UNC-WRRI-94-287

Anaerobic Biodegradation of Hazardous Organics in Groundwater Down Gradient of a Sanitary Landfill

by
James J. Johnston, Morton A. Barlaz and Robert C. Borden
Department of Civil Engineering
College of Engineering
North Carolina State University
Raleigh, North Carolina 28695

The research on which this report is based was financed in part by the United States Department of Interior, Geological Survey, through the N.C. Water Resources Research Institute.

Contents of this publication do not necessarily reflect the views and policies of the United States Department of Interior, nor does mention of trade names or commercial products constitute their endorsement by the United States Government.

WRRI Project No. 70116
Agreement No. 14-08-0001-G2037
USGS Project No. 08 (Fy '92)
November 1994

One hundred fifty copies of this report were printed at a cost of $897.00 or $5.98 per copy.

ABSTRACT

The primary objectives of this investigation were to evaluate the distribution and anaerobic biodegradability of selected organic contaminants leaching from the Wilder's Grove landfill in Raleigh, NC. This facility is typical of sanitary landfills located in the Piedmont region of North Carolina and was constructed without an engineered liner or leachate control system.

The aquifer studied was shallow and unconfined and consisted of a saprolite material. The major organic compounds identified were c-dichloroethylene (c-DCE) and toluene. A tracer test utilizing chloride and bromide was conducted to characterize the hydraulic properties of the aquifer. The groundwater velocity was determined to be 6.4 cm/day in the vicinity of the study area.

A series of monitoring wells was installed along a single streamline and monitored to determine if there was evidence of contaminant biodegradation. Large temporal and spatial variations were observed in groundwater chemistry. These large variations are typical of leachate impacted aquifers and made it impossible to determine if biodegradation was a significant process in limiting contaminant migration.

The biodegradative potential of anaerobic aquifer sediment was further explored in laboratory experiments. Anaerobic aquifer sediment was obtained down gradient of the landfill and used to construct microcosms in which the degradability of benzene, toluene, ethylbenzene, meta- and ortho-xylene (BTEX) and trichloroethylene (TCE) was examined under ambient and amended conditions. The ambient condition study consisted of live microcosms and killed controls constructed using groundwater and aquifer sediment from each of three boreholes. Aquifer sediment and groundwater from one borehole were used to construct microcosms in which the potentially stimulatory effects of buffering, nutrient addition and availability of readily degradable carbon sources were tested. Benzene, ethylbenzene and xylene isomers were recalcitrant in both ambient and amendment experiments. Variations in TCE and toluene degradation in the ambient condition study indicated varied affinity for these compounds within the aquifer. TCE exhibited an inhibitory effect on toluene degradation at one location. Stimulatory effects of the three amendments tested were minimal if not negligible with respect to BTEX. Biotransformation of TCE was stimulated by buffering with calcium carbonate. TCE was converted to ethylene, a harmless byproduct, in several tests.

key words: anaerobic biodegradation, biotransformation, benzene, toluene, ethylbenzene, meta- and ortho-xylene, trichloroethylene, landfills, groundwater

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1.0 SUMMARY AND CONCLUSIONS

The anaerobic biodegradability of alkylbenzenes (benzene, toluene, ethylbenzene and xylene isomers or BTEX) and chlorinated aliphatic hydrocarbons (CAHs) leaching from a sanitary landfill was studied to evaluate the potential for these contaminants to naturally attenuate during transport through the subsurface. The site selected for study was the Wilder's Grove Sanitary Landfill in Raleigh, NC. This facility is typical of sanitary landfills located in the Piedmont region of North Carolina and was constructed without an engineered liner or leachate control system.

Field Characterization

The hydrogeology and contaminant distribution immediately down gradient of the refuse disposal area was characterized by a tracer test and periodic groundwater sampling. A series of wells was installed and monitored over a 6-month period to determine the spatial and temporal variation in BTEX, CAHs and indicator parameters. A non-reactive tracer test using chloride and bromide was performed to estimate the groundwater velocity in the study area and allow comparison of the laboratory degradation rates with the field monitoring results. While the chloride pulse was masked by the high background chloride concentrations, the appearance of bromide at a monitoring well immediately down gradient was used to estimate the groundwater velocity. The groundwater velocity estimated from the tracer test (0.07 m/d) is very similar to preliminary estimates of groundwater velocity (0.11 m/d) based on measurements of hydraulic conductivity and water table gradient.

Groundwater within the study area is highly reduced as evidenced by the absence of dissolved oxygen and nitrate, negative redox potential, and high concentrations of dissolved iron and manganese. The chemical oxygen demand (COD) is highest near the edge of refuse and lowest near the adjoining drainage channel indicating some attenuation of organic contaminants during transport through the subsurface.

Groundwater throughout the study area is contaminated with a variety of hazardous organic contaminants including benzene, toluene, ethylbenzene, xylene isomers, tetrachloroethylene (PCE), and cis- and trans-dichloroethylene (c-DCE and t-DCE). Benzene, toluene, PCE, and both c-DCE and t-DCE all exceed groundwater standards at one or more wells although the maximum benzene concentration is 5 µg/l. There was no evidence during the study of an increase or decrease in overall contaminant concentrations in the study area, although concentrations in individual wells did occasionally show trends.

A series of wells was installed and monitored along a single streamline to evaluate potential for contaminant attenuation during transport through the subsurface. Based on 6 months of monitoring over a 12-m distance, there is no evidence of significant contaminant attenuation. Over a larger scale, contaminant attenuation may or may not be evident.

Laboratory Studies

A laboratory microcosm study was conducted to evaluate the potential for natural attenuation of alkylbenzenes and chlorinated aliphatics under anaerobic conditions in the aquifer immediately down gradient of the landfill. Attenuation of compounds under ambient aquifer conditions was monitored in three sets of microcosms constructed using aquifer sediment and groundwater collected three meters apart along a groundwater flow path. In a second experiment, attempts were made to enhance biotransformation of compounds through the following amendments: buffering with calcium carbonate to pH 7.5, addition of nutrients

(ammonia chloride and potassium phosphate) and addition of easily degradable carbon (acetate, formate, benzoate, glucose and yeast extract).

Benzene, ethylbenzene and xylene isomers (BEX) appeared recalcitrant in microcosms in both sets of experiments. There may have been limited and localized biological activity towards BEX; however, any such activity was minimal (statistically insignificant in all but one case). Given the low total organic carbon (TOC) content of the soil (0.028%), sorption to the aquifer material in microcosms was negligible in comparison to sorption/diffusion into the stoppers. Buffering, nutrient addition and carbon supplements did not significantly stimulate biodegradation of BEX.

There was a varying affinity for toluene in the three sets of ambient microcosms. Pockets of toluene degraders apparently exist within the aquifer. However, complete biodegradation of toluene was rarely observed. Toluene-degrading microcosms rapidly degraded toluene to the 30 μg/l range after which degradation ceased. Toluene is one of the predominant hydrocarbons in the groundwater at the research site and the toluene degrading ability in the aquifer is highly variable.

TCE was completely removed from microcosms with complete or near complete conversion to ethylene through the process of reductive dechlorination. TCE removal was not observed in microcosms containing bromoethanosulfonic acid, a methanogen inhibitor, indicating that methanogens were involved in TCE removal. The groundwater contains sufficient carbon sources (electron donors) to support methanogenesis without external carbon addition. Buffering the groundwater to approximately pH 7.5 significantly enhanced the TCE biotransformation rate but was not necessary to achieve complete removal of TCE. Nutrient addition and supplemental carbon addition did not stimulate TCE biotransformation beyond buffering alone, although the rate of complete conversion to ethylene was increased.

CONCLUSIONS

1. The aquifer down gradient of the refuse disposal area at the Wilder's Grove Sanitary Landfill is contaminated with a variety of hazardous organic contaminants including benzene, toluene, ethylbenzene, xylene isomers, tetrachloroethylene (PCE), and cis- and trans-dichloroethylene (c-DCE and t-DCE). Benzene, toluene, PCE, and both c-DCE and t-DCE all exceed groundwater standards at one or more wells although benzene is present at very low concentrations (maximum concentration = 5 µg/l).

2. Conventional field monitoring techniques were not adequate to determine if anaerobic biotransformation processes are significant in limiting the migration of hazardous organics in the subsurface. Large variations in contaminant concentration were observed in groundwater down gradient from waste deposits. Because of these large variations, it would not be possible to detect a slow, but environmentally significant, decline in contaminant concentration without extensive repeated sampling over a large area. This level of monitoring is not practical in most cases.

3. General conditions are appropriate in the aquifer down gradient of the Wilder's Grove landfill to allow the anaerobic biodegradation of TCE and toluene in the contaminated groundwater. Microorganisms are present that can carry out these reactions, and there is no indication that environmental conditions (pH, nutrients, etc.) would inhibit the biodegradation of these compounds.

4. If laboratory biodegradation rates under ambient conditions can be extrapolated to the field, naturally occurring anaerobic biotransformation reactions should prevent the off-site migration of TCE and toluene at this site. It is not yet known whether laboratory-derived biodegradation rates can be reliably used to estimate contaminant transport in the field.

5. Laboratory microcosm results indicate that the rate of TCE biotransformation can be enhanced by buffering the groundwater to approximately pH 7.5. Complete conversion of chlorinated compounds to ethylene may be further enhanced through the addition of inorganic nutrients and an easily degradable carbon source.

6. Benzene, ethylbenzene, meta- and ortho-xylene (BEX) were recalcitrant in the first destructive microcosm study under ambient conditions. While one set of microcosms (LFM) exhibited statistically greater benzene degradation rates in live replicates than in abiotic replicates, this phenomenon was likely the result of greater scatter in the live data. Accelerated BEX loss was observed in a few microcosms in the amendment study. Biodegradation of BEX may have occurred in these isolated cases, but further research will be required to confirm this result.

2.0 RECOMMENDATIONS

1. The North Carolina groundwater quality standards have recently been modified to allow consideration of natural bioremediation in the management of contaminated groundwater. Our results clearly show that anaerobic biotransformation processes have the potential to significantly reduce the migration of alkylbenzenes and chlorinated aliphatic hydrocarbons in the subsurface. Conventional monitoring techniques are not sufficiently sensitive to estimate anaerobic degradation rates in the field at a reasonable cost. If natural attenuation processes are to be considered in the management of contaminated groundwater, better methods will be needed to evaluate the rate and extent of biotransformation under in-situ conditions. Additional research should be performed to understand the factors which influence biodegradation in the field and to develop more accurate and precise methods for estimating degradation rates under in-situ conditions.

2. Our laboratory studies indicate that benzene, ethylbenzene and xylenes were anaerobically biodegraded in a few isolated microcosms. There are very few well-documented cases of anaerobic benzene biodegradation. Additional studies should be performed to determine if this observation can be repeated and to identify the factors that control this process.

3.0 INTRODUCTION

Sanitary landfills constructed without engineered liners release leachate into the subsurface, resulting in groundwater contamination (Hughes et al., 1971; Reinhard et al., 1984; Baedecker and Apgar, 1984; Douglass and Borden, 1992). Leachate composition is highly variable and can contain high concentrations of dissolved ions, ammonia-nitrogen, phosphorus, dissolved solids, heavy metals and hazardous organics. The focus of this study is on the movement and biotransformation of two groups of hazardous organic compounds in groundwater down gradient of the Wilder's Grove sanitary landfill near Raleigh, NC. This landfill is typical of many landfills constructed in the Piedmont of North Carolina prior to 1980. The two groups of compounds to be examined are chlorinated aliphatic hydrocarbons and alkylbenzenes.

Over the past 50 years, chlorinated aliphatic hydrocarbons (CAH) have been widely used as solvents and degreasers and as intermediates in chemical synthesis. Poor disposal practices have led to widespread contamination of groundwater supplies. CAHs are persistent in the environment due in part to their resistance to aerobic biotic and abiotic degradation (Sewell et al., 1990; Suflita and Sewell, 1991; Semprini et al., 1992). The low retardation factors of CAHs increase their environmental impact relative to other hydrocarbons (Suflita and Sewell, 1991).

Another class of compounds of concern are the alkylbenzenes including benzene, toluene, ethylbenzene and xylene isomers (BTEX). Poor disposal practices have made alkylbenzenes a leading contaminant of groundwater supplies. Alkylbenzenes have low solubilities and are relatively resistant to anaerobic degradation (Suflita and Sewell, 1991). Alkylbenzenes sorb easily to organic aquifer material, making it particularly difficult to remove them to acceptable levels by traditional pump-and-treat technologies.

Some CAHs and BTEX components such as vinyl chloride and benzene are known carcinogens (Merck and Co., Inc., 1989). Due to the risk to public health, concentrations of BTEX and a variety of CAHs [tetrachloroethylene (PCE), trichloroethylene (TCE), cis-1,2-dichloroethylene (c-DCE), trans-1,2-dichloroethylene (t-DCE), and vinyl chloride (VC)] in groundwater are regulated under subchapter 2L of the North Carolina Administrative Code (Classifications and Water Quality Standards Applicable to the Groundwaters of North Carolina, sections .0100, .0200 and .0300).

Effective techniques for remediation of groundwater contaminated by CAHs and BTEX are needed. Pump-and-treat technology is often expensive to implement and maintain over the period of time necessary to restore a contaminated site. In addition, pump and treat may not reduce contamination to an acceptable level. In-situ biological treatment is an attractive alternative for aquifer restoration because complete mineralization to non-toxic end products or less toxic intermediates may be achieved without the removal of large volumes of groundwater. However, it is necessary to understand and possibly control the process because harmful intermediates such as dichlororethylenes or vinyl chloride may be produced from PCE and TCE biodegradation.

Aerobic biological treatment of organic compounds is widely documented and practiced. Aerobic metabolism is energetically more favorable to organisms and generally proceeds faster than anaerobic processes (Brock and Madigan, 1991). However, contaminated aquifers are typically anaerobic due to the biological oxygen demand (BOD) placed on them by the contaminant load. Leachate contains numerous organic contaminants including products of refuse decomposition, BTEX, CAHs and a variety of other hazardous compounds. Groundwater contaminated with leachate will quickly become anaerobic as aerobic respiration by

microorganisms depletes the available dissolved oxygen. Bioremediation processes based on external addition of oxygen have been demonstrated. However, oxygen addition is expensive. Thus, to the extent that natural bioremediation is to be successful, contaminants must be primarily degraded under anaerobic conditions.

The objective of this study was to measure the biodegradability of BTEX and TCE in a landfill leachate contaminated aquifer. A series of monitoring wells was installed along a single flow line immediately down gradient from the edge of the landfill to determine the spatial variation in these contaminants and, if possible, estimate ambient degradation rates. Laboratory-scale tests were conducted to measure biodegradability under conditions which simulated ambient conditions in the aquifer. Additional laboratory tests were conducted to evaluate the potential to enhance biodegradation by alteration of environmental conditions. Buffer, nutrients and addition of easily degradable carbon were evaluated for their potential to stimulate BTEX and TCE biodegradation.

4.0 ANAEROBIC DEGRADATION OF HAZARDOUS ORGANICS

4.1 Anaerobic Microbiology of the Subsurface

Most of the subsurface microorganisms identified to date are aerobes, but obligate anaerobes have been identified from a few sites (Ghiorse and Wilson, 1988). Microbially mediated denitrification was observed in a sand and gravel aquifer contaminated with treated sewage (Smith and Duff, 1988). Anaerobic bacteria were recovered by Van Beelen and Fleuren-Kemila (1989) from two sandy aquifers, a saturated peat soil and a river sediment.

Several recent studies have shown that obligate anaerobes are present in deep sediments. Chapelle et al. (1987) identified methanogenic and sulfate-reducing bacteria from sediments collected 20 to 180 m below grade in the Maryland coastal plain. More recent work by Jones et al. (1989) has shown that methanogens are present at over 300 m below grade in sediments at the Savannah River Plant near Aiken, SC. Although the microbial community was dominated by aerobic microorganisms, sulfate-reducing and methanogenic organisms could be identified from most sediments throughout the depth profile. In most cases, the total number of methanogens was very low, but the anaerobic organisms present were capable of degrading a wide variety of organic (benzoate, phenol, lactate, formate, acetate).

Additional evidence for the presence of methanogens in the subsurface comes from research on contaminated aquifers. Microbiologists from the U.S. Geological Survey have studied two different creosote contaminated aquifers where methanogenic degradation of organic compounds has been observed. Field studies at a contaminated aquifer in St. Louis Park, MN showed that methane production was occurring in zones within the aquifer that had been contaminated with creosote (Godsey et al., 1983). Later studies demonstrated that the presence of anaerobes (denitrifiers, iron reducers, sulfate reducers and methanogens) was highly correlated with the presence of creosote. More recent work at an abandoned creosote plant in Pensacola, FL has shown a wide variety of organic compounds present in the aquifer were undergoing methanogenic biodegradation and that transport distances in the aquifer could be correlated with biodegradation rates observed in laboratory microcosms (Goerlitz et al., 1985; Troutman et al., 1984).

Monitoring at petroleum-contaminated sites also provides evidence of methanogenic biotransformation of petroleum-related compounds. Ehrlich et al. (1985) observed elevated numbers of sulfate-reducing and methanogenic bacteria in a jet-fuel-contaminated aquifer. Evans and Thompson (1986) and Marrin (1987) monitored methane concentrations in soil gas to map subsurface hydrocarbon contamination. In a study of soil gas concentrations near

underground storage tanks, Payne and Durgin (1988) found elevated methane concentrations at over 20% of the 36 sites surveyed. Methane gas production can be so rapid that safety problems occur at some sites. Hayman et al. (1988) had to develop a special apparatus to remove the large quantities of methane generated from a fuel spill at the Miami, FL, airport.

Hult (1987) observed the production of large volumes of methane in the unsaturated zone immediately below a crude oil spill at the U.S. Geological Survey research site in Bemiji, Minnesota. At this same site, Eganhouse et al. (1987) observed a two-order-of-magnitude decrease in alkylbenzene concentration over 150 m. This decrease was accompanied by elevated concentrations of aliphatic and aromatic acids in the groundwater (Baedecker et al., 1987). The acids identified in the groundwater included benzoic, methylbenzoic, trimethylbenzoic, toluic, cyclohexanoic, and dimethylcyclohexanoic. These are the same acids identified by Grbic-Galic and Vogel (1987) as intermediates in the anaerobic degradation of alkylbenzenes. Groundwater and sediment analyses demonstrated that methanogenic biodegradation was resulting in a pH decrease and a rise in bicarbonate concentrations in the groundwater. The actual drop in groundwater pH appears to have been limited by dissolution of carbonate minerals (and possibly aluminosilicates) (Siegel, 1987). Most recently, dissolved methane has been detected in alkylbenzene contaminated aquifers at Sleeping Bear Dunes in Michigan. This was accompanied by a concurrent decline in dissolved alkylbenzenes (Wilson et al., 1994).

Adaptation is defined as the ability of microorganisms to degrade a chemical at an increasing rate with exposure to the chemical (Aelion et al., 1987). Such adaptation has been reported for groundwater microorganisms. Wilson et al. (1985) compared the polynuclear aromatic hydrocarbon (PAH) degrading capability of microorganisms in pristine aquifer material to microorganisms at the margin of a creosote contaminated plume. In a demonstration of adaptation, microorganisms with prior exposure were able to degrade the PAHs in laboratory microcosms while those from the pristine area exhibited no such activity. However, adaptation does not occur for every chemical in every ecosystem. Of nine chemicals tested by Aelion et al. (1987), only one exhibited a typical adaptation response and the time of the response varied from a few days to 6 weeks in different samples. An additional six of the nine chemicals were biotransformed by pristine aquifer sediment. These data on adaptation suggest that the prior exposure of aquifer microorganisms increases the potential for biodegradation of the target compounds in aquifer sediment.

4.2 Anaerobic Biodegradation of Alkylbenzenes

Early studies of hydrocarbon biotransformation indicated that aromatic hydrocarbons were refractory under anaerobic conditions (Atlas, 1988). More recent research has shown that a wide variety of organics may be biodegraded by methanogenic consortia. These compounds include substituted monoaromatic compounds including creosol isomers (Healy and Young, 1979; Smoleski and Suflita, 1987), homocyclic and heterocyclic aromatics (Berry et al., 1987), nitrogen containing compounds (Godsy et al., 1983), benzothiophene (Godsy and Grbic-Galic, 1989), phthalates and ketones (Shelton and Tiedje, 1984), and phenols (Boyd et al., 1983). While earlier work had indicated that alkylbenzenes are recalcitrant under methanogenic conditions, recently there have been a few reports of the successful anaerobic biodegradation of alkylbenzenes.

Wilson et al. (1986) observed 99% removal of benzene, toluene, ethylbenzene and o-xylene as well as TCE, DCE isomers and 1,2-dibromoethane in microcosms constructed with methanogenic aquifer material from a landfill site. Long lag periods were required for significant removal of all compounds except toluene. Toluene removal occurred within the first six weeks of incubation. Significant removal of benzene, ethylbenzene and o-xylene did

not occur through 20 weeks of incubation; however, after 40 weeks, approximately 25% of the original material remained. After 120 weeks, less than 1% of the initial BTEX remained.

Wilson et al. (1990) performed microcosm studies using aquifer material from the Traverse City, MI, field site. In these studies, microcosms containing aquifer material from an anaerobic portion of the aquifer were amended with an alkylbenzene dosing solution and incubated anaerobically at 12°C for 2 months. At the end of the incubation, the concentrations of benzene, toluene, m,p-xylene and o-xylene (BTX) had dropped from 450, 420, 440, and 410 µg/l to 6, 40, 17, and 6 µg/l, respectively. The disappearance of BTX in the anaerobic microcosms was accompanied by the production of methane, indicating that anaerobic conditions were maintained. The alkylbenzenes were removed even more rapidly in microcosms prepared using material from an aerobic portion of the aquifer (stations B and C) and incubated under aerobic conditions. In a sterile control, there was a 40 to 50% drop in each of the alkylbenzenes, presumably due to irreversible adsorption. These results clearly demonstrate that anaerobic biotransformation of alkylbenzenes is occurring in the subsurface at the Traverse City site.

Barker et al. (1986) observed decreases in o-xylene concentrations relative to ethylbenzene in a leachate-contaminated aquifer. Their data were suggestive of anaerobic transformation. In anaerobic groundwater down gradient from a Raleigh, NC, landfill, Douglass and Borden (1992) reported a decrease in toluene from 813 to 10 µg/l and in xylenes from 125 to <5 µg/l, while chloride decreased from 90 to 59 mg/l. Again, these data are suggestive of anaerobic transformation.

Grbic-Galic and Vogel (1987) observed benzene and toluene degradation under methanogenic conditions in an enrichment culture fed ferulic acid for 5 years. Unlabeled and ^{14}C-labeled substrates (ring-labeled toluene and benzene and methyl-labeled toluene) were used. More than 50% of the substrates were converted to CO_2 and methane, with methane accounting for over 60% of the mineralized carbon. A high percentage of CO_2 was recovered from the methyl-labeled toluene, suggesting nearly complete conversion of the methyl group to CO_2 and not methane. Low percentages of CO_2 were produced from ring labeled substrates, indicating incomplete conversion of ring carbon to CO_2. Phenol, cresols and aromatic alcohols were observed as intermediates (Vogel and Grbic-Galic, 1986). Most recently, Sewell and Gibson (1991) showed toluene to be degraded under methanogenic conditions.

Edwards and Grbic-Galic (1992) observed anaerobic benzene degradation in microcosms constructed with material from an anaerobic gasoline contaminated aquifer. Benzene was degraded in all microcosms after a minimum 30-day lag. Radio-labeled carbon was used to verify mineralization of benzene. Approximately 90% of the ^{14}C was recovered as $^{14}CO_2$. The electron acceptor was not established, although the data suggest that sulfate reduction was involved. Methane was produced, but not enough to account for the benzene disappearance, thus ruling out methanogenesis as the dominant electron sink.

4.3 Anaerobic Biodegradation of Chlorinated Aliphatics

Many highly chlorinated hydrocarbons, including most CAHs, are resistant to aerobic biodegradation (Suflita and Sewell, 1991). TCE, which is both a widespread contaminant and an anaerobic biotransformation product of PCE degradation, can be degraded aerobically by co-metabolism with toluene or an alkane (Neilson, 1990) but is also degradable under anaerobic conditions (Sewell et al., 1990). Given the recalcitrance of CAHs to aerobic metabolism, anaerobic processes are particularly important. Since chlorinated compounds are relatively oxidized by the presence of chlorine substituents, they are susceptible to reduction (Vogel et

al., 1987). Anaerobic degradation of CAHs may occur through a process termed reductive dechlorination. During reductive dechlorination, the CAH serves as the electron acceptor when the chloride moiety is removed and replaced by a hydrogen, forming a less chlorinated and more reduced intermediate (Suflita and Sewell, 1991).

Reductive dechlorination requires the presence of an organic electron donor. In the presence of an organic electron donor, PCE can be reductively dechlorinated to TCE. TCE can be sequentially dechlorinated to DCE isomers, then vinyl chloride, and possibly completely dechlorinated to ethylene or ethane (Freedman and Gossett, 1989; De Bruin et al., 1992). Acetate, benzoate, glucose, lactate, methanol, toluene and hydrogen have been shown to be suitable electron donors (Vogel and McCarty, 1985; Scholz-Muramatsu et al., 1989; De Bruin et al., 1992; Freedman and Gossett, 1989; Sewell and Gibson, 1991; DiStephano et al., 1992). In addition, the natural background organic carbon present in many ecosystems may serve as an electron donor for reductive dechlorination. Reductive dechlorination has been shown to occur primarily under methanogenic conditions. Although sulfate and nitrate may inhibit reductive dehalogenation (Mohn and Tiedje, 1992), reductive dechlorination under denitrifiying and sulfate-reducing conditions has also been reported (Bouwer and McCarty, 1983; Semprini et al., 1992).

Obligate anaerobes, though not necessarily methanogens, appear to be the primary organisms responsible for reductive dechlorination and require varying acclimation periods. Reductive dechlorination has been reported to occur in several natural anaerobic environments including freshwater sediment (De Bruin et al., 1992; Gibson and Suflita, 1986), marine sediment (King, 1988), anaerobic sewage sludge (Gibson and Suflita, 1986) and aquifer sediment (Sewell et al., 1990). Reductive dechlorination has been demonstrated for a variety of compounds including chlorobenzenes (Fathepure and Boyd, 1988; Bosma et al., 1988), chloroanilines (Kuhn and Suflita, 1989), trichlorophenoxyacetic acid (Gibson and Suflita, 1989), polychlorinated biphenyls (Quensen et al., 1988), and chlorophenols (Woods et al., 1988).

The reductive dehalogenation of chlorinated aliphatics has also been widely reported. Bouwer and McCarty (1983) were among the first to note reductive dechlorination of halogenated compounds, under both methanogenic and denitrifiying conditions. In batch experiments using a methanogenic mixed culture in a deoxygenated anaerobic medium, PCE and carbon tetrachloride (CT) were reductively dechlorinated within 16 days and 8 weeks, respectively. TCE was an intermediate in the reductive dechlorination of PCE. Of chloroform (CF), CT and 1,1,1-TCE tested in batch experiments under denitrifying conditions, only CT was removed. Final end products were not reported. In work with a pure Methanosarcina culture, Fathepure and Boyd (1988) showed a direct linkage between PCE dechlorination and methane production. In the absence of a growth substrate, the methanogen culture did not dechlorinate PCE, thereby demonstrating the need for a supplementary carbon source.

In batch experiments using anaerobic enrichment cultures from a sludge digester, Freedman and Gossett (1989) observed complete dechlorination of PCE to ethylene under methanogenic conditions. After an initial dose of PCE was completely removed, serum bottles were respiked with PCE or TCE. Samples from first-generation cultures that degraded PCE were used to seed second-generation cultures and so on until sixth-generation cultures were produced. Early cultures exhibited accumulations of DCE isomers. However, in successive cultures, PCE was rapidly degraded to vinyl chloride, which accumulated prior to conversion to ethylene. The dechlorinating step from vinyl chloride to ethylene was the rate-limiting step. Complete conversion of PCE to vinyl chloride and ethylene was not observed apparently due to leakage losses. Radio tracer studies with ^{14}C-PCE indicated ^{14}C-ethylene was the terminal product. Significant conversion to $^{14}CO_2$ or $^{14}CH4$ was not observed. Although methanol was the most

effective electron donor, hydrogen, formate, acetate and glucose also supported reductive dechlorination.

Sewell and Gibson (1991) observed reductive dechlorination of PCE in batch experiments using aquifer solids exposed to both alkylkbenzenes and chlorinated ethenes. TCE and DCE isomers were detected after 120 and 140 days, respectively. DCE isomers and PCE were never detected together at the same sampling time. PCE reduction was not observed in microcosms that did not also receive an inorganic nutrient spike of ammonium phosphate. Acetate was the only metabolic intermediate of toluene degradation observed, reaching a maximum concentration of 180 μM (11 ppm) by 184 days. PCE reduction lagged behind acetate production, which lagged behind toluene removal, which is consistent with the idea that an intermediate of toluene degradation serves as the immediate electron donor for PCE reduction (Scholz-Muramatsu et al., 1989). The reaction stalled at DCE, with DCE reaching a maximum concentration of 20 μM versus an initial PCE concentration of approximately 35.8 μM. The failure to complete a mass balance was attributed to losses during sampling.

De Bruin et al. (1992) observed complete reductive dechlorination of PCE to ethylene and ethane in column studies using anaerobic Rhine River sediment mixed with anaerobic granular sludge and lactate as the electron donor. TCE, DCE isomers and vinyl chloride were observed as dechlorination intermediates. However, after 105 days only ethylene and ethane were observed in the column effluent. The transformation did not occur in the presence of bromoethanesulfonic acid (BES), a methanogen inhibitor, indicating that methanogens play an important role in the transformation in this ecosystem. PCE was not reduced in columns containing Rhine River sediment or granular sludge individually.

Semprini et al. (1992) observed in-situ transformations of carbon tetrachloride (CT), 1,1,1-trichloroethane (TCA), trichloroflouromethane (CFC-11) and 1,1,2-trichloro-1,2,2-triflouroethane (CFC-113) in the presence of acetate as a growth substrate and nitrate and sulfate as potential electron acceptors. CT disappearance began 2 weeks after active denitrification and gradually increased over the 10-week study. Chloroform appeared as an intermediate product, representing only 30-60% of the CT transformed. When nitrate was removed from the injection fluid, CT transformation increased, indicating either that (a) microorganisms other than denitrifiers mediate the transformation of CAHs or (b) a secondary microbial population that grew slowly was responsible for the transformation and its growth was inhibited in the presence of nitrate. Isolated denitrifiers from the test zone failed to transform CT in subsequent studies. Sulfate reducers may have been responsible for the observed removal, but there were insufficient data to confirm this. Methanogens were ruled out since methane production was not observed.

DiStephano et al. (1992) examined hydrogen as a potential electron donor to the reductive dechlorination of PCE in batch experiments using a PCE/MeOH enrichment culture. In culture suspensions fed hydrogen, PCE was completely reduced to vinyl chloride and ethylene in 14 to 40 days. However, this culture could not sustain reductive dechlorination for extended periods. In identically prepared methanol-fed suspensions, reductive dechlorination of PCE was sustainable over time. Further work showed that hydrogen was the direct electron donor for reductive dechlorination but both acetogenic and methanogenic activity were required for the process to proceed.

4.4 ANAEROBIC BIODEGRADATION SUMMARY

The presence of anaerobic bacteria in aquifers has been documented for both pristine and contaminated sites. Preliminary evidence indicates that alkylbenzenes (benzene, toluene, ethylbenzene and xylene isomers) are biodegradable under highly reducing anaerobic

conditions typical of leachate-contaminated aquifers. Toluene appears to be the most degradable of the alkylbenzenes while benzene appears to be the most recalcitrant. Acclimation periods for biodegradation range from several days to several months and are highly variable. It is not yet known under what conditions alkylbenzenes will degrade anaerobically or at what rate biodegradation may occur in leachate-contaminated aquifers.

Highly chlorinated ethenes are often degraded in anaerobic settings via reductive dechlorination, given sufficient electron donors. The ease of dechlorination appears to decrease as the number of chlorine atoms decreases. Recent work has shown that under certain circumstances, chlorinated ethenes may be completely dechlorinated to ethylene and ethane. Most previous research on anaerobic degradation of chlorinated ethenes has used readily degradable carbon sources (e.g., acetate or lactate). The rate and extent of reductive dechorination in a leachate-contaminated aquifer, where the most readily degradable electron donors have been depleted, is unknown.

5.0 STUDY SITE

5.1 Location and Characteristics

The Wilder's Grove Sanitary Landfill was chosen for study and is located in the Piedmont physiographic province of North Carolina in eastern Wake County. The facility has been in operation since 1972 and receives approximately 1000 tons of municipal refuse per day including domestic, commercial and industrial solid wastes. Disposal of hazardous waste within the landfill is not permitted, although prior to 1989 an active program was not in place to exclude hazardous materials.

This landfill has no design features intended to prevent movement of leachate into the groundwater. The landfill does not have an engineered cover system. In operating the landfill, general nonsegregated solid waste is placed in lifts with 6 to 12 inches of daily cover. After the site is filled to the approved elevation, an additional 24-inch final cover of compacted soil is applied. The refuse disposal area is divided into two regions by a small stream channel passing through the center of the landfill. The portion of the landfill to the west of the channel was deposited between about 1972 and about 1982. Refuse on the eastern side is younger, having been buried from about 1982 to present. Our research has been conducted in a small area on the eastern side of the site immediately adjoining the drainage channel. A site plan showing the monitoring well and soil boring locations is shown in Figure 1.

5.2 Hydrogeology

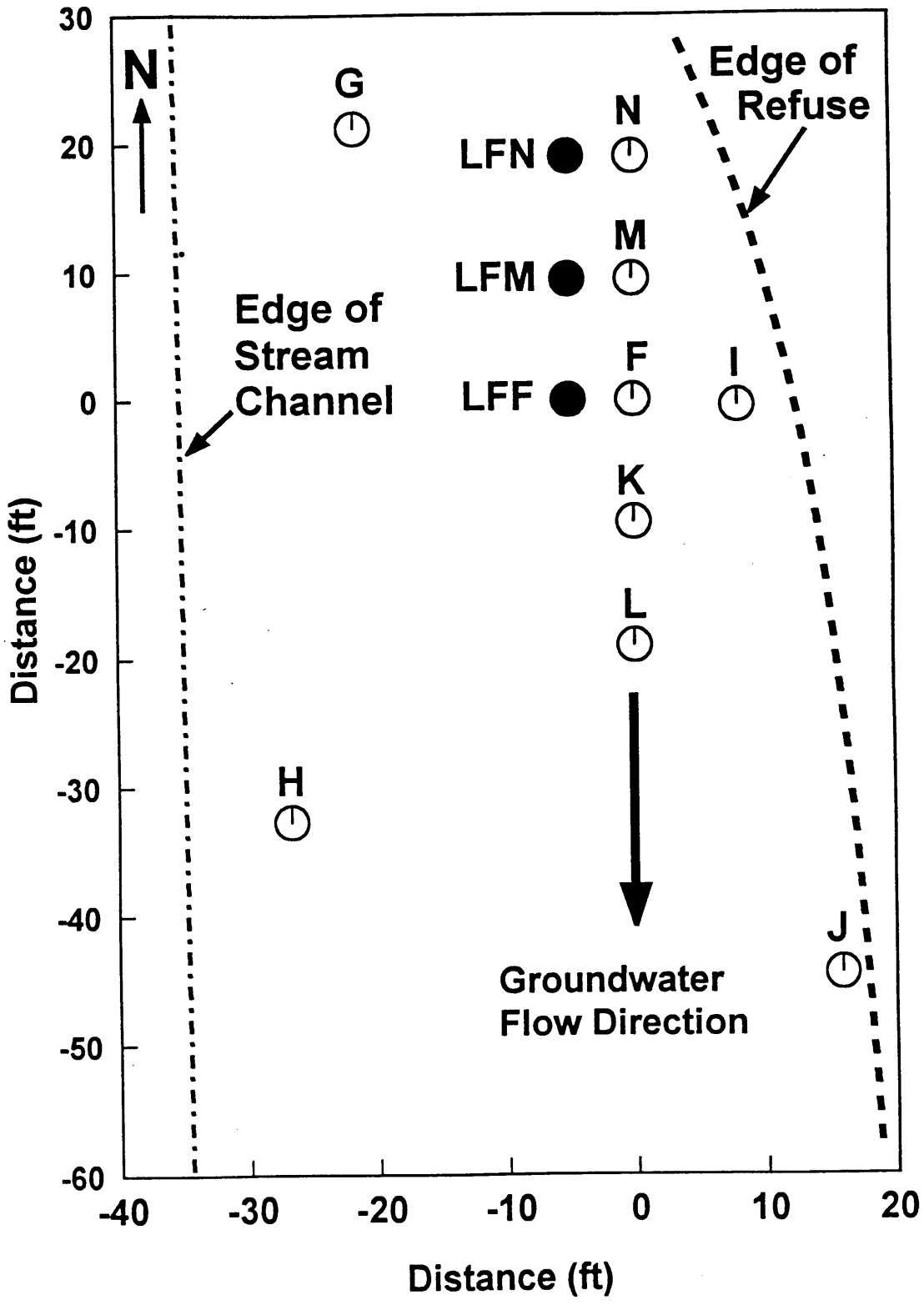
The landfill is located over a granite intrusion known as the Rolesville Batholith which was probably formed during the Middle Paleozoic Era and is described as being an intrusion of Adamellite (Parker, 1979). Adamellite is a massive, gray, granite rock that is composed of subequal parts of sodic plagioclase and potassium feldspars with quartz, biotite, muscovite and other accessory minerals. To the immediate west of the landfill are injected gneiss and schist that may underlie a portion of the site. The gneiss and schist are described as being layered and having numerous dikes and sills of granite, pegmatite and aplite.

Core samples taken from the study area during drilling have revealed 6 to 9 m of colluvial sedimentary deposits. These deposits vary widely in composition from silty sand to sandy clay, and are often similar in appearance to surrounding granitic saprolite. Part of the upper colluvium was noted to have a high organic fraction. The hydraulic conductivity of this area was determined to be approximately 0.8 m/d from short term specific capacity tests. This is similar to hydraulic conductivity measurements in other wells around the site (Britt and Van Tassel, 1988; Marshall, 1989; Roudebush and Whitman, 1989).

Typically, groundwater flows from higher elevations, converging toward the lower lying drainages. Monitoring wells in the immediate vicinity of the study area indicate that groundwater flow is almost due south, parallel to the natural stream channel and toward a small pond-wetland. The water table in the study area has a constant slope of 4% which is approximately equal to the stream channel slope. Using the measured water table gradient and hydraulic conductivity, the average groundwater velocity was estimated to be 0.11 m/day.

Measurement of the piezometric surface in nearby wells screened in the fractured bedrock and transition zone indicates a very weak downward hydraulic gradient. This finding indicates that the study area is not in a groundwater discharge area.

Figure 1 - Monitoring Well and Soil-Boring Locations



5.3 Tracer Test

As part of the site characterization, a non-reactive tracer test was performed to estimate the groundwater velocity in the study area. This velocity was needed to allow comparison of the laboratory degradation rates with the field monitoring results. The tracers selected for the test were chloride and bromide. Chloride and bromide are non-toxic in low concentrations, are inexpensive, move with the water, can be detected at low concentrations, are chemically stable, and are generally not filtered or absorbed by the porous media.

The up gradient Well N was used as the point of injection. A 1040-liter capacity pillow tank was filled with water from Well N at a pumping rate of between 85 and 100 l/hr over a period of 12 hrs. The pillow tank was constructed of a polyester fabric based membrane with a PVC coating. Prior to the injection, the pillow tank was soaked for 48 hrs with well water to allow equilibration of the tank lining with any contaminants present. The tank was placed flat on a mat on the ground surface's up gradient side to create a positive head for the injection. The tank was filled and flushed with argon to remove oxygen in the tank. Argon gas was continuously injected into the well void space to displace oxygen during the pump-out and injection.

After the tank was filled with well water, a concentrated 2-liter stock solution of chloride and bromide was pumped into the tank and thoroughly mixed. This stock solution consisted of 95 gm/l $CaCl_2$, 100 gm/l NaCl, 10 gm/l NaBr and 11.9 gm/l KBr mixed in anaerobic D.I. water. The solution in the tank was sampled at several intervals during the injection period and was found to have average concentrations of chloride and bromide of approximately 590 mg/l (s.d.=54) and 102 mg/l (s.d.=7.1), respectively. The injection concentrations were selected to ensure that the NC groundwater quality standards would not be violated due to the introduction of foreign compounds (chloride and bromide). Wells down gradient of the injection point were then monitored for chloride and bromide 5 times over the next 43 days to determine the rate of tracer migration.

In reviewing the monitoring data, there were no significant trends in the chloride concentrations over the 43-day test period. Monitoring could not be continued beyond this time because the study area was covered by refuse. It appears that the injected chloride solution was masked by the high background concentration of 100 to 200 mg chloride/l. A measurable increase in the bromide concentration was observed at well M, 43 days after the injection. The bromide concentration increased from 2.5 mg/l to 11.0 mg/l over a 7-day period. This would indicate a groundwater velocity of 0.07 m/day, very near the predicted velocity of 0.11 m/day.

5.4 Results of Previous Groundwater Monitoring

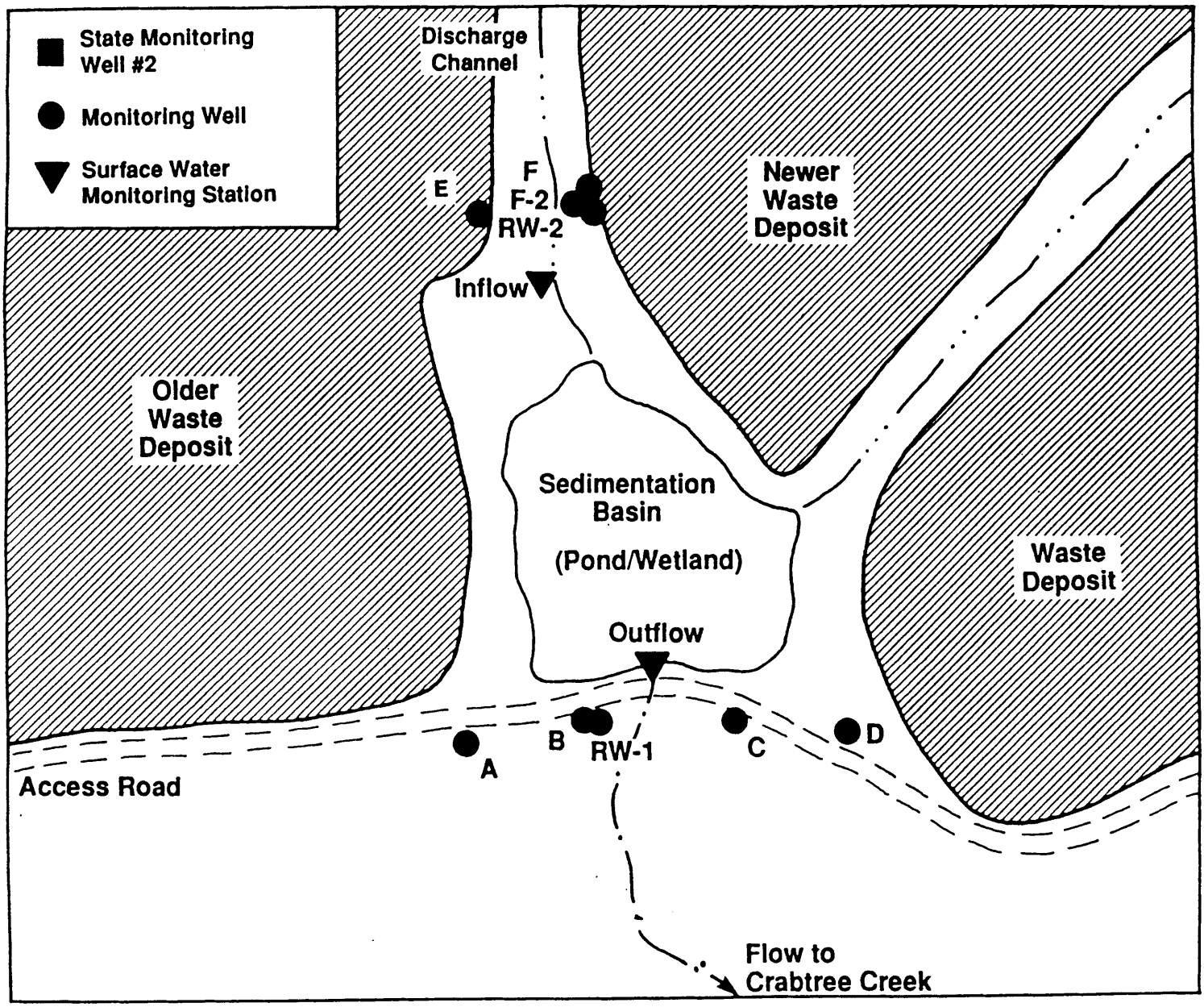
Previous monitoring at the landfill by Douglass and Borden (1992) has shown the groundwater to be contaminated with a wide variety of pollutants. Average concentrations of selected organic and inorganic pollutants at wells in 1989 are shown in Table 1. Monitoring well locations are shown in Figure 2. Elevated concentrations of chemical oxygen demand (COD), total organic carbon (TOC), iron, manganese and synthetic organic chemicals (SOCs) were observed in groundwater at the study site immediately down gradient from the refuse. In 1989, groundwater in the study area (monitoring well F) contained elevated concentrations of phenol, diethyl phthalate, 4-methylphenol, vinyl chloride, methylene chloride, 1,1-dichloroethane, 1,2-dichloroethene, benzene, toluene, ethylbenzene, xylenes, acetone, 2-butanone, 2-hexanone, MIBK, caprolactam and a variety of organic acids. At the down gradient edge of the working area (wells A, B and D), approximately 1,000 ft up gradient from the

Table 1. Average Ground Water Quality Monitoring Results - 1989*

	MONITORING LOCATION							
	A	B	D	E	F	F-2	RW-1	RW-2
pH[1]	6.23	5.60	6.10	5.67	4.62	ND	ND	ND
SPECIFIC COND.[2]	619	213	229	294	1351	754	451	685
CHLORIDE	32.9	39.0	10.4	58.5	90.0	59	16	59
NITRATE-N (mg/l)	0.1	ND	0.1	0.15	0.2	0.06	0.11	0.1
AMMONIA-N (mg/l)	7.5	1.3	0.72	0.95	0.64	ND	ND	ND
SULFATES (mg/l)	<3.0	ND	<3.0	<3.0	<3.0	<3.0	<3.0	3.0
COD (mg/l)	126.5	23	49	35	893	84	13	23
TOC (mg/l)	86	ND	21	63	385.5	60	25	60
TDS (mg/l)	268	ND	236	172	1080	484	280	448
IRON (mg/l)	96.2	31.3	2.2	13.9	109.8	3.5	2.5	3.8
MANGANESE (mg/l)	7.98	<0.1	6.1	1.9	64.11	19.7	8	8.3
ZINC (mg/l)	0.07	ND	<0.05	<0.05	0.05	0.16	<0.05	<0.05
LEAD (mg/l)	<0.03	ND	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Phenol (ug/l)	<5.0	<5.0	<5.0	<5.0	32	<5.0	<5.0	<5.0
Diethyl Pthalate (ug/l)	<5.0	<5.0	<5.0	<5.0	20	<5.0	<5.0	<5.0
4-Methylphenol (ug/l)	<5.0	<5.0	<5.0	20	253	<5.0	<5.0	<5.0
Vinyl Chloride (ug/l)	<5.0	<5.0	<5.0	69	37	26	3E	8
Methylene Chloride (ug/l)	<5.0	<5.0	<5.0	18	13	<5.0	2E	<5.0
1,1-dichloroethane (ug/l)	<5.0	<5.0	<5.0	39	92	60	3E	26
1,2-dichloroethene (ug/l)	<5.0	<5.0	<5.0	109	83	12	68	<5.0
Trichloroethylene (ug/l)	<5.0	<5.0	<5.0	10	<5.0	<5.0	<5.0	<5.0
Benzene (ug/l)	<5.0	<5.0	<5.0	6	13	8	<5.0	2E
Toluene (ug/l)	<5.0	<5.0	<5.0	61	813	126	<5.0	10
Xylenes (total) (ug/l)	<5.0	<5.0	<5.0	12	125	12	<5.0	<5.0
Tetrachloroethene (ug/l)	<5.0	<5.0	<5.0	14	<5.0	<5.0	<5.0	<5.0
Ethylbenzene (ug/l)	<5.0	<5.0	<5.0	7	38	3E	<5.0	<5.0
Acetone (ug/l)	<5.0	<5.0	<5.0	<5.0	1524	181	<5.0	<5.0
2-Butanone (MEK) (ug/l)	<5.0	<5.0	<5.0	111	1575	102	<5.0	<5.0
2-Hexanone (ug/l)	<5.0	<5.0	<5.0	<5.0	70	<5.0	<5.0	<5.0
MIBK (ug/l)	<5.0	<5.0	<5.0	6	84	<5.0	<5.0	<5.0
Caprolactam (ug/l)	995	95	133	78	88	<5.0	<5.0	<5.0
Hexanoic Acid[3]	-	-	-	+	+	ND	ND	ND
Heptanoic Acid[3]	-	-	-	+	+	ND	ND	ND
Octanoic Acid[3]	-	-	-	+	+	ND	ND	ND

* From Douglass and Borden, 1992
1 Standard pH units
2 umho/cm
3 + means detected, - means not detected
E estimated concentration

Figure 2 - Monitoring Well Locations from Douglass and Borden (1992)



property boundary, concentrations of most pollutants were much lower. Concentrations of many of the organic contaminants decreased with travel distance much more rapidly than chloride suggesting that some attenuation mechanism other than dilution was occurring. The relative order and rates of disappearance of the different compounds suggest that biotransformation may result in significant removal of some compounds.

6.0 CHEMICAL CHARACTERIZATION OF THE AQUIFER

6.1 Groundwater Monitoring

A total of nine monitoring wells were installed at the site. Initially, five wells (F, G, H, I, and J) were located to determine the groundwater flow direction and hydraulic gradient. Upon determining the direction of flow, four additional wells were located on a streamline passing through well F. These wells were designed to allow the estimation of contaminant attenuation during transport along this streamline. Two wells, M and N, were located up gradient of well F; and the two wells, K and L, were located down gradient of well F. The five wells (F, K, L, M, and N) were located approximately 3 m (9 ft) apart.

All the wells with exception of K and L were constructed using a 5-cm (2-inch) diameter PVC well casing with a 1.5-m (5-foot) long, number-10-slot well screen. Wells K and L were constructed of 2.5-cm (1-inch) diameter PVC well casing utilizing a 15-cm (6-inch) long well screen (10 slot). All wells were installed in the saturated shallow saprolite-colluvium soil zone. The wells were drilled using a 7-cm (2.75-inch) diameter hollow-stem auger and a soil sampling hydraulic probe. The screens were packed with native material that formed as the borehole collapsed after drilling. Bentonite seals were placed above the water table. Steel protective covers were set in concrete above the bentonite seals and fitted with locking caps. The monitoring wells were developed by repeated surging and bailing to remove fine-grained sediments.

Water table elevation and flow direction were remarkably constant over the study period. Groundwater flow direction was consistently due south down the line of monitoring wells N to L. Depth to the water table in well M varied from 2.08 to 2.30 m (6.8 to 7.5 ft) below ground surface.

6.2 Sampling and Analytical Procedures

Dissolved BTEX, CAHs and related parameters were monitored in groundwater six times from March to July 1992. The original intent was to continue monitoring into the late fall of 1992. Unfortunately, the study area was covered with refuse in August 1992. Prior to sampling, all wells were filled with pre-purified argon gas to minimize oxygen exchange with the sample and then purged of at least five well volumes using a dedicated inertial pump (Waterra). Groundwater samples were then collected in 40-ml vials with Teflon-lined septa and no headspace. Samples for metals and ions were pumped through a 0.45-μm filter prior to collection. Temperature, pH, oxidation-reduction (redox) potential, and dissolved oxygen were measured in the field with standard probes. Carbon dioxide was measured in the field by titrating with NaOH to pH 8.3 and is reported as total CO_2 (carbonate, bicarbonate and free CO_2). When dissolved iron exceeded 1 mg/l, CO_2 results were corrected for NaOH consumed during the reaction with iron. BTEX and CAH concentrations were analyzed as described in section 7.2.4.1. Inorganic nutrients and ions (NO_3, NH_4, PO_4, SO_4) and metals (Ca, Cd, Zn, Ni, Fe, Mn, Mg, Na, K, Cu, S) were analyzed by ion chromatography and inductively coupled plasma emission spectroscopy following Standard Methods (American Public Health Association, 1992).

6.3 Geochemical Conditions in the Aquifer

Results of the geochemical monitoring are summarized in Tables 2 through 4. Overall geochemical conditions in the aquifer were fairly uniform. The aquifer was strongly reducing (Eh <-100 mV) except in those wells directly adjoining the stream channel. Dissolved oxygen was consistently below 1 mg/l in all wells. The small amount of oxygen that was present is believed to be due to oxygen exchange at the water table interface. Alkalinity and dissolved carbon dioxide were high and pH ranged from 6.2 to 6.8. The parameter that showed the greatest variability was chemical oxygen demand (COD), which varied from over 300 mg/l directly adjoining the refuse (well I) to less than 30 mg/l near the drainage channel. High concentrations of iron and manganese were present due to the strongly reducing conditions.

6.3.1 Temporal Variation in BTEX and CAHs. As the first step in evaluating the monitoring data for the hazardous organics, an analysis was conducted to determine if there had been any systematic variation in the BTEX and CAH concentrations with time. Correlation coefficients were calculated between the concentration of individual compounds and time. Whenever the correlation coefficient exceeded 0.5, the data were plotted and examined to evaluate the significance of any trends. In general, only toluene showed a systematic variation with time. Toluene concentrations in four representative wells (F, G, I, and N) are shown in Figure 3. Wells nearest the stream (G and H) had consistently low toluene concentrations with little variability. In contrast, the wells nearest the refuse (I and J) had the highest toluene concentrations and these concentrations increased over the monitoring period. Along the intensively monitored streamline, toluene concentrations in wells N and M decreased, whereas toluene concentrations in wells F, K and L were highly variable with no apparent trend. The decline in toluene in N and M appeared to follow an exponential decay curve. To evaluate this, toluene concentration in each well was fit to the equation $C_t = C_o^{-Kt}$ where C_t is the toluene concentration at time t and K is the effective first-order decay coefficient. The effective first-order decay coefficients for toluene were 0.022 ($\pm$ 0.003) per day for well N and 0.025 ($\pm$ 0.006) per day for well M (value in parentheses is the standard error). Figure 4 shows the comparison between the field monitoring results and the regression lines.

In summary, most wells did not show any systematic trends for BTEX or CAHs with time. A few wells did show a systematic trend of decreasing toluene while others showed a consistent trend of increasing toluene. There was no overall trend of increasing or decreasing contaminant concentrations throughout the study area. On the basis of these results, we have chosen to use the arithmetic average of all concentration measurements in all further analyses. Average concentrations of BTEX and CAHs are presented in Table 5.

6.3.2 Spatial Distribution of BTEX and CAHs. The highest contaminant concentrations are present in wells I and J, immediately adjoining the refuse. The lowest concentrations are in wells G and H, closest to the drainage channel. There were no consistent trends in the average concentrations of toluene, cis-DCE or PCE along the intensively monitored streamline between wells N and K (Figure 5). At well L, there was a significant increase in the trans-DCE concentration and a decrease in the toluene concentration. The reason for this shift is unclear. The decline in toluene and increase in trans-DCE could be due to biological activity or could be due to minor shifts in the groundwater flow path. The aquifer has a higher clay content in the area of well L that could cause the flow line to bypass well L. Also, wells K and L have shorter 15-cm (6-inch) screens, which could affect the measured contaminant concentration.

The monitoring data were evaluated to identify any evidence of biodegradation during transport along the groundwater flow path. If biodegradation were occurring, the contaminant concentrations should decrease as groundwater migrates from well N to M to F to K. The absence of a measurable decrease indicates that either biodegradation is not occurring or is

Table 2.

Average Concentrations of Electron Acceptors and Nutrients

Well ID	O_2 (mg/l)	Nitrate NO_3-N (mg/l)	Total Phosphorus ((mg/l)	Ammonia NH_4-N (mg/l)	Total Kjeldahl Nitrogen (mg/l)
F	.3	<0.2	<0.2	4.1	NA
G	.4	<0.2	<0.2	5.8	4.1
H	.7	<0.2	<0.2	.2	NA
I	.6	<0.2	<0.2	1.7	5.1
J	.7	<0.2	<0.2	.5	NA
K	NA	<0.2	<0.2	.7	4.9
L	NA	<0.2	<0.2	<0.2	1.4
M	.4	<0.2	<0.2	4.8	NA
N	.6	<0.2	<0.2	3.2	NA

BDL - Below Detectable Limit
NA - Not Available

Table 4.

Average Concentrations of Dissolved Metals and Ions in Groundwater

WELL ID	Fe (mg/l)	Mn (mg/l)	Ca (mg/l)	Mg (mg/l)	Na (mg/l)	Cl (mg/l)	Al (mg/l)	Zn (mg/l)	Cu (mg/l)	K (mg/l)	Si (mg/l)	Br (mg/l)
F	31.2	49.4	246.3	43.7	87.8	162.	0.10	0.04	0.00	5.9	3.0	1.2
G	27.6	24.0	82.0	24.2	54.2	64.	0.10	0.02	0.00	8.1	1.9	0.0
H	0.5	12.4	85.3	37.4	54.2	56.	0.10	0.04	0.02	2.2	2.7	0.3
I	40.7	48.5	242.4	42.9	103.0	118.	0.02	0.03	0.01	5.3	3.9	0.7
J	12.9	44.1	168.0	37.3	79.7	132.	0.06	0.10	0.00	1.7	7.7	0.7
K	33.2	33.4	76.4	23.4	57.2	106.	0.10	0.17	0.00	2.0	0.8	0.4
L	34.5	19.8	38.2	9.7	57.6	72.	0.00	0.23	0.00	2.0	0.3	0.0
M	34.1	36.3	235.5	55.4	111.8	186.	0.10	0.10	0.00	6.4	4.3	0.9
N	30.5	43.0	186.5	48.5	101.5	151.	0.10	0.00	0.00	7.8	8.8	1.2

Figure 3 - Variation in Toluene Concentration in Monitoring Wells with Time

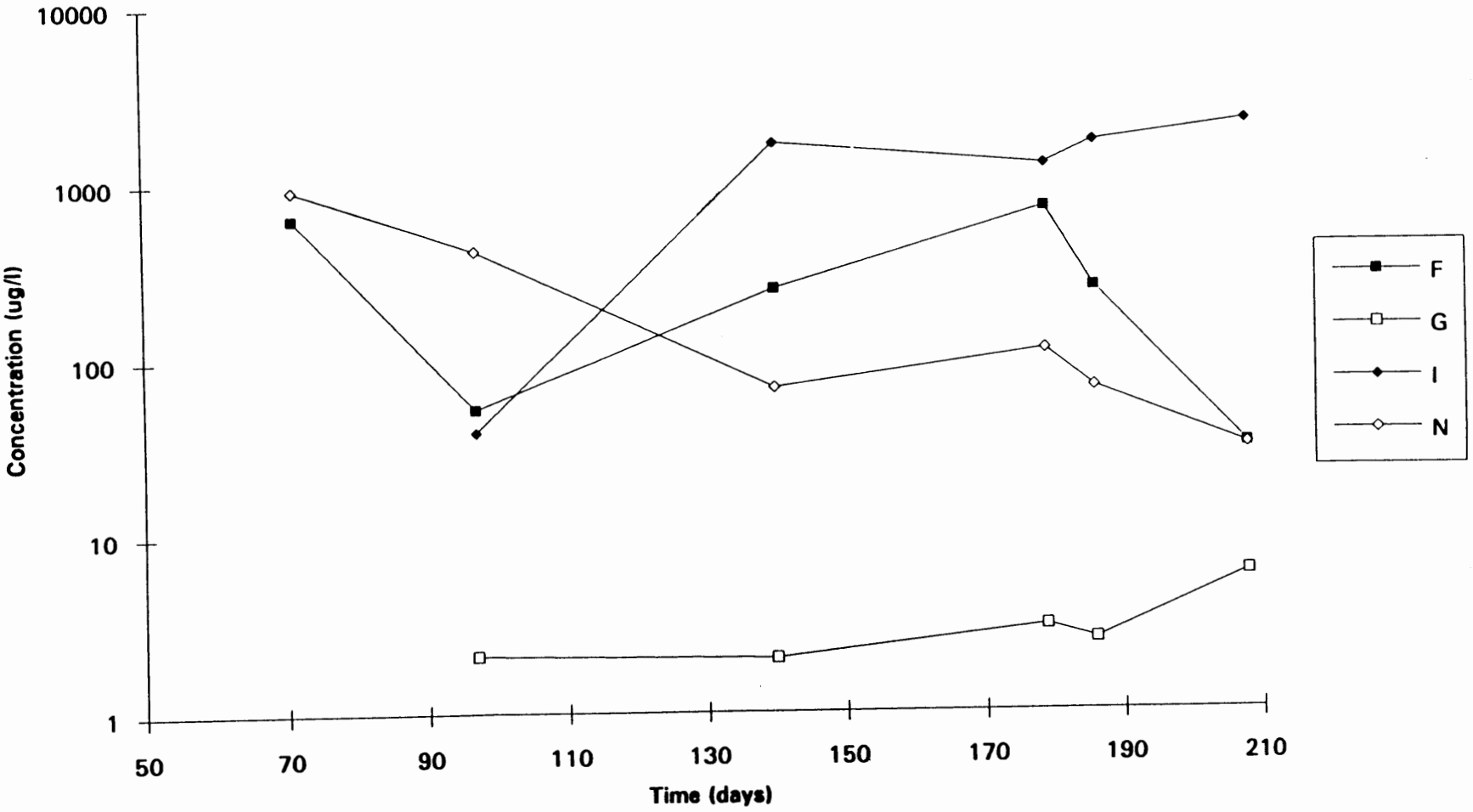


Figure 4 - Comparison of Toluene in Monitoring Wells M and N with First-Order Regression

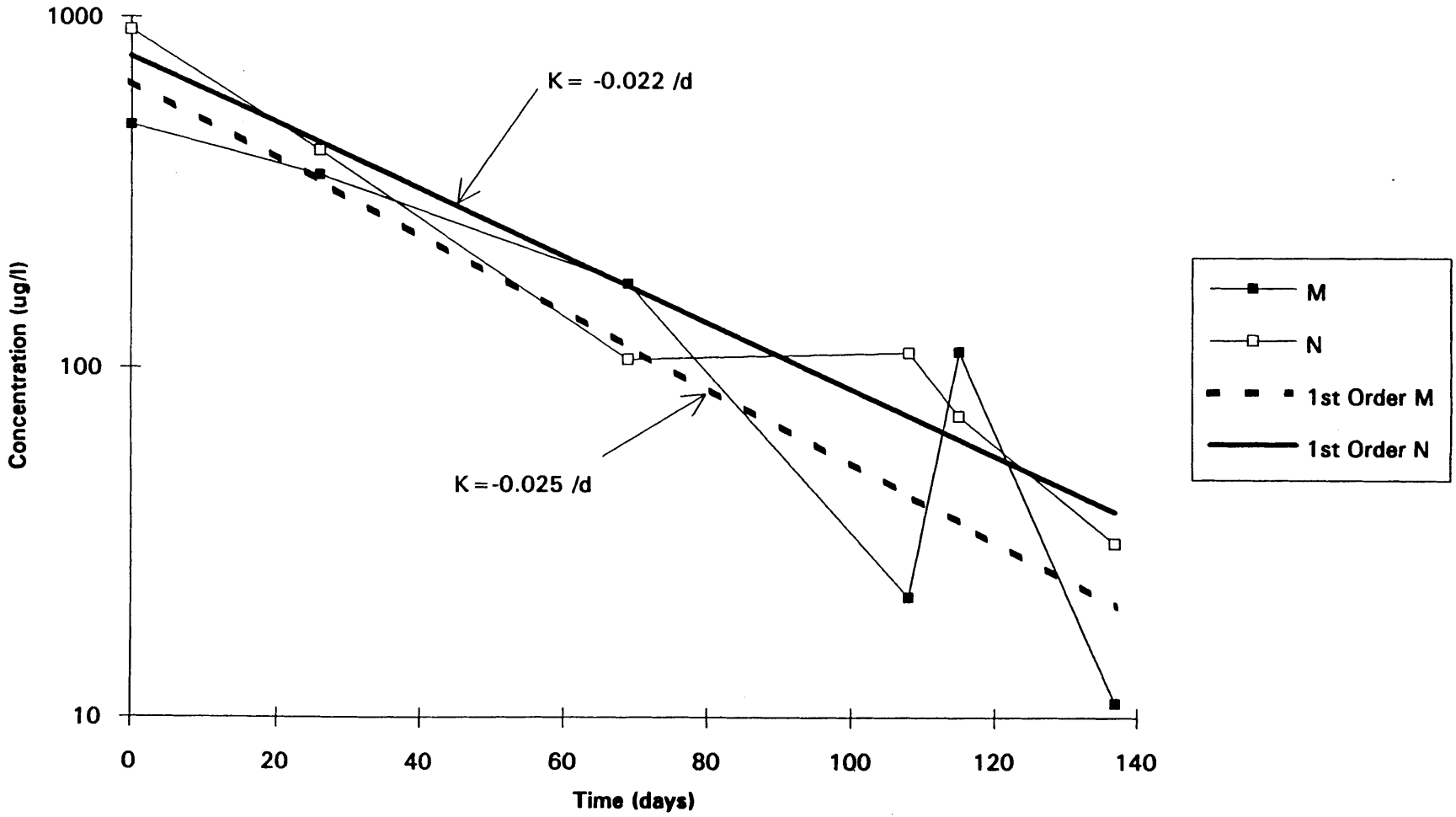


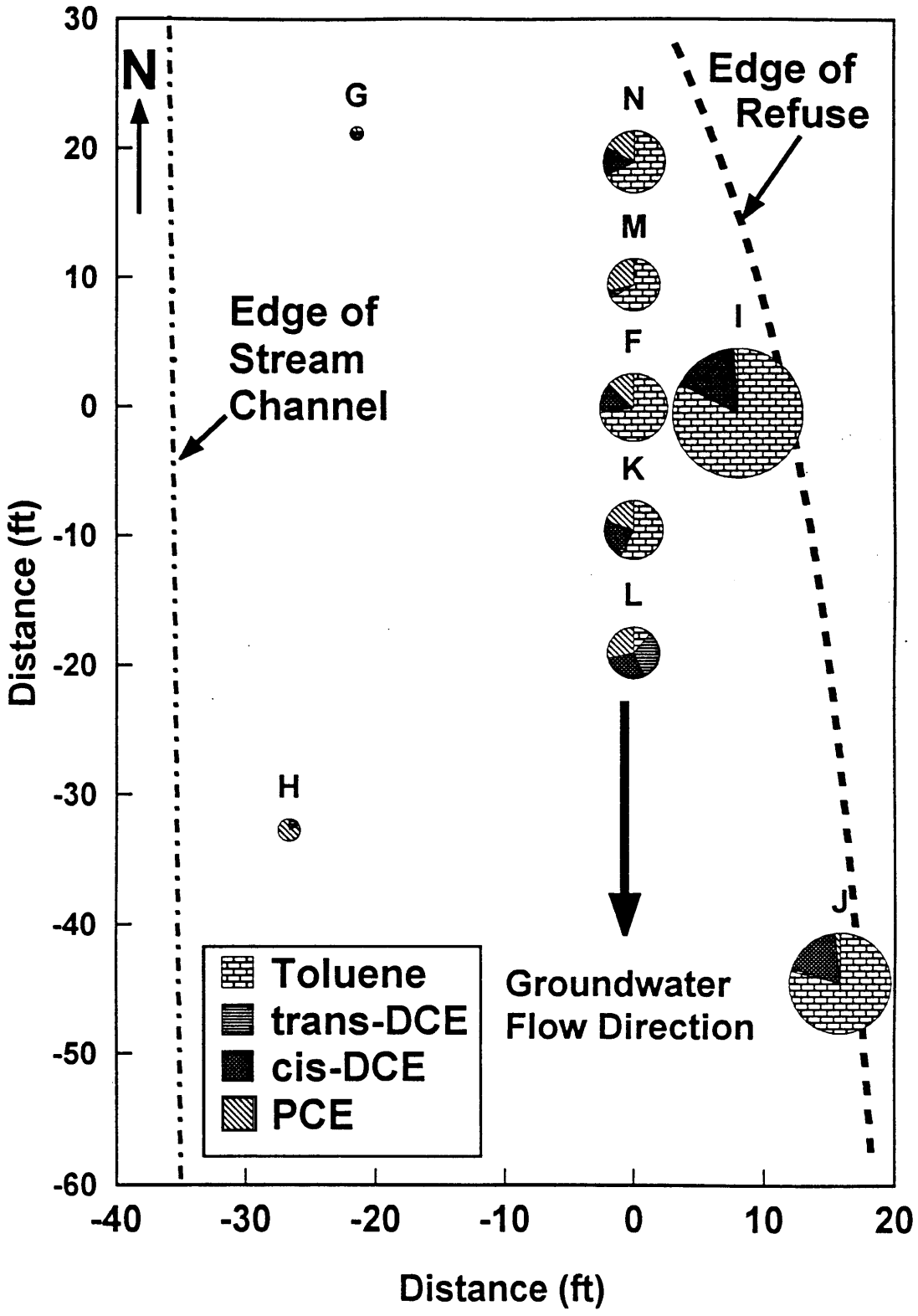
Table 5.
Average Concentration of Organics Detected in Groundwater (all concentrations in μg/l)

	--------F--------		--------G--------		--------H--------		--------I--------		--------J--------	
	AVG.	STD.	AVG.	STD.	AVG.	STD.	AVG.	STD.	AVG.	STD.
Benzene	2.	1.	2.	0.	1.	1.	1.	1.	2.	1.
Toluene	326.	286.	3.	2.	1.	1.	1358.	807.	772.	482.
Ethlybenzene	4.	3.	2.	1.	0.	0.	2.	3.	5.	4.
m,p-Xylene	6.	3.	2.	1.	1.	1.	6.	5.	6.	3.
o-Xylene	5.	2.	1.	1.	1.	0.	4.	3.	3.	2.
trans-Dichloroethylene	6.	5.	9.	9.	6.	NA	10.	NA	14.	NA
cis-Dichloroethylene	67.	104.	5.	4.	3.	NA	340.	NA	241.	NA
Trichloroethylene	0.	0.	2.	2.	2.	NA	0.	NA	0.	NA
Tetrachloroethylene	68.	119.	7.	7.	57.	NA	23.	NA	24.	NA

	--------K--------		--------L--------		--------M--------		--------N--------	
	AVG.	STD.	AVG.	STD.	AVG.	STD.	AVG.	STD.
Benzene	3.	1.	4.	3.	3.	2.	4.	4.
Toluene	211.	201.	24.	21.	184.	196.	270.	348.
Ethlybenzene	5.	3.	5.	4.	7.	4.	12.	11.
m,p-Xylene	12.	4.	7.	6.	15.	3.	24.	9.
o-Xylene	6.	1.	6.	2.	8.	1.	13.	5.
trans-Dichloroethylene	11.	8.	76.	120.	12.	8.	0.	0.
cis-Dichloroethylene	82.	93.	64.	105.	3.	2.	59.	111.
Trichloroethylene	0.	0.	0.	0.	0.	0.	2.	2.
Tetrachloroethylene	77.	111.	73.	112.	92.	103.	76.	117.

BDL - below detection limit of 1 μg/l
AVG. - average concentration
STD. - standard deviation
NA - not available

Figure 5 - Spatial Variation in Average Concentrations of Toluene, c-DCE, t-DCE and PCE



occurring too slowly to be detected given the high variability in contaminant concentrations. The coefficient of variation typically exceeded 100% for the chlorinated solvents and ranged from 60 to 130% for toluene. Coefficients of variation for benzene, ethylbenzene and the xylenes were lower because the averages were close to the analytical detection limit. Because of this high variation in contaminant concentrations, the rate of biodegradation would have to be very high to be detectable. Field monitoring results indicate that there is no evidence of significant biodegradation over the 4- to 5-month travel time from well N to K. Previous laboratory studies had suggested that significant biodegradation would occur during this transport period. The lack of measurable biodegradation in this test suggests that it may not be feasible to accurately estimate in situ biodegradation rates using conventional monitoring well networks when the biodegradation rate is low. While monitoring wells could be spaced further apart to increase the travel time, this would increase the uncertainty about whether the wells are on the same streamline and could further increase the temporal variability in contaminant concentrations.

7.0 LABORATORY STUDIES OF ANAEROBIC BIODEGRADATION

7.1 Experimental Design and Methods

The objective of the laboratory experiments was to study the methanogenic biodegradation of BTEX and TCE in an aquifer contaminated by municipal solid waste landfill leachate. BTEX and TCE were selected for study because these compounds (1) are of regulatory concern, (2) are typical components of landfill leachate and (3) have been reported in Raleigh landfill leachate. Studies were conducted under controlled laboratory conditions to obtain biodegradation data for our target compounds. Two sets of microcosms were constructed for measurement of anaerobic degradation of BTEX and TCE. The first set was designed to simulate in-situ conditions as closely as possible. A second set of microcosms was designed to evaluate the effects of potentially stimulating amendments on biodegradation. These amendments include calcium carbonate buffer, inorganic nutrients (ammonia and phosphate) and readily degradable organic carbon.

Microcosms were constructed using aquifer sediment and groundwater from the Wilder's Grove landfill in Raleigh, NC. Aquifer sediment was obtained from three distinct boreholes located along a groundwater flow line using anaerobic and aseptic techniques (Figure 1). Boreholes were separated by 3 m, which represent approximately 1.5 months of travel time. Groundwater was obtained from monitoring wells located immediately adjacent to each borehole. Microcosms simulating in-situ conditions were constructed using material from each individual borehole. The second set of microcosms, which contained the aforementioned amendments, was constructed using aquifer sediment and groundwater from the middle borehole (LFM).

BTEX and TCE were added to the ambient microcosms. BTEX and TCE concentrations were selected to be representative of conditions in sanitary landfills and to provide concentrations which could be easily measured. Abiotic controls were operated to differentiate between biological and abiotic losses. Abiotic conditions were achieved by autoclaving the microcosms after soil and groundwater addition but before the BTEX/TCE addition. Mercuric chloride (500 mg/l) was added after autoclaving. Abiotic controls containing groundwater but no aquifer sediment were used to differentiate between sorption to the aquifer material and sorption-diffusion into the stopper.

The ambient condition microcosms were constructed in 10-ml serum bottles using 20 g of wet aquifer sediment and approximately 10 ml of groundwater. The serum bottles were sealed without headspace to prevent volatilization of dissolved compounds. Thirty-four replicates of the live and abiotic microcosms were constructed initially. Microcosms were destructively sampled in triplicate at each time point.

The second experiment was designed to test potentially stimulatory effects of amendments on biodegradation of our target compounds. In one set of microcosms, the pH was adjusted and controlled with calcium carbonate. A second set of microcosms contained both buffer and nutrients while a third set contained buffer, nutrients and readily degradable carbon sources. Two controls containing all amendments were also constructed. The first contained bromoethanosulfonic acid (BES), a methanogen inhibitor, to study the role of methanogens in the anaerobic biotransformation of BTEX/TCE. The second set of controls contained no BTEX/TCE to measure methane production from background carbon sources. Microcosms were constructed in triplicate, except the live and abiotic ambient condition microcosms where five replicates were constructed. In all, ten sets of microcosms were constructed as described in Table 6.

TABLE 6 - Experimental Design for Amended Microcosms

Set	Description
A	Live with BTEX/TCE
B	Abiotic with BTEX/TCE
C	Live with BTEX/TCE and CaCO3
D	Live with BTEX/TCE, CaCO3 and nutrients
E	Live with BTEX/TCE, CaCO3, nutrients and supplemental carbon
F	Abiotic with BTEX/TCE, CaCO3, nutrients and supplemental carbon
G	Live with BTEX/TCE, CaCO3, nutrients, supplemental carbon and BES
H	Abiotic water (without aquifer sediment) with BTEX/TCE
Controls for Background Methane Production	
I	Live with CaCO3, nutrients and supplemental carbon
J	Live containing aquifer sediment and groundwater only

The amended microcosms were constructed in "125-ml" serum bottles (working volume of roughly 160 ml) using approximately 50 ml (75 g) of wet aquifer sediment and 52 ml of groundwater and dissolved amendments, leaving approximately 55 ml of headspace. The headspace was repetitively sampled over time. Microcosms were incubated in anaerobic jars in the dark at 61^{o}F (16^{o}C). Microcosms were monitored for changes in BTEX/TCE, methane and ethylene concentrations over time by GC. Details of the construction and analysis of microcosms are presented in the following section.

7.2 Experimental Methods

Aquifer sediment and groundwater from each borehole were required for microcosm construction. The methods used to obtain sediment and groundwater and to construct microcosms are described in this section.

7.2.1 Soil and Water Collection and Preparation. Soil cores were obtained under anaerobic and aseptic conditions by drilling to approximately 0.61 m below the water table (approximately 3.7 m below the ground surface) with an 11.4-cm (4.5-inch) hollow stem auger. A sterile split-spoon sampler was then advanced through the middle of the hollow stem auger flights to the bottom of the borehole and driven to sample the 3.7- to 4.3-m interval of the aquifer and retrieved. The split-spoon sampler contained a sterile polyethylene liner (Brainard-Kilman, Raleigh, NC) which was removed with the intact soil core. The core was then wrapped in plastic wrap, placed in a cooler and transported to the laboratory where it was placed in an anaerobic chamber. In the anaerobic chamber, the polyethylene liner was cut away with a sterile scalpel and the outer portions of the soil pared away using a sterile spatula. The remaining soil was transferred into one-quart Mason jars in the chamber and stored at 4^{o}C. Prior to use, soil from the Mason jars was thoroughly mixed in a sterile aluminum pan in the anaerobic chamber. Soil for both sets of microcosms was stored for less than two weeks between removal from the ground and microcosm construction.

Groundwater was collected under anaerobic and aseptic conditions from monitoring wells located adjacent to each borehole. Prior to collection, sampling apparatus was autoclaved, sample bottles were sparged with nitrogen and wells were purged with argon. Water was pumped through a closed system of tubing by a dedicated Waterra pump (Waterra, Buffalo, NY) through tygon tube equipped with a 0.45-μm filter and into a collection bottle. Water was stored at 4°C. Prior to use, the groundwater was sparged with nitrogen using a stone bubble diffuser to strip volatile compounds.

7.2.2 Preparation of Spike Solutions. BTEX, TCE, sodium sulfide and resazurin were added to all ambient microcosms and mercuric chloride was added to the abiotic controls. Amended microcosms contained various combinations of calcium carbonate, ammonia and phosphate, readily degradable carbon sources and bromoethanesulfonic acid (BES). Spike preparation was done either at the anaerobic gassing station or inside an anaerobic chamber. With the exception of the BTEX, all solutions were autoclaved.

Two tenfold concentrated spike solutions were used for the ambient microcosms. The first solution contained sodium sulfide (Na_2S), resazurin and BTEX. The final concentrations of sodium sulfide and resazurin in the microcosms were 2.7 mM and 0.0002%, respectively. The initial target concentration for benzene and toluene was 2 mg/l each while the target concentration for the other compounds was 1 mg/l. Actual initial concentrations were measured and are reported with the results. The second spike contained mercuric chloride ($HgCl_2$). The final concentration of $HgCl_2$ was 500 mg/l.

Six spike solutions were used for the amended microcosms: (1) BTEX and TCE, (2) Na_2S and resazurin, (3) $HgCl_2$ in abiotic controls only, (4) ammonia and phosphate, (5) supplemental carbon and (6) BES. The final sulfide, resazurin and $HgCl_2$ concentrations were given above. Ammonium chloride (NH_4Cl) and monobasic potassium phosphate (KH_2PO_4) were added to the microcosms at final concentrations of 10 mM and 5 mM, respectively. The target concentrations of BTEX and TCE in the microcosms were 5 mg/l each. Actual initial concentrations are reported with the results. The final concentration of each supplemental organic carbon source in the microcosms was 50 mg/l for each of sodium acetate, sodium formate, sodium benzoate, glucose and yeast extract. Where added, the final BES concentration in the microcosms was 5 mM.

7.2.3 Microcosm Construction. The ambient microcosms were constructed without headspace to ensure all compounds remained in the aqueous phase. First, 20 g of soil were added to a sterile, 10-ml serum bottle. Next, groundwater was added to the serum bottles until nearly full. One ml of BTEX/TCE spike was then added by syringe at the bottom of the bottle to minimize evaporative losses. Groundwater was then added at the top of the serum bottle until full and the bottle sealed with a teflon-faced butyl rubber stopper (West Co., Phoenixville, PA). Aluminum crimp caps secured the stoppers. In the case of abiotic microcosms, sufficient groundwater was added to cover the soil after which microcosms were sealed and autoclaved for 1 hour on 2 consecutive days. Then, mercuric chloride and BTEX spikes were added, the bottles were filled with groundwater and sealed.

Amended microcosms were constructed in the anaerobic chamber using aquifer sediment and groundwater from a borehole near LFM. First, 75 g of soil were added to a sterile 125-ml serum bottle. Groundwater was then added to saturate the soil and the bottle was shaken to release trapped gases. The excess water was poured off leaving approximately 50 ml of saturated soil in the microcosm. At this point, microcosms scheduled for buffer addition received 3.7 g of powdered calcium carbonate. In preliminary experiments, it was determined that this quantity of calcium carbonate would buffer the sediment at pH 7.5. Next,

a predetermined volume of groundwater was added to each microcosm in consideration of the other additions to be made. The final volume of free liquid, including groundwater and spike solutions, was 50 ml in each microcosm. After the appropriate volume of groundwater was added the microcosms were sealed with black butyl stoppers (Belco Biotechnology, Vineland, NJ) and aluminum crimp caps. BTEX/TCE and treatment spikes were then added by syringe through the stopper. The volume of each spike addition is presented in Table 7. The abiotic microcosms were autoclaved for 1 hour on 3 consecutive days before the BTEX , treatment spikes and mercuric chloride were added.

Once construction of a set of microcosms was complete, the microcosms were placed in an anaerobic incubation jar with oxygen-scavenging catalyst envelopes (BBL GasPak Jar System, Fisher Scientific, Raleigh, NC) and dry redox indicator strips. The jars were then evacuated and refilled with nitrogen three times, and then sealed and stored in the dark at 61°F (16°C).

Table 7 -- Spikes Added to Each Set of Microcosms

	Treatment						
Microcosm Set	BTEX/ TCE (ml)	Na_2S, Resazurin (ml)	$CaCO_3$ (gms)	Nutrients (ml)	Supplemental Carbon (ml)	$HgCl_2$ (ml)	BES (ml)
A	7	1					
B	7	1				1	
C	7	1	3.7				
D	7	1	3.7	1			
E	7	1	3.7	1	1		
F	7	1	3.7	1	1	1	
G	7	1	3.7	1	1		1
H	7	1				1	
I		1	3.7	1	1		
J		1					

7.2.4 Sampling and Analysis of Microcosms. In the ambient microcosms, triplicate live and abiotic microcosms were destructively sampled at each time point. The liquid above the soil-water interface was sampled for BTEX/TCE and methane/ethylene analyses. Dissolved methane and ethylene were measured by injecting a 1-ml liquid sample to a 10-ml headspace sampling vial previously sealed with a black butyl rubber stopper and aluminum crimp cap. To prepare a sample for analysis, 3 ml of deionized water were injected to pressurize the vial slightly, after which 1 ml of headspace gas was withdrawn and immediately analyzed by GC. Methane and ethylene concentrations were calculated assuming all dissolved methane and ethylene from the sample volatilized in the headspace of the sample vial. A second aliquot of liquid, (0.25 - 1.0 ml), for BTEX and TCE analysis, was diluted to 5.0 ml in a 5.0 ml Hamilton Gastight syringe for injection into the Tekmar purge-and-trap apparatus for BTEX and CAH analysis. In the amended microcosms, both liquid and headspace samples were analyzed. For liquid sampling, the bottle was gently placed on its side to allow the liquid to cover the stopper. The stopper was then punctured and 0.1 ml of liquid was withdrawn, diluted to 5.0 ml as above and injected into the purge and trap. To obtain headspace samples for both BTEX, TCE, methane and ethylene, 1.0 ml of nitrogen gas was injected into the

microcosm through the stopper after which 1.0 ml of headspace was removed using the same syringe for each GC injection. Headspace samples were analyzed immediately after removal from a microcosm. After sampling, microcosms were returned to the anaerobic incubation jars for incubation as described above.

7.2.4.1. Analytical Methods. BTEX concentrations were analyzed by a Perkin-Elmer 8500 or a Perkin-Elmer Autosystem gas chromatograph (GC). Each GC was equipped with a DB-624 megabore capillary column (J & W Scientific, Folsom, CA) and flame ionization detector (FID). The column was 75 m long by 0.53-mm inside diameter with a 3.0-m film thickness. For liquid samples, the GCs were equipped with a Tekmar LSC 2000 purge-and-trap injection system. The GC oven temperature was initially held at 35°C for five minutes, then increased to 220°C at 10°C/minute and finally held at 220°C for 7 minutes to bake any residual analytes off the column before the next sample injection. The flow rate of helium carrier gas was 10 ml/min. Methane was analyzed by injection of 1 ml of headspace gas onto a Shimadzu GC-9A equipped with a 1.5-m by 3.2-mm stainless steel column packed with Hayesep T 100/120 mesh (Alltech, Deerfield, IL) and an FID. The column was operated isothermally at 32°C and a helium carrier gas flow rate of 30 ml/min.

7.2.4.2 Statistical Analysis of Monitoring Data. Concentrations of the added organics were monitored as a function of time. Degradation rates were calculated assuming that degradation was governed by the first-order rate equation,
$dC/dt = -KC$, or in integrated form:

$C_t = C_o(e^{-Kt})$ where,
C_t = concentration in mg/l at time t
C_o = initial concentration in g/l
K = rate constant in $days^{-1}$
t = time in days

The rate constant was calculated from the slope of the regression line between log concentration and time. In the ambient microcosms, Students T-tests were employed to evaluate whether differences in the slopes between live and abiotic regression lines were significant (Moore and McCabe, 1993). A significantly higher loss rate in a set of live microcosms denoted biodegradation. The experimental design in the amended study did not permit use of the Student's T-distribution because multiple comparisons between treatments and controls as well as between treatments were necessary. The Dunnett's T-distribution (SAS Institute Inc., 1991) was applied between the average first-order rates of any two sets of microcosms for which a comparison was desired.

8.0 RESULTS OF LABORATORY STUDIES

Two sets of microcosms were constructed using groundwater and aquifer sediment from the Wilder's Grove landfill in Raleigh, NC. The first set, referred to as the ambient microcosms, was constructed to simulate as nearly as possible in-situ conditions. These microcosms were destructively sampled over time. The second set, referred to as the amended microcosms, evaluated the effects of buffer, nutrients and easily degradable carbon source addition on BTEX/TCE biodegradation. These microcosms were repetitively sampled over time. The BTEX, TCE, methane and ethylene concentrations were monitored for 306, 318 and 395 days at locations LFN, LFM and LFF, respectively and for 197 days in the amended microcosms. Monitoring results and data analyses are presented in this section.

8.1 Ambient Microcosms

8.1.1 Biodegradation in LFN Microcosms. Borehole LFN is the most up gradient location and is approximately 1.5 m from the edge of the buried refuse. Concentrations of each target compound over time in the LFN microcosms are presented in Table 8. Biodegradation of each compound will be discussed individually in this section.

Microorganisms in the LFN aquifer sediment exhibited limited affinity for TCE. From an initial concentration of 2710 µg/l, TCE was degraded to below detection (10 µg/l) in one of three microcosms by Day 41, a trend that continued at each of the next two samplings (Days 99 and 208). At all three time points where one of three microcosms degraded TCE, the remaining two microcosms exhibited no biological losses of TCE. By Day 306, TCE was below detection in two of three microcosms (Figure 6). Microcosms that completely degraded TCE subsequent to the Day 41 sample may have experienced a lag phase that was not detected due to our sampling schedule. The TCE biodegradation data show the disappearance of TCE in individual microcosms while other, seemingly replicate microcosms, either did not have the appropriate microorganisms or were unable to adapt to TCE degradation.

Daughter products of TCE biodegradation did not appear regularly. c-DCE appeared only once, at Day 99 (1205 µg/l) in the microcosm that degraded TCE. Based on the presence of 2709 µg TCE/l initially, 1997 µg/l of DCE would be expected if all the TCE were present as DCE. However, further dechlorination may also have occurred. Vinyl chloride, a likely daughter product, would not have been detected using the analytical techniques employed here.

Degradation rate constants are presented in Table 9. The rate constant for TCE degradation had a large standard error in the live data set because TCE was completely degraded in some microcosms and not degraded in others. This caused the T-statistic to indicate no significant difference between the first order rates of the live and abiotic microcosms, despite the fact that the first order rate of the live microcosms was 3.3 times greater than the first-order abiotic rate. Despite this statistical result, there were clearly biological losses of TCE in selected microcosms.

Ethylene was observed at Day 306 (150 and 140 µg/l) in the two microcosms that degraded TCE. Based on the initial TCE concentration, four times this amount of ethylene would be expected had it been the only daughter product produced. There are many factors which could explain the failure to account for all the TCE as ethylene including (1) the presence of vinyl chloride, (2) losses of ethylene during sampling or into the stopper during incubation, (3) abiotic losses of TCE through the stopper such that not all TCE present initially was

Table 8 BTEX, TCE and Methane Concentrations in LFN Microcosms (µg/l)

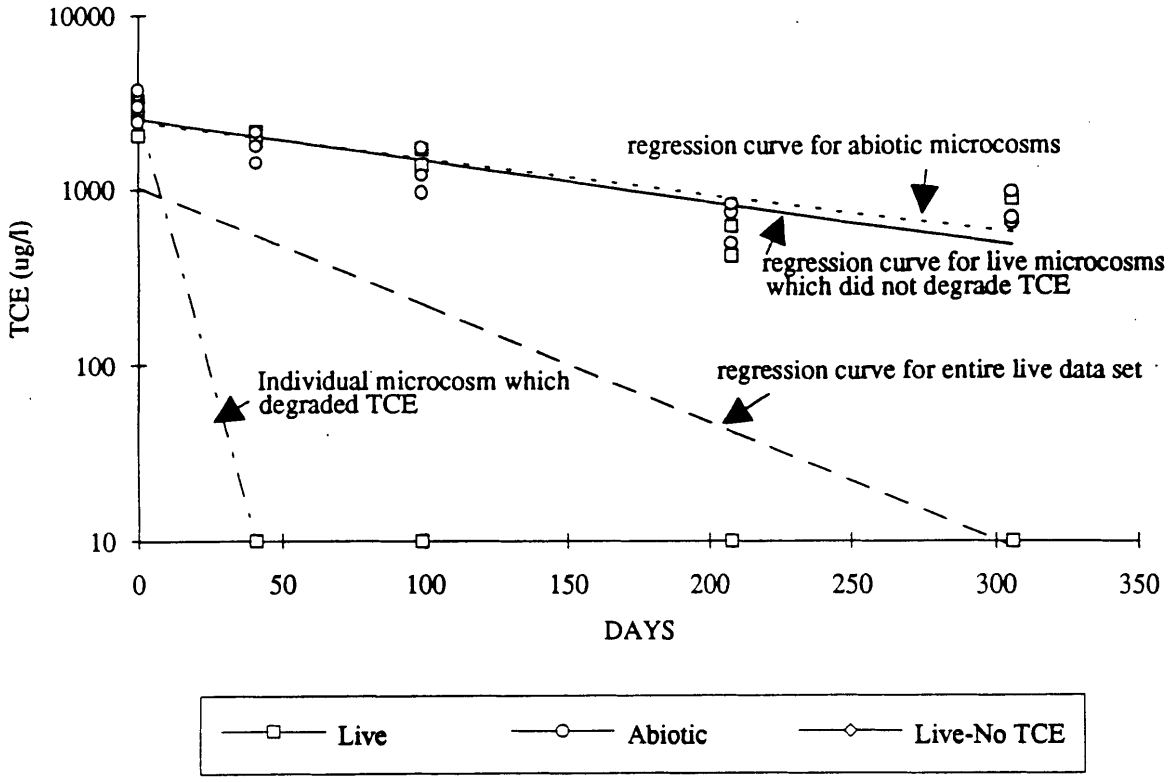
Day	0		41		99		208		306	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
TCE										
Live	2709	480	1349	1176	1023	900	349	319	293	507
Abiotic	3123	566	1777	355	1299	391	689	174	764	177
Abiotic Water	1837	89			1129	31	771	34	716	41
Toluene										
Live	2158	404	2893	414	1426	953	685	102	924	416
Abiotic	2533	452	1869	446	1195	337	780	232	818	264
Abiotic Water	1812	334			1297	309	903	219	843	139
Live - no TCE	3439	661			1803	449	33	6	37	9
Live - no added BTEX/TCE	686	699					318	246	16	2
Benzene										
Live	4532	817	4156	32	3531	344	2181	482	3084	310
Abiotic	5218	880	3318	363	3132	618	2210	458	2946	503
Abiotic Water	3052	104			2466	43	2159	110	2363	120
Live - no TCE	4395	817			2843	265	2557	277	2616	181
Ethylbenzene										
Live	685	98	553	82	398	92	137	43	195	73
Abiotic	867	167	337	110	263	160	212	87	178	98
Abiotic Water	533	36			237	26	176	16	134	11
Live - no TCE	1707	360			559	66	520	285	320	130
m-Xylene										
Live	727	100	603	89	413	92	141	44	193	74
Abiotic	959	155	344	123	272	179	222	97	174	97
Abiotic Water	573	46			240	25	177	15	131	13
Live - no TCE	1763	373			547	64	502	282	301	125

Table 8 BTEX, TCE and Methane Concentrations in LFN Microcosms (µg/l)

Day	0		41		99		208		306	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
o-Xylene										
Live	1162	181	1066	131	767	143	297	81	449	160
Abiotic	1548	235	628	198	541	326	464	198	412	217
Abiotic Water	865	69			468	24	365	22	302	28
Live - no TCE	2335	513			904	111	865	438	577	226
Methane (mg/l)										
Live	1.0	0.7	0.6	0.1	12.4	7.4	29.8	2.0	25.7	3.2
Abiotic	0.3	0.2	0.2	0.1	0.3	0.2	0.5	0.6	0.1	0.03
Abiotic Water	1.5	0.1			1.2		1.2	0.1	1.1	0.1
Live - no BTEX	1.7	1.1			27.0	2.3	33.4	2.3	29.9	2.3
Live - no TCE	0.0	0.4			31.8	3.6	30.0	5.1	27.3	2.9

SD = Standard Deviation

Figure 6 - TCE Degradation in LFN Microcosms



biologically converted and (4) biological conversion of ethylene to ethane (De Bruin et al., 1992).

Table 9 -- First-Order Degradation Rate Constants in Live and Abiotic LFN Microcosms

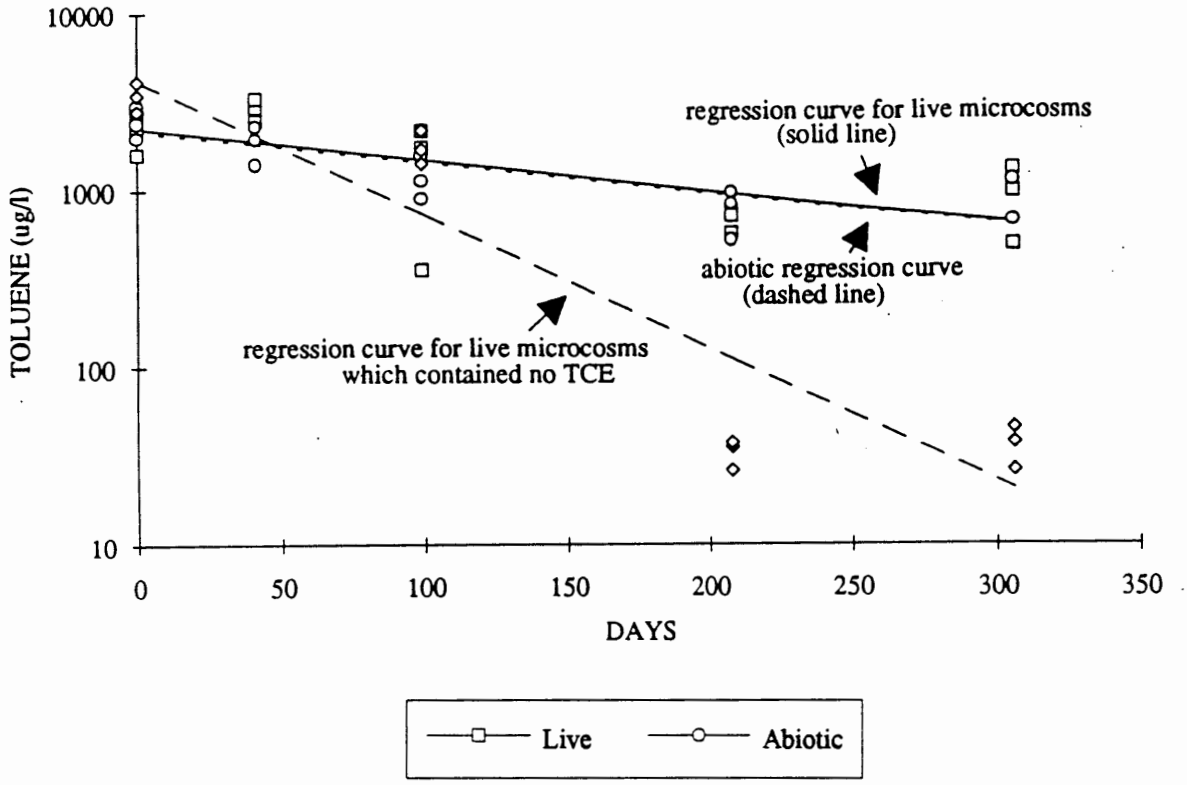
Compound	First Order Rate Constant[1] Live	Abiotic	Adjusted[2]	95% Confidence Interval[3]
Benzene	-1.76	-1.9	0.14	(-1.73, 1.44)
Ethylbenzene	-5.1	-4.69	-0.41	(-3.29, 2.45)
m-Xylene	-5.36	-4.98	-0.38	(-3.36, 2.59)
o-Xylene	-4.16	-3.84	-0.32	(-3.07, 2.43)
Toluene	-4.02	-3.96	-0.06	(-2.88, 2.75)
Toluene (no TCE)	-17.34	-3.96	-13.38*	(-18.26, -8.50)
TCE (overall)[4]	-15.4	-4.72	-10.68	(-25.12, 3.75)
TCE (anomaly)[5]	-192.5	-4.72	-187.78*	NA

1 Units of day^{-1} x 1000; negative sign indicates loss of compound
2 Adjusted = live rate minus abiotic rate
3 95% confidence interval of adjusted rate constant
4 Rate constant calculated between 0 and 306 days
5 Rate constant calculated between average initial concentration and single microcosm that degraded TCE by Day 41
* Significant difference between live and abiotic microcosms indicated in T-test
NA Not applicable since rate was calculated between time zero replicates and an individual microcosm at Day 41

Toluene biodegradation was monitored in sets of microcosms with and without TCE. There was no biological loss of toluene in the presence of TCE as the rate of toluene disappearance in live and abiotic LFN microcosms was not statistically different (Table 9). In the absence of TCE, there was no apparent biological loss of toluene through day 99. However, as illustrated in Table 8 and Figure 7, measurable toluene losses were observed by Day 208. The first-order degradation rate of toluene between Days 99 and 208 was -0.036 day^{-1} compared to -0.013 day^{-1} for the entire 306-day monitoring period.

Toluene degradation was also monitored in microcosms to which no BTEX or TCE was added. These microcosms were initiated to measure background methane production and had small amounts of toluene associated with the sediment or groundwater. Here, toluene was degraded to an average of 16 µg/l (Table 8). In two sets of microcosms, the lower limit of toluene degradation was 16 to 34 µg/l. The presence of TCE appeared to inhibit toluene degradation in

Figure 7 - Toluene Degradation in LFN Microcosms



the LFN microcosms. TCE inhibition amongst all microcosms is discussed in the following section.

Benzene, ethylbenzene and xylene isomer concentrations in the LFN microcosms are presented in Table 8. Regression coefficients and T-statistics are presented in Table 9. There were no significant differences between the losses measured in live and abiotic microcosms. Thus, these compounds were recalcitrant under the conditions of this study.

Abiotic or killed control microcosms were monitored to differentiate between biological and abiotic losses of the target compounds. Abiotic losses have been reported in Table 8 and calculated biodegradation rates have been corrected for abiotic losses in Table 9. Abiotic microcosms with water but no aquifer sediment were used to differentiate between sorptive and diffusive losses. Statistical analysis indicated that loss rates between abiotic microcosms containing aquifer sediment and abiotic microcosms containing groundwater only were not significantly different. Therefore, sorption to the aquifer sediment was insignificant compared with sorption and diffusion through the stopper. An average of 0.31 mg methane/l was present in abiotic microcosms on Day 0 (Table 8). The most reasonable explanation for this observation is that methane was produced between the time that microcosm construction was complete but prior to autoclaving and mercuric chloride addition. No further methane was produced after Day 0 and the abiotic microcosms were judged to be biologically inactive. In judging microcosms to be biologically inactive, we assumed that the absence of methanogenic activity corresponded to the absence of all biological activity.

Methane concentrations in LFN microcosms are presented in Table 8. LFN included three different groups of live microcosms containing (1) BTEX and TCE, (2) BTEX but no TCE and (3) no added BTEX or TCE. On Day 99, average methane concentrations in these microcosm sets were 12, 31 and 27 mg /l, respectively. By Day 208, methane concentrations were 30, 30 and 33 mg/l in these microcosms, respectively, and remained fairly constant through the last sampling on Day 306. Since similar amounts of methane were produced in microcosms to which organic additions were and were not made, methane was produced from biodegradation of background carbon in the groundwater and could not be attributed to biodegradation of our target compounds.

Approximately 3.4 mg/l of toluene were biodegraded in the LFN microcosms containing no TCE. Assuming complete conversion of the toluene to methane and carbon dioxide as described by Eqn. 1, 2.7 mg/l methane would have been expected. This is significantly less than the methane produced due to naturally occurring carbon. Thus, methane production cannot be definitively attributed to toluene degradation. Methane production was observed earlier in the two sets of microcosms containing no TCE, suggesting that TCE partially inhibited methanogenesis.

$$C_6H_5CH_3 + 5H_2O \text{ ---> } 4.5CH_4 + 2.5CO_2 \qquad (1)$$

8.1.2 Biodegradation in LFM Microcosms. The concentrations of BTEX, TCE and methane in microcosms constructed with aquifer sediment from borehole LFM are presented in Table 10. The biodegradation of each compound will be discussed individually in this section.

The concentration profile for TCE is presented in Figure 8. There was a lag phase of between 50 and 110 days before the onset of TCE removal. Once TCE removal began it was uniform in the live microcosms. Between Days 110 and 222, TCE was degraded to less than 10 µg/l in all live microcosms. Biodegradation rates are presented in Table 11.

Table 10 BTEX, TCE and Methane Concentrations in LFM Microcosms (µg/l)

Day	0		55		110		222		318	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
TCE										
Live	1313	150	926	152	612	149	<10		<10	
Abiotic	1587	244	895	83	622	38	408	59	530	114
Abiotic Water	1362	27			764	70	434	54	464	61
Toluene										
Live	1016	181	26	5	29	9	34	4	20	2
Abiotic	1323	325	707	89	302	238	363	54	358	103
Abiotic Water	1486	354			690	117	453	61	466	45
Live - no added BTEX/TCE	161	63			30	11	8	3	13	1
Benzene										
Live	2700	264	2351	401	1869	305	1425	217	1368	205
Abiotic	3069	432	2249	85	1907	96	1642	147	2287	428
Abiotic Water	2576	39			1981	78	1565	107	1806	145
Ethylbenzene										
Live	205	40	136	26	80	41	46	16	26	2
Abiotic	244	52	125	25	61	8	50	10	62	17
Abiotic Water	233	2			106	29	68	14	62	18
m-Xylene										
Live	303	59	194	38	108	58	62	22	37	2
Abiotic	360	71	178	39	84	12	64	14	83	22
Abiotic Water	343	4			149	44	89	20	99	54
o-Xylene										
Live	495	97	315	68	194	93	143	46	90	2
Abiotic	573	115	328	61	179	25	143	33	195	45
Abiotic Water	530	12			290	69	190	36	166	24

Table 10 BTEX, TCE and Methane Concentrations in LFM Microcosms (µg/l)

Day	0		55		110		222		318	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Methane (mg/l)										
Live	0.06	0.01	0.53		39.64	9.32	51.54	5.09	42.48	7.28
Abiotic	0.00	0.00	0.20		0.90	1.28	0.20	0.07	0.45	0.42
Abiotic Water	0.33	0.57			1.28	0.21	0.62	0.07	1.38	1.30
Line - no BTEX	0.27	0.23			54.66	0.98	42.76	5.60	42.46	4.31

SD = Standard Deviation

Figure 8 - TCE Degradation in LFM Microcosms

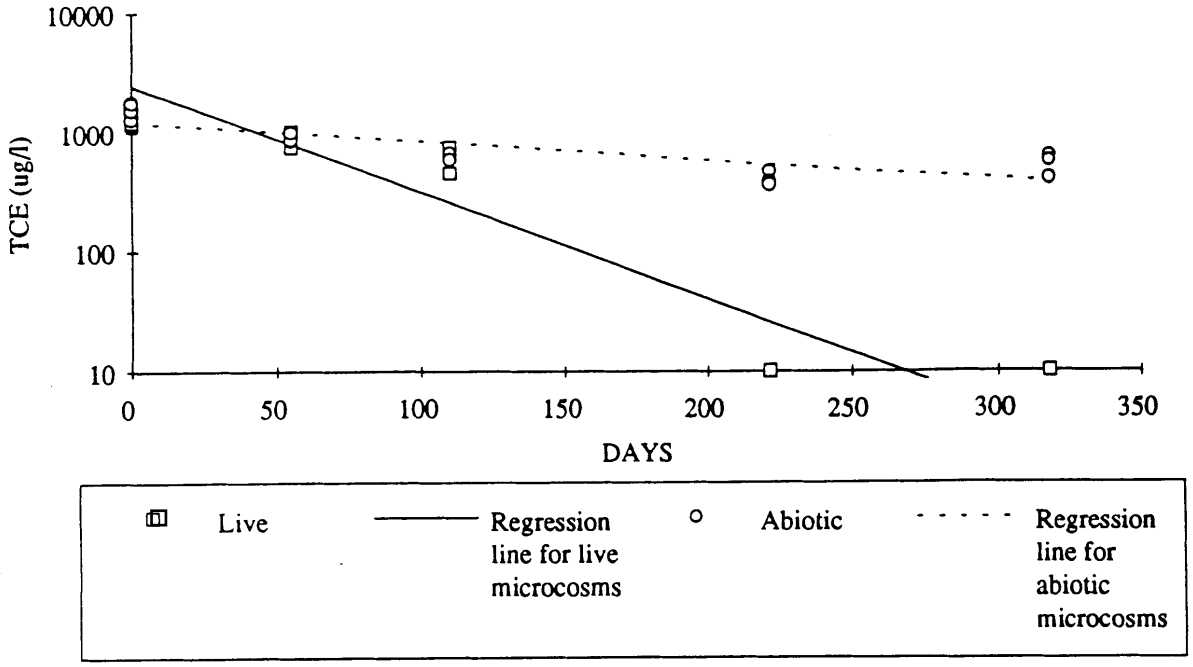


Table 11 -- First-Order Degradation Rate Constants in Live and Abiotic LFM Microcosms

Compound	First Order Rate Constant[1] Live	Abiotic	Adjusted[2]	95% Confidence Interval[3]
Benzene	-2.28	-1.01	-1.27*	(-2.38, 0.05)
Ethylbenzene	-6.38	-4.35	-2.03	(-4.32, 0.31)
m-Xylene	-6.56	-4.73	-1.83	(-4.32, 0.66)
o-Xylene	-5.11	-3.56	-1.55	(-3.78, 0.69)
Toluene	-9.32	-3.71	-5.61	(-12.25, 1.03)
Toluene (0-55)[4]	-66.95	-3.71	-63.24*	
TCE	-26.72	-3.63	-23.09*	(-28.81, 17.58)
TCE (110-222)[5]	-57.11	-3.63	-53.48*	

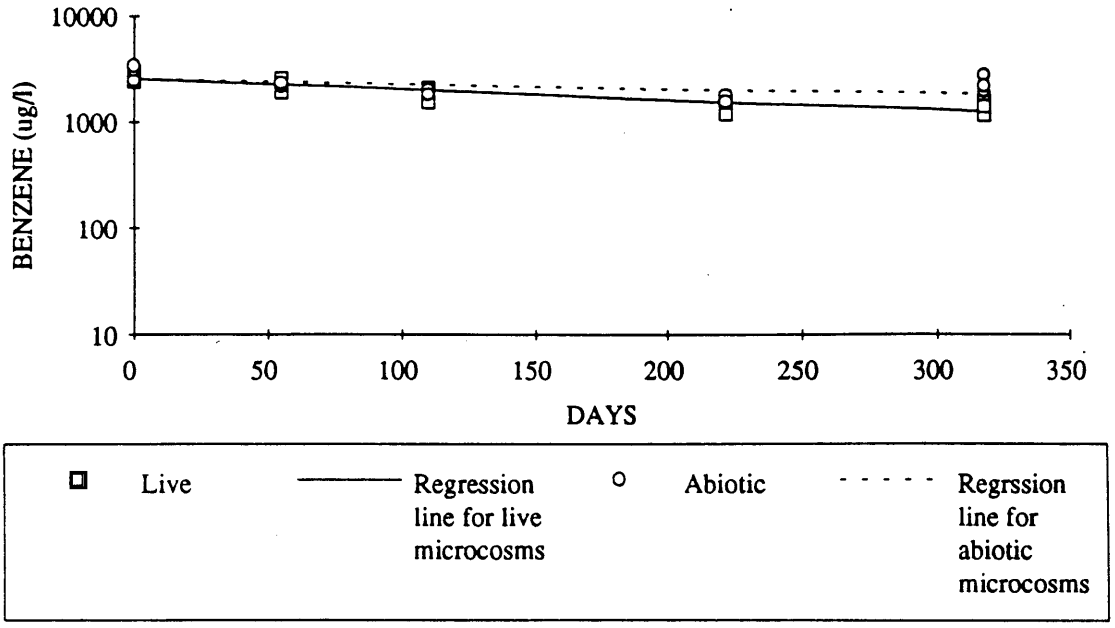
1 Units of day^{-1} x 1000; negative sign indicates loss of compound
2 Adjusted = live rate minus abiotic rate
3 95% confidence interval of adjusted rate constant
4 Rate constant calculated between 0 and 55 days
5 Rate constant calculated between 110 and 222 days
* Significant difference between live and abiotic microcosms indicated in T-test

Ethylene was the only daughter product of reductive dechlorination observed over 318 days of monitoring. In the three microcosms sampled on Day 318, 80, 70 and 50 µg ethylene/l were measured . From an initial 1313 µg TCE/l, 280 µg/l ethylene would be expected if all the TCE were converted to ethylene. Potential explanations for the failure to recover all TCE as ethylene were discussed earlier. Actual ethylene recovery may have been higher than it appeared based on the observed TCE losses in abiotic microcosms, which contained only 33% of the initial TCE dose by Day 318. TCE losses may have occurred in live microcosms as well. This would result in less than the initial TCE concentration being available for biodegradation.

Toluene was degraded from an initial concentration of 1016 µg/l to 26 µg/l by Day 55 (Table 10). Between Days 55 and 318, toluene concentrations varied between 20 and 34 µg/l. The first-order biological loss rate for toluene is reported in Table 11. In microcosms with no added BTEX or TCE, toluene concentrations decreased from 161 to 30 µg/l by the first sampling on Day 110. These microcosms were initiated to measure background methane production and had small amounts of toluene associated with the sediment or groundwater.

Benzene concentrations in the LFM microcosms are presented in Table 10 and Figure 9. The first-order loss rate for benzene in live microcosms was significantly greater than in abiotic controls (P = 0.95). Inspection of the data in Table 10 shows that the slope of the regression line may be heavily influenced by the Day 318 data where benzene concentrations remained nearly constant in the live microcosms but increased in the abiotic set. This increase is likely

Figure 9 - Benzene Degradation in LFM Microcosms



random variation but influenced the statistical analysis. As presented in the literature review, there are very few reports of anaerobic benzene degradation. Thus, the result here warrants further monitoring to verify anaerobic benzene degradation.

Ethylbenzene and xylene data for the LFM microcosms are presented in Table 10. There were no significant differences in losses of ethylbenzene and xylene isomers between live and abiotic microcosms. Concentration profiles for these compounds are very similar to those of benzene where the rate of degradation was reported to be statistically significant. The reason for the different statistical conclusion with respect to benzene versus ethylbenzene and xylene is the increased variability in the concentration data for the latter two compounds and the trend in the abiotic benzene data discussed above. The high abiotic losses for ethylbenzene and m-xylene make it difficult to statistically validate small biological losses. It is interesting to note that in the case of benzene, ethylbenzene and m- and o-xylene, the slope of the regression line for the live microcosms is always slightly steeper than that of the abiotic microcosms.

BTEX and TCE concentrations in abiotic microcosms with and without aquifer sediment are presented in Table 10. As in the LFN microcosms, there was no significant difference in the loss rate between abiotic microcosms containing aquifer sediment and abiotic microcosms containing groundwater only. Thus, sorption to the aquifer sediment was insignificant compared with sorption and diffusion through the stopper. Methane was observed in one abiotic microcosm (0.99 mg/l) on Day 0. By the last sampling on Day 318, five of six abiotic microcosms sampled contained an average of 0.52 mg methane/l and a sixth microcosm contained 2.9 mg/l. Thus, there was slight methane production over the test period indicating that abiotic microcosms were not completely killed by autoclaving and addition of mercuric chloride. However, methane production in abiotic microcosms was less than 5% of that observed for live microcosms. Thus, the abiotic microcosms were still useful to differentiate between biological and other losses.

Methane concentrations in LFM microcosms are presented in Table 10. Concentrations greater than 1.0 mg methane/l were first observed on Day 110 in the live microcosms. On Day 318, the last sampling point, the average concentrations were 42.5 mg/l in microcosms both with and without added BTEX and TCE. Thus, methane production must be attributed to background carbon as opposed to the added BTEX and TCE.

8.1.3 Biodegradation in LFF Microcosms. The concentrations of BTEX, TCE and methane in microcosms constructed with aquifer sediment from borehole LFF are presented in Table 12. The biodegradation of each compound will be discussed individually in this section. No biological losses of TCE were observed through the first 108 days of incubation. From an average concentration of 604 μg/l on Day 108, TCE was degraded to below detection (10 μg/l) in two microcosms and 74 μg/l in the third microcosm by Day 218. By Day 302, TCE was below detection in all three microcosms sampled. Biodegradation rates are presented in Table 13. Ethylene was the only daughter product of reductive dechlorination observed in LFF microcosms. Ethylene was observed on Day 302 at 48.5 μg/l (s.d. 14.8 μg/l) and on Day 395 at 39.8 μg/l (s.d. 29.4 μg/l). Based on an initial TCE concentration of 730 μg/l, 156 μg ethylene/l would be expected if all the TCE were converted to ethylene. Potential explanations for these deficiencies were discussed in section 8.1.1.

Table 12 BTEX, TCE and Methane Concentrations in LFF Microcosms (µg/l)

Day	0		75		108		164		218		302		395	
	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd
TCE														
Live	730	115	688	27	604	69	285	130	25	43	<10		<10	
Abiotic	750	163	595	37	613	62	456	36	361	55	270	8	322	97
Abiotic Water	823	81			650	63			398	23	304	31	374	76
Toluene														
Live	1439	190	1351	27	1235	26	933	142	704	222	502	93	509	110
Abiotic	1578	341	1157	97	1157	140	885	82	648	153	499	32	664	307
Abiotic Water	1632	240			1235	81			851	84	578	37	805	201
Live - no added BTEX/TCE	189	38			119	13			60	42	176	206	5	8
Benzene														
Live	1643	300	1850	155	1612	251	1235	48	1151	255	1024	142	1099	299
Abiotic	1662	269	1639	74	1617	51	1468	78	1217	170	1096	27	1317	256
Abiotic Water	1702	188			1455	98			1173	72	1069	88	1287	171
Ethylbenzene														
Live	415	54	416	51	318	30	189	43	116	60	84	23	87	21
Abiotic	411	58	324	36	309	71	169	44	118	32	93	7	138	110
Abiotic Water	481	51			308	41			159	34	91	10	167	82
m-Xylene														
Live	436	55	503	120	324	34	182	42	110	59	80	22	82	19
Abiotic	439	55	332	35	313	74	160	44	113	27	88	7	130	104
Abiotic Water	507	54			317	43			153	35	85	10	157	78
***o*-Xylene**														
Live	566	59	575	52	480	44	294	64	199	95	153	39	169	38
Abiotic	569	55	476	50	461	99	276	62	207	50	173	13	253	194
Abiotic Water	635	68			451	57			260	51	164	17	297	140

Table 12 BTEX, TCE and Methane Concentrations in LFF Microcosms (µg/l)

Day	0		75		108		164		218		302		395	
	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd
Methane														
Live	0.0		3.3	1.2	11.6	5.5	24.7	5.5	27.7	8.3	18.3	8.7	24.1	5.7
Abiotic	0.3	0.3	0.4	0.1	1.6	1.9	0.3	0.3	2.7	3.6	0.3	0.1	0.5	0.2
Abiotic Water	0.7	0.6			1.6	0.3			1.6	0.3	1.5	0.5	1.3	0.5
Live-no BTEX	0.6	1.0			6.6	5.0			29.9	2.0	31.2	3.8	27.8	3.2

SD = Standard Deviation

Table 13 -- First-Order Degradation Rate Constants in Live and Abiotic LFF Microcosms

Compound	First-Order Rate Constant[1] Live	Abiotic	Adjusted[2]	95% Confidence Interval[3]
Benzene	-1.47	-0.96	-0.51	(-1.28, 0.26)
Ethylbenzene	-4.93	-3.99	-0.94	(-2.58, 0.70)
m-Xylene	-5.36	-4.32	-1.04	(-2.80, 0.72)
o-Xylene	-3.95	-3.16	-0.79	(-2.29, 0.70)
Toluene	-3.18	-2.79	-0.39	(-1.42, 0.65)
TCE	-20.81	-2.61	-18.20*	(-23.03, -13.38)
TCE (108-218)[4]	-44.94	-2.61	-42.33*	

1 Units of day^{-1} x 1000; negative sign indicates loss of compound
2 Adjusted = live rate minus abiotic rate
3 95% confidence interval of adjusted rate constant
4 Rate constant calculated between 108 and 218 days
* Significant difference between live and abiotic microcosms indicated in T-test

Toluene was not biodegraded in LFF microcosms (Table 12) as there was not a significant difference between the live and abiotic loss rates (Table 13). Toluene degradation in the absence of TCE was not monitored in LFF microcosms. In microcosms with no added BTEX or TCE, toluene was reduced from an initial concentration of 189 µg/l to less than 10 µg/l by Day 395. These microcosms were initiated to measure background methane production and had small amounts of toluene associated with the sediment or groundwater. Toluene losses in excess of abiotic losses were not apparent in these microcosms until the Day 395 sample. This long lag time is consistent with the absence of measured biodegradation in the microcosms with added BTEX and suggests that limited capacity for toluene biodegradation is present in the LFF aquifer sediment.

Benzene, ethylbenzene and xylene concentrations in the LFF microcosms are presented in Table 12. Loss rates are presented in Table 13. There were no significant differences between the losses measured in live and abiotic microcosms. Thus, these compounds were recalcitrant under the conditions of this study.

Organic concentrations in abiotic microcosms with and without aquifer sediment were similar. Thus, sorption and diffusion through the stopper appear to be the dominant abiotic losses. As observed in LFN and LFM microcosms, LFF abiotic microcosms with and without aquifer sediment contained methane at the initial sampling (0.31 and 0.69 mg/l, respectively). No further methane was produced through 395 days of observation. Thus, these microcosms served their purpose as abiotic controls.

Methane concentrations are presented in Table 12. Methane production in live microcosms containing BTEX and TCE and in microcosms without added organics were similar on Days 108,

218, 302 and 395. Since increased methane production was not observed in microcosms with added BTEX and TCE, it is apparent that the observed methane resulted primarily from background carbon in the groundwater and aquifer sediment.

8.1.4 Biodegradation of BTEX and TCE in the Presence of Buffer, Nutrients and Readily Degradable Carbon. The effects buffer addition, nutrient addition and addition of readily degradable carbon on BTEX and TCE biodegradation were studied in what are referred to as the amended microcosms. In contrast to the 20-ml serum bottles used for the ambient microcosms, these microcosms were constructed in 125-ml serum bottles using aquifer sediment and groundwater from borehole LFM. Thirty four microcosms were constructed as described in Table 1. Microcosms were repetitively sampled over time and analyzed for BTEX, TCE, methane and ethylene. Results of these analyses are discussed in this section.

There was some variation in the initial concentrations of the respective target compounds in the amended microcosm study. Upon construction of the amended microcosms, each microcosm was thoroughly shaken by hand and stored at 16^{o}C for 12 to 24 hrs. A 0.1-ml aliquot of the free liquid was then removed from the microcosm by syringe for analysis. Concentrations are presented as initial concentrations in Tables 14 and 17 through 21. After another 12 to 24 hrs, allowing time to analyze the liquid in each of the 34 microcosms, an initial headspace gas sample (1.0 ml) was analyzed. This value was adjusted to the concentration in the liquid and does not represent a concentration in the headspace gas. This concentration is presented as "headspace analysis" in Tables 14 and 17 through 21. A more accurate description of the headspace data would be "liquid concentration by headspace analysis." The variation between microcosms and between the initial liquid analysis and the initial headspace gas analysis can be explained by differing states of equilibrium between the liquid and the headspace in the microcosms. The initial headspace gas analyses consistently showed lower liquid concentrations of target compounds than the direct liquid analyses. This may have been due to the short time period allowed for the liquid and gas to reach equilibrium. More time should have been allowed for the microcosms to equilibrate before an initial analysis was made to reduce the variability in the initial concentrations. However, for uniformity in reporting and discussing results, the initial headspace analysis is used when referring to an initial concentration in a microcosm or group of microcosms.

TCE concentration profiles are illustrated in Figure 10. TCE losses were greater in live microcosms (sets A, C, D and E) than in the abiotic controls (sets B, F and H) or the BES inhibited live controls (set G). The regression lines for microcosm sets B and F overlap in Figure 10 and the data for the BES amended microcosms are presented in Table 14 only. The degradation rate in sets C, D and E, which were amended with potential stimulants, was greater than the rate in the unamended live controls (set A). However, the first-order loss rates were not significantly different among sets C, D and E.

Calcium carbonate was a component of each of the three amendments (sets C, D and E). It served to adjust the pH from 6.7 under ambient aquifer conditions to 7.5. None of the treatments beyond $CaCO_3$ further stimulated biological removal of TCE to a greater extent than the stimulation attributable to $CaCO_3$. Since there was no additional stimulation of TCE degradation in the supplemental carbon-amended microcosms, there was apparently sufficient carbon in the groundwater to support reductive dechlorination. As described in the literature review, reductive dechlorination requires an oxidizable carbon source. Although TOC measurements were not made on groundwater collected for this study, Douglass and Borden (1992) reported 893 mg/l COD and 386 mg/l TOC in well LFF during 1988 and 1989.

Figure 10 - Best Fit Regression Lines for TCE Degradation in Amended Microcosms

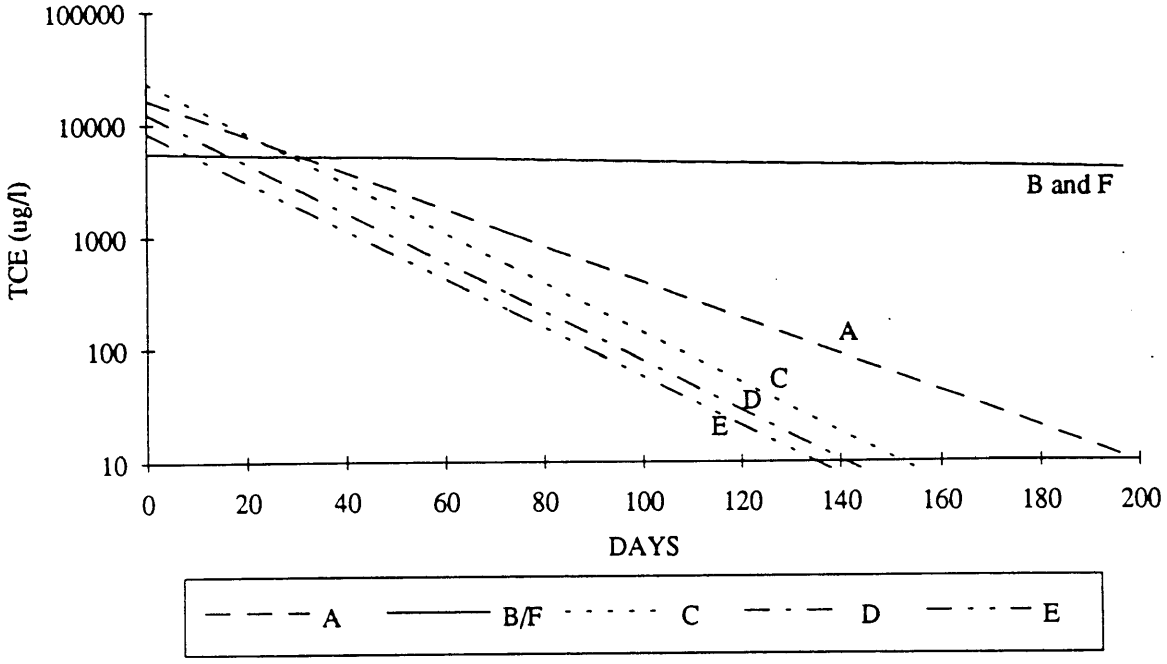


Table 14 TCE Concentrations in Amended Microcosms (µg/l)

Day	0		0		30		73		160		197	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Description	Liquid		Headspace Analysis									
A) Live with BTEX/TCE	7215	948	5634	929	4644	1175	3970	1126	858	521	<10	
B) Abiotic with BTEX/TCE	7219	480	5840	186	5116	312	4484	274	4359	340	3838	339
C) Live with CaCO3 and BTEX/TCE	7460	1118	6959	836	5466	640	4532	857	<10		<10	
D) Live with CaCO3, Nutrients and BTEX/TCE	6425	1112	5219	1296	4056	1468	1433	1108	<10		<10	
E) Live with CaCO3, Nutrients, Carbon and BTEX/TCE	6065	1539	6105	1581	4509	1680	1651	1897	<10		<10	
F) Abiotic as in E	7338	189	7075	266	5802	96	5523	329	5394	556	4665	316
G) Live as in E with BES	5823	177	5970	173	5286	417	4606	260	4492	372	3653	634
H) Abiotic Water with BTEX/TCE	7691	1399	6892	852	3348	2170	5331	640	5443	457	4728	527
I) Live as in E with no amendments and no BTEX/TCE	<10		<10		<10		<10		<10		<10	
J) Live with no Amendments and no BTEX/TCE	<10		<10		<10		<10		<10		<10	

SD = standard deviation

Neither biological loss of TCE nor methane production was observed in BES inhibited microcosms. Since methane was produced and TCE was reduced in non-BES microcosms, it appears that methanogens were involved in TCE reduction. Whether methanogens participated directly in TCE reduction, or performed some critical function for another group of organisms which participated in reductive dehalogenation, could not be determined from these data.

Ethylene concentrations are presented in Table 15. An average of 10, 225 and 761 µg ethylene/l was observed in microcosm sets C, D and E, respectively by Day 73. Ethylene production was less than 1 µg/l in unamended live microcosms (set A) during the same period and no ethylene was observed in the live controls (sets I and J). Thus, ethylene production was stimulated by the amendments in sets C, D and E, and the addition of nutrients and buffer (Set D) was more stimulatory at Day 73 than buffer alone. By Day 197, ethylene production was similar in the unamended (set A) and amended (sets C, D and E) microcosms. The data show that most of the TCE degraded was recovered as ethylene. From an average initial TCE concentration of 6093 µg/l in the three amended microcosm sets, 1300 µg/l ethylene would be produced if all the TCE were converted to ethylene. As presented Table 16, recovery of TCE as ethylene ranged from 82 to 95% with one outlier (123%). The largest ethylene concentration observed in an abiotic microcosm was 6.4 µg/l; 0.4% of the expected value if the initial TCE was completely converted ethylene.

Table 16 -- Expected and Observed Ethylene Recovery

Description and Bottle #		Initial TCE (µg/l)	Expected Eth (mg/l)	Cum. Eth at 197 days (mg/l)	TCE Recovered as Eth (%)
A) Live with	1	5509	1.18	0.97	82.7
BTEX/TCE	2	4072	0.87	0.77	89.1
	3	6073	1.3	1.15	88.4
	4	6367	1.36	1.12	82.6
	5	6148	1.31	1.16	88.4
C) Live with $CaCO_3$	1	7248	1.55	1.47	95.0
	2	7611	1.62	1.33	82.0
	3	6017	1.28	1.08	84.1
D) Live with $CaCO_3$	1	6043	1.29	1.15	89.2
and nutrients	2	3725	0.79	0.98	122.8
	3	5887	1.26	1.1	87.8
E) Live with	1	6427	1.37	1.27	92.6
all amendments	2	4388	0.94	0.84	89.8
	3	7499	1.6	1.38	86.1

Table 15 Ethylene Concentrations in Amended Microcosms (mg/l)

Day	0		30		73		160		197	
Description	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
A) Live with BTEX/TCE Average	0.0		0.0		0.8	0.1	643.3	97.8	1034.5	163.6
B) Abiotic with BTEX/TCE Average	0.0		0.0		1.5	1.0	4.3	1.5	4.2	1.6
C) Live with CaCO3 and BTEX/TCE Average	0.0		0		10.3	16.3	1388.5	203.7	1293.3	197.5
D) Live with CaCO3, Nutrients and BTEX/TCE Average	0.0		0.0		225.0	257.9	1109.1	93.7	1076.0	89.9
E) Live with CaCO3, Nutrients, Carbon and BTEX/TCE Average	0.0		0		760.8	400.0	1258.0	324.4	1161.8	283.8
F) Abiotic as in E Average	0.0		0		1.5	0.6	3.4	1.9	3.7	2.4
G) Live as in E with BES Average	0.0		0.0		0.9	0.1	1.8	0.5	87.4	109.8
H) Abiotic Water with BTEX/TCE Average	0.0		0.0		0.0		0.1	0.2	0.1	0.1
I) Live as in E with no amendments and no BTEX/TCE Average	0.0		0.0		0.0		1.4	0.8	0.8	0.2
J) Live with no Amendments and no BTEX/TCE Average	0.0		0.0		0.0		1.4	0.7	1.1	0.1

SD = standard deviation

The behavior of toluene in the amendment microcosms was inconsistent between treatments and between individual microcosms within a treatment set. Toluene concentration data are presented in Table 17. Two distinctly different phenomena occurred. Several amended and unamended live microcosms exhibited biological losses of toluene during the 197 days of observation. However, several other microcosms spiked with hydrocarbons and one microcosm to which no hydrocarbon was added displayed increases in toluene over time.

Further research demonstrated that the apparent increase in toluene concentration was due to leaching of a contaminant from the black butyl rubber stoppers. The contaminant had approximately the same retention time as toluene using our analytical protocol. Release of the contaminant from the stoppers appears to have been the result of poor quality control in stopper production. Another type of stopper will be used in future research.

Four of the live controls that contained BTEX but no amendments (set A) exhibited degradation of toluene. These microcosms degraded toluene to between 5 and 28% of the average initial toluene concentration of 2057 μg/l. However, in a fifth microcosm in this group, the apparent toluene concentration increased from 3506 μg/l to 11,093 μg/l by Day 197. Given the contaminant problem, toluene concentrations were not known with certainty. Even in microcosms exhibiting a decrease in toluene concentration, the measured concentration may have been impacted by the presence of contaminant. Thus, toluene degradation rates were not calculated.

There was not a measurable loss of toluene that could be attributed to biodegradation in microcosms amended with buffer (set C). Two of three microcosms amended with buffer and nutrients (set D) removed 90 and 94% of the initial toluene by Day 197. The third microcosm exhibited slightly less toluene degradation (67%). One microcosm in the live group that received all three amendments (set E), degraded toluene from 2497 μg/l initially to 298 μg/l (88% removal). There was no measurable biological toluene loss in two other microcosms in set E. However, this may have been due to interference of the contaminant in the toluene analyses.

The three live controls containing no added BTEX and no amendments (set J) degraded an average of 98% of the initial background concentration of toluene. One of three live controls containing all of the amendments but no added BTEX (set I) degraded 96% of the background toluene by Day 160. Another microcosm in this set exhibited only abiotic losses over time and the third microcosm had an increase in apparent toluene from 3185 μg/l initially to 13,328 μg/l by Day 197. Again, this may have been due to interference of the contaminant in the toluene analyses.

Apparent toluene concentration increases occurred in 7 of 34 live and abiotic microcosms. Toluene was not a metabolic byproduct since increases also occurred in abiotic controls. Despite the confusing data, it is evident that microorganisms indigenous to this aquifer sediment biodegrade toluene. This is consistent with the results from the ambient microcosm data. However, given the contaminant input, it is not possible to determine a degradation rate or whether the amendments in Sets C, D and E had an effect on toluene biodegradation.

Concentration data for benzene, ethylbenzene and xylene isomers are presented in Tables 18 to 21. These compounds did not exhibit statistically significant biological losses and appeared recalcitrant. A regression of the benzene degradation data is presented in Figure 11. Note that the regression lines for the abiotic microcosms (sets B and F) overlap and that the vertical scale has been expanded in order to present small differences in regression slopes. The data for ethylbenzene and o- and m-xylene exhibit similar trends and were not plotted.

Table 17 Toluene Concentrations in Amended Microcosms (µg/l)

Day	0		0		30		73		160		197	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Description	Liquid		Headspace Analysis									
A) Live with BTEX/TCE	3387	517	2347	712	2450	1676	3011	2820	3515	5008	2482	4817
B) Abiotic with BTEX/TCE	8018	5892	7307	5074	6403	4901	7001	5982	10030	9706	9338	9061
C) Live with CaCO3 and BTEX/TCE	3640	564	2756	428	2235	306	1968	390	1787	293	1499	454
D) Live with CaCO3, Nutrients and BTEX/TCE	3345	411	1831	445	1524	413	1097	251	636	536	333	357
E) Live with CaCO3, Nutrients, Carbon and BTEX/TCE	3305	946	3416	851	2731	899	2048	804	2283	1193	1687	1212
F) Abiotic as in E	4447	318	4210	278	3692	339	3703	204	3460	373	3034	241
G) Live as in E with BES	4402	649	5329	1137	7184	2705	8473	4498	13919	8802	13511	8756
H) Abiotic Water with BTEX/TCE	5809	3370	5079	2486	2186	848	5555	4411	7646	7540	7564	8217
I) Live as in E with no amendments and no BTEX/TCE	1256	642	2325	1237	3827	2789	7273	5296	4925	7177	4991	7263
J) Live with no Amendments and no BTEX/TCE	740	505	1116	679	1709	918	1581	848	17	29	17	30

SD = standard deviation

Table 18 Benzene Concentrations in Amended Microcosms (µg/l)

Day	0		0		30		73		160		197	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Description	Liquid		Headspace Analysis									
A) Live with BTEX/TCE	7682	1238	6829	677	6067	1025	5501	1065	5161	971	4657	1005
B) Abiotic with BTEX/TCE	7571	437	6732	211	6176	269	5867	140	5956	249	5395	102
C) Live with CaCO3 and BTEX/TCE	7538	792	7617	623	6421	414	5960	437	5642	646	5338	521
D) Live with CaCO3, Nutrients and BTEX/TCE	7364	565	6375	822	5411	1018	4613	1169	4285	1126	4051	1220
E) Live with CaCO3, Nutrients, Carbon and BTEX/TCE	7075	757	7054	596	5838	905	5184	1406	5049	1814	4302	1368
F) Abiotic as in E	7260	99	6923	210	6318	273	6206	14	6259	140	5585	111
G) Live as in E with BES	6704	384	6568	331	6273	445	6001	312	6082	265	5538	231
H) Abiotic Water with BTEX/TCE	7352	1048	6522	432	3461	2363	5779	435	6050	268	5469	465
I) Live as in E with no amendments and no BTEX/TCE	<5		<5		<5		<5		<5		<5	
J) Live with no Amendments and no BTEX/TCE	<5		<5		<5		<5		<5		<5	

SD = standard deviation

Table 19 Ethylbenzene Concentrations in Amended Microcosms (µg/l)

Day	0		0		30		73		160		197	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Description	Liquid		Headspace Analysis									
A) Live with BTEX/TCE	205	38	289	87	201	72	155	59	103	43	87	37
B) Abiotic with BTEX/TCE	205	20	326	28	239	25	175	11	137	20	114	14
C) Live with CaCO3 and BTEX/TCE	271	111	427	73	284	56	218	50	149	41	127	32
D) Live with CaCO3, Nutrients and BTEX/TCE	687	736	323	173	214	113	151	91	96	70	89	63
E) Live with CaCO3, Nutrients, Carbon and BTEX/TCE	765	725	389	179	217	134	175	113	122	97	91	67
F) Abiotic as in E	498	111	532	100	323	51	290	66	207	58	171	45
G) Live as in E with BES	471	38	501	35	335	50	234	28	189	41	161	35
H) Abiotic Water with BTEX/TCE	1373	274	574	152	200	115	298	50	246	35	190	30
I) Live as in E with no amendments and no BTEX/TCE	<5		<5		<5		<5		<5		<5	
J) Live with no Amendments and no BTEX/TCE	<5		<5		<5		<5		<5		<5	

SD = standard deviation

Table 20 M-Xylene Concentrations in Amended Microcosms (µg/l)

Day	0		0		30		73		160		197	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Description	Liquid		Headspace Analysis									
A) Live with BTEX/TCE	171	29	256	79	170	65	133	52	89	36	76	32
B) Abiotic with BTEX/TCE	168	20	301	28	206	14	158	5	124	18	104	8
C) Live with CaCO3 and BTEX/TCE	220	95	378	62	248	47	190	43	123	34	106	27
D) Live with CaCO3, Nutrients and BTEX/TCE	266	71	260	113	164	83	109	63	70	43	65	40
E) Live with CaCO3, Nutrients, Carbon and BTEX/TCE	355	185	352	177	185	115	147	94	101	81	77	57
F) Abiotic as in E	444	112	491	93	281	49	256	59	176	49	148	39
G) Live as in E with BES	423	36	442	54	299	46	207	32	170	32	146	24
H) Abiotic Water with BTEX/TCE	557	171	514	112	174	100	261	50	214	35	168	31
I) Live as in E with no amendments and no BTEX/TCE	<5		<5		<5		<5		<5		<5	
J) Live with no Amendments and no BTEX/TCE	<5		<5		<5		<5		<5		<5	

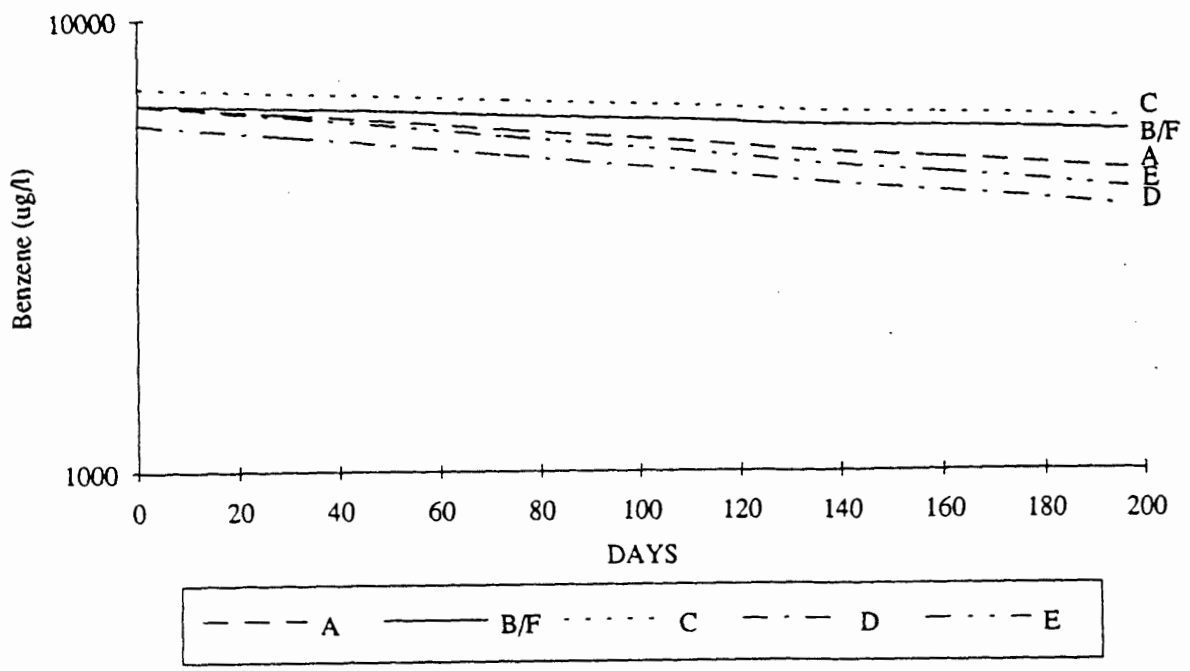
SD = standard deviation

Table 21 O-Xylene Concentrations in Amended Microcosms (µg/l)

Day	0		0		30		73		160		197	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Description	Liquid		Headspace Analysis									
A) Live with BTEX/TCE	225	25	363	115	253	97	193	77	140	58	118	50
B) Abiotic with BTEX/TCE	210	14	427	64	348	33	231	23	184	28	159	19
C) Live with CaCO3 and BTEX/TCE	274	82	504	79	359	29	264	51	197	45	171	36
D) Live with CaCO3, Nutrients and BTEX/TCE	389	60	351	140	267	21	161	90	114	69	103	64
E) Live with CaCO3, Nutrients, Carbon and BTEX/TCE	439	212	512	192	260	149	236	114	157	120	118	83
F) Abiotic as in E	571	165	626	81	375	63	357	63	255	62	218	51
G) Live as in E with BES	529	15	621	84	371	47	266	43	229	48	198	41
H) Abiotic Water with BTEX/TCE	654	179	608	140	263	100	351	54	296	37	234	36
I) Live as in E with no amendments and no BTEX/TCE	<5		<5		<5		<5		<5		<5	
J) Live with no Amendments and no BTEX/TCE	<5		<5		<5		<5		<5		<5	

SD = standard deviation

Figure 11 - Best Fit Regression Lines for Benzene Degradation in Amended Microcosms



Non-biological losses of BTEX were measured in all microcosm sets for each compound. Although some of the losses were attributable to sorption to the aquifer sediment, analysis revealed that the total organic carbon (TOC) content of the aquifer sediment was only 0.028% (280 mg/kg). Losses from abiotic microcosms containing aquifer sediment were only slightly greater than from abiotic microcosms containing no aquifer sediment. Therefore, sorption to the aquifer sediment was small relative to the overall losses observed. This is consistent with the low organic carbon content of the aquifer material (0.028%). Instead, the losses appeared to be the result of sorption/diffusion through the stoppers. Nonetheless, repeated punctures of the stoppers did not increase abiotic losses compared to the ambient microcosms which were destructively sampled.

Although statistical analysis indicated no biological removal of benzene, ethylbenzene and xylene isomers (BEX), there was an individual microcosm in each of sets A, D and E in which these compounds appeared to degrade. Noticeable toluene losses (as opposed to increases) also occurred in these three microcosms. In addition, TCE removal was accelerated in these three microcosms relative to replicate microcosms of the same set. However, ethylene production was slightly lower in these microcosms on Day 197. This suggests that there may have been a higher than expected abiotic loss of BEX in these microcosms as opposed to a biological loss. Another possible explanation for an atypically high abiotic loss is the presence of wood fragments in the clayey soil. Such fragments were observed occasionally and efforts were made to remove all such fragments prior to filling microcosms. However, the presence of a small piece of wood would dramatically increase losses due to sorption.

Losses of target compounds occurred in abiotic microcosms. Losses were not significantly different between abiotic and abiotic water microcosms. Abiotic losses occurred primarily by sorption and diffusion through the stopper. As presented in Table 22, all abiotic microcosms in the amended study contained between 0.01 and 1.98 mg methane/l at the initial sampling on Day 0. However, over 197 days of monitoring, no abiotic microcosms exhibited methane production. The initial methane observed was likely produced before the microcosms were autoclaved and spiked with mercuric chloride. Abiotic microcosms containing BTEX and TCE produced between 0.041 and 0.014 mg ethylene/l by Day 197 (Table 15), compared to between 4.99 and 9.48 mg ethylene/l in amended and unamended live microcosms containing BTEX and TCE. Apparently, there was limited biological activity in the abiotic microcosms.

Methane concentrations in amended microcosms are presented in Table 22. Microcosms in sets D and E produced more methane on Days 30 and 73 than microcosms in sets A and C. However, methane production in sets D and E was similar to that in sets I and J, neither of which contained added BTEX or TCE. By Day 197, five sets of live microcosms (A, C, D, E and J) contained between 21.2 and 25.0 mg methane/l. Microcosms in set I contained an average of 29.4 mg methane/l in two microcosms analyzed. Abiotic microcosms and live microcosms containing the methanogen inhibitor, BES, each produced less than 0.29 mg methane/l by Day 197, indicating that BES did inhibit methanogen activity.

Degradation of BTEX did not significantly contribute to methane production since statistically similar methane production was observed in microcosms both with and without added BTEX. Since neither calcium carbonate, nutrients nor the addition of readily degradable carbon stimulated methane production, there appears to be another factor limiting methane production in the amended microcosms.

Table 22 Methane Concentrations in Amended Microcosms (mg/l)

Day	0		30		73		160		197	
Description	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
A) Live with BTEX/TCE	0.02	0.003	0.47	0.22	17.95	1.79	27.08	0.52	22.45	2.56
B) Abiotic with BTEX/TCE	0.15	0.14	0.15	0.14	0.12	0.12	0.13	0.12	0.13	0.12
C) Live with CaCO3 and BTEX/TCE	0.07	0.01	0.65	0.10	18.51	2.62	23.11	0.97	21.32	1.36
D) Live with CaCO3, Nutrients and BTEX/TCE	0.01	0.01	1.23	0.54	24.01	5.13	23.01	0.62	24.27	1.40
E) Live with CaCO3, Nutrients, Carbon and BTEX/TCE	0.01	0.01	0.97	0.34	27.01	3.05	23.62	0.64	24.98	1.58
F) Abiotic as in E	0.19	0.03	0.19	0.03	0.16	0.02	0.16	0.02	0.17	0.03
G) Live as in E with BES	0.02	0.002	0.02	0.003	0.02	0.01	0.01	0.001	0.08	0.07
H) Abiotic Water with BTEX/TCE	0.00	0.00	0.002	0	0.00	0.00	0.02	0.01	0.00	0.00
I) Live as in E with no amendments and no BTEX/TCE	0.07	0.01	1.19	0.26	21.77	1.40	25.44	1.02	19.61	17.46
J) Live with no Amendments and no BTEX/TCE	0.07	0.01	1.55	0.51	24.86	4.02	23.51	0.36	21.23	1.68

SD = standard deviation

9.0 DISCUSSION

An extensive field and laboratory investigation was conducted to evaluate the significance of anaerobic biodegradation on the fate of hazardous organics in the subsurface down gradient from a sanitary landfill. Concentrations of benzene, toluene, ethylbenzene, and xylene isomers (BTEX) and chlorinated organics [tetrachloroethylene (PCE), trichloroethylene (TCE), and cis-dichloroethylene (c-DCE), and trans-dichloroethylene (t-DCE)] were measured in a series of monitoring wells down gradient of the refuse disposal area over a 6-month period. Two sets of laboratory experiments were conducted to measure the biodegradative activity of anaerobic aquifer sediment and to facilitate our understanding of trends observed in the field. Ambient microcosms were constructed to simulate in-situ conditions using aquifer sediment from each of three boreholes adjacent to the monitoring wells (Figure 1). A second set of microcosms was constructed using aquifer sediment from the middle borehole to evaluate the effects of adding buffer, nutrients and easily degradable carbon. A summary of biodegradation rates for the organic compounds tested in this microcosm study is presented in Table 23. In this chapter, trends observed in the field and laboratory experiments are summarized and discussed.

9.1 Field Monitoring

Groundwater in the study area, immediately down gradient from the refuse disposal area, is contaminated with a variety of organic compounds. Benzene, toluene, ethylbenzene, xylene isomers, PCE, c-DCE and t-DCE were consistently detected in several wells throughout the study area. In the one well with a longer monitoring history (well F), contaminant concentrations have not changed significantly since the well was first installed in 1988. Of the compounds detected, benzene, toluene, c-DCE, t-DCE and PCE consistently exceeded NC groundwater quality standards. With the exception of toluene, no systematic trends in concentration with time were detected. In several wells, toluene concentrations declined steadily over the monitoring period while in other wells toluene concentrations increased.

On a small scale, there was no detectable decrease in the average concentration of BTEX, PCE or c-DCE in a series of wells installed along a single streamline (wells N to L). This indicates that during the 4- to 5-month travel time along this streamline, biotransformation processes did not measurably affect the concentration of these contaminants. This may be due to (1) an absence of biodegradation or (2) the high variability in contaminant concentrations. At slow rates of biodegradation, the random variations in contaminant concentration could obscure any long term spatial trends. Average contaminant concentrations were highest in monitoring wells closest to the refuse and lowest near the drainage channel suggesting that over a larger scale, biotransformation processes could be important.

9.2 Biodegradation in Ambient and Amended Microcosms

Ambient microcosms were constructed using aquifer material collected adjacent to monitoring wells N, M and F (boreholes LFN, LFM and LFF). These microcosms were constructed to represent ambient conditions within the aquifer to the maximum extent possible. Since the microcosms were destructively sampled, each of the 153 bottles sampled represents an independent measure of compound loss. While there were similarities in results between the three sets of microcosms, there were also significant differences.

Amended microcosms were constructed using aquifer material collected adjacent to monitoring well M (borehole LFM) and treated with various amendments to examine the effect of these treatments on the rate of biodegradation. Since the microcosms were repetitively sampled, measurements at later time points are not independent of earlier observations.

Table 23 - Summary of Biodegradation Rates ($days^{-1}$)[a]

	Compound					
	Benzene	Ethyl-benzene	m-Xylene	o-Xylene	TCE	Toluene
Microcosm Set						
LFN	x	x	x	x	-0.011	-0.013[b]
LFM	0.001	x	x	x	-0.023	-0.0632[c]
LFF	x	x	x	x	-0.018	x
Amended Microcosms						
A	x	x	x	x	-0.036	d
B	x	x	x	x	x	x
C	x	x	x	x	-0.049	d
D	x	x	x	x	-0.049	d
E	x	x	x	x	-0.048	d
F	x	x	x	x	x	x
G	x	x	x	x	x	x
H	x	x	x	x	x	x
I	x	x	x	x	x	d
J	x	x	x	x	x	d

a A negative sign denotes loss of the compound.
b Toluene degradation occurred in the absence of TCE. No toluene degradation observed in microcosms containing TCE.
c Rate calculated between 0 and 55 days.
d Toluene degradation observed but not quantified.
x Statistically insignificant degradation rate.

9.2.1 TCE Biodegradation in Ambient Microcosms. TCE degradation occurred in microcosms from all three locations. However, the lag period prior to biodegradation was extremely variable at any given borehole and between boreholes. Typically, there was an extended lag period followed a very rapid decline in TCE. In the LFN microcosms at 41 days, the TCE concentration in one of the microcosms was below detection (<10 µg/l) while in the other two, the TCE concentration was approximately equal to the abiotic controls. This pattern continued at days 99 and 208. By day 306, TCE was depleted in two of three microcosms. A similar pattern occurred in the LFM and LFF microcosms although the lag periods differed. In the LFM and LFF microcosms, TCE transformation did not begin until days 110 and 108, respectively, and TCE was depleted in all LFM and LFF microcosms by days 222 and 302, respectively.

With one minor exception, ethylene was the only daughter product observed during the reductive dechlorination of TCE. Ethylene did not appear for over 300 days in the ambient microcosms and less than 25% of the theoretical ethylene was recovered. Another possible fate for dechlorinated ethenes is ethane (De Bruin et al., 1992). Ethane was not detected indicating that further reduction of ethylene did not occur. This implies that vinyl chloride accumulated in the dechlorinating microcosms and ethylene was produced slowly in a

subsequent dechlorination step. While ethylene is a harmless byproduct of TCE biotransformation, vinyl chloride is not.

Effective first-order decay rates for TCE (adjusted for abiotic loss) in the three sets of microcosms are presented in Table 24. Average degradation rates for TCE in the LFN, LFM and LFF microcosms over 306, 318 and 395 days were 0.011, 0.0223 and 0.018 day^{-1}, respectively. The reader is cautioned against over-interpretation of these results. None of the microcosms followed a classic first-order decay. For example, the average decay rate for TCE in the LFM microcosms is approximately double the average decay rate in the LFN microcosms even though, TCE was completely removed in one of the LFN microcosms on day 41. This illustrates the importance of the lag time on the average decay rate. In general, once degradation started, TCE concentrations dropped from the initial concentration to below detection in one sampling interval. Calculated decay rates during the period of active degradation are also reported in Table 24. Because of the rapid drop in TCE, the calculated degradation rates will be affected by the time interval between sampling and the initial TCE concentration in the microcosm. This concentration varied between LFN, LFM and LFF. Production of ethylene indicated that at least a portion of the TCE is being converted to non-toxic end products.

9.2.2 TCE Biodegradation in Amended Microcosms. One set of microcosms was constructed and repetitively sampled which did not receive any amendments (Set A - live control). The rate of TCE removal in these microcosms was comparable to the previous ambient microcosms constructed with aquifer material from this same location (LFM) (Table 24). The lag period was comparable in both sets (160 days for repetitively sampled, 110 days for destructively sampled microcosms). The apparent degradation rate was higher in the repetitively sampled microcosms, due to the higher initial TCE concentration.

Addition of calcium carbonate to the microcosms reduced the lag period for TCE degradation from 160 to 73 days, resulting in a higher apparent degradation rate. Addition of nutrients and supplemental carbon sources did not further reduce this lag period although it did reduce the time required for conversion to ethylene. In all of the amended microcosms, between 82% and 95% of the initial TCE was recovered as ethylene. The reason for the higher recovery of ethylene in the amended microcosms relative to the ambients is not clear. Accumulation of DCE isomers was observed on only one occasion, implying that conversion from vinyl chloride to ethylene is the rate-limiting step. This is consistent with previous work by Freedman and Gossett (1989) who found dechlorination of vinyl chloride to ethylene to be the rate-limiting step in the complete dechlorination of PCE. In the amended microcosms, nutrient and supplemental carbon additions apparently increased the rate of this critical reaction.

The laboratory monitoring data indicate that TCE was removed by reductive dechlorination. Field monitoring indicates that sulfate and nitrate are below the analytical detection limit in all wells in the area. The large amounts of methane produced in the microcosms and the absence of alternative electron acceptors indicates that methanogenesis is the dominant microbial process. The large amounts of methane produced in the microcosms that did not receive BTEX, TCE or supplementary carbon indicates that degradable carbon was available for use as an electron donor for the reductive dehalogenation of TCE. The inhibition of TCE disappearance in the presence of BES also points to the importance of methanogenic processes in this aquifer.

Table 24 - Comparison of TCE Biodegradation Rates at Various Time Intervals

Microcosm Set	Degradation Rate (day^{-1})	Time Interval (days)	Below Detection Occurred (days)	Previous Sampling (days)
LFN	-0.011	0-306	41, 99, 208[1]	306[2]
LFN	-0.188	0-41[1]		
LFM	-0.023	0-318	222	110
LFM	-0.053	110-222		
LFF	-0.018	0-395	218[3]	164
LFF	-0.042	108-218		
Amended Microcosms (constructed with LFM aquifer material)				
A	-0.036	0-197	197	160
A	-0.059	73-197		
C	-0.049	0-197	160[4]	73
C	-0.087	73-16		
D	-0.049	0-197	160	73
D	-0.066	30-160		
E	-0.048	0-197	160[5]	73
E	-0.064	30-160		
E	-0.076	30-73		

1 TCE below detection limit in 1 of 3 microcosms on Days 41, 99 and 208

2 TCE below detection limit in 2 of 3 microcosms on Day 306 (879 g/l in third microcosm)

3 TCE below detection limit in 2 of 3 microcosms on Day 218 (74 g/l in third microcosm)

4 TCE below detection limit in 2 of 3 microcosms on Day 160 (14 g/l in third microcosm)

5 TCE below detection limit in 1 of 3 microcosms on Day 73

9.2.3 Methane Production in Microcosms. Methane production was monitored in both the ambient and amended microcosms. In the ambient microcosms, there was an initial lag period where significant methane production did not occur by the first time point (41 to 75 days). However, substantial amounts of methane (12 to 40 mg/l CH4) had been produced by the second time point (99 to 110 days). TCE initially inhibited methane production, as evidenced by the reduced amount of methane produced through day 99 in LFN microcosms which received TCE compared to LFN microcosms that did not receive TCE. Methane production typically plateaued at 30 to 40 mg/l after 200 days. There was no significant difference between methane production in microcosms which received BTEX/TCE and those that did not, indicating that the large majority of methane was produced from organic carbon already present in the contaminated groundwater.

Methane production was also monitored in the amended microcosms. Results in the amended microcosms were similar to those in the ambient microcosms. Methane production plateaued between 23 and 27 mg/l at 160 days. Addition of buffer ($CaCO_3$) appeared to slightly enhance the rate of methane production but did not alter the final amount produced. The absence of additional methane production in the microcosms provided with supplemental carbon (50 mg/l each of sodium acetate, sodium formate, sodium benzoate, glucose and yeast extract) was surprising. This should have resulted in over 50 mg/l of additional methane yet no detectable increase was observed. There is no apparent explanation for this observation.

9.2.4 BTEX Biodegradation in the Ambient Microcosms. Toluene was degraded in two of three sets of ambient microcosms although the pattern of degradation differed. In the LFN microcosms, toluene biodegradation was completely inhibited in the presence of TCE, but gradually degraded in the absence of TCE after a 99-day lag. In contrast, toluene was rapidly degraded in LFM but not in LFF microcosms, both in the presence of TCE. Tests in the absence of TCE were not conducted at the LFM and LFF boreholes. It will be interesting to observe whether toluene degradation proceeds in the LFF microcosms once TCE is depleted.

There appears to be a minimum concentration below which toluene degradation ceases. In all microcosms where toluene degradation occurred, the toluene concentration dropped to roughly 30 µg/l and then leveled off. For example, in the LFM microcosms, toluene dropped from 1,016 µg/l to 26 µg/l in 55 days and then varied between 20 and 34 µg/l over the next 263 days.

Benzene, ethylbenzene, m-xylene and o-xylene were not degraded in any of the ambient microcosms. An analysis of the effective first-order decay rates indicated that the benzene decay rate in the LFM microcosms was greater than the abiotic rate. This was due to an apparently random increase in the benzene concentration in the abiotic microcosms and is not believed to be significant.

The greater anaerobic degradability of toluene than the other BTEX components is consistent with results of previous investigators. Of the commonly monitored BTEX components, toluene is the component which most frequently undergoes anaerobic biodegradation. Investigators have shown toluene to be degraded under denitrifying (Kuhn et al., 1985), iron-reducing (Lovley and Lonergan, 1990), sulfate-reducing (Beller et al., 1992) and methanogenic conditions (Grbic-Galic and Vogel, 1987; Sewell and Gibson, 1991). In contrast, benzene appears to be the most difficult to degrade (Grbic-Galic, 1990).

The lack of benzene, ethylbenzene and xylenes degradation may also be related to the low concentrations of these compounds present in the aquifer at the Wilder's Grove landfill. Monitoring in the study area has shown that of the BTEX components, only toluene is present in

groundwater at concentrations above 30 µg/l. While benzene, ethylbenzene and the xylene isomers are present in low concentrations (between 1 and 20 µg/l), the concentrations of these compounds in the aquifer may be too low to induce the required enzymes. As measured in the ambient microcosms, once the toluene concentration dropped below 30 µg/l, biotransformation ceased. While the BEX concentrations in the microcosms were substantially higher, the incubation time may not have been sufficient to allow adaptation to occur in the microcosms.

9.2.5 BTEX Biodegradation in the Amended Microcosms. Toluene biodegradation was observed in several of the amended microcosms. However, rates of degradation were not calculated because of the confounding effects of a contaminant which was released from the stoppers and measured as toluene. The calcium carbonate, nutrients and supplemental carbon amendments did not appear to reduce the lag period or increase the rate of toluene degradation. However, interpretation is difficult because of uncertainty in the toluene concentration data.

Interpretation of the BEX degradation data is also difficult because of the potential inhibition of BEX degradation by the contaminant. Nonetheless, it is apparent that benzene, ethylbenzene, m-xylene and o-xylene decreased in several of the microcosms with active toluene degradation. Table 25 shows ethylbenzene concentrations in replicate microcosms of sets that (a) did not receive any amendments, (b) received $CaCO_3$ and nutrients, and (c) received $CaCO_3$, nutrients and supplemental carbon. The final ethylbenzene concentration in the second microcosm from each group is roughly 20 µg/l; approximately five times lower than the concentrations in the other microcosms. This indicates that ethylbenzene biodegradation did occur in isolated microcosms. These same microcosms showed losses of benzene, toluene, m-xylene and o-xylene. The cause of this highly variable response is unknown but suggestive of biodegradation of compounds that are typically recalcitrant. Further investigation of the potential for BEX biodegradation in warranted based on this result.

9.3 Effect of Biotransformation on Contaminant Fate and Transport

Projecting the effects of anaerobic degradation on the fate and transport of BTEX and CAHs in the field from the laboratory results is difficult, in part, because of the highly variable lag periods prior to the start of biodegradation. For example, in the ambient microcosms prepared using aquifer material from borehole LFN, the apparent lag period for TCE degradation varied from 0 to over 306 days. This borehole is the most up gradient, located closest to the refuse and presumably exposed to the highest CAH concentrations. The aquifer material from this borehole was sieved and blended prior to construction of the microcosms to generate uniform conditions. There are at least three possible explanations for the highly variable lag period observed in the microcosms: (1) minute differences in construction techniques in a single set of microcosms strongly influence the lag period; (2) there is tremendous variability in the metabolic capabilities and/or level of adaptation of subsurface microorganisms to individual contaminants; or (3) spatial relationships between microorganisms are critical for biodegradation, and these relationships are disturbed as soil is processed for microcosm construction. Review of the field data can provide some assistance in understanding the lag period.

Table 25 Ethylbenzene Concentrations in Individual Amended Microcosms (µg/l)

	Liquid Concentration	Headspace Analysis				
Day	0	0	30	73	160	197
A) Live with BTEX and TCE						
1	214	317	233	179	119	102
2	161	136	75	51	28	22
3	208	307	210	172	113	96
4	262	350	249	196	140	118
5	182	336	235	178	116	96
Avg	205	289	201	155	103	87
SD	38	87	72	59	43	37
B) Abiotic with BTEX/TCE						
1	226	360	219	174	126	109
2	192	344	230	182	125	121
3	226	325	249	185	171	127
4	199	314	278	176	137	119
5	183	287	218	157	124	92
Avg	205	326	239	175	137	114
SD	20	28	25	11	20	14
C) Live with CaCO3 and BTEX/TCE						
1	399	487	340	274	191	159
2	204	448	283	204	144	127
3	210	346	228	177	111	96
Avg	271	427	284	218	149	127
SD	111	73	56	50	41	32
D) Live with CaCO3, nutrients and BTEX/TCE						
1	235	328	219	175	101	99
2	291	147	100	50	24	21
3	1537	492	325	227	163	146
Avg	687	323	214	151	96	89
SD	736	173	113	91	70	63
E) Live with CaCO3, nutrients, carbon and BTEX/TCE						
1	1570	415	233	201	126	107
2	163	198	76	52	23	17
3	561	553	343	274	217	148
Avg	765	389	217	175	122	91
SD	725	179	134	113	97	67

In the laboratory, c-DCE and t-DCE did not accumulate to any measurable extent although the slower production of ethylene suggests that vinyl chloride did accumulate, at least temporarily. In contrast, in the field c-DCE and t-DCE persist to some extent. While vinyl chloride was not monitored in this study, previous monitoring has shown the presence of vinyl chloride in the vicinity of borehole LFN. One possible hypothesis to explain these apparently contradictory results is that organisms with the ability to reductively dehalogenate CAHs are not widely distributed in the aquifer but are present in discrete, widely spaced microcolonies. If this occurred, then transient intermediates could accumulate in the aquifer as biodegradation intermediates were transported away from a microcolony by the flowing groundwater. In a stagnant microcosm, such transport would not occur. The average degradation rates in the field will depend on the spatial heterogeneity of the appropriate microorganisms, the spatial variability in contaminant concentrations and the groundwater transport velocity.

Laboratory degradation data indicate that toluene is biodegradable under the conditions present in the aquifer yet field data did not show any consistent trend in toluene with distance. Laboratory degradation rates for toluene varied from zero (no significant degradation in LFF) to 0.06 per day in LFM. If we assume the spatially averaged degradation rate is 0.01 per day, toluene should have declined by roughly 70% during transport from well N to L. Average toluene concentrations increased from 270 μg/l at well N to 326 μg/l at F, then decreased to 24 μg/l at L. While the field data did show an overall decline, the data are too variable to draw any definite conclusions. This illustrates the problem with using field data to estimate biodegradation rates. Because of the large temporal variations in contaminant concentrations, it often impossible to detect slow, but environmentally significant declines in contaminant concentration without extensive and repeated sampling over a large area. This level of monitoring is not practical in most cases. In contrast, laboratory microcosms can provide definite proof of contaminant biodegradation under representative conditions, although there will always be questions raised when attempting to use laboratory results to estimate field degradation rates.

One important result of this study was the observed effect of the amendments on CAH degradation. Addition of calcium carbonate buffer clearly enhanced the rate of TCE removal while addition of nutrients and supplemental carbon further enhanced the rate of complete dechlorination to ethylene, a harmless end product. Remediation of this aquifer and other sanitary landfill aquifers by pump and treat is not technologically feasible because the source of contamination, the landfill, cannot easily be eliminated. One alternative would be to alter conditions in the aquifer to enhance CAH degradation by increasing the pH and/or addition of nutrients. If this could be accomplished over the long term at a reasonable cost, then the migration of CAHs (and possibly BTEX) could be controlled.

REFERENCES

American Public Health Association. 1985. Standard methods for the examination of water and wastewater, 16th ed. Washington, DC: American Public Health Association.

Aelion, C. M., C. M. Swindoll, and F. K. Pfaender. 1987. Adaptation to and biodegradation of xenobiotic compounds by microbial communities from a pristine aquifer. Appl. Environ. Microbiol. 53:2212-17.

Atlas, R.M., 1988. Microbiology: fundamentals and applications, 2nd ed. New York, NY: MacMillan Publishing Co.

Baedecker, M. J., I. M. Cozzarelli, and J. A. Hopple. 1987. The composition and fate of hydrocarbons in a shallow glacial-outwash aquifer. USGS Open File Report 87-109.

Baedecker, M. J., and M. A. Apgar. 1984. Hydrogeochemical studies at a landfill in Delaware. In: Groundwater contamination. Washington, DC: National Academy Press.

Barker, J. F., J. S. Tessmann, P. E. Plotz, and M. Reinhard. 1986. The organic geochemistry of a sanitary landfill leachate plume. J. Contam. Hydrol. 1:171-89.

Beller, H. R., D. Grbic-Galic, and M. Reinhard. 1992. Microbial degradation of toluene under sulfate-reducing conditions and the influence of iron on the process. Appl. Environ. Microbiol. 58(3):786-93.

Berry, D. F., A. J. Francis, and J. M. Bollag. 1987. Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions. Microbiol. Rev. 51:43-59.

Bosma T. N. P., J. R. van deer Meer, G. Schraa, M. E. Tros, and A. J. B. Zehnder. 1988. Reductive dechlorination of all trichloro- and dichlorobenzene isomers. FEMS Microbiol. Ecol. 53:223-29.

Boyd, S. A., D. R. Shelton, D. Berry, and J. M. Tiedje. 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Appl. Environ. Microbiol. 46:50-54.

Bouwer, E. J., and P. L. McCarty. 1983. Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. 45:1286-94.

Britt, J. M., and F. M. Van Tassel. 1988. Measurement of hydraulic conductivity: field results versus lab results. CE 543 project, North Carolina State University (unpublished).

Brock, T. D., and M. T. Madigan. 1991. Biology of microorganisms, 6th ed. Englewood Cliffs, NJ: Prentice Hall.

Chapelle, F. H., J. L. Zelibor, D. J. Grimes, and L. L. Knobel. 1987. Bacteria in deep coastal plain sediments of Maryland: a possible source of CO_2 to groundwater. Water Resour. Res. 23:1625-32.

De Bruin, W. P., M. J. J. Kotterman, M. A. Posthumus, G. Schraa, and A. J. B. Zehnder. 1992. Complete biological reductive transformation of tetrachloroethylene to ethane. Appl. Environ. Microbiol. 58:1196-2000.

DiStephano, T. D., J. M. Gossett, and S. H. Zinder. 1992. Hydrogen as an electron donor for dechlorination of tetrachloroethylene by an anaerobic mixed culture. Appl. Environ. Microbiol. 58:3622-29.

Douglass, J. L., and R. C. Borden. 1992. The impact of a piedmont sanitary landfill on surface and ground water quality. N.C. Water Resour. Res. Inst. Rep. no. 266.

Edwards, E. A., and D. Grbic-Galic. 1992. Complete mineralization of benzene by aquifer microcosms under strictly anaerobic conditions. Appl. Environ. Microbiol. 58:2663-66.

Eganhouse, R. P., T. F. Dorsey, and C. S. Phinney. 1987. Transport and fate of monoaromatic hydrocarbons in the subsurface at the Bemidji. Minnesota, research site. U.S. Geol. Surv. Open File Rep. 87-109.

Ehrlich, G. G., R. A. Schroeder, and P. Martin. 1985. Microbial populations in a jet-fuel-contaminated shallow aquifer at Tustin, California. U.S. Geol. Surv. Open File Rep. 85-335.

Evans, O. D., and G. M. Thompson. 1986. Field and interpretation techniques for delineating subsurface petroleum hydrocarbon spills using soil gas. In: Proceedings of petroleum hydrocarbons and organic chemicals in ground water: prevention, detection and restoration. Dublin, OH: National Water Well Association.

Fathepure, B. Z., and S. A. Boyd. 1988. Dependence of tetrachloroethylene dechlorination on methanogenic substrate consumption by Methanosarcina sp. strain DCM. Appl. Environ. Microbiol. 54:2976-80.

Freedman, D. C., and J. M. Gossett. 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Appl. Environ. Microbiol. 55:2144-51.

Ghiorse, W. C., and J. T. Wilson. 1988. Microbial ecology of the terrestrial subsurface. Adv. Appl. Microbiol. 33:107-72.

Gibson, S. A., and J. M. Suflita. 1986. Extrapolation of biodegradation results to groundwater aquifers: reductive dehalogenation of aromatic compounds. Appl. Environ. Microbiol. 52:681-88.

Gibson, S. A., and J. M. Suflita. 1989. Anaerobic biodegradation of 2,4,5-trichloro-phenoxyacetic acid in samples from a methanogenic aquifer: stimulation by short-chain organic acids and alcohols. Appl. Environ. Microbiol. 56:1825-32.

Godsy, E. M., D. F. Goerlitz, and G. G. Ehrlich. 1983. Methanogenesis of phenolic compounds by a bacterial consortium from a contaminated aquifer in St. Louis Park, Minnesota. Bull. Environ. Contam. Toxicol. 30:261-68.

Godsy, E. M., and D. Grbic-Galic. 1989. Biodegradation pathways for benzothiophene in methanogenic microcosms. U.S. Geol. Surv. Water Res. Invest. Rep. 88-4220.

Goerlitz D. F., D. E. Troutman, E. M. Godsy, and B. J. Franks. 1985. Migration of wood preserving chemicals in contaminated groundwater in a sand aquifer at Pensacola, Florida. Environ. Sci. Technol. 19:955-61.

Grbic-Galic, D. 1990. Anaerobic microbial transformation of nonoxygenated aromatic and alicyclic compounds in soil, subsurface and freshwater sediments. In: Bollag, J. M. and G. Stotzky, eds. Soil biochemistry. New York, NY: Marcel Dekker.

Grbic-Galic, D., and T. M. Vogel. 1987. Transformation of toluene and benzene by mixed methanogenic cultures. Appl. Environ. Microbiol. 53:254-60.

Hayman, J. W., R. B. Adams, and J. J. McNally. 1988. Anaerobic biodegradation of hydrocarbons in confined soils beneath busy places: a unique problem of methane control. In: Proceedings of petroleum hydrocarbons and organic chemicals in ground water: prevention, detection and restoration. Dublin, OH: National Water Well Association.

Healy, J. B., and L. Y. Young. 1979. Anaerobic biodegradation of eleven aromatic compounds to methane. Appl. Environ. Microbiol. 38:84-89.

Hughes, G. M., R. A. Landon, and R. N. Farvolden, 1971. Hydrogeology of solid waste disposal sites in northeastern Illinois. U.S. Environmental Protection Agency.

Hult, M. F. 1987. Introduction, Chapter C - Movement and fate of crude oil contaminants on the subsurface environment at Bemidji, Minnesota. U.S. Geol. Surv. Open File Rep. 87-109.

Jones, R. E., R. E. Beeman, and J. M. Suflita. 1989. Anaerobic metabolic processes in the deep terrestrial subsurface. Geomicrobiol. J. 7:117-30.

King, G. M. 1988. Dehalogenation in marine sediments containing natural sources of halophenols. Appl. Environ. Microbiol. 54: 3079-85.

Kuhn, E. P., and J. M. Suflita. 1989. Sequential reductive dehalogenation of chloroanilines by microorganisms from a methanogenic aquifer. Environ. Sci. Technol. 23:848-52.

Kuhn E. P., P. J. Colberg, and J. L. Schnoor. 1985. Microbial transformation of substituted benzene during infiltration of river water to groundwater: laboratory column studies. Environ. Sci. Technol. 19:961-68.

Lovley, D. R., and D. J. Lonergan. 1990. Anaerobic oxidation of toluene, phenol, and p-cresol by the dissimilatory iron-reducing organisms, GS-15. Appl. Environ. Microbiol. 56:1858-64.

Marshall, N. E. 1989. Slug test performance and analysis methods. CE 584 Project. North Carolina State Univ.

Marrin, D. L. 1987. Soil gas analysis of methane and carbon dioxide: delineating and monitoring petroleum hydrocarbons. In: Proceedings of petroleum hydrocarbons and organic chemicals in ground water: prevention, detection and restoration. Dublin, OH: National Water Well Association.

Merck and Co., Inc. 1989. Merck index, an encyclopedia of chemicals, drugs, and biologicals, 11th ed. Rahway, NJ: Merck and Co., Inc.

Mohn, W. W., and J. M. Tiedje. 1992. Microbial reductive dehalogenation. Microbiol. Rev. 56:482-507.

Moore, D. S., and G. P. McCabe. 1993. Introduction to the practice of statistics, 2nd ed. New York, NY: W. H. Freeman and Company.

Neilson, A. H. 1990. The biodegradation of halogenated organic compounds. J. Appl. Bacteriol. 69:445-70.

North Carolina Division of Environmental Management. 1989. Classifications and water quality standards applicable to the groundwaters of North Carolina, Title 15, Subchapter 2L, Sec. .0200. Raleigh, NC: N.C. Department of Environment, Health, and Natural Resources.

Parker, J. M., III. 1979. Geology and mineral resources of Wake County. N. C. Div. Land Resour., Geol. Surv. Sect. Bull. 86.

Payne, T. Brent, and P. B. Durgin. 1988. Hydrocarbon vapor concentrations adjacent to tight underground gasoline storage tanks. In: Proceedings of 2nd outdoor action conference on aquifer restoration, ground water monitoring and geophysical methods. Dublin, OH: National Water Well Association.

Quensen, J. F., J. M. Tiedje, and S. A. Boyd. 1988. Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. Science 242:752-54.

Reinhard, M., N. L. Goodman, and J. F. Barker. 1984. Occurrence and distribution of organic chemicals in two landfill leachate plumes. Environ. Sci. Technol. 18:953-61.

Roudebush, S. T., and E. J. Whitman. 1989. Evaluation of pump test data deep rock monitoring well. CE 584 project, North Carolina State University (unpublished).

SAS Institute Inc. 1991. SAS/STAT User's Guide, version six, fourth edition, volume 2. Cary, NC: SAS Institute Inc.

Scholz-Muramatsu, H., R. Szewzyk, U. Szewzyk, and S. Gaiser. 1989. Tetrachloroethylene as electron acceptor for the anaerobic degradation of benzoate. FEMS Microbiol. Lett. 66:81-86.

Semprini, L., G. D. Hopkins, P. L. McCarty, and P. V. Roberts. 1992. In-situ transformation of carbon tetrachloride and other halogenated compounds resulting from biostimulation under anoxic conditions. Environ. Sci. Technol. 26: 2454-61.

Sewell, G. W., S. A. Gibson, and H. H. Russell. 1990. Anaerobic in-situ treatment of chlorinated ethenes. In: Proceedings of the 1990 Water Pollution Control Federation annual conference: in-situ bioremediation of groundwater and contaminated soil. pp. 68-79.

Sewell, G. W., and S. A. Gibson. 1991. Simulation of the reductive dechlorination of tetrachloroethylene in anaerobic microcosms by the addition of toluene. Environ. Sci. Technol. 25:982-84.

Shelton, D. R., and J. M. Tiedje. 1984. General method for determining anaerobic biodegradation potential. Appl. Environ. Microbiol. 47:850-57.

Siegel, D. I. 1987. Geochemical facies and mineral dissolution - Bemidji, Minnesota, research site. U.S. Geol. Surv. Open File Rep. 87-109.

Smith, R. L., and J. H. Duff. 1988 Denitrification in a sand and gravel aquifer. Appl. Environ. Microbiol. 54:1071-78.

Smolenski, W. J., and J. M. Suflita. 1987. Biodegradation of cresol isomers in anoxic aquifers. Appl. Environ. Microbiol. 53(4):710-16.

Suflita, J. M., and G. W. Sewell. 1991. Anaerobic biotransformation of contaminants in the subsurface. EPA Environ. Res. Brief EPA/600/M-90/024.

Troutman, D. E., E. M. Godsy, D. F. Goerlitz, and G. G. Ehrlich. 1984. Phenolic contamination in the sand-and-gravel aquifer from a surface impoundment of wood treatment wastes Pensacola, Florida. U.S. Geol. Surv. Water Resour. Invest. Rep. 84-4230.

Van Beelen, P., and A. K. Fleuren-Kemila. 1989. Enumeration of anaerobic and oligotrophic bacteria in subsoils and sediments. J. Contam. Hydrol. 4:275-84.

Vogel, T. M., and P. L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, vinyl chloride and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49:1080.

Vogel, T. M., and D. Grbic-Galic. 1986. Incorporation of oxygen from water into toluene and benzene during anaerobic fermentative transformation. Appl. Environ. Microbiol. 52:200-02.

Vogel, T. M., C. S. Criddle, and P. L. McCarty. 1987. Transformations of halogenated aliphatic compounds. Environ. Sci. Technol. 21:722-36.

Wilson, J. L., J. F. McNabb, J. W. Cochran, T. W. Wang, M. B. Tomson, and P. B. Bedient. 1985. Influence of microbial adaptation on the fate of organic pollutants in groundwater. Environ. Toxicol. Chem. 4:721-26.

Wilson, B. H., G. B. Smith, and J. F. Rees. 1986. Biotransformations of selected alkylbenzenes and halogenated aliphatic hydrocarbons in methanogenic aquifer material: a microcosm study. Environ. Sci. Technol. 20:997-1002.

Wilson, B. H., J. T. Wilson, D. H. Kampbell, B. E. Bledsoe, and J. M. Armstrong. 1990. Biotransformation of monoaromatic and chlorinated hydrocarbons at an aviation spill site. Geomicrobiol. J. 8: 225-40.

Wilson, J. T., D. H. Kampbell, and J. Armstrong. 1994. Natural bioreclamation of alkybenzenes (BTEX) from a gasoline spill in methanogenic ground water. In: Hinchee, R. E., B. C. Alleman, R. E. Hoeppel, and R. N. Miller, eds. Hydrocarbon bioremediation. Boca Raton, FL: CRC Press, Inc.; pp. 201-218.

Woods, S. L., J. F. Ferguson, and M. M. Benjamin. 1988. Characterization of chlorophenol and chloromethoxybenzene biodegradation during anaerobic treatment. Environ. Sci. Technol. 23: 62-68.