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TEST PLAN FOR IN SITU BIOREMEDIATION DEMONSTRATION OF THE SAVANNAH RIVER INTEGRATED DEMONSTRATION PROJECT DOE/OTD TTP NO.: SR 0566-01 (U)

by

Terry C. Hazen

Westinghouse Savannah River Company
Savannah River Laboratory
Aiken, South Carolina 29808

This is a Technical Report

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Derivative Classifier
D.B. Moore-Shedrow, Section Manager
Authorized Derivative Classifier

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Terry C. Hazen

September 18, 1991 Revison 3; April 23, 1992

Authentication:

Approved by:

D.B. Moore-Shedrow, Manager
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Prepared for the U.S. Department of Energy under Contact No. DE-AC09-89R180035

Test Plan for In Situ Bioremediation Demonstration of the Savannah River Integrated Demonstration Program DOE/OTD TTP No.: SR 0566-01 (U)

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Test Plan for In Situ Bioremediation Demonstration of the Savannah River Integrated Demonstration Project DOE/OTD TTP No.: SR 0566-01

1.0 Test Plan Summary

This project is designed to demonstrate in situ bioremediation of groundwater and sediment contaminated with chlorinated solvents. Indigenous microorganisms will be stimulated to degrade trichloroethylene (TCE), tetrachloroethylene (PCE) and their daughter products in situ by addition of nutrients to the contaminated zone. 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 and public and regulatory acceptability. Bioremediation has been found to be among the least costly technologies in applications where it will work.

The horizontal wells that form the basis for the SRS Integrated Demonstration are expected to provide significant advantages over conventional bioremediation nutrient delivery techniques. The increased surface area will allow better delivery of nutrients and easier recovery of gas and water, as well as minimizing formation clogging and plugging phenomena. The principal nutrient to be supplied via the horizontal wells in this test is methane, at a low concentration in air (4%). The lower horizontal well will provide a very efficient delivery of gas throughout the contaminated region. A vacuum will be applied to the upper well (vadose zone) to encourage air/methane movement through the upper saturated zone and lower vadose zone and inhibit spreading of the plume. Air/methane mixtures have been demonstrated to stimulate selected members of the indigenous microbial community that have the capability to degrade TCE. An extensive characterization and monitoring program using existing monitoring wells and periodic borings for sediment will be used to measure the response of the soil and water following injection of air/methane. In addition, off-gas from the upper horizontal well will be assayed for methane, total VOC, TCE, PCE, potential break down products of TCE/PCE (eg. DCE, VC, and carbon dioxide). Data from the previous demonstration of in situ air stripping, where air alone was injected at different rates for 19 weeks will be used to provide base line geological, hydrological, chemical, and biological characteristics. An extensive pre-test and post-test characterization of the site via sediment borings was done for the in situ air stripping test. The post-test characterization study for the in situ air stripping test will also serve as the pre-test characterization for the in situ bioremediation test. The previous characterization and monitoring data will also establish the effect of air injection without nutrients on the hydrological, chemical, and biological characteristics of the site, in effect providing a unique and dramatic control experiment for the first bioremediation demonstration. After the test is complete another post-test characterization will be done at the site.

At startup air will be extracted from the upper well for two weeks, or until steady state concentrations of VOC are reached in the off-gases, then air alone will be injected along with extraction from the upper well, again for two weeks or until steady state concentrations of VOC are reached in the off-gases from the extraction well. Initially 4% methane/air will be injected continuously in the lower well; however, in order to insure process optimization, ie. to further stimulate the indigenous microorganisms to peak biodegradation rates and efficiencies, the injection protocol may be altered. At three month intervals during the twelve month demonstration the data from the test and process support activities will be examined by the technical support group and a decision made as to whether the injection protocol should be altered. These alterations could include changes

in injection rates, extraction rates, concentrations of methane, pulsing of air/methane to stimulate stress biodegradation, vadose zone wetting to inhibit the potential drying effect of vacuum extraction from the vadose zone, and periodic addition of other nutrients, such as phosphate, that may prove to be limiting factors. It is anticipated that more than one of these alternatives will be tried during the twelve month demonstration.

2.0 Test Objectives: The principal objective is to demonstrate the utility of in situ methanotrophic bioremediation for cleanup of non-arid waste sites contaminated with chlorinated solvents. The ancillary objectives are, 1. to establish the optimal conditions for complete biodegradation of chlorinated solvents by in situ nutrient stimulation of microorganisms, 2. to demonstrate the utility of horizontal wells as a nutrient delivery technique for in situ bioremediation, 3. to demonstrate the utility of biomolecular probes (nucleic acids, fluorescent antibodies and enzymes) and other direct analysis assays for characterization, monitoring and controlling the biological aspects of an in situ bioremediation, and 3. to establish, via process optimization studies (bioreactors) compared with in situ data, an explanatory and deterministic environmental model of the in situ methanotrophic bioremediation process.

3.0 Background: Technology and Site.

3.1 Technology Background. Biodegradation of TCE by methanotrophs (methane-oxidizing bacteria) has been demonstrated in microbiological studies and in methanotrophic laboratory-scale bioreactors. J. T. Wilson at the U.S. Environmental Protection Agency laboratory in Ada, Oklahoma was among the first to observe TCE degradation in laboratory soil columns in the presence of methane (Wilson and Wilson, 1985; Wilson et al., 1986). Investigators at Stanford University demonstrated TCE degradation by methanotrophs in laboratory columns of saturated aquifer material (Mayer et al., 1988). Little et al. (1988) at ORNL isolated a mixed methanotrophic culture from a TCE-contaminated well on the Oak Ridge Reservation. This culture was subsequently used in a prototype lab-scale continuous flow bioreactor at ORNL (Donaldson et al., 1988). Fliermans et al. (1988) at SRL isolated consortia and species capable of aerobic degradation of TCE with methane as the primary nutrient from TCE contaminated soil and groundwater from the Savannah River Site. These organisms have also been successfully used in laboratory scale fluidized bed bioreactors to treat TCE/PCE contaminated groundwater (Phelps et al., 1990).

Methanotrophs, methane-oxidizing bacteria, oxidize methane via a series of enzymes that are unique to this group. The primary enzyme in this oxidation chain is methane monooxygenase. Methane monooxygenase is an extremely powerful oxidizer, thus giving it the capability of oxidizing a wide variety of normally recalcitrant compounds including TCE. Wackett (Newman and Wackett, 1991; Tsien et al., 1989) and others (Chaudhry and Chapalamadugu, 1991; Wilson and Wilson, 1985; Fogel et al., 1986; Little et al., 1988) have shown that the soluble methane monooxygenase type I induces formation of TCE-epoxide from TCE. TCE-epoxide is extremely unstable and therefore spontaneously breaks down to simpler compounds like formate, etc. All of the daughter compounds are either unstable or small and easily metabolizable compounds, thus making the final and almost immediate end products of TCE-epoxide formation, carbon dioxide and chloride salts. Several investigators have also shown that even though TCE is degraded by methanotrophs they achieve no measurable benefit from the reaction making it a fortuitous metabolism or as some investigators prefer, co-metabolism/co-oxidation.

Other leading investigators in the development of TCE bioremediation technology include W. Jewell at Cornell University, P. McCarty at Stanford University, D. White and T. Phelps at The University of Tennessee (UT), S. Fogel at CAA, Inc., and a group at Battelle Columbus. These investigators comprise a consortium for development of this methanotrophic treatment technology under the auspices of the Gas Research Institute and the Savannah River Laboratory (SRL). The investigators meet regularly to exchange technical information, and Radian Corporation is serving as a data repository and process evaluation function under contract to the Gas Research Institute. The combined expertise and knowledge base of this consortium will be essentially an ad hoc resource to this present DOE in situ remediation project since Oak Ridge National Laboratory (ORNL), UT, and SRL are charter members of the consortium.

In addition to the laboratory bioreactor studies at ORNL, UT, and elsewhere, one pilot-scale bioreactor system has been operated by Battelle Columbus at Tinker Air Force Base, Oklahoma (Wickramanayake et al., 1990). This project was funded by the Air Force Engineering and Services Center, Tyndall Air Force Base, Florida. This pilot-scale study demonstrated that actual TCE-contaminated groundwater can be treated in a trickle-bed bioreactor. The culture used in this test was provided by ORNL. Tyndall AFB is continuing to support development of TCE bioreactor technology at ORNL and UT and Savannah River Site (SRS). The bioreactors used at Tinker Air Force Base are

being provided by the Air Force for further field tests at Oak Ridge and Savannah River.

Although development of methanotrophic bioreactors for TCE bioremediation is progressing well, in situ biodegradation of TCE is an emerging technology that has not yet been demonstrated at a practical scale. Tests on a small area of a shallow aquifer at the Moffett Naval Air Station in California (Semprini et al., 1988) have shown that indigenous microorganisms can be stimulated with methane and oxygen to degrade TCE. These results are very encouraging. Their experiences in these studies are a large part of the basis for the process design for this in situ demonstration at the SRS.

Methane itself is generally recognized as a natural compound found universally in subsurface environments. Years of experience by the Oil and Gas industries have shown that subsurface environments and groundwater can be exposed to high concentrations of methane for many years with no adverse effects. In addition, the U.S. Geological Survey has used methane as a conservative tracer in groundwater at Cape Cod for several years at their Groundwater Flow Study Facility with no adverse effects (Harvey and George, 1987; Garabedian, 1990). Thus we are confident that methane can be injected safely with extremely low probability of any adverse environmental effects of any kind.

3.2 Technical Need. Organic xenobiotic chemical contamination of groundwater has become the most important pollution problem of industrialized nations of the world. More than 15% of community drinking water supplies in the United States are contaminated with carcinogenic, chlorinated hydrocarbons (Craun, 1986; Patrick et al., 1983). Identification of previously unknown waste disposal sites that are impacting groundwater occurs almost daily, thus the extent of the problem is undoubtedly greater than any of the current data suggest. Indeed, our reliance on groundwater in the United States has steadily increased over the past 30 years, not only for drinking water, but also for industrial processes, agricultural irrigation, etc. (Craun, 1986; Patrick et al., 1983). As sources of clean surface water steadily decline, our reliance on groundwater will undoubtedly continue to increase far into the next century. Thus, with increasing urgency ways have been sought to clean-up, i.e. remediate, contaminated groundwater. The major organic contaminant of waste sites at DOE facilities is also chlorinated solvents.

Subsurface soils and water adjacent to an abandoned process sewer line at the SRS have been found to have elevated levels of TCE/PCE. This area of subsurface and groundwater contamination is the focus of a current integrated demonstration of in situ air stripping technology utilizing horizontal wells. Bioremediation has the potential to enhance the performance of in situ air stripping as well as offering stand-alone remediation of this and other contaminated sites. Horizontal wells could also be used to enhance the recovery of groundwater contaminants for bioreactor conversions from deep or inaccessible areas (e.g., under buildings) and to enhance the distribution of nutrient or microorganism additions in an in situ bioremediation.

The basic concepts of this technology are expected to be applicable to other sites having TCE-contaminated soils and water. However, the particular process designs will be site specific. The experience gained at the SRS Integrated Demonstration will provide the basis for designs for other sites. The generic needs for this technology are described in Sections 3.1.3 and 3.1.4 of the USDOE Environmental Restoration and Waste Management Five-Year Plan (1989). Regulatory drivers for this activity are RCRA (40 CFR 264 and 265 Subparts F and G), CERCLA (40 CFR 300 1986 Amendments Section 122) and SDWA (40 CFR 141).

3.3 Alternatives. The principal existing technology for remediation of TCE-contaminated groundwater is pumping followed by air stripping. Unsaturated sediment contamination can only be remediated by vapor extraction. Neither of these are TCE destruction technologies; in both cases the TCE is either discharged to the atmosphere or captured on activated carbon for subsequent disposal. At the SRS no air emission restrictions are presently in force, and air stripping is being used already. However, the lack of emission restrictions is not the usual case, and may well change at the SRS in the very near future.

Preliminary economic evaluations have shown that while air stripping without emissions control is the least costly technique, biodegradation will be very competitive with air stripping with emissions control.

3.4 Benefits. in situ bioremediation technology is based on biological destruction of the contaminants at the site. Therefore, risks normally associated with handling, transporting, and treating or storing contaminated residuals are avoided. In this sense there is a very significant reduction of risk.

Costs for in situ bioremediation of TCE are not known since this is an emerging technology. However, current in situ bioremediation technologies for other organics (such as gasoline) are nearly always less expensive than alternative technologies that provide destruction of the contaminant (and hence permanent remediation). Cost analysis of methanotrophic bioreactors compared with air stripping combined with carbon adsorption of the air stream and direct carbon adsorption from the water have suggested that for several TCE concentrations and flow rates that the methanotrophic system would save 40-60% over conventional technologies (Radian, 1989). We expect that these observations will also be the case for in situ bioremediation of TCE alone or in combination with bioreactors.

in situ bioremediation coupled with air stripping is expected to lead to a significant reduction in the time required to complete the remediation because bioremediation provides a second simultaneous pathway for removal (destruction) of the TCE. Furthermore, the stimulated indigenous microorganisms will gain access to TCE in the vadose zone and aquifer matrices that may be very difficult to remove by air stripping. Thus a "cleaner" endpoint should be reached in less time.

The enzymes induced in the microorganism by the methane cometabolically oxidize a host of other organic compounds, including toluene, benzene, etc. Since many contaminated sites also contain these or similar compounds, in situ bioremediation and bioreactor systems also address their degradation. Preliminary laboratory studies have demonstrated the proof of this principle (Phelps et al., 1990)

3.5 Acceptability. Bioremediation technologies enjoy relatively high regulatory acceptability in cases where the technology has been demonstrated to be effective. Regulatory agencies are also showing interest in the addition of specialized microbial cultures to the site. California has granted permits for demonstration projects that inject nutrients and TCE-degrading bacteria into a contaminated aquifer. California, Texas and Michigan have allowed field project injection of methane and nutrients for in situ bioremediation of TCE contaminated aquifers. Massachusetts and other states have also allowed methane to be injected into aquifers as a tracer for several years. There is a clear precedence for this type of project in the field. This general environment bodes well for approval to use in situ bioremediation at the SRS.

Bioremediation enjoys relatively favorable societal acceptance, in part because it is perceived to be "natural." Essentially ambient process conditions and the lack of unsightly large equipment also contribute to societal acceptability. Use of genetically engineered organisms is not yet socially acceptable. However, such organisms will not be needed at the SRS (although they may offer process advantages at a later date when the acceptability issue has been resolved).

3.6 Site Description. The Savannah River Site is a 300 square mile facility owned by the U.S. Department of Energy and operated under contract DE-AC09-89R180035 by the Westinghouse Savannah River Company. The site is near Aiken, South Carolina (Figure 3.1). The site has been operated as a nuclear production facility for DOE since 1950. The production processes carried out over the past 40 years have generated considerable waste and waste sites. This waste includes radiological, heavy metals, organic solvents, sanitary landfills and other types of mixed wastes. Many contaminated environments at SRS have been identified including both surface water and soils, subsurface sediment and groundwater. Cleanup of these wastes and waste sites has become a top priority for DOE. Due to the large number of waste sites and large volume of contaminants at many of these sites a considerable amount of time and money will be required to complete the mandated cleanup. Thus, another priority stemming from this cleanup program is to develop and demonstrate new and innovative technologies that may decrease costs, decrease time, decrease environmental impact and/or result in a cleaner end point.

The 300-M Area operations of SRS were used to fabricate fuel and target elements that were later irradiated in SRS reactors (Figure 3.1). During these operations the elements are degreased at several stages in the process. These degreasing operation generated large amounts of metal-degreasing solvent wastes. From 1952 to 1982, M Area used an estimated 13 million pounds of chlorinated degreasing solvents (Marine and Bledsoe, 1984). Evaporation alone accounted for 50 to 95% loss, while the remainder went to the M Area process sewer system. Marine and Bledsoe (1984) estimate that as much as 2 million pounds may have been released to the sewer that leads to the M Area Settling Basin; another 1.5 million pounds went directly to the A-14 outfall at Tims Branch. The discharges to the M Area Settling Basin consisted primarily of trichloroethylene (TCE: 317,000 lb.), tetrachloroethylene (PCE: 1,800,000 lb.), and 1,1,1-trichloroethane (TCA: 19,000 lb.) (Marine and Bledsoe, 1984). From 1952 until 1962 TCE was used; in 1962 the process in one of the facilities was changed and PCE was substituted for TCE, TCE continued to be used until 1971. In 1979 PCE was replaced by TCA. By 1976 all discharges from the area were disposed of via direct release into the M Area seepage basin (Figure 3.1). Solvents were detected in the groundwater below M Area Basin in 1981 and visual inspection of the terra cotta pipe of the process sewer line revealed cracks and root penetration; this pipe was relined in 1984. The solvents discharged into the settling basin spread through the vadose zone and entered the groundwater below the basin. The leaking process sewer line used to convey these wastes to the basin also released large quantities of the solvents into the surrounding vadose zone sediments. The process sewer line was abandoned and removed in 1986. The seepage basin was contained via a clay cap closure (RCRA) completed in 1991 (DPSPU 84-11-11), State accepted and closed 9/91. Groundwater and sediment contamination in M Area is extensive, however, vadose zone (surface to water table) contamination is confined to the linear source associated with the leaking process sewer line, solvent storage tank area, settling basin, and the A-14 outfall. A conventional groundwater extraction and treatment system (air stripper) has been in operation since 1984 and has removed more than 230,000 pounds of solvents from the groundwater. For detailed descriptions of discharges from the area see Marine and Bledsoe (1984), Christensen and Brendell (1982), and Pickett (1985).

The residual solvents in the vadose zone associated with the abandoned process sewer line and the settling basin continue to leach into the groundwater covering more than 1 square mile. Since the plume caused by the leaking process sewer line was linear, horizontal wells were selected as the injection and extraction system that would best remediate the site. The horizontal wells were installed in 1988 (Kaback et al., 1989) and the area has been extensively characterized in terms of its hydrology, geology and ecology. For a complete characterization of the site see Eddy et al. (1991). From July 1990 to December 1990 the site was used to demonstrate in situ air stripping via the horizontal wells; for a complete description of the test see Looney et al. (1991).

SAVANNAH RIVER SITE

INTEGRATED DEMONSTRATION PROJECT

AREA LOCATION MAP

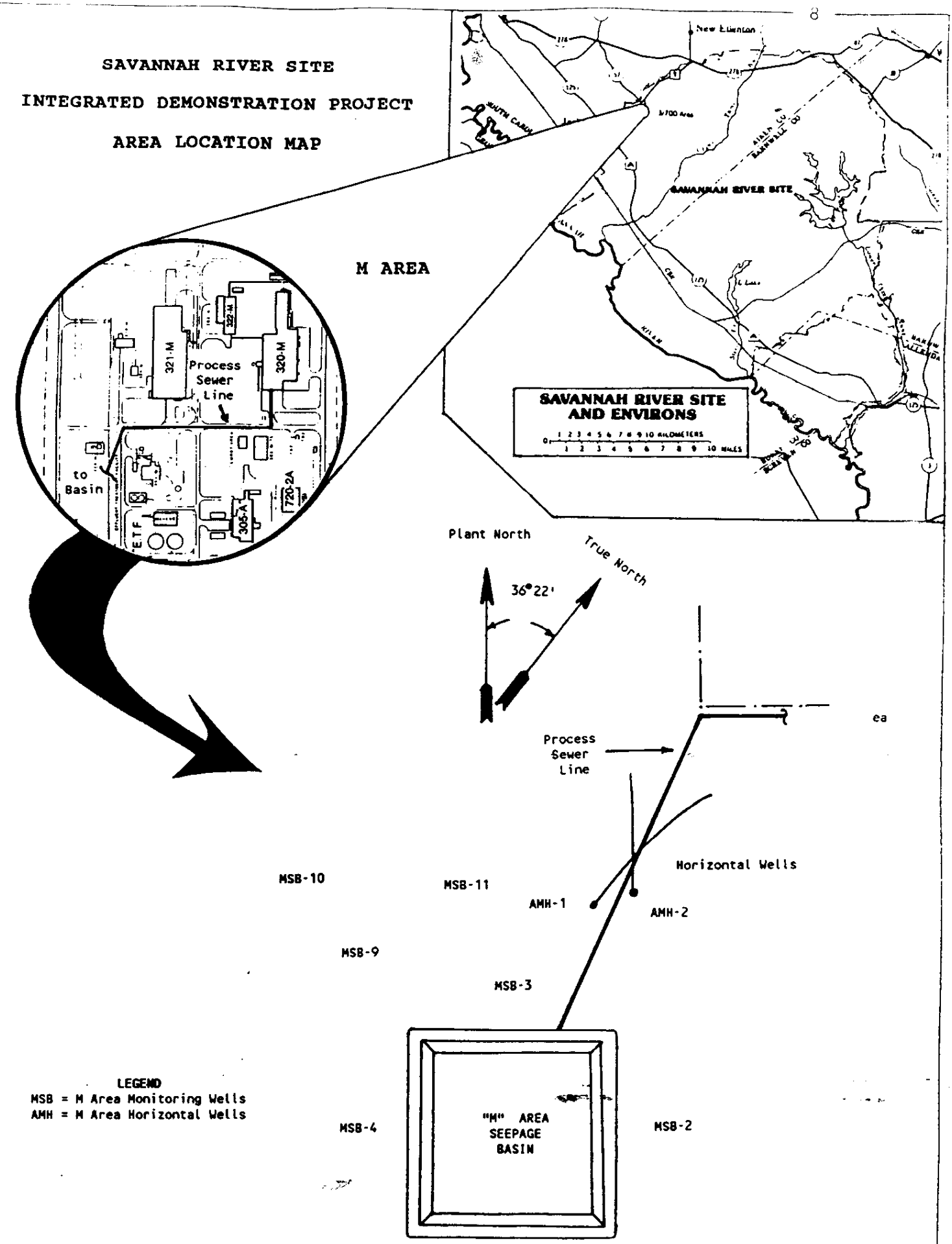


Figure 3.1

4.0 Test Plan

4.1 Criteria for Success. There are four primary criteria by which the overall success of this demonstration will be evaluated:

1. Evidence of biological destruction (biodegradation) of TCE from the contaminated soils and water. Since a major advantage of bioremediation is destruction, it is important and significant to demonstrate that biodegradation is occurring. The evidence is expected to come primarily from comparison of the compositions of the off-gases before and after addition of methane to stimulate biodegradation, and from laboratory studies in soil columns using soil cores from the site. In the latter case we expect to show that radiolabeled TCE is degraded under conditions similar to those in the field.

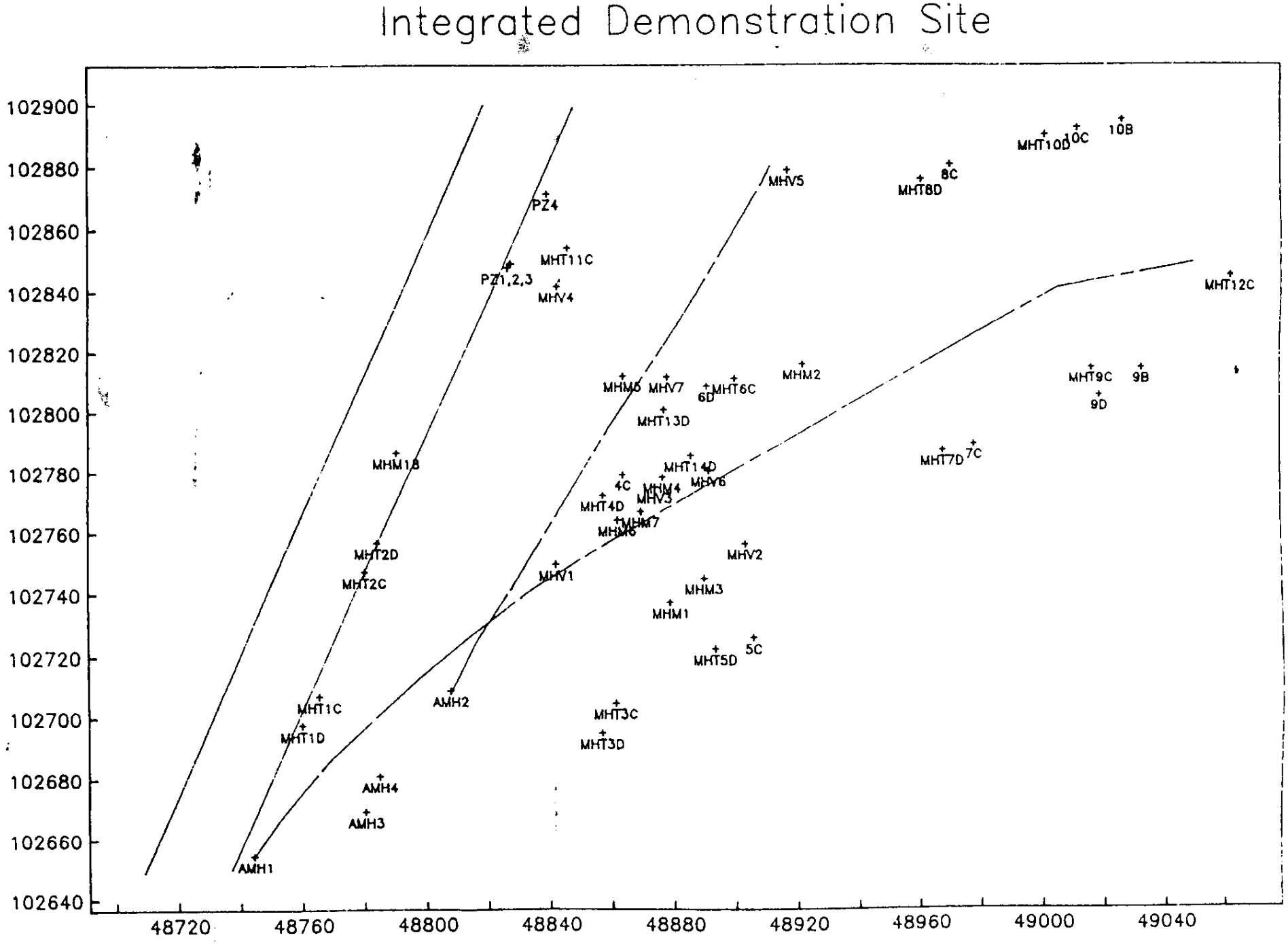
2. Increased reductions of TCE in soil and water samples from the site during periods of biostimulation. The technology is expected to accelerate the removal of TCE over in situ air stripping alone, which is the focus of the first phase of the integrated demonstration.

3. Reduced cost over comparable conventional technologies. Comparison of costs of air stripping currently in use at the site and cost of in situ air stripping from the first demonstration. Costs of air stripping, in situ air stripping operations and the bioremediation can be compared to rates of removal and/or degradation to arrive at normalized costs for all three processes for the same site.

4. Relatively simple and trouble-free operation. These characteristics contribute to favorable economics. A critical assumption for the successful demonstration is that gases can be successfully injected via the lower horizontal well and recovered via the upper well. This ability has been demonstrated in phase 1 of the integrated demonstration project. The wealth of data from phase 1 can be compared and used as a control for the bioremediation project.

The **principal uncertainties** concern the rate of TCE removal/degradation--how long it will take. The permeability of the soil will influence the delivery of nutrients (gases and potentially liquids) to the bacteria. Slow delivery will mean slow bioactivity. Similarly, heterogeneity's in the strata may cause some regions to be bypassed; however, if the contaminants infiltrated these zones, then nutrients will too, but it may occur slowly. We do not believe there will be a danger of plugging the soil around the wells by the growth of biomass. This phenomenon has occurred in the past at other bioremediation sites; however, we now know how to avoid this problem by the proper addition and/or omission of nutrients.

4.2 Pre-Test Characterization and Monitoring. Continuous cores were collected to a depth of approximately 200 feet from one borehole in each of the ten two well MHT clusters (Figure 4.1). Above the water table, samples were collected using a split spoon sampler with a hollow stem auger. Below the water table, a punch core was used in conjunction with mud rotary drilling to collect the core samples. Geophysical logging of the MHT boreholes included natural gamma ray, caliper-resistivity (16" and 64"), density, and neutron logs. The MHT and MHV cores were geologically logged in the field: samples were collected at 5 foot intervals and major lithology changes for VOC analysis; and samples for microbiological analysis were collected every 10 feet. The MHT cores were microscopically examined in the SRL core-logging laboratory. Sand (grains 2 mm - 0.0625 mm), gravel (grains > 2 mm),


Integrated Demonstration Site
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102660
102640
48720
48760
48800
48840
48880
48920
48960
49000
49040
MHT10D
10C
10B
MHT8D
8C
MHV5
PZ4
MHT11C
PZ1,2,3
MHV4
MHT12C
MHM2
MHM5
MHV7
6D
MHT6C
MHT13D
MHT9C
9B
9D
MHM1B
MHT14D
MHT7D
7C
4C
MHV6
MHV4
MHT4D
MHV3
MHM7
MHM6
MHT2D
MHV2
MHT2C
MHV1
MHM3
MHM1
5C
MHT5D
AMH2
MHT1C
MHT3C
MHT1D
MHT3D
AMH4
AMH3
AMH1

Figure 4.1.

clay (grains < 0.0625 mm), and carbonate percentages were determined, as were the muscovite, lignite, glauconite and sulfide content of the cores. Selected samples were sieved for grain size analysis. The MHT clusters were completed as four inch monitoring wells and consist of a well screened in the water table (designated with the suffix D) and a well screened with five foot screens in the underlying semiconfined aquifer at elevations ranging from 204 to 214 feet (designated with a suffix C). Ideally, the water table wells were to be screened with twenty foot screens with 5 feet of the screen above the water table and 15 feet below the water table. Since the water table zone is approximately 5 to 10 feet thick, the twenty foot screens were installed with more than 5 feet of the screen above the water table to avoid screening into the underlying semiconfined aquifer. Specific well construction details are given in Eddy et al. (1991). Five borings (designated by the prefix MHV) were cored in order to install piezometer clusters in the vadose zone. MHV4 is located west of the injection and extraction wells, MHV1, MHV3 and MHV5 are located between the vapor extraction and injection wells, and MHV2 is located east the injection and extraction wells (Figure 4.1). These borings were drilled with 6-1/4 inch hollow stem auger and sampled with a split spoon sampler to at least 120 feet. Continuous sediment cores were collected and sampled for VOC analysis. Each of the MHV holes was completed as a multiple piezometer cluster. Three piezometer tubes were installed in each hole: each tube was completed with a one inch tee, one inch ball valve, an access port, and a five foot screen. Specific well construction details are given in Eddy et al. (1991). A HydroPunch sampler was used to collect groundwater samples at discrete depths. Samples collected with the HydroPunch are designated with the prefix MHP and were collected adjacent to the well clusters at MHT2, MHT3, MHT4, MHT5, MHT7, MHT8, MHT9, and MHT10. Each sample was analyzed for VOC content and baseline microbial characteristics. All collection methods were designed to minimize microbial contamination of cores from adjacent depths and drilling fluids. Barrels were steam cleaned between collections.

Data reported from this pre-test characterization for the in situ air stripping demonstration provides much of the baseline data for the demonstration site in terms of geology, hydrology and biology (Eddy et al., 1991). Data on the following parameters are included in this report: elevation of geological picks, monitoring well completion details, stratigraphy, geologic cross sections, 3-D mapping, geophysical logs, conceptual description of SRS groundwater system, aquifer characteristics, high and low nutrient heterotrophic plate counts, TCE/PCE concentration, acridine orange direct counts, phospholipid fatty acids, DNA probes, fluorescent antibody probes, plasmid frequency, methanotroph counts and hydropunch samples of water coincident with sediment sampling at the time of boring. Additional analyses not included in this report are currently in progress; sulfate, sulfide, total sulfur, phosphate, total phosphorus, total organic carbon, total Kjeldahl nitrogen, nitrate, nitrite, ammonia, cation exchange, chloride, and iron. Methods are summarized in Section 4.3.4.

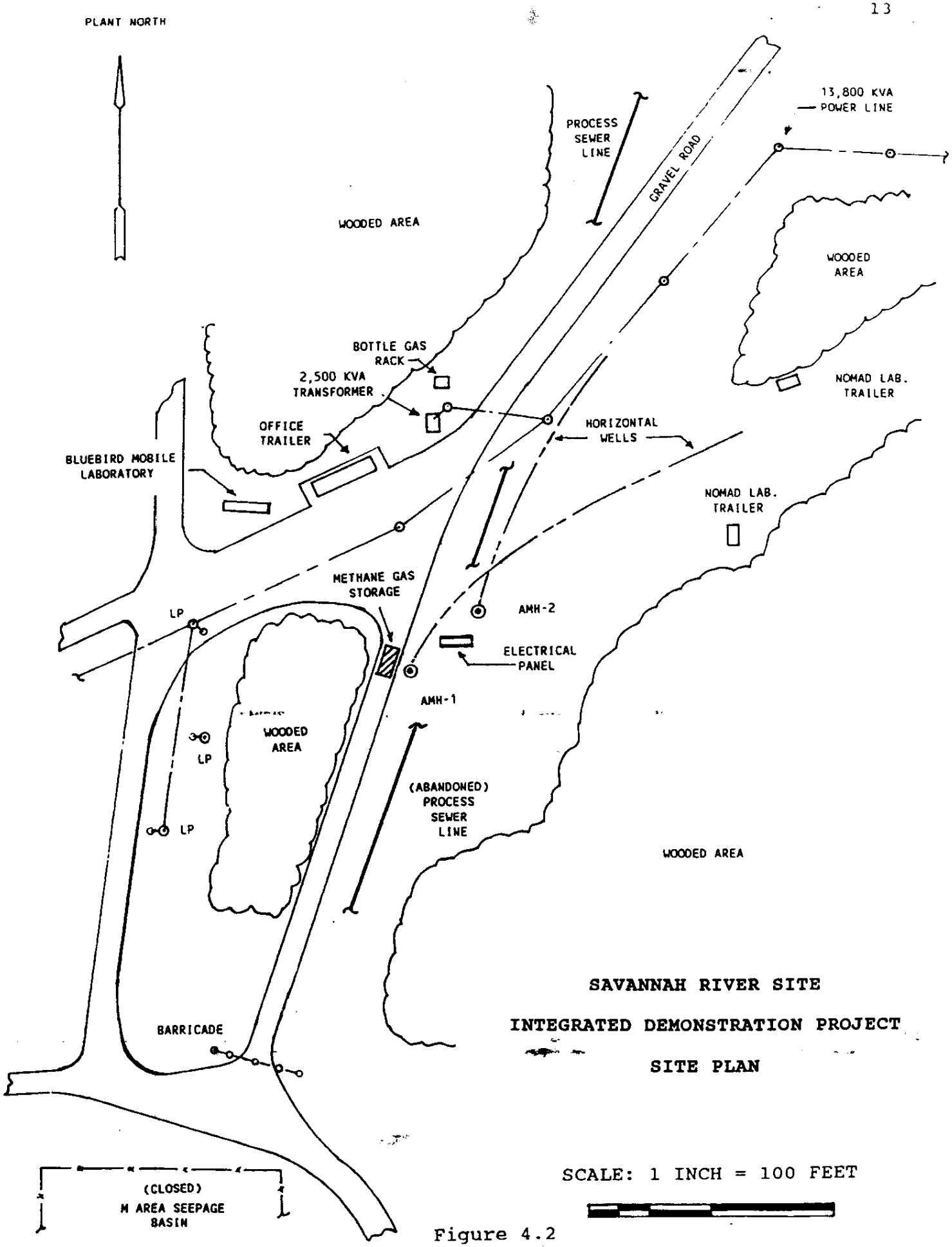
The post-test characterization for the in situ air stripping demonstration will also be used to provide the most recent pre-test characterization data for the geology, hydrology and biology data for the in situ bioremediation demonstration. Sampling for the in situ air stripping post-test/ in situ bioremediation pre-test characterization began in March 1991 and was completed in June 1991. Eight additional boreholes (MHT-10B, MHT-9B, MHT-11C, MHT-5V, MHT-7T, MHT-3T, MHT-1V, MHT-2T) were drilled and sediment samples taken as described above in this section 4.2. The sample analyses were done as described above. Data analysis for these samples has not yet been completed, but will be added to the characterization data as another report by March, 1992. See Eddy et al. (1991) and section 4.3.4 below for all methods and procedures.

In situ bioremediation pre-test monitoring from groundwater began August 15, 1991. Water samples are being collected using dedicated submersible pumps according to documented SRS well sampling protocols (WSRC 3Q5). Bulk water parameters including temperature, pH, dissolved oxygen (DO), conductivity, and oxidation-reduction potential (ORP) are measured using a Hydrolab Surveyor model (Hydrolab Inc., Houston, TX). Samples are collected for microbiological studies and VOC analyses. Based upon previous sampling (Looney et al., 1991) the following wells will be sampled every 2 weeks for the duration of the project: MHT-1C, MHT-2C, MHT-3C, MHT-4C, MHT-5C, MHT-6C, MHT-7C, MHT-8C, MHT-9C, MHT-11C, MHT-9B, MHT-10B. All sampling and analysis are as described below in section 4.3.3.

4.3 Test Description.

(Note: The principal parties responsible for an activity are noted in italics at the end of each section)

4.3.1 Injection Protocol. Site plan layout is shown in Figure 4.2. Extraction of the upper horizontal well (AMH2) in the vadose zone, will begin first at a rate of 240 SCFM for 2 weeks. This initial extraction-only operation should encourage flow of air through the vadose zone. After 2 weeks 100% air will be injected at 200 SCFM (100 psig) for 2 weeks into the lower horizontal well (AMH1) in the saturated zone. This is as per requirements of the SRS-IDP Monitoring Technical Support Group, to establish baseline injection data for in situ flow sensors, etc. After 2 weeks of 100% air, the injection will be changed to 4% methane in air at 100 psig injected at the rate of 200 SCFM. Initially 4% will be used to provide the greatest quantity of methane possible (includes a margin of safety of 20% relative to the lower explosive limit of 5% methane). Should lower injection or extraction rates be required for any reason, both will be adjusted so that the extraction rate is no more than 20% higher than the injection rate. This strategy is used to insure that we are preventing plume spreading resulting from injection but also substantially decreasing abiotic processes, ie. in situ air stripping. The rates and pressures were determined from the previous in situ air injection test with the same wells (Looney et al., 1991). The in situ air stripping test demonstrated that injection rates of 170 SCFM stimulated bacteria density increases in the groundwater, the lower rate of 65 SCFM had no effect and 270 SCFM stimulated bacteria only marginally in some parts of the formation over the medium rate (Looney et al., 1991). The gaseous residence times varied from several hours to several weeks. Given the long residence times and apparently tortuous paths that the air can follow in this subsurface site, the methane/air mixture will be injected continuously at concentrations of 4% of the air flow. Thus methane will not reach explosive concentrations, and aerobic conditions will be maintained within the subsurface sediments. After three months of operation, if biodegradation rates are significantly increased and methane does not appear to be limiting then a pulsing regime may be initiated. Operation protocol will be reviewed every 3 months by the SRS-IDP Bioremediation Technical Support Group to determine if any changes are necessary to better accomplish the demonstration objectives and criteria for success. Pulsing of the methane flow could have two advantageous effects: (1) an increase in degradative efficiency by decreasing competition for TCE and methane at enzyme sites and then provision of methane for growth and cellular maintenance; and (2) eliminating the constant availability of methane near the injection well to expand the breadth of the biomass enriched zone.



SAVANNAH RIVER SITE

INTEGRATED DEMONSTRATION PROJECT

SITE PLAN

SCALE: 1 INCH = 100 FEET

Figure 4.2

Another possibility is if monitoring of inorganic compounds during the initial bioremediation test operating campaign suggests that one or more of these compounds is limiting microbial degradation of TCE, then the later part of the bioremediation test operating campaign may also employ supplementation of limiting nutrients, eg. nitrogen. Ammonia could be added as a source of nitrogen. Delivery of ammonia will be at low levels (less than 0.1%) and will be pulsed counter to methane so that zones around the injection well will not see an abundance of nitrogen and energy source simultaneously, thereby increasing the zone of influence of biomass stimulation. Another advantage to ammonia additions could be the alkaline buffering capacity. Waters in the vicinity are slightly acidic (pH 5-6.5), so the addition of ammonia will assist in maintaining a pH more suitable to methanotrophic bioremediation (pH 5-7.5). However process control experiments are currently underway to determine if nitrous oxide may have more advantages over ammonia.

Other parameters which can be tested in the bioremediation gaseous substrate injection test include changes in influent flow and pressure, alteration in extraction vacuum, and enrichment with propane. In previous studies TCE degraders from nearby subsurface sediments were greatly stimulated in microcosm studies after the addition of propane. Propane supplements at the level of 5-30% of the methane additions (>2% total flow) may be tested based upon results of laboratory process control experiments (Section 4.3.6). Input from the results of these process control studies will be critical in deciding what alterations will be made over the course of the test.

The equipment, maintenance and operations of the compressor, vacuum blower, methane blending systems, and offgas treatment system will be handled by a subcontractor as specified in the Scope of Work, Appendix C.

4.3.2 Tracer Studies. Every month 3 standard cylinders of helium (250 cu ft) will be added to the injected air over a 24 hour period using a regulator and flow meter. All of the identifiable potential exit points for gas to leave the system will be sampled. The procedure for these studies is as described by Looney et al. (1991). Helium analysis methods are described below in section 4.3.5. By comparing the times until breakthrough and rates of recovery of helium from the extraction well (AMH2) and monitoring wells, changes in geohydrological structure that could effect flow rate can be detected and/or confirmed. In addition, at the beginning of the injection and near the end of the injection helium will be injected continuously until steady state concentrations are reached in all the off gas sampling sites. This information will be essential to model mass balance of methane during the remediation. In this case helium will act as an indirect measure of success and infer mass of TCE biotransformed. Since helium is not transformed it can be assumed that if the ratio of TCE/He in the off gas changes then the methane is being degraded in the subsurface. Since the volume of off-gas and injected-gas is known than the quantity of methane that is being degraded in the subsurface can be calculated. *SRL and subcontractor.*

4.3.3 Ground Water Monitoring. Water samples will be collected every two weeks from MHT-1C, MHT-2C, MHT-3C, MHT-4C, MHT-5C, MHT-6C, MHT-7C, MHT-8C, MHT-9C, MHT-11C, MHT-9B, MHT-10B using dedicated submersible pumps according to documented SRS well sampling protocols (WSRC 3Q5). Bulk water parameters including temperature, pH, dissolved oxygen (DO), conductivity, and oxidation-reduction potential (ORP) will be measured using a Hydrolab Surveyor model (Hydrolab Inc., Houston, TX). Samples will be collected for microbiological studies in 4 sterile 250 ml capped, disposable

Erlenmeyer flasks (Corning Inc.) and VOC analysis in a headspace vial (Hewlett-Packard Inc.). The 2 flasks for microbiological analysis will be fixed in the field with 1 ml of formalin (37% vol/vol), these samples will be used for direct counts. The remaining two flasks are to be used for viable counts, PLFA, etc. analyses. Water is filtered in the field with charged microporous cartridges (Virosorb, Cuno Inc.). These filters are transported in sterile bags for extraction of nucleic acids in the laboratory (Hazen et al., 1990). All samples shipped off site for analysis will have a chain of custody. *SRL*

4.3.3.1 Analysis of VOCs. TCE, PCE, CH_4 and all of the potential daughter products (c-DCE, t-DCE, VC, and CO_2) will be measured. VOC analyses will be performed on a Hewlett-Packard 5890 Gas Chromatograph with an electron capture detector, an HP 19395A Headspace Sampler, an HP 3392A Networking Integrator, computer controlled data control and acquisition via Chemstation software, and a 60 m x 0.75 mm ID Supelco VOCOL wide bore capillary column coated with a 1.5 µm film. The instrument is calibrated using samples spiked with standard solution. Within the headspace sampler, the teflon-lined vials are punctured, and the gases are released into the gas chromatograph. The gases are analyzed in the gas chromatograph, and the analysis is printed out (EPA Method 524.2). Total inorganic carbon will be measured in ground water by acidifying samples in a serum bottle with a crimp sealed septa. 30 ml of ground water will be added to an amber serum bottle, capped and crimped in the field and held on ice until analyzed. 1 ml of concentrated HCl will be added to serum bottles with a syringe allowed to equilibrate and then 2.5 ml of headspace injected onto a GC with a thermal conductivity detector (TCD). Standards will be made with sodium bicarbonate solutions (EPA Method 524.2). *SRL*

4.3.3.2 Acridine Orange Direct Counts (AODC). AODC will provide a direct estimate of the total number of bacteria in the environment, regardless of ability to grow on any media that might be used. Samples fixed with formalin in the field are concentrated by continuous flow centrifugation (Serval, E. I. DuPont de Nemours Company) at 6000 RPM from a initial volume of 500 ml to 10 ml final volume. Ten microliters of supernatant is spotted onto each well of a toxoplasmosis microscope slide (Celline Inc.), stained 2 min with 0.01% acridine orange (Difco, Detroit, MI), then rinsed with distilled water. The number of cells stained with acridine orange are counted by epifluorescent microscopy (Hazen et al., 1991; Sinclair and Ghiorse, 1989). *SRL*

4.3.3.3 Aerobic Heterotrophic Plate Count. This method will provide an estimate of the total number of viable aerobic and facultatively anaerobic bacteria in the groundwater. Low and high nutrient concentrations of a medium will be used to indicate differences in bacteria adapted to oligotrophic and eutrophic conditions. Unfixed samples of 1, 10 and 100 ml are filtered through 0.45 µm pore size, 47 mm diameter membrane filters (HAWG, Millipore Co., Bedford, MA). Media of 1% and full strength formulation of peptone trypticase yeast extract (PTYG) with 0.1% cycloheximide to inhibit fungal growth will be used (Balkwill, 1989). Plates are incubated at room temperature (25°C) for at least two weeks prior to counting. Bacterial colonies are counted with the aid of low power magnification. *SRL*

4.3.3.4 Methane Enrichment Most Probable Number Enumeration. This method will provide an estimate of the total number of viable aerobic and facultatively anaerobic bacteria capable of living in an enriched methane groundwater. Successful bioremediation of TCE/PCE can also be in terms of increased microbial activity, increased biomass; particularly biomass which contains TCE degrading machinery, increased biomass capable of consuming methane as evidence of stimulation by treatments. Most probable number enumeration techniques will be used to enumerate methanotrophic microorganisms in both ground water and sediments. Minimal salts media (Fogel et al., 1986) will be used with a 10% methane 90% air headspace in Balch tubes sealed with black butyl rubber stoppers. A 3 tube 4 dilution MPN will be done on water samples. For water samples the first tube will have 1 ml of sample and if counts are too high then higher dilutions will be made on the first tube. Tubes will be incubated for 4-6 weeks depending upon initial results from control tubes. A set of 4-5 control tubes will be set up at the same time MPNs are set up. The headspace methane concentration in the control tubes will be averaged and the standard deviation will represent the lower limit of methane removal needed to count as a positive tube in the MPNs. *SRL, ORNL, UT*

4.3.3.5 Enzyme Analysis. Enzymes are the principal biologically active compounds responsible for nearly all biodegradation and cell metabolic and catabolic activities. The concentrations of these enzymes found in a sample are indicative of the biological activity of a particular soil or water sample. Phosphatases are important adaptive enzymes produced by a wide variety of organisms in response to phosphorous limitation, and are generally extracellular. The method measures the hydrolysis of a surrogate substrate, disodium p-nitrophenyl phosphate (PNPP) under either acidic or alkaline conditions. Generally samples are added to the substrate in an appropriate buffer solution and allowed to incubate. Hydrolysis of the colorless substrate liberates free p-nitrophenol, a yellow colored substance which can be measured quite readily photometrically (Dougherty & Lanza, 1989; Lanza & Dougherty, 1991). Specifically, 4 ml of PNPP (in 0.2 M TRIS Buffer, pH 8.5 for Alkaline Phosphatase; in 0.1 M Citrate Buffer, pH 4.8 for Acid Phosphatase) are added to 4 ml of sample in a sterile culture tube. After mixing, the samples are incubated at room temperature for 7 days. The pH is adjusted to 8.5 (for Acid Phosphatase only), and the absorbance of the solution is read from a Spectronic 20 at 420 nm. The amount of p-nitrophenol liberated is determined from a standard curve.

The dehydrogenase assay provides a broad spectrum measure of general microbial activity since these enzymes are responsible for the transfer of electrons from substrates to acceptors. They are exclusively intracellular. Dehydrogenase activity is assayed by measuring the reduction of an organic electron acceptor using the procedure originally described by Lenhard as modified by Ryssov-Nielsen (Dougherty & Lanza, 1989; Lanza & Dougherty, 1991). In this procedure the colorless substrate, 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is reduced to the colored product MTT-Formazan which can be measured photometrically. Specifically, 4 ml of MTT (in 0.06 M Phosphate Buffer, pH 7.2) are added to 4 ml of sample in a sterile culture tube. After mixing, the samples are

incubated at room temperature for 7 days. After incubation, 5 ml of xylene are added. The samples are vigorously mixed then centrifuged. The xylene is carefully removed, and its absorbance is measured on a Spectronic 20 at 570 nm. The amount of MTT-Formazan liberated is determined from a standard curve. *SRL*

4.3.3.6 Community Diversity/Functionality. Changes in relative community structure may be important in determining: 1. the overall stability of the biological community, 2. the potential for producing unwanted effects, and 3. the relative changes in the functional capability of the community related to nutrient input and contaminant degradation. Community diversity will be determined via colony morphology and biochemical/physiological characterization. Every bacterial colony type is noted, counted, and cataloged for calculation of diversity indices (Shannon) and measurement of structural diversity. Representatives of these isolates are grown in pure culture and frozen for future biochemical studies and measurement of functional diversity. Biochemical/physiological traits will be catalogued by inoculating pure cultures of bacteria into a 96 well microtiter screening plate (MT and GN type Biolog Inc.) Similarity and cluster analysis will be used to compare groups of random isolates overtime within and between sampled wells. *SRL, USC*

4.3.3.7 Fluorescent Antibody Direct Counts. Since nitrogen is believed to be limiting in situ autecological probes will be used to directly estimate whether certain types of nitrogen transformers are changing. It has been found that these bacteria are critical to activity in the soil (Dommergues et al., 1978). It will also provide direct measurements of a TCE degrader isolated from the site. Samples are prepared as for AODC described above in section 4.3.3.2. Samples fixed on slides, blocked for non-specific adsorption are then stained by incubation with fluorescein isothiocyanate labeled antibodies (specific for a particular bacteria, eg. TCE-degrading bacteria isolated from M area sediment) for 20 min, and then excess stain is washed away with buffer. The stained slides are then examined with a fluorescent microscope and the number of yellow/green fluorescing cells enumerated as with AODC. Fluorescent antibodies for several nitrogen transforming organisms are also being tested: Nitrosomonas eurpoea, Nitrobacter agilis and winogradsky combined, Ferrobacillus ferrooxidans, Nitrosolobus sp. (AV), Azotobacter chroococum, and Beijerinckia japonicum; a SRL-TCE degrader, and a methanotroph. All antibodies were prepared and supplied by E. L. Schmidt, University of Minnesota. For details on preparation of antibodies and staining technique see Fliermans et al. (1974) and Bohlool and Schmidt (1980). *SRL*

4.3.3.8 Signature Biomarkers: Phospholipid Fatty Acid Analysis (PLFA) and Other Physiological Measurements. Culturing techniques are severely limited in determining the overall community structure, microbial biomass and nutritional status, since these techniques rely upon a general media and incubation conditions that are totally unlike anything that the microbial community may have been exposed to before. Signature biomarker compounds overcome many of these limitations by allowing direct determination of sub-femptomolar quantities of microbial cellular constituents and

compound used for energy storage (White et al., 1990). One such group of compounds is the PLFA. Ester-linked PLFAs are extracted from filtered samples via inverse serial extraction, fractionated, and methylated by microtechniques. Identifications are made by comparison of retention times to standards after extracting specific ions from a total ion chromatogram obtained with electron impact GC/MS. These techniques minimized the input of contaminants while maximizing sample input.

Additional techniques could be used to identify and determine nutritional status and metabolic activity. Water samples (10 ml) will be incubated with ^{14}C-acetate for 24 hours at in situ temperatures. The samples will then be fixed with chloroform-methanol and filtered through 0.2 µm pore size filters. The acetate incubated samples will be extracted with chloroform-methanol, dried, resuspended in 2.0 ml chloroform and aliquots counted by liquid scintillation counting to determine the amount of radioactivity incorporated into microbial lipids. See Phelps et al. (1989, 1991) for details. *UT*

4.3.3.9 Nucleic Acid Analysis. Recent techniques for probing environmental samples with nucleic acid probes have allowed for the first time truly synecological studies (Hazen and Jiménez, 1989). The section of genomic structure that codes for enzymes involved in biodegradation, regardless of species, can finally be assayed. These probes allow a nearly direct estimate of the functional capability of the environment being tested. Direct extraction of the DNA from filtered water allows direct determination of the presence and amount of certain conserved nucleic acid sequences that code for the enzymes involved in contaminant degradation. These probes should allow direct assessment of the amount of methanotrophs and other groups of organisms capable of degrading TCE/PCE and/or providing essential conditions, eg. nitrogen, pH for optimal in situ bioremediation. By filtering large quantities of DNA water to obtain high concentration of cells/DNA a number of probes can be tested simultaneously.

Total DNA is extracted from filters by placing the sample into a solution of 2.5% Sodium Dodecyl Sulfate (SDS) in (0.1 M) sodium phosphate buffer, pH 8.0 for 1 hour to lyse the cells. After a 1 hour incubation at 70°C proteins and cell debris are separated from the DNA by the addition of 0.5 volume of sodium acetate or ammonium acetate. The sample was then incubated for 30 min at -20°C. After incubation the mixture was centrifuged at 12,000 x g for 15 min. The supernatants are pooled and transferred to another container and 2 volumes of 95% ethanol are added, then DNA was precipitated overnight at room temperature. Samples are centrifuged at 12,000 x g for 30 minutes to recover the DNA. Buoyant density centrifugation in Cesium Chloride-Ethidium Bromide Gradients was performed as described elsewhere (Maniatis et al., 1987). DNA was extracted and purified from the gradients as described by Maniatis et al. (1987). Concentration of DNA and purity was determined by absorbance at 260 nm and 280 nm. If the ratio of 260/280 was lower than 1.8 the solution was purified by a cesium chloride-ethidium bromide gradient. DNA concentration per gram sediment was then calculated from the initial dry weight used. Slot blots are used to further purify genomic fragments. The resultant purified DNA is then hybridized under stringent conditions with specific DNA probes. For DNA probes with known primers, polymerase chain reaction (PCR) will

be used to amplify samples with low concentrations. Total DNA is also being subjected to thermal melting point determinations via a melting point spectrophotometer and subsequent calculation of mol% G+C for diversity estimates. RNA will be extracted in a similar fashion (Sayler et al., 1989). *ORNL, UT*

The following probes have been chosen as being important and readily available:

1. A TCE-degrading type I methanotroph (68-1) probe. The probe is DNA fragment that encodes a putative gamma subunit of methane monooxygenase and 16S rRNA. *ORNL, UT, UM*
2. A type II B gene methanotroph 16S rRNA probe. *U M*
3. A potentially TCE-degrading Tod(C_2C_1BA) toluene diooxygenase complex, Pseudomonas putida F1. *ORNL, UT*
4. A potentially TCE-degrading nahA Naphthalene diooxygenase Pseudomonas putida NAH7. *ORNL, UT*
5. A potentially TCE-degrading TOL upper pathway xylene oxidase, Pseudomonas putida mt2, pWWO. *ORNL, UT*
6. 16S rRNA sequences from SRS subsurface bacteria. *U I*
7. Acetogen specific DNA and RNA probes from Clostridium thermoaceticum. *USCC*
8. A nitrogen fixing, aromatic degrading nifH fragment from Klebsiella pneumoniae. *USCC*
9. A aromatic degrading catechol diooxygenase fragment from Rhizobium leguminosarum. *USCC*
10. A potentially TCE-degrading TOL plasmid probes. *USCA*
11. A TCE-degrading toluene diooxygenase (Tod C2C1BA) from Pseudomonas putida. This probes is also being used by UT, thus data from UT and PNL/WSU can be compared. *PNL, WSU*
12. A cytochrome P450cam (camC) from Pseudomonas putida. Dechlorinates alkanes oxidatively and reductively. P450's are a family of enzymes known to be involved in xenobiotic degradation. *PNL, WSU*
13. A TCE-degrading toluene monooxygenase (tmoABCDE) from Pseudomonas mendocina KR1. *PNL, WSU*
14. A haloalkaline dehalogenase (dhlA) from Xanthobacter autotrophicus. Broad substrate specificity, hydrolytically dechlorinates alkanes. May have activity against PCE/TCE metabolites. *PNL, WSU*
15. A haloakanoate dehalogenase (dhlB) from Xanthobacter autotrophicus. Broad substrate specificity may have activity against PCE/TCE metabolites. *PNL, WSU*

4.3.3.10 TCE/PCE Mineralization Analysis. The greatest measure of success would be demonstration of ^{14}C-TCE disappearance in microcosms within hours of collection of water samples and continuing for days, as compared to controls. Second best measure of success would be substantial loss of TCE in enrichments as compared to controls and background samples. ^{14}C-labeled TCE and PCE is injected into sealed tubes with 10 ml of groundwater sample and incubated at in situ temperature for 48 h. The non radioactive and radioactive carbon dioxide concentration in the sample is determined with gas chromatography-gas proportional counting as described by Phelps et al. (1989). *SRL, ORNL, UT*

4.3.3.11 Microbial Inhibition/Toxicity Analysis. Disappearance of target compounds, i.e. TCE/PCE, may not correlate with decrease in health hazards associated with the treated material (Mueller et al., 1991), thus a site may appear to be remediated when in fact it still represents a health or environmental risk. In situ bioremediation involves manipulation of an extremely complex milieu. Various biodegradation products and substances transformed by the changes caused in the physical/chemical environment of the contaminated soil and groundwater could go undetected by the standard analytical procedures employed. In order to monitor changes in the health hazard of the groundwater a microbial bioassay will be employed. Water samples will be inoculated into a Microtox model 500 toxicity autoanalyzer (Microbics Carp., Carlsbad, CA). This assay evaluates toxicity by measuring the change in light of viable luminescent bacteria upon their exposure to test substrates. The Microtox testing will be conducted in accordance with the manufacturers recommendations (Microbics Corp., Carlsbad, CA) adding a range of amounts of water from the samples to yield dose-response data. Toxicity is expressed relative to positive control substances, eg. phenol, as EC_{50} values. Inhibition of microbial metabolism will be assessed at the same concentrations as used for the Microtox testing. For water samples, the test organisms (unimpacted, unadapted microbes) will be collected by centrifugation from clean ground water from the reference site. The organisms will be added as a standard inoculum (10^6 bacteria/ml based on AODC) to filtered test well water diluted with clean ground water to produce the desired concentration range. $^{32}PO_4$ will be added at a concentration high enough to saturate uptake. Using a high concentration also reduces isotope dilution effects of natural PO_4. After an appropriate period of incubation the samples will be filtered and the filters counted to assess uptake into biomass. Results will be compared to water from the reference sites and toxicity expressed as IC_{50} values (the concentration required to inhibit metabolism 50%). All radiolabelling techniques according to Dobbins and Pfaender (1988). *SRL, ORNL, UT*

4.3.3.12 Physical/Chemical Analysis. The physical and chemical nature of the environment is critical to understanding biological phenomena, eg. degradation rates. Many inorganic elements can become limiting nutrients, eg. P, N, S, Fe). Physical parameters, eg. pH, dissolved oxygen, conductivity, oxidation/reduction potential, can effect rates and extent of enzymatic reactions; these parameters can also be changed by biological activity. These measurements could be critical to a thorough understanding of the in situ bioremediation process and the potential for controlling degradation rates, destruction efficiency and adverse phenomena. All methods will be EPA approved and/or in Standard Methods (APHA, 1989). Temperature, dissolved oxygen, pH, conductivity, oxidation-reduction potential, and salinity will be determined at the well head by specific probes on a Hydrolab Surveyor II equipped with a data logger and flow through cell (Hydrolab Inc., Austin, TX). The pH and dissolved oxygen probe are calibrated daily, and the remaining probes calibrated monthly. The remainder of the assays will be performed by a subcontractor with EPA approved methods in an EPA certified laboratory. Iron will be determined by inductively coupled plasma-atomic emission spectroscopy with pre acid digestion (EPA SW-846). Total Organic Carbon (TOC) will be determined by the

ultraviolet oxidation method (EPA 415.1). Samples will be acidified and stored at 4°C prior to analysis. Soluble reactive phosphate concentrations will be measured by the ascorbic acid colorimetric determination method (EPA 365.2). Total Phosphorus will be determined by the persulfate digestion and ascorbic acid colorimetric determination (EPA 365.2). Total Kjeldahl Nitrogen (TKN), which includes free-ammonia plus organic nitrogen will be determined colorimetrically following digestion, distillation and Nesslerization method (EPA 351.3). Ammonia as distilled ammonia nitrogen will be determined colorimetrically following distillation and Nesslerization method (EPA 350.2). Chloride, Nitrate, Nitrite, and Sulfate will be determined by the ion chromatography method (EPA 300.0). Alkalinity will be done by the pH 4.5 titrametric method (EPA 625/410). *SRL and subcontractor*

4.3.4 Sediment Monitoring. Every three months two bore holes will be drilled and sampled from the surface to 200 ft. in the area of expected remediation influence (see DSOP 254 and Eddy et al., 1991 for methods and procedures). An additional two bore holes will be drilled in adjacent areas not expected to be influenced as a control. Sediment samples will be collected at ten foot intervals and, in addition, at all significant lithologic changes in the core. All collection methods are designed to minimize microbial contamination of cores from adjacent depths and drilling fluids. Barrels will be steam cleaned between collections. Samples will be collected using a modified syringe tube and plunger. This technique results in the collection of a consistent volume of sediment. Immediately after collection, the sediment sample is placed in a headspace vial. Five milliliters of solution, comprised of 10 grams of sodium sulfate and 0.3 ml of phosphoric acid (0.15%) in 200 ml of distilled water, will be added to the vial. The vials will be sealed with crimped aluminum rings over teflon-lined septa. Samples will be placed in a cooler on ice. The samples will be collected daily and refrigerated in the lab. Prior to sample analysis, the samples will be weighed in order to determine the mass of the sample. Samples will then be placed in the sonic dismembrator for fifteen minutes in order to disaggregate the sediment.

Core specimens for microbial analysis are obtained directly from the split spoon or barrel. Cores are sectioned into 3 inch lengths with sterile spatulas and the outermost layer (about 1/4 the diameter of the core) is scraped off using a sterile scoopula. The sample is then placed in a sterile Whirl-Pak bag (Ft. Wilkinson, WI) for immediate transport to the laboratory for analysis. All samples shipped off site for analysis are under a chain of custody. *SRL*

4.3.4.1 Analysis of VOC. TCE, PCE, CH_4 and all of the potential daughter products (c-DCE, t-DCE, VC, and CO_2) will be measured. VOC analyses will be performed on a Hewlett-Packard 5890 Gas Chromatograph with an electron capture detector, an HP 19395A Headspace Sampler, an HP 3392A Networking Integrator, Computer controlled data control and acquisition via Chemstation software, and a 60 m x 0.75 mm ID Supelco VOCOL wide bore capillary column coated with a 1.5 µm film. The instrument is calibrated using samples spiked with standard solution. Within the headspace sampler, the teflon-lined vials are punctured, and the gases are released into the gas chromatograph. The gases are analyzed in the gas chromatograph, and the analysis is printed out (EPA Method 524.2; Sims et al., 1991). Total inorganic carbon will be measured in ground water be acidifying samples in a serum bottle with a crimp sealed septa. 30 ml of ground water will be added to an amber serum bottle, capped and crimped in the field and held on ice

until analyzed. 1 ml of concentrated HCl will be added to serum bottles with a syringe allowed to equilibrate and then 2.5 ml of headspace injected onto a GC with a thermal conductivity detector (TCD). Standards will be made with sodium bicarbonate solutions (EPA Method 524.2). *SRL*

4.3.4.2 Acridine Orange Direct Counts (AODC). AODC will provide a direct estimate of the total number of bacteria in the environment, regardless of ability to grow on any media that might be used. Samples are preserved in phosphate buffered formalin. Samples (1 to 3 grams) are extracted three times with a non-ionic homogenizing detergent to remove bacteria from the sediment particles. Homogenates are cleared by low speed centrifugation and the supernatants pooled. Ten microliters of supernatant is spotted onto each well of a toxoplasmosis microscope slide, stained with 0.01% acridine orange, then rinsed with distilled water. The number of cells stained with acridine orange are counted by epifluorescence microscopy. The number of cells per sample is normalized by dividing by the dry weight of the sediment. Counts are reported as cells per gram (Sinclair and Ghiorse, 1989). *SRL*

4.3.4.3 Aerobic Heterotrophic Plate Count. This method will provide an estimate of the total number of viable aerobic and facultatively anaerobic bacteria in the groundwater. Low and high nutrient concentrations of a medium will be used to indicate differences in bacteria adapted to oligotrophic and eutrophic conditions. Samples (1 to 3 grams) are weighed directly into 15 ml conical centrifuge tubes containing 9 ml of pyrophosphate buffer. Subsequent serial dilutions are made in phosphate buffered saline. 0.1 ml of each appropriate dilution was inoculated onto a corresponding plate of appropriate medium. For this study, 1% and full strength formulation of peptone-trypticase-yeast extract-glucose (PTYG) are used (Balkwill, 1989). A glass rake and turntable are used to spread the inoculum evenly over the entire surface of the agar. Plates are incubated at room temperature for at least two weeks prior to counting. Bacterial colonies are counted with the aid of low power magnification. Counts are normalized to sediment dry weights and reported as colony forming units (CFU) per gram. *SRL*

4.3.4.4 Methane Enrichment Most Probable Number Enumeration. This method will provide an estimate of the total number of viable aerobic and facultatively anaerobic bacteria capable of living in an enriched methane sediment. Successful bioremediation of TCE/PCE can also be in terms of increased microbial activity, increased biomass; particularly biomass which contains TCE degrading machinery, increased biomass capable of consuming methane as evidence of stimulation by treatments. Most probable number enumeration techniques will be used to enumerate methanotrophic microorganisms in sediments. Minimal salts media (Fogel et al. , 1986; AEM 51(4): 720-724) will be used with a 10% methane 90% air headspace in Balch tubes sealed with black butyl rubber stoppers. A 3 tube 5 dilution MPN will be done on sediment samples. For sediments, 5 grams of sediment, possibly 10 grams, will be used in the first tube of the dilution. Tubes will be incubated for 4-6 weeks depending upon initial results from control tubes. A set of 4-5 control tubes will be set up at the same time MPNs are set up. The headspace methane concentration in the control tubes will be averaged and the standard deviation will

represent the lower limit of methane removal needed to count as a positive tube in the MPNs. *SRL, ORNL, UT*

4.3.4.5 Enzyme Analysis. Enzymes are the principal biologically active compounds responsible for nearly all biodegradation and cell metabolic and catabolic activities. The concentrations of these enzymes found in a sample are indicative of the biological activity of a particular soil or water sample. The urease method is used to measure the nitrogen scavenging activity of bacteria. Urea is used as the substrate. Ammonia released during the hydrolysis of the urea is measured colorimetrically (Lloyd and Sheaffe, 1973). Ten grams of soil is incubated in 10 ml of 0.1 M phosphate buffer (pH 6.7) and 10 ml of a 20% urea solution for 4 hours at 30° C. Controls consist of distilled water. Microbial activity is arrested with mercuric chloride and the ammonia is released by addition of potassium chloride. All samples are brought up to 50 ml volume with water and the amount of ammonia is determined colorimetrically.

Phosphatases are important adaptive enzymes produced by a wide variety of organisms in response to phosphorous limitation, and are generally extracellular. The method measures the hydrolysis of a surrogate substrate, disodium p-nitrophenyl phosphate (PNPP) under either acidic or alkaline conditions. Generally samples are added to the substrate in an appropriate buffer solution and allowed to incubate. Hydrolysis of the colorless substrate liberates free p-nitrophenol, a yellow colored substance which can be measured quite readily photometrically (Dougherty & Lanza, 1989; Lanza & Dougherty, 1991). Specifically, 6 ml of PNPP (in 0.2 M TRIS Buffer, pH 8.5 for Alkaline Phosphatase; in 0.1 M Citrate Buffer, pH 4.8 for Acid Phosphatase) are added to approximately 2 grams of pre-weighed sample in a sterile culture tube. After mixing, the samples are incubated at room temperature for 24 hours. The samples are centrifuged to remove the soil particles. The supernatant is carefully removed, and the pH is adjusted to 8.5 (for Acid Phosphatase only). The absorbance of the solution is then read from a Spectronic 20 at 420 nm. The amount of p-nitrophenol liberated is determined from a standard curve, and normalized to the weight of dry soil.

The dehydrogenase assay provides a broad spectrum measure of general microbial activity since these enzymes are responsible for the transfer of electrons from substrates to acceptors. They are exclusively intracellular. Dehydrogenase activity is assayed by measuring the reduction of an organic electron acceptor using the procedure originally described by Lenhard as modified by Ryssov-Nielsen (Dougherty & Lanza, 1989; Lanza & Dougherty, 1991). In this procedure the colorless substrate, 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is reduced to the colored product MTT-Formazan which can be measured photometrically. Specifically, 6 ml of MTT (in 0.06 M Phosphate Buffer, pH 7.2) are added to approximately 2 grams of pre-weighed sample in a sterile culture tube. After mixing, the samples are incubated at room temperature for 7 days. After incubation, the samples are centrifuged to remove soil particles. The supernatants are carefully decanted and 6 ml of methanol are added. The samples are vigorously mixed and re-centrifuged. The methanol is carefully removed, and its absorbance is measured on a Spectronic 20 at

570 nm. The amount of MTT-Formazan liberated is determined from a standard curve and normalized to the dry soil weight. *SRL, ORNL, UT*

4.3.4.6 Community Diversity/Functionality. Changes in relative community structure may be important in determining: 1. the overall stability of the biological community, 2. the potential for producing unwanted effects, and 3. the relative changes in the functional capability of the community related to nutrient input and contaminant degradation. Community diversity will be determined via colony morphology and biochemical/physiological characterization. Every bacterial colony type is noted, counted, and cataloged for calculation of diversity indices (Shannon) and measurement of structural diversity. Representatives of these isolates are grown in pure culture and frozen for future biochemical studies and measurement of functional diversity. Biochemical/physiological traits will be catalogued by inoculating pure cultures of bacteria into a 96 well microtiter screening plate (MT and GN type Biolog Inc.) Similarity and cluster analysis will be used to compare groups of random isolates overtime by wells. *SRL, USC*

4.3.4.7 Fluorescent Antibody Direct Counts. Since nitrogen is believed to be limiting in situ, autecological probes will be used to directly estimate whether certain types of nitrogen transformers are changing. It has been found that these bacteria are critical to activity in the soil (Dommergues et al., 1978). It will also provide direct measurements of a TCE degrader isolated from the site. Samples are prepared as for AODC described above in Section 4.3.4.2. Samples fixed on slides, blocked for nonspecific adsorption, are stained by incubation with fluorescein isothiocyanate labeled antibodies (specific for a particular bacteria, eg. TCE-degrading bacteria isolated from M area sediment) for 20 min, and then excess stain was washed away with buffer. The stained slides are then examined with a fluorescent microscope and the number of yellow/green fluorescing cells enumerated as with AODC. Fluorescent antibodies for several nitrogen transforming organisms are also being tested: Nitrosomonas europea, Nitrobacter agilis and winogradsky combined, Ferrobacillus ferrooxidans, Nitrosolobus sp (AV), Azotobacter chroococum, and Beijerinckia japonicum; a SRL-TCE degrader, and a methanotroph. All antibodies were prepared and supplied by E. L. Schmidt, University of Minnesota. For details on preparation of antibodies and staining technique see Fliermans et al. (1974) and Bohlool and Schmidt (1980). *SRL*

4.3.4.8 Phospholipid Fatty Acid Analysis (PLFA) and Other Physiological Measurements. Culturing techniques are severely limited in determining the overall community structure, microbial biomass and nutritional status, since these techniques rely upon a general media and incubation conditions that are totally unlike anything that the microbial community may have been exposed to before. Signature biomarker compounds overcome many of these limitations by allowing direct determination of sub-femptomolar quantities of compounds used for energy storage, metabolic intermediaries and enzymes (White et al., 1990). One such group of compounds is the PLFA. Ester-linked PLFAs are extracted from filtered samples via inverse serial extraction, fractionated, and methylated by microtechniques. Identifications are made by comparison of retention

times to standards after extracting specific ions from a total ion chromatogram obtained with electron impact GC/MS. These techniques minimized the input of contaminants while maximizing sample input.

Additional techniques could be used to identify and determine nutritional status and metabolic activity. Sediment samples (10 ml) will be incubated with ^{14}C-acetate for 24 hours at <u>in situ</u> temperatures. The samples would then be fixed with chloroform-methanol and filtered through 0.2 µm pore size filters. The acetate incubated samples will be extracted with chloroform-methanol, dried, resuspended in 2.0 ml chloroform and aliquots counted by liquid scintillation counting to determine the amount of radioactivity incorporated into microbial lipids. *UT*

4.3.4.9 Nucleic Acid Analysis. Recent techniques for probing environmental samples with nucleic acid probes have allowed for the first time truly synecological studies(Hazen and Jiménez, 1989). The section of genomic structure that codes for enzymes involved in biodegradation, regardless of species can finally be assayed. These probes allow a nearly direct estimate of the functional capability of the environment being tested. Direct extraction of the DNA from filtered water allows direct determination of the presence and amount of certain conserved nucleic acid sequences that code for the enzymes involved in contaminate degradation. These probes should allow direct assessment of the amount of methanotrophs and other groups of organisms capable of degrading TCE/PCE and/or providing essential conditions, eg. nitrogen, pH for optimal <u>in situ</u> bioremediation.

Total DNA will be extracted from sediment samples by direct lysis, alkaline extraction procedure, and bead homogenization with a bead beater. Cell lysis will be achieved by incubation in a solution of 1% sodium dodecyl sulfate (SDS) in 0.12 M sodium phosphate buffer (pH 8.0) for 1 hour at 70°C. Lysis will be further increased by homogenization in the bead beater for 5 min. Sediments will be washed three times with 0.12 M sodium phosphate buffer, and supernatants will be pooled and transferred to another container where 0.5 volumes of polyethylene glycol (50%) will be added to precipitate the DNA overnight. After centrifugation the pellets will be suspended in Tris EDTA (TE) buffer (pH 7.0) and DNA will be purified by two phenol and one chloroform isoamyl alcohol extraction's in order to separate sediment particles, protein, and carbohydrates from the DNA. Final precipitation will be done adding two volumes of ethanol and one tenth volumes of sodium acetate. Concentration and purity of DNA will be determined by absorbance at 260 nm and 280 nm and by ethidium bromide quantification (Maniatis et al., 1987). The resultant purified DNA will be fixed on nylon filters and hybridized under stringent conditions with specific DNA probes. RNA will be extracted in a similar fashion (Sayler et al., 1989). *ORNL, UT*

The following probes have been chosen as being important and readily available:

1. A TCE-degrading type I methanotroph (68-1) probe. The probe is DNA fragment that encodes a putative gamma subunit of methane monooxygenase and 16S rRNA. *ORNL, UT, UM*
2. A type II B gene methanotroph 16S rRNA probe. *U M*

3. A potentially TCE-degrading Tod(C_2C_1BA) toluene diooxygenase complex, Pseudomonas putida F1. *ORNL, UT*
4. A potentially TCE-degrading nahA Naphthalene diooxygenase Pseudomonas putida NAH7. *ORNL, UT*
5. A potentially TCE-degrading TOL upper pathway xylene oxidase, Pseudomonas putida mt2, pWWO. *ORNL, UT*
6. 16S rRNA sequences from SRS subsurface bacteria. *UI*
7. Acetogen specific DNA and RNA probes from Clostridium thermoaceticum. *USCC*
8. A nitrogen fixing, aromatic degrading nifH fragment from Klebsiella pneumoniae. *USCC*
9. A aromatic degrading catechol diooxygenase fragment from Rhizobium leguminosarum. *USCC*
10. A potentially TCE-degrading TOL plasmid probes. *USCA*
11. A TCE-degrading toluene diooxygenase (Tod C2C1BA) from Pseudomonas putida. This probes is also being used by UT, thus data from UT and PNL/WSU can be compared. *PNL, WSU*
12. A cytochrome P450cam (camC) from Pseudomonas putida. Dechlorinates alkanes oxidatively and reductively. P450's are a family of enzymes known to be involved in xenobiotic degradation. *PNL, WSU*
13. A TCE-degrading toluene monooxygenase (tmoABCDE) from Pseudomonas mendocina KR1. *PNL, WSU*
14. A haloalkaline dehalogenase (dhlA) from Xanthobacter autotrophicus. Broad substrate specificity, hydrolytically dechlorinates alkanes. May have activity against PCE/TCE metabolites. *PNL, WSU*
15. A haloakanoate dehalogenase (dhlB) from Xanthobacter autotrophicus. Broad substrate specificity may have activity against PCE/TCE metabolites. *PNL, WSU*

4.3.4.10 TCE/PCE Mineralization Analysis. The greatest measure of success would be demonstration of ^{14}C-TCE disappearance in microcosms within hours of collection of sediment samples and continuing for days, as compared to controls. Second best measure of success would be substantial loss of TCE in enrichments as compared to controls and background samples. ^{14}C-labeled TCE and PCE is injected into sealed tubes with 1-3 g of sediment sample in 10 ml distilled water and incubated at in situ temperature for 48 h. The non radioactive and radioactive carbon dioxide concentration in the sample is determined with gas chromatography-gas proportional counting as described by Phelps et al. (1989). *SRL, ORNL, UT*

4.3.4.11 Microbial Inhibition/Toxicity Analysis. Disappearance of target compounds, eg. TCE/PCE, may not correlate with decreases in health hazards associated with the treated material (Mueller et al., 1991). In situ bioremediation involves manipulation of an extremely complex milieu. Various biodegradation products and substances transformed by the changes caused in the physical/chemical environment of the contaminated soil and groundwater could go undetected by the standard analytical procedures employed. In order to monitor changes in the health hazard of the sediment a microbial bioassay will be employed. Sediment samples will be inoculated into a Microtox model 500 toxicity autoanalyzer (Microbics

Carp., Carlsbad, CA). This assay evaluates the toxicity of a sample by measuring the change in light level of viable luminescent bacteria upon their exposure to test substrates. The Microtox testing will be conducted in accordance with the manufacturers recommendations (Microbics Corp., Carlsbad, CA) adding a range of amounts of sediment from the samples to yield dose-response data. A new solids testing protocol is available for Microtox that will be used for the sediment samples and produces results directly comparable to other Microtox data. Toxicity is expressed relative to positive control substances, eg. phenol, as EC_{50} values. Inhibition of microbial metabolism will be assessed at the same concentrations as used for the Microtox testing. For aquifer solids samples, test organisms will be obtained from clean solids by removing them with polyvinlylpyrrolidone and polyphosphate washing, which removes microbes from soil without impact on their metabolic abilities (Dobbins and Pfaender, 1988; Konopka and Turco, 1991). An inoculum of these organisms will be added (10^7 cells/gm sediment) to heat sterilized test sediment mixed with different quantities of sterile reference site sediment, to produce a range of test soil concentrations. Inhibition of incorporation of $^{32}PO_4$ into biomass will be assessed by counting aliquots of the soil after washing to remove unincorporated phosphate. Results will be compared to sediment from the reference sites and toxicity expressed as IC_{50} values (the concentration required to inhibit metabolism 50%)*SRL, UNC*

4.3.4.12 Physical/Chemical Analysis. The physical and chemical nature of the environment is critical to understanding biological phenomena, eg. degradation rates. In addition, some of these parameters have implication on nutrient requirements (P, N, S, Fe), effects that the biomass may be having on the environments, eg. pH, conductivity, TOC. These measurements could be critical to a thorough understanding of <u>in situ</u> bioremediation process and the potential for controlling degradation rates, destruction efficiency and adverse phenomena. All methods will be EPA approved and/or in Standard Methods (APHA, 1989). The following assays will be performed by a subcontractor with EPA approved methods in an EPA certified laboratory. Iron will be determined by inductively coupled plasma-atomic emission spectroscopy with pre acid digestion (EPA SW-846). Total Organic Carbon (TOC) will be determined by the ultraviolet oxidation method (EPA 415.1). Samples will acidified and stored at 4°C prior to analysis. Ortho Phosphate concentrations will be measured by the ascorbic acid colorimetric determination method (EPA 365.2). Total Phosphorus will be determined by the persulfate digestion and ascorbic acid colorimetric determination (EPA 365.2). Total Kjeldahl Nitrogen (TKN), which includes free-ammonia plus organic nitrogen will be determined by the colorimetric, following digestion, distillation and Nesslerization method (EPA 351.3). Ammonia as distilled ammonia nitrogen will be determined by the colorimetric, following distillation and Nesslerization method (EPA 350.2). Chloride, Nitrate, Nitrite, and Sulfate will be determined by the ion chromatography method (EPA 300.0). *SRL and subcontractor*

4.3.4.13 Protozoan Analysis. Recent work has indicated that small numbers of protozoa commonly inhabit subsurface soils at pristine sites at various geographical locations. The ubiquitous distribution of protozoa in the subsurface have important implications

in bioremediation operations. When nutrients are added to increase bacteria biomass, concomitant increases in protozoan populations occur. These protozoa could be important in removing bacterial biomass and cycling of contaminants that were only adsorbed to biomass and not degraded. Protozoa also could be important in maintaining hydraulic conductivity and ensuring proper flow of nutrients into contaminated zones. Protozoa also may be important in maintaining balanced growth thus facilitating greater metabolic efficiency. Samples will be diluted and plated inside plastic rings imbedded in non-nutrient agar base. One milliliter of water will be added to each ring, and replenished as needed. Non-growing cells of Enterobacter aerogenes will be supplied as a food source. Cultures will be checked between 3 days and 2 months by making a wet mount and examining the slides with phase microscopy. Protozoan counts will be expressed as counts per gram dry weight. Basic identification of representative protozoa will also be done. *ManTech Environmental Technology Inc., EPA*

4.3.4.14 Fungal and Actinomycete Analysis. Large increases in biomass during remediation projects may also cause increases in fungal biomass. Some yeast have been implicated in TCE degradation (Wackett et al., 1989). The importance of fungi in contaminated environments has largely gone unstudied. Fungi and actinomycetes in sediment samples will be enumerated with acidified mycological agar and acidified actinomycetes isolation agar. Colonies isolated on these median will be screened for their ability to degrade TCE/PCE in vials in the presence of air supplemented with methane and propane. *PNL*

4.3.5 Offgas Monitoring. Offgas from the extraction well (AMH2) and from the vadose zone piezometers will be collected and analyzed daily by the operations subcontractor as specified in the scope of work for this contract (see Appendix C). The subcontractor shall collect pressure and vacuum data from approximately 20 vadose zone piezometers and 22 groundwater wells, as well as from the vacuum and pressure well heads and 6 downhole tubes in each horizontal well. The subcontractor shall provide the gas concentration measurements for gases collected from the approximately 20 vadose zone piezometers, gases collected from the 6 downhole tubes in the vacuum horizontal well (AMH-2), and for the vacuum well head. Up to 10 samples will be collected each day for chemical analysis of trichloroethylene (TCE), tetrachloroethylene (PCE), cis- and trans- dichloroethylene (DCE), vinyl chloride (VC), methylene chloride (MC), methane (CH_4) and carbon dioxide (CO_2). Detection limits of less than or equal to 5 ppm by volume for chlorinated compounds and less than or equal to 0.1 % for methane and carbon dioxide are required. Moisture content and oxygen concentration will also be monitored continuously from the extraction well off gas stream. *SRL, Subcontractor*

WSRC personnel will collect and analyze helium from the approximately 20 vadose zone piezometers, gases collected from the 6 downhole tubes in the vacuum horizontal well (AMH-2), and for the vacuum well head. The procedure to be used is as described previously by Looney et al. (1991) Samples are collected using a 50 ml disposable syringe and the samples are placed in 30 ml preevacuated serum vials. Contents of these vials are analyzed using a helium mass spectrometer that has been modified to sample the serum vials at a constant rate. The mass spectrometer is calibrated in two steps. First, the mass spectrometer is tuned and the sensitivity adjusted to an internal calibrated leak (diffusion) standard in units of standard ml of He per second;

after this step, gas standards prepared in the serum vials are used to convert the instrument reading to ppm (volume) and check the stability of the tuning.

4.3.6 Laboratory Process Control Studies. These studies are done using soil columns with sediments from the subsurface and liquid and gas phase bioreactors to recommend injection protocol, feeding regimens, and test treatments that would provide faster and/or more complete biodegradation of more recalcitrant species like PCE. Concentrations, flows, vacuum, and pulsing will be varied to examine the impact on TCE and PCE degradation in laboratory soil reactors. Limitations with respect to moisture, phosphate, and nitrogen will also be examined. Success of these studies will be provision of data for modeling efforts and determination of factors limiting TCE/PCE degradation, and identification of treatment regimes that could compromise continued operation of the wells. The operation of the bioreactors will yield an evaluation of the potential effects of alterations on TCE degradation rate. Treatment regimens investigated in the laboratory soil reactors found to be potentially more successful than current field operating conditions could then be tried in the field and thus significantly improve the rates of bioremediation. The data could also be used in subsurface models by identifying factors controlling the degradation rate. The soil columns and bioreactors may be critical in identifying regimes which could lead to sediment plugging. In addition, optimization of the bioreactors and soil columns will provide ex-situ treatments that could be used in later demonstrations and process design information for still other demonstrations.

4.3.6.1 SRL. Studies at SRL will utilize two pilot-scale (1 gpm) methanotrophic trickle filter bioreactors for treatment of TCE/PCE contaminated groundwater. These systems will operate with contaminated water from SRS wells and suggest what groundwater parameters are important to optimal degradation by SRS microorganisms. Later in the year a pilot-scale (2-3 gpm) fluidized expanded bed bioreactor will be tested with the same water in cooperation with GRI (Radian, MBI and Envirex). Another major task will focus on stimulation of TCE/PCE degradation in surface soils by vegetation. Field plots at SRS will provide evidence of the effectiveness of this treatment and the potential to couple this with methane injection. See TTP No. SR 0308-AA. *SRL, GRI, USAF, UT, MBI, Radian, Envirex, ORNL, Stanford University*

4.3.6.2 ORNL. Studies at ORNL will utilize a pilot-scale (1 gpm) methanotrophic trickle filter bioreactor to treat TCE/PCE contaminated groundwater at ORNL that also has high concentrations of other organics and metals. A steam stripper will be used to pretreat the groundwater and provide better control over reactor operating conditions (TTP No.: OR 0369-ABD). An additional bioreactor will be set up for soil column testing using SRS contaminated soils. This system will specifically test the strategies for biodegradation of PCE under methanotrophic conditions. This project will provide vital information on methods for effecting simultaneous degradation of TCE and PCE in the vadose zone and water. (TTP No.: OR 0368-AL). *ORNL, UT*

4.3.6.3 INEL. Studies at INEL will use the differential soil bioreactor (DSBR) to develop real time measurements of microbial activity (growth and contaminant degradation) to be followed with time under realistic subsurface conditions. The realism includes the use of samples of subsurface material and groundwater from SRS, so that

indigenous microbial activity is studied and all of the many geochemical variable that affect microbial metabolism are correct. (TTP No.: ID 0566-AA) *INEL*

Some of the parameters which can be tested in the initial bioremediation (gaseous substrate injection) test in the above bioreactors include changes in influent flow and pressure, alteration in extraction vacuum, and enrichment with propane. In previous studies, TCE degraders from nearby subsurface sediments were greatly stimulated in microcosm studies after the addition of propane. Propane supplements at the level of 5-30% of the methane additions (>2% total flow) may be tested based upon results of laboratory process control experiments. Input from the results of these studies will be critical in deciding what alterations will be made over the course of the test.

4.3.7 Ancillary IDP Monitoring Activities. These activities include in situ flow sensors, seismic tomography, electrical resistance tomography, depth discrete samplers, special chemical samplers and sensors, and other activities covered in TTP No.: SR 0566-2. *SNL, ORNL, LLNL, BNL*

4.3.8 Meteorological Data. SRS is the Southeastern Emergency Weather Station. The weather station is physically less than one (1) mile from the demonstration site. Data is available on rainfall, temperature, barometric pressure, humidity, etc. on a daily basis. This station is manned by the Environmental Technology Section of the Savannah River Laboratory.

4.3.9 Ancillary Groundwater Data. Water is collected quarterly (SRS Quarterly Groundwater Monitoring Report) from more than 50 wells within 1 mile of the demonstration site and hydrogeological summaries (Bledsoe 1984, 1986, 1988). Parameters include basic physical/chemical parameters, VOC's (including TCE/PCE), radionuclides, and heavy metals. In addition, data are also available on operation of the M-Area above ground air stripper system which is part of the groundwater corrective action plan for M-area. These data include operating costs, VOC removals and amounts stripped (Christensen and Gordon, 1983).

4.4 Post-Test Characterization and Monitoring. The objective of these measurements is to determine how rapidly the environment returns to pre-stimulation conditions and/or how long contaminant changes persist. This monitoring will also allow monitoring for adverse changes in the environment, eg. toxic daughter products, anaerobic conditions, acidic conditions.

4.4.1 Groundwater Monitoring. All parameters (Section 4.3.3) will be measured every two weeks as during the test for two (2) months. The sampling interval will than be changed to monthly for two (2) more months. All methods used will be as described above in Section 4.3.3.

4.4.2 Sediment Characterization. Ten boreholes will be drilled from the surface to 200 ft., one borehole adjacent to each of the 10 existing clusters. All techniques and methods will be as those described above in Section 4.3.4 and Section 4.2.

4.5 Modeling and data interpretation including hydrological modeling, modeling of the degradative processes and evaluation of the data. The objective is to compare results with theoretical models, interpret data, and facilitate communications between investigators. Models will be developed for methane dispersion, TCE loss, bioremediation, air, water and gas flows with pressure, impact of bioremediation.

This work is a continuation of the modeling activity initiated in the in situ Air Stripping Demonstration (Looney et al., 1991). *SRL, ORNL, LLNL, INEL, LANL*

4.6 Schedule of Events and Reports.

4.6.1 Project Planning and Management. January 1990. The Bioremediation Technical Support Group (BTSG) was selected. They were presented with the characterization data and discuss appropriate biotechnologies. A draft Technical Task Plan (TTP) was prepared by the BTSG and reviewed. A final meeting was held to discuss the TTP and look for fatal flaws. Based on recommendations of the BTSG sampling and research programs for various laboratories were scoped, prepared and submitted as TTPs or requisitioned as contracts. In addition appropriate personnel from SRS and from participating institutions were contacted and asked to prepare the essential documentation associated with the detailed test plan, quality assurance, operational and worker safety, site access and security, sample handling/analysis procedures, and waste handling disposal. This task was begun in July 1990 and is on going.

4.6.2 Process Design and Modeling. June 1990. This task will entail a review of the existing air stripping system including the physical arrangement of the piping and other hardware, horizontal wells, site hydrology, soil permeability, etc., so that the bioremediation system and sampling requirements can be effectively integrated with the existing physical equipment with minimal effort. Based on site characteristics, initial modeling studies were carried out to estimate potentially affected zones surrounding the primary injection and recovery points to assist planning of the sampling campaign. Operational parameters deemed necessary to promote biodegradation yet compatible with site characteristics were selected.

4.6.3 Operations Contract. May 1991. Operation scope of work (see appendix C) prepared 5/91, submitted 6/91, sent out for bid by procurement 7/91, proposals received 8/91, technical evaluation 8/91, contract currently in negotiation by procurement.

4.6.4 Permitting. November 1990. Appropriate personnel at SRS were contacted and permits prepared for the Underground Injection Permit Modificaiton. Submitted to SCDHEC 5/91, approved 7/91, NEPA approved 8/91, Air Permit submitted 11/91, appropriate site use, clearance, etc. completed 12/91.

4.6.5 Analytical Facilities. January 1990. An on-site analytical and laboratory facility will be established. A trailer (climate controlled for instrument operation) will be obtained and equipped with a gas chromatograph for monitoring chlorinated alkenes, methane, oxygen and carbon dioxide in the water and influent and effluent gas phases, a pH meter, dissolved oxygen probe and specific ion electrode(s) for determining water quality, and miscellaneous small equipment (e.g. bench-top centrifuge) for sample handling and preparation. The SRL Mobile Microbial Ecology Laboratory (MMEL) is satisfactorily equipped and was used for the duration of the phase 1 task. Access to two additional gas chromatographs in a nearby support laboratory will also be required to handle the extensive volatile organic analysis needed for both water and off-gas monitoring during operating campaigns. Additional support trailer has been obtained and an additional GC has been purchased and is operational as of 9/91.

4.6.6 Pre-Test Characterization. March 1991. Completed July 1991, analysis still in progress.

4.6.7 Finalization of Test Plan by BTSG. October 1991.

4.6.8 Ground Water Monitoring. September 1991. In Progress.

4.6.9 QA and Safety Report. December 1991. Must be submitted and approved prior to mobilization (appendix C).

4.6.10 Mobilization. January 1992. Dependent on operations contract equipment procurement and final setup and inspection. Contract awarded to ECOVA 11/91.

4.6.11 Test Schedule. January 1992. Dependent on operations contract equipment procurement and final setup and inspection. Contract awarded to ECOVA 11/91. Operations contractor submits weekly operations report. Monthly reports submitted by all investigators to Principal Investigators. Monthly reports submitted by Principal Investigators to WSRC Procurement, SRS-ER, DOE-OTD and SCDHEC. Quarterly reports submitted by Principal Investigators to Technical Support Group. If the technology is determined to be sound 3-5 months before the end of the project a recommendation will be sent to ER to begin permitting and contracting so that full-scale use of the technology can be started at the end of the project.

1/92	Start Extraction. 2 weeks
1/92	Start Air Injection. 2 weeks
2/92	Start Air/Methane Injection. 3 months
4/92	First Quarterly Sediment Sampling.
4/92	First BTSG Quarterly Review.
4/92	First Change Injection and Continue Monitoring.
7/92	Second Quarterly Sediment Sampling.
7/92	Second BTSG Quarterly Review.
7/92	Second Change Injection, Continue Monitoring.
10/92	Third Quarterly Sediment Sampling.
10/92	Third BTSG Quarterly Review.
10/92	Third Change Injection, Continue Monitoring.
12/92	Stop Methane injection.
1/93	Stop Air Injection.
1/93	Stop Extraction.

4.6.12 Post-Test Monitoring. January 1993.

4.6.13 Post-Test Characterization. January 1993.

4.6.14 Demobilization. January 1993.

4.6.15 Final Operations Report. February 1993.

4.6.16 BTSG Review of Test and Final Test Report. March 1993.

4.6.17 Post-Test Characterization Report. April 1993.

4.6.18 BTSG Review and Recommendations. April 1993.

4.6.19 Final Technology Report. July 1993.

4.7 Communications and Technology Transfer.

4.7.1 WIN. All members of the Bioremediation Technical Support Group (BTSG) and investigators will be given a WIN account to allow direct electronic mail communications of meetings, reports, reviews and support information.

4.7.2 Reports and Sample Shipments. All monthly and quarterly reports will be sent to all BTSG members and Investigators. Investigators will be notified by WIN and/or telephone when a sample has been shipped. All sample shipments will be by overnight mail scheduled to arrive on weekdays. Should weekend arrival be necessary then the investigator will be notified by phone and WIN 48 h in advance of when the sample will arrive.

4.7.3 Bioremediation Technical Support Group. The BTSG will meet at a minimum of every 3 months to review progress of the test and make recommendations on new courses of action and future directions.

4.7.4 In Situ Bioremediation Demonstration Project Symposium. At the end of the project all investigators and the BTSG will give presentations and publish a proceedings of project work. The Symposium will be internationally advertised to the academic, government and industrial communities.

4.7.5 Publications and Presentations. All investigators will be encouraged and assisted in presenting and publishing investigations conducted during the project. In addition, press releases will be sent periodically to radio, TV, and newspapers throughout the tenure of the project.

5.0 Organizational Structure and Funding. For more details of the Organization Structure of the Integrated Demonstration Program see " Integrated Demonstration for Cleanup of Organics in Soils and Groundwater at Non-Arid Sites Project Management Plan (IDP-0566). For details on funding see individual Technical Task Plans (OTD). For WSRC organizational structure see WSRC Management Policies, WSRC-1-01

5.1 U.S. Department of Energy. As per direction of the Secretary of Energy as outlined in the Environmental Restoration and Waste Management Five-Year Plan (1989) the U.S. Department of Energy is striving to implement initiatives for environmental protection and waste management at DOE facilities. The **Office of Technology Development** (0TD) through the Division of Demonstration, Testing, and Evaluation (DT&E) provides programmatic direction, and overview of the Integrated Demonstration Program (IDP). Funding and DOE Oversight of the individual tasks associated with IDP will be through Technical Program Officers (TPO) at the respective operations offices, eg. **Savannah River Operations.** The prime contractor at the DOE office, eg. **Westinghouse Savannah River Company,** will appoint a Technical Program Manager (TPM) for the site to manage the tasks at that site.

5.2 Westinghouse Savannah River Company. WSRC has designated the TPM to be in the **Savannah River Laboratory** division of the company. The TPM acts as manager for all Technical Task Plans for WSRC. The TPM with the concurrence of the TPO selects an Integrated Demonstration Coordinator.

5.3 Integrated Demonstration Project. The Integrated Demonstration Planning Group is responsible for acting as a steering committee for the program and advising the Project Manager. The Planning Group is also responsible for selecting Technical Support Groups (TSG) and their chairpersons. The Program has TSGs for Analysis and Evaluation, Monitoring, Characterization, Drilling, Regulatory and Remediation. The Remediation TSG is further subdivided into Bioremediation and Physical Chemical. The TSGs provide technical guidance to the Planning Group and Technical Support for the program in their area of expertise.

5.4 Bioremediation Technical Support Group. The BTSG was established in January 1990. The following are currently members: Terry C. Hazen, SRL (chairman); Fred Brockman, PNL; Carl Fliermans, SRL; John Wilson, USEPA; Jim Spain, USAF; Rashalee Levine, USDOE; Graham Andrews, INEL; Perry McCarty, Stanford U.; John Knezovich, LLNL; Gary Sayler, U. Tennessee; Tom Phelps, U. Tennessee; Carl Gehrs, ORNL; Tony Palumbo, ORNL; Frank Chappelle, USGS; Brian Looney, SRL; Terry Donaldson, ORNL; Tom Hayes, GRI; Paul Wichlacz, INEL; and Tom Brouns, PNL; exoficio: Joel Dougherty, SRL, Mike Enzien, SRL (ORAU post-doctoral fellows).

5.5 Primary and Ancillary Technical Task Plans and Funding. Only those ancillary TTPs that have been identified with Bioremediation Demonstrations at the SRS Integrated Demonstration are listed. Other ancillary TTPs associated with these and other laboratories have been identified with the other TSG of the program. Only requested FY92 budgets are presented.

5.5.1 Savannah River Site

5.5.1.1 SR 0566-01. SRS Integrated Demo: Remediation Tasks. $2,090K. PI: T. C. Hazen and B. B. Looney.

5.5.1.2 SR 0366-02. SRS Integrated Demonstration Directional Drilling & Characterization. $2,400K. PI: C. A. Eddy and D. S. Kaback

5.5.1.3 SR 0566-03. Integrated Demonstration for Cleanup of Soils and Groundwater at Non-Arid Site: Off-Gas Treatment. $1,100K. PI: J. Haselow and B. B. Looney.

5.5.1.4 SR 0308-AA. TCE Biodegradation Demonstration. $1,150K. PI: T. C. Hazen.

5.5.2 Oak Ridge National Laboratory

5.5.2.1 OR 0369-ABD. Demonstration of Co-Metabolic Technology. $320K. PI: A. Palumbo and S. Herbes.

5.5.2.2 OR 0368-AL. Bioremediation of Groundwater (PCE). $250K. PI: S. Herbes.

5.5.2.3 OR 0369-AH. TCE Degradation Demo Support. $625K. PI: A. Palumbo.

5.5.2.4 OR 0566 AC. Vegetation Enhancement. $400K. PI: B. Walton and N. Edwards.

5.5.3 Idaho National Engineering Laboratory

5.5.3.1 ID 0533-RD. Biodegradation Screening of Microbes. $345K. PI: F. Colwell.

5.5.3.2 ID 0566-AA. Soil Bioreactor Studies. $100K. PI: G. Andrews.

5.5.4 Pacific Northwest Laboratory

5.5.4.1 RL 0566-AB. Biomolecular Probe Analysis. $70K. PI: F. Brockman.

5.5.5 Lawrence Livermore National Laboratory

5.5.5.1 SF 0566-AA. Design, Performance Assessment, and Risk/Benefit Analysis. $400K. PI: D. Chestnut.

5.5.6 Los Alamos National Laboratory

5.5.6.1 AL 2202-R. Cost/Benefit Analysis. $500K. PI: L. Trocki.

5.6 Participants: Government, Industry, Academic

5.6.1 Government: Department of Energy, Environmental Protection Agency, Geological Survey, Air Force, Army Corps of Engineers, South Carolina Department of Health and Environmental Control.

5.6.2 Academia: Stanford University, University of South Carolina, University of Illinois, University of Washington, Utah State University, Georgia State University, University of Minnesota, University of Cincinnati.

5.6.3 Industry: Gas Research Institute, Radian Corp., Eastman Christiensen, Westinghouse, E. I. duPont de Nemours Inc., Michigan Biotech Institute,

Envirex Inc., Bechtel Inc., Graves, O'Brien and Gere, Monitoring Testing Service, General Engineering Lab, Tren Fuels, South Carolina Electric and Gas Co., Terra-Vac

6.0 Permits, Patents, Licenses and Contracts.

6.1 Permits

6.1.1 National Environmental Policy Act NEPA Environmental Evaluation Checklist completed 4/8/91. DOE-SR approved as a categorical exclusion SR CX9105008, 8/8/91.

6.1.2 Underground Injection Control Permit from South Carolina Department of Health and Environmental Control. Modification of UIC Permit #103 (WSRC-RP-91-354) prepared and submitted SCDHEC 4/91. Approved by SCDHEC 6/91.

6.1.3 Air Permit from South Carolina Department of Health and Environmental Control. Required to meet Clean Air Act Regulations. Prepared and submitted 11/91.

6.1.4 US Department of Transportation Certification. Required for transporting methane from the filling station to field site for both the vehicle and drivers. SRS obtained.

6.1.5 DOE SRS Site Use, Site Clearance, and Work Clearance Permits. Obtained from WSRC and US DOE Savannah River Operations. See WSRC Engineering and Engineered Services Procedure Manual (1E) Procedure 3.02 for obtaining these permits and clearances. SRS obtained.

6.2 Patents and Licenses: Patent search on 9/6/91 reveals only 4 patents that could have any relationship to demonstration being done.

6.2.1 Patent US 4660639 issued 4/28/87, Removal of Volatile contaminants from the Vadose Zone of Contaminated Ground. The vapor extraction from the upper horizontal well is covered by this patent and WSRC has a paid-up one time license with the assignee; The UpJohn Company, for use of this process with the horizontal wells.

6.2.2 Patent US 4832122, issued 5/23/89, In-Situ Remediation System and Method for Contaminated Groundwater The project will also use In Situ Air Stripping. This patent is assigned to WSRC/DOE.

6.2.3 Patent US 4713343, issued 12/15/87, Biodegradation of halogenated aliphatic hydrocarbons; water purification using microorganism capable of aerobic degradation of low molecular weight alkanes. The demonstration will also use this process but since the assignee for this patent is US EPA no license is necessary since DOE is also a US government agency.

6.2.4 Patent US 5006250, issued 4/9/91, Pulsing of electron donor and electron acceptor for enhanced biotransformation of chemicals. One of the supplemental injection strategies to be used could be pulsing; however, the patent specifically covers only pulsing of electron donors in "aliquots of water". It does not specifically cover pulsing of electron donors in air, thus no licenses should be necessary. Prior to initiating a pulsing regimen we will have the legal department examine this patent and if necessary obtain a license from the assignee: Stanford, Leland Jr University Trustees.

6.2.5 Catalytic Destruction of Offgas Contaminants. It shall be the responsibility of the suppliers of this treatment unit to obtain licenses for the process used as appropriate.

6.3 Contracts and Agreements This does not include the specific tasks covered by joint participants TTP's at ORNL, PNL and INEL, see Section 5.5 above.

6.3.1 Operations Contract: Injection/Extraction and Offgas Treatment Equipment. A single competitively bid contract will cover procurement, maintenance and operation of the following equipment: air compressor, vacuum blower, air/methane blending system, field engineered manifold, and offgas catalytic treatment. The successful bidder will provide 24 h/day coverage of field operations including monitoring of pressure and offgas chemical analysis of trichloroethylene (TCE), tetrachloroethylene (PCE), cis- and trans- dichloroethylene (DCE), vinyl chloride (VC), methylene chloride (MC), methane (CH_4) and carbon dioxide (CO_2). Detection limits of less than or equal to 5 ppmV for chlorinated compounds and less than or equal to 0.1 % for methane and carbon dioxide are required. See Appendix C for the scope of work for this contract.

6.3.2 Methane Supply and Storage. Gas Research Institute has agreed to specify in their contract with Radian Corp. (Houston, TX) that Radian contract Tren Fuels Inc. (Denver, Co.) to fabricate a trailer with a tank capable of supplying compressed natural gas for the demonstration. The two trailers containing CNG cylinders will be DOT approved and certified. Each trailer will have a filled capacity of 12,480 std. cu ft at 3000 psi. Radian Corp. will also contract South Carolina Electric and Gas Company for a fill station to be located on SCE&G property in Jackson, South Carolina. The fill station will have a fill rate of 25 scf/min, enabling a fill time of 8-8.3 hours. Radian will provide vehicles and certified drivers for refilling the tank trailer at the filling station when required.

6.3.3 Chemical Analysis of Water and Soil. Samples to be analyzed for sulfur, nitrogen, nitrate+nitrite, sulfate, sulfide, dissolved organic carbon, ammonia, phosphorus, phosphate, chloride, iron, and cation exchange capacity will be done under a WSRC task order contract (AX00659N) to General Engineering Laboratory (Charleston, SC). Procedures to be used are all EPA approved or recommended.

6.3.4 Stanford University. Scope of Work for Kinetics of Methanotrophic Reactions, Principal Investigator: Dr. Perry McCarty, AA46349T, October 1, 1991 - September 31, 1992. The objective of this investigation is to determine the kinetics of methanotrophic transformations of trichloroethylene (TCE) and tetrachloroethylene (PCE). This will allow the determination of the important coefficients for growth and substrate utilization by methanotrophs, and will also permit determining the important coefficients for substrate (methane) and inhibitor (TCE) utilization.

6.3.5 ManTech Environmental Technology Inc. - USEPA. All protozoan analysis from sediments will be done by sole source contract (C11591) to Dr. James Sinclair, ManTech Environmental Technology Inc. - USEPA, Kerr Research Lab, Ada, OK. The requisition has been placed and is waiting final contract award.

6.3.6 SCUREF - University of South Carolina. Task orders for three specific projects have been started through the WSRC contract with the South Carolina Research and Undergraduate Education Foundation.

6.3.6.1 Dr. James Yates. Development of procedures for identification of organisms capable of degrading trichloroethylene in the environment, Started 8/90, AA00900T task 12, Currently in renewal. Development of DNA probes specific for chromosomal sequences present in the host bacterium (designated T1) from which TOL-1 was isolated. Development of a procedure to introduce DNA into T1. Detection of TOL-1 cells after entrapment on filters. The current scope of work also calls for analysis of isolates using Biolog plates.

6.3.6.2 Dr. Charles Lovell. Development of Functional Group Probes: Acetogens, Nitrogen Fixers and Aromatic Degraders, Started 6/90, AA00900T task 10, Currently in renewal. The objective of this investigation is to develop DNA probes for quantitative measurement of acetogen, nitrogen-fixing, and aromatic degrader bacterial populations and genes involved in these activities. To evaluate the sensitivity and specificity of nucleic acid probes for measuring these functional bacterial populations in mixed microbial culture and to correlate these measurements with rates of degradation and/or fixation. To determine these functional bacterial population dynamics in contaminated and uncontaminated environments.

6.3.6.3 Dr. John Morse. Experimental Bioreactor for Treatment of TCE and PCE-Contaminated SRS Groundwater, Started 6/90, AA00900T task 8, Currently in renewal. The objective of this investigation is to evaluate the use of a bench-scale, fluidized expanded-bed, bioreactor for the degradation of trichloroethylene (TCE) and tetrachloroethylene (PCE) in SRS groundwaters. The ultimate goal of the investigation is to demonstrate feasibility of biological remediation of TCE and PCE contaminated groundwater at the Savannah River Site, Aiken, South Carolina.

7.0 Safety, Quality Assurance and Security.

7.1 Safety.

7.1.1 Savannah River Site. General safety rules for the Savannah River Site are documented in the Savannah River Site (SRS) Safety Manual (8Q) and in compliance with DOE order 5483.1A.

7.1.2 Savannah River Laboratory. Savannah River Laboratory Safety Practices and Procedures Manual (8Q8) documents safety procedures for all activities for SRL Employees, SRL visitors, and Vendors/Subcontractors.

7.1.3 Process Hazards Review. As defined in Savannah River Site (SRS) Safety Manual (8Q) in Procedure 10-1. To be performed on site with cognizant functional personnel required.

7.1.4 Other Safety Information. Other sources of safety information include: SRP Industrial Hygiene (DPSOP 158 Series), SRP Engineering Standards and Specifications (DPSOP 208-1), SRL Occupational Health Control Procedures (DPSTP-R), and SRL Engineering Practices (DPSTOM-51).

7.1.5 Subcontractor. While on SRS, the Successful offerer will be responsible for adhering to the safety regulations of "WSRC Safety Guidelines for Subcontractors". All members of the successful offerer project staff must attend a site safety and security orientation (approximately 8 hours) prior to beginning work at SRS. The orientation includes information about handling chemicals on site, emergency signals, and security.

7.2 Quality Assurance/Quality Control: All activities at SRS are governed by WSRC Quality Assurance Program as outlined in WSRC Management Policies, WSRC-1-01 MP 4.2. Specific Quality Assurance Procedures are documented by organization as required.

7.2.1 Westinghouse Savannah River Company. WSRC Quality Assurance is documented in WSRC Quality Assurance Manual (1Q).

7.2.2 Chemical Processes and Environmental Technology Department. Quality Assurance implementation procedures for the CP&ET Department are documented in CP&ET Quality Assurance Implementation Procedures (1Q31).

7.2.3 Environmental Sciences Section. Quality Assurance implementation procedures for the section are found in ESS Quality Assurance Implementation Procedures (1Q31-1). Operating procedures for the section are documented in ESS Operating Procedures Manual (WSRC-L-14-1).

7.2.4 Subcontractor. All subcontractors will adhere to WSRC Quality Assurance program and submit all document and records in the Quality Assurance Report and in the Final Report.

7.3 Security. Westinghouse Savannah River Company security requirements and procedures are documented in the WSRC Security Manual (7Q). These procedures are as required by Federal Laws and applicable DOE Orders, eg. DOE Order 5631.1A.

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Appendix A. Lists of Analytical Parameters.

Appendix A. Lists of Parameters

Parameter	Frequency	Type (G/W/S)	Amount	Laboratory	Priority	Reference
AODC	Biweekly	W	500 ml	SRL	1	Sinclair and Ghiorse, 1989
	Quarterly	S	10 g	SRL	1	Sinclair and Ghiorse, 1989
Heterotrophic Count						
1%PTYG & PTYG	Biweekly	W	10 ml	SRL	1	Balkwill, 1989
	Quarterly	S	10 g	SRL	1	Balkwill, 1989
Methanotrophs	Biweekly	W	100 ml	SRL/UT	1	MPN
	Quarterly	S	10 g	SRL/UT	1	MPN
Fluorescent Antibodies	Biweekly	W	500 ml	SRL	1	Fliermans et al., 1974
(p. 16)	Quarterly	S	10 g	SRL	1	Fliermans et al., 1974
^{14}C-Acetate to lipids	Biweekly	W	10 ml	SRL	2	Phelps et al., 1989, 1991
	Quarterly	S	10 g	SRL	2	Phelps et al., 1989, 1991
Nucleic Acid Probes			1000 ml			
(see p. 17)			100 g			
Probe 1	Biweekly	W		UT/UM	1	Sayler et al., 1989
	Quarterly	S		UT/UM	1	Sayler et al., 1989
Probe 2	Biweekly	W		UT/UM	1	Sayler et al., 1989
	Quarterly	S		UT/UM	1	Sayler et al., 1989
Probe 3	Biweekly	W		UT/ORNL	1	Sayler et al., 1989
	Quarterly	S		UT/ORNL	1	Sayler et al., 1989
Probe 4	Biweekly	W		UT/ORNL	1	Sayler et al., 1989
	Quarterly	S		UT/ORNL	1	Sayler et al., 1989
Probe 5	Biweekly	W		UT/ORNL	1	Sayler et al., 1989
	Quarterly	S	10 g	UT/ORNL	1	Sayler et al., 1989
Probe 6	Biweekly	W		UI	2	Sayler et al., 1989
	Quarterly	S		UI	2	Sayler et al., 1989

Probe 7	Biweekly	W		USCC	2	Sayler et al., 1989
	Quarterly	S		USCC	2	Sayler et al., 1989
Probe 8	Biweekly	W		USCC	2	Sayler et al., 1989
	Quarterly	S		USCC	2	Sayler et al., 1989
Probe 9	Biweekly	W		USCC	2	Sayler et al., 1989
	Quarterly	S		USCC	2	Sayler et al., 1989
Probe 10	Biweekly	W		USCA	2	Sayler et al., 1989
	Quarterly	S		USCA	2	Sayler et al., 1989
Probe 11	Biweekly	W		PNL/WSU	2	Sayler et al., 1989
	Quarterly	S		PNL/WSU	2	Sayler et al., 1989
Probe 12	Biweekly	W		PNL/WSU	2	Sayler et al., 1989
	Quarterly	S		PNL/WSU	2	Sayler et al., 1989
Probe 13	Biweekly	W		PNL/WSU	2	Sayler et al., 1989
	Quarterly	S		PNL/WSU	2	Sayler et al., 1989
Probe 14	Biweekly	W		PNL/WSU	2	Sayler et al., 1989
	Quarterly	S		PNL/WSU	2	Sayler et al., 1989
Probe 15	Biweekly	W		PNL/WSU	2	Sayler et al., 1989
	Quarterly	S		PNL/WSU	2	Sayler et al., 1989
Protozoa	Quarterly	S	50 g	MT/EPA	2	Sinclair et al., 1989
Fungi	Quarterly	W	100 ml	PNL	3	PNL
	Quarterly	S	10 g	PNL	3	PNL
Actinomycetes	Quarterly	S	10 g	PNL	3	PNL
Community Diversity	Biweekly	W	10 ml	SRL/INEL	2	Balkwill, 1989
	Quarterly	S	10 g	SRL/INEL	2	Balkwill, 1989

TCE Mineralization	Biweekly	W	10 ml	SRL/UT	1	Phelps et al., 1989, 1991
	Quarterly	S	10 g	SRL/UT	1	Phelps et al., 1989, 1991
PCE Mineralization	Biweekly	W	10 ml	SRL/UT	1	Phelps et al., 1989, 1991
	Quarterly	S	10 g	SRL/UT	1	Phelps et al., 1989, 1991
PLFA/PHA	Biweekly	W	500 ml	ORNL/UT	2	Phelps et al., 1989, 1991
	Quarterly	S	100 g	ORNL/UT	2	Phelps et al., 1989, 1991
Enzymes						
acid phosphatase	Biweekly	W	10 ml	SRL	1	Dougherty, 1990
	Quarterly	S	10 g	SRL	1	Dougherty, 1990
alkaline phosphatase	Biweekly	W	10 ml	SRL	2	Dougherty, 1990
	Quarterly	S	10 g	SRL	2	Dougherty, 1990
urease	Biweekly	W	10 ml	ORNL	2	Lloyd et al., 1973
	Quarterly	S	10 g	ORNL	2	Lloyd et al., 1973
Microbe Inhibition/ Toxicity (Microtox)	Biweekly	W	10 ml	SRL/UNC	2	Dobbins et al., 1989
	Quarterly	S	10 g	SRL/UNC	2	Dobbins et al., 1989
Dissolved Oxygen	Biweekly	W	In situ	SRL	1	Hazen et al., 1991
Temperature	Biweekly	W	In situ	SRL	1	Hazen et al., 1991
Conductivity	Biweekly	W	In situ	SRL	1	Hazen et al., 1991
Redox Potential	Biweekly	W	In situ	SRL	1	Hazen et al., 1991
pH	Biweekly	W	In situ	SRL	1	Hazen et al., 1991
Chloride	Biweekly	W	10 ml	SRL/GEL	1	EPA300.0
	Quarterly	S	10 g	SRL/GEL	1	EPA300.0
Fe	Biweekly	W	10 ml	SRL/GEL	2	EPA SW-846
	Quarterly	S	10 g	SRL/GEL	2	EPA SW-846

TKN	Biweekly	W	10 ml	SRL/GEL	1	EPA351.3
	Quarterly	S	10 g	SRL/GEL	1	EPA351.3
NO_2	Biweekly	W	10 ml	SRL/GEL	1	EPA300.0
	Quarterly	S	10 g	SRL/GEL	1	EPA300.0
NO_3	Biweekly	W	10 ml	SRL/GEL	1	EPA300.0
	Quarterly	S	10 g	SRL/GEL	1	EPA300.0
SO_4	Biweekly	W	10 ml	SRL/GEL	1	EPA300.0
	Quarterly	S	10 g	SRL/GEL	1	EPA300.0
Total Phosphorus	Biweekly	W	10 ml	SRL/GEL	1	EPA365.2
	Quarterly	S	10 g	SRL/GEL	1	EPA365.2
PO_4	Biweekly	W	10 ml	SRL/GEL	1	EPA365.2
	Quarterly	S	10 g	SRL/GEL	1	EPA365.2
TOC	Biweekly	W	10 ml	SRL/GEL	2	EPA415.1
	Quarterly	S	10 g	SRL/GEL	2	EPA415.1
NH_4	Biweekly	W	10 ml	SRL/GEL	2	EPA350.2
	Quarterly	S	10 g	SRL/GEL	2	EPA350.2
He	Monthly	G	10 ml	SRL	1	Looney et al., 1991
TCE	Bidaily	G	10 ml	SRL/Sub	1	EPA 524.2
	Biweekly	W	10 ml	SRL/Sub	1	EPA 524.2
	Quarterly	S	10 g	SRL/Sub	1	EPA 524.2
CH4	Bidaily	G	10 ml	SRL/Sub	1	EPA 524.2
	Biweekly	W	10 ml	SRL/Sub	1	EPA 524.2
	Quarterly	S	10 g	SRL/Sub	1	EPA 524.2
PCE	Bidaily	G	10 ml	SRL/Sub	1	EPA 524.2
	Biweekly	W	10 ml	SRL/Sub	1	EPA 524.2
	Quarterly	S	10 g	SRL/Sub	1	EPA 524.2

c-DCE	Bidaily	G	10 ml	SRL/Sub	1	EPA 524.2
	Biweekly	W	10 ml	SRL/Sub	1	EPA 524.2
	Quarterly	S	10 g	SRL/Sub	1	EPA 524.2
t-DCE	Bidaily	G	10 ml	SRL/Sub	1	EPA 524.2
	Biweekly	W	10 ml	SRL/Sub	1	EPA 524.2
	Quarterly	S	10 g	SRL/Sub	1	EPA 524.2
VC	Bidaily	G	10 ml	SRL/Sub	1	EPA 524.2
	Biweekly	W	10 ml	SRL/Sub	1	EPA 524.2
	Quarterly	S	10 g	SRL/Sub	1	EPA 524.2
CO_2	Bidaily	G	10 ml	SRL/Sub	1	EPA 524.2
	Biweekly	W	10 ml	SRL/Sub	1	EPA 524.2
	Quarterly	S	10 g	SRL/Sub	1	EPA 524.2
Alkalinity	Quarterly	S	10 g	SRL/Sub	1	EPA 625/401
Moisture	Continuous	G	offgas	SRL/Sub	1	TBD
Oxygen	Continuous	G	offgas	SRL/Sub	1	TBD

Appendix B. Technical Task Plan.

OFFICE OF TECHNOLOGY DEVELOPMENT

Technical Task Plan
Summary
Title: SRS Integrated Demo: Remediation Tasks

Operations Office:	SR	TTP No.:	SR 0566-01
Contractor:	WSRC	Date:	10/15/91
Division:	DT&E	Revision:	2
Fiscal Year:	1992	Old TTP No.:	SR 0566-AA

Technical Program Officer: M. G. O'Rear, DOE-SR (803) 725-5541

Principal Investigators: T. C. Hazen and B. B. Looney, WSRC (803) 952-7517

Technical Program Manager: J. L. Steele (803) 725-1830

Joint Participants: Oak Ridge National Laboratory (OR 0369-ABD, OR 0368-AL, OR 0369-AH, OR 0566 AC)
Idaho National Engineering Laboratory (ID 0533-RD, ID 0566-AA)
The University of Tennessee (OR 0369-AH)
Lawrence Livermore National Laboratory (SF 0566-AA)
Pacific Northwest Laboratory (RL 0566-AB)

Jointly Funded Program: 01

Integrated Demonstration or Program: A1

Primary Technology Area: 02, 06, 09

Work Breakdown Structure Element: 1.14.02, Capital 1.14.06.01

B&R Code: EW 40 10 40

Task Summary

This technical task plan includes selection, demonstration, and evaluation of innovative remediation technologies, with an emphasis on in situ methods. Phase I of the SRS Integrated Demonstration involved demonstration of in situ air stripping to remediate both soils and groundwater contaminated with volatile organics. The next phase of the demonstration will involve a full-scale demonstration of in situ bioremediation using methane injection at the same site where in situ air stripping was demonstrated last year. Other tasks included under this technical task plan are oversight for the remediation technology technical support group and scoping work to prepare for future chemical/thermal in situ remediations. This project provides a unique blend of collaborative partners from industry, academia and government. It also builds on the funding of related research projects. The Gas Research Institute, in collaboration with SRL, has been funding research and development of a methanotrophic treatment process for trichloroethylene-contaminated groundwater for the past 3 years. The university and industry investigators funded by GRI as well as scientists from numerous national laboratories (DOE and EPA) have been integrated into this project

to provide the greatest experience and resource of bioremediation expertise that has ever been assembled for any bioremediation demonstration.

In the first phase of the bioremediation demonstration indigenous microorganisms will be stimulated to degrade trichloroethylene (TCE), tetrachloroethylene (PCE) and their daughter products in situ by addition of nutrients to the contaminated zone and by surface treatment of contaminated off-gas and water. The horizontal wells that form the basis for the SRS Integrated Demonstration are expected to provide significant advantages over conventional in situ bioremediation with vertical wells or infiltration galleries. The increased surface area will allow better delivery of nutrients and easier recovery of gas and water, as well as minimizing formation clogging and plugging phenomena. Biodegradation is a highly attractive technology for remediation because contaminants are destroyed, not simply moved to another location or immobilized. Bioremediation has been found to be among the least costly technologies in applications where it will work.

An extensive monitoring program using existing monitoring wells will serve to observe the response of the soil and water following injection of air/methane, and the off-gas from the upper horizontal well will be assayed for methane, total VOC, TCE, PCE, potential break down products of TCE/PCE (eg. DCE, VC), and carbon dioxide. Data from the previous demonstration of in situ air stripping, where air alone was injected at different rates for 20 weeks will be used to provide base line geological, hydrological, chemical, and biological characteristics. These data will also establish the effect of air injection without nutrients on the hydrological, chemical, and biological characteristics of the site, in effect providing a unique and dramatic control experiment for the first bioremediation demonstration.

Following the initial continuous air/methane injection campaign, process optimization will be pursued to further stimulate the indigenous microorganisms and enhance biodegradation. This action will include pulsing of air/methane to stimulate stress biodegradation, vadose zone wetting to inhibit the potential drying effect of vacuum extraction from the vadose zone, and periodic addition of other nutrients, such as phosphate, that may prove to be limiting factors. Data will be reviewed and changes made to injection protocol, if necessary, at 3 month intervals.

The second phase of the bioremediation demonstration will focus on injection of multiple nutrients as indicated by the first demonstration. The third phase of the demonstration will inject microorganisms with and without nutrients and/or inducer substances. The fourth phase of the demonstration will combine above ground off-gas and groundwater bioreactors with nutrient and microbe injection strategies demonstrated in the previous demonstrations to maximize treatment efficiency and speed.

Scoping studies and site preparation work for a thermally enhanced demonstration are planned for the next FY in collaboration with PNL. A major element in the scoping studies will be a Preliminary Technology Status Report. This report will be assembled by the remediation technical support group, in close collaboration with the analysis and evaluation technical support group. An important part of the process will be identification of potential problems. This identification will take advantage of all of the technical support groups and the many scientific/technical disciplines on these groups (e.g., hydrologists, biologists, physicists, chemists, engineers, etc.). The identified problems will be assessed to determined if they are significant, or if they can be unambiguously addressed using straightforward experiments. This process will be the basis for planning and will have several go/no go decision points -- these will be based on the demonstration meeting a specific ER need and the potential for significant overall savings in cost of remediation.

Budget Summary = (Dollars in Thousands)

	PY ACT	FY 91CO	FY 92R	FY 93R	FY 94R	FY 95R
OP	1538	62	1500	1500	1500	600
CE	2	498	590	950		
GPP						
LI						
Total	1540	560	2090	2450	1500	600

Task/Milestone Summary:

Task	FY 91	FY 92	FY 93	FY 94	FY 95
1. Project Start up	o---o[a]				
Process Design	o----o[b]				
Test Plan	o---------o[c]				
2. Air/Methane Injection		o--o[d]--o[d]--o[e]			
3. Multiple Nutrient Injection			o--o[f]--o[f]--o[g]		
4. Microbe/Nutrient Injection				o--o[h]--o[h]--o[i]	
5. Combined In situ/Pump and Treat					o--o[j]--o[j]--o[k]
6. Technology evaluation by the technical support group and planning for thermal demonstration.		--------o[l]----------			

Milestone Explanation:

a. Complete equipment modifications; ready to start Task 2.
b. Complete detailed process design.
c. Complete detailed test plan, with QA/QC, health and safety, etc.
d. Decision point for protocol change to first injection campaign (KEY).
e. Complete first injection campaign (KEY). Decision point for protocol to be used in second injection campaign Task 3.
f. Decision point for protocol change to second injection campaign (KEY).
g. Evaluate multiple nutrient injection campaign (KEY). Decision point for need/benefits of Task 5.
h. Decision point for protocol change to third injection campaign (KEY).
i. Evaluate microbe injection campaign (KEY). Decision point for need/benefits of Task 5.
j. Decision point for protocol change to fourth injection campaign (KEY).
k. Evaluate combined in situ/pump and treat campaign.
l. Complete Preliminary Technology Status Report for thermal treatments

Part 2: Technical Task Description

Technology Description:

Task 1. Project Startup

Subtask 1.1. Project Planning and Management

This task will consist of contacting and interacting with appropriate personnel from SRS and from participating institutions to prepare the essential documentation associated with the detailed test plan, quality assurance, operational and worker safety, site access and security, sample handling/analysis procedures, and waste handling disposal. Also included will be the preparation and submission of the permit applications for subsurface injection of air/methane and trace salts (see Task 2).

Subtask 1.2. Process Design and Modeling

This task will entail a review of the existing air stripping system including the physical arrangement of the piping and other hardware, horizontal wells, site hydrology, soil permeability, etc., so that the bioremediation system and sampling requirements can be effectively integrated with the existing physical equipment with minimal effort. Based on site characteristics, initial modeling studies will be carried out to estimate potentially affected zones surrounding the primary injection and recovery points to assist planning of the sampling campaign. Operational parameters deemed necessary to promote biodegradation yet compatible with site characteristics will be selected.

Subtask 1.3. System Modification

The existing in situ air stripping system will be modified to allow for the injection of the air/methane mixture. It is planned to introduce methane and air through the same inlet port that were used for the air stripping demonstration, so system modification should be relatively minimal. The projected methane injection rate (approximately 2 to 6 SCFM to make 1-3% of the total gas injection rate of no more than 200 SCFM) will necessitate the use of a tanker truck kept on-site. An air compressor capable of delivering approximately 200 SCFM will be required as well as a vacuum system to remove off-gas from the recovery well. The vacuum extraction will be operated at a 20% higher value than the air injection system or no more than 240 SCFM (Note: In terms of size and type, the air stripping and compressor equipment used in phase 1 will be satisfactory.) A methane monitor, alarm, and automatic shut-off system will be installed for protection in the event that the methane level exceeds explosive limits (>5%).

Subtask 1.4. Analytical Facilities

An on-site analytical and laboratory facility will be established. A trailer (climate controlled for instrument operation) will be obtained and equipped with a gas chromatograph for monitoring chlorinated alkenes, methane, oxygen and carbon dioxide in the water and influent and effluent gas phases, a pH meter, dissolved oxygen probe and specific ion electrode(s) for determining water quality, and miscellaneous small equipment (e.g. bench-top centrifuge) for sample handling and preparation. The SRL Mobile Microbial Ecology Laboratory (MMEL) is satisfactorily equipped and was used for the duration of the phase 1 task. Access to two additional gas chromatographs in a nearby support laboratory will also

be required to handle the extensive volatile organic analysis needed for both water and off-gas monitoring during operating campaigns.

Subtask 1.5. Microbial Characterization

Direct measurement of reductions in TCE concentrations in the off-gas and/or increases in biodegradation end-products (carbon dioxide and chloride) in the water during the bioremediation test as compared to baseline values during the phase 1 in situ air stripping would be considered evidence for the effectiveness of the bioremediation effort. However, this may not occur. Other indirect evidence will be obtained to verify that the air/methane injection campaign is having the desired positive effect on the aquifer system. Such evidence will include the determination of an increase in methanotrophic populations and concurrent biological TCE degrading activity within the aquifer and vadose zone. Baseline determinations of existing methanotrophic biomass/activity levels will thus be required. Several techniques will be utilized including DNA/RNA probes for functional groups with the community, membrane lipids and other enzymes for physiological and biomass characterization, methanotrophic cell number determinations, fluorescent antibody staining for specific populations, soil microcosm activity levels, and enrichment tests for methanotrophs. The latter two techniques will be used to assess TCE degrading activity in samples from the aquifer and vadose zone using radiolabeled TCE. Included in this task will be a determination of the ability of the in situ microbial populations to degrade other chlorinated alkenes (e.g. DCE isomers and PCE) which are present. In addition to providing information on the status of the bioremediation campaign, the results of this subtask will provide a unique in-depth assessment of specific microbial population and community changes in the aquifer and vadose zone resulting from directed alterations of subsurface conditions.

Task 2. Air/Methane Injection Campaign

The purpose of this task is to test the bioremediation of the site by injection of gases (air and methane) into the aquifer to stimulate growth of methane utilizing bacteria and the resulting degradation of the contaminants. Liquid and or gas nutrients may also be injected with methane/air under this task if additional stimulation is needed during the first campaign If other nutrients are not added during this campaign they will be deferred to Task 3 (below) which will optimize multiple nutrient injection. Task 2 will involve the actual air/methane injection as well as monitoring of results, and supporting process analysis and development.

Task 2 has been divided into 5 subtasks as follows:

2.1 **Operations**, including process engineering and operations at the site, (e.g. regulation of gas flow regimens);

2.2 **Chemical** analyses of influent and off-gases, water samples, and maintenance of on-line detection systems;

2.3 **Biological** monitoring of biomass, physiological state, and degradative activities, of populations, functional groups, and communities;

2.4 **Laboratory process control studies** using soil columns with sediments from the subsurface and liquid and gas phase bioreactors to recommend injection protocol, feeding regimens, and test treatments that would provide faster and/or more complete biodegradation of more recalcitrant species like PCE.

2.5 **Modeling** and data interpretation including hydrological modeling, modeling of the degradative processes and evaluation of the data.

Details of these tasks are given below.

Subtask 2.1. Operations

This task will cover the actual injection of the methane in air. The phase 1 air injection demonstrated that injection rates of 170 SCFM stimulated bacteria density increases in the groundwater, the lower rate of 65 SCFM had no effect and 270 SCFM stimulated bacteria only marginally over the medium rate. The gaseous residence times varyed from several hours to several weeks. Given the long residence times and apparently tortuous paths that the air can follow in this subsurface site the Air/Methane mixture will be injected continuously at concentrations of 1-3% of the air flow. Thus methane will not reach explosive concentrations, and aerobic conditions will be maintained within the subsurface sediments. After three months of operation, if biodegradation rates are significantly increased and methane does not appear to be limiting then a pulsing regime will be initiated. Pulsing of the methane flow will have two advantageous effects; an increase in degradative efficiency by decreasing competition for TCE and methane at enzyme sites and then provision of methane for growth and cellular maintenance; and eliminating the constant availability of methane near the injection well to expand the breadth of the biomass enriched zone.

If monitoring of inorganic compounds during the initial operating campaign suggests that one or more of these compounds is limiting microbial degradation of TCE then the later part of the operating campaign may also employ supplementation of limiting nutrients, eg. nitrogen. Ammonia will likely be added as a source of nitrogen. Delivery of ammonia will be at low levels (less than 0.1%) and it will be pulsed counter to methane so that zones around the injection well will not see an abundance of nitrogen and energy source simultaneously, thereby increasing the zone of influence of the biomass stimulation. Another advantage to ammonia additions will be the alkaline buffering capacity. Waters in the vicinity are slightly acidic pH 5-6.5, so the addition of ammonia will assist in maintaining a pH more suitable to methanotrophic bioremediation (pH = 5 - 7.5). Process control experiments are currently underway to determine if nitrous oxide may have more advantages over ammonia.

Other parameters that can be tested in the gaseous substrate injection task include changes in influent flow and pressure, alteration in extraction vacuum, and enrichment with propane. In previous studies TCE degraders from nearby subsurface sediments were greatly stimulated in microcosm studies after the addition of propane. Propane supplements at the level of 5-30% of the methane additions (>2% total flow) may be tested based upon results of laboratory process control experiments (Subtask 2.4). Input from the results of Subtask 2.4 will be critical in deciding what alterations will be made over the course of task 2.

Subtask 2.2. Chemical Analyses

The purpose of this task is to provide an evaluation of the changes in the chemical composition of the effluent gas and the groundwater in order to determine if remediation is occurring at a rate rapid enough to detect. Chemical analyses will be used to monitor toxicants, nutrients, and water chemistry in the zone of bioremediation. Values will be compared with monitoring wells, background information, and models.

Sampling will include influent and off gases, which will be analyzed for methane, carbon dioxide, TCE, PCE, DCE, DCE epoxide, VC, vinylidine chloride, and other related compounds. Daily sampling is scheduled for many components, some of which may be analyzed on-line. Three gas chromatographs (GCs) will be on or near-site, with thermal conductivity, flame ionization, electron capture, and electrolytic detectors. A software and integrating package with spreadsheet and modem capabilities will also be available. GC-mass spectroscopy, more detailed analyses, and frequent back-up analyses will be provided by participating laboratories. Monitoring wells will also be examined for soluble concentrations of the compounds listed above on a weekly basis. In addition the waters will be examined biweekly for mineral concentrations such as nitrogen, ammonia, nitrate, phosphate, total phosphorus, dissolved organic carbon, and sulfate, in addition to weekly measurements of temperature, dissolved oxygen, redox, conductivity, pH, chloride, TCE and PCE from all monitoring wells. All or the VOC parameters and mineral parameters will be measured every 3 months from two sediment profiles within and outside of the treatment influence zone.

Success of the chemical analyses task will be judged by the availability of sufficient high quality data to evaluate the effects of the injection on concentrations of contaminants in the water and effluent gas. Data will be incorporated into a model of the remediation process; if changes are rapid enough, the chemical analyses could show that the rate of toxicant decreases in off-gases is far greater than expected by air infusion and hence the result of bioremediation. Data will also be available to indicate the dispersion rates of methane and the effects of the injection on monitoring well chemistry in a large zone of influence. Quality assurance will be accomplished by standards, blanks, blind samples, chain of custody, and independent verification of random samples in other laboratories. Helium gas tracer studies of both groundwater and off-gas samples will be conducted bimonthly to determine if dispersion pathways and rates are changing in the subsurface relative to microbial activity.

Subtask 2.3. Biological Analyses.

The goal of this subtask is to provide definitive information demonstrating the effects of treatment regimens on the abundance of microorganisms, abundance of physiological groups, toxicant degrading capabilities and activities of the resident subsurface microorganisms as a consequence of the treatments. In addition one or more bioassays of toxicity will also be done to determine what effect the treatment has on overall environmental toxicity. Sampling would be biweekly, and coordinated with the in depth chemical sampling and experiments. As with chemical analyses, sediments will be sampled at 3 month intervals.

Parameters to be measured include viable biomass, microbial activities, and toxicant degrading capabilities. Viable biomass will be determined by plate counts, MPN enumeration experiments, microscopy, and phospholipid biomass. General microbial activity will be assayed by acetate incorporation rate into microorganisms. Abundance of physiological groups related to toxicant degradation will be assayed by molecular biology probes for DNA and or RNA, and fluorescent antibodies. This represents a major advance in the application of molecular biology probes for environmental uses. These probes will enable enumerating and following over time the abundance of microorganisms possessing unique enzyme systems known to impact toxicant degradation. The increase in these bacteria will be an effect of treatment regimens and bioremediation and can be used to judge the success of the project. Thus characterization, monitoring and control will occur by determining the functional capability of the microbial community to degrade TCE/PCE.

Microcosms will be used to assess the biodegradation of radiolabeled TCE over time as a measure of bioremediation potential of the sediments and as an indicator of possible in situ rates. Again changes in this potential can be used to judge the success of the project. Enrichments (microcosms plus nutrients) will be used to assess the rate of radiolabeled TCE loss when nutrient conditions are optimized. The enrichment rates will be compared to the microcosm rates, and to models of the sediments as well as serving as a measure of potential of bioremediation. If degradation occurs within hours and continues for days, substantially lowering the ^{14}C-TCE in the microcosm, then it would be a direct demonstration of bioremediation or as a potential if it occurred within days to weeks.

The greatest measure of success would be demonstration of ^{14}C-TCE disappearance in microcosms within hours of collection of both water and sediment samples, as compared to controls. Second best measure of success would be substantial loss of TCE in enrichments as compared to controls and background samples. Success can also be in terms of increased microbial activity, increased biomass; particularly biomass which contains TCE degrading machinery, increased biomass capable of consuming methane as evidence of stimulation by treatments.

Subtask 2.4. Process Control Bioreactors and Soil Columns.

The objective of this subtask is to provide a testing and validation capability for design and implementation of treatments considered for the subsurface. It will be a continuation of baseline biological activity measurements initiated in Task 1.

Concentrations, flows, vacuum, and pulsing will be varied to examine the impact on TCE and PCE degradation in laboratory soil reactors. Limitations with respect to moisture, phosphate, nitrogen will also be examined. Success of the subtask will be provision of data for modeling efforts and determination of factors limiting TCE/PCE degradation, and identification of treatment regimes that could compromise continued operation of the wells. The operation of the bioreactors will yield an evaluation of the potential effects of alterations on TCE degradation rate. Treatment regimens investigated in the lab soil reactors and found to be potentially more successful than current field operating conditions could than be tried in the field and thus significantly improve the rates of bioremediation. The data could also be used in subsurface models by identifying factors controlling the degradation rate. The soil columns and bioreactors may be critical in identifying regimens which could lead to plugging the sediments. In addition, optimization of the bioreactors and soil columns will provide ex-situ treatments that could be used in Task 5 and process design information for tasks 3 and 4.

Subtask 2.5. Modeling and Data Interpretation.

This subtask objective is to compare results with theoretical models, interpret data, and facilitate communications between investigators. Models will be developed for methane dispersion, TCE loss, bioremediation, air, water and gas flows with pressure, impact of bioremediation. This work is a continuation of the modeling activity initiated in Task 1 for process design guidance.

Task 3. Multiple Nutrient Injection Campaign

The goal of the second campaign is to further develop bioremediation by overcoming limitations indicated by the first injection program and the enrichments, and the soil column/bioreactor studies. The chief hypothesis for the second injection program is that an

additional layer of technology (e.g. liquid nutrient addition) will likely be required to enhance bioremediation to the highest possible rate and efficiency. In freshwater environments phosphate is typically the limiting nutrient; and in groundwater analyses throughout the site indicated that phosphate is near or below 50 ppb. (< 1 ppm may be microbially limiting). Other nutrients will likely become limiting as the biomass is increased, placing a higher demand on the several required micronutrients. Sulfate is typically 1-10 ppm , nitrate plus ammonia is often <1 ppm, and lesser nutrients such as Mn, borate, etc. may become limiting, and thus require pulsing of a dilute mineral solutions periodically. Laboratory soil column/bioreactor tests (Task 2) will facilitate elucidation of nutrients required, concentration and pulsing frequency. A nutrient delivery system may consist of a ground irrigation system, or perhaps periodic backwashing of both through the extraction and injection well with mineral solutions. Air would then be pulsed after the injection of minerals and only after a longer period of time would the methane be returned. Hence, growth will be reduced next to the injection and extraction wells resulting in increased opportunity for microbial growth in the vadose and distal regions of the zone of influence. In addition, combinations of propane and methane and/or other energy sources will be tried to enhance biodegradation rates and efficiencies of TCE and the more recalcitrant PCE.

Subtasks for this second injection experiments are as in Task 2, with the addition of a design and site modification function and additional supply costs for the added minerals and nutrients.

Parameters to be tested include those of Task 2, and over the same range, with the emphasis being the effects of added micronutrients and energy sources to the subsurface sediments.

The same monitoring parameters will be tested with a bit more emphasis on the water ionic chemistry to see if we are relieving the nutrient limitations as judged by groundwater chemistry from nearby monitoring wells.

Task 4. Microbe/Nutrient Injection

The objective of this task is to demonstrate that addition of certain TCE/PCE degrading microbes to the subsurface environment will improve rates and/or efficiencies of TCE/PCE biodegradation over indigenous stimulation alone. Species and consortia have been isolated that under optimal conditions (bioreactors) will degrade TCE/PCE extremely rapidly, perhaps much more rapidly than the indigenous microflora. Thus by growing large quantities of these microbes in surface bioreactors and then injecting them with inducer substances and/or nutrients we could greatly improve the time and efficiency of a waste site bioremediation. Preliminary studies by ECOVA inc. in California using the G4 bacteria and a natural inducer substance (tryptophan) with a TCE contaminated aquifer have suggested that this technique has merit.

Subtasks for this second injection experiments are as in Tasks 2 and 3, with the addition of a design and site modification function and additional supply costs for the microbe producing bioreactor and nutrient/inducer supplementation.

Parameters to be tested include those of Task 2, and over the same range, with the emphasis being the effects of added microbes and energy/inducer sources to the subsurface sediments.

The same monitoring parameters will be tested with a bit more emphasis on the biological parameters especially those that track the injected microbes and the effects that they have on the biological community.

Task 5. Combined in situ with pump and treat technologies

A realistic possibility is that the in situ air stripping and bioremediation technologies will successfully remove some (perhaps most) of the contaminants from the soil and water, but may not achieve the ultimate desired decontamination. The primary reason for this expectation is that gas injection alone may not create sufficient liquid contact and mixing to clean the water. Therefore, some kind of water pumping may be needed.

Task 5 could pump water from the lower well and reinject it via the upper well. While the water is on the surface, it will be treated biologically to remove organics. For reinjection, addition of nutrients and perhaps microorganisms will be evaluated to enhance the in situ degradation. The result of this continuous water recirculation will be to move the water through the aquifer strata in the vicinity of the wells and thus promote contacting with microorganisms both on the surface and in the subsurface. Degradation rates should be significantly enhanced, and accessibility to otherwise dead/stagnant zones should be improved.

This task would appear to be relatively straightforward and utilize the existing process equipment at the site. Only relatively minor modifications would be necessary. The main need is probably for a pump of sufficient capacity to circulate the water.

Task 6 - Technology Evaluation by Technical Support Group

The remediation Technology Technical Support Group (TSG) has the following Charter:

> Identification, evaluation and recommendation of emerging remediation technologies applicable to subsurface organic contamination at non-arid sites. A systems approach dictates that all facets of remediation will be considered, including in situ methods, surface and secondary waste treatment, and disposal. The TSG shall perform preliminary technology status evaluations to identify potentially high return/cost effective technologies that are ready for full scale demonstration. Further, the TSG shall advise the planning committee on near term and future remediation activities, coordinate field demonstrations as they occur, and coordinate with the other TSGs to assure maximum benefit from the field demonstrations.

The charter identifies the two pronged roles of the Remediation Technology TSG within the integrated demonstration. The first of these is a large scale planning and evaluation function; the second is the detailed planning associated with near term demonstrations. Importantly, this TSG, as well as the others in the integrated demonstration, serve as a technical filter to assist the planning group in each of the broad areas of expertise. The particular activities identified for this funding period are: review and reaffirmation of the charter, critical evaluation of alternative thermal technologies, generation of preliminary technology status reports for selected technologies, coordinate with PNL for SRS specific thermal demonstration, and develop details of next full scale demonstration. Several areas will be addressed in generating the first preliminary technical status report - assessment of need (using ER needs Assessment), identification of criteria for success, measures and goals (based on CERCLA,

OSHA, etc.), an assessment of limitations or flaws (technical, regulatory, etc), and a preliminary engineering/cost assessment. The outline of the report will be based on DOE HQ guidance for final technology status reports. Importantly, generation of the document has been set up in such a way that it will take advantage of the infrastructure of the ID (e.g., the cost analysis group will perform independent cost analysis, the various TTPs will perform multidisciplinary flaw analysis). Specific flaws and concerns identified in the planning process will be addressed by designing simple experiments to provide data to make go/no go decisions.

Background:

Biodegradation of TCE by methanotrophs has been demonstrated in microbiological studies and in methanotrophic laboratory-scale bioreactors. J. T. Wilson at the U.S. Environmental Protection Agency laboratory in Ada, Oklahoma was among the first to observe TCE degradation in laboratory soil columns in the presence of methane. Little et al. at ORNL isolated a mixed methanotrophic culture from a TCE-contaminated well on the Oak Ridge Reservation. This culture was subsequently used in a prototype lab-scale continuous flow bioreactor at ORNL.

Other leading investigators in the development of TCE bioremediation technology include W. Jewell at Cornell University, P. McCarty at Stanford University, D. White and T. Phelps at The University of Tennessee (UT), S. Fogel at CAA, Inc., and a group at Battelle Columbus. These investigators comprise a consortium for development of this technology under the auspices of the Gas Research Institute and the Savannah River Laboratory (SRL). The investigators meet regularly to exchange technical information, and Radian Corporation is serving as a data repository and process evaluation function under contract to the Gas Research Institute. The combined expertise and knowledge base of this consortium will be essentially an ad hoc resource to this present DOE in situ remediation project since Oak Ridge National Laboratory (ORNL), UT, and SRL are charter members of the consortium.

In addition to the laboratory bioreactor studies at ORNL, UT, and elsewhere, one pilot-scale bioreactor system has been operated by Battelle Columbus at Tinker Air Force Base, Oklahoma. This project was funded by the Air Force Engineering and Services Center, Tyndall Air Force Base, Florida. This study demonstrated that actual TCE-contaminated groundwater can be treated in a trickle-bed bioreactor. The culture used in this test was provided by ORNL.

Tyndall AFB is continuing to support development of TCE bioreactor technology at ORNL and UT and Savannah River Site (SRS). The bioreactors used at Tinker Air Force Base are being provided by Tyndall Air Force Base for further field tests at Oak Ridge and Savannah River.

Although development of methanotrophic bioreactors for TCE bioremediation is progressing well, in situ biodegradation of TCE is an emerging technology that has not yet been demonstrated at a practical scale. Tests on a small area of a shallow aquifer at the Moffett Naval Air Station in California (McCarty et al.) have shown that indigenous microorganisms can be stimulated with methane and oxygen to degrade TCE. These results are very encouraging. McCarty's experiences in these studies are a large part of the basis for the process design for this in situ demonstration at the SRS.

Technical Progress/Milestones:

Task 1 (Project Start-up) is projected to take six to nine months. During this time the permitting and regulatory requirements will be met, and detailed test plans will be developed, along with QA/QC, health and safety plans, etc. Necessary system equipment modifications will be accomplished, including set up of an analytical trailer at the site. A large amount of microbial characterization baseline work will be done during this period. This will involve obtaining field samples of soils and water from the site and background locations, followed by laboratory studies described in Subtask 1.5. A detailed Test Plan was completed and approved by the Technical Support Group 10/91. NEPA and the Underground Injection Permits were obtained 8/91. The air permit application was submitted 10/91 and all support contracts are either in place or in contract negotiation.

Task 2, the methane injection campaign, is projected to begin in FY92 and last twelve months. The completion of this Task is a key milestone in that the performance of the system during this task will determine the technical objectives for the next task.

Task 3 will begin upon the completion of Task 2 in late FY92. It is a second bioremediation campaign to further enhance the performance over Task 2. We anticipate that Task 2 will lead to identification of limiting factors that can be addressed in Task 3. Task 3 is scheduled to last twelve months, at which time an evaluation for the needs for Tasks 4 and 5 will be completed.

Task 4, Microbe and inducer/nutrient injection, is scheduled to begin in late-FY93 with an evaluation and design subtask for one month, followed by construction of a bioreactor for microbe supplementation. This injection campaign will be operated for about twelve months, and the Task will be finished in late-FY94.

Task 5, Combined in situ/ pump and treat technologies is scheduled to begin in late-FY94 with an evaluation and design subtask for one month, followed by construction of one or more bioreactors and injection, stripping systems. This task will rely upon capital expenditures from previous tasks and minimization of sampling and control parameters as indicated by the previous tasks. This campaign will be operated for about twelve months, and the the Task will be finished in late-FY95.

Task 6, Remediation Technology Technical Support Group. At least two meetings of the technical support group will be held. One Preliminary Technology Status Report will be prepared.

Funding Basis:

Task	Cost (K$) FY-92	FY-93	FY-94	FY-95
1. Project Start-up				
1.1 Planning and Management				
1.2 Process Design/Modeling				
1.3 System Modification				
1.4 Analytical Facilities				
1.5 Microbial Characterization (R&D)				
SRL				
PNL				
ORNL/UT				
2. Air/Methane Injection Campaign				
2.1 Operation	800			
2.2 Chemical Analyses	200			
2.3 Biological Analyses	400			
2.4 Bioreactor/Soil Columns (R&D)				
ORNL				
INEL				
2.5 Modeling/Interpretation	100			
3. Multiple Nutrient Addition Campaign				
3.1 Operation		700		
3.2 Chemical Analyses		200		
3.3 Biological Analyses		300		
3.4 Modeling/Interpretation		100		
3.5 Design Upgrades		50		
3.6 Equipment Modification		50		
3.7 Additional Supplies		50		
3.8 Additional Maintenance		50		
4. Microbe Nutrient Addition Campaign				
4.1 Operation			700	
4.2 Chemical Analyses			100	
4.3 Biological Analyses			300	
4.4 Modeling/Interpretation			100	
4.5 Design Upgrades			50	
4.6 Equipment Modification			50	
4.7 Additional Supplies			50	
4.8 Additional Maintenance			50	
5. Combined In Situ/Pump and Treat			100	600
6. Technical Support Group Support				
Operating Costs	1500	1500	1500	600
Capital Costs	590	950		
Total	2090	2450	1500	600

Funding for Task 3 is essentially identical to Task 2, plus additional funding for upgrading the system to add nutrients and increased supplies and maintenance. This task is scheduled for an initial campaign of twelve months

Funding for Task 4 is essentially identical to Task 2 and 3, plus additional funding for upgrading the system to add microbes and increased supplies and maintenance. This task is scheduled for an initial campaign of twelve months

At this time funding estimates for Task 5 are simply extensions of the funding level needed for the field work in Tasks 2-4. More detailed funding requirements will have to be developed later when more information is available on the nature of the tasks.

Funding for Task 6 is included in Tasks 1 through 5 and in the project management funding for the integrated demonstration.

Work Breakdown Structure Element: 1.14.02, 1.14.06

Technical Need:

Subsurface soils and water adjacent to an abandoned process sewer line at the SRS have been found to have elevated levels of TCE. This area of subsurface and groundwater contamination is the focus of a current integrated demonstration of new remediation technologies utilizing horizontal wells. Bioremediation has the potential to enhance the performance of in situ air stripping as well as offering stand-alone remediation of this and other contaminated sites. Horizontal wells could also be used to enhance the recovery of groundwater contaminants for bioreactor conversions from deep or unaccessible areas (e.g., under buildings) and to enhance the distribution of nutrient or microbe additions in an in situ bioremediation.

The basic concepts of this technology are expected to be applicable to other sites having TCE-contaminated soils and water. However, the particular process designs will be site specific. The experience gained at the SRS Integrated Demonstration will provide the basis for designs for other sites. The generic needs for this technology are described in Sections 2.2.3 and 2.2.4 of the RDDT&E Plan. Regulatory drivers for this activity are RCRA (40 CFR 264 and 265 Subparts F and G), CERCLA (40 CFR 300 1986 Amendments Section 122) and SDWA (40 CFR 141).

Alternatives:

The principal existing technology for remediation of TCE-contaminated groundwater is pumping followed by air stripping. This is not a TCE destruction technology; the TCE is either discharged to the atmosphere or captured on activated carbon for subsequent disposal. At the SRS no air emission restrictions are presently in force, and air stripping is being used already. However, the lack of emission restrictions is not the usual case, and will change at the SRS.

Preliminary economic evaluations have shown that while air stripping without emissions control is the least costly technique, biodegradation will be very competitive with air stripping with emissions control.

Benefits:

In situ bioremediation technology is based on biological destruction of the contaminants at the site. Therefore, risks normally associated with handling, transporting, and treating or storing contaminated residuals are avoided. In this sense there is a very significant reduction of risk.

Costs for in situ bioremediation of TCE are not known since this is an emerging technology. However, current in situ bioremediation technologies for other organics (such as gasoline)

are nearly always less expensive than alternative technologies that provide destruction of the contaminant (and hence permanent remediation). Cost analysis of methanotrophic bioreactors compared with air stripping combined with carbon adsorption of the air stream and direct carbon adsorption from the water have suggested that for several TCE concentrations and flow rates that the methanotrophic system would save 40-60% over conventional technologies. We expect that these observations will also be the case for in situ bioremediation of TCE alone or in combination with bioreactors.

In situ bioremediation coupled with air stripping is expected to lead to a significant reduction in the time required to complete the remediation because bioremediation provides a second simultaneous pathway for removal (destruction) of the TCE. Furthermore, the stimulated indigenous microorganisms will gain access to TCE in the vadose zone and aquifer matrices that may be very difficult to remove by air stripping. Thus a "cleaner" endpoint should be reached in less time.

The enzymes induced in the microbe by the methane cometabolically oxidize a host of other organic compounds, including toluene, benzene, etc. Since many contaminated sites also contain these or similar compounds, in situ bioremediation and bioreactor systems also address their degradation. Preliminary laboratory studies have demonstrated the proof of this principle (Phelps et al.)

Criteria for Success:

There are three primary criteria by which the overall success of this demonstration will be evaluated:

1. Evidence of biological destruction (biodegradation) of TCE from the contaminated soils and water. Since a major advantage of bioremediation is destruction, it is important and significant to demonstrate that biodegradation is occurring. The evidence is expected to come primarily from comparison of the compositions of the off-gases before and after addition of methane to stimulate biodegradation, and from laboratory studies in soil columns using soil cores from the site. In the latter case we expect to show that radiolabeled TCE is degraded under conditions similar to those in the field.

2. Increased reductions of TCE in soil and water samples from the site during periods of biostimulation. The technology is expected to accelerate the removal of TCE over in situ air stripping alone, which was the focus of the first phase of the integrated demonstration.

3. Reduced cost over comparable conventional technologies. Comparison of costs of air stripping currently in use at the site and cost of in situ air stripping from the first demonstration. Costs of both operations and the bioremediation can be compared to rates of removal and/or degradation to arrive at normalized costs for both processes for the same site.

4. Relatively simple and trouble-free operation. These characteristics contribute to favorable economics. A critical assumption for the successful demonstration is that gases can be successfully injected via the lower horizontal well and recovered via the upper well. This ability has been demonstrated in phase 1 of the integrated demonstration project. The wealth of data from phase 1 can be compared and used as a control for the bioremediation project.

The **principal uncertainties** concern the rate of TCE removal/degradation--how long it will take. The permeability of the soil will influence the delivery of nutrients (gases and potentially

liquids) to the bacteria. Slow delivery will mean slow bioactivity. Similarly, heterogeneities in the strata may cause some regions to be bypassed; however, if the contaminants infiltrated these zones, then nutrients will too, but it may occur slowly.

We do not believe there will be a danger of plugging the soil around the wells by the growth of biomass. This phenomenon has occurred in the past at other bioremediation sites; however, we now know how to avoid this problem by the proper addition of nutrients.

Regulatory Requirements:

The demonstration will provide timely transfer technology to Environmental Restoration to assist in meeting all applicable environmental regulations. Specific permits for the demonstrations have been negotiated (e.g., underground injection control, air quality etc.).

Technology Transfer:

Bioremediation of TCE contamination has already captured the imagination of several progressive private firms. They have already established contact with us, and are watching carefully as DOE pursues above-ground bioreactors and in situ technology. They simply do not have the resources (nor the expertise) to assume the risks associated with technology demonstration. However, based on the excitement and anticipation we have experienced with these companies to date, we expect that successful demonstration of in situ bioremediation at the SRS will do much to encourage these companies to utilize this technology.

In addition to these existing professional contacts and relationships, the formal technology transfer functions at SRS, ORNL, and INEL will be utilized. Several investigators will also participate in professional/technical symposia to disseminate information on the performance of this technology.

Acceptability:

Bioremediation technologies enjoy relatively high regulatory acceptability in cases where the technology has been demonstrated to be effective. Regulatory agencies are also showing interest in the addition of specialized microbial cultures to the site. California has already granted permits for demonstration projects that inject nutrients and TCE-degrading bacteria into a contaminated aquifer. California, Texas and Michigan have also allowed field project injection of methane and nutrients for in situ bioremediation of TCE contaminated aquifers. Massachusetts and other states have also allowed methane to be injected into aquifers as a tracer for several years. There is a clear precedence for this type of project in the field. This general environment bodes well for approval to use in situ bioremediation at the SRS.

Methane itself is generally recognized as a natural compound found universally in subsurface environments. Years of experience by the Oil and Gas industries has shown that subsurface environments and groundwater can be exposed to high concentrations of methane for many years with no adverse effects. In addition, the U.S. Geological Survey has used methane as a conservative tracer in groundwater at Cape Cod for several years at their Groundwater Flow Study Facility with no adverse effects (Harvey et al.). Thus we are confident that methane can be injected safely with extremely low probability of any adverse environmental effects of any kind.

Bioremediation enjoys relatively favorable societal acceptance, in part because it is perceived to be "natural." Essentially ambient process conditions and the lack of unsightly large equipment also contribute to societal acceptability. Use of genetically engineered organisms is not yet socially acceptable. However, such organisms will not be needed at the SRS (although they may offer process advantages at a later date when the acceptability issue has been resolved).

Appendix C. Scope of Work.

Scope of Work for Operational Support of Phase 2 Integrated Demonstration In Situ Bioremediation

Background

Two horizontal wells have been installed below an abandoned process sewer line that leaked volatile organic compounds during the 1960's through the 1970's. The VOC's primarily consisted of trichloroethylene (TCE) and tetrachloroethylene (PCE). The wells were installed to test new methods of in situ remediation of soils and ground water.

A deep horizontal well, installed below the water table, has been used as an air-injection well. The shallow horizontal well, installed in the vadose zone, has been used to vacuum extract volatiles from the vadose zone and upper saturated zone in the area. These wells were initially used to demonstrate a simple mass transfer process to remove VOC's from both the groundwater and vadose zone. During an 20 week test 16,000 lbs of VOC's were extracted from the subsurface. The entire demonstration site was carefully characterized and monitored. The technologies used include surface and borehole geophysics, cross hole geophysical tomography, pressure monitoring in all affected areas, fluid flow sensors, microbial characterization of soil and water, and chemical analysis of soil, soil gas and groundwater. We are currently planning a test of in situ bioremediation using these same test wells to remediate the residual VOC's at the site.

Specific nutrients have been demonstrated to stimulate selected members of the indigenous microbial community that can degrade TCE/PCE. Laboratory studies using TCE/PCE contaminated sediment and groundwater from M area (at SRS) have demonstrated that a variety simple carbon sources, e. g. methane, will stimulate a microbial community that will completely degrade TCE/PCE to carbon dioxide and chloride. Small pilot studies in other parts of the U.S. have shown that injection of methane/air mixtures into groundwater could significantly increase the biodegradation rate of the indigenous microflora. Use of methane has the advantage of being cheap and abundant (natural gas), naturally occurring, and having a distribution system that corresponds to major contaminated areas in the U.S.. It also has the advantage of stimulating specifically those organisms that are capable of degrading TCE. This will greatly reduce the possibility of aquifer plugging and of proliferation of unwanted microbes, giving greater control and better biodegradation monitoring capability. Biodegradation is a highly attractive technology for remediation because contaminants are destroyed, not simply moved to another location or immobilized. Bioremediation is also among the least costly technologies in applications where it will work.

The horizontal wells that form the basis for the SRS Integrated Demonstration should provide significant advantages over conventional bioremediation techniques. The increased surface area will allow better delivery of nutrients and easier recovery of gas and water, as well as minimizing formation clogging and plugging phenomena.

Purpose and Objectives

The overall objective of this project is to demonstrate the utility of stimulating indigenous microorganisms that will degrade trichloroethylene (TCE), tetrachloroethylene (PCE) and their daughter products as a treatment (remediation) process for contaminated groundwater and sediment. An additional objective is to demonstrate that horizontal wells can be used as an effective delivery and recovery mechanism for bioremediation. The specific objective of this scope of work is to identify and procure the engineering and field support for the test. This support includes field coordination, data acquisition, and the provision and operation maintenance of the air/gas delivery and extraction equipment as discussed below.

The purpose of the project is to develop a more efficient, more cost effective, faster, and more environmentally sound method of cleaning up wastes sites that are contaminated with TCE/PCE. Because this is a rapidly developing technology, there are limited data related to system operation. The effects of various design and operating parameters will be evaluated. The system must have maximum flexibility to allow changes during operation that can take advantage of findings and developments that occur after start up.

General Test Description

Treatment will occur in situ and above ground. The above ground treatment will process off-gas and water through optional and/or experimental treatment units (e.g. experimental bioreactors). Below ground treatment (in situ) will consist of injecting one or more simple nutrients to stimulate biodegradation of TCE/PCE to inorganic components. The principal nutrient to be supplied via the horizontal wells is methane, at a low concentration in air (1-3% by volume). The lower horizontal well will provide a very efficient delivery of gas throughout the contaminated region. A vacuum will be applied to the upper well (vadose zone) to encourage air/methane movement through the upper saturated zone and lower vadose zone, thus inhibiting spreading of the contaminant plume. An extensive monitoring program using existing monitoring wells will observe the response of the soil and water during injection of air and methane. The off-gas from the upper horizontal well will be assayed for methane, total VOC, TCE, PCE, potential break down products of TCE/PCE, e.g., dichloroethylenes (DCE), vinyl chloride (VC), and carbon dioxide. Data from the phase 1 demonstration of in situ air stripping will determine base line geological, hydrological, chemical, and biological characteristics. only air was injected during phase 1. These data will establish the effect of air injection without nutrients on the hydrological, chemical, and biological characteristics of the site. In effect, it provides a unique and dramatic control experiment for the phase 2 bioremediation.

Subcontractor Tasks

1. The Successful offerer will furnish an **air compressor** capable of delivering 300 scfm at 100 psi. Unit must have standard safety equipment and interlocks (e.g., pressure limit at 125 psi). These safety features must be inspected according to the vendor's safety program as approved by the **Subcontract Technical Representative (STR)**. Unit must be skid or trailer mounted and delivered to the test site. Unit cost must include all supplies and maintenance. Services must begin within 12 hours of operational problem identification and be completed within 72 hours. Periodic maintenance schedule requirements must be identified in the proposal from the offerer. Vendor must provide all supplies, spare parts and maintenance for all subcontractor equipment. The unit must be equipped with an hour meter to allow accurate records of operating times.

2. The Successful offerer must provide a **vacuum blower** capable of extracting 500 SCFM at 10 inches Hg (inlet). Unit must have an inlet filter and outlet silencers and be mounted in an enclosure to reduce noise level to 90 decibels during operation. The outlet must allow discharge through a 15 foot high dispersion stack, to a subcontractor supplied off gas treatment unit if present (Task 7), or to WSRC supplied experimental offgas treatment units. The experimental offgas treatment unit's back pressure will be limited to a maximum of 5 inches of water. Unit must have standard safety equipment and interlocks (e. g., pressure limit at 125 psi). These safety features must be inspected according to the vendor's safety program as approved by the STR. Unit must be skid or trailer mounted and delivered to the test site. Unit cost must include all supplies and maintenance. Service must begin within 12 hours of operational problem identification and be completed within 72 hours. Periodic maintenance schedule requirements must be identified in the proposal from the offerer.

Vendor must provide all supplies, spare parts, and maintenance for all subcontractor equipment. In addition the offgas outlet must have an audible and visible recorder for monitoring when offgas methane concentration exceeds 5% volume/volume. The unit must be equipped with an hour meter to allow accurate records of operating times.

3. The Successful offerer must furnish an **air/methane blending system.** This system must integrate with the air compressor and be capable of providing controllable methane/air mixtures in the range of 0 to 4% (vol/vol). The blender must have a redundant safety system that automatically stops the flow of methane if the methane exceeds 5% methane (vol/vol). The system must generate a visible and audible signal of the alarm condition. The flow of methane must be controlled using a microprocessor that allows either constant mixtures or time programmed operation. A recording of the methane concentration over time is required. The calibration of the blender must be documented by the vendor in the final Quality Assurance report and the final project report. SRS will provide a low pressure (20 to 30 psi) methane source using industry standard connections from support organizations. Vendor must provide all supplies, spare parts, and maintenance for all subcontractor equipment.

4. The Successful offerer must provide **field engineered manifold and connections.** The vacuum unit in Task 2 will be connected to extraction well AMH2. AMH2 is a horizontal well screened in the vadose zone with a four and one-half inch diameter stainless steel wire-wrapped screen. This horizontal well's screened section is approximately 200 feet in length and has a vertical depth of 70 to 80 feet.

The air/methane blend injector (Task 1 & 3) must be connected to well AMH1, the injection well. AMH1 is a horizontal well screened in the saturated zone with two and 3/8 inch diameter stainless steel perforated tubing. The well is approximately 310 feet long with a true vertical depth of 150 to 180 feet. Vendor must provide all supplies and maintenance for all subcontractor equipment.

5. **Chemical analyses.** The Successful offerer must provide on site concentration measurements in offgas from well head and other gas sampling points for trichloroethylene (TCE), tetrachloroethylene (PCE), cis- and trans- dichloroethylene (DCE), vinyl chloride (VC), methylene chloride (MC), methane (CH_4) and carbon dioxide (CO_2). Detection limits of less than or equal to 5 ppmV for chlorinated compounds and less than or equal to 0.1 % for methane and carbon dioxide are required. The Successful offerer shall supply all sampling equipment, and on site capillary column gas chromatograph, all standards, and any other equipment needed to complete the sampling and analysis. The offerer must exactly describe and document the performance of the proposed sampling and analysis methods in the final Quality Assurance Report and Final Project Report, as specified in the Quality Assurance Section below.

6. **On-Site Project Management.** The Successful offerer shall supply (as required by the subcontract terms and conditions) an on site manager and support staff, to oversee the project, collect data, produce the weekly operations reports, produce the delivery inspection and approval report, and operate/maintain the subcontractor equipment. These activities must be performed by the successful offerer 24 hrs/day, 7 days/week. The Successful offerer shall collect pressure and vacuum data from approximately 20 vadose zone piezometers and 22 groundwater wells, as well as from the vacuum and pressure well heads and 6 downhole tubes in each horizontal well. The successful offerer shall provide the gas concentration measurements for gases collected from the approximately 20 vadose zone piezometers, gases collected from the 6 downhole tubes in the vacuum horizontal well (AMH-2), and for the vacuum well head. All sampling procedures and controls must be strictly documented, approved by the STR prior to use and conform to site quality assurance. The Successful offerer shall assure the housekeeping and condition of the site and generate the weekly operations reports on site. The on site management shall assure the continued

operation of subcontractor equipment and assure rapid correction of operational problems, as described in subcontractor performance requirement section. The on site project manager shall maintain safety and security records and oversee adherence to safety and security rules by all subcontractor personnel.

7. **Offgas Treatment (option).** If WSRC exercises its' option for offgas treatment at any time after subcontract execution, the successful offerer must provide a system to destroy chlorinated solvents in the offgas stream from the vacuum blower. The destruction must be performed on site. The system must reduce the total VOC concentration to less than 5 ppm (vol/vol) and generate nonhazardous/inorganic compounds, e. g. CO_2, H_2O, HCl. A properly sized Allied Signal HDC catalytic oxidation system or an Air Resources Inc. (ARI) fluidized bed catalytic oxidation system, or equal are acceptable. WSRC will exercise the option to include this task based on the permit negotiations that are currently underway. The offgas is expected to be similar to the offgas stream generated during the Phase I test (180°F, 400 ppm total VOC); details on the expected offgas concentrations are provided in the Phase I operational report provided by WSRC. If selected, unit must be skid or trailer mounted and delivered to the test site. The unit cost must include all supplies and maintenance. Service must begin with 12 hours of operational problem identification. Periodic maintenance schedule requirement must be identified in the proposal from the offerer. As specified in items 1 - 4 the vendor must provide all supplies, spare parts, and maintenance for all subcontractor equipment.

Government Furnished Property and Facilities

1. Power (3 phase 440v and normal 240 and 120 v will be available) Site procedures for connection to power must be followed.
2. Methane
3. Permits: Site Use/Site Clearance/NEPA/Work Clearance
4. Safety inspection of:
   Compressor/Blender and Vacuum Blower by CSWE
   Schematic diagrams are required from the vendor for CSWE. This will facilitate inspection of the system.
5. Fork lift truck for mobilization and demobilization
6. Safety and Security Training.
7. On site mobile laboratory space (approximately 50 ft^2) to set up analytical instrumentation.
8. Continuous ambient barometric pressure measurements.

WSRC furnished data

1. Phase 1 operational report (attachment #4).
2. SRS Quality Assurance Policy. STR shall discuss applicable sections of the QA program prior to the start of work (attachment #3).
3. SRS Safety procedures (attachment #2).

Subcontractor Deliverables (hardware and data):

1. Health and Safety Report. (must be approved by STR before mobilization). The subcontractor health and safety plan must be summarized. Also, this document shall include: a) schematic diagrams of all subcontractor equipment and approximate design of field engineering manifold/connection., b) a table of pressure expected in each component and the rating for that component, c) test and inspection procedure for electrical and pressure subcontractor equipment and safety devices, and d) MSDS data for all potentially hazardous chemical to be used during the project, and specific handling procedures for these chemicals. The report must be supplied as both a hard copy and on diskette.

2. Quality Assurance Report. The successful offerer shall abide by WSRC Quality Assurance Program as outlined in WSRC Management Policies, WSRC-1-01 MP 4.2 throughout the period of performance (attachment #3). In accordance with these policies, the successful offerer shall maintain records to document high quality work. The report containing statements of how the successful offerer will certify documents, personnel and training in accordance with WSRC Quality Assurance policies shall be submitted in draft form with the proposal and must be submitted in final form prior to initiation of work as described below. Field work shall include blank and duplicate sampling (duplicate samples will be collected at approximately 10% of the sampling locations). Analytical work shall be referenced to industry accepted standard materials that are approved by the STR. After comment and revision in collaboration with the STR the final report must be issued. The report must be supplied as both a hard copy and on diskette within 14 days of comment by the STR. The successful offerer must follow the approved quality assurance procedures throughout the contract period, unless written approval for changes are provided by procurement.

3. Delivery, inspection and approval of field subcontractor equipment report. At the time of mobilization all subcontractor equipment must be inspected as required by Health/Safety and Security. The STR must inspect and approve that all subcontractor equipment,

operational plans and personnel involved meet the specifications of the contract. When mobilization is complete and all inspections are complete and operation is ready to begin, and the STR has approved, then this report will be issued immediately prior to operation. The report must be supplied as both a hard copy and on diskette.

4. Weekly Operations report. This report must be issued weekly and include a summary, operations listing and all analytical results. The report must be supplied as both a hard copy and on diskette.

5. Final Report. This report must summarize the weekly operations report and include as appendices the Delivery/Inspection/Approval report, the Quality Assurance Report, and the Health and Safety Report. It must also include complete listing of operating parameters for the contract period and all analytical results. The report shall also contain as appendices any chain-of-custody sheets, verification of standards and any quality assurance documentation that may directly impact the analytical results presented. The report must be supplied as both a hard copy (5 copies) and on diskette.

Subcontractor performance requirements

1. Successful offerer shall be capable of mobilizing within 21 days of award of contract.

2. The health and Safety Report shall be submitted within 10 days of award of contract. This report shall be issued in final form after STR concurrence prior to mobilization.

2. Delivery, inspection and approval of field subcontractor equipment report shall be issued immediately prior to operation.

2. Weekly reports shall be provided by Wednesday of the following week for the week ending on the preceding Sunday.

3. Correction of operational problems including maintenance, repairs, and modifications shall begin within 12 hours of problem identification and be completed within 72 hours.

4. Final Report is due within 30 days of demobilization.

5. Following approval by the STR, all reports and data shall be provided both as paper copies and on disk.

WSRC quality assurance requirements and activities

The successful offerer must adhere to the WSRC Quality Assurance program and submit all document and records in the Quality Assurance Report and submit a Health and Safety Report. These are described in detail in the section titled Deliverables.

Safety

While on SRS, the Successful offerer will be responsible for adhering to the safety regulations of "WSRC Safety Guidelines for Subcontractors" (Attachment #2). All members of the successful offerer project staff must attend a site safety and security orientation (approximately 8 hours) prior to beginning work at SRS. The orientation includes information about handling chemicals on site, emergency signals, and security.

WSRC requirements unique to the SOW for safety, health protection, environmental, waste management, security, and badging which are not covered in SRS policies.

NONE

Technical/Cost Proposal Requirements and Evaluation

Selection of the successful offerer will be based on cost among the offerers that meet the technical requirements of the scope of work. The requirements for the technical proposal and the cost proposal are outlined below. Note that this is a procurement to qualify a vendor for engineering support based on anticipated needs. The estimated quantities of items listed in the cost proposal section are for comparison purposes only, and do not represent guaranteed future work. The actual amount of funding allocated to be used for each unit during the period of performance will be determined by the STR. The technical proposal shall address the item listed in the sections below.

Technical Proposal

The technical proposal will be evaluated by at least three qualified SRL/SRS staff members. Each reviewer will score each proposal using the following categories (a more detailed description of each category is provided in the Qualifications, Task Descriptions, and Quality Assurance sections).

I. Capability of the company - This section must demonstrate the ability of the company to carry out all phases of the work. This section shall include: documentation of successful past studies, documentation of necessary equipment and resources, documentation of reliable reporting capability.

II. Capability of key personnel - This section must identify and document the capability of the primary onsite manager, and other key personnel. The manager must have documented practical experience in performing field remediation work, and in analytical chemistry. The capabilities of other support staff persons (e. g., samplers) must be documented. All personnel working at SRS must be U. S. citizens. Any changes in personnel during the project must be approved in writing by the STR.

III. Detailed description of proposed subcontractor equipment (vacuum blower, compressor, methane blender, field engineering and offgas treatment systems). This section shall include process diagrams, equipment specifications, site requirements, and define maintenance requirements. See the Subcontractor Tasks and Subcontractor Deliverables Sections for more information.

IV. Proposed sampling and analytical methods - This includes type of sample, instrument, column, detector, detection limits to be used for each of the analytes.

V. Quality Assurance - A draft report describing the offerers ability to conform to the WSRC Quality Assurance policies. This section shall document the ability of the proposed analytical methods to meet the requirements of the Scope of Work. See the Subcontractor Tasks and Subcontractor Deliverables Sections for more information.

The results of the individual reviewers will be combined and the overall scores evaluated to qualify a group of offerers.

Cost Proposal

The comparison of the cost for the various vendors will be based on the following table of unit costs and quantities (See note 1):

	Cost (Estimated)	Units		
Mobilization/Demobilization (supply a cost breakdown to justify listed cost see note 2)	____	x 2	=	____
On site management	____	x 52wks	=	___
QA plan (note 1&4)	____	lump sum	=	___
Health & Safety plan (note 1&4)	____	lump sum	=	___
Air compressor	____	x 52wks	=	___
Vacuum Blower	____	x 52wks	=	___
Air/Methane Blending System	____	x 52wks	=	___
Field Engineered Manifold	____	lump sum	=	___
Sample analysis - chlorinated solvents (listed in Scope of Work)	____	x 2000	=	___
Sample analysis - Methane & CO_2 (listed in Scope of Work)	____	x 2000	=	___
Off gas treatment system (option)	____	x 52wks	=	___
Final Report (note 1&4)	____	lump sum	=	___

Total (All items) _____

Total (less off gas treatment system) _____

Notes to cost proposal on next page

Notes to Cost Proposal:

1. The cost proposal is to be bid in a unit price format. All of the unit prices bid shall be fully loaded to include overhead, administrative costs, travel, per diem, etc. The invoices submitted shall correspond to the description and prices listed. No payment will be made for standby time. Partial units are to be billed based on fraction of overall unit (e. g. 3 days = 3/7 of week unit). Report costs will be paid only after an approved report is received by the STR.

2. The mobilization/demobilization price shall include all travel, shipping of instruments, and other expenses associated with beginning the project and ending the project.

3. The unit cost per sample shall include collection of samples and analyses of these samples for the listed constituents. The unit price is for each sample location and shall be fully loaded as describe in note 1. Duplicate analysis may be billed at the unit cost, however, analytical standards and other quality assurance samples are considered routine services that are not billed.

4. The unit cost for each Report, shall also be fully loaded as described in note 1. A description of reports are provided in the Subcontractor Deliverables Section.