Characterization of redox conditions in a petroleum contaminated aquifer: Implications for bioremediation potential

by

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Abstract

Currently, the application of bioremediation requires extensive and costly monitoring due to limited understanding of the terminal electron accepting processes (TEAPs) that control biodegradation, which impairs the accurate quantification of contaminant mass loss. The measurement of redox conditions and evaluation of TEAPs are critical in assessing the capacity for bioremediation at any site. A series of batch microcosm experiments, using sediment collected from a gasoline-contaminated aquifer at Fort McCoy, Wisconsin, was designed to: 1) evaluate the role of Fe(III) in the development of TEAPs during biodegradation of benzene, toluene, ethylbenzene, and the xylenes (BTEX); 2) examine the biodegradation potential in different portions of the plume; and 3) compare methods of TEAP characterization. In general, the presence of Fe-oxides in microcosms inhibited methanogenesis. Although Fe-reducers did not actively degrade BTEX, Fe-reduction did occur, and most probable number (MPN) counts showed that added Fe(III) increased numbers of Fe-reducers in the microcosms. Methane production in microcosms constructed from sediment near the source area was ~5 times lower than levels produced by the mid-plume sediment. No Fe-reduction occurred in microcosms containing sediment from the source area. These results suggest that the source area has much lower biological activity than the mid-plume. TEAP characterization was conducted using a variety of methods, including geochemical indicators, redox dyes, MPN, and hydrogen concentrations. Monitoring of CH_4

concentration yielded useful information in delineation of redox processes; Fe(II) monitoring was unreliable as a geochemical indicator. Redox dyes supplied basic information on reducing environments. MPN counts estimated microbial populations in lieu of faulty geochemical indicators, i.e., Fe(II). The measurement of H_2 proved to be one of the more simple and reliable methods for TEAP identification. Results of this study indicate that TEAP characterization should include use of multiple methods; relying on geochemical indicators alone is not sufficient.

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Introduction

The undesired release of petroleum products via leaking underground storage tanks is a widespread and costly problem. In 1993, EPA estimated that approximately 300,000 sites in the U.S. are contaminated in this manner (EPA, 1993). Of particular concern are the BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds because of their toxicity and relative high water solubility, which makes them very mobile in groundwater. Intrinsic bioremediation, the use of naturally occurring microorganisms to degrade contaminants, has been identified as an innovative and cost efficient strategy in cleaning up petroleum contaminated sites. As a result, there has been increasing interest in application of bioremediation for *in situ* treatment of contaminated groundwater (EPA, 1999). Currently, the application of bioremediation requires extensive and costly monitoring due to limited understanding of the oxidation-reduction reactions that control biodegradation, which impairs the accurate quantification of mass loss.

BTEX compounds are degraded via a variety of microbially-mediated oxidation-reduction (redox) reactions. Redox reactions couple the oxidation of an electron donor (e.g. BTEX) and reduction of an electron acceptor (e.g. oxygen). Oxygen, nitrate, manganese, iron, sulfate, and carbon dioxide can all be used as electron acceptors for microbially-mediated reactions involving BTEX. Microbes gain the most energy by using oxygen as the electron acceptor and obtain the least energy by using carbon dioxide as the electron acceptor (methanogenesis). Theoretically, these terminal electron-accepting processes (TEAPs) are thought to proceed in a sequential manner with respect to the electron acceptor, beginning with the reaction that yields the most energy to the bacteria and ending with the reaction that yields the least amount of energy.

Table 1 shows the amount of free energy microorganisms would gain during the coupling of benzene oxidation with reduction of NO_3, Fe(III), SO_4, and CO_2. Based on these free energy calculations, if nitrate is present, the nitrate-reducers have a competitive advantage over the Fe(III)-reducers, sulfate-reducers, and methanogens. However, if nitrate concentrations decrease and Fe(III) is available, conditions will favor the Fe(III)-reducers. This process continues in such a manner until all electron acceptors except for carbon dioxide are depleted. This sequential utilization of electron acceptors creates the classic spatial arrangement of TEAP zones shown in Figure 1.

Table 1. Anaerobic Benzene Oxidation/Reduction Reactions and Free Energy Produced. (Modified from Wiedemeier et al., 1995)

Benzene oxidation/denitrification	
$6NO^-_3 + 6H^+ + C_6H_6 = 6CO_{2,g} + 6H_2O + 3N_2$	ΔG°_r= -775.75 kcal/mole
Benzene oxidation/iron reduction	
$60H^+ + 30Fe(OH)_{3,a} + C_6H_6 = 6CO_2 + 30Fe^{2+} + 78H_2O$	ΔG°_r= -560.10 kcal/mole
Benzene oxidation/sulfate reduction	
$7.5H^+ + 3.75SO_2^{4-} + C_6H_6 = 6CO_{2,g} + 3.75H_2S^\circ + 3H_2O$	ΔG°_r= -122.93 kcal/mole
Benzene oxidation/methanogenesis	
$4.5H_2O + C_6H_6 = 2.25CO_{2,g} + 3.75CH_4$	ΔG°_r= -32.40 kcal/mole

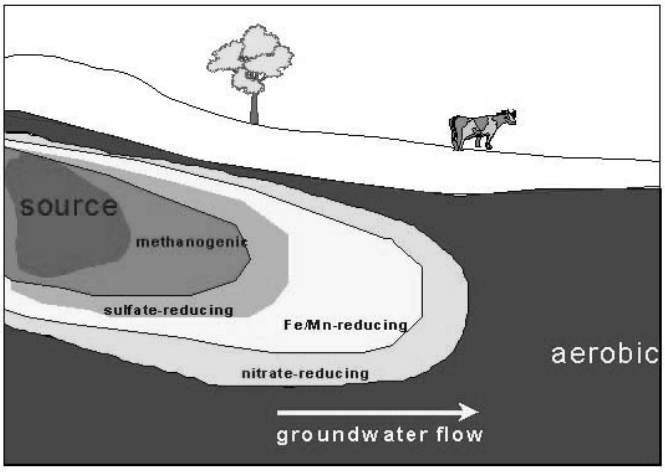

Figure 1. Classic representation of spatial delineation in redox zones in relation to a contaminant source.

Studies have shown that TEAPs exert a strong control over biodegradation rates (e.g., Christensen et al., 2000, Chapelle and Bradley, 1998, Borden et al., 1995). Thus, the measurement of redox conditions and evaluation of TEAPs are critical in assessing the capacity for bioremediation at any site. A wide range of methods have been applied in the delineation of TEAPs, including measurement of: 1) redox state; 2) dissolved (DO, NO_3, SO_4) or solid-phase (Fe(III)) electron acceptors; 3) redox byproducts (Fe(II), CH_4); 4) microbial population counts; and 5) hydrogen concentrations. However, no standardized or generalized approach exists (Christensen et al., 2000).

Redox state is often assessed through measurement of E_h or use of redox dyes. E_h is a general measure of the ratio of reduced/oxidized ions in a solution. Measurement of E_h can easily be taken from groundwaters with a platinum electrode (Chapelle et al., 1996). However, several problems arise when using E_h measurements to characterize redox conditions in contaminant plumes. Electron tower theory only indicates that the reaction is possible at a given redox potential from a thermodynamic point of view, not that the reaction will actually occur (Christensen et al., 2000). It has been found that electrode-measured E_h values do not agree with E_h values calculated from measured concentrations of redox couples (Lindberg and Runnells, 1984). Microbially-mediated redox reactions in contaminant plumes involve organic matter, and the actual pathways may be much more complex than suggested by the half-reactions (Chapelle et al., 1996). Also, contaminant plumes are unlikely to be in internal equilibrium (Christensen et al., 2000). Despite its problems, E_h measurements do provide valuable information on strongly reducing portions of contaminant plumes, or areas of a plume where one TEAP

is dominant (Chapelle et al., 1996, Christensen et al., 2000). In general, redox dyes are colored in oxidized form and colorless in reduced form (Jacob, 1970) (Table 2). Each redox dye has a known potential at which it is reduced, allowing the estimation of redox potential when water containing the dye is colorless. These dyes have been used to evaluate changes in redox potential caused by a methanogenic population during reductive dechlorination of pentachlorophenol and degradation of acetate (Stuart et al., 1999). Redox dyes are easy to prepare, and the analysis is quick and simple because the color change can be seen with the naked eye.

Table 2. Redox potentials for redox dyes (Jacob, 1970) and selected redox pairs (Madigan, 2000).

Redox dye	Eh (mV) at which dye is reduced (turns colorless)		Selected redox pairs	Standard redox potentials (mV) for redox pairs
			Aerobic respiration (O_2/H_2O)	+820
			Nitrate Reduction (NO_3/N_2)	+430
Thionin	+66		Fe-Reduction ($Fe(OH_3)/Fe(II)$)	+50
Cresyl Violet	-75			
Resazurin	-110			
			Sulfate-reduction (SO_4/HS)	-210
Phenosafranin	-244		Methanogenesis (CO_2/CH_4)	-250

Evaluation of electron acceptor concentrations within a plume allow for delineation of redox zones when compared to electron acceptor concentrations outside of the plume boundaries (Borden et al., 1995). The concentration of nitrate, for instance, will be lower in areas of the plume where nitrate reduction is occurring than it is in pristine aquifer conditions. However, previous studies have shown that naturally

occurring organic matter and other reduced compounds can also act as electron donors in contaminant plumes (e.g., Schreiber and Bahr, 1999), thus limiting the applicability of using electron acceptor concentrations for delineation of TEAPs.

Byproducts of electron-accepting processes can also help in identifying redox zones, where production with respect to pristine aquifer conditions indicates utilization (Borden et al., 1995). However, patterns of byproduct concentrations can complicate TEAP delineation. For example, Fe(II), the byproduct of Fe(III) reduction, and CH_4, the byproduct of methanogenesis, can be transported with groundwater, creating an apparent "overlap" of redox zones (Albrechtsen et al., 1995). In addition, vertical variations in TEAPs can also attribute to erroneous delineation when data collected from standard monitoring wells is vertically averaged (Borden et al., 1997, Schreiber and Bahr, 1999). Thus, redox byproducts must be used in combination with other methods for TEAP delineation.

Most probable number (MPN) counts aid in identifying and enumerating microbial populations responsible for the redox processes occurring in different portions of the plume. Microorganisms are quantified by growth on synthetic media, and population density can be calculated from the positive and negative results in a series of dilutions, each dilution consisting of a set of replicate tubes (Bekins et al., 1999). MPN counts are a useful tool, but the presence of culturable organisms of a given physiologic type does not prove that the associated process was indeed active in the aquifer (Bekins et al., 1999). The process also relies on the assumption that the indigenous population is culturable in the synthetic media and that a representative number of microorganisms can be removed from the sediment.

Hydrogen concentrations can also be used as an indicator of the predominant TEAP reaction in aquifer sediment. Hydrogen is a key intermediate for the electron flow involving reduction of organic matter by microorganisms (Lovley and Goodwin, 1988). In theory, the hydrogen concentration is constant and controlled by parameters related to the physiology of the mediating bacteria (Christensen et al., 2000). Each TEAP therefore is related to a unique hydrogen concentration (Table 3). However, hydrogen concentrations alone cannot be used to delineate TEAPs, as recent studies have shown that a given hydrogen concentration does not necessarily indicate a given redox process (Hoehler et al., 1998, Jakobsen et al., 1998), but reflects the partial equilibrium of redox processes in the plume.

Table 3. Characteristic hydrogen concentrations associated with TEAPs (Lovley and Goodwin, 1988)

TEAP	H_2 concentration
Nitrate or Manganese Reduction	<0.05 nM
Iron Reduction	0.2 nM
Sulfate Reduction	1-1.5 nM
Methanogenesis	7-10 nM

In addition to problems arising from the various methods in identifying redox zonation, several studies have identified instances where TEAPs do not proceed in the classical sequential manner. The heterogeneity in Fe(III) concentrations and bioavailability of Fe(III) minerals allowed sulfate reduction to occur in the presence of Fe(III) in a landfill plume in Grinsted, Denmark (Jakobsen et al., 1998). Simultaneous utilization of more than one electron acceptor has been proposed in several studies to aid in modeling biodegradation at the site (Essaid et al., 1995). Lovley and Goodwin (1988) proposed that while an electron donor (e.g. H_2) may dominantly control TEAPs, more

than one TEAP could occur if hydrogen is not limiting. Concomitant utilization of electron acceptors was also observed in the transition between electron accepting processes (Lovley and Goodwin, 1988). When availability of the more energetically favorable reaction is limited, more than one process can occur.

Drawbacks in identifying the predominant TEAP and the realization that more than one TEAP can occur simultaneously impede the modeling and design of appropriate remediation plans. The main objective of this thesis was to use microcosm experiments to evaluate the geochemical and biological controls on TEAP utilization in petroleum-contaminated aquifers . More specific goals were to: 1) examine the role of Fe(III) heterogeneity in the development of redox conditions, 2) compare bioremediation potential of different portions of the plume, and 3) evaluate different experimental techniques for TEAP characterization. To examine the impact of Fe(III) heterogeneity on TEAP utilization, a series of microcosm experiments were constructed in which Fe(III) heterogeneity was simulated using mixed vs. layered arrangements of Fe(III)-coated beads and/or sediment. Comparison of bioremediation potential of the aquifer was also examined through microcosm experiments, in which sediment from different portions of the plume was used. Evaluation of TEAP characterization techniques was accomplished by comparing a variety of methods, including redox dyes, concentrations of electron acceptors and redox byproducts, MPN, and hydrogen concentrations to track TEAPs in the microcosms.

Methods

A series of batch microcosm experiments, using sediment collected from a gasoline-contaminated aquifer at Fort McCoy, Wisconsin (Fig. 2), was designed to address the research objectives. The experiments were specifically designed to: 1) evaluate the role of Fe(III) in the development of TEAPs during BTEX biodegradation; 2) examine the biodegradation potential in different portions of the plume; and 3) compare methods of TEAP characterization.

Sediment was collected at the site and transported back to the laboratory to create microcosms, glass serum vials containing sediment, synthetic Fe(III)-oxides, and simulated anaerobic groundwater. Simulated groundwater contains vitamins and nutrients needed by the bacteria to ensure a proper environment for growth. A reducing agent, cysteine, was also used in conjunction with a colorimetric redox indicator in some trials to ensure that the simulated groundwater was anaerobic. The microcosms were sampled for electron donors, electron acceptors, byproducts of electron accepting processes, and hydrogen in order to characterize the TEAPs occurring in each microcosm. Redox dyes were added to selected microcosms to evaluate the overall redox potential. In addition, sediment from selected microcosms was used for MPN analysis.

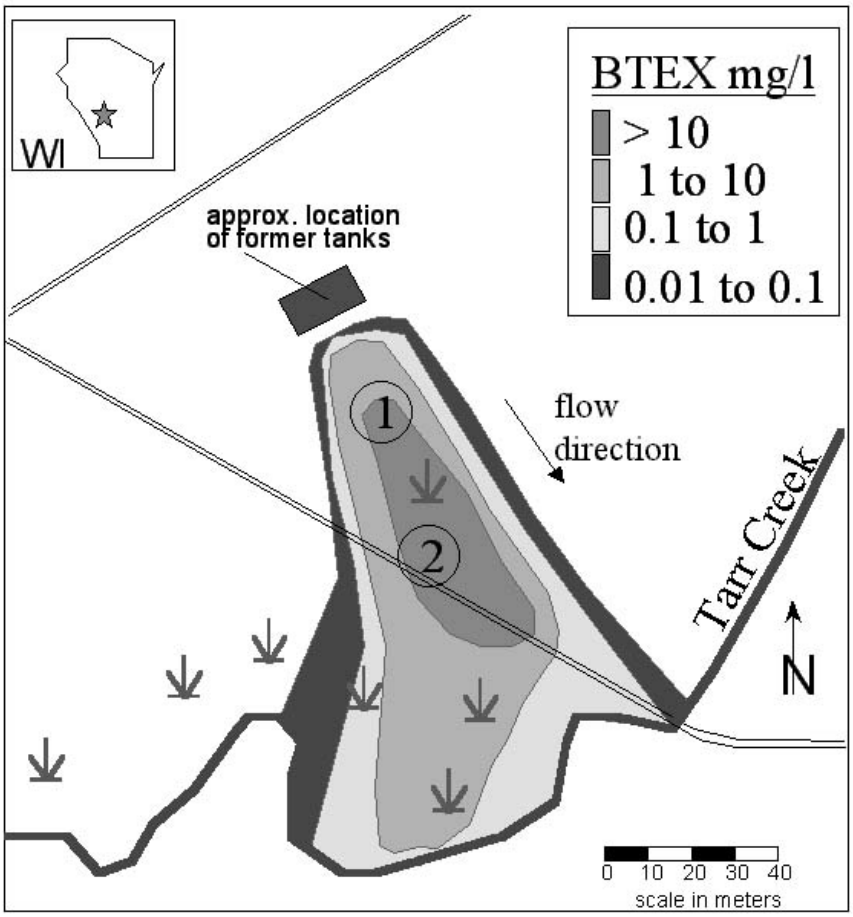

Figure 2. Site map from Ft. McCoy, WI, with sediment sampling locations noted 1: source area; 2: mid-plume.

Field sample collection

Sediment. The Ft. McCoy site (Fig. 2) was previously a Petroleum, Oils, and Lubricants (POL) station, and is contaminated with BTEX from two 12,000-gallon leaking underground storage tanks (USTs) that were installed in 1943 and removed in 1989. Dissolved BTEX concentrations are generally less than 20 mg/L (Schreiber, 1999). The upgradient portion of the plume is located in a grass-covered wetland and the downgradient section of the plume migrates through a forested wetland and ultimately

discharges to Tarr Creek, a shallow stream (Schreiber and Bahr, 1999). Sediment cores were collected at selected multilevel well sampling locations according to concentrations of geochemical indicators and BTEX.

The vertical intervals selected for collection of sediment contained groundwater with depleted oxygen and nitrate concentrations and elevated total dissolved Fe, methane, and BTEX concentrations (Fig 3). The sediment was collected aseptically at three locations in 3 ft. acetate liners using a Geoprobe. The acetate cores were capped immediately after retrieval from the core barrel, wrapped in foil, refrigerated, and transported to the laboratory.

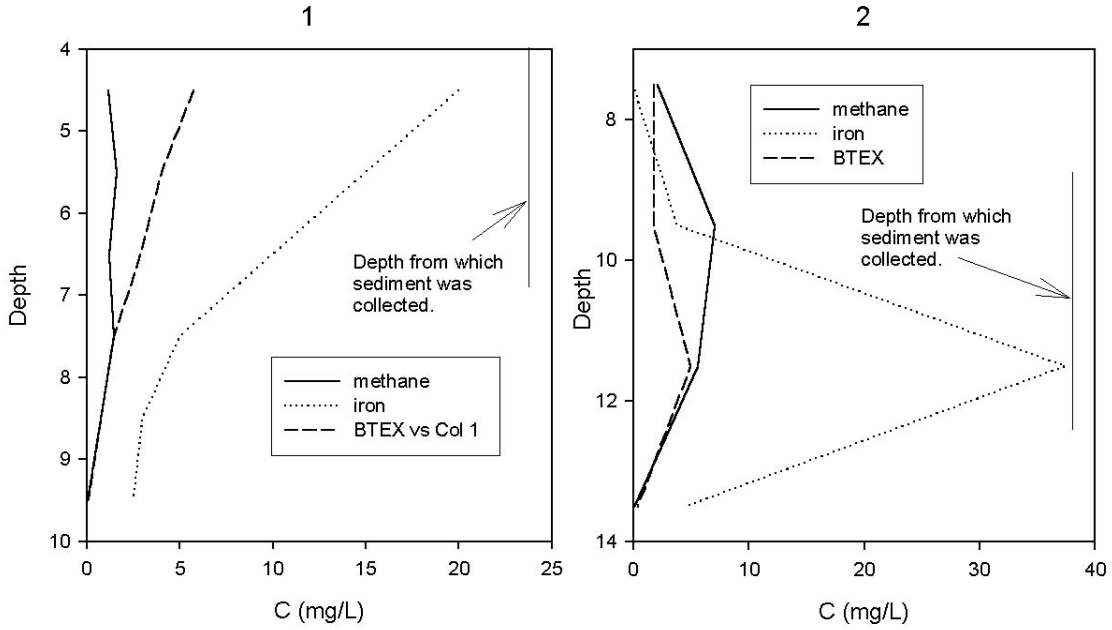

Figure 3. Plots exhibiting mg/L concentrations of geochemical indicators and BTEX vs. depth (feet below ground surface) for sediment sampling locations 1 and 2 (see Figure 2).

Groundwater. Prior to sampling, we purged each level of the multilevel samplers with a peristaltic pump at a rate of approx. 100 ml/min for 4-6 min. to allow for temperature and

specific conductance to stabilize. BTEX samples were collected under low-flow (<10 ml/min) conditions into pre-acidified (HCl) 40 ml VOC vials. Samples for methane analysis were collected in anaerobic, sterilized 10 ml serum vials. Multilevels were then sampled for field analysis of DO, nitrate and Fe(II) using colorimetric methods (Chemetrics, Inc).

Field analytical methods

In addition to the field colorimetric analysis, total iron and Fe(II) were measured using the ferrozine method (Stookey 1970). A 200 µL aliquot was taken from each sample and added to a cuvette, and 1.5 mL of ferrozine was then added to each cuvette. To analyze Fe(II), the 562 nm absorbance was measured for each sample using a spectrophotometer. Total dissolved Fe was analyzed after adding 150 µL of 5N hydroxylamine hydrochloric acid to the sample, waiting 5 minutes, and measuring the 562 nm absorbance. BTEX and CH_4 were measured on an SRI gas chromatograph (GC) equipped with a flame ionization detector (FID). Headspace samples (500 µL) were injected on a 30m long, 0.53mm ID, 5.0 µm df, MXT-1 (crossbond 100% dimethyl polysiloxane) column with a temperature program that began at 40˚ C and ramped 20˚ C/min until the temperature was 140˚ C. The temperature was then held at 140˚ C for 5 minutes. External standards were used to calculate the BTEX concentrations. Methane concentrations were calculated using the Henry's Law method, where $C_{L,f} = C_{g,f}/K_{H'}$, where $C_{L,f}$ = final concentration in the liquid, $C_{g,f}$ = final gaseous concentration, and $K_{H'}$ = 30.33, the dimensionless Henry's Law constant. Using this headspace method, the detection limit is estimated to be 25 ppb for BTEX and 2 ppb for CH_4.

Microcosm construction

Sediment. Once in the laboratory, the sediment cores were transferred to a Coy© anaerobic chamber which contained an atmosphere of ~99% N_2 and 1% H_2. Only sediment from the interior of the cores was used for microcosm construction. The sediment was homogenized in a stainless steel bowl and allowed to sit within the chamber for 7 days to facilitate the diffusion and volatilization of BTEX

Media. After homogenizing and sieving, sediment (45 g) was placed in 150 mL serum vials along with simulated groundwater, trace salts, resazurin (a redox indicator; Trials 2,3), and cysteine (reducing agent; Trials 2,3). Sediment for abiotic controls was autoclaved (120˚C) for 1 hour for 3 subsequent days prior to addition. One liter of simulated groundwater contained 38.21 mg NH_4Cl, 43.02 mg KH_2PO_4, and 2.5 g $NaHCO_3$ in deionized (DI) water. Media for the killed controls also contained 1 g of Na-azide. The trace salts stock solution consisted of 30 mg $CoCl_2*6H_2O$, 0.15 mg $CuCl_2$, 5.7 mg H_3BO_3, 20 mg $MoCl_2*4H_2O$, 2.5 mg $Na_2MoO_4*2H_2O$, 1.5 mg $NiCl_2*2H_2O$, and 2.1 mg $ZnCl_2$. 15 mL of trace salts were added to 1 L of simulated groundwater. The resazurin stock solution was prepared by adding 100 mg of resazurin salt to 100 mL of deionized (DI) water, and 0.1 mL of stock solution was added per 100 mL of medium. Stock solutions of redox dyes, thionin, cresyl violet, and phenosafranin, were prepared by adding 50 mg of the respective salt to 100 mL of DI. Seventy-five mL of the solution was then transferred to each microcosm, creating a 2 mM concentration. The cysteine stock solution was prepared by boiling 100 mL of DI and then bubbling the water with N_2

as it cooled; 2.5 g of cysteine was then added. The cysteine solution was then autoclaved (120˚C) and neutralized to a pH of 7 with $NaHCO_3$ (Ljungdahl and Wiegel, 1986). The microcosms ultimately contained 45 g of sand, 75 mL of the media, salts, and resazurin solution, and 1.5 mL cysteine solution. The microcosms that contained BTX were spiked with 3 mL of a 250 mg/L BTX stock solution to create a final concentration of 1 mg/L in each microcosm.

Due to problems with using cysteine as a reducing agent, a method for creating anaerobic water without using a reducing agent was developed during Trial 3. Deionized water with resazurin was first autoclaved (120˚C) for 70 minutes and then bubbled with N_2 and aspirated simultaneously until the water was cool to the touch. The water was then autoclaved for an additional 70 minutes resulting in a clear color that indicated the water was anaerobic. The experimental matrix for the microcosm experiments is presented in Table 4.

Table 4. Experimental matrix for microcosm experiments

Trial 1	Mid-plume sediment (Site #2;Fig. 2)				
treatment	BTX	Fe(III) source (ferrihydrite on glass beads)	Fe(III) mixed/layered	killed	reducing agent
white	no	no	n/a	no	no
pink	yes	no	n/a	no	no
green	yes	yes	layered	no	no
blue	yes	yes	mixed	no	no
yellow	yes	yes	layered	no	no
orange	yes	yes	mixed	no	no
red	yes	yes	layered	yes	no

Trial 2	Sediment from Trial 1 used				
treatment	BTX	Fe(III) source (ferrihydrite on quartz sand)	Fe(III) mixed/layered	killed	reducing agent

white	no	no	n/a	no	yes
pink	yes	no	n/a	no	yes
green	yes	yes	layered	no	yes
blue	yes	yes	mixed	no	yes
yellow	yes	yes	layered	no	yes
orange	yes	yes	mixed	no	yes
red	yes	yes	layered	yes	yes

Trial 3	Source area sediment (site 1;Fig. 2)				
treatment	BTX	Fe(III) source (ferrihydrite on quartz sand)	Fe(III) mixed	killed	reducing agent
yellow	no	no	n/a	no	yes
pink	yes	no	n/a	no	yes
orange	yes	yes	yes	no	yes
red	yes	yes	yes	yes	yes

Table 4. Experimental matrix for microcosm experiments. Trial 1 used sediment collected from the mid-plume. Glass beads coated with Fe-oxides used in Trial 1 in mixed and layered fashion. Trial 2 used the same sediment as Trial 1 but quartz sand grains are coated with Fe-oxides and arranged in a mixed/layered arrangement. Trial 3 contains sediment from the source area and all ferrihydrite coated sand grains are mixed thoroughly throughout the sediment.

Fe-oxides. Several types of Fe(III) were added to the microcosms: 1) ferrihydrite-coated glass beads, 2) ferrihydrite-coated sand, and 3) pure goethite. Borosilicate glass beads were coated with amorphous Fe(III)-oxides for the first trial using the method of Grantham and Dove (1996). The 1mm diameter glass beads were added to 125 mL of DI, 1.7375 g $FeSO_4$, and 13.75 mL $NaHCO_3$ in an Erlenmeyer flask after being etched overnight in 5 M NaOH. Five drops of H_2O_2 were then added to oxidize Fe(II), and the solution was mixed thoroughly. The beads were allowed to dry in an oven at 40°C and were rinsed thoroughly prior to use (Grantham and Dove, 1996).

Quartz sand grains coated with ferrihydrite were added to some Trials 2, and 3 as a source of Fe(III) to bacteria using the method of Grafe (2000). The sand was treated with HCl and dried prior to coating with ferrihydrite. One hundred grams of sand, 8.08 g

of $Fe(NO_3)_3*9H_2O$, and 50 mL of DI were combined in a 250 mL ceramic evaporation dish and mixed thoroughly. Subsequently, 12 mL of 5 M NaOH was added and mixed well, and the pH was then adjusted to 7.5 with 5M NaOH or 6M HCl. The mixture was allowed to dry in an oven at 40° C overnight (Grafe, 2000). After drying, the sand was rinsed with DI and placed back in the drying oven. This process was continued until the eluent from rinsing was clear. Microcosms requiring amendment with Fe(III) received five grams of ferrihydrite coated sand.

Goethite (α-FeOOH) was synthesized from $Fe(NO_3)_3$ using the method by Schwertmann and Cornell (1991) and modified by Grafe (2001).

X-ray diffraction (XRD) was used to identify the iron phases used in the experiments. Because the coatings on the glass and quartz sand were very thin, it was difficult to collect enough sample for an x-ray pattern. Although the appropriate methods were used to make ferrihydrite and goethite, a definitive identification was not made.

Microcosm sampling

The microcosms were sampled continuously, calling for unique equipment in stoppering the serum bottles. Mininert© valves, which are stoppered with a Teflon bar so that that the septa can be replaced, were utilized in order to repeatedly sample the same microcosm. Microcosms were sampled approximately every seven days for BTX, CH_4, and total dissolved Fe.

Microcosm analytical methods

BTX and CH_4 concentrations were measured on the GC-FID (under the same conditions in the field), by withdrawing 500 µl of headspace from each microcosm and injecting the headspace gas into the GC. Total dissolved Fe was analyzed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES) at the Virginia Tech Soil Testing Laboratory. A 250 µl liquid sample from each microcosm was filtered through a 0.2 micron filter, preserved with HNO_3 (pH<2) and analyzed for total Fe.

Hydrogen was measured on a Trace Analytical GC equipped with a reduced gas detector (RGD). Five mL of headspace from the microcosm was injected into the GC. External standards were used. Hydrogen concentrations were calculated using the following equation (Mazur and Jones, 2002), assuming a temperature of 20˚C: H_2 (liquid,nM) = [H_2, gas, ppb] * [0.04157 moles/liter] * [0.0194 (Ostwald coefficient for H_2)]. The minimum and maximum detection limits for hydrogen were 0.1 and 10 ppm, respectively. Peak areas for samples containing more than 10 ppb H_2 were used to calculate estimated hydrogen concentrations.

Most Probable Number (MPN)

Bacterial populations of Fe(III)-reducers and methanogens were estimated using a Most Probable Number (MPN) procedure (Bekins et al., 1999). Approximately 10 g of sediment was added to an anaerobic isolation roll streak tube filled with 20 mL of mineral salts solution. Each liter of mineral salts contains 0.75 g KH_2PO_4, 0.89 g

K_2HPO_4, 0.36 g $MgCl_2*6H_2O$, 0.9 g NH_4Cl, 9 mL trace metal solution, 5 mL of vitamin solution, and 10 mg of Tween 80. The vitamin solution contains 4 mg biotin, 10 mg p-aminobenzoic acid, 4 mg folic acid, 10 mg pantothenic acid, 10 mg nicotinic acid, 0.2 mg vitamin B13, 10 mg thiamine hydrochloride, 20 mg pyridoxine hydrochloride, 10 mg thioctic acid, and 1 mg riboflavin per 100 mL DI. The trace metal solution consists of 1500 mg nitriloacetic acid, 3000 mg $MgSO_4*7H_2O$, 500 mg $MnSO_4*7H_2O$, 1000 mg NaCl, 100 mg $Co(NO_3)_2*6H_20$, 100 mg $CaCl_2$ (anhydrous), 100 mg $ZnSO_4*7H_2O$, 10 mg $CaSO_4*5H_2O$, 10 mg $AlK_2(SO_4)_3$ (anhydrous), 10 mg boric acid, and 10 mg $Na_2MoO_4*2H_2O$ per L DI. The pH was adjusted to 7 and the solution was autoclaved (120˚C), and cysteine was added. The tube was sealed and mixed well after the addition of sediment, and then allowed to stand for 2 hours to allow the penetration of Tween into the sample. Tubes were opened and sonicated with a Branson Sonifier, Model 200, with a microtip (10 W for 30 s) to dislodge bacteria from the sediment. The samples were not stored for longer than 4 hr before inoculation into growth media.

Iron-reducing media contained 2.5 g of sodium acetate*$3H_2O$, 2.5 g of $NaHCO_3$, 0.1 g of $CaCl_2*2H_2O$, 0.1 g of KCl, 1.5 g of NH_4Cl, 1 g of KH_2PO_4, 0.1 g of $MgCl_2*6H_2O$, 0.1 g of $MgSO_4*7H_2O$, 9 mL of trace metal solution, 5 mL of vitamin solution, and 200 mL of 0.5 M Fe^{3+} per liter. The pH was adjusted to 7.0, and the media was dispensed into serum vials and autoclaved (120˚C). After inoculation, the serum vials were pressurized to 140 kPa with a 70:30 mix of H_2:CO_2. The serum bottles were allowed to incubate for 6 weeks at room temperature.

Methanogenic bacteria were enumerated on mineral dilution salts media containing 2.5 g sodium formate and 2.5 g sodium acetate per liter under an atmosphere

of H_2 and CO_2 at a 70:30 ratio. Each serum vial was pressurized to 140 kPa with the H_2:CO_2 mix and inoculated for 6 weeks at room temperature.

The MPN was calculated from 6 serial dilutions, each with five replicate tubes. MPN numbers were determined using a MPN chart, which assigns a number based on the positive and negative results in each dilution series. The number was then multiplied by the dilution factor of the dilution series with the second highest number of positive tubes.

Results

Trial 1.

The objective of Trial 1 was to determine the role of spatial Fe(III) heterogeneity in controlling TEAP development. Trial 1 used sediment from site #2 (Fig. 2), in the middle portion of the plume. Microcosms contained Fe(III)-coated borosilicate glass beads in either a layered arrangement or mixed throughout the sediment.

Figure 4 shows a plot of total BTX concentrations. A slight decrease of BTX was observed in all microcosms, likely from losses due to sampling. Comparing the live trials to the abiotic trials, the only treatment that showed significant BTEX loss was the trial containing BTEX and no added iron (pink).

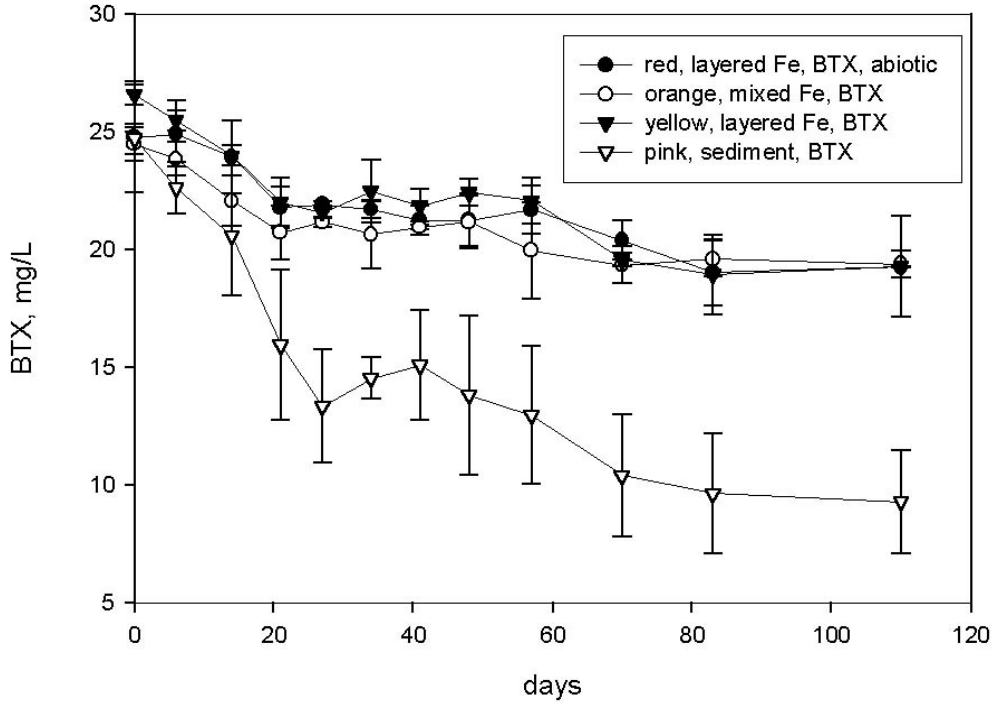

Figure 4. Average total BTX concentrations (mg/L) for the Trial 1. Error bars represent standard deviation of triplicate measurements.

Methane concentrations are shown in Figure 5. Significant methane production was measured in the trials with BTX and no added iron (pink), and the buildup was coincident with BTX loss (Figure 4), suggesting that methanogenesis coupled to BTX oxidation was active in this group. Methanogenesis was expected to be the dominant terminal electron accepting process in this group because no iron was added, and no other electron acceptors (DO, NO_3, Mn, SO_4) were present. At around 80 days, methane production decreased, and BTX concentrations stabilized. The sediment control (white) also produced some methane, indicating the presence of some natural organic matter or other reduced compound that the methanogens were able to utilize as an electron donor.

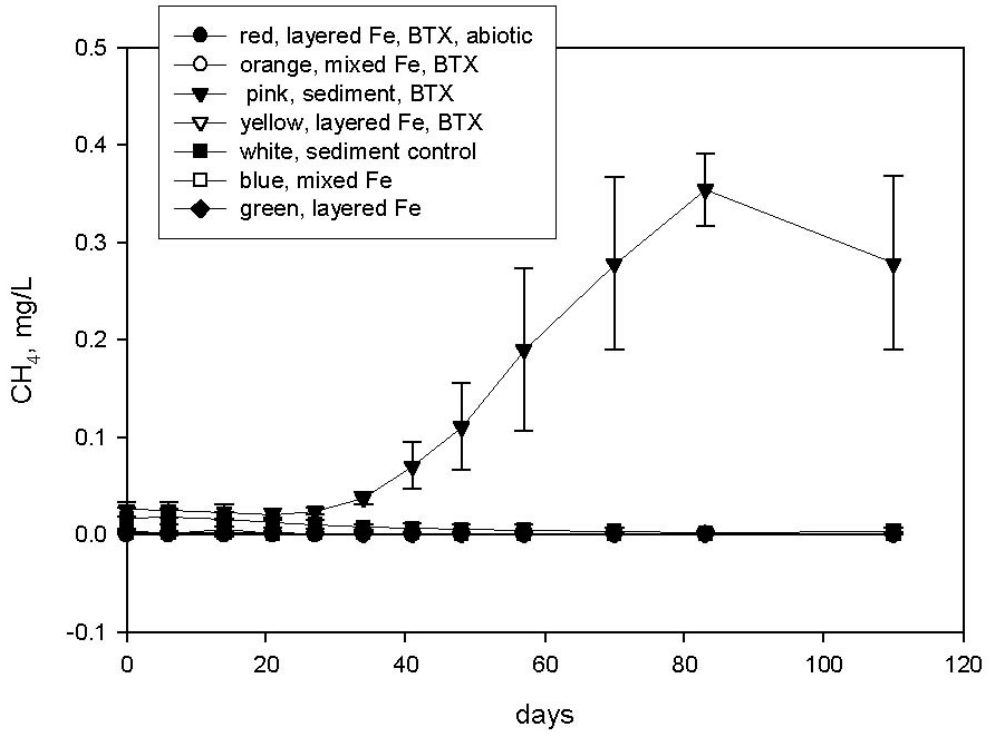

Figure 5. Average total CH_4 concentrations (mg/L) for Trial 1. Error bars represent standard deviation of triplicate measurements.

Total dissolved Fe concentrations exhibited wide fluctuations, but were elevated in three of the four groups of microcosms that contained added Fe(III) (Fig. 6). The two groups with a mixed arrangement of Fe(III)-oxide coated beads (one with BTX, one without) sustained a higher level of total dissolved Fe than the group with a layered arrangement of beads and no BTX. The microcosms that contained added Fe(III) in a layered fashion, as well as BTX, did not show a significant increase in total dissolved Fe. The abiotic control, which also contained added Fe(III), exhibited no increases in total dissolved Fe concentration. Although increases in dissolved Fe were apparent, there was no observed BTX degradation coupled to any of the increases (Fig 4). There are two possible explanations for this: 1) as with methanogenesis, some other reduced compound in the sediment is readily available as an electron donor in the microcosms while Fe was

used as the electron acceptor; or 2) iron was released to solution not from reduction but from sloughing of the Fe(III) oxide coating. Aqueous samples were filtered using a 0.2 μm membrane, but some particles less than 0.2 μm may have passed through the membrane. Total dissolved Fe concentrations in the white and pink groups remained low. Because iron was not added to these groups, the production of some dissolved Fe indicates that enough Fe(III) was present in the original sediment to support limited Fe(III) reduction.

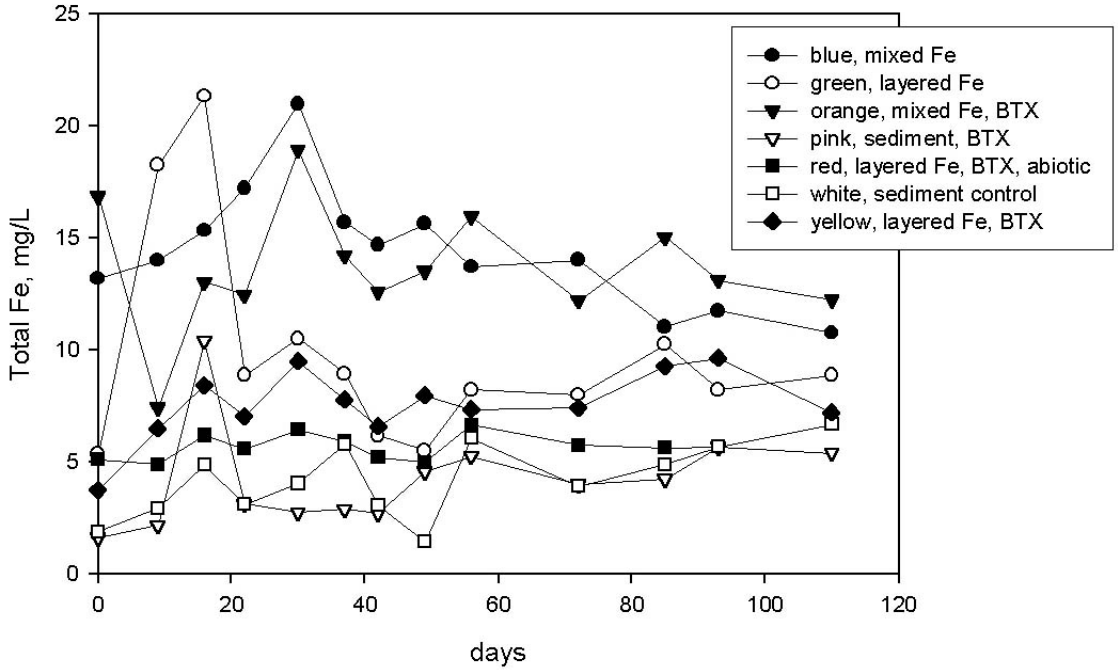

Figure 6. Average total dissolved (< 0.2 micron) Fe concentrations (mg/L) for Trial 1.

The decrease in methane production after 80 days in the BTX only trial and the lack of significant evidence for Fe(III) reduction in microcosms containing added Fe(III) raised concerns that the media may not have been properly stripped of dissolved oxygen. Because no redox indicator or reducing agent was used, it was difficult to document the redox state of the media. In addition, there was concern that boron, released from the

borosilicate beads, was interfering with Fe(III) reduction. Boron, which reached concentrations of 50 mg/L in the microcosms, acts as a weak base in solution and can potentially sorb to the sites on the Fe-oxides where the bacteria potentially attach (Leblanc, 1984).

Trial 2.

In an effort to stimulate bacterial activity by removing the borosilicate glass beads and ensure that the simulated groundwater was in fact anaerobic, the microcosms from Trial 1 were destroyed, and the sediment was retained to start a new trial. Resazurin, a colorimetric redox indicator, and cysteine, a reducing agent, were added to the media. Ferrihydrite-coated sediment was prepared using HCl-cleaned quartz grains instead of the borosilicate beads.

Similar to Trial 1, BTX concentrations in Trial 2 decreased slowly over time due to sampling losses (Fig. 7a). In comparison to the abiotic control, the only groups to show BTX losses were the pink (BTX, no added Fe) and the orange (BTX, mixed Fe) microcosms. Due to excessive BTX losses in the orange group (Fig. 7b), these microcosms were re-spiked with BTX on day 35, but significant BTX degradation did not resume after the re-spiking (Fig. 7a).

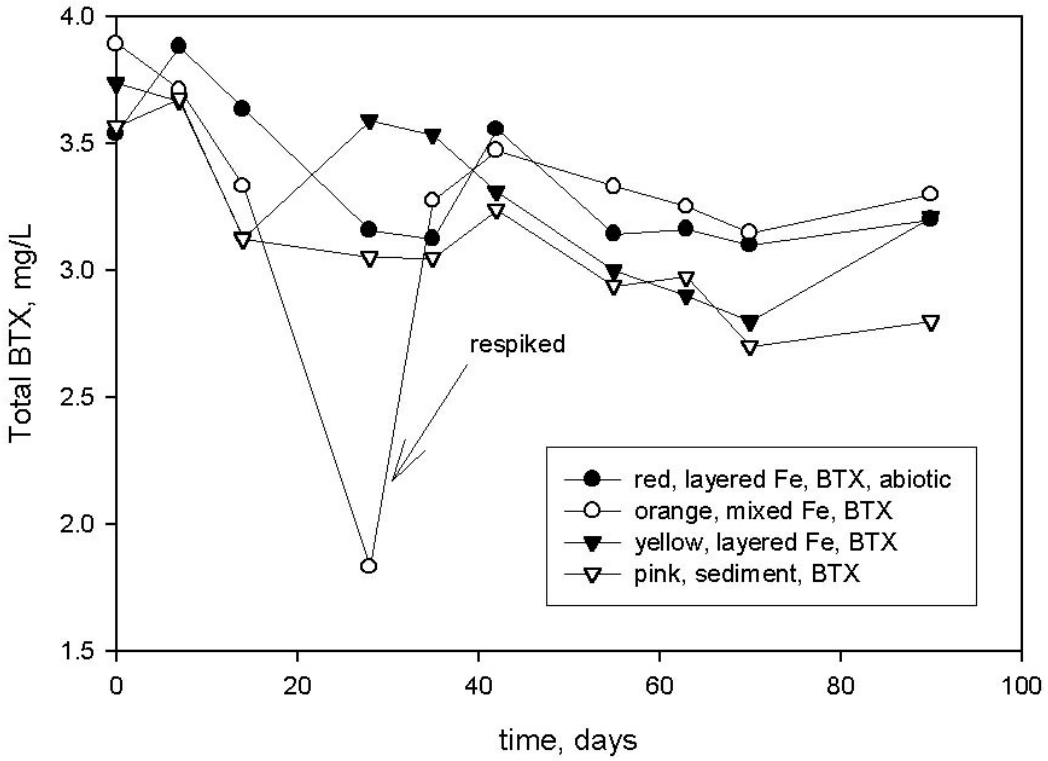

Figure 7a. Average total BTX concentrations (mg/L) in Trial 2. The orange trials were re-spiked on day 36. This trial ended on day 90.

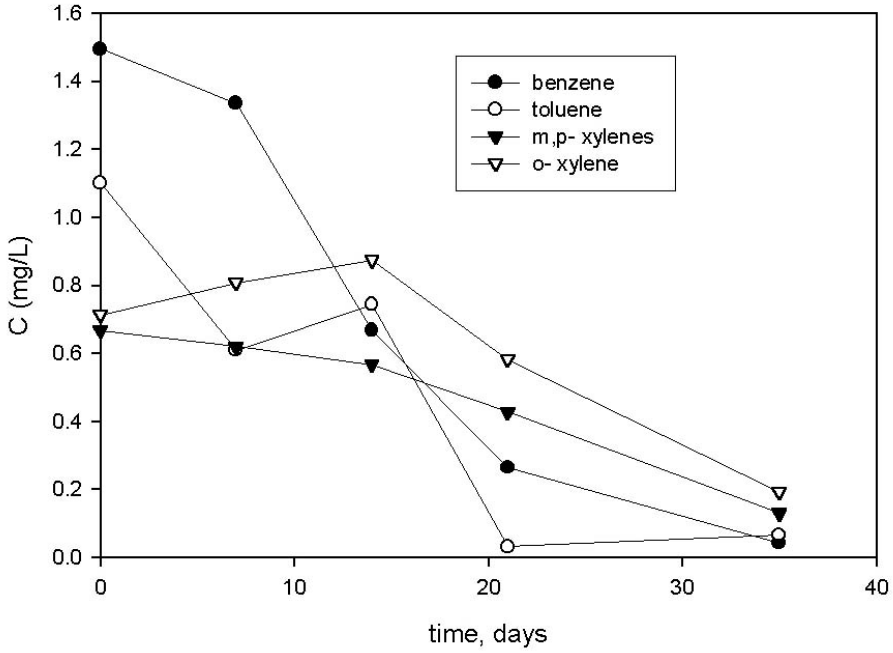

Figure 7b. Individual BTX concentrations (mg/L) in Trial 2, orange (mixed Fe+BTEX) treatments before re-spiking on day 36.

Methane production was observed in the groups of microcosms that did not contain added Fe (Fig. 8). Methane was produced within 7 days in the microcosms containing sediment and BTX. Unlike Trial 1, both groups of microcosms lacking iron produced basically the same amount of methane (Fig. 8). Similar to the results of Trial 1, however, methanogenesis started earlier, and at a faster rate, in the group that contained BTX and no iron, suggesting that the presence of BTX stimulated methanogenesis, even though the BTX compounds were not significantly degraded (Fig. 7a). Also similar to Trial 1, methanogenesis did not occur at all in microcosms containing added Fe.

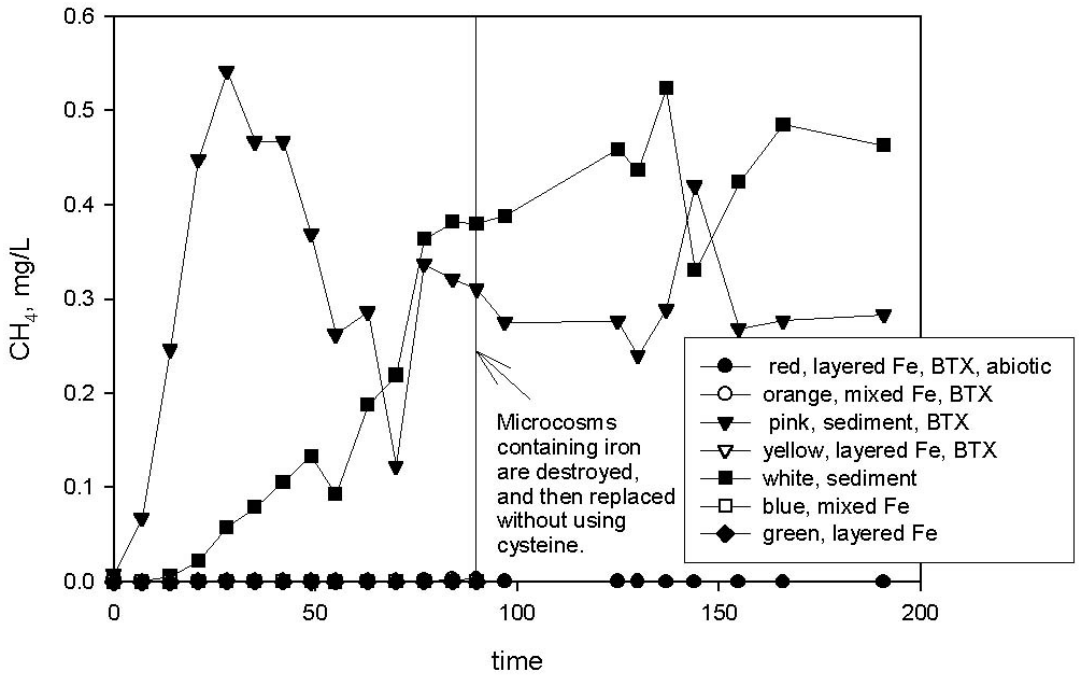

Figure 8. Average total CH_4 for Trial 2. Microcosms containing iron, with the exception of the abiotic control, were taken apart on day 90 and reconstructed using anaerobic media without cysteine and freshly precipitated iron-coated sand. The white, red, and pink groups remained unchanged; methane concentrations in these microcosms were monitored until day 191.

A significant increase in total dissolved Fe concentrations was observed for three of the four biotic groups of microcosms that contained added iron (Fig. 9). The two groups of microcosms with a mixed Fe arrangement (orange, blue) and the group with a layered Fe (green) arrangement but no BTX began producing dissolved Fe after approximately 50 days (Fig. 9). Although the concentrations are variable, analysis of variance (ANOVA) resulted in an f value of 8.1 at significance level of $p = 4.06e-07$. The large f value ($>> 1$) and low p value (<0.05) confirm that the increases in iron in the biotic trials are statistically significant.

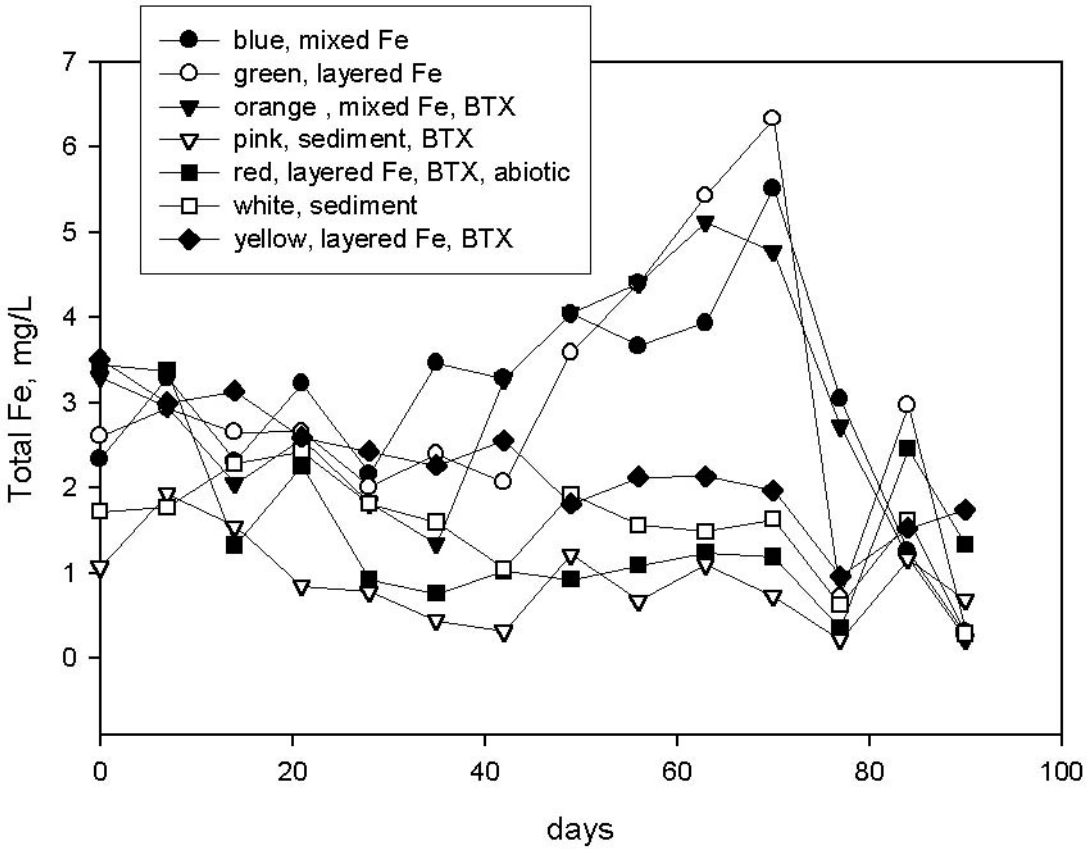

Figure 9. Average total dissolved (< 0.2 micron) Fe concentrations for Trial 2.

As shown in Figure 9, dissolved iron concentrations decreased abruptly at day 78. Coincident with this decrease, a black precipitate began to form. The black precipitate was observed to be heaviest in microcosms that contained added iron, and was accompanied by a sulfur smell that was noticed during sampling the microcosms. Analysis of the precipitate by ICP after acid digestion revealed that the compound contained 4713 mg/kg sulfur and 10,940 mg/kg iron, suggesting that the precipitate was likely FeS phase, created by H_2S, a byproduct of sulfur reduction, and the dissolved Fe(II), a byproduct of Fe(III) reduction, in the microcosms. Sulfate was not added to the microcosms; however, cysteine, a sulfur containing reducing agent, was added to the simulated anaerobic groundwater for this trial as a reducing agent.

Results of hydrogen analyses are shown in Figure 10. It should be noted that all microcosms originally contained an abundance of H_2, since the atmosphere in the anaerobic chamber is 1% H_2. The abiotic controls (red) exhibit the highest hydrogen concentrations (>> 8 nM), while the live microcosms all exhibit lower values. Within the group of live microcosms, the microcosms that exhibit methanogenesis (i.e., do not contain added Fe; white, pink) exhibit hydrogen concentrations that fall within the transition between sulfate reduction and methanogenesis (see Table 3). Microcosms containing Fe (blue, green, yellow, orange) have higher hydrogen concentrations than those without added Fe, but lower than the abiotic controls. These patterns suggest that hydrogen can be used as an indicator of microbial activity; the most active microcosms exhibit the lowest hydrogen, while the least active (abiotic) have the highest hydrogen concentrations.

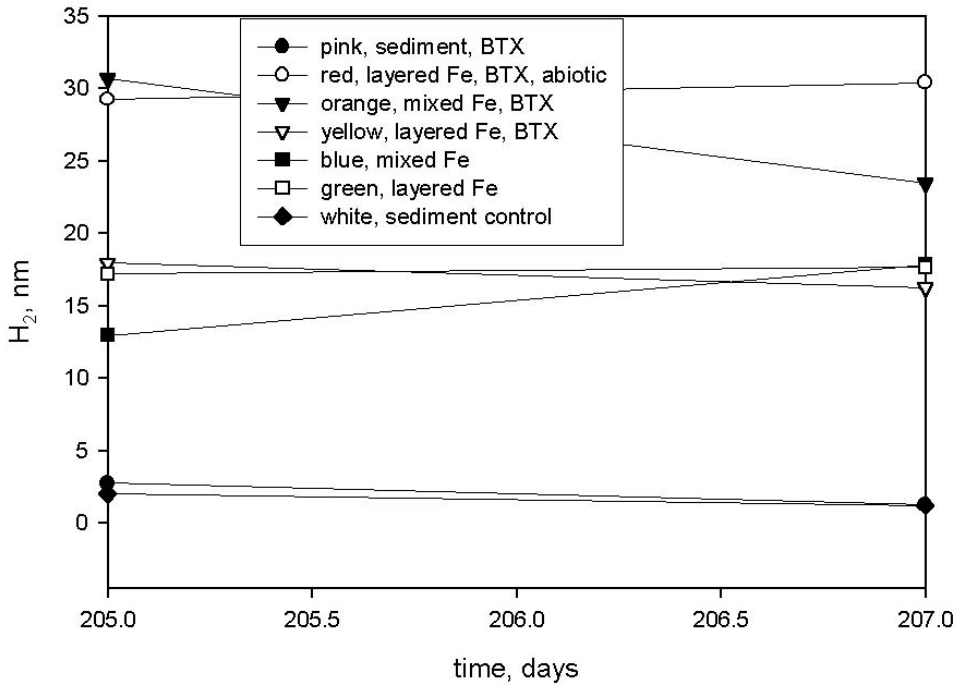

Figure 10. Average total H_2 concentrations for Trial 2. The upper detection limit for H_2 is 8 nM; reported values above 8 nM are estimates. The only trials that contained H_2 concentrations lower than 8 nM were the pink (BTX, no iron) and the white (sediment control), in which methanogenesis is occurring.

Redox dyes were injected into Trial 2 microcosms to estimate the redox potential. Results are shown in Table 5. The abiotic microcosms exhibit an Eh of less than -244 mV, indicating that the cysteine was working properly, creating fully reducing conditions in the microcosms. The groups without added iron (pink and white) had redox potentials between –110 and –224 mV, in the theoretical range between sulfate reduction and methanogenesis (Table 5).

Table 5. Redox potentials of microcosms as determined by redox dyes for Trial 2.

Group	Thionin (66mV)	Cresyl Violet (-75mV)	Resazurin (-110mV)	Phenosafranin (-244mV)	Interpreted redox range (mV)
Red (abiotic, BTX, Fe)	No change	No change	No change	No change	<-244
Pink (BTX, no Fe)	No change	No change	No change	Color change	-244 < Eh < -110
White (sediment control)	No change	No change	No change	Color change	-244 < Eh < -110

As shown in Figure 11, MPN counts of sediment from site #2 and from microcosms with and without added Fe reveal how the different treatments influence microbial populations. Sediment collected from site #2 contains $7.7x10^7$ Fe-reducers/g; no methanogens were detected. After incubating for 48 days, microcosm sediment without added Fe contained 560 methanogens/g and 73 Fe-reducers/g. Microcosm sediment to which Fe was added contained 120 methanogens/g and $2.3x10^6$ Fe-reducers/g. Results indicate that Fe-reducing bacteria were present in the original sample, and the addition of Fe-oxides enabled continuation of these populations in the microcosms, albeit at lower numbers. Without added iron, the Fe-reducing population decreased, giving rise to methanogenic populations.

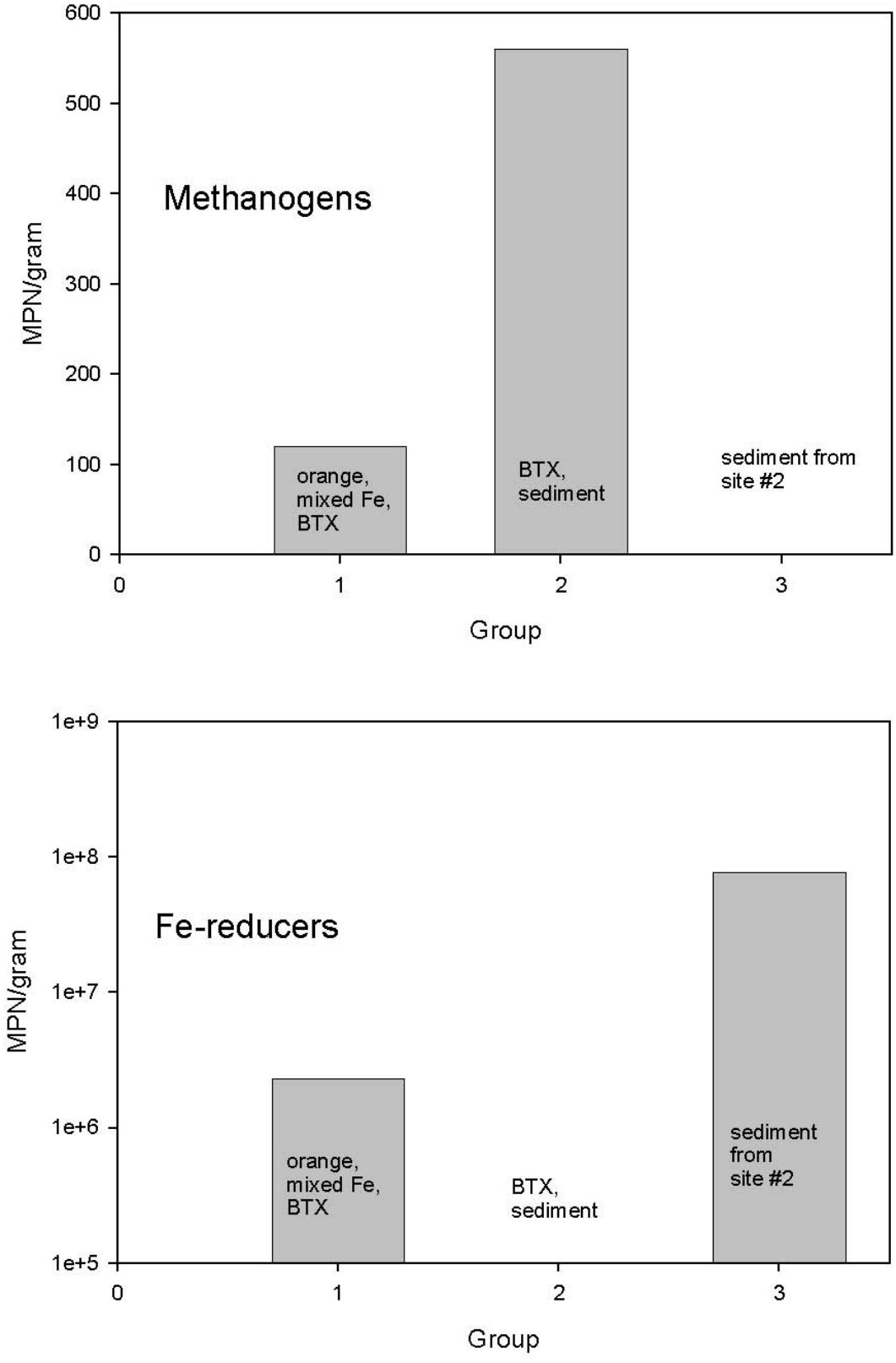

Figure 11. Most Probable Number estimations of selected sediment for Trial 2.

As mentioned previously, cysteine, the reducing agent used, is a sulfur-base compound, and the formation of the black precipitate, tentatively identified as an amorphous Fe-S compound, suggested that sulfur was actively being reduced. Sulfur concentrations, measured at the end of the experiment, are shown in Figure 12. Concentrations were highest (46 mg/L) in the abiotic control. Microcosms not containing added Fe had the next highest concentrations (~15 mg/L), and microcosms containing added Fe exhibited the lowest concentrations (~1.5 mg/L). These results indicate that sulfur was actively reduced in all of the biotic microcosms, but not in the abiotic control. The presence of dissolved Fe in microcosms (blue, green, orange, yellow) allowed for greater removal of S through precipitation of an amorphous FeS phase in comparison with trials that did not contain added Fe (white, pink).

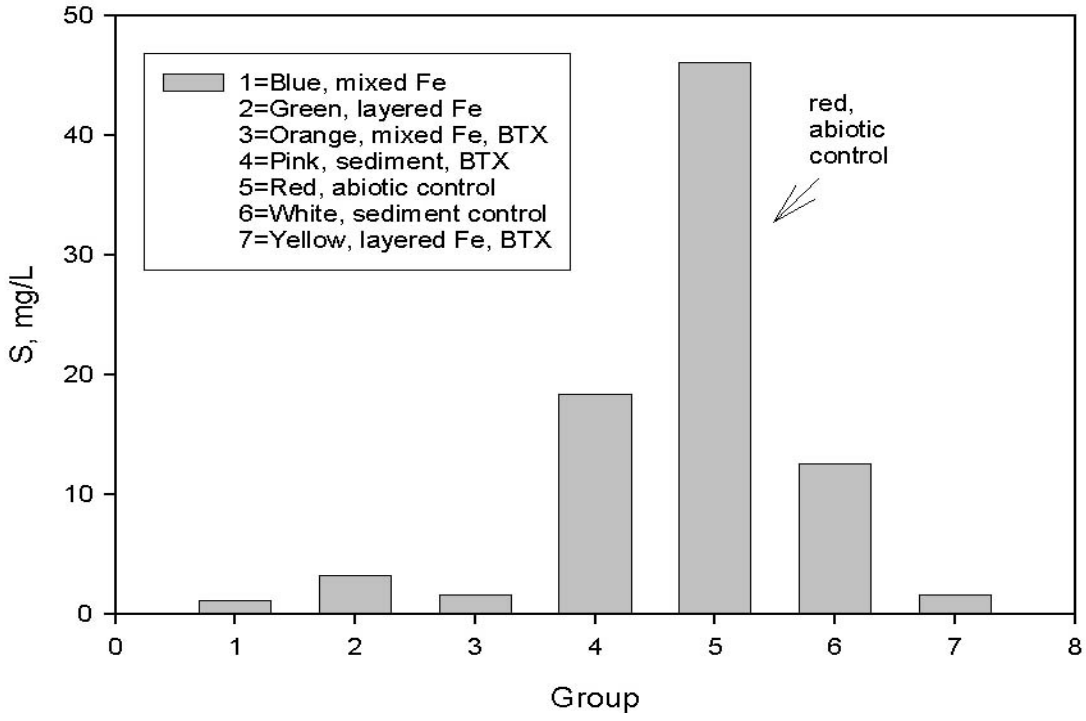

Figure 12. Average total sulfur concentrations for Trial 2 at day 200.

Trial 3.

Trial 3 was constructed with sediment from the source area (Fig. 2) to evaluate differences in biodegradation potential in highly vs. moderately contaminated portions of the plume. Sediment digestion revealed that sediment in the source area contained low concentrations of iron oxides (average 20 mg/kg) in comparison to pristine sediment (average 226 mg/kg), indicating that the source area was depleted with respect to available Fe(III) Microcosms were constructed in a similar manner to Trial 2; cysteine and resazurin were utilized to ensure anaerobic conditions, and ferrihydrite coated quartz grains were used as the source of Fe(III).

Results of BTEX analyses are shown in Figure 13. No BTX losses were observed in the abiotic control or the microcosms containing only BTX. Slight decreases in BTEX were observed in the orange (BTX, Fe) group.

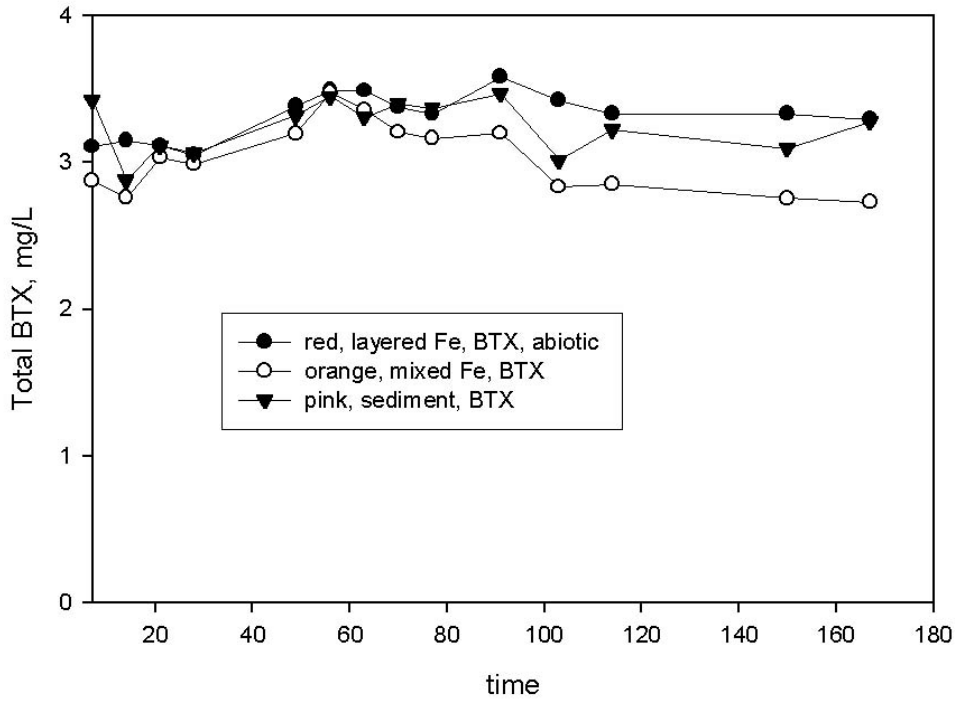

Figure 13. Average total BTX concentrations (mg/L) for Trial 3.

Methane concentrations for Trial 2 are shown in Figure 14. As with the previous two trials of microcosms, the pink group containing BTX showed significant increases in CH_4 production. However, methane concentrations in Trial 3 were not as high (0.2 mg/L maximum) as in Trials 1 and 2 (0.5 mg/L maximum). Similar to Trial 1, the sediment control (white) is also undergoing methanogenesis, as shown by methane production, but to a much lesser extent than the group containing BTX (pink). In contrast to Trials 1 and 2, the orange group of microcosms (BTX+Fe) also exhibited a significant increase in methane production, more in fact, than the sediment control. Although it contains added Fe, this group showed no observed increase in Fe prior to the increase in methane (Fig. 15). Fe concentrations are fluctuating and variable, and there is no obvious pattern of consistent increase in total Fe concentrations over the abiotic control as in the previous trials.

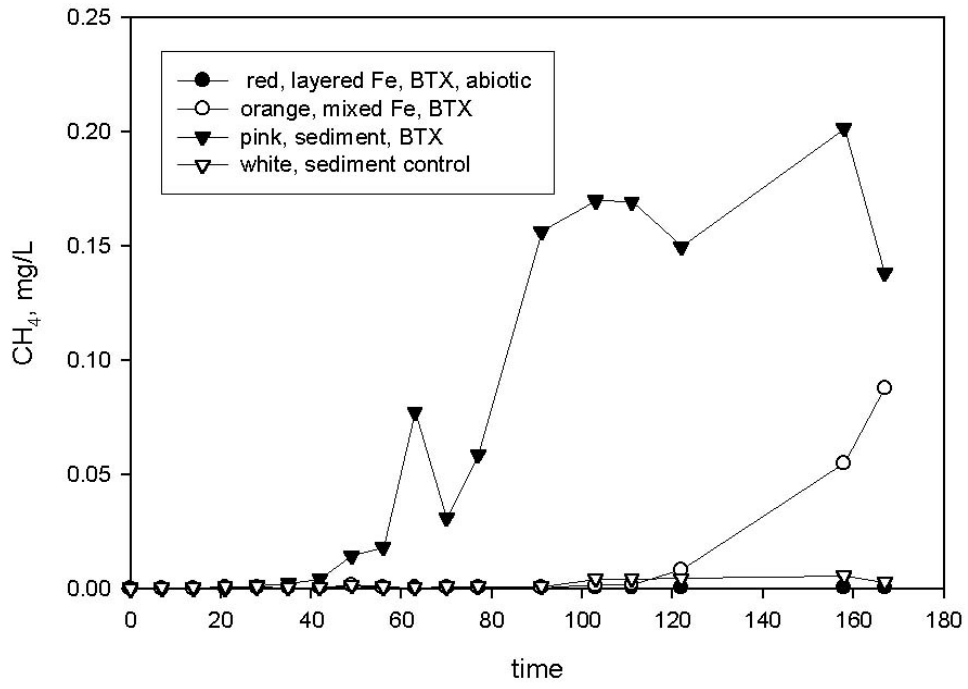

Figure 14. Average total CH_4 concentrations (mg/L) for Trial 3.

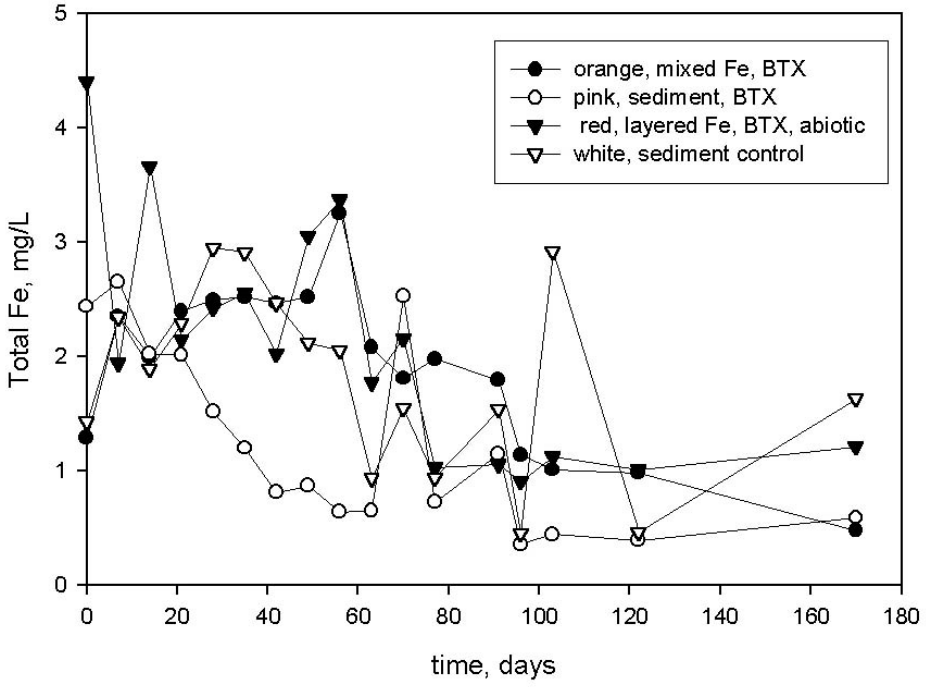

Figure 15. Average total dissolved (<0.2 micron) Fe concentrations (mg/L) for Trial 3.

Hydrogen concentrations were measured in Trial 3 at 172 and 176 days (Fig. 16). In theory, hydrogen concentrations are kept low enough by the bacteria utilizing the energetically favorable reaction that other bacterial populations cannot compete (see Table 3). Hydrogen concentrations in the microcosms with BTX but no added Fe (pink) are indicative of the transition zone between sulfate reduction and methanogenesis. The sediment control (white) exhibits hydrogen concentrations characteristic of a methanogenic population in steady-state. Similar to Trial 2, the hydrogen concentrations in the abiotic control were high due to the 1% H_2 contained in the atmosphere of the anaerobic chamber (estimated 35 nM), while all live microcosms were less than 15 nM.

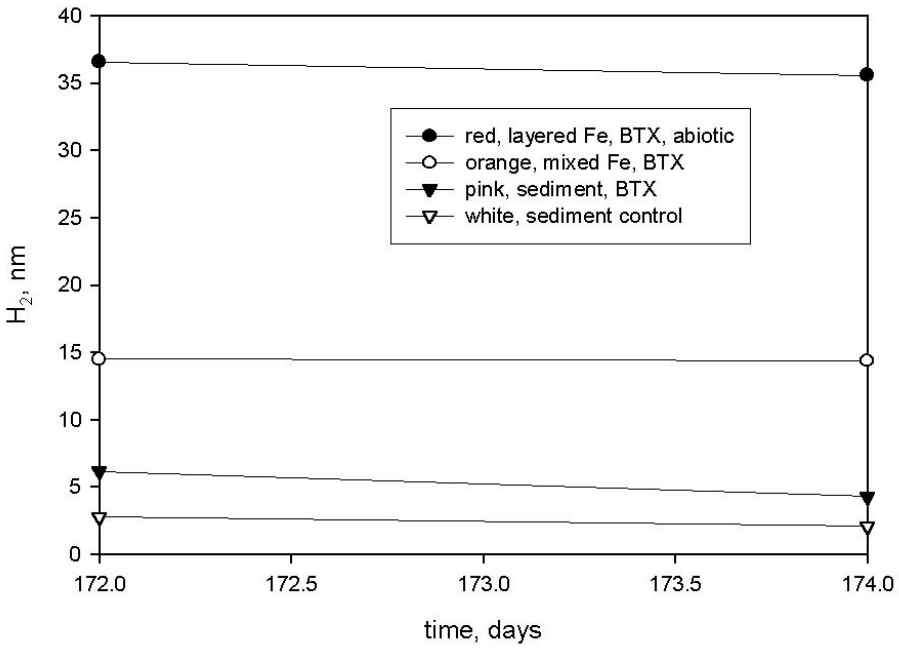

Figure 16. Hydrogen concentrations (nM) in Trial 3. Upper detection limit is 8 nM; reported values above 8 nM are estimates.

Redox dyes were also used in Trial 3 to determine relative redox potentials in the different groups (Table 6). The abiotic group exhibited a potential between –75 and 66 mV, which indicates that conditions were not fully reducing. The groups exhibiting methanogenesis, white (sediment control) and pink (BTX, no Fe), have redox potentials somewhere between -110 mV and -244 mV, which fall in the theoretical range of sulfate reduction and methanogenesis.

Table 6. Redox potentials of microcosms as determined by redox dyes for Trial 3.

Group	Thionin (66mV)	Cresyl Violet (-75mV)	Resazurin (-110mV)	Phenosafranin (-244mV)	Interpreted redox range (mV)
Red (abiotic, BTX, Fe)	No change	Color change	Color change	Color change	-75 < Eh < 66
White (sediment control)	No change	No change	No change	Color change	-244 < Eh < -110
Pink (BTX, no Fe)	No change	No change	No change	Color change	-244 < Eh < -110
Orange (BTX, Fe)	No change	No change	No change	Color change	-244 < Eh < -110

MPN counts were conducted on the sediment control, and the BTX+Fe (orange) microcosms. The sediment control, which can be used as a proxy for the original sediment, contained 220 methanogens/g and 1500 Fe-reducers/g (Figure 17). Sediment from microcosms with added Fe (orange) contained 550 methanogens/g and $2x10^5$ Fe-reducers/g. These results suggest that the presence of Fe-oxides stimulated growth of the Fe-reducing population in the microcosms. However, active methanogenesis is also occurring in microcosms containing added Fe, as shown by the production of methane (Fig. 14). The MPN counts show an increase in the methanogenic population between the original sediment and sediment to which Fe was added. Fe-reducing populations are not as prevalent in this trial, when compared to Trial 2, indicating spatial differences in microbial populations at the site.

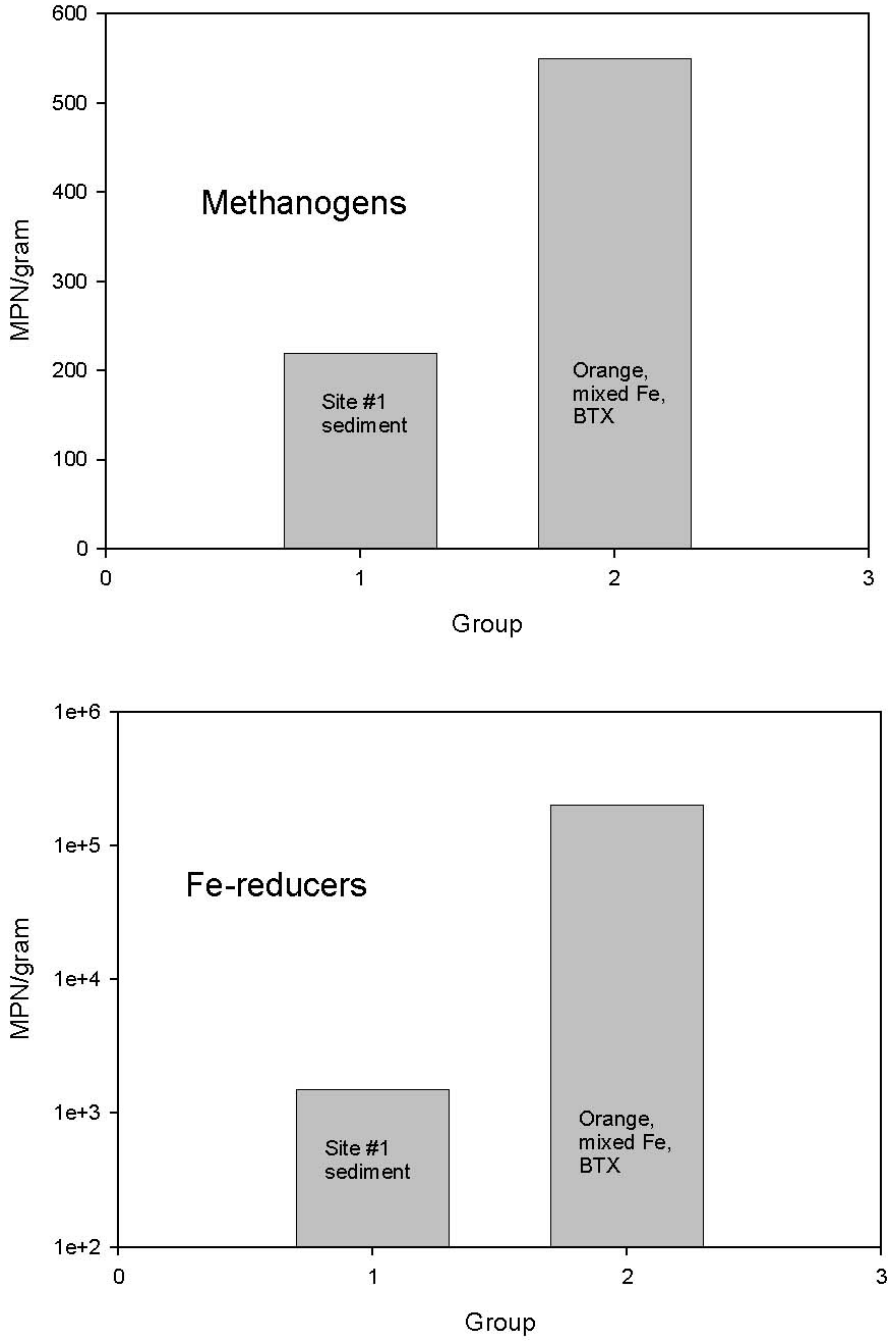

Figure 17. Most probable number estimations of selected sediment from Trial 3.

Similar to Trial 2, the abiotic control exhibited the highest sulfur concentrations (Fig. 18). The sediment control contained 22 mg/L sulfur, and the microcosms with added Fe exhibited sulfur concentrations of ~9 mg/L. The microcosms with BTX and no

added Fe (pink) averaged ~8 mg/L. Sulfur was actively reduced in all biotic microcosms, but not in the abiotic control.

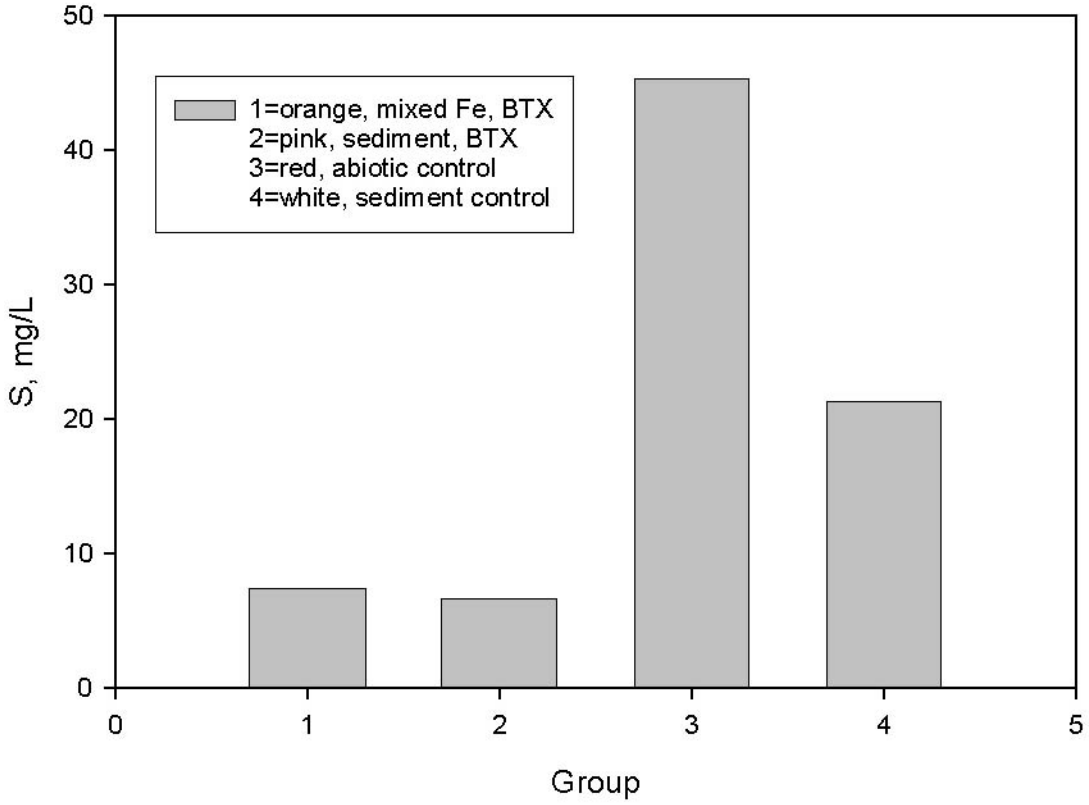

Figure 18. Average total Sulfur concentrations for Trial 3.

Discussion

The role of Fe(III)-oxides in promoting Fe(III) reduction

Trials 1 and 2 contained Fe(III)-oxides in two spatial arrangements, layered and mixed, to create heterogeneous and homogeneous conditions, respectively. This experiment was designed to evaluate if the spatial arrangement (heterogeneous vs. homogeneous) of Fe(III)-oxides would control TEAP development; a layered arrangement (heterogeneous) would promote Fe-reduction in the layer of Fe(III)-oxides and methanogenesis would dominate the sediment beneath the Fe(III)-oxides while

mixing of Fe(III)-oxides throughout the sediment (homogeneous) would allow Fe-reducers to outcompete methanogens.

In all trials, total dissolved Fe concentrations in biotic microcosms were consistently higher than those in the abiotic control, suggesting that the addition of Fe did promote biotic Fe-reduction. The occurrence of Fe-reduction in the microcosms is also indicated by the development of a black FeS precipitate in all of the biotic microcosms. As mentioned previously, reduced sulfur was present in the microcosm through microbial degradation of the reducing agent, cysteine. Black precipitate did not form in the abiotic controls because Fe(II) and reduced S were not produced in the absence of microbial activity.

Although added Fe(III) did promote Fe-reduction in the biotic microcosms, the spatial (homogeneous vs. heterogeneous) arrangement of Fe(III)-oxides in the microcosm experiments did not produce the anticipated effect of the homogeneous system promoting only Fe-reduction, and the heterogeneous system allowing for both Fe-reduction and methanogenesis. The only notable difference between layered and mixed arrangements was that microcosms with a mixed arrangement of Fe(III)-oxides sustained higher total dissolved Fe concentrations on average than did the microcosms with a layered arrangement, suggesting that the homogeneous system may have allowed for more Fe-reduction than did the heterogeneous system.

The addition of Fe(III)-oxides to the microcosms did not promote the level of Fe-reduction expected in Trials 1 and 2 (mid-plume). Previous work showed that Fe-reduction was occurring in this area and Fe(III) was still present in aquifer sediment (Schreiber, 1999). The lower level of Fe-reduction in the microcosms as compared with

the field could be explained in several ways: 1) the Fe(III) added to Trials 1 and 2, an amorphous Fe(III)-hydroxide and ferrihydrite, respectively, was not readily reducible by the Fe-reducers at the site; 2) the added Fe(III) scavenged an essential nutrient (e.g. PO_4) or vitamin needed by the Fe-reducers; or 3) the Fe(II) produced by Fe(III) reduction adsorbed onto the Fe(III)-oxide, thus preventing microorganisms from attaching to the surface. Because both types of Fe(III)-hydroxides added have high surface area and have been shown to be bioavailable (e.g., Lovley and Phillips, 1987), it is unlikely that these mineral phases would have limited Fe(III) reduction. The other two possibilities will be explored in further work. In Trial 3, the addition of Fe(III)-oxides to sediment from the source area also did not promote Fe(III)-reduction, but this is expected, because Fe-reducing populations were not as prevalent in the source area.

While no significant amount of BTX degradation could be attributed to Fe-reduction, Fe-reducers were active in the microcosms, as indicated by MPN. The addition of Fe(III)-oxides, no matter what spatial arrangement, played an important role in TEAP development in the microcosms.

Inhibition of methanogenesis by Fe(III)

Biotic microcosms containing added Fe(III)were affected in two distinct ways. First, in Trials 1 and 2, no methane was produced in any microcosm containing added Fe, and in Trial 3 there was an 80 day lag period in methane production compared to methane production in groups not containing Fe. Both of these observations suggest that Fe(III)-oxides inhibited methanogenesis. Fe addition most likely inhibited methanogenesis by allowing the Fe-reducing populations to outcompete methanogens. MPN counts reveal

that Fe-reducing populations were much more prevalent in microcosms containing added Fe than in microcosms with only sediment from the site. And, in turn, methanogenic populations were much higher in microcosms that did not have added Fe.

Second, hydrogen concentrations were much higher in microcosms containing added Fe(III) than those with active methanogenic populations. This is interpreted to mean that the methanogens utilized hydrogen as an electron donor. In all of the methane-generating microcosms, the production of methane declined after a certain period of time. This may have been caused by the depletion of hydrogen by the methanogens; once the hydrogen reached a certain minimum concentration, it was no longer available as an electron donor, and methanogenesis was curtailed.

Interestingly, although the presence of BTX in microcosms allowed for a greater production of methane, no significant BTX losses were observed in these microcosms. The reason for this is not entirely clear; however it is possible that low levels of BTX were degraded, releasing hydrogen, which could then be used as an electron donor by the methanogens. This would explain the difference in methane production in the sediment controls and microcosms containing BTX that underwent methanogenesis.

Biodegradation potential: source area vs. mid-plume

Trials 1 and 2 were created with sediment from the mid-plume, while Trial 3 contained sediment near the source area.. There are two noticeable differences in the potential for degradation between the two sites: 1) microorganisms nearer the source area are less active than those in the middle of the plume, and 2) Fe-reducing bacteria are not as prevalent near the source area as they are in the middle of the plume.

The highest total methane production in Trial 3 (source area) was 0.1 mg/L, whereas up to 0.5 mg/L methane was produced in Trials 1 and 2 (mid-plume). MPN counts reveal that sediment from the source area contained smaller populations of Fe-reducers and methanogens. Toxicity effects from the high concentrations of BTEX in the source area (non-aqueous phase liquid, NAPL, was previously identified in the source area by Schreiber, 1999) could explain the sluggishness of Trial 3 bacteria compared to Trials 1 and 2.

Unlike Trials 1 and 2, in which added Fe(III) inhibited methanogenesis throughout the entire trial, in Trial 3, methane was produced in all biotic microcosms containing added Fe. This observation suggests that Fe-reducers in the source area cannot outcompete methanogens, even though Fe(III) is present in the microcosms. This is due to a lack of Fe(III) in the source area sediment, causing a decline in the presence of Fe-reducing populations capable of dominating their environment.

TEAP Characterization

Geochemical indicators, specifically Fe(II) and methane (byproducts of iron reduction and methanogenesis), were used as the "first cut" for characterizing TEAPs. CH_4 production provided a good indication of methanogenesis occurring in the microcosms because CH_4 is generally conservative (it can be oxidized but only under aerobic conditions). In contrast, the Fe(II) analysis was not very reliable because Fe(II) is highly reactive. It is unclear how dissolved Fe(II) behaves in the subsurface, but Fe(II) is known to form a variety of minerals, most notably oxides and sulfides. Due to the

precipitation of the Fe-S phase in the microcosms, using changes in dissolved Fe(II) concentrations was not a reliable indicator for iron reduction.

Redox dyes estimate ranges of redox conditions accurately, but more detailed information is often needed. The wide range of potentials estimated for the microcosms cannot be used to identify a single redox process. However, the dyes did provide a quick and easy way to determine the general redox potential of media.

MPN counts give a good estimate of microbial populations and information as to which redox processes can occur at a site. However, MPN counts cannot distinguish between active and inactive populations, and it is possible that some microbes are not culturable. As noted in the counts for this study, methanogenic populations appear to be very low, but the numbers compare well with those of Bekins (1999), whose method was used for the MPN experiments.

Hydrogen concentrations provide very valuable data on redox processes in two ways. First, an overabundance of hydrogen can indicate a lack of microbial activity. The initial overabundance of hydrogen in all microcosms is due to the 1% H_2 that is present in the anaerobic chamber. Because hydrogen is used as an electron donor, microbial activity would result in decreased concentrations. In contrast, if the microbial populations were inactive or killed, the hydrogen concentrations would remain high. Second, the steady state hydrogen concentrations in active microcosms can be used to delineate the dominant TEAP. In this study, the hydrogen concentrations of microcosms producing methane were in the range of methanogenic conditions, indicating that the hydrogen data correlated well with the geochemical indicator and the MPN data, which also documented methanogenic conditions in these microcosms. The major disadvantage to

using hydrogen measurements for delineating TEAPs in microcosms is that conditions are constantly changing, and determining a steady-state hydrogen concentration is not possible. Thus, although hydrogen should only be used in conjunction with other geochemical data, it is one of the more simple and reliable methods for TEAP identification.

Implications

The results of this study have several implications for bioremediation research. First, and most important, microbial populations, both in the numbers and distribution (e.g., Fe-reducers vs. methanogens) must be taken into account when estimating biodegradation rates. As illustrated by the differing rates of methanogenesis in two unique locations within the contaminant plume (source area and mid-plume) documented in this study, a single decay constant cannot be applied to an entire plume or even a single redox process. Not only do contaminants degrade at different rates under different redox conditions, a single redox process can have differing rates according to spatial location in the plume. First order kinetics cannot model these biological processes. Monod kinetics, which incorporate microbial populations, in terms of both numbers and growth, into the decay equation, are better suited for estimating contaminant mass loss.

Second, because Fe(II) is reactive and CH_4 can potentially be oxidized, concentrations of geochemical indicators (e.g. CH_4, Fe(II)) are not a good source for stoichiometric calculations of contaminant mass loss. Even in the controlled laboratory environment, the fate of Fe(II) is difficult to quantify. Thus, any mass loss estimate relying on the concentration of Fe(II) could grossly underestimate or overestimate contaminant mass

loss. Another difficulty in using byproducts for mass loss calculations is that there are other compounds, both in the field (e.g., other non-BTEX compounds, natural organic matter) and in the laboratory (natural organic matter, H_2) that can be used as electron donors. Contaminant mass loss would be incorrectly calculated if the contaminant was assumed to be the only electron donor present. As a result, geochemical indicators should only be used in an effort to delineate overall redox conditions, and should not be used for mass loss calculations unless other data on the behavior of Fe(II) and CH_4 are available.

Summary

Microcosm experiments using sediment from a petroleum-contaminated aquifer were designed to evaluate the role of Fe-oxides in development of redox conditions. The sediment was collected from areas at the site that exhibited elevated Fe(II) and CH_4 concentrations. These areas potentially contained active methanogenic and Fe-reducing bacterial populations as noted by the presence of the byproducts of both TEAPs.
The spatial (homogeneous vs. heterogeneous) arrangement of Fe-oxides was varied in the first two trials of microcosms, which were created using sediment from a mid-plume location. A third trial of microcosms was created using sediment near the source area. It was thought that the spatial variations of Fe-oxides in the microcosms might promote concomitant utilization of available electron acceptors by Fe-reducers and methanogens. Although this did not occur, results showed that Fe-oxides played a significant role in the development of redox conditions, most notably by completely inhibiting methanogenesis.

Comparison of results from microcosms constructed with sediment from the source area and from the mid-plume showed the effects of very high BTEX concentrations on microbial populations. The same types of bacteria, Fe-reducers and methanogens, were present near the source as were in mid-plume, but populations near the source operated at much slower rates. In addition, because Fe(III) oxides were depleted in the source area, the numbers of Fe-reducers in the source area were much lower than those in the mid-plume.

Several methods were used to examine redox conditions in the microcosms. Methane worked well as a geochemical indicator, but Fe(II) performed poorly. Estimation of redox potential with redox dyes revealed general information about reducing conditions, but individual redox processes cannot be delineated by this method. MPN counts contribute valuable information on the microorganisms that are present and the relative size of different populations, but cannot distinguish between active and inactive populations. The most reliable method for TEAP characterization was the use of hydrogen in combination with the other geochemical data.

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Appendix Key
r-red, layered Fe, BTX, abiotic
o-orange, mixed Fe, BTX
y-yellow, layered Fe, BTX
p-pink, sediment, BTX
w-white, sediment control
b-blue, mixed Fe
g-green, layered Fe
1,2,3-denotes microcosm triplicates

Appendix A. BTX concentrations

Trial 1 BTX

day 0	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1		r2		r3	
benzene	5109.269	6.9097579	6073.381	8.2253411	5999.923	8.125104
toluene	4362.523	5.6486776	5107.675	6.6262338	5119.6635	6.641961
m,p	7203.705	4.6154618	8592.956	5.5147116	8522.524	5.469122
o	2748.37	4.8700619	3270.0105	5.8031958	3308.959	5.872869
		22.043959		26.169482		26.10906
	o1	conc.	o2	conc.	o3	conc.
benzene	5636.717	7.6294894	5576.379	7.5471549	5525.0285	7.477084
toluene	4840.276	6.2754362	4629.117	5.9984192	4636.181	6.007686
m,p	8434.468	5.4121238	7738.856	4.9618603	7951.8565	5.099734
o	3626.256	6.4404637	2919.698	5.1765411	3028.926	5.371933
		25.757513		23.683975		23.95644
	y1	conc.	y2	conc.	y3	conc.
benzene	6082.095	8.2372319	6668.5735	9.0375136	6031.414	8.168075
toluene	5332.265	6.9208708	5108.7925	6.6276999	5061.876	6.566151
m,p	8575.79	5.5036002	8616.252	5.5297909	8712.3725	5.592009
o	3307.571	5.8703857	3237.642	5.7452935	3334.2575	5.918124
		26.532089		26.940298		26.24436
	p1	conc.	p2	conc.	p3	conc.
benzene	6092.4165	8.2513161	5881.888	7.9640385	6004.5535	8.131422
toluene	4220.55	5.4624249	4746.815	6.1528258	4926.1865	6.388141
m,p	7747.463	4.9674315	7630.419	4.89167	7913.082	5.074635
o	3189.885	5.6598637	3007.1105	5.3329085	3303.19	5.862549
		24.341036		24.341443		25.45675
day 6	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	6329.9845	8.5754906	5733	7.7608728	5541	7.498878
toluene	5225	6.7801511	4789	6.2081678	4615	5.979899
m,p	8454	5.4247667	8010	5.1373694	7694	4.932825
o	3212	5.699424	3094	5.4883403	2983	5.289779
		26.479832		24.59475		23.70138
	o1	conc.	o2	conc.	o3	conc.
benzene	5769	7.8099967	5608	7.5903035	5522	7.472952
toluene	4900	6.3537874	4605	5.9667804	4544	5.886755

m,p		7868	5.0454541		7627	4.8894569		7399	4.741875
o		3056	5.4203642		2971	5.2683124		2882	5.109105
			24.629602			23.714853			23.21069
	y1		conc.	y2		conc.	y3		conc.
benzene		6667	9.0353665		5869	7.9464522		5962	8.073356
toluene		4939	6.4049511		4928	6.3905203		4997	6.481041
m,p		7700	4.9367092		8027	5.1483734		8441	5.416352
o		3006	5.330922		3090	5.4811849		3257	5.779922
			25.707949			24.966531			25.75067
	p1		conc.	p2		conc.	p3		conc.
benzene		5958	8.0678975		5829	7.89187		5327	7.206864
toluene		3678	4.7506599		4672	6.0546769		4168	5.393485
m,p		6886	4.4098142		7359	4.7159829		6453	4.129537
o		2982	5.2879897		2880	5.1055275		2722	4.82289
			22.516361			23.768057			21.55278
day 14	**peak area**		**C (mg/L)**	**peak area**		**C (mg/L)**	**peak area**		**C (mg/L)**
	r1		conc.	r2		conc.	r3		conc.
benzene		5421	7.0663856		5403	7.0431747	6752.893		8.783849
toluene		4601	5.884547		4563	5.8365326	4927.057		6.296532
m,p		7912	5.0192785		7774	4.9328448	8175.4725		5.184299
o		3063	5.1581873		3057	5.1482129	3232.264		5.439572
			23.128398			22.960765			25.70425
	o1		conc.	o2		conc.	o3		conc.
benzene		5331.756	6.9513063		5386.214	7.0215293		4959	6.470641
toluene		4531.884	5.7972164		4478.734	5.7300595		4130	5.289422
m,p		7790.328	4.9430715		7584.778	4.8143292		7017	4.458712
o		3041.373	5.1222346		2954.008	4.976999		2755	4.646168
			22.813829			22.542917			20.86494
	y1		conc.	y2		conc.	y3		conc.
benzene		5644	7.353942		5730	7.4648382		5786	7.53705
toluene		4648	5.9439331		4769	6.0968209		4824	6.166315
m,p		7833	4.9697983		8141	5.1627083		8252	5.232231
o		3116	5.2462945		3173	5.3410513		3233	5.440795
			23.513968			24.065419			24.37639
	p1		conc.	p2		conc.	p3		conc.
benzene		4984.096	6.5030019		5738	7.4751541	4996.081		6.518456
toluene		2994.73	3.8549676		4669	5.9704674	3533.9		4.536228
m,p		5833.7255	3.7175908		7672	4.868959	5650.723		3.602971
o		2910.4645	4.9046123		3084	5.1930977	2717.826		4.58437
			18.980173			23.507678			19.24203
day 21	**peak area**		**C (mg/L)**	**peak area**		**C (mg/L)**	**peak area**		**C (mg/L)**
	r1		conc.	r2		conc.	r3		conc.
benzene		5782.158	6.828029		5853.148	6.9123201	5806.61		6.857062
toluene		4979.84	5.6946815		4917.328	5.6224208	4872.112		5.570153
m,p		8644.9625	4.8370885		8330.9445	4.658222	6210.327		3.450306
o		3420.7355	5.1048195		3307.668	4.9340743	3280.544		4.893114
			22.464618			22.127037			20.77064

	o1	conc.	o2	conc.	o3	conc.
benzene	5822.15	6.8755141	5325.116	6.2853526	5455.008	6.439582
toluene	4894.79	5.596368	4464.56	5.099044	4536.197	5.181853
m,p	8306.9	4.6445261	7411.59	4.1345523	7605.0645	4.244756
o	3262.98	4.8665902	2886.18	4.2975778	3010.242	4.484926
		21.982998		19.816527		20.35112
	y1	conc.	y2	conc.	y3	conc.
benzene	5811.398	6.8627476	5777.44	6.822427	6251.163	7.38491
toluene	4731.6	5.4077287	4790.232	5.4755043	5167.298	5.911373
m,p	7785.634	4.3476099	8095.4865	4.5241037	8624.953	4.825691
o	3126.115	4.6599079	3171.3285	4.7281856	3393.679	5.063961
		21.277994		21.550221		23.18594
	p1	conc.		conc.	p3	conc.
benzene	4575.814	5.3956566	5662.252	6.6856566	4407.194	5.195443
toluene	2124.533	2.3940919	3958.721	4.5143199	2500.544	2.828742
m,p	3964.144	2.1708669	6809.328	3.7915003	4260.446	2.339642
o	2769.706	4.1216883	3106.912	4.6309091	2516.242	3.738928
		14.082304		19.622386		14.10275
day 27	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	5699.7	6.7283635	5771.886	6.8140524	5788.693	6.834003
toluene	4884.09	5.5839994	4835.148	5.5274249	4871.26	5.569169
m,p	8451.6715	4.7269888	8191.1025	4.5785672	8194.3155	4.580397
o	3361.326	5.0151042	3268.119	4.8743506	3294.6715	4.914448
		22.054456		21.794395		21.89802
	o1	conc.	o2	conc.	o3	conc.
benzene	5647.01	6.6658175	5632.008	6.6480093	5658.654	6.67964
toluene	4752.321	5.4316811	4699.758	5.3709209	4665.263	5.331046
m,p	8116.879	4.536289	7967.58	4.4512474	7785.688	4.347641
o	3204.725	4.7786182	3127.7	4.6623014	3099.133	4.619162
		21.412406		21.132479		20.97749
	y1	conc.	y2	conc.	y3	conc.
benzene	5779.864	6.8235227	5860.366	6.9190831	5756.726	6.796057
toluene	4724.576	5.3996093	4848.509	5.5428695	4773.2035	5.45582
m,p	7878.548	4.4005343	8199.427	4.5833088	8086.065	4.518737
o	3164.857	4.7184129	3225.0815	4.809359	3194.5405	4.763238
		21.342079		21.85462		21.53385
	p1	conc.	p2	conc.	p3	conc.
benzene	3773.16	4.441452	4894.512	5.77256	3886.476	4.575964
toluene	1536.458	1.7143072	2631.812	2.9804806	2085.203	2.348628
m,p	2980.45	1.6105491	5487.46	3.0385566	3510.333	1.912374
o	2581.588	3.837608	2893.093	4.3080172	2373.157	3.522853
		11.603916		16.099614		12.35982
day 34	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	6271.2665	6.6462182	6613.846	7.0081044	6373.1775	6.753873
toluene	5397.85	5.4951079	5568.576	5.6686682	5379.314	5.476264

m,p	9355.984	4.5460477	9342.718	4.5396103	9075.854	4.410115
o	3727.376	4.8744714	3751.1005	4.9054871	3677.784	4.809639
		21.561845		22.12187		21.44989
	o1	conc.	o2	conc.	o3	conc.
benzene	6479.43	6.8661131	6068.0655	6.4315655	5983.712	6.342458
toluene	5565.971	5.6660199	5097.6875	5.1899624	5025.953	5.117037
m,p	9721.011	4.7231764	7151.742	3.4764426	8519.5465	4.140168
o	3860.6225	5.0486681	3423.57	4.4772979	3404.658	4.452574
		22.303978		19.575268		20.05224
	y1	conc.	y2	conc.	y3	conc.
benzene	6368.216	6.7486315	6563.505	6.9549263	7039.634	7.457888
toluene	5320.952	5.4169333	5558.0735	5.6579913	6015.7505	6.123266
m,p	9032.546	4.3890999	9552.2	4.6412612	10307.746	5.007889
o	3640.988	4.7615341	3768.443	4.9281594	4087.235	5.344925
		21.316199		22.182338		23.93397
	p1	conc.	p2	conc.	p3	conc.
benzene	4603.755	4.8847314	5145.85	5.4573771	4829.688	5.123397
toluene	2209.131	2.2534527	2713.538	2.7662334	2835.718	2.890442
m,p	4720.201	2.2965411	6196.142	3.0127392	4723.8495	2.298312
o	3370.3605	4.4077357	3267.022	4.2726386	3018.2385	3.947397
		13.842461		15.508988		14.25955
day 41	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	6132.996	6.7507048	6072.534	6.6839616	5980.757	6.58265
toluene	5276.15	5.578478	5067.339	5.3562985	5051.636	5.33959
m,p	9171.361	4.636968	8362.306	4.224269	8589.83	4.340329
o	3674.758	4.97957	3358.464	4.5475098	3479.991	4.713517
		21.945721		20.812039		20.97609
	o1	conc.	o2	conc.	o3	conc.
benzene	5978.978	6.5806864	6014.058	6.6194107	6087.196	6.700147
toluene	5077.7575	5.367384	5033.885	5.3207027	5058.428	5.346817
m,p	8750.33	4.4222001	8551.402	4.3207269	8448.2185	4.268093
o	3478.072	4.7108953	3393.32	4.5951234	3392.921	4.594578
		21.081166		20.855964		20.90964
	y1	conc.	y2	conc.	y3	conc.
benzene	6218.888	6.8455199	6222.586	6.849602	6488.0915	7.14269
toluene	5135.404	5.4287212	5176.778	5.472744	5472.604	5.787509
m,p	8650.243	4.3711457	8814.603	4.4549857	9296.442	4.700772
o	3512.362	4.7577357	3513.9605	4.7599193	3722.728	5.045097
		21.403122		21.537251		22.67607
	p1	conc.	p2	conc.	p3	conc.
benzene	4394.4365	4.831532	5645.605	6.2126803	4685.811	5.153176
toluene	2074.819	2.1721907	3487.88	3.675719	2782.9	2.925605
m,p	4279.846	2.1418063	6669.5415	3.3607899	4634.566	2.322749
o	3030.766	4.0998716	3282.642	4.4439363	2909.557	3.934299
		13.245401		17.693126		14.33583
day 48	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**

	r1	conc.	r2	conc.	r3	conc.
benzene	6324.0765	6.9616361	6069.822	6.6809679	5826.295	6.412142
toluene	5443.958	5.7570295	5082.597	5.3725333	4911.172	5.190133
m,p	9346.974	4.7265482	8448.441	4.2682065	8269.142	4.176746
o	3717.87	5.0384613	3397.158	4.6003661	3347.255	4.532198
		22.483675		20.922074		20.31122
	o1	conc.	o2	conc.	o3	conc.
benzene	6391.1375	7.0356638	6094.843	6.7085882	5857.4355	6.446517
toluene	5400.4685	5.7107557	5115.058	5.4070726	4864.974	5.140978
m,p	9157.5275	4.6299115	8656.3695	4.3742708	8119.723	4.100527
o	3636.6525	4.9275176	3414.212	4.623662	3246.802	4.394979
		22.303849		21.113594		20.083
	y1	conc.	y2	conc.	y3	conc.
benzene	6392.8535	7.0375581	6658.3975	7.3306886	6378.252	7.02144
toluene	5296.389	5.6000128	5563.8325	5.8845786	5374.841	5.683487
m,p	8867.948	4.482197	9418.8895	4.7632322	9113.41	4.607407
o	3598.8285	4.8758497	3738.3205	5.0663969	3627.856	4.915501
		21.995618		23.044896		22.22784
	p1	conc.	p2	conc.	p3	conc.
benzene	3047.009	3.3441246	5332.866	5.8674519	4620.508	5.081089
toluene	1380.897	1.4338423	3187.3	3.3558952	2692.518	2.829436
m,p	3030.492	1.5045108	6445.761	3.2466395	4599.0345	2.304624
o	2916.688	3.9440401	3288.644	4.4521351	3017.307	4.081486
		10.226518		16.922122		14.29664
day 57	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	6356.929	6.9979015	6220.6355	6.8474489	5979.628	6.581404
toluene	5437.779	5.7504549	5184.4875	5.4809471	4946.066	5.227261
m,p	9216.894	4.6601943	8612.604	4.351946	8102.2525	4.091616
o	3713.977	5.0331435	3472.553	4.7033563	3289.876	4.453818
		22.441694		21.383698		20.3541
	o1	conc.	o2	conc.	o3	conc.
benzene	6122.614	6.7392443	5121.923	5.6345947	6094.748	6.708483
toluene	5145.657	5.4396306	4228.563	4.4638222	5024.347	5.310554
m,p	8653.9845	4.3730542	7066.4585	3.5632572	8261.165	4.172677
o	3435.658	4.6529574	2823.71	3.8170314	3343.122	4.526552
		21.204886		17.478706		20.71827
	y1	conc.	y2	conc.	y3	conc.
benzene	6194.894	6.8190332	6586.842	7.2516994	6671.254	7.344881
toluene	5106.8685	5.3983587	5471.882	5.7867412	5569.3255	5.890423
m,p	8504.558	4.2968318	9166.331	4.6344022	9356.694	4.731506
o	3452.4575	4.6759057	3641.804	4.9345545	3741.136	5.070243
		21.190129		22.607397		23.03705
	p1	conc.	p2	conc.	p3	conc.
benzene	2732.2035	2.9966149	4888.288	5.3766881	4360.5065	4.794077
toluene	1260.683	1.3059319	2722.121	2.8609344	2398.058	2.516124
m,p	2888.105	1.4318792	5879.606	2.9578438	4092.693	2.04634

o	2890.462	3.9082152	3126.939	4.2312447	2851.1515	3.854517
		9.6426412		15.426711		13.21106
day 70	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	5641.715	6.2083829	2414.504	2.5492272	5688.14	6.022028
toluene	4922.904	5.2026164	2093.02	2.0484268	4885.17	4.819792
m,p	8573.409	4.3319527	3731.946	1.7161779	8365.916	3.920731
o	3433.377	4.6498415	1529.684	1.9695661	3422.784	4.46111
		20.392794		8.283398		19.22366
	o1	conc.	o2	conc.	o3	conc.
benzene	5958.494	6.3088304	5744.851	6.0821896	5536.232	5.860878
toluene	5038.343	4.9718243	4845.286	4.7802045	4638.7905	4.575246
m,p	8538.0105	4.0026025	8232.324	3.857176	7753.912	3.629578
o	3407.166	4.4405549	3264.2675	4.2524835	3129.188	4.074703
		19.723812		18.972054		18.1404
	y1	conc.	y2	conc.	y3	conc.
benzene	5953.635	6.3036758	5933.565	6.2823848	5836.734	6.179663
toluene	4930.255	4.8645409	5043.1135	4.9765593	4926.5335	4.860847
m,p	8222.48	3.8524929	8571.3875	4.0184812	8258.194	3.869483
o	3362.509	4.381781	3419.4835	4.4567662	3299.742	4.299172
		19.402491		19.734191		19.20917
	p1	conc.	p2	conc.	p3	conc.
benzene	2282.3165	2.4089975	4254.9455	4.5016395	3108.074	3.284993
toluene	1002.856	0.9663782	2239.224	2.1935424	1649.855	1.608561
m,p	2502.512	1.1312902	5158.029	2.3946189	3083.8155	1.407838
o	2599.164	3.3771285	2945.794	3.8333346	2449.273	3.179854
		7.8837944		12.923136		9.481246
day 83	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	5918.4925	6.2663953	5323.438	5.6351382	5894.352	6.240786
toluene	5081.626	5.0147851	4473.837	4.4115206	5021.684	4.955289
m,p	8642.991	4.0525457	7392.984	3.4578706	8513.878	3.991122
o	3494.904	4.5560285	3011.542	3.9198668	3510.5805	4.576661
		19.889755		17.424396		19.76386
	o1	conc.	o2	conc.	o3	conc.
benzene	6075.368	6.4328149	5794.176	6.1345155	5947.748	6.297431
toluene	5185.447	5.1178333	4850.31	4.7851911	4951.607	4.885734
m,p	8945.092	4.1962664	8126.278	3.806726	8187.902	3.836043
o	3613.653	4.7123162	3261.8035	4.2492406	3345.395	4.359257
		20.459231		18.975673		19.37846
	y1	conc.	y2	conc.	y3	conc.
benzene	5689.1245	6.0230727	5331.132	5.6433003	6332.648	6.705748
toluene	4695.0355	4.6310725	4482.0635	4.4196859	5308.069	5.239542
m,p	7736.254	3.621177	7603.362	3.5579553	8922.13	4.185343
o	3170.147	4.1286098	3043.017	3.9612916	3609.124	4.706356
		18.403932		17.582233		20.83699
	p1	conc.	p2	conc.	p3	conc.

benzene	2213.7835	2.336295	3940.2155	4.1677616	2657.464	2.806969
toluene	961.457	0.9252873	2008.138	1.9641767	1373.566	1.334329
m,p	2449.99	1.1063035	5167.888	2.3993092	2865.348	1.303905
o	2560.316	3.3259999	3080.457	4.0105671	2508.81	3.258212
		7.6938858		12.541815		8.703414

day 110

	r1	conc.	r2	conc.	r3	conc.
benzene	5720.637	6.0565024	5810.697	6.1520416	5754.87	6.092818
toluene	4911.456	4.8458819	4924.966	4.8592913	4901.268	4.83577
m,p	8345.466	3.9110019	8149.856	3.8179429	8267.475	3.873899
o	3409.947	4.444215	3400.433	4.4316934	3432.504	4.473903
		19.257601		19.260969		19.27639
	o1	conc.	o2	conc.	o3	conc.
benzene	5985.188	6.3371485	5878.26	6.2237151	5788.544	6.128541
toluene	5102.0815	5.0350883	4927.426	4.861733	4813.337	4.748493
m,p	8634.33	4.0484253	8208.3315	3.8457619	7910.894	3.70426
o	3527.048	4.5983338	3323.33	4.3302168	3280.9505	4.27444
		20.018996		19.261427		18.85573
	y1	conc.	y2	conc.	y3	conc.
benzene	5211.664	5.5165639	6097.249	6.4560272	6258.486	6.627074
toluene	4286.91	4.2259851	5153.782	5.086404	5282.3	5.213965
m,p	7062.916	3.3008449	8749.209	4.1030775	8909.274	4.179226
o	2894.242	3.7654861	3564.852	4.6480883	3634.46	4.739701
		16.80888		20.293597		20.75997
	p1	conc.	p2	conc.	p3	conc.
benzene	2275.518	2.4017854	3690.4265	3.9027757	2343.537	2.473943
toluene	969.2955	0.9330675	1799.799	1.7573886	1169.122	1.131406
m,p	2626.376	1.1902169	4862.724	2.2541313	2681.484	1.216434
o	2715.256	3.5299193	3015.413	3.9249615	2467.172	3.203411
		8.0549891		11.839257		8.025194

Trial 2 BTX

day 0	**peak area**		**peak area**		**peak area**	
	r1	conc.	r2	conc.	r3	conc.
benzene	1689.682	1.5062239	1255.782	1.1194348	1521.008	1.355864
toluene	1286.74	1.0854901	985.886	0.8316906	1213.885	1.02403
m,p	1527.322	0.6480216	1166.329	0.4948572	1498.8165	0.635927
o	684.388	0.6967483	527.417	0.5369424	676.211	0.688424
		3.9364839		2.982925		3.704244
	o1	conc.	o2	conc.	o3	conc.
benzene	1677.178	1.4950776	1723.222	1.5361223	2171.446	1.93568
toluene	1305.408	1.1012384	1354.206	1.1424043	1577.633	1.330887
m,p	1573.175	0.6674763	1643.055	0.6971255	1735.655	0.736414
o	701.126	0.7137886	735.399	0.7486806	762.2285	0.775995
		3.9775809		4.1243326		4.778976
	y1	conc.	y2	conc.	y3	conc.

benzene	1689.94	1.5064539	0	0	1675.269	1.493376
toluene	1323.573	1.1165623	0	0	1324.193	1.117085
m,p	1599.1355	0.678491	0	0	1621.708	0.688068
o	720.798	0.7338159	0	0	726.948	0.740077
		4.0353232		0		4.038606
	p1	conc.	p2	conc.	p3	conc.
benzene	1507.914	1.3441915	1479.092	1.3184988	1477.028	1.316659
toluene	1188.2875	1.0024359	1143.971	0.9650506	1144.236	0.965274
m,p	1460.263	0.6195693	1368.481	0.5806275	1385.0145	0.587642
o	673.833	0.6860027	639.826	0.6513815	649.776	0.661511
		3.6521994		3.5155585		3.531087
day 7	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	1504.792	1.4745634	1506.123	1.4758677	1493.572	1.463569
toluene	1178.592	1.0629437	1164.804	1.0505087	1173.886	1.058699
m,p	1443.7075	0.5958839	1399.563	0.5776634	1430.2315	0.590322
o	660.882	0.7721396	653.671	0.7637146	649.742	0.759124
		3.9055307		3.8677545		3.871714
	o1	conc.	o2	conc.	o3	conc.
benzene	1362.546	1.3351749	1709.108	1.6747751	1309.418	1.283114
toluene	676.555	0.6101687	1341.298	1.2096843	1010.0395	0.91093
m,p	1502.446	0.620128	1649.02	0.6806257	1229.418	0.507437
o	691.142	0.8074938	736.534	0.8605274	544.56	0.636235
		3.3729653		4.4256126		3.337716
	y1	conc.	y2	conc.	y3	conc.
benzene	1594.531	1.5624998	1601.254	1.5690877	1518.154	1.487657
toluene	1246.231	1.1239457	1243.492	1.1214755	1232.1425	1.11124
m,p	1528.146	0.6307355	1520.995	0.627784	1456.694	0.601244
o	699.769	0.8175731	671.41	0.78444	652.028	0.761795
		4.1347541		4.1027871		3.961936
	p1	conc.	p2	conc.	p3	conc.
benzene	1486.536	1.4566742	1663.9855	1.630559	1388.779	1.360881
toluene	1272.3595	1.1475104	1159.646	1.0458568	1073.672	0.968319
m,p	1439.622	0.5941976	1398.89	0.5773857	1340.544	0.553304
o	659.355	0.7703555	671.093	0.7840696	629.886	0.735926
		3.9687377		4.0378711		3.618429
day 14	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	1182.512	1.2117267	1355.26	1.3887426	1422.064	1.457197
toluene	1001.888	0.9529085	997.793	0.9490137	1296.591	1.233204
m,p	978.7315	0.4405724	1188.54	0.5350169	1294.7835	0.582842
o	560.436	0.694942	590.861	0.7326691	590.0165	0.731622
		3.3001496		3.6054423		4.004865
	o1	conc.	o2	conc.	o3	conc.
benzene	652.136	0.6682474	1584.32	1.6234617	1232.174	1.262616
toluene	782.1975	0.7439581	1105.2	1.0511699	954.378	0.907721
m,p	1261.5605	0.5678868	1098.65	0.4945532	1103.221	0.496611

o	704.855	0.8740219	503.75	0.6246512	550.866	0.683075
		2.8541142		3.793836		3.350023
	y1	conc.	y2	conc.	y3	conc.
benzene	1472.972	1.5093627	754.629	0.7732726	850.24	0.871246
toluene	1321.9725	1.257345	1360.046	1.2935572	620.396	0.590067
m,p	1585.6625	0.7137801	673.958	0.3033797	711.052	0.320077
o	711.008	0.8816517	344.132	0.4267245	357.7275	0.443583
		4.3621395		2.796934		2.224973
	p1	conc.	p2	conc.	p3	conc.
benzene	1229.808	1.2601912	492.612	0.5047823	1966.428	2.01501
toluene	950.624	0.9041507	378.106	0.3596215	1160.379	1.103651
m,p	736.228	0.3314103	737.437	0.3319545	1122.775	0.505413
o	446.543	0.5537144	334.907	0.4152855	631.113	0.782582
		3.0494666		1.6116438		4.406656
day 28	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	883.124	0.9049422	1410.144	1.4449825	1410.144	1.444983
toluene	684.118	0.6506734	1053.15	1.0016644	1053.15	1.001664
m,p	827.126	0.3723277	1231.928	0.5545478	1231.928	0.554548
o	380.814	0.4722103	551.146	0.6834224	551.146	0.683422
		2.4001536		3.6846172		3.684617
	o1	conc.	o2	conc.	o3	conc.
benzene	257.97	0.2643433	1351.587	1.3849788	1500.574	1.537647
toluene	34.026	0.0323626	1047.362	0.9961594	1137.703	1.082084
m,p	951.09	0.4281296	1273.916	0.5734486	1300.282	0.585317
o	469.678	0.5824019	558.858	0.6929853	605.9935	0.751433
		1.3072374		3.6475721		3.956481
	y1	conc.	y2	conc.	y3	conc.
benzene	1529.18	1.5669594	1535.545	1.5734816	1619.849	1.659868
toluene	1160.709	1.1039652	1168.288	1.1111737	1221.8	1.16207
m,p	1398.114	0.6293558	1417.542	0.6381013	1474.1175	0.663569
o	620.907	0.7699262	619.206	0.767817	665.7635	0.825548
		4.0702066		4.0905736		4.311055
	p1	conc.	p2	conc.	p3	conc.
benzene	1305.007	1.3372481	1387.142	1.4214122	1231.965	1.262402
toluene	980.122	0.9322066	1038.114	0.9873635	915.984	0.871204
m,p	1123.272	0.5056367	1194.07	0.5375062	1071.126	0.482163
o	532.47	0.6602641	564.859	0.7004266	520.607	0.645554
		3.4353555		3.6467085		3.261323
day 35	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	1401.814	1.4745227	1187.104	1.2486762	753.608	0.792696
toluene	1083.586	1.0578795	901.036	0.8796603	580.94	0.567158
m,p	1375.002	0.6300124	1066.366	0.4885984	893.526	0.409405
o	608.3645	0.8010066	463.972	0.6108914	389.64	0.513022
		3.9634212		3.2278263		2.28228
	o1	conc.	o2	conc.	o3	conc.

benzene	96.406	0.1014063	728.592	0.7663823	968.836	1.019087
toluene	0	0	560.208	0.5469179	701.774	0.685125
m,p	657.224	0.3011336	688.148	0.3153026	893.884	0.409569
o	341.738	0.4499513	304.934	0.4014931	382.348	0.503421
		0.8524912		2.0300959		2.617202
	y1	conc.	y2	conc.	y3	conc.
benzene	1497.814	1.575502	1078.178	1.1341005	1523.732	1.602764
toluene	1151.413	1.1240974	809.358	0.7901572	1171.868	1.144067
m,p	1338.956	0.6134964	959.846	0.439792	1364.926	0.625396
o	636.706	0.8383226	501.262	0.6599895	629.761	0.829178
		4.1514185		3.0240391		4.201406
	p1	conc.	p2	conc.	p3	conc.
benzene	1317.352	1.3856799	964.6495	1.0146835	969.232	1.019504
toluene	1002.208	0.9784321	700.722	0.6840984	715.54	0.698565
m,p	1244.658	0.57029	779.185	0.3570149	885.107	0.405547
o	548.244	0.7218486	378.579	0.4984582	394.47	0.519381
		3.6562506		2.554255		2.642997
day 42	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	875.37	0.91885	1289.17	1.3532036	1328.236	1.39421
toluene	659.99	0.6032815	975.911	0.8920576	1036.5115	0.947451
m,p	826.591	0.3671943	1122.61	0.498694	1237.528	0.549744
o	364.858	0.4694338	523.112	0.6730466	549.814	0.707402
		2.3587595		3.4170017		3.598807
	o1	conc.	o2	conc.	o3	conc.
benzene	41.508	0.0435697	1599.664	1.67912	1521.052	1.596603
toluene	73.299	0.0670009	1199.227	1.0961856	1105.124	1.010168
m,p	293.4535	0.13036	1437.474	0.6385651	1309.536	0.581732
o	149.857	0.1928091	641.097	0.8248485	595.819	0.766593
		0.4337398		4.2387192		3.955096
	y1	conc.	y2	conc.	y3	conc.
benzene	1431.859	1.50298	1579.495	1.6579492	1503.721	1.578411
toluene	1093.724	0.9997477	1203.417	1.1000155	1134.241	1.036783
m,p	1342.164	0.5962258	1489.098	0.6614979	1329.85	0.590756
o	591.728	0.7613293	643.56	0.8280174	612.5235	0.788085
		3.8602829		4.2474801		3.994036
	p1	conc.	p2	conc.	p3	conc.
benzene	826.484	0.8675358	1370.634	1.4387139	1113.827	1.169151
toluene	628.806	0.574777	1025.549	0.9374305	827.672	0.756556
m,p	837.2655	0.3719362	1150.801	0.5112172	991.034	0.440244
o	354.864	0.4565753	561.664	0.7226484	465.022	0.598307
		2.2708242		3.61001		2.964258
day 55	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	1336.068	1.402431	1326.368	1.3922492	1308.484	1.373477
toluene	1037.422	0.9482834	1002.006	0.9159104	1007.982	0.921373
m,p	1237.966	0.5499383	1125.732	0.5000808	1209.941	0.537489

o	559.63	0.7200314	530.672	0.6827734	564.514	0.726315
		3.620684		3.4910139		3.558654
	o1	conc.	o2	conc.	o3	conc.
benzene	2478.588	2.6017005	1302.625	1.3673269	1416.654	1.48702
toluene	1685.656	1.540819	972.14	0.8886106	1043.738	0.954057
m,p	2210.688	0.9820479	1157.79	0.5143219	1237.3655	0.549671
o	1093.696	1.4071716	515.438	0.6631731	594.3825	0.764745
		6.531739		3.4334325		3.755493
	y1	conc.	y2	conc.	y3	conc.
benzene	1480.654	1.5541987	1477.461	1.5508471	1583.739	1.662404
toluene	1125.285	1.0285969	1137.44	1.0397075	1289.056	1.178296
m,p	1427.2	0.6340012	1325.1495	0.5886675	1493.9605	0.663658
o	616.0125	0.7925743	595.762	0.7665196	677.2825	0.871406
		4.009371		3.9457417		4.375764
	p1	conc.	p2	conc.	p3	conc.
benzene	1244.088	1.3058824	1178.43	1.2369631	1236.69	1.298117
toluene	924.836	0.8453711	885.492	0.8094077	942.9525	0.861931
m,p	1066.76	0.4738839	1060.37	0.4710453	1045.263	0.464334
o	519.869	0.6688741	487.326	0.6270036	509.74	0.655842
		3.2940114		3.1444196		3.280224
day 63	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	1045.102	1.1045955	1119.652	1.1833894	995.271	1.051928
toluene	798.441	0.7757126	875.074	0.8501642	764.47	0.742709
m,p	977.3065	0.4460957	968.585	0.4421148	927.945	0.423564
o	437.8865	0.5721165	436.1545	0.5698535	405.476	0.529771
		2.8985203		3.0455218		2.747972
	o1	conc.	o2	conc.	o3	conc.
benzene	1859.034	1.9648614	1070.874	1.1318346	1080.686	1.142205
toluene	1234.796	1.1996464	802.524	0.7796794	801.56	0.778743
m,p	1605.282	0.7327378	949.783	0.4335325	943.9215	0.430857
o	786.688	1.0278398	418.194	0.5463874	413.434	0.540168
		4.9250854		2.8914339		2.891973
	y1	conc.	y2	conc.	y3	conc.
benzene	970.471	1.0257161	960.475	1.015151	1055.624	1.115716
toluene	727.253	0.7065511	719.869	0.6993772	787.362	0.764949
m,p	888.778	0.4056865	853.242	0.3894659	939.326	0.428759
o	383.508	0.5010688	381.315	0.4982035	426.17	0.556808
		2.6390224		2.6021977		2.866233
	p1	conc.	p2	conc.	p3	conc.
benzene	1283.26	1.3563109	904.92	0.9564335	946.482	1.000361
toluene	874.708	0.8498086	675.987	0.6567444	517.228	0.502505
m,p	1014.952	0.4632792	756.3315	0.3452307	600.836	0.274254
o	487.548	0.6370012	363.358	0.474742	297.46	0.388644
		3.3063999		2.4331506		2.165764
day 70	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.

benzene	1113.862	1.1772697	1051.638	1.1115036	1297.616	1.371484
toluene	860.225	0.8357379	772.5	0.7505101	984.882	0.956846
m,p	1033.119	0.4715716	865.16	0.394906	1179.697	0.538478
o	467.373	0.6106418	401.336	0.5243618	526.442	0.687818
		3.095221		2.7812814		3.554626
	o1	conc.	o2	conc.	o3	conc.
benzene	1914.166	2.0231319	991.999	1.0484696	812.69	0.858953
toluene	1285.3515	1.2487628	752.506	0.7310852	568.942	0.552747
m,p	1708.376	0.7797955	911.82	0.4162041	679.4465	0.310136
o	849.708	1.110178	390.63	0.5103739	307.9965	0.40241
		5.1618681		2.7061328		2.124246
	y1	conc.	y2	conc.	y3	conc.
benzene	1201.554	1.2699537	931.682	0.984719	1164.948	1.231264
toluene	899.829	0.8742145	716.049	0.695666	882.575	0.857452
m,p	1098.07	0.5012187	822.4235	0.3753987	1031.5265	0.470845
o	498.83	0.6517416	366.3045	0.4785917	464.7585	0.607226
		3.2971286		2.5343753		3.166786
	p1	conc.	p2	conc.	p3	conc.
benzene	888.574	0.939157	1050.764	1.1105798	925.346	0.978022
toluene	723.061	0.7024784	838.084	0.8142271	532.599	0.517438
m,p	833.602	0.3805012	952.7545	0.4348889	624.697	0.285146
o	497.952	0.6505945	546.8075	0.7144262	295.883	0.386583
		2.672731		3.074122		2.167189
day 90	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	1136.068	0.9802986	1126.368	0.9719286	1308.484	1.129074
toluene	937.422	1.0079806	802.006	0.862372	1007.982	1.083852
m,p	1037.966	0.5121206	925.732	0.4567456	1209.941	0.596971
o	359.63	0.5019541	430.672	0.601111	564.514	0.787921
		3.0023538		2.8921572		3.597818
	o1	conc.	o2	conc.	o3	conc.
benzene	1638.033	1.4134377	1053.826	0.909333	1011.186	0.872539
toluene	1103.222	1.1862602	792.622	0.8522817	697.355	0.749844
m,p	1450.9	0.7158575	962.326	0.4748007	832.086	0.410542
o	741.126	1.0344276	427.986	0.597362	386.422	0.539349
		4.3499831		2.8337774		2.572274
	y1	conc.	y2	conc.	y3	conc.
benzene	1240.573	1.0704746	1078.469	0.9305971	923.326	0.796726
toluene	934.713	1.0050677	812.659	0.8738269	743.816	0.799802
m,p	1144.057	0.5644647	960.359	0.4738302	762.966	0.376439
o	505.212	0.7051503	432.841	0.6041384	358.71	0.50067
		3.3451573		2.8823926		2.473637
	p1	conc.	p2	conc.	p3	conc.
benzene	1021.0225	0.8810273	937.096	0.8086082	824.98	0.711865
toluene	815.798	0.8772022	693.442	0.7456366	609.408	0.655277
m,p	878.991	0.4336841	776.782	0.3832554	697.7535	0.344264
o	414.71	0.578832	376.2115	0.5250977	342.4105	0.47792

		2.7707456		2.4625978		2.189326
day 97	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**	**peak area**	**C (mg/L)**
	r1	conc.	r2	conc.	r3	conc.
benzene	673.313	0.5809932	753.481	0.6501691	656.03	0.56608
toluene	513.149	0.5517731	564.612	0.6071097	503.662	0.541572
m,p	617.46	0.3046477	636.456	0.3140201	616.572	0.30421
o	284.559	0.3971736	305.852	0.4268933	275.711	0.384824
		1.8345876		1.9981923		1.796686
	o1	conc.	o2	conc.	o3	conc.
benzene	976.799	0.8428674	720.951	0.6220994	845.993	0.729997
toluene	656.112	0.7054968	543.022	0.5838946	592.498	0.637095
m,p	862.066	0.4253335	658.976	0.3251312	711.246	0.350921
o	443.286	0.618717	292.298	0.4079753	333.019	0.464812
		2.5924147		1.9391006		2.182824
	y1	conc.	y2	conc.	y3	conc.
benzene	1005.49	0.8676245	1039.079	0.896608	837.676	0.72282
toluene	751.532	0.8080989	789.599	0.8490312	628.064	0.675338
m,p	926.651	0.457199	957.496	0.4724176	711.989	0.351287
o	415.631	0.5801175	431.508	0.6022779	335.891	0.46882
		2.71304		2.8203346		2.218265
	p1	conc.	p2	conc.	p3	conc.
benzene	1088.466	0.9392234	687.245	0.5930149	784.552	0.67698
toluene	771.63	0.8297097	506.75	0.5448925	578.198	0.621718
m,p	924.948	0.4563588	569.889	0.2811767	660.283	0.325776
o	442.803	0.6180429	278.262	0.3883846	328.625	0.458679
		2.8433347		1.8074687		2.083153

Trial 3 BTX

day 7	**peak area**		**peak area**		**peak area**	
	r1	conc.	r2	conc.	r3	conc.
benzene	817.209	0.8595957	716.998	0.754187	819.942	0.86247
toluene	628.527	0.6136161	469.017	0.4578903	562.584	0.549238
m,p	568.892	0.2606607	522.4495	0.2393812	562.668	0.257809
o	249.348	0.3283055	230.5015	0.3034911	250.371	0.329652
		2.062178		1.7549496		1.999169
	o1	conc.	o2	conc.	o3	conc.
benzene	415.094	0.4366239	641.72	0.6750045	808.524	0.85046
toluene	404.413	0.3948189	451.158	0.4404549	564.899	0.551498
m,p	414.4985	0.1899191	441.251	0.2021769	770.051	0.35283
o	125.98	0.1658723	190.3515	0.2506274	260.503	0.342993
		1.1872342		1.5682637		2.09778
	b1	conc.	b2	conc.	b3	conc.
benzene	869.878	0.9149965	1223.344	1.2867959	1313.001	1.381103
toluene	604.974	0.5906219	918.306	0.8965206	888.5	0.867422
m,p	707.9385	0.3243704	859.2025	0.3936781	898.534	0.411699
o	303.8735	0.4000968	389.565	0.512923	423.7675	0.557956

		2.2300856		3.0899176		3.21818
	p1	conc.	p2	conc.	p3	conc.
benzene	543.062	0.5712293	1206.726	1.269316	831.665	0.874801
toluene	367.007	0.3583003	810.108	0.7908894	548.876	0.535855
m,p	380.94	0.174543	921.4985	0.4222215	614.596	0.281602
o	155.49	0.2047268	381.5045	0.5023101	276.459	0.364001
		1.3087994		2.984737		2.056259

day 14

	r1	conc.	r2	conc.	r3	conc.
benzene	1438.895	1.6818781	1152.121	1.331778	1152.121	1.331778
toluene	972.666	1.0729307	777.8245	0.8376944	652.9	0.68687
m,p	955.01	0.4567568	780.915	0.3603373	650.907	0.288335
o	420.81	0.5370062	339.598	0.4157201	275.716	0.320315
		3.7485717		2.9455297		2.627298
	o1	conc.	o2	conc.	o3	conc.
benzene	1131.599	1.3067243	1131.599	1.3067243	1379.342	1.609174
toluene	761.0205	0.8174066	532.212	0.5411612	917.262	1.00604
m,p	749.374	0.3428689	503.089	0.2064682	906.1	0.429669
o	295.06	0.3492047	200.846	0.2085007	392.215	0.494301
		2.8162044		2.2628544		3.539185
	b1	conc.	b2	conc.	b3	conc.
benzene	1317.133	1.5332283	1079.562	1.2431964	1331.114	1.550297
toluene	1069.458	1.1897897	740.157	0.7922176	913.6165	1.001639
m,p	1151.672	0.5656746	779.289	0.3594368	877.2	0.413663
o	448.107	0.577773	334.11	0.407524	413.099	0.52549
		3.8664656		2.8023747		3.491089
	p1	conc.	p2	conc.	p3	conc.
benzene	1368.658	1.5961312	1297.019	1.5086727	1297.019	1.508673
toluene	921.804	1.0115239	864.714	0.9425979	864.714	0.942598
m,p	906.067	0.4296505	909.17	0.4313691	909.17	0.431369
o	408.799	0.5190684	377.668	0.4725758	377.668	0.472576
		3.556374		3.3552155		3.355215

day 21

	r1	conc.	r2	conc.	r3	conc.
benzene	1310.839	1.3759489	1350.737	1.4178287	1350.737	1.417829
toluene	903.382	0.8257605	919.949	0.840904	919.949	0.840904
m,p	930.726	0.4134539	894.676	0.3974395	894.676	0.397439
o	381.756	0.4911751	398.509	0.5127298	398.509	0.51273
		3.1063384		3.168902		3.168902
	o1	conc.	o2	conc.	o3	conc.
benzene	1238.79	1.3003212	1008.11	1.0581832	1248.064	1.310056
toluene	836.716	0.7648227	720.396	0.6584973	889.493	0.813065
m,p	836.03	0.3713873	678.101	0.301231	817.016	0.362941
o	372.382	0.4791143	301.402	0.38779	367.876	0.473317
		2.9156455		2.4057014		2.959378
	b1	conc.	b2	conc.	b3	conc.
benzene	1449.133	1.521112	1227.036	1.2879834	1250.143	1.312238

toluene	1137.012	1.0393163	831.9375	0.7604548	860.504	0.786567
m,p	1438.278	0.6389223	830.393	0.3688832	830.99	0.369148
o	540.862	0.6958841	380.008	0.4889261	385.448	0.495925
		3.8952347		2.9062474		2.963879
	p1	conc.	p2	conc.	p3	conc.
benzene	1259.159	1.3217019	1197.056	1.2565143	1238.79	1.300321
toluene	861.95	0.7878885	801.782	0.7328903	836.716	0.764823
m,p	818.296	0.3635094	777.736	0.3454915	836.03	0.371387
o	360.418	0.4637212	350.589	0.451075	372.382	0.479114
		2.936821		2.7859711		2.915645

day 28

	r1	conc.	r2	conc.	r3	conc.
benzene	1130.42	1.3363505	1220.294	1.4486018	1130.42	1.33635
toluene	770.332	0.8763366	825.913	0.9457678	770.332	0.876337
m,p	730.108	0.3515705	793.016	0.3908856	730.108	0.351571
o	332.1765	0.4656853	353.842	0.5026735	332.1765	0.465685
		3.0299429		3.2879286		3.029943
	o1	conc.	o2	conc.	o3	conc.
benzene	1571.924	1.8877824	1220.294	1.4486018	1203.247	1.42731
toluene	805.7335	0.9205598	825.913	0.9457678	836.284	0.958723
m,p	739.44	0.3574027	793.016	0.3908856	870.71	0.439441
o	334.621	0.4698586	353.842	0.5026735	382.202	0.551091
		3.6356035		3.2879286		3.376566
	b1	conc.	b2	conc.	b3	conc.
benzene	1433.372	1.714733	1037.263	1.2199988	1203.247	1.42731
toluene	1167.882	1.3729513	709.364	0.8001761	836.284	0.958723
m,p	1542.53	0.8593025	700.664	0.3331692	870.71	0.439441
o	558.418	0.8519343	324.643	0.4528238	382.202	0.551091
		4.7989212		2.8061678		3.376566
	p1	conc.	p2	conc.	p3	conc.
benzene	1469.2425	1.7595348	1037.263	1.2199988	1089.385	1.285098
toluene	840.3035	0.9637442	709.364	0.8001761	740.302	0.838824
m,p	815.725	0.4050778	700.664	0.3331692	725.388	0.348621
o	326.963	0.4567846	324.643	0.4528238	339.896	0.478864
		3.5851413		2.8061678		2.951407

day 49

	r1	conc.	r2	conc.	r3	conc.
benzene	1217.079	1.3407616	1005.112	1.0950455	1222.344	1.346865
toluene	1025.5	1.0743083	677.224	0.6832154	816.191	0.839267
m,p	1422.843	0.7127835	663.8635	0.2818145	800.582	0.359447
o	493.3275	0.6327785	300.2435	0.3512082	354.533	0.430377
		3.7606318		2.4112836		2.975956
	o1	conc.	o2	conc.	o3	conc.
benzene	1207.23	1.3293445	1116.19	1.2238092	1127.618	1.237057
toluene	781.924	0.8007872	833.922	0.8591778	789.441	0.809228
m,p	793.3325	0.3553305	858.0595	0.3920842	805.9225	0.362479
o	416.7665	0.5211312	446.693	0.5647724	407.775	0.508019

		3.0065933		3.0398435		2.916784
	b1	conc.	b2	conc.	b3	conc.
benzene	1217.079	1.3407616	1007.23	1.0645676	1116.19	1.17973
toluene	1025.5	1.0743083	681.924	0.6625124	753.922	0.732461
m,p	1422.843	0.7127835	693.3325	0.3164746	758.0595	0.346019
o	493.3275	0.6327785	316.7665	0.4138683	346.693	0.452968
		3.7606318		2.4574228		2.711179
	p1	conc.	p2	conc.	p3	conc.
benzene	1181.327	1.2993172	1216.19	1.3397311	1203.07	1.324522
toluene	771.19	0.7887335	853.922	0.8816366	831.179	0.856098
m,p	771.912	0.3431673	858.0595	0.3920842	812.699	0.366327
o	403.917	0.502393	446.693	0.5647724	418.6485	0.523876
		2.9336111		3.1782242		3.070823

day 56

	r1	conc.	r2	conc.	r3	conc.
benzene	603.869	0.5210708	642.153	0.5541056	1142.082	0.985488
toluene	402.054	0.4323161	426.809	0.4589344	756.609	0.813558
m,p	377.818	0.1864111	400.248	0.1974778	720.963	0.355715
o	171.055	0.2387502	181.498	0.2533261	329.964	0.460548
		1.3785483		1.4638439		2.615309
	o1	conc.	o2	conc.	o3	conc.
benzene	1135.516	0.9798222	731.637	0.6313202	601.229	0.518793
toluene	753.176	0.8098667	485.184	0.5217032	396.096	0.42591
m,p	734.28	0.3622854	456.11	0.2250395	370.0915	0.182599
o	334.461	0.4668244	205.927	0.2874229	167.15	0.2333
		2.6187987		1.6654858		1.360601
	b1	conc.	b2	conc.	b3	conc.
benzene	1232.818	1.0637829	553.094	0.4772577	414.838	0.357958
toluene	1051.688	1.1308473	371.738	0.3997183	281.582	0.302776
m,p	1484.936	0.7326505	360.754	0.1779919	273.673	0.135027
o	504.936	0.7047651	167.35	0.233579	127.316	0.177701
		3.6320458		1.2885469		0.973463
	p1	conc.	p2	conc.	p3	conc.
benzene	1069.533	0.9228864	580.818	0.5011804	256.873	0.221652
toluene	633.268	0.6809333	380.648	0.4092989	168.82	0.181527
m,p	679.975	0.3354919	370.686	0.1828922	175.2735	0.086478
o	309.634	0.4321721	167.328	0.2335483	74.024	0.103319
		2.3714837		1.3269199		0.592976

day 63

	r1	conc.	r2	conc.	r3	conc.
benzene	575.236	1.199121	492.691	0.9935807	473.2205	0.945098
toluene	381.928	0.6636621	330.547	0.5436775	319.202	0.517185
m,p	359.647	0.1738773	312.339	0.1233184	277.1775	0.085741
o	166.356	0.2796967	143.314	0.2118463	122.467	0.150459
		2.3163571		1.8724228		1.698483
	o1	conc.	o2	conc.	o3	conc.
benzene	596.529	1.2521414	527.284	1.0797186	649.148	1.383165

toluene	400.581	0.7072204	346.978	0.582047	319.36	0.517554
m,p	372.074	0.1871583	322.136	0.1337886	306.4415	0.117016
o	169.6675	0.2894479	145.89	0.2194317	134.6415	0.186309
		2.435968		2.0149859		2.204043
	b1	conc.	b2	conc.	b3	conc.
benzene	670.132	1.4354158	553.094	1.1439866	414.838	0.799724
toluene	584.8985	1.1376375	371.738	0.6398664	281.582	0.429335
m,p	812.18	0.6575077	360.754	0.1750604	273.673	0.081995
o	271.01	0.5878651	167.35	0.2826237	127.316	0.164738
		3.8184262		2.241537		1.475792
	p1	conc.	p2	conc.	p3	conc.
benzene	930.832	2.0845692	673.8505	1.444675	628.284	1.331213
toluene	542.4435	1.0384968	427.888	0.7709876	380.994	0.661481
m,p	610.984	0.4424858	432.092	0.2513006	358.623	0.172783
o	269.196	0.5825236	196.617	0.3688045	164.125	0.273127
		4.1480755		2.8357677		2.438604

day 70

	r1	conc.	r2	conc.	r3	conc.
benzene	1417.849	1.4913894	1448.118	1.5232284	1354.044	1.424275
toluene	970.3525	0.9473323	976.093	0.9529366	904.504	0.883046
m,p	951.4705	0.4359544	945.44	0.4331913	862.32	0.395107
o	431.755	0.5684727	437.5	0.5760369	396.904	0.522586
		3.4431488		3.4853932		3.225013
	o1	conc.	o2	conc.	o3	conc.
benzene	1301.151	1.3686386	1236.766	1.3009141	1438.734	1.513358
toluene	878.6875	0.8578419	829.972	0.8102821	961.783	0.938966
m,p	877.7	0.4021535	807.534	0.3700041	940.119	0.430753
o	405.188	0.5334931	373.674	0.492	435.236	0.573056
		3.1621271		2.9732003		3.456133
	b1	conc.	b2	conc.	b3	conc.
benzene	934.714	1.038948	1265.921	1.401426	1129.6465	1.252285
toluene	863.896	0.8923215	696.34	0.729772	771.481	0.802668
m,p	1316.232	0.6465651	692.382	0.368557	766.916	0.401772
o	440.949	0.6232287	327.32	0.4778283	364.285	0.525129
		3.2010633		2.9775833		2.981854
	p1	conc.	p2	conc.	p3	conc.
benzene	1338.1365	1.4075424	1369.905	1.4409587	1524.592	1.603669
toluene	780.388	0.7618745	869.466	0.8488392	990.114	0.966625
m,p	857.781	0.3930268	903.2945	0.4138806	981.7195	0.449814
o	399.419	0.5258973	413.9115	0.5449789	454.211	0.598039
		3.088341		3.2486575		3.618148

day 77

	r1	conc.	r2	conc.	r3	conc.
benzene	1618.5845	1.7025366	1618.5845	1.7025366	1605.985	1.689284
toluene	1085.566	0.9655483	1085.566	0.9655483	1068.474	0.950346
m,p	1020.01	0.4898007	1020.01	0.4898007	1018.016	0.488843
o	468.02	0.5445259	468.02	0.5445259	465.422	0.541503

		3.7024115		3.7024115		3.669976
	o1	conc.	o2	conc.	o3	conc.
benzene	1574.6215	1.6562933	1637.2155	1.7221339	1660.76	1.7469
toluene	1047.751	0.9319141	1065.45	0.9476563	1084.464	0.964568
m,p	1013.246	0.4865527	989.566	0.4751818	1013.146	0.486505
o	465.518	0.5416149	453.876	0.5280698	464.902	0.540898
		3.616375		3.6730418		3.738871
	b1	conc.	b2	conc.	b3	conc.
benzene	2214.946	2.3298299	1584.277	1.6664496	1584.82	1.667021
toluene	1975.774	1.757337	1025.1725	0.9118318	1049.0675	0.933085
m,p	2900.47	1.3927827	956.339	0.4592264	997.17	0.478833
o	949.51	1.1047237	445.77	0.5186387	467.492	0.543912
		6.5846733		3.5561466		3.622851
	p1	conc.	p2	conc.	p3	conc.
benzene	1515.096	1.5936804	1717.572	1.8066583	1714.423	1.803346
toluene	866.805	0.770973	1069.214	0.9510042	1061.394	0.944049
m,p	926.585	0.4449388	1071.282	0.5144211	1035.234	0.497111
o	428.866	0.4989715	488.732	0.5686236	471.89	0.549029
		3.3085637		3.8407072		3.793534

day 91

	r1	conc.	r2	conc.	r3	conc.
benzene	1511.8575	1.657134	1568.751	1.7314366	1522.172	1.670605
toluene	1026.662	0.9585564	1031.626	0.9642729	997.04	0.924444
m,p	952.236	0.3809631	959.98	0.3847799	931.964	0.370971
o	436.911	0.4745013	441.124	0.4800262	431.116	0.466902
		3.4711548		3.5605156		3.432922
	o1	conc.	o2	conc.	o3	conc.
benzene	1541.1905	1.6954427	1389.44	1.4972574	1577.02	1.742236
toluene	994.084	0.9210397	823.107	0.7241432	1053.876	0.989896
m,p	971.3375	0.3903778	944.233	0.3770186	1005.794	0.407361
o	442.694	0.4820851	435.522	0.4726798	456.844	0.500641
		3.4889453		3.071099		3.640134
	b1	conc.	b2	conc.	b3	conc.
benzene	2255.2405	2.6279881	1328.931	1.418233	1609.11	1.784145
toluene	2021.926	2.1046985	877.486	0.7867659	1055.535	0.991806
m,p	2978.956	1.3798886	845.293	0.3282532	1005.316	0.407125
o	969.016	1.1722982	395.793	0.4205796	471.694	0.520115
		7.2848734		2.9538317		3.703192
	p1	conc.	p2	conc.	p3	conc.
benzene	1330.89	1.4207914	1468.022	1.5998851	1559.831	1.719787
toluene	965.0665	0.8876232	839.731	0.7432873	946	0.865666
m,p	1068.635	0.4383336	894.592	0.3525516	951.084	0.380395
o	531.26	0.5982296	415.826	0.4468507	436.381	0.473806
		3.3449779		3.1425747		3.439655

day 103

	r1	conc.	r2	conc.	r3	conc.
benzene	1455.644	1.5103587	1470.725	1.5271672	1378.048	1.423874

toluene	937.332	1.0043409	963.891	1.0365266	899.142	0.95806
m,p	854.4895	0.4084096	884.6925	0.4244452	826.232	0.393407
o	394.051	0.4808523	409.528	0.5017175	381.394	0.463789
		3.4039614		3.4898565		3.23913
	o1	conc.	o2	conc.	o3	conc.
benzene	1443.7	1.4970464	1315.94	1.354651	1521.392	1.583638
toluene	934.594	1.0010228	700.84	0.7177464	939.611	1.007103
m,p	882.0125	0.4230223	832.628	0.3968028	852.552	0.407381
o	394.9385	0.4820488	385.282	0.4690304	391.164	0.47696
		3.4031403		2.9382306		3.475082
	b1	conc.	b2	conc.	b3	conc.
benzene	2013.816	2.1324714	1485.8915	1.5440711	1549.888	1.615399
toluene	1822.987	2.0776279	945.978	1.0148186	1023.528	1.108798
m,p	2710.535	1.3938333	885.088	0.4246552	966.12	0.467677
o	869.489	1.1218116	412.858	0.5062069	455.788	0.564083
		6.7257442		3.4897517		3.755957
	p1	conc.	p2	conc.	p3	conc.
benzene	1337.3005	1.3784585	1574.884	1.6432581	1527.1915	1.590102
toluene	764.9365	0.7954222	955.8505	1.0267826	918.804	0.981888
m,p	801.8155	0.3804436	946.82	0.4574303	930.8	0.448925
o	366.432	0.4436179	430.072	0.5294138	425.557	0.523327
		2.9979421		3.6568848		3.544242

day 114

	r1	conc.	r2	conc.	r3	conc.
benzene	1329.823	1.4714566	1328.09	1.4694476	1481.556	1.647348
toluene	869.924	0.8996058	872.61	0.9026221	970.1495	1.012153
m,p	808.974	0.3642121	815.587	0.3679672	902.763	0.417468
o	374.554	0.4595736	375.766	0.461341	416.82	0.521209
		3.1948481		3.2013779		3.598179
	o1	conc.	o2	conc.	o3	conc.
benzene	1291.29	1.4267884	1202.1165	1.3234168	1464.274	1.627315
toluene	890.992	0.9232639	591.0625	0.5864613	951.654	0.991384
m,p	872.057	0.4000324	854.094	0.3898325	891.119	0.410856
o	401.904	0.4994575	393.4835	0.4871781	410.145	0.511475
		3.2495422		2.7868887		3.54103
	b1	conc.	b2	conc.	b3	conc.
benzene	2076.1695	2.3366354	1378.712	1.5281296	1527.561	1.700678
toluene	1910.888	2.0685453	942.372	0.9809606	1010.428	1.057383
m,p	2902.2095	1.5528076	892.7005	0.4117543	964.094	0.452293
o	923.6475	1.2603049	420.699	0.5268659	458.686	0.582261
		7.2182933		3.4477103		3.792616
	p1	conc.	p2	conc.	p3	conc.
benzene	1314.0575	1.4531809	1515.934	1.6871999	1469.898	1.633834
toluene	741.9655	0.7559162	934.876	0.972543	884.039	0.915456
m,p	784.266	0.3501823	926.768	0.4310987	899.296	0.415499
o	360.69	0.439356	425.098	0.5332808	412.7115	0.515218
		2.9986354		3.6241225		3.480008

day 150

	r1	conc.	r2	conc.	r3	conc.
benzene	1672.4405	1.5917545	1635.256	1.5563639	1635.046	1.556164
toluene	1116.732	0.9932687	1092.514	0.9717282	1095.726	0.974585
m,p	1059.3895	0.4641356	1041.48	0.4562892	1042.1355	0.456576
o	500.525	0.5823444	490.534	0.5707202	491.6525	0.572022
		3.6315031		3.5551014		3.559347
	o1	conc.	o2	conc.	o3	conc.
benzene	1532.868	1.4589156	1209.586	1.1512301	1736.578	1.652798
toluene	1086.308	0.9662083	602.112	0.5355439	1167.851	1.038736
m,p	1073.313	0.4702357	956.024	0.4188495	1101.792	0.482713
o	508.4215	0.5915317	444.021	0.5166038	515.5445	0.599819
		3.4868913		2.6222274		3.774066
	b1	conc.	b2	conc.	b3	conc.
benzene	3013.314	3.6180279	1581.8505	1.7485445	1760.398	1.981727
toluene	2929.364	3.1497006	1076.674	1.01615	1182.784	1.138346
m,p	4733.9965	2.2449093	1076.238	0.4420809	1143.721	0.475342
o	1535.087	1.9146377	521.711	0.5857072	555.33	0.629795
		10.927276		3.7924826		4.225209
	p1	conc.	p2	conc.	p3	conc.
benzene	1483.641	1.4120635	1771.399	1.6859388	1716.2985	1.633497
toluene	856.242	0.7615779	1087.746	0.9674873	1044.073	0.928643
m,p	918.187	0.4022725	1084.1725	0.4749934	1070.118	0.468836
o	435.1105	0.5062368	497.266	0.5785526	498.9775	0.580544
		3.0821506		3.7069722		3.611519

day 167

	r1	conc.	r2	conc.	r3	conc.
benzene	1221.897	1.3645576	1390.232	1.5783923	1635.046	1.889378
toluene	789.339	0.7729393	900.216	0.8825882	1095.726	1.075933
m,p	713.792	0.3128597	819.983	0.3610232	1042.1355	0.461782
o	335.076	0.4313136	384.5015	0.49693	491.6525	0.639182
		2.8816701		3.3189337		4.066274
	o1	conc.	o2	conc.	o3	conc.
benzene	1145.765	1.2678476	1209.586	1.348919	1147.039	1.269466
toluene	782.906	0.7665775	602.112	0.587786	753.345	0.737344
m,p	758.059	0.3329372	956.024	0.4227254	698.054	0.305722
o	356.243	0.4594145	444.021	0.5759469	326.651	0.420129
		2.8267769		2.9353773		2.73266
	b1	conc.	b2	conc.	b3	conc.
benzene	912.3885	0.861904	912.3885	0.861904	1329.975	1.332573
toluene	600.797	0.5337061	600.797	0.5337061	863.826	0.770413
m,p	569.708	0.2367707	569.708	0.2367707	799.96	0.336672
o	269.5935	0.3040193	269.5935	0.3040193	383.82	0.437892
		1.9364001		1.9364001		2.877549
	p1	conc.	p2	conc.	p3	conc.
benzene	1038.798	1.1319682	1471.4935	1.6816182	1292.244	1.453919
toluene	616.2215	0.6017392	905.4665	0.8877806	778.174	0.761898

m,p	939.428	0.4151982	886.129	0.3910241	780.246	0.343
o	288.183	0.3690594	411.146	0.5323027	362.971	0.468346
		2.517965		3.4927256		3.027164

Appendix B. Methane concentrations

Trial 1

	day 0			day 6			day 14		
	C water			C water			C water		
	mg/L	Average	Std dev	mg/L	Average	Std dev	mg/L	Average	Std dev
r1	0	0	0	0	0	0	0	0	0
r2	0			0			0		
r3	0			0			0		
o1	0.003602	0.002993	0.00116	0.018518	0.006173	0.01069	0.17106	0.05702	0.098761
o2	0.003719			0			0		
o3	0.001659			0			0		
p1	0.031516	0.026175	0.00708	0.113201	0.037734	0.06536	1.045707	0.348569	0.603739
p2	0.028859			0			0		
p3	0.018151			0			0		
y1	0	0.002251	0.00212	0.033996	0.011332	0.01963	0.31404	0.10468	0.181311
y2	0.00253			0			0		
y3	0.004222			0			0		
w1	0.020564	0.017245	0.0111	0.177552	0.059184	0.10251	1.640154	0.546718	0.946944
w2	0.004867			0			0		
w3	0.026304			0			0		
b1	0.001223	0.002139	0.00188	0.030143	0.010048	0.0174	0.278451	0.092817	0.160763
b2	0.000888			0			0		
b3	0.004306			0			0		
g1	0.003267	0.003401	0.00076	0.012205	0.004068	0.00705	0.112741	0.03758	0.065091
g2	0.002714			0			0		
g3	0.004222			0			0		

	day 21			day 27			day 34		
	C water			C water			C water		
	mg/L	Average	Std dev	mg/L	Average	Std dev	mg/L	Average	Std dev
r1	0.000519	0.000173	0.0003	0	0	0	0	0	0
r2	0			0			0		
r3	0			0			0		
o1	0.004255	0.003233	0.0011	0.002463	0.002167	0.00032	0.002245	0.001737	0.000511
o2	0.002077			0.002211			0.001742		
o3	0.003367			0.001826			0.001223		
p1	0.024242	0.021137	0.0061	0.024711	0.024367	0.00338	0.040594	0.037841	0.006103
p2	0.025063			0.027561			0.030847		
p3	0.014106			0.020828			0.042082		
y1	0.000653	0.001804	0.00107	0.000892	0.001783	0.0008	0.000568	0.001569	0.00099
y2	0.001977			0.00201			0.001592		
y3	0.002781			0.002446			0.002547		
w1	0.013922	0.012733	0.00486	0.012331	0.01087	0.00423	0.011845	0.008052	0.003291
w2	0.007388			0.006104			0.005962		
w3	0.016887			0.014176			0.006348		
b1	0.000821	0.001625	0.00136	0.001055	0.001815	0.00101	0.001095	0.001722	0.000963
b2	0.000854			0.001424			0.00124		
b3	0.0032			0.002965			0.002831		
g1	0.002128	0.002256	0.001	0.001994	0.001809	0.00083	0.001726	0.001659	0.000856

g2	0.001324			0.000905			0.000771		
g3	0.003317			0.00253			0.00248		
	day 41			**day 48**			**day 57**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0	0	0	0	0	0	0	0	0
r2	0			0			0		
r3	0			0			0		
o1	0.002094	0.001754	0.0003	0.002061	0.001586	0.00049	0.001927	0.001497	0.000375
o2	0.001625			0.001608			0.001324		
o3	0.001541			0.001089			0.00124		
p1	0.08236	0.071043	0.02426	0.137814	0.111315	0.04467	0.232035	0.190469	0.083646
p2	0.043197			0.059746			0.094182		
p3	0.087571			0.136386			0.245191		
y1	0.00069	0.001419	0.00068	0.000994	0.001565	0.00065	0.000669	0.001435	0.000807
y2	0.001525			0.001424			0.001357		
y3	0.002044			0.002278			0.002278		
w1	0.012046	0.00701	0.00452	0.010772	0.005768	0.00452	0.010689	0.005456	0.004849
w2	0.005679			0.004563			0.004563		
w3	0.003306			0.001967			0.001115		
b1	0.001217	0.001707	0.0009	0.001014	0.001617	0.00075	0.000872	0.001436	0.000849
b2	0.001156			0.001374			0.001022		
b3	0.002748			0.002463			0.002412		
g1	0.001793	0.001726	0.00079	0.001826	0.001664	0.00096	0.001642	0.001519	0.000844
g2	0.000905			0.000637			0.00062		
g3	0.00248			0.00253			0.002295		
	day 70			**day 83**			**day 110**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0	0	0	0	0	0	0	0	0
r2	0			0			0		
r3	0			0			0		
o1	0.001927	0.00148	0.0004	0.001927	0.001631	0.00033	0.00196	0.001575	0.000394
o2	0.001374			0.001692			0.001592		
o3	0.001139			0.001273			0.001173		
p1	0.337096	0.278753	0.08901	0.352609	0.354541	0.03698	0.337514	0.279007	0.089119
p2	0.176298			0.318565			0.17644		
p3	0.322865			0.392447			0.323068		
y1	0.000933	0.00135	0.00047	0.000953	0.001412	0.0006	0.000913	0.001477	0.000655
y2	0.001257			0.001189			0.001324		
y3	0.00186			0.002094			0.002195		
w1	0.007857	0.004255	0.00338	0.003384	0.002487	0.00127	0.008008	0.004299	0.003425
w2	0.003752			0.003042			0.00363		
w3	0.001156			0.001034			0.001257		
b1	0.000588	0.001358	0.00081	0.001156	0.001552	0.00072	0.001075	0.001559	0.000661
b2	0.00129			0.001122			0.00129		
b3	0.002195			0.002379			0.002312		
g1	0.001508	0.001519	0.00077	0.001608	0.001508	0.00081	0.001592	0.001619	0.000662

g2	0.000754	0.000653	0.000972
g3	0.002295	0.002262	0.002295

Trial 2

	day 0 C water mg/L	Average	Std dev	day 7 C water mg/L	Average	Std dev	day 14 C water mg/L	Average	Std dev
r1	0	0	0	0	0	0	0	0	0
r2	0			0			0		
r3	0			0			0		
o1	0	0	0	0	0	0	0	0	0
o2	0			0			0		
o3	0			0			0		
p1	0.010384	0.008642	0.00181	0.059127	0.068194	0.01397	0.313875	0.246647	0.130767
p2	0.006762			0.061177			0.095942		
p3	0.008779			0.08428			0.330124		
y1	0	0	0	0	0	0	0	0	0
y2	0			0			0		
y3	0			0			0		
w1	0	0	0	0	0.00072	0.00125	0.003436	0.005756	0.007201
w2	0			0			0		
w3	0			0.002161			0.01383		
b1	0	0	0	0	0	0	0	0	0
b2	0			0			0		
b3	0			0			0		
g1	0	0	0	0	0	0	0	0	0
g2	0			0			0		
g3	0			0			0		

	day 28 C water mg/L	Average	Std dev	day 35 C water mg/L	Average	Std dev	day 42 C water mg/L	Average	Std dev
r1	0.000552	0.000354	0.00018	0.000766	0.000357	0.00039	0.000255	0.000304	5.34E-05
r2	0.000297			0			0.000361		
r3	0.000212			0.000306			0.000297		
o1	0.000403	0.000368	4.4E-05	0.000382	0.000263	0.00023	0.000148	0.000339	0.000168
o2	0.000318			0.000408			0.000403		
o3	0.000382			0			0.000467		
p1	0.440596	0.448155	0.02716	0.593287	0.542195	0.05786	0.391681	0.467161	0.076193
p2	0.47829			0.553937			0.544047		
p3	0.425578			0.479362			0.465754		
y1	0.000382	0.000417	0.00012	0	0.000399	0.00035	0.000339	0.000332	5.34E-05
y2	0.000552			0.000611			0.000382		
y3	0.000318			0.000586			0.000276		
w1	0.026982	0.022117	0.0197	0.081095	0.057739	0.04998	0.106676	0.079036	0.069052
w2	0.000445			0.000357			0.000445		

w3	0.038924			0.091767			0.129988		
b1	0	0.000198	0.00017	0.000357	0.000475	0.00011	0.000467	0.000417	6.82E-05
b2	0.000318			0.000484			0.000445		
b3	0.000276			0.000586			0.000339		
g1	0.000276	0.000212	0.00019	0.000917	0.000798	0.00016	0.000382	0.000375	1.22E-05
g2	0.000361			0.000611			0.000361		
g3	0			0.000866			0.000382		
	day 55			**day 63**			**day 70**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0.000382	0.000361	3.7E-05	0.000467	0.000424	9.2E-05	0	0.000304	0.000332
r2	0.000382			0.000488			0.000658		
r3	0.000318			0.000318			0.000255		
o1	0.000339	0.000325	6.5E-05	0.000255	8.48E-05	0.00015	0.000424	0.000424	0.000148
o2	0.000382			0			0.000573		
o3	0.000255			0			0.000276		
p1	0.443226	0.467203	0.03131	0.402075	0.369437	0.09717	0.203912	0.262598	0.098451
p2	0.50262			0.44609			0.37626		
p3	0.455763			0.260145			0.207624		
y1	0	0.000304	0.00027	0	0	0	0.000488	0.000509	0.000223
y2	0.000424			0			0.000742		
y3	0.000488			0			0.000297		
w1	0.143966	0.1057	0.09186	0.165603	0.133495	0.12025	0.091445	0.092902	0.093639
w2	0.000891			0.000445			0		
w3	0.172242			0.234436			0.18726		
b1	0.000382	0.000361	3.7E-05	0.000403	0.000134	0.00023	0	0	0
b2	0.000318			0			0		
b3	0.000382			0			0		
g1	0.000403	0.000332	7.4E-05	0.000382	0.000127	0.00022	0.000488	0.000276	0.00025
g2	0.000339			0			0.000339		
g3	0.000255			0			0		
	day 90			**day 97**			**day 125**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0.000361	0.000354	5.3E-05	0.000535	0.00045	0.00011	0.001044	0.000628	0.000554
r2	0.000404			0.000331			0.00084		
r3	0.000297			0.000484			0		
o1	0.000446	0.000432	0.00015	0.000255	0.000688	0.00071	0.000713	0.001324	0.001216
o2	0.000574			0.001503			0.002725		
o3	0.000276			0.000306			0.000535		
p1	0.276778	0.286758	0.09757	0.263508	0.291134	0.12292	0.221458	0.337268	0.182864
p2	0.388934			0.425519			0.548079		
p3	0.194563			0.184374			0.242266		
y1	0.000404	0.000347	5.3E-05	0.000331	0.000391	8.2E-05	0.000764	0.000424	0.000311
y2	0.00034			0.000484			0.000153		
y3	0.000297			0.000357			0.000357		
w1	0.298516	0.18755	0.16268	0.392587	0.254194	0.21938	0.627187	0.363705	0.321216
w2	0.000807			0.001248			0.005883		

w3	0.263327			0.368748			0.458044		
b1	0.000276	0.000361	8.5E-05	0.000331	0.000323	8.9E-05	0.000739	0.000246	0.000426
b2	0.000446			0.000408			0		
b3	0.000361			0.000229			0		
g1	0.000297	0.000297	4.2E-05	0.000306	0.000331	2.5E-05	0.000637	0.000475	0.00014
g2	0.000255			0.000331			0.000408		
g3	0.00034			0.000357			0.000382		
	day 130			**day 137**			**day 144**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0.000892	0.000545	0.00048	0.000892	0.000545	0.00048	0.000892	0.000545	0.000478
r2	0.000744			0.000744			0.000744		
r3	0			0			0		
o1	0.000637	0.002642	0.0036	0.000637	0.003138	0.00446	0.000637	0.004399	0.006644
o2	0.0068			0.008287			0.01207		
o3	0.000489			0.000489			0.000489		
p1	0.186126	0.321225	0.24195	0.160265	0.310522	0.25915	0.122845	0.275382	0.262181
p2	0.60056			0.609761			0.57812		
p3	0.176989			0.16154			0.125182		
y1	0.000659	0.000531	0.00013	0.000659	0.000531	0.00013	0.000659	0.000531	0.000127
y2	0.000531			0.000531			0.000531		
y3	0.000404			0.000404			0.000404		
w1	0.584963	0.382113	0.31468	0.548052	0.379903	0.2908	0.583539	0.388091	0.293913
w2	0.019614			0.044114			0.050086		
w3	0.541762			0.547542			0.530648		
b1	0.000616	0.000517	0.00011	0.000616	0.000517	0.00011	0.000616	0.000517	0.000107
b2	0.000531			0.000531			0.000531		
b3	0.000404			0.000404			0.000404		
g1	0.00051	0.000453	6.5E-05	0.00051	0.000453	6.5E-05	0.00051	0.000453	6.49E-05
g2	0.000467			0.000467			0.000467		
g3	0.000382			0.000382			0.000382		
	day 155			**day 166**			**day 191**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0.000892	0.000545	0.00048	0.000892	0.000545	0.00048	0.000892	0.000545	0.000478
r2	0.000744			0.000744			0.000744		
r3	0			0			0		
o1	0.000637	0.004399	0.00664	0.000637	0.004399	0.00664	0.000637	0.004399	0.006644
o2	0.01207			0.01207			0.01207		
o3	0.000489			0.000489			0.000489		
p1	0.097133	0.276891	0.29909	0.084616	0.240398	0.26489	0.084616	0.240398	0.365226
p2	0.62215			0.546246			0.546246		
p3	0.111391			0.090333			0.090333		
y1	0.000659	0.000531	0.00013	0.000659	0.000531	0.00013	0.000659	0.000531	0.000127
y2	0.000531			0.000531			0.000531		
y3	0.000404			0.000404			0.000404		
w1	0.599944	0.458952	0.1892	0.553619	0.437008	0.17378	0.553619	0.437008	0.185688
w2	0.243926			0.237274			0.237274		

w3	0.532986			0.52013			0.52013		
b1	0.000616	0.000517	0.00011	0.000616	0.000517	0.00011	0.000616	0.000517	0.000107
b2	0.000531			0.000531			0.000531		
b3	0.000404			0.000404			0.000404		
g1	0.00051	0.000453	6.5E-05	0.00051	0.000453	6.5E-05	0.00051	0.000453	6.49E-05
g2	0.000467			0.000467			0.000467		
g3	0.000382			0.000382			0.000382		

Trial 3

	day 7			**day 14**			**day 21**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0	0	0	0.000535	0.000458	7.6E-05	0.000382	0.000433	5.09E-05
r2	0			0.000458			0.000484		
r3	0			0.000382			0.000433		
o1	0	0.000102	0.00018	0.000408	0.000272	0.00024	0.000255	0.000289	8.19E-05
o2	0			0			0.000229		
o3	0.000306			0.000408			0.000382		
p1	0.000484	0.00045	0.00018	0	0.000246	0.00023	0.000382	0.000408	4.41E-05
p2	0.000255			0.000458			0.000458		
p3	0.000611			0.00028			0.000382		
y1	0	0	0	0.00084	0.000484	0.00031	0.000433	0.000509	7.64E-05
y2	0			0.000331			0.000586		
y3	0			0.00028			0.000509		
b1	0.00056	0.000543	5.3E-05	0.000306	0.000382	0.00015	0.000306	0.000391	0.000106
b2	0.000484			0.00028			0.000357		
b3	0.000586			0.00056			0.000509		
	day 28			**day 49**			**day 56**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0.000718	0.000532	0.00047	0	0.000599	0.00052	0.000758	0.000599	0.000183
r2	0.000878			0.000838			0.000638		
r3	0			0.000958			0.000399		
o1	0	0.000306	0.00027	0.001197	0.000904	0.00032	0.000599	0.000611	5.88E-05
o2	0.000439			0.000559			0.000559		
o3	0.000479			0.000958			0.000674		
p1	0.000559	0.000186	0.00032	0.001716	0.001423	0.00047	0.00415	0.002235	0.001666
p2	0			0.000878			0.001117		
p3	0			0.001676			0.001436		
y1	0.001157	0.000838	0.00028	0.001157	0.000838	0.00028	0.000279	0.000585	0.000283
y2	0.000638			0.000638			0.000638		
y3	0.000718			0.000718			0.000838		
b1	0	0.000239	0.00041	0.001237	0.001184	0.00013	0.000918	0.000692	0.000241
b2	0.000718			0.001277			0.000439		

b3	0			0.001037			0.000718		
	day 63			**day 70**			**day 77**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0.000599	0.000519	6.9E-05	0.001317	0.001397	8E-05	0.00085	0.000623	0.000197
r2	0.000479			0.001476			0.000531		
r3	0.000479			0.001397			0.000489		
o1	0.000559	0.000479	8E-05	0.001436	0.001436	0.00028	0.000595	0.000489	0.000106
o2	0.000479			0.001157			0.000489		
o3	0.000399			0.001716			0.000382		
p1	0.007741	0.004403	0.00314	0.025577	0.014564	0.01093	0.038611	0.018197	0.017853
p2	0.001516			0.003711			0.005504		
p3	0.00395			0.014405			0.010476		
y1	0.000379	0.000446	7E-05	0.001955	0.001649	0.00028	0.001169	0.000928	0.000213
y2	0.000519			0.001596			0.000765		
y3	0.000439			0.001397			0.00085		
b1	0.000519	0.000505	6.1E-05	0.000519	0.000771	0.00024	0.00085	0.000659	0.000245
b2	0.000559			0.000798			0.000744		
b3	0.000439			0.000998			0.000382		
	day 91			**day 103**			**day 114**		
	C water			**C water**			**C water**		
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**
r1	0.00085	0.000623	0.0002	0.00085	0.000623	0.0002	0.00085	0.000623	0.000197
r2	0.000531			0.000531			0.000531		
r3	0.000489			0.000489			0.000489		
o1	0.000595	0.000489	0.00011	0.000595	0.000489	0.00011	0.000595	0.000489	0.000106
o2	0.000489			0.000489			0.000489		
o3	0.000382			0.000382			0.000382		
p1	0.165727	0.077455	0.07916	0.052444	0.031117	0.01885	0.058118	0.058614	0.034873
p2	0.053868			0.024203			0.093733		
p3	0.012771			0.016702			0.023991		
y1	0.000956	0.000701	0.00023	0.001169	0.000928	0.00021	0.001169	0.001041	0.000239
y2	0.00051			0.000765			0.000765		
y3	0.000637			0.00085			0.00119		
b1	0.00085	0.000659	0.00025	0.00085	0.000659	0.00025	0.00085	0.000659	0.000245
b2	0.000744			0.000744			0.000744		
b3	0.000382			0.000382			0.000382		
	day 150			**day 167**					
	C water			**C water**					
	mg/L	**Average**	**Std dev**	**mg/L**	**Average**	**Std dev**			
r1	0.00085	0.000623	0.0002	0.00085	0.000623	0.0002			
r2	0.000531			0.000531					
r3	0.000489			0.000489					
o1	0.000595	0.000489	0.00011	0.003804	0.001679	0.00184			
o2	0.000489			0.000701					
o3	0.000382			0.000531					
p1	0.072632	0.156455	0.12113	0.072504	0.169906	0.14568			
p2	0.295329			0.337382					

p3	0.101404			0.099831		
y1	0.001169	0.001041	0.00024	0.004292	0.004292	0.00074
y2	0.000765			0.005036		
y3	0.00119			0.003549		
b1	0.00085	0.000659	0.00025	0.00085	0.000659	0.00025
b2	0.000744			0.000744		
b3	0.000382			0.000382		

Appendix C. ICP Fe

Trial 1	**day 0**		**day 6**		**day 14**	
	Fe mg/L	**avg**	**Fe mg/L**	**avg**	**Fe mg/L**	**avg**
b1	6.776	15.332	18.04	17.22	22.32	20.98
b2	18.8		17.82		23.28	
b3	20.42		15.8		17.34	
g1	18.48	21.33333	10	8.860667	8.342	10.50067
g2	23.96		7.582		11.6	
g3	21.56		9		11.56	
o1	20.54	13.03533	14.02	12.45333	16.08	18.92
o2	5.246		11.96		21.8	
o3	13.32		11.38		18.88	
p1	17.6	10.38	3.302	3.119333	2.162	2.746667
p2	6.674		3.446		3.234	
p3	6.866		2.61		2.844	
r1	5.554	6.19	4.758	5.574	5.428	6.444
r2	6.196		5.172		6.224	
r3	6.82		6.792		7.68	
w1	8.446	4.870667	1.232	3.11	1.034	4.048667
w2	3.246		2.838		4.392	
w3	2.92		5.26		6.72	
y1	5.468	8.423333	8.17	7.025333	7.322	9.491333
y2	12.96		8.312		9.952	
y3	6.842		4.594		11.2	
	day 21		**day 27**		**day 34**	
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg
b1	14.38	13.71333	12.42	14.01333	9.746	11.022
b2	12.76		14.78		12.38	
b3	14		14.84		10.94	
g1	4.94	8.226667	4.332	7.997333	9.948	10.24
g2	10.74		8.02		8.292	
g3	9		11.64		12.48	
o1	16.74	15.94667	11.64	12.20667	18	15.02667
o2	15.6		11.98		11.4	
o3	15.5		13		15.68	
p1	5.27	5.248	4.45	3.996667	5.238	4.232
p2	3.958		3.526		2.926	
p3	6.516		4.014		4.532	
r1	6.594	6.652667	4.96	5.752667	5.114	5.636667
r2	6.704		5.608		5.128	
r3	6.66		6.69		6.668	
w1	6.526	6.062667	1.874	3.913333	2.206	4.882667
w2	5.3		3.272		3.182	
w3	6.362		6.594		9.26	
y1	4.542	7.348	5.486	7.406	8.648	9.271333
y2	8.192		8.786		9.396	
y3	9.31		7.946		9.77	

	day 41		**day 48**		**day 57**	
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg
b1	14.66	15.68667	16.72	14.67333	11.56	15.64
b2	15.54		15.9		19.2	
b3	16.86		11.4		16.16	
g1	11.24	8.933333	5.032	6.174	4.786	5.512
g2	8.37		7.002		6.116	
g3	7.19		6.488		5.634	
o1	13.38	14.19333	10.78	12.58667	9.808	13.50933
o2	12.78		13.42		12.92	
o3	16.42		13.56		17.8	
p1	2.37	2.879333	2.618	2.698	4.396	4.570667
p2	2.53		1.596		4.676	
p3	3.738		3.88		4.64	
r1	6.018	5.908667	5.112	5.193333	4.172	4.972667
r2	5.854		4.83		4.948	
r3	5.854		5.638		5.798	
w1	0.644	5.796	0.774	3.059333	0.842	1.448667
w2	1.024		2.782		1.756	
w3	15.72		5.622		1.748	
y1	7.418	7.776	5.232	6.592	12.52	7.966667
y2	7.966		7.318		3.746	
y3	7.944		7.226		7.634	

	day 70	
	Fe mg/L	avg
b1	9.746	11.022
b2	12.38	
b3	10.94	
g1	9.948	10.24
g2	8.292	
g3	12.48	
o1	18	15.02667
o2	11.4	
o3	15.68	
p1	5.238	4.232
p2	2.926	
p3	4.532	
r1	5.114	5.636667
r2	5.128	
r3	6.668	
w1	2.206	4.882667
w2	3.182	
w3	9.26	
y1	8.648	9.271333
y2	9.396	
y3	9.77	

Trial 2

	day 0		**day 7**		**day 14**		**day 28**	
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg
b1	3.376	2.344	3.842	3.29	3.428	2.317333	4.152	3.226
b2	2.266		3.16		2.058		2.79	
b3	1.39		2.868		1.466		2.736	
g1	2.544	2.610667	2.804	2.941333	3.546	2.654	3.654	2.662667
g2	3.09		3.706		2.502		2.164	
g3	2.198		2.314		1.914		2.17	
o1	2.44	3.298667	4.24	2.950667	1.364	2.048	1.246	2.57
o2	3.71		2.36		2.628		3.856	
o3	3.746		2.252		2.152		2.608	
p1	0.784	1.068667	1.846	1.924	1.406	1.547333	1.272	0.848667
p2	0.978		1.762		1.744		0.646	
p3	1.444		2.164		1.492		0.628	
r1	3.058	3.444	1.064	3.374667	0.708	1.326	1.524	2.258
r2	3.684		3.568		35.62		2.138	
r3	3.59		5.492		1.944		3.112	
w1	2.064	1.719333	3.018	1.772667	2.484	2.289333	3.176	2.428
w2	2.178		1.77		1.822		2.378	
w3	0.916		0.53		2.562		1.73	
y1	2.786	3.513333	3.264	3.004	3.55	3.134667	3.584	2.597333
y2	4.09		2.756		2.702		2.44	
y3	3.664		2.992		3.152		1.768	
	day 35		**day 42**		**day 55**		**day 63**	
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg
b1	2.84	2.166667	3.132	3.468	2.036	3.286	2.708	4.044667
b2	2.562		3.818		4.518		5.12	
b3	1.098		3.454		3.304		4.306	
g1	3.196	2.012	2.676	2.400667	2.256	2.065333	5.072	3.592
g2	1.49		1.496		1.402		1.662	
g3	1.35		3.03		2.538		4.042	
o1	0.784	1.820667	0.996	1.347333	5.488	3.280667	6.332	4.055333
o2	2.566		1.8		2.49		3.278	
o3	2.112		1.246		1.864		2.556	
p1	0.604	0.778	0.492	0.444	0.458	0.318667	0.566	1.215333
p2	0.66		0.234		0.186		1.698	
p3	1.07		0.606		0.312		1.382	
r1	0.656	0.924	0.66	0.76	0.658	1.019333	0.636	0.924667
r2	0.83		1.092		0.916		1	
r3	1.286		0.528		1.484		1.138	
w1	3.126	1.811333	2.262	1.597333	2.092	1.051333	3.66	1.926667
w2	1.53		1.698		0.752		1.308	
w3	0.778		0.832		0.31		0.812	
y1	3.162	2.427333	3.136	2.266667	3.102	2.558667	2.728	1.810667
y2	2.064		1.992		2.556		1.186	

y3	2.056		1.672		2.018		1.518	
	day 70		**day 90**		**day 97**		**day 125**	
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg
b1	2.534	3.667333	0.332	3.939333	2.858	5.520667	2.458	5.94
b2	6.464		7.144		8.038		8.138	
b3	2.004		4.342		5.666		7.224	
g1	7.13	4.408667	7.382	5.433333	9.03	6.334667	4.568	4.79
g2	2.14		4.564		4.768		4.206	
g3	3.956		4.354		5.206		5.596	
o1	6.76	4.410667	8.894	5.120667	7.148	4.778	7.608	4.560667
o2	4.364		4.76		5.332		2.362	
o3	2.108		1.708		1.854		3.712	
p1	0.746	0.674	1.682	1.098667	0.766	0.728	0.738	0.511333
p2	0.478		0.904		0.7		0.486	
p3	0.798		0.71		0.718		0.31	
r1	0.694	1.087333	1.048	1.238	0.758	1.190667	0.996	1.319333
r2	1.152		1.174		0.944		1.688	
r3	1.416		1.492		1.87		1.274	
w1	2.384	1.566	2.58	1.487333	2.658	1.633333	2.314	1.596667
w2	1.252		1.254		1.046		1.166	
w3	1.062		0.628		1.196		1.31	
y1	3.622	2.130667	3.256	2.132667	3.422	1.972667	3.146	2.034
y2	1.282		1.636		1.098		1.428	
y3	1.488		1.506		1.398		1.528	
	day 130		**day 137**		**day 144**			
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg		
b1	1.648	1.252667	0.31	0.278667	2.896	7.443333		
b2	0.994		0.336		10.58			
b3	1.116		0.19		8.854			
g1	6.456	2.971333	0.438	0.318667	10.42	8.576667		
g2	1.5		0.246		7.422			
g3	0.958		0.272		7.888			
o1	0.794	1.174	0.158	0.224	8.382	6.443333		
o2	1.114		0.35		4.612			
o3	1.614		0.164		6.336			
p1	0.934	1.165333	0.554	0.682667	0.624	0.866667		
p2	1.016		0.678		0.968			
p3	1.546		0.816		1.008			
r1	2.552	2.458667	0.776	1.334	0.996	1.357333		
r2	2.154		1.264		1.252			
r3	2.67		1.962		1.824			
w1	1.458	1.619333	0.174	0.287333	2.082	1.222667		
w2	1.812		0.396		0.978			
w3	1.588		0.292		0.608			
y1	1.932	1.528667	2.318	1.741333	5.206	4.753333		
y2	1.814		2.3		2.196			
y3	0.84		0.606		6.858			

Trial 3

	day 7		**day 14**		**day 21**		**day 28**	
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg
b1	0.048	0.047333	0.0005	0.023	0.518	0.408667	0.288	0.362
b2	0.044		0.0005		0.222		0.412	
b3	0.05		0.068		0.486		0.386	
o1	1.432	1.288	1.796	2.352	1.916	1.996	2.248	2.396667
o2	1.012		1.846		2.344		2.672	
o3	1.42		3.414		1.728		2.27	
p1	1.47	2.436667	1.934	2.652	1.78	2.02	2.258	2.014667
p2	1.652		3.694		2.718		1.968	
p3	4.188		2.328		1.562		1.818	
r1	2.478	4.404667	2.17	1.942	2.626	3.662667	2.418	2.146667
r2	4.954		1.488		3.68		1.65	
r3	5.782		2.168		4.682		2.372	
w1	1.57	1.427	2.004	2.341	1.44	1.891	2.42	2.286
w2	1.528		2.044		2.038		2.698	
w3	1.284		2.678		2.342		2.152	
	day 49		**day 56**		**day 63**		**day 70**	
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg
b1	0.326	0.294	0.33	0.368	0.17	0.155333	0.232	0.252
b2	0.248		0.404		0.176		0.214	
b3	0.308		0.37		0.12		0.31	
o1	2.27	2.493333	2.018	2.521333	2.618	2.464667	2.564	2.521333
o2	2.912		3.148		2.796		2.784	
o3	2.298		2.398		1.98		2.216	
p1	1.238	1.514667	1.078	1.198	0.81	0.81	0.872	0.867333
p2	1.274		0.912		0.554		0.782	
p3	2.032		1.604		1.066		0.948	
r1	2.702	2.422667	2.446	2.556	1.844	2.024	2.812	3.052667
r2	1.972		2.188		2.04		2.586	
r3	2.594		3.034		2.188		3.76	
w1	2.736	2.949	2.748	2.91	2.504	2.465	1.344	2.116
w2	3.192		2.944		2.806		2.806	
w3	3.162		3.072		2.426		2.888	
	day 77		**day 91**		**day 103**		**day 114**	
	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg	Fe mg/L	avg
b1	1.028	0.968	0.394	0.142667	0.13	0.117333	0.472	0.355333
b2	0.652		0		0.098		0.314	
b3	1.224		0.034		0.124		0.28	
o1	3.252	3.252	2.038	2.076	0.708	1.809333	1	1.976
o2	3.66		2.076		0.546		2.394	
o3	2.844		2.114		4.174		2.534	
p1	1.056	0.642	0.468	0.653333	2.626	2.525333	0.686	0.726
p2	0.29		0.986		2.464		1.058	

p3	0.58		0.506		2.486		0.434	
r1	2.916	3.368667	1.894	1.769333	2.308	2.158	2.138	1.027333
r2	4.308		2.014		2.372		0.422	
r3	2.882		1.4		1.794		0.522	
w1	0.668	2.052	0.242	0.931	1.406	1.545	0.924	0.938
w2	3.408		0.246		0.916		6.852	
w3	3.436		1.62		1.684		0.952	

	day 150		**day 167**	
	Fe mg/L	avg	Fe mg/L	avg
b1	0.0735	0.620667	<0.0011	0
b2	0.008		<0.0011	
b3	0.0116		<0.0011	
o1	0.0388	1.792	0.0493	1.138667
o2	0.1194		0.0738	
o3	0.1106		0.0477	
p1	0.0392	1.148667	0.0189	0.357333
p2	0.0793		0.0165	
p3	0.0538		0.0182	
r1	0.1019	1.056667	0.1074	0.906
r2	0.0394		0.011	
r3	0.0172		0.0175	
w1	0.0529	1.536	0.0215	0.453333
w2	0.0832		0.0202	
w3	0.0943		0.0263	

Appendix D. Hydrogen concentration

Trial 2

day 205	H2(aq)nm	avg	day 207	H2(aq)nm	avg
r1	8.06458	>8.06458	r1	8.06458	>8.06458
r2	8.06458		r2	8.06458	
r3	8.06458		r3	8.06458	
o1	8.06458	>8.06458	o1	8.06458	>8.06458
o2	8.06458		o2	8.06458	
o3	8.06458		o3	8.06458	
p1	2.332196	2.764377	p1	0.432987	1.264042
p2	1.733885		p2	0.885491	
p3	4.22705		p3	2.473649	
y1	8.06458	>8.06458	y1	8.06458	>8.06458
y2	8.06458		y2	8.06458	
y3	8.06458		y3	8.06458	
w1	1.244365	2.048699	w1	0.457504	1.24587
w2	2.153082		w2	1.373317	
w3	2.748651		w3	1.906789	
b1	8.06458	>8.06458	b1	8.06458	>8.06458
b2	8.06458		b2	8.06458	
b3	8.06458		b3	8.06458	
g1	8.06458	>8.06458	g1	8.06458	>8.06458
g2	8.06458		g2	8.06458	
g3	8.06458		g3	8.06458	

Trial 3

day 172	H2(aq)nm	avg	day 174	H2(aq)nm	avg
r1	8.06458	>8.06458	r1	8.06458	>8.06458
r2	8.06458		r2	8.06458	
r3	8.06458		r3	8.06458	
o1	8.06458	>8.06458	o1	8.06458	>8.06458
o2	8.06458		o2	8.06458	
o3	8.06458		o3	8.06458	
p1	8.06458	6.190802	p1	3.376478	4.307615
p2	2.443245		p2	1.481786	
p3	8.06458		p3	8.06458	
y1	2.64486	2.793463	y1	1.765659	2.105716
y2	2.590908		y2	2.351148	
y3	3.144622		y3	2.20034	

Appendix E. Trial 2 Sulfur

Trial 2	day 200		Trial 3	day 180	
	S(aq)mg/L	Avg		S(aq)mg/L	Avg
b1	0	1.146667	o1	0.414	7.34
b2	0.059		o2	0.687	
b3	0.113		o3	0	
g1	0.0664	3.268	p1	0.714	6.634667
g2	0.0684		p2	0.1663	
g3	0.3554		p3	0.1149	
o1	0.0516	1.636	r1	1.629	45.33333
o2	0.1058		r2	2.204	
o3	0.088		r3	2.967	
p1	2.375	18.34267	y1	1.734	21.34667
p2	0.2985		y2	1.029	
p3	0.0779		y3	0.439	
r1	1.323	46.10667			
r2	3.022				
r3	2.571				
w1	0	11.98067			
w2	1.453				
w3	0.3441				
y1	0.1407	2.737333			
y2	0.1329				
y3	0.137				

Appendix F. Most Probable Number

Trial 2	# methanogens	#Fe-reducers
orange	120	2300000
pink	560	73
site 2	0	77000000

Trial 3	# methanogens	#Fe-reducers
site 1	220	1500
orange	550	200000

JACKSON M. SPAIN

EDUCATION:

B.S., Geology, Auburn University, 2000
M.S., Geological Sciences, Virginia Tech, 2002

WORK SUMMARY:

Mr. Spain has 2 years experience in hydrogeology involving a bioremediation investigation of benzene, toluene, ethylbenzene, and xylene (BTEX) degradation in a petroleum-contaminated aquifer in Ft. McCoy, WI. The investigation involved installation of multilevel sampling wells, monitoring of groundwater from multilevel sampling wells, collection of sediment from the site using a Geoprobe, and construction of microcosms using sediment from the site. He performed a variety of laboratory analyses for this project and integrated novel approaches into the construction of microcosms under anaerobic conditions. Mr. Spain also has experience with numerical modeling of groundwater flow and contaminant transport.

PROFESSIONAL EXPERIENCE:

August 2002 to present, Hydrogeologist, SAIC. Mr. Spain has supported preparation of CERCLA RI/FS reports for the Ravenna Army Ammunition Plant, including preparation of potentiometric maps, evaluation of groundwater flow direction, and groundwater contaminant distribution. He has also assisted in preparing RI/FS reports for the Memphis International Airport(ANG-UST5), including numerical modeling of contaminant transport.

September 2000 to August 2002 Graduate Research Assistant, Virginia Tech University. As a master's degree student at Virginia Tech, Mr. Spain designed a series of batch microcosm experiments to evaluate the role of FeIII heterogeneity in the development of redox conditions in a petroleum-contaminated aquifer with funding provided by the Petroleum Research Fund (PRF). The goal of his research was to better understand temporal changes in redox zonation, allowing for more accurate estimation of contaminant mass loss. Mr. Spain will be the primary author of a paper reporting the results of his research and a paper detailing his approach in simulating anaerobic conditions in the laboratory. Conducted gas chromatograph (GC) analyses of BTX and CH_4, and performed ferrozine analyses, which analyzed for dissolved FeII and FeIII. Hydrogen concentrations were also measured on a GC, along with estimation of redox potential using colorimetric redox dyes, in order to delineate terminal electron accepting processes (TEAPs) inside the microcosms. Designed a method for creating simulated anaerobic groundwater without using reducing agents, which were found to interfere with microbial processes in the microcosms. Analyzed arsenic-bearing iron-sulfide minerals from an abandoned arsenic mine with x-ray diffraction.

COMPUTER PROFICIENCY:

Document production and spreadsheet software experience includes Microsoft Word, Excel, Adobe Photoshop and Illustrator. Web page design experience includes Microsoft FrontPage and Macromedia Dreamweaver. Mr. Spain also has experience with Arcview GIS 3.2. Numerical modeling software experience includes Argus One, MT3D, and Groundwater Vistas, including export to Modflow88 and 2000.

MISCELLANEOUS:

Bernould M. "Bruno" Hanson Environmental Grant Recipient, AAPG Grants-in-Aid; Article in AAPG Explorer, June 2002.

CUSTOMERS:

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