To the Graduate Council:

I am submitting herewith a thesis written by John Joseph Niedzielski entitled "Aerobic Trichloroethylene Biodegradation in Continuous-Recycle Expanded-Bed Bioreactors." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Life Sciences.

David C. White, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that the accurate acknowledgement of the source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature John J. Tikkl

Date November 30, 1988

AEROBIC TRICHLOROETHYLENE BIODEGRADATION IN CONTINUOUS-RECYCLE EXPANDED-BED BIOREACTORS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

John Joseph Niedzielski

December 1988

To my mother and father

ACNOWLEDGEMENTS

During the course of my thesis research, a number of scholars have assisted me generously. In particular I am indebted to David C. White, Tommy J. Phelps, Stephen E. Herbes, and Walter Farkas, who served as my director and committee respectively. I also thank Richard Schram, Arpad Vass, David Nivens, and Richard Jack for technical assistance and insight. With their consultation, criticism, and encouragement, I was able to achieve results otherwise impossible.

Research was supported by the Oak Ridge National Laboratory Director's Research and Development Fund; by the Y-12 Plant Remedial Action Project, U.S. Department of Energy, under contract DE-AC05-84OR21400; Savannah River Laboratory, under contract AX681901 with E.I. duPont deNemours and Co., Aiken, SC; and a grant from the Gas Research Institute, Chicago, IL.

ABSTRACT

Chlorinated aliphatic hydrocarbons pose a serious threat to our fresh water supplies. Their toxic effects are well documented and, their abundant use in industrial processes and commercial products has led to widespread environmental contamination. Present water treatment processes are not desirable since the chlorinated compounds are not destroyed, but are merely removed and transferred to other portions of the environment. In the atmosphere, chlorinated hydrocarbons undergo transformations in the ozone layer leading to the formation of other chlorinated intermediates.

Recently, it has been discovered that certain microorganisms have the ability to biologically transform some chlorinated aliphatics to non-toxic compounds such as carbon dioxide. Test cultures capable of degrading trichloroethylene (TCE) at concentrations up to 100 mg/L have been maintained successfully in this laboratory. The present research has investigated the feasibility of utilizing these TCE-degrading cultures in continuous-recycle expanded-bed bioreactors capable of degrading TCE aerobically at concentrations of 20 mg/L. Two solid support matrices were compared and two different mixed culture consortia were utilized for bioreactor degradation studies. Operating reactors were subjected to a number of

test conditions in which parameters including energy source, pH, and nutrient levels were altered. The reactors were shown to be an effective means of monitoring the biological transformation of TCE occurring within these enclosed systems. Greater than 90% removal of TCE at initial liquid phase concentrations of 20 mg/L was observed in test reactors after 5 days, while control reactors contained 98% of the initial TCE added.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PART I

INTRODUCTION

INTRODUCTION

Occurrence

Trichloroethylene (TCE) and other related chlorinated-hydrocarbon solvents are common groundwater contaminants and are significant contributors to landfill runoffs (44). These compounds do not occur naturally, and because of their volatility, are rarely detected at concentrations exceeding a few micrograms per liter in surface waters (27). These compounds are persistent in subsurface environments, are transported rapidly in groundwater, and can enter the atmosphere by evaporation which can lead to even greater dispersal (22).

In general, TCE and the other related chlorinated aliphatics are volatile, nonflammable in air, and are poorly soluble in water (28)(Table 1). These characteristics make them ideal candidates for industrial solvents. Commercial and industrial uses served as the primary vectors for their accumulation in soil and groundwater (10). Chlorinated ethenes and ethanes were commonly used as degreasers and cleaning solvents in the dry-cleaning industry and in semiconductor manufacturing (44). Over two million tons of these compounds were used by American industry in 1985 and TCE comprised 89,000 tons of this total (45). A decline in usage of TCE has resulted from more stringent regulations (Table 2).

Table 1
Physical Properties of Chlorinated Ethenes

Compound	B.P.	V.P.	Solubility	Partition Coefficient	Evaporation Rate
VC	-13.9	2320	60 (10°C)	0.02 (10°C)	26
DCE	83.6	63.9	8800	26.4	22
TCE	87.0	57.9	1100	2.74	21
PCE	121.2	14.0	150	1.22	25

VC=vinyl chloride, DCE=dichloroethylene,
TCE=trichloroethylene,
PCE=tetrachloroethylene.
B.P.=boiling point, V.P.=vapor pressure (mm Hg/20°C), Solubility in water (20°C), partition coefficient in water, evaporation rate (halflife, minutes), typical environmental conditions.

Table 2
1988 EPA Drinking Water Standards for Chlorinated Ethenes

Compound	Maximum Concentration Level (MCL, ug/L)	MCL Goal (ug/L)
Vinyl Chloride	2.0	0
Dichloroethylene (1,1)	7.0	5.0
Trichloroethylene	5.0	0
Tetrachloroethylene	10.0	-

Obtained from Tennessee Department of Health and Environment, Division of Water pollution and Supply.

However, it has been a common ingredient in many household products including spot-removers, rug cleaners, air fresheners, industrial metal cleaners, dry cleaning fluids, refrigerants, polishers and anesthetics (26,30). The fact that TCE is the most commonly reported groundwater contaminant can probably be attributed to its ubiquitous use (2,28).

Nearly everyone has been exposed to these solvents as a result of their widespread use and their persistence as environmental contaminants. The volatility of chlorinated ethenes suggests that a major route of exposure is by the respiratory system (16). Occupational exposures can involve applications ranging from a secretary using correctional fluid to an industrial worker cleaning factory equipment (2,44). Domestic exposure can occur from taking a hot shower with tainted water or from dry-cleaned clothes (2,28). Clearly, exposure should not be equated with toxicity. The dose-response relationship is the fundamental principle of toxicology and requires that there be an exposure and a toxic effect (2). The potential for toxicologic interaction increases as the exposure increases, and exposure to mixtures leads to the possibility of unpredictable additivity, synergism, or potentiation of effects (2).

Trichloroethylene and other haloalkenes are characterized by both fatty liver production and necrosis

in mammals (8). The hepatoxicity activity of chlorinated aliphatics has been associated with the ease with which a chlorine atom can be removed to produce a reactive metabolite (25). The first step in the biotransformation of TCE in mammalian systems has been proposed to involve microsomal oxidation leading to epoxide formation across the double bond (2). The resulting oxiranes are highly reactive and can bind covalently to nucleic acids leading to mutations and cancer (2,40). The metabolic activation of hepatic microsomal cytochrome P-450 (a mixed function oxidase) appears to be a prerequisite for the mutagenicity and carcinogenicity of chlorinated ethylenes (8). Vinylidine chloride (VDC) is the most hepatoxic followed by trichloroethylene (TCE) and vinyl chloride (VC), with perchloroethylene (PCE) and cis- and trans-dichloroethylene (DCE) being of lower hepatotoxicity (15). With regard to carcinogenicity, VC has been found to be carcinogenic in mammals, while TCE and VDC appear to be weakly carcinogenic and PCE non-carcinogenic even though PCE may be degraded to TCE, VDC and VC (2,8).

Water Treatment Methods

At present, two water treatment processes are used to remove halogenated hydrocarbons (46): aeration or air stripping, and adsorption (28). Aeration can effectively lower the concentration of volatile organics in waste

water. Mixtures of solvents often exist in contaminated water; even though the efficiency of the process varies for each solvent, aerating to remove one specific contaminant will reduce the concentrations of the others (28). The ease of removing a contaminant by aeration generally increases with an increasing Henry's Law constant and by adjusting the air to water ratio, it is possible to remove up to 99% of a given contaminant with respect to the original influent concentration (28).

The adsorption process is more complex than aeration, and water quality can greatly influence its performance (28). Granular activated carbon has typically been used as a water treatment adsorbent. The adsorption capacity for each contaminant can vary from 1 to 100 mg/g carbon at an equilibrium concentration of 500 ug/L (28). Adsorption isotherms vary with equilibrium concentrations (influent concentrations) which can pose a problem when treating contaminated waters containing varying concentrations of respective pollutants (13,28). An increased effluent concentration can occur when the influent concentration of the adsorbate declines, and the result is desorption or "leakage" which can be explained by adsorption equilibrium theory (13,28).

One problem with both of these water treatment systems is that they do not change or decompose the contaminant, but merely transfer it elsewhere. Aeration

releases the halocarbons into the atmosphere and adsorption transfers them to the site where the spent adsorbent is disposed. A process that permits treatment of contaminated water resulting in destruction of the contaminants rather than simple transfer may have many environmental and economic advantages.

Abiotic Transformations

Radiation-induced degradation of trichloroethylene and tetrachloroethylene in drinking water has been demonstrated using ^{60}Co gamma rays (14). The radiation dose appears to be a function of the ratio between the pollutant and the mineral content of the water. The required dose decreases with decreasing concentrations of the contaminants and is lower for distilled water than for drinking water. The necessary dose is not dependent on initial pollutant concentration, but rather on the content of mineral constituents of the water (14).

Most abiotic transformations are slow, but may be important when considering the time frames commonly associated with groundwater movement (44). Abiotic half-lives of TCE and PCE as a result of hydrolysis or dehydrohalogenation are 0.7 and 0.9 years respectively in water under typical environmental conditions (44). Most experiments that are used to determine abiotic reaction rates in water are performed at elevated temperatures

since reaction rates are too slow at environmental temperatures (44). Rates measured at higher temperatures can then be extrapolated to lower temperatures. For TCE and other chlorinated aliphatic compounds, a 3.5-fold decrease in reaction rates has been observed for each 10°C decrease in temperature (44). A different type of reaction may prevail at a certain temperature which leads to differences in reported reaction rates.

Biotic and abiotic systems have similarities in reaction mechanisms and transformation products (37,38,44). The transformations of halogenated aliphatics for each system can generally be divided into two classes (44). Oxidations and reductions are one class and require that external electrons be transferred (25). Substitutions and dehydrohalogenations are the other class and do not require external electron transfer (44). External electron transfer indicates that electrons are transferred from some moiety other than the halocarbon itself (25,38,44). Examples of the various reactions are shown in Table 3.

The concern over the effects of chlorofluoro-carbons on the ozone layer has led to a rapid increase of the understanding of atmospheric breakdown mechanisms, particularly those involving chlorine atoms. Although UV light plays a crucial part in the balance of the ozone layer, direct photodegradation is not a major mechanism in

Table 3
Abiotic and Biotic Reactions of Halogenated Aliphatic Compounds

Reactions	Examples
I. Substitution	
a.) solvolysis, hydrolysis	
$RX + H_2O \longrightarrow ROH + HX$	$CH_3CH_2Cl + H_2O \longrightarrow CH_3CH2OH + HCl$
b.) conjugation and other nucleophilic reactions	
$RX + N^- \longrightarrow RN + X^-$	$CH_3CH_2Cl + HS^- \longrightarrow CH_3CH_2SH + Cl^-$
II. Dehydrohalogenation	
$-\overset{\vert}{\underset{X}{C}}-\overset{\vert}{\underset{H}{C}}- \longrightarrow C{=}C + HX$	$CCl_3CH_3 \longrightarrow CCl_2CH_2 + HCl$
III. Oxidation	
a.) -hydroxylation	
$-\overset{\vert}{\underset{H}{C}}-X + H_2O \longrightarrow -C-X + 2H^+ + 2e^-$	$CH_3CHCl_2 + H_2O \longrightarrow CH_3CCl_2OH + 2H^+ + 2e^-$
b.) halosyl oxidation	
$-\overset{\vert}{C}-X + H_2O \longrightarrow -C-X^+O^- + 2H^+ + 2e^-$	$CH_3CHCl_2 + H_2O \longrightarrow CHClCl^+O^- + 2H^+ + 2e^-$
c.) epoxidation	
$\backslash C{=}C \overset{X}{/} + H_2O \longrightarrow \overset{O\ X}{C-C} + 2H^+ + 2e^-$	$CHClCCl_2 + H_2O \longrightarrow CHClOCCl_2 + 2H^+ + 2e^-$
d.) biohalogenation (alkenes)	
$\backslash C{=}C + X^- + H_2O \longrightarrow -\overset{OH\ X}{C-C}- + 2H^+ + 2e^-$	$CH_2CH_2 + Cl^- + H_2O \longrightarrow CH_2OHCH_2Cl + 2H^+ + 2e^-$
IV. Reduction	
a.) hydrogenolysis	
$RX + H^+ + 2e^- \longrightarrow RH + X^-$	$CCl_4 + H^+ + 2e^- \longrightarrow CHCl_3 + Cl^-$
b.) dihalo-elimination	
$-\overset{\vert}{\underset{X}{C}}-\overset{\vert}{\underset{X}{C}}- + 2e^- \longrightarrow C{=}C + 2X^-$	$CCl_3CCl_3 + 2e^- \longrightarrow CCl_2CCl_2 + 2Cl^-$
c.) coupling	
$2RX + 2e^- \longrightarrow R-R + 2X^-$	$2CCl_4 + 2e^- \longrightarrow CCl_3CCl_3 + 2Cl$

the disappearance of chlorocarbons in the atmosphere. More significant is the reaction with hydroxyl radicals which are themselves formed photolytically. The major mechanism for tropospheric destruction is attack by OH·. This to the abstraction of H· from molecules which are saturated and incompletely halogenated or to elimination of a chlorine atom if the molecule is unsaturated (38).

Biotic Transformations

Several researchers have investigated biological degradation of chlorinated ethenes (1,3-4,6-7,10-12,30-33,41-46). Bouwer and McCarty (7) have demonstrated that 300 ug/L concentrations of TCE and PCE can be degraded anaerobically by mixed anaerobic sludge cultures. Tetrachloroethylene was degraded to TCE by reductive dehalogenation and then to vinyl chloride, which is highly carcinogenic and recalcitrant. Over 99% of the PCE was degraded after 10 days, however, the VC produced represented nearly all of the initial PCE and TCE molar concentrations.

The fate of vinyl chloride produced anaerobically from PCE is not well understood. Complete mineralization to carbon dioxide may occur depending on initial PCE concentrations and length of sampling times since vinyl chloride concentrations decreased by 75% after 22 days in this study (6).

Aerobic degradation of chlorinated ethenes has been reported by many investigators (11-12,27,30-32,42,45-46). Wilson and Wilson (46) showed that TCE at an initial concentration of 150 ug/L was converted to carbon dioxide after a two day period in an unsaturated soil column which was exposed to a natural gas/air mixture. The observed biodegradation was attributed to the metabolic activities of methane-oxidizing bacteria. Nelson et al. (31) have demonstrated aerobic biodegradation of TCE by a gram-negative, rod-shaped bacterium (strain G4) at concentrations of 0.84 ug/L. These investigators have suggested that the biodegradation elicited from the G4 organism involves induction of enzymes of an aromatic degradative pathway (32). Results indicated that the G4 bacterium was not a methanotroph and that phenol or toluene addition induced TCE degradation via the aromatic degradative pathway (32). Little et al. (27) have isolated Type-I pure strain methanotrophs from groundwater samples drawn from monitoring wells at a waste disposal site capable of degrading TCE at initial concentrations of 400 ug/L to carbon dioxide and water-soluble products (identified as glyoxylic acid and dichloroacetic acid).

Heterotrophic enrichment cultures capable of degrading more than 99% of exogenous TCE aerobically to carbon dioxide at concentrations exceeding 50 mg/L have been cultured from TCE-contaminated subsurface sediments

(11). These enrichment cultures were main
various combinations of yeast extract, try
methane, propane and methanol (11). To da
highest TCE concentration at which signifi
degradation of TCE has been shown to occur.

Analytical Problems

A difficulty that arises when studying TCE degradation in a given system is quantifying the absolute concentration of TCE (45). Chlorinated aliphatics partition between the aqueous phase, the headspace, and the substratum. Henry's Law allows one to estimate the concentration in the phases with respect to the phase ratios being employed (20). The accuracy of this calculation is affected both by the deviation of TCE from ideal gas behavior, and by the fact that volatilization from the aqueous phase into the headspace is not instantaneous, which may lead to deviations from equilibrium between aqueous and gaseous TCE concentrations (13). Adsorption of TCE may also occur onto bacterial cells or surfaces of the reaction vessel. Plastics and rubber compounds are not desirable materials for TCE quantification studies since they adsorb halogenated compounds (13,45). Any TCE adsorption would increase the effective concentration in the liquid phase as a result of equilibrium shift (13,20).

Objectives

The research objectives of the work described herein were threefold:

1. Demonstrate the feasibility of continuous-recycle expanded-bed bioreactors for aerobic TCE degradation and quantification of volatile chlorinated aliphatic compounds, volatile organic substrates and carbon dioxide.

2. Examine parameters which optimize TCE degradation.

3. Determine monitoring criteria for bioreactors to assess efficient operation and stability.

LITERATURE CITED

1. Anders, M. W., and I. Jakabson. 1985. Biotransformation of halogenated solvents. Scand. J. Environ. Health. 11:23-32.

2. Andrews, L. S., and R. Snyder. 1986. Toxic effects of solvents and vapors, p. 636-668. In Klaassen, C. D., M. O. Amdur, and J. Doull (ed.), Toxicology. Macmillan Publishing Company, New York.

3. Barrio-Lage, G., F. Z. Parsons, and R. S. Nassar. 1987. Kinetics of the depletion of trichloroethylene. Environ. Sci. Technol. 21:366-370.

4. Barrio-Lage, G., F. Z. Parsons, R. S. Nassar, and P. A. Lorenzo. 1986. Sequential dehalogenation of chlorinated ethenes. Environ. Sci. Technol. 20:96-99.

5. Best, R. C., Hammond, and D. Scott. 1981. Methane-oxidizing microorganisms. Microbiol. Rev. 45:556-590.

6. Bouwer, E. J., P. L. McCarty, and J. C. Lance. 1981. Trace organic behavior in soil columns during rapid infiltration of secondary wastewater. Water Research. 15:151-159.

7. Bouwer, E. J., and P. L. McCarty. 1983. Transformation of 1- and 2- carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. 45:1286-1294.

8. Costa, A. K., and K. M. Ivanetich. 1984. Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes. Carcinogenesis. 6:1629-1636.

9. Dilling, W. L., N. B. Tefertiller, and G. J. Kallos. 1975. Evaporation rates of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and other chlorinated compounds in dilute aqueous solutions. Environ. Sci. Technol. 9(9):833-837.

10. Fathepure, B.Z., J. P. Nenju, and S. A. Boyd. 1987. Anaerobic bacteria that dechlorinate perchloroethylene. Appl. Environ. Microbiol. 53(1):2671-2674.

11. Fliermans, C. B., T. J. Phelps, D. Ringleberg, A. T. Mikell, and D. C. White. 1988. Mineralization of trichloroethylene by heterotrophic enrichment cultures. Appl. Environ. Microbiol. 54(7):1709-1714.

12. Fogel, M. M., A. R. Taddeo, and S. Fogel. 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. 51:720-724.

13. Garbarini, D. R., and L. W. Lion. 1986. Influence of the nature of soil organics on the sorption of toluene and trichloroethylene. Environ. Sci. Technol. 20:1263-1269.

14. Gehringer, P., E. Proksh, and W. Szinovatz. 1985. Radiation-induced degradation of trichloroethylene and tetrachloroethylene in drinking water. Int. J. Appl. Radiat. Isot. 36(4):313-314.

15. Ghantos, H., B. R. G. Danielsson, L. Dencker, J. Gorczak, and O. Vesterberg. 1986. Trichloroacetic acid accumulates in murine amniotic fluid after tri- and tetrachloroethylene inhalation. Acta Pharmacol. et Toxicol. 58:105-114.

16. Greim, H., T. Wolff, M. Hofler, and E. Lahaniatis. 1984. Formation of dichloroacetylene from trichloroethylene in the presence of alkaline material - possible cause of intoxication after abundant use of chloroethylene containing solvents. Arch. Tox. 56:74-77.

17. Hartmans, S., J. A. M. deBont, J. Tramper, and K. CH. A. M. Luyben. 1985. Bacterial degradation of vinyl chloride. Biotechnol. Let. 7(6):383-388.

18. Henson, J. M., M. V. Yates, J. W. Cochran, and D. L. Shackleford. 1988. Microbial removal of halogenated methanes, ethanes, and ethylenes in an aerobic soil exposed to methane. FEMS Microbiol. Ecol. 53:193-201.

19. Hou, C. T., R. Patel, A. I. Laskin, N. Barnabe, and I. Barist. 1983. Epoxidation of short-chain alkenes by resting-cell-suspensions of propane-grown bacteria. Appl. Environ. Microbiol. 46:171-177.

20. Ioffe, B. V., and A. G. Vittenberg. 1984. Headspace analysis and related methods in gas chromatography. John Wiley and Sons, Inc., New York.

21. Janssen, D. B., G. Grobben, and B. Witholt. 1987. Toxicity of chlorinated aliphatic hydrocarbons and degradation by methanotrophic consortia, p.515-518. In O. M. Neijssel (ed.), Proceedings of the 4th European Congress on Biotechnology. Elsevier Science Publishers B. V., Amsterdam, Netherlands.

22. Kanazawa, S., and Z. Filip. 1986. Effects of trichloroethylene, tetrachloroethylene and dichloromethane on enzymatic activities in soil. Appl. Microbiol. Biotechnol. 25:76-81.

23. Kanazawa, S., and Z. Filip. 1987. Effects of trichloroethylene, tetrachloroethylene and dichloromethane on soil biomass and microbial counts. Zbl. Bakt. Hyg. B. 184:24-33.

24. Kleopfer, R. D., D. M. Easley, B. B. Haas, Jr., and T. G. Deihl. 1985. Anaerobic degradation of trichloroethylene in soil. Environ. Sci. Technol. 19(3):277-280.

25. Kline, S. A., J. J. Solomon, and B. J. Van Duuren. 1978. Synthesis and reactions of chloroalkene epoxides. J. Org. Chem. 43(18):3596-3600.

26. Kobayashi, H., and B. E. Rittman. 1982. Microbial removal of hazardous organic compounds. Environ. Sci. Technol. 16(3):170A-182A.

27. Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54(4):951-956.

28. Love, T. Jr., and R. G. Eilers. 1982. Treatment of drinking water containing trichloroethylene and related industrial solvents. J. Am. Water Works Assoc. 74:413-425.

29. Lynd, L., R. Kerby, and J. G. Zeikus. 1982. Carbon monoxide metabolism of the methylotrophic acetogen Butyribacterium methylotrophicum. J. Bacteriol. 149:255-263.

30. McConnell, G., D. M. Ferguson, and C. R. Pearson. 1974. Chlorinated hydrocarbons and the environment. Endeavor. 34:13-18.

31. Nelson, M. J. K., S. O. Montgomery, E. J. O'Neill, and P. H. Pritchard. 1986. Aerobic metabolism of trichloroethylene by a bacterial isolate. Appl. Environ. Microbiol. 52:383-384.

32. Nelson, M. J. K., S. O. Montgomery, W. R. Mahaffey, and P. H. Pritchard. 1987. Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 53:949-954.

33. Nichols, P. D., J. M. Henson, C. P. Antworth, J. Parsons, J. T. Wilson, and D. C. White. 1987. Detection of a microbial consortium, including Type II Methanotrophs, by use of phospholipid fatty acids in an aerobic halogenated hydrocarbon-degrading soil column enriched with natural gas. Environ. Tox. Chem. 6:89-97.

34. Nichols, P. D., G. A. Smith, C. P. Antworth, R. S. Hanson, and D. C. White. 1985. Phospholipid and lipopolysaccharide normal and hydroxy fatty acids as potential signatures for methane-oxidizing bacteria. FEMS Microbiol. Ecol. 31:327-335.

35. Okouchi, S. 1986. Volatilization coefficient for stripping trichloroethylene, 1,1,1, trichloroethane and tetrachloroethylene from water. Wat. Sci. Tech. 18:137-138.

36. Parsons, F., and G. B. Lage. 1985. Chlorinated organics in simulated groundwater environments. J. Am. Water Works Assoc. 77:52-59.

37. Parsons, F., P. R. Wood, and J. DeMarco. 1984. Transformations of tetrachloroethene and trichloroethene in microcosms and groundwater. J. Am. Water Works Assoc. 76:56-59.

38. Pearson, C. R. 1981. C_1 and C_2 Halocarbons, p. 69-88. In O. Hutzinger (ed.), The handbook of environmental chemistry,. Imperial Chemical Industries Ltd., Brixham Laboratory, Devon, England.

39. Politzer, P., and T. R. Proctor. 1982. Calculated properties of some possible vinyl chloride metabolites. Internat. J. Quant. Chem. 22:1271-1279.

40. Politzer, P., P. Trefonas III, I. R. Politzer, and B. Elfman. 1981. Molecular properties of the chlorinated ethylenes and their epoxide metabolites. Ann. N.Y. Aca. Sci. 367:478-492.

41. Roberts, L. 1987. Discovering microbes with a taste for PCBs. Res. News. 237:975-977.

42. Vandenbergh, P. A., and B. S. Kunka. 1988. Metabolism of volatile chlorinated aliphatic hydrocarbons by Pseudomonas fluorescens. Appl. Environ. Microbiol. 54:2578-2579.

43. Vogel, T. M., and P. L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49:1080-1083.

44. Vogel, T. M., C. S. Criddle, and P. L. McCarty. 1987. Transformations of halogenated aliphatic compounds. Environ. Sci. Technol. 21(8):722-736.

45. Wackett, L. P., and D. T. Gibson. 1988. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with Pseudomonas putida F1. Appl. Environ. Microbiol. 54(7):1703-1708.

46. Wilson, J. T., and B. H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49:242-243.

47. Ziglio, G., G. Beltramelli, F. Pregliasco, and G. Ferrari. 1985. Metabolites of chlorinated solvents in blood and urine of subjects exposed at environmental level. The Sci. Tot. Environ. 47:473-477.

PART II

AN EXPANDED-BED CONTINUOUS-RECYCLE BIOREACTOR DESIGN WHICH ALLOWS DIRECT HEADSPACE SAMPLING OF VOLATILE CHLORINATED ALIPHATIC COMPOUNDS

ABSTRACT

A two phase column system was developed which allowed direct head-space sampling of volatile chlorinated aliphatic compounds including trichloroethylene, cis- and trans-1,2-dichloroethylene, vinyl chloride, propane, methane and carbon dioxide. A comparison of two possible solid support matrixes (crushed glass and anthracite coal) was performed. Over 98±2% of the TCE added to control systems was still present following 12 days of operation. The bioreactor consisted of a liquid-phase expanded-bed column containing 70 grams of 60-80 mesh substratum. The liquid-phase was recirculated through the expanded-bed column into a gas recharge column fitted with a headspace sampling port which allowed direct sampling and subsequent gas chromatography analysis.

INTRODUCTION

The ever increasing contamination of environmental groundwaters with halogenated compounds has generated public and scientific concern (9,11). Researchers have demonstrated that aerobic and anaerobic biotransformation of chlorinated ethenes can occur both in the laboratory and in the field (1-4,6,8,12-17).

Analysis of short-chain chlorinated compounds can be performed by using various gas chromatography (GC) techniques including headspace chromatography, solvent extraction and purge and trap methods (2-7,17). Difficulty arises in quantifying these compounds since chlorinated ethenes partition between the gas and liquid phases (4,5,16).

Typical batch culture experiments are performed in glass vials equipped with teflon-faced silicone septa since chlorinated aliphatics tend to adsorb to materials such as butyl rubber, making quantitative analyses difficult (4,8,12,13). Liquid phase samples have been analyzed by allowing a measured amount of effluent to equilibrate in a vessel, as described above, and then performing headspace gas chromatography to quantitate the volatile components (2). Solvent extraction of liquid phase samples has also been utilized in quantification procedures (3). The purge and trap method is another

technique used to concentrate volatile compounds for quantification (6,17). Purge and trap methods are quite reliable, but like equilibration and solvent extraction procedures, they require liquid phase removal from the test system (15).

Batch cultures of bacterial consortia have been maintained in this laboratory which are capable of degrading trichloroethylene (TCE) (4). The purpose of designing a continuous-flow bioreactor was to determine the feasibility of utilizing the degrading capabilities of these bacterial cultures to treat environmentally contaminated waters. An important consideration in design of the bioreactor was providing a means for rapid sample withdrawal and subsequent quantification of chlorinated ethenes by GC techniques. We report here a bioreactor design which utilizes the direct headspace sampling method, and thus allows volatile component sampling without changing the volume of the liquid or gas phase of the continuous-recycle bioreactor system.

MATERIALS AND METHODS

Design of the Continuous-Recycle Bioreactor System

The continuous-recycle bioreactor system consisted of two organic resistant borosilicate glass chromatography columns (Pharmacia, Piscataway, NJ) linked in series providing a flow loop for the liquid phase (Fig.1). The gas recharge column provided a known headspace volume which allowed partitioning of volatile compounds (methane, propane, TCE) that afforded the use of GC techniques for quantitative analysis. The recharge column was 100 cm in height, 2.7 cm in diameter and displaced 525 mL total volume. The top end cap was equipped with two fittings, one of which provided a direct link from the recharge column to the expanded-bed column, while the second fitting served as a port for headspace sampling. The expanded-bed column was 44 cm in height, 2.7 cm in diameter and displaced a total volume of 235 mL. This column contained 70 grams of 60-80 mesh substratum (crushed glass or anthracite coal) which provided a large surface area for bacterial attachment. The substratum had a volume displacement of 45 mL. Similarly, this column had a top end cap equipped with two fittings, one of which provided a direct link to the gas recharge column, while the other was utilized as a port for sampling from or adding to the liquid phase. The end cap O-rings were

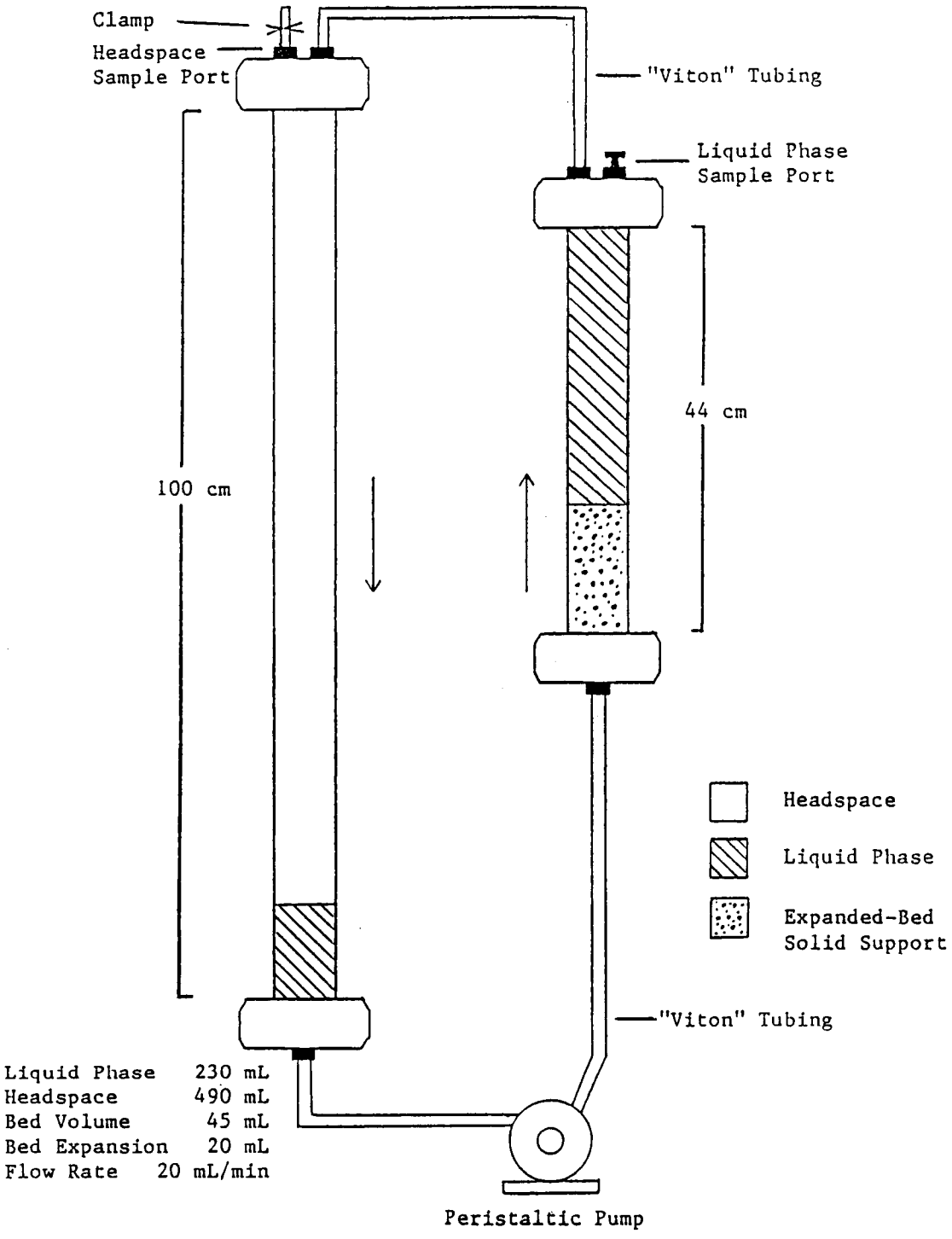

Fig.1 Continuous-recycle expanded-bed bioreactor design used for headspace analysis

teflon-coated to provide a gas-tight seal and all threaded fittings employed were sealed with teflon tape. All tubing connections consisted of either teflon tubing or organic-resistant tubing (Cole-Parmer, Chicago, IL). A continuous recycle flow rate was maintained at 20 mL/min. with a peristaltic pump. A septum was constructed for the gas recharge column by utilizing a 10 cm section of size 14 organic resistant tubing which was pierced with a 26 gauge needle. Clamps were placed above and below the pierced hole to prevent head-space leakage. The columns were mounted vertically in a closed metal cabinet to prevent light exposure which could foster growth of photosynthetic microorganisms and induce photolysis of the chlorinated aliphatic compounds. All reactors were operated at room temperature (25°C).

Medium

The liquid phase in the reactors was the same medium that was employed for maintaining TCE-degrading test cultures. The medium contained: $MgSO_4$, 0.055 g; $CaSO_4 \cdot 2H_2O$, 0.054 g; NH_4SO_3, 1.48 g; trace minerals II (10), 10 mL; 10X vitamin solution (10), 1 mL; 10% yeast extract/trypticase (1 culture only), 2 mL; 200 mM phosphate/bicarbonate buffer solution, 10 mL; nanopure filtered water, 1000 mL. Resazurin was added (1 mL of a 0.2% solution per liter media) to monitor the

aerobic status of the liquid phase. In all cases, the pH of the medium was maintained at 7.2.

Gases and Chemicals

Propane and oxygen were more than 99.5% pure, while methane was over 98% pure. All chemicals were of reagent grade and were obtained from Mallinkrodt, Inc. (Paris, KY) or Sigma Chemical Co. (St. Louis, MO). Glass distilled solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ).

Operation of the Continuous-Recycle Bioreactor System

The total liquid phase volume of the system was 230 mL. The effluent flowed upward through the expanded-bed column containing the expanded crushed glass substratum. It then exited the top of the expanded-bed column and entered the top of the gas recharge column which contained 40 mL of the liquid phase. The expanded-bed column (saturated) contained 190 mL of the effluent. The headspace in the gas recharge column was then 485 mL, which resulted in a headspace:liquid volume ratio of 2.1:1. The flow rate of the effluent was maintained at 20 mL/min with a peristaltic pump creating a bed expansion of 25-30% (10-15 mL in volume).

A TCE-saturated water solution (1100 mg/L)(1,3) was added to the medium which made the total nominal liquid

phase concentration of TCE in the reactor 20 mg/L. Methane and propane were also added to the system through the headspace sample port and were maintained at 5% and 3% of the gas phase (v/v) respectively. Reactor headspaces were monitored for several weeks by GC before and after inoculation of the two test test columns. The remaining column served as a control and contained 0.5% formalin and 0.2% sodium azide in order to inhibit microbial contamination.

The columns were tested for leaks by pressurizing them to 10 pounds per square inch overnight and checking for pressure loss after 24 hours prior to subsequent experiments. No pressure loss was observed which indicated that the reactors were gas-tight.

For GC quantitative analyses, gas phase aliquots were sampled directly from the head-space of the recharge column. This was done by inserting a GC syringe needle through the fabricated septum so that the needle tip was placed directly inside of the headspace of the recharge column. The syringe inserted into the column was flushed 5 times prior to sample withdrawal. Following sample removal, the tubing was clamped below the point of needle entry to prevent any gas loss.

Gas Chromatography

TCE was analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a 50-m Hewlett Packard Ultra Performance (Ultra 1) cross-linked methyl silicone capillary column (internal diameter of 0.2 mm, 0.33 um film) and an ECD detector. The following conditions were used: oven temperature, 60°C; injector temperature, 150°C; detector temperature, 200°C. The split ratio was set at 10:1. The carrier gas was hydrogen and the makeup gas was nitrogen. Vinyl chloride, cis- and trans-1,2-dichloroethylene (DCE) were analyzed with a Schimadzu GC-9A gas chromatograph equipped with a 2.44 m, 3.2 mm diameter Poropak T packed column and a PID detector (HNU Systems, Newton, MA). The following conditions were used: oven temperature, 150°C; injector temperature, 160°C; detector temperature, 230°C. Methane and CO_2 were assayed with a Schimadzu GC-8A gas chromatograph equipped with a 2.74 m, 3.2 mm diameter Carbosieve 8000 packed column and a TCD detector. The following conditions were used: oven temperature, 130°C; injector/detector temperature, 140°C. Propane was analyzed with a Schimadzu GC-9A gas chromatograph equipped with a 2.44 m, 3.2 mm diameter Poropak N packed column and an FID detector. The following conditions were used: oven temperature, 80°C; detector/injector temperature, 220°C.

Quantitative analyses were made for propane, methane and CO_2 by relating peak heights of column samples to those of prepared gas standard curves. Chlorinated volatile compounds sampled from the columns were compared to standard curves (peak areas) of the respective compounds using a Nelson 2600 analytical chromatography system with version 4.0 (Nelson Analytical, Cupertino, CA). The standards were prepared to have the same headspace:liquid phase ratio as that of the reactors using the principles of Henry's Gas Laws (4,6).

The limits of detection for sampled compounds were as follows; TCE, 0.1 ug/L; cis-1,2-DCE, 10 ug/L; trans-1,2-DCE, 10 ug/L; VC, 100 ug/L; propane, CH_4, and CO_2, 0.05% (v/v). Detection limits for chlorinated compounds are nominal liquid phase concentrations. Reproducibility was obtained at a signal to noise ratio (S/N) greater than 3.0.

Quantitative Determination of TCE in Bioreactors

When the distribution coefficient (K) or the Henry's Law constant (H) for a volatile organic compound (VOC) are known, the equilibrium gas concentration (C_G) of a VOC in a closed system serves as a direct measure of its liquid concentration (C_L) under conditions of constant temperature and pressure since $C_L^o = C_G(K+V_G/V_L)$ (note C_L^o is the initial liquid phase concentration) (4,6). The

terms V_G and V_L correspond to gas phase and liquid phase volumes respectively. The distribution coefficient and Henry's Law constant for a given system are inversely proportional (6). If gas chromatographic peak areas are normalized to a uniform ratio of gas and liquid phase volume irregardless of total system volume, then the gas concentration of the VOC will be the same for either system as long as conditions of constant temperature and pressure are met and no significant VOC adsorption is observed which would shift the phase equilibrium (5).

RESULTS

TCE Partitioning and Quantification

Two support materials were evaluated for their possible utility in the bioreactors. Anthracite coal and crushed glass, both at 60/80 mesh, have given favorable results for bacterial attachment and TCE degradation using bacterial test consortia (data not shown). Seventy grams of the respective substrata were added to the saturated fluidized-bed column. Table 1 shows the results of the comparison of the two support materials in the bioreactors following TCE addition at a total nominal liquid phase concentration of 20 mg/L. Partitioning of TCE into the coal was extensive, resulting in decreased headspace concentrations compared to those of standards prepared at the same phase ratios without coal. The crushed glass showed no indication of significant TCE adsorption over the 24 hour period (Table 1).

The insignificant adsorption of the crushed glass substrata ($P>0.01$) led to further investigation of its possible utility as a solid support matrix for bacterial studies. The liquid-phase TCE concentration in the bioreactors was evaluated relative to external TCE standards prepared at the same phase ratios as the reactor

Table 1
Comparison of Support Materials and TCE Partitioning in Bioreactors Using Headspace Sampling Technique

Substratum	TCE Concentration (mg/L) 5 minutes	45 minutes	24 hours
Anthracite Coal (60/80 mesh)	19.2 ± 0.8	13.0 ± 0.6	5.0 ± 0.3
Crushed Glass (60/80 mesh)	20.0 ± 0.8	20.0 ± 0.8	19.9 ± 0.7

Results are the mean of duplicate experiments. TCE-saturated water (1100 mg/L) was added to make the initial total nominal liquid concentration 20 mg/L. Liquid phase was medium, pH 7.2.

systems (Figure 2). The ECD detector peak area responses at nominal TCE concentrations are virtually identical, which indicates that the TCE concentration in the bioreactors is comparable to that seen in the standard solutions. A mass distribution calculation for TCE (K=2.74)(11) revealed that approximately 45% of the total nominal amount of TCE in the liquid phase migrates to the gas phase of the reactors.

Similar results were obtained for the other chlorinated compounds with the crushed glass substratum (data not shown). The results indicated that GC headspace analysis can be used to monitor the subsequent change

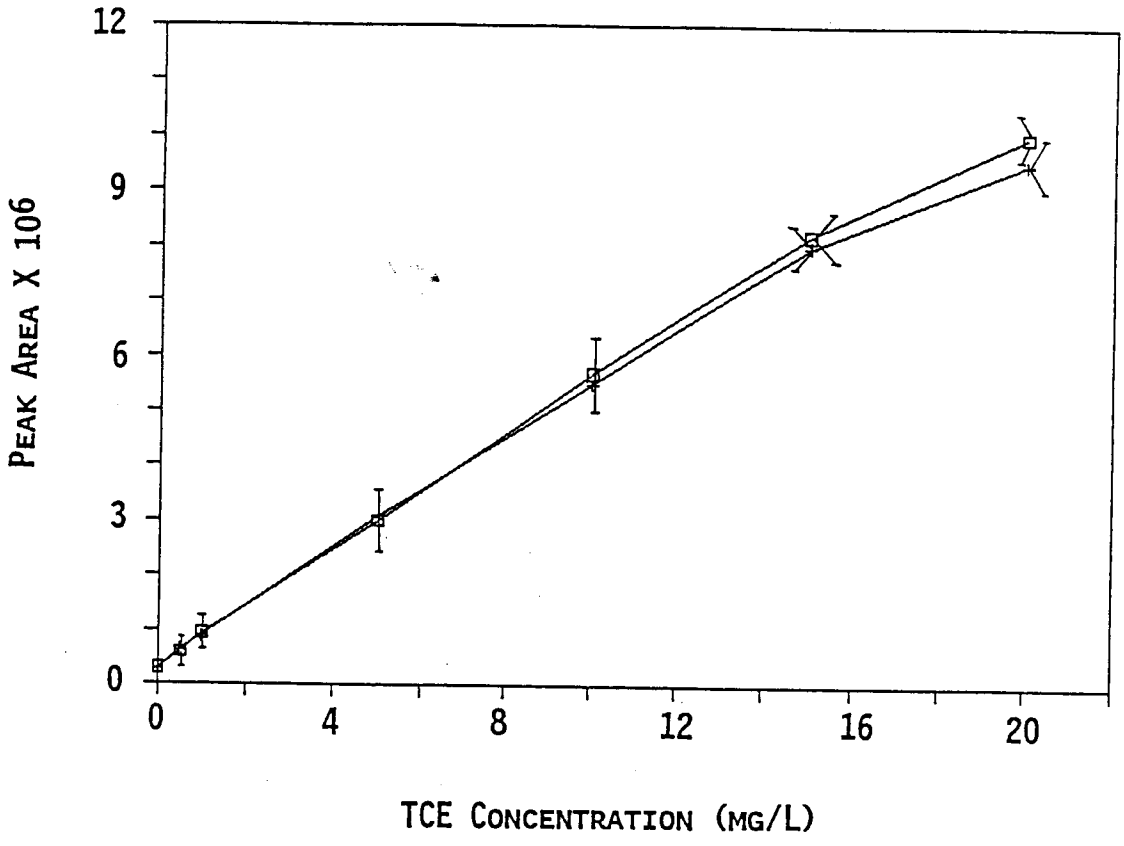

Fig.2 Electron Capture Detector response to increasing concentrations of TCE from standard headspace (□) and reactor headspace (+) utilizing crushed glass substratum. Results are the mean of duplicate experiments.

of TCE concentration in the bioreactors when studying the degradative capabilities of bacterial consortia following their introduction into the system.

The observed TCE equilibration time differed greatly when analyzing standard and reactor samples. Static standard TCE solutions prepared in 45 mL EPA vials required approximately one hour of equilibration before headspace analysis. Reactor headspace samples reached equilibrium within 15 minutes after TCE addition. The difference in equilibration time was justified by the fact that the reactor is operated under continuous-recycle conditions, allowing a more rapid mass transfer.

Bioreactor Gas Dynamics: Before Inoculation

Medium was added to reactors along with TCE, propane and methane prior to inoculation with test cultures to determine that all reactors behaved in the same fashion. Table 2 shows the results of headspace analysis over a 5 day period. No appreciable loss of any of the analytes was observed, and concentrations measured in all of the reactors were identical within analytical uncertainty. There was no CO_2 formation in any of the reactors, which indicated that there was no significant loss or degradation.

Table 2
Concentration of Volatile Components In Bioreactors Prior To Inoculation

Sample	TCE	Propane	Methane	Carbon Dioxide
	(mg/L)		(mMoles/L)	
Day 0				
Reactor 1	20.0±0.8	0.93±0.06	2.25±0.10	n.d.
Reactor 2	19.8±0.9	0.93±0.06	2.25±0.10	n.d.
Reactor 3	19.9±0.9	-	2.24±0.10	n.d.
Day 5				
Reactor 1	19.9±0.7	0.93±0.04	2.25±0.12	n.d.
Reactor 2	19.7±0.8	0.91±0.06	2.25±0.10	n.d.
Reactor 3	19.8±0.8	-	2.23±0.14	n.d.

Liquid phase was medium, pH 7.2, mean of 2 sample injections, n.d. = not detected

Bioreactor Gas Dynamics: After Inoculation

Two reactors were inoculated with test cultures by recirculating 1L (approximately 240 mg/L dry weight of biomass) of each of the respective cultures through the reactor substrata for 5 hours. Reactors were drained following inoculation. At this time, medium (500 mL) was recirculated through each of the reactors for one hour to flush each system. The reactors were then drained and 230 mL of fresh medium was added to each reactor. Column 1 served as a control and contained 0.5% formalin and 0.2% sodium azide. The headspace of each recharge column was then purged with pure oxygen for 1 hour. TCE

was then added to each reactor at a total nominal liquid phase concentration of 20 mg/L, followed by methane and propane, which were 5% and 3% (v/v) of the head-space respectively. Table 3 shows the results of headspace analysis over a 9 day period. Methane and propane consumption and CO_2 formation were observed in the test columns. The control reactor showed no loss of any of the analytes and no CO_2 production. The test systems indicated that no loss of TCE had occurred during the initial four day time period. At day nine, reactors 2 and 3 indicated TCE losses of 70% and 50% respectively of the initial contaminant as compared to the inhibited control.

Table 3
Concentration Of Volatile Components in Bioreactors After Inoculation

Sample	TCE (mg/L)	Propane	Methane (mMoles/L)	Carbon Dioxide
Day 0				
Reactor 1	20.0±0.9	0.93±0.06	2.25±0.12	n.d.
Reactor 2	19.7±0.8	0.91±0.04	2.25±0.12	n.d.
Reactor 3	19.8±0.8	-	2.25±0.14	n.d.
Day 4				
Reactor 1	19.9±0.9	0.91±0.06	2.25±0.12	n.d.
Reactor 2	19.7±0.7	0.23±0.02	1.13±0.06	0.85±0.04
Reactor 3	19.7±0.7	-	0.57±0.04	0.76±0.04
Day 9				
Reactor 1	19.0±0.9	0.93±0.04	2.25±0.14	n.d.
Reactor 2	5.5±0.3	n.d.	0.66±0.02	0.91±0.04
Reactor 3	9.0±0.5	n.d.	0.23±0.02	0.89±0.04

Liquid phase was medium, pH 7.2, mean of 2 sample injections, n.d. = not detected
Reactor 1 was an uninoculated control containing 0.5% formalin 0.2% sodium azide. Reactors 2 and 3 were inoculated with test consortia.

DISCUSSION

Over 96% of the initial TCE added to the bioreactors at initial nominal liquid phase concentrations of 20 mg/L was still present following 12 days of operation. Discrepancies in total TCE applied to inhibited soil columns were observed by researchers studying continuous-flow systems and subsequent mass balance determinations (17). Anaerobic continuous-flow column studies involving biotransformation of chlorinated ethenes did not include results for comparable inhibited controls (15). The lack of comparable controls in previous endeavors gives rise to analytical questions and data interpretation.

The mass distribution of TCE in the bioreactors revealed that approximately 45% of the total nominal amount of TCE in the liquid phase migrates to the gas phase (11). Intuitively, the precision of the subsequent GC headspace analysis performed on reactor samples should be acceptable since roughly half of the added mass of TCE is in each phase (5,16).

Partitioning of TCE into the solid support (Table 1) demonstrates that care needs to be taken in the choice of substratum. Approximately 75% of the TCE added to reactors containing anthracite coal at initial liquid phase concentrations of 20 mg/L was absorbed to the solid support. Over 85% of this TCE could be retrieved

following thermal desorption (data not shown). Similar results were obtained in TCE adsorption studies involving soils (5).

In choosing a solid support for a bioreactor of this type, three things should be considered: bacterial attachment to the support, the ability of the attached bacteria to actively degrade the compound of interest, and the inert characteristics of the support. Previous studies with glass beads indicated that the beads were inert to chlorinated ethenes, however, the smooth glass beads did not promote bacterial attachment to create the necessary biomass. The crushed glass substratum showed no indication of TCE adsorption and displayed positive characteristics for microbial attachment and biomass formation. This substratum was therefore chosen for further studies in which microbial consortia capable of biologically degrading TCE are introduced into the bioreactor system.

It is shown here that liquid phase sample removal from the reactor is not necessary to determine the concentration of volatile compounds such as TCE. Avoidance of removal of liquid phase samples has multiple advantages. First, the ratio of headspace to liquid phase volume remains undisturbed as a result of sampling only a small volume of the reactor gas phase, thus simplifying mass balance calculations and interpretation

of bioreactor operation. Second, the ease of direct headspace GC sampling allows for rapid analysis as opposed to time-consuming solvent extraction procedures or purge and trap techniques used by other investigators (3,6,15).

A continuous-recycle system, similar to the one described here, should be beneficial for quantitative analysis of other volatile organic compounds that display liquid phase:headspace partitioning behavior similar to that of TCE. This technique should be useful for researchers investigating microbial degradation of volatile contaminants in efforts to design pilot-scale processes. Various operational parameters could be studied simultaneously, including: test cultures, substrates, solid supports and waste composition. The techniques described herein may serve useful for monitoring and maintaining the performance of other on-site bioreactors of this type.

LITERATURE CITED

1. Anders, M. W., and I. Jakabson. 1985. Biotransformation of halogenated solvents. Scand. J. Environ. Health. 11:23-32.

2. Bouwer, E. J., P. L. McCarty, and J. C. Lance. 1981. Trace organic behavior in soil columns during rapid infiltration of secondary wastewater. Water Research. 15:151-159.

3. Bouwer, E. J., and P. L. McCarty. 1983. Transformation of 1- and 2- carbon halogenated aliphatic compounds under methanogenic conditions. Appl. Environ. Microbiol. 45:1286-1294.

4. Fliermans, C. B., T. J. Phelps, D. Ringleberg, A. T. Mikell, and D. C. White. 1988. Mineralization of trichloroethylene by heterotrophic enrichment cultures. Appl. Environ. Microbiol. 54(7):1709-1714.

5. Garbarini, D. R., and L. W. Lion. 1986. Influence of the nature of soil organics on the sorption of toluene and trichloroethylene. Environ. Sci. Technol. 20:1263-1269.

6. Kleopfer, R. D., D. M. Easely, B. B. Haas, Jr., and T. G. Deihl. 1985. Anaerobic degradation of trichloroethylene in soil. Environ. Sci. Technol. 19(3):277-280.

7. Ioffe, B. V., and A. G. Vittenberg. 1984. Headspace analysis and related methods in gas chromatography. John Wiley and Sons, Inc., New York.

8. Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54(4):951-956.

9. Love, T. Jr., and R. G. Eilers. 1982. Treatment of drinking water containing trichloroethylene and related industrial solvents. J. Am. Water Works Assoc. 74:413-425.

10. Lynd, L., R. Kerby, and J. G. Zeikus. 1982. Carbon monoxide metabolism of the methylotrophic acetogen Butyribacterium methylotrophicum. J. Bacteriol. 149:255-263.

11. McConnell, G. D., D. M. Ferguson, and C. R. Pearson. 1974. Chlorinated hydrocarbons and the environment. Endeavor. 34:13-18.

12. Nelson, M. J. K., S. O. Montgomery, E. J. O'Neill, and P. H. Pritchard. 1986. Aerobic metabolism of trichloroethylene by a bacterial isolate. Appl. Environ. Microbiol. 52:383-384.

13. Nelson, M. J. K., S. O. Montgomery, W. R. Mahaffey, and P. H. Pritchard. 1987. Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 53:949-954.

14. Nichols, P. D., J. M. Henson, C. P. Antworth, J. Parsons, J. T. Wilson, and D. C. White. 1987. Detection of a microbial consortium, including Type II Methanotrophs, by use of phospholipid fatty acids in an aerobic halogenated hydrocarbon-degrading soil column enriched with natural gas. Environ. Tox. Chem. 6:89-97.

15. Vogel, T. M., and P. L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49:1080-1083.

16. Wackett, L. P., and D. T. Gibson. 1988. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with Pseudomonas putida F1. Appl. Environ. Microbiol. 54(7):1703-1708.

17. Wilson, J. T., and B. H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49:242-243.

PART III

BIODEGRADATION OF TRICHLOROETHYLENE IN CONTINUOUS-RECYCLE EXPANDED-BED BIOREACTORS

ABSTRACT

Experimental bioreactors were inoculated with bacterial cultures which utilized methane, propane and tryptone-yeast extract as energy sources. Uninoculated bioreactors were inhibited with 0.5% formalin and 0.2% sodium azide as controls for mass balance determinations. Each bioreactor consisted of a saturated expanded-bed column containing 70 grams of 60-80 mesh crushed glass substratum. The liquid phase was recirculated through the expanded-bed column into a gas recharge column which allowed direct headspace sampling. Trichloroethylene (TCE) (20 mg/L) was added to each bioreactor and GC techniques were used to monitor TCE, propane, methane and carbon dioxide. Greater than 95% removal of TCE was observed in experimental bioreactors after 6 days.

INTRODUCTION

United States industry used approximately 90 thousand tons of trichloroethylene (TCE) in 1985 and the Environmental Protection Agency has listed this compound as a priority pollutant (18). Many subsurface aquifers in this country are contaminated with chlorinated ethenes and some industrial sites are reported to contain chlorocarbons in excess of 1,000 mg per liter (4,5,11,). Chloroalkenes are suspected carcinogens and are generally resistant to degradation in the environment (3). The present technologies available for reclaiming groundwater polluted with these compounds involve pumping the contaminated water to the surface and either air-stripping the compounds in aeration towers or adsorbing the pollutants on a sorbent (11). A process that results in the destruction of these compounds, rather than simply transferring them to other portions of the environment, would be more desirable.

Biological degradation of TCE has been shown to occur with pure cultures and mixed microbial consortia (4-5, 13-20). Chlorinated alkenes may be converted anaerobically via reductive dehalogenation to known carcinogens such as vinyl chloride (2). Natural gas has been shown to stimulate aerobic TCE degradation in unsaturated soil columns leading to carbon dioxide

formation (20). In column experiments, the methanotroph biomass increased, and it was hypothesized that methanotrophs were a major constituent in the TCE mineralization process (15). The mixed-function oxidases of methanotrophs were thought to be responsible for the mineralization of TCE and other chlorinated organic compounds (4,10).

Heterotrophic microorganisms have been shown to degrade TCE aerobically when enriched with an aromatic compound, such as toluene or phenol (14). Although TCE was mineralized to carbon dioxide, stimulation by an aromatic compound was required. Toluene dioxygenase has been implicated in the TCE metabolic activities of these organisms (19).

The purpose of this study was to examine the utility of a continuous-recycle bioreactor capable of degrading TCE aerobically using mixed culture consortia obtained from environmental sites contaminated with short-chain chlorinated hydrocarbons. The construction and maintenance of such a system and its performance for accurately measuring the volatile components of a given microbial community are discussed elsewhere in Part II. Bacterial cultures capable of degrading TCE consistently at concentrations of 50 mg/L have been maintained under aerobic conditions in this laboratory (4). This report describes the performance of bioreactors inoculated with

TCE-degrading mixed cultures. The bioreactors were monitored five to twelve days following subjection to various conditions of energy source, pH, and nutrient levels.

MATERIALS AND METHODS

Gases and Chemicals

All gases were supplied from MG Industries (Chattanooga, TN). Propane and oxygen were greater than 99.5% pure and methane was more than 98% pure. All chemicals were of reagent grade and were obtained from Mallinkrodt, Inc. (Paris, KY) or Sigma Chemical Co. (St. Louis, MO). Glass-distilled solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, N.J.).

Bacterial Cultures

Two separate mixed culture consortia which displayed TCE-degrading capabilities were used as inoculum for bioreactors. Culture PM-M contained mixtures of propane and methane-oxidizing bacteria obtained from Ada, OK (15) along with a TCE-degrading consortia isolated from the Savannah River Plant, Aiken, SC capable of degrading TCE at concentrations in excess of 50 mg/L (4) and a type I methanotroph isolated from a waste disposal site near Oak Ridge, TN capable of degrading TCE at concentrations less than 1 mg/L (10). Culture SM-1 contained only Savannah River Plant consortia and the type I methanotroph. Culture PM-M was grown solely on methane and propane, 5% and 3% respectively (v/v, headspace).

Culture SM-1 was amended with 100 mg/L yeast extract trypticase (one addition) and 5% methane, 3% propane (v/v, headspace).

Laboratory Techniques

Construction, maintenance and operation of the bioreactors used in this study are discussed in Part II. The liquid phase in the reactors was the same medium that was employed for maintaining TCE-degrading test cultures. The medium contained per liter: $MgSO_4$, 0.055 g; $CaSO_4 \cdot 2H_2O$, 0.054 g; NH_4NO_3, 1.48 g; trace minerals II, 10 mL (12); 10X vitamin solution, 1 mL (12); 10% yeast extract/trypticase (100 mg/L, 1 culture only), 2 mL; 200 mM phosphate/bicarbonate buffer solution, 10 mL; deionized water, 1000 mL. Resazurin was added (1 mL of a 0.2% solution per liter media) to monitor the redox of the liquid phase.

The bioreactors were subjected to a variety of experimental conditions and the headspace concentration of analytes was measured over time. For each experiment, the liquid phase was a phosphate-buffered medium set at pH 7.2 (except the pH 7.5 experiment). The total liquid phase volume of each reactor during all experiments was 230 mL, while the total headspace volume remained at 490 mL. The nominal liquid phase TCE concentration at the onset of each experiment was 20 mg/L. Control reactors

contained 0.2% sodium azide and 0.5% formalin to deter microbial contamination. Please note that for the data presented in the remainder of the text, the following designations are used; reactor 1 was the uninoculated control, reactor 2 was inoculated with culture PM-M and reactor 3 was inoculated with culture SM-1.

Bioreactor Equilibration

Experimental reactors inoculated with test cultures did not show any indication of TCE loss until several weeks after inoculation. Once test reactors had repeatedly displayed TCE removal, experimental parameters were altered in an effort to maximize the microbial degradation. All experimental degradation studies described herein were initiated at nominal liquid phase TCE concentrations of 20 mg/L.

Bioreactor Experiments

Pulsed substrate addition studies were performed by adding energy source (propane, methane) to test reactors only at day zero. Continuous substrate experiments were conducted by replenishing the energy sources (methane, propane) utilized daily after determining their respective concentrations in the headspace of the test reactors. Experiments where no energy source was added to the test reactors were termed starvation. The liquid phase pH

remained at 7.2 throughout time course experiments with the exception of the pH 7.5 experiment.

Analytical Procedures

TCE was analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a 50-m Ultra Performance (Ultra 1, Hewlett Packard) cross-linked methyl silicone capillary column (internal diameter of 0.2 mm, 0.33 um film) and an electron capture detector (Hewlett Packard). The following conditions were used: oven temperature, 60° C; injector temperature, 150°C; detector temperature, 200° C. The split ratio was set at 10:1. Hydrogen served as the carrier gas and the makeup gas was nitrogen. Vinyl chloride, cis- and trans-1,2-dichloroethylene (DCE) were analyzed using a Schimadzu GC-9A gas chromatograph equipped with an 2.44 m, 3.2 mm diameter Poropak T packed column and a photoionization detector (HNU Systems, Newton, MA). The following conditions were used: oven temperature, 150°C; injector temperature, 160°C; detector temperature, 230°C. Methane and CO_2 were assayed using a Schimadzu GC-8A gas chromatograph equipped with a 2.74 m, 3.2 mm diameter Carbosieve 8000 packed column and a TCD detector. The following conditions were used: oven temperature, 130°C; injector/detector temperature, 140°C. Propane was analyzed using a Schimadzu GC-9A gas chromatograph equipped with an 2.44 m, 3.2 mm diameter

Poropak N packed column and a flame ionization detector. The following conditions were used: oven temperature, 80° C; detector/injector temperature, 220°C.

Concentration analyses were determined for propane, methane and CO_2 by relating peak heights of reactor samples to those of prepared gas standard calibration curves. Chlorinated volatile compounds sampled from the columns were compared to standard curves (peak areas) of the respective compounds using a Nelson 2600 analytical chromatography system with version 4.0. (Nelson Systems, Cupertino, CA). The standards were prepared to have the same headspace:liquid phase ratio as that of the columns using the principles of Henry's Gas Laws (6).

The limits of detection for sampled compounds were as follows; TCE, 0.1 ug/L; cis-1,2-DCE, 10 ug/L; trans-1,2-DCE, 10 ug/L; VC, 100 ug/L; propane, CH_4, and CO_2, 0.05% (v/v). Detection limits for chlorinated compounds are nominal liquid phase concentrations. Reproducibility was obtained at a signal to noise ratio (S/N) greater than 3.0.

RESULTS

TCE Biodegradation

Previous studies performed with the bioreactor indicated that in the absence of added microbial cultures, over 98±2% of the TCE added to the systems at nominal liquid concentrations of 20 mg/L was still present after 5 days (Part II). Experimental reactors inoculated with test cultures did not show any indication of TCE loss until several weeks after inoculation.

The first set of experiments examined pulsed, daily and starved feeding (Table 1). Reactor 3 displayed over 86% loss in TCE, while reactor 2 showed over 43% loss after 5 days in pulsed feeding experiments. Reactor 2 was fed only on day zero with 5% methane in the headspace and 200 mg/L tryptone/yeast extract (YE/TRY) while reactor 3 initially contained 5% methane and 3% propane (v/v) in the headspace. Reactor 1 (control) showed no loss of analytes. The liquid phase pH remained at 7.2 in all reactors throughout the time course of the experiment. The results of continuous substrate experiments indicated 90% and 85% removal of the initial TCE added to reactors 2 and 3 respectively after five days (Fig.1). Carbon dioxide production increased as compared to the pulsed addition experiments. The results for daily carbon dioxide headspace analyses are shown in Fig.2.

Table 1. Comparison of TCE degradation in bioreactors under conditions of pulsed feeding, continuous feeding and starvation

Experiment	Run (Days)	Reactor	Propane Consumed (mmoles)	Methane Consumed (mmoles)	Carbon Dioxide (mmoles)	TCE Loss (umoles)	% TCE Loss	Substr. /TCE Loss (umoles/ umoles)
Fed Day 1, Starved	5	1	0.05	0.05	<0.01	1.24	3.5%	-
		2	0.67	1.10	0.40	31.43	90.3%	54.0
		3	0.64	1.10	0.42	15.74	45.2%	110.7
	5							
		1	0.04	0.05	<0.01	1.07	3.1%	-
		2	0.67	1.01	0.43	29.90	86.0%	55.2
		3	0.62	1.10	0.51	14.94	43.0%	115.1
Continuous Substrate	5	1	0.05	0.06	<0.01	1.11	3.1%	-
		2	2.10	1.33	1.20	31.41	90.3%	109.2
		3	1.83	2.15	3.50	29.62	85.1%	135.1
Starved	5	1	-	-	<0.01	1.01	2.9%	-
		2	-	-	0.20	2.61	7.5%	-
		3	-	-	0.75	3.48	10.0%	-

Reactors: #1 = control, #2 = culture PM-M, #3 = culture SM-1
Initial TCE concentration of 20 mg/L (34.8 umoles/reactor)
Replicate for Continuous Substrate experiment shown in Table 2 for comparison

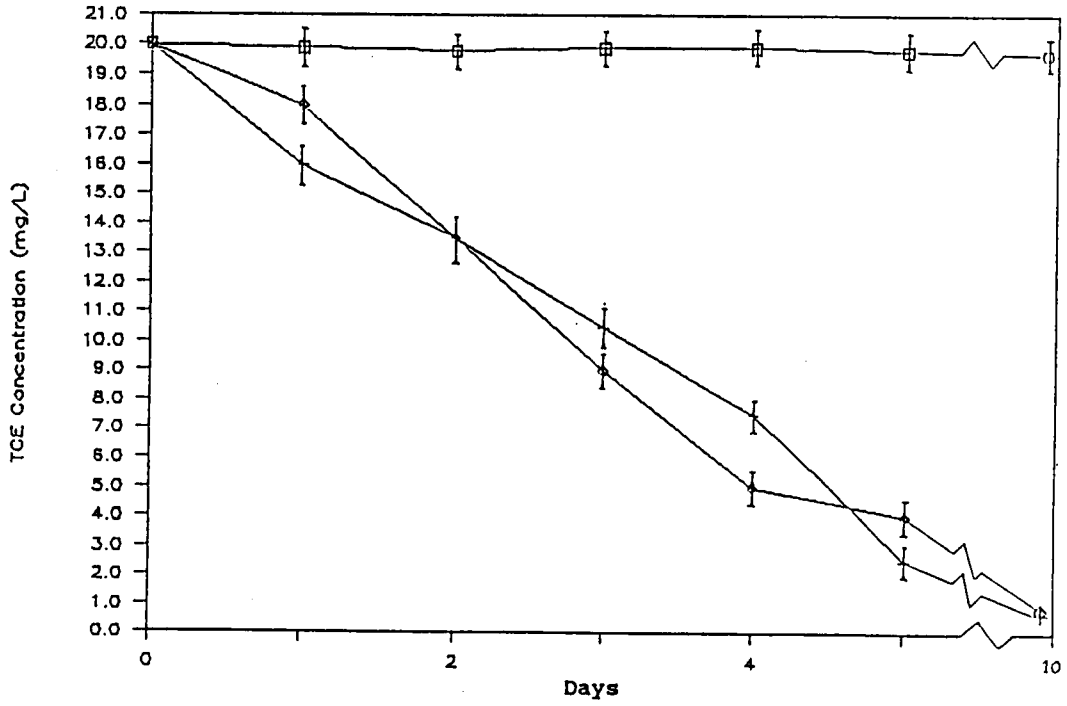

Fig.1 Utilization of TCE during methane/propane continuous substrate experiments. The control reactor (□) was inhibited with 0.2% sodium azide and 0.5% formalin. Experimental reactors contained test culture PM-M (+) and test culture SM-1 (◇). Results are the mean of duplicate experiments.

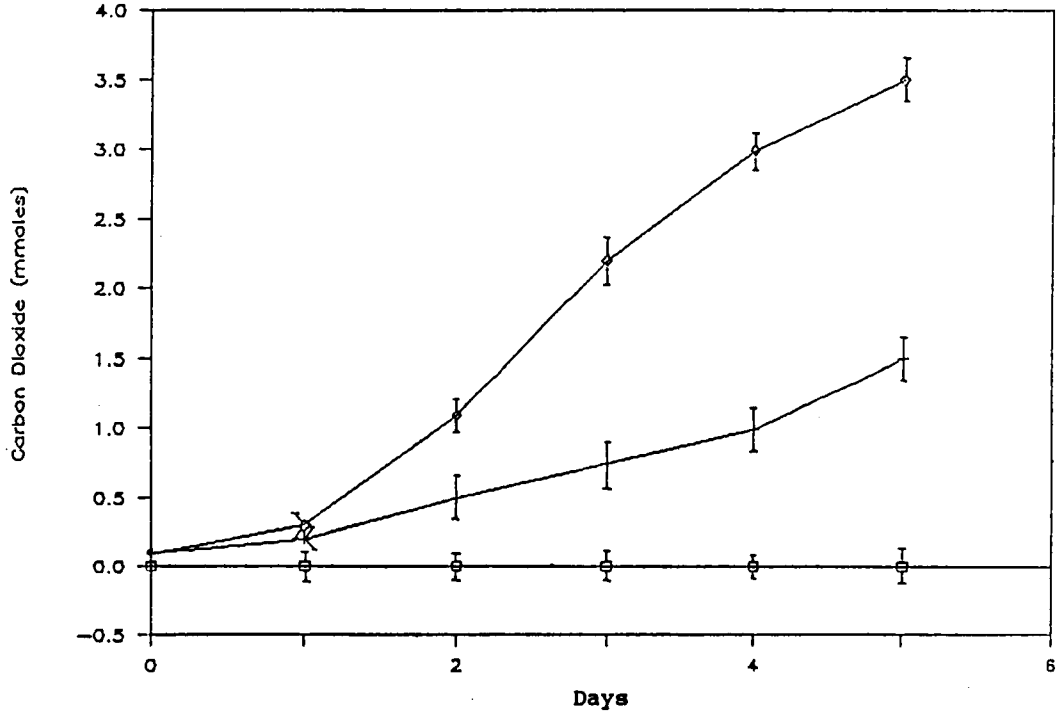

Fig.2 Observed carbon dioxide production during methane/propane continuous substrate experiments. The control reactor (□) was inhibited with 0.2% sodium azide and 0.5% formalin. Experimental reactors contained test culture PM-M (+) and test culture SM-1 (◇). Results are the mean of duplicate experiments.

When substrate was not added to the reactors (starvation), the rates of TCE removal were less than 10% of those seen in either the "propane only" or mixed substrate propane/methane experiments (Table 1).

An increased pH was attempted to decrease headspace pressure resulting from carbon dioxide production. When the media pH was changed from 7.2 to 7.5, only 15% of the initial TCE concentration was degraded (Table 2). The amount of methane and propane utilized as well as the amount of carbon dioxide produced also declined sharply. This showed that the bioreactors were sensitive to a small increase in pH.

Doubling the nutrient level in the reactor medium in terms of phosphates, nitrates, vitamins and trace minerals did not increase TCE degradation when maintained at continuous substrate levels of propane and methane (Table 2). This increase in macro and micro nutrients resulted in TCE removals up to 33% less than those observed with standard medium concentrations (Table 1). Reactor 2 showed a 25% increase in methane consumption, while reactor 3 displayed a 50% decrease in methane consumption. Total carbon dioxide production increased by 50% in reactor 2, but did not increase in reactor 3.

When methane alone was added to the reactors as an energy source (3%,v/v), the total TCE degraded decreased by approximately 60% in both systems as compared to

Table 2. Comparison of TCE degradation in bioreactors under conditions of continuous substrate, elevated pH and increased nutrients

Experiment	Run (Days)	Reactor	Propane Consumed (mmoles)	Methane Consumed (mmoles)	Carbon Dioxide (mmoles)	TCE Loss (umoles)	% TCE Loss	Substr. /TCE Loss (umoles/ umoles)
Continuous Substrate	5	1	0.06	0.05	<0.01	1.07	3.0%	-
		2	2.16	1.30	1.47	32.27	92.7%	108.5
		3	1.73	2.22	3.92	28.21	81.1%	139.3
pH 7.5	5	1	0.03	0.03	<0.01	1.15	3.3%	-
		2	0.45	0.44	0.22	5.23	15.0%	170.2
		3	0.29	0.41	0.77	6.10	17.5%	114.8
2X Nutrient	5	1	0.04	0.05	<0.01	1.19	3.4%	-
		2	2.05	1.77	2.10	26.14	75.1%	145.8
		3	2.13	1.04	3.40	20.04	57.6%	158.2
	5	1	0.06	0.05	<0.01	1.01	2.9%	-
		2	2.20	1.70	2.42	25.27	72.6%	154.3
		3	1.94	1.10	3.21	19.41	55.8%	156.7

Reactors: #1 = control, #2 = culture PM-M, #3 = culture SM-1

Initial TCE concentration of 20 mg/L (34.8 umoles/reactor)

2X nutrients = doubling of micronutrients, vitamins, phosphates and nitrates in medium

experiments where both methane and propane were maintained at 5% and 3% (v/v) respectively (Table 3). Carbon dioxide production also decreased by 65% as compared to experiments with mixed methane/propane energy sources.

When propane was provided as the sole energy source (Table 3), the total TCE loss was very similar to that observed when methane and propane were maintained at constant levels in the headspace of the reactors. Carbon dioxide production was also similar to that observed with mixed methane/propane substrates.

The results for daily analysis of TCE and carbon dioxide in the reactors corresponding to methane experiments and propane experiments are shown in figures 3-6. After day one, the TCE was degraded more rapidly with propane than with methane as an energy source in both test reactors (Fig.3,Fig.5). Daily carbon dioxide production increased in both test reactors during propane experiments as compared to methane experiments (Fig.4,Fig.6).

No volatile TCE intermediates were detected in either of the test reactors during any of the experiments performed. The control reactor, however, typically contained 0.5 mg/L of trans-1,2-DCE and 20 ug/L vinyl chloride following a five day time course experiment. This was possibly a result of chemical decomposition of TCE in the presence of the microbial growth inhibitors

Table 3. Comparison of TCE degradation in bioreactors under conditions of continuous methane or continuous propane substrate

Experiment	Run (Days)	Reactor	Propane Consumed (mmoles)	Methane Consumed (mmoles)	Carbon Dioxide (mmoles)	TCE Loss (umoles)	% TCE Loss	Substr. /TCE Loss (umoles/ umoles)
Propane	5	1	0.04	-	<0.01	1.09	3.1%	-
		2	2.37	-	1.80	30.50	87.6%	77.7
		3	2.82	-	2.70	27.88	80.1%	101.2
	5	1	0.06	-	<0.01	1.08	3.1%	-
		2	2.56	-	2.10	31.70	91.1%	82.0
		3	2.67	-	2.59	26.13	75.1%	102.4
Methane	5	1	-	0.03	<0.01	1.05	3.0%	-
		2	-	1.61	0.36	12.20	35.1%	132.0
		3	-	1.65	1.10	10.45	30.0%	157.9
	5	1	-	0.04	<0.01	1.11	3.1%	-
		2	-	1.65	0.41	11.95	34.3%	138.1
		3	-	1.70	1.32	10.19	29.3	166.8

Reactors: #1 = control, #2 = culture PM-M, #3 = culture SM-1
Initial TCE concentration of 20 mg/L (34.8 umoles/reactor)
Propane maintained at 3% (v/v) in headspace
Methane maintained at 5% (v/v) in headspace

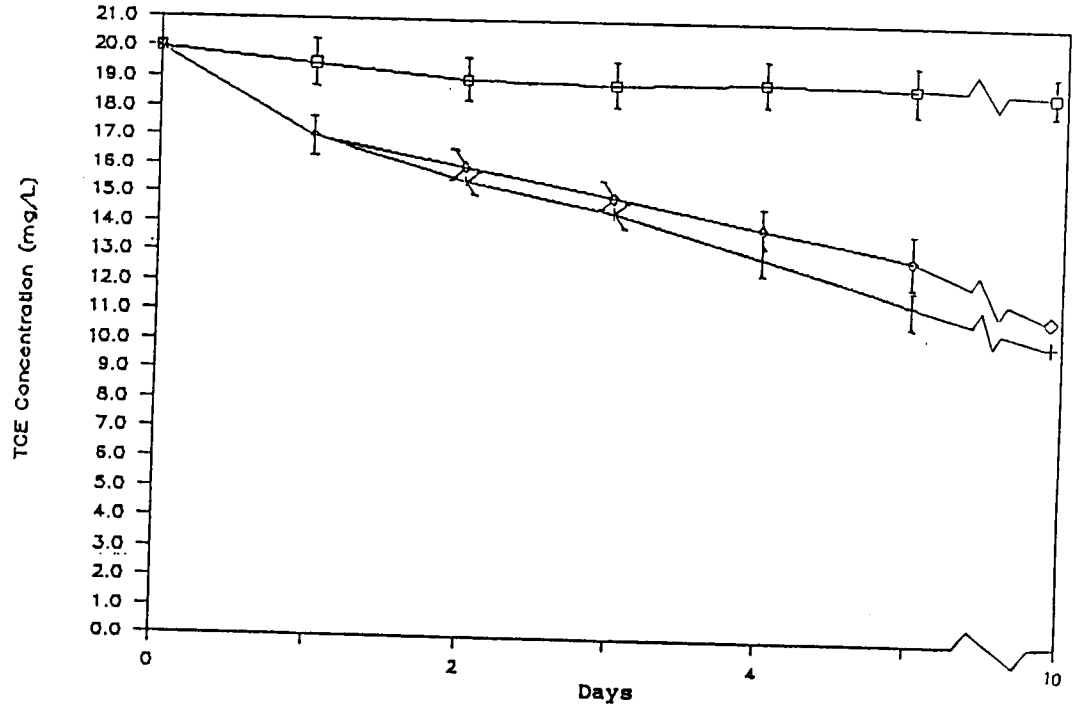

Fig.3 Utilization of TCE during methane continuous substrate experiments. The control reactor (□) was inhibited with 0.2% sodium azide and 0.5% formalin. Experimental reactors contained test culture PM-M (+) and test culture SM-1 (◇). Results are the mean of duplicate experiments.

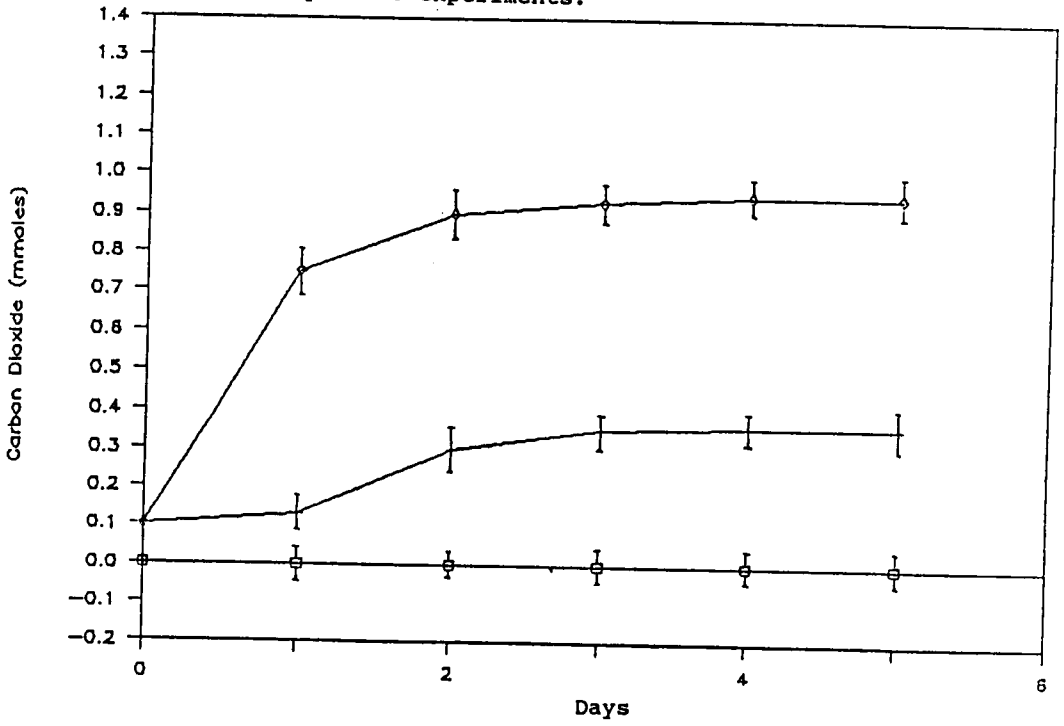

Fig.4 Observed carbon dioxide production during methane continuous substrate experiments. The control reactor (□) was inhibited with 0.2% sodium azide and 0.5% formalin. Experimental reactors contained test culture PM-M (+) and test culture SM-1 (◇). Results are the mean of duplicate experiments.

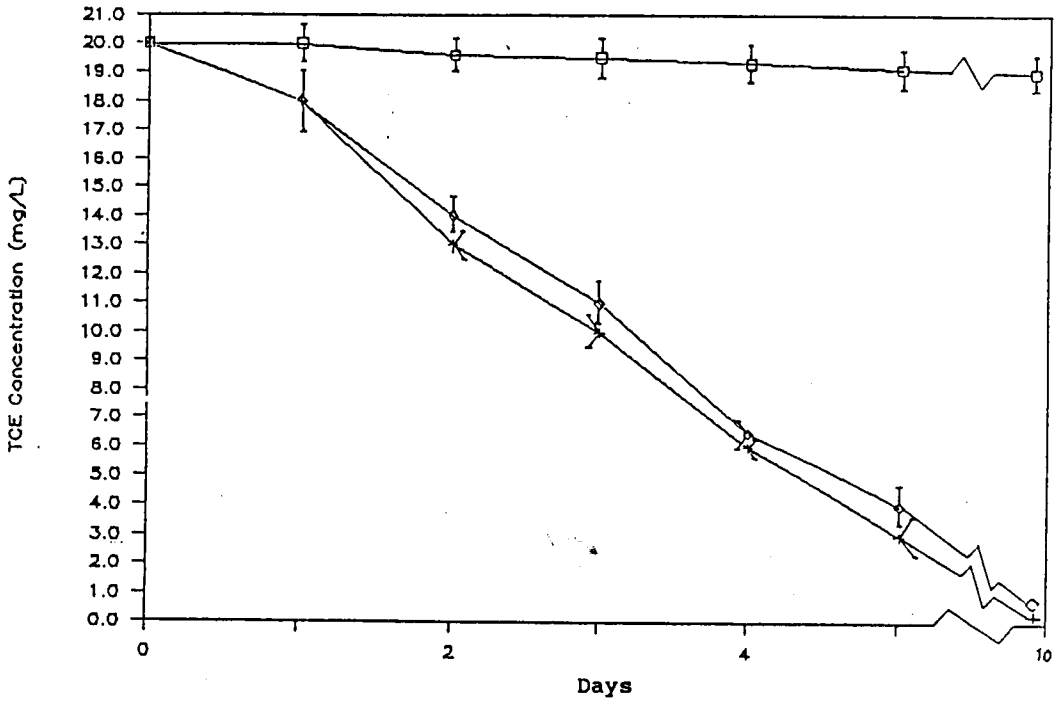

Fig.5 Utilization of TCE during propane continuous substrate experiments. The control reactor (□) was inhibited with 0.2% sodium azide and 0.5% formalin. Experimental reactors contained test culture PM-M (+) and test culture SM-1 (◇). Results are the mean of duplicate experiments.

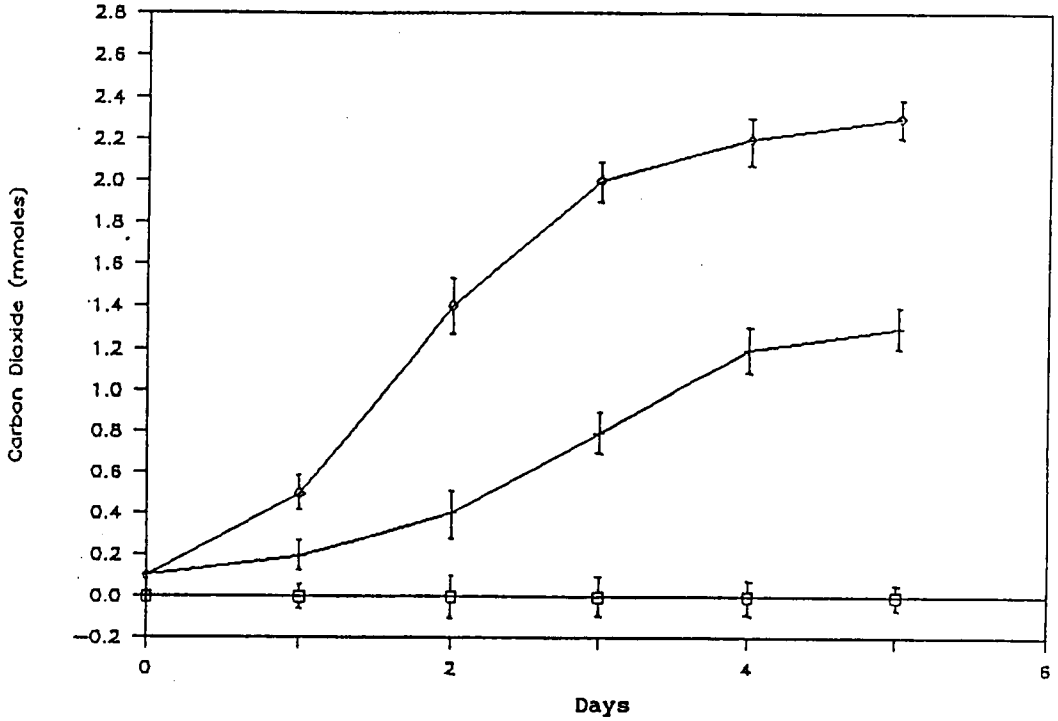

Fig.6 Observed carbon dioxide production during propane continuous substrate experiments. The control reactor (□) was inhibited with 0.2% sodium azide and 0.5% formalin. Experimental reactors contained test culture PM-M (+) and test culture SM-1 (◇). Results are the mean of duplicate experiments.

in the control reactor liquid phase (sodium azide, formalin), or trace contaminants found in the reagent TCE used in this study.

DISCUSSION

Results of these experiments demonstrate that inoculated bioreactors containing aerobic mixed culture consortia are capable of biologically degrading over 90% of the TCE present in a 20 mg/L solution while utilizing propane or methane/propane energy sources. Control reactors still contained 98±2% of the initial TCE added following 5 days of operation.

When only propane was provided as a substrate in the bioreactors, the extents and rates of TCE degradation were similar to methane/propane substrate mixtures. However, when methane was the only substrate available, the total amount of TCE degraded decreased by approximately 60% in both bioreactors. This suggests that the same group of organisms use propane more efficiently as a growth substrate or that propane does not compete as effectively as methane at TCE-transforming enzyme sites.

In terms of TCE degradation efficiency (substrate utilized/TCE degraded), propane as a sole carbon source was the most efficient when considering the total amount of TCE removed from the system during any of the daily substrate replenishment experiments. When the bioreactors were starved (no substrate added), TCE degradation decreased significantly. This finding is in accord with the results presented by other researchers (4,10).

Pulsed substrate additions (fed, starved conditions) showed that the bioreactor inoculated with culture PM-M had a substrate/TCE degraded ratio of approximately 55 while degrading over 85% of the initial nominal liquid phase TCE concentration of 20 mg/L. In pulsed and daily substrate replenishment experiments, the threshold TCE concentration was approximately 0.5 mg/L. This limit of TCE degradation was observed if experiments were allowed to proceed for 8 to 10 days. It is not known why the initial TCE concentration was not degraded to levels below the stated threshold limit considering that cell suspensions degraded 99.9% of the TCE present at liquid phase concentrations of 20 mg/L (data not shown).

Differences in microbial populations present in the reactors were evident as a result of substrate utilized, carbon dioxide produced and TCE consumed. The TCE removal efficiency was higher in the reactor inoculated with culture PM-M under all conditions with the exception of elevated pH. Culture PM-M was represented by a more diverse microbial community than was culture SM-1 at the time of reactor inoculation. This suggests that a diverse community structure may be more desirable in terms of stability for TCE degradation under various conditions of operation.

Anaerobic TCE degradation results in vinyl chloride formation which is mutagenic and recalcitrant (2,17).

Volatile TCE metabolite formation, including vinyl chloride, was not observed at our limits of detection. Resazurin was used as a redox indicator in our experiments to monitor the aerobic status of the liquid phase in the bioreactors. The media in the bioreactors remained blue through five day experimental runs. When experiments were performed for periods longer than one week, the media would turn pink unless additional oxygen was added to the headspace of the experimental reactors. This indicates that the test consortia were capable of reducing the redox potential of the liquid phase.

In previous studies involving methane or natural gas stimulation of degradation, TCE was converted to CO_2, cell-bound material or water-soluble products (5,10,20). The methanotrophic biomass increased in methane-enriched soil columns which led workers to believe that methanotrophs played a major role in decreasing the TCE concentration (15). It is believed that the monooxygenase of methanotrophs can oxidize and dechlorinate TCE (10). In mixed groundwater cultures, water-soluble TCE metabolites produced by methanotrophs are apparently mineralized to CO_2 by heterotrophic bacteria (10). The determination of possible water-soluble TCE breakdown products was unsuccessful in our experiments due to interference by some component in the medium.

The utility of the continuous-flow bioreactor for quantitative analysis of volatile organic compounds is discussed in Part II. Bioreactors inoculated with test consortia which aerobically oxidize gaseous hydrocarbons demonstrated successful TCE removal over one order of magnitude at initial media concentrations of 20 mg/L.

Several researchers have observed chlorinated alkene biodegradation in methane or natural gas-enriched mixed cultures obtained from various environmental sources (5,7,10,20). Our results suggest that propane may be a more suitable substrate for promoting TCE degradation and mixed microbial consortia may offer greater stability and promise for pilot scale studies. Propane-oxidizing bacteria can epoxidate ethylene (8) and investigators have hypothesized that the enzyme that epoxidates ethylene may transform TCE (20).

Previous studies and the work presented here indicate the potential for bioremediation of environments contaminated with chlorinated ethenes. A system similar to the bioreactors described here may prove useful for treating environmentally contaminated waters containing higher levels of chlorinated ethylenes.

LITERATURE CITED

1. Barrio-Lage, G., F. Z. Parsons, R. S. Nassar, and P. A. Lorenzo. 1986. Sequential dehalogenation of chlorinated ethenes. Environ. Sci. Technol. 20:96-99.

2. Bouwer, E. J., and P. L. McCarty. 1983. Transformation of 1- and 2- carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. 45:1286-1294.

3. Costa, A. K., and K. M. Ivanetich. 1984. Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes. Carcinogenesis. 6:1629-1636.

4. Fliermans, C. B., T. J. Phelps, D. Ringleberg, A. T. Mikell, and D. C. White. 1988. Mineralization of trichloroethylene by heterotrophic enrichment cultures. Appl. Environ. Microbiol. 54(7):1709-1714.

5. Fogel, M. M., A. R. Taddeo, and S. Fogel. 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. 51:720-724.

6. Garbarini, D. R., and L. W. Lion. 1986. Influence of the nature of soil organics on the sorption of toluene and trichloroethylene. Environ. Sci. Technol. 20:1263-1269.

7. Henson, J. M., M. V. Yates, J. W. Cochran, and D. L. Shackleford. 1988. Microbial removal of halogenated methanes, ethanes, and ethylenes in an aerobic soil exposed to methane. FEMS Microbiol. Ecol. 53:193-201.

8. Hou, C. T., R. Patel, A. I. Laskin, N. Barnabe, and I. Barist. 1983. Epoxidation of short-chain alkenes by resting-cell-suspensions of propane-grown bacteria. Appl. Environ. Microbiol. 46:171-177.

9. Kleopfer, R. D., D. M. Easley, B. B. Haas, Jr., and T. G. Deihl. 1985. Anaerobic degradation of trichloroethylene in soil. Environ. Sci. Technol. 19(3):277-280.

10. Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54(4):951-956.

11. Love, T. Jr., and R. G. Eilers. 1982. Treatment of drinking water containing trichloroethylene and related industrial solvents. J. Am. Water Works Assoc. 74:413-425.

12. Lynd, L., R. Kerby, and J. G. Zeikus. 1982. Carbon monoxide metabolism of the methylotrophic acetogen Butyribacterium methylotrophicum. J. Bacteriol. 149:255-263.

13. Nelson, M. J. K., S. O. Montgomery, E. J. O'Neill, and P. H. Pritchard. 1986. Aerobic metabolism of trichloroethylene by a bacterial isolate. Appl. Environ. Microbiol. 52:383-384.

14. Nelson, M. J. K., S. O. Montgomery, W. R. Mahaffey, and P. H. Pritchard. 1987. Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 53:949-954.

15. Nichols, P. D., G. A. Smith, C. P. Antworth, J. Parsons J. T. Wilson, and D. C. White. 1987. Detection of a microbial consortium including Type II Methanotrophs, by use of phospholipid fatty acids in an aerobic halogenated hydrocarbon-degrading soil column enriched with natural gas. Environ. Tox. Chem. 6:89-97.

16. Vandenbergh, P. A., and B. S. Kunka. 1988. Metabolism of volatile chlorinated aliphatic hydrocarbons by Pseudomonas fluorescens. Appl. Environ. Microbiol. 54:2578-2579.

17. Vogel, T. M., and P. L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49:1080-1083.

18. Vogel, T. M., C. S. Criddle, and P. L. McCarty. 1987. Transformations of halogenated aliphatic compounds. Environ. Sci. Technol. 21(8):722-736.

19. Wackett, L. P., and D. T. Gibson. 1988. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with Pseudomonas putida F1. Appl. Environ. Microbiol. 54(7):1703-1708.

20. Wilson, J. T., and B. H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49:242-243.

PART IV

SUMMARY

SUMMARY

Conclusions

The validation of the continuous-recycle bioreactor head space sampling technique for accurately quantifying TCE and other chlorinated aliphatics should prove useful for current bioremediation technology development. It would now be feasible to investigate the degradative capabilities of several microbial enrichments or solid support materials concurrently as long as the substratum used is found to be inert to the xenobiotics being studied. Experimental parameters such as pH, substrate, redox-potential, nutrient levels, and contaminants can be altered and analyzed quite readily using a similar system design and should prove useful for research involving other volatile halogenated compounds.

The microbial removal of halogenated aliphatics by volatile hydrocarbon-utilizing organisms has been shown by several investigators (6,7,9,14). Degradation of short-chain chlorinated compounds has been observed to occur with enrichment studies of soils or groundwater samples exposed to methane or natural gas (9,14). These researchers report degradation of chlorinated compounds at concentrations below one milligram per liter and the consensus has been that the methanotrophs are a major factor in the resulting contaminant removal. The results

given in Part III of this text illustrate that propane-oxidizing consortia are capable of removing over 95% of the TCE presented at initial nominal liquid phase concentrations of 20 mg/L in time frames much shorter than those reported by other workers. Clearly, alternative substrates should be studied for their stimulation of microbial halogenated compound removal in light of these results and those given by other investigators studying heterotrophic cultures (5,10,11,13). The findings for significant biological removal of high concentrations of chlorinated ethylenes provides great promise for the development of strategies to be used in remediation of extremely contaminated environments.

Recommendations

Some of the major issues that must be addressed to improve biological treatment of man-made environmental contaminants are microbial population selection, development and retention. The relative refractiveness of the compound must also be considered.

Population selection can be based upon the use of genetic engineering or enrichment techniques (5,8,9). Nutrient availability, pH, substrate utilization, and redox potential are key control parameters. The goals are to determine which organism carries out the limiting reaction, and to assure its proliferation. The needs of

the interacting organisms must also be considered. In some cases, a series of biological reactors (each maintained under defined conditions) may be required to provide the wide variety of microorganisms necessary for complete biodegradation.

The biodegradability of various organic contaminants and mixed wastes needs to be considered to plan for selective organism use. Compounds that require longer detention times, extending to several weeks, are considered refractory. Refractory compounds are those which cannot be degraded over time periods considered practical for a waste treatment process.

The use of microorganisms for biological treatment of xenobiotics is a relatively new concept. Several advancements, however, are required before large-scale application is possible. Among the most important is an improved knowledge of metabolic pathways for the biodegradation of specific compounds by different organisms. This understanding is necessary if limiting reactions are to be determined and the proper types of organisms are to be selected for specific applications. More information regarding the types of microorganisms to be selected and maintained in actual treatment systems is needed as well as the key parameters to monitor and insure efficient operation of the implemented system.

LITERATURE CITED

1. Barrio-Lage, G., F. Z. Parsons, R. S. Nassar, and P. A. Lorenzo. 1986. Sequential dehalogenation of chlorinated ethenes. Environ. Sci. Technol. 20:96-99.

2. Bouwer, E. J., P. L. McCarty, and J. C. Lance. 1981. Trace organic behavior in soil columns during rapid infiltration of secondary wastewater. Water Research. 15:151-159.

3. Bouwer, E. J., and P. L. McCarty. 1983. Transformation of 1- and 2- carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. 45:1286-1294.

4. Fathepure, B.Z., J. P. Nenju, and S. A. Boyd. 1987. Anaerobic bacteria that dechlorinate perchloroethylene. Appl. Environ. Microbiol. 53(1):2671-2674.

5. Fliermans, C. B., T. J. Phelps, D. Ringleberg, A. T. Mikell, and D. C. White. 1988. Mineralization of trichloroethylene by heterotrophic enrichment cultures. Appl. Environ. Microbiol. 54(7):1709-1714.

6. Fogel, M. M., A. R. Taddeo, and S. Fogel. 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. 51:720-724.

7. Henson, J. M., M. V. Yates, J. W. Cochran, and D. L. Shackleford. 1988. Microbial removal of halogenated methanes, ethanes, and ethylenes in an aerobic soil exposed to methane. FEMS Microbiol. Ecol. 53:193-201.

8. Kobayashi, H., and B. E. Rittman. 1982. Microbial removal of hazardous organic compounds. Environ. Sci. Technol. 16(3):170A-182A.

9. Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54(4):951-956.

10. Nelson, M. J. K., S. O. Montgomery, E. J. O'Neill, and P. H. Pritchard. 1986. Aerobic metabolism of trichloroethylene by a bacterial isolate. Appl. Environ. Microbiol. 52:383-384.

11. Nelson, M. J. K., S. O. Montgomery, W. R. Mahaffey, and P. H. Pritchard. 1987. Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 53:949-954.

12. Nichols, P. D., J. M. Henson, C. P. Antworth, J. Parsons, J. T. Wilson, and D. C. White. 1987. Detection of a microbial consortium, including Type II Methanotrophs, by use of phospholipid fatty acids in an aerobic halogenated hydrocarbon-degrading soil column enriched with natural gas. Environ. Tox. Chem. 6:89-97.

13. Wackett, L. P., and D. T. Gibson. 1988. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with Pseudomonas putida F1. Appl. Environ. Microbiol. 54(7):1703-1708.

14. Wilson, J. T., and B. H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49:242-243.

APPENDIXES

APPENDIX A

Additional Experimentation

Several experiments were performed involving the bioreactors which were not mentioned in previous sections of this text. Preliminary studies were performed with the bioreactors using anthracite coal as a solid support for microbial attachment and methane as a growth substrate. Test culture SM-1 was used as an inoculum and daily headspace monitoring of volatile components revealed a 70% difference in nominal liquid phase TCE concentration (20 mg/L) when comparing uninoculated control and inoculated experimental bioreactors following daily TCE additions. Acridine orange staining performed on solid support subsamples taken from both control and experimental bioreactors revealed extensive bacterial attachment on the inoculated substratum and virtually no cell mass on the subsample from the inhibited control reactor.

A difficulty arose in the interpretation of results for TCE degradation studies with these experiments since it was determined that the coal was adsorbing a significant fraction of the TCE added to each of the reactors. Thermal desorption of TCE from reactor subsamples showed that over 85% of the added TCE could be accounted for in the control reactor. To determine whether microbial activity was responsible for the large

differences in observed TCE concentrations between control and experimental reactors, subsamples were removed from the respective reactors and spiked with 1,2 ^{14}C-TCE at nominal liquid phase concentrations equivalent to 20 mg/L. Subsequent GC-Gas Proportional Counter (GPC) analysis revealed that over 40% of the TCE degraded in subsamples from the experimental reactor was converted to carbon dioxide (Table 1).

Problems Encountered

The primary problem encountered in working with anthracite coal as a solid support was determining the true volatile organic carbon concentration in the bioreactors. Since coal is organic in nature, it readily adsorbs chlorinated ethylenes which is explained by adsorption isotherm equilibration theory. This analytical difficulty imposed by the anthracite coal led to the use of a more inert substrata which would alleviate VOC analytical problems associated with more reactive solid supports. The crushed glass substratum described in Part II of this text was found to be acceptable for quantitative analysis and therefore was used for the remainder of the bioreactor experimentation.

The results listed in Parts II and III indicate that the TCE removal from the bioreactors is not merely a result of adsorption to the cellular biomass. When

Results of pulsed TCE additions in bioreactors containing Anthracite Coal substratum

Reactor	Total TCE Added (umoles)	Total TCE Remaining (umoles)	% TCE Loss *
Control	174.0	156.6	10
Experimental	174.0	34.8	80

Results are the mean of duplicate five day experiments. 10% methane (v/v) headspace substrate concentration. Daily TCE additions were 34.8 umoles per reactor (20 mg/L liquid phase concentration). Control reactor was inhibited with 0.5% formalin. Total TCE remaining was calculated after TCE was desorbed (thermal desorption) from coal subsamples.

* 1 g. subsamples were taken from reactors, placed in 12 mL vials and tested with ^{14}TCE under conditions similar to reactor treatment. Results indicated that 50% of TCE present was degraded and 40% was converted to $^{14}CO_2$. Carbon dioxide was analyzed using GC-GPC. Samples acidified to pH 6.7 prior to GC analysis.

substrate (methane or propane) was not provided to the bioreactors, TCE removal in the experimental reactors was only 5% greater than the loss seen in the control reactor which is within the confines of analytical error. The loss in the experimental reactors seen here may be the result of heterotrophic activities of the microbial consortia. The results of experiments with substrate additions showed that over 95% of the added TCE was degraded in inoculated experimental systems which supports the concept of cometabolism.

A point not addressed in this study was what portion of the microbial population is responsible for the observed TCE degradation. Since mixed culture consortia were employed in both of the inoculated experimental reactors, it may be that there are several microbial processes occurring simultaneously which results in the ultimate removal of TCE from the system. A better understanding of these biochemical transformations is deemed appropriate before any scale-up endeavors are pursued.

APPENDIX B

Henry's Law Calculation for TCE Concentration in Bioreactors

Henry's Constant for TCE	= 0.36
K (distribution coefficent)	= 2.74
V_G (gas phase volume)	= 490 mL
V_L (liquid phase volume)	= 230 mL
C_L^o (initial liquid phase concentration, TCE)	= 20 mg/L (4.6 mg/0.23L)
TCE solubility in water	= 1100 mg/L

Solving for [TCE] in gas phase of the reactor:

$$C_L^o = C_G\ (K + V_G/V_L)$$

$$C_G = C_L^o/(K + V_G/V_L)$$

$$C_G = (20\ mg/L)/[2.74 + (0.49L/0.23L)]$$

$$C_G = 4.13\ mg/L$$

Total amount of TCE in headspace of reactor:

$$\text{TCE (gas phase)} = C_G \times V_G$$

$$= (4.13\ mg/L) \times (0.49\ L)$$

$$= 2.02\ mg \text{ total TCE in headspace}$$

Total amount of TCE in liquid phase of reactor:

(Total TCE added to system) - (Total TCE in gas phase)

4.6 mg - 2.02 mg = 2.58 mg TCE in liquid phase

Equilibrated liquid phase TCE concentration:

2.58 mg/ 0.23 L = 11.2 mg/L TCE

VITA

John Joseph Niedzielski was born in Northampton, Massachusetts on December 22, 1963. He attended elementary schools in the town of West Springfield and graduated from West Springfield Senior High School in June 1982. The following August he entered Worcester Polytechnic Institute located in Worcester, Massachusetts. In June 1986 he received a Bachelor of Science degree in Biology/Biotechnology with distinction. In the fall of 1986, he accepted a teaching assistantship at The University of Tennessee, Knoxville and began study towards a Master's degree in Environmental Toxicology. As a second year graduate student, he accepted a graduate assistantship through Oak Ridge National Laboratory and the Ecology Department at The University of Tennessee. A Master's degree in Life Sciences was awarded in December 1988.

The author is a member of Sigma Xi, The Society of Environmental Toxicology and Chemistry (SETAC), and The American Society for Microbiology (ASM).