposure time of the soil microbial population to anthracene could account for the elevated microbial activity over that

acting in the unacclimated marine sediments investigated by Bauer and Capone.

Keck et al (1989) reported a half-life of 99 days for fluorene biodegradation in Kidman soil. Fluorene was present as part of a refinery waste and its initial concentration was 29 µg/g. The half-life for fluorene biodegradation under aerobic conditions obtained here is 100 days.

The biodegradation rates for acenaphthene under aerobic and denitrifying conditions (see Table 4.1) are substantially higher than those reported by Mihelcic and Luthy (1988) which were 0.125 mg/l-day and 0.016 mg/l-day, respectively. The reason for this difference could be attributed to the much lower initial acenaphthene concentrations used by those researchers, 1 mg/l for the aerobic environment and 0.4 mg/l for the denitrifying one. Equally important is the fact that Mihelcic and Luthy used a more diluted soil suspension (1:25 and 1:50) than the one used here. The lower soil-to-water ratio probably gave rise to a lower concentration of the microbial population and in turn to lower biodegradation rates of PAHs.

4.4.2. Bench-scale bioreactor. The criteria for selecting a particular redox condition for a soil bioremediation technology have to be based, in the first place, on the ability of that redox environment to support a biological activity that is capable of both biodegrading and detoxifying the contaminants, and to do so within a reasonable time frame. Another equally important consideration is economical feasibility; unless the treatment process is economical, there is little chance for it to be developed into a full-scale application.

The redox-condition experiment has shown that both the oxic and the denitrifying conditions could act as a suitable environment for treating PAH-contaminated soil. However from a practical point of view, the denitrifying environment could provide a better prospect for developing a bioremediation technology than the aerobic one, for the following reasons. First, an aerobically operated bioreactor would result in substantial volatilisation of the PAH compounds, thus creating a potential air pollution problem. Second, due to the relatively high density of the soil slurry, aerobic treatment is bound to face a mass transfer problem with respect to oxygen diffusion. Energy demand to overcome limitations on oxygen diffusion might turn out to be high enough to render the process uneconomical.

For the above reasons, the denitrifying environment was chosen for conducting a further study to investigate the performance of a more advanced bench-scale bioreactor.

The disappearance of the four PAH-compounds from the bioreactor and its control, along with nitrate and pH measurements, are presented in Fig. 4.4. A statistical analysis was also performed to determine the rate of disappearance due to biotic processes (see Appendix D). These rates are presented in Table 4.1, along with the results obtained from the redox-conditions experiments. The confidence limits corresponding to the difference between the observed volatilisation rate in the control and the rate of disappearance in the bioreactor for each PAH compound exceeded 95%.

As shown by Table 4.1, the biotransformation rates obtained in the bench-scale bioreactor are very close to those obtained from the denitrifying erlenmeyer flask, thus suggesting that scaling up the

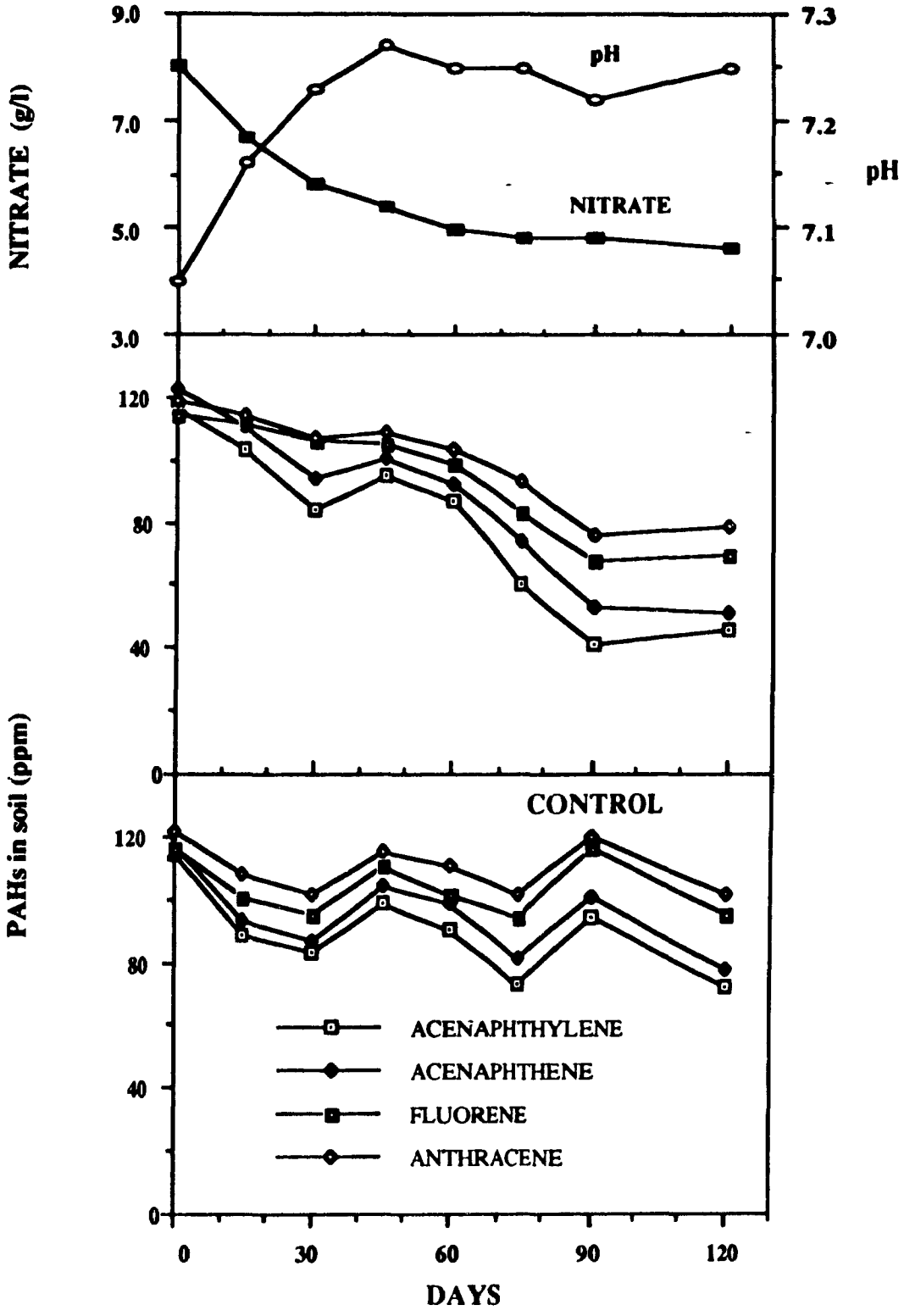

Figure 4.4. Disappearance of PAHs in the bench-scale bioreactors with pH and nitrate measurements.

treatment process and simultaneously reducing the mixing intensity, from continuous mixing to intermittent one, did not result in any significant change in PAH-biodegradation rates.

Finally, the naphthalene biodegradation study (chapter 3) has shown that the denitrifying environment can support significant biodegradation of naphthalene. This finding is further confirmed by the present study and extends it to include other PAH-compounds with higher molecular weight. Furthermore, besides its capacity to support a PAH-utilizing microbial population, the denitrifying environment could provide an environmentally safer and a more economical alternative to aerobic treatment processes.

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CHAPTER 5

5.1. CONCLUSIONS

In developing a remediation technique capable of treating PAH-contaminated soils, there would be two main objectives:

1. Optimisation of the different factors influencing the biological reaction in order to create favorable, and ideally optimum environment for the growth of the biodegrading microbial population.

2. Under optimal conditions, the rate limiting factors have to be identified and in light of this, appropriate methods can be devised to speed the reaction rate.

With regard to the first objective, this study has investigated the role and significance of the redox environment in the biological treatment of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). The results showed that the four PAH compounds studied, acenaphthene, acenaphthylene, fluorene and anthracene, were biodegradable under both aerobic and denitrifying conditions, but all four were recalcitrant under the sulfate-reducing and methanogenic environments. Furthermore, the biodegradation rates under the denitrifying environment compare favorably with those obtained under the oxic one. Moreover, aerobic treatment of contaminated soil has resulted in the volatilisation of 74% of the total amount of PAHs initially added, thus rendering them unavailable to soil bacteria.

With regard to the second objective, this study has examined the

effect of the initial concentration of naphthalene and its adsorptive/desorptive behavior on its degradation rate under denitrifying conditions. The results showed that when naphthalene concentration in the soil slurry is maintained close to or higher than the aqueous phase saturation limit, the mineralization kinetics are zero order with respect to substrate total concentration. However, once the concentration of naphthalene in the liquid phase drops below its solubility limit, the mineralization rate becomes substrate dependent and exhibits higher order kinetics. In this latter case, the desorption of naphthalene from the solid phase controls the reaction speed.

In conclusion, this study proposes first, that the denitrifying environment could be the one to favour in the development of a bioreactor system for treating PAH-contaminated soils. Second, the bioavailability of the PAH contaminants to microorganisms in the soil/water matrix could constitute a rate limiting step for the biological reaction.

Future research should investigate, with respect to the first of these propositions, the susceptibility of PAHs with more than three benzene-rings to biodegrade under the denitrifying environment. Also, the performance of bioreactors should be evaluated when the soil to be treated has been contaminated with a complex mixture of industrial compounds including PAHs.

However, to determine the extent to which PAH bioavailability can constitute the limiting step to the biological reaction, it is necessary to perform further experiments, investigating the effect of emulsifiers, soil-to-water ratio, organic content, pH, temperature, and chemical substitution on the soil-PAH adsorptive/desorptive properties.

Finally a note is made here on the experimental procedure. In this

study both radioactive-tracer and chromatographic analysis have been used to determine the biodegradation rates of the PAH compounds. While the former monitors PAH biodegradation through CO_2 evolution which is exclusively a product of biological activity, the latter monitors the concentration of the PAH compounds and their rate of disappearance which are due to both biotic and abiotic processes. Radioactive analysis provides a more accurate method than chromatography for PAH determination in soils, for the following reasons:

1. There is an error arising from the inconsistency of slurry sampling. In the case of chromatographic analysis, soil sampling acts as a major source of error. This is due to the fact that the finer the soil particles are the larger their organic content and also the higher their PAH adsorptive capacity. Hence, slight variations in the particle size distribution of the sample could lead to considerable fluctuations in the results.

2. Analysis on GC or HPLC instruments requires prior extraction, purification (for a complex waste), fractioning and concentration of the sample. Each of these steps could act as a source of error, especially when dealing with low concentrations and with compounds having appreciable volatilisation rates.

APPENDIX A

Results from the naphthalene biodegradation and adsorption study (from Chapter 3).

Table A.1. Naphthalene mineralized at 500 ppm initial concentration and using soil slurry type A.

DAYS	First sample (ppm)	Second sample (ppm)	Average (ppm)
0	0	0	0
5	0	0	0
10	6.1	5.6	5.9
18	21.3	17.5	19.4
25	35.4	29.5	32.5
35	51.0	42.6	46.8
42	67.6	59.7	63.6
60	78.3	60.3	69.3
75	72.1	80.6	76.3
100	80.7	88.6	84.6
160	113.7	86.4	100.1

Table A.2. Naphthalene mineralized at 200 ppm initial concentration and using soil slurry type A.

DAYS	First sample (ppm)	Second sample (ppm)	Average (ppm)
0	0	0	0
5	0	0	0
10	4.5	5.2	4.8
18	23.1	19.9	21.5
25	38.6	41.1	39.8
35	44.9	43.4	44.2
42	57.3	55.0	56.1
60	72.3	62.7	67.5
75	81.3	78.0	79.7
100	91.0	87.7	89.4
160	106.6	95.2	100.9

Table A.3. Naphthalene mineralized at 50 ppm initial concentration and using soil slurry type A.

DAYS	First sample (ppm)	Second sample (ppm)	Average (ppm)
0	0	0	0
5	0	0	0
10	3.2	4.6	3.9
18	11.5	15.2	13.4
25	22.0	19.7	20.9
35	41.7	32.2	37.0
42	40.6	44.6	42.6
60	45.8	45.3	45.6
75	43.6	45.8	44.7
100	45.7	45.2	45.4
160	45.3	44.8	45.0

Table A.4. Naphthalene mineralized at 200 ppm initial concentration and using soil slurry type B.

DAYS	First sample (ppm)	Second sample (ppm)	Average (ppm)
0	0	0	0
5	0	0	0
10	3.9	5.3	4.6
18	17.7	14.7	16.2
25	38.3	23.0	30.6
35	56.3	34.1	45.2
42	54.4	43.1	48.7
60	58.5	51.2	54.8
75	65.3	58.8	62.0
100	71.6	77.6	74.6
160	96.8	84.8	90.8

Table A.5. Naphthalene partitioning between the solid and liquid phases.

Total concentration ppm	liquid phase concentration ppm	solid phase concentration ppm
0.0	0.0	0.0
10.0	4.8	22.1
20.0	10.3	42.7
35.0	22.4	64.4
50.0	31.3	93.9
100.0	43.2	232.5
200.0	48.7	553.8
500.0	49.8	1550.8

APPENDIX B

Langmuir, Freundlich and B.E.T isotherms describing the adsorption of naphthalene by soil (Chapter 3).

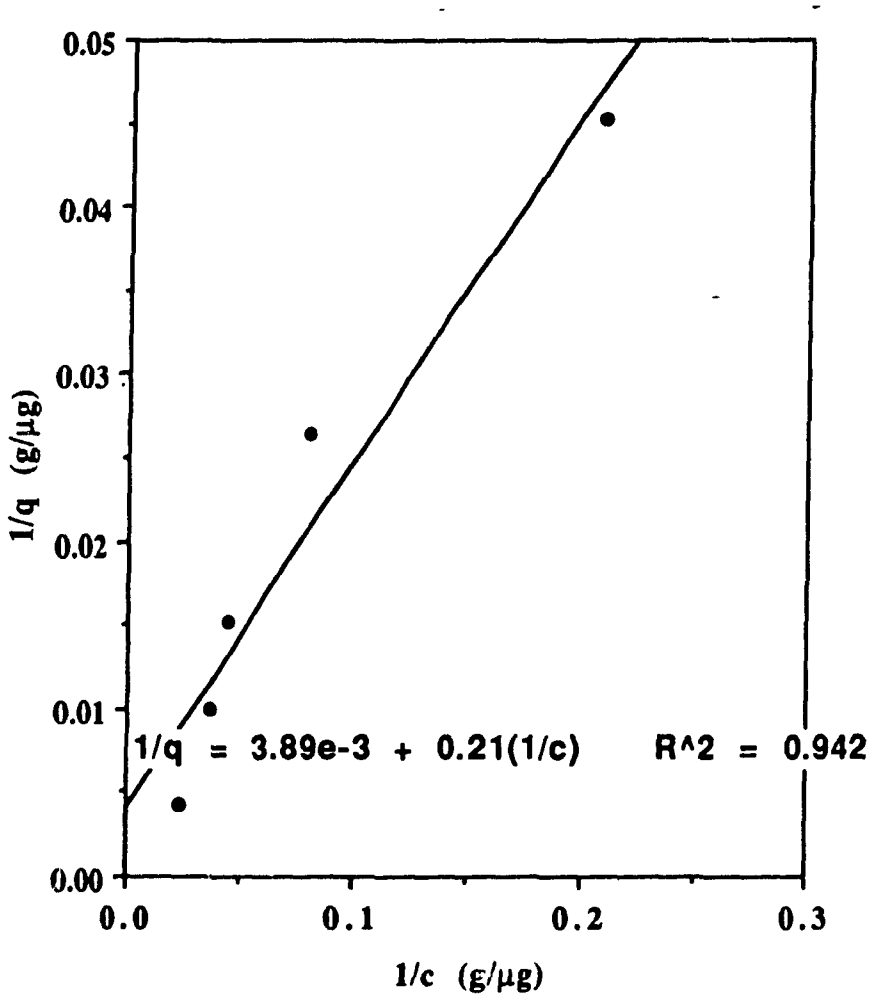

Fig. B.1. Langmuir model fitted to naphthalene aqueous concentrations below saturation limit.
q = µg naphthalene adsorbed/g soil at equilibrium.
c = µg naphthalene in solution/ml water at equilibrium.

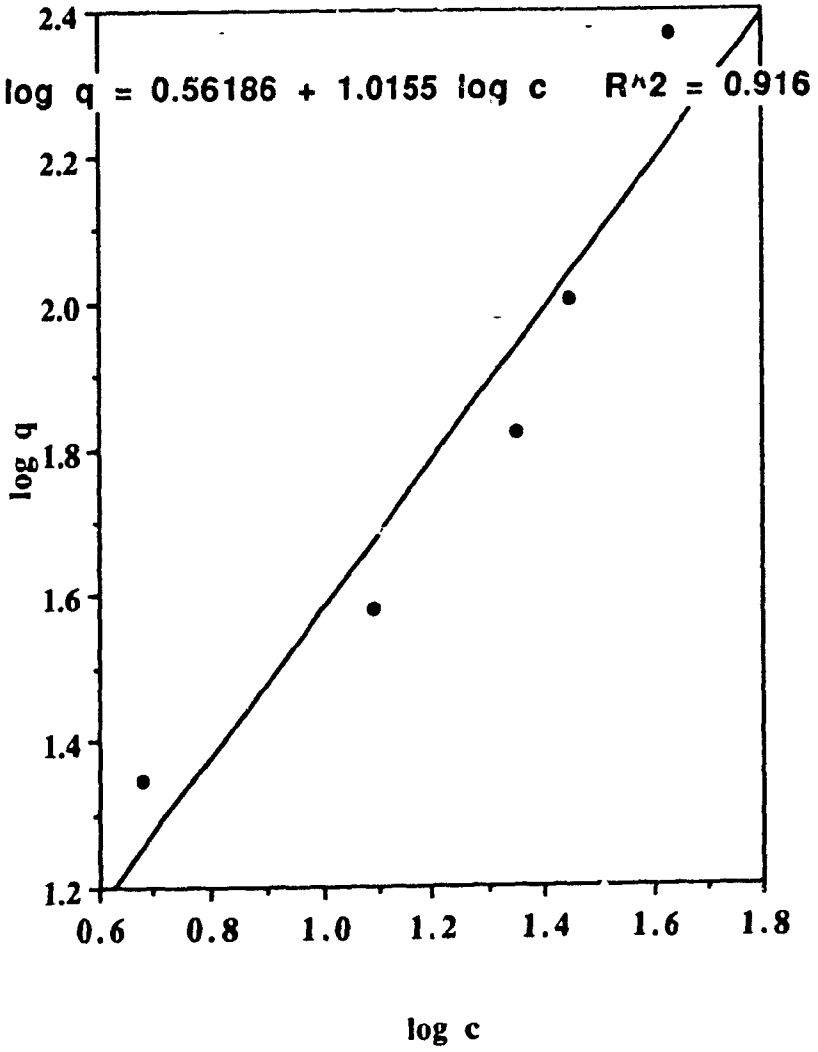

Fig. B.2. Freundlich model fitted to naphthalene aqueous concentrations lower than the saturation level.
q = µg naphthalene adsorbed/g soil at equilibrium.
c = µg naphthalene in solution/ml water at equilibrium.

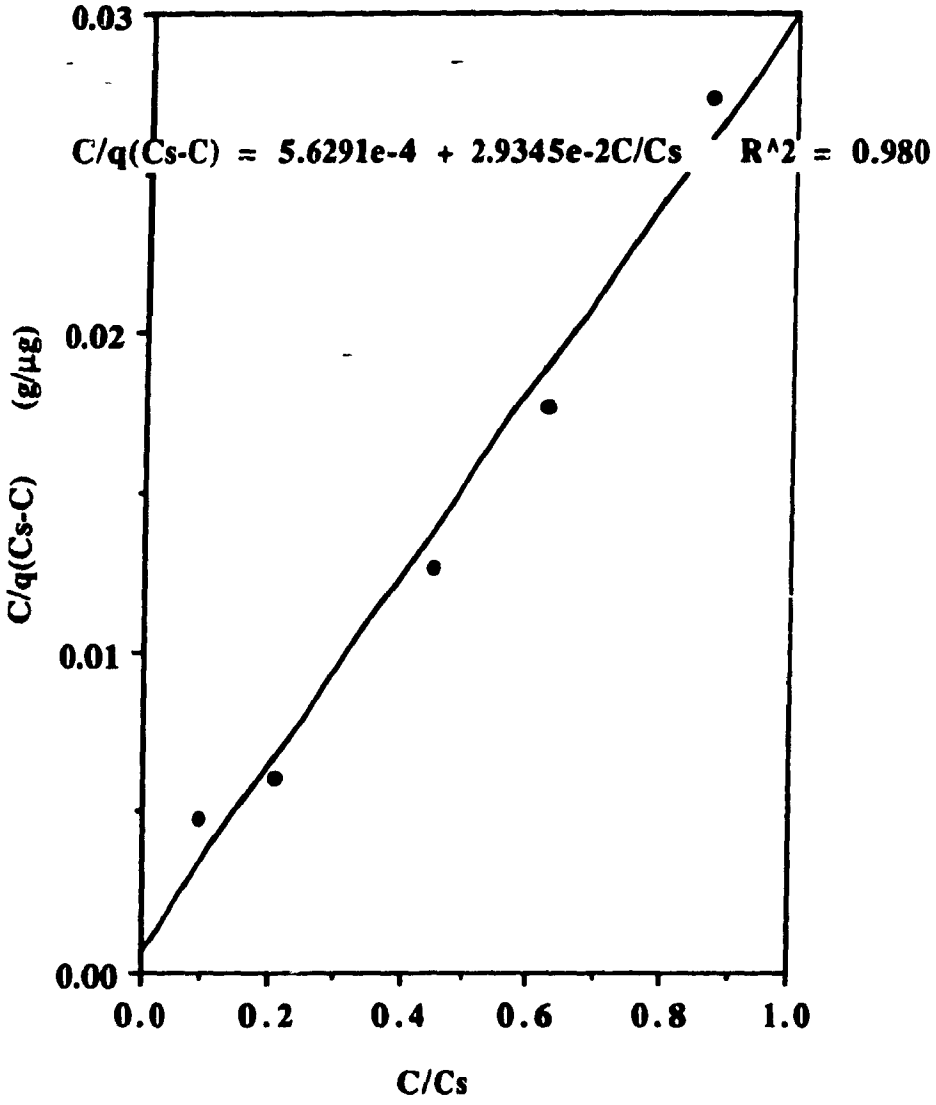

Fig. B.3. B.E.T. model fitted to naphthalene aqueous concentrations below saturation limit.
q = µg naphthalene adsorbed/g soil at equilibrium.
C = µg naphthalene in solution/ml water at equilibrium.
Cs = saturation concentration of naphthalene in water (µg/ml).

APPENDIX C

Results from the biodegradation of acenaphthylene, acenaphthene, fluorene and anthracene under various redox conditions (from Chapter 4).

Table C.1. Changes in acenaphthylene concentration (ppm) with time under various redox conditions.

DAYS	AEROBIC	DENITRI-FYING	SULFATE-REDUCING	METHANO-GENIC
0	101.6	98.3	99.5	96.7
15	58.2	66.3	72.4	87.3
30	37.9	63.6	92.8	97.7
45.	7.5	69.7	82.3	81.2
60	0.0	53.9	83.9	85.6
75	0.0	38.2	71.4	77.9
90	0.0	45.7	67.0	86.4
120	0.0	32.1	78.1	66.4

Table C.2. Changes in acenaphthylene concentration (ppm) with time under various redox conditions in the control samples.

DAYS	AEROBIC	DENITRI-FYING	SULFATE-REDUCING	METHANO-GENIC
0	96.3	97.8	102.1	91.2
15	61.2	77.6	89.5	93.6
30	54.7	85.3	79.6	101.4
45	23.9	92.8	71.7	86.7
60	11.4	71.6	82.3	92.3
75	0.0	72.4	85.5	75.8
90	0.0	66.5	68.6	71.5
120	0.0	73.1	65.8	87.9

Table C.3. Changes in acenaphthene concentration (ppm) with time under various redox conditions.

DAYS	AEROBIC	DENITRI-FYING	SULFATE-REDUCING	METHANO-GENIC
0	99.6	104.5	92.2	96.5
15	67.3	72.3	75.8	91.3
30	41.6	75.8	95.7	101.9
45	16.9	78.7	82.9	84.6
60	0.8	55.3	86.5	88.4
75	0.5	45.1	74.7	82.5
90	0.4	56.2	72.2	91 2
120	0.6	38.7	78.0	68.8

Table C.4. Changes in acenaphthene concentration (ppm) with time under various redox conditions in the control samples.

DAYS	AEROBIC	DENITRI-FYING	SULFATE-REDUCING	METHANO-GENIC
0	96.7	92.6	105.8	94.8
15	67.8	82.4	94.4	96.0
30	55.4	91.3	86.3	102.5
45	33.5	96.8	75.7	87.4
60	19.1	71.6	86.5	94.3
75	0.0	78.5	91.3	78.6
90	0.0	69.2	73.1	76.5
120	0.0	81.1	74.2	89.9

Table C.5. Changes in fluorene concentration (ppm) with time under various redox conditions.

DAYS	AEROBIC	DENITRI-FYING	SULFATE-REDUCING	METHANO-GENIC
0	97.2	101.7	95.1	101.8
15	78.4	84.5	78.6	95.2
30	47.6	83.8	98.5	107.4
45	24.5	84.6	87.2	89.1
60	0.0	66.3	91.3	97.6
75	0.0	59.1	82.5	87.9
90	0.0	68.4	76.8	102.0
120	0.0	48.5	84.3	78.2

Table C.6. Changes in fluorene concentration (ppm) with time under various redox conditions in the control samples

DAYS	AEROBIC	DENITRI-FYING	SULFATE-REDUCING	METHANO-GENIC
0	101.7	101.2	99.6	92.1
15	71.9	83.8	102.8	93.5
30	77.4	97.9	93.6	103.4
45	46.1	106.6	87.3	91.7
60	39.6	78.4	92.7	96.3
75	11.8	86.5	98.3	83.3
90	0.0	77.3	84.2	83.7
120	0.0	92.1	82.8	92.5

Table C.7. Changes in anthracene concentration (ppm) with time the various redox conditions.

DAYS	AEROBIC	DENITRI-FYING	SULFATE-REDUCING	METHANO-GENIC
0	102.3	103.6	95.6	106.4
15	88.5	91.7	85.9	102.2
30	58.7	83.4	105.4	108.8
45	36.5	86.3	101.3	93.5
60	11.8	68.8	93.8	98.2
75	0.0	62.7	97.8	88.6
90	0.0	75.5	82.1	106.4
120	0.0	54.2	89.0	81.5

Table C.8. Changes in anthracene concentration (ppm) with time under various redox conditions in the control samples.

DAYS	AEROBIC	DENITRI-FYING	SULFATE-REDUCING	METHANO-GENIC
0	98.5	94.8	101.1	94.3
15	91.3	85.2	105.2	95.6
30	83.9	103.1	98.6	103.5
45	54.7	106.6	91.3	95.8
60	48.7	82.5	97.7	99.6
75	23.3	93.9	102.0	87.4
90	0.0	80.5	87.2	88.2
120	0.0	97.3	89.9	96.1

Table C.9. Changes in the concentration of PAH compounds (ppm) with time in the bench-scale bioreactor.

DAYS	Acenaph-thylene	Acenaph-thene	Fluorene	Anthracene
0	118.3	123.3	117.8	119.4
15	103.6	111.3	112.2	114.7
30	84.1	94.2	106.5	107.5
45	95.5	101.1	105.8	1092
60	87.2	92.6	99.6	103.7
75	60.3	74. 8	83.4	93.6
90	41.6	53.6	68.6	76.4
120	46.4	51.5	70.2	79.7

Table C.10. Changes in the concentration of PAH compounds (ppm) with time in the control bench-scale bioreactor.

DAYS	Acenaph-thylene	Acenaph-thene	Fluorene	Anthracene
0	118.8	118.4	119.3	122.7
15	89.3	93.5	101.6	108.3
30	83.7	87.0	95.8	102.7
45	99.2	104.9	111.6	115.5
60	91.7	99.5	102.5	111.8
75	73.6	81.3	94.8	102.4
90	94.9	101.7	116.2	120.3
120	72.4	78.1	95.4	102.4

APPENDIX D

Statistical analysis to determine the disappearance rates of the PAH compounds under the various redox conditions (from Chapter 4).

A straight line is best fitted to the set of data representing the disappearance of the PAH compounds under a particular redox environment. The straight-line fit was fixed at one point, that which corresponds to the initial concentration of the PAH compound. The slope of the fitted line, which indicates the rate of the disappearance of the compound, is denoted as M_1. Similarly, M_2 indicates the rate of disappearance of the compound from the control sample.

Also let μ_1 and μ_2 represent the slopes of the straight line fits, corresponding to M_1 and M_2, respectively, that might be obtained from an infinitely large number of sampling points.

Now, to construct the confidence interval for $\mu_1 - \mu_2$, do the following:

1. Test the hypothesis that

$$H_0 : \mu_1 - \mu_2 = 0 \text{ or } \mu_1 = \mu_2$$

using the following statistical values:

$$t' = \frac{(M_1 - M_2) - d_0}{\sqrt{S_1^2 + S_2^2}}$$

$$\nu = \frac{(S_1^2 + S_2^2)^2}{\frac{(S_1^2)^2}{n_1 - 2} + \frac{(S_2^2)^2}{n_2 - 2}}$$

where $\sigma_1 \neq \sigma_2$ and **unknown**

if t' lies in the critical region: $t' < -t_{\alpha/2}$ and $t' > t_{\alpha/2}$

then reject H_0 in favor of the alternative hypothesis:

$$H_1 : \mu_1 - \mu_2 = d_0 \text{ or } \mu_1 \neq \mu_2$$

2. The confidence interval for $\mu_1 - \mu_2$, where $\sigma_1 \neq \sigma_2$ and **unknown**:

$$M_1 - M_2 \pm t_{\alpha/2} \sqrt{S_1^2 + S_2^2}$$

3. Confidence interval for μ_1:

$$M_1 \pm t_{\alpha/2} S_1$$

and $\nu_1 = n_1 - 2$

where

variance of slope (M)	$S^2 = \frac{\hat{\sigma}^2}{SXX}$
residual mean square	$\hat{\sigma}^2 = \frac{RSS}{n-2}$
residual sum of squares	$RSS = SYY - \frac{(SXY)^2}{SXX}$
corrected sum of squares for the x_i's	$SXX = \Sigma(x_i - \bar{x})^2$
corrected sum of squares for the y_i's	$SYY = \Sigma(y_i - \bar{y})^2$
corrected sum of cross products	$SXY = \Sigma(x_i - \bar{x})(y_i - \bar{y})$
Sample average for the x_i's	$\bar{x} = \Sigma x_i/n$
Sample average for the y_i's	$\bar{y} = \Sigma y_i/n$
(days, concentration) for the i^{th} data point	(x_i, y_i)
number of data points	n

Table D.1. Statistical analysis to determine rates of biotic and abiotic losses for the four PAH compounds in the 500 ml-erlenmeyer flasks.

	ACYN. AERO.	ACYN. DENIT.	ACYN. SULF.	ACYN. METAH.	ACEN. AERO.	ACEN. DENIT.	ACEN. SULF.	ACEN. METAH.
M1 (ppm/day)	2.13	0.68	0.30	0.26	1.78	0.58	0.27	0.22
M2 (ppm/day)	1.60	0.32	0.33	0.21	1.39	0.26	0.25	0.17
VAR1	0.09	0.17	0.29	0.04	0.02	0.15	0.23	0.03
VAR2	0.18	0.09	0.03	0.03	0.10	0.07	0.02	0.02
n1	4.00	7.00	7.00	7.00	4.00	7.00	7.00	7.00
n2	5.00	7.00	7.00	7.00	5.00	7.00	7.00	7.00
t	2.21	1.83	-0.14	0.50	2.54	1.81	0.09	0.49
v	18.90	14.03	7.41	14.76	85.48	12.91	6.98	18.56
M1-M2 (ppm)	0.53	0.35	-0.03	0.05	0.39	0.32	0.02	0.04
conf. limit	>95%	>90%	•	•	>95%	>90%	•	•

	FLUOR. AERO.	FLUOR. DENIT.	FLUOR. SULF.	FLUOR. METAH.	ANTH. AERO.	ANTH. DENIT.	ANTH. SULF.	ANTH. METAH.
M1 (ppm/day)	1.68	0.45	0.20	0.11	1.39	0.40	0.10	0.08
M2 (ppm/day)	1.18	0.16	0.14	0.13	1.01	0.10	0.08	0.08
VAR1	0.06	0.04	0.19	0.03	0.16	0.01	0.05	0.05
VAR2	0.15	0.13	0.03	0.02	0.14	0.12	0.03	0.03
n1	4.00	7.00	7.00	7.00	5.00	7.00	7.00	7.00
n2	5.00	7.00	7.00	7.00	6.00	7.00	7.00	7.00
t	2.36	1.90	0.31	-0.15	1.62	2.15	0.19	0.00
v	27.86	127.57	8.34	17.28	11.96	825.17	16.81	15.60
M1-M2 (ppm)	0.50	0.29	0.06	-0.01	0.38	0.29	0.02	0.00
conf. limit	>95%	>90%	•	•	>85%	>95%	•	•

Table D.2. Statistical analysis to determine biotic and abiotic losses for the four PAH compounds in the bench-scale bioreactor.

	ACYN.	ACEN.	FLUO.	ANTH.
M1 (ppm/day)	0.73	0.64	0.48	0.39
M2 (ppm/day)	0.36	0.34	0.15	0.11
VAR1	0.07	0.11	0.13	0.07
VAR2	0.24	0.10	0.07	0.02
n1	7.00	7.00	7.00	7.00
n2	7.00	7.00	7.00	7.00
t	1.76	1.73	1.99	2.43
v	123.09	21.87	14.08	9.68
M1-M2 (ppm)	0.37	0.30	0.33	0.28
Conf. limit	>90%	>90%	>90%	>95%

APPENDIX E

Reprint of the published manuscript on the biodegradation of naphthalene in soil slurry.

Appl Microbiol Biotechnol (1990) 34 414-419

Applied and Microbiology Biotechnology

Effect of soil/contaminant interactions on the biodegradation of naphthalene in flooded soil under denitrifying conditions

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Received 1 March 1990 Accepted 3 August 1990

Summary. The mineralization of ^{14}C-labelled naphthalene was studied in pristine and oil-contaminated soil slurry (30% solids) under denitrifying conditions using a range of concentrations from below to above the aqueous phase saturation level. Results from sorption-desorption experiments indicated that naphthalene desorption was highly irreversible and decreased with an increase in the soil organic content, thus influencing the availability for microbial consumption. Under denitrifying conditions, the mineralization of naphthalene to CO_2 occurred in parallel with the consumption of nitrate and an increase in pH from 7.0 to 8.6. When the initial substrate concentration was 50 ppm (i.e. close to the aqueous phase saturation level), about 90% of the total naphthalene was mineralized within 50 days, and a maximum mineralization rate of 1.3 ppm day^{-1} was achieved after a lag period of approx. 18 days. When added at concentrations higher than the aqueous phase saturation level (200 and 500 ppm), similar mineralization rates (1.8 ppm day^{-1}) occurred until about 50 ppm of the naphthalene was mineralized. After that the mineralization rates decreased logarithmically to a minimum of 0.24 ppm day^{-1} for the rest of the 160 days of the experiments. For both of these higher concentrations, the reaction kinetics were independent of the concentration, indicating that desorption of the substrate governs the mineralization rate. Other results indicated that pre-exposure of soil to oil contamination did not improve the degradation rates nor reduce the lag periods. This study clearly shows the potential of denitrifying conditions for the biodegradation of low molecular weight PAHs.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds that are composed of two or more fused benzene rings, and have been found to exhibit mutagenic and carcinogenic effects (Heitkamp and Cerniglia 1988, Zedeck 1980). They are introduced into the environment through natural and anthropogenic processes, such as the incomplete combustion of fossil fuel and organic matter in coke production and petroleum refining (Lee 1981, Sims and Overcash 1983). In the last few decades, contamination with PAHs has received increased attention as a result of the unprecedented levels they have reached in the environment, particularly in soils and sediments (Jones 1988, Yland 1986). Due to their lipophilic properties, PAHs tend to accumulate and persist in soils for a relatively long time (Bulman et al. 1988). One of the PAHs that has been extensively studied is naphthalene, being with its methylated derivatives among the most toxic compounds found in the water-soluble fraction of petroleum (Heitkamp et al. 1987). A great amount of work in this field has focused on the mechanisms and the effects of various environmental factors on the biodegradation of naphthalene. Most of these studies have related to naphthalene biodegradation under aerobic conditions in which free oxygen acts as the final electron acceptor (Bauer and Capone 1985, Hosler et al. 1988).

Only a few authors have investigated naphthalene biodegradation in a soil-water system under denitrifying conditions. Furthermore, only few research workers have considered the sorption-desorption pattern of a pollutant to the soil matrix as a variable controlling the kinetics of biodegradation. The information available indicates that the dissolution rate of some PAHs, including naphthalene, is an important factor in controlling the rate of biodegradation (Thomas et al. 1986, Stucki and Alexander 1987). Miheloic and Luthy (1988a, b) showed that the soil desorption kinetics and the reversibility of desorption influences the rate of biodegradation of naphthalene. However their studies were conducted with concentrations much below the aqueous phase saturation level. This is of particular importance because at concentrations higher than the aqueous phase saturation level the sorption-desorption

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pattern of the contaminant is expected to be somewhat different. Furthermore, sites where soil is contaminated with very high concentrations of PAHs are common Such information can help design better processes for remediation of subsurface soils and ground-waters when denitrifying conditions are present. A study that has investigated naphthalene biodegradation in primary sewage effluent under denitrifying conditions reported no significant biodegradation rates (Bouwer and McCarty 1983) In another study, naphthalene was mineralized to a very low concentration (7 mg l^{-1}), in a soil-to-water ratio of 1:50 (Mihelcic and Luthy 1988b).

The objective of this work was to pursue the subject further by studying the biodegradation of a wide range of napthalene concentrations (50 to 500 ppm) under denitrifying conditions in a flooded system with a high soil-to-water ratio (1:3). Furthermore the study took into consideration the naphthalene sorption-desorption pattern as one of the parameters that possibly influence the biodegradation rate.

Materials and methods

Soil Two bulk soil samples were used in this study. First, a pristine soil obtained from the Biotechnology Research Institute land premises, Montreal, Quebec, and second, a weathered oil-contaminated soil sample obtained from the vicinity of a petroleum oil refinery in Montreal In both cases, the top 2-5 cm of soil was scraped off and the soil samples were collected at 5-10 cm depth The latter site has been contaminated for a long period of time (approximately 40 years) Both soils were dried at 35° C for 24 h and large particles removed with a 500 μ mesh sieve The sieved soils were analysed for total organic content and particle size distribution using the U S Department of Agriculture Scheme (Gee and Bauder 1986). These soils were further analysed for extractable oil content by extracting 5 g of soil with benzene in a Soxhlet extractor. The solvent was then evaporated (Buchi Rotavapor RE 120, Laboratorium-Technic, Flawil, Switzerland) until the oil was brought to dryness The weight of the oil remaining was then measured The characteristics of the two soils were as follows for the pristine soil the content of sand, silt and clay expressed as a percentage of the dry weight was 74, 21 and 5%, respectively, and the organic content was 2 1% No extractable oil was measured For the oil-contaminated soil, the sand, silt and clay contents were 78, 20 and 2% respectively and the organic content 4 3% In this soil the extractable oil content represented 1 2% of the dry weight

The two soils tested in both the sorption-desorption and biodegradation experiments were (1) a soil mixture consisting of 90% pristine soil and 10% weathered oil-contaminated soil (Soil 1) and (2), a 100% weathered oil-contaminated soil (Soil 2)

Mineral medium A mineral medium was used to flood the soil and to provide an excess of nutrients. This medium was prepared by dissolving the following salts in tap water KH_2PO_4, 0 170 g l^{-1}; K_2HPO_4, 0 430 g l^{-1}, Na_2HPO_4 $7H_2O$, 0 660 g l^{-1}, $FeCl_3 \cdot 6H_2O$, 0 003 g l^{-1}, NH_4Cl, 0 034 g l^{-1}, $MgSO_4$ $7H_2O$, 0 450 g l^{-1}, $NaNO_3$, 20 g l^{-1} The final pH of the medium was adjusted to 7 0

Naphthalene solution Naphthalene was obtained from Aldrich (Milwaukee, Wisc, USA) as a scintillation-grade crystalline powder with 99%+ purity The uniformly radio-labelled [^{14}C]naphthalene was obtained from Sigma (St-Louis, Mo, USA) with 98%+ purity and 4 7 mCi mmol^{-1} specific activity A solution with a specific activity of 10000 dpm mg^{-1} of naphthalene was prepared by adding the ratio-labelled [^{14}C]naphthalene to a stock solution This solution contained 66 7 g l^{-1} naphthalene dissolved in methanol

Sorption-desorption experiment This experiment was conducted according to the procedure described by DiToro and Horzempa (1982) Centrifugation vials (16 ml) with Teflon-lined screw caps were used for this experiment They were filled with a slurry made from a mixture of Soil 1 and mineral medium in a 30% (w v) ratio The naphthalene concentration in these vials was adjusted to 10, 25, 35, 50, 100, 200 and 500 ppm Another vial containing 200 ppm naphthalene was prepared as above, but in this case Soil was used To verify the soil sorption capacity a control experiment, using the same procedure but without adding soil was made All vials were placed on a Wrist-Action® Shaker, Model 75 (Burrell Co, Pittsburg, Pa, USA) and incubated at 35 C After 24 h of shaking, the vials were centrifuged for 10 min at 2000 g (Centra 4, International Equipment Co, Needham Heights, MA USA) To determine the naphthalene concentration 1 ml supernatant was added to 15 ml scintillation cocktail (AC S, Amersham Arlington Heights, Ill, USA) and tested in a scintillation counter (Packard Tri-Carb model 4530, Packard, Downersgrove Ill USA) The rest of the supernatant was discharged and replaced with fresh mineral medium The vials were returned to the shaker for a further 24 h incubation The procedure of introducing fresh amounts of mineral medium was repeated twice more, such that each time a new equilibrium state was determined

Biodegradation experiments The soil samples were incubated in 50-ml glass vials containing 9 g soil and 21 g mineral medium Each vial was equipped with a CO_2 trap containing 0 7 ml of 5 N NaOH in a 1 2-ml glass cup Each glass cup was supported by a steel rod suspending the cup above the slurry level in the head space of the vial The soil sample and mineral medium were added to the vial, the NaOH cup was put in place and a known amount of [^{14}C]-labelled naphthalene solution was injected into the soil slurry to obtain the required initial naphthalene concentration in terms of ppm total slurry weight Each vial was then sealed with a Teflon-lined stopper, wrapped in aluminum foil to prevent photodegradation of naphthalene, and then placed in a shaking water bath set at 100 rpm and kept at 35 C To avoid oxygen contamination, the above procedure was conducted in an anaerobic chamber filled with nitrogen gas A total of 100 vials were prepared in this manner, covering the four different series of experimental conditions For each series, 20 vials were prepared using the procedure described above and 5 vials received 500 ppm of $HgCl_2$ to halt bacterial activity (Wolf et al 1989) These controls were also used to verify the results obtained from the other experiments The incubating conditions, in terms of naphthalene initial total concentration and soil type, were (A) 50 ppm in Soil 1, (B) 200 ppm in Soil 1, (C) 500 ppm in Soil 1 and (D) 200 ppm in Soil 2

To avoid adding large amounts of methanol when the initial naphthalene concentration was 500 ppm, spiking was performed in two steps First, a known amount of non-labelled naphthalene was dissolved in ether in a boiling flask and 10% of the soil sample designated for this experiment was added to the prepared solution After mixing the content of the flask vigorously, the organic solvent was evaporated using a rotoevaporator until the soil fraction was dry The spiked soil was then mixed thoroughly with the rest of the soil sample to produce a homogeneous mixture One gram of the resulting soil was extracted with benzene in a Soxhlet apparatus and the naphthalene concentration was determined by HPLC Since the measured concentration was approximately 300 ppm, [^{14}C]naphthalene was added to reach a final concentration of 500 ppm

Sampling and analysis At given time intervals, two vials (duplicates) were terminated by the addition of 2 ml concentrated phosphoric acid, by syringe, to halt bacterial activity as well as to liberate any CO_2 trapped in the soil slurry The punctured hole in the stopper was sealed with silicone and the vials were returned to

the shaker for another 24 h The vials were then connected through a silicone tube to an external CO_2 trap containing a solution of 10 % NaOH The gas in the headspace of each vial was bubbled through the solution to recover all CO_2 This procedure was continued until no radioactivity was detected in the trap To remove any traces of [^{14}C]naphthalene, the NaOH solution in the glass cup was extracted with hexane The residual solution was then weighed and 0 1 ml was mixed with 15 ml scintillant The radioactivity of the trapped CO_2 was quantified using a liquid scintillation counter Correction for colour quenching was applied using an external standard The nitrate concentrations in the test samples were measured using an HPLC (model SP8100 with integrator model SP4200, Spectra-Physics, San Jose, Calif , USA) equipped with conductivity type detector (model 430, Waters Chromatography Division, Milford, Mass , USA) and an anion exchange column (model PRP-X100, Hamilton Comp , Reno, Nev , USA) Naphthalene concentration in the benzene extract was measured using a 25-cm reverse-phase column (model RP8, Brownlee, Santa Clara, Calif , USA) on the above HPLC equipped with a fluorescence detector (model Spectroflow 980, Kratos Co , Ramsey, N J , USA)

Results

Results from the sorption-desorption experiment are shown in Fig 1 The aqueous phase saturation concentration of naphthalene in the soil slurry was about 50 ppm When naphthalene was added at concentrations higher than the aqueous phase saturation level the sorption isotherm bent upwards indicating a substantial reduction in solubility and a significant naphthalene increase in the soil fraction (Fig 1A) For these high concentrations, naphthalene in the solid phase would probably be found both sorbed to soil and precipitated At concentrations lower than the saturation level, the sorption isotherm is best described by the trinomial function ($r^2 = 0.99$)

$$y = -1\,68 + 7\,15x - 0\,35x^2 + 7.33\ 10^{-3}x^3,$$

where x represents the fraction of naphthalene dissolved in the aqueous phase and y the fraction sorbed in the soil (see Fig 1B) The inflection point for the above function corresponds to an aqueous phase concentration of 16 mg l^{-1}. This function provided a better fit than the Langmuir ($r^2 = 0\,95$) or Freunlich ($r^2 = 0.90$) isotherm functions (Green 1984).

Desorption isotherms (Soils 1 and 2) were established from the seven data points used to construct the sorption isotherm For each of these points a desorption curve was obtained, suggesting that desorption is an irreversible process dependent on the amount of naphthalene previously sorbed (Fig. 1). The control desorption experiment, realized without soil, showed that the equilibrium concentration in the aqueous phase after each of the four desorption sequences always remained close to the saturation limit (50 ppm). This phenomenon demonstrates the strong sorptive capacity of these soils for naphthalene It also shows that the solubilisation of naphthalene in solid form (precipitate) never reached the saturation limit when soil was present The desorption behaviour of the weathered oil-contaminated soil (Soil 2) was studied only for the 200 ppm naphthalene concentration, which was the concentration used later in the mineralization experiments. Comparison between the desorption isotherm for Soils 1 and 2 indicated that the naphthalene partition of the weathered oil-contaminated soil shifted more towards the solid phase and that the concentration found in the aqueous phase decreased significantly below the saturation concentration The latter soil exhibited a greater degree of sorption (smaller slope) suggesting that the desorption is influenced by the soil organic content. In fact, the organic content of the weathered oil-contaminated soil was approx twice the concentration of the pristine soil (Table 1) The results also showed that the methanol introduced in different amounts in the system did not influence the solubility of naphthalene. In a separate experiment, it was found that when the total naphthalene concentration was increased to 500 ppm, the solubility of naphthalene in the aqueous phase increased by only 2 ppm As a result, the impact of the variations in methanol concentration on the sorption-desorption process was neglected

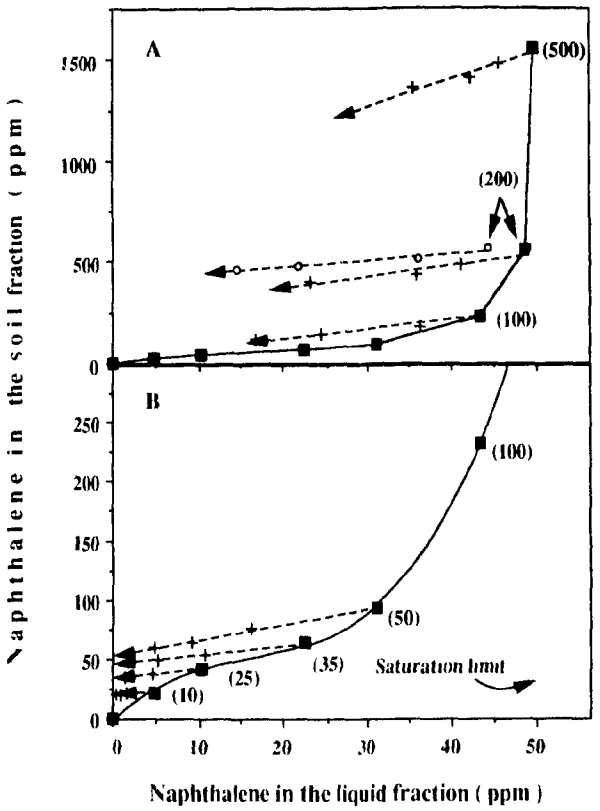

Fig. 1 A, B. Partition of naphthalene between the liquid and the solid phase after addition of a known amount *Numbers in parentheses* indicate the concentration of naphthalene injected (ppm) For the desorption experiment, each data point represents the new equilibrium obtained after 24 h of mixing with fresh medium **A** Full-scale presentation **B** Enlarged area for lower concentrations – ■ –, adsorption isotherm for soil slurry (Soil 1), – + – desorption isotherm for soil slurry (Soil 1), – ○ – desorption isotherm for oil-contaminated slurry (Soil 2) Values on the ordinate were calculated from the dissolved naphthalene measured in the liquid phase and by taking into account that the solid fraction represented 30% of the total slurry weight

Table 1. Decomposition of naphthalene under aerobic and denitrifying conditions

Type of ecosystem	Initial naphthalene concentration (ppm)	Temperature (°C)	Redox condition	Maximum decomposition rate (ppm day)	Reference
Marine sediment slurry	100	23	Aerobic	1.87[a]	(Bauer and Capone 1988)
Marine sediment slurry	100	23	Aerobic	11.70[a,b]	(Bauer and Capone 1988)
Soil-water slurry	7	Room temp	Aerobic	Approx. 1.5[c]	(Mihelcic and Luthy 1988a)
Soil-water slurry	7	Room temp	Denitrification	Approx. 0.25[c]	(Mihelcic and Luthy 1988a)
Soil-water slurry	500	35	Denitrification	1.7–1.8	This study
Soil-water slurry	200	35	Denitrification	1.7–1.8[c]	This study
Soil-water slurry	50	35	Denitrification	1.3[c]	This study

[a] Mineralized fraction
[b] Previous pre-exposure to naphthalene
[c] Measured upon disappearance from sample

Biodegradation of naphthalene under denitrifying conditions was monitored for 160 days (Fig. 2). The results obtained from the analysis of trapped $^{14}CO_2$ corresponded to the amounts of naphthalene mineralized and represented the average of two readings. The vertical lines indicate the standard deviation. Analysis of the traps from the control samples showed radioactivity levels similar to those of the background, indicating that mineralization due to abiotic processes is negligible.

Mineralization occurred under all experimental conditions. An adaptation period of approx. 18 days was observed in all cases. When the initial naphthalene concentration was 50 ppm (Fig. 2A) the rate of mineralization reached a maximum of 1.3 ppm day^{-1} after 18 days. The same rate was observed until about 80% of the naphthalene was mineralized. Following this period, the mineralization rate decreased rapidly. The total amount of naphthalene recovered in the form of $^{14}CO_2$ attained 90% after a 50-day period.

In the experiments where naphthalene was added at concentrations higher than the aqueous phase saturation level (200 and 500 ppm, Soils 1 and 2) mineralization resulted in similar curve profiles (see Figs. 2B, C and D). After the adaptation period, all curves showed an initial region (almost linear) followed by a gradual logarithmic decrease in the mineralization rate. This indicated that the factors controlling the mineralization process were similar for these three different experiments. The first region lasted until approximately 50 ppm was mineralized. The highest mineralization rates, corresponding to these sections, were 1.8 ppm day^{-1}. These values decreased to 0.24 ppm day^{-1} on the 160th day. The mineralization rate curves indicated that preexposure of the soil to hydrocarbon contamination did not effect the initial rate of biodegradation, but the subsequent biodegradation was less rapid (see Fig. 2D), with a minimum rate of 0.15 ppm day^{-1} observed on the 160th day.

Nitrate consumption and pH increase were associated with the denitrification process (Figs. 2A–D). In all cases, nitrate was in excess of the biological demand and did not act as a limiting factor to the mineralization process. The lowest nitrate consumption was observed with 50 ppm naphthalene and the highest with the oil-contaminated soil. Moreover, there was no detectable nitrate consumption in the control experiment indicating that denitrification was the dominant biological process. This was in agreement with the expected nitrate demand as a result of the biodegradation of methanol and naturally occurring organic carbon in the soil samples. Also, the nitrate consumption rate was highest in the initial stages of the experiment, when the easily biodegradable compounds (such as the introduced methanol) were metabolized. In all experiments, the pH increased in parallel with the utilization of nitrate. The highest pH value (8.6) was recorded for the experiment with the weathered oil-contaminated soil at 200 ppm naphthalene, while the lowest pH (8.0) was observed for Soil 1 with 50 ppm.

Discussion

The main objective of this work was to study the biodegradation of naphthalene in a soil-slurry system under denitrifying conditions. More specifically, it was to investigate the mineralization of ^{14}C-labelled naphthalene when it was present at concentrations close to or above the aqueous phase saturation levels, and the effect of the naphthalene sorption-desorption pattern on the biodegradation rate.

The results obtained in this study illustrate that naphthalene mineralization under denitrifying conditions occurred in all the experimental set-ups. These results also show that different mineralization rates were obtained in response to the induced changes in naphthalene concentration and soil type (Soil 1 vs Soil 2). In order to understand these differences it was necessary to study the naphthalene sorption-desorption pattern for the two soil compositions. The sorption isotherms presented in Fig. 1 show that addition of various amounts of naphthalene to the soil slurry results in different concentrations in the aqueous phase. Hence when the initial naphthalene concentrations were 200 and 500 ppm, the corresponding concentration in the aqueous phase never exceeded 50 ppm. Also, it was

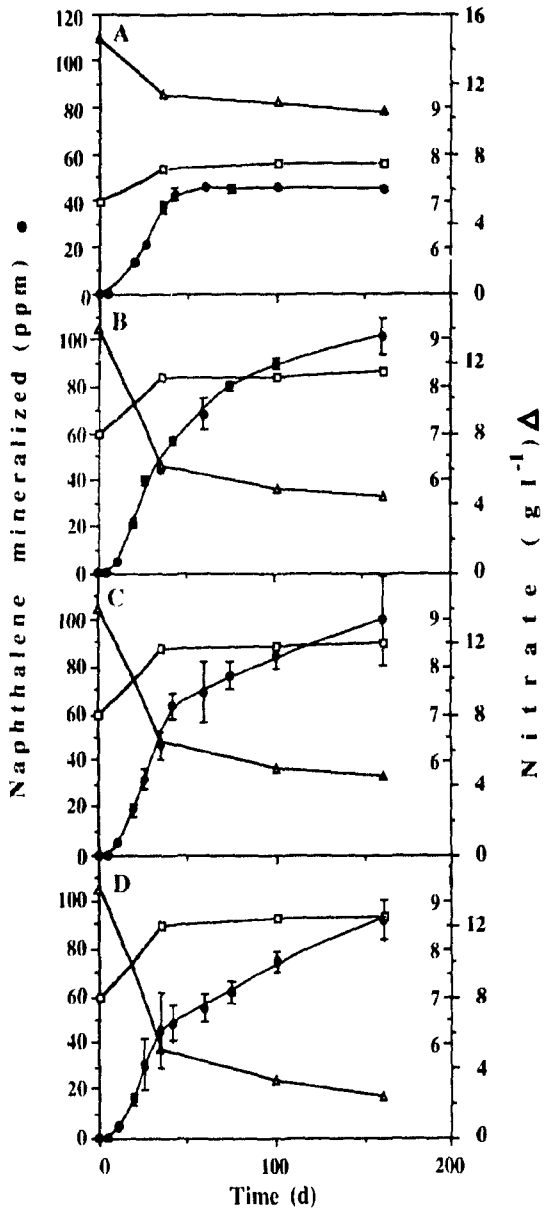

Fig. 2A–D. Mineralization of [^{14}C]naphthalene in soil slurries **A** 50 ppm in Soil 1 **B** 200 ppm in Soil 1. **C** 500 ppm total naphthalene concentration in Soil 1 **D** 200 ppm total naphthalene concentration, using oil-contaminated soil (Soil 2) □ pH

noted that the biodegradation rates obtained for these two different concentrations were similar Therefore, it is concluded that when naphthalene concentration in a soil slurry is maintained close to, or higher than the aqueous phase saturation level, the kinetics of mineralization are zero order with respect to the total substrate concentration In this case the desorption is controlling the rate of biodegradation However, in the experiment where the initial concentration was equal to the aqueous pha saturation level (50 ppm) the actual concentration available in the aqueous phase was significantly less (about 32 ppm) and the mineralization rate represented about 60% of that obtained for the 200 and 500 ppm experiments This decrease in the mineralization rate indicates that the availability of naphthalene as a carbon source for the microorganisms was an important factor in controlling the reaction speed This also suggests that once the concentration of naphthalene in the aqueous phase decreases below the saturation level, the mineralization rate becomes substrate dependent and exhibits higher order kinetics.

The low biodegradation rate (0 24 ppm day^{-1}), as observed after the first 50 ppm were mineralized for the 200 and 500 ppm naphthalene total concentration experiments (Figs. 2B, C and D) could be attributed to the following reasons Firstly, it could be due to sorption of naphthalene on the relatively high specific surface area of the solid material. For instance, Fig. 1 shows that the sorption of naphthalene on soil was very strong, thus limiting the amount available in the liquid phase for microbial attack. Therefore, in spite of the high amounts of naphthalene added, the aqueous concentration dropped quickly below the saturation level. In the experiment with the weathered oil-contaminated soil (Soil 2), the decrease in the reaction rate was found to be higher than with Soil 1, which was mostly pristine soil However, for the same time period, the naphthalene mineralized in Soil 2 was 13% less than in Soil 1 (Figs. 2B and D) This could probably be attributed to increased sorption due to the higher organic content of the contaminated soil Secondly, increase in pH probably inhibited the microbial activity.

Expectedly, when compared with pristine soil the weathered oil-contaminated soil did not support higher naphthalene-degrading activity nor a shorter lag period. This lack of difference cannot be associated with a possible lack of denitrifying activity because denitrifiers are ubiquitous in soil. On the other hand the higher concentration of organic matter (4 3% versus 2.1%) in the oil contaminated soil led to a quick pH rise (up to 8 6, see Fig. 2D) This increased pH could have reduced the activity of the denitrifiers

Comparisons between the results of this study and studies conducted by other researchers are difficult to evaluate partly because the soils used were different and did not exhibit identical sorption-desorption patterns (Table 1). From that table, it appears that naphthalene biodegradation rates obtained under denitrifying conditions were in the same range as those obtained under aerobic conditions. It also indicates that denitrifying conditions could play an important role in the decomposition of PAHs in nature. From an engineering perspective, it would be easier and probably more economical to supply the needed amounts of electron acceptors for the decontamination reaction to take place in the soil-water system in the form of nitrate, rather than as molecular oxygen. These results show also that, in order to reach a better understanding of the biodegradation process of an organic pollutant in a soil-water system, a certain amount of knowledge is needed about the interaction of the contaminant with the solid phase. Work is being pursued to assess the best operating conditions for using denitrification as a means to treat PAH-contaminated soils and to better understand the influence of PAH sorption-desorption patterns on

the biodegradation kinetics using soils of various composition.

Acknowledgements The authors gratefully acknowledge the technical assistance of Chantale Beaulieu and Sylvie Sanschagrin and the helpful comments obtained from Drs Yves Comeau and Charles W Greer

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