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Test Plan for Methanotrophic Bioreactor at Savannah River Site–TNX (U)

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DISCLAIMER

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1.0 Executive Summary

The primary purpose of this project is to demonstrate the feasibility and practicality of operating a methanotrophic mobile trickle filter bioreactor (MMB) unit to effectively reduce or eliminate trichloroethylene (TCE) and associated hydrocarbons from contaminated groundwater. The two-column trickle filter system can process 1.67 gallons per minute (gpm) of contaminated groundwater. During this project, the pilot system will evaluate, optimize, and demonstrate methanotrophic treatment technology (MTT). The mobile system will receive a 1–4% methane to air mixture for stimulating the methanotrophic TCE degrading bacteria, thereby increasing the rates of degradation of these contaminants. This project will also evaluate the efficacy of different bacteria for degrading TCE for use in the system at the laboratory-scale sample groundwater monitoring wells at TNX and set up the system for continued operation. The trickle filter system may be used to inexpensively treat other small-scale organic waste streams at SRS after the initial start-up.

The MTT was demonstrated as an effective and efficient method of degrading TCE in the laboratory and during a field-scale *in situ* demonstration for degrading TCE in a groundwater plume at SRS. The methanotrophic bacteria increase significantly in population numbers and in the production of methane monooxygenase (MMO), an extremely powerful oxidizer. MMO was demonstrated as effective in oxidizing TCE and other recalcitrant compounds in laboratory studies. In the presence of MMO, TCE is oxidized to TCE-epoxide, which breaks down spontaneously into simple, easily degraded, daughter compounds. The system will receive a 1–4% methane to air mixture, which will effectively grow and maintain the methanotrophic bacteria that will degrade TCE.

This demonstration will have broad applications to bioremediating contaminated groundwater systems where *in situ* bioremediation is not practical. Groundwater contamination is widespread in the United States. In locations where *in situ* air stripping and methanotrophic bioremediation are not practical, it may be more practical to pump the groundwater from one or more wells and process it using an onsite MMB.

Onsite bioremediation offers the following benefits of *in situ* bioremediation: onsite terminal destruction without the added costs and risks associated with transporting and storing at another site and it is an environmentally sound, socially, and politically acceptable mechanism for destroying toxic chemicals that is cost-effective and safer than many alternative methods. The MTT offers additional bioremediation advantages of more rapid and complete degradation with less toxic by-products, and it is a simple, easy to use, and cost-effective technology.

The mobile system is the next stage in a series of bioremediation demonstrations being conducted at SRS by the Biotechnology Group. Development of MTT occurred in conjunction with a consortium of engineers and scientists from Radian, Envirex, Michigan Biotechnology Institute, Vanderbilt, Oak Ridge National Laboratory, Cornell Universities, and the Gas Research Institute (GRI). GRI, the Department of Energy, Bechtel Savannah River, Inc., the Savannah River Technology Center, Westinghouse Savannah River Company, and the Environmental Remediation Division provided funding, logistical and technical support. The demonstration of the mobile system will occur at the SRS TNX Area (funded by the Environmental Restoration Division).

2.0 Test Objectives

The following list outlines principal test objectives:

- demonstrating the utility of onsite methanotrophic bioremediation for cleaning up groundwater sites contaminated with chlorinated solvents
- determining the rates of degradation of trichloroethylene (TCE) and associated contaminants under different concentrations of contaminants and methane enrichment, thereby optimizing the standard operation of the mobile bioreactor unit
- measuring the responses of microbial populations to the different regimens imposed on the bioreactors
- determining the effects of long-term constant and interrupted exposure to contaminants on the degradation rates and microbial populations
- demonstrating the feasibility, both economically and environmentally, of the mobile bioreactor for alternative applications onsite

Ancillary objectives include:

- determining the feasibility of using additional energy sources to stimulate and enhance degradation rates
- determining factors that influence and regulate microbial populations and their functional capabilities (degradation rates) under different test conditions
- demonstrating the utility of immunological probes (fluorescent antibodies) and other direct analysis/assays for characterization, monitoring, and controlling the biological aspects of Methanotrophic Treatment Technology (MTT) bioremediation
- establishing an explanatory and deterministic process model of the mobile bioremediation process via process optimization studies

2.1 Benefits

Success in this demonstration has potential benefits for the gas industry and government and private sites challenged with the need to contain plumes of pollutants while implementing *in situ* remedial efforts. Other benefits include establishing a working mobile bioreaction system that could cut the cost of remediating other contaminated sites and reducing future liabilities and costs of managing organic wastes at SRS.

3.0 Background, Technology Review, and Process and Site Descriptions

3.1 Technology Background

Biodegradation of TCE by methanotrophs (methane-oxidizing bacteria) was demonstrated in microbiological studies and 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 Oak Ridge National Laboratory (ORNL) isolated a mixed methanotrophic culture from a TCE-contaminated well on the Oak Ridge Reservation. This culture was subsequently used in a prototype laboratory-scale continuous flow bioreactor at ORNL (Donaldson et al. 1988). At SRTC, Fliermans et al. (1988) isolated consortia and species capable of aerobic degradation of TCE with methane as the primary nutrient from TCE-contaminated soil and groundwater from SRS. These organisms were also successfully used in laboratory-scale fluidized bed bioreactors to treat TCE/PCE-contaminated groundwater (Phelps et al. 1990).

Although field demonstrations of *in situ* bioremediation of chlorinated solvents are few (Semprini et al. 1990; Nelson et al. 1990; Hazen et al. 1994), the biodegradation of TCE and PCE has been demonstrated in pure cultures, (Fliermans et al. 1988), mixed cultures (Fliermans et al. 1988; Little et al. 1988), microcosms (Donaldson et al. 1988), soil columns (Wilson and Wilson 1985; Wilson et al. 1986), and laboratory bioreactors (Phelps et al. 1990; Jewell et al. 1991). A MMB has been used for degrading BTEX compounds (Wu et al 1992). Aerobic degradation of TCE was stimulated by adding 1–4% methane (Hazen et al. 1994; Jewell 1991; Enzien et al. 1993) although concentrations of methane above 3–5 mg/L (Jewell et al. 1991) also inhibited some microbial degradation efforts.

Methane to air mixtures stimulated indigenous microbial communities and consortia designed to degrade TCE. Such a mixture was effective in the *in situ* demonstration conducted at SRS (Hazen et al. 1994). Methane is a readily available, low-cost gas in abundant supply in the United States and internationally. It may be the most cost-effective carbon/energy stimulant for TCE degradation.

Methanotrophs, methane-oxidizing bacteria, oxidize methane via a series of enzymes unique to this group. The primary enzyme in this oxidation chain is methane monooxygenase (MMO). MMO is an extremely powerful oxidizer, thus giving it the capability to oxidize many 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 MMO, Type I, induces the formation of TCE-epoxide from TCE. TCE-epoxide is extremely unstable, therefore spontaneously breaks down to simpler compounds like formate, etc. The daughter compounds are either unstable or small and easily metabolized 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, it achieves no measurable benefit from the reaction, making it a fortuitous metabolism or, as some investigators prefer, co-metabolism/co-oxidation.

In addition to the laboratory bioreactor studies at ORNL, UT, and elsewhere, Batelle-Columbus operated one pilot-scale bioreactor system 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 study demonstrated that actual TCE-contaminated groundwater can be treated in a trickle-bed bioreactor. ORNL provided the culture used in this test. Tyndall Air Force Base is continuing to support development of TCE bioreactor technology at ORNL, UT, and SRS. The bioreactors used at Tinker Air Force Base are being provided by the Air Force for further field tests at ORNL and SRS.

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. A large-scale demonstration of *in situ* degradation of TCE at SRS clearly demonstrated the feasibility of MTT in bioremediating groundwater (Hazen et al. 1994).

Methane is 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 used safely with an extremely low probability of adverse environmental effects.

Fliermans et al. (1994) demonstrated the importance of selective microbial monitoring tools to characterize bacterial communities during *in situ* bioremediation activities. The size of bacterial populations changed according to treatment, whether air, 1% CH_4, 4% CH_4, or pulsed CH_4 additions. Further, the responses of the populations in each of the 12 monitoring wells, when inoculated into Biolog plates, showed clear differences among the wells. Specifically, fluorescent antibody methods and Biolog techniques were valuable tools for autecological and synecological studies of groundwater microbial populations. Interestingly, the bacteria expected to proliferate in the TCE-contaminated system (SRL-MIFF) did not show a change in population numbers in response to perturbation of the system. Similar methods will also be employed in this test.

Enzien et al. (1993) observed degradation rates of >75% of TCE and PCE in a soil column that received TCE and PCE in concentrations of 500 µg/L in a methanol-based solution. Aerobic and anaerobic sites were present in the column and the most significant reduction of the TCE and PCE occurred from days 338 to 436. Bacterial numbers were determined using MPN and methanotrophic populations did not exceed 100 MPN/gdw. Air/methane mixtures were demonstrated to stimulate key members of the indigenous and selected consortia microbial communities that can degrade TCE. An extensive characterization and monitoring program using accepted methods will be

used to measure the response of the bacteria on the ceramic substrate and in the water after injecting air/methane into the bioreactor. In addition, offgas from the bioreactor will be assayed for methane, total volatile organic compounds (VOC), TCE, PCE, and potential break down products of TCE/PCE (e.g., CIS-and trans-dichloroethylene (DCE), vinyl chloride (VC), and carbon dioxide). Data from previous bioreactor demonstrations and laboratory experiments will be used to provide baseline hydrological, chemical, and biological characteristics, in effect, providing control experiments for the bioremediation demonstration. This bioremediation technology offers an environmentally sound, onsite method of terminal destruction of TCE.

3.2 Process Summary

The GRI, ORNL, and the Environmental Sciences Section (ESS) of SRTC combined forces in researching and developing methanotrophic bioreactors. These bioreactors have a potential use in remediating groundwater contaminated with TCE and PCE. ESS worked with ORNL to design and construct a trickling filter bioreactor unit, similar in design to the Air Force Bioreactor Project #JK659 (South Carolina Department of Health and Environmental Control (SCDHEC) Operating Permit #17,434-IW) operating at TNX. The new reactor is a mobile experimental treatment unit that can move to various sites for testing. Each test will check the durability and the flexibility of the bioreactor system. These tests provide baseline data for methanotrophic destruction of VOCs (TCE and PCE). The mobile trickle flow bioreactor unit will be used to handle simulated feed and actual groundwater from existing groundwater monitoring wells at SRS. The goal of the investigation is to demonstrate the feasibility of biological remediation of TCE- and PCE-contaminated groundwater at SRS. This unit and its performance will be used as a basis for comparing the fluidized bed bioreactor design and trickling filter bioreactor design. Once this comparison is made, design and construction of a full-scale system may be investigated.

The mobile bioreactor system is an improvement on the Air Force system tested previously. Improvements to the packing material, liquid distribution, methane feed control, and automation of the system, make this system more efficient, and easy to operate and control.

This unit may also be used to test the ability of the system to degrade various organic wastes generated at SRS. The unit would serve as a future test system to prove the capability of treating waste organic streams in a biological system. SCDHEC's approval will be obtained prior to introducing new pollutants and before using the unit in this expanded capacity.

Contamination of groundwater aquifers by organic chemicals is a major national problem. Currently, groundwater is pumped to the surface where air stripping and carbon adsorption technologies are proven methods for removing organics from the water. However, these techniques simply move the contaminant from one phase to another; the organics remain and a disposal problem still exists. Photolytic and chemical destruction methods are under development, but are not yet proven. Many microorganisms can break down organic chemicals by way of metabolic degradation to carbon dioxide, water, and simple inorganic acids, bases, and salts. Aerobic

biodegradation requires a primary carbon source and oxygen. The organic concentrations in groundwater are often too low to support microbial growth. Methanotrophic microorganisms can degrade halogenated organics such as TCE and PCE in the presence of methane (the primary carbon source) and oxygen. TCE degradation was demonstrated in the laboratory by several investigators, including groups at ORNL and Stanford University.

3.3 Process Description

The mobile trickle flow bioreactor system is housed on a portable trailer unit and can treat a maximum of 100 gph of contaminated water from a monitoring well. The unit primarily consists of two bioreactor columns, an air/methane blending unit, a nutrient feed system, a portable diesel generator, and an effluent hold tank.

The system is operated by passing a water stream through the two bioreactor vessels. The influent stream, predominately contaminated with TCE, mixes with nutrients (<100 ppm phosphate and ammonium nitrate) and/or buffer and titrate solutions to control pH. The nutrient and pH control is provided by the nutrient feed system before entering the first column. Nutrients are required by the microbes for growth. Once the water stream enters the column, it trickles over the biofilm-covered ceramic packing material (Koch Flexeramic® Type 48), while methane and air are being pumped concurrently into the column by the air/methane blending unit. The halogenated organics (TCE) are degraded while trickling over the biofilm. Once the water moves through the packing material in the first column, it is pumped to the second column and recycled or pumped out of the system to a hold tank. Recycling may be necessary when operating the system to control the residence time of the groundwater due to changing process kinetics caused by different influent streams. Finally, the water is pumped from the holding tank to a transfer truck for final disposal at the M-Area air stripper (SCDHEC Operating Permit #10253). The MMB is powered by the diesel generator.

The MMB consists of two columns connected in a series. The columns are constructed of stainless steel with borosilicate glass viewing and sampling areas. Each column is filled with four feet of waffled ceramic packing material, Koch Flexeramic® Type 48, which provides a growing surface for microorganisms. This packing material will increase residence time and the gas/liquid distribution and interactions in the system. This is an improvement on the Air Force Bioreactor, Project #JK659 (SCDHEC Operating Permit #17,434-IW). The system is controlled by four Anafaze™ units that manipulate the system and are connected to data acquisition and control software on a portable computer. The proportional-integral-derivative controllers are connected to actuated valves, flowmeters, level meters, pumps, and an air to methane blending unit. The air to methane blending system has an infrared detector connected to an audible alarm that triggers and automatic shutdown if the methane concentrations exceed the 5.4% combustible limit. The maximum air to methane flow is 0.75 standard cubic feet per minute. The gas is supplied from compressed gas cylinders on the trailer or a

process air line, if available. The system also includes devices to measure bulk water parameters, including pH, and dissolved oxygen. This data is automatically acquired using the Anafaze control system. The nutrient delivery system delivers concentrated nutrient media to the system at a flow of 150–300 ml/hr.

A mixture of different methane utilizing and TCE degrading bacteria will be grown in bulk for inoculation of the system Once a cell density of greater than 1×10^8 per milliliter. Once the inoculum has reached this density the mixture will be poured into the system and allowed to recycle for a period of 24–48 hours in a 3–4% methane environment. The 24–48 hour period allows the bacteria to stick to the ceramic matrix. Nutrients will then be added as required to make up for evaporative loses and utilization by the microorganisms as required until a visible active biofilm is formed. This process is expected to take one to two weeks.

When using actual groundwater, the influent will be pumped directly from existing wells in TNX areas through the MMB. The groundwater will be fed into the system by connecting the process water line from the inlet to the columns and pumping directly from the well to the columns. The treated groundwater will then be pumped to a hold tank before final disposal at the M-Area air stripper (Permit #10253). The water will be pumped from a hold tank to the purgewater collection truck for transport to M-Area. TCE concentrations during testing should not exceed 4000 µg/l.

When using actual groundwater, the system will operate around the clock for two- to three-week campaigns. Various campaigns will test the system's ability to treat diverse types of groundwater. The pH of the system will remain between 6 and 8. (The optimal pH for methanotrophic bacteria is 7.) The pH of the system will be automatically controlled using a buffer solution or a titrant such as sodium hydroxide. These solutions will be introduced to the system using the nutrient feed system, consisting of a 50-gallon stainless steel tank and a positive displacement pump. If necessary, hydrogen peroxide, pure oxygen, or a solid phase peroxide may be used to increase the oxygen content of the system. The results from these campaigns will provide an accurate baseline for methanotrophic destruction of VOCs (TCE and PCE). (Section 5 discusses waste disposal and spill response procedures.) The control system will be configured to shutdown the transfer pumps if there is a loss of influent flow from a well.

3.4 Technical Need

Organic xenobiotic chemical contamination of groundwater has become the most important pollution problem for industrialized nations. 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 impacting groundwater occurs almost daily; thus, the extent of the problem is undoubtedly greater than 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 (or remediate) contaminated groundwater. The major organic contaminates of waste sites at DOE facilities are also chlorinated solvents.

3.5 Alternatives

The principal existing technology for remediating trichloroethylene (TCE) contaminated groundwater is pumping followed by air stripping or granular activated carbon. The TCE is either discharged to the atmosphere or captured on another material. Neither of these are TCE destruction technologies. Subsequent disposal of activated carbon is necessary. At SRS, no air emission restrictions are in force and air stripping is being used. However, the lack of emission restrictions is not usual and may change in the near future.

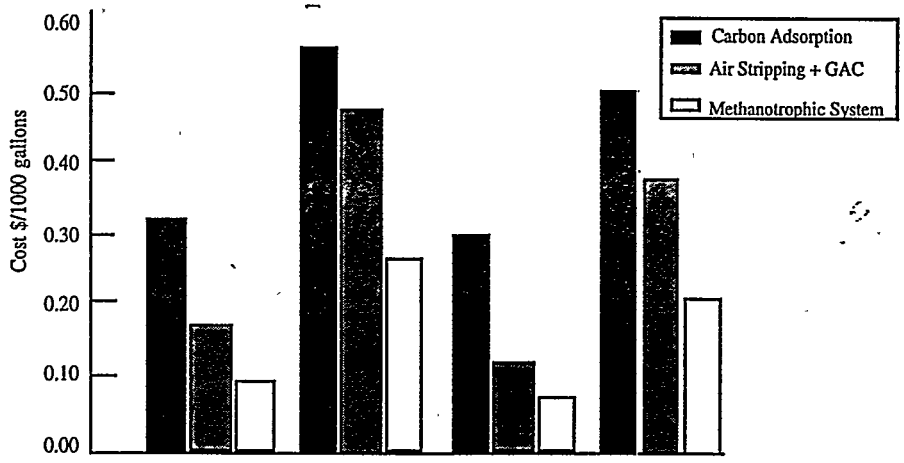

Figure 1. Cost Comparison of Alternative Technologies

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

3.6 Benefits

Successful development and use of the methanotrophic treatment technology (MTT) and MMB benefits SRS and other governmental and gas industry sites challenged with TCE plumes. Benefit considerations are listed below.

- MTT offers the practical use of a powerful oxidase enzyme system which, when combined with ancillary biological and chemical treatment processes, has the potential of reducing the cost of remediating contaminated sites.
- Future liabilities and costs associated with managing wastes will be reduced.
    - In removing chlorinated hydrocarbon pollutants from groundwater, a successfully developed MTT is expected to reduce

remediation costs by more than 50%, compared to conventional technologies of activated carbon and air stripping in treating groundwater.

- Transportation costs are minimal.
- A significant new high-form use for natural gas will result because MTT represents a potentially important new market opportunity for the gas industry, conservatively estimated at 600 bcf/yr (Radian 1989).
- Onsite MTT bioremediation technology is based on biological destruction of the contaminants. Therefore, risks normally associated with handling, transporting, and treating or storing contaminated residuals are avoided. In this sense, there is a significant reduction of risk.
- Costs for MMB 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 to air stripping combined with carbon adsorption of the air stream and direct carbon adsorption from the water suggested that the methanotrophic system would save 40–60% over conventional technologies for several TCE concentrations and flow rates (Radian 1989). We expect that these observations will also hold true for field-scale MMB bioremediation of TCE alone or combined with anaerobic bioreactors for destroying tetrachloroethylene (PCE).

3.7 Acceptability

Bioremediation technologies enjoy relatively high regulatory acceptability in cases where the technology has been effective. Regulatory agencies are also showing interest in adding specialized microbial cultures to the site. California granted permits for demonstration projects that inject nutrients and TCE-degrading bacteria into a contaminated aquifer. California, Texas, and Michigan 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. Above-ground bioreactors are self-contained units with effluents being recycled or passed through final granulated activated carbon filters to remove traces of toxic contaminants. Therefore, the planned MMB offers little or no possibility for above-ground contamination of surface waters or soils under normal operating conditions.

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

3.8 Site Description

SRS is a 300-square mile facility owned by the U.S. Department of Energy (DOE) (operated under contract DE-AC09-89R180035) by Westinghouse Savannah River Company. SRS is near Aiken, South Carolina and has operated as a nuclear production

facility for DOE since 1950. The production processes carried out over the past 40 years generated considerable waste and waste sites. This waste includes radiological waste, heavy metals, organic solvents, sanitary landfills, and other types of mixed wastes. Many contaminated environments at SRS have been identified, including surface water and soils, subsurface sediment, and groundwater. Cleanup has become a top priority for DOE. Due to the large number of waste sites and the large volume of contaminants, 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, time, environmental impacts, and/or result in a cleaner end-point.

3.9 TNX Site Description

The TNX Area was selected for the MMB demonstration site for the following reasons:

- It is easily accessible and readily convenient to the Biotechnology laboratory.
- It is contaminated with the target TCE and associated toxins.
- Data are available on the history of the site and the target contaminants.
- Wells are available to provide contaminated water directly from the site to the MMB.

Detailed information regarding the hydrogeologic framework at TNX is available (Nichols 1993). The groundwater monitoring system at TNX contains 51 wells that monitor six aquifers. Most of the monitoring wells were drilled using mud rotary techniques and constructed of four-inch, schedule 40 PVC casing and slotted screens. The 12 piezometers in the flood plain are constructed of two-inch, 316 stainless steel casing and wire wrapped screens with PVC risers. One-half horse-power Grundfos submersible pumps were installed in the wells and are sampled quarterly. Groundwater samples are collected and analyzed from 46 of the wells on a quarterly basis. The project will sample four of the remaining five wells at TNX. These wells have not been sampled because the purge water requires containerization.

In 1980, the first series of groundwater monitoring wells was installed in the TNX Area. This series was determined inadequate and the wells were abandoned and replaced in 1984. The groundwater sampling data from the new wells indicated that seepage from the unlined basins, leakage from the process sewers, and leachate from other waste disposal activities in the area resulted in various degrees of soil and groundwater contamination throughout the area. Analysis of surface water samples collected from the swamp adjacent to the Savannah River indicate that groundwater contaminated with volatile organic compounds (VOC) is outcropping in the swamp before it reaches the river. An Environmental Impact Statement addressing groundwater contamination caused by SRS operations was submitted for public comment in 1987.

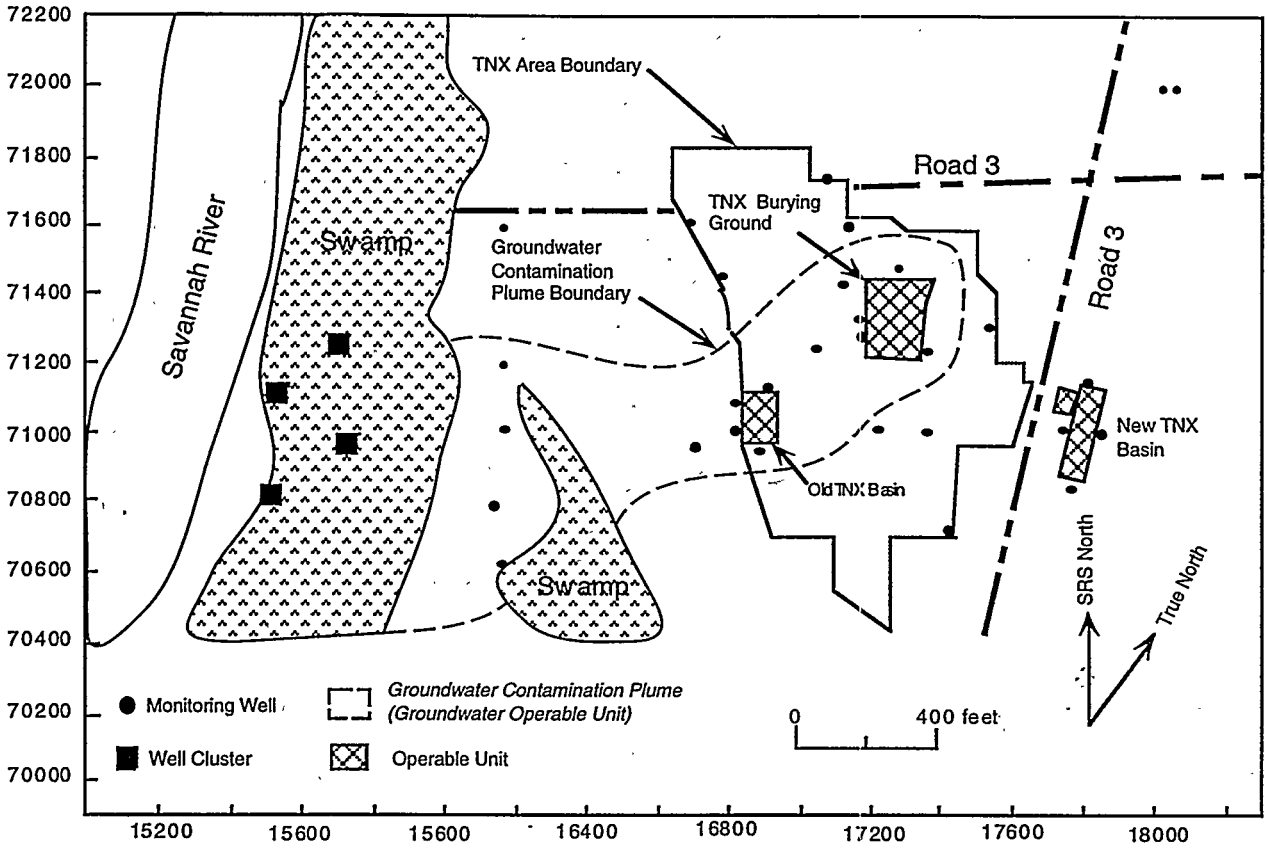

Figure 2. Wells and Boreholes at TNX

The water table aquifer at TNX is contaminated with chlorinated VOCs, primarily trichloroethylene (TCE), tetrachloroethylene (PCE), and carbon tetrachloride, and to a lesser extent, gross alpha, mercury, and nitrate(Simmoms 1985). The VOC contamination underlays eight acres, has a maximum thickness of 20 feet and contains approximately six gallons of TCE. Gross alpha and mercury were detected above the Primary Drinking Water Standards (PDWS) in one of the monitoring wells. Mercury and gross alpha do not pose a threat in the down gradient portion of the plume and, as a result the interim remedial action will focus on the recovery and treatment of VOC-contaminated groundwater.

Groundwater contaminated with VOCs is migrating from the TNX Area into the adjacent swamp. The strategy for remediating the contaminated groundwater is to prevent further migration of the contamination in the short-term and to remove the source of VOCs in the long-term. The proposed MMB action may help reduce the migration of contaminated groundwater into the swamp. Specifically, this treatment will address the long-term goal of destroying the VOCs through bioremediation.

4.0 Test Plan

4.1 Criteria for Success

The overall success of this demonstration will be evaluated by the following primary criteria:

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 from a comparison of the influent and effluent waters before and after adding methane to the MMB to stimulate biodegradation. The evidence of biodegradation for this test will consist of a mass balance on the liquid and gas streams and a mass balance on the chloride species.
2. *Reduced cost over comparable conventional technologies (comparison of costs of air stripping and activated carbon adsorption at SRS.).* The costs of a large-scale system will be extrapolated using the current costing in industry.
3. *Relatively simple and trouble-free operation.* These characteristics contribute to favorable economics. A critical assumption for a successful demonstration is that gases can be successfully injected into the MMB and will stimulate the degradation of the TCE by the indigenous and consortia microorganisms. This ability has been demonstrated in laboratory bioreactor experiments and is expected to work equally well with field-scale bioreactors. The wealth of data from *in situ* demonstrations can be compared and used as a control for the MMB bioremediation project.

4.2 General Comments

Sampling times and frequencies must depend on the parameter, the organism, or the analytical procedure to be tested. Additional factors, such as the operational protocol, length of time the operation has proceeded successfully, dramatic changes in activity, and catastrophic and other unforeseen events, will have a direct impact on sample frequency once each operation is in progress.

In general, sampling will be more frequent during the early stages of the operation as the microbial populations increase, become established, and key organisms compete for space and substrates; during this interval of log growth, changes in TCE degradation may also be dramatic. When the bioreactor community is well established and optimal maximum degradation rates of TCE and other toxics are achieved, the sampling frequency may be decreased, thus the frequency only needs to be changed in response to operational changes or changes in the function of the reactor.

In each event, technical services are limited to a basic, five-day, 40 hour-work week, thereby restricting sampling times to daylight-weekday periods. Further, the number of demonstration projects in progress and the number of technicians available have a direct effect on the sampling frequency. Sampling and processing samples for many parameters are labor-intensive and time-consuming, therefore sampling frequency will be limited by time and technician services available.

The following key points were gathered from literature regarding MMB degradation of TCE, PCE, BTEX, etc. during bioremediation studies:

- Total degradation of TCE is more likely using mixed cultures.
- Indigenous cultures appear to be more likely to succeed.

Low levels of methane (1% or less) are more likely to stimulate growth and degradation of TCE than concentrations greater than 1%. Concentrations of 3–5% are inhibitory to degradation, but stimulate bacterial growth. Optimal pH ranges from 6.4 to 6.8, but good degradation is recorded at greater than 6.0 pH. A pH less than 5.5 caused a crash in a small bioreactor (anaerobic). The bioreactor did not recover within a 43-day period. Key nutrients, including nitrogen and phosphate sources, may have to be added to stimulate growth and degradation.

4.3 Optimal Operating Conditions

The MMB system will connect to TBG-4 a monitoring well at TNX and treat chlorinated compounds in the liquid stream. The system will pump the liquid from the ground past a nutrient injector system into two columns connected in a series, or operated independently. Samples and measurements of the stream inside the columns will be taken. The system measurements include: pH, dissolved oxygen, temperature, solvent levels, and microbial and air samples. (The ranges for these measurements are shown in Table 1.) Once the stream moves through the column, it will directly discharge to the effluent hold tank or recycle with the influent stream. The recycle ratio is determined by the ratio of the recycle stream to the amount of groundwater entering the system. The total flow through the system, including the recycle stream, is limited to 6.31 liters per minute.

For optimal operation, the system will operate at no recycle. However, the goal of the project is to determine the rates of degradation and to have no chlorinated solvents in the effluent. We will test the affect of different groundwater on the ability of the system to degrade the intended compounds.

Table 1. Optimal Operating Conditions

System Parameter	Optimal Value	Low	High	Units
General Conditions				
pH	7	6.5	7.5	-log[h+]+]
Temperature	28	16	32	Celsius
Dissolved Oxygen	7.5	2	9	mg/l
Gas and Liquid Flow Rates				
Nutrient Flow Rate	200	0	200	ml/min
Liquid Flow Rate	3	2	3.8	lpm
Space Time	60	45	90	minutes
Recycle Ratio		1	5	Qr/Q
Organic Levels				
TCE Influent	4,000	500	10,000	µg/l
TCE Effluent	5	0	100	µg/l
PCE Influent	100	0	1,000	µg/l
PCE Effluent	5	0	1,000	µg/l
Nutrient Tank Concentrations				
Nitrate	7500	300	40,500	mg/l
Phosphate	7500	300	40,500	mg/l
Sulfate	7500	300	40,500	mg/l
Nutrient Levels in System				
Nitrate	250	1	1500	mg/l as $NO3^-$
Phosphate	250	1	1500	mg/l as $PO4^-$
Sulfate	250	1	1500	mg/l as SO4
Methane Levels				
Influent Methane levels	1–3	1	4	vol %
Effluent Methane levels	0	0	4	vol %

4.3.1 Dissolved Oxygen

Description: The dissolved oxygen readings in the liquid phase should be saturated. The amount of oxygen that dissolves in water is a function of temperature and pressure. Typical saturated values for temperatures ranging from 10 to 35°C and atmospheric pressure are 6.5–7.8 mg/l.

Measurement: The dissolved oxygen levels are measured using an online-Rosemount probe. The level can be viewed on the control panel or the computer display.

Correction: To correct a low dissolved oxygen reading, the inlet flow rate of the air/methane mixture must be increased. If this does not work, adjustments in the inlet liquid flow rate may be needed. Because of the design of the system, low dissolved oxygen readings should not develop. (Before making adjustments, the calibration of the probe or the dissolved oxygen levels must be checked with a portable meter.)

4.3.2 Gas and Liquid Flows

Description: The nutrient delivery system is used to control the pH and provide nutrients for bacterial biofilm growth and maintain cellular activity.

Measurement: Nutrient flow rate of the system is controlled at the nutrient feed pump by the operator. The pH is also operator controlled. The flow rate of the delivery system is measured by an online-flowmeter and controlled by the control panel using a control valve. The maximum flow rate of the system is 300 ml/min. The minimum accurate and dependable flow rate is 150 ml/min.

Correction: Correcting the concentration of nutrients of the pH in the tank is operator-intensive. Corrections may also be done by changing the flow rate of the system at the control panel.

4.3.3 Influent Flow Rate

Description: The influent flow rate is the amount of water treated on a per unit time basis. The maximum amount of water the pump is rated for pumping is 6.3 L/hr.

Measurement: The influent flow rate is measured online by a flowmeter, this flowmeter is connected with a control loop that adjusts the flow rate to match the set point established by the control and set by the operating engineer or operator.

Correction: To correct the influent flow rate, either the set point must be adjusted on the control panel or the calibrate of the flow rate must be calibrated and checked. The inlet flow rate may also be adjusted by changing the flow rate of the well pump.

4.3.4 Hydraulic Retention Time

Description: The space-time, or residence-time, is the hydraulic retention time for a liquid that experiences no hold-up due to chemical or physical interactions. It is equal to the amount of time that an average molecule of water spends inside the system. The space-time is affected by the amount of biofilm, channeling, and flow rate.

Measurement: The space-time is usually measured using tracer tests. These tests add a non-reactive tracer to the inlet stream and then measure the tracer as it exits the unit or along the reactor's path. The data can give information on the space-time distribution and may indicate a flow problem within the system.

Correction: If the space-time in the system decreases, the recycle rate may be increased or the packing material may be cleaned using chemical or physical methods.

4.3.5 Recycle Ratio

Description: The recycle ratio is the amount of recycled liquid divided by the amount of liquid entering the system.

Measurement: Recycle and influent flows can be measured using flowmeters on the mobile bioreactor control panel. The recycle ratio is set on the control panel and controlled using a electronic valve and a feedback control loop. Flow rate measurements on the effluent, influent, and recycle line are compared to set points established on the control unit and the control valve adjusts to match those set points.

Correction: The recycle ratio may be corrected by changing the set point and or the tolerance on the control panel. If this does not fix the recycle ratio, the control valves and flowmeters may require calibration.

4.3.6 Methane Levels

Description The concentration of methane in the system determined weather the bacterial system feeds on methane or degrade TCE. Changing and varying the methane inlet concentration is an important operational variable.

Measurement Inlet methane levels are measured by the air methane blending unit. The unit is accurately calibrated to read concentration's from 0–5% vol/vol. Outlet concentrations from the system will also be monitored using a CES Landfill portable gas analyzer.

Correction The flow rate and methane concentration may be adjusted at the control panel.

4.4 Microbial Monitoring

4.4.1 Acridine Orange Direct Counts

Acridine Orange Direct Counts (AODC) will provide a direct estimate of the total number of bacteria in the environment, regardless of the ability to grow on any media that might be used. Different sample volumes will be used to get an accurate AODC count when filtering is used. However, with high bacterial concentrations expected in the MMB toxoplasmosis will predominantly be used. Ten microliters of supernatant are spotted onto each well of a toxoplasmosis microscope slide (Celline, Inc.), stained two minutes with 0.01% acridine orange (Difco of 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). Counts are reported as cells per milliliter.

4.4.2 Aerobic Heterotrophic Plate Count

This method will provide an estimate of the total number of viable aerobic and facultative 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 with 0.1% cycloheximide to inhibit fungal growth will be used (Balkwill 1989). Plates are incubated at room temperature (25°C) for at least one day prior to counting. Bacterial colonies are counted with the aid of low power magnification. The counts are then used to calculate a bacterial density in colony-forming units per gram.

4.4.3 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 may be indicated from microbial activity, increased biomass (particularly biomass that contains TCE degrading machinery), and increased biomass capable of consuming methane as evidence of stimulation by treatments. The most probable number enumeration techniques will be used to enumerate methanotrophic microorganisms from the effluent and packing material. 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 4 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 four to six weeks, depending upon initial results from control tubes. A set of four to five 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. Again, the results from these samples will not be available until after the test is complete, but it will provide useful information when coupled with fluorescent antibody data and the other microbial monitors.

4.4.4 Community Diversity and Functionality

Changes in relative community structure may be important in determining the following:

- the overall stability of the biological community
- the potential for producing unwanted effects
- 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 calculating diversity indices (Shannon) and measuring structural diversity. Representatives of these isolates are grown in pure culture and frozen for future biochemical studies and measuring functional diversity. Biochemical/physiological traits will be catalogued by inoculating pure cultures of bacteria into a 96-well microliter screening plate (MT and GN type, Biolog, Inc.). Similarity and cluster analyses will be used to compare groups of random isolates over time within and between sampled wells.

4.4.5 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 for the AODC described above). Samples fixed on slides and blocked for non-specific adsorption are then stained by incubation with fluorescein isothiocyanate-labeled antibodies (specific for a particular bacteria, e.g., TCE-degrading bacteria isolated from M-Area sediment) for 20 minutes, 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 several methanotrophs are under development. Samples will be held for analysis with these antibodies when they become available. This will allow monitoring of the methanotrophic populations of the bacteria over time in the system. For details on preparing antibodies and staining techniques see Fliermans et al. (1974) and Bohlool and Schmidt (1980).

4.4.6 Substrate (Ceramic Packing) Monitoring

The ceramic substrate in the mobile bioreactor system will be analyzed weekly to determine the biofilm microbial composition and health.

The collection methods are designed to minimize microbial contamination of the sample populations. Samples will be collected using sterile techniques to obtain a representative sample of the biofilm.

4.5 Analysis of Volatile Organic Compounds

TCE, PCE, methane (CH_4), and all of the potential daughter products [c-DCE, t-DCE, vinyl chloride (VC), and CO_2] will be measured. Volatile organic compound (VOC) analyses will be performed on a Hewlett-Packard 5890 gas chromatograph with an electron capture and flame ionization detector, a Hewlett-Packard 19395A Headspace Sampler, a Hewlett-Packard 3392A Networking Integrator, a computer-controlled data control and acquisition via Chemstation software, and a 60 m × 0.75 mm ID Supelco VOCOL wide bore capillary column coated with a 1.5 mm 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 (EPA Method 524.2; Sims et al. 1991). Total inorganic carbon will be measured in groundwater by acidifying samples in a serum bottle with a crimp-sealed septa. Thirty milliliters of groundwater will be added to an amber serum bottle, capped and crimped in the field, and held on ice until analyzed. One milliliter of concentrated HCl will be added to serum bottles with a syringe allowed to equilibrate and then 2.5 ml of headspace will be injected onto a gas chromatograph with a thermal conductivity detector. Standards will be made with sodium bicarbonate solutions (EPA Method 524.2).

4.6 Physical and Chemical Analysis

The physical and chemical nature of the environment is critical to understanding biological phenomena (e.g., degradation rates). In addition, some of these parameters have implications on nutrient requirements (phosphorous, nitrogen, sulfur, and iron)—effects that the biomass may be having on the environments total organic compound (TOC) (e.g., pH, conductivity). These measurements could be critical to the potential for controlling degradation rates, destruction efficiency, and adverse phenomena. All methods will be EPA approved and/or in Standard Methods (APHA 1989). TOC will be determined by the ultraviolet oxidation method (EPA 415.1). Samples will acidified and stored at 4°C prior to analysis. Chloride, phosphate, nitrate, nitrite, and sulfate will be determined by the ion chromatography method (EPA 300.0; APHA 4100).

4.7 Offgas Monitoring

Offgas from the mobile bioreactor will be collected and analyzed daily [up to ten samples 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.

WSRC personnel will collect and analyze other gases that must be analyzed from each mobile bioreactor. The procedure to be used is as described previously by Looney et al. (1991). Samples are collected using a 50 ml disposable syringe and then placed in 30 ml pre-evacuated serum vials. Contents of these vials are analyzed using a mass spectrometer modified to sample the serum vials at a constant rate. The mass spectrometer is calibrated in two steps. First, it is tuned and the sensitivity is adjusted to an internal calibrated leak (diffusion) standard in units of standard milliliter of gas per second. Second, gas standards prepared in the serum vials are used to convert the instrument reading to parts per million (volume) and check the stability of the tuning.

4.8 Potential Problems Associated with Mobile Bioreactor Operation

The principal uncertainty concerns the rate of TCE removal/degradation, specifically how long it will take to degrade the contaminants in the MMB and whether it is necessary to recycle the contaminated water one or more times through the MMB, are outlined below.

Pilot-scale studies, coupled with earlier laboratory experiments, suggest that problems may be encountered during the startup and daily operation and may reduce the efficiency and efficacy of bioreactor operations. The following information is provided in an attempt to reduce the number and severity of these potential problems and to increase the efficiency of the operation of the MMB.

Ideally, MMB will be capable of continuous efficient and effective operation and degradation of TCE and associated toxic compounds with minimum maintenance and attention. There are numerous physical and biological factors to monitor and regulate to maintain optimal operating conditions.

The microbial components and the microbial environment are crucial to understand in operating any bioreactor. More specifically, the bacteria, even the methanotrophs, vary in their requirements and their capacity to adapt to various conditions. (Often the literature may suggest that a single factor is responsible for a decrease in efficiency in bioreactor operation when it may have been due to a series of factors not identified at the time.)

It is clearly stated in several studies that a single pass of a TCE influent through aerobic bioreactor systems result in the degradation of 50% or less of the TCE (Wickramanayake et al. 1990).

4.9 Modeling

Modeling and data interpretation include hydrological modeling, modeling of the degradative processes, and evaluating the data. The objective is to compare results with theoretical models, interpret data, and determine values for building a full scale unit. Models will be developed for, TCE loss, biodegradation, water, and gas flows.

4.10 Permits

Refer to WSRC Engineering and Engineered Services Procedure Manual (1E), Procedure 3.02, for obtaining permits and clearances. Appropriate SRS personnel were contacted and permits were prepared for the methanotrophic bioreactor; the Wastewater Treatment Facility Permit was submitted to SCDHEC in August 1993; the construction permit was approved in October 1993; the National Environmental Policy Act was granted a categorical exclusion (B. 3. 10) for pilot scale demonstration of research activities in January 1991; and the Air Permit variance was granted in September 1993.

5.0 Waste Disposal and Best Management Practices Plan

5.1 Projected Effluent Quality and Disposal

Treated effluent from the bioreactor will vary in contaminant concentration depending on the operating scheme. Normal discharge from the facility is anticipated to be approximately 1 gpm or less and intermittent since recycling will be common during operation. The waste stream will be transferred to the M-Area Air Stripper (Operating Permit #10253) where remaining TCE and PCE will be removed prior to release to the environment. This additional treatment acts as a safeguard for the bioreactor system during testing or in the event of a biofilm failure during biofilm development and during control runs without a biofilm. The effluent from the stripper will be discharged out of a National Pollution Discharge Elimination System outfall permitted for TCE and PCE.

Initial testing of the system will occur at TNX. This testing will use uncontaminated water and simulated contaminated water. The tests include control runs to measure absorption by the packing material and runs to measure volatility losses. Tests will also run on the system without a biofilm. During these tests, the total TCE and PCE concentrations in the effluent will be 4000 µg/l or less. These tests will operate in a batch mode with approximately ten gallons of water. The effluent will be containerized for disposal using the TNX Bioreactor System (Operating Permit #17,434-IW).

5.2 Solid Waste Disposal

Solid waste will consist of spent packing from the bioreactor and possibly spill cleanup materials. The spent packing material will undergo toxicity characteristic leaching procedure testing for characterization. This will occur at the end of testing or if the packing material is replaced. If the waste is hazardous, it will be handled as such and sent to the SRS Non-Radioactive Hazardous Waste Storage building before offsite disposal. Nonhazardous solid wastes will be disposed of in the SRS Sanitary Landfill. Spill cleanup materials will be addressed in a similar manner.

5.3 Best Management Practices Plan

A complete copy of the Best Management Practices (BMP) plan (WSRC-IM-90-49) for the MMB will be maintained by the WSRC Environmental Protection Department BMP coordinator, the permit custodian, and near the MMB system. This BMP plan will be made available to the EPA, Region IV, and the South Carolina Department of Health and Environmental Control for onsite review during normal working hours.

A description of risk identification and assessment is described below. Reporting of incidents, materials compatibility, good housekeeping, preventative maintenance, inspections and records, security, and employee training are detailed in the plan (WSRC-IM-90-49).

5.3.1 Risk Identification and Assessment

SRTC will demonstrate and test a liquid phase bioreactor system at various wells at TNX. The aqueous treatment system utilizes natural methane degrading bacteria to treat an aqueous stream containing chlorinated organics. The primary pollutants in this inlet stream are TCE and PCE. This contaminated stream is pumped from a groundwater monitoring well to the mobile system. This system consists of two trickle flow columns connected in a series. The system can hold 720 liters, but during normal operation a level switch maintains the liquid volume at or below 50 liters. The bacteria grow on methane and inorganic nutrients, such as sodium nitrate and potassium phosphate. The methane is supplied using compressed gas cylinders and a methane delivery system that mixes air and methane to desired levels. The nutrients are delivered using a nutrient delivery system, which consists of a 225 liter tank and a pump capable of delivering 200 ml/min. Once the groundwater leaves the bioreactor system, it is transferred to a 11,300 liter holding tank. The tank contents are disposed of at the M-Area air stripper. The entire process is powered using a diesel generator with a 290 liter fuel tank. The liquid pumps are filled with lubricating oil.

5.3.2 Identified Hazardous Substances

Trichloroethylene contaminated water. Lubricating oils and diesel fuel.

5.3.3 Contaminated Water

The contaminated water could discharge to the environment at the inlet and outlet from the system, within the system to the hold tank, and from the hold tank. The hold tank is surrounded by a sandbagged diked area with an impermeable plastic cover. The area of the diked area is 25 m^2 and 60 cm in height, adequate to hold 11.36 m^3 of water plus 15 centimeters (six inches) of rainwater. Figure 2 illustrates the typical site layout with respect to well, system, tank, and containment placement.

5.3.4 Transfer of Influent

The maximum inlet flow rate to the system is 6.31 liters per minute. A flexible hose with a quick-connect type fastener links the system to the well head. The submersible pump in the well and the positive displacement pump on the system transfers the groundwater to the trailer through another quick connector. Both connections have a catch basin under the fittings during initial startup and are checked daily. The system control unit shuts down operation and produces an alarm condition if the influent line flow changes, indicating a leak. The entire system is monitored daily and any leaks are barricaded.

5.3.5 Transfer of Effluent

Bi-weekly, or as needed, 11,300 liters of fluid from the hold tank are transferred to the M-Area stripper. The water is transferred using the purged water disposal truck using Procedure SOP LETF-194 or -190. The truck operator transfers water to the M-Area stripper or the purged water disposal station using Procedure SOP LETF-191 or -192. The Liquid Effluent Treatment Facility group in Reactor Materials Production is responsible for transferring and disposing of the effluent once it is placed in the effluent hold tank. Copies of the procedures may be found in Section 4, Volume 1, of the Operations and Maintenance manual for this system. The transfer is done using a flexible hose assembly. During transfer, spills are barricaded and/or containerized and supervision is notified.

5.3.6 Lubricating and Oils Diesel Fuel

Petroleum sources on the trailer unit are monitored daily for leaks. The diesel tank holds 290 liters of fuel. Each of the three pumps holds approximately 1 liter of lubricating oil. Administrative controls are in place for operating the system. This includes, checking the system daily and having crushed corn cobs available. If a leak occurs on the diesel tank and the generator fails, the system shuts off and an alarm condition is triggered. The oil goes to the surface of the trailer unit or the ground where it is excavated, as required for disposal.

6.0 Safety, Quality Assurance and Security

6.1 Safety

General safety rules for SRS are documented in SRS Safety Manual (8Q), in compliance with DOE Order 5483.1A.

6.2 Process Hazards Review

As defined in the SRS Safety Manual (8Q) in Procedure 10-1. Completed April 1990.

6.3 Safety Inspection

A safety inspection of the unit was completed according to Manual (8Q). 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).

6.4 Quality Assurance and Quality Control

The Environmental Science Section's Biotechnology Group (ESS/BG) is working on a Work Authorization Document written through WSRC's Environmental Remediation Division. All Quality Assurance (QA) activities performed by ESS/BG associated with this project will be in accordance with WSRC L1, 8.01, Revision 0, and the SRTC QA Implementation Matrix. A QA checklist and QA task technical will be written for the project.

6.5 Security

WSRC security requirements and procedures are documented in the WSRC Security Manual (6Q). These procedures are as required by federal laws and are applicable DOE orders (e.g., DOE Order 5631.1A).

7.0 References

----- , 1985, *Closure Plan for the M-Area Settling Basin and Vicinity at the Savannah River Plant*, DPSPU 84-11-11, Rev. November 1985, E. I. du Pont de Nemours and Company, Savannah River Laboratory, Aiken, SC.

----- , 1989, *United States Department of Energy Environmental Restoration and Waste Management Five-Year Plan*, DOE/S-0070, NTIS, Springfield, VA.

----- , 1991, *Hydrogeologic Data Collection Methods, Procedures, and Specifications*, WSRC 3Q5, Westinghouse Savannah River Company, Aiken, SC.

----- , 1991, *Modification of UIC Permit #103*, WSRC-RP-91-354, Westinghouse Savannah River Company, Aiken, SC.

----- , 1991, *Integrated Demonstration Project: Project Management Plan, March 1991*, IDP-0566, Westinghouse Savannah River Company, Aiken, SC.

----- , 1992, *The Savannah River Site's Groundwater Monitoring Program, Third Quarter 1992*, ESH-EMS-920037, prepared for the U. S. Department of Energy under Contract No. AA46317P.

APHA, 1989, *Standard Methods for the Examination of Water and Wastewater*, 17th ed., American Public Health Association, Washington, DC.

Balkwill, D. L., 1989, "Numbers, Diversity, and Morphological Characteristics of Aerobic, Chemoheterotrophic Bacteria in Deep Subsurface Sediments from a Site in South Carolina", *Geomicrobiol. J.*, 7:33–52.

Bohlool, B. B. and E. L. Schmidt, 1980, "The Immunofluorescence Approach in Microbial Ecology", *Adv. Microb. Ecol.*, 4:203–241.

Chaudhry, G. R. and S. Chapalamadugu, 1991, "Biodegradation of Halogenated Organic Compounds", *Microbiol.*, Rev. 55:59-79.

Craun, G. F., 1986, "Waterborne Diseases in the United States", 295 pp., *CRC Press*, Boca Raton, FL.

Dommergues, Y. R., L. W. Belser, and E. L. Schmidt, 1978, "Limiting Factors for Microbial Growth and Activity in Soil", *Adv. Microb. Ecol.*, 2:49–104.

Donaldson, T. L., G. W. Strandberg, et al., 1988, "Degradation of Trichloroethylene and Trans-1,2-Dichloroethylene by a Methanotrophic Consortia in a Trickle-type Bioreactor", *Proceedings DOE Model Conference*, Oak Ridge, TN, October 3–7, 1988.

Enzien et al., 1994, "Reductive Dechlorination of Trichloroethylene and Tetrachloroethylene under Aerobic Conditions in a Sediment Column", *Appl. Environ. Microbiol.*, 60:220–2204.

Fliermans, C. B., B. B. Bohlool, and E. L. Schmidt, 1974, "Autecological Study of the Chemautotroph Nitrobacter by Immunofluorescence", *Appl. Microbiol.*, 27:124–129.

Fliermans, C. B., W. B. Cherry, L. H. Orrison, and L. Thicker, 1979, "Isolation of Legionella Pneumophila from Nonepidemic Related Aquatic Habitats", *Appl. Environ. Microbiol.*, 37:1239–1242.

Fliermans, C. B., T. J. Phelps, D. Ringelberg, A. T. Mikell, and D. C. White, 1988, "Mineralization of Trichloroethylene by Heterotrophic Enrichment Cultures", *Appl. Environ. Microbiol.*, 54:1709–1714.

Fliermans, C. B, et al. 1994, "Imunological Techniques as Tools to Characterize the Subsurface Microbial Community at a Trichloroethylene Contaminated Site", *Applied Biotechnology for Site Remediation, 186–203., 1994.*

Fogel, M. M., A. R. Taddeo, and S. Fogel, 1986, "Biodegradation of Chlorinated Ethenes by a Methane-Utilizing Mixed Culture", *Appl. Environ. Microbiol.*, 51:720–724.

Garabedian, S. P., 1990, "A Comparison of Results from Small- and Large-Scale Tracer Test in a Heterogeneous Aquifer, Cape Code, Massachusetts", *Proceedings of the First International Symposium on Microbiology of the Deep Subsurface*, January 15–19, 1990, WSRC Information Services, Aiken, SC.

Harvey, R. W. and L. H. George, 1987, "Growth Determinations for Unattached Bacteria in a Contaminated Aquifer", *Appl. Environ. Microbiol.*, 53:2992–2996.

Hazen, T. C., 1990, *Test Plan for FY92 In Situ Bioremediation Demonstration of DOE/OTD TTP No.: SR 0566-01*, WSRC Information Services, Aiken, SC.

Hazen et al., 1991, "Comparison of Bacteria Density and Activity in Groundwater and Adjacent Sediments", *Microb. Ecol.*, 22:293–304.

Hazen et al., 1994, "Full-Scale Demonstration of *In Situ* Bioremediation of Chlorinated Solvents at SRS", *South Carolina Engineer.*

Jewell et al., 1991, "Methanotrophs for Biological Pollution Control: TCE Removal and Nutrient Removal with the Expanded Bed, Contract No. 5089-260-1798, *Ann. Rep. for 1990*, Cornell Univ. for GRI.

Lanza, G. R. and J. M. Dougherty, 1991, "Microbial Enzyme Activity and Biomass Relationships in Soil Ecotoxicology", *Environmental Toxicology and Water Quality*, 6:165–176.

Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer, 1988, "Trichloroethylene Biodegradation by a Methane-Oxidizing Bacterium", *Appl. Environ. Microbiol.*, 54:951–956.

Looney, B. B., T. C. Hazen, D. S. Kaback, and C. A. Eddy, 1991, "Full-Scale Field Test of the *In Situ* Air Stripping Process at the Savannah River Integrated Demonstration Test Site", WSRC-RD-91-22, Westinghouse Savannah River Company, Aiken, SC.

Mayer, K. P., D. Grbic-Galic, L. Semprini, and P. L. McCarty, 1988, "Degradation of Trichloroethylene by Methanotrophic Bacteria in a Laboratory Column of Saturated Aquifer Material", *Water Sci. Tech.*, 20:175–178.

Newman, L. M. and L. P. Wackett, 1991, "Fate of 2,2,2-Trichloroacetaldehyde (Chloral hydrate) Produced during Trichloroethylene Oxidation by Methanotrophs", *Appl. Environ. Microbiol.*, 57:2399–2402.

Nichols, 1993, *Program for TNX Area Groundwater Characterization Wells*, WSRC-RP-89-269, Westinghouse Savannah River Company, Aiken, SC.

Patrick, R., E. Ford, and J. Luarles, 1983, *Groundwater Contamination in the United States,* 2nd ed., University of Pennsylvania Press, Philadelphia, PA.

Phelps, T. J., D. Ringelberg, D. Hedrick, J. Davis, C. B. Fliermans, and D. C. White, 1989, "Microbial Biomass and Activities Associated with Subsurface Environments Contaminated with Chlorinated Hydrocarbons", *Geomicrobiol. J.*, 6:157–170.

Phelps, T. J., J. J. Niedzielski, R. M. Schram, S. E. Herbes, and D. C. White, 1990, "Biodegradation of Trichloroethylene in Continuous-Recycle Expanded-Bed Bioreactors", *Appl. Environ. Microbiol.*, 56:1702–1709.

Phelps, T. J., K. Malachowsky, R. M. Schram, and D. C. White, 1991, "Aerobic Mineralization of Vinyl-Chloride by a Bacterium of the Order Actinomycetales", *Appl. Environ. Microbiol.*, 57:1252–1254.

Radian, 1989, *Phase 1—Technical Feasibility of Methane Use for Water Treatment: Task 2—Preliminary Economic Analysis*, Gas Research Institute, Chicago, IL.

Semprini, Lewis, Roberts, P. V. et al., 1988, *A Field Evaluation of In- Situ Biodegradation Methodologies for the Restoration of Aquifers contaminated with Chlorinated Aliphatic Compounds: Results of a Preliminary Demonstration, 1987*, Technical Report No. 302, Department of Civil Engineering, Stanford University.

Sims, W. R., B. B. Looney, and C. A. Eddy, 1991, "Evaluation of a Rapid Headspace Analysis Method for Analysis of Volatile Constituents in Soils and Sediments", *Proceedings of the Fifth Outdoor Action Conference, Association of Groundwater Scientists and Engineers*, Las Vegas, NV, May 1991.

Simmons, R. V, et al., 1985, Technical Data Summary: Chemical and Radionuclide Characterization Study of the Old TNX Seepage Basin, DOE SRS DPSTD-85-115.

Sinclair, J. L., and W. C. Ghiorse, 1989, "Distribution of Aerobic Bacteria, Protozoa, Algae, and Fungi in Deep Subsurface Sediments", *Geomicrobiol. J.*, 7:15–32.

Tsien, H. C., G. A. Brusseau, R. S. Hanson, and L. P. Wackett, 1989, "Biodegradation of Trichloroethylene by Methylosinus Trichosporium OB3B", *Appl. Environ. Microbiol.*, 55:3155–3161.

Vogel, T. M. and P. L. McCarty, 1985, "Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl-Chloride, and Carbon-Dioxide under Methanogenic Conditions", *Appl. Environ. Microbiol.*, 49:1080–1083.

Wackett, L. P., G. A. Brusseau, S. R. Householder, and R. S. Hansen, 1989, "Survey of Microbial Oxygenases: Trichloroethylene Degradation by Propane Oxidizing Bacteria", *Appl. Environ. Microbiol.*, 55:2960–2964.

White, D. C., D. B. Ringelberg, J. B. Guckert, and T. J. Phelps, 1990, "Biochemical Markers for *In Situ* Microbial Community Structure", C. B. Fliermans and T. C. Hazen (eds.), *Proceedings of the First International Symposium on Deep Terrestrial Subsurface Microbiology*, WSRC Information Services, Aiken, SC.

Wickramanayake, G. B., H. Nack, E. H. Hagen, D. T. Palmer, and C. L. Criner, 1990, "Aerobic Biotreatment of Trichloroethylene-Contaminated Groundwater", *Proc. Haz. Wastes Haz. Mat.*, (HMCRI) 7:359–362.

Wilson, J. T. and B. H. Wilson, 1985, "Biotransformation of Trichloroethylene in Soil", *Appl. Environ. Microbiol.*, 49:242–243.

Wilson, B. H., G. B. Smith, and J. F. Rees, 1986, "Biotransformations of Selected Alkylbenzenes and Halogenated Aliphatic Hydrocarbons in Methanogenic Aquifer Material a Microcosm Study", *Environ. Sci. Technol.*, 20:997–1002.

Wilson, J. T., 1988, "Degradation of Halogenated Hydrocarbons", *Biosensors and Environmental Biotechnology*, (Gustav Fisher).

Wu, W. M., D. Wagner, J. Krzewinski, and R. Hickey, 1992, "Pilot Test of the Granular Activated Carbon Fluidized Bed Reactor (GACFBR) and Pelletized Diatomaceous Earth Fluidized Bed Reactor (PDE-FBR) for Methanotrophic TCE Degradation", *Quar. Rep.*, MBI; Radian Corp.