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Microcosm Studies of Acetone and
Benzene at the West KL Avenue Landfill

presented by

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MICROCOSM STUDIES OF ACETONE AND BENZENE DEGRADATION AT THE WEST KL AVENUE LANDFILL

By

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Abstract

Microcosm Studies of Acetone and Benzene

Degradation at the West KL Avenue Landfill

By

Michael S. Apgar

The West KL Landfill is a disposal facility that was used by Kalamazoo county and the surrounding area for 20 years. It was closed in 1979 when volatile organic compounds were discovered in domestic wells near the landfill. High levels of compounds such as acetone and benzene were detected in the shallow aquifer associated with the KL site. To evaluate the potential for biodegradation of these compounds, a carbon balance was performed in microcosms containing KL landfill material. Two electron donors, acetone and benzene, and four different electron acceptors, nitrate, oxygen, ferric iron, and sulfate, were evaluated. A non-amended microcosm and a killed control were used to assess intrinsic remediation potential and sorption/volatilization loses.

Forty percent of the acetone was mineralized when no electron acceptors were added to the system. Under denitrifying and aerobic conditions, 45 and 60% of the acetone was mineralized respectively. Benzene was not mineralized without the addition

of an electron acceptor. Under nitrate reducing conditions, 30% of the benzene was mineralized to CO_2. The results of an isolation experiment were inconclusive.

To my parents, Judith and Peter Apgar.

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Chapter 1

Introduction

The KL landfill is located in Ishtemo Township (T2S, R12W) in the south central portion of section 21 in Kalamazoo county, Michigan. Opened in the early 1960's, the KL landfill was used primarily for municipal waste disposal. In 1968, Kalamazoo county assumed management of the site and the landfill began accepting commercial and industrial waste. The landfill was closed in 1979 when volatile organic compounds were discovered in nearby residential wells. Closure included regrading, installation of a bentonite-enhanced soil cover, gas vents and re-vegetation.

The site is situated near the crest of the Kalamazoo moraine, a glacial deposit containing sediments from the Wisconsin age. Site stratigraphy includes alternating layers of poorly sorted glacial till composed of clay and silt and moderately sorted glacial outwash interbedded with clay or silty clay beds and lenses. Two aquifers are present in the glacial sediments: an upper, poorly confined shallow aquifer, and a lower, more confined aquifer. The upper boundary of the shallow aquifer is approximately 865 feet above mean sea level (AMSL) and the lower boundary is at about 700 feet AMSL. The mean hydraulic conductivity of the shallow aquifer is 0.015 cm/sec. The hydraulic conductivity of the clay beds was found to be $1x\ 10^{-6}$ cm/sec.

Analysis of leachate from several wells revealed widespread contamination of the

shallow aquifer. Detected contaminants included benzene, toluene, xylene, acetone, 2-butonone, 4-methyl-2-pentanone, 1,1-dichloroethane, and 1,2-dichloroethane.

Contaminant distribution is quite complex at this site. There are four identified contaminant plumes, one extending to the northwest, the other, towards the southwest. These two plumes are divided into two sub-plumes. Contaminants are distributed laterally in the northwest plume. Contaminants detected in wells 379 and MW-16 designate plume N1 and include benzene, toluene, 1,1-dichloroethane and 1,2-dichloroethane. No acetone was detected in plume N1. Plume N2, defined by contaminants detected in wells MW12 and 90E, contains benzene, toluene, acetone, 2-butonone, 4-methyl-2-pentanone, 1,1-dichloroethane, and 1,2-dichloroethane. (Figure 1.)

The southwest plume is differentiated into an upper and a lower plume. The upper plume is characterized by contaminants detected in wells 90D and contains 1,1-dichloroethane and high (5 mg/l) concentrations of acetone,. The lower plume is characterized by contaminants detected in wells 90F. Contaminants include benzene, toluene and 1,2-dichloroethane. No acetone was detected in 90D or 90F. Benzene concentrations ranged from 4 micrograms/L to 1800 micrograms/L and acetone concentrations ranged from less than 0.15 mg/L (detection limit) to 5 mg/L. The concentrations of benzene and acetone are given in Tables 1 and 2.

Concern that the plumes would reach Dustin Lake, a ground water lake west of

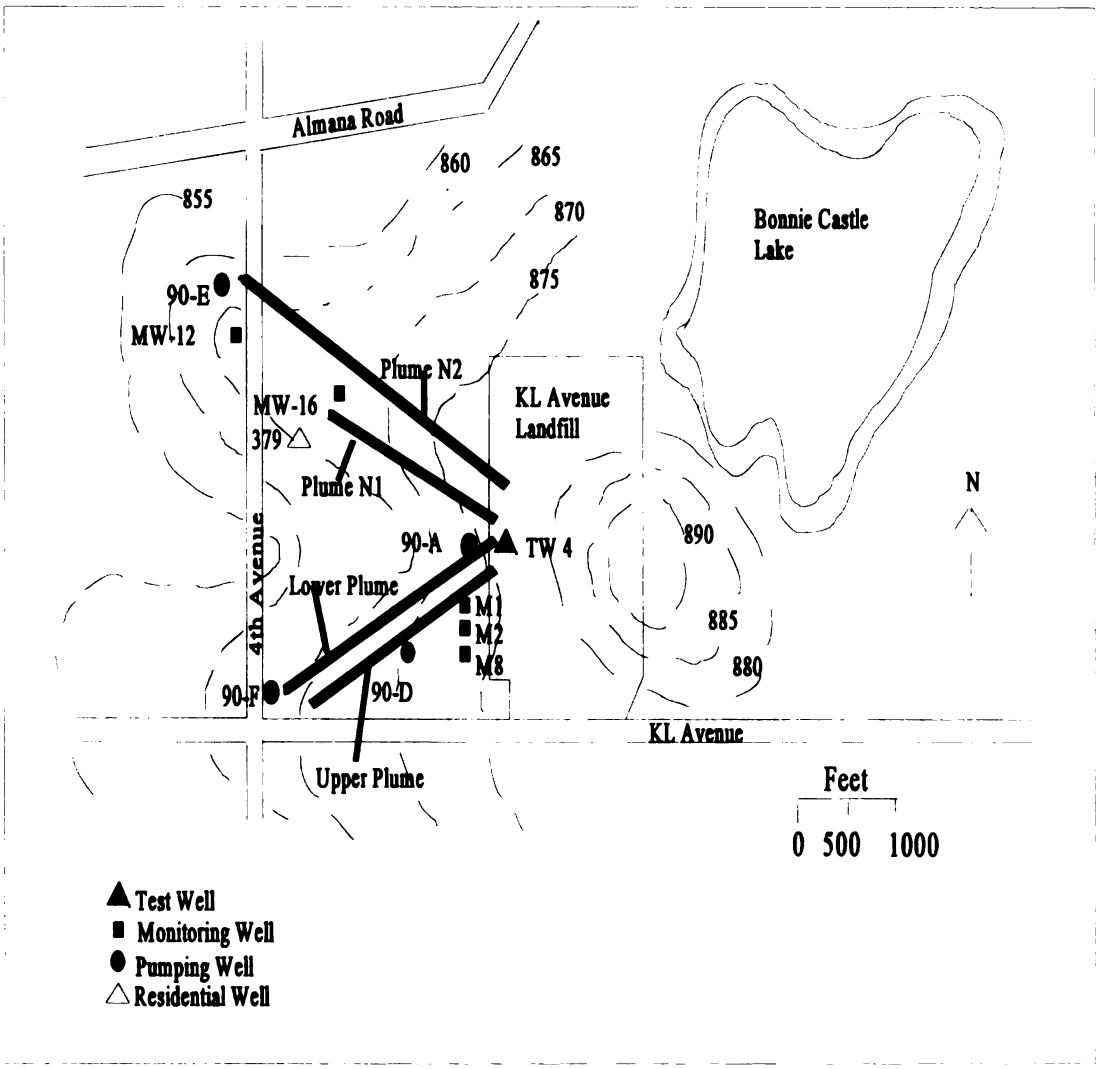

Figure 1. A Map of the West KL Landfill and the Surrounding Area. (Johnson and Varadhan, 1994)

Table 1. Fluctuation of Benzene Concentrations at Several KL Monitoring Wells.

Date	Benzene Concentration (mg/l)			
	Location			
	M1[a]	M2[b]	M8[c]	8794 W. KL
March, 1980	0.03	0.08	1.80	N.D.
June, 1980	0.01	0.03	0.40	N.D.
September, 1980	0.05	0.12	0.33	N.D.
December, 1980	0.07	0.14	0.50	N.D.

a. Monitoring Well #1, depth below mean water level, 31.63 feet.
b. Monitoring Well #2, depth below mean water level, 21.88 feet.
c. Monitoring Well #8, depth below mean water level, 70.70 feet.
d. Domestic well, depth below mean water level, 260 feet.
Note. The concentration of benzene at Test Well 4 was 0.76 mg/l in 1993.

Table 2. Acetone Concentrations at Several Monitoring Wells and at Test Well 4 (T.W.4) in 1993.

Location	Concentration (mg/L)
Test Well 4	less than 0.15
Monitoring Well 1	less than 0.15
Monitoring Well 2	0.17
Monitoring Well 8	less than 0.15

the landfill, and contaminate residential wells as well as the lake, prompted remediation feasibility studies of the shallow KL aquifer. Further analysis indicated that benzene concentrations in the upper plume were decreasing with respect to time. It was postulated that attenuation of the contaminant plume at KL might be biologically mediated, but studies are needed to establish the mechanism of attenuation. Rates of degradation of specific contaminants are also needed in order to predict when the contaminant plumes would reach a specified point and what the steady state concentrations of the constituent contaminants might be.

Research Problem

The primary objective of this project was to perform a carbon balance for two electron donors, (benzene and acetone) with four electron acceptors (nitrate, oxygen, sulfate and ferric iron) using aquifer material collected from the shallow aquifer at the West KL Avenue Landfill. A secondary objective was to estimate degradation rates of the electron donors and to estimate the intrinsic remediation potential of the column microcosms.

Chapter 2

Review of the Literature

Aerobic Benzene Degradation

The earliest evidence of benzene degradation was reported by Sohngen (1913, as cited by Shirei 1986). Subsequently, Marr and Stone (1961) reported a small amount of catechol, a common intermediate compound of aerobic benzene degradation. Later, Gibson (1968) and Hogn (1972) confirmed Marr and Stone's results.

Axcell and Geary (1975) proposed an aerobic degradation pathway and described the constitutive enzymes involved in the degradation of benzene. Several species of *Arthrobacter* and *Pseudomonas* were isolated and grown on a benzene-supplemented mineral salts medium. The *Arthrobactors* and most of the *Pseudomonads* did not degrade benzene efficiently. *Pseudomonas putida* grew rapidly in good yield in 10 mM benzene. This organism produced a stable, soluble benzene dioxygenase system.

Hal'ama and Augustín (1980) further defined the pathway that was associated with *P. putida*. Their medium contained only benzene (800 mg/L) and mineral salts. A very high affinity for O_2 was observed with benzene degradation. From 0 to 1.5% O_2 saturation, O_2 consumption was proportional to O_2 concentration. Above 2% O_2

saturation, no dependence on O_2 concentration was observed and some other nutrient was limiting. Benzoate inhibited benzene degradation at concentrations above 1200 ppm.

Further elaboration of the aerobic degradation pathway was presented by van den Tweel et al. (1988). Van den Tweel's group isolated a *Pseudomonas* species capable of growing on solid media with benzene as the sole carbon and energy source. Studies demonstrated that benzene was oxidized to *cis*-benzene glycol (CBG), supporting earlier work by Gibson (1970). *cis*-Benzene glycol was further metabolized to catechol. A mutant strain of this particular *Pseudomonad* produced no CBG dehydrogenase and was unable to form catechol from CBG.

A novel aerobic benzene degradation pathway was proposed by Hyman et al. (1985). They reported the oxidation of benzene to phenol by whole cells of *Nitrosomonas europaea.* The oxidation, catalyzed by ammonia mono-oxygenase and hydrazine (as a reductant), gave the highest rate of benzene oxidation at 300 mg/L benzene and equaled 6 micromoles per hour per mg of protein. However, these organisms transform the benzene cometabolically. As a result, phenol accumulates in the culture.

A complicating factor in aerobic benzene degradation is substrate interaction during degradation. Alvarez and Vogel (1991) reported on the substrate interactions during aerobic degradation of benzene, toluene and xylenes (BTX). Enhanced degradation of benzene and *p*-xylene, by *Pseudomonas* sp. strain CFS-215 in the presence

of toluene was observed. Benzene and toluene degradation by *Pseudomonas* in aquifer slurries was inhibited by the presence of *p*-xylene.

In 1993, Chang et al. isolated two aerobic BTX degraders, *Pseudomonas* strain B1 and *Pseudomonas* strain X1, from a pilot scale fluidized-bed reactor. Strain B1 grew with benzene and toluene as the sole carbon and energy sources, and cometabolized *p*-xylene in the presence of toluene. Strain X1 grew on *p*-xylene and toluene, but not on benzene. Chang et al. were able to model competitive inhibition and cometabolic transformation by adding a competitive term to the Monod expression and defining two transformation capacity terms, one relating consumption of growth substrate to the consumption of non-growth substrate, the other relating the consumption of biomass to the consumption of non-growth substrate, for the cometabolizing culture.

In the late 1980's, work on benzene degradation moved from a laboratory setting to contaminated sites in the field. Oxidation of benzene at contaminated sites by in-situ microcosms was reported by Chaing et al.(1989). Results from 10 sampling periods over three years showed a significant reduction in the total benzene mass with respect to time in the ground water. A natural attenuation rate for benzene was calculated at 0.95% per day. Areas of benzene degradation showed low levels of dissolved oxygen, supporting work of Hal'ama and Augustín (1980). Dissolved oxygen ranged from 0.0 mg/L in the plume to 10.0 mg/L outside of the plume.

Hadley and Armstrong (1991) investigated benzene contamination in domestic water supply wells contaminated by leaking underground storage tanks. Over seven

thousand wells were analyzed. The authors had expected to find high concentrations of benzene in the contaminated wells because benzene is the most water soluble (1780 mg/l at 25° C) component of gasoline. It also accounts for two to five percent of the total mass of gasoline and should be the most mobile in the saturated zone. Only 10 wells had detectable (microgram /liter) levels of benzene. Biodegradation (intrinsic) and volatilization were proposed as mechanisms to account for the absence of benzene in gasoline contaminated ground water. These aquifers had measurable amounts of oxygen.

Hutchins (1991 a, b, c) reported on the degradation of JP-4 jet fuel in an aquifer located in Traverse City, Michigan. Benzene and alkylbenzenes were degraded within 7 days under aerobic conditions. Alkylbenzenes only degraded when nitrate or nitrous oxide were the terminal electron acceptor. With limited oxygen, monoaromatic hydrocarbons were degraded. However, degradation ceased after oxygen was depleted. If nitrate was present, degradation of the alkylbenzenes continued. Benzene did show some concentration reduction when exposed to environments containing low levels of oxygen and sufficient nitrate. Hutchins concluded that benzene was recalcitrant under anaerobic conditions at this particular site.

Anaerobic Benzene Degradation.

Benzene has a reputation for recalcitrance in an anaerobic environment. Khun et al. (1988) studied columns filled with 30% aquifer material and 70% expanded slate grain

material fed continuously with BTEX and other compounds as the electron donors, and nitrate as an electron acceptor. Toluene and *m*-xylene were rapidly mineralized. Benzene, naphthalene, methyl-cyclohexane, and 1,3-dimethylcyclohexane were not degraded. Benzoate was formed as an intermediate of toluene metabolism. Oxygen inhibited degradation of toluene and *m*-xylene when it was substituted for nitrate.

Hutchins (1991 a,b,c) concluded that benzene did not degrade anaerobically. Uncontaminated and JP-4 (a jet fuel) contaminated cores were prepared and incubated in an anaerobic glove box and amended with nitrate. The uncontaminated cores were able to degrade toluene with no timelag. After a 30 day timelag, activity was observed with xylene, ethyl benzene and 1,2,4-trimethylbenzene. The contaminated cores showed lower activity and exhibited a much longer timelag. Benzene (8 mg/L) was not significantly degraded within a 6 month period. It was suggested that benzene inhibited the degradation of toluene and other biodegradable substances. Denitrification occurred in all the cultures. In a follow up study, Hutchins (1991 b) reported that benzene was not degraded in one year regardless of whether or not it was available as the sole carbon source. Microcosms were prepared anaerobically and amended with nitrate. Hutchins observed that nitrate concentrations above 500 mg/L appeared to be inhibitory. In a later report, Hutchins (1991 c), observed that benzene and alkylbenzenes were degraded within 7 days under aerobic conditions. When either nitrate or nitrous oxide was provided as the terminal electron acceptor, only alkylbenzenes were degraded. With limited oxygen, monoaromatic hydrocarbons were degraded, but degradation ceased after oxygen was depleted. If nitrate was present, degradation of the alkylbenzenes continued. Benzene

did show some concentration reduction when exposed to environments containing low levels of oxygen and sufficient nitrate.

In 1986, Vogel and Grbić-Galić demonstrated that benzene could be anaerobically degraded. They observed the incorporation of ^{18}O from ^{18}O labeled water, forming phenol as the initial step in the anaerobic oxidation of benzene by acclimated methanogenic cultures. They concluded that toluene and benzene were fermentativly oxidized by an as-yet-unknown mechanism with water as the source of oxygen. Subsequently, Grbić-Galić and Vogel (1987) better defined the anaerobic pathway for benzene degradation under methanogenic conditions. Toluene and benzene were anaerobically transformed to methane and carbon dioxide (partially) by mixed methanogenic cultures derived from ferulic acid degrading sewage sludge enrichments. There was no *cis*-benzene glycol or catechol detected during the degradation.

In 1992, Edwards and Grbić-Galić reported anaerobic degradation of benzene in a microcosm found in a gasoline contaminated aquifer. Gasoline contaminated aquifer solids from Seal Beach, CA, were prepared in a sulfide reduced defined mineral medium and supplemented with 20 mM sulfate. Benzene concentrations ranged from 40 to 200 micromolar. Under these conditions, 90 % of the radiolabeled benzene was transformed to CO_2.

The first report of benzene oxidation in an iron reducing environment came from

Lovely et al. (1994). *Shewanella putreficans* degraded benzene using nitrilotriacetic acid-chelated iron as the electron acceptor with lactate amendment.

Degradation of benzene by in-situ microcosms was studied by Cozzarelli et al. (1990) in anoxic groundwater of a shallow glacial outwash aquifer near Bemidji, Minnesota. The contaminant plume was defined in three sections with respect to an observer moving down-gradient from the point source of contamination: zone one with an O_2 concentration equal to 0; zone two with an O_2 concentration ranging from 0 to 1 mg/L; zone three with an O_2 concentration greater than 1 mg/L (approaching background levels of 6 mg/L). Mono aromatic hydrocarbons were transported downgradient. Organic acids that were not original components of the contamination were identified in the ground water down-gradient from the contamination site. These acids were presumed to be degradation products. High concentrations of organic acids were found associated with low concentrations of the original monoaromatics.

Morgan et al. (1993) reported on a BTEX contaminated site located in Uiterburen, Netherlands, where benzene concentrations approached 20 mg/L. All compounds degraded aerobically. Under denitrifing conditions, decreasing levels of benzene, toluene, ethylbenzene, *m*-xylene and *p*-xylene were observed. Degradation rates under denitrifying conditions were much slower than aerobically mediated degradation.

Aerobic Acetone Degradation

Research on the biodegradation of acetone is not as extensive as that for benzene. This may be due to the fact that acetone is produced in industrial quantities by microbial fermentation (Taylor et al., 1980) and is not considered the environmental hazard that benzene presents. ~~Several reports considering aerobic biodegradation of acetone~~ were ~~found~~.

Taylor et al. (1980) have described the earliest works on acetone degradation. Supnieski (1923) and Goepfert (1941) observed the formation of formate and formaldehyde by *Bacillus* and *Fusarium* cultures during acetone degradation. Levine and Krampitz (1952) reported the production of acetaldehyde by a soil diphtheroid and Vestial and Perry (1969) reported acetone as an intermediate in the degradation of propane.

Taylor et al. (1980) isolated four Gram-positive bacteria from soil that utilized acetone as a sole carbon source. All of the organisms tested oxidized acetone aerobically. Taylor et al. suggest a pathway starting with isopropanol and ending with pyruvate that includes acetone as an intermediate. Acetone is regarded as easily degradable during aerobic waste water treatment. Platen and Schink (1989) discovered that aerobically, bacteria degrade acetone via oxygenation.

Anaerobic Degradation of Acetone

Many of the papers that reported aerobic degradation of acetone also reported anaerobic degradation of acetone. It was known as early as 1905 (Schardinger as reported in Platen and Schink, 1989) that acetone was formed fermentativly in anoxic environments. Taylor et al. (1980) observed that in the absence of molecular oxygen, acetone is completely degraded to methane. Platen and Schink (1987) discovered that anaerobic growth with acetone depends on carbon dioxide, and the degradation of acetone involves a carboxylation reaction as a primary step. Later, Platen and Schink (1989) reported that an anaerobic enrichment culture degraded 1 mole of acetone to 2 moles of methane and 1 mole of carbon dioxide. The degradation was performed by two organisms, an organism similar to *Methanothrix sp.* and an as yet unidentified bacillus. Cultures were collected from a polluted fresh water creek located near Konstanz, FRG.

In summary, degradation of benzene and acetone in the laboratory and benzene in the field is fairly well documented. Isolates of benzene and acetone degrading aerobes as well as isolates of acetone degrading anaerobes have been obtained. A benzene degrading anaerobe is yet to be isolated.

Chapter 3

Materials and Methods

The KL landfill is a disposal facility used by Kalamazoo county and the surrounding community for 20 years. It was closed in 1979 when volatile organic compounds were discovered in domestic wells in the vicinity of the landfill. High levels of compounds such as acetone and benzene were detected in the aquifer within and outside of the landfill's boundaries. To assess the fate of these compounds in the aquifer, it was proposed that a carbon balance be performed using two electron donors, acetone and benzene, in four different electron accepting environments; nitrate, oxygen, ferric iron and sulfate. This chapter presents the materials and methods which were utilized in the microcosm assessment.

Chemicals

Radiolabeled acetone (Sigma Chemical Company, St. Louis) with all of the carbon as ^{14}C, was diluted to 778 mg/L and utilized at 1 mg/L concentrations. Radiolabeled benzene (Sigma) with all of the carbon as ^{14}C, was diluted to 500 mg/L and utilized at 1 mg/L concentrations. Sodium nitrate, sodium sulfate and ferric ammonium sulfate ($FeNH_4(SO_4)_2$) were used for electron acceptors. Potassium hydroxide and hydrochloric acid were used for pH adjustment. All of the above salts and the acid were obtained from Baker Chemical Company (Phillipsburg).

Equipment

Kontes (Vineland) 30 cm x 1 cm glass columns equipped with the appropriate Kontes flow adaptor were utilized as reaction chambers. Harvard 20 (South Natick) syringe pumps equipped with Popper and Sons (Hyde Park) 50 ml glass syringes were used to exchange column volumes. Polytetraflouroethylene (PTFE) tubing (1/16 O.D.), obtained from Kontes, was used throughout the experiment. All connectors (Altech (Deerfield), Kontes) were polypropylene. Two way valves (Kontes) were polycarbonate with polypropylene stopcocks. (See Figure 2).

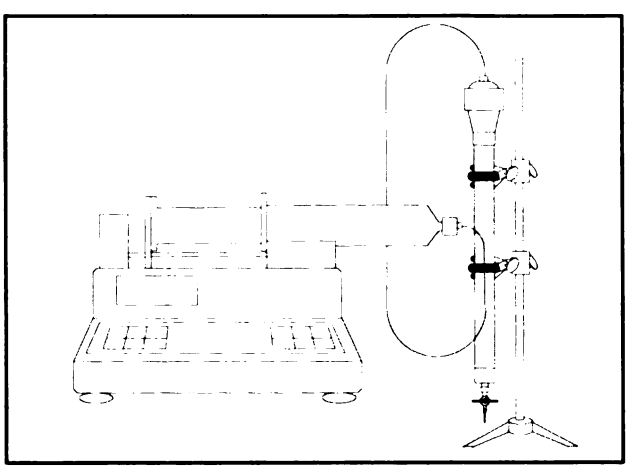

Figure 2. Apparatus Used to Exchange Columns

Experimental Design

Duplicate columns of aquifer material from the West KL Avenue Landfill were prepared in an anaerobic glovebox (4% hydrogen, 90% nitrogen and 6% carbon dioxide atmosphere). These columns were eluted with a mixture of site water, 1 mg/L of radiolabeled electron donor (acetone or benzene) and two times the stoichiometric

equivalent of electron acceptor required to completely oxidize the acetone or the benzene. S^{2-} was added for O_2 scavenging, where appropriate, at 1/50 the concentration of the electron acceptor. Columns were incubated at 15 C° and exchanged at one week intervals. Inhibited controls consisted of duplicate columns exchanged with 100 mg/L $HgCl_2$.

The sample preparation utilized pH adjustment and purging with nitrogen gas to track $^{14}CO_2$ and other radiolabled metabolic products. The carbon balance was generated using ratios of disintegrations per minute per ml of the effluent samples to the disintegrations per minute per ml for the influent standard.

General Procedure

Aquifer material was collected aseptically at well 90-A from the shallow aquifer at the West KL Landfill and stored at 4°C (Figure 1, pg 3). Eleven columns were packed with the aquifer material. Two electron donors were tested in three reducing and one oxidizing environment. Killed controls were utilized to assess sorption losses. All columns were duplicated. Column designations were Nitrate 1 and 2 (NO31 and NO32), Oxygen 1 and 2 (O21 and O22), Ferric Iron 1 and 2 (Fe31 and Fe32), Sulfate 1 and 2 (SO41 and SO42,) Kill 1 and 2 (Kil1 and Kil2) and a Non-amended control (Non-A). Kill 1 and 2 were abiotic controls. Preparation for the control columns consisted of an initial gamma irradiation, after loading of aquifer material, (University of Michigan Cyclotron, 5 Mrads over a 13 hour period). After irradiation, 100 mg/L of $HgCl_2$ was

added to the eluent for each exchange.

Site Water Collection and Storage

Water was collected from Test Well 4 (Figure 1, pg 3), approximately 70 feet from the surface. At least 50 gallons of water were removed from the well before samples were collected. This water was stored in autoclaved 20 liter polyethylene carboys in a 4°C walk-in incubator. Before use in the columns, site water was filtered through an 8 micron filter to remove any solids that might plug the columns.

Preparation and Storage of Donors

Radiolabeled acetone was diluted to 788 mg/L, transferred to a nitrogen evacuated 60 ml crimp top serum vial and stored at 15° C. Radiolabeled benzene was diluted to 500 mg/L and stored in a nitrogen evacuated 60 ml crimp top vial at 15°C.

Preparation of Electron Acceptors

Nitrate, ferric iron and sulfate solutions were prepared in deionized (18 Mohm or greater) water. Air (on line Michigan State University) was bubbled through site water for at least 20 minutes to prepare O_2 saturated water.

Calibration of Columns

The pore volume in each column was determined using a tritium (Sigma) tracer added to site water. Elution fluid was prepared in site water with no electron acceptors or donors added. One hundred mg/L of $HgCl_2$ was added to eluent for the killed controls. After pore volume calibration with tritiated water, columns were calibrated with radiolabled acetone and then radiolabled benzene, with no electron acceptors added to eluent. Samples were analyzed on a Packard liquid scintillation counter (Canberra). The data were used to generate breakthrough curves of C_t/C_o vs total volume eluted where C_t = disintegrations per minute per ml at time t of the effluent and C_o = disintegrations per minute per ml at time t =0 of the influent. The pore volume was calculated using interpolation of breakthrough curves at $C_t/C_o = 0.50$.

Specific Procedures

Acetone Exchange Events

Pore volume exchanges were performed at one week intervals. Site water was degassed for at least 20 minutes with nitrogen gas, and cooled simultaneously to 4°C in an ice bath. Water was transferred to a 50 ml glass volumetric flask that had also been cooled in an ice bath. Approximately 60 microliters of the radiolabeled acetone was spiked into the site water with a Hamilton (Las Vagas) 100 microliter glass syringe. The

appropriate amount of electron acceptor was added (2 times the stoichiometric amount required for complete mineralization of the electron donor) to the KL water/radio-labeled acetone mixture. For example, the balanced chemical equation for the oxidation of acetone with nitrate is:

$$10\ C_3H_6O + 32\ NO_3^- + 32\ H^+ \leftrightarrows 30\ CO_2 + 16\ N_2 + 46\ H_2O$$

One milligram of acetone per liter of water is equivalent to 1.72×10^{-5} moles of acetone per liter of water. The molar ratio of acetone to nitrate in the balanced equation is 1 : 3.2. Since the desired amount of nitrate in the eluent is two times the stoichiometric equivalent of nitrate required to completely oxidize 1 mole of acetone, the concentration of nitrate in the eluent is $(((1.72 \times 10^{-5}) \times 3.2) \times 2)$ or 4.6×10^{-4} moles of nitrate per liter of water. Each eluent was prepared using these calculations.

Exchange fluid was transferred to a 50 ml glass syringe. Exchange fluid was eluted through the column at 2.5 cm/min (~ 2 ml/min). Approximately 20 ml were used for a complete exchange of fluid in the column. Preparation of the elution fluid for the killed control included the addition of 100 microliters of 50 g/L $HgCl_2$ and nitrate at the same concentration used in the nitrate columns.

Six ml of column effluent were collected for analysis. Approximately 0.5 ml (15 seconds) of column fluid was allowed to drain prior to sample collection. Samples were pooled in 20 ml scintillation vials placed in an ice bath. Duplicate samples were collected from each column and assayed for radioactivity. Four sample designations were used: Filter/Acid/Purge (FAP), Acid/Purge (AP), Base (B) and Base/Purge (BP). Sample

FAP was prepared by filtering a sample through a 0.45 micron filter, adjusting the pH to < 2 and purging. Sample AP was adjusted to pH < 2 and purged. Sample B was adjusted to pH > 10. Sample BP was adjusted to pH >10 and purged (see equations 1-4 page 25, and Figure 3, pg 23).

Twenty nine 8 ml scintillation vials were prepared. Seventy five microliters of 2 molar HCl were added to 15 of the vials and 75 microliters of 2 molar KOH was added to 12 of the vials. The two remaining vials were used for background ^{14}C analysis. Vials were tared. Three vials were reserved for total activity (initial activity) analysis of the eluent and designated Co. One-half ml sub samples were collected from the sample pool and distributed into the prepared scintillation vials. Following sample addition to a scintillation vial, the vial was weighed to determine the mass of sample in that vial.

One set of base-treated samples was analyzed without purging. All other samples were purged for ten minutes with water saturated nitrogen gas and weighed. Five milliliters of Saftysolve® scintillation cocktail (Mount Prospect) were added to the samples and the samples were analyzed on a Packard liquid scintillation counter.

Benzene Exchange Events

Benzene exchange events followed the same protocol as the acetone exchange events with the following exceptions. Site water was degassed for at least 20 minutes

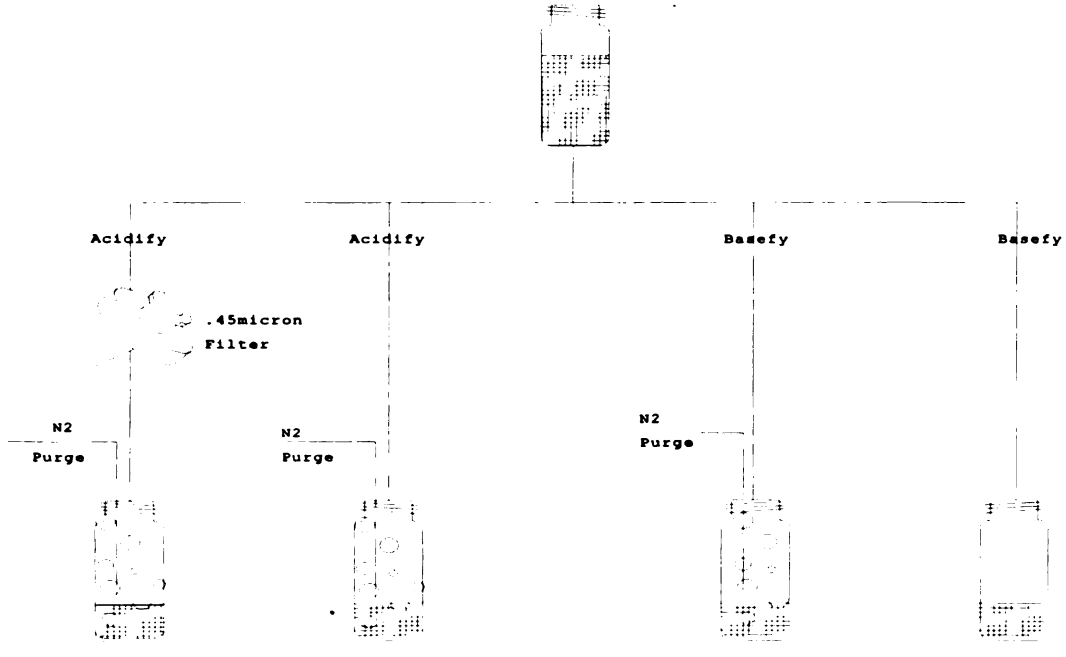

Figure 3. A Flow Chart Representation of the Sample Treatment Procedure.

with helium gas before any preparation. Approximately 0.2 milliliter of 500 mg/L radiolabeled benzene was transferred to the site water with a Becton Dickinson (Franklin Lakes) 1 milliliter disposable syringe. Instead of 20 milliliters of exchange fluid, approximately 100 milliliters were used for a complete exchange of fluid in the benzene columns. Eluant was not cooled prior to elution.

The benzene experiment was repeated and followed the same procedure as the original benzene experiment with the following exceptions. The amount of nitrate supplied to the columns was increased to meet the C.O.D. of the leachate (70 mg/L) as measured with low range C.O.D. vials obtained from Fisher Chemical Company (Pittsburgh) and the purge interval was increased from ten to twenty minutes. Four identical columns were used to access the nitrate amended microcosm.

Triplicate samples were collected from each column using the same treatments as the acetone exchanges. Approximately 0.5 milliliter of column fluid was allowed to drain before sample collection. Samples were pooled in 20 milliliter scintillation vials containing 0.90 milliliters of 2 M KOH in an effort to trap all of the dissolved CO_2 in the sample. The vials were placed in an ice bath and 6 milliliters of column effluent were collected for analysis. Twenty seven 8 milliliter scintillation vials were prepared, tared, and cooled on an ice bath before sample collection. Ninety microliters of 2 M HCl were added to 12 of the vials. Forty five microliters of KOH were added to the vials designated Co (the total radioactivity of the eluent). One third milliliter sub samples were collected from the sample pool and distributed into the 8 milliliter scintillation vials. After a sample had been added to a scintillation vial, the vial was weighed to determine the mass of sample added to that vial. One set of base-treated samples was analyzed without

purging. All other samples were purged for twenty minutes with water saturated nitrogen gas and weighed. Five milliliters of scintillation cocktail were added to each sample, and samples were analyzed on a scintillation counter.

Data Collection and Analysis

Carbon balances were generated using the following assumptions:

1. Any carbon dioxide generated in the column can be trapped in a basic solution and purged in an acidic solution.
2. Non-transformed acetone/benzene can be removed from a sample by purging with nitrogen gas.
3. ^{14}C incorporated into cell biomass can be removed from solution using a 0.45 micron filter.
4. All other counts are non-volatile products of acetone/benzene degradation.

These assumptions generated the following set of equations:

1. CO_2 = base treated/purged - acidified/purged (BP-AP) [1]
2. Cells = base treated/purged - filtered/acidified/purged (BP-FAP) [2]
3. Volatile products = base treated - base treated/purged (B-BP) [3]
4. Non-volatile products = filtered/acidified/purged (FAP) [4]

Results of the calculations were converted to percentages by dividing the result of

each equation by the disintegrations per minute of the eluent , Co (total activity). Sorption to columns and volatilization losses were calculated by subtracting the sum of the equations from 1. Nitrate was analyzed using a Shimatzu High performance liquid chromatographer (HPLC),(Kyoto). See Appendix A for specific calculation procedures.

Calculation of Zero and First order rates

Zero and first order rates were calculated using the following equations. Where $\partial C/\partial t=0$, the following equation can be used to calculate the rate of donor degradation and the rate of CO_2 production:

$$C_t-C_o/t_o-t_t=k \qquad [5]$$

where C_t is the concentration at time t, C_o is the concentration at time t=0, t_t is the end time of incubation, t_o is the start time of the incubation and k is the zero order rate coefficient.

Where $\partial C/\partial T=-kC$ [6], first order rates for donor degradation can be calculated. Separating variables and integrating [6] yields:

$$C_t=C_o \times e^{-k"t} \qquad [7]$$

where C_t is the concentration of donor at time = t, C_o is the concentration of donor at time=0, t is the time interval and k" is the first order rate coefficient. The techniques for analyzing the acetone fractions did not allow for first order calculations for rate of degradation of the radiolabled acetone and the rate of evolution of $^{14}CO_2$. First order rates for the degradation of radiolabled benzene and the evolution of $^{14}CO_2$ from the benzene

could be calculated. Since the rate of evolution of $^{14}CO_2$ is dependent on the initial concentration of radiolabled benzene, we can write:

$$d(^{14}CO_2)/dt=\gamma k"B_t \quad [8]$$

where γ is a constant that relates the rate of benzene degradation to the rate of $^{14}CO_2$ evolution and B_t is the benzene concentration at time = t. Substituting the RHS of equation [7] for Bt in equation [8], we can write:

$$d^{14}CO_2/dt=\gamma k"B_o e^{-k"t} \quad [9]$$

Integration yields:

$$^{14}CO_{2t}=\gamma B_o(1-e^{-k"t}) \quad [10]$$

where $^{14}CO_{2t}$ is the concentration of $^{14}CO_2$ at time = t. Sample calculations are given in Appendix B.

Viable Cell Count

Solid growth media was prepared using R2A (Difco, Detroit) solid media with distilled, deionized water and 2% of 1000 mg/L phosphate buffer solution. Nitrate was added from a 15 000 mg/L stock of sodium nitrate prepared in D.I. water at 0.16 mg/L. Anaerobic plates were degassed for 72 hours in an anaerobic glovebox (Coy Manufacturing, Ann Arbor) prior to inoculation. Eppendorf tubes were autoclaved and used to collect effluent from the column being enumerated. Effluent from the column was plated on the prepared plates at 10x, 100x and 1000x dilution for the live columns

and at 1x, 10x and 100x dilutions for the killed control in a laminar flow hood in triplicate. Anaerobic plates were placed in an anaerobic glovebox and incubated for at least 72 hours at 20°C. Plates for the aerobic assay were placed in a 15°C incubator and incubated for at least 72 hours. Plates were placed on a manual plate counter, counted three times and reported as colony forming units (CFU's).

Chapter 4

Results and Discussion

This chapter reports the results from the experiments performed on the KL microcosm. The acetone results are reported first, followed by the benzene results. The discussion concludes with the results of the viable cell count.

Column Experiments Utilizing Acetone As The Electron Donor

Columns utilizing acetone as the electron donor and various electron acceptors were studied for a minimum of 42 days. Table 3 presents the results of the acetone assessment column experiments. The nitrate and the non-amended microcosms demonstrated the highest activity with respect to $^{14}CO_2$ production. Only one non-amended column was utilized, and the purge and trap technique did not allow for the calculation of a volatile fraction.

Problems with the fractionation method for sample treatment described in Chapter 2 were encountered with acetone. An analysis of purging efficiency for acetone was performed. Radiolabled acetone was placed in the various mineral matrices. Samples were purged with water saturated nitrogen gas. Subsamples of the acetone/mineral matrix were collected every 5 minutes and analyzed on a liquid scintillation counter. After 20 minutes of purging, only 70% of untransformed acetone was stripped from the samples in

the various mineral matrices used during this experiment. As a result of the poor purging efficiency, numeric values for the volatile fraction were erroneous and had to be discarded. Since the calculations for the percentages of $^{14}CO_2$ and particulate fractions did not rely upon the removal of untransformed acetone, only these fractions could be quantified.

Table 3. Percentage of Total Radioactivity Recovered in Indicated Fraction Compared to Influent Concentration of ^{14}C-Labeled Acetone.

Fraction	Column	Electron Accepting Environment					
		NO_3	O_2	Fe^{3+}	SO_4^{2-}	Non-amended	Control
$^{14}CO_2$	Column 1	23%	25%	13%	<1%	31%	<1%
	Column 2	56%	20%	5%	<1%	NA	<1%
Particulate	Column 1	9%	<1%	3%	<1%	1%	<1%
	Column 2	3%	<1%	5%	<1%	NA	<1%

Estimated rates of mineralization of radiolabled acetone are reported in Table 4. The greatest rate of mineralization was in the Non-amended column after day 45. See Appendix B for sample calculations.

Table 4. Rate of Mineralization of ^{14}C-Radiolabeled Acetone in Four Electron Accepting Environments.

Column		CO_2 Zero Order Production Rate[a] k[b] (millimoles Carbon/ml/Day)	% Mineralization	R^2
Nitrate	Col. 1	4.3e-5	23	0.79
	Col. 2	7.5e-5	56	0.99
Oxygen	Col. 1	3.6e-5	25	0.96
	Col. 2	3.1e-5	20	0.92
Sulfate	Col. 1	3.0e-6	<1	.99
	Col. 2	2.0e-6	<1	.35
Ferric Iron	Col. 1	5.0e-8	13	0.71
	Col. 2	4.1e-7	5	0.77
Non-amended[b]	Day 0-35	5.6e-6	31	0.81
	Day 35-75	9.3e-5	31	0.96
Control	Col. 1	2.6e-6	<1	0.99
	Col. 2	2.6e-6	<1	0.99

a. rates are reported as millimoles of carbon / ml / day.
b. Equation for zero order kinetics: $k=(C_t-C_o)/(t_o-t_t)$; k is the zero order rate, C_t is the final concentration of CO_2, C_o is the initial concentration of CO_2, t_t is the end time of incubation, and t_o is the start time of incubation.

Column Experiments Utilizing Benzene as the Electron Donor

Results of the first set of experiments utilizing benzene as the electron donor are summarized in Table 5 and Table 6. The nitrate columns demonstrated the greatest mineralization of benzene. The ferric iron environments showed little or no activity.

Table 5. Percentage of Total Radioactivity Recovered in Indicated Fraction Compared to Influent Concentration of ^{14}C-Labeled Benzene, First Experiment.

Electron Accepting Environment	Fraction			
	14CO2	Particulate	Volatile	Non-volatile
Nitrate	58%[a] 40%[b]	3% 3%	21% 31%	11% 13%
Oxygen	64%[c] 23%[d]	9% 10%	33% 48%	12% 12%
Ferric Iron	10%	1%	48%	15%
Sulfate	25%[e] 20%[f]	<1% <1%	42% 47%	12% 20%
Non-amended	8%	2%	34%	24%
Control	2%	<1%	75%	7%

a. Nitrate results after 49 days.
b. Nitrate results from 49 to 70 days.
c. Oxygen results to 50 days.
d. Oxygen results from 50 to 99 days.
e. Sulfate results to 50 days.
f. Sulfate results from 50 to 90 days.
Note. Numbers presented are the average of two replicates.

The oxygen columns showed little activity, presumably because they were oxygen limited. The technique used for oxygen saturation of the elution water allowed for only 8-10 mg/L of oxygen to be dissolved in the water. Table 6 summarizes the rates of $^{14}CO_2$ production and benzene degradation in the first benzene experiment.

Table 6. Summary of the First Benzene Experiment.

Electron Acceptor	Column	Rate of 14CO2 Production		
		0 order[a] (k)	γ^c	R^2
Nitrate	Col.1	4.51e-4	6.59e-1	.94
	Col.2	5.32e-4	1.09	.93
Oxygen	Col.1	3.27e-4	4.25e-1	.74
	Col.2	2.35e-4	8.90e-2	.80
Ferric Iron	Col.1	6.90e-5	1.32e-1	.79
	Col.2	7.23e-5	1.46e-1	.44
Sulfate	Col.1	2.02e-4	7.90e-2	.81
	Col.2	2.53e-5	6.00e-3	.86
Control[b]	Col.1	1.85e-5	4.70e-2	.84

Electron Acceptor	Column	Rate of Benzene Disappearance					
		0 order (k)	R^2		First Order[d] (k")	$t_{½}$[e]	% mineralization
			Vol	Co			
Nitrate	Col.1	7.57e-4	.97	.99	1.38e-1	5.1	58
	Col.2	7.9e-4	.98	.99	1.517e-1	4.8	40
Oxygen	Col.1	7.92-4	.93	.99	1.09e-1	6.3	64
	Col.2	9.09e-4	.93	.99	1.36e-1	5.1	23
erric Iron	Col.1	5.86e-4	.97	.99	1.12e-1	6.2	10
	Col.2	5.54e-4	.96	.99	1.03e-1	6.7	10
Sulfate	Col.1	5.02e-4	.98	.99	8.41e-2	8.2	25
	Col.2	4.55e-4	.99	.99	6.03e-2	11.5	20
Control		4.26e-4	.97	.99	5.84e-2	11.9	2

a. Zero order rates are reported as millimoles of carbon / ml / day.
Equation for zero order kinetics: $k=(B_o-B_f)/(t_o-t_i)$; k is the zero order rate constant, B_f is the final concentration of benzene, B_o is the initial concentration of benzene t_i is the end time of incubation, and t_o is the start time of incubation.
b. Only one column was used to assess losses and abiotic transformation
c. First order rates are reported as / day. Equation for first order kinetics for CO2 production: $CO_{2t} = \gamma B_o(1-e^{-k"t})$. k" is the first order rate constant for benzene degradation, CO_{2t} is the concentration of CO_2 at time= t, γ is the conversion factor relating benzene disappearance to CO_2 production, B_o is the concentration of benzene at time=0.
d. Equation for first order kinetics for benzene disappearance: $k"=\ln(B_f/B_o)/(t_o-t_i)$. k" is the first order rate constant, B_o is the concentration of benzene at t=o, B_f is the concentration of benzene at time = f.
e. $t_{½}$ is the half-life of benzene in each particular microcosm.

The results of the second set of experiments are summarized in Table 7 and Table 8. Nitrate was the electron acceptor. Nitrate analyses were performed at each exchange. The first nitrate analysis of columns 3 and 4 indicated that denitrification was occurring. Subsequent analysis of these columns revealed no further significant denitrification. Mineralization of benzene continued until the experiment was stopped at 54 days. Results reported in Table 7 are the average of four replicates. The standard deviations were calculated using four replicates. The control carbon balance is from one column. R^2 from a regression analysis of the data generated by the control are reported in the last column. See Appendix A for sample calculations.

Table 7. Percentage of Total Radioactivity Recovered in Indicated Fraction Compared to Influent Concentration of ^{14}C- Labeled Benzene, Second Experiment .

Fraction	Percentage of total radioactivity recovered in indicated fraction		
	Denitrifying Environment	Control	
		% Fraction	R^2
$^{14}CO_2$	29±5%	1%	.98
Particulate	2±1%	1%	.83
Volatile	65±5%	82%	.97
Non-volatile	7±3%	16%	.94

Note. Standard deviations were calculated from four columns run simultaneously.

Rates of degradation and mineralization for the second benzene experiment are presented in Table 8.

Table 8. Summary of the Second Benzene Experiment.

Electron Acceptor	Column	Rate of $^{14}CO_2$ Production		
		0 order[a] (k)	γ	R^2
Nitrate	NO31	4.49e-4	9.19e-1	.98
	NO32	4.12e-4	6.89e-1	.98
	NO33	6.06e-4	1.00	.98
	NO34	7.68e-4	1.18	.98
Non-amended		9.05e-5	1.22e-1	.81
Control[b]		2.99e-5	1.04e-1	.53

Electron Acceptor	Column	Rate of Benzene Disappearance					
		0 order	R^{2e}		First Order	t½[f]	% mineralization
			vol	Co			
Nitrate	NO31	5.29e-4	.96	.96	6.18e-2	11.2	26
	NO32	6.47e-4	.97	.96	7.00e-2	9.9	22
	NO33	6.56e-4	.96	.95	6.23e-2	11.1	31
	NO34	7.04e-4	.95	.95	6.66e-2	10.4	39
Non-amended[g]		8.04e-4	.72	.76	1.94e-2	3.6	8
Control[b]		3.11e-4	.97	.95	4.17e-2	16.6	2

a. Zero order rates are reported as millimoles of carbon / ml / day.
Equation for zero order kinetics: $k=(B_o-B_t)/(t_o-t_t)$; k is the zero order rate constant, B_t is the final concentration of benzene, B_o is the initial concentration of benzene t_t is the end time of incubation, and t_o is the start time of incubation.
b. Only one column was used to assess losses and abiotic transformation.
c. First order rates are reported as / day. Equation for first order kinetics for CO2 production: $CO_{2t} = \gamma B_o(1-e^{-k"t})$. k" is the first order rate constant for benzene degradation, CO_{2t} is the concentration of CO_2 at time= t, γ is the conversion factor relating benzene disappearance to CO_2 production, B_o is the concentration of benzene at time=0.
d. Equation for first order kinetics for benzene disappearance: $k"=\ln(B_t/B_o)/(t_o-t_t)$. k" is the first order rate constant, B_o is the concentration of benzene at t=o, B_t is the concentration of benzene at time = t.
e. R^2 was calculated by a regression along the data indicated. The regression equation for the volatile concentration was subtracted from the regression equation for the influent concentration to yield a regression equation for the zero order rate.
f. t½ is the half-life and is reported as days
g. Recovery of radiolabeled benzene from the non-amended column was 55%. The rest of the columns had 100% recovery.

The column that demonstrated the most rapid zero and first order rate of benzene degradation coupled with the highest percent mineralization was NO34. See Appendix B for sample calculations.

Viable cell counts were performed on each column. The results and a comparison to the inhibited control of each of the nitrate columns are presented in Table 10. When eluant from the control was incubated aerobically at 20°C, two distinct cell morphologies were found on the plates. The same eluant incubated anaerobically showed no growth after two weeks in an anaerobic environment at 20°C. Column 3 and column 4 had suspended population counts 10 times greater than the suspended population counts in columns 1 and 2. The highest concentration of organisms was found in column 3. This suggests that the reason for the higher zero and first order rates of benzene degradation in columns 3 and 4, when compared to columns 1 and 2, is simply the higher population of organisms in columns 3 and 4. The higher zero order rates in columns 3 and 4 may also be a result of the history of columns 3 and 4. When the second benzene experiment was started, columns 3 and 4 were the aerobic columns from the first benzene experiment. In addition to the history of columns 3 and 4, columns 1 and 2 were the original benzene degrading microcosms and may have exhibited "column fatigue", a build up of inhibitory toxins or organism attenuation (the population of benzene degrading organisms was worn out).

Table 9. Viable Cell Counts* of the Nitrate/Benzene Microcosms.

Column	Incubating environment	
	aerobic	anaerobic
NO31	2e5 ± 6e4	2e5 ± 8e3
NO32	7e5 ± 5e5	3e5 ± 2e4
NO33	3e6 ± 3.5e4	2e5 ± 1e4
NO34	1e6 ± 6e4	7e5 ± 2e4
Control	3000 ± 50	No visible colonies

* Counts are CFU's / ml.

In summary, acetone degradation was the most rapid in the Non-amended column. Mineralization of acetone was most extensive in the nitrate microcosm. Activities for the rest of the columns, from most to least efficient, in terms of rate of mineralization and percent mineralization were Nitrate > Oxygen > Ferric iron > Sulfate. The inhibited control exhibited mineralization rates almost two times lower than the nitrate microcosms in both of the benzene experiments.

In the first set of benzene column experiments, the order of efficiency (taking into account zero order production of CO_2, zero and first order disappearance rates of

benzene, and percent mineralization of benzene) was nitrate > oxygen > sulfate > ferric iron. The nitrate microcosms had the highest zero and first order degradation rates and the highest zero and first order mineralization rates. All of the columns in the second benzene experiment showed similar activities. Zero order rates of degradation in the second experiment ranged from 5.3e-4 to 8.0e-4 millimoles of carbon / ml / day. First order rates for benzene degradation ranged from 6.2e-2 to 7.0e-2 / day. The viable organism count indicated that the columns demonstrating the highest rate of benzene degradation had the highest concentrations of organisms.

A significant rate of disappearance for benzene was calculated for both of the inhibited control microcosms in the benzene experiments. This disappearance could be due to volatilization losses, sorption losses, abiotic transformation or cometabolism since low numbers of organisms were isolated from the inhibited control effluent. In the first benzene experiment, the non-volatile fraction for the inhibited control and the nitrate amended microcosm was the same. The percent of radiolabled fraction unaccounted for was 10% for the nitrate amended microcosm and 15% for the inhibited control, implying that sorption or volatilization losses played a role in the disappearance of benzene. In the second benzene experiment, recovery of radiolabled product was 100%. However, the non-volatile fraction in the inhibited control was two times the amount of non-volatile fraction in the nitrate amended microcosm, indicating that abiotic transformation or cometabolism played a role in the rate of benzene disappearance in the inhibited control.

Chapter 5

Engineering Significance

Bench-top microcosms derived from material collected from the shallow aquifer at the KL landfill and placed in 30 cm x 1 cm columns were used to achieve several goals in this experiment: assessment of the column microcosm's ability to degrade acetone and benzene, the generation of carbon balances, and the estimation of zero order CO_2 production for acetone and CO_2 production rates for benzene and zero and first order rates for benzene degradation.

Assessment

Acetone

The column microcosms were able to mineralize acetone under aerobic and nitrate reducing conditions. Most notably, acetone was mineralized without the addition of a specific electron acceptor in the Non-amended microcosm. This implies that no additional compounds would need to be added to the shallow aquifer for in-situ remediation of acetone. The ferric iron (iron III) microcosm showed some activity but was probably limited by iron III availability (Lovely et al. 1994). The sulfate microcosms

showed no significant activity.

Benzene

The column microcosms were able to degrade benzene under aerobic and nitrate reducing conditions. The results of the non-amended column indicated benzene had a half life of 36 days without additional compounds (2.04 e-4 millimoles of benzene as carbon / ml / day zero order and 1.94 e-2 / day as first order). In the nitrate amended columns, benzene had a half life of 10 days (6.5 e-4 millimoles of benzene as carbon / ml / day and 6.5 e-2 / day for zero and first order rates of degradation respectively). Analysis of the effluent from the benzene/nitrate columns indicated no significant change in the nitrate concentration over the incubation period. However, the non-amended column demonstrated significantly lower rates of benzene degradation, implying that nitrate may be necessary for anaerobic degradation of benzene. Other anaerobic environments, methanogenic conditions for example, could explain the results obtained in the benzene/nitrate microcosms. Further study of the benzene/nitrate microcosm is required to draw any further conclusions.

The sulfate microcosms demonstrated some activity, but the design of the first experiment did not allow for statistical calculations. There are recent publications (Edwards and Grbić-Galić 1992, and Young et al., 95th annual meeting for the American Society for Microbiology, Washington D.C., 1995) that have demonstrated

benzene mineralization under sulfate reducing conditions. In order for sulfate amended degradation to be considered as a potential remediation strategy, more studies of the benzene/sulfate microcosm must be performed.

The benzene/iron III microcosm showed no significant activity and was judged inappropriate for a remediation strategy. However, Lovely et al. (1994), demonstrated mineralization of benzene under iron III reducing conditions with the addition of a chelating agent, nitralotriacetic acid (NTA). The iron III system should not be discarded as a potential remediation strategy until an assessment of a benzene/iron III/ NTA microcosm can be performed.

It is recommended that if a specific remediation strategy for benzene degradation is to be adopted and that strategy is to include aquifer amendment, that the order of consideration be oxygen, nitrate, sulfate, and iron III amendment. The two strategies that should be considered and further investigated are the oxygen and nitrate amendment strategies. It is further suggested that because of the cost of oxygen amendment, nitrate amendment should be targeted for a pilot scale study and pursued as the amendment strategy of choice.

Mineralization

Acetone was mineralized in all but the sulfate reducing microcosms.

Mineralization of benzene occurred in all of the tested microcosms. The amount of mineralization in both acetone and benzene aerobic microcosms was less than expected, probably because of competition for oxygen within the microcosms. Table 10 presents a summary of the donor:acceptor stoichiometric ratios used in this experiment, and gives recommended ratios for remediation procedures. Recommendations for the ratio of acetone and benzene to oxygen in a remediation procedure should be considered conservative.

Table 10. Stoichiometric Ratios of Donor : Acceptor Recommended for In-situ or Ex-situ Remediation.

	Benzene			Acetone		
	required[a] ratio	ratio in[b] columns	recommended[c] ratio	required ratio	ratio in columns	recommended ratio
O_2	1:7.5	1:19	≤1:19	1:4	1:14	≤1:14
NO_3^-	1:6	1:12	1:12	1:3.2	1:6.4	≤1:6.4

a. Stoichiometric amount necessary for complete mineralization of the substrate (theoretical)
b. Twice the required ratio.
c. Based on bench top microcosm performance.

Since there was 100% recovery of nitrate in the benzene/nitrate microcosm, the recommended ratio of benzene to nitrate is the same as was used in the benzene/nitrate experiment. There were no breakthrough data available for the acetone/nitrate microcosms. Recommendations for acetone/nitrate ratios should be considered preliminary, and used as a minimum amount required for mineralization. The acetone/nitrate microcosm mineralized 50% of the acetone. Platen and Schink (1989)

reported 71% mineralization of acetone under denitrifying conditions. It is understood that the conditions of the Platen/Schink experiment were different from conditions in this experiment, but it should be noted that the acetone/nitrate microcosms could have been limited by available nitrate and could be capable of higher mineralization percentages.

Rates

Table 11 summarizes some of the benzene degradation rates presented by the authors of various papers cited in chapter 2. The zero order rates calculated for Edwards and Grbić-Galić, Holm, and Hutchins were calculated with the concentrations given by the authors. These were one thousandth of the concentrations used by Morgan and this work. Rates of benzene degradation calculated in this study compare favorably with the rates calculated from the results reported in Table 11. Apparently, the first order rate of benzene degradation under aerobic and nitrate reducing conditions is consistently on the order of 10^{-2} to 10^{-3} per day.

Table 11. A Summary of Rates of Benzene Degradation Under Aerobic and Denitrifying Conditions From Data Presented in Cited Literature.

Authors	Acceptor	Zero Order milliMoles Carbon/ml/day	First Order per day
Hutchins, 1991a	O_2	$5x10^{-6}$	NA[a]
Edwards and Grbic-Galic, 1992	SO_4^{2-}	$1x10^{-6}$ to $3x10^{-6}$	NA[b]
Holm, 1992, in-situ	O_2	$3x10^{-8}$	$6x10^{-2}$
Morgan, 1993, in-situ	O_2	$2x10^{-4}$	$4x10^{-2}$
Morgan, 1993, in-situ	NO_3^-	$1x10^{-10}$	$4x10^{-2}$
Lovely, 1994	Fe^{3+}	NA	$1x10^{-3}$
This thesis	O_2	$8.5x10^{-4}$	$1.2x10^{-1}$
This thesis	NO_3^-	$6.3x10^{-4}$	$6.5x10^{-2}$

a. Not applicable. No benzene degradation under Denitrifying conditions was reported.
b..Not applicable. The article presented only zero order rates of degradation.

Johnson and Varadhan (1994) used the reported concentration of contaminants and hydro-geologic data from the KL landfill to enable a modeling program entitled Bioplume II. Among the predictions of extent of the plume and position of the plume with respect to time, were first order degradation rate predictions for acetone and benzene. The first order degradation rates predicted for acetone ranged from $1.67x10^{-4}$ to $4.73x10^{-4}$ per day. The first order degradation rates predicted for benzene ranged from $1.34x10^{-3}$ to $3.87x10^{-3}$ per day. The rates reported by this study and the rates calculated from Morgan's study on benzene degradation under denitrifying conditions are 10 times

faster than the rates predicted by Bioplume II. Unfortunately, in order to calculate first order degradation rate estimates for acetone in this study, the volatile and the non-volatile fractions from the acetone mass balance experiment would have to be treated as one fraction. There is no basis for comparison of the calculated zero order rates for acetone generated by this study with the rates predicted by Bioplume II or with literature reviewed for this work. However, Platen and Schink (1989) reported that an anaerobic enrichment culture produced 2 moles of methane and 1 mole of carbon dioxide from one mole of acetone. Assuming that in an anaerobic system, the majority of the acetone is used to produce energy for the microcosm, and that the ratio of the rate of acetone degradation to the rate of CH_4 and CO_2 production is similar to the stoichiometric ratio for acetone degradation producing CH_4 and CO_2, an estimate of the zero order rate for acetone degradation can be calculated. The mineralization of acetone in the Non-amended column in this work was 31%, the expected stoichiometric amount of CO_2 according to Platen and Schink's work. Therefore, the rate of acetone degradation should be 3 times the rate of carbon dioxide production. This gives a value for the zero order rate of acetone degradation of 2.8 e-4 milliMoles of acetone as carbon/ml/day.

Summary

The column technique proved to be a simple method for gathering the following information:

- The assessment of the column microcosms ability to degrade specific

electron donors with modifications in the fractionation method for volatility of the substrate

- The assessment of donor/acceptor couples for mineralization efficiency
- The calculation of mass balances

Upon comparison with degradation rate values obtained in other studies, the degradation rates calculated using the methods summarized in Appendix B appear to be valid and may present a relatively easy method for calculating zero and first order degradation rates for specific substrates in a column microcosm.

Chapter 6

Summary and Recommendations

The effectiveness of the techniques developed during this study are discussed in this chapter. Recommendations for further study with respect to the KL landfill and the organisms making up the microcosm associated with the site are also presented.

Effectiveness of Techniques

The question "How well does the microcosm in these columns represent the microcosm found at the site?" must be addressed. These aquifer samples were collected aseptically and stored at 4°C for one year before they were distributed into columns. After the columns were prepared and sealed in an anaerobic glove box, they were stored in a 4°C incubator for nine months. It needs to be stressed that this work presents results of experiments performed on a microcosm derived from the contaminated shallow aquifer associated with the KL landfill. Holm et al. (1992) performed a column study where a column with two compartments was prepared and then inserted into the ground at the site being assessed. This procedure eliminated the need for storage, inoculation, temperature control and light control. Holm et al. reported higher degradation rates in the on-site columns than in the columns assessed in the laboratory.

The column studies proved to be a simple method that allowed for long-term

analysis of a bench top column microcosm that led to the calculation of the mass balances and degradation rates for acetone and benzene. In addition to generating data that resulted in the mass balances, the column technique proved to be a suitable method for screening various combinations of electron donors and acceptors.

The purge and trap method for assessing radiolabeled carbon dioxide, particulate matter, non-transformed donor and non-volatile fractions, worked well for the analysis of benzene, but not for acetone. Acetone is simply too soluble, at the concentrations utilized in this study, to be readily volatilized. In summary, these techniques, with modification for each instance, show potential for the complete assessment of individual sites.

Summary of Results

A mass balance was generated for microcosms created using subsurface material from a contaminated shallow aquifer at the KL landfill. In conjunction with the mass balance, the microcosm's ability to degrade acetone and benzene was assessed. Rates of degradation of benzene and rates of mineralization of acetone were calculated.

Denitrifing environments for acetone and benzene were the most efficient in mineralizing the electron donors. Forty five percent of the acetone and 30 % of the benzene was oxidized to $^{14}CO_2$. In a column containing no additional electron acceptor, 31 % of the acetone was mineralized after an acclimation period of 40 days. There was

little mineralization (10%) in a non-amended benzene column. Columns with oxygen as the electron acceptor did not perform as well (with respect to mineralization) as the nitrate amended columns with benzene or acetone as the electron donor, probably as a result of insufficient oxygen in the elution water.

Rates for the production of CO_2 from benzene and acetone in the first experiments were greatest in the nitrate amended columns, 5e-4 millimoles of benzene as carbon/ml/day and 6e-5 millimoles of acetone as carbon/ml/day, respectively. In the inhibited control, rates of production of CO_2 were one tenth the rates of CO_2 production in the benzene and acetone nitrate amended columns. The zero order rate for benzene disappearance in the first experiment was 8e-4 millimoles of benzene as carbon / ml / day for the nitrate amended microcosm and the first order rate of disapearence was 5e-1 / day.

In the second benzene experiment, the zero order $^{14}CO_2$ production was 5e-4 millimoles of CO_2 as carbon / ml / day in the nitrate amended microcosms and 9e-5 millimoles of CO_2 as carbon / ml / day in the inhibited column. The zero and first order disappearance rates for benzene were 6e-4 millimoles of benzene / ml / day and 6e-2 / day. The inhibited control exhibited zero and first order rates of 3e-4 millimoles of benzene / ml / day and 4e-2 / day respectivly.

Direct plate counts on nitrate amended R2A showed at least 4 different colony morphologies and cell concentrations of at least 1e6 cells/ml on plates incubated at room

temperature in an aerobic environment. Direct plate counts of the inhibited control produced two colony morphologies and cell concentrations of 3e3 cells/ml. Plates incubated anaerobically indicated cell/ml concentrations one tenth that of the non-control, and one one thousandth that of the control.

In summary, the following findings/conclusions were made:

- There is evidence of intrinsic degradation of acetone at the KL landfill.
- Zero order rates of $^{14}CO_2$ production from radiolabled acetone degradation ranged from 5.0e-8 millimoles of CO_2 as carbon / ml / day (ferric iron environment) to 9.3e-5 millimoles of CO_2 as carbon / ml / day (non-amended environment).
- Benzene degraded anaerobically in a microcosm containing landfill material.
- Zero order rates for benzene degradation in a nitrate amended environment ranged from 5.3e-4 millimoles of benzene as carbon/ ml / day to 7.9e-4 millimoles of benzene as carbon/ ml / day. First order rates of benzene degradation ranged from 6.2e-2 to 1.5e-2 / day.
- Zero order rates for $^{14}CO_2$ evolution from radiolabled benzene in a nitrate amended environment ranged from 4.1e-4 millimoles of CO_2 as carbon / ml / day to 5.3e-4 millimoles of CO_2 as carbon / ml / day.

Recommendations

The column method of Siegrist and McCarty (1986) was easy to manipulate and modify. Storage of the columns, elutions and sample collection proved to be simple

procedures. Generally, in future studies, a method of monitoring the oxygen levels within the column might be employed. Electrodes could be placed at different heights in the column before it is filled with aquifer solids. In addition, shorter columns with a larger diameter might be used to increase the sample size without significantly increasing run time. With larger samples, it might be possible to perform several different analyses of the individual samples. Acetone was ineffectually removed with gas purging, indicating that some other method of acetone sample preparation and analysis was required. Sample analysis utilizing a combination of high performance liquid chromatography and a ^{14}C detector is indicated for the assessment of acetone. In this particular study, the specific activity of the radio-labeled acetone was not high enough for a ^{14}C detector. Substrates with a higher specific activity would have allowed a more detailed analysis of each sample. Dilutions of each sample could have been performed, allowing for a more complete analysis of each sample.

The trap and purge method proved to be an effective tool in analyzing the samples collected from the benzene amended columns. Ninety percent of the untransformed benzene was removed from the elution matrix after 10 minutes when purged with nitrogen gas at a flow rate of 450 ml/min.

Organisms found on plates from a gas pack isolation experiment have been archived and are stored at the Center for Microbial Ecology, Michigan State University, East Lansing, Michigan. There is a precedent for isolating the gene sequence that is

responsible for the suite of enzymes that degrade benzene aerobically (Irie et al. 1987). It is suggested that an attempt be made to isolate the suite of enzymes responsible for the anaerobic degradation of benzene. Once this gene sequence is isolated it might be possible to move it to an organism that is easier to grow and isolate

In conclusion, this study has provided evidence that supports the hypothesis that the microcosm associated with the contaminated shallow aquifer at the West KL Avenue Landfill is capable of degrading acetone without amendment. Organisms capable of degrading benzene (aerobically and anaerobically) exist at the site and, with amendment, may be able to degrade the benzene on site. Assessment of the microcosm's ability to degrade other contaminants on site (chlorinated solvents in particular) was not performed in this series of experiments. This assessment is necessary in order to make predictions as to the fate of these other compounds.

Unfortunately, each site has its own particular microcosm and each site must be individually characterized. At present, there is no way to judge the ability of the microcosm present at a different site to degrade benzene or acetone by considering the results of this study. However, this work does provide a protocol for the conduct of such studies.

An assessment of the ability of organisms indigenous to the KL aquifer to degrade chlorinated solvents is needed. In addition, the interactions of the organisms and

contaminants associated with the KL landfill should be examined. A pilot scale, in-situ collection of columns is recommended after Holm et al. (1992) to examine the fates of a wider range anthropogenic substances located at the landfill.

Appendices

Appendix A

Calculation of the Mass Balances

Appendix A

Calculation of Mass Balances

The following describes the calculation of the mass balances.
Specific activity of benzene=1.215e9 dpm/millimole

1. Raw counts, as dpm/ml, are recorded on a per exchange basis.

Mass balance		NO31	vol=8.8 ml			
days					Co-vol/8.8/days	
date	day	14CO2	Partic	Volatile	Nonvola.	Co
11/30/94	0	0	0	0	0	0
12/6/94	6	83	49	772	186	901
12/12/94	12	210	62	573	99	915
12/17/94	17	293	27	622	88	986
12/22/94	22	235	12	660	77	1496
12/26/94	26	201	25	1173	86	1276
01/02/95	33	506	40	696	101	784
01/09/95	40	302	21	429	90	665
01/16/95	47	265	18	363	66	567
01/23/95	54	111	22	394	58	631

2. Raw counts are converted to millimoles of carbon.

constant = 1e6/1.215e9*1/78.112*72.07/78.11= 9.72E-06
as millimoles of carbon

day	14CO2	Partic	Volatile	Nonvola.	Co
0	0.00	0.00	0.00	0.00	0.00
6	0.01	0.00	0.07	0.02	0.08
12	0.02	0.01	0.05	0.01	0.08
17	0.02	0.00	0.05	0.01	0.08
22	0.02	0.00	0.06	0.01	0.13
26	0.02	0.00	0.10	0.01	0.11
33	0.04	0.00	0.06	0.01	0.07
40	0.03	0.00	0.04	0.01	0.06
47	0.02	0.00	0.03	0.01	0.05
54	0.01	0.00	0.03	0.00	0.05

3. millimoles of carbon are recorded as an accumulation of millimoles of carbon by adding the amount of carbon from the latest exchange to the accumulation of the earlier exchanges. accumulation as millimoles of carbon

day	14CO2	Partic	Volatile	Nonvola.	Co
0	0.00000	0.00000	0.00000	0.00000	0.00000
6	0.00709	0.00419	0.06586	0.01588	0.07693
12	0.02505	0.00950	0.11479	0.02433	0.15500
17	0.05003	0.01180	0.16789	0.03186	0.23920
22	0.07010	0.01283	0.22419	0.03842	0.36686
26	0.08728	0.01495	0.32434	0.04579	0.47574
33	0.13049	0.01835	0.38378	0.05441	0.54263
40	0.15628	0.02015	0.42043	0.06207	0.59939
47	0.17889	0.02170	0.45143	0.06771	0.64777
54	0.18838	0.02361	0.48504	0.07270	0.70162

4. The reduced data is graphed (accumulation of carbon vs. Time) to generate the following chart:

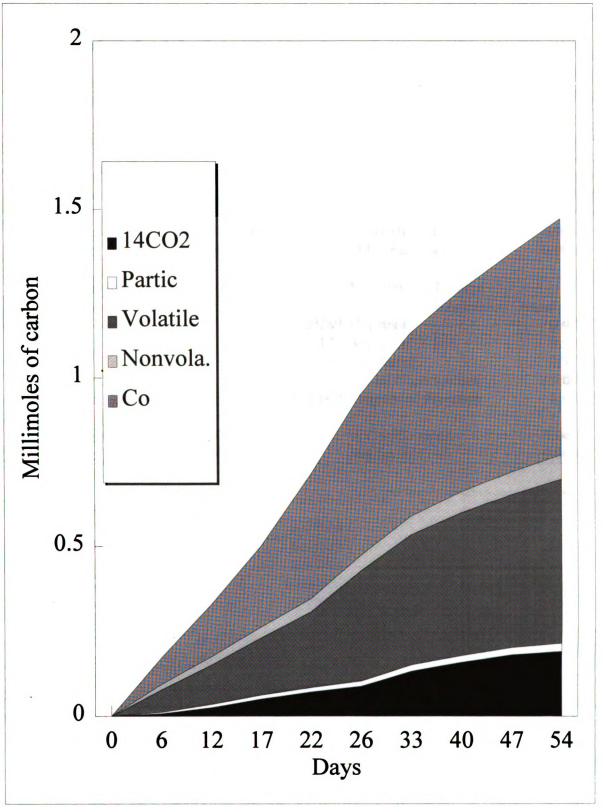

Figure 4. Chart of Accumulations for Raw Benzene Data.

5 .The data from the accumulation graph are regressed along the interface of each fraction.

14CO2 fraction

Regression Output:		
Constant		-0.01186
Std Err of Y Est		0.00988
R Squared		0.98258
No. of Observations		10.00000
Degrees of Freedom		8.00000
X Coefficients)	0.003938	
Std Err of Coef.	0.000185	

Volatile fractionl.

Regression Output:	
Constant	0.01844
Std Err of Y Est	0.03510
R Squared	0.96329
No. of Observations	10.00000
Degrees of Freedom	8.00000
X Coefficients)	0.009546
Std Err of Coef.	0.000659

Particulate fraction

Regression Output:		
Constant		0.00289
Std Err of Y Est		0.00176
R Squared		0.95317
No. of Observations		10.00000
Degrees of Freedom		8.00000
X Coefficients)	0.000421	
Std Err of Coef.	0.000033	

Non-volatile fraction

Regression Output:	
Constant	0.00752
Std Err of Y Est	0.00411
R Squared	0.97326
No. of Observations	10.00000
Degrees of Freedom	8.00000
X Coefficient(s)	0.001315
Std Err of Coef.	0.000077

Total activity of eluent (Co)

Regression Output:		
Constant		0.02508
Std Err of Y Est		0.05352
R Squared		0.95950
No. of Observations		10.00000
Degrees of Freedom		8.00000
X Coefficient(s)	0.013830	
Std Err of Coef.	0.001005	

6. The data are regenerated, using the regression equations for each fraction. The regression lines are forced through zero.

Calculations

	day	14CO2	Partic	Volatile	Nonvola.	Co
0	0	0.00000	0.00000	0.00000	0.00000	0.00000
6	6	0.02363	0.00252	0.05728	0.00789	0.08298
12	12	0.04726	0.00505	0.11455	0.01578	0.16596
18	17	0.07089	0.00757	0.17183	0.02367	0.24894
24	22	0.09452	0.01010	0.22911	0.03157	0.33192
30	26	0.11815	0.01262	0.28638	0.03946	0.41490
36	33	0.14178	0.01515	0.34366	0.04735	0.49788
42	40	0.16542	0.01767	0.40094	0.05524	0.58086
48	47	0.18905	0.02020	0.45821	0.06313	0.66384
54	54	0.21268	0.02272	0.51549	0.07102	0.74682

7. The data is graphed against a liner time axis and generates the following chart:

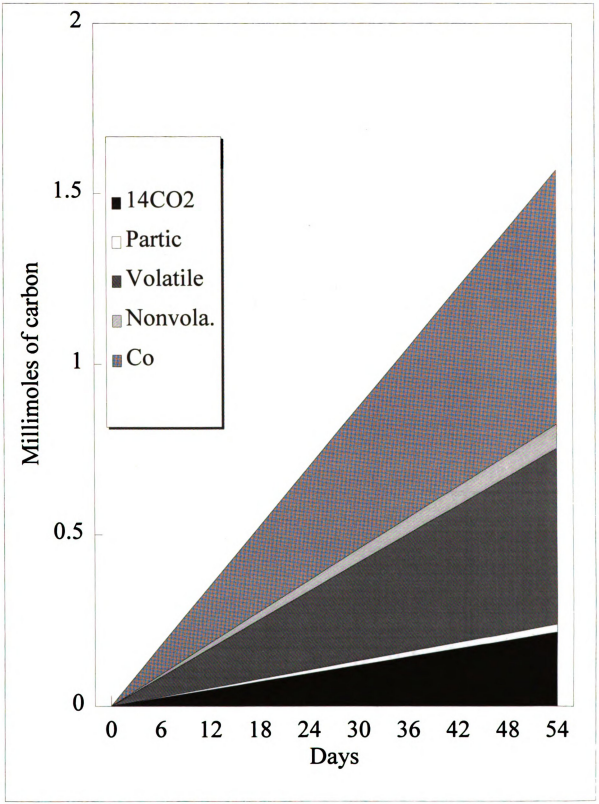

Figure 5. Chart of Accumulations for Regressed Benzene Data.

8. The mass balance is calculated by dividing the individual fractions by the Co fraction. The errors recorded in the first row are a result of division by zero.

Mass balance calculated from regression

day	14CO2	Partic	Volatile	Nonvola.	recovery
0					
6	0.28	0.03	0.69	0.10	1.10
12	0.28	0.03	0.69	0.10	1.10
17	0.28	0.03	0.69	0.10	1.10
22	0.28	0.03	0.69	0.10	1.10
26	0.28	0.03	0.69	0.10	1.10
33	0.28	0.03	0.69	0.10	1.10
40	0.28	0.03	0.69	0.10	1.10
47	0.28	0.03	0.69	0.10	1.10
54	0.28	0.03	0.69	0.10	1.10

Appendix B

Calculation of the Zero and First Order Rates

Appendix B

Calculation of the zero and first order rates.

1) Raw numbers from the sample analysis are recorded:

NO31 Calculation of zero and first order rates for CO2 production and benzene disappearance

date	day	14CO2	Partic	Volatile	Nonvola.	Co
11/30/94	0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
12/6/94	6	8.30E+01	4.91E+01	7.72E+02	1.86E+02	9.01E+02
12/12/94	12	2.10E+02	6.22E+01	5.73E+02	9.90E+01	9.15E+02
12/17/94	17	2.93E+02	2.69E+01	6.22E+02	8.83E+01	9.86E+02
12/22/94	22	2.35E+02	1.21E+01	6.60E+02	7.69E+01	1.50E+03
12/26/94	26	2.01E+02	2.49E+01	1.17E+03	8.63E+01	1.28E+03
01/02/95	33	5.06E+02	3.98E+01	6.96E+02	1.01E+02	7.84E+02
01/09/95	40	3.02E+02	2.11E+01	4.29E+02	8.97E+01	6.65E+02
01/16/95	47	2.65E+02	1.81E+01	3.63E+02	6.61E+01	5.67E+02
01/23/95	54	1.11E+02	2.24E+01	3.94E+02	5.85E+01	6.31E+02

2) Raw numbers are converted from dpms/ml to millimoles of carbon/ml for 14CO2, and to millimoles of benzene /ml for volatile and Co fractions

constant = 1e6/1.215e9*1/78.112*72.07/78.11= 9.72E-06 millimoles Carbon/dpm
constant = 1e6/1.215e9*1/78.112 = 1.05E-05 millimoles Benzene/dpm

day	14CO2 millimoles carbon /ml	Partic	Volatile millimoles of benzene/ml	Nonvola.	Co millimoles of benzene /ml
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
6	8.07E-04	4.77E-04	8.13E-03	1.96E-03	9.50E-03
12	2.05E-03	6.05E-04	6.04E-03	1.04E-03	9.64E-03
17	2.84E-03	2.62E-04	6.56E-03	9.30E-04	1.04E-02
22	2.29E-03	1.17E-04	6.95E-03	8.10E-04	1.58E-02
26	1.96E-03	2.42E-04	1.24E-02	9.10E-04	1.34E-02
33	4.92E-03	3.87E-04	7.34E-03	1.06E-03	8.26E-03
40	2.94E-03	2.05E-04	4.52E-03	9.45E-04	7.01E-03
47	2.58E-03	1.76E-04	3.83E-03	6.97E-04	5.97E-03
54	1.08E-03	2.18E-04	4.15E-03	6.16E-04	6.65E-03

3) Converted numbers are recorded as accumulation by adding the latest number to the sum of the previous numbers down a row:

accumulation

day	14CO2	Partic	Volatile	Nonvola.	Co
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
6	8.07E-04	4.77E-04	8.13E-03	1.96E-03	9.50E-03
12	2.85E-03	1.08E-03	1.42E-02	3.00E-03	1.91E-02
17	5.70E-03	1.34E-03	2.07E-02	3.93E-03	2.95E-02
22	7.98E-03	1.46E-03	2.77E-02	4.74E-03	4.53E-02
26	9.94E-03	1.70E-03	4.00E-02	5.65E-03	5.87E-02
33	1.49E-02	2.09E-03	4.74E-02	6.72E-03	6.70E-02
40	1.78E-02	2.30E-03	5.19E-02	7.66E-03	7.40E-02
47	2.04E-02	2.47E-03	5.57E-02	8.36E-03	8.00E-02
54	2.15E-02	2.69E-03	5.99E-02	8.97E-03	8.66E-02

4) 14CO2, Volatile and Co columns are regressed with respect to time:

14CO2 fraction

Regression Output:

Constant	-1.35E-03
Std Err of Y Est	1.12E-03
R Squared	9.83E-01
No. of Observations	1.00E+01
Degrees of Freedom	8.00E+00
X Coefficients)	4.49E-04
Std Err of Coef.	2.11E-05

Volatile fraction

Regression Output:

Constant	2.28E-03
Std Err of Y Est	4.33E-03
R Squared	9.63E-01
No. of Observations	1.00E+01
Degrees of Freedom	8.00E+00
X Coefficients)	1.18E-03
Std Err of Coef.	8.13E-05

Total activity of the eluent (Co)

Regression Output:

Constant	3.10E-03
Std Err of Y Est	6.61E-03
R Squared	9.59E-01
No. of Observations	1.00E+01
Degrees of Freedom	8.00E+00
X Coefficients)	1.71E-03
Std Err of Coef.	1.24E-04

5) Calculate new concentrations based on the regressed data, and force the resulting lines through zero:

As Carbon/ml

days	14CO2	Volatile	Co	Co-Vol	Time
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
6	2.69E-03	6.53E-03	9.46E-03	2.93E-03	6.00E+00
12	5.38E-03	1.31E-02	1.89E-02	5.86E-03	1.20E+01
18	8.07E-03	1.96E-02	2.84E-02	8.79E-03	1.70E+01
24	1.08E-02	2.61E-02	3.78E-02	1.17E-02	2.20E+01
30	1.35E-02	3.26E-02	4.73E-02	1.46E-02	2.60E+01
36	1.61E-02	3.92E-02	5.67E-02	1.76E-02	3.30E+01
42	1.88E-02	4.57E-02	6.62E-02	2.05E-02	4.00E+01
48	2.15E-02	5.22E-02	7.56E-02	2.34E-02	4.70E+01
54	2.42E-02	5.87E-02	8.51E-02	2.64E-02	5.40E+01

6. The following chart results:

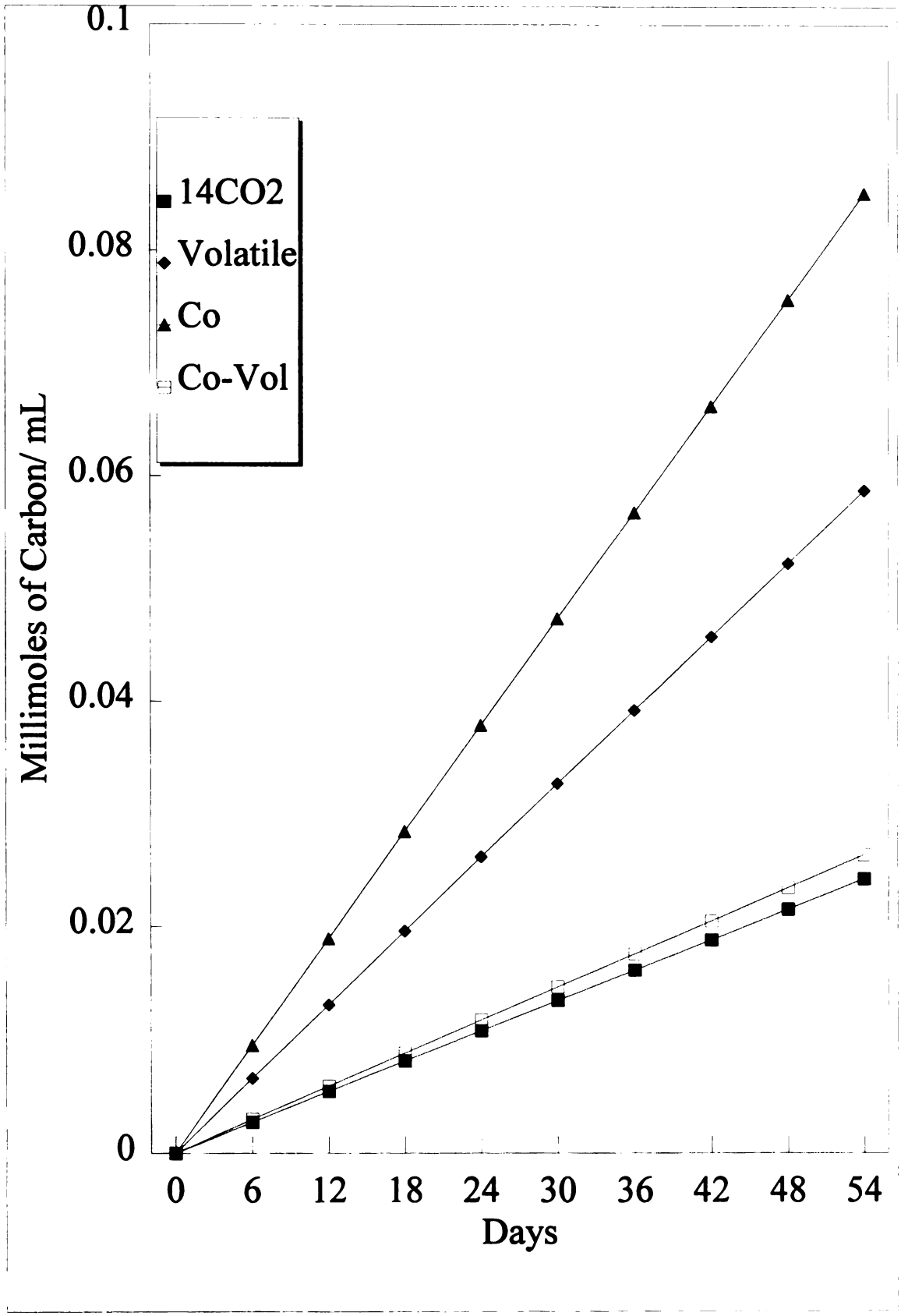

Figure 6. Chart of Regressed Benzene Data.

7) Calculate zero and first order rates of benzene degradation and CO2 production using the regressed data and the given equations. Assume that the concentration of CO2 is 1.00e-12 at time t=0. Calculate k zero order, k" first order, t1/2, the half-life of benzene, and 2t, the doubling time for CO2 production.
The zero order rate for benzene degradation can be represented by:

$$k=(Bo-Bf)/(Tf-Ti) \quad [1]$$

The first order degradation rate is modeled by:

$$-dB/dt = k"B \quad [2]$$

where k" is the rate constant for benzene degradation.
Integration by separation of variables and solving for k" yields:

$$k"=\ln(Bf/Bo)/(Tf-Ti) \quad [3]$$

Since CO2 production is dependent on benzene degradation, we write:

$$dCO2/dt=g(-dB/dt) \quad [4]$$

where g is the multiplication factor that relates the rate of benzene degradation to the rate of CO2 evolution.
Substituting k"B for -dB/dt, in eq.[4], we get:

$$dCO2/dt=gk"B \quad [5]$$

Integrating eq.[2] results in:

$$B=Boe^{-k"t} \quad [6]$$

where Bo is the initial benzene concentration and B is the benzene concentration at time t.
Substituting eq.[6] into eq.[5] results in:

$$dCO2/dt=gk"Bo(e^{-k"t})dt \quad [7]$$

Integration of eq.[7] yields:

$$CO2=g*Bo*(1-(e^{(-k"t)})) \text{ evaluated from } t=0 \text{ to } t=t \quad [8]$$

8) Calculate benzene zero and first order rates of degradation:

As Benzene/ml time(T)	Bf	Bo	Bo-Bf	zero order k k=(Bo-Bf)/(	first order k" k"=ln(Bf/Bo)/Tf-To
0	0.00E+00	0.00E+00	0.00E+00	5.29E-04	6.18E-02
6	7.07E-03	1.02E-02	3.17E-03	5.29E-04	6.18E-02
12	1.41E-02	2.05E-02	6.35E-03	5.29E-04	6.18E-02
18	2.12E-02	3.07E-02	9.52E-03	5.29E-04	6.18E-02
24	2.83E-02	4.10E-02	1.27E-02	5.29E-04	6.18E-02
30	3.54E-02	5.12E-02	1.59E-02	5.29E-04	6.18E-02
36	4.24E-02	6.15E-02	1.90E-02	5.29E-04	6.18E-02
42	4.95E-02	7.17E-02	2.22E-02	5.29E-04	6.18E-02
48	5.66E-02	8.19E-02	2.54E-02	5.29E-04	6.18E-02
54	6.36E-02	9.22E-02	2.86E-02	5.29E-04	6.18E-02

Calculate benzene concentration over the first 6 day period:

time(days)	[Ben] 0 order	[Ben] 1st order
0	1.02E-02	1.02E-02
1	9.71E-03	9.63E-03
2	9.19E-03	9.05E-03
3	8.66E-03	8.51E-03
4	8.13E-03	8.00E-03
5	7.60E-03	7.52E-03
6	7.07E-03	7.07E-03

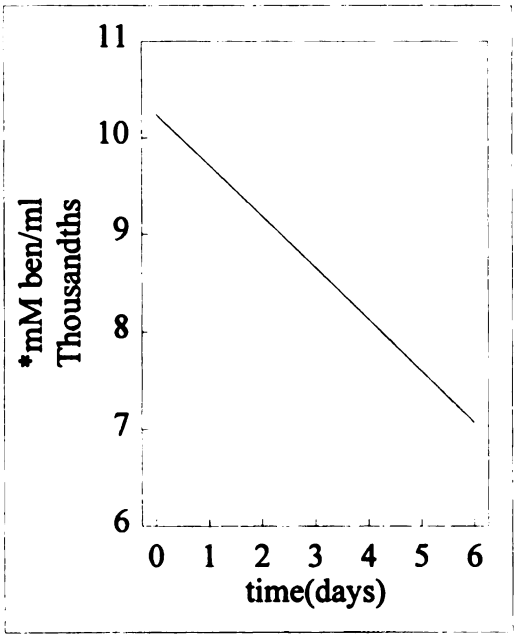

Figure 7. Zero Order Benzene. Degradation.

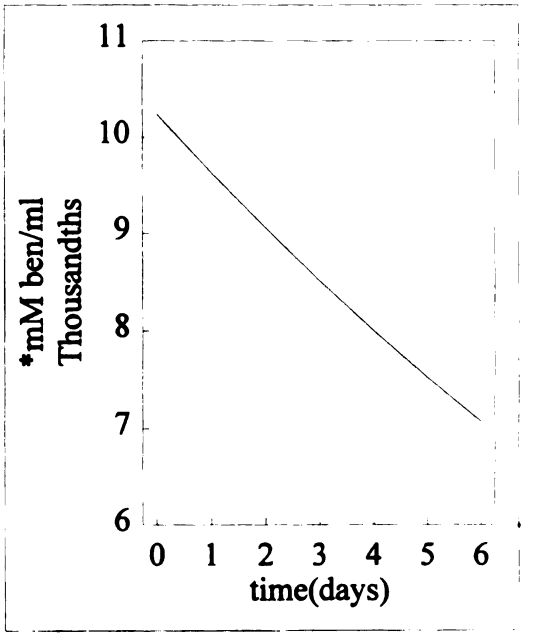

Figure 8. First Order Benzene Degradation.

* millimoles of benzene

9) Calculate zero and first order rates of CO2 production:
Solving eq. [8] for g yields:

g=(CO2f/(BoC*(1-e^(-1*k"t)))

14CO2				kCO2 o orde benzene as carbon		
		14CO2		Boc		
time(Ti)	time(Tf)	CO2f	CO2o	CO2f-CO2o/Tf-To		k"CO2
0	6	0.00E+00	0.00E+00	4.49E-04	0.00E+00	1.95E-01
6	12	2.69E-03	1.00E-12	4.49E-04	9.46E-03	1.95E-01
12	18	5.38E-03	1.00E-12	4.49E-04	1.89E-02	1.95E-01
18	24	8.07E-03	1.00E-12	4.49E-04	2.84E-02	1.95E-01
24	30	1.08E-02	1.00E-12	4.49E-04	3.78E-02	1.95E-01
30	36	1.35E-02	1.00E-12	4.49E-04	4.73E-02	1.95E-01
36	42	1.61E-02	1.00E-12	4.49E-04	5.67E-02	1.95E-01
42	48	1.88E-02	1.00E-12	4.49E-04	6.62E-02	1.95E-01
48	54	2.15E-02	1.00E-12	4.49E-04	7.56E-02	1.95E-01
54		2.42E-02	1.00E-12	4.49E-04	8.51E-02	1.95E-01

Calculate Concentration of CO2 for the first 6 day period:

day	CO2 0 order	g*Bo	1-(exp(-kt))	Cf Calc.
0	1.00E-12	0.00E+00	0.00E+00	0.00E+00
1	4.49E-04	9.41E-03	5.99E-02	5.64E-04
2	8.97E-04	9.41E-03	1.16E-01	1.09E-03
3	1.35E-03	9.41E-03	1.69E-01	1.59E-03
4	1.79E-03	9.41E-03	2.19E-01	2.06E-03
5	2.24E-03	9.41E-03	2.66E-01	2.50E-03
6	2.69E-03	9.41E-03	3.10E-01	2.92E-03

time(Ti)	g
0	9.19E-01
6	9.19E-01
12	9.19E-01
18	9.19E-01
24	9.19E-01
30	9.19E-01
36	9.19E-01
42	9.19E-01
48	9.19E-01
54	9.19E-01

Limit of CO2 production from given benzene concentration :[CO2]=g*Bo

10) Calculations generate the following charts:

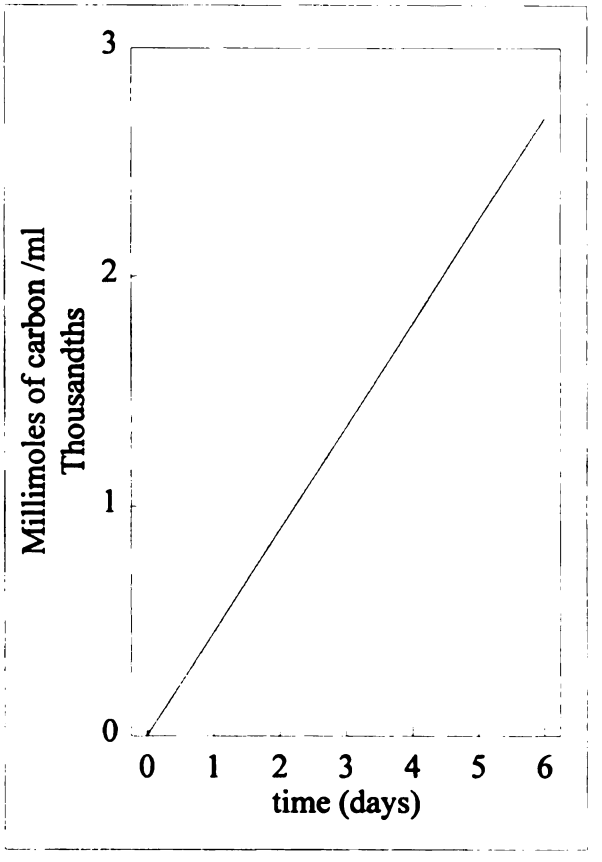

Figure 9. Zero Order CO2 Production.

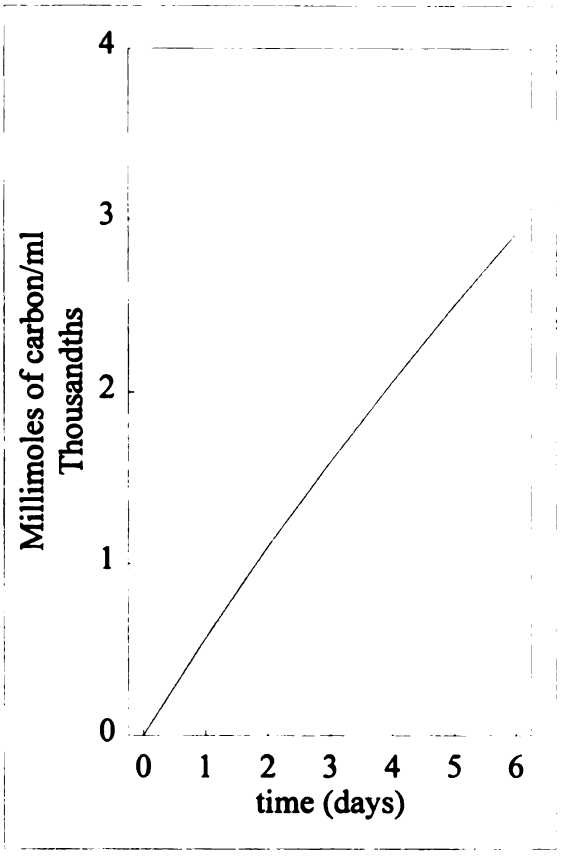

Figure 10. First Order CO2 Production

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List of References

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