

Field Studies of In-situ Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons

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<Abstract>

Results will be presented from two field studies that evaluated the in-situ treatment of chlorinated aliphatic hydrocarbons (CAHs) using aerobic cometabolism. In the first study, a cometabolic air sparging (CAS) demonstration was conducted at McClellan Air Force Base (AFB), California, to treat chlorinated aliphatic hydrocarbons (CAHs) in groundwater using propane as the cometabolic substrate. A propane-biostimulated zone was sparged with a propane/air mixture and a control zone was sparged with air alone. Propane-utilizers were effectively stimulated in the saturated zone with repeated intermediate sparging of propane and air. Propane delivery, however, was not uniform, with propane mainly observed in down-gradient observation wells. Trichloroethene (TCE), *cis*-1,2-dichloroethene (*c*-DCE), and dissolved oxygen (DO) concentration levels decreased in proportion with propane usage, with *c*-DCE decreasing more rapidly than TCE. The more rapid removal of *c*-DCE indicated biotransformation and not just physical removal by stripping. Propane utilization rates and rates of CAH removal slowed after three to four months of repeated propane additions, which coincided with the depletion of nitrogen (as nitrate). Ammonia was then added to the propane/air mixture as a nitrogen source. After a six-month period between propane additions, rapid propane-utilization was observed. Nitrate was present due to groundwater flow into the treatment zone and/or by the oxidation of the previously injected ammonia. In the propane-stimulated zone, *c*-DCE concentrations decreased below the detection limit (1 µg/L), and TCE concentrations ranged from less than 5 µg/L to 30 µg/L, representing removals of 90 to 97%. In the air sparged control zone, TCE was removed at only two monitoring locations nearest the sparge-well, to concentrations of 15 µg/L and 60 µg/L. The responses indicate that stripping as well as biological treatment were responsible for the removal of contaminants in the biostimulated zone, with biostimulation enhancing removals to lower contaminant levels. As part of that study bacterial population shifts that occurred in the groundwater during CAS and air sparging control were evaluated by length heterogeneity polymerase chain reaction (LH-PCR) fragment analysis. The results showed that an organism(s) that had a fragment size of 385 base pairs (385 bp) was positively correlated with propane removal rates. The 385 bp fragment consisted of up to 83% of the total fragments in the analysis when propane removal rates peaked. A 16S rRNA clone library made from the bacteria sampled in propane sparged groundwater included

clones of a TM7 division bacterium that had a 385bp LH-PCR fragment; no other bacterial species with this fragment size were detected. Both propane removal rates and the 385bp LH-PCR fragment decreased as nitrate levels in the groundwater decreased.

In the second study the potential for bioaugmentation of a butane culture was evaluated in a series of field tests conducted at the Moffett Field Air Station in California. A butane-utilizing mixed culture that was effective in transforming 1,1-dichloroethene (1,1-DCE), 1,1,1-trichloroethane (1,1,1-TCA), and 1,1-dichloroethane (1,1-DCA) was added to the saturated zone at the test site. This mixture of contaminants was evaluated since they are often present as together as the result of 1,1,1-TCA contamination and the abiotic and biotic transformation of 1,1,1-TCA to 1,1-DCE and 1,1-DCA. Model simulations were performed prior to the initiation of the field study. The simulations were performed with a transport code that included processes for in-situ cometabolism, including microbial growth and decay, substrate and oxygen utilization, and the cometabolism of dual contaminants (1,1-DCE and 1,1,1-TCA). Based on the results of detailed kinetic studies with the culture, cometabolic transformation kinetics were incorporated that butane mixed-inhibition on 1,1-DCE and 1,1,1-TCA transformation, and competitive inhibition of 1,1-DCE and 1,1,1-TCA on butane utilization. A transformation capacity term was also included in the model formation that results in cell loss due to contaminant transformation. Parameters for the model simulations were determined independently in kinetic studies with the butane-utilizing culture and through batch microcosm tests with groundwater and aquifer solids from the field test zone with the butane-utilizing culture added. In microcosm tests, the model simulated well the repetitive utilization of butane and cometabolism of 1,1,1-TCA and 1,1-DCE, as well as the transformation of 1,1-DCE as it was repeatedly transformed at increased aqueous concentrations. Model simulations were then performed under the transport conditions of the field test to explore the effects of the bioaugmentation dose and the response of the system to the biostimulation with alternating pulses of dissolved butane and oxygen in the presence of 1,1-DCE (50 µg/L) and 1,1,1-TCA (250 µg/L). A uniform aquifer bioaugmentation dose of 0.5 mg/L of cells resulted in complete utilization of the butane 2-meters downgradient of the injection well within 200-hrs of bioaugmentation and butane addition. 1,1-DCE was much more rapidly transformed than 1,1,1-TCA, and efficient 1,1,1-TCA removal occurred only after 1,1-DCE and butane were decreased in concentration. The simulations demonstrated the strong inhibition of both 1,1-DCE and butane on 1,1,1-TCA transformation, and the more rapid 1,1-DCE transformation kinetics. Results of the field demonstration indicated that bioaugmentation was successfully implemented; however it was difficult to maintain effective treatment for long periods of time (50 days or more). The demonstration showed that the bioaugmented experimental leg effectively transformed 1,1-DCE and 1,1-DCA, and was somewhat effective in transforming 1,1,1-TCA. The indigenous experimental leg treated in the same way as the bioaugmented leg was much less effective in treating the contaminant mixture. The best operating performance was achieved in the bioaugmented leg with about over 90%, 80%, 60 % removal for 1,1-DCE, 1,1-DCA, and 1,1,1-TCA, respectively. Molecular methods were used to track and enumerate the bioaugmented culture in the test zone. Real Time PCR analysis was used to enumerate the bioaugmented culture. The results show higher numbers of the bioaugmented microorganisms were present in the treatment zone groundwater when the contaminants were being effectively transformed. A decrease in these numbers was associated with a reduction in treatment performance. The results of the field tests indicated that although bioaugmentation can be successfully implemented, competition for the growth substrate (butane) by the indigenous microorganisms likely lead to the decrease in long-term performance.