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Biodegradation of Ethylene Dibromide (EDB) Under In Situ and Biostimulated Conditions at MMR

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A Masters Project Presented

by

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ABSTRACT

An extensive microcosm study was conducted to investigate the biodegradation of 1,2-dibromoethane (EDB) under *in situ* and biostimulated conditions within a plume at the Massachusetts Military Reservation in Cape Cod, MA. This particular EDB plume is unique because it has persisted for over 38 years, is more than 61 m below the ground surface, and has both aerobic and anaerobic zones with EDB levels above the maximum contaminant level (MCL) of 0.05 μg/L (ppb). Microcosms were constructed with *in situ* materials and conducted under environmentally relevant conditions (field EDB concentrations; incubated at 12°C). The results showed that natural attenuation occurred under anaerobic conditions but not under aerobic conditions. The lack of natural attenuation occurring in the aerobic zone, which is much larger than the anaerobic zone, offers valuable insight as to why EDB is so persistent at this site. EDB degradation rates were greater under biostimulated conditions for both the aerobic and anaerobic microcosms. On average, methane-amended aerobic microcosms degraded EDB at a first order rate eight times faster than unamended microcosms with the best performing replicate showing EDB degradation at a rate of 7.0 yr^{-1} (half-life ($t_{1/2}$) = 0.10 yr). The lactate amended anaerobic microcosms degraded EDB at an average first order rate of 3.5 yr^{-1} ($t_{1/2}$ = 0.20 yr) which was 19% faster than the unamended anaerobic microcosms. These results indicate potential for enhanced natural attenuation at the FS-12 site, especially under aerobic conditions.

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ABBREVIATIONS

1,2-DCA	1,2-dichlorethane
1,3-D	1,3-dichloropropene
AFB	Air Force Base
AvGas	Aviation gasoline
BGS	Below ground surface
BTEX	Benzene, toluene, ethylbenzene, and xylenes
CAH	Chlorinated aliphatic hydrocarbon
CAS	Cometabolic air sparging
CP	chloropicrin
DCE	1,2-dichloroethene
ECD	Electron capture detector
EDB	1,2-dibromoethane, ethylene dibromide
EDTA	Ethylenediaminetetraacetic acid
ETH	Ethylene
FID	Flame ionization detector
FS-12	Fuel Spill-12
GC	Gas chromatograph
MADEP	Massachusetts Department of Environmental Protection
MCL	Maximum contaminant level
MITC	methyl isothiocyanate
MMR	Massachusetts Military Reservation
MTBE	Methyl tert-butyl ether
PCE	Tetrachloroethylene

QuEChERS   Quick, easy, cheap, effective, rugged, and safe

REMChlor   Remediation evaluation model for chlorinated solvents

$t_{1/2}$ Half-life

TCD   Thermal conductivity detector

TCE   Trichloroethylene

TOC   Total organic carbon

USEPA   United States Environmental Protection Agency

UST   Underground storage tank

VC   Vinyl chloride

1.0 BACKGROUND

This project was completed in three phases. Phase I consisted of a preliminary literature review, methodology development and analytical technique advancement. A preliminary microcosm study was performed during the second phase, investigating the effects of aeration, EDB concentration, biostimulation, and nutrient addition on EDB degradation. This phase was used to develop experimental protocols and identify the primary electron donor (anaerobic biostimulation) and cosubstrate (aerobic biostimulation) to be studied further during Phase III of the project. Phase II results are shown in Appendix A. Important findings from Phase II were as follows: EDB degradation at the FS-12 site was not nutrient limited (nutrient enhanced microcosms were not used during Phase III) and lactate and methane were identified as the most promising electron donor and cosubstrate for further investigation[1]. Phase III was the bulk of the first year of this project and consisted of the construction and operation of the primary microcosms. Data obtained from this microcosm study was used to describe the kinetics of EDB degradation in a way that could be incorporated into existing groundwater fate and transport models developed by CH2MHill. This report focused on the Phase III microcosm study and the transition to the second year of this project.

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[1] Although JP-8 (jet fuel) induced the highest rate of EDB degradation under aerobic conditions it is not a feasible cosubstrate for full scale *in situ* application because it contains toxic regulated BTEX compounds which would not be allowed to enter the environment.

2.0 INTRODUCTION

The lead scavenger 1,2-dibromoethane (EDB) is a common additive to leaded gasoline, which is used to form volatile dihalides with lead deposits and reduce engine fouling [1]. Lead is no longer added to conventional motor vehicle gasoline, due to its 1980's phase-out in the United States. EDB is still used in aviation gasoline (AvGas) and other fuels used for high performance applications, such as automobile racing [2]. The current widespread presence of EDB in the subsurface is mainly from old releases, such as past underground storage tank (UST) and pipeline leaks. EDB is highly toxic, a probable carcinogen, and causes both acute and chronic health effects. Acute effects can include damage to the liver, stomach, and reproductive system while chronic health effects include damage to the respiratory system, nervous system, liver, heart, and kidneys [3]. The current United States Environmental Protection Agency (USEPA) maximum contaminant level (MCL) for EDB, of 0.05 μg/L (ppb), is the second lowest for all drinking water contaminants [4]. The Massachusetts Department of Environmental Protection (MADEP) has set an even more stringent MCL of 20 ppb [5].

EDB is one of the most commonly detected contaminants in public drinking water systems reliant on groundwater [6]. EDB is especially problematic in groundwater systems due to its mobility and persistence under certain conditions. The physical properties of EDB, including a water solubility of 4300 mg/L [7] and a low gasoline-water partition coefficient, indicate that EDB can rapidly dissolve out of free-phase gasoline. EDB is relatively hydrophilic, K_{ow} (octanol-water partition coefficient) = 58 [7], and often mobile in groundwater systems. Therefore, extensive EDB plumes can be formed downgradient of the source zone, especially in areas where natural attenuation processes are not robust and fast groundwater flows exist [8].

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EDB is particularly persistent in these downgradient zones where BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds are no longer present and aerobic conditions exist.

One particular site where EDB is persistent is Fuel Spill-12 (FS-12) on the Massachusetts Military Reservation (MMR). FS-12 is one of several long EDB plumes (>1 km), which have separated from the source zone and are no longer in the presence of dissolved BTEX compounds [9]. FS-12 was formed by a pipeline leak of approximately 265,000 L of AvGas in 1972 [10]. While petroleum hydrocarbons from the AvGas leak have been remediated, EDB still remains in the soil and groundwater [11]. This plume ranges in depth from 150 ft to 250 ft (45.7 m to 76.2 m), below the ground surface, and has both aerobic and anaerobic zones with EDB concentrations above the Massachusetts MCL of 0.02 μg/L [10]. At the start of this project (September, 2009) the EDB concentrations within the aerobic zone ranged from just above the Massachusetts MCL to approximately 30 μg/L. Several characteristics of the plume, such as its age, depth, distance from the source zone and variety of aeration conditions make this plume unique. An extremely low natural attenuation rate, of 0.04 yr^{-1} ($t_{1/2}$ = 17.33 yr) [12], has been calculated for this site using a simple mass balance and assuming first-order kinetics. However, prior to this study, the level of natural attenuation within this plume has not yet been validated through more in-depth studies and the potential for enhanced natural attenuation was not known.

Despite the importance of EDB as a groundwater contaminant, literature on EDB biodegradation is limited. Available literature on the biological degradation of EDB has shown that EDB can be degraded under both aerobic [13, 14, 15, 16, 17] and anaerobic conditions [18, 19, 20, 21]. These biological mechanisms can be divided into several categories: anaerobic reductive dehalogenation, aerobic metabolism, and aerobic co-metabolism. In general, EDB degradation rates are more rapid under anaerobic conditions and in the presence of BTEX

compounds. EDB dehalogenation has been shown to be especially favorable under methanogenic anaerobic conditions [18], where methanogens were able to degrade EDB by utilizing hydrogen and carbon dioxide [19]. Aerobic EDB degradation, especially by indigenous soil microorganisms, has been far less studied but has previously been shown to occur within soil material obtained from a shallow stream bed contaminated with EDB [16].

Although in some instances moderate to rapid EDB degradation rates have been reported, many of these studies were conducted under strictly anaerobic conditions, at relatively high temperatures (>20°C), with non-environmentally relevant EDB concentrations, or with materials not representative of conditions found within a deep aquifer. To our knowledge, no aerobic EDB degradation study has been conducted at environmentally relevant temperatures and concentrations with *in situ* materials from a deep contaminated aquifer.

The purpose of this study was to evaluate the biodegradation of EDB under natural and biostimulated conditions within a detached plume void of BTEX compounds at the MMR. Microcosms were constructed with groundwater and native soil material obtained from soil cores from the aerobic and anaerobic zones of an EDB plume at the MMR and incubated at the average *in situ* groundwater temperature of 12 ± 2°C. Microcosms were set up with varying conditions to understand the natural attenuation of EDB at the FS-12 site and to investigate the effect of aeration and EDB concentration on unamended EDB biodegradation. In addition, biostimulated microcosms using lactate for anaerobic and methane for aerobic conditions were also constructed to investigate the potential for enhanced natural attenuation at the FS-12 site. Considering the importance of EDB and the lack of knowledge surrounding EDB biodegradation within deep aquifers this study is very important.

3.0 OBJECTIVES

This project investigated the biodegradation of EDB under the natural conditions at the MMR site (natural attenuation) in order to: (a) provide kinetic data that could be incorporated into groundwater models used to predict EDB fate and transport, (b) determine whether EDB is biodegradable by members of the indigenous microbial community, and (c) determine factors that limit biodegradation of EDB by indigenous microorganisms. The specific objectives that have been achieved during this interdisciplinary research project are as follows:

1. Perform a literature review on EDB degradation.

2. Conduct microcosm studies under varying conditions to understand the natural attenuation of EDB at the FS-12 site and effects of aeration, EDB concentration, and addition of electron donors, cosubstrates, and nutrients (Phase II only) on EDB biodegradation.

3. Describe the kinetics of EDB degradation in a way that could be incorporated into groundwater fate and transport models.

4. Recommend methods for enhanced natural attenuation.

4.0 LITERATURE REVIEW

This literature review is divided into six sections including: EDB degradation (aerobic biodegradation, aerobic cometabolic biodegradation, anaerobic biodegradation, anaerobic biostimulation, and abiotic degradation), degradation of similar compounds (biodegradation, cometabolic biodegradation, and abiotic degradation), bioremediation of similar compounds (using methane and lactate in the field), EDB extraction from soil, toxicity of EDB, and EDB fate and transport modeling.

4.1 Degradation of EDB

4.1.1 Aerobic EDB Biodegradation:
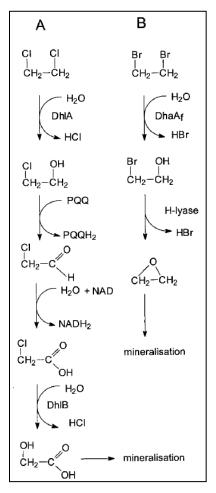
A number of researchers have shown the potential for EDB degradation under aerobic conditions. Freitas et al. [14] showed that EDB-enriched mixed cultures were able to degrade EDB at concentrations up to 1,000 mg/L as a sole source of carbon and energy without any inhibitory growth effects. The authors also indicated that mixed cultures led to the complete conversion of EDB to Br^-, CO_2, H_2O, and biomass. Freitas et al. [14] also specified that the culture could be obtained from these authors.

By studying the substrate range for the culture, the authors concluded that the aerobic EDB degradation pathway should be similar to the pathway for 1,2 dichloroethane (1,2-DCA), which is:

1,2-DCA => Chloroethanol => Chloroacetaldehyde => Chloroacetate => Glycolate

Therefore, for EDB:

EDB => Bromoethanol => Bromoacetaldehyde => Bromoacetate => Glycolate

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In the above listed analogy of pathways, the intermediate bromoacetaldehyde is cell toxic and formation of this toxic compound is circumvented in Mycobacteria. Poelarends et al. [17] described the first isolation of a microorganism that utilizes EDB as a growth substrate (GP1). Degradation occurs via a pathway in which 2-bromoethanol is converted to epoxyethane instead of the toxic bromoacetaldehyde (Figure 1).

Figure 1: (A) Proposed route of the metabolism of 1,2-DCA in *X.autotrophicus* and *A. aquaticus* strains. (B) Proposed route of the metabolism of EDB in *Mycobacterium* sp. strain GP1 from [17].

Pignatello et al. [16] examined the biodegradation of EDB by soil microorganisms under aerobic conditions at both environmentally relevant (6-8 µg/L EDB) and high (15-18 mg/L EDB)

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concentrations without the addition of supplements. Samples were collected from a site, near Windsor Locks, Connecticut, which overlies an aquifer consisting of 10 to 20 meters of sandy soil above bedrock. Samples were chosen from two sites, which represented the extremes of organic compound content and microbial activity. The first soil was composed of organic carbon-poor (0.24% total organic carbon (TOC) by weight), medium-to-coarse sand taken from the stream bed at a location where the stream was swift and shallow. Experiments with this soil (S1) were conducted with a 3:2 mixture (dry wt/vol) of solid material and accompanying stream water. The second soil sample was composed of organic-rich (14% TOC by weight), muddy soil from an area of groundwater upwelling adjacent to the stream. This soil was partially anaerobic and contained between 4.2 and 8.6 µg of EDB per kg.

Experiments with S2 were conducted after it had been mixed with distilled water and passed through a 250-µm sieve, resulting in a slurry composed of 7% solids by weight. S1 microcosms consisted of 60g of solids and 40 mL of stream water in a 125-mL screw-cap Erlenmeyer flask (actual volume, 143 mL) leaving 75 mL of headspace. Flasks were capped with Teflon Mininert valves. They were then spiked through the valves with a small volume of stock EDB (prepared in autoclaved distilled water) and incubated inverted in a thermostatically controlled Psycotherm shaker at 70 rpm and $23 \pm 3°C$. Incubations were carried out at either 6-8 µg/L EDB or 15-18 mg/L EDB. Subsamples were withdrawn, after brief shaking, using a calibrated syringe and an 18-gauge needle. The subsamples were extracted by shaking with hexane containing 1,2-dibromopropane as an internal standard (recovery of EDB ranged from 91-95%). S2 samples containing the lower concentration of EDB (6-8 µg/L) required preextraction with 2 volumes of acetone followed by partitioning between 1 volume of hexane and 5 volumes of water (recovery of EDB ranged from 86-92%). EDB concentrations were

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determined by gas chromatography on 15% OV-17 on Chromsorb W HP (80/100 mesh) with a ^{63}Ni detector.

EDB was almost completely degraded in both the S1 (sand, low organic carbon content) and S2 (muddy soil, high organic carbon content) at initial concentrations of 6-8 µg/L (environmentally relevant concentrations) with the autoclaved controls showing only minor losses. Additional spikes were also degraded. At termination the environmental relevant concentration flasks showed at least 99% removal to at or below the detection limit (0.02 µg/L). At high concentrations, EDB losses from S2 controls were coupled with stoichiometric evolution of Br$^-$. No measurable EDB loss or bromide evolution occurred in water controls. This showed that soil components can catalyze EDB hydrolosis or chemically react with EDB. Rates were considerably slower at 15-18 ppm concentrations that they were at 6-8 ppb. This was attributed to a possible toxic effect at higher concentrations.

4.1.2 Aerobic Cometabolic EDB Biodegradation:

Hartzell et al. [15] conducted three batch reactor studies to determine whether soil microbes could degrade EDB through cometabolic mechanisms. Methane, propane, and natural gas were the three growth substrates used during this study. The bacterial consortium used in their reactors was isolated from soils collected from the Department of Defense Housing Facility (Novato, CA). The reactors were constructed using 160 mL serum bottles and had initial EDB concentrations of 200 µg/L. EDB concentrations were reduced by more than 99% in all of their live reactors within 11 days. These results demonstrated that EDB can be degraded in the presence of propane, methane, and natural gas. From these results they concluded that

bioremediation techniques such as cometabolic air sparging may show promising results for EDB degradation when applied in the field.

4.1.3 Anaerobic EDB Biodegradation:

There are three available pathways for anaerobic biodegradation of EDB (Figure 2). These include hydrolic debromination, where EDB is converted to 2-bromoethanol, reductive dehalogenation (hydrogenolysis), where EDB is converted to bromoethane, and dibromoelimination (dihaloelimination), where EDB is converted to ethylene. Given the three different available pathways for anaerobic EDB biodegradation, significant variance in the rate of EDB degradation can be expected under anaerobic conditions.
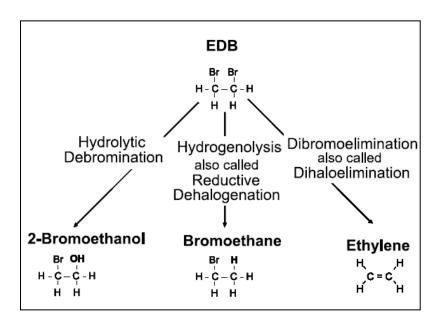
Figure 2: Biological transformations of EDB under anaerobic conditions from [2].

In a study by Bouwer and McCarty [18], EDB was transformed by reductive dehalogenation under methanogenic conditions at EDB concentrations below 100 µg/L.

However, unlike other chloroaliphatics that were completely mineralized to CO_2 under methanogenic conditions, EDB was reduced to a highly volatile hydrocarbon with the liberation of Br^-. Although no data were directly provided, it is presumed that ethylene was produced and escaped by volatilization. Overall, the authors suggested that transformation and volatilization is an important route to EDB removal from highly reducing environments.

4.1.4 Anaerobic EDB Biodegradation and Biostimulation:

Henderson et al. [20] designed a microcosm study to evaluate natural attenuation and biostimulation of EDB and 1,2-DCA in the source (343 ± 186 µg/L EDB, anaerobic) and midgradient (10.5 ± 1.9 µg/L EDB) zones of an underground storage tank (UST) leak site in Clemson, SC. Three treatments were prepared for the source and midgradient zones, in triplicate or quadruplet bottles per treatment: no amendments (to simulate natural attenuation), amended lactate (0.14 mM, to simulate biostimulation), and killed controls (autoclaved for 1 hour on three consecutive days). Biological activity identified during first 76 days. On day 76, 70 mg of 50% syrup/g soil glutaraldehyde was added and no further biological activity occurred.

The microcosms were constructed using 1.5 L of groundwater (with 1 mg/L resazurin added) and 400 grams of soil (well-mixed), leaving 0.3 L of headspace. The microcosms were incubated, undisturbed, at 23 ± 1°C in an inverted position with un-punctured septa. EDB, bromoethane, vinyl bromide, 1,2-DCA, BTEX and MTBE (methyl tert-butyl ether) were monitored by headspace analysis on a gas chromatograph (GC) equipped with both an ECD (electron capture detector) and a FID (flame ionization detector). This method was chosen over EPA method 8011 (liquid extraction using hexane) because it avoided the need for liquid

extraction and did not disturb the liquid to headspace ratio within the microcosms. Prior to sampling the headspace, the microcosms were shaken, placed in the upright position and allowed to sit overnight.

For the source zone microcosms, the average results indicated significantly better removal of EDB in the biostimulated treatment compared to the natural attenuation treatment. Less than 1% of the initial EDB concentration remained after 380 days, versus 22% in the natural attenuation treatment and 50% in the killed controls. For the midgradient microcosms, the biostimulated treatment also outperformed the natural attenuation in terms of EDB removal. Less abiotic loss occurred in the midgradient killed controls ($11.1 \pm 10.5\%$). The midgradient biostimulation treatment degraded EDB to below the MCL (0.05 µg/L) in all replicates. Lactate concentrations were monitored twice a month and when all the lactate had been consumed more was added. Approximately 12 times more lactate was consumed in the midgradient bottles. Psedofirst order degradation rates (yr^{-1}) for EDB degradation were determined to be as follows: source natural attenuation (1.5 ± 1.0), source biostimulation (5.5 ± 1.2), midgradient natural attenuation (5.4 ± 0.3), and midgradient biostimulation (9.4 ± 0.2). The EDB biodegradation observed in this study was primarily through reductive dehalogenation with a minor amount of hydrolytic debromination.

4.1.5 Abiotic EDB Degradation:

Barbash and Reinhard [22] studied the reactions of 1,2-DCA and EDB with H_2O and HS^-. Flame-sealed glass ampules were spiked with either EDB or 1,2-DCA and a 50 mM phosphate buffer containing 0.67 mM Na_2S (in the cases where reactions with HS^- were studied) and were held in the dark at 4°C for the duration of the kinetic run. 1,2-DCA and EDB concentrations

were monitored using a Carlo Erba GC equipped with a 30-m capillary column and an ECD. Using these methods, it was found that both 1,2-DCA and EDB were susceptible to abiotic dehalogenation by both H_2O and HS^- under the environmentally relevant conditions of a pH of 7, a temperature of 15°C, and a concentration of 10^{-6} to 10^{-3} M of total sulfide. The activation energies for the substitution reactions were found to be smaller when HS^- was the nucleophile than when H_2O was the nucelophile and, at 25°C and pH 7, the rate of reaction of EDB with HS^- was greater than the rate of reaction with H_2O for total sulfide concentrations greater than 40 μM. The half lives of each compound were found to be significant with respect to time scales that are typical of groundwater remediation processes under conditions of 1 mM total sulfide, pH 7, and a temperature of 15°C and, according to the authors, should be considered when determining strategies for the removal of 1,2-DCA and EDB from contaminated aquifers.

In the study by Pignatello et al. [16], an abiotic pathway of EDB degradation was theorized. Autoclaved controls containing S1 and S2 soil were found to degrade EDB, though EDB losses in these microcosms were smaller than in non-autoclaved microcosms. EDB degradation in the S2 autoclaved control was coupled with the evolution of bromide (2.0 +/- 0.3 molar equivalents), while no EDB loss or bromide was detected in autoclaved stream or distilled water controls. The authors determined that this indicated soil components could catalyze EDB hydrolysis and chemically react with EDB. The observed irreversible incorporation of ^{14}C into the soil particles of the sterile controls supports this. Products of abiotic EDB degradation were thought to include EDB hydrolysis products such as 2-bromoethanol and ethylene glycol while products of biotic EDB degradation were thought to include soluble cell components and metabolic intermediates of EDB.

4.2 Degradation of Similar Compounds - Laboratory Studies and Protocols

4.2.1 Biodegradation of Similar Compounds:

Microcosms have been used in studies investigating the biodegradation of compounds similar to EDB. In a study by Kane et al. [23], the biodegradation of MTBE was studied. Killed controls for the experiments were created by autoclaving sediments taken from the site being investigated and then adding groundwater from the same site. Sodium azide was then added to the groundwater at a concentration of two grams per liter in order to kill any remaining microorganisms. Two microcosm configurations were used. In the first microcosm set-up, 15 grams of sediment and 72 milliliters of groundwater were placed in a 125 milliliter amber glass bottle while, in the second set-up, 30 grams of sediment and 144 milliliters of groundwater were placed in a 250 milliliter amber glass bottle. The concentration of MTBE in the bottles ranged from 4.2 mg/L to 4.8 mg/L and the assembled microcosms were subjected to end-over-end mixing while being stored at a constant temperature of 4°C. The method of detection used to monitor the degradation of MTBE was purge-and-trap gas chromatography-mass spectrometry with selected ion monitoring and some of the microcosms were amended with growth medium instead of groundwater. This growth medium consisted of vitamins, trace elements, and salts. At the end of the experiment, it was determined that degradation of MTBE was hindered by the presence of water-soluble gasoline components.

In a study by van der Zaan et al. [24], microcosms were constructed in order to study the biodegradation of 1,2-DCA in selected rivers. The controls used in the experiment included sediment free microcosms, which were used to determine whether the microorganisms were linked to the river water or sediment, and autoclaved killed controls. Microcosms were prepared in 120 milliliter serum bottles with a headspace to water and soil ratio of 7:5. Each microcosm

contained 50 milliliters of river water and 1-10% (w/w) river sediments. The river water used in the microcosms contained a concentration of 1 to 2 μg/L of 1,2-DCA and, in addition, each microcosm was spiked with 100 μM 1,2-DCA. A nitrogen atmosphere was created in each anaerobic microcosm. The completed microcosms were mixed by shaking at 150 rpm for 12 months and the degradation of 1,2-DCA was monitored by headspace analysis. In order to monitor the concentration of 1,2-DCA and its dechlorination products, a 500 microliter sample was taken from the headspace of each microcosm and injected into a GC with a FID and a Porabond-Q column. Concentrations of CO_2 were determined using a GC equipped with a thermal conductivity detector with a Poraplot-Q column. Selected microcosms were amended with acetate and lactate and, at the end of the experiment, it was found that the transformation of 1,2-DCA occurred only under anaerobic conditions and that, under methanogenic conditions, degradation occurred via reductive dechlorination.

In a study by Bradley et al. [25], the degradation of 1,2-dichloroethene (DCE) and vinyl chloride (VC) was tracked. Sediment-free controls, as well as duplicate killed controls that had been autoclaved twice, served as the controls for the experiment and each microcosm was constructed in a 30 milliliter serum bottle. Fifteen grams of saturated aquifer or stream bed sediments (25% water w/w) were placed in each serum bottle and each microcosm was spiked with 5 μM DCE and 1 μM VC. Microcosms for each experimental condition (aerobic, Fe (III)-reducing, SO_4-reducing, and methanogenic conditions) were created in triplicate and all of the microcosms were stored in the dark at room temperature for 50 days. Aerobic microcosms were created with a headspace of air, while anaerobic microcosms were created with a headspace of 100% helium. One milliliter of anoxic, sterile, distilled water was added to each of the aerobic and methanogenic treatments while one milliliter of anoxic, sterile Fe-EDTA (Fe-

ethylenediaminetetraacetic acid) was added to the Fe (III)- reducing treatments and one milliliter of sterile, anoxic $MgSO_4$ was added to the SO_4-reducing treatments.

Degradation of DCE and VC were monitored by sampling for daughter products of reductive dechlorination in the headspace of each microcosm, as well as by monitoring for $^{14}CO_2$. Concentrations of degradation products of DCE and VC (including ethene, ethane, and methane) were determined using thermal conductivity detection gas chromatography and $^{14}CO_2$ was monitored by scintillation counting after entrapment in a 3 M KOH base trap attached to each microcosm. The presence of *cis*-DCE, *trans*-DCE, and VC in each microcosm at the end of the experiment was determined by shaking each microcosm vigorously and analyzing the headspace using flame ionization detection gas chromatography. No additional electron donors were added to the microcosms and, overall, the more reducing conditions proved to be the least effective for DCE and VC mineralization. However, significant mineralization occurred under both aerobic and anaerobic conditions and even under methanogenic conditions.

Freedman et al. [26] conducted a microcosm study in order to observe the degradation of tetrachloroethylene (PCE) and trichloroethylene (TCE) by microbial populations. The controls used in the experiment included water controls and killed controls. Water controls were created by adding 100 milliliters of DI water containing PCE or TCE to a 160 milliliter serum bottle while the killed controls were created by adding 100 milliliters of mixed liquor samples to 160 milliliter serum bottles and then autoclaving. Once the bottles were autoclaved, either PCE or TCE was added. The experimental microcosms were created by adding 100 milliliters of liquid obtained from an anaerobic digester (15 liters, stirred, and semicontinuous with a residence time of 20 days) to a 160 milliliter serum bottle. The liquid maintained contact with the Teflon-lined septa, closing off the serum bottle in order to minimize the loss of volatile chemicals. The

microcosms were stored at 35°C and contained 0.50 to 0.75 mg/L of PCE or 0.72 to 0.92 mg/L of TCE. Samples taken from the original bottles created were used to seed the second and third generation serum bottles and 2-10% inoculum (vol/vol) was transferred to the new bottle each time.

Volatile organic compounds (methane, ethylene (ETH), VC, DCE, PCE, TCE) were detected by injecting a 0.5 milliliter headspace sample into a GC with a FID and a stainless steel column packed with 1% SP-1000 on 60/80 Carbopack-B. Volatile compounds labeled with ^{14}C were also detected by injecting a 0.5 milliliter headspace sample into a GC, though a GC combustion technique was used instead of the flame ionization detector. Glucose, methanol, acetic acid, hydrogen gas and sodium formate were added to serve as electron donors and, by the end of the experiment, PCE and TCE had been degraded to ETH rather than the more harmful VC. EDB has also been shown to be degraded to ETH by studies cited in this article. The most effective electron donor of those used was methanol.

Wilson and Wilson [27] also investigated the microbial degradation of TCE. A soil column study was performed to study the biotransformation of TCE in soil. Sandy soil was packed into a 150 cm long glass column (5 cm, inner diameter) and TCE contaminated water was applied at a rate of 21 cm per day. Air containing 0.6% natural gas by volume was fed over the head of the column. After a three week acclimation phase, the soil column received water containing TCE at an average concentration of 150 µg/L. Extensive removal of TCE was observed; >95%. This biological activity lowered the TCE concentration by almost 1 order of magnitude during the 2 day residence time within the column. To ensure that this degradation was microbial, the column was subsequently poisoned with sodium azide (2 g/L). The amount of TCE in the effluent increased drastically after the poisoning, thus verifying that the
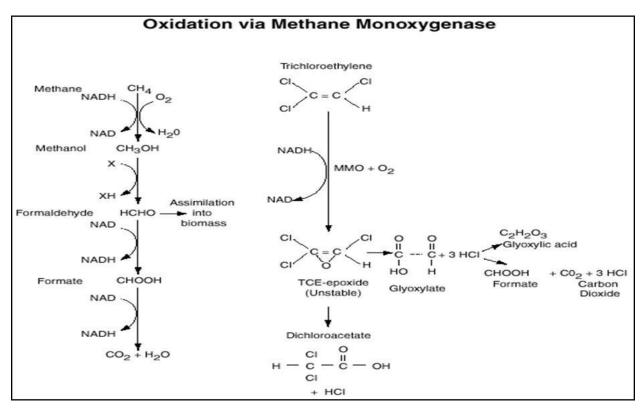
degradation was in fact biological.   Based on these results it was concluded that a TCE degradation rate of that magnitude would be adequate for *in situ* reclamation.

4.2.2 Cometabolic Degradation of Similar Compounds:

Tovanabootr et al. [28] conducted a cometabolic air sparging (CAS) demonstration at the McClellan Air Force Base (AFB) in California to treat chlorinated aliphatic hydrocarbons (CAHs) in groundwater using propane as the cometabolic substrate. The treatment system was operated as a "passive treatment system" which entailed sparging for a short duration followed by extensive periods without sparging. A designated propane-biostimulated zone was sparged with a 4% propane:air mixture at a rate of 5 scfm twice a week for a duration of 5 to 10 hours and compared to a control area which received air alone. Indigenous propane-utilizing microorganisms were successfully stimulated in the saturated zone with repeated intermediate sparging.   TCE, 1,2-*cis*-dichloroethylene (c-DCE), and dissolved oxygen concentrations decreased in proportion with propane utilization. c-DCE concentrations decreased more rapidly than TCE. After four months of repeated sparging, propane utilization rates and rates of CAH transformation decreased.   This decrease was identified as the result of nitrogen depletion; ammonia was then added with the propane:air mixture as a nitrogen source. Over a six month period, rapid propane utilization and CAH degradation was observed. c-DCE concentrations were reduced to below the detection limit of 1 ppb (from >500 ppb) and TCE concentrations ranged from less than 5 ppb to 30 ppb. TCE concentrations were also decreased in the control zone indicating that both stripping and biological activity were responsible for the contaminant removal.

In conjunction with Tovanabootr et al's work [28], Timmins et al. [29] created a 15-microcosm matrix to determine the potential for using propane-utilizing microorganisms to aerobically cometabolize TCE and c-DCE at the McClellan AFB in California. Their microcosms were constructed using soil and groundwater from a treatment location on the base where Tovanabootr et al. [28] were conducting their CAS tests using propane as the primary substrate. Environmentally relevant concentrations were used for their experiments. The results from their microcosm study were very similar to results obtained by Tovanabootr et al. [28] during their field experiment. The indigenous microorganisms at the McClellan AFB treatment area have the ability to utilize propane and transform c-DCE and TCE. Rates of transformation were more rapid for c-DCE than TCE. Nitrogen limitation occurred quickly when propane was used as a carbon source and once nitrogen levels were low propane utilization decreased and TCE transformation ceased. Given these results, they identified the amount of bioavailable nitrogen at the site as a limiting factor for CAS implementation as a form of bioremediation. Ammonia was identified as an adequate nitrogen source but the possible long-term effects of using ammonia as the sole nitrogen source were not investigated in this study.

A proposed cometabolic pathway for TCE (oxidation via the enzyme methane monoxygenase) is shown in Figure 3. Though not yet defined, it is hypothesized that EDB would undergo a similar cometabolic degradation pathway as TCE but with brominated intermediates rather than chlorinated.

Figure 3: Cometabolic TCE degradation pathway via methane monoxygenase from [30].

4.2.3 Abiotic Degradation of Similar Compounds:

He et al. [31] studied the abiotic degradation of TCE in groundwater. A column was constructed to simulate field conditions at a site where an OU-1 biowall was used to treat a TCE groundwater plume. The biowall used in the field contained 50% (v/v) tree mulch, 10% (v/v) cotton gin trash, and 40% (v/v) sand, while the column created in the lab replaced the 40% sand with 36% sand and 4% hematite in order to determine the effect of an addition of reactive Fe (III). The authors concluded that supplying the biowall with reactive iron would promote the formation of FeS, which in turn would promote an abiotic dechlorination pathway over biological reductive dechlorination. The abiotic degradation pathway results in the formation of acetylene instead of the more toxic vinyl chloride and will be most prominent in engineered systems that contain high concentrations of reactive iron.

Overall, in the microcosm studies reviewed, detection of contaminants and degradation products was accomplished through headspace sampling and the microcosms were often thoroughly mixed before sampling occurred. Killed controls were often killed by autoclaving, though sodium azide was also used in some instances, and sediment-free water controls were used in some cases. All microcosms constructed were smaller than those being used in the current EDB study and most contained higher concentrations of contaminant than those used in the current EDB study. The electron donors studied were often the same as those being used in the EDB study (methanol, lactate, hydrogen gas) and reductive dehalogenation was the most common degradation pathway of the chlorinated contaminants studied. Degradation of the chlorinated compounds was achieved under aerobic and anaerobic conditions.

4.3 Bioremediation of Similar Compounds: Use of Methane and Lactate in the Field:

In a study by Hirschorn et al. [32], lactate was used as an electron donor in an aquifer in California contaminated with 1,2-DCA and TCE. In the study, emulsified soybean oil and lactate (4%) were injected into six wells that reached an aquifer at depths ranging from 32.0 m to 35.0 m (approximately 105 feet to 115 feet). The mixture was added to water that had been removed via a well and then re-injected into the aquifer. The aquifer used in the field study was anaerobic. Dechlorination was found to account for 10.7 to 35.9%, 21.9 to 74.9%, and 54.4 to 67.8 % of 1,2-DCA, TCE and cDCE concentration loss, respectively, at the pilot test area.

Scheutz et al. [33] conducted a field study of the biodegradation of DCE and VC. Sodium lactate was injected into the contaminated aquifer at a time-weighted average concentration of 400 mg/L. The depth of the aquifer ranged from 10 m to 14 m (approximately 33 feet to 46 feet) and the aquifer was moderately anaerobic with an ORP of about +29 mV.

About 15 days after the lactate was added to the system, samples taken from a monitoring well within a 10-day groundwater travel time of the injection well indicated that DCE was being dechlorinated to VC.

Hazen et al. [34] injected mixtures of 1% methane:air and 4% methane:air into an aquifer contaminated by TCE. The depth of the contaminated aquifer ranged from 0 m to 46 m (approximately 0 feet to 151 feet) and the pulses of gas were injected via a horizontal well installed on site. Levels of TCE dropped rapidly and reached levels as low as 10 ppb after the injection of 1% methane:air. Methanotrophs were thought to be responsible for the degradation of TCE.

In a study by Semprini [35], water was drawn from a shallow aquifer contaminated with various chlorinated aliphatic hydrocarbons and methane was added to the water at the surface before being injected back into the aquifer. The methane was added at a time-averaged concentration of 6 mg/L and was determined to be the best growth substrate of those tested for degrading *t*-DCE.

4.4 Extraction of EDB and Similar Compounds from Soil:

Several methods for extracting EDB from soil were described by Steinberg et al. [36]. In order to determine the amount of EDB present in a soil sample, the soil was extracted with methanol at 75° C in a glass screw-cap vial with a Teflon-lined silicone septum. The extracted material was then diluted with water and hexane was added to the mixture in order to facilitate the transfer of the EDB into the hexane. The hexane was analyzed by gas chromatography in order to determine the concentration of EDB present in the soil.

Two methods of extracting EDB from soil into an aqueous phase were described. In order to purge EDB from the soil into an aqueous phase, a 250 mL glass gas washing bottle was filled with 100 mL of distilled water or 10 grams of soil and 100 mL of 200 mg/L NaN_3 in distilled water. An aliquot of $[^{14}C]$ EDB was added to each bottle and the contents were stirred at room temperature. Stirring took place for 5 minutes for the distilled water samples and for 3 hours for the soil suspension samples. Purging was then initiated by passing gas through an 8 mm diameter tube into the bottle. Gas exiting the bottle was carried into two 14 mL glass screw-cap septum vials containing 10 mL of hexane via Teflon tubing. Gas flow was stopped after 10 minutes and an aliquot of the hexane was added to 10 mL of scintillation fluid so that the amount of radioactive EDB removed could be determined.

In order to transfer EDB from soil to an aqueous phase using a batch method, a soil suspension containing 1 gram of soil and 5mL of 0.01M $CaCl_2$ solution was placed in a Teflon-lined screw-cap test tube and incubated. The suspensions were not shaken during the incubation period. Vials were then centrifuged and the supernatant was transferred to a vial containing 2 mL of hexane. Following this, while remaining in the same vial, the remaining soil was washed with 5 mL of 0.01M $CaCl_2$ and centrifuged again. The supernatant was extracted and combined with the initial supernatant in the original 2 mL of hexane. The hexane was analyzed by gas chromatography and the concentration of EDB leaving the soil was determined.

The soils used by Steinberg et al. [36] were obtained from agricultural areas that had once been fumigated by EDB and were located in Connecticut. The native EDB present in the soils was extremely resistant to removal by purging. While 100% of EDB newly added to the soil in the lab was removed within 100 minutes, less than 5% of native EDB was removed in that time. Native EDB was also not found to be readily available for degradation by microorganisms in the

environment. Microcosm studies were performed with each soil type and, in every case, transformation of radioactive EDB added in the lab was observed while there was no evidence of microbial degradation of native EDB. Only when soil samples were mechanically broken up in a ball mill were observed concentrations of EDB released by the soil. One sample that previously had shown a 0.1% release of EDB after a 15 minute extraction with water showed a 30% release of EDB after ten minutes of pulverization. This may mean that EDB becomes trapped in soil micropores over time, indicating that EDB once applied to a soil is not readily available for volatilization, release into aqueous solution, and degradation by microorganisms in the soil.

Pinto et al. [37] proposed a modified version of the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method that would allow the extraction of chlorinated compounds from soil. In the proposed method, 2.5 g of the soil sample were weighed in a 15 mL glass centrifuge tube with a screw cap. 1.5 mL of ultrapure water was then added to the soil in the centrifuge tube in order to make the pores in the soil more accessible to the extraction solvent and to homogenize the water content of the soil sample. The mixture of soil and water was shaken for 1 minute with a Vortex device and, following this, 2.5 mL of ethyl acetate (EtOAc) was added. Both MeCN and EtOAc were evaluated for use with EtOAc presenting advantages over MeCN. This was followed by another minute of shaking and the addition of 1 g of magnesium sulfate. Once the magnesium sulfate had been added, the tube was shaken again for one minute as quickly as possible (in order to avoid the formation of $MgSO_4$ conglomerates). The tube was centrifuged at 5000 rpm for 5 minutes and the supernatant was analyzed by gas chromatography.

GC analysis of the samples in the study by Pinto et al. [37] was performed with an Agilent 7890A chromatograph equipped with a [63]Ni micro-electron-capture detector. A DB-

VRX capillary column intended for fast gas chromatography was used with helium as the carrier gas. Splitless injection was used for the volatile compounds tested (such as chloroform) and solvent vent injection was used for the semi-volatile compounds tested (such as 1,2-dichlorobenzene and hexachlorobenzene). The splitless injection technique involved injecting 0.2 uL of sample into an injector set to 250°C and kept at that temperature throughout the analysis time. 5.0 uL of sample was used for the solvent vent injection method. In this method, the vent flow was adjusted to 20 mL/ min and the vent pressure was set to 5.00 psi. Once 30 seconds had passed, the split valve was closed and the liner was flash-heated to 300°C at a rate of 12°C/s. The analytes were then transferred from the liner to the capillary column with an injection time of 1.5 minutes and the split valve was opened.

Guo et al. [38] examined the effect of time, temperature, and extraction solvent on the extraction of fumigant residues from soils. The solvents tested were acetone, acetonitrile, ethyl acetate, hexane, and methanol while the temperatures tested were 20, 50, and 80°C. Extraction times evaluated were 1, 12, and 24 hours. Samples extracted at 20°C were shaken continuously while those extracted at 50°C and 80°C were mixed occasionally. The fumigant residues used in the experiment were 1,3-D (1,3-dichloropropene), MITC (methyl isothiocyanate), and CP (chloropicrin).

Extraction with acetonitrile in sealed vials at 80°C with a 1:1 soil/solvent ratio over a period of 24 hours was found to be the most effective. In general, the extraction efficiency increased as time and temperature increased. At lower temperatures, methanol proved to be the most effective solvent, while hexane was the least effective solvent, though more of the sample residue was extracted using acetonitrile at 80°C than methanol at 20°C. At 50°C, methanol was found to be the most effective solvent until the extraction time was extended to 12 hours or more,

at which point the acetonitrile was again determined to be the most efficient. The authors found little change in the concentration extracted after a time period of 24 hours and little change when the soil/solvent ratio decreased from 1:1, indicating that the extraction was completed in a 24 hour time period and that only a 1:1 soil/solvent ratio is required for a complete extraction. A second extraction taken after the soil sample had been washed three times with acetonitrile resulted in little fumigant extracted, indicating that the extraction process is complete after a single extraction.

4.5 Toxicity of EDB

Kszos et al. [39] studied the toxicity of EDB by placing *Daphnia magna* and *Ceriodaphnia dubia* in a 40 mL glass vial filled with dilute mineral water and injected with EDB. Based on the concentration of EDB at which half of the living organisms died after 48 hours, 48 hour LC_{50}s were calculated for *D. magna* and *C. dubia* and were determined to be 6.5 mg/L and 3.6 mg/L respectively.

4.6 EDB Fate and Transport Modeling:

Henderson et al. [8] used an analytical model to simulate the effects of partial source removal and plume remediation on EDB and 1,2-DCA plumes at contaminated underground storage tank (UST) sites. REMChlor (Remediation Evaluation Model for Chlorinated Solvents) was the model used for this study; this model accounts for variable source and plume remediation. Two extremes in UST plume behavior were considered for this model. The first was termed a "short" plume which represented the plume type likely to exist at sites where groundwater flows are on the slow side (Darcy velocity of 10 m/yr) and natural attenuation

processes are vigorous. The second was termed a "long" plume which represented the plume type likely to exist at sites with high groundwater flows (Darcy velocity of 20 m/yr) and lacking natural attenuation processes. The FS-12 plume would be considered a long plume. Unfortunately, this study focused more on the effects of source remediation, rather than the treatment of existing plumes. Yet, several pertinent conclusions were made in their discussion section. They mentioned the importance of investigating aerobic remedial techniques for EDB (and 1,2-DCA) at UST sites given that EDB (and 1,2-DCA) can serve as a growth substrate under aerobic conditions. However, they also mentioned that since there is no established S_{min} (the minimum substrate concentration that supports growth) for EDB, it is unclear whether aerobic biodegradation can be sustained at the low levels required to attain its MCL.

5.0 MATERIALS AND METHODS

5.1 Soil and Groundwater Collection

Soil core samples (15 cm diameter by 3 m length) were obtained, from the FS-12 site, by sonic core drilling in October 2009. A cross section of the FS-12 plume is shown in the Appendix. Aerobic samples were taken from the 61 m to 64 m, below ground surface (BGS), core which represented an aerobic zone with high EDB concentrations (13.1 µg/L measured on May 20, 2009). Upon retrieval of the cores, visual inspection revealed a mix of coarse and fine grained sand with a light to medium brown color, indicating an aerobic zone. Samples were extruded, in a manner as to minimize disturbance, in 30 cm intervals into headspace free sterile Pyrex® glass containers (volume 1.8 L) and stored in the dark at 10°C until use. Anaerobic samples were taken from the 67 m to 70 m BGS core which represented an anaerobic zone with low, but still over the maximum contaminant level (0.02 µg/L for MA), EDB concentrations (~0.7 µg/L). Soil material from this zone was comprised of a dark gray, very fine and densely packed silt, signifying an anaerobic zone.   Samples were again extruded with minimal disturbance in 30 cm intervals into nitrogen sparged, headspace free sterile Pyrex® glass containers, and stored in the dark at 10°C until use. Groundwater was obtained from an adjacent monitoring well with screens corresponding to the depth of both the aerobic and anaerobic zones of the plume (Table 1).

Table 1: Groundwater Monitoring Data (90MW0106)

Location	Environment	Depth (ft)	Analyses Date (DO, Temp)	Dissolved Oxygen (DO) (mg/L)	Temp (°C)	Sampling Date (EDB)	EDB Conc (µg/L) EPA Method 504.1
106A	Anaerobic	69	4/10/2008	0.31	12.5	5/7/2009	0.705
106B	Aerobic	65	5/20/2009	4.95	13.8	5/20/2009	13.1
106C	Aerobic	59	4/10/2008	10.11	13.46	5/7/2009	0.571
106D	Aerobic	54	5/9/2007	11.35	14.5	5/20/2009	ND

ND: Not Determined

5.2 Microcosms

5.2.1 Construction

Microcosms were constructed in Pyrex® glass bottles with a total working volume 1.2 L. Each bottle was fitted with an attached glass stem onto which Mininert™ valves were attached. Mininert™ valves are Teflon-lined and gas tight, which allows for liquid and/or gas sampling through replaceable septa, without allowing volatile chemicals (i.e., EDB) to escape during the sampling process. Microcosms were constructed in triplicate using soil and groundwater corresponding to the environment each set was mimicking, i.e., aerobic or anaerobic. For each aerobic microcosm, 200 grams of soil from the aerobic zone, which was homogenized by thorough mixing, was added along with 900 mL of groundwater obtained from well screen B (65 m BGS). Anaerobic microcosms were set up in a similar fashion but were continually sparged with nitrogen during the process. Each anaerobic microcosm received 200 grams of soil from the anaerobic zone, which was also homogenized by thorough mixing, along with 900 mL of groundwater obtained from well screen A (69 m BGS). The microcosms were incubated at 12 ±

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2°C (corresponding to the average groundwater temperature at the FS-12 site), without agitation, in the dark.

5.2.2 Methodology

A summary of the microcosm experiments performed in this study is shown in Table 2. An orthogonal matrix was used for our experimental design to ensure that all results were statistically independent. The base case (aerobic *in situ* unamended set), used to investigate aerobic natural attenuation at the FS-12 site, mimicked field conditions as closely as possible. Environmental conditions within the microcosms were systematically altered from the base case to examine the effects of EDB concentration, aeration (aerobic, microaerophilic, anaerobic), and substrate addition on EDB biodegradation.

Every microcosm set, excluding the base case, received a spike of EDB to bring the concentration to 50 µg/L (5X *in situ*) or 100 µg/L (10X *in situ*) which was rapidly injected through the installed Teflon lined Mininert™ valve. The higher concentration aerobic unamended microcosms were compared to the base case to investigate the effect of EDB concentration on degradation. Unamended anaerobic microcosms were compared to the 5X aerobic and microaerophilic microcosms to determine the effect of varying aeration conditions on EDB natural attenuation. Amended microcosm sets, used to investigate the effect of biostimulation on EDB degradation, received subsequent spikes of methane (aerobic) and lactate (anaerobic) to bring the starting concentration to 35 mg/L (saturation; 12.3% CH_4 in headspace) and 180 mg/L respectively.

Table 2: Primary (Phase III) Microcosm Configurations

Environment	Description	Amendments	Number of Microcosms	Target EDB Conc. (µg/L)	Comments
Aerobic	Killed Control	None	2	*In situ* (~10)	2 g/L Sodium Azide
Aerobic	*In situ*	None	3	*In situ* (~10)	Base Case (Aerobic Natural Attenuation)
Aerobic	Killed Control	None	2	5X *In situ* (~50)	10 mg/L Sodium Azide (2 g/L after Day 28)
Aerobic	50 µg/L EDB *In situ*	None	3	5X *In situ* (~50)	Effect of EDB Concentration, Effect of Aeration
Microaerophilic	50 µg/L EDB *In situ*	None	3	5X *In situ* (~50)	Effect of Aeration
Anaerobic	Killed Control	None	2	5X *In situ* (~50)	10 mg/L Sodium Azide (2 g/L after Day 28)
Anaerobic	50 µg/L EDB *In situ*	None	3	5X *In situ* (~50)	Anaerobic Natural Attenuation, Effect of Aeration
Aerobic	Killed Control	Methane (35 mg/L)	2	10X *In situ* (~100)	10 mg/L Sodium Azide (2 g/L after Day 28)
Aerobic	Co-substrate	Methane (35 mg/L)	3	10X *In situ* (~100)	Methane induced Cometabolism
Anaerobic	Killed Control	Lactate (180 mg/L)	2	10X *In situ* (~100)	10 mg/L Sodium Azide (2 g/L after Day 28)
Anaerobic	Reductive Dehalogenation	Lactate (180 mg/L)	3	10X *In situ* (~100)	Lactate induced Reductive Dehalogentation

Methane was chosen as the co-substrate for the aerobic biostimulated microcosm set because previous studies have shown that methane addition supported aerobic co-metabolism of EDB [15]. Lactate was chosen as the substrate for the anaerobic biostimulated microcosm set because it has been used in the field to induce enhanced natural attenuation of compounds similar to EDB [31, 32] and has proven potential for enhanced EDB degradation [20]. These

factors made methane and lactate two promising substrates to investigate for enhanced natural attenuation within the FS-12 plume.

Abiotic degradation was identified by comparing each set to a corresponding set of killed controls. The killed controls were inactivated using sodium azide (10 mg/L). Significant EDB losses were noted in the anaerobic killed controls during the first 28 days of incubation. On day 28, the sodium azide concentration was increased to 2 g/L in all killed controls to halt any biological activity occurring within the controls as shown in Kane et al. [23]. A sterile water control set was also operated in this study to identify any possible abiotic losses not related to the soil matrix [36]. Three microcosms at varying EDB concentrations, 1X, 5X and 10X *in situ*, were prepared in the same manner as all other microcosms but with autoclaved glass beads and filter sterilized groundwater replacing the soil medium and unfiltered groundwater, respectively. No sodium azide was added to these sterile water controls.

5.3 Chemicals

The chemicals and substrates used in this study, 1,2-dibromoethane (> 99% purity), methane (> 99.9%), and lactate were purchased from Sigma-Aldrich (St. Louis, MO). All solvents (hexane and methanol) were HPLC grade and purchased from Thermo Fisher Scientific (Waltham, MA).

5.4 Analytical Methods

EDB determination was performed using USEPA method 504.1 [40] with a Hewlett Packard 5890 Plus Gas Chromatograph (GC) equipped with an auto-sampler, capillary column (DB-1, 30 m x 0.25 mm ID, 1.0 μm film thickness), and an electron capture detector (ECD). Sample

volume was reduced from 35 mL to 10 mL in order to conserve liquid volume within each microcosm. EDB quantification during this study was not affected by using the smaller sample volume as compared with the larger volume (Figure 4). Methane levels were determined through manual headspace injection (500 μL sample volume) using a Hewlett Packard 5890 Plus GC equipped with a thermal conductivity detector (TCD). Standard operating procedures were used for measuring pH and ORP in the microcosms based on Standard Method [41] protocols.
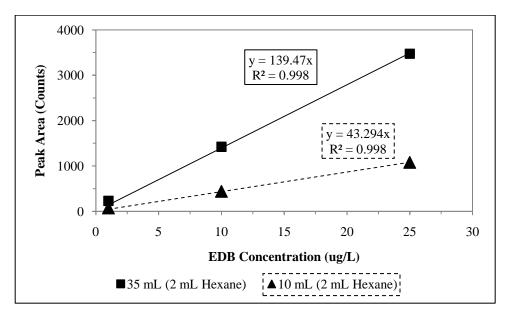
Figure 4: USEPA Method 504.1 Sample Size Reduction

5.5 Statistical Analysis

For each individual microcosm, EDB concentrations over time were plotted and fit with an exponential regression curve. Outliers were then identified, as points falling more than two standard deviations away from the regression curve, and removed. Remaining data for each set were then combined and plotted as average EDB concentrations over time with the standard deviation at each point represented by error bars. The statistical significance of each set was

determined by performing a paired Student t test ($\alpha = 0.05$) on the slopes of the regression lines, at each time step, versus the corresponding killed control set. This test determined, with a 95% confidence interval, whether the degradation seen in the live set (i.e., total degradation; biotic + abiotic degradation) was significantly different than the killed control set (i.e., solely abiotic degradation).

6.0 RESULTS AND DISCUSSION
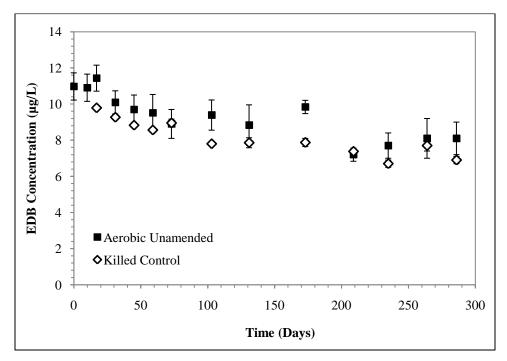
This section presents and discusses the results obtained from the primary (Phase III) microcosm experiments. Raw data tables are shown in the Appendix. The first-order decay rates reported in this section were determined from duplicate killed control and triplicate experiment microcosm bottles. The error bars shown on the plots, at each individual time step, represent the standard deviation of that set.

6.1 Natural Attenuation

6.1.1 In Situ Aerobic Conditions

EDB concentrations over time for the unamended aerobic *in situ* (base case) microcosm set and corresponding killed control set are shown in Figure 5. Total EDB loss over the 286 day incubation period averaged 26% and 29% for the base case and killed control set, respectively. Losses within filter sterilized water controls averaged 30% over the same time period (data not shown). The first-order decay rates calculated for the unamended *in situ* aerobic microcosm set and its corresponding killed control set were determined to be 0.43 ± 0.09 yr^{-1} ($t_{1/2} = 1.61$ yr) and 0.38 ± 0.03 yr^{-1} ($t_{1/2} = 1.82$ yr), respectively. These data indicate that there was no statistically significant difference (Student's *t* test, $\alpha = 0.05$) between the two rates and that any losses observed were likely to be associated with abiotic degradation. These results suggest that natural attenuation of EDB, in the aerobic zone, is not occurring at a significant rate. The results agree with previous findings by Falta [12], who found a very slow EDB natural attenuation rate of 0.04 yr^{-1} ($t_{1/2} = 17.33$ yr), based on a simple mass balance, for the entire FS-12 plume at MMR. The lack of significant EDB natural attenuation under aerobic conditions, coupled with the fact that the vast majority of the FS-12 plume is under aerobic conditions, explains why EDB is so

persistent at the current investigation site.
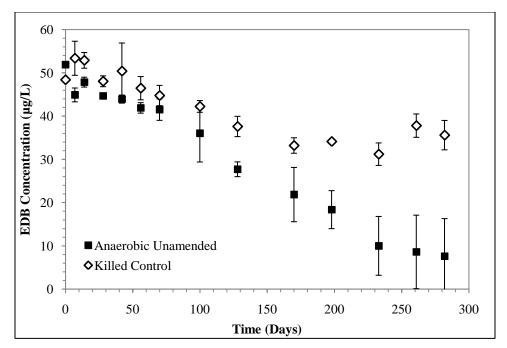
Figure 5: Average EDB concentrations for the unamended aerobic base case (*in situ*) microcosm set.

6.1.2 In Situ Anaerobic Conditions

EDB concentrations over time, for the unamended EDB spiked anaerobic microcosm set and corresponding killed control set, are shown in Figure 6. Over the 282 day incubation period, EDB losses averaged 85% and 26% for the unamended anaerobic and killed control set, respectively. The first-order decay rates calculated for the unamended anaerobic microcosm set and its corresponding killed control set were 2.96 ± 1.35 yr^{-1} ($t_{1/2} = 0.23$ yr) and 0.60 ± 0.03 yr^{-1} ($t_{1/2} = 1.16$ yr), respectively. Although a fair amount of EDB was lost in the killed controls for this set, the two rates are significantly different (Student's *t* test, $\alpha = 0.05$) which indicates the presence of anaerobic EDB biodegradation (Table 3). These data also suggest that natural

attenuation is occurring in the anaerobic zone of the FS-12 plume. The natural attenuation rate observed in this study is comparable to the rate of 5.4 ± 0.3 yr^{-1} ($t_{1/2} = 0.13$ yr) found by Henderson et al. [20] for a similar site under anaerobic conditions, but with the presence of a significant concentration of fuel hydrocarbons.
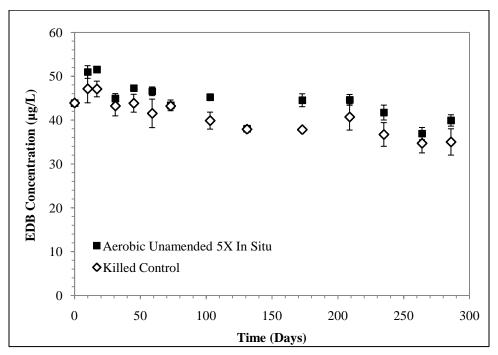
Figure 6: Average EDB concentrations for the unamended anaerobic (50 µg/L EDB spiked) microcosm set

6.2 Effect of EDB Concentration

Average EDB concentrations over time for the unamended aerobic EDB spiked microcosm set, and corresponding killed control set, are shown in Figure 7. After 286 days of incubation, EDB losses of 9% and 20% were observed in the aerobic EDB spiked and corresponding killed control set, respectively. EDB was degraded at a first-order rate of 0.26 ± 0.06 yr^{-1} ($t_{1/2} = 2.67$ yr) and 0.33 ± 0.05 yr^{-1} ($t_{1/2} = 2.10$ yr) within the live and killed control set,

respectively. Statistical analysis revealed that there was no significant difference between the two rates indicating that losses were through abiotic mechanisms. This result corresponds with the base case indicating that the presence of higher, but still environmentally relevant, EDB concentrations does not induce significant biological degradation. EDB degradation within the EDB spiked set occurred at a rate 40% slower than the *in situ* set but there was no statistically significant difference between the two rates. Given that the rates are not statistically different, it can be concluded that a five-fold increase in starting concentration had no significant impact on EDB degradation under aerobic conditions (Table 3).

Figure 7: Average EDB concentrations for the unamended aerobic (50 µg/L EDB spiked) microcosm set

6.3 Effect of Aeration

The FS-12 plume is unique because, although the majority of EDB lies within the aerobic zones of the plume, there are parts of the plume which are microaerophilic and anaerobic. The EDB concentrations within the zones lacking significant dissolved oxygen tend to be lower than those found within the aerobic zone; however, they are above the Massachusetts MCL of 0.02 µg/L. Therefore, it was important to understand the effect of aeration on EDB degradation to determine whether it has an effect on EDB degradation within the FS-12 plume. In order to perform this investigation, a microaerophilic microcosm set was constructed with water and soil from the aerobic and anaerobic zones of the FS-12 plume, respectively, and compared with the EDB spiked (5X) unamended aerobic and anaerobic sets. The microaerophilic set started with the same EDB concentration (~50 µg/L) as both of these sets. After 282 days, a first-order decay rate of 1.14 ± 0.26 yr^{-1} ($t_{1/2} = 0.61$ yr) was determined for the microaerophilic set. Comparing the high concentration (5X *in situ*) unamended aerobic, anaerobic, and microaerophilic rates to each other yielded a strong positive correlation (Figure 8). The data suggest that the more anaerobic the system, the higher the EDB degradation rate. However, it also seems to suggest that the more anaerobic the system the less predictable the rate of degradation is (higher error bars; larger standard deviation).
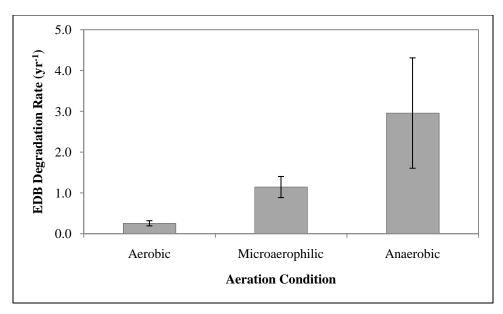
Figure 8: Effect of aeration on EDB degradation rates within unamended microcosm sets.

Statistical analysis of the live aerobic and anaerobic unamended EDB spiked (5X *in situ*) microcosm sets showed a significant difference between the two rates of EDB degradation. This indicates that the presence of oxygen inhibits EDB natural attenuation at the FS-12 site; EDB natural attenuation only occurs under strictly anaerobic conditions. The level of EDB natural attenuation occurring within the anaerobic zone of the FS-12 plume, and lack thereof within the aerobic zone, coincides with the varying concentrations and total amount of EDB amongst the two zones at the site. The vast majority and highest concentrations of EDB is located within the aerobic zone of the plume where significant EDB natural attenuation is not occurring. Therefore, it is not surprising that EDB is degrading *in situ* under anaerobic conditions; however the presence of significant natural attenuation within the anaerobic portion alone will not be able to bring the EDB concentration to the MCL at the current investigation site.

6.4 Effect of Biostimulation
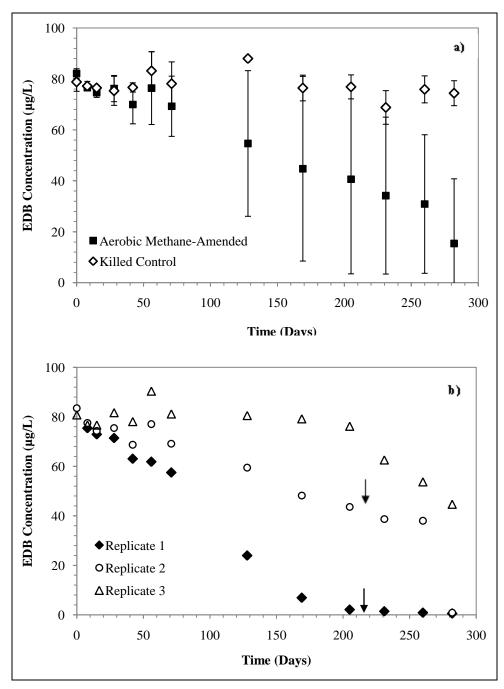
6.4.1 Effect of Co-substrate Addition under Aerobic Conditions

Concentrations of EDB over time, for the methane amended aerobic microcosm set and individual replicates from this set, are shown in Figure 9 (a) and (b), respectively. The average percent loss of EDB observed over the 282 day incubation period for this set was 81% compared to only 6% in the killed control set. The first-order decay rates calculated for the methane amended microcosm set and its corresponding killed control set were found to be 3.49 ± 3.29 yr^{-1} ($t_{1/2} = 0.20$ yr) and 0.07 ± 0.05 yr^{-1} ($t_{1/2} = 9.90$ yr), respectively. The methane amended degradation rate was approximately eight times higher than the unamended *in situ* rate of 0.43 ± 0.09 yr^{-1} ($t_{1/2} = 1.61$ yr) (Figure 5).

However, there was large variance observed amongst this microcosm set (Figure 9 (b)). After 282 days of incubation, both replicates 1 and 2 contained less than 1% of the initial EDB concentration (82 µg/L) but replicate 3 contained more than 55% of the starting EDB concentration. The corresponding decay rates for each replicate were 7.04 yr^{-1} ($t_{1/2} = 0.10$ yr), 2.88 yr^{-1} ($t_{1/2} = 0.24$ yr), and 0.55 yr^{-1} ($t_{1/2} = 1.26$ yr), respectively. In order to investigate the cause of this high variation, methane levels within each microcosm bottle of the experimental and corresponding killed control set were measured on day 220. Methane was not detected in replicates 1 and 2 but was detected in replicate 3 and two killed controls. Replicates 1 and 2 were re-spiked with methane to bring the concentration in the headspace to 10% methane. Subsequent methane measurements, taken on the last day of incubation for this study (day 282), showed no methane remaining in replicate 1, less than half of the methane spike remained in replicate 2, and more than 75% of the intital methane remained in replicate 3 and the killed controls. In addition, the emergence of a microbial mat, unique to only replicates 1 and 2, was a

strong indication of enhanced microbial activity within these bottles. Collectively, these data suggest that the EDB degradation within these replicates was the result of methane-induced cometabolism.

It is worth noting that towards the end of the incubation period, samples from replicate 3 smelled of hydrogen sulfide and black matter appeared within the top layer of the soil matrix. These are indications that this microcosm bottle (replicate 3) was not properly maintained under aerobic conditions, partly accounting for why aerobic methane-mediated EDB degradation was not occurring within replicate 3. Both replicate 1 and 2 contained less than 1% of the initial 82 µg/L of EDB but the rate of EDB degradation was much slower in replicate 2. It is possible that the microbial community within replicate 2 required a longer acclimation period than replicate 1; significant EDB degradation occurred within replicate 2 between the last two sampling events (38 µg/L on day 260 to 0.9 µg/L on day 282). Replicate 2 was re-sampled on day 282 to confirm this sudden decrease in EDB concentration and the same results were obtained.

Figure 9: EDB concentrations in aerobic methane-amended (3.4 mM) microcosm set. a) Average EDB concentrations from triplicate live replicates and duplicate killed controls and b) individual EDB concentrations from live replicates. Downward arrows signify a re-spike of methane to bring the concentration back to 3.4 mM.
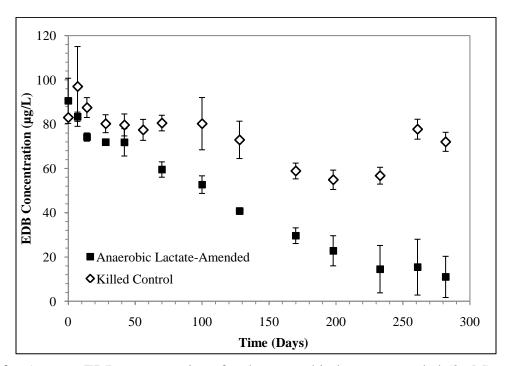
Significant variance among individual replicate EDB microcosm bottles was also observed by Henderson et al. [20], although their study was for EDB degradation under

anaerobic biostimulated conditions. In both studies, thorough mixing of the soil prior to construction was done in an effort to homogenize the material and minimize differences among replicates. However, given that microbial populations are present in such small numbers in deep soils below the water table [16] it is quite possible that varying microbial communities and densities existed among the bottles and might have caused the variation observed. Nonetheless, it needs to be emphasized that the methane amended set was the only aerobic microcosm set whose degradation rate differed significantly from its respective killed controls degradation rate, indicating the presence of enhanced biological EDB degradation under cometabolic aerobic conditions (Table 3). In addition, observations of the growth of a microbial mat coupled with the disappearance of methane in the working microcosms indicate that methane is a very promising co-substrate. A new phase of this study, reinvestigating methane and investigating more aerobic co-substrates, will be conducted in the near future by our research team.

6.4.2 Effect of Electron Donor Addition under Anaerobic Conditions

EDB concentrations over the incubation period for the lactate amended anaerobic microcosm set and corresponding killed control set are plotted in Figure 10. The average percent loss of EDB observed over the 282 day incubation period for this biostimulated set was 88% compared to only 13% in the killed control set. The first-order decay rates determined for the anaerobic lactate amended microcosm set and its corresponding killed control set were 3.52 ± 2.46 yr^{-1} ($t_{1/2} = 0.20$ yr) and 0.42 ± 0.13 yr^{-1} ($t_{1/2} = 1.65$ yr), respectively. The lactate amended set varied significantly from its corresponding killed control set however, like the methane-amended aerobic set, significant variance among individual live replicates was observed within this biostimulated set. However, within this set all replicates performed well and two of the

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replicates (replicate 2 and 3) degraded EDB at very similar rates (2.12 yr^{-1} ($t_{1/2}$ = 0.33 yr) and 2.08 yr^{-1} ($t_{1/2}$ = 0.33 yr), respectively). The best performing replicate (replicate 1) degraded EDB at a rate of 6.35 yr^{-1} ($t_{1/2}$ = 0.11 yr) over the entire incubation period but only began varying significantly from the other two replicates sometime after 128 days of incubation (Figure 11). For the first 128 days, the average EDB degradation rate amongst this set was 1.98 ± 0.21 yr^{-1} ($t_{1/2}$ = 0.35 yr) which showed significantly less deviation. However, from day 128 to day 282 (last day of incubation) EDB was degraded at an incredibly fast rate of 11.94 yr^{-1} ($t_{1/2}$ = 0.06 yr) within replicate 1[2].   Over the entire 282 day incubation period, EDB levels were reduced from 97.7 µg/L to just 0.4 µg/L in replicate 1. Despite the impressive results of replicate 1 the average rate of EDB reduction was just 19% greater than the un-amended anaerobic set (Table 3).

Figure 10: Average EDB concentrations for the anaerobic lactate-amended (2mM) microcosm set.

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[2] Please note that this rate was calculated using only 5 sampling points and is not representative of the rate of EDB degradation within replicate 1 over the entirety of the incubation period.
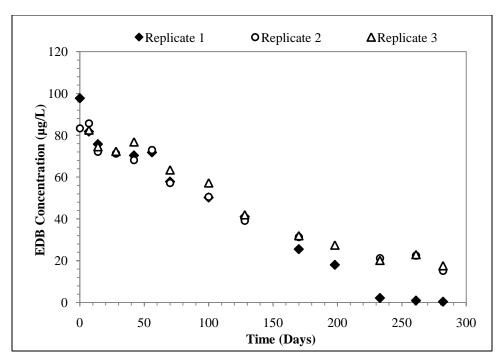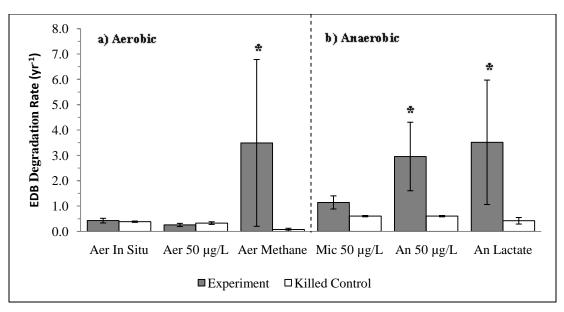
Figure 11: Individual Live Anaerobic Lactate-amended Microcosms

The improvement of lactate addition on EDB degradation versus the unamended set is significantly less than the 400% shown by Henderson et al. [20]. The difference can most likely be attributed to some differing site characteristics and incubation conditions. Fuel hydrocarbons, such as BTEX, are not found at the FS-12 site because it is far down-gradient from the initial EDB release, whereas the site investigated by Henderson et al. [20] contained significant levels of BTEX compounds, especially within the source zone. Also, microcosms in the current study were incubated undisturbed at the average groundwater temperature of the FS-12 site, 12°C, to mimic *in situ* conditions, whereas Henderson et al.'s samples were incubated at 22-24°C and shaken prior to sampling. The presence of BTEX compounds, higher temperature, and vigorous mixing at each sampling point could have contributed to both the faster EDB degradation rates and greater effectiveness of lactate addition on EDB degradation. This finding is significant because it shows that microbial populations far down-gradient from the source area of an EDB

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release, void of hydrocarbons, may be less stimulated by lactate addition under anaerobic conditions.

6.5 Overall Rate Comparison

First-order degradation rates for all *in situ* and enhanced microcosm sets performed in this study are shown in Figure 12. A summary of the primary microcosm results (Phase III) is shown in Table 3. Significant degradation was not observed within either of the unamended aerobic microcosm sets and no statistically significant difference was found between the two rates. However, under aerobic conditions, methane biostimulation showed significant EDB degradation with an eight-fold increase over the unamended aerobic microcosm set (base case). Under anaerobic conditions, significant EDB degradation occurred in both the unamended and lactate-biostimulated sets. Lactate biostimulation increased the EDB degradation rate by only 19% versus the unamended anaerobic set, and produced more variance among replicates.

Figure 12: First-order EDB decay rates for the a) aerobic and b) anaerobic (and microaerophilic) microcosm sets. Rates were calculated from the combined triplicate (experiment) and duplicate (killed control) bottles. Error bars represent the standard error of the slope of the regression line used to determine the rates. Rates marked with a single asterisk (*) indicate that there is a statistically significant difference between it and its adjacent killed control (Student's *t* test on the slopes of the regression lines, $\alpha = 0.05$). The absence of an asterisk indicates no statistically significant difference.

Table 3: Primary Microcosm Results Summary

Purpose		Effect of EDB Concentration (a,b)		Effect of Aeration (b,c,d)		Effect of Biostimulation (Aer: a,e) (Ana: d,f)	
Case		a	b	c	d	e	f
Aeration		Aerobic	Aerobic	Microaero	Anaerobic	Aerobic	Anaerobic
Amendments		None	None	None	None	Methane (3.4 mM)	Lactate (2 mM)
Starting EDB Concentration (µg/L)		11	44	51	52	82	91
First Order Degradation Rate (yr⁻¹)	**Experiment**	0.43 ± 0.09	0.26 ± 0.06	1.14 ± 0.26	2.96 ± 1.35	3.49 ± 3.29	3.52 ± 2.46
	Killed Control	0.38 ± 0.03	0.33 ± 0.05	0.60 ± 0.03	0.60 ± 0.03	0.07 ± 0.05	0.42 ± 0.13
Evidence of Significant Biological Degradation?		No	No	No	**Yes**	**Yes**	**Yes**

7.0 CONCLUSIONS

This study investigated the degradation of EDB under natural and biostimulated conditions, at environmentally relevant concentrations, in soil microcosms constructed with material from a deep aquifer void of fuel hydrocarbons. Microcosm experiments, constructed with native materials from both an aerobic and anaerobic zone of the plume, were used to investigate both the level of EDB natural attenuation occurring at the FS-12 site as well as the effect of biostimulation on EDB degradation. Specific conclusions of this research are as follows:

- Natural attenuation is occurring in the anaerobic zone but not in the aerobic zone of FS-12. Since the majority of EDB is located in the aerobic zone, monitored natural attenuation may not be feasible for this site.

- A five-fold increase in initial EDB concentration had no significant effect on EDB natural attenuation within the aerobic zone.

- Increased aeration had a negative effect on EDB degradation rates within unamended anaerobic (microaerophilic) microcosm sets; significant EDB degradation only occurred under strictly anaerobic conditions.

- Methane amended aerobic microcosms showed promising results; however further research is needed to conclude if methane is the best co-substrate for the FS-12 site or to identify other more promising co-substrates with more predictable results.

- Lactate amended anaerobic microcosms consistently produced degradation rates faster than unamended anaerobic microcosms. This confirms the potential using lactate as an electron donor for biostimulation within the anaerobic zone of the FS-12 plume.

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However, due to the fairly small degree of improvement over the unamended anaerobic microcosms and the fact that the majority of EDB lies in the aerobic portion of the plume, more investigation on aerobic biostimulation is needed.

8.0 FURTHER RESEARCH

During the first year of this project, significant knowledge was gained on EDB degradation at the FS-12 site. However, given the significant variance amongst the methane-amended microcosm set, further research is required in order to provide recommendations for enhanced natural attenuation of the aerobic zone. A new microcosm study, focusing on investigating the potential for enhanced natural attenuation of the aerobic zone, was proposed and accepted. This new study is investigating many different cosubstrates and all microcosm sets are being performed under *in situ* conditions. This research is focusing on determining the most effective cosubstrate for aerobic biostimulation and whether the implementation of enhanced natural attenuation is capable of bringing EDB concentrations to below the MCL. The first quarterly report prepared for our client can be found in the Appendix.

After the first 84 days of static incubation, only the aerobic phenol-amended, anaerobic unamended, and 37°C (body temperature) unamended aerobic microcosm sets have shown statistically significant EDB degradation. EDB biodegradation under unamended anaerobic conditions was shown in our previous study, so this result was expected. In addition, the increased levels of EDB degradation in the unamended aerobic body temperature microcosm set was also expected as it is widely known that microbial degradation rates are increased at higher temperatures. However, the significant biological degradation observed in the aerobic phenol-amended microcosm set is novel and offers great promise for enhanced natural attenuation within the aerobic zone of the FS-12 plume.

The rate of degradation observed within the phenol-amended set, so far, is not only comparable to the rate observed previously in the aerobic-methane amended set but has shown much less variance (3.69 ± 0.92 yr^{-1} ($t_{1/2} = 0.19$ yr) versus 3.49 ± 3.29 yr^{-1} ($t_{1/2} = 0.20$ yr)).

Consistent phenol degradation has also been observed within this set indicating that the EDB degradation is very likely cometabolic phenol-mediated. After 42 days, phenol levels had dropped 93% (from the starting concentration of 0.90 ± 0.14 mg/L) versus only 22% in the corresponding killed controls. Phenol levels were respiked in the live microcosms to 0.96 ± 0.18 mg/L on day 49 and were subsequently decreased by 29% over the next 35 days.

Once the remaining phenol is consumed in the live microcosms it will be imperative to investigate the effect the absence of phenol has on the rate of EDB degradation. Ideally, rapid EDB degradation will continue in the absence of phenol but it is likely that the EDB degradation in these microcosms is occurring cometabolically though the microbial utilization of phenol. Nevertheless, the concentration of phenol being used in this study (< 1 ppm) meets the lifetime drinking water health advisory level of 2 ppm [42], is readily degradable in the subsurface, and is therefore not an environmental concern [43].

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APPENDICES
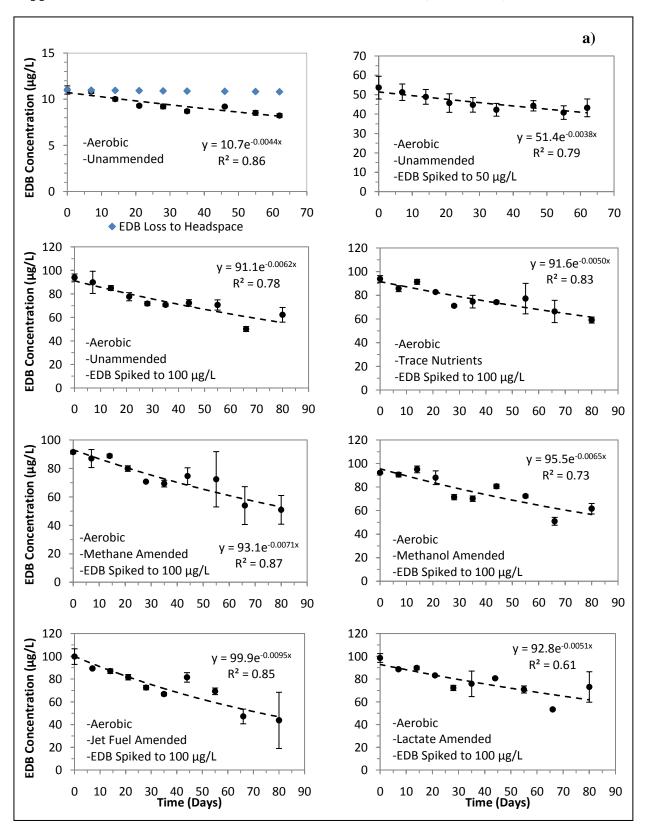
Appendix A: Phase II EDB Concentration over Time Plots
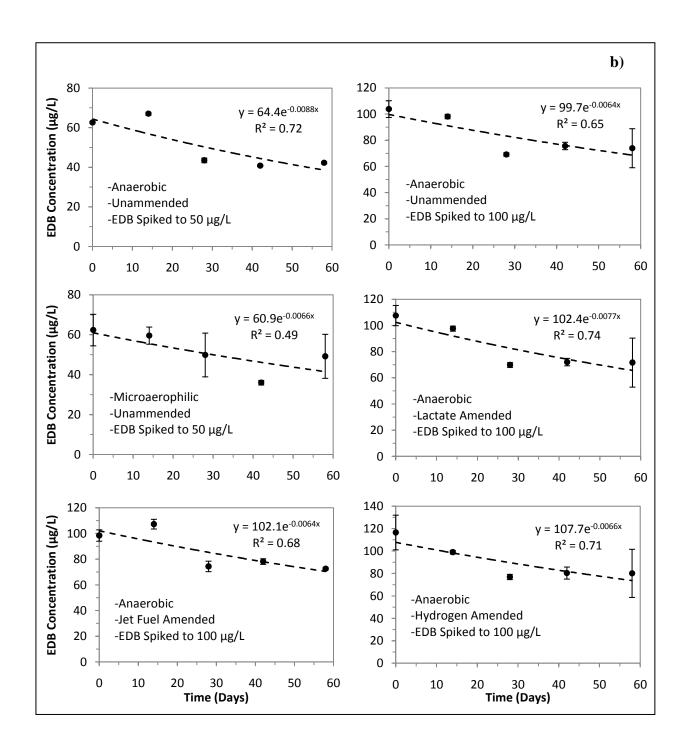
Appendix B: Year 2 Quarter 1 Report

Appendix C: Primary Microcosm (Phase III) Raw Data Tables

Appendix D: FS-12 Plume Cross Section

Appendix A: Phase II EDB Concentration Over Time Plots: a) Aerobic, b) Anaerobic

a)

b)

Panel 1 (top left): $y = 64.4e^{-0.0088x}$, $R^2 = 0.72$
-Anaerobic
-Unammended
-EDB Spiked to 50 µg/L

Panel 2 (top right): $y = 99.7e^{-0.0064x}$, $R^2 = 0.65$
-Anaerobic
-Unammended
-EDB Spiked to 100 µg/L

Panel 3 (middle left): $y = 60.9e^{-0.0066x}$, $R^2 = 0.49$
-Microaerophilic
-Unammended
-EDB Spiked to 50 µg/L

Panel 4 (middle right): $y = 102.4e^{-0.0077x}$, $R^2 = 0.74$
-Anaerobic
-Lactate Amended
-EDB Spiked to 100 µg/L

Panel 5 (bottom left): $y = 102.1e^{-0.0064x}$, $R^2 = 0.68$
-Anaerobic
-Jet Fuel Amended
-EDB Spiked to 100 µg/L

Panel 6 (bottom right): $y = 107.7e^{-0.0066x}$, $R^2 = 0.71$
-Anaerobic
-Hydrogen Amended
-EDB Spiked to 100 µg/L

Axis labels: EDB Concentration (µg/L); Time (Days)

Appendix B: Year 2 Quarter 1 Report

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Enhanced Natural Attenuation of Ethylene Dibromide (1,2-Dibromoethane [EDB]) in the Subsurface at MMR

1.0 INTRODUCTION

This project investigates the potential for enhanced natural attenuation within the aerobic zone of the FS-12 plume at MMR. This study focuses on determining the most effective cosubstrate for aerobic biostimulation and whether the implementation of enhanced natural attenuation is capable of bringing EDB concentrations to below the Massachusetts maximum contaminant level (MCL) of 0.02 µg/L. Soil core and groundwater samples were obtained from the Camp Good News site on Wednesday December 1st. Soil samples and groundwater from the site were used to construct microcosms aimed at exploring EDB degradation under *in situ* field conditions, as well as examining the effects of cosubstrate addition, temperature, and aeration on EDB degradation. An orthogonal matrix was used for our experimental design to ensure that all results will be statistically independent.

Specific objectives of the interdisciplinary research project are to:

1. Investigate the effect of differing cosubstrate (methane, butane, propane, and phenol) addition on *in situ* EDB degradation at the FS-12 site.

   a. Identify the ideal cosubstrate for enhanced natural attenuation at the FS-12 site.

   b. Determine whether EDB can be degraded to below its MCL under biostimulated conditions (i.e. $S_{min} < MCL$?).

2. Investigate the effect of initial EDB concentrations on natural attenuation under aerobic conditions.

3. Investigate the effect of temperature on natural attenuation of EDB under aerobic conditions.

4. Expand the analytical capacity to gain a better insight of *in situ* EDB biodegradation, which will be achieved by:

    a. Tracking intermediates of microbial EDB degradation.

    b. Developing dehalogenase as a microbial functional marker for degradation potential.

    c. Identifying EDB-cometabolizing organisms and related genes.

5. Recommend methods for enhanced natural attenuation of the aerobic zone of the FS-12 plume.

2.0 ACTIVITIES

2.1 Analytical Methods

EDB determination is currently performed using USEPA method 504.1 (USEPA, 1995) with a Hewlett Packard 5890 Plus Gas Chromatograph (GC) equipped with an auto-sampler, capillary column (DB-1, 30 m x 0.25 mm ID, 1.0 µm film thickness), and an electron capture detector (ECD). Sample size volume has been reduced from 35 mL to 10 mL for all microcosm sets. This sample volume reduction allows for additional sampling points throughout the entirety of the experiment without significant loss of liquid volume within each microcosm. There is no effect on EDB quantification with the smaller sample volume compared with the larger volume at EDB levels greater than 1 µg/L.

Methane and oxygen levels are currently being monitored through manual headspace injection using a Hewlett Packard 5890 Plus GC equipped with a thermal conductivity detector (TCD). Phenol determination is currently being performed by the amino-antipyrine method (Grifols-Lucas, 1951) using a Thermo Spectronic Genesys 10uv spectrometer at 510 nm. Standard operating procedures are currently being used for measuring pH and ORP based on Standard Method (APHA *et al.*, 2000) protocols.

2.2 Chemicals

EDB (> 99% purity) was purchased from Sigma-Aldrich. Methane (>99.9%), Propane (>99.9%), and Butane (>99.9%) were purchased from Supelco (Sigma-Aldrich). Phenol (5 ± .05% w/v) was purchased from Fisher Scientific. All solvents (Hexane and Methanol) were HPLC grade. All other chemicals were ACS reagent grade.

2.3 Microcosms

2.3.1 Soil Core Sampling: Soil core samples (6 inch diameter by 5 foot length) were obtained by sonic core drilling from the Camp Good News site in early December, 2010. Aerobic samples were taken from the 170-175' core (below ground surface) which, based on the groundwater monitoring data taken on the day of collection (Table 1), represents an aerobic zone with DO and EDB concentrations of 9.44 mg/L and 10.2 µg/L, respectively. Upon retrieval of the cores, visual inspection revealed a mix of coarse and fine grained sand with a light to medium brown color, indicating an aerobic zone. Samples were extruded, in a manner as to minimize disturbance, in 1 foot intervals into headspace free sterile pyrex glass containers (volume 1.8 L) and stored in the dark at 10°C until use. Anaerobic samples were taken from the

190'-195' core which was composed of very fine and densely packed silt with a dark gray color, indicating an anaerobic zone. Samples were again extruded with minimal disturbance in 1 foot intervals into nitrogen sparged, headspace free sterile pyrex glass containers, and stored in the dark at 10°C until use. Later analysis (Table 1) revealed unexpectedly high DO and EDB concentrations of 5.12 mg/L and 39 µg/L within this zone. However, microcosms prepared from these materials were still treated as anaerobic and were sparged with nitrogen during construction.

Table 1: Groundwater monitoring data (90MW0206A)

Date Sampled	Depth TOS (ft bgs)	Depth BOS (ft bgs)	EDB Conc (µg/L) EPA Method 504.1	Dissolved Oxygen (DO) (mg/L)	Temp (°C)
12/1/2010	170	175	10.2	9.44	10.28
12/1/2010	190	195	39	5.12	10.46

2.3.2 Microcosm Configurations: Microcosms were constructed in 2 liter Pyrex® glass bottles (total working volume 2.4 L), each with an attached glass stem (modified by the UMass Glass Shop), onto which Mininert™ valves have been attached. Mininert™ valves are Teflon-lined and gas tight, which allows for liquid and/or gas sampling through replaceable septa, without allowing volatile chemicals (i.e., EDB) to escape during the sampling process.

A summary of the microcosm experiments being performed in this study are shown in Table 2. Microcosms were constructed in triplicate using soil and groundwater corresponding to the environment each set was mimicking, i.e., aerobic and anaerobic.

For each aerobic microcosm, 400 grams of soil from the aerobic zone (170' − 175' BGS), which was homogenized by thorough mixing, was added along with 1800 mL of groundwater obtained during sample collection. This soil/water ratio was based off of a similar EDB microcosm experiment performed by Henderson et al. (2008). The anaerobic microcosm set was set up in a similar fashion but was continually sparged with nitrogen during the process. Each anaerobic microcosm received 400 grams of soil from the anaerobic zone (190' − 195' BGS), which was also homogenized by thorough mixing, along with 1800 mL of groundwater obtained from the anaerobic zone during sample collection.

Unique treatments were prepared with amendments or with different headspace gases, and compared to a killed control. Starting concentrations for these amendments are listed in Table 2. The killed controls were deactivated using 1 g/L sodium azide (Kane et al., 2001). In order to track abiotic EDB losses not associated with the soil matrix, a set of water control microcosms were constructed. These microcosms were prepared with filter sterilized aerobic groundwater and autoclaved glass beads. This set was also poisoned with 1 g/L sodium azide to eliminate biological activity. All microcosms were incubated at 12±2°C (Figure 1), corresponding to the average groundwater temperature at MMR, without agitation in the dark. Every microcosm experiment was performed in parallel triplicates.

Figure 1: Incubated Microcosms (12±2°C)

Table 2: Microcosm configurations

Microcosm Type	# of Sets*	Metabolic Condition (Aeration)	Inoculum (Soil & GW)	Starting EDB Concentration (µg/L)	Amendments	Temp (°C)
In Situ Base Case	2	Aerobic	High *In Situ* Concentration	10	None	12
			Low *In Situ* Concentration	1	None	
	1	Anaerobic	*In Situ* Concentration	20	None	
Aerobic Cometabolism	4	Aerobic	High *In Situ* Concentration	10	Methane (200 mL)	
					Propane (200 mL)	
					Butane (200 mL)	
					Phenol (1 mg/L)	
Temperature Effect	2	Aerobic	High *In Situ* Concentration	10	None	20
						37
Water Controls	1	Aerobic	High *In Situ* Concentration	10	Sodium Azide (1 g/L) No KC's	12

* For each set, microcosms were set up in triplicate with corresponding killed controls

3.0 FINDINGS

3.1 EDB Degradation

Average EDB concentrations over time, for the first 84 days of incubation, for all microcosm experiments are shown in Figures 1 and 2. Error bars represent the standard deviation of the measured EDB concentration, amongst each set, at the respective time point. Figure 1 compares the aerobic high unamended set (*in situ* base case) to the four aerobic cometabolism microcosm sets to investigate the effect of enhanced natural attenuation using each

of the respective cosubstrates. The water control set shown in this figure represents abiotic EDB degradation that is not associated with the soil matrix. Figure 2 compares the aerobic high unamended set to the rest of the microcosms of this study used to investigate the effect of EDB concentration, temperature, and aeration. Again, the water control set is shown to represent abiotic EDB degradation that is not associated with the soil matrix.

3.1.1 Aerobic Natural Attenuation: After 84 days of static incubation, the base case microcosm set (aerobic high unamended) showed 18% EDB degradation compared to 21% in the killed control. The degradation rate constant (k) for the base case was found to be 0.0023 d^{-1} (0.84 yr^{-1}), as shown in Figure 1. Given that the corresponding killed control microcosm had similar EDB degradation it can be concluded that as of this point all EDB degradation observed was abiotic. In addition, EDB levels within the water control microcosm set averaged 17% indicating that the abiotic degradation observed in the aerobic high unamended set was not associated with the soil matix. As expected, statistical analysis determined that significant biological degradation has not yet occurred within the aerobic high unamended set.

3.1.2 Aerobic Cometabolism (Enhanced Aerobic Natural Attenuation): EDB degradation observed after 84 days in the methane, propane, butane, and phenol amended live microcosms equaled 25%, 18%, 22%, and 56%, respectively (compared to 25%, 17%, 21%, and 16% in the killed controls, respectively). These rates at which EDB was degraded within these cometabolic microcosms, up until this point, are 0.0035 d^{-1} (1.28 yr^{-1}), 0.0031 d^{-1} (1.13 yr^{-1}), 0.0035 d^{-1} (1.28 yr^{-1}), and 0.0101 d^{-1} (3.69 yr^{-1}), respectively. Statistical analysis revealed that after 84 days the phenol amended microcosm set was the only set which showed significant biological

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degradation[3]. The rate of EDB degradation within the phenol-amended set is comparable to the rate observed within the methane-amended set from our previous studies, but with less variance (3.69 ± 0.92 yr^{-1} versus 3.49 ± 3.29 yr^{-1}). Phenol degradation, consistent with EDB degradation, within this set has also been observed (Figure #). After 42 days, 93% of the starting phenol concentration (0.90 ± 0.14 mg/L) was degraded in the live set compared to only 22% in the killed control. Phenol levels were respiked to 0.96 ± 0.18 mg/L on day 49 in the live set and were subsequently degraded by 29% over the next 35 days. Up until this point, EDB degradation within the live phenol microcosms has been very rapid and consistent in the presence of low dose phenol. The next step for our research is to allow the phenol levels to completely degrade and evaluate the impact this will have on EDB degradation. We need to identify whether the EDB degradation is dependent on the presence of phenol or if the system will continue to degrade EDB once the phenol has depleted.

3.1.3 Concentration and Temperature Effect: Thus far there has been no statistically significant difference between the rate of EDB degradation in the aerobic high unamended (10 µg/L) and aerobic low unamended (2 µg/L) microcosm sets. EDB concentrations have dropped from 1.9 µg/L to 1.7 µg/L and 2.2 µg/L to 1.5 µg/L in the live and killed aerobic low unamended microcosm sets, respectively. The effect of temperature has yielded similar results when comparing the aerobic high unamended set, incubated at the average groundwater temperature of 12°C, to the aerobic high unamended set incubated at room temperature (20°C). The rate of EDB degradation within the live room temperature set (0.62 yr^{-1}) was only marginally lower (-

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[3] Given that it is still very early in the incubation period it is very likely that the methane, propane, and butane microcosm sets are currently in a "lag phase". It has been previously documented that some cometabolic applications encounter a lag phase, where the contaminant of concern is not rapidly degraded, while the microbial community adapts to the addition of the cosubstrate (Tovanabootr, 2000).

26%) than the rate observed in the live groundwater temperature set (0.84 yr^{-1}). Additionally, the rate of degradation within both of their respective killed control sets was identical (1.17 yr^{-1}). However, there was a statistically significant difference at 37°C (body temperature). The rate of EDB degradation within the live 37°C set was 10.48 yr^{-1}, which was 1148% faster than the live groundwater temperature set. The degradation rate within the killed control set, of 1.42 yr^{-1}, was only slightly faster than the killed groundwater temperature set (1.17 yr^{-1}) and was significantly lower than the rate within the live body temperature set. These comparisons strongly indicated a significant amount of biological EDB degradation at 37°C. Statistical analysis confirmed that the live body temperature set varied significantly from its respective killed control and both the groundwater and room temperature live aerobic unamended sets.
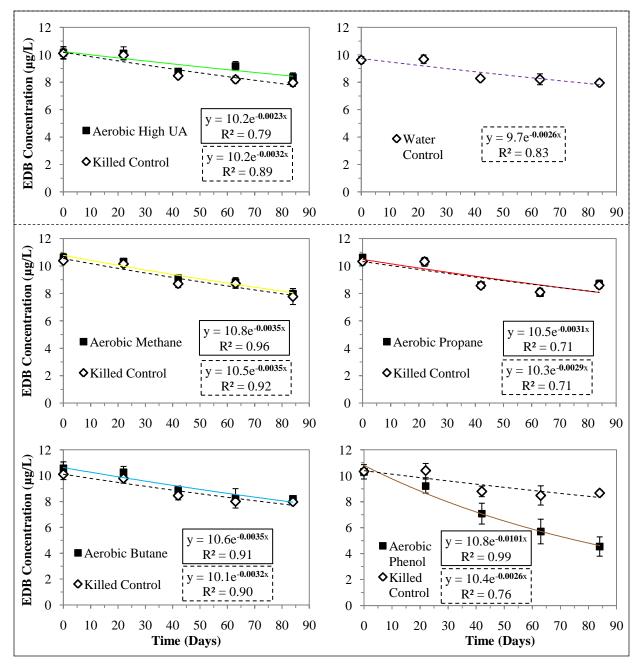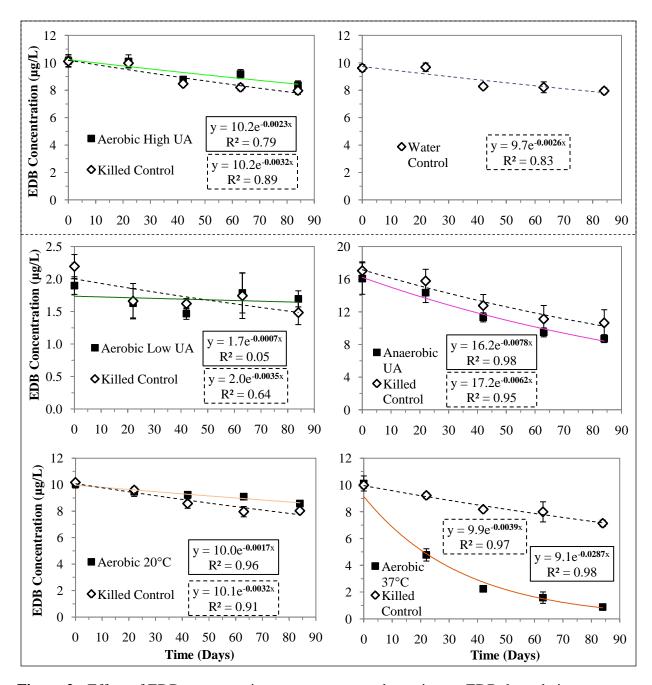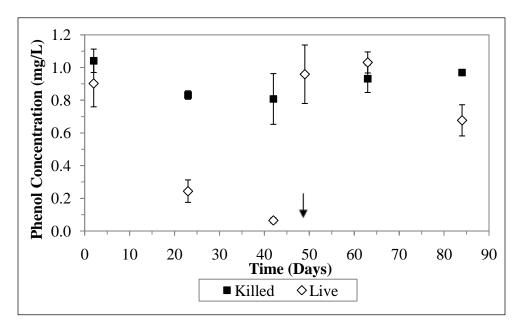
Figure 1: Effect of cometabolism on EDB degradation under *in situ* aerobic conditions

Figure 2: Effect of EDB concentration, temperature, and aeration on EDB degradation.

Table 3: EDB degradation rate summary and comparison.

	Degradation Rate (yr^{-1})				Rate Difference from Aerobic High UA	Rate Difference from Killed Control	Significant Biological Degradation?
	Live Set	R^2	Killed Control	R^2			
Aerobic High Unamended (UA)	**0.84**	0.79	**1.17**	0.89	n/a	-28%	No
Aerobic Methane Amended	**1.28**	0.96	**1.28**	0.92	+52%	0%	No
Aerobic Propane Amended	**1.13**	0.71	**1.06**	0.71	+35%	+7%	No
Aerobic Butane Amended	**1.28**	0.91	**1.17**	0.90	+52%	+9%	No
Aerobic Phenol Amended	**3.69**	0.99	**0.95**	0.76	+339%	+288%	**Yes**
Anaerobic Unamended	**2.85**	0.98	**2.26**	0.95	+239%	+26%	**Yes**
Aerobic Low Unamended	**0.26**	0.05	**1.28**	0.64	-70%	-80%	No
Aerobic Room Temperature	**0.62**	0.96	**1.17**	0.91	-26%	-47%	No
Aerobic Body Temperature	**10.48**	0.98	**1.42**	0.97	+1148%	+636%	**Yes**
Water Controls (WC)	-	-	**0.95**	0.83			
Killed Controls (not including WC)	Avg	**1.31**					
	Std	0.38					

Figure 3: Average phenol concentration over time in the live and killed aerobic phenol amended microcosm set.

4.0 FUTURE PLANS

4.1 Engineering

Short term goals of the Engineering team are to: (1) continue measurements of water quality parameters in the current microcosms and (2) continue statistical analysis of the data to determine whether degradation trends are significant.

5.0 REFERENCES

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Appendix C: Primary Microcosms (Phase III) Raw Data Tables

Aerobic Unamended (Average EDB Concentrations [ug/L])													
Day	0	10	17	31	45	59	73	103	131	173	209	235	264	286
Aer KC10	-	-	9.8	9.3	8.8	8.6	9.0	7.8	7.9	7.9	7.4	6.7	7.7	6.9
Error	-	-	0.1	0.1	0.1	0.0	0.2	0.0	0.3	0.2	0.0	0.2	0.3	0.2
Aer KC50	43.9	47.1	47.1	43.2	43.8	41.5	43.2	39.9	37.9	37.8	40.7	36.7	34.7	35
Error	0.1	3.2	1.8	2.3	2.0	3.3	0.6	1.9	0.0	0.0	3.0	2.7	2.2	3
Aer 50	43.8	50.9	51.5	44.9	47.3	46.6	43.3	45.2	38.0	44.5	44.6	41.7	36.9	39.9
Error	0.7	1.4	0.7	1.1	0.2	1.0	1.2	0.8	0.4	1.5	1.2	1.7	1.4	1.3
Aer 10	11.0	10.9	11.4	10.1	9.7	9.5	8.9	9.4	8.8	9.8	7.2	7.7	8.1	8.1
Error	0.8	0.8	0.7	0.6	0.8	1.0	0.8	0.8	1.1	0.4	0.4	0.7	1.1	0.9
Henrys	11.0	11.0	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9

Aerobic Methane-amended (Average EDB Concentrations [ug/L])												
Day	0	8	15	28	42	56	71	128	169	205	231	260	282
Aer KC 100	78.8	77.2	76.5	75.3	76.7	83.2	78.1	88.0	76.4	76.9	68.8	75.9	74.4
Error	3.7	1.8	0.9	5.7	1.8	7.5	8.6	0.0	5.1	4.7	6.6	5.3	4.9
Methane	82.1	76.6	74.6	76.2	70.0	76.4	69.3	54.7	44.8	40.6	34.2	30.9	15.4
Error	1.9	1.0	1.9	5.2	7.6	14.3	11.8	28.6	36.3	37.1	30.8	27.2	25.4

Anaerobic Unamended and Lactate-amended (Average EDB Concentrations [ug/L])													
Day	0	7	14	28	42	56	70	100	128	170	198	233	261	282
An KC50	48.4	53.4	52.9	48.1	50.4	46.5	44.8	42.2	37.6	33.2	34.1	31.2	37.8	35.6
Error	0.0	3.9	1.8	1.3	6.5	2.7	2.3	1.4	2.3	1.8	0.0	2.6	2.7	3.4
An KC100	83.0	97.0	87.5	80.2	79.6	77.4	80.5	80.2	72.9	58.8	54.9	56.7	77.7	72
Error	0.0	18.0	4.5	4.0	5.0	4.7	3.5	11.8	8.4	3.6	4.4	3.8	4.5	4.3
An 50	51.9	44.9	47.9	44.7	43.9	41.9	41.5	36.0	27.7	21.9	18.4	10.0	8.6	7.6
Error	0.0	1.6	1.1	0.5	0.9	1.2	2.5	6.7	1.7	6.3	4.4	6.8	8.5	8.7
Micro 50	50.5	49.3	44.9	46.0	43.7	43.7	41.9	39.4	35.9	29.6	25.4	25.1	22	23.1
Error	3.2	1.6	1.6	0.1	4.8	2.5	1.7	3.5	1.8	3.1	3.2	1.4	5.7	5.5
Lactate	90.5	83.3	74.1	71.8	71.8	72.4	59.5	52.7	40.7	29.6	22.8	14.5	15.4	11.0
Error	10.2	2.1	1.9	0.8	6.2	0.8	3.5	4.0	1.5	3.5	6.8	10.7	12.6	9.3

Water Controls (EDB Concentrations [ug/L])													
Day	0	10	17	31	45	59	73	103	131	174	209	237	265	286
Ster KC 10	15.8	15.1	15.1	15.0	14.7	13.7	16.1	14.0	12.0	14.4	11.9	10.1	10.7	10.5
Ster KC 50	42.1	44.3	44.0	44.5	42.5	42.2	48.7	44.8	36.3	33.9	38.6	28.7	32.3	32.1
Ster KC 100	82.0	91.7	95.3	90.6	91.2	85.0	94.9	79.5	70.3	46.8	63.6	50.3	54.8	54.0

Aerobic Unamended In Situ (10 ug/L)				
EDB Concentrations (ug/L)				
Day	4.1	5.1	6.1	KC 10	KC 10 (2)
0	11.8	10.6	10.5	-	-
10	11.8	10.5	10.4	-	-
17	12.3	11.0	11.1	9.8	9.7
31	10.8	9.7	9.8	9.4	9.2
45	10.6	9.1	9.3	8.7	8.9
59	10.7	8.9	8.9	8.6	8.6
73	9.8	8.3	8.7	8.8	9.1
103	10.3	8.8	9.0	9.6	7.8
131	10.1	7.9	8.5	8.1	7.7
173	10.8	8.8	4.6	8.0	7.4
209	-	6.9	7.5	7.7	-
235	8.4	7.1	7.7	6.9	6.6
264	9.3	7.2	7.7	8	7.5
286	9.1	7.3	7.8	6.8	7.1

Aerobic Unamended EDB Spiked (50 ug/L)				
EDB Concentrations (ug/L)				
Day	1.1	2.1	3.1	KC 50	KC 50 (2)
0	43.9	44.5	43.2	43.8	44.0
10	49.3	51.6	52.0	44.9	49.4
17	51.3	50.9	52.3	45.8	48.3
31	44.3	44.2	46.2	41.6	44.8
45	47.5	47.0	47.3	42.4	45.3
59	47.3	45.4	46.9	39.2	43.8
73	42.9	42.4	44.7	43.6	42.8
103	44.5	45.0	46.0	38.5	41.2
131	38.2	38.3	37.8	32.9	37.9
173	45.2	42.8	45.5	37.8	-
209	45.7	44.8	43.3	38.6	42.8
235	42.6	39.7	42.7	34.8	38.7
264	38.5	36.3	35.9	33.2	36.2
286	41.3	39.3	39.0	32.9	37.1

Aerobic Methane-amended				
EDB Concentrations (ug/L)				
Day	19.1	20.1	21.1	KC 100	KC 100 (2)
0	82.5	83.5	80.8	76.2	81.4
8	75.5	77.5	76.7	75.9	78.5
15	72.9	74.3	76.7	75.8	77.1
28	71.5	75.5	81.7	71.3	79.4
42	63.1	68.7	78.1	75.4	77.9
56	61.9	77.1	90.4	77.9	88.5
71	57.5	69.1	81.2	72.1	84.2
100	55.9	68.7	76.3	70.9	84.5
128	24.0	59.5	80.5	84.2	88.0
169	6.9	48.2	79.2	72.9	80
205	2.1	43.6	76.2	73.5	80.2
231	1.4	38.7	62.6	64.1	73.5
260	0.9	38.0	53.7	72.1	79.6
282	0.5	0.9	44.7	71.0	77.9

Anaerobic Unamended				
EDB Concentrations (ug/L)				
Day	7.1	8.1	9.1	An KC 50	An KC 50 (2)
0	57.3	51.9	44.1	48.4	68.0
7	46.0	45.9	43.7	50.6	56.2
14	46.6	48.8	48.2	51.6	54.2
28	44.2	44.8	45.1	48.9	47.2
42	42.9	44.8	44.1	55.0	45.8
56	40.7	41.9	43.1	48.4	44.5
70	40.8	39.4	44.3	46.4	43.1
100	39.2	28.4	40.5	43.2	41.3
128	28.9	-	26.5	39.3	35.9
170	26.4	14.7	24.5	34.5	31.9
198	23.4	15.4	16.3	34.1	-
233	17.8	6.6	5.5	29.4	33.1
261	18.4	4.4	3.0	39.7	35.9
282	17.6	3.5	1.8	38.0	33.2

Microaerophilic Unamended			
EDB Concentrations (ug/L)			
Day	10.1	11.1	12.1
0	54.2	48.8	48.6
7	50.5	49.9	47.4
14	46.0	44.2	43.7
28	23.4	46.1	45.9
42	47.1	-	40.3
56	45.7	44.5	41.0
70	42.7	43.1	40.0
100	41.9	40.9	35.4
128	37.0	36.9	33.9
170	32.9	29.1	26.8
198	28.4	25.6	22.1
233	26.1	24.1	-
261	27.3	22.8	16.0
282	28.2	23.7	17.2

Anaerobic Lactate-amended					
EDB Concentrations (ug/L)					
Day	34.1	35.1	36.1	An KC 100	An KC 100 (2)
0	97.7	83.3	105.0	83.0	121.3
7	81.8	85.7	82.5	84.3	109.8
14	75.7	72.1	74.6	84.3	90.7
28	-	71.3	72.4	83.0	77.3
42	70.3	68.1	76.9	83.1	76.1
56	71.8	73.0	-	80.7	74.0
70	57.8	57.2	63.5	83.0	78.0
100	50.2	50.6	57.3	88.6	71.9
128	41.1	39.1	42.0	78.8	66.9
170	25.5	31.3	32	61.4	56.3
198	18.0	11.9	27.5	57.9	51.8
233	2.1	21.2	20.2	59.4	54.0
261	0.9	22.4	23.0	80.9	74.5
282	0.4	15.1	17.6	75.0	68.9

Appendix D: FS-12 Plume Cross Section

FIGURE 3

FS-12 EDB CROSS-SECTION A-A'
AFCEE - Massachusetts Military Reservation
10 September 2008 Technical Update Meeting