US Army Corps of Engineers

Toxic and Hazardous Materials Agency

Task Order 8
Biotreatment of Gaseous-Phase Volatile Organic Compounds

Final Report

Contract DAAA15-88-D-0010

January 1991

Prepared for the:

United States Army
Toxic and Hazardous Materials Agency
(USATHAMA)

Prepared by:

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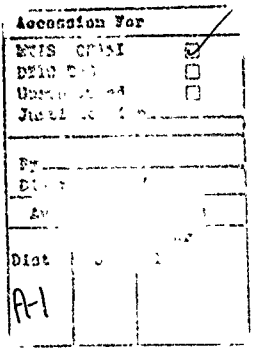

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Past disposal of volatile organic solvents has resulted in contamination of soils at U.S. Army installations. Technologies that involve volatilizing or air stripping contaminants from contaminated soils are used for remediation of these sites. The resulting stream of gaseous volatile organic compounds (VOCs) must be treated before the air stream is released to the atmosphere. Thermal treatment or carbon adsorption of gaseous VOCs is effective but expensive. The present study was conducted to evaluate the utility of using fixed populations of VOC-degrading microorganisms to destroy (mineralize to inorganic products) gaseous VOCs. This technology holds potential as a cost-effective alternative or supplement to thermal treatment or carbon adsorption.

The VOCs investigated include benzene, carbon tetrachloride, chlorobenzene, methyl ethyl ketone (MEK), toluene, and trichloroethylene (TCE).

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ABSTRACT

Radiolabeled (^{14}C) VOCs were used in the experiments to determine VOC destruction efficiency (conversion of organic ^{14}C to $^{14}CO_2$).

Continuous-flow and static microcosm experiments were conducted. A laboratory-scale gas generator was used to produce a defined stream of volatilized ^{14}C-VOC in air for the continuous-flow experiments. The gas stream entered a glass column that was filled with a solid matrix that supported a fixed population of microorganisms. Exhaust gasses from the column passed through a series of two impingers that absorbed VOCs and CO_2, respectively. The amounts of ^{14}C-organics and $^{14}CO_2$ evolved from the test matrix were periodically determined by liquid scintillation counting (LSC). The VOC destruction efficiency of the test matrix was evaluated by comparing the amounts of ^{14}C activity evolved as $^{14}CO_2$ to the total ^{14}C activity evolved ($^{14}CO_2$ plus ^{14}C-organics).

Static experiments were conducted using glass vials with teflon-lined caps as microcosms. Each microcosm contained a solid test matrix to support microbial growth and a known amount of ^{14}C-VOC. Some microcosms also received a known volume of biodegradable non-labeled organic solvent to serve as a co-substrate for ^{14}C-VOC biodegradation. Microcosms were incubated under ambient conditions. The VOC destruction efficiency of the test matrix was evaluated by comparing the amounts of ^{14}C activity evolved as $^{14}CO_2$ to the total ^{14}C activity evolved ($^{14}CO_2$ plus ^{14}C-organics).

All of the non-chlorinated VOCs tested (benzene, toluene, and methyl ethyl ketone) were extensively mineralized in uncontaminated soil. Under continuous-flow conditions, complete VOC destruction (100 percent conversion of ^{14}C activity to $^{14}CO_2$) was observed for all three non-chlorinated VOCs. The chlorinated VOCs tested (chlorobenzene, TCE, and carbon tetrachloride) were mineralized to lesser degrees than the non-chlorinated VOCs. The mineralization of TCE and chlorobenzene was enhanced by the presence of toluene as a co-substrate. Under continuous-flow conditions, destruction of chlorobenzene reached 100 percent for a short time. Carbon tetrachloride was not significantly mineralized under any conditions tested.

The experimental data suggest that biological filters may be a feasible technology for treating gaseous-phase VOCs, particularly non-chlorinated solvents such as benzene, toluene, and MEK.

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SECTION 1

EXECUTIVE SUMMARY

Past disposal of volatile organic solvents has resulted in contamination of soils at U.S. Army installations. Technologies that involve volatilizing or air stripping contaminants from contaminated soils are used for remediation of these sites. The resulting stream of gaseous volatile organic compounds (VOCs) must be treated before the air stream is released to the atmosphere. Thermal treatment or carbon adsorption of gaseous VOCs is effective but expensive. The present study was conducted to evaluate the utility of using fixed populations of VOC-degrading microorganisms to destroy (mineralize to inorganic products) gaseous VOCs. This technology holds potential as a cost-effective alternative or supplement to thermal treatment or carbon adsorption.

The VOCs investigated include benzene, carbon tetrachloride, chlorobenzene, methyl ethyl ketone (MEK), toluene, and trichloroethylene (TCE). Radiolabeled (^{14}C) VOCs were used in the experiments to determine VOC destruction efficiency (conversion of organic ^{14}C to $^{14}CO_2$).

Continuous-flow and static microcosm experiments were conducted. A laboratory-scale gas generator was used to produce a defined stream of volatilized ^{14}C-VOC in air for the continuous-flow experiments. The gas stream entered a glass column that was filled with a solid matrix that supported a fixed population of microorganisms. Exhaust gases from the column passed through a series of two impingers that absorbed VOCs and CO_2, respectively. The amounts of ^{14}C-organics and $^{14}CO_2$ evolved from the test matrix were periodically determined by liquid scintillation counting (LSC). The VOC destruction efficiency of the test matrix was evaluated by comparing the amounts of ^{14}C activity evolved as $^{14}CO_2$ to the total ^{14}C activity evolved ($^{14}CO_2$ plus ^{14}C-organics).

Static experiments were conducted using glass vials with teflon-lined caps as microcosms. Each microcosm contained a solid test matrix to support microbial growth and a known amount of ^{14}C-VOC. Some microcosms also received a known volume of biodegradable non-labelled organic solvent to serve as a co-substrate for ^{14}C-VOC biodegradation. Microcosms were incubated under ambient conditions. The VOC destruction efficiency of the test matrix was evaluated by comparing the amounts of ^{14}C activity evolved as $^{14}CO_2$ to the total ^{14}C activity evolved ($^{14}CO_2$ plus ^{14}C-organics).

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The experimental data suggest that biological filters may be a feasible technology for treating gaseous-phase VOCs, particularly non-chlorinated solvents such as benzene, toluene, and MEK.

SECTION 2

INTRODUCTION

2.1 LITERATURE REVIEW

As a result of past solvent handling practices, soils at several U.S. Army installations are contaminated with volatile organic compounds (VOCs). These compounds include benzene, trichloroethylene (TCE), toluene, methyl ethyl ketone (MEK), carbon tetrachloride, and chlorobenzene. If left untreated, VOCs may eventually migrate and contaminate groundwater supplies. Several treatment alternatives are available, including:

- Soil excavation and subsequent disposal in a hazardous waste landfill.
- In situ volatilization.
- Thermal stripping (combustion or activated carbon adsorption of the off gasses is typically required).

However, these technologies have distinct disadvantages, including high cost, transfer of the contaminant from one medium to another, and lack of being a final solution in an increasingly more restrictive regulatory climate.

The U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) is investigating the ability of microorganisms to biologically degrade gaseous-phase VOCs. Organic chemicals, including several contaminating U.S. Army sites, are susceptible to biodegradation by microorganisms. Biodegradation can be defined as the molecular degradation of an organic substance resulting from the action of living organisms. Ideally, the compound of interest will be completely mineralized, and only carbon dioxide, simple inorganics such as nitrogen and phosphate, and water will remain as end products.

Environmental variables and complex chemical structures may result, and instead, an intermediate compound may be produced that may be less, equally, or even more toxic than the parent compound from which it was derived (EPA, 1989). Therefore, laboratory-scale studies must be carefully conducted to determine whether a compound can be degraded, under what conditions a chemical is optimally degraded, and what metabolites are produced as a result of this degradation.

Mineralization is the conversion of an inorganic chemical to inorganic products such as CO_2, H_2O, SO_4, and N_2. Biodegradation and biotransformation are less precise terms that may describe either mineralization or transformation of a parent compound to another organic. Radiolabeled (^{14}C) test compounds were used to determine the mineralization of the VOCs of interest, with degradation being determined by the production of $^{14}CO_2$. The use of radioisotopes is the most sensitive and precise technology available for mineralization studies.

Contaminant-degrading organisms are often found at contaminated waste sites (EPA, 1989). Organisms present as part of the natural microflora can often be augmented with rate-limiting elements and nutrients so that their metabolic activity is increased. Alternatively, commercially-available organisms may be used, or microbes can be isolated from the waste site, produced in batch culture, and reintroduced into the matrix on site.

Many factors of the waste-containing substrate affect the efficiency of biodegradation. These factors must be optimized and continually controlled either in situ or in some type of reactor system so that microbial activity is maximized. Critical factors include available soil moisture, oxidation/reduction potential, temperature, oxygen, pH, and nutrient concentrations and availability (EPA, 1989; Paul and Clark, 1989). The following variables were examined during this study:

- Substrate and co-substrate concentrations.
- Inorganic nutrient amendments.
- Solid matrix composition and moisture addition.
- Gas flow rate through the biological filter (residence time of VOCs in the filter).

Microorganisms investigated include:

- Unacclimated soil microbes.
- Methanotrophs.
- Aromatic hydrocarbon degraders.
- White-rot fungi (Phanerochaete chrysosporium).

The following VOCs were investigated:

- Benzene.
- Carbon tetrachloride.
- Chlorobenzene.
- Methyl ethyl ketone.
- Toluene.
- Trichloroethylene.

2.1.1 Benzene

Benzene (C_6H_6; CAS Registry No. 71-43-2) is a toxic, carcinogenic substance, and its physical properties are shown in Table 2-1. Even though it is produced in extremely large amounts, measured environmental concentrations are low, reflecting benzene's efficient degradation (Dagley, 1971).

Although volatile, released product that does not evaporate may migrate to groundwater. Biodegradation of liquid product does occur in soil, although half-lives vary

Table 2-1. Physical properties of VOCs used during these studies.

Chemical	Molecular Weight	Melting Point (°C)	Boiling Point (°C)	Water Solubility (mg/L)	Vapor Pressure (mmHg; 25°C)
Benzene	78.1	5.5	80.1	1,791; 25°C	95.2
Carbon tetrachloride	153.8	-23	76.5	805; 20°C	113.8
Chlorobenzene	112.6	-45	132	472; 25°C	11.9
Methyl ethyl ketone	72.1	-86	79.6	239,000; temp unknown	90.6
Toluene	92.1	-95	110.6	534; 25°C	28.4
Trichloroethylene	131.4	-73	87	1,100; 25°C	69.0

considerably, depending upon environmental conditions, and range from 2 to 28 days (Dagley, 1971; Howard, 1990).

2.1.2 Carbon Tetrachloride

Carbon tetrachloride (CCl_4; CAS Registry No. 56-23-5) is a toxic, flammable chemical often used in industry. Few data exist regarding biological degradation, but it apparently did degrade in 16 days under anaerobic conditions during one study (Howard, 1990). Because of its low soil adsorption coefficient (a measurement of how readily a substance is sorbed to soil), it is expected to migrate in groundwater (Howard, 1990). Additional physical data are presented in Table 2-1.

2.1.3 Chlorobenzene

Chlorobenzene (C_6H_5Cl; CAS Registry No. 108-90-7) is used as an industrial solvent and pesticide. It is fairly volatile and is somewhat degradable under proper environmental conditions, especially warmer weather. However, as with all chlorinated aromatics, degradation is more difficult than for non-chlorinated aromatics. It can be expected to leach into groundwater (Howard, 1990).

2.1.4 Methyl Ethyl Ketone

Methyl ethyl ketone (C_4H_8O; CAS Registry No. 78-93-3) is a frequently used solvent in many industrial processes. When released into soil, it will partially evaporate and may leach into groundwater because it is poorly hydrolyzed. Biological degradation appears to be excellent (Howard, 1990); physical constants are given in Table 2-1.

2.1.5 Toluene

Toluene (C_7H_8; CAS Registry No. 108-88-3) is often accidentally released into the environment as a by-product of industrial processes. Like benzene, toluene is readily and ultimately biodegradable. Depending upon environmental conditions, toluene is often degraded in 8 days, with a 3- to 4-day acclimation period; the biodegradation processes are well understood (Gibson, 1980). Upon release, toluene is susceptible to evaporation and biodegradation (Howard, 1990).

2.1.6 Trichloroethylene

Trichloroethylene (C_2HCl_3; CAS Registry No. 79-01-6) is often used in degreasing metals and may be released into the environment in air, wastewater, or spills. While liquid product may rapidly evaporate, it will also leach into groundwater. Trichloroethylene degrades poorly in soil and water (Howard, 1990).

2.2 REPORT OBJECTIVE

This report evaluates the potential for degrading gaseous-phase organic contaminants of interest to the U.S. Army, using biotreatment technologies. The purpose of the study was

to determine whether biotreatment of vapor-phase VOCs is feasible. Specifically, the objectives of this study were to:

- Determine whether mineralization of gaseous-phase VOCs of interest can occur.
- If mineralization does occur, determine the extent of mineralization for VOCs of interest in the gaseous phase.
- Determine optimal conditions for the mineralization of gaseous-phase VOCs while minimizing emissions of VOCs from the biological filter.

SECTION 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Test Chemicals

The following uniformly labeled (UL) organic chemicals were used to measure the amount of biodegradation:

- [UL-^{14}C] benzene (Chemsyn Science Labs).
- [UL-^{14}C] carbon tetrachloride (New England Nuclear).
- [UL-^{14}C] trichloroethylene (Sigma Chemical Co.).
- [UL-^{14}C] methyl ethyl ketone (Chemsyn Labs).
- [ring-UL-^{14}C] toluene (Chemsyn Labs).
- [UL-^{14}C] chlorobenzene (Sigma Chemical Co.).

All compounds were uniformly labeled, including aromatics. This gives a representative indication of the mineralization of all carbon atoms in a given molecule, not just those in a particular location.

3.1.2 Non-Labeled VOCs

Non-labeled test compounds of the highest obtainable purity (> 99%) were obtained from Aldrich Chemical Co., Milwaukee, WI. Non-labeled VOCs were used to prepare dilute stock solutions of ^{14}C-VOCs and as co-substrates during some experiments.

3.1.3 Stock Solutions

Stock solutions of VOCs were prepared for use in mineralization studies. Radioisotopes were diluted in a volume of the corresponding non-labeled VOC. The specific activity of each stock solution was determined based upon the volume of non-labeled compound added and the measured ^{14}C activity in the resulting stock solution. These specific activity values (units of radioactivity/unit volume) relate the amount of ^{14}C activity to a volume or mass of total (^{14}C plus non-labeled) stock solution and were used to convert the concentrations of ^{14}C activity detected in test samples to units of mass or volume concentration. Stock solutions were prepared so that the smallest mass of VOC that could be detected by radioassay was approximately 1 nl (nanoliter). Stock solutions were prepared at a nominal ^{14}C concentration of 10,000 dpm/ml, so that the presence of 1 nl in a scintillation vial would be detected as 10 dpm.

3.1.4 Liquid Scintillation

A Tracor Analytic 6895 liquid scintillation counter (LSC) was used to quantify ^{14}C activity in experimental samples. The LSC was calibrated by counting a set of eight sealed ^{14}C standards of varying degrees of quench. This calibration provided quench correction and

standardization of the counter to a known ^{14}C standard. One of the sealed standards was counted on a daily basis when test samples were run. If ^{14}C activity deviated more than 5 percent from the certified ^{14}C level, the counter was recalibrated.

Three background samples that contained only the appropriate LSC cocktail and blank sample matrix were counted before each batch of test samples. Samples were counted for at least 2 minutes each. Average background activity was subtracted from test samples, and counts per minute (cpm) was converted to disintegrations per minute (dpm) by the external standards ratio (ESR) method.

3.1.5 Test Matrices

Two types of soil were collected for use as solid test matrices during the experiments. Soil contaminated with fuel oil was chosen because of the presence of organisms already exposed to organic compounds. Uncontaminated soil was obtained from a nearby site. Soil characteristics are shown in Table 3-1.

In addition to soil, wood chips inoculated with white-rot fungus (Phanerochaete chrysosporium) were used as a test matrix. Untreated wood chips were saturated with a mineral salts solution (see Subsection 3.2.4), autoclaved (121°C, 30 minutes), and inoculated with the fungus from agar slants. The culture was incubated at 35°C for 3 weeks until the fungus visibly grew on the wood chips. These wood chips were then used as a test matrix.

3.1.6 Temperature

All experiments were conducted at ambient temperature as specified in the test plan (approximately 20° to 25°C).

3.1.7 Continuous-Flow Test Apparatus

Continuous-flow experiments were conducted using an apparatus that consisted of the following components: a gas generator vessel, a test column, a bubble flow meter, and a variable-speed air pump. Air flowed through the apparatus by negative pressure in the following order: bubble flow meter, gas generator, test column, volatile compound trapping apparatus, air pump.

3.1.7.1 Gas Generator

A gas generator was used to produce gas streams for use in continuous-flow experiments. The gas generator consisted of glass and teflon diffusion chambers and gas diffusion tubes. A gas diffusion tube is a liquid-filled (liquid VOC in this instance) reservoir with a long open-top capillary neck. The mass diffusion rate of gaseous material from the end of the capillary tube neck is a function of the inside diameter and length of the neck. The amount of chemical diffused into the gaseous phase over time was determined by weight loss of the diffusion tube. Gas diffusion tubes were placed inside the diffusion chambers.

Table 3-1. Characteristics of test soils.

Soil Type	TPH (mg/kg)	Solids (%)	Moisture (%)	TOC (mg/kg)	pH
Contaminated	24,000	73.5	26.5	53,500	5.8
Uncontaminated	11	85.3	14.7	8,520	7.0

TPH: Total petroleum hydrocarbons
TOC: Total organic carbon

The gas generator worked by passing a stream of air into the diffusion chamber (which contains a diffusion tube filled with the VOC of interest). Air flowing out of the chamber contained a concentration of gaseous test chemical that was dependent upon the characteristics of the individual diffusion tube employed and the air flow rate. The air flowed directly into the test column by means of glass and/or teflon tubing (see Figure 3-1).

Diffusion tubes were characterized before use in mineralization experiments by documenting the mass loss of VOC from the tube as a function of time. Each VOC exhibited a characteristic mass transfer rate in a given diffusion tube. Mass transfer rates (in terms of mass of VOC evolved as vapor per unit time) were used to calculate the concentration of gaseous VOC in a gas stream emitted by the gas generator, using Equation 1.

$$C_a = K_m / F_a \qquad \text{Equation 1}$$

where:

C_a = Concentration of VOC in air stream leaving the diffusion chamber (μg/mL).

K_m = Mass loss of VOC as vapor from permeation or diffusion tube (μg/min).

F_a = Flow rate of air through the gas generator diffusion chamber (mL/min).

The calculated value of C_a was confirmed by trapping the evolved gaseous VOC in the gas trapping device (see Subsection 3.1.9). The amount of ^{14}C activity recovered in the trapping device was converted to mass or volume of VOC by using the specific activity of the VOC stock solution.

3.1.8 Test Columns

Test columns consisted of liquid chromatography columns with threaded connectors on both ends. All internal surfaces of the columns were constructed of glass or teflon. The gas flow rate for individual experiments was determined by the internal volume of the column and the flow rate capability of the gas generator. The columns were packed with test matrix appropriate for the particular experiment (see Subsection 3.2.8). A defined gas stream, consisting of a known amount of ^{14}C-VOC in air, was passed through the column at a measured flow rate. Flow rates were measured with a bubble flow meter. Exhaust gases passed through the gas sampling apparatus described in Subsection 3.1.9.

3.1.9 Volatile Compound Trapping Apparatus

Exhaust gases from the columns passed through a gas sampling apparatus to separate and trap volatilized ^{14}C-organics and $^{14}CO_2$. The gas sampling apparatus consisted of two LSC vial impingers that contained LSC cocktail. The first contained Betafluor, which retains only organic compounds. The second impinger contained Oxasol-^{14}C LSC cocktail. Oxasol-^{14}C LSC cocktail is an alcohol/amine-based cocktail that absorbs CO_2 from gases. Impinger vials were removed from the sampling apparatus and placed directly into the

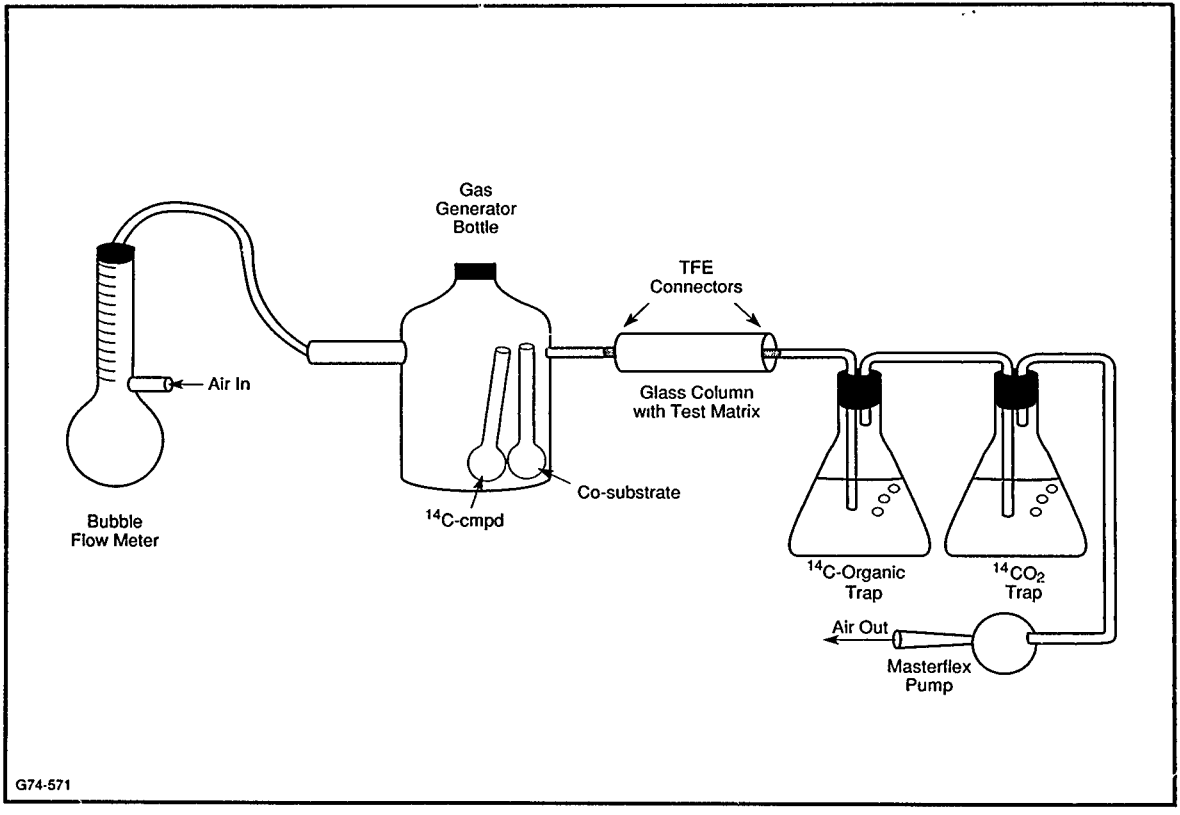


FIGURE 3-1 DIAGRAM OF APPARATUS USED IN FLOW THROUGH EXPERIMENTS

liquid scintillation counter for ^{14}C determination. The system is a modified version of the apparatus described by Marinucci and Bartha (1979).

3.2 METHODS

3.2.1 Continuous-Flow Experiments

Continuous-flow experiments were conducted as follows. A test column was weighed, filled with appropriate solid test matrix, reweighed, and attached between a gas generator and a volatile compound trapping apparatus. A diffusion tube was weighed, filled with approximately 1 mL of ^{14}C-VOC stock solution, reweighed, and placed inside the gas diffusion vessel Two impinger vials were filled with the appropriate trapping solutions and attached to the trapping apparatus. Air flow was then initiated through the apparatus.

The impinger traps were removed and replaced with fresh traps daily during the experiments. Used vials were placed directly into the LSC for ^{14}C determination. The flow rate of air into each gas generator vessel was determined weekly with the bubble flow meter.

Data were reduced as follows. On a daily basis, the total ^{14}C activity evolved from the test column was calculated as the sum of the ^{14}C-organics and $^{14}CO_2$ detected by the LSC in the impinger vials. The ^{14}C activity as ^{14}C-organics and $^{14}CO_2$ was expressed as a percent of the total ^{14}C activity evolved. The daily percent $^{14}CO_2$ evolved was plotted as a function of time for each experiment. Evolution of 100 percent $^{14}CO_2$ indicated that no detectable ^{14}C-organic was evolved from the column at that point in time, indicating complete VOC destruction in the column.

Each experiment was run for a sufficient period of time to reach equilibrium for the gas flow rate, the VOC loading rate, and the resulting extent of $^{14}CO_2$ production.

The primary objective of the continuous-flow-experiments was to demonstrate mineralization of the VOCs. Once this was established for a given VOC, the second objective was to determine the optimal conditions for VOC mineralization. During some experiments an attempt was made to determine the maximum air flow and VOC loading rate for a given volume of test matrix while maintaining near-optimal contaminant destruction and minimizing break-through of parent compound. Maximum acceptable air and VOC flow rates may be used as parameters for evaluating the feasibility of full-scale VOC biotreatment.

3.2.2 Static Microcosm Experiments

Experiments also were conducted in static microcosms with chlorinated ^{14}C-VOCs. Static experiments were conducted because they required less time to conduct than continuous-flow experiments. All treatments were run in triplicate.

Static microcosm experiments were conducted as follows. For experiments with unacclimated soils, ten grams of solid test matrix was added to autoclaved 40 mL glass vials with teflon-lined septa. For the experiments with pre-acclimated soils, five grams of solid

test matrix was added to autoclaved 25 mL glass vials with teflon-lined septa. The solid matrix was then directly spiked with a measured amount of stock solution that contained a known ratio of ^{14}C-VOC to non-labeled co-substrate. The vials were quickly sealed and incubated in the dark at ambient temperature before sampling.

After incubation, the headspace in the microcosms was sampled for ^{14}C-organics and $^{14}CO_2$ as follows. The vial was attached to a volatile compound trapping apparatus (^{14}C-organic and $^{14}CO_2$ impingers, Figure 3-1) by means of a hypodermic needle and plastic tubing. For destructive samplings, approximately 15 mL of 10% HCl solution was injected into the microcosm through the TFE septum (the microcosms were acidified to drive off ^{14}C-carbonates as $^{14}CO_2$). For non-destructive samplings, the microcosms were not acidified (non-destructive samplings were conducted to replenish oxygen in the microcosms). A second hypodermic needle was inserted through the septum to allow air to flow into the microcosm. A negative pressure air pump was used to pull air through the microcosm and the trapping apparatus, retaining ^{14}C-organics and $^{14}CO_2$ in the respective impinger vials. Each microcosm was sampled in this manner for 15 minutes.

Data were reduced in the same manner as for continuous-flow experiments, the amount of $^{14}CO_2$ evolved being expressed as a percentage of the total ^{14}C activity ($^{14}CO_2$ plus ^{14}C-organics) evolved.

Some static experiments were conducted using soil that had been pre-acclimated to specific gas-phase VOCs. Pre-acclimation was accomplished as follows. Contaminated and uncontaminated soil (Table 3-1) were mixed at a ratio of 50 parts uncontaminated to 1 part contaminated (to obtain microorganisms that had been previously exposed to hydrocarbons). The moisture content of the soil mixture was determined by mass loss upon drying at 105°C and the soil moisture content was raised to 50 weight percent by the addition of a mineral salts medium solution. Four wide-mouth glass jars were used as pre-acclimation vessels. The jars were 2-L in volume and were sealed with teflon-lined screw caps. The jars contained approximately 400 g of soil mixture. Gaseous VOCs were introduced to the jars by placing a 25 mL glass vial that contained either toluene, phenol, or methanol inside. The fourth jar contained no VOC and served as a source of control soil. The VOCs were allowed to contact the soil only in the vapor phase. The jars were sealed and incubated at ambient temperature until the soils were used in microcosm experiments. Twice weekly during the pre-acclimation period, the jars were opened (to replenish oxygen in the headspace), the soils stirred, and the VOC vial refilled if necessary. Pre-acclimation was conducted for two weeks before the soils were used in the first microcosm experiment.

Non-destructive samplings were utilized only during experiments with pre-acclimated soils. For data reduction purposes, the total amounts of ^{14}C-organic and $^{14}CO_2$ activity evolved when two sampling points were used was combined.

A summary of the static microcosm experimental designs is presented in Table 3-2.

Table 3-2

Summary of Static Microcosm Experimental Designs

Soil	^{14}C-substrate	Non-labeled Co-substrate	Non-destructive Sampling	Destructive Sampling
unacclimated*	carbon tetrachloride	methanol	-	day 5
unacclimated*	carbon tetrachloride	toluene	-	day 5
unacclimated*	TCE	methanol	-	day 5
unacclimated*	TCE	toluene	-	day 5
unacclimated*	chlorobenzene	methanol	-	day 5
unacclimated*	chlorobenzene	toluene	-	day 5
pre-acclimated**	TCE	methanol	day 6	day 9
pre-acclimated**	TCE	toluene	day 6	day 9
pre-acclimated**	chlorobenzene	methanol	day 7	day 10
pre-acclimated**	chlorobenzene	toluene	day 7	day 10

* - conducted with 10 g of soil in 40 mL microcosms.
** - conducted with 5 g of soil in 25 mL microcosms.

3.2.3 Test Conditions and Variables

3.2.3.1 Fixed Parameters

The following parameters were fixed and not subject to experimental investigation:

Temperature. Temperature markedly affects biological reaction rates. However, temperature was not investigated as an experimental variable. Experiments were conducted at ambient temperature (20° to 26°C).

Redox Potential and pH. The redox potential and pH dependency for mineralization of the test compounds were not subject to experimental investigation, and the pH of the test matrices was not altered.

3.2.3.2 Variable Parameters

The following parameters were investigated as experimental variables.

Sterile Controls versus Active Test Matrix. To ensure that test compound mineralization was of biological origin, sterile controls were used for comparison with viable test matrices. The test matrices for sterile controls were sterilized by autoclaving (121°C, 15 psi, 30 minutes).

Source of Microflora. Microorganisms used in the mineralization studies were obtained from several sources. However, emphasis was placed on enhancing the naturally occurring microflora in the test matrices. The micro-organism used for each experiment was determined on an experiment-by-experiment basis. Organisms tested include unacclimated soil microorganisms; soil organisms used in static experiments that were preacclimated to methanol, toluene, or phenol; and white-rot fungus (Phanerochaete chrysosporium). Preacclimated organisms and white-rot fungus were used only for VOCs that were poorly mineralized by unacclimated soil microorganisms.

If applicable, test matrices were inoculated with specific organisms and/or pre-acclimated to a specific VOC before initiating mineralization experiments. Test matrices were preacclimated by exposing the matrix to the appropriate gaseous non-labeled VOC in a glass jar.

Co-Substrates. Phenol and toluene facilitated the mineralization of TCE by an organism isolated by Nelson et al. (1987). This organism has been referred to as G4. This ability to degrade TCE while metabolizing phenol or toluene subsequently has been observed in a number of microorganisms. Consequently, phenol and toluene were investigated as co-substrates for chlorinated ^{14}C-VOCs.

Methanotrophs use compounds such as methane and methanol as growth substrates. Naturally occurring methanotrophs have been found to co-metabolize TCE. However, TCE is not used as a growth substrate and competes with methane for the monooxygenase. Consequently, methane can be used as a co-substrate for TCE degradation, but its concentration must be controlled. The concentration ratio of VOC to co-substrate was

investigated. Methanotrophs have not, to date, proven effective on C_1 or C_2 compounds containing greater than three chlorine atoms.

Inorganic Nutrients. Inorganic nutrients were added to test matrices to determine if indigenous nutrients were rate-limiting. Inorganic nutrients were added to the solid test matrices in the form of a dilute mineral salts medium. The mineral salts medium contained per liter of reagent grade water; 400 mg KH_2PO_4, 1,130 mg Na_2HPO_4, 1,000 mg NH_4NO_3, 200 mg $MgSO_4*7H_2O$, 117 mg $Na_2CO_3*H_2O$, 10 mg $CaCl_2*2H_2O$, 23 mg $MnSO_4*H_2O$, and 5 mg $FeSO_4*7H_2O$. This media has been previously used to grow hydrocarbon-degrading microorganisms (Jamison et al., 1976).

Moisture Content. The natural moisture content of the test matrices varied. A moisture content of approximately 50 percent (by weight) is generally accepted as beneficial to bacteriological activity in soils and similar substrates. Fungi generally require a lower moisture content. Increased moisture content in porous media decreases the permeability of the matrix to air. The moisture content of the test matrices was adjusted by addition of reagent grade water or a mineral salts medium solution as detailed for individual experiments. Comparisons were made between flow-through columns tested at ambient moisture content and amended to 50 wt% during experiment No. 3.

Test Matrices. Soil and wood chips were investigated as solid test matrices.

Exposure Time. Experiment length varied depending upon the nature of the test compounds, matrix, and air flow rates. The contact time between the gaseous VOCs and test matrix was investigated as an experimental variable. Experiments were terminated when equilibrium for air flow rate, VOC loading rate, and VOC mineralization became established for a given set of experimental conditions.

3.3 EXPERIMENTAL DESIGNS

The experimental work in this project was split into seven experimental phases. Each of the phases/experiments is described in the following subsections.

3.3.1 Experiment 1

Experiment 1 was conducted to characterize the VOC volatilization rate from the diffusion tubes. Diffusion tubes with inside neck diameters of 0.5, 1, 2, and 4 mm were used. The experiments were conducted by filling a tared diffusion tube with approximately 0.5 mL of non-labelled VOC and reweighing the tube periodically to determine the mass of volatilized VOC.

3.3.2 Experiment 2

This experiment was aborted and the data not used because several microcosms were broken during incubation.

3.3.3 Experiment 3

Experiment 3 was conducted using continuous-flow test systems to investigate the mineralization of ^{14}C-toluene. The experimental design is described in Table 3-3.

The objectives of the experiment were to validate the test apparatus and to determine the mineralization of ^{14}C-toluene under ambient (column 1), enhanced (columns 2 and 3), and sterile/negative (columns 4 and 5) conditions. The proper separation of ^{14}C-organics and $^{14}CO_2$ was demonstrated through the use of column 5. If the trapping apparatus functioned properly, no $^{14}CO_2$ should have been detected from column 5.

3.3.4 Experiment 4

Experiment 4 was conducted to determine the mineralization of ^{14}C-benzene, ^{14}C-carbon tetrachloride, ^{14}C-chlorobenzene, ^{14}C-toluene, and ^{14}C-TCE in uncontaminated soil. All treatments were run using 10-cm columns (31.4 cc) with uncontaminated soil that was amended with 0.17 mL/g sterile mineral salts medium solution. An additional column was run without any gaseous ^{14}C-VOC loading to determine background ^{14}C levels.

3.3.5 Experiment 5

Experiment 5 was conducted to determine the mineralization of ^{14}C-TCE in the presence of non-labeled toluene co-substrate. Toluene was added to the test gas stream by including a second diffusion tube in the gas generator vessel for each column. Test conditions were as noted in Subsection 3.3.4. Four columns were run, as noted in Table 3-4.

The objective was to determine whether ^{14}C-TCE mineralization was enhanced (as compared to Experiment 4) in the presence of varying amounts of toluene co-substrate.

3.3.6 Experiment 6

Experiment 6 was conducted to determine:

- The mineralization of ^{14}C-MEK in uncontaminated soil.
- The mineralization of ^{14}C-carbon tetrachloride in the presence of methanol and toluene co-substrates in soil.
- The mineralization of ^{14}C-chlorobenzene in the presence of methanol and toluene co-substrates in soil.
- The mineralization of ^{14}C-chlorobenzene by white-rot fungus in wood chips.

All gaseous ^{14}C-VOCs were produced from 2-mm ID diffusion tubes. The toluene and methanol co-substrates were produced from 4-mm diffusion tubes. All test matrices received 0.17 mL/g of sterile mineral salts medium solution.

Table 3-3. Design of Experiment 3.

Column	Matrix
1	Uncontaminated soil -- unamended.
2	Uncontaminated soil -- 0.17 mL/g water added.
3	Uncontaminated soil -- 0.17 mL/g MM added
4	Uncontaminated soil -- 0.17 mL/g MM added, autoclaved to serve as sterile control.
5	Column left empty to serve a no matrix control

Table 3-4. Design of Experiment 5.

Column	Diffusion Tube 1		Diffusion Tube 2	
	Compound	Diameter (mm)	Compound	Diameter (mm)
14	Toluene	4	^{14}C-TCE	2
15	Toluene	2	^{14}C-TCE	2
16	Toluene	1	^{14}C-TCE	2
17	Toluene	0.5	^{14}C-TCE	2

3.3.7 Experiments 7 and 8

Experiments 7 and 8 consisted of a series of static microcosm experiments to evaluate the mineralization of ^{14}C-TCE, ^{14}C-chlorobenzene, and ^{14}C-carbon tetrachloride in soil in the presence of varying amounts of toluene and methanol co-substrates. During some experiments, soils were preacclimated to phenol, methanol, and toluene (Table 3-2). All test soils were amended with 0.10 mL/g of sterile mineral salts medium solution (see Subsection 3.2.2).

SECTION 4

RESULTS

4.1 PERFORMANCE OF TEST APPARATUS

Data from Experiment 3 (the first continuous-flow experiment) were used to demonstrate the proper operation of the test apparatus. Toluene was chosen as the ^{14}C-VOC substrate because it was expected to be readily mineralized under favorable conditions. Column 5 (no matrix control) produced only background levels of ^{14}C activity in the $^{14}CO_2$ impinger (see Figure 4-1), demonstrating negligible carryover of organic ^{14}C activity to the $^{14}CO_2$ impinger. Column 4 (autoclaved control) also produced only background levels of ^{14}C activity in the $^{14}CO_2$ trap (see Figure 4-2).

Increased levels of $^{14}CO_2$ were produced in columns that contained active soil relative to the no matrix and autoclaved controls, demonstrating the role of microbial activity in the appearance of ^{14}C activity in the $^{14}CO_2$ impingers (see Figures 4-3, 4-4, and 4-5).

The mass of ^{14}C-toluene that volatilized from the diffusion tube was compared to the mass of ^{14}C-toluene that was recovered in the impingers for Experiment 3. These comparisons were conducted to determine the transfer efficiency of VOCs from the diffusion tubes to the impingers. Comparisons were run two ways:

- By comparing the recovery of ^{14}C-toluene (which can be converted to mass) in the impingers to the mass loss of the diffusion tubes.
- By comparing the recovery of ^{14}C-toluene in the impingers to the measured diffusion rate of toluene from a 4-mm diffusion tube extrapolated to the length of the experiment (see Subsection 4.2).

Results are summarized in Table 4-1.

Based on the mass loss of the diffusion tubes, the average recovery of ^{14}C-toluene in the impingers was 77 percent (range 67 to 84 percent, standard deviation 7.2 percent). (These values were calculated as [column (2) / column (1) * 100 percent] in Table 4-1). Recovery of ^{14}C activity was highest in column No. 5, which contained no test matrix.

Based on the previously measured diffusion rate of toluene from a 4-mm diffusion tube, the average recovery of ^{14}C-toluene in the impingers was 69 percent (range 59 to 76 percent, standard deviation 7 percent). (These values were calculated as [column (2) / column (3) * 100 percent] in Table 4-1).

4.2 VOC VOLATILIZATION RATES

The mean diffusion rates for the six VOCs (as measured by mass loss during Experiment 1) are presented in Table 4-2. All mineralization data are presented in tabular form in Appendix A.

FIGURE 4-1 MINERALIZATION OF TOLUENE IN EMPTY SOIL COLUMN
(NO-SOIL CONTROL)

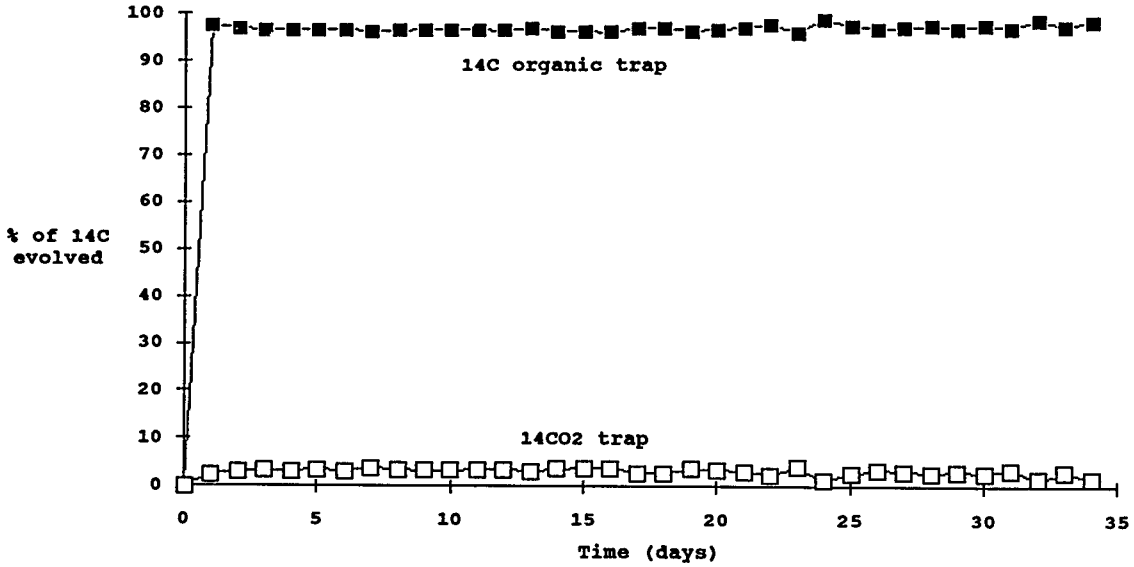


FIGURE 4-2 MINERALIZATION OF TOLUENE IN AUTOCLAVED SOIL

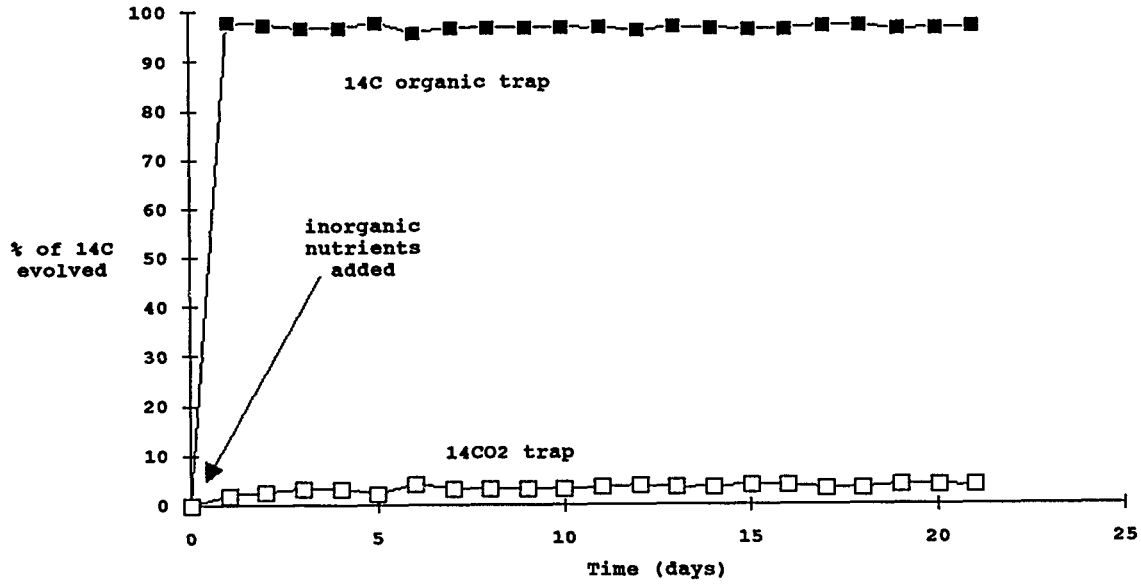


FIGURE 4-3 MINERALIZATION OF TOLUENE IN UNAMENDED SOIL

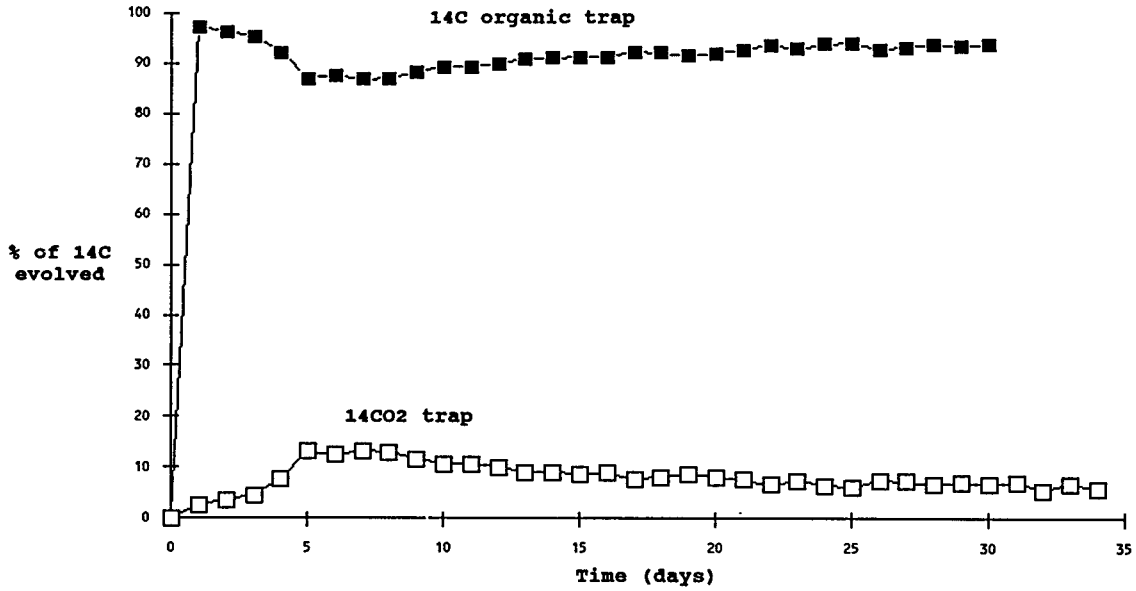


FIGURE 4-4 MINERALIZATION OF TOLUENE IN AMENDED SOIL

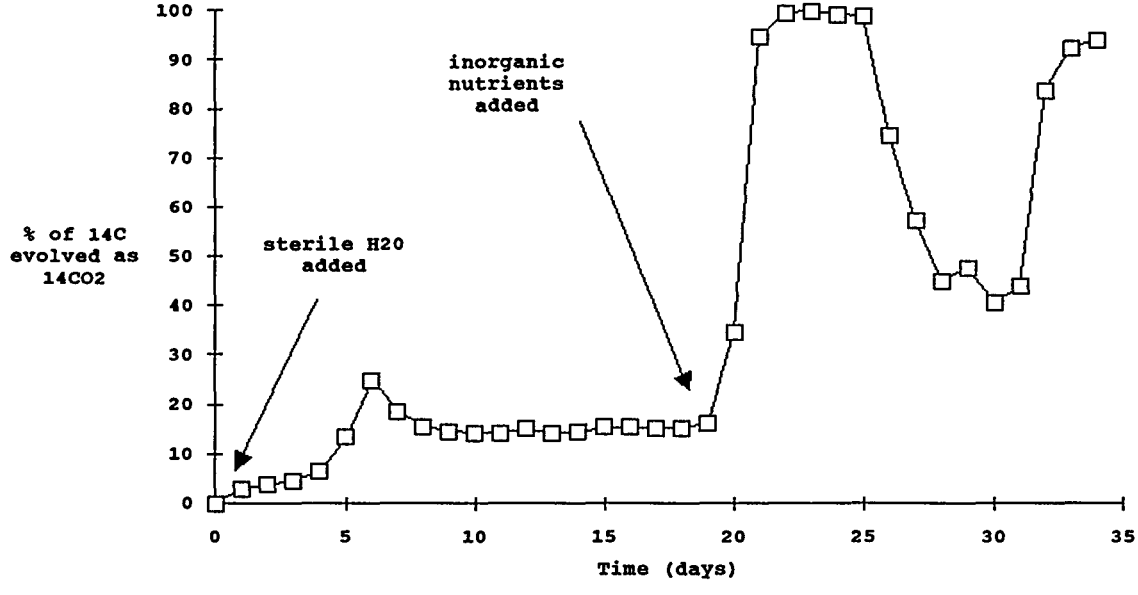


FIGURE 4-5 MINERALIZATION OF TOLUENE IN AMENDED SOIL

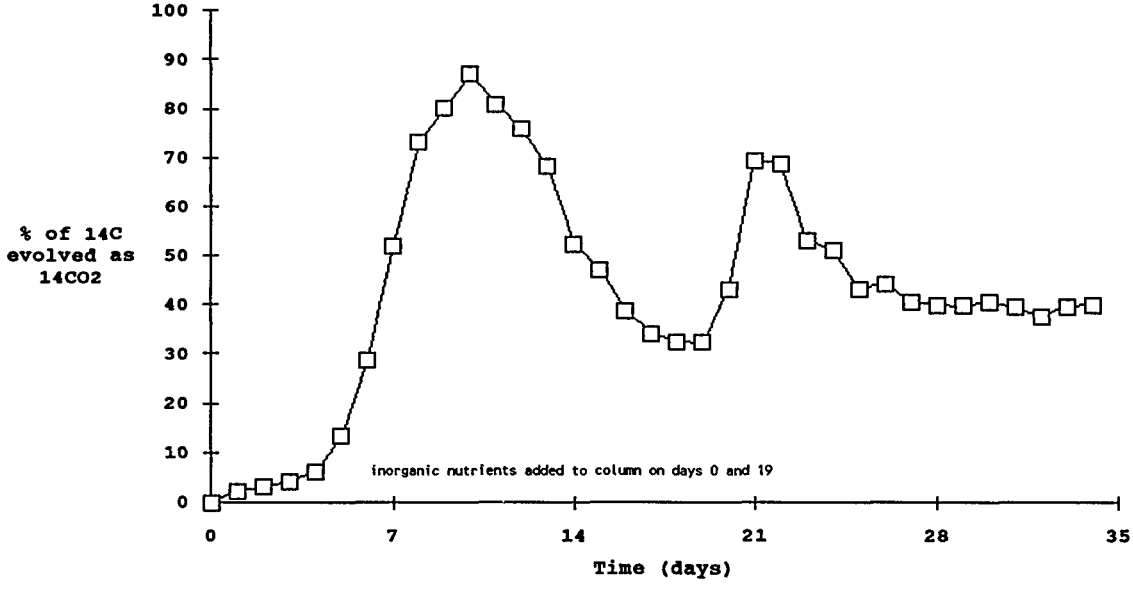


Table 4-1. Transfer efficiency of ^{14}C-toluene from diffusion tubes to impingers during Experiment 3.

Column Number	Mass Loss of Diffusion Tube (mg)	Mass Recovered in Impinger (mg)	Extrapolated from Diffusion Rate (mg)
1	337	269	381
2	336	226	381
3	347	251	381
4	218	179	235
5	339	286	381

Table 4-2. Mean diffusion rates of ^{14}C-VOCs from 1-, 2-, and 4-mm ID diffusion tubes (data in mg/hr).

VOC	Diffusion Tube ID (mm)		
	1	2	4
Benzene	0.163	0.323	1.563
Toluene	0.122	0.138	0.467
MEK	0.192	0.485	1.205
TCE	0.748	0.569	1.895
Carbon tetrachloride	0.463	0.848	3.329
Chlorobenzene	0.094	0.071	0.307

4.3 VOC MINERALIZATION UNDER FLOW-THROUGH CONDITIONS

4.3.1 Toluene Mineralization

Toluene was poorly mineralized in unamended soil columns (see Figure 4-3). A small increase was observed subsequent to day 5 after an initial acclimation period. This increase was sustained through day 10 and was followed by a gradual decrease over the next 7 days. Toluene mineralization rates then stabilized at about 5 to 7 percent $^{14}CO_2$ for the remainder of the experiment.

When sterile water was added to soil, a similar rate profile to that shown in Figure 4-3 was observed, except mineralization rates were higher and peaked at day 7 (see Figure 4-4). This transient rate increase declined to approximately 12 percent by day 10. The extent of mineralization was constant through day 19, at which time 6.0 mL of the mineral salts medium (inorganic nutrients) was added to the test matrix. $^{14}CO_2$ evolution then increased to nearly 100 percent in less than 48 hours (see Figure 4-4), followed by a gradual decline by day 30.

Addition of the mineral salts medium at the start of an experiment yielded mineralization rates of 90 percent by day 10 after an initial acclimation period (see Figure 4-5). $^{14}CO_2$ production then declined until day 20, at which time sterile mineral salts medium was again introduced. This nutrient addition more than doubled mineralization rates within 48 hours (see Figure 4-5).

Sterilization of column soil prior to toluene addition resulted in essentially no toluene mineralization (see Figure 4-2). This column was terminated at day 20 because of column leakage. Finally, toluene mineralization did not occur in the column containing no text matrix (see Figure 4-1).

During Experiment 4, toluene mineralization reached 100 percent by day 4 in unacclimated, uncontaminated soil that was amended with the mineral salts medium (see Figure 4-6). Two spikes occurred in the data at days 27 and 33. The extent of mineralization returned to 100 percent after these spikes. The cause of these spikes is described in Section 5.

4.3.2 Benzene Mineralization

During Experiment 4, benzene mineralization reached 100 percent after 5 days (see Figure 4-7). However, after 12 days $^{14}CO_2$ evolution started a decrease-increase-decrease cycle that continued for the duration of the experiment. Decreases in mineralization were observed at days 13, 20, and 27. The air flow rate was reduced by half on day 34 to determine the effect of reduced air flow on mineralization, if any. After reducing the air flow, mineralization decreased, averaging approximately 65 percent, with a range of 50 to 85 percent.

FIGURE 4-6 MINERALIZATION OF TOLUENE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

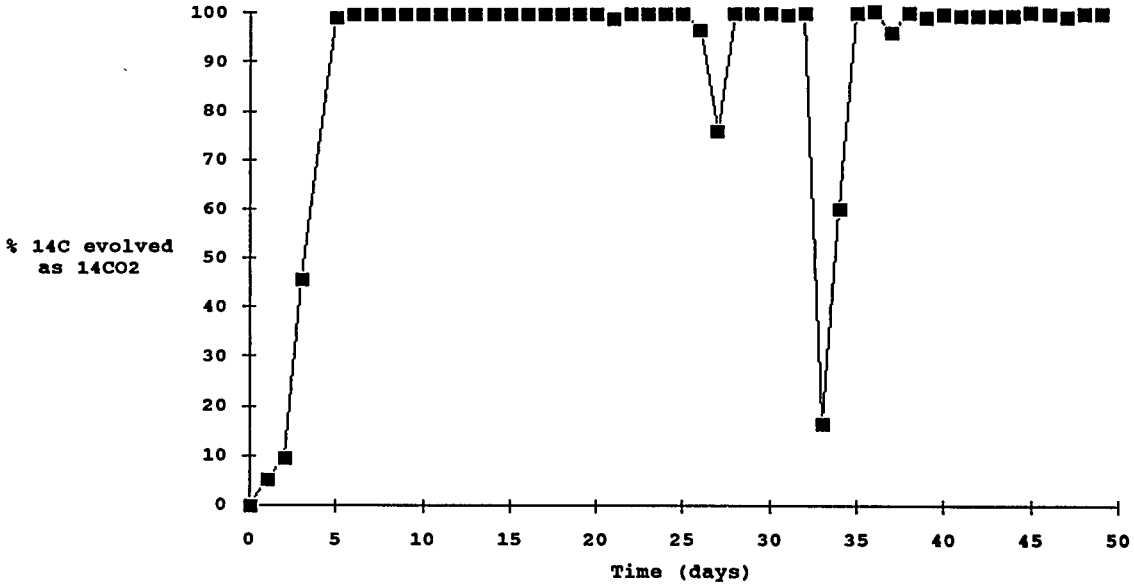
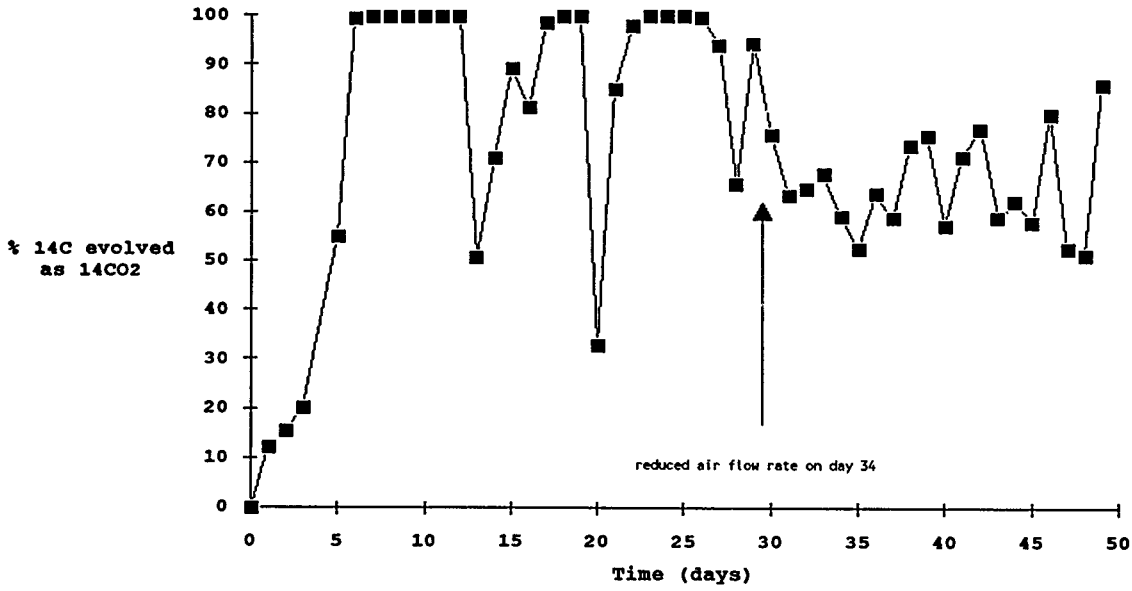


FIGURE 4-7 MINERALIZATION OF BENZENE IN SOIL AMENDED WITH INORGANIC NUTRIENTS



4.3.3 Trichloroethylene Mineralization

4.3.3.1 Trichloroethylene Mineralization Without Co-Substrates

In unacclimated soil amended with inorganic nutrients, ^{14}C-TCE mineralization reached 20 to 25 percent within 4 days, followed by a gradual decline to background levels by day 49 (see Figure 4-8). A spike in $^{14}CO_2$ evolution occurred on day 18. This was a transient increase observed on day 18 only. The cause is unknown.

4.3.3.2 Trichloroethylene Mineralization With Co-Substrates

Toluene was tested as a co-substrate for ^{14}C-TCE mineralization during Experiment 5. Four test columns were used; the diffusion tubes containing TCE had 2-mm ID necks, while those containing toluene had 4-, 2-, 1-, and 0.5-mm ID necks. Data were compared to ^{14}C-TCE mineralization without co-substrate (see Experiment 4).

All columns exhibited similar mineralization curves (see Figures 4-9a and 4-9b). Columns exposed to toluene vapor from the 0.5-mm (see Figure 4-10), 2-mm (see Figure 4-11), and 4-mm (see Figure 4-12) diffusion tubes exhibit nearly identical mineralization rates of ^{14}C-TCE over the course of the experiment. The column exposed to toluene vapor from the 1-mm diffusion tube exhibited the highest amount of ^{14}C-TCE mineralization of the columns tested (see Figure 4-13).

4.3.4 Chlorobenzene Mineralization

4.3.4.1 Chlorobenzene Mineralization Without Co-Substrate

Chlorobenzene (see Experiment 4) exhibited poor mineralization as the sole source of carbon in soil amended with the mineral salts medium (see Figure 4-14). Average $^{14}CO_2$ evolution was less than 5 percent of total ^{14}C production during the first 30 days.

During Experiment 5, chlorobenzene also was poorly mineralized in soil as the sole source of carbon. Initial mineralization values of 60 percent during Experiment 5 were apparently due to contamination of the impingers from a previous experiment (see Figure 4-15).

4.3.4.2 Chlorobenzene Mineralization With Co-Substrates

When toluene was used as a co-substrate in soil amended with inorganic nutrients, chlorobenzene mineralization increased significantly within 2 weeks (see Figure 4-16). Mineralization reached nearly 100 percent on day 13 and then declined to approximately 40 percent by day 20 of the study. Methanol did not enhance chlorobenzene mineralization when used as a co-substrate, (see Figure 4-17).

4.3.4.3 Phanerochaete chrysosporium Effect on Mineralization

The fungi Phanerochaete chrysosporium, inoculated into wood chips that were used as the test matrix, had no effect on chlorobenzene mineralization (see Figure 4-18). The

FIGURE 4-8 MINERALIZATION OF TCE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

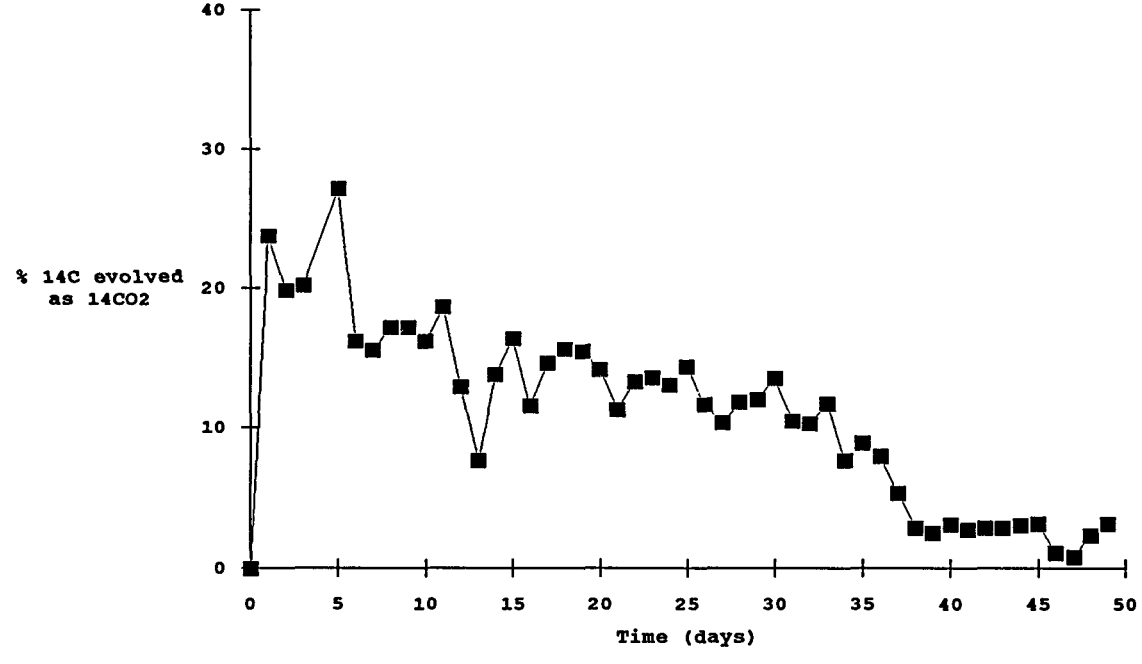


FIGURE 4-9a MINERALIZATION OF TCE WITH TOLUENE CO-SUBSTRATE

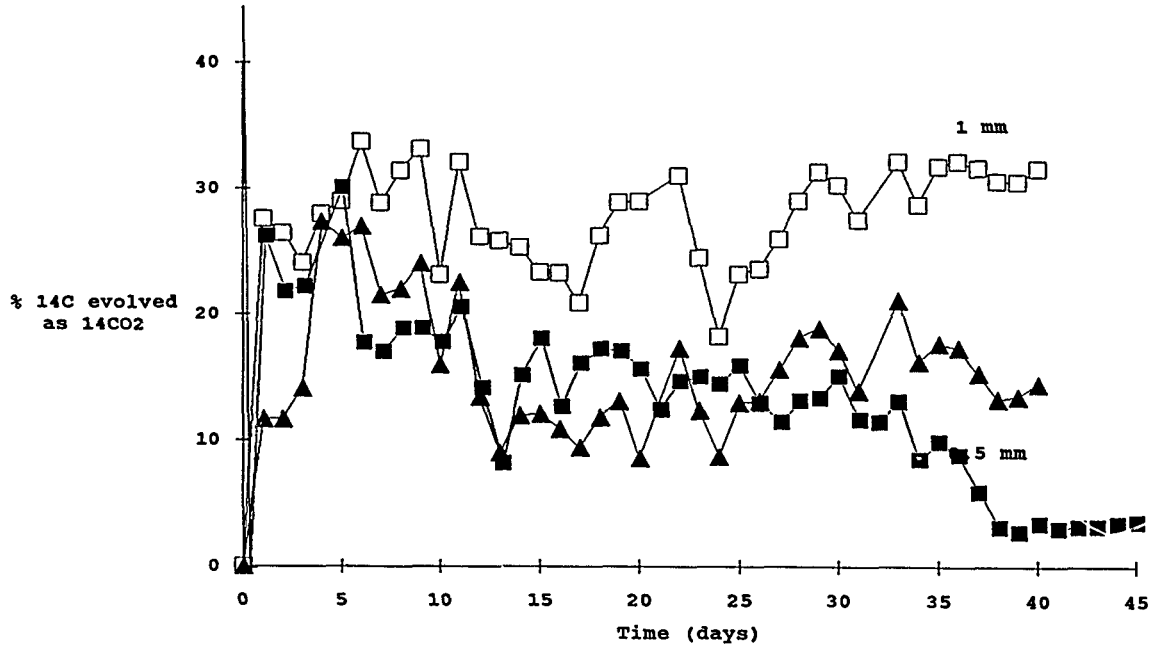


FIGURE 4-9b MINERALIZATION OF TCE WITH TOLUENE CO-SUBSTRATE

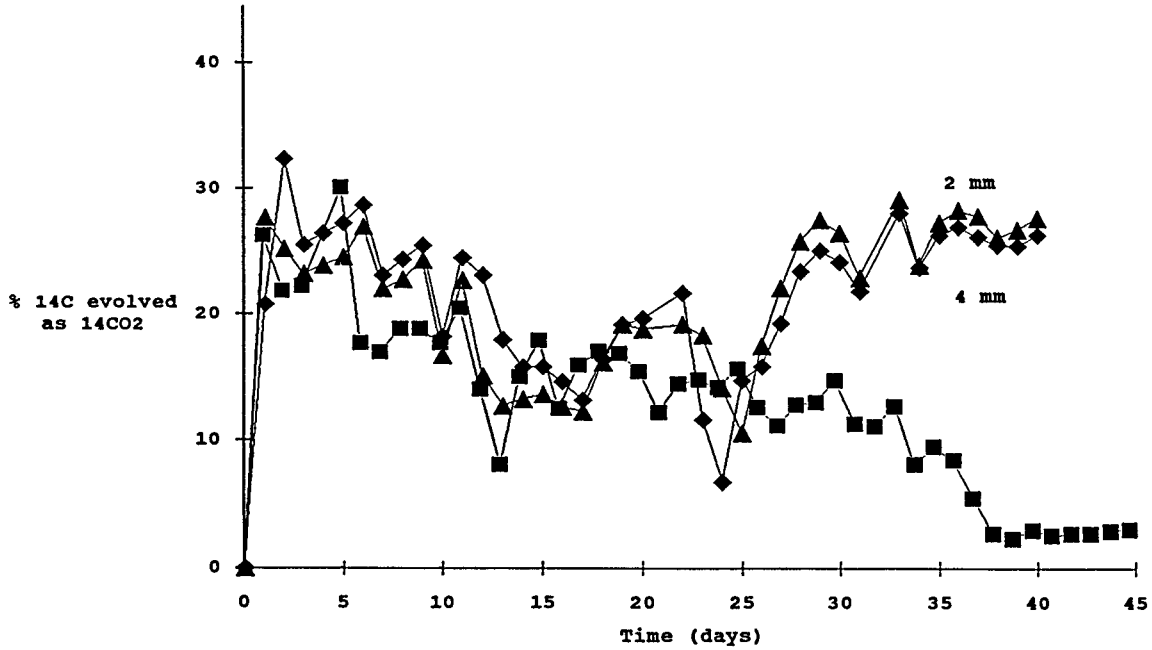


FIGURE 4-10 MINERALIZATION OF ^{14}C-TCE IN SOIL IN THE PRESENCE OF TOLUENE CO-SUBSTRATE

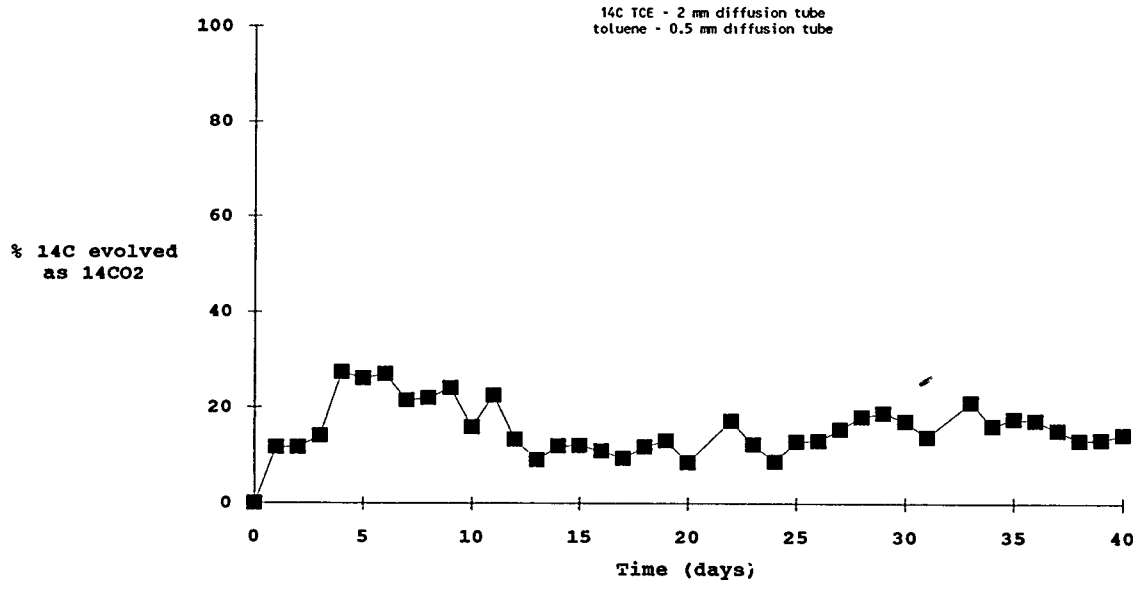


FIGURE 4-11 MINERALIZATION OF ^{14}C-TCE IN SOIL IN THE PRESENCE OF TOLUENE CO-SUBSTRATE

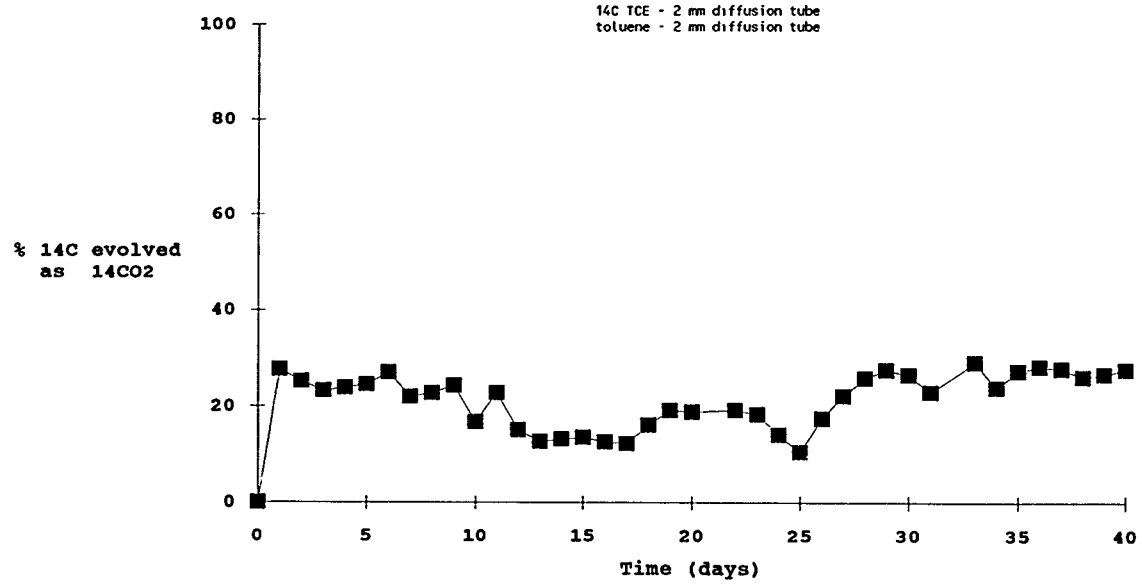


FIGURE 4-12 MINERALIZATION OF ^{14}C-TCE IN SOIL IN THE PRESENCE OF TOLUENE CO-SUBSTRATE

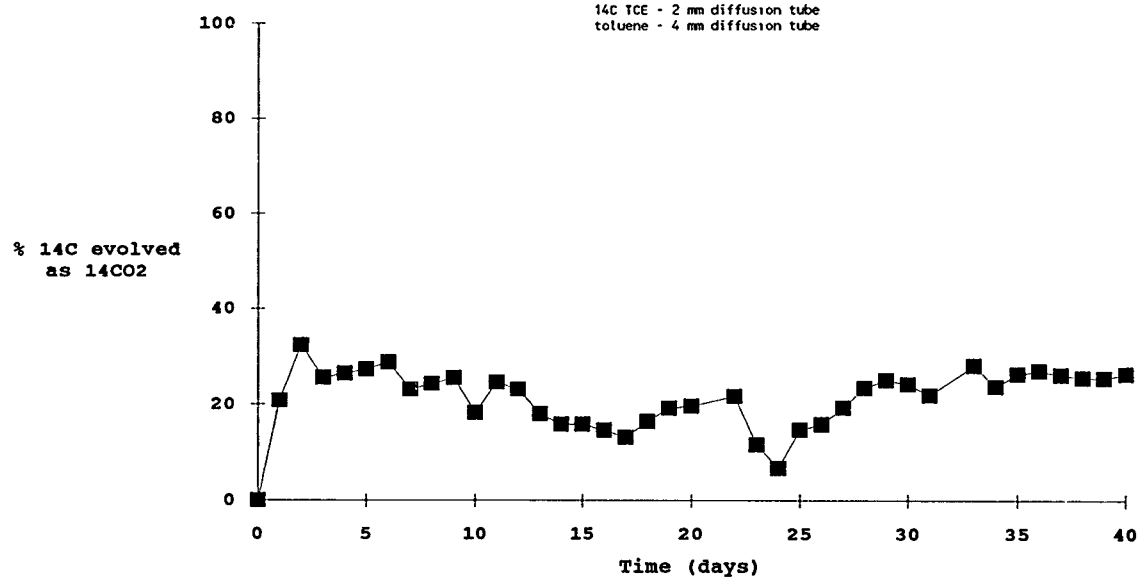


FIGURE 4-13 MINERALIZATION OF ^{14}C-TCE IN SOIL IN THE PRESENCE OF TOLUENE CO-SUBSTRATE

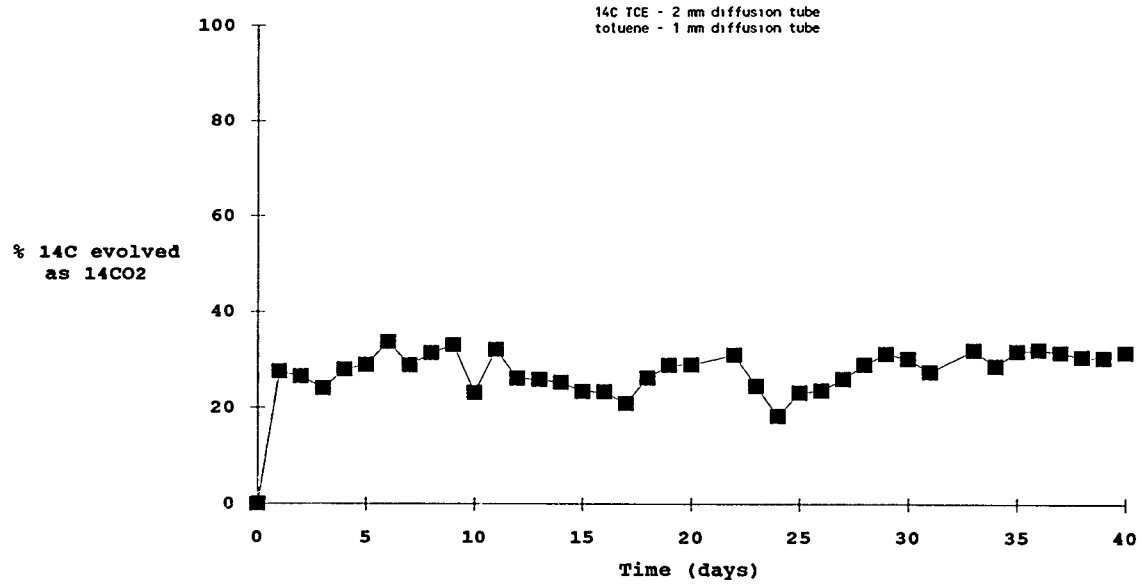


FIGURE 4-14 MINERALIZATION OF ^{14}C-CHLOROBENZENE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

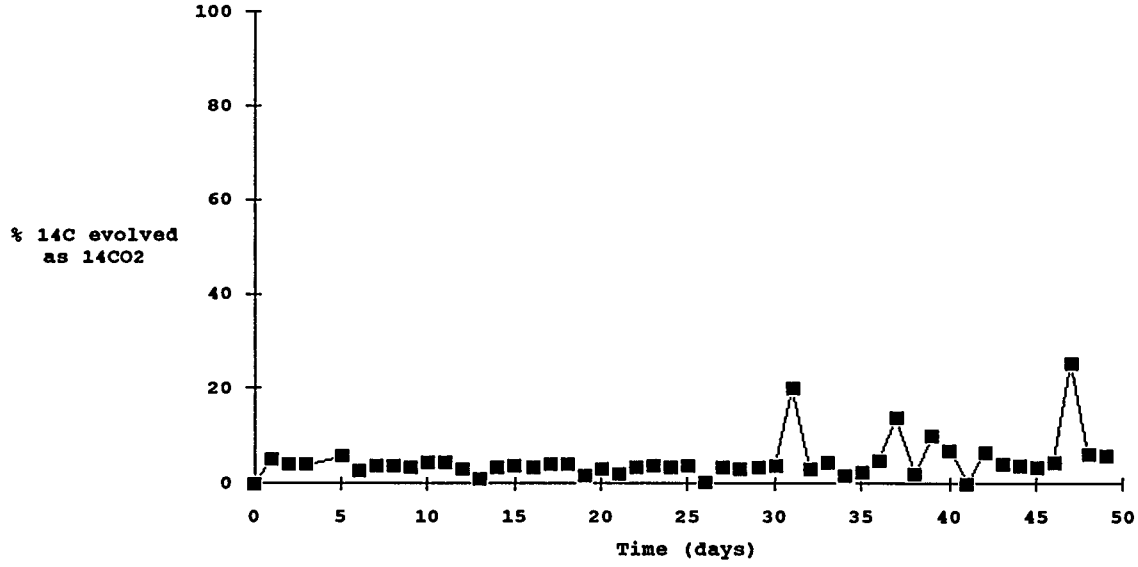


FIGURE 4-15 MINERALIZATION OF ^{14}C-CHLOROBENZENE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

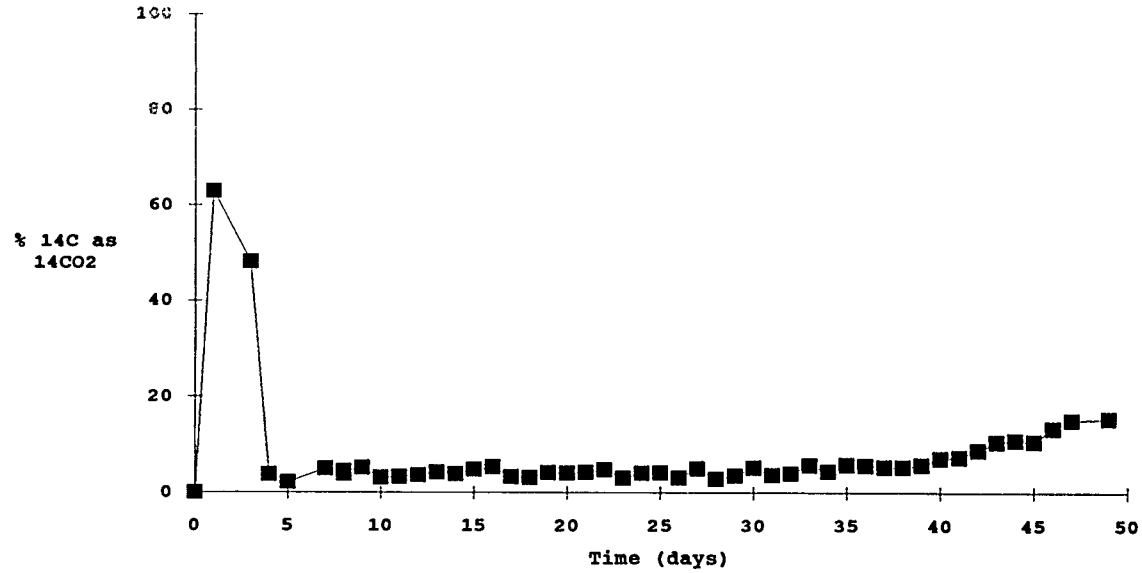


FIGURE 4-16 MINERALIZATION OF ^{14}C-CHLOROBENZENE WITH TOLUENE CO-SUBSTRATE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

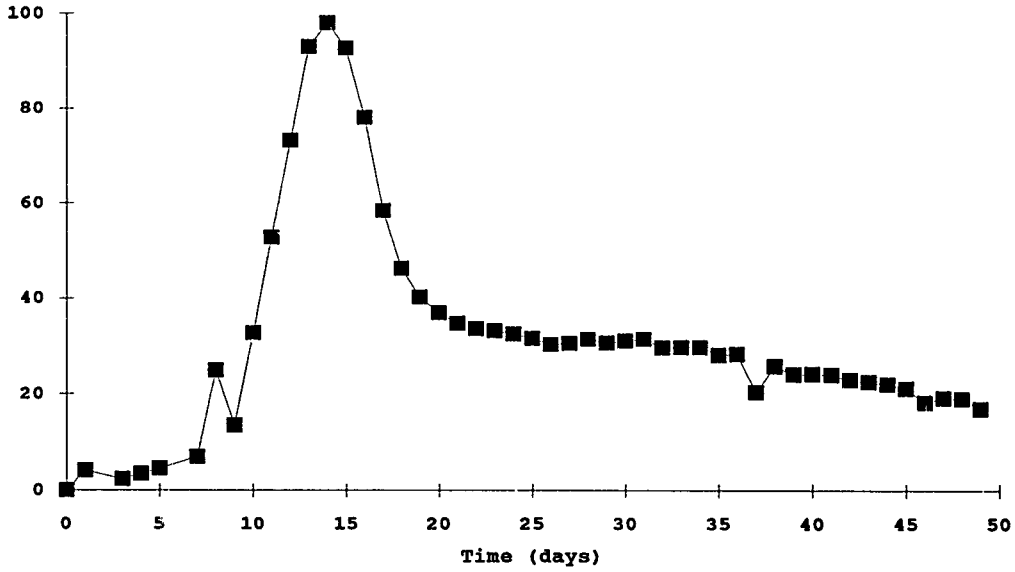


FIGURE 4-17 MINERALIZATION OF ^{14}C-CHLOROBENZENE WITH METHANOL CO-SUBSTRATE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

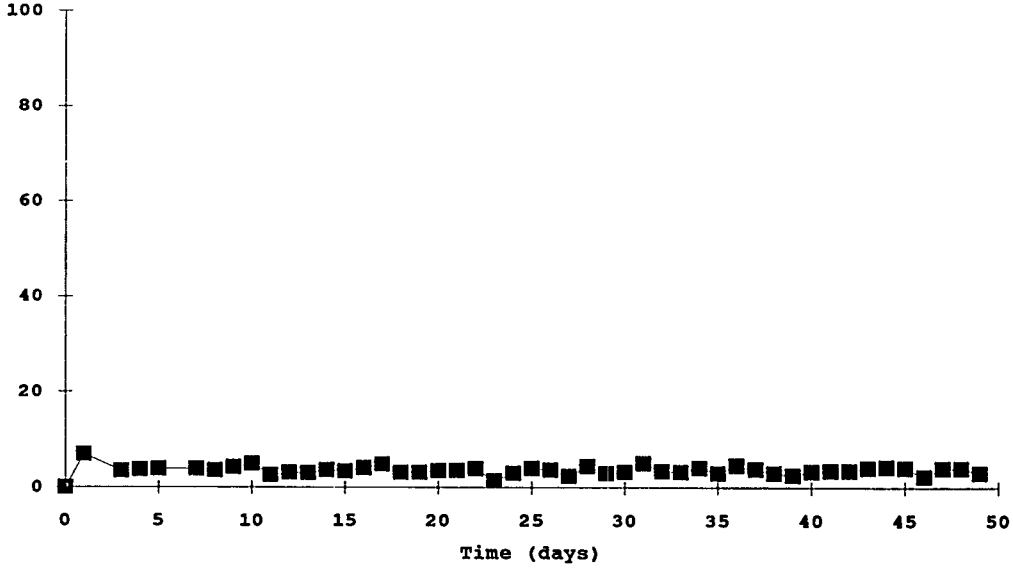
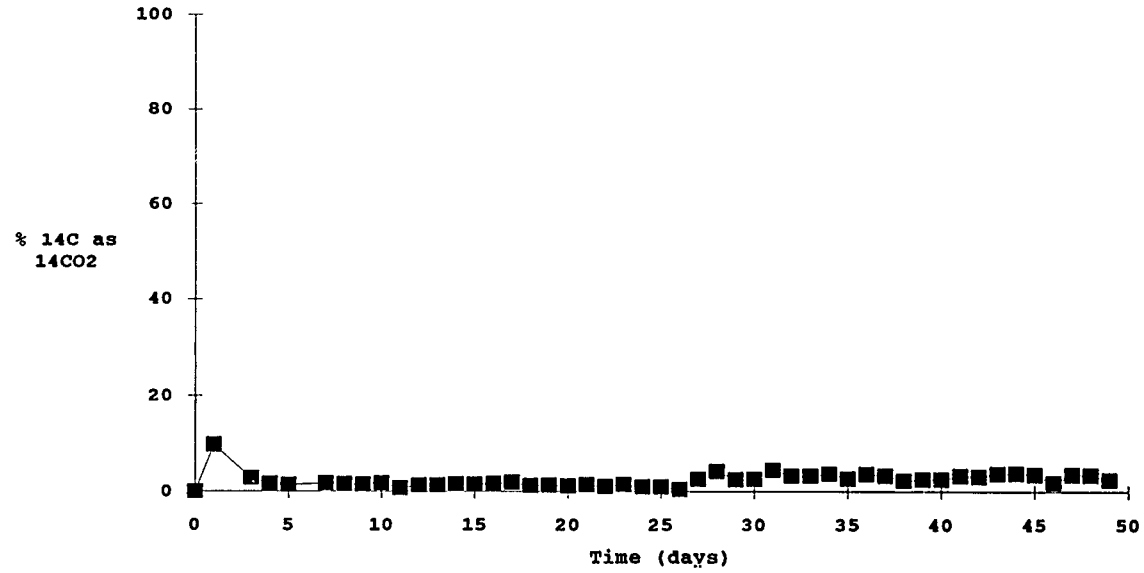


FIGURE 4-18 MINERALIZATION OF ^{14}C-CHLOROBENZENE IN WOOD CHIPS INOCULATED WITH WHITE ROT FUNGUS (P. CHRYSOSPORIUM)



hi[illegible]ial mineralization rate was apparently due to contamination of the impingers from [illegible]us experiment.

4.3.5 Carbon Tetrachloride Mineralization

4.3.5.1 Unamended Carbon Tetrachloride Mineralization

During Experiment 4 (soil amended with inorganic nutrients), ^{14}C-carbon tetrachloride mineralization increased to 27 percent by day 4 after an initial acclimation period (see Figure 4-19). Mineralization for the duration of the experiment was sporadic and eventually declined to background levels by day 40.

4.3.5.2 Carbon Tetrachloride Mineralization With Co-Substrates

The addition of toluene (see Figure 4-20) or methanol (see Figure 4-21) as co-substrates did not enhance microbial mineralization of ^{14}C-carbon tetrachloride above that observed during an inorganic nutrient amended control (see Figure 4-22). All three columns experienced mineralization rates of 5 to 15 percent for the duration of the study.

4.3.6 Methyl Ethyl Ketone Mineralization

During Experiment 6 (unacclimated soil amended with inorganic nutrients), mineralization of methyl ethyl ketone reached 100 percent within 3 days (see Figure 4-23) and remained at 100 percent for the duration of the experiment (47 days).

4.4 MINERALIZATION OF CHLORINATED VOCS IN STATIC MICROCOSMS

4.4.1 ^{14}C-Carbon Tetrachloride

In unacclimated, uncontaminated soil, ^{14}C-carbon tetrachloride was not mineralized beyond the extent observed for the sterilized control under any treatment conditions (Figure 4-24). The presence or absence of toluene or methanol as co-substrates had no measurable effect on mineralization (Figure 4-24).

4.4.2 ^{14}C-TCE

The presence of methanol as a co-substrate enhanced mineralization of ^{14}C-TCE in static microcosms with unacclimated, uncontaminated soils. The data suggest that mineralization was enhanced the most at low ratios of co-substrate to ^{14}C-TCE (Figure 4-25) At the 90 percent confidence level, ^{14}C-TCE mineralization was significantly enhanced by the presence of 0.5.1 and 1.1 volumetric ratios of methanol to ^{14}C-TCE (Figure 4-25).

In pre-acclimated soils, no statistically significant enhancement of ^{14}C-TCE mineralization was attributable to the presence of methanol or toluene co-substrates (Figure 4-26).

FIGURE 4-19 MINERALIZATION OF ^{14}C-CARBON TETRACHLORIDE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

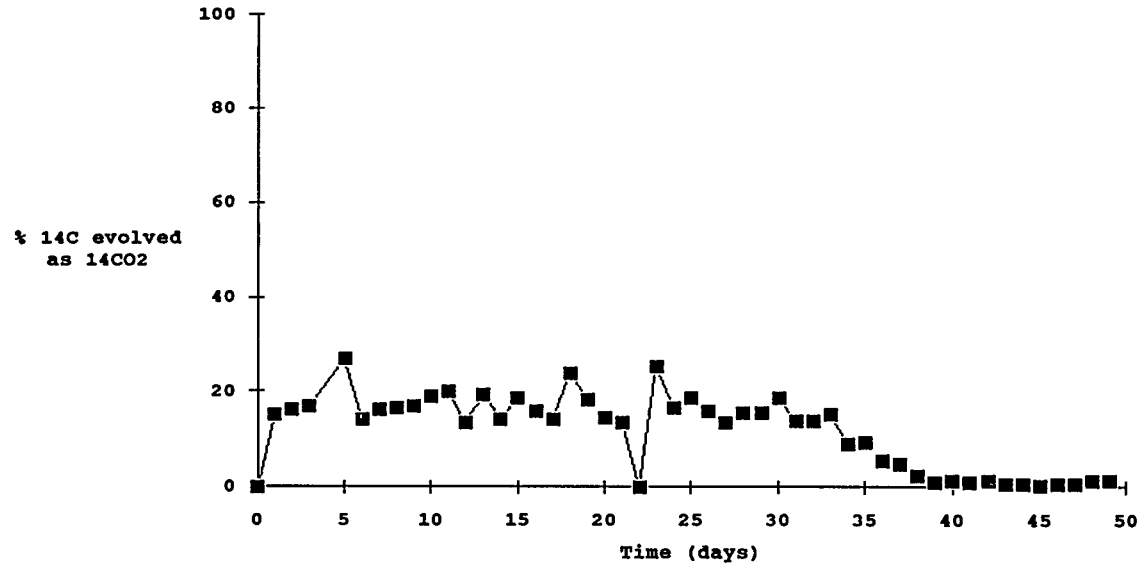


FIGURE 4-20 MINERALIZATION OF ^{14}C-CARBON TETRACHLORIDE WITH TOLUENE CO-SUBSTRATE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

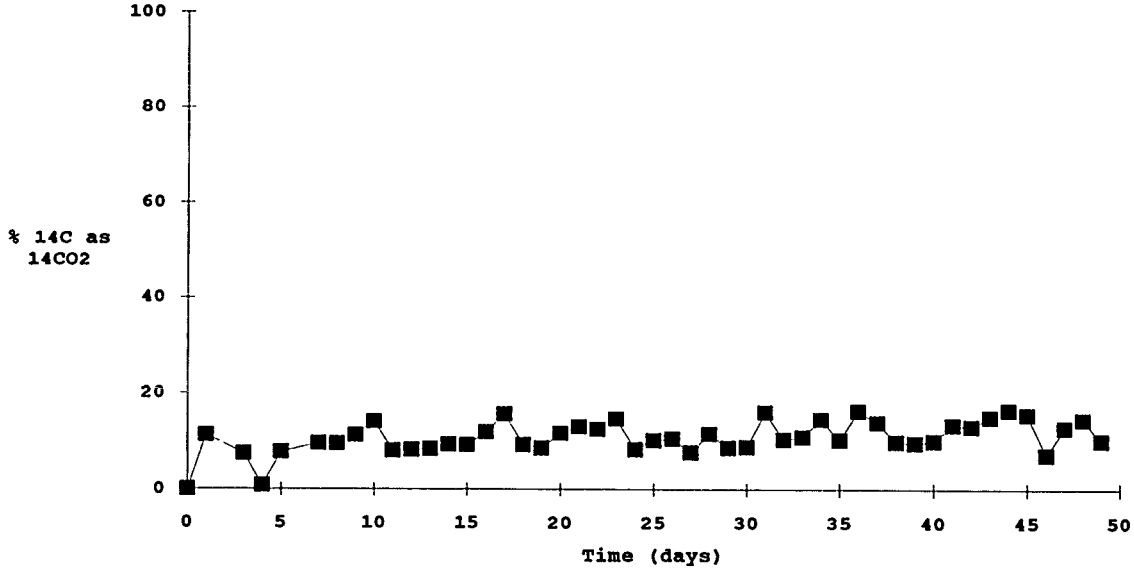


FIGURE 4-21 MINERALIZATION OF ^{14}C-CARBON TETRACHLORIDE WITH METHANOL CO-SUBSTRATE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

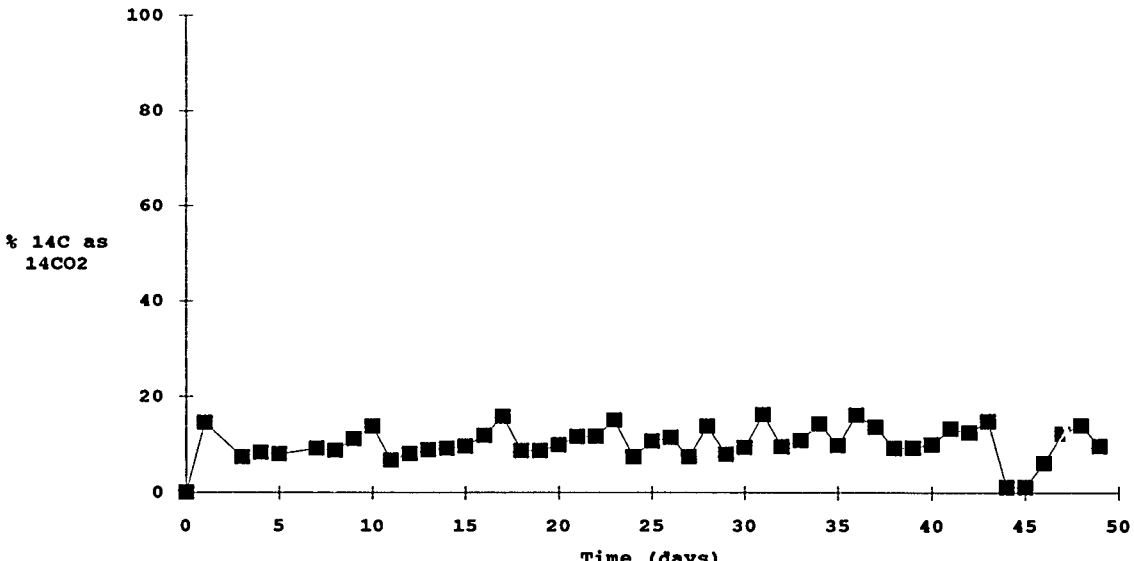


FIGURE 4-22 MINERALIZATION OF ^{14}C-CARBON TETRACHLORIDE IN SOIL AMENDED WITH INORGANIC NUTRIENTS

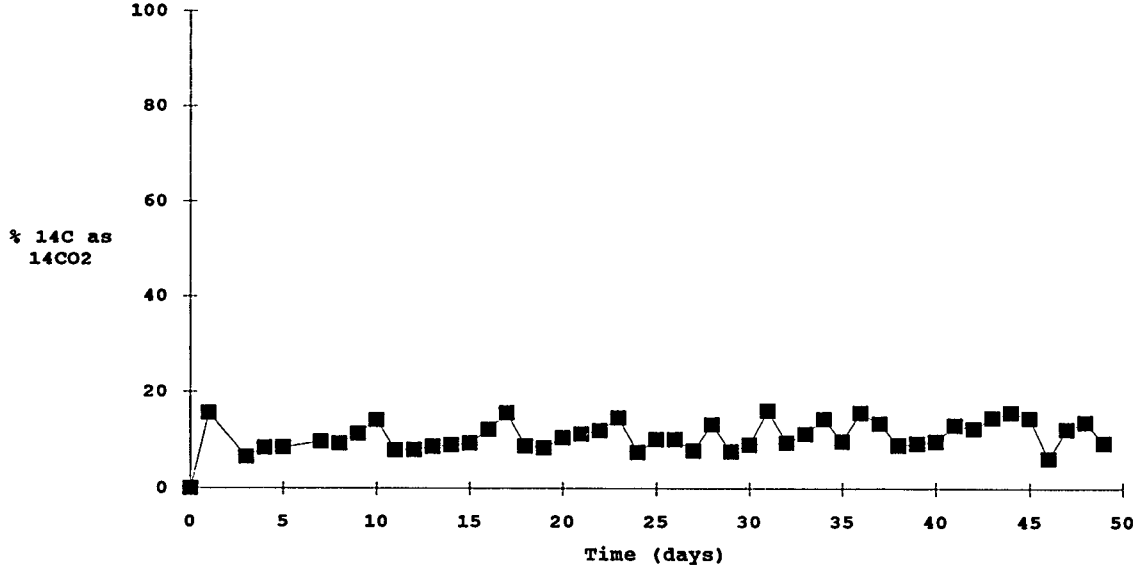


FIGURE 4-23 MINERALIZATION OF ^{14}C-MEK IN SOIL AMENDED WITH INORGANIC NUTRIENTS

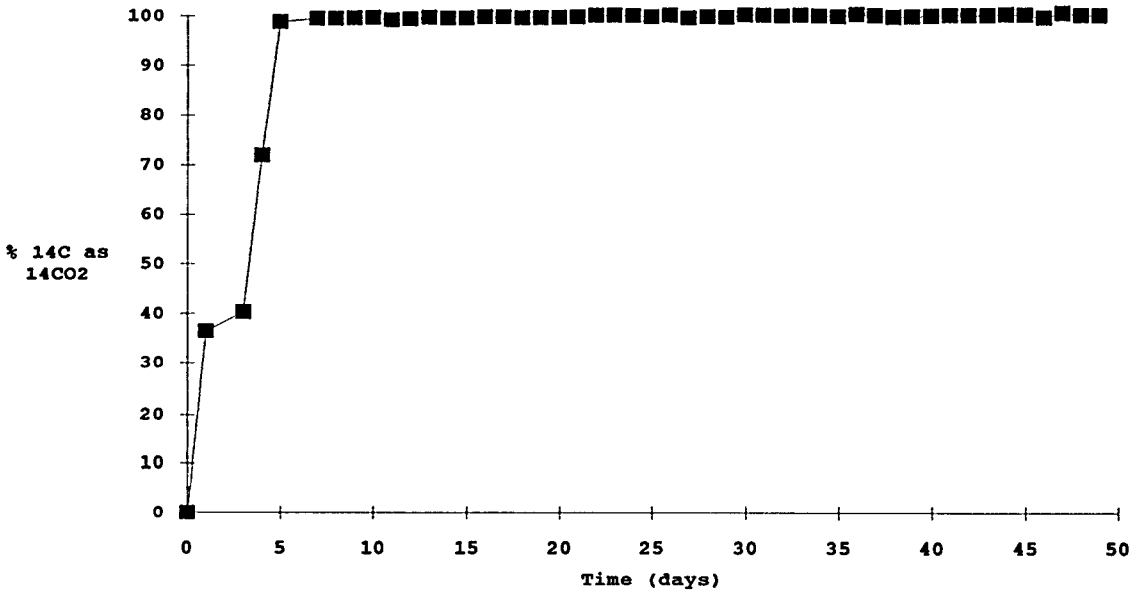


FIGURE 4-24 EVOLUTION OF $^{14}CO_2$ FROM ^{14}C-CARBON TETRACHLORIDE IN STATIC SOIL MICROCOSMS (ERROR BARS INDICATE MEAN ± 90 PERCENT CONFIDENCE INTERVAL)

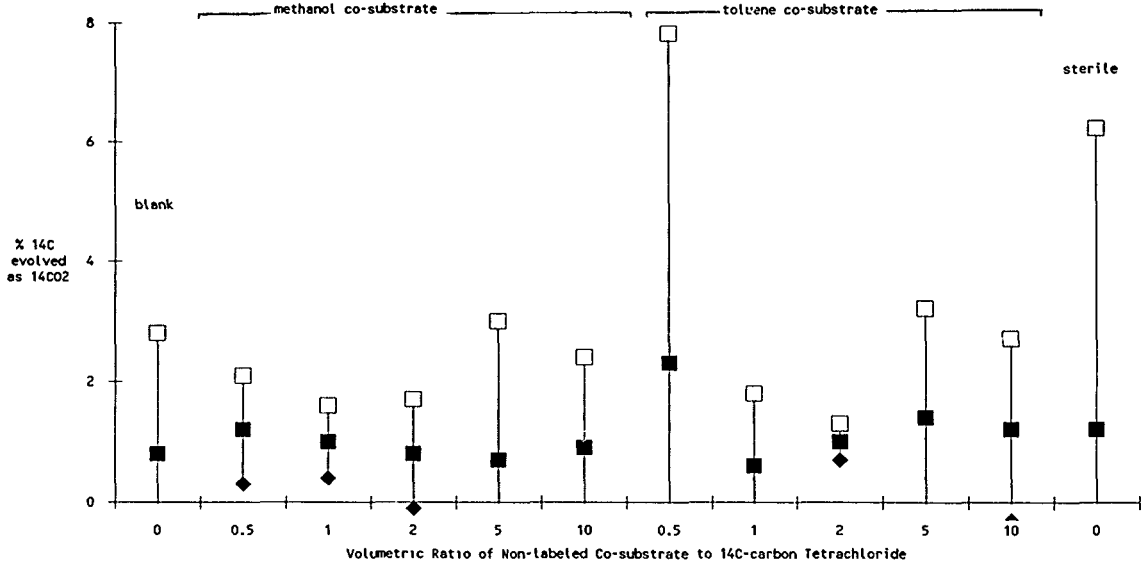


FIGURE 4-25 EVOLUTION OF $^{14}CO_2$ FROM ^{14}C-TCE IN STATIC SOIL MICROCOSMS (ERROR BARS INDICATE MEAN ± 90 PERCENT CONFIDENCE INTERVAL)

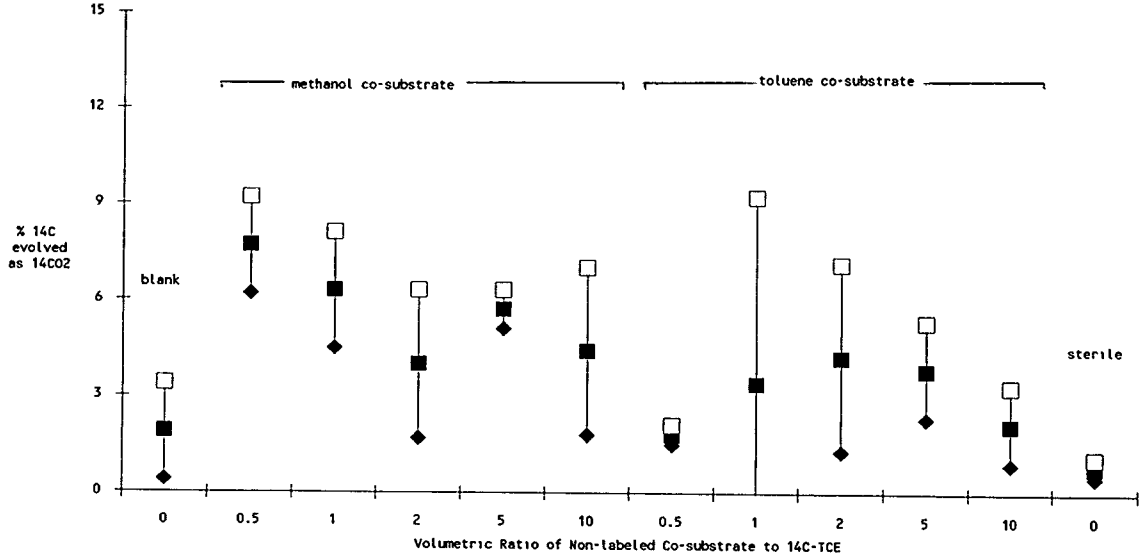
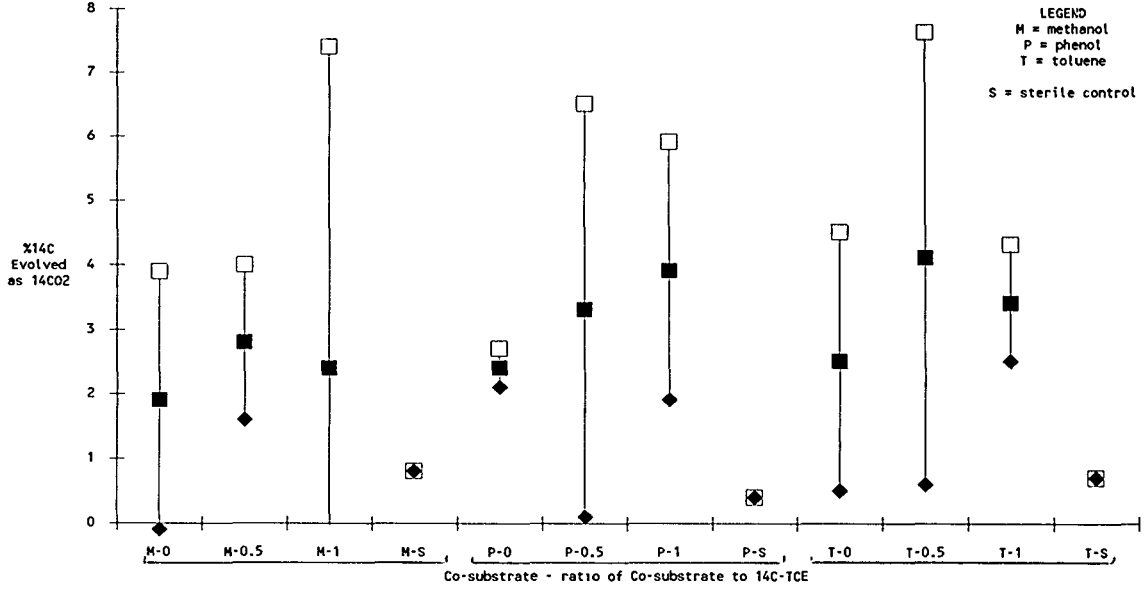


FIGURE 4-26 MINERALIZATION OF ^{14}C-TCE IN STATIC SOIL MICROCOSMS WITH PRE-ACCLIMATED SOILS (ERROR BARS INDICATE MEAN ± 90 PERCENT CONFIDENCE INTERVAL)



4.4.3 ^{14}C-Chlorobenzene

The presence of methanol or toluene co-substrates had no statistically significant effect on ^{14}C-chlorobenzene mineralization in either unacclimated or pre-acclimated soils (Figures 4-27 and 4-28). The maximum extent of mineralization observed under any treatment conditions was approximately 14 percent (Figure 4-27).

FIGURE 4-27 EVOLUTION OF $^{14}CO_2$ FROM ^{14}C-CHLOROBENZENE IN STATIC SOIL MICROCOSMS (ERROR BARS INDICATE MEAN ± 90 PERCENT CONFIDENCE INTERVAL)

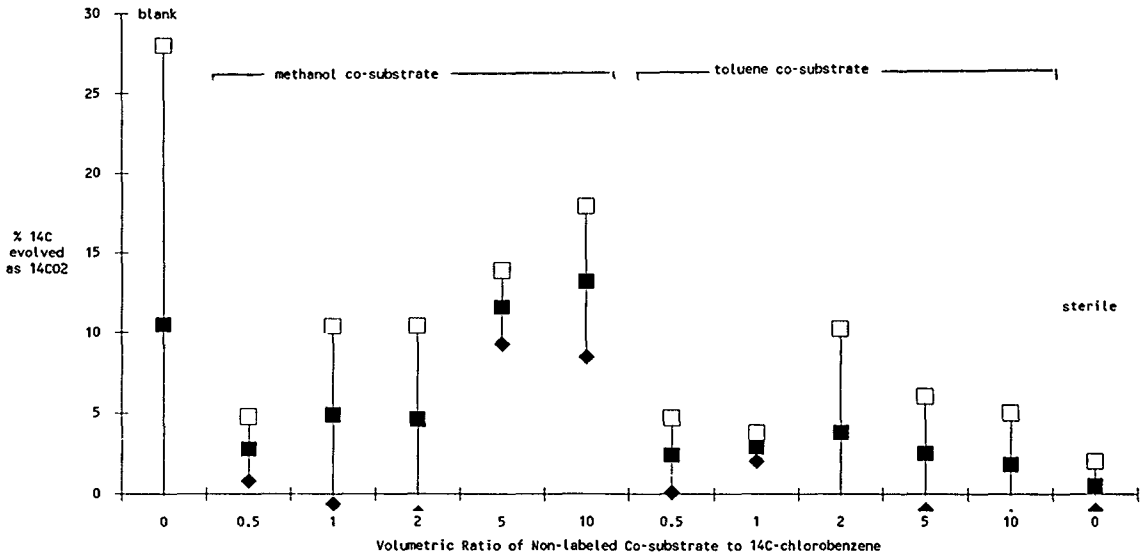
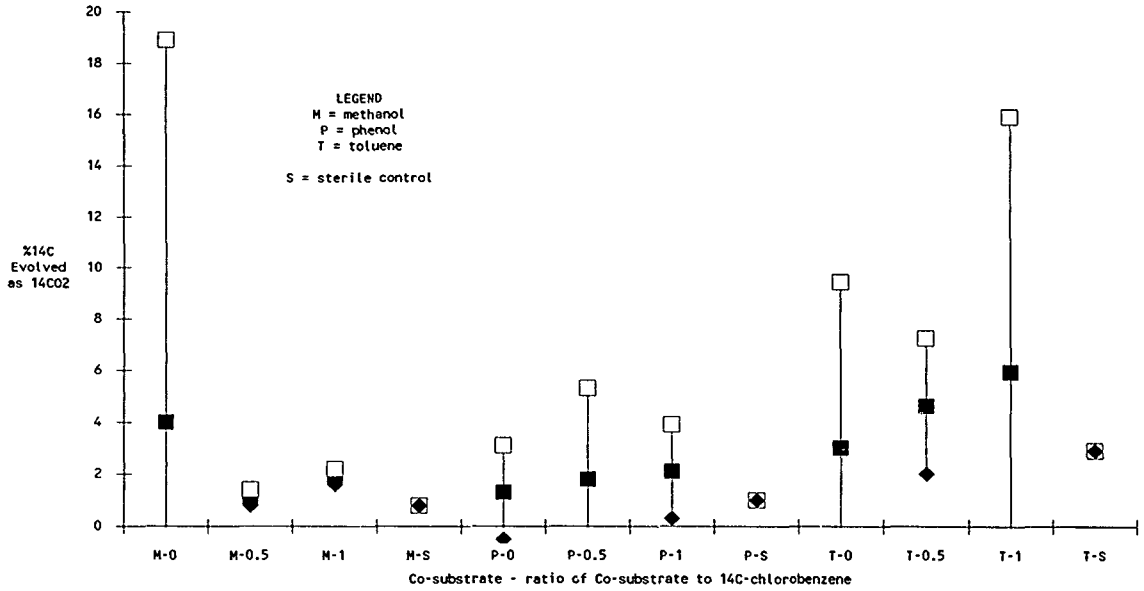


FIGURE 4-28 MINERALIZATION OF ^{14}C-CHLOROBENZENE IN STATIC SOIL MICROCOSMS WITH PRE-ACCLIMATED SOILS (ERROR BARS INDICATE MEAN ± 90 PERCENT CONFIDENCE INTERVAL)



SECTION 5

DISCUSSION

5.1 TOLUENE MINERALIZATION

Toluene was poorly mineralized in unamended soil (Figure 4-3). Increasing the soil moisture content by adding sterile water to the columns produced a greater rate of toluene mineralization (Figure 4-4). This increase was expected, since microbial activity increases with soil moisture as long as adequate oxygen is available to the microorganisms (EPA, 1989). Excessive moisture addition becomes detrimental to aerobic microorganisms as water displaces available oxygen in soil pore spaces. This detrimental effect usually becomes apparent at moisture concentrations above 70 percent of water holding capacity.

The addition of inorganic nutrients to soil in the form of the inorganic salts media resulted in a significant increase in toluene mineralization (Figures 4-4 and 4-5). These data indicate that inorganic nutrients were limiting in the soil and that additional nutrients were required to obtain optimal conditions for VOC mineralization with this soil type. Mineralization of toluene was clearly a result of microbial activity, as demonstrated by the results obtained in the no matrix and sterilized control columns during Experiment 3 (Figures 4-1 and 4-2).

Toluene is readily and, usually, completely degradable in standard sewage tests, although only 1-2 percent degraded in the subsurface environment (Howard, 1990). Wilson et al. (1981) found that toluene degraded in some soil columns but not others and Grbic-Galic and Vogel (1986) determined that toluene is degradable by methanogenic cultures under strict anaerobic conditions.

The spikes in $^{14}CO_2$ evolution in Figure 4-6 were apparently due to leakage in the impingers on those specific days and the manner in which the data were collected. The continuous-flow apparatus (Figure 3-1) worked by negative pressure, so that leaks in the impingers caused less VOC to be drawn through the test apparatus. When impinger vials were changed each day, the impingers were not cleaned before new vials were installed. The residual ^{14}C activity on the impingers was left in place and was collected with the next sample. Each set of impingers, however, was dedicated to a single column for the duration of each experiment, so that no cross-contamination occurred between experiments as a result of this procedure. The amount of residual ^{14}C activity on the impingers was insignificant when compared with the ^{14}C activity trapped during the sampling of the column exhaust gases.

As previously noted, mineralization data are expressed as a percentage of total ^{14}C activity evolved from the column for each day. In essence, the ratio of $^{14}CO_2$ evolved to ^{14}C-organics evolved was calculated. When leaks occurred in the impingers and less ^{14}C-gas was pulled through the test apparatus, the residual ^{14}C activity from the previous day represented a larger portion of the activity trapped. In some cases, the residual ^{14}C activity on the impingers from the previous day (when no leaks occurred) was high enough to change the observed ratio of evolved ^{14}C-organic to $^{14}CO_2$ on those days when leaks did occur, resulting in the spikes observed in some of the figures.

During experiment 3, the mean flow rate of gas through the test column during days 1 through 29 was approximately 4.6 mL/min. The internal volume of the soil columns was 31.4 cm^3, and the columns contained an average of 38 grams of soil. Assuming a soil porosity of 0.30, the residence time of gas in the column was 2.0 minutes.

This residence time of 2.0 minutes was sufficient to achieve 90-100 percent mineralization (Figures 4-4 and 4-5). Using the diffusion rate of toluene from a 4 mm i.d. diffusion tube (0.467 mg/hr, Table 4-2), the approximate extent of toluene degradation (at 100 percent mineralization) was:

$$0.467 \text{ mg/hr}/38 \text{ g soil} = 0.012 \text{ mg/hr/g soil}$$
$$= 0.288 \text{ mg/day/g soil.}$$

While this value is valid only for the test apparatus and experimental parameters used in the present study, it represents a conservative estimate of the potential destruction rate of gaseous toluene in a soil column. This destruction rate may potentially be increased by modifying the length to diameter ratio of the column, the gas flow rate, and/or the VOC loading rate.

5.2 BENZENE MINERALIZATION

Benzene was readily mineralized as shown in Figure 4-7. An acclimation period of five days preceded the complete mineralization of benzene vapor. However, $^{14}CO_2$ production exhibited large fluctuations following day 12 of the experiment. These observations were likely the result of leaks in the test apparatus, as discussed in Subsection 5.1.

Benzene appears to be biodegradable in aerobic but not anaerobic environments. In soil, 20 ppm benzene was 24 percent degraded in one week and 47 percent degraded after ten weeks (Howard, 1990). Sheehan et al. (1987) found benzene at a gasoline-contaminated site to be even more degradable than toluene and xylene. Grbic-Galic and Vogel (1986) determined benzene to be degradable by methanogenic cultures acclimated to lignin-derived aromatic acids under strict anaerobic conditions.

5.3 CARBON TETRACHLORIDE MINERALIZATION

Carbon tetrachloride was not extensively mineralized when present as the sole source of carbon in nutrient amended soils (Figures 4-19 and 4-22; while the tests represented in both figures had identical treatments, the experiment shown in Figure 4-22 served as a control for the experiments in Figures 4-20 and 4-21). The addition of toluene and methanol as co-substrates did not enhance carbon tetrachloride mineralization (Figures 4-20 and 4-21) as compared with the negative control (Figure 4-22).

Data are not readily available regarding carbon tetrachloride biodegradation aerobically in soil, but it does biodegrade under anaerobic conditions (Howard, 1990). Bouwer and McCarty (1983) found that carbon tetrachloride was both mineralized and converted to carbon dioxide under anoxic conditions when nitrate was present as the terminal electron acceptor. Bouwer and McCarty stressed that this is an unusual observation since carbon tetrachloride is usually hydrolyzed to carbon dioxide.

5.4 CHLOROBENZENE MINERALIZATION

Chlorobenzene exhibited minimal mineralization when present as the sole source of carbon, even in nutrient-amended soil (Figures 4-14 and 4-15; the data in Figure 4-15 is the negative control for the data in Figures 4-16 and 4-17). The use of methanol as a co-substrate was ineffective for enhancing mineralization (Figure 4-17); however, using toluene as a co-substrate substantially increased ^{14}C-chlorobenzene mineralization (Figure 4-16). A maximum mineralization rate of nearly 100 percent was achieved by day 13. The use of the white rot fungus Phanerochaete chrysosporium did not enhance chlorobenzene mineralization.

The data demonstrated that utilization of co-substrates such as toluene improved chlorobenzene degradation. Previous studies with chlorobenzene have shown that degradation is temperature dependent and even slower than other recalcitrant compounds such as cresol (Pfaender and Bartholomew, 1982). Wilson et al. (1981) found that 20-40 percent of chlorobenzene biodegraded or could not be accounted for and that the percentage degraded was directly proportional to the initial amount applied. In groundwater, chlorobenzene was degraded in some samples but not others

5.5 METHYL ETHYL KETONE MINERALIZATION

Methyl ethyl ketone mineralization rapidly reached 100 percent and maintained that level for 47 days (Figure 4-23). This compares favorably with previous studies that cite an 87 percent total mineralization of MEK in five days (Howard, 1990). Methyl ethyl ketone appears to be readily degradable, in part, due to its chemical structure and abiotic degradation.

5.6 TRICHLOROETHYLENE MINERALIZATION

Mineralization of trichloroethylene (TCE) was only 20 to 25 percent of the total ^{14}C evolved, and required a two-day acclimation period prior to reaching that level (Figure 4-8). This rate declined to background levels by day 40. An increase in $^{14}CO_2$ evolution was observed on day 18, t' is was only a transient increase and was probably caused by leaks in the impingers.

In the present study, toluene from the 1-mm diameter vials served as an excellent co-substrate and produced a higher TCE mineralization rate, when compared with controls, for the majority of the experiment (Figure 4-9a). Other concentrations of toluene were less effective (Figures 4-9a, 4-9b, 4-10 through 4-13). It was difficult to quantify these effects since only single replicate columns were used during Experiment 5.

While TCE biodegrades slowly (Howard, 1990), it has been shown that TCE is transformed aerobically by a consortia of microorganisms (Fliermans et al., 1988) and converted to carbon dioxide by microbes exposed to natural gas (77 percent methane) (Wilson and Wilson, 1985). Degradation did not occur in the presence of methanol, but did occur in the presence of methane (Henry and Grbic-Galic, 1986). Methanotrophs, such as Methylosinus trichosporium, have been demonstrated to degrade TCE at rates up to 56 percent in one hour (initial [TCE] = 20 μM), although the rate of TCE removal decreases with time (Tsien et al., 1989).

Henry and Grbic-Galic (1987) determined that three methane-degrading mixed bacterial cultures rapidly transformed TCE into intermediates, carbon dioxide, and cell carbon. Excessive methane and depleted oxygen were inhibitory to TCE transformation. Lanzarone and McCarty (1987) determined that optimal TCE degradation occurred when either a low methane concentration (1.5 mg/mL) or an alternating pulse of methane (4.5 mg/mL) one week and oxygen the next produced maximum TCE degradation. Little et al. (1987) also found methane-oxidizing bacteria that degrade TCE.

Nelson et al. (1986) found an aerobic bacteria, tentatively identified as an Acinetobacter, that degrades TCE via dechlorination. However, the bacteria required an unknown co-factor present in the water at the site from which the TCE-degrading bacteria was isolated. This co-factor was subsequently found to be phenol, although toluene also proved effective. The organism was given the designation G4. Subsequently, organisms with similar capabilities have been isolated by a number of researchers.

5.7 OPTIMIZATION OF CONDITIONS

During continuous-flow experiments, the average retention time of the gas stream in the columns was approximately 12 minutes, although retention times as short as 6.8 minutes were used successfully during Experiment 3.

The importance of inorganic nutrients in the solid test matrix was clearly demonstrated by the experiments conducted with ^{14}C-toluene. Likewise, the addition of moisture to more favorable levels substantially increase ^{14}C-toluene destruction.

Environmental conditions that were adequate to obtain complete destruction of gaseous ^{14}C-benzene, ^{14}C-toluene, and ^{14}C-MEK were attained by the present study. Further refinements may allow for the use of shorter VOC retention times, an important consideration for pilot- or full-scale implementation.

The uncontaminated soil used as a solid test matrix during most of the experiments proved to be entirely adequate for non-chlorinated ^{14}C-VOC destruction. In terms of pilot- to full-scale implementation, soil has the advantages of being easily and cheaply obtained. Modification of the soil porosity through the addition of bulking agents may be desirable for larger scale operation, however.

The need is apparent for a non-chlorinated co-substrate to be present in the gas stream to obtain significant destruction of chlorinated VOCs. For sites that contain mixtures of chlorinated and non-chlorinated solvents, the correct type and amount of non-stripping VOC may or may not be present in the gas stream from an air stripping operation.

SECTION 6

CONCLUSIONS

The three non-chlorinated VOCs tested (benzene, methyl ethyl ketone, and toluene) were all completely mineralized in the vapor phase under continuous-flow conditions in uncontaminated, unacclimated soil that had been amended with additional inorganic nutrients. Data from this study indicate that specially-acclimated microorganisms are not required for mineralization of these non-chlorinated VOCs. Biological filters may be a viable treatment technology for removing these substances from contaminated air streams.

Two of the three chlorinated VOCs tested (trichloroethylene and carbon tetrachloride) were not completely mineralized under any conditions tested. The use of toluene as a co-substrate appeared to enhance TCE mineralization, but not carbon tetrachloride mineralization. Chlorobenzene was completely mineralized for a short period of time in the presence of toluene as a co-substrate, and data from this experiment suggest that the enzymes involved in degrading the aromatic ring of toluene are effective against chlorobenzene as well.

For gaseous chlorinated VOCs, it appears unlikely that biological filters could be satisfactory as the sole treatment process. However, the use of a biological filter as a pre-treatment stage in series with an activated carbon absorber may reduce the VOC load on the carbon unit and the costs associated with its operation.

This study indicates that biological filters should be further investigated for use in the treatment of gaseous VOCs, particularly non-chlorinated solvents. Parameters in need of refinement include the minimum acceptable gas retention time, the acceptable range of VOC concentrations, the optimal treatment conditions, and the maximum period of time for which a solid matrix treatment vessel can sustain complete VOC destruction.

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