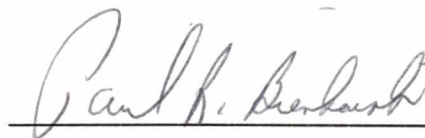


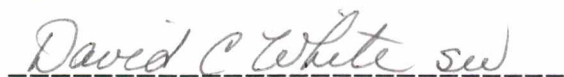
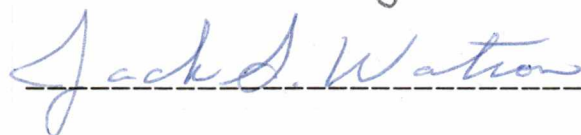
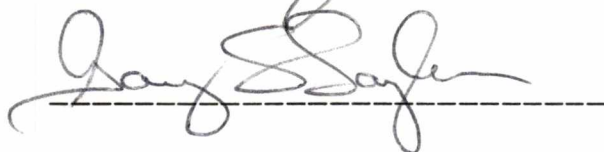
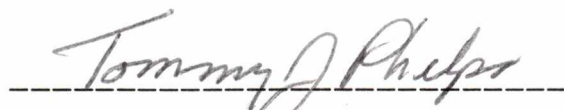
To the Graduate Council:

I am submitting herewith a dissertation written by Oren Francis Webb entitled " Analysis of *Pseudomonas fluorescens* HK44 for use as a biosensor for PAH pollutant degradation and migration. " I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemical Engineering.



Paul R. Bienkowski, Chairperson

I have read this dissertation and
recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor
and Dean of the Graduate School

Analysis of *Pseudomonas fluorescens* HK44 for use as a biosensor
for PAH pollutant degradation and migration.

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Oren Francis Webb
December 1992

DEDICATION

This dissertation is dedicated to my wife

Mary Carville Webb,

my son Jacob Stephen Webb,

and

to my parents Francis L. and Janet T. Webb.

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ABSTRACT

Alginate immobilized *Pseudomonas fluorescens* HK44 was investigated for use as a biosensor organism. Mass transfer, adsorption, and multiple substrate effects on alginate immobilized *Pseudomonas fluorescens* HK44 were investigated. Biological and physical processes were analyzed using simulated subsurface conditions and material balances. A structured model was developed to describe physical and biological processes occurring in the reactor system. The structured model consisted of systems of coupled ordinary and partial differential equations. Orthogonal collocation and finite difference methods were used for numerical solution of the model equations when analytical solutions were unavailable.

Model parameters were measured using steady and unsteady state experiments. Least square techniques indicated that an overall second order rate equation, first order rate in salicylate and first order in biomass, adequately described salicylate degradation by *P. fluorescens* HK44. The reaction rate constants obtained using this model ranged from 2.30×10^{-2} to $3.45 \times 10^{-2} \text{ dm}^3/\text{g}/\text{min}$. Alginate proved to be an excellent support material for evaluating the degradation kinetics of salicylate by *P. fluorescens* HK44. Salicylate did not adsorb to alginate while naphthalene adsorption on alginate was linear. Bioluminescent activity was significant in the presence of salicylate and naphthalene. Light emission was not

significant when exposed to non-inducing substrates. Steady state light emission was found to be a function of salicylate concentration.

Adsorption of PAH affected the availability of PAH compounds in soils. The varying physical structure of soils and distribution of pollutants greatly complicated analysis of adsorption using traditional step and pulse perturbation methods. Sinusoidal perturbation methods proved to be an effective method for dynamic analysis of polycyclic aromatic hydrocarbon adsorption in soils.

A previously developed packed bed reactor (PBR) system was used in this research for simulating the soil environment. On-line analytical tools for measuring steady and unsteady state behavior are described.

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TABLE OF ABBREVIATIONS

FID_Flame Ionization Detector

GC_Gas Chromatography

HPLC_High Performance Liquid Chromatography

LC_Liquid Chromatography

PAH_Polycyclic Aromatic Hydrocarbon

PBR_Packed Bed Reactor

PFR_Plug Flow Reactor

RPLC_Reverse Phase Liquid Chromatography

SRM_Standard Reference Material

TPB_Three-Point Backward Finite Difference Formula

TABLE OF SYMBOLS

a_i	Dimensionless constant in Langmuir adsorption isotherm
A	Surface Area of solid phase per unit volume of reactor (dm^2/dm^3)
b_i	Langmuir equilibrium constant (dm^3/mol)
C_B	Biomass concentration (mol/dm^3)
C_i	Concentration of component i in liquid phase (mol/dm^3)
$C_{i0_{\text{Max}}}$	Maximum feed concentration of component i (mol/dm^3)
C_{Di}	Dimensionless concentration of component i in liquid phase (dimensionless)
C_{DPi}	Dimensionless concentration of component i in the liquid phase inside the pores of the particle (dimensionless)
C_{DS_i}	Dimensionless adsorbed solid phase concentration of component i (dimensionless)
C_{p_i}	Concentration of component i in liquid phase inside the pores of the particle (mol/dm^3)
C_{s_i}	Adsorbed solid phase concentration inside the particle (mol/dm^3)
$C_{s_i}^*$	Equilibrium adsorbed solid phase concentration inside the particle (mol/dm^3)
D_m	Molecular diffusivity (dm^2/s)
D_c	Microcrystal diffusivity (dm^2/s)
Da	Damkohler number (Dimensionless)
D_p	Pore diffusivity (dm^2/s)
D_{s_i}	Solid diffusion coefficient (dm^2/s)
D_{l_i}	Dispersion coefficient of component i (mol/dm^2)

f_{L_i} —Flux from liquid to solid phase (mol/dm²-s)
 f_{i_p} —Flux in liquid void of the particle (mol/dm²-s)
 f_{i_s} —Flux on solid surface of particle (mol/dm²-s)
 $g(x)$ —Function
 j_{D_i} —Flux of component i (mol/dm²-s)
 k —Second order rate constant (dm³/mol-s)
 k_e —Adjustable constant for exponential function in equation (40)
 k_f —External fluid film mass transfer coefficient (dm/s)
 k_m —Michaelis constant in Equation (14) (mol/dm³)
 K_c —Equilibrium constant (dimensionless)
 K_L —Overall mass transfer coefficient (dm/s)
 L —Bed length (dm)
 P —Pressure (atmospheres)
 Pe —Peclet number with bed length as the characteristic length
(Dimensionless)
 $P_i(x)$ —Orthogonal polynomial
 P_i —Period of sin wave
 q_i —Solid phase concentration of component i (mol/dm³)
 q_s —Saturation coverage (mol/dm³)
 r —Position in particle (dm)
 r_c —Microcrystal radius (dm)
 R —Dimensionless radius of particle in spherical coordinates
 Re —Reynold's number (Dimensionless)
 R_i —Reaction rate (mol/dm³-s)
 R_p —Particle radius (dm)
 S —Cross sectional area of conduit or reactor (dm²)

t _Time (s)
 t_r _Mean residence time (s)
 u_f _Fluid velocity (dm/s)
 v _Fluid velocity (dm/s)
 $V_{\max}$ _Maximum rate of reaction in Equation (14) (mol/dm³-s)
 w_i _Wave velocity of component i (dm/s)
 $W(x)$ _Weighting function
 Y _Dependent variable in equation (28)
 Y_i _Mole ratio of component i (Dimensionless)
 z -Position in column (dm)
 Z _Dimensionless position in column
 α _Reaction order in Equation (13) (Dimensionless)
 α_i _Adjustable constants used in collocation method
 β _Reaction order in Equation (13) (Dimensionless)
 β_i _Constants in equation (28)
 γ_1 _Constant Equation (4) (Dimensionless)
 γ_2 _Constant Equation (4) (Dimensionless)
 ε _Bed void volume (Dimensionless)
 ε_p _Particle void volume (Dimensionless)
 θ_{s_i} _Ratio of of maximum feed concentration of component i to the
 maximum ratio of component 1 (Dimensionless)
 μ _Mean residence time (s)
 ρ_f _Fluid phase density (g/dm³)
 σ _Variance (s)
 τ _Dimensioless time
 φ _Dimensionless position in the particle

1 INTRODUCTION

1.1 OVERVIEW

1.1A BACKGROUND AND GENERAL STATEMENT OF PROBLEM

Microorganisms can potentially decontaminate many types of sites contaminated with hazardous waste. The effective use of bacteria for restoration of contaminated sites depends upon a number of important factors including: 1) availability of substrates, 2) maintenance of long term microbial degradation activity, and 3) survival of useful microbial populations. Availability depends in part upon a number of coupled transport processes. Genetic capability, viability, and a host of other factors affect the long term degradation activity of the organisms. Survival of degrading bacteria depends upon factors such as availability of nutrients, temperature, and ecology.

Past production of synthetic natural gas (town gas) from the pyrolysis of coal has produced several thousand sites nationwide which are contaminated with polycyclic aromatic hydrocarbons

(PAH) termed manufacturers gas plant (MGP) soil. These sites range in size from less than one acre to over 100 acres, with contamination at depths exceeding 15 feet. Much concern exists over these sites since many PAH compounds such as benzo(a)pyrene, benzo(j)fluoranthene, benzo(b)fluoranthene, benzo(e)pyrene and others are believed to be carcinogenic (Harrison, et al., 1974). PAH readily adsorb onto soil matrices (Karickhoff, 1979) and also diffuse into pores and the solid matrix of soil particles.

Adsorption, liquid phase diffusion, and solid phase diffusion occur by different mechanisms and occur at significantly different rates. PAH distribution in and on the soil greatly affects the remediation process. If biodegradation occurs in the liquid phase, then solid phase diffusion of the pollutant through the solid matrix to the bulk liquid phase may easily be the rate controlling step.

Adsorption is the movement of a chemical species from a fluid phase onto the surface of a solid. In many cases the solid has a high affinity for the compound and reduces the liquid phase concentration. Adsorption and degradation act as competing sinks for the compound and are termed coupled processes, i.e., the rates of adsorption and degradation affect one another. Adsorption complicates analysis of bioremediation technologies both mathematically and physically. Separate description of biological and physical processes result in complicated but reliable models for prediction of remediation effectiveness.

The physical structure of the adsorbing solid may profoundly affect the liquid phase availability of a compound. If the pollutant

is distributed in a relatively nonporous solid, then significant contaminant transport may occur by solid diffusion. The decreased porosity of the particle reduces the area for liquid diffusion to occur and, hence, reduces liquid diffusion rates. The main transport mechanism for the pollutant then becomes the solid diffusion process. Solid diffusion is orders of magnitude slower than the liquid diffusion process.

New kinetic tools are needed for analysis of the complicated biological, ecological and physical processes occurring in the subsurface. Without proper tools evaluation of the potential for use of bioremediation technologies is inadequate. Developed bioluminescent reporter strains show great promise for use as kinetic tools and biosensors (King et al., 1990; Burlage et al., 1990; and Dunbar et al., 1991) because they are "non invasive, nondestructive, rapid and population specific" (DiGrazia, 1991). DiGrazia (1991) and Heitzer et al. (1992) demonstrated strong relationships between bioluminescence and pollutant degradation activity for *Pseudomonas fluorescens* HK44 (King et. al., 1990). *P. fluorescens* HK44, is an environmental isolate from a manufacturer's gas plant soil that received the constructed bioluminescent reporter plasmid pUTK21 (King et. al., 1990). DiGrazia (1991) found that bioluminescence and degradation were nonlinear processes using frequency response analysis. Heitzer (1992) demonstrated in batch experiments that bioluminescence is correlated with naphthalene concentration.

1.1B INTENT OF RESEARCH

The overall intent of this research was to provide new tools for environmental research. For researchers to understand the environment, a host of challenges present themselves due to the complex nature of physical, biological and ecological processes. Without proper tools, evaluation of the potential usefulness and effectiveness of bioremediation is not possible. Bioluminescent reporter strains are potentially a powerful tool for environmental researchers because they have the potential to indicate optimal degradation conditions and to provide important environmental insights.

The effects of mass transfer, adsorption, and multiple substrates on alginate immobilized *P. fluorescens* HK44 were investigated using steady and unsteady state experiments. Microbial activity was affected by dispersion, adsorption, mass transfer and the presence of multiple substrates in the subsurface environment. If bioluminescence is to be used as a quantitative indicator of PAH degradation activity, present investigations must be expanded to determine the effects of physical and chemical processes on PAH degradation and bioluminescence. Sinusoidal and step perturbations were investigated for studying soil adsorption processes. The varying physical structure of soils and distribution of pollutants dampen the system response to perturbations in feed concentrations.

1.1C GENERAL RESEARCH APPROACH

Biological and physical processes were analyzed using simulated subsurface conditions and material balances. Mass transfer, dispersion, convection, and adsorption were assumed to be important physical processes in the subsurface environment. A structured model was developed to describe physical and biological processes occurring in the reactor system. The structured model consists of systems of coupled ordinary and partial differential equations. Orthogonal collocation and finite difference methods are used for numerical solution of the model equations when analytical solutions are unavailable.

Model parameters were measured using steady and unsteady state experiments. Single component degradation kinetics were investigated under steady state conditions. Unsteady state experiments were used to measure physical processes. Steady state experiments were used to measure reaction rates while unsteady state experiments were used to measure physical processes.

A previously developed packed bed reactor (PBR) system was also proposed for use as a tool in this research (Webb, et al, 1991; Webb, 1991). A packed bed reactor is well suited for studying physical and microbial interactions because the reactor configuration simulates the soil environment. On-line analytical tools were available for measuring steady and unsteady state

behavior (Webb, et al, 1991; Webb, 1991; Dunbar et al., 1991). Real time monitoring facilitated analysis.

Bacterial degradative activities are similar to chemical heterogeneous reaction schemes although bacterial degradative activity may be complicated by growth, induction, inhibition and other biological processes. Under appropriate conditions degradation in soil matrices may be modeled using heterogeneous reaction schemes. Heterogeneous reactions take place in multiple liquid and solid phases and are slowed by inter- and extra-particulate fluid diffusional resistance, adsorption, and solid phase diffusional processes. In contaminated soil the biodegradation of xenobiotic chemicals by microorganisms may be influenced by one or more of these processes. A hydrophobic compound such as naphthalene predominantly adsorbs onto the organic components of the soil (Karickhoff, 1984). At equilibrium the naphthalene will be partitioned throughout the solid matrix of the organic matter, on the surface of the organic matter, and in the liquid and gas phases. Theoretically the bioavailability of naphthalene and hence the biodegradation of naphthalene is influenced by one or all of these processes.

1.1D OBJECTIVES OF RESEARCH

The overall objective of this work was to provide insight into how mass transfer, adsorption, and multiple substrates affect degradation and bioluminescence of alginate immobilized *P. fluorescens* HK44 in simulated aquifer systems. Mass transfer and fluid dynamics of the reactor system were characterized using different support media. The degradative activities of bacteria were investigated with respect to measurable dependent variables. Control variables were examined to determine their role in degradative processes. Different models were evaluated for describing adsorption and reaction processes. Methods were investigated for analyzing adsorption in soils contaminated with complex mixtures of organic wastes. Specifically the objectives of this work were:

- 1) Characterize degradation kinetics of engineered bioluminescent bacteria,
- 2) Analyze the relation between reporter degradative activities and bioluminescence,
- 3) Investigate methods for analysis of adsorption in heavily contaminated soils,
- 4) Investigate the role of adsorption in the bioremediation of heavily contaminated PAH soils.

1.2 POLYCYCLIC AROMATIC HYDROCARBON PHYSICAL CHARACTERISTICS

PAH refers to a broad class of chemicals that are characterized by the presence of fused aromatic rings. PAHs have similar chemical and physical properties to one another. Many physical properties of PAHs such as solubility are greatly influenced by the absence of electronegative atoms, their high molecular weights and their conjugation. PAH chemical properties are also greatly influenced by their highly conjugated ring systems. Unlike aliphatic compounds which tend to undergo addition reactions, PAH compounds are very stable and tend to undergo substitution reactions (Morrison & Boyd, 1973). Spectral properties of PAHs are also highly affected by the presence of their conjugated systems (Morrison & Boyd, 1973).

According to Lee *et al.* (1979), the presence of high energy π bonding orbitals and lower energy π^* anti-bonding orbitals produce the characteristic electronic spectra of PAH molecules. In unconjugated molecules the π^* anti-bonding orbitals generally have lower energy than π bonding orbitals (Brittain, *et al.*, 1970). Brittain *et al.* (1970) report that increasing conjugation causes a red shift in the absorption spectra. Solvent polarity may also cause a red shift in the absorption spectra.

PAHs exhibit fluorescence and phosphorescence. After a PAH molecule absorbs a photon, an electron is elevated to an excited

state (Figure 1). Lee *et al.* (1979) maintain that the electron will drop to the lowest available vibrational level, S_1 , through non-radiative processes. If the spin of the electron was changed by absorption of the electron, then the electron will drop to the lowest available triplet state, T_1 . The movement of the electron from the excited state (S_1 or T_1) to the ground state, S_0 , is termed fluorescence or phosphorescence, respectively. Fluorescence is a relatively fast process (10^{-8} seconds) compared to phosphorescence (10^{-4} to 10^2 seconds) (Calder, 1969).

Lee *et al.* (1979) state that the availability of π electrons and the highly conjugated structures of PAHs greatly affect their solubility. The π electrons allow PAHs to act as electron donors in charge transfer complexes. Acceptor molecules must possess low-energy vacant orbitals. Some complexes, such as picric acid and 2,4,7-trinitrofluoren-9-one, are stable enough for melting point measurement (Lee *et al.*, 1979). Also, the polarizability of the conjugated π electron systems may preferentially increase solubility in different solvents. Unsubstituted PAHs of moderate to high molecular weight are generally soluble at less than 1 milligram/liter in water.

Because many pesticides are similar to PAHs, information available about adsorption and degradation of some pesticides in soils may be applied to PAH interactions in soils. Saltzman and Yaron (1986) review key papers on interactions between pesticides and soils. They make the observation that the adsorption of

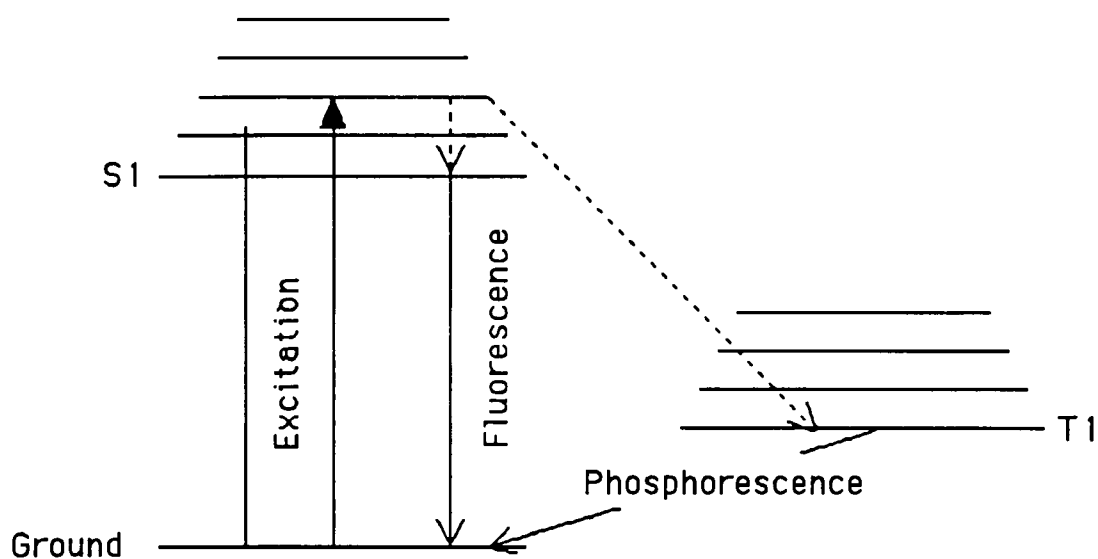


Figure 1. Excitation Emission Diagram. Adapted from Lee, M. L., Novotny, M. V., and Bartle, K. D., *Analytic Chemistry of Polycyclic Aromatic Hydrocarbons* Academic Press, New York, 1979.

pesticides correlated best with the organic matter content in soil although functional groups of the organic matter may affect the ratio of available hydrophobic/hydrophilic adsorption sites (p.66). Thermodynamic evidence and other observations indicate that hydrophobic bonding may play an important role in adsorption of nonpolar pesticides in soils. Saltzman and Yaron (p. 64) note that adsorption of nonpolar pesticides to charged clay surfaces is very low. Pesticide adsorption in soils is usually described by the empirical Freundlich equation or the Langmuir equation.

Linear and nonlinear PAHs differ in chemical reactivity under environmental conditions (Lee *et al.* 1979). Lee *et al.* (1979) state that reactivity of linear PAHs increases with ring number up to heptacene. Both linear and angular PAHs undergo Diels-Alder substitution to form endoperoxides in the presence of oxygen and light. Upon irradiation under anaerobic conditions, linear PAHs dimerize. Angular PAHs do not have an increased tendency for reactivity with increasing ring number, nor for photodimerization. Adsorption onto solids in certain cases increases reactivity (Sawicki, 1970). Also, several PAHs react in the presence of light when dissolved in water (Katz *et al.*, 1979, Zepp & Schlotzauer, 1979).

1.3 BIOLOGICAL DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS

Cerniglia and Heitkamp (1989), in their review of PAH metabolism by eukaryotes and prokaryotes, observes that several general statements may be made about degradative pathways. Prokaryotes use a dioxygenase attack with further oxidation to dihydroxy compounds through cis-dihydrodiols. Eukaryotes use a monooxygenase attack with further oxidation to trans-dihydroxydiols. Bacteria may use PAHs as a source of carbon and energy. Fungi metabolize PAHs to detoxify them but have not been shown to use them as a source of carbon or energy. Recalcitrance of PAHs generally increase with ring number.

Pseudomonas strains and a number of bacterial species are capable of aerobic naphthalene degradation. Continuing their review, Cerniglia and Heitkamp, (1989) comment that many of the degradative and regulatory genes are plasmid encoded for the *Pseudomonas* species. Initial attack consists of oxygen incorporation forming cis-1,2-dihydro-1,2-dihydroxynaphthalene (Jefferey *et al.*, 1975; Catterall *et al.*, 1971; and Ensley *et al.*, 1982). Further oxidation forms 1,2-dihydroxynaphthalene (Patel *et al.*, 1974). The ring is then cleaved with the formation of cis-o-hydroxybenzal pyruvate (Davies & Evans, 1964). Salicylic aldehyde and pyruvic acid are formed by cleavage of the side chain. The aldehyde is converted to salicylic acid (Davies & Evans, 1964;

Rockind *et al.*, 1986). Catechol is then formed from the salicylic acid. Further metabolism occurs through the meta or ortho pathways. The ortho pathway consists of intradiol cleavage of the ring. The meta pathway consists of extradiol cleavage of the ring. If the bacteria uses the ortho pathway, acetyl-CoA and succinic acid are formed (Ornston & Stanier, 1966; Stanier & Ornston, 1973). If the bacteria uses the meta pathway, acetaldehyde and pyruvic acid are formed (Bayly & Dagley, 1969; Dagley & Gibson, 1965; Sala-Trepat & Evans, 1971; Sala-Trepat *et al.*, 1971; Williams *et al.*, 1971).

Cerniglia and Heitkamp (1989) also discuss the similarities of the anthracene and phenanthrene degradative pathways to naphthalene degradation (Cerniglia & Heitkamp, 1989). *Pseudomonas* species attack anthracene at the 1,2 position (Jerina *et al.*, 1976; Akhtar, 1975) whereas phenanthrene is attacked at the 1,2 or the 3,4 position (Evans *et al.*, 1965; Jerina *et al.*, 1976; Kiyohara & Nagao, 1978; Barnsley, 1983; Colla *et al.*, 1959; Kiyohara *et al.*, 1976; Kiyohara *et al.*, 1982; Ghosh & Mishra, 1983).

Dihydroxyanthracene is formed (Jerina *et al.*, 1976; Akhtar, 1975) and is followed by ring cleavage (Evans *et al.*, 1965). Metabolism of anthracene continues with the formation of 2-hydroxy-3-naphthoic acid. Thus the metabolite of anthracene, 2-hydroxy-3-naphthoic acid enters the naphthalene pathway. Cis-3,4-dihydro-3,4-dihydroxyphenanthrene is converted to 1,2-dihydroxynaphthalene and then enters the naphthalene pathway.

Prolonged exposure of bacteria to PAHs may increase the ability of the microbial community to metabolize PAHs (Cerniglia & Heitkamp, 1989). Cerniglia & Heitkamp (1989) also indicate that some members of a bacterial community may be eliminated by the introduction of toxic PAHs. Other members that are resistant to or are able to metabolize the PAHs may increase.

Many other factors may affect the degradation of PAHs in the environment. Oxygen has been shown to be important for degradation of PAHs although anaerobic degradation may occur. Also, PAH may isomerize or form endoperoxides in the presence of colloids and light. These reaction products may be more recalcitrant than the parent compounds. Temperature and other physical and chemical factors as well as biological community factors such as predation may also affect PAH degradation rates. Also, nutrient status of the cells, the presence of metabolic genes and cometabolites are also important factors affecting degradation.

A number of methods may be used to examine the ecology of a community. Gene probes (Jain, *et al.*, 1988) allow detection of specific genotypes. Gene probes have the advantage of detecting sub-populations with the genetic capability for the process in question. Lipid analysis may be used to produce a finger print of the community. Also, the lipid phosphate content is a reproducible measure of biomass. (White *et al.*, 1979).

1.4 BIOLUMINESCENCE

Bioluminescence in a number of species has been genetically (Meighen, 1988; and Meighen, 1991) and physiologically (Hastings *et al.*, 1985) characterized. Light production depends upon two key reactions (Meighen, 1991):



The actual light reaction (1) is catalyzed by heterodimeric luciferase which is encoded by the *luxA* and *luxB* genes. The second reaction, (2) responsible for aldehyde synthesis and fatty acid recycling, is catalyzed by a reductase, transferase, and synthetase. The required enzymes for the second reaction are encoded by the *luxC*, *luxD*, and *luxE* genes, respectively. The *luxCDABE* genes thus code for the light reaction and also for transformation of the product fatty acid back to the aldehyde substrate. The production of light depends on the availability of aldehyde, oxygen, NADPH, ATP, and FMNH₂.

The degradation of many pollutants including naphthalene and salicylate degradation (and many other pollutants) is often mediated by expression of plasmid-encoded, inducible catabolic operons

(Sayler et. al, 1990). The naphthalene degradative genes on the catabolic plasmid NAH7 are organized in two operons which are positively regulated and inducible by salicylate. The arrangement of the NAH operons is shown in Figure 2. The regulatory protein encoded by the nahR gene binds to the promoter of the lower and upper naphthalene operons. The upper naphthalene operon codes for enzymes responsible for degradation of naphthalene to salicylate. The lower salicylate operon codes for enzymes responsible for degradation of salicylate to compounds appropriate for entrance into the TCA cycle. After the critical cellular concentration of salicylate is reached, the upper and lower pathways are induced by salicylate binding to the regulatory protein (Schell, 1990). The expression of these genes may be monitored by a number of methods including direct measurement of mRNA (Erickson et al., 1987; and Sayler et al., 1991), specific enzyme activity, or by the use of gene fusions (Heitzer et al., 1990; Jain et al., 1988; and Silhavy et al., 1985).

The genetic characterization of the lux system made it possible to use the lux genes as a reporter for the measurement of gene expression (Engbrecht et al., 1985) The fusion of lux and catabolic NAH genes provides an attractive method for measuring specific degradative activities of target bacteria populations. The fusion of lux genes from bioluminescent bacteria and catabolic plasmids, may provide a rapid in situ and on-line method for monitoring gene expression.

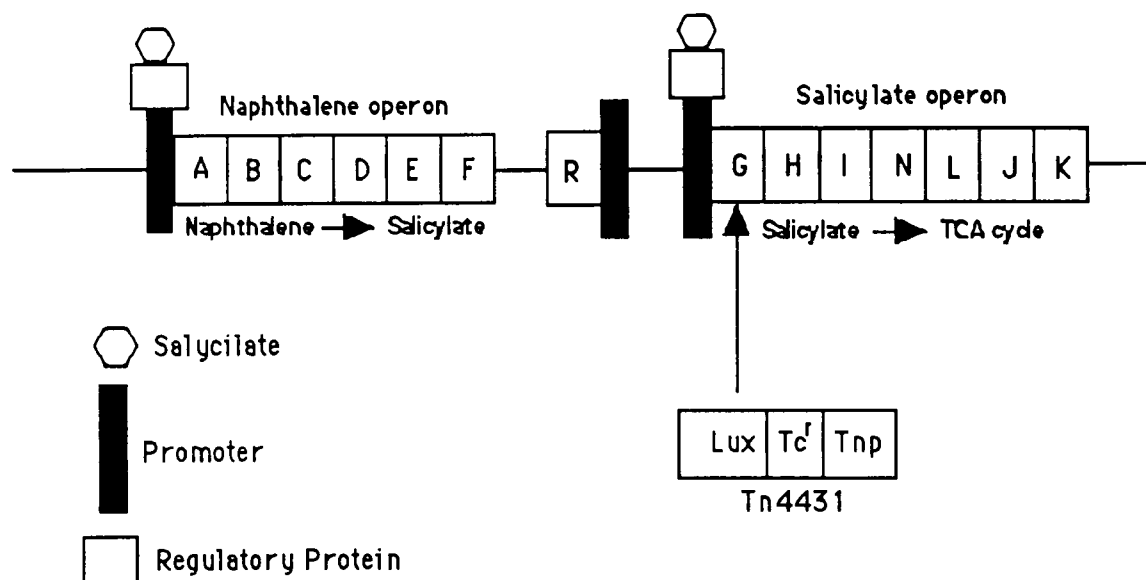


Figure 2. Arrangement of nah genes on NAH7 plasmid and the insertion site of the lux transposon Tn4431. (Adapted from Saylor & Fox, 1991).

The bioluminescent reporter plasmid pUTK21 was constructed by transposon mutagenesis of the naphthalene catabolic plasmid pKA1 (King et al., 1990). The lower NAH pathway in pKA1 was disabled by insertion of the lux transposon Tn4431 (containing the luxCDABE genes) into the nahG gene (Figure 2) resulting in pUTK21. *P. fluorescens* HK44, an environmental isolate from a MGP soil received the bioluminescent reporter plasmid, pUTK21 (King et. al., 1990). *P. fluorescens* HK44 has the ability to degrade salicylate independent of the Nah pathway (DiGrazia, 1991) supplementing the disabled lower pathway. Plasmid pKA1 was previously shown to contain a NAH type operon as found on the plasmid NAH7 by southern hybridization (Applegate et al., unpublished data).

P. fluorescens HK44 shows promise as a bioluminescent reporter strain (Sayler et. al, 1989; King et al., 1990; Dunbar et al., 1991; DiGrazia, 1991; Heitzer et al., 1992). The bioluminescent response of *P. fluorescens* HK44 correlated well with naphthalene and sodium salicylate concentration. DiGrazia (1991) demonstrated that bioluminescence and degradation in mixed cultures were nonlinear processes.

1.5 TRANSPORT, MULTICOMPONENT ADSORPTION AND CHEMICAL REACTION UNDER ISOTHERMAL CONDITIONS (GENERAL MODEL)

Component movement through a packed reactor bed occurs by convection and dispersion. As components move through the bed, molecules also diffuse into and out of the packing, adsorb onto the packing and are degraded by cells present in the packing material. Figure 3 illustrates the movements of chemical components in the packed bed reactor. The model used in this work uses a mass balance on the bulk fluid phase and a mass balance on the packing. The liquid phase unsteady state mass balance for component i yields:

$$\int_t^{t+\Delta t} \left\{ (S\epsilon u_f C_i)_{z,t} - (S\epsilon u_f C_i)_{z+\Delta z,t} + (S\epsilon j_{D_i})_{z,t} - (S\epsilon j_{D_i})_{z+\Delta z,t} + \int_z^{z+\Delta z} f_{L_i} (1-\epsilon) dz \right\} dt$$

$$= \int_z^{z+\Delta z} \{ (S\epsilon C_i)_{z,t+\Delta t} - (S\epsilon C_i)_{z,t} \} dz \quad (1).$$

Compound moves into the balance region through boundary A at position z by convection, $S\epsilon u_f$, and dispersion, $S\epsilon j_D$, (Figure 3) and then out of the balance region across boundary B at position $z + \Delta z$ by convection and dispersion. $S\epsilon$ is the surface area available for convection or dispersion. Compound can also move from the liquid

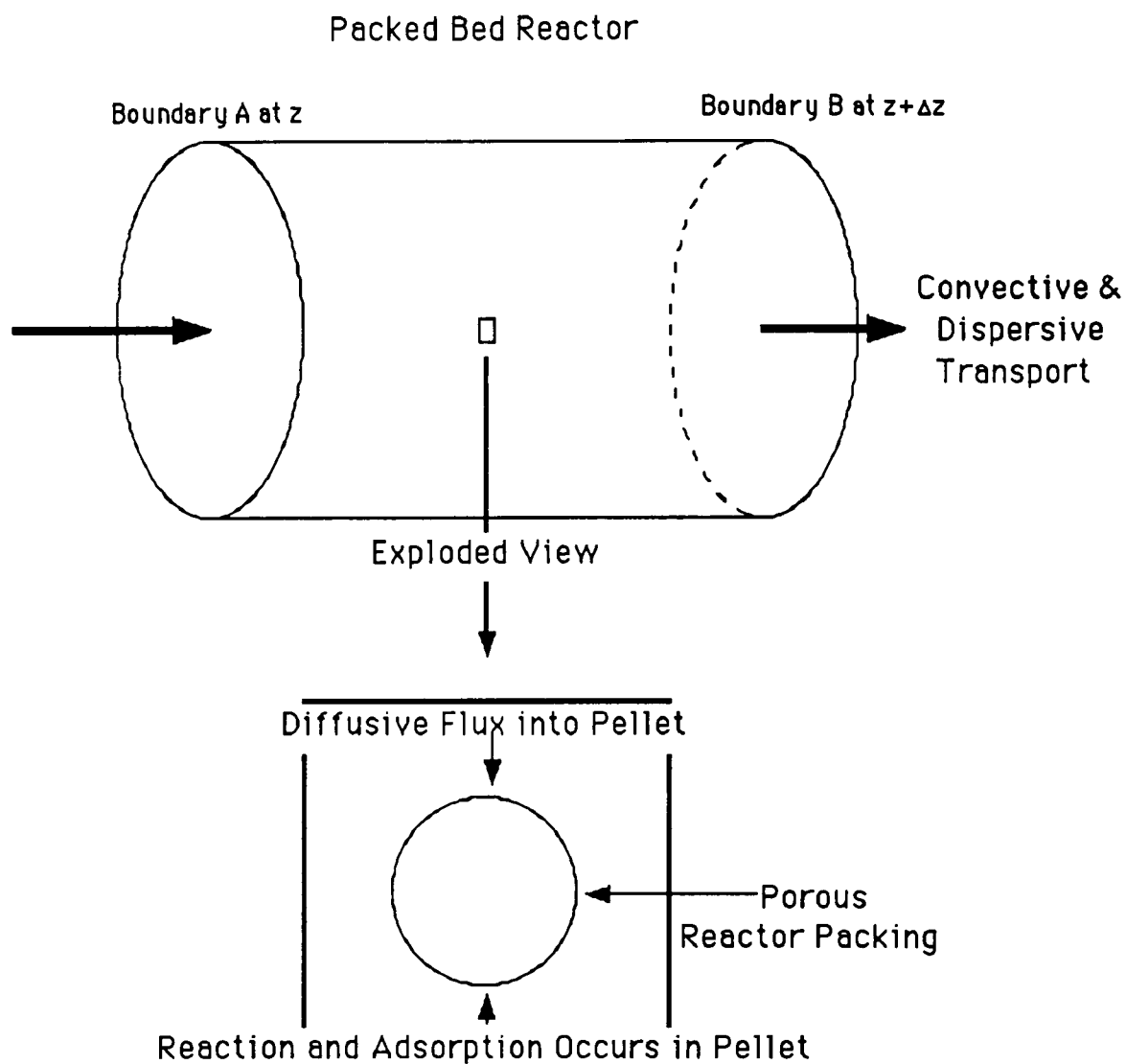


Figure 3. Liquid phase model for packed bed reactor.

phase to the solid phase, $f_{L_i}(1-\varepsilon)$. The $(1-\varepsilon)$ is the bed fraction that is solid and available for accumulation and reaction. The total movement of the compound across the boundaries is integrated over time to account for changes in convection and dispersion at the boundaries. The difference in movement across boundaries A and B is due to accumulation and/or reaction in the solid phase. The bulk phase mass balance is produced by subtracting the movement across boundary B from the movement across boundary A. The changes in concentration at a single position and at the times t and $t + \Delta t$ in the balance region are accounted for by the right hand side of equation (1). The right hand side is integrated over the whole balance region to account for changes at different points.

Equation (1) is transformed into a differential equation by applying the mean value theorem of integral calculus to both sides. Equation (1) then becomes

$$\begin{aligned} & \{ (S\varepsilon u_f C_i)_{z,t} - (S\varepsilon u_f C_i)_{z+\Delta z,t} + (S\varepsilon j_{D_i})_{z,t} - (S\varepsilon j_{D_i})_{z+\Delta z,t} + f_{L_i}(1-\varepsilon)\Delta z \} \Delta t \\ & = \{ (S\varepsilon C_i)_{z,t+\Delta t} - (S\varepsilon C_i)_{z,t} \} \end{aligned} \quad (2).$$

Both sides are divided by $\Delta z \Delta t$. Upon simplification and taking the limit as Δt and Δz go to 0, equation (1) yields:

$$D_i \frac{\partial^2 C_i}{\partial z^2} - \frac{\partial}{\partial z} (v C_i) - \frac{\partial C_i}{\partial t} + \frac{A}{\varepsilon} f_{i_L} = 0 \quad (3).$$

Significant flow is assumed to occur only in the axial direction. The first term on the left accounts for movement of species i due to dispersion. D_l is an effective dispersion coefficient that accounts for radial and axial dispersion (Aris, 1956). In many cases the axial dispersion term is neglected. The second term describes movement of species i due to convective forces. The third term from the left accounts for the change in concentration of species i in the liquid phase as a function of time. The fourth term accounts for the flux of species i to the solid phase from the liquid phase.

For fully developed flow, dispersion can be split into two parts (Ruthven, 1984):

$$D_l = \gamma_1 D_m + 2\gamma_2 R_p v \quad (4).$$

Equation (4) indicates that dispersion includes affects from both molecular diffusion and fluid movement around bed particles. In many cases the molecular diffusion term is negligible for liquids (Ruthven, 1984). Levenspiel (1972) presents a compilation of experimental Peclet numbers plotted as a function of Reynolds' Number. The Peclet number is a dimensionless parameter comparing the movement of a species by diffusion to movement by convection. For Reynolds' numbers (laminar flow conditions) as low as 0.1, Levenspiel indicates that the Peclet number approaches a limiting value of 1.8.

The fourth term on the right hand side of equation (3) accounts for flux to the solid phase. The flux is caused by adsorption,

reaction and concentration gradients in and around the pellet. The flux term may be replaced by

$$f_{i_L} = K_{L_i} (C_i - C_{P_i})_{r=R} \quad (5).$$

Dispersion and mass transfer may be measured using analytical expressions for the first and second moments of the column response to a pulse (Haynes & Sarma 1973). The model is very general and may be used to determine mass transfer resistances and dispersion coefficients. Pellets are assumed to be aggregates of smaller porous particles. Model assumptions include a linear isotherm, liquid phase mass transfer resistance, dispersion, macropore (diffusion in the aggregate), and micropore (diffusion in the small particles) diffusion. Equations (6) and (7) relate the first and second moments of the column response to the model parameters:

$$\mu = \frac{L}{v} \left[1 + \left(\frac{1-\varepsilon}{\varepsilon} \right) \varepsilon_p + \left(\frac{1-\varepsilon}{\varepsilon} \right) (1-\varepsilon_p) K_c \right] \quad (6),$$

$$\begin{aligned}
\sigma^2 = & \frac{2L}{v} \frac{D_L}{v^2} \left[1 + \left(\frac{1-\varepsilon}{\varepsilon} \right) \varepsilon_p + \left(\frac{1-\varepsilon}{\varepsilon} \right) (1-\varepsilon_p) K_c \right]^2 \\
& + \frac{2}{3} \frac{L}{v} \frac{R_p}{k_f} \left(\frac{1-\varepsilon}{\varepsilon} \right) [\varepsilon_p + (1-\varepsilon_p) K_c]^2 \\
& + \frac{2}{15} \frac{L}{v} \frac{R_p^2}{\varepsilon_p D_p} \left(\frac{1-\varepsilon}{\varepsilon} \right) [\varepsilon_p + (1-\varepsilon_p) K_c]^2 \\
& + \frac{2}{15} \frac{L}{v} \frac{r_c^2}{D_c} \left(\frac{1-\varepsilon}{\varepsilon} \right) (1-\varepsilon_p) K_c
\end{aligned} \tag{7}.$$

Convection may be the largest contributor to the movement of species *i*. When the convective term is highly dominant, the dispersion term may be ignored. Under appropriate conditions the error introduced by ignoring the dispersion term is insignificant.

Equation (8) accounts for adsorption, degradation, and diffusion into the particles of the bed. The development presented is similar to that of Holland & Liapis (1983). Performing an unsteady state material balance over a spherical shell in a single pellet of the packed bed results in the following equation:

$$\begin{aligned}
& \int_t^{t+\Delta t} \{ [(f_{i_s} + \varepsilon_p f_{i_p}) 4 \pi r^2]_{r,t} - [(f_{i_s} + \varepsilon_p f_{i_p}) 4 \pi r^2]_{r+Dr,t} + [r_i 4 \pi r_m^2 \Delta r]_{r,t} \} dt \\
& = \int_r^{r+\Delta r} \{ 4 \pi r^2 [(\varepsilon_p C_{P_i} + C_{S_i})_{t+Dt,r} - (\varepsilon_p C_{P_i} + C_{S_i})_{t,r}] \} dr
\end{aligned} \tag{8}.$$

Applying the Mean Value Theorem of Calculus to both sides of equation (8), taking the limit of Δt and Δx and simplifying:

$$\frac{1}{r^2} \frac{\partial}{\partial r} [r^2 (f_{i_s} + \epsilon_p f_{i_p})] + R_i = \frac{\partial}{\partial t} (\epsilon_p C_{p_i} + C_{s_i}) \quad (9).$$

Noting that the flux in the pellet occurs only by diffusion, equation (9) becomes:

$$\frac{1}{r^2} \frac{\partial}{\partial r} \left[r^2 \left(D_{s_i} \frac{\partial C_{s_i}}{\partial r} + \epsilon_p D_{p_i} \frac{\partial C_{p_i}}{\partial r} \right) \right] + R_i = \frac{\partial}{\partial t} (C_{s_i} + \epsilon_p C_{p_i}) \quad (10).$$

The solid phase diffusion term, the unsteady state pellet liquid and/or solid phase concentrations in equation (10) are sometimes ignored. Solid diffusion is generally several orders of magnitude less than liquid diffusion (Treyball, 1980), and may be considered insignificant. The pellet liquid phase concentration may also be insignificant to the solid phase concentration if the adsorbent has a high capacity. If the adsorption capacity of the pellet is small compared to the liquid phase, then the solid phase terms may be ignored. Applying the chain rule, equation (10) may take on any of the following forms:

$$D_{s_i} \left[\frac{2}{r} \frac{\partial C_{s_i}}{\partial r} + \frac{\partial^2 C_{s_i}}{\partial r^2} \right] + \epsilon_p D_{p_i} \left[\frac{2}{r} \frac{\partial C_{p_i}}{\partial r} + \frac{\partial^2 C_{p_i}}{\partial r^2} \right] + R_i$$

$$= \varepsilon_p \frac{\partial C_{p_i}}{\partial t} + \frac{dC_{s_i}^*}{dC_{p_i}} \frac{\partial C_{p_i}}{\partial t} \quad (11a),$$

$$D_{s_i} \left[\frac{2}{r} \frac{\partial C_{s_i}}{\partial r} + \frac{\partial^2 C_{s_i}}{\partial r^2} \right] + \varepsilon_p D_{p_i} \left[\frac{2}{r} \frac{\partial C_{p_i}}{\partial r} + \frac{\partial^2 C_{p_i}}{\partial r^2} \right] + R_i = \frac{dC_{s_i}^*}{dC_{p_i}} \frac{\partial C_{p_i}}{\partial t} \quad (11b),$$

$$\frac{1}{r^2} \frac{\partial}{\partial r} \left[r^2 (\varepsilon_p D_{p_i} \frac{\partial C_{p_i}}{\partial r}) \right] + R_i = \varepsilon_p \frac{\partial C_{p_i}}{\partial t} + \frac{dC_{s_i}^*}{dC_{p_i}} \frac{\partial C_{p_i}}{\partial t} \quad (11c),$$

$$\frac{1}{r^2} \frac{\partial}{\partial r} \left[r^2 (\varepsilon_p D_{p_i} \frac{\partial C_{p_i}}{\partial r}) \right] + R_i = \frac{dC_{s_i}^*}{dC_{p_i}} \frac{\partial C_{p_i}}{\partial t} \quad (11d),$$

$$\frac{1}{r^2} \frac{\partial}{\partial r} \left[r^2 (\varepsilon_p D_{p_i} \frac{\partial C_{p_i}}{\partial r}) \right] + R_i = \varepsilon_p \frac{\partial C_{p_i}}{\partial t} \quad (11e).$$

In deriving equations (11a-e) the solid phase and liquid phase concentrations inside the pellet are assumed to be in equilibrium, thus the adsorption isotherm relates the solid and liquid phase concentrations inside the pellet. For multicomponent scenarios, the component pellet mass balances maybe coupled through the reaction and adsorption terms.

Appropriate initial and boundary conditions for equations (3) and (11) are:

$$C_i(t < 0, z) = f(z) \quad (12a),$$

$$C_{p_i}(t < 0, r) = C_{s_i}^*(t < 0, r) = 0 \quad (12b),$$

$$\left[\frac{\partial C_i}{\partial z}\right]_{z=L} = 0 \quad (12c),$$

$$K_{L_i}(C_i - C_{P_i})_{r=R} = \varepsilon_p D_{i_p} \left[\frac{\partial C_{P_i}}{\partial r}\right]_{r=R} \quad (12d),$$

$$\left[\frac{\partial C_{P_i}}{\partial r}\right]_{r=0} = \left[\frac{\partial C_{S_i}}{\partial r}\right]_{r=0} = 0 \quad (12e).$$

R_i of equation (11) accounts for generation of component i from chemical and/or biological reaction and can be modeled using a number of forms. Two commonly used reaction terms are:

$$R_i = -kC_i^\alpha C_B^\beta \quad (13),$$

$$R_i = -C_B \frac{V_{\max} C_i}{k_m + C_i} \quad (14).$$

Equation (13) is commonly used to describe chemical reaction rates and indicates that the rate of reaction of i will continue to increase as the concentrations of i and B increase. The superscripts, a and b , in equation (13) indicate that the rate of reaction is proportional to the concentration of i raised to the a power and that the rate is proportional to the concentration of B raised to the b power.

Equation (14) is the familiar Michaelis-Menten rate equation and indicates that the rate of reaction will approach the limiting value, $V_{\max}$. The equations predict similar results at low concentrations ($\alpha = \beta = 1$) but predict significantly different maximum rates. Other mathematical forms may be used to represent conversion of i .

Equations (3), (11), and (12) may be made nondimensional. Let

$$C_i = C_{Di} C_{i0_{Max}} \quad (15a),$$

$$LZ = z \quad (15b),$$

$$C_{P_i} = C_{DP_i} C_{i0_{Max}} \quad (15c),$$

$$C_{S_i} = \theta_{S_i} C_{DS_i} C_{i0_{Max}} \quad (15d),$$

$$t_R \tau = t \quad (15e),$$

$$\phi R_p = r \quad (15f).$$

Substitution of equations (14a-f) and their derivatives produces the dimensionless form of equations (3), (11), and (12a-e):

$$\frac{t_r D_{Li}}{L^2} \frac{\partial^2 C_{Di}}{\partial Z^2} - \frac{\partial C_{Di}}{\partial Z} - \frac{\partial C_{Di}}{\partial \tau} + \frac{(1-\varepsilon)}{\varepsilon} \frac{3t_r}{r_0} K_{L_i} (C_{Di} - C_{DP_i})_{r=r_0} = 0 \quad (16),$$

$$\begin{aligned} & \frac{2}{R_p^2 \phi} \left(D_{S_i} \theta_{S_i} \frac{\partial C_{DS_i}}{\partial \phi} + \varepsilon_P D_{P_i} \frac{\partial C_{DP_i}}{\partial \phi} \right) + \frac{1}{R_p^2} \left(D_{S_i} \theta_{S_i} \frac{\partial^2 C_{DS_i}}{\partial \phi^2} + \varepsilon_P D_{P_i} \frac{\partial^2 C_{DP_i}}{\partial \phi^2} \right) \\ & = \frac{dC_{DP_i}}{dt} \left(\frac{\varepsilon_P}{\tau} + \frac{\theta_{S_i}}{\tau} \frac{dC_{DS_i}}{dC_{DP_i}} \right) + R_i = \frac{\partial C_{DP_i}}{\partial t} \left(\frac{\varepsilon_P}{\tau} + \frac{\theta_{S_i}}{\tau} \frac{dC_{DS_i}}{dC_{DP_i}} \right) \quad (17), \end{aligned}$$

$$C_{D_i}(t < 0, z) = \frac{f(z)}{C_{0i_{max}}} \quad (18a),$$

$$C_{DP_i}(t < 0, r) = C_{DS_i}^1(t < 0, r) = f(z) \quad (18b),$$

$$\left[\frac{\partial C_{D_i}}{\partial Z} \right]_{z=L} = 0 \quad (18c),$$

$$\frac{R_p K_{L_i}}{\varepsilon_p D_{i_p}} (C_{D_i} - C_{DP_i})_{\varphi=1} = \left[\frac{\partial C_{DP_i}}{\partial r} \right]_{\varphi=1} \quad (18d),$$

$$\left[\frac{\partial C_{DP_i}}{\partial \varphi} \right]_{\varphi=0} = \left[\frac{\partial C_{DS_i}}{\partial \varphi} \right]_{\varphi=0} = 0 \quad (18e).$$

These equations form the general model that will be used in this work. The model relates transport processes such as convection, dispersion, and mass transfer with adsorption and biological reaction. These equations do not take into account biological processes such as growth, ecology, or maintenance.

1.6 ADSORPTION UNDER EQUILIBRIUM CONDITIONS

Adsorption is an important process for environmental cleanup of PAH. The bioavailability of PAH and hence the treatment of contaminated sites may be profoundly affected by adsorption processes.

When mass transfer resistance is negligible, equilibrium is quickly reached between the solid and liquid phases. Mass transfer processes are defined here as the movement of a species onto, into,

and/or through the bed packing material from the bulk fluid phase. When mass transfer resistance is insignificant, the balance equations simplify and are amenable to analytical solution. Fundamental knowledge of adsorption phenomena may be gained by examination of the analytical solution for equilibrium multicomponent adsorption. Although few real world situations approach the equilibrium case (negligible mass transfer resistance and dispersion), the solutions are useful in that they supply a theoretical model to compare other solutions to.

Component adsorption isotherms may greatly affect unsteady state column behavior (see equations (16) and (17)). Isotherms may be classed into two general types. These two general types may be further divided into five groups depending upon whether there is mono- or multilayer coverage (Branauer *et al.*, 1940). A type I isotherm is termed a favorable isotherm and indicates that the adsorbed solute-solute affinity is less than the solute-adsorbent affinity. Type III is termed an unfavorable isotherm and indicates that adsorbed solute-solute affinity is less than the solute-adsorbent affinity. (See Figure 4). Type II and type IV isotherm are multilayer analogues of a type I isotherm. A type V isotherm is a multilayer analogue of a type III isotherm. Favorable isotherms are all concave downward while unfavorable are concave upward. The concavity of the isotherm has a profound affect upon column behavior.

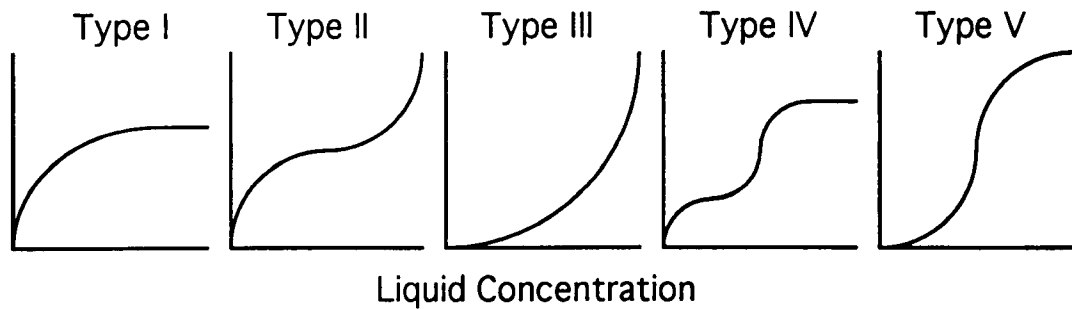


Figure 4. Types of Isotherms as defined by Brannauer *et al.* (1940).

Adapted from Ruthven, D. M., *Principles of Adsorption and Adsorption Processes*, John Wiley & Sons, New York, 1984.

Qualitative adsorption effects may be studied using the analytical solution for adsorption when mass transfer and dispersion are negligible. Equation (19) is a mass balance on component i assuming convective transport, negligible dispersion, negligible accumulation in the fluid phase, and negligible mass transfer:

$$v \frac{\partial C_i}{\partial z} + \left(\frac{1 - \varepsilon}{\varepsilon} \right) \frac{\partial q_i}{\partial t} = 0 \quad (19)$$

Equations (20) and (21) are boundary and initial conditions for solution of the partial differential equation (PDE) system;

$$C_i = C_{i_0} \quad t \geq 0 \quad (20),$$

$$C_i = 0 \quad t < 0 \quad (21).$$

A system containing j components is described by a coupled system of j PDEs. The j PDEs describe a surface in $j+2$ dimensions. When mathematical equations are coupled, solution is greatly complicated. The $j+2$ dimensioned surface described by the coupled PDE system may be transformed to a j dimension system consisting only of ordinary differential equations (ODE) using the Method of Characteristics (Gluekauf, 1949; Ruthven, 1984; Coroyannakis, 1987; and Yang, 1987). The system of ODEs is then numerically solved to produce a solution in Y_j space. The predicted adsorption phenomena is dampened by mass transfer resistance and dispersion. The Method of Characteristics is only applicable to Riemann problems (uniform feed and initial conditions with a single step change). Much of this discussion is summarized from Coroyannakis (1987).

The multicomponent Langmuir adsorption isotherm (equation (22)) is used for general representation of favorable type isotherms:

$$q_i = \frac{q_s b_i C_i}{1 + \sum_{i=1}^n b_i C_i} \quad (22).$$

The mathematical development of the isotherm includes the following important assumptions:

- 1) Surface coverage occurs at levels no greater than a monolayer,
- 2) Adsorbed molecules do not interact,

- 3) Adsorption occurs at a finite number of sites on the solid surface, and
- 4) All sites are open to adsorption by all adsorbing molecular species.

For multicomponent Langmuirian systems all solution characteristics in the transformed space are lines tangent to a parabolic solution envelop (Figure 5) (Coroyannakis, 1987). The solution envelope is tangent to the system axes. Prediction of front velocities, wave front types, and displacement is possible using a simple geometric approach suggested by Coroyannakis (1987) and Gluekauf (1949). The solution in Y_i space then can be transformed back to q_i - Y_i and q_i - Z - t space using the isotherm model.

Figure 5 shows characteristic solutions for a Langmuirian system (Coroyannakis, 1987). Positive and negative solution occur from the transformation from the $j+2$ dimensioned space to the j dimensioned space. Coroyannakis (1987) describes the solution for a two component Langmuirian system (Figure 5). The parabolic solution envelop is tangent to the axes at points termed watershed points. Equation (23) defines the parabolic envelope;

$$\left[\frac{a_2 - a_1}{a_1 b_2} + Y_1 - Y_2 \right]^2 + 4 Y_1 Y_2 = 0 \quad (23)$$

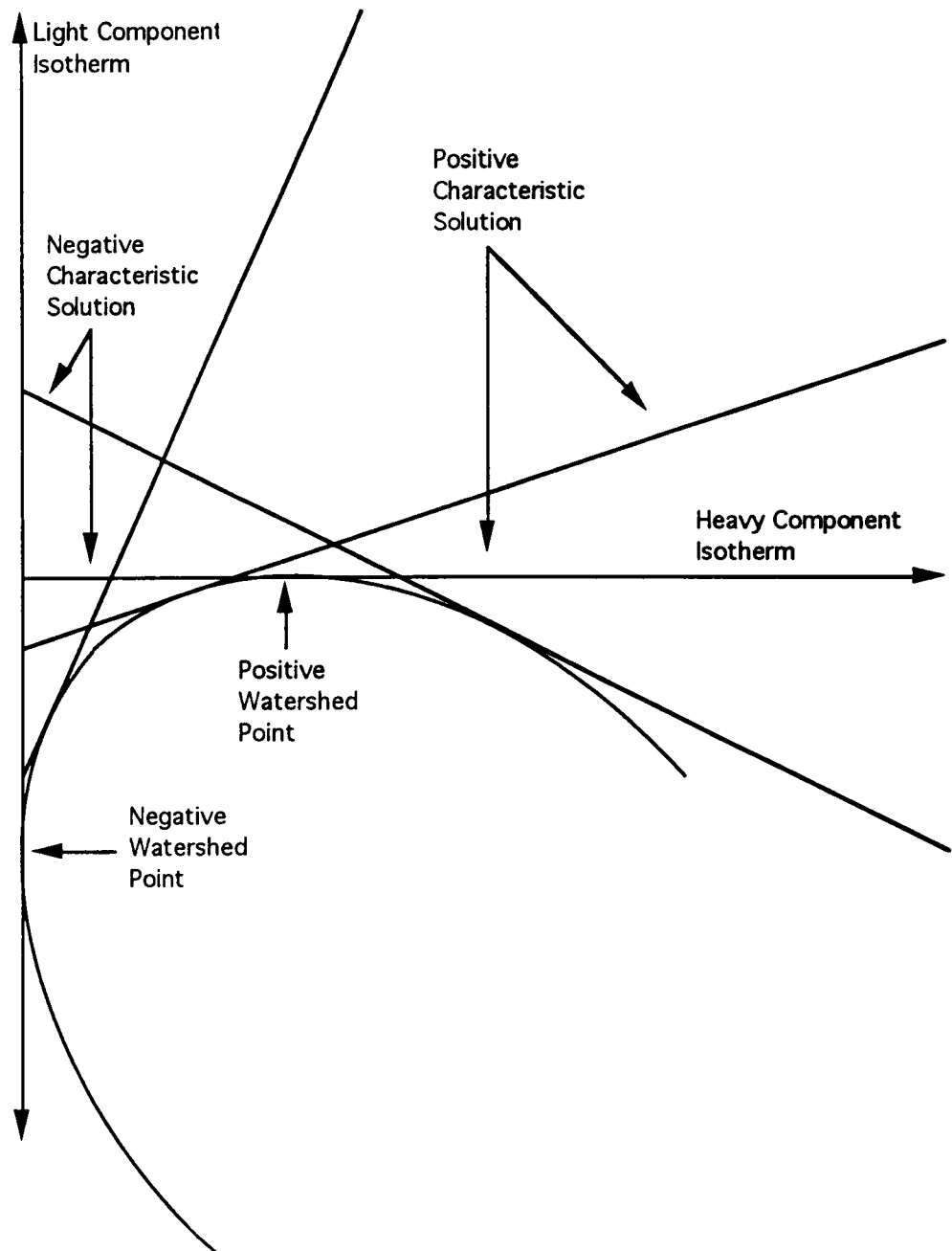


Figure 5. Concentration relationship between two adsorbing components in a packed bed with negligible mass transfer and dispersion. Adapted from Coroyannakis, Thesis, University of Toronto, 1990.

The physical significance of the watershed point can be examined by considering the relationship between isotherm slope and bed velocity for the dual component case. Component velocities are inversely proportional to their isotherm slopes (equation 24):

$$w_1 = \frac{v}{1 + \left(\frac{1-\varepsilon}{\varepsilon} \right) \left(\frac{dq_1^*}{dc_1} \right)} \quad (24).$$

The greater the affinity of the solute for the adsorbent (the greater the isotherm slope), the longer the solute stays adsorbed to the solid phase. Species movement occurs through convection in the liquid phase. Components that are strongly adsorbed move slowly through an adsorption bed because they spend little of their time in the liquid phase where movement can occur. The watershed point is the intersection of the limiting slope of the light component isotherm at the origin and the heavy component isotherm. A unique watershed point exists for each pair of Langmuirian solutes. If the heavy component concentration is less than the watershed point then the heavy component isotherm slope is always greater than the light component slope; therefore, the heavy component velocity is always less than the light component velocity. For example, simultaneous introduction of two square wave pulses of benzene and benzo(a)pyrene might result in two totally separated pulses in the effluent because the benzo(a)pyrene pulse may travel much slower than the benzene pulse.

The axes in the hodogram correspond to pure component isotherms in $Y-q$ space. The watershed point splits the heavy component isotherm into negative and positive characteristic solutions. The negative watershed point does not have physical significance.

Figure 6 shows the relation between concentrations in Figure 5 (hodogram) and the individual component isotherms. The curves on the isotherm were produced by taking the points from a characteristic solution and plugging them into the isotherm relation (equation 21). The solid phase concentration is then plotted as a function of the liquid phase concentration read from the characteristic solution curve in Figure 5.

The bed concentration (as a function of time) can be read from the hodogram. The positive and negative characteristic solutions correspond to concentration fronts in the bed (Figure 7). There is a single positive characteristic that passes through the initial bed concentration and indicates the leading concentration front in the bed. There is also a single negative characteristic that passes through the feed concentration and indicates a second front in the bed. The intersection of these positive and negative solution characteristics, the plateau point, corresponds to a zone of constant concentration between the two concentration fronts. The transition zones may be gradual increases or discontinuities referred to as shocks. The transition will be a shock if dq/dY (isotherm slope) decreases on the adsorption pathway from the initial concentration

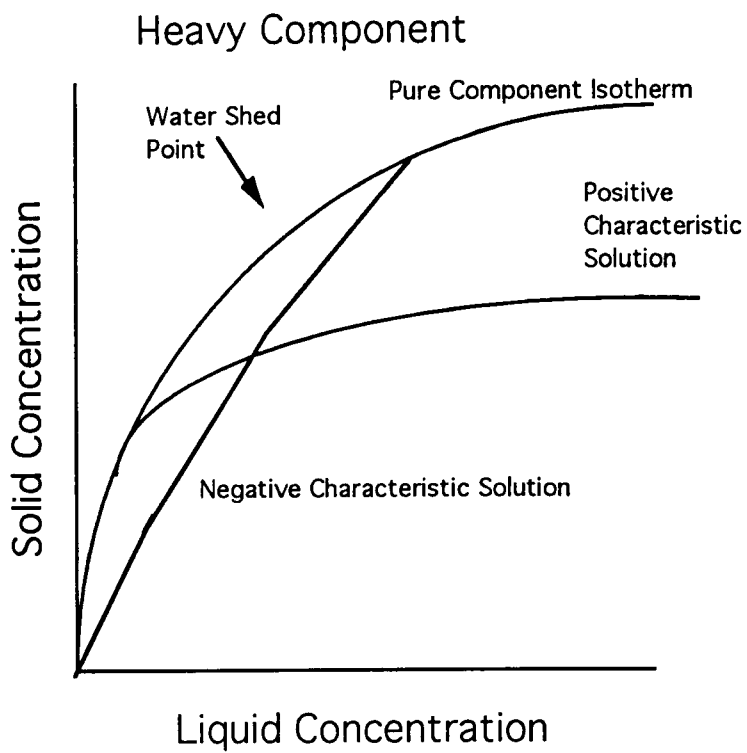


Figure 6. Solid concentration as a function of liquid concentration.

The heavy component solid phase concentration is plotted as a function of the component isotherm and solution pathways from Figure 5.

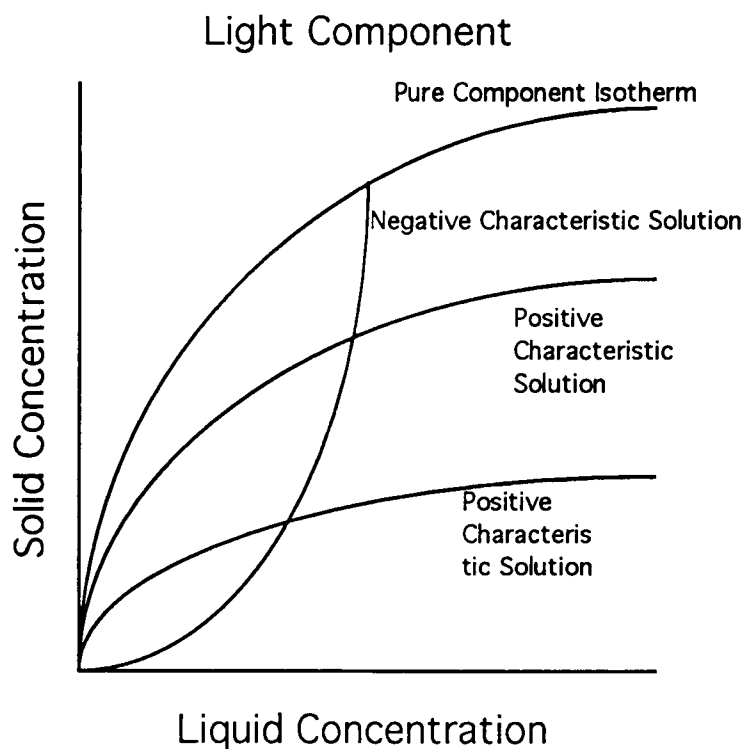


Figure 6 (Contd.). The solid phase concentration of the least strongly adsorbed component is plotted as a function of the component isotherm and solution pathways from Figure 5.

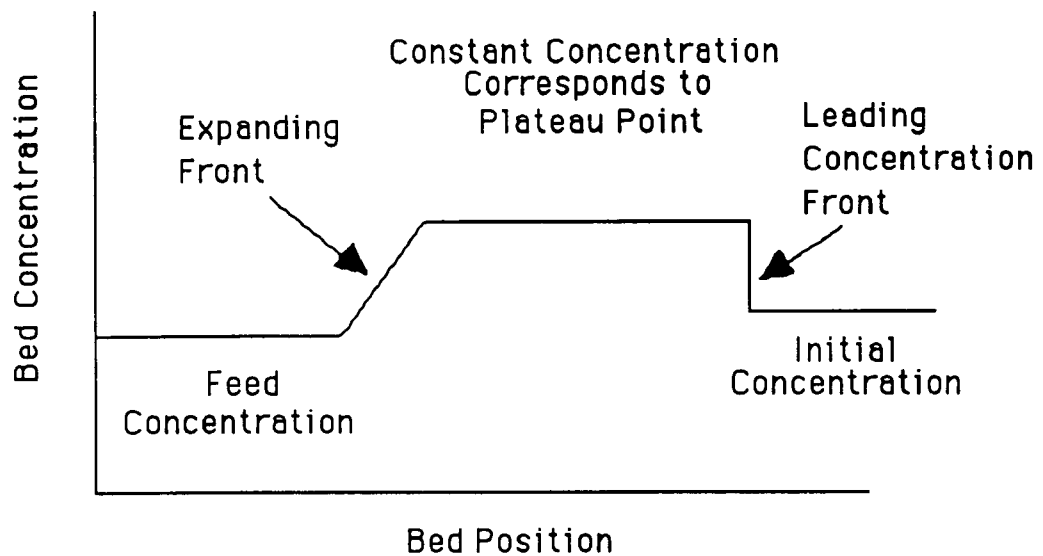


Figure 7. Concentration as a function of position in a packed bed. Different types of concentration fronts are shown that may be encountered in adsorption processes. Adapted from Coroyannakis, Thesis, University of Toronto, 1990.

to the plateau point or from the plateau point to the feed concentration. For example in Figure 8, the slope from initial concentration to the plateau point is decreasing and would correspond to a shock transition. The slope from the plateau to the feed concentration is increasing and would correspond to an expanding front.

Coroyannakis (1987) states that the use of geometrical representations is applicable to systems that are Langmuirian near the origin without recourse to their full isotherms. The next few paragraphs discuss analysis of various situations using this information.

For favorable isotherms (types I, II and IV), adsorption of a binary feed on a clean bed produces pure component and mixed component shock fronts (Coroyannakis, 1987). For desorption of a bed loaded with two solutes, an expanding front occurs when using a clean carrier.

For unfavorable isotherms (types III and V), a shock occurs when desorbing a bed loaded with two solutes. An expanding front occurs with adsorption onto a clean bed using a binary feed. For other isotherms, mixed fronts occur (Basmadjian *et al.*, 1987). For more than two components, Yang (1987) indicates that there are the same number of transition zones as components and that the velocity of the transition zones follow the order of adsorption strength.

Initial, Feed and Plateau Positions

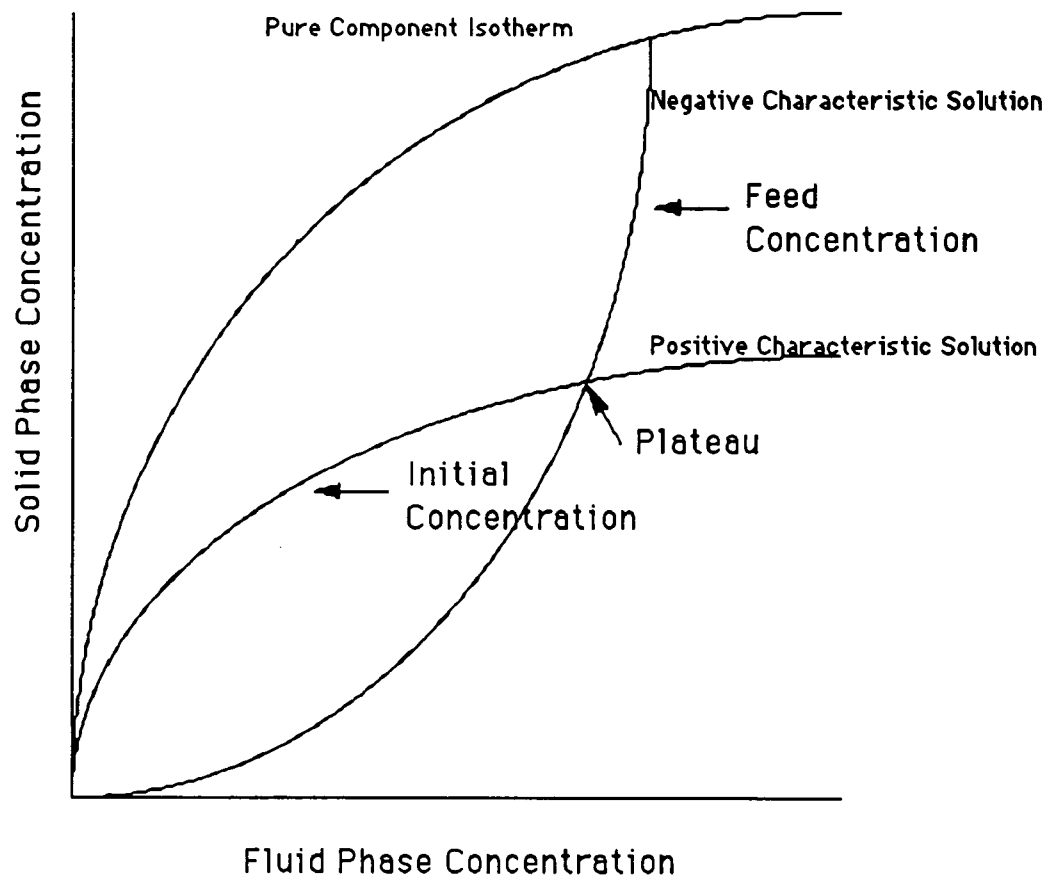


Figure 8. Solid phase concentration as a function of liquid phase for the light component. The plateau point, positive and negative characteristics for the light component from Figure 5 are overlaid on the component isotherm.

There are several cases using single component feeds and single component adsorbed phases that can be analyzed using the approach outlined by Gluekauf (1949). Table 1 outlines possible cases for components with type I isotherms. Table 2 relates transition zones to the cases indicated in Table 1. Case 1 represents the interaction of a light component with a heavy component. The feed concentration of the heavy component is below the watershed point. The only negative characteristic passing through the feed point is the left half of the Y2 axis. The only positive characteristic passing through the initial condition is the Y1 axis. The intersection of the negative and positive characteristics is the origin, i.e., there is a region of clean bed between the pure and heavy component fronts. Thus the plateau point is the origin. The slope in q-Y space increases from the initial condition to the plateau concentration; therefore the first transition is continuous from initial condition to plateau condition. The slope then decreases from the plateau concentration to the feed condition. The transition from plateau to feed is a shock. The other situations outlined below can be found using this method.

Table 1. Possible Adsorption Cases.

Case	Feed Component	Feed Concentration	Adsorbed Component	Adsorbed Concentration	Displacement Occurs
1	Heavy	< Watershed	Light	Open	Does Not Occur
2a	Heavy	> Watershed	Light	Less than Plateau Point	Displacement Occurs
2b	Heavy	> Watershed	Light	Greater than Plateau Point	Displacement Occurs
3	Light	Open	Heavy	< Watershed	Displacement Occurs
4a	Light	Less than Negative Feed Characteristic	Heavy	> Watershed	Displacement Occurs
4b	Light	Greater than Negative Feed Characteristic	Heavy	> Watershed	Displacement Occurs

Table 2. Transition zones for Table 1.

Case	Initial-Plateau	Plateau-Feed
1	Continuous	Shock
2a	Shock	Shock
2b	Continuous	Shock
3	Shock	Continuous
4a	Continuous	Continuous
4b	Shock	Continuous

1.7 SOLUTION METHODS FOR CONVECTION-DIFFUSION PARTIAL DIFFERENTIAL EQUATIONS

According to the authors knowledge, general analytical solutions for coupled systems of the form of equations (16) and (17) do not exist. Equations (16) and (17) may be simplified by eliminating dispersion and/or assuming simplified reaction terms, mass transfer terms or isotherms. Ruthven (1984) outlines various analytical solutions for different simplifications of equation (16) and no reaction. Most of the analytical solutions for these simplified cases are difficult to evaluate. For example many of the analytical solutions for the Henry's Law adsorption case involve numerical integration of complex functions and are subject to severe numerical oscillation problems. Evaluation generally becomes more difficult as the physical situation is better represented.

The following assumptions are made in modeling the reactor system:

- a) Constant density of liquid and solid phases in the reactor;
- b) Velocity in the radial direction is zero;
- c) Fully developed flow;
- d) N_{ir} is zero;
- e) N_{iq} is zero;
- f) Cross sectional area is constant;
- g) Isothermal operation;
- h) Constant volumetric flow rate;

i) Void fraction is constant;

Solid and liquid phase densities are assumed to be constant since reactor pressures greater than several hundred psig are not usually exceeded. Velocities in angular ϕ and radial direction are assumed to be zero due to reactor symmetry. Fully developed flow is calculated to occur at less than 1 mm of the bed length (McCabe *et al.*, 1986). The metal frit installed at the inlet is approximately 6 mm thick and allows a fully developed flow profile to develop prior to entrance into the soil wafer (see section 2.2). A void volume of 0.5 and a maximum volumetric flow rate of $0.4 \text{ cm}^3 \text{ min}^{-1}$ were used for calculation of the bed length required for fully developed flow to occur. N_{ir} and N_{iq} are assumed to be zero due to reactor symmetry. Cross sectional area of the bed, void fraction and volumetric flow rate are assumed to be constant since soil for the reactor is sized prior to use. Isothermal operation is assumed since the total heat of the reactor body is large compared to the maximum amount of heat generated through reaction in the soil wafer. Controls may be used to estimate the required time for steady state operation to be achieved. These are the major modeling assumptions.

Techniques available for simulation of equation (16) and (17) with mass transfer resistance fall into three major categories. The first category employs finite difference, finite element, orthogonal collocation (or a combination) algorithm. The second method makes use of a simplifying assumption such as the differential volume assumption to simplify the mathematics. The mathematics are

greatly simplified when steady state behavior may be assumed.

These techniques are discussed below.

A finite difference, orthogonal, (or combination) algorithm may be employed to solve a coupled system of equations (16) and (17). Finite difference methods are very powerful for simulating behavior of reaction systems. Unfortunately, a number of stability problems may be encountered when employing finite difference algorithms. Khanna & Seinfeld (1987) outline the various strengths and weaknesses of the different numerical techniques for simulating PDE systems. Table 3 is adapted from Khanna & Seinfeld (1987) and lists the strengths and weaknesses of the various techniques.

Finite difference methods use difference formulas to approximate differential terms in the differential equations. The difference formulas are generally based on Taylor series expansions. The algorithms may be single or multistep in time and/or space. Finite difference algorithms provide a powerful tool for simulation of PDE behavior but are subject to stability problems. To improve stability the finite difference spatial and time grid may be decreased or an implicit algorithm may be used. Using smaller grid step sizes may increase round off error and may reduced algorithm efficiency. Implicit methods are numerically less efficiency than explicit methods. Wu (1989) discusses various problems encountered with a number of finite difference algorithms.

Table 3. Strengths and weaknesses of different simulation algorithms.

Algorithm	Strengths	Weaknesses
Finite difference	Simple approach	May require very fine grid for sharp fronts or gradients
Orthogonal collocation	Generally computationally more efficient than finite difference	Assumes a smooth solution-sharp fronts may cause problems
Orthogonal collocation on finite elements	Solution not assumed to be smooth.	Sharp fronts may cause problems if collocation points are not picked well.

Adapted from Khanna & Seinfeld in Advances in Chemical Engineering, Wei, J., J. L. Anderson, K. B. Bischoff, M. M. Denn, and J. H. Seinfeld, Eds., 1987.

Mathematical description of transport and reaction processes in packed bed reactors result in systems of equations that are termed mixed hyperbolic-parabolic PDEs (Wu, 1989) or convection-diffusion PDEs. If the convection term is dominant, numerical diffusion or oscillation may cause a finite difference algorithm to fail. The numerical problems arise from spatial and temporal truncation and round off error. Therefore, choice of technique is very important. Round error is caused when the computer represents a real value by a machine number. Real numbers are represented by machine language with a finite number of digits. Because simulation of a problem may require millions of mathematical operations, round off error may become a serious consideration. A number of difference formulas are available for simulation of transport reaction PDE systems. Wu (1989) proposes replacing the spatial and temporal first order differentials in the difference formula by a three-point backward finite difference formula (TPB). TPB in combination with the centered difference formula for the second order spatial differential produces an algorithm of overall second order truncation error. Since the algorithm is multistep in time and space the algorithm shows promise but requires intensive programming efforts.

Collocation techniques, and specifically orthogonal collocation and collocation on finite elements, are useful for simulation of convection-dispersion PDE systems (Holland & Liapis, 1983; Villadsen & Michelsen, 1978; and Finlayson, 1972). Orthogonal

collocation is based on the concepts of orthogonal polynomials, Gaussian quadrature, and the Method of Weighted Residuals.

Orthogonal polynomials, $P_n(x)$, have the mathematical property for all family members:

$$\int_a^b W(x)P_i(x)P_j(x)dx = 0 \quad \text{if } (i \neq j) \quad (25),$$

$$\int_a^b W(x)P_i(x)P_j(x)dx > 0 \quad \text{if } (i = j) \quad (26).$$

where $W(x)$ are weighting functions. All roots of orthogonal polynomials are real and positive. Jacobi polynomials are commonly used for orthogonal collocation although any family of orthogonal polynomials may be used. Several common orthogonal families, their recursion formulas, and the interval upon which they are orthogonal are listed in Table 4 (Holland & Liapis, 1983). In the Jacobi recursion formula, $\Gamma(n+1)$ is the gamma function. If n is an integer, then $\Gamma(n+1) = n!$. A family of Jacobi polynomials, $P_n^{(\alpha,\beta)}$, results from each choice of the parameters a and b .

Table 4. Orthogonal polynomial families and general recursion formula.

Family (Orthogonality Interval)	Recursion Formula
Legendre [-1,1]	$L_n(x) = \left(\frac{2n-1}{n}\right)xL_{n-1}(x) - \left(\frac{n-1}{n}\right)xL_{n-2}(x) \quad (n \geq 2)$
Laguerre [0, ∞]	$l_n(x) = (2n-x-1)l_{n-1}(x) - (n-1)^2l_{n-2}(x) \quad (n \geq 2)$
Chebyshev [-1,1]	$T_n(x) = 2xT_{n-1}(x) - T_{n-2}(x) \quad (n \geq 2)$
Hermite [- ∞ , ∞]	$H_n(x) = 2xH_{n-1}(x) - 2(n-1)H_{n-2}(x) \quad (n \geq 2)$
Jacobi [0,1]	$(1-x)^\alpha x^\beta P_n^{(\alpha,\beta)}(x) = \frac{(-1)^n \Gamma(\beta+1)}{\Gamma(n+\beta+1)} \frac{d^n[(1-x)^{n+\alpha} x^{n+\beta}]}{dx^n}$

Gaussian quadrature is a numerical integration method. For example, assume that the function for integration is costly or difficult to integrate. Given the function values at several independent points and the corresponding independent variable values, there exist a set of constants, c_i , such that

$$\sum_{i=0}^n c_i g(x_i) \equiv \int_a^b g(x) dx \quad (27).$$

where x_i are the independent variable values (the collocation points) at which the function values are known. The Gaussian quadrature method provides a means for calculating the c_i 's and choosing the collocation points at which to measure the function so that the error in integrating the function is minimized. Equation (27) is exact (using Gaussian quadrature) if the following conditions are met:

- 1) $g(x)$ is a polynomial of order $2n+1$,
- 2) the $n+1$ points at which the function values will be measured are the roots of an orthogonal polynomial,
- 3) the orthogonal polynomial is of degree $n+1$.

Gaussian quadrature approaches the exact solution for any continuous function, as the number of collocation points are increased to infinity. Depending upon which orthogonal polynomial is used for the quadrature, the method may be described as Gauss-Laguerre, Gauss-Chebyshev, Gauss-Hermite, or Gauss-Jacobi.

Collocation uses a polynomial function as a trial solution for the differential equation. The trial solution is made to fit the

boundary conditions by use of adjustable parameters α_i . For example, consider the differential equation

$$\beta_1 \frac{d^2 Y}{dx^2} + \frac{\beta_2}{x} \frac{dY}{dx} - \beta_3 Y = 0 \quad (28a)$$

with boundary conditions

$$\frac{dY}{dx} \Big|_{x=1} = 0 \quad (28b),$$

$$Y(0) = 1 \quad (28c).$$

Let the trial solution be

$$Y_{n=nodes}(x) = 1 + (x-1)^2 \sum_{i=1}^n a_i x^{i-1} \quad (29).$$

Using 2 equally spaced collocation points ($n=2$), the trial solution and it's derivatives are:

$$Y_{n=2}(x) = 1 + (x-1)^2 (a_1 + a_2 x) \quad (30a),$$

$$\frac{dY_{n=2}}{dx} = 2(x-1)(a_1 + a_2 x) + (x-1)^2 (a_2) \quad (30b),$$

$$\frac{d^2 Y_{n=2}}{dx^2} = 2a_2(x-1) + 2(a_1 + a_2 x) + 2(x-1)(a_2) \quad (30c).$$

Equation (30a-c) are then used to replace terms in equation (28). Two collocation points (values of x between $x=0$ and $x=1$) are then chosen. Since the differential equation requires that

$$\alpha_1 \frac{d^2 Y}{dx^2} + \frac{\alpha_2}{x} \frac{dY}{dx} - \alpha_3 Y = 0 \quad (31)$$

be equal to zero at every point between $x=0$ and $x=1$, the values of the differential equation must be equal to zero at each of the collocation points. Use of the collocation points then lead to a system of equations (a system of two equations in this case) that can be solved for the α_i 's. The polynomial may then be used to estimate values on the exact solution of (28). Increasing the number of collocation points decreases the error of the collocation solution. For a continuous function, the exact solution is approached when the number of collocation points are increased to infinity. Use of orthogonal polynomials as trial functions and their roots as collocation points has been shown to minimize the error of the algorithm.

A number of commercial numerical simulation packages are available for numerical solution of PDE systems. There are also a number of public domain packages available that maybe useful for simulation of the dispersion-convection transport equation.

A second technique is to assume that the changes occurring in the reactor are small. This assumption, if appropriate, generally reduces effort and may allow analytical solutions of physically

meaningful models to describe the system. This approach is known as the differential volume assumption.

The differential volume assumption, if used correctly, introduces a small amount of error. A number of researchers have discussed the assumption. Levenspiel (1972) discusses the general use of the assumption. Massaldi and Maymo' (1969) investigated the error produced for the steady state case of equation (1). Ludwig *et al.* (1985) discuss the use of the assumption for the case of the shrinking core model without dispersion. Bienkowski *et al.* (1989) suggest use of the assumption for studying biological degradation of PAH in soils. Dunbar *et al.*, (1989) suggest use of the assumption with biosensors for kinetic investigation of naphthalene degradation. For the steady state case, equation (16) becomes

$$-D_l \frac{\partial^2 C_A}{\partial z^2} + \frac{\partial}{\partial z}(vC_A) = R_A \quad (32).$$

If the true reaction rate

$$R_A = f(C_A, C_B, \dots) \quad (33)$$

is replaced by the average of the inlet and outlet concentrations then the error in using the differential volume assumption can be calculated as suggested by Massaldi and Maymo' (1969).

A third approach is to assume steady state behavior. At steady state, ODEs are obtained which may be analytically solved. Fogler (1986) and Levenspiel (1972) provide general discussions on steady

state reaction. The following equations assume negligible mass transfer and an evenly distributed population throughout the soil wafer. Equation (34) is the second order ODE from the mass balance. Equation (35) is the steady state analytical solution for first order reaction kinetics ($-R_i = kC_{Bacteria}C_i$) with respect to pollutant concentration,

$$\frac{D_l}{v} \frac{\partial^2 C_i}{\partial z^2} + \frac{\partial C_i}{\partial z} - R_i = 0 \quad (34),$$

$$C = \frac{4q \exp(Pe_r / 2)}{(1+q)^2 \exp(Pe_r q / 2) - (1-q)^2 \exp(-Pe_r q / 2)} \quad (35)$$

where

$$q = \sqrt{1 + 4Da / Pe} \quad (36).$$

Pressure drop through a packed bed is generally calculated using the Ergun equation (McCabe *et al.*, 1985):

$$\Delta P = \rho_f (\varepsilon v)^2 \left(\frac{L}{2R_p} \right) \left(\frac{1-\varepsilon}{\varepsilon^3} \right) \left[\frac{150(1-\varepsilon)}{Re_p} + 1.75 \right] \quad (37).$$

For low Reynolds Numbers, the Ergun Equation predicts that pressure drop is a linear function of flow rate. The Ergun equation is an empirical formula for predicting pressure drop through packed beds.

Equation (16) is typically used to describe reaction and adsorption in packed bed reactors and may be applied to biological soil systems under appropriate conditions. Equation (16) accounts for dispersion and convective flow, adsorption, and reaction.

Description of more complex biological situations accounting for inhibition growth, etc., may be accomplished by modifying the reaction term or developing differential equations for biomass description. Full or partial solution of equation (16) may require great effort. As the physical situation is better described, finite difference or analytical solutions become more complex. Analytical solutions may not be easily evaluated when available. Use of the differential volume assumption simplifies equation (16) and greatly reduces effort for the non-steady state case. Analytical solutions are available for the steady state case, equation (9), and first order reaction kinetics. Use of the differential volume assumption may be useful for solution of equation (16) for analysis of biological systems in soils.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

A bioluminescent reporter strain, *P. fluorescens* HK44, was investigated for use as a biosensor for subsurface pollutant degradation and migration. Attempts were made to relate bioluminescence to degradation processes for the single and multicomponent cases (salicylate, naphthalene, and non-inducing substrates). The required tasks involved kinetic characterization of bacteria and investigation of the bioluminescence as a function of PAH degradation and transport processes.

Models were developed to describe multicomponent adsorption and degradation of hazardous xenobiotics by immobilized (alginate beads) bioluminescent reporter strains. Model parameters were measured using residence time distribution, adsorption and steady state degradation experiments. The models were validated by conducting unsteady state experiments and comparing to simulations using developed transport-reaction models and measured parameters. The role of adsorption in soil bioremediation was investigated by comparing a dispersive-convective-transport, adsorption model (numerical solution) to adsorption experiments conducted with heavily PAH contaminated soils (naphthalene(aq) used as a displacer). Sinusoidal perturbation methods for measurement and analysis of adsorption processes were also

investigated and compared to traditional step and pulse analysis methods.

Performing a mass balance around the reactor produced a system of coupled partial differential equations (see equations (16) and (17)). Measurement of dispersion, convection, void volume, component isotherms, and the form of the reaction terms were required for a full description of the degradative system.

Rate terms with a theoretical basis were primarily investigated.

Convection and void volume were calculated by measuring the system flow rate and comparing the first moment of the residence time distribution to the measured volume of the unpacked reactor.

Total dispersion and mass transfer were measured for the reactor. Dispersion coefficients for the inlet and outlet of the bed were assumed to be equal. The total liquid phase mass transfer coefficient was calculated using several empirical correlations or measured using the method of Haynes & Sarma (1973). (Note that the total mass transfer resistance and dispersion was a function of the bed size, particle diameter, and particle porosity).

Adsorption isotherms of naphthalene and sodium salicylate were measured using concentration step experiments or batch experiments. Simulation using measured parameters were compared with actual unsteady state behavior.

Initial experiments were conducted to determine the time for achieving lux and degradation steady states. Experiments were conducted to differentiate between the different kinetic models. The models mentioned in equations (12) and (13) differed the most

at high conversions. The appropriate form of the rate term was chosen by fitting a wide range of conversion data to different degradation models and then comparing least square residuals. In this way, the models were clearly differentiated.

Analytic solutions for zero and first order kinetic degradation models were used to calculate the response using salicylate (steady state conditions). Priority was given to salicylate because of its expected low adsorption affinity for alginate. Compounds with highly unfavorable isotherms had long breakthrough times and resulted in long experiments. Long experiments were undesirable since growth limiting conditions might have adversely affected bacteria. Alginate bead structure might have also been significantly altered during long experiments.

2.2 REACTOR SPECIFICATIONS

The PBR was designed to retain the soil wafer, reduce dispersion effects, and simulate low flow rates in soil. Figure 9 and Figure 10 are cut-away diagrams of the PBR. The reactor was constructed from 316 stainless steel and consisted of two parts: 1) a piston which fits into 2) an annulus with one closed end. The PBR had an inner diameter of 2.54 cm and a variable soil depth of 0.8 to 11.0 cm. An n-butyl rubber o-ring was used to produce a gas tight

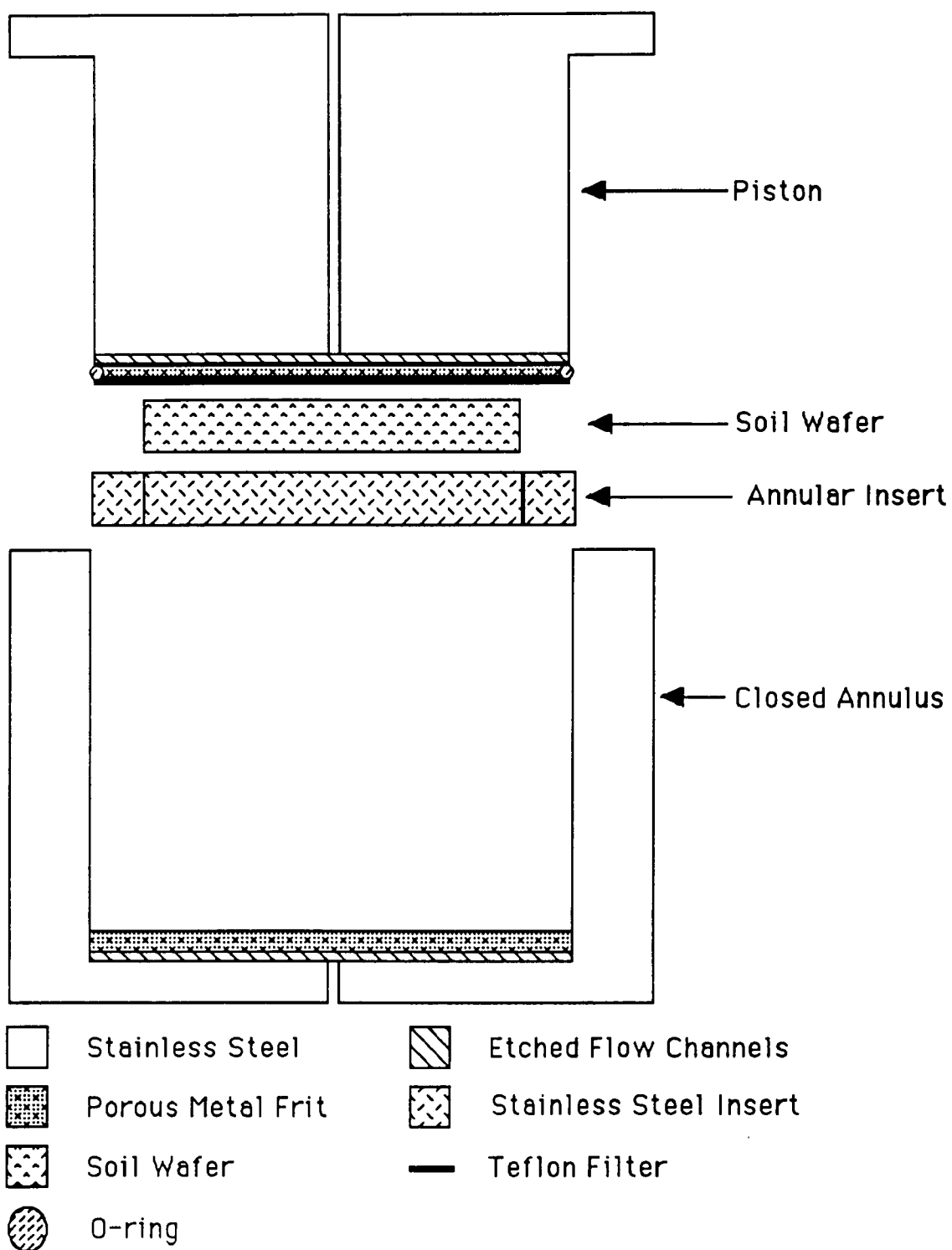


Figure 9. Cut away view of the PBR (expanded view). The PBR was constructed of stainless steel with stainless steel frits on the reactor inlet and outlet.

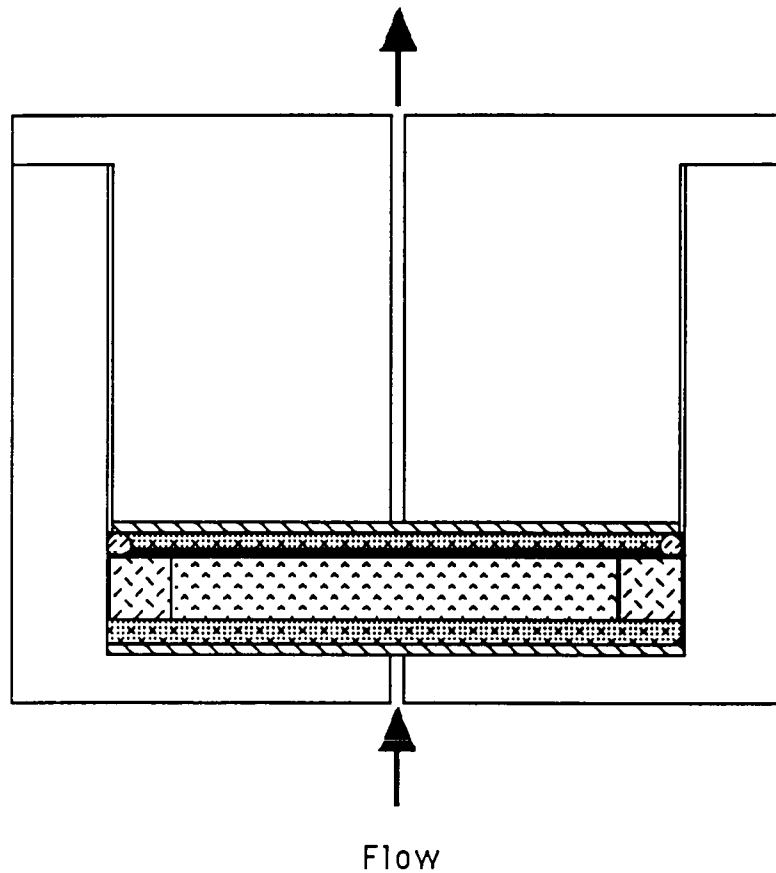


Figure 10. Cut away view of the PBR (assembled view). The diagram depicts an assembled PBR with a soil sample in the cavity. The outlet filter was held in place by the annular insert and the top half of the reactor. Media passed up through the base of the PBR, through the soil sample, and out through the filter and outlet of the PBR.

seal between the two sections. After assembly, the reactor formed a cavity that could be filled with a support media with an attached community of organisms. Metal frits were welded onto the piston and closed annulus. The metal frits retain the soil wafer in the cavity and conducted feed to the soil wafer. The exit metal frit attached to the piston was fitted with a 0.2 mm membrane. With the addition of an inter-cavity insert, a large variety of filters, including inorganic filters, could be used with the reactor. Without the insert, a polypropylene reinforced PTFE filter was used during reactor operation. (See Figure 9 and Figure 10). All reactor parts were made of 316 stainless steel and were manufactured in the Department of Chemical Engineering machine shop at the University of Tennessee.

The PBR was designed to minimize dispersion and channeling effects. Fluid entered at the base and proceeded upwards through a set of channels etched in the base of the PBR (see Figure 10). The channels distributed the fluid to a porous metal frit (Pall Metal Company, Cortland, NY), 0.32 cm thickness, 316 stainless steel with 0.4-4 micron pores. The fluid exited the wafer and passed through two 0.2 micron PTFE filters (Gelman TF-200). The use of exit filters produced a uniform resistance to flow across the reactor and enhanced the flow distribution. The fluid passed through a second metal frit (0.32 cm thickness, 316 stainless steel, 0.4-4 micron pores) and into a second set of channels etched in the top portion of the reactor which collected the fluid.

As can be seen from Figures 5 and 6, the reactor feed expanded from a small inlet to fill the reactor bed (to effect the low flow rates observed in the subsurface). The reactor bed was approximately one to two orders of magnitude greater in diameter than the inlet. If the resistance to flow was not uniform through out the soil wafer, as might be expected in a soil bed, then the flow pattern would not be reproducible. Addition of a large uniform resistance to flow was then desirable. Because the pressure drop (resistance to flow) caused by the filter was large, the smaller resistances to flow in the soil bed became insignificant. Also, the filter protected the metal frit from clogging.

An annular insert was designed for reactor use because the o-ring was not well suited for holding the membrane in place. After soil was placed inside the cavity of the annular insert, the membrane was placed on top of the insert. The insert allowed use of many types of membranes. For example, an inorganic filter was used instead of the PTFE reinforced membrane for adsorption studies.

2.3 REACTOR OPERATION

The PBR support systems were designed to provide operating safety and flexibility. All PBR system equipment were rated at or above 8500 kPa. Substrate was stored in stainless steel pressure vessels (Millipore) rated to 690 kPa. An emergency adjustable

pressure relief valve was installed to assure maintenance of safe operating pressures.

The reactor system with support equipment is pictured in Figure 11. Liquid feeds were kept in the pressurized substrate containers. Two Gilson 301 HPLC pumps were available to supply media to each reactor. The reactor was equipped with a light detection system (Oriel, Stratford, CT) for monitoring of bioluminescent activity (Dunbar, et al., 1992). The reactor effluent stream was split for dual analysis using high performance liquid chromatography (HPLC) with fluorescent detection and gas chromatography (GC) with flame ionization detection (FID). Computers were available for recording and measuring concentration and light data and for control of the Gilson HPLC pumps.

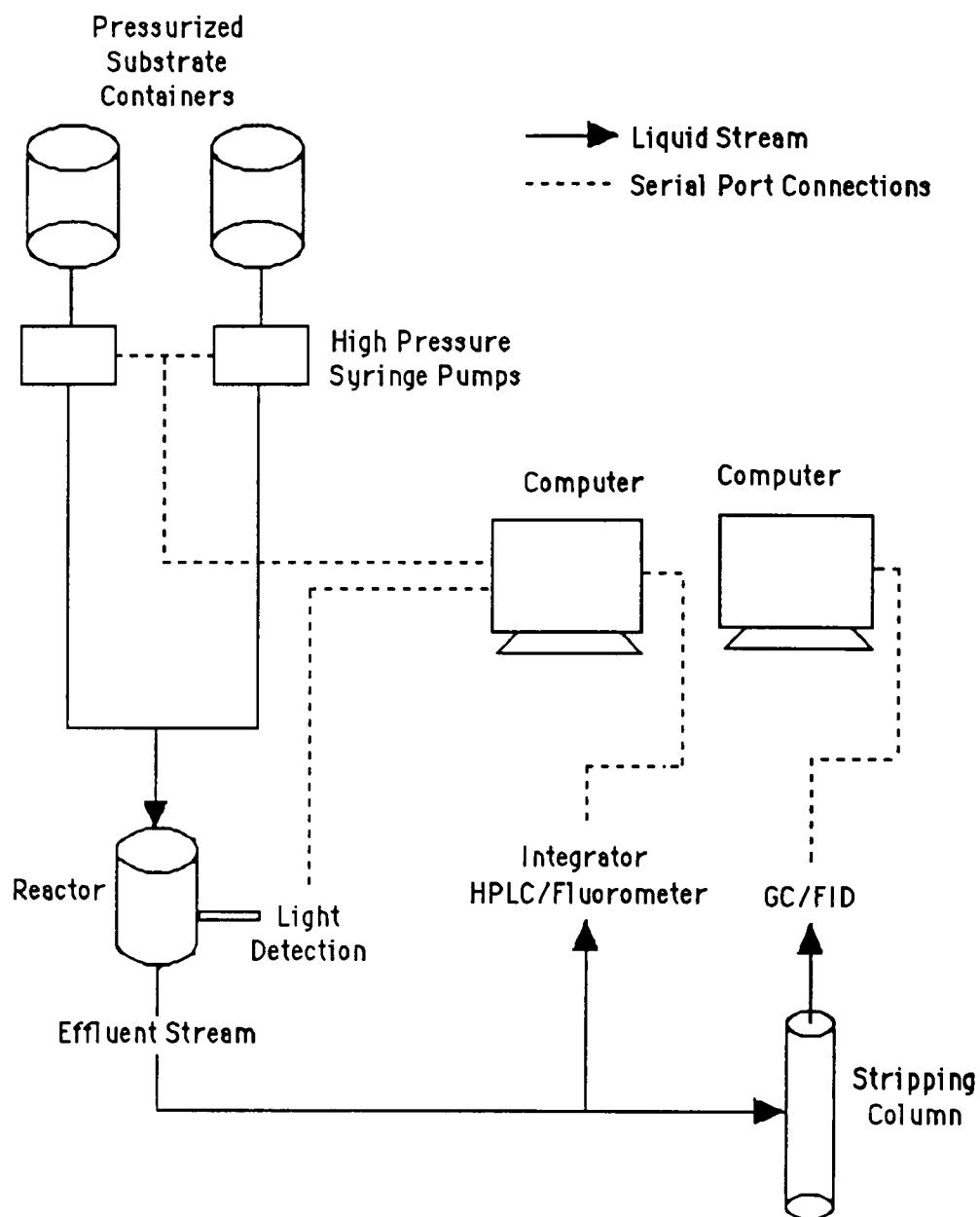


Figure 11. Block diagram of PBR with support equipment.

PAH conversion depended upon a number of parameters and variables. Table 5 lists reactor control and dependent variables, and reactor parameters used for experiments.

The reactor system was designed to allow control of a number of reactor variables. Concentrations of dissolved gases in the feed, including oxygen, were controlled by adjusting their partial pressures in stainless steel pressurized substrate containers. All nutrients including dissolved gases entered the reactor in the liquid phase. Gilson 301 HPLC pumps were used to control the feed rate of substrate to the reactor system with a high degree of accuracy. Two HPLC pumps supplied substrate to the reactor in a sinusoidal manner and were controlled by a personal computer using serial port communications. A constant hydraulic flow to the reactor was maintained by operating the pumps 180° out of phase. The reactor temperature was controlled by a Lauda RMT-6 recirculating bath with a heat exchanger coil that enclosed the reactor.

Table 5. Control Variables.

Control Variables	Parameters	Dependent Variables
Dissolved Oxygen	Pressure	PAH Concentration
PAH Feed Concentration	Organic Carbon	Total Biomass
Total Biomass	Void Volume	NahA Frequency
Feed Flow Rate	Reactor Bed Diameter	Mineralization Rate
Inhibitors	Dispersion Coefficient	
Reactor Bed Volume	Bead Diameter	
NahA Frequency		
pH		
Temperature		

A number of support media were available for use with the PBR including weathered contaminated soils, uncontaminated soils and immobilization gels. Manufacturers gas plant soil (MGP soil), a weathered contaminated soil, was characterized as a coal tar soil contaminated with high concentrations of PAH. Hydraulic conductivity for MGP soil after treatment was between 5.2×10^{-3} and $5.2 \text{ mL cm}^{-2} \text{ min}^{-1}$ (Freeze & Cherry, 1979). Hydraulic conductivity for HPLC grade sand was between 5.2×10^{-2} and $50 \text{ mL cm}^{-2} \text{ min}^{-1}$ (Freeze & Cherry, 1979). Flow rates for experiment using these soils were held between 2.4×10^{-3} and $2.0 \times 10^{-2} \text{ mL cm}^{-2} \text{ min}^{-1}$.

MGP soils were sieved ($0.18 < D_p < 1.0 \text{ mm}$) and washed with 1 percent sodium pyrophosphate solution. The pyrophosphate treatment removed loosely associated organic particles from soil surfaces. The use of sized soils and the 1 percent sodium pyrophosphate treatment prevented excessive pressure buildup within the reactor. Soil was added to the reactor in a slurry.

0.05 percent HgCl_2 was used as an inhibitor for abiotic experiments performed using MGP soil. A second inhibition route was to reduce the feed to anaerobic conditions blocking aerobic degradation pathways. One percent sodium azide solution was added downstream of the biotic reactor to inhibit growth in the stripping column and effluent tubing.

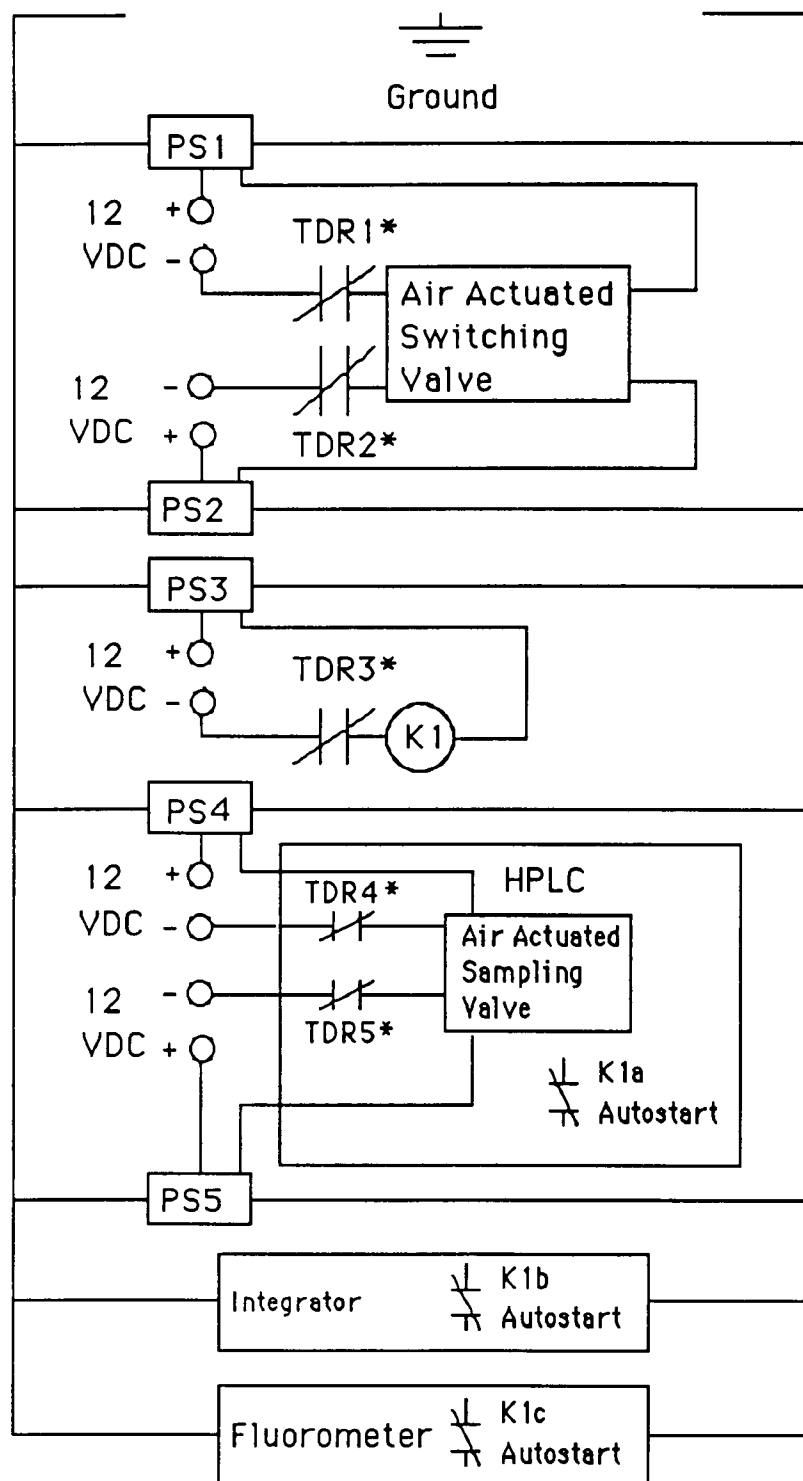
2.4 REACTOR PHYSICAL CHARACTERIZATION

A residence time distribution (RTD) (Haynes & Sarma 1973) was used to ascertain the PBR's departure from ideal behavior. The RTD was used to determine an effective dispersion coefficient which accounted not only for axial and radial dispersion but also for diffusion effects (Aris, 1956). Sodium salicylate or potassium bromide tracer was used for the RTD procedure. 50 microliters of 0.1 molar sodium salicylate or 1 molar potassium bromide tracer were introduced to the reactor through a six port HPLC valve directly upstream of the reactor. Effluent samples were analyzed for bromide or potassium tracer ion using a Waters Ion chromatography system with a series 510 HPLC pump, IC-PAK anion exchange column and 431 conductance detector (Waters, Cambridge, Mass.). Salicylate tracer ion was measured on-line by passing the reactor effluent directly through a fluorometer with a 3 mL sample cell (Perkin Elmer SL-4 fluorometer, Norwalk, CT).

2.5 ANALYTICAL REACTOR METHODS

The analytical systems control network is pictured in Figure 12. The abbreviations, symbols and sequence of events for Figure 12 are listed in the Appendix A. The computer for feed pump control was also double tasked for data collection from the high performance liquid chromatography (HPLC) system integrator. Switching valves were installed to allow simultaneous monitoring of up to four reactors. The reactor effluent streams were split and monitored simultaneously by GC and HPLC. If two reactors were operated at the same time, both were analyzed sequentially by HPLC with only one reactor system being analyzed by GC. (See Figure 12). The timed relay controlled the operation of the HPLC gradient elution system, the fluorescent detector, and the switching valve. The HPLC gradient system was programmed to perform sequentially up to 10 different gradients and allowed great flexibility in analysis. Automatic sampling was controlled by the HPLC gradient system.

On-line high performance liquid chromatography (HPLC) with fluorescent detection was available for analysis of PAH compounds. The choice of the HPLC column was crucial (Sander, 1988). A Vydac 201TP 25 cm column (Sep-ar-a-tions Group, Hesparia, CA), shown to be one of the best columns for separating complex mixtures of PAHs,



* Programable Time Delay Relay

Figure 12. Control network for PBR.

was used for analysis (Katz & Ogan, 1980; Ogan & Katz, 1980; Sander & Wise, 1988). A gradient program was developed that resolved many of the compounds desorbing from a sample of Town Gas soil. The HPLC system consisted of a model 5560 liquid chromatograph (Varian, Palo Alto, CA), a 25 cm Vydac 201TP5 column and a LS-4 fluorescent spectrophotometer. The fluorometer was equipped with a 3 microliter flow sample cell. The gradient conditions used for the separation are listed in Table 6. Eluant flow rate was maintained at 1.5 mL/minute. Sample injection occurred at 1 minute in the HPLC gradient program. This program effectively separated PAH compounds present in Standard Reference Material (SRM) 1647a obtained from the National Institute of Standards and Technology (Gaithersburg, MD). A typical chromatogram of components that leached from MGP soil is shown in Figure 13. The chromatogram of SRM 1647a is shown in Figure 14.

Differences in the resolution of Figure 13 and Figure 14 were due to the phase in which they were prepared and injected. The gradient program was optimized to resolve components from aqueous solutions. Retention times of compounds in Figure 13 were determined by injecting internal standards. The components in SRM 1647a were dissolved in acetonitrile. Components dissolved in

Table 6. HPLC gradient program used for PAH compounds leaching
from MGP soil.

Time	% H ₂ O	% CH ₃ CN	Flow Rate
0	100	0	0.01
1	100	0	1.5
2	50	50	1.5
10	50	50	1.5
19	0	100	1.5
25	0	100	1.5
26	100	0	1.5
28.5	100	0	0.01

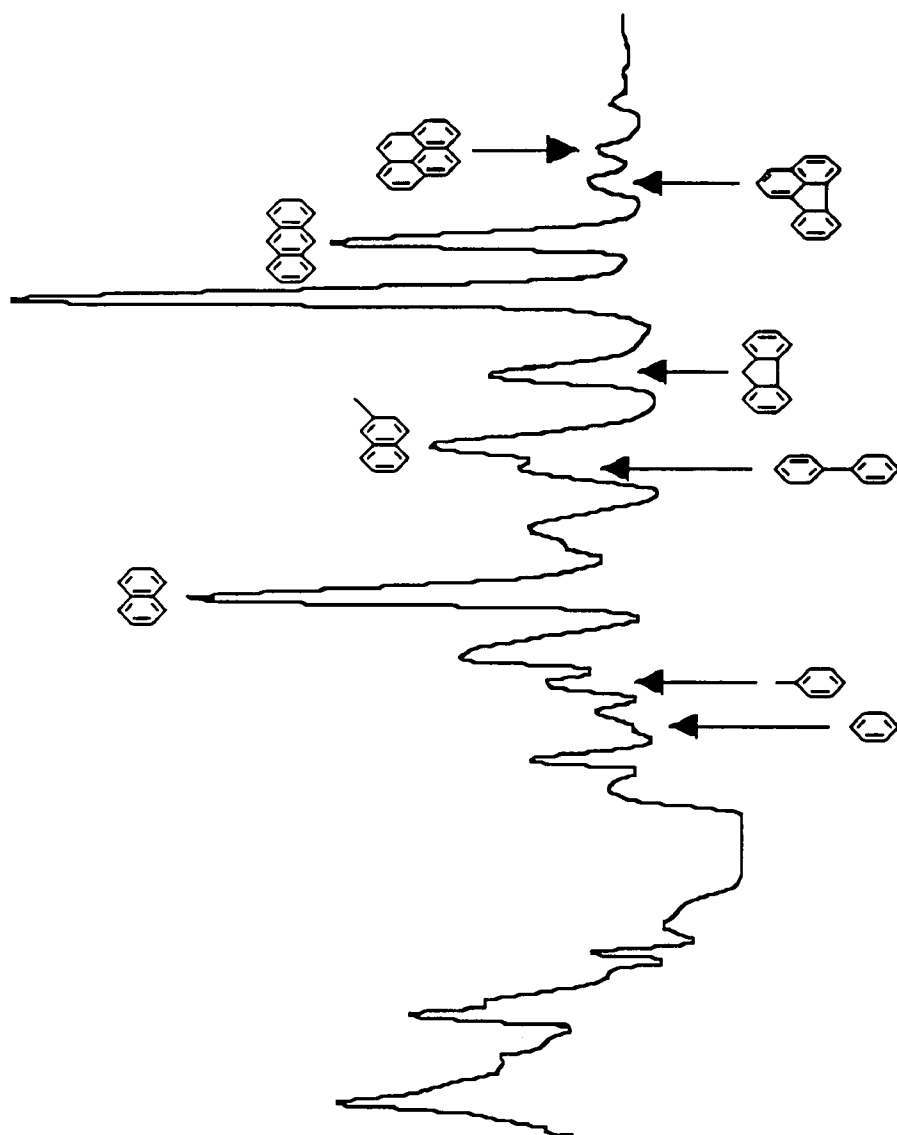


Figure 13. Leachates from MGP soil. A. UV

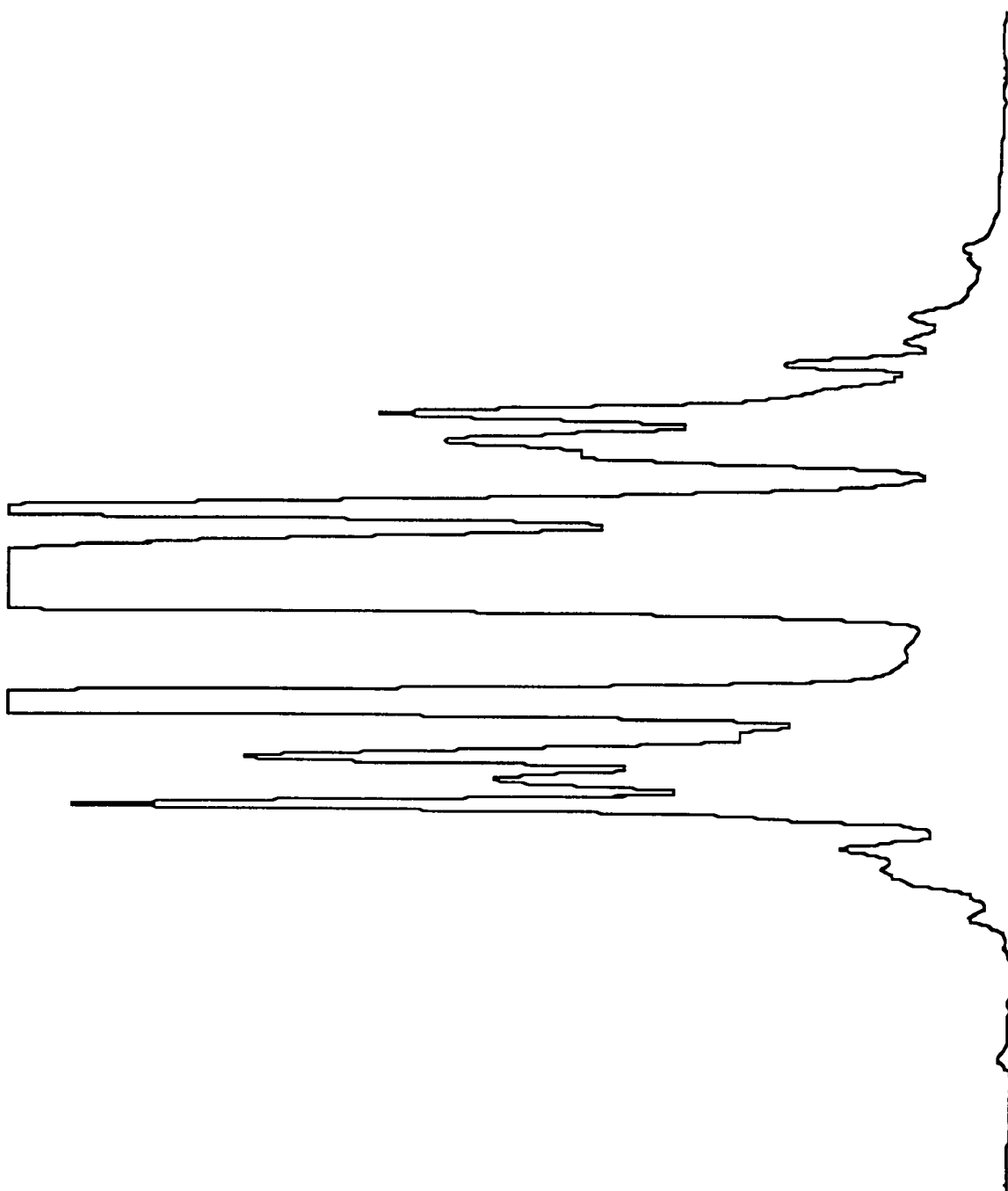


Figure 13. Continued . B. Fluorescence.

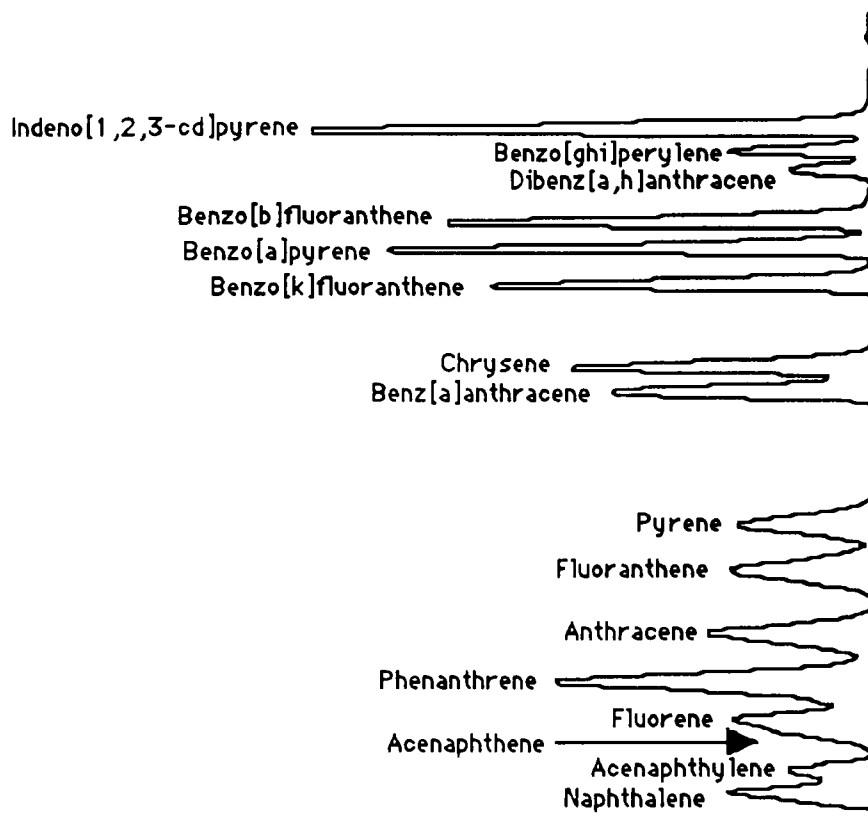


Figure 14. Liquid chromatogram of Standard Reference Material 1647a.

acetonitrile do not adsorb on the column in a sharp front. The peak shape was a function of the initial adsorption distribution in the column. Figure 13 was produced using ultraviolet (UV) and fluorescent detectors. Figure 14 was produced using UV absorption detection at 254 nanometers.

Compounds that fluoresce had individual excitation and emission spectra determined by structure. By changing the fluorometer excitation and emission wavelengths, fluorescence was used to selectively analyze compounds in complex mixtures. This approach was used for analysis of complex effluent streams.

Volatiles from the reactor effluent were analyzed by gas chromatography. After flowing through the reactor the effluent entered the stripping column, mixed with helium, and then flowed to waste collection. The effluent volatiles in the helium stream entered the GC (Hewlett Packard 5890 Series II) through a heated sample line. GC operation was controlled by computer and support software (Hewlett Packard computer, HP Chem Station software). The reactor system was equipped with a reactor bypass valve for calibration of the stripping column.

A stripping column was designed and optimized to remove PAH volatiles for GC analysis. A 0.533 cm inside diameter tube packed with 3 mm glass beads was used as the analytical stripping column. Naphthalene was used as the model compound for stripping column design. A constant molar flow rate was assumed since naphthalene solution was dilute. Column operation was isothermal. A Henry's Law Constant of 327.8 torr-L/gmole (Dunbar, 1990) coupled with an

estimated overall mass transfer coefficient (Yoshida *et al.*, 1962) was used to calculate a stripper height. After inclusion of a safety factor, a 20 cm column was built.

Solubility of PAH compounds ranged from several mg/L to low microgram/L. Detection by the stripping technique required the compound to have a large Henry's Law constant if the compound was present in low concentrations. One, two, and some three ring aromatic compounds were detectable by the stripping technique. The stripping technique was used to analyze low weight PAH compounds. The naphthalene detection limit approached several micrograms/l. Combining the selectivity of the stripping technique with the high resolution available from a capillary column provided a functional method for monitoring volatile compounds.

Gas and liquid streams entered through the bottom of the column and passed upward through the stripper packing. High purity helium gas flow at a rate of 2 mL/min was maintained by flow restriction using a low length of small bore tubing (0.004 inch ID) and regulating the upstream pressure. The two streams separated in a 1.90 cm tee where the volatiles passed through a heated line into a GC. The heated gas line was maintained above 100°C. The GC used an automatically controlled six- port gas sampling valve. The gas sampling valve temperature was maintained at 142° C by the GC ensuring a constant amount of sample for injection.

Capillary Gas Chromatography (HP 5890, series II) was used as the separation method for the compounds of interest. Helium was used as the carrier gas at 4 mL/min. Table 7 lists the temperature program used for analysis of volatile compounds. The GC was equipped with a flame ionization detector (FID). The temperature program was optimized for detection and resolution of PAHs (Table 7). A 5% phenylmethylsilicon cross-linked 25 meter, 0.33 mm inside diameter, 0.5 micron film thickness column (Lee *et al.*, 1979) was used for separation of the volatiles from the reactor effluent. A typical chromatogram showing volatile components leaching from Town Gas soil is pictured in Figure 15.

Gas Chromatography-Mass Spectroscopy (GC-MS) was used to identify compounds desorbing from the soil. The effluent passed through a column containing 0.1 grams of Tenax-GC (Lee *et al.*, 1979). The Tenax-GC method was routinely used for isolation and identification of PAH compounds occurring in the atmosphere or in ground or drinking water (Lee *et al.*, 1979). The Tenax-GC was then extracted with GC-MS grade isooctane (Baxter). One microliter of the isooctane solution was injected onto a GC-MS (Hewlett Packard 5996) using a HP1 methylsilicon cross-linked, 50 meter, 0.2 mm, 20 micron film thickness column. The Tenax-GC was prepared for use by heating at 375 °C for 2 hours under a nitrogen stream. Retention times and mass spectra were identified using SRM 1491 obtained from the National Institute of Standards and Technology. The chromatogram of SRM 1491 is shown in Figure 16.

Table 7. Temperature Program for Volatile Component Analysis by GC.

Initial Temperature ° C	Initial Time Min	Rate ° C/Min	Final Temperature ° C	Final Time Min
35	2	40	105	1
		10	135	0
		20	250	2

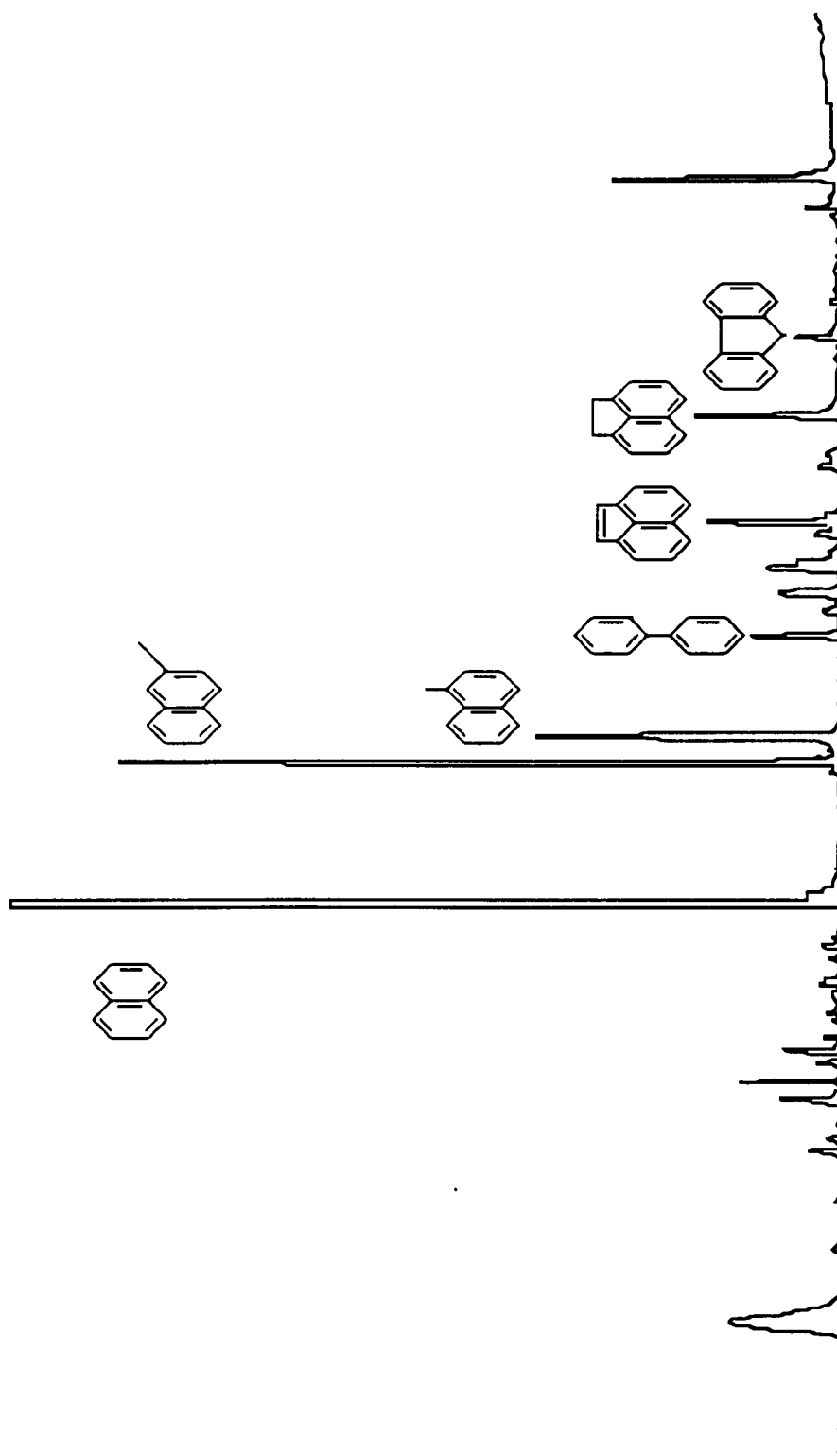


Figure 15. Volatile PAH compounds from PBR containing MGP soil.

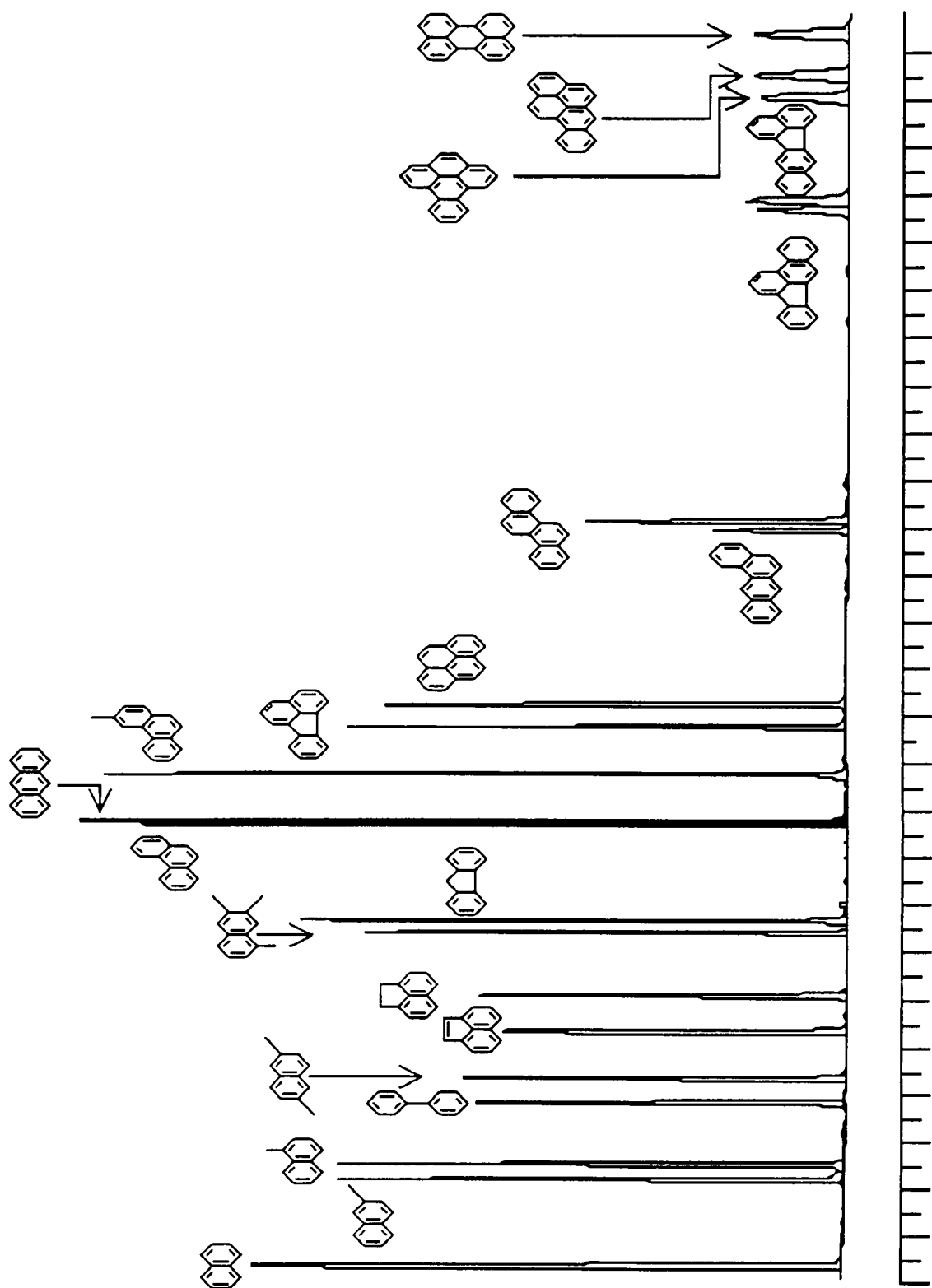


Figure 16. Gas chromatogram of SRM 1491 using a capillary gas chromatograph with a flame ionization detector.

2.6 NUMERICAL SIMULATION METHODS

Orthogonal collocation on finite elements was used for simulation of reactor processes. A public domain software program, PDECOL (Madsen & Sincovec, 1979), was used for integration of the partial differential equations describing processes in the reactor system. A copy of the driver routines and partial differential equation with appropriate boundary equation are included in Appendix C.

2.7 BIOLOGICAL ORGANISMS

P. fluorescens HK44, was used in pure culture and consortial degradation studies. *P. fluorescens* HK44, an MGP isolate, maintained a *nah-lux* reporter plasmid, pUTK21 (King et al., 1990). *P. fluorescens* HK44 was able to mineralize salicylate independent of the *nah* pathway. Inoculum were frozen at -70° C until use.

The consortia used for PAH degradation were prepared by enrichment from MGP soil (DiGrazia, 1991). Inoculum were frozen at -70° C until use.

2.8 MEDIA FOR BIOLOGICAL EXPERIMENTS

Yeast extract-peptone-glucose (YEPG) or yeast extract-peptone-salicylate (YEPS) were used for growth of cultures. Basal salts, mineral salts, or soil extract media were used for degradation experiments. Media recipes are listed in Table 8. Soil extract concentrate was prepared by autoclaving 10 gallons of distilled water with 1 gallon of characterized uncontaminated soil. After cooling the supernatant was passed twice through a 0.2 micron filter.

Table 8. Media recipes.

	Component	Concentration
YEPG, pH 7.0	Glucose	1.0 g/l
	Polypeptone	2.0 g/l
	Yeast Extract	0.2g/l
	NH ₄ NO ₃	0.2 g/l
YEPSS, pH 7.0	Sodium Salycilate	0.5 g/l
	Sodium Succinate	2.7 g/l
	Polypeptone	1.0 g/l
	Yeast Extract	0.2g/l
	NH ₄ NO ₃	0.2 g/l
Basal Salts pH 7.0	NaNO ₃	4.0 g/l
	KH ₂ PO ₄	1.5 g/l
	FeCl ₃	5.0X10 ⁻³ g/l
	MgSO ₄	0.2 g/l
	CaCl ₂	1.0X10 ⁻² g/l
	Na ₂ HPO ₄	0.5 g/l

Table 8. (Contd.).

	Component	Concentration
Soil Extract, pH 7.0	Soil Extract	200 mL/l
	Concentrate	
	NaNO ₃	4.0 g/l
	KH ₂ PO ₄	1.5 g/l
	FeCl ₃	5.0X10 ⁻³ g/l
	MgSO ₄	0.2 g/l
	CaCl ₂	1.0X10 ⁻² g/l
	Na ₂ HPO ₄	0.5 g/l
Mineral Salts, pH 7.2	MgSO ₄ ·7H ₂ O	0.1 g/l
	NH ₄ NO ₃	0.2 g/l
	trizma base	3.03 g/L
	MgO	1.0X10 ⁻³ g/l
	CaCl ₂	2.9X10 ⁻⁴ g/l
	FeCl ₃ ·6H ₂ O	5.4X10 ⁻⁴ g/l
	ZnSO ₄ ·7H ₂ O	1.4X10 ⁻⁴ g/l
	CuSO ₄	2.5X10 ⁻⁵ g/l
	H ₃ BO ₄	6.2X10 ⁻⁶ g/l
	Na ₂ MoO ₄ ·H ₂ O	4.9X10 ⁻⁵ g/l

2.9 CULTIVATION CONDITIONS

For pure culture experiments, a freshly thawed vial of *P. fluorescens* HK44 was added to 100 mL of YEPG media containing 14 mg/l of tetracycline (Heitzer et. al, 1992). The culture was then shaken for 15 hours at 25° C. The culture was then centrifuged at 8,000 rpm for 10 minutes. The pellet was then washed with 0.9% NaCl solution three times before use.

For mixed consortia experiments, cultures were grown in YEPG media for 12 hours. Soil was then added to the culture flask and incubated for 24 hours. Sand was autoclaved before incubation; however, MGP soil was not autoclaved prior to incubation with the subject bacteria population. After incubation and removal from the broth, the soils were washed twice with phosphate buffered saline, assayed, and then placed in the reactor.

Pure cultures were immobilized by the following procedure:

- 1) Centrifuge broth at 8,000 rpm for 10 minutes,
- 2) Wash pellet three times with 0.9% NaCl solution,
- 3) Suspend pellet in 10% NaCl solution,
- 4) Mix cell suspension and alginate solution (28 g low viscosity alginate, 9 g NaCl and 1 l water) in a ratio of 1 to 2 by volume,
- 5) Form alginate cell suspension beads by drop-wise addition to 0.1 M CaCl₂ solution using an alginate-

cell suspension to CaCl₂ solution ratio of 1 to 5 by volume (stir for 15 minutes),

- 6) Replace old CaCl₂ solution with an equal volume of fresh CaCl₂ solution with an equal volume of fresh CaCl₂ solution and stir for an additional 30 minutes.

Immobilized cultures were analyzed by dissolving the gels with 50 mM sodium hexametaphosphate. Pure culture populations are enumerated using optical density.

Biological analyses of cultures attached to soils were prepared by adding phosphate buffered saline (pH 7.0) and sodium pyrophosphate (0.1% w/v) and vortexing for 2 minutes. Total viable cells were enumerated by the serial dilution technique and spread plate inoculation onto non-selective 1/4 strength YEPG agar. All plates were incubated for 5 days at 20° C. The naphthalene genotype population was enumerated by using DNA:DNA colony hybridization (Applegate & Uphoff, 1990) using a gene probe for the iron-sulfur protein of naphthalene dioxygenase (nahA).

Because sampling during the experiment might induce substantial disturbance of the system, biological samples were collected at the start and end of each experiment.

3 RESULTS

3.1 PACKED BED REACTOR AND DIFFUSION PROCESSES

3.1A REACTOR HYDRODYNAMIC PERFORMANCE & DIFFUSION PROCESSES

Dispersion and mass transfer were analyzed using the method of Haynes & Sarma (1973) with potassium bromide or sodium salicylate tracer. The overall dispersion and liquid phase mass transfer values were measured as a function of flow rate with reactor volume and particle size as parameters. The complete data set is listed in Table A in Appendix B. The slope is a measure of dispersion while the intercept indicates total mass transfer resistance. Table 9 lists slopes at typical flow rates used for degradation experiments. At lower flow rates, the slope of the curves approached limiting values of between 1.0×10^{-2} and 2.5×10^{-2} cm²/min (Table 9) indicating that dispersion is of the order of 10^{-2} cm²/min. The particles used were either glass beads ($D_p=0.01$) or HPLC grade sand ($D_p=0.025$). Four different reactor volumes and two particle sizes were used for the experiments.

Table 9. Slopes of experimental dispersion and mass transfer curves. At flow rates typically used for experiments, dispersion was equal to the slope of the dispersion mass transfer curve.

Fluid Velocity	Reactor Volume	Dp	Asymptotic Slope (Dispersion)
cm/min	cm ³	cm	cm ² /min
0.1	3.62	1.00×10^{-2}	1.03×10^{-2}
0.1	7.24	1.00×10^{-2}	2.43×10^{-2}
0.1	14.48	1.00×10^{-2}	2.42×10^{-2}
0.1	3.62	2.50×10^{-2}	2.49×10^{-2}

Figure 17 is a plot of dispersion and mass transfer as a function of fluid velocity, expressed as $\frac{L\sigma^2}{2\mu^2v}$. Data points shown in Figure 17 are averages of raw data sets. The dispersion mass transfer curves approached asymptotic values at flow rates generally used for experiments but were concave downwards at higher flow rates. The curves all indicate that as fluid velocity increased, mass transfer resistance decreased toward a limiting low overall mass transfer resistance (y-axis intercept) while dispersion increased. For the low flow rate range, total mass transfer resistance became larger as the size of the reactor was increased as indicated by the intercept value. Because the particles were all solid, the mass transfer coefficients represent liquid phase resistance only.

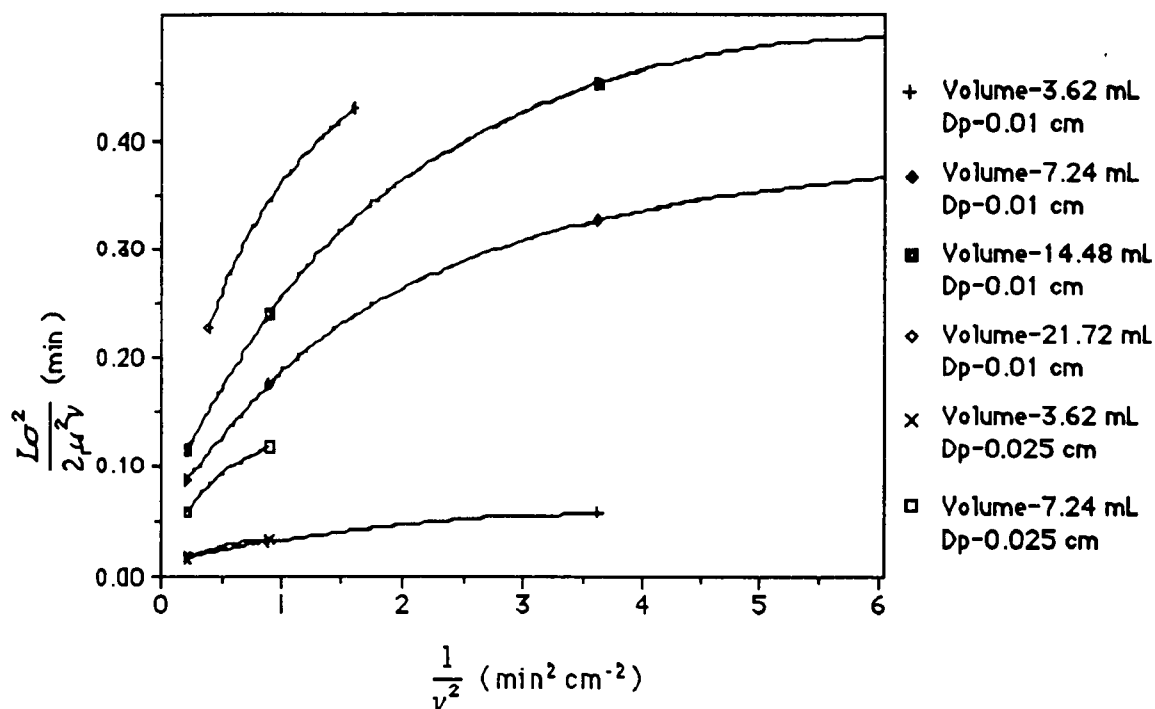


Figure 17. Mass transfer and dispersion as a function of fluid velocity with particle size and reactor volume as parameters. The slope of the curves approached asymptotic values at flow rates typically used for experiments. Very low flow rate data are not shown.

3.1B SIMULATIONS OF DIFFUSIONAL PROCESSES OCCURRING IN ALGINATE BEADS & SOILS

Figure 18 is a comparison of a simulation using PDECOL (Madsen & Sincovec, 1979) and an analytical series solution (Crank, 1975) for predicting diffusion into a porous sphere. The simulation was conducted to partially evaluate the performance of PDECOL (Madsen & Sincovec, 1979) for numerically solving diffusion problems encountered in the analysis of packed beds. Figure 18 relates concentration at the center of a sphere as a function of time. The series solution was carried through 1000 terms. The difference between the simulation predictions and the series solution was generally less than one percent except at very low concentrations where round off error was significant.

Figure 19 shows the time course of the concentration distribution inside a porous pellet for different values of the solid diffusion coefficient. The simulation was conducted to evaluate the role of solid diffusion in transport of solutes in packing or soil particles. Solid diffusion coefficients may easily be two or more orders of magnitude less than liquid diffusion coefficients (Treyball, 1980). Liquid diffusion is the dominant process for

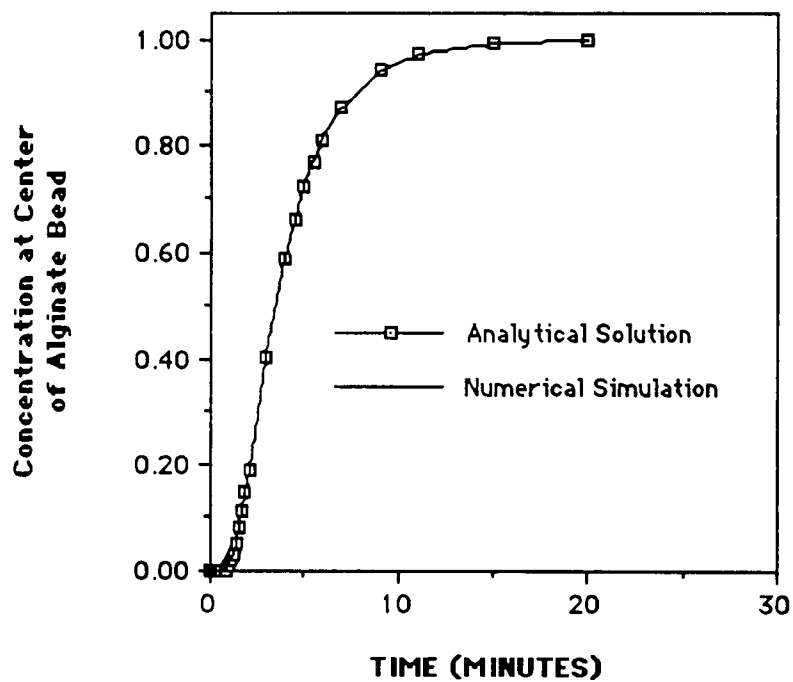


Figure 18. Comparison of an analytical and a numerical solution for predicting solute concentration at the center of a sphere as a function of time. Pellet surface concentration and the diffusion coefficient were held constant. The numerical technique predictions were within a percent of the analytical solution values. Adsorption was negligible.

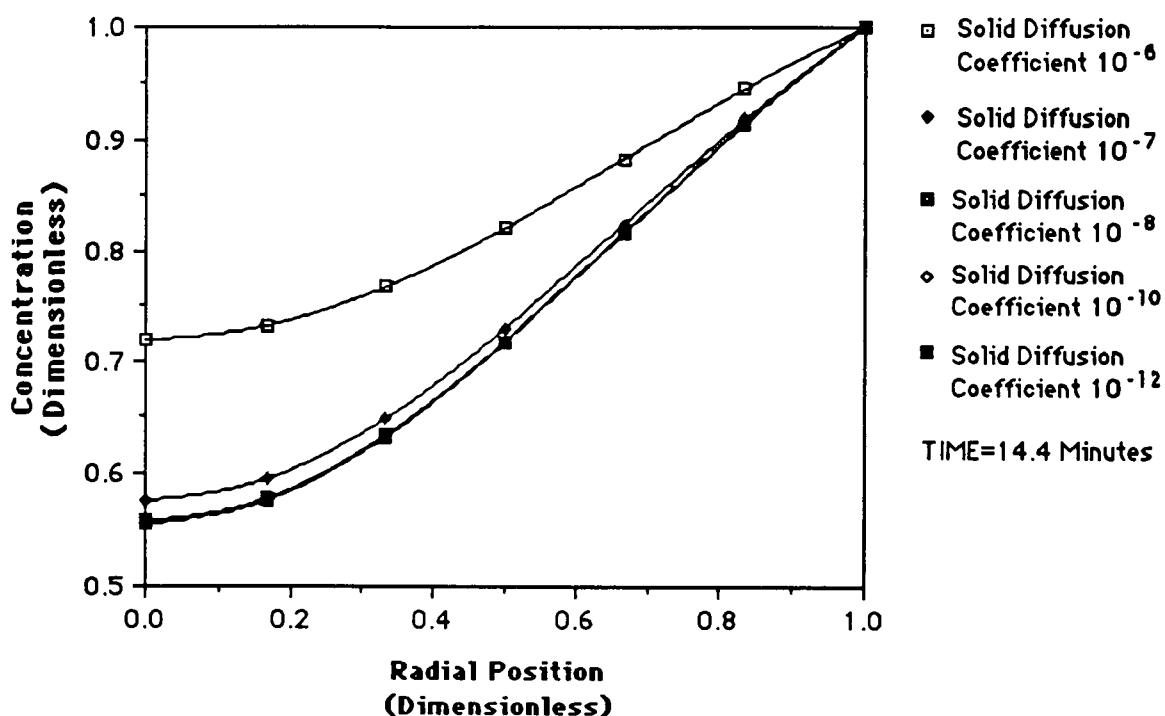


Figure 19. Simulated effects of solid diffusion coefficient upon concentration distribution inside a porous pellet. The distributions shown occurred at 14.4 minutes. The lower three curves were almost identical and overlapped. A different solid diffusion coefficient was used for each curve, varying between 10^{-6} and 10^{-12} cm^2/min . The liquid diffusion coefficient was held constant at 8.97×10^{-6} cm^2/min for each of the simulations. The pellet was initially free of solute. Adsorption was negligible.

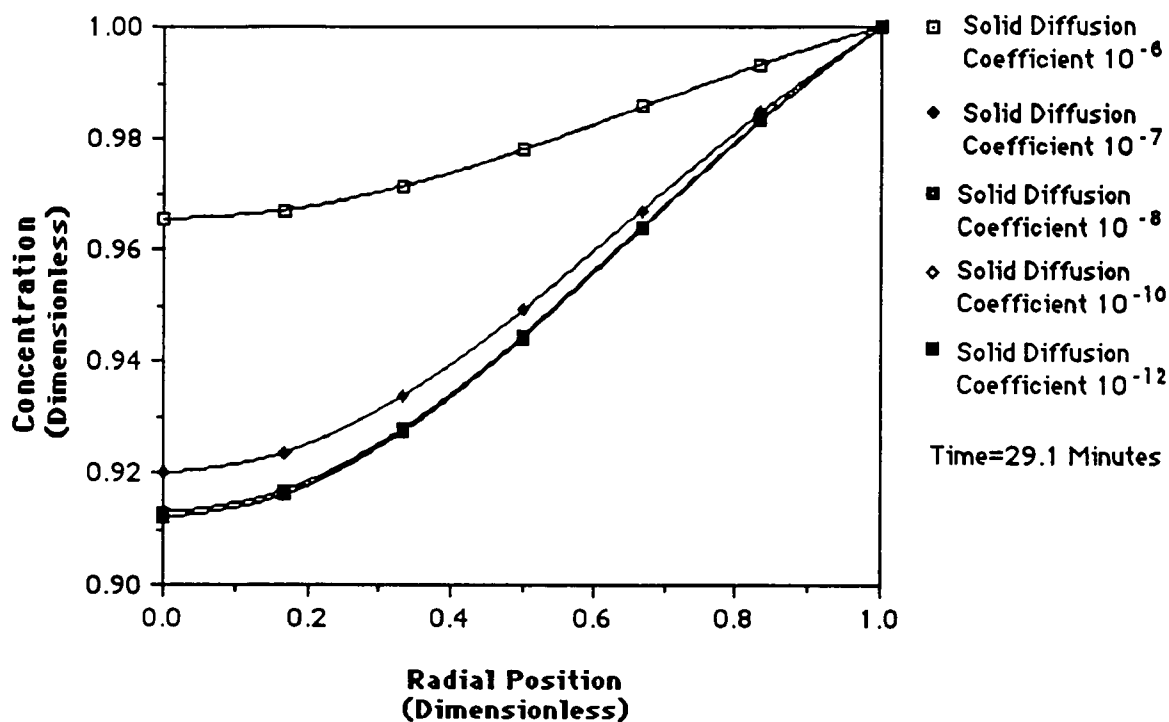


Figure 19 (Contd.). The distributions shown occurred at 29.1 minutes. The lower three curves were almost identical and overlapped. A different solid diffusion coefficient was used for each curve, varying between 10^{-6} and 10^{-12} cm^2/min . All other parameters were identical to those used to produce Figure 19A.

transport of solute through porous solids although solid diffusion (a much slower process) may also contribute. Figures 19A and 19B indicate the concentration distributions after 14.4 and 29.1 minutes, respectively. The simulations show the concentrations inside a pellet after immersion into a solute solution. The simulation did not include adsorption or degradation effects. The liquid diffusion coefficient and the particle void volume were kept constant for all simulations at $8.97 \times 10^{-6} \text{ cm}^2/\text{min}$ and 0.5, respectively. Each curve represents the cumulative effects of the solid and liquid diffusion processes (the solid diffusion coefficient was varied from $1.0 \times 10^{-6} \text{ cm}^2/\text{min}$ to $1.0 \times 10^{-12} \text{ cm}^2/\text{min}$ while the liquid diffusion coefficient was kept constant) upon solute transport in a porous solid. As the solid diffusion coefficient was increased, solute transport occurred more rapidly. The concentrations in Figure 19A ranged from 0.56 to 1.0 while the concentrations in Figure 19B ranged from 0.91 to 1.0. Figures 19A and 19B indicate that the solid diffusion transport mechanism was insignificant unless the solid diffusion coefficient was within several orders of magnitude of the liquid diffusion coefficient.

3.2 ADSORPTION PROCESSES

3.2A ADSORPTION OF NAPHTHALENE AND SODIUM SALICYLATE ON CALCIUM ALGINATE

The adsorption isotherms of naphthalene and sodium salicylate on calcium alginate were measured in batch and continuous systems. The isotherm type and magnitude were very important in determining liquid phase availability. The solid component and liquid solutions were placed together until the liquid phase concentration no longer changed. The isotherm was determined by measuring the ending liquid phase concentration (after equilibrium between the liquid and solid phases was reached) and calculating the solid phase concentration by subtracting starting and ending liquid concentrations.

Figure 20 represents the adsorption isotherm of salicylate and naphthalene on calcium alginate. The horizontal axis indicates the final liquid phase concentration in contact with the alginate after reaching equilibrium. The vertical axis represents the solid phase concentration that was in equilibrium with the liquid phase. The solid phase concentration of naphthalene was proportional to the

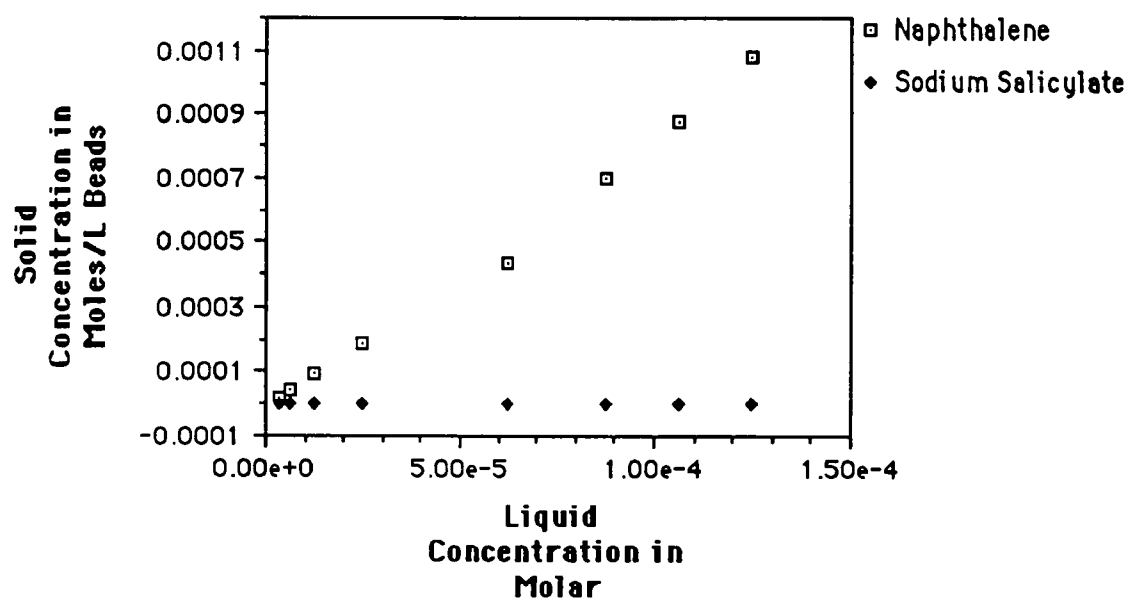


Figure 20. Adsorption isotherms of naphthalene and sodium salicylate on calcium alginate.

liquid phase concentration. The ratio of naphthalene solid to liquid phase concentration was 8.4. Salicylate did not partition onto the solid phase. The solid concentrations ranged from 0.0 to 1.1×10^{-3} moles adsorbate/liter beads. The liquid phase concentration ranged from 0.0 to 1.5×10^{-4} moles of adsorbate/liter of solution. The isotherms for both Salicylate and naphthalene did not exhibit nonlinear behavior. Nonlinear behavior could significantly complicate analysis and prediction of availability and degradation.

Table 10 indicates the time required to achieve 95 % saturation of an alginate bead with naphthalene for different adsorption and diffusion parameters. The simulation was conducted to evaluate the effects of model parameter error on achieving steady state behavior. For example, how much would a 50 percent error in the diffusion coefficient increase the time required to reach steady state. The base case corresponded to the average measured or estimated values and the corresponding saturation time. Pellet void volume and adsorption coefficient were measured. The liquid diffusion was reported by Dunbar (1990). Estimated saturation times for an alginate bead ranged from 7 minutes to almost 2 hours. Variation of the liquid diffusion coefficient had a significant effect upon the saturation time with values ranging between 7 and 99 minutes. Decreasing the solid diffusion coefficient had little effect. Increasing the solid diffusion coefficient reduced the saturation time by 30 percent. Increasing the adsorption capacity doubled the saturation time. Decreasing the adsorption capacity by half reduced the saturation time by 60 percent.

Table 10. Effect of adsorption and liquid diffusion parameters on naphthalene saturation of a calcium alginate bead. The bead diameter used for the simulation was 0.04 cm.

Liquid Diffusion coefficient D_f cm^2/s	Solid Diffusion coefficient D_f cm^2/s	Langmuir equilibrium constant $b_{\text{naphthalene}}$ $\text{cm}^3\text{s}/\text{mol}$	95 % saturation time min
Base case			
* 8.97×10^{-6}	* 9.0×10^{-8}	*8.4	63
8.97×10^{-5}	9.0×10^{-8}	8.4	7
8.97×10^{-7}	9.0×10^{-8}	8.4	99
8.97×10^{-6}	9.0×10^{-7}	8.4	47
8.97×10^{-6}	9.0×10^{-9}	8.4	65
8.97×10^{-6}	9.0×10^{-8}	4.2	26
8.97×10^{-6}	9.0×10^{-8}	12.6	119

*Parameters are measured or calculated experimental average values.

3.2B SIMULATION OF ADSORPTION PROCESSES

For modeling adsorption in heavily contaminated coal tar soil, the soil particles were assumed to be relatively nonporous. Fluid phase resistance was also assumed to be negligible. The simplified mathematical description of the packed bed consisted of n partial differential equations, one equation for each component:

$$\frac{1}{P_{e_i}} \frac{\partial^2 C_i}{\partial z^2} - \frac{\partial C_i}{\partial z} = \frac{\partial C_i}{\partial t} \left(1 + \left(\frac{1-\varepsilon}{\varepsilon} \right) \frac{dC_{s_i}^*}{dC_i} \right) \quad (38).$$

The required effort for solving these equations increases dramatically as more components are added due to the coupling of terms.

To measure the effectiveness of PDECOL (Madsen & Sincovec, 1979) for predicting adsorption in packed beds, two simulations were conducted using a sinusoidal feed and a step change in feed concentration. Equation (38) and the following boundary and initial conditions were used for the simulations using the sinusoidal feed:

$$C_i|_{z=0} = 0.5 + \frac{\sin(2\pi t / P_i - \pi / 2)}{2} \quad (39a),$$

$$\left. \frac{\partial C_i}{\partial z} \right|_{z=1} = 0 \quad (39b),$$

$$C_i = 0 \quad (t < 0) \quad (39c).$$

The first boundary condition, equation (39a), indicated that the feed concentration was varied in a sinusoidal manner. The second boundary condition, equation (39b), indicated that mass transfer by diffusion ceased as the component flowed out of the PBR. The initial condition, equation (39c) indicated that there was no feed component inside the PBR in the liquid phase at the start of the experiment.

The first simulation was conducted to partially evaluate the performance of PDECOL (Madsen & Sincovec, 1979) for numerically solving convection-adsorption problems encountered in the analysis of packed bed processes. Negligible mass transfer resistance, dispersion, and adsorption were assumed. Under these conditions, a sine wave with the same amplitude and frequency of the input wave would move through the bed (Figure 21). The horizontal axis is dimensionless feed concentration. The vertical axis is the dimensionless effluent concentration. The concentration distribution through the bed was a sinusoidal wave form with an amplitude and period equal to the feed wave. The numerical technique produced the expected behavior.

A simulation using a step change in the feed concentration was also conducted to measure the effectiveness of PDECOL (Madsen & Sincovec, 1979) for use in modeling adsorption in packed beds. Equation (38) and the following initial and boundary conditions were used for the simulation using the step change:

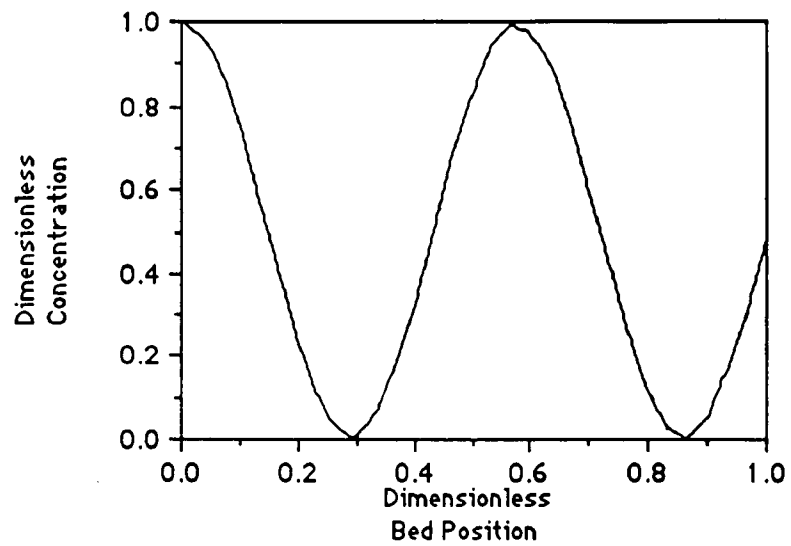


Figure 21. Concentration distribution in a packed bed with a sinusoidally varying feed concentration. The numerical solution included negligible adsorption, negligible mass transfer resistance, and negligible dispersion.

$$C_i|_{z=0} = 1 - \exp(-k_e t \tau) - \frac{1}{P_e} \frac{\partial C_i}{\partial Z} \quad (40a),$$

$$\left. \frac{\partial C_i}{\partial z} \right|_{z=1} = 0 \quad (40b),$$

$$C_i = 0 \quad (t < 0) \quad (40c).$$

The first boundary condition, equation (40a), indicated that the feed concentration increased rapidly from zero to a dimensionless concentration of 1.0 at a time close to zero. A perfect step change has an infinite slope and can not be represented on a digital computer. Changing the feed concentration from 0.0 to 1.0 at time zero introduces a discontinuity (infinite first derivative value) and generally causes erroneous results. The exponential function is used to rapidly increase the feed concentration to 1.0 without introducing a discontinuity. A value of 200 was assigned to k_e for the simulations (the greater the value of k_e , the greater the slope). The boundary conditions (40b) and equation (40c) are identical to the previous case.

Figure 22 shows the predicted concentration distribution inside the packed bed. The horizontal axis is dimensionless bed position. The vertical axis is dimensionless concentration inside the bed. The concentration through the bed approached a step function. Two anomalies were present in the simulation, the negative concentration dip at the bottom of the step, and the

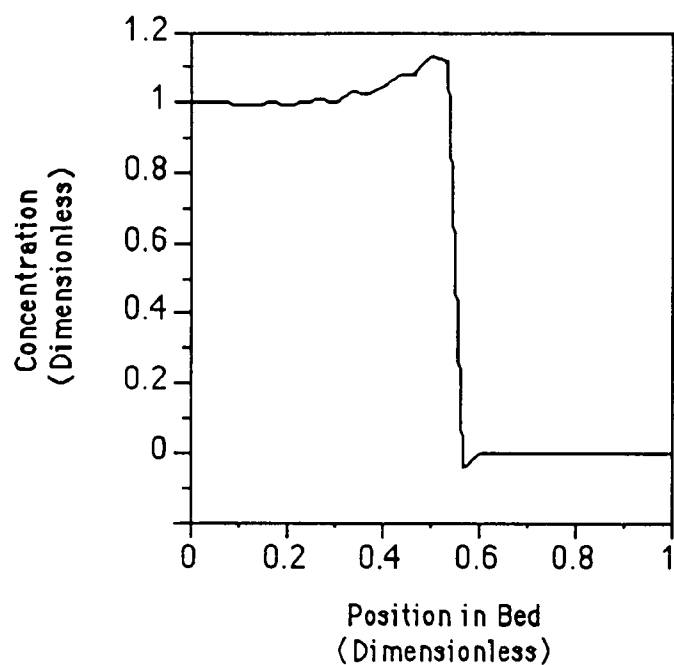


Figure 22. Simulated response of a packed bed adsorber to a step increase in feed concentration. The model includes dispersion and favorable nonlinear adsorption.

concentrations greater than 1.0 at the top of the step. These anomalies arise from the orthogonal collocation technique.

Two single component limiting cases were investigated using the simulation techniques mentioned above. The first case corresponded to a low capacity adsorption bed sinusoidally loaded with a relatively high concentration feed. The second case involved the addition of a relatively low concentration feed to a high capacity bed.

Figure 23 depicts the simulated loading of a low capacity bed with a high concentration (sinusoidal wave) feed. The simulation was conducted to analyze the effects that favorable adsorption has on sine wave form. A favorable isotherm was used for the simulation. The horizontal axis indicates the dimensionless bed position. The vertical axis indicates dimensionless concentration. A position of 0.0 corresponded to the entrance of the bed while the exit corresponded to a value of 1.0. Three feed cycles were observed in the bed. The left most wave had been in the column the least time while the right hand wave had been in the column the longest time. As the waves continued through the bed, adsorption effects become more pronounced. The first wave, being in the column the least amount of time, retained most of its sinusoidal character. The second wave was altered due to it's longer residence in the bed. The shape of the third wave included a sharp leading front while the trailing edge continued to expand. The period and amplitude of the

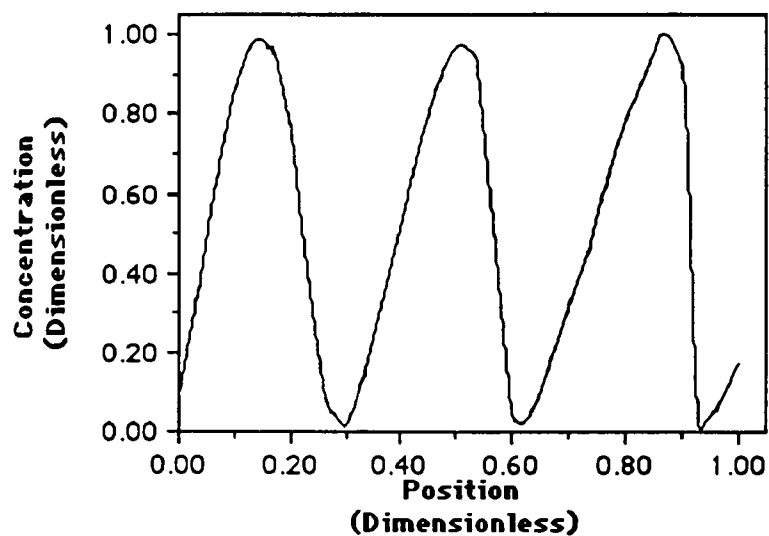


Figure 23. Formation of a saw tooth wave due to adsorption in a packed bed reactor. The shape of the sinusoidal feed waves in the packed bed reactor were changed by nonlinear favorable adsorption.

third wave was approximately equal to the first two waves. The simulation suggested that loading a low capacity bed with a high concentration feed produced an outlet concentration distribution with the same frequency and amplitude as the input wave, but with a saw tooth shape.

Figure 24 depicts the simulated loading of a high capacity bed with a low concentration sinusoidal feed. Many physical situations correspond to this scenario. Each diagram depicts the cut away view of the adsorption column at different times. The horizontal axes are all the same scale and indicate dimensionless position in a packed bed. The vertical axes are all the same scale and indicate dimensionless liquid phase concentration. Operation times are reported in cycles of the feed sine wave in the top center of each diagram. For example, the top diagram depicts the concentration in a packed bed after the feed concentration has cycled through two periods. Because the solute had a large affinity for the solid phase, the bed loaded from the inlet forward to the outlet. The adsorbent bed in the last frame of Figure 24 is almost saturated with solute as indicated by the deep penetration of solute in the bed. Continued addition of feed resulted in complete loading of the solid phase. Because the feed wave was severely damped, the liquid phase effluent concentration was equal to the average feed concentration.

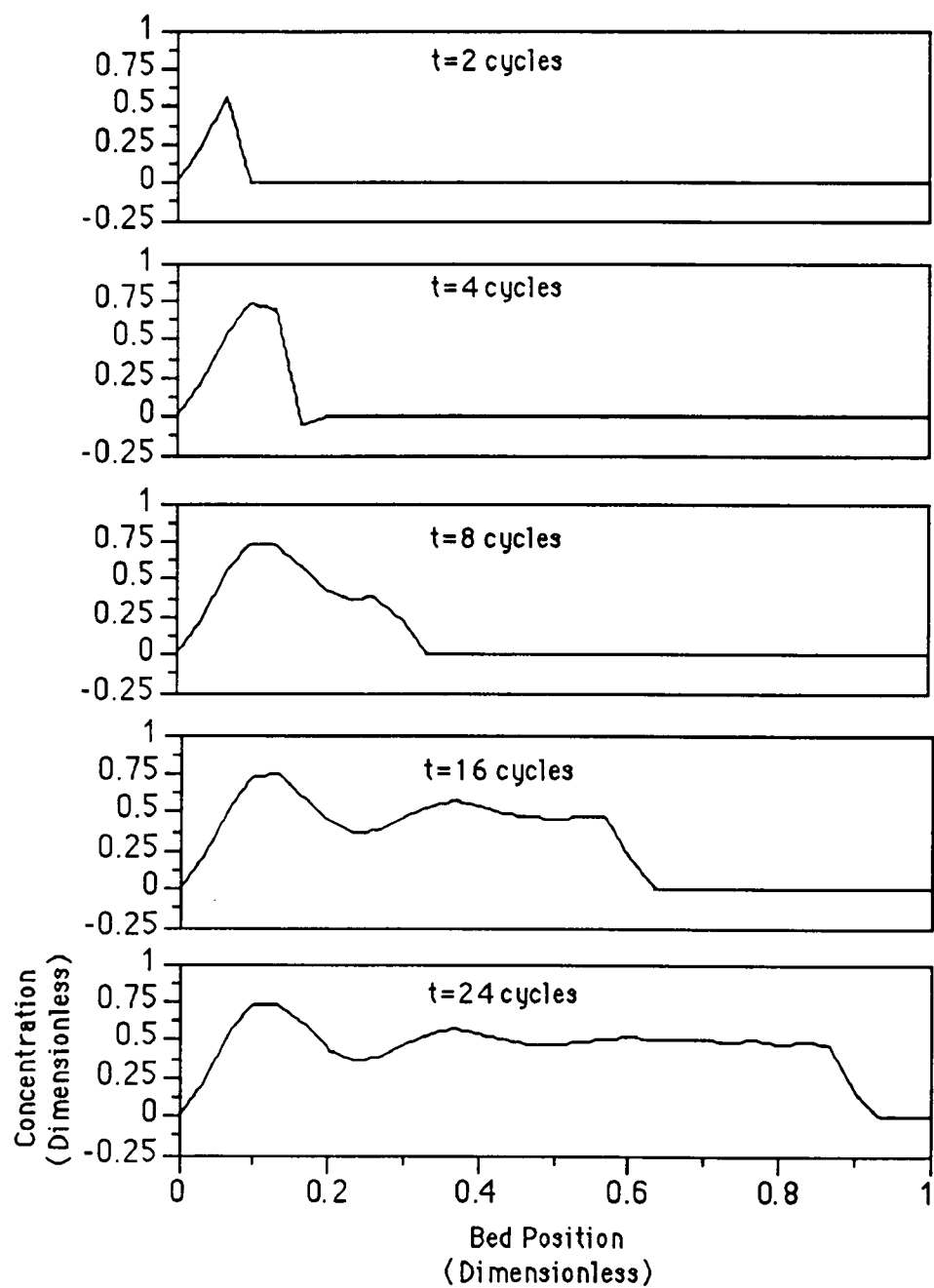


Figure 24. Time course study of the input of a low concentration sine wave to a high capacity adsorption bed.

The dampening of the sine wave in Figure 24 deserves further discussion. Initially, in the top diagrams, the sine wave was severely damped. After the solid phase in the front part of the column became loaded, the input sine wave began to be evident. Although less evident in the last frames of Figure 24, the sine wave was prevalent near the inlet but was damped as the wave moved through the bed. The observed dampening effect of the column depended upon the adsorption isotherm, mass transfer, and dispersion.

Figure 25 depicts the simulated response of a packed column to a sinusoidal feed wave after the bed is fully loaded. For a high capacity bed, the dampening of the sine wave depended mostly on adsorption. Figure 25 first figure depicts liquid phase concentration in the packed column as a function of position and time. The vertical axis in the first figure indicates concentration. The axis projecting out of the plane of the paper indicates time in cycles of the feed wave. The horizontal axis in the plane of the paper indicates position in the bed. A damped feed sine wave is shown moving through the bed (first frame). The second frame indicates the feed concentration as a function of time over one period and is also in the first frame (feed sine wave). The third frame indicates liquid phase concentration as a function of position in the bed at the beginning of a feed cycle. The dampening effect of adsorption is clearly observable in the last frame.

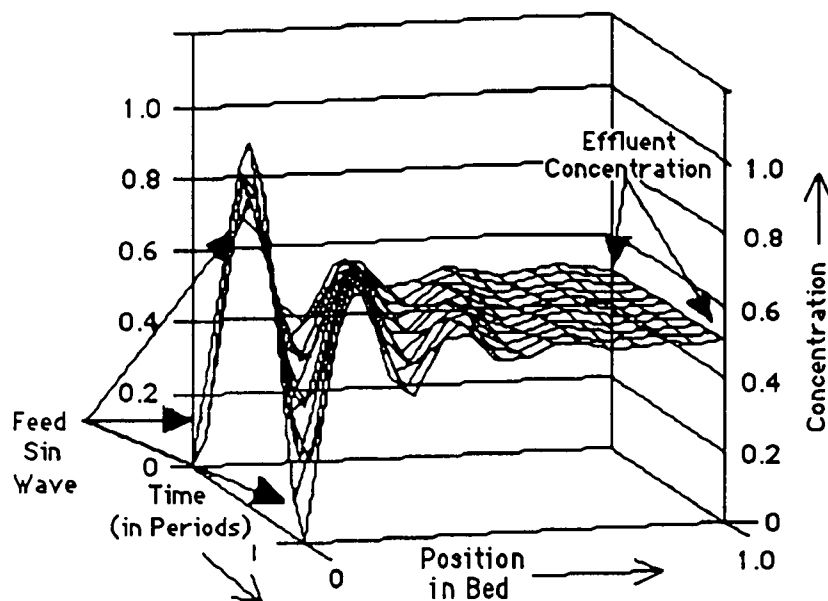


Figure 25. Dampening of a sinusoidal feed wave. The sinusoidal feed wave was damped by adsorption. The numerical solution included effects of favorable single component adsorption and dispersion.

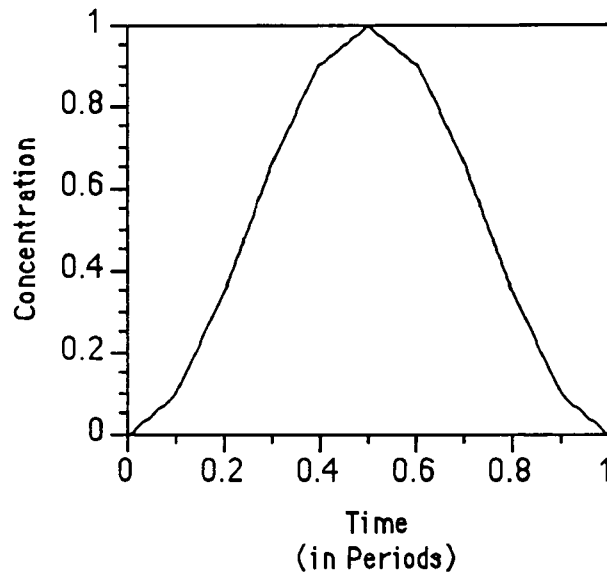


Figure 25 (Contd.). Sinusoidal feed concentration over one period.
The feed concentration varied from 0 to 1.0 over one cycle.

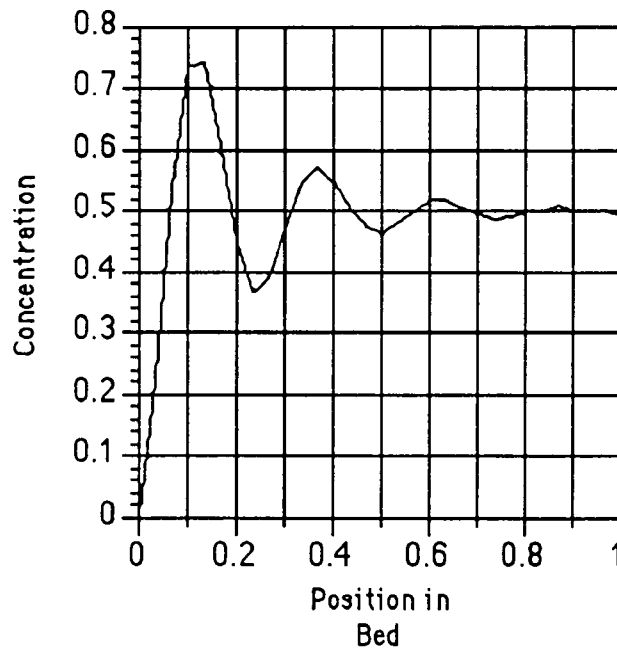


Figure 25 (Contd.). The sinusoidal feed wave was damped by adsorption. The inlet concentration (position=0) is shown at the beginning of a cycle. The feed concentration varied between a concentration of 0 and 1.0.

In summary, PDECOL (Madsen & Sincovec, 1979) was effective in simulating adsorption processes in packed beds using sinusoidal boundary conditions. PDECOL (Madsen & Sincovec, 1979) was less effective in simulating adsorption bed behavior using a step boundary condition. Adsorption was important in determining the shape and dampening of sine feed waves. The shape of the feed sine wave was altered as it passed through a low capacity bed to include a steep leading front and an expanding trailing front. The feed sine wave was severely dampened as it passed through a high capacity bed. After the high capacity bed was loaded, the sin wave could be observed near the inlet. The amount of dampening was a strong function of the bed capacity.

3.2C SOIL ADSORPTION STUDIES

Initial experiments investigated sorption processes within the PBR equipment. Adsorption to structural parts of the reactor was negligible. The reactor effluent concentration tracked the inlet when feed was diverted through the reactor bypass valve while varying the feed concentration in a sinusoidal manner (Figure 26).

A PBR was loaded with 16.2 mL of MGP soil. HPLC grade water was then passed through the reactor at a volumetric flow rate of 0.3 cm³/min. Over a period of several days, concentrations of leachates

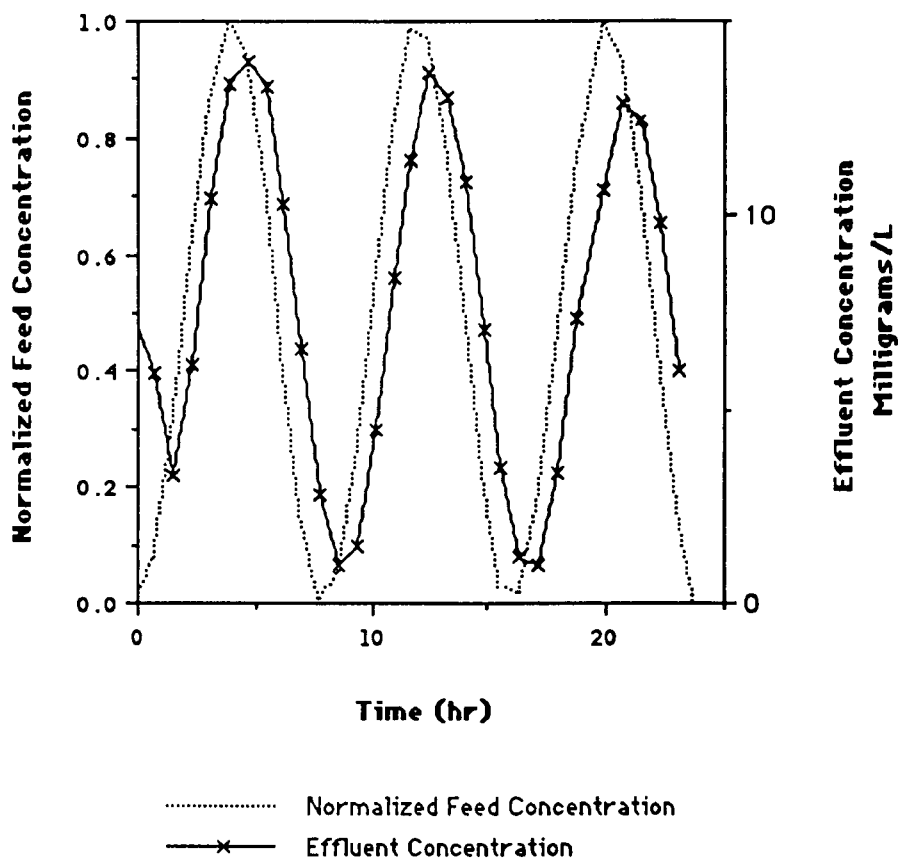


Figure 26. Response to sinusoidal perturbation with feed bypassing the soil wafer. Naphthalene effluent concentration tracked the feed concentration when the feed did not contact the soil.

in the effluent stream decreased due to depletion of contaminants in the soil. Microbial activity was inhibited by continuous addition of 0.05 percent HgCl_2 and anaerobic conditions.

Soil leachates identified using GC-MS and retention times from Standard Reference Material (SRM) 1491 (National Institute of Standards and Technology, Gaithersburg, MD) were biphenyl, naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, acenaphthalene, acenaphthene, 9-h-flourene, phenanthrene, and benzo(e)pyrene. 1,3,5-trimethylbenzene, ethenylmethylbenzene, 1,6-Dimethylnaphthalene, and dibenzofuran were identified using GC-MS only. Table 11 contains concentrations of PAH found in the MGP soil as measured by Cushy and Morgan, 1990. Two ring compounds and benzene derivatives were generally volatile enough to be detected by the on-line GC method.

Table 11. Components and concentrations of contaminants in MGP soil (Soil A).

Component	Concentration mg/kg Soil	Component	Concentration mg/kg Soil
Benzene	1.1	Ethylbenzene	2.9
Styrene	1.1	Toluene	1.8
Xylenes	15	Carbazole	89
Naphthalene	1200	Acenaphthylene	540
Acenaphthene	120	Fluorene	190
Anthracene	320	Phenanthrene	240
Fluoranthene	266	Pyrene	220
Benzo(b)- fluoranthene	39	Benz(a)- anthracene	170
Benzo(a)pyrene	86	Chrysene	100
Benzo(k)- fluoranthene	56	Dibenzo(a,h)- anthracene	210
Indeno(123- cd)pyrene	125	Benzo(g,h,i)- perylene	59
2-methyl- naphthalene	510		

Adapted from Cushy, M. A. and Morgan, D. J., Biological Treatment of Soils Containing Manufactured Gas Plant Residues, Topical Report, 1990, Gas Research Institute, Contract No. 5086-254-1334.

The normalized FID responses for leachates were measured using SRM 1491. Because FID response was proportional to mass, approximate concentrations were calculated from the peak area. The normalized FID response factors of the GC were measured and compared to literature values (Table 12). The response factors were relative to fluoranthene. The measured response factors for most of the compounds were within about 10 percent of the literature values.

Naphthalene solution was fed to the reactor in a sinusoidal manner. Sinusoidally perturbing the system forced the system into a continual response pattern. The frequency of the input sine wave was 8 hr with a maximum naphthalene concentration of 14 mg/l. The naphthalene feed solution (pH of 7.0) volumetric flow rate was 0.30 cm³/min. The experiments were conducted at 20° C. Leachate and naphthalene responses to the naphthalene feed concentration are shown in Figures 27-33. Only naphthalene was present in the feed. Normalized naphthalene feed concentration, experimental time and effluent concentrations are shown on the left hand, horizontal and right hand axis, respectively. The HPLC method was not developed for on-line use at the time of initial sinusoidal perturbation experiments. Later the HPLC method was used to verify that the effluent leachate concentrations were affected by perturbations in the naphthalene feed concentration.

Table 12. Literature and measured normalized FID response factors.

Compound	Literature*	Empirical
Naphthalene	Not listed	9.50×10^{-1}
2-methylnaphthalene	Not listed	9.22×10^{-1}
1-methylnaphthalene	Not listed	9.810×10^{-1}
Biphenyl	7.51×10^{-1}	9.36×10^{-1}
2,6-dimethyl-naphthalene	Not listed	9.46×10^{-1}
Acenaphthalene	Not listed	9.541×10^{-1}
Acenaphthene	Not listed	9.122×10^{-1}

Table 12. (Contd.)

Compound	Literature*	Empirical
2,3,5-trimethyl-naphthalene	Not listed	9.4×10^{-1}
Fluorene	8.640×10^{-1}	9.513×10^{-1}
Phenanthrene	9.20×10^{-1}	9.648×10^{-1}
Anthracene	8.80×10^{-1}	9.703×10^{-1}
1-methyl-phenanthren	Not listed	9.65×10^{-1}
Fluoranthene	1.000	1.00
Pyrene	1.067	9.82×10^{-1}

*Contents of Literature Column from Source (Lao, *et al.*, *Analytical Chemistry*, 45, 908-915, 1973.)

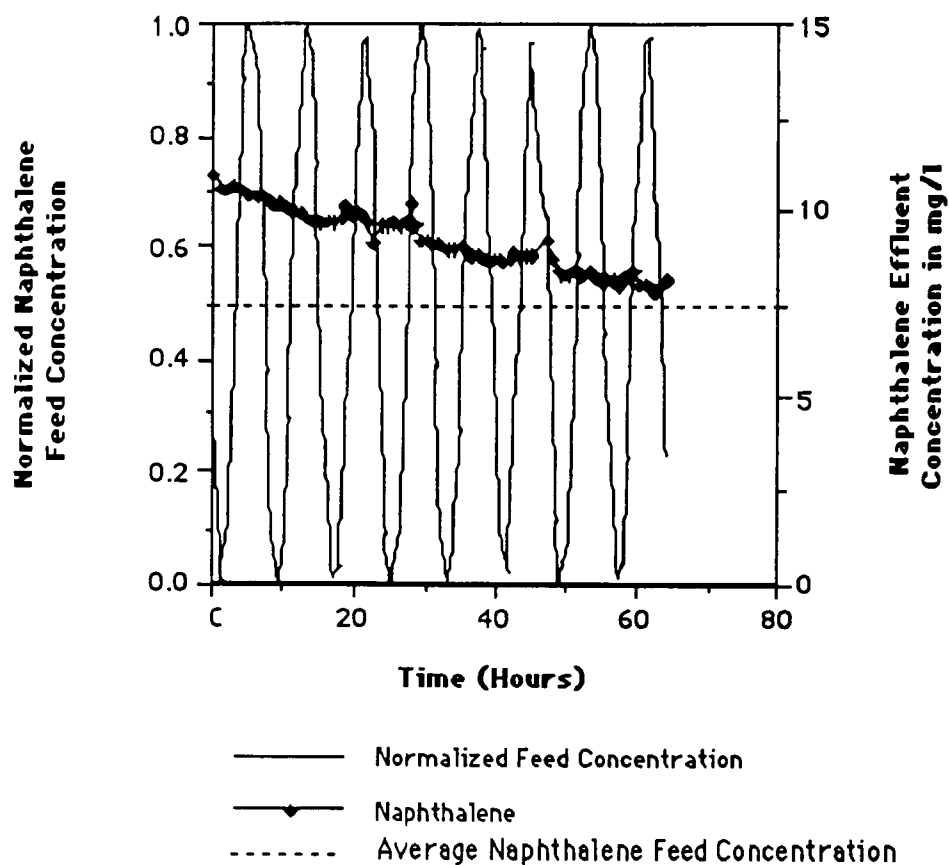


Figure 27. Effluent naphthalene response to sinusoidal feed perturbations. The naphthalene feed concentration was supplied to the reactor in a sinusoidal manner. The PBR was packed with 16.2 mL of MGP soil.

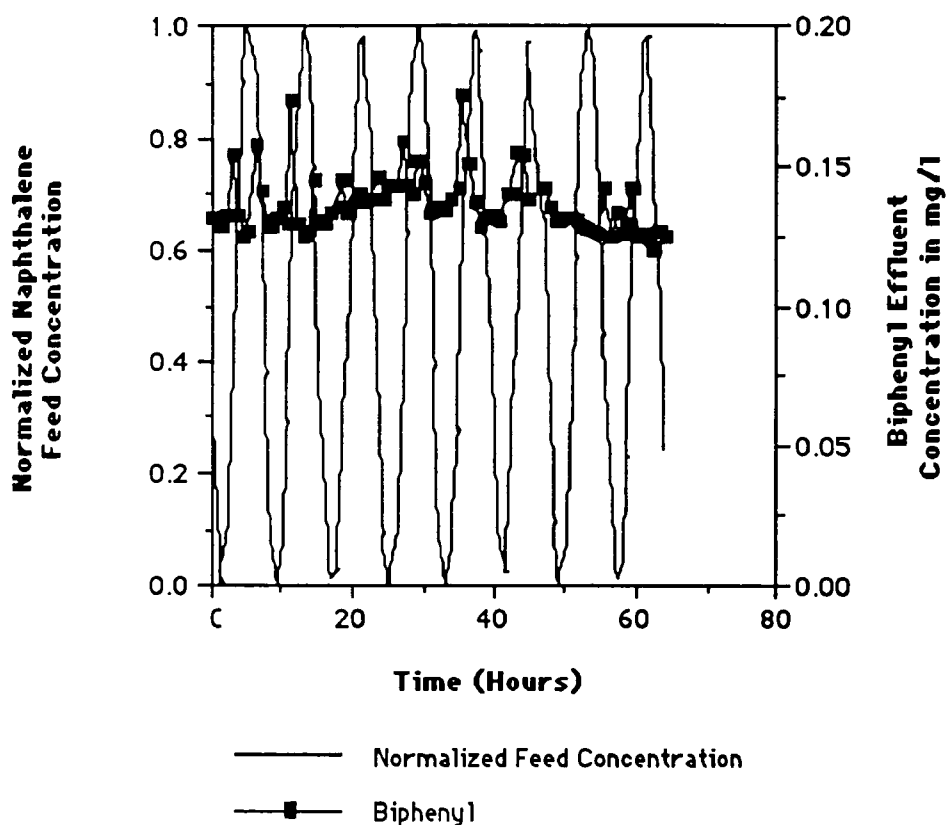


Figure 28. Biphenyl effluent concentration as function of sinusoidally varying naphthalene feed concentration. The PBR was packed with 16.2 mL of MGP soil. Biphenyl was not present in the feed and leached from the soil.

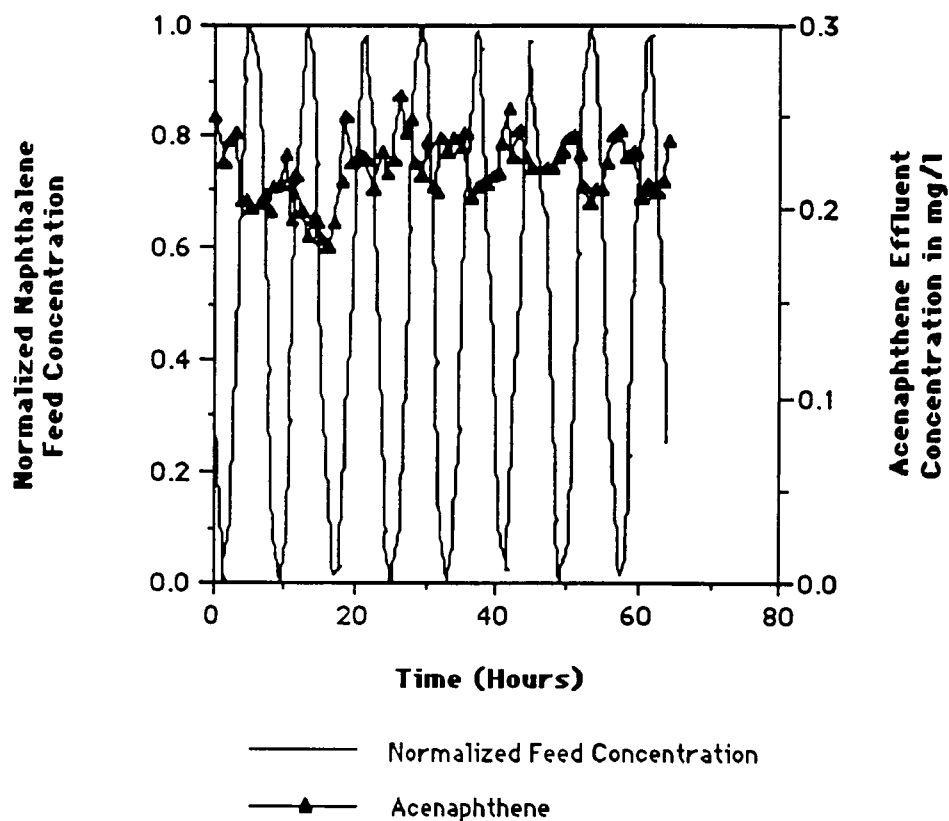


Figure 29. Acenaphthene effluent response to sinusoidal naphthalene feed perturbations. The PBR was packed with 16.2 mL of MGP soil. The acenaphthene was not present in the feed and leached from the soil.

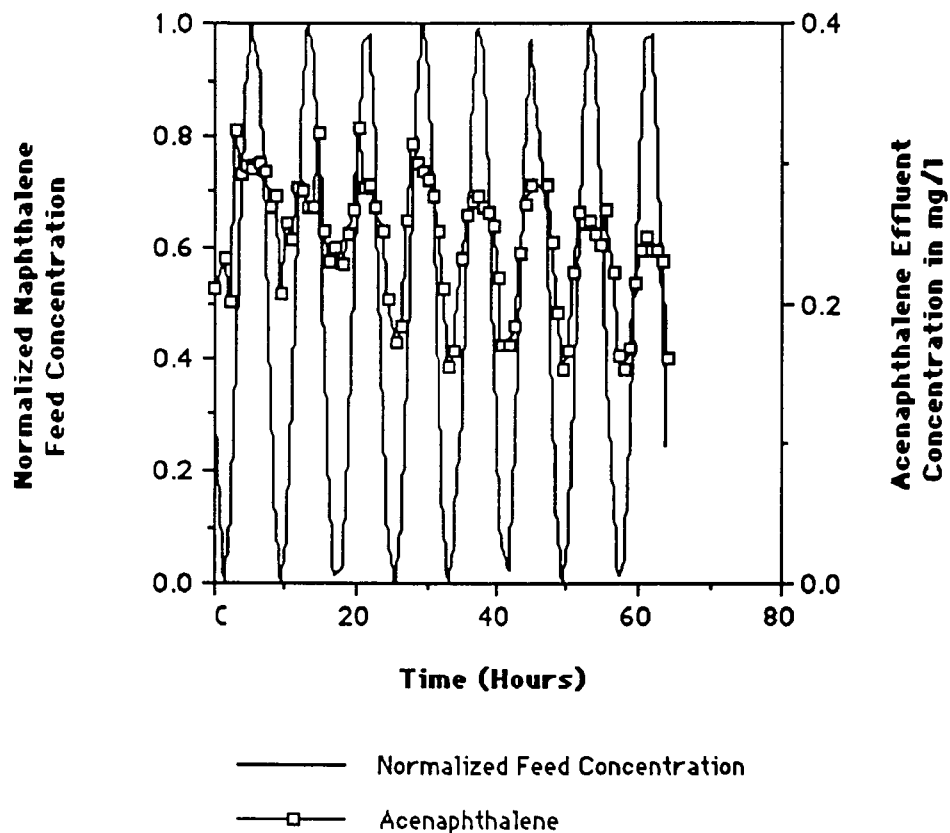


Figure 30. Acenaphthalene effluent response to sinusoidal naphthalene feed perturbations. The PBR was packed with 16.2 mL of MGP soil. The acenaphthalene was not present in the feed and leached from the soil.

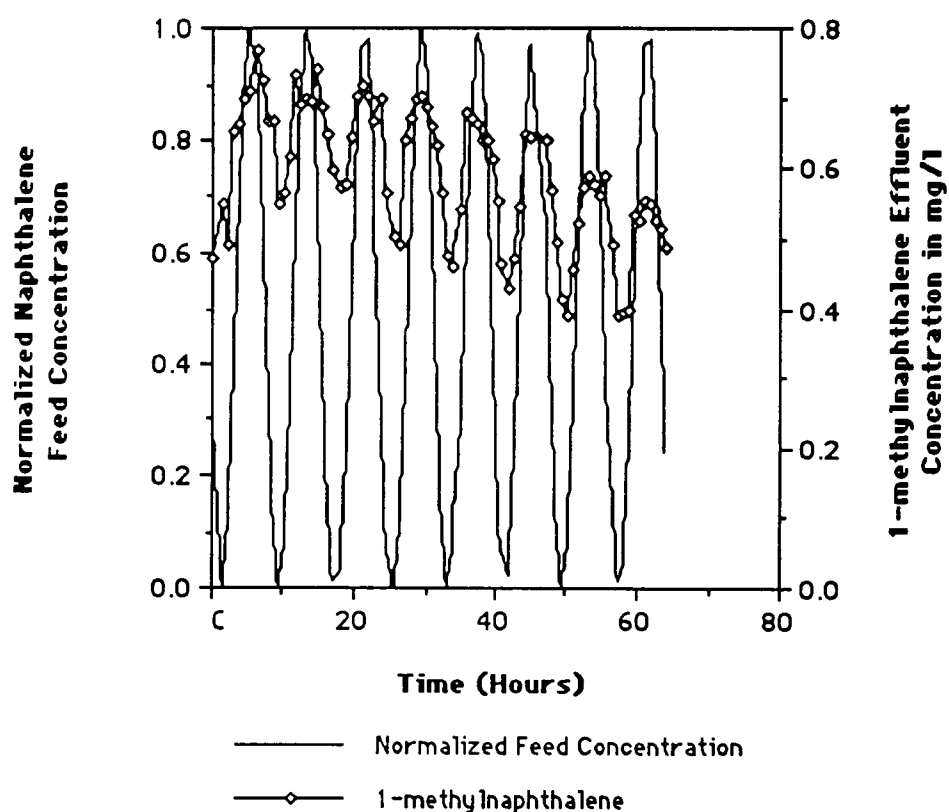


Figure 31. 1-methylnaphthalene effluent response to sinusoidal naphthalene feed perturbations. The PBR was packed with 16.2 mL of MGP soil. The 1-methylnaphthalene was not present in the feed and leached from the soil.

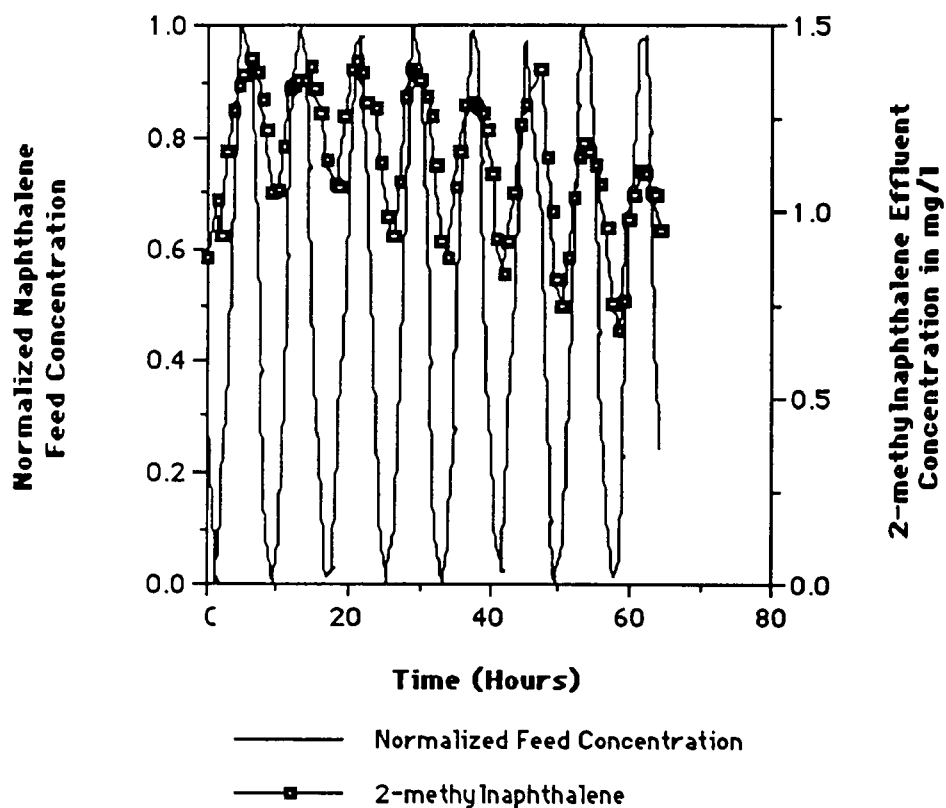


Figure 32. 2-methylnaphthalene effluent response to sinusoidal naphthalene feed perturbations. The PBR was packed with 16.2 mL of MGP soil. The 2-methylnaphthalene was not present in the feed and leached from the soil.

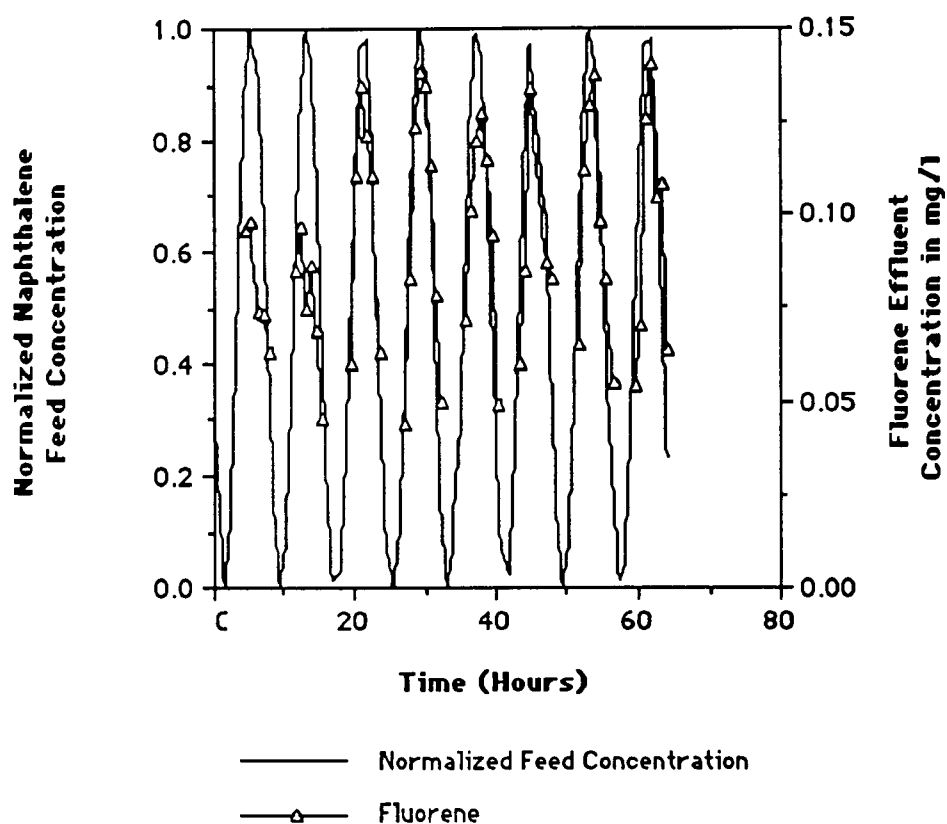


Figure 33. Fluorene effluent response to sinusoidal naphthalene feed perturbations. The PBR was packed with 16.2 mL of MGP soil. The fluorene was not present in the feed and leached from the soil.

Figure 27 illustrates the differences between input and output naphthalene concentration waves from the MGP soil. The effluent naphthalene concentration is shown on the right hand axis. The dotted horizontal line indicates the average feed concentration of the naphthalene. Initial naphthalene effluent concentrations were above average feed levels indicating leaching of pre-existing naphthalene from the soil matrix. The output naphthalene sine wave was severely damped and was always greater than the mean concentration of the feed. Complete dampening of the feed pulse would result in an effluent concentration equal to the mean of the feed concentration. In other experiments where water was passed through the column, naphthalene was a major effluent component.

Figures 28 and 29 depict the effluent biphenyl and acenaphthene concentrations when the PBR was pulsed with the sinusoidal naphthalene feed. In Figure 28 and 29 the right axis is the respective effluent biphenyl or acenaphthene concentration. Peak areas were well above noise levels. Some scattering of data occurred although the effluent concentrations of acenaphthene and biphenyl were approximately constant indicating that biphenyl and acenaphthene were not greatly influenced by the sinusoidal addition of naphthalene. Because no biphenyl or acenaphthene were present in the feed, the effluent concentrations are the result of leaching and sorption processes occurring in the soil.

Figures 30-33 depict the respective effluent concentrations of acenaphthalene, 1-methyl, 2-methylnaphthalene and fluorene concentrations as the PBR was pulsed with a sinusoidal naphthalene

feed (constant hydrodynamic flow). The left hand axis is the normalized concentration of the naphthalene feed with the experiment time shown on the horizontal axis. The right axis is the effluent acenaphthalene concentration were the result of leaching and sorption processes occurring in the soil. In contrast to acenaphthalene and biphenyl, the effluent concentrations exhibited periodicity similar to the naphthalene feed concentration perturbations. Peak areas for calculation of the acenaphthalene and alkylated naphthalene concentrations were well above noise levels. Fluorene concentrations at noise levels were not included in Figure 33. Figures 30-33 demonstrated that acenaphthalene, 1 and 2-methylnaphthalene and fluorene concentrations were affected by the naphthalene feed concentration.

Figures 27-33 illustrated the differences between input naphthalene concentration and output leachate concentration fronts from the MGP soil. A number of the compounds exhibited sinusoidal responses similar to the input naphthalene feed. Acenaphthene and biphenyl did not appear to exhibit the sinusoidal behavior that many of the other compounds did. For leachate concentration calculations using the GC method, Henry's Law constants for two ring PAH were assumed equal to that of naphthalene. Henry's Law constants for most components were not readily available. Concentrations were measured using on-line HPLC methods.

Step perturbation experiments were conducted to compare the sinusoidal response to the step change response and to further investigate the effect of naphthalene on the desorption process of

PAH in the MGP soil. The experiments were conducted by running distilled water through the reactor bed followed by a constant input of aqueous naphthalene solution at a flow rate of 0.3 mL/min. The experiment was conducted at 20^o C. The feed concentration for the naphthalene step perturbation was 16 mg/l. Naphthalene and several leachate responses to the step change in naphthalene feed concentration are shown in Figures 34-36. Normalized naphthalene feed concentration, experimental time and effluent concentrations are shown on the left hand, horizontal and right hand axis, respectively.

Figure 34 illustrates the response of phenanthrene to the step perturbation in naphthalene feed. The left hand axis is the normalized concentration of the naphthalene feed with the experiment time shown on the horizontal axis. The right axis is the effluent phenanthrene concentration. Because only naphthalene was present in the feed, the effluent concentration was the result of leaching and sorption processes occurring in the soil. The phenanthrene concentration initially reached a maximum when distilled water was passed through the reactor bed and then displayed a continuous decay pattern. When naphthalene solution was passed through the bed, the effluent phenanthrene concentration

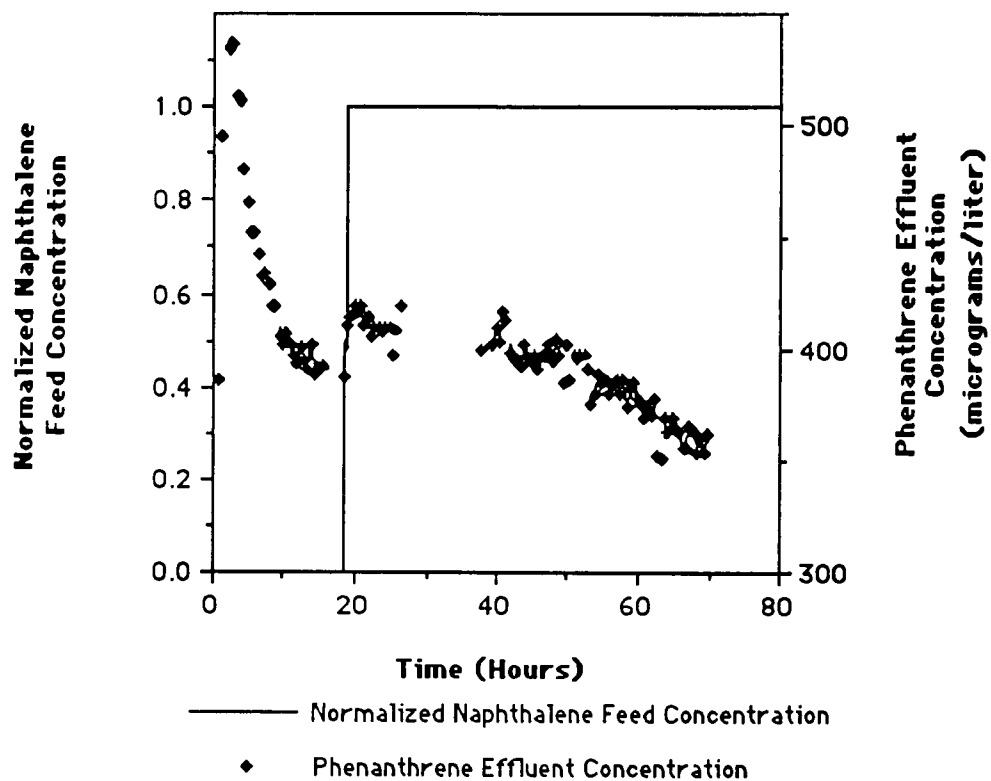


Figure 34. Phenanthrene effluent response to step perturbation in the naphthalene feed concentration. The PBR was packed with 45 mL of MGP soil. The phenanthrene was not present in the feed and leached from the soil.

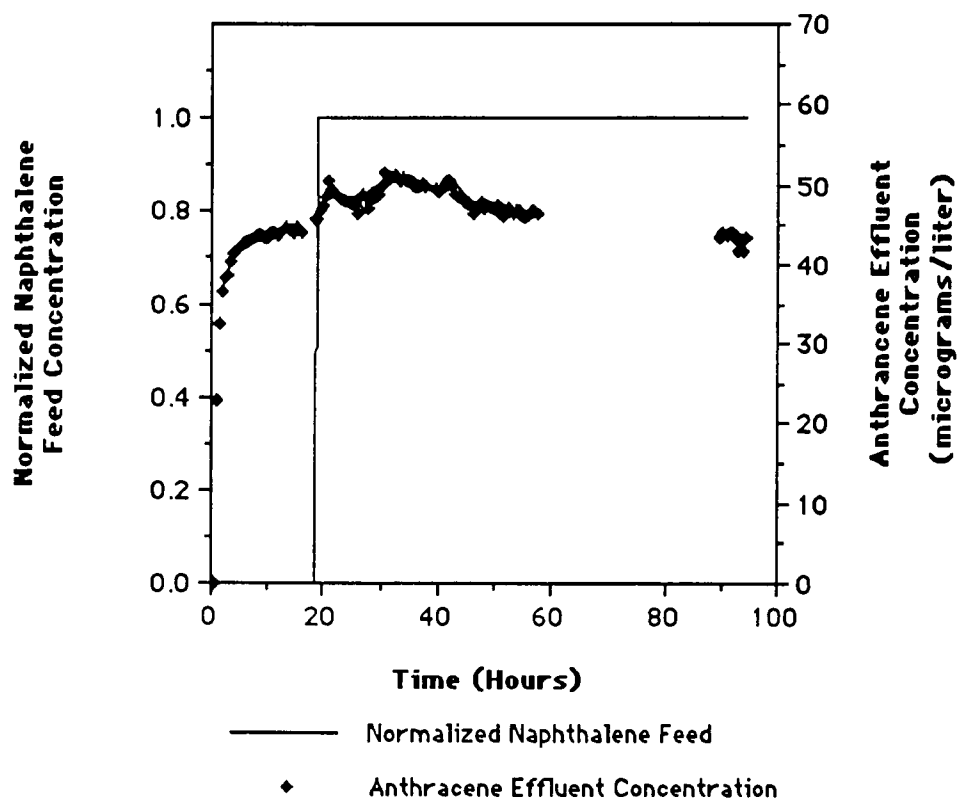


Figure 35. Anthracene effluent response to step perturbation in the naphthalene feed concentration. The PBR was packed with 45 mL of MGP soil. The anthracene was not present in the feed and leached from the soil.

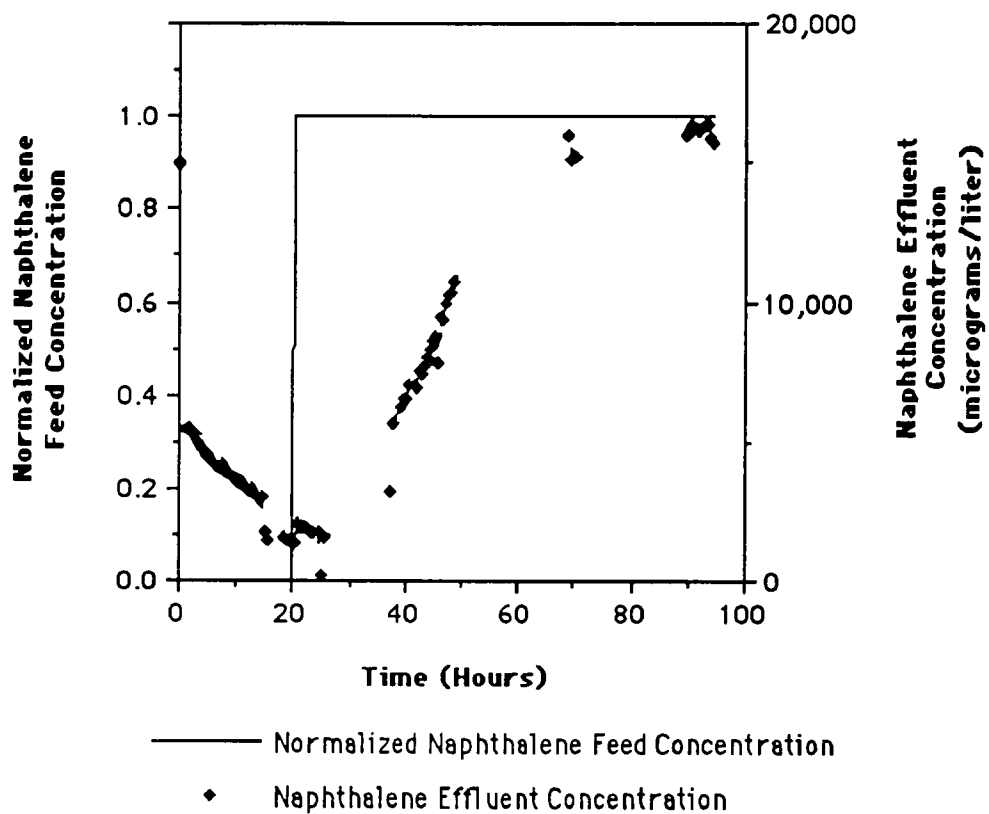


Figure 36. Naphthalene effluent response to a step change in naphthalene feed concentration. The PBR was packed with 45 mL of MGP soil. Naphthalene was not initially in the feed and leached from the soil.

increased by 10 percent. The phenanthrene concentration continued to decay although possibly at a reduced slope. The effluent phenanthrene concentration was affected by the addition of naphthalene.

Figure 35 illustrates the response of anthracene to the step perturbation in naphthalene feed. The left hand axis is the normalized concentration of the naphthalene feed with experiment time shown on the horizontal axis. The right axis is the effluent anthracene concentration. Because only naphthalene was present in the feed, the effluent concentration was the result of leaching and sorption processes occurring in the soil. When distilled water was passed through the soil bed, anthracene concentration was constant. The effluent concentration increased by approximately 10 percent when naphthalene solution was added. The anthracene effluent concentration continued at or above concentrations previous to the step increase in naphthalene feed concentration.

Figure 36 illustrates the response of naphthalene to the step change in naphthalene feed concentration. The left hand axis is the normalized concentration of the naphthalene feed with the experiment time shown on the horizontal axis. The right axis is the effluent naphthalene concentration. When water was passed through the bed, the naphthalene effluent concentration initially reached a maximum and then began decreasing. When naphthalene solution began flowing through the bed, the effluent naphthalene concentration began increasing (estimated to be within several bed volumes). Gaps appear in the data of Figure 36 because of required

maintenance and equipment problems. Approximately 50 hours (40 bed volumes) after the step change in the feed concentration, the effluent concentration was equal to the feed concentration. The effluent naphthalene concentration was equal to one half the feed concentration approximately 30 hours (24 bed volumes) after the addition of naphthalene solution. The long breakthrough time indicates that the soil had a relatively high adsorption capacity for naphthalene.

Leachate responses to step perturbations were less easily interpreted than responses to sinusoidal perturbations. Figures 23-28 illustrated the differences between input naphthalene concentration and output leachate concentration fronts from the MGP soil using sinusoidal perturbations. A number of the compounds exhibited sinusoidal responses similar to the input naphthalene feed. Acenaphthene and biphenyl did not appear to exhibit the sinusoidal behavior that many of the other compounds did. Figures 34-35 indicate that anthracene and phenanthrene effluent concentrations were affected by the addition of naphthalene solution. Figure 36 indicates that the soil had a high capacity for adsorption of naphthalene.

3.3 KINETIC EVALUATION OF *P. fluorescens* HK44

The degradation properties of *P. fluorescens* HK44 were investigated. The form of the rate equation and associated kinetic

constants were determined from experiments using alginate immobilized *P. fluorescens* HK44. Feed and biomass concentrations and flow rates were varied to determine the form of the rate equation and associated constants.

Degradation experiments, their parameters, and the degradation results are listed in Table 13. Feed concentrations, biomass concentrations, and flow rates were varied to determine the form of the rate term and rate term constants. Experiments were conducted by varying experimental conditions over the wide ranges to affect large responses by bacteria. Experiments were generally carried out over a three day period and were begun using a high flow rate (1.0 mL/minute). After steady state (with respect to degradation) was achieved, the flow rate was reduced. The degradation was then allowed to reach steady state again. After repeating this procedure several times, the flow rate was increased to a previously used flow rate (0.6 mL/min) and allowed to attain steady state. All experiments were limited in phosphate in an attempt to minimize growth. Oxygen (used in aerobic degradation) at high concentrations may prove toxic to bacteria. Because oxygen is supplied to the reactor dissolved in the aqueous phase, total amounts of organic compounds were limited to less than 15 mg/L. PAH aqueous solubility ranges up to several mg/L.

Table 13. Kinetic investigation of *P. Fluorescens* HK44. Biomass, feed concentrations, residence times, and effluent concentrations are listed for the different experiments.

Experiment	Biomass	Sodium Salicylate Feed	Residence Time	Salicylate Effluent	Standard Deviation of Effluent
	cells/mL	mg/L	min	mg/L	% of Effluent Concentration
1a	3.3×10^9	2.25	14	1.43	1.99
1b	3.3×10^9	2.25	58	0.46	1.82
1c	3.3×10^9	2.25	14	1.65	2.89
2a	3.5×10^9	2.47	24	1.03	2.75
2b	3.5×10^9	2.47	26	0.86	1.08
2c	3.5×10^9	2.47	94	0.20	1.40
3a	1.9×10^9	4.45	14	4.06	2.18
3b	1.9×10^9	4.45	18	3.84	1.82
3c	1.9×10^9	4.45	24	3.42	2.50
3d	1.9×10^9	4.45	36	2.76	1.53
3e	1.9×10^9	4.45	72	1.81	2.96
3f	1.9×10^9	4.45	24	3.12	1.31
4a	1.9×10^9	4.45	29	3.07	2.10
4b	1.9×10^9	4.45	36	2.76	1.43
4c	1.9×10^9	4.45	48	2.25	1.54
4d	1.9×10^9	4.45	72	1.75	1.16
4e	1.9×10^9	4.45	150	0.89	1.73
4f	1.9×10^9	4.45	48	2.24	1.56

Experiments in Table 13 were numbered 1 to 4 and were further grouped according to residence time (indicated by a lower case letter). The experiments are identified through out the rest of the text using the notation in Table 13. The first column of Table 13 identifies the experiment. The second and third column indicate cell concentrations and feed concentrations. The fourth, fifth and sixth columns indicate reactor residence time, normalized effluent concentrations, and the standard deviation of the normalized effluent concentrations in percent of the average effluent concentration. Substrate concentrations are reported in milligrams/liter.

The second, third, and fourth columns of Table 13 lists important parameters that were varied to kinetically characterize *P. fluorescens* HK44. Biomass concentrations listed in the second column were varied from 1.9×10^9 to 3.5×10^9 cells/mL. Feed concentrations are listed in the third column and ranged from 2.25 to 4.45 milligrams of sodium salicylate/liter. Residence times are listed in the fourth column of Table 13 and ranged over one order of magnitude from 14 to 150 minutes.

Effluent concentrations are listed in the fifth column. Effluent concentrations for all experiments ranged from 8 to 91 percent of the feed concentrations. The range of respective effluent concentrations in percent of the feed concentration for experiments 1, 2, 3, and 4 varied from: 70 to 20; 40 to 8; 90 to 40; and 70 to 20.

Effluent concentration standard deviations for each of the experiments are listed in column six of Table 13 and ranged between 1.08 and 2.96 percent of the respective effluent concentrations. Simulations indicated that degradative steady state behavior was achieved approximately one hour after the system was perturbed. Unsteady state data was not included in Table 13. Large numbers of samples (between 30 and 250) were used to determine each of the standard deviations listed in Table 13.

Several trends may be observed in Table 13. The ratios of salicylate to biomass in the different experiments varied by a factor of 3.5. Varying the ratio of salicylate to biomass is important because bacteria have a finite ability to degrade salicylate. Experiments 1a-c had the lowest ratio, while experiments 3a-f and 4a-f had the greatest ratio of salicylate to biomass concentration. A second trend in Table 13 is that the standard deviation of the effluent concentrations were approximately 2 percent of the effluent concentrations. The standard deviation was small and consistent for all experiments. Bacteria degradative activity was generally steady over time although a small amount of drift was noticed in some experiments. Degradation is a function of the contact time between bacteria and the target compound. Thus residence times were varied over one order of magnitude. A greater range of residence times was not used because of equipment and material restrictions.

A number of experiments were conducted using the same biomass concentrations, substrate concentrations, and residence

times to investigate the reproducibility of experiments. The effluent concentrations are normalized to simplify comparisons. These pairs are listed in Table 14 for ease of study. The effluent concentration is a function of the time that the bacterial are in contact with the substrate concentration (residence time), the feed concentration, and the number of bacteria present to degrade the compound (biomass concentration).

Table 14 highlights pairs of experiments conducted using identical feed concentrations, biomass concentrations, and residence times. The first through third columns respectively identifies experiments, lists feed concentrations, and lists residence times. Feed concentrations varied from 2.25 to 4.45 milligrams of sodium salicylate/L. Biomass concentrations varied between 1.9×10^9 to 3.3×10^9 cells/mL. Residence times varied from 1.4×10^1 to 7.2×10^1 minutes. The last two columns list dimensionless effluent concentrations (effluent concentration divided by feed concentration) and standard deviations. Effluent concentrations were normalized to allow direct comparison between experiments. Dimensionless effluent concentrations ranged from 2.32×10^{-1} to 7.70×10^{-1} . Standard deviations ranged between 1.31 to 2.96 percent of the feed concentration.

Table 14. Comparison of repeated degradation experiments.

Experiment	Biomass	Sodium Salicylate Feed	Residence Time	Normalized Salicylate Effluent	Standard deviation in Percent of the Effluent
	cells/mL	milligrams /liter	minutes		
3c	1.9×10^9	4.45	24	7.70×10^{-1}	2.50
3f	1.9×10^9	4.45	24	7.01×10^{-1}	1.31
3d	1.9×10^9	4.45	36	6.21×10^{-1}	1.53
4b	1.9×10^9	4.45	36	6.21×10^{-1}	1.43
4c	1.9×10^9	4.45	48	4.94×10^{-1}	1.54
4f	1.9×10^9	4.45	48	4.97×10^{-1}	1.56
3e	1.9×10^9	4.45	72	4.07×10^{-1}	2.96
4d	1.9×10^9	4.45	72	3.92×10^{-1}	1.16
1a	3.3×10^9	2.25	14	6.37×10^{-1}	1.99
1c	3.3×10^9	2.25	14	7.31×10^{-1}	2.89

Degradation is a function of the substrate concentration, the biomass in contact with the substrate, and the contact time between the bacteria and the substrate. Table 14 suggests that the degradation results were repeatable. The values shown in Table 14 are the averages of hundreds of individual measurements. Experiments with significantly different conditions (residence time, substrate concentration, and biomass concentration) had significantly different normalized effluent concentrations. The experiments (Table 13 and 14) suggest that degradation is a function of biomass concentration, substrate concentration, and contact time between bacteria and substrate.

The dispersed plug flow model, equation (32), was used to describe the steady state degradation of sodium salicylate in the PBR with immobilized *P. fluorescens* HK44 (in alginate beads). Analytical solutions exist for the dispersed plug flow model when the degradation term takes either of the following forms;

$$-R_{\text{Salicylate}} = kC_{\text{Biomass}}C_{\text{Salicylate}} \quad (41)$$

$$-R_{\text{Salicylate}} = kC_{\text{Biomass}} \quad (42).$$

The author knows of no analytical solution for the dispersed plug flow model when the degradation rate is described by the Michaelis-Menten rate equation. The boundary conditions used for solution of the dispersed plug flow model are

$$C_{Salicylate} \Big|_{z=0} = C_{Salicylate_{Feed}} - \frac{1}{Pe} \frac{\partial C_{Salicylate}}{\partial Z} \quad (43),$$

$$\frac{dC_{Salicylate}}{dZ} \Big|_{z=1} = 0 \quad (44).$$

The first boundary condition (43) indicates that the feed is immediately dispersed as soon as it enters the reactor. The second boundary condition (44) indicates that mass transfer by diffusion ceases when the component exits the reactor. The solution (Danckwerts, 1953) for the plug flow model with equation (41) describing salicylate degradation and assuming negligible growth is

$$\frac{C_{Salicylate}}{C_{Salicyte_{Feed}}} \Big|_{Z=1} = \frac{4\eta \exp(Pe/2)}{(1+\eta)^2 \exp(Pe\eta/2) - (1-\eta)^2 \exp(-Pe\eta/2)} \quad (45)$$

where

$$\eta = \sqrt{1 + \frac{4LkC_{Biomass}}{vPe}} \quad (46).$$

The solution for the dispersed plug flow model with equation (44) describing salicylate degradation is

$$C_{Salicylate} = \frac{\phi \exp[Pe(Z-1)]}{Pe} - \phi Z - \frac{\phi}{Pe} + 1 \quad (47)$$

where

$$\phi = \frac{Lk}{v} \frac{C_{Biomass}}{C_{Salicylate_{Feed}}} \quad (48).$$

Equations (45) and (47) were fit to the data in Table 13 using least square techniques and are listed in Tables 15 and 16, respectively. A number of regressions were conducted based on different criteria in an attempt to discern the form of the true rate equation and rate constants. Regressions were conducted using the full data set, individual experiments, and conversion extrema from individual experiments. The degradation rate constant was varied for the regression. Table 17 lists the number of data points from each experiment used for the regressions, and their percentage of the total data points.

Table 15. Regression curve of overall second order (first order in biomass and first order in substrate concentration) degradation model on salicylate degradation data.

Regression Series	Best fit rate Constant dm ³ /g/min	Residual Experiment 1a-c	Residual Experiment 2a-c	Residual Experiment 3a-f	Residual Experiment 4a-f
Full Data Set (1a-c, 2a-c, 3a-f, 4a-f)	2.68 X 10 ⁻²	1.10 X 10 ⁻¹	1.38 X 10 ⁻¹	1.19 X 10 ⁻¹	6.51 X 10 ⁻²
Conversion Maxima (1b, 2c, 3e, 4e)	2.30 X 10 ⁻²	6.82 X 10 ⁻²	2.10 X 10 ⁻¹	1.17 X 10 ⁻¹	1.44 X 10 ⁻¹
Conversion Minima (1a, 2a, 3a, 4a)	2.78 X 10 ⁻²	1.25 X 10 ⁻¹	1.22 X 10 ⁻¹	1.29 X 10 ⁻¹	6.49 X 10 ⁻²
Experiment 1a-c	2.26 X 10 ⁻²	6.75 X 10 ⁻²	2.20 X 10 ⁻¹	1.22 X 10 ⁻¹	1.56 X 10 ⁻¹
Experiment 2a-c	3.45 X 10 ⁻²	2.26 X 10 ⁻¹	5.99 X 10 ⁻²	2.39 X 10 ⁻¹	1.91 X 10 ⁻¹
Experiment 3a-f	2.48 X 10 ⁻²	8.24 X 10 ⁻²	1.79 X 10 ⁻¹	1.09 X 10 ⁻¹	9.74 X 10 ⁻²
Experiment 4a-f	2.73 X 10 ⁻²	1.18 X 10 ⁻¹	1.33 X 10 ⁻¹	1.24 X 10 ⁻¹	6.36 X 10 ⁻²

Table 16. Results from regression of overall first order (first order in biomass and zero order in substrate concentration) degradation model on salicylate degradation data.

Regression Series	Best fit Reaction Rate Constant min^{-1}	Residual Experiment 1a-c	Residual Experiment 2a-c	Residual Experiment 3a-f	Residual Experiment 4a-f
Full Data Set (1a-c, 2a-c, 3a-f, 4a-f)	5.98×10^{-5}	2.22×10^{-1}	5.71×10^{-1}	5.18×10^{-1}	7.55×10^{-1}
Conversion Maxima (1b, 2c, 3e, 4e)	5.29×10^{-5}	1.72×10^{-1}	5.01×10^{-1}	5.64×10^{-1}	8.40×10^{-1}
Conversion Minima (1a, 2a, 3a, 4a)	1.08×10^{-4}	9.15×10^{-1}	1.41	3.09×10^{-1}	4.27×10^{-1}
Experiment 1a-c	5.56×10^{-5}	1.70×10^{-1}	5.05×10^{-1}	5.59×10^{-1}	8.30×10^{-1}
Experiment 2a-c	5.20×10^{-5}	1.79×10^{-1}	4.99×10^{-1}	5.74×10^{-1}	8.59×10^{-1}
Experiment 3a-f	1.65×10^{-4}	1.87	2.71	1.23×10^{-1}	4.98×10^{-1}
Experiment 4a-f	1.32×10^{-4}	1.29	1.93	2.10×10^{-1}	3.64×10^{-1}

Table 17. Number of data points used for regression by experiment.

Experiment	Data Points	Percentage of Total
1a	73	3.8
1b	89	4.7
1c	59	3.1
2a	248	13
2b	380	20
2c	104	5.4
3a	50	2.6
3b	42	2.2
3c	126	6.6
3d	60	3.1
3e	179	9.4
3f	32	1.7
4a	35	1.8
4b	36	1.9
4c	114	6.0
4d	72	3.8
4e	176	9.2
4f	36	1.9

Table 15 indicates the results from a least square fit of equation (45) on the degradation data. Least square fits were performed using the complete data set and combinations of individual experiments. The first column of Table 15 indicates the experiments (experiment numbers identified in Table 13) that were used for the least square fit. The second column indicates the rate constant value obtained. The third, fourth, fifth, and sixth columns list the respective residual from experiments 1a-c, 2a-c, 3a-f, and 4a-f. For example, the first row in Table 15 was generated by fitting the complete data set to equation (45). The minimum residual corresponded to a rate constant value of 2.68×10^{-2} grams/L/min (complete data set). The residual values in the last four columns were then calculated using the previously determined reaction rate constant.

Rate constants ranged from 2.26×10^{-2} to 3.45×10^{-2} dm³/g/min. The residuals for experiments 1a-c, listed in column 3, ranged from 6.75×10^{-2} to 2.26×10^{-1} . The residuals for experiment 2a-c, listed in column 4, ranged from 5.99×10^{-2} to 2.20×10^{-1} . The residuals for experiments 3a-f, listed in column 5, ranged from 1.09×10^{-1} to 2.39×10^{-1} . The residuals for experiments 4a-f, listed in column 6, ranged from 6.36×10^{-2} to 1.91×10^{-1} .

The first three rows of Table 15 suggest an overall goodness of fit of the reaction rate form and the regressed rate constant. If obtained rate constants vary greatly and residuals are large, then the regressed rate equation might be deemed inappropriate. The rate

constants reported in Table 15 (obtained by regressing equation (45) on the salicylate degradation data) were within plus or minus 15 percent of $2.86 \times 10^{-2} \text{ dm}^3/\text{g}/\text{min}$. When equation (45) was regressed on the full data set, the rate constant was within 7 percent of $2.86 \times 10^{-2} \text{ dm}^3/\text{g}/\text{min}$. If the rate law is significantly different than the regression form (e.g. Michaelis-Menten), regression of equation (45) on degradation minima and maxima may produce significantly different degradation coefficients. The extrema rate constants were within 15 percent of one another. Although more analysis is required, the first three rows of Table 15 appear to suggest that a first order reaction rate form may be appropriate for describing the degradation kinetics of *P. fluorescens* HK44.

The final four rows of Table 15 indicate the relative contribution of each experimental set to the overall residual. The second column reports rate constants when equation (45) was regressed on degradation data from individual sets of experiments. The average of the four rate constants in column two was 2.75×10^{-2} . The regressed degradation rate constants from experimental sets 1, 3, and 4 differed from the mean by no more than 17 percent. The regressed degradation rate constant for experiment 2 was differed from the mean by 26 percent. Of the four experimental sets, experiment 2 appears to have contributed the most to the overall residual. This observation is confirmed by studying the different rows of Table 15. In every row except the third and fifth,

experiment 2 has the greatest residual (Row five reports the results of regressing equation (45) on experimental series 2).

Table 16 indicates the results from a least square fit of equation (47) on the degradation data. Least square techniques were used to fit equation (47) to the complete data set and to combinations of individual experiments. The format of Table 16 is the same as the format of Table 15. The best fit reaction rate constants listed in column 2, varied from 1.65×10^{-5} to $5.20 \times 10^{-4} \text{ min}^{-1}$. The residuals for experiments 1a-c, listed in column 3, ranged from 1.71×10^{-1} to 1.41. The residuals for experiments 2a-c, listed in column 4, ranged from 1.79×10^{-1} to 2.71. The residuals for experiments 3a-f, listed in column 5, ranged from 1.23×10^{-1} to 5.74×10^{-1} . The residuals for experiments 4a-f, listed in column 6, ranged from 3.64×10^{-2} to 8.59×10^{-1} .

The first three rows of Table 16 suggest the overall goodness of fit of a zero order reaction rate, equation (47), and the value of the rate constant. The rate constants reported in Table 16 (obtained by regressing equation (47) on the salicylate degradation data) were within plus or minus 53 percent of $8.93 \times 10^{-5} \text{ min}^{-1}$. If the rate law is significantly different than the regressed reaction rate form (e.g. Michaelis-Menten), then regression of equation (47) on degradation minima and maxima should produce significantly different degradation coefficients. The regressed minima conversion reaction constant was approximately double the regressed maxima conversion reaction constant. The first three rows of Table 16 appear to suggest that a zero order reaction rate

form may not be appropriate for describing the degradation kinetics of *P. fluorescens* HK44.

The final four rows of Table 16 suggest the relative contribution that each set of experiments contributed to the overall residual. The second column reports rate constants when equation (47) was regressed on degradation data from individual sets of experiments. The average of the four rate constants in column two was 1.01×10^{-4} . The top two and the bottom two experiments appear to be grouped together.

Except for one case, the residuals using equation (45) were less than those obtained with equation (47) for identical data sets and were, in many instances, one order of magnitude less. The comparison of the two tables would suggest that equation (45) describes the degradation kinetics of salicylate by *P. fluorescens* HK44.

Bead diameters were tightly grouped around 4.5×10^{-2} cm. Table 18 lists typical bead diameters of alginate beads used for degradation experiments. Dispersion and mass transfer are a function of particle size. Bead diameters were fractionated using sieves. The first column lists bead diameter in cm. The second column lists bead size distribution. The beads had an average diameter of 4.5×10^{-2} cm. A heterogeneous particle size distribution may lead to non-uniform conditions through out the reactor.

Table 18. Typical particle sizes of alginate beads used for degradation experiments.

Average diameter (cm)	Percent of total beads by weight
1.1	0
7.5×10^{-2}	4.3
4.6×10^{-2}	65.
3.9×10^{-2}	29.
3.3×10^{-2}	1.6
2.8×10^{-2}	negligible
2.3×10^{-2}	negligible

3.3 BIOLUMINESCENCE

3.3A BIOLUMINESCENCE (CONTROLS)

Experiments were performed to investigate the relationship between degradation and bioluminescence. Non-inducing and inducing substrates were used for degradation and bioluminescence experiments.

Control experiments were conducted to determine the effect of glucose, a *nah* non-inducing substrate, and mineral salts media (no carbon) on the light emission of *P. fluorescens* HK44.

Bioluminescent activity was minimal using mineral salts media. In the second series of control experiments, glucose was supplied to *P. fluorescens* HK44 at 5 mg/L. Feed was supplied to the reactor at a flow rate of 1.0 mL/min. The temperature and pH were 20° C and 7.2, respectively. The typical response of *P. fluorescens* HK44 to glucose is shown in Figure 37. The horizontal axis is time in hours.

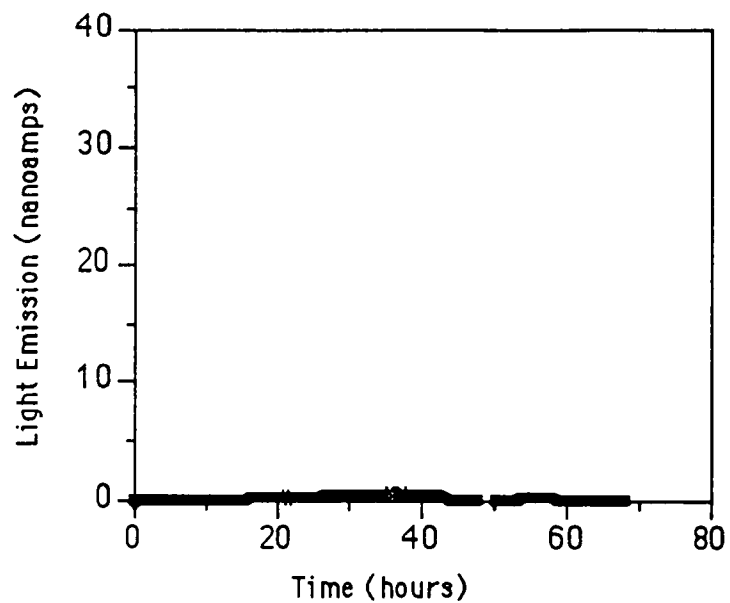


Figure 37. Light emission (regular scale) from alginate-immobilized *P. fluorescens* HK44 when exposed to 5 mg/l glucose solution in a packed bed reactor. Glucose was added at one hour.

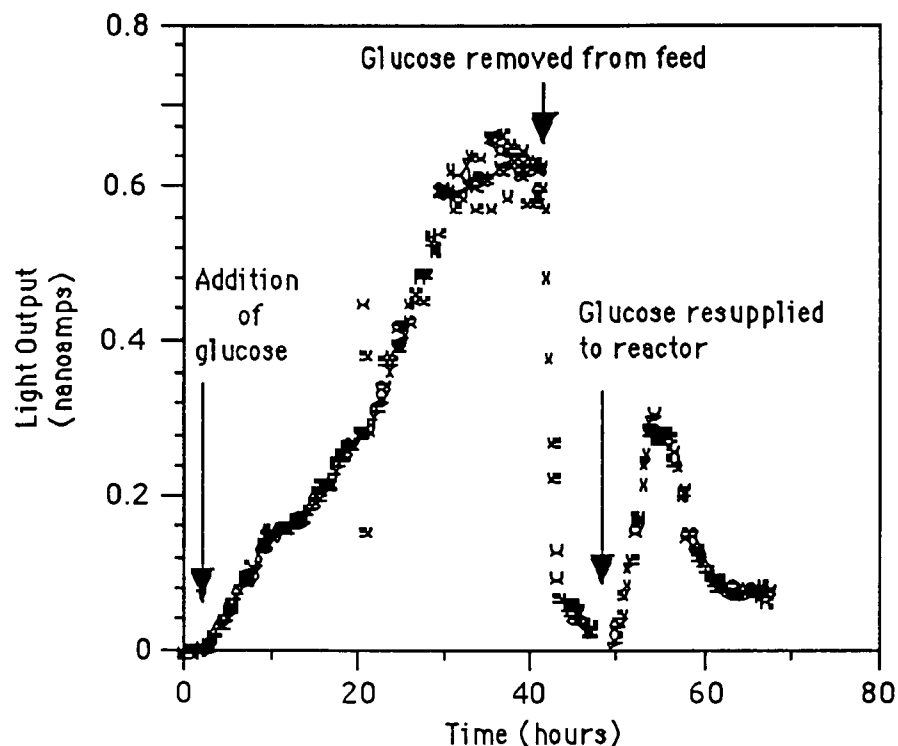


Figure 37 (Contd.). Light emission (expanded scale) from *P.*

fluorescens HK44 using 5 ppm glucose. The maximum light emission by *P. fluorescens* HK44 when exposed to glucose solution was orders of magnitude lower than the maximum light emission when *P. fluorescens* HK44 was exposed to substrates that induced the *Nah* and *lux* pathways.

The vertical axis is light emission (nanoamps) at the inlet of the bed. The light emitted by *P. fluorescens* HK44 when exposed to glucose is orders of magnitude less than light given off during induction. Figure 37A shows light output at a typical scale used for induction experiments. Figure 37B depicts the light output at a reduced scale to show the emission behavior in detail. Prior to addition of glucose, light emission was minimal and steady. Light emission rose slowly and achieved a maximum of 0.6 nanoamps 26 hours after initial addition of glucose. Steady state emission occurred for 12 hours until glucose was removed from the feed. After removal of glucose from the feed, light emission dropped to minimal levels. After reintroduction of glucose, a transitory peak was observed. Light emission then dropped to a reduced steady state level.

3.3B BIOLUMINESCENCE (STEADY STATE)

Experiments were conducted to investigate the steady state light emission of *P. fluorescens* HK44. Salicylate and naphthalene were used as inducer substrates.

The steady state light emission of *P. fluorescens* HK44 was characterized with respect to liquid phase salicylate concentration. Table 19 lists average light emission of *P. fluorescens* HK44 and experimental parameters. The first column lists the experiment number as listed in Table 13. The second, third, fourth, and fifth columns list biomass concentrations, feed concentrations, residence times, and effluent concentrations. The sixth column lists steady state light emission. The seventh column lists the standard deviation of steady state light emission. Biomass concentrations listed in the second column were varied from 1.9×10^9 to 3.5×10^9 cells/mL. Feed concentrations are listed in the third column and ranged from 2.25 to 4.45 milligrams of sodium salicylate/liter. Residence times ranged over one order of magnitude from 1.4×10^1 to 1.5×10^2 minutes. Effluent concentrations for all experiments ranged from 8 to 91 percent of the feed concentrations. Light emission ranged from 8.28×10^{-1} to 4.79 nanoamps. Standard

Table 19. Steady state light production by *P. fluorescens* HK44 as a function of biomass, feed concentration and residence time.

Experiment	Biomass	Sodium Salicylate Feed	Residence Time	Salicylate Effluent	Light Production	Standard Deviation of Light Production
	cells/mL	mg/L	min	mg/L	nanoamps	nanoamps
1a	3.3×10^9	2.25	1.4×10^1	1.43	*0.27	1.53×10^{-2}
1b	3.3×10^9	2.25	5.8×10^1	0.46	*0.89	1.69×10^{-2}
1c	3.3×10^9	2.25	1.4×10^1	1.65	*0.25	7.66×10^{-2}
2a	3.5×10^9	2.47	2.4×10^1	1.03	2.71	3.60×10^{-1}
2b	3.5×10^9	2.47	2.6×10^1	0.86	1.81	2.26×10^{-1}
2c	3.5×10^9	2.47	9.4×10^1	0.20	0.16	1.59×10^{-2}
3a	1.9×10^9	4.45	1.5×10^1	4.06	**	**
3b	1.9×10^9	4.45	1.8×10^1	3.84	**	**
3c	1.9×10^9	4.45	2.4×10^1	3.42	3.07	1.10×10^{-1}
3d	1.9×10^9	4.45	3.6×10^1	2.77	2.26	1.00×10^{-2}
3e	1.9×10^9	4.45	7.2×10^1	1.81	0.95	5.10×10^{-2}
3f	1.9×10^9	4.45	2.4×10^1	3.12	**	**
4a	1.9×10^9	4.45	2.9×10^1	3.07	**	**
4b	1.9×10^9	4.45	3.6×10^1	2.76	**	**
4c	1.9×10^9	4.45	4.8×10^1	2.25	2.17	7.70×10^{-2}
4d	1.9×10^9	4.45	7.2×10^1	1.75	1.61	6.61×10^{-2}
4e	1.9×10^9	4.45	1.5×10^2	0.89	0.83	5.86×10^{-2}
4f	1.9×10^9	4.45	4.8×10^1	2.24	2.53	4.68×10^{-1}
5a	1.9×10^9	2.5	15×10^0	***	1.60	1.90×10^{-1}
5b	1.9×10^9	2.5 salicylate, 8.0 naphthalene	15×10^0	***	4.79	3.18×10^{-1}

* Light probe blocked.

** No steady state light data available.

*** Concentration data not available.

deviations for all experiments ranged 1.0×10^{-2} to 4.68×10^{-1} nanoamps. Because only light emission data was available, experiments 5a-b were not listed in Table 13.

Table 19 indicates experiments that achieved steady state light emission. Standard deviations of the light output varied from 3 to 12 percent of the signal. This large standard deviation was probably due to reflection inside the reactor, electrical noise, and equipment performance.

Table 20 highlights experiments that were repeated. The first through third columns respectively lists experiments, feed concentrations, and residence times. Feed concentrations varied from 2.25 to 4.45 milligrams of sodium salicylate/L. Biomass concentrations varied between 1.9 to 3.3×10 cells/mL. Residence times varied from 1.4×10^1 to 7.2×10^1 minutes. The last column lists steady state light emission. Light emission varied between 1.61 and 3.42 nanoamps. Table 20 suggests that results from light emission experiments were repeatable.

Table 20. Comparison of steady state light emission in experiments with identical feed concentrations, biomass concentrations, and residence times.

Experiment	Biomass	Sodium Salicylate Feed	Residence Time	Light emission
	cells/mL	mg/liter	min	nanoamps
1a	3.3×10^9	2.25	14	*0.27
1c	3.3×10^9	2.25	14	*0.25
3e	1.9×10^9	4.45	72	1.81
4d	1.9×10^9	4.45	72	1.61
3c	1.9×10^9	4.45	24	3.42
3f	1.9×10^9	4.45	24	3.12
4c	1.9×10^9	4.45	48	2.17
4f	1.9×10^9	4.45	48	2.53

Table 21 lists light emission data as a function of concentration inside the PBR. Comparison of light emission with feed or effluent concentration might lead to erroneous results because concentration changes with axial position inside the reactor. Light emission is reported as a specific light response and was divided by the biomass concentration for comparison between experiments. The salicylate concentration inside the reactor was calculated using equation (45) and a reaction rate constant of $2.68 \times 10^{-2} \text{ dm}^3/\text{g}/\text{mol}$ (see column1, row 1 of Table 15). The reaction rate constant was obtained by fitting equation (45) to the full data set in Table 13. Estimated concentrations ranged from 1.8 to 4.2 milligrams of salicylate/liter. The third column lists specific light emission with values ranging between 4.7×10^{-2} to $1.6 \text{ nanoamps}/10^9 \text{ cells}$.

Table 21. Specific steady state light emission by alginate-immobilized *P. fluorescens* HK44 as a function of estimated concentration inside the PBR.

Experiment	Biomass	Predicted Salicylate Concentration at light pipe	Normalized Light Emission
	cells/mL	mg/L	nanoamps /10 ⁹ cells
2a	3.5 X 10 ⁹	2.31	7.7 X 10 ⁻¹
2b	3.5 X 10 ⁹	2.29	5.2 X 10 ⁻¹
2c	3.5 X 10 ⁹	1.88	4.7 X 10 ⁻²
3c	1.9 X 10 ⁹	4.19	1.6 X 10 ⁰
3d	1.9 X 10 ⁹	4.06	1.2 X 10 ⁰
3e	1.9 X 10 ⁹	3.70	5.0 X 10 ⁻¹
4c	1.9 X 10 ⁹	4.19	1.2 X 10 ⁰
4d	1.9 X 10 ⁹	4.06	9.2 X 10 ⁻¹
4e	1.9 X 10 ⁹	3.70	4.7 X 10 ⁻¹
4f	1.9 X 10 ⁹	4.19	1.2 X 10 ⁰
5a	1.9 X 10 ⁹	2.41	8.4 X 10 ⁻¹

Table 21 lists the steady state light emission for several experiments. Each of the data points are the culmination of hundreds of individual measurements (see Table 17). The data demonstrated a positive correlation between light emission and concentration. These results must be treated qualitatively because there is no available reference light source for comparing emission intensity between experiments. Opacity of the alginate immobilization substrate may also vary from experiment to experiment. Table 21 indicates that light emission at steady state was a function of inducer concentration. Quantitative comparisons may not be made because of the lack of an available reference emission source for standardization of experiments and possible variance in the opacity of the alginate immobilization medium.

3.3C BIOLUMINESCENCE (UNSTEADY STATE)

The unsteady state response of *P. fluorescens* HK44 to salicylate inducer concentration was investigated. In general, degradation rates (reflected by effluent concentration) became constant approximately one hour after a perturbation. Light response became steady after approximately 8 hours (including an approximate one hour induction period).

The typical response of *P. fluorescens* HK44 to sodium salicylate is shown in Figure 38. The intensity and duration of unsteady state light emission affects the use of *P. fluorescens* HK44 as a biosensor. Glucose solution was generally added to the reactor 15 hours prior to inducer addition. No significant differences in response were observed when glucose was added 1 to 15 hours prior to addition of the inducing substrate. Salicylate (glucose continued to be supplied at initial concentrations) was then supplied continuously to the PBR. The light response to glucose at the beginning of the experiment was minimal. Upon the addition of sodium salicylate, light production initially increased orders of magnitudes and reached the maximum output at approximately 1 hour after addition of salicylate. Four hours after maximum light emission occurred, light emission dropped to steady state levels.

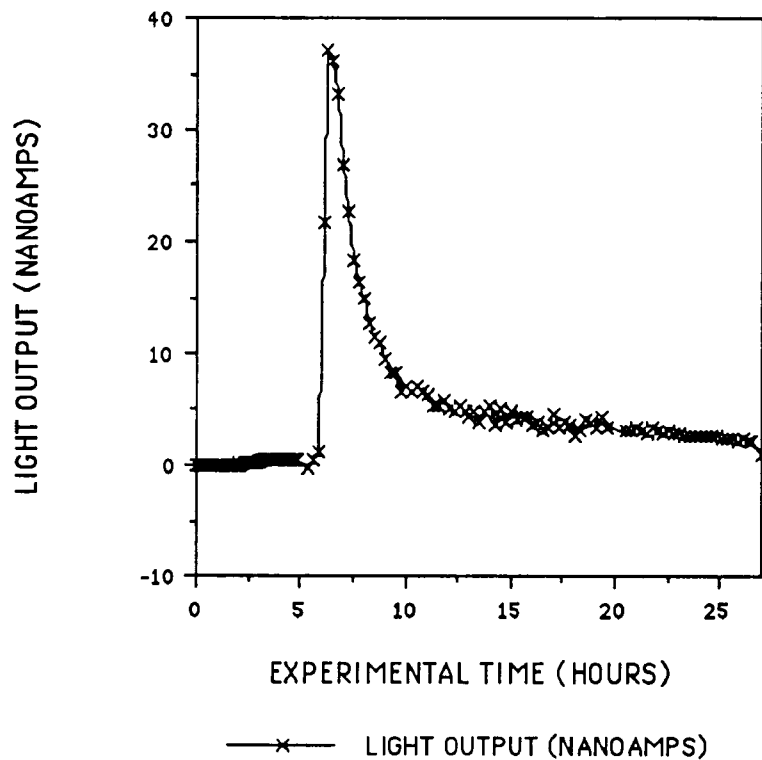


Figure 38. Light emission as a function of time after induction of alginate-immobilized *P. fluorescens* HK44 by the addition of sodium salicylate. 5 mg/l of sodium salicylate and glucose were supplied at the third hour of the experiment.

In general, light emission approached steady state levels between 4 and 8 hours after addition of an inducing substrate. Light emission was approximately constant for the next 20 hours although some drift was observed. Light emission was approximately two orders of magnitude over uninduced levels.

The general response of *P. fluorescens* HK44 to salicylate and naphthalene included a maximum emission that occurred shortly after addition of an inducing substrate. Table 22 list maximum light emission by alginate-immobilized *P. fluorescens* HK44. The experiments listed in Table 22 were all conducted at identical flow rates, temperatures, pressures, and were supplied with identical concentrations of minerals and glucose (5 mg/l). Flow rates,

Table 22. Maximum light emission by alginate-immobilized *P.*

fluorescens HK44 .

Experiment	Feed Salicylate (Naphthalene) Concentration	Biomass Concentration	Maximum Light Emission
	mg/liter	cells/mL	nanoamps
1a	2.25	3.3×10^9	*6.2
2a	2.47	3.5×10^9	37
3a	4.45	1.9×10^9	13.9
4a	4.45	1.9×10^9	15.9
5a	2.5	1.9×10^9	13.4
5b	(naphthalene) 8	1.9×10^9	870

*Light probe blocked.

temperature, and pressure for the experiments listed below were 1.0 mL/minute, 20° C, and 125 psig, respectively. Oxygen was always kept at concentrations in large excess of that required for complete degradation. Effluent oxygen concentrations measured when degradation of salicylate was almost complete ranged from 10 to 12 mg/L. The first column lists experiment number as indicated in Table 13. Degradation data was not collected in experiment 5 because of equipment malfunctions and was not included in Table 13. The second column of Table 22 lists biomass concentration. The third column of Table 22 lists feed concentrations used for the experiments. The last column lists maximum light emission levels. Light emission levels for salicylate were proportional to biomass concentration. No relationship between feed concentration and maximum light emission was found. Experiment 5b was conducted with all conditions identical to those noted above except that naphthalene was used as the inducing substrate and was supplied at 8 mg/L. The light response of *P. fluorescens* HK44 to naphthalene was orders of magnitude greater than to salicylate.

Figure 39 depicts the time course of light emission from *P. fluorescens* HK44 when exposed to naphthalene. The bottom axis is time in hours. The vertical axis is light emission in nanoamps. At 16 hours, salicylate was added to the PBR. Light response is listed in Table 19 as experiment 5a. Light emission reached a steady state after addition of salicylate and before naphthalene addition.

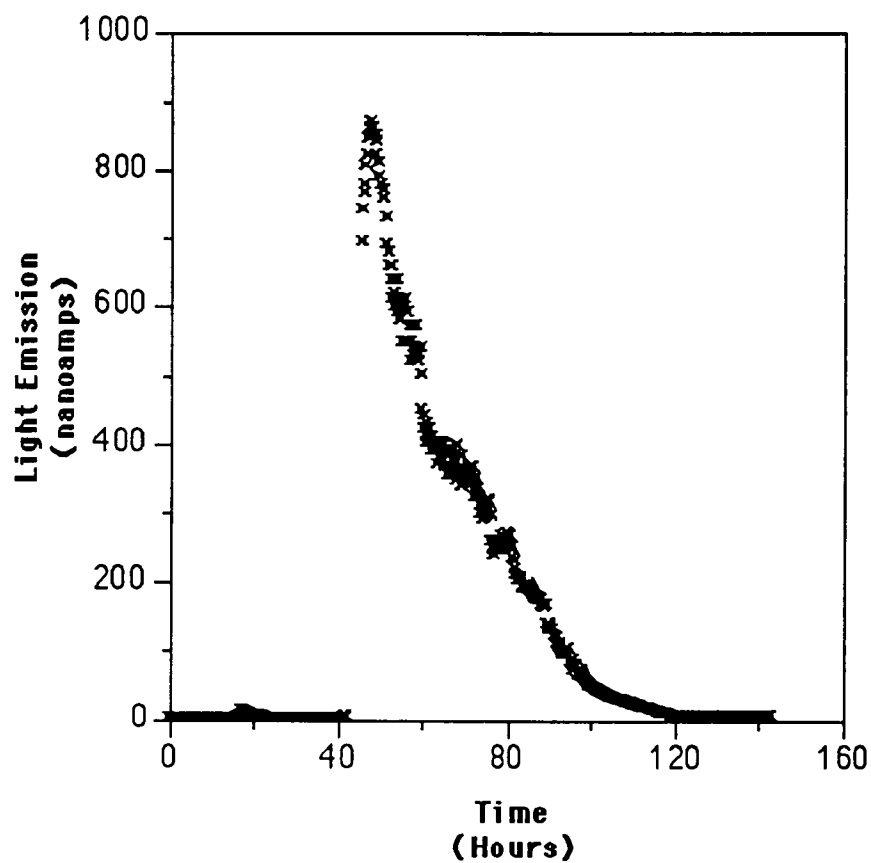


Figure 39. Light emission of alginate-immobilized *P. fluorescens* HK44 when exposed to naphthalene. Naphthalene was added at 45 hours.

The light response of alginate-immobilized *P. fluorescens* HK44 was immediate and intense upon the addition of naphthalene at 45 hours. The response of *P. fluorescens* HK44 to naphthalene differed in two respects from its response to salicylate. The response of *P. fluorescens* HK44 to naphthalene was approximately two orders of magnitudes greater than the response to salicylate under identical conditions. Secondly, the transitory period (unsteady state light emission from the exposure to naphthalene) varied over a period of days as compared to a period of hours for salicylate. When the experiment was repeated, *P. fluorescens* HK44 again emitted orders of magnitude more light when exposed to naphthalene than when exposed to salicylate. The transitory period (when exposed to naphthalene) was again days instead of hours (when exposed to salicylate).

4 DISCUSSION

4.1 PACKED BED REACTOR AND DIFFUSION PROCESSES

4.1A REACTOR HYDRODYNAMIC PERFORMANCE & DIFFUSION PROCESSES

Dispersion and total mass transfer resistance were measured using the method of Haynes & Sarma (1973). The measured dispersion coefficients (at flow rates typically used for experiments) asymptotically approached 10^{-2} cm²/min. Measured residence times when compared to the measured reactor volume and measured flow rates demonstrated that channeling was not significant. Total liquid phase mass transfer resistance was a function of reactor volume and ranged from $4. \times 10^{-2}$ to $5. \times 10^{-1}$ min. Because solid particles were used for this set of experiments, the measured mass transfer resistances are due to the fluid phase only. At typical flow rates used for measurement of degradation and adsorption, there was a linear relationship between mass transfer and dispersion (represented as $\frac{L\sigma^2}{2\mu^2v}$), and the inverse square of velocity as predicted by Ruthven (1984). At higher flow

rates, not used for kinetic and adsorption experiments, the curves in Figure 17 were concave downwards.

The measured and predicted dispersion coefficients were within an order of magnitude of one another (equation (4)). The difference in the two values was possibly due to the sudden expansion in the flow lines at the inlet and outlet of the reactor. Equation (4) suggests that dispersion was a function of liquid phase diffusion, particle diameter, and fluid velocity:

$$D_t = \gamma_1 D_m + 2\gamma_2 R_p v \quad (4).$$

For liquids, diffusion coefficients are of the order of 10^{-5} cm²/s and may not contribute significant to dispersion. The two constants, γ_1 and γ_2 , in equation (4) are close to unity. Thus the second term in equation (4), which accounts for mixing (caused by fluid movement around the particles), is generally dominant for liquids. Expansion of the flow stream from the inlet diameter (0.010 inches) to the bed diameter (0.75 or 1.00 inches) influenced the measured dispersion coefficient. To achieve the low flow rates observed in ground water and to maintain low residence times in the reactor tubing, the bed diameter was several orders of magnitude greater than the inlet and outlet tube diameters (see Figure 20). The dispersion caused by expansion at the entrance and outlet of the reactor was not directly measured because of the investment required for modifying the reactor and developing a measurement protocol. The measured dispersion coefficients accounted not only

for dispersion in the reactor but also for dispersion at the inlet and outlet of the reactor.

The curves in Figure 17 indicated that as fluid velocity increased, mass transfer resistance decreased toward a limiting low overall mass transfer resistance (y-axis intercept). Solute transport occurs in the bulk phase by convection. Mass transfer from the bulk fluid phase to solid particles occurs by diffusion across a stagnant layer of fluid surrounding individual particles. The thickness of this sublayer decreases as fluid velocity increases (McCabe, *et al.*, 1985). Solute flux from the bulk phase to the solid phase increased because the diffusive path length was reduced. As was previously discussed, dispersion is a linear function of velocity. Thus as velocity increased, dispersion increased (due to mixing) and mass transfer resistance decreased (the sublayer thickness decreased with increasing velocity).

4.1B SIMULATIONS OF DIFFUSIONAL PROCESSES OCCURRING IN ALGINATE BEADS & SOILS

PDECOL (Madsen & Sincovec, 1979) was evaluated for solving reactor diffusion problems. The numerical solution obtained using PDECOL (Madsen & Sincovec, 1979) was within one percent of the analytical series solution (Crank, 1975) for solute diffusing into a

porous sphere. Very steep gradients are difficult to represent numerically and pose a potential source of error. Initially a steep concentration gradient existed at the surface of the pellet (due to the sudden increase in liquid concentration) and could have caused excessive error propagation in later stages of the simulation. This initial steep gradient did not cause significant inaccuracy in later stages of the simulation. Because orthogonal collocation techniques employed continuous polynomials to represent the actual solution, the true solution is assumed to be continuous. Significant inaccuracies may have occurred if the real solution contained a discontinuity(s). One approach to reducing many sources of inaccuracy was to increase the number of collocation points. 30 collocation points provided an accurate prediction of reactor processes for which there were analytical solutions to compare to. Many researchers have found that 8 collocation points generally provide an accurate solution to many adsorption problems (Liapis & Rippin, 1978; Holland & Liapis, 1983).

Solid diffusion was probably not a significant PAH transport mechanism in porous solids such as alginate beads. Solid diffusion coefficients are generally several orders of magnitudes less than their liquid phase counterparts (Treybal, 1980). Figures 19A and 19B indicated that solid diffusion did not contribute significantly to solute flux through porous media unless the solid coefficient was within two orders of magnitude of the liquid diffusion coefficient.

For nonporous solids such as soils heavily contaminated with oily solids or tars, solid diffusion may be the dominant process.

Equation (49) relates solid and liquid diffusion

$$D_{s_i} \left[\frac{2}{r} \frac{\partial C_{s_i}}{\partial r} + \frac{\partial^2 C_{s_i}}{\partial r^2} \right] + \varepsilon_p D_{p_i} \left[\frac{2}{r} \frac{\partial C_{p_i}}{\partial r} + \frac{\partial^2 C_{p_i}}{\partial r^2} \right] = \varepsilon_p \frac{\partial C_{p_i}}{\partial t} + \frac{\partial C_{s_i}}{\partial C_{p_i}} \frac{\partial C_{p_i}}{\partial t} \quad (49).$$

Ordinarily, liquid diffusion is dominant (second bracketed term on the left hand side of the equation); however, if the particle porosity is small (ε_p), then the solid diffusion term becomes dominant (first bracketed term). The porosity dictates which process, liquid or solid diffusion, is dominant. Solid diffusion is an inherently slow process compared to liquid diffusion. Solutes would be relatively unavailable for liquid phase degradation if they are distributed inside a non-porous solid .

4.2 ADSORPTION PROCESSES

4.2A ADSORPTION ISOTHERMS OF NAPHTHALENE AND SODIUM SALICYLATE ON CALCIUM ALGINATE

The adsorption isotherms of sodium salicylate and naphthalene on calcium alginate were measured. A measurable amount of sodium salicylate did not adsorb to alginate at concentrations used for

kinetic evaluation of *P. fluorescens* HK44. The naphthalene adsorption isotherm was linear.

Adsorption slows solute movement through solids. Alginate appears to be an ideal support material when using sodium salicylate. Salicylate moves through the alginate quickly because alginate is very porous and does not adsorb salicylate. Most adsorbents typically used for collection of PAH from ground water and air samples have hydrophobic and/or aromatic chemical groups (Lee et al., 1979). Alginate has little or no aromatic chemical groups and has a chemical structure that includes hydrophilic groups.

Table 10 indicates that adsorption significantly reduces solute movement through alginate. Naphthalene adsorbed linearly and preferentially on alginate. The solid to liquid phase concentration ratio was 8.4 at equilibrium. Naphthalene transport through alginate is hindered because of adsorption.

For larger PAH that strongly adsorb to alginate, solute movement may be significantly hindered. For strong adsorbed components, the time required to approach saturation of the bead may be very long. In this scenario, interior bacteria may be exposed to only a fraction of the target compound present in the bulk fluid. Degradation and bioluminescent results may be significantly biased if this behavior is not accounted for. For larger PAH, the PAH-immobilization media adsorption isotherm may significantly influence bioluminescent and degradative behavior.

4.2B SIMULATION OF ADSORPTION PROCESSES

PDECOL (Madsen & Sincovec, 1979) was an effective method for numerically solving adsorber problems. PDECOL provided a very accurate simulation of the passage of a sine wave tracer through a packed bed. Two anomalies were present when PDECOL was used to simulate the passage of a step perturbation through an adsorption bed (favorable isotherm). The real differential equation solution is assumed to be continuous because orthogonal collocation uses continuous polynomials as trial solutions. The anomalies were caused by the inability to represent a very steep gradient with a smooth polynomial function. Caution is appropriate when interpreting results from simulations where sharp fronts or discontinuities are expected or encountered. Increasing the number of collocation points reduces error although the tendency for error will always be greater near discontinuities than in smooth sections of the solution.

Figure 23 depicts the simulated loading of a low capacity bed with a high concentration (sinusoidal wave and favorable isotherm) feed. Adsorption changed the shape of the sine wave. The leading concentration front became sharp while the trailing front expanded. The wave amplitudes and periods were essentially unchanged as they passed through the column. The simulation suggests that loading a

low capacity bed with a high concentration feed produces an outlet concentration distribution with the same frequency and amplitude as the input wave, but with a saw tooth shape.

The formation of a saw tooth wave was an example of constant pattern behavior. Concentration front velocity is a function of the isotherm (equation (24)). For favorable isotherms, low concentration zones move slowly through packed beds because the molecules spend most of their time adsorbed in the solid phase (see isotherm relationship). High concentration zones move rapidly through adsorption beds because a greater proportion of the solute is distributed in the liquid phase (where movement can occur). A sharp leading front occurred because the low concentration zone at the front of the sine wave was overtaken by the higher concentration zone in the middle of the cycle. Mass transfer acted to disperse the sharp front while adsorption acted to sharpen the front. After a period of time, the opposing processes of adsorption and mass transfer balanced one another and the leading front achieved a shape that did not change. The expanding front of the saw tooth wave was formed because the high concentration zone in the center of the wave pulls away from the slower moving low concentration front at the rear of the sine wave. Formation of sharp leading fronts and expanding trailing fronts is also observed with square wave perturbations.

Figure 24 and 25A-C depicts the simulated loading of a high capacity bed with a low concentration (sinusoidal wave and favorable isotherm) solute. The high capacity bed loaded from

entrance to the back in a continuous manner. The feed sine wave was severely damped by adsorption.

The dampening of the feed wave in Figures 24 and 25A-C are explained by equation (24) as are the formation of the saw tooth waves in Figure 23. Most of the solute from the first feed cycle was adsorbed in the solid phase of the high capacity bed. Because the solute was adsorbed, little or no movement occurred. Because the bed was already partially saturated, solute from the next cycle advanced farther into the bed. This process continued with subsequent cycles until the adsorption bed was saturated.

Sinusoidal perturbation methods are useful for adsorption analysis because two parameters, period and amplitude, were available for dynamically testing the system. Constant pattern behavior (favorable adsorption only) also is predicted and may be used to study mass transfer and adsorption effects. The relationship between adsorbed solute bed distribution, dispersion, isotherm form and adsorption present potential areas of future study.

4.2C SOIL ADSORPTION STUDIES

The effective use of reporter bacteria for monitoring restoration of PAH contaminated sites is affected by a number of physical processes including adsorption. Adsorption and biodegradation are coupled processes. PAH readily adsorb to soils (Karickhoff, 1979) and become unavailable for liquid phase biodegradation. Heterogeneous soil structure and pollutant distribution hinder analysis of adsorption by dampening many types of perturbations. Two methods for studying PAH adsorption in soils, sinusoidal and step perturbations, were investigated.

PAH adsorption was studied using step perturbation methods. A number of compounds exhibited increased liquid phase concentrations when naphthalene was supplied to the reactor in a continuous fashion. Microbial activity was inhibited by addition of mercuric chloride and by the maintenance of anaerobic conditions in the reactor. PAH are generally assumed to be favorably adsorbed in soils (Saltzman & Yaron, 1986). Displacement occurs when two or more components compete for adsorption sites. An increase in liquid phase concentration is then observed. Leachate responses are due to adsorption (displacement of solutes from the solid phase by the feed component). Figures 34-35 indicate that anthracene and phenanthrene effluent concentrations were displaced by naphthalene. Because of the very long breakthrough time, Figure 36 indicates that the soil had a high adsorption capacity for naphthalene.

Acenaphthene and biphenyl did not appear to exhibit the sinusoidal behavior that many of the other compounds did. Using the same reasoning mentioned above, possibly the naphthalene concentration was not great enough to displace acenaphthalene (in this scenario, naphthalene is the heavy component). Because there is no analytical solution for adsorption with a sinusoidal feed boundary condition, the role of the watershed point is unclear in this case.

Adsorption and leaching of pre-existing naphthalene dampened the naphthalene sine wave. Total dampening of the feed sine wave should produce an effluent concentration equal to the average of the feed concentration. Naphthalene effluent concentrations were always above average feed levels indicating leaching of pre-existing naphthalene from the soil matrix. In high capacity adsorption beds, simulations indicate that adsorption significantly dampens sine wave perturbations (Figure 25). Adsorption processes and leaching of preexisting naphthalene from the soil dampened the effluent concentration response.

Careful examination of Figures 30-33 reveals that leachate responses in the first or second perturbation period were much different than responses in subsequent periods. The difference in response between the first two periods and later periods may have been due to loading of the adsorber with naphthalene (Figure 24) and/or mass transfer processes. As perturbations continued, the system was forced into a characteristic response. Sinusoidally perturbing the system forced the system into a continual response pattern which could then be studied in detail.

Adsorption effects were important in determining the liquid phase concentration of naphthalene and of other PAH leaching from the soil. Because microbial activity was inhibited, leachate responses are due to adsorption (displacement of solutes from the solid phase by the feed component). Displacement commonly occurs for favorable type systems (step boundary condition) when a previous saturated bed is eluted with a second component provided the feed concentration is greater than the watershed concentration (Coroyannakis, 1987).

In this investigation step perturbations were shown to produce displacement. Adsorption effects from the soil were important not only in determining the liquid-phase concentration of naphthalene but also of other PAH leaching from the soil. In contaminated soils, multicomponent availability may not be adequately described assuming Henry's Law behavior. For example, addition of dissolved naphthalene may increase the aqueous phase concentration of fluorene. The liquid phase concentrations of multiple PAH contaminants are important for the bioremediation processes because degradation of the PAH is thought to go through common enzymatic pathways.

Besides being an effective tool for studying adsorption in soils, sinusoidal perturbations are similar to washing of pollutants from one position to another through natural forces such as rain storms. Sinusoidal perturbations may be used for analysis of adsorption and may simulate environmental conditions. For example, degradation of target components may be affected by the presence of

pollutants that are displaced by rain. Displaced components may slow degradation and enhance target compound migration.

Sinusoidal perturbations produced continual system response patterns that could easily be differentiated from normal system behavior. Step and pulse perturbations produced only transient responses which were more difficult to interpret than sinusoidal perturbations. Total concentration of PAHs in soils may not correlate well with availability. Sinusoidal perturbations provided two parameters, period and amplitude, while only one parameter, magnitude of the step function, was available for dynamic studies using traditional step perturbation methods. Both sinusoidal and step pulses displayed constant pattern behavior which could be used for analysis of mass transfer and adsorption. Clearly lack of an isotherm model describing multicomponent adsorption of PAH in this soil greatly hampers analysis of biological degradation and bioluminescent response.

A number of possibilities exist for further investigation of PAH adsorption in soils, namely:

- 1) analytical solution of the equilibrium (negligible mass transfer and dispersion) multicomponent adsorption case with a sinusoidal feed boundary condition,
- 2) isotherm truncation due to limited solubility,
- 3) effect of isotherm type on the shape of displaced component response using sinusoidal boundary conditions,

- 4) heterogeneous contaminant distribution,
- 5) effects of surfactants and other components upon isotherms and solubility, and
- 6) effect of heterogeneous soil physical structure on adsorption.

Because of their reduced solubility, PAH may be best described by truncated isotherm models. Most isotherm models do not take into account limited solubility. Figure 40 illustrates how an isotherm might be truncated due to solubility restrictions. The vertical dotted line indicates solubility of the component. The right hand section is not valid because the component is not soluble at concentrations greater than the dotted vertical line. Isotherm truncation may have unusual effects upon adsorption behavior. Mass transfer is affected by soil structure. What effects would one expect if the soil structure was not homogeneous. Heterogeneous pollutant distribution may also have unusual effects on adsorption and degradation processes.

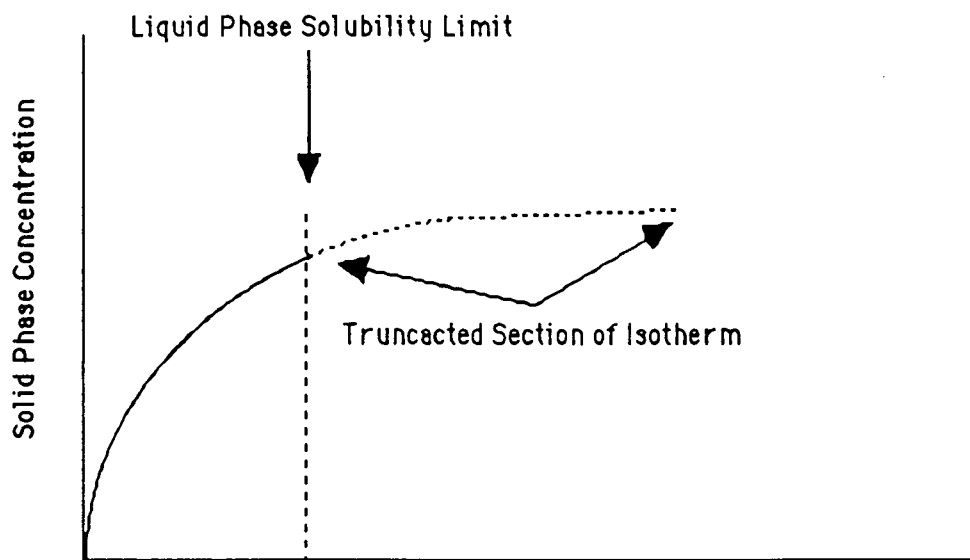


Figure 40. Truncation of isotherm by solute solubility limitation.

The right hand section of the isotherm is not valid because the component is not soluble at concentrations greater than the dotted line.

4.3 KINETIC EVALUATION OF *P. FLUORESCENS*, HK44

Alginate immobilized *P. fluorescens* HK44 was characterized with respect to its salicylate degradation kinetics. Least square techniques indicated that a first order rate equation adequately described salicylate degradation by *P. fluorescens* HK44. DiGrazia (1991) found that naphthalene degradation by *P. fluorescens* HK44 was described by first order kinetics. The form of the rate equation and the kinetic constants were determined from experiments using alginate immobilized *P. fluorescens* HK44. Feed and biomass concentrations and flow rates were varied to determine the form of the degradation rate equation and associated constants.

Experimental conditions were widely varied to produce large differences in bacterial response. A total of 18 experiments (experiments 1a-c, 2a-c, 3a-f, and 4a-f) were conducted to determine the relationship between degradation, substrate concentration, biomass, and contact time (between bacteria and substrates). More than 1900 individual measurements of the effluent concentration were made. Biomass concentrations were varied between 1.9×10^9 and 3.5×10^9 cells/mL. Sodium salicylate

concentration was varied between 2.25 and 4.45 mg/l. Higher substrate concentrations were not used due to concerns about toxicity of high oxygen concentrations required for substrate mineralization. Residence times were varied between 14 and 150 minutes. A number of duplicate experiments were included in the experimental design.

Measurements of effluent concentrations were consistent through out the investigation. Standard deviations of the effluent concentrations for the individual experiments are listed in Table 13 and ranged between 1.08 and 2.96 percent of the respective effluent concentrations. Bacteria degradative activity was generally constant over time although a small amount of drift was noticed in some experiments. Unsteady state behavior occurred for 1 hour after a perturbation occurred. Unsteady state behavior was not included in the kinetic analysis.

Pairs of experiments conducted under identical conditions are listed in Table 14. Generally these independent experiments had roughly the same conversion values. Generally these results suggest that results from degradation experiments were repeatable. Along with the results form Table 13, Table 14 indicates that degradation is a function of biomass, reactor residence time, and substrate concentration. The slight disagreement between duplicate experiments may be due to inaccuracies biomass measurements, varying bacterial activity, and/or heterogeneous mass transfer resistance due to non-uniformity of the alginate immobilization media. Experiments were extensively standardized to minimize

error. Alginate is a natural product and is subject to chemical and physical heterogeneity from batch to batch of the purchased material. Autoclaving (required for sterilizing) may further alter chemical and physical properties of the alginate. Laboratory alginate processing was standardized in an attempt to reduce experimental variability. The uniformity of alginate was not addressed in the present investigation and may warrant investigation in future studies.

Kinetic experiments were conducted to investigate the applicability of two reaction rate models for describing degradation of salicylate. The first model was first order in substrate and first order in biomass (equation (41)). The second model was zero order in substrate concentration and first order in biomass concentration (equation (42)). Both models had one adjustable parameter, the reaction rate constant. The best fit of the different degradation models was achieved using a degradation equation of first order in biomass and first order in substrate concentration model. This model was tested with a maximum ratio of substrate to biomass of 4.45 mg/l of salicylate to 1.0×10^9 cells/mL. This first order model should not be extended to higher ratios unless additional experiments are conducted. Higher substrate concentrations were not used due to concerns about toxicity of high oxygen concentrations required for degradation. Very high concentrations of oxygen may be toxic to bacteria. If high concentrations of substrate are expected, then the biosensor biomass should be

increased. Most PAH are soluble at several mg/L concentrations or below.

Least square techniques indicated that equation (45) provided a superior description of the degradation of salicylate by *P. fluorescens* HK44. The residuals from fitting equation (45) to the degradation data using least square methods were significantly lower than fitting equation (46) to the degradation data. Except for one case, the residuals using equation (45) were less than those obtained with equation (47) for identical data sets and were, in many instances, one order of magnitude less. The range of regressed degradation constants was significantly smaller when using equation (45) than when using equation (46). The reaction rate constants obtained using equation (45) ranged from 2.30×10^{-2} to $3.45 \times 10^{-2} \text{ dm}^3/\text{g}/\text{min}$ while the reaction rate constants obtained using equation (46) ranged over half an order of magnitude, 5.20×10^{-5} to $1.65 \times 10^{-4} \text{ min}^{-1}$. These results strongly indicate that a first order reaction term is more appropriate for describing degradation of salicylate by *P. fluorescens* HK44 than a zero order reaction term.

The final four rows of Table 15 indicated the contribution of each experiment to the overall residual. The regressed rate constants from experimental sets 1, 3, and 4 differed by no more than 17 percent. The regressed degradation constant for experiment 2 differed significantly from the other experiments. Residuals were greatest for experiment 2 in most of the rows of Table 15. These

results suggest that experiment 2 contributed the most to the residuals of Table 15.

The ranges of bead diameters used for experiments were tightly grouped around the average bead diameter , 4.5×10^{-2} cm. Overall mass transfer resistance and dispersion are functions of particle diameter. Bead generation procedures were optimized to produce the smallest possible beads.

4.4 BIOLUMINESCENCE

4.4A BIOLUMINESCENCE (CONTROLS)

Control experiments were conducted to determine the effect of glucose, a *nah* non inducing substrate, and mineral salts media (no carbon) on the light emission of *P. fluorescens* HK44. Bioluminescent activity was minimal using mineral salts media. *P. fluorescens* HK44 emitted light when exposed to glucose although the quantity was orders of magnitude less than light given off in the presence of inducing substrates.

When *P. fluorescens* HK44 was exposed to glucose, light emission was probably due to constitutive levels of *lux* pathway enzymes. Schell (1985) found that low levels of NAH mRNA were present in uninduced cells indicating that low levels of NAH degradative enzymes are present in uninduced cells. Thus low

constitutive levels of *lux* enzymes are expected in *P. fluorescens* HK44. Light emission by *P. fluorescens* HK44 is dependent upon the availability of aldehyde, oxygen, NADPH, ATP, and FMNH₂ as well as induction of the *lux* pathway. Light emission may be limited by availability of any of these substrates. Measured effluent oxygen levels were typically greater than 10 mg/l even after 90 percent of the salicylate was degraded. Oxygen was not limiting. NADPH, ATP and FMNH₂ are common compounds used for cellular energy storage. Glucose is an excellent carbon and energy source for bacteria. Glucose addition increased light production by possibly increasing pools of one or more of the light substrates (NADPH, ATP, FMNH₂, or aldehyde).

A number of potential areas for future study include investigation of cellular production of NADPH, ATP, FMNH₂, or aldehyde when *P. fluorescens* HK44 is exposed to non-inducing substrates.

4.4B BIOLUMINESCENCE (STEADY STATE)

Experiments were conducted to investigate the steady state light emission of *P. fluorescens* HK44. Salicylate and naphthalene were used as inducer substrates.

Light emission of *P. fluorescens* HK44 was a function of salicylate concentration. Table 20 lists light emission by *P. fluorescens* HK44 when exposed to different concentrations of salicylate. Light emission was reported as a specific light response. The salicylate concentration inside the reactor was calculated using equation (45) and a reaction rate constant of $2.68 \times 10^{-2} \text{ dm}^3/\text{g}/\text{mol}$ (see column1, row 1 of Table 15). The reaction rate constant was obtained by fitting equation (45) to the full data set in Table 13. The data demonstrate a positive correlation between light emission and concentration.

4.4C BIOLUMINESCENCE (UNSTEADY STATE)

The unsteady state response of *P. fluorescens* HK44 to inducer concentration was investigated. Salicylate and naphthalene were used as inducing substrates.

Several trends in Table 21 and Figures 38-39 are reiterated here. After addition of an inducing substrate, light emission

initially increased to a maximum level and then decayed to steady state. Steady state light emission occurred 8 hours after addition of salicylate and 60 hours after addition of naphthalene. Maximum light emission was proportional to biomass but could not be correlated with inducing substrate concentration. Maximum light emission after addition of naphthalene was significantly greater than after addition of salicylate.

Maximum light emission was correlated with biomass but was not a function of salicylate concentration. Possibly the reason that light emission was a function of biomass was that one pathway of the light reaction was limited in substrate. If the light reaction is limited in some way, then addition of extraneous substrates would affect the maximum light output. This phenomena should be further investigated.

Light emission upon naphthalene addition was orders of magnitude greater than upon salicylate addition. (No concentration data was available for the naphthalene experiments because of equipment failures). Two explanations occur to the author explaining the large difference in the effects of naphthalene and salicylate. Uptake of the two substrate may occur by very different mechanisms. Naphthalene uptake is passive because naphthalene preferentially adsorbs to the lipid fraction of the cell (Bateman *et al.*, 1986). Salicylate uptake may occur by active transport (a slower process than adsorption) because salicylate is a charged ion. Thus transport may affect light emission. Naphthalene preferentially adsorbs to the lipid fraction of the cell. Because

naphthalene adsorbs to the lipid fraction, cellular lipid synthesis may be increased to maintain lipid integrity and counteract the effect of naphthalene in the lipid phase of the cell. The increased synthesis of lipids would then increase the aldehyde substrate for the light reaction. The increased availability of aldehyde substrate leads to elevated light production upon exposure to naphthalene. In this scenario, the light reaction would be limited in aldehyde substrate upon salicylate addition. Validation of this hypothesis (aldehyde substrate limitation in the case of salicylate addition) would partially explain the relationship between maximum light output and biomass observed in the salicylate experiments. This information also indicates that *P. fluorescens* HK44 may be far more sensitive to inducer concentration than suggested by the salicylate results. *P. fluorescens* HK44 continues to show promise as a biosensor organism. These relationships require further investigation.

Because light emission is a function of intercellular inducer concentration, light emission should reach a steady state after degradation reaches a steady state. In general, degradation rates (reflected by effluent concentration) became constant approximately one hour after a change in flow rate or feed concentration; whereas, light emission became steady after significantly longer periods of time (8 hours for salicylate and 60 hours for naphthalene). This large lag time may also indicate that the light reaction is limited in aldehyde substrate. Further investigation is required.

5 CONCLUSIONS

5.1 PACKED BED REACTOR AND DIFFUSION PROCESSES

5.1A REACTOR HYDRODYNAMIC PERFORMANCE & DIFFUSION PROCESSES

The reactor could simulate the very low flow rates observed in ground water. The residence time in the tubing to and from the reactor was small even at low flow rates.

Dispersion and mass transfer resistance approached minimal values. Measured residence times when compared to the measured reactor volume and measured flow rates demonstrated that channeling was not significant.

The reactor had a small residence time and allowed rapid evaluation of kinetics. Because the adsorber could be easily modified for various types of perturbation analysis, the adsorber may be used to address the question of component availability in soil systems.

5.1B SIMULATIONS OF DIFFUSIONAL PROCESSES OCCURRING IN ALGINATE BEADS & SOILS

PDECOL (Madsen & Sincovec, 1979) effectively solved reactor diffusion problems. Numerical solutions obtained using PDECOL (Madsen & Sincovec, 1979) closely approximated analytical solution values. 30 collocation points provided an accurate prediction of reactor processes for which there were analytical solutions to compare to. A minimum of 30 collocation points were used in all numerical simulations in this investigation.

Solid diffusion was not a significant PAH transport mechanism in alginate.

5.2 ADSORPTION PROCESSES

5.2A ADSORPTION ISOTHERMS OF NAPHTHALENE AND SODIUM SALICYLATE ON CALCIUM ALGINATE

Sodium salicylate did not measurably adsorb to alginate. Alginate appears to be an ideal support material when evaluating degradation of sodium salicylate by *P. fluorescens* HK44. Salicylate moved through the alginate quickly because alginate is very porous and did not adsorb salicylate.

The naphthalene adsorption isotherm was linear. The solid to liquid phase concentration ratio was 8.4 at equilibrium. Naphthalene transport through alginate is hindered because of adsorption. For larger PAH, the PAH-immobilization media adsorption isotherm may significantly influence bioluminescent and degradative behavior.

Measured unsteady state behavior (adsorption and degradation) of naphthalene and salicylate was consistent with predicted behavior (using PDECOL(Madsen & Sincovec, 1979)).

5.2B SIMULATION OF ADSORPTION PROCESSES

PDECOL (Madsen & Sincovec, 1979) was an effective method for numerically solving adsorber problems. PDECOL provided a very accurate simulation of the passage of a sine wave tracer through a packed bed. PDECOL was less effective at simulating the breakthrough curve for a step feed perturbation with a very steep leading front. simulation of the passage of a sine wave tracer through a packed bed. Caution is appropriate when interpreting results from simulations where sharp fronts or discontinuities are expected or encountered. Increasing the number of collocation points reduced error although the tendency for error was always greater near sections with very steep fronts.

Adsorbing sine wave fronts displayed constant pattern behavior as did square waves.

Dampening of sine waves in high capacity adsorption beds was due to adsorption. Complete dampening of the sine feed wave produced a constant effluent concentration equal to the average of the feed concentration.

Equation (24) could be used to explain sine wave behavior in low and high capacity adsorption beds.

Sinusoidal perturbation methods proved to be useful for adsorption analysis because two parameters, period and amplitude, were available for dynamically testing the system. Constant pattern behavior (favorable adsorption only) could also be used to study mass transfer and adsorption effects.

5.2C SOIL ADSORPTION STUDIES

Adsorption effects were important in determining the liquid phase concentration of naphthalene and of other PAH leaching from the soil. MGP soil had a large naphthalene adsorption capacity. PAH compounds adsorbed in the soil were displaced by naphthalene addition using both a constant feed (step change perturbation) and a sinusoidal feed. Adsorption and leaching of pre-existing naphthalene dampened the naphthalene sine wave.

Sinusoidal perturbation methods proved to be more versatile than step perturbations for studying soil adsorption processes. Sinusoidal perturbations produced continual system response patterns that could be studied in detail and could easily be

differentiated from normal system behavior. Step and pulse perturbations produced only transient responses which were more difficult to interpret than sinusoidal perturbations. Total concentration of PAHs in soils may not correlate well with availability. Sinusoidal perturbations provided two parameters, period and amplitude, while only one parameter, magnitude of the step function, was available for dynamic studies using traditional step perturbation methods. Both sinusoidal and step pulses displayed constant pattern behavior which could be used for analysis of mass transfer and adsorption.

Several analytical methods were used for off-line and automated on-line measurement of PAH. The on-line GC technique effectively resolved complex mixtures of volatile PAH. HPLC with fluorescent detection proved to be a selective and highly sensitive method for analysis of PAH mixtures (detection limit for naphthalene <100 ng/L). Use of polymeric C₁₈ columns provided good separation of PAH and could be used to separate the priority pollutants in SRM 1647. Use of GC and HPLC methods allowed qualitative and quantitative monitoring of complex PAH mixtures leaching from soils in real time.

The continuous flow-packed bed system with on-line GC (FID detection)/HPLC (Fluorescence detection) is well suited for study of adsorption of volatile and non-volatile PAH compounds in soil systems.

The reactor was used to characterize PAH and PAH-related compounds leaching from contaminated MGP soil. Total

concentration of PAH in soils may not correlate well with availability. The reactor had a small residence time and allowed rapid evaluation of kinetics. Reactor metal parts and adsorber inorganic filters provided few sites for PAH adsorption. Because the reactor could be easily modified for various types of perturbation analysis, the adsorber may be used to address the question of component availability in soil systems.

5.3 KINETIC EVALUATION OF *P. FLUORESCENS*, HK44

Least square techniques indicated that an overall second order rate equation, first order rate in salicylate and first order in biomass, adequately described salicylate degradation by *P. fluorescens* HK44. The reaction rate constants obtained using equation (45) ranged from 2.30×10^{-2} to 3.45×10^{-2} dm³/g/min. The second order model, equation (45) proved to be far superior to equation (46) for describing degradation of salicylate by *P. fluorescens* HK44. Bacterial degradative activity was constant over time. Degradation results were repeatable.

5.4 BIOLUMINESCENCE

5.4A BIOLUMINESCENCE (CONTROLS)

Bioluminescent activity was minimal using mineral salts media.

P. fluorescens HK44 emitted light when exposed to glucose although the quantity was orders of magnitude less than light given off in the presence of inducing substrates. Glucose addition increased light production by increasing pools of one or more of the light substrates (NADPH, ATP, FMNH₂, or aldehyde).

5.4B BIOLUMINESCENCE (STEADY STATE)

Light emission of *P. fluorescens* HK44 was a function of salicylate concentration. Further work is required to standardize methods to allow direct quantitative comparison between experiments.

5.4C BIOLUMINESCENCE (UNSTEADY STATE)

The response of *P. fluorescens* HK44 to salicylate was very different than the response to naphthalene. Unsteady state light emission behavior occurred for 8 hours after addition of salicylate and 60 hours after addition of naphthalene. Light emission upon addition of naphthalene was orders of magnitude greater than light emission when salicylate was added. *P. fluorescens* HK44 continues to show promise as a sensitive biosensor organism.

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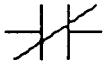
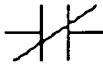
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APPENDICES

APPENDIX A

1. SYMBOLS AND SEQUENCE OF EVENTS FOR PBR CONTROL NETWORK

	Ground
$\varnothing$	Positive Line
N	Neutral Line
PS1	Power Supply
TDR1	Time Delay Relay
	Usually Open Unless Energized
TDR1*	Programmable Time Delay Relay
	Usually Open Unless Energized
K1	Relay
 K1a	Contact Closure Autostart
 Autostart	Connected to Relay K1, Contact a

Sequence of Events

TDR1*, TDR2*, and TDR3* operate independently of one another.

Sequence for TDR1*

TDR1* is usually open.

At programmed time, TDR1* closes and supplies voltage to solenoid 1 in the switching valve.

Switching valve changes position.

TDR1* opens.

Sequence for TDR2*

TDR2* is usually open.

At programmed time, TDR2* closes and supplies voltage to
solenoid 1 in the switching valve.

Switching valve changes position.

TDR2* opens.

Sequence for TDR3*

TDR3* is usually open.

At program time, TDR3* closes and supplies voltage to K1.

K1 is energized and K1a, K1b and K1c close. These contacts
are usually open. The HPLC, integrator and fluorometer
programs start when contacts K1a, K1b and K1c close,
TDR4* and TDR5* are time programmed relative to HPLC
operation and work in the same manner described for
TDR1* and TDR2*.

TDR3* closes.

APPENDIX B

FULL DATA SETS

Table A: Mass transfer and dispersion as a function of particle size, flow rate and reactor volume.

Reactor Volume (cm ³)	D _p (cm)	v (cm/min)	$\frac{1}{v^2}$ (min ² /cm ²)	$\frac{L\sigma^2}{2\mu^2v}$ (min)	NRE
7.24	1.00E-02	2.11E+00	2.26E-01	8.51E-02	3.57E-02
7.24	1.00E-02	2.11E+00	2.26E-01	9.35E-02	3.57E-02
7.24	1.00E-02	2.11E+00	2.26E-01	8.64E-02	3.57E-02
7.24	1.00E-02	2.11E+00	2.26E-01	9.27E-02	3.57E-02
7.24	1.00E-02	2.11E+00	2.26E-01	7.69E-02	3.57E-02
7.24	1.00E-02	1.05E+00	9.03E-01	1.86E-01	1.79E-02
7.24	1.00E-02	1.05E+00	9.03E-01	1.77E-01	1.79E-02
7.24	1.00E-02	1.05E+00	9.03E-01	1.61E-01	1.79E-02
7.24	1.00E-02	5.26E-01	3.61E+00	3.46E-01	8.93E-03
7.24	1.00E-02	5.26E-01	3.61E+00	3.08E-01	8.93E-03
7.24	1.00E-02	2.63E-01	1.44E+01	5.90E-01	4.47E-03
14.48	1.00E-02	2.11E+00	2.26E-01	9.95E-02	3.57E-02
14.48	1.00E-02	2.11E+00	2.26E-01	1.18E-01	3.57E-02
14.48	1.00E-02	2.11E+00	2.26E-01	1.29E-01	3.57E-02
14.48	1.00E-02	1.05E+00	9.03E-01	2.34E-01	1.79E-02
14.48	1.00E-02	1.05E+00	9.03E-01	2.45E-01	1.79E-02
14.48	1.00E-02	5.26E-02	3.61E+02	9.10E+00	8.93E-04
14.48	1.00E-02	5.26E-01	3.61E+00	4.52E-01	8.93E-03
21.72	1.00E-02	1.58E+00	4.01E-01	2.24E-01	2.68E-02
21.72	1.00E-02	1.58E+00	4.01E-01	2.45E-01	2.68E-02
21.72	1.00E-02	1.58E+00	4.01E-01	2.09E-01	2.68E-02
21.72	1.00E-02	7.93E-01	1.59E+00	4.60E-01	1.35E-02
21.72	1.00E-02	7.93E-01	1.59E+00	3.95E-01	1.35E-02
21.72	1.00E-02	7.93E-01	1.59E+00	3.51E-01	1.35E-02

Table 1: (continued)

Reactor Volume cm ³	D _p cm	v cm/min	$\frac{1}{v^2}$ min ² /cm ²	$\frac{L\sigma^2}{2\mu^2v}$ min	NRE
3.62	1.00E-02	2.11E+00	2.26E-01	2.05E-02	3.57E-02
3.62	1.00E-02	2.11E+00	2.26E-01	1.82E-02	3.57E-02
3.62	1.00E-02	2.11E+00	2.26E-01	1.61E-02	3.57E-02
3.62	1.00E-02	2.11E+00	2.26E-01	1.77E-02	3.57E-02
3.62	1.00E-02	1.05E+00	9.03E-01	3.07E-02	1.79E-02
3.62	1.00E-02	1.05E+00	9.03E-01	3.06E-02	1.79E-02
3.62	1.00E-02	1.05E+00	9.03E-01	3.35E-02	1.79E-02
3.62	1.00E-02	5.26E-01	3.61E+00	5.95E-02	8.93E-03
7.24	2.50E-02	2.11E+00	2.26E-01	5.79E-02	8.93E-02
7.24	2.50E-02	2.11E+00	2.26E-01	6.05E-02	8.93E-02
7.24	2.50E-02	2.11E+00	2.26E-01	5.56E-02	8.93E-02
7.24	2.50E-02	1.05E+00	9.03E-01	1.21E-01	4.47E-02
7.24	2.50E-02	1.05E+00	9.03E-01	1.19E-01	4.47E-02
7.24	2.50E-02	1.05E+00	9.03E-01	1.12E-01	4.47E-02
21.72	2.50E-02	1.05E+00	9.03E-01	5.71E-01	4.47E-02
3.62	2.50E-02	2.11E+00	2.26E-01	1.77E-02	8.93E-02
3.62	2.50E-02	2.11E+00	2.26E-01	1.71E-02	8.93E-02
3.62	2.50E-02	1.05E+00	9.03E-01	3.41E-02	4.47E-02
3.62	2.50E-02	1.05E+00	9.03E-01	3.44E-02	4.47E-02

APPENDIX C

PARTIAL DIFFERENTIAL EQUATIONS USED TO DESCRIBE REACTOR ADSORPTION, REACTION AND TRANSPORT PROCESSES

I. Michaelis-Menten Reaction without mass transfer resistance.

Mass Balance on Total Bed.

Program MMRX.FOR

$$\frac{\partial C}{\partial t} = \frac{1}{P_e} \frac{\partial^2 C}{\partial z^2} - \frac{\partial C}{\partial z} - \frac{kL}{V} \left(\frac{C}{1 + k_2 C_{i_o} C} \right)$$

II. Alginate Pellet with Michealis-Menton Kinetics, Langmuirian Adsorption and Liquid Diffusion.

Program PMMRX.FOR

$$\begin{aligned} \frac{\partial C_{Dpi}}{\partial t} = & \frac{2 \left(\epsilon_p D_{p_i} \frac{\partial C_{Dpi}}{\partial r} \right)}{R^2 r \left[\frac{\epsilon_p}{\tau} + (1 - \epsilon_p) \frac{\theta_{S_i}}{\tau} \frac{dC_{Dsi}}{dC_{Dpi}} \right]} + \frac{\epsilon_p D_{p_i} \frac{\partial^2 C_{Dpi}}{\partial r^2}}{R^2 \left[\frac{\epsilon_p}{\tau} + (1 - \epsilon_p) \frac{\theta_{S_i}}{\tau} \frac{dC_{Dsi}}{dC_{Dpi}} \right]} \\ & - \frac{k_3 C_{Dpi}}{1 + k_4 C_{i_o} C_{Dpi} \left[\frac{\epsilon_p}{\tau} + (1 - \epsilon_p) \frac{\theta_{S_i}}{\tau} \frac{dC_{Dsi}}{dC_{Dpi}} \right]} \end{aligned}$$

- III. Pellet with Michealis-Menton Kinetics, Langmuirian Adsorption, and Liquid and Solid Diffusion.

Program PM2MMRX.FOR

$$\begin{aligned} \frac{\partial C_{Dpi}}{\partial t} \left[\frac{\epsilon_p}{\tau} + (1 - \epsilon_p) \frac{\theta_{S_i}}{\tau} \frac{dC_{Dsi}}{dC_{Dpi}} \right] &= \frac{2}{R^2 r} \left(D_{S_i} \theta_{S_i} (1 - \epsilon_p) \frac{\partial C_{Dsi}}{\partial r} + \epsilon_p D_{P_i} \frac{\partial C_{Dpi}}{\partial r} \right) \\ &+ \frac{1}{R^2} \left(D_{S_i} \theta_{S_i} (1 - \epsilon_p) \frac{\partial^2 C_{Dsi}}{\partial r^2} + \epsilon_p D_{P_i} \frac{\partial^2 C_{Dpi}}{\partial r^2} \right) - \frac{k_3 C_{Dpi}}{1 + k_4 C_{i_o} C_{Dpi}} \end{aligned}$$

- IV. Plug Flow Reactor with Dispersion, Convection, and Single Component Langmuirian Adsorption.

Program SCADS.FOR

$$\frac{\partial C}{\partial t} \left[1 + \frac{1 - \epsilon}{\epsilon} \frac{k}{(1 + k_2 C_{i_o} C)^2} \right] = \frac{1}{P_e} \frac{\partial^2 C}{\partial z^2} - \frac{\partial C}{\partial z}$$

- V. Plug Flow Reactor with Dispersion, Convection, Mass Transfer, and Two Component Langmuirian Adsorption with Linear and Favorable Isotherms.

TCADSLF.FOR

$$\frac{\partial C_1}{\partial t} \left[1 + \frac{1 - \varepsilon}{\varepsilon} \frac{dC_{Dsi1}}{dC_1} \right] = \frac{1}{Pe} \frac{\partial^2 C_1}{\partial z^2} - \frac{\partial C_1}{\partial z}$$

$$\frac{\partial C_2}{\partial t} \left[1 + \frac{1 - \varepsilon}{\varepsilon} \frac{dC_{Dsi2}}{dC_2} \right] = \frac{1}{Pe_2} \frac{\partial^2 C_2}{\partial z^2} - \frac{\partial C_2}{\partial z}$$

VITA

Oren Francis Webb was born on March 1, 1961, in Waynesville, North Carolina. He received his elementary and secondary education in the Haywood County public school system and graduated from Tuscola Senior High School in June 1979. He studied chemistry at Western Carolina University and graduated with honors with a BA in August 1984. After working as an ultrafiltration technician and a chemist, he entered the graduate program in chemical engineering at the University of Tennessee in August 1988, where in December 1991 he received his MS in chemical engineering. He received his PhD in chemical engineering at the University of Tennessee in December 1992.