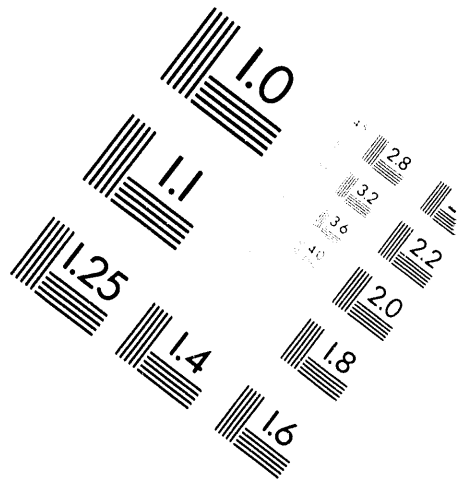
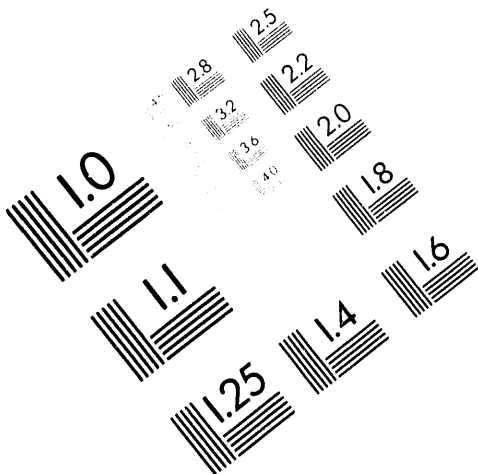




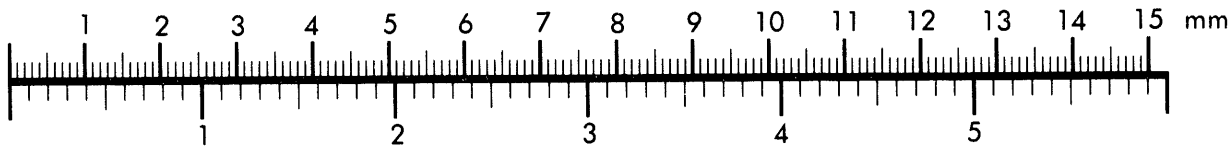
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**Association for Information and Image Management**

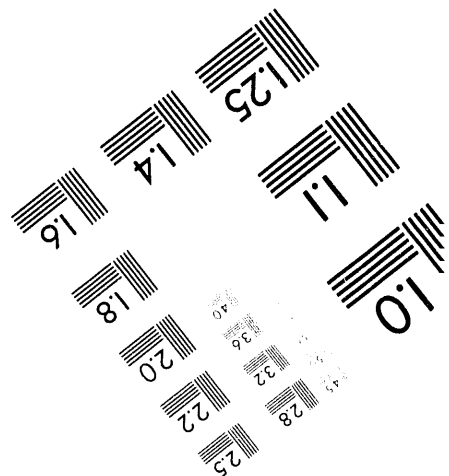
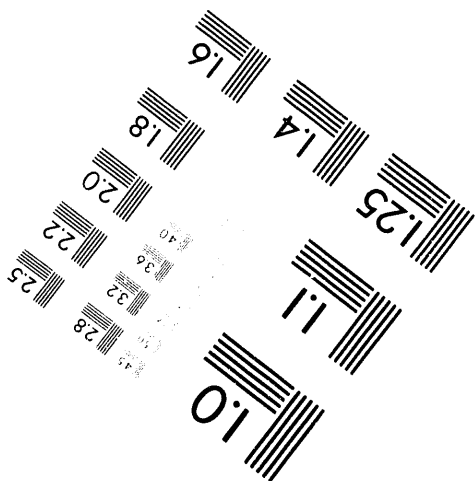
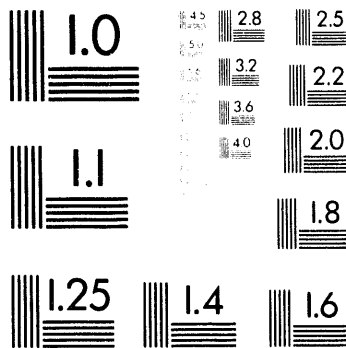
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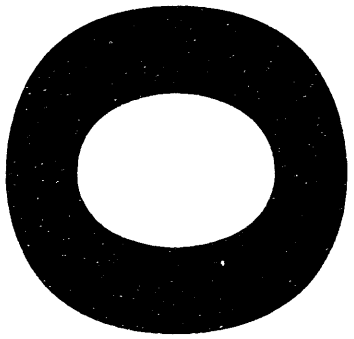
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Inches



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TECHNOLOGY SUMMARY OF THE IN SITU BIOREMEDIATION  
DEMONSTRATION (METHANE BIOSIMULATION) VIA HORIZONTAL  
WELLS AT THE SAVANNAH RIVER SITE INTEGRATED  
DEMONSTRATION PROJECT

by

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A document prepared for SPECTRUM '94 at Atlanta, GA from 8/14/94 thru 8/18/94.

DOE Contract No. DE-AC09-89SR18035

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**Technology Summary of the In Situ Bioremediation Demonstration  
(Methane Biostimulation) Via Horizontal Wells at the Savannah River  
Site Integrated Demonstration Project**

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## **Technology Summary of the 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, Office of Technology Development, has been sponsoring full-scale environmental restoration technology demonstrations for the past 4 years. The Savannah River Site Integrated Demonstration focuses on "Clean-up of Soils and Groundwater Contaminated with Chlorinated VOCs". Several laboratories including our own had demonstrated the ability of methanotrophic bacteria to completely degrade or mineralize chlorinated solvents, and these bacteria were naturally found in soil and aquifer material. Thus the test consisted of injection of methane mixed with air into the contaminated aquifer via a horizontal well and extraction from the vadose zone via a parallel horizontal well. Groundwater was monitored biweekly from 13 wells for a variety of chemical and microbiological parameters. Groundwater from wells in effected areas showed increases in methanotrophs of more than 1 order of magnitude every 2 weeks for several weeks after 1% methane in air injection was started. Simultaneous with the increase in methanotrophs was a decrease in water and soil gas concentrations of trichloroethylene and tetrachloroethylene. Two wells declined in TCE/PCE concentration in the water 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 month. Four of five vadose zone piezometers (each with 3 sampling depths) declined from concentrations 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 microbial parameters increased with methane injection indicating the extent and type of stimulation that had occurred. History-matching models by LANL has shown that 41% more TCE is removed by biodegradation than by just 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 and that the time required to reach 95% removal is less than half the time of the physical process.

## I. 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 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. 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 does intend to provide a basis for further evaluating the technology and for practitioners to immediately apply some parts of the technology. This is the first of seven Technology Reports that will be issued regarding this demonstration. The other reports will deal with Nucleic Acid Probe Analyses, Community Structure Analyses, Biodegradation Analyses, Site Preparation and Field Engineering, Modeling, and the Final Summary Report.

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 DOE, USGS, EPA, industry, and academia met on a regular basis for the last 3 years and 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 2 horizontal wells for injection and extraction at a site contaminated with chlorinated solvents (TCE/PCE) from a leaking process sewer line. Figure 1 shows the relationship between the horizontal wells and the now abandoned 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. 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 month demonstration as shown in Table 1. Air, water, and sediment samples were taken before, during and after the demonstration as per the Test Plan for this demonstration (WSRC-RD-91-23).

## II. CRITERIA FOR SUCCESS.

As decided upon by the Bioremediation Technical Support Group the measures of success for the project were 1. biostimulation/biodegradation, 2. bioremediation, 3. cost effectiveness, and 4. ease of use and operation.

### 1. 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. Densities of methanotrophs, the functional group that the process was trying to stimulate, 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 rarely 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. Increases in methanotroph densities were only observed after methane injection started. Phospholipid fatty acid analyses (PLFA) done by the University of Tennessee (UT) and Oak Ridge National Laboratory (ORNL) also indicated biostimulation of methanotrophs, and that methanotrophs were being stimulated to become the dominant population in the total microbial community. Studies by Savannah River Technology Center (SRTC) and UT using soil columns and mineralization assays demonstrated that PCE was being biodegraded even under bulk aerobic conditions. This later 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 two different laboratories, Pacific Northwest Laboratory (PNL), Washington State University (WSU), University of Minnesota (UM), SRTC and UT 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 the consumption of methane (50%), calculated via measurements of methane and helium, as a tracer, 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 as 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 but indirect. Increased biodegradation was demonstrated by increases seen by three different labs (ORNL, UT, PNL) measuring TCE and PCE mineralization potential and by measurements of nucleic acid probes. 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. 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. Detectable toxicity differences were seen only temporarily in two wells during the period of greatest biostimulation. Water analyses by SRTC also indicted 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.

## 2. Bioremediation

Though a mass balance was difficult to determine, several measurement 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 (air and water) was constantly moving from outside the treatment zone used for inventories to the inside. Higher 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. Higher contaminated soil gas was constantly moving in to 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 though as the test progressed even wells that showed 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 ground water, 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 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/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. 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.

### **3. Cost Effectiveness**

Los Alamos National Laboratory (LANL) analyses to date have shown that in situ air stripping is more cost effective than the baseline technology of soil vapor extraction and ground water pump and treat. The in situ bioremediation process tested was 10% less expensive than the baseline technology even if no TCE/PCE was biodegraded. LANL history matching models

suggest that 43% more TCE/PCE is biodegraded/removed as compared to in situ air stripping alone. The in situ bioremediation process employed in this demonstration has only the cost of the natural gas, trace nutrients and methane monitoring equipment as an additional cost. As little as 900 lbs of TCE/PCE needs to be biodegraded to off set 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 \$1.5 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.

#### **4. 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 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.

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.

### III. 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 with out production of toxic daughter products, 3) that the process is easy to use and can be automated, 4) that the cost for adding on the methane injection capability is relatively low and easily recovered, 5) that gaseous nutrient injection represents a significant new delivery technique for in situ bioremediation, and 6) that 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.

### ACKNOWLEDGMENTS

This work was funded by the Office of Technology Development, within the Department of Energy's Office of Environmental Management, under the Integrated Demonstration Program and the In Situ Remediation Integrated Program.

We are extremely grateful to nearly 100 scientists and engineers from the following organizations that participated in this demonstration: Bechtel Savannah River Incorporated, University of Tennessee, Stanford University, Idaho National Engineering Laboratory, Gas Research Institute, Radian Corporation, Eaves Oil Company, Oak Ridge National Laboratory, University of South Carolina, Pacific Northwest Laboratory, Savannah River Technology Center, Westinghouse Electric Company, ECOVA Corporation, University of North Carolina, Washington State University, University of Minnesota, US Air Force, US EPA Kerr Laboratory, DOE-HAZWRAP, ManTech, and the US Geological Survey.

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**Table 1. Operational Testing Modes**

	<u>Injection Operations</u>	<u>Start Date</u>	<u>End Date</u>
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



Table 2. GROUNDWATER DATA - PEARSON CORRELATION MATRIX

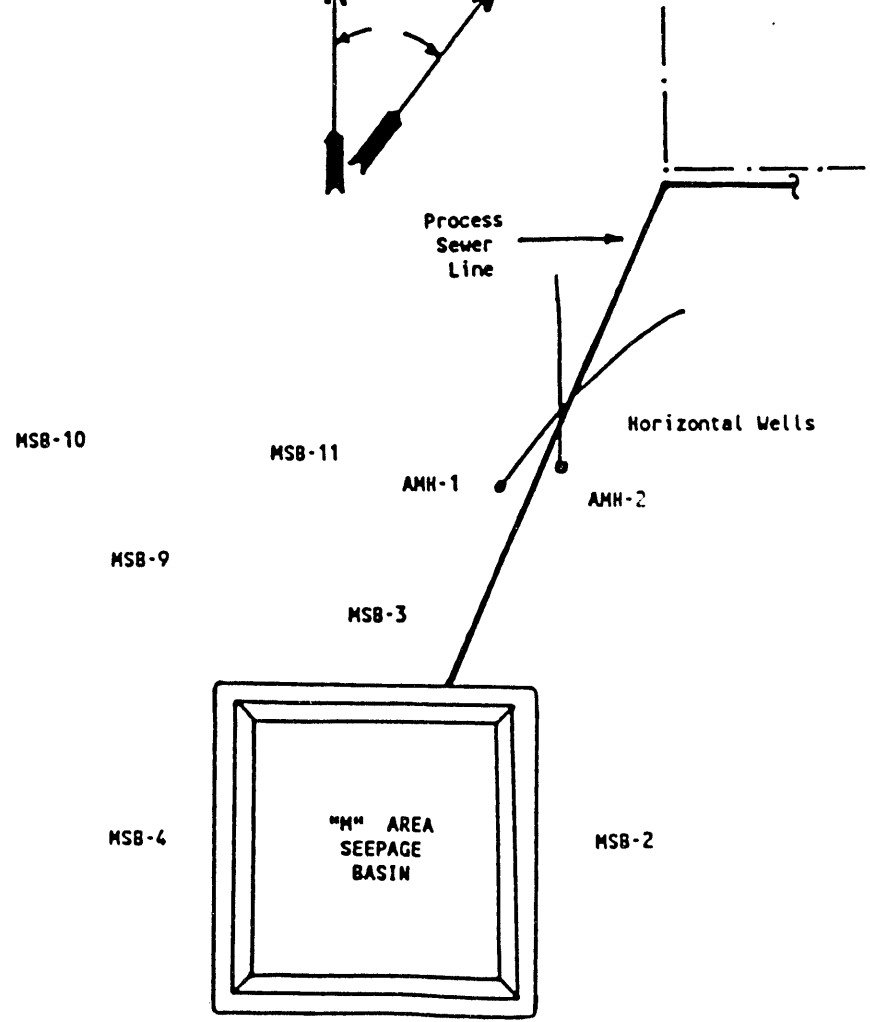
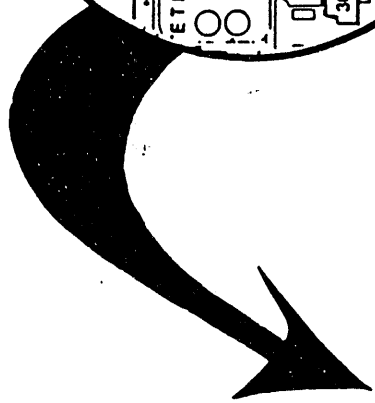
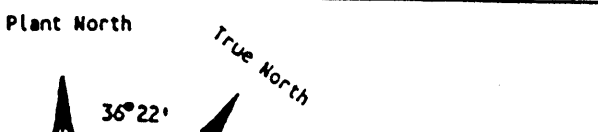
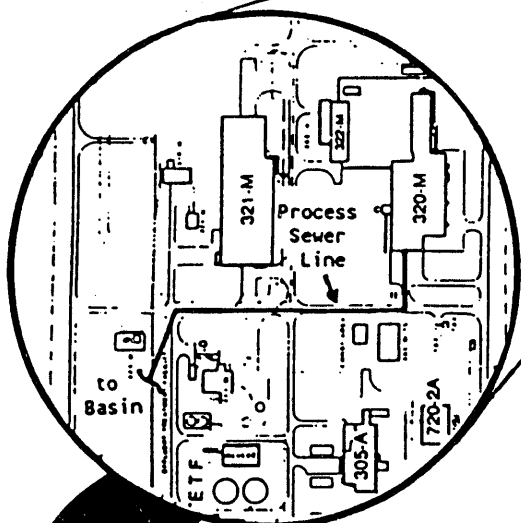
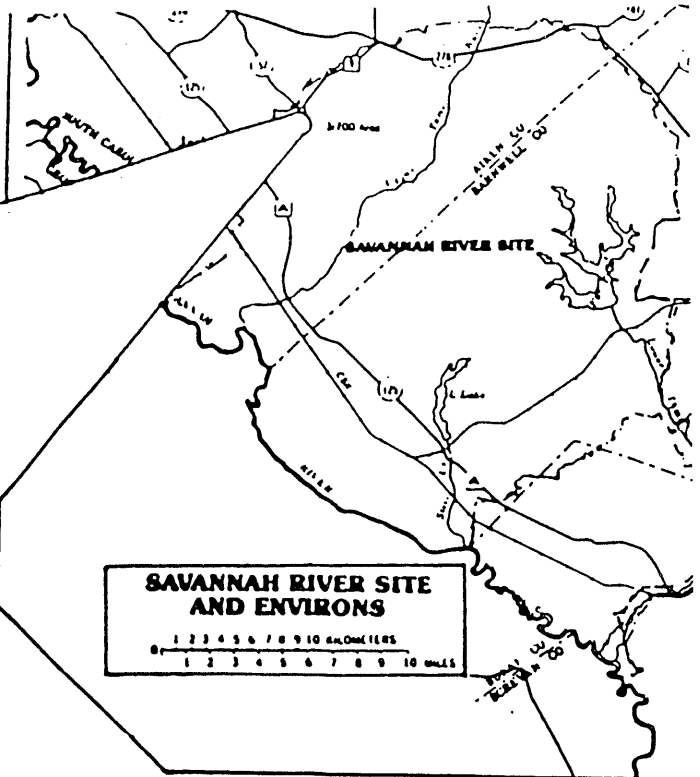
ALPHA = 0.01

	TCE	PCE	VIABLE <sub>Log</sub>	AODC <sub>Log</sub>	SRTC CH <sub>4</sub> <sub>Log</sub>	UT CH <sub>4</sub> <sub>Log</sub>	UT METHY
ACID_PO <sub>4</sub>	<u>0.182</u>	0.012	<u>0.164</u>	0.018	<u>-0.206</u>	<u>-0.270</u>	-0.093
ALK_PO <sub>4</sub>	<u>-0.177</u>	-0.131	<u>0.483</u>	<u>0.434</u>	<u>0.197</u>	0.114	0.125
DHA_MTT	0.094	-0.017	0.059	0.099	<u>-0.191</u>	<u>-0.199</u>	-0.085
ACTIVE	-0.045	-0.012	<u>-0.236</u>	<u>-0.487</u>	-0.033	-0.111	-0.116
TCE		<u>0.230</u>	-0.114	<u>-0.274</u>	<u>-0.398</u>	<u>-0.385</u>	-0.150
PCE	<u>0.230</u>		0.099	<u>-0.133</u>	-0.096	<u>-0.170</u>	-0.042
ACETATE	0.079	-0.117	<u>0.243</u>	<u>0.193</u>	0.133	-0.077	0.003
TCE MIN.	-0.079	-0.110	<u>-0.197</u>	<u>0.207</u>	<u>-0.282</u>	<u>-0.337</u>	<u>-0.296</u>
PCE MIN. EN	0.043	-0.045	0.013	0.131	0.074	0.108	<u>0.147</u>
Cl <sup>-</sup>	<u>-0.321</u>	0.087	<u>0.178</u>	-0.071	0.116	0.099	-0.054
NO <sub>3</sub>	<u>0.145</u>	<u>0.436</u>	<u>-0.192</u>	<u>-0.290</u>	<u>-0.316</u>	<u>-0.391</u>	<u>-0.226</u>
PO <sub>4</sub>	-0.116	0.033	0.066	<u>0.141</u>	-0.053	-0.112	-0.103
VIABLE <sub>Log</sub>	-0.114	0.099		<u>0.371</u>	<u>0.188</u>	<u>0.229</u>	<u>0.194</u>
AODC <sub>Log</sub>	<u>-0.274</u>	<u>-0.133</u>	<u>0.371</u>		<u>0.156</u>	<u>0.165</u>	<u>0.144</u>
SRTC CH <sub>4</sub> <sub>Log</sub>	<u>-0.398</u>	-0.096	<u>0.188</u>	<u>0.156</u>		<u>0.688</u>	<u>0.444</u>
UT CH <sub>4</sub> <sub>Log</sub>	<u>-0.385</u>	<u>-0.170</u>	<u>0.229</u>	<u>0.165</u>	<u>0.688</u>		<u>0.465</u>

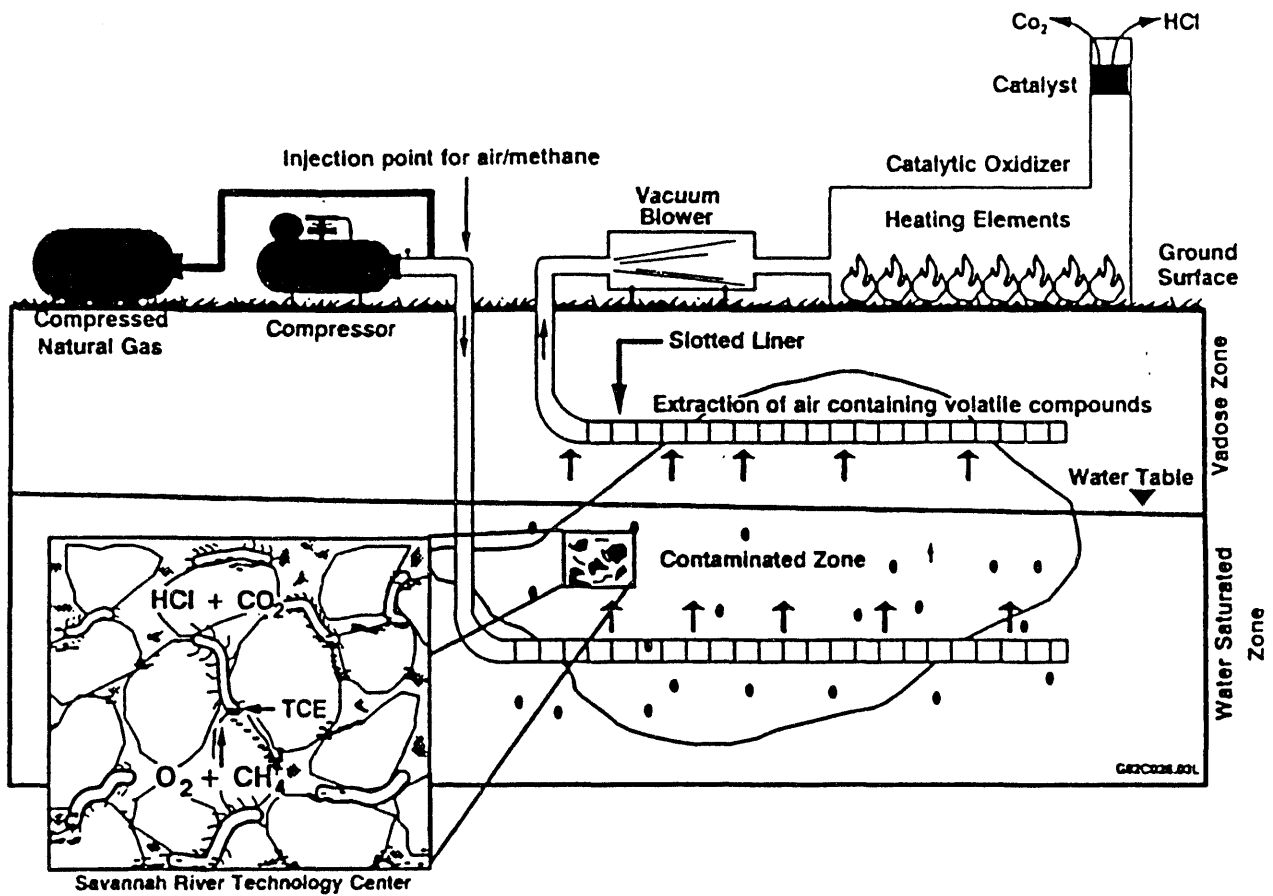
## KEY:

ACID.PO<sub>4</sub> = Acid Phosphatase Assay; ALK.PO<sub>4</sub> = Alkaline Phosphatase Assay; DHA-MTT = Dehydrogenase Assay; TCE = Trichloroethylene; PCE = Tetrachloroethylene; TCE or PCE MIN. = TCE or PCE Mineralization Assay; VIABLE <sub>Log</sub> = Viable Counts, Log Scale; AODC <sub>Log</sub> = Acridine Orange Direct Counts, Log Scale; SRTC CH<sub>4</sub> <sub>Log</sub> ; Savannah River Technology Center, Methanotrophs Log Scale; UT CH<sub>4</sub> <sub>Log</sub> = University of Tennessee, Methanotrophs Log Scale; UT METHY = Unniversity of Tennessee, Methylootrophs.

SAVANNAH RIVER SITE  
 INTEGRATED DEMONSTRATION PROJECT  
 AREA LOCATION MAP



**LEGEND**  
 MSB = M Area Monitoring Wells  
 AMH = M Area Horizontal Wells



**Captions:**

**Table 1.** Six different operational modes were tested during the 14 month demonstration.

**Table 2.** 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.

**Figure 1.** The SRS Integrated Demonstration Project Area Location Map shows the relationship between the horizontal wells and the now abandoned process sewer line.

**Figure 2.** This illustration provides a pictorial view of the horizontal wells in relation to the surface mounted equipment.

**Key Words:**

**Horizontal Wells**

**In Situ Bioremediation**

**Biostimulation**

**Trichloroethylene**

**Tetrachloroethylene**

**Running Head:**

**Technology Summary; In Situ Bioremediation**

**DATE**

**FILMED**

7/26/94

**END**

