Inovative Site Characteristics of Microbes

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Abstract

Bioremediation has proven to be one of the most cost effective and environmentally sound remediation technologies available at sites where it will work. The Savannah River Site has just completed an Integrated Demonstration on "Clean-up of Soils and Groundwater Contaminated with Chlorinated VOCs." More than 20 laboratories, several companies and several government agencies were involved in the planning, execution and evaluation of this demonstration. The demonstration showed how gaseous nutrients (methane, nitrous oxide and triethyl-phosphate) could be injected into a aquifer via a horizontal well to stimulate indigenous bacteria (methanotrophs) to degrade trichloroethylene and other microbes to reduce tetrachloroethylene to trichloroethylene. Sediment, water, and soil gas samples were taken before, during and after the demonstration. Indeed, more than 90 measurements were done on over 2000 sediment samples, 173 different analyses of more than 1000 ground water samples, and over 30 different measurements of more than 3000 soil gas samples. The 14 month demonstration showed how nucleic acid probes, fluorescent antibodies, and phospholipid fatty acid analyses could be used to directly characterize and monitor bioremediation in the sediment and groundwater. A number other assays were cross compared with varying degrees of success. The direct functional group assays were extremely effective at showing quickly, who was present, how important they were to the remediation and how "happy" they were. Evaluations and modeling by several laboratories showed that this aerobic methane stimulation in situ bioremediation process was at least 40% more effective than any

physical stripping process (also tested at this site), and 5 times more effective than any pump and treat process. The process removed 78% of all of the TCE and PCE present during the demonstration, with initial concentrations higher than 1000 ppb and final concentrations in the most effected areas reaching less than 2 ppb.

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Introduction

This project was designed to demonstrate in situ bioremediation of ground water and sediment contaminated with chlorinated solvents. Indigenous microorganisms were stimulated to degrade trichloroethylene (TCE), tetrachloroethylene (PCE) and their daughter products in situ by addition of nutrients to the contaminated aquifer and adjacent vadose zone (Figure 1). The principle carbon/energy source nutrient used in this demonstration was methane (natural gas). In situ biodegradation is a highly attractive technology for remediation because contaminants are destroyed, not simply moved to another location or immobilized, thus decreasing costs, risks, and time, while increasing efficiency, safety, and public and regulatory acceptability [1, 2, 3, 4, 5].

It is important to note that the criteria for success, the measurements taken, the nature of each operating campaign during the test, data analysis and evaluation, the test plan, and the final report and conclusions were a consensus of the Bioremediation Technical Support Group (Expert Panel). This group of experts from DOE, USGS, EPA, industry, and academia met on a regular basis for the last 3 years and provided unique and valuable in sights for the planning, execution and evaluation of this demonstration. This group is responsible for the successes of this demonstration which is the largest and most technically comprehensive full-scale in situ bioremediation demonstration ever done.

The demonstration consisted of using 2 horizontal wells for injection and extraction at a site contaminated with chlorinated solvents (TCE/PCE) from a leaking process sewer line. The lower (injection) well (175 ft depth) was installed below the water table (120 ft) and the upper (extraction) well (80 ft depth) was in the vadose zone above the water table [6]. Air was extracted from the upper well during all operating campaigns at 240 scfm. Extracted air was treated by a thermal catalytic oxidizer. Air was injected into the lower well at a constant rate of 200 scfm during all operating campaigns. Six different operational modes were tested during the 14 month demonstration as follows:

Table 1. Injection Operations

Injection Operations	Start Date	End Date
1. No air injection (air extraction only)	02/26/92	03/18/92
2. Air injection	03/18/92	04/20/92
3. 1% methane/air	04/20/92	08/05/92
4. 4% methane/air	08/05/92	10/23/92

5. Pulsed methane/air	10/23/92	01/25/93
Long intervals (5-14 days air/5 days 1% methane)	10/23/92	12/20/92
Short intervals (36 h air/8 h 4% methane)	12/20/92	01/25/93
6. Pulsed 4% methane (short intervals), continuous 0.07% nitrous oxide and 0.007% triethyl phosphate	01/25/93	04/30/93

Air, water, and sediment samples were taken before, during and after the demonstration as per the Test Plan for this demonstration [7].

The measures of success for the project were 1. biostimulation/biodegradation, 2. bioremediation, 3. cost effectiveness, and 4. ease of use and operation.

Biostimulation/Biodegradation

The evidence for biostimulation and biodegradation of TCE/PCE was both overwhelming and unequivocal. No less than 26 separate measurements of sediment and ground water done by 6 different laboratories indirectly demonstrated biostimulation and biodegradation in situ by the processes tested.

Biostimulation was measured in terms of increases in the numbers of methanotrophs, the functional group that the process was trying to stimulate. Increases in methanotroph densities were only observed after methane injection started, (Figure 2). Densities of methanotrophs increased in the ground water by as much as 7 orders of magnitude. This stimulation occurred first in the wells that were closest to the injection point and later moved farther and farther away. Densities of methanotrophs in the sediment closest to the injection well increased from barely detectable to over a million cells/gdw. The methanotroph enumerations were done by 3 different laboratories (University of Tennessee, Pacific Northwest Laboratory, Savannah River Technology Center) using 3 different methods and all obtained nearly identical results. Phospholipid fatty acid (PLFA) analyses done by the University of Tennessee (UT) and Oak Ridge National Laboratory (ORNL) also indicated biostimulation of methanotrophs, and that methanotrophs were stimulated to become the dominant population in the total microbial community [8]. Studies by Savannah River Technology Center (SRTC) and UT using soil columns and mineralization assays demonstrated that PCE was biodegraded even under bulk aerobic conditions [9]. This latter observation is particularly significant since PCE can only be degraded anaerobically. Their data suggests that enough anaerobic pockets are created by the increased biomass to allow a significant amount of anaerobic reductive dechlorination of PCE to TCE, which can then be oxidized by the methanotrophs. Nucleic acid probe analyses by five different laboratories, Pacific Northwest Laboratory (PNL), Washington State University (WSU), University of Minnesota (UM), SRTC and UT showed very specifically that methanotrophs were stimulated in the sediment. Biostimulation was also indirectly indicated by the depletion of nitrate (a limiting nutrient) in the ground water as stimulation continued, by the increase in carbon dioxide observed in the extraction air after injection was started and by the consumption of methane (50%), calculated via measurements of methane and helium tracer in injection well and extraction well. It is important to note that community changes caused by a biostimulation process were reversible as demonstrated for nitrogen-transforming bacteria which were measured using

fluorescent antibody probes by SRTC. In general pulsing and multiple nutrient injection were found to give the greatest biostimulation. The continuous 4% methane injection was not as stimulatory as continuous 1% methane injection or pulsing of 4% methane.

The evidence for biodegradation is also convincing. Increased biodegradation was demonstrated by increases in TCE and PCE mineralization potential and by measurements of nucleic acid probes, as seen by three different labs (ORNL, UT, PNL) . The nucleic acid probe analyses demonstrated that the methanotrophs being stimulated were those possessing soluble methane monooxygenase (sMMO), the form of MMO most active in TCE oxidation [10]. Methanotroph isolates from the water that were positive for sMMO were tested for their ability to oxidize both TCE and naphthalene by UT. Those isolates from wells most effected by the injection process were shown to have rates of TCE oxidation that were more than 3 times greater than the rates for *Methylosinus trichosporium* OB3b, the type culture for methanotrophs and reputed best TCE oxidizer. Studies by the University of North Carolina (UNC) using MICROTOX and MUTATOX assays demonstrated that both sediment and water samples were not significantly toxic before, during or after the stimulation processes tested [11]. Detectable toxicity differences were seen only temporarily in two wells during the period of greatest biostimulation. Water analyses by SRTC also indicated a strong inverse correlation between TCE concentration and chloride concentration. Thus as TCE concentration declined in the ground water the chloride concentration increased. The only mechanism known that could result in this correlation is the biodegradation of TCE to carbon dioxide and chloride.

Bioremediation

Though a mass balance was difficult to determine, several measurements provide both direct and indirect evidence that very significant amounts of bioremediation occurred in situ. The evidence for bioremediation is linked by necessity to changes in TCE/PCE inventories in the soil gas, sediment and ground water and the evidence for biodegradation and biostimulation discussed above. TCE/PCE concentrations declined in all media examined; however, the amount degraded and original amount present were difficult to determine. The problem with inventories at the site was a lack of source control, (ie. more contaminated material [soil gas and water] was constantly moving from outside the treatment zone used for inventories to the inside). More highly contaminated water could move in to the saturated zone treatment area from below, due to water flow created by the injection, the sides and from above. Highly contaminated soil gas was constantly moving into the treatment area due to the much larger area influenced by the extraction well. Even given these limitations, concentrations of TCE and PCE declined in all well samples coincident with the onset of injection. Water concentrations of TCE/PCE decreased by as much as 95%, reaching concentrations below detectable limits, ie. < 2 ppb in some wells, well below drinking water standards of 5 ppb. Those wells closest to the injection well showed the greatest decline; however, as the test progressed even wells that had shown no effect during the previous in situ air stripping demonstration showed significant decline. Soil gas TCE/PCE declined by more than 99%, with the piezometers closest to the injection well having consistent undetectable concentrations by the end of the demonstration. Sediment concentrations were significantly lower after only 3 months of 1% methane

injection. Total sediment concentrations of TCE/PCE declined from 100 ppb to non-detectable concentrations in most areas. Densities of methanotrophs also were inversely correlated with the concentration of TCE in groundwater, ie. as densities of methanotrophs increased the concentration of TCE decreased. Soil gas, ground water and sediment were constantly monitored for vinyl chloride and dichloroethylene, toxic daughter products of anaerobic biodegradation. Neither of these compounds was detected except transiently at concentrations below drinking water standards (< 5 ppb). Thus, unlike anaerobic processes the methanotrophic process did not generate toxic daughter products. This further suggests that the disappearance of volatile organic compounds (VOCs) in situ was due primarily to aerobic processes. Studies by Idaho National Engineering Laboratory (INEL) using sediment/ground water chambers with material from the SRS demo site showed that high rates of biodegradation of TCE could be stimulated by the injection strategies used and that the amount of TCE biodegraded was directly proportional to the amount of chloride being produced [12]. During the field demonstration, chloride, the end product of TCE/PCE biodegradation, was measured directly in the ground water. Chloride concentration in the water was inversely correlated to TCE concentration in the same sample, (Table 2). This observation provides direct chemical evidence that bioremediation was occurring during the demonstration.

Cost Effectiveness

Los Alamos National Laboratory (LANL) analyses have shown that in situ air stripping is more cost effective, or 40-42% cheaper than the baseline technology of soil vapor extraction and ground water pump and treat [13]. The in situ bioremediation process tested was only 8% more expensive than in situ air stripping even if no TCE/PCE was biodegraded. LANL history matching models suggest that 41% more TCE/PCE is biodegraded/removed as compared to in situ air stripping alone [14]. The only costs for the in situ bioremediation process employed in this demonstration were those of the natural gas, trace nutrients and methane monitoring equipment. As little as 1,570 lbs of TCE/PCE needs to be biodegraded to offset the additional costs to the in situ air stripping system. In addition, the LANL analyses indicate that it would take in situ air stripping more than 10 years to achieve 95% removal of the contaminants, while the in situ bioremediation process would take < 4 years. This difference alone would result in a $1.6 million cost savings over the conventional system for just this one site. Indeed, the bioremediation process may be the only one that can achieve drinking water standards (< 5 ppb) in many scenarios. The bioremediation process also destroys contaminants in situ, thereby reducing the cost of any pump and treat system (gas or liquid) with which it is combined.

Ease of Use and Operation

The system was nearly completely automated and was trouble free, once the initial shake-down period (2 weeks) was complete. It was so easy to use that one full-time technician, also responsible for required analytical performance monitoring, could operate at least six of these systems simultaneously. Out of the total number of days the system could have operated, 429, it actually operated 384, or 90% of the time. Thus the system was down for only 1,097 hours, 344 h for power outages, 258 h for electrical repairs, 120 h for experiments, 285 h for maintenance, and 90 h due to inclement weather. Excluding weather, experiments. and scheduled power outages, the system was operational 95% of the

time. The electrical repairs all occurred during the first week of operation and after a lightening strike disabled a microprocessor board. All repairs were completed within 72 h [15].

This demonstration represents the first time ever that multiple nutrients (carbon, nitrogen, phosphorus) have all been injected as gases. The horizontal wells that form the basis for the SRS Integrated Demonstration provided significant advantages over conventional bioremediation nutrient delivery techniques. The increased surface area allowed better delivery of nutrients and easier recovery of gas, as well as minimizing formation clogging and plugging phenomena. There was never any indication of reduced flow or plugging during any of the six operational conditions employed. Indeed the zone of effect was far greater than that ever reported for liquid nutrient injection systems.

Summary

In Summary this demonstration has shown the following:

_ Bacteria capable of degrading TCE/PCE can be stimulated in situ using relatively simple nutrients.
_ Biostimulation and biodegradation occurred in situ with out production of toxic daughter products.
_ The process is easy to use and can be automated.
_ The cost for adding on the methane injection capability is relatively low and easily recovered.
_ Gaseous nutrient injection represents a significant new delivery technique for in situ bioremediation.
_ Combined with in situ air stripping this technology represents a significant improvement in terms of cost and efficiency over conventional baseline technologies used for remediation of chlorinated solvents.

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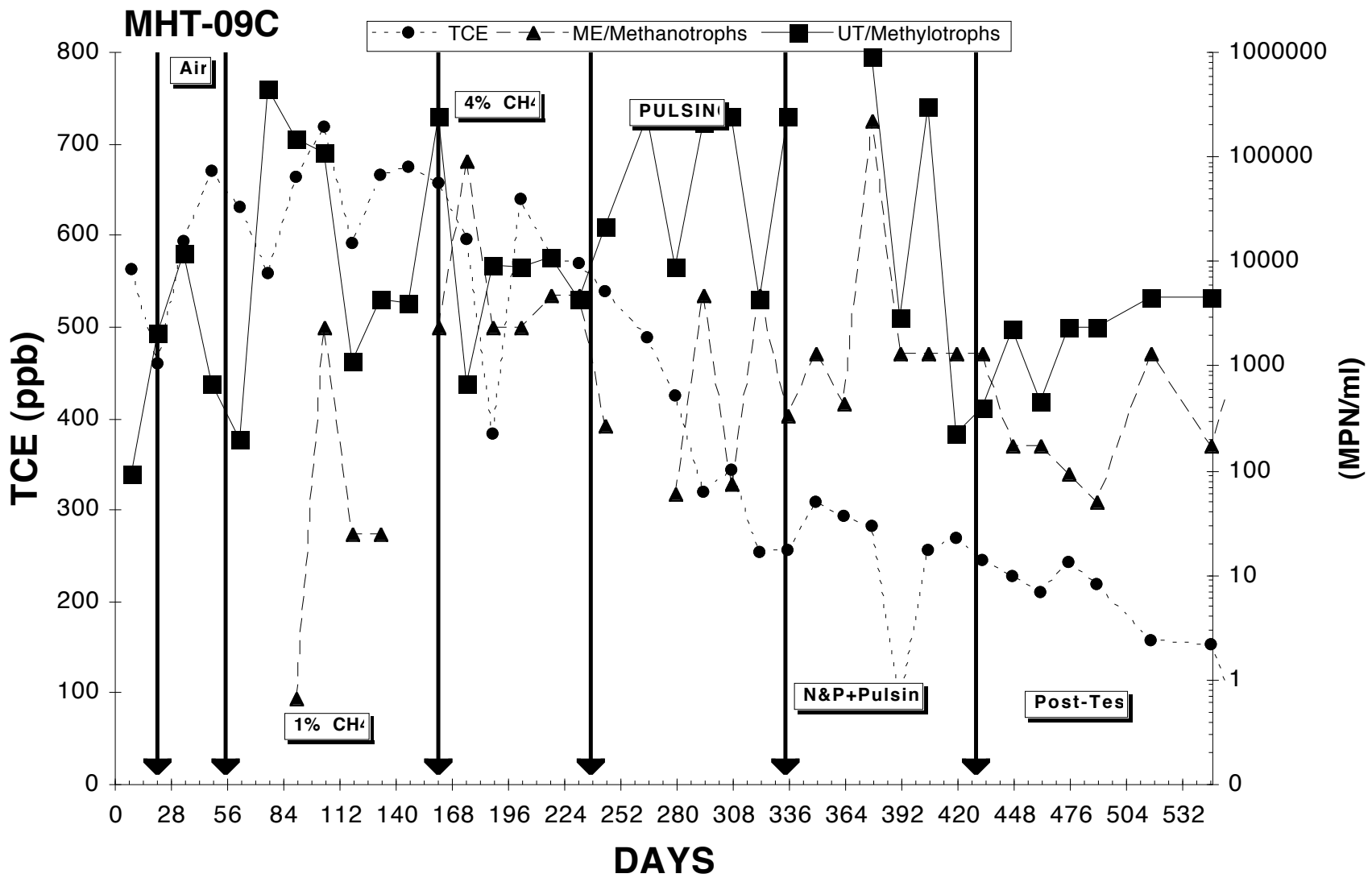
Table 2. GROUNDWATER DATA - PEARSON CORRELATION MATRIX ALPHA=0.01

	TCE	PCE	$VIABLE_{LOG}$	$AODC_{LOG}$	SRTC $CH_{4\ LOG}$	UT $CH_{4\ LOG}$	UTMETHY
$ACID\_PO_4$	0.182	0.012	0.164	0.018	-0.206	-0.270	-0.093
ALK_PO_4	-0.177	-0.131	0.483	0.434	0.197	0.114	0.125
DHA_MTT	0.094	-0.017	0.059	0.099	-0.191	-0.199	-0.085
ACTIVE	-0.045	-0.012	-0.236	-0.487	-0.033	-0.111	-0.116
TCE		0.230	-0.114	-0.274	-0.398	-0.385	-0.150
PCE	0.230		0.099	-0.133	-0.096	-0.170	-0.042
ACETATE	0.079	-0.117	0.243	0.193	0.133	-0.077	0.003
TCE MIN.	-0.079	-0.110	-0.197	0.207	-0.282	-0.337	-0.296
PCE MIN. EN	0.043	-0.045	0.013	0.131	0.074	0.108	0.147
CI^-	-0.321	0.087	0.178	-0.071	0.116	0.099	-0.054
NO_3	0.145	0.436	-0.192	-0.290	-0.316	-0.391	-0.226
PO_4	-0.116	0.033	0.066	0.141	-0.053	-0.112	-0.103
$VIABLE_{LOG}$	-0.114	0.099		0.371	0.188	0.229	0.194
$AODC_{LOG}$	-0.274	-0.133	0.371		0.156	0.165	0.144
SRTC $CH_{4\ LOG}$	-0.398	-0.096	0.188	0.156		0.688	0.444
UT $CH_{4\ LOG}$	-0.385	-0.170	0.229	0.165	0.688		0.465

KEY:
$ACID\_PO_4$ = Acid Phosphatase Assay; $ALK\ PO_4$ = Alkaline Phosphatase Assay; DHA-MTT = Dehydrogenase Assay; TCE = Trichloroethylene; PCE = Tetrachloroethylene; TCE or PCE MIN. = TCE or PCE Mineralization Assay; $VIABLE_{log}$ = Viable Counts, Log Scale; $AODC_{log}$ = Acridine Orange Direct Counts, Log Scale; SRTC $CH_{4\ log}$;Savannah River Technology Center, Methanotrophs Log Scale; UT $CH_{4\ log}$ = University of Tennessee, Methanotrophs Log Scale; UT METHY = University of Tennessee, Methylotrophs.

Figure 2. Densities of Methanotrophs and Methylotrophs vs. trichloroethylene concentrations in groundwater over time.



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