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Biodegradation of ethylene dibromide (1,2-dibromoethane [EDB]) in microcosms simulating *in situ* and biostimulated conditions

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ABSTRACT

Although 1,2-dibromoethane (EDB) is a common groundwater contaminant, there is the lack of knowledge surrounding EDB biodegradation, especially under aerobic conditions. We have performed an extensive microcosm study to investigate the biodegradation of EDB under simulated *in situ* and biostimulated conditions. The materials for soil microcosms were collected from an EDB-contaminated aquifer at the Massachusetts Military Reservation in Cape Cod, MA. This EDB plume has persisted for nearly 40 years in both aerobic and anaerobic EDB zones of the aquifer. Microcosms were constructed under environmentally relevant conditions (field EDB and DO concentrations; incubated at 12 °C). The results showed that natural attenuation occurred under anaerobic conditions but not under aerobic conditions, explaining why aerobic EDB contamination is so persistent. EDB degradation rates were greater under biostimulated conditions for both the aerobic and anaerobic microcosms. Particularly for aerobic biostimulation, methane-amended microcosms degraded EDB, on average, at a first order rate eight times faster than unamended microcosms. The best performing replicate achieved an EDB degradation rate of 7.0 yr^{-1} (half-life ($t_{1/2}$) = 0.10 yr). Residual methane concentrations and the emergence of methanotrophic bacteria, measured by culture independent bacterial analysis, provided strong indications that EDB degradation in aerobic methane-amended microcosms occurred via cometabolic degradation. These results indicate the potential for enhanced natural attenuation of EDB and that methane could be considered co-substrate for EDB bioremediation for the EDB-contaminated groundwater in aerobic zone.

1. 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], and also as a pesticide for termite and Japanese beetle control within wood structures. The current widespread presence of EDB in the subsurface is mainly from historic releases, such as past underground storage tank 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 maximum contaminant level (MCL) for EDB, of 0.05 μg/L, is the second lowest for all drinking water contaminants [4]. The Massachusetts Department of Environmental Protection has set an even more stringent MCL of 0.02 μg/L [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

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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 found downgradient of source zones, especially in areas where natural attenuation processes are not robust and fast groundwater flows exist [8]. 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) at the Massachusetts Military Reservation (MMR) on Cape Cod Massachusetts (Fig. 1S). The geology of this site consists of sandy glacial outwash deposits with groundwater flow velocities ranging from 0.3 to 0.6 m/day [9]. FS-12 is one of several long EDB plumes (>1 km) that have separated from the source zone and are no longer containing dissolved BTEX compounds as co-contaminants [10]. FS-12 resulted from an underground 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 groundwater [11]. This plume has migrated approximately 4500 feet downgradient of the source area, ranges in depth from 46 m to 76 m, below the ground surface, and has both aerobic and anaerobic zones with EDB concentrations above the Massachusetts MCL of 0.02 μg/L [12] (Fig. 2S). At the start of this project (October 2009) the EDB concentrations within the aerobic zone ranged from just above the Massachusetts MCL to approximately 23 μg/L [10]. An extremely low natural attenuation rate, of 0.04 yr^{-1} ($t_{1/2}$ = 17.33 yr), was calculated for this site using a simple mass balance approach and assuming first-order kinetics [13]. However, prior to this study, the level of natural attenuation within the plume had 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 [14–18] and anaerobic conditions [19–22]. 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 [19,20]. 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 [17].

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), or with non-environmentally relevant EDB concentrations. To our knowledge, no aerobic EDB degradation study has been conducted at environmentally relevant temperatures and concentrations with *in situ* materials, especially 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 absent 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 the FS-12 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 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 of EDB.

2. Materials and methods

2.1. Soil and groundwater collection

Soil core samples (15 cm diameter × 3 m length) were obtained from the FS-12 site using rotosonic drilling methods in October 2009. Aerobic soil samples were collected from the 61 m to 64 m BGS core, which represented an aerobic zone within the aquifer that is characterized by high EDB concentrations (13.1 μg/L reported at a nearby monitoring well 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 from the drilling core barrel into a disposable polyethylene core liner, then transferred 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 collected from the 67 m to 70 m BGS core which represented an anaerobic zone within the aquifer that is characterized by low EDB concentrations (0.7 μg/L). Soil material from this zone comprised of a dark gray, fine grained sand with 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. Native groundwater was used to construct the microcosms. Groundwater was collected from adjacent monitoring wells, which have screen settings that correspond to the depth of both the aerobic and anaerobic zones of the plume. Both geochemical and water quality data for these groundwater sets are listed in Table 1S.

2.2. Microcosms

2.2.1. Construction

Microcosms were constructed in sterilized Pyrex® glass bottles with a total working volume of 1.2 L. Each bottle was fitted with an attached glass stem onto which Mininert™ valves were attached (at UMass Glassblowing Laboratory). Mininert™ valves are Teflon-lined and gas tight, which allows for liquid and/or gas sampling through replaceable septa, without allowing volatile chemicals to escape during the sampling process. Microcosms were constructed in triplicate using FS-12 site soil and groundwater corresponding to the environment each set was representing, i.e., aerobic or anaerobic. For each aerobic microcosm 200 g of soil from the aerobic zone, which was homogenized by thorough mixing, was added along with 800 mL of groundwater obtained from well screen 90MW0106B (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 g of soil from the anaerobic zone, which was also homogenized by thorough mixing, along with 800 mL of groundwater obtained from well screen 90MW0106A (69 m BGS). The initial headspace volume within all microcosms was 300 mL at the start of incubation. The microcosms were incubated at 12 ± 2 °C (corresponding to the average groundwater temperature at the FS-12 site), without agitation, in the dark.

2.2.2. Methods

A summary of the microcosm experiments performed in this study is shown in Table 1. 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 or anaerobic), and substrate addition on EDB biodegradation.

Table 1
Microcosm configurations.

Environment	Description[a]	Amendments	Target EDB conc. (μg/L)
Aerobic	*In situ* (aerobic base case)	None	*In situ* (~10)
Aerobic	Killed control	None	*In situ* (~10)
Aerobic	*In situ* (50 μg/L EDB)	None	5× *In situ* (~50)
Aerobic	Killed control	None	5× *In situ* (~50)
Aerobic	Methane addition	Methane (0.15 mM)	10× *In situ* (~100)
Aerobic	Killed control	Methane (0.15 mM)	10× *In situ* (~100)
Anaerobic	*In situ* (50 μg/L EDB)	None	5× *In situ* (~50)
Anaerobic	Killed control	None	5× *In situ* (~50)
Anaerobic	Lactate addition	Lactate (2 mM)	10× *In situ* (~100)
Anaerobic	Killed control	Lactate (2 mM)	10× *In situ* (~100)

[a] Number of microcosm replicates: for live microcosms 3 each, for killed controls 2 each.

Every microcosm set, excluding the base case, received a spike of EDB to bring the concentration to 50 μg/L (5× *in situ*) or 100 μg/L (10× *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 5× aerobic microcosms to determine the effect of varying aeration conditions on EDB natural attenuation. For the methane amended sets, 35 mL of methane was dissolved into 800 mL of water in the microcosm bottle to target approximately 10% CH_4 in the headspace or 0.15 mM in the water at 12 °C. For the lactate-amended sets, sodium lactate (syrup, 60%, w/w, Fisher Chemical) was added into water to bring the starting concentration to 2 mM. Methane was chosen as the co-substrate for the aerobic biostimulated microcosm set because previous studies had shown that methane addition supported aerobic co-metabolism of EDB [16]. 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 [23,24] and has proven potential for enhanced EDB degradation [21].

Abiotic degradation was identified by comparing each set to a corresponding set of killed controls. The killed controls were initially 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. [25]. A sterile water control set was also operated in this study to identify any possible abiotic losses not related to the soil matrix [26]. Three microcosms at varying EDB concentrations, 1×, 5×, and 10× *in situ*, were prepared in the same manner as all other microcosms but with autoclaved glass beads and filter sterilized (0.22 μm pore size) groundwater replacing the soil medium and unfiltered groundwater, respectively. No sodium azide was added to these sterile water controls.

2.3. Chemicals

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

2.4. Analytical methods

EDB determination was performed using USEPA method 504.1 [27] with a Hewlett Packard 5890 Plus Gas Chromatograph (GC) equipped with an auto-sampler, capillary column (DB-1, 30 m × 0.25 mm ID, 1.0 μm film thickness), and an electron capture detector (ECD). Sample size 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 (data not shown). The analytical capacity in our laboratory yielded an effective method detection limit of approximately 0.08 μg/L of EDB. Methane levels were determined through manual headspace injection (500 μL sample volume) using a Hewlett Packard 5890 Plus GC equipped with an HP-Molesieve column (30 m × 0.320 mm ID, 12.0 μm film thickness) and a thermal conductivity detector (TCD). Standard operating procedures were used for measuring pH and ORP in the microcosms based on Standard Method [28] protocols.

2.5. 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's *t* test ($\alpha = 0.05$) on the slopes of the regression lines, at each time step, vs. 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 and abiotic degradation) was significantly different than the killed control set (i.e. solely abiotic degradation).

3. Results and discussion

The use of the term "biodegradation" in this study refers to the removal of the parent compound, EDB, but no information is provided on the respective daughter products of this degradation under varying microcosm conditions.

3.1. Natural attenuation

3.1.1. 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 Fig. 1. 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 $0.43 \pm 0.09\ yr^{-1}$ ($t_{1/2} = 1.61$ yr) and $0.38 \pm 0.03\ yr^{-1}$ ($t_{1/2} = 1.82$ 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

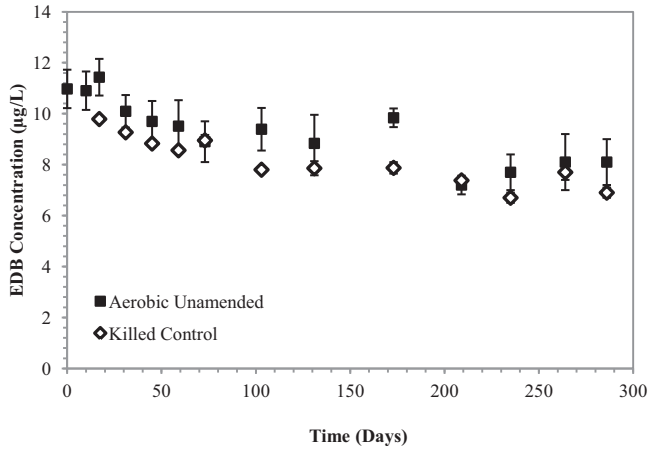

Fig. 1. Average EDB concentrations over time for the unamended aerobic base case (*in situ*) microcosm set.

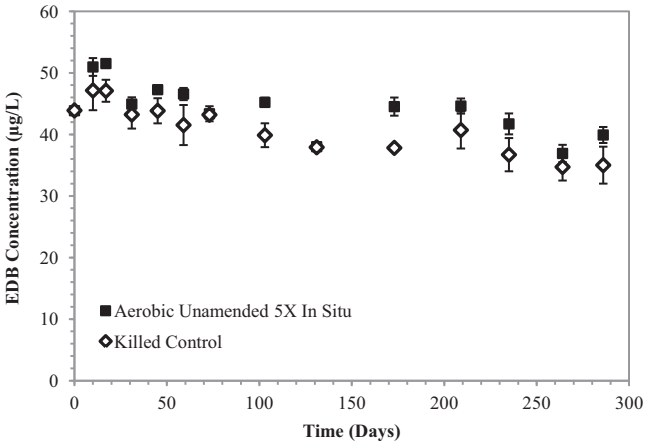

Fig. 3. Average EDB concentrations over time for the unamended aerobic (50 µg/L EDB spiked) microcosm set.

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 [13], 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 natural attenuation of EDB 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 MMR site.

3.1.2. *In situ anaerobic conditions*

EDB concentrations over time, for the unamended EDB spiked anaerobic microcosm set and corresponding killed control set, are shown in Fig. 2. Over the 282 day incubation period, EDB losses averaged 85% and 26% for the unamended anaerobic and killed control sets, respectively. The first-order decay rates calculated for the unamended anaerobic microcosm set and its 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 (α = 0.05) indicating active anaerobic biodegradation of EDB. 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. [21] for a similar site under anaerobic conditions, but in the presence of a significant concentration of fuel hydrocarbons.

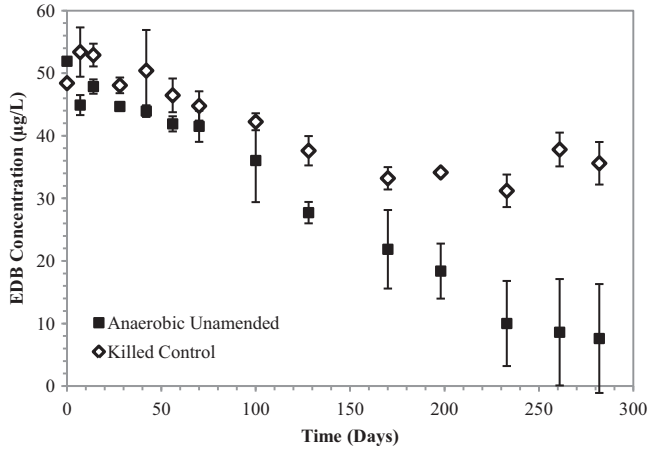

Fig. 2. Average EDB concentrations over time for the unamended anaerobic (50 µg/L EDB spiked) microcosm set.

3.2. *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 Fig. 3. After 286 days of incubation, EDB losses of 9% and 20% were observed in the aerobic EDB spiked and killed control sets, 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 sets, respectively. Statistical analysis revealed that there was no significant difference between the two rates indicating that the observed losses were due to 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 under aerobic conditions. There was no statistically significant difference between the rate of EDB degradation within the EDB spiked set and the *in situ* base case set. 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.

3.3. *Effect of aeration*

Statistical analysis of the live anaerobic and aerobic unamended EDB microcosm sets (Fig. 2 vs. Fig. 3) revealed a significant difference between the two rates of EDB degradation. This indicates that aerobic conditions do not favor natural attenuation of EDB at the FS-12 site. The level of EDB natural attenuation occurring within the anaerobic zone, 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 of EDB mass and highest concentrations of EDB are 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 study site.

3.4. *Effect of biostimulation*

3.4.1. *Effect of methane addition under aerobic conditions*

Concentrations of EDB over time, for the individual methane amended aerobic replicates and corresponding killed control set,

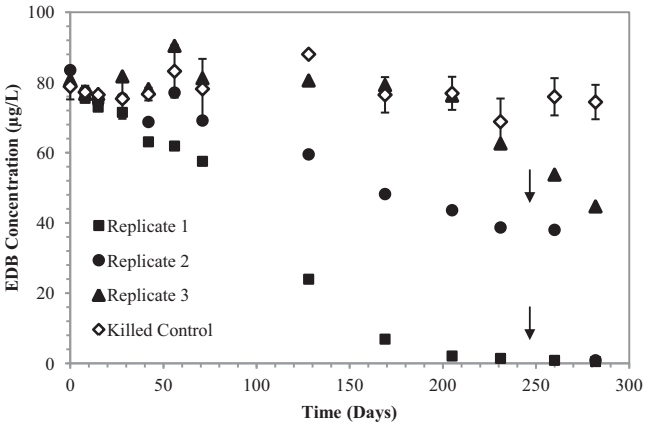

Fig. 4. EDB concentrations over time in the individual aerobic methane-amended live replicates and corresponding killed control set. Downward arrows signify a re-spike of methane to bring the concentration back to 10% headspace.

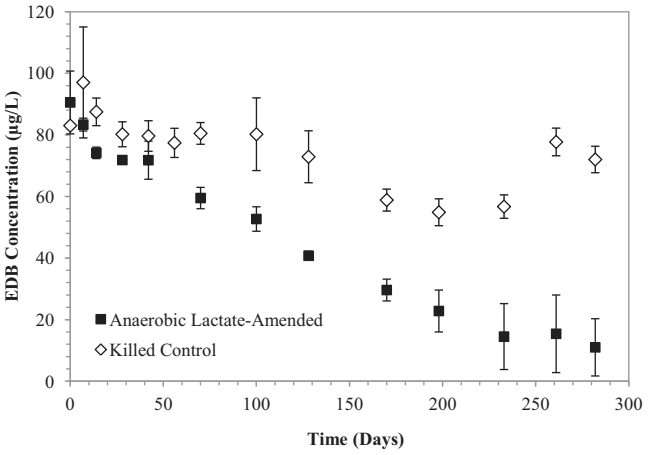

Fig. 5. Average EDB concentrations for the anaerobic lactate-amended (2 mM) microcosm set.

are shown in Fig. 4. The average percent loss of EDB 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) (Fig. 1).

However, a large variation was observed amongst the biostimulated microcosm set. After 282 days of incubation, both replicates 1 (R1) and 2 (R2) contained less than 1% (0.5 µg/L and 0.7 µg/L, respectively) of the initial EDB concentration (82 µg/L) but replicate 3 (R3) still contained more than 55% (44.7 µg/L) 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 R1 and R2 but was detected in R3 and both killed controls. R1 and R2 were re-spiked with methane to bring the methane concentration in the headspace back to 10%. Subsequent methane measurements, taken on the last day of incubation for this study (day 282), showed no methane remaining in R1, less than half of the methane spike remained in R2, and more than 75% of the initial methane remained in R3 and the killed controls. The transformation yield (T_y) for this set of microcosms was calculated to be 0.81 mg CH_4 utilized for each µg of EDB consumed.

In addition, the emergence of a visible microbial mat, unique to only R1 and R2, was a good indication of enhanced microbial activity within these bottles. Subsequent microbial analyses (Figs. 3S and 4S) showed that R1 was dominated by members of the Alphaproteobacteria belonging to *Beijernickiaceae* sp., which produce particulate methane monooxygenase. Microcosm R2 was dominated by methylotrophs belonging to the Betaproteobacteria while the microbial community in R3 was more diverse. In the presence of methane, most methanotrophs produce methane monooxygenases, which oxidize methane to methanol and can also co-oxidize chlorinated compounds [29]. Our data suggest that the difference between the community of methanotrophs in each methane-amended microcosm affected the degradation rate of EDB.

It is worth noting that towards the end of the incubation period, samples from R3 smelled of hydrogen sulfide, and some black matter appeared within the top layer of the soil matrix. These are indications of sulfate reduction under aerobic conditions, partly accounting for why aerobic methane-mediated EDB degradation was not occurring within R3. Though DO was not monitored along the incubation of microcosms we measured O_2 from the headspace on day 222 to see if the lack of O_2 was the cause of inconsistency seen with R3. The measured headspace O_2 values showed only 3% in R3 which is equivalent to 1.3 mg/L DO at 12 °C. Two active sets and killed control sets showed head space O_2 in the range of 7 and 9%, equal to 3.1–3.9 mg/L DO. This indicates that R3 bottle did not somehow properly retain the good aerobic conditions. Also, this set of information further indicates that O_2 was not limited in active bottles R1 and R2 for aerobic EDB degradation. Both R1 and R2 contained less than 1% of the initial 82 µg/L of EDB but the rate of EDB degradation was much slower in R2. It is possible that the microbial community within R2 required a longer acclimation period than R1; significant EDB degradation occurred in R2 between the last two sampling events, a reduction from 38 µg/L on day 260 to 0.9 µg/L on day 282. A re-sampling effort of R2 on day 282 confirmed this sudden decrease in EDB concentration.

Significant variance amongst individual EDB microcosm bottles was also observed by Henderson et al. [21], although their study focused on 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 to minimize differences among replicates. Given that microbial populations in deep soils below the water table are present in such small numbers [17], it is quite possible that varying microbial communities existed at differing cell densities among the replicates, which might have caused the observed variation. 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 control, indicating the presence of enhanced biological EDB degradation under aerobic cometabolic conditions. 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 promising co-substrate. A new phase of this study, reinvestigating methane and an exploration of more aerobic co-substrates, will be conducted in the near future by our research team.

3.4.2. *Effect of lactate 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 Fig. 5. The average percent loss of EDB observed over the 282 day incubation period for this biostimulated set was 88% compared to just 13% in the killed control set. The first-order decay rates determined for the anaerobic lactate

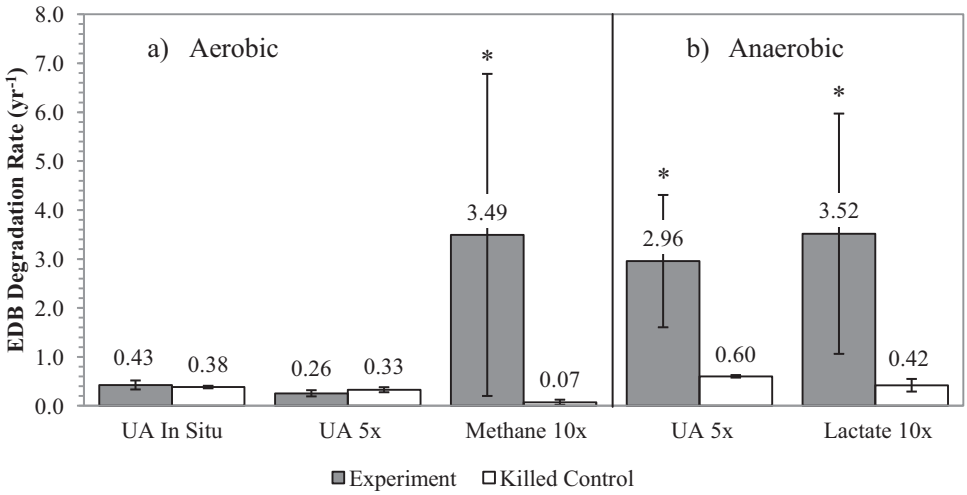

Fig. 6. First-order EDB decay rates for the (a) aerobic and (b) anaerobic microcosm sets. Rates were calculated from the combined triplicate (experiment) and duplicate (killed control) bottles. Error bars represent the standard error of the slope of the regression line used to determine the rates. Rates 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. UA: un-amended.

amended microcosm set and its 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. These two rates were significantly different ($\alpha = 0.05$) which indicate active anaerobic biodegradation of EDB in presence of lactate.

In spite of enhanced EDB biodegradation in presence of lactate, this improvement is significantly less than 400% shown by Henderson et al. [21]. The difference can most likely be attributed to 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. [21] 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, 12 °C, to mimic *in situ* conditions, whereas samples in the Henderson et al. study were incubated at 22–24 °C and shaken prior to sampling. The current study's finding is significant because it shows that microbial populations far down-gradient from the source area of an EDB release, also void of hydrocarbons, may be less stimulated by lactate addition under anaerobic conditions.

3.5. Overall comparison

First-order degradation rates for all *in situ* and enhanced microcosm sets performed in this study are shown in Fig. 6. 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, methane biostimulation under aerobic conditions resulted in 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 produced more variation amongst replicates and subsequently, on average, no statistically significant difference was observed in the rate of EDB degradation rate compared to the unamended anaerobic set.

4. Conclusions

This study investigated the degradation of EDB under natural and biostimulated conditions, at environmentally relevant concentrations, under conditions simulating 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 as well as the effect of biostimulation on EDB degradation under *in situ* conditions.

Specific conclusions resulting from this research are as follows:

- EDB degraded in unstimulated laboratory microcosms, using material obtained from the FS-12 plume, under anaerobic conditions but not under aerobic conditions. These results are consistent with field data reported in other studies. Given that the majority of EDB is located in the aerobic zone of FS-12 plume, monitored natural attenuation may not be feasible for this site.
- A five-fold increase in initial EDB concentration had no significant effect on the natural attenuation of EDB under aerobic conditions.
- Methane addition into the aerobic microcosms led to effective biodegradation. Residual methane concentrations and emergence of methanotrophic bacteria within these sets provided evidence that this aerobic EDB degradation was achieved via aerobic cometabolism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.12.067.

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