BIODEGRADATION OF SELECTED ORGANIC COMPOUNDS BY A PURE CULTURE WITH ELEVATED ENZYME ACTIVITY IN AN AQUIFER MATRIX

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 1992

OKLAHOMA STATE UNIVERSITY

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Thesis Approved:

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DEDICATION

This work is dedicated to

Ahmed Bolaji and Yagana Hadiza-Noro

ACKNOWLEDGEMENTS

I wish to express my deepest appreciation to Dr. J.N. Veenstra, my major advisor, for his guidance, support, and motivation during my study at Oklahoma State University. My appreciation is also extended to Dr. M. Grula, Dr. M. Bates, Dr. W. Clarkson, and Dr. W. McTernan who served as members of my committee. I also wish to thank the former Vice President of Student Services, Mr. Pat Hofler, for his assistance and friendship.

Thanks to Dr. S. Simkins, of the University of Massachusetts, who made his computer programs available and who also assisted in modifying the programs for use in this research, Dr. Douglas Kent who assisted with sampling at the Oklahoma State University Agronomy Research Station site, Dr. D.R. Snethen who assisted with the characterization of the aquifer materials, and Dr. P.L. Claypool, Department of Statistics, Oklahoma State University, for his assistance with the statistical analysis of my data. A special note of thanks also goes to Dr. J.P. Chandler, Department of Computer Science, Oklahoma State University, for his assistance. I would also like to thank Ms. Iris McPherson of the University Computer Center at Oklahoma State University for her assistance. The Soil Forage Laboratory at Oklahoma State University was responsible for the analysis of the metal concentration in the subsurface samples.

Special thanks goes to the Oklahoma Water Resources Board for providing the funding for this study and the Water Quality Research Laboratory, Oklahoma State University, Stillwater, for providing the job opportunities.

I would also like to thank Don Spoonmore and Fred Meyers and my colleagues at Civil Engineering for their assistance and friendship. Special thanks go to Shashi Nayak,

Rakesh Chaubey, Suresh Subramaniam, and Rashid Abdulla for their assistance with the cell cultures and kinetic experiments.

My thanks goes to my secretary, Ms. Sherl Holesko, for the care taken in typing this manuscript and my friend, Patricia Onoja, for her assistance.

Finally, I wish to thank my parents, Mr. and Mrs. Akolade, and other members of my extended family, Omoyemi and Jide Aluko, Kyari and Hadiza Abba Bukar, Ahmed Bolaji, Yagana Hadiza-Noro, Baba Adam, Ronnie, and Stephanie Williams for their understanding and support.

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NOMENCLATURE

A	=	absorbance	dimensionless
B	=	concentration of metal in digested solution	M/L^3
b	=	microbial decay rate	1/T
C	=	equilibrium concentration of solute	M/L^3
C*	=	concentration of enzyme	mM
D	=	dispersion coefficient	L^2/T
D_d	=	molecular diffusion coefficient	L^3/T
D_m	=	mechanical dispersion coefficient	L^2/T
D_o	=	solution diffusion coefficient	L^2/T
E*	=	molar coefficient of extinction	$mM^{-1}cm^{-1}$
F	=	final volume of digested solution	L^3
dh/dx	=	hydraulic gradient in the direction of groundwater flow	L/L
K*	=	hydraulic conductivity of the formation in the direction of groundwater flow	L/T
K_1	=	first order rate constant	1/T
K	=	$\mu_{max}X_o$	$M/L^3 \cdot T$
K_f	=	Freundlich constant	$(\frac{L^3}{M})^{1/n}$
K_{oc}	=	organic carbon partition coefficient	dimensionless
K_p	=	partition coefficient between whole soil and water	dimensionless
K_s	=	affinity or half-velocity coefficient or half-saturation coefficient	M/L^3
K_{zz}	=	hydraulic conductivity in the vertical direction	L/T
L	=	light path	L
m	=	mass of adsorbent	M

N	=	number of data points	dimensionless
n	=	slope of a line for Freundlich isotherm	dimensionless
OC	=	organic content of soil	M/M
q	=	mass of solute per mass of sorbent	M/M
R	=	substrate production term	M/L^3T
R*	=	retardation factor	dimensionless
RXN	=	reaction term	$M/L^3 \cdot T$
S	=	concentration of substrate at time t	M/L^3
S_o	=	initial substrate concentration	M/L^3
S_{min}	=	minimum substrate concentration	M/L^3
Sp	=	substrate concentration at which persistence occurs	M/L^3
t	=	time	T
v	=	groundwater velocity	L/T
V_{max}	=	maximum rate of production	M^3/T
Vg	=	vertical groundwater velocity	L/T
W	=	percent moisture content	%
W_w	=	weight of water present in soil mass	M
W_s	=	weight of soil solids	M
x	=	spatial coordinate	L
X_o	=	initial concentration of active bacteria	M/L^3
x*	=	amount of solute adsorbed	M
Y	=	yield coefficient	dimensionless
α	=	dispersivity parameter	L
Θ_t	=	porosity of the medium	dimensionless
μ_{max}	=	the maximum rate of substrate utilization	1/T
ρ, ρ_b, ρ_o	=	density	M/L^3

τ	=	toruosity of the medium	dimensionless
cm	=	centimeter	
g	=	gram	
L	=	liter	
mg	=	milligram	
ml	=	milliliter	
μl	=	microliter	

ACRONYMS

ATCC	American Type Culture Collection
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
NCP	National Oil and Hazardous Substances Pollution Contingency Plan
RCRA	Resources Conservation and Recovery Act
SARA	Superfund Reauthorization Act
USEPA	United States Environmental Protection Agency

CHAPTER 1

INTRODUCTION

Treatment of contaminated groundwater can be accomplished using various techniques. *In situ* biological treatment has various advantages when compared to other treatment techniques. Such advantages include low cleanup costs and the possibility of complete transformation of organic contaminants to harmless end products. Traditional *in situ* biorestoration can be accomplished by either enhancing the indigenous microbial population or by introducing microorganisms that have been acclimated to the compounds of interest. In cases using introduced pure microorganisms or enhancing growth of indigenous microorganisms, growth of the microorganisms is expected to take place within the subsurface systems. In order for this growth to take place, carbon and energy sources, nitrogen, phosphorus, sulfur and in some cases elements such as magnesium are required. In addition, a terminal electron acceptor may be required under aerobic and anaerobic conditions. Traditional *in situ* biorestoration techniques require all of these growth components to be present within the subsurface for biorestoration to take place. Subsurface environments are defined as those which occur beneath the soil zones of the earth's crust; including both unsaturated and saturated zones (Ghiorse and Balkwill, 1983). Each of the required nutrients and the bacteria introduced into the subsurface environment have different transport properties and will move differently. Therefore, transportation of all required nutrients to appropriate sites within the aquifer becomes a limiting factor to *in situ* biorestoration of hazardous chemicals (McCarty, 1988).

Restoration techniques requiring growth of microorganisms within the subsurface have been proven when the organic compounds of interest are easily biodegraded and when the environmental conditions are ideal for growth and maintenance of the microbial population

(Lee *et al.*, 1988). Growth of microorganisms within the subsurface, however, has shortcomings: for example, easily biodegradable compounds resulting in the production of high concentrations of cells that could plug the aquifer and/or create taste and odor problems. Organic compounds present at high concentration may also inhibit growth of the microorganisms within the subsurface.

New and innovative treatment methods are needed to overcome the shortcomings of traditional *in situ* biorestoration. These problems can be resolved by separating growth of microorganisms from catalysis and substrate conversion. The growth of the microorganisms could take place in surface reactors under optimum conditions. Catalysis and substrate conversion are expected to take place within the subsurface. One approach that can be used to separate growth of microorganisms from substrate conversion is to culture microorganisms with high enzyme activities in surface fermentors in the presence of an enzyme-inducing substrate. The enzyme rich microbial culture could then be introduced into the subsurface in a high enough concentration to be able to effect appreciable conversion within the time frame desired. An important deviation from the traditional approach is that growth of the microorganism within the subsurface is no longer a limiting factor to biodegradation since growth of the microorganism takes place in surface reactors.

Biological transformations depend on the presence of a suitable enzyme or enzyme system produced by the microorganisms. Enzymes are biocatalysts that lower the activation energy of biological reactions, thereby allowing very slow reactions to proceed at a significant rate. By stimulating the production of enzymes in surface reactors, the first stage involved in the biodegradation of organic compounds is accomplished. The enzyme or enzymes may be extracellular or intracellular. Extracellular enzymes are released into the environment by microorganisms and substrate conversion takes place outside the microbial cells. In the case of intracellular enzymes, the organic compounds must be transported through passive, facilitated, or active transport mechanisms into the microbial cells before conversion can take place. Cytochrome P-450 is an example of an intracellular enzyme with broad substrate

specificity. D(+)-camphor is an inducer of the cytochrome P-450 enzyme system in *Pseudomonas putida* PpG-786 (Smith and Davis, 1980). D(+)-camphor is a bicyclic saturated terpene ketone that exists in optically active dextroform. Dextrorotatory is defined as rotating the polarization of a wave traveling through a medium in a clockwise direction as seen by an eye observing (as observed by someone facing the oncoming radiation) the light (McGraw-Hill, 1984). Once the enzyme system is present, it is capable of catalizing the biodehalogenation of a wide variety of organic compounds.

Previous studies demonstrated that 1,2-dichloropropane is difficult to biodegrade in activated sludge reactors with mixed cultures of microorganisms (Kincannon *et al.*, 1982). After one month of acclimation of a mixed culture of microorganisms followed by 60 days of sampling, no biodegradation of 1,2-dichloropropane was observed. 1,2-dichloropropane was also shown to be resistant to biodegradation by a mixed inoculum of soil, surface water, and sludge (Kawasaki, 1980). Experiments by Roberts and Stoydin (1976) showed 98% of 1,2-dichloropropane applied to a sandy loam remained after 12 weeks.

Objectives

The main objectives of this study were to demonstrate biodegradation of selected low molecular weight halogenated compounds and provide kinetic data for their biodegradation by the resting cells of *Pseudomonas putida* PpG-786 under various environmental conditions in the presence of an aquifer matrix.

The environmental factors that were varied were temperature, pH, dissolved oxygen, and the presence of one heavy metal at different concentrations. The effects of substrate and cell concentrations were also evaluated.

Limitations

This report deals with the effect of selected environmental parameters on biodegradation of organic compounds by resting cells of *Pseudomonas putida* PpG-786 in the groundwater

aquifer matrix. Biodegradation using microorganisms grown to induce high enzyme activities was investigated. While only pure cultures were used, the concepts are equally applicable to mixed cultures. Rates of biodegradation were evaluated as affected by selected environmental parameters. All experiments were conducted in batch reactors.

Chemical Type

The low molecular weight halogenated compounds used in this study are EPA priority pollutants (USEPA, 1979). Halogenated aliphatic compounds are capable of oxidation-reduction reactions in the presence of an external electron acceptor. When an electron acceptor is absent, substitution and dehydrohalogenation occurs (Vogel, 1987).

The three chemicals used in this research were 1,2-dichloroethane (DCE), 1,2-dichloropropane (DCP) and 1,2-dibromo-3-chloropropane (DBCP). DCE is widely used in the manufacture of vinyl chloride and tetraethyl lead. It is a constituent of paint, varnish and finish removers. Its major use is in extracting spices such as annatto, paprika and turmeric (Verschueren, 1983).

Bouwer and McCarty (1983) reported removal of 65 μg/l 1,2-dichloroethane by a methanogenic mixed culture. A 63% removal was reported after 25 weeks of incubation with the acclimated culture.

The environmental fate of 1,2-dichloropropane (DCP) was reviewed by Howard (1990) as follows. DCP is an intermediate for the manufacture of perchloro-ethylene and carbon tetrachloride, lead scavengers for antiknock fluids solvent and soil fumigant for nematodes (Verschueren, 1983). It is also used as a solvent for oils and fats, a solvent for dry cleaning and degreasing operations (USEPA, 1988). DCP is released into soil and eventually into groundwater during its use as soil fumigant for nematodes, chemical intermediate, solvent, insecticide for stored grain, and from municipal landfill leachates. DCP readily leaches into the groundwater, especially in the sandy soils of Georgia, South Carolina, North Carolina, and Virginia where it is used as a nematocidal fumigant (Howard, 1990). It is lost from soil

through volatilization (USEPA, 1983). No evidence of biodegradation of 1,2-dichloropropane in sandy soil has been noted although minor removal was reported in medium loam soil in 20 weeks in a closed glass container (Howard, 1990). It was also resistant in a two week screening test that utilized a mixed inoculum of soil, surface water, and sludge (Kawasaki, 1980). DCP is reported as likely to be persistent and mobile in the soil environment (USEPA, 1979). A study by Cohen (1983) showed levels of DCP as high as 1200 μg/L in shallow wells near sites were DCP was used as a fumigant.

1,2-dibromo-3-chloropropane (DBCP) was used as a nematocidal fumigant for more than 40 crops until 1977. Between 1977 and 1979, the USEPA canceled all uses of DBCP except on pineapples in Hawaii (USEPA, 1988). Castro and Belser (1968) reported a maximum conversion of DBCP of 63% in soils containing active microbial populations from orchards and fields from Southern California over a period of four weeks.

CHAPTER II

LITERATURE REVIEW

Introduction

Groundwater is a precious, exhaustible resource providing water for domestic, industrial and agricultural uses. Consequently, various laws and regulations are available to protect groundwater supplies. Three of the laws of the United States of America that are applicable to the protection of the groundwater environment from hazardous materials are the Safe Drinking Water Act (1974), Safe Drinking Water Act Amendment (1986), Resource Conservation and Recovery Act (RCRA) (1976) and Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) or Superfund (1980, 1986). The Safe Drinking Water Act was enacted to protect underground sources of drinking water. RCRA regulates the disposal of hazardous wastes in response to a growing public awareness of the serious problems relating to their disposal. CERCLA of 1980 provided a statutory basis for dealing with the threat posed by hazardous waste sites to human health and the environment (Anderson, 1990). The Superfund Amendment and Reauthorization Act (SARA) of 1986 provided more funds for cleanup of hazardous waste sites. It established cleanup standards, addressed long term solutions to land disposal, persistence, toxicity, mobility and bioaccumulation of hazardous materials and established a preference for remedial action. The National Oil and Hazardous Substances Pollution Contingency Plan (NCP) allowed use of innovative technologies in remediation of hazardous waste sites (USEPA, 1990).

This chapter deals with a review of literature of the microorganisms inhabiting the groundwater environment and the roles they play in determining the persistence of contaminants within this environment. Secondly, it discusses the factors influencing the transport of

microorganisms and contaminants and physicochemical reactions that play a role in determining the fate and persistence of contaminants within the groundwater environment. Thirdly, various techniques available for restoration of aquifers are discussed and a theoretical basis is provided for biodegradation of organic compounds.

Aquifer Environment

The deep subsurface environment has been shown to be sparsely populated by oligotrophic microorganisms adapted for survival under nutrient limited conditions (Wilson *et al.*, 1983a; Ghiorse and Balkwill, 1983). Microbial composition of the subsurface community is predominantly bacteria (Wilson and McNabb, 1983; Wilson *et al.*, 1983a). These microorganisms are metabolically active and nutritionally diverse (Lee *et al.*, 1988). In a study conducted at Lula, Oklahoma by Beloin *et al.* (1988), the distribution and activity of the subsurface microorganisms were observed to show a site-dependent variation with depth. Another study conducted using microorganisms from a pristine aquifer indicated that a lag phase might be required before biodegradation can be observed (Aelion *et al.*, 1987). This period is required for adaptation of the microbial communities indigenous to the groundwater environment to the contaminants and might be critical to the success of *in situ* biorestoration. Although some of the subsurface microorganisms may adapt to the presence of xenobiotic compounds, their ability to adapt and their adaptation time become limiting factors to biodegradation (Aelion, 1987). Surface-type protozoa and cyanobacteria were detected in the saturated zone of the Lula aquifer indicating hydrological connection to a nearby river (Beloin *et al.*, 1988). In this case, a situation analogous to surface waters might develop in which eucaryotic microorganisms graze on the bacteria. Consequently, the growth rate of the bacteria must exceed those of the predators for biodegradation to be sustainable. Microorganisms populating deep subsurface environments are exposed mostly to recalcitrant compounds that have percolated through the biologically active surface layers. Due to the limited amount of easily biodegradable materials in deeper aquifers, microorganisms living in this environment

may have low metabolic rates. These low metabolic rates, coupled with the stability of the groundwater environment, may result in indigenous microorganisms that tend to be highly specialized in capturing metabolizable organic compounds from very dilute solutions (Wilson *et al.*, 1983b). In addition, in cases where predation from eucaryotic microorganisms is absent, Wilson *et al.* proposed that these microbes may develop slower rates of growth and metabolism. Consequently, in the event of groundwater pollution, the microbial population indigenous to the subsurface may be easily inundated by an influx of a high concentration of highly toxic organic compounds. When bacterial cells with elevated enzyme activities are introduced into the subsurface, the cell concentration must be high enough to overcome the effect of predation such as in situations when eucaryotic microorganisms graze on the bacteria. Elevated enzyme activities can be due to prior exposure of microorganisms to inducers such as D(+) camphor.

Transport of Microorganisms and Contaminants

Negatively charged microorganisms adsorbed onto positively charged mineral surfaces and can become detached under high nutrient and carbon concentrations (McCarthy and Zachara, 1989) probably due to competition for adsorption sites on the mineral surface. In addition, predominantly negatively charged groundwater matrices permit rapid transport of negatively charged particles such as microorganisms. The transport of microorganisms is further aided by their ability to move through channels and secondary pore structures instead of spreading through intergranular pore spaces (Harvey, *et al.*, 1989; Smith *et al.*, 1985).

The behavior of organic contaminants within the groundwater aquifer environment is highly dependent on the physicochemical characteristics of the contaminants, such as the aqueous solubility, Henry's law constant, specific gravity, octanol-water partition coefficient or organic carbon partition coefficient. A two-phase approach to modeling contaminant transport in the groundwater environment describes the partitioning of the contaminants between the immobile solids and mobile aqueous phases. This process represents a balance between

their tendency to remain in the aqueous phase (estimated using their solubilities) and the tendency to partition onto aquifer materials (estimated using their partition coefficient). The degree of partitioning affects their rate of movement with groundwater flow. The Henry's law constant describes the tendency of the organic contaminants to volatilize from the aqueous phase into the gas phase. The specific gravity of the contaminants determines if the contaminants will sink to the bottom of the aquifer or float on top of the saturated zone.

The movement of an unreactive contaminant in saturated porous media is predominantly influenced by advection and secondarily by hydrodynamic dispersion. Advection is due to the movement of the groundwater while hydrodynamic dispersion is due to mechanical mixing and molecular diffusion (Freeze and Cherry, 1979). Advection causes the contaminants to move with the groundwater while hydrodynamic dispersion causes the spreading out of the contaminant plume.

Advection of a pollutant through porous media depends on the average linear velocity of the groundwater, v, represented as (Freeze and Cherry, 1979):

$$v = -\left(\frac{K^*}{\Theta_t}\right)\left(\frac{dh}{dx}\right) \tag{1}$$

where

K^* = the hydraulic conductivity of the formation in the direction of the groundwater flow

Θ_t = the porosity of the formation

dh/dx = the hydraulic gradient in the direction of the groundwater flow

A one dimensional representation of the transport of the solute in saturated porous media is given by:

$$\frac{\partial}{\partial x}\left(D\frac{C}{\partial x}\right) - v\frac{\partial C}{\partial x} = \frac{\partial C}{\partial t} \pm RXN \tag{2}$$

where

D = the dispersion coefficient

v = the groundwater velocity

C = the concentration of the solute

x = the spatial coordinate

RXN = the reaction

t = the time

Although the dispersion coefficient is constant at low velocity, it increases linearly as the groundwater velocity increases (Palmer and Johnson, 1989). The dispersion coefficient D is composed of two parts: an effective molecular diffusion coefficient, D_d and a mechanical dispersion coefficient, D_m. D is represented by:

$$D = D_m + D_d \tag{3}$$

D_m is a function of the groundwater velocity and is expressed as:

$$D_m = \alpha v \tag{4}$$

where

α = the dispersivity parameter (L)

Molecular diffusion is given by:

$$D_d = \tau D_o \tag{5}$$

where

τ = the tortuosity of the medium

D_o = the solution diffusion coefficient

Tortuosity is defined as the increased distance a diffusing ion must travel to get around the sand grains (Palmer and Johnson, 1989).

Reactive contaminants are influenced by sorption/desorption, physical and biochemical

reactions. When a pollutant is adsorbed, D is replaced by D' (D'=D/R*); where R*, retardation factor, is defined as (Freeze and Cherry, 1979):

$$R^* = 1 + \frac{\rho_b K_p}{\theta_t} \quad (6)$$

where

ρ_b = the soil bulk density (g/cm^3)

θ_t = the porosity of the media

R* = retardation factor

K_p is given by:

$$K_p = K_{oc} \frac{(\%OC)}{100} \quad (7)$$

where

OC = the organic carbon content of the soil

K_{oc} = the organic carbon partition coefficient

K_p = the partition coefficient between whole soil and water

Sorption to aquifer materials is due to the hydrophobic bond between an organic compound and natural organic matter associated with the media (Karickhoff, *et al.*, 1979; Tanford, 1973; Mackay and Powers, 1987; Chiou, *et al.*, 1985). Aquifer materials are characterized by lower organic carbon content, therefore, have lower sorption of organic components when compared to soil from the top layer.

Physicochemical and biological reactions such as hydrolysis or biodegradation can affect the persistence of the organic contaminants in the environment. This is accounted for by the RXN term of equation 2. Hydrolysis of halogenated aliphatic compounds is represented by (Siegrist and McCarty, 1987):

$$RX + HOH \rightarrow ROH + HX \quad (8)$$

and

$$RCHCH_2X \xrightarrow{H^+} RHC=CH_2 + HX \quad (9)$$

The environmental half-lives (at 20°C, degradation from abiotic hydrolysis) for some halogenated aliphatic compounds are quite high. For instance, environmental half-lives are 35 years for 1,2-dibromo-3-chloropropane and 50 years for 1,2-dichloroethane. The hydrolytic half-life for 1,2-dichloropropane could be from six months to several years (Howard, 1990). Groundwater contaminated by compounds such as these require other remediation techniques.

The effects of the density of the contaminants on the vertical groundwater velocity (V_g) can be estimated using (Frind, 1982):

$$V_g = -\frac{K_{zz}}{\theta_t}\left(\frac{\rho_b}{\rho_o} - 1\right) \quad (10)$$

where

K_{zz} = the hydraulic conductivity in the vertical direction

ρ_b ρ_o = densities of the pollutant and the groundwater, respectively

If the contaminant is denser than water, it has a tendency to move towards the bottom of the aquifer. If it is less dense, the tendency is to move toward the top and spreads on the surface of the groundwater table as the water moves down gradient through the aquifer. Halogenated aliphatic compounds such as 1,2-dichloropropane, 1,2-dichloroethane, and 1,2-dibromo-3-chloropropane are denser than water and will tend to sink to the bottom. The depth of the monitoring wells is dependent on the location of the contaminants within the aquifer environment.

Aquifer Restoration

Once contaminated, an aquifer can either be abandoned for alternative water supply or restored. With dwindling water supplies, the trend is to restore the contaminated aquifer.

The contaminated soil or aquifer material can be stabilized and contained using techniques such as sorption, lime-fly ash pozzolana processes, thermoplastic microencapsulation or macroencapsulation (Spooner, 1985). Alternate restoration techniques are shown in Figure 1. Two options are available for restoring the water in a contaminated aquifer. The groundwater can either be pumped to the surface and treated or *in situ* restoration techniques can be used. Pump and treat systems can be based on physical, chemical, biological or a combination of the above techniques. Physical treatment techniques include phase separation, filtration and gravity sedimentation, air stripping, and steam stripping. Chemical treatment techniques include chemical coagulation, pH adjustment, carbon adsorption, resin adsorption and chemical oxidation. Biological pump and treat systems include using traditional techniques such as aerobic fixed film, suspended growth or anaerobic treatment methodologies.

In Situ Treatment

In situ treatment is a potential cost effective alternative to pump and treat systems (Anonymous, 1989). *In situ* treatment of hazardous wastes involves the use of physical, chemical or biological techniques to remove or immobilize the contaminant within the subsurface. *In situ* physical treatment methods include heating or freezing, *in situ* stripping, and vacuum removal, while chemical methods involve injection of a specific chemical into the subsurface to either immobilize or increase the mobility of the contaminants. Various techniques applicable to *in situ* treatment were reviewed by Wagner and Kosin (1985) and Pennington (1985). According to these reviews, *in situ* chemical treatments include water or surfactant flushing, oxidation, reduction, hydrolysis, polymerization and sorption. Compounds that can be flushed from the aquifer with water are hydrophilic; with high solubility in water. Otherwise, surfactant flushing can enhance the solubility of organic compounds. Chemical oxidation of contaminants involves use of ozone, hypochlorite, or hydrogen peroxide. Chemical oxidation is limited by being nonspecific, and results in the possible formation of more toxic end products. Chemical reduction of halogenated compounds can be

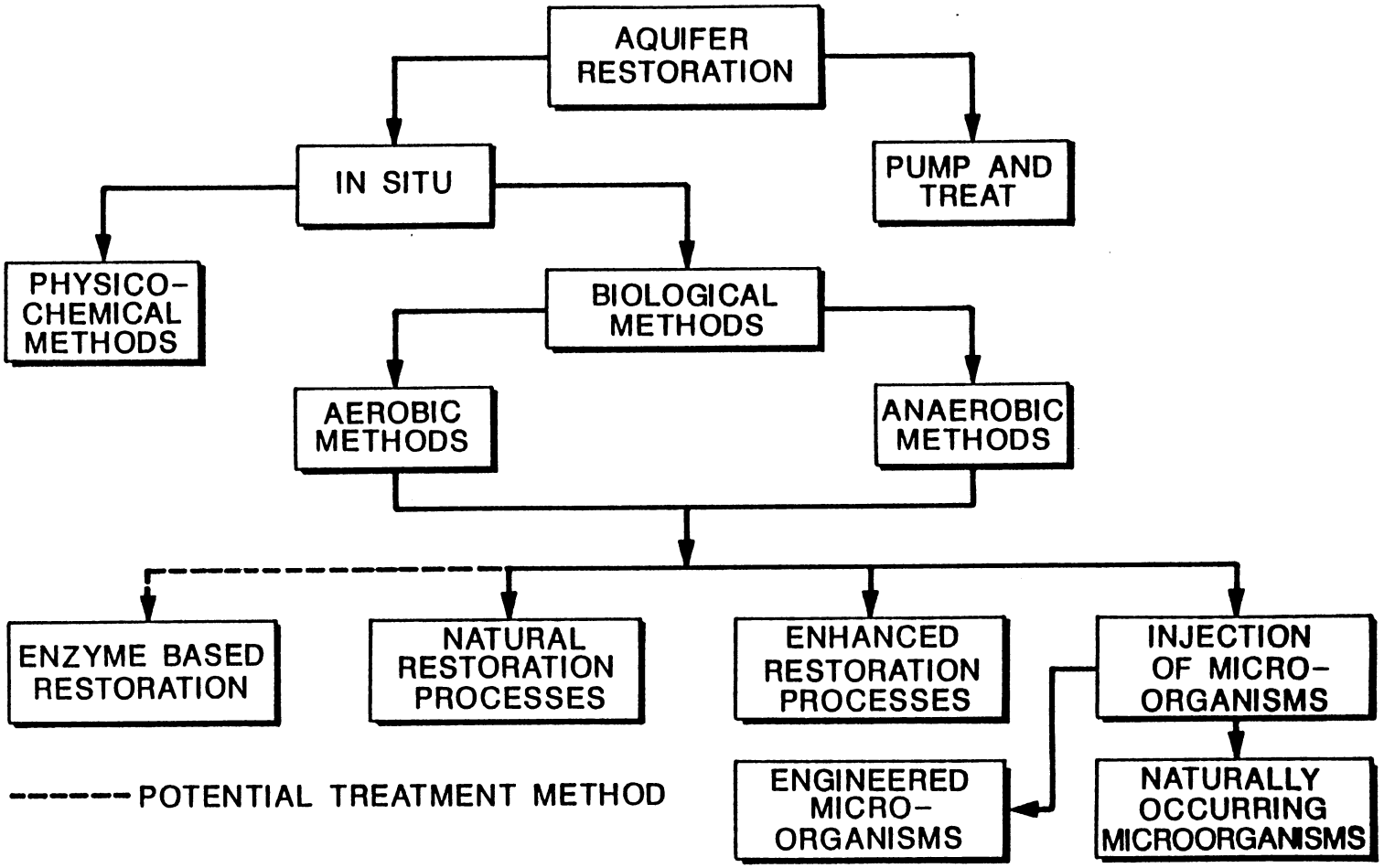


Figure 1. Conceptualization of *In Situ* Biorestoration of a Contaminated Aquifer.

accomplished in the presence of a catalyst such as nickel. This is limited by the cost of the catalyst and because of the very few research studies on this method that have been done to date (Wagner and Kosin, 1985). Hydrolysis reactions are possible for pesticides such as parathion. This is limited by a difficulty in hydrolyzing some sorbed organic compounds. Polymerization involves injection of a catalyst capable of polymerizing organic monomers such as vinyl chloride in the subsurface. This process results in the immobilization of a once fluid substance. Polymerization has only limited application in hazardous waste sites containing a mixture of compounds. *In situ* biodegradation usually involves either enhancement of indigenous microbial population or the introduction of acclimated microorganisms. Of these methods only air stripping, surfactant flushing, and *in situ* biodegradation are applicable to low molecular weight halogenated aliphatic hydrocarbons (Wagner and Kosin, 1985).

Biodegradation of Organic Compounds

Biodegradation is defined as "the biological transformation of an organic chemical to another form, no extent implied" (Grady, 1985). When organic compounds are present in the environment, they can either be used as a sole source of carbon and energy, cometabolized or transformed through gratuitous biodegradation. Cometabolism (a subcategory of secondary substrate utilization) is defined as "the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound" (Dalton and Stirling, 1982). Cometabolism by definition does not yield energy for growth or intermediates for the synthesis of biomass (Stirling and Dalton, 1979; Slater and Bull, 1982). Gratuitous metabolism occurs because the required enzyme(s) present can catabolize the conversion of other substrates. Unlike cometabolism, gratuitous metabolism does not require the presence of a growth substrate. The following factors are important for biodegradation of organic compounds (Grady, 1985):

1) Microorganisms capable of biodegradation must be present
2) Enzyme synthesis must be possible

3) Environmental conditions such as temperature, pH, and dissolved oxygen must be ideal for enzyme catalyzed reaction(s)

The subsurface, although sparsely populated, contains microorganisms that metabolize synthetic compounds. This ability is highly dependent on the types of microorganisms, the types of chemical and the subsurface environment. Biorestoration can take place under two major environmental conditions; aerobic and anaerobic. Under aerobic conditions oxygen is the terminal electron acceptor while under anaerobic condition nitrate or other organic components serve as the terminal electron acceptor. For example, under aerobic conditions, methane oxidizing bacteria (methanotrophs) have been shown to transform halogenated aliphatic compounds such as trichloroethylene (TCE) (Wilson and Wilson, 1985). In nature, a clear-cut distinction between types of removal mechanisms may not exist as pockets of anaerobic zones exist due to rapid depletion of oxygen by biologically active microorganisms or due to other types of reducing environments.

Biodegradation of organic compounds occurs either through primary substrate utilization, secondary substrate utilization, or through gratuitous metabolism. Another substrate serves as the source of carbon and energy for the microorganisms under cometabolism. Energy generated through endogenous metabolism may adequately maintain enzyme systems in an active state thereby allowing biotransformation to proceed for a short time (McCarty, 1988). However, in the absence of energy-yielding substrates, microorganisms may undergo endogenous respiration for a limited time, providing a possible survival mechanism for the population of organisms within the subsurface.

In Situ Biorestoration Techniques

Traditional techniques for biorestoration of contaminated aquifers depend on the growth of the required microorganisms within the aquifer systems. Environmental factors likely to influence the growth of microorganisms within the subsurface include (Lee *et al*, 1988; Johnston and Robinson, 1984):

1) Dissolved oxygen
2) Availability of required nutrients and growth factors (influenced by transport and adsorption phenomena)
3) pH
4) Presence of toxicants such as heavy metals
5) Temperature
6) Concentration and chemical structure of contaminant

Others include the toxicity of the pollutants, the presence of suitable primary substrates, and the resistance of the compounds to microbial degradation. Pollutants within the aquifer are only biodegradable when the required enzymes are present or inducible. In a technique pioneered by Raymond and co-workers (1974, 1975, 1976a, 1976b, 1978) to stimulate *in situ* biodegradation, nutrients and oxygen are introduced into the subsurface environment. Swindoll *et al.* (1988) observed enhanced biodegradation of ethylene dibromide in an aquifer when multiple inorganic nutrients are added.

Limitations of Traditional *In Situ* Biorestoration Techniques

Microorganisms may be adapted to degrade a particular compound through induction of the appropriate enzyme systems, production of a new metabolic capability through genetic engineering or increasing the numbers of microorganisms able to catalyze a particular reaction (Spain *et al.*, 1980). The survival of the microorganisms introduced into any environment depends on their ability to tolerate abiotic stresses, remain viable when starved and coexist with antagonists (Liang *et al.*, 1982).

When microorganisms are introduced into a polluted environment for the purpose of *in situ* restoration, the organisms may fail to function due to one or more of the following reasons (Goldstein *et al.*, 1985; Zaidi *et al.*, 1988,1989):

1) Presence of predators and growth inhibitors

2) Concentration of the pollutant is below the minimum substrate concentration required to support growth
3) Inoculated organisms may use organic compounds other than the one selected to metabolize
4) Concentration of the inorganic nutrients are low
5) Organic compounds may not be accessible to the organism

Several factors limit the biodegradation of organic compounds using the traditional *in situ* biorestoration approach. During traditional *in situ* biorestoration, nutrients are introduced into the subsurface environment in an attempt to enhance the growth of indigenous microbial population. As previously stated, the rate of transport of the nutrients, oxygen and micro-organisms within the groundwater environment are different. Thus, there exists a problem of having all of the components required for growth simultaneously present at the same location. In addition, variability among and within sites makes it difficult to extrapolate data on growth of microorganisms within the subsurface from site to site.

Advantages and Disadvantages of *In Situ* Biorestoration

The following are the advantages of *in situ* biorestoration (Lee *et al.*, 1988; Staps, 1989):

1) Often applicable where other techniques cannot be applied (e.g., under buildings)
2) Applicable for treatment of both top soil and aquifer materials
3) Environmentally sound, no transfer to other phases
4) Relatively fast, safe and cost effective (compared to pump and treat systems)
5) Treatment can move with the plume of contaminants in the groundwater
6) Can be used to treat some organic compounds such as hydrocarbons

The following are the disadvantages of biorestoration (Lee *et al.*, 1988; Staps, 1989):

1) Applicable to only biodegradable components

2) May not work with subsoils with low permeability that do not permit adequate circulation
3) Can be inhibited by toxic components such as heavy metals
4) Excessive bacterial growth may clog soils and wells
5) Residual concentrations of pollutants may remain in the subsoil
6) Treatment may require relatively long time (for compounds requiring long periods of acclimation)
7) Long-term effects insufficiently understood
8) Residuals of pollutants may cause taste and odor problems

Possible sources of oxygen for *in situ* restoration are air, pure oxygen, and peroxides. These sources are listed in Table I.

Microorganism Type

Winslow *et al.* (1917) first described the genus *Pseudomonas* as the predominant member of the family *Pseudomonadaceae*. *Pseudomonas* sp. are prevalent in the natural environment. They are typically aerobic and have developed a remarkably diverse physiological capacity. This is because *Pseudomonas* sp. can produce may different enzymes in response to different substrates (Clarke and Slater, 1986). For example, a *Pseudomonas* strain obtained from enrichment culture with fluoroacetate as the carbon source showed dehalogenation of chloroacetate, fluoroacetate and iodoacetate (Goldman, 1965).

A degradative plasmid in *Pseudomonas* specifies the biodegradation of a diverse group of compounds such as aliphatic and aromatic hydrocarbons, terpene, alkaloid, chlorinated aliphatic and chlorinated aromatic compounds as indicated in the next paragraph. The plasmid CAM specifies degradation of camphor in *Pseudomonas putida* (Clarke and Slater, 1986). Since the genes that encode for the desired degradative capacity reside in plasmids their transfer to other organisms is possible (Weightman and Slater, 1988; Sayler *et al.*, 1990). As such, the survival of *Pseudomonas putida* is not critical. Instead, the ability to

TABLE I

OXYGEN SUPPLY ALTERNATIVES

Substance	Application Method	Advantages	Disadvantages
Air	In-line	▪ most economical	▪ not practical except for trace contamination (< 10mg/L COD)
	In situ wells	▪ constant supply of oxygen possible	▪ wells subject to blow out
Oxygen-Enriched Air or Pure Oxygen	In-line	▪ provides considerably higher O_2 solubility than does aeration	▪ not practical except for low levels contamination (< 25 mg/L COD)
Hydrogen Peroxide	In-line	▪ moderate cost ▪ intimate mixing with groundwater ▪ greater oxygen concentration can be supplied to the subsurface (100 mg/L H_2O_2 provides 50 mg/L O_2) ▪ helps to keep well free of heavy biogrowth	▪ H_2O_2 decomposes rapidly upon contact with soil, and oxygen may bubble out prematurely unless properly stabilized ▪ H_2O_2 is cytotoxic; however organisms can be acclimate to high concentrations

(Source: Wagner K. and Kosin, Z., 1985)

maintain the *in situ* degradative capacity within the subsurface microbial community is. For instance, transfer of large mercury resistant plasmids to *Pseudomonas putida* was reported for a surface environment such as river epilithon (Bale *et al.*, 1988). The river epilithon is a mixture of bacteria forming a slimy community on the surfaces of submerged stones (Bale *et al.*, 1988). Gene probes can be used to track the organisms in which the degradative plasmid resides. This aspect was not pursued further in this research.

Pseudomonas putida PgG-786 was originally isolated from soil by enrichment with D(+)-camphor (Hedegaard *et al.*, 1961). When grown in the presence of D(+)-camphor, an intracellular cytochrome enzyme system (cytochrome P-450$_{cam}$) is induced. Although enzymes are specific for the *type of reaction* they catalyze, they are less specific for the *types of substrate* involved in binding (Knackmuss, 1981). Thus, the cytochrome P-450$_{cam}$ enzyme system enhanced by D(+)-Camphor is capable of catalyzing the conversion of a wide variety of compounds such as 1,2 dibromo-3-chloropropane (DBCP) (Castro and Belser, 1968), chloropicrin (Cl_3CNO_2), trichloronitromethane, bromotrichloromethane (BTM), ethylene dibromide and carbon tetrachloride (Lam and Vilker, 1987; Castro *et al.*, 1989) and 1,1,2-trichloroethane (Castro and Belser, 1990). Castro and co-worker (1990) observed that the cytochrome P-450$_{cam}$ was capable of both reductive and oxidative dehalogenation in soil under aerobic conditions.

Induction of Microbial Enzymes

Induction is the synthesis of a specific enzyme brought about by exposure to the inducer (Grula, 1991). The detoxification of xenobiotics in the environment by microorganisms is made possible by the presence of the required enzymes. The rapid induction of such an enzyme system capable of catalyzing a wide variety of reactions is of great importance to the design of an enzyme based *in situ* biorestoration technology. The monoterpene D-(+) camphor induces a high concentration of the cytochrome P-450$_{cam}$ of *Pseudomonas putida* PpG-786 when it is used as a sole carbon source. The enzyme system is intracellular and is a

non-bound (to membranes) form of cytochrome P-450 (Wiseman, 1977). This allows the cytochrome $P\text{-}450_{cam}$ to be purified and crystallized as an enzyme-substrate complex with D-(+) camphor (Yu and Gunsalus,1970).

Cytochrome $P\text{-}450_{cam}$ consists of 3 proteins (Smith and Davis, 1980):

1) Putidaredoxin reductase (NADH specific, contains FAD)
2) Putidaredoxin (a nonheme iron-sulfur protein)
3) A soluble cytochrome P-450 (b-type heme)

Pseudomonas putida and the Cytochrome $P\text{-}450_{cam}$ System

Microorganisms contain enzymes referred to as monooxygenases. Monooxygenases are responsible for initiating oxidation of organic compounds by incorporating oxygen from the atmosphere. Monooxygenases incorporate one atom of oxygen while dioxygenases incorporate two atoms. Klingenberg (1958) and Garfinkel (1958) first observed the unique spectral properties of cytochrome P-450s. They reported a broad but intense absorption band at 450 nm after bubbling carbon monoxide into a dithionite-reduced mammalian microsomal suspension. *Pseudomonas putida* was first described as a source of cytochrome $P\text{-}450_{cam}$ by Gunsalus *et al.* (1965). The enzyme catalyzes a stereospecific hydroxylation of camphor to 5-exoalcohol and requires molecular oxygen and NADH.

In order for a compound to serve as an inducer of the cytochrome $P\text{-}450_{cam}$ enzyme system, it should be easily transported through the microbial cell membrane and serve as substrates required to bind P-450 monooxygenase (Parke, 1975). An earlier study showed that the intrinsic enzyme reaction rate for cytochrome $P\text{-}450_{cam}$ embedded in the cellular cytoplasm is higher than enzyme extracted from the bacterial cells (Castro *et al.*, 1983). This eliminated the need to go through extensive enzyme extraction processes. Instead, the enzyme systems remained immobilized within the microbial cells. Further support of the use of whole resting cells rather than extracted enzymes is provided by Lam and Vilker (1987) and Vilker and Khan (1989). These studies showed that intrinsic enzyme kinetics rather than

transport of halogenated compounds through the cell membrane or other diffusion processes is rate limiting.

Castro *et al.* (1989) proposed that in the presence of cytochrome P-450$_{cam}$, polyhalomethanes undergo reactive hydrogenolysis according to the following reaction:

$$RCX_n + 2PFe^{II} + H^+ \rightarrow RCX_{n-1} + 2PFe^{III} + X^{-1}$$

Vicinal halides are converted to the corresponding olefins according to the following reaction:

$$-\overset{|}{\underset{|}{C}}X-\overset{|}{\underset{|}{C}}X + 2PFe^{II} \rightarrow \overset{\backslash}{\underset{/}{C}}=\overset{/}{\underset{\backslash}{C}} + 2PFe^{III} + 2X^{-1}$$

P = cytochrome P-450$_{cam}$

X = halogen

A generalized pathway for biological dehalogenation of chlorinated aliphatic compounds is shown in Figure 2.

Theoretical Basis

Adsorption Kinetics

The *Freundlich adsorption isotherm* is an empirical equation used to describe the adsorption of a solute to an adsorbent. The Freundlich equation has the form (Freundlich, 1926):

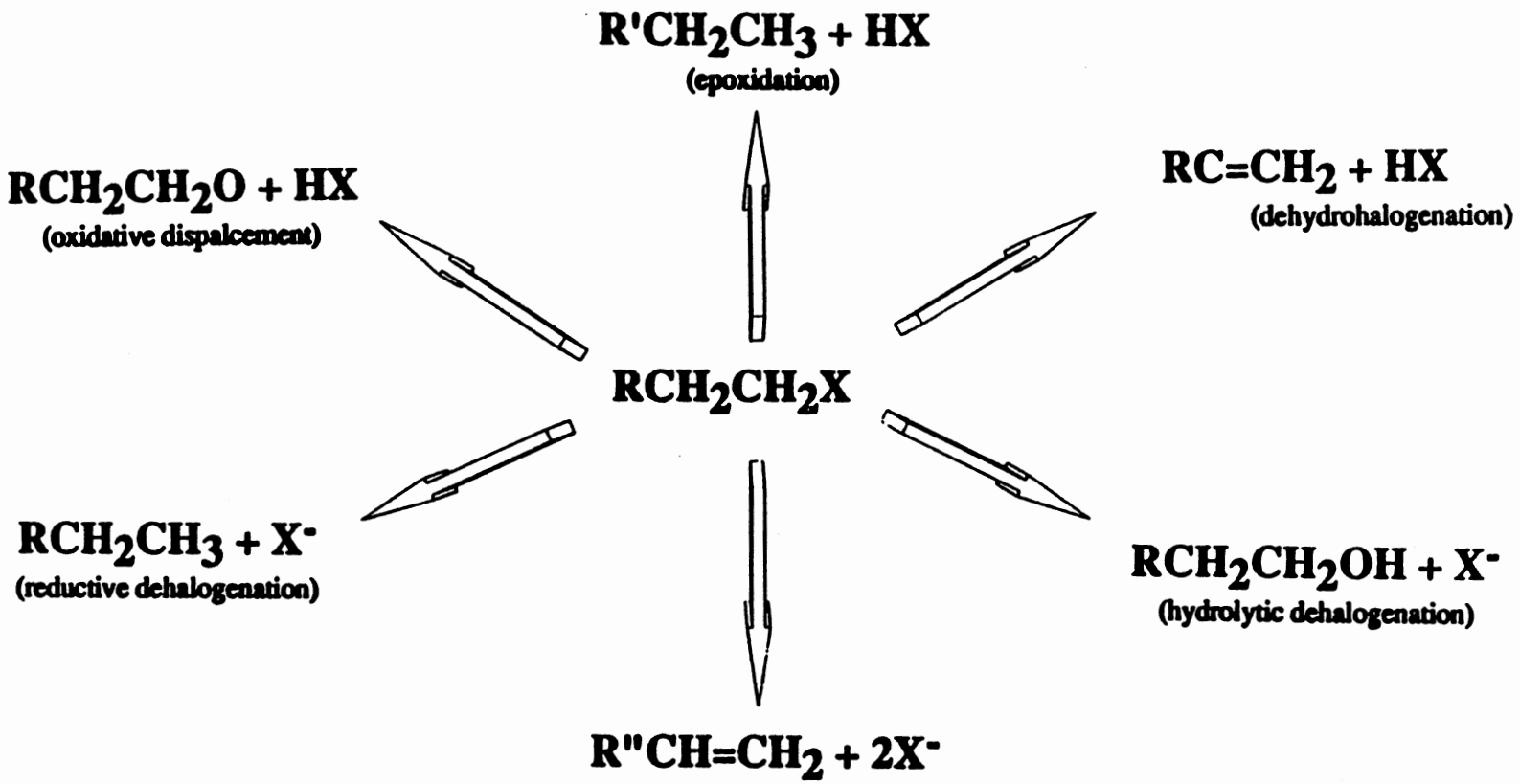
$$q = \left(\frac{x^*}{m}\right) = K_f C^{1/n} \qquad (11)$$

where

x^* = the amount of the solute adsorbed

q = mass of solute per mass of sorbent

m = the mass of the adsorbent



X = halogen substituent R =parent compound

R' = R with a loss of an X group R" = R with a loss of an X^- group

(modified from Niedleman and Geigert, 1986 and Vogel *et al.*, 1987)

Figure 2. Possible Routes of Biological Dehalogenation of Chlorinated Aliphatic Compounds.

C = the equilibrium concentration of the solute

n = slope of a line

K_f = intercept at log 1 concentration

K_f is the Freundlich distribution or partition coefficient between the solute sorbed and the solute remaining in solution.

The equation is linearized using a log-log plot or the expression:

$$\log \left(\frac{x^*}{m} \right) = \log K_f + \frac{1}{n} \log C \qquad (12)$$

where

$$\frac{x^*}{m} = \frac{\text{(initial conc. - equil. conc.) (volume of solution)}}{\text{weight of absorbent}}$$

Equation 12 is analogous to a linear equation relating a dependent variable y to an independent variable x of the form:

$$y = b + ax \qquad (13)$$

where

a = 1/n (slope)

b = log K_f (intercept)

Biodegradation Kinetics

The relationship between the growth and decay of microorganisms and substrate consumption can be expressed by the equations described by Monod (1949) and modified by Herbert *et al.* (1956). The equations are:

$$\frac{dX}{dt} = Y\mu_{max}X \frac{S}{(K_s + S)} - bX \qquad (14)$$

and

$$-\frac{dS}{dt} = \mu_{max}X \frac{S}{(K_s + S)} \qquad (15)$$

where

μ_{max} = the maximum rate of substrate utilization

Y = yield coefficient

X = concentration of active bacteria

K_s = the half-saturation constant

t = time

b = the microbial decay rate

S = the concentration of the primary substrate

Detailed description of various modifications of Monod kinetics was previously given by Simkins and Alexander (1984), Alexander (1985), and Schmidt *et al.* (1985). These authors proposed that although many environmental factors are likely to influence pattern of mineralization kinetics, the variability in the substrate disappearance curves can be explained with only the initial concentration of the compound, the population density, and the parameters of the Monod equation. They proposed six models which incorporate only initial substrate concentration and initial cell density (Figure 3). The two vertical and one diagonal lines correspond to the divisions between the six regions. The first vertical line is placed at $K_s = S_o$ while the second vertical line is placed at S_o corresponding to one and one-half orders of magnitude greater than K_s. Points along the diagonal line represent substrate concentrations supporting one division of active cells. Initial cell density above the diagonal lines are assumed to be constant during substrate conversion. Figure 3 shows guidelines under which the various kinetic models can be used. Selection of the appropriate biodegradation kinetic model can be made only after a careful examination of the necessary conditions applicable to each model. At an initial cell concentration below the diagonal line as indicated in Figure 3, removal of substrates is coupled with growth. The applicable models are logistic, Monod with growth and logarithmic. When the initial cell concentration is high enough that changes in cell concentration during the experiment are low compared to initial cell concentration (above the diagonal line), the zero order, first order, or Monod with no growth are used.

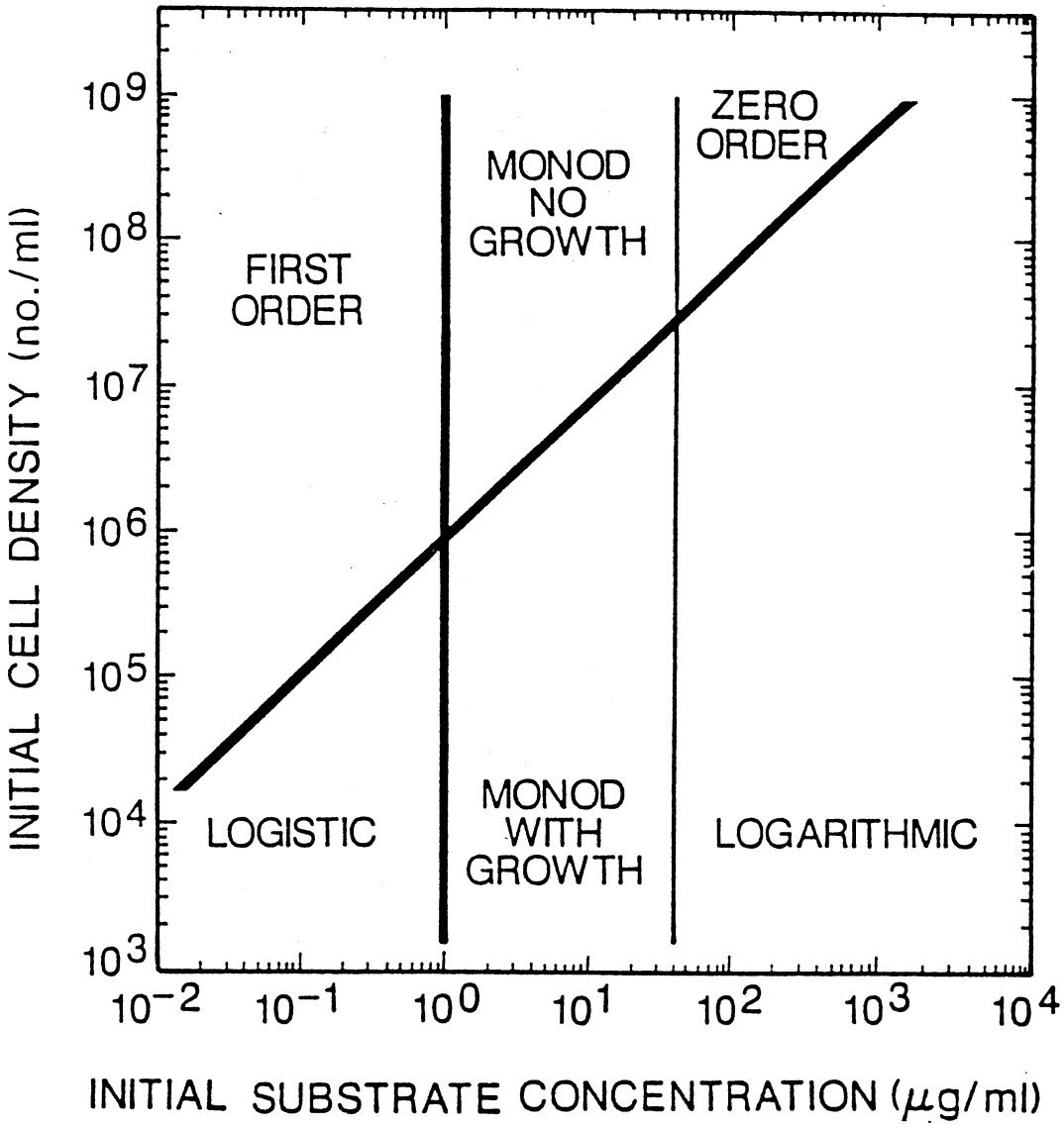


Figure 3. Kinetic Models as a Function of Initial Substrate Concentration and Initial Cell Concentration (after Simkins and Alexander, 1984).

Biodegradation of substrates not supporting growth is most typically modeled by Monod no growth kinetics (Simkins and Alexander, 1984; Schmidt *et al.*, 1985). Figure 4 illustrates the shape of the disappearance curves for the various approximations. Figure 4 is useful in visually selecting the models applicable to a particular study.

When a compound supporting growth obeys equations 14 and 15, its concentration may not fall below a minimum concentration, S_{min} which is obtained by setting the derivative of X with respect to time in equation 14 to zero and solving for S (McCarty, 1985). The minimum substrate concentration is described by:

$$S_{min} = K_s \frac{b}{(\mu_{max} Y - b)} \tag{16}$$

This suggests that concentrations of contaminants present below S_{min} may persist. In the presence of other growth supporting compounds, it is possible to have reduction below S_{min} (McCarty, 1985). This reduction is possible during cometabolism or gratuitous metabolism. However, the use of more easily biodegradable compounds may prevent conversion of substrate of interest.

When the initial concentration of cells is high, the change in biomass with respect to time is negligible, X is approximated by X_o, substrate removal can be modeled by using:

$$-\frac{dS}{dt} = K \frac{S}{(K_s + S)} \tag{17}$$

where

$$K = \mu_{max} X_o$$

When X is constant, equation 17 can be rearranged thus:

$$\frac{dS}{dt} = -K \frac{S}{(K_s + S)} \tag{18}$$

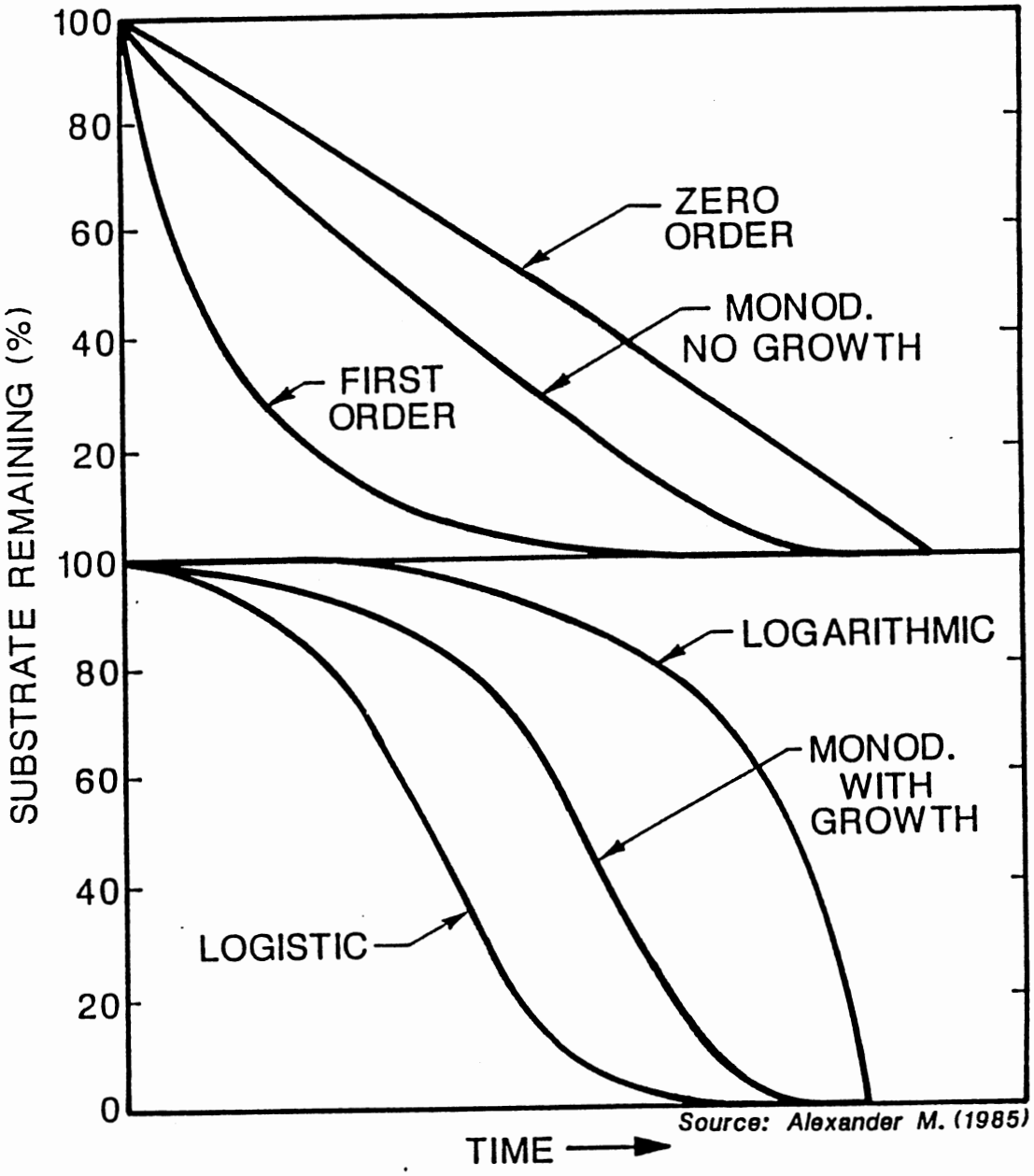


Figure 4. Disappearance Curves for Chemicals that are Mineralized as Related to Individual Kinetic Models.

Equation 18 can be solved implicitly using separation of variables and expressed as:

$$K_s \ln \left(\frac{S}{S_o}\right) + (S - S_o) = -Kt \qquad (19)$$

Since S cannot be expressed explicitly, an alternative method used to describe variation of substrate concentration with time is to use numerical techniques. Detailed derivation of this equation is shown in Appendix A.

When change in biomass concentration is negligible relative to the initial cell concentration, further simplifications of equation 18 are possible (Simkins and Alexander, 1984). At high substrate concentration, equation 18 reduces to a zero order equation dependent on only the biomass and independent of the substrate concentration. This is expressed as:

$$\frac{dS}{dt} = -K_1 \qquad \text{(differential form) (20)}$$

$$S = S_o - K_1 t \qquad \text{(integral form) (21)}$$

necessary conditions:

$$K_1 = \mu_{max} X_o$$

$$S_o >> K_s \quad \text{and} \quad X_o >> S_o$$

At low substrate concentration, equation 15 reduces to a first order equation. Thus,

$$\frac{dS}{dt} = -K_1 S \qquad \text{(differential form) (22)}$$

$$S = S_o \exp^{(-K_1 t)} \qquad \text{(integral form) (23)}$$

necessary conditions:

$$K_1 = \left(\frac{\mu_{max}}{K_s}\right) X_o$$

$$S_o << K_s \text{ and } X_o >> S_o$$

Due to the dependence of removal rate on concentration of microorganisms, increasing

the microorganism concentration increases the substrate utilization rate. At high cell and enzyme concentrations, the distinction between Michaelis-Menten equation and Monod equation describing substrate removal rates blurs. Michaelis-Menten equation is analogous to Monod equation shown in equations 18 and 19. Both equations are of the same hyperbolic form describing the removal of a substrate with time. The derivation of Michaelis-Menten equation is shown in Appendix B.

Kinetic Models

Kinetic models used for modeling the transformation of compounds by microorganisms can be divided into two major categories:

1. Those used for modeling substrate transformation coupled with growth. These include logistic (Schmidt *et al.*, 1985), logarithmic (Schmidt *et al.*, 1985), Monod with growth (Robinson and Tiedje, 1984; Schmidt *et al.*, 1985), compartment model (Scow, 1989), and three-half-order models (Brunner and Focht, 1984). These models require that the compounds removed are the growth limiting substrate. Product inhibition and cometabolism were modeled by Alvarez-Cohen and McCarty (1991).
2. Those used for modeling substrate transformation not supporting appreciable change in cell concentration. This can be because:
    a. Transformation involved resting microbial cells and no appreciable growth occurred at this stage.
    b. The initial cell concentration was high relative to the initial substrate concentration and/or the transformation of the initial substrate did not result in appreciable change in cell concentration.

    The models include Michaelis-Menten equation or Monod Kinetics (Simkins and Alexander, 1984; Schmidt *et al.*, 1985) and their zero order and first order approximations (Oldenhuis *et al.*, 1989; Strand *et al.*, 1990).

CHAPTER III

MATERIALS AND METHODS

Introduction

The experiments in this study were designed to meet the objectives discussed in Chapter I. The main objectives of this study were to demonstrate biodegradation of selected low molecular weight compounds and provide kinetic data for their biodegradation by the resting cells of *Pseudomonas putida* PpG-786 under various environmental conditions in the presence of an aquifer matrix. Environmental factors that were varied were temperature, pH, dissolved oxygen, and presence of one heavy metal at different concentrations. The effects of different substrate and cell concentrations were also evaluated. The primary purpose of these experiments was to demonstrate the biodegradation of a recalcitrant halogenated compound by microorganisms with high enzyme activities and to evaluate the effect of sterilized subsurface materials and selected environmental parameters on the rate of conversion of these types of compounds.

Subsurface materials are all materials removed from the sampling sites at the depths indicated in Table II, air dried and passed through a size 40 mesh sieve (0.425 mm openings). High enzyme activity was induced by culturing *Pseudomonas putida* in the presence of D(+)-Camphor, an inducer of the cytochrome P-450$_{cam}$ enzyme system. Such a study will provide the background work necessary to design an *in situ* biorestoration system for contaminated subsurface materials using microorganisms with elevated enzyme activities. The experimental procedure (Figure 5) together with the materials used are described below.

TABLE II

SAMPLE SITES FROM SAND SPRINGS PETROCHEMICAL COMPLEX SITES AND OKLAHOMA STATE UNIVERSITY AGRONOMY STATION

Identification Number	Site Number	Location	*Sampling Depth (inches)
T - 29	1	About 30 - 35 ft. southwest of South Glen Wynn Lagoon, Sand Springs Petrochemical Complex	20, 30
T - 22	2	In an old tank battery, 200 ft. northeast of South Glenn Wynn Lagoon, Sand Springs Petrochemical Complex	11, 24
T - 32	3	Within 4 ft. of the southwest corner of South Glen Wynn Lagoon, Sand Springs Petrochemical Complex	24, 68
	4	Oklahoma State University Agronomy Station, Perkins, Oklahoma	174

*Depth below ground surface.

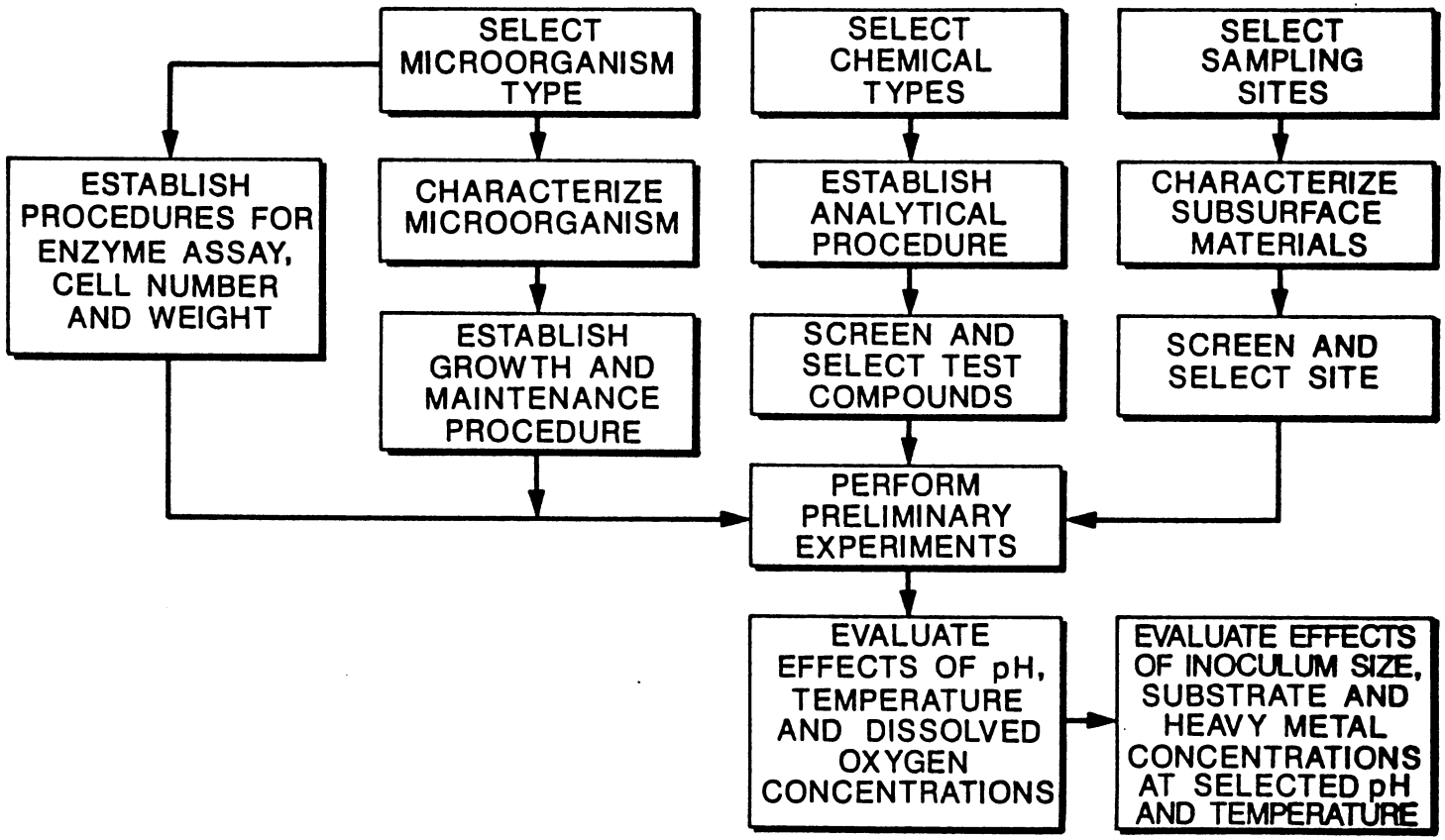


Figure 5. Overview of Experimental Procedures.

Source of Pure Culture

A strain of *Pseudomonas putida* PpG-786 which has demonstrated high enzyme concentration when grown in the presence of D(+) camphor was obtained from the American Type Culture Collection (ATCC). *Pseudomonas putida* PpG-786 (ATCC 29607) was initially isolated from soil by Hedegaard *et al.* (1961) and is therefore an ideal candidate for restoration of groundwater. The procedure for cultivating, handling, and maintaining the pure culture is shown in Figure 6. The characteristics of the *Pseudomonas sp.* used are listed in Appendix C.

Maintenance of Pure Culture

Pseudomonas putida acquired from ATCC was in a freeze-dried form and was rehydrated using camphor minimal medium. The culture was maintained by weekly transfer into agar plates containing D(+) camphor inside the top cover of the petri dish. The agar media was composed of 20 g Difco Bacto agar/L and potassium phosphate-ammonium chloride solution. Phosphate ammonium solution was 100 parts phosphate-ammonium (PA) solution [10.7 g/L K_2PO_4, 3.1 g/L KH_2PO_4, 8.0 g/L NH_4Cl, pH 7.4] mixed with one part mineral salt solution. The mineral salt solution consisted of 19.5 g/L $FeSO_4.7H_2O$, 0.3 g/L $CaCl_2.H_2O$, and 1.0 g/L ascorbic acid (Lam and Vilker, 1987).

Growth Procedure for *Pseudomonas putida*

Pseudomonas putida PpG-786 obtained from ATCC was cultured according to the procedure of Lam and Vilker (1987). This involved a three staged growth procedure:

1) Stage 1

   P. putida was transferred from the agar plates into a flask containing 50 ml of L-broth (1.0 g/L Bacto tryptone, 0.5 g/L yeast extract, 0.5 g/L NaCl, 0.1 g/L glucose, pH 7.4). The flask was agitated continuously at room temperature for 24 h.

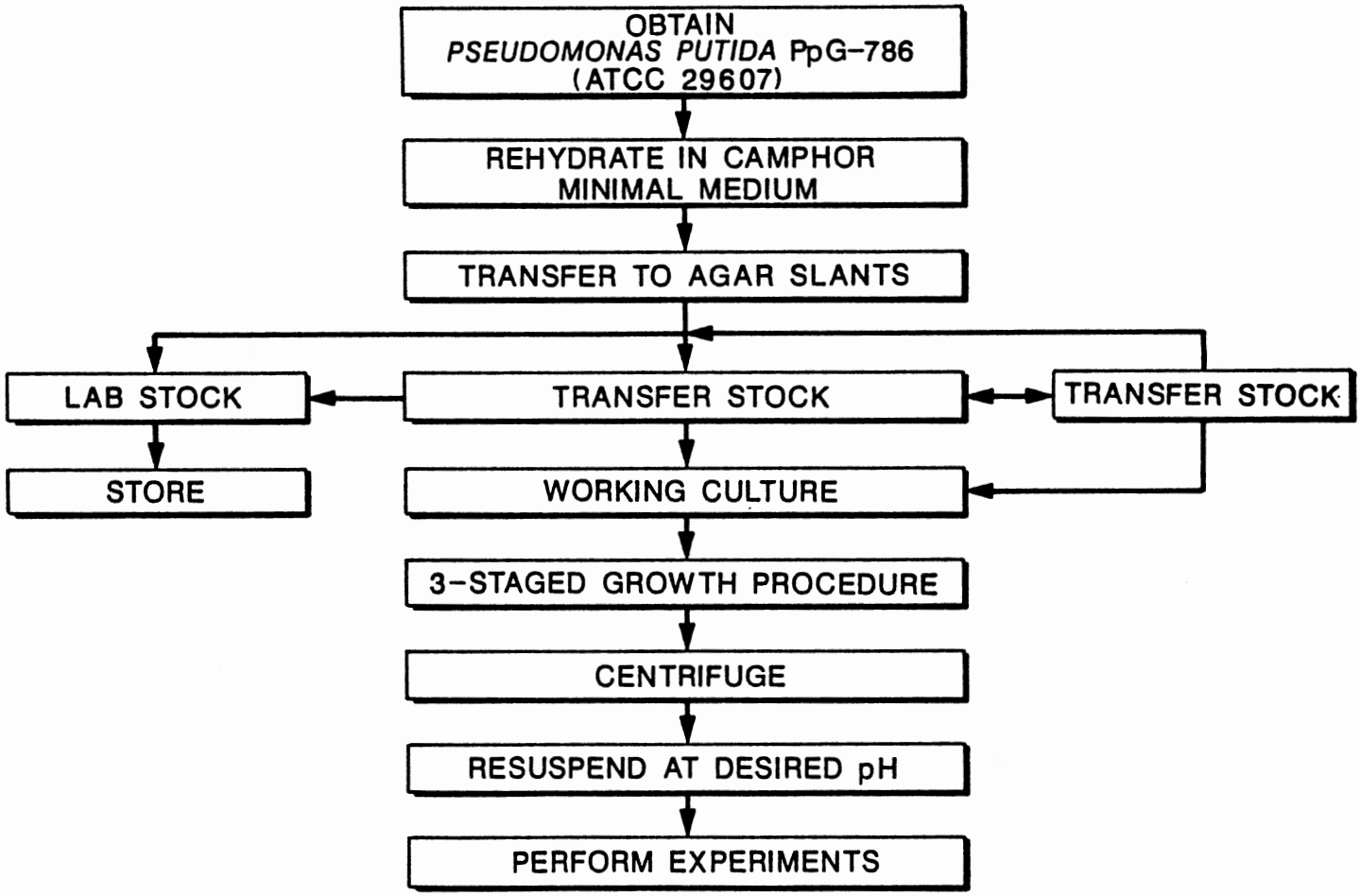


Figure 6. Flowchart for Cultivation, Handling, and Maintenance of *Pseudomonas putida* PpG-786.

2) Stage 2

Another flask containing 100 ml phosphate ammonium salt (PAS), containing phosphate-ammonium (PA) and mineral salt solutions, with 10mM sodium glutamate was incubated with 0.5 ml of the contents of the flasks from Stage 1. This flask was agitated at room temperature for 8 h. Then 0.15 ml of 3M camphor in N,N-dimethylformamide (stock camphor) was added. This was followed 12 h later by another addition of 0.15 ml of stock camphor with incubation and agitation for 4-6 h. The flask was continuously agitated throughout Stage 2.

3) Stage 3

The entire content of the flask from Stage 2 (100 ml) were transferred to a fermenter containing 10 L PA solution, 50 ml 10% Bacto yeast extract solution and 10 ml stock camphor solution. No antifoam agent was used. Laboratory air was filtered through an activated carbon column and air filter (pore size $< 0.45\ \mu m$). Purified laboratory air was required to prevent contamination of the pure culture growing in the fermenter. The air was introduced into the fermenter at the rate of 7000 cm^3/ minute. This stage lasted for 12 h.

Enzyme-rich resting cells from the fermenter were harvested by centrifuging at 3550 G on a Beckman J-21B for 10 minutes. The cell paste was then washed twice with reagent water to remove residual camphor. Cells were suspended in 0.1 M mono and dibasic phosphate buffer adjusted to the required pH.

Reagent water was prepared by boiling reverse osmosis water from an Autostill 5 distiller (Wheaton Co.) for 15 minutes. While maintaining the temperature at 90°C, nitrogen was bubbled through activated carbon column and cotton swabs into the water for one hour. The pure water was cooled, then transferred to tightly capped containers for storage. Procedure used in cleaning the containers was previously outlined by Betsill (1990).

Preliminary Experiments

Preliminary experiments were conducted to determine appropriate methods for estimating cell density, determine the stability of the cytochrome P-450$_{cam}$ enzyme system, and account for losses due to abiotic mechanisms such as volatilization, effect of laboratory light, adsorption onto glassware, and adsorption onto aquifer materials. The subsurface materials were also characterized using pH, moisture content, particle size distribution, heavy metal concentration, porosity, and density. Experiments were conducted to evaluate adsorption of test compounds onto cellular materials.

The Determination of Cell Concentration

The concentration of resting *Pseudomonas putida* cells suspended in 0.1 M phosphate buffer (mono and dibasic phosphate) was determined using total solids, optical density and viable and total cell counts. To determine the total solids, clean evaporating dishes were ignited at 550°C for 1 hour in a muffle furnace, cooled, dessicated, and weighed. The evaporating dishes were cleaned using Microclean^{R} and rinsed with double distilled deionized water. Ten milliliters of cell suspended in phosphate buffer were placed into each dish and dried at 56°C (Vilker and Khan, 1989). Phosphate buffer controls were set up to correct for inorganic dissolved solids in the experimental medium. The dry weights of the cells were determined as the average weights of samples with cell suspended in phosphate buffer control minus the average weights of the samples with phosphate buffer control.

Optical density measurements were taken on serial dilutions of cell suspension. Measurements were taken at 600 nm with Baush and Lomb Spectronic 100. Viable counts were done by plating out serial dilutions of original stock in camphor minimal medium or Trypticase-Soy Agar (TSA). Total count was determined using direct microscopic count.

The Determination of Enzyme Concentration

Cytochrome P-450$_{cam}$ content of whole *Pseudomonas putida* PpG-786 cells was deter-

mined using the procedure of O'Keeffe *et al.* (1978). A 6 ml aliquot of whole cells of *Pseudomonas putida* was deoxygenated by gently bubbling argon into the vial. A few grains of sodium dithionate was added to totally reduce the haeme iron present in the cytochrome P-450 to its ferrous form. The cell suspension was then evenly divided into sample and reference cuvettes (1 cm pathlength). A spectral baseline was recorded from 400 to 500 nm. The sample cells were then gently bubbled with carbon monoxide for 15 seconds. The ferrous-carbon monoxide versus ferrous cytochrome P-450$_{cam}$ difference spectrum was recorded. A differential extinction coefficient of 91 mM^{-1} cm^{-1} between 446 nm and 490 nm for the Soret band of the ferrous carbon monoxide and ferrous forms was used to determine the amount of cytochrome P-450$_{cam}$ present (Omura and Sato, 1964). The equation used was:

$$A = E^*C^*L \tag{24}$$

where

A = absorbance

E^* = molar coefficient of extinction (91 $mM^{-1}Cm^{-1}$)

C^* = concentration

L = light path, usually 1cm

Equation 24 was solved for the concentration of the enzyme, C^*. Measurements were taken on a Shimadzu UV-160A, a microcomputer-controlled double-beam recording spectrophotometer.

Site Description

Subsurface materials were collected from two locations in the State of Oklahoma. The first site was the Sand Springs Petrochemical Complex, Sand Springs, Oklahoma, an EPA superfund site. Samples were collected from three sampling points around the South Glen Wynn Lagoon as listed in Table II. The Glen Wynn sampling sites are indicated in Figure 7. The South Glenn Wynn lagoon covers an area of 4769 sq. ft. This lagoon is unlined and was

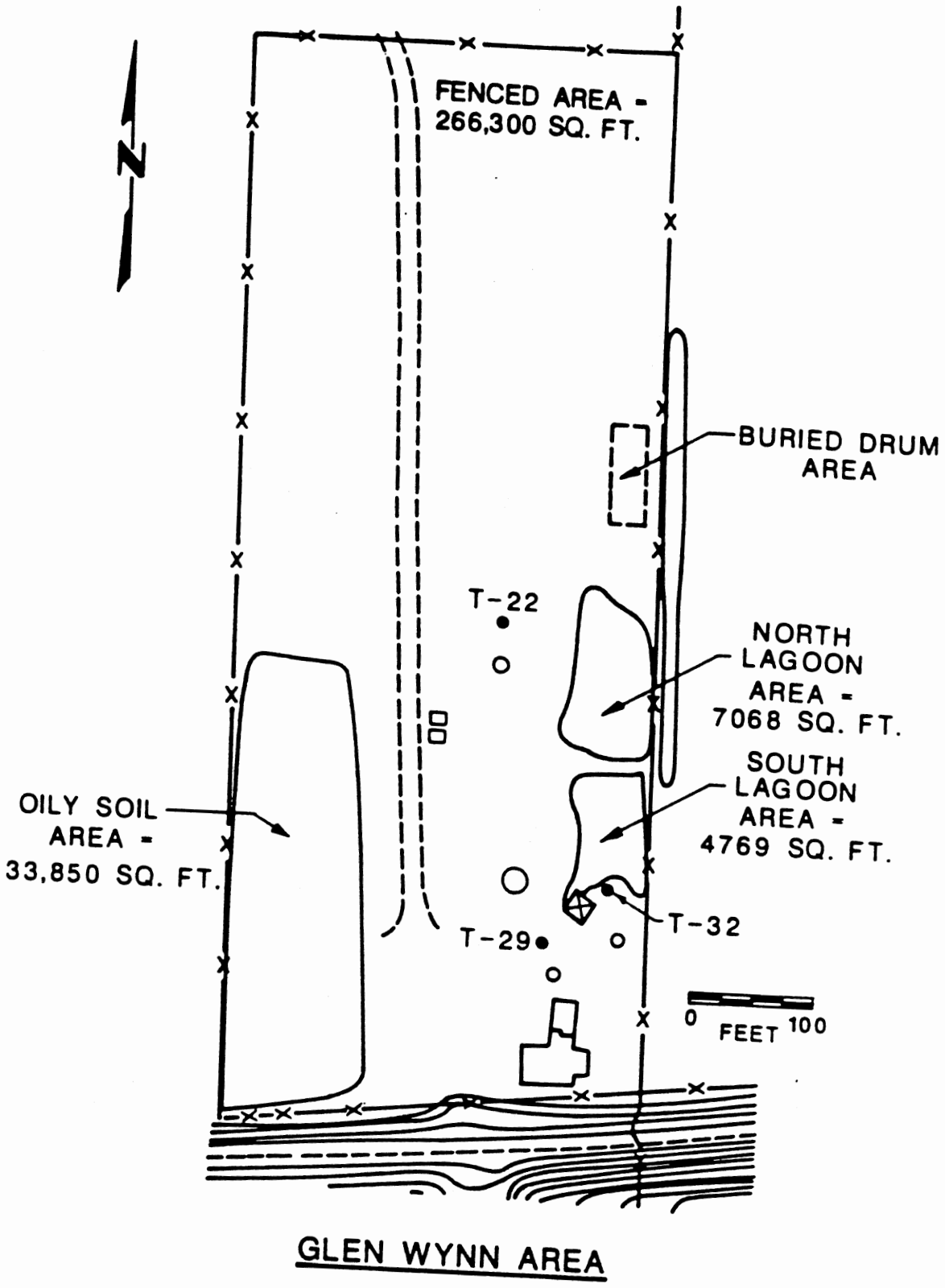


Figure 7. Location of Sampling Point at Sand Springs Petrochemical Complex, Sand Springs, Oklahoma

used as a dumping pit for wastes from oil and solvent recovery operations between 1964 and 1983 (Bechtel, 1989). Samples were collected by drilling with a hand auger above the groundwater table because most of the contaminants from the petrochemical complex site were located in this region (John Mathes and Associates, Inc., 1987). Earlier estimate of the depth to groundwater surface was about 22 ft (John Mathes and Associates, Inc., 1987).

The Oklahoma State University Agronomy Research Station located at Perkins, Oklahoma was the source of the fourth sample (Figure 8). The sampling depth at this site was 14.5 ft which is below the groundwater table. The groundwater table was observed to be at a depth 11 - 12 ft.

Samples were collected at the two sites by drilling using a hand auger to the appropriate depth and were transported to the Oklahoma State University Environmental Engineering Laboratory for the determination of total organic matter, pH, moisture content, particle size distribution, porosity, and density. The procedures used are described in detail below. The hand auger was cleaned with Microclean^{R} and rinsed with double distilled deionized water. The auger was also wiped with cotton swabs dipped in methanol prior to sampling.

Determination of Organic Matter of Subsurface Materials

Organic matter content of subsurface materials was estimated using a Hach DR/3 spectrophotometer with a precalibrated meter scale. The organic matter was oxidized using the dichromate method. Hexavalent chromium (Cr^{6+}) was reduced to trivalent form (Cr^{3+}) while the organic matter was converted to carbon dioxide. The reduction of chromium was accompanied by a change in color from orange to green. The procedure was as follows (Hach Company, 1985):

One-half to one gram of subsurface materials were oxidized with 10 ml of 1.00 N potassium dichromate solution in a 250-ml Erlenmeyer flask. Twenty ml of concentrated sulfuric acid was added to each flask. The flasks were then covered with inverted 50-ml Erlenmeyer flasks. The 250-ml flasks were placed on asbestos hot plates for 10 minutes.

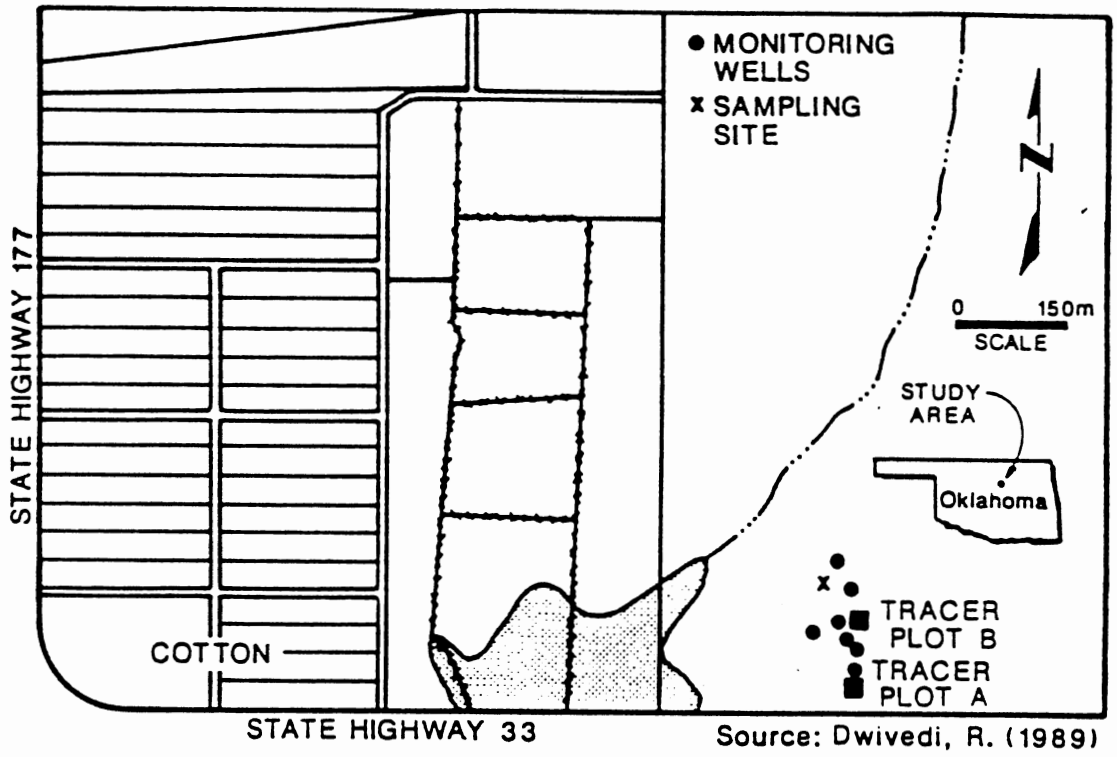


Figure 8. Location of Sampling Point at Oklahoma State University Agronomy Research Station.

The flasks were removed from the hot plate at 10 minutes as indicated by a timer and 100 ml of demineralized water was added to each flask. The flasks were allowed to stand overnight to allow the soil residue to settle.

Twenty-five ml of clear solution was pipetted into the sample cell of the Hach DR/3 spectrophotometer set at a wavelength of 610 nm. The absorbance was read for each sample and a control prepared with the same procedure outlined above except no subsurface materials were added. The concentration of organic matter in the sample was either read directly (if sample size was 1.0 g) or adjusted using the correction factor:

$$\frac{1.0\ g}{\text{actual sample size (g)}}$$

Determination of pH, Moisture Content, and Particle Size Distribution

The subsurface materials were further characterized for pH, moisture content, and particle size distribution on both original and air dried samples. pH was determined using 10 gm of subsurface materials blended with 50 ml of demineralized water in a blender. The pH of the soil slurry was determined using a Fisher Scientific Accumet 900 pH meter. The moisture content of the materials was determined at 103°C in a Precision Scientific Thelco model 17 oven. Samples were dried overnight in the oven.

The percent moisture content w was calculated using:

$$w = \frac{W_w}{W_s}\ 100 \text{ percent} \tag{25}$$

where

W_w = weight of water present in the aquifer materials

W_s = weight of soil solids

The weight of water present in the aquifer materials (W_w) was the difference between

the weight of the sample before and after oven drying.

Particle size distribution of the subsurface materials was determined using a wet method (Snethen, 1990) for the Sand Springs Petrochemical complex sample, while a dry method was used on the Oklahoma State University Agronomy Station sample (Bowles, 1986). A wet method was required for the Sand Springs Petrochemical Complex site because of the level and nature of pollutants at the site. The samples from the Sand Springs Petrochemical Complex were sticky and not easily air-dried. For the wet method, samples were initially dispersed in either acetone, hexane, or methanol. Acetone gave the best visual dispersion of subsurface materials and was chosen for the particle size analysis. One hundred grams of subsurface material was washed through a stack of sieves (numbers 4, 20, 40, 100, 140, 200), dried, and weighed. The amount of samples retained on each sieve was calculated. For the dry method, 500 g of unsieved subsurface material was washed through a No. 200 sieve with tap water. The residue was poured, using backwashing, into a large weighed dish and allowed to sit for a short period of time until the top suspension became clear. The top clear water was poured off and the remaining soil-water suspension was dried in an oven at 103°C for 24 hours. The weight of the oven dried residual was determined. Cooled, dried samples were poured through the stack of sieves (40 to 200) indicated above, shaken, and weighed. Air dried subsurface materials were sieved through a 40 mesh sieve (0.425 mm openings) and stored in air-tight containers until used.

Determination of Heavy Metal Concentrations in the Subsurface Materials

One gram of air dried subsurface materials was digested in 250-ml beakers with watch glass covers (Plumb, 1981). The subsurface materials were moistened with 0.5 - 1.0 ml deionized distilled water. Ten ml of concentrated (15 N) HNO_3 was added to the beaker and swirled. The beakers were placed on hot plates. The samples were brought to slow boil and boiled continuously until the solution approached dryness. More HNO_3, in 5-ml increments,

was added until all visible organic matter was destroyed and the solution began to clear. Boiling continued until the evolution of reddish-brown fumes ceased.

The breakers were removed from hot plates, cooled to room temperature, and 20 ml double distilled water was added to rinse the beakers and watch glasses clear. These were transferred and made up to 100 ml with double distilled deionized water and analyzed for heavy metal concentration using inductively coupled plasma atomic absorption spectrophotometry by the Soil Forage Laboratory, Oklahoma State University. The instrument used was a Thermo Jarrel Ash 9000. The metals analyzed for were cadmium, chromium, copper, nickel, lead, and zinc.

The detection limits were 0.01 mg/l for cadmium, 0.02 mg/l for chromium, 0.01 mg/l for copper, 0.03 mg/l for nickel, 0.08 mg/l for lead, and 0.01 mg/l for zinc.

The concentrations of metals in the air dried aquifer materials were calculated using:

$$\text{Metal concentration, mg/g} = \frac{B \times F}{\text{g of air dried samples}} \qquad (26)$$

where

B = concentration of metal in digested solution, mg/L

F = final volume of digested solution, L

Determination of Porosity and Density

Further characterization of aquifer materials from Oklahoma State University Agronomy Research Station was done using porosity and density. The procedures for the determination of porosity and density used in this research were as follows (Association of Environmental Engineering Professors, 1975).

Total Porosity

Two 1000 milliliter graduated cylinders were used. Two-hundred milliliters of air-dried

sample were placed in one cylinder while the other cylinder contained 500 milliliters of distilled water. The sample was slowly poured into the cylinder containing the 500 milliliters of water. The total volume of the sample, the water and the apparent volume of the sample were observed and recorded. Then to simulate fluidization of the particles, enough water was added to completely fill the cylinder, which was then plugged on top with parafilm. The cylinder was rapidly inverted several times, then quickly set down to allow the particles to settle. The apparent volume of the sample was measured. A duplicate sample was similarly analyzed.

Density or Specific Gravity

The density of triplicate air-dried samples was determined using water displacement technique in a 250 ml BOD bottle. The bottles were weighed empty, then after completely filling with water at 24°C, were weighed again. The bottles were dried and partially filled with air-dried samples and weighted. Water was added to fill the bottle, capped, then shaken vigorously to drive out air bubbles in or on the media. The samples were allowed to soak overnight with occasional shaking to expel air bubbles. At 24 hours, the bottles were filled with water, capped, then weighed again.

Criteria for the Selection of Organic Compounds

The biodegradation of low molecular weight halocarbons found at two sites in the State of Oklahoma was investigated. The sites surveyed were the EPA Superfund Site at Sand Springs, Oklahoma, and Tinker Air Force Base Waste Site. Two chemicals, 1,2-dichloropropane and 1,2-dichloroethane, were selected from the lists in Appendix D, and an initial screening of the biodegradation potential of the two compounds was done. Further experiments were done with 1,2-dichloropropane which showed appreciable removal relative to the control within 4 hours. No appreciable removal of 1,2-dichloroethane was observed within the four hour test period. 1,2-dibromo-3-chloropropane, previously shown to be biodegraded

by *Pseudomonas putida* PpG-786 by Lam and Vilker (1987), was also tested with and without aquifer materials.

Materials

As previously stated, the chemicals used in the biodegradation study are prevalent in groundwater aquifers around the United States and are potential pollutants in the State of Oklahoma (John Mathes and Associates, Inc., 1987; Combs, 1987). Possible sources of groundwater pollution by chlorinated halogenated compounds together with pollutants previously identified at two different sites are shown in Appendix D. The physical properties of the chemicals selected are listed in Table III. All organic compounds used in the study are reagent grade ACS certified. The list of chemicals used in this research is given in Appendix E.

Gas Chromatographic Analysis

The disappearance of the parent compounds in batch reactors was monitored using a gas chromatographic technique. A Perkin-Elmer Sigma 2000 model equipped with a nickel^{63} electron-capture detector was used. The GC was fitted with the appropriate column set out below. The carrier gas used was 95% argon and 5% methane supplied by Big Three Industries, Grand Prairie, Texas.

COMPOUND: 1,2-dichloropropane and 1,2-dichloroethane

COLUMN: 3% SP 1000 on 100/120 Supelcoport

GAS: 95% argon and 5% methane (on a mole percent basis).

GC OPERATING CONDITION:

OVEN TEMPERATURE:	68°C
INJECTION PORT TEMPERATURE:	100°C
ELECTRON CAPTURE DETECTOR:	350°C

TABLE III

CHARACTERISTICS OF ORGANIC COMPOUNDS

Properties	1,2-dichloro-propane*	1,2-dichloro-ethane**	1,2-dibromo-3-chloro propane*
Molecular Weight	112.99	98.96	236.36
Boiling Point (°C)	96.4	83.5	196
Melting Point (°C)	-100.4	-35.3	---
Vapor Pressure (mm Hg)	50 (20°C)	64 mm (20°C)	0.8 (21°C)
Henry's Law Constant ($atm.m^3/mol$)	0.00294 (25°C)	0.00131 (25°C)	0.000126 (20°C)
Solubility	2700 mg/l (20°C)	8300 mg/l (20°C)	1230 mg/l (20°C)
Specific Gravity	1.15 (20°C)	1.235 (20°C/4°C)	2.08 (20°C)
Log Octanol/Water Partition Coefficient	2.28	1.15***	2.43
CAS Number	78-87-5	107-06-2	96-12-8

Source: *USEPA (1988).
**Montgomery, J.H. and Welkorn, L.M. (1990).
***USEPA (1988)

COMPOUND: 1,2-dibromo-3-chloropropane

COLUMN: OV 101 on 80/100 Chromosorb WHP

GAS: 95% argon and 5% methane (on a mole percent basis)

GC OPERATING CONDITION:

OVEN TEMPERATURE: 115°C

INJECTION PORT TEMPERATURE: 150°C

ELECTRON CAPTURE DETECTOR: 250°C

The oven temperature for analysis of 1,2-dichloropropane was sometimes adjusted downwards to prevent interference from the reaction matrix.

Experimental Systems

All subsurface materials and vials were sterilized twice in an Amsco medalist 200 autoclave at 121°C and 18.5 psi. The sterilization of the subsurface materials was done for 2 hours each time in 15 ml vials covered with aluminum foil placed in autoclavable bags to prevent moisture from the autoclave from condensing onto the sample. All glassware was cleaned according to the cleanup procedure outlined by Betsill (1990). Three-tenths gram of subsurface material was used to evaluate biodegradation rates in the presence of aquifer materials. The sterilization procedure adequacy was verified by plating out sterile aquifer materials in a rich medium (TSA). During the experiments, the foil was replaced by sterile teflon-coated screw caps with mininert valves from Supelco. Cells in 0.1 M phosphate buffer were introduced into the vials, capped and when necessary allowed to equilibrate to the experimental temperature (usually at least 10 minutes for measured temperature in batch reactors to be at experimental temperature). The biodegradation reaction was initiated by introducing the chemical of interest into the vials. Each vial was shaken on shakers for a specified time (between zero and four hours) and then terminated by injecting 2 ml of hexane into the vial. The samples were then shaken for 10 minutes followed by centrifugation at 2600 rpm for 45 minutes. This speed was chosen to prevent breakage of vials. Hexane extracts of chemicals

of interest were transferred into 2 ml storage vials with teflon lined caps for subsequent gas chromatographic analysis. The sediments obtained during centrifugation were discarded. Samples were stored at $<4°C$ and analyzed as soon as possible. Concentrations of the parent compounds were monitored using the gas chromatographic method of peak retention time for identification. Samples were set in duplicate or triplicate.

Stock solutions of each compound were prepared and adjusted to the required concentration using ACS certified methanol. The stocks were compared to EPA quality control standards. A calibration curve was prepared for each analysis and the concentrations of the samples were read off the calibration curve.

The detection limits established for 1,2-dichloropropane, 1,2-dibromo-3-chloropropane, and 1,2-dichloroethane were 11 μg/L, 3 μg/L, and 3 μg/L, respectively.

The removal of organic compounds from the liquid phase in the presence of groundwater aquifer material from the OSU Agronomy Station was determined while environmental parameters such as pH, temperature, dissolved oxygen and heavy metals concentration were varied. Various inoculum sizes were used to evaluate how removal rates changed with cell concentration. The effect of initial substrate concentration and subsurface materials from Sand Springs Petrochemical Complex Site were investigated at pH of 7.4 and temperature of 25°C. Although samples were collected form T-22, T-29, and T-32 at the Sand Springs Petrochemical Complex, Sand Springs, Oklahoma, only samples from T-32 were used for the kinetic study. Site T-32 was closest to the South Lagoon area where the wastes from the petrochemical industry were dumped. An initial screening of biodegradation of 1,2-dichloroethane, 1,2-dichloropropane, and 1,2-dibromo-3-chloropropane was also conducted.

In order to fulfill the objectives of this study, experiments were performed under various environmental conditions to evaluate the biodegradation of low molecular weight halogenated compounds by *Pseudomonas putida* in an aquifer matrix. The environmental factors that were examined were:

1) **Effect of pH**

Most enzymes are affected by proton concentration (usually expressed as pH). Three pH values ranging from 5.4 - 8.9 were used in the degradation kinetic study of 1,2-dichloropropane in the presence of Oklahoma State University aquifer matrix. Specific pH values used were 5.4, 7.4, and 8.9. Buffers were prepared by titrating 0.1 M mono and dibasic phosphate buffer.

2) **Effect of Temperature at Different pHs**

The range of temperatures used in this study was 15°C - 30°C. Specific temperatures used were 15°C, 25°C, and 30°C. Based on the initial results from pH experiments, the corresponding pH used were 6.4, 7.4, and 7.8. This was used to evaluate the effect of temperature on the biodegradation of 1,2-dichloropropane. For experiments conducted at pH 6.4, 7.4, and 7.8, various concentrations of ACS certified sodium chloride were added as shown in Appendix F. The concentrations of chloride added were 2.102 g/L, 0.871 g/L, and 0.151 g/L at pH 6.4, 7.4, and 7.8, respectively for common ionic strength. Concentrations of chloride in groundwater is highly dependent on the region and varies from 0.010 g/L in humid regions to 200 g/L in brines (Todd, 1990). The typical chloride ion in groundwater is 0.200 g/L (Tchobanolous and Schroeder, 1985).

3) **Effect of Heavy Metal**

The effect of lead (as lead acetate) at concentrations of 0 mg/L, 2.2 mg/L, 5.8 mg/L, and 10 mg/L on the kinetics of biodegradation of 1,2-dichloropropane was investigated in this study. Based on the pH and temperature results, the effect of lead (as lead acetate) was evaluated at pH of 7.4 and temperature of 25°C. Previous measurements of metals present at the South Glen Wynn Lagoon, Sand Springs Petrochemical Complex indicated that the inorganic priority pollutant having the highest concentration was lead which was present at 2,022 mg/kg in the solids and 0.593 mg/L in the liquid phase (John Mathes and Associates, Inc., 1987).

4) **Effect of Dissolved Oxygen**

Various levels of initial dissolved oxygen were investigated in this study. The dissolved oxygen ranged from 6.0 mg/L to 16.0 mg/L. Specific dissolved oxygen concentrations were 6.0 mg/L, 8.2 mg/L, and 16.0 mg/L. Based on the pH and temperature results, the effect of dissolved oxygen was evaluated at pH of 7.4 and temperature of 25°C. Dissolved oxygen was measured with an YSI Model 54A dissolved oxygen meter.

5) **Effect of Inoculum Size**

A series of experiments was designed to investigate the effect of initial concentration of microorganisms required to effect removal of 1,2-dichloropropane in the presence of subsurface materials within a reasonably short period of time. The dry cell weights (at 56°C) used were 1.455 g/L, 3.317 g/L, 6.470 g/L, and 8.017 g/L.

6) **Effect of Substrate Concentration**

The effect of varying substrate concentration of 1,2-dichloropropane was also investigated. The concentrations of 1,2-dichloropropane used were 732 μg/L, 1209 μg/L, and 4907 μg/L.

A series of experiments designed to account for losses due to abiotic processes was conducted. They were to evaluate losses of test compounds due to:

a) sorption of compounds onto glassware

b) volatilization of the compounds

c) sorption of organic compounds onto the subsurface materials

d) loss due to adsorption onto cells

e) effect of laboratory light

The experimental set-up is shown in Table IV. Other experiments included an estimate of how long the enzyme stayed active and adsorption of the microbial cells onto the OSU aquifer material. The *Freundlich adsorption isotherm* (Freundlich, 1926) was used to describe the adsorption of *Pseudomonas putida* onto Oklahoma State University Agronomy

Station aquifer materials. A stock concentration of *Pseudomonas putida* at 4.8 x 10^{11} cells was diluted using 0, 100, 1000, and 10,000 dilution factors and the adsorption of *Pseudomonas putida* to three-tenth gram of Oklahoma State University Agronomy Station aquifer materials was observed. Adsorption of test compounds onto cellular materials was also accounted for by adding silver sulfate or potassium cyanide to the reaction vials to inactivate the cells. Experiments were conducted at pH 6.4, 7.4, 7.8, and room temperature.

TABLE IV

EXPERIMENTAL SET-UP FOR PRELIMINARY EXPERIMENTS TO ACCOUNT FOR ABIOTIC LOSSES

Experiment	Condition
Glassware adsorption	Dark, no headspace, no aquifer matrix
Volatilization	Dark, headspace, no aquifer matrix
Adsorption onto aquifer matrix	Dark, no headspace, with aquifer matrix
Effect of laboratory light	Light, no headspace, no aquifer matrix

Samples from a United States Environmental Protection Agency (USEPA) Superfund site were also spiked with the compound of interest (1,2-dichloropropane) and its removal with time was monitored to evaluate how biodegradation of the compound of interest was influenced by the presence of subsurface materials while environmental parameters were varied. The Superfund site was Sand Springs Petrochemical Complex, Sand Springs, Oklahoma. Table V shows the experimental conditions under which the different experiments were conducted.

TABLE V

EXPERIMENTAL CONDITIONS FOR KINETIC EXPERIMENTS

Experiment/ID #**	pH	Temp °C	Subs Mat.	Wet Weight g/L	Dry Weight g/L	Optical Density
EFFECT OF PRESENCE OF AQUIFER MATERIALS (1,2-dibromo-3-chloropropane)						
DBCPDF	7.4	25	NONE	17.5	3.000	0.7
DBCPDF2	7.4	25	OSU	17.5	3.000	0.7
EFFECT OF AQUIFER						
AQUIFDF	7.4	25	OSU	28.8	5.753	*1.2
AQUIFDF2	7.4	25	SS	28.8	5.753	*1.2
EFFECT OF pH						
pH54(25)	5.4	25	OSU	26.6	*5.200	1.4
pH74(25)	7.4	25	OSU	26.6	*5.200	1.4
pH89(25)	8.9	25	OSU	26.6	*5.200	1.4
EFFECT OF DISSOLVED OXYGEN[1]						
DOCOM3	7.4	25	OSU	26.6	5.527	1.5
DOCOM4	7.4	25	OSU	26.6	5.527	1.5
DOCOM5	7.4	25	OSU	26.6	5.527	1.5
EFFECT OF TEMPERATURE[2]						
pH64(15)	6.4	15	OSU	26.6	6.223	1.6
pH64(25)	6.4	25	OSU	26.6	7.907	1.6
pH64(30)	6.4	30	OSU	26.6	7.417	1.6
pH74(15)	7.4	15	OSU	26.6	6.573	1.6
pH74/25	7.4	25	OSU	26.6	7.743	1.6
pH74(30)	7.4	30	OSU	26.6	8.107	1.6
pH7815c	7.8	15	OSU	26.6	4.747	1.6
pH7825c	7.8	25	OSU	26.6	6.617	1.6
pH7830c	7.8	30	OSU	26.6	*5.200	1.5
EFFECT OF INOCULUM SIZE						
INNODES1	7.4	25	OSU	17.2	1.455	0.6
INNODES2	7.4	25	OSU	20.6	3.317	0.7
INNODES3	7.4	25	OSU	32.7	6.470	1.0
INNODES4	7.4	25	OSU	41.1	8.017	1.2
EFFECT OF SUBSTRATE CONCENTRATION						
SUSTIDF	7.4	25	OSU	26.6	5.713	0.9
SUST2DF	7.4	25	OSU	26.6	5.713	0.9
SUST3DF	7.4	25	OSU	26.6	5.713	0.9

TABLE V (continued)

Experiment/ID #**	pH	Temp °C	Subs Mat.	Wet Weight g/L	Dry Weight g/L	Optical Density
EFFECT OF HEAVY METAL CONCENTRATION[3]						
LEADDES1	7.4	25	OSU	26.6	5.995	1.3
LEADDES2	7.4	25	OSU	26.6	5.245	1.4
LEADDES3	7.4	25	OSU	26.6	5.842	1.2
LEADDES4	7.4	25	OSU	26.6	5.995	1.3

*Estimated from cell concentration curves.
**Experiments performed at DO 16 mg/L and 1,2 dichloropropane except where indicated.
[1]Experiments conducted at 16.0 mg/L, 8.2 mg/L and 6.0 mg/L dissolved oxygen for DOCOM3, DOCOM4, DOCOM5 respectively.
[2]Experiments were conducted with chloride concentration of 2.102 g/L, 0.871 g/L and 0.151 g/L at pH 6.4, 7.4 and 7.8 respectively.
[3]Lead concentrations were 0 mg/L, 10 mg/L, 5.8 mg/L and 2.2 mg/L for LEADDES1, LEADDES2, LEADDES3, and LEADDES4 respectively.
SUBS MAT - Subsurface Materials
OSU - Oklahoma State University Agronomy Research Station aquifer
SS - Sand Springs Petrochemical Complex subsurface materials

Statistical Analysis

Statistical analyses were performed on experimental data obtained for the following:

- Experiments to account for abiotic losses of test compounds
- Cellular adsorption of test compounds
- Buffer controls for kinetic experiments
- Concentration of test compounds remaining at the end of four hours experiments in microcosm containing *Pseudomonas putida* PpG-786.

Analysis of variance (ANOVA) procedure was applied to experimental data to account for abiotic losses of test compounds. The hypothesis tested was if the mean concentrations obtained at the end of the four hour test period were equal under different experimental conditions shown in Table IV. The different test conditions were designed to account for effect of laboratory light, volatilization, aquifer adsorption (where applicable), and adsorption onto glassware.

The General Linear Model (GLM) procedure found in the SAS program (SAS Institute Inc., 1985) was applied to data obtained to account for cellular adsorption of test compounds. The GLM procedure used a method of least squares to test a time dependent difference in the mean concentrations of test compounds in the microcosm containing cells and those containing buffer only. The Mean procedure found in SAS, together with a T-test (Lotus Development Corporation, 1985) on the coefficients of linear regression model, were used to evaluate the variation in concentration of test compounds in the buffer controls of the kinetic experiments.

The different kinetic experiments are shown in Table V. A paired T-test procedure available in SAS was used to determine if the mean difference in concentrations of test compounds obtained in the control were equal to zero for the entire test period starting from 30 seconds after initiation of the kinetic tests to 4 hours. A paired T-test available in SAS was also used to evaluate the mean concentrations of test compounds obtained from the microcosm containing cells for each of the groups of experiments tested on Table V. The hypothesis

tested was: are the mean concentrations of the test compounds under the different experimental conditions were equal for the different groups of experiments?

Numerical Routine

A numerical model developed by Simkins (1991) was modified for use in this study. The kinetic models appropriate for use are the first order and the Monod with no growth or Michaelis-Menten models previously described in Chapter II and an author derived equation that accounted for endogenous substrate production. Their selection was based on the high initial cell concentration relative to initial substrate used in this study (see Figure 3). Since equation 19 cannot be solved explicitly, a nonlinear regression approach using Newton's method and Marquardt routine for error minimization in the parameters was used. Robinson and Characklis (1984) evaluated the effect of errors in S_o on the estimation of the parameters of Michaelis-Menten equation using linearized and integrated forms of the equation. They concluded that the nonlinear form of the integrated Michaelis-Menten equation was superior to traditional linearized forms for estimating of V_{max} and K_m when S_o is not error free.

First Order Model

Fates of contaminants in the environment are typically modeled using first order approximations (Baughman *et al.*, 1980; Paris *et al.*, 1982; Horowitz *et al.*, 1983; Suflita *et al.*, 1983; Oldenhuis *et al.*, 1989; Strand *et al.*, 1990). This equation is given by:

$$S = S_o e^{(-K_1 t)} \tag{27}$$

Equation 27 has two parameters; K_1 and S_o, with the initial concentration treated as another parameter to be approximated. The Marquardt routine requires the input of the partial derivative of the individual equation with respect to each of the parameters, given by:

$$\frac{\partial S}{\partial S_o} = e^{(-K_1 t)} \tag{28}$$

and

$$\frac{\partial S}{\partial K_1} = -S_o\, t\, e^{(-K_1 t)} \tag{29}$$

Equations 28 and 29 show that the first order approximation is nonlinear with respect to its parameters and could be solved by nonlinear approximations. A sensitivity equation was defined as the first derivative of the dependent variable with respect to a parameter of the chosen nonlinear model (Robinson, 1985). The dependent variable for all the kinetic models used in this study are the substrate concentrations measured in the batch reactors containing cells of *Pseudomonas putida* PpG-786.

Modified First Order Model

The author hereby proposes a modified first order equation. Suppose the first order reaction were modified to account for other reactions occurring in the system that are independent of the initial substrate concentration as follows:

$$\frac{dS}{dt} = -K_1 S + R \tag{30}$$

where R is a reaction term introduced to account for reactions occurring in the medium independent of initial substrate concentration.

Equation 30 is a linear first order differential equation that has a solution:

$$S = \frac{R}{K_1}\,(1-e^{-K_1 t}) + S_o e^{-K_1 t} \tag{31}$$

Equation 31 has three parameters; R, K_1, and S_o. The partial derivatives of equation 31 with respect to each one of its parameters required by the Marquardt routine and sensitivity analysis are given by;

$$\frac{\partial S}{\partial S_o} = e^{-K_1 t} \tag{32}$$

$$\frac{\partial S}{\partial R} = \frac{1}{K_1}(1-e^{-K_1 t}) \tag{33}$$

$$\frac{\partial S}{\partial K_1} = \frac{-R}{K_1^2}(1-e^{-K_1 t}) + \frac{R}{K_1}(te^{-K_1 t}) - S_o te^{-K_1 t} \qquad (34)$$

Michaelis-Menten Model

Biodegradation of low substrates by resting cells is typically modeled by Michaelis-Menten or Monod no growth kinetics and their approximations (Robinson and Tiedje, 1982, 1984; Simkins and Alexander, 1984; Schmidt *et al.*, 1985). The Michaelis-Menten equation is analogous to Monod no growth kinetics as indicated earlier with both equations having a hyperbolic form. The integrated form of the Michaelis-Menten equation is given by:

$$K_m \ln(\frac{S}{S_o}) + (S-S_o) + V_{max} t = 0 \qquad (35)$$

The numerical routine used for parameter updating required input of the partial derivative of equation 35 with respect to each of the three parameters K_m, S_o, and V_{max}. These are given by:

$$\frac{\partial S}{\partial K_m} = \frac{-\ln(\frac{S_o}{S})}{(1+\frac{K_m}{S})} \qquad (36)$$

$$\frac{\partial S}{\partial V_{max}} = \frac{-t}{(1+\frac{K_m}{S})} \qquad (37)$$

$$\frac{\partial S}{\partial S_o} = \frac{(1+\frac{K_m}{S_o})}{(1+\frac{K_m}{S})} \qquad (38)$$

The Michaelis-Menten equation is a nonlinear equation which is also nonlinear with respect to its parameters. Detailed sensitivity analysis of equation 35 is given by Robinson and Characklis (1984).

Newton's method solves an equation in the form of $f(x) = 0$ provided that the first derivative $f'(x) \neq 0$ (Burden and Faires, 1985):

$$g(x) = x - \frac{f(x)}{f'(x)} \tag{39}$$

Newton's method converges quadratically and requires an initial estimate of the parameter x. Michaelis-Menten or Monod no growth equations have no explicit analytical solutions but can be solved numerically. When applied to the Monod no growth equation f(x) is given by:

$$f(time,pr,K_m,S_o) = pr - K_m 1n\left(\frac{\left(\frac{K_m}{S_o - pr}\right)}{S_o}\right) - V_{max}t \tag{40}$$

The derivative of equation 40 is given by:

$$f'(pr,K_m,S_o) = \frac{K_m}{S_o - pr} + 1 \tag{41}$$

where pr is the parameter to be estimated.

Function To Be Minimized

The function minimized in the numerical routine was:

$$RSS = SUM(Y_{obs} - Y_{pred})^2 \tag{42}$$

where

RSS = residual sums-of-squares

Y_{obs} = observed values of the dependent variable

Y_{pred} = predicted Y values

Residual sums-of-squares (RSS) and the corrected sums of squares (CSS) obtained from the routine is used to compute the F values according to the following equation:

$$F = \left(\frac{CSS - RSS}{RSS}\right) \left(\frac{N-P}{P}\right) \quad (43)$$

where

N = number of data points

P = number of parameters

The calculated F value was compared with F in a statistics table with P degrees of freedom in the numerator and N - P degrees of freedom in the denominator.

Model Selection

Using the guidelines established by Simkins and Alexander (1984), and visual estimation, two models were selected as possible candidates for modeling the disappearance of the low molecular weight compounds used in this study. The model with the lower number of parameters was selected unless a more complicated model with a higher number of parameters provided a significantly better fit at 95% confidence level or higher ($P < 0.05$) using a standard F-test (Robinson, 1985). Comparison of fit between the two models is done using the following equation (Beck and Arnold, 1977):

$$F = \frac{(RSS_1 - RSS_2)(N - p_2)}{RSS_2} \quad (44)$$

where

RSS_1 = the residual sums of squares from a less complicated model

RSS_2 = the residual sums of squares from a more complicated model

N = the number of data points

p_2 = the number of parameters from the more complicated model

The calculated F value is then compared with the value of F from a statistics table for one degree of freedom in the numerator and $n - p_2$ degrees of freedom in the denominator. A lower calculated F-value relative to the tabular F-value indicated the model with the lower

number of parameters was adequate in describing the data (Robinson, 1985).

Models were also evaluated in terms of the correlation between the parameters and the residuals between the observed and the predicted values. The correlation between the parameters is usually not a problem until the value is greater than 99.9% (Simkins, 1991).

Sensitivity Analysis

Sensitivity analysis was performed on the modified first order and Michaelis-Menten models using simulated data sets. Error free data sets were generated using known parameter values. Random errors of the simple type (constant standard deviation) with normal distribution were generated using the @RISK computer program and were introduced into the error free data sets using Monte Carlo simulation techniques contained in the @RISK program (Harbaugh and Bonham-Carter, 1970) to perturb the error free data set. The random errors had a mean of zero and a standard deviation of one. Ordinary least squares methods assumed that the dependent variable contains normally distributed errors that have a mean of zero and a standard deviation of one (Beck and Arnold, 1977). As a previous study by Robinson and Characklis (1984) showed, both simple and relative errors (constant coefficient of variation) gave the same results. The sensitivity equations are the partial derivatives of the model equations with respect to each one of the parameters as given above. The sensitivity analysis was conducted on the simulated data sets at test concentrations to ensure convergence did not occur due to poor initial estimates. The sensitivity analysis is also used to check correlation between each model's parameters.

CHAPTER IV

RESULTS

The main objectives of this study were to demonstrate biodegradation of selected low molecular weight halogenated compounds and provide kinetic data for their biodegradation by the resting cells of *Pseudomonas putida* under various environmental conditions in the presence of an aquifer matrix. The results are presented under experimental results and numerical modeling sections. Under the experimental results section, there are seven categories. The categories are:

- Preliminary Experiments
- Effect of pH
- Effect of Dissolved Oxygen
- Effect of Temperature
- Effect of Inoculum Size
- Effect of Initial Substrate Concentration
- Effect of Lead Concentrations

Under the numerical modeling section, all the above categories except Preliminary Experiments are presented.

Experimental Results

Preliminary Experiments

Preliminary experiments were conducted to characterize the subsurface materials, establish a procedure for estimating cell density, establish the stability of the cytochrome $P\text{-}450_{cam}$

enzyme, and account for losses due to abiotic mechanisms. Experiments were also conducted to investigate any adsorption of selected organic compounds onto cellular materials.

a) **Characterization of Subsurface Materials**

The results of the characterization of samples from Sand Springs Petrochemical Complex and Oklahoma State University Agronomy Research Station (OSU Agronomy Station) are shown in Table VI, Appendices G-L, and Figures 9 and 10. Moisture content and pH were determined for the samples immediately on arrival at the Oklahoma State University Environmental Engineering Laboratory as shown in Appendices I and J. These measurements were repeated for air dried subsamples from site T-32 of Sand Springs Petrochemical Complex and OSU Agronomy Research Station (site 4). Table VI shows that the moisture content of the samples from both Oklahoma State University Agronomy Research Station and Sand Springs Petrochemical Complex samples were substantially reduced using air drying techniques. Air dried samples were used in subsequent experiments.

The pH values varied from 5.9 to 6.3 for Sand Springs Petrochemical Complex sites while a value of 7.2 was obtained for the OSU Agronomy Research Station. The mean total organic matter in air dried samples obtained from site T-32 of Sand Springs Petrochemical Complex was 10.5 times higher than the mean total organic matter from OSU Agronomy Research Station. This was probably due to the presence of pollutants in the former site. The total organic matter in the air dried sample from T-32 (2.2%) was also reduced relative to the wet sample (6%) analyzed immediately on arrival at the Oklahoma State University Environmental Engineering Laboratory from the sampling sites. The concentrations of cadmium and lead were below detection level for T-32 and those of cadmium, nickel and lead were below detection levels for OSU Agronomy Research Station sample. Higher amounts of chromium (19.2 mg/kg), copper (13.6 mg/kg) and zinc (142.2 mg/kg) were found in the Sand Springs Petrochemical Complex site (T-32) as compared to OSU Agronomy Station site which contained 6.8 mg/kg chromium, 3.6 mg/kg copper and 12.5 mg/kg zinc. Samples from OSU Agronomy Research Station were also characterized in

TABLE VI

CHARACTERISTICS OF SUBSURFACE MATERIALS
(AIR DRIED SAMPLES)

Parameters	Sand Springs Petrochemical Complex Site 3 (T-32)	Oklahoma State University Agronomy Research Station
Moisture Content %	0.77	0.30
pH	5.9 - 6.3	7.2
Total Organic Matter %	2.2	0.21
Metals (mg/kg)		
Cadmium	< 1.0	< 1.0
Chromium	19.2	6.8
Copper	13.6	3.6
Nickel	9.4	< 3.0
Lead	< 8.0	< 8.0
Zinc	142.2	12.5
Porosity	ND	43
Density (24°C)(g/ml)	ND	2.6

ND = Not determined.

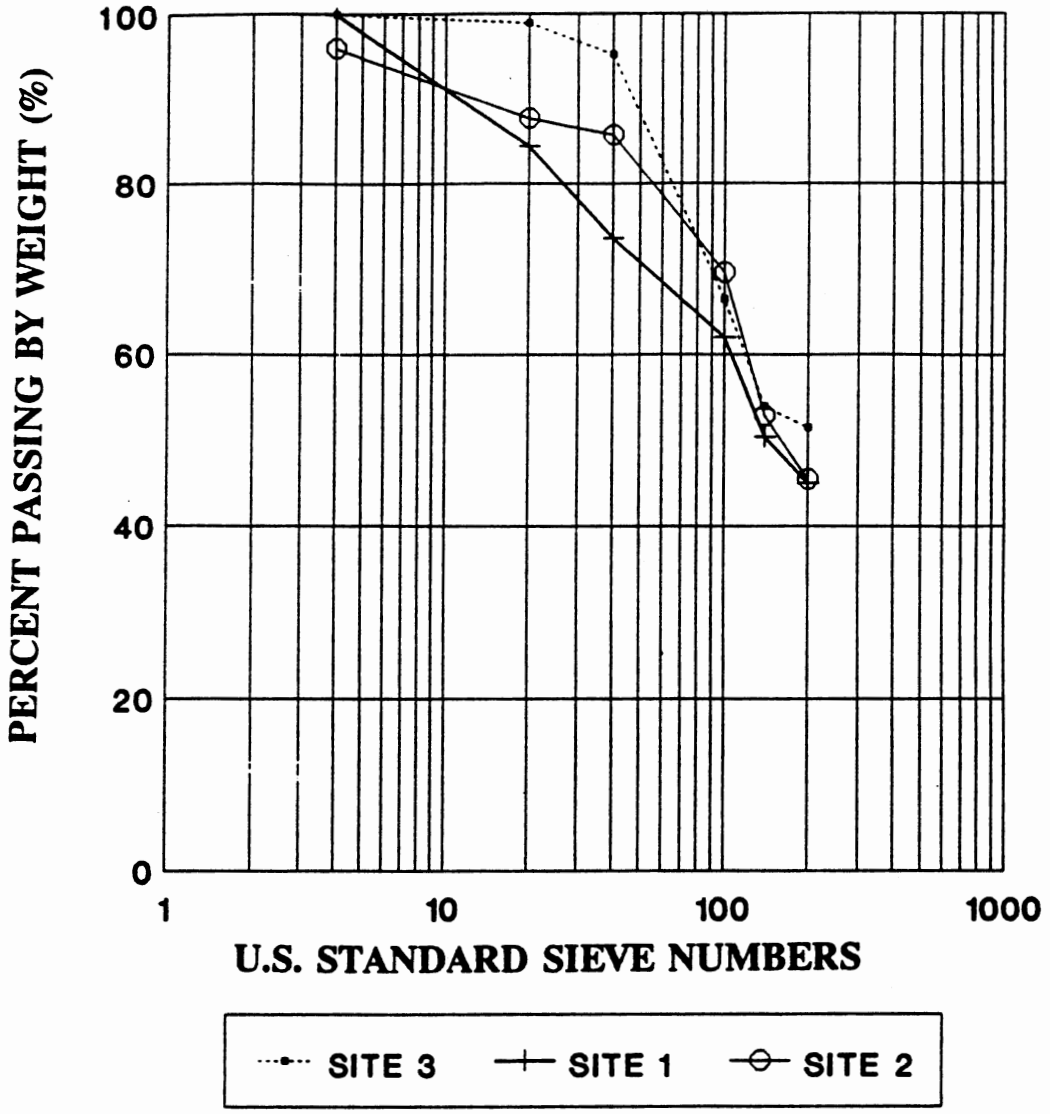


Figure 9. Particle Size Distribution of Samples from Sand Springs Petrochemical Complex Sites

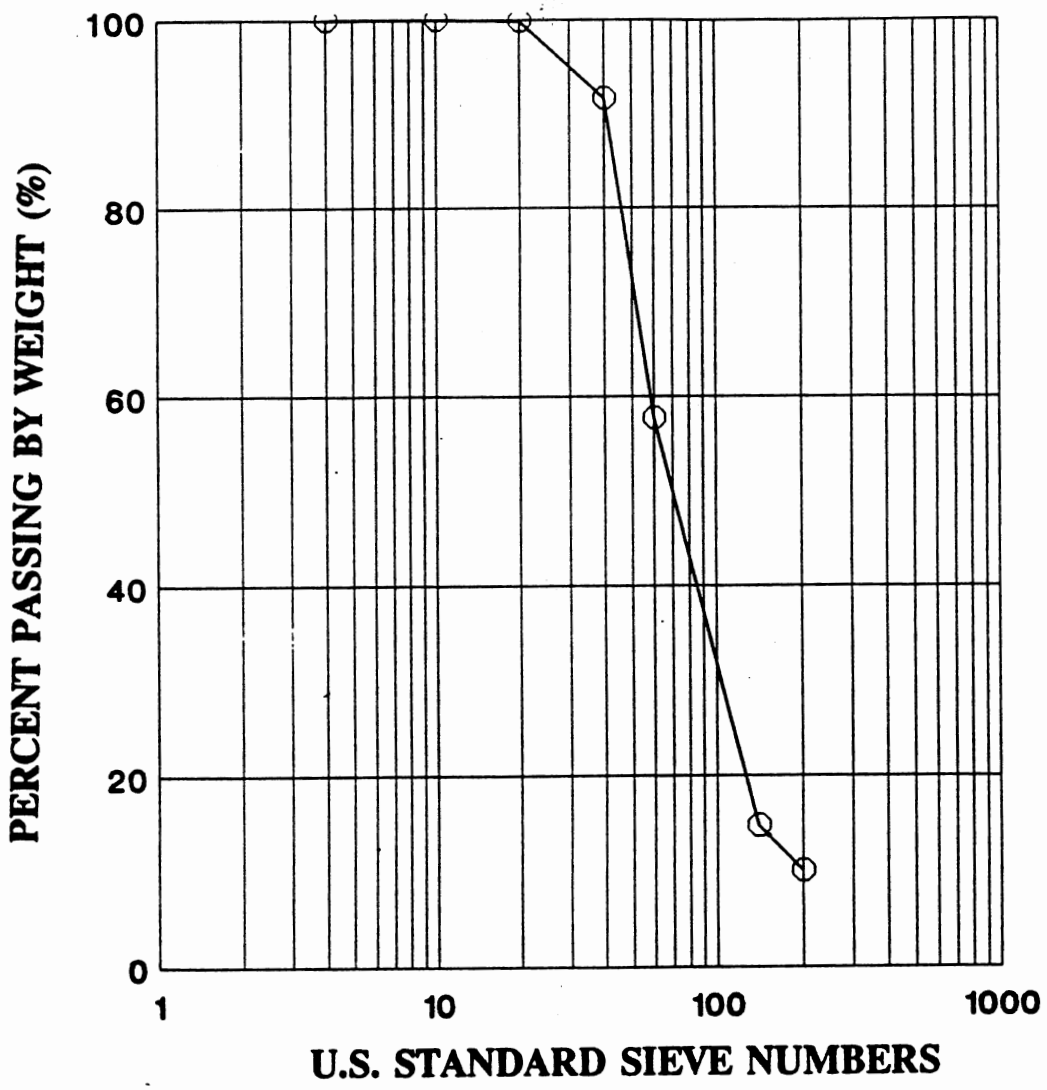


Figure 10. Particle Size Distribution of Samples from Oklahoma State University Agronomy Research Station.

terms of porosity and density. The porosity of the OSU Agronomy Station samples was 43 while the density was 2.6 g/ml.

b) **Characterization of Cell Culture of *Pseudomonas putida* PpG-786**

The results of the characterization of cell cultures used in the experiments are shown in Appendix C. The characterization was based on growth on Camphor minimum media, gram stain, motility, colony shape, UV fluorescence, growth temperature, and growth in oxidation fermentation tubes. These characteristics were observed in subsamples tested throughout the period of the study and was used as an indicator of the purity of the cell culture.

c) **Cell Density Determination**

The amounts of cells used in this experiment were determined using dry weight (at 56°C and 103°C) and wet weight, optical density and total cell count. The relationships between dry weight and wet weight and optical density and wet weight are shown in Figures 11 and 12. Negative cell mass was obtained at lower corresponding wet weights at 103°C when compared to measurements at 56°C. This result is consistent with an earlier assertion by Monod (1949) that dry weight determination at high temperature in the presence of high dissolved solids gives accurate results only at high cell concentrations. Subsequent measurements of cell dry weights were taken at 56°C. At high cell concentrations, the dry weight to wet weight ratio varied from 0.01 to 0.3 at 103°C and 0.1 to 0.3 at 56°C when the pH was varied from 5.4 to 8.9 (Appendices M - O). Negative cell mass values obtained at low cell concentrations were discarded.

d) **Enzyme Assay**

Figure 13 shows the concentration and the activity of the cytochrome P-450$_{cam}$ over a 74.5 hour period. The activity was measured in terms of the percent removal of 1,2-dibromo-3-chloropropane achieved one hour after starting the biodegradation experiment. The x-axis indicates the time lapse that occurred after the cells were harvested. The level of activity

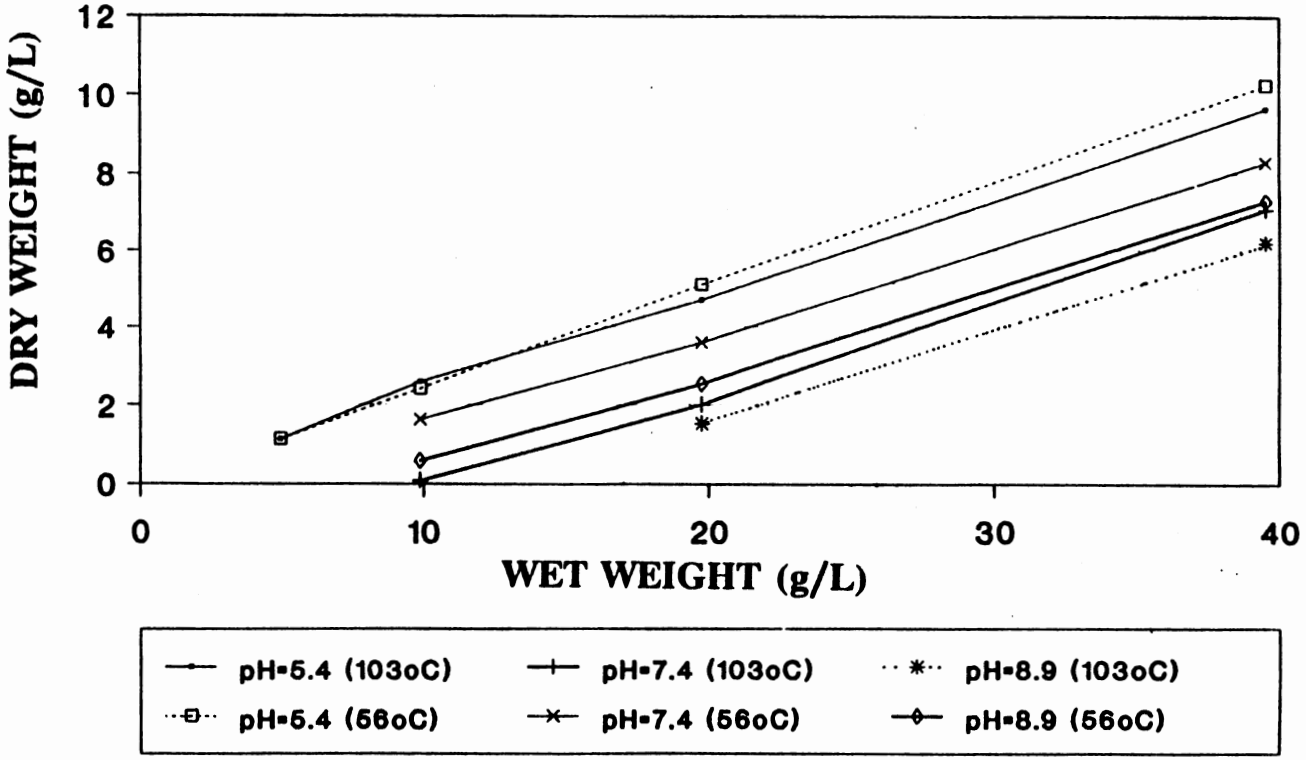


Figure 11. Relationship Between Dry and Wet Weights.

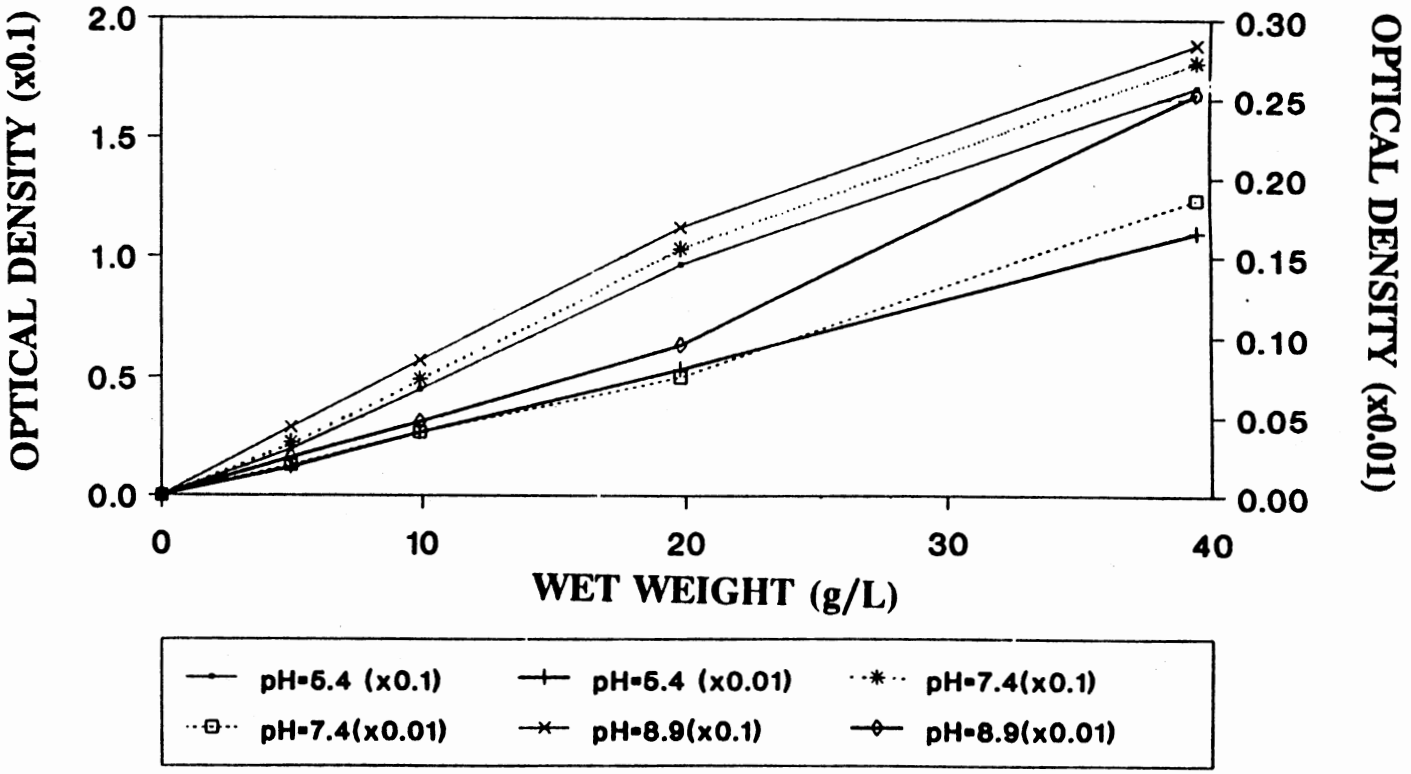


Figure 12. Relationship Between Optical Density and Wet Weight.

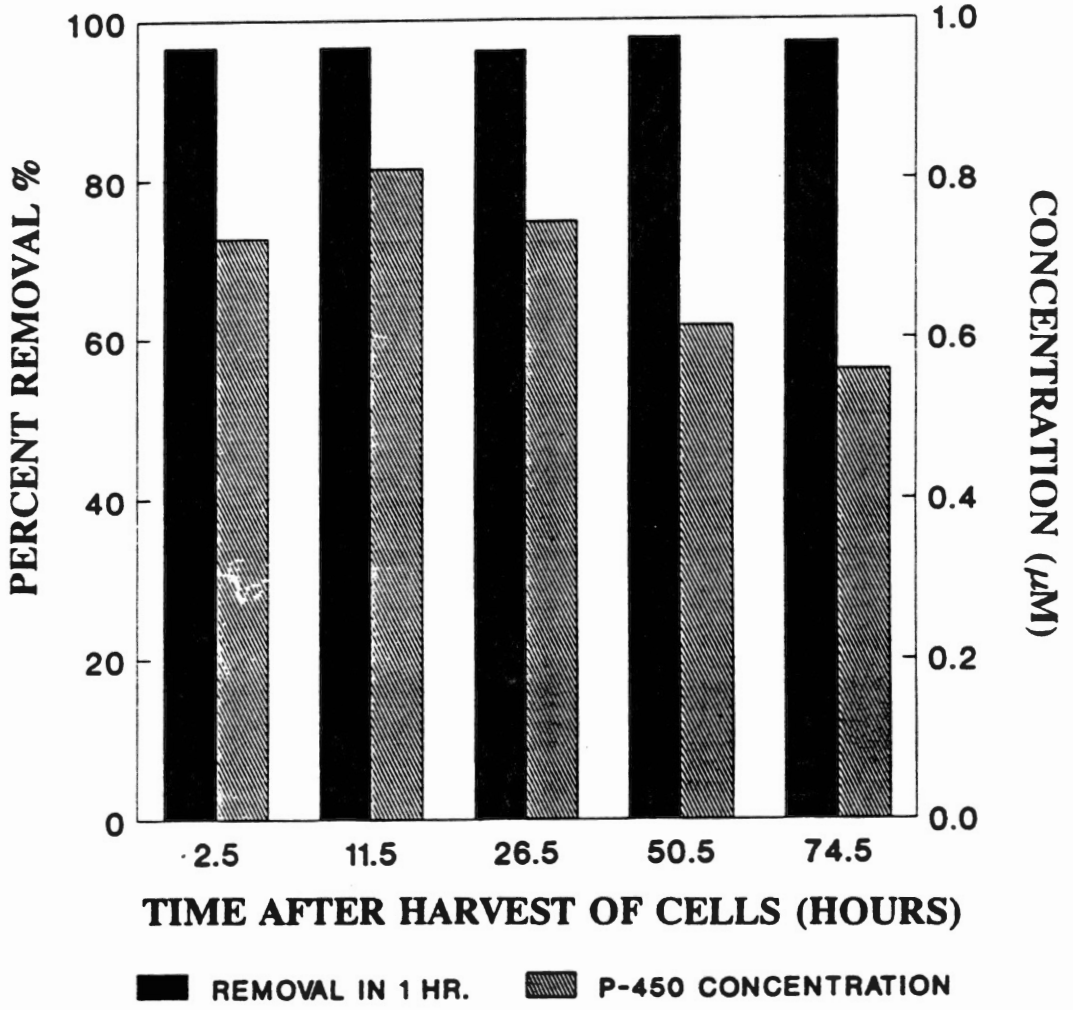


Figure 13. Percent Removal of 1,2-dibromo-3-chloropropane and Concentration of Cytochrome P-450$_{cam}$.

of P-450$_{cam}$ reported in this study can be compared to those reported by Vilker and Khan (1989). They reported a decrease to one-third of the original enzyme concentration at room temperature after aging the cells at room temperature for 24 hours. In contrast to the experimental set up by Vilker and Khan, cells used in this study were not agitated in between measurements to prevent the possible exhaustion of the cytochrome P-450$_{cam}$. Instead, aeration of the cells was initiated only just prior to the experiments. Although the concentration of the cytochrome P-450$_{cam}$ was reduced from 0.73 to .56 μM, 74.5 hours after the cells were harvested, the activity remained essentially constant. The concentration of cells used in this study was 26.6 g/L wet weight or 7.390 g/L dry weight (56°C).

e) **Adsorption of Cells onto Aquifer Materials**

The adsorption of *Pseudomonas putida* PpG-786 onto a sandy aquifer was evaluated using aquifer materials from Oklahoma State University Agronomy Research Station. This experiment was performed using three-tenth gram of Oklahoma State University Agronomy Station aquifer materials. A *Freundlich* equation (equation 12) was used and the constants solved via simple linear regression. The graph of the adsorption isotherm of *Pseudomonas putida* onto the aquifer materials is shown in Figure 14 with corresponding experimental data shown in Appendix P. The isotherm equation (equation 11) is present with the estimated constants as:

$$\left(\frac{x^*}{m}\right) = 0.001C^{1.5} \quad (r^2 = 0.998) \tag{45}$$

A slope (n) less than one indicated unfavorable adsorption isotherm between the sandy aquifer materials and *Pseudomonas putida* PpG-786.

e) **Abiotic Losses**

The preliminary experiments were designed to investigate if any significant abiotic losses or cellular adsorption occurred during the experiment. The results of the abiotic experiments are shown in Tables VII - IX.

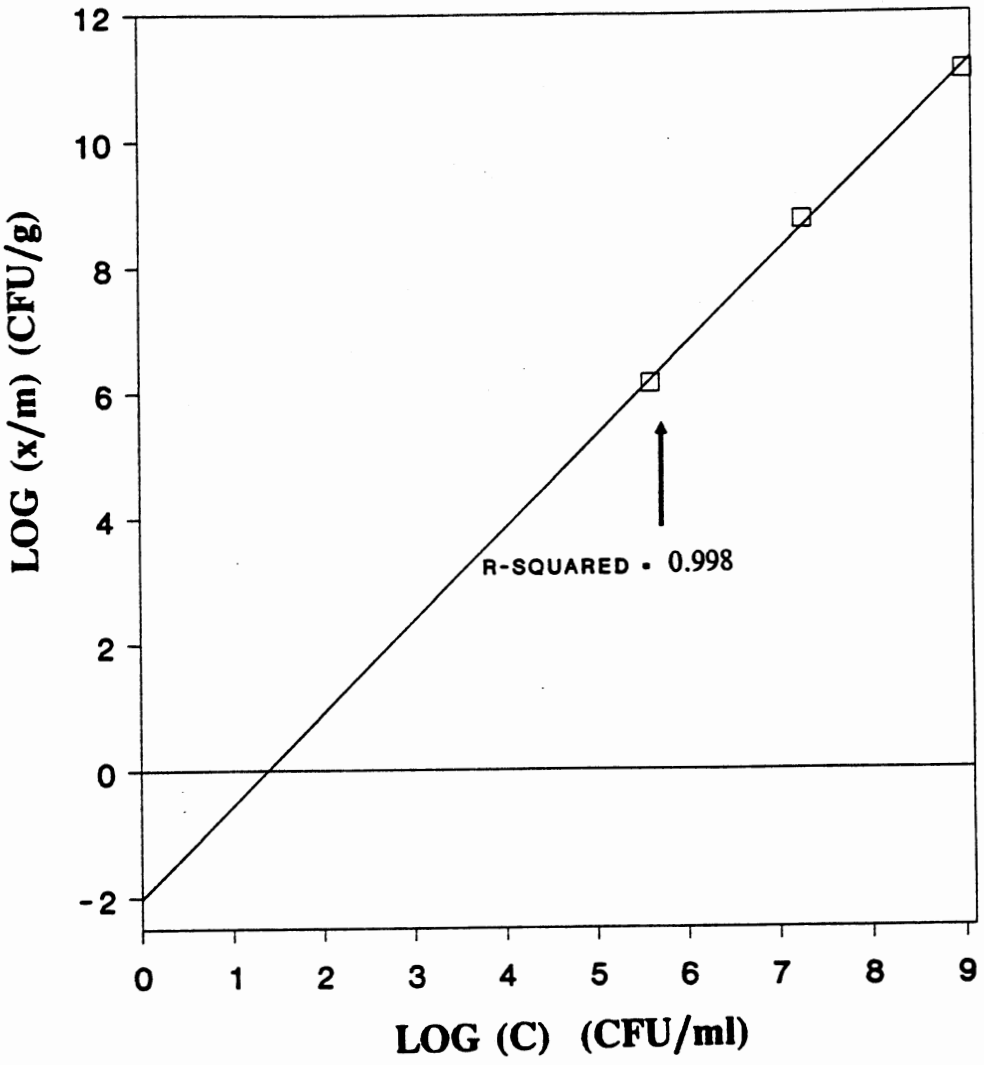


Figure 14. Adsorption Isotherm of *Pseudomonas putida* onto Oklahoma State University Agronomy Research Station Aquifer Materials.

TABLE VII

A SUMMARY OF ANOVA COMPARISON OF MEAN CONCENTRATION OF 1,2-DICHLOROPROPANE OBTAINED AT THE END OF A FOUR HOUR TEST PERIOD UNDER DIFFERENT EXPERIMENTAL CONDITIONS (TEMPERATURE, 25°C)

EXPERIMENT	COMPARISON TO CONTROL*
(pH = 6.4)	
Glassware adsorption	Not significant
Volatilization	Not significant
Adsorption onto Subsurface Materials	
OSU Agronomy Research Station	Not significant
Sand Springs Subsurface Materials	Not significant
Effect of Laboratory Light	Not significant
(pH = 7.4)	
Glassware adsorption	Not significant
Volatilization	Not significant
Adsorption onto Subsurface Materials	
OSU Agronomy Research Station	Not significant
Sand Springs Subsurface Materials	Not significant
Effect of Laboratory Light	Not significant
(pH = 7.8)	
Glassware adsorption	Not significant
Volatilization	Not significant
Adsorption onto Subsurface Materials	
OSU Agronomy Research Station	Not significant
Sand Springs Subsurface Materials	Not significant
Effect of Laboratory Light	Not significant

*Control is initial test sample. Comparison is at 0.05 level of significance.

TABLE VIII

A SUMMARY OF ANOVA COMPARISON OF MEAN CONCENTRATION OF 1,2-DIBROMO-3-CHLORO-PROPANE OBTAINED AT FOUR HOUR TEST PERIOD AT ROOM TEMPERATURE (25°C, pH 7.4)

EXPERIMENT	COMPARISON TO CONTROL*
Glassware adsorption	Not significant
Volatilization	Not significant
Adsorption onto Subsurface Materials	
OSU Agronomy Research Station	Not significant
Effect of Laboratory Light	Not significant

*Control is initial test sample. Comparison is at 0.05 level of significance.

TABLE IX

A SUMMARY OF ANOVA COMPARISON OF MEAN CONCENTRATION OF 1,2-DICHLOROETHANE OBTAINED AT FOUR HOUR TEST PERIOD AT ROOM TEMPERATURE (25°C, pH 7.4)

EXPERIMENT	COMPARISON TO CONTROL*
Glassware adsorption	Not significant
Volatilization	Not significant
Effect of Laboratory Light	Not significant

*Control is initial test sample. Comparison is at 0.05 level of significance.

The extraction efficiency was determined for the different compounds at their experimental pH. Extraction efficiencies ranged from 82% - 99% for 1,2-dichloropropane and 82% - 113% for 1,2-dibromo-3-chloropropane. The extraction efficiency for 1,2-dichloroethane was 93%. All data gathered during the study were subsequently corrected using extraction efficiencies generated for the individual experiment at the time the experiments were conducted. The extraction efficiencies are listed in Appendix Q.

The SAS program and output of the statistical analysis of the preliminary experiments are shown in Appendices R and S. The hypothesis tested was: are the mean concentrations obtained at the end of the four hour test period equal under different experimental conditions? The results are shown in Table IV. There was no significant abiotic loss at the end of the four hour experiments for 1,2-dibromo-3-chloropropane, 1,2-dichloropropane or 1,2-dichloroethane at any of the pHs investigated at a level of significance of at least 0.05. The statistical analysis was performed on data obtained at the end of the four hour experiments. The results also indicated that loss due to cellular adsorption is not significant for any of these compounds. Inhibition of enzyme activity was accomplished using potassium cyanide and silver sulfate. The experiments were set up with buffer controls and the concentration of compounds was recorded over time. An analysis of variance (ANOVA) procedure available in Statistical Analysis System (SAS) (1985) was also used to test whether any significant cellular adsorption of the test compounds occurred during the experiments (alpha of at least 0.05 level of significance) relative to the buffer controls. There was no significant difference between concentrations in the batch reactors containing cells relative to the buffer for all pHs tested (Table X). Although no significant difference was observed at the end of the four hour test period at pH 7.8 in the presence of chloride ions, there was a significant time dependent variation (Table XI) in the concentration of 1,2-dichloropropane in the buffer (alpha of at least 0.05). This variation in concentration was determined using an ANOVA procedure and tested at a level of significance of at least 0.05. Consequently, the concentrations of DCP in the batch reactors containing microbial

TABLE X

A SUMMARY OF ANOVA COMPARISON OF MEAN CONCENTRATION OF SELECTED COMPOUNDS IN BUFFER AND INHIBITED CELLS (EVALUATION OF CELLULAR ADSORPTION AT 25°C)

COMPOUND	pH	COMPARISON TO CONTROL*
1,2-dichloropropane	6.4	Not significant
	7.4	Not significant
	7.8	Not significant
1,2-dibromo-3-chloropropane	7.4	Not significant

*Control is initial test sample. Comparison is at least 0.05 level of significance.

TABLE XI

STATISTICAL ANALYSIS OF THE BUFFER CONTROLS DATA USED DURING KINETIC EXPERIMENTS

Experiment/ID Number	pH	Temp °C	Subs Mat.	Paired T-Test T-calc	Prob>|T|	SIG	T-Test on Regression Coefficient T-CALC	DF	T-TABLE	SIG
EFFECT OF PRESENCE OF AQUIFER MATERIALS (1,2-dibromo-3-chloropropane)										
DBCPDF	7.4	25	NONE	0.617	0.571	NS	0.885	15	2.131	NS
DBCPDF2	7.4	25	OSU	0.152	0.889	NS	0.246	13	2.160	NS
EFFECT OF AQUIFER										
AQUIFDF	7.4	25	OSU	-0.973	0.402	NS	-0.977	12	2.179	NS
AQUIFDF2	7.4	25	SS	-0.163	0.881	NS	-1.191	9	2.262	NS
EFFECT OF pH										
pH54(25)	5.4	25	OSU	0.268	0.802	NS	0.563	14	2.145	NS
pH74(25)	7.4	25	OSU	0.229	0.830	NS	0.704	14	2.145	NS
pH89(25)	8.9	25	OSU	0.213	0.842	NS	0.801	15	2.131	NS
EFFECT OF DISSOLVED OXYGEN[1]										
DOCOM3	7.4	25	OSU	-0.632	0.572	NS	-2.260	6	2.447	NS
DOCOM4	7.4	25	OSU	-0.004	0.997	NS	0.108	7	2.365	NS
DOCOM5	7.4	25	OSU	-0.052	0.960	NS	0.471	15	2.131	NS
EFFECT OF TEMPERATURE[2]										
pH64(15)	6.4	15	OSU	-0.918	0.411	NS	-1.836	14	2.145	NS
pH64(25)	6.4	25	OSU	-0.814	0.461	NS	-0.940	10	2.228	NS
pH64(30)	6.4	30	OSU	1.483	0.212	NS	2.045	15	2.131	NS
pH74(15)	7.4	15	OSU	0.754	0.493	NS	2.105	14	2.145	NS
pH74/25	7.4	25	OSU	-0.388	0.718	NS	-0.799	11	2.201	NS
pH74(30)	7.4	30	OSU	0.288	0.788	NS	0.085	13	2.160	NS
pH7815c	7.8	15	OSU	0.277	0.796	NS	0.039	14	2.145	NS
pH7825c	7.8	25	OSU	0.404	0.707	NS	2.017	15	2.131	NS
pH7830c	7.8	30	OSU	1.199	0.297	NS	3.842	12	2.179	S
EFFECT OF INOCULUM SIZE[3]										
INNODES1	7.4	25	OSU	0.407	0.705	NS	2.002	12	2.179	NS
INNODES2	7.4	25	OSU	0.407	0.705	NS	2.002	12	2.179	NS
INNODES3	7.4	25	OSU	0.407	0.705	NS	2.002	12	2.179	NS
INNODES4	7.4	25	OSU	0.407	0.705	NS	2.002	12	2.179	NS
EFFECT OF SUBSTRATE CONCENTRATION										
SUSTIDF	7.4	25	OSU	-0.357	0.745	NS	-3.281	13	2.160	S
SUST2DF	7.4	25	OSU	-0.771	0.497	NS	-0.625	11	2.201	NS
SUST3DF	7.4	25	OSU	0.379	0.730	NS	1.231	9	2.262	NS

TABLE XI (Continued)

Experiment/ID Number	pH	Temp °C	Subs Mat.	Paired T-Test T-calc	Prob > |T|	SIG	T-Test on Regression Coefficient T-CALC	DF	T-TABLE	SIG
EFFECT OF HEAVY METAL CONCENTRATION[4]										
LEADDES1	7.4	25	OSU	0.103	0.923	NS	-0.215	9	2.626	NS
LEADDES2	7.4	25	OSU	-0.292	0.785	NS	-0.516	9	2.626	NS
LEADDES3	7.4	25	OSU	0.528	0.625	NS	0.022	9	2.626	NS
LEADDES4	7.4	25	OSU	-0.095	0.929	NS	-1.024	8	2.626	NS

*Experiments performed at DO 16.0 mg/L and 1,2-dichloropropane except where indicated.
[1]Experiments were performed using inoculum sizes of 1.455 g/L, 3.317 g/L, 6.470 g/L and 8.017 g/L for INNODES1, INNODES2, INNODES3 AND INNODES4, respectively.
[2]Experiments were conducted with chloride concentrations of 2.120 g/L, 0.871 g/L and 0.151 g/L at pH 6.4, 7.4 and 7.8, respectively.
[3]Experiments conducted at 16.0 mg/L, 8.2 mg/L and 6.0 mg/L dissolved oxygen for DOCOM3, DOCOM4, DOCOM5, respectively.
[4]Experiments conducted at 732 μg/L, 1209 μg/L, and 4907 μg/L 1,2-dichloropropane for SUSTIDF, SUST2DF, and SUST3DF, respectively.
[5]Lead concentrations where 0.0 mg/L, 10.0 mg/L, 5.8 mg/L and 2.2 mg/L for LEADDES1, LEADDES2, LEADDES3, and LEADDES4, respectively.
SS - Sand Springs Petrochemical Complex Subsurface Materials
OSU - Oklahoma State University Agronomy Station Aquifer materials
S - Significant
NS - Nonsignificant
T-CALC - Calculated T Value, T-TABLE - Table value of T
DF - Degrees of freedom compared to alpha of at least 0.05
SUBS MAT - Subsurface Materials
TEMP - Temperature
Prob > | T | - Probability level at which statistical test is significant.

cells for all experiments conducted at pH 7.8 wereadjusted for variation in the buffer. The adjustment was made by first calculating the average change in concentration of 1,2-dichloropropane in the buffer controls relative to the initial value for each time measurement of 1,2-dichloropropane. The average change in the concentration of 1,2-dichloropropane in the control was then subtracted from the corresponding average concentrations of the test compounds contained in the batch reactors with microbial cells.

Experimental Data and Analysis

Several batch reactors were set up to evaluate the effects of selected environmental factors on the biodegradation of test compounds by a pure culture of *Pseudomonas putida* PpG-786. Each set of four hour experiments had a set of buffer controls to account for any abiotic losses that might occur during the experiments. The four hour test period was considered adequate based on previous studies by Lam and Vilker (1987) in which biodegradation of 1,2-dibromo-3-chloropropane was demonstrated using *Pseudomonas putida* PpG-786. An initial screening test with 1,2-dichloropropane was also conducted which indicated steady state could be accomplished within four hours. The time frame for the experiment also ensured minimal change in enzyme activity. A summary of the statistical analysis performed on the concentration of test compounds measured in the buffer controls is shown in Table XI. Statistical analyses were compared to an alpha value of at least 0.05. Statistical comparisons involved a paired T-test comparison of all buffer control data (columns 4-6, Table XI) and a T-test on the coefficient of a linear regression on the control data (columns 7-10, Table XI) over the time of the experiment. The paired T-test evaluates the variations in adjacent paired groups within the time frame of the experiments while the T-test on the regression tests if the slope of a linear regression model is significantly different from zero. Most of the experiments showed no significant difference between the mean concentration of the test compounds in the controls over the time period of the experiments. A significant variation over time was observed for the controls in one of the experiments set up to evaluate the effect of temperature

(pH of 7.8 and temperature of 30°C) as indicated by a T-test on the coefficient of the linear regression of concentrations against time. A significant variation was also observed in the controls set up for evaluating the effect of substrate concentration (S_o = 732 μg/L). Corrections were made to the concentrations of 1,2-dichloropropane measured in all experiments conducted at pH of 7.8 (experiment to evaluate effect of temperature) in order to directly compare the results obtained at this pH for all temperatures used in the study. A similar significant change in concentrations of test compound was previously observed in the experiment set up to account for cellular adsorption of 1,2-dichloropropane presented earlier. No adjustments were however made for the experiments to evaluate the effect of substrate at pH 7.4 since no other sets of experiments performed at this pH showed any significant decrease. The variation was attributed to bad controls.

Figures 15 - 44 show the removal of the test compounds under the different experimental conditions presented in Table V. The percent removal of the test compounds at the end of the four hour experiments corresponding to the different experimental conditions in Table V are presented in Table XII. The results of the paired T-test performed on the concentrations of test compounds remaining at the end of the four hour experiments are presented in Table XIII.

Effect of Aquifer and Chemical Types

Figures 15 - 18 and Tables XII to XIII show the effect of the presence of aquifer materials and aquifer types on the biodegradation of low molecular weight halogenated aliphatic compounds used in this study. The data obtained in the experiment conducted in the absence of aquifer material is compared to that obtained in the presence of Oklahoma State University Agronomy Research Station aquifer materials in Tables XII and XIII and in Figures 15 and 16. Figure 15 shows the removal of 1,2-dibromo-3-chloropropane in the absence of aquifer materials while Figure 16 shows its removal in the presence of Oklahoma State University Agronomy Research Station aquifer materials. The percent removal of 1,2-dibromo-3-chlo-

TABLE XII

PERCENT REMOVAL OF TEST SUBSTRATES UNDER DIFFERENT EXPERIMENTAL CONDITIONS (FOUR HOUR TEST PERIOD)

Experiment/ID Number*	pH	Temperature °C	Subsurface Materials	Removal at Four Hours %
EFFECT OF PRESENCE OF AQUIFER MATERIALS (1,2-dibromo-3-chloropropane)				
DBCPDF	7.4	25	NONE	98 ± 0
DBCPDF2	7.4	25	OSU	98 ± 0
EFFECT OF AQUIFER				
AQUIFDF	7.4	25	OSU	76 ± 8
AQUIFDF2	7.4	25	SS	76 ± 3
EFFECT OF pH				
pH54(25)	5.4	25	OSU	95 ± 3
pH74(25)	7.4	25	OSU	95 ± 1
pH89(25)	8.9	25	OSU	92 ± 5
EFFECT OF DISSOLVED OXYGEN[1]				
DOCOM3	7.4	25	OSU	82 ± 5
DOCOM4	7.4	25	OSU	60 ± 3
DOCOM5	7.4	25	OSU	65 ± 3
EFFECT OF TEMPERATURE[2]				
pH64(15)	6.4	15	OSU	57 ± 3
pH64(25)	6.4	25	OSU	58 ± 4
pH64(30)	6.4	30	OSU	38 ± 6
pH74(15)	7.4	15	OSU	11 ± 3
pH74(25)	7.4	25	OSU	51 ± 8
pH74(30)	7.4	30	OSU	28 ± 4
pH78(15)	7.8	15	OSU	38 ± 0
pH78(25)	7.8	25	OSU	70 ± 1
pH78(30)	7.8	30	OSU	59 ± 2
EFFECT OF INOCULUM SIZE[3]				
INNODES1	7.4	25	OSU	28 ± 13
INNODES2	7.4	25	OSU	58 ± 3
INNODES3	7.4	25	OSU	67 ± 1
INNODES4	7.4	25	OSU	90 ± 12

TABLE XII (Continued)

Experiment/ID Number*	pH	Temperature °C	Subsurface Materials	Removal at Four Hours %
EFFECT OF SUBSTRATE CONCENTRATION[4]				
SUSTIDF	7.4	25	OSU	98 ± 0
SUST2DF	7.4	25	OSU	97 ± 3
SUST3DF	7.4	25	OSU	26 ± 13
EFFECT OF HEAVY METAL CONCENTRATION[5]				
LEADDES1	7.4	25	OSU	52 ± 2
LEADDES2	7.4	25	OSU	61 ± 5
LEADDES3	7.4	25	OSU	61 ± 4
LEADDES4	7.4	25	OSU	63 ± 1

*Experiments performed at DO 16.0 mg/L and 1,2-dichloropropane except where indicated.

[1]Experiments conducted at 16.0 mg/L, 8.2 mg/L and 6.0 mg/L dissolved oxygen for DOCOM3, DOCOM4, DOCOM5, respectively.

[2]Experiments were conducted with chloride concentrations of 2.120 g/L, 0.871 g/L and 0.151 g/L AT pH 6.4, 7.4 and 7.8, respectively.

[3]Experiments were performed using inoculum sizes of 1.455 g/L, 3.317 g/L, 6.470 g/L and 8.017 g/L for INNODES1, INNODES2, INNODES3 and INNODES4, respectively.

[4]Experiments conducted at 732 μg/L, 1209 μg/L, and 4907 μg/L 1,2-dichloropropane for SUSTIDF, SUST2DF, and SUST3DF, respectively.

[5]Lead concentrations were 0.0 mg/L, 10.0 mg/L, 5.8 mg/L and 2.2 mg/L for LEADDES1, LEADDES2, LEADDES3, and LEADDES4, respectively.

OSU - Oklahoma State University Agronomy Research Station Aquifer (0.3 g)

SS - Sand Springs Petrochemical Complex Subsurface materials (0.3 g)

TABLE XIII

STATISTICAL ANALYSIS OF CONCENTRATION OF TEST COMPOUND REMAINING AT THE END OF FOUR HOURS UNDER DIFFERENT EXPERIMENTAL CONDITIONS

EXPERIMENT/ID NUMBER*	pH	Temp °C	SUBS MAT	DF	T	Prob>|T|	SIG
EFFECT OF PRESENCE OF AQUIFER MATERIALS (1,2-dibromo-3-chloropropane)							
DBCPDF VS. DBCPDF2	7.4	25	NONE /OSU	4.0	-4.9497	0.0078	S
EFFECT OF AQUIFER							
AQUIFDF VS. AQUIFDF2	7.4	25	OSU	4.0	-0.0795	0.9424	NS
EFFECT OF pH							
pH54(25) VS. pH74(25)	5.4/7.4	25	OSU	4.0	-0.3739	0.7274	NS
pH54(25) VS. pH89(25)	5.4/8.9	25	OSU	4.0	-0.8019	0.4676	NS
pH74(25) VS. pH89(25)	7.4/8.9	25	OSU	2.1	-0.6978	0.5558	NS
EFFECT OF DISSOLVED OXYGEN[1]							
DOCOM3 VS. DOCOM4	7.4	25	OSU	2.0	-3.9880	0.0575	NS
DOCOM3 VS. DOCOM5	7.4	25	OSU	2.0	-4.6483	0.0433	S
DOCOM4 VS. DOCOM5	7.4	25	OSU	2.0	-0.1690	0.8813	NS
EFFECT OF TEMPERATURE[2]							
pH64(15) VS. pH64(25)	6.4	15/25	OSU	4.0	0.2326	0.8275	NS
pH64(15) VS. pH64(30)	6.4	15/30	OSU	4.0	-1.7976	0.1466	NS
pH64(25) VS. pH64(30)	6.4	25/30	OSU	4.0	-1.8425	0.1392	NS
pH74(15) VS. pH74/25	7.4	15/25	OSU	4.0	2.4378	0.0714	NS
pH74(15) VS. pH74(30)	7.4	15/30	OSU	4.0	1.6289	0.1787	NS
pH74/25 VS. pH74(30)	7.4	25/30	OSU	4.0	1.6544	0.1734	NS
pH7815c VS. pH7825c	7.8	15/25	OSU	4.0	29.9868	0.0010	S
pH7815c VS. pH7830c	7.8	15/30	OSU	4.0	33.7076	0.0000	S
pH7825c VS. pH7830c	7.8	25/30	OSU	4.0	0.1073	0.9197	NS
EFFECT OF INOCULUM SIZE[3]							
INNODES1 VS. INNODES2	7.4	25	OSU	4.0	3.1046	0.0361	S
INNODES1 VS. INNODES3	7.4	25	OSU	2.0	4.1508	0.0530	S
INNODES2 VS. INNODES3	7.4	25	OSU	4.0	3.7636	0.0197	S
INNODES1 VS. INNODES4	7.4	25	OSU	4.0	4.8273	0.0085	S
INNODES2 VS. INNODES4	7.4	25	OSU	4.0	3.5264	0.0243	S
INNODES3 VS. INNODES4	7.4	25	OSU	2.0	2.6119	0.1199	NS

TABLE XIII (Continued)

EXPERIMENT/ID NUMBER*	pH	Temp °C	SUBS MAT	DF	T	Prob>|T|	SIG
EFFECT OF SUBSTRATE CONCENTRATION[4]							
SUSTIDF VS. SUST2DF	7.4	25	OSU	2.0	-1.0000	0.4226	NS
SUST1DF VS. SUST3DF	7.4	25	OSU	2.0	-8.1224	0.0148	S
SUST2DF VS. SUST3DF	7.4	25	OSU	2.0	-8.0439	0.0149	S
EFFECT OF HEAVY METAL CONCENTRATION[5]							
LEADDES1 VS. LEADDES2	7.4	25	OSU	1.2	-0.8077	0.5560	NS
LEADDES1 VS. LEADDES3	7.4	25	OSU	2.0	2.1029	0.1702	NS
LEADDES2 VS. LEADDES3	7.4	25	OSU	2.0	2.0696	0.1743	NS
LEADDES1 VS. LEADDES4	7.4	25	OSU	2.0	-2.2802	0.1502	NS
LEADDES2 VS. LEADDES4	7.4	25	OSU	2.0	-0.0203	0.9856	NS
LEADDES3 VS. LEADDES4	7.4	25	OSU	2.0	-3.1835	0.0861	NS

*Experiments performed at DO 16.0 mg/L and 1,2-dichloropropane except where indicated.
[1]Experiments conducted at 16.0 mg/L, 8.2 mg/L and 6.0 mg/L dissolved oxygen for DOCOM3, DOCOM4, DOCOM5, respectively.
[2]Experiments conducted with chloride concentrations of 2.102 g/L, 0.871 g/L and 0.151 g/L at pH 6.4, 7.4 and 7.8, respectively.
[3]Experiments performed using inoculum sizes of 1.455 g/L, 3.317 g/L, 6.470 g/L and 8.017 g/L for INNODES1, INNODES2, INNODES3, and INNODES4, respectively.
[4]Experiments conducted at 732 μg/L, 1209 μg/L, and 4907 μg/L 1,2-dichloropropane for SUSTIDF, SUST2DF, and SUST3DF, respectively.
[5]Lead concentrations were 0.0 mg/L, 10.0 mg/L, 5.8 mg/L and 2.2 mg/L for LEADDES1, LEADDES2, LEADDES3, and LEADDES4, respectively.
SS - Sand Springs Petrochemical Complex subsurface materials
OSU - Oklahoma State University Agronomy Station aquifer materials
S - Significant
NS - Nonsignificant
TEMP - Temperature
SUBS MAT - Subsurface Materials
DF - Degrees of Freedom
SIG - Significance of test compared to alpha=0.05

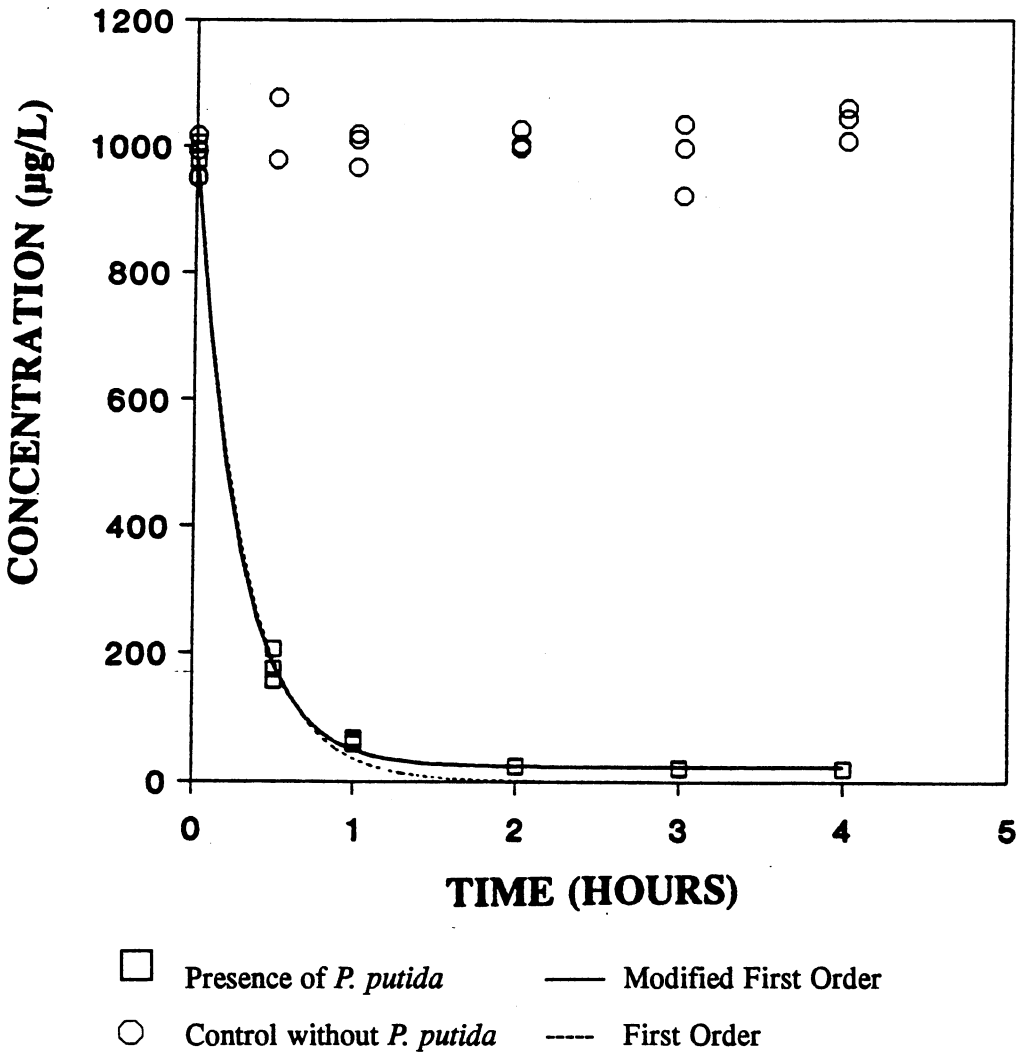


Figure 15. Removal of 1,2-dibromo-3-chloropropane by *Pseudomonas putida* PpG-786 in the Absence of Aquifer Materials (pH 7.4, temperature 25°C)

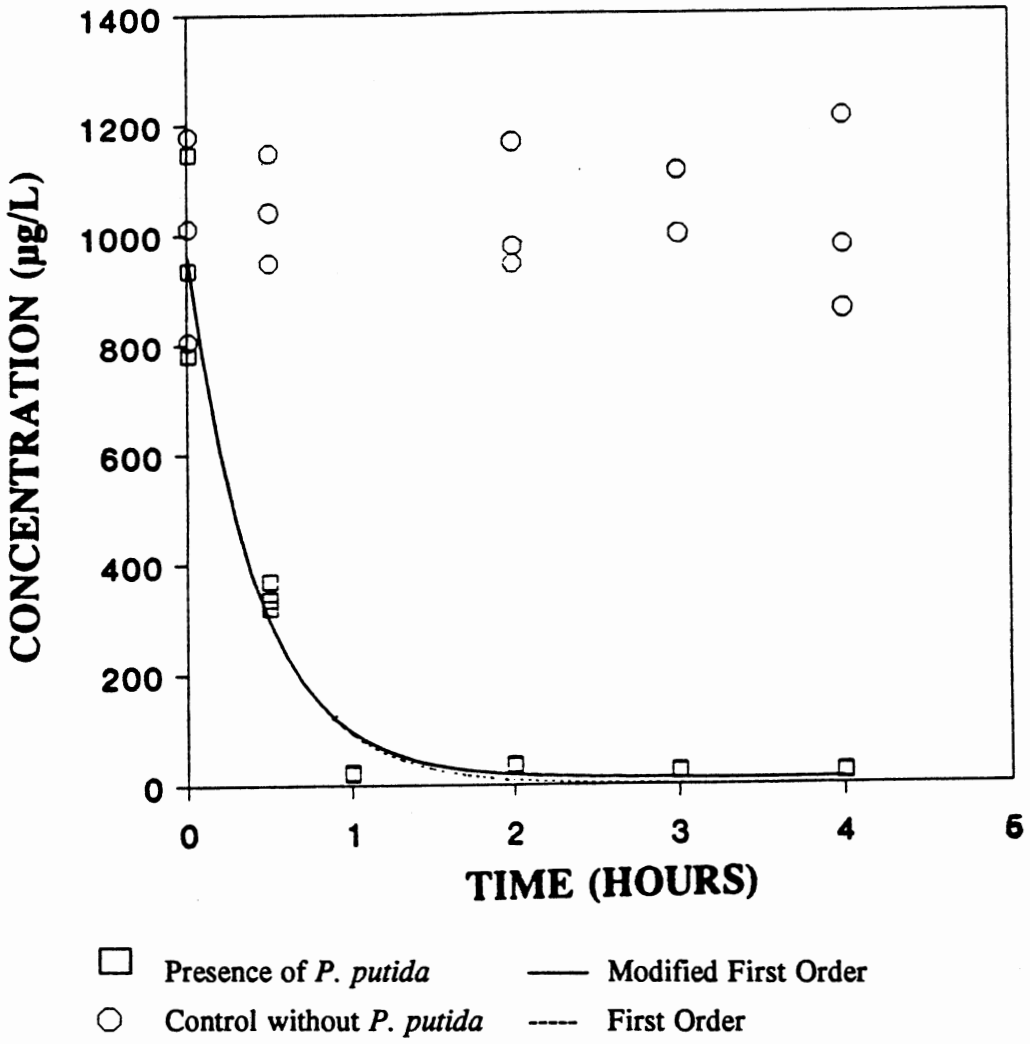


Figure 16. Removal of 1,2-dibromo-3-chloropropane by *Pseudomonas putida* PpG-786 in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.4, temperature 25°C)

ropropane with and without aquifer materials was 98%. The experiments were performed at a mean initial substrate concentration of 994 μg/L. Based on the T-test performed on the concentration of 1,2-dibromo-3-chloropropane remaining at the end of the four hour experiment, the final concentration of the test compound obtained at in the absence of aquifer materials (20 $\pm$ 1 μg/L) was significantly different from those obtained in the presence of Oklahoma State University Agronomy Station aquifer material (22 $\pm$ 1 μg/L). Further analysis of the data collected throughout the entire test period was required in order to fully explain the observed result. This was done by running numerical models to determine kinetic constants for the experimental data collected in this study. This was done using nonlinear parameter estimation technique for curve fitting on all experimental data obtained in the batch reactors containing cells of *Pseudomonas putida* PpG-786. No removal of 1,2-dichloroethane was observed during the four hour test period.

The removal of 1,2-dichloropropane in the presence of subsurface materials obtained from Oklahoma State University Agronomy Research Station and the Sand Springs Petrochemical Complex subsurface materials are shown in Figures 17 and 18, respectively. A 76% removal of test substrates was observed in these experiments for both sites. There was also no significant difference between Oklahoma State University Agronomy Station and Sand Springs Petrochemical Complex subsurface materials in the final concentrations obtained at the end of the four hour experiments. The mean initial substrate concentration used in these experiments was 1162 μg/L. These results indicate that although the rate of biodegradation of low molecular weight halogenated aliphatic compounds by *Pseudomonas putida* PpG-786 is decreased by the presence of aquifer materials, extent of decrease was virtually the same two quite different aquifer materials. Biodegradation of low molecular weight halogenated compounds by this microorganism is, however, dependent on the specific low molecular weight halogenated compound.

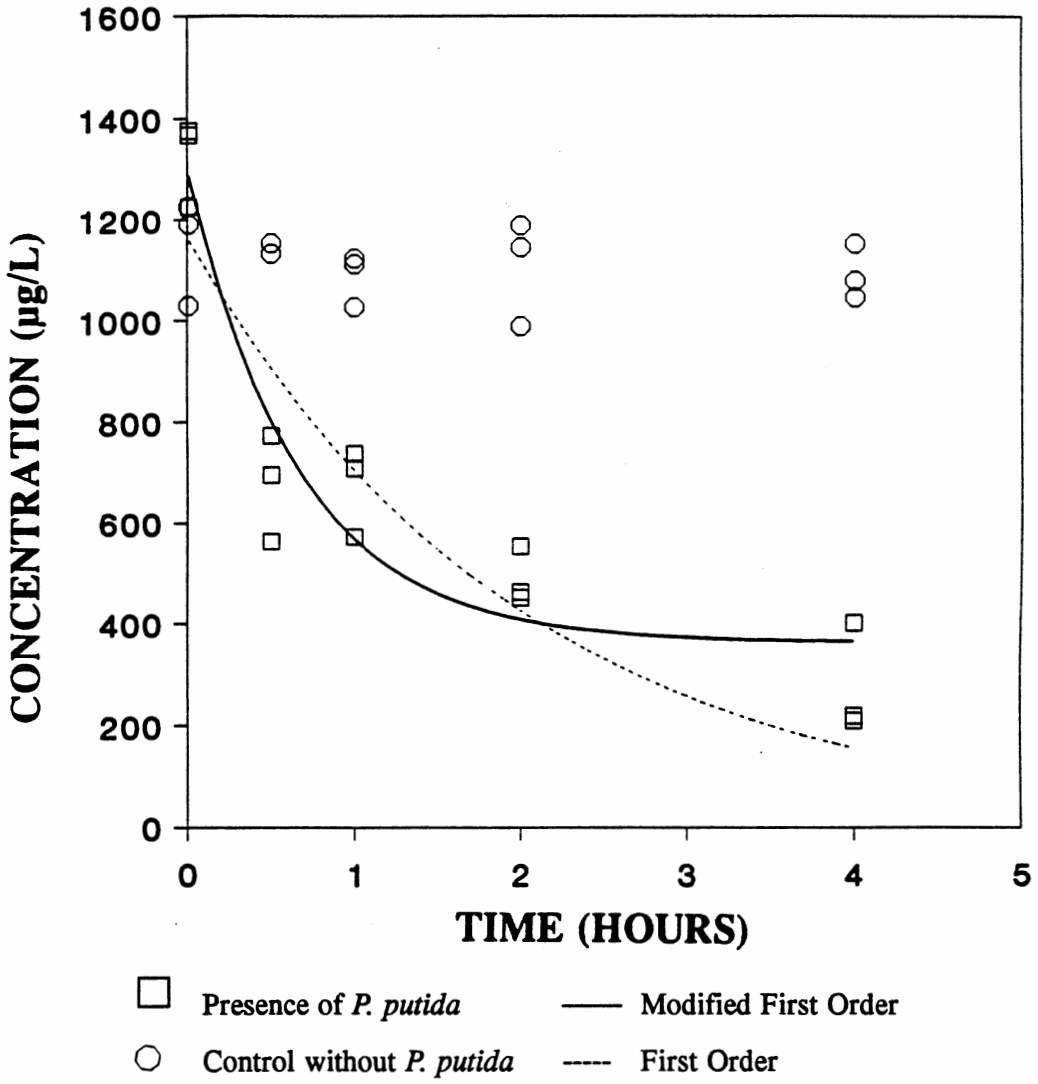


Figure 17. Removal of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.4, Temperature 25°C)

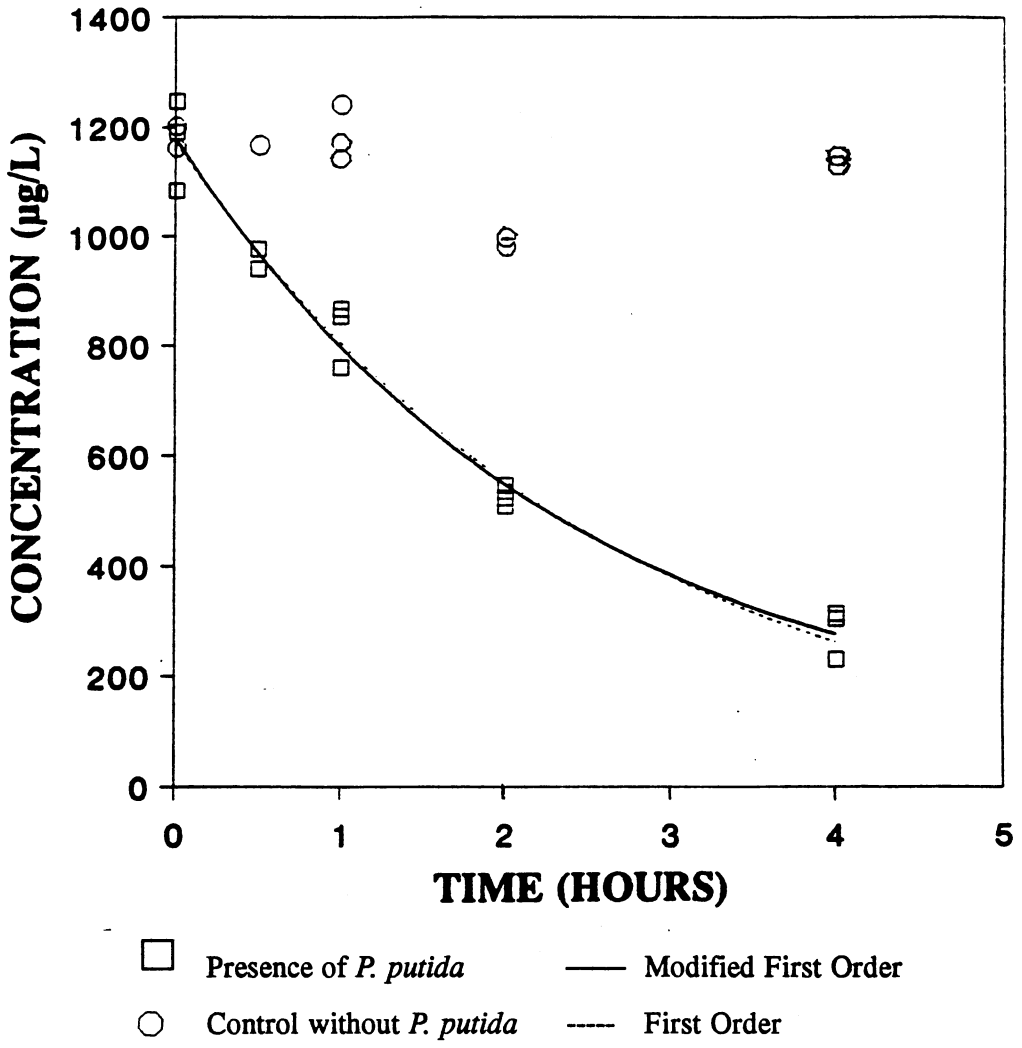


Figure 18. Removal of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 in the Presence of Sand Springs Petrochemical Complex Subsurface Materials (pH 7.4, Temperature 25°C)

Effect of pH

The results of the effects of pH at room temperature are shown in Figures 19 - 21 and Tables XII and XIII. The percent removals of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 were 95% at pH 5.4 and pH 7.4, and 92% at pH of 8.9. Analysis of test concentrations remaining at the end of the four hour experiments showed no significant difference for all three pH values. The mean initial substrate concentration of 1,2-dichloropropane used was 1090 μg/L. This indicates it has no effect on the removal of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 when the pH is varied from 5.4 - 8.9 at 25°C in the presence of aquifer materials obtained from the Oklahoma State University Agronomy Research Station.

Effect of Dissolved Oxygen

Figures 22 - 24 show the effect of initial dissolved oxygen on the removal of 1,2-dichloropropane by a pure culture of *Pseudomonas putida* PpG-786. Percent removals of the test compounds were 82% at an initial dissolved oxygen of 16.0 mg/L (Figure 22), 60% at initial dissolved oxygen of 8.5 mg/L (Figure 23), and 65% at an initial dissolved oxygen of 6.0 mg/L (Figure 24). The mean initial substrate concentration used was 1030 μg/L. There was a significant difference in the final mean concentration of 1,2-dichloropropane observed at the end of the four hour experiment when the initial dissolved oxygen was 6.0 mg/L compared to when it was 16 mg/L. Although the initial dissolved oxygen level of 8.5 mg/L showed no significant difference from that obtained at 16.0 mg/L, the probability level observed (0.0575) was very close to the assumed alpha level of significance. Dissolved oxygen levels of 16.0 mg/L, 8.5 mg/L, and 6.0 mg/L correspond to 2.9 mg DO/g cell, 1.5 mg DO/g cell, and 1.1 mg DO/g cell. An initial dissolved oxygen of 16.0 mg/L was used for all other experiments conducted outside these three experiments because this allows a final measured dissolved oxygen level of at least 1.5 mg/L at the end of the experiments.

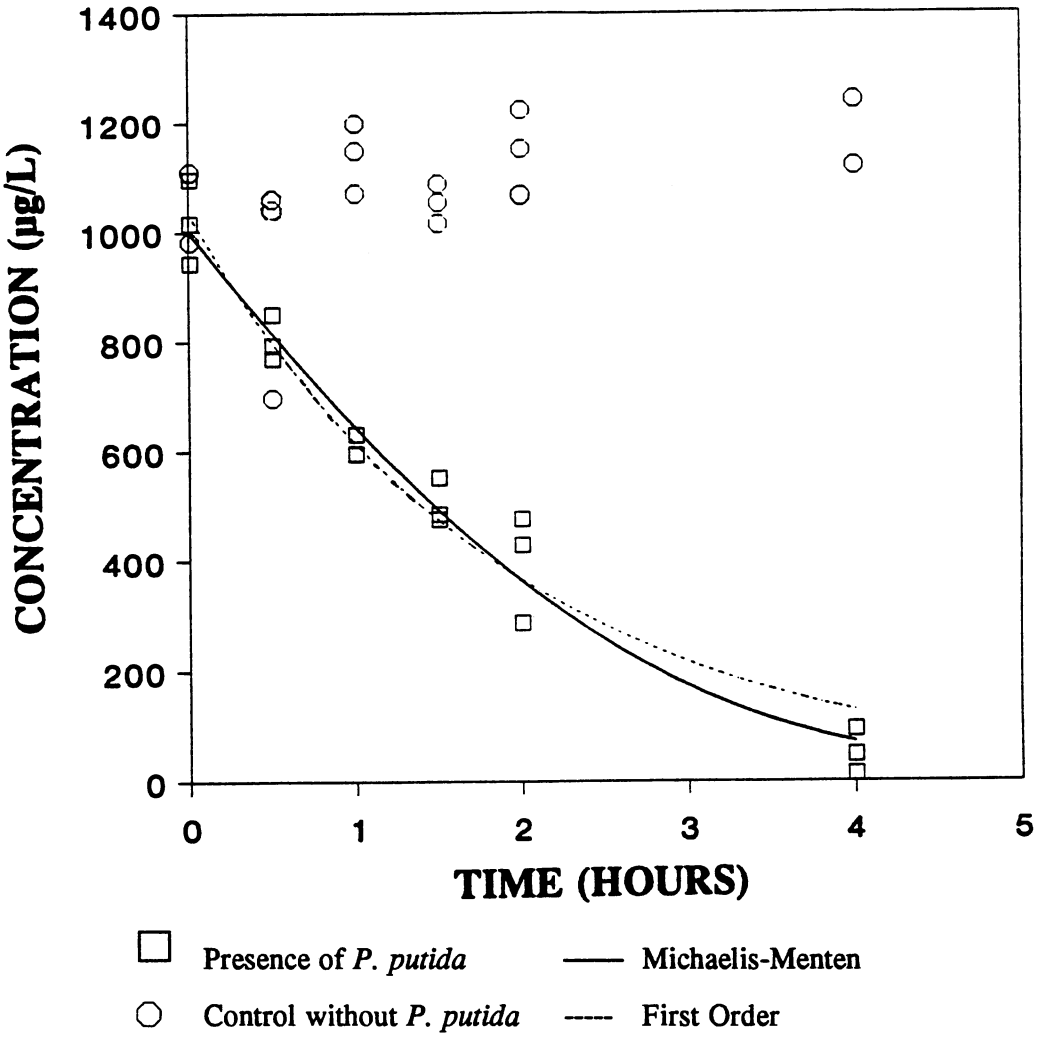


Figure 19. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 5.4, Temperature 25°C)

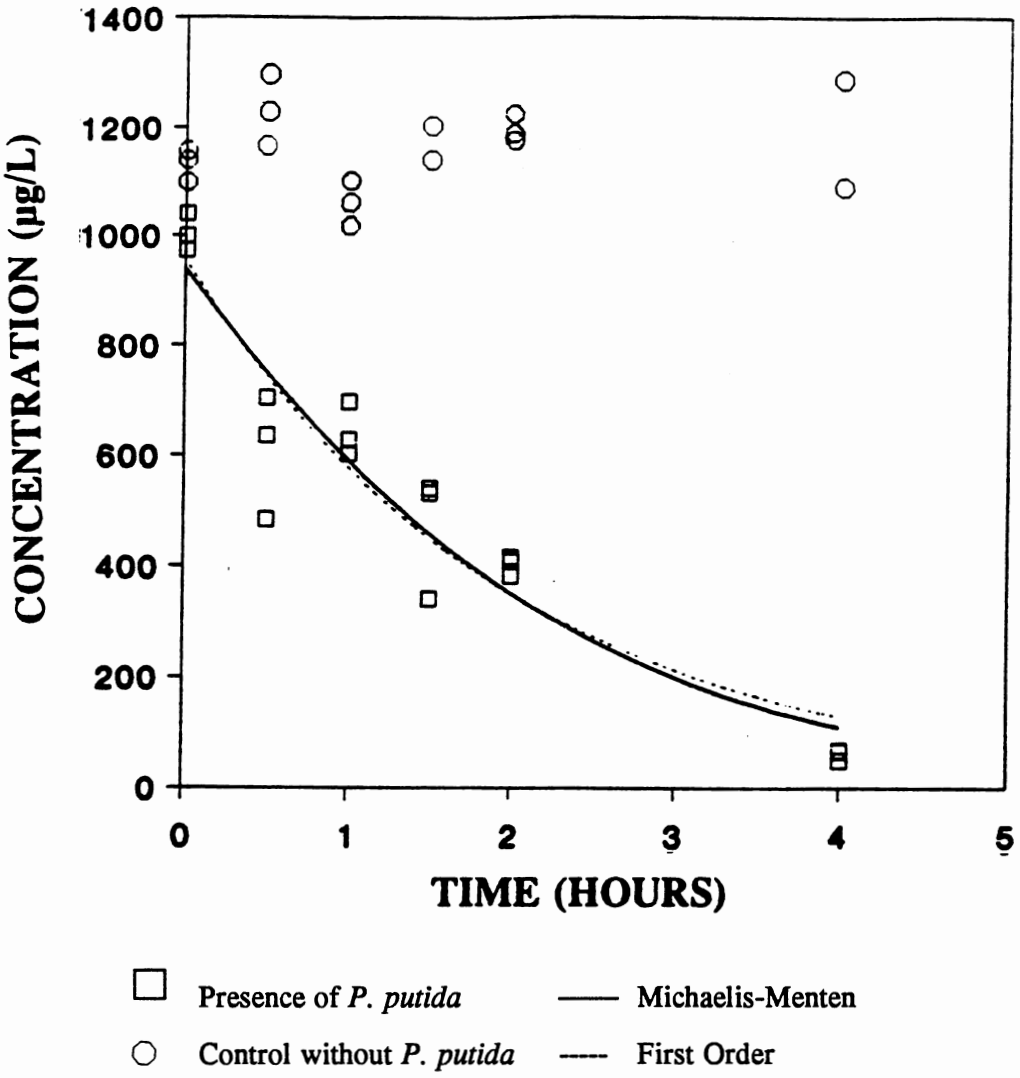


Figure 20. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.4, Temperature 25°C)

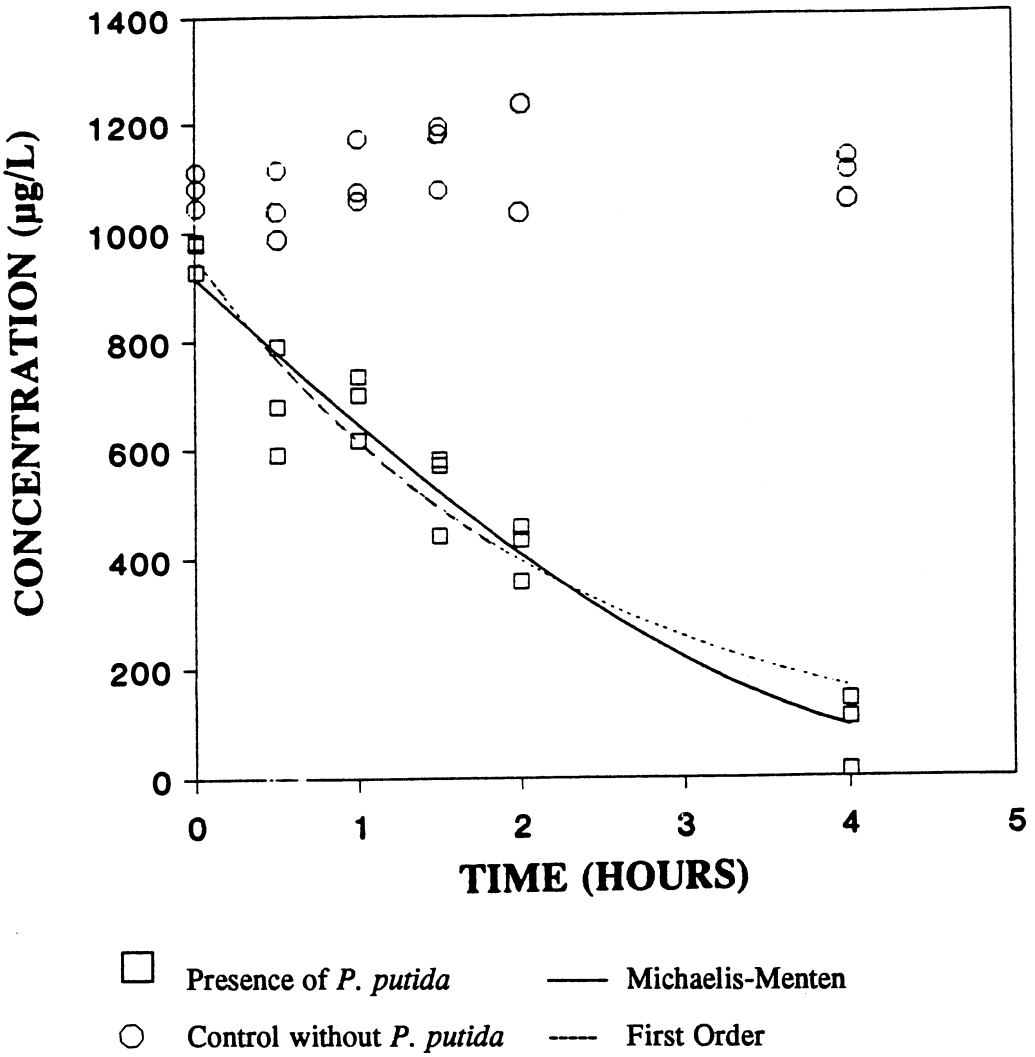


Figure 21. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 8.9, Temperature 25°C)

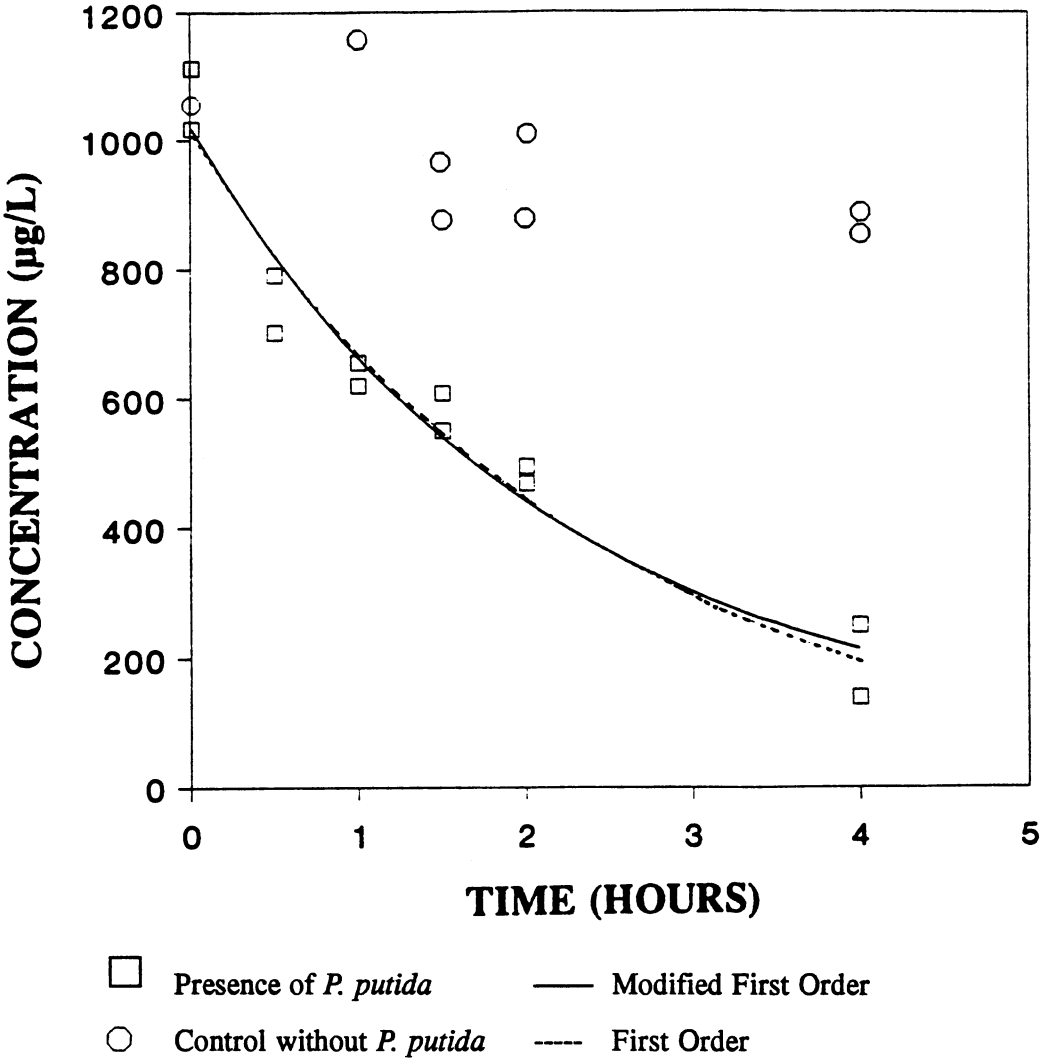


Figure 22. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Dissolved Oxygen 16.0 mg/L, pH 7.4, Temperature 25°C)

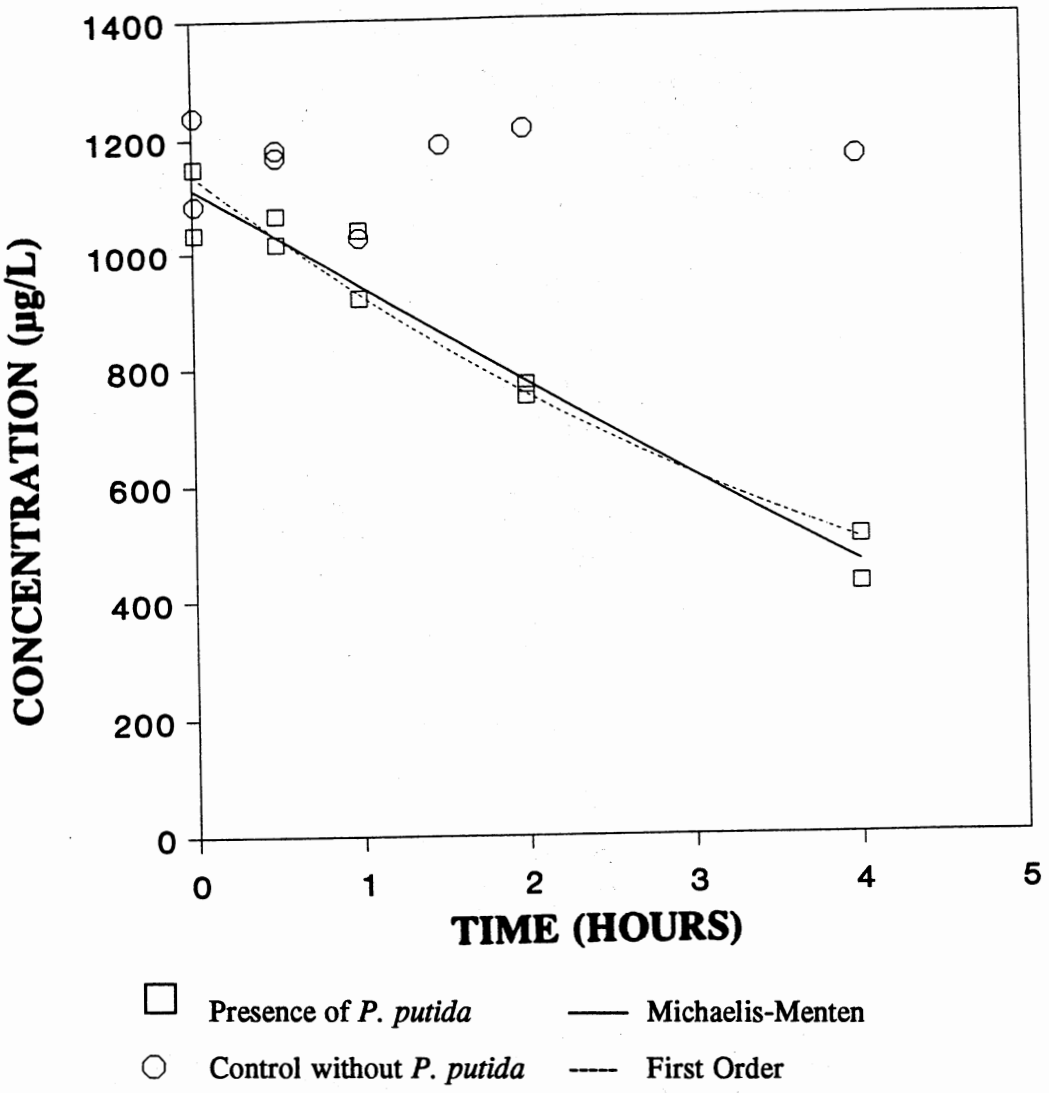


Figure 23. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Dissolved oxygen 8.5 mg/L, pH 7.4, Temperature 25°C)

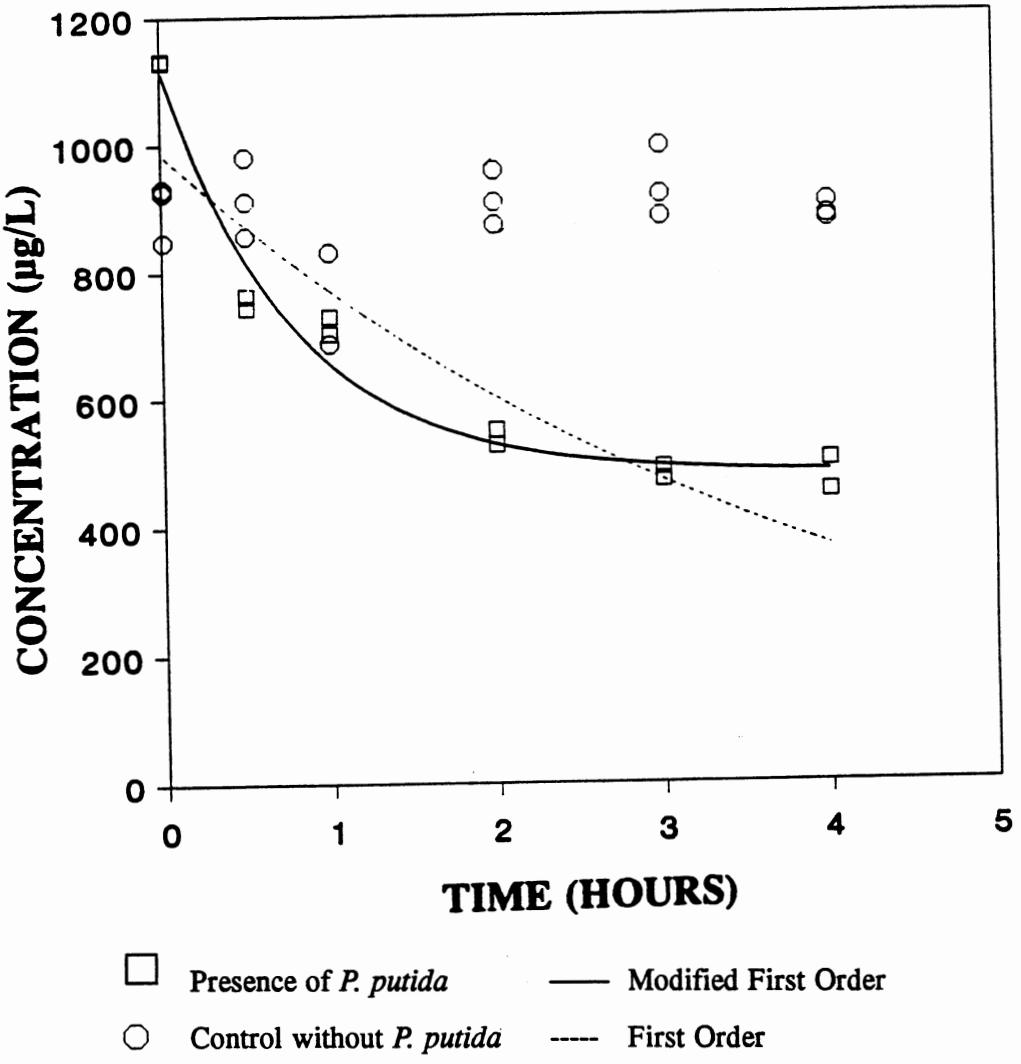


Figure 24. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Dissolved oxygen 6.0 mg/L, pH 7.4, Temperature 25°C)

Effect of Temperature

Since there was no significant difference in the removal of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 when the pH was varied from 5.4 to 8.9, temperature effects could be evaluated at any point within this pH interval. Experiments conducted to evaluate the effect of temperature were carried out at pH 6.4, 7.4, and 7.8, close to the maximum buffering capacity which is about a pH of 7.2 for 0.1 M phosphate buffer used in the experiments. This ensured that minimal change in the pH values occurred during the course of the experiments as the temperature varied. Figures 25 - 33 and Tables XII and XIII show the effect of temperature on the removal of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 at pH of 6.4 (chloride concentration of 2.102 g/L), 7.4 (chloride concentration of 0.871 g/L), and pH of 7.8 (chloride concentration of 0.151 g/L). Chloride ion was added to these sets of experiments to equalize the ionic strength of the buffer solution. The buffer solution was prepared with double distilled, dionized water. At the end of the four hour experiments, removals of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 were 57%, 11%, and 38% for pH 6.4, 7.4, and 7.8, respectively at 15°C. At 25°C, the removal of the test compound increased to 58%, 51%, and 70% for pH 6.4, 7.4, and 7.8, respectively. At 30°C, the removals of the test compound were lowered when compared to measurements at 25°C to 38%, 28%, and 59% for pH 6.4, 7.4, and 7.8, respectively. The effect of temperature was, however, not significant at 6.4 and 7.4 under these experimental conditions. At pH 7.8, there was a significantly higher concentration of 1,2-dichloropropane remaining at 15°C when compared to 25°C and 30°C. The mean initial concentrations of 1,2-dichloropropane used were 1195 μg/L at pH 6.4, 1102 μg/L at pH 7.4, and 1387 μg/L at pH of 7.8. The presence of chloride ions (experiment identified as pH74/25 in Table V) significantly (alpha of at least 0.05) affects the removal of 1,2-dichloropropane when compared to a similar experiment with no chloride ions (identified as pH74(25) in Table V) using a T-test.

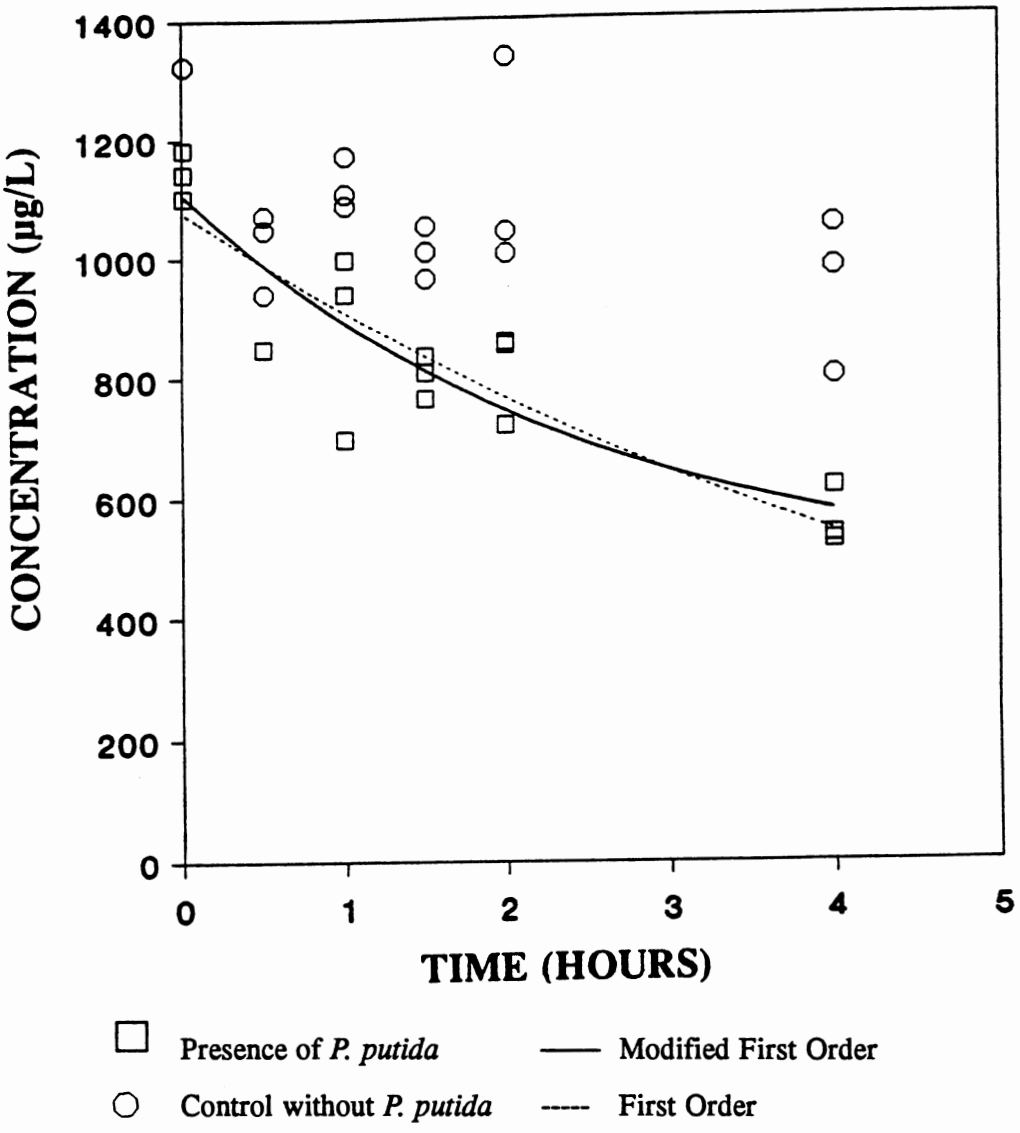


Figure 25. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 6.4, Temperature 15°C, Chloride 2.102 g/L)

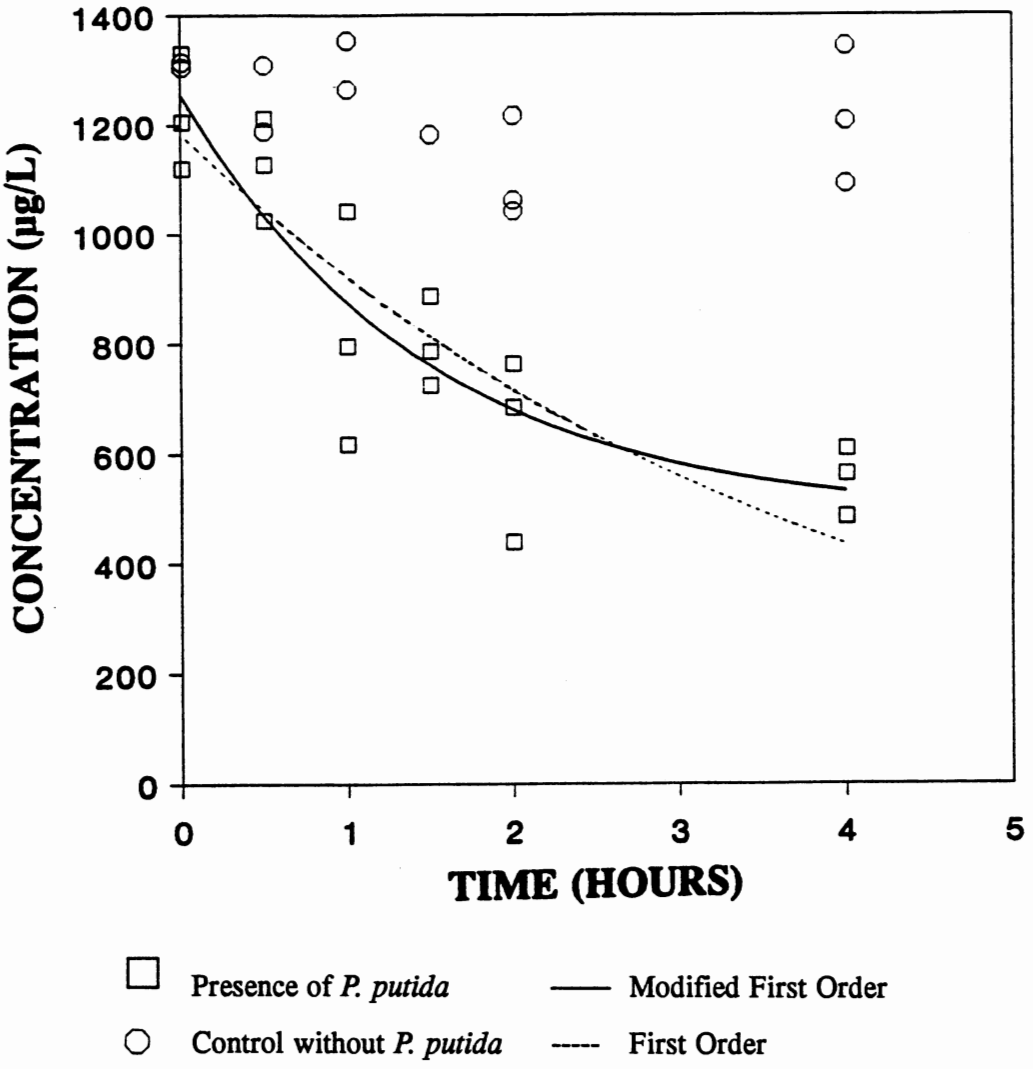


Figure 26. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 6.4, Temperature 25°C, Chloride 2.102 g/L)

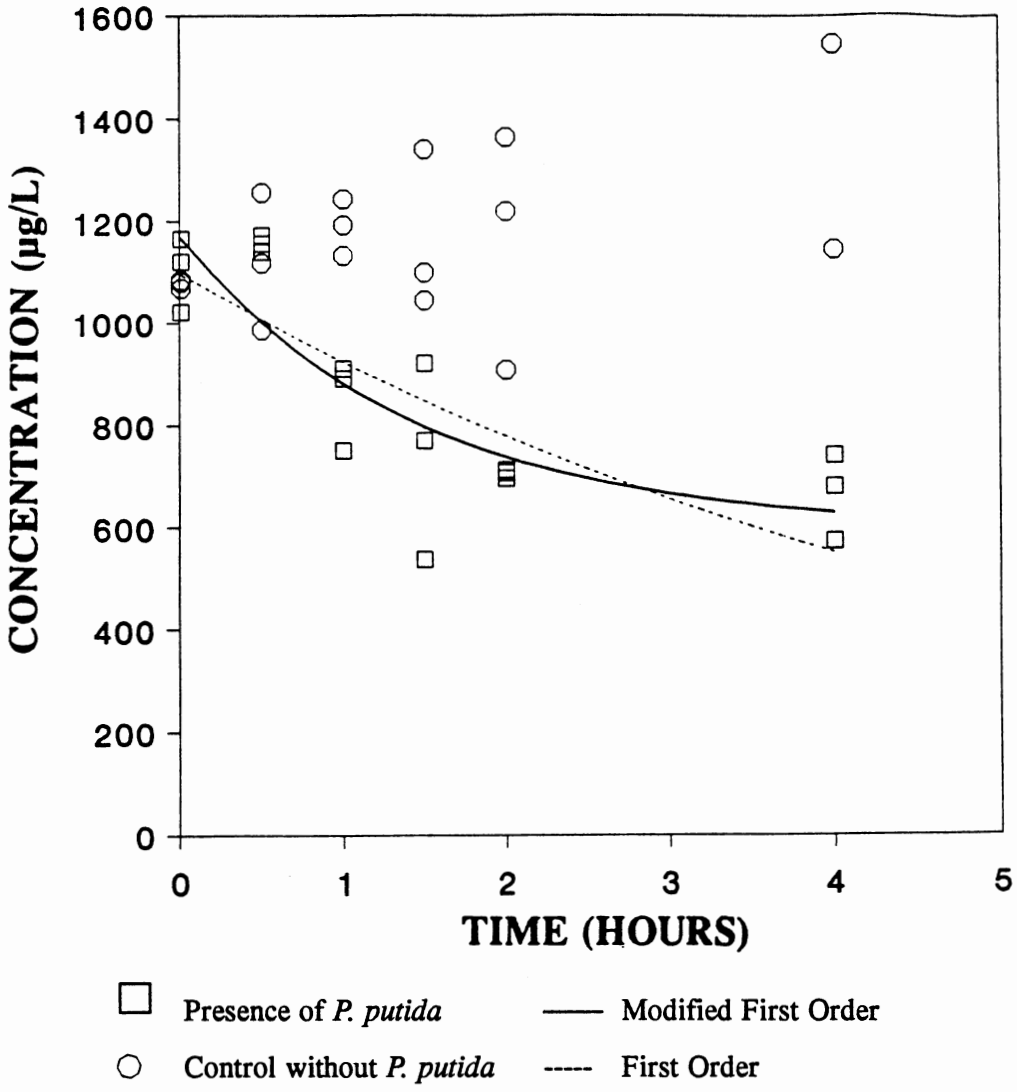


Figure 27. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 6.4, Temperature 30°C, Chloride 2.102 g/L)

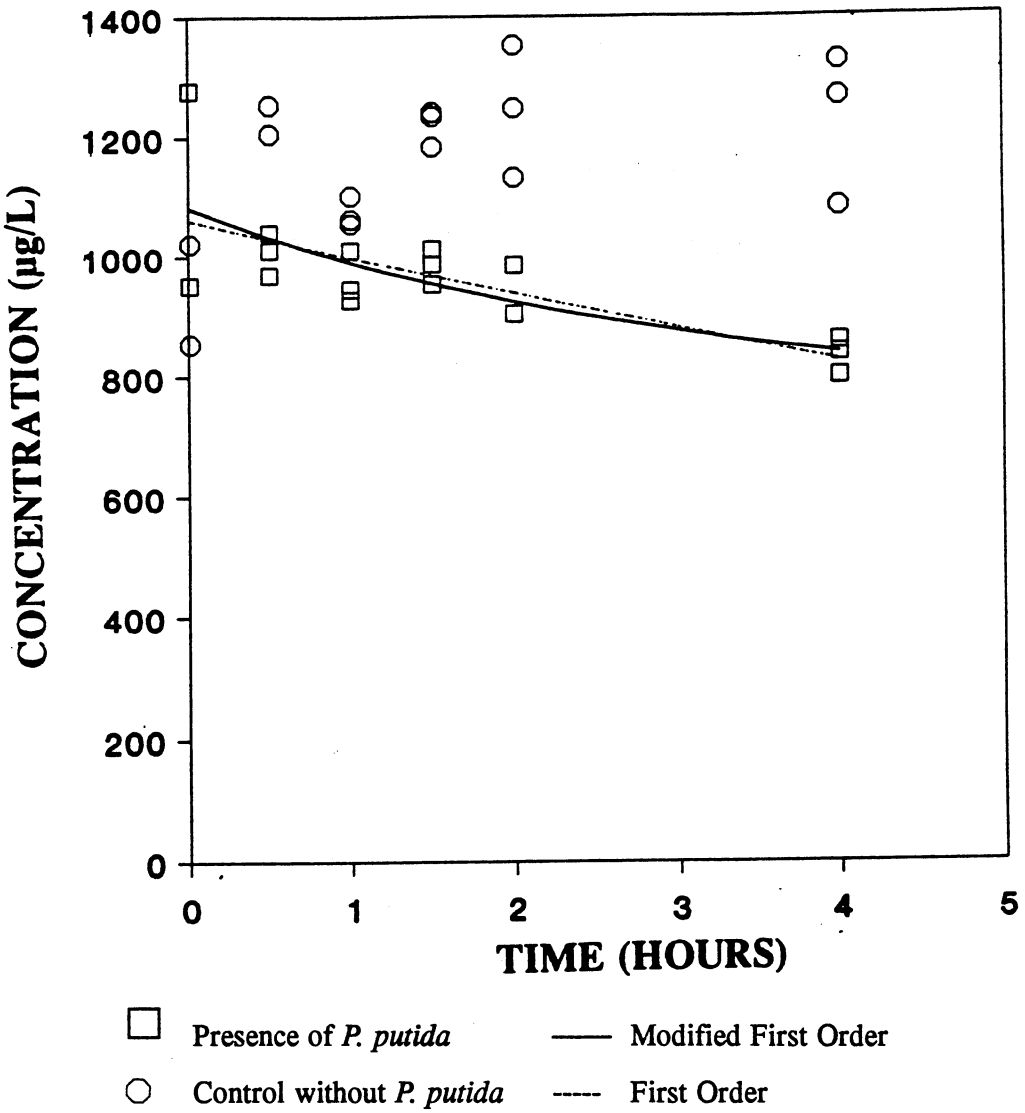


Figure 28. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.4, Temperature 15°C, Chloride 0.871 g/L)

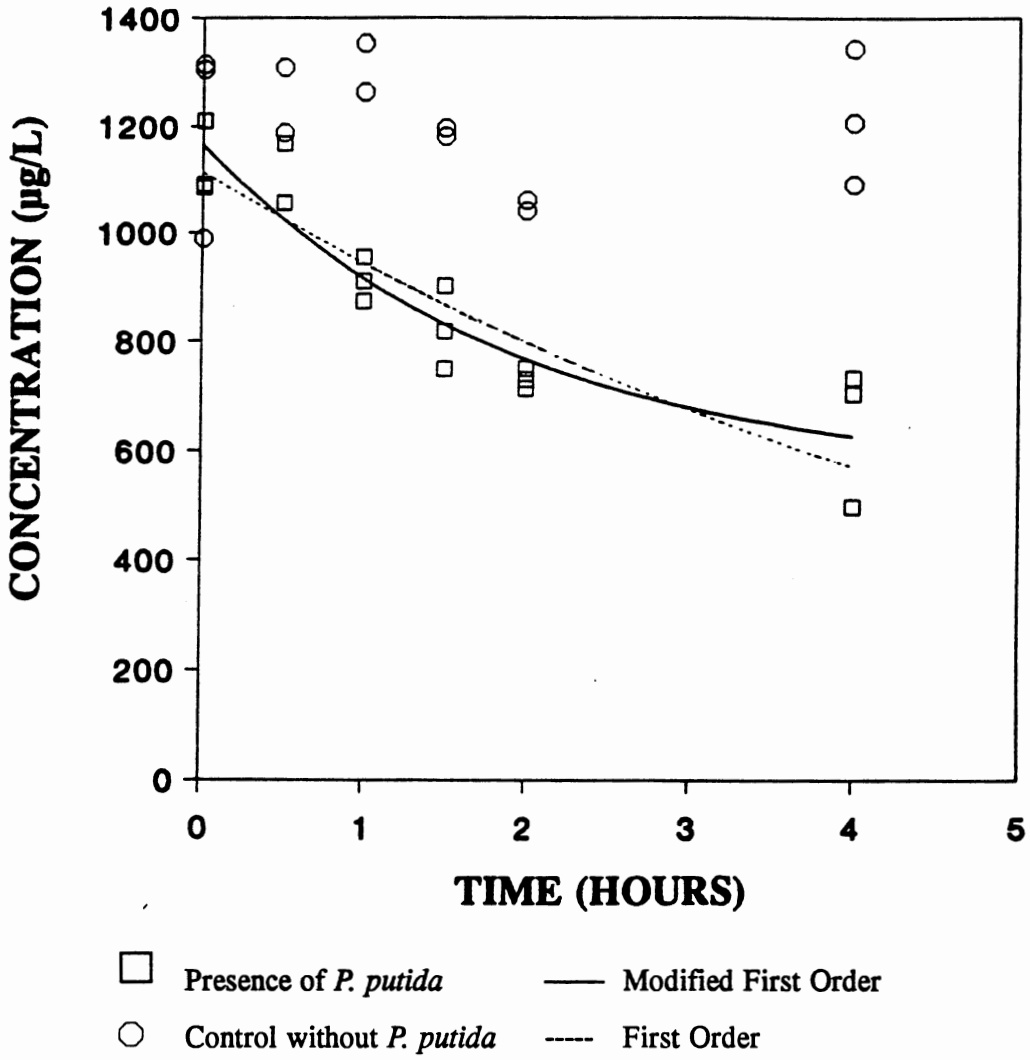


Figure 29. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.4, Temperature 25°C, Chloride 0.871 g/L)

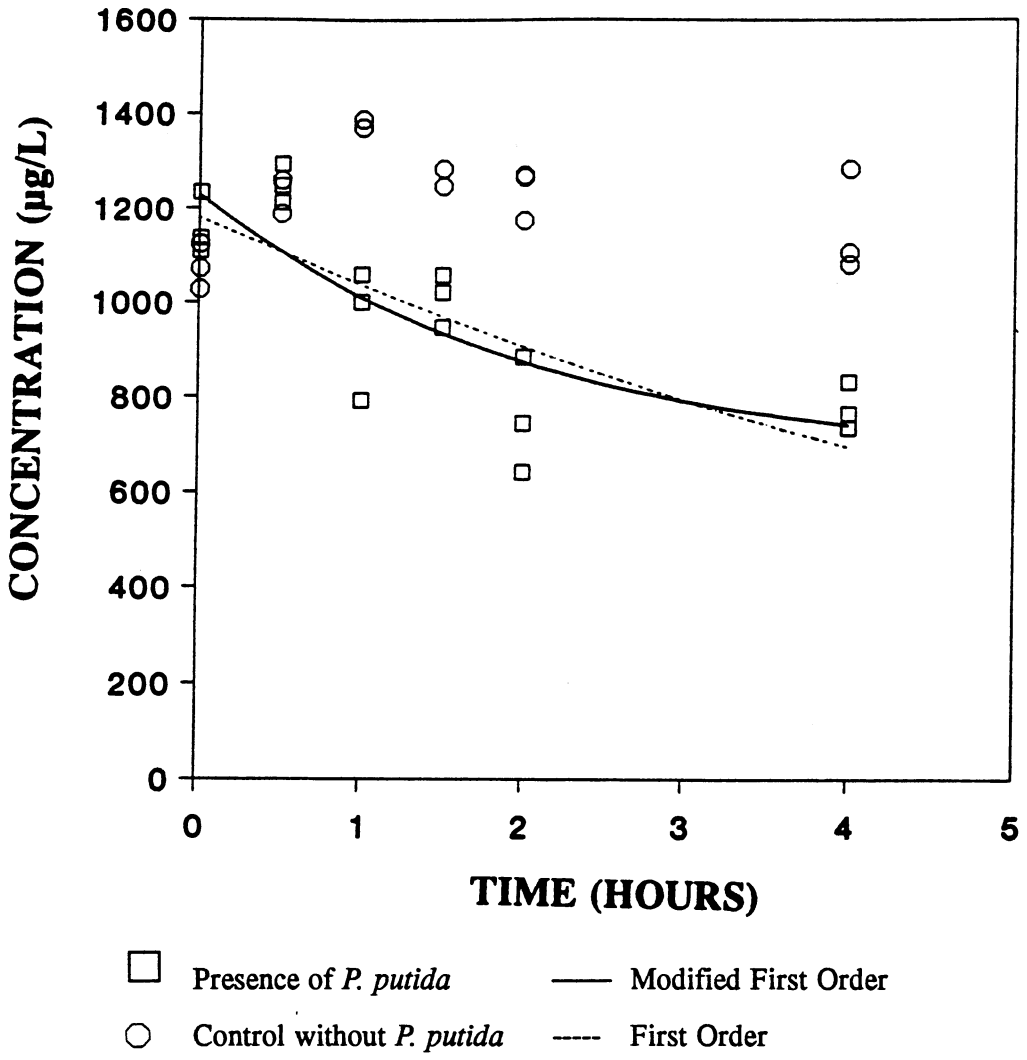


Figure 30. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.4, Temperature 30°C, Chloride 0.871 g/L)

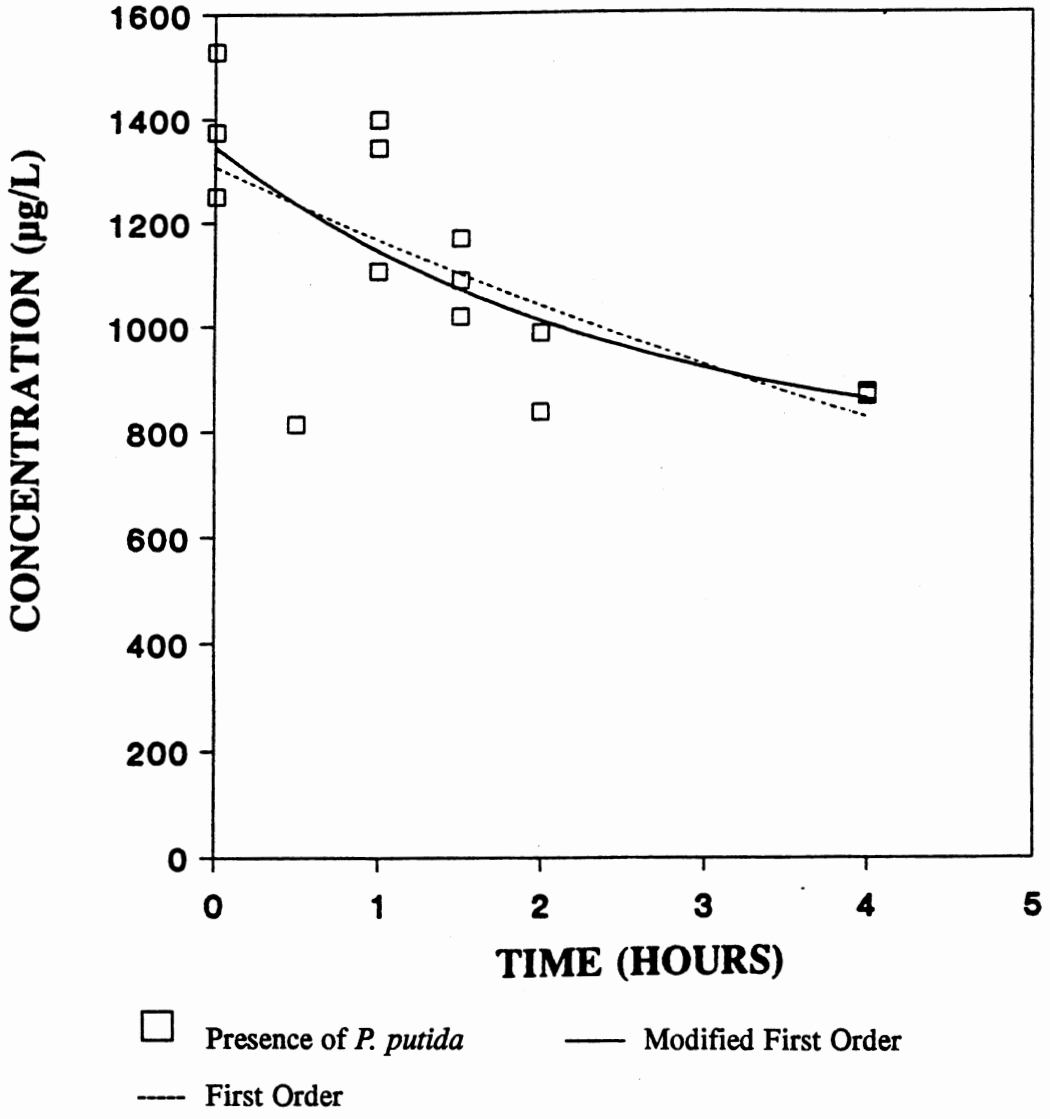


Figure 31. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.8, Temperature 15°C, Chloride 0.151 g/L)

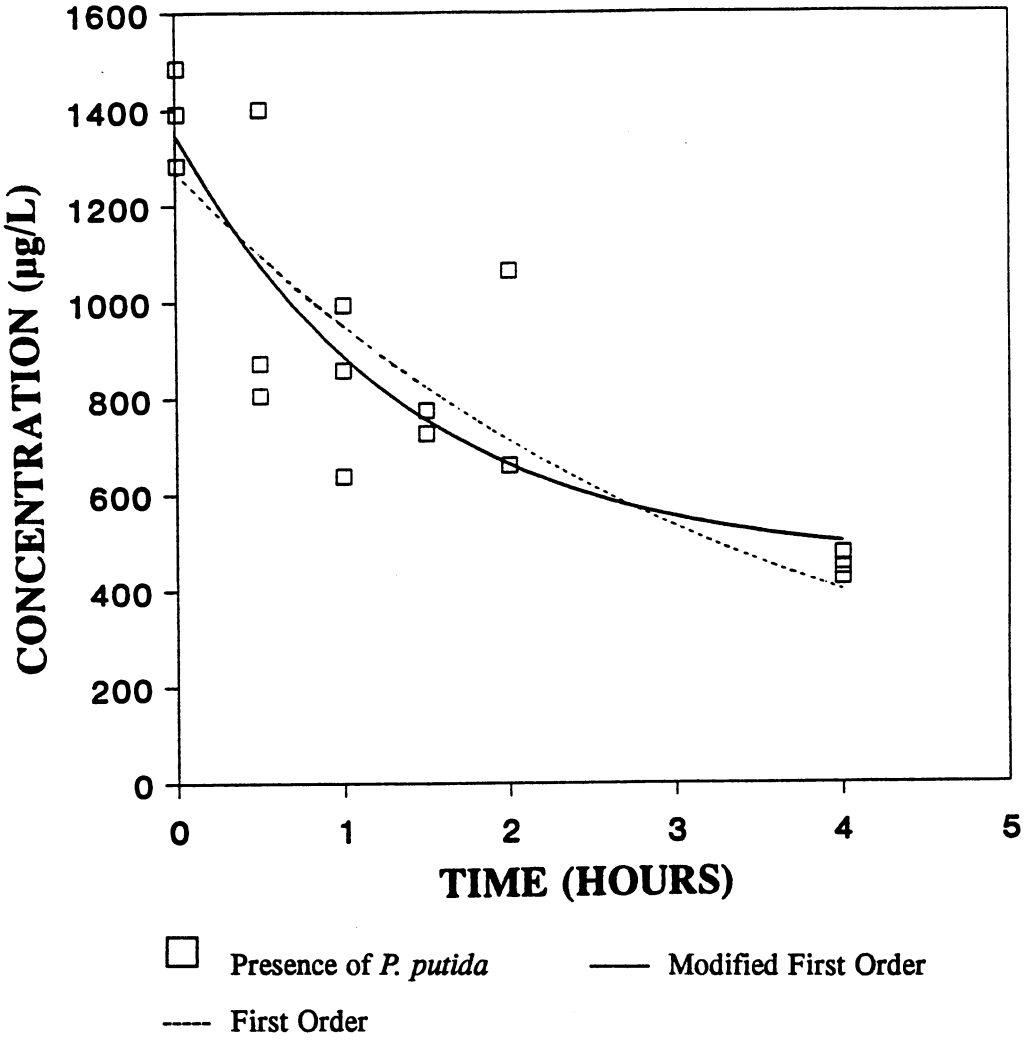


Figure 32. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.8, Temperature 25°C, Chloride 0.151 g/L)

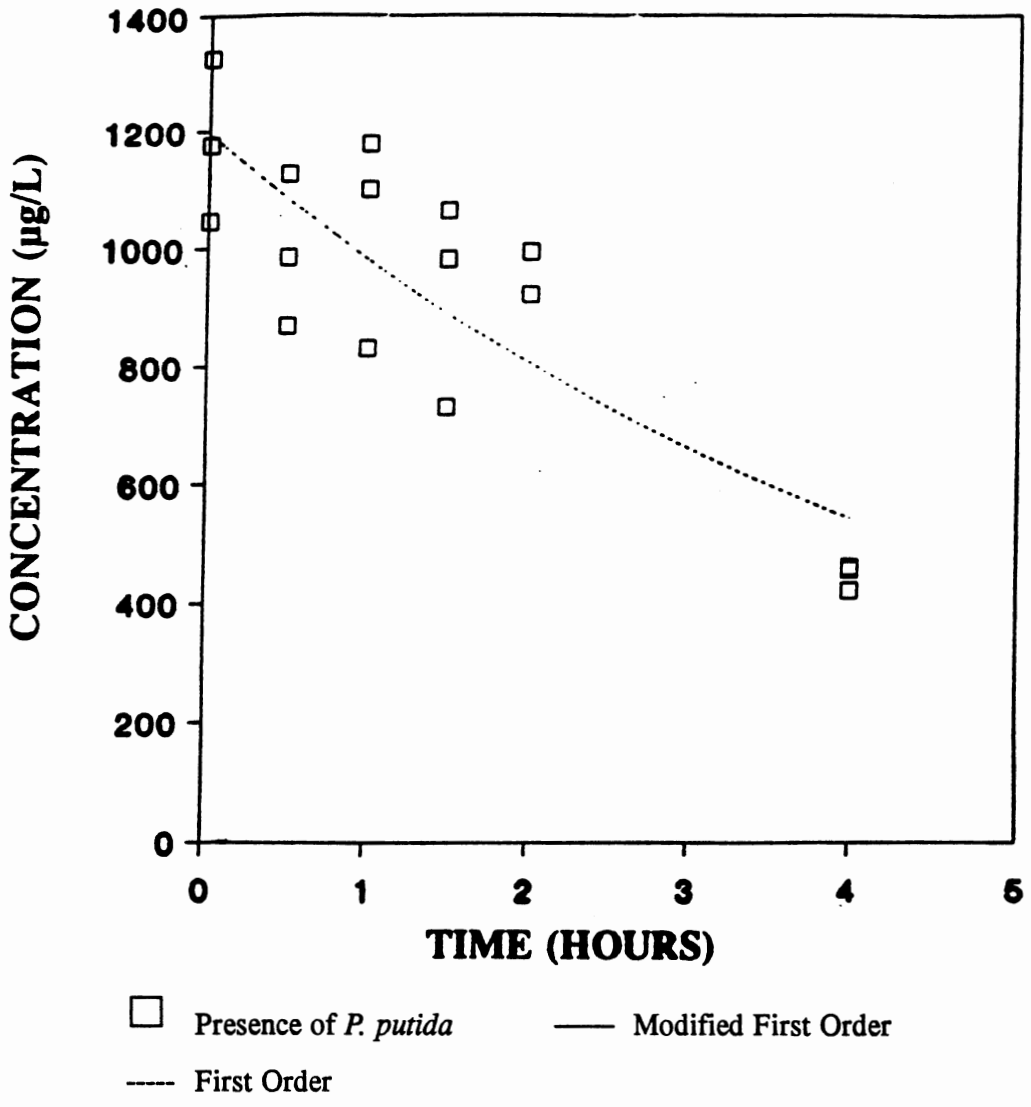


Figure 33. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (pH 7.8, Temperature 30°C, Chloride 0.151 g/L)

Effect of Inoculum Sizes

The results of the experiment to evaluate the effect of varying inoculum sizes on biodegradation of 1,2-dichloropropane are shown in Tables XII and XIII and Figures 34 - 37. The inoculum sizes used were 1.455 g/L (Figure 34), 3.317 g/L (Figure 35), 6.470 g/L (Figure 36), and 8.017 g/L (Figure 37). The percent removals observed were 28%, 58%, 67%, and 90% for inoculum sizes of 1.455 g/L, 3.317 g/L, 6.470 g/L, and 8.017 g/L, respectively. Cell measurements were made using a Mettler AE-160 model digital balance (0.0000 g digital display). The mean initial substrate concentration used in these experiments was 992 μg/L.

Effect of Substrate Concentration

The effect of different initial substrate concentrations on the biodegradation of 1,2 dichloropropane by *Pseudomonas putida* PpG-786 was evaluated and the results are shown in Figures 38 - 40 and in Tables XII and XIII. The percent removals observed were 98%, 97%, and 26% corresponding to initial substrate concentrations of 732 μg/L, 1209 μg/L, and 4907 μg/L, respectively. There was no significant difference between the substrate concentrations remaining at the end of the experiments using 732 μg/L and 1209 μg/L. However, there was a significant difference between the substrate concentration remaining at 4907 μg/L and the other two initial concentrations tested.

Effect of Lead Concentration

Results of the experiments to evaluate effects of lead (added as lead acetate) on the biodegradation of 1,2-dichloropropane in the batch reactors containing cells of *Pseudomonas putida* PpG-786 are shown in Figures 41 - 44 and Tables XII and XIII. The percent removals of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 in the presence of Oklahoma State University Agronomy Research Station aquifer materials and lead were 52%, 63%, 61%, and 61% at lead concentrations of 0 mg/L, 2.2 mg/L, 5.5 mg/L, and 10.0 mg/L, respectively.

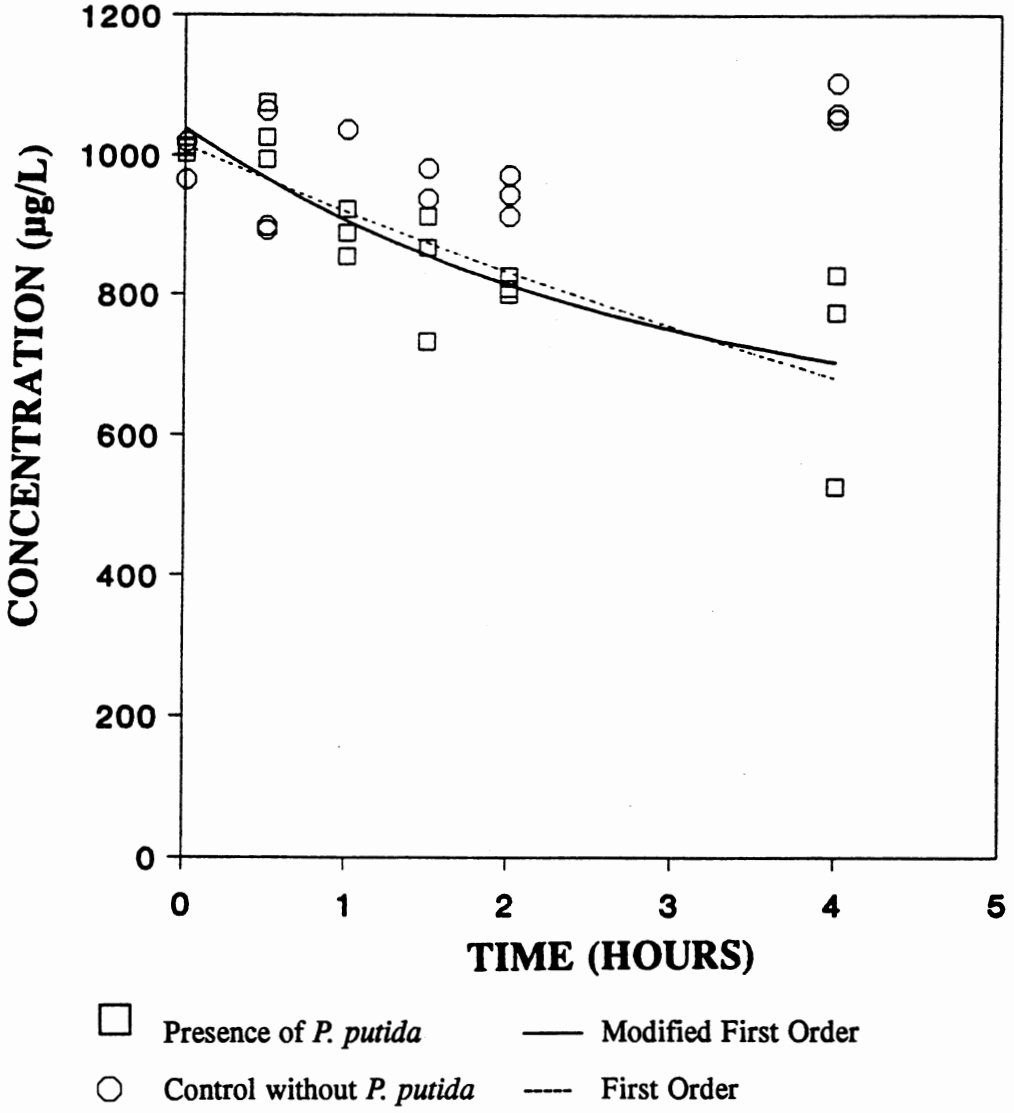


Figure 34. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Inoculum Size 1.455 g/L, pH 7.4, Temperature 25°C)

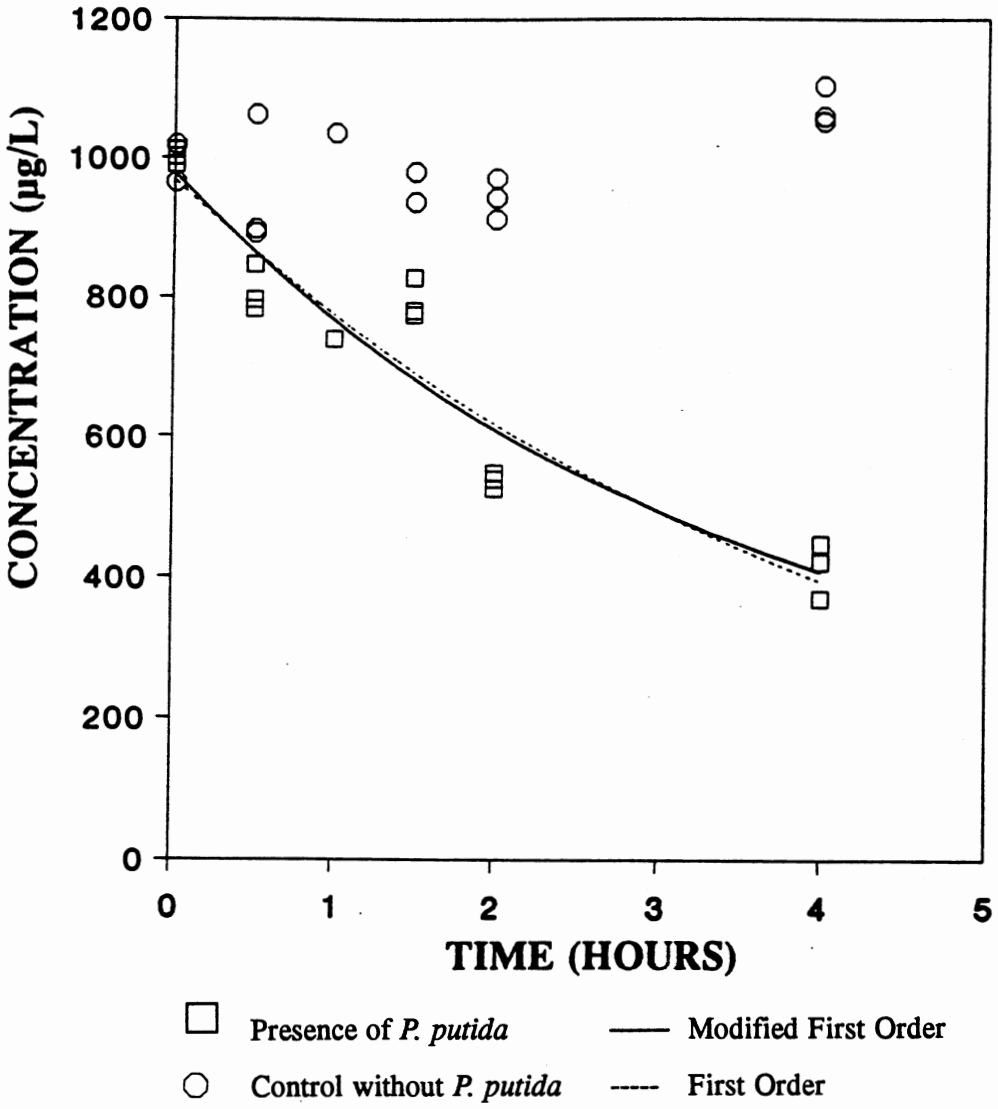


Figure 35. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Inoculum Size 3.317 g/L, pH 7.4, Temperature 25°C)

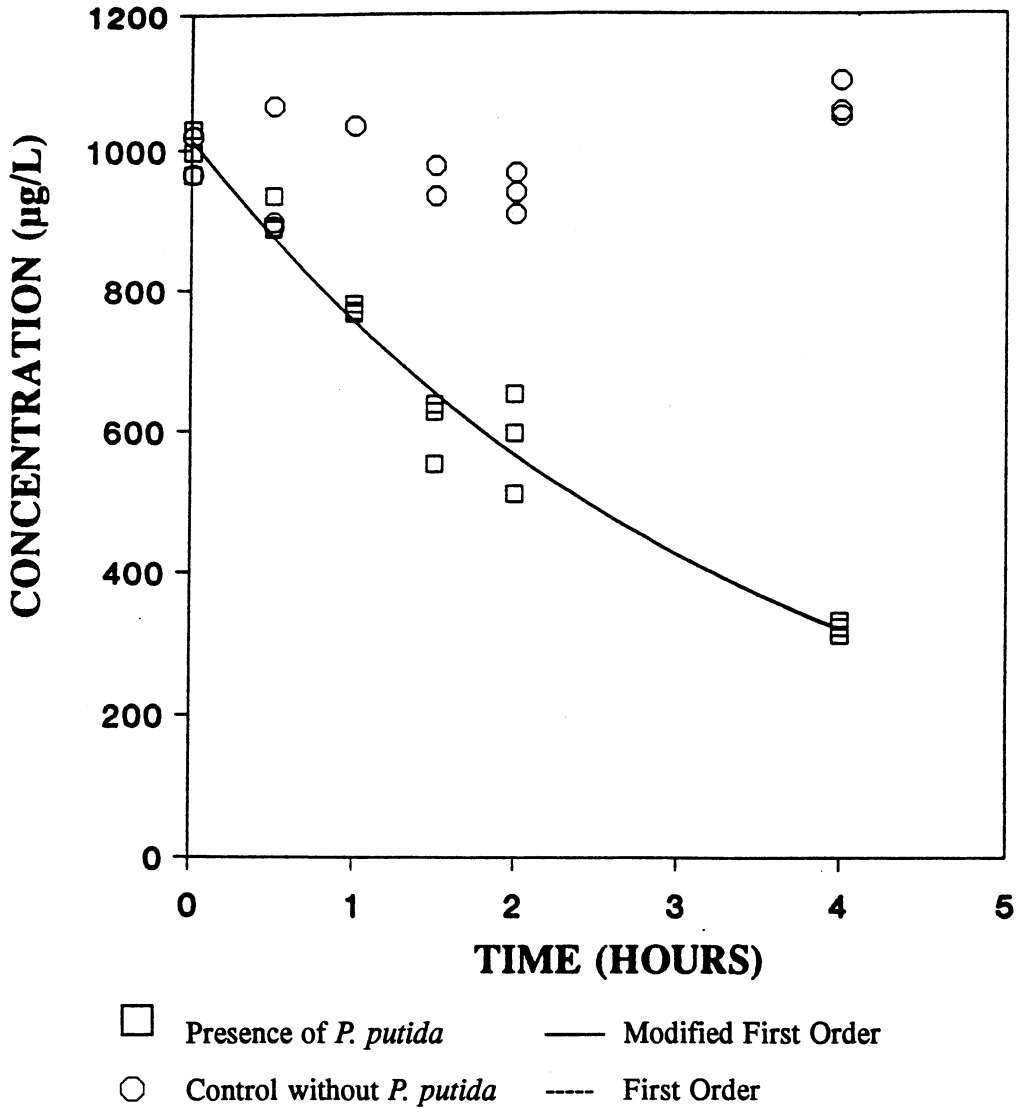


Figure 36. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Inoculum Size 6.470 g/L, pH 7.4, Temperature 25°C)

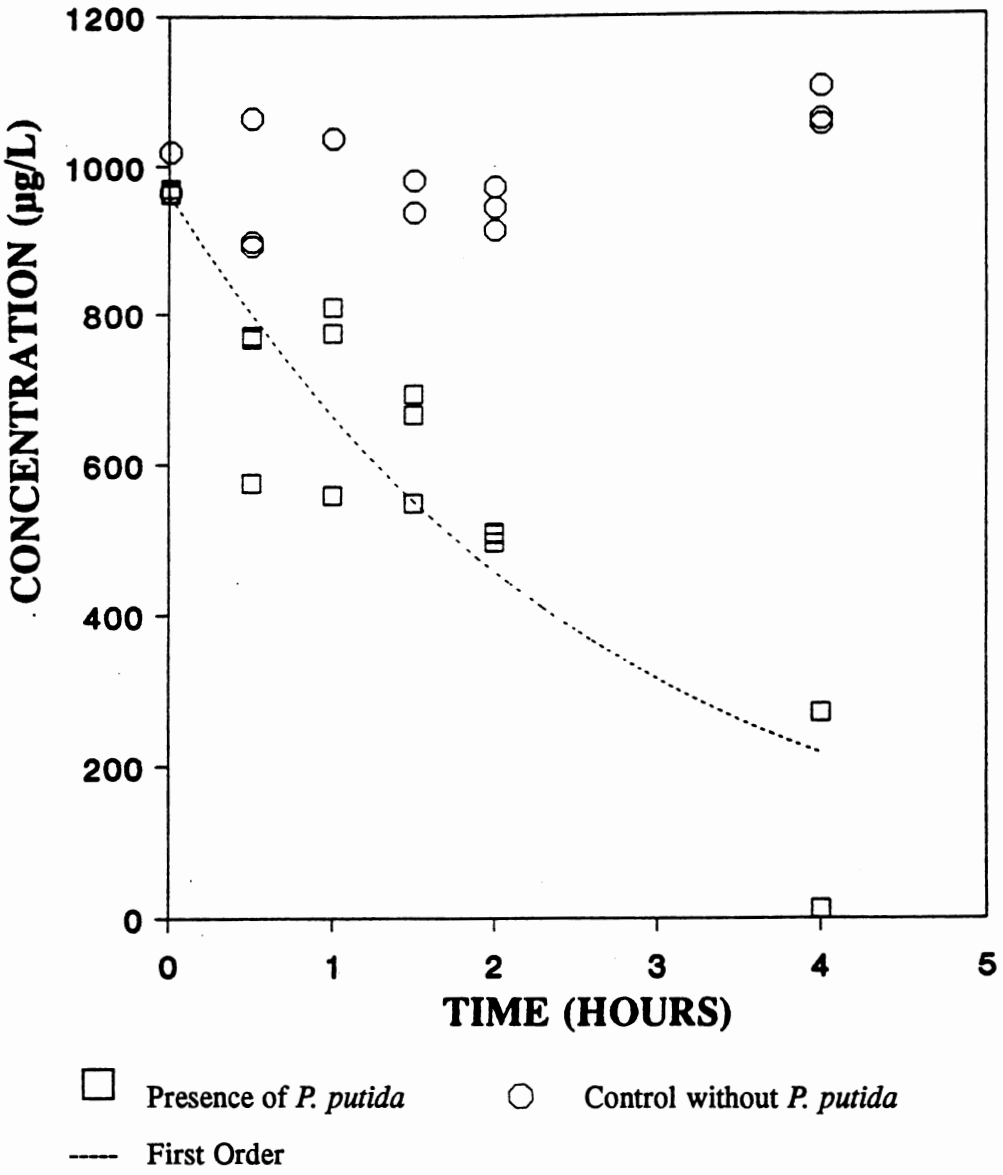


Figure 37. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Inoculum Size 8.017 g/L, pH 7.4, Temperature 25°C)

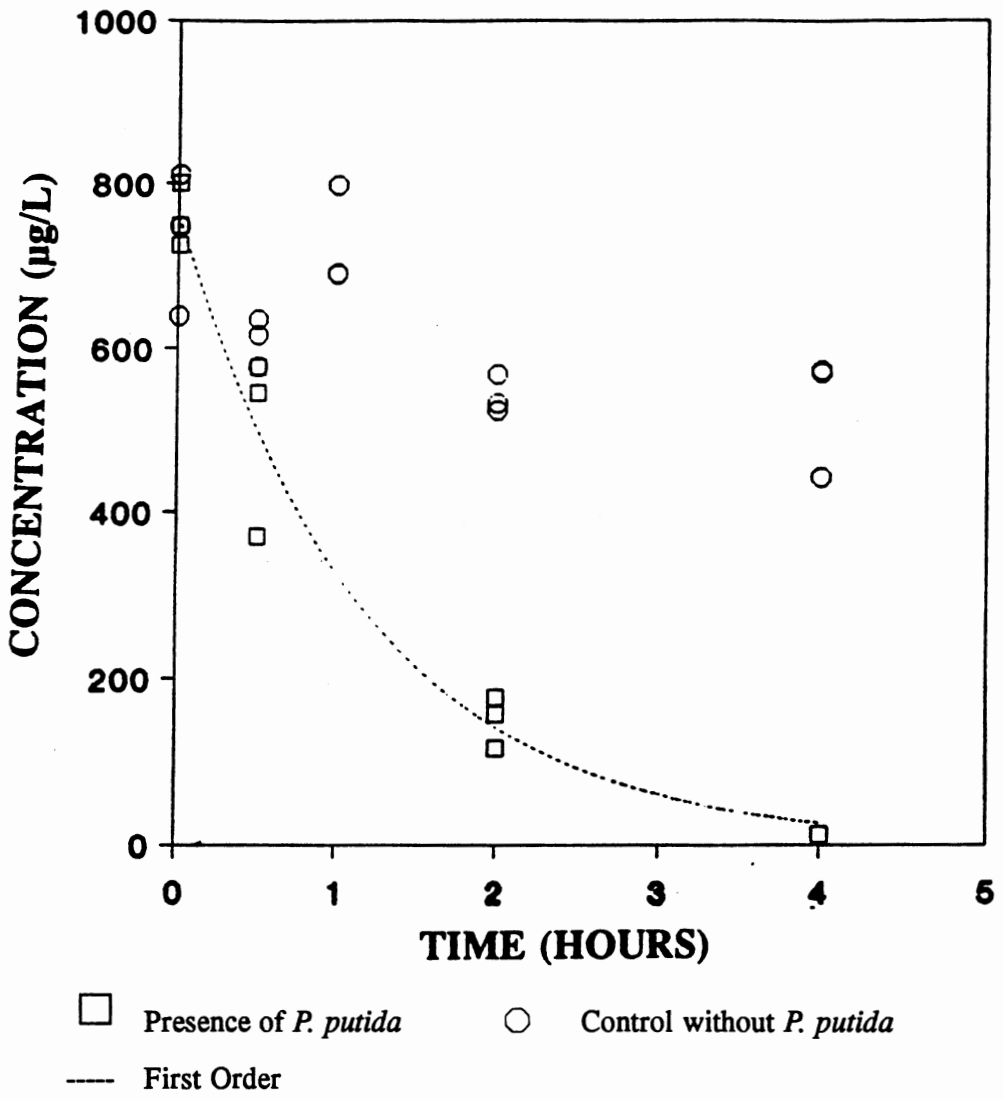


Figure 38. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Initial Substrate Concentration 732 µg/L, pH 7.4, Temperature 25°C)

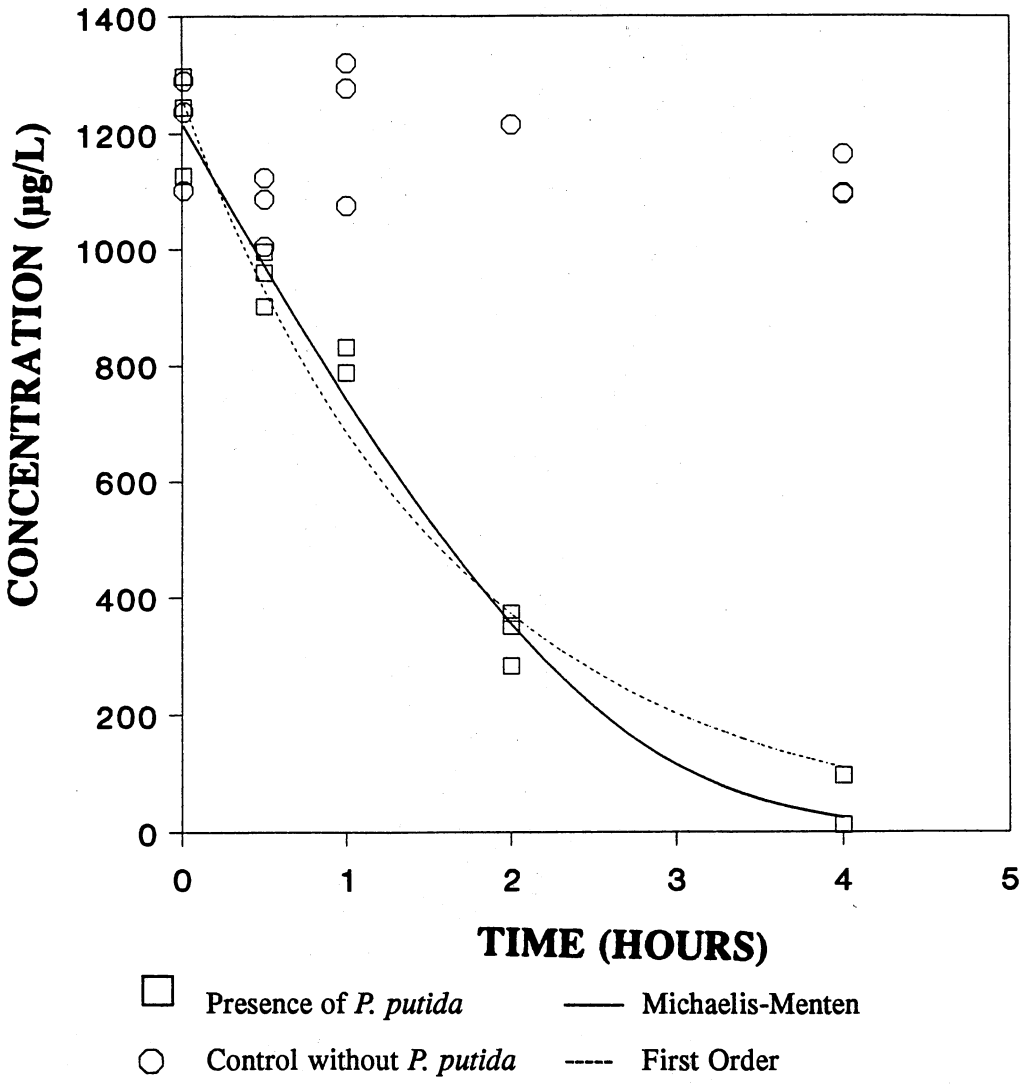


Figure 39. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Initial Substrate Concentration 1209 µg/L, pH 7.4, Temperature 25°C)

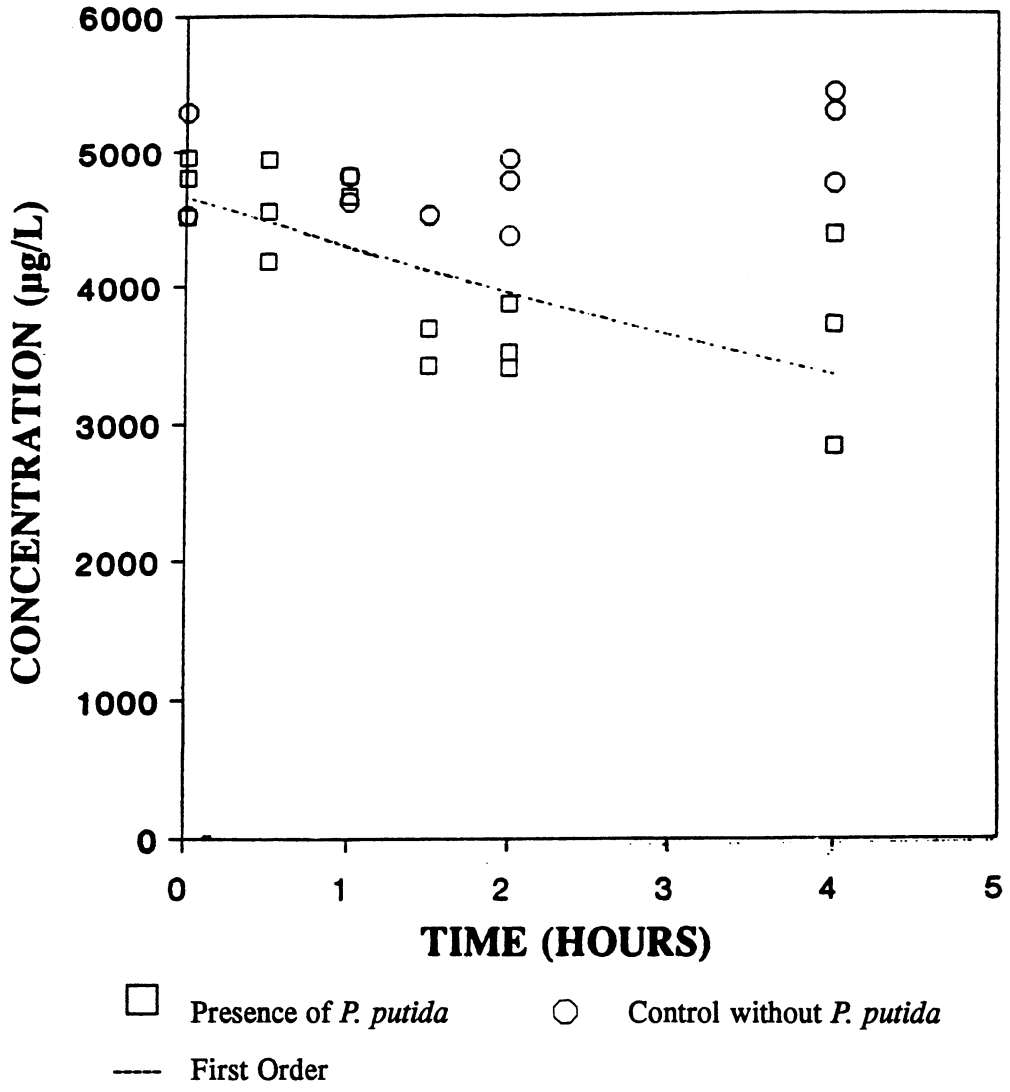


Figure 40. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Initial Substrate Concentration 4907 µg/L, pH 7.4, Temperature 25°C)

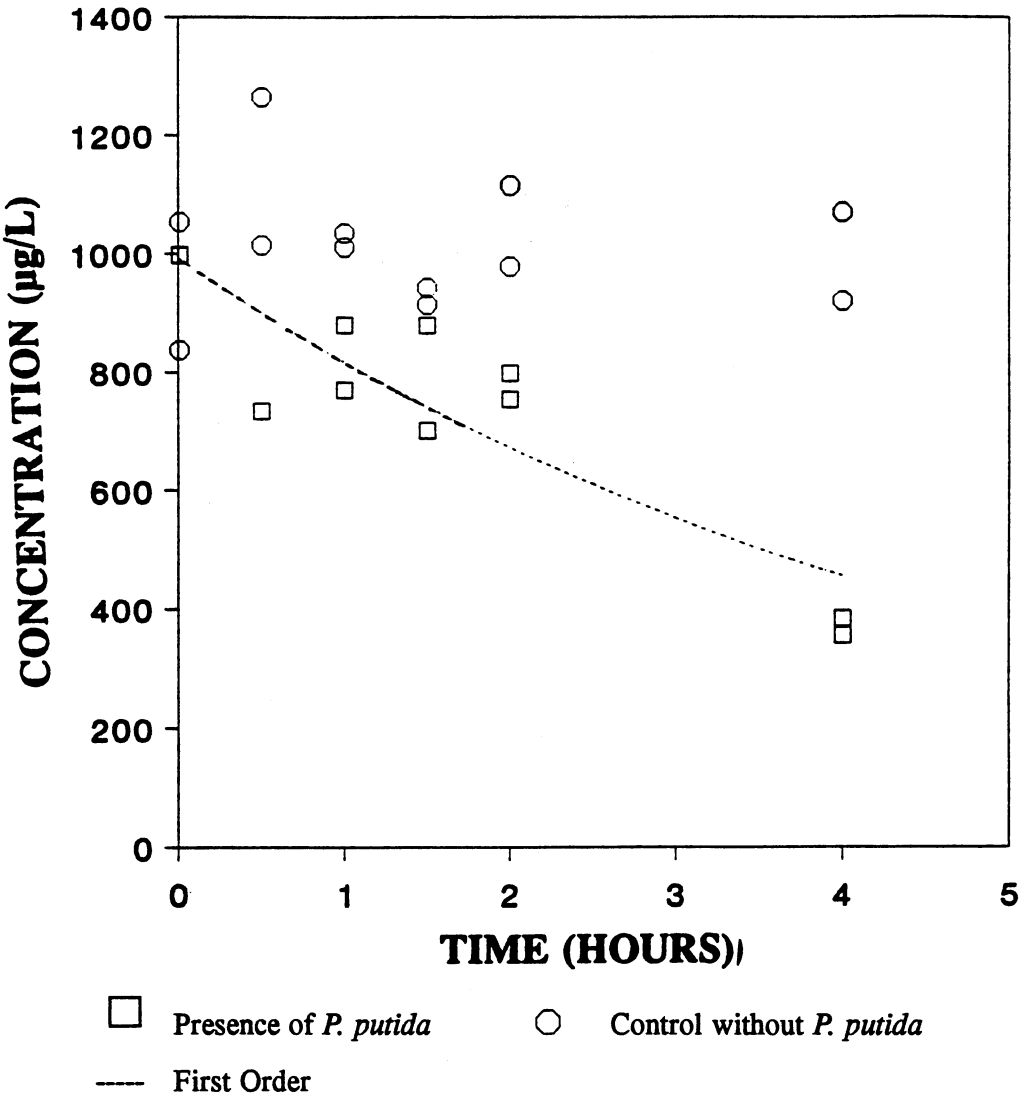


Figure 41. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Lead Concentration 0 mg/L, pH 7.4, Temperature 25°C)

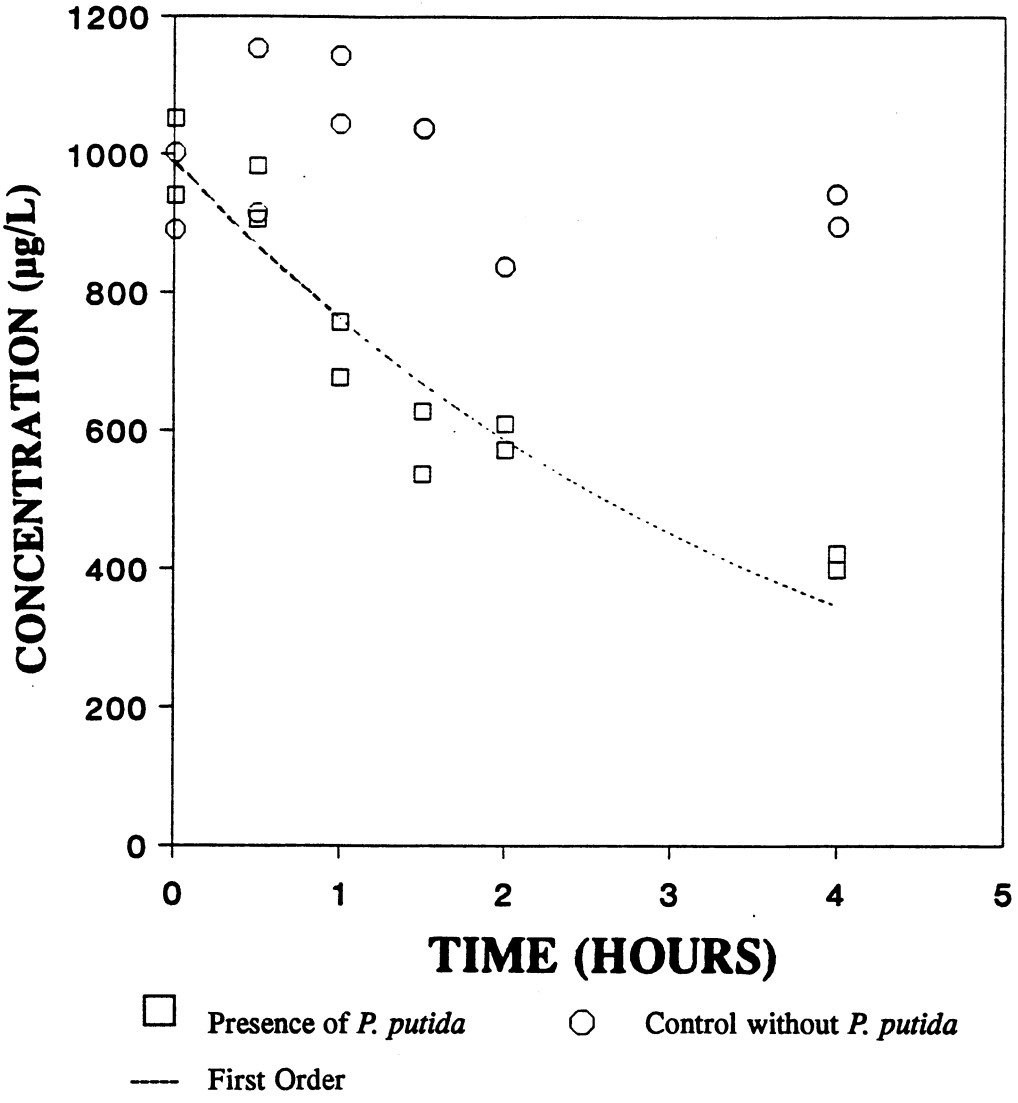


Figure 42. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Lead Concentration 2.2 mg/L, pH 7.4, Temperature 25°C)

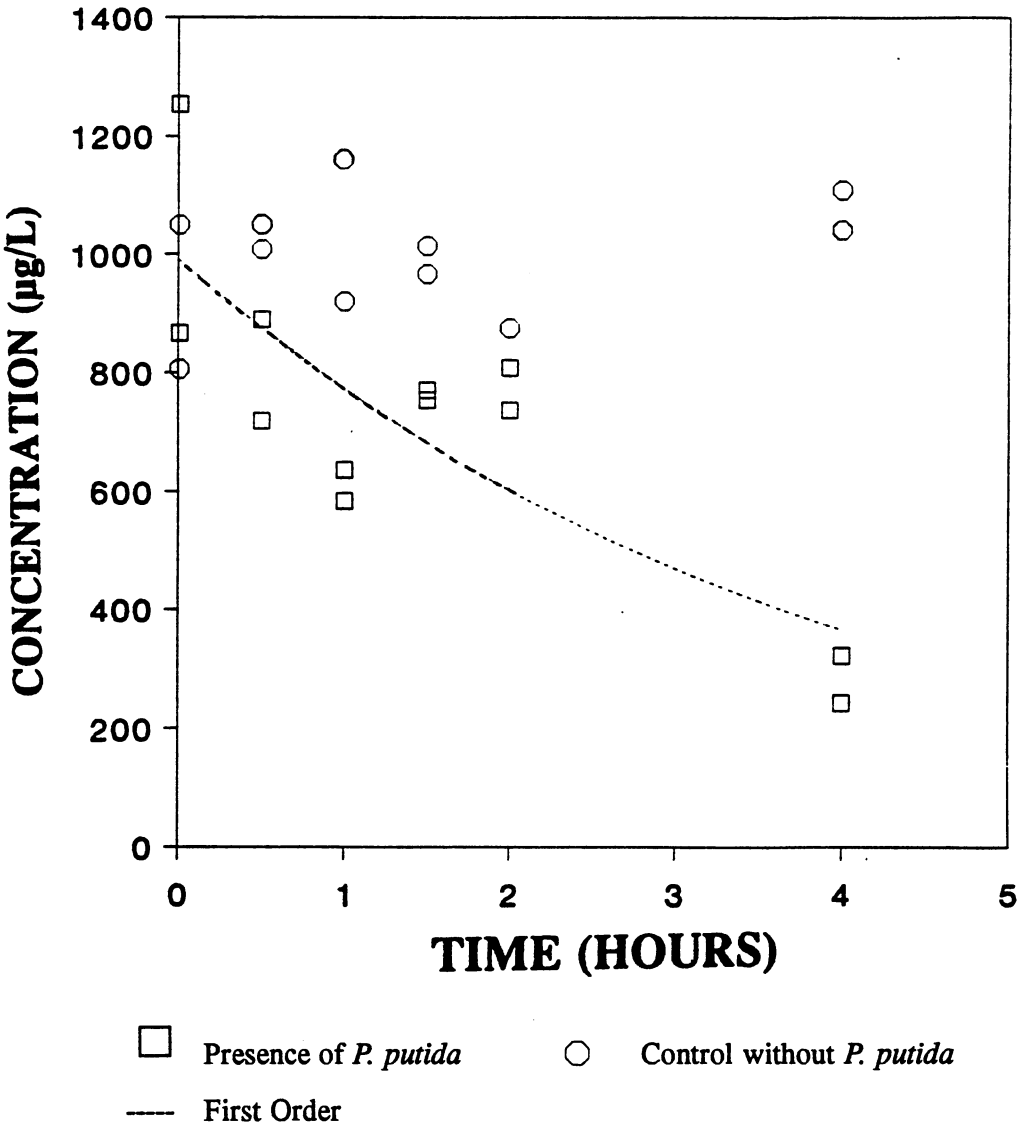


Figure 43. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Lead Concentration 5.8 mg/L, pH 7.4, Temperature 25°C)

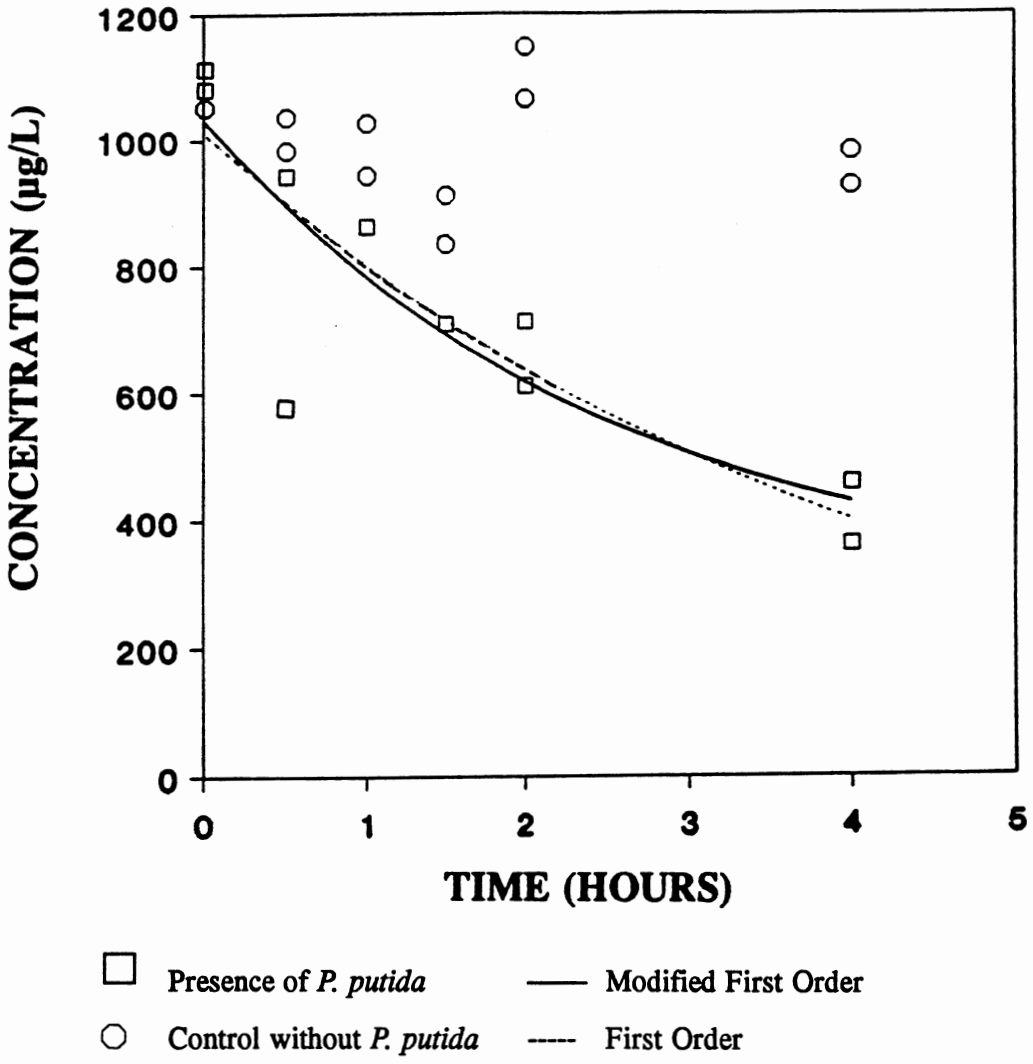


Figure 44. Removal of 1,2-dichloropropane in the Presence of Oklahoma State University Agronomy Station Aquifer Materials (Lead Concentration 10 mg/L, pH 7.4, Temperature 25°C)

The biodegradation of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 was not significantly affected by lead levels between 0.0 - 10.0 mg/L in the batch reactors.

The ability to obtain reproducible results was tested by performing a T-test on experimental data obtained on different test dates but set up under identical conditions. These experiments were identified as pH 74(25) and SUST2DF in Table V (dry weight 5.2 g/L, pH 7.4, and temperature 25°C). The concentrations of the test compound remaining at the end of the four hour experiments were not signficantly different for the two experiments (identified as pH 74(25) and SUST2DF in Table V) when compared at an alpha level of at least 0.05.

Numerical Modeling

The rates of biodegradation of low molecular weight halogenated aliphatic compounds used in this study were determined using nonlinear parameter estimation methods. The rate constants determined are used to evaluate the effects of the various environmental conditions. The overall rate of biodegradation was estimated using all experimental data collected in the reactors containing cells of *Pseudomonas putida* PpG-786 for each experiment.

Modified First Order Model

The first order model was modified as shown in equation 30 to allow for substrate production term, R, independent of the substrate concentration as follows:

$$\frac{dS}{dt} = -K_1 S + R$$

The modified first order model predicts that there is a concentration of the substrate at which a chemical is likely to persist. This occurred when the derivative of S with respect to time is equal to zero, a situation analogous to the Monod equation presented in Chapter 2. In the case of the modified first order equation, this persistence level is given by:

$$S_p = \frac{R}{K_1} \qquad (46)$$

where S_p is the substrate concentration at which persistence occurs. S_p represents an asympotically stable equilibrium position. For a given R/K_1 value, different initial substrate concentrations will tend toward the same level of persistence as shown in Figure 45. Figure 45 also indicates that if the initial substrate concentration is higher than R/K_1, then the concentration will be driven down toward the persistence level. If however, the initial substrate concentration is less than R/K_1, the tendency is for the concentration to increase to the level of persistence, S_p, under environmental conditions where persistence is observed. The level of persistence is defined as the concentration of the compound at which no change in concentration is observable with time.

The modified first order equation has the solution as shown in equation 30:

$$S = \frac{R}{K_1}(1-e^{-K_1 t}) + S_o e^{-K_1 t}$$

part 1 part 2

This indicates that the modified first order equation has a production part (part 1) and a first order part (part 2) as shown in Figures 46 and 47 for 1,2-dibromo-3-chloropropane and 1,2-dichloropropane, respectively as examples where the terms are plotted separately using selected K_1 and R values. Part 1 represents any reaction such as endogenous substrate production that act to slow down the rate of reaction as predictable from first order removal rate equation (part 2). Biosynthesis of halogenated compounds have been demonstrated in microorganisms containing haloperoxidase enzymes which are widely distributed in nature (Neidleman and Geigert, 1986).

The computer printouts of all model fits are shown in Appendix V.

Sensitivity Analysis

Sensitivity analysis was performed on the three parameter models, namely Michaelis-Menten and modified first order models. Previous studies by Robinson and Characklis (1984) indicated there are three regions over which various approximations could be made to the

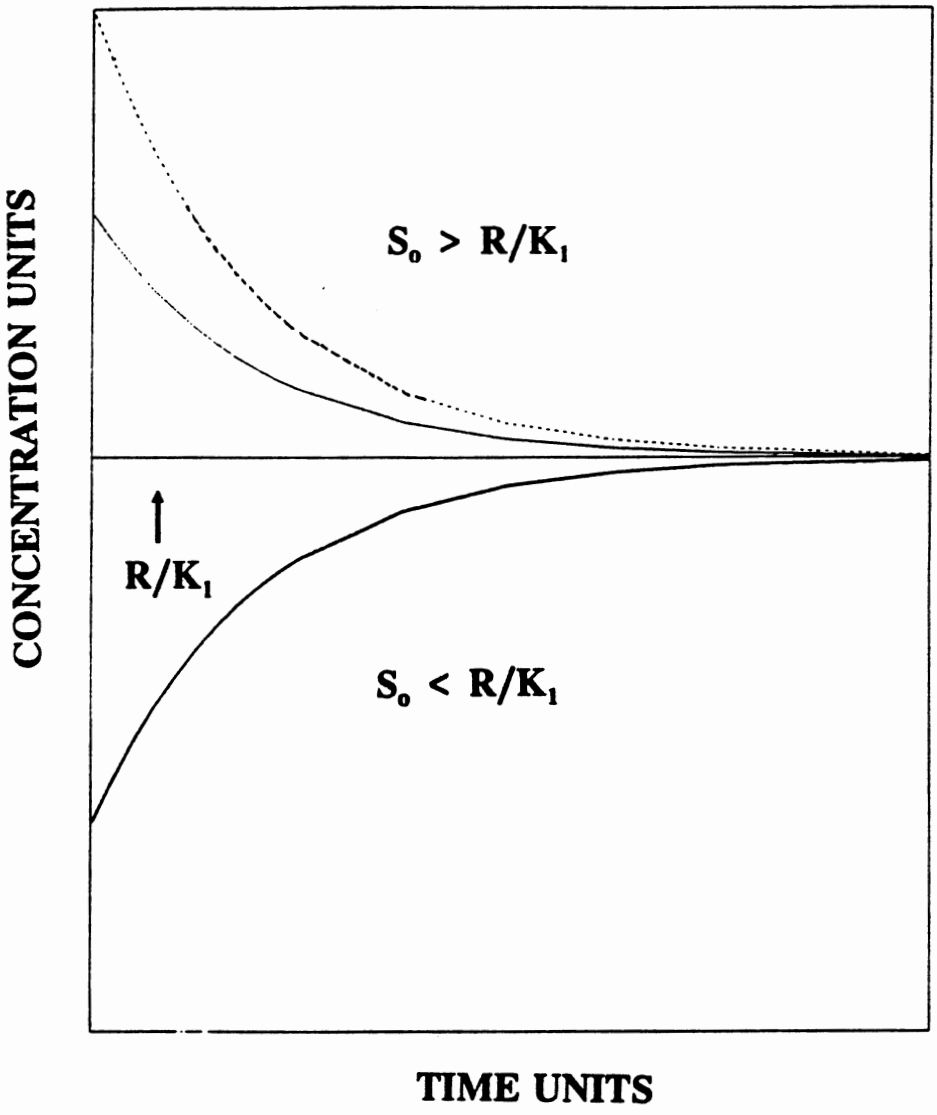


Figure 45. Hypothetical Substrate Concentration Vs. Time for the Modified First Order Model at Different initial Substrate Concentrations with Same R and K_1 values.

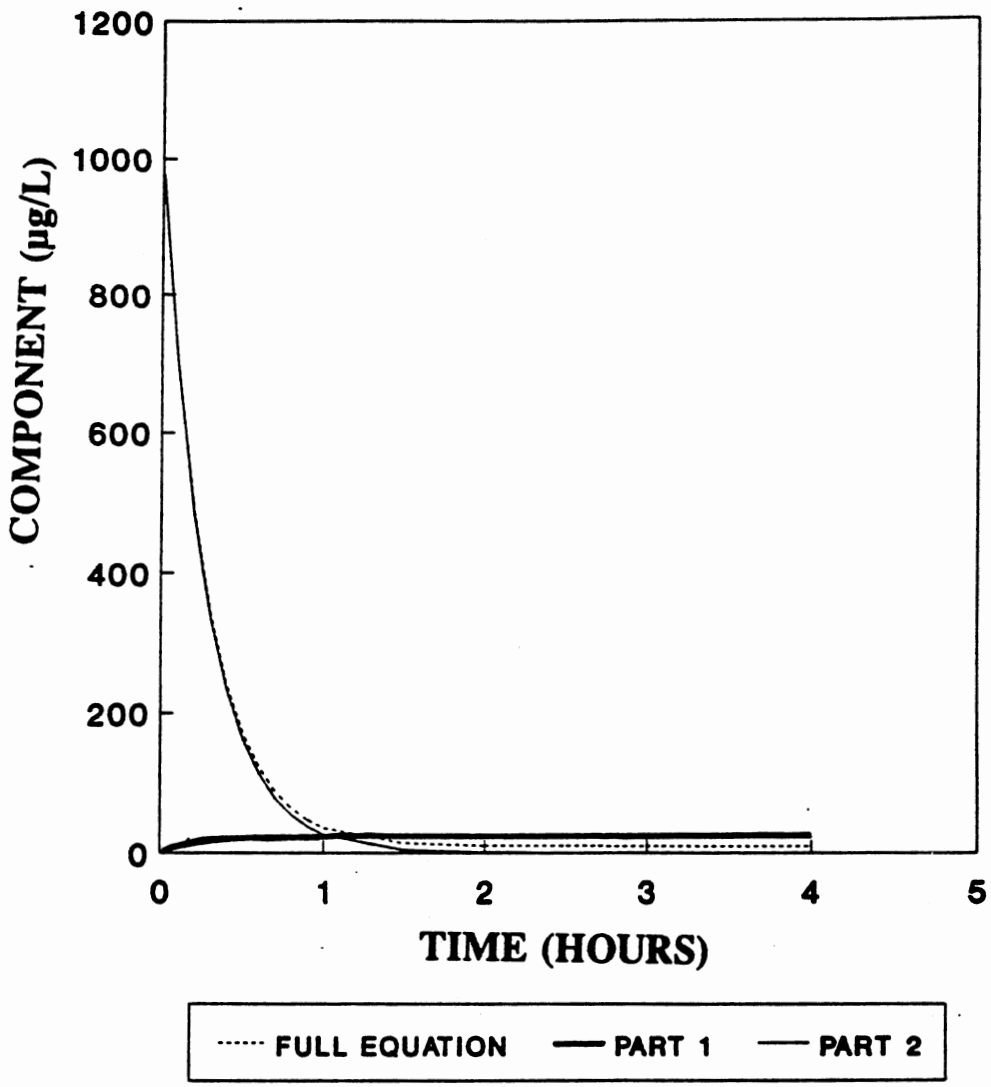


Figure 46. The Two Parts of the Modified First Order Model Applied to the Biodegradation of 1,2-dibromo-3-chloropropane (K_1 = 3.64/hr, S_o = 1005 µg/L, R = 85.31 µg/L·hr).

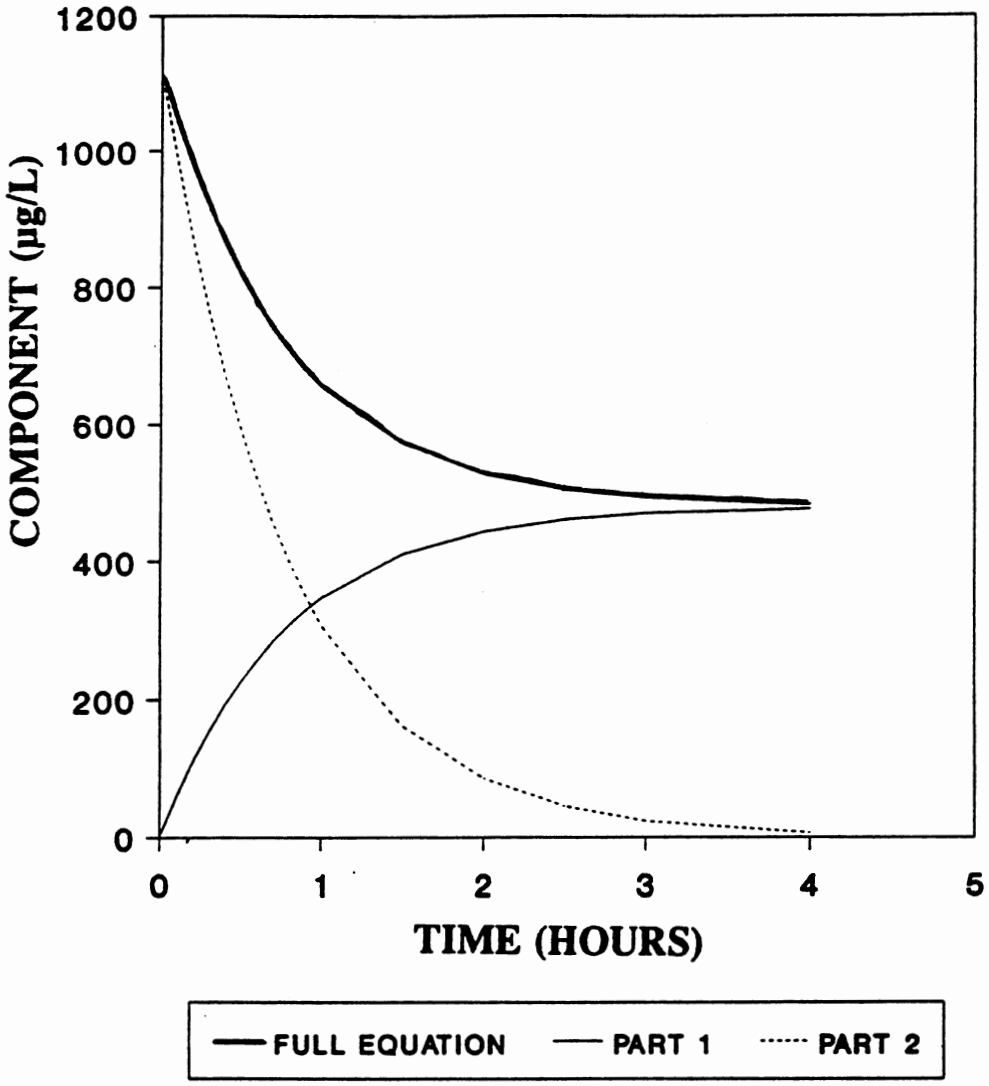


Figure 47. Modified First Order Model and Its Two Parts for Biodegradation of 1,2-dichloropropane (Initial Dissolved Oxygen of 6.0 mg/L, K_1 = 1.29/hr, S_o = 1119 µg/L, R = µg/Lhr).

Michaelis-Menten equation. The first order region occurs when S_o is less (about 10-fold) than K_m while the zero order occurs when the initial substrate is at or above saturating. The mixed order zone falls in between the zero order and first order zones. Zero order and first order regions are where the Michaelis-Menten equation can not be used because the sensitivity equations are approximately proportional while the mixed order region is where the Michaelis-Menten equations can be used. A sensitivity analysis for the Michaelis-Menten equation in the mixed order range is shown in Figure 48. This shows that the parameters in this region are not highly correlated and could be estimated using nonlinear regression techniques.

The

sensitivity analysis of the modified first order equation was performed at initial concentrations of 100 μg/L, 200 μg/L, 400 μg/L, 600 μg/L, 800 μg/L, 900 μg/L and 1005 μg/L at R of 85.47 ug/L.hr and K_1 of 3.64/hr and are shown in Figures 49 - 55. These figures illustrate the relationships between the product of the sensitivity parameters ($\partial/\partial K_1$, $\partial/\partial S_o$, and $\partial/\partial R$) and the corresponding parameter estimates for the parameters (K_1, S_o, and R) of the modified first order model. These plots allow a visualization of the relationship between the parameters of the respective model. A high relationship between R and K_1 for initial concentration of 100 - 300 ug/L indicates a simpler model, such as the two parameter first order model could be used in this region if nonlinear regression analysis is to be used for parameter estimation. At higher concentrations (about 400 μg/L), the parameters of the modified first order equation are less dependent and can be obtained using nonlinear regression techniques (Figures 52-55).

Different initial guesses were used to evaluate the effects of different starting points on the final values of the parameters for both the modified first order and Michaelis-Menten models. Three simulated data sets were prepared using known values of the parameters while simple errors, with a mean of zero and standard deviation of one, were introduced using the Monte Carlo method available in the @Risk computer program. The Michaelis-Menten equation was numerically integrated for the following parameter values: K_m = 373.29 μg/L, V_{max} = 660.27 μg/L·hr, S_o = 1218 μg/L. The modified first order equation data set was

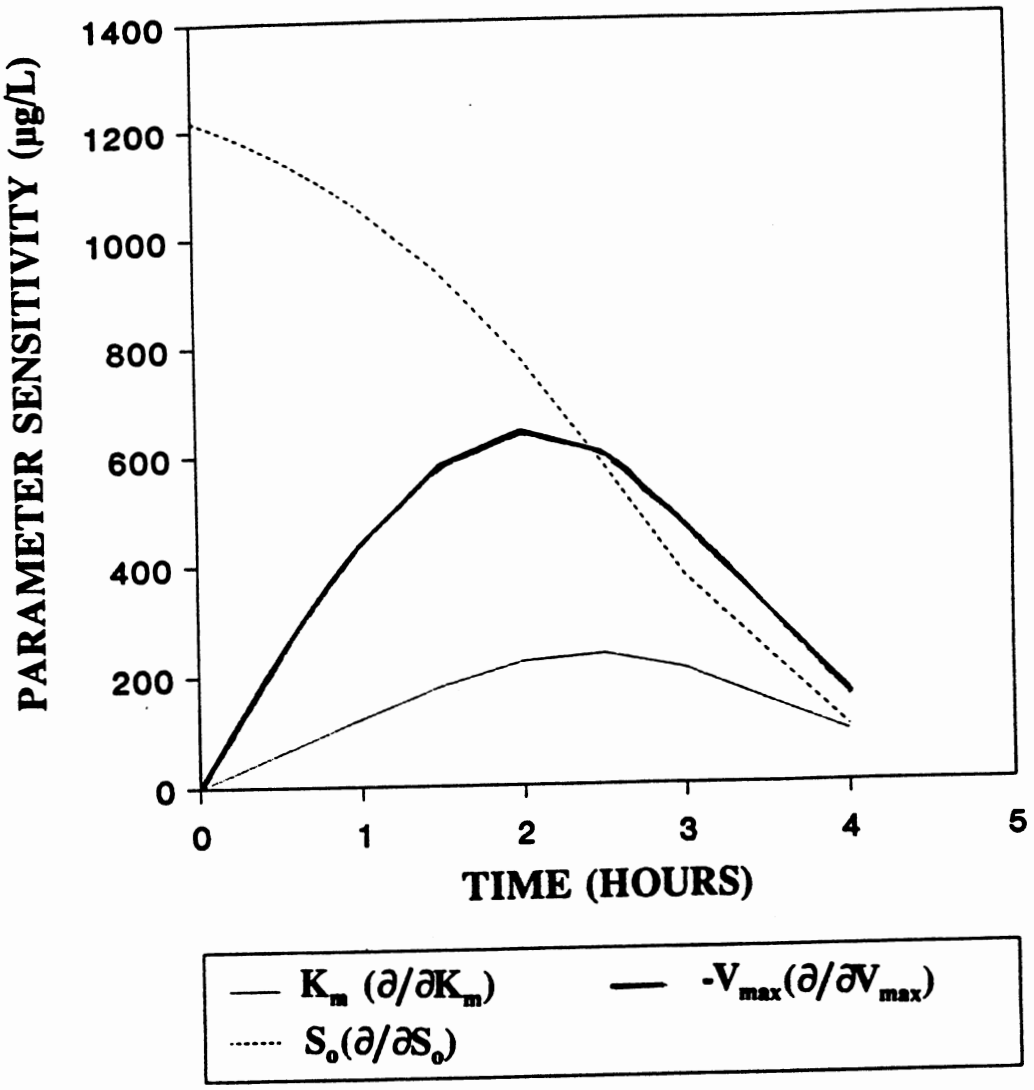


Figure 48. Sensitivity Analysis of Michaelis-Menten Model at Initial Substrate Concentration of 1,2-dichloropropane of 1218 µg/L.

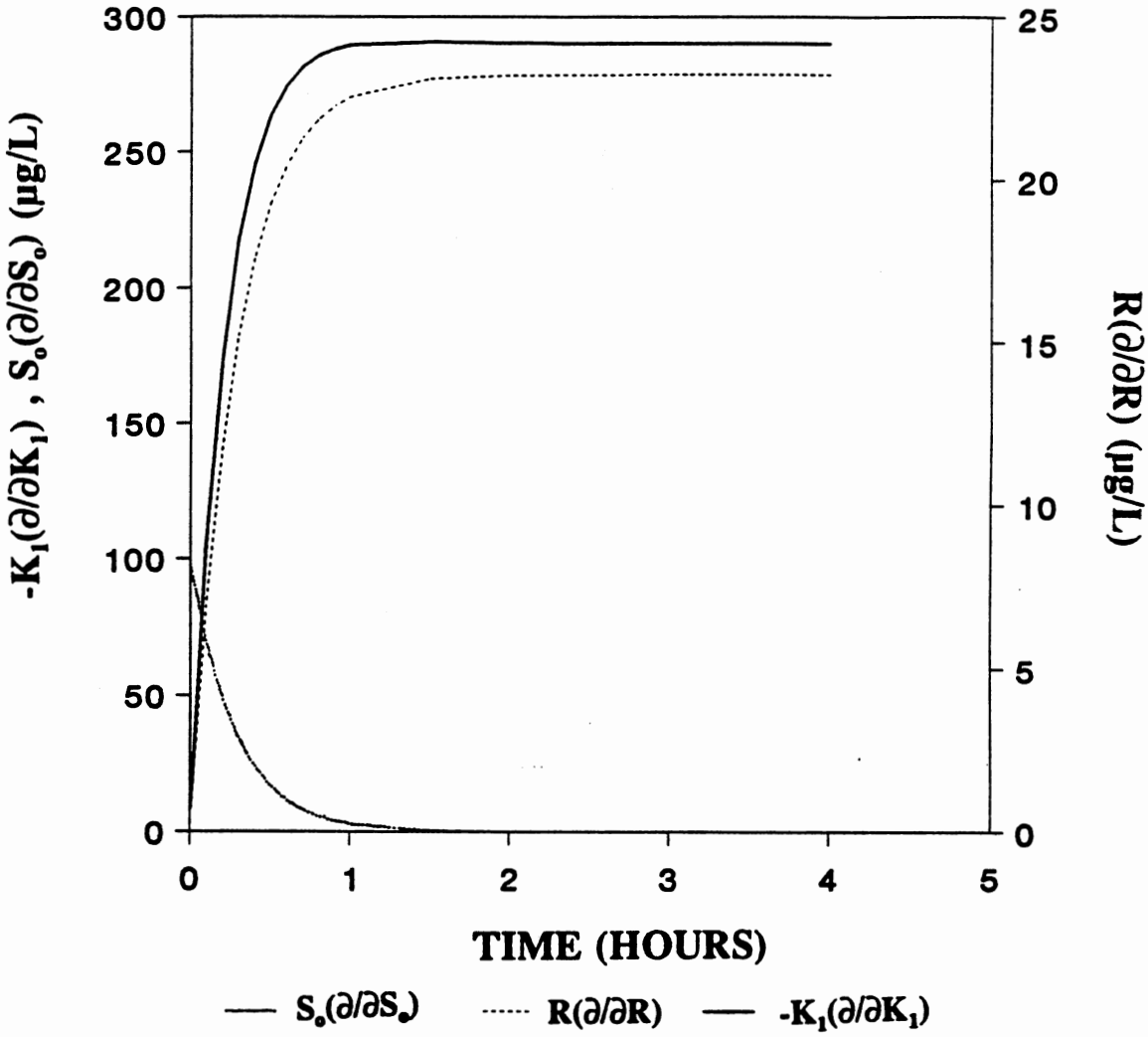


Figure 49. Sensitivity Analysis of Modified First Order Equation at Initial Substrate Concentration of 100 μg/L, K_1 of 3.64/hr and R of 85.47 μg/L·hr.

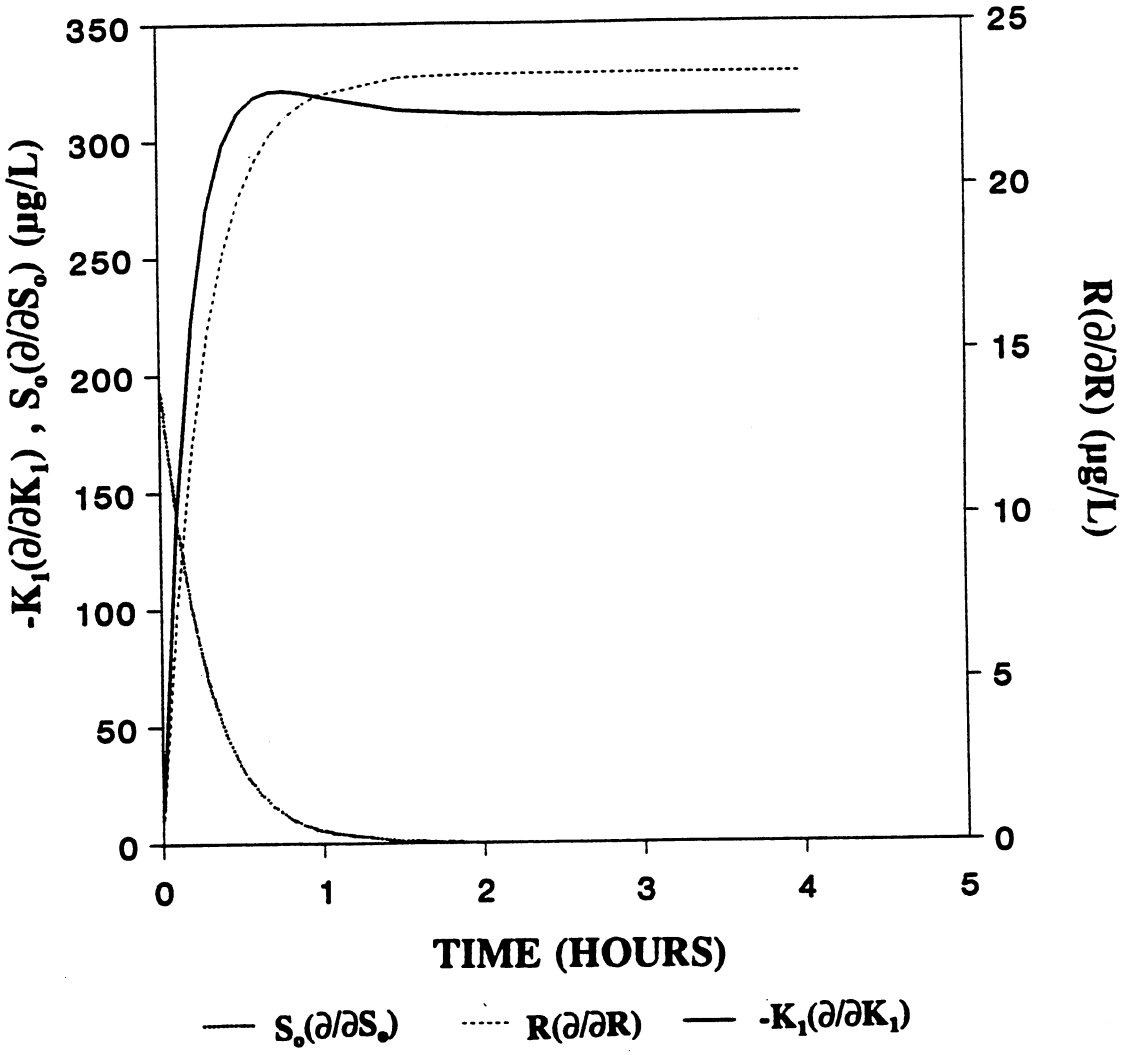


Figure 50. Sensitivity Analysis of Modified First Order Equation of Initial Substrate Concentration of 200 µg/L, K_1 of 3.64/hr and R of 85.47 µg/L·hr.

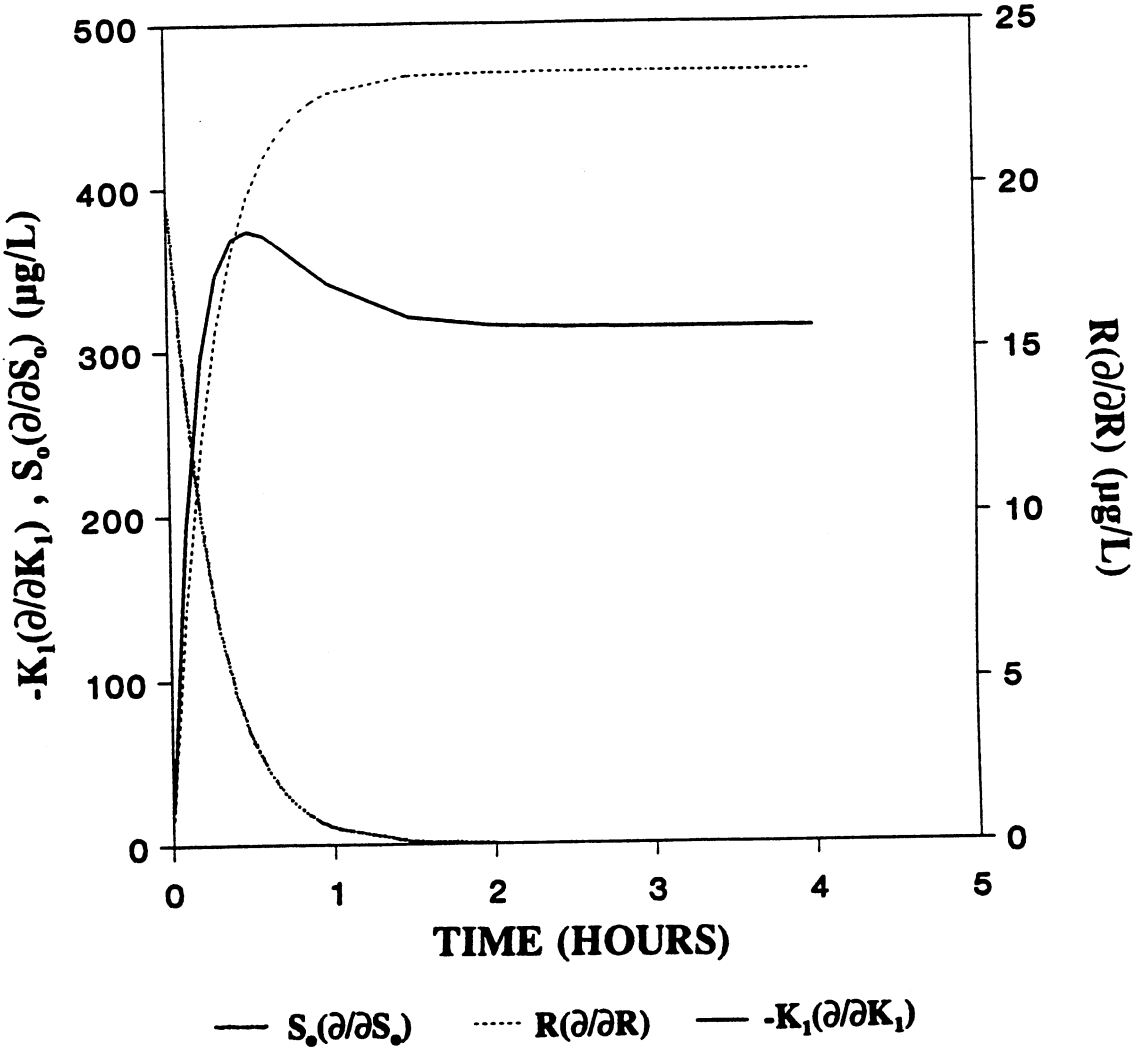


Figure 51. Sensitivity Analysis of Modified First Order Equation of Initial Substrate Concentration of 400 μg/L, K_1 of 3.64/hr and R of 85.47 μg/L·hr.

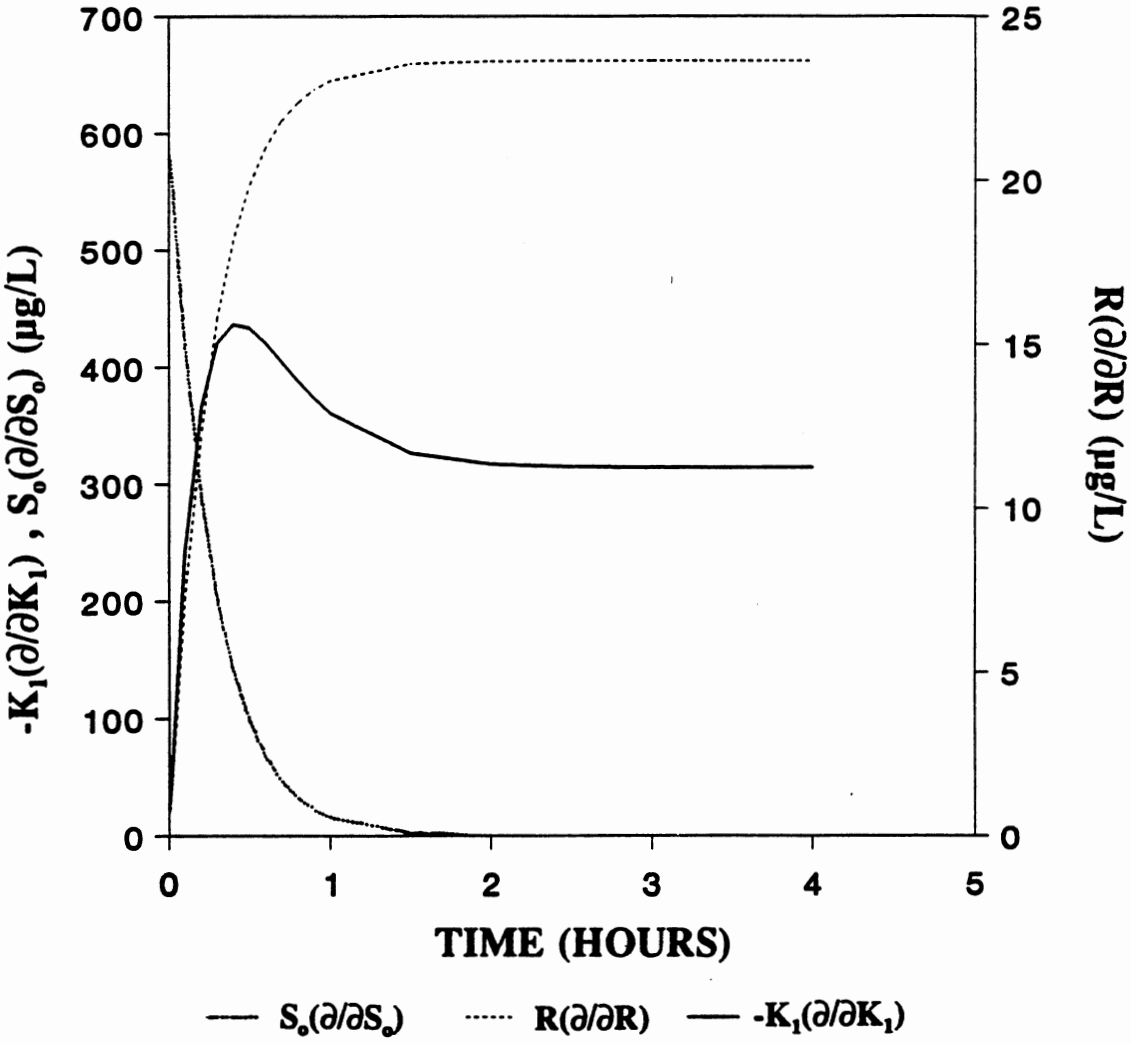


Figure 52. Sensitivity Analysis of Modified First Order Equation of Initial Substrate Concentration of 600 μg/L, K_1 of 3.64/hr, and R of 85.47 μg/L·hr.

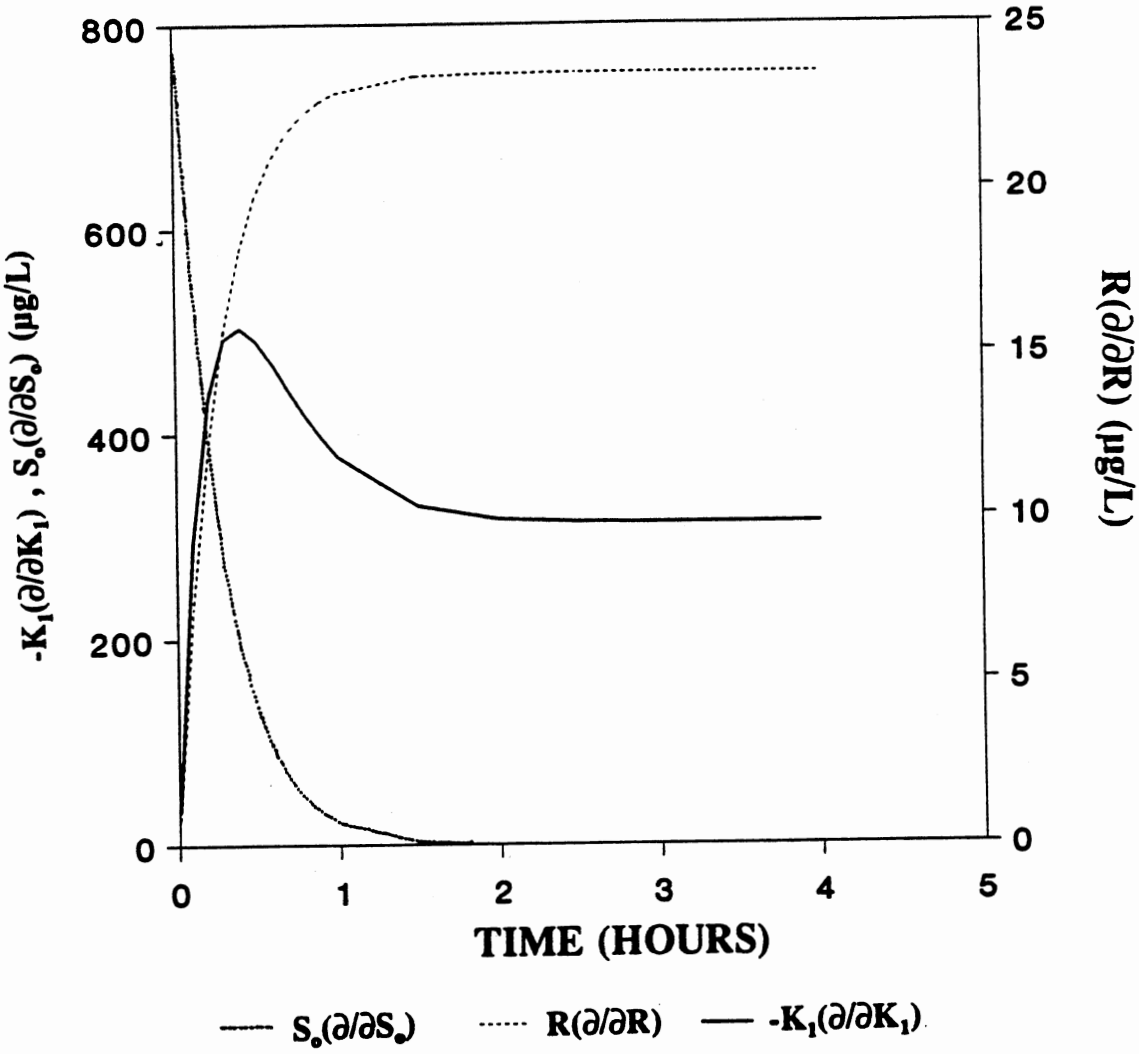


Figure 53. Sensitivity Analysis of Modified First Order Equation of Initial Substrate Concentration of 800 μg/L, K_1 of 3.64/hr, and R of 85.47 μg/L·hr.

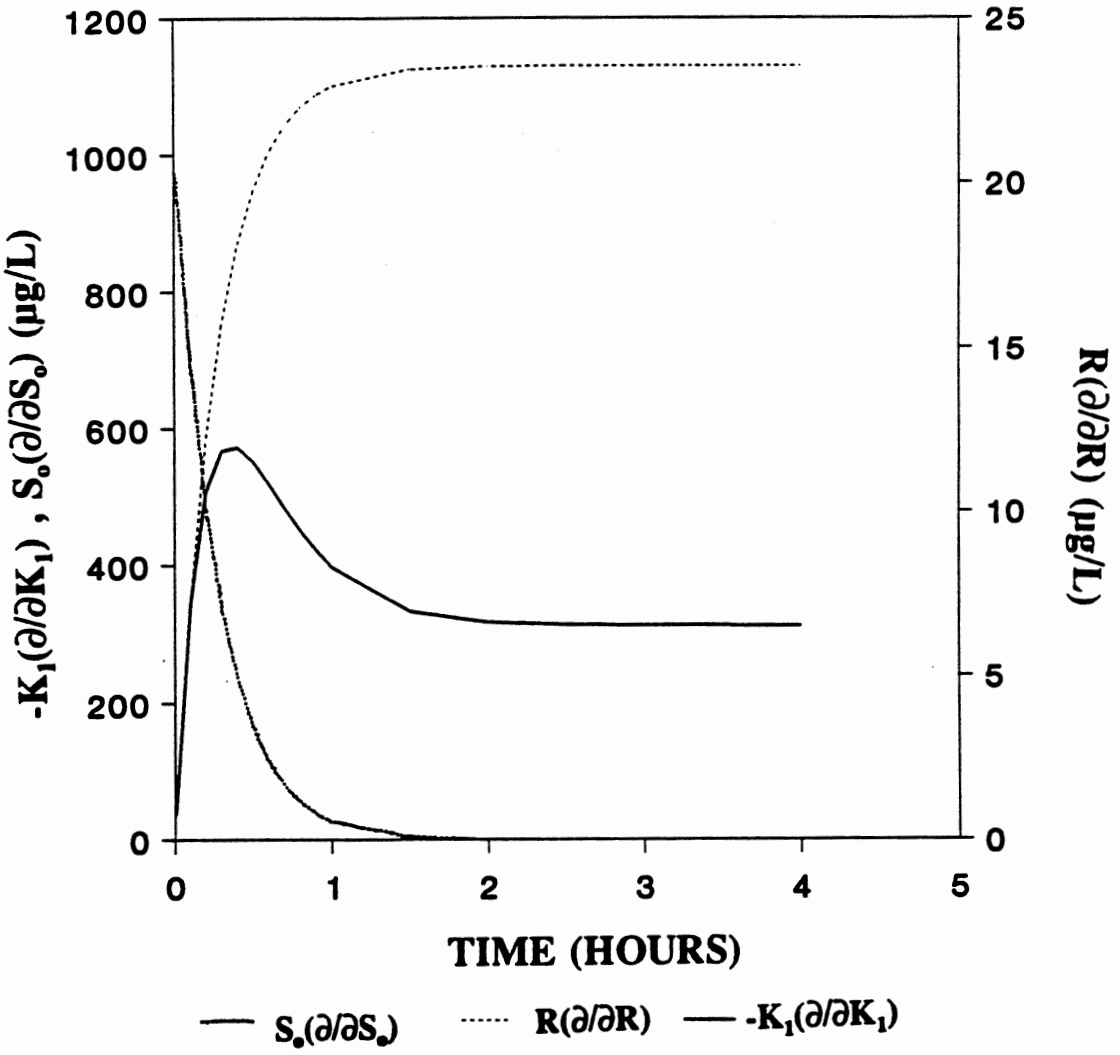


Figure 54. Sensitivity Analysis of Modified First Order Equation of Initial Substrate Concentration of 900 µg/L, K_1 of 3.64/hr, and R of 85.47 µg/L·hr.

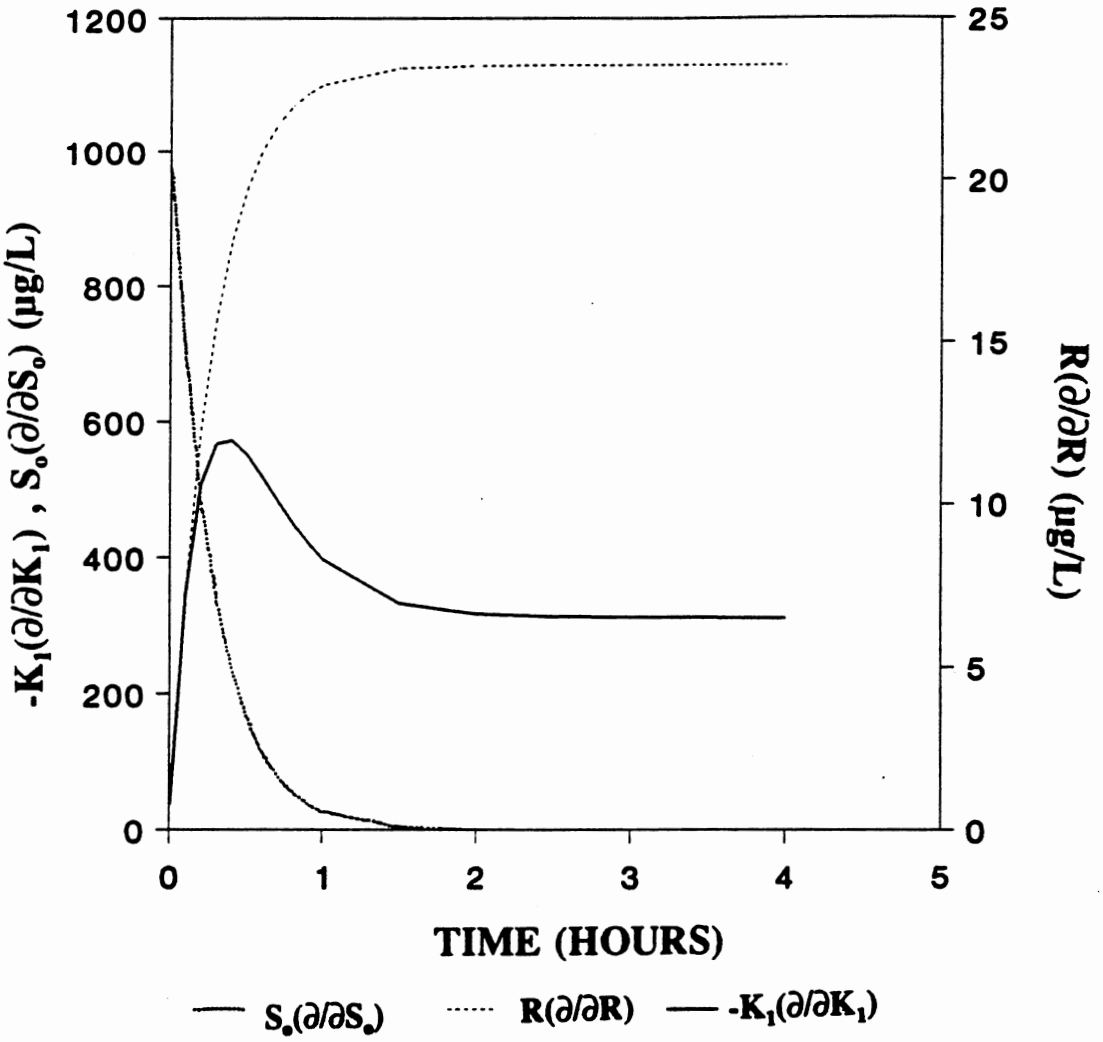


Figure 55. Sensitivity Analysis of Modified First Order Equation of Initial Substrate Concentration of 1005 µg/L, K_1 of 3.64/hr, and R of 85.47 µg/L·hr.

generated using K_1 = 3.64/hr, S_o = 1005 μg/L, and R = 85.47 μg/L·hr. The relative errors of both the initial and final estimates of the parameters are shown in Tables XIV and XV. Provided convergence occurred, the parameters were well estimated for a large range of initial guesses. The ratio of residual sums-of-squares of the initial guesses to the final parameter estimates indicated 10^6 reduction in error from the initial guess to the final guess.

Effect of Aquifer and Chemical Types

The rate of biodegradation is dependent on the individual chemicals and independent of the type of aquifer materials. Tables XVI and XVII showed the biodegradation of 1,2-dibromo-3-chloropropane and 1,2-dichloropropane. The effect of the presence of aquifer materials on the biodegradation of 1,2-dibromo-3-chloropropane is shown in Table XVI. The addition of three tenths of one gram of aquifer materials from the Oklahoma State Agronomy Research Station to the reactor resulted in the reduction of the first order rate constant by 29%. The results also indicated that the modified first order model provided a significantly better fit (compared using equation 44) to the data obtained in the absence of aquifer materials when compared to the first order model (Table XVI) when compared at alpha of at least 0.05. Variation in rate of degradation in the presence of different types of aquifer materials was investigated using 1,2-dichloropropane. The first order rate constant for aquifer materials obtained from the Oklahoma State University Agronomy Research Station was 1.3 times higher than those obtained from the Sand Springs Petrochemical Complex. A statistical test on the concentration of 1,2-dichloropropane remaining at the end of the experiments in reactors containing subsurface materials from the Sand Springs Petrochemical Complex were, however, not significantly different from those with the Oklahoma State University Agronomy Station. Although the modified first order equation provided a significantly better fit than the first order equation (alpha = 0.05) for biodegradation of 1,2-dichloropropane in the presence of Oklahoma State University Agronomy Research Station Aquifer materials, the first order

TABLE XIV

ERRORS IN PARAMETER ESTIMATE USING DIFFERENT INITIAL GUESSES FOR THE MODIFIED FIRST ORDER MODEL

data[a] set #	% error in: initial estimates K_1	S_o	R	final estimates K_1	S_o	R	RSS_i/RSS_f[b]
1	0.0	0.0	-900.0	0.0	0.0	-1.6	356
1	0.0	0.0	90.0	0.0	0.0	-1.6	36839
1	0.0	0.0	-11.1	0.0	0.0	-1.6	4
1	0.0	0.0	9.1	0.0	0.0	-1.6	7
1	0.0	0.0	-25.0	0.0	0.0	-1.6	16
1	0.0	0.0	16.7	0.0	0.0	-1.6	21
1	-11.1	0.0	0.0	0.0	0.0	-1.6	629
1	9.1	0.0	0.0	0.0	0.0	-1.6	452
1	-25.0	0.0	0.0	0.0	0.0	-1.6	2941
1	16.7	0.0	0.0	0.0	0.0	-1.6	1597
1	-42.9	0.0	0.0	0.0	0.0	-1.6	7893
1	23.1	0.0	0.0	0.0	0.0	-1.6	3178
1	-66.7	0.0	0.0	0.0	0.0	-1.6	17055
1	28.6	0.0	0.0	0.0	0.0	-1.6	5044
1	0.0	-900.0	0.0	0.0	0.0	-1.6	109953
1	0.0	90.0	0.0	0.0	0.0	-1.6	11003499
1	0.0	-11.1	0.0	0.0	0.0	-1.6	1351
1	0.0	9.1	0.0	0.0	0.0	-1.6	1367
1	0.0	-25.0	0.0	0.0	0.0	-1.6	5417
1	0.0	16.7	0.0	0.0	0.0	-1.6	5451
1	0.0	0.0	0.0	0.0	0.0	-1.6	1
2	0.0	0.0	-900.0	-0.1	0.0	0.1	351
2	0.0	0.0	90.0	-0.1	0.0	0.1	34318
2	0.0	0.0	-11.1	-0.1	0.0	0.1	6
2	0.0	0.0	9.1	-0.1	0.0	0.1	4
2	0.0	0.0	-25.0	-0.1	0.0	0.1	19
2	0.0	0.0	16.7	-0.1	0.0	0.1	16
2	-11.1	0.0	0.0	-0.1	0.0	0.1	568
2	9.1	0.0	0.0	-0.1	0.0	0.1	441
2	-25.0	0.0	0.0	-0.1	0.0	0.1	2707
2	16.7	0.0	0.0	-0.1	0.0	0.1	1529
2	-42.9	0.0	0.0	-0.1	0.0	0.1	7313
2	23.1	0.0	0.0	-0.1	0.0	0.1	3023
2	-66.7	0.0	0.0	-0.1	0.0	0.1	15854
2	28.6	0.0	0.0	-0.1	0.0	0.1	4781
2	0.0	-900.0	0.0	-0.1	0.0	0.1	103083
2	0.0	90.0	0.0	-0.1	0.0	0.1	10302406
2	0.0	-11.1	0.0	-0.1	0.0	0.1	1278
2	0.0	9.1	0.0	-0.1	0.0	0.1	1267

TABLE XIV (Continued)

data[a] set #	% error in: initial estimates K_1	S_o	R	final estimates K_1	S_o	R	RSS_i/RSS_f[b]
2	0.0	-25.0	0.0	-0.1	0.0	0.1	5100
2	0.0	16.7	0.0	-0.1	0.0	0.1	5077
2	0.0	0.0	0.0	-0.1	0.0	0.1	1
3	16.7	0.0	0.0	0.1	0.1	-0.6	1739
3	0.0	0.0	-900.0	0.1	0.1	-0.6	391
3	0.0	0.0	-11.1	0.1	0.1	-0.6	5
3	0.0	0.0	9.1	0.1	0.1	-0.7	6
3	0.0	0.0	-25.0	0.1	0.1	-0.6	19
3	-11.1	0.0	0.0	0.1	0.1	-0.7	665
3	9.1	0.0	0.0	0.1	0.1	-0.6	497
3	-25.0	0.0	0.0	0.1	0.1	-0.7	3139
3	-42.9	0.0	0.0	0.1	0.1	-0.7	8451
3	23.1	0.0	0.0	0.1	0.1	-0.6	3450
3	-66.7	0.0	0.0	0.1	0.1	-0.7	18289
3	28.6	0.0	0.0	0.1	0.1	-0.7	5465
3	-0.1	-900.0	0.0	0.1	0.1	-0.7	118579
3	0.0	90.0	0.0	0.1	0.1	-0.6	11837443
3	0.0	-11.1	0.0	0.1	0.1	-0.6	1484
3	0.0	9.1	0.0	0.1	0.1	-0.7	1441
3	0.0	-25.0	0.0	0.1	0.1	-0.7	5891
3	0.0	16.7	0.0	0.1	0.1	-0.7	5804
3	0.0	0.0	0.0	0.1	0.1	-0.7	1

[a]Simulations contain simple errors with mean of zero and standard deviation of one. True values of the parameter for all simulations were K_1 = 3.64/hr, S_o = 1005 μg/L, and R = 85.47 μg/L·hr.

[b]Ratio of residual sums-of-squares for initial parameter estimates to sums-of-squares for the final parameter estimates.

TABLE XV

ERROR IN PARAMETER ESTIMATE USING DIFFERENT INITIAL GUESSES FOR THE MICHAELIS-MENTEN EQUATION

data set[a] #	% error in: initial estimates K_m	V_{max}	S_o	final estimates K_m	V_{max}	S_o	RSS_i/RSS_f[b]
1	0.46	0.86	0.16	0.60	0.32	0.05	5
1	37.91	0.86	0.16	0.60	0.32	0.05	6481
1	61.45	0.86	0.16	0.60	0.32	0.05	31997
1	-186.80	0.86	0.16	0.60	0.32	0.05	14727
1	0.46	37.91	0.16	0.60	0.32	0.05	45925
1	0.46	61.45	0.16	0.60	0.32	0.05	186633
1	0.46	-69.35	0.16	0.60	0.32	0.05	55532
1	0.00	-109.08	0.16	0.59	0.32	0.05	100977
2	0.46	0.86	0.16	0.60	0.32	0.05	5
2	0.46	0.86	61.45	0.60	0.32	0.05	4822532
2	0.46	0.86	-69.35	0.60	0.32	0.05	242829
2	0.46	0.86	-88.17	0.60	0.32	0.05	311724
2	0.46	0.86	-109.08	0.60	0.32	0.05	379291
2	-158.12	0.86	0.16	0.58	0.31	0.05	12818
2	0.46	-88.17	0.16	0.60	0.32	0.05	77335
2	0.46	-0.04	-52.42	0.60	0.32	0.05	173078
2	-158.12	0.86	0.16	-0.28	-0.05	0.04	9211
2	0.46	-88.17	0.16	-0.27	-0.05	0.04	55272
2	0.46	0.86	31.70	-0.26	-0.05	0.04	260695
2	0.46	0.86	57.59	-0.26	-0.05	0.04	2462782
3	0.46	0.86	0.16	-1.98	-0.86	-0.04	5
3	37.91	0.86	0.16	-1.98	-0.86	-0.04	3430
3	61.45	0.86	0.16	-1.98	-0.86	-0.04	17066
3	-158.12	0.86	0.16	-1.99	-0.86	-0.04	6954
3	0.46	38.20	0.16	-1.98	-0.86	-0.04	25300
3	0.46	61.45	0.16	-1.98	-0.86	-0.04	100479
3	0.46	-69.35	0.16	-1.98	-0.86	-0.04	29656
3	0.46	-88.17	0.16	-1.98	-0.86	-0.04	41332
3	0.46	0.86	37.91	-1.98	-0.86	-0.04	345490
3	0.46	0.86	57.59	-1.98	-0.86	-0.04	1846395
3	0.46	0.86	-69.35	-1.98	-0.86	-0.04	130433
3	0.46	0.86	-88.17	-1.98	-0.86	-0.04	167415
3	0.46	0.86	-109.08	-1.98	-0.86	-0.04	203683

[a]Data set contains triplicate simulated data points with simple errors actual values: K_m = 373.29 μg/L; V_{max} = 660.27 μg/L·hr; S_o = 1218 μg/L.
The simple errors have a constant standard deviation of one and mean of zero.
[b]RSS_i/RSS_f ratio of initial residual sums-of-squares to final residual sums-of-squares.

TABLE XVI

EVALUATION OF THE EFFECT OF *PRESENCE OF AQUIFER MATERIALS* ON THE REMOVAL OF 1,2-DIBROMO-3-CHLOROPROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 AT 25°C

ID NUMBER	EXPERIMENTAL CONDITION	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	R (μg/L·hr)	STD* (μg/L·hr)	F	N_p
First Order Fit									
DBCPDF	NONE	3.34	0.14	1003	14	NA	NA	1941.01	18
DBCPDF2	OSU	2.37	0.27	979	46	NA	NA	177.73	18
Modified First Order Fit									
DBCPDF	NONE	3.64	0.12	1005	9	85.31	49.54	3194.09	18

*Asymptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right)N^{-1}}$

F = (CSS-RSS)/RSS(N_p-p)/p
CSS = Corrected sums-of-squares
RSS = Residual sums-of-squares
N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.
N_p = Number of data points
p = Number of parameters
NA - Not applicable
OSU - Oklahoma State University Agronomy Research Station
$F_{0.05,2,16} = 3.63$; $F_{0.05,3,16} = 3.24$

TABLE XVII

EVALUATION OF THE EFFECT OF *AQUIFER MATERIAL TYPE* ON PARAMETER ESTIMATES USING *FIRST ORDER AND MODIFIED FIRST ORDER* KINETIC FITS ON THE BIODEGRADATION OF 1,2-DICHLOROPROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 AT 25°C

ID NUMBER	EXPERIMENTAL CONDITION	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	R (μg/L·hr)	STD* (μg/L·hr)	F	N_p
First Order Fit									
AQUIFDF	OSU	0.50	0.09	1165	85	NA	NA	26.54	15
AQUIFDF2	SS	0.38	0.02	1173	25	NA	NA	273.60	14
Modified First Order Fit									
AQUIFDF	OSU	1.51	0.04	1300	77	550.51	384.75	32.97	15

*Asymptoptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right) N^{-1}}$

$F = (CSS-RSS)/RSS(N_p-p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

OSU - Oklahoma State University Agronomy Research Station

SS - Sand Springs Petrochemical Complex

NA - Not applicable

$F_{0.05,2,13} = 3.81$; $F_{0.05,2,12} = 3.89$; $F_{0.05,3,15} = 3.29$

model was selected because the S_o was closer to the initial substrate concentration used in the study.

1,2-dibromo-3-chloropropane was biodegraded faster than 1,2-dichloropropane. 1,2-dichloroethane was not biodegraded during the incubation period by *Pseudomonas putida* PpG-786.

Effect of pH

The effect of pH on the biodegradation of 1,2-dichloropropane was investigated and the result is displayed in Table XVIII and Figure 56. A slight decrease in first order rate constant (15.5%) was observed when the pH was varied from 5.4 to 8.9. The range of pH selected reflected the pH that was observed earlier for the different sites. Further statistical tests using split time analysis were conducted on the data obtained in this experiment to determine if the rate constants obtained from linearizing first order fits to the data are significantly different. The result indicated that the rate constants are not significantly different over the pH range tested (Appendix V).

Effect of Temperature at Different pHs

The effects of temperature at different pH values were investigated under the experimental conditions outlined in Appendix F and Table V. The first order rate constants for the experiments to determine the effect of temperature at 15°C, 25°C and 30°C are shown in Tables XIX, XX, and XXI and Figure 57. For the three pH values tested, the highest rate of reaction occurred at 25°C. This is the temperature under which the microorganism was cultured prior to the experiments.

Effect of Dissolved Oxygen

The result of the effect of the dissolved oxygen on the biodegradation rate of 1,2-dichloropropane is shown in Table XXIV and Figure 58. The rate of reaction was increased 2.0

TABLE XVIII

EVALUATION OF THE EFFECT OF *pH* ON PARAMETER ESTIMATES USING *FIRST ORDER* KINETIC FITS ON THE BIODEGRADATION OF 1,2-DICHLORO-PROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 AT 25°C

ID Number	pH	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	F	N_P
pH54(25)	5.4	0.52	0.04	1036	32	198.67	18
pH74(25)	7.4	0.50	0.06	957	50	68.00	18
pH89(25)	8.9	0.44	0.04	956	41	89.34	18

*Asymptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right) N^{-1}}$

$F = (CSS-RSS)/RSS(N_p-p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

$F_{0.05,2,16} = 3.63$.

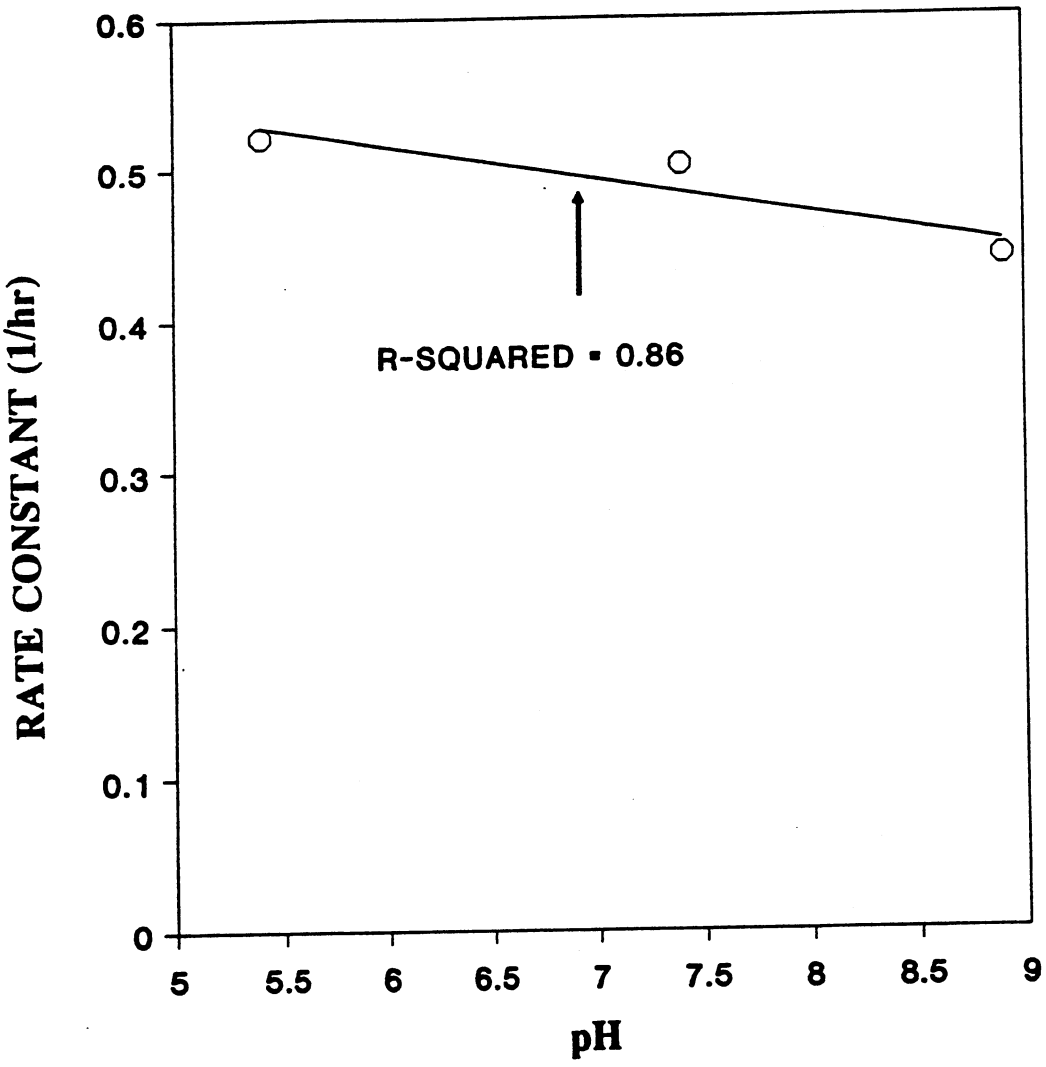


Figure 56. Variation of First Order Rate Constant with pH at 25°C Determined for Biodegradation of 1,2-dichloropropane by *Pseudomonas putida* PpG-786.

TABLE XIX

EVALUATION OF THE EFFECT OF *TEMPERATURE* ON PARAMETER ESTIMATES USING *FIRST ORDER KINETIC* FITS ON THE BIODEGRADATION OF 1,2-DICHLOROPROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 (*pH=6.4*)

ID Number	pH	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	F	N_p
pH64(15)	6.4	0.17	0.03	1078	43	26.65	16
pH64(25)	6.4	0.25	0.04	1184	63	19.40	18
pH64(30)	6.4	0.17	0.03	1097	55	13.90	18

*Asymptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right)} N^{-1}$

$F = (CSS-RSS)/RSS(N_p-p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

$F_{0.05,2,14} = 3.74$, $F_{0.05,2,16} = 3.63$.

TABLE XX

EVALUATION OF THE EFFECT OF *TEMPERATURE* ON PARAMETER ESTIMATES USING *FIRST ORDER KINETIC* FITS ON THE BIODEGRADATION OF 1,2-DICHLOROPROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 *(pH=7.4)*

ID Number	pH	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	F	N_p
pH74(15)	7.4	0.06	0.02	1062	43	26.65	16
pH74/25	7.4	0.17	0.02	1115	36	30.04	18
pH74(30)	7.4	0.13	0.03	1181	52	12.30	18

*Asymptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right)N^{-1}}$

$F = (CSS-RSS)/RSS(N_p-p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

$F_{0.05,2,14} = 3.74$; $F_{0.05,2,16} = 3.63$.

TABLE XXI

EVALUATION OF THE EFFECT OF *TEMPERATURE* ON PARAMETER ESTIMATES USING *FIRST ORDER KINETIC* FITS ON THE BIODEGRADATION OF 1,2-DICHLOROPROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 (*pH=7.8*)

ID Number	pH	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	F	N_p
pH7815c	7.8	0.11	0.03	1309	75	6.34	17
pH7825c	7.8	0.33	0.06	1284	82	11.71	15
pH7830c	7.8	0.20	0.03	1196	61	19.79	17

*Asymptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right)} N^{-1}$

$F = (CSS-RSS)/RSS(N_p-p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

$F_{0.05,3,15} = 3.68$; $F_{0.05,1,13} = 3.81$.

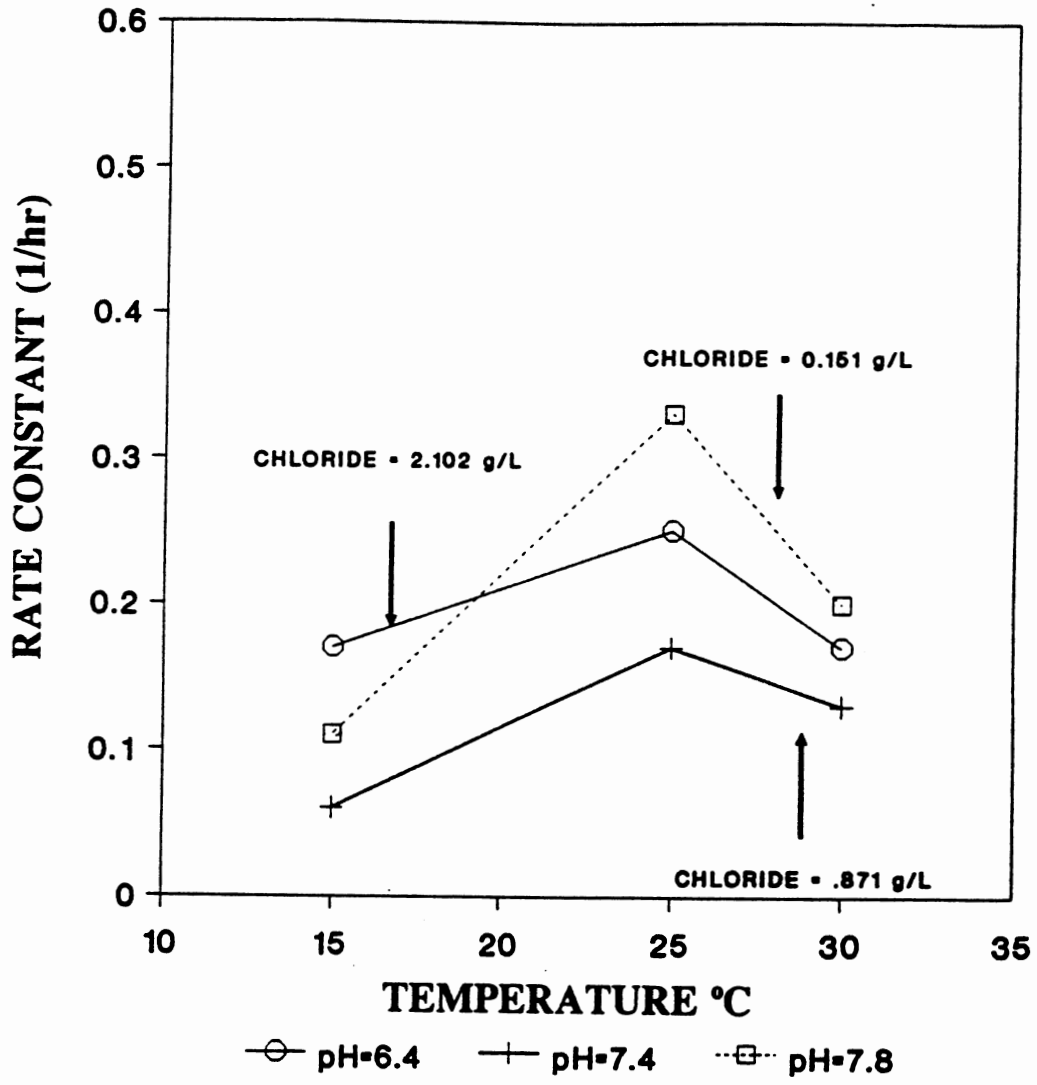


Figure 57. Effect of Temperature at Different pH on First Order Rate Constant for the Biodegradation of 1,2-dichloropropane by *Pseudomonas putida* Ppg-786.

TABLE XXIV

EVALUATION OF THE EFFECT OF *DISSOLVED OXYGEN* ON PARAMETER ESTIMATES USING *FIRST ORDER* AND *MODIFIED FIRST ORDER* KINETIC FITS ON THE BIODEGRADATION OF 1,2-DICHLORO-PROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 AT 25°C

ID NUMBER	DO (mg/L)	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	R (μg/L·hr)	STD* (μg/L·hr)	k_M (μg/L)	STD* (μg/L)	V_{max} (μg/L·hr)	STD* (μg/L·hr)	F	N_p
First Order Fits													
DOCOM3	16.0	0.41	0.04	1014	38	NA	NA	NA	NA	NA	NA	95.65	12
DOCOM4	8.2	0.20	0.02	1139	32	NA	NA	NA	NA	NA	NA	68.24	12
DOCOM5	6.0	0.25	0.04	987	56	NA	NA	NA	NA	NA	NA	22.98	12
Modified First Order Fits													
DOCOM5	6.0	1.29	0.19	1119	32	618.99	174.74	NA	NA	NA	NA	97.74	12
Michaelis-Menten Fit													
DOCOM4	8.2	1.44**	NA	1113	34	NA	NA	133.17	627.92	191.16	137.77	52.18	12

*Asymptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right) N^{-1}}$

**$K_1 = U_{max}(X_o/K_m)$

$F = (CSS\text{-}RSS)/RSS(N_p\text{-}p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

NA - Not applicable

DO - Initial dissolved oxygen level

$F_{0.05,2,10} = 4.10$; $F_{0.05,3,10} = 3.71$

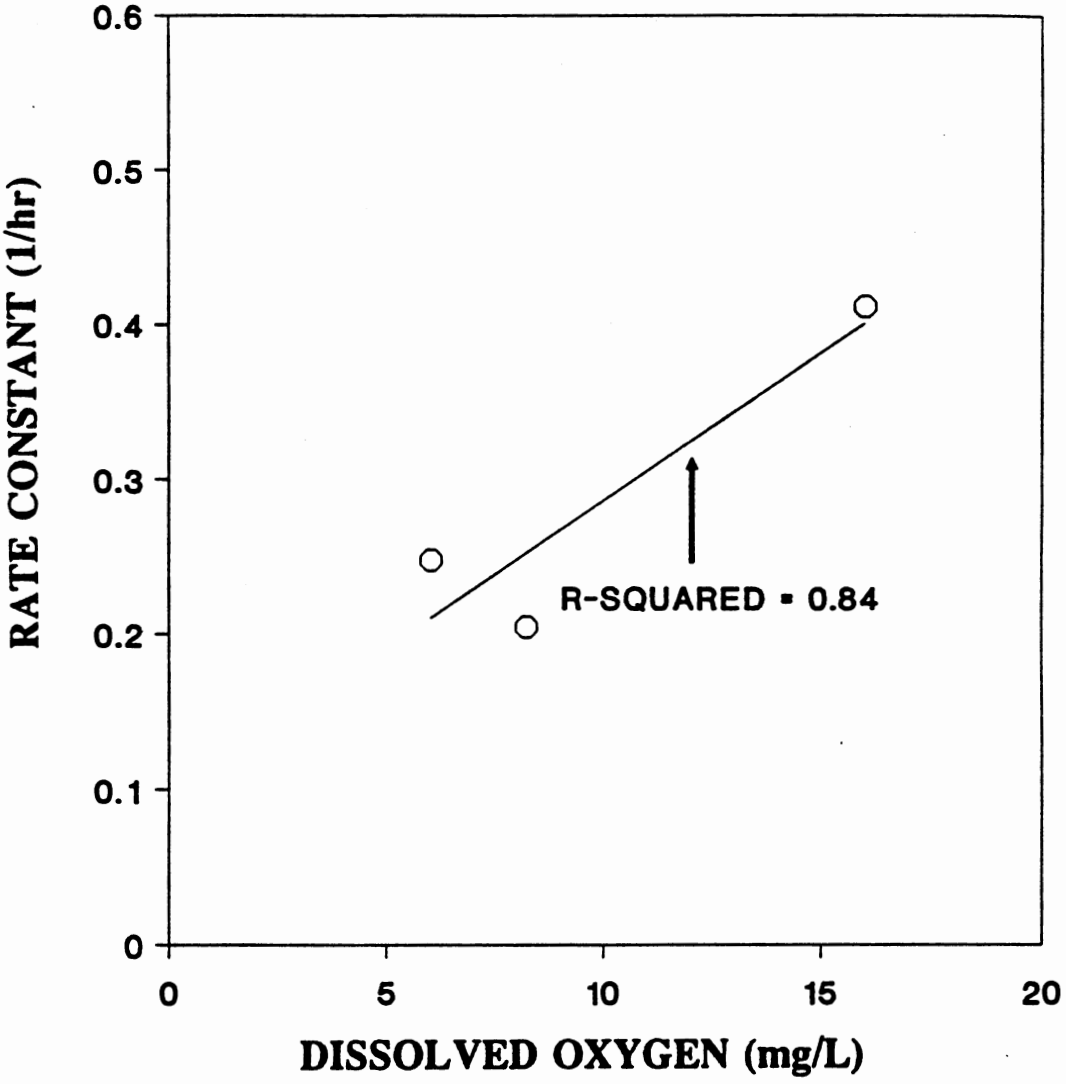


Figure 58. Effect of Dissolved Oxygen on First Order Rate Constant for the Biodegradation of 1,2-dichloropropane by *Pseudomonas putida* PpG-786.

times when the initial dissolved oxygen concentration was increased to 16 mg/L from 8.2 mg/L. At a dissolved oxygen of 6 mg/L, the modified first order model provided a significantly better fit than the first order model (alpha of at least 0.05). This indicates that the compound will persist at a level of R/K_1 corresponding to 479 μg/L (using R = 618.99 μg/L·hr, K_1 = 1.29/hr, and Figure 24). The experiments were conducted with 26.6 g/L wet weight of cells (corresponding to 5.2 g/L dry weight at 56°C) and initial substrate concentration of 1030 μg/L.

Inoculum Size

The effect of inoculum size on the biodegradation rate of 1,2-dichloropropane by *Pseudomonas putida* PpG-786 is shown in Table XXV and Figure 59. The first order rate of biodegradation increased linearly as the concentration of cells increased from 1.455 g/L to 8.017 g/L (dry weight determined at 56°C). The first order rate of reaction increased 3.71 times from 0.1/hr at a cell concentration of 1.455 g/L cells to 0.37/hr at 8.017 g/L over the four hour period used for the experiments.

Substrate and Heavy Metal Concentrations

The effects of initial substrate concentrations and different concentrations of lead (added as lead acetate) are shown in Tables XXVI and XXVII and Figures 60 and 61. The first order rate of reaction was reduced from 0.84/hr at an initial substrate concentration of 1,2-dichloropropane of 761 μg/L to 0.08/hr at an initial concentration of 4660 μg/L. This indicates toxicity by 1,2-dichloropropane to *Pseudomonas putida* PpG-786 at high concentration. Only at an initial concentration of 1218 μg/L of 1,2-dichloropropane did the Michaelis-Menten model provide a significantly better fit than the first order model when compared using a F-test of alpha of at least 0.05. Increasing the concentration of lead from 0.0 mg/L to 10.0 mg/L has only a slight effect on the first order rate constant as shown in Figure 31 and Table XXVI. The first order rate of reaction was 0.20/hr at 0.0 mg/L and 0.23/hr at 10.0 mg/L lead.

TABLE XXV

EVALUATION OF THE EFFECT OF *INOCULUM SIZE* ON PARAMETER ESTIMATES USING FIRST ORDER KINETIC FITS ON THE BIODEGRADATION OF 1,2-DICHLOROPROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 AT 25°C

ID Number	INOCULUM SIZE (g/L)	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	F	N_p
INNODES1	1.455	0.10	0.02	1015	31	16.34	18
INNODES2	3.317	0.22	0.02	969	32	55.77	16
INNODES3	6.470	0.29	0.02	1012	20	231.34	18
INNODES4	8.017	0.37	0.05	963	56	41.31	18

*Asymptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right)N^{-1}}$

$F = (CSS-RSS)/RSS(N_p-p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

$F_{0.05,2,16} = 3.63$; $F_{0.05,2,14} = 3.74$.

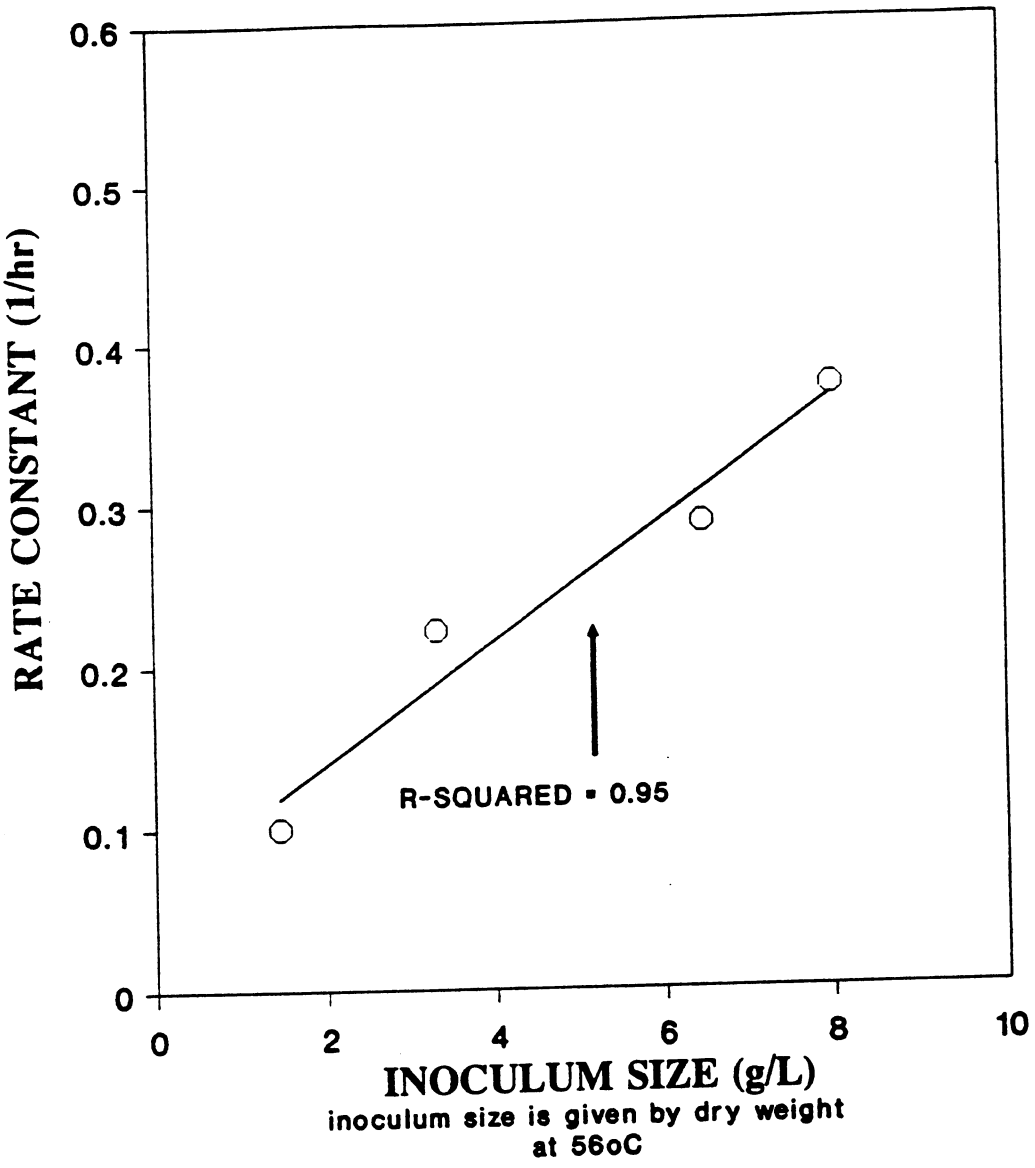


Figure 59. Effect of Inoculum Size on First Order Rate Constant for the Biodegradation of 1,2-dichloropropane by *Pseudomonas putida* PpG-786.

TABLE XXVI

EVALUATION OF THE EFFECT OF *INITIAL SUBSTRATE CONCENTRATION* ON PARAMETER ESTIMATES USING FIRST ORDER AND MICHAELIS-MENTEN KINETIC FITS ON THE BIODEGRADATION OF 1,2-DICHLOROPROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 AT 25°C

ID Number	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	K_m (μg/L)	STD* (μg/L)	V_{max} (μg/L·hr)	STD* (μg/L·hr)	F	N_p
First Order Equation										
SUST1DF	0.84	0.01	761	30	NA	NA	NA	NA	168.45	12
SUST2DF	0.61	0.05	1260	42	NA	NA	NA	NA	198.14	14
SUST3DF	0.08	0.02	4660	199	NA	NA	NA	NA	6.00	16
Michaelis-Menten Equation										
SUST2DF	1.77**	NA	1218	32	373.29	323.41	660.27	208.82	231.08	14

*Asymptotic standard deviation $\approx \sqrt{2\left(\frac{RSS}{N_p - p}\right)} N^{-1}$

**$K_1 = u_{max}(X_o/K_m)$

$F = (CSS-RSS)/RSS(N_p-p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

NA - Not applicable

$F_{0.05,2,10} = 4.10$; $F_{0.05,2,12} = 3.89$; $F_{0.05,2,14} = 3.74$; $F_{0.05,3,12} = 3.41$

TABLE XXVII

EVALUATION OF THE EFFECT OF *LEAD CONCENTRATIONS* ON PARAMETER ESTIMATES USING FIRST ORDER KINETIC FITS ON THE BIODEGRADATION OF 1,2-DICHLOROPROPANE BY *PSEUDOMONAS PUTIDA* PpG-786 AT 25°C

ID Number	LEAD (mg/L)	K_1 (1/hr)	STD* (1/hr)	S_o (μg/L)	STD* (μg/L)	F	N_p
LEADDES1	0.0	0.20	0.04	993	56	17.47	11
LEADDES2	10.0	0.23	0.05	1011	71	12.95	10
LEADDES3	5.8	0.25	0.07	992	86	9.88	12
LEADDES4	2.2	0.26	0.03	992	41	42.45	12

*Asymptotic standard deviation $\approx \sqrt{2}\left(\frac{RSS}{N_p - p}\right) N^{-1}$

$F = (CSS-RSS)/RSS(N_p-p)/p$

CSS = Corrected sums-of-squares

RSS = Residual sums-of-squares

N^{-1} = Diagonal elements of the inverse matrix containing the sum of the product of the partial derivatives of the equation and corresponding to each parameter.

N_p = Number of data points

p = Number of parameters

$F_{0.05,2,9} = 4.26$; $F_{0.05,2,8} = 4.46$; $F_{0.05,2,10} = 4.10$.

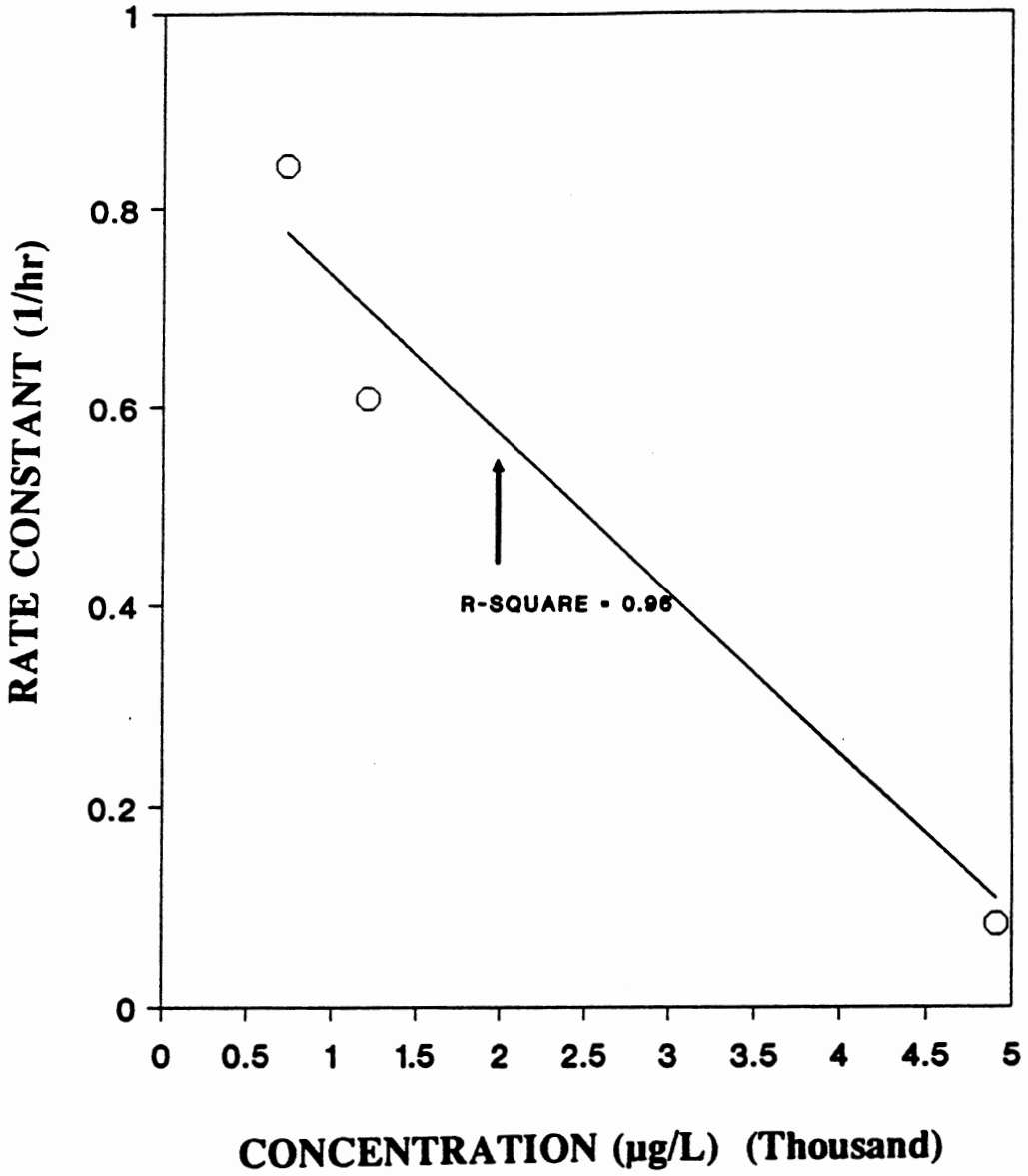


Figure 60. Effect of Initial Substrate Concentration on First Order Rate Constant for the Biodegradation of 1,2-dichloropropane by *Pseudomonas putida* PpG-786.

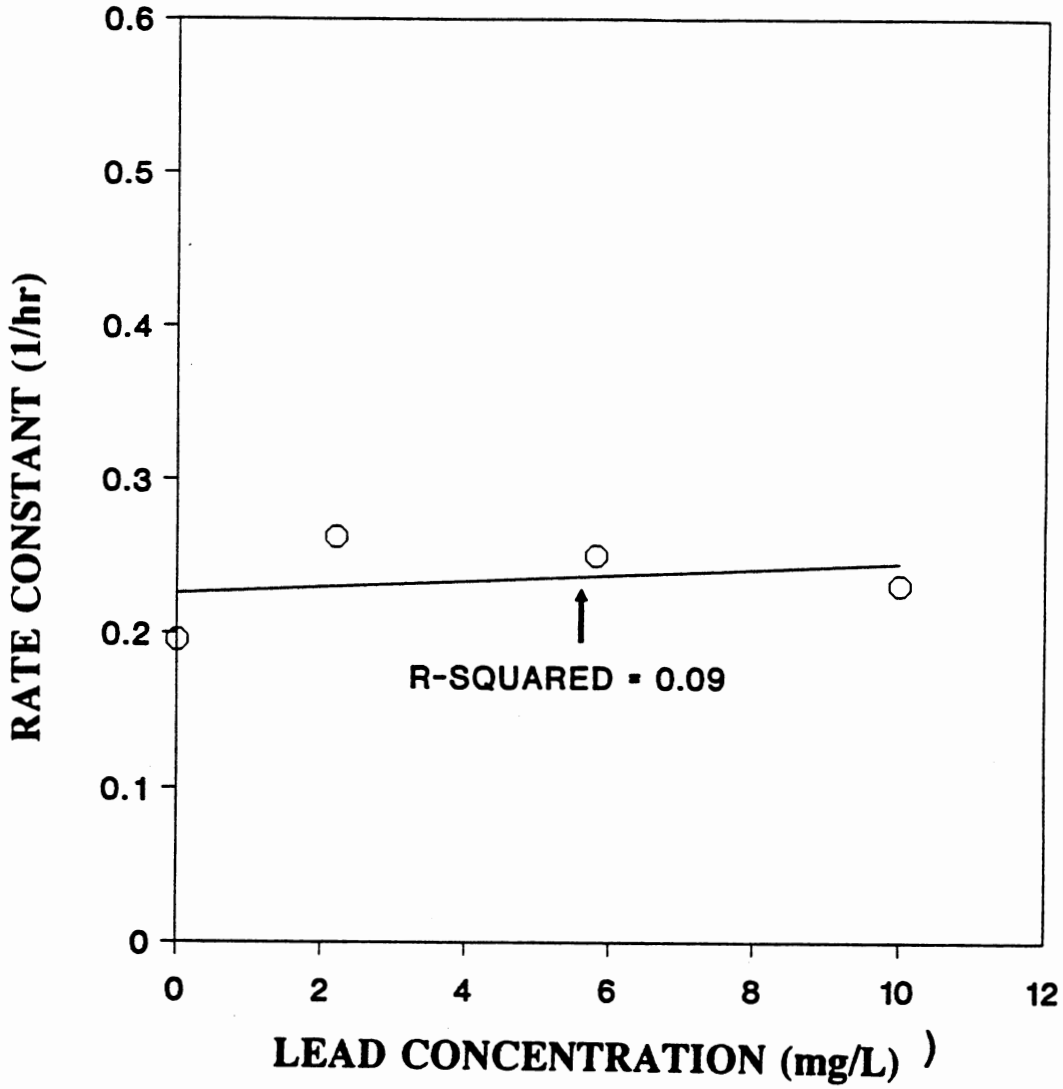


Figure 61. Effect of Lead Concentration on First Order Rate Constant for the Biodegradation of 1,2-dichloropropane by *Pseudomonas putida* PpG-786.

CHAPTER V

DISCUSSION

The main objectives of this study were to demonstrate biodegradation of selected low molecular weight halogenated compounds and provide kinetic data for their biodegradation by the resting cells of *Pseudomonas putida* PpG-786 under various environmental conditions in the presence of an aquifer matrix. In this study, some of the factors that influence the rate of biodegradation of selected halogenated compounds by the resting cells of *Pseudomonas putida* PpG-786 were investigated. The cytochrome P-450$_{cam}$ enzyme system consisting of three interacting proteins was induced by culturing *Pseudomonas putida* in the presence of camphor. The enzyme system has been implicated in the biodegradation of chlorinated aliphatic compounds such as 1,2-dibromo-3-chloropropane (Vilker and Khan, 1989). The concentration and activities of cytochrome P-450$_{cam}$ in whole cells were tested and shown to be stable over the entire test period.

Few studies have been done on the biodegradation of recalcitrant compounds by resting cells of pure cultures of microorganisms. Pure cultures of bacteria with highly specialized enzyme systems, such as the cytochrome P-450$_{cam}$ system, and degradative capacities have unique roles to play in the conversion of toxic substances which are otherwise resistant to biodegradation. DCP has been previously shown to be resistant to biodegradation by mixed cultures in activated sludge reactors (Kincannon, *et al.*, 1982) or mixed inoculum from soil, surface water, or sludge (Kawasaki, 1980). DCP was also observed to persist in the environment up to 12 weeks when applied to sandy loam (Roberts and Stoydin, 1976). In order for biodegradation of recalcitrant compounds such as 1,2-dichloropropane to be effected, highly specialized microorganisms such as *Pseudomonas putida* PpG-786 need to be used.

The use of resting cells of microorganisms rather than growing cells allows separation and optimization of growth conditions from catalysis and substrate conversion. Cells are cultured under optimum conditions of temperature, pH, nutrients, and the inducer of the required enzyme system. Resting cells are subsequently harvested and the enzymes immobilized within the bacterial cells serve as biocatalysts for the conversion of the compounds. Factors which may affect growth of microorganisms may not necessarily affect the conversion of substrates by enzymes immobilized within the bacterial cells. Nutrients and substrates critical for growth and survival of cells may not be necessary for conversion of substrates by the enzyme system. The discussion of the various experimental results conducted during the study are presented as follows.

Preliminary Experiments

The preliminary experiments were designed to account for any significant abiotic losses during the study. Results shown in Tables VII - IX indicated that abiotic losses of 1,2-dichloropropane, 1,2-dibromo-3-chloropropane and 1,2-dichloroethane were not significant (alpha of at least 0.05) during the experiment. Loss due to volatilization was minimized by conducting the experiments in closed systems with minimal headspace. In open glass containers, about 99% of 1,2-dichloropropane was reported lost due to volatilization (Roberts and Stoydin, 1976). Cellular adsorption was also found not to be significant (alpha of at least 0.05) during the experiment. Enzyme activity was maintained at a high level by aerating the cells only prior to the beginning of the experiment rather than continuously. More reliable cell density was obtained at 56°C than at 103°C because at low cell concentration, cell mass determined at 103°C could result in negative cell weight values.

Model Comparison

Most of the data collected in this research could be fitted by the two-parameter first order model. The more complex model typically lowers the residual sums-of-squares below

that obtained for a simpler model (Robinson, 1985). However, in order to select a three parameter model, the more complicated model must provide a significantly better fit when compared to the two parameter model. Equation 44 is used to calculate F values that are then compared to tabulated F-values (numerator degree of freedom, one; denominator degree of freedom, n-p; where p is the number of parameters of the more complicated model while n is the number of data points). If the calculated F-value is less than the tabulated F-value, then the simpler model is selected as the appropriate model. This is illustrated in Figures 15 - 44. The Figures show comparisons between first order and/or modified first order and/or Michaelis-Menten models when a convergence to a solution is obtained. Table XXVIII shows the models selected to fit the data obtained during the different experiments. The majority of the experiments were fitted using the first order model. Schmidt *et al.* (1985) also concluded that in the presence of adequate number of microbial cells and low substrate concentration, the rate of conversion of substrate not supporting growth is first order.

Modified First Order Model

Much research attention is focused on biodegradation of recalcitrant compounds in the environment. However, the modified first order equation addresses the possibility of biosynthesis of these compounds under certain environmental conditions. Figure 45 shows that if the initial concentration of the compound is below R/K_1, the tendency is for the concentration to increase and approach R/K_1 asymptotically. Given that enzyme catalyzed reactions are reversible with one direction of reaction being predominant over the other, the environmental condition can result in the formation of, rather than, or along side removal of, the compounds of interest. Biosynthesis of halogenated compounds have been demonstrated in microorganisms containing haloperoxidase enzymes which are widely distributed in nature (Neidleman and Geigert, 1986). Haloperoxidase can convert alkenes into halogenated compounds in the presence of hydrogen peroxide. The modified first order equation provided a significantly

TABLE XXVIII

MODEL FITS OF DATA COLLECTED DURING DIFFERENT KINETIC EXPERIMENTS

Experiment/ ID Number	pH	Temperature °C	Subsurface Materials	Selected Model
Effect of Presence of Aquifer Materials (1,2-dibromo-3-chloropropane)				
DBCPDF	7.4	25	None	Modified First Order
DBCPDF2	7.4	25	OSU	First Order
Effect of Aquifer				
AQUIFDF	7.4	25	OSU	First Order
AQUIFDF2	7.4	25	SS	First Order
Effect of pH				
pH54(25)	5.4	25	OSU	First Order
pH74(25)	7.4	25	OSU	First Order
pH89(25)	8.9	25	OSU	First Order
Effect of Dissolved Oxygen[1]				
DOCOM3	7.4	25	OSU	First Order
DOCOM4	7.4	25	OSU	First Order
DOCOM5	7.4	25	OSU	Modified First Order
Effect of Temperature[2]				
pH64(15)	6.4	15	OSU	First Order
pH64(25)	6.4	25	OSU	First Order
pH64(30)	6.4	30	OSU	First Order
pH74(15)	7.4	15	OSU	First Order
pH74/25	7.4	25	OSU	First Order
pH74(30)	7.4	30	OSU	First Order
pH7815c	7.8	15	OSU	First Order
pH7825c	7.8	25	OSU	First Order
pH7830c	7.8	30	OSU	First Order

TABLE XXVIII, Continued

Experiment/ ID Number	pH	Temperature °C	Subsurface Materials	Selected Model
Effect of Inoculum Size[3]				
INNODES1	7.4	25	OSU	First Order
INNODES2	7.4	25	OSU	First Order
INNODES3	7.4	25	OSU	First Order
INNODES4	7.4	25	OSU	First Order
Effect of Substrate Concentration[4]				
SUST1DF	7.4	25	OSU	First Order
SUST2DF	7.4	25	OSU	Michaelis-Menten
SUST3DF	7.4	25	OSU	First Order
Effect of Heavy Metal Concentration[5]				
LEADDES1	7.4	25	OSU	First Order
LEADDES2	7.4	25	OSU	First Order
LEADDES3	7.4	25	OSU	First Order
LEADDES4	7.4	25	OSU	First Order

*Experiments performed at DO 16.0 mg/L and using 1,2-dichloropropane except where indicated.

[1]Experiments were performed using inoculum sizes of 1.455 g/L, 3.317 g/L, 6.470 g/L, and 8.017 g/L for INNODES1, INNODES2, INNODES3, and INNODES4, respectively.

[2]Experiments were conducted with chloride concentrations of 2.120 g/L, 0.871 g/L, and 0.151 g/L at pH 6.4, 7.4, and 7.8, respectively.

[3]Experiments conducted at 16.0 mg/L, 8.2 mg/L, and 6.0 mg/L dissolved oxygen for DOCOM3, DOCOM4, and DOCOM5, respectively.

[4]Experiments conducted at 732 μg/L, 1209 μg/L, and 4907 μg/L 1,2-dichloropropane for SUST1DF, SUST2DF, and SUST3DF, respectively.

[5]Lead concentrations were 0.0 mg/L, 10.0 mg/L, 5.8 mg/L, and 2.2 mg/L for LEADDES1, LEADDES2, LEADDES3, and LEADDES4, respectively.

SS - Sand Springs Petrochemical Complex subsurface materials.

OSU - Oklahoma State University Agronomy Station aquifer materials.

better fit (alpha of at least 0.05) compared to the first order fit when initial dissolved oxygen serving as a terminal electron acceptor is at 6.0 mg/L.

Modeling of Kinetic Experiments

Most of the experiments conducted during this research can be fitted to the first order model. A three parameter model must provide a significantly better fit (alpha = 0.05) before it can be selected in place of the two parameter first order model. In all cases, the three parameter models resulted in the reduction of the residual sums of squares of the nonlinear regression as shown in Appendix V. Nonlinear approximation of the Michaelis-Menten equation is useful only in the mixed order zone, corresponding to K_m that is within five times the initial substrate concentration. When K_m is much larger than S_o, the sensitivity equations using partial derivatives of K_m and V_{max} are proportional (multiples of one another), therefore, the parameters of the Michaelis-Menten equation cannot be independently estimated using nonlinear techniques. The Michaelis-Menten equation provided the best fit when compared to the first order and modified first order models for the experiment identified as SUST2DF, an experiment conducted using 1,2-dichloropropane.

Sensitivity Analysis

Sensitivity analysis of the modified first order model indicated that the model is applicable in the region where S_o is greater than R/K_1 . As S_o gets closer to S_p, the correlation between two of the three parameters, namely K_1 and R becomes very large. This implies that nonlinear regression will not result in unique parameter estimation and that a simpler model such as the two parameter first order model should be considered. The Michaelis-Menten model was applicable in the mixed order region as indicated by previous studies (Robinson and Charaklis, 1984; Robinson, 1985). Use of nonlinear parameter estimation in regions outside of the mixed order zone is prevented by the correlation between two of the three parameters of the Michaelis-Menten model.

Effect of Aquifer and Chemical Types

The rate of biodegradation of 1,2-dichloropropane was dependent on the presence of aquifer materials and the type of subsurface materials. Biodegradation is also found to be compound specific. The presence of Oklahoma State University Agronomy Research Station aquifer materials reduced the rate of removal of 1,2-dibromo-3-chloropropane by *Pseudomonas putida* PpG-786 when compared to a similar experiment when no aquifer materials were present. The rate of removal in the absence of aquifer materials was faster (3.3/hr) than in the presence of the materials (2.4/hr). No appreciable removal of 1,2-dichloroethane was observed during the 4 hour incubation period used in this experiment. 1,2-dichloroethane was, however, observed to be biodegradable by *Xanthobacter autotrophicus* GJ-10 (Jensen *et al.*, 1987), *Pseudomonas fluorescens* (Vandenberg and Kunka, 1988), and *Methylosinus trichosporium* OB-3b (Riebeth *et al.*, 1992).

Biodegradation of 1,2-dibromo-3-chloropropane in the absence of aquifer materials was predicted by the modified first order model while in the presence of aquifer materials from Oklahoma State University Agronomy Research Station, removal of 1,2-dibromo-3-chloropropane was predicted by the first order model. In the absence of aquifer materials, 1,2-dibromo-3-chloropropane was initially rapidly removed until the concentration reached a level at which it persisted. A persistence level predicted by equation 45 ($R/K_1 = 23$ μg/L) was observed for the kinetic experiment conducted in the absence of aquifer materials. Over a longer period, the complete removal of 1,2-dibromo-3-chloropropane was reported by Lam and Vilker (1986). Although the rate of removal of 1,2-dibromo-3-chloropropane was faster in the absence of aquifer materials when compared to its rate of removal in the presence of aquifer materials, mean concentrations of test compounds remaining at the end of the four hour experiments were 20 ± 1 μg/L in the absence of aquifer materials and 22 ± 1 μg/L in the presence of aquifer materials from Oklahoma State University Agronomy Station.

Effect of pH and Temperature

The influence of pH and temperature on the kinetics of 1,2-dichoropropane's biodegradation was shown in Figures 56 and 57. Analysis of substrate concentrations remaining at the end of the four hour experiments conducted to evaluate the effect of pH showed no significant difference for all three pH values. The first order rate constant dropped by 15.5% while the pH was varied from 5.4 to 8.9. There was no significant difference in the test concentrations measured at the end of the four hour experiments conducted to evaluate the effect of temperature at pH 6.4 and 7.4 for temperatures of 15°C, 25°C, and 30°C. However, at pH 7.8, there was a significant difference in the concentrations of 1,2-dichloropropane remaining at 15°C when compared to 25°C and 30°C. The first order rate constants obtained for all pH values was highest at temperature of 25°C.

The result of this research can be directly compared to those of Dibble and Bartha (1979) who observed an optimum temperature for oily sludge degradation in soil was 20°C, with negligible microbial activity occurring at 5°C. *Pseudomonas putida* PpG-786 cultivated at 25°C was able to maintain its biodegradative activities when the temperature was varied from 15°C to 25°C.

Effect of Dissolved Oxygen

Due to the heterogenous nature of the subsurface and the existence of pockets of anaerobic zones, biodegradation may occur at a rate less than will be observed under ideal conditions when oxygen is available in abundance and therefore not rate limiting. Doubling the dissolved oxygen doubles the rate of removal of 1,2-dichlorpropane in the presence of Oklahoma State Agronomy Station aquifer materials (Figure 58). At a dissolved oxygen of 6 mg/L, 1,2-dichloropropane was observed to persist due to the depletion of dissolved oxygen by the end of the experiment. This provides a possible explanation for the better fit of the modified first order model when compared to the first order model. The metabolism of other pollutants such as pentachlorophenol (PCP) by a pure culture of *Arthrobacter* sp. Strain

ATCC 33790 with dehalogenating activity was found to also be influenced by dissolved oxygen, decreasing to 0 - 24% of that obtained in the presence of dissolved oxygen (Schenk *et al.*, 1989). Anaerobic biodegradation of m-cresol was inhibited by oxygen in anoxic aquifer slurries (Ramanand and Suflita, 1991).

Effect of Inoculum Size

Inoculum size was previously identified as a factor limiting biodegradation of synthetic compounds in the natural environment (Ramadan *et al.*, 1990). The lag phase observed during the biodegradation of 2,4-dichloro-phenoxyacetic acid by Greer *et al.* (1990) was significantly reduced by increasing the inoculum size.

The rate of degradation of 1,2-dichloropropane by resting cells of *Pseudomonas putida* PpG-786 used in this study increased linearly with increase in inoculum size (Figure 59). This indicated that for biorestoration, the more cells introduced into the aquifer the faster the clean-up that can be effected. The biodegradation of 1,2-dichloropropane in the presence of Oklahoma State University Agronomy Research Station aquifer materials can be enhanced by increasing the inoculum size of *Pseudomonas putida* PpG-786. Although biodegradation is significantly affected by at least doubling inoculum sizes, high cell concentrations can result in plugging of wells and result in rapid depletion of the dissolved oxygen. At low dissolved oxygen concentration, the removal rate is slower and the compound may persist because a terminal electron acceptor was not available.

Effect of Substrate Concentration

High substrate concentration resulted in a decrease in the first order rate of reaction (Figure 60). This could be due to the toxicity of the substrate to the microorganism. However 1,2-dichloropropane was reported (Cohen, 1983) in groundwater at a level at which appreciable conversion of 1,2-dichloropropane by resting cells of *Pseudomonas putida* PpG-786 could be observed. The previous studies by Castro and Belser (1990) also indicated

substrate toxicity by 1,1,2-trichloroethane to *Pseudomonas putida* PpG-786. Vilker and Khan (1989) also showed that at concentrations of 1,2-dibromo-3-chloropropane above 100 μM, the degradation activity of *Pseudomonas putida* PpG-786 was almost completely inhibited and proposed that 1,2-dibromo-3-chloropropane acts as a metabolic poison at high concentrations.

Effect of Heavy Metal

Analysis of substrate concentrations remaining at the end of the four hour experiments conducted to evaluate the effect of lead (added as lead acetate) indicated that biodegradation of 1,2-dichloropropane was not significantly affected by lead concentrations measured at 0 mg/L, 2.2 mg/L, 5.8 mg/L, and 10.0 mg/L.

The first order rate constant varied only slightly when the lead concentration was increased from 0.0 mg/L to 10.0 mg/L (Figure 61). High lead concentration was previously observed to inhibit growth of mixed cultures of microorganisms (Stover and Kincannon, 1983). This indicated an advantage of not requiring growth to occur in order for the substrate to be converted especially in hazardous wastes sites where a variety of pollutants could be present.

A possible reason why lead inhibited growth and survival of microorganisms but not conversion by the cytochrome P-450$_{cam}$ enzyme system was provided by Tornabene and Edwards (1973). They showed that the continuous culture of a bacterium *Micrococcus luteus* in the presence of 600 μg/L lead caused a disruption of cytoplasmic material. In addition, lead was largely concentrated in the cytoplasmic cell membranes of the bacteria. Cytochrome P-450$_{cam}$ has, however, been shown to maintain its biodegradative capacity outside a bacterial cell (Castro *et al.*, 1985).

Significance of Research

This research demonstrated the biodegradation of recalcitrant low molecular weight halogenated compounds under various environmental conditions. Compounds such as 1,2-

dichloropropane which have so far been resistant to biodegradation by mixed cultures of microorganisms require specialized microorganisms for conversion in the environment. This research provided some background work required for the design of *in-situ* biorestoration of contaminated sites with enzyme rich microorganisms. A conceptualization of a treatment system using enzyme rich microorganisms is presented in Figure 62. The separation of growth of microorganisms from substrate conversion has advantages such as eliminating the need to introduce nutrients into the subsurface and being less susceptible to conditions where growth can be inhibited in the subsurface. If the required microorganisms are introduced at high enough concentrations then the effects of predation can be overcome. Care must be taken however to ensure that the level of microorganisms introduced into the aquifer is not so high that the treatment system can be plugged or that dissolved oxygen level drops low enough to limit biodegradation. The rate of biodegradation is affected by the dissolved oxygen. As such, the design of *in-situ* biorestoration systems using enzyme rich microorganisms in some cases require a terminal electron acceptor to be present for biodegradation to be effected. The temperature of the aquifer can also be increased by injecting steam to raise the temperature and to increase the rate at which biodegradation occurs when economically viable. The use of enzyme rich microorganisms isolated from the soil environment in biodegradation of otherwise recalcitrant compounds might lead to eliminating some of the problems associated with groundwater pollution.

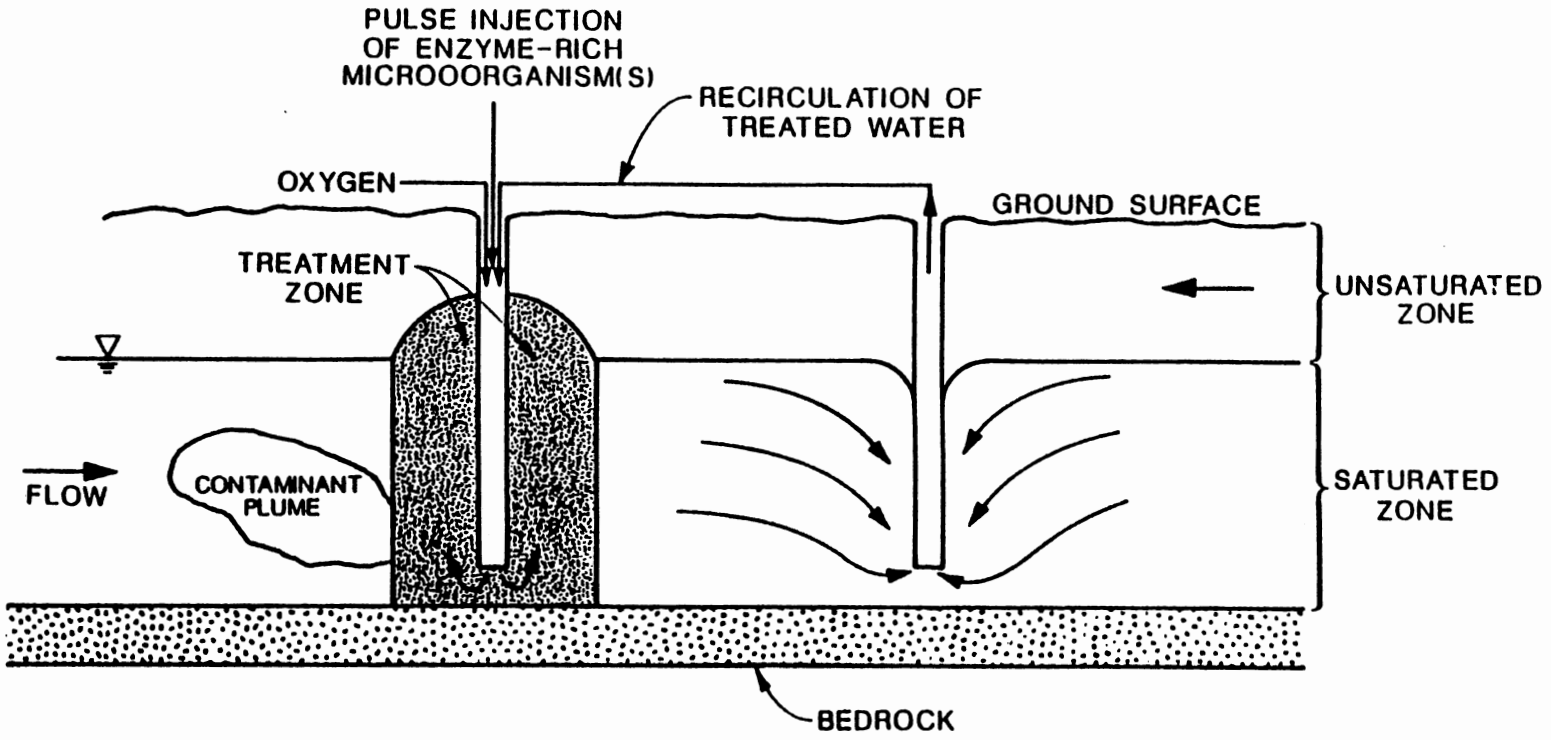


Figure 62. Conceptualization of Treatment of Contaminated Aquifer with Enzyme-Rich Microorganism(s).

CHAPTER VI

CONCLUSIONS

The results of this research corroborated previous studies as well as provided new insights into the problems associated with biological conversion of low molecular weight halogenated aliphatic compounds. The conclusion that can be drawn from this research are:

- The biodegradation of low molecular weight halogenated compounds by resting cells of a pure culture of *Pseudomonas putida* PpG-786 was dependent on the individual compounds. The rate of removal of 1,2-dibromo-3-chloropropane was greater than 1,2-dichloropropane while no appreciable removal of 1,2-dichloroethane was observed within the four hour incubation time used in this research.

- The first order rate constant for the removal of 1,2-dichloropropane was only slightly decreased (15.5%) when the pH was varied from 5.4 to 8.9.

- The highest biodegradation rate occurred at temperature of 25°C for experiments conducted at pH 6.4 (chloride = 2.102 g/L), 7.4 (chloride = 0.871 g/L), and 7.8 (chloride = 0.151 g/L). This optimum temperature is close to the temperature under which the microorganisms were cultured. The rate of biodegradation of 1,2-dichloropropane was significantly decreased by the presence of chloride ions at pH 7.4 when compared to rate obtained in the absence of chloride ions.

- The first order rate of biodegradation of 1,2-dichloropropane was approximately doubled when the initial dissolved oxygen was doubled. At a low dissolved oxygen

concentration of 6.0 mg/L, the compound tended to persist during the experiment and was satisfactorily fitted by the modified first order equation.

- The modified first order model provided a significantly better fit compared to a first order fit, for the biodegradation of 1,2-dibromo-3-chloropropane in the absence of aquifer materials from Oklahoma State University Agronomy Station. Its biodegradation in the presence of the aquifer materials was fitted by the first order model. A T-test on the concentration of 1,2-dibromo-3-chloropropane remaining in the reactors from the two experiments was found to be statistically significantly different. The concentrations of 1,2-dibromo-3-chloropropane remaining at the end of the four hour experiments was 20 $\pm$ 1 μg/L in the absence of aquifer materials and 22 $\pm$ 1 μg/L in its presence. The standard deviation from both experiments (based on triplicate samples) was so small that there was no overlap between the two sets of experimental results, hence will always result in statistically different tests.

  There was less than 10% difference in the final concentration of 1,2-dibromo-3-chloropropane remaining in the reactors in the presence of the aquifer materials, compared to its absence.

- The biodegradation of 1,2-dichloropropane was independent of the type of subsurface materials tested. About 76% removal of 1,2-dichloropropane was observed in the presence of Oklahoma State University Agronomy Station aquifer materials and subsurface materials from Sand Springs Petrochemical Complex (Site T-32).

- Inoculum size influenced the rate at which biodegradation occurred with higher inoculum size resulting in higher removal rate.

- The initial substrate concentration influenced the first order rate of degradation. The rate of dropped from 0.84/hr to 0.08/hr when the substrate concentration was increased from 761 μg/L to 4660 μg/L indicating inhibition of substrate removal at high substrate concentration.

- Biodegradation of 1,2-dichloropropane by resting cells of a pure culture of *Pseudomonas putida* PpG-786 was only slightly affected by lead (added as lead acetate) when the concentration of lead was varied from 0.0 mg/L to 10.0 mg/L.

CHAPTER VII

RECOMMENDATIONS FOR FURTHER STUDIES

The potential for the use of resting cells of a pure culture of *Pseudomonas putida* PpG-786 in biodegradation of low molecular weight halogenated compound that are persistent in the environment has been demonstrated in this research. Important environmental factors likely to influence the rate at which this biodegradation occurs were also investigated. Pure cultures containing high enzyme activity capable of immediate removal of toxic compound are important for *in-situ* restoration. Therefore, the following recommendation for future work is proposed:

- Investigate the mechanism of substrate inhibition by 1,2-dichloropropane.
- Investigate toxicity of the chlorinated aliphatic compounds to the enzyme systems of *Pseudomonas putida* or other pure cultures capable of biodegradation of persistent compounds of environmental importance.
- Use columns with aquifer materials to investigate long term biodegradation of 1,2-dichloropropane and 1,2-dichloroethane.
- Screen more recalcitrant organic compounds for biodegradation by pure cultures with high enzyme activity.
- Use field studies to evaluate the potential for enzyme-rich pure cultures for bioresto-ration of aquifers contaminated by recalcitrant compounds.

- Screen several concentrations of organic compounds for their levels of persistence.
- Investigate the possibility of biosynthesis of halogenated aliphatic compounds under selected environmental conditions.
- Determine pathways for biosynthesis and biodegradation of low molecular weight aliphatic compounds.
- Evaluate the use of other sources of oxygen such as hydrogen peroxide for biodegradation of halogenated compounds.

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APPENDICES

APPENDIX A

DERIVATION OF INTEGRATED FORM OF MONOD KINETICS

The derivation of different forms of Monod Kinetics was shown by Simkins and Alexander (1984) and reproduced in greater details here. Monod kinetics describing the specific growth rate of a microorganism is frequently described as:

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{1}$$

where:

$\mu = \frac{1}{B}\frac{dB}{dt}$ = the specific growth rate

μ_{max} = the maximum specific growth rate

In addition, the mass balance on a batch reactor containing substrate utilizing bacteria is given by:

$$S_o + qB_o = S + qB \tag{2}$$

where:

S_o = the initial substrate concentration

B_o = initial population density

q = inverse yield or cell quota

S = final substrate concentration

B = final cell concentration

In cases where q is independent of time and concentration, q is replaced by X and is given by:

$$X = qB \tag{3}$$

where:

X = amount of substrate required to produce a population density equal to B

Consequently,

$$\frac{dx}{dt} = q\frac{dB}{dt} \tag{4}$$

and

$$x_o = qB_o$$

Equations 1 and 2 can be rewritten as:

$$\frac{1}{x}\frac{dx}{dt} = \frac{\mu_{max}S}{K_s + S} \tag{5}$$

and

$$S_o + X_o = S + X \tag{6}$$

Solving equation 6 for X gives:

$$X = S_o + X_o - S \tag{7}$$

hence,

$$\frac{dx}{dt} = -\frac{ds}{dt} \tag{8}$$

Substituting equations 7 and 8 into equation 5 gives:

$$-\frac{ds}{dt} = \frac{\mu_{max} S (S_o + X_o - S)}{K_s + S} \tag{9}$$

Equation 9 indicates that only the initial cell and substrate concentrations determine the kinetics of biodegradation. Various approximations can be made to equation 9. For instance, when $X_o >> S$, the term $(S_o + X_o - S)$ can be replaced by X_o, hence equation 9 becomes:

$$-\frac{ds}{dt} = \frac{\mu_{max}SX_o}{K_s + S} \tag{10}$$

This is the same as equation 15 on page 25. Other approximations that can be made are discussed by Simkins and Alexander (1984).

Equation 10 can be solved by separation of variables:

$$\int_{So}^{S} \left(\frac{K_s + S}{S}\right) ds = -\int_{O}^{t} \mu_{max} S_o dt$$

$$K_s \ln\left(\frac{S}{S_o}\right) + (S - S_o) = -\mu_{max} X_o t \qquad (10)$$

If $\mu_{max} X_o = K$, equation 10 becomes

$$K_s \ln\left(\frac{S}{S_o}\right) + (S - S_o) = -Kt \qquad (11)$$

APPENDIX B

DERIVATION OF MICHAELIS-MENTEN EQUATION

Michaelis-Menten enzyme kinetic equation models the conversion of a substrate such as an organic compound to a product according to the following concepts (Aiba, Humphrey, and Millis, 1965):

$$E + S \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} E - S \xrightarrow{K_{+2}} E + P \qquad (1)$$

where:

E = enzyme

S = substrate concentration

E - S = enzyme - substrate complex

P = product

K_{+1} = forward reaction rate constant

K_{-1} = reverse reaction rate constant

K_{+2} = reaction rate constant

e, s, and c = concentration of total enzyme, substrate, and enzyme - substrate complex

dC/dt = rate of change

The rate of change of enzyme - substrate complex is given by:

$$\frac{dc}{dt} = K_{+1}(e-c)S - K_{-1}C - K_{+2}C \qquad (2)$$

It is assumed that $S >> e$.

At steady state, left hand of equation 2 becomes zero, the

$$c = \frac{eS}{\left(\frac{K_{-1} + K_{+2}}{K_{+1}}\right) + S} \qquad (3)$$

The rate of product formation, v*, in the enzyme reaction (equation 1) is given by:

$$v^* = K_{+2}C = \frac{K_{+2}eS}{\left(\frac{K_{-1} + K_{+2}}{K_{+1}}\right) + S} = \frac{V_{max}S}{\left(\frac{K_s + K_{+2}}{K_{+1}}\right) + S}$$

$$v^* = \frac{V_{max}S}{K_m + S} \qquad \text{(Michaelis-Menten equation) (4)}$$

where:

V = maximum rate of production, all of enzyme forms the enzyme - substrate complex

$K_2^* = \frac{K_{-1}}{K_{+1}}$ = equilibrium constant in the dissociation of the enzyme - substrate complex, E - S

$$K_m = K_s^* + \frac{K_{+2}}{K_{+1}}$$

If $K_{+2} << K_{+1}$, rate of production is controlled by the specific rate, K_{+2}, then $K_m = K_s^*$.

Equation 4 is analogous to Monod equation describing product formation with respect to time.

APPENDIX C

CHARACTERISTICS OF *PSEUDOMONAS PUTIDA* PpG-786

Characterization	Result
Growth on Camphor Minimum Media	+
Gram Stain	- (rod)
Motility	motile
Colony	round raised
UV Fluorescence	+
Temperature Growth	
25°C	+
37°C	+
Oxidation Fermentation Tubes	Acid top + Acid bottom + (delayed) Aerobic-facultative

APPENDIX D

LOW-MOLECULAR WEIGHT HALOCARBONS IN SAND SPRINGS PETROCHEMICAL COMPLEX, TULSA, OKLAHOMA AND IN GROUNDWATER AT TINKER AIR FORCE BASE, MIDWEST CITY, OKLAHOMA

TABLE I

Compound	Maximum Concentration in Sediment mg/kg
1,2-Dichloroethane	33.0
Methylene chloride	340.0
1,1-Dichloroethane	110.0
1,1,1-Trichloroethane	14000.0
Trans 1,2-Dichloroethane	330.0
1,2-Dichloropropane	180.0
Trichloroethane	1200.0
Chloroform	10.0
Tetrachloroethane	19000.0

Source: John Mathes and Associates, Inc., 1987.

TABLE I

Compound	Average Concentration ug/L
1,2-Dichloroethane	86.3
1,1-Dichloroethane	3.0
1,1,1-Trichloroethane	43.0
1,1,2-Trichloroethane	60.0
1,2-Dichloropropane	36.0
Chloroethane	116.2
Trichloroethylene	10820.0
Carbon tetrachloride	9.5
Tetrachloroethane	167.7
Tetrachloroethylene	164.4

Source: Combs, D.L., 1987.

APPENDIX E

LIST OF CHEMICALS

Chemicals	Company/CAS/Model Number	Company	Certification
Ferrous Ammonium Sulfate	2054	JT Baker Chemical Co., Tulsa, OK	Baker analyzed reagent/ACS
Ammonium Chloride	A-5666	Sigma Chemical Co., St. Louis, MO	ACS
Sodium Phosphate Tribasic	S 377	Fisher Scientific, Atlanta, GA	ACS
L-Glutamic Acid (monosodium salt)	G 1626	Sigma Chemical Co., St. Louis, MO	ACS
Calcium Chloride	C79-500	Fisher Scientific, Atlanta, GA	ACS
Magnesium Sulfate Heptahydrate GR Crystals	MX0070	EM, Cherry Hill, NJ	None
Manganese Sulfate GR Monohydrate	M-117	Fisher Scientific, Atlanta, GA	None
L-Ascorbic Acid	A 1417	Sigma Chemical Co., St. Louis, MO	ACS
Ferrous Sulfate Crystal	I146-500	Fisher Scientific, Atlanta, GA	ACS
Buffer Solution pH 7.0	SB107	Fisher Scientific, Atlanta, GA	Fisher
Buffer Solution pH 10.0	1600-16*UK	Baxter, McGraw Park, IL	None
Sodium Hydroxide N/5	SS274-4	Fisher Scientific, Atlanta, GA	Fisher
Hexane	H-292-4	Fisher Scientific,Atlanta, GA	ACS
Acetone	A19-1	Fisher Scientifi, Atlanta, GA	ACS
Methanol	A408-1	Fisher Scientific, Atlanta, GA	ACS
1,2 Dichloropropane	A16B	Kodak, Rochester, NY	ACS
1,2 Dichloroethane (lot 712644)	E-175	Fisher Scientific, Atlanta, GA	ACS
n-Pentane lot 884094	P 393-1	Fisher Scientific, Atlanta, GA	Infra-Red Spectranalyzed
Sodium Chloride	S-271	Fisher Scientific, Atlanta, GA	ACS
Potassium Phosphate Monobasic	P-284	Fisher Scientific, Atlanta, GA	None
Potassium Phosphate Dibasic	S-374	Fisher Scientific, Atlanta, GA	ACS
VWR Buffer Solution pH 7.0	34180	VWR Scientific Union	None
(IR)-(+) Camphor 99% (lot#49F3438)	11F3409	Sigma Chemical Co., St. Louis, MO	None
Difco Bacto-Agar	0140	Difco Laboratories, Detroit, MI	None
Calcium Chloride Anhydrous	C614-500	Fisher Scientific, Atlanta, GA	None
Methanol	A408-1	Fisher Scientific, Atlanta, GA	ACS

Chemicals, continued

Chemicals	Company/CAS/Model Number	Company	Certification
1,2 dichloroethane	E-175	Fisher Scientific, Atlanta, GA	ACS
n-pentane (lot #884094)	P393-1	Fisher Scientific, Atlanta, GA	Infra-Red Spectranalyzed
Potassium Dichromate (1.0 N)	21971-53	Hach Company, Loveland, CO	ACS
Zinc Sulfate 7-Hydrate Crystal	4882	JT Baker Chemical Co., Tulsa, OK	Baker Analyzed Reagent
Lead Acetate, Trihydrate	I-2271	JT Baker Chemical Co., Tulsa, OK	Baker Analyzed Reagent
Bactotryptone			
Glucose (dextrose)	D16	Fisher Scientific, Atlanta, GA	ACS
Yeast Extract	9127-01-7	Difco Laboratories, Detroit, MI	Difco

APPENDIX F

CHLORIDE ADDED AT DIFFERENT pHs

pH of Buffer	Chloride Ion Concentration Average g/L	Standard Deviation g/L
6.4	2.1021	0.0928
7.4	0.8708	0.0311
7.8	0.1506	0.0355

NOTE:

Typical chloride ion concentration in groundwater:

0.200 g/L (Tchobanoglous and Schroeder, 1985. *Water Quality*. Addison-Wesley Publishing Company, Inc., page 164)

Chloride ion concentration:

Chloride ion concentration in groundwater is variable and highly dependent on the region.

less than 0.010 g/L in humid regions
1.000 g/L in arid regions
19.300 g/L in seawater
200.00 g/L in brines

(David Keith Todd, 1980. John Wiley and Sons, New York, page 274)

APPENDIX G

PARTICLE SIZE ANALYSIS OF SUBSURFACE MATERIALS FROM SAND SPRINGS PETROCHEMICAL COMPLEX (WET METHOD)

Sieves #	Wt of Sample Retained + Seive (g)	Weight of Sieve (g)	Weight of Sample (g)	Percent Passing %
Site 1:				
4	705.7	705.6	0.1	99.9
20	616.5	601.0	15.5	84.4
40	473.0	462.1	10.9	73.5
100	463.6	452.1	11.5	62.0
140	502.8	491.2	11.6	50.4
200	498.2	492.8	5.4	45.0
SUM			55.0	
Site 2				
4	707.3	703.2	4.1	95.9
20	607.2	599.0	8.2	87.7
40	463.6	461.6	2.0	85.7
100	465.4	449.2	16.2	69.5
140	505.3	488.7	16.6	52.9
200	498.7	491.2	7.5	45.4
SUM			54.6	
Site 3:				
4	705.7	705.6	0.1	99.9
20	602.0	601.0	1.0	98.9
40	465.8	462.1	3.7	95.2
100	480.9	452.1	28.8	66.4
140	503.6	491.2	12.4	54.0
200	495.3	492.8	2.5	51.5
SUM			48.5	

APPENDIX H

PARTICLE SIZE ANALYSIS OF SUBSURFACE MATERIALS FROM OKLAHOMA STATE UNIVERSITY AGRONOMY RESEARCH STATION

Sieves #	Wt. of Sample Retained + Sieve (g)	Weight of Sieve (g)	Weight of Sample (g)	Percent Passing %
4	703.0	703.0	0.0	100.0
10	453.1	453.1	0.0	100.0
20	472.7	472.1	0.6	99.9
40	596.6	555.4	41.2	91.6
60	678.5	508.9	169.6	57.7
140	703.1	488.3	214.8	14.8
200	502.2	478.8	23.4	10.1
			———	
SUM			426.2	

APPENDIX I

MOISTURE CONTENT FOR SAND SPRINGS PETROCHEMICAL COMPLEX AND OSU AGRONOMY RESEARCH STATION SITES

Identification Number	Site Number	Moisture Content (%)	Average (%)	Standard Deviation
T - 29	1	24.18		
		24.86		
		34.08	27.71	4.51
T - 22	2	19.35		
		21.38		
		21.12	20.62	0.90
T - 32	3	10.84		
		7.68		
		14.79	11.10	2.91
T - 32	3*	0.81		
	*	0.74	0.77	0.03
	4	25.79		
		26.03		
		28.11		
		28.14	27.02	1.11
	4*	0.29		
	*	0.31	0.30	0.01

*air dried sample

APPENDIX J

pH VALUES FOR SAND SPRINGS PETROCHEMICAL COMPLEX AND OSU AGRONOMY RESEARCH STATION SITES

Identification Number	Site Number	Mass of Soil (g)	Volume of Water (ml)	pH
T - 29	1	10	50	7.4
		10	50	7.0
		10	50	6.0
T - 22	2	10	50	6.3
		10	50	6.2
		10	50	6.2
T - 32	3	10	50	6.3
		10	50	6.4
		10	50	5.9
	4	10	50	7.2
		10	50	7.2

APPENDIX K

DETERMINATION OF PERCENT ORGANIC MATTER FOR SAND SPRINGS PETROCHEMICAL COMPLEX AND OSU AGRONOMY STATION SITES

Identification Number	Site Number	Mass of Sample (g)	Measure Organic Matter %	Calculated Organic Matter %	Mean %
T - 29	1	0.5004	2.40	4.80	
		0.5004	2.20	4.40	4.60
T - 22	2	0.5001	1.90	3.80	
		0.5004	1.70	3.40	3.60
T - 32	3	0.5000	3.10	6.20	
		0.5007	3.00	6.00	6.10
T - 32	3 *	1.0000	2.30	2.30	
	3 *	1.0000	2.10	2.10	
	3 *	1.0000	2.20	2.20	2.20
	4 *	1.0000	0.20	0.20	
	4 *	1.0000	0.22	0.22	0.21

*air dried sample

APPENDIX L

METAL CONCENTRATION IN SAMPLES FROM SAND SPRINGS PETROCHEMICAL COMPLEX (T-32) AND OSU AGRONOMY RESEARCH STATION SITES

Metal	Sand Springs Petrochemical Complex (Site 3) mg/kg	OSU Agronomy Station mg/kg	Sand Springs Petrochemical Complex (Site 3) Mean mg/kg	OSU Agronomy Station Standard Deviation mg/kg	OSU Agronomy Station Mean mg/kg	Sand Springs Petrochemical Complex Standard Deviation mg/kg
CADMIUM	<1.0	<1.0				
	<1.0	<1.0				
	<1.0	<1.0				
	<1.0	<1.0	<1.0	------	<1.0	------
CHROMIUM	21.9	8.3				
	20.1	6.5				
	18.2	6.3				
	16.7	6.2	19.2	0.9	6.8	1.9
COPPER	12.6	3.4				
	13.2	3.3				
	11.3	3.8				
	17.2	4.0	13.6	0.3	3.6	2.2
NICKEL	9.4	<3.0				
	11.2	<3.0				
	8.6	<3.0				
	8.2	<3.0	9.4	------	<3.0	1.1
LEAD	<8.0	<8.0				
	<8.0	<8.0				
	<8.0	<8.0				
	<8.0	<8.0	<8.0	------	<8.0	<8.0
ZINC	143.9	11.1				
	155.0	11.5				
	151.3	14.8				
	118.5	12.7	142.2	1.5	12.5	14.2

APPENDIX M

RELATIONSHIP BETWEEN WET WEIGHT AND DRY WEIGHT AT DIFFERENT pHs USING OVEN TEMPERATURE OF 103°C

pH	Volume ml	Conc. (Wet) g/L	Mass of Dish g/L	Mass of Dish + Sample g/L	Mass of Sample g/L	Mass of Sample - Buffer g/L	Dry/Wet
5.4	20	0.0	43.8358	44.1184	14.1300		
5.4	20	0.0	44.5469	44.8261	13.9600		
7.4	20	0.0	21.1383	21.5471	20.4400		
7.4	20	0.0	22.0195	22.4329	20.6700		
8.9	20	0.0	44.7004	45.1343	21.6950		
8.9	20	0.0	43.8352	44.2732	21.9000		
5.4	20	4.9	47.5150	47.8172	15.1100	1.1275	0.2284
5.4	20	4.9	46.1991	46.5038	15.2350		
5.4	20	9.9	1.5425	1.8711	16.4300	2.5700	0.2603
5.4	20	9.9	1.5395	1.8755	16.8000		
5.4	10	19.8	1.5347	1.7206	18.5900	4.6900	0.2375
5.4	10	19.8	1.5211	1.7099	18.8800		
5.4	10	39.5	1.5164	1.7566	24.0200	9.6500	0.2443
5.4	10	39.5	1.5458	1.7795	23.3700		
7.4	20	4.9	38.7020	39.0735	18.5750	-1.9650	-0.3980
7.4	20	4.9	45.2752	45.6473	18.6050		
7.4	10	9.9	1.5237	1.7346	21.0900	0.0800	0.0081
7.4	10	9.9	1.5285	1.7303	20.1800		
7.4	10	19.8	1.5280	1.7578	22.980	2.0000	0.1013
7.4	10	19.8	1.5427	1.7640	22.1300		
7.4	10	39.5	1.5335	1.8138	28.0300	7.0550	0.1786
7.4	10	39.5	1.5210	1.7929	27.1900		
8.9	20	4.9	44.8965	45.2805	19.2000	-2.6150	-0.5296
8.9	20	4.9	52.3940	52.7773	19.1650		
8.9	10	9.9	1.5092	1.7150	20.5800	-0.8675	-0.0878
8.9	10	9.9	1.5114	1.7242	21.2800		
8.9	10	19.8	1.5239	1.7566	23.2700	1.5225	0.0771
8.9	10	19.8	1.5397	1.7734	23.3700		
8.9	10	39.5	1.5306	1.8086	27.8000	6.1925	0.1568
8.9	10	39.5	1.5426	1.8244	28.1800		

APPENDIX N

RELATIONSHIP BETWEEN WET WEIGHT AND DRY WEIGHT AT DIFFERENT pHs USING OVEN TEMPERATURE OF 56°C

pH	Volume ml	Conc. (Wet) g/L	Mass of Dish g/L	Mass of Dish + Sample g/L	Mass of Sample g/L	Mass of Sample - Buffer g/L	Dry/Wet
5.4	20	0.0	39.4539	39.7419	14.4000		
5.4	20	0.0	75.2003	75.4797	13.9700		
7.4	20	0.0	68.4407	68.8315	19.5400		
7.4	20	0.0	47.2990	47.7429	22.1950		
8.9	20	0.0	48.8717	49.3436	23.5950		
8.9	20	0.0	73.9123	74.3870	23.7350		
5.4	20	4.9	79.1665	79.4716	15.2550	1.1300	0.2289
5.4	20	4.9	48.3553	48.6628	15.3750		
5.4	20	9.9	43.2513	43.5870	16.7850	2.4000	0.2430
5.4	20	9.9	66.3592	66.6869	16.3850		
5.4	10	19.8	1.5279	1.7195	19.1600	5.0900	0.2577
5.4	10	19.8	1.5436	1.7375	19.3900		
5.4	10	39.5	1.5389	1.7838	24.4900	10.2500	0.2595
5.4	10	39.5	1.5466	1.7904	24.3800		
7.4	20	4.9	74.6079	75.0182	20.5150	-0.3775	-0.0765
7.4	20	4.9	45.6047	46.0139	20.4600		
7.4	10	9.9	1.5362	1.7613	22.5100	1.6300	0.1651
7.4	10	9.9	1.5131	1.7379	22.4800		
7.4	10	19.8	1.5104	1.7592	24.8800	3.5750	0.1810
7.4	10	19.8	1.5460	1.7860	24.0000		
7.4	10	19.5	1.5218	1.8083	28.6500	8.2900	0.2099
7.4	10	39.5	1.5684	1.8650	29.6600		
8.9	20	4.9	46.4389	46.9180	23.9550	-0.0075	-0.0015
8.9	20	4.9	43.9144	44.3816	23.3600		
8.9	10	9.9	1.5164	1.7525	23.6100	0.5800	0.0587
8.9	10	9.9	1.5087	1.7575	24.8800		
8.9	10	19.8	1.5389	1.8004	26.1500	2.5200	0.1276
8.9	10	19.8	1.5414	1.8036	26.2200		
8.9	10	39.5	1.5418	1.8536	31.1800	7.2800	0.1843
8.9	10	39.5	1.5333	1.8404	30.7100		

APPENDIX O

RELATIONSHIP BETWEEN WET WEIGHT, DRY WEIGHT AND OPTICAL DENSITY AT pH = 5.4, 7.4 AND 8.9 AND OVEN TEMPERATURE OF 56°C

Average Wet Wt. (pH = 5.4) g/L	Dry Wt. (56°C) g/L	Optical Density (x0.1)	Optical density (x0.01)
0.0000	0.0000	0.00	0.00
4.9375	1.1300	0.20	0.02
9.8750	2.4000	0.45	0.04
19.7500	5.0900	0.97	0.08
39.5000	10.2500	1.71	0.17

Average Wet Wt. (pH = 7.4) g/L	Dry Wt. (56°C) g/L	Optical Density (x0.1)	Optical Density (x0.01)
0.0000	0.0000	0.00	0.00
4.9375	-0.3775	0.22	0.02
9.8750	1.6300	0.49	0.04
19.7500	3.5750	1.03	0.08
39.5000	8.2900	1.82	0.19

Average Wet Wt. (pH = 8.9) g/L	Dry Wt. (56°C) g/L	Optical Density (x0.1)	Optical Density (x0.01)
0.0000	0.0000	0.00	0.00
4.9375	-0.0075	0.29	0.02
9.8750	0.5800	0.57	0.05
19.7500	2.5200	1.12	0.10
39.5000	7.2800	1.90	0.25

APPENDIX P

EVALUATION OF ADSORPTION OF MICROORGANISMS ONTO AQUIFER MATERIAL USING pH 7.4 AT 25°C (ROOM TEMPERATURE)

TEMPERATURE 25°C OPTICAL DENSITY 1.141

pH: 7.4 (1:10 dilution)

TOTAL SOLIDS:

initial # of microorganisms/ml:	4.80E+12
Initial concentration in 0.1 ml:	4.80E+11
Stock (1:10) dilution	4.80E+11

# of microorganisms/ml:

Dilution Factor	Blank #	(OSU Agronomy) #	Blank CFU/ml	Soil 1 (OSU Agronomy) CFU/ml
0	—	0	—	0.00E+00
0	0	0	0.00E+00	0.00E+00
0	0	0	0.00E+00	0.00E+00
100	4864	4600	4.86E+05	4.60E+05
100	3280	3624	3.28E+05	3.62E+05
100	5360	—	5.36E+05	—
1000	3328	2080	3.33E+06	2.08E+06
1000	3560	2328	3.56E+06	2.33E+06
1000	3184	1140	3.18E+06	1.14E+06
10000	3360	1336	3.36E+07	1.34E+07
10000	5648	1092	5.65E+07	1.09E+07
10000	TMTC	620	TMTC	6.20E+06

AVERAGE CELLS REMAINING IN SOLUTION (COUNTED)

Blank CFU/ml	OSU CFU/ml
0	0
450133	411200
3357333	1849333
45040000	10160000

AVERAGE CELLS REMAINING IN SOLUTION AND x^*/m (CORRECTED FOR DILUTION)

Cell Concentration	x^*/m	
Blank (CFU/ml)	OSU (CFU/ml)	OSU (CFU/g)
0.00E+00	0.00E+00	0.00E+00
4.50E+05	4.11E+05	1.30E+06
3.36E+07	1.85E+07	5.03E+08
4.50E+09	1.02E+09	1.16E+11

LOG (C) AND LOG (x^*/m) FOR OKLAHOMA STATE UNIVERSITY AGRONOMY RESEARCH STATION AQUIFER MATERIALS

LOG(C) (CFU/ml)	LOG(x^*/m) (CFU/g)
---	---
5.61	6.11
7.27	8.70
9.01	11.07

RELATIONSHIP BETWEEN LOG C (CFU/ml) AND LOG x^*/m (CFU/g) OSU AGRONOMY STATION USING COLONY FORMING UNITS

Regression Output:

Constant	-2.0162774492
Standard Error of Y Est	0.143185381
R Squared	0.9983291958
Number of Observations	3
Degrees of Freedom	1
X Coefficient(s)	1.4587364494
Standard Error of Coefficient	0.0596764148

SLOPE = 1.458736449 =(1/N)
===> N = 0.685524791

APPENDIX Q

EXTRACTION EFFICIENCIES

RANGE OF EXTRACTION EFFICIES UNDER DIFFERENT EXPERIMENTAL CONDITIONS FOR TEST COMPOUNDS

Compound	pH	Temperature °C	Extraction Efficiency % Blank	OSU	Sand Springs
DCP	6.4	15	82.74 - 92.45		
	7.4	15	85.96 - 93.40		
	7.8	15	91.72		
DCP	6.4	25	81.78 - 85.92	79.98 - 94.04	
	7.4	25	84.19 - 98.80	84.19	85.24 - 88.96
	7.8	25	77.70 - 87.49	90.31 - 99.80	
DCP	6.4	30	81.70 - 89.12		
	7.4	30	85.04 - 88.61		
	7.8	30	87.93 - 90.40		
DCE	7.4	25	93.44		
DBCP	7.4	25	81.76 - 113.00	106 - 109	

EXTRACTION EFFICIENCIES THE DIFFERENT KINETIC EXPERIMENTS

EXPERIMENT/ID NUMBER**	SUBSURFACE MATERIALS	pH	TEMPERATURE °C	EXTRACTION EFFICIENCY %
EFFECT OF PRESENCE OF AQUIFER MATERIALS (1,2 dibromo-3-chloropropane)				
DBCPDF	NONE	7.4	25	113
DBCPDF2	OSU	7.4	25	87
EFFECT OF AQUIFER				
AQUIFDF	OSU	7.4	25	99
AQUIFDF2	SS	7.4	25	99
EFFECT OF pH				
pH54(25)	OSU	5.4	25	99
pH74(25)	OSU	7.4	25	99
pH89(25)	OSU	8.9	25	99
EFFECT OF DISSOLVED OXYGEN[1]				
DOCOM3	OSU	7.4	25	99
DOCOM4	OSU	7.4	25	99
DOCOM5	OSU	7.4	25	99
EFFECT OF TEMPERATURE[2]				
pH64(15)	OSU	6.4	15	88
pH64(25)	OSU	6.4	25	85
pH64(30)	OSU	6.4	30	85
pH74(15)	OSU	7.4	15	90
pH74/25	OSU	7.4	25	99
pH74(30)	OSU	7.4	30	87
pH78(15)	OSU	7.8	15	92
pH78(25)	OSU	7.8	25	82
pH78(30)	OSU	7.8	30	89
EFFECT OF INOCULUM SIZE[3]				
INNODES1	OSU	7.4	25	99
INNODES2	OSU	7.4	25	99
INNODES3	OSU	7.4	25	99
INNODES4	OSU	7.4	25	99
EFFECT OF SUBSTRATE CONCENTRATIONS				
SUST1DF	OSU	7.4	25	99
SUST2DF	OSU	7.4	25	99
SUST3DF	OSU	7.4	25	99

TABLE continued

EXPERIMENT/ID NUMBER**	SUBSURFACE MATERIALS	pH	TEMPERATURE °C	EXTRACTION EFFICIENCY %
EFFECT OF HEAVY METAL CONCENTRATION[4]				
LEADDES1	OSU	7.4	25	99
LEADDES2	OSU	7.4	25	99
LEADDES3	OSU	7.4	25	99
LEADDES4	OSU	7.4	25	99

**Experiments performed at 16 mg/l dissolved oxygen and 1,2-chloropropane except where indicated.

[1]Dissolved oxygen was 6.0 mg/l, 8.2 mg/l and 16.0 mg/l for DOCOM3, DOCOM4, and DOCOM5 respectively.

[2]Experiments were conducted with chloride concentrations of 2.120 g/l, 0.871 g/l and 0.151 g/l at ph 6.4, 7.4 and 7.8 respectively.

[3]Experiments were performed using inoculum sizes of 1.455 g/l, 3.317 g/l, 6.470 g/l and 8.017 g/l for INNODES1, INNODES2, INNODES3 and INNODES 4 respectively.

[4]Lead concentrations were 0 mg/l, 10 mg/l, 5.8 mg/l and 2.2 mg/l for LEADDES1, LEADDES2 and LEADDES4 respectively

NA - not applicable

OSU - Oklahoma State University Agronomy Research Station

SS - Sand Springs Petrochemical Complex

APPENDIX R

STATISTICAL ANALYSIS OF EXPERIMENTS TO ACCOUNT FOR ABIOTIC LOSSES OF TEST COMPOUNDS

-- SAS PROGRAM AND ANALYSIS --

```
option PS=60 LS=64 NODATE NONUMBER;
*  ABIOTIC.CTL;
DATA ABI;
    INFILE 'B:PRELIM.DAT';
    INPUT IDN $1-8 FACTOR $ PH COMPND $ CONC;
PROC SORT DATA=ABI;
    BY PH COMPND;
PROC PRINT DATA=ABI;
    TITLE 'DATA FOR ABIOTIC EXPERIMENT';
PROC ANOVA DATA=ABI;
    BY PH COMPND;
    CLASSES FACTOR;
    MODEL CONC=FACTOR;
    MEANS FACTOR;
TITLE 'ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR AS A CRD';
TITLE2 'FOR EACH PH LEVEL AND COMPOUND!';
RUN;
```

DATA FOR ABIOTIC EXPERIMENT

OBS	IDN	FACTOR	PH	COMPND	CONC
1	1	OSU	6.4	DCP	870
2	2	OSU	6.4	DCP	871
3	3	OSU	6.4	DCP	834
4	1B	OSU	6.4	DCP	886
5	3B	OSU	6.4	DCP	901
6	50	PHOTO.	6.4	DCP	945
7	51	PHOTO.	6.4	DCP	806
8	53	GLASS	6.4	DCP	901
9	54	GLASS	6.4	DCP	835
10	57	VOLAT	6.4	DCP	826
11	58	VOLAT	6.4	DCP	861
12	60	VOLAT	6.4	DCP	871
13	60B	VOLAT	6.4	DCP	924
14	61B	VOLAT	6.4	DCP	965
15	C01	CONTR	6.4	DCP	803
16	C02	CONTR	6.4	DCP	912
17	C03	CONTR	6.4	DCP	997
18	34	OSU	7.4	DBCP	1043
19	35	OSU	7.4	DBCP	1025
20	35B	OSU	7.4	DBCP	956
21	36B	OSU	7.4	DBCP	930
22	98	PHOTO	7.4	DBCP	1037
23	99	PHOTO	7.4	DBCP	843
24	102	GLASS	7.4	DBCP	1006
25	103	GLASS	7.4	DBCP	1122
26	105	VOLAT	7.4	DBCP	1023
27	106	VOLAT	7.4	DBCP	1280
28	107	VOLAT	7.4	DBCP	982
29	108B	VOLAT	7.4	DBCP	846
30	C13	CONTR	7.4	DBCP	1113
31	C14	CONTR	7.4	DBCP	1002
32	C15	CONTR	7.4	DBCP	1101
33	121	PHOTO	7.4	DCE	1064
34	122	PHOTO	7.4	DCE	998
35	125	GLASS	7.4	DCE	1008
36	126	GLASS	7.4	DCE	1076
37	129	VOLAT	7.4	DCE	991
38	130	VOLAT	7.4	DCE	1028
39	C10	CONTR	7.4	DCE	1099
40	C11	CONTR	7.4	DCE	1015
41	C12	CONTR	7.4	DCE	1118
42	9	OSU	7.4	DCP	1097
43	11	OSU	7.4	DCP	1064
44	11B	OSU	7.4	DCP	1007
45	12B	OSU	7.4	DCP	1031

Table continued

OBS	IDN	FACTOR	PH	COMPND	CONC
46	13	SANDSP	7.4	DCP	1169
47	15	SANDSP	7.4	DCP	1088
48	15B	SANDSP	7.4	DCP	982
49	16B	SANDSP	7.4	DCP	971
50	17B	SANDSP	7.4	DCP	914
51	61	PHOTO.	7.4	DCP	1034
52	62	PHOTO.	7.4	DCP	1064
53	63	PHOTO.	7.4	DCP	1107
54	65	GLASS	7.4	DCP	1044
55	67	GLASS	7.4	DCP	1136
56	69	VOLAT	7.4	DCP	1000
57	70	VOLAT	7.4	DCP	1141
58	71	VOLAT	7.4	DCP	1156
59	C04	CONTR	7.4	DCP	1112
60	C05	CONTR	7.4	DCP	1080
61	C06	CONTR	7.4	DCP	1075
62	18	OSU	7.8	DCP	816
63	20	OSU	7.8	DCP	960
64	20B	OSU	7.8	DCP	942
65	73	PHOTO.	7.8	DCP	973
66	74	PHOTO.	7.8	DCP	1007
67	77	GLASS	7.8	DCP	988
68	78	GLASS	7.8	DCP	1014
69	83	VOLAT	7.8	DCP	1003
70	83B	VOLAT	7.8	DCP	883
71	85B	VOLAT	7.8	DCP	891
72	C07	CONTR	7.8	DCP	900
73	C08	CONTR	7.8	DCP	1044
74	C09	CONTR	7.8	DCP	1001

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=6.4 COMPND=DCP ----------

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
FACTOR	5	CONTR GLASS OSU PHOTO. VOLAT

Number of observations in by group = 17

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=6.4 COMPND=DCP ----------

Analysis of Variance Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	4	2651/57058824	0.18	0.9467
Error	12	45314.90000000		
Corrected Total	16	57966.47058824		

R-Square	C.V.	CONC Mean
0.055280	6.960749	882.82352941

Source	DF	Anova SS	F Value	Pr > F
FACTOR	4	2651.57058824	0.18	0.9467

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=6.4 COMPND=DCP ----------

Analysis of Variance Procedure

Level of FACTOR	N	CONC Mean	CONC SD
CONTR	3	904.000000	97.2471079
GLASS	2	868.000000	46.6690476
OSU	5	872.400000	24.9258902
PHOTO.	2	875.500000	98.2878426
VOLAT	5	889.400000	54.9481574

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DBCP ----------

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
FACTOR	5	CONTR GLASS OSU PHOTO VOLAT

Number of observations in by group = 15

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DBCP ----------

Analysis of Variance Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	4	29397.8500000	0.52	0.7212
Error	10	140427.7500000		
Corrected Total	14	169825.6000000		

R-Square	C.V.	CONC Mean
0.173106	11.61103	1020.6000000

Source	DF	Anova SS	F Value	Pr > F
FACTOR	4	29397.8500000	0.52	0.7212

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DBCP ----------

Analysis of Variance Procedure

Level of FACTOR	N	CONC Mean	CONC SD
CONTR	3	1072.00000	60.917978
GLASS	2	1064.00000	82.024387
OSU	4	988.50000	54.101756
PHOTO	2	940.00000	137.178716
VOLAT	4	1032.75000	181.364045

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DCE ----------

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
FACTOR	4	CONTR GLASS PHOTO VOLAT

Number of observations in by group = 9

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DCE ----------

Analysis of Variance Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	6059.72222222	0.90	0.5016
Error	5	11183.16666667		
Corrected Total	8	17242.88888889		

R-Square	C.V.	CONC Mean
0.351433	4.529504	1044.1111111

Source	DF	Anova SS	F Value	Pr > F
FACTOR	3	6059.72222222	0.90	0.5016

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DCE ----------

Analysis of Variance Procedure

Level of FACTOR	N	CONC Mean	CONC SD
CONTR	3	1077.33333	54.8117992
GLASS	2	1042.00000	48.0832611
PHOTO	2	1031.00000	46.6690476
VOLAT	2	1009.50000	26.1629509

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DCP ----------

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
FACTOR	6	CONTR GLASS OSU PHOTO. SANDSP VOLAT

Number of observations in by group = 20

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DCP ----------

Analysis of Variance Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	5	15450.5833333	0.63	0.6819
Error	14	68950.2166667		
Corrected Total	19	84400.8000000		

R-Square	C.V.	CONC Mean
0.183062	6.598200	1063.6000000

Source	DF	Anova SS	F Value	Pr > F
FACTOR	5	15450.5833333	0.63	0.6819

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.4 COMPND=DCP ----------

Analysis of Variance Procedure

Level of FACTOR	N	CONC Mean	CONC SD
CONTR	3	1089.00000	20.074860
GLASS	2	1090.00000	65.053824
OSU	4	1049.75000	39.220530
PHOTO.	3	1068.33333	36.692415
SANDSP	5	1024.80000	102.213991
VOLAT	3	1099.00000	86.063930

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.8 COMPND=DCP ----------

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
FACTOR	5	CONTR GLASS OSU PHOTO. VOLAT

Number of observations in by group = 13

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.8 COMPND=DCP ----------

Analysis of Variance Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	4	18595.8974359	1.12	0.4107
Error	8	33159.3333333		
Corrected Total	12	51755.2307692		

R-Square	C.V.	CONC Mean
0.359305	6.737670	955.53846154

Source	DF	Anova SS	F Value	Pr > F
FACTOR	4	18595.8974359	1.12	0.4107

ANALYSIS OF CONCENTRATION (μg/L)-VS- FACTOR
AS A CRD FOR EACH PH LEVEL AND COMPOUND!

---------- PH=7.8 COMPND=DCP ----------

Analysis of Variance Procedure

Level of FACTOR	N	CONC Mean	CONC SD
CONTR	3	981.66667	73.9211291
GLASS	2	1001.00000	18.3847763
OSU	3	906.00000	78.4601810
PHOTO.	2	990.00000	24.0416306
VOLAT	3	925.66667	67.0919767

APPENDIX S

STATISTICAL ANALYSIS OF EXPERIMENTS TO ACCOUNT FOR CELLULAR ADSORPTION OF TEST COMPOUNDS

-- SAS PROGRAM AND ANALYSIS --

```
option PS=60 LS=64 NODATE NONUMBER;
*  INHIBIT.CTL;
DATA ADS;
    INFILE 'B:INHIBIT.DAT';
    INPUT TIME COMPND $ FACTOR $ CONC IDN $;
PROC SORT DATA=ADS;
    BY IDN COMPND FACTOR TIME;
PROC PRINT DATA=ADS;
    TITLE 'DATA FOR CELL ADSORPTION EXPERIMENT';
PROC GLM DATA=ADS;
    BY IDN COMPND;
    CLASSES FACTOR TIME;
    MODEL CONC=FACTOR|TIME / SS1;
    MEANS FACTOR|TIME;
TITLE 'ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD';
TITLE2 'FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!';
RUN;
DATA DCP78KC; SET ADS;
    IF IDN='DCP78KC';
    T2=TIME**2; T3=TIME**3;
    T4=TIME**4; T5=TIME**5;
PROC GLM DATA=DCP78KC;
    BY IDN COMPND FACTOR;
    MODEL CONC =TIME T2 T3 T4 T5 / SS1;
    TITLE '5-TH DEGREE POLYNOMIAL OF CONCENTRATION OVER TIME FOR
EACH';
    TITLE2 'FACTOR AT IDN=DCP78KC AND COMPOUND=DCP';
RUN;
```

DATA FOR ADENIKE'S CELL ADSORPTION EXPERIMENT

OBS	TIME	COMPND	FACTOR	CONC	IDN
1	0.0083	dcp	buffer	1012	agdcp74
2	0.0083	dcp	buffer	798	agdcp74
3	1.5000	dcp	buffer	757	agdcp74
4	1.5000	dcp	buffer	791	agdcp74
5	4.0000	dcp	buffer	600	agdcp74
6	4.0000	dcp	buffer	792	agdcp74
7	0.0083	dcp	cell	525	agdcp74
8	0.0083	dcp	cell	683	agdcp74
9	1.5000	dcp	cell	801	agdcp74
10	1.5000	dcp	cell	740	agdcp74
11	4.0000	dcp	cell	639	agdcp74
12	4.0000	dcp	cell	772	agdcp74
13	0.0083	dbcp	buffer	856	dbcp74kc
14	0.0083	dbcp	buffer	963	dbcp74kc
15	0.5000	dbcp	buffer	995	dbcp74kc
16	0.5000	dbcp	buffer	991	dbcp74kc
17	1.0000	dbcp	buffer	885	dbcp74kc
18	0.0083	dbcp	cell	851	dbcp74kc
19	0.0083	dbcp	cell	859	dbcp74kc
20	0.5000	dbcp	cell	910	dbcp74kc
21	0.5000	dbcp	cell	948	dbcp74kc
22	1.0000	dbcp	cell	1021	dbcp74kc
23	1.0000	dbcp	cell	795	dbcp74kc
24	0.0083	dcp	buffer	508	dcp64kc
25	0.0083	dcp	buffer	1025	dcp64kc
26	0.5000	dcp	buffer	575	dcp64kc
27	0.5000	dcp	buffer	443	dcp64kc
28	1.0000	dcp	buffer	916	dcp64kc
29	1.0000	dcp	buffer	923	dcp64kc
30	1.5000	dcp	buffer	694	dcp64kc
31	1.5000	dcp	buffer	748	dcp64kc
32	2.0000	dcp	buffer	598	dcp64kc
33	2.0000	dcp	buffer	558	dcp64kc
34	4.0000	dcp	buffer	964	dcp64kc
35	4.0000	dcp	buffer	1061	dcp64kc
36	0.0083	dcp	cell	689	dcp64kc
37	0.0083	dcp	cell	650	dcp64kc
38	0.5000	dcp	cell	817	dcp64kc
39	0.5000	dcp	cell	748	dcp64kc
40	1.0000	dcp	cell	738	dcp64kc
41	1.0000	dcp	cell	600	dcp64kc
42	1.5000	dcp	cell	759	dcp64kc
43	1.5000	dcp	cell	706	dcp64kc
44	2.0000	dcp	cell	547	dcp64kc
45	2.0000	dcp	cell	704	dcp64kc
46	4.0000	dcp	cell	1062	dcp64kc
47	4.0000	dcp	cell	415	dcp64kc

Table continued

OBS	TIME	COMPND	FACTOR	CONC	IDN
48	0.0083	dcp	buffer	1003	dcp74kc
49	0.0083	dcp	buffer	653	dcp74kc
50	0.5000	dcp	buffer	643	dcp74kc
51	0.5000	dcp	buffer	820	dcp74kc
52	1.0000	dcp	buffer	1191	dcp74kc
53	1.0000	dcp	buffer	660	dcp74kc
54	1.5000	dcp	buffer	665	dcp74kc
55	1.5000	dcp	buffer	698	dcp74kc
56	2.0000	dcp	buffer	727	dcp74kc
57	2.0000	dcp	buffer	770	dcp74kc
58	4.0000	dcp	buffer	813	dcp74kc
59	4.0000	dcp	buffer	701	dcp74kc
60	0.0083	dcp	cell	663	dcp74kc
61	0.0083	dcp	cell	735	dcp74kc
62	0.5000	dcp	cell	777	dcp74kc
63	0.5000	dcp	cell	772	dcp74kc
64	1.0000	dcp	cell	897	dcp74kc
65	1.0000	dcp	cell	1059	dcp74kc
66	1.5000	dcp	cell	506	dcp74kc
67	1.5000	dcp	cell	655	dcp74kc
68	2.0000	dcp	cell	559	dcp74kc
69	2.0000	dcp	cell	588	dcp74kc
70	4.0000	dcp	cell	790	dcp74kc
71	4.0000	dcp	cell	696	dcp74kc
72	0.0083	dcp	buffer	603	dcp78kc
73	0.0083	dcp	buffer	631	dcp78kc
74	0.5000	dcp	buffer	657	dcp78kc
75	1.0000	dcp	buffer	665	dcp78kc
76	1.0000	dcp	buffer	967	dcp78kc
77	1.5000	dcp	buffer	1056	dcp78kc
78	1.5000	dcp	buffer	1185	dcp78kc
79	2.0000	dcp	buffer	785	dcp78kc
80	2.0000	dcp	buffer	1069	dcp78kc
81	4.0000	dcp	buffer	546	dcp78kc
82	4.0000	dcp	buffer	512	dcp78kc
83	0.0083	dcp	cell	796	dcp78kc
84	0.0083	dcp	cell	773	dcp78kc
85	0.5000	dcp	cell	1134	dcp78kc
86	0.5000	dcp	cell	608	dcp78kc
87	1.0000	dcp	cell	500	dcp78kc
88	1.0000	dcp	cell	910	dcp78kc
89	1.5000	dcp	cell	695	dcp78kc
90	1.5000	dcp	cell	697	dcp78kc
91	2.0000	dcp	cell	411	dcp78kc
92	2.0000	dcp	cell	561	dcp78kc
93	4.0000	dcp	cell	642	dcp78kc
94	4.0000	dcp	cell	587	dcp78kc

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=agdcp74 COMPND=dcp ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
FACTOR	2	buffer cell
TIME	3	4 1.5 0.0083

Number of observations in by group = 12

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=agdcp74 COMPND=dcp ----------

General Linear Models Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	5	101792.000000	1.88	0.2327
Error	6	65095.000000		
Corrected Total	11	166887.000000		

R-Square	C.V.	CONC Mean
0.609946	14.02819	742.50000000

Source	DF	Type I SS	F Value	Pr > F
FACTOR	1	29008.3333333	2.67	0.1531
TIME	2	11088.5000000	0.51	0.6238
FACTOR*TIME	2	61695.1666667	2.84	0.1353

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=agdcp74 COMPND=dcp ----------

General Linear Models Procedure

Level of FACTOR	N	CONC Mean	CONC SD
buffer	6	791.666667	131.548724
cell	6	693.333333	101.344298

Level of TIME	N	CONC Mean	CONC SD
4	4	700.750000	95.510471
1.5	4	772.250000	28.581754
0.0083	4	754.500000	204.923563

Level of FACTOR	Level of TIME	N	CONC Mean	CONC SD
buffer	4	2	696.000000	135.764502
buffer	1.5	2	774.000000	24.041631
buffer	0.0083	2	905.000000	151.320851
cell	4	2	705.500000	94.045202
cell	1.5	2	770.500000	43.133514
cell	0.0083	2	604.000000	111.722871

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dbcp74kc COMPND=dbcp ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
FACTOR	2	buffer cell
TIME	3	1 0.5 0.0083

Number of observations in by group = 11

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dbcp74kc COMPND=dbcp ----------

General Linear Models Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	5	20811.1363636	0.65	0.6761
Error	5	32024.5000000		
Corrected Total	10	52835.6363636		

R-Square	C.V.	CONC Mean
0.393884	8.738702	915.81818182

Source	DF	Type I SS	F Value	Pr > F
FACTOR	1	4510.3030303	0.70	0.4396
TIME	2	12873.1770833	1.00	0.4297
FACTOR*TIME	2	3427.6562500	0.27	0.7755

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dbcp74kc COMPND=dbcp ----------

General Linear Models Procedure

Level of FACTOR	N	CONC Mean	CONC SD
buffer	5	938.000000	63.6710295
cell	6	897.333333	80.1365501

Level of TIME	N	CONC Mean	CONC SD
1	3	900.333333	113.777561
0.5	4	961.000000	40.108187
0.0083	4	882.250000	53.934374

Level of FACTOR	Level of TIME	N	CONC Mean	CONC SD
buffer	1	1	885.000000	
buffer	0.5	2	993.000000	2.828427
buffer	0.0083	2	909.500000	75.660426
cell	1	2	908.000000	159.806133
cell	0.5	2	929.000000	26.870058
cell	0.0083	2	855.000000	5.656854

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp64kc COMPND=dcp ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
FACTOR	2	buffer cell
TIME	6	1 2 4 0.5 1.5 0.0083

Number of observations in by group = 24

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp64kc COMPND=dcp ----------

General Linear Models Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	420206.000000	1.19	0.3829
Error	12	385040.000000		
Corrected Total	23	805246.000000		

R-Square	C.V.	CONC Mean
0.521836	24.63927	727.00000000

Source	DF	Type I SS	F Value	Pr > F
FACTOR	1	13920.166667	0.43	0.5226
TIME	5	195780.000000	1.22	0.3577
FACTOR*TIME	5	210505.833333	1.31	0.3225

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp64kc COMPND=dcp ----------

General Linear Models Procedure

Level of		CONC	
FACTOR	N	Mean	SD
buffer	12	751.083333	217.873799
cell	12	702.916667	156.428002

Level of		CONC	
TIME	N	Mean	SD
1	4	794.250000	155.238258
2	4	601.750000	71.602491
4	4	875.500000	310.421756
0.5	4	645.750000	169.208698
1.5	4	726.750000	31.595095
0.0083	4	718.000000	218.947482

Level of	Level of		CONC	
FACTOR	TIME	N	Mean	SD
buffer	1	2	919.50000	4.949747
buffer	2	2	578.00000	28.284271
buffer	4	2	1012.50000	68.589358
buffer	0.5	2	509.00000	93.338095
buffer	1.5	2	721.00000	38.183766
buffer	0.0083	2	766.50000	365.574206
cell	1	2	669.00000	97.580736
cell	2	2	625.50000	111.015765
cell	4	2	738.50000	457.498087
cell	0.5	2	782.50000	48.790368
cell	1.5	2	732.50000	37.476659
cell	0.0083	2	669.50000	27.577164

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp74kc COMPND=dcp ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
FACTOR	2	buffer cell
TIME	6	1 2 4 0.5 1.5 0.0083

Number of observations in by group = 24

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp74kc COMPND=dcp ----------

General Linear Models Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	314103.458333	1.33	0.3143
Error	12	257301.500000		
Corrected Total	23	571404.958333		

R-Square	C.V.	CONC Mean
0.549704	19.47965	751.70833333

Source	DF	Type I SS	F Value	Pr > F
FACTOR	1	17442.041667	0.81	0.3848
TIME	5	251835.208333	2.35	0.1047
FACTOR*TIME	5	44826.208333	0.42	0.8274

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp74kc COMPND=dcp ----------

General Linear Models Procedure

Level of FACTOR	N	CONC Mean	CONC SD
buffer	12	778.666667	165.066011
cell	12	724.750000	152.031172

Level of TIME	N	CONC Mean	CONC SD
1	4	951.750000	228.661868
2	4	661.000000	103.231132
4	4	750.000000	60.238415
0.5	4	753.000000	76.432977
1.5	4	631.000000	85.334635
0.0083	4	763.500000	163.791534

Level of FACTOR	Level of TIME	N	CONC Mean	CONC SD
buffer	1	2	925.500000	375.473701
buffer	2	2	748.500000	30.405592
buffer	4	2	757.000000	79.195959
buffer	0.5	2	731.500000	125.157900
buffer	1.5	2	681.500000	23.334524
buffer	0.0083	2	828.000000	247.487373
cell	1	2	978.000000	114.551299
cell	2	2	573.500000	20.506097
cell	4	2	743.000000	66.468037
cell	0.5	2	774.500000	3.535534
cell	1.5	2	580.500000	105.358910
cell	0.0083	2	699.000000	50.911688

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp78kc COMPND=dcp ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
FACTOR	2	buffer cell
TIME	6	1 2 4 0.5 1.5 0.0083

Number of observations in by group = 23

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp78kc COMPND=dcp ----------

General Linear Models Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	702337.369565	2.12	0.1136
Error	11	330637.500000		
Corrected Total	22	1032974.869565		

R-Square	C.V.	CONC Mean
0.679917	23.47006	738.69565217

Source	DF	Type I SS	F Value	Pr > F
FACTOR	1	52775.021080	1.76	0.2120
TIME	5	256198.348485	1.70	0.2140
FACTOR*TIME	5	393364.000000	2.62	0.0853

ANALYSIS OF CONCENTRATION AS A FAT (FACTOR*TIME) IN A CRD
FOR EACH COMBINATION OF ID BLOCK AND COMPOUND!

---------- IDN=dcp78kc COMPND=dcp ----------

General Linear Models Procedure

Level of FACTOR	N	CONC Mean	CONC SD
buffer	11	788.727273	237.979449
cell	12	692.833333	193.967586

Level of TIME	N	CONC Mean	CONC SD
1	4	760.500000	217.541567
2	4	706.500000	286.390759
4	4	571.750000	55.978418
0.5	3	799.666667	290.575865
1.5	4	908.250000	250.680906
0.0083	4	700.750000	97.831062

Level of FACTOR	Level of TIME	N	CONC Mean	CONC SD
buffer	1	2	816.00000	213.546248
buffer	2	2	927.00000	200.818326
buffer	4	2	529.00000	24.041631
buffer	0.5	1	657.00000	
buffer	1.5	2	1120.50000	91.216775
buffer	0.0083	2	617.00000	19.798990
cell	1	2	705.00000	289.913780
cell	2	2	486.00000	106.066017
cell	4	2	614.50000	38.890873
cell	0.5	2	871.00000	371.938167
cell	1.5	2	696.00000	1.414214
cell	0.0083	2	784.50000	16.263456

APPENDIX T

STATISTICAL ANALYSIS OF BUFFER CONTROLS FOR THE KINETIC EXPERIMENTS

-- SAS PROGRAM AND ANALYSIS --

PROGRAM

```
OPTIONS PS=60 LS=64;
* BUFFER.CTL;
DATA BUFFER2;
  INFILE 'BUFFER2.DAT';
  INPUT TIME CONC IDN $ TABLE;
PROC SORT DATA=BUFFER2;
  BY IDN time;
PROC MEANS; VAR CONC; BY IDN TIME;
OUTPUT OUT=MEANS MEAN=MCON;
DATA DIFF; SET MEANS; BY IDN;
RETAIN OLD;
IF FIRST.IDN THEN DO;
  OLD=MCON;
RETURN;
END;
DIFFCON=MCON-OLD;
OUTPUT;
KEEP DIFFCON IDN;
OLD=MCON;
PROC MEANS DATA=DIFF N MEAN STD T PRT; VAR DIFFCON; BY IDN;
RUN;
```

ANALYSIS

SAS
Analysis Variable : CONC

---------- IDN=AQUIFDF TIME=0.0083 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	1029.00	1226.00	1148.67

N Obs	Std Dev
3	105.1015382

---------- IDN=AQUIFDF TIME=0.5 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	1131.00	1152.00	1141.50

N Obs	Std Dev
2	14.8492424

---------- IDN=AQUIFDF TIME=1 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	1025.00	1121.00	1085.33

N Obs	Std Dev
3	52.5388745

---------- IDN=AQUIFDF TIME=2 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	986.0000000	1185.00	1104.33

N Obs	Std Dev
3	104.7107126

SAS
Analysis Variable : CONC

---------- IDN=AQUIFDF TIME=4 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	1041.00	1148.00	1088.00

N Obs	Std Dev
3	54.6717477

---------- IDN=AQUIFDF2 TIME=0.0083 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	1162.00	1202.00	1182.00

N Obs	Std Dev
2	28.2842712

---------- IDN=AQUIFDF2 TIME=0.5 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	1162.00	1164.00	1163.00

N Obs	Std Dev
2	1.4142136

---------- IDN=AQUIFDF2 TIME=1 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	1142.00	1239.00	1184.33

N Obs	Std Dev
3	49.6621922

SAS
Analysis Variable : CONC

IDN=AQUIFDF2 TIME=2

N Obs	N	Minimum	Maximum	Mean
2	2	978.0000000	996.00	987.0000000

N Obs	Std Dev
2	12.7279221

IDN=AQUIFDF2 TIME=4

N Obs	N	Minimum	Maximum	Mean
2	2	1127.00	1144.00	1135.50

N Obs	Std Dev
2	12.0208153

IDN=DOCOM3 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
1	1	1055.00	1055.00	1055.00

N Obs	Std Dev
1	.

IDN=DOCOM3 TIME=1

N Obs	N	Minimum	Maximum	Mean
1	1	1157.00	1157.00	1157.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

IDN=DOCOM3 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	875.0000000	963.0000000	919.0000000

N Obs	Std Dev
2	62.2253967

IDN=DOCOM3 TIME=2

N Obs	N	Minimum	Maximum	Mean
2	2	879.0000000	1009.00	944.0000000

N Obs	Std Dev
2	91.9238816

IDN=DOCOM3 TIME=4

N Obs	N	Minimum	Maximum	Mean
2	2	854.0000000	885.0000000	869.5000000

N Obs	Std Dev
2	21.9203102

IDN=DOCOM4 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	1084.00	1238.00	1161.00

N Obs	Std Dev
2	108.8944443

SAS
Analysis Variable : CONC

---------- IDN=DOCOM4 TIME=0.5 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	1167.00	1180.00	1173.50

N Obs	Std Dev
2	9.1923882

---------- IDN=DOCOM4 TIME=1 ----------

N Obs	N	Minimum	Maximum	Mean
1	1	1025.00	1025.00	1025.00

N Obs	Std Dev
1	.

---------- IDN=DOCOM4 TIME=1.5 ----------

N Obs	N	Minimum	Maximum	Mean
1	1	1185.00	1185.00	1185.00

N Obs	Std Dev
1	.

---------- IDN=DOCOM4 TIME=2 ----------

N Obs	N	Minimum	Maximum	Mean
1	1	1211.00	1211.00	1211.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

---------- IDN=DOCOM4 TIME=4 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	952.0000000	1368.00	1160.00

N Obs	Std Dev
2	294.1564210

---------- IDN=DOCOM5 TIME=0.0083 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	848.0000000	931.0000000	901.6666667

N Obs	Std Dev
3	46.5438861

---------- IDN=DOCOM5 TIME=0.5 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	856.0000000	980.0000000	915.6666667

N Obs	Std Dev
3	62.1315808

---------- IDN=DOCOM5 TIME=1 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	686.0000000	830.0000000	758.0000000

N Obs	Std Dev
2	101.8233765

SAS
Analysis Variable : CONC

IDN=DOCOM5 TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	871.0000000	955.0000000	911.0000000

N Obs	Std Dev
3	42.1426150

IDN=DOCOM5 TIME=3

N Obs	N	Minimum	Maximum	Mean
3	3	883.0000000	993.00	931.0000000

N Obs	Std Dev
3	56.3205114

IDN=DOCOM5 TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	878.0000000	904.0000000	888.3333333

N Obs	Std Dev
3	13.7961347

IDN=INNODES1 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	965.0000000	1018.00	991.50

N Obs	Std Dev
2	37.4766594

SAS
Analysis Variable : CONC

IDN=INNODES1 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	891.0000000	1063.00	950.3333333

N Obs	Std Dev
3	97.6183043

IDN=INNODES1 TIME=1

N Obs	N	Minimum	Maximum	Mean
1	1	1036.00	1036.00	1036.00

N Obs	Std Dev
1	.

IDN=INNODES1 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	936.0000000	980.0000000	958.0000000

N Obs	Std Dev
2	31.1126984

IDN=INNODES1 TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	911.0000000	970.0000000	941.3333333

N Obs	Std Dev
3	29.5352896

SAS
Analysis Variable : CONC

IDN=INNODES1 TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1052.00	1103.00	1071.67

N Obs	Std Dev
3	27.4286954

IDN=INNODES2 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	965.0000000	1018.00	991.50

N Obs	Std Dev
2	37.4766594

IDN=INNODES2 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	891.0000000	1063.00	950.3333333

N Obs	Std Dev
3	97.6183043

IDN=INNODES2 TIME=1

N Obs	N	Minimum	Maximum	Mean
1	1	1036.00	1036.00	1036.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

IDN=INNODES2 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	936.0000000	980.0000000	958.0000000

N Obs	Std Dev
2	31.1126984

IDN=INNODES2 TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	911.0000000	970.0000000	941.3333333

N Obs	Std Dev
3	29.5352896

IDN=INNODES2 TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1052.00	1103.00	1071.67

N Obs	Std Dev
3	27.4286954

IDN=INNODES3 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	965.0000000	1018.00	991.50

N Obs	Std Dev
2	37.4766594

SAS
Analysis Variable : CONC

IDN=INNODES3 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	891.0000000	1063.00	950.3333333

N Obs	Std Dev
3	97.6183043

IDN=INNODES3 TIME=1

N Obs	N	Minimum	Maximum	Mean
1	1	1036.00	1036.00	1036.00

N Obs	Std Dev
1	.

IDN=INNODES3 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	936.0000000	980.0000000	958.0000000

N Obs	Std Dev
2	31.1126984

IDN=INNODES3 TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	911.0000000	970.0000000	941.3333333

N Obs	Std Dev
3	29.5352896

SAS
Analysis Variable : CONC

IDN=INNODES3 TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1052.00	1103.00	1071.67

N Obs	Std Dev
3	27.4286954

IDN=INNODES4 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	965.0000000	1018.00	991.50

N Obs	Std Dev
2	37.4766594

IDN=INNODES4 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	891.0000000	1063.00	950.3333333

N Obs	Std Dev
3	97.6183043

IDN=INNODES4 TIME=1

N Obs	N	Minimum	Maximum	Mean
1	1	1036.00	1036.00	1036.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

---------- IDN=INNODES4 TIME=1.5 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	936.0000000	980.0000000	958.0000000

N Obs	Std Dev
2	31.1126984

---------- IDN=INNODES4 TIME=2 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	911.0000000	970.0000000	941.3333333

N Obs	Std Dev
3	29.5352896

---------- IDN=INNODES4 TIME=4 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	1052.00	1103.00	1071.67

N Obs	Std Dev
3	27.4286954

---------- IDN=LEADDES1 TIME=0.0083 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	837.0000000	1053.00	945.0000000

N Obs	Std Dev
2	152.7350647

SAS
Analysis Variable : CONC

IDN=LEADDES1 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
1	1	1264.00	1264.00	1264.00

N Obs	Std Dev
1	.

IDN=LEADDES1 TIME=1

N Obs	N	Minimum	Maximum	Mean
2	2	1010.00	1034.00	1022.00

N Obs	Std Dev
2	16.9705627

IDN=LEADDES1 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	914.0000000	942.0000000	928.0000000

N Obs	Std Dev
2	19.7989899

IDN=LEADDES1 TIME=2

N Obs	N	Minimum	Maximum	Mean
2	2	978.0000000	1115.00	1046.50

N Obs	Std Dev
2	96.8736290

SAS
Analysis Variable : CONC

IDN=LEADDES1 TIME=4

N Obs	N	Minimum	Maximum	Mean
2	2	919.0000000	1070.00	994.50

N Obs	Std Dev
2	106.7731240

IDN=LEADDES2 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
1	1	1051.00	1051.00	1051.00

N Obs	Std Dev
1	.

IDN=LEADDES2 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	982.0000000	1035.00	1008.50

N Obs	Std Dev
2	37.4766594

IDN=LEADDES2 TIME=1

N Obs	N	Minimum	Maximum	Mean
2	2	943.0000000	1026.00	984.5000000

N Obs	Std Dev
2	58.6898628

SAS
Analysis Variable : CONC

IDN=LEADDES2 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	834.0000000	912.0000000	873.0000000

N Obs	Std Dev
2	55.1543289

IDN=LEADDES2 TIME=2

N Obs	N	Minimum	Maximum	Mean
2	2	1064.00	1147.00	1105.50

N Obs	Std Dev
2	58.6898628

IDN=LEADDES2 TIME=4

N Obs	N	Minimum	Maximum	Mean
2	2	925.0000000	981.0000000	953.0000000

N Obs	Std Dev
2	39.5979797

IDN=LEADDES3 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	805.0000000	1050.00	927.5000000

N Obs	Std Dev
2	173.2411614

SAS
Analysis Variable : CONC

IDN=LEADDES3 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	1008.00	1050.00	1029.00

N Obs	Std Dev
2	29.6984848

IDN=LEADDES3 TIME=1

N Obs	N	Minimum	Maximum	Mean
2	2	919.0000000	1162.00	1040.50

N Obs	Std Dev
2	171.8269478

IDN=LEADDES3 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	966.0000000	1013.00	989.5000000

N Obs	Std Dev
2	33.2340187

IDN=LEADDES3 TIME=2

N Obs	N	Minimum	Maximum	Mean
1	1	874.0000000	874.0000000	874.0000000

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

IDN=LEADDES3 TIME=4

N Obs	N	Minimum	Maximum	Mean
2	2	1042.00	1108.00	1075.00

N Obs	Std Dev
2	46.6690476

IDN=LEADDES4 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	890.0000000	1002.00	946.0000000

N Obs	Std Dev
2	79.1959595

IDN=LEADDES4 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	914.0000000	1153.00	1033.50

N Obs	Std Dev
2	168.9985207

IDN=LEADDES4 TIME=1

N Obs	N	Minimum	Maximum	Mean
2	2	1044.00	1144.00	1094.00

N Obs	Std Dev
2	70.7106781

SAS
Analysis Variable : CONC

IDN=LEADDES4 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
1	1	1036.00	1036.00	1036.00

N Obs	Std Dev
1	.

IDN=LEADDES4 TIME=2

N Obs	N	Minimum	Maximum	Mean
1	1	840.0000000	840.0000000	840.0000000

N Obs	Std Dev
1	.

IDN=LEADDES4 TIME=4

N Obs	N	Minimum	Maximum	Mean
2	2	895.0000000	945.0000000	920.0000000

N Obs	Std Dev
2	35.3553391

IDN=SUST1DF TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	639.0000000	810.0000000	732.0000000

N Obs	Std Dev
3	86.4812118

SAS
Analysis Variable : CONC

---------- IDN=SUST1DF TIME=0.5 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	576.0000000	633.0000000	607.6666667

N Obs	Std Dev
3	29.0229794

---------- IDN=SUST1DF TIME=1 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	688.0000000	796.0000000	724.6666667

N Obs	Std Dev
3	61.7845720

---------- IDN=SUST1DF TIME=2 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	523.0000000	568.0000000	541.0000000

N Obs	Std Dev
3	23.8117618

---------- IDN=SUST1DF TIME=4 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	442.0000000	572.0000000	527.6666667

N Obs	Std Dev
3	74.2046719

SAS
Analysis Variable : CONC

IDN=SUST2DF TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	1102.00	1289.00	1209.00

N Obs	Std Dev
3	96.3794584

IDN=SUST2DF TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	1003.00	1122.00	1070.00

N Obs	Std Dev
3	60.9015599

IDN=SUST2DF TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1075.00	1319.00	1223.00

N Obs	Std Dev
3	130.0461457

IDN=SUST2DF TIME=2

N Obs	N	Minimum	Maximum	Mean
1	1	1213.00	1213.00	1213.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

IDN=SUST2DF TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1093.00	1162.00	1117.00

N Obs	Std Dev
3	39.0000000

IDN=SUST3DF TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	4520.00	5294.00	4907.00

N Obs	Std Dev
2	547.3006486

IDN=SUST3DF TIME=1

N Obs	N	Minimum	Maximum	Mean
2	2	4620.00	4810.00	4715.00

N Obs	Std Dev
2	134.3502884

IDN=SUST3DF TIME=1.5

N Obs	N	Minimum	Maximum	Mean
1	1	4514.00	4514.00	4514.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

IDN=SUST3DF TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	4370.00	4942.00	4696.67

N Obs	Std Dev
3	294.5459783

IDN=SUST3DF TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	4743.00	5420.00	5146.33

N Obs	Std Dev
3	356.6403417

IDN=dbcpdf TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	950.0000000	1016.00	986.3333333

N Obs	Std Dev
3	33.5012438

IDN=dbcpdf TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	976.0000000	1076.00	1026.00

N Obs	Std Dev
2	70.7106781

SAS
Analysis Variable : CONC

IDN=dbcpdf TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	965.0000000	1018.00	997.33

N Obs	Std Dev
3	28.3607710

IDN=dbcpdf TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	995.00	1026.00	1007.33

N Obs	Std Dev
3	16.4418166

IDN=dbcpdf TIME=3

N Obs	N	Minimum	Maximum	Mean
3	3	920.0000000	1034.00	983.3333333

N Obs	Std Dev
3	58.0459588

IDN=dbcpdf TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1008.00	1060.00	1037.33

N Obs	Std Dev
3	26.6333125

SAS
Analysis Variable : CONC

---------- IDN=dbcpdf2 TIME=0.0083 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	806.0000000	1178.00	1001.00

N Obs	Std Dev
3	186.6520828

---------- IDN=dbcpdf2 TIME=0.5 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	948.0000000	1148.00	1044.67

N Obs	Std Dev
3	100.1665280

---------- IDN=dbcpdf2 TIME=2 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	944.0000000	1162.00	1027.33

N Obs	Std Dev
3	117.7171752

---------- IDN=dbcpdf2 TIME=3 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	996.00	1113.00	1073.67

N Obs	Std Dev
3	67.2631648

SAS
Analysis Variable : CONC

IDN=dbcpdf2 TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	860.0000000	1211.00	1016.33

N Obs	Std Dev
3	178.6122430

IDN=pH54(25) TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	982.0000000	1109.00	1045.50

N Obs	Std Dev
2	89.8025612

IDN=pH54(25) TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	1040.00	1059.00	1049.50

N Obs	Std Dev
2	13.4350288

IDN=pH54(25) TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1069.00	1198.00	1138.33

N Obs	Std Dev
3	65.0410127

SAS
Analysis Variable : CONC

---------- IDN=pH54(25) TIME=1.5 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	1015.00	1087.00	1051.67

N Obs	Std Dev
3	36.0185138

---------- IDN=pH54(25) TIME=2 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	1065.00	1223.00	1146.33

N Obs	Std Dev
3	79.1033080

---------- IDN=pH54(25) TIME=4 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	925.0000000	1240.00	1094.33

N Obs	Std Dev
3	158.8279992

---------- IDN=pH64(15) TIME=0.0083 ----------

N Obs	N	Minimum	Maximum	Mean
1	1	1322.00	1322.00	1322.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

IDN=pH64(15) TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	939.0000000	1071.00	1019.00

N Obs	Std Dev
3	70.3135833

IDN=pH64(15) TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1088.00	1172.00	1122.67

N Obs	Std Dev
3	43.8786205

IDN=pH64(15) TIME=1.5

N Obs	N	Minimum	Maximum	Mean
3	3	965.0000000	1053.00	1009.33

N Obs	Std Dev
3	44.0037877

IDN=pH64(15) TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	1007.00	1335.00	1128.33

N Obs	Std Dev
3	179.8814424

SAS
Analysis Variable : CONC

---------- IDN=pH64(15) TIME=4 ----------

N Obs	N	Minimum	Maximum	Mean
3	3	801.0000000	1053.00	945.3333333

N Obs	Std Dev
3	129.9397296

---------- IDN=pH64(25) TIME=0.0083 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	1305.00	1315.00	1310.00

N Obs	Std Dev
2	7.0710678

---------- IDN=pH64(25) TIME=0.5 ----------

N Obs	N	Minimum	Maximum	Mean
2	2	1309.00	1356.00	1332.50

N Obs	Std Dev
2	33.2340187

---------- IDN=pH64(25) TIME=1 ----------

N Obs	N	Minimum	Maximum	Mean
1	1	1263.00	1263.00	1263.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

IDN=pH64(25) TIME=1.5

N Obs	N	Minimum	Maximum	Mean
1	1	1181.00	1181.00	1181.00

N Obs	Std Dev
1	.

IDN=pH64(25) TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	1041.00	1544.00	1215.33

N Obs	Std Dev
3	284.8092929

IDN=pH64(25) TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1091.00	1343.00	1213.00

N Obs	Std Dev
3	126.1903324

IDN=pH64(30) TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	1067.00	1083.00	1076.33

N Obs	Std Dev
3	8.3266640

SAS
Analysis Variable : CONC

IDN=pH64(30) TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	984.0000000	1254.00	1117.67

N Obs	Std Dev
3	135.0197516

IDN=pH64(30) TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1130.00	1240.00	1186.67

N Obs	Std Dev
3	55.0757055

IDN=pH64(30) TIME=1.5

N Obs	N	Minimum	Maximum	Mean
3	3	1042.00	1338.00	1158.67

N Obs	Std Dev
3	157.6367132

IDN=pH64(30) TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	906.0000000	1362.00	1161.67

N Obs	Std Dev
3	232.9814013

SAS
Analysis Variable : CONC

IDN=pH64(30) TIME=4

N Obs	N	Minimum	Maximum	Mean
2	2	1140.00	1544.00	1342.00

N Obs	Std Dev
2	285.6711396

IDN=pH74(15) TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	853.0000000	1021.00	937.0000000

N Obs	Std Dev
2	118.7939392

IDN=pH74(15) TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	1205.00	1253.00	1229.00

N Obs	Std Dev
2	33.9411255

IDN=pH74(15) TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1053.00	1101.00	1071.67

N Obs	Std Dev
3	25.7164020

SAS
Analysis Variable : CONC

IDN=pH74(15) TIME=1.5

N Obs	N	Minimum	Maximum	Mean
3	3	1183.00	1240.00	1219.00

N Obs	Std Dev
3	31.3209195

IDN=pH74(15) TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	1130.00	1351.00	1242.33

N Obs	Std Dev
3	110.5456165

IDN=pH74(15) TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1082.00	1325.00	1224.33

N Obs	Std Dev
3	126.7451511

IDN=pH74(25) TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	1097.00	1156.00	1130.67

N Obs	Std Dev
3	30.3699413

SAS
Analysis Variable : CONC

IDN=pH74(25) TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	1165.00	1293.00	1228.00

N Obs	Std Dev
3	64.0234332

IDN=pH74(25) TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1019.00	1102.00	1061.00

N Obs	Std Dev
3	41.5090352

IDN=pH74(25) TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	1140.00	1199.00	1169.50

N Obs	Std Dev
2	41.7193001

IDN=pH74(25) TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	1174.00	1223.00	1194.67

N Obs	Std Dev
3	25.3837218

SAS
Analysis Variable : CONC

IDN=pH74(25) TIME=4

N Obs	N	Minimum	Maximum	Mean
2	2	1090.00	1285.00	1187.50

N Obs	Std Dev
2	137.8858223

IDN=pH74(30) TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	1027.00	1123.00	1073.33

N Obs	Std Dev
3	48.0867272

IDN=pH74(30) TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	1186.00	1258.00	1222.00

N Obs	Std Dev
2	50.9116882

IDN=pH74(30) TIME=1

N Obs	N	Minimum	Maximum	Mean
2	2	1369.00	1387.00	1378.00

N Obs	Std Dev
2	12.7279221

SAS
Analysis Variable : CONC

IDN=pH74(30) TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	1244.00	1281.00	1262.50

N Obs	Std Dev
2	26.1629509

IDN=pH74(30) TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	1171.00	1268.00	1234.33

N Obs	Std Dev
3	54.8847277

IDN=pH74(30) TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1079.00	1283.00	1155.67

N Obs	Std Dev
3	111.0375312

IDN=pH74/25 TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	1305.00	1315.00	1310.00

N Obs	Std Dev
2	7.0710678

SAS
Analysis Variable : CONC

IDN=pH74/25 TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	1019.00	1356.00	1228.00

N Obs	Std Dev
3	182.5184922

IDN=pH74/25 TIME=1

N Obs	N	Minimum	Maximum	Mean
2	2	1353.00	1459.00	1406.00

N Obs	Std Dev
2	74.9533188

IDN=pH74/25 TIME=1.5

N Obs	N	Minimum	Maximum	Mean
2	2	1197.00	1494.00	1345.50

N Obs	Std Dev
2	210.0107140

IDN=pH74/25 TIME=2

N Obs	N	Minimum	Maximum	Mean
1	1	1303.00	1303.00	1303.00

N Obs	Std Dev
1	.

SAS
Analysis Variable : CONC

IDN=pH74/25 TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1091.00	1343.00	1213.00

N Obs	Std Dev
3	126.1903324

IDN=pH7815c TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	1296.00	1460.00	1404.67

N Obs	Std Dev
3	94.1134068

IDN=pH7815c TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	1595.00	1712.00	1653.50

N Obs	Std Dev
2	82.7314934

IDN=pH7815c TIME=1

N Obs	N	Minimum	Maximum	Mean
2	2	1523.00	1591.00	1557.00

N Obs	Std Dev
2	48.0832611

SAS
Analysis Variable : CONC

IDN=pH7815c TIME=1.5

N Obs	N	Minimum	Maximum	Mean
3	3	1049.00	1701.00	1370.33

N Obs	Std Dev
3	326.1001891

IDN=pH7815c TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	1298.00	1457.00	1397.00

N Obs	Std Dev
3	86.3770803

IDN=pH7815c TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1409.00	1651.00	1510.67

N Obs	Std Dev
3	125.5481315

IDN=pH7825c TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	1384.00	1587.00	1468.67

N Obs	Std Dev
3	105.6046085

SAS
Analysis Variable : CONC

IDN=pH7825c TIME=0.5

N Obs	N	Minimum	Maximum	Mean
2	2	894.0000000	1536.00	1215.00

N Obs	Std Dev
2	453.9625535

IDN=pH7825c TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1208.00	1770.00	1446.67

N Obs	Std Dev
3	290.4089071

IDN=pH7825c TIME=1.5

N Obs	N	Minimum	Maximum	Mean
3	3	1630.00	1679.00	1650.33

N Obs	Std Dev
3	25.5408170

IDN=pH7825c TIME=2

N Obs	N	Minimum	Maximum	Mean
3	3	1016.00	1577.00	1247.00

N Obs	Std Dev
3	293.3104158

SAS
Analysis Variable : CONC

IDN=pH7825c TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1707.00	1928.00	1828.67

N Obs	Std Dev
3	112.1799150

IDN=pH89(25) TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
3	3	1045.00	1110.00	1078.67

N Obs	Std Dev
3	32.5627599

IDN=pH89(25) TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	988.0000000	1114.00	1046.33

N Obs	Std Dev
3	63.5164021

IDN=pH89(25) TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1057.00	1171.00	1100.33

N Obs	Std Dev
3	61.7197969

SAS
Analysis Variable : CONC

IDN=pH89(25) TIME=1.5

N Obs	N	Minimum	Maximum	Mean
3	3	1076.00	1193.00	1149.67

N Obs	Std Dev
3	64.1274772

IDN=pH89(25) TIME=2

N Obs	N	Minimum	Maximum	Mean
2	2	1036.00	1233.00	1134.50

N Obs	Std Dev
2	139.3000359

IDN=pH89(25) TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1055.00	1135.00	1099.67

N Obs	Std Dev
3	40.8084958

IDN=ph7830c TIME=0.0083

N Obs	N	Minimum	Maximum	Mean
2	2	1089.00	1153.00	1121.00

N Obs	Std Dev
2	45.2548340

SAS
Analysis Variable : CONC

IDN=ph7830c TIME=0.5

N Obs	N	Minimum	Maximum	Mean
3	3	1089.00	1273.00	1155.00

N Obs	Std Dev
3	102.4304642

IDN=ph7830c TIME=1

N Obs	N	Minimum	Maximum	Mean
3	3	1126.00	1217.00	1167.67

N Obs	Std Dev
3	45.9818805

IDN=ph7830c TIME=1.5

N Obs	N	Minimum	Maximum	Mean
1	1	1105.00	1105.00	1105.00

N Obs	Std Dev
1	.

IDN=ph7830c TIME=2

N Obs	N	Minimum	Maximum	Mean
2	2	1151.00	1220.00	1185.50

N Obs	Std Dev
2	48.7903679

SAS
Analysis Variable : CONC

IDN=ph7830c TIME=4

N Obs	N	Minimum	Maximum	Mean
3	3	1234.00	1356.00	1315.33

N Obs	Std Dev
3	70.4367328

SAS
Analysis Variable : DIFFCON

IDN=AQUIFDF

N Obs	N	Mean	Std Dev	T	Prob > |T|
4	4	-15.1666667	31.1647355	-0.9733223	0.4022

IDN=AQUIFDF2

N Obs	N	Mean	Std Dev	T	Prob > |T|
4	4	-11.6250000	142.9081792	-0.1626919	0.8811

IDN=DOCOM3

N Obs	N	Mean	Std Dev	T	Prob > |T|
4	4	-46.3750000	146.7658992	-0.6319588	0.5723

IDN=DOCOM4

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	-0.2000000	113.0102871	-0.0039573	0.9970

IDN=DOCOM5

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	-2.6666667	112.5262932	-0.0529907	0.9603

IDN=INNODES1

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	16.0333333	88.1712664	0.4066135	0.7051

IDN=INNODES2

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	16.0333333	88.1712664	0.4066135	0.7051

SAS
Analysis Variable : DIFFCON

IDN=INNODES3

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	16.0333333	88.1712664	0.4066135	0.7051

IDN=INNODES4

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	16.0333333	88.1712664	0.4066135	0.7051

IDN=LEADDES1

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	9.9000000	215.3004877	0.1028194	0.9231

IDN=LEADDES2

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	-19.6000000	150.1717350	-0.2918454	0.7849

IDN=LEADDES3

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	29.5000000	124.9254778	0.5280268	0.6254

IDN=LEADDES4

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	-5.2000000	121.8208726	-0.0954480	0.9285

IDN=SUST1DF

N Obs	N	Mean	Std Dev	T	Prob > |T|
4	4	-51.0833333	132.4398885	-0.7714192	0.4966

SAS
Analysis Variable : DIFFCON

IDN=SUST2DF

N Obs	N	Mean	Std Dev	T	Prob > |T|
4	4	-23.0000000	129.0090436	-0.3565642	0.7450

IDN=SUST3DF

N Obs	N	Mean	Std Dev	T	Prob > |T|
4	4	59.8333333	315.4426050	0.3793611	0.7297

IDN=dbcpdf

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	10.2000000	36.9696572	0.6169355	0.5707

IDN=dbcpdf2

N Obs	N	Mean	Std Dev	T	Prob > |T|
4	4	3.8333333	50.2736954	0.1524986	0.8885

IDN=pH54(25)

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	9.7666667	81.5587382	0.2677694	0.8021

IDN=pH64(15)

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	-75.3333333	183.4902359	-0.9180350	0.4105

IDN=pH64(25)

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	-19.4000000	53.2985043	-0.8139012	0.4614

SAS
Analysis Variable : DIFFCON

IDN=pH64(30)

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	53.1333333	80.1043070	1.4831880	0.2122

IDN=pH74(15)

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	57.4666667	170.3534887	0.7543102	0.4926

IDN=pH74(25)

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	11.3666667	110.8757688	0.2292353	0.8299

IDN=pH74(30)

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	16.4666667	127.8711048	0.2879508	0.7877

IDN=pH74/25

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	-19.4000000	111.9069926	-0.3876408	0.7180

IDN=pH7815c

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	21.2000000	171.3612737	0.2766357	0.7958

IDN=pH7825c

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	72.0000000	398.3058568	0.4042042	0.7067

SAS
Analysis Variable : DIFFCON

IDN=pH89(25)

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	4.2000000	44.0182096	0.2133546	0.8415

IDN=ph7830c

N Obs	N	Mean	Std Dev	T	Prob > |T|
5	5	38.8666667	72.5041570	1.1986693	0.2968

APPENDIX U

STATISTICAL ANALYSIS OF DATA OBTAINED AT THE END OF FOUR HOURS EXPERIMENTS

-- SAS PROGRAM AND ANALYSIS --

SAS PROGRAM

```
OPTIONS PS=60 LS=64 NODATE NONUMBER;
*  fourhr.CTL;
DATA four;
   INFILE 'FOURHR.DAT';
   INPUT TIME  CONC TEMP PH IDN $ TABLE;
proc sort data =four;
by idn;
RUN;
DATA one; set four;
if idn='dbcpdf' or idn='dbcpdf2';
proc ttest data=one;
class idn;
var conc;
data two; set four;
IF IDN='aquifdf' OR IDN='aquifdf2';
PROC TTEST DATA=two;
class idn;
VAR CONC;
RUN;
DATA three; SET four;
if idn='docom3' or idn='docom4';
proc ttest data=three;
class idn;
var conc;
run;
DATA five; SET four;
IF IDN='docom3' OR IDN='docom5';
PROC TTEST data=five;
CLASS IDN;
VAR CONC;
RUN;
DATA six; SET four;
if idn='docom4' or idn='docom5';
proc ttest data=six;
class idn;
var conc;
run;
DATA seven; SET four;
IF IDN='innodes1' OR IDN='innodes2';
PROC TTEST data=seven;
CLASS IDN;
VAR CONC;
RUN;
DATA eight; SET four;
if idn='innodes1' or idn='innodes3';
proc ttest data=eight;
class idn;
var conc;
run;
DATA nine; SET four;
```

```
IF IDN='innodes2' OR IDN='innodes3';
PROC TTEST data=nine;
CLASS IDN;
VAR CONC;
RUN;
DATA ten; SET four;
if idn='innodes1' or idn='innodes4';
proc ttest data=ten;
class idn;
var conc;
run;
DATA eleven; SET four;
IF IDN='innodes2' OR IDN='innodes4';
PROC TTEST data=eleven;
CLASS IDN;
VAR CONC;
RUN;
DATA twelve; SET four;
if idn='innodes3' or idn='innodes4';
proc ttest data=twelve;
class idn;
var conc;
run;
DATA twob; SET four;
IF IDN='leaddes1' OR IDN='leaddes2';
PROC TTEST data=twob;
CLASS IDN;
VAR CONC;
RUN;
DATA threeb; SET four;
if idn='leaddes2' or idn='leaddes3';
proc ttest data=threeb;
class idn;
var conc;
run;
DATA fourb; SET four;
IF IDN='leaddes1' OR IDN='leaddes3';
PROC TTEST data=fourb;
CLASS IDN;
VAR CONC;
RUN;
DATA fiveb; SET four;
if idn='leaddes1' or idn='leaddes4';
proc ttest data=fiveb;
class idn;
var conc;
run;
DATA sixb; SET four;
IF IDN='leaddes2' OR IDN='leaddes4';
PROC TTEST data=sixb;
CLASS IDN;
VAR CONC;
RUN;
DATA sevenb; SET four;
```

```
if idn='leaddes3' or idn='leaddes4';
proc ttest data=sevenb;
class idn;
var conc;
run;
DATA eightb; SET four;
IF IDN='pH54(25)' OR IDN='pH74(25)';
PROC TTEST data=eightb;
CLASS IDN;
VAR CONC;
RUN;
DATA nineb; SET four;
if idn='pH54(25)' or idn='pH89(25)';
proc ttest data=nineb;
class idn;
var conc;
run;
DATA tenb; SET four;
IF IDN='pH74(25)' OR IDN='pH89(25)';
PROC TTEST data=tenb;
CLASS IDN;
VAR CONC;
RUN;
DATA elevenb; SET four;
IF IDN='pH64(15)' OR IDN='pH74(15)';
PROC TTEST data=elevenb;
CLASS IDN;
VAR CONC;
RUN;
DATA twelveb; SET four;
if idn='pH64(15)' or idn='pH7815c';
proc ttest data=twelveb;
class idn;
var conc;
run;
DATA onec; SET four;
IF IDN='pH74(15)' OR IDN='pH7815c';
PROC TTEST data=onec;
CLASS IDN;
VAR CONC;
RUN;
DATA twoc; SET four;
if idn='pH64(25)' or idn='pH74/25';
proc ttest data=twoc;
class idn;
var conc;
run;
DATA threec; SET four;
IF IDN='pH64(25)' OR IDN='pH7825c';
PROC TTEST data=threec;
CLASS IDN;
VAR CONC;
RUN;
DATA fourc; SET four;
```

```
if idn='pH74/25' or idn='pH7825c';
proc ttest data=fourc;
class idn;
var conc;
run;
DATA fivec; SET four;
IF IDN='pH64(30)' OR IDN='pH74(30)';
PROC TTEST data =fivec;
CLASS IDN;
VAR CONC;
RUN;
DATA sixc; SET four;
if idn='pH64(30)' or idn='pH7830c';
proc ttest data=sixc;
class idn;
var conc;
run;
DATA sevenc; SET four;
IF IDN='pH74(30)' OR IDN='pH7830c';
PROC TTEST data=sevenc;
CLASS IDN;
VAR CONC;
RUN;
DATA eightc; SET four;
IF IDN='sust1df' OR IDN='sust2df';
PROC TTEST data=eightc;
CLASS IDN;
VAR CONC;
RUN;
DATA ninec; SET four;
if idn='sust1df' or idn='sust3df';
proc ttest data=ninec;
class idn;
var conc;
run;
DATA tenc; SET four;
IF IDN='sust2df' OR IDN='sust3df';
PROC TTEST;
CLASS IDN;
VAR CONC;
RUN;
```

PROGRAM

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
dbcpdf	3	19.33333333	0.57735027	0.33333333
dbcpdf2	3	21.66666667	0.57735027	0.33333333

Variances	T	DF	Prob>|T|
Unequal	-4.9497	4.0	0.0078
Equal	-4.9497	4.0	0.0078

For H0: Variances are equal, F' = 1.00 DF = (2,2)
Prob>F' = 1.0000

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
aquifdf	3	276.33333333	107.19297241	61.88789147
aquifdf2	3	281.66666667	44.97036061	25.96364980

Variances	T	DF	Prob>|T|
Unequal	-0.0795	2.7	0.9424
Equal	-0.0795	4.0	0.9405

For H0: Variances are equal, F' = 5.68 DF = (2,2)
Prob>F' = 0.2993

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
docom3	2	193.50000000	78.48885271	55.50000000
docom4	2	467.50000000	57.27564928	40.50000000

Variances	T	DF	Prob>|T|
Unequal	-3.9880	1.8	0.0743
Equal	-3.9880	2.0	0.0575

For H0: Variances are equal, F' = 1.88 DF = (1,1)
Prob>F' = 0.8027

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
docom3	2	193.50000000	78.48885271	55.50000000
docom5	2	475.50000000	34.64823228	24.50000000

Variances	T	DF	Prob>|T|
Unequal	-4.6483	1.4	0.1005
Equal	-4.6483	2.0	0.0433

For H0: Variances are equal, F' = 5.13 DF = (1,1)
Prob>F' = 0.5293

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
docom4	2	467.50000000	57.27564928	40.50000000
docom5	2	475.50000000	34.64823228	24.50000000

Variances	T	DF	Prob>|T|
Unequal	-0.1690	1.6	0.8856
Equal	-0.1690	2.0	0.8813

For H0: Variances are equal, F' = 2.73 DF = (1,1)
Prob>F' = 0.6927

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
innodes1	3	709.66666667	160.48156696	92.65407588
innodes2	3	413.33333333	39.71565602	22.92984470

Variances	T	DF	Prob>|T|
Unequal	3.1046	2.2	0.0810
Equal	3.1046	4.0	0.0361

For H0: Variances are equal, F' = 16.33 DF = (2,2)
Prob>F' = 0.1154

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
innodes1	3	709.66666667	160.48156696	92.65407588
innodes3	3	324.33333333	10.01665280	5.78311719

Variances	T	DF	Prob>|T|
Unequal	4.1508	2.0	0.0530
Equal	4.1508	4.0	0.0143

For H0: Variances are equal, F' = 256.69 DF = (2,2)
Prob>F' = 0.0078

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
innodes2	3	413.33333333	39.71565602	22.92984470
innodes3	3	324.33333333	10.01665280	5.78311719

Variances	T	DF	Prob>|T|
Unequal	3.7636	2.3	0.0560
Equal	3.7636	4.0	0.0197

For H0: Variances are equal, F' = 15.72 DF = (2,2)
Prob>F' = 0.1196

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
innodes1	3	709.66666667	160.48156696	92.65407588
innodes4	3	98.33333333	149.53371972	86.33333333

Variances	T	DF	Prob>|T|
Unequal	4.8273	4.0	0.0086
Equal	4.8273	4.0	0.0085

For H0: Variances are equal, F' = 1.15 DF = (2,2)
Prob>F' = 0.9295

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
innodes2	3	413.33333333	39.71565602	22.92984470
innodes4	3	98.33333333	149.53371972	86.33333333

Variances	T	DF	Prob>|T|
Unequal	3.5264	2.3	0.0626
Equal	3.5264	4.0	0.0243

For H0: Variances are equal, F' = 14.18 DF = (2,2)
Prob>F' = 0.1318

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
innodes3	3	324.33333333	10.01665280	5.78311719
innodes4	3	98.33333333	149.53371972	86.33333333

Variances	T	DF	Prob>|T|
Unequal	2.6119	2.0	0.1199
Equal	2.6119	4.0	0.0593

For H0: Variances are equal, F' = 222.86 DF = (2,2)
Prob>F' = 0.0089

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
leaddes1	2	368.50000000	20.50609665	14.50000000
leaddes2	2	409.00000000	67.88225099	48.00000000

Variances	T	DF	Prob>|T|
Unequal	-0.8077	1.2	0.5560
Equal	-0.8077	2.0	0.5041

For H0: Variances are equal, F' = 10.96 DF = (1,1)
Prob>F' = 0.3735

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
leaddes2	2	409.00000000	67.88225099	48.00000000
leaddes3	2	281.00000000	55.15432893	39.00000000

Variances	T	DF	Prob>|T|
Unequal	2.0696	1.9	0.1834
Equal	2.0696	2.0	0.1743

For H0: Variances are equal, F' = 1.51 DF = (1,1)
Prob>F' = 0.8688

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
leaddes1	2	368.50000000	20.50609665	14.50000000
leaddes3	2	281.00000000	55.15432893	39.00000000

Variances	T	DF	Prob>|T|
Unequal	2.1029	1.3	0.2521
Equal	2.1029	2.0	0.1702

For H0: Variances are equal, F' = 7.23 DF = (1,1)
Prob>F' = 0.4532

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
leaddes1	2	368.50000000	20.50609665	14.50000000
leaddes4	2	410.00000000	15.55634919	11.00000000

Variances	T	DF	Prob>|T|
Unequal	-2.2802	1.9	0.1655
Equal	-2.2802	2.0	0.1502

For H0: Variances are equal, F' = 1.74 DF = (1,1)
Prob>F' = 0.8263

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
leaddes2	2	409.00000000	67.88225099	48.00000000
leaddes4	2	410.00000000	15.55634919	11.00000000

Variances	T	DF	Prob>|T|
Unequal	-0.0203	1.1	0.9869
Equal	-0.0203	2.0	0.9856

For H0: Variances are equal, F' = 19.04 DF = (1,1)
Prob>F' = 0.2868

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
leaddes3	2	281.00000000	55.15432893	39.00000000
leaddes4	2	410.00000000	15.55634919	11.00000000

Variances	T	DF	Prob>|T|
Unequal	-3.1835	1.2	0.1767
Equal	-3.1835	2.0	0.0861

For H0: Variances are equal, F' = 12.57 DF = (1,1)
Prob>F' = 0.3500

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH54(25)	3	51.00000000	40.58324778	23.43074903
pH74(25)	3	60.00000000	9.53939201	5.50757055

Variances	T	DF	Prob>|T|
Unequal	-0.3739	2.2	0.7419
Equal	-0.3739	4.0	0.7274

For H0: Variances are equal, F' = 18.10 DF = (2,2)
Prob>F' = 0.1047

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH54(25)	3	51.00000000	40.58324778	23.43074903
pH89(25)	3	87.33333333	67.17390366	38.78287136

Variances	T	DF	Prob>|T|
Unequal	-0.8019	3.3	0.4773
Equal	-0.8019	4.0	0.4676

For H0: Variances are equal, F' = 2.74 DF = (2,2)
Prob>F' = 0.5348

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH74(25)	3	60.00000000	9.53939201	5.50757055
pH89(25)	3	87.33333333	67.17390366	38.78287136

Variances	T	DF	Prob>|T|
Unequal	-0.6978	2.1	0.5558
Equal	-0.6978	4.0	0.5237

For H0: Variances are equal, F' = 49.59 DF = (2,2)
Prob>F' = 0.0395

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH64(15)	3	562.00000000	48.75448697	28.14841618
pH74(15)	3	831.66666667	30.00555504	17.32371528

Variances	T	DF	Prob>|T|
Unequal	-8.1588	3.3	0.0030
Equal	-8.1588	4.0	0.0012

For H0: Variances are equal, F' = 2.64 DF = (2,2)
Prob>F' = 0.5494

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH64(15)	3	562.00000000	48.75448697	28.14841618
pH7815c	3	872.33333333	4.50924975	2.60341656

Variances	T	DF	Prob>|T|
Unequal	-10.9780	2.0	0.0080
Equal	-10.9780	4.0	0.0004

For H0: Variances are equal, F' = 116.90 DF = (2,2)
Prob>F' = 0.0170

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH74(15)	3	831.66666667	30.00555504	17.32371528
pH7815c	3	872.33333333	4.50924975	2.60341656

Variances	T	DF	Prob>|T|
Unequal	-2.3214	2.1	0.1421
Equal	-2.3214	4.0	0.0810

For H0: Variances are equal, F' = 44.28 DF = (2,2)
Prob>F' = 0.0442

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH64(25)	3	551.33333333	62.68439466	36.19085213
pH74/25	3	645.66666667	128.70249933	74.30642263

Variances	T	DF	Prob>|T|
Unequal	-1.1413	2.9	0.3402
Equal	-1.1413	4.0	0.3174

For H0: Variances are equal, F' = 4.22 DF = (2,2)
Prob>F' = 0.3835

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH64(25)	3	551.33333333	62.68439466	36.19085213
pH7825c	3	447.66666667	24.11085509	13.92040868

Variances	T	DF	Prob>|T|
Unequal	2.6735	2.6	0.0925
Equal	2.6735	4.0	0.0556

For H0: Variances are equal, F' = 6.76 DF = (2,2)
Prob>F' = 0.2578

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH74/25	3	645.66666667	128.70249933	74.30642263
pH7825c	3	447.66666667	24.11085509	13.92040868

Variances	T	DF	Prob>|T|
Unequal	2.6191	2.1	0.1143
Equal	2.6191	4.0	0.0589

For H0: Variances are equal, F' = 28.49 DF = (2,2)
Prob>F' = 0.0678

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH64(30)	3	663.33333333	84.59511412	48.84101191
pH74(30)	3	777.33333333	49.36935622	28.50341110

Variances	T	DF	Prob>|T|
Unequal	-2.0159	3.2	0.1321
Equal	-2.0159	4.0	0.1140

For H0: Variances are equal, F' = 2.94 DF = (2,2)
Prob>F' = 0.5081

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH64(30)	3	663.33333333	84.59511412	48.84101191
pH7830c	3	445.66666667	21.45538006	12.38726945

Variances	T	DF	Prob>|T|
Unequal	4.3199	2.3	0.0428
Equal	4.3199	4.0	0.0124

For H0: Variances are equal, F' = 15.55 DF = (2,2)
Prob>F' = 0.1209

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
pH74(30)	3	777.33333333	49.36935622	28.50341110
pH7830c	3	445.66666667	21.45538006	12.38726945

Variances	T	DF	Prob>|T|
Unequal	10.6718	2.7	0.0036
Equal	10.6718	4.0	0.0004

For H0: Variances are equal, F' = 5.29 DF = (2,2)
Prob>F' = 0.3177

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
sust1df	3	12.00000000	0.00000000	0.00000000
sust2df	3	40.00000000	48.49742261	28.00000000

Variances	T	DF	Prob>|T|
Unequal	-1.0000	2.0	0.4226
Equal	-1.0000	4.0	0.3739

NOTE: All values are the same for one CLASS level.

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
sust1df	3	12.00000000	0.00000000	0.00000000
sust3df	3	3638.33333333	773.28994131	446.45915578

Variances	T	DF	Prob>|T|
Unequal	-8.1224	2.0	0.0148
Equal	-8.1224	4.0	0.0012

NOTE: All values are the same for one CLASS level.

SAS
TTEST PROCEDURE

Variable: CONC

IDN	N	Mean	Std Dev	Std Error
sust2df	3	40.00000000	48.49742261	28.00000000
sust3df	3	3638.33333333	773.28994131	446.45915578

Variances	T	DF	Prob>|T|
Unequal	-8.0439	2.0	0.0149
Equal	-8.0439	4.0	0.0013

For H0: Variances are equal, F' = 254.24 DF = (2,2)
Prob>F' = 0.0078

APPENDIX V

COMPUTER PRINTOUTS OF NUMERICAL ANALYSIS

FIRST ORDER MODEL

NUMERICAL DETERMINATION OF RATE CONSTANTS
FOR FIRST ORDER REACTION FOR 1,2 DIBROMO
-3-CHLOROPROPANE (pH 7.4,
TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: DBCPDF.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1539903.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =  244218.400000
Prms ->       1.581147      931.653200

IT#          2Lambda =   9.999999E-04 RSS =   37306.230000
Prms ->       2.517750      976.790700

IT#          3Lambda =   9.999999E-05 RSS =   10118.150000
Prms ->       3.135292      999.444000

IT#          4Lambda =   9.999999E-06 RSS =    8874.547000
Prms ->       3.316698     1002.897000

IT#          5Lambda =   9.999999E-07 RSS =    8861.100000
Prms ->       3.337486     1003.317000

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =    8861.046000
       3.338820     1003.352000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.337486	1.390844E-01
S0	1003.317000	14.226130

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .2683 1.0000
```

ANOVA ingredients

```
   RSS =     8861.046000
   CSS =  2158781.000000
     N =       18
```

RESULTS

DDBCPDF.OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	977	976	1
0.0083	1004	976	28
0.0083	950	976	-26
0.5	156	189	-33
0.5	174	189	-15
0.5	205	189	16
1	61	36	25
1	67	36	31
1	58	36	22
2	23	1	22
2	23	1	22
2	23	1	22
3	21	0	21
3	19	0	19
3	19	0	19
4	20	0	20
4	19	0	19
4	19	0	19

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2 DIBROMO-3-CHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: DBCPDF2.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1271863.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =  212561.500000
Prms ->       1.509906      949.421100

IT#          2Lambda =   9.999999E-04 RSS =   98551.800000
Prms ->       2.156477      972.826100

IT#          3Lambda =   9.999999E-05 RSS =   93882.770000
Prms ->       2.368484      979.338600

IT#          4Lambda =   9.999999E-06 RSS =   93880.900000
Prms ->       2.366216      978.516500

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   93880.880000
       2.366415      978.528100
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.366216	2.662537E-01
S0	978.516500	45.632020

CORRELATION OF PARAMETER ESTIMATES

1.0000	
.3426	1.0000

ANOVA ingredients

RSS =	93880.880000
CSS =	2179605.000000
N =	18

RESULTS

DBCPDF2.OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1145	959	186
0.0083	780	959	-180
0.0083	935	959	-25
0.5	319	300	20
0.5	334	300	34
0.5	368	300	68
1	21	92	-70
1	21	92	-71
1	19	92	-73
2	34	9	26
2	33	9	25
2	36	9	27
3	24	1	23
3	24	1	23
3	22	1	21
4	22	0	21
4	21	0	21
4	22	0	22

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS
(pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: aquifdf.dat

IT#          0Lambda =   1.000000E-01 RSS =  814147.400000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =  397630.000000
Prms ->   5.912457E-01     1232.648000

IT#          2Lambda =   9.999999E-04 RSS =  378933.100000
Prms ->   5.269957E-01     1179.142000

IT#          3Lambda =   9.999999E-05 RSS =  377440.400000
Prms ->   5.081994E-01     1168.844000

IT#          4Lambda =   9.999999E-06 RSS =  377323.400000
Prms ->   5.030391E-01     1165.950000

IT#          5Lambda =   9.999999E-07 RSS =  377314.200000
Prms ->   5.015976E-01     1165.142000

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =  377313.400000
   5.011925E-01     1164.915000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	5.015976E-01	9.130809E-02
S0	1165.142000	84.820170

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6039 1.0000
```

ANOVA ingredients

```
   RSS =   377313.400000
   CSS =  1917710.000000
     N =        15
```

RESULTS

DAQUIFDF.OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1224	1160	64
0.0083	1367	1160	207
0.0083	1376	1160	216
0.5	563	907	-344
0.5	694	907	-213
0.5	771	907	-136
1	708	706	2
1	572	706	-134
1	736	706	30
2	462	427	35
2	553	427	126
2	450	427	23
4	219	157	62
4	210	157	53
4	400	157	243

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF SAND SPRINGS PETROCHEMICAL COMPLEX AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: aquifdf2.dat

IT#          0Lambda =   1.000000E-01 RSS = 1051568.000000
Prms ->       1.000000     1300.000000

IT#          1Lambda =   1.000000E-02 RSS =  436167.600000
Prms ->   2.124457E-01     1183.095000

IT#          2Lambda =   9.999999E-04 RSS =   44798.460000
Prms ->   3.321856E-01     1155.357000

IT#          3Lambda =   9.999999E-05 RSS =   31716.330000
Prms ->   3.723721E-01     1171.819000

IT#          4Lambda =   9.999999E-06 RSS =   31668.400000
Prms ->   3.751067E-01     1173.221000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   31668.380000
   3.751607E-01     1173.258000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.751067E-01	2.106931E-02
S0	1173.221000	25.153610

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6059 1.0000
```

ANOVA ingredients

```
   RSS =    31668.380000
   CSS =  1475762.000000
     N =          14
```

RESULTS

DAQUIFDF2.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1247	1170	77
0.0083	1191	1170	21
0.0083	1083	1170	-87
0.5	975	973	2
0.5	939	973	-34
1	867	806	61
1	853	806	47
1	759	806	-47
2	545	554	-9
2	522	554	-32
2	508	554	-46
4	312	262	50
4	303	262	41
4	230	262	-32

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 5.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH54(25).DAT

IT#          0Lambda =   1.000000E-01 RSS = 1705876.000000
Prms ->   1.000000E-01      900.000000

IT#          1Lambda =   1.000000E-02 RSS =  256886.400000
Prms ->   3.145021E-01      912.831700

IT#          2Lambda =   9.999999E-04 RSS =   67797.980000
Prms ->   5.024105E-01     1025.503000

IT#          3Lambda =   9.999999E-05 RSS =   66576.090000
Prms ->   5.222598E-01     1036.396000

IT#          4Lambda =   9.999999E-06 RSS =   66570.380000
Prms ->   5.208665E-01     1035.559000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   66570.340000
   5.209820E-01     1035.626000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	5.208665E-01	3.598063E-02
S0	1035.559000	32.297370

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6472 1.0000
```

ANOVA ingredients

```
   RSS =    66570.340000
   CSS =  1721402.000000
     N =          18
```

RESULTS

pH54(25).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	942	1031	-89
0.0083	1015	1031	-16
0.0083	1095	1031	64
0.5	769	798	-29
0.5	850	798	52
0.5	792	798	-6
1	631	615	16
1	594	615	-21
1	595	615	-20
1.5	484	474	10
1.5	475	474	1
1.5	550	474	76
2	286	365	-79
2	428	365	63
2	475	365	110
4	93	129	-36
4	48	129	-81
4	12	129	-117

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR FIRST ORDER
REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF
OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS
(pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH74(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  740727.700000
Prms ->       1.000000     1300.000000

IT#          1Lambda =   1.000000E-02 RSS =  171650.600000
Prms ->   5.673094E-01     1002.192000

IT#          2Lambda =   9.999999E-04 RSS =  158700.200000
Prms ->   4.997267E-01      956.225200

IT#          3Lambda =   9.999999E-05 RSS =  158687.900000
Prms ->   5.017233E-01      956.960200

MARQUART: Convergence criterion met
IT#          4Lambda =   9.999999E-05 RSS =  158687.900000
   5.016518E-01      956.920700
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	5.017233E-01	5.827999E-02
S0	956.960200	49.529900

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6509 1.0000
```

ANOVA ingredients

```
   RSS =   158687.900000
   CSS =  1508387.000000
     N  =         18
```

RESULTS

pH74(25).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1039	953	86
0.0083	997	953	44
0.0083	972	953	19
0.5	483	745	-262
0.5	704	745	-41
0.5	635	745	-110
1	629	579	50
1	698	579	119
1	603	579	24
1.5	339	451	-112
1.5	540	451	89
1.5	531	451	80
2	382	351	31
2	416	351	65
2	409	351	58
4	65	129	-64
4	66	129	-63
4	49	129	-80

NUMERICAL DETERMINATION OF EFFECT OF pH ON RATE CONSTANTS FOR FIRST ORDER REACTIONS FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 8.9, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH89(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  907752.600000
Prms ->       1.000000     1000.000000

IT#          1Lambda =   1.000000E-02 RSS =  472964.400000
Prms ->   2.473567E-01      948.290400

IT#          2Lambda =   9.999999E-04 RSS =  119758.500000
Prms ->   4.034807E-01      948.372300

IT#          3Lambda =   9.999999E-05 RSS =  112644.200000
Prms ->   4.414184E-01      957.806600

IT#          4Lambda =   9.999999E-06 RSS =  112630.000000
Prms ->   4.394470E-01      956.152400

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =  112629.800000
   4.396270E-01      956.262400
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	4.394470E-01	4.417812E-02
S0	956.152400	40.721000

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6625 1.0000
```

ANOVA ingredients

```
   RSS =   112629.800000
   CSS =  1370586.000000
     N =          18
```

RESULTS

pH89(25).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	978	953	25
0.0083	983	953	30
0.0083	927	953	-26
0.5	589	768	-179
0.5	678	768	-90
0.5	790	768	22
1	616	616	0
1	699	616	83
1	734	616	118
1.5	580	495	85
1.5	441	495	-54
1.5	569	495	74
2	357	397	-40
2	433	397	36
2	457	397	60
4	109	165	-56
4	141	165	-24
4	12	165	-153

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS
FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE
PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER
MATERIALS (pH 6.4, TEMPERATURE 15°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH64(15).DAT

IT#          0Lambda =   1.000000E-01 RSS = 3640867.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-01 RSS =  614787.600000
Prms ->   3.887880E-01     1242.574000

IT#          2Lambda =   1.000000E-02 RSS =  130194.000000
Prms ->   1.880303E-01     1119.558000

IT#          3Lambda =   9.999999E-04 RSS =  122123.300000
Prms ->   1.712129E-01     1079.700000

IT#          4Lambda =   9.999999E-05 RSS =  122095.200000
Prms ->   1.698130E-01     1077.991000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-05 RSS =  122095.100000
   1.697611E-01     1077.926000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.698130E-01	2.512912E-02
S0	1077.991000	42.540860

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7220 1.0000
```

ANOVA ingredients

```
   RSS =   122095.100000
   CSS =   586971.800000
     N =          16
```

RESULTS

DP64(15).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1102	1076	26
0.0083	1183	1076	107
0.0083	1142	1076	66
0.5	839	990	-151
1	931	910	21
1	987	910	77
1	692	910	-218
1.5	836	836	0
1.5	765	836	-71
1.5	808	836	-28
2	722	768	-46
2	854	768	86
2	859	768	91
4	529	547	-18
4	539	547	-8
4	618	547	71

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS
FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE
PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER
MATERIALS (pH 7.4, TEMPERATURE 15°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH74(15).DAT

IT#          0Lambda =   1.000000E-01 RSS = 3003875.000000
Prms ->   5.000000E-01     1100.000000

IT#          1Lambda =   1.000000E-01 RSS =  539832.600000
Prms ->   2.193850E-01     1186.589000

IT#          2Lambda =   1.000000E-02 RSS =   84786.840000
Prms ->   7.410091E-02     1093.719000

IT#          3Lambda =   9.999999E-04 RSS =   78838.680000
Prms ->   6.369306E-02     1062.807000

IT#          4Lambda =   9.999999E-05 RSS =   78827.950000
Prms ->   6.299920E-02     1061.749000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-05 RSS =   78827.950000
   6.298929E-02     1061.734000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	6.299920E-02	1.604842E-02
S0	1061.749000	31.034490

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7488 1.0000
```

ANOVA ingredients

```
   RSS =    78827.950000
   CSS =   171901.800000
     N =         16
```

RESULTS

DP74(15).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	951	1061	-110
0.0083	1277	1061	216
0.5	968	1029	-61
0.5	1040	1029	11
0.5	1010	1029	-19
1	925	997	-72
1	1009	997	12
1	944	997	-53
1.5	951	966	-15
1.5	1010	966	44
1.5	985	966	19
2	982	936	46
2	901	936	-35
4	837	825	12
4	858	825	33
4	798	825	-27

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS
FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE
PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER
MATERIALS (pH 7.8, TEMPERATURE 15°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH7815c.DAT

IT#          0Lambda =   1.000000E-01 RSS = 7704100.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-01 RSS =  427930.600000
Prms ->   1.127629E-01     1368.970000

IT#          2Lambda =   1.000000E-02 RSS =  385596.500000
Prms ->   1.164330E-01     1317.707000

IT#          3Lambda =   9.999999E-04 RSS =  385130.300000
Prms ->   1.141913E-01     1308.750000

MARQUART: Convergence criterion met
IT#          4Lambda =   9.999999E-04 RSS =  385129.300000
   1.140056E-01     1308.377000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.141913E-01	3.372177E-02
S0	1308.750000	75.381130

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7215 1.0000
```

ANOVA ingredients

```
   RSS =   385129.300000
   CSS =   760790.900000
     N =          15
```

RESULTS

PH7815c.OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1250	1308	-58
0.0083	1528	1308	220
0.0083	1375	1308	67
0.5	814	1236	-422
1	1400	1168	232
1	1107	1168	-61
1	1345	1168	177
1.5	1171	1103	68
1.5	1090	1103	-13
1.5	1020	1103	-83
2	988	1042	-54
2	839	1042	-203
4	868	829	39
4	872	829	43
4	877	829	48

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 6.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH64(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  624500.000000
Prms ->   1.000000E-01     1000.000000

IT#          1Lambda =   1.000000E-02 RSS =  369469.800000
Prms ->   1.926653E-01     1103.647000

IT#          2Lambda =   9.999999E-04 RSS =  329026.400000
Prms ->   2.413011E-01     1172.486000

IT#          3Lambda =   9.999999E-05 RSS =  328082.300000
Prms ->   2.494913E-01     1182.902000

IT#          4Lambda =   9.999999E-06 RSS =  328069.100000
Prms ->   2.504830E-01     1183.974000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =  328069.000000
   2.505967E-01     1184.094000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.504830E-01	4.149483E-02
S0	1183.974000	62.842430

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6957 1.0000
```

ANOVA ingredients

```
   RSS =   328069.000000
   CSS =  1260309.000000
     N =          18
```

RESULTS

PH64(25).OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1329	1182	147
0.0083	1204	1182	22
0.0083	1120	1182	-62
0.5	1211	1045	166
0.5	1127	1045	82
0.5	1024	1045	-21
1	1041	922	119
1	795	922	-127
1	616	922	-306
1.5	885	813	72
1.5	785	813	-28
1.5	723	813	-90
2	762	717	45
2	683	717	-34
2	439	717	-278
4	608	435	173
4	562	435	127
4	484	435	49

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH74/25.DAT

IT#          0Lambda =   1.000000E-01 RSS = 2184502.000000
Prms ->   5.000000E-01     1010.000000

IT#          1Lambda =   1.000000E-02 RSS =  798653.800000
Prms ->   4.495552E-02     1119.216000

IT#          2Lambda =   9.999999E-04 RSS =  139893.600000
Prms ->   1.333519E-01     1090.747000

IT#          3Lambda =   9.999999E-05 RSS =  120210.400000
Prms ->   1.629995E-01     1111.582000

IT#          4Lambda =   9.999999E-06 RSS =  120047.600000
Prms ->   1.660811E-01     1114.822000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =  120046.900000
   1.662899E-01     1115.067000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.660811E-01	2.180508E-02
S0	1114.822000	35.858260

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7123 1.0000
```

ANOVA ingredients

```
   RSS =   120046.900000
   CSS =   629184.300000
     N =          18
```

RESULTS

PH74/25.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1210	1113	97
0.0083	1084	1113	-29
0.0083	1090	1113	-23
0.5	1167	1026	141
0.5	1055	1026	29
0.5	1054	1026	28
1	954	944	10
1	909	944	-35
1	873	944	-71
1.5	901	869	32
1.5	818	869	-51
1.5	749	869	-120
2	750	800	-50
2	730	800	-70
2	714	800	-86
4	734	574	160
4	705	574	131
4	498	574	-76

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.8, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH7825C.DAT

IT#          0Lambda =   1.000000E-01 RSS = 2383092.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS = 1121985.000000
Prms ->   1.682007E-01     1336.655000

IT#          2Lambda =   9.999999E-04 RSS =  437597.800000
Prms ->   2.692613E-01     1247.008000

IT#          3Lambda =   9.999999E-05 RSS =  406821.400000
Prms ->   3.182711E-01     1273.850000

IT#          4Lambda =   9.999999E-06 RSS =  405660.200000
Prms ->   3.291820E-01     1282.451000

IT#          5Lambda =   9.999999E-07 RSS =  405626.800000
Prms ->   3.310713E-01     1284.023000

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =  405625.800000
   3.313839E-01     1284.284000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.310713E-01	6.234707E-02
S0	1284.023000	81.602830

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6381 1.0000
```

ANOVA ingredients

```
   RSS =   405625.800000
   CSS =  1782760.000000
     N =          15
```

RESULTS

PH7825C.DAT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1391	1280	111
0.0083	1283	1280	3
0.0083	1485	1280	205
0.5	1399	1088	311
0.5	872	1088	-216
0.5	806	1088	-282
1	992	922	70
1	637	922	-285
1	857	922	-65
1.5	773	781	-8
1.5	725	781	-56
2	659	662	-3
4	425	342	83
4	473	342	131
4	445	342	103

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS FOR FIRST ORDER ORDER REACTION FOR 1,2 - DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 6.4, TEMPERATURE 30°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: ph64(30).dat

IT#          0Lambda =   1.000000E-01 RSS = 3966203.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-01 RSS =  915965.100000
Prms ->   4.284675E-01     1264.009000

IT#          2Lambda =   1.000000E-02 RSS =  299992.400000
Prms ->   2.005777E-01     1149.292000

IT#          3Lambda =   9.999999E-04 RSS =  284660.400000
Prms ->   1.754236E-01     1100.726000

IT#          4Lambda =   9.999999E-05 RSS =  284455.700000
Prms ->   1.718833E-01     1096.799000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-05 RSS =  284453.400000
   1.715126E-01     1096.373000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.718833E-01	3.460849E-02
S0	1096.799000	55.433080

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7111 1.0000
```

ANOVA ingredients

```
   RSS =   284453.400000
   CSS =   778747.600000
     N =          18
```

RESULTS

DP64(30).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1165	1095	70
0.0083	1119	1095	24
0.0083	1021	1095	-74
0.5	1137	1006	131
0.5	1169	1006	163
0.5	1153	1006	147
1	909	924	-15
1	890	924	-34
1	749	924	-175
1.5	919	848	71
1.5	768	848	-80
1.5	536	848	-312
2	711	778	-67
2	706	778	-72
2	695	778	-83
4	572	551	21
4	679	551	128
4	739	551	188

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS FOR FIRST ORDER ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 30°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH74(30).DAT

IT#          0Lambda =   1.000000E-01 RSS = 7277731.000000
Prms ->   9.000000E-01       900.000000

IT#          1Lambda =   1.000000E-01 RSS =  995185.600000
Prms ->   7.161915E-03      1136.740000

IT#          2Lambda =   1.000000E-02 RSS =  317410.200000
Prms ->   8.591705E-02      1127.373000

IT#          3Lambda =   9.999999E-04 RSS =  270038.200000
Prms ->   1.270942E-01      1173.671000

IT#          4Lambda =   9.999999E-05 RSS =  269390.200000
Prms ->   1.324407E-01      1181.140000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-05 RSS =  269387.500000
   1.327863E-01     1181.612000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.324407E-01	2.834019E-02
S0	1181.140000	52.370990

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7200 1.0000
```

ANOVA ingredients

```
   RSS =   269387.500000
   CSS =   683402.600000
     N =          18
```

RESULTS

PH74(30).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1233	1180	53
0.0083	1135	1180	-45
0.0083	1108	1180	-72
0.5	1292	1105	187
0.5	1246	1105	141
0.5	1210	1105	105
1	1056	1035	21
1	997	1035	-38
1	790	1035	-245
1.5	1017	968	49
1.5	1053	968	85
1.5	944	968	-24
2	883	906	-23
2	743	906	-163
2	642	906	-264
4	764	695	69
4	736	695	41
4	832	695	137

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS
FOR FIRST ORDER ORDER REACTION FOR 1,2-DICHLOROPROPANE IN
THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER
MATERIALS (pH 7.8, TEMPERATURE 30°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: PH7830C.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1389751.000000
Prms ->   5.000000E-01     1300.000000

IT#          1Lambda =   1.000000E-02 RSS =  457058.000000
Prms ->   1.302813E-01     1186.428000

IT#          2Lambda =   9.999999E-04 RSS =  313861.800000
Prms ->   1.933597E-01     1195.529000

IT#          3Lambda =   9.999999E-05 RSS =  313433.900000
Prms ->   1.973035E-01     1195.584000

MARQUART: Convergence criterion met
IT#          4Lambda =   9.999999E-05 RSS =  313432.700000
   1.969981E-01     1195.222000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.973035E-01	3.746255E-02
S0	1195.584000	61.203450

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6905 1.0000
```

ANOVA ingredients

```
   RSS =   313432.700000
   CSS =  1140675.000000
     N =          17
```

RESULT

DPH7830C.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1324	1194	130
0.0083	1175	1194	-19
0.0083	1045	1194	-149
0.5	1126	1083	43
0.5	984	1083	-99
0.5	869	1083	-214
1	1177	982	195
1	1099	982	117
1	830	982	-152
1.5	1063	889	174
1.5	981	889	92
1.5	730	889	-159
2	920	806	114
2	993	806	187
4	460	543	-83
4	456	543	-87
4	421	543	-122

NUMERICAL DETERMINATION OF EFFECT OF DISSOLVED OXYGEN ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DISSOLVED OXYGEN 16 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: docom3.dat

IT#          0Lambda =   1.000000E-01 RSS = 2337271.000000
Prms ->       3.000000     1200.000000

IT#          1Lambda =   1.000000E-01 RSS =  114505.000000
Prms ->   5.541768E-01     1169.732000

IT#          2Lambda =   1.000000E-02 RSS =   46328.930000
Prms ->   4.475058E-01     1045.021000

IT#          3Lambda =   9.999999E-04 RSS =   42342.660000
Prms ->   4.126864E-01     1014.981000

IT#          4Lambda =   9.999999E-05 RSS =   42332.300000
Prms ->   4.108771E-01     1013.570000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-05 RSS =   42332.290000
   4.108084E-01     1013.522000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	4.108771E-01	3.757835E-02
S0	1013.570000	38.192540

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6677 1.0000
```

ANOVA ingredients

```
   RSS =    42332.290000
   CSS =   852136.900000
     N =         12
```

RESULTS

DDOCOM3.OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1113	1010	103
0.0083	1017	1010	7
0.5	791	825	-34
0.5	703	825	-122
1	656	672	-16
1	620	672	-52
1.5	607	547	60
1.5	549	547	2
2	468	446	22
2	494	446	48
4	138	196	-58
4	249	196	53

NUMERICAL DETERMINATION OF DISSOLVED OXYGEN ON RATE CONSTANT FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DISSOLVED OXYGEN 8.2 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: DOCOM4.DAT

IT#          0Lambda =   1.000000E-01 RSS = 5893676.000000
Prms ->       3.000000     1200.000000

IT#          1Lambda =     1.000000 RSS = 5277167.000000
Prms ->       2.331236     1210.135000

IT#          2Lambda =     1.000000 RSS = 4644891.000000
Prms ->       1.882147     1225.012000

IT#          3Lambda =   1.000000E-01 RSS = 2601459.000000
Prms ->  -1.480770E-02     1236.069000

IT#          4Lambda =   1.000000E-02 RSS =  190095.600000
Prms ->   1.037370E-01     1083.300000

IT#          5Lambda =   9.999999E-04 RSS =   39156.220000
Prms ->   1.929906E-01     1130.614000

IT#          6Lambda =   9.999999E-05 RSS =   37717.030000
Prms ->   2.047635E-01     1139.035000

IT#          7Lambda =   9.999999E-06 RSS =   37716.030000
Prms ->   2.044275E-01     1138.614000

MARQUART: Convergence criterion met
IT#          8Lambda =   9.999999E-06 RSS =   37716.020000
   2.044423E-01     1138.630000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.044275E-01	2.035169E-02
S0	1138.614000	32.005380

CORRELATION OF PARAMETER ESTIMATES

1.0000
.7044 1.0000

ANOVA ingredients

RSS = 37716.020000
CSS = 552476.900000
N = 12

RESULT

DOCOM4.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1149	1137	12
0.0083	1033	1137	-104
0.5	1015	1028	-13
0.5	1064	1028	36
1	1038	928	110
1	920	928	-8
1.5	905	838	67
1.5	786	838	-52
2	772	757	15
2	752	757	-5
4	508	503	5
4	427	503	-76

NUMERICAL DETERMINATION OF EFFECT OF DISSOLVED OXYGEN ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DISSOLVED OXYGEN 6.0 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: DOCOM5.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1205308.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =  302368.200000
Prms ->   1.756754E-01     1059.255000

IT#          2Lambda =   9.999999E-04 RSS =  114633.800000
Prms ->   2.272747E-01      976.381800

IT#          3Lambda =   9.999999E-05 RSS =  111634.900000
Prms ->   2.442525E-01      984.295500

IT#          4Lambda =   9.999999E-06 RSS =  111583.600000
Prms ->   2.467546E-01      986.520800

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =  111582.800000
   2.470729E-01      986.820900
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.467546E-01	3.935205E-02
S0	986.520800	56.259060

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6605 1.0000
```

ANOVA ingredients

```
   RSS =   111582.800000
   CSS =   624467.000000
     N  =          12
```

RESULTS

DDOCOM5.OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1131	985	146
0.0083	1129	985	144
0.5	761	872	-111
0.5	742	872	-130
1	702	771	-69
1	728	771	-43
2	526	602	-76
2	549	602	-53
3	489	471	18
3	470	471	-1
4	500	368	132
4	451	368	83

NUMERICAL DETERMINATION OF EFFECT OF INOCULUM SIZE ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DRY CELL WT. 1.455 g/L, pH 7.4, TEMPERATURE 25 °C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: innodes1.dat

IT#          0Lambda =   1.000000E-01 RSS =  569199.900000
Prms ->   1.000000E-01     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =  104541.200000
Prms ->   1.098215E-01     1044.708000

IT#          2Lambda =   9.999999E-04 RSS =   98505.580000
Prms ->   1.005939E-01     1015.766000

IT#          3Lambda =   9.999999E-05 RSS =   98493.410000
Prms ->   9.979358E-02     1014.707000

MARQUART: Convergence criterion met
IT#          4Lambda =   9.999999E-05 RSS =   98493.400000
   9.977005E-02     1014.677000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	9.979358E-02	1.835012E-02
S0	1014.707000	30.865640

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7282 1.0000
```

ANOVA ingredients

```
   RSS =    98493.400000
   CSS =   299652.000000
     N =          18
```

RESULTS

DINODES1.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1002	1014	-12
0.0083	1012	1014	-2
0.0083	1010	1014	-4
0.5	1025	965	60
0.5	993	965	28
0.5	1074	965	109
1	923	918	5
1	889	918	-29
1	854	918	-64
1.5	911	874	37
1.5	866	874	-8
1.5	732	874	-142
2	826	831	-5
2	799	831	-32
2	807	831	-24
4	828	681	147
4	774	681	93
4	527	681	-154

NUMERICAL DETERMINATION OF EFFECT OF INOCULUM SIZE ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DRY CELL WT. 3.317 g/L, pH 7.4, TEMPERATURE 25 °C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: innodes2.dat

IT#          0Lambda =   1.000000E-01 RSS =  655754.900000
Prms ->   5.000000E-01     1000.000000

IT#          1Lambda =   1.000000E-02 RSS =  117269.100000
Prms ->   1.814700E-01      979.108500

IT#          2Lambda =   9.999999E-04 RSS =   74407.730000
Prms ->   2.197305E-01      966.956600

IT#          3Lambda =   9.999999E-05 RSS =   74171.720000
Prms ->   2.243306E-01      969.102600

MARQUART: Convergence criterion met
IT#          4Lambda =   9.999999E-05 RSS =   74171.600000
   2.244460E-01      969.198400
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.243306E-01	2.426405E-02
S0	969.102600	32.343350

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6738 1.0000
```

ANOVA ingredients

```
   RSS =    74171.600000
   CSS =   665129.900000
     N =          16
```

RESULTS

DINODES2.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	990	967	23
0.0083	1011	967	44
0.0083	1002	967	35
0.5	782	866	-84
0.5	846	866	-20
0.5	795	866	-71
1	739	774	-35
1.5	774	692	82
1.5	827	692	135
1.5	780	692	88
2	526	619	-93
2	548	619	-71
2	539	619	-80
4	422	395	27
4	448	395	53
4	370	395	-25

NUMERICAL DETERMINATION OF EFFECT OF INOCULUM SIZE ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DRY CELL WT. 6.470 g/L, pH 7.4, TEMPERATURE 25 °C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: innodes3.dat

IT#          0Lambda =   1.000000E-01 RSS = 1725926.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS = 1077292.000000
Prms ->   8.477449E-02     1020.425000

IT#          2Lambda =   9.999999E-04 RSS =   73283.730000
Prms ->   2.213262E-01      980.818400

IT#          3Lambda =   9.999999E-05 RSS =   30550.840000
Prms ->   2.821728E-01     1009.547000

IT#          4Lambda =   9.999999E-06 RSS =   30347.910000
Prms ->   2.871310E-01     1012.377000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   30347.890000
   2.871650E-01     1012.399000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.871310E-01	1.601823E-02
S0	1012.377000	19.554620

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6892 1.0000
```

ANOVA ingredients

```
   RSS =    30347.890000
   CSS =   907948.300000
     N =          18
```

RESULTS

DINODSE3.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	996	1010	-14
0.0083	1028	1010	18
0.0083	964	1010	-46
0.5	935	877	58
0.5	887	877	10
0.5	890	877	13
1	769	760	9
1	782	760	22
1	772	760	12
1.5	630	658	-28
1.5	640	658	-18
1.5	555	658	-103
2	513	570	-57
2	655	570	85
2	600	570	30
4	314	321	-7
4	325	321	4
4	334	321	13

NUMERICAL DETERMINATION OF EFFECT OF INOCULUM SIZE ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DRY CELL WT. 8.017 g/L, pH 7.4, TEMPERATURE 25 °C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: innodes4.dat

IT#          0Lambda =   1.000000E-01 RSS = 1264896.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =  417666.900000
Prms ->   2.454778E-01      961.753300

IT#          2Lambda =   9.999999E-04 RSS =  223952.100000
Prms ->   3.614818E-01      965.150300

IT#          3Lambda =   9.999999E-05 RSS =  222755.500000
Prms ->   3.725623E-01      964.008200

IT#          4Lambda =   9.999999E-06 RSS =  222748.200000
Prms ->   3.712203E-01      963.001600

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =  222748.000000
   3.714063E-01      963.129700
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.712203E-01	5.430147E-02
S0	963.001600	55.489590

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6746 1.0000
```

ANOVA ingredients

```
   RSS =   222748.000000
   CSS =  1372836.000000
     N =          18
```

RESULTS

DINODES4.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	969	960	9
0.0083	963	960	3
0.0083	962	960	2
0.5	769	800	-31
0.5	765	800	-35
0.5	575	800	-225
1	808	664	144
1	773	664	109
1	559	664	-105
1.5	692	552	140
1.5	664	552	112
1.5	548	552	-4
2	507	458	49
2	496	458	38
2	509	458	51
4	271	218	53
4	12	218	-206
4	12	218	-206

NUMERICAL DETERMINATION OF EFFECT OF SUBSTRATE CONCENTRATION ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

sust1df.dat

IT#          0Lambda =   1.000000E-01 RSS =  124943.200000
Prms ->   5.000000E-01      750.000000

IT#          1Lambda =   1.000000E-02 RSS =   38078.510000
Prms ->   7.103555E-01      743.269700

IT#          2Lambda =   9.999999E-04 RSS =   30479.340000
Prms ->   8.295648E-01      760.059000

IT#          3Lambda =   9.999999E-05 RSS =   30419.280000
Prms ->   8.423246E-01      761.580600

MARQUART: Convergence criterion met
IT#          4Lambda =   9.999999E-05 RSS =   30419.280000
   8.422604E-01      761.564600
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	8.423246E-01	9.266944E-02
S0	761.580600	30.108910

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .4795 1.0000
```

ANOVA ingredients

```
   RSS =    30419.280000
   CSS =  1055238.000000
     N =       12
```

RESULTS

DSUST1DF.OUT

TIME (hr)	CONCENTRATION ACTUAL (µg/L)	PREDICTED (µg/L)	RESIDUAL (µg/L)
0.0083	800	756	44
0.0083	724	756	-32
0.0083	748	756	-8
0.5	544	500	44
0.5	370	500	-130
0.5	576	500	76
2	156	141	15
2	115	141	-26
2	177	141	36
4	12	26	-14
4	12	26	-14
4	12	26	-14

NUMERICAL DETERMINATION OF EFFECT OF SUBSTRATE CONCENTRATION ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: SUST2DF.DAT

IT#          0Lambda =   1.000000E-01 RSS =  520420.600000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =  202444.200000
Prms ->   4.497098E-01     1237.305000

IT#          2Lambda =   9.999999E-04 RSS =   80404.090000
Prms ->   5.975601E-01     1261.010000

IT#          3Lambda =   9.999999E-05 RSS =   79810.880000
Prms ->   6.103737E-01     1261.093000

IT#          4Lambda =   9.999999E-06 RSS =   79805.150000
Prms ->   6.087792E-01     1260.293000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   79805.030000
   6.090020E-01     1260.398000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	6.087792E-01	5.033879E-02
S0	1260.293000	42.155640

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5631 1.0000
```

ANOVA ingredients

```
   RSS =    79805.030000
   CSS =  2715253.000000
     N =          14
```

RESULTS

DSUST2DF.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	1297	1254	43
0.0083	1126	1254	-127
0.0083	1244	1254	-10
0.5	958	930	29
0.5	944	930	15
0.5	900	930	-29
1	788	686	102
1	831	686	145
2	374	373	1
2	283	373	-90
2	352	373	-21
4	96	110	-14
4	12	110	-99
4	12	110	-99

NUMERICAL DETERMINATION OF EFFECT OF SUBSTRATE CONCENTRATION ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: SUST3DF.DAT

IT#          0Lambda =   1.000000E-01 RSS =   1.007327E+08
Prms ->       1.000000     5000.000000

IT#          1Lambda =   1.000000E-01 RSS =   1.194278E+07
Prms ->   2.444313E-01     5270.688000

IT#          2Lambda =   1.000000E-02 RSS = 3622213.000000
Prms ->   9.887937E-02     4822.080000

IT#          3Lambda =   9.999999E-04 RSS = 3459088.000000
Prms ->   8.334802E-02     4668.632000

IT#          4Lambda =   9.999999E-05 RSS = 3458215.000000
Prms ->   8.194482E-02     4660.232000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-05 RSS = 3458212.000000
   8.187024E-02     4659.789000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	8.194482E-02	2.423111E-02
S0	4660.232000	198.764200

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7136 1.0000
```

ANOVA ingredients

```
   RSS =  3458212.000000
   CSS =  6420811.000000
     N =          16
```

RESULTS

SUST3DF.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	4795	4657	138
0.0083	4954	4657	297
0.0083	4504	4657	-153
0.5	4544	4473	71
0.5	4930	4473	457
0.5	4174	4473	-299
1	4661	4294	368
1	4810	4294	517
1.5	3690	4121	-431
1.5	3419	4121	-702
2	3514	3956	-442
2	3870	3956	-86
2	3400	3956	-556
4	3707	3358	349
4	4375	3358	1017
4	2833	3358	-525

NUMERICAL DETERMINATION OF EFFECT OF LEAD CONCENTRATION ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (LEAD 0.0 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: LEADDES1.DAT

IT#          0Lambda =   1.000000E-01 RSS = 3608956.000000
Prms ->       2.000000     1010.000000

IT#          1Lambda =     1.000000 RSS = 3169827.000000
Prms ->       1.595288     1024.674000

IT#          2Lambda =   1.000000E-01 RSS = 2462748.000000
Prms ->  -4.593921E-02     1056.509000

IT#          3Lambda =   1.000000E-02 RSS =  248267.900000
Prms ->   8.115439E-02      930.411900

IT#          4Lambda =   9.999999E-04 RSS =   93774.450000
Prms ->   1.841919E-01      987.850800

IT#          5Lambda =   9.999999E-05 RSS =   92677.480000
Prms ->   1.955125E-01      994.046000

IT#          6Lambda =   9.999999E-06 RSS =   92673.660000
Prms ->   1.947466E-01      993.180400

MARQUART: Convergence criterion met
IT#          7Lambda =   9.999999E-06 RSS =   92673.630000
   1.948105E-01      993.247300
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.947466E-01	3.825142E-02
S0	993.180400	56.161020

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .7122 1.0000
```

ANOVA ingredients

```
   RSS =    92673.630000
   CSS =   452426.200000
     N =          11
```

RESULTS

LEADDES1.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	997	992	5
0.0083	997	992	5
0.5	733	901	-168
1	769	817	-48
1	880	817	63
1.5	700	742	-42
1.5	879	742	137
2	797	673	124
2	753	673	80
4	383	456	-73
4	354	456	-102

NUMERICAL DETERMINATION OF EFFECT OF LEAD CONCENTRATION ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (LEAD 10.0 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: LEADDES2.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1261256.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS = 1066759.000000
Prms ->   2.778095E-02     1036.025000

IT#          2Lambda =   9.999999E-04 RSS =  181638.400000
Prms ->   1.579467E-01      976.677300

IT#          3Lambda =   9.999999E-05 RSS =  137284.800000
Prms ->   2.228384E-01     1006.079000

IT#          4Lambda =   9.999999E-06 RSS =  136807.900000
Prms ->   2.310864E-01     1011.045000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =  136807.100000
   2.314246E-01     1011.317000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.310864E-01	5.278651E-02
S0	1011.045000	71.232080

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6486 1.0000
```

ANOVA ingredients

```
   RSS =   136807.100000
   CSS =   579606.900000
     N =          10
```

RESULTS

LEADDES2.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1113	1009	104
0.0083	1080	1009	71
0.5	941	901	40
0.5	577	901	-324
1	863	802	61
1.5	708	715	-7
2	609	637	-28
2	710	637	73
4	457	401	56
4	361	401	-40

NUMERICAL DETERMINATION OF EFFECT OF LEAD CONCENTRATION ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (LEAD 5.8 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: LEADDES3.DAT

IT#          0Lambda =   1.000000E-01 RSS =  597764.200000
Prms ->   5.000000E-01     1300.000000

IT#          1Lambda =   1.000000E-02 RSS =  267503.600000
Prms ->   2.970871E-01     1038.151000

IT#          2Lambda =   9.999999E-04 RSS =  254996.000000
Prms ->   2.478422E-01      991.119100

IT#          3Lambda =   9.999999E-05 RSS =  254978.800000
Prms ->   2.495765E-01      992.293300

MARQUART: Convergence criterion met
IT#          4Lambda =   9.999999E-05 RSS =  254978.800000
   2.495135E-01      992.238200
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.495765E-01	6.747965E-02
S0	992.293300	85.777650

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6958 1.0000
```

ANOVA ingredients

```
   RSS =   254978.800000
   CSS =   758598.700000
     N =          12
```

RESULTS

LEADDES3.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	865	990	-125
0.0083	1253	990	263
0.5	716	876	-160
0.5	888	876	12
1	583	773	-190
1	634	773	-139
1.5	769	682	87
1.5	752	682	70
2	806	602	204
2	736	602	134
4	320	366	-46
4	242	366	-124

NUMERICAL DETERMINATION OF EFFECT OF LEAD CONCENTRATION ON RATE CONSTANTS FOR FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (LEAD 2.2 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with no background
    1 independents and  2 parameters.
EXECUTION BEGINS

FILE #: LEADDES4.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1201225.000000
Prms ->       1.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =  496974.200000
Prms ->   1.118805E-01     1027.541000

IT#          2Lambda =   9.999999E-04 RSS =   70817.470000
Prms ->   2.136049E-01      966.061300

IT#          3Lambda =   9.999999E-05 RSS =   56170.950000
Prms ->   2.562000E-01      986.887900

IT#          4Lambda =   9.999999E-06 RSS =   55984.120000
Prms ->   2.618422E-01      991.175400

IT#          5Lambda =   9.999999E-07 RSS =   55982.880000
Prms ->   2.623089E-01      991.575900

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-05 RSS =   55982.870000
   2.623454E-01      991.607400
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.623089E-01	3.256828E-02
S0	991.575900	40.520810

CORRELATION OF PARAMETER ESTIMATES

```
   1.0000
  .6935 1.0000
```

ANOVA ingredients

```
   RSS =    55982.870000
   CSS =   531307.700000
     N =          12
```

RESULTS

LEADDES4.OUT

TIME (hr)	ACTUAL CONCENTRATION (µg/L)	PREDICTED CONCENTRATION (µg/L)	RESIDUAL (µg/L)
0.0083	1051	989	62
0.0083	940	989	-49
0.5	982	870	112
0.5	905	870	35
1	757	763	-6
1	676	763	-87
1.5	627	669	-42
1.5	536	669	-133
2	609	587	22
2	571	587	-16
4	421	347	74
4	399	347	52

MODIFIED FIRST ORDER MODEL

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2 DIBROMO-3-CHLOROPROPANE (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: DBCPDF.DAT

IT#          0Lambda =   1.000000E-01 RSS =  106012.100000
Prms ->       4.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =    5889.573000
Prms ->       3.920163     1027.377000       80.539120

IT#          2Lambda =   1.000000E-02 RSS =    4581.394000
Prms ->       3.816935     1010.576000       72.694940

IT#          3Lambda =   1.000000E-02 RSS =    3920.649000
Prms ->       3.734701     1007.800000       73.351050

IT#          4Lambda =   9.999999E-04 RSS =    3804.238000
Prms ->       3.653057     1006.124000       66.180810

IT#          5Lambda =   9.999999E-05 RSS =    3380.615000
Prms ->       3.641701     1005.904000       82.857320

IT#          6Lambda =   9.999999E-06 RSS =    3374.056000
Prms ->       3.641570     1005.902000       85.305440

MARQUART: Convergence criterion met
IT#          7Lambda =   9.999999E-06 RSS =    3374.055000
       3.641572     1005.902000       85.343080
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.641570	1.241730E-01
S0	1005.902000	9.117508
R	85.305440	49.542730

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .2467 1.0000
  .9551  .2098 1.0000
```

ANOVA ingredients

```
   RSS =     3374.055000
   CSS =  2158781.000000
     N =          18
```

RESULTS

DDBCPDF.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	977	977	0
0.0083	1004	977	27
0.0083	950	977	-27
0.5	156	182	-26
0.5	174	182	-8
0.5	205	182	23
1	61	49	12
1	67	49	18
1	58	49	9
2	23	24	-1
2	23	24	-1
2	23	24	-1
3	21	23	-2
3	19	23	-4
3	19	23	-4
4	20	23	-3
4	19	23	-4
4	19	23	-4

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2 DIBROMO-3-CHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPEARTURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: DBCPDF2.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1706554.000000
Prms ->       1.000000     1200.000000       100.000000

IT#          1Lambda =   1.000000E-02 RSS =  283427.500000
Prms ->       1.611323      945.587000       104.871700

IT#          2Lambda =   9.999999E-04 RSS =  143893.100000
Prms ->       2.363165      976.821400       166.803000

IT#          3Lambda =   9.999999E-05 RSS =   95286.470000
Prms ->       2.452305      979.651100        62.429550

IT#          4Lambda =   9.999999E-06 RSS =   92807.380000
Prms ->       2.432881      979.040600        23.546900

IT#          5Lambda =   9.999999E-07 RSS =   92790.880000
Prms ->       2.436271      979.168400        26.804980

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =   92790.820000
       2.435742      979.145900       26.558210
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.436271	3.451036E-01
S0	979.168400	46.975530
R	26.804980	79.158940

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .3080 1.0000
  .7327  .1298 1.0000
```

ANOVA ingredients

```
   RSS =    92790.820000
   CSS =  2179605.000000
     N =          18
```

RESULTS

EDBCPDF2.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	1145	960	186
0.0083	780	960	-180
0.0083	935	960	-25
0.5	319	297	22
0.5	334	297	36
0.5	368	297	71
1	21	96	-74
1	21	96	-75
1	19	96	-77
2	34	18	16
2	33	18	15
2	36	18	18
3	24	12	12
3	24	12	12
3	22	12	10
4	22	11	11
4	21	11	10
4	22	11	11

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: AQUIFDF.DAT

IT#          0Lambda =   1.000000E-01 RSS =  487354.200000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  232517.300000
Prms ->       1.046023     1250.563000      278.155500

IT#          2Lambda =   9.999999E-04 RSS =  213999.100000
Prms ->       1.236544     1273.436000      402.735400

IT#          3Lambda =   9.999999E-05 RSS =  211451.800000
Prms ->       1.376335     1288.008000      500.044500

IT#          4Lambda =   9.999999E-06 RSS =  210299.400000
Prms ->       1.451448     1294.869000      541.588800

IT#          5Lambda =   9.999999E-07 RSS =  208378.600000
Prms ->       1.486603     1297.986000      550.128000

IT#          6Lambda =   9.999999E-08 RSS =  207675.900000
Prms ->       1.502294     1299.352000      550.776700

IT#          7Lambda =   9.999999E-08 RSS =  207516.500000
Prms ->       1.509270     1299.945000      550.653900

IT#          8Lambda =   9.999999E-08 RSS =  207483.900000
Prms ->       1.512382     1300.206000      550.559700

IT#          9Lambda =   9.999999E-08 RSS =  207477.400000
Prms ->       1.513772     1300.321000      550.512100

MARQUART: Convergence criterion met
IT#         10Lambda =   9.999999E-08 RSS =  207476.000000
       1.514394     1300.373000      550.489800
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.513772	3.729396E-01
S0	1300.321000	76.852230
R	550.512100	384.750300

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .4012 1.0000
  .9827  .3505 1.0000
```

ANOVA ingredients

```
   RSS =   207476.000000
   CSS =  1917710.000000
     N =          15
```

RESULTS

EAQUIFDF.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1224	1289	-65
0.0083	1367	1289	78
0.0083	1376	1289	87
0.5	563	803	-240
0.5	694	803	-109
0.5	771	803	-32
1	708	570	138
1	572	570	2
1	736	570	166
2	462	409	53
2	553	409	144
2	450	409	41
4	219	366	-147
4	210	366	-156
4	400	366	34

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF SAND SPRINGS PETROCHEMICAL COMPLEX SUBSURFACE MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: AQUIFDF2.DAT

IT#          0Lambda =   1.000000E-01 RSS =  716558.100000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  166340.600000
Prms ->   5.494657E-01     1192.795000      201.848300

IT#          2Lambda =   9.999999E-04 RSS =   38535.750000
Prms ->   4.344375E-01     1185.511000       56.953870

IT#          3Lambda =   9.999999E-05 RSS =   30547.710000
Prms ->   4.227578E-01     1180.773000       31.118310

IT#          4Lambda =   9.999999E-06 RSS =   30528.880000
Prms ->   4.229082E-01     1180.734000       30.222440

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   30528.880000
   4.229006E-01     1180.733000       30.227220
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	4.229082E-01	7.981525E-02
S0	1180.734000	28.555210
R	30.222440	44.160480

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5436 1.0000
  .9506  .3932 1.0000
```

ANOVA ingredients

```
   RSS =    30528.880000
   CSS =  1475762.000000
     N =          14
```

RESULTS

EAQUIFD2.OUT

TIME (hr)	ACTUAL CONCENTRATION (µg/L)	PREDICTED CONCENTRATION (µg/L)	RESIDUAL CONCENTRATION (µg/L)
0.0083	1247	1177	70
0.0083	1191	1177	14
0.0083	1083	1177	-94
0.5	975	969	6
0.5	939	969	-30
1	867	798	69
1	853	798	55
1	759	798	-39
2	545	548	-3
2	522	548	-26
2	508	548	-40
4	312	276	36
4	303	276	27
4	230	276	-46

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 5.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE : PH54(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  766444.200000
Prms ->   5.000000E-01     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =   69073.650000
Prms ->   5.350869E-01     1058.445000    8.863983E-01

IT#          2Lambda =   9.999999E-04 RSS =   47575.360000
Prms ->   3.765352E-01     1016.861000      -77.769870

IT#          3Lambda =   9.999999E-04 RSS =   47442.220000
Prms ->   3.578130E-01     1012.471000      -88.713250

IT#          4Lambda =   9.999999E-05 RSS =   47204.290000
Prms ->   3.502319E-01     1010.732000      -90.390290

IT#          5Lambda =   9.999999E-06 RSS =   47134.880000
Prms ->   3.496736E-01     1010.608000      -89.148220

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-06 RSS =   47134.450000
   3.496518E-01     1010.603000      -89.036870
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.496736E-01	7.007660E-02
S0	1010.608000	29.129320
R	-89.148220	47.151790

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5925 1.0000
  .9599  .4384 1.0000
```

ANOVA ingredients

```
   RSS =    47134.450000
   CSS =  1721402.000000
     N =          18
```

RESULTS

PH54(25).OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	CONCENTRATION RESIDUAL (μg/L)
0.0083	942	1007	-65
0.0083	1015	1007	8
0.0083	1095	1007	88
0.5	769	808	-39
0.5	850	808	42
0.5	792	808	-16
1	631	637	-6
1	594	637	-43
1	595	637	-42
1.5	484	494	-10
1.5	475	494	-19
1.5	550	494	56
2	286	374	-88
2	428	374	54
2	475	374	101
4	93	58	35
4	48	58	-10
4	12	58	-46

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH74(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  427986.300000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  198439.500000
Prms ->   7.307835E-01     1003.249000       99.850270

IT#          2Lambda =   9.999999E-04 RSS =  160043.900000
Prms ->   4.257095E-01      951.082800      -21.239310

IT#          3Lambda =   9.999999E-05 RSS =  149440.800000
Prms ->   3.667896E-01      938.511300      -68.690060

IT#          4Lambda =   9.999999E-06 RSS =  149121.300000
Prms ->   3.596344E-01      936.445600      -69.038790

IT#          5Lambda =   9.999999E-07 RSS =  149087.800000
Prms ->   3.585575E-01      936.222800      -68.527460

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =  149087.000000
   3.584040E-01      936.189600      -68.436740
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.585575E-01	1.387731E-01
S0	936.222800	51.874300
R	-68.527460	82.671810

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5903 1.0000
  .9581  .4330 1.0000
```

ANOVA ingredients

```
   RSS =   149087.000000
   CSS =  1508387.000000
     N =          18
```

RESULTS

EP74(25).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1039	933	106
0.0083	997	933	64
0.0083	972	933	39
0.5	483	751	-268
0.5	704	751	-47
0.5	635	751	-116
1	629	597	32
1	698	597	101
1	603	597	6
1.5	339	467	-128
1.5	540	467	73
1.5	531	467	64
2	382	359	23
2	416	359	57
2	409	359	50
4	65	78	-13
4	66	78	-12
4	49	78	-29

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 8.9, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH89(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  651355.100000
Prms ->       1.000000     1300.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  166997.300000
Prms ->   6.592033E-01      999.475500      106.001700

IT#          2Lambda =   9.999999E-04 RSS =  133970.100000
Prms ->   2.627396E-01      935.328100      -56.614650

IT#          3Lambda =   9.999999E-05 RSS =   92014.410000
Prms ->   2.342587E-01      926.988600     -111.381600

IT#          4Lambda =   9.999999E-06 RSS =   90333.400000
Prms ->   2.319133E-01      925.890700     -106.318500

IT#          5Lambda =   9.999999E-07 RSS =   90295.140000
Prms ->   2.317596E-01      925.866500     -105.421900

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =   90294.980000
   2.317521E-01      925.864800     -105.362100
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.317596E-01	1.073526E-01
S0	925.866500	39.598630
R	-105.421900	94.405140

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6220 1.0000
  .9843  .5249 1.0000
```

ANOVA ingredients

```
   RSS =    90294.980000
   CSS =  1370586.000000
     N =          18
```

RESULTS

EP89(25).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	978	923	55
0.0083	983	923	60
0.0083	927	923	4
0.5	589	775	-186
0.5	678	775	-97
0.5	790	775	15
1	616	640	-24
1	699	640	59
1	734	640	94
1.5	580	520	60
1.5	441	520	-79
1.5	569	520	49
2	357	414	-57
2	433	414	19
2	457	414	43
4	109	92	17
4	141	92	49
4	12	92	-80

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 6.4, TEMPERATURE 15°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH64(15).DAT

IT#          0Lambda =   1.000000E-01 RSS = 2648400.000000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  326797.900000
Prms ->   6.065620E-01     1130.781000      457.461200

IT#          2Lambda =   9.999999E-04 RSS =  134940.200000
Prms ->   5.259051E-01     1124.267000      309.893300

IT#          3Lambda =   9.999999E-04 RSS =  132014.000000
Prms ->   4.552882E-01     1116.050000      251.557100

IT#          4Lambda =   9.999999E-05 RSS =  126608.900000
Prms ->   3.991981E-01     1110.041000      201.785300

IT#          5Lambda =   9.999999E-06 RSS =  114371.400000
Prms ->   3.901162E-01     1108.323000      175.519900

IT#          6Lambda =   9.999999E-07 RSS =  114056.500000
Prms ->   3.889664E-01     1108.081000      171.456300

IT#          7Lambda =   9.999999E-08 RSS =  114052.000000
Prms ->   3.887565E-01     1108.052000      170.938000

MARQUART: Convergence criterion met
IT#          8Lambda =   9.999999E-08 RSS =  114051.900000
   3.887163E-01     1108.047000      170.843300
```

NAME	VALUE	PARAMETER STD (asymptotic)
K1	3.887565E-01	2.150805E-01
S0	1108.052000	51.712460
R	170.938000	90.134950

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5372 1.0000
  .9670  .3986 1.0000
```

ANOVA ingredients

```
   RSS =   114051.900000
   CSS =   586971.800000
     N =          16
```

RESULTS

EP64(15).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1102	1106	-4
0.0083	1183	1106	77
0.0083	1142	1106	36
0.5	839	990	-151
1	931	893	38
1	987	893	94
1	692	893	-201
1.5	836	813	23
1.5	765	813	-48
1.5	808	813	-5
2	722	747	-25
2	854	747	107
2	859	747	112
4	529	581	-52
4	539	581	-42
4	618	581	37

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 15°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH74(15).DAT

IT#          0Lambda =   1.000000E-01 RSS =  900882.400000
Prms ->   3.000000E-01     1093.646000      386.499000

IT#          1Lambda =   1.000000E-02 RSS =  188209.900000
Prms ->   3.866501E-01     1056.057000      241.462800

IT#          2Lambda =   9.999999E-04 RSS =  104770.800000
Prms ->   3.325846E-01     1082.002000      282.877200

IT#          3Lambda =   9.999999E-05 RSS =   77731.460000
Prms ->   3.409574E-01     1083.679000      251.498700

IT#          4Lambda =   9.999999E-06 RSS =   77335.050000
Prms ->   3.341852E-01     1083.386000      255.590100

IT#          5Lambda =   9.999999E-07 RSS =   76866.720000
Prms ->   3.322019E-01     1083.267000      250.615700

IT#          6Lambda =   9.999999E-08 RSS =   76822.180000
Prms ->   3.317997E-01     1083.231000      249.177100

IT#          7Lambda =   9.999999E-08 RSS =   76820.380000
Prms ->   3.317095E-01     1083.224000      248.884600

MARQUART: Convergence criterion met
IT#          8Lambda =   9.999999E-08 RSS =   76820.300000
   3.316884E-01     1083.222000      248.818800
```

NAME	PARAMETER VALUE	STD (asymptotic)
K1	3.317095E-01	4.049670E-01
S0	1083.224000	46.826150
R	248.884600	106.573900

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6618 1.0000
  .9841  .5710 1.0000
```

ANOVA ingredients

```
   RSS =    76820.300000
   CSS =   171901.800000
     N =          16
```

RESULTS

EP74(15).OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	951	1082	-131
0.0083	1277	1082	195
0.5	968	1032	-64
0.5	1040	1032	8
0.5	1010	1032	-22
1	925	989	-64
1	1009	989	20
1	944	989	-45
1.5	951	953	-2
1.5	1010	953	57
1.5	985	953	32
2	982	922	60
2	901	922	-21
4	837	839	-2
4	858	839	19
4	798	839	-41

NUMERICAL DETERMINATION OF RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.8, TEMPERATURE 15°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH7815C.DAT

IT#          0Lambda =   1.000000E-01 RSS =  406331.600000
Prms ->   5.000000E-01     1400.000000      350.000000

IT#          1Lambda =   1.000000E-02 RSS =  382688.700000
Prms ->   4.529565E-01     1358.860000      373.491000

IT#          2Lambda =   9.999999E-04 RSS =  375859.900000
Prms ->   4.292375E-01     1349.487000      342.020200

IT#          3Lambda =   9.999999E-05 RSS =  375685.000000
Prms ->   4.060639E-01     1347.122000      317.321200

IT#          4Lambda =   9.999999E-06 RSS =  371033.100000
Prms ->   4.058639E-01     1346.964000      302.571200

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =  371032.900000
   4.057886E-01     1346.956000      302.411000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	4.058639E-01	4.530294E-01
S0	1346.964000	97.602330
R	302.571200	193.797200

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5364 1.0000
  .9728  .4174 1.0000
```

ANOVA ingredients

```
   RSS =   371032.900000
   CSS =   760790.900000
     N =          15
```

RESULTS

EPH7815c.OUT

TIME (hr)	ACTUAL CONCENTRATION (µg/L)	PREDICTED CONCENTRATION (µg/L)	RESIDUAL (µg/L)
0.0083	1250	1345	-95
0.0083	1528	1345	183
0.0083	1375	1345	30
0.5	814	1236	-422
1	1400	1146	254
1	1107	1146	-39
1	1345	1146	199
1.5	1171	1073	98
1.5	1090	1073	17
1.5	1020	1073	-53
2	988	1013	-25
2	839	1013	-174
4	868	864	4
4	872	864	8
4	877	864	13

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 6.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH64(25).DAT

IT#          0Lambda =   1.000000E-01 RSS = 2481432.000000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  407149.900000
Prms ->   6.383608E-01     1246.435000      395.881800

IT#          2Lambda =   9.999999E-04 RSS =  262703.200000
Prms ->   7.040982E-01     1257.772000      328.418400

IT#          3Lambda =   9.999999E-05 RSS =  259058.400000
Prms ->   6.734653E-01     1255.920000      329.723700

IT#          4Lambda =   9.999999E-06 RSS =  258231.200000
Prms ->   6.770479E-01     1256.518000      323.812000

IT#          5Lambda =   9.999999E-07 RSS =  258219.100000
Prms ->   6.764661E-01     1256.442000      324.451500

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =  258218.800000
   6.765506E-01     1256.454000      324.342800
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	6.764661E-01	2.576561E-01
S0	1256.442000	71.267220
R	324.451500	135.258000

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5175 1.0000
  .9567  .3805 1.0000
```

ANOVA ingredients

```
   RSS =   258218.800000
   CSS =  1260309.000000
     N =          18
```

RESULTS

EPH6425.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1329	1252	77
0.0083	1204	1252	-48
0.0083	1120	1252	-132
0.5	1211	1034	177
0.5	1127	1034	93
0.5	1024	1034	-10
1	1041	875	166
1	795	875	-80
1	616	875	-259
1.5	885	761	124
1.5	785	761	24
1.5	723	761	-38
2	762	680	82
2	683	680	3
2	439	680	-241
4	608	532	76
4	562	532	30
4	484	532	-48

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH74/25.DAT

IT#          0Lambda =   1.000000E-01 RSS =  487422.300000
Prms ->   5.000000E-01     1200.000000      400.000000

IT#          1Lambda =   1.000000E-02 RSS =  140732.400000
Prms ->   6.402360E-01     1169.745000      331.149800

IT#          2Lambda =   9.999999E-04 RSS =  115521.600000
Prms ->   5.352798E-01     1165.472000      337.056500

IT#          3Lambda =   9.999999E-05 RSS =   90065.850000
Prms ->   5.257912E-01     1165.832000      295.275300

IT#          4Lambda =   9.999999E-06 RSS =   89774.870000
Prms ->   5.263767E-01     1165.813000      291.581300

IT#          5Lambda =   9.999999E-07 RSS =   89773.880000
Prms ->   5.262500E-01     1165.798000      291.763800

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =   89773.840000
   5.262726E-01     1165.801000      291.722400
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	5.262500E-01	1.865225E-01
S0	1165.798000	41.219190
R	291.763800	79.907490

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5500 1.0000
  .9653  .4198 1.0000
```

ANOVA ingredients

```
   RSS =    89773.840000
   CSS =   629184.300000
     N =          18
```

RESULTS

EPH74/25.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1210	1163	47
0.0083	1084	1163	-79
0.0083	1090	1163	-73
0.5	1167	1024	143
0.5	1055	1024	31
0.5	1054	1024	30
1	954	916	38
1	909	916	-7
1	873	916	-43
1.5	901	832	69
1.5	818	832	-14
1.5	749	832	-83
2	750	768	-18
2	730	768	-38
2	714	768	-54
4	734	629	105
4	705	629	76
4	498	629	-131

NUMERICAL DETERMINATION OF EFFECT OF TEMPERATURE ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.8, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH7825C.DAT

IT#          0Lambda =   1.000000E-01 RSS = 2403313.000000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  551914.800000
Prms ->   7.129216E-01     1342.242000      395.032700

IT#          2Lambda =   9.999999E-04 RSS =  489980.300000
Prms ->   7.302067E-01     1352.041000      328.935200

IT#          3Lambda =   9.999999E-05 RSS =  489886.100000
Prms ->   7.215320E-01     1351.592000      326.966300

IT#          4Lambda =   9.999999E-06 RSS =  489848.800000
Prms ->   7.188469E-01     1351.168000      323.677300

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =  489845.200000
   7.180635E-01     1351.041000      322.680000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	7.188469E-01	3.445810E-01
S0	1351.168000	106.724400
R	323.677300	194.732600

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5175 1.0000
  .9443  .3702 1.0000
```

ANOVA ingredients

```
   RSS =   489845.200000
   CSS =  1813957.000000
     N =          16
```

RESULTS

EPH7825C.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1391	1346	45
0.0083	1283	1346	-63
0.0083	1485	1346	139
0.5	1399	1079	320
0.5	872	1079	-207
0.5	806	1079	-274
1	992	889	103
1	637	889	-253
1	857	889	-32
1.5	773	757	16
1.5	725	757	-32
2	659	664	-6
2	1061	664	397
4	425	501	-76
4	473	501	-28
4	445	501	-56

NUMERICAL DETERMINATION OF EFFECT OF pH ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 6.4, TEMPERATURE 30°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: ph64(30).dat

IT#          0Lambda =   1.000000E-01 RSS = 2918317.000000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  387587.500000
Prms ->   6.285968E-01     1162.465000      464.671100

IT#          2Lambda =   9.999999E-04 RSS =  238289.500000
Prms ->   7.456406E-01     1172.275000      412.910500

IT#          3Lambda =   9.999999E-05 RSS =  221404.600000
Prms ->   6.813832E-01     1169.070000      419.231200

IT#          4Lambda =   9.999999E-06 RSS =  217413.700000
Prms ->   7.020132E-01     1171.611000      411.145700

IT#          5Lambda =   9.999999E-07 RSS =  216934.000000
Prms ->   6.933462E-01     1170.781000      413.293700

IT#          6Lambda =   9.999999E-08 RSS =  216852.600000
Prms ->   6.966161E-01     1171.135000      412.278400

IT#          7Lambda =   9.999999E-08 RSS =  216840.800000
Prms ->   6.953343E-01     1171.002000      412.649200

MARQUART: Convergence criterion met
IT#          8Lambda =   9.999999E-08 RSS =  216839.000000
   6.958290E-01     1171.054000      412.501700
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	6.953343E-01	3.200823E-01
S0	1171.002000	65.456830
R	412.649200	162.132900

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5137 1.0000
  .9743  .4126 1.0000
```

ANOVA ingredients

```
   RSS =   216839.000000
   CSS =   778747.600000
     N =          18
```

RESULTS

EP64(30).OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1165	1168	-3
0.0083	1119	1168	-49
0.0083	1021	1168	-147
0.5	1137	1001	136
0.5	1169	1001	168
0.5	1153	1001	152
1	909	882	27
1	890	882	8
1	749	882	-133
1.5	919	797	122
1.5	768	797	-29
1.5	536	797	-261
2	711	737	-26
2	706	737	-31
2	695	737	-42
4	572	629	-57
4	679	629	50
4	739	629	110

NUMERICAL DETERMINATION OF EFFECT OF pH ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 30°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: ph74(30).dat

IT#          0Lambda =   1.000000E-01 RSS = 4751620.000000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS = 1161985.000000
Prms ->   4.861752E-01     1208.464000      541.134300

IT#          2Lambda =   9.999999E-04 RSS =  741532.400000
Prms ->   8.096764E-01     1233.579000      421.359100

IT#          3Lambda =   9.999999E-05 RSS =  539061.800000
Prms ->   4.643812E-01     1214.836000      423.727300

IT#          4Lambda =   9.999999E-06 RSS =  285691.800000
Prms ->   5.493628E-01     1231.770000      329.363300

IT#          5Lambda =   9.999999E-07 RSS =  257929.400000
Prms ->   4.850882E-01     1228.038000      350.151700

IT#          6Lambda =   9.999999E-08 RSS =  241348.200000
Prms ->   5.029770E-01     1230.875000      326.818100

IT#          7Lambda =   9.999999E-08 RSS =  239990.200000
Prms ->   4.945739E-01     1230.117000      332.566300

IT#          8Lambda =   9.999999E-08 RSS =  239691.300000
Prms ->   4.975293E-01     1230.476000      329.613100

IT#          9Lambda =   9.999999E-08 RSS =  239654.200000
Prms ->   4.963817E-01     1230.350000      330.625600

IT#         10Lambda =   9.999999E-08 RSS =  239648.500000
Prms ->   4.968087E-01     1230.399000      330.227800

MARQUART: Convergence criterion met
IT#         11Lambda =   9.999999E-08 RSS =  239647.800000
   4.966471E-01     1230.381000      330.375100
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	4.968087E-01	3.297395E-01
S0	1230.399000	67.077070
R	330.227800	140.228400

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5569 1.0000
  .9713  .4381 1.0000
```

ANOVA ingredients

```
   RSS =   239647.800000
   CSS =   683402.600000
     N =          18
```

RESULTS

PH74(30).OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	1233	1228	5
0.0083	1135	1228	-93
0.0083	1108	1228	-120
0.5	1292	1106	186
0.5	1246	1106	140
0.5	1210	1106	104
1	1056	1009	47
1	997	1009	-12
1	790	1009	-219
1.5	1017	933	84
1.5	1053	933	120
1.5	944	933	11
2	883	874	9
2	743	874	-131
2	642	874	-232
4	764	742	22
4	736	742	-6
4	832	742	90

NUMERICAL DETERMINATION OF EFFECT OF DISSOLVED OXYGEN ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DISSOLVED OXYGEN 16 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: DOCOM3.DAT

IT#          0Lambda =   1.000000E-01 RSS =  312080.400000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =   59477.510000
Prms ->   7.337564E-01     1068.529000      190.940800

IT#          2Lambda =   9.999999E-04 RSS =   48298.720000
Prms ->   5.321135E-01     1034.573000       85.759700

IT#          3Lambda =   9.999999E-05 RSS =   41785.190000
Prms ->   4.778384E-01     1024.679000       41.259780

IT#          4Lambda =   9.999999E-06 RSS =   41403.250000
Prms ->   4.710903E-01     1022.975000       33.205040

IT#          5Lambda =   9.999999E-07 RSS =   41400.620000
Prms ->   4.701283E-01     1022.777000       32.362830

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =   41400.570000
   4.699843E-01     1022.750000       32.245850
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	4.701283E-01	1.290265E-01
S0	1022.777000	43.919730
R	32.362830	61.784780

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5632 1.0000
  .9376  .3757 1.0000
```

ANOVA ingredients

```
   RSS =    41400.570000
   CSS =   852136.900000
     N =          12
```

RESULTS

EDOCOM3.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1113	1019	94
0.0083	1017	1019	-2
0.5	791	823	-32
0.5	703	823	-120
1	656	665	-9
1	620	665	-45
1.5	607	540	67
1.5	549	540	9
2	468	441	27
2	494	441	53
4	138	214	-76
4	249	214	35

NUMERICAL DETERMINATION OF EFFECT OF DISSOLVED OXYGEN ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DISSOLVED OXYGEN 8.2 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: DOCOM4.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1838092.000000
Prms ->   5.000000E-01     1146.724000     -125.000000

IT#          1Lambda =   1.000000E-02 RSS =   64562.480000
Prms ->   2.462874E-01     1132.558000       65.867840

IT#          2Lambda =   9.999999E-04 RSS =   57534.800000
Prms ->   1.257299E-01     1129.484000      -36.595220

IT#          3Lambda =   9.999999E-05 RSS =   55833.390000
Prms ->   4.744172E-02     1117.012000     -150.792800

IT#          4Lambda =   9.999999E-05 RSS =   46650.450000
Prms ->   4.106691E-02     1114.444000     -150.014600

IT#          5Lambda =   9.999999E-05 RSS =   39304.500000
Prms ->   3.697973E-02     1113.784000     -148.013000

IT#          6Lambda =   9.999999E-05 RSS =   35221.000000
Prms ->   3.422450E-02     1113.320000     -146.327600

IT#          7Lambda =   9.999999E-05 RSS =   33064.800000
Prms ->   3.227895E-02     1112.980000     -145.161900

IT#          8Lambda =   9.999999E-05 RSS =   31913.340000
Prms ->   3.084521E-02     1112.724000     -144.418500

IT#          9Lambda =   9.999999E-05 RSS =   31272.840000
Prms ->   2.974875E-02     1112.526000     -143.961200

IT#         10Lambda =   9.999999E-05 RSS =   30898.590000
Prms ->   2.888364E-02     1112.368000     -143.687300

IT#         11Lambda =   9.999999E-05 RSS =   30668.160000
Prms ->   2.818381E-02     1112.240000     -143.525800

IT#         12Lambda =   9.999999E-05 RSS =   30519.660000
Prms ->   2.760619E-02     1112.134000     -143.433700
```

```
IT#          13Lambda =   9.999999E-05 RSS =   30420.460000
Prms ->   2.712141E-02     1112.044000     -143.386400

IT#          14Lambda =   9.999999E-05 RSS =   30351.200000
Prms ->   2.670968E-02     1111.968000     -143.362900

IT#          15Lambda =   9.999999E-05 RSS =   30301.950000
Prms ->   2.635623E-02     1111.903000     -143.356700

IT#          16Lambda =   9.999999E-05 RSS =   30266.060000
Prms ->   2.605019E-02     1111.846000     -143.360800

IT#          17Lambda =   9.999999E-05 RSS =   30239.350000
Prms ->   2.578339E-02     1111.797000     -143.370700

IT#          18Lambda =   9.999999E-05 RSS =   30219.290000
Prms ->   2.554927E-02     1111.754000     -143.385200

IT#          19Lambda =   9.999999E-05 RSS =   30203.810000
Prms ->   2.534304E-02     1111.716000     -143.400000

IT#          20Lambda =   9.999999E-05 RSS =   30191.900000
Prms ->   2.516052E-02     1111.682000     -143.416400

IT#          21Lambda =   9.999999E-05 RSS =   30182.610000
Prms ->   2.499841E-02     1111.652000     -143.432900

IT#          22Lambda =   9.999999E-05 RSS =   30175.300000
Prms ->   2.485397E-02     1111.625000     -143.449200

IT#          23Lambda =   9.999999E-05 RSS =   30169.560000
Prms ->   2.472480E-02     1111.601000     -143.465700

IT#          24Lambda =   9.999999E-05 RSS =   30164.930000
Prms ->   2.460919E-02     1111.580000     -143.480200

IT#          25Lambda =   9.999999E-05 RSS =   30161.220000
Prms ->   2.450550E-02     1111.560000     -143.494000

IT#          26Lambda =   9.999999E-05 RSS =   30158.250000
Prms ->   2.441230E-02     1111.543000     -143.507100

IT#          27Lambda =   9.999999E-05 RSS =   30155.810000
Prms ->   2.432861E-02     1111.528000     -143.518000

IT#          28Lambda =   9.999999E-05 RSS =   30153.910000
Prms ->   2.425298E-02     1111.514000     -143.530500

IT#          29Lambda =   9.999999E-05 RSS =   30152.340000
Prms ->   2.418471E-02     1111.501000     -143.541100

IT#          30Lambda =   9.999999E-05 RSS =   30151.060000
Prms ->   2.412302E-02     1111.490000     -143.550800
```

```
IT#          31Lambda =   9.999999E-05 RSS =   30150.030000
Prms ->   2.406712E-02     1111.479000     -143.560500

IT#          32Lambda =   9.999999E-05 RSS =   30149.180000
Prms ->   2.401643E-02     1111.470000     -143.569300

IT#          33Lambda =   9.999999E-05 RSS =   30148.460000
Prms ->   2.397060E-02     1111.461000     -143.576300

IT#          34Lambda =   9.999999E-05 RSS =   30147.910000
Prms ->   2.392884E-02     1111.453000     -143.584700

IT#          35Lambda =   9.999999E-05 RSS =   30147.440000
Prms ->   2.389086E-02     1111.446000     -143.591800

IT#          36Lambda =   9.999999E-05 RSS =   30147.040000
Prms ->   2.385643E-02     1111.440000     -143.597400

IT#          37Lambda =   9.999999E-05 RSS =   30146.720000
Prms ->   2.382507E-02     1111.434000     -143.603400

MARQUART: Convergence criterion met
IT#          38Lambda =   9.999999E-04 RSS =   30146.450000
   2.379657E-02     1111.429000     -143.608400
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.382507E-02	1.263457E-01
S0	1111.434000	34.968190
R	-143.603400	858.604800

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6715 1.0000
  .9999  .6629 1.0000
```

ANOVA ingredients

```
   RSS =    30146.450000
   CSS =   552476.900000
     N =          12
```

RESULTS

EDOCOM4.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL (μg/L)
0.0083	1149	1110	39
0.0083	1033	1110	-77
0.5	1015	1027	-12
0.5	1064	1027	37
1	1038	943	95
1	920	943	-23
1.5	905	861	44
1.5	786	861	-75
2	772	779	-7
2	752	779	-27
4	508	463	45
4	427	463	-36

NUMERICAL DETERMINATION OF EFFECT OF DISSOLVED OXYGEN ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DISSOLVED OXYGEN 6.0 mg/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: DOCOM5.DAT

IT#          0Lambda =   1.000000E-01 RSS =  728628.900000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =   28726.530000
Prms ->   9.230413E-01     1104.682000      388.111700

IT#          2Lambda =   9.999999E-04 RSS =   21269.010000
Prms ->       1.106068     1104.661000      497.767700

IT#          3Lambda =   9.999999E-05 RSS =   19434.570000
Prms ->       1.240446     1116.106000      602.063900

IT#          4Lambda =   9.999999E-06 RSS =   19134.190000
Prms ->       1.281679     1119.026000      625.073200

IT#          5Lambda =   9.999999E-07 RSS =   18618.640000
Prms ->       1.288831     1119.602000      620.459300

IT#          6Lambda =   9.999999E-08 RSS =   18596.630000
Prms ->       1.289939     1119.693000      619.176300

IT#          7Lambda =   9.999999E-08 RSS =   18596.100000
Prms ->       1.290121     1119.707000      618.987200

MARQUART: Convergence criterion met
IT#          8Lambda =   9.999999E-08 RSS =  18596.090000
       1.290151     1119.709000      618.956800
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.290121	1.902598E-01
S0	1119.707000	32.202820
R	618.987200	174.743200

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .4500 1.0000
  .9915  .4165 1.0000
```

ANOVA ingredients

```
   RSS =    18596.090000
   CSS =   624467.000000
     N =          12
```

RESULTS

EDOCOM5.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	1131	1113	18
0.0083	1129	1113	16
0.5	761	816	-55
0.5	742	816	-74
1	702	656	46
1	728	656	72
2	526	528	-2
2	549	528	21
3	489	493	-4
3	470	493	-23
4	500	483	17
4	451	483	-32

NUMERICAL DETERMINATION OF EFFECT OF INOCULUM SIZE ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DRY CELL WT. 1.455 g/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: INNODES1.DAT

IT#          0Lambda =   1.000000E-01 RSS =  514588.500000
Prms ->   5.000000E-01     1010.000000      200.000000

IT#          1Lambda =   1.000000E-02 RSS =  175619.000000
Prms ->   3.599933E-01     1037.660000      272.640200

IT#          2Lambda =   9.999999E-04 RSS =  117112.900000
Prms ->   4.153605E-01     1041.491000      230.135100

IT#          3Lambda =   9.999999E-05 RSS =  109577.700000
Prms ->   3.631140E-01     1038.648000      244.542100

IT#          4Lambda =   9.999999E-06 RSS =   92153.030000
Prms ->   3.610656E-01     1039.266000      217.894000

IT#          5Lambda =   9.999999E-07 RSS =   92119.520000
Prms ->   3.613788E-01     1039.267000      216.857900

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =   92118.830000
   3.613336E-01     1039.264000      217.007800
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.613788E-01	2.793208E-01
S0	1039.267000	40.793170
R	216.857900	88.400320

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5896 1.0000
  .9775  .4788 1.0000
```

ANOVA ingredients

```
   RSS =    92118.830000
   CSS =   299652.000000
     N =          18
```

RESULTS

EINODES1.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	1002	1038	-36
0.0083	1012	1038	-26
0.0083	1010	1038	-28
0.5	1025	967	58
0.5	993	967	26
0.5	1074	967	107
1	923	906	17
1	889	906	-17
1	854	906	-52
1.5	911	855	56
1.5	866	855	11
1.5	732	855	-123
2	826	813	13
2	799	813	-14
2	807	813	-6
4	828	704	124
4	774	704	70
4	527	704	-177

NUMERICAL DETERMINATION OF EFFECT OF INOCULUM SIZE ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DRY CELL WT. 3.317 g/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: INNODES2.DAT

IT#          0Lambda =   1.000000E-01 RSS = 3956178.000000
Prms ->   3.000000E-01     1280.000000      300.000000

IT#          1Lambda =   1.000000E-02 RSS =  440625.000000
Prms ->   7.496759E-01     1017.766000      169.494100

IT#          2Lambda =   9.999999E-04 RSS =  237689.500000
Prms ->   4.099766E-01      979.993800      207.761800

IT#          3Lambda =   9.999999E-05 RSS =  149398.300000
Prms ->   2.630246E-01      979.824400       72.592120

IT#          4Lambda =   9.999999E-06 RSS =   75188.420000
Prms ->   3.114764E-01      978.789000       47.379780

IT#          5Lambda =   9.999999E-07 RSS =   72823.800000
Prms ->   3.027560E-01      978.874500       52.005190

IT#          6Lambda =   9.999999E-08 RSS =   72775.300000
Prms ->   3.024088E-01      978.867700       50.524700

MARQUART: Convergence criterion met
IT#          7Lambda =   9.999999E-08 RSS =   72775.220000
   3.024083E-01      978.867400       50.470900
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.024088E-01	1.598699E-01
S0	978.867700	38.688720
R	50.524700	78.770060

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5640 1.0000
  .9764  .4491 1.0000
```

ANOVA ingredients

```
   RSS =    72775.220000
   CSS =   665129.900000
     N =          16
```

RESULTS

EINODES2.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	990	977	13
0.0083	1011	977	34
0.0083	1002	977	25
0.5	782	865	-83
0.5	846	865	-19
0.5	795	865	-70
1	739	767	-28
1.5	774	683	91
1.5	827	683	144
1.5	780	683	97
2	526	610	-84
2	548	610	-62
2	539	610	-71
4	422	409	13
4	448	409	39
4	370	409	-39

NUMERICAL DETERMINATION OF EFFECT OF INOCULUM SIZE ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DRY CELL WT. 6.470 g/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: INNODES3.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1086797.000000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  155161.100000
Prms ->   5.739310E-01     1045.041000      255.676700

IT#          2Lambda =   9.999999E-04 RSS =  150608.200000
Prms ->   3.100207E-01     1022.666000       75.466980

IT#          3Lambda =   9.999999E-05 RSS =   30429.340000
Prms ->   2.971236E-01     1014.302000        7.999489

IT#          4Lambda =   9.999999E-06 RSS =   30316.790000
Prms ->   2.962580E-01     1013.779000        5.712188

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   30316.780000
   2.962700E-01     1013.781000        5.699880
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	2.962580E-01	7.627419E-02
S0	1013.779000	23.174820
R	5.712188	45.291510

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6059 1.0000
  .9745  .4831 1.0000
```

ANOVA ingredients

```
   RSS =    30316.780000
   CSS =   907948.300000
     N =          18
```

RESULTS

EINODES3.OUT

TIME (hr)	CONCENTRATION ACTUAL (µg/L)	PREDICTED (µg/L)	RESIDUAL (µg/L)
0.0083	996	1011	-15
0.0083	1028	1011	17
0.0083	964	1011	-47
0.5	935	877	58
0.5	887	877	10
0.5	890	877	13
1	769	759	10
1	782	759	23
1	772	759	13
1.5	630	657	-27
1.5	640	657	-17
1.5	555	657	-102
2	513	569	-56
2	655	569	86
2	600	569	31
4	314	323	-9
4	325	323	2
4	334	323	11

NUMERICAL DETERMINATION OF EFFECT OF INOCULUM SIZE ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (DRY CELL WT. 8.017 g/L, pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: INNODES4.DAT

IT#          0Lambda =   1.000000E-01 RSS =   1.462902E+07
Prms ->   2.000000E-02     1100.000000      200.000000

IT#          1Lambda =   1.000000E-02 RSS = 3426038.000000
Prms ->  -1.358954E-03      897.914600       15.469600

IT#          2Lambda =   9.999999E-04 RSS =  898237.800000
Prms ->   7.357841E-03      900.547700      -91.832870

IT#          3Lambda =   9.999999E-05 RSS =  341415.800000
Prms ->   1.140924E-02      906.860400     -143.968900

IT#          4Lambda =   9.999999E-06 RSS =  223506.000000
Prms ->   1.377357E-02      907.942600     -164.275300

IT#          5Lambda =   9.999999E-07 RSS =  207212.100000
Prms ->   1.592683E-02      908.511100     -167.498500

IT#          6Lambda =   9.999999E-08 RSS =  164083.400000
Prms ->   1.635715E-02      908.670600     -188.549900

IT#          7Lambda =   9.999999E-08 RSS =  162736.000000
Prms ->   1.629689E-02      908.669400     -193.821300

IT#          8Lambda =   9.999999E-08 RSS =  162710.000000
Prms ->   1.627098E-02      908.663100     -193.446900

IT#          9Lambda =   9.999999E-08 RSS =  162707.900000
Prms ->   1.629214E-02      908.668000     -192.877000

IT#         10Lambda =   9.999999E-08 RSS =  162704.000000
Prms ->   1.629853E-02      908.669500     -193.049800

MARQUART: Convergence criterion met
IT#         11Lambda =   9.999999E-08 RSS =  162703.800000
   1.629432E-02      908.668500     -193.177400
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	1.629853E-02	1.497598E-01
S0	908.669500	51.291580
R	-193.049800	1846.611000

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .6728 1.0000
  .9999  .6670 1.0000
```

ANOVA ingredients

```
   RSS =   162703.800000
   CSS =  1372836.000000
     N =          18
```

RESULTS

INNODES4.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	969	907	62
0.0083	963	907	56
0.0083	962	907	55
0.5	769	805	-36
0.5	765	805	-40
0.5	575	805	-230
1	808	702	106
1	773	702	71
1	559	702	-143
1.5	692	601	91
1.5	664	601	63
1.5	548	601	-53
2	507	500	7
2	496	500	-4
2	509	500	9
4	271	104	167
4	12	104	-92
4	12	104	-92

NUMERICAL DETERMINATION OF EFFECT OF SUBSTRATE CONCENTRATIONS ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

sust1df.dat

IT#          0Lambda =   1.000000E-01 RSS =   72681.030000
Prms ->       1.000000      800.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =   32716.720000
Prms ->   9.462953E-01      772.464500       19.617260

IT#          2Lambda =   9.999999E-04 RSS =   30041.630000
Prms ->   8.031760E-01      759.667900      -10.889680

IT#          3Lambda =   9.999999E-05 RSS =   30030.700000
Prms ->   7.962719E-01      758.748000      -12.870430

MARQUART: Convergence criterion met
IT#          4Lambda =   9.999999E-05 RSS =   30030.670000
   7.959378E-01      758.714800      -12.899820
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	7.962719E-01	1.594328E-01
S0	758.748000	32.361170
R	-12.870430	37.128340

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .4743 1.0000
  .8168  .2486 1.0000
```

ANOVA ingredients

```
   RSS =    30030.670000
   CSS =  1055238.000000
     N =          12
```

RESULTS

ESUSt1DF.OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	800	754	46
0.0083	724	754	-30
0.0083	748	754	-6
0.5	544	504	40
0.5	370	504	-134
0.5	576	504	72
2	156	141	15
2	115	141	-26
2	177	141	36
4	12	16	-4
4	12	16	-4
4	12	16	-4

NUMERICAL DETERMINATION OF EFFECT OF SUBSTRATE CONCENTRATIONS ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: sust2df.dat

IT#          0Lambda =   1.000000E-01 RSS =  341841.200000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  106750.600000
Prms ->   5.186434E-01     1248.817000       -4.511223

IT#          2Lambda =   9.999999E-04 RSS =   52037.540000
Prms ->   4.363090E-01     1237.479000     -103.498100

IT#          3Lambda =   9.999999E-05 RSS =   51750.340000
Prms ->   4.313000E-01     1235.271000     -102.381400

IT#          4Lambda =   9.999999E-06 RSS =   51734.290000
Prms ->   4.315406E-01     1235.336000     -101.294300

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   51734.260000
   4.315274E-01     1235.333000     -101.348600
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	4.315406E-01	8.094497E-02
S0	1235.336000	36.458550
R	-101.294300	53.428190

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5523 1.0000
  .9414  .3927 1.0000
```

ANOVA ingredients

```
   RSS =    51734.260000
   CSS =  2715253.000000
     N =          14
```

RESULTS

ESUST2DF.OUT

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1297	1230	67
0.0083	1126	1230	-104
0.0083	1244	1230	14
0.5	958	950	8
0.5	944	950	-6
0.5	900	950	-50
1	788	720	67
1	831	720	111
2	374	385	-12
2	283	385	-102
2	352	385	-34
4	96	27	69
4	12	27	-15
4	12	27	-15

NUMERICAL DETERMINATION OF EFFECT OF SUBSTRATE CONCENTRATIONS
ON RATE CONSTANTS FOR MODIFIED FIRST ORDER REACTION FOR
1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY
RESEARCH STATION AQUIFER MATERIALS (pH 7.4,
TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: sust3df.dat

IT#          0Lambda =   1.000000E-01 RSS =   9.535878E+07
Prms ->       1.000000     5000.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =   1.202345E+07
Prms ->   5.783475E-01     4807.480000     2714.769000

IT#          2Lambda =   9.999999E-04 RSS = 6402505.000000
Prms ->   8.264834E-01     4851.422000     2337.227000

IT#          3Lambda =   9.999999E-05 RSS = 3846498.000000
Prms ->   6.404395E-01     4849.813000     2436.205000

IT#          4Lambda =   9.999999E-06 RSS = 3008633.000000
Prms ->   6.720266E-01     4873.234000     2236.524000

IT#          5Lambda =   9.999999E-07 RSS = 2984018.000000
Prms ->   6.480404E-01     4869.115000     2250.350000

IT#          6Lambda =   9.999999E-08 RSS = 2963071.000000
Prms ->   6.591959E-01     4872.161000     2226.966000

IT#          7Lambda =   9.999999E-08 RSS = 2958451.000000
Prms ->   6.532654E-01     4870.756000     2237.083000

IT#          8Lambda =   9.999999E-08 RSS = 2957126.000000
Prms ->   6.561301E-01     4871.506000     2231.294000

IT#          9Lambda =   9.999999E-08 RSS = 2956818.000000
Prms ->   6.546788E-01     4871.145000     2234.000000

IT#         10Lambda =   9.999999E-08 RSS = 2956739.000000
Prms ->   6.553960E-01     4871.328000     2232.604000

MARQUART: Convergence criterion met
IT#         11Lambda =   9.999999E-08 RSS = 2956720.000000
   6.550375E-01     4871.237000     2233.288000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	6.553960E-01	5.211373E-01
S0	4871.328000	259.153700
R	2232.604000	1045.096000

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5100 1.0000
  .9903  .4545 1.0000
```

ANOVA ingredients

```
   RSS =  2956720.000000
   CSS =  6420811.000000
     N =          16
```

RESULTS

ESUST3DF.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	4795	4863	-68
0.0083	4954	4863	90
0.0083	4504	4863	-359
0.5	4544	4462	82
0.5	4930	4462	468
0.5	4174	4462	-288
1	4661	4167	494
1	4810	4167	643
1.5	3690	3955	-265
1.5	3419	3955	-535
2	3514	3801	-288
2	3870	3801	68
2	3400	3801	-402
4	3707	3513	194
4	4375	3513	862
4	2833	3513	-680

NUMERICAL DETERMINATION OF EFFECT OF LEAD ON RATE CONSTANTS
FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE
IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER
MATERIALS (LEAD 10.0 mg/L, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: LEADDES2.DAT

IT#          0Lambda =   1.000000E-01 RSS =  858418.100000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =  163738.700000
Prms ->   7.004005E-01     1069.226000      358.353300

IT#          2Lambda =   9.999999E-04 RSS =  151241.700000
Prms ->   5.301979E-01     1049.446000      232.884600

IT#          3Lambda =   9.999999E-05 RSS =  147077.700000
Prms ->   4.186746E-01     1036.930000      153.401400

IT#          4Lambda =   9.999999E-06 RSS =  134251.600000
Prms ->   3.971245E-01     1033.046000      114.298800

IT#          5Lambda =   9.999999E-07 RSS =  133787.700000
Prms ->   3.937005E-01     1032.337000      107.215600

IT#          6Lambda =   9.999999E-08 RSS =  133778.700000
Prms ->   3.929212E-01     1032.226000      106.086900

MARQUART: Convergence criterion met
IT#          7Lambda =   9.999999E-08 RSS = 133778.300000
   3.927326E-01     1032.201000      105.829900
```

PARAMETER

NAME	VALUE	STD (asymptotic)
K1	3.929212E-01	3.710112E-01
S0	1032.226000	88.947140
R	106.086900	160.037100

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5493 1.0000
  .9648  .4205 1.0000
```

ANOVA ingredients

```
   RSS =   133778.300000
   CSS =   579606.900000
     N =          10
```

RESULTS

ELEADES2.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	1113	1030	83
0.0083	1080	1030	50
0.5	941	896	45
0.5	577	896	-319
1	863	785	78
1.5	708	693	15
2	609	617	-8
2	710	617	93
4	457	428	29
4	361	428	-67

NUMERICAL DETERMINATION OF EFFECT OF LEAD ON RATE CONSTANTS
FOR MODIFIED FIRST ORDER REACTION FOR 1,2-DICHLOROPROPANE
IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER
MATERIALS (LEAD 2.2 mg/L, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = First-order with R term
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: LEADDES4.DAT

IT#          0Lambda =   1.000000E-01 RSS =  758015.300000
Prms ->       1.000000     1200.000000      100.000000

IT#          1Lambda =   1.000000E-02 RSS =   67943.800000
Prms ->   6.752918E-01     1052.588000      308.996600

IT#          2Lambda =   9.999999E-04 RSS =   48041.610000
Prms ->   5.614123E-01     1035.404000      212.378100

IT#          3Lambda =   9.999999E-05 RSS =   41408.150000
Prms ->   5.363171E-01     1031.451000      178.337200

IT#          4Lambda =   9.999999E-06 RSS =   40980.420000
Prms ->   5.387923E-01     1031.602000      173.403700

IT#          5Lambda =   9.999999E-07 RSS =   40976.500000
Prms ->   5.385308E-01     1031.571000      173.871200

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =   40976.460000
   5.385532E-01     1031.575000      173.820000
```

NAME	PARAMETER VALUE	STD (asymptotic)
K1	5.385308E-01	1.719589E-01
S0	1031.571000	44.103940
R	173.871200	70.874580

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .5472 1.0000
  .9483  .3847 1.0000
```

ANOVA ingredients

```
   RSS =    40976.460000
   CSS =   531307.700000
     N =          12
```

RESULTS

ELEADES4.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	1051	1028	23
0.0083	940	1028	-88
0.5	982	864	118
0.5	905	864	41
1	757	736	21
1	676	736	-60
1.5	627	639	-12
1.5	536	639	-103
2	609	564	45
2	571	564	7
4	421	405	16
4	399	405	-6

NUMERICAL DETERMINATION OF THE EFFECT OF DISSOLVED OXYGEN
CONCENTRATION ON RATE CONSTANTS FOR MICHAELIS-MENTEN
FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU
AGRONOMY RESEARCH STATION AQUIFER MATERIALS
(DISSOLVED OXYGEN 8.2 mg/L, pH 7.4,
TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = MICHAELIS-MENTEN (SUBSTRATE FORM
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: DOCOM4.DAT

IT#          0Lambda =   1.000000E-01 RSS = 1395997.000000
Prms ->     800.000000      900.000000      1100.000000

IT#          1Lambda =   1.000000E-02 RSS =   85323.710000
Prms ->    1595.361000      582.887500      1097.665000

IT#          2Lambda =   9.999999E-04 RSS =   33270.210000
Prms ->    1519.466000      469.620700      1127.641000

IT#          3Lambda =   9.999999E-04 RSS =   32469.440000
Prms ->    1270.578000      432.258600      1128.889000

IT#          4Lambda =   9.999999E-05 RSS =   31941.960000
Prms ->     -35.839110      156.767900      1120.129000

IT#          5Lambda =   9.999999E-06 RSS =   30050.750000
Prms ->     110.816800      186.708700      1112.990000

IT#          6Lambda =   9.999999E-07 RSS =   30038.880000
Prms ->     133.166500      191.156100      1112.578000

MARQUART: Convergence criterion met
IT#          7Lambda =   9.999999E-07 RSS =   30038.850000
     130.862100      190.641900      1112.479000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
Km	133.166500	627.919700
Vmax	191.156100	137.768400
So	1112.578000	34.082080

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .9934 1.0000
  .6389  .7010 1.0000
```

ANOVA ingredients

```
   RSS =    30038.850000
   CSS =   552476.900000
     N =          12
```

RESULTS

MDOCOM4.OUT

TIME (hr)	ACTUAL (μg/L)	CONCENTRATION PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1149	1111	38
0.0083	1033	1111	-78
0.5	1015	1028	-13
0.5	1064	1028	36
1	1038	943	95
1	920	943	-23
1.5	905	860	45
1.5	786	860	-74
2	772	778	-6
2	752	778	-26
4	508	464	44
4	427	464	-37

NUMERICAL DETERMINATION OF THE EFFECT OF pH ON RATE CONSTANTS FOR MICHAELIS-MENTEN FOR 1,2-DICHLORO-PROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 5.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = MICHAELIS-MENTEN (SUBSTRATE FORM
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH54(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  142382.500000
Prms ->     800.000000      900.000000     1000.000000

IT#          1Lambda =   1.000000E-02 RSS =   53871.960000
Prms ->     952.593800      818.041000     1009.541000

IT#          2Lambda =   9.999999E-04 RSS =   53378.410000
Prms ->     857.842000      763.896900     1010.032000

IT#          3Lambda =   9.999999E-05 RSS =   52784.790000
Prms ->     632.721000      644.245700     1005.346000

IT#          4Lambda =   9.999999E-06 RSS =   52736.360000
Prms ->     586.108100      617.619300     1002.696000

IT#          5Lambda =   9.999999E-07 RSS =   52734.230000
Prms ->     576.380500      612.166200     1002.240000

MARQUART: Convergence criterion met
IT#          6Lambda =   9.999999E-07 RSS =   52734.110000
     574.195800      610.934200     1002.145000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
Km	576.380500	459.146500
Vmax	612.166200	247.242400
So	1002.240000	30.554980

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .9870 1.0000
  .4458  .5468 1.0000
```

ANOVA ingredients

```
   RSS =    52734.110000
   CSS =  1721402.000000
     N =          18
```

RESULTS

MPH5425.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	942	999	-57
0.0083	1015	999	16
0.0083	1095	999	96
0.5	769	815	-46
0.5	850	815	35
0.5	792	815	-23
1	631	645	-14
1	594	645	-51
1	595	645	-50
1.5	484	493	-9
1.5	475	493	-18
1.5	550	493	57
2	286	363	-77
2	428	363	65
2	475	363	112
4	93	72	21
4	48	72	-24
4	12	72	-60

NUMERICAL DETERMINATION OF THE EFFECT OF pH ON RATE CONSTANTS FOR MICHAELIS-MENTEN FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = MICHAELIS-MENTEN (SUBSTRATE FORM
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH74(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  229585.600000
Prms ->     800.000000      900.000000     1000.000000

IT#          1Lambda =   1.000000E-02 RSS =  160460.900000
Prms ->    1017.624000      823.985500      942.816700

IT#          2Lambda =   9.999999E-04 RSS =  158303.800000
Prms ->    1123.467000      836.342200      932.685900

IT#          3Lambda =   9.999999E-05 RSS =  158233.100000
Prms ->    1235.561000      892.499500      934.672200

IT#          4Lambda =   9.999999E-06 RSS =  158178.800000
Prms ->    1370.666000      960.876000      936.282300

IT#          5Lambda =   9.999999E-07 RSS =  158147.800000
Prms ->    1490.269000     1021.569000      937.514600

IT#          6Lambda =   9.999999E-08 RSS =  158130.700000
Prms ->    1589.975000     1072.153000      938.438300

IT#          7Lambda =   9.999999E-08 RSS =  158121.500000
Prms ->    1670.990000     1113.230000      939.131200

IT#          8Lambda =   9.999999E-08 RSS =  158116.400000
Prms ->    1735.578000     1145.962000      939.650900

IT#          9Lambda =   9.999999E-08 RSS =  158113.600000
Prms ->    1786.300000     1171.659000      940.040300

IT#         10Lambda =   9.999999E-08 RSS =  158112.000000
Prms ->    1825.639000     1191.583000      940.331800

MARQUART: Convergence criterion met
IT#         11Lambda =   9.999999E-08 RSS =  158111.100000
    1855.850000     1206.882000      940.549800
```

PARAMETER

NAME	VALUE	STD (asymptotic)
Km	1825.639000	4535.344000
Vmax	1191.583000	2264.939000
So	940.331800	55.444820

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .9980 1.0000
  .4704  .5086 1.0000
```

ANOVA ingredients

```
   RSS =   158111.100000
   CSS =  1508387.000000
     N =          18
```

RESULTS

MPH7425.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	1039	937	102
0.0083	997	937	60
0.0083	972	937	35
0.5	483	752	-269
0.5	704	752	-48
0.5	635	752	-117
1	629	592	37
1	698	592	106
1	603	592	11
1.5	339	460	-121
1.5	540	460	80
1.5	531	460	71
2	382	352	30
2	416	352	64
2	409	352	57
4	65	109	-44
4	66	109	-43
4	49	109	-60

NUMERICAL DETERMINATION OF THE EFFECT OF pH ON RATE CONSTANTS FOR MICHAELIS-MENTEN FOR 1,2-DICHLOROPROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 8.9, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = MICHAELIS-MENTEN (SUBSTRATE FORM
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: PH89(25).DAT

IT#          0Lambda =   1.000000E-01 RSS =  254447.200000
Prms ->     800.000000      900.000000     1100.000000

IT#          1Lambda =   1.000000E-02 RSS =  105821.100000
Prms ->    1035.503000      782.125100      963.963400

IT#          2Lambda =   9.999999E-04 RSS =   97979.290000
Prms ->     885.933800      634.860400      935.032700

IT#          3Lambda =   9.999999E-05 RSS =   95486.670000
Prms ->     190.353000      337.912800      924.536900

IT#          4Lambda =   9.999999E-06 RSS =   93261.590000
Prms ->     262.684800      359.864000      916.296600

IT#          5Lambda =   9.999999E-07 RSS =   93200.910000
Prms ->     286.535800      369.925600      917.211500

IT#          6Lambda =   9.999999E-08 RSS =   93199.340000
Prms ->     291.104700      372.094000      917.465800

MARQUART: Convergence criterion met
IT#          7Lambda =   9.999999E-08 RSS =   93199.310000
     291.844100      372.454700      917.512900
```

PARAMETER

NAME	VALUE	STD (asymptotic)
Km	291.104700	317.349300
Vmax	372.094000	154.877600
So	917.465800	38.811850

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .9738 1.0000
  .4874  .6240 1.0000
```

ANOVA ingredients

```
   RSS =     93199.310000
   CSS =   1370586.000000
     N =           18
```

RESULTS

MPH8925.OUT

TIME (hr)	ACTUAL CONCENTRATION (μg/L)	PREDICTED CONCENTRATION (μg/L)	RESIDUAL CONCENTRATION (μg/L)
0.0083	978	915	63
0.0083	983	915	68
0.0083	927	915	12
0.5	589	779	-190
0.5	678	779	-101
0.5	790	779	11
1	616	647	-31
1	699	647	52
1	734	647	87
1.5	580	523	57
1.5	441	523	-82
1.5	569	523	46
2	357	409	-52
2	433	409	24
2	457	409	48
4	109	94	15
4	141	94	47
4	12	94	-82

NUMERICAL DETERMINATION OF THE EFFECT OF SUBSTRATE CONCENTRATION ON RATE CONSTANTS FOR MICHAELIS-MENTEN FOR 1,2-DICHLORO-PROPANE IN THE PRESENCE OF OSU AGRONOMY RESEARCH STATION AQUIFER MATERIALS (pH 7.4, TEMPERATURE 25°C)

```
OVERFIT:
  CONFIGURED WITH MODEL = MICHAELIS-MENTEN (SUBSTRATE FORM
    1 independents and  3 parameters.
EXECUTION BEGINS

FILE #: SUST2DF.DAT

IT#          0Lambda =   1.000000E-01 RSS =   73658.240000
Prms ->    1000.000000      900.000000     1200.000000

IT#          1Lambda =   1.000000E-02 RSS =   46454.800000
Prms ->     842.107700      947.724800     1224.327000

IT#          2Lambda =   9.999999E-04 RSS =   43618.180000
Prms ->     593.639800      799.456500     1226.666000

IT#          3Lambda =   9.999999E-05 RSS =   42439.370000
Prms ->     364.101700      652.731400     1218.279000

IT#          4Lambda =   9.999999E-06 RSS =   42411.000000
Prms ->     373.290800      660.271700     1218.056000

MARQUART: Convergence criterion met
IT#          5Lambda =   9.999999E-06 RSS =   42410.930000
     372.083300      659.440900     1217.971000
```

PARAMETER

NAME	VALUE	STD (asymptotic)
Km	373.290800	323.410600
Vmax	660.271700	208.281100
So	1218.056000	32.380570

CORRELATION OF PARAMETER ESTIMATES

```
 1.0000
  .9805 1.0000
  .4235  .5370 1.0000
```

ANOVA ingredients

```
   RSS =    42410.930000
   CSS =  2715253.000000
     N =          14
```

RESULTS

TIME (hr)	CONCENTRATION ACTUAL (μg/L)	PREDICTED (μg/L)	RESIDUAL (μg/L)
0.0083	1297	1214	83
0.0083	1126	1214	-87
0.0083	1244	1214	30
0.5	958	972	-14
0.5	944	972	-28
0.5	900	972	-72
1	788	743	45
1	831	743	88
2	374	356	17
2	283	356	-73
2	352	356	-5
4	96	25	71
4	12	25	-14
4	12	25	-14

GENERAL LINEAR MODEL OF DATA FROM pH (TEMPERATURE
= 25°C) EXPERIMENTS
- SAS PROGRAM AND ANALYSIS -

```
OPTIONS  PS = 62   LS = 132;
*ENZYME.CTL;
DATA ENZ;
   INFILE 'A:STATIST.DAT';
   INPUT TIME CONC TEMP PH IDN $ TABLE;
      LNCONC = LOG(CONC);
      TLOF = TIME;
      TIME2 = TIME**2; TIME3 = TIME**3;
      PROC SORT DATA = ENZ;
           BY TABLE IDN TLOF;
DATA TB8; SET ENZ;
   IF TABLE = 8;
   K22=0; K23=0; K24=0
      IF IDN = 'pH5425' THEN K22=1
      IF IDN = 'pH74(25)' THEN K23=1
      IF IDN = 'pH89(25)' THEN K24=1
      K22T - K22*TIME; K23T = K23*TIME; K24T = K24*TIME;
PROC GLM DATA = TB8;
   CLASSES IDN TLOF;
   MODEL LNCONC = K22 K23 TIME K22T K23T IND*TLOF/SS1;
   TITLE "K22T" & "K23T" MEASURE DIFF IN SLOPES (K VALUES) OF THE ';
   TITLE2/THREE ID GROUPS IN TABLE 8!/;
```

'K22T' & 'K23T' MEASURE DIFF IN SLOPES (K VALUES) OF THE THREE ID GROUPS IN TABLE 8!

General Linear Models Procedure
Class Level Information

Class	Levels	Values
ID	3	ph5425 ph74(25) ph89(25)
TLOF	6	1 2 4 0.5 1.5 0.0083

Number of observations in data set = 54

'K22T' & 'K23T" MEASURE DIFF IN SLOPES (K VALUES) OF THE THREE ID GROUPS IN TABLE 8!

General Linear Models Procedure

Dependent Variable: LNCONC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	52.13121615	3.06654213	16.60	0.0001
Error	36	6.61823271	0.18383980		
Corrected Total	53	58.74944886			

R-Square	CV	Root MSE	LNCONC Mean
0.887348	7.144149	0.4876543	6.00163097

Source	DF	Type I SS	Mean Square	F Value	Pr > F
k22	1	0.02894980	0.02894980	0.16	0.6938
k23	1	0.01607927	0.01607927	0.09	0.7691
TIME	1	48.64130452	48.64130452	264.59	0.0001
K22T	1	0.36990217	0.36990217	2.01	0.1646
K23T	1	0.00719641	0.00719641	0.04	0.8443
IDN*TLOF	12	3.06778398	0.25564866	1.39	0.2152

VITA

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Doctor of Philosophy

Thesis: BIODEGRADATION OF SELECTED ORGANIC COMPOUNDS BY A PURE CULTURE WITH ELEVATED ENZYME ACTIVITY IN AN AQUIFER MATRIX

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