Bioremediation of TCE Source Area at the Mobile Launch Platform Rehabilitation Sites and Vehicle Assembly Building Area

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***Abstract*:** Microcosm tests were conducted to evaluate the potential to biodegrade trichloroethene (TCE) in groundwater at Kennedy Space Center (KSC), Florida. Microcosm treatments were constructed to: (i) evaluate if indigenous microorganisms at the site could be stimulated with electron donors to completely dechlorinate TCE; and (ii) assess the potential for bioaugmentation with KB-1, a natural, non-pathogenic, dehalorespiring microbial culture that improves the rate and extent of dechlorination. The microcosm treatments amended with electron donors indicated that reducing conditions were present at incubation day 36. Although natural microbial populations had developed, TCE was only slowly degrading to cis-1,2-dichloroethene (cis-1,2-DCE) after 126 days of incubation. These results tend to indicate that the natural population of dehalorespiring bacteria is low and that the electron donor enhancement alone did not sufficiently stimulate growth. At day 36, microcosm treatments amended with electron donors were bioaugmented with the KB-1 culture. Complete TCE dechlorination via cis-1,2-DCE and vinyl chloride (VC) to ethene was observed in the bioaugmented treatments. Microcosm results indicate that the average time required to degrade TCE and cis-1,2-DCE by one half (half-life) was 4 and 15 days, respectively. The results, after 205 days of incubation, indicated that bioaugmentation with KB-1 can greatly improve the rate and extent of TCE dechlorination. Complete biodegradation of TCE and its degradation products to ethene was observed in the microcosms bioaugmented with KB-1.

***Site Background*:** The Mobile Launch Platform Rehabilitation Sites (MLP) and Vehicle Assembly Building (VAB) are located in northern Brevard County at KSC, Florida. The MLP and VAB sites have been active for over 30 years. The MLP Rehab Sites were the original construction sites for the three Apollo mobile launchers. The VAB was originally built for assembly of Apollo/Saturn vehicles. Both the MLP and VAB were modified during the 1970s to accommodate the Space Transportation System (Shuttle). Groundwater underlying the site is contaminated primarily with TCE, cis-1,2-DCE, and VC. Based on the observed pattern of contamination, it is believed that chlorinated solvents were discharged to ground surface in the vicinity of the northeast corner of the VAB. The chlorinated solvent plume is elongated in the direction of groundwater flow (towards the north) and extends over an area of approximately 8 acres. Elevated chlorinated solvents were detected in the parking lot immediately north-northeast of the VAB. In this area, TCE concentrations range from 3 to 50 milligrams per liter (mg/L); cis-1,2-DCE from 2 to 17 mg/L; and VC from 5 to 19 mg/L. These elevated chlorinated solvent concentrations are generally present at depths between 40 and 50 ft below land surface. An investigation performed at the site utilizing a membrane interface probe (MIP) indicated that dense non-aqueous phase liquids (DNAPL's) were not present in

site soils at the 19 boreholes tested. Based on conditions observed at the site, it appears that only small amounts of TCE may be present as ganglia trapped in pore spaces. The laboratory biotreatability microcosm study discussed in this paper is being conducted to support an ongoing Corrective Measures Study (CMS). Bioaugmentation is being evaluated as a potential treatment technology to remediate impacted groundwater at the site.

Dechlorination Background: In anaerobic-reducing environments, the main biodegradation mechanism for TCE is reductive dechlorination, which involves the sequential replacement of chlorine atoms on the alkene molecule by hydrogen atoms. The chlorinated ethenes serve as electron acceptors and simple organic carbon compounds (i.e., sugars, alcohols, fatty acids) serve as electron donors. Sequential dechlorination of TCE proceeds to either trans-1,2-dichloroethene or cis-1,2-DCE, and VC to ethene.

Microcosm Construction and Results: A laboratory microcosm study was used to evaluate whether indigenous microorganisms present in site soil could be stimulated to dechlorinate TCE completely to ethene under anaerobic conditions through: (i) the addition of electron donors; (ii) addition of methane; (iii) addition of alternative electron acceptors (nitrate); and/or (iv) bioaugmenting with the KB-1 culture. A series of treatment and control microcosms were constructed in triplicate using site soil and groundwater. Each microcosm was amended with TCE to achieve initial target concentrations in the range of 20 to 25 mg/L.

Anaerobic Intrinsic Control microcosms were not amended with any substrates. All anaerobic Sterile Control microcosms were autoclaved and amended with 1.5 milliliters (mL) of mercuric chloride (5 percent) and 0.5 mL of sodium azide (5 percent) to inhibit microbial activity. All treatments and controls were constructed in triplicate, incubated in an anaerobic glove box, in the dark, and in an inverted position to minimize VOC loss via the microcosm closure.

Anaerobic microcosms consisted of 250 mL sterile glass bottles filled with 60 grams (g) of homogenized site soil and 150 mL of site groundwater. One replicate for all treatments was amended with 0.15 mL of resazurin to monitor groundwater redox conditions (resazurin is clear under anaerobic conditions but turns pink when exposed to oxygen). Electron donor amended microcosms contained target concentrations of about 100 mg/L for each electron donor (methanol, ethanol, acetate, lactate [MEAL]). These microcosms were amended with MEAL on a regular basis during the course of the investigation. Electron acceptor treatment microcosms were amended with nitrate to a final concentration of 5 millimoles (mM). The dehalorespiring consortium KB-1 was added (5 mL) to the microcosm designated for bioaugmentation once reducing conditions were attained (40 days of incubation).

Microcosms were incubated for a period of up to 205 days. Each microcosm was sampled periodically during the incubation period. The samples were analysed to determine the presence of VOC's, headspace gases (e.g., oxygen, methane, ethene,

ethane), electron donors, and electron acceptors. As expected, TCE degradation was not observed in the sterile, anaerobic intrinsic, or nitrate-amended microcosm treatments. The MEAL-amended microcosm showed variable TCE degradation corresponding with an increase in cis-1,2-DCE concentrations, with no VC or ethene production. Analytical results indicate that methanol was rapidly metabolized in all MEAL-amended microcosms, indicating that indigenous microbial populations were active. The analytical data obtained from the microcosm sampling suggest that it may be possible to stimulate TCE biodegradation at the site by adding MEAL after a long acclimation period.

A triplicate set of MEAL-amended microcosms was bioaugmented with KB-1 at incubation day 36. TCE degradation was accompanied by an increase in cis-1,2-DCE production in both the MEAL-amended and KB-1 bioaugmented microcosms at around incubation day 21. In the microcosms bioaugmented with KB-1, once TCE concentrations declined (day 40) cis-1,2-DCE and VC concentrations increased. VC concentrations decreased rapidly (day 60) in one of the KB-1 replicates, whereas in the other two replicates, VC did not begin to decrease until after 120 days of incubation. The longer VC half-life calculated in replicates 2 and 3 may be attributed to the elevated sulfate concentrations (up to 650 mg/L) detected. Sulfate can be microbially reduced to hydrogen sulfide. It may be possible that elevated hydrogen sulfide levels built up in these two microcosms inhibited the degradation of VC to ethene.

A summary of the calculated half-lives for TCE, cis, 1-2,DCE, and VC are provided in Table 1.

Table 1. Summary Of Calculated Anaerobic Biodegradation Half-Lives.

		Half-Life in Days		
Treatment	Incubation Days	TCE	Cis-1,2-DCE	VC
Sterile Control	0-180	>500	>500	>500
Active Control	0-180	331	>500	>500
Electron Donor (MEAL)-amended	0-180	>500	203	>500
Nitrate-Amended	0-180	>500	>500	>500
Bioaugmented (Rep.1)	21-42	3	-	-
	42-77	-	3	-
	60-77	-	-	4
Bioaugmented (Rep.2)	21-36	6	-	-
	63-205	-	26	-
	126-205	-	-	60
Bioaugmented (Rep.3)	21-58	4	-	-
	42-180	-	14	-
	80-205	-	-	53
Average Bioaugmented		4	15	39

Conclusions: Based on the microcosm study results, it may be possible to stimulate existing microorganisms to dechlorinate TCE through electron donor addition after a long acclimation period. Rapid and complete dechlorination of TCE to ethene via cis-1,2-DCE and VC can be quickly achieved through the addition of the KB-1 microorganisms.