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**PHASE III TEST PLAN FOR THE
BIODEGRADATION OF CHLORINATED SOLVENTS
IN A SOIL-COLUMN REACTOR**

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**ROCKY FLATS ENVIRONMENTAL TECHNOLOGY SITE
Environmental Restoration Program**

**U.S. Department of Energy
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"REVIEWED FOR CLASSIFICATION"

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List of Acronyms, Abbreviations, and Chemical Formulas

amps/torr	ampere/torr
ANOVA	Analysis of Variance
BFB	bromofluorobenzene
° C	degrees Celsius
CH ₂ Cl ₂	methylene chloride
CH ₃ Cl ₃	chloroform
CH ₄	methane
CCl ₄	carbon tetrachloride
CO ₂	carbon dioxide
CMS/FS	Corrective Measures Study/Feasibility Study
C/N/P	carbon to nitrogen to phosphorus ratio
EPA	Environmental Protection Agency
FY	Fiscal Year
g	gram
GC/MS	gas chromatograph/mass spectrometer
H ₂ S	hydrogen sulfide gas
IHSS	Individual Hazardous Substance Site
kg	kilogram
lbs	pounds
mg	milligram
ml	milliliter
N ₂	nitrogen gas
O ₂	oxygen
OU	operable unit
PCE	perchloroethylene or tetrachloroethylene
ppm	parts-per-million
QAPjP	Quality Assurance Project Plan
RFP	Rocky Flats Plan
Site	Rocky Flats Environmental Technology Site
TCA	trichloroethane
TCE	trichloroethylene
TSP	RFETS Environmental Restoration Sitewide Treatability Study Program
μ l	microliter

1.0 INTRODUCTION

This test plan describes Phase III of the remedy-screening level biodegradation study to be performed at the Rocky Flats Environmental Technology Site (Site) during fiscal year 1995 (FY-95). This test plan supplements the FY-94 Biodegradation Treatability Study Work Plan (RFP/ERM-94-0007) which describes Phases I and II of this project. Potential primary contaminants under evaluation are six chlorinated solvents including tetrachloroethylene (PCE), trichloroethylene (TCE), methylene chloride, carbon tetrachloride, 1,1,1-trichloroethane (1,1,1 TCA), and 1,1,2-trichloroethane (1,1,2 TCA).

1.1 Application/Scope

This multi-phase biodegradation treatability study directly contributes to the Operable Unit 2 (OU 2) Corrective Measures Study/Feasibility Study (CMS/FS) and is a part of the Environmental Restoration Site-wide Treatability Study Program (TSP). The study supports evaluation of treatment options for solvent contaminated subsurface soil in the OU 2 east trenches area and contaminated groundwater in OU 2. The results of this study may also be applicable for solvent contaminated media in other OUs.

The OU 2 Biodegradation Treatability Study began with Phase I which examined chemical changes of solvent contaminants caused by the abiotic (nonliving) components of OU 2 soil and by the resident microbial soil population which was not stimulated to grow. This provided an understanding of the underlying changes expected when solvent mixtures contact the surficial Site soil which had not been altered to enhance biological activity. Phase II involved enhancing the conditions for several mixed microbial populations under aerobic and anaerobic conditions and evaluating their ability to transform six solvents of interest for OU 2.

Phase III will be conducted as a laboratory-scale study of the biotreatability of a mixture of the six chlorinated solvents described above. Phase III applies the successful biodegrading microbial strains from Phase II to solvent contaminated soil and determines the extent to which complete solvent degradation is achievable using sequential anaerobic/aerobic treatment. Phase IV evaluates the application of findings from Phases I through III for the Site's needs and determines scale-up requirements.

This treatability study focuses on low concentrations of these solvents on soil in the range of 25 ppm (mg/kg) and below. A wide range of solvent concentrations exist in OU 2 soil from free-product to nondetectable levels. If successful, this technology will be most suited to treat moderate to low concentrations of solvents and will be applicable for free product treatment only if the contaminated media can be mixed with less contaminated soil or water. If free-product treatment is required, ex-situ remediation in a bioreactor will most likely be required to control contaminant loading and toxicity. If residual solvent treatment is required in which solvents are at low to moderate concentrations, in-situ treatment may be considered.

2.0 TREATMENT TECHNOLOGY DESCRIPTION

The following is an overview of the microbial mechanisms for solvent transformation. A more in-depth explanation is provided in the Biodegradation Treatability Study Work Plan for FY-94 (RFP-ERM-94-0007)

Prior to 1983, chlorinated aliphatic solvents such as TCE were thought to be recalcitrant or unable to be degraded microbially. Since then, numerous studies have shown that important biochemical pathways are available for degradation of TCE, TCA, carbon tetrachloride, methylene chloride, and the other chlorinated solvents, and that degradation or transformations will occur when conditions are appropriate (Egli et al 1988, Egli et al 1990, Fogel et al 1986, Gossett 1985, Nelson et al 1987, Vogel et al 1987, Wackett et al 1989)

2.1 Anaerobic Treatment

Anaerobic treatments show that microbes are able to reductively dechlorinate solvents such as carbon tetrachloride and PCE as well as other highly chlorinated solvents. For some degradation pathways, the rate of reaction for chlorine removal increases as the number of chlorines on the solvent molecule increases (Hughes and Parkin 1991). For example, carbon tetrachloride (CCl_4) is dechlorinated faster than chloroform (CHCl_3) which, in turn, is dechlorinated faster than methylene chloride (CH_2Cl_2) under this pathway (Hughes and Parkin 1991). Highly chlorinated solvents such as TCE, TCA, and PCE are good targets for anaerobic treatment.

While highly chlorinated compounds tend to dechlorinate rapidly, the degradation products of dechlorination are themselves less chlorinated compounds which may tend to accumulate and not dechlorinate further under anaerobic conditions (Kästner 1991, Vogel and McCarty 1985). Anaerobic treatment can be used to convert highly chlorinated solvents to less chlorinated compounds which are then susceptible to aerobic biodegradation as discussed below.

2.2 Aerobic Treatment

Research in aerobic treatment of chlorinated solvents shows that compounds such as TCE, TCA, methylene chloride, and less chlorinated solvents can be degraded via oxidation of the solvent molecules by methanotrophic bacteria and other aerobic microorganisms (Fogel et al. 1986, Janssen et al. 1988, Little et al 1988, Oldenhuis et al 1989). Methanotrophs are bacteria which use methane as an electron donor (or energy source) in the presence of oxygen. The enzymes produced by methanotrophs which incorporate oxygen into the methane molecule during metabolism can also oxidize many mildly chlorinated solvent molecules. Highly chlorinated solvents such as PCE and carbon tetrachloride tend to be resistant to methanotrophic treatment (Janssen et al. 1991).

Only a few aliphatic (straight-chain) chlorinated solvents have been found to serve as primary growth substrates for microbial populations (Janssen et al. 1991). Typically, aliphatic chlorinated solvents are not directly oxidized as a food or energy source for microbes. Oxidation

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of solvents occurs incidentally to other metabolic processes and the microbes obtain no energy from the transformation. This usually involves the production of nonspecific enzymes which coincidentally oxidize compounds which are not the target energy source. This process is called cometabolism (Brock 1984).

The previous section briefly describes a few of the many pathways available for biotreatment of chlorinated solvents. This study examines several of these pathways for use in treating solvent contaminated soil at the Site. Specifically, one aerobic and three anaerobic pathways are examined.

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3.0 TEST OBJECTIVES

The overall objective of a treatability study for remedy screening is to provide data so that regulators and responsible parties can determine if a particular treatment technology shows enough promise to warrant further investigation or development

Figure 3.1 shows the logical progression of information which must be obtained in order to meet this objective for the Bioremediation Treatability Study. The information discussed in this section will be gathered for Phase III during FY-95 to evaluate whether bioremediation meets this objective for treatment of chlorinated solvent mixtures in soil and groundwater

3.1 Phase III Objectives

Items E and F on Figure 3.1 concern the degree to which the populations chosen from Phase II can be optimized for solvent degradation, and whether sequential anaerobic/aerobic treatment leads to conversion of the solvents to nonregulated products

The objectives of Phase III are to evaluate

- 1) the extent successful microbial populations from Phase II can biotransform PCE, TCE, methylene chloride, carbon tetrachloride, 1,1,1 TCA, and 1,1,2 TCA in a soil-column,
- 2) the effect of electron donor, electron acceptor, and nutrient loading on the biotransformations of the chlorinated solvents in soil in order to determine optimum treatment conditions, and
- 3) whether complete solvent degradation is achievable under optimal conditions using either one microbial population (or treatment condition) or several microbial populations sequentially to effect complete solvent destruction

The objectives will be met by

- 1) growing microbial populations from Phase II in a soil-column reactor spiked with chlorinated solvents and quantitatively analyzing chlorinated solvents and degradation products over time;
- 2) comparing the concentration changes in chlorinated solvents and degradation products in batch cultures when electron donor, electron acceptor, and nutrients are varied in a controlled manner to determine the optimum conditions for maximum biotransformation potential, and
- 3) growing anaerobic cultures and the aerobic culture along different lengths of a soil-column and measuring biotransformations of solvents in the aqueous media passing through the two cultures

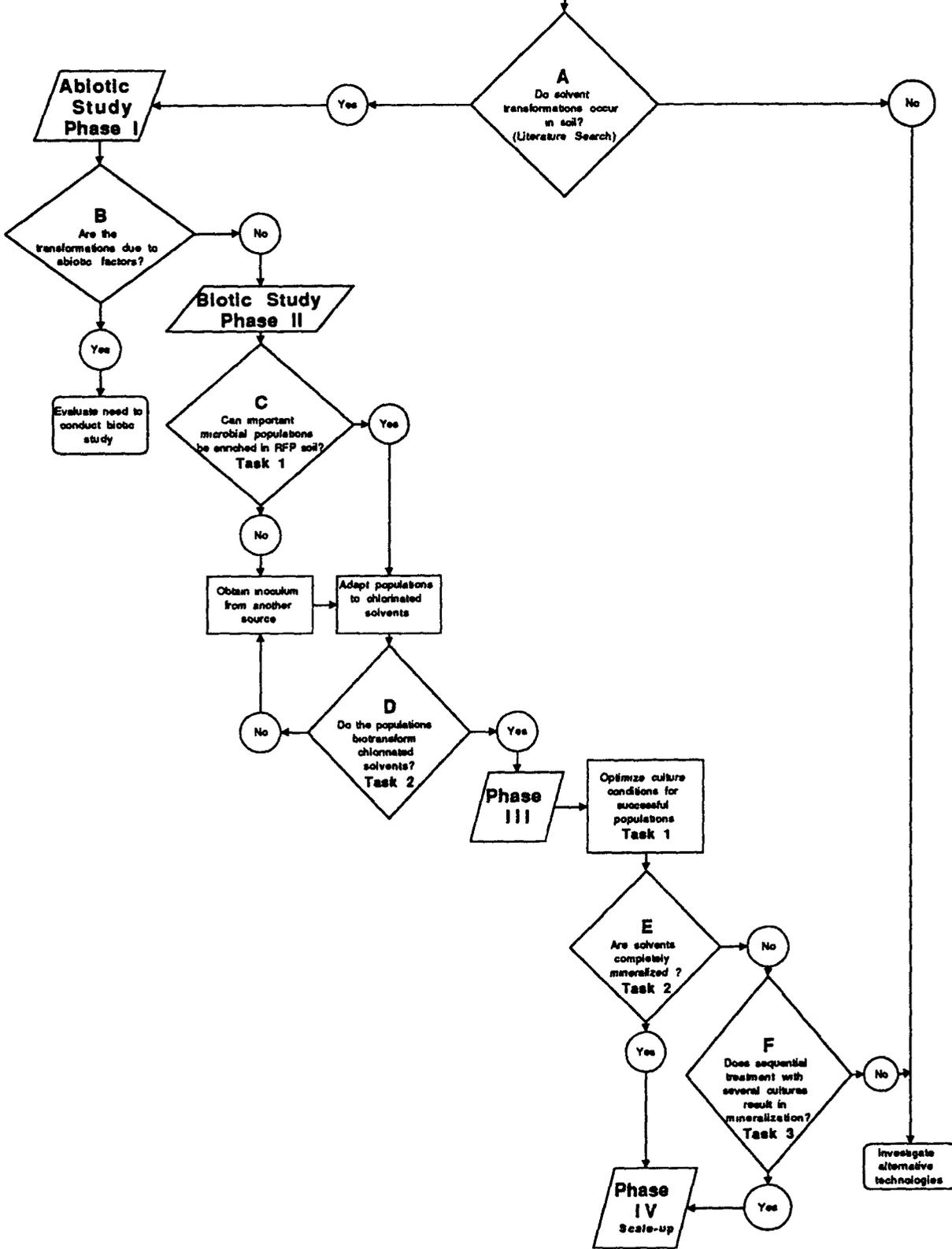


Figure 3 1 Flow diagram for the Biodegradation Treatability Study

4.0 EXPERIMENTAL DESIGN

The Phase III experimental plan includes soil-column reactor design and construction, the reactor operational plan, implementation of reactor experiments, and biotransformation optimization studies. The following sections describe these activities.

4.1 Design and Construction of Soil-Column Reactor

This study requires the design and construction of a soil-column reactor to test the rate and extent of solvent biotransformations using soil microbes from the Site. Soil-column reactors are used to simulate in-situ treatment. There is not a standard soil-reactor used for experimentation because soil, contaminants, and site-specific data needs dictate the design of the reactor. The following describes the initial concept for the soil-column reactor planned for this study. The final design may vary from this preliminary model.

Figure 4.1 shows the conceptual design of the soil-column reactor. This reactor is composed of a glass column approximately 2 inches in diameter. The column will be separated into sections which are joined by clamps. In this way the column can be lengthened or shortened depending on the needs of this and future experiments.

Each section will be approximately 6 inches in length and equipped with 2 to 4 septa-ports for removal of liquid samples and addition of nutrients and electron acceptors into each section of the column. Septa-ports consist of small openings into the soil-column which are sealed with screw-caps equipped with Teflon septa.

The soil packing material will be held in place in the column by screens which allow only passage of liquid. Soil-free zones will be established after each soil section using two screens held in place by clamps separated by 1 inch of column length. The soil-free portions of the column will be used as locations to remove liquid samples and add nutrients and electron acceptors. Hence, adding and removing liquid from the column will occur in these areas free of soil packing-material. This should allow collection of representative samples and uniform dispersion of nutrients injected into the column.

4.2 General Operation of the Soil-Column Reactor During Experimentation

4.2.1 Packing the Soil-Column

Each section of the column will be sterilized at 121° C for 45 minutes. The sections will be packed with soil (including culture material from an enrichment culture of interest for the particular experiment). The soil packing will consist of culture soil mixed with equal amounts of sand (which has been washed and sterilized at 121° C for 45 minutes on three consecutive days). This should provide a packing in which nutrient media and chlorinated solvents may pass through at a reasonable flow-rate. The achievable flow-rates with a sand and culture mixture will be tested during evaluation of the soil-column.

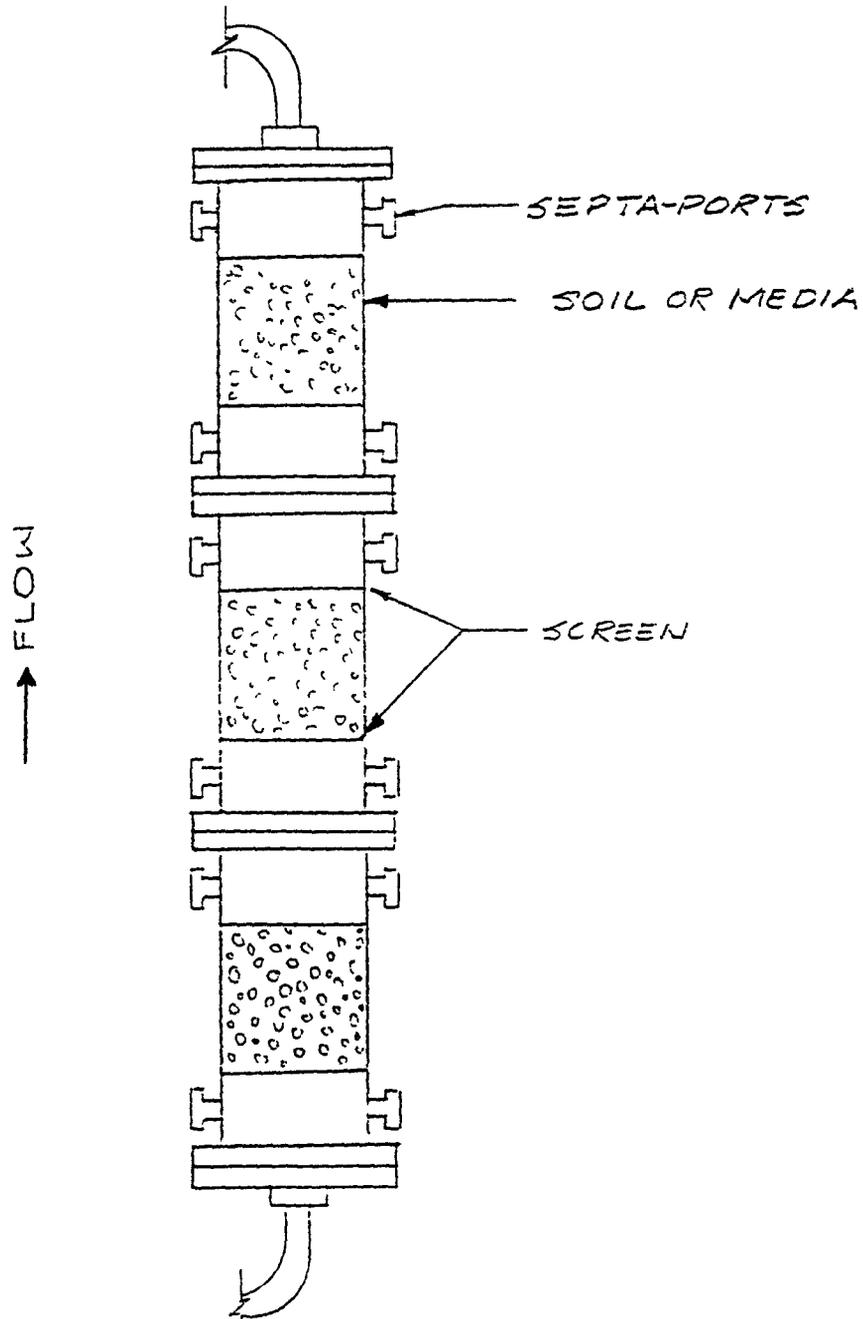


Figure 4 1 Preliminary design of soil-column reactor

4 2.2 Controlling the Type of Microbial Population in the Soil-Column

The type of microbial population operating in these solvent degradation experiments is controlled by the electron acceptor provided for growth. When oxygen is present (and methane is provided as the only food source) methanotrophs will grow. When oxygen is absent and nitrate is provided, denitrifiers will grow. When oxygen and nitrate are absent and sulfate is provided, sulfate reducers will grow. When oxygen, nitrate, and sulfate are absent and carbonate or carbon dioxide is provided, methanogens will grow. The type of microbial population being studied will be controlled by the electron acceptor supplied to the column in the aqueous nutrient media or along the length of the soil-column through addition of electron acceptors in water via the septa-ports.

4 2.3 Aqueous Growth Media and Delivery to Soil-Column

Sterile nutrient media will be prepared and stored in a sterile vessel. The nutrient media used will depend on the population of interest for the particular experiment (Table 4.1). The aqueous media will be delivered to the soil-column via sterile Teflon tubing and pumped into the front-end of the soil-column in an up-flow configuration. The nutrient media will be spiked with a mixture of solvents at 5 ppm each. The solvent mixture is described below in Section 4.2.5.

4 2.4 Flow-rates of Media and Solvents Through Soil-Column

Flow-rates will be regulated so that media passing through the length of each section of the column will not disturb soil and microbial growth. The goal is to adjust the flow-rate to achieve the minimum liquid retention time and maximum biotransformation extent. A suitable flow-rate will be selected during experimentation.

Once media has passed the previous section of column, the next appropriate electron acceptor will be added via the septa port for the next section of column. In this way growth of several populations in the same column may be possible, and sequential treatment of solvents by different populations can be tested in the length of one soil-column.

Flow-rates will be controlled by pumping water containing nutrients, electron acceptors, energy sources, and solvents through the column in an up-flow direction. The exact configuration of the column will be determined during set-up and evaluation of the system.

Liquid samples will be removed for electron acceptor analysis via a syringe from the septa-ports. Electron acceptor concentrations will be measured as necessary using standard colorimetric techniques (Section 6.3).

Table 4.1 Nutrient media used for enrichment culturing

Organism	Chemical	Quantity per liter	Final pH at 25°C
Methanotrophs	NH ₄ Cl	0.290 g	6.8 to 7.2
	KH ₂ PO ₄	0.040 g	
	MgSO ₄ ·7H ₂ O	0.150 g	
	CaCl ₂ ·2H ₂ O	0.0250 g	
	FeSO ₄ ·7H ₂ O	0.60 mg	
	CH ₄	2.5% Volume in Headspace	
	O ₂	20.4% Volume in Headspace	
Denitrifiers	NH ₄ Cl	0.290 g	6.8 to 7.2
	KH ₂ PO ₄	0.040 g	
	MgSO ₄ ·7H ₂ O	0.150 g	
	CaCl ₂ ·2H ₂ O	0.0250 g	
	FeSO ₄ ·7H ₂ O	0.60 mg	
	KNO ₃	4.040 g	
	Sodium Acetate	5.67 g	
	Sodium Lactate	5 ml of 60% solution	
Sulfate Reducers	NH ₄ Cl	0.290 g	7.2 to 7.5
	KH ₂ PO ₄	0.040 g	
	MgSO ₄ ·7H ₂ O	0.150 g	
	CaCl ₂ ·2H ₂ O	0.0250 g	
	Ascorbic Acid	0.100 g	
	Na ₂ SO ₄ ·7H ₂ O	2.680 g	
	Sodium Acetate	5.67 g	
	Sodium Lactate	5 ml of 60% solution	
Methanogens	NH ₄ Cl	0.290 g	6.8 to 7.2
	KH ₂ PO ₄	0.040 g	
	MgSO ₄ ·7H ₂ O	0.150 g	
	CaCl ₂ ·2H ₂ O	0.0250 g	
	Sodium Acetate	5.67 g	
	Sodium Lactate	5 ml of 60% solution	

4.2.5 Solvent Mixture

The solvent mixture of interest for OU 2 contains PCE, TCE, carbon tetrachloride, 1,1,1 TCA, 1,1,2 TCA, and methylene chloride. These solvents will be delivered to the soil-column at 5 ppm each in the growth media. Delivery will be continuous during experimentation along with delivery of growth media.

4.2.6 Preparation of Soil-Column for Quantitative Evaluation of Solvent Degradation

4.2.6.1 Leak-Testing of Empty Column

A fully assembled empty column will be leak tested prior to beginning the experiment. The empty column will be pressurized with helium. The helium leak rate will be measured by removing gas samples from the septa-ports and measuring the change in helium concentration over time using a mass spectrometer (Section 6.4.1). This should show whether the reactor design is air-tight and capable of retaining volatile chlorinated solvents.

4.2.6.2 Leak-Testing and Equilibration of Soil-Column

Prior to investigation of biodegradation of solvents in the soil-column, the amount of solvent lost due to leaks and adsorption onto the reactor and packing material will be evaluated. The soil-column will be packed as described in Section 4.2.1. Deionized water containing the solvent mixture at 5 ppm will be delivered to the column and allowed to pass through at the flow-rate chosen for experimentation. The aqueous solvent concentration entering the column will be compared to the aqueous solvent concentration leaving the column. Solvents will be analyzed on a daily basis or as necessary.

When the concentration of the solvents leaving the column has stabilized or is equal to the concentration entering the column, degradation experimentation will begin. Degradation experiments entail replacing the deionized water in which the solvents were delivered with a growth media which will stimulate biological activity.

4.2.7 Quantitative Evaluation of Solvent Degradation and Transformations in a Soil-Column with Maximum Biological Activity

Once the soil-column has equilibrated, the deionized water will be replaced with an appropriate growth media. This will stimulate the target biological activity in the soil-column. The solvent mixture will continue to be delivered to the soil-column. The concentration and types of solvents entering the column, leaving the first microbial section and entering the second section, and leaving the second section will be quantified by removing 1 ml samples of liquid media from each section and analyzing the solvents using a purge-and-trap system coupled with a gas chromatograph/mass spectrometer (GC/MS). The analysis method is described in Section 6.2.3. (More than two microbial sections may be employed during experimentation depending on the findings of Phase II, such sections will be sampled and analyzed as described above.)

Duplicate samples will be taken from each column section using a gas-tight syringe to remove liquid media through the septa-ports. Samples will be injected into double-septa purge-and-trap vials and analyzed immediately.

Biodegradation and biotransformation will be evaluated based on the disappearance of the parent solvents from the liquid medium and formation of degradation products which were not originally added to the column. The disappearance of the parent solvents and formation of degradation products will be compared to that found in a control soil-column which is described in Section 4.2.8. Statistical analysis of the rate or extent to which parent solvents disappear and degradation products form within the living soil-column versus the control column will be used to conclude whether biotransformations of the solvents is significant.

4.2.8 Control Soil-Column with Minimum Biological Activity

In order to evaluate the influence of stimulated biological activity on the removal of solvents in the soil-column, a control soil-column will be prepared in which biological activity is at a minimum. The only difference between the experimental column and the control column will be in the packing material and the nutrient solution used to deliver solvents. Leak testing and equilibration will be performed as described in Section 4.2.6.1.

The packing material used for the control column will consist of OU 2 soil mixed with equal parts of washed sand. The soil will be packed into the column sections and will be sterilized at 121° C for 45 minutes on three consecutive days. The column will be assembled and tested as described above.

Solvents will be delivered to the control column using sterile deionized water in place of a nutrient medium. It is possible that sterile conditions may not be sustainable in the control column since numerous samples will be withdrawn from the column over the life of the study and there is the chance that contamination with bacteria may occur. However, the biological activity of the control column should be significantly less than the experimental column, which is continuously supplied with nutrients and energy sources.

The concentration and types of solvents entering the column, leaving the first packed section and entering the second section, and leaving the second section will be measured using a purge-and-trap system coupled with a gas chromatograph/mass spectrometer (GC/MS). The analysis method is described in Section 6.2.3.

Liquid samples will be taken from each column section using a gas-tight syringe and removing liquid media through the septa-ports. Samples will be injected into double-septa purge-and-trap vials and analyzed immediately.

4.3 Plan of Soil-Column Experiments

At the time of the Phase III Test Plan preparation, Phase II experiments were not completed. Without this information, it is not possible to rule out any of the four microbial populations studied in Phase II as being incapable of performing solvent transformations. Therefore, it is assumed that Phase III will require testing all four populations in the soil-column described above. Data from Phase II will be available prior to initiation of Phase III experiments, so, the actual plan of experiments may be modified from the one described here. Table 4.2 shows the type of microbial populations each column will contain.

Table 4.2 Configuration and sampling events of soil-columns

Column	1st Population (1st Section)	Second Population (2nd Section)	Samples Per Event	Sampling Events
1	Denitrifiers	Methanotrophs	6	30
2	Sulfate Reducers	Methanotrophs	6	30
3	Methanogens	Methanotrophs	6	30
Control	Sterile Soil	Sterile Soil	6	30

Total Samples= 720

The number of samples and sampling events may vary depending on the initial findings of these experiments. It is expected that samples will be taken every 3 days for 3 months. If Phase II results show that any of the above microbial populations are not capable of transforming the solvents of interest, they will be removed from the experimental plan.

4.4 Optimization Study

Small batch-studies will be performed to determine the influence of electron donor concentration, electron acceptor concentration, and added nutrients on the biotransformation of the solvents of interest. These parameters will be varied in a systematic manner based on the Taguchi Method for design of experiments (Elliot 1987).

The variables of study for all four cultures include 1) the electron donor to electron acceptor ratio (e-donor/e-acceptor) in the culture, and 2) the carbon to nitrogen to phosphorus ratio (C/N/P) in the culture. The effects of interest include the individual influences of items 1 and 2 and the combined influence of items 1 and 2 on the extent of biotransformation of chlorinated solvents over a two-month period.

Both variables will be tested at three levels. The e-donor e-acceptor variable will be tested at 1, 0.5, and 1.5 times the standard ratio (based on weight) provided for each culture by the media described in Table 4.1. The C:N:P variable will be tested at 100:10:1, 100:25:1, and 100:10:10 based on weight. With this design, there are three effects of interest (the effect of the e-donor e-acceptor, the effect of the C:N:P, and the interaction between the two variables). The Taguchi Method prescribes an L9 orthogonal array for the design of an experiment with two independent variables, three variable levels, and three effects of interest.

Large batch cultures of each population will be prepared as described in the Biodegradation Treatability Study Work Plan (RFP/ERM-94-0007). Once stationary phase growth is reached, the cultures will be subdivided into double septa purge-and-trap vials with 1.5 grams of culture material to 20 milliliters liquid nutrient media. The independent variables and their levels are shown in Table 4.3. All other conditions of the culture test vials will be maintained at constant levels as described in the Phase II culturing procedure for this study (RFP/ER-94-0007). One culture test vial will be prepared for each of the following test conditions shown in Table 4.3.

Table 4.3 Level of each variable in each test vial

Culture Test Vial (Sample #)	e-donor e-acceptor (by weight) in Culture Vial	C:N:P (by weight) in Culture Vial
1	1X*	100:10:1
2	1X*	100:25:1
3	1X*	100:10:10
4	0.5X*	100:10:1
5	0.5X*	100:25:1
6	0.5X*	100:10:10
7	1.5X*	100:10:1
8	1.5X*	100:25:1
9	1.5X*	100:10:10

*For denitrifiers, X is the ratio of lactate + acetate to nitrate shown in Table 4.1

*For sulfate reducers, X is the ratio of lactate + acetate to sulfate shown in Table 4.1

*For methanogens, X is the ratio of lactate + acetate to carbonate shown in Table 4.1

*For methanotrophs, X is the % volume ratio of methane to oxygen shown in Table 4.1

The optimization experiment will be run in duplicate for each of the four cultures. So there will be 9 test vials per culture in duplicate (18 test vials) and 4 cultures to be tested. This requires a total of 72 analyses. The end-point of interest is the extent of biotransformation of TCE at 5 ppm at the end of two months incubation. Samples will be analyzed directly from the test vial using purge-and-trap GC/MS analysis described in Section 6.2.3.

5.0 EQUIPMENT AND MATERIALS

5.1 Phase III Equipment/Supplies

- a 40 ml Teflon septa vials
- b Gas chromatograph/mass spectrometer (GC/MS)
- c Purge-and-trap system
- d GC/MS column J&W DB-624 capillary column
- e Parent solvent standards 5000 µg/ml in methanol
 - Carbon Tetrachloride -1,1,2 Trichloroethane
 - Trichloroethylene -1,1,1 Trichloroethane
 - Tetrachloroethylene -Methylene Chloride
- f Parent and degradation product solvent standards 200 µg/ml in methanol
 - Purgeable A (Supelco, Inc)
 - Purgeable C (Supelco, Inc)
- g 10 µl, 100 µl, 10 ml, 25 ml gas-tight syringes
- h Analytical balance
- i Common laboratory glassware
- j Temperature controlled incubator
- k pH meter
- l Deionized water
- m Methanol
- n Mass spectrometer (Spectra Link Model 100)
- o Air-tight culture flasks with valve lid ports
- p Autoclave
- q Growth media nutrient solutions described in Table 4.1
- r Gas cylinders containing methane in air, N₂, H₂S in helium, and CO₂ in helium
- s Buffers/acids/bases
- t Soil-column reactors (4)
- u Spectrophotometer
- v HACH kits for nitrate and sulfate determination

6.0 SAMPLING AND ANALYSIS

6.1 Soil Sample Collection

Surficial soil was collected from within and around Individual Hazardous Substance Site (IHSS) 110 at OU 2. The sample includes soil down to approximately 6 inches. Approximately 100 pounds of bulk soil was collected by removing grasses and removing soil with a shovel from down to approximately six inches depth outside the boundary of the IHSS. The same procedure was used to collect a 10 pound sample from within the IHSS.

Subsurface soil and groundwater sediment was collected from OU 2 in a solvent contaminated area by drilling down approximately 20 feet using standard nonsterile methods to retrieve sediment cores. The exterior portion of the cores were removed using a sterile knife. The interior core material was purged with argon, placed in sterile containers, and stored at 4° C until experimentation.

6.2 Chlorinated Solvent Sampling and Analysis

6.2.1 Soil-Column Chlorinated Solvent Sampling

One ml aqueous samples will be removed from the soil-columns through the septa-ports as described in Section 4.0. Liquid will be injected into double septa vials and analyzed using purge-and-trap sample preparation and GC/MS analysis described in Section 6.2.3. Duplicate samples will be collected.

The number of samples and sampling events may vary depending on the initial findings of these experiments. It is expected that samples will be taken every 3 days for 3 months. If two populations are tested per column for four columns with all samples run in duplicate, then a total of 720 samples will be collected and analyzed (Table 4.2).

6.2.2 Optimization Study Chlorinated Solvent Sampling

The optimization study consists of preparing separate purge-and-trap vials for each test condition shown in Table 4.3. Entire purge-and-trap test vials will be sacrificed for analysis in duplicate. No subsampling will occur. The optimization experiment will be run in duplicate for each of the four cultures. A total of 72 test vials will be analyzed.

6.2.3 Purge-and-Trap GC/MS Analysis of Solvent Samples

All volatile solvent analyses will be performed using a Varian Star Saturn 3 gas chromatograph/mass spectrometer (GC/MS) with a Dynatech purge-and-trap system. Controls, blanks, and internal and external standards will be run with each sample set. Standard curves will be produced to establish the validity of the analytical method and to quantify GC/MS data.

Since this is an experimental process, EPA method 8260 has been modified to suit the instrument and needs of the study. The following deviations from method 8260 will occur during analysis: 1) a bromofluorobenzene (BFB) standard of 5 ng total will be used rather than the specified 25 ng; 2) a matrix spike of the solvents on the sample matrix (i.e., soil) will not be individually performed since all of the samples and standards being analyzed are, effectively, matrix spikes, 3) all the analytes identified with method 8260 will be detected and identified but only those of interest for the study will be quantified with standards. Precise and accurate quantitative data is required from chlorinated solvent analyses.

6.3 Soil-Column Electron Acceptor Samples and Analyses

6.3.1 Sampling for Electron Acceptors in the Soil-Column

The purpose of sampling and analyzing nitrate and sulfate is to ensure that the flow-rate of aqueous media through the soil-column is sufficiently slow to allow complete removal of these electron acceptors prior to the liquid moving into the next column section. The number of samples required and frequency of sampling will depend on the column operator's ability to properly adjust the flow-rate to achieve the desired electron acceptor removal. For the first column preparation, this will be based on trial-and-error.

The electron acceptors of interest for analysis are nitrate and sulfate. One ml aqueous samples from the soil-columns will be removed through the septa-ports. Samples will be analyzed immediately for either nitrate or sulfate as described in Section 6.3.2.

6.3.2 Analysis of Electron Acceptors

Colorimetric analysis of nitrate and sulfate is suitable to evaluate their concentrations in the aqueous phase of the columns.

Nitrate will be analyzed using a Cadmium Reduction Method HACH procedure DR/3000 N.6 for nitrate in the range of 0-5.0 mg/l (HACH 1986). A HACH DR/3000 Spectrophotometer will be used to quantify the concentration of nitrate in the samples.

Sulfate will be analyzed using a modified Barium Sulfate Turbidimetric Method described in HACH procedure DR/3000 S.6 for sulfate in the range of 0-50 mg/l (HACH 1986). A HACH DR/3000 Spectrophotometer will be used to quantify the concentration of sulfate in the samples.

A four-point standard curve will be prepared for both nitrate and sulfate to verify the performance of the instrument. One standard and one blank will be run with each analysis event.

6.4 Verifying Microbial Growth With Headspace Gas Analysis

During preparation of batch cultures for the optimization study, cultures will be grown in air-tight culture flasks equipped with sampling ports for removal of headspace gases. During growth, the populations of interest should generate or remove characteristic headspace gases in proportion to their growth rate. Measurement of these gases can be used as an indication of the growth rate of each population.

The methanotrophic culture will be supplied with an atmosphere of 2.5% methane in air as a growth substrate. The removal of methane from the headspace will indicate methanotrophic activity.

Denitrifying bacteria convert nitrate to nitrogen gas. The denitrifying culture will be supplied with an inert argon atmosphere so that changes in nitrogen concentrations in the headspace can be measured.

The sulfate reducing culture produces hydrogen sulfide during metabolism. Sulfate reduction will be verified by visual inspection for the presence of ferric sulfide precipitate. Carbon dioxide generation in the headspace will be used to quantify growth.

Finally, the methanogenic culture produces methane, which can be measured in the headspace samples.

6.4.1 Sampling and Analysis of Culture Headspace Gases

The growth curves for each population were established and verified in Phase II (RF/ER-94-0022 UN). Hence, the generation rates of the key gases over time can be predicted from past data for each population. Sampling and analysis of headspace gases will occur once during the expected log-phase growth and once during stationary-phase growth to verify the cultures are matching expected gas generation rates for the appropriate populations.

Such sampling will occur each time a new batch culture is prepared for either the optimization study or for subculturing to maintain active laboratory populations.

During sampling, a 10 ml gas sample will be pulled directly from the culture flask headspace via the lid-valve into a mass spectrometer for identification and quantification of gases. This 10 ml sample will provide enough volume for several successive analysis sweeps on the mass spectrometer. Gas sensitivities (amps/torr) will be evaluated for gas standards including carbon dioxide, oxygen, nitrogen, and methane prior to each analysis event.

The mass spectrometer used for analysis is a quadrupole mass spectrometer equipped with an electron bombardment source.

7.0 DATA ANALYSIS AND INTERPRETATION

Upon completion of the treatability study experiments, data will be presented and interpreted in accordance with Section 3.0 of the RFP Quality Assurance Project Plan (RFP QAPjP) which describes the requirements for data reduction, validation, useability criteria, and reporting of data (see Appendix A of RFP/ERM-94-0007). Data will be summarized and evaluated to determine the validity of measurements and performance of the treatment processes.

7.1 Interpretation of Chlorinated Solvent Data for Biotransformation Evaluation

The change in concentration of the solvents over time and the formation of degradation products indicates the occurrence of transformations. Significant disappearance of parent solvents and appearance of degradation products from active cultures or columns versus sterilized controls indicates biotransformations. Disappearance of parent solvents and appearance of degradation products in the sterilized control indicates abiotic losses or transformations when biological activity is minimized.

There is the possibility that some degradation products may not be volatile organics, and therefore not detectable using the gas chromatograph/mass spectrometer GC/MS. However, if transformations are occurring, it is expected that some degradation products would be volatile and detectable. The rate and extent to which volatile daughters are produced will be determined.

Data from this phase will be presented as curves showing the changes in parent and daughter product concentration versus time for each solvent. The rates of parent loss and degradation product formation will be calculated.

7.2 Interpretation of Optimization Study Results

The optimization study evaluates whether varying the electron donor to electron acceptor ratio and the carbon to nitrogen to phosphorus ratio will significantly affect the extent of biotransformation of TCE (at 5 ppm) after two months of incubation. The Taguchi Method for design of experiments is applied to the design of the optimization study (Allied-Signal 1987).

The combination of variables and the manner in which data is evaluated via statistical means is dictated by the L9 orthogonal array in which data is input at the conclusion of the experiment. The L9 array provides the means to easily compare data so that Analysis of Variance (ANOVA) can be applied to determine the significance of the singular effect of each variable and the combined effect of the variables versus noise. The significance of effect of each variable and the combined effect of the variables will be evaluated in this manner. The extent of biotransformation of TCE is the effect of interest.

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7.3 Headspace Data Analysis for Microbial Culture Growth

During culturing, headspace gas production will indicate culture growth. Mass spectrometer data for the concentration of methane, nitrogen gas, and carbon dioxide in the appropriate cultures will be determined at two points during culturing. The presence of the key headspace gases at the expected levels based on growth curves (i.e., natural logarithm of headspace gas production versus time) generated in Phase II will indicate the presence of the target populations.

8.0 DATA MANAGEMENT

Work performed, observations, and data for this study will be documented in bound notebooks and/or in detailed logbooks. All bound laboratory notebooks will be project specific.

All samples generated during the study will be labelled with unique sample identifiers. Sample identifiers and data will be documented. All other relevant details will be recorded in the notebooks. Data will be maintained in hard-copy and electronic forms. The following will be documented in notebooks and reports:

- Testing procedures
- Departure from protocols and reasons for departure
- Instrument calibration
- Sampling methods
- Chemical additions
- Test observations

It is anticipated that all analytical work will be performed by the Environmental Technologies Group of Technology Development.

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9.0 REPORTS

A final report will be prepared in accordance with Section 3.12 of The Guidance for Conducting Treatability Studies under CERCLA (EPA 1992) following the completion of the FY-95 portion of Phase II and Phase III testing. The report will document the results obtained through Phase III. The report will include recommendations and scope for future activities. The following outline will be used as a guide when preparing this report.

OUTLINE FOR TREATABILITY STUDY REPORT

1 0 INTRODUCTION

- 1 1 Site Description
- 1 2 Contaminant Description
- 1 3 Treatment Technology Description

2 0 TREATABILITY STUDY APPROACH/METHODS AND MATERIALS

- 2 1 Test Objectives and Rationale
- 2 2 Experimental Design and Procedures
- 2 3 Equipment and Materials
- 2 4 Sampling and Analysis
- 2 5 Data Management

3 0 RESULTS AND DISCUSSIONS

- 3 1 Data Analysis and Interpretation
- 3 2 Quality Assurance/Quality Control

4 0 CONCLUSIONS AND RECOMMENDATIONS

- 4 1 Conclusions
- 4 2 Recommendations
- 4 3 Key Contacts

References

Appendices

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10.0 QUALITY ASSURANCE, HEALTH & SAFETY, AND WASTE DISPOSAL

The Quality Assurance, Health and Safety, and waste disposal guidelines and documentation which apply to this study are discussed in the Biodegradation Treatability Study Work Plan in Section 9, Section 10, and Appendix A (RFP/ERM-94-0007).

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