# SUMMARY OF IN-SITU BIOREMEDIATION DEMONSTRATION (METHANE BIOSTIMULATION) VIA HORIZONTAL WELLS AT THE SAVANNAH RIVER SITE INTEGRATED DEMONSTRATION PROJECT

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## ABSTRACT

The U.S. Department of Energy's Office of Technology Development has been sponsoring full-scale environmental restoration technology demonstrations for the past 4 years. The Savannah River Site (SRS) Integrated Demonstration focuses on "Clean-up of Soils and Groundwater Contaminated with Chlorinated VOC." Several laboratories, including SRS, had demonstrated the ability of methanotrophic bacteria (found in soil and aquifer material) to completely degrade or mineralize chlorinated solvents. The test consisted of injecting methane mixed with air into the contaminated aquifer via a horizontal well and extracting it from the vadose zone via a parallel horizontal well. Ground water was monitored biweekly from 13 wells for a variety of chemical and microbiological parameters. The water from wells in affected areas showed increases in methanotrophs of more than 1 order of magnitude every 2 weeks for several weeks after 1% methane in air injection started. Simultaneous with the increase in methanotrophs was a decrease in water and soil gas concentrations of trichloroethylene (TCE) and tetrachloroethylene (PCE). In two wells, the TCE/PCE concentration in the water declined by more than 90%, to below 2 ppb. All of the wells in the zone of effect showed significant decreases in contaminants in less than 1 mo. In four of five vadose-zone piezometers (each with three sampling depths) concentrations declined from as high as 10,000 ppm (vol/vol) to less than 5 ppm in less than 6 weeks. The fifth cluster also declined by more than 95%. A variety of other micro-

bial parameters increased with methane injection, indicating the extent and type of stimulation that had occurred. History-matching models constructed by Los Alamos National Laboratories (LANL) have shown that 41% more TCE is removed by biodegradation than by physical stripping alone. The LANL model has also shown that in-situ bioremediation can reach a lower concentration than in-situ air-stripping or pump-and-treat methods and that the time required to reach 95% removal is less than half the time required by the physical process.

## 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. The principal 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. This report describes the preliminary results of the demonstration and provides conclusions only for those measures that the Bioremediation Technical Support Group (Expert Panel) felt were so overwhelmingly convincing that they do not require further analyses. Though this report is necessarily superficial, it is intended to provide a basis for further evaluating the technology and to give practitioners a means to immediately apply some parts of the technology.

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. This group of experts from the U.S. Department of Energy, U.S. Geological Survey, U.S. Environmental Protection Agency, industry, and academia has met on a regular basis for the last 3 yr and has provided unique and valuable insights for the planning, execution, and evaluation of this demonstration. This group is responsible for the striking successes of this demonstration which is, without a doubt, the largest and most technically comprehensive full-scale, in-situ bioremediation demonstration ever done.

The demonstration consisted of using two horizontal wells for injection and extraction of nutrients at a site contaminated with chlorinated solvents (TCE/PCE) from a leaking process sewer line. Figure 1 shows the

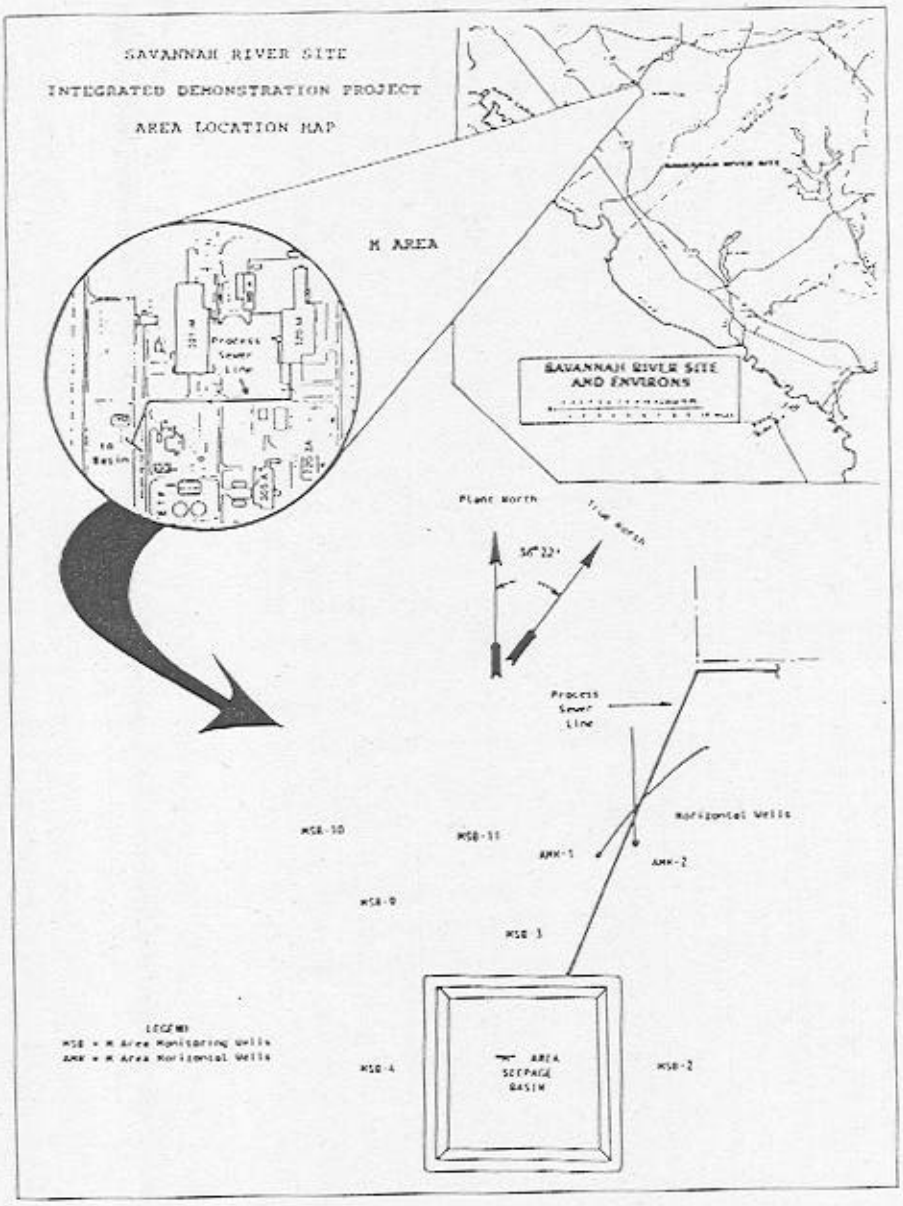


Figure 1. Savannah River Site integrated demonstration project area location map

relationship between the horizontal wells and the now-abandoned process sewer line. The lower (injection) well (175 ft deep) was installed below the water table (120 ft), and the upper (extraction) well (80 ft deep) was in the vadose zone above the water table. 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.

Figure 2 provides a pictorial view of the horizontal wells in relation to the surface-mounted equipment. Six different operational modes were tested during the 14-mo demonstration, as shown in Table 1. Air, water, and sediment samples were taken before, during, and after the demonstration as prescribed in the test plan for this demonstration.

## CRITERIA FOR SUCCESS

As decided by the Bioremediation Technical Support Group, the measures of success for the project were: (1) biostimulation/biodegradation,

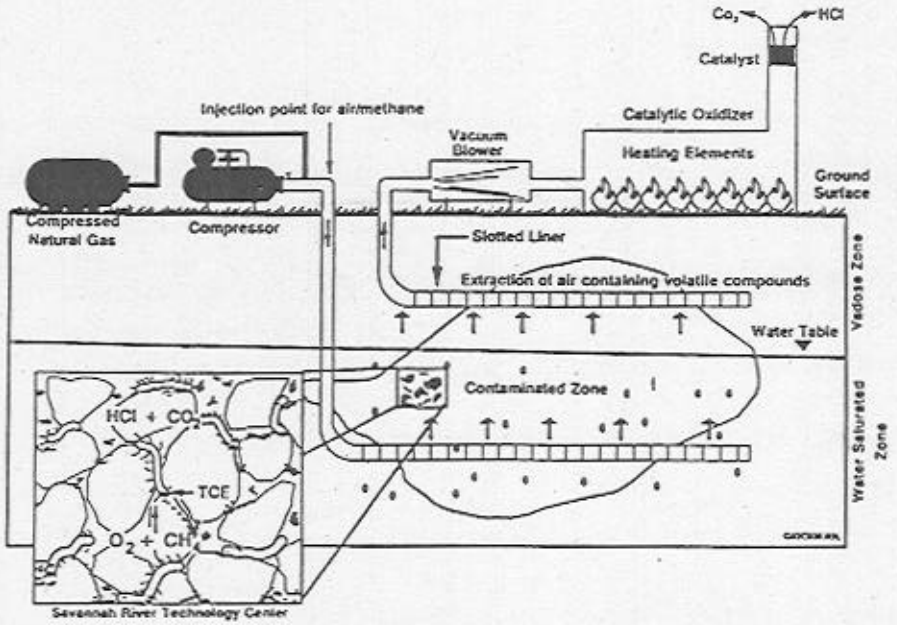


Figure 2. Relationship of horizontal wells in relation to surface-mounted equipment.

Table 1. Six different operational modes tested during 14-mo demonstration

| | 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 hr air/8 hr 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 |

(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, performed by six different laboratories, indirectly demonstrated biostimulation and biodegradation in situ by the processes tested. Densities of methanotrophs, the functional group that the process was trying to stimulate, increased in the ground water by as much as seven orders of magnitude. This stimulation occurred first in the wells that were closest to the injection point and later in more distant wells. Densities of methanotrophs in the sediment closest to the injection well increased from rarely detectable to over a million cells per gram dry weight.

The methanotroph enumerations were performed by three different laboratories (University of Tennessee [UT], Pacific Northwest Laboratory [PNL], Savannah River Technology Center) using three different methods, and all obtained nearly identical results. Increases in methanotroph densities were observed only after methane injection started. Phospholipid fatty acid analyses (PLFA) performed by the Uni-

versity of Tennessee (UT) and Oak Ridge National Laboratory (ORNL) indicated biostimulation of methanotrophs, and also that methanotrophs were being stimulated to become the dominant population in the total microbial community. Studies by Savannah River Technology Center (SRTC), UT, and PNL, using soil columns and mineralization assays, demonstrated that PCE was being biodegraded even under bulk aerobic conditions. The latter observation is particularly significant since PCE can only be degraded anaerobically. Their data suggest 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 than be oxidized by the methanotrophs.

Nucleic acid probe analyses by Washington State University (WSU), University of Minnesota (UM), SRTC, UT, and PNL showed very specifically that methanotrophs were being stimulated in the sediment. Biostimulation was also indirectly shown by the depletion of nitrate (a limiting nutrient) in the ground water as stimulation continued. Biostimulation was indirectly indicated by the increase in carbon dioxide observed in the extraction air after injection was started and by 50% consumption of injected methane, based on injection of the tracer helium and measurements of methane and helium in the 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, 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 convincing even though indirect. Increased biodegradation was demonstrated by increases seen by three different labs (ORNL, UT, PNL) that measured TCE and PCE mineralization potential and by measurements using nucleic acid probes. The latter demonstrated that the methanotrophs being stimulated were those possessing soluble methane monooxygenase (sMMO), the form of MMO most active in TCE oxidation. Methanotroph isolates from the water that were positive for sMMO were tested by UT for their ability to oxidize both TCE and naphthalene. Those isolates from wells most affected by the injection process were shown to have rates of TCE oxidation that were more than three 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. 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 ion concentration, as shown in Table 2. 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 the original amount present were difficult to determine. The problem with inventories at the site was a lack of source control, i.e., more contaminated material (air and water) was constantly moving from outside the treatment zone used for inventories to the inside. More-contaminated water could move into the saturated zone treatment area from below (due to water flow created by the injection), from the sides, and from above (due to stabilization of TCE in groundwater recharge). More-contaminated soil gas was constantly moving into the treatment area due to the much larger area influenced by the extraction well. Even with these limitations concentrations of TCE and PCE declined in all well samples with the onset of injection. Water concentrations of TCE and PCE decreased by as much as 95%, reaching concentrations below detectable limits, i.e., <2 ppb in some wells—well below drinking-water standards of 5 ppb. Those wells closest to the injection well showed the greatest decline, although, as the test progressed, even wells that showed no effect during the previous in-situ air-stripping demonstration showed significant decline. Soil gas TCE and PCE declined by more than 99%, with the piezometers closest to the injection well having consistently undetectable concentrations by the end of the demon-

**Table 2.** Measurements of sediment and ground water by 6 different laboratories during in-situ bioremediation demonstration at Savannah River Site. [a]

| | TCE | PCE | $VIABLE_{Log}$ | $AODC_{Log}$ | SRTC $CH_{4\,Log}$ | UT $CH_{4\,Log}$ | UT METHY |
|---|---|---|---|---|---|---|---|
| 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 |
| Cl- | -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 |

[a] underlining = significant ($p < 0.05$)

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.

stration. Sediment concentrations were significantly lower after only 3 mo of 1% methane injection. Total sediment concentrations of TCE and PCE declined from 100 ppb to nondetectable concentrations in most areas. Densities of methanotrophs also were inversely correlated with the concentration of TCE in ground water, i.e., 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 compound was detected, except transiently at concentrations below drinking-water concentrations (<5 ppb). Thus, unlike anaerobic processes, the methanotrophic process did not generate toxic daughter products. This further suggests that the disappearance of VOC in situ was due primarily to aerobic processes.

Studies by Idaho National Engineering Laboratory (INEL) using sediment/groundwater chambers with material from the SRS demonstration 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. 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. This observation provides direct chemical evidence that bioremediation was occurring during the demonstration.

## Cost Effectiveness

Los Alamos National Laboratory analyses to date have shown that in-situ air-stripping is more cost-effective than the baseline technology of soil vapor extraction and groundwater pump-and-treat. The in-situ bioremediation process tested was 10% less expensive then the baseline technology, even when no TCE/PCE was biodegraded. LANL history-matching models suggest that 43% more TCE/PCE is biodegraded/removed compared to in-situ air-stripping alone. The additional cost of the in-situ bioremediation process employed in this demonstration included only the natural gas, trace nutrients, and methane monitoring equipment. As little as 900 lb 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 yr to achieve 95% removal of the contami-

nants, while the in-situ bioremediation process would take <4 yr. This difference alone would result in a $1.5 million cost savings over the conventional system for just this one site. Indeed, the bioremediation process may be the only one that can reach 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) that it is combined with.

### Ease of Use and Operation

The system was nearly completely automated and extremely 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. The total number of days the system could have operated was 429; it actually operated 384, or 90% of the time. Thus the system was down for 1097 hr, 344 hr for power outages, 258 hr for electrical repairs, 120 hr for experiments, 285 hr for maintenance, and 90 hr 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, after a lightning strike disabled a microprocessor board. All repairs were completed within 72 hr.

This demonstration represents the first time that multiple nutrients (carbon, nitrogen, phosphorus) have 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 the formation of 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

The preliminary technology evaluation of this demonstration has shown that: (1) bacteria capable of degrading TCE/PCE can be stimulated in situ using relatively simple nutrients; (2) biostimulation and biodegradation occurred in situ without production of toxic daughter

products; (3) the process is easy to use and can be automated; (4) the cost for adding on the methane injection capability is relatively low and easily recovered; (5) gaseous nutrient injection represents a significant new delivery technique for in-situ bioremediation; and (6) 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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