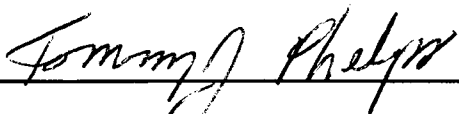


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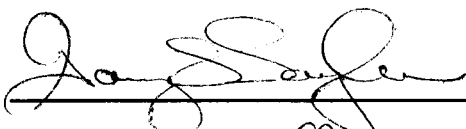


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We have read this dissertation
and recommend its acceptance:




Accepted for the Council:



Associate Vice Chancellor
and Dean of The Graduate School

**Utilization Of Packed-bed Bioreactor Systems
For Fate Analysis of Trichloroethylene
and Mixtures of Organic Wastes**

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

**Laura Waneta Lackey
May 1993**

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ACKNOWLEDGEMENTS

I would like to thank my major professors, Dr. Paul R. Bienkowski and Dr. Tommy J. Phelps, for their help and support. I would also like to thank my other committee members, Dr. Gary S. Sayler and Dr. Terrence L. Donaldson, for their comments and expertise. In addition, I would like to express sincere thanks to Lisa Kohring and Dr. Marc Mittleman for occasionally playing Santa Claus, to Bruce Applegate and Jack McPherson for their interdisciplinary collaboration, and to Vivek Korde, Robert Mackowski, Oren Webb for their companionship and technical support.

I would especially like to thank my parents, Jack and Betty Lackey, and my sister, Amy, for their support and encouragement.

ABSTRACT

This study investigated the feasibility of utilizing native microbial consortia for the on-site bioremediation of trichlorethylene and other chlorinated organic groundwater contaminants. Experimental bioreactors, including a single-pass packed-bed reactor, a differential volume reactor system and a recirculating expanded-bed reactor, were constructed and used to study TCE and mixed-organic waste degradation. A mathematical simulation was developed to describe the fate of TCE in packed-bed soil systems. Additional batch studies were used to monitor toxicant degradation, and the changes in the microbial community structure by monitoring signature fatty acid biomarker trends during TCE degradation. Pure and multi-species microbial consortia and bioluminescent reporter technology were used in both reactor and batch studies to increase the fundamental understanding of the microbial fortuitous metabolism of TCE (and metabolites) and the biodegradation of the BTEX compounds (benzene, toluene, xylene, and ethyl benzene).

Significant data were obtained from the construction of a 1.9 L single-pass horizontal packed-bed reactor using sand as a solid support substratum for a TCE-degrading consortia. With inlet TCE concentrations ranging from 1 to 2 mg/L, and 1.9 to 3.2 days residence time, effluent concentrations of

5 to 10 $\mu\text{g/L}$ were obtained, which approach drinking water quality standards. Pulse feeding regimes were typically necessary for the TCE effluent concentrations to be less than 10 $\mu\text{g/L}$. Introducing mixed organic wastes into the system showed an increased recalcitrance of TCE as the complexity of the mixture increased, with first order TCE degradation rate constants varying from 0.20 with TCE the only toxicant present to 0.16 when there were 12 organic wastes in the system.

Data from the differential volume reactor system verified the increased recalcitrance of TCE when experiments were conducted with mixed organic wastes. The reactor also provided a rapid and effective tool for abiotically determining dispersion and mass-transfer coefficients. Mass-transfer coefficients were found to decrease as the diameter of the particles in the bed increased. Such variables are critical for describing flow characteristics in packed-bed soil or sand systems.

The mathematical simulation developed describes transport properties such as dispersion, advection, and sorption. It describes the fortuitous metabolism process and the microbial cometabolic degradative properties of TCE in packed-bed reactors. The simulation portrayed the ability to correlate reactor TCE effluent concentrations in mixed organic-waste systems very accurately. The largest error observed when comparing experimental to simulation results was $\sim 9\%$. A parameter sensitivity analysis performed on the simulation showed the variables Peclet Number (Pe), the donor

saturation constant (K_{SD}) and the electron donor concentration (C_D) to have a large effect on simulation predictions.

Successful on-site or *in-situ* bioremediation is often limited by the scarcity of internal biodegradation markers and the inability to rapidly assess catabolic gene expression. Preliminary results are presented addressing the feasibility of using bioluminescent reporter technology to provide continuous information on microbial biodegradation activity. To correlate microbial activity and/or inducer concentration with bioluminescence, batch studies, expanded-bed bioreactor and differential volume reactor experiments were conducted using bacteria containing the *lux* gene cassette. The results indicate that engineered bacteria containing the *lux* gene cassette show promise as a continuous monitor of the biodegradation process.

This study endeavors to increase the fundamental understanding of the microbial fortuitous metabolism of TCE and the biodegradation of the BTEX compounds, utilizing microbial consortia. Through the complementary methodologies demonstrated in this report, integrated bioremediation techniques could be realized that remediate mixed-organic wastes on-site rather than first transferring the toxicants from one environment to another.

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NOMENCLATURE

b	Langmuir equilibrium constant or cell decay coefficient, d^{-1}
b_o	pre-exponential factor
C_2	concentration of secondary substrate
C_A	concentration of electron acceptor
C_{Ao}	initial concentration of species A
C_D	concentration of electron donor
C_i	concentration in the fluid phase of a single species, mg/L
D, D_L	axial dispersion coefficient, cm^2/s
d_c	oxygen demand for cell decay, g O_2 / g cells
D_c	intercrystalline diffusivity
D_h	hydrodynamic dispersion coefficient
D_m	molecular diffusivity, cm^2/s
D_p	pore diffusivity, cm^2/s
F	stoichiometric ratio of electron acceptor to electron donor utilization for biomass synthesis, g acceptor/ g donor
f_d	fraction of cells that is biodegradable
$-\Delta H_o$	limiting heat of adsorption at low coverage
k	first-order reaction rate coefficient, day^{-1}
k_2	secondary substrate maximum utilization rate, g/g cell-d
K	Dimensionless Henry's law adsorption equilibrium constant

K_d	partition coefficient
K_s	saturation constant, mg/L
K_{s2}	secondary substrate saturation coefficient, mg/L
K_{SA}	acceptor saturation constant, mg/L
K_{SD}	donor saturation constant, mg/L
L	reactor bed length, m
n	order of reaction rate
Pe	dimensionless Peclet number
q	sorbate concentration, mg/L
q_s	saturation limit, mg/L
R	retardation factor for the solute
r_c	crystal or microparticle radius, m
R_i	Reaction rate of species i
R_p	Particle radius, m
Sh	dimensionless Sherwood number
t	time, s
v	superficial velocity, m/s
V	flow rate, ml/hr
x	spatial dimension, m
X	reactor cell concentration, mg/L
Y	yield coefficient, g cells/ g donor
z	spatial dimension, length

Greek Letters

α	mass transfer coefficient, sec^{-1}
ϵ	void volume or porosity
θ_c	mean
μ	mean retention time
ρ	density, g/cm^3
σ	standard deviation of chromatographic response peak
σ_θ^2	variance
τ	reactor residence time, day

CHAPTER I

INTRODUCTION

BACKGROUND

Chlorinated short chain aliphatic compounds such as trichloroethylene (TCE), perchloroethylene (PCE), and dichloroethylene (DCE) are widely used industrial solvents and degreasing agents often utilized in the dry cleaning business and other industrial settings. These compounds represent one of the largest contaminants of groundwater (Vogel et al., 1987). The National Priority List of the U.S. Environmental Protection Agency lists TCE as one of the most frequently reported contaminant at hazardous waste sites (Little et al., 1988). Illegal dumping by direct discharge, landfill leaching and accidental spills are ways that these priority pollutants are introduced into the environment (Richards and Shiek, 1986).

Typically, short-chain aliphatic compounds cannot be removed from groundwater using conventional water treatment techniques such as coagulation, sedimentation, or filtration (Wood and DeMarco, 1980). Instead, they are generally removed by air stripping and adsorption (Love and Eilers, 1982). Unfortunately, these methods of removal do not destroy the potentially unsafe compounds, but have only maneuvered them from one phase to another.

An alternate method for removing these aliphatic compounds from water systems is biodegradation. Biodegradation has shown the potential to transform toxic compounds to non-toxic compounds such as carbon dioxide, water, bases, salts, and inorganic acids. A variety of experimental methods and bioreactor designs have demonstrated the ability to bioremediate such compounds. TCE degradation has been observed in the laboratory by several investigators (Phelps et al., 1990; Little et al., 1988; Fathepure et al., 1987; Bouwer and McCarty, 1983; Fliermans et al., 1988) under both aerobic and anaerobic conditions. Also, a wide variety of reactor designs have been used for TCE degradation, including, fluidized bed, trickling filter, activated sludge, anaerobic continuous stirred tank, and an upflow biofilter.

HYPOTHESIS

Subsurface microorganisms capable of degrading TCE have been isolated from contaminated sites. It is hypothesized that the microbial cometabolism of TCE is dependent on the specific conditions in the degradative environment (*in-situ* or on-site). Potentially key variables controlling rates and degradation potentials are as follows: nutrients and trace mineral availability, electron donor and acceptor concentrations, and the complexity of mixed-organic wastes in the specific environment. Specifically, the maximum degradative potential can be achieved when these critical parameters are optimized. Furthermore, the *lux* bioreporter offers a

promising new technology that potentially provides a method for monitoring shifts in microbial activity by bioluminescence when the above mentioned key variables are altered.

RESEARCH OBJECTIVES

1. A 2 liter, horizontal, single-pass, packed-bed columnar reactor will be developed for use as a polishing column with the goal of degrading inlet TCE concentrations of ≤ 2 mg/L and other mixed-organic wastes to concentrations approaching drinking water quality standards (approximately 0.01 mg/l). Additionally, efforts will be made to find optimal levels of critical system variables by perturbing the inlet concentration of such parameters.

2. Critical system variables will be perturbed in a stepwise fashion in a differential volume reactor containing sand and a TCE-degrading consortia. Also, this reactor system will be utilized in an abiotic fashion to apply a pulse perturbation scheme to quickly estimate dispersion and mass transfer coefficients that contribute to the description of flow characteristic within a packed-bed system. The rapid responses available in the DVR system will greatly increase the amount of data obtained.

3. A mathematical simulation will be developed describing the fate of TCE in packed-bed systems, with critical parameters such as electron donor and acceptor concentrations represented by variables.

4. The DVR system will be utilized in conjunction with the *lux* bioreporter B2 (*Pseudomonas putida* F1 containing a *tod-lux* gene fusion) immobilized in alginate beads in an effort to analyze the relationship between reporter degradative activities and bioluminescence. Bioluminescence will be correlated with the microbial cometabolism and metabolism of TCE and toluene, respectfully. Batch vial experiments will be used to relate the light response of the reporter strain B2 to the concentration of toluene in the system.

5. Existing expanded-bed reactors containing methane and propane oxidizing bacteria, and an engineered bacteria (Rb1401) containing the *tol-lux* gene cassette will be monitored. Efforts will be made to demonstrate the applicability of inserting a molecular bioreporter into a previously established microbial consortium to monitor bacterial activity, degradation ability, and bioluminescence in relation to system perturbations.

DOCUMENT ORGANIZATION

This document was written and organized both traditionally and in a moderately unorthodox dissertation fashion. The document is divided into major divisions by the use of chapters. Each chapter is then subdivided in an orderly and meaningful manner to aid and simplify comprehension for the reader. Chapters I, II, III are the Introduction, Literature Review, and Materials and Methods sections, respectfully. Chapters IV, V, and VI

represent a deviation from the typical dissertation. It was suggested to the author that these chapters contain manuscripts submitted in their entirety to peer reviewed journals. Chapter IV is entitled "Biodegradation of Chlorinated Aliphatic Hydrocarbon Mixtures in a Single-Pass Packed-Bed Reactor" and was submitted to *Applied Biochemistry and Biotechnology*. Chapter V represents a paper submitted to the journal *Biodegradation* entitled "Feasibility Testing for the On-Site Bioremediation of Organic Wastes by Native Microbial Consortia." Chapter VI contains a paper submitted to *Biotechnology Progress* having the title "Utilization of Packed-Bed Bioreactor Systems for Fate Analysis of Trichloroethylene in Subsurface Environments." Each of these manuscripts addresses one or more of the research objectives listed above. The only disadvantage the author sees in this type of chapter organization is occasional repetitive text. This distraction was held to a minimum. The remaining sections include Chapter VII entitled "Analysis of a Mathematical Simulation Describing Biodegradation of Mixed-Organic Wastes in a Packed-Bed Bioreactor," Chapter VIII, "Bioluminescent Reporter Technology for Monitoring the Fate of TCE and Toluene" and Chapter IX, "Conclusions" in the customary dissertation format.

CHAPTER II

LITERATURE REVIEW

ANAEROBIC/AEROBIC DEGRADATION

Anaerobic studies have clearly shown biotransformations of PCE and TCE. In all cases, the biotransformation is the result of a reductive dehalogenation (Vogel and McCarty, 1985; Bouwer and McCarty, 1983; Kleopfer et al., 1985; Fathepure et al., 1987). All of these anaerobic studies show the following sequential reductive dechlorination pathway: PCE → TCE → DCE → VC (vinyl chloride) → ethene. Under anaerobic conditions, the rate of dechlorination decreases as chlorine molecules are removed (Vogel and McCarty, 1985). To date, no data are available indicating PCE mineralization to carbon dioxide under aerobic conditions (Shields and Reagin, 1992).

Several different environments have shown the ability to support anaerobic biodegradation. For example, transformations have occurred in continuous-flow methanogenic fixed film reactors (Bouwer and McCarty, 1983; Vogel and McCarty, 1985). In the fixed-film reactor system, PCE concentrations were reduced by 98 to 99 percent, while TCE and VC concentrations increased. Typically, a two day retention time was adequate to degrade concentrations of PCE from 20.5 mg/L to 4.4 µg/L. Some mixed methanogenic bacterial cultures exhibit the ability to biodegrade anaerobically (Bouwer et al., 1981). Experiments with the mixed cultures

demonstrated near complete transformation with a two day retention time for all compounds (TCE, PCE, etc.) except 1,2-DCE.

Anaerobic soil (Kleopfer et al., 1985) and organic sediment from the Everglades (Barrio-Lage et al., 1985) show the ability to house methanogenic bacterial cultures that degrade chlorinated ethenes. Vials containing TCE contaminated soil have been used to dechlorinate TCE to 1,2-DCE when inoculated with specific degrading microorganisms. Isomer specificity was observed; no 1,1-DCE was detected. Similarly, vials containing soil from two sites in the Everglades were spiked with 1,1-DCE, cis-1,2-dichloroethene, or trans-1,2-dichloroethene. Vinyl chloride was produced after one to two weeks in all vials.

Degradation of TCE to CO_2 by aerobic mixed cultures has been observed. In such an environment, the degradation pathway has not been clearly identified (Fogel et al., 1986). Pure strains of aerobic methotrophs have also been shown to degrade TCE (Colby et al., 1977; Little et al., 1988). The maximum rate of TCE degradation was found to occur during the active phase of cell growth (days 2 to 6) and drops to zero after methane is depleted. Thus, active metabolism of a suitable growth substrate such as methane, methanol, or propane seems necessary for TCE biodegradation to occur (Little et al., 1988).

Methane monooxygenase, the enzyme which normally oxidizes methane to methanol, is apparently used by methanotrophs to degrade TCE,

as well. Researchers hypothesize that this enzyme also oxidizes TCE to form a trichloroethene epoxide which is then rapidly hydrolyzed. In support of this argument, Colby et al., (1977) noted that TCE degradation is inhibited by acetylene, a specific inhibitor of methane monooxygenase. TCE degradation with this enzyme begins with the epoxidation of the double bond, resulting in the formation of water soluble TCE breakdown products. These products include acetic, formic, oxalic, glyoxylic, and mono-, di-, and trichloroacetic acids. Fortunately, most of the breakdown products can be metabolized by heterotrophic bacteria in mixed methane-enriched cultures. Little et al. (1988) have suggested a possible mechanism for this biodegradation pathway. It is also known that methanotrophs convert ethene to ethene epoxide in a similar fashion (Hou et al., 1979).

TCE degradation has also been observed in cultures of *Pseudomonas* that are typically associated with aromatic degradative pathways that utilize *meta* fission. More specifically, *Pseudomonas mendocina* (Winter et al., 1989), *Pseudomonas putida* F1 (Wackett and Gibson 1988), and *Pseudomonas cepacia* G4 (Folsom et al., 1990) have all shown the ability to degrade TCE. Degradation rates by *Pseudomonas* are similar and greater than rates seen by methanotrophic cultures (Wackett and Gibson, 1988). This aromatic pathway suggests that the toluene dioxygenase enzyme is responsible for degradation. In fact, toluene or phenol was always required to induce degradation (Nelson et al., 1986).

The aerobic processes of biodegradation differ from the anaerobic process in two distinct ways. The aerobic degradation products do not include other volatile chlorinated compounds. Secondly, it is recalled that in anaerobic degradation, the rate of transformation decreased for less-chlorinated compounds. However, in the aerobic process the opposite trend is observed; the degradation rates decreased for more-chlorinated compounds. This trend of aerobic degradation allows vinyl chloride to be degraded more rapidly than TCE, but does not allow PCE to be degraded at all (Fathepure et al., 1987). These two points led Fathepure et al. (1987) to suggest a practical application of a two-stage biological process for the decontamination of waters containing complex mixtures of C_1 and C_2 chlorinated solvents. The process would consist of anaerobic reductive dechlorination followed by methanotrophic bio-oxidation.

REACTOR TYPES

Several different reactor designs have been implemented to study aerobic degradation of chlorinated compounds. Three reactor types, namely the continuous stirred-tank, fluidized bed, and packed bed, were analyzed by Holladay et al. (1978) for their usefulness in the study of phenol degradation. The foremost advantage of the continuous stirred-tank reactor was found to be the simplicity of its operation. However, the CSTR was found to be very vulnerable to shock. That is, the CSTR was unable to

prevent the microbe community from being exposed to either a high concentration of a toxic compound or by a new toxic compound, which are both inhibiting and potentially lethal. Of the three reactors studied, the fluidized bed reactor has the highest biodegradation rates. This finding was attributed to the high surface areas associated with fluidized bed reactors. Other advantages of using a fluidized bed reactor include: its small shear forces, the possibility to control biofilm thickness, lack of dead zones and its insensitivity to clogging (Galli, 1987).

DEGRADATION IN REACTORS

Trickling Filters

Degradation of TCE has been studied in many reactor configurations. A trickling filter packed with 6 mm Berl saddles (reactor volume = 2.16 L) was used for research conducted by the Oak Ridge National Laboratory (Strandberg et al., 1989; Garland et al., 1989). The reactor was operated continuously at a rate of 14 L/day in a downflow fashion with an average fluid retention time of 0.8 hr. The average influent TCE concentration was 1 mg/L. Experiments were constructed to allow the methane concentration in the reactor to vary (2 - 20% v/v CH₄) with the average being 4%. The remainder of the gas flow in the reactor was air (36 L/day). The degradation rate of TCE was observed to be 2.3 - 6.9 mg/L TCE per reactor day, with 13.4 mg/L per reactor day being the maximum degradation rate obtained.

The first order reaction rate coefficient ranged from $0.023 - 0.047 \text{ min}^{-1}$.

The reactor efficiency was determined by comparing the amount of methane that was utilized in the system versus the amount of TCE that was degraded. The reactor showed an efficiency of $165 - 55 \text{ g CH}_4$ used per gram of TCE degraded.

Fluidized Bed Reactors

The fluidized bed reactor was used in TCE degradation research at Cornell University (Jewell et al., 1990) and at the University of Tennessee (Phelps et al., 1990; Phelps and White, 1989; Niedzielski et al., 1989). Both of these studies indicated that the fluidized bed reactor was able to degrade TCE to concentrations asymptotically approaching $0.2 - 0.5 \text{ mg/L}$.

Experiments at Cornell University were operated in both batch and continuous modes. The total liquid flow rates for both reactor types ranged from 1.5 to 5.0 L/day . Total gas flow rate (methane plus oxygen) on the reactors was $0 - 17 \text{ L/day}$. Methane flow ranged from $0 - 8 \text{ L/day}$. The TCE degradation rate was found to be $27.6 - 852 \text{ mg/L bed day}^{-1}$ (batch) and $42 - 159 \text{ mg/L bed day}^{-1}$ (continuous). Final concentrations of TCE were $0.2 - 4 \text{ mg/L}$ (batch) and $0.9 - 2.0 \text{ mg/L}$ (continuous). The first order reaction rate coefficients were 0.175 day^{-1} (batch) and $0.1 - 1.05 \text{ day}^{-1}$ (continuous). The efficiency of the reactors with respect to CH_4 consumption was $675 - 50 \text{ g CH}_4 \text{ used/ g TCE degraded}$ (batch) and $22 - 76 \text{ g CH}_4 \text{ used/g TCE degraded}$ (continuous).

Phelps et al. (1990) looked at an expanded bed reactor that operated in batch cycles. While the average TCE inlet concentration was 20 mg/L, the medium flow rate was maintained at 20 ml/min. Batch experimental runs lasted from 120 - 216 hr with an initial methane, propane, and oxygen concentration of 10, 5, and 3% (v/v), respectively. The rate of TCE degradation was observed to be 8.5 mg/L reactor day⁻¹ and the lowest final TCE concentration achievable was 0.5 mg/L. The first order reaction rate coefficient was 0.15 day⁻¹ and the grams of methane consumed per gram of TCE degraded were 12 - 19.5. In this study it was noticed that propane, even in small amounts, stimulated conversion of TCE to degradation products and that TCE degradation occurred best at a pH = 7.2 and showed drastic deterioration at pH = 7.5.

Rotating Biological Contactor

A system of rotating biological contactors (Leahy et al., 1989) with constant head space and 32 disks was utilized in examining PCE, TCE, and cis-DCE degradation. Total reactor volume was 12 L with retention times ranging between 4.5 and 25 hr. Influent methane and oxygen concentrations for both gases were 2 - 20%. The TCE degradation rate was found to range from 0.10 - 0.22 mg/L feed day⁻¹ and the lowest achievable TCE concentration for this system was found to be 0.04 mg/L. The first order rate coefficient was 6 - 23 day⁻¹. In this system, methane utilization was found to increase when greater initial concentrations of methane and

oxygen were used, and the degradation of TCE was observed to be much faster when the initial concentration of methane was 2% rather than 15%.

Packed Bed Soil Columns

In-situ bioremediation and the use of soil columns in degradation were the focus of the work of Roberts et al. (1989) and Mayer et al. (1988) at Stanford University. The column (volume = 0.133 L) was run continuously at a liquid flow rate of 66 ml/day. The methane input was 0.0031 L/day, with the oxygen rate being 0.00104 L/day. TCE concentration was reduced by 23 - 35% in this column.

TCE contaminated water, at a flow rate of 21 cm/day, was pumped through an aerobic glass column reactor (5 cm inner diameter with length of 150 cm) packed with sandy soil. The soil system (Wilson and Wilson 1985) was exposed to natural gases and compared to a different columnar system (Wilson et al. 1981) that was also subjected to doses of TCE, but was not exposed to natural gases. The natural gas used to expose that soil column had a composition of at least 96% hydrocarbons. The hydrocarbon mixture included the following gases in order of decreasing percentages; methane, ethane, propane, n-butane, iso-butane, pentanes, and hexanes. It was found that in the column containing the hydrocarbon gases, less than 5% of the applied TCE passed through the soil. In contrast, the only evidence of TCE loss in the column that did not contain the gas mixture came as a result of volatilization.

Mayer et al. (1988) also studied the packed bed reactor to investigate TCE degradation. A glass column (40 cm by 2 cm diameter) was packed with soil from an uncontaminated shallow aquifer, and maintained under laboratory conditions that simulated the subsurface system. The initial objective of this study was to determine whether methanotrophic bacteria could be enriched from a previously uncontaminated shallow confined aquifer. After treating the column for three months with methane and oxygen, the column showed the presence of methanotrophs and demonstrated the ability to degrade TCE.

IN-SITU BIORESTORATION

Many techniques are available that remediate organic contaminated groundwater. One feasible remediation alternative is *in-situ* treatment with chemicals or microbes. *In-situ* chemical treatment uses chemicals to inactivate or immobilize the contaminant. Biological *in-situ* treatment is accomplished by stimulating the existing microbial population. Many researchers have written reviews discussing such techniques (Boutwell et al., 1986, Sims et al., 1986, Mackay and Cherry 1989, Lee et al., 1988, McCarty 1988, and Wilson et al., 1986).

In-situ bioremediation techniques were pioneered by Raymond et al. (1976). The *in-situ* bioremediation approach of halogenated aliphatic compounds requires a slightly different approach than the more studied *in-*

situ techniques where the contaminants are metabolized as primary substrates. In this case where the wastes are halogenated aliphatic compounds, the contaminants cannot be metabolized by the native bacterial population as a primary growth substrate. Such compounds are degraded cometabolically (Horvath, 1972). To degrade contaminants cometabolically, or as secondary substrates, a primary substrate, electron acceptor, plus nutrients must be added to the treatment zone. Thus, a more complex system results than one in which the contaminant is degraded as a primary substrate. Recent success in bioremediating sites contaminated with chlorinated ethenes have been reported (Roberts et al., 1991; Semprini et al., 1991; Wilson et al., 1987, Nelson et al., 1990).

MODELING SOIL SYSTEMS

The fate of liquid transport through soil systems is typically governed by three processes; convective-dispersive flux, sorption, and chemical-biological transformations. The one-dimensional partial differential equation typically termed the convective-dispersion transport equation is conventionally implemented to describe solute transport through porous media and is represented by equation (2.1) (Lapidus and Amundson, 1952):

$$-D_L \frac{\partial^2 c_i}{\partial z^2} + \frac{\partial}{\partial z} (v c_i) + \frac{\partial c_i}{\partial t} + \left(\frac{1 - \epsilon}{\epsilon} \right) \frac{\partial q_i}{\partial t} = R_i \quad (2.1)$$

where z is a spatial dimension, v is the interstitial velocity, D_{Li} is the hydrodynamic dispersion coefficient, c_i is the concentration in the fluid of a single species, and ϵ is the porosity. From left to right, the terms in equation (2.1) are defined as follows: 1. accounts for the movement of component i by dispersion, 2. describes the movement of component i by convective forces, 3. describes the change of concentration of component i in the liquid phase, 4. describes the accumulation of component i in the solid phase, and 5. accounts for the production of species i by reaction.

When the reaction term is neglected, analytical solutions for equation (2.1) are available and summarized (Ruthven, 1984). Typically, computational difficulties often occur when applying these analytical solutions.

There are several feasible approaches to solving systems of partial differential equations (PDE's) that are similar in form to equation (2.1). Viable options include; (1) using an algorithm that incorporates the methods of finite difference or finite element, (2) applying the differential volume (DVR) assumption, (3) or when reaction is too great to apply the DVR assumption, incorporate the integral volume assumption.

Biodegradation within a bioreactor system can be mathematically described by a system of convection-dispersion partial differential equations similar to equation (2.1). Such systems of equations have been used by investigators to simulate solute transport through a porous medium

describing a variety of systems which incorporate practical initial and boundary conditions. Using numerical algorithms based on finite difference or finite element techniques to solve this mathematical system has displayed a history of instability and numerical oscillations that tend to occur most frequently when the PDE's are convection dominant (Finlayson, 1980; Allen et al., 1988; Wu, 1989). Successful modeling has been achieved by several researchers. When the reaction term is neglected, numerous equilibrium and non-equilibrium transport models have been developed that represent the transport of the contaminants through porous media (Goltz and Roberts, 1986; Sleep and Sykes, 1989; Miller and Weber, 1986). Other simulations incorporating the biodegradation reaction term have been developed (Celia et al., 1989; Baveye and Valocchi, 1989; Rifai and Bedient, 1990; Yeh and Tripathi, 1991; Widdowson et al., 1988; Molz et al., 1986; Widdowson et al., 1988; Borden and Bedient, 1986; Semprini and McCarty, 1991; Wu, 1982).

The second method mentioned to solve equation (2.1) is the differential volume reactor (DVR) assumption. If applicable, the DVR assumption can simplify the system of equations and may justify the use of analytical equations to effectively describe the reactor system. The DVR assumption can be properly implemented when the reaction within the reactor is small. The broad usage of this assumption has been discussed (Levenspiel, 1972; Fogler, 1986). The error in the use of this method has

been discussed by Massaldi (1969) and Ludwig (1985). Massaldi and Maymo (1969) found that when the conversion within the reactor was less than 30%, the DVR assumption was valid. Recently this assumption has been suggested to aid in the modeling of biological systems (Bienkowski et al., 1989) and by Dunbar et al. (1989) to study the kinetics of a biological reactor capable of degrading naphthalene.

The integral method is a third possible procedure that could be rationally implemented to describe the biological soil reactor system. This method is used when conversion within the reactor is large. This procedure is developed by making a material balance for any reaction component on a cross-section of the reactor. The governing relationships in dimensionless form for this procedure is given in equation (2.2).

$$\frac{D}{vL} \frac{d^2 C_A}{dz^2} - \frac{dC_A}{dz} - k\tau C_A^n = 0 \quad (2.2)$$

Wehner and Wilhelm (1956) solved this equation analytically for first-order reaction in vessels having any inlet and outlet boundary conditions. The solution is as follows:

$$\frac{C_A}{C_{Ao}} = 1 - X_A = \frac{4a \exp(\frac{1}{2} \frac{vL}{D})}{(1 + a)^2 \exp(\frac{a}{2} \frac{vL}{D}) - (1 - a)^2 \exp(-\frac{a}{2} \frac{vL}{D})} \quad (2.3)$$

where

$$a = \sqrt{1 + 4k\tau(D/vL)} \quad (2.4)$$

Within equation (2.1), the terms relating to dispersion, adsorption and biological reaction rate can be theoretically represented or determined by a variety of methods and assumptions. The dispersion coefficient characterizes the degree of backmixing during flow. Within a packed bed reactor, this dispersion coefficient can be easily determined by utilizing a pulse injection perturbation on the system. The outlet response to the pulse could be characterized, typically as gaussian, and the mean and variance describing the outlet response can be computed. The assumed system boundary conditions control how the mean and variance are computed. Many of these cases are reported by van der Laan (1958). If the measurements are taken at least 2 or 3 particle distances inside the bed, Bischoff (1961) has shown that the open vessel boundary conditions are applicable. The open vessel boundary condition mean and variance were developed by Levenspiel and Smith (1957) and are represented as follows:

$$mean = \theta_c = 1 + 2 \frac{D}{vL} \quad (2.5)$$

$$\text{variance} = \sigma_{\theta}^2 = 2 \frac{D}{vL} + 8 \left(\frac{D}{vL} \right)^2 \quad (2.6)$$

In equation (2.6), the dimensionless group D/vL , called the vessel dispersion number, represents the amount of axial dispersion in the reactor. Thus,

$$\frac{D}{vL} \rightarrow 0 \quad \text{negligible dispersion, hence plug flow} \quad (2.7)$$

$$\frac{D}{vL} \rightarrow \infty \quad \text{large dispersion, hence mixed flow} \quad (2.8)$$

The inverse of the dimensionless group D/vL is called the Peclet Number, "Pe".

An alternate method that is capable of separating the dispersion within the system from the adsorption effects was developed by Haynes and Sarma (1973). The method utilizes simple analytic expressions for the first and second moments of the pulse response and is capable of providing a realistic representation of a packed bed. When several simplifying assumptions are made within the laminar flow regime, the resulting equation is as follows:

$$\frac{\sigma^2}{2\mu^2} \frac{L}{v} = \frac{D_L}{v^2} + \left(\frac{\epsilon}{1-\epsilon} \right) \left(\frac{R_p^2}{3D_m} + \frac{R_p^2}{15\epsilon_p D_p} + \frac{r_c^2}{15K D_c} \right) \left(1 + \frac{\epsilon}{(1-\epsilon)K} \right)^{-2} \quad (2.9)$$

From this equation, it is observed that the macropore resistances and the external mass transfer resistance are directly proportional to the square of the particle radius. Thus, if small particles are used, these terms can be considered insignificant. It is observed from equation (2.9) that if a plot is made of $(\sigma^2/2\mu^2)(L/v)$ versus $1/v^2$, a line is produced with a slope D_L and an intercept which corresponds to the total mass transfer resistance.

Adsorption characterizes the solutes accumulation at a solid interface. A common and simple method for describing adsorption is the Langmuir isotherm.

$$\frac{q}{q_s} = \frac{bc}{1+bc} \quad (2.10)$$

Although the Langmuir isotherm has many simplifying assumptions, it typically describes a system adequately when small concentration ranges are studied. When concentrations of the organics are low (solution concentration is below 10^{-5} M or below one half the compound solubility), adsorption to sediments is linear (Karickhoff et al.). Linear adsorption can be described by Henry's law, which is represented in terms of concentration as follows:

$$q = Kc \quad (2.11)$$

The Langmuir isotherm behaves like Henry's law at low concentrations. The Langmuir isotherm becomes

$$q = bq_s c$$

where bq_s represents the Henry's law constant K . In this case, q_s represents the specific number of adsorption sites available and is, therefore, temperature independent. However, the temperature dependent constant, b , should follow the vant Hoff equation:

$$b = b_o \exp\left(\frac{-\Delta H_o}{RT}\right) \quad (2.12)$$

Since adsorption is exothermic, the value of b should decrease as the temperature increases.

Most chlorinated compounds are thought to adsorb due to their hydrophobic interactions (Baughman and Paris 1981), and Henry's law constants have been established for several hydrophobic organic compounds and microorganisms. Steen and Karickhoff (1981) found that several bacterial cultures had similar partition coefficients for pyrene and phenanthrene while other investigations (Grimes and Morrision 1975, Tsezos and Seto 1986) have shown partition coefficients between pure cultures of

bacteria to vary by two orders of magnitude for chlorinated insecticides or chloroethanes.

If the adsorption isotherm is linear, analytic solutions for the concentration front and breakthrough curves can be derived. Solutions are attainable in closed form only when the boundary conditions are simple, but, if analytic solutions are available, they provide a good understanding of the behavior of the adsorption column. Levenspiel and Bischoff (1963) developed an analytical solution for the dispersed, plug flow model of adsorption in a packed bed reactor. When the column is assumed to be relatively long, the asymptotic solution of the breakthrough curve is as follows:

$$\frac{C}{C_o} = \frac{1}{2} \operatorname{erfc}\left(\frac{1 - \frac{t}{t_1}}{2\left[\frac{D_L}{vZ}\left(\frac{t}{t_1}\right)\right]^{\frac{1}{2}}}\right) \quad (2.13)$$

$$t_1 = \frac{Z}{v} \left\{ 1 + K\left(\frac{1-\epsilon}{\epsilon}\right) \right\} \quad (2.14)$$

The biological reaction rate, represented by R_i in equation (2.1), can be portrayed in a variety of ways. The form most often used to represent chemical reaction rate can also be used to predict biological reaction as is illustrated in equation (2.15).

$$-r_A = -\frac{dC_A}{dt} = k C_A^\alpha C_B^\beta \quad (2.15)$$

Several models have been proposed to describe systems where secondary substrates are oxidized by cometabolism (no energy for growth). One of the simplest models has been expressed by McCarty (1984) in the following manner:

$$\frac{dC_2}{dt} = -\frac{k}{K_s} X_a C_2 \quad (2.16)$$

where C_2 is the concentration of the secondary substrate, X_a is the microorganism concentration, and k/K_s is a ratio of constants that represents a second-order rate constant. K_s depicts the organisms' affinity for the substrate and is called the half velocity constant (mass/volume). The constant k (time^{-1}) represents the maximum specific substrate utilization rate per unit mass of microorganisms per unit time.

The Monod kinetic model has also been used to describe the transformation rate of the secondary compound to its concentration in solution (McCarty, 1984):

$$\frac{dC_2}{dt} = -X_A k_2 \left(\frac{C_2}{K_{s2} + C_2} \right) \quad (2.17)$$

where C_2 is the concentration of the cometabolized contaminant in the solution (mg/L), k_2 is the maximum rate of contaminant transformation (mg of C_2 (mg of cells) $^{-1}$ day $^{-1}$), X_A is the active microbial concentration, and K_{s2} is the half-velocity constant (mg/L).

The rate of cometabolic degradation for resting cells (organisms in the absence of a primary growth substrate), was given by Alvarez-Cohen and McCarty (1991a) as follows:

$$\frac{dC_2}{dt} = - \frac{k (X_o - \frac{1}{T_c} (C_{20} - C_2)) C_2}{(K_s + C_2)} \quad (2.18)$$

where C_{20} is the initial concentration of cometabolized contaminant (mg/L), C_2 is the concentration of contaminant at time t , and T_c is the transformation capacity coefficient that represents the maximum mass of cometabolized compound that can be transformed per unit mass of resting cells (Alvarez-Cohen and McCarty, 1991b).

An equation developed by Bailey and Ollis (1986) also describes cometabolism as the competitive inhibition between a secondary substrate and the electron donor:

$$\frac{dC_2}{dt} = -X_A k_2 \left(\frac{C_2}{K_{S2} + C_2 + \frac{K_{S2}}{K_{SD}} C_D} \right) \quad (2.19)$$

Other examples of rate equations are seen in equations (2.20) and (2.21).

These equations describe the reactions of two different substrates catalyzed by one enzyme (Bailey and Ollis, 1986).

$$-\frac{dC_A}{dt} = r_A = \frac{k_1 e_o C_A / K_A}{1 + C_A / K_A + C_B / K_B} \quad (2.20)$$

$$-\frac{dC_B}{dt} = r_A = \frac{k_B e_o C_B / K_B}{1 + C_A / K_A + C_B / K_B} \quad (2.21)$$

CHAPTER III

EXPERIMENTAL METHODS

SINGLE-PASS PACKED-BED REACTOR SYSTEM

A single-pass packed-bed bioreactor system, illustrated in Figure 3.1, consisted of two borosilicate-glass columns (Sectrum Medical Industries, Inc., Los Angeles, CA). The two columns (ID = 5 cm, length = 100 cm, total volume = 1960 ml) were sealed using stainless steel end caps equipped with teflon O-rings. These columns provided a closed system. The lower column was inoculated with a TCE degrading consortia and 50 - 70 mesh white quartz sand (Sigma Chemical Co., St Louis, MO). The upper column provided oxygen, methane, and propane to the packed-bed on a recirculating basis (Figure 3.1). The gases were pumped using an FMI lab pump model RP-G400 (Fluid Metering, Inc., Oyster Bay, NY) that distributed the gases to three equally spaced regions on the bottom of the packed bed. A 3% hydrogen peroxide solution was pumped by a Gilson Miniplus 2 peristaltic pump at the rate of 1-2 ml/hr as an additional electron acceptor source. The flow was evenly distributed to three regions in the bottom of the reactor bisecting the gas flow inlets. The top of the packed-bed column was equipped with a gas outlet stream that recirculated into the upper column. All column connections were made with Swagelok® fittings.

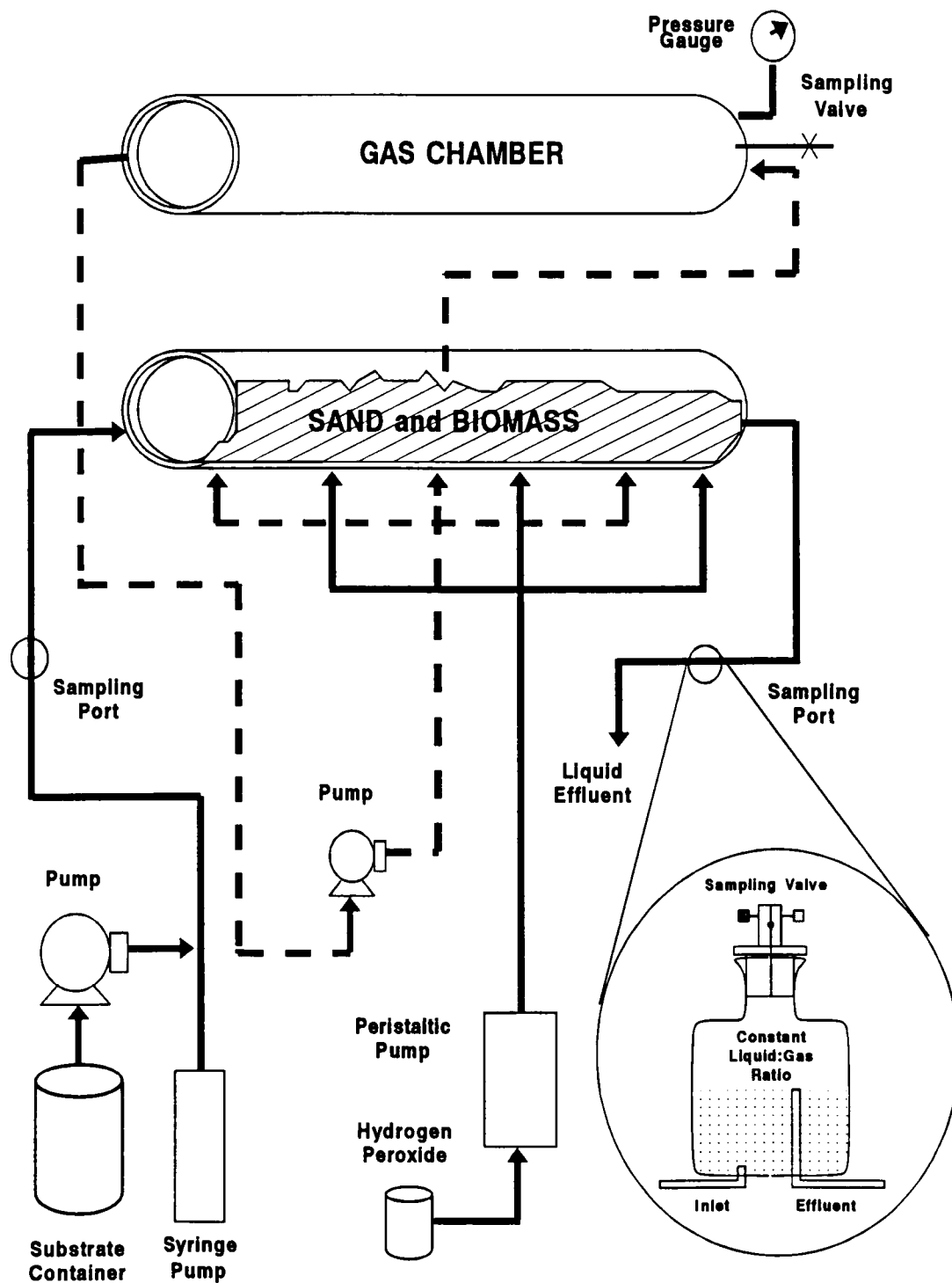


Figure 3.1 Single-Pass Packed-Bed Bioreactor System with Sampling Port Inset

Chlorinated hydrocarbons were introduced into the reactor by an ISCO, model 314, high pressure syringe metering pump equipped with a pressure monitor model 1590 (ISCO Instrument Specialties Co., Lincoln, Nebraska). The syringe pump, having no headspace, allowed a constant concentration of the contaminants to enter the reactor. Medium recipes are shown in Tables 3.1, a through e. Phosphate/Bicarbonate Buffered Medium, PBBM, (recipe shown in Table 3.1, a.) was added to the reactor by the use of a Beckman model 11A pump. Medium and the organic contaminants were mixed in a tee and the mixture initially entered a glass sampling port. The inlet and effluent sampling ports, detailed in Figure 3.1, allowed for a constant liquid to gas ratio, from which GC gas headspace analyses were performed. A Teflon Mininert valve (Scientific Products, McGaw Park, IL) was attached to the glass sampling port providing a leak tight sampling point. Liquid samples were taken from the effluent sampling port for oxygen concentration analysis. An MI-730 Micro-Oxygen Electrode equipped with an OM-4 oxygen meter (Microelectrodes, Inc., Londonderry, NH) was used to determine the concentration of oxygen in the effluent. All tubing connections in the reactor system were of stainless-steel. Connections to the inlet and outlet sampling ports utilized organic resistant Viton tubing (Cole-Parmer, Chicago, Illinois). The columns were covered with black sheeting to reduce growth of photosynthetic microorganisms and to prevent photolysis of the chlorinated compounds.

Prior to each degradation experiment, the inlet and outlet of the bioreactor were sealed and the reactor was pressurized to 3 psi. The system was able to hold pressure for the 24 hr test period allotted at the beginning of the run.

Table 3.1 Medium recipes

a. PBBM	
Compound	Amount Added
MgSO ₄	0.055 g
CaSO ₄ ·2H ₂ O	0.054 g
NaNO ₃	0.50 g
Trace Minerals II	10 ml
10x Vitamin Solution	1 ml
200-mM phosphate bicarbonate buffer	20 ml
distilled H ₂ O	1000 ml

Table 3.1, Medium Recipes, continued

b. Trace Mineral II Solution	
Compound	mg/L
Nitrilotriacetic acid	1500
$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	200
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	100
Na Tungstate	20
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	100
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	100
ZnCl_2	50
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	2
H_3BO_3	5
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1000
Na Molybdate	10
NaCl	1000
Na_2SeO_3	17
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	24

Table 3.1, Medium Recipes, continued

c. Vitamin Solution	
Compound	grams
Biotin	0.002
Folic Acid	0.002
B ₆ (pyridoxine) HCl	0.010
B ₁ (thiamine) HCl	0.005
B ₂ (riboflavin)	0.005
Nicotinic Acid (niacin)	0.005
Pantothenic Acid	0.005
B ₁₂ (cyanocobalamine crystalline)	0.0001
PABA (P-aminobenzoic acid)	0.005
Lipoic acid (thioctic)	0.005
Distilled H ₂ O	1000 ml

Table 3.1, Medium Recipes, continued

d. Luria Broth (LB)	
Compound	grams
Bacto Tryptone	10
yeast extract	5
NaCl	10
Distilled H ₂ O	1000 ml

Add NaOH to bring ph to 7

Table 3.1, Medium recipes, continued

e. Modified Trace Mineral Media	
Compound	Amount Added
MgSO ₄	0.055 g
CaSO ₄ ·2H ₂ O	0.054 g
NaNO ₃	1.0 g
Trace Mineral II	10 ml
10x Vitamin Solution	1 ml
Pyruvic acid	0.03 g
Trizma Base	6.05 g
CaCl ₂ ·2H ₂ O	8.0 g
Distilled H ₂ O	1000 ml

Add CaCl₂·2H₂O by sterile filterization after autoclaving.

DIFFERENTIAL VOLUME REACTOR (DVR) SYSTEM

The DVR system was designed to simulate a section of an ideal plug flow reactor (Webb et al., 1991). Figures 3.2 and 3.3 represent a cut away view of the reactor while assembled and expanded, respectively. A complete description of this reactor can be found in Webb et al., 1991. In this investigation, the reactor was utilized in biotic and abiotic experiments.

Trichloroethylene biodegradation and abiotic studies were performed utilizing the DVR. A system was designed, and is illustrated in Figure 3.4, incorporating the DVR. The system was equipped with two Millipore stainless steel substrate containers rated to 690 kPa. The feed from the substrate vessels to the reactor inlet was controlled by two Gilson 301 HPLC pumps. The medium used for these experiments was PBBM, previously shown in Table 3.1, a. The reactor effluent entered a 1/4 in. stripping column packed with 3mm glass beads to provide adequate surface area for the volatile separation process. The volatiles were stripped with helium in a cocurrent fashion through the stripper. The outlet of the stripping column was attached directly to the GC/ECD (Hewlett-Packard 5890 Series II) by a heated sampleline. Automatic injections were made on the GC by supporting computer and software (Hewlett Packard computer, HP Chem Station software). This system was equipped with a bypass line around the reactor in order to calibrate the stripping column. Liquid sampling ports for measuring oxygen concentrations were available on the

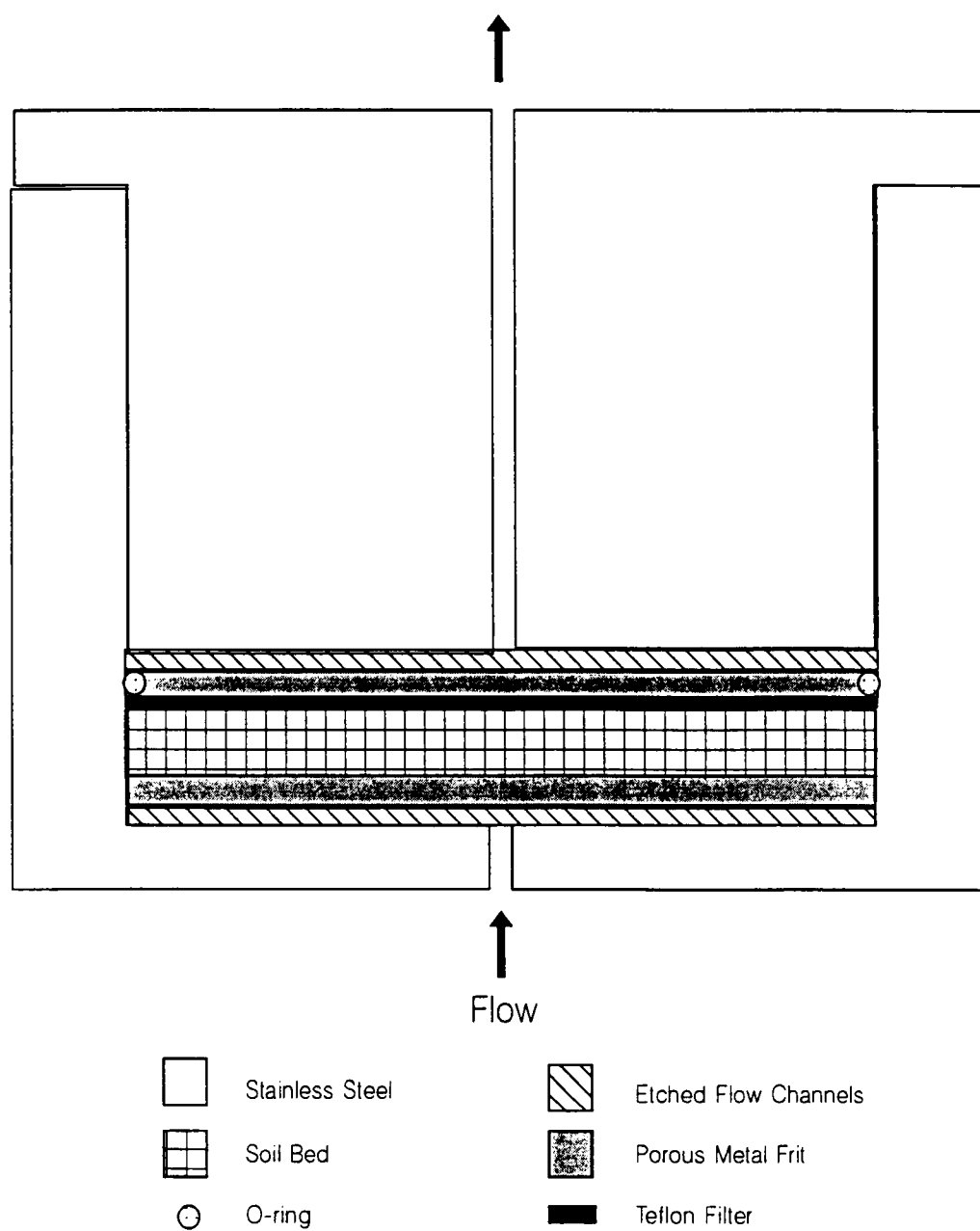


Figure 3.2 Cut Away View of the DVR (Assembled View)

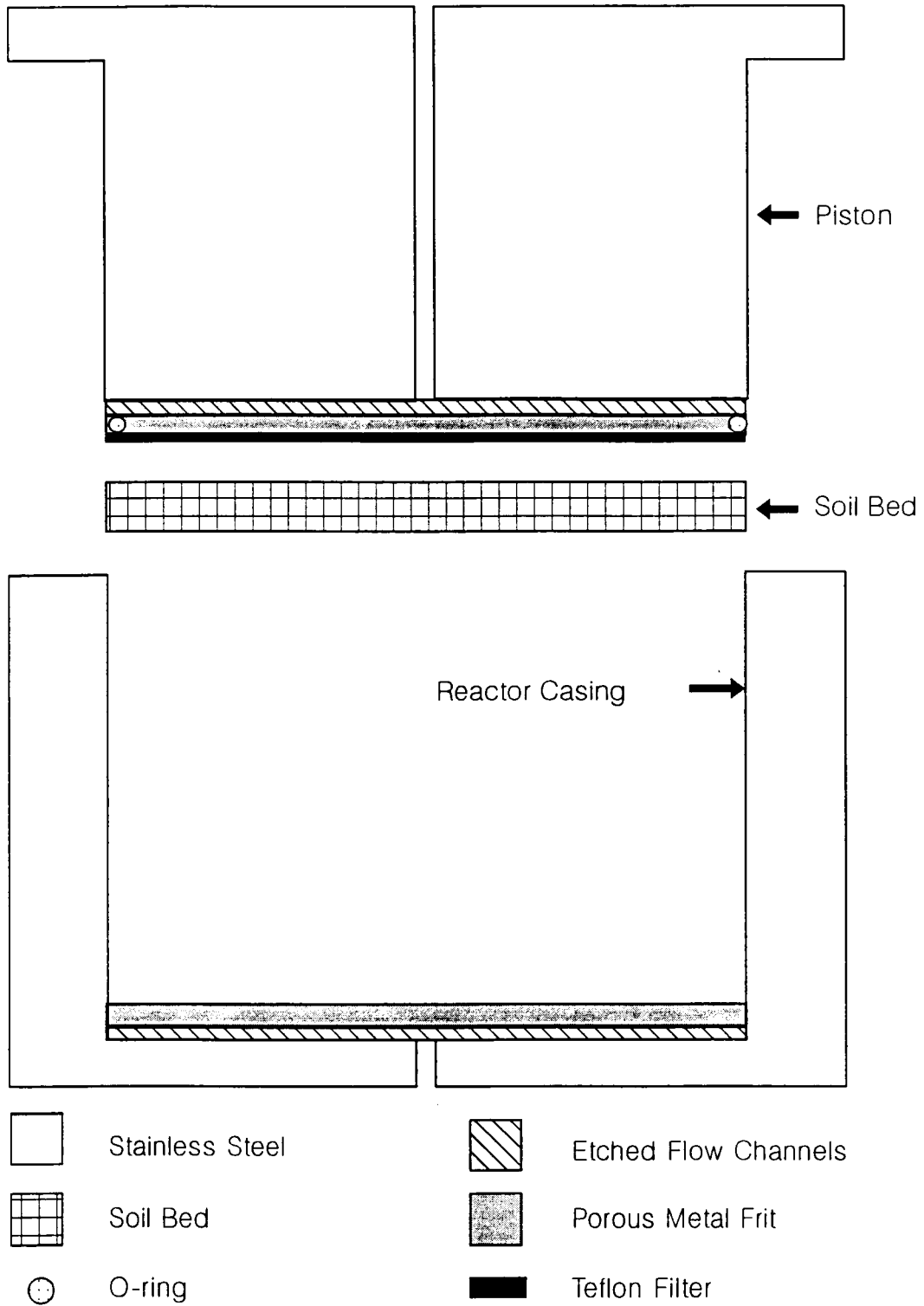


Figure 3.3 Cut Away View of the DVR (Expanded View)

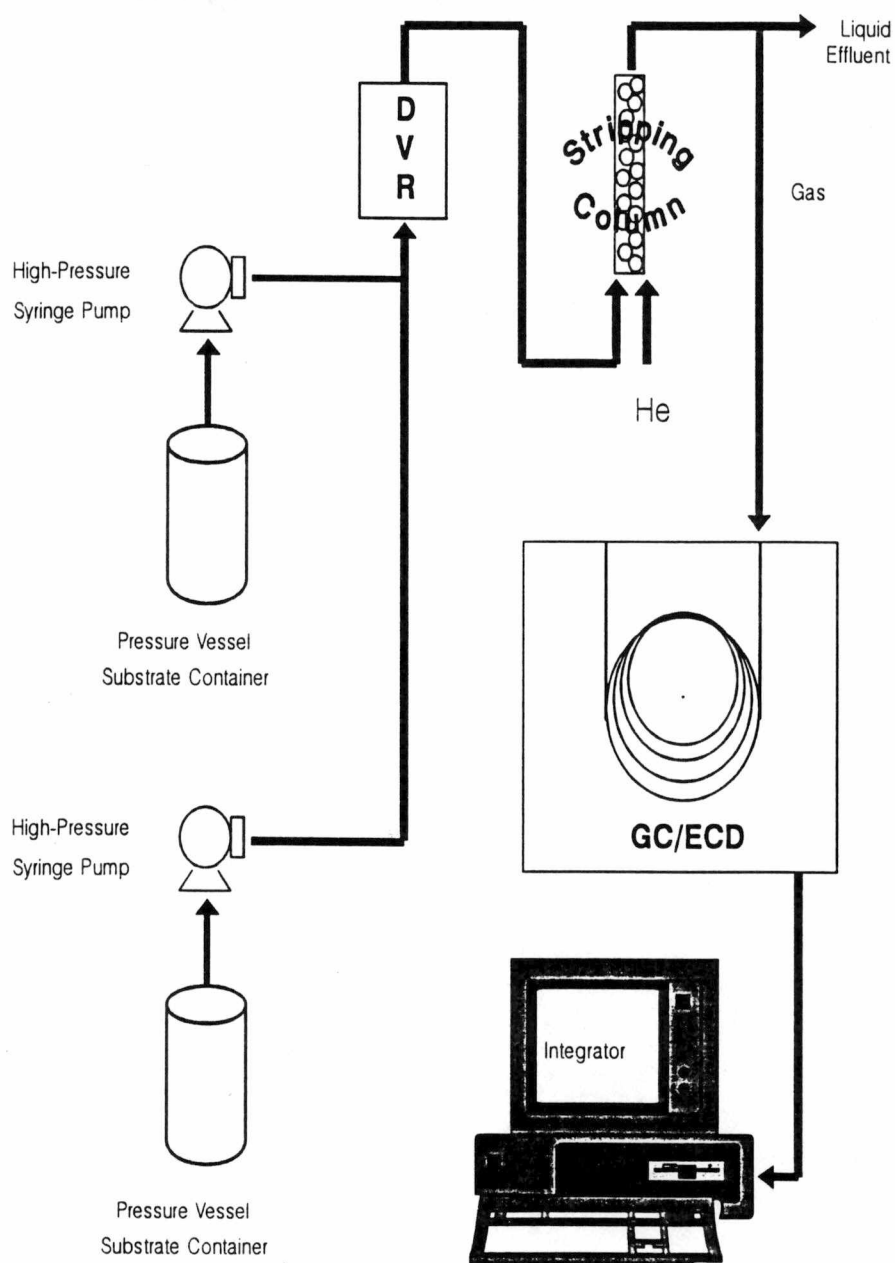


Figure 3.4 Differential Volume Reactor System

inlet and outlet lines of the system. This degradation DVR system was used in systems where soil or Sigma sand was utilized as the biomass support matrix.

BIOLUMINESCENCE DVR SYSTEM

A similar system, as viewed in Figure 3.5, was utilized to study the bioluminescence response of *Pseudomonas putida* F1 (Wackett and Gibson, 1988), which is capable of TCE degradation and has been engineered to contain the lux gene cassette. This system incorporates or traps the bacteria in an alginate bead (Sigma) matrix. The system differs from the previous DVR system by the addition of a photomultiplier and a computer to collect the bioluminescence data. This system used a two pump scheme to provide TCE and medium (trace mineral medium; refer to Table 3.1, e.) in a continuous fashion into the DVR while toluene was introduced using a step perturbation. The flow rate through the reactor was 0.4 ml/min with inlet TCE and toluene concentrations being 20 and 10 mg/l respectively. As in the above DVR system, effluent volatiles from the reactor are stripped by helium in a cocurrent fashion through a packed stripping column. A sampling port affixed to the gas line running to the GC/ECD is available for the extraction of gas phase samples. The sampling port is a 1/8" Swagelok® ball valve having one end attached to the effluent line and the other equipped with a teflon septa fitting. Use of the septa allows minimal leaking while valve is opened as closed during a

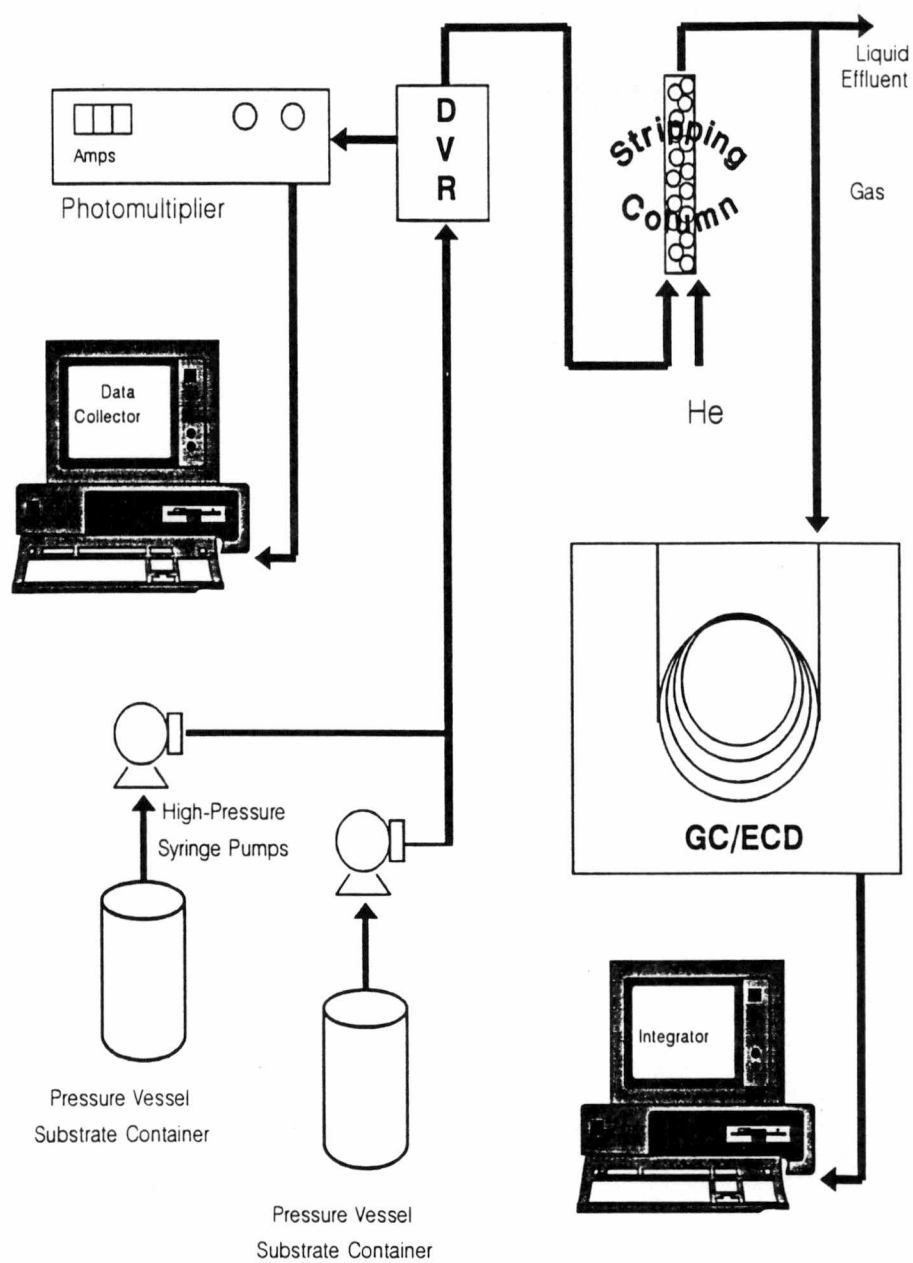


Figure 3.5 Bioluminescent Differential Volume Reactor System

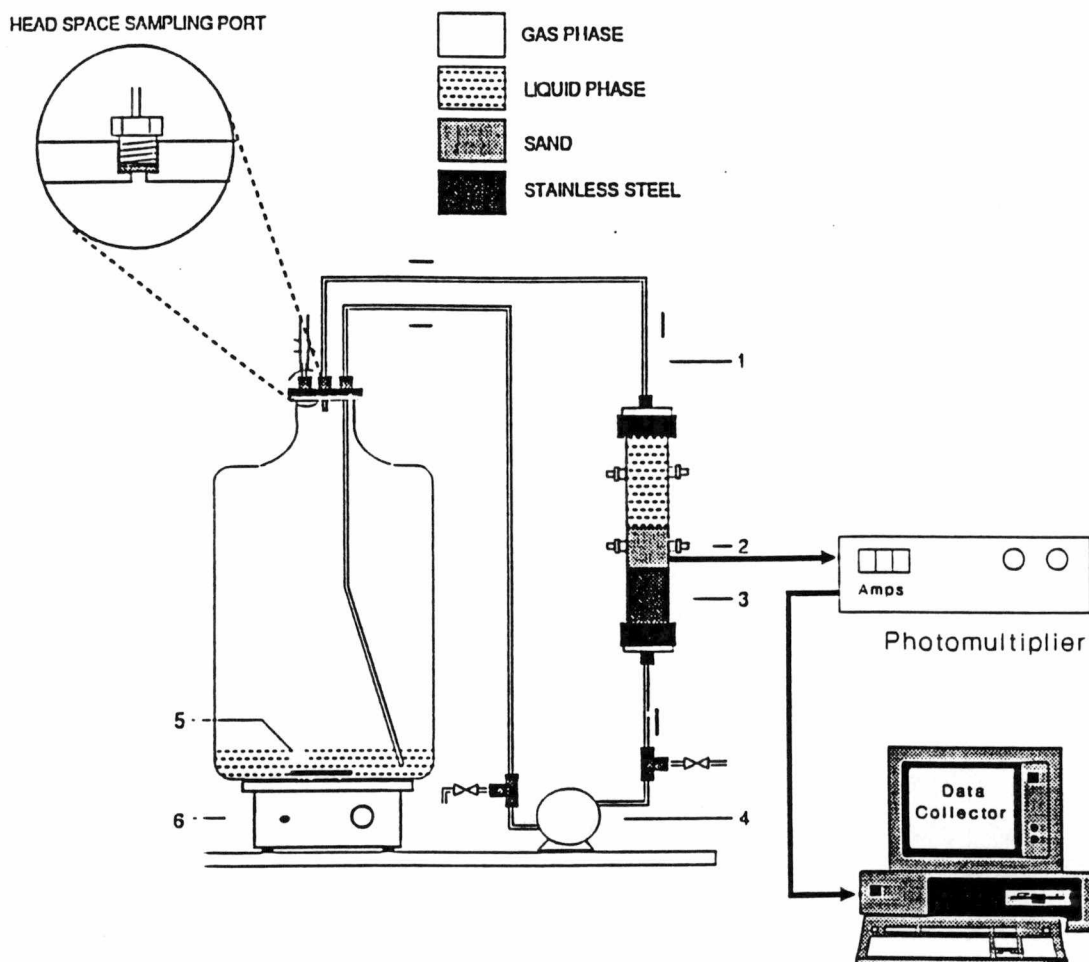
sampling period. Volatiles, such as toluene, that are not readily detectable on the GC/ECD are removed with a gas tight syringe from the port and manual injections are performed on the GC equipped with an FID detector needed for compound detection.

ALGINATE IMMOBILIZATION OF BIOREPORTER B2

The frozen culture of the bioreporter strain *P.putida* F1 was used to inoculate 1 liter of LB media (refer to Table 3.1, d.) containing 50 mg/L tetracycline and was allowed to grow while continuously shaking at 27°C and a pH of 7. The culture was harvested when the OD₅₄₆ was 1.0 and washed three times in a 0.9% NaCl solution. Washing consisted of the centrifugation of the cells and then resuspension in the NaCl solution. After washing, the cells were resuspended in the 0.9% NaCl mixture and a low viscosity alginate solution in a 1:2 ratio by volume, respectively. The alginate solution consisted of 4.2 g low viscosity alginate in 150 ml 0.9% NaCl solution. The alginate cell suspension beads were formed by using a syringe to drop-wise add the cell/alginate mixture to a 0.1 M CaCl₂ solution. The formed beads were stirred for 30 min. before adding to the DVR reactor. This immobilization technique was developed by Webb, 1992.

EXPANDED-BED BIOREACTOR

The total-recycle expanded-bed bioreactor was developed by V. Korde in 1992. Further explanation of the design and operation of this reactor can be found in Korde et al., 1993. The reactor was based on a design previously developed by Niedzielski et al., 1989. Figure 3.6 is a schematic of the reactor system. The expanded-bed portion of the reactor system utilized sand as a solid support matrix for the TCE degrading consortia. The column was a 30 x 5 cm borosilicate glass chromatography column (Spectrum Medical Industries, Inc., Los Angeles, CA) sealed with stainless steel end-caps equipped with teflon o-rings. A 2.5 gal. glass carboy served as a gas-recharge vessel. The carboy was sealed with a $\frac{3}{8}$ " stainless steel plate equipped with a teflon o-ring and fastened by a clamp. A teflon coated stir bar spinning at a high rate of speed provided high gas-liquid mass transfer rates of oxygen, methane, and propane into the liquid medium. Stainless steel fitting were available on the steel plate covering the carboy that allowed the expanded-bed reactor to be joined with the gas recharge unit. Ports were also available on the plate to provide a method of semi-continuous headspace sampling. An Oriel digital display (model 7070) photomultiplier and a liquid light cable were connected to the reactor to measure the bioluminescent response of the bioreporter in the sand phase.



Schematic of Total-Recycle Expanded-Bed Bioreactor

- 1 - 1/8" stainless steel tubing
- 2 - Solids sampling port with Mininert valves
- 3 - Stainless steel lower end cap
- 4 - Metering pump
- 5 - Stir bar
- 6 - Stir plate

Figure 3.6 Schematic of Total-Recycle Expanded-Bed Bioreactor System

BIOLUMINESCENT BATCH VIAL EXPERIMENTS

An overnight culture prepared from a frozen stock of bioreporter strain *Pseudomonas putida* F1 (B2) was grown in a two liter flask containing 1000 ml of LB media (refer to Table 3.1, d.) and 50 mg/L tetracycline for the positive selection of the *lux* transposon. The culture was grown and maintained at 27°C and at a pH of 7. From this culture, 30 ml was transferred to one liter of fresh medium as described above. The culture was harvested when growing exponentially and was at an OD₅₄₆ of approximately 0.175. Once reaching the desired OD₅₄₆, the culture was transferred to triplicate test vials. The vials were sealed and continuously agitated. The desired solvents were added to the vials and the emitted light was measured in 15-min intervals with an Oriel digital display (model 7070), and a liquid light cable. After a 1 hr period, the final OD₅₄₆ of all the test cultures in the vials were taken.

ANALYTICAL PROCEDURES

A Hewlett-Packard 5890A capillary gas chromatograph with a split-splitless injector was used to analyze the chlorinated hydrocarbons during both manual and automated injections. The GC was equipped with an electron capture detector (Hewlett-Packard) and a cross-linked methyl silicone capillary column (internal diameter of 0.2 mm, 0.33 μ m film thickness). The oven temperature was operated isothermally at 60°C.

Other operating parameters include the injection temperature of 150°C and the detector temperature of 200°C. The split ratio was set at 10:1. Helium was used as the carrier gas and nitrogen as the makeup gas.

Methane and carbon dioxide percentages were analyzed using a Shimadzu GC-8A gas chromatograph. The GC was equipped with a 2.74m, 3.2mm diameter Carbosieve 8000 packed column and a thermal conductivity detector. Both the injector and detector temperatures were 140°C, while the oven was operated isothermally at 130°C. Propane was analyzed using a Shimadzu GC-9A gas chromatograph equipped with a 2.44 m, 3.2 mm diameter Poropak N packed column and a flame ionization detector. The isothermal temperature of the oven was 80°C and the detector and injector temperature were 220°C.

The concentration of methane, propane, and carbon dioxide were determined by comparing peak heights of prepared gas standard calibration curves to samples taken from the reactor. For halogenated compounds, concentrations were determined by comparing peak areas integrated by using a Nelson 2600 analytical chromatography system with version 4.0 (Nelson Systems, Cupertino, CA). Standard curves were obtained using the same liquid to gas ratio used in the experimental reactors or batch vial systems. By using a constant ratio, this allowed for the direct calculation of volatile compound concentrations in the liquid phase by implementing Henry's Law.

A Waters model 431 conductivity detector (Waters, Milford, Mass.) was used for bromide ion detection. The eluent used was lithium borate/gluconate in conjunction with a Waters IC-PAK anion column. A Waters model 510 HPLC pump transported the eluent through the system at a rate of 1.2 ml/min. A Waters model WISP 710B intelligent sample processor was used as an automatic injection module for this high performance liquid chromatography (HPLC) system. The system held a maximum of 48 samples and could inject a 1 to 2000 μ l sample size.

CHAPTER IV

**BIODEGRADATION OF CHLORINATED ALIPHATIC
HYDROCARBON MIXTURES IN A SINGLE-PASS
PACKED-BED REACTOR**

ABSTRACT

Aliphatic chlorinated compounds such as trichloroethylene (TCE) and tetrachloroethylene (PCE) are major contaminants of groundwater. A single-pass packed-bed bioreactor was utilized to study the biodegradation of organic waste mixtures consisting of PCE, TCE and other short-chain chlorinated organics. The bioreactor consisted of two 1960 ml glass columns joined in series. One column was packed with sand containing a microbial consortia enriched from a contaminated site. The other column provided a reservoir for oxygen and a carbon source of methane/propane that was recirculated through the reactor. Sampling was accomplished by both direct headspace and liquid effluent concentration analyses. The reactor was operated in a single-pass mode. Greater than 99% degradation of trichloroethylene, approaching drinking water standards, was observed when the bioreactor residence time ranged from 1.9 to 3.2 days. Typically, when the reactor was pulse-fed with methane, propane, and air, 1 mole of TCE was degraded per 110 moles of substrate utilized. Perturbation studies were performed to characterize reactor behavior. The system's degradation

behavior was affected by providing different carbon sources, a pulse feeding regime, supplementing microbial biomass, and by altering flow rates.

INTRODUCTION

Chlorinated short-chain aliphatic compounds such as trichloroethylene (TCE), tetrachloroethylene (PCE), and dichloroethylene (DCE) are some of the most prevalent contaminants of groundwater (Vogel et al. 1987). The National Priority List of the U.S. Environmental Protection Agency lists TCE as the most frequently reported contaminant at hazardous waste sites (Little et al. 1988). Recently, EPA has determined these halogenated organics to be carcinogenic and regulation on their existence in groundwater is close to analytically detectable limits.

Typically, these compounds cannot be removed from groundwater using conventional water treatment techniques such as coagulation, sedimentation, or filtration (Wood & DeMarco, 1980). Instead, chlorinated aliphatics are generally removed by air stripping and adsorption (Love & Eilers, 1982). Unfortunately, these methods of removal have not destroyed the dangerous compounds, but have just moved them from one phase to another. An alternate method for removing these aliphatic compounds from water is biodegradation. Biodegradation has shown the potential to transform toxic compounds to non-toxic compounds such as carbon dioxide, water, bases, salts, and inorganic acids.

The ability to degrade TCE has been observed in both pure cultures and mixed microbial consortia (Vogel et al., 1987; Little et al., 1988; Colby et al., 1977; Fliermans et al., 1986; Fogel et al., 1986; Nelson et al., 1987; Phelps & White 1989; Strandburg et al., 1989; Vandenberg & Kunka 1988; Wackett & Gibson 1988; Wilson & Wilson 1985). Methanotrophs show the ability to degrade TCE and other chlorinated compounds (Little et al., 1988; Colby et al., 1977). By utilizing methane monooxygenase, the enzyme which normally oxidized methane to methanol, the contaminant is oxidized and water soluble TCE breakdown products are utilized. Fortunately, most of the breakdown products can be metabolized by heterotrophic bacteria in mixed methane-enriched cultures. Other oxygenases, such as those formed during the oxidation of propane (Fliermans et al., 1988; Wackett et al., 1989), toluene and other aromatics (Wackett & Gibson, 1988; Nelson et al., 1986), and ammonia (Arciero et al., 1989), have been found that are capable of contributing to TCE degradation.

TCE degradation has also been observed in cultures of *Pseudomonas* that are typically associated with aromatic degradative pathways that utilize *meta* fission (Wackett & Gibson, 1988; Winter et al., 1989; Folson et al., 1990). This aromatic pathway suggests that the toluene dioxygenase enzyme is responsible for degradation. Toluene or phenol was required to induce TCE degradation (Nelson et al., 1986). Anaerobic studies have clearly shown biotransformation of PCE and TCE. In all cases, the

transformation is the result of a reductive dehalogenation (Vogel & McCarty, 1985; Bower & McCarty, 1983; Kleopfer et al., 1985; Fathepure et al., 1987). Under anaerobic conditions, the rate of dechlorination decreases as chlorine molecules are removed (Vogel & McCarty, 1985).

Although degradation of the chlorinated organics is possible, there is a minimum concentration to which a single organic material can be decomposed under steady-state conditions (Stranderg et al., 1989; Phelps et al., 1990; Phelps et al., 1991). Biodegradation of such trace compounds can occur in general only if they are used as secondary substrates. The kinetics of degradation of low concentrations of mixed wastes is relatively unknown. Little knowledge has been assessed concerning the interaction of the primary and secondary substrate or of the impact sorption has on the ability to degrade trace organics.

The purpose of this study was to further explore and understand the cometabolic degradation abilities of microorganisms biodegrading mixed wastes in a packed-bed reactor. The reactor was seeded with a microbial community which exhibited the ability to degrade TCE when fed methane and/or propane under various conditions.

MATERIALS AND METHODS

Gases and chemicals

National Welders (Charlotte, NC) supplied all gases. Methane was greater than 98% pure, and oxygen and propane were greater than 99.5% pure. All chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) or from Mallinckrodt, Inc. (Paris, KY). Glass distilled solvents and reagents were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ).

Bacterial culture

The consortium used in seeding the bioreactor was obtained from several sites. A TCE degrading consortium capable of degrading concentrations greater than 100 mg/L was isolated from the Savannah River Plant, Aiken SC (Fliermans et al., 1988). Methane- and propane-utilizing consortium from Ada, OK was added to the reactor (Nichols et al., 1985) along with a methanotroph isolate from a waste disposal site near Oak Ridge, TN.

Bioreactor configuration

The single-pass packed-bed bioreactor system, illustrated in Figure 3.1, consisted of two borosilicate-glass columns (Sectrum Medical Industries, Inc., Los Angeles, CA). The two columns (ID = 5 cm, length = 100 cm, total volume = 1960 ml) were sealed using stainless steel end caps equipped with teflon O-rings. These columns provided a closed

system. The lower column was inoculated with the consortia mentioned above and 50 - 70 mesh white quartz sand (Sigma Chemical Co., St Louis, MO). The upper column provided oxygen, methane, and propane to the packed-bed on a recirculating basis (Figure 3.1). The gases were pumped using an FMI lab pump model RP-G400 (Fluid Metering, Inc., Oyster Bay, NY) that distributed the gases to three equally spaced regions on the bottom of the packed bed. A 3% hydrogen peroxide solution was pumped by a Gilson Miniplus 2 peristaltic pump at the rate of 1-2 ml/hr as an additional electron acceptor source. The flow was evenly distributed to three regions in the bottom of the reactor bisecting the gas flow inlets. The top of the packed-bed column was equipped with a gas outlet stream that recirculated into the upper column. All column connections were made with Swagelok® fittings.

Chlorinated hydrocarbons were introduced into the reactor by an ISCO, model 314, high pressure syringe metering pump equipped with a pressure monitor model 1590 (ISCO Instrument Specialties Co., Lincoln, Nebraska). The syringe pump, having no headspace, allowed a constant concentration of the contaminants to enter the reactor. Medium was added to the reactor by the use of a Beckman model 11A pump. Medium and the organic contaminants were mixed in a tee and the mixture initially entered a glass sampling port. The inlet and effluent sampling ports, detailed in Figure 3.1, allowed for a constant liquid to gas ratio, from which GC gas

headspace analyses were performed. A Teflon Mininert valve (Scientific Products, McGaw Park, IL) was attached to the glass sampling port providing a leak tight sampling point. Liquid samples were taken from the effluent sampling port for oxygen concentration analysis. An MI-730 Micro-Oxygen Electrode equipped with an OM-4 oxygen meter (Microelectrodes, Inc., Londonderry, NH) was used to determine the concentration of oxygen in the effluent. All tubing connections in the reactor system were of stainless-steel. Connections to the inlet and outlet sampling ports utilized organic resistant Viton tubing (Cole-Parmer, Chicago, Illinois). The columns were covered with black sheeting to reduce growth of photosynthetic microorganisms and to prevent photolysis of the chlorinated compounds.

Prior to each degradation experiment, the inlet and outlet of the bioreactor were sealed and the reactor was pressurized to 3 psi. The system was able to hold pressure for the 24 hr test period allotted at the beginning of the run.

Medium

The medium entering the reactor system contained the following: MgSO_4 , 0.055 g; $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.054 g; NaNO_3 , 1.0 g; trace minerals II (Lynd et al., 1982), 20 ml; 10x vitamin solution (Lynd et al., 1982), 1 ml; 200-mM phosphate/bicarbonate buffer solution, 30 ml; nanopure-filtered water, 1000 ml. The pH of the medium was kept at 7.0.

Analytical procedures

A Hewlett-Packard 5890A capillary gas chromatograph with a split-splitless injector was used to analyze the chlorinated hydrocarbons. The GC was equipped with an electron capture detector (Hewlett-Packard) and a cross-linked methyl silicone capillary column (internal diameter of 0.2 mm, 0.33 μ m film thickness). The oven temperature was operated isothermally at 60°C. Other operating parameters include the injection temperature of 150°C and the detector temperature of 200°C. The split ratio was set at 10:1. Helium was used as the carrier gas and nitrogen as the makeup gas.

Methane and carbon dioxide percentages were analyzed using a Schimadzu GC-8A gas chromatograph. The GC was equipped with a 2.74 m, 3.2 mm diameter Carbosieve 8000 packed column and a thermal conductivity detector. Both the injector and detector temperatures were 140°C, while the oven was operated isothermally at 130°C. Propane was analyzed using a Schimadzu GC-9A gas chromatograph equipped with a 2.44 m, 3.2 mm diameter Poropak N packed column and a flame ionization detector. The isothermal temperature of the oven was 80°C and the detector and injector temperatures were 220°C.

The concentration of methane, propane, and carbon dioxide were determined by comparing peak heights of prepared gas standard calibration curves to samples taken from the reactor. For halogenated compounds, this was done using a Nelson 2600 analytical chromatography system with

version 4.0 (Nelson Systems, Cupertino, CA). Standard curves were obtained using the same liquid to gas ratio found in the reactor system. By using a constant ratio, this allowed for the direct calculation of volatile compound concentrations in the liquid phase by implementing Henry's Law.

A Waters model 431 conductivity detector (Waters, Milford, Mass.) was used for bromide ion detection. The eluent used was lithium borate/gluconate in conjunction with a Waters IC-PAK anion column. A Waters model 510 HPLC pump transported the eluent through the system at a rate of 1.2 ml/min. A Waters model WISP 710B intelligent sample processor was used as an automatic injection module for this high performance liquid chromatography (HPLC) system. The system held a maximum of 48 samples and could inject 1 to 2000 μ l samples.

RESULTS AND DISCUSSION

Abiotic Results

Residence time distribution (RTD). The non-ideality of the bioreactor's flow behavior was defined by pulse injection perturbations. Potassium bromide served as the inert tracer in the RTD study. The reactor portrayed a typical Gaussian-shaped distribution curve (Figure 4.1). Appendix A contains a sample complete dataset in Table A.1. Pulse perturbation experiments were conducted at different flow rates. These abiotic experiments provided information on the sand column's residence time

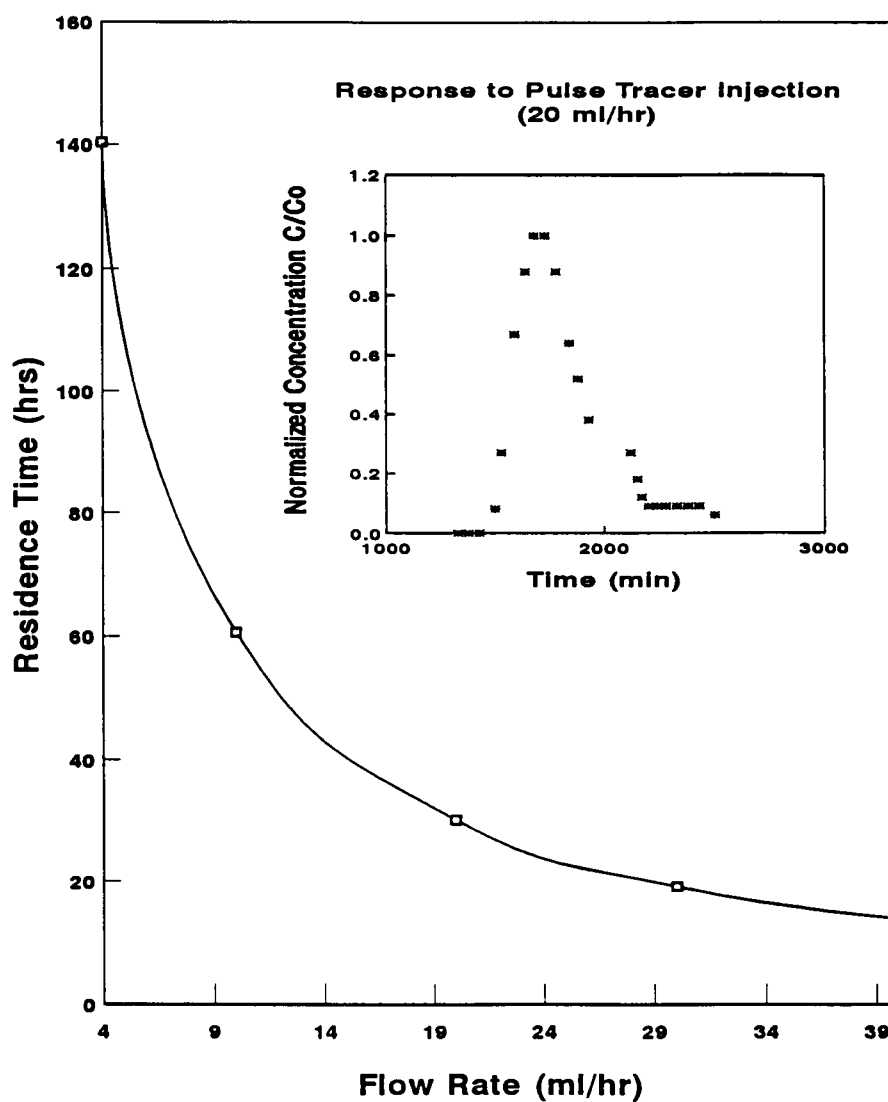


Figure 4.1 Bioreactor Residence Time Distribution with Pulse Tracer Injection Response

distribution, Peclet number, and the void volume (porosity) within the reactor bed. Figure 4.1 represents the reactor's residence time distribution. The residence time was defined as the time that corresponded to the peak of the dimensionless effluent concentration (effluent concentration/feed concentration). The calculated average reactor dispersion coefficient was $3.9 \times 10^{-3} \text{ cm}^2/\text{s}$, and the void volume was determined as $\epsilon = 0.42$.

Biotic Results

The bioreactor exhibited the ability to degrade greater than 99% of the TCE introduced into the reactor. Although this phenomenon did not occur during each experiment, the effluent from the bioreactor approached drinking water quality when the residence time was ≥ 1.9 days. Table 4.1 summarizes the typical results for TCE biodegradation. It should be noted that greater than 99% degradation was observed in both the starvation and feeding regimes. Because drinking water quality was not always attainable within the system, different perturbations were made on the system in order to optimize the degradation process. Data from these experiments are presented.

Pulsed feeding. Table 4.2 compares degradation of waste mixtures including trichloroethylene (TCE), chloroform, 111-trichloroethane (111-TCA), 112-trichloroethane (112-TCA), and tetrachloroethylene (PCE). During the period of continuous feeding, methane and propane were always present in the reactor. During the starvation period, no energy source was

Table 4.1 TCE Degradation Approaching Drinking Water Quality in Single-Pass Sand Column

Flow Rate (ml/hr)	TCE Conc. Influent (mg/l)	TCE Conc. Effluent (mg/l)	Propane Consumed (mmol)	Methane (mmol)	Rate of TCE degr. (mg/L day ⁻¹)	Substrate/ TCE loss (μ mol/ μ mol)
18	0.75	0.08	0.9	0.6	0.36	292
16	0.45	0.04	0.01	0.08	0.19	*28
10	1.13	0.01	0.4	0.15	0.35	*62
18	1.73	0.017	0.4	0.27	0.91	*46

*Loss of substrate during TCE degradation-starvation phase. Reactor was fed methane plus propane for 1 day prior to experiment. If the substrate utilization during the feeding stage were included the substrate/TCE loss would be ≈ 150 .

Table 4.2 Comparison of Mixed Waste Degradation in Bioreactor under Conditions of Pulsed Feeding and Continuous Feeding

	CHCl ₃	111-TCA	TCE	112-TCA	PCE
Influent Conc. (mg/l)	2.0	2.0	2.0	2.0	2.0
Effluent Conc. for Continuous feeding (mg/l)	0.41	0.35	1.1	0.32	1.18
Effluent Conc. for Starvation (mg/l)	0.40	0.37	0.75	0.32	0.88
Rate of Organic Degradation (mg/l day) Continuous feeding	0.60	0.62	0.34	0.64	0.31
Rate of Organic Degradation (mg/l day) Starvation	0.61	0.62	0.48	0.64	0.43

The flowrate through the bioreactor was 13 ml/hr. Steady-state was reached after 5 days of operation. Oxygen concentration in the reactor was 3.14 mg/l after eight days of operation in feeding mode. In starvation phase, after 10 days of operation, oxygen concentration was 4 mg/l.

added to the reactor. During these intervals, only the phosphate-bicarbonate buffer medium and the chlorinated hydrocarbons were being added to the reactor. Table 4.2 shows that there was comparable degradation ability during both the feeding and starvation periods. It is also noted that the μ mole of substrate required per μ mole of contaminant removed during the starvation phase is essentially halved. During the feeding period 209 μ mol of methane plus propane were required per μ mol of chlorinated organic degraded versus 110 mol of primary substrate required during starvation feeding periods. This result indicates that the supply of a primary carbon source can be essentially halved while system degradation potential shows no adverse affects. It was observed that after five days, degradation activity in the reactor decreased. Phelps et al. (Phelps et al., 1991) observed similar results in an expanded-bed bioreactor. This loss in degradation potential suggests a depletion of the enzyme responsible for the epoxidation of the chlorinated compounds. This observation is expected in the absence of a primary substrate. Typical reactor results of methane and propane removal and carbon-dioxide production can be seen in Appendix A, Figures A.1 and A.2.

Similar experiments were conducted which utilized the mixed-waste system seen in Table 4.2 with the addition of carbon tetrachloride. Preliminary results indicate 99% degradation of carbon tetrachloride is achievable when initial concentrations of the toxicant were ≤ 50 mg/l (data

not shown here). Previous results indicated carbon tetrachloride degradation only under anaerobic conditions (Fogel et al., 1985; Bouwer & McCarty, 1983a; Bouwer & McCarty 1983b).

Propane vs. methane. Although methane facilitates TCE and other waste degradation (Little et al., 1988), it is shown in Table 4.3 that the rates or percents of degradation when methane was the only electron donor were retarded when compared with those with propane as the carbon source. Similar results were observed in expanded-bed reactors (Phelps et al., 1990). When propane was the only available primary substrate, results similar to that seen in the system containing both methane and propane were observed.

Mixed-waste degradation. A series of experiments were performed testing the ability of the bioreactor to degrade a mixture of aliphatic chlorinated compounds and aromatic compounds. Table 4.4 indicates the bioreactor was robust to such a mixture of compounds, and that degradation abilities are comparable to those observed in single-component or small-mixture waste systems. It should be noted that tetrachloroethylene was the only compound showing a drastic decrease in degradation potential. This result implies that more highly chlorinated wastes, such as PCE, are degraded less efficiently than the less chlorinated hydrocarbons in highly mixed-waste systems and that longer residence times are required to achieve

Table 4.3. Comparison of Degradation in Bioreactor when Substrate Limited to Only Methane or Only Propane

Substrate	Percent Degradation (%)				
	CHCl ₃	111-TCA	TCE	112-TCA	PCE
Propane Only	98	96	84	92	84
Methane Only	58	53	39	49	35

Table 4.4 Degradation of Mixed Organic Wastes in Bioreactor

Compound	Concentration of Toxicant	
	In (mg/l)	Out (mg/l)
Trichloroethylene	5.5	0.57
cis-1,2-Dichloroethylene	15.8	≤ 0.005
trans-1,2-Dichloroethylene	2.7	≤ 0.005
1,1,2-Trichloroethane	2.1	0.22
1,1,1-Trichloroethane	7.2	0.68
Chloroform	10.9	1.7
Tetrachloroethylene	0.91	0.80
Methylene Chloride	27.5	2.3
Benzene	3.57	0.40
Toluene	9.8	0.22
Xylene	1.8	≤ 0.005
Ethyl Benzene	3.5	≤ 0.005

The flow rate through the bioreactor was 7 ml/hr.

desirable bioremediation potentials. Although TCE is considered a highly chlorinated compound, results indicate that TCE does not retard the metabolic efficiency within this mixed-waste system. Phelps et al., 1991, suggested that many components in a mixed-waste system do not require additional nutrients or degradation time. This result is supported here.

Modeling. Utilizing abiotic and biotic experiments performed at various flow rates, a model was developed to describe degradation within the packed-bed bioreactor. A first order reaction rate was assumed ($-r_A = kC_A$) and the rate constant found for TCE degradation was $k = 0.023 \text{ hr}^{-1}$. The rate constants for other compounds in a mixed-waste system were found and summarized in Table 4.5. These results suggest that the degradation rate of chlorinated aliphatics is controlled by the extent of chlorination and length of the carbon chain. The degradation abilities of the five compounds listed in Table 4.5 were compared with degradation observed for the large-mixture waste system composed of the compounds listed in Table 4.4, which include aromatic and aliphatic wastes. There were no data indicating that the highly mixed-waste system inhibited any compounds' ability to be degraded except that of PCE.

Table 4.5. First-order Rate Constants of Chlorinated Aliphatics in a Mixed-Waste System

Compound	k(hr ⁻¹)
TCE	0.023
112-TCA	0.025
111-TCA	0.027
Chloroform	0.032
PCE	0.019

Utilizing a dispersed plug-flow reactor model and first-order reaction kinetics, a material balance across the reactor can be solved analytically (Wehner & Wilhelm, 1956). The solution is as follows:

$$\frac{C_A}{C_{A_0}} = \frac{4a \exp\left(\frac{1}{2} \frac{vL}{D}\right)}{(1 + a)^2 \exp\left(\frac{a}{2} \frac{vL}{D}\right) - (1 - a)^2 \exp\left(-\frac{a}{2} \frac{vL}{D}\right)} \quad (4.1)$$

where

$$a = \sqrt{1 + 4k\tau(D/vL)} \quad (4.2)$$

and where C_A is the effluent concentration of the toxicant (mg/l), C_{A_0} is the inlet toxicant concentration (mg/l), v is the superficial velocity in the reactor (ml/hr), L is the reactor bed length (m), D is the reactor dispersion coefficient (cm²/s), k is the first order reaction rate (hr), and τ represents the reactor

residence time (hr). Figure 4.2 compares model predictions with experimental results. The experimental points plotted represent an average of the data obtained from 5-10 runs at each flow rate. During each experiment, 3 data points were collected daily over a 5 day period and then all 15 were averaged. Although chlorinated aliphatics are degraded cometabolically, these data suggest first-order rate equations can be used to model such mixed-waste systems effectively.

SUMMARY

The single-pass packed-bed reactor system was utilized in this investigation to facilitate the understanding of chlorinated aliphatic hydrocarbon biodegradation. The reactor system represents a low technology apparatus (system has been maintenance free for two years) that exhibits the potential to consistently degrade chlorinated aliphatics to levels approaching drinking water quality. Possible field application could be as simple as a culvert filled with sand; residence time, and therefore degradation potential, being controlled by the physical dimensions of the culvert.

The reactor was subjected to perturbations with the intent of degradation optimization. The bioreactor system showed resiliency when subjected to mixed wastes including both aromatics and aliphatics. The

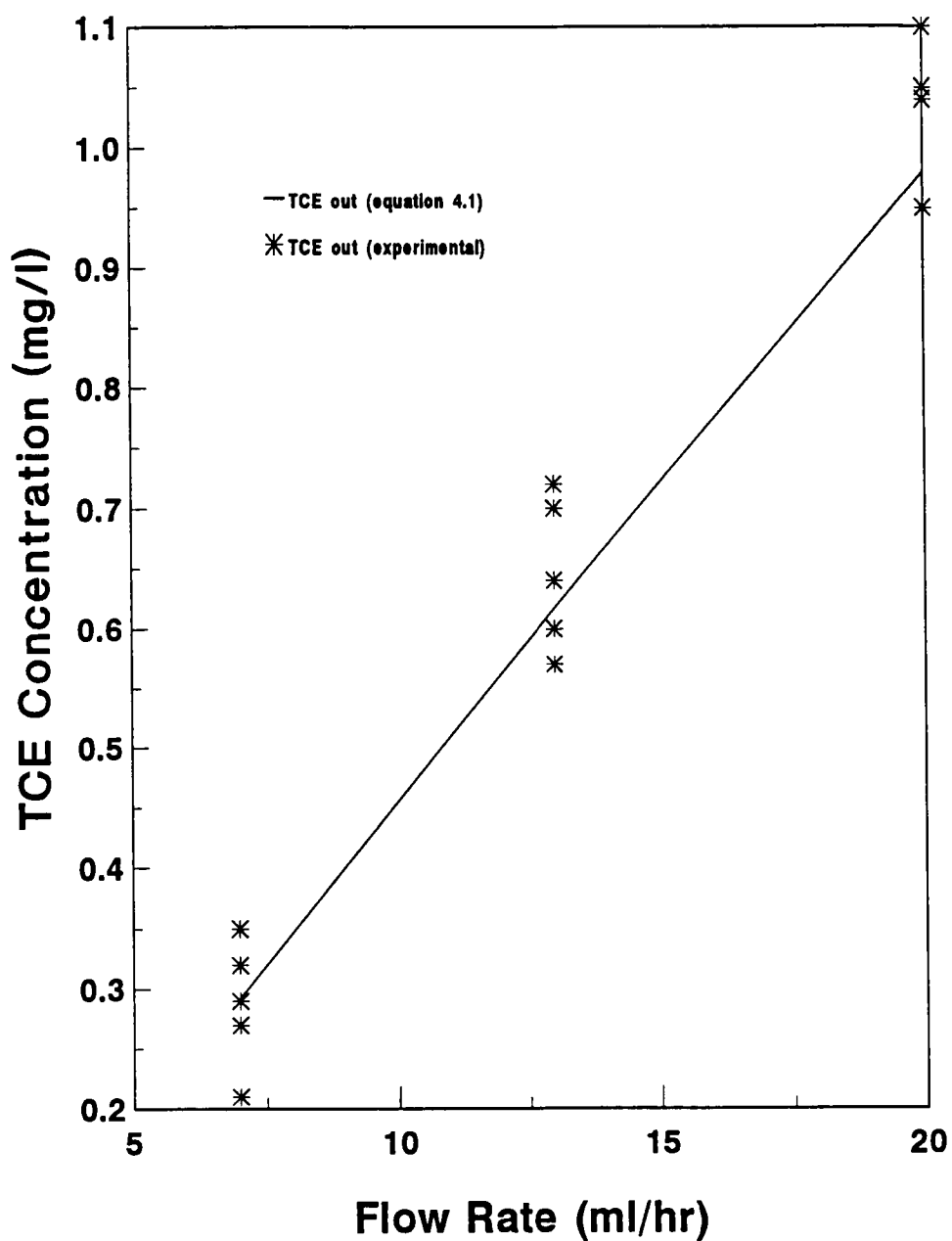


Figure 4.2 Comparison of Model Predictions with Experimental Results
TCE Inlet Concentration = 2 mg/L

aromatics, xylene and ethyl benzene, in a mixed-waste system displayed the potential to consistently be degraded to below detectable limits when the residence time in the reactor was ≥ 2.5 days. In a highly-mixed waste system, PCE was the only compound exhibiting a depressed metabolism rate. Preliminary results of this study indicate that 99% degradation of carbon tetrachloride is achievable when initial concentrations of the toxicant is ≤ 50 mg/l. In conclusion, although the system contained many process variables, the ability to accurately predict degradation potentials was attainable by a simple analytical solution, the dispersion model, for packed-bed reactors assuming first-order reaction rate characteristics.

CHAPTER V

FEASIBILITY TESTING FOR THE ON-SITE BIOREMEDIATION OF ORGANIC WASTES BY NATIVE MICROBIAL CONSORTIA

ABSTRACT

Microbial consortia capable of degrading chlorinated hydrocarbons and organic solvents may exist in many contaminated sediments. These native microbial communities with the capability to bioremediate toxicants on-site may prove to be a resource during remediation efforts. For this study, microbial consortia capable of degrading trichloroethylene (TCE) and mixtures of chlorinated and aromatic toxicants were enriched from contaminated sediments and the feasibility of their participation in on-site bioremediation was examined. Batch studies were used to monitor changes in the microbial community structure by monitoring signature fatty acid biomarker trends during TCE degradation. Experimental bioreactors, which utilized sand as a support matrix for the microbial community, were constructed to study TCE and organic mixed-waste degradation. In continuously recycled expanded-bed bioreactors and a single-pass packed-bed reactor, mixtures of organic wastes were degraded including: benzene, xylene, toluene, tetrachloroethylene, trichloroethylene, dichloroethylenes and vinyl chloride. Degradation proceeded to >99% depletion for many contaminants. Bioreactors were stable over an 18 month period of operation

while using propane or methane plus propane as energy sources and oxygen as the electron acceptor. Biodegradation was most efficient when the bioreactors were pulsed-fed, maintaining the consortia in suboptimal conditions. For the single-pass packed-bed reactor, metabolic efficiencies of 20 - 70 μmol substrate per μmol TCE degraded were observed for pulse-feeding regimes while continuous substrate availability experiments required > 180 μmol substrate per μmol TCE. Continuous feeding experiments utilizing mixtures of organic wastes showed metabolic efficiencies of 83 - 240 μmol substrate utilization per μmol total organic wastes degraded. These results demonstrated efficient and simultaneous degradation of organic solvents and chlorinated hydrocarbons by environmentally derived microorganisms in laboratory reactors.

INTRODUCTION

Chlorinated hydrocarbons and organic solvents are widespread contaminants of ground water. The combination of high usage and disposal practices has resulted in the contamination of the subsurface beneath some sites (Fliermans et al., 1988; Phelps et al., 1989). Remediating contaminated aquifers will be a formidable task. Processes capable of mineralizing contaminants on-site may be more desirable than transferring toxicants to another site or to the atmosphere.

Biological transformation of trichloroethylene has been demonstrated aerobically with pure cultures of methanotrophs (Little et al., 1988; Wackett & Gibson, 1988), heterotrophs (Wackett & Gibson, 1988; Nelson et al., 1987; Vandenberg & Kunka, 1988; Wackett et al., 1989) and mixed microbial consortia (Fliermans et al., 1988; Fogel et al., 1986; Phelps et al., 1990; Bouwer & McCarty, 1983). Anaerobic biodegradation of chlorinated aliphatics occurs by reductive dechlorination which can result in the formation and accumulation of vinyl chloride (Bouwer & McCarty, 1983; Barrio-Lage et al., 1986; Kleopfer et al., 1985; Vogel & McCarty, 1985). Natural gas stimulates aerobic TCE degradation in unsaturated soil columns (Henson et al., 1988; Wilson & Wilson, 1985), and various types of bioreactors (Phelps et al., 1990; Strandberg et al., 1989). Within these soil columns and reactors, TCE was degraded and the methanotrophic biomass that participated in the TCE mineralization process increased over time (Henson et al., 1988; Wilson & Wilson, 1985). Heterotrophic consortia obtained from severely contaminated subsurface sediments (Fliermans et al., 1988; Phelps et al., 1989) degraded TCE at concentrations exceeding 100 mg/L with propane, methanol or yeast extract as energy sources. Combining native heterotrophic consortia with methane utilizing microorganisms facilitated the aerobic degradation of TCE (Phelps et al., 1991).

The potential role of native microbial consortia for the on-site bioremediation of subsurface contaminants is of considerable interest. While the enrichment of consortia capable of detoxifying TCE demonstrates degradation potential, it does not assess the feasibility of on-site bioremediation of a contaminant plume. Feasibility testing should include assessment of co-contaminant degradation, degradation of expected intermediates, degrading efficiency, stability, and parameters necessary for upscaling the bioremediation process. Recirculating bioreactors were utilized for the initial examination of degradation potential and efficiency over several 11 day periods. Degradation efficiency, kinetics, and long term stability were further examined using single-pass sand column experiments over an 18 month period. In addition to substrate toxicant degradation, the ester-linked phospholipid fatty acid (PLFA) profile of the consortium was analyzed in growth experiments to ascertain physiological changes within the microbial consortia during toxicant degradation. Feasibility testing for the bioremediation of organic waste mixtures by microbial consortia was accomplished using laboratory bioreactors which examined degradation capabilities, reactor and consortia stability, and biological reaction kinetics. Batch experiments verified degradation potentials and analyzed the toxicant degrading microbial community structure. These studies are useful in assessing the bioremediation potential of native microorganisms at

contaminated sites and provide experimentally measured parameters for process scale-up and modeling activities.

EXPERIMENTAL

Selection of microbial consortia

Two consortia were used in this study. Consortium SM-1 contained the TCE degrading consortium enriched from the Savannah River Plant, Aiken, SC (Fliermans et al., 1988; Phelps et al., 1989) plus a methanotroph isolated from a waste disposal site near Oak Ridge, TN. The TCE degrading consortium was capable of degrading >99.5% of 100 mg/L TCE (Fliermans et al., 1988) while the methanotroph was capable of degrading 1 mg/L TCE (Little et al., 1988). The PM-M consortium contained mixtures of propane and methane-oxidizing bacteria obtained from the vicinity of Ada, OK (Wilson & Wilson, 1985), the TCE-degrading consortia isolated from the Savannah River Plant plus the methanotroph isolated from a waste disposal site near Oak Ridge, TN. Consortia were maintained on a phosphate and bicarbonate buffered mineral salts medium (Fliermans et al., 1988) supplemented with 5% methane and 3% propane (vol/vol, headspace) and the SM-1 consortium was additionally supplemented with 100 mg/L of yeast extract and Trypticase.

Laboratory Techniques

All manipulations utilized syringe techniques for transferring solutions and inocula. Batch experiments utilized crimp-top tubes or serum vials (Bellco Glass, Inc. Vineland, NJ) sealed with Teflon-lined septa (Altech, Deerfield, IL). All incubations were at ambient temperature (23°C). Construction, maintenance, and operation of the total-recycle expanded-bed bioreactors (Niedzielski et al., 1989) and the single-pass packed-bed reactor (Lackey et al., 1993) used in this study are described elsewhere. The expanded-bed reactor had a constant liquid volume of 230 ml, while the total headspace volume remained at 490 ml. In all experiments the headspace to liquid volume ratio was greater than 2.0. The TCE concentration varied between 2.0 and 150 mg/L. Control reactors contained 0.2% sodium azide and 0.5% formalin to deter microbial contamination.

Gases, chemicals and reagents

Gases were supplied by MG Industries (Chattanooga, TN) and were greater than 99.5% pure. All chemicals were of reagent grade and were obtained from Mallinckrodt, Inc. (Paris, KY) or Sigma Chemical Co. (St. Louis, MO). Glass distilled solvents and reagents were obtained from Burdick & Jackson Division (Muskegon, MI). Vinylidene chloride and tetrachloroethylene were obtained from Aldrich (Milwaukee, WI) and vinyl chloride was purchased from Supelco (Bellefonte, PA). The [1,2-¹⁴C]TCE

(10 mCi/mmol) and [1,2- ^{14}C]vinyl chloride (0.53 mCi/mmol) were obtained from New England Nuclear (Boston, MA).

Analytical procedures

Analytical procedures are described in detail elsewhere (Fliermans et al., 1988; Phelps et al., 1989; Phelps et al., 1990; Niedzielski et al., 1989). TCE and many chlorinated hydrocarbons were analyzed using a Hewlett-Packard 5890 gas chromatograph equipped with a 50 m Ultra Performance (Ultra 1, Hewlett-Packard) cross-linked methyl silicone capillary column (0.2 mm, 0.33 μm film internal diameter) and an electron capture detector (Hewlett Packard) as previously described (Fliermans et al., 1988). Vinyl chloride, cis- and trans-1,2-dichloroethylene (DCE) were analyzed using a Shimadzu GC-9A gas chromatograph equipped with a 2.24 m, 3.2 mm diameter Poropak T packed column and a photoionization detector (HNU Systems, Newton, MA). Methane and CO_2 were assayed using a Shimadzu GC-8A gas chromatograph with a 2.74 m, 3.2 mm diameter Carbosieve 8000 packed column thermal conductivity detector while radioactive CO_2 was determined by a gas proportional counter as described previously (Fliermans et al., 1988). Propane was analyzed using a Shimadzu GC-9A gas chromatograph equipped with a 2.44 m, 3.2 mm diameter Poropak N packed column and a flame ionization detector.

Batch vial culture conditions and analytics

The microbial consortium was grown as previously described by Phelps et al., 1990. The pH of the medium was kept at 7.0. Culture tubes were inoculated and propane was added (10% v/v) as the sole carbon source for triplicate samples at each of six sampling time points. TCE was added for a final concentration of 20 ppm to half of the tubes, while the remainder were left as controls. At each time point, three controls, three treated samples, and one uninoculated blank were sacrificed for analysis of PLFA synthesis, TCE and propane loss.

Lipid extraction and fractionation

A sample volume (20 ml) was lyophilized then extracted in a Bligh & Dyer (1955) single-phase solvent system modified to include phosphate buffer (White et al., 1979) as described in Guckert et al., 1991. General lipid classes were separated from the total lipid extract by silicic acid column chromatography as detailed in Guckert et al., 1985. The PHA-containing acetone fraction was retained for analysis of microbial physiological status. PHA was prepared for gas chromatography (GC) analysis by forming ethyl esters of the constituent PHA monomers by acid ethanolysis as described by Findlay and White, 1983. The phospholipid-containing methanol fraction was used in the evaluation of bacterial membrane lipid profiles. The ester-linked PLFA were methylated, and the resultant fatty acid methyl esters

(FAME) separated, quantified, and identified as described by Guckert et al., 1991.

Fatty acid nomenclature

Fatty acids are reported as '**A:B ω C**' where '**A**' designates the total number of carbon atoms, '**B**' the number of double bonds, and '**C**' the distance of the closest unsaturation from the aliphatic (ω) end of the molecule. The suffixes '**c**' for cis and '**t**' for trans refer to geometric isomers. The prefixes '**i**' and '**a**' refer to iso and anteiso methyl-branching, respectively (Kates, 1986). Cyclopropyl fatty acids are designated as '**cy**' with the ring position in parentheses relative to the aliphatic end.

RESULTS

Expanded-bed bioreactor experiments

Total-recycle expanded-bed bioreactors were well suited for evaluating the degradation potential for microorganisms (Niedzielski et al., 1989; Phelps et al., 1990, Phelps et al., 1991). The bioreactors were used to monitor the response of the microbial community during perturbation experiments as well as to provide information concerning short term (days-weeks) stability on a microbial community attached to the substratum. In these studies toxicants were recirculated for 5 - 21 days during which time substrate, toxicants, and products could be monitored. Table 5.1 shows results from methane and propane fed bioreactors which contained microbial inocula enriched from a

contaminated site. After 11 days of recirculation, 93% of the 20 mg/L TCE was removed from the recirculating expanded-bed bioreactors, propane and methane were consumed, and carbon dioxide produced. In the absence of added microbial cultures, > 95% of the TCE added to the systems was still present after 11 days. An average of 4.9 mmol propane plus 1.2 mmol methane were oxidized as substrates for a substrate/TCE loss ratio of 43 ($\mu\text{mol}/\mu\text{mol}$).

When mixtures of organic toxicants, including tetrachloroethylene, TCE, vinylidene chloride, vinyl chloride, benzene, toluene, and xylene were recirculated through the bioreactor, an average of 6.63 mmol propane plus 2.21 mmol methane were oxidized for a substrate/toxicant loss ratio of 40 ($\mu\text{mol}/\mu\text{mol}$). Benzene and xylene were degraded to detectable limits on the eighth day and toluene was depleted on the tenth day. Vinyl chloride was not detected within the reactor on the final day of the experiment. The system degraded PCE, TCE, and vinylidene chloride to 58, 81, and 78 %, respectively. When the single ring aromatics as well as both high and low chlorinated compounds were added to the system, the aromatics and the lesser chlorinated compounds were rapidly degraded at little or no change in metabolic efficiency as represented in Table 5.1 by the foodstock utilized/toxicant loss ratio. However, the more highly chlorinated compounds such as TCE were degraded to a lesser extent during the 11 day experiments.

Table 5.1 Comparison of TCE and Mixed-Organic Waste Degradation in the Expanded-Bed Bioreactor

Experiment	Initial TCE Conc. (mg/L)	Average Degradation in Reactor (%)	Foodstock Consumed (μ mol)	Foodstock/ toxicant loss (μ mol/ μ mol)	Toxicant Consumed μ mol/ day/gdw biomass
TCE control	20	<5	n.a.	n.a.	n.a.
TCE	20	93	6100	43	107
Mixed organics	30	85	8840	40	167

The expanded-bed bioreactor was continuously operated for an 11 day period.

Propane and methane were maintained at 3% and 5% v/v, respectively.

n.a., not applicable.

Sand column experiments

Several bench-scale studies using fluidized beds, expanded beds and trickling filter bioreactors for TCE degradation experienced difficulty in achieving effluent drinking water standards (Phelps et al., 1990; Strandberg et al., 1989). Table 5.2 shows results from a single-pass sand column capable of lowering 1-2 mg/L influent TCE concentrations to near drinking water quality. In these experiments a control reactor was not utilized, rather a perfluorocarbon (perfluoromethylcyclohexane) was added as a conservative tracer. Effluent perfluorocarbons averaged $90 \pm 1\%$ of influent concentrations demonstrating retention of volatile chlorinated hydrocarbons. Flow rates of 18 ml/hr resulted in $>90\%$ removal of 0.75 - 1.7 mg/L TCE in a single pass, in turn resulting in approximately 1 mg of TCE degraded each day per liter of reactor volume with a substrate/TCE loss ratio of 127 - 292 ($\mu\text{mol}/\mu\text{mol}$). Experiments within the single-pass sand-column typically used lower inlet concentrations (1-2 mg/L) than those used in the expanded-bed bioreactor (≈ 20 mg/L). The degradation of the lower versus higher concentrations of TCE was more energy intensive, exhibiting a foodstock consumed/TCE degraded ratio 6.5 times larger than observed when initial concentrations were higher in the expanded-bed bioreactor. At lower flow rates utilizing pulsed feeding regimes, 90 - 99% of the TCE was

Table 5.2 Comparison of TCE and Mixed-Organic Waste Degradation in the Single-Pass Packed-Bed Bioreactor

Experiment	Initial TCE Conc. (mg/L)	Average TCE Degradation in Reactor (%)	Substrate Consumed (μ mol)	Substrate/ toxicant loss (μ mol/ μ mol)
TCE with CH ₄ and C ₃ H ₈ at day = 0 only (7 ml/hr) ¹	1.53	99	670	*58
TCE with CH ₄ and C ₃ H ₈ at day = 0 only (10 ml/hr)	1.13	99	550	*62
TCE (18 ml/hr)	0.75	89	1500	282
Mixed organics (7 ml/hr)	10	84 (79) ²	4140	83
Mixed organics (13 ml/hr)	10	45 (33) ²	3960	79

The single-pass packed-bed reactor was operated in both a continuous and pulse feeding regime.

*The packed-bed reactor was fed methane plus propane for at least 1 day prior to the experiment. If the substrate utilization during the feeding stage was included, the substrate/organic waste loss would be > 150.

¹ Flow rate (ml/hr) through the reactor is indicated in parentheses. Mixed organics included TCE, PCE, chloroform, 1,1,1-trichloroethane, and 1,1,2-trichloroethane. The initial concentration of all toxicants was 2 mg/L.

² Percent of mixture degraded.

degraded resulting in molar degradation efficiencies of 28-62 ($\mu\text{mol}/\mu\text{mol}$).

To obtain lower effluent concentrations of toxicants and maintain degradative efficiencies it was necessary to feed the reactor for a day, provide a several hour starvation period during which time propane and methane were utilized, and then add TCE without additional propane or methane. TCE degradative activity remained for two or more days before replenishing with substrates was necessary. If one included the substrate concentrations consumed during the non-TCE degrading periods then the substrate/TCE ratio would likely be > 150 . Results from a mixed-organic waste experiment showed a metabolic efficiency of 83 μmol substrate/ μmol organic waste and a decrease in the overall degradation percent within the reactor when compared to a single toxicant system (Table 5.2). When the flowrate within the reactor was both 7 and 10 ml/hr, and TCE was the only degradable toxicant, the average TCE degradation was 99%. In contrast, during mixed organic-waste experiments, the average TCE degradation at 7 and 13 ml/hr were 84 and 45%, respectively. During the mixed-organic waste experiments, the aromatics and the lower chlorinated compounds were degraded to 90 - 99%. Thus, the retarded degradation potentials were a result of a pronounced decrease in degradation of the highly chlorinated organics in the waste mixture.

Comparison of mixed organic wastes degradation in test tube experiments, recirculating and single-pass bioreactors

Batch studies initially demonstrated toxicant removal by the native microbial consortia. Reactors inoculated with the consortia portrayed similar degradation trends, but differences between batch and reactor studies were observed. Degradation of mixed organic wastes by the hydrocarbon fed consortium in bioreactors and cell suspensions are shown in Table 5.3. The bioreactors were fed both methane and propane while batch test tube experiments were fed only propane. A variety of chlorinated compounds were degraded, many to detection limits in both reactors and test tube experiments. Typical of the total-recycle bioreactors, TCE degradation ceased at 0.3 mg/L, resulting in a loss of only 90% of the initial TCE concentration while batch studies indicated a 99% degradation potential of TCE. Both reactors showed less extensive and less rapid TCE degradation than was expected from batch experiments. Degradation of tetrachloroethylene in batch studies by these consortia failed. In contrast, within the recirculating bioreactor and the single-pass packed-bed reactor 60% and 12%, respectively, of the tetrachloroethylene was lost despite the fact that degradation had not occurred in cell suspension or growth cultures. Current studies are reexamining tetrachloroethylene degradation by these consortia. Dichloroethylenes and vinyl chloride, suspected intermediates of TCE degradation, were degraded to detectable limits. Benzene, toluene and xylene, common groundwater contaminants associated with petroleum

Table 5.3 Degradation of mixed-organic wastes by propane fed microbial consortia

Organic Waste	Conc. range in expanded-bed bioreactor Day 0 (mg/l)	Average loss in reactor after 21 days (%)	Inlet sand-column conc. (mg/l)	Average loss in sand-column 7 ml/hr ⁴	Day 0 Test tube exp. conc. (mg/l)	Average loss in test tube exp. after 30 days (%)
Vinyl Chloride	4 - 20	>99			5	>99
Vinylidene Chloride	20	99				
1,1-dichloroethane	1.1	>99				
1,1-dichloroethylene	0.7	>99	15.8	>99	1	95
1,2-dichloroethylene	3.0	>99	2.7	>99	1	95
1,1,1-trichloroethane	1.2	>99	7.2	91	1	>99
tetrachloroethylene	2.1 - 20	60 - 80	0.91	12		
trichloroethylene	2.6 - 20	81 - 90	5.5	89	50	>99
benzene	0.1 - 1	>99	3.57	89	1	>99
xylene	0.1 - 5	>99	1.8	>99	1	>99
toluene	0.05 - 5	>99	9.8	98	1	>99

Ranges are depicted by a dash (-). The residence time in the single-pass sand-column was 5 days.

⁴At 7 ml/hr, the degradation rate coefficient when TCE was the only toxicant was 0.20 day⁻¹. Upon addition of organic-waste mixtures the TCE degradation rate coefficient decreased to 0.16 day⁻¹.

solvents, were also degraded to detectable limits. The single-pass sand column degraded 1,1-dichloroethylene, 1,2-dichloroethylene, and xylene to detectable limits.

Biological degradation rate constants associated with complex mixed-organic waste experiments in the recirculating expanded-bed and the single-pass packed-bed bioreactors are compiled in Table 5.4. Rate constants in both bioreactors showed common trends. Higher first order rate constants were calculated for the aromatics than for the chlorinated organics in the mixtures. It was also noted that the rate constants for the lesser chlorinated compounds were larger than those of the more highly chlorinated toxicants. For example, in the expanded-bed bioreactor, the rate constants for benzene, vinyl chloride, and, trichloroethylene were 1.67, 0.91, and 0.79 day^{-1} , respectively.

Rate constants derived from the single-pass sand column appear to be similar. Representing degradation potentials in this manner can be deceiving if the longevity of the residence times within the reactor system are not noted. For example, compare the rate constants for TCE, 0.20 day^{-1} , and PCE, 0.16 day^{-1} . After a 10 day residence time, this correlates to a 87% degradation of TCE and a 79% of PCE; a significant difference.

Table 5.4 Biological rate constants of organic contaminants associated with the recirculating expanded-bed and single-pass packed-bed bioreactors

Compound	Rate constants for Recirculating expanded-bed bioreactor (day ⁻¹)	Rate constants for single-pass packed-bed bioreactor (day ⁻¹)
Benzene	1.67 ± 0.25	0.59 ± 0.11
Xylene	1.67 ± 0.08	0.67 ± 0.13
Toluene	1.0 ± 0.11	0.42 ± 0.09
Vinyl chloride	0.91 ± 0.08	-
1,2-dichloroethylene	-	0.37 ± 0.06
Trichloroethylene	0.79 ± 0.02	0.20 ± 0.06
Tetrachloroethylene	0.59 ± 0.05	0.16 ± 0.05

Benzene
Toluene
Xylene

$\ll$

VC
DCE
VDC

$\ll$

PCE
TCE

Community structure of toxicant degrading microorganisms and consortia

Table 5.5 shows ester-linked phospholipid fatty acid profiles (PLFAME) of methanotrophic TCE degraders and bioreactor contents. The methanotrophic TCE degrading consortium exhibited 16:1 ω 7t and 16:1 ω 5c PLFAME found in Type I methanotrophs (Nichols et al., 1985) plus a small contribution of 18:1 ω 8c/t synthesized by some Type II methanotrophs. When contaminated groundwaters were treated in bioreactors, PLFAME characteristic of protozoa appeared. Upon microscopic examination, protozoan cysts were observed in the bioreactor contents. These results suggested successful colonization of methanotrophic consortia by protozoan species. Preliminary results (data not shown) suggest that protozoan grazing upon bacteria can result in decreased efficiency of bioremediation. Quantifying PLFAME in bioreactors or during bioremediation may enable monitoring and modeling of the abundance and impact of protozoan species upon toxicant degrading consortia.

Signature fatty acid biomarkers

Dissection of the signature PLFA biomarkers provided evidence that the presence of TCE affected community structure. Individual fatty acids were extracted from the entire fatty acid profile and summed for each microbial classification. Refer to Figure 5.1 (a-d).

Table 5.5 Phospholipid fatty acid profiles of mixed cultures and bioreactors contents

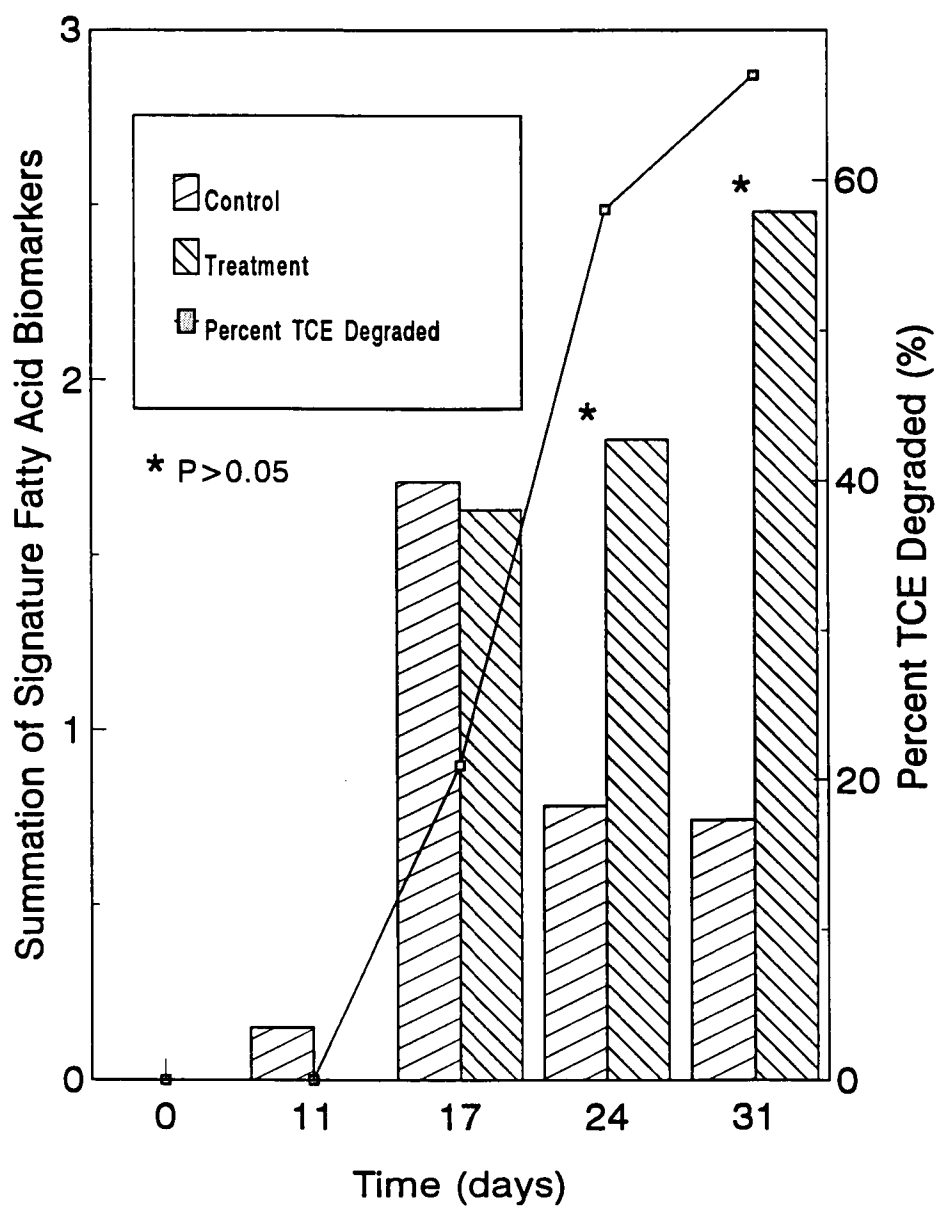
PLFAME ¹	Methanotrophic TCE degrading consortia	Native methylotrophic TCE degrading consortia	Microbial contribution ²
i15:0		2.0	A
15:0		2.2	A
i16:0		0.7	A
16:1w7c	30	2.9	Ps, I, & II
16:1w7t	12.3	1.5	Ps & I
16:1w5c	0.1	0.4	Ps & I
16:0	34	7.0	
10 me 16:0		1.1	Act
i17:1		0.4	
i17:0		1.4	A
a17:0		1.8	A
cy17:0	5.1	3.2	Ps
17:0		1.5	
18:2w6		0.3	P
18:1w9c		3.8	Ps, P & II
18:1w8c/t		15.1	Ps & II
18:1w7c	16.9	13.1	Ps & II
18:1w7t	0.6	0.6	Ps & II
18:0	0.3	8.6	
10me18:0		4.6	Act
br19:1		0.8	
19:1w12c		0.7	
cy19:0	0.3	23.4	Ps
20:3w6			P
20:5w3		0.3	P
20:4w6		0.7	P
20:0		1.2	P

¹ Analyzed as phospholipid-fatty-acid methyl-esters (PLFAME) identified by gas chromatography.

² I = common PLFAME associated with Type I methanotrophs; II = Type II methanotrophs; P = PLFAME typically associated with protozoa; A = PLFAME typically associated with Arthrobacter; Act = PLFAME typically associated with Actinomycetes; Ps = PLFAME typically associated with Pseudomonas; B&P = PLFAME typical of both bacteria and protozoa.

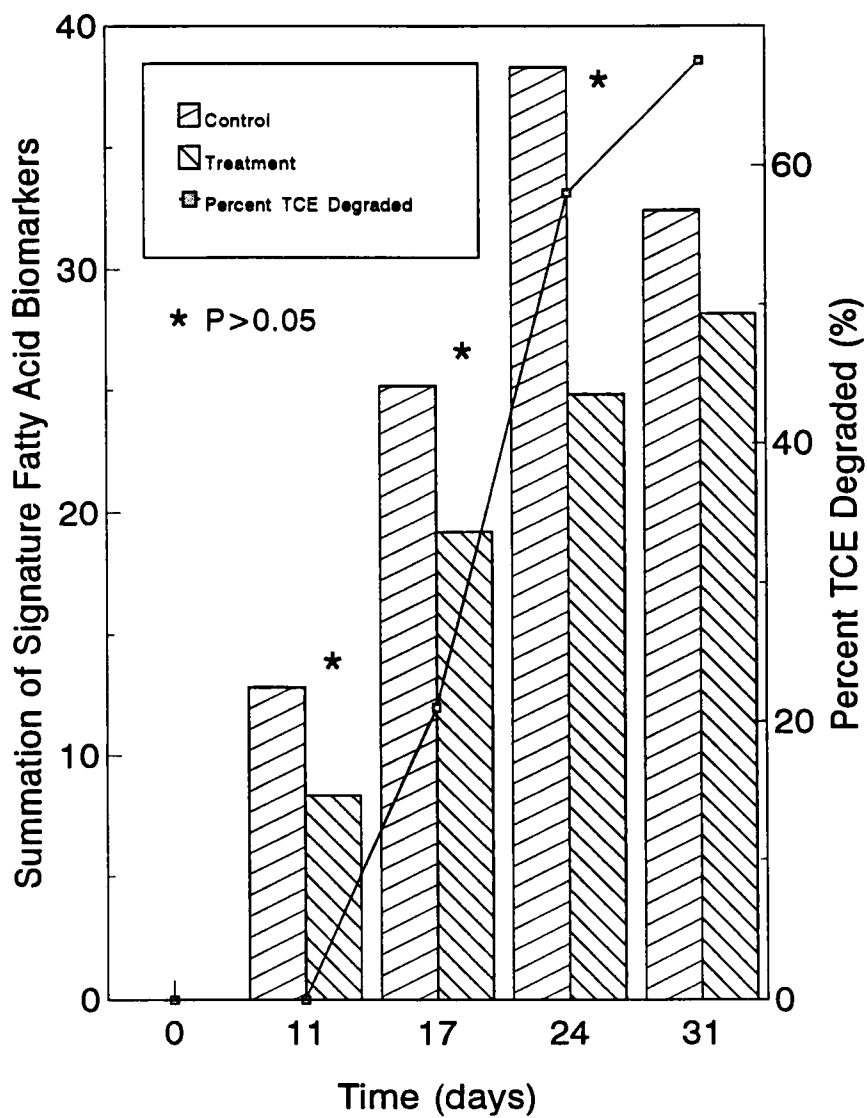
Gram(+) Arthrobacter-type signature biomarkers (terminally branched saturates) exhibited a statistically significant increase over the other fatty acid profile constituents as TCE was degraded in stationary phase (Figure 5.1, a). Gram(+) Actinomycete-type biomarkers (10Me17:0, 10Me18:0) exhibited an increase in the control culture (Figure 5.1, b). Gram(-) Pseudomonad-type biomarkers (monoenoic and cyclopropyl PLFA) peaked during the first week and declined to a constant level in both the treated and control consortium (Figure 5.1, c). There was little correlation between TCE degradation and changes in gram(-) biomarkers. A propane utilizing bacterial biomarker (16:1 ω 6) increased as TCE was degraded and propane consumed (Figure 5.1, d). This biomarker was increased in the control consortia during log phase, but this trend was reversed during stationary phase when the TCE was degraded.

These results indicate that reactor studies as well as test tube experiments are useful in feasibility testing, scale-up, and modeling of on-site bioremediation. Results indicate that reactors should be designed for the compound with the smallest rate constant. Batch studies provided microbial community structure and toxicant degradation potentials while reactor experiments provided a system response to perturbations, stability information, and degradation kinetics required for process scale-up.



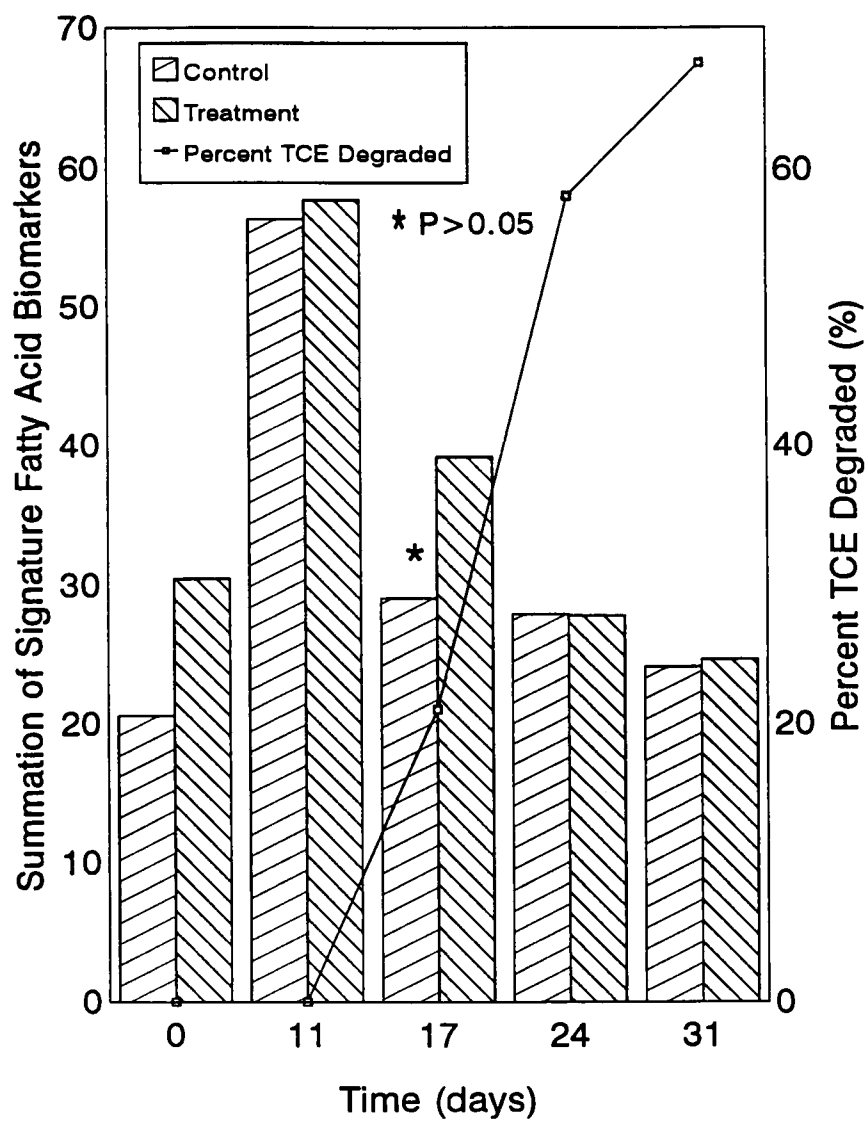
a. Gram(+) *Arthrobacter* sp.

Figure 5.1 Changes in microbial community structure during TCE degradation



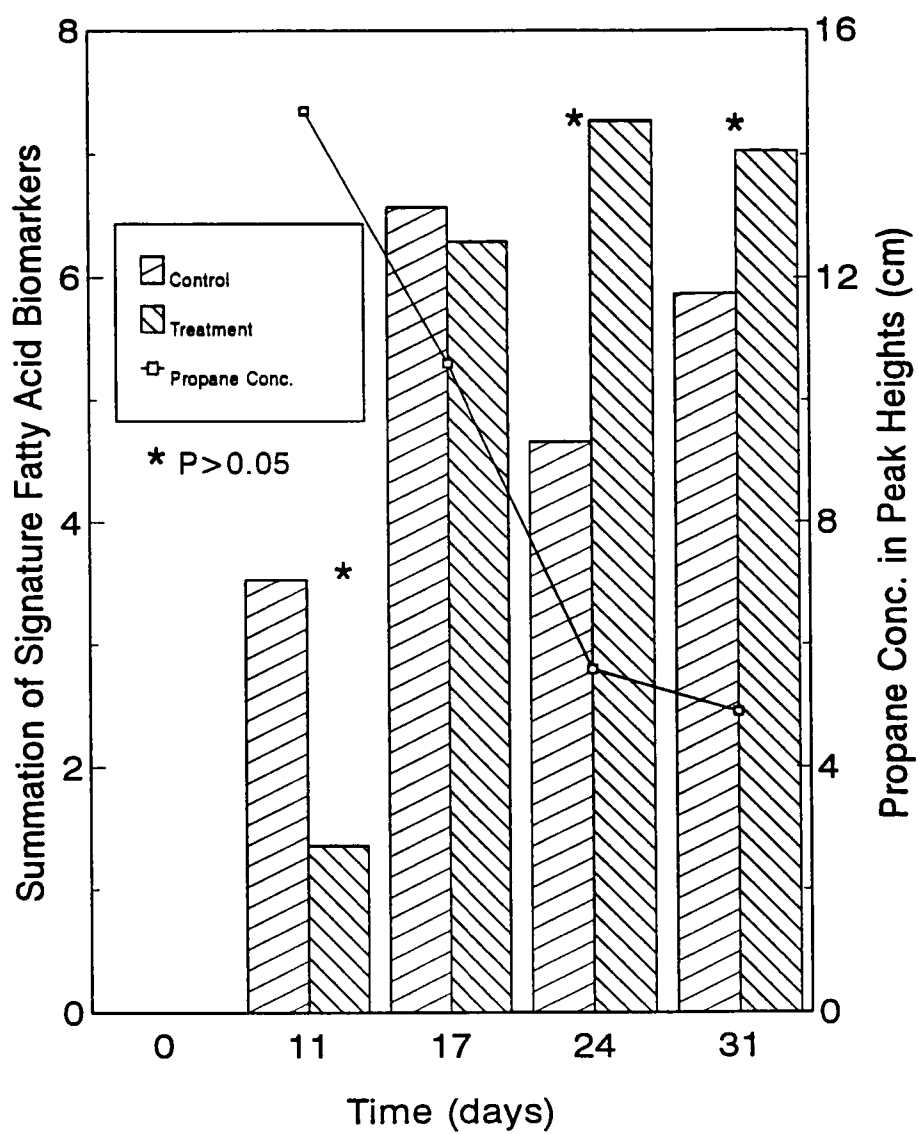
b. Gram(+) Actinomycetes sp.

Figure 5.1 (continued)



c. Gram(-) *Pseudomonias* sp.

Figure 5.1 (continued)



d. Propane utilizing sp.

Figure 5.1 (continued)

DISCUSSION

Results demonstrated that bioreactors containing mixed consortia enriched from contaminated subsurface environments were capable of degrading TCE and mixed organic wastes while utilizing propane or methane plus propane as energy sources. Phospholipid fatty acid profiles characterized the microbial community structure of the TCE degrading microbial consortium and provided evidence of protozoan species colonizing with the consortia.

Reactor studies

In terms of TCE or mixed-organic waste degradation efficiency (substrate utilized/toxicant degraded), pulsed feeding of microbial consortia resulted in the highest degradation rates and efficiency in both recycled bioreactors and single-pass sand columns. When the bioreactors were starved for several days (no substrate added), TCE degradation decreased significantly. This finding is in accord with the results presented by other researchers (Fliermans et al., 1988; Little et al., 1988; Phelps et al., 1990; Strandberg et al., 1989). This study, as well as previous work (Phelps et al., 1990; Niedzielski et al., 1989; Phelps et al., 1991) shows the potential usefulness of recirculating expanded-bed bioreactors for examining degradation potentials and resiliency of the microbial community under various different operating conditions such as pH, medium, primary substrate availability, and organic-waste mixtures.

Biological rate constants and degradation efficiencies were compared between reactor types. Higher first order rate constants were observed in the recirculating expanded-bed bioreactor when rates were compared to those found in the single-pass sand column. Feasible explanations of the rate differences might include the different reactor configurations or that the lower inlet concentrations studied in the single-pass column were more difficult to degrade. Although rate constants between reactor types differed, trends associated with toxicant groups (benzene, toluene, and xylene; high and low chlorinated organics) were similar. Degradation was more efficient ($\mu\text{mol substrate}/\mu\text{mol toxicant}$) in the expanded-bed reactor but lower effluent TCE concentrations were achievable in the single-pass sand column. The single-pass packed-bed bioreactor provided a low-technological method for determining microbial stability as well as biodegradation rates associated with single toxicant and mixed-organic waste environments.

Bioreactors currently appear inefficient at lowering concentrations of highly chlorinated hydrocarbons to drinking water standards (Phelps et al., 1990; Strandberg et al., 1989). Incorporation of pulse-fed sand columns as an additional stage to bioreactors appears complimentary for complete biodegradation of residual chlorocarbons. Surprisingly, the sand columns lowered TCE concentrations to low part per billion levels at substrate/TCE ratios less than 300 ($\mu\text{mol}/\mu\text{mol}$). It could be hypothesized that sand

columns may be used to model in-situ degradation efficiencies. Accordingly, it may be possible to bioremediate chlorinated hydrocarbons within confined subsurface aquifers at substrate to TCE ratios less than 500.

Lipid biomarkers indicate protozoan and toxicant degraders

Lipid analysis provided a method to measure protozoan colonization, the stressed status of the microbial community, as well as provide signature lipid biomarkers yielding qualitative TCE degradation potentials. Monitoring the PLFAME provides a quantitative tool for assessing the abundance of protozoa within bioreactors as well as changes in the microbial community structure. It is possible to monitor the ratios of methanotrophic lipid markers (Nichols et al., 1985) to those lipids typical of methylotrophs, arthrobacters, actinomycetes, or protozoa. In this study, signature lipid biomarkers provided information concerning the activity of the TCE degrading consortia. This provides the potential to qualitatively identify degradation activity during an on-site or *in-situ* remediation program.

Idealized on-site bioremediation

Figure 5.2 is an artistic representation of on-site bioremediation of mixed organic wastes. Solid black circles represent concentrated wastes, including chlorinated hydrocarbons, with cumulative concentrations exceeding 1 g/L. Excessive oxygen demand and nutrient costs may dictate pump-and-treat or evaporative extraction techniques coupled with surface

bioreactors for remediation of concentrated wastes. Upon arrival at the surface, concentrated wastes could enter an anaerobic digester where readily digestible organics could be fermented without the cost of aeration. Reductive dechlorinations could also be accomplished anaerobically to facilitate more efficient aerobic degradation. Aerobic biological treatment in either up- or downward flow could result in >95% reduction of the toxicants, many being reduced to drinking water standards. Residual toxicants such as the final 0.2 mg/L TCE could be removed in the polishing columns. This third stage could utilize biological treatment or chemical-physical means. Multiple polishing columns are depicted because biological treatment may require pulsed-feeding with nutrients followed by a brief starvation and then toxicant degradation. Alternating between three polishing columns would enable simultaneous feeding, starving and toxicant degradation for continuous operations. Water exiting the polishing columns may be recycled through the system or utilized for in-situ bioremediation. Coupling of bioreactor designs enables complete detoxification of many contaminant mixtures.

The striped circles in Figure 5.2 represent lesser contaminated subsurface aquifers suitable for in-situ bioremediation. Optimization of three dimensional hydrological control and nutrient delivery may be accomplished by application of shallow horizontal wells. Horizontal wells may be suitable for upward or downward flow of nutrients during in-situ bioremediation and

On-Site Biological Remediation
of Mixed Organic Wastes

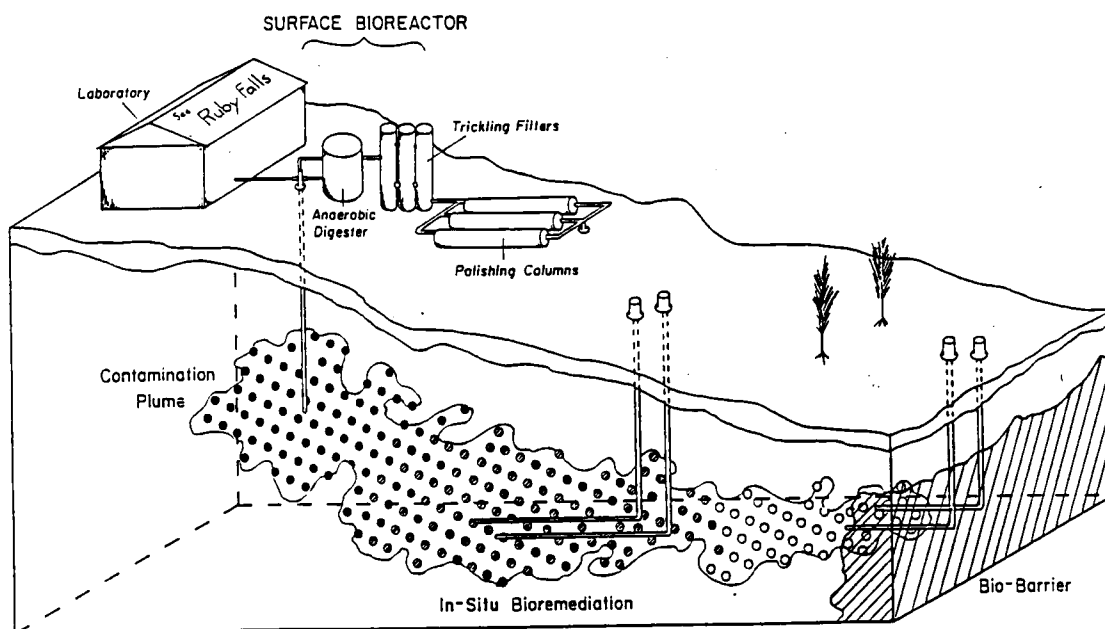


Figure 5.2 Artistic representation of on-site and *in-situ* bioremediation. degradation for continuous operations

may additionally be suitable for vacuum extraction regimes. The open circles represent residual toxicant concentrations which could be degraded or immobilized by a down-gradient biological barrier. In addition to degrading organics, a biological curtain or wall at the plume boundary may be useful in binding or immobilizing heavy metals in exopolymers.

Previous studies and work presented here indicate the potential for bioremediation of environments contaminated with chlorinated ethenes and mixed-organic wastes on-site and *in-situ*. This study suggests the use of both an expanded-bed and a single-pass packed-bed bioreactor to assess the feasibility of bioremediating a contaminated plume. Exploration of the degradative potential and the resiliency of the native microbial community could be examined in a recirculating expanded-bed bioreactor. The biodegradation rates associated with single toxicant and mixed organic-waste degradation can be examined in single-pass packed-bed reactors. Lipid analysis provides useful information concerning protozoan grazing on toxicant degrading organisms as well as insight on the native populations ability to degrade TCE. Through complementing methodologies integrated bioremediation technologies could be realized which remediate mixed-organic wastes on-site rather than transferring the toxicants from one environment to another.

CHAPTER VI

UTILIZATION OF PACKED-BED BIOREACTOR SYSTEMS

FOR FATE ANALYSIS OF TRICHLOROETHYLENE

IN SUBSURFACE ENVIRONMENTS

ABSTRACT

Trichloroethylene (TCE) and other organic solvents constitute one of the major sources of groundwater contamination. The biotic and abiotic fate of these contaminants in the subsurface is of utmost importance for understanding *in-situ* biodegradation. In this study a 1.9 L packed-bed sand-column and a differential volume soil reactor were used to simulate the subsurface environment for transport and degradation of TCE and other chlorinated and aromatic organics. The differential volume reactor (DVR) system was equipped with on-line detection devices for chlorinated organics. From abiotic experiments the DVR system was characterized as to axial dispersion and aqueous/soil mass transfer using perturbation experiments while varying flow rate, pressure drop and soil characteristics. The experimentally derived dispersion and mass transfer coefficients were used to solve the convection-dispersion equation. The coefficients derived from the DVR system were used in modeling the larger 1.9 L packed-bed sand column.

INTRODUCTION

Trichloroethylene, a commonly used degreaser, is utilized in the manufacturing of metals, electronic components, and rubber products as well as in the dry cleaning industry. Due to the widespread usage and disposal of TCE and other chlorinated compounds, these toxicants have become major contaminants of subsurface groundwater. Common remediation techniques such as air stripping and adsorption of chlorinated compounds have proven to be time consuming and/or costly. An alternative approach presently being examined is bioremediation utilizing both *in-situ* and pump and treat technologies. For bioremediation to compete with the more accepted, traditional removal techniques, there must be an increased understanding of groundwater flow, toxicant sorption/desorption processes, biological reaction rates, and the physical properties associated with bioreactors.

The ability to degrade TCE and other chlorinated compounds has been observed in both pure and mixed culture applications (Fliermans et al., 1988, Little et al., 1988, and Vogel et al., 1987). Such compounds are degraded cometabolically, and typically there is a methane, propane, or toluene oxygenase enzyme responsible for degradation (Little et al., 1988, Wackett, et al., 1989, and Wackett and Gibson, 1988). To degrade contaminants cometabolically, a primary substrate, electron acceptor, plus nutrients must

be added. Thus, a more complex system results than one in which the contaminant is degraded as a primary substrate.

There has been much success biodegrading TCE in laboratory batch experiments (Little et al., 1988), reactor studies (Phelps et al., 1991, Strandberg et al., 1989), and in soils (Roberts et al., 1991; Semprini et al., 1991; Wilson et al., 1987, Nelson et al., 1990).

Mathematical models have been developed that describe the fate of TCE in soil systems (Semprini & McCarty, 1991). When modeling, necessary parameters are collected from the literature and by experimentation, while others are simply fitted in order for the simulation to produce reasonable results. In this research, an effort was made to minimize the number of fitted parameters necessary for modeling. This research utilized two packed-bed reactor systems, a differential volume reactor, and a 1.9 L sand column, to aid in the understanding of biodegradation in soil columns and subsurface environments. A simulation was developed which modeled the transport and degradative properties of TCE in the packed-bed reactors. The model incorporated transport properties such as dispersion, advection, and sorption. Attention was given to several microbial processes including; the biodegradation of aliphatic chlorinated compounds, microbial growth, and the utilization of electron donors (methane/propane) and an electron acceptor (oxygen). The current model is similar in design to those previously developed (Molz et al., 1986; Borden and Bedient, 1986;

Semprini and McCarty, 1991; Wu, 1982) in that it is based on the traditional convection/dispersion equation.

MATERIALS AND METHODS

Differential Volume Reactor

Two reactor systems, a differential volume reactor, and a 1.9 liter sand column, were utilized in this study. The DVR system was designed to simulate a section of an ideal plug flow reactor or a soil segment within a contaminated site. A complete description of this reactor can be found in Webb et al., 1991. The reactor was utilized in biotic and abiotic experiments.

Trichloroethylene biodegradation and abiotic studies were performed utilizing the DVR as illustrated in Figure 3.4. The system was equipped with two Millipore stainless steel substrate containers rated to 690 kPa. The feed from the substrate vessels to the reactor inlet was controlled by two Gilson 301 HPLC pumps. The substrate vessels were pressurized with either oxygen or propane. Thus, electron donor and acceptor were provided to the system through the aqueous phase. Concentrations of these gases in the liquid phase was controlled by the pressure within the substrate vessels. The reactor effluent entered a 1/4 in. stripping column packed with 3 mm glass beads to provide adequate surface area for the volatile separation process. The volatiles were stripped with helium in a cocurrent fashion

through the stripper. The outlet of the stripping column was attached directly to a gas chromatograph (GC, Hewlett Packard 5890 Series II) with a flame ionization detector (FID) or electron capture detector (ECD) by a heated sample line maintained at 75°C. Automatic injections (25 μ l sample loop) were made on the GC by a computerized control process (Hewlett Packard computer, HP Chem Station software). This system was equipped with a bypass line around the reactor in order to calibrate the stripping column. Liquid sampling ports for measuring oxygen concentrations were available on the inlet and outlet lines of the system. This DVR system was used in systems where soil or sand was utilized as the biomass support matrix.

For the abiotic experiments, soil from a TCE contaminated site was wet sieved and washed with 1 percent sodium pyrophosphate solution. Organic particles loosely affixed to soil surfaces were removed during the treatment with sodium pyrophosphate solution.

1.9 L Sand Column.

Figure 3.1 is an illustration of the single-pass packed-bed bioreactor system. The reactor was composed of two borosilicate-glass columns (Spectrum Medical Industries, Inc., Los Angeles, CA). The two columns (ID = 5 cm, length = 100 cm, total volume = 1960 ml) were sealed using stainless steel end caps equipped with teflon O-rings. These columns were joined by 1/8 in. stainless steel lines that provided a closed system. The lower

column was inoculated with a TCE degrading consortium and size 50 to 70 mesh white quartz sand (Sigma Chemical Co., St Louis, MO). The upper column provided oxygen, methane, and propane to the packed-bed on a recirculating basis (Figure 3.1). The gases were pumped from the upper gas recharge column into the lower bioreactor column using an FMI lab pump model RP-G400 (Fluid Metering, Inc., Oyster Bay, NY) that distributed the gases to three equally spaced regions on the bottom of the packed bed. A 3% hydrogen peroxide solution was pumped into the bioreactor column by a Gilson Miniplus 2 peristaltic pump at the rate of 1-2 ml/hr as an additional electron acceptor source. The flow was evenly distributed to three regions in the bottom of the reactor bisecting the gas flow inlets. The top of the packed-bed column was equipped with a gas outlet stream that recirculated into the upper column. All column connections were made with Swagelok® fittings.

The inlet and outlet of the single-pass sand bioreactor was sealed and the reactor was pressurized to 3 psi prior to each experiment. The system consistently held pressure for a 24 hr test period prior to each experiment. A more complete description of this system can be found in Lackey et al., 1993.

Analytical procedures.

A Hewlett-Packard 5890A capillary gas chromatograph with a split-splitless injector was used to analyze the chlorinated hydrocarbons in the

1.9 L column system. The GC was equipped with an electron capture detector (Hewlett-Packard) and a cross-linked methyl silicone capillary column (internal diameter of 0.2 mm, 0.33 μm film thickness). The oven temperature was operated isothermally at 60°C. Other operating parameters include the injection temperature of 150°C and the detector temperature of 200°C. The split ratio was set at 10:1. Helium was used as the carrier gas and nitrogen as the makeup gas.

Methane and carbon dioxide present in the gas phase were analyzed using a Shimadzu GC-8A gas chromatograph. The GC was equipped with a 2.74 m, 3.2 mm diameter Carbosieve 8000 packed column and a thermal conductivity detector. Both the injector and detector temperatures were 140°C, while the oven was operated isothermally at 130°C. Propane was analyzed using a Shimadzu GC-9A gas chromatograph equipped with a 2.44 m, 3.2 mm diameter Poropak N packed column and a flame ionization detector. The isothermal temperature of the oven was 80°C and the detector and injector temperature were 220°C.

Medium

The medium used in both bioreactor systems was the following: MgSO_4 , 0.055 g; $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.054 g; NaNO_3 , 1.0 g; trace minerals II (Lynd et al., 1982), 20 ml; 10x vitamin solution (Lynd et al., 1982), 1 ml; 200-mM phosphate/bicarbonate buffer solution, 30 ml; nanopure-filtered water, 1000 ml. The pH of the medium was kept at 7.0.

Gases and chemicals

All gases were supplied by National Welders (Charlotte, NC).

Methane was greater than 98% pure, and oxygen and propane were greater than 99.5% pure. Sigma Chemical Co. (St. Louis, MO) or Mallinckrodt, Inc. (Paris, KY) provided chemicals and reagents. Glass distilled solvents and reagents were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ).

Bacterial culture

The consortium used in inoculating both the bioreactors was obtained from several sites. A TCE degrading consortium was isolated from the Savannah River Plant, Aiken SC (Fliermans et al., 1988). A methane and propane utilizing consortium from Ada, OK, was added to the reactor (Nichols et al., 1985) along with a methanotroph isolate from a waste disposal site near Oak Ridge, TN.

MODELING SOLUTE TRANSPORT IN SOIL SYSTEMS

Liquid transport through soil systems is typically governed by three processes; convective-dispersive flux, sorption, and chemical-biological transformations. The one-dimensional partial differential equation typically termed the convective-dispersion transport equation is conventionally implemented to describe solute transport through porous media and is represented by equation (6.1) (Lapidus and Amundson, 1952):

$$-D \frac{\partial^2 C_i}{\partial x^2} + V \frac{\partial C_i}{\partial x} + \frac{\partial C_i}{\partial t} + \frac{\rho}{\epsilon} \frac{\partial \bar{C}_i}{\partial t} = R_i \quad (6.1)$$

From left to right, the terms in equation (6.1) account for the movement of component i by dispersion; describe the movement of component i by convective forces; describe the change of concentration of component i in the liquid phase; describe the accumulation of component i in the solid phase; and account for the production or disappearance of species i by reaction.

Within equation (6.1), the terms relating to dispersion, adsorption and biological reaction rate can be theoretically represented or determined by a variety of methods and assumptions. The dispersion coefficient characterizes the degree of backmixing during flow. Within a packed bed reactor, the dispersion coefficient can be easily determined by utilizing a pulse injection perturbation on the system. The outlet response to the pulse can be characterized, typically as gaussian, and the mean and variance describing the outlet response can be computed.

Haynes and Sarma, 1973, developed a method capable of separating the dispersion within a packed-bed system from adsorption effects. The method utilizes simple analytic expressions for the first and second moments of the pulse response and is capable of providing a realistic representation of a packed bed. When several simplifying assumptions are made within the laminar flow regime, the resulting equation is as follows:

$$\frac{\sigma^2}{2\mu^2} \frac{L}{v} = \frac{D_L}{v^2} + \left(\frac{\epsilon}{1-\epsilon}\right)\left(\frac{R_p^2}{3D_m} + \frac{R_p^2}{15\epsilon_p D_p} + \frac{r_c^2}{15K D_c}\right)\left(1 + \frac{\epsilon}{(1-\epsilon)K}\right)^{-2} \quad (6.2)$$

From this equation, it is observed that the macropore resistances and the external mass transfer resistance are directly proportional to the square of the particle radius. Thus, if small particles are used, these terms can be considered insignificant. It is observed from equation (6.2) that if a plot is made of $(\sigma^2/2\mu^2)(L/v)$ versus $1/v^2$, a straight line is produced with values of slope D_L and intercept which correspond to the total mass transfer resistance.

A simulation was developed that modeled the transport and degradative properties of TCE in a packed-bed reactor. The model incorporates transport properties such as dispersion, advection, and sorption in conjunction with several microbial processes including; the biodegradation of aliphatic chlorinated compounds, microbial growth, and the utilization of electron donor (propane) and acceptor (oxygen). This model is similar to those previously developed (Molz et al., 1986; Borden & Bedient, 1986; Semprini & McCarty, 1991; Wu, 1982).

The rates of microbial growth and the utilization of hydrocarbon substrates and oxygen were simulated by using multiplicative Monod kinetics (Borden & Bedient, 1986). This type of modeling, which uses the product of the terms described in a Monod kinetic fashion for each limiting

substrate, has been widely accepted and implemented in microbial systems.

This altered Monod relationship was developed by Rich, 1973.

Within the system, it is assumed that both electron donor and acceptor contribute to microbial growth and decay rates. (Kissel et al., 1984; Molz et al., 1986; Borden & Bidient, 1986; Semprini & McCarty, 1991). The equation for microbial growth and decay rate is as follows.

$$\frac{\partial X}{\partial t} = XkY\left(\frac{C_D}{K_{SD} + C_D}\right)\left(\frac{C_A}{K_{SA} + C_A}\right) - bX\left(\frac{C_A}{K_{SA} + C_A}\right) \quad (6.3)$$

Equations (6.4) and (6.5) represent the rates of utilization of electron donor and acceptor, respectively. This simulation assumes the secondary substrate's demand for dissolved oxygen is much lower than that of the primary substrate (Roberts et al., 1989), thus this term has been neglected in equation (6.5).

$$\frac{\partial C_D}{\partial t} = -kX\left(\frac{C_D}{K_{SD} + C_D}\right)\left(\frac{C_A}{K_{SA} + C_A}\right) \quad (6.4)$$

$$\frac{\partial C_A}{\partial t} = -kFX\left(\frac{C_D}{K_{SD} + C_D}\right)\left(\frac{C_A}{K_{SA} + C_A}\right) - d_c f_d b X\left(\frac{C_A}{K_{SA} + C_A}\right) \quad (6.5)$$

Equation (6.6) represents secondary substrate utilization (Bailey & Ollis, 1986). In this equation, K_{SD} is an empirical parameter used for the modeling of complex substrate systems. This modeling approach compensates for the retarded degradation potentials of such chlorinated compounds due to the competitive inhibition between the secondary substrate and electron donor.

$$\frac{dC_2}{dt} = - \frac{Xk_2C_2}{K_{S2} + C_2 + \frac{K_{S2}}{K_{SD}}C_D} \quad (6.6)$$

The Freundlich isotherm is often used to describe adsorption when equilibrium is assumed between the adsorbed and solution concentration. Lapidus & Amundson (1952) suggested using a kinetic approach towards equilibrium, and the following rate equations were proposed.

$$\frac{d\overline{C}_2}{dt} = \alpha (K_{d_2}C_2 - \overline{C}_2) \quad (6.7a)$$

$$\frac{d\overline{C}_D}{dt} = \alpha (K_{d_D}C_D - \overline{C}_D) \quad (6.7b)$$

$$\frac{d\overline{C}_A}{dt} = \alpha (K_{d_A}C_A - \overline{C}_A) \quad (6.7c)$$

The above first-order model, which utilized the product of the overall mass transfer coefficient and the concentration driving force, was used to describe the fate of solute adsorption on the solid phase. Solving this kinetic adsorption scenario, the expressions for biotransformation of the secondary substrate, and for the utilization of the electron donor and acceptor are added to the transport equation [equations (6.4), (6.5), and (6.6), are added to equation (6.1)]. Results are as follows:

$$\begin{aligned} \frac{\partial C_2}{\partial t} = & D \frac{\partial^2 C_2}{\partial x^2} - V \frac{\partial C_2}{\partial x} - \frac{\partial \bar{C}_2}{\partial t} \\ & - X k_2 \left[\frac{C_2}{K_{S2} + C_2 + \frac{K_{S2} C_D}{K_{SD}}} \right] \left(\frac{C_A}{K_{SA} + C_A} \right) \end{aligned} \quad (6.8)$$

$$\frac{\partial C_D}{\partial t} = D_h \frac{\partial^2 C_D}{\partial x^2} - V \frac{\partial C_D}{\partial x} - \frac{\partial \bar{C}_D}{\partial t} - k X \left(\frac{C_D}{K_{SD} + C_D} \right) \left(\frac{C_A}{K_{SA} + C_A} \right) \quad (6.9)$$

$$\begin{aligned} \frac{\partial C_A}{\partial t} = & D_h \frac{\partial^2 C_A}{\partial x^2} - V \frac{\partial C_A}{\partial x} - \frac{\partial \bar{C}_A}{\partial t} \\ & - k F X \left(\frac{C_D}{K_{SD} + C_D} \right) \left(\frac{C_A}{K_{SA} + C_A} \right) - d_c f_d X \left(\frac{C_A}{K_{SA} + C_A} \right) \end{aligned} \quad (6.10)$$

The seven equations, (6.3), (6.7a), (6.7b), (6.7c), (6.8), (6.9), and (6.10), adequately describe the packed-bed soil system. These equations

were put into dimensionless form by utilizing the following relationships and then solved by manipulating the IMSL routine MOLCH (Sewell, 1982).

$$C_i^* = \frac{C_i}{C_{i0}} \quad (6.11)$$

$$t^* = \frac{t V}{L} \quad (6.12)$$

$$x^* = \frac{x}{L} \quad (6.13)$$

$$Pe = \frac{VL}{D} \quad (6.14)$$

RESULTS AND DISCUSSION

Experimental Results

Soil from a TCE contaminated aquifer was packed in the DVR and abiotic sodium salicilate (NaSal) pulse perturbation experiments (varying flow rate and particle size) were performed. Within each experiment, the flow rate varied from 0.5 to 3.5 ml/min and the NaSal effluent response to the perturbations were monitored. Experiments were performed utilizing four

different particle diameters, obtained using the following mesh sizes: size 35 to 40 mesh (average $d_p = 460 \mu\text{m}$), size 50 to 60 mesh (average $d_p = 270 \mu\text{m}$), size 70 to 80 mesh (average $d_p = 190 \mu\text{m}$) and size 100 to 120 mesh (average $d_p = 140 \mu\text{m}$).

Results were analyzed using the method of moments developed by Haynes and Sarma (1973). A linear relationship was observed from a plot made of $(\sigma^2/2\mu^2)(L/v)$ versus $1/v^2$. This was the expected result indicated by equation (6.2). Figure 6.1 is an example of such a plot when the average particle size in the reactor was $140 \mu\text{m}$. The insert in Figure 6.1 represent the Gaussian shaped response to a pulse perturbation. This curve is a representative data set used to comprise one of the points creating the line in the figure. The slope of the line produced provided the dispersion coefficient for the system, D_L , while the intercept was representative of the total mass transfer resistance in the system. Results of this development can be seen in Figure 6.1. A listing of the data sets used in this analysis can be found in the tables in Appendix C.

In the analysis of trends observed in this system, it is useful to note the dimensionless Sherwood Number, Sh , as it is defined in the low Reynolds number regime.

$$Sh \equiv \frac{d_p k_f}{D_m} = 2 \quad (6.15)$$

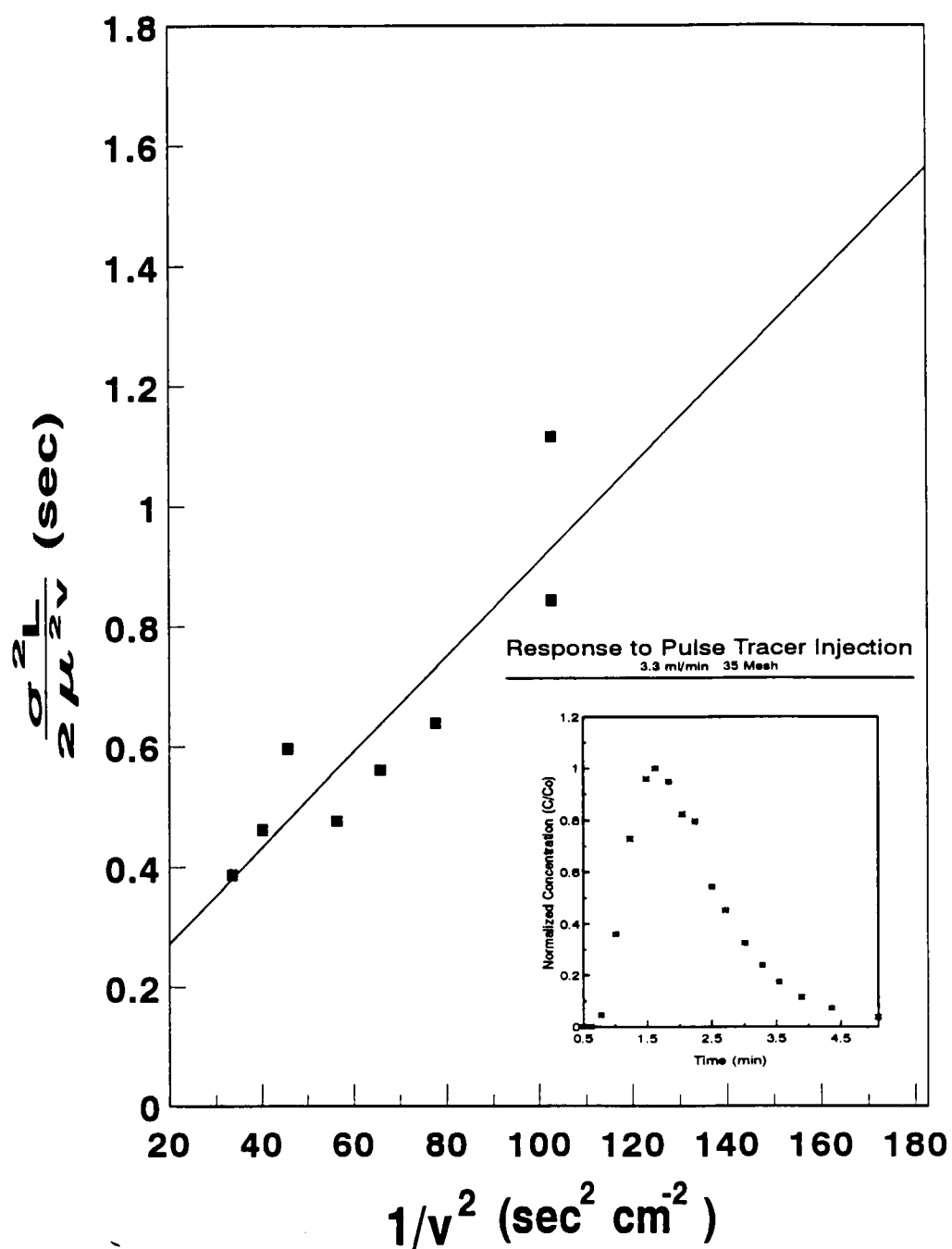


Figure 6.1 Determination of mass-transfer and dispersion coefficients by method of Haynes and Sarma, 1973. Insert represents reactor effluent response to sodium salicylate tracer pulse injection.

Theory predicted a decrease in the mass transfer coefficient when particle diameter was increased. Such a trend is presented in Table 6.1. The data predicted a range in mass transfer coefficient of 0.15 to 0.06 sec^{-1} as the particle diameter increased from an average of 140 to 460 μm . The same general trend with respect to particle size occurred with the dispersion coefficient showing a range of values from 1.3×10^{-4} to $3.1 \times 10^{-5} \text{ cm}^2/\text{sec}$. The total pressure drop of the system was monitored and artificially changed by using check valves with a range of 50 - 200 psi. Total system pressure showed no effect on overall mass transfer resistance or dispersion.

Table 6.1 Mass transfer and dispersion coefficients associated with different soil particle sizes

Particle Diameter (μm)	k_f (sec^{-1})	D (cm^2/sec)
140	0.15	1.3×10^{-4}
190	0.13	5.4×10^{-5}
270	0.08	3.7×10^{-5}
460	0.06	3.1×10^{-5}

Single component and mixed-organic waste experiments were performed in both the DVR and the single-pass sand-column reactors. Table 6.2 lists experimentally measured TCE and PCE biodegradation rate constants, within the single-pass sand column, while the number of organics introduced to the reactor was altered. Rate constants were derived assuming first order kinetics, therefore, $\ln C_{out} = \ln C_{in} - kt$. A representative graph of this nature can be found in Figure C.2 in Appendix C. The reaction rate constant decreased from 0.20 to 0.16 day⁻¹ and 0.18 to 0.02 day⁻¹ for TCE and PCE respectively, as the number of organics in the system increased from 1 to 12. For PCE, an order of magnitude difference in the rate constants was observed. Results from this reactor indicated an increased recalcitrance of TCE and PCE with an increased complexity of organic wastes.

Table 6.2 TCE and PCE rate constants associated with mixed-organic waste systems in the single pass sand column.

Number of Compounds in Organic Mixed-Waste System	TCE k (day ⁻¹)	PCE k (day ⁻¹)
1	0.20 ± 0.06	0.16 ± 0.05
5	0.17 ± 0.05	0.17 ± 0.07
12	0.16 ± 0.05	0.02 ± 0.05

The differential volume reactor was operated in a single-component fashion and under two different organic-mixed waste conditions. Single-component experiments were performed using TCE as the sole toxicant. Experiments were run using two different mixed waste systems defined as follows:

Mixture 1. TCE, 1,1,1-trichloroethane, benzene,
toluene,

Mixture 2. TCE, 1,1,2-trichloroethane, 1,1,1-
trichloroethane, chloroform, and
tetrachloroethylene.

The degradation rate constants found were $0.42 \pm 0.06 \text{ day}^{-1}$, $0.30 \pm 0.10 \text{ day}^{-1}$, and $0.14 \pm 0.06 \text{ day}^{-1}$ for the single component TCE experiment, mixture 1, and mixture 2, respectively. The DVR system showed an increase in the recalcitrance of TCE as the complexity of the organic-mixtures increased. These data suggest that possibly benzene and toluene are primary substrates for non-methanotrophic organisms in the reactor consortia (Wackett & Gibson, 1988; Nelson et al., 1990; Winter et al., 1989), and therefore they minimally influence an increase in TCE recalcitrance in mixed-organic waste systems. Information concerning abiotic controls within the DVR can be found in Figure C.1 in Appendix C.

Simulation Predictions

The dispersion and mass-transfer coefficients determined were utilized in modeling the DVR and the 1.9 L sand column. Simulation results were compared to experimental degradation values of toxicants, biomass availability, and electron donor and acceptor concentrations. Table 6.3 lists simulation input parameter values. These input parameters, when possible, were determined using experimental results, while others were obtained from the literature. Three parameters (refer to Table 6.3) were altered when necessary to allow the simulation to provide a good fit to the experimental data shown in Appendix C, Table C.4.

Electron donor and acceptor concentrations were monitored in the reactor system. Within the DVR system, the inlet and effluent oxygen concentrations were measured after the system reached a steady state operation. The inlet oxygen concentration was 2.97 mg/L with an effluent concentration of 1.65 mg/L. Therefore, 44% of the oxygen within the system was utilized. Figure 6.2a represents simulation predictions of oxygen concentration through the DVR soil bed during steady-state operating conditions.

The simulation predicts a 40% oxygen removal, which compares well with experimental results. Over a period of 5 days, experimental results showed 62% of the propane depleted from the system. Figure 6.2b shows the simulation prediction of propane utilization. The simulation predicts

Table 6.3 Model simulation input parameters

Parameter	Value	Source
X_0 (mg/L)	350	E
K day ⁻¹	2.3	E
K_{SD} (mg/L)	5.5	F
K_{SA} (mg/L)	1.0	L ¹
K_{S2} (mg/L)	1.0	L ²
L (m)	1.0	E
F (mg/mg)	2.6	F
d_c (mg/mg)	0.42	L ³
f_d	0.8	L ³
k_2 (day ⁻¹)	0.0096	E
B (day ⁻¹)	0.55	F
Y (mg/mg)	0.5	L ³
ρ (g/L)	1.8	E
ϵ	0.4	E

Source:

E = Experimentally measured

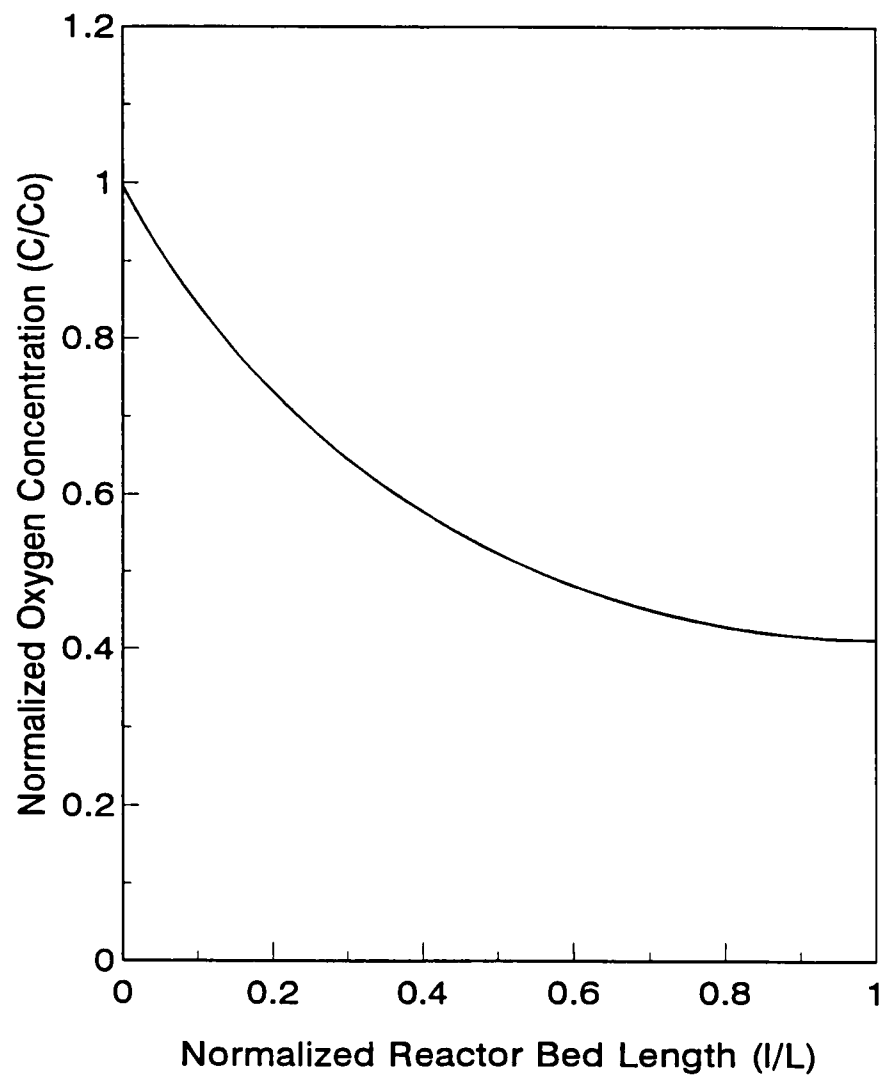
L = Literature

F = Fitted

¹Wilkinson, T.G. et al., 1974

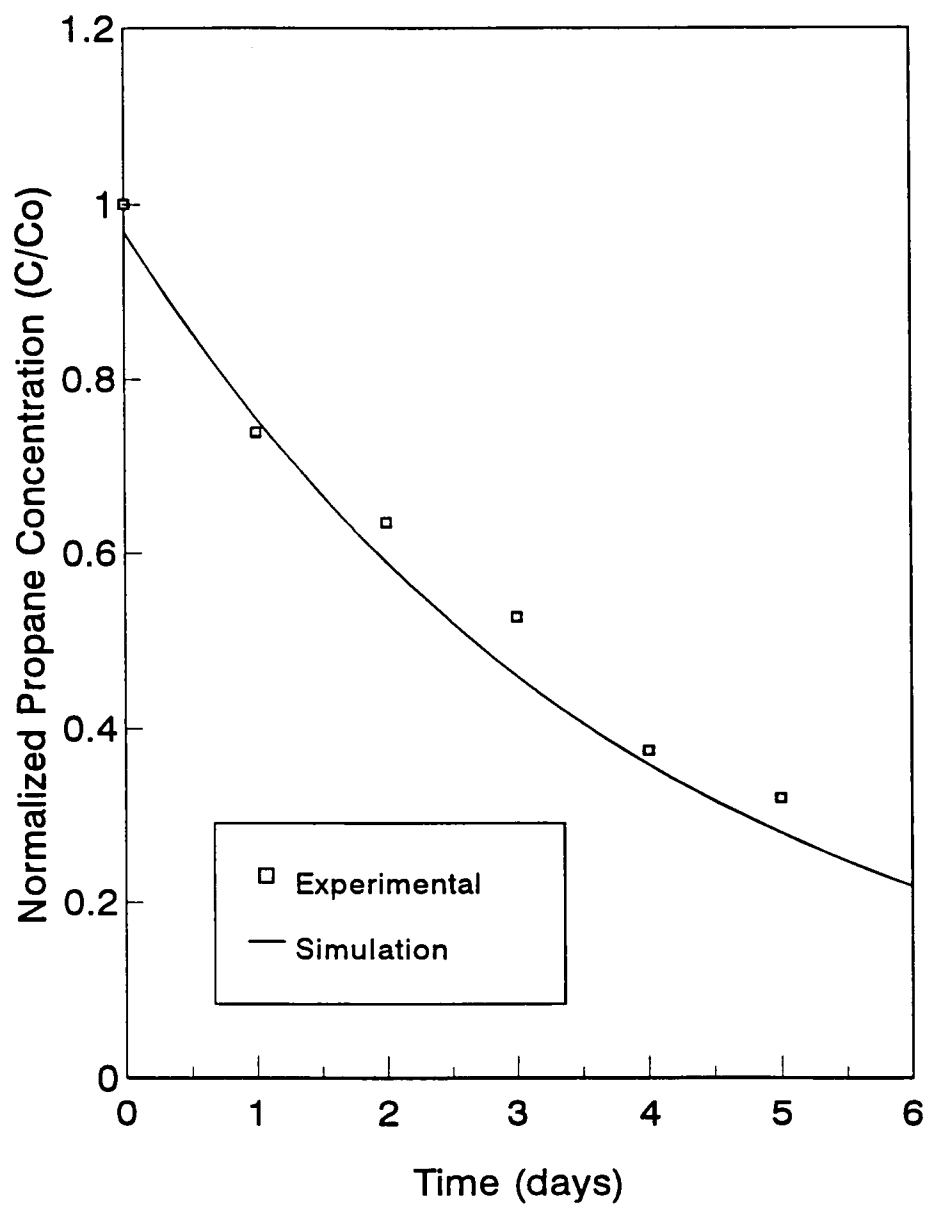
²Semprini et al., 1988

³Semprini and McCarty, 1991



a. oxygen utilization

Figure 6.2 Simulation results for oxygen and propane utilization in the single-pass packed-bed bioreactor



b. propane utilization

Figure 6.2 (continued)

values comparable to experimental results for both oxygen and propane utilization, thus providing a mechanism to keep the system optimally supplied with oxygen and a primary carbon source.

Liquid velocity within the reactor is a key variable controlling TCE degradation. Figure 6.3 shows simulation predictions in a single secondary substrate system of TCE degradation as a function of flow rate. By only changing the velocity input parameter, the simulation was capable of accurately predicting TCE effluent concentrations. In contrast, when the reactors were operated in a mixed-organic waste mode, altering the velocity was not adequate to successfully model effluent TCE concentrations. As the complexity of the mixture increased, the simulations ability to accurately describe TCE degradation declined. From the experimental results observed, which predicted an increased recalcitrance of TCE with increased complexity of organic mixed-wastes, this was not an unexpected result.

SUMMARY

A single-pass, 1.9 L packed-bed reactor system and a differential volume reactor system were utilized to study the abiotic and biological fate of chlorinated organics. Abiotic studies provided information concerning mass transfer and dispersion coefficients under various conditions such as soil particle size and flow rate. Consistent with theory, the mass transfer coefficient decreased with an increase in particle diameter.

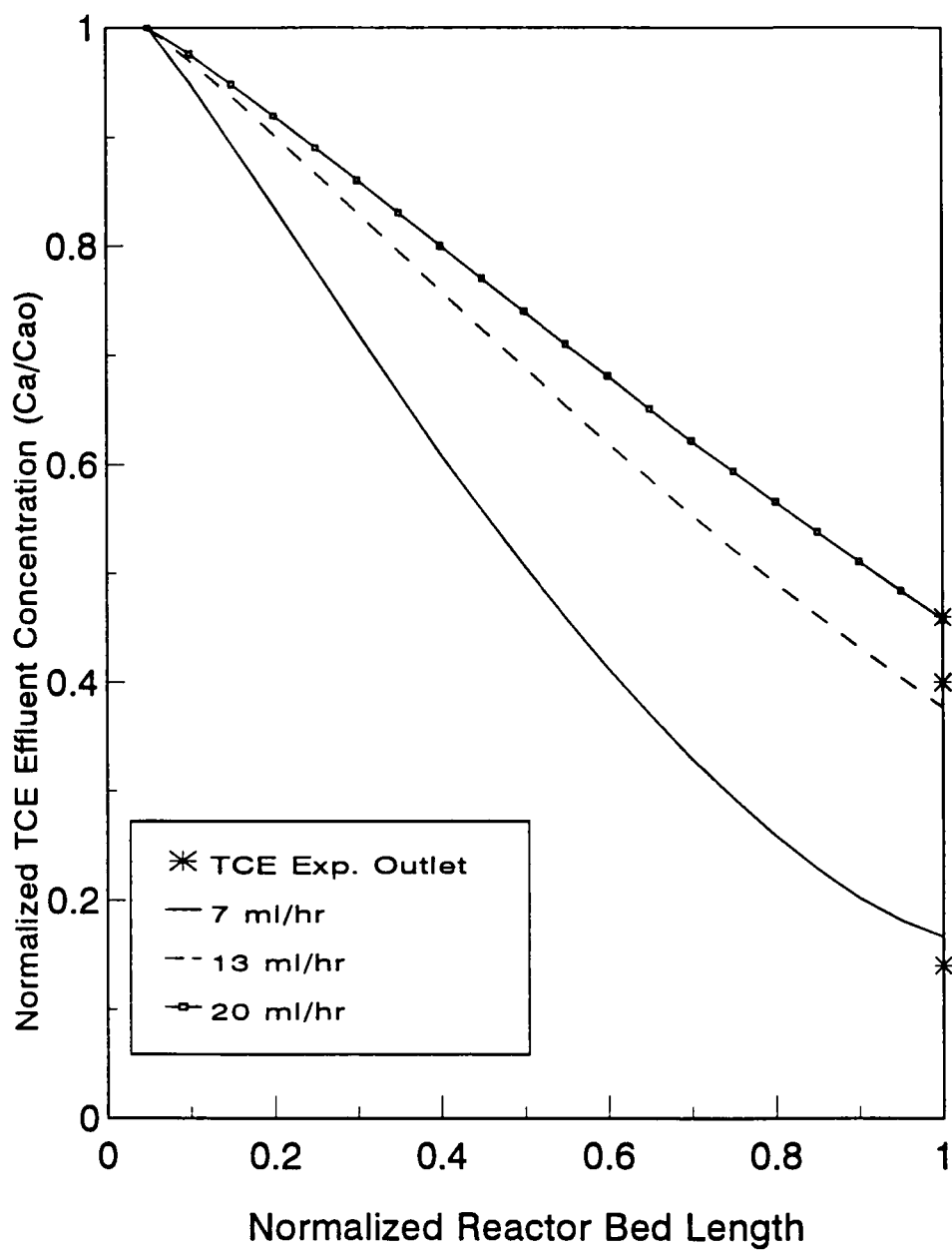


Figure 6.3 TCE degradation at varying flow rates vs. simulation results

This information aided in the ability to mathematically model degradation studies within the DVR system. The coefficients determined in the DVR reactor were also useful in modeling the larger 1.9 L reactor, thus showing the potential use of the DVR system to aid in scale-up and design of *in-situ* and pump and treat bioremediation techniques. TCE degradation rate constants were determined in both single toxicant and organic mixed-waste experiments. Results showed an increased recalcitrance of TCE and PCE with an increased complexity of organic mixed-wastes. Preliminary results indicate that the addition of benzene and toluene to the mixtures have less recalcitrance effect than other chlorinated compounds. Simulation predictions provided accurate information concerning electron donor and acceptor utilization as well as degradation potentials during reactor conditions which varied in biomass support matrix, reactor dimensions, and flow rates. Future work will concentrate on theoretically altering the input parameter representing the electron donor saturation constant and the electron donor concentration to advance modeling techniques of soil systems containing organic-mixed wastes.

CHAPTER VII

ANALYSIS OF A MATHEMATICAL SIMULATION DESCRIBING BIODEGRADATION OF MIXED-ORGANIC WASTES IN A PACKED-BED BIOREACTOR

INTRODUCTION

The mathematical simulation, reported in the previous chapter, that describes the fate of trichloroethylene in packed-bed reactor systems was expanded to further explore the usefulness of a mathematical representation to describe *in-situ* and on-site remediation techniques of mixed-organic wastes. The following approaches were made to improve simulation biodegradation predictions. (1) Three simulation parameters were varied to determine their relative effects on the degradation prediction of the mathematical simulation. The parameters varied were the Peclet number, the concentration of electron donor, and the donor saturation constant. (2) The ability of the simulation to predict effluent TCE concentrations in a mixed-organic waste system was improved. (3) The potential of the simulation to predict other researchers' results was investigated.

SIMULATION PARAMETER EFFECTS

A parameter sensitivity analysis of the solved equations [equations (6.3), (6.7a), (6.7b), (6.7c), (6.8), (6.9) and (6.10)] demonstrated the effects of flow characteristics described by the Peclet number (Pe), electron donor concentration (C_D), and the electron donor saturation constant (K_{SD}) on bioremediation potentials of mixed-organic wastes. Such a sensitivity analysis was performed to better understand the effects of the parameters on the solution.

The simulation was allowed to converge to an outlet TCE tolerance of $< 1\%$. The effects of varying the parameters over a single order of magnitude was monitored by observing the changes in TCE concentration over the length of the reactor bed as predicted by the simulation.

Peclet Number

The Peclet number is the dimensionless group defined as (interstitial velocity x reactor bed length)/(dispersion). This group typifies flow characteristics within a packed-bed reactor system. Theory predicts that as Pe approaches infinity, the closer the reactor behaves in a plug flow manner, and as Pe approaches zero, the reactor behaves as a CSTR. To study the effects of this parameter, Pe was varied within the reactor between 1 and 25 within the simulation. The Pe number was altered by changing the interstitial velocity. Results seen in Figure 7.1 show how TCE effluent concentration changed with respect to bed position.

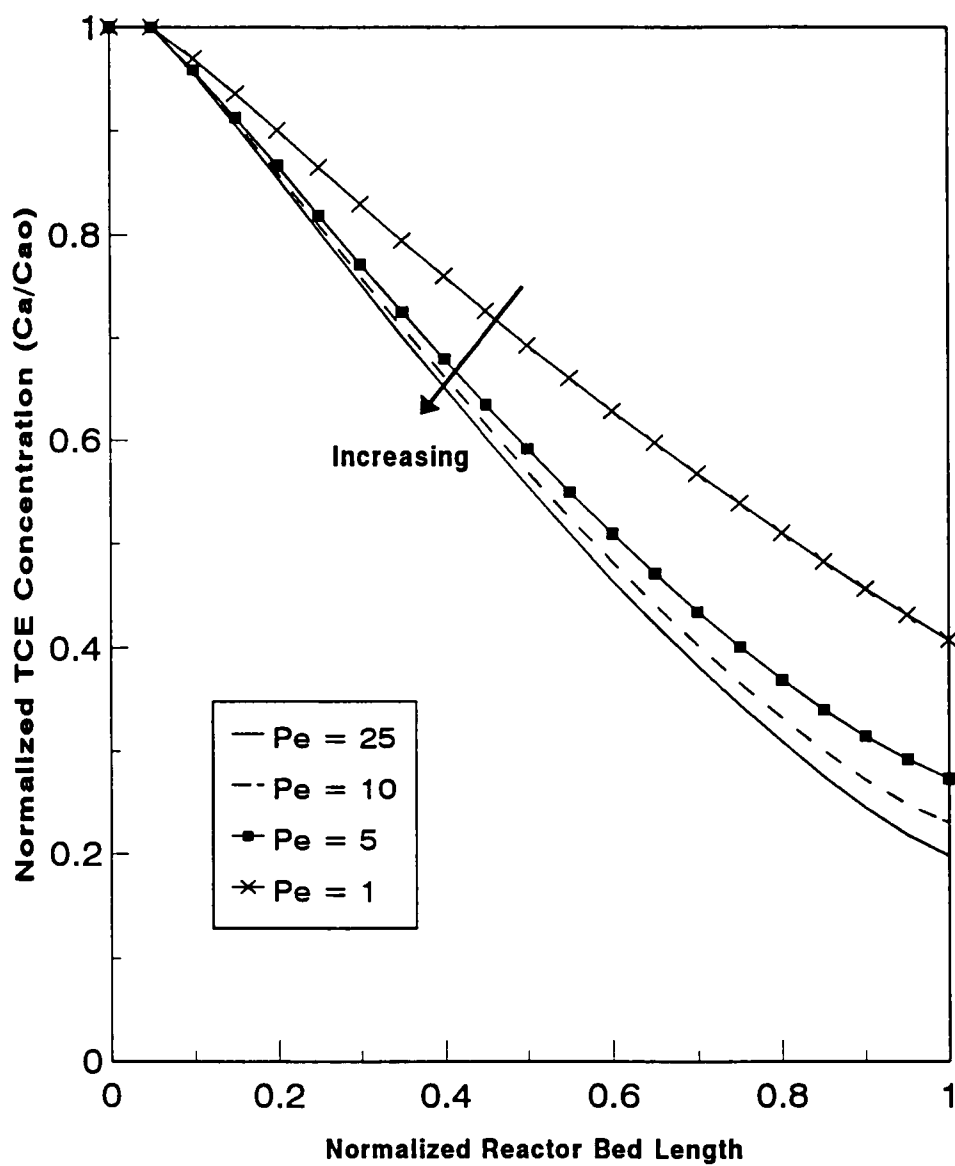


Figure 7.1 Simulation sensitivity to changing Peclet number, Pe

Figure 7.1 shows that as the Pe number increased, the final effluent concentration of TCE in the reactor decreased, thus implying that the TCE reaction rate within the bed is a function of the Pe number.

Donor saturation constant.

The numerical value assigned to the donor saturation constant has an effect on TCE degradation as can be seen in equation (6.6). The equation predicts that as K_{SD} decreases, there is an increased inhibitory effect on TCE degradation within the reactor. Values of this constant were altered within the simulation ranging from 1 to 25 mg/L. The magnitude of effect on TCE degradation with respect to reactor bed position can be seen in Figure 7.2. The simulation predicted that as K_{SD} increased from 1 to 25 mg/L, degradation within the reactor increased ~5%

Electron donor concentration

The electron donor concentration, C_D , also has an effect on the degradation potential within a packed-bed reactor. As the concentration of the electron donor increases, the competition between donor and TCE increases for active enzyme sites. As seen in equation (6.6), as the electron donor concentration increases, the simulation predicts less degradation of the compound degraded cometabolically. Figure 7.3 shows that as the electron donor concentration increased from 5 to 50%, degradation within the reactor decreased by 14%.

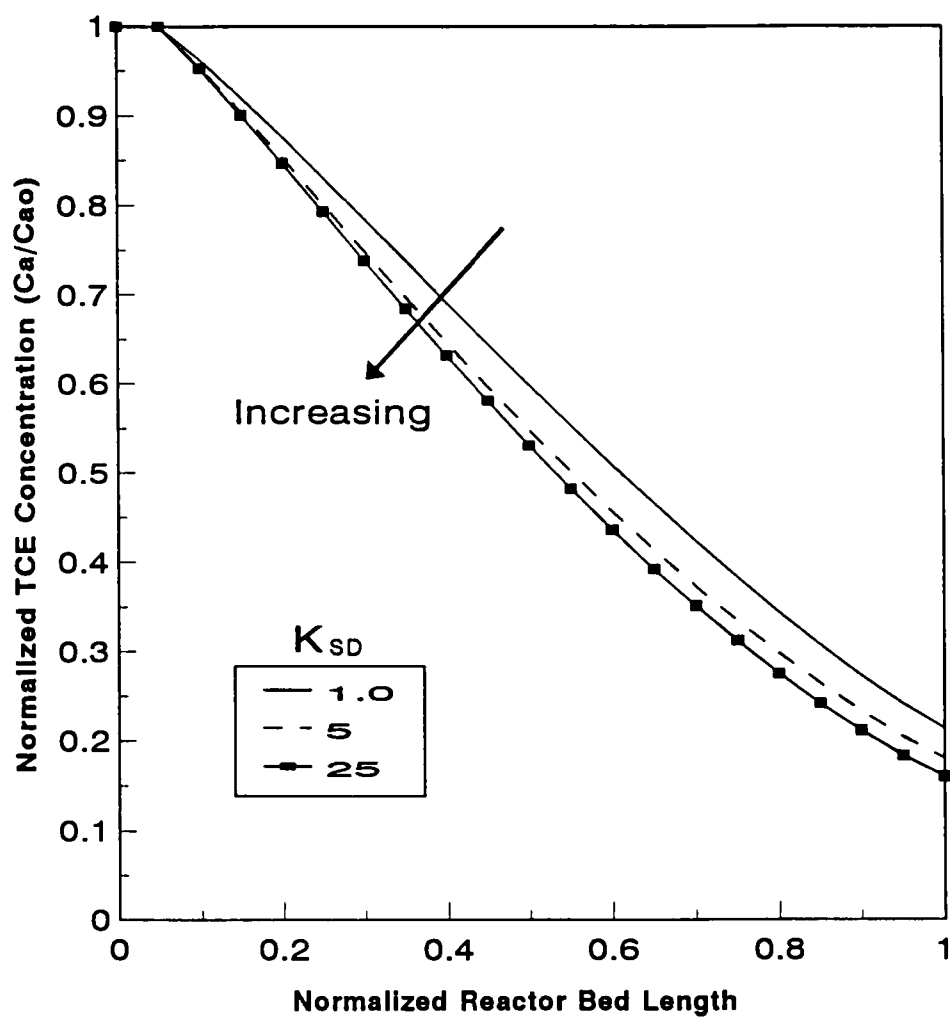


Figure 7.2 Simulation sensitivity to changing donor saturation constant, K_{SD}

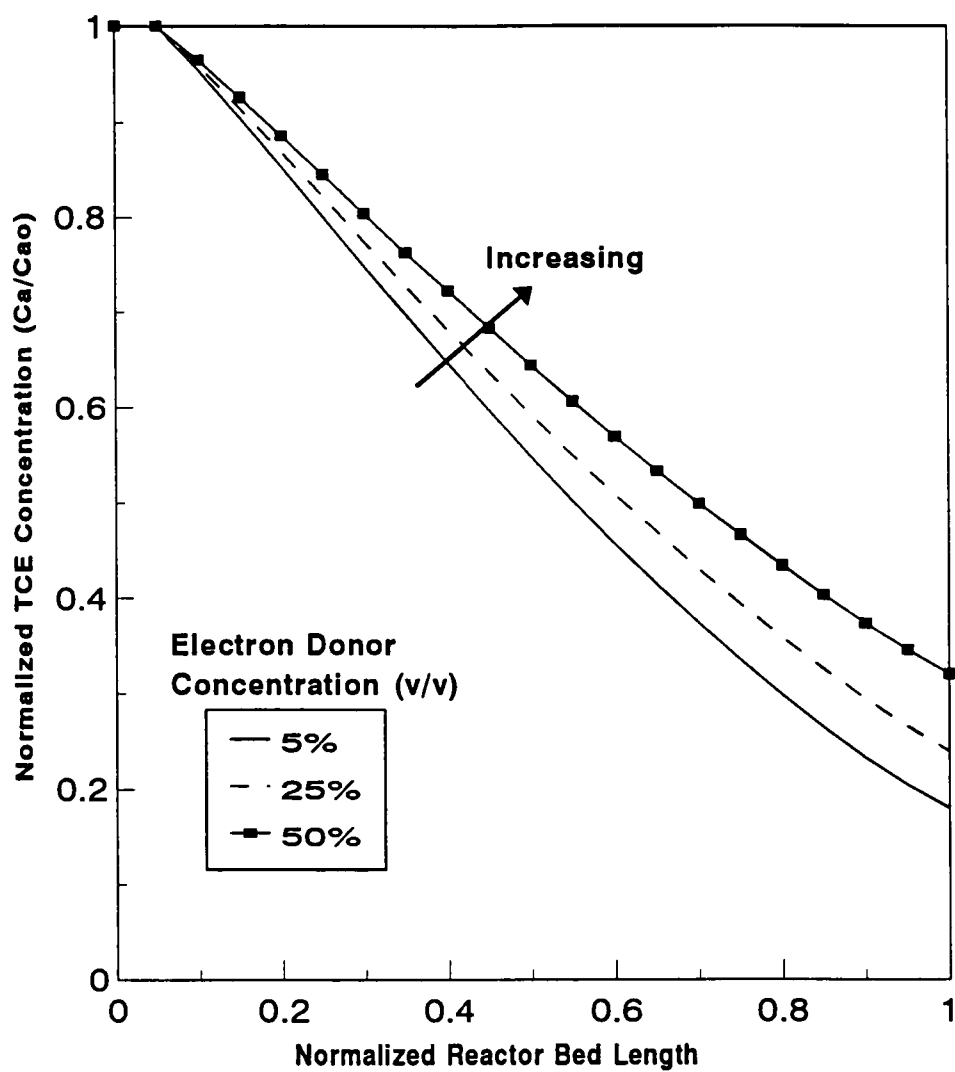


Figure 7.3 Simulation sensitivity to changing electron donor concentration, C_D

SIMULATION PREDICTIONS FOR MIXED-ORGANIC WASTE SYSTEMS

As previously noted, experimental results indicated an increased recalcitrance of TCE when other toxicants were present in the bioreactor. Therefore, the simulation was adapted to better predict TCE effluent concentrations under such circumstances. The simulation was altered by adjusting the donor saturation constant parameter with respect to the number of compounds in the system.

The necessary adjustments on the K_{SD} parameter were made in accordance to the following observations and manipulations. The effect of K_{SD} on TCE concentration throughout the bioreactor bed was observed in Figure 7.2. Figure 7.4 shows the effect of varying K_{SD} on TCE effluent concentrations. Experimental results provided knowledge of effluent TCE concentrations when there were different numbers of chlorinated aliphatics present in the system. As shown in Figure 7.4, experimentally derived TCE effluent concentrations associated with specific numbers of chlorinated organics were utilized to produce a relationship between K_{SD} and the number of organic compounds in the system. For example, when 10 chlorinated compounds were present during an experimental run, the average normalized (C_{outlet}/C_{feed}) effluent TCE concentration was 0.19. This effluent concentration is represented by a K_{SD} value of 4.5 (Figure 7.4). Figure 7.5 shows the results of the procedure comparing K_{SD} with the number of chlorinated organic compounds in an experimental system.

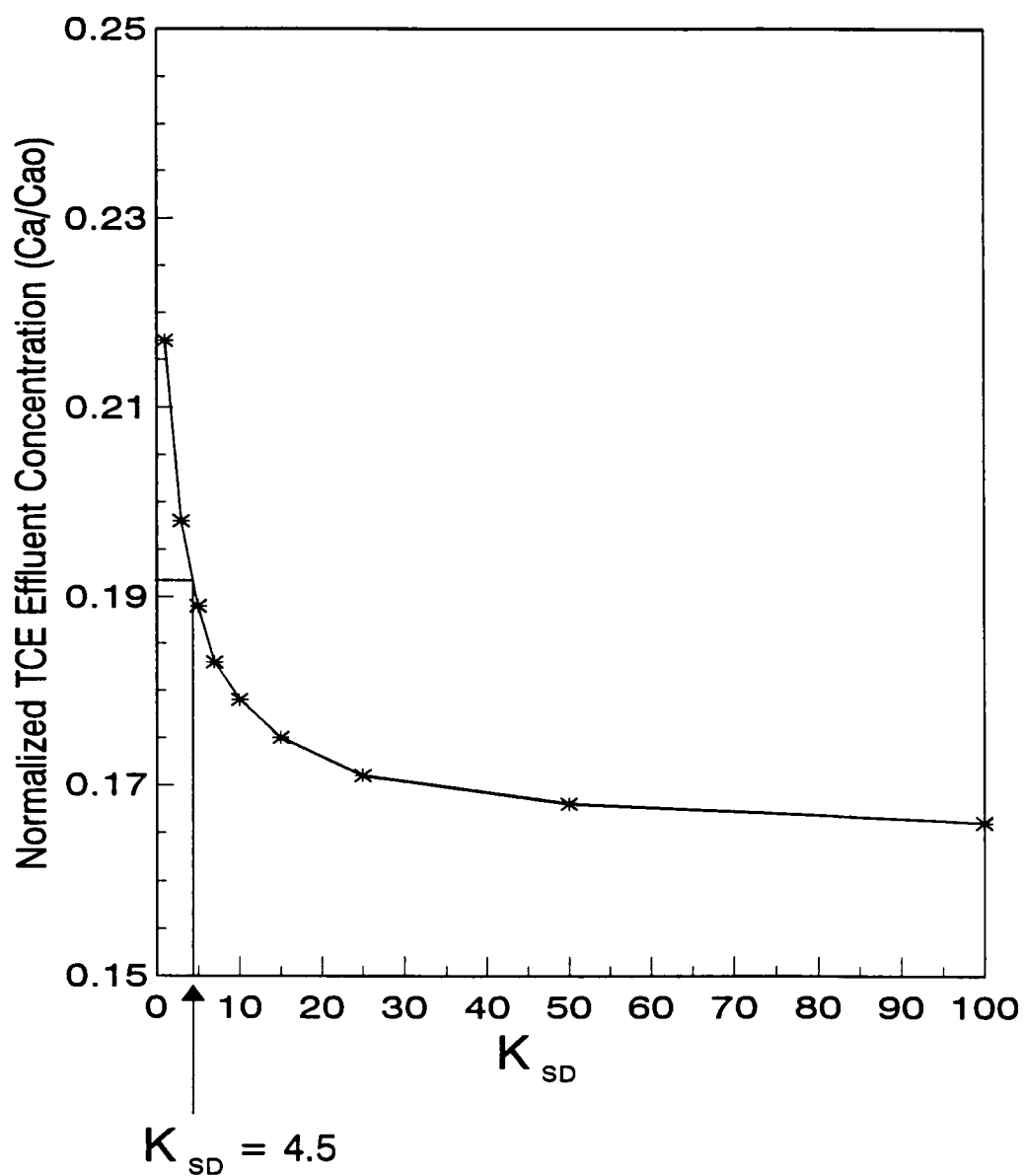


Figure 7.4 TCE effluent concentration as a function of the donor saturation constant, K_{SD}

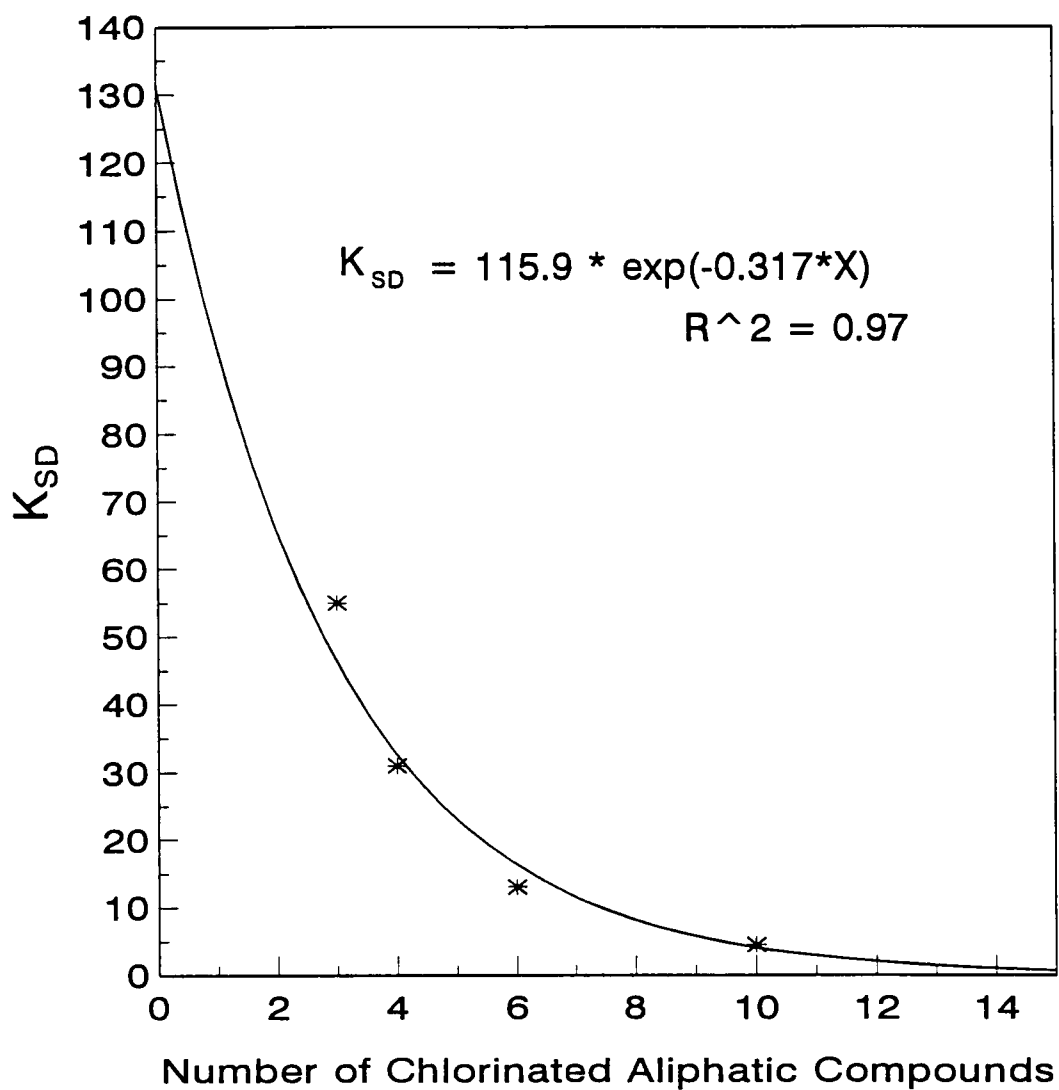


Figure 7.5 Relationship between donor saturation constant and the number of chlorinated aliphatics in a mixed organic-waste system

Least-squares analysis was used to correlate the data into the following relationship:

$$K_{SD} = 115.9 \times \exp(-0.317 \times \text{number of compounds}) \quad (7.1)$$

$$R^2 = 0.97$$

Figures 7.6 and 7.7 show simulation predictions for TCE concentrations as the number of chlorinated experimental compounds vary between 1 and 12. The superficial velocities in the bioreactors are represented at 7 and 13 ml/hr, respectively. Experimental TCE effluent concentrations in mixed organic-waste systems are compared to simulation predictions in Table 7.1. The TCE effluent concentrations are accurately correlated, with the largest error ~9% when comparing experimental versus simulation results.

Table 7.1 Experimental TCE Effluent Concentrations in Mixed Organic-Waste Systems Compared to Simulation Predictions

Number of Compounds	Experimental TCE Effluent Concentration (mg/l)	Simulation Predicted TCE Effluent Concentration (mg/l)
1	0.06 ± 0.01	0.066
5	0.23 ± 0.07	0.226
12	0.27 ± 0.08	0.294

The inlet TCE concentration was 2.0 mg/l.

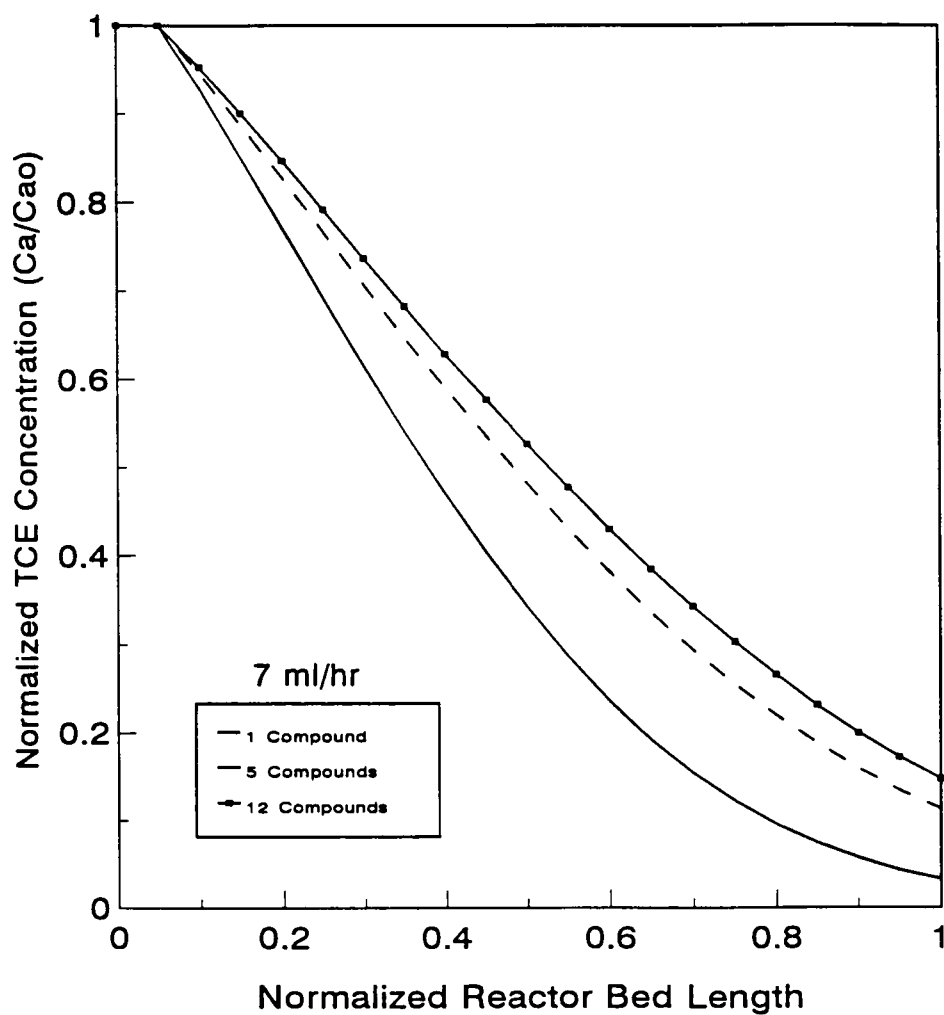


Figure 7.6 Simulation predictions of TCE effluent concentration in mixed organic-waste systems at a flow rate of 7 ml/hr

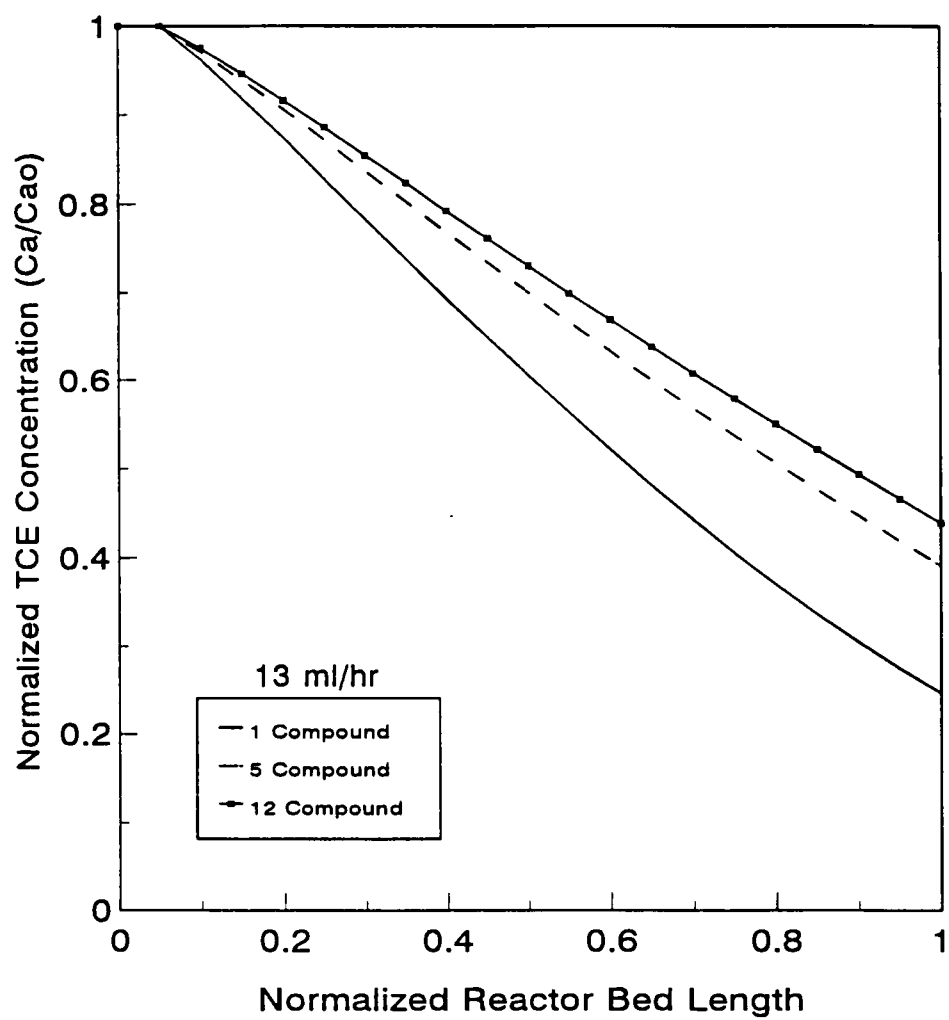


Figure 7.7 Simulation predictions of TCE effluent concentration in mixed organic-waste systems at a flow rate of 13 ml/hr

APPLICATION OF THE SIMULATION TO PRIOR RESEARCH

The primary intent for the simulation development was to reliably estimate the experimental reactor results of the current study. But, the utility of such a simulation is limited, unless it is applicable to packed-bed reactor systems used by other researchers to study the cometabolic degradation of chlorinated aliphatic compounds. The effort of testing the flexibility or the predictive nature of the simulation when applied to the results of others was hampered by insufficient descriptions of experiments reported in the literature. The author could find no available published material that provided all of the parameter values that described experimental design and results which are critical to simulation operation. As a result, it was not possible to accurately estimate the ability of the simulation to describe the work of others. Variables within the simulation can be chosen to force the simulation to exactly predict the solute fate of any experimental result within a packed-bed system. The broad predictive nature of the simulation is confirmed by the range of TCE effluent concentrations achievable by altering one variable with the simulation. This can be verified by recalling Figures 7.1-7.3 and 7.6-7.7.

Although no available text provided all the needed simulation variables, several provided most of the needed parameters. In such instances, remaining required parameters were estimated or theoretically calculated by the author. In no instance were the parameters altered just to

give a best fit to other researchers results. Results were utilized from a specific manuscript. Table 7.2 gives the reference manuscript, a summary of variables not specifically addressed in the manuscript, and a comparison of simulation versus experimental results.

A brief description of the experimental apparatus used in the manuscripts listed in Table 7.2 is provided. A fixed-film, packed-bed bioreactor utilizing a methanotrophic TCE degrading consortium was studied by Strandberg et al., 1989. The bioreactor consisted of a glass column with a 5 cm inner diameter and 110 cm in length. The column was packed with 0.6 cm ceramic berl saddles (Scientific Products, McGaw Park, IL) to serve as a support matrix for the biofilm. Methane was the primary substrate (4% v/v) introduced with air into the reactor in a concurrent fashion. Liquid flow rates ranged between 0.05 and 0.005 L/min giving mean residence times of 11 to 75 min. The typical inlet TCE concentration was 1 mg/L.

Variables required for the simulation that were not available in the two manuscripts listed in Table 7.2 are as follows: void volume, ϵ ; biomass concentration, X ; and the Peclet Number, Pe . Perry et al., suggested a void volume of $\epsilon = 0.6$ for reactors packed with the Berl saddles described above. A conservative steady-state biomass concentration, $X = 0.2$ g/L, was chosen from a literature review (Bouwer & McCarty, 1984) discussing similar reactors. A Peclet number of 40 was chosen to represent an intermediate amount of dispersion (Levenspiel, 1972).

Table 7.2 Application of the Simulation to Prior Research

Reference	Estimated Parameters	Simulation Error ^a %
Garland et al., 1989 and Strandberg et al., 1989	$\epsilon = 0.6$ $X = 0.2 \text{ g/L}$ $Pe = 40$	18.9 ± 15.8

^aSimulation error calculated by:

$$\Delta \text{Exp.} = \frac{\text{Experimental inlet conc.} - \text{Experimental effluent conc.}}{\text{Experimental inlet conc.}}$$

$$\Delta \text{Simul.} = \frac{\text{Experimental inlet conc.} - \text{Simulation effluent conc.}}{\text{Experimental inlet conc.}}$$

$$\text{Simulation err\%} = \frac{\Delta \text{Exp.} - \Delta \text{Simul.}}{\Delta \text{Exp.}} \times 100$$

The simulation was compared to experimental results using six different flow rates. Table 7.3 shows the comparison of experimental results with simulation predictions. The absolute simulation error ranged from 5.0 to 43.3% having a mean value of $18.9 \pm 15.8\%$. Even with the insufficient knowledge of variables, the simulation provided a very reasonable tool to estimate TCE effluent concentrations in this type of reactor system.

Table 7.3 Comparison of Experimental Results with Simulation Predictions

Flow rate	Experimental TCE		Simulation	Simulation
	inlet	effluent	effluent	error
L/min	mg/L	mg/L	mg/L	%
0.005	0.9	0.2	0.32	17.1
0.010	1.0	0.3	0.43	18.6
0.010	1.1	0.5	0.47	-5.0
0.020	1.0	0.5	0.54	8.0
0.035	1.1	0.8	0.67	-43.3
0.050	1.0	0.6	0.75	37.5

CHAPTER VIII

**BIOLUMINESCENT REPORTER TECHNOLOGY FOR
MONITORING THE FATE OF TCE AND TOLUENE**

ABSTRACT

Experiments were conducted which focused on the utilization of genetically engineered bacteria containing the *lux* gene cassette to monitor the biodegradation of toluene and/or trichloroethylene. Three different systems, utilizing either bioreactor or batch vials, were used. This chapter presents preliminary results addressing the feasibility of using bioluminescent reporter technology as a technique to rapidly measure catabolic gene expression in mixed- and pure-culture environments.

A differential volume reactor system was used to monitor the bioluminescent and degradative response of the reporter B2. B2 is *Pseudomonas putida* F1 that contains the *tod-lux* fusion plasmid. The strain produces bioluminescence in the presence of toluene and has the ability to degrade the compound as well as to fortuitously metabolize TCE. This reactor system used alginate beads to encapsulate the bioreporter.

Batch vial studies were performed with B2 to quantitatively predict toluene concentration by bioluminescence monitoring of catabolic gene expression.

An expanded-bed bioreactor used sand as a solid support matrix for a TCE-degrading consortia and the engineered reporter strain Rb1401. This bioreactor system was used to demonstrate the applicability of inserting a molecular bioreporter into a previously established microbial consortium to monitor bacterial activity, degradation ability, and bioluminescence in relation to system perturbations.

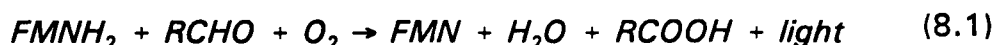
INTRODUCTION AND BACKGROUND

The measured success and optimization of field application bioremediation could be improved by the availability of better internal biodegradation markers and the ability to rapidly assess catabolic gene expression. Recent advancement in monitoring relevant microbial activity has been enhanced through the utilization of bioluminescent *lux* "reporter" strains.

Luminous bacteria have been found in marine, freshwater and terrestrial environments and are commonly isolated growing on dead fish or meat (Gordon, 1992). Classification of almost all luminous bacteria fall into the three genera *Vibrio*, *Photobacterium*, and *Xenorhabdus* (Meighen, 1991). Many of these bacteria can be found living in sea water or as parasites in the hemolymph of different crustaceans.

The oxidation of reduced riboflavin phosphate (FMNH₂) and a long chain fatty aldehyde is necessary for the light-emitting reaction in bacteria to

occur. The reaction is highly specific for FMNH₂, produces a blue-green light, and is catalyzed by bacterial luciferases. The reaction is as follows:



A number of bacteria have been engineered to contain the *lux* gene cassette in critical operons of processes that are involved in the biodegradation of mixed organic wastes. Some advantages for using these bioluminescent bacteria as reporters for gene expression include their ability to economically detect microbial degradation activity in a rapid, on-line, and real-time fashion. Researchers have shown these bacterial tools to successfully determine organic concentrations and the bioavailability of specific compounds (Burlage et al., 1990; King et al., 1990; Heitzer et al., 1992). Others have used either CSTR/soil slurry reactors (DiGrazia, 1991) or packed-bed reactors (Dunbar, 1992; Webb, 1992) to characterize and model bioluminescent and biodegradation processes.

The general objective of these preliminary experiments was to test the feasibility of using bioluminescent bacteria as a tool to gain insight into microbial metabolism and cometabolism in mixed and pure microbial consortia while degrading mixed organic wastes. Specifically, the DVR and the expanded-bed bioreactors were used to monitor correlations between (a) toluene concentration and bioluminescence and (b) TCE and toluene degradation and bioluminescence. The results are discussed in this chapter.

RESULTS AND DISCUSSION

Differential Volume Reactor

Preliminary experimental results display a very rapid and seemingly instantaneous bioluminescent response within the differential volume reactor upon the introduction of toluene. The light emitted is a function of the number of genes, the active RNA polymerases and ribosomes, and the number of luciferase molecules in the cell. This light reaction is often described using first order processes characterized by the following three differential equations (Freifelder, 1987).

$$\frac{dE_i}{dt} = k_{qL}^o \xi_i R_L - k_{eN} E_L - \mu E_{Li} \quad (8.2)$$

$$\frac{dR_L}{dt} = k_{pL}^o \eta_i G_N - k_{dL} R_L - \mu R_L \quad (8.3)$$

$$L = q \times 2.376 \times 10^{-12} \times E_L \quad (8.4)$$

The curve depicted in Figure 8.1 shows the typical first-order light-emitting response predicted by the above equations. The shape of the curve does not change as the parameters in the equations are altered, only the maximum predicted light is altered. Other researchers have shown this light emitting reaction to be of second order or higher (DiGrazia, 1991; Webb, 1992; Dunbar, 1992). Figure 8.1 also shows the light response of reporter B2 in the DVR when induced by 10 mg/L toluene at a flow rate of 0.4 ml/min.

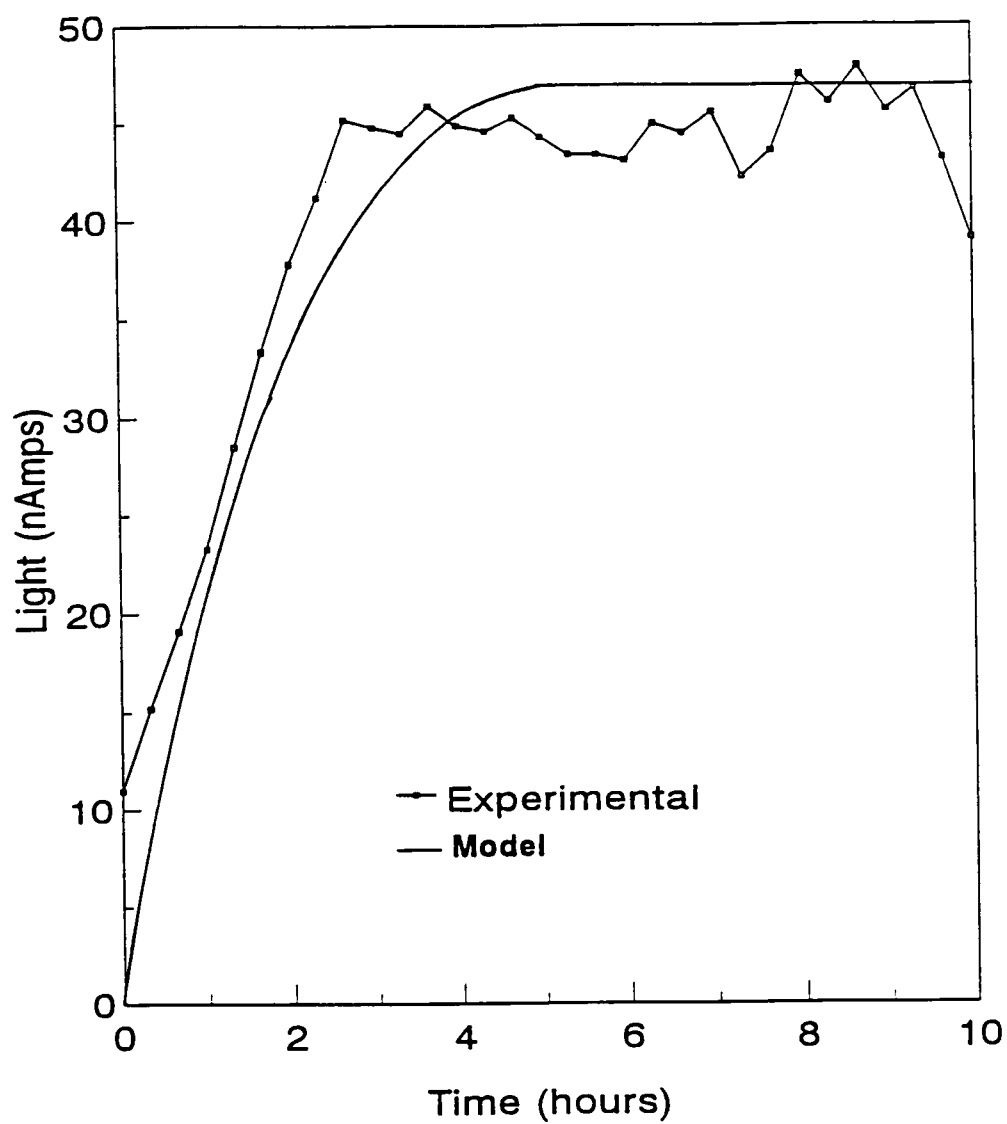


Figure 8.1 Typical first-order light-emitting response predicted and experimental bioluminescent response of reporter B2 in a DVR bioreactor

The maximum steady-state light production observed under these conditions was 43.4 ± 6.8 nAmps. Note the similarities between the shape of the theoretical and experimental bioluminescent curves upon induction.

Experimental results are similar to the curve shown in Figure 8.1, which is typical of the first order response predicted by Freifelder and equations (8.2), (8.3), and (8.4). It is hypothesized that different cellular physiology and regulatory systems account for the contrasts in bioluminescent responses between each unique reporter strain.

The DVR system inoculated with B2 immobilized in alginate beads was used to determine the light response of the strain when induced by toluene. The inlet concentration of toluene was altered by using square-wave perturbations having 20 hr cycles. During feed portions of the cycle, 10 mg/L toluene was introduced into the inlet of the reactor. The system response to such perturbations are depicted in Figure 8.2 which shows both the light response of the reporter strain in the reactor and the step changes made with the inlet concentration of the inducer. This data suggests a direct correlation between bioluminescence of B2 and toluene presence in the system. During the cycle, light emission increased by 16.3 ± 1.2 nA/hr. The toluene effluent concentration approached zero after the toluene feed was stopped, and the light response in the reactor decreased at a rate of 3.4 ± 0.8 nA/hr.

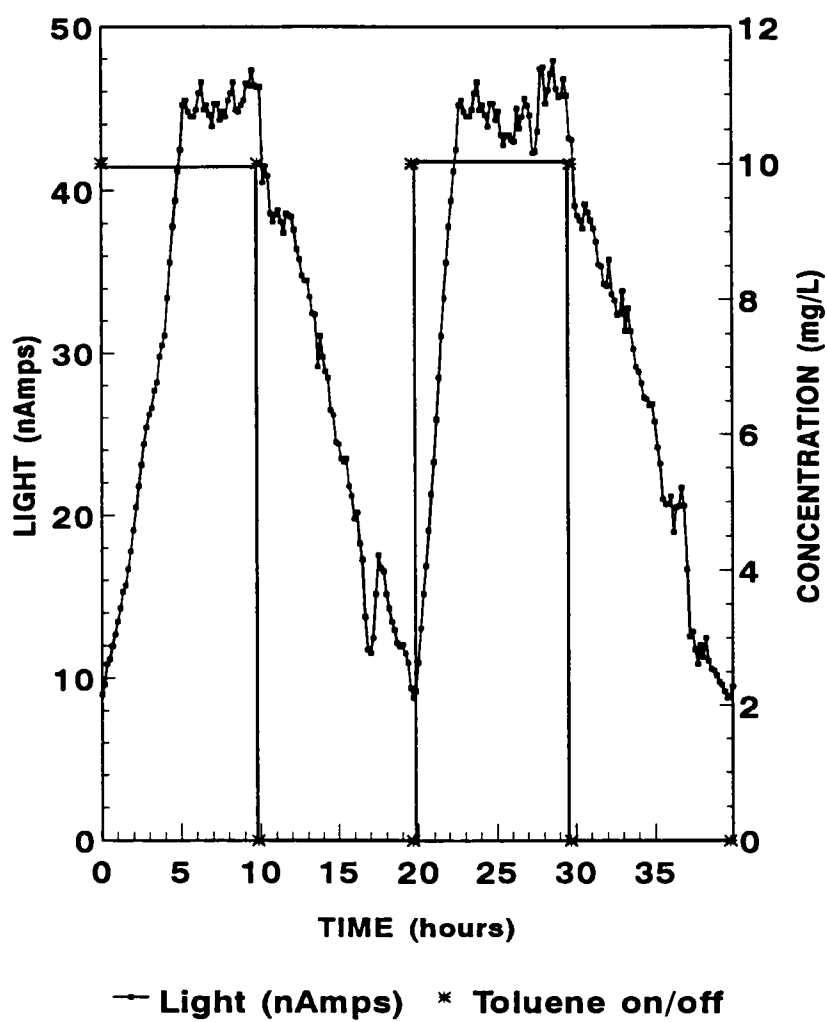


Figure 8.2 Bioluminescent light response of bioreporter strain B2 during square-wave perturbation of 10 mg/L toluene in 20 hr cycles

Figure 8.3 depicts the response of the DVR system described above when 20 mg/L of TCE is also continuously added to the feed stream. As the system is induced with toluene, light is emitted and TCE is degraded. These data shows a direct correlation between light emission and TCE degradation. The maximum light response was 43.4 ± 6.8 nAmps. The system degraded approximately 20% of the TCE and 50% of the toluene. This gave a steady-state TCE effluent concentration when toluene was being introduced into the system of 16.5 ± 0.2 mg/L, and an effluent toluene concentration of 5.8 ± 0.1 mg/L. This represents a ratio of $1.7 \mu\text{mol}$ toluene degraded/ μmol TCE degraded.

A control experiment was conducted in the DVR system using the wild strain *Pseudomonas putida* F1. Conditions of this experiment match those of the experimental design described above. Results showed approximately 15% of the TCE and 80% of the toluene were degraded. This gives a steady-state concentration of TCE and toluene of 17.1 ± 0.3 and 1.8 ± 0.2 , respectively, when toluene is being introduced into the reactor. Therefore, $89 \mu\text{mol}$ TCE and $22 \mu\text{mol}$ of toluene were degraded giving a ratio of $4.0 \pm 0.6 \mu\text{mol}$ toluene degraded/ μmol TCE degraded.

The anomaly between the toluene removal rate of the wild type strain *P.putida* F1 and that of the bioluminescent reporter strain B2 have several plausible explanations (Applegate, 1992) and are listed as follows.

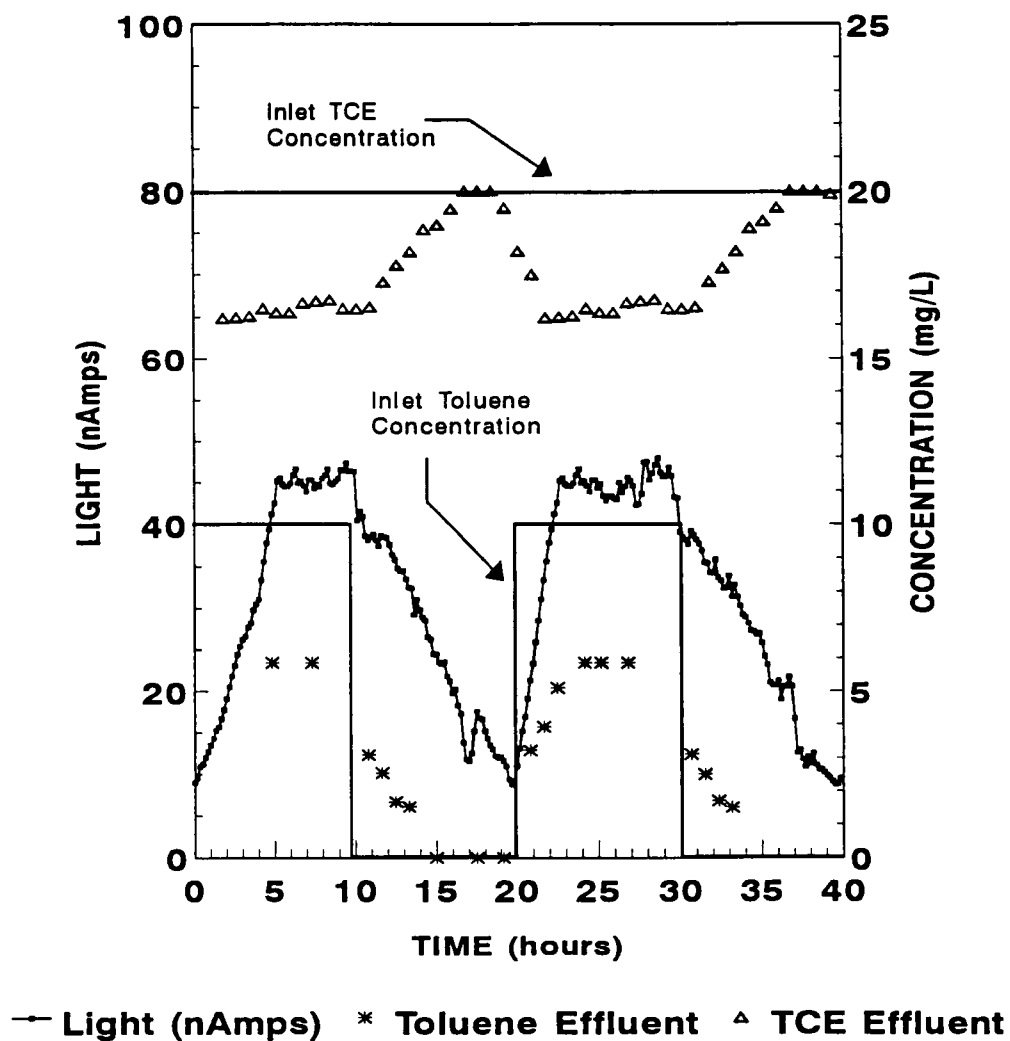


Figure 8.3 Bioluminescent light response and TCE and toluene degradation in the DVR system containing B2. Reactor was subjected to square-wave perturbations of 10 mg/L toluene.

1. The light reaction competes for oxygen and cofactors associated with toluene degradation.
2. The binding site (promoter) for the positive regulatory protein of the *tod* operon is out-competed by the same site found on the reporter plasmids, reducing the level of transcription.

Batch Vial Experiments

Preliminary batch vial experiments were also conducted with the reporter B2. A correlation was observed between toluene concentration and bioluminescent light response in the vial. Figure 8.4 represents a time-course of toluene-induced bioluminescence in the growing reporter strain B2. Results are presented for vials labeled control, 1 mg/L, and 25 mg/L, representing the concentration of toluene added to the culture growing in LB media. The control had no toluene added to the media. The maximum light emission from the three sets of experimental vials occurred at the experimental endpoint time of one hour. The light emission data for the three sets of labeled vials are represented in Figure 8.4 and are 1.053 ± 0.001 , 1.222 ± 0.004 , and 2.038 ± 0.010 nAmps, respectively. The initial OD_{546} of the growing culture added to the vials was 0.187 ± 0.001 . The OD_{546} after one hour was 0.878 ± 0.016 . The final OD_{546} of the control vials where no toluene was added was 0.860 ± 0.006 .

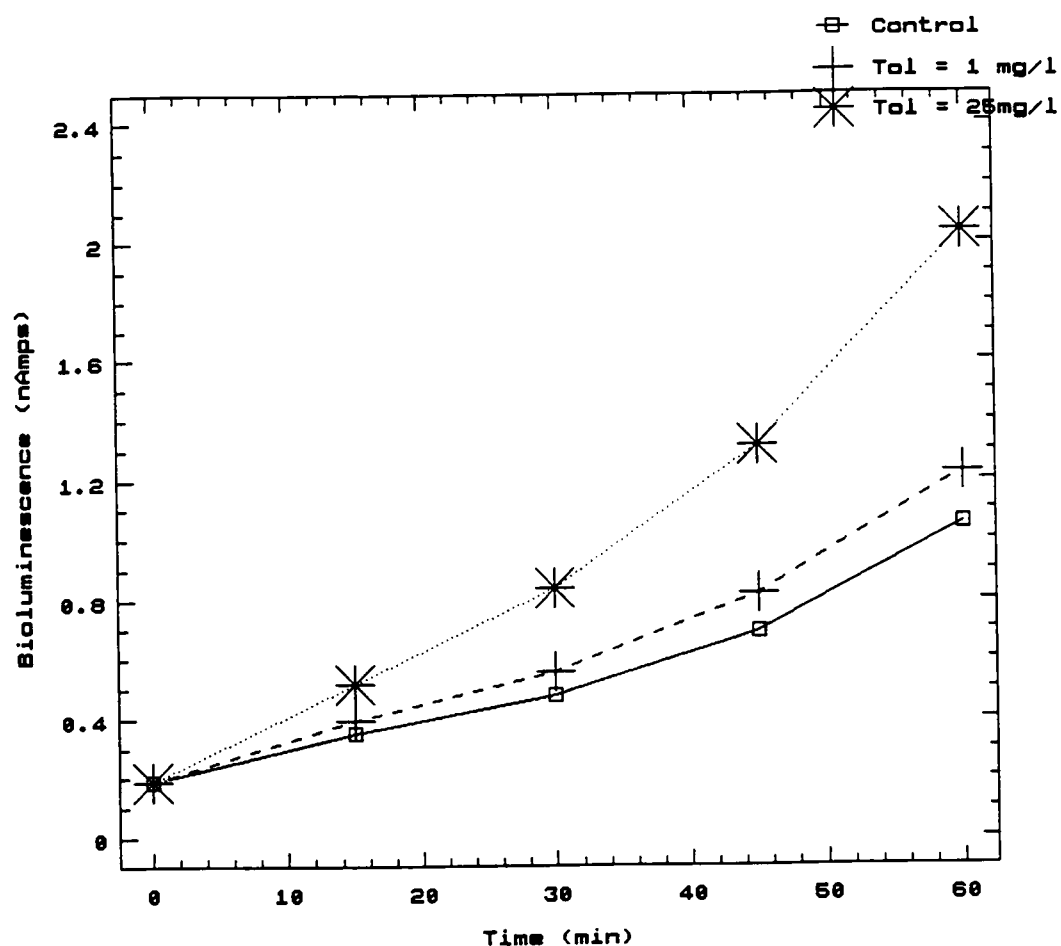


Figure 8.4 Time-course of toluene-induced bioluminescence in growing *P. putida* B2. Samples include 1 mg/L and 25 mg/L toluene added to triplicate vials, as well as control samples.

Figure 8.5 shows the relationship between initial toluene concentration and the bioluminescent response of B2 or the *tod* gene expression. Results are shown of light emitted versus toluene concentration after a one hour incubation of a growing culture. Bioluminescence reached a plateau above toluene concentrations of 25 to 50 mg/L and showed a maximum light of 2.18 ± 0.15 nA. This preliminary finding indicates a linear relationship between toluene concentration and bioluminescence when toluene concentrations are between 0 to 5 or 10 mg/L. This result is similar to that observed by Heitzer et al., 1992 when using the bioluminescent reporter strain *Pseudomonas fluorescens* HK44 for monitoring the bioavailability of naphthalene and salicylate. Further experimentation is needed to verify the linear relationship between toluene and light emission for the reporter B2.

Expanded-Bed Bioreactor Experiments

Preliminary studies were conducted using an expanded-bed reactor to study the feasibility of introducing a molecular bioreporter (Rb1401) into a previously established TCE degrading microbial consortium (SRP) to correlate bioluminescence with bacterial degradation activity. Evidence from this study indicates that inserting a molecular bioreporter into an existing steady-state microbial community is a difficult task.

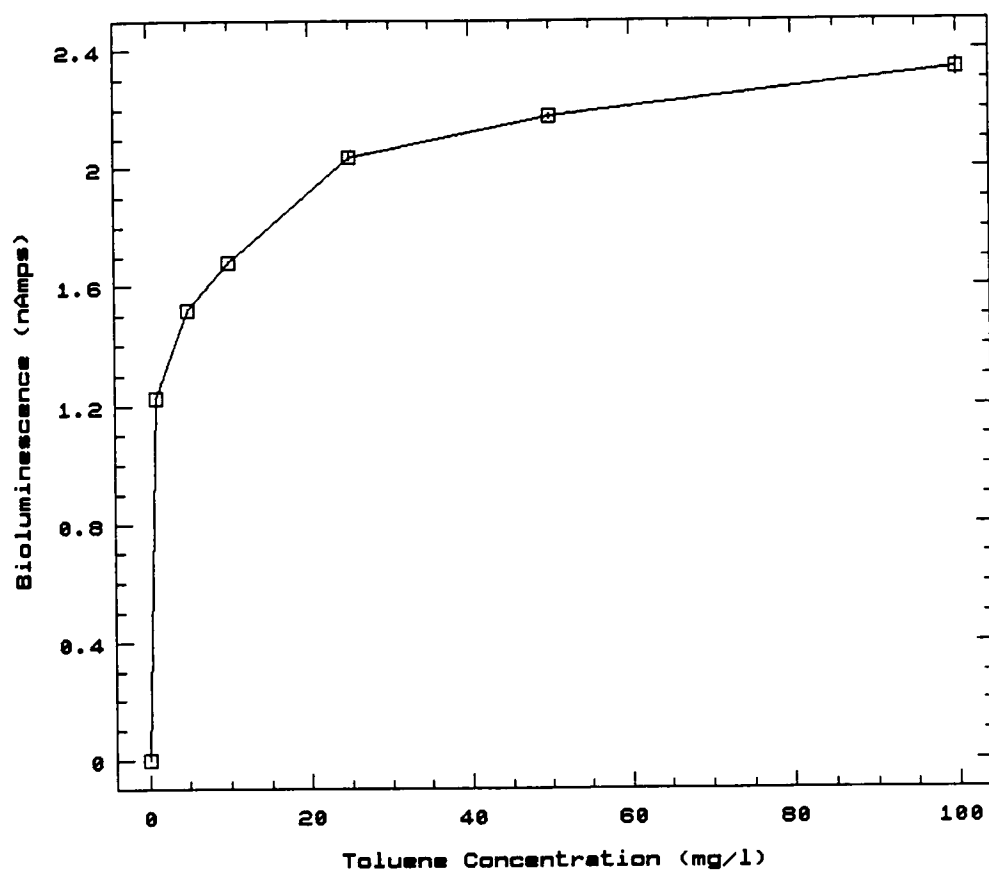


Figure 8.5 Relationship between toluene concentration and bioluminescence of *P.putida* after a 1 hr incubation

Figure 8.6 shows the average bioluminescent light in three different phases within the bioreactor two hours after induction with toluene. Each bar in the graph represents the average of triplicate data points taken at three different ports in each phase. Table 8.1 summarizes such data taken at two and 12 hours after inoculating. Notice from Figure 8.6 and Table 8.1 that two hours after introducing toluene into the system, the bioluminescence in the liquid phase is much higher than that observed in the solid sand and biomass phase. The average light produced in the liquid versus sand phase was 96.0 ± 9.1 and 4.0 ± 0.6 nAmps, respectively. But after 12 hours, as seen in Table 8.1, the only bioluminescence response detected in the reactor was in the sand phase. Because of this finding, the bioluminescence data presented in Figures 8.7 and 8.8 were taken from the sand phase of the reactor.

Figure 8.7 represents a three-day experiment in which concentration and bioluminescent measurement data were taken once daily. The experiment was conducted using initial concentrations of 20 mg/L

Table 8.1 The variation of light production as a function of time in the phases of the expanded-bed bioreactor

Time (hours)	Phase		
	Sand (nAmps)	Sand/Liquid (nAmps)	Liquid (nAmps)
2	4.03 ± 0.60	80.80 ± 14.97	96.00 ± 9.06
12	3.90 ± 0.01	0.10 ± 0.01	0.10 ± 0.01

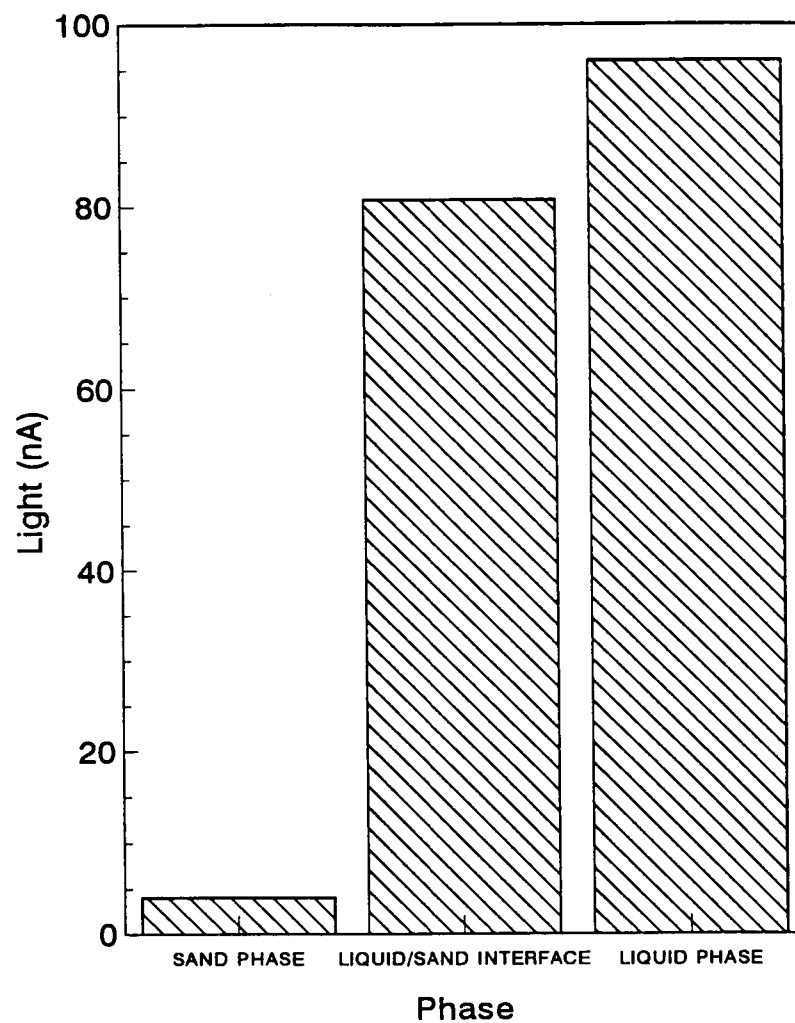


Figure 8.6 Variation of light production with respect to phase in the expanded-bed bioreaction 2 hr after the addition of 20 mg/L toluene

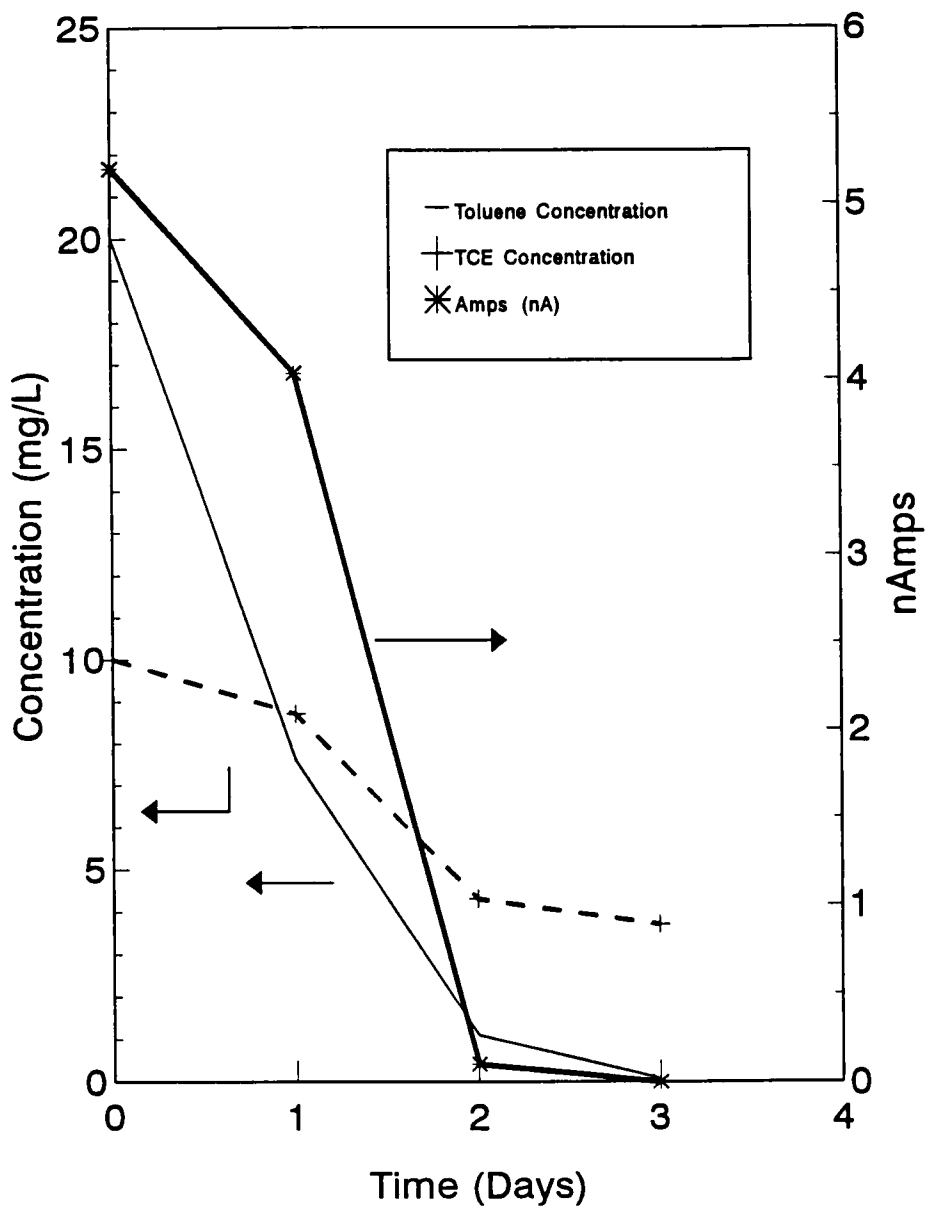


Figure 8.7 Bioluminescence of reporter Rb1401 and degradation of TCE and toluene in the expanded-bed bioreactor induced with 20 mg/L toluene

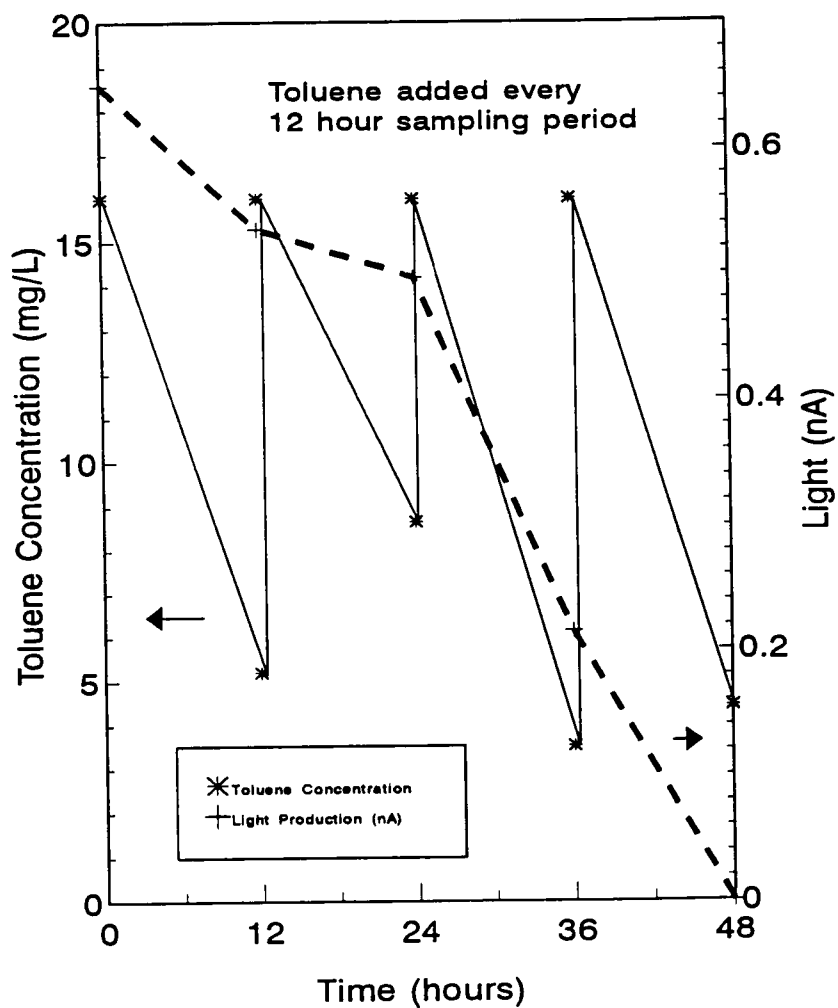


Figure 8.8 Bioreactor toluene concentration and bioluminescent response of reporter Rb1401 monitored at 12 hr intervals. During each sample period, toluene concentration was returned to the initial concentration of 16 mg/L.

toluene and 10 mg/L TCE. The maximum bioluminescence measurement was observed on day one and had a value of 5.20 ± 0.91 . For the first two days, the light response and toluene concentration decreased at a rate of 2.55 ± 0.47 nAmps/day and 9.45 ± 1.05 mg/L day⁻¹, respectively. From such an experiment there appeared to be some correlation between bioluminescent response and the microbial degradation or concentration of toluene.

Results from a follow-up experiment are shown in Figure 8.8. The initial concentration of toluene in the reactor was 16 mg/L and the maximum light response was 0.65 ± 0.17 nAmps. Toluene concentration and bioluminescent response data were taken every 12 hours, at which time toluene was added to return the concentration to the initial concentration of 16 mg/L. Results over a two day period when toluene was continually present in the reactor show that the light response in the sand phase steadily decreased at a rate of 0.33 ± 0.07 nAmps/day, until no light was detected at the end of 48 hours. Toluene was degraded at an average rate of 5.27 ± 1.12 mg/L per day. Such results indicate the reporter's inability to provide activity measurements within this previously established consortium. There are several explanations for such results: 1. The reporter plasmid is unstable, 2. there is no antibiotic in the medium to provide the selective pressure needed for the reporter to keep the plasmid, and 3. the reporter is being out-competed, or selected against.

CHAPTER IX

CONCLUSIONS

This work showed that a heterogeneous bacteria consortia, taken from a trichloroethylene-contaminated subsurface environment, was effective for degrading chlorinated aliphatic hydrocarbons. Engineered luminescent bacteria were employed to analyze the relationship between reporter degradative activities and bioluminescence. Additionally, preliminary results from *lux* bioreporter experiments show that such a technology potentially provides a method for monitoring microbial activity by bioluminescence. This capability would provide an on-site bioreactor or an *in-situ* remediation scheme with a much needed internal biodegradation monitor.

SINGLE-PASS PACKED-BED BIOREACTOR

The horizontal, single-pass packed-bed bioreactor system represented a low technology apparatus that exhibited the potential to consistently degrade chlorinated aliphatics to levels approaching drinking water quality. The reactor was subjected to concentration perturbations of critical system parameters such as electron donor and acceptor. Drinking water quality standard concentrations for TCE was approached during the starvation regime when pulsed feeding experiments were performed. The bioreactor

both aromatics and aliphatics. The aromatics in such a mixed-waste system displayed the potential to consistently be degraded to detectable limits when the hydraulic residence time in the reactor was ≥ 2.5 days. An increased recalcitrance of TCE was observed when mixed-organic wastes were present in the system as contrasted to a single-toxicant system. Under aerobic conditions, preliminary results also indicate 99% carbon tetrachloride degradation when initial concentrations of the toxicant was ≤ 50 mg/L. Previous studies indicate carbon tetrachloride degradation only under anaerobic conditions.

FEASIBILITY STUDIES

Results demonstrated that bioreactors, both recirculating expanded-bed and single-pass packed-bed, that contain a mixed consortia were capable of degrading TCE and mixed-organic wastes while utilizing methane and propane as primary carbon sources. Pulsed feeding of the microbial consortia resulted in the highest degradation efficiencies (μmol substrate utilized/ μmol toxicant degraded) in both reactor types. When the bioreactors were starved for several days (no substrate added), TCE degradation decreased significantly. Biological rate constants and degradation efficiencies were compared between reactor types. Higher first order rate constants were observed in the recirculating expanded-bed bioreactor when rates were compared to those found in the single-pass sand column. Results

from this study showed the potential usefulness of recirculating expanded-bed bioreactor for examining degradation potentials and resiliency of the microbial community under various different operating conditions such as Ph, medium, primary substrate availability, and organic-waste mixtures.

Phospholipid fatty acid profiles can characterize the microbial community structure of a TCE degrading consortium and provide evidence of protozoan species colonizing with the consortia. Monitoring the PLFAME provided a quantitative tool for assessing the abundance of protozoa within bioreactors as well as changes in the microbial community structure. Signature lipid biomarkers provided information concerning the activity of a TCE degrading consortia, thus providing the potential to qualitatively identify degradation activity during an on-site or *in-situ* remediation program.

MATHEMATICAL SIMULATION

A mathematical simulation was developed to describe the fate of solute transport through porous media. The simulation considered sorption, dispersion, advection, as well as incorporation of the microbial processes of substrate utilization, growth, secondary substrate degradation, competitive inhibition between primary and secondary substrate, and an increased recalcitrance of TCE in the presence of mixed-organic wastes. Solute transport was described by assuming one-dimensional, uniform flow. Input parameters were based on laboratory research or from the literature. The

simulation was able to correlate the performance of the single-pass packed-bed and the differential volume reactor systems very closely.

BIOREPORTER TECHNOLOGY

Preliminary experiments were conducted to determine the feasibility of using bioluminescent bacteria as a tool to gain insight into microbial metabolism and cometabolism. Insertion of the reporter Rb1401 into an existing microbial community in an expanded-bed reactor showed this specific reporter's inability in the current experimental environment to provide knowledge of consortia activity measurements. With additional experimentation, a more suitable environment for the reporter could possibly be determined, and the reporter could then monitor bacterial activity, degradation ability and bioluminescence in relation to system perturbations.

When the reporter B2 was encapsulated in alginate beads and packed in the differential volume reactor a first-order light response was observed when induced with toluene. When the system is induced with toluene, light is emitted and TCE and toluene are degraded. The data showed a direct correlation between light emission and TCE and toluene degradation. Batch vial experiments showed a correlation between toluene concentration and bioluminescent light response.

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APPENDICES

APPENDIX A
SINGLE-PASS PACKED-BED REACTOR DATA

Table A.1 Example data set from single-pass sand column KBr tracer experiments.

Sand Column Tracer Exp. (5 Molar KBr) 20 ml/hr		
Time	Peak Height	Normalized Conc.
(min)	(cm)	(C/C _{max})
1330	0.0	0.00
1380	0.0	0.00
1430	0.25	0.08
1500	0.9	0.27
1530	2.2	0.67
1590	2.9	0.88
1640	3.3	1.00
1680	3.3	1.00
1730	2.9	0.88
1780	2.1	0.64
1840	1.7	0.52
1880	1.25	0.38
1930	0.9	0.27
1980	0.6	0.18
2120	0.5	0.15
2150	0.4	0.12
2170	0.3	0.09
2200	0.3	0.09
2240	0.3	0.09
2280	0.3	0.09
2330	0.3	0.09
2380	0.3	0.09
2430	0.3	0.09
2500	0.2	0.06

Inlet concentration of KBr = 5M. Flow rate for this data set was 20 ml/hr.

Table A.2 Typical Carbon Dioxide Production in the Single-Pass Sand Column

Typical Carbon Dioxide Production in the Single-Pass Sand Column		
Time (days)	PBBM medium only (mmol produced)	PBBM + 50mg/L Glucose (mmol produced)
0	0	0
1	2.08	3.56
2	2.24	7.69
3	2.48	10.6
4	2.50	14.6
5	2.58	15.7
6	2.30	15.9
7	1.80	16.9

Table A.3 Typical Methane Utilization in the Single-Pass Sand Column

Typical Methane Utilization in the Single-Pass Sand-Column		
Time (days)	PBBM medium Only (mmol consumed)	PBBM + 50 mg/L Glucose (mmol consumed)
0	0.00	0.00
1	0.44	0.02
2	0.87	0.05
3	1.16	0.08
4	1.32	0.12
5	1.51	0.12
6	1.59	0.21
7	1.68	0.18

Table A.4 Typical Propane Utilization in the Single-Pass Sand Column

Typical Propane Utilization in the Single-Pass Sand-Column		
Time (days)	PBBM Medium Only (mmol consumed)	PBBM + 50 mg/L Glucose (mmol consumed)
0	0.00	0.00
1	0.78	0.21
2	1.09	0.42
3	1.58	0.53
4	1.87	0.66
5	2.04	0.95
6	2.24	1.26
7	2.32	1.61

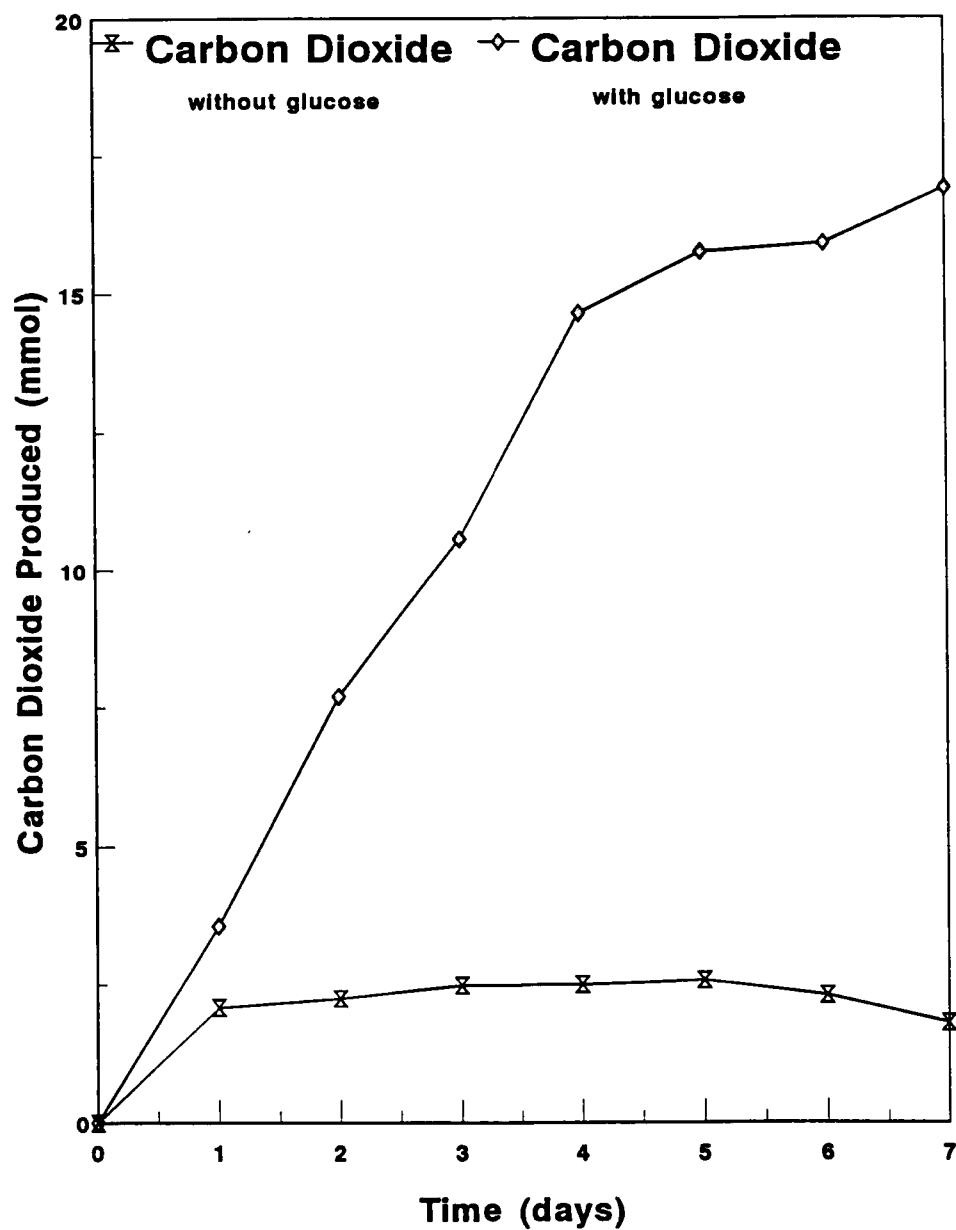


Figure A.1 Typical carbon dioxide production in the single-pass packed-bed bioreactor. Note increase in carbon dioxide production when glucose is present in the system.

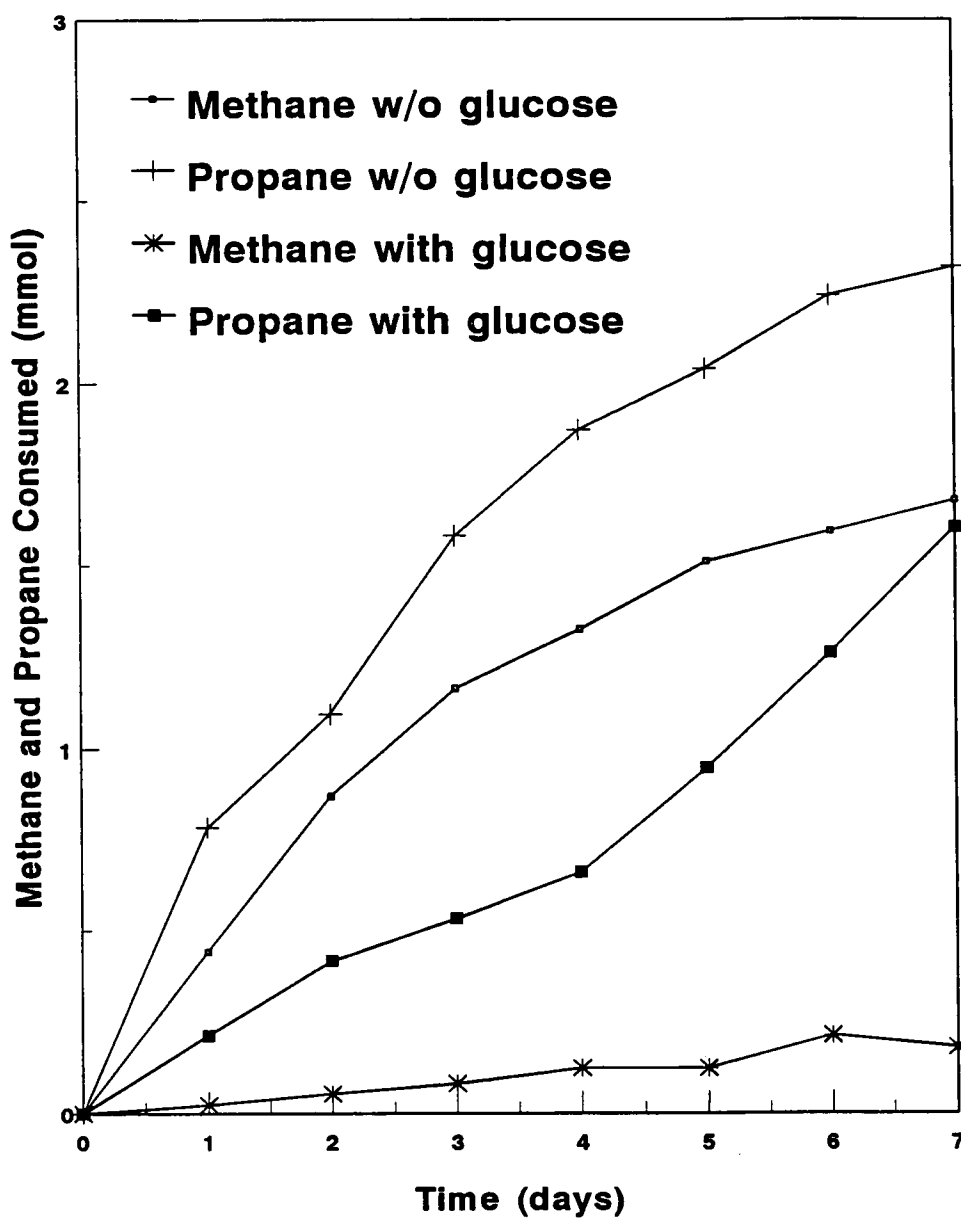


Figure A.2 Typical methane and propane consumption in the single-pass sand-column. Data shown for glucose present and absent from the system. Note the decrease in methane and propane consumed in the presence of glucose.

APPENDIX B***DATA SETS FOR SIGNATURE FATTY ACID BIOMARKERS***

Table B.1 Changes in microbial community structure during TCE degradation

Control	Error	Treatment	Error
Propane Utilizing Species (16:1 ω 6)			
0.000	0.000	0.000	0.000
3.530	0.420	1.360	1.930
6.570	0.240	6.290	0.180
4.660	1.440	7.270	0.320
5.870	0.600	7.030	0.180
Gram(+) Actinomycetes Sp. (Σ 10Me17:0, 10Me18:0)			
0.000	0.000	0.000	0.000
12.850	1.479	8.37	0.877
25.20	1.686	19.226	3.860
38.29	6.003	24.856	1.723
32.437	1.705	28.185	5.605

Table B.1 (continued)

Control	Error	Treatment	Error
Gram(+) <i>Arthrobacter</i> Sp. (Σ i14:0, i15:0, a15:0, i16:0, i17:0)			
0.000	0.000	0.000	0.000
0.15	0.15	0.000	0.000
1.710	0.349	1.63	0.187
0.783	0.339	1.83	0.156
0.943	0.189	2.48	0.72
Gram(-) <i>Pseudomonas</i> Sp. (Σ 16,18:1 ω F.A., cy17,19:0, i17:1 ω 7,17:1 ω 6)			
20.530	11.030	30.44	5.36
56.32	1.430	57.71	3.8
29.05	0.604	39.15	3.63
27.88	2.240	27.78	1.81
24.07	0.070	23.985	3.275

Table B.2 Utilization of TCE and Propane during
time course of experiment

Compound Degraded and 95% Confidence Intervals			
TCE		Propane	
% Degraded	Error	Peak Height (cm)	Error
0.000	0.000	-	-
0.000	0.000	14.7	0.17
21.00	0.000	10.6	0.95
58.00	3.460	5.6	0.45
67.50	4.950	4.9	0.71

APPENDIX C

BIOLOGICAL AND ABIOTIC TRACER RESULTS FROM DVR

Table C.1 Full data sets used for Haynes and Sarma (1973) analysis

X	Y
MESH 35-40	
33.535	0.3525
45.645	0.4110
65.729	0.4179
102.701	0.4928
MESH 50-60	
33.535	0.3252
45.646	0.3207
65.729	0.3314
102.701	0.4001
182.579	0.6415
MESH 78-80	
52.398	0.2678
56.352	0.3187
77.657	0.4075
102.701	0.4396
MESH 100-120	
33.535	0.3871
40.118	0.4613
45.645	0.5961
56.352	0.4756
65.729	0.5602
77.657	0.6385
102.701	0.8433
102.701	1.1148

Table C.2 Representative data set for effluent Gaussian shaped curves observed after a pulse perturbation of NaSal was introduced into the DVR

Representative Data Set for Gaussian Shaped Curves					
time (cm)	time (min)	conc (cm) ^a	time ²	time x conc	time ² x conc
0.5	1	0	1	0	0
0.6	1.2	0	1.44	0	0
0.69	1.38	1	1.90	1.38	1.90
0.79	1.58	3.5	2.50	5.53	8.74
0.9	1.8	6.2	3.24	11.16	20.09
0.97	1.94	7.05	3.76	13.68	26.53
1.12	2.24	5.55	5.02	12.43	27.85
1.37	2.74	3.25	7.51	8.91	24.40
1.64	3.28	1.75	10.76	5.74	18.83
2	4	1.05	16	4.20	16.80
2.3	4.6	0.82	21.16	3.77	17.35
2.9	5.8	0.5	33.64	2.90	16.82
3.32	6.64	0.4	44.09	2.66	17.64
4.2	8.4	0.3	70.56	2.52	21.17
		31.37		74.87	218.11
		tau ²		sigma ²	
		5.696		1.25	
		tau		sigma	
		2.39		1.12	

^a concentrations were measured as peak heights.

Abiotic controls within the DVR system provided an excellent mass balance. In Figure C.1 depicting data collected during a non-biological experiment, essentially 100% of the TCE introduced into the reactor was accounted for in the effluent stream. The inlet TCE concentration was 5.05 ± 0.1 while the effluent concentration was 5.07 ± 0.1 . In a subsequent experiment after the sand was inoculated with a TCE-degrading consortia, the TCE effluent concentration was 4.25 ± 0.07 , giving a 15.8% reduction.

Table C.3 Typical Steady-State Abiotic DVR Control Data and Biological TCE Degradation Data

Typical Steady-State Abiotic DVR Control Data and Biological TCE Degradation Data			
Time	TCE Inlet	Abiotic TCE Out	Biologic TCE out ^a
(hours)	(mg/L)	(mg/L)	(mg/L)
0.0	5.0	4.99	4.36
0.5	5.11	5.20	4.23
1.0	5.09	5.22	4.42
1.5	5.26	5.17	4.32
2.0	5.04	5.23	4.21
2.5	5.13	5.11	4.20
3.0	5.04	5.09	4.18
3.5	5.04	5.04	4.03
4.0	5.01	5.13	4.29
4.5	4.80	5.10	4.27
5.0	4.97	4.97	4.15
5.5	4.99	4.95	4.26
6.0	5.15	4.99	4.21
6.5	5.04	4.91	4.18
7.0	5.02	5.05	4.30
7.5	5.10	5.04	4.20
8.0	4.99	5.02	4.24

^a Subsequent experiment with TCE inlet concentration = 5.05
± 0.01 mg/L

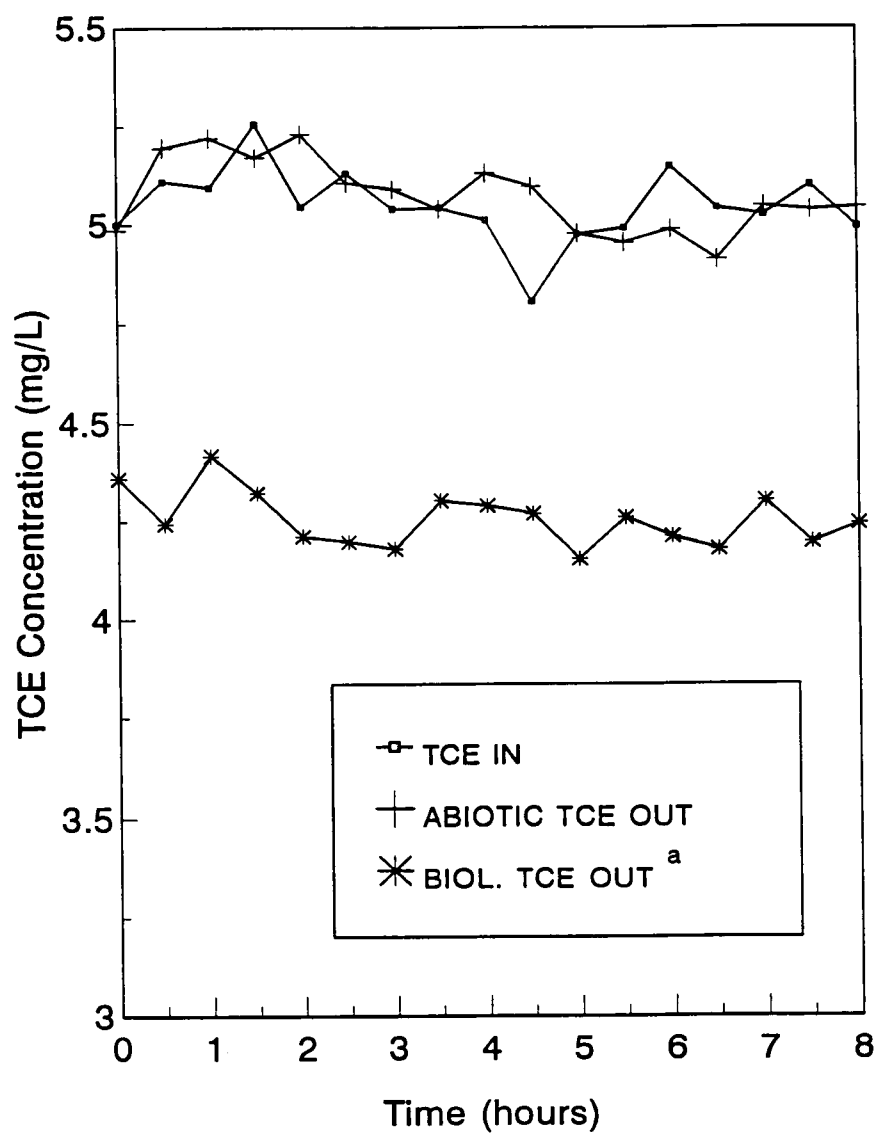


Figure C.1 Typical Steady-State Abiotic DVR Control Data and Biological TCE Degradation Data
* Subsequent experiment with TCE inlet concentration = 5.05 ± 0.01 mg/L

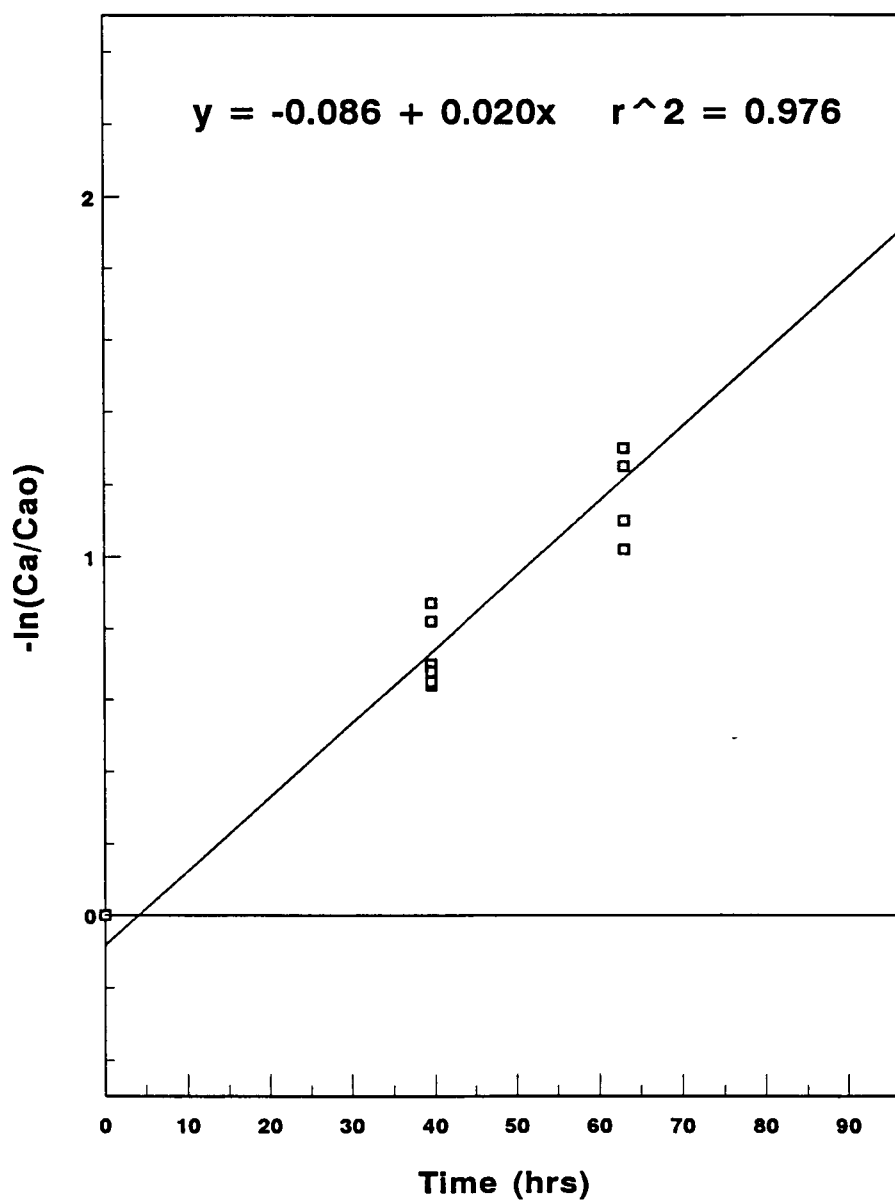


Figure C.2 Test for first-order TCE reaction rate constant. The initial feed concentration of TCE was 2 mg/L. During steady state operation in an experiment, 3 data points were collected daily over a 5 day period and then averaged. Each point represents the average data point from a single experiment.

Table C.4 TCE degradation data at
varying flow rates

Flow Rate (ml/hr)	Normalized TCE ^a Effluent Conc. C_A/C_{A0}
7	0.11
7	0.15
7	0.16
7	0.14
13	0.38
13	0.42
13	0.4
13	0.39
20	0.45
20	0.46
20	0.45
20	0.47

^a Inlet TCE concentration for all experiments was 2 ± 0.3 mg/L

APPENDIX D
SIMULATION ALGORITHM (FORTRAN CODE)

```

C      * PROGRAM: PDE1.FOR
C      * AUTHOR: LAURA LACKEY
C      * SUBROUTINES CALLED:
C      *   IMSL-MOLCH
C      * DESCRIPTION:
C      *   THIS SIMULATION WAS DEVELOPED TO MODEL THE
C      *   TRANSPORT AND DEGRADATIVE PROPERTIES OF TCE IN
C      *   PACKED-BED REACTORS. THE MODEL INCORPORATES
C      *   TRANSPORT PROPERTIES SUCH AS DISPERSION,
C      *   ADVECTION, AND SORPTION.
C      *   ATTENTION WAS GIVEN TO SEVERAL MICROBIAL
C      *   PROCESSES INCLUDING THE BIODEGRADATION OF
C      *   ALIPHATIC CHLORINATED COMPOUNDS, MICROBIAL
C      *   GROWTH, AND THE UTILIZATION OF ELECTRON DONORS
C      *   (METHANE/PROPANE) AND ELECTRON ACCEPTOR (OXYGEN).
C      * *****
C
C      INTEGER          LDY, NPDES, NX
C      PARAMETER        (NPDES=5, NX=20, LDY=NPDES)
C
C      INTEGER          I, IDO, J, NOUT, NSTEP
C      REAL             FLOAT, HINIT, T, TEND, TOL, XBREAK(NX),
C      &                Y(LDY, NX)
C      CHARACTER        TITLE*19
C      INTRINSIC        FLOAT
C      EXTERNAL         FCNBC, FCNUT, MOLCH, UMACH, WRRRN
C      common/worksp/rwksp
C      real rwksp(104820)
C      call iwkin(104820)
C
C      CD00 = 1
C      CA00 = 1
C      C200 = 1.0
C      X00 = 1
C      C20B0 = 1
C      Y(1,1) = cd00
C      Y(2,1) = ca00
C      Y(3,1) = c200
C      Y(4,1) = x00
C      Y(5,1) = c20b0
C
C      DO 10 I=1, NX
C      XBREAK(I) = (FLOAT(I-1)/FLOAT(NX-1))*1.1
C      Y(1,0) = cd00
C      Y(2,0) = CA00
C      Y(3,0) = C200
C      Y(4,i) = X00
C      Y(5,i) = C20B0
C
C      10 CONTINUE
C
C      TOL = 1.E-4
C      HINIT = 0.001
C      IDO = 1
C      NSTEP = 40
C      CALL UMACH (2, NOUT)

```

```

DO 20 J=1, NSTEP
    TEND = FLOAT(J)/20
C
    CALL MOLCH (IDO, FCNUT, FCNBC, NPDES, T, TEND, NX, XBREAK, TOL,
&             HINIT, Y, LDY)
    WRITE(TITLE, '(A,F4.2)') 'SOLUTION AT T =', T
    CALL WRRRN (TITLE, NPDES, NX, Y, -LDY, 0)
20 CONTINUE
C
    IDO = 3
    CALL MOLCH (IDO, FCNUT, FCNBC, NPDES, T, TEND, NX, XBREAK, TOL,
&             HINIT, Y, LDY)
    END
    SUBROUTINE FCNUT (NPDES, X, T, U, UX, UXX, UT)
    INTEGER      NPDES
    REAL         X, T, U(5), UX(5), UXX(5), UT(5)
C
C     Define the PDE system
C
C
C
C             List of Parameters
C
RD = 1.0
RA = 1.0
R2 = 12
CD0 = 0.865
CA0 = 10.7
C20 = 2.0
C20B = 0.01
K = 1.0
X0 = 0.9
C     v = 7ml/hr
C     v = 0.204
C     v = 13ml/hr
C     v = 0.378
C     v = 20ml/hr
C     v = 0.582
NC = 12
IF (NC.EQ.1) then
    ksd = 500
ELSE
    KSD = 115.9*EXP(-0.317*NC)
ENDIF
KSA = 1.0
KS2 = 1.0
L = 1
F = 2.4
DC = 0.42
FD = 0.8
K2 = 0.201
D = 0.01
C     b = 4.4 for 7ml/hr
B = 2.37
Y = 0.511
y = 0.9
RHO = 2.2
MTC = .33
THETA = 0.42

```

C List of grouped parameters

C PE = V*L/D
 PE = 500
 GR = (K*L*X0*U(4))/(V)
 GRD = GR/RD
 GRA = GR/RA
 GR2 = GR/R2
 CONSTD = U(1)/(KSD+U(1)*CD0)
 CONSTA = U(2)/(KSA+U(2)*CA0)
 CONST2 = U(3)/(KS2+U(3)*C20)

C Define the PDE's
 UT(1) = (1/(RD*PE))*UXX(1) - (1/RD) * UX(1)
 & - GRD*CONSTD*(CONSTA*CA0)
 UT(2) = (1/(RA*PE))*UXX(2) - (1/RA)*UX(2) - ((GRA*F)*
 & (CONSTD*CD0)*CONSTA) - ((GRA/K)*DC*FD*CONSTA)
 UT(3) = (1/PE)*UXX(3) - UX(3) - (L*RHO*MT)/(THETA*C20*V)
 & *(KD2*C20*U(3) - C20B*U(5))
 & - GR*(U(3)/((KS2+C20*U(3)) + ((KS2*CD0*U(1))/KSD))
 & *(CONSTA*CA0))
 UT(4) = (U(4)*K*Y*L/V)*(CONSTA*CA0) - B*U(4)*CONSTA*CA0
 UT(5) = (MTC*L)/(V*C20B)*(KD2*C20*U(3) - C20B*U(5))
 RETURN
 END

 SUBROUTINE FCNBC (NPDES, X, T, ALPHA, BETA, GAMP)
 INTEGER NPDES
 REAL X, T, ALPHA(5), BETA(5), GAMP(5)

C
 C

 IF (X .eq. 0) THEN
 ALPHA(1) = 1.0
 BETA(1) = 0.0
 GAMP(1) = 0.0
 ALPHA(2) = 1.0
 BETA(2) = 0.0
 GAMP(2) = 0.0
 ALPHA(3) = 1.0
 BETA(3) = 0.0
 GAMP(3) = 0.0
 ALPHA(4) = 1.0
 BETA(4) = 0.0
 GAMP(4) = 0.0
 ALPHA(5) = 1.0
 BETA(5) = 0.0
 GAMP(5) = 0.0
 ELSE
 ALPHA(1) = 0.0
 BETA(1) = 1.0
 GAMP(1) = 0.0
 ALPHA(2) = 0.0
 BETA(2) = 1.0
 GAMP(2) = 0.0
 ALPHA(3) = 1.0
 BETA(3) = 1.0
 GAMP(3) = 0.0
 ALPHA(4) = 0.0
 BETA(4) = 1.0
 GAMP(4) = 0.0

```
      ALPHA(5) = 0.0  
      BETA(5) = 1.0  
      GAMP(5) = 0.0  
END IF  
RETURN  
END
```

VITA

Laura W. Lackey was born in Richland, Washington on May 11, 1965. Her primary and secondary education was received through the public school system in Oak Ridge, Tennessee. She graduated from Oak Ridge High School in June 1983. In June 1987, she graduated from the University of Tennessee with a Bachelor of Science degree in Chemical Engineering.

The author entered graduate school immediately after completing the requirements for her B.S. degree. She received her Master's degree in Chemical Engineering in May 1992 and her Doctor of Philosophy in May 1993. She is currently employed with the Tennessee Valley Authority in Muscle Shoals, Alabama.