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Biodegradation of Desorption -Resistant Organic Contaminants in Wetland Soils.

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**BIODEGRADATION OF DESORPTION-RESISTANT ORGANIC
CONTAMINANTS IN WETLAND SOILS**

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The Department of Civil and Environmental Engineering

by

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ABSTRACT

Contaminant bioavailability depends on physicochemical processes such as adsorption/desorption, diffusion, and dissolution. The rates of sorption and desorption of trichloroethylene (TCE) and 1,3-Dichlorobenzene (1,3-DCB) were studied. Desorption kinetics of 3-month and 5-month old contaminated soils showed that progressively less amount of contaminant was available for facile desorption compared to freshly contaminated soil in an empirical non-linear model.

Microcosm batch studies were conducted to study the biodegradation of 1,3-DCB in the aqueous and soil phase containing a freshly contaminated soil and a soil containing only the desorption resistant fraction. The presence of the soil reduced the rates of biodegradation. It is clear that from the freshly contaminated soil, 1,3-DCB readily desorbed into the aqueous phase and was bioavailable from microbial consumption whereas for the soils containing the desorption-resistant 1,3-DCB, mass transfer into the aqueous phase limited the contaminant availability.

Biodegradation of TCE by toluene-degrading bacteria was measured under aerobic conditions in aqueous and soil-slurry batch microcosms containing a freshly contaminated soil and a soil containing only the desorption resistant fraction of TCE. Presence of soil resulted in biodegradation rates substantially lower than those determined in the absence of soil. An appreciable increase in the rate and extent of TCE biodegradation was observed in microcosms when toluene was added multiple times.

The availability of CB-sorbed soils to CB degrading bacteria was assessed using a kinetic mineralization assay. It was demonstrated that soil-sorbed CB was available to

CB-degrading bacteria, and that the extents of bioavailability of soil-sorbed CB decreased with prolonged aging.

The rates of sorption and desorption of 1,4-DCB depend on aging, and soil types, and were investigated with the aid of Freundlich isotherm. According to a hysteresis index, hysteresis was not necessarily correlated with aging. Desorption isotherms demonstrated that desorption patterns in marsh soil were linear than in PPI soil even when the contaminant was aged. Bioavailability of 1,4-DCB was occurred both in freshly bounded into soil as well as in desorption resistant fraction in soils without a distinctive difference in two fractions. No significant differences in biodegradation were monitored in different wetland soil types containing various organic fractions.

CHAPTER 1 INTRODUCTION

Bioavailability is defined as “the extent to which a toxic contaminant is available for biologically-mediated transformation and biological actions in an aquatic environment” (Hamelink *et al.*, 1994). The concept of bioavailability also extends to the soil and sediment environment. Bioavailability is a function of numerous factors intrinsic to the biological system as well as environmental conditions. Specifically, the bioavailability of a chemical depends on physicochemical processes such as adsorption, desorption, diffusion, and dissolution (Ogram *et al.*, 1985; Luthy *et al.*, 1994). Bioavailability of contaminants is a critical factor affecting the success of biologically-based remediation systems.

Understanding sorption and desorption behavior is crucial to understanding bioavailability. Considerable experimental and theoretical work has been done on the transport of hydrophobic organic chemicals and partitioning of organic chemicals to sediments and saturated soils. Simple two-phase partitioning between the soil solid and the aqueous phase is commonly assumed in these systems. Reversible sorption of chlorinated solvents in soils and sediments has been widely used in assessing risk and determining remedial endpoints. However, reversible sorption models are not able to explain the long-term persistence of organic contaminants at many sites (Kan *et al.*, 1998). A two stage (bi-phasic) desorption of organic chemicals from soils and sediments have been observed by many researchers (Di Toro and Horzempa, 1982; Karickhoff *et al.*, 1985; Pignatello and Xing, 1996). A rapid release of a labile fraction is followed by the slow release of a non-labile fraction. The bi-phasic desorption phenomenon has been reported also for polycyclic aromatic hydrocarbons, and chlorinated

organic compounds (Gess and Pavlostathis, 1997; Cornelissen *et al.*, 1997; Berg *et al.*, 1998; Williamson *et al.*, 1998). The rate and extent of desorption were dependent of soil and sorbate properties such as soil organic carbon content, carbon-exchange capacity, specific surface area, and water solubility, as was also reported by other researchers (Pignatello, 1990a, b; Pavlostathis and Mathavan, 1992, Fu *et al.*, 1994; Kan *et al.*, 1998; Valsaraj *et al.*, 1999).

The processes by which organic compounds become increasingly desorption-resistant in soils and sediments, usually referred to as contaminant “aging”, are poorly understood. Some investigators have theorized that the aged or desorption-resistant fraction of organic compounds may result from the slow diffusion of these molecules within some components of soil organic matter in soils (Brusseau *et al.*, 1991). Others have suggested that desorption-resistance results when the chemicals slowly diffuse into and become entrapped within small pores in soil aggregates (Steinberg, *et al.*, 1987). It is also possible that the formation of strong bonds between organic compounds and soil or sediment constituents may account for their resistance. Other researchers have demonstrated that the irreversible compartment has been found to contain a finite maximum capacity (Kan *et al.*, 1997). They have speculated that the adsorbent surface layer can undergo rearrangement upon adsorption and that this physical-chemical rearrangement can be the cause of non-reversible adsorption (Fu *et al.*, 1994; Kan *et al.*, 1997, 1998, 1998, 2000). Recently, Huang and Weber (1996, 1997, 1998) concluded that the entrapment of sorbing molecules within condensed soil organic matter matrices contributes significantly to sorption-desorption hysteresis or sorption irreversibility. The persistent release of contaminants in this phase has been explained by the

heterogeneous adsorption with varied adsorption sites (Leboeuf and Weber, 2000). Although the presence of this desorption-resistant fraction has been demonstrated, there is no agreement on the mechanism of sorption of contaminants in this phase.

How does the presence of this desorption-resistant fraction affect bioavailability? Biodegradation is commonly thought to be an aqueous-phase reaction only, where only chemicals present in the aqueous phase are directly available for intracellular uptake by biodegradative bacteria (Ogram *et al.*, 1985; Greer and Shelton, 1992; Mihelcic *et al.*, 1993). Several researchers have confirmed that biodegradation can be limited by the slow rate of desorption of organic compounds (Bosma *et al.*, 1997; Robinson *et al.*, 1990; Al-Bashir *et al.*, 1994; Steinberg *et al.*, 1987; Hughes *et al.*, 1997). When desorption is rapid and reversible, it is possible for biodegradation processes to rapidly decrease solid phase contaminant concentrations (Hughes *et al.*, 1997). If, however, contaminants release slowly, or if the desorption process is not entirely reversible, biodegradation processes may have limited impact. During slow desorption, the flux of contaminants from the soil/sediment surface may not be sufficient to sustain the activity of the bacteria and degradation rates will decrease over time. If desorption ceases, biodegradation may cease (Hughes *et al.*, 1997).

Robinson *et al.*, (1990) observed that although most of the toluene in soil-water slurries were biodegraded rapidly, a small fraction was biodegraded much more slowly and at a rate limited by desorption. Bioavailability limitations caused by slow desorption was also observed in the study of phenanthrene contaminated soil (Lahlou and Ortega-Calvo, 1999). However, recent studies (Feng *et al.*, 2000; Park, *et al.*, In press) have demonstrated that naphthalene, which could not be removed by exhaustive

aqueous extraction, was removed in the presence of bacteria. These studies also found that the desorption-resistant fraction was bioavailable. These studies proposed two critical hypotheses. First, the presence of bacteria may serve to extract the non-desorbable fraction, much as organic solvent would by producing a wide array of soluble organic materials. Second, bacteria may be able to degrade contaminants directly on the soil or sediment surface.

Several methodologies have been developed to assess the bioavailability of sorbed organics to bacteria. , These methods all involve the use of ^{14}C -labeled organic compounds as tracers. The mineralization of these compounds is followed by recovery of $^{14}\text{CO}_2$. A coupled degradation-desorption model based on first order kinetics was developed by Boyd and Guerin, (1992, 1997) and will be used in the current study. Mineralization data expressed as the percentages of the initial activity converted to $^{14}\text{CO}_2$ as a function of time can be fitted to a first order production equation in the following form:

$$P = P_{\max} (1 - e^{-kt}) \quad (1-1)$$

Estimation of P_{\max} (the maximum percentage mineralized) and k (the first-order rate constant) can be made by the curve-fitting. A second model, the coupled degradation-desorption (CDD) model has the following form:

$$P = v_1 t + \left[(v_1 + v_2) (1 - e^{-kt}) \right] / k \quad (1-2)$$

The parameter v_1 represents the initial reaction rate (percentage hour⁻¹), which gives the initial mineralization rates. The v_2 parameter represents the mineralization rate resulting from desorption of bound contaminants and is related to the kinetics of contaminant desorption. The relative magnitude of the initial rate (based on v_1) relative to the rate of

an aqueous culture with no soil has been used to assess whether soil-sorbed organics are bioavailable.

A second approach uses a “bioavailability factor” to determine whether sorbed organics are bioavailable. The bioavailability factor (B_f , dimensionless) was used by Zhang *et al.*, (1998) to simulate the contaminant biotransformation rate in a batch system. The equation was formed as follows:

$$B_f = \frac{1}{1 + K_d \cdot m / v_l} \quad (1-3)$$

The parameter K_d represents distribution coefficients (L/kg), which can be obtained from sorption isotherms. The parameters of m and v_l refer to soil mass (kg) and volume of aqueous phase (L), respectively. Using the bioavailability factor and the first-order mineralization equations, two additional models can be derived, the P_d and P_{nd} models (Feng *et al.*, 2000). These models have the assumption that mineralization follows instantaneous desorption and no desorption, respectively. The equations are as follows:

$$P_d = P_{\max} \cdot (1 - e^{-B_f \cdot k \cdot t}) \quad (1-4)$$

$$P_{nd} = B_f \cdot P_{\max} \cdot (1 - e^{-k \cdot t}) \quad (1-5)$$

If biodegradation of sorbed compounds is observed the mineralization curve will plot above the P_d model. If desorption proceeds degradation, the curve will plot between the P_d and P_{nd} curves.

The relationship between bioavailability and sorption-desorption is least understood in the highly organic soils found in wetlands. Wetlands are commonly contaminated with organic contaminants because of their position in the landscape. If

the extent of contaminant desorption is dependent on the organic carbon content of soil as reported in earlier studies (Cornelissen *et al.*, 1997; Berg *et al.*, 1998), bioavailability may be greatly decreased in these systems. Other studies (Huang and Weber, 1998; Pardue *et al.*, 1993) have suggested that desorption-resistance may be less important in highly organic wetland soils. Since microbial degradation of organic contaminants may represent a remedial strategy for many wetlands, the bioavailability of these contaminants is of concern.

Based on this introduction, the following hypotheses were proposed:

The first hypothesis is that biphasic, desorption-resistance is observed in wetland soils but hysteresis is less in highly organic soils. A second hypothesis is that bioavailability to bacteria is greatly reduced in the desorption-resistant fraction. Finally, it is hypothesized that bioavailability is further reduced in the highly organic wetland soils. This dissertation is directed toward investigating the interactions of between microorganisms and sorbed contaminants on wetland soil. Specifically, the effect of sorption on bioavailability is investigated. The scope of the research is a series of laboratory bioavailability investigating the remediation of desorption-resistant, aged fraction of chlorinated organic compounds in wetland soils. The first objective of this research is to investigate the interactions of organic compounds and soil, especially sorption and desorption phenomenon. In order to investigate the desorption phenomenon with freshly contaminated and aged soil, desorption kinetics are covered in this study. The second objective is to determine the effect of sorption and desorption on bioavailability in contaminated wetland soils to understand processes limiting in bioavailability in soils that are candidates for biological remediation. Finally,

appropriate models, which can explain the effect of sorption in bioavailability, are applied, and verified based on the results of these experiments and literature review.

The organization of the dissertation is in journal style. Chapter 1 gives a general introduction to the objectives and background of the study. Chapter 2 is a study of the kinetics of desorption of two chlorinated compounds, trichloroethylene (TCE) and 1,3-dichlorobenzene (1,3-DCB) in two wetland soils. An empirical 2 site model developed by Opdyke and Loehr (1999) is used to describe the data on desorption kinetics. The results of this study were published in the *Journal of Environmental Monitoring and Assessment*.

The bioavailability of 1,3-DCB in the aqueous and sorbed phase is investigated and is presented in Chapter 3. Significant differences in bioavailability in soils containing readily desorbable fraction and only desorption-resistant fraction were observed. A manuscript is in preparation describing this study. The bioavailability study was extended with soil containing freshly contaminated and desorption-resistant trichloroethene (TCE) with a microbial culture capable of cometabolically degrading the compound. The cometabolic biodegradation of TCE by toluene degrading bacteria in wetland soil containing both fractions was investigated, including measurements of toluene dioxygenase activity. This is presented in Chapter 4, which is in review on *Journal of Environmental Toxicology and Chemistry*.

In Chapter 5, the mineralization of ^{14}C -labeled chlorobenzene (CB) was investigated with soils containing reversible and desorption-resistant fractions. Sorption isotherms in soils with different contact time were performed to obtain partition coefficients and to understand the behavior of organic compounds in wetland soils

containing different organic content. $^{14}\text{CO}_2$ production from CB was fit to several models including the first-order and coupled desorption/degradation model proposed by Boyd and Guerin (1992, 1997). Application of these models allows separation of degradation of the sorbed and dissolved pools of contaminants provided that a soil-free control is also used. The effects of sorption and aging were demonstrated and the results were submitted as a manuscript to *Water Research*.

In a Chapter 6, the sorption and desorption hysteresis of 1,4-dichlorobenzene (1,4-DCB) was determined by performing sorption/desorption isotherms. Wetland soils containing different organic carbon contents and contaminant contact time were used. Similar to the previous chapter, bioavailability was determined by measuring CO_2 production in soils containing both fractions (easily desorbable and desorption-resistant). The effect of sorption and desorption-resistance on the fate of this compound was determined. Finally, some general conclusion about the work and recommendations about the future applications are presented in Chapter 7.

CHAPTER 2

RATE-LIMITED DESORPTION OF VOLATILE ORGANIC COMPOUNDS¹

Introduction

There is increasing evidence that contaminants incorporated into soils are not easily desorbed (Pavlostathis and Mathavan, 1992; Linz and Nakles, 1997; Opdyke and Loehr, 1999). The rates of disappearance of persistent organic pollutants (POPs) have been shown to slow markedly upon prolonged contact with the soil, presumably due to sequestration within the soil. Pollutant sequestration also reduces the bioavailability to microorganisms in soil (Alexander, 1994). Studies suggest a two stage (bi-phasic) desorption of organic chemicals from soils and sediments. A rapid release of a labile fraction is followed by the slow release of a non-labile fraction (Di Toro and Horzempa, 1982; Karickhoff, *et al.*, 1985; Pignatello and Xing, 1996). A two-site model with a fast and a slow binding site often characterizes this behavior. The bi-phasic desorption phenomenon has been reported for polyaromatic hydrocarbons, and chlorinated organic compounds (Gess and Pavlostathis, 1997; Cornelissen *et al.*, 1997; Berg *et al.*, 1998; Williamson *et al.*, 1998). The slow diffusion within the sediment particles is suggested as one of the reasons for the kinetic limitations in desorption of the non-labile fraction (Pignatello and Xing, 1996). The diffusional retardation could be due to microscale partitioning of the contaminant in the sediment particle pores (Miller and Pedit, 1992; Ball and Roberts, 1991) or to diffusion in the sediment organic matter (Pignatello *et al.*, 1993; Brusseau, *et al.*, 1991). Slow desorption has also been attributed to entrapment in micropores in combination with slow diffusion through narrow particle pores

¹The author acknowledges the permission of the Journal of Environmental Monitoring and Assessment to reprint the material of Chapter 2.

(Steinberg, *et al.*, 1987) or to the re-arrangement of soil organic matter around the pollutant (Kan *et al.*, 1997). In contrast, the rapidly desorbing fraction is thought to be present in the outer regions of the sediment aggregates, and is readily accessible. Quantitative models have been only partly successful in explaining desorption hysteresis, irreversibility and slow reversible, non-equilibrium behavior. The traditional one-site model cannot describe the bi-phasic sorption/desorption kinetic data (Connaughton *et al.*, 1993). A two-site model proposed by Coats and Elzerman (1986) is also not capable of explaining the biphasic nature of desorption. Models such as the radial diffusion or pore diffusion model proposed by Wu and Gschwend (1986) can address some of the deficiencies. An empirical model such as the one suggested by Opdyke and Loehr (1999) is better suited to describe the data on desorption.

Chemical desorption from soil determines the contaminant transport and mobility which are key factors in their fate assessment in the sub-surface. This paper is part of an ongoing research work in support of the modeling activities at a Louisiana Superfund site (Petro Processors, Inc. site or PPI) that is presently undergoing clean-up. This site was operated in the mid 60's and 70's to dispose petrochemical waste composed mainly of chlorinated solvents. In earlier papers, we reported our findings on the soil/water partition coefficient (K_d) and desorption hysteresis in both laboratory-spiked and field-contaminated soils from the site (Valsaraj *et al.*, 1999; Kommalapati *et al.*, 2000). The determination of K_d was done using a standard method that required a 72-hour equilibrium before determining the soil and aqueous phase concentrations. The K_d values are to be used in the MODFLOW groundwater model to ascertain the efficacy of the pump and treat (P&T) remediation scheme presently employed at the site. If

sorption is kinetically limited, the K_d values obtained may be vastly over-predicted and can introduce substantial uncertainties in assessing the transport of compounds away from the site. Therefore, we undertook an investigation of the kinetics of desorption of two of the compounds, trichloroethylene (TCE) and 1,3-dichlorobenzene (1,3-DCB) from the site soil. This work is also part of an on-going study of the bioavailability of these contaminants and their biodegradation rates in the site soil.

Materials and Methods

Soils

Two different soils were used in this study, one from an uncontaminated region of the Brooklawn site (one of the two within the PPI site), located north of Baton Rouge, La., and another from the Bluebonnet swamp, a bottomland hardwood forest surrounded by a residential community in Baton Rouge, La. Large lumps of soil were broken and oven dried at 55°C for 24 hours. The soils were then pulverized before sieving through a 40µm sieve (US standard sieve No. 40). Samples from these soils were methanol-extracted and analyzed and found to be free of the test contaminants. Soil analyses were performed as per the standard methods in the LSU Soil Science laboratory and the data for both the soils are presented in Table 2.1. The soil from the PPI site is classified as silty soil and the one from the Bluebonnet swamp is characterized as a silty-clay.

Chemicals

The test contaminants selected for this study were trichloroethylene (TCE), and 1,3-dichlorobenzene (1,3-DCB or DCB). TCE (99.5% purity, spectrophotometric grade), and 1,3-DCB (98% purity) were purchased from Aldrich Chemical Co.

(Milwaukee, WI) and were used as supplied. Some of the properties of these two chemicals are provided in Table 2.2.

Table 2.1. Physicochemical characteristics of the various soils used in the study

<i>Soil Type</i>		Silty Soil	Silty Clay Soil
<i>Location of Collection</i>		PPI site, Baton Rouge, LA	Bluebonnet swamp, Baton Rouge, LA
<i>Texture Analysis</i>	Clay (%)	10	37.5
	Silt (%)	82.2	54.9
	Sand (%)	7.9	7.6
	Organic Carbon (%)	1.35	2.6
<i>Elemental Analysis</i>	Hydrogen (%)	0.33	
	Oxygen (%)	2.01	
	Nitrogen (%)	0.06	
	Sulfur (%)	0.04	
	Ash (%)	96.38	

Table 2.2. Properties of the test contaminants (TCE and DCB)*

Property	Trichloroethylene (TCE)	1,3-Dichlorobenzene (DCB)
Molecular weight	131.39	147.01
Aqueous solubility (mg/L @ 25°C)	1000	123
Vapor pressure (mm Hg @ 25°C)	74	2.3
Henry's constant (atm m ³ /mol @ 25°C)	9.1 x 10 ⁻³	3.6 x 10 ⁻³
Log K _{ow}	2.4	3.4
Density (g/cm ³ @ 20°C)	1.46	1.28

From Montgomery, J.M., 1997.

A 1000 mg/l standard solution was prepared by dissolving aliquots of neat TCE and 1,3-DCB in HPLC grade methanol (Fisher Scientific, Rochester, NY) and pre-determined quantity of this standard solution was used to spike deionized water to obtain aqueous solutions with the desired concentration of the test contaminants. All working solutions also contained 200 mg/L sodium azide, NaN_3 (Sigma Chemical Co., St. Louis, MO) to serve as a bacteriological inhibitor or biocide.

Glassware

EPA certified clean amber vials of 40-ml capacity (VWR Scientific, Sugarland, TX) were used for conducting all the experiments. These vials could be centrifuged at low speeds and thus eliminate potential volatilization losses during the transfer of contents into a centrifuge tube. Similar vials of 20-ml size were used to collect and store the aqueous solutions before analysis.

Soil Contamination Step

A measured quantity (5 grams) of dried uncontaminated soil was added to pre-weighed 40-ml EPA certified amber vials. Water spiked with the test chemical was added to these vials and mixed vigorously to wet the entire soil and then filled to capacity. Three different initial spike concentrations of contaminants (3 mg/L, 10 mg/L and 20 mg/L) were used. The vials were set aside for a few minutes and examined for air bubbles. If air bubbles were present, the vials were opened carefully and filled with the same water as above and recapped. This procedure was repeated until no air bubbles were observed. The vials were equilibrated for 72 hours on a tumbler at 80 rpm. At the end of the equilibration period, centrifuging the sample at 1500 rpm for 25 minutes separated the aqueous phase. The aqueous phase thus obtained was analyzed

immediately. The soil left in the vials was used for the subsequent desorption kinetic studies. Twenty-seven vials were prepared initially, 3 of which were sacrificed at the end of the sorption step for soil extraction. The remaining 24 vials were used for desorption kinetics study as described below.

Desorption Kinetics Study

A sacrificial batch method was used to obtain the rate of release (ROR) of compounds from the soil obtained after the sorption step (Williamson *et al.*, 1998). The aqueous phase was replaced with 35-40 ml (to fill the vial without air bubbles) of fresh deionized water and equilibrated on a tumbler. Care was exercised to minimize the time during withdrawal and refilling of the aqueous solution to avoid any losses due to volatilization. At the end of the desired interval, 3 vials were sacrificed for analysis of contaminants in the aqueous phase. This experiment was continued for a period of approximately 30 days, sacrificing 3 vials at each interval. Thus at every time step, the pollutant released from the soil was obtained and plotted to obtain the rate of contaminant release from the laboratory spiked soil. The difference between our method and that of Williamson *et al.* (1998) was that we did not add any tenax particles in the water. Tenax particles help to maintain a zero contaminant concentration in the water and thereby maximize the concentration gradient between the soil and water, thus providing the maximum rate of release from soil. Our experiments are, however, more reflective of the natural conditions that exist during desorption.

Effect of Contaminant Aging within Soil

To address the effect of aging of the contaminant within the soil, a desorption kinetics experiment similar to the one above was designed with a 3 month old and a 5

month old contaminated soil. Five grams of sterilized (autoclaved) soil was added to the EPA certified vials and filled with water spiked with the test contaminants (3 mg/l) and 200 mg/l of sodium azide. Care was taken to avoid any air bubbles and the vials were tumbled for 72 hours at 80 rpm. The soil-water mixture was then set aside at room temperature in a dark area for 3 months and 5 months respectively. At the end of the 3-month incubation period the aqueous phase was separated and replaced with deionized water and the rate of release experiment was conducted as described above. The same experiment was repeated for the 5-month aged soil as well.

Soil Phase Extraction

A hot extraction method (Sawhney, *et al*, 1987; Pignatello, 1990) was used to extract TCE bound to soil. Twenty mL of HPLC grade methanol was added to the soil remaining in the vials after centrifugation and separation of aqueous phase to obtain the total concentration of contaminant in the soil before desorption kinetics was initiated. The vials were mixed vigorously for a few minutes with hand before placement in a water bath maintained at a constant temperature (75°C) for 20 hours. The supernatant was separated after centrifugation and analyzed for the test contaminants. For DCB, however, a sonication method was used. Twenty mL of HPLC grade methanol was added to the vials and the vials were sonicated for 30 minutes before separating the methanol and analyzing for DCB.

Analysis of Test Compounds

Aqueous phase TCE and methanol extracts containing TCE were analyzed using a purge-and- trap unit (HP 7695) attached to a HP 6890 series Gas Chromatograph equipped with a mass selective (MS) detector. The samples were purged with nitrogen

gas. The method detection limit (MDL) was 1 µg/L. The samples were manually injected and the total length of the run was 21 minutes.

Aqueous phase DCB and methanol extracts containing DCB were analyzed using a purge-and-trap liquid sample concentrator (Tekmar, Model LSC-2) attached to a HP 5890A gas chromatograph equipped with a HP5971 mass selective detector (MS) and an auto sampler. The samples were purged with helium gas. The method detection limit (MDL) was 1 µg/L.

Results and Discussion

Mass Balance

The mass of contaminant sorbed on the soil during the sorption step can be determined either from the difference in the concentration of the test contaminant in aqueous solutions before and after the sorption step or, by directly extracting the soil and analyzing for the test compound. This provided a check for the mass balance of contaminant within the system. Table 2.3 shows the mass balance with column 3 showing the total mass of the contaminant in the system based on initial aqueous phase spike concentration and column 6 showing the total mass in the system based on the measured soil phase and equilibrium aqueous phase concentrations. Column 7 shows the percent mass accounted for by the measured concentrations. As is evident from the table, reasonable mass balances within 85 – 96% were obtained in all cases. No significant losses by volatilization in experimental procedures were found with the involvement of extreme care. This provided us confidence in the measurement of the adsorbed concentration on the soil that is necessary for the subsequent desorption kinetics experiments.

Desorption Kinetics from Soils with 3 days Contact With Contaminant

Desorption kinetic studies with freshly contaminated soils (i.e., only 72 hours contact time during the sorption step) were conducted using the soil that was spiked with test contaminants during the sorption study. Triplicate vials were sacrificed at each predetermined interval, the time interval varied for each set of experiments based on the preliminary kinetic studies. Three levels of soil phase concentrations were obtained by contaminating the soils with 3, 10 and 20 mg/L of aqueous concentration of the test contaminants. Thus the designation, 3 mg/L TCE indicates that the soil is contaminated initially using 3 mg/L aqueous solution of TCE.

Soil desorption kinetics data for TCE was collected using both the soils and for DCB, only silty soil from the PPI site was used. The fraction of TCE released, as a function of time is plotted in Figure 2.1a for silty soil (PPI site) and Figure 2.1b for the silty clayey (Bluebonnet swamp) soil, respectively. The data corresponding to all three initial aqueous spike levels is plotted together. As evident from both figures, a substantial portion of the contaminant is released within the first 20-30 hours, followed by a very slow release over a very long period. This slow release was observed over the entire duration of the experiment (100 to 450 hours). Approximately 57, 42 and 51% of TCE was desorbed in the first 24 hours for soils contaminated respectively with 3, 10 and 20 mg/L initial aqueous spike concentrations during the sorption step for the silty PPI soil. The fraction was about 52% for TCE when all the data for the PPI soil was collapsed into one set.

Desorption was higher as the soil contamination level increased for the silty clayey soil (Figure 2.1b). About 55, 60 and 65 of TCE (62% for all the data combined)

was released in the first 24 hours for soils corresponding to the three initial aqueous spike concentrations. Again, very little was desorbed in the subsequent period of desorption.

Table 2.3. Mass balance for TCE and DCB in silty soil

Contaminant	Initial Spike Aqueous Concentration (mg/L)	Total Mass ¹ (µg)	Measured Total Mass ² (µg)	Recovery (%)
TCE	3	110	100.7	91.6
		110	105.3	95.7
		110	100.9	91.7
	10	370	332.8	90.0
		370	342.5	92.6
		370	321.9	87.0
DCB	3	110	98.3	89.4
		110	99.7	90.6
		110	93.4	84.9
	10	370	324.1	87.6
		370	295.3	79.8
		370	309.7	83.7

¹ Total mass introduced into the system calculated based on the initial spike aqueous phase concentration.

² Total mass in the system calculated based on measured soil and aqueous concentrations after equilibration for 72 hours.

A comparison of the two soils showed that the silty soil (PPI) had an organic carbon content of 1.5% and the silty clayey soil had about 2.6% organic carbon. The silty clayey soil with the higher organic carbon content released a higher fraction of TCE. Typically when the contaminant laden aqueous phase is equilibrated with soil, the contaminant is adsorbed quickly to the easily reversible sites and then further

diffuses to the non-labile sites. We hypothesize that the soil with higher silty fraction tend to bind a larger fraction of the contaminant to reversible sites in the limited time available for sorption (72 hours) and thus a higher fraction of contaminant is released in a relatively shorter period of time (higher desorption rate). There is evidence to suggest that soil organic carbon is not the only factor in determining the desorption rates of organic compounds to soils (Pavlostathis and Mathavan, 1992; Ahlert and Uchirin, 1990).

Figure 2.2 shows the data for 1,3-dichlorobenzene on the silty soil from the PPI site, in which case we also observed a similar, desorption behavior, a fast labile phase followed by a slow phase. The fraction of DCB released from the silty soil was slightly higher than that for TCE. 59, 62 and 67% of DCB (60% when all the data is collapsed into one set) was desorbed from soils contaminated with initial aqueous spike concentrations of 3, 10 and 20 mg/L respectively.

An empirical model was used by Opdyke and Loehr (1999) to describe the contaminant rate of release (ROR) similar to what we observed; a relatively rapid release of the chemical followed by a much slower release of the remaining chemical. The non-linear equation used to describe the bi-phasic behavior during desorption was given by:

$$\frac{S_t}{S_0} = 1 - Fe^{-k_1t} - (1 - F)e^{-k_2t} \quad (2.1)$$

where t is time (h), S_t/S_0 is the fraction of chemical released after time t . F is the fraction of chemical released quickly (labile phase) and $(1-F)$ is the fraction of chemical released slowly (non-labile phase). k_1 and k_2 are the first order rate constants describing the desorption of the labile fraction and slowly released (non-labile) fraction (h^{-1}).

The equation contains two exponential terms with k_1 and k_2 as unknowns and another unknown F , the labile fraction of the chemical. A software package, Curve Expert (Version 1.34, a free download from <http://www.ebicom.net/~dhyams/cvxpt>) was used to fit the experimental data to the above equation and to determine the model parameters, F , k_1 , and k_2 .

The results of the model fit, the values for the parameters, F , k_1 and k_2 the corresponding r^2 values and standard error of estimate, S are presented in Table 2.4 for TCE (both silty soil and silty clay soil) and DCB (only silty soil). The data were collapsed into one set corresponding to all the three contamination levels and the model parameters were determined. The lines in the figures are obtained using equation 2.1 with the model parameters determined from the non-linear fit for the composite data. As can be seen from the figures and Table 2.4, the first order rate constant (k_2) for the slowly desorbing fraction was in the range of 3.5×10^{-6} to $2.4 \times 10^{-3} \text{ h}^{-1}$ ($5 \times 10^{-4} \text{ h}^{-1}$ for both soils with the composite data) for TCE and 4.1×10^{-5} to $7.8 \times 10^{-4} \text{ h}^{-1}$ ($6 \times 10^{-4} \text{ h}^{-1}$ for the composite data) for DCB. The first order rate constant for the labile fraction (k_1), however, ranged from 0.08 to 0.55 h^{-1} (0.52 and 0.62 h^{-1} using the composite data for silty soil and silty clay soil respectively) for TCE. The first order rate constant k_1 was almost constant, 0.022-0.038 h^{-1} (0.028 h^{-1} using the entire data) for DCB on silty soil. In both cases k_1 increased slightly as the initial soil contaminant concentration increased. Thus there are at least two orders of magnitude difference in the values of rate constants for the labile and non-labile fractions.

As discussed by Opdyke and Loehr (1999), the precision in k_2 is good only if many samples are taken near the time corresponding to $1/k_2$. For the cases studied, this

means a k_2 of approximately 10^{-4} h^{-1} would translate to 10,000 hours. This is somewhat impractical for volatile compounds such as the ones studied here, since losses by volatilization and biodegradation are inevitable for such long-term studies.

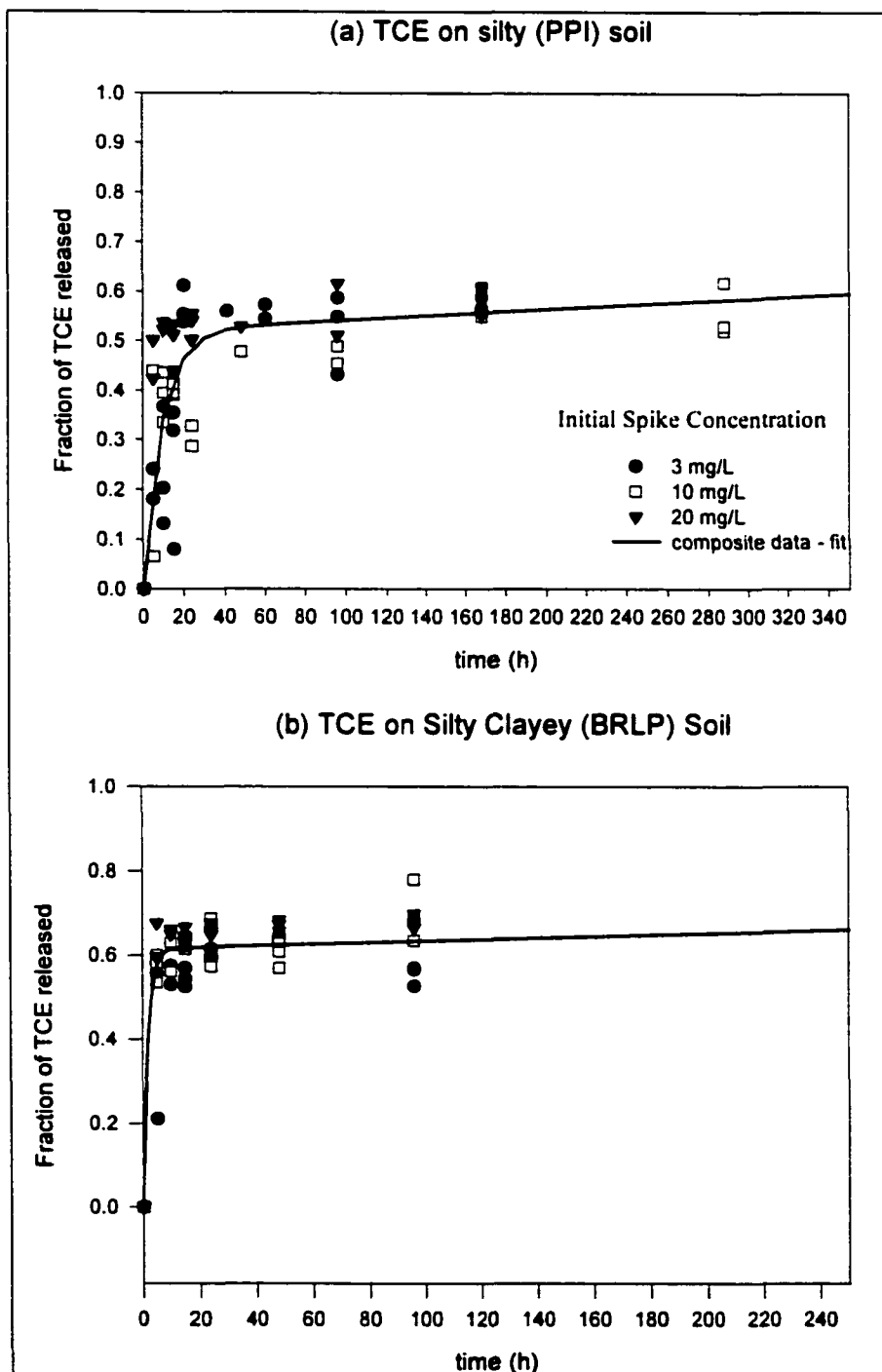


Figure 2.1. Desorption kinetics for TCE contaminated soil.

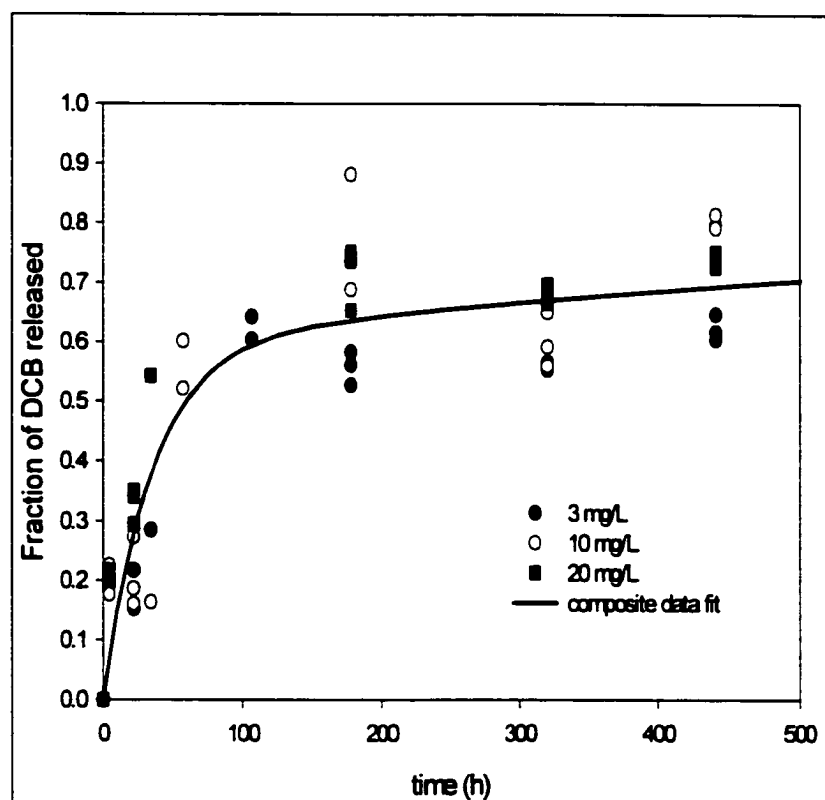


Figure 2.2. Fraction of 1,3-DCB released as a function of time in PPI soil.

As a result, the precision in k_2 values reported here are presumed unsatisfactory and only order of magnitude estimates are useful in interpreting the time frame for the desorption of the non-labile fraction. To increase the precision in k_2 , one needs to redesign the experiments with the ROR procedure as described by Opdyke and Loehr (1999) and extend the duration of the experiment. The precision in k_1 is deemed good since its determination relies on sufficient data points during the early part of the desorption curve. In the present experiment, the number of samples collected towards the beginning of the experiment are sufficient for good estimates of k_1 for both TCE and DCB on the silty (PPI) soil, whereas it was not so for TCE from silty clayey (Bluebonnet swamp) soil. The fraction of contaminant that is rapidly released (F) provides an indication of the amount of organic contaminant that is available for

immediate transport or uptake by microorganisms in the soil. The comparison of release rate constants for TCE with the two different soils indicate that silty clay that has a higher fraction (F) of TCE also desorbed quickly (higher k_1) compared to silty soil. The fast release rate constant, k_1 in silty clay soil was 5 to 7 times greater than that for silty soil except for the soil contaminated with higher initial aqueous phase concentration (20 mg/L) where it is lower for the silty clay soil. It is to be noted that the silty clay soil has more organic carbon (2.6%) compared to the silty soil (1.5%). The data indicates that the release of TCE is dependent not only on the soil organic carbon and contaminant properties but also on the soil composition, the fraction of silt, clay and sand. Thus the distribution of the contaminant among the labile and non-labile fractions is dependent on the type of chemical, soil organic carbon content and other factors including aging as discussed below. Pavlostathis and Mathavan (1992) made similar observations for TCE from various soils, DiToro and Horzempa (1982) for PCB, Locke (1992) for Alachlor and time-dependent isotherm studies for several organic compounds by Xing and Pignatello (1996).

The comparison between DCB and TCE for the silty soil from the PPI site indicates that the labile fraction (F) for DCB was higher compared to TCE. However, k_1 values were significantly lower for DCB compared to TCE. As can be seen by the comparison between the desorption curves for TCE and DCB (Figures 1 and 2), although the labile fraction of DCB was higher it took considerably longer time to release that fraction (about 100 hours) compared to about 24 hours or less for TCE. This can be explained by the fact that DCB is more hydrophobic and thus has stronger affinity to soil organic carbon than the aqueous phase. However, if enough equilibration

time is provided one would expect a larger F and a smaller k_1 for DCB compared to TCE. This can be seen from the data for aged soil as described in the next section, where F and k_1 were higher for TCE than DCB. The first order rate parameter (k_2) values for the slowly released fraction are not significantly different for TCE and DCB when the values obtained for the composite data are compared.

It was clear that an equilibrium desorption time of 24 hours is adequate for ascertaining the batch desorption partition coefficient for TCE, whereas at least 100 hours or larger is required for DCB. Accordingly, three triplicate samples each of the PPI soil at three different initial soil concentrations were kept stirred with 40 mL of pure distilled water for 24 hours in the case of TCE and 178 hours in the case of DCB. The aqueous supernatant and soil were then analyzed separately for the compounds. The desorption isotherm obtained in this manner is plotted in Figures 3a and 3b. It is clear that the desorption isotherm indicates that a portion of the compounds remains bound to the soil. The linear isotherm representing this would consist of: (i) a reversible portion obeying the conventional linear isotherm, $W_{rev} = K_{sw}C_w$, where W_{rev} is the soil concentration ($\mu\text{g/g}$), K_{sw} is the partition constant (L/g) and C_w is the aqueous phase concentration ($\mu\text{g/L}$), and (ii) a desorption resistant component W_{irr} ($\mu\text{g/g}$). Thus $W = W_{irr} + K_{sw}C_w$. A plot of W versus C_w will give W_{irr} as the intercept and K_{sw} as the slope. This is shown in Figure 2.3a for TCE and 2.3b for DCB on the silty (PPI) soil. For TCE, the W_{irr} obtained was $0.9 \mu\text{g/g}$ and $K_{sw} = 0.00372 \text{ L/g} = 3.72 \text{ L/kg}$, which gives $K_{oc} = K_{sw}/f_{oc} = 275 \text{ L/kg}$. This is substantially larger than the reported K_{oc} of 63 L/kg (Montgomery, 1997) obtained from adsorption measurements done using 24 hour equilibration between soil and water.

Table 2.4. Comparison of the empirical non-linear model parameters (F, k_1 , k_2) for TCE (two soils) and DCB (one soil).

Soil Age (Initial Spike Concen.)	Silty Soil (PPI) TCE				Silty-Clayey Soil (BRPL) TCE				Silty-Soil (PPI) DCB			
	F	k_1 (h^{-1})	k_2 (h^{-1})	R^2 (S)	F	k_1 (h^{-1})	k_2 (h^{-1})	R^2 (S)	F	k_1 (h^{-1})	k_2 (h^{-1})	R^2 (S)
3 days (3 mg/L)	0.57	0.075	3.5×10^{-6}	0.93 (0.10)	0.55	0.335	1.9×10^{-3}	0.95 (0.07)	0.59	0.022	4.1×10^{-5}	0.97 (0.07)
3 days (10 mg/L)	0.44	0.122	1.1×10^{-3}	0.94 (0.08)	0.60	0.582	2.4×10^{-3}	0.98 (0.04)	0.64	0.023	7.8×10^{-4}	0.93 (0.09)
3 days (20 mg/L)	0.51	0.460	9.4×10^{-4}	0.99 (0.03)	0.65	0.389	9.0×10^{-4}	0.99 (0.02)	0.67	0.038	4.3×10^{-4}	0.97 (0.06)
Consolidated data for 3 day aged soil	0.51	0.108	5.4×10^{-4}	0.79 (0.15)	0.62	0.533	5.4×10^{-4}	0.96 (0.06)	0.60	0.028	6.0×10^{-4}	0.91 (0.09)
3 months (3 mg/l)	0.43	0.344	7.9×10^{-4}	0.99 (0.02)					0.38	0.085	2.0×10^{-4}	0.97 (0.04)
5 months (3 mg/L)	0.30	0.118	1.1×10^{-3}	0.97 (0.03)					0.32	0.217	2.0×10^{-4}	0.99 (0.02)

Note: S is the standard error of estimate. As S approaches 0, the quality of the fit increases. R^2 is the correlation coefficient

Pavlostathis and Mathavan (1992) reported K_{oc} for TCE desorption from three types of field-contaminated soils. Values ranging from 46 to 438 L/kg were reported. For DCB the value of W_{irr} obtained was 9.1 L/kg, which corresponded to a K_{oc} of 674 L/kg. The reported K_{oc} from adsorption measurements was 293 L/kg (Montgomery, 1997). Many others (Pavlostathis and Jaglal, 1991; Steinberg et al, 1987) also have reported that desorption partition coefficients are much larger than adsorption partition coefficients.

Desorption Kinetics from Soils with Extended Contact with the Contaminant

Experiments were conducted to study the effect of aging of contaminants sorbed on the soil on desorption characteristics. Soils contaminated with aqueous solutions containing 3 mg/L each of TCE and DCB were stored for three months and five months at room temperature. Subsequently, the aqueous phase was replaced with deionized water and the desorption kinetics was initiated. The data was compared with that from the 3 days aged contaminated soil. The desorption kinetics data are presented in Figures 2.3 and 2.4 for TCE and DCB respectively along with those for freshly contaminated soil for comparison purposes. The values of the model parameters, F , k_1 and k_2 for this study are provided in the bottom rows along with those from earlier experiments in Table 2.4. As expected, the labile fraction was reduced from 62 to 38% for DCB and 57 to 34% for TCE for the 3 month aged soil. For the 5 month aged soil the labile fraction was further reduced to 30% for TCE and 32% for DCB. The longer incubation period in the case of the aged soil allows organic molecules to diffuse and sorb onto the compartments or regions in the sorbent that exhibit slow adsorption/desorption kinetics (Pignatello, *et. al*, 1993; Connaughton, *et al.*, 1993; Scribner, *et al.*, 1992). However,

the desorption rate constants k_1 for the labile fraction was higher for the aged contaminated soil than the freshly contaminated soil for both TCE and DCB in the case of the 3 month aged soil. This suggests that the initial release from the aged soil is faster than that for the freshly contaminated soil. For DCB this trend is clear for both 3 and 5 month aged soils. During adsorption, all the reversible sites on the soil and the organic carbon are first occupied by the contaminant before it starts to accumulate in the irreversible sites. For freshly contaminated soil, within the short equilibrium time of 3 days, the contaminant should be mainly in the reversible sites and diffusion of contaminant into irreversible sites might still be occurring. Thus, both processes of desorption into water and migration to the non-labile sites are occurring simultaneously and the overall desorption rate is lowered. However, for aged soils, the diffusion of contaminant from the reversible sites to non-labile sites would be nearly complete in the 3 and 5-month periods. Thus, when the desorption process was initiated, the fraction of contaminant in the labile sites, though smaller, desorbs at a faster rate than from the freshly contaminated soil. This suggests that the 72-hour equilibration time used in the experiment is not enough for the contaminant to diffuse into the soil organic matter and micropores and thus larger fractions would be released when subjected to desorption. The aged soil study also further reinforces the need to assess F with k_1 and k_2 when comparing fast and slow release rates, as the rate coefficients also may be misleading if the aging process is not well known.

Implications for the Remediation of the PPI Site

As per the Consent Decree entered into by the industry and government in the U.S. District Court, Middle District of Louisiana the current remedy is: (i) to cover the

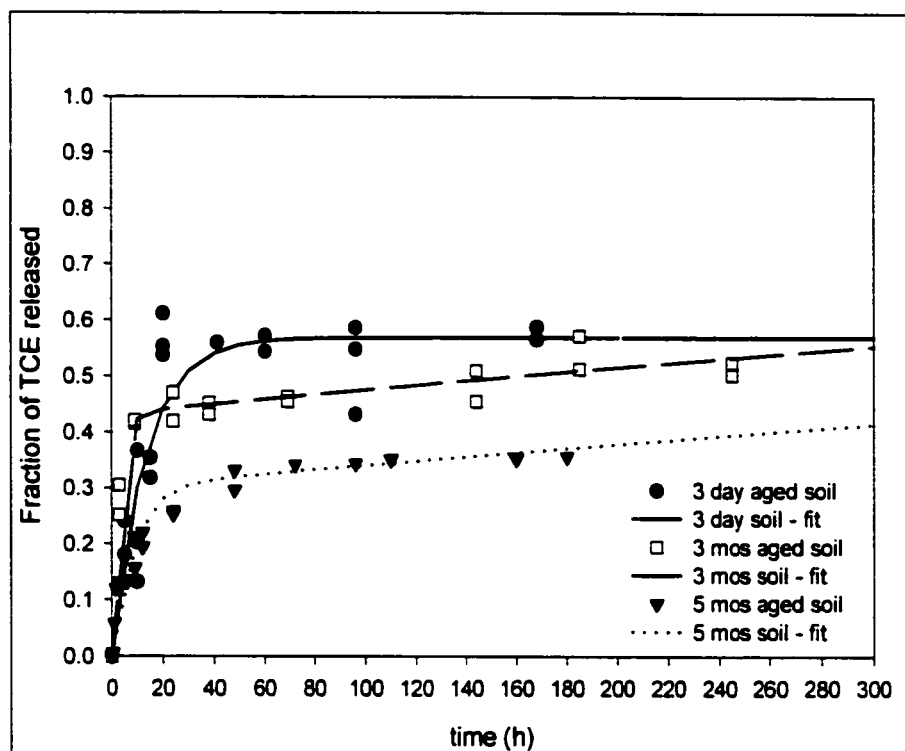


Figure 2.3. Fraction of TCE released in aged soil as a function of time.

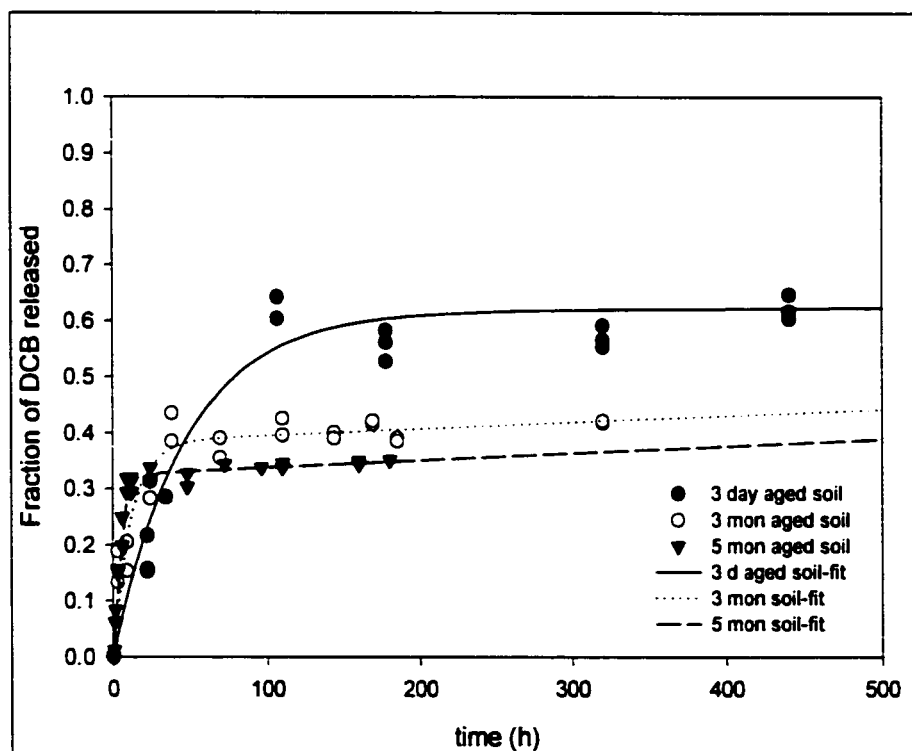


Figure 2.4. Fraction of 1,3-DCB released in aged soil.

site with a clay cap, and (ii) to install a series of wells as part of a containment/recovery facility for free phase liquids. This technology, commonly called pump-and-treat requires that a hydraulic gradient be maintained to prevent lateral and vertical migration of the waste. A groundwater flow model (MODFLOW) is used to predict the movement of the contaminant plume. Our work is in support of this effort. In this activity, the measured K_d values reported earlier by us (Valsaraj *et al*, 1999; Kommalapati *et al*, 2000) for the organic compounds are used as input values for the MODFLOW model. These measurements assumed equilibrium for both adsorption and desorption in 72 hours. The current data, however, suggests that at most only 60% of the compound is labile and participate in the reversible sorption equilibrium. The half-life for desorption of this fraction is of the order of a few hours (2 to 24) hours for TCE and larger for DCB. The labile fraction becomes less as the contaminant ages within the soil. Our earlier work (Kommalapati *et al*, 2000) showed that even when the water in contact with the soil is replaced with fresh water at every step after 24-hours of equilibration, only a small percentage of the material is recovered from the contaminated soil. Hence, sequential desorption also is incapable of removing the non-labile fraction from the soil. The projections from the MODFLOW model based on the 72-hour equilibrium data only pertain to the labile fraction of pollutant. Hence, the long term predictions using the batch K_d values will significantly over-predict the movement of contaminants that have been in contact with the soil for decades at the PPI site. On the other hand, we conclude that a significant portion of the contaminant in the aged site soil is inaccessible to water in a P&T scheme and hence remain within the soil to pose no significant threat of migration away from the site. Moreover, the slowly released

fraction can probably be managed by the natural assimilative capacity of the soil. Thus the desorption resistant concentration may be considered an environmentally acceptable end point (EAE) in the soil that can be managed by the so-called natural attenuation scheme.

Conclusions

The rates of desorption of trichloroethylene (TCE) and 1,3-dichlorobenzene (DCB) from a silty soil at a Superfund site and a silty-clayey soil from an uncontaminated bottomland hardwood swamp in Baton Rouge, Louisiana were studied in laboratory batch systems. The effect of the age of soil contamination was studied using a laboratory-spiked soil incubated for 3 days, 3 months and 5 months. An empirical non-linear model was used to describe the bi-phasic nature of desorption with one fraction (labile) being released in relatively short periods of time (typically 24 - 100 hours) and a second fraction (non-labile or irreversible) being resistant to desorption. The non-linear model parameters, viz., the fraction of the chemical released rapidly (F), and the first order desorption rate coefficients, k_1 and k_2 respectively for the labile and slowly released fractions were determined by fitting the experimental data to the model. The data fit the model well as indicated by the high r^2 values. The estimate of k_1 was good. However, the values of k_2 are known with less precision due to the limited duration of the experiment and number of samples taken at long times. In addition, desorption kinetics of 3-month and 5-month old contaminated soils showed that progressively less amount of contaminant was available for facile desorption (lower F) compared to freshly contaminated soil. The labile fraction had desorption rate constants of the order of 10^{-1} h^{-1} , whereas the slowly released fraction had rate constants of the

order of 10^{-4} h^{-1} in accord with literature reported values for a variety of other compounds and soils. Possible mechanisms describing these rates and implications for the site clean up are discussed.

CHAPTER 3

BIOAVAILABILITY OF REVERSIBLY SORBED AND DESORPTION RESISTANT 1,3-DICHLOROBENZENE

Introduction

Hydrophobic organic contaminants (HOCs) are known to desorb slowly from contaminated soil and sediment (Steinberg *et al.* 1987, Connaughton *et al.* 1993, Valsaraj *et al.* 1991, Lee *et al.* 2000a). Recent research shows that HOCs, and particularly aromatic compounds, may be biodegraded by microorganisms to a residual concentration that no longer decreases or which decreases only very slowly over time with continued treatment (Linz and Nakles, 1997). It is widely believed that further reductions are limited by the availability of hydrocarbons to microorganisms and these limitations are severe for soils contaminated for a long time period (Bosma *et al.* 1997). Zhang and Bouwer (1997) studied the bioavailability of benzene, toluene and naphthalene in a soil-water slurry, and noted that the rate of biodegradation decreased with increasing organic compound hydrophobicity, soil/water ratio, soil particle size, and soil organic carbon content suggesting that the bioavailability of the contaminant was affected by soil characteristics and the rate of desorption.

The bioavailability of a chemical depends on physicochemical processes such as adsorption/desorption, diffusion, and dissolution (Ogram *et al.* 1985 and Luthy *et al.* 1994). In particular, for aged contaminated soils, a fraction of the contaminant appears to be inaccessible for biodegradation. All evidence seems to indicate a reduced availability of contaminant in soils and sediments contaminated for a prolonged period of time, and pollutant, not nutrient availability being the cause. This aging or “weathering” as it is usually referred to may result from (i) chemical oxidation reactions incorporating contaminants into soil organic matter (ii) slow diffusion into small pores

and absorption into organic matter or (iii) the formation of semi-rigid films around non-aqueous phase liquids with a high resistance toward organic-to-water mass transfer (Bosma, *et al.* 1997).

Several researchers have confirmed that biodegradation can be limited by the slow rate of desorption of organic compounds (Robinson *et al.* 1990, Al-Bashir *et al.* 1994, Steinberg *et al.* 1987, Pignatello, 1989). Robinson *et al.* (1990) observed that although most of the toluene in soil-water slurries were biodegraded rapidly, a small fraction was biodegraded much more slowly and at a rate limited by desorption. Steinberg *et al.* (1987) reported that ethylenedibromide (1,2-Dibromoethane, EDB), a soil fumigant with relatively high water solubility, volatility and biodegradation persisted in top soils for as long as 19 years after its last application. However, laboratory experiments with freshly added EDB showed rapid biodegradation (Scribner *et al.* 1992). It has been suggested that residues of EDB in the top few centimeters were unavailable for microbes because it was sorbed in to soil micropores and unavailable for biodegradation. Salkinoja-Salonen *et al.* (1989) reported that no degradation of chlorophenols was observed in soil that was polluted for over 40 years while chlorophenol degradation proceeded rapidly in freshly polluted soil. Freshly added pentachlorophenol in a previously contaminated soil was, however, mineralized instantaneously suggesting that enough chlorophenol degrading bacteria and nutrients are available for biodegradation.

The current paper is part of ongoing research conducted to support the remediation activities at a Louisiana Superfund site known as Petro Processors Inc., (PPI) site located in north Baton Rouge, LA. Contaminants at the site include a wide

range of halogenated hydrocarbons. In earlier papers we reported the adsorption/desorption hysteresis and desorption kinetics for some of the prevalent contaminants at the site (Valsaraj *et al.* 1999, Kommalapati *et al.* 2000 and Lee *et al.* 2001a). Trichloroethylene and 1,3-dichlorobenzene desorption exhibited a biphasic desorption pattern with one fraction that is readily desorbed and a second fraction that was resistant to desorption. Also, the age of contamination had an effect on the fraction that was readily desorbed. 1,3-DCB is a prevalent contaminant at the PPI site. Several studies reported the degradation of DCBs in the aqueous phase using mixed and pure cultures (Reineke and Knackmuss 1984, deBont *et al.* 1986 and Jackson *et al.* 1999). In this study, we conducted aerobic microcosm batch experiments with aqueous phase (soil free) and soil-waster slurries to determine the extent of 1,3-DCB biodegradation over a 4-week period. Another soil that contained only the desorption-resistant fraction (i.e. soil that has been repeatedly extracted to remove the readily desorbing fraction) was also used to study the bioavailability of the contaminant.

Monitored natural attenuation (MNA) is presently being considered as an alternative to the current pump and treat remediation scheme for the PPI site. The results of the study could have implications in addressing the question of an acceptable end point for meeting the clean-up goals at the site.

Materials and Methods

Soil

An uncontaminated soil from the Brooklawn portion of the PPI site, located in north Baton Rouge, LA was used for microcosm studies. Large lumps of soil were broken and oven dried at 55°C for 24 hours. The soil was then pulverized before

sieving through a 150 μm sieve (US standard sieve No. 100). Samples from this soil were methanol-extracted and analyzed and found to be free of the test contaminant, 1,3-dichlorobenzene (1,3-DCB). Soil analysis was performed as per the standard methods by the LSU Soil Science laboratory and the soil characteristics are presented in Table 3.1. The soil is classified as silty soil. All soil samples were autoclaved before using them in the biodegradation experiments.

Table 3.1. Physicochemical characteristics of the soil used in the study

Characterization of Soil		Silty Soil
Location of Collection		PPI site
Texture Analysis	Clay (%)	10
	Silt (%)	82.2
	Sand (%)	7.9
	Organic Carbon	1.35
Elemental Analysis	Hydrogen (%)	0.33
	Oxygen (%)	2.01
	Nitrogen (%)	0.06
	Sulfur (%)	0.04
	Ash (%)	96.38

Chemicals

The test contaminant for this study was 1,3-DCB. Previously we had studied its adsorption/desorption kinetics from the same soil (Lee *et al.* 2001). 1,3-DCB with 98 % purity was purchased from Aldrich Chemical Co. (Milwaukee, WI) and was used as supplied. Relevant properties of 1,3-DCB are provided in Table 3.2. Sodium azide, NaN_3 (Sigma Chemical Co., St Louis, MO) was used as a bacteriological inhibitor (biocide). The mineral salts medium used for the biodegradation experiments and contained the following ingredients (in grams liter⁻¹): $(\text{NH}_4)_2\text{SO}_4$ (3); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1); K_2HPO_4 (1.55); $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.85) and 2 ml of trace mineral solution.

Glassware

Trace Clean bottles of 125 ml or 40 ml capacity (VWR Scientific, Sugar Land, TX) which were certified to be free of trace organics were used for this study. The caps were blue, polypropylene open-top caps with bonded Teflon fluorocarbon resin/silicon septum. The bottles could be centrifuged at low speeds and thus eliminate the potential volatilization losses during the transfer of contents into a centrifuge tube.

Table 3.2. Properties of the test contaminant (DCB)*

Property	1,3-Dichlorobenzene (DCB)
Molecular weight	147.01
Aqueous solubility (mg/L @ 25 ⁰ C)	123
Vapor pressure (Pa @ 25 ⁰ C)	287
Henry's constant (Pa m ³ /mol @ 25 ⁰ C)	365
Log K _{ow}	3.60
Density (g/cm ³ @ 20 ⁰ C)	1.2884

*From Montgomery, J.M., 1997

Bioavailability Studies

Biodegradation experiments were conducted in three different sets using (i) aqueous phase that is representative of soil porewater, (ii) freshly contaminated soil and (iii) soil containing the desorption resistant fraction of the contaminant prepared by sequential desorption using water before performing the bioavailability experiments. For the preparation of soil phase experiment, soils were subjected to sequential desorption steps to obtain soils containing desorption resistant fraction.

Enrichment of 1,3-DCB Degrading Bacterial Culture

Aerobic enrichment cultures were prepared with mixed cultures obtained from sludge samples at the municipal wastewater treatment plant in Baton Rouge, Louisiana. About 100 ml of mineral salts media and a predetermined amount of 1,3-DCB stock solution were added to give a final aqueous concentration of 200 µg/L. The solution was equilibrated under aerobic conditions at room temperature (22~24°C). An appropriate amount of potassium phosphate buffer solution was added to maintain a pH of 7.0. Biological growth was monitored using volatile suspended solids concentration and turbidity measurements with UV-VIS Spectrophotometer (Shimadzu, Model UV-1201) as recommended by the standard methods (Eaton et al. 1995). Aqueous 1,3-DCB concentrations were measured using GC-MS to monitor the degradation. The culture thus developed was used during its growth phase as the seed inoculum for the biodegradation experiments.

Preparation of Contaminated Soil and Soil Pore Water for Biodegradation Studies

Figure 3.1 shows the various steps involved in preparing soil and soil pore water for biodegradation experiments. A measured amount (250 g) of dry uncontaminated soil was added to a 1 L glass jar with a Teflon lined cap. Aqueous solution spiked with 1,3-DCB (2mg/L) was added to these jars and mixed vigorously and then filled to capacity with minimum headspace. The jars were equilibrated on a tumbler for 72 hours at 80 rpm. After equilibration, the jars were allowed to stand for about 1 day to obtain a clear soil-water interface and the aqueous phase and the contaminated soil were separated. For the experiments using freshly contaminated soil, the soil obtained in the above step was used directly after determining the soil concentration of 1,3-DCB.

However, the above soil was subjected to sequential desorption using water as described in detail in our earlier papers (Valsaraj *et al.* 2000, Kommalapati *et al.* 2000 and Lee *et al.* 2001a). Briefly, after separating the aqueous phase from the soil, the jar containing the contaminated soil was filled with distilled water and equilibrated for 24 hours. The aqueous phase was replaced again with distilled water and the process was repeated 4 more times (1 total of five desorption steps). From our earlier experiments, we observed that the contaminant remaining on the soil after 5 desorption steps could be assumed to be predominantly desorption resistant. The soil concentration of 1,3-DCB was measured and the soil used for the biodegradation experiments. The aqueous phase from the initial contamination step and all the subsequent desorption steps was collected and stored. The aqueous solution soil pore water thus accumulated was analyzed for 1,3-DCB and used for the aqueous phase biodegradation studies after adding appropriate amendments.

Biodegradation of 1,3-DCB in the Aqueous Phase

The soil pore water obtained as above was analyzed for 1,3-DCB. Ten milliliters of mineral salts medium prepared as mentioned in the earlier section was added with 40 ml of soil pore water to the 125 ml Trace Clean bottle. A predetermined amount of 1,3-DCB stock solution (1000 mg/l) was also added to obtain an initial 1,3-DCB aqueous concentration around 100 µg/L. Five milliliters of DCB degrading bacterial culture was added to each bottle. However, for control experiments, 15ml of sodium azide solution (200 mg/l) was added instead of nutrient solution and seed inoculum. The total volume of the aqueous phase was about 55 ml for the aqueous phase biodegradation studies. A total of 42 bottles (21 controls and 21 with bacterial

cultures) were equilibrated on a tumbler at 80 rpm and at room temperature. At predetermined time intervals, three bottles containing the bacterial cultures and 3 control bottles were sacrificed and analyzed for 1,3-DCB. The experiment was continued for about four weeks.

Biodegradation of 1,3-DCB in Freshly Contaminated Soil

The contaminated soil obtained after equilibration of uncontaminated soil with 2 mg/L of aqueous 1,3-DCB solutions as mentioned above was used for this study. At the end of the equilibration period, contaminated soil was separated and analyzed for 1,3-DCB. Approximately 7 grams of the wet contaminated soil was added into each 125 ml Trace Clean *I-Chem* bottle. Extreme care was taken during the soil transfer to prevent possible volatilization losses. Twenty-five ml of nutrient solution prepared as above and 26 ml of DCB degrading bacterial culture was added to each bottle. For control bottles, however, 26 ml of biocide, sodium azide (NaN_3 solution at 200 mg/l) was added instead of bacterial culture. Twenty-eight bottles (7 controls and 21 with bacterial cultures) were equilibrated on a tumbler at 80 rpm and at room temperature. At predetermined time intervals 3 bottles containing the bacterial cultures and 1 control were sacrificed and analyzed for total 1,3-DCB using sonication method described below with 10 ml methanol. The experiment was continued for about 4 weeks.

Biodegradation of 1,3-DCB in Soil Containing only Desorption Resistant Fraction

The contaminated soil remaining after 5 sequential desorption steps was used. The set-up, sampling and analysis procedures were exactly the same as that for freshly contaminated soil described above. Prior to using soils, the measurement of concentrations in soils was conducted by GC-MS.

Extraction and Analysis of 1,3-DCB

Extraction of 1,3-DCB from the test bottles containing the soil and the aqueous solutions was done using a sonication method. HPLC grade methanol (10 ml) was added to the bottles and sonicated for 30 minutes. The aqueous solution was separated after centrifugation and the supernatant was analyzed for DCB. Aqueous solutions and the soil extracts which contained methanol and water were analyzed for 1,3-DCB using a purge-and –trap liquid sample concentrator (Tekmar, Model LSC-2) attached to a HP 5890a gas chromatograph equipped with a HP 5971 mass selective detector (MS) and an auto sampler. The total run time for the method was 20minutes and the method detection limit (MDL) was 1 µg/L.

Results and Discussion

1,3-DCB Desorption

As reported in our earlier papers (Valsaraj *et al.* 1999, Kommalapati *et al.* 2000, and Lee *et al.* 2001a), the DCB sorbed on the soil can be conceptualized to be in two compartments, one from which it is readily desorbed (labile or reversibly bound) and second where the contaminant is tightly bound and is resistant to desorption.

The freshly contaminated soil was subjected to a sequential desorption. The objective was to obtain a soil that contained only the tightly bound contaminant (non labile, desorption resistant) fraction and determine whether it is available for microorganisms to degrade. Figure 3.2 shows a plot of the soil phase concentration and the corresponding aqueous concentration during the sequential desorption. The first point corresponding to the initial equilibrium sorption step when the soil was contaminated with 1,3-DCB and set aside for 24 hours. The next five points correspond

to the 5 successive desorption steps. As seen from the figure, after 5 desorption steps, most the readily desorbed fraction of 1,3-DCB was removed from soil. This is in agreement with earlier reports (Valsaraj *et al.* 1999, Lee *et al.* 2001a, DiToro *et al.* 1982). As noted in Figure 3.1, for freshly contaminated soil, the initial 1,3-DCB concentration was about 18,000 mg/kg soil compared to about 1650 mg/kg soil for the experiments using soil containing the desorption resistant fraction of the contaminant.

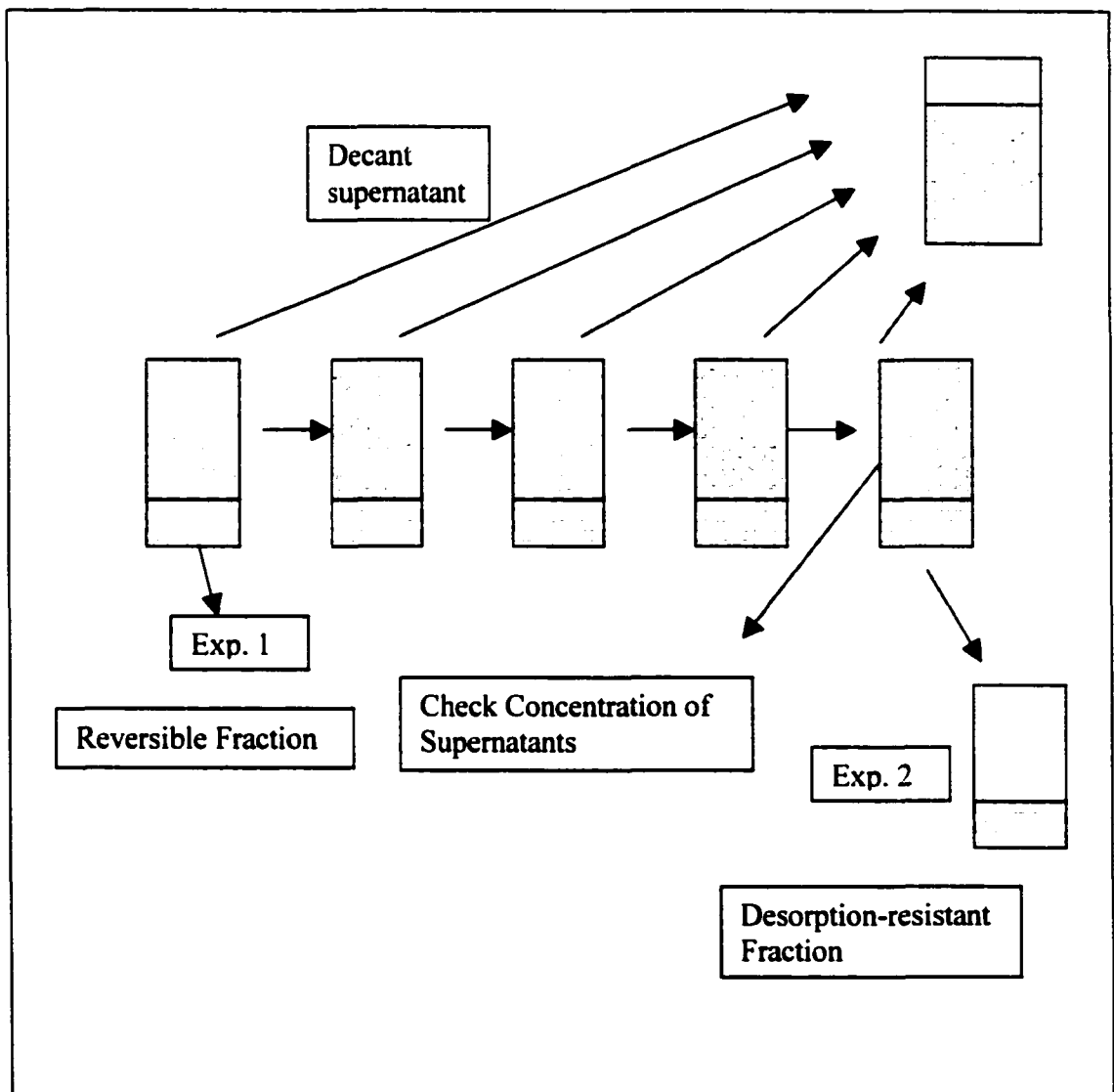


Figure 3.1. Different steps involved in preparing soils and water for bioavailability experiments

Biodegradation in the Aqueous Phase

Aqueous phase biodegradation of 1,3-dichlorobenzene (1,3-DCB) was investigated using soil pore water obtained from the desorption of freshly contaminated soil. Hydrogen peroxide was added to maintain the oxygen necessary for the bacterial metabolism of 1,3-DCB. Data from the controls indicated that the loss of 1,3-DCB by volatilization was negligible. The initial aqueous phase concentration (about 5 mg/l) remained constant for all the sampling intervals.

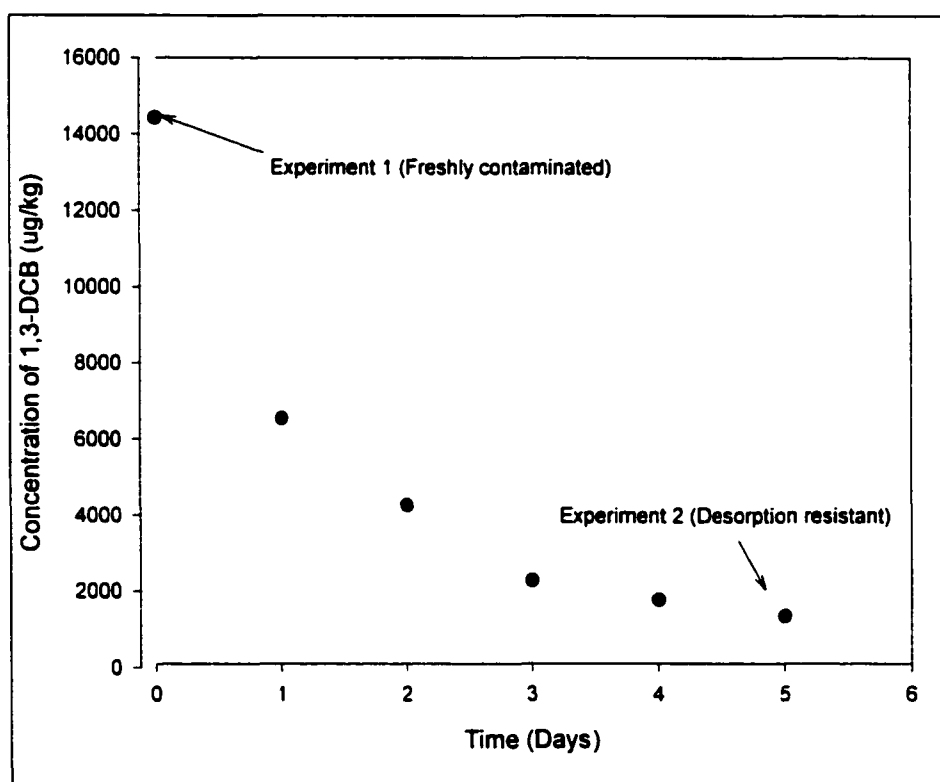


Figure 3.2. Sequential desorption of 1,3-DCB from soil.

Aqueous phase concentration of 1,3-DCB in both controls and treatments shown in Figure 3.3. As mentioned earlier, all the control and test samples were run in triplicate and the averages are used for the plots. As can be seen from the figure, the

1,3-DCB concentrations decreased by 80% in 3 days and nearly complete biodegradation was accomplished within 7 days. The growth and metabolism of microorganisms appeared to be sustained during the duration of the experiment. This is indicated by the protein concentration that reached a maximum and remained constant at 18 mg/L towards the final days of the experiment.

Several researchers have noted that the rate of biodegradation in the aqueous phase (soil free) was faster than that in the presence of soil (Zhang and Bouwer, 1997,

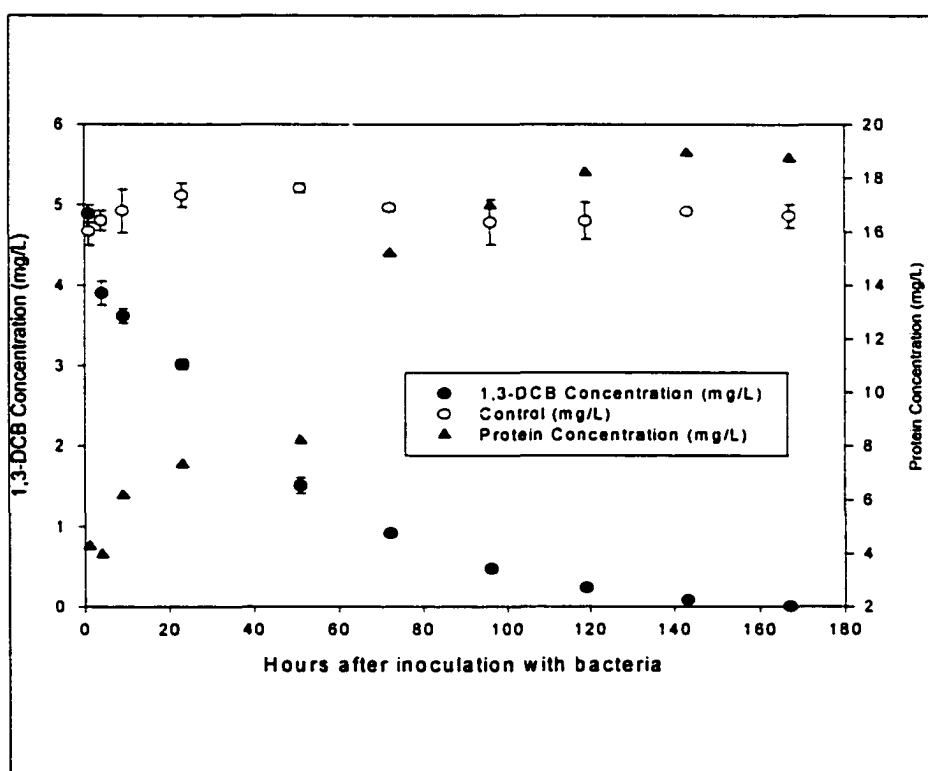


Figure 3.3. Biodegradation of 1,3-DCB in aqueous phase.

As will be seen later from the results of the experiments with soil, our results corroborate those observations. The initial concentration of 1,3-DCB is lower in our studies compared to those reported in the above studies.

Biodegradation of 1,3-DCB in Freshly Contaminated Soil

The uncontaminated soil was equilibrated with an aqueous solution spiked with 1,3-DCB, the water was separated and the soil directly used to study the biodegradation of 1,3-DCB. Figure 3.4 shows the percent 1,3-DCB degraded as a function of incubation time after accounting for all the losses in the controls. As can be seen from the figure, the percent biodegradation was 23% after 1 day of incubation in the microcosm. Thereafter the biodegradation did not seem to contribute to any more loss of DCB from the microcosms. From the positive slope of the biodegradation curve it appears that oxygen was not a limiting factor for these experiments. Also, if the experiment had been continued beyond the 6-week period it is possible that we might have seen more biodegradation. However, it was noted earlier that about 70-80% of the sorbed contaminant is the reversible compartment and the rest is tightly bound to soil (Valsaraj *et al.* 2000 and Lee *et al.* 2001a). It appears from the figure, that bacteria were able to degrade a significant fraction of the readily available fraction of 1,3-DCB.

The rate of biodegradation of DCB was much slower in the soil phase than that than in the absence of soil (aqueous phase). For example, nearly 80% of 1,3-DCB was biodegraded within 3 days of incubation and nearly complete in 7 days for the aqueous phase (soil free) DCB compared to about 55% degradation from freshly contaminated soil. This is in agreement with the literature (Zhang and Bouwer, 1997 and Robinson *et al.* 1990). It is also reported that microorganisms utilize the substrate (contaminant) in the aqueous phase rather than metabolize the contaminant directly sorbed to the soil. Hence mass transfer of 1,3-DCB from soil surface to the aqueous phase could play a significant role and potentially limit biodegradation (Robinson *et al.* 1990).

Biodegradation of 1,3-DCB from Soils Containing the Desorption-Resistant Fraction

A known amount of the soil containing the desorption-resistant fraction of DCB was added to the microcosms prepared as above and 1,3-DCB biodegradation was monitored. Figure 3.4 shows the percent biodegradation as a function of incubation time for the soil containing the tightly bound 1,3-DCB along with that for freshly contaminated soil. As seen from the figure, for the soil containing the non-labile phase

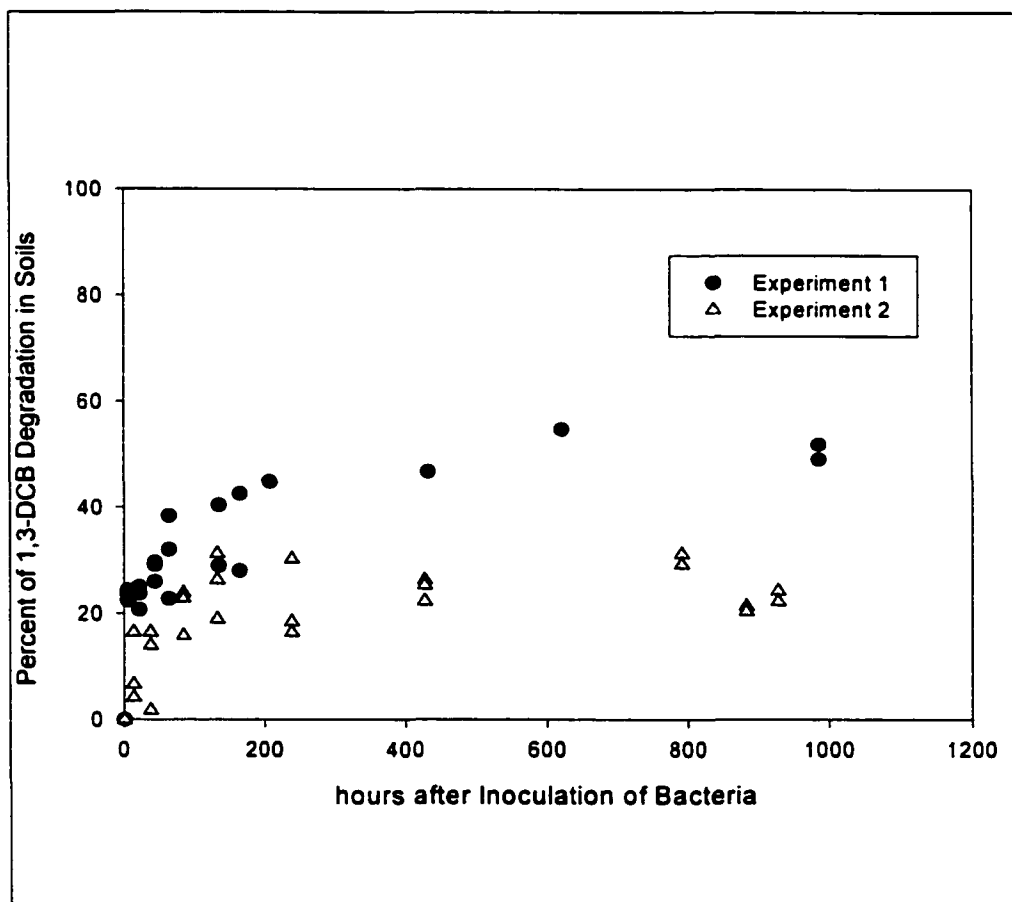


Figure 3.4. Biodegradation of 1,3-DCB from the soil.

DCB the degradation was significantly lower. For example, the degraded portion was ~22% during the first week with only an additional 10% thereafter. It should be noted that samples were sacrificed at each sampling period and slight variations in data are not

uncommon. The soil that was subjected to 5 sequential desorption steps might still contain some DCB fraction which was readily used by the microbes during the first week and beyond that 1,3-DCB was not available for biodegradation since desorption was limited by the tightly bound fraction.

The figure also provides a comparison with the percent degraded from freshly contaminated soils. As evident from the figure, the degradation in soil containing irreversibly bound contaminant was 30% compared to about 55% for freshly contaminated soil. A number of investigators have shown that the desorption resistant fraction in the soil is unavailable for microorganisms even under optimal conditions for biodegradation (Zhang and Bouwer, 1997, Robinson *et al.*, 1990, Al-Bashir *et al.*, 1994, Pignatello, 1989). The irreversibly bound contaminant could be present in the soil micropores or chemically bound to soil humic matter and thus microorganisms may not be able to access this fraction. This is supported by data from Robinson *et al.* (1990) who observed that acclimated bacteria readily degraded the extractable fraction of sorbed toluene, which accounted for about 90% of the total toluene. However, small unextractable portion biodegraded at a rate limited by desorption. Ogram *et al.* (1985) reported that acclimated bacteria could biodegrade only the aqueous phase contaminant and could not utilize the sorbed phase contaminant. Our microcosm batch studies showed that microorganisms readily degraded the easily extracted fraction of the sorbed DCB and the desorption mass transfer is limiting the biodegradation.

Conclusions

The bioavailability of 1,3-DCB from freshly contaminated soils and soils containing only irreversibly bound (non-labile phase) fraction was studied along with

that in aqueous phase. About 99% of 1,3-DCB was degraded in the aqueous phase within 7 days. About 55% of the total 1,3-DCB was degraded within the 6-week incubation period for freshly contaminated soils compared to about 30% for soils containing tightly bound 1,3-DCB. Biodegradation in the aqueous phase was significantly faster than that for the soil phase. From our earlier desorption studies, we reported that about 20-30% of the sorbed contaminant is in the irreversibly bound compartment and is not easily extractable. This fraction could be even larger for aged or weathered soils. It appears from our microcosm batch studies, that microorganisms can readily degrade the easily extracted fraction of the sorbed DCB but the desorption-resistant fraction is not readily bioavailable to the microorganisms and thus the biodegradation is limited. This study raises an intriguing question. If the desorption resistant fraction is not available for biodegradation and is not extracted with water as in a pump and treat (P&T) technology, is it necessarily harmful to the environment and should we revise cleanup goals for soils?

CHAPTER 4

EFFECT OF SORPTION AND DESORPTION-RESISTANCE ON TRICHLOROETHYLENE BIODEGRADATION IN SOILS

Introduction

Trichloroethylene (TCE), a suspected carcinogen and U.S. Environmental Protection Agency priority pollutant, is a common groundwater and soil contaminant in the United States. Various types of aerobic bacteria including methanotrophs (Moran and Hickey, 1997; Chu and Alvarez-Cohen, 1998; Fox, *et al.*, 1990), toluene-oxidizers (Heald *et al.*, 1994; Cox *et al.*, 1998), phenol-oxidizers (Lee and Chang, 1998) and ammonia oxidizers (Moran and Hickey, 1997) have been shown to cometabolically degrade TCE. Growth of these bacteria on substrates other than chlorinated aliphatic compounds (CACs) involve non-specific oxygenase enzymes that are able to fortuitously degrade the CACs. For toluene degrading microorganisms, for example, TCE can be degraded by means of both toluene dioxygenase (Tod) (Heald *et al.*, 1994; Cox *et al.*, 1998; Wackett and Gibson, 1988; Li and Wackett, 1992) and monooxygenase (Shields *et al.*, 1991) enzyme systems. The relationship between cometabolic TCE transformation and toluene consumption has been described qualitatively by many researchers over the past decade (Fliermans *et al.*, 1988; Fan and Scow, 1993; Leahy, *et al.*, 1996; Cox *et al.*, 1998; Mars *et al.*, 1998; Bielefeldt *et al.*, 1995; El-Farhan *et al.*, 2000).

Most previous studies have been performed in the absence of soil, and little is known about how sorption of TCE to soil affects its degradation rate by cometabolic reaction pathways. Although degradation of organic pollutants in soil and water is often the direct result of microbial activity, sorption of the organic compounds to soil or sediments may reduce availability of the organic molecules to microorganisms and

thereby slow biodegradation. This phenomenon has been observed for a variety of organic contaminants in soil. Zhang and Bouwer (1997) studied the bioavailability of benzene, toluene, and naphthalene in a soil-water slurry, and noted that the rate of biodegradation decreased with increasing organic compound hydrophobicity, soil/water ratio, soil particle size, and soil organic carbon content suggesting that the contaminant bioavailability was affected by soil characteristics and the rate of contaminant desorption.

Contaminant bioavailability depends on physicochemical processes such as adsorption/desorption, diffusion, and dissolution (Ogram *et al.*, 1985; Luthy *et al.*, 1994). A substantial body of evidence indicates that a fraction of the contaminants are inaccessible for biodegradation in soils and sediments contaminated for prolonged periods of time. This “aging” as it is usually referred to, may result from a number of processes including: (i) chemical oxidation reactions incorporating contaminants into soil organic matter (ii) slow diffusion into very small pores and absorption into organic matter or (iii) the formation of semi-rigid films around non-aqueous phase liquids with a high resistance toward organic-to-water mass transfer (Bosma *et al.*, 1997).

Several researchers have confirmed that biodegradation can be limited by the slow rate of desorption of organic compounds (Steinberg *et al.*, 1987, Pignatello, 1989, Robinson *et al.*, 1990). In earlier papers, we reported that desorption kinetics for TCE showed a biphasic desorption pattern with one fraction that is readily desorbed and a second fraction that was resistant to desorption (Lee *et al.*, 2001a). The rate and extent of desorption were dependent of soil and sorbate properties such as soil organic carbon content, cation-exchange capacity, specific surface area, and water solubility, as was

also reported by other researchers (Pavlostathis and Mathavan, 1992, Kan *et al.*, 1997, 1998, Valsaraj *et al.*, 1999). Robinson *et al.*, (1990) observed that although most of the toluene in soil-water slurries was biodegraded rapidly, a small fraction was biodegraded much more slowly and at a rate limited by desorption. Steinberg *et al.*, (1987) reported that ethylenedibromide (1,2-Dibromoethane, EDB), a soil fumigant with relatively high water solubility, volatility and biodegradability persisted in top soils for as long as 19 years after its last application. However, laboratory experiments with freshly added EDB showed rapid biodegradation (Scribner *et al.*, 1992). It has been suggested that residues of EDB in the top few centimeters were unavailable for microbes because it was sorbed in to soil micropores and unavailable for biodegradation. Hatzinger and Alexander (1995) reported that when phenanthrene or 4-nitrophenol was aged in loam or muck for long periods (up to 315 days), the biodegradation rate and extent decreased with the amount of time aged. The contaminants become irreversibly bound to soil components to an increasing extent with increasing sorption time (Alexander, 1995; Pignatello and Xing, 1996).

For the soil phase, little information on the role of toluene-degrading bacteria and degree of bioavailability of TCE in contaminated soil is available, to the best of our knowledge. Although numerous studies on TCE cometabolism by toluene-degrading microorganisms have appeared in the literature, few studies quantifying enzyme activity in the TCE degradation processes of aqueous or soil phases have been conducted to date. In terms of engineering applications, effective use of co-metabolic processes by maximizing enzyme production may be an important issue for remediation of TCE contaminated groundwater and soil.

Studies described herein were conducted to determine the effect of toluene in TCE degradation in both aqueous and soil-slurry phases. For the aqueous phase, TCE degradation was investigated in the presence and absence of toluene with different concentrations of toluene as a primary substrate. For soil-slurry experiments, a freshly contaminated soil and a soil containing soil that has artificially “aged” containing only the desorption-resistant fraction of TCE (i.e., soil that was repeatedly extracted to remove the readily desorbing fraction) were used to study bioavailability of the contaminant. Research described herein is part of ongoing research conducted in support of remediation activities at a Louisiana Superfund site known as the Petro Processors Inc., (PPI) site located in north Baton Rouge, LA. Results of the studies described herein could have implications in addressing the question of an acceptable end point for meeting the clean-up goals for TCE contamination at the site.

Materials and Methods

Chemicals

TCE (99.5 % purity, spectrophotometric grade) purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as supplied. TCE solutions were prepared by dissolving aliquots of TCE (neat) in deionized water to obtain the desired contaminant concentration. Toluene (99.8 % purity, nano grade, Fisher Scientific) was used as a primary growth substrate. 2-propanol (99.8 % purity, nano grade, Fisher Scientific) and methanol (99.9 % purity, Fisher Scientific) were used for extraction of TCE from soil.

Soils

Soil was collected from an uncontaminated region of the Brooklawn site (one of the two Superfund sites collectively known as the PPI site) located in north Baton

Rouge, LA. Large lumps of soil were broken apart and the material was oven dried at 55 °C for 24 hours. The soil was then pulverized and homogenized by passage through a sieve with 150 µm openings (US standard sieve No.100). Samples from this soil were methanol-extracted, analyzed, and found to be free of TCE and toluene.

Soil classification was performed according to standard methods in the LSU Soil Science laboratory. The soil is classified as silty soil, with approximately 82% (by mass) silt, 10% clay, 8% sand, and 1.35 % organic carbon. All soil samples were autoclaved before use in subsequent experiments.

Biodegradation experiments were conducted in three different sets using (i) aqueous phase TCE solution, (ii) soil freshly contaminated with TCE, and (iii) soil containing desorption resistant fraction of TCE contamination. Preparation of the aqueous TCE solution and two types of TCE contaminated soil are described below. For aqueous phase experiments, an aqueous solution spiked with TCE (~6 mg/L) was prepared and added to the vials for degradation experiments.

For experiments using freshly contaminated soil, five grams of dry, uncontaminated soil was added to 40-mL capacity vials. An aqueous solution spiked with TCE (~6 mg/L) was added to the vials, mixed vigorously, filled to capacity without headspace and capped with Teflon-lined silicone rubber septa. The vials were equilibrated in a tumbler for 3 days at 80 rpm and were then centrifuged to obtain a clear soil-water interface. The soil obtained was used directly after determining the concentration of TCE. For experiments with soil containing only desorption resistant fraction of TCE, soil was further prepared by following the iso-propanol co-solvent extraction method developed by Liu *et al.* (1999). In this process, equal volumes of

isopropanol and electrolyte solution (0.01 M NaCl, 0.01 M CaCl₂) were used. Contaminated soil was mixed vigorously with co-solvent in a tumbler for 24 hours, and the co-solvent was separated from the soil via centrifugation. The separated soil was rinsed with electrolyte solution two times to remove the residual iso-propanol and then mixed with co-solvent again, and placed in a tumbler for another 24 hours. An additional rinse with electrolyte solution was conducted, and TCE concentration in the aqueous phase was measured. The soil containing only the desorption resistant fraction was obtained after rinsing with electrolyte solution twice again.

The TCE concentration in the soil was measured by a hot extraction method (Sawney *et al.*, 1987; Pignatello, 1990) to determine the mass of TCE bound in soil. In this process, 10 mL of methanol was added to the soil remaining in the vials. The vials were shaken vigorously for approximately one minute before placement in a water bath maintained at a constant temperature of 75°C for 20 hours. The supernatant, after centrifuging, was analyzed for the test contaminant.

Glassware

Degradation experiments were conducted using 40-mL capacity EPA certified clean vials (VWR Scientific, Sugar Land, TX) that were sealed with Teflon-lined silicone rubber septa. These vials could be centrifuged at low speeds and thus eliminate the potential volatilization losses during the transfer of content into a centrifuge tube. An initial study was conducted to quantify abiotic losses following puncturing of the septa. No significant abiotic losses were found in vials with septa punctured two times by the needle of a 10 µl-size gas tight syringe (Hamilton, Baton Rouge, LA). However, as a precautionary measure, Teflon tape attached with laboratory tape was placed over

punctured septa in degradation experiments to prevent possible losses of TCE through the puncture.

TCE and Toluene Analysis

Aqueous-phase TCE and methanol extracts containing TCE were analyzed using a purge-and-trap attached to a HP 1530A gas chromatograph equipped with a capillary column and mass selective detector (MSD, Model HP 6890) and an auto-sampler (Tekmar, Model 2016). The injection port was operated in EPC splitless inlet mode, and flow was purged to split vent 50 mL/min for 2 min with N₂ gas. Pressure was set at 17 psi and the flow rate of N₂ carrier gas was 54 mL/min. A capillary column (30.0 m x 250 µm x 0.25 µm, Model HP 19091S-433) filled with 5 % Phenyl Methyl Siloxane was used with the pressure 17.3 psi and flow rate 2.0 mL/min. The oven temperature was set at 55 °C for 1 minute, 20 °C for 3.33 minutes in ramp 1, 80 °C for 9.33 minutes in ramp 2, and 220 °C for 16.33 minutes in ramp 3.

Toluene concentrations were measured using a HP 5890A gas chromatograph (GC) equipped with a HP 5971 mass spectrometer (MS) detector. Samples were introduced into the GC by an auto sampler and purge-and-trap method. The purge-and-trap of aqueous samples was performed at ambient temperature using helium as a carrier gas. The GC employed a 30 m capillary column, with a 0.25 ~ 0.32 mm internal diameter and 1.0 µm film thickness. The injector temperature was between 200 and 225 °C, the transfer line temperature between 250 and 300 °C, and oven temperature was isothermal at 80 °C. The total cycle time per sample was 32 minutes.

Culture of Toluene-degrading Microorganisms

A schematic diagram of the sparged-gas reactor used to grow toluene-degrading microorganisms is provided in Figure 4.1. Compressed air from the laboratory flowed through an activated carbon filter and then a pressure regulator before being passed through a glass tube equipped with a septum-filled injection port. A KD Scientific model 1000 syringe pump (Boston, MA) delivered toluene from a gas-tight syringe (Hamilton Co., Reno, NV) through a needle that pierced the septum into the injection port and into the air stream at a rate of 0.2 mL toluene per hour. The toluene-contaminated air then passed through an aeration stone submerged in a 4.0 L glass kettle reactor (Pyrex, Acton, MA) with a working liquid volume of 3.0 L. A flow meter (Manostat, New York, NY) measured and regulated the air flow rate at 200 mL per minute.

The reactor was filled with 3.0 L of nutrient solution containing the following constituents added (per liter) to distilled water: 5.3 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 1.4 g KH_2PO_4 , 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g $(\text{NH}_4)_2\text{SO}_4$, and 5 mL of a trace element solution as described by Zeikus (1979). The trace elemental solution contained the following constituents added to 1.0 L of distilled water: 0.2 g $\text{FeCl}_3 \cdot 4\text{H}_2\text{O}$, 0.1 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.17 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 g ZnCl_2 , 0.02 g CuCl_2 , 0.01 g H_3BO_3 , 0.01 g $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 1 g NaCl , and 0.02 g Na_2SeO_3 . The reactor was maintained at ambient room temperature (approximately 23°C). A preliminary experiment conducted before inoculation of microbes indicated that the toluene concentration in the reactor was 45 mg/L in the absence of microbial populations.

The reactor was inoculated with 50 mL of activated sludge with a total suspended solids (TSS) concentration of 350 mg/L obtained from an ongoing

experiment which utilized a culture originally obtained from a domestic wastewater treatment facility in Baton Rouge, LA. The reactor was maintained without nutrient addition or sludge wasting for a period of 15 days. Starting on day 16 of reactor operation, 300 mL of MLSS was removed from the reactor on a daily basis and 300 mL of nutrient solution was added to produce a mean cell residence of 10 days. The toluene concentration in the reactor was found to be approximately 15 mg/L in the presence of toluene-degrading bacteria on day 30 of operation.

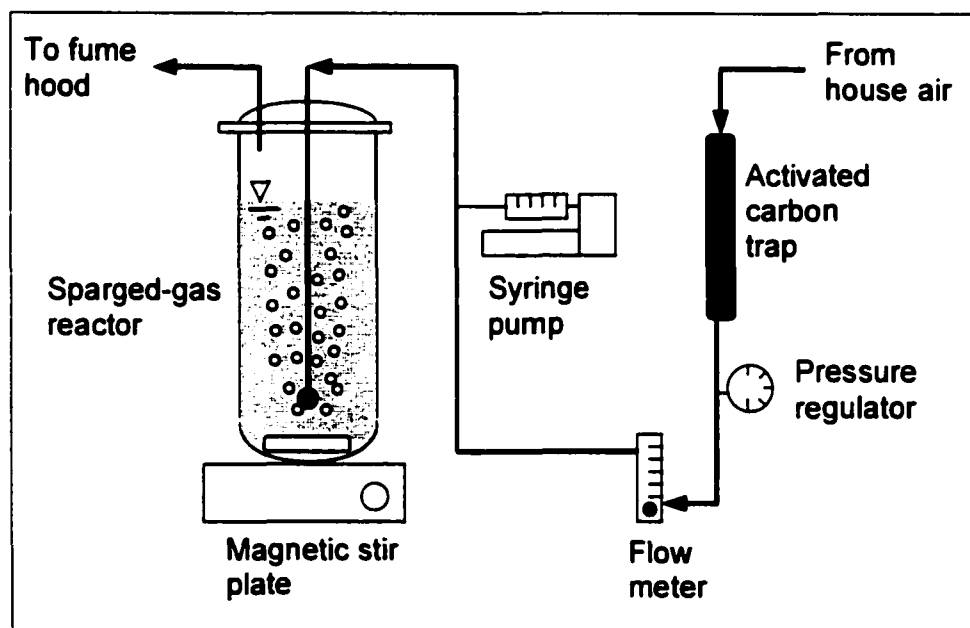


Figure 4.1. Gas-sparged bioreactor for cultivating toluene-degrading bacteria.

On the days when microbes were taken from the reactor for use in experiments described in the subsequent sections, a sample was withdrawn from the reactor and measurements were made to determine total suspended solids (TSS), volatile suspended solids (VSS), oxygen uptake rate (OUR), and protein content. TSS and VSS were measured following standard methods (Eaton *et al.*, 1995). Oxygen uptake rate was measured using a YSI Model 5300 Biological Oxygen Monitor.

Protein Content

Biomass samples were collected for protein analysis by placing 10 mL of microbial suspension in a 15 mL sterile centrifuge tube (Nalge Co.). The tube was centrifuged at 8000 rpm for 20 minutes, and then the supernatant was decanted. Next, protein was extracted from the biomass by adding 10 mL of 0.5 N NaOH and heating to 90 °C for 10 minutes following a method similar to that of Herbert *et al.* (1971) and Daniels *et al.* (1994). A 1.0 mL sample was removed from the centrifuge tube and placed in a 1.5 mL tube that was centrifuged at 10,000 rpm for 10 minutes to remove particulates. Protein concentration was measured using a modified Lowry method using pre-prepared reagents with Bovine serum albumin (BSA) used as a standard (Bio-Rad Laboratory, Inc.) following the manufacturer's instructions. Absorbance was measured at 750 nm in a Shimadzu Model UV-1201 Spectrophotometer (Shimadzu Co., Columbia, MD).

Toluene Dioxygenase (Tod) Enzyme Assay

For experiments conducted in the absence of soil, cells were collected by centrifugation and washed once in potassium phosphate buffer, (8.72 g/L of KH_2PO_4 , pH 7.2). Cell pellets were resuspended in extraction fluid containing potassium phosphate buffer (100 mL) containing 10% glycol, 10% ethanol, 0.355 g of NADH, and 0.154 g of dithiothreitol (DTT). Cells were disrupted by sonification (Branson Sonifier Model 450) and centrifuged for 10 minutes in an Eppendorf centrifuge (10,000 x g, 4 °C) to remove cellular debris.

The clear supernatant solution was used as a source of crude cell extract in the enzyme assay. Toluene dioxygenase (Tod) enzyme activity was determined by

adapting the indole oxidation assay developed by Jenkins *et al.* (1985). All enzyme assays were carried out at room temperature (~24 °C). Reaction mixtures containing (1 L) 4.36 g of potassium phosphate buffer, pH 7.2, 0.0278 g of FeSO₄·7H₂O, 0.234 g of NADH were prepared. Toluene dioxygenase was assayed spectrophotometrically. The reaction was initiated by addition of 5 µl of indole solution with a concentration of 11.7 g/L. Oxidation of indole to cis-indole dihydrodiol and in turn to indigo was monitored as a function of time by measuring the increase in absorbance at 400 nm against a blank containing all compounds except indole. The rate of indole oxidation was related to the Tod enzyme concentration. Results of Tod enzyme activity measurements made at various time intervals are reported relative to the concentration at time zero by dividing the enzyme activity by the initial enzyme activity value.

Biodegradation Studies

Biodegradation experiments were conducted in three different sets using (i) aqueous phase, (ii) freshly contaminated soil, and (iii) soil containing desorption resistant fraction of the contaminant. Table 4.1 summarizes preparation of microcosm studies in aqueous and soil phases. As shown in the table, eleven different treatment conditions, arbitrarily named A through K, were used. Aqueous phase experiments were composed of 2 categories: those that received low (4.5 mg/L) and high (27 mg/L) concentration toluene spike. Soil phase experiments utilizing freshly contaminated and desorption resistant fractions of TCE received the same treatment as aqueous phase experiments with two exceptions. First, the TCE solution used to prepare contaminated soil initially contained approximately 10 mg/L TCE while the aqueous phase experiments used approximately 6 mg/L. Second, the soil-slurry microcosms received

either a toluene spike of 4.5 mg/L or no toluene addition. H₂O₂ (30 %) was added as an oxygen supply. Treatments A and B, were spiked with toluene concentrations of 4.5 mg/L while Treatments D and E received 27 mg/L toluene.

Table 4.1. Summary of microcosm preparation for biodegradation studies in aqueous and soil-slurry phases.

	Aqueous Phase					Soil Phase					
	Toluene spike with low concentration			Toluene spike with high concentration		Freshly contaminant Fraction			Desorption resistance fraction		
	A	B	C	D	E	F	G	H	I	J	K
Volume of TCE solution (6 mg/L) added (mL)	10	10	10	10	10	41 ¹	41 ¹	41 ¹	41 ¹	41 ¹	41 ¹
Volume of cell suspension added (mL)	10	10	10	10	10	10	10	10	10	10	10
Volume of nutrient solution added (mL)	10	10	10	10	10	10	10	10	10	10	10
Volume of H ₂ O ₂ added (mL)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Toluene concentrations spiked (mg/L)	0	4.5 ²	4.5 ³	27 ²	27 ³	0	4.5 ²	4.5 ³	0	4.5 ²	4.5 ³
Volume of NaN ₃ solution (1g/L) added (mL)	2	-	-	-	-	2	-	-	2	-	-
Amount of dry soil added (g)	-	-	-	-	-	5.0	5.0	5.0	5.0	5.0	5.0

¹, The volume of TCE solution (10 mg/L) added into each vial containing soil was the one when the sorption experiment was initiated.

², Toluene was spiked only one time.

³, Toluene was spiked two times.

Treatments A and D were spiked with toluene only once (at time zero). Treatments B and E were spiked with toluene at time zero as well as 3.8 hours into the experiment. Meanwhile, 4.5 mg/L of toluene was spiked in Treatments F, I and then, a second toluene spike was added 120 hours after the experiment began. Treatments C, H, and K were prepared as killed-cell controls with addition of sodium azide as a biocide.

For the microcosm studies, 300 mL of toluene-degrading bacteria were removed from the reactor and placed in a beaker. The microbial suspension had a TSS of 315 mg/L, a VSS of 260 mg/L, an OUR of 16.6 mg O₂/L·hr, and a specific oxygen uptake rate (SOUR) of 63.8 mg O₂/g VSS·hr. The protein concentration was 49 mg/L, which corresponds to a biomass content of 19% protein based on VSS. After the microbial suspension was aerated for 30 minutes to volatilize residual toluene, 10.0 mL of the microbial suspension (2.6 mg of biomass measured as VSS) was added to each vial and vials were capped. In treatments A and D, toluene was injected a second time through the septa using a microsyringe. In such cases, the septa were immediately covered with Teflon lined tape as described previously. The vials were put on tumbler immediately and were maintained there until vials were sacrificed at designated time intervals. TCE biodegradation in soil phase was calculated after according for TCE losses measured in control microcosms.

Results and Discussion

Biodegradation of TCE in Aqueous Phase Experiments

Figure 4.2 depicts TCE and toluene concentrations as a function of time in aqueous-phase degradation experiments. As clearly shown in the figure, the TCE

concentration was essentially constant in the Treatment A microcosms where sodium azide was added as an inhibitor. This provides an indication that the observed decrease in TCE in other microcosms was due to microbial activity rather than abiotic processes.

For microcosms receiving Treatment B (toluene added only at time zero), toluene degradation proceeded rapidly with the concentration decreasing from an initial concentration of approximately 4.5 mg/L at time zero to a concentration of near 0 mg/L after 1 hour. TCE decreased from an initial concentration of approximately 1 mg/L at time zero to approximately 0.56 mg/L at 2 hours. From time 2 hours to 7 hours (when the experiment was terminated), the TCE concentration decreased steadily, though at a rate somewhat less than during the first two hours. After a total of 7 hours of incubation, approximately 0.3 mg/L of TCE remained in Treatment B microcosms.

For microcosms receiving Treatment C (same as Treatment B but with a second addition of toluene at time 3.8 hours), the toluene concentration increased from a concentration of approximately zero to a concentration of approximately 4.7 mg/L during the 15-minute time period following the second addition of toluene. During the next hour, the toluene concentration decreased rapidly to near 0 mg/L. Immediately following the second addition of toluene, the TCE concentration decreased at a much faster rate, comparable to that during the first two hours of the study. Unlike Treatment B microcosms where residual TCE was present at the end of the experiment, the TCE concentration in Treatment C microcosms could no longer be detected after approximately 6 hours. The second addition of toluene (present in Treatment B, but not in C) had an obvious positive effect on the rate and extent of TCE degradation. As shown in Figure 4.3, a similar pattern was observed in aqueous-phase microcosms that

received a higher concentration (approximately 27 mg/L vs. 4.5 mg/L) toluene spike. For microcosms receiving Treatment D (toluene added only at time zero), toluene concentration decreased rapidly from an initial level of approximately 27 mg/L at time zero to near 0 mg/L after 2 hours. Likewise, TCE degradation was rapid during the first two hours, decreasing from an initial concentration of approximately 1.07 mg/L to a concentration of approximately 0.2 mg/L after two hours.

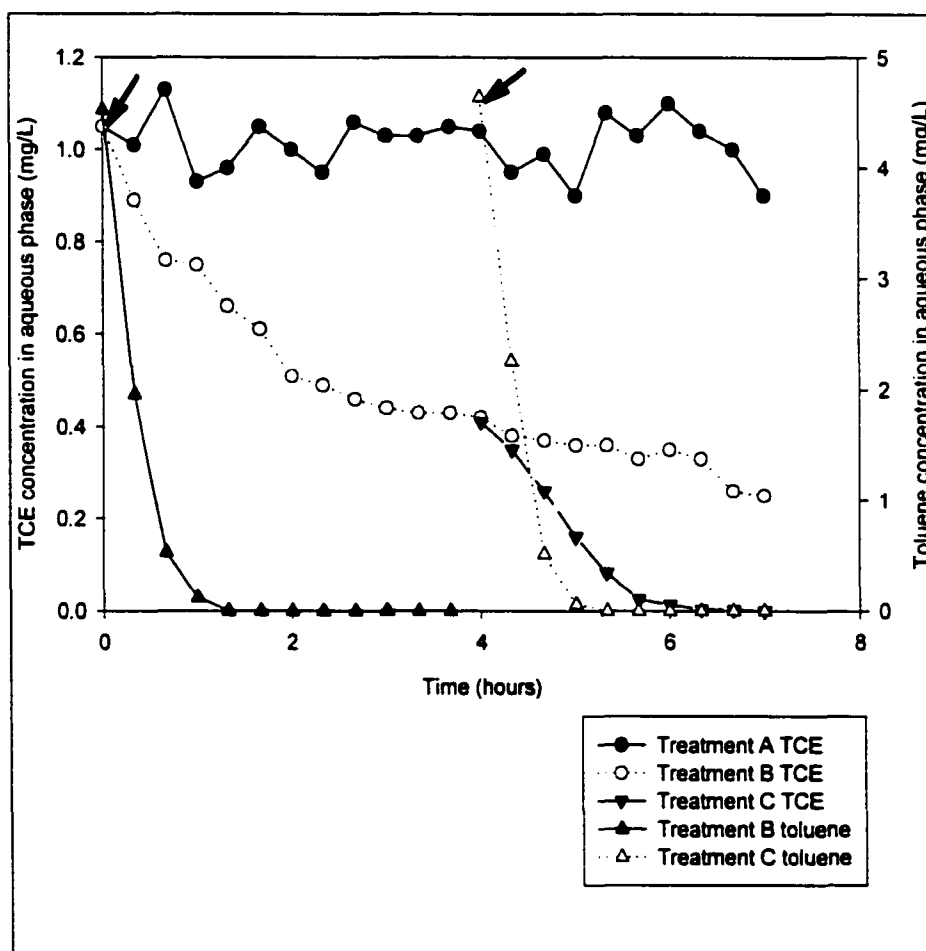


Figure 4.2. TCE and toluene degradation in aqueous-phase microcosms when toluene was added in low (~4.5 mg/L) concentration (Treatment A, B, and C).

Shortly after the toluene concentration approached zero, the rate of TCE degradation became relatively constant but at a rate much less than that observed during the first two hours. Approximately 0.1 mg/L of TCE was still present in Treatment D microcosms

when the experiment was terminated at time equal to 10 hours. This observed result is very similar to that from Treatment B, which was identical to Treatment D in all respects except for the initial toluene concentration.

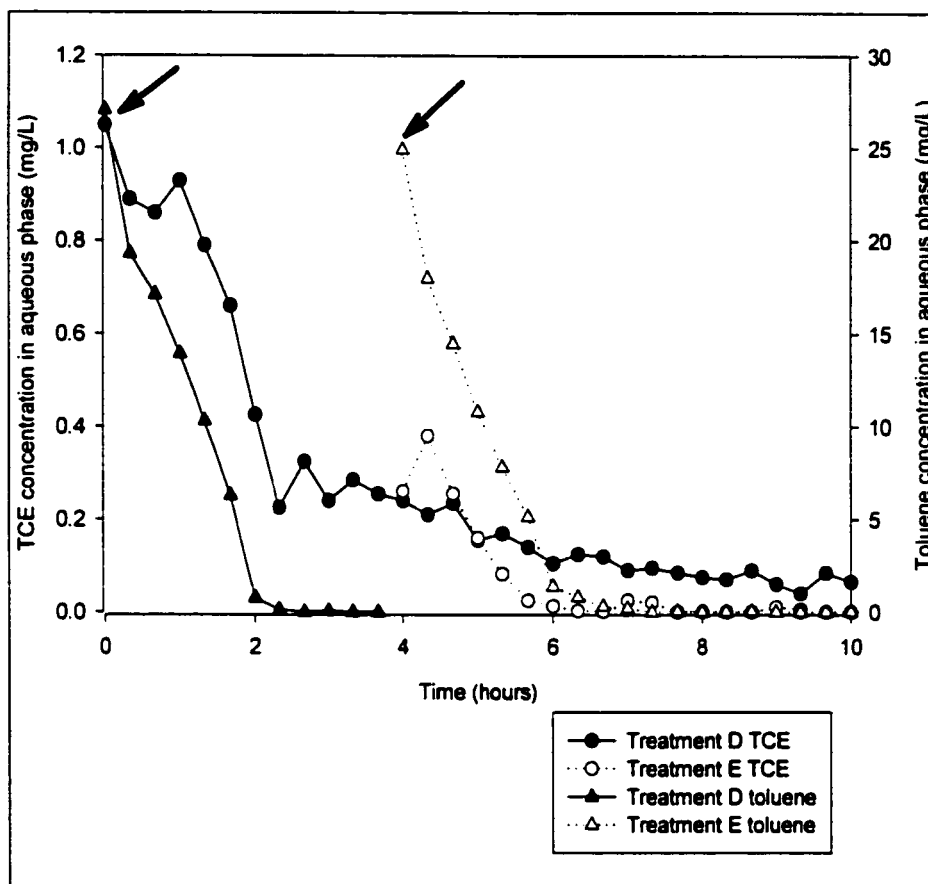


Figure 4.3. TCE and toluene degradation in aqueous-phase microcosms when toluene was added in high (~27 mg/L) concentration (Treatment D and E).

Similar to Treatment C microcosms, Treatment E microcosms received toluene additions at 0 and 3.8 hours. Following the second addition of toluene, the concentration increased rapidly to 27 mg/L within 30 minutes after addition. It took approximately 2 hours for toluene to degrade to a concentration near zero. Similar to the pattern observed for TCE degradation in Treatment C, the TCE degradation rate increased following the second toluene addition, and TCE concentration reached

approximately zero after 6 hours. The TCE biodegradation rates for the first 2 hours of treatment and two hours following the second addition were comparable. As was observed in Treatment C, the second addition of toluene had an obviously positive impact on the rate and extent of TCE degradation.

Biodegradation rates were calculated to quantify the effects of toluene on TCE degradation. Because biomass concentrations were not measured after time zero, degradation rates are reported in terms of concentration of contaminant per unit time rather than in terms of specific contaminant removal rate. The toluene biodegradation rates in Treatment B and C microcosms (initial toluene concentration of 4.7 mg/L) were approximately 5.97 and 6.16 mg toluene·L⁻¹·hr⁻¹, respectively, during the period immediately following toluene addition and lasting approximately 1 hour. Toluene biodegradation rates in Treatment D and E microcosms (initial toluene concentration of 27 mg/L) were approximately 13.2 and 11.8 mg toluene·L⁻¹·hr⁻¹, respectively, during the period immediately following addition of toluene.

The TCE biodegradation rates were approximately 270 and 313 µg TCE·L⁻¹·hr⁻¹ for the first 2 hours following toluene addition in experiments with low (4.5 mg/L) and high (27 mg/L) initial toluene concentration (see Figures 4.2 and 4.3). Shortly after toluene was depleted from the system, the TCE degradation rate slowed considerably to 52 and 35 µg·TCE·L⁻¹·hr⁻¹, respectively. Significant differences in TCE biodegradation rates were observed in the presence of toluene in microcosms when a second toluene spike was added (Treatment C and E). Biodegradation rates in Treatments C and E were 231 and 265 µg·TCE·L⁻¹·hr⁻¹ during the one and a half hours after the second toluene spike. Meanwhile, the TCE biodegradation rates in microcosms not receiving a

second addition of toluene (Treatments B and D) remained at approximately 52 and 35 $\mu\text{g}\cdot\text{TCE}\cdot\text{L}^{-1}\cdot\text{hr}^{-1}$, respectively, during the same time period.

Interestingly, the initial concentration and total mass of toluene supplied had less of an effect on extent of TCE degradation in the microcosms than did the time interval during which toluene was supplied. This is clearly evident when one compares the TCE degradation in Treatment C and D microcosms. Treatment C microcosms received only one sixth of the toluene concentration (4.5 mg/L vs. 27 mg/L) and only one third of the toluene mass (9 mg/L vs. 27 mg/L), but it still degraded more of the TCE. This observation suggests that periodic addition of low levels of toluene can effectively maintain sufficient enzyme levels to yield high rate and extent of cometabolic TCE degradation while minimizing the mass of toluene that must be added. This concept, previously suggested but not experimentally verified by Fan and Scow (1993), is further supported by Tod enzyme measurements described below. The TCE concentration in Treatments B and D decreased to the method detection level after the second addition of toluene; however, if the initial TCE concentration had been higher, it is expected that the rate of TCE degradation would slow again shortly after all of the toluene from the system.

The increase in TCE degradation rate following toluene addition and the subsequent slowing of TCE degradation rate following toluene depletion can be readily explained by changes in the Tod enzyme activity. Tod enzyme activity as a function of time for microorganisms subjected to Treatments B and C is depicted in Figure 4.4. As clearly shown in the figure, the Tod enzyme activity was initially high following addition of toluene at time zero. After toluene was depleted from the system

(approximately one hour -- see Figure 4.2), the enzyme concentration decreased rapidly to a level only a fraction of its original value. This time period, when Tod enzyme level decreased, corresponds to the time period during which TCE degradation rate slowed appreciably. The decreased TCE degradation rate was likely due to decreased levels of Tod enzyme available to catalyze cometabolic reactions.

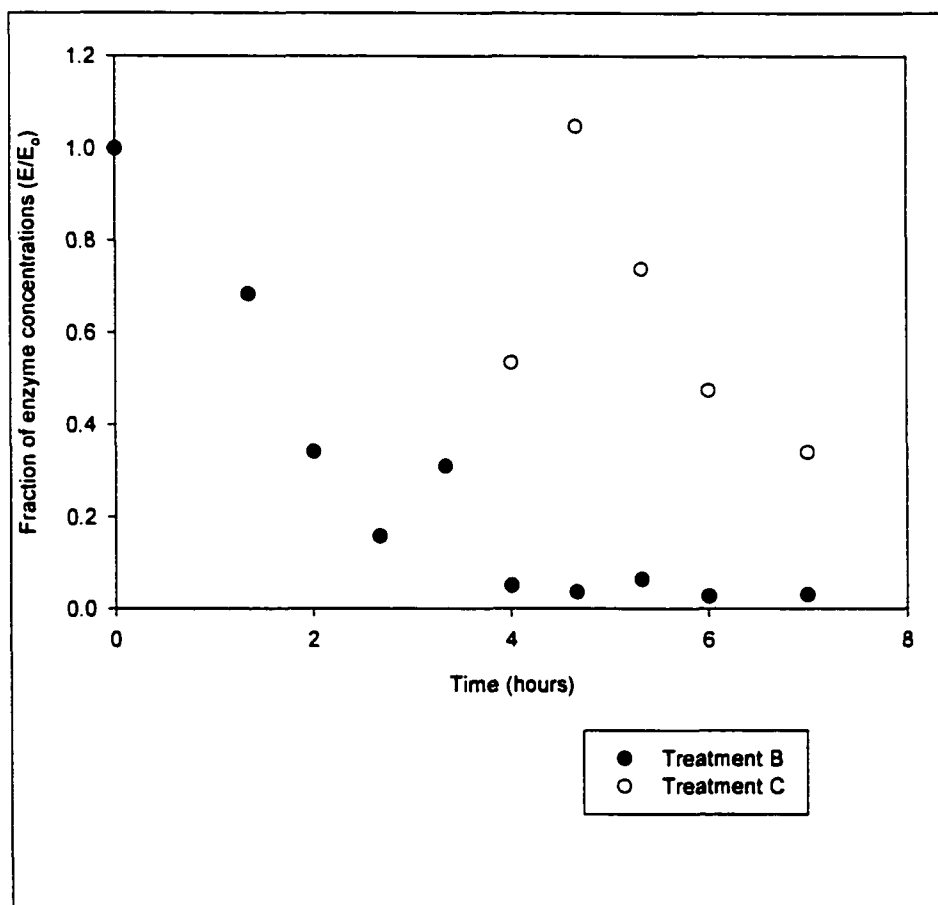


Figure 4.4. Relative toluene dioxygenase enzyme activity over time in microcosms with one and two additions of toluene (Treatment B and C).

Addition of toluene at a time of 3.7 hours in Treatment C microcosms caused a rapid increase in Tod enzyme concentration to a level approximately the same as its initial value. At the same time, a rapid increase in the rate of TCE degradation also occurred (see Figure 4.2). Compared with the treatment that did not receive a second

toluene spike (Treatment B), the treatment with toluene spike (Treatment C) had more than five times higher Tod enzyme concentration 30 minutes after toluene addition, and more than twenty times higher 55 minutes after toluene addition. At the same time, Treatment C microcosms had a much higher TCE biodegradation rate. Enzyme concentration decreased again after all toluene from the second addition was biodegraded. TCE degradation by toluene-degrading bacteria was clearly correlated with the toluene dioxygenase concentrations over time. Although enzymes other than Tod (e.g., toluene monooxygenase) have been reported to cometabolically degrade TCE, and the presence of these enzymes was not measured in the studies described herein, changes in Tod concentrations reasonably account for the observed changes in TCE degradation rates over time. Furthermore, it is expected that enzymes from toluene degradation pathways not containing Tod would follow a similar pattern of increase and decrease in the experimental system tested.

Protein concentration measured as a function of time in Treatment B and C microcosms are depicted in Figure 4.5. Although data were somewhat scattered, a general pattern of increasing protein concentration over time was observed. This was likely caused by biomass growth in the microcosms as toluene was degraded. No appreciable difference was observed between microcosms that received a second addition of toluene (Treatment C) and those that did not (Treatment D). This was not unexpected considering the relatively small amount of biomass increase expected.

Biodegradation of TCE in Freshly Contaminant Soil

Figure 4.6 depicts TCE concentration as a function of time in microcosms that contained soil freshly-contaminated by TCE. As clearly shown in the figure, the TCE

concentration was essentially constant in the Treatment F microcosms where sodium azide was added as a biocide. Similar to the results of Treatment A microcosm studies, this indicates that the observed decrease in TCE concentration in other microcosms was due to microbial activity rather than abiotic processes.

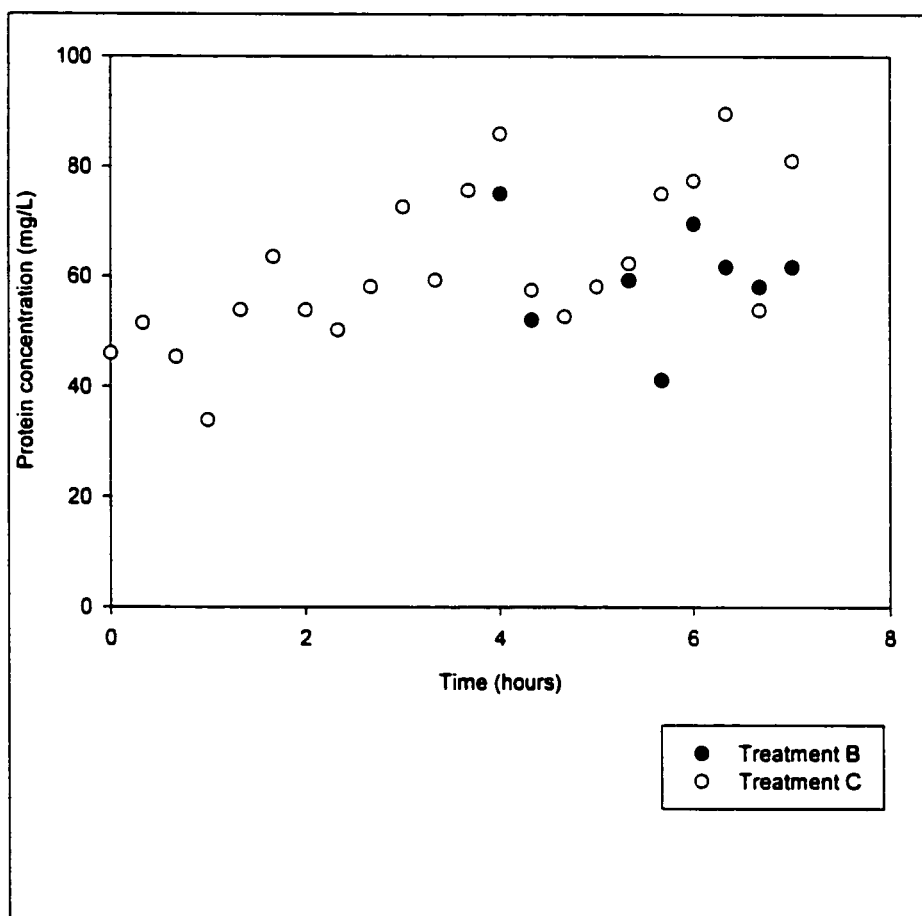


Figure 4.5. Protein concentrations as a function of time in Treatment B and C microcosms.

Treatment G (toluene added only at time 0) and H (toluene added at time 0 and 120 hours) microcosm studies were conducted for approximately 480 hours. In both treatments, the TCE concentration decreased from an initial concentration of approximately 7.0 mg/kg (mass TCE per mass dry solids) to a concentration of approximately 1.2 mg/kg after 39 hours. This corresponds to a TCE degradation rate of

approximately $150 \mu\text{g TCE kg}^{-1}\cdot\text{hr}^{-1}$ during the 39-hour period following addition of toluene at time zero. The TCE degradation rates then slowed considerably in both treatments. The TCE biodegradation rates in Treatment G and H microcosms were approximately 0.59 and $0.84 \mu\text{g TCE kg}^{-1}\cdot\text{hr}^{-1}$, respectively for the period from 39 to 480 hours.

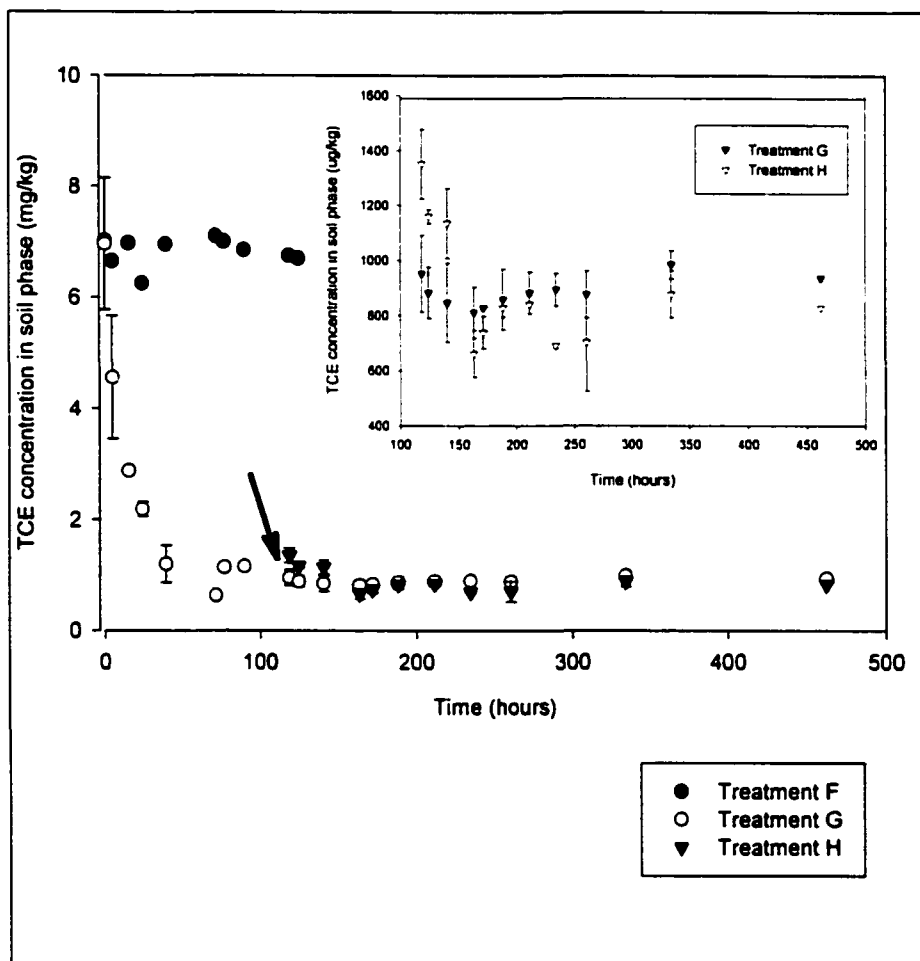


Figure 4.6. Biodegradation of TCE in soil-slurry microcosms containing freshly contaminated soil (Treatment F, G, and H).

The second addition of toluene to microcosms that received Treatment H resulted in a distinct pattern of TCE removal as depicted in the inner plot in Figure 4.6. Compared to Treatment G, the TCE concentration observed in Treatment H soil extracts

increased appreciably during the 30 hours following the second addition of toluene. A likely explanation for this is the role of toluene as a co-solvent through which presence of toluene enhances desorption of TCE from soil particles. This phenomenon is supported by previous findings that aromatic compounds compete with TCE for sorption sites on soil particles (Fan and Scow, 1993).

Following the period during which an increase in TCE concentration was observed in soil extracts, the TCE concentration in Treatment H microcosms decreased to a level less than that in Treatment G microcosms. Although the differences were relatively small, the extent of TCE degradation in Treatment H was greater than in Treatment G. When the experiment was terminated after 480 hours, the TCE concentrations were 0.94 mg/kg and 0.83 mg/kg in microcosms that received Treatments G and H, respectively. This corresponds to overall TCE removal of approximately 86.6 and 88.1%, respectively. In both cases, the rate and extent of TCE degradation was much less than that observed in microcosm experiments conducted in the absence of soil. It is interesting to note that approximately 96% of the TCE removal occurred during the first 39 hours of the treatment, and only about 4% of the removal occurred during the subsequent 441 hours.

Because toluene concentration was not measured in either the aqueous phase of the slurry or from soil extracts, it is not known whether toluene added to the Treatment G and H microcosms was present in solution or if it was sorbed to the soil. Likewise, because Tod enzyme measurements were not conducted on extracts from these microcosms, it is unknown whether toluene induced toluene dioxygenase activity. Thus, from experiments described herein, it cannot be concluded unequivocally whether the

slow degradation rate was caused because toluene was not bioavailable to induce enzymes necessary for cometabolism or if the slow degradation rate was caused because TCE was not bioavailable. In either case, lack of bioavailability limited the TCE degradation rate in the contaminated soil. This is consistent with previous reports that physical occlusions in soil due to entrapment in soil micropores limits bioavailability of organic compounds (Pignatello, 1989). This process can be caused by the rate-limited diffusion in intraparticle water or diffusion through the solid-phase organic matter in soil particles (Wu and Gschwend, 1986). Limited access of microorganisms to matrix bound organic compounds thereby limits the biodegradation rate. This is further discussed in the following section.

Error bars depicted in Figures 4.6 and 4.8 represent one standard deviation from the triplicate analyses. Error may have been caused by analytical error associated with the methanol extraction process, GC analysis, or the soil content, which was not completely homogenized.

Biodegradation of TCE in Soil Containing the Desorption-Resistant Fraction

For biodegradation studies with soil containing only the desorption resistant fraction of TCE, soil was prepared by subjecting TCE contaminated soil, prepared in the same manner as that used for experiments described in the previous section, to a co-solvent extraction process. TCE concentration in the soil was measured following each extraction step, and results are depicted in Figure 4.7. As shown in the figure, the TCE concentration decreased from its initial level of approximately 4.9 mg/kg in the freshly contaminated soil to a concentration of 1.1 mg/kg after one extraction cycle. After the extraction process was complete (total of 6 desorption cycles), 500 µg/kg remained in

the soil. The method described by Liu *et al.* (1999) has been demonstrated and exhibited the same partitioning behavior as compounds subjected to multiple sequential desorption steps in the laboratory. The observed partitioning is the same as described in papers by Kan *et al.* (1997, 1998).

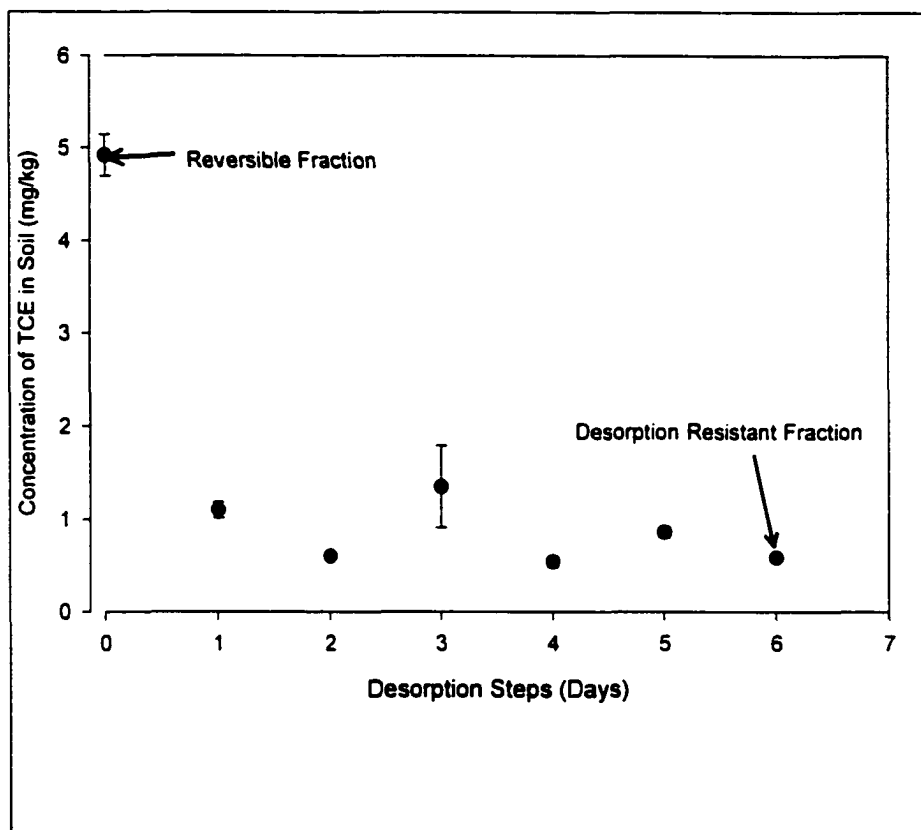


Figure 4.7. Desorption of TCE in soil during the co-solvent (iso-propanol) extraction process.

Figure 4.8 depicts TCE concentrations as a function of time in microcosms that contained soil with only the desorption resistant fraction of TCE. As shown in the figure, the TCE concentration was essentially constant in the Treatment I microcosms where sodium azide was added as a biocide. Similar to the results of Treatments A and F, results indicate that the observed decrease in TCE concentration in other microcosms was due to microbial activity rather than abiotic processes. Biodegradation of TCE

observed in microcosms receiving Treatments J (toluene added only at time 0) and K (toluene added at time 0 and 120 hours) is shown in Figure 4.8.

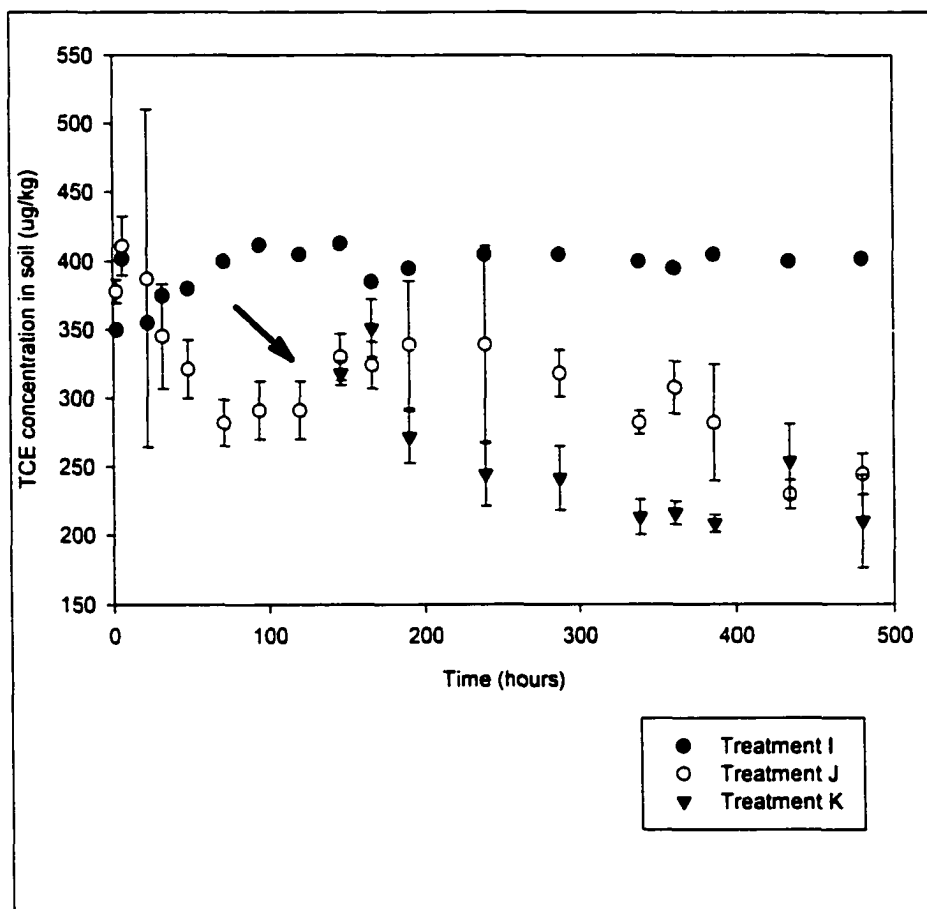


Figure 4.8. Biodegradation of TCE in soil-slurry microcosms containing only the desorption-resistant fraction of TCE.

TCE concentration decreased from an initial level of 500 µg/kg at time zero to near 280 µg/kg after 75 hours. Similar to the pattern observed in Treatment H microcosms, the TCE concentration in soil extracts in Treatment K microcosms increased during the period following addition of toluene compared to the microcosms that did not receive a second toluene addition (Treatment J). Subsequently, the TCE concentration decreased from approximately 350 µg/kg to 210 µg/kg within 200 hours after the second toluene addition. By comparison, nearly twice as much TCE remained in the Treatment J

microcosms. As was shown in microcosms receiving Treatment H, a co-solvent effect was observed. When the experiment was terminated after a total of 480 hours, approximately 260 $\mu\text{g/kg}$ and 220 $\mu\text{g/kg}$ TCE remained in the soil receiving Treatments J and K, respectively. This corresponds to removal of 48 and 56 %, respectively. As with the freshly contaminated soil, the second addition of toluene produced a small but noticeable increase in the overall extent of TCE degradation.

Because data were somewhat scattered, the average TCE degradation rate over the entire experimental duration (480 hours) was calculated for comparison purposes rather than TCE degradation rates for sub-intervals of the treatment process. The TCE degradation rates in soil-slurry microcosms containing the desorption-resistant fraction of TCE contaminated soil were approximately 0.27 and 0.32 $\mu\text{g TCE} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$, respectively in microcosms receiving one and two additions of toluene (Treatment J and K, respectively).

The rate and extent of TCE degradation was much lower in microcosms that contained TCE contaminated soil compared to microcosms that contained only aqueous-phase TCE. Additionally, the rate of TCE degradation was lower in microcosms that contained only the desorption-resistant fraction of TCE compared to microcosms that contained freshly-contaminated soil. Similar reports of limited biodegradation in the soil phase caused by the limited rate of organic compound desorption from soil into the aqueous phase have been reported previously (Zhang and Bouwer, 1997). Thus, TCE mass transfer from soil to the aqueous phase could play a significant role in limiting biodegradation (Robinson *et al.*, 1990). VOCs present in soils are subject to sorption, volatilization, and solubilization and are distributed among

the soil solution, atmosphere, and solid phase. Microbial populations in soil are concentrated in the water films that fill pores and coat soil particles, and only the aqueous phase of a chemical is considered to be directly available for uptake by microorganisms.

The lower rate and extent of biodegradation in soil-slurry microcosms (compared to aqueous-phase microcosms) can be attributed to bioavailability limitations caused by desorption rate limitations of TCE, toluene, or both. The higher TCE degradation rates observed in microcosms containing freshly-contaminated soils compared to those containing only the desorption-resistant fraction of TCE suggest that bioavailability of TCE rather than toluene caused the decreased degradation rate. On the other hand, the increased degradation rates observed with multiple additions of toluene suggest that limited bioavailability of toluene needed to induce enzymes necessary for cometabolism may be a factor. Interpretation of these results is somewhat complicated by the role that toluene may play as a co-solvent, thereby increasing solubilization and/or extractability of TCE. From a practical perspective, further research is needed to ascertain whether addition of toluene to the soil resulted in residual non-bioavailable toluene contamination in the soil.

In terms of engineering applications, to obtain high TCE removal efficiencies, appropriate strategies are needed for maximizing Tod enzyme production. According to the results in this study, multiple additions of toluene could stimulate Tod enzyme production and subsequently TCE biodegradation. Consequently, addition of toluene may be a useful tool for enhancing TCE removal and degradation at contaminated sites.

Conclusions

Biodegradation of trichloroethylene (TCE) by toluene-degrading bacteria was measured under aerobic conditions in aqueous and soil-slurry batch microcosms. For soil-phase experiments, a freshly-contaminated soil and a soil containing only the desorption-resistant fraction of TCE were tested. In both cases, presence of soil resulted in biodegradation rates substantially lower than those determined in the absence of soil.

In aqueous phase experiments, the TCE biodegradation rates were approximately 270 and 310 $\mu\text{g TCE}\cdot\text{L}^{-1}\cdot\text{hr}^{-1}$ for the first 2 hours following toluene addition in experiments with low (4.5 mg/L) and high (27 mg/L) initial toluene concentrations. Shortly after toluene was depleted from the system, the TCE degradation rates slowed considerably to 52 and 35 $\mu\text{g}\cdot\text{TCE}\cdot\text{L}^{-1}\cdot\text{hr}^{-1}$, respectively. An appreciable increase in the rate and extent of TCE biodegradation was observed in microcosms when toluene was added multiple times. Toluene dioxygenase (Tod) enzyme activity was monitored, and TCE degradation was clearly correlated with Tod enzyme activity over time. In soil-slurry experiments containing freshly-contaminant soil, a TCE degradation rate of approximately 150 $\mu\text{g TCE kg}^{-1}\cdot\text{hr}^{-1}$ was observed during the first 39-hour period, and then the TCE degradation rate slowed considerably to 0.59 and 0.84 $\mu\text{g TCE kg}^{-1}\cdot\text{hr}^{-1}$ for microcosms receiving one and two additions of toluene, respectively.

The TCE degradation rates in soil-slurry microcosms containing the desorption-resistant fraction of TCE contaminated soil were approximately 0.27 and 0.32 $\mu\text{g TCE kg}^{-1}\cdot\text{hr}^{-1}$ in microcosms receiving one and two additions of toluene, respectively. It is

clear from these results that mass transfer into the aqueous phase limited bioavailability of TCE in the contaminated soil. These observations are in agreement with results reported in the literature for other organic contaminants, and they support the general theory that the longer a contaminant is aged with soil, the lower the fraction of contaminant will be bioavailable.

CHAPTER 5

EFFECT OF SORPTION AND DESORPTION-RESISTANCE ON BIODEGRADATION OF CHLOROBENZENE IN TWO WETLAND SOILS

Introduction

Understanding the relationship between the sorption-desorption phenomenon and contaminant transport is crucial for site remediation. Sorption processes have been shown to influence the biodegradation, bioavailability, and subsurface transport of various organic chemicals (Guerin and Boyd, 1992; Pignatello and Xing, 1996; Zhang, 1995; Zhang and Bouwer, 1998). In general, sorption increases as the molecular weight of nonionic organic compounds increases, as water solubility decreases and as the organic matter content in soils increases. Sorbed organic contaminants are thought to be more resistant to biodegradation than soluble organic contaminant, as it is generally considered that sorbed chemicals are unavailable to microorganisms unless desorption occurs first (Ogram *et al.*, 1985). Although bacteria have been reported that are capable of degrading soil-sorbed naphthalene (Guerin and Boyd, 1992, 1993, 1997), several researchers have confirmed that biodegradation can be limited by the slow rate of desorption of organic compounds (Steinberg *et al.*, 1987; Pignatello, 1989; Robinson *et al.*, 1990). In the latter studies, the rate and extent of desorption were dependent on soil and sorbate properties such as soil organic carbon content, cation-exchange capacity, specific surface area, and water solubility, as reported by other researchers (Pavlostathis and Mathavan, 1992; Huang, *et al.*, 1998; Williamson, *et al.*, 1998; Valsaraj, *et al.*, 1999; Lee *et al.*, 2001a).

Reversible sorption of chlorinated solvents in soils and sediments has been commonly used in assessing risk and determining remedial endpoints. However, reversible sorption models are not able to explain the long-term persistence of organic

contaminants at many sites (Kan *et al.*, 1998). A “desorption-resistance” has been observed that is characterized by compounds that do not desorb normally from soil and sediment organic matter. Desorption has been repeatedly observed to be biphasic, the bulk of the contaminant is readily desorbed but a second fraction is highly resistant to desorption. Like sorption, contaminant aging is another critical factor limiting bioavailability. The aged fraction of organic compounds may result from the slow diffusion of these molecules within some components of solid organic matter in soils (Brusseau and Rao, 1991; Brusseau *et al.*, 1991). The “aging” effect can be a major factor in controlling the desorption phenomenon because of enrichment in a slow fraction owing to partial dissipation or degradation of more labile fractions before collection (Pignatello and Xing, 1996). Pignatello and Xing extensively observed mechanisms of slow sorption of organic compounds, their causes, and its effect on biodegradation. Many research endeavors have proven that the extent of bioavailability of soil-sorbed contaminants decreases with increased aging by conducting experiments with various contaminants such as biphenyl (Feng *et al.*, 2000), styrene (Fu *et al.*, 1994), phenanthrene (Hatzinger and Alexander, 1995; Kelsey *et al.*, 1997; Nam *et al.*, 1998), and DDT (Morrison *et al.*, 2000).

In these studies, chlorobenzene (CB) was chosen as a representative non-ionic contaminant. Chlorobenzene is present in the environment from its use as a solvent and as a biodegradation daughter product of higher chlorinated benzenes. CB-degrading aerobic strains have been isolated (Reineke and Knackmuss, 1984; Nishino *et al.*, 1992, 1994). CB degraders are readily observed in samples with previous exposure histories of CB and these indigenous organisms appear to have a competitive advantage over

inoculated strains. CB-degrading organisms may also possess the ability to degrade dichlorobenzenes including 1,2-dichlorobenzene (Haigler *et al.*, 1988) and 1,4-dichlorobenzene (Spain and Nishino, 1987). Investigation on a laboratory scale showed the feasibility of removing CB from soil using these CB-degraders (Brunsbach and Reineke, 1994; Nishino, *et al.*, 1994). Studies have suggested that CB-degradation may result from horizontal gene transfer between organisms possessing aromatic ring dioxygenases and chlorocatechol degradation (van der Meer *et al.*, 1998).

Bioavailability of non-ionic organics to bacteria is commonly determined using a mineralization assay to quantify the liberation of $^{14}\text{CO}_2$ from a soil previously spiked with a ^{14}C -labeled compound. Three mineralization experiments were conducted: (i) an experiment comparing CB mineralization in soil slurries with different moisture contents; (ii) an experiment examining the mineralization of CB in soil freshly contaminated with CB; and (iii) an experiment measuring the mineralization of the desorption-resistant fraction of CB in soil. The research described herein is part of ongoing research conducted in support of remediation activities at a Superfund site known as Petro Processors Inc. (PPI) site located in north Baton Rouge, LA. These findings could have implications in addressing the question of an acceptable end point for meeting the clean-up goals for CB contamination at that site.

Materials and Methods

Chemical Preparation and Analysis

Non-labeled CB (99.5 % purity, spectrophotometric grade) was purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as supplied. CB solutions were prepared by dissolving aliquots of CB (neat) in deionized water to obtain the desired

contaminant concentrations. Radiolabeled chlorobenzene (Sigma Chemical Co., St. Louis, Missouri, 27 mCi/mmol) was used as a tracer. For the preparation of extraction co-solvent, 2-propanol (99.8 % purity, nanograde, Fisher Scientific) and methanol (99 % purity, Fisher Scientific) were used.

Soils

Soil was collected from the Brooklawn site, one of two Superfund sites collectively known as the Petro Processors Inc. (PPI) site located in north Baton Rouge. Soil was collected from the surface (0-20 cm) of a bottomland hardwood swamp located adjacent to the site. Soil was also collected from the top layer (0-20 cm) of a freshwater marsh located in Madisonville, LA. For the preparation of the PPI soil, large lumps of soil were broken apart and the material was oven dried at 55°C for 24 hours. The soil was then pulverized and homogenized by passage through a sieve with 150 µm openings (US standard sieve No. 100). The soil was then autoclaved under 123.9°C and 20 psi for 30 minutes in Electric Pressure Steam Sterilizer (Model 25X, Wisconsin Aluminum Foundry Co. Inc., Manitowoc, WI). Meanwhile, the marsh soil samples were first chopped evenly with scissors and then sterilized by autoclaving as described above. Samples from these soils were methanol-extracted, analyzed, and found to be free of CB. All soil samples were autoclaved again before use in subsequent experiments. Soil properties were determined according to standard methods in the Huffman Laboratories Inc., Golden, MO (Table 5.1).

Sorption Isotherms

An equilibrium batch sorption isotherm procedure was conducted with soils that had undergone different aging times. Briefly, approximately 5 g of sterilized PPI soil

Table 5.1. Elemental analysis of the soils used in this study

Soils	% C	% H	% O	%N	%S	%Ash	% OC
PPI	1.35	0.33	2.01	0.06	0.04	96.38	1.35
Marsh	27.58	3.93	23.32	2.06	1.00	42.40	27.58

and 0.8 g of marsh soil, on a dry weight basis, were placed in 45-mL Teflon centrifuge tubes. The solution matrixes consisted of 0.001M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.001M NaHCO_3 , and 0.0004M MgCl_2 used to mimic the composition of porewater with 0.1M NaN_3 as a biocide (Stumm and Morgan, 1990). CB was also added to reach concentrations of 1, 2, 5, 20 $\text{mg} \cdot \text{L}^{-1}$. Finally, solutions were spiked with tracer activities of $[^{14}\text{C}]\text{CB}$. All tubes were capped with Teflon-lined screw caps and the slurries were equilibrated by mixing in a tumbler for 48 hours. Tubes were then centrifuged at 3000g for 30 minutes, after which 1-mL portions of supernatant were removed, transferred to liquid scintillation vials containing 10 ml of a scintillation counting cocktail (EcoLite (+)TM, ICN Biomedical Company, Costa Mesa, CA), then analyzed in a liquid scintillation analyzer (Beckman, Model LS6000SC, Fullerton, CA). The distribution coefficients were obtained according to the linear equation

$$C_s = K_d \cdot C_a \quad (5.1)$$

where K_d is the distribution coefficient, C_s is the sorbed phase concentration, and C_a is the solution phase concentration (Karickhoff and Brown, 1979).

In addition to K_d in freshly contaminated soils, partitioning in soils containing only the desorption-resistant fraction was calculated. The approach of Kan and Tomson (Kan *et al.*, 1997, 1998) was used to estimate partitioning to this phase. The biphasic irreversible adsorption model is as follows:

$$q = q^{rev} + q^{irr} \quad (5.2)$$

where, q^{rev} = the concentration of sorbed compound in the reversible fraction

q^{irr} = the concentration of sorbed compound in the desorption-resistant fraction

The sorption to desorption-resistant fraction is represented by a Langmuir-type sorption isotherm:

$$q^{irr} = \frac{K_{oc}^{irr} \cdot OC \cdot q_{max}^{irr} \cdot f \cdot C}{q_{max}^{irr} \cdot f + K_{oc}^{irr} \cdot OC \cdot C} \quad (5.3)$$

where, K_{oc}^{irr} = partition coefficient of the desorption-resistant fraction

OC = organic carbon content (as a fraction)

f = fraction of the compound residing in the desorption-resistant compartment

q_{max}^{irr} = maximum capacity of the desorption-resistant fraction.

A semi-empirical relationship was used to estimate q_{max}^{irr} as follows:

$$q_{max}^{irr} \approx 37765 \cdot OC \cdot K_{ow}^{-0.23} \quad (5.4)$$

The K_{oc}^{irr} values obtained from equation 3 were used to obtain K_d^{irr} , the partition coefficient between the desorption-resistant fraction and water.

Microbial Culture

The source of the microbial culture was a 1,3 dichlorobenzene culture degrading originally isolated in liquid media (Dorn *et al.*, 1974) from sewage sludge from the Central Wastewater Treatment Plant in Baton Rouge, La. Prior to use in bioavailability assays, mixed cultures of 1,3-DCB degrading bacteria were grown in the same mineral media substituting 3 mg of CB per liter for 1,3-DCB. Organisms were repeatedly transferred to fresh liquid mineral medium and cultured for a month prior to the

initiation of the mineralization studies. Cultivated organisms were capable of utilizing CB as a sole carbon and energy source.

Mineralization Studies

The mineralization experiments were set up to evaluate the availability of soil-sorbed CB to the CB-degrading bacteria. These assays were adapted from the method described by Guerin and Boyd (1992) and Feng *et al.* (2000). All treatments for these experiments were set up in 200-mL capacity flasks, capped with Teflon-lined silicon rubber septa and then placed on a rotary shaker and maintained at approximately 25°C. Table 2 summarizes the preparation of mineralization studies in the aqueous and soil phases. Each reactor had a CO₂ trap inside, which contained 2 ml of 1N NaOH. The reactors were set for a designated time to collect a sample, and 1 ml of ¹⁴CO₂ was sampled and counted by liquid scintillation. Hionic-Fluor (Packard Bioscience Company, Meriden, CT) was the scintillant in a 1:10 sample-to-scintillant ratio for the mineralization study. 2 ml of 1N NaOH were replaced each time sampling was conducted. This procedure was always conducted in a temperature-controlled room at a setting of 5°C to minimize possible CB evaporation.

A summary of the experimental treatments is presented in Table 5.2. Three mineralization experiments were conducted: (i) an experiment comparing CB mineralization in soil slurries with different moisture contents; (ii) an experiment examining the mineralization of CB in soil freshly contaminated with CB; and (iii) an experiment measuring the mineralization of the desorption-resistant fraction of CB in soil. For the experiment comparing the effect of moisture contents, three sets of soil slurries containing various moisture contents were prepared. For the PPI soil, soil:water

ratios of 0.04, 0.44 and 0.89 (w/v) were used. For the marsh soil, soil:water ratios of 0.01, 0.04 and 0.08 (w/v) were used. The weights of the soils were based on the dry weight. Ratios were selected to result in approximately the same aqueous concentration range between the two soils. Because of the high moisture content in the marsh soil (approximately 91% water) and the high partition coefficient, the soil weights for these microcosms are comparatively light.

For experiments comparing the effect of sorption on mineralization, soil, 60 grams of PPI soil and 60 grams of marsh soil were added into each replicate 125ml-capacity glass bottles (I-Chem). An aqueous solution spiked with CB (~5 mg/L) was added to each bottle, and the bottles were mixed vigorously, then filled to capacity with minimal headspace, and capped with Teflon fluorocarbon resin/silicon septa. These bottles allowed centrifugation at low speeds to eliminate potential volatilization losses. The bottles were equilibrated in a tumbler for 2 days at 80 rpm. The bottles were then centrifuged to obtain a clear soil-water interface. The supernatant was then decanted off. The soil was then used directly, after determining the soil concentration of CB on a separate aliquot using a biological oxidizer (Model OX600, R.J. Harvey Instrument Corporation, Hillsdale, NJ). Soil was added to flasks as to assay mineralization as described above. Soil (8.01 g and 1.64 g of PPI and marsh soil, respectively, on a dry weight basis) was added into the reactor immediately in a temperature-controlled room to minimize volatilization. Mineralization was then determined as described above.

For the experiment measuring the mineralization of the desorption-resistant fraction of CB, the soil was further prepared by following the isopropanol cosolvent extraction method developed by Liu *et al.* (1999).

In this method, equal volumes of isopropanol and electrolytes (0.01 M NaCl, 0.01 M CaCl₂) were used to prepare an extraction solution. Soils were contaminated as described above (20 mg/L of initial concentration of CB). In order to make the soil “aged” artificially, the CB sorbed soil, in *I-Chem* bottles, was stored in the dark area for a designated time before it was used. Contaminated soil was mixed vigorously with the co-solvents in a tumbler for 24 hours, and then the co-solvents were separated from the soil via centrifugation (120 ml/g of soil). The separated soil was rinsed with electrolyte two times to remove the residual iso-propanol and then mixed with co-solvents again, and placed in a tumbler for another 24 hours. An additional rinse with electrolytes was conducted, and CB concentration in the aqueous phase was measured. The CB concentrations in the soil phase were determined using a Biological Oxidizer (Model OX600, R.J. Harvey Instrument Corporation, Hillsdale, NJ).

Data Analysis

In order to quantify the mineralization extent and rate, experimental data were applied to a first-order CO₂ production equation,

$$P = P_{\max} \cdot (1 - e^{-k \cdot t}) \quad (5.5)$$

where P is the percentage of initial radioactivity mineralized, P_{max} is the maximal percentile mineralized, k is the first-order rate constant (h⁻¹), and t is time (hour). Since ¹⁴C-labeled CB conversion to biomass was not accounted for, the results are conservative estimates of degradation.

An alternative model, the coupled degradation-desorption (CDD) model, suggested by Guerin and Boyd (1992, 1997) was also applied for comparison.

Table 5.2. Summary for the preparation of mineralization study

Treatments	Soil Type	Aging (days)	Soil Wt. (g)	Nutrients (mL)	¹⁴ CB solution (mL)	μCi of ¹⁴ C-CB per reactor	R _{s/w} (kg/L)
Soil-free		0	0	20	10 [*]	3.0x10 ⁻³	0
Soil-Slurry	PPI	0	40	30	10 ^{**}	4.17x10 ⁻³	0.89
		0	20	30	10 ^{**}	4.17x10 ⁻³	0.44
		0	2	30	10 ^{**}	4.17x10 ⁻³	0.04
	Marsh	0	3.68	30	10 ^{**}	4.17x10 ⁻³	0.08
		0	1.84	30	10 ^{**}	4.17x10 ⁻³	0.04
		0	0.18	30	10 ^{**}	4.17x10 ⁻³	0.01
Freshly-contaminated	PPI	2	8.01	15	99.47 ^{***}	8.1x10 ⁻³	0.43
	Marsh	2	1.64	15	71.48 ^{**}	7.9x10 ⁻³	0.08
Desorption-resistant	PPI	2	7.19	15	111.3 ^{***}	4.64x10 ⁻⁴	0.32
		7	2.81	15	110.1 ^{***}	4.47x10 ⁻⁴	0.40
		31	6.6	15	110.7 ^{***}	1.00x10 ⁻³	0.28
	Marsh	2	0.92	15	86.5 ^{***}	3.35x10 ⁻⁴	0.03
		7	6.59	15	83.4 ^{***}	3.13x10 ⁻⁴	0.03
		31	0.95	15	82.3 ^{***}	4.05x10 ⁻⁴	0.03

* 2 mg/L of initial concentration was added.

** 5 mg/L of initial concentration was added.

***, The volume of CB solution (20 mg/L) added into each bottle containing soil was the one when the sorption experiment was initiated.

Its equation is,

$$P = v_2 t + [(v_1 + v_2)(1 - e^{-k't})]/k \quad (5.6)$$

where v_1 represents the initial rate (percent hour⁻¹) which, when normalized to the initial CB concentration (μg·L⁻¹), gives initial mineralization rates (μg·L⁻¹·hour⁻¹) directly comparable to those derived by using the first-order model (equation 5.5). The

parameter of v_1/k is the equivalent of P_{\max} and v_2 represents the mineralization rate resulting from the desorption of bound CB, related to the kinetics of CB desorption. P is the percentile of CB mineralized as a function of time, t .

Zhang *et al.* (1998) used a bioavailability factor (B_f , dimensionless) to understand the impact of sorption and desorption behavior in a batch system, given by the following equation

$$B_f = \frac{1}{1 + K_d \cdot \frac{m}{v_1}} \quad (5.7)$$

where K_d can be obtained from a sorption isotherm study, and m/v_1 is the soil/water ratio ($\text{kg} \cdot \text{L}^{-1}$).

Feng *et al.* (2000) developed additional equations to explain mineralization kinetics by hypothesizing relationships between biodegradation and desorption behavior. These are expressed as:

$$P_d = P_{\max} \cdot (1 - e^{-B_f \cdot k \cdot t}) \quad (5.8)$$

$$P_{nd} = B_f \cdot P_{\max} \cdot (1 - e^{-k \cdot t}) \quad (5.9)$$

The assumption for equation 8 is a soil slurry with instantaneous desorption, while a slurry without desorption is assumed for equation 9. P_d and P_{nd} (%) are the percentile of CO_2 production in the mineralization assay.

Mineralization data for the experiments used to determine biodegradability of freshly-added and desorption-resistant CB was plotted together with the theoretical lines, P_d (equation 5.8) and P_{nd} (equation 5.9). If sorbed CB was unavailable to bacteria, the CO_2 production values should be equal to or less than P_d values.

Cumulative CO₂ production levels lying above P_{nd} values indicate that bacteria have access to sorbed CB. Cumulative CO₂ production rates falling below P_{nd} values indicate that bacteria have no access to sorbed CB.

Results and Discussion

Sorption Isotherms

CB sorption was measured on two types of soils with different lengths of contaminant aging. As expected, the measured K_{ds} in soils increased as aging and the fraction of organic carbon in the soil increased. The linear partition coefficients, K_{ds}, increased from 3.24 to 33.3 L/kg in the PPI soil and from 71.3 to 311 L/kg in the marsh soil when aged from 2 to 31 days. Sorption isotherms for CB were found to be relatively linear with R² ranging from 0.74 to 0.98. These results are in good agreement with previous findings (Fu *et al.*, 1994; Kan *et al.*, 1997, 1998). The increased partition coefficients as CB ages may result from slow diffusion into and entrapment within small pores in soil aggregates (Steinberg *et al.*, 1987). A conformational rearrangement of the organic matter following sorption may also be responsible for the observed effect (Kan *et al.*, 1998). The exact mechanism of contaminant aging is unknown, however, the end result is a substantial decrease in porewater concentrations of CB in these soils.

Mineralization Studies

Effect of Soil:Water Ratios

Mineralization of CB in the treatments with various soil:water ratios is presented in Figure 5.1. In the absence of soil, approximately 30% of the carbon was converted to CO₂. Mineralization was higher in the presence of soil with approximately 45% and 50% of the carbon converted into CO₂ in the PPI and marsh soil, respectively.

Mineralization curves were fit to the first-order CO₂ production model (eq. 5.5). Parameters are presented in Table 5.3. The soil:water ratio did not substantially affect mineralization of CB in the PPI soil (Figure 5.1). There were no statistical differences in P_{\max} , a measure of the extent of mineralization, or the first-order CO₂ production rate constant (k), between treatments when compared using Bonferroni's inequality at $\alpha=0.05$. No further increase in CO₂ production was detected after approximately 150 hours.

In contrast, moisture content dramatically affected mineralization in the marsh soil. The k values decreased as the soil:water ratio increased and rates were statistically different between all three treatments ($\alpha=0.05$). This is attributed to easier accessibility of microorganisms to CB in the aqueous phase than in the sorbed phase in the early stage of exposure. The extent of mineralization (P_{\max}) in the lowest soil:water ratio (0.01) was not statistically different from the soil-free control, however, the addition of only 0.18 g of marsh soil more than doubled the rate constant (from 0.01 hr⁻¹ to 0.023 hr⁻¹).

Differences in the patterns of mineralization between the PPI and marsh soils are interesting because soil:water ratios were selected to result in the same range of CB porewater concentrations in both soils. Despite similar porewater concentrations and the same initial microbial populations, the presence of soil affected the mineralization differently. This suggests that other soil properties affect the rate and extent of mineralization and that the sorption-desorption/mineralization relationship in soils is not simply a function of the porewater concentration and the initial microbial population size. Previous studies reported that biodegradation rates have been influenced by soil

moisture conditions through several mechanisms, including reduced oxygen availability at higher moisture contents and reduced diffusion of the substrate at lower moisture contents (Helweg, 1987; Hatzinger and Alexander, 1995; Flint *et al.*, 1997;

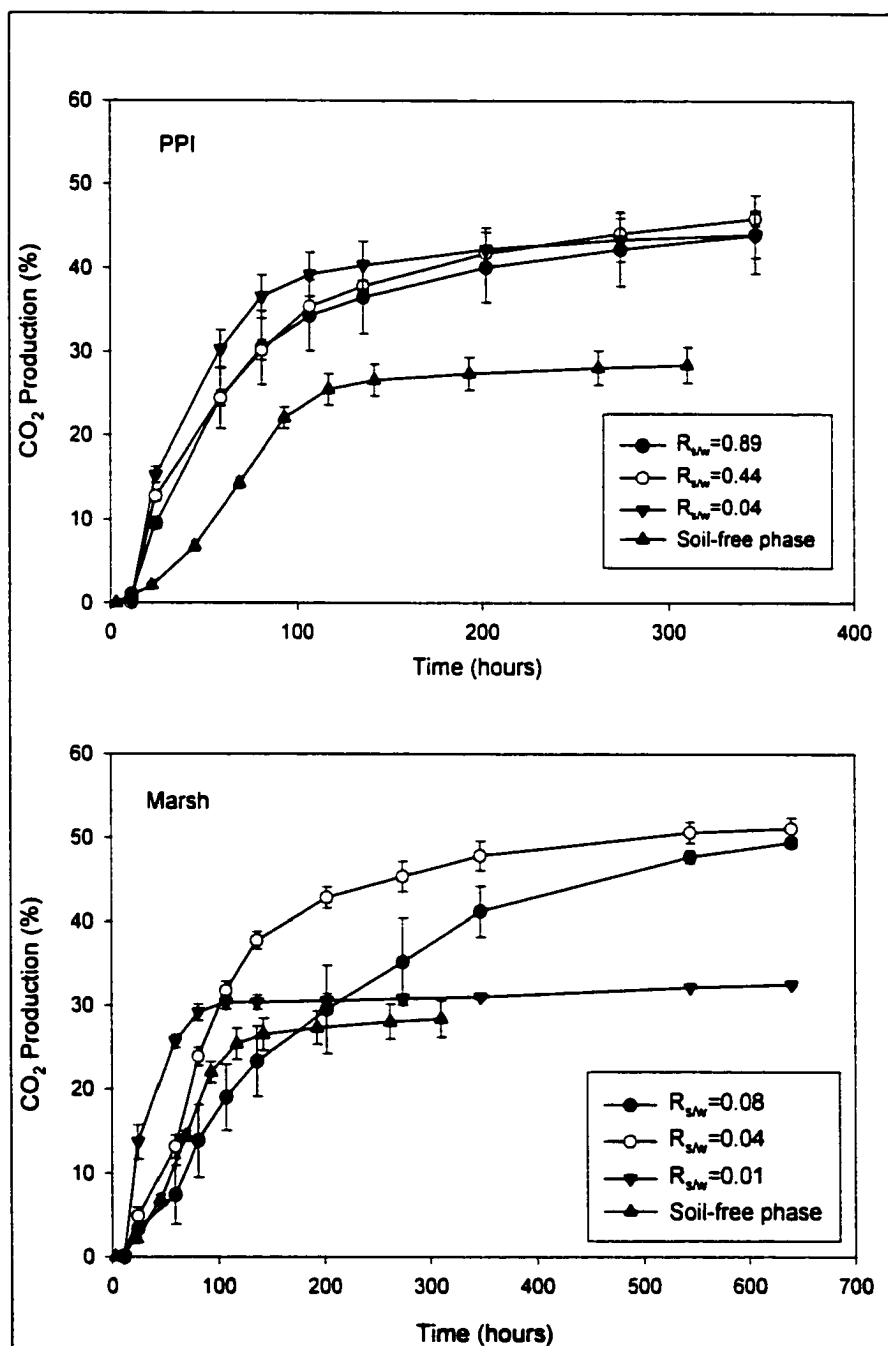


Figure 5.1. Mineralization of CB in soil-slurry microcosms containing various moisture contents and soil free phase.

Cupples, *et al.*, 2000). It is not clear which mechanism was operating in these experiments. Follow-up studies were conducted to assess the effect of sorption on mineralization rates.

Table 5.3. Parameter estimates of CB mineralization as a function of moisture contents based on the simple first-order model and the coupled degradation-desorption model

Soils	$R_{s/w}$ *	First-order model			Coupled degradation-desorption model			
		P_{max} (%)	k (hr^{-1})	R^2	V_1 (%/hr $^{-1}$)	V_2 (%/hr $^{-1}$)	k (hr^{-1})	R^2
PPI	0.04	44.33 (1.93)**	0.018 (0.002)	0.95	0.79 (0.11)	3.01×10^{-6} (0.035)	0.018 (0.006)	0.94
	0.44	45.20 (1.77)	0.013 (0.001)	0.97	0.50 (0.09)	1.66×10^{-7} (0.068)	0.011 (0.005)	0.95
	0.89	44.20 (1.67)	0.013 (0.001)	0.97	0.49 (0.08)	1.38×10^{-7} (0.064)	0.011 (0.006)	0.95
Wetland	0.01	31.94 (1.14)	0.023 (0.003)	0.92	0.75 (0.13)	2.62×10^{-6} (0.007)	0.024 (0.005)	0.91
	0.04	52.08 (2.20)	0.008 (0.001)	0.97	0.41 (0.04)	6.78×10^{-6} (0.022)	0.008 (0.003)	0.96
	0.08	55.186 (2.299)	0.004 (0.001)	0.99	0.21 (0.06)	4.85×10^{-6} (0.039)	0.004 (0.002)	0.99
Soil-free Phase		31.44 (3.03)	0.010 (0.002)	0.92	0.31 (0.13)	1.24×10^{-6} (0.099)	0.010 (0.010)	0.90

* Refers to ratio of soil to water.

** Values in parentheses are standard errors of parameter estimates

Effect of Sorption

To assess the possible role of mass transfer in governing the rate of biodegradation, the rate and extent of mineralization was measured in soils containing freshly added CB. Soil:water ratios were selected that are more typical of these soils under field conditions (0.43 for PPI and 0.08 for marsh). As shown in Figure 5.2, experimental data were applied to the first-order CO₂ production model and it proved to fit reasonably well ($R^2=0.96$ in PPI and 0.87 in marsh). The observed values of P_{max}

were 58.1% and 49.9% in the PPI and the marsh soil, respectively. First-order rate constants were $\sim 0.01 \text{ hr}^{-1}$ for both soils.

Experimental data were plotted together with theoretical lines, P_d (equation 5.8) and P_{nd} (equation 5.9), to evaluate sorbed CB bioavailability. If sorbed CB is not available to bacteria and if only aqueous-phase CB can be degraded in soil slurries, the amount of initial CO_2 production should be equal or less than P_d , which accounts for instantaneous desorption. The amount of initial CO_2 production should be above P_{nd} , which assumes no desorption. As shown in Figure 5.2, the initial CO_2 production levels in soil slurries are above P_d values, indicating that mineralization rates are faster than would be expected, signifying that bacteria have access to sorbed CB. In other words, the degradation rates exceeded those expected with instantaneous desorption.

To further investigate the bioavailability of sorbed CB, data were fit to the CDD model. Parameter estimates ($P_{\max} \approx v_1/k$) were obtained that are consistent with the simple first-order model (equation 5.5). Despite the presence of more adjustable parameters ($n=3$) for the CDD model, a significantly higher residual sum of squares was observed when compared with the first-order model. Nevertheless, theoretical curves provided by CDD models show reasonable fits for both soils. No significant differences were found in the values of initial mineralization rates, v_1 , in either soil. Values of v_2 were low relative to v_1 , confirming that desorption did not contribute significantly to the CB mineralization observed in both soils during the initial stage of transformation. Surprisingly, there were no statistical differences between fitted parameters for the two soils despite large differences in soil properties.

To obtain further evidence of the bioavailability of sorbed CB in soils, initial mineralization rates were calculated from parameters obtained from CDD model fits. The initial mineralization rate (IMR, $\mu\text{g}\cdot\text{L}^{-1}\cdot\text{hr}^{-1}$) is the product of P_{max} , k (hr^{-1}), and the initial CB concentration ($\mu\text{g}\cdot\text{L}^{-1}$) and is an estimate of the initial slope of the CO_2 production curve (Guerin and Boyd, 1992, 1997; Feng *et al.*, 2000). IMR values were calculated from mineralization data and plotted as a function of equilibrium aqueous phase CB concentration (Figure 5.3). Measurements of porewater CB concentration were conducted in control microcosms containing a bacterial inhibitor. A reference line was constructed using the IMR from the soil-free control and the corresponding initial CB concentration (Guerin and Boyd, 1992). IMRs plotting above the reference line indicating rates faster than would be predicted from porewater degradation alone proving further evidence for direct degradation of sorbed CB. As shown in Figure 5.3, experimental data for both the PPI soil and the marsh soil were above the reference line based on the aqueous phase concentration. This provides further evidence that sorbed CB in both soils was available to microorganisms. In conclusion, mineralization of CB occurred, and bacteria appeared to have access to the pool of freshly added, sorbed CB.

Effect of Desorption-Resistance and Aging

To understand the effects of desorption-resistance and contaminant aging on the rate of biodegradation, an experiment was conducted with soils where the sorbed CB was “aged” in the laboratory and then extracted with an iso-propanol cosolvent to remove the remaining reversibly-bound portion of CB. Two hypotheses were tested from this experiment. An initial hypothesis was that extraction of the “aged” CB with iso-propanol would result in the same concentration regardless of aging time. Secondly,

we hypothesized that removing the readily-desorbable CB would result in significantly decreased rates and extent of mineralization. As described above, the iso-propanol extraction is designed to remove an easily desorbed fraction of the contaminant on the soil.

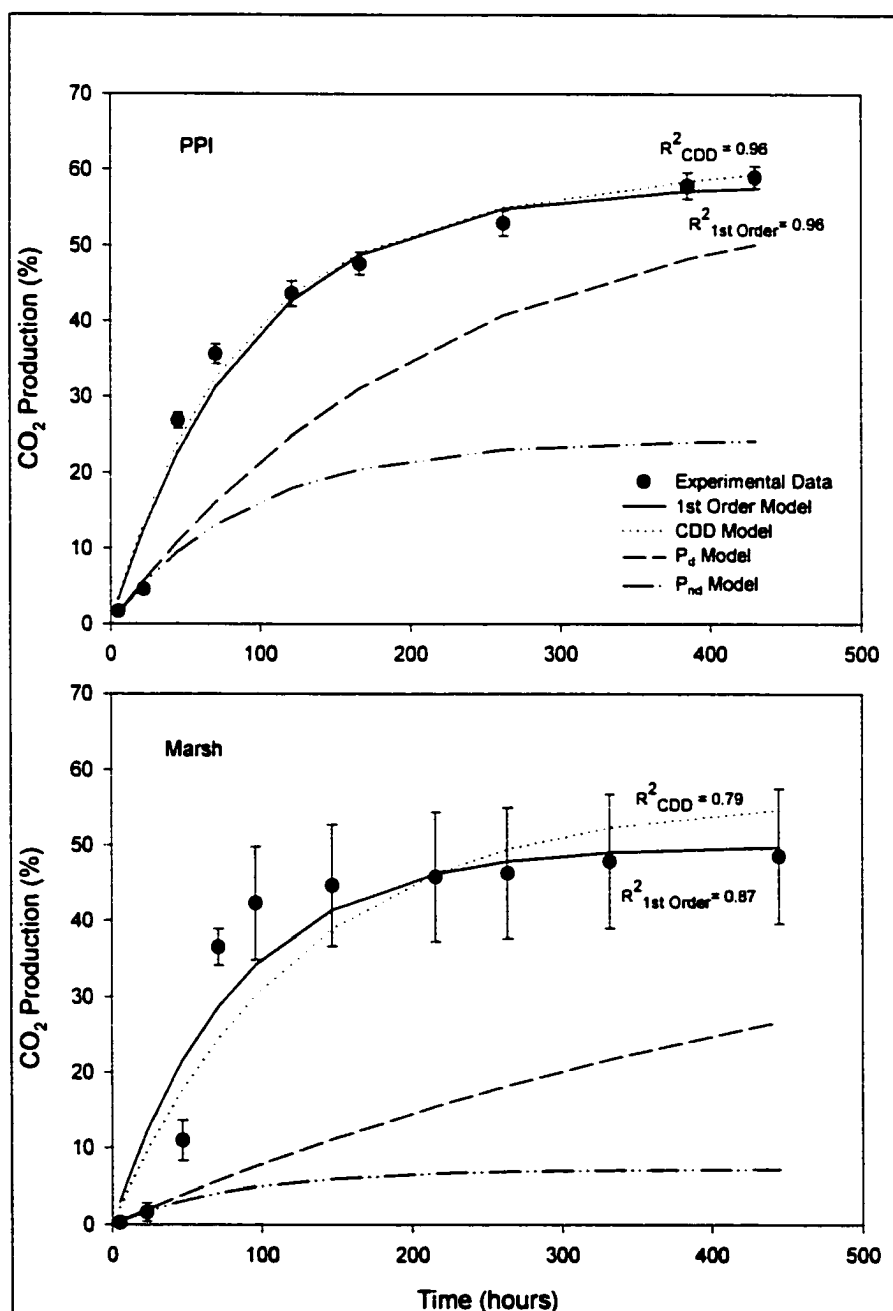


Figure 5.2. Plot of mineralization versus incubation time in freshly contaminated PPI and marsh soil.

The isopropanol extraction has resulted in observed partition coefficient that are very similar to those obtained after long-term multiple desorption steps (Liu *et al.*, 1999). These experiments were performed with both soils because they yielded various fractions of desorption-resistant CB with different contact times.

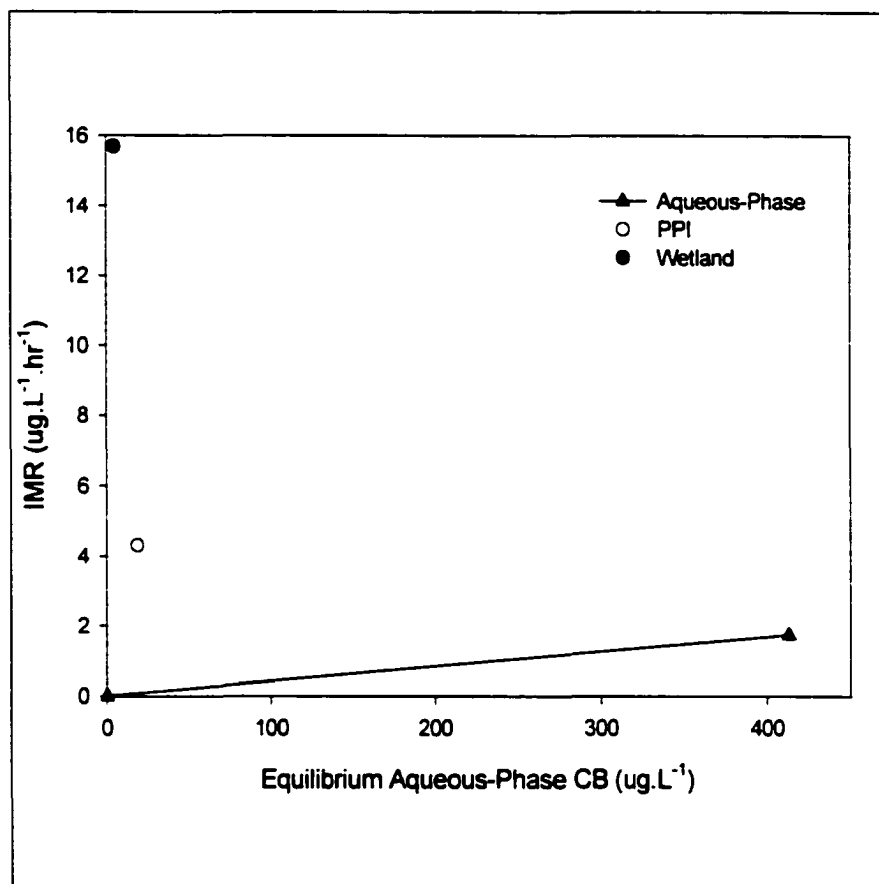


Figure 5.3. Plot of initial mineralization rates versus the equilibrium aqueous-phase CB concentrations in soils.

After aging for 2, 7, and 31 days, iso-propanol extraction yielded different concentrations for the PPI soil (1.1 mg/kg, 2.89 mg/kg and 6.22 mg/kg for 2, 7 and 31 days, respectively). Marsh soil concentrations were more similar with 16.1, 13.1 and 17.9 mg/kg after 2, 7 and 31 days, respectively. Our initial hypothesis was proved incorrect for PPI soil but not for the marsh soil. Iso-propanol extraction did not result in

the same concentrations for each aging time for the PPI soil. Results can be explained by the time necessary to reach equilibrium with the desorption-resistant fraction. Equilibrium with the desorption-resistant fraction has been observed to require 3 to 8 days (Chen *et al.*, 2000). Extracting prior to this will not result in the same concentrations in the desorption-resistant fraction. The behavior of the marsh soil indicates that equilibrium with this phase may be faster in highly organic marsh soils.

The effects of aging on CB mineralization were significant in both soils. Figures 5.5 and 5.6 depict mineralization experiments in both soils with theoretical curves developed from first-order, CDD, P_d , and P_{nd} models. In contrast to the freshly-added CB sorption experiments, data from both soils were not well fitted with the first-order and CDD models (Table 5.4). Although R^2 ranged from 0.85 to 0.97, parameter estimates were often poor with standard errors exceeding the value of the parameter, itself. Much of the problem in fit appears to result from the lag observed in degradation in most of the treatments. Mineralization curves from the desorption resistant treatments had distinctly different sigmoidal shapes when compared with the freshly-added CB curves. The extent of mineralization (P_{max}) was different as soil aged, ranging from approximately 50.3% to 28.6% in PPI soil and from 58.4% to 14.1% in wetland soil, when the soils were aged from 2 to 31 days. Due to the standard errors, however, only the P_{max} from the marsh soil after 31 days of aging was statistically lower. No statistical difference was observed in the rates of mineralization, however, rates were an order of magnitude lower than in the previous experiment where CB was freshly added ($\sim 0.001 \text{ day}^{-1}$ versus $\sim 0.01 \text{ day}^{-1}$). As shown in Figures 5.5 and 5.6, experimental data plotted above theoretical mineralization curves derived from the P_d and P_{nd} models.

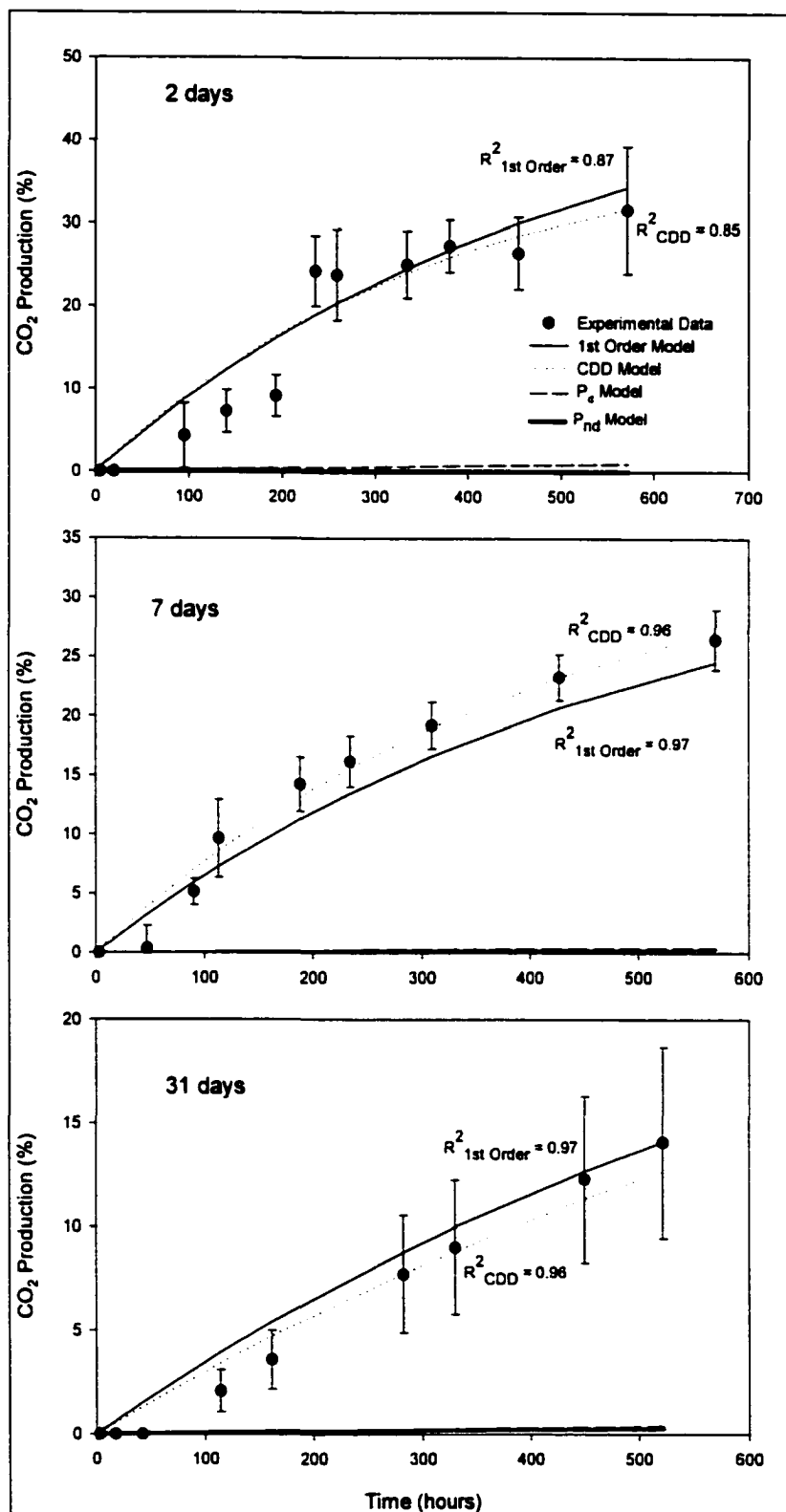


Figure 5.4. Mineralization of CB in PPI soil containing only desorption-resistant fraction with various contact times.

This indicates that CB degrading bacteria have access to desorption-resistant CB in both soil types. For these calculations, the bioavailability factor, B_f , was calculated from equation 5.7. Partition coefficients, K_d^{nr} , were determined both experimentally and calculated from equations 5.2. through 5.4 and used to estimate B_f values. The higher partition coefficient for the desorption-resistant fraction results in the low very P_d and P_{nd} curves. According to the results of this experiment, it is concluded that desorption-resistant CB was still accessible by this degrading culture despite decreases in porewater concentration of 2 orders of magnitude (0.0056 mg/L for PPI, 0.0079 mg/L for marsh soil).

Numerous studies have been devoted to understanding the effect of aging in various soil types (Hatzinger and Alexander, 1995; Kelsey *et al.*, 1997; Nam *et al.*, 1998). Previous studies have suggested that bioavailability of sorbed nonionic organic contaminants may be influenced by organic matter content of the soils (Guerin and Boyd, 1993; Hatzinger and Alexander, 1995). Those studies suggested that if CB were present in a desorption resistant fraction in soil containing high organic carbon, the CB would be less accessible to degrading organisms when compared with a soil with low organic carbon. In the present study, few differences were observed between these soils despite large differences in organic carbon content. Results from the freshly-added and desorption-resistant mineralization assays were remarkably similar. Differences in the mineralization assays with different soil:water ratio were really due to a single treatment in the marsh soil, the very lowest ratio tested. Based on these results, there is little evidence that difference in organic carbon content alone results in different mineralization behavior.

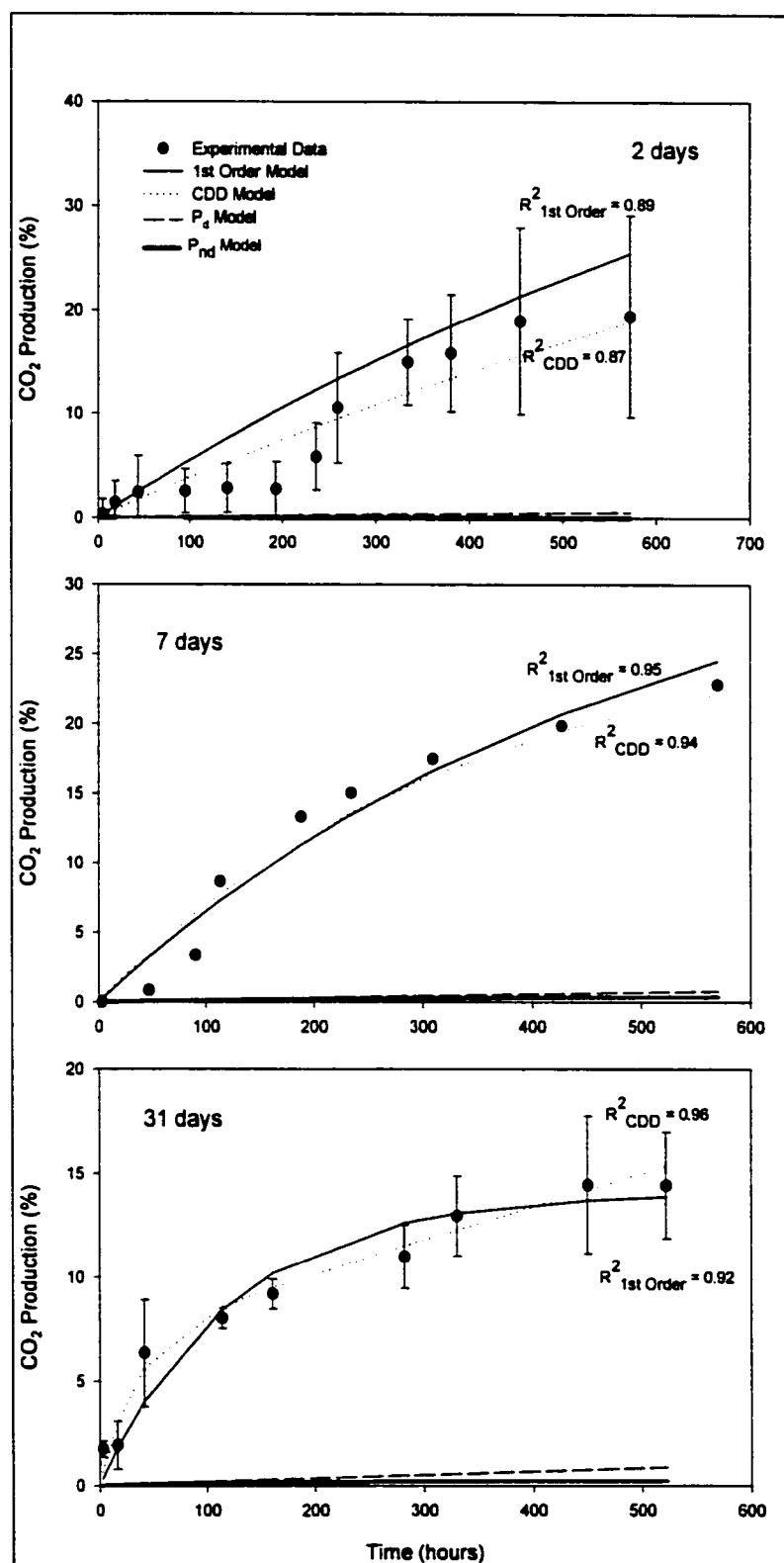


Figure 5.5. Mineralization of CB in marsh soil containing only desorption-resistant fraction with various contact times.

Table 5.4. Parameter estimates of CB mineralization based on the simple first-order model and the coupled degradation-desorption model for the freshly contaminated PPI and marsh soil.

Soils	First-order model			Coupled degradation-desorption model			
	$P_{\max}(\%)$	$k(\text{hr}^{-1})$	R^2	V_1 (%/hr ⁻¹)	V_2 (%/hr ⁻¹)	k (hr ⁻¹)	R^2
PPI	58.065 (2.647)*	0.011 (0.002)	0.962 (3.920)	0.662 (0.092)	0.017 (0.036)	0.013 (0.005)	0.956 (4.161)
Marsh	49.944 (4.285)	0.012 (0.003)	0.867 (6.816)	0.450 (0.180)	5×10^{-6} (0.130)	0.008 (0.009)	0.788 (8.50)

*Values in parentheses are standard errors of parameter estimates

This study was in agreement with previous findings (Hatzinger and Alexander, 1995; Kelsey, *et al.*, 1997; Nam *et al.*, 1998) that aging served to decrease overall mineralization rate and extent. The present study demonstrated that soil-sorbed CB was available to CB degrading bacteria.

The extent of bioavailability of soil-sorbed CB decreased with aging but despite very large decreases in the porewater concentration, CB mineralization was still a significant fate process. Aging may decrease bioavailability and reduce rates, but degradation can potentially still proceed at environmentally relevant rates.

Conclusions

Based on these experiments, the following conclusions were drawn.

Differences in soil:water ratios did not affect mineralization rate and extent in the PPI soil despite aqueous phase concentrations. Differences were observed in the marsh soil particularly when the soil:water ratio was very low. In both soils, the presence of soil solids had a stimulatory effect on the rate of mineralization. Mineralization results under different soil:water ratios could not simply be explained as

a function of the initial porewater concentration and the initial microbial population size. CB-degrading microorganisms biodegraded sorbed CB in both wetland soils. Evidence for biodegradation of sorbed CB includes mineralization curves that plotted well above theoretical mineralization curves assuming instantaneous desorption preceding biodegradation. Other evidence was also obtained from computation of initial mineralization rates (IMRs). IMRs were 0.14 $\mu\text{g/L-hr}$ and 1.92 $\mu\text{g/L-hr}$ for the marsh soil and mineral PPI soil, respectively. IMRs plotted above a reference line defined by the mineralization rate of a soil-free control, also indicating that CB-degrading bacteria had access to sorbed CB.

After aging CB-contaminated soil and the subsequent extraction of a readily-desorbable CB fraction, microorganisms still mineralized the remaining desorption-resistant CB. Despite reductions of porewater CB concentrations of 3 orders of magnitude, mineralization of desorption-resistant CB was observed even though the rate and extent of mineralization were reduced.

Biodegradation of desorption-resistant contaminants is slower but still occurs with environmentally relevant rates in wetland soils where the contaminant was artificially aged using isopropanol. This suggests that microbial degradation could proceed in soils where the chlorobenzene was present in a desorption-resistant form with a much higher partition coefficient. Biological treatment may remain an option at sites with long exposure histories and a significant mass of contaminant in the desorption-resistant form.

CHAPTER 6

SORPTION AND DESORPTION HYSTERESIS OF 1,4-DICHLOROBENZENE AND EFFECT OF SORPTION IN BIODEGRADATION IN WETLAND SOILS

Introduction

Extensive studies have been conducted to understand sorption and desorption processes in the fate of contaminants in soil (Pavlostathis and Mathavan, 1992; Huang *et al.*, 1998; Opdyke and Loehr, 1999; Lee *et al.*, 2001a). In order to optimize the effectiveness in bioremediation processes, understanding of sorption and desorption become crucial.

Most solute-sorbent systems require contact periods of weeks or months to approach true sorption/desorption equilibrium conditions (Huang *et al.*, 1998). And the effects of contact time in sorption process were quantitatively demonstrated in the experiment with phenanthrene contaminated soils by observed K_d values in soils containing 0.02, and 30 days contact times were 12.06, and 53.87 L/kg in Kan *et al.* (1994). Under conditions having long contact time of contaminants with soils, measured K_D^d values are usually greater than their respective value K_D^s values, and this phenomenon is considered as hysteresis (Huang *et al.*, 1998; Lueking *et al.*, 2000). This condition is particularly associated with systems for which so called 'aging effect' are evident. Sorption and desorption hysteresis is traditionally evaluated by comparing the solute phase-distribution coefficient, K_D^s , measured in the sorption step to that measured in desorption step, K_D^d . The variable K_d is defined as the ratio of the equilibrium solid- and aqueous-phase solute concentrations, q_e and C_e , respectively.

It is often observed that a small fraction of the sorbed contaminant remains in the soil and sediment even with various treatments. Lots of researches have been

reported to explain the existence of the resistant fraction and a significant fraction of some contaminants is bound into sediments irreversibly (Fu *et al.*, 1994a, b; Kan *et al.*, 1994, 1997, 1998; Pignatello and Xing, 1996; Huang *et al.*, 1998; Chen *et al.*, 2000). This irreversible fraction, so-called “desorption-resistant fraction”, is often persistent in natural environment, and limit bioavailability. Several researchers have confirmed that biodegradation can be limited by the slow rate of desorption of organic compounds (Steinberg *et al.*, 1987; Pignatello, 1989; Robinson *et al.*, 1990). Sorbed NOCs are thought to be more resistant to biodegradation than soluble NOCs as it is generally considered that sorbed chemicals are unavailable to microorganisms unless desorption occurs first (Ogram *et al.*, 1985). However, bacteria thought to be capable of degrading soil-sorbed naphthalene (Guerin and Boyd, 1992, 1997). The slow diffusion within the sediment particles is suggested as one of the reasons for the limitation in desorption of non-labile fraction (Pignatello and Xing, 1996).

As one of factors influencing bioavailability, aging is critical factor to limit bioavailability. And the prolonged contact with pollutant in soil and sediment cause sequestration that drives to slow desorption and irreversibility. And this sequestration eventually causes reduced bioavailability (Alexander, 1994). Polychlorinated biphenyls (Di Toro and Horzempa, 1982), pesticides (Scribner *et al.*, 1992), and halogenated aliphatic hydrocarbons (Pignatello, 1990) have been found to exist in soils and sediments partially in a strongly sorbed, and the amount of desorption-resistant fraction may increase dramatically with time as the chemical remains in the soil or sediment.

In our previous study, we reported that CB existing in easily desorbed and desorption-resistant phases was both bioavailable (Lee *et al.*, 2001b, c). And the rates

and extents of biodegradation were dependent of soil and sorbate properties, as was also reported by other researchers (Pavlostathis and Mathavan, 1992; Lee *et. al.*, 2001b, c).

Most contaminated sites have soils and sediment containing contaminants for a prolonged time. In order to mimic this situation, this study was performed with soils various aging duration and containing desorption resistant 1,4-DCB. Sorption and desorption isotherms in various aged soils from PPI Superfund and Madisonville site were attempted. And also CO₂ production (%) was measured in 1,4-DCB contaminated soils to quantify the extents of bioavailability depending on soil types containing a various aging period. To observe the possible effect of mass transfer in degradation process, a freshly contaminated soil and a soil containing only the desorption resistant 1,4-DCB were used for mineralization study.

Research described herein is part of ongoing research conducted in support of remediation activities at a Louisiana Superfund site known as the Petro Processors Inc., (PPI) site located in north Baton Rouge, LA. Findings of this study may have implications for interpretation of sorption/ desorption phenomenon and bioavailability of DCB in contaminated soil.

Materials and Methods

Sorbents

Three sorbents were utilized in the study: a mineral-dominated soil collected adjacent to the Brooklawn site (one of the two Superfund sites collectively known as the Petro Processors site), a marsh soil collected from Madisonville. Two types of soil were collected from uncontaminated region of the Brooklawn site (one of the two Superfund sites collectively known as the PPI site) located in north Baton Rouge, and top 20 cm and below 30 cm layer of freshwater marsh soil in Madisonville located in south Baton

Rouge, LA. For the preparation of the PPI soil, large lumps of soil were broken apart and the material was oven dried at 55°C for 24 hours. The soil was then pulverized and homogenized by passage through a sieve with 150 µm openings (US standard sieve No. 100). The soil was sterilized by autoclaving at 123.9°C and 20 psi for 30 minutes in Electric Pressure Steam Sterilizer (Model 25X, Wisconsin Aluminum Foundry Co. Inc., Manitowoc, WI). For the marsh sorbents, soil was first chopped evenly with scissors to homogenize the material and then the soil was sterilized as described above. Sorbents were stored in the refrigerator prior to use. Soil samples were methanol-extracted, analyzed, and found to be free of 1,4-DCB. All soils were autoclaved again before use in subsequent experiments. Soil properties were determined according to standard methods in the Huffman Laboratories Inc. (Golden, CO). Soil characteristics are summarized in Table 6.1.

Table 6.1. Elemental analysis of soils used in this study

Soils	% C	% H	% O	%N	%S	%Ash	% OC
PPI	1.35	0.33	2.01	0.06	0.04	96.38	1.35
Marsh (top)*	27.58	3.93	23.32	2.06	1.00	42.40	27.58
Marsh (deeper)*	23.14	3.38	21.52	1.65	1.01	52.42	23.14

* Refers to layers collected from Madisonville site, LA

Chemical Preparation and Analysis

[¹⁴C]1,4-DCB (19.99 mCi mmol⁻¹, >95% purity) and cold 1,4-DCB (99.5 % purity, spectrophotometric grade) were purchased from Sigma Chemical Co. (St. Louis, MO) and were used as supplied. For the preparation of extraction cosolvent, 2-propanol (99.8 % purity, nanograde, Fisher Scientific) and methanol (99 % purity, Fisher Scientific) were used.

^{14}C -labeled CO_2 concentrations in NaOH traps were determined using a liquid scintillation counter (Beckman, Model LS6000SC, Fullerton, CA), Hionic-Fluor (Packard Bioscience Company, Meriden, CT) was used as the scintillant in a 1:10 sample-to scintillant ratio for the mineralization studies. EcoLite (+)TM(ICN Biomedical Company, Costa Mesa, CA) was used as a scintillant for the sorption/desorption isotherm study. Carbon-14 Cocktail (R.J. Harvey Instrument Corporation, Hillsdale, NJ) was used for biological oxidizer to measure concentrations of 1,4-DCB in soil phase. Blank samples were run in triplicates for every sample run and were used as a background radiation correction. Blanks consisted of 1 ml of D.I. water suspended in 10 ml of EcoLite (+)TM scintillation cocktail.

Sorption /Desorption Isotherms

Equilibrium batch sorption and desorption isotherm procedures were conducted with soils that underwent different aging times. At the beginning of the sorption experiment, approximately 5 grams of dried and sterilized PPI soil and approximately 0.8 grams of freshwater marsh soil, on a dry weight basis, were placed in 45-mL Teflon centrifuge tubes. The solution mixtures consisted of 0.001 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.001 M NaHCO_3 , and 0.0004 M MgCl_2 used to mimic the composition of porewater with 0.1 M NaN_3 as a biocide (Stumm and Morgan, 1990). 1,4-DCB was added at initial concentrations of 1, 2, and 5 $\text{mg} \cdot \text{L}^{-1}$ in the aqueous phase. Finally, the solution was spiked with tracer activities of [^{14}C]1,4-DCB. All tubes were capped with Teflon-lined screw caps and the slurries were equilibrated by mixing in a tumbler for 48 hours. Tubes were then centrifuged at 3000g for 30 minutes, after which 1-mL portions of supernatant were removed, transferred to liquid scintillation vials and analyzed using an

LSC as described above. Desorption experiments were conducted using withdraw-refill batch techniques developed by Huang *et al.* (1998). Soils that had been equilibrated with the 1,4-DCB were centrifuged to remove any unsettled particles. Desorption was induced by successive replacement of 30 to 35 ml supernatant with electrolytes, described previously, and allowed to equilibrate by mixing in a tumbler for 24 hours. Partitioning was determined as in previous sorption runs. Six to ten successive desorption steps were employed in these experiments.

The mass of chemicals that disappeared from the solution phase at the end of sorption experiment was assumed to be sorbed onto the sediment. In each desorption step, the amount of chemical desorbed was deduced from the change in the solution-phase concentration during consecutive steps. This is based on the assumption that the changes in solution-phase concentration were due solely to sorption/desorption. This assumption was tested by mass-balance analysis in which the activity of ^{14}C present at the completion of the test was compared to the activity recovered by biological oxidizer.

Sorption and desorption data were fitted to the linear and Freundlich isotherms to determine partition coefficients and to assess linearity. Fits were performed using the curve-fitting software, Tablecurve (Version 4, AISN software). To facilitate direct comparisons of sorption affinities among the samples tested, organic carbon-normalized single-point distribution coefficient values [$K_{oc} = (q_e / C_e) / f_{oc}$] at $C_e = 0.2 \text{ mg/L}$ were calculated for all samples.

The sorption-desorption hysteresis was quantified for each sorbent/solute-solution using the Hysteresis Index (HI) (Huang *et al.*, 1997, 1998; Leboeuf and Weber, 2000) defined as:

$$\text{Hysteresis Index (HI)} = \frac{q_e^d - q_e^s}{q_e^s} | T, C_e \quad (6.1)$$

where q_e^s and q_e^d are solid-phase solute concentrations for the single-cycle sorption and desorption experiments, respectively, and the subscripts T and C_e specify conditions of constant room temperature (approximately 23°C) and residual solution phase concentration

In addition to partitioning in freshly contaminated soils, partitioning in soils containing a desorption-resistant fraction was also calculated. More detailed calculation was described in the previous CB study (Lee *et al.*, 2001c). The approach of Kan and Tomson (Kan *et al.*, 1997, 1998) was used to estimate partitioning. Their proposed biphasic adsorption model is as follows:

$$q = q^{rv} + q''' \quad (6.2)$$

where, q^{rv} = the concentration of sorbed compound in the reversible fraction

q''' = the concentration of sorbed compound in the desorption-resistant fraction

The sorption to desorption-resistant fraction is represented by a Langmuir-type sorption isotherm:

$$q''' = \frac{K_{OC}''' \cdot OC \cdot q_{\max}''' \cdot f \cdot C}{q_{\max}''' \cdot f + K_{OC}''' \cdot OC \cdot C} \quad (6.3)$$

where, K_{OC}''' = partition coefficient of the desorption-resistant fraction

OC = organic carbon content (as a fraction)

f = fraction of the compound residing in the desorption-resistant compartment

q_{\max}''' = maximum capacity of the desorption-resistant fraction.

A semi-empirical relationship was used to estimate q_{\max}^{irr} as follows:

$$q_{\max}^{irr} \approx 37765 \cdot OC \cdot K_{ow}^{-0.23} \quad (6.4)$$

Microbial Culture

The source of the microbial culture was 1,3-dichlorobenzene-degrading culture enriched from sewage sludge from the Central Wastewater Treatment Plant in Baton Rouge, LA using liquid nutrient media previously described by Dorn *et al.* (1974). Prior to use in bioavailability assays, the undefined mixed culture of 1,3-DCB degrading microorganisms were grown in the same mineral media substituting 3 mg of 1,4-DCB per liter for 1,3-DCB. Organisms were repeatedly transferred to fresh liquid mineral medium and cultured for approximately one-month prior to the initiation of mineralization studies. A preliminary experiment (data not shown) indicated that the cultivated organisms were capable of utilizing 1,4-DCB as a sole carbon and energy source.

Mineralization Studies

The mineralization experiments were set up to evaluate the availability of soil-sorbed 1,4-DCB to 1,4-DCB-degrading bacteria. These assays were adapted from the method described by Guerin and Boyd (1992) and Feng *et al.* (2000). More details are provided in our previous paper (Lee *et al.*, 2001c). All treatments for the mineralization experiments were set up in 200-mL capacity flasks capped with Teflon-lined silicon rubber septa (VWR Scientific, Sugar Land, TX). Flasks were placed on a rotary shaker at 100 rpm and maintained at approximately 25°C.

The sampling procedure was conducted in a temperature-controlled room at 5°C to minimize 1,4-DCB volatilization losses. At various time intervals, the contents of the

base trap were removed from the reactor, and 1 ml of the base solution was removed and counted by liquid scintillation to quantify $^{14}\text{CO}_2$. Two ml of 1N NaOH were replaced each time sampling was conducted.

Table 6.2. Summary for the preparation of mineralization study

Treatments	Soil Type	Aging (d)	Soil Wt.(g)	Nutrients (mL)	Total mass of 1,4-DCB per reactor (mg)	R _{s/w} (kg/L)
Soil-free		0	0	20	0.020	0
Freshly-contaminated	PPI	2	8.25	15	0.045	0.35
	Top*	2	1.76	15	0.911	0.05
	Deeper*	2	1.76	15	0.784	0.05
Desorption-resistant	PPI	2	7.52	15	0.025	0.32
		7	7.10	15	0.034	0.31
		31	8.01	15	0.094	0.34
	Marsh (Top* layer)	2	1.24	15	1.770	0.04
		7	1.14	15	0.898	0.04
		31	1.23	15	0.750	0.04
	Marsh (Deeper* layer)	2	1.24	15	0.822	0.04
		7	1.14	15	0.923	0.04
		31	1.23	15	0.810	0.04

* Refers to layer of Marsh soil, Madisonville, LA.

Table 6.2 summarizes the details of the mineralization studies. Two mineralization experiments were conducted: (i) an experiment determining the mineralization of freshly added 1,4-DCB in the three soils; (ii) an experiment determining the mineralization of the desorption-resistant fraction of 1,4-DCB in the three soils.

For experiments comparing the effect of sorption on mineralization, 60 g of PPI soil and 60 g of the two marsh soils were added into replicate 125-mL capacity glass

bottles (I-Chem). An aqueous solution spiked with 1,4-DCB (~5 mg/L) and ^{14}C -1,4-DCB as a tracer was added to each bottle, and the bottles were mixed vigorously, then filled to capacity with minimal headspace, and capped with Teflon fluorocarbon resin/silicon septa. The bottles were equilibrated in a tumbler for 2 days at 80 rpm. The bottles were then centrifuged to obtain a clear soil-water interface. These bottles allowed centrifugation at low speeds to eliminate volatilization during subsequent extraction and analysis (see previous section). The supernatant was then decanted off. The soil was then used directly, after determining the soil activity of ^{14}C -1,4-DCB on a separate aliquot using a biological oxidizer (Model OX600, R.J. Harvey Instrument Corporation, Hillsdale, NJ). Soil (8.25 g and 1.76 g of PPI and marsh soils, respectively, on a dry weight basis) was added into the reactor immediately in a temperature-controlled room to minimize volatilization. Mineralization was then determined as described above.

For the experiment measuring the mineralization of the desorption-resistant fraction of 1,4-DCB, the soil was further prepared using the isopropanol cosolvent extraction method described by Liu *et al.* (1999). In this method, equal volumes of isopropanol and electrolytes (0.01 M NaCl, 0.01 M CaCl_2) were used to prepare an extraction solution. Soils were contaminated as described above using 20 mg/L of initial concentration of 1,4-DCB. The soil containing sorbed 1,4-DCB was stored in the dark in *I-Chem* bottles for a designated time before it was used. Following the aging period, contaminated soil was mixed vigorously with the co-solvent solution (120 ml/g of soil) in a tumbler for 24 hours, and then the soil separated via centrifugation. The separated soil was rinsed with electrolyte two times to remove the residual iso-propanol and then

mixed with the co-solvent solution again, and placed in a tumbler for another 24 hours. An additional rinse with electrolytes was conducted, and 1,4-DCB concentration in the aqueous phase was measured. The 1,4-DCB concentration in the soil phase was determined using the biological oxidizer.

Data Analysis for Biodegradation Studies

Mineralization data were fit to first-order CO₂ production equation:

$$P = P_{\max} \cdot (1 - e^{-k \cdot t}) \quad (6.5)$$

where P is the percentage of initial radioactivity mineralized, P_{\max} is the maximal percentile mineralized, k is the first-order rate constant, and t is time (hour). The parameters derived from this model were used to describe the mineralization kinetics of sorbed and nonsorbed 1,4-DCB in systems. Since ¹⁴C-labeled 1,4-DCB present in biomass was not measured at each sampling interval, results are minimal estimates of degradation.

An alternative model, the coupled degradation-desorption (CDD) model, suggested by Guerin and Boyd (1992, 1997) was also utilized. It is expressed as:

$$P = v_1 t + [(v_1 + v_2)(1 - e^{-k \cdot t})] / k \quad (6.6)$$

where v_1 represents the initial rate (percent hour⁻¹). The value of v_1/k (%) gives the extent of mineralization after the initial, exponential phase of mineralization. The parameter of v_1/k is the equivalent of P_{\max} and v_2 represents the mineralization rate resulting from desorption of bound 1,4-DCB and is related to the kinetics of 1,4-DCB desorption. And P is the percentile of 1,4-DCB mineralized as a function of time, t . The parameter k (hour⁻¹) is the first-order rate constant.

Zhang *et al.* (1998) used a bioavailability factor (B_f , dimensionless) to understand the impact of sorption and desorption behavior on biodegradation in batch systems. It is expressed using the following equation:

$$B_f = \frac{1}{1 + K_d \cdot m/v_l} \quad (6.7)$$

where K_d can be obtained from sorption isotherms described in our previous CB study, and m/v_l is the soil/water ratio ($\text{kg} \cdot \text{L}^{-1}$). Fitted parameters from sorption isotherms were used as estimates of K_d for mineralization experiments using freshly added 1,4-DCB. For the mineralization experiments using desorption-resistant 1,4-DCB, partition coefficients of the desorption-resistant fraction K_d^{irr} , were obtained from the sorption models proposed by Kan *et al.*, (1997 and 1998).

Feng *et al.* (2000) developed equations by hypothesizing possible relationships between desorption behavior and mineralization. These equations are expressed as:

$$P_d = P_{\max} \cdot (1 - e^{-B_f \cdot k \cdot t}) \quad (6.8)$$

$$P_{nd} = B_f \cdot P_{\max} \cdot (1 - e^{-k \cdot t}) \quad (6.9)$$

Both equations assume that only the aqueous phase contaminant can be mineralized. The assumption for equation 6.8 is that the contaminant is desorbed instantaneously while equation 6.9 assumes that no desorption occurs. P_d and P_{nd} (%) are the percentile of CO_2 production in the mineralization assay for instantaneous desorption case and the no desorption case, respectively.

Mineralization data for the experiments using freshly added and desorption-resistant 1,4-DCB was plotted together with the theoretical lines, P_d (equation 6.8) and P_{nd} (equation 6.9). If sorbed 1,4-DCB is unavailable to bacteria, the CO_2 production

values should be equal or less than P_d values. If the CO_2 production levels in the microcosms are above P_d values, this indicates that bacteria have access to sorbed 1,4-DCB. If the CO_2 production rate is below P_d values, this indicates bacteria do not have access to sorbed 1,4-DCB.

Results and Discussion

Sorption/ Desorption Isotherms

In the present study, both sorption/desorption isotherms of 1,4-DCB were not linear showing n values less than 1 with high correlation coefficients ranging from 0.87 to 0.95 (Table 6.3). And the nonlinear patterns of desorption isotherms presented significantly showing all values less than 0.7. The sorption times as a time that chemical contact with soil have been set up with 3 different equilibrium times, 2, 7, and 31 days. The contact times used to generate sorption isotherms typically range from 4 to 48 hours. However, these times may be inadequate for true equilibrium to be attained resulting in the determination of erroneous “equilibrium” constants (Brusseau *et al.*, 1991). Organic solutes have been found to exhibit a two-stage approach to equilibrium, with a short initial phase of rapid uptake/release followed by an extended period of much slower uptake/release occurring over periods of days or months.

For the soils contaminated with 1,4-DCB, partition coefficients, (K_{ds}) increased significantly from 13.24 to 39 L/kg in PPI soil, 219.62 to 332.90 L/kg in the surface marsh soil and 338.54 to 462.22 L/kg in the deeper marsh soil when the soil was aged from 2 to 31 days (Table 6.4). The measured organic carbon based distribution coefficients (K_{oc}) are 981, 796.3 and 1463 L/kg in the soil from PPI, top and deeper layer of Madisonville site, respectively. These values are in the range reported K_{oc} of

1258 L/kg (Montgomery, 1997) obtained from adsorption measurements done using 24 hr equilibration between soil and water.

Figures 6.1 through 6.3 show the sorption/desorption isotherms of 1,4-DCB, for the PPI soil, surface marsh soil and deeper marsh soil, respectively. Sorption conducted for 2, 7, 31 days. The desorbed amounts of 1,4-DCB decreased as the initial contact time with the 1,4-DCB increased regardless of soil types. For the PPI soil, approximately 71% of sorbed 1,4-DCB desorbed following 2 days contact time, whereas 55%, and 40% of 1,4-DCB in soil desorbed after 7 and 31 days contact time, respectively. The similar phenomenon was observed in both layers of soil from Madisonville site. As contact times increase from 2, 7 and 31 days in the top layer of soil in Madisonville site, 74%, 65%, and 45% of the sorbed fraction could be desorbed, respectively. Same phenomenon was observed in the deeper layer of soil from Madisonville site showing 50%, 50%, and 33% of desorbed percentages in three different aging period. It is noteworthy that desorbed fraction in soil phase was affected by the aging period accordingly. This “aging effect” was well described by Pignatello and Xing (1996) as a major controlling factor in desorption phenomenon because of enrichment of slow fraction. Further observation of aging effect in biodegradation was attempted by mineralization in the following section. This was clearly demonstrated by the Figures 6.1 through 6.3 that the amounts of 1,4-DCB, which is not easily desorbed fraction, were steadily increased as contact time increased.

As shown in figures 6.1 through 6.3, desorption hysteresis was observed in all tested soils, and results were demonstrated in Table 6.3. As increase of contact time soil has, more desorption hysteresis was observed except top layer of Madisonville soil

Table 6.3. Sorption and desorption isotherm parameters, calculated hysteresis indices

Soils	Sorption			Desorption					K _{oc} (L/kg)	Hysteresis Index
	Aging	logK _f	n	R ²	C _e range (mg/L)	N [*]	logK _f	n	R ²	N [*]
PPI	2	1.001	0.827	0.991	0.3-2.5	9	1.158	0.393	0.971	18
	7	1.163	0.801	0.991	0.2-2.0	9	1.315	0.283	0.980	18
	31	1.425	0.762	0.998	0.1-1.2	9	1.472	0.209	0.900	21
Marsh (Top layer)	2	2.168	0.752	0.900	0.1-1.0	9	2.228	0.628	0.927	33
	7	2.337	0.924	0.934	0.1-0.7	9	2.232	0.485	0.874	29
	31	2.484	0.945	0.999	0.1-0.5	9	2.344	0.527	0.972	24
Marsh (Deeper layer)	2	2.459	0.899	0.995	0.05-0.4	9	2.362	0.689	0.972	33
	7	2.431	0.817	0.962	0.05-0.4	9	2.342	0.590	0.978	39
	31	2.623	0.940	0.973	0.05-0.3	9	2.337	0.464	0.987	30

* Number of observations

Table 6.4. Parameter estimates of 1,4-DCB mineralization based on the simple first-order model and the coupled degradation-desorption model for the freshly contaminated PPI and Madisonville sites.

Soils	First-order model			Coupled degradation-desorption model				
	P_{\max}	k	R^2	v_1	v_2	k	v_1/k	R^2
PPI	28.206 (0.812)*	0.017 (0.002)	0.977	0.513 (0.041)	0.015 (0.004)	0.023 (0.003)	22.304	0.99
Marsh (Top layer)	61.159 (2.475)	0.012 (0.002)	0.973	0.764 (0.089)	1.34×10^{-6} (0.034)	0.013 (0.004)	60.689	0.97
Marsh (Deeper layer)	46.186 (2.729)	0.011 (0.002)	0.952	0.553 (0.091)	1.28×10^{-6} (0.038)	0.012 (0.005)	45.275	0.94
Soil-free phase	30.762 (1.241)	0.026 (0.004)	0.938					

*Values in parentheses are standard errors of parameter estimates

equilibrated with test compound only for 2 days. It is interesting that most of desorption in PPI soil occurs in the first 5 desorption steps and the amount of desorbed test compounds decreases with incremental desorption steps. After fifth step, a very small amount (<5% per desorption step) of test compounds desorbs with each dilution. This fact is in accordance with the observation in phenanthrene and naphthalene studies by Fu *et al.*, (1994), who found desorption hysteresis in soils. However, more desorption steps were required for the freshwater marsh soil located in Madisonville, LA.

It is noteworthy that the contact time of 1,4-DCB on soil increases, more amounts of the chemicals sorbed and thus the amounts of desorption resistant fraction increased. And this phenomenon might drive desorption hysteresis. In this study, hysteresis was occurred, however, did not increase as equilibrium times increases accordingly. This might due to the sorbed fraction of 1,4-DCB in soil particle was also increased as equilibrium time increases.

The phenomenon showing the increased extents of desorption-resistant fraction in soil containing longer contact time with test compounds may be explained by the following hypotheses proposed by several previous studies. The aged or desorption-resistant fraction of organic compounds may result from the slow diffusion of molecules within some components of solid organic matter in soils (Brusseau *et al.*, 1991), or entrapment, limited diffusion and retardation of chemicals from micropores in soil aggregates by partitioning of chemical between pore water and organic matter on pore walls (Steinberg, *et al.*, 1987). Kan *et al.*, (1997) proposed that the phenomenon may be due to the occlusion of chemicals by a cooperative conformational changes of the

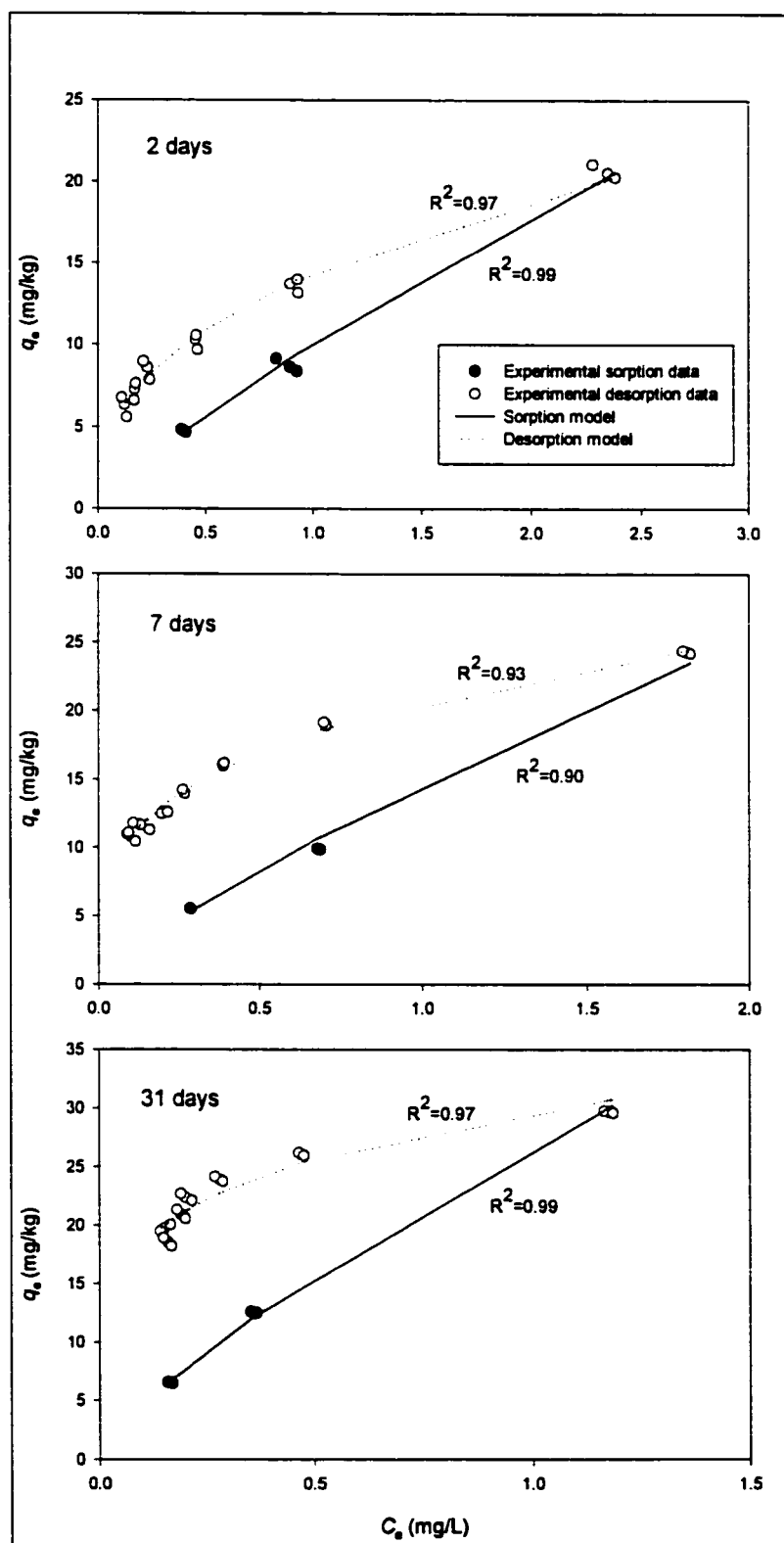


Figure 6.1. Sorption and desorption isotherms for the soil from PPI site as a function of contact times.

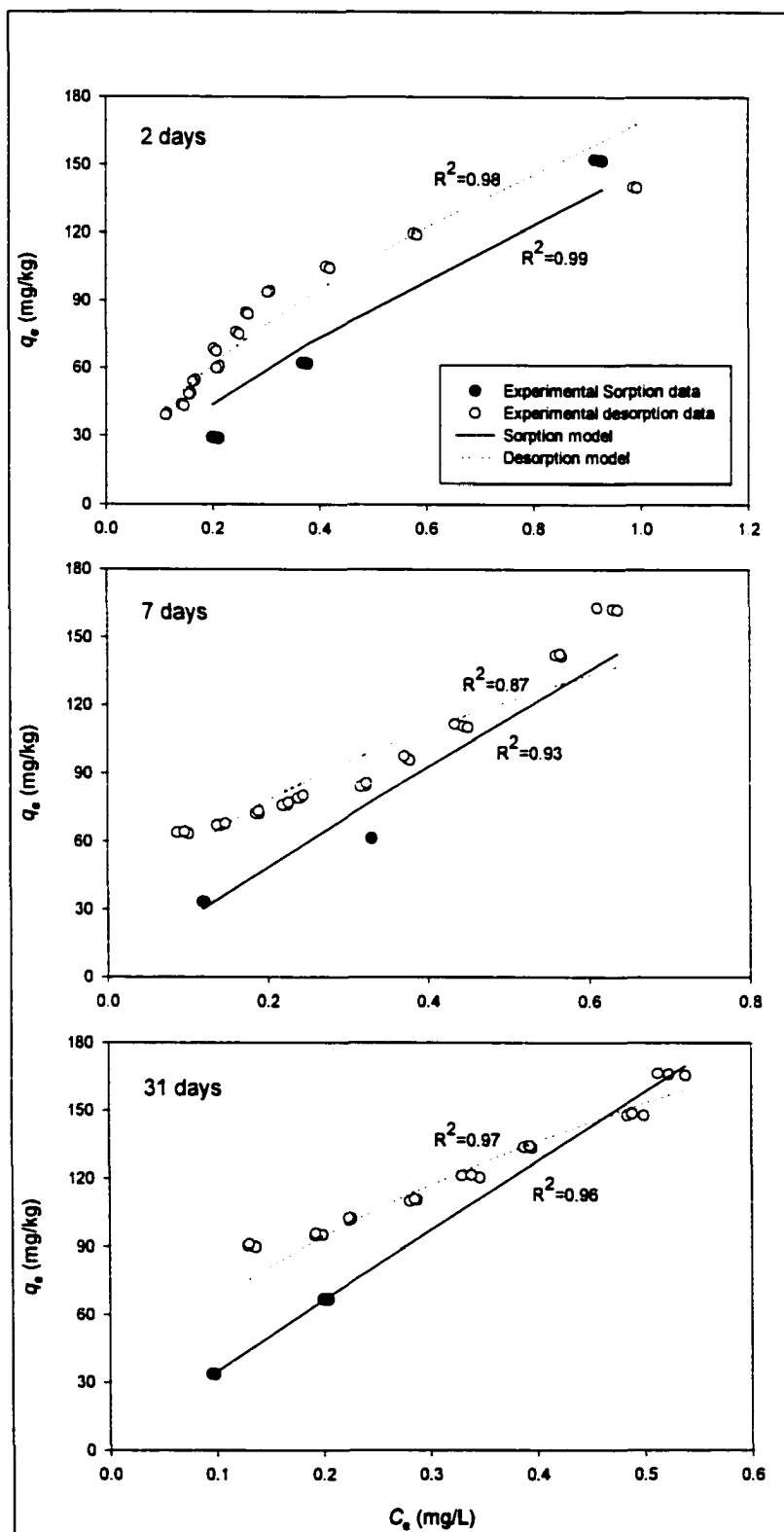


Figure 6.2. Sorption and desorption isotherms for the top layer of Madisonville site as a function of contact times

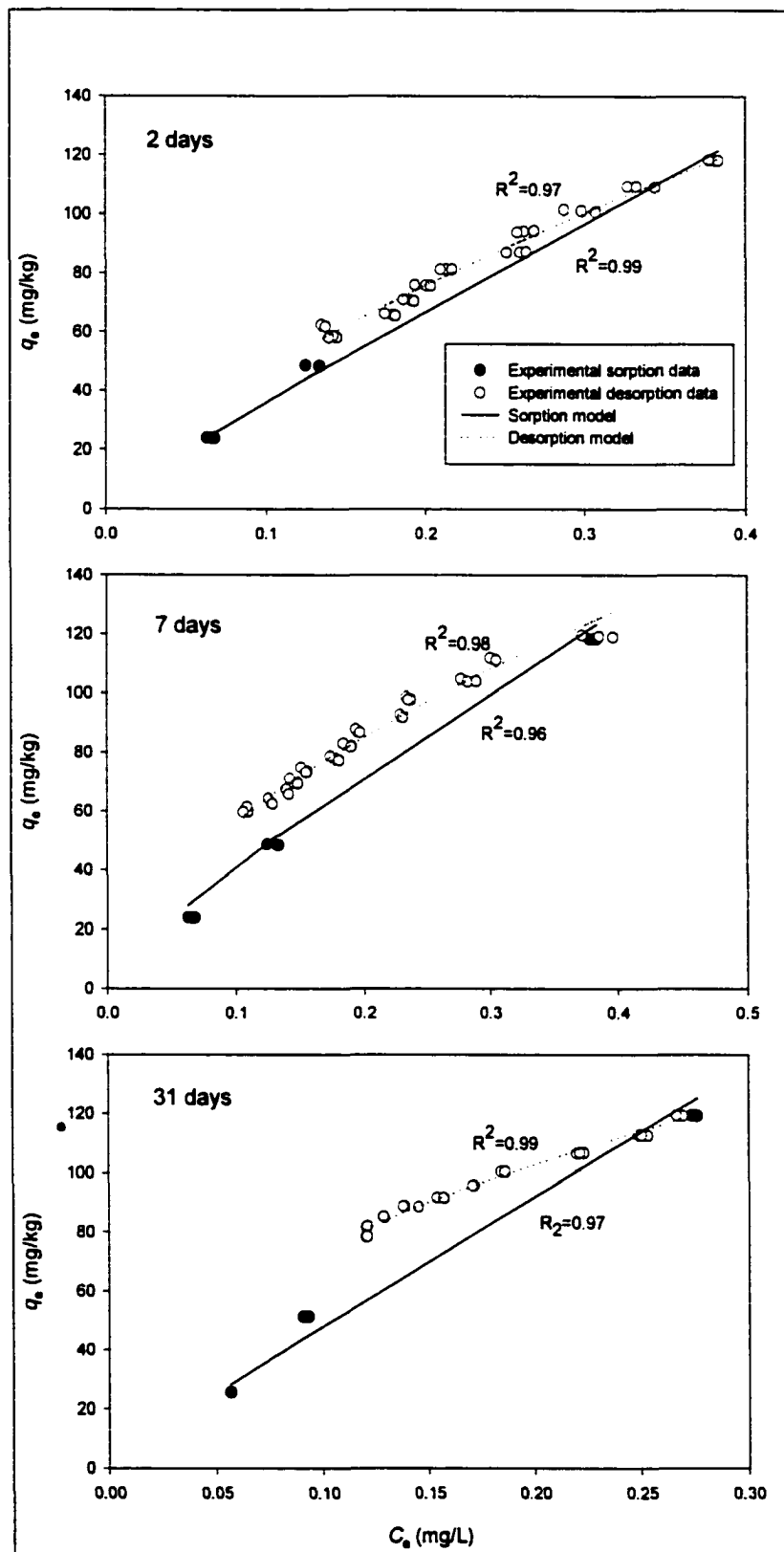


Figure 6.3. Sorption and desorption isotherms for the deeper layer of Madisonville site as a function of contact times.

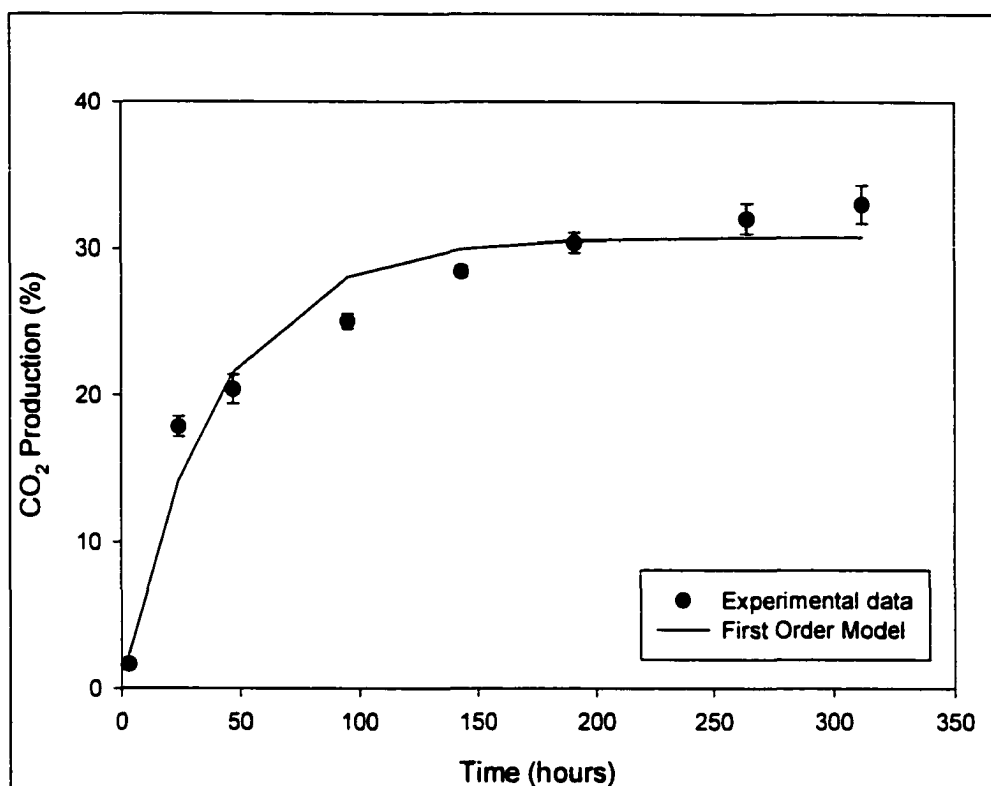


Figure 6.4. 1,4-DCB mineralization in soil-free phase as a function of time.

organic matter during the adsorption process or due to physical rearrangement of the organic matter phase. And also the physical characteristics of soil organic matter in soil, whether it composed of soft carbon domains or hard carbon domains, can attribute to sorption/desorption rates (Lueking *et al.*, 2000).

Mineralization Studies

Effect of sorption

To monitor the effect of sorption on biodegradation of 1,4-DCB, the rate and extent of mineralization was measured in soils containing freshly added 1,4-DCB. Results were analyzed with the aid of empirical first order and CDD models developed by Guerin and Boyd (1992, 1997). These two models fit very well with experimental data in tested soils ($R^2 > 0.94$).

In the absence of soil, the 1,4-DCB degrading culture mineralized the compound with the following fitted parameters ($P_{\max} =$; $k = 0.026 \text{ hr}^{-1}$). The observed maximum mineralization rates, P_{\max} , were 28.2, 61.2, and 46.2 % in PPI and surface and deeper layer of marsh soil, respectively (Figure 6.5). First order rate constants were 0.017 hr^{-1} , 0.012 hr^{-1} , 0.011 hr^{-1} for PPI soil, surface and deeper layer of marsh soil, respectively. Rate constants were not statistically different from one another although all were lower .

Mineralization data was also fit to the coupled desorption and degradation (CDD) model. Values for v_1/k were similar to P_{\max} , in all soil types indicating agreement with the fitted first-order parameters. As shown in Table 6.4, the initial mineralization rate, v_1 , showed a higher rate in marsh soil versus the PPI soil, indicating a faster rate of degradation in the immediately accessible pool of substrate in marsh soil. Values of v_2 were low relative to v_1 , confirming that desorption did not contribute significantly to the 1,4-DCB mineralization observed in both soils, especially in marsh soil as observed in previous study (Lee *et al.*, 2001c). This simple three-parameter model provides excellent fits to our experimental data for the PPI soil ($R^2 > 0.99$), and a reasonably good fit ($R^2 > 0.94$) in freshwater marsh soil (Table 6.4). Despite the good fits, decreases in the residual sum of squares from this 3-parameter model were not substantial enough to improve the prediction obtained from the 2-parameter first-order CO_2 production model.

To evaluate sorbed 1,4-DCB bioavailability, experimental data were plotted together with theoretical lines, P_d (eq. 6.8) and P_{nd} (eq. 6.9) as shown in Figure 6.5. If sorbed 1,4-DCB is not available to bacteria and only 1,4-DCB in the aqueous phase can be degraded in soil slurries, the amount of initial CO_2 production should be equal or less

than P_d , which accounts for instantaneous desorption, and should be above P_{nd} , which assumes no desorption. As shown in Figure 6.5, the initial CO_2 production levels in soil slurries are above P_d and P_{nd} values, this indicates that mineralization rates are faster than would be expected based on liquid-phase concentrations, possibly indicating that bacteria have access to sorbed 1,4-DCB in all soil phases. In other words, the degradation rates exceeded that which would be expected with instantaneous desorption.

Effect of Desorption-Resistance and Aging

To understand the effects of desorption-resistance and aging on the rate of biodegradation, an experiment was conducted with soils that was artificially “aged” containing only desorption resistant 1,4-DCB by extracted with an iso-propanol co-solvent to remove the remaining reversibly-bound portion of 1,4-DCB. Two hypotheses were tested in this experiment. An initial hypothesis was that removing the readily desorbable 1,4-DCB would result in significantly decreased rates and extent of mineralization. Secondly, we hypothesized that aging time would cause decreased extents of mineralization.

The initial hypothesis was proved by comparing of P_{\max} and k values derived from the freshly added 1,4-DCB experiment with the desorption-resistant experiment (Tables 6.4 and 6.5). Maximum mineralization rates in freshly contaminated soil and soil containing desorption-resistant fraction going through same equilibrium times as a 2 days were approximately 28% and 24% in PPI soil, 61% and 37% in soil from top layer, and 46%, and 38% in soil from a deeper layer in marsh soil (Table 6.4 and 6.5). No significant differences in k , first order rate constant, in freshly contaminated fraction

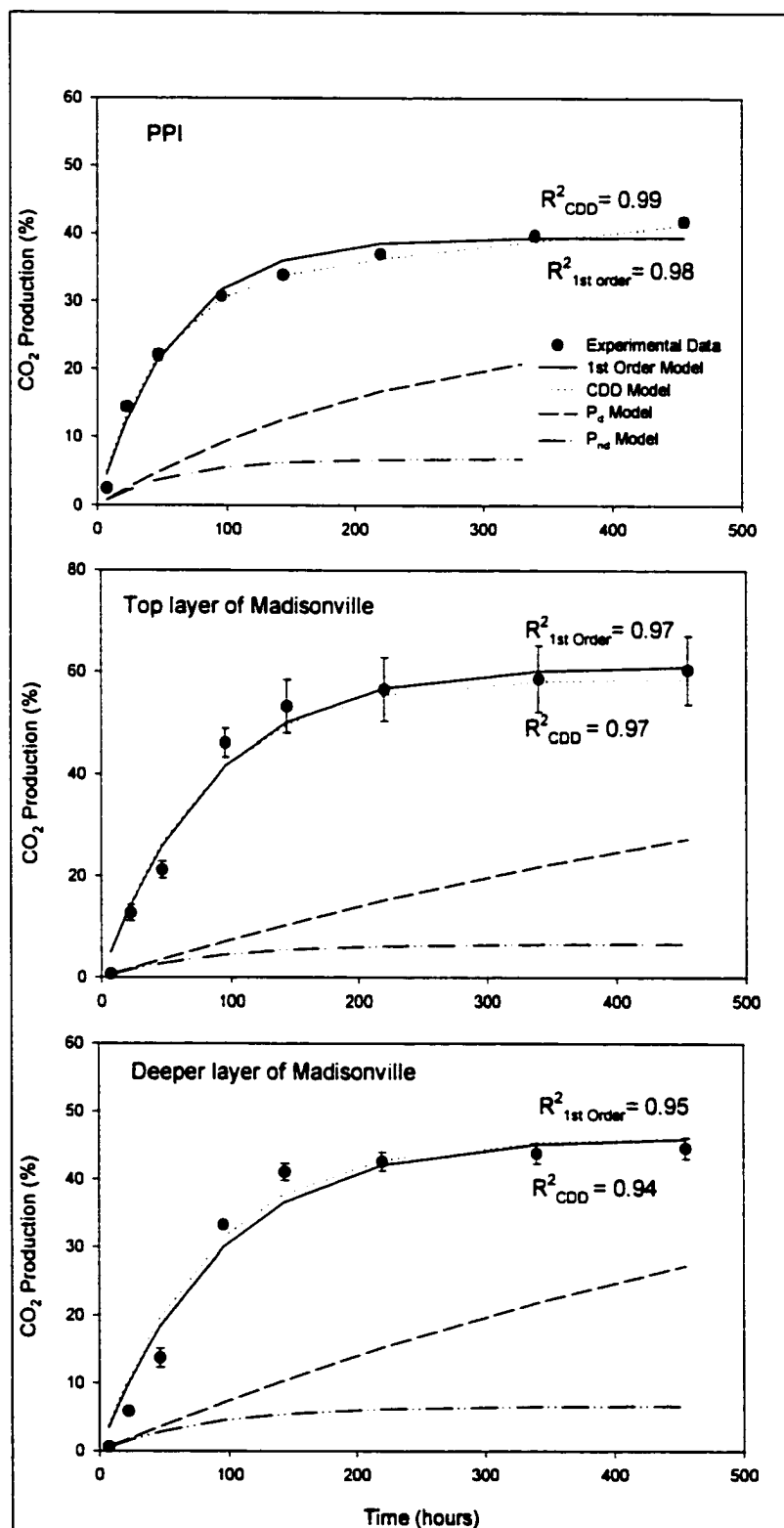


Figure 6.5. Plot for the mineralization versus incubation time in freshly contaminated soil with 1,4-DCB from PPI and Madisonville sites.

and desorption resistant fraction were observed in marsh soil, but in PPI soil showing higher value in easily desorbed fraction in soil than the other. This may indicate that the mass transfer of 1,4-DCB in PPI soil affected significantly on biodegradation rate.

The effect of mass transfer in soil phase was well described in CDD model. The values of initial mineralization rates, v_i , were higher in freshly contaminated soil than the one containing only desorption-resistant fraction (Tables 6.4 and 6.5). This might explain that mass transfer of 1,4-DCB into bulk phase would cause decrease in mineralization rates. In other words, this indicates that the mineralization extents were decreased as limitations of desorption occurs. It would be concluded that bioavailability can be affected by desorption process.

After aging for 2, 7, and 31 days, iso-propanol extraction yielded different final concentrations for the PPI soil (2.25 mg/kg, 3.3 mg/kg and 8.0 mg/kg). And marsh soil concentrations were more similar with 57.1 mg/kg, 66.2 mg/kg and 51.2 mg/kg for top layer, and 61.0 mg/kg, 74.5 mg/kg and 60.6 mg/kg for deeper layer of marsh soil after 2, 7 and 31 days, respectively. As aging time increased, the extents of 1,4-DCB in desorption-resistant fraction in PPI soil increased gradually, but not in marsh soil. This result was in accordance with our previous study conducted with chlorobenzene (Lee *et al.* 2001c).

The effects of aging on 1,4-DCB mineralization were significant in both soils. Figures 6.6, 6.7 and 6.8 depict mineralization rates in each soil containing only artificially “aged” and desorption-resistant fraction with theoretical curves developed from first-order, CDD, P_d , and P_{nd} models. Experimental data were well fitted with first-order and CDD models with high R^2 . However, parameter estimates were often

poor with high standard errors exceeding the value of the parameter, itself. Estimated parameters (P_{\max} and k) from the first-order CO_2 production model also indicated significant differences depending on age of the contaminant. The P_{\max} values are 23.9, 22.1, 21.6% for PPI soil, 37.4, 27.0, 21.9% for top layer and 38.3%, 22.0%, 21.6% for the deeper layer of marsh soil with aging of 2, 7, 31 days, respectively (Table 6.5). Significant decreases in the values of k , first-order rate constants, were observed in marsh soils, but not in PPI as aging time increases.

However, estimated parameters (v_1 , v_2 , k) from CDD model explained the differences in each soil type more in detail. The values of initial mineralization rates, v_1 , decrease as the age of 1,4-DCB increases in all soil types. Values of v_2 representing the mineralization rate resulting from the desorption of bound 1,4-DCB, were low relative to v_1 , confirming the desorption did not contribute significantly to the 1,4-DCB mineralization. And the values of v_2 , was also decreased as aging increases in PPI soil, not in marsh soil. Values of k , representing rate constant, decreases as aging of 1,4-DCB bound to soil increases. This indicates mass transfer of 1,4-DCB tightly bounded soils affected by decreasing mineralization rate. Based on these findings, consensus may have been reached with general theory that aging served to decrease overall mineralization rates and extents.

And sorbed 1,4-DCB in soils was still available to bacteria that have accessed to sorbed 1,4-DCB even if sequestration caused by aging was occurred. This was again proved by the experimental data located above the P_d and P_{nd} theoretical curves in Figures 6.6, 6.7, and 6.8. The higher partitioning coefficient for the desorption-resistant fraction results in the very low P_d and P_{nd} curves. This indicates that the tightly

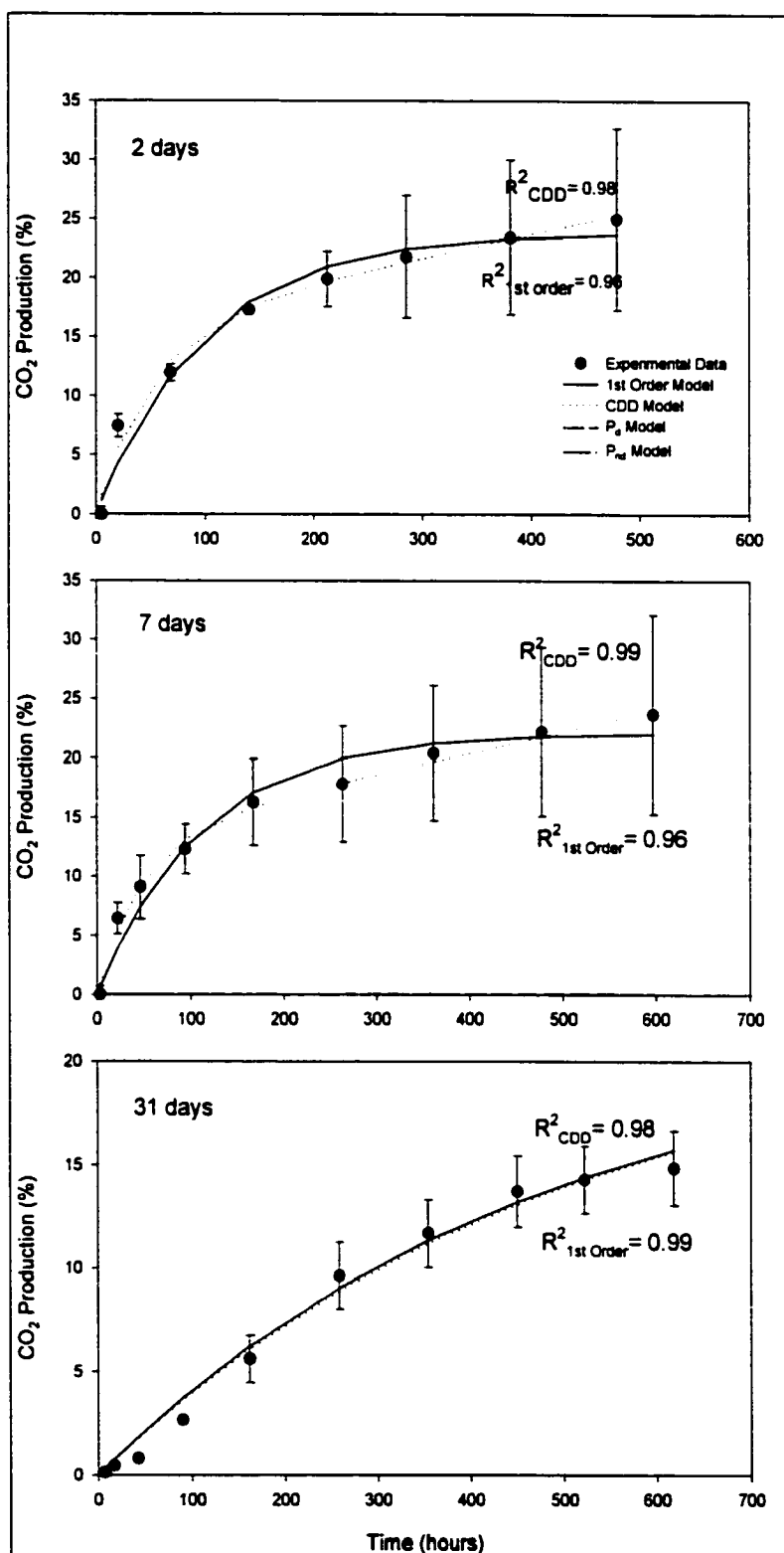


Figure 6.6. Mineralization of 1,4-DCB in soil from PPI site containing only desorption-resistant fraction as a function of contact times.

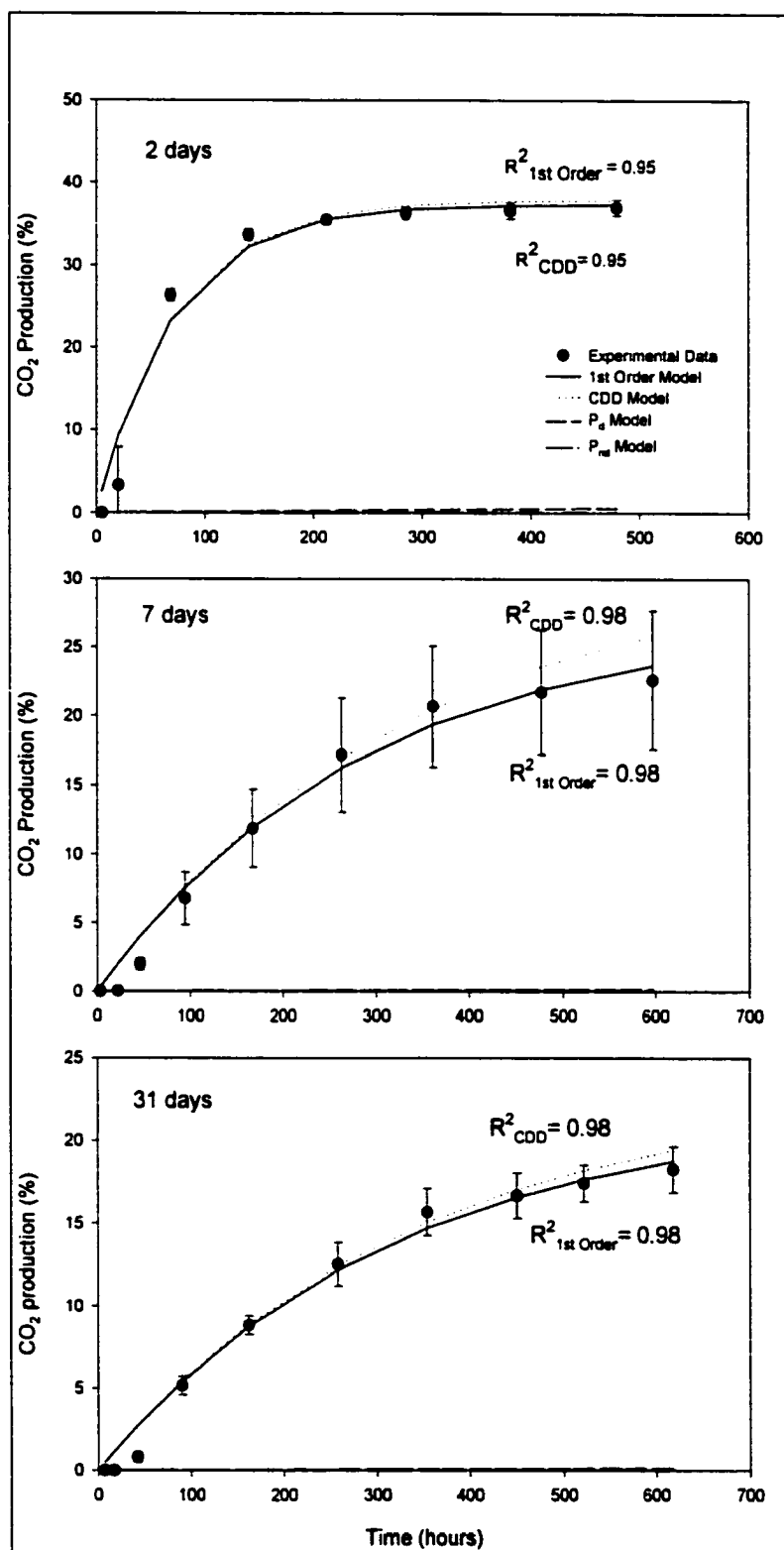


Figure 6.7. Mineralization of 1,4-DCB in deeper layer of soil from Madisonville site containing only desorption-resistant fraction as a function of contact times.

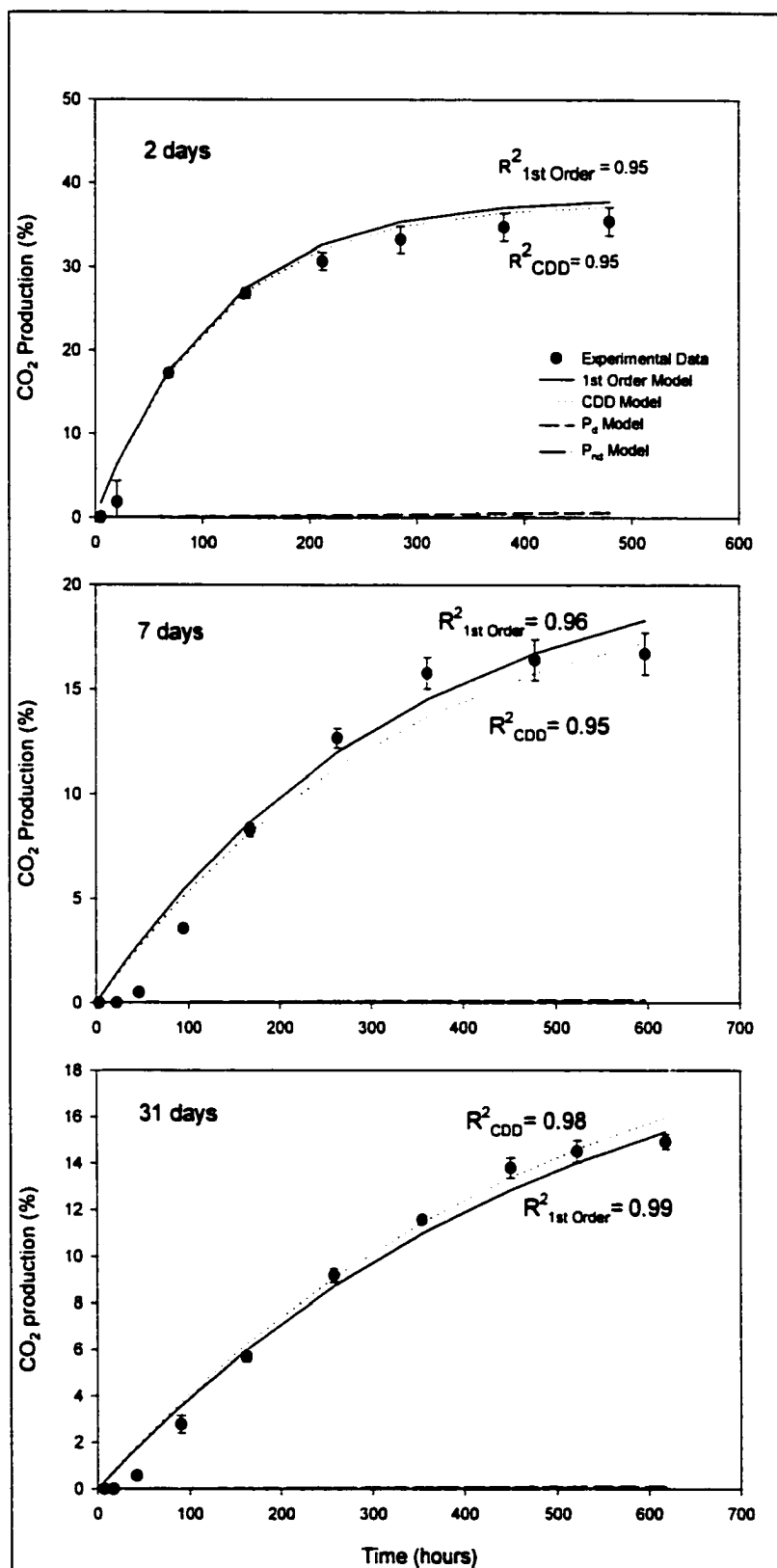


Figure 6.8. Mineralization of 1,4-DCB in deeper layer of soil from Madisonville site containing only desorption-resistant fraction as a function of contact times.

sorbed 1,4-DCB in aged soils was still available by 1,4-DCB degrading bacteria and the extents and rates of availability were decreased by increases in aging. For the calculation of partition coefficients, K_d''' , were determined both experimentally and calculated from equations 6.2 through 6.4 and used to estimate B_f values. According to the results of this experiment, it is concluded that desorption-resistant 1,4-DCB was still accessible by this degrading culture despite decreases in porewater 1,4-DCB concentrations by 3 orders of magnitude (0.41 to 1.79×10^{-4} mg/L for PPI, 0.265 to 0.0052 mg/L for top layer and 0.282 to 0.0056 mg/L for deeper layer of marsh soil)

This study suggest that a larger portion of 1,4-DCB was present in a desorption resistant fraction in marsh soil containing high organic carbon contents. However, soil organic matter in marsh soil in this study might stimulate the biodegradation rates than in the PPI soil containing less organic carbon contents as a carbon source. This indicates that other factors besides of organic carbon contents might be involved in biodegradation processes in this study. In addition, desorption rates in marsh soil was more linear than PPI soil when the soil aging proceeds. This indicates that higher amounts of 1,4-DCB in bulk phase could be available for bacteria in soil-slurry phase in marsh soil. No significant differences in biodegradation were found in each layer in marsh soil.

As a conclusion, this study demonstrated that soil-sorbed 1,4-DCB in easily desorbed phase as well as desorption-resistant phase was both available to 1,4-DCB degrading bacteria. The extent of bioavailability of soil-sorbed 1,4-DCB decreased as increases in aging, which was in a good agreement with previous findings (Hatzinger and Alexander, 1995; Kelsey, *et al.*, 1997; Nam *et al.*, 1998; Lee *et al.*, 2001c).

Table 6.5. Parameter estimates of 1,4-DCB mineralization based on the simple first-order model and coupled degradation-desorption model for the soils containing only desorption-resistant 1,4-DCB and artificially “aged”.

Soils	Aging (d)	First-order model				Coupled degradation-desorption model					
		P_{\max} (%)	k (hr ⁻¹)	RSS	R ²	v_1	v_2	k	v_1/k	RSS	R ²
PPI	2	23.871 (1.150)*	0.001 (0.002)	1.588	0.960	0.293 (0.058)	0.020 (0.006)	0.020 (0.006)	14.65	1.232	0.975
	7	22.116 (0.873)	0.009 (0.001)	1.524	0.957	0.267 (0.031)	0.018 (0.002)	0.022 (0.003)	12.14	0.709	0.990
	31	21.618 (2.996)	0.002 (0.001)	0.710	0.985	0.044 (0.105)	2.06x10 ⁻⁵ (0.056)	0.002 (0.004)	21.649	0.759	0.982
Marsh (top layer)	2	37.356 (1.736)	0.014 (0.003)	3.032	0.954	0.530 (0.104)	5.69x10 ⁻⁶ (0.022)	0.014 (0.006)	37.348	3.223	0.942
	7	26.992 (2.263)	0.004 (0.001)	1.244	0.980	0.093 (0.069)	4.36x10 ⁻⁶ (0.041)	0.003 (0.003)	31	1.330	0.977
	31	21.872 (1.828)	0.003 (0.001)	0.907	0.984	0.069 (0.055)	7.67x10 ⁻⁶ (0.033)	0.003 (0.003)	21.89	0.969	0.981
Marsh (deeper layer)	2	38.313 (1.646)	0.009 (0.001)	2.055	0.979	0.339 (0.042)	3.32x10 ⁻⁶ (0.028)	0.009 (0.003)	38.292	2.251	0.973
	7	21.952 (3.847)	0.003 (0.001)	1.446	0.955	0.062 (0.131)	6.57x10 ⁻⁶ (0.074)	0.003 (0.007)	22.049	1.562	0.946
	31	21.618 (2.996)	0.002 (0.001)	0.710	0.985	0.045 (0.105)	1.22x10 ⁻⁵ (0.056)	0.002 (0.005)	21.710 2	0.759	0.982

* Values in parentheses are standard errors of parameter estimates

Conclusions

Sorption and desorption isotherm study with the aid of Freundlich sorption model was conducted in soils containing different organic carbon contents with various aging. Non-linear isotherms patterns were observed in sorption and desorption by finding n values less than 1. This study did not support general theory that the desorption hysteresis in soils containing high organic matter occurs more significantly, and drives limitation of biodegradation. Hysteresis was occurred not in accordance with the aging based on the values of hysteresis index, but was occurred more significantly in PPI soil containing low contents of organic carbon contents than marsh soil. Desorption isotherm study proved that desorption pattern in marsh soil were more linear than in PPI soil regardless of aging. And no distinctive differences in sorption and desorption isotherms were observed in top and deeper layer in marsh soil. These relatively linear desorption behavior would drive higher biodegradation rates in marsh soil by increasing 1,4-DCB concentrations in bulk phase which was available to bacteria more easily. Reduced availability in aged soil, suggested by greater sorption in both soils, may explain persistence of 1,4-DCB in soils.

The facts that extents of mineralization in marsh soil containing freshly added 1,4-DCB were higher than in the PPI soil can indicate that organic carbon originated from soil organic matter can involved both negatively and positively in biodegradation process by providing energy source for bacteria or interference of bioavailability through sorption process.

This study was also proved that extents and rates of biodegradation was affected by desorption and aging. Both rates and extents of 1,4-DCB mineralization by bacteria

that were initially able to degrade sorbed 1,4-DCB, decreased as aging periods increased, for all soils. This suggests an increase in the nonlabile pool of 1,4-DCB during aging and that access to this pool is limited. This study may have some implications for bioremediation in sites contaminated with chlorinated hydrophobic compounds that existed for a prolonged time.

CHAPTER 7 CONCLUSIONS

A considerable amount of research has shown that sorbed organic contaminants are more resistant to biodegradation than soluble organic contaminants. Until recently, it was generally considered that sorbed chemicals were unavailable to microorganisms unless desorption occurred first. Although, microorganisms have been recently observed that degrade soil-sorbed contaminants, several researchers have confirmed that biodegradation can be limited by the slow rate of desorption of organic compounds.

Two separate but related desorption effects have received much recent interest. First, an “aging” effect is observed where the rate and extent of desorption decreases as the age of contamination increases. This phenomenon occurs because of enrichment of a slowly-desorbing fraction owing to partial dissipation or biodegradation of more labile fractions. The aged fraction of organic compounds may result from the slow diffusion of these molecules within some components of solid organic matter in soils. A second effect is the “desorption-resistance” observed where the rate and extent of biodegradation decreases because of slow release of desorption. Studies were conducted to assess the bioavailability of these desorption-resistant contaminants.

Studies were initiated to understand estimate rates of sorption and desorption processes of hydrophobic organic compounds in 2 different types of wetland soils: a silty PPI soil and silty-clayey Bluebonnet swamp soil. Trichloroethylene (TCE) and 1,3-dichlorobenzene (1,3-DCB) were used as test compounds. The effect of the age of soil contamination was studied using a laboratory-spiked soil incubated for 3 days, 3 months, and 5 months with the aid of an empirical non-linear model. The model described the biphasic nature of desorption with one fraction (labile) being released in

relatively short periods of time (typically 24-100 hr) and a second fraction (non-labile or irreversible) being resistant to desorption. The labile fraction had desorption rate constants of the order of 10^{-1} h^{-1} , whereas the slowly released fraction had rate constants of the order of 10^{-4} h^{-1} in accord with literature reported values for a variety of other compounds and soils.

Microcosm batch studies were conducted to study the biodegradation of 1,3-DCB in the aqueous (soil-free) and soil phase. Nearly all of the 1,3-DCB in the aqueous phase was biodegraded by enriched bacterial cultures within 7 days. The extent of biodegradation was different in freshly contaminated soil and soil containing only the desorption-resistant fraction. Only 55% of 1,3-DCB was degraded over a 4-week incubation period from the freshly contaminated soil. Degradation in the soil containing the desorption-resistant fraction of the contaminants was considerably lower (32%). It was clear that from the freshly-contaminated soil, 1,3-DCB readily desorbed into the aqueous phase and was bioavailable for microbial consumption whereas for the soils containing mostly the desorption-resistant 1,3-DCB, mass transfer into the aqueous phase limited the contaminant availability.

Biodegradation of TCE by toluene-degrading bacteria was measured under aerobic conditions in aqueous and soil-slurry batch microcosms. In aqueous phase experiments, the TCE biodegradation rates were approximately 270 and $310 \mu\text{g TCE} \cdot \text{L}^{-1} \cdot \text{hr}^{-1}$ for the first 2 hours following toluene addition in experiments with low (4.5 mg/L) and high (27 mg/L) initial toluene concentrations. An appreciable increase in the rate and extent of TCE biodegradation was observed in microcosms when toluene was added multiple times. Toluene dioxygenase (Tod) enzyme activity was monitored, and

TCE degradation was clearly correlated with Tod enzyme activity over time. In soil-slurry experiments containing freshly-contaminated soil, a TCE degradation rate of approximately $150 \mu\text{g TCE kg}^{-1}\cdot\text{hr}^{-1}$ was observed during the first 39-hour period, and then the TCE degradation rate slowed considerably to 0.59 and $0.89 \mu\text{g TCE kg}^{-1}\cdot\text{hr}^{-1}$ for microcosms receiving one and two additions of toluene, respectively. The TCE degradation rates in soil-slurry microcosms containing the desorption-resistant fraction of TCE contaminated soil were approximately 0.27 and $0.32 \mu\text{g TCE kg}^{-1}\cdot\text{hr}^{-1}$ in microcosms receiving one and two additions of toluene, respectively. It was clear from these results that mass transfer into the aqueous phase limited bioavailability of TCE in the contaminated soil. These results support the general hypothesis that the longer a contaminant is aged with soil, the lower the fraction of contaminant that is bioavailable.

The availability of sorbed chlorobenzene (CB) to degrading bacteria was assessed using mineralization assays in two wetland soils with contrasting properties. ^{14}C mineralization was influenced by moisture, with response depending on soil type. Increasing moisture resulted in more ^{14}C mineralization in PPI soil, but not in wetland soil. Mineralization curves for CB plotted above theoretical lines assuming instantaneous desorption followed by mineralization, providing evidence that CB-degrading populations have access to sorbed CB. Sorbed CB in soils was also accessible to bacteria due to its higher values of initial mineralization rates (IMRs) than that in the soil-free phase. Maximum mineralization rates (P_{max}) were higher in soils containing less organic carbon contents, but decreased as soil aged, suggesting that availability of sorbed CB was affected by prolonged aging and organic carbon content.

This study demonstrated that soil-sorbed CB was available to CB-degrading bacteria, and that the extent of bioavailability of soil-sorbed CB decreased with prolonged aging.

Sorption and desorption isotherm studies and microbial mineralization studies were also conducted in 1,4-DCB contaminated soils containing different organic carbon contents with various degrees of aging. According to a hysteresis index, hysteresis was not necessarily correlated with aging. Results also indicated that hysteresis occurred more significantly in PPI soil than marsh soil. Desorption isotherms demonstrated that desorption patterns in marsh soil were more linear than in PPI soil even when the contaminant was aged. These relatively linear desorption behavior would drive higher biodegradation rates in marsh soil by maintaining certain 1,4-DCB concentrations in bulk phase which were more easily available to bacteria. Sorbed 1,4-DCB was also found to be bioavailable to degrading populations. Both the rate and extent of 1,4-DCB mineralization decreased as aging periods increased, for all soils. Results indicated that the desorption-resistant 1,4-DCB was also bioavailable in both wetland soils.

The mechanisms for bioavailability were not addressed in these studies. However, several explanations are possible. First, bacterial populations may act as competitive sorption media, particularly those possessing hydrophobic cell surfaces (Guerin and Boyd, 1997). Bacterial cells may also acquire the substrate by physical contact with sorbed substrate, and sorbed contaminants on surface may also dissolve in the lipids at the bacterial surface (Feng *et al.*, 2000). Park *et al.* (2001) extended the role of bacteria with sorbed contaminants and suggested that the presence of bacteria may serve to extract the desorption-resistant fraction by producing a wide array of soluble organic materials that facilitate desorption. They also proposed an hypothesis

that the bacteria are able to degrade this material directly, without desorption through direct partitioning to the cell membrane or via degradation by extracellular enzymes. However, all these hypotheses have not been proven experimentally. In order to prove the proposed hypotheses, the surface chemistry and geometry of both cells and sorbents may be important determinants of the physical interactions between the mass transfer of organic contaminants and biodegradative bacteria.

In this study, mechanistic explanations for biodegradation process in sorbed phase were not demonstrated. However, some possible explanations can be suggested based on the experimental results. Diffusion of contaminants from micropores in desorption-resistant compartments may be retarded by the tortuous path through the pores. This retarded diffusion process can be a major limiting factor, which can determine bioavailability in aged soil containing desorption-resistant compartment. According to our results sorbed contaminants such as chlorobenzene and dichlorobenzenes were bioavailable, even when in the desorption-resistant phase. One mechanistic explanation is that bacterial cells were able to physically contact sorbed contaminants within these pores. Bacteria might be able to degrade directly sorbed contaminants on the pore wall surface that has relatively higher concentrations than in the bulk phase. As described in previous study (Feng *et al.*, 2000), understanding the bacterial physiology to degrade sorbed contaminants to elucidate the mechanisms of availability of soil-sorbed chemicals will be a crucial role in interpreting how physicochemical factors influence bioavailability in soils.

Few studies have addressed the biodegradation of contaminants in desorption-resistant fraction soils. In this study, biodegradation studies in soils containing a

defined desorption-resistant fraction were conducted. Experimental results suggest that biodegradation occurred both in freshly added, reversible contaminants in wetland soil as well as in desorption resistant fraction in soils. No significant differences in biodegradation were observed in different wetland soil types containing various organic fractions. The magnitude of the desorption-resistant effect was less in marsh soils containing higher organic carbon content. Experimental results show little evidence that difference in organic carbon content alone result in different mineralization behavior and this disproved our hypothesis that soil organic carbon content will decrease the bioavailability of freshly added and desorption-resistant contaminants. Studies also showed that the extent of bioavailability of soil-sorbed organic compounds decreased with aging but despite very large decreases in the porewater concentration biodegradation of organic compounds was still a significant fate process. Aging may decrease bioavailability and reduce rates, but degradation can potentially still proceed at environmentally relevant rates. This result is in accordance with the previous studies (Feng *et al.*, 2000) dealing with sorbed biphenyl that found decreased availability by aging.

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VITA

Sangjin Lee was born in October 12, 1965 in Pusan, South Korea. He attended Pukyung National University in Pusan, South Korea and received his B.S. degree in Marine Biology in February 1992.

In 1993, he ventured to attend the University of Louisiana in Lafayette, Louisiana majoring in Biology. He spent 2 years as a Lab instructor and research assistant. In 1996, he attended Northeastern University in Boston, Massachusetts where he obtained a Master of Science in Civil and Environmental Engineering in July 1998. He also spent a year as an Environmental Engineer in Deer Island Wastewater Treatment Plant in Winthrop, Massachusetts. He conducted several projects with project and program managers to assist operation of secondary treatment plant.

In 1998, he came to Louisiana State University to pursue a Ph.D. degree in department of Civil and Environmental Engineering under the direction of Professor John Pardue.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

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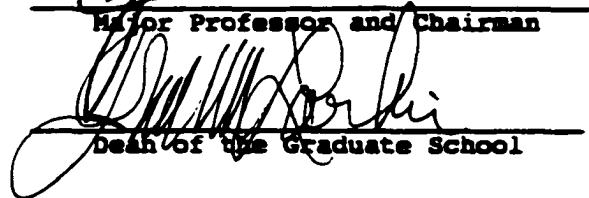
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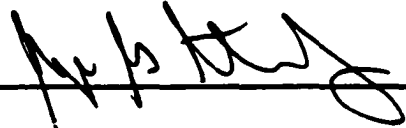


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