Preliminary Technology Report for Southern Sector Bioremediation (U)

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Executive Summary

This project was designed to demonstrate the potential of intrinsic bioremediation and phytoremediation in the Southern Sector of the A/M-Area at the Savannah River Site. A subsurface plume of trichloroethylene (TCE) and perchloroethylene (PCE) is present in the Lost Lake aquifer upgradient of the study site and is predicted to impact the area at some point in the future. The surface area along the Lost Lake aquifer seep line where the plume is estimated to emerge was identified. Ten sites along the seep line were selected for biological, chemical, and contaminant treatability analyses. A survey was undertaken in this area to quantify the microbial and plant population known to be capable of remediating TCE and PCE. The current groundwater quality upgradient and downgradient of the zone of influence was determined. No TCE or PCE was found in the soils or surface water from the area tested at this time. A TCE biodegradation treatability test was done on soil from the 10 selected locations. From an initial exposure of 25 ppm of TCE, eight of the samples biodegraded up to 99.9% of all the compound within 6 weeks. This biodegradation of TCE appears to be a combination of aerobic and anaerobic microbial activity as intermediates that were detected in the treatability test include vinyl chloride (VC) and the dichloroethenes (DCE) 1,2-cis-dichloroethylene and 1,1-dichloroethylene. The TCE biological treatability studies were combined with microbiological and chemical analyses. The soils were found through immunological analysis with direct fluorescent antibodies (DFA) and microbiological analysis to have a microbial population of methanotrophic bacteria that utilize the enzyme methane monooxygenase (MMO) and cometabolize TCE. The project has determined that there are indigenous microbes and plants that can potentially bioremediate the TCE and PCE moving in groundwater through the soil before the contaminant can break through to surface receptors. This work has extreme cost significance since it confirmed intrinsic or natural bioremediation potential in the soils of the Southern Sector.

Background

From the 1950s to 1985, M-Area process wastes were discharged to the M-Area Settling Basin and the A-014 Outfall (Looney, 1994). The unlined, eight million gallon capacity basin was designed to settle and contain metallic waste discharged from the fuel and target fabrication processes. The M-Area waste stream contained nickel, aluminum, uranium, lead, acids, caustics, and solvents. Under the Resource Conservation and Recovery Act (RCRA) Hazardous Wastes Listings the waste stream at this location was designated as F006-electroplating waste.

SRS submitted a RCRA Part A permit application in 1980, which initiated facility inspections, personnel training, and groundwater monitoring. In September 1984 SRS submitted a closure plan for the area that was approved by the South Carolina Department of Health and Environmental Control (SCDHEC) in July, 1987. After approval of associated National Pollutant Discharge Elimination System (NPDES) and wastewater construction permits, the settling basin was closed by dewatering and stabilizing the sludge at the basin bottom in April, 1988. The basin was then back-filled with surrounding soils and covered by a cap with a synthetic liner and clay barrier. Closure of the basin was completed in 1990 and final approval was received from SCDHEC in 1991.

Groundwater cleanup was initiated in February, 1983 with an experimental pump and treat system. In February, 1985, SRS submitted a RCRA Part B permit application addressing post closure maintenance of the area including groundwater monitoring and corrective action. The permit application committed SRS to remediate solvent contaminated groundwater, including TCE and PCE, in the A/M-area. This required system evaluation, monitoring, and any modifications to

improve remediation efficiency. SRS initiated the operation of a full scale pump (500-600 gpm) and treat system (the M1 system) in November, 1985. This RCRA Part B permit was approved in September, 1987. SRS subsequently agreed to submit a 5 year renewal of the permit to designate the proposed modifications. The renewal permit, known as the "1992 RCRA Part B Renewal Application" was submitted in 1991.

Through the extensive groundwater monitoring program it was found that TCE and PCE had contaminated the subsurface aquifer in the area. Both TCE and PCE have higher densities than water as well as very low water solubility and are thus referred to as dense non-aqueous phase liquids (DNAPLs). The contaminants tend to move downward and come to rest in a pool at the bottom of the aquifer. The DNAPL represents a long term source of water contamination because the material will continue to enter the water phase to replace DNAPL transported away from the site, biodegraded, or removed by some remediation technology (Hazen et al., 1993). Continued migration of this groundwater as it moves toward hydrologic boundaries such as surface streams has resulted in TCE and PCE-contaminated subsurface plumes that can potentially result in contamination of surface soils and water.

Trichloroethylene (TCE) is the most frequently observed volatile organic contaminant at Resource Conservation and Recovery Act (RCRA) sites (Alvarez-Cohen and McCarty, 1991) and in groundwater (Westrick et al. 1984). Methanotrophic bacteria have been isolated and characterized from TCE-contaminated soils (Bowman *et al.* 1993). Fliermans *et al.*, (1988) and others demonstrated that cultures enriched with methane and propane could cometabolically degrade a wide variety of chlorinated aliphatic hydrocarbons including ethylene; 1,2-cis-dichloroethylene (c-DCE); 1,2-trans-dichloroethylene (t-DCE); vinyl chloride

(VC); toluene; phenol and cresol. Wilson and Wilson (1985) demonstrated that TCE is susceptible to cometabolism by soil communities enriched with natural gas. While a number of bacterial types have been isolated that produce methane monooxygenase (MMO) and participate in the degradation of TCE, the numbers of organisms containing the MMO are relatively few (Koh et al., 1993). Soluble MMO (sMMO) is present in selected methanotrophic bacteria and has been found to be responsible for oxidizing a wide range of carbon substrates including TCE and PCE (Cardy et al., 1991).

It is now well recognized that TCE and other chlorinated aliphatic compounds can be degraded by selected methanogens (Bouwer and McCarty, 1984), methanotrophs (Little et al., 1988) and species of *Pseudomonas* (*P. cepacia, P. mendocina* and *P. putida*) (Nelson et al., 1988). Methanotrophic bacteria are fastidious, tedious to culture, and isolation of pure cultures can require several months. TCE degradation rates by methanotrophs can vary significantly depending on the species and culture conditions (DiSpirito et al., 1992).

The basic concept behind intrinsic bioremediation and phytoremediation is to use the ability of naturally occurring microorganisms and plants to degrade contaminants that have been released into the subsurface and at the same time minimize risks to public health and the environment. Vegetation has the ability to degrade TCE present in the associated soils through the metabolic interactions of bacteria in the root zone or rhizosphere. The use of vegetation to actively promote microbial restoration of chemically contaminated soils has been previously demonstrated at the Savannah River Site (SRS) (Walton and Anderson, 1990). A comparison of the environmental fate of ^{14}C labeled TCE in vegetated and non-vegetated soils from a TCE contaminated field indicated increased mineralization ($^{14}CO_2$ production) in soils containing vegetation (Anderson and Walton, 1995).

While soil horizons below 1 meter may not significantly be affected by herbaceous plant rooting, larger plants, and in particular woody plants such as phreatophytes, have

shown to be able to extend their root systems in search of water to significantly deeper horizons. Concern over the possibility that tree roots may reach buried waste is confirmed in a number of studies, among which that of Murphy and Tuckfield (1994) who investigated root penetration at a Savannah River Site.

The role of plant roots in contaminants chemistry and microbial ecology is controversial. On one side, biodegradation of contaminants by free-living soil microorganisms can be stimulated by several mechanisms of plant/soil interaction, including: 1) improvement of the physical and chemical properties of the contaminated soil, 2) an increase in soil microbial activity due to the presence of root exudates, 3) an increase in the contact between microbes associated with the root and the toxic compounds in the contaminated soil, and 4) an increase in rhizosphere microorganism populations as the growing root tips provide new, favorable, growth sites.

Conversely, plant roots can have a detrimental effect on microbial activities that are responsible for contaminants biodegradation and/or sequestration, by competing with microorganisms for essential nutrients such as nitrogen and phosphate, for oxygen, and for water. As previously mentioned, plants can also have a significant role in altering the existing distribution pattern of the contaminants in the soil profile

Water movement and availability in the rhizosphere is a critical factor because most compounds in the soil (nutrients and contaminants) must be in solution to be affected (absorbed, modified, degraded, sequestered, etc.) by either microorganisms or plant roots. Plants take up many times the volume of water needed for metabolism and growth. This additional water is transpired out of the leaves as the final step in nutrient transport and cooling processes in the plant. However, all of this water and the compounds dissolved in it (the soil solution) moves through the rhizosphere, where it is subjected to processing by microorganisms before it enters the root. There is often considerable spatial variation in water availability, emphasizing the importance of coordinated rhizotron and field studies.

Plants need molecular oxygen, and roots, as well as soil organisms, capture energy from the oxidation of organic substances. Large plants have shown to affect the groundwater levels by transpiration (USDA, 1955) so that gas phase diffusion can occur in the soil. Some plants have the ability to enhance oxygen transfer to aerobic microbial communities by transporting oxygen within the plant and releasing it in the subsurface, but frequently plants and microorganisms may compete for the oxygen in the soil. As discussed, a decrease of the oxygen level in the soil has profound effects on soil chemical and physical characteristics. Of the most relevant, organic acids and volatile compounds, such as ethylene, may accumulate to levels that are toxic to plants or influence root growth, and metal mobility is drastically changed. Few models have been generated that analyze the relationship between oxygen diffusion rates, root growth and function (Luxmoor et al, 1970, Jones et al, 1991), but there is a lack of comprehensive, integrated information on root-microorganism interaction in oxygen use and contaminant mobility.

Nutrient uptake by roots include: a) nutrient demand for plant growth, b) operation of metabolically driven uptake and transport mechanisms, and c) functioning of physical and chemical processes in the root environment (Brar and Reynolds, 1996). Item c above includes root exudates and the complex chemical communication between plant roots and rhizosphere microorganisms. One of the primary functions of root exudates is to mobilize inorganic nutrients required by the plant.

Root exudates, these complex root secretions which "feed" the microorganisms by providing carbohydrates, also contain natural chelating agents (citric, acetic, and other organic acids) that make the ions of both nutrients and contaminants more mobile in the soil. Root exudates may also include enzymes, such as nitroreductases, dehalogenases, and laccases. These enzymes have important natural functions, but they may also degrade organic contaminants that contain nitro groups or halogenated compounds (Fliermans et al., 1988). Some rhizosphere microorganisms secrete plant hormones that increase root growth,

and thereby the secretion of root exudates that contain metabolites they use as an energy source.

It is common knowledge that plants can take up significant amounts of nitrogen and phosphate. Competition between roots and microorganisms for both nutrients and contaminants is a definite possibility, depending on the density of roots, numbers of microorganisms, and the diffusion rate and form of the mobilized compound in the restricted rhizosphere soil mass surrounding the roots. Certain species of bacteria form a symbiotic relationship with roots to fix atmospheric nitrogen (Wagner et al., 1995).

Characterization of selected microorganisms in the natural setting is important for the evaluation of bioremediation potential and its effectiveness. This realization has necessitated techniques that are direct, selective, sensitive and easily applicable to soils, sediments, and groundwater that can identify and quantify microbial types *in situ* in real time (Fliermans, et al., 1994). It has been shown with experimental soil columns that TCE and PCE can be biodegraded under aerobic and anaerobic conditions (Enzien et al., 1994). The goal of this project is an assessment of the potential for the intrinsic bioremediation and phytoremediation potential of TCE in soils from the Southern Sector of A/M-Area.

Materials and Methods

Environmental Sampling.

Compliance and research activities have included the evaluation of the nature and extent of residual solvent in the A/M-Area through extensive groundwater monitoring. As a result of that work a surface impact area was identified where a plume of TCE and PCE contaminated groundwater is estimated to emerge as a result of geological/topographical interactions in the Southern Sector in the next 10 years.

Soil in the rhizosphere was sampled at ten sites throughout the predicted impacted area (1 ft. depth) with a stainless steel soil core. Cores included all soil and plant material (leaves, roots, etc.) present at each site. These sites were in wooded areas alongside streams in the estimated impact zone. Water samples were collected from adjacent ponds and streams for chemical analysis where applicable. Dominant plant and soil types were noted at sites of collection. All soil and water samples were placed in a cooler immediately after collection and processed in the laboratory within 2 h. Each sample was mixed and split for microbiological and chemical testing.

TCE Treatability Study

Approximately 10 grams of soil obtained from each of ten sites was placed into twenty 22 mL headspace vials. One set of vials with soil from each site were autoclaved. Five ml of filter sterilized water was added to all vials. The weight of the soil was recorded and then 1 µl of TCE was added to all vials to yield a final concentration of 25 ppm TCE. These samples were immediately crimped with aluminum ring and Teflon® lined septa using a hand crimper. Each vial was then vortexed for one to two minutes. All vials were stored at room temperature in a shaking incubator. Duplicate vials were removed for readings at 2 week intervals.

Analytical Method Description

Volatile organic compounds were measured on a Hewlett-Packard 5890 Series II gas chromatograph (GC) equipped with a Hewlett-Packard #7694 headspace sampler, an electron capture detector, a flame ionization detector, a 60-m, 0.32-id, 3 µm-thick Vocol™ (Cat # 2-4157, Supelco, Bellefonte, PA) column, and a 0.22 mm, 1:1 ratio, column splitter

(Hewlett Packard Model #0101-0594). Column temperature was held at 35°C for 10 minutes then increased to 130°C (7°C/min) and finally to 200°C (4°/min). Helium was used as the carrier gas at a flow rate of 1.04 mL/min. Flow was kept constant using automatic pressure control of the Series II 5890. Samples were equilibrated at 90°C for 15 minutes prior to injection. The headspace of each vial was pressurized at 8 psi prior to injection. Injection into the series II 5890 lasted one minute. The split ratio of sample to vent was about 14.4:1.

Detector response was measured and quantified using a Hewlett-Packard 3365 Series II Chemstation, software package, version A.03.34. A linear fit was used to correlate the concentration amount with detector response. This was done for the electron capture detector and the flame ionization detector.

The calibration data was entered into the Chemstation software package and configured to automatically calculate concentrations. The software was configured to automatically generate a report listing the component name, retention time, concentration in µg/l, area of response, and peak characteristics. In addition a chromatogram was automatically generated with each individual sample run.

Determination of microbial biomass.

Total heterotrophic bacteria were determined using the heterotrophic plate count technique that provides an estimate of the total number of viable aerobic and facultatively anaerobic bacteria in the soils. Low (1%) concentrations of Peptone-Trypticase-Yeast extract-Glucose (PTYG) media were used to indicate bacteria colony forming units (CFUs) (Balkwill, 1989). Samples (1-2 grams) were weighed directly into 15 mL sterile conical centrifuge tubes containing 9 mL of sodium pyrophosphate (NaPP). Subsequent serial dilutions were made in phosphate

buffered saline (PBS). Each dilution (0.1 mL) was inoculated onto a corresponding plate of 1 % formulation of PTYG (Balkwill 1989). The inoculum was evenly spread over duplicate agar plates and incubated at room temperature for 1 week prior to counting.

Methanotrophic bacteria from environmental samples were quantified in soil samples using the MPN enumeration method. Minimal salts media (MSM) (Fogel et al. 1986) were supplemented with 10 % methane 90 % air headspace in Balch tubes sealed with black butyl rubber stoppers. Triplicate tubes were run for each dilution. The first dilution contained 3 g of soil into 20 mL MSM with 3 subsequent 1 to 10 dilutions. Tubes were incubated for 6 weeks at ambient temperature along with a set of 4 control tubes. The concentrations of methane and carbon dioxide in the headspace of control tubes were determined. One half the area of the chromatograms represented the lower limits of methane removal and carbon dioxide production needed to count as a positive tube in the MPNs.

The direct fluorescent antibody (DFA) was also employed to were quantify methanotrophic bacteria in soil samples. One to two grams of soil were added to 5 ml. of NPP, mixed well, sonicated for 30 seconds and vortexed for 30 sec. The mixture was then centrifuged at 3000 rpm for 5 minutes. The supernatant was diluted 1:10 in PBS. Two ml of the diluted supernatant was filtered through a 0.2 micron filter (Nucleopore, Pleasantview, CA) in a vacuum manifold. Ten µl of the DFA were used to flood the filter, the filter apparatus was then covered with aluminum foil and allowed to incubate for 30 min. at room temperature. Following incubation, 20 ml. of PBS were filtered through the filter to remove any unattached DFA (background). The filter was then mounted on a microscope slide using drop of elvanol with another drop of elvanol placed an top of the filter and a cover slip applied (Rodriguez, 1960). Methanotrophic bacteria labeled with the

specific DFA were then counted using an epifluorescent microscope (Axioscope, Carl Zeiss Inc., NY, NY) .

Total bacterial counts were accomplished by the Acridine Orange Direct Count Method (AODC). One to two grams of soil was added to 5 mL NPP, mixed well, sonicated for 30 sec. to separate bacteria from soil particles, and vortexed for 30 sec. The mixture was then centrifuged at 3000 rpms for 5 min. The supernatant was removed and diluted 1:10 with PBS. One mL of this dilution was mixed with 0.5 mL acridine orange for 2 minutes on a 0.2 μm filter (Nucleopore) in a vacuum manifold, and then filtered. The filter was then mounted on a slide on top of 1 drop of immersion oil. A second drop of immersion oil was added on top of the filter and a cover slip applied.

Results and Discussion

Results of Southern Sector Soils TCE Treatability Test for soils from sites 1-10 are summarized in Table 1. Technical difficulties caused exclusion of certain samples from the table as indicated by ND (not determined). Note that samples from sites 1 to 8 decreased from an initial TCE concentration of 25 ppm to $\leq$ 5 ppb within 6 weeks. Bioremediation at sites 1, 2, 4, 6, 7, 8, 9, and 10 by microbial dechlorination was proven by the production of significant quantities of cis-DCE. Vinyl chloride (VC), a daughter product of TCE biodegradation, was produced in all samples in significant quantities either on days 14, 28, or day 42, which is indicative of anaerobic dechlorination. In samples 9 and 10, it is not clear why TCE concentrations were lower after 6 weeks but not significantly reduced as compared to the other soil samples (Table 1). Soil from site 10 demonstrated limited production of DCE and VC (Table 1).

Results of Southern Sector Soils TCE Treatability Test for autoclaved soils from sites 1-10 are summarized in Table 2. Although initially these soil samples were autoclaved as controls it became evident that there was microbial activity and subsequent TCE biodegradation. It is interesting that samples from sites 1 to 8 similarly decreased from an initial TCE concentration of 25 ppm to $\leq$ 5 ppb within 6 weeks. In samples 9 and 10, as in unautoclaved soils, the TCE concentrations were still elevated after week 6 (Table 2). Sites 9 and 10 also showed production of DCE and VC respectively (Table 2).

Table 3 summarizes total heterotrophic bacteria (1% PYTG plates), total counts (AODC), methanotrophic bacteria (DFA) counts, and the most probable number (MPN) of methanotrophic bacteria. Site 7 had the highest density total microbial cells (AODC)/gram dry weight while site 10 was the lowest. Site 6 had the highest total colony forming microbial cells (CFU)/gram dry weight grown on 1% PTYG while site 1 was the lowest. Total methanotrophic microbial cells/gram dry weight counted by DFA were greatest in site 9 and lowest in site 2. The MPN for methanotrophic microbial cells/gram dry weights were found to be highest concentration at Site 1 and lowest at site 6.

From the data shown here, there is not a clear pattern or correlation between significant biodegradation of TCE (Sites 1-8) and limited biodegradation of TCE so far as the microbiological results. Specific bacteria were found in the natural soils in significant numbers for bioremediation of TCE to occur (WSRC, 1996). In this study the DFA technique found the methanotrophic microbial population on the average to be several orders of magnitude greater than the culture based technique (MPN). Results from testing gene probes with TCE contaminated sediments have shown that cultural enrichment procedures commonly underestimate methane-oxidizing bacteria by several orders of magnitude (Brockman et al., 1994). It is possible that there are natural occurring materials (clays, plant products, minerals, etc.) in the soils of sites 9 and 10 which interfered with microbiological biodegradation of TCE. In addition, there could be other bacteria not tested for in this study

(i.e. ammonia oxidizers) that could be significant so far as aerobic biodegradation of TCE is concerned in these soils (Brockman et al., 1994). The results shown here demonstrate the effective use of immunoassays for the purpose of studying select microorganisms in soil samples. Preliminary work with these DFAs indicates they can be used to monitor methanotrophic bacteria in active TCE bioremediation work (Brigmon et al., 1995).

In our experiment, soils and associated plant material were mixed with TCE and water and then tightly capped during the experiment. Because this was an airtight container and VC was formed we believe these systems went anaerobic due to microbial metabolism and associated oxygen demand as previously described (Enzien et al., 1994). Therefore, bacteria used all available oxygen and the population shifted to anaerobic or anoxic metabolism, leaving the DCE and VC as a residual. In the natural soil column it is presumed there would be a combination of aerobic and anaerobic metabolism due to seasonal and environmental effects that would completely eliminate these compounds (Fliermans et al., 1988). These microbial/geochemical interactions have been proven to be a natural process in soils that can be enhanced to increase bioremediation of TCE in the subsurface (Hazen et al., 1993). The rhizosphere samples contained soil as well as plant material from the root zone. An analysis of which plant material in the samples may have stimulated or inhibited biodegradation of TCE was not done.

Plants were identified in the Southern Sector which have shown to facilitate the microbial degradation of TCE in the rhizosphere. These plants include *Fabacae* (Lespedeza), *Pinaceae* (Loblolly pine), *Gramineae* (Bahia grass), and *Compositae* (Goldenrod) (Anderson, et al., 1993). Several other plant samples from the Southern Sector that appear to be the dominant species at those sites were identified include bracken ferns, *Pteridium aquilinum*, from site 4, Japanese grass, *Microstegnum vimineum*, from site 5, and cattails, *Tyhpa Latifolia* from site 7. Methanotrophic

bacteria which can degrade TCE were identified in the rhizosphere of these plants andthe analysis of these samples in continuing.

CONCLUSIONS

The preliminary technology of this treatability test has shown that 1) bacteria capable of degrading TCE are present in most of these soils, 2) in a closed system 99.9% biodegradation of TCE occurred with production of daughter products VC and DCE , 3) the process occurs without added nutrients or bacteria, 4) plants that can bioremediate TCE have been identified growing in the area, 5) that combined with land management practices phytoremediation can represent a significant improvement over conventional baseline technologies used for remediation of chlorinated solvents.

The treatability test showed that bioremediation is linked to changes in TCE concentrations and its by-products. However, the test was based on higher TCE levels (ppm range) than those estimated to impact at the area of concern (ppb range). These results demonstrate the 8 out of 10 select rhizosphere samples from this area can bioremediate any level of TCE to which they could be exposed in this environment.

This work has proven that there exists in the soil at select sites the biology to degrade TCE. This natural or intrinsic bioremediation will certainly reduce the TCE before allowing contaminant breakthrough to surface waters at Tims Branch Creek in the sector. Continued research will help determine the rate at which indigenous microbes in the soil will degrade TCE as the plume moves. Development of an enhanced phytoremediation system incorporating the natural occurring microorganisms for complete degradation of groundwater contaminants could be accomplished with minimum cost and environmental impact.

Several recommendations are made on the basis of this work. First, long term monitoring seasonal influence (Rainfall, Temperature, etc.) on biological activity (species density and diversity) as it will affect bioremediation. Installation of vadose zone piezometers is planned to measure seasonal soil gas changes (i.e. carbon dioxide and methane) which can be indicative of soil biological activity. This specific activity is related to methane analysis as it applies to quantifying the activity level of TCE degrading bacteria in the Southern Sector. Methane could influence bioremediation by stimulating methanotrophic bacteria in sediments and rhizosphere (King, 1996). Recommendations of steps to be taken to optimize phytoremediation in the area were examined and discussed in collaboration with Richard Davalos, Gloria Nielsen, and Don Imm of the U.S. Forest Service Station at SRS. The sites tested here are amenable to operations facilitating bioremediation that may include planting, fertilization, irrigation, forest management, etc. Studies with soil from these sites are being designed to examine the potential for simultaneous aerobic and anaerobic biotransformation processes for PCE and TCE under bulk aerobic conditions (Enzien et al., 1994). Future recommendations for a plan to monitor the zone of influence as the contaminated plume approaches and moves into the zone of biological influence will be evaluated. We recommend the design of a phytoremediation system for complete degradation of groundwater contaminants that can be based on analysis of data from environmental monitoring wells, soil column studies, and future treatability studies to examine soil amendments (i.e. fertilizer additions) and TCE bioremediation for the Southern Sector.

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TABLE 1. Results of Southern Sector Soils trichloroethylene (TCE) Treatability Test. Daughter products include dichloroethylene (DCE) and vinyl chloride (VC).

	TIME 0	DAY 14			DAY 28			DAY 42		
SS sites	TCE (DAY 1)	TCE (DAY 14)	DCE	VC	TCE (DAY 28)	DCE	VC	TCE (DAY 42)	DCE	VC
	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
SS1	24514	5666	3	10	16	1114	18	1	1	56
SS2	24514	9761	4	1	ND*	ND	ND	1	78	103
SS3	24514	14060	3	6	ND	ND	ND	1	8.0	51
SS4	24514	7483	3	1	ND	ND	ND	1	95	143
SS5	24514	ND	ND	ND	1	9	66	ND	ND	ND
SS6	24514	13889	4	1	1	9625	62	ND	ND	ND
SS7	24514	10649	1	5	1	67	208	ND	ND	ND
SS8	24514	6126	3	23	2	255	128	ND	ND	ND
SS9	24514	ND	ND	ND	ND	ND	ND	14928	4	4
SS10	24514	ND	ND	ND	ND	ND	ND	22077	166	127

ND*-Not determined

TABLE 2. Results of autoclaved Southern Sector Soils trichloroethylene (TCE) Treatability Test. Daughter products include dichloroethylene (DCE) and vinyl chloride (VC).

	TIME 0	DAY 14			DAY 28			DAY 42		
SS sites	TCE (DAY 1)	TCE (DAY 14)	DCE	VC	TCE (DAY 28)	DCE	VC	TCE (DAY 42)	DCE	VC
	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
SS1	24514	2584	2	3	2937	1	7	1	3	53
SS2	24514	17859	1	1	8751	2	21	1	1	36
SS3	24514	10106	1	7	ND*	ND	ND	1	7	53
SS4	24514	11568	4	12	ND	ND	ND	1	1	72
SS5	24514	7163	2	9	1	3	72	ND	ND	ND
SS6	24514	17714	4	1	2	5500	141	ND	ND	ND
SS7	24514	9170	3	1	1	1	119	ND	ND	ND
SS8	24514	3593	1	36	1	246	152	ND	ND	ND
SS9	24514	ND	ND	ND	ND	ND	ND	20404	62	4
SS10	24514	ND	ND	ND	10008	2	65	6774	4	6

ND*-Not determined

TABLE 3. Results from enumerating the total colony forming units (CFU) (1% PYTG plates), total counts (AODC), methanotroph DFA counts, and the most probable number (MPN) of viable methanotrophs from all ten sites.

SAMPLE #	DATE	TOTAL BACTERIA CELLS/GDW	1% PTYG CFU/GDW	DFA Methanotrophs /GDW	MPN Methanotrophs /GDW
SITE 1	6/17/96	5.39E+08	5.39E+05	2.28E+05	5.52E+04
SITE 2	6/17/96	7.60E+08	6.83E+05	1.09E+05	8.50E+02
SITE 3	6/17/96	9.57E+08	2.09E+06	2.11E+05	8.44E+01
SITE 4	6/20/96	9.23E+08	2.25E+07	4.27E+05	8.50E+02
SITE 5	6/20/96	5.72E+08	1.10E+07	8.23E+05	1.47E+02
SITE 6	6/20/96	6.24E+08	2.90E+07	3.68E+05	4.60E+00
SITE 7	6/20/96	1.13E+09	1.87E+06	4.56E+05	8.50E+03
SITE 8	6/24/96	8.27E+08	3.03E+06	2.86E+06	2.95E+03
SITE 9	6/24/96	1.11E+09	7.80E+06	3.13E+06	2.93E+01
SITE 10	6/24/96	2.45E+08	5.44E+06	1.83E+06	8.50E+02