APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1988, p. 951–956
0099-2240/88/040951-06$02.00/0

Vol. 54, No. 4

Trichloroethylene Biodegradation by a Methane-Oxidizing Bacterium†

C. DEANE LITTLE,[1,2]* ANTHONY V. PALUMBO,[1] STEPHEN E. HERBES,[1] MARY E. LIDSTROM,[3] RICHARD L. TYNDALL,[1] AND PENNY J. GILMER[2]

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831[1]; Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306[2]; and Environmental Engineering Science, California Institute of Technology, Pasadena, California 91125[3]

Received 5 October 1987/Accepted 6 January 1988

Trichloroethylene (TCE), a common groundwater contaminant, is a suspected carcinogen that is highly resistant to aerobic biodegradation. An aerobic, methane-oxidizing bacterium was isolated that degrades TCE in pure culture at concentrations commonly observed in contaminated groundwater. Strain 46-1, a type I methanotrophic bacterium, degraded TCE if grown on methane or methanol, producing CO_2 and water-soluble products. Gas chromatography and ^{14}C radiotracer techniques were used to determine the rate, methane dependence, and mechanism of TCE biodegradation. TCE biodegradation by strain 46-1 appears to be a cometabolic process that occurs when the organism is actively metabolizing a suitable growth substrate such as methane or methanol. It is proposed that TCE biodegradation by methanotrophs occurs by formation of TCE epoxide, which breaks down spontaneously in water to form dichloroacetic and glyoxylic acids and one-carbon products.

Trichloroethylene (TCE) is the most frequently reported contaminant at hazardous waste sites on the National Priority List of the U.S. Environmental Protection Agency (18). It and other chlorinated alkenes present a serious groundwater contamination problem to industrial societies; they are suspected carcinogens and generally resist biodegradation in the environment (9). In anaerobic subsurface sediments and aquifers, chlorinated alkenes may be converted by reductive dehalogenation to dichloroethylene and ultimately to more potent carcinogens such as vinyl chloride (2, 16). In aerobic environments, TCE resists biodegradation under a variety of conditions (2, 20), although one aerobic microorganism has been shown to degrade TCE when the organism is simultaneously exposed to phenol (13).

Recent research has shown that natural gas may stimulate TCE degradation in aerobic sediment samples and mixed bacterial cultures (5, 21). These results suggest that methane-utilizing bacteria (methanotrophs) may be able to biodegrade chlorinated alkenes such as TCE (14). Methanotrophic bacteria are ubiquitous organisms that possess a unique methane monooxygenase enzyme system which enables them to utilize methane as a sole carbon and energy source (3). The methane monooxygenase enzyme complex has a low substrate specificity and is able to oxidize or dechlorinate a wide variety of economically and environmentally important organic compounds (7).

We report here the first observation of TCE biodegradation by pure cultures of methanotrophic bacteria. Two TCE-degrading methanotrophs, strains 46-1 and 68-1, were isolated from groundwater samples drawn from monitoring wells at a waste disposal site near Oak Ridge, Tenn. Because of favorable growth characteristics, strain 46-1 was selected for studies to determine the rate and methane dependence of TCE degradation. On the basis of these data, a tentative mechanism for TCE degradation by the organism was proposed.

MATERIALS AND METHODS

Groundwater sampling. Groundwater samples were obtained from monitoring wells at a waste disposal site near Oak Ridge, Tenn. This site was used for direct dumping of chlorinated organic-solvent wastes from 1970 to 1981. Levels of individual chlorinated organics in groundwater at the site range from a few micrograms per liter to over 100 mg/liter.

Water samples were collected by nitrogen displacement with a sampling device (Well Wizard 3013; Q.E.D. Environmental Systems, Inc., Ann Arbor, Mich.). Water samples were collected in sterile flasks after well lines had been pumped forcibly for several cycles. Samples were stirred and vented for several hours in a laboratory hood to remove volatile organic contaminants.

Strain 46-1 was isolated from partially oxygenated groundwater containing TCE and other organic contaminants drawn from a 6-m-deep well. Analyses of water samples collected from this well from 1984 to 1986 showed the following ranges in composition: dissolved oxygen, 3.6 to 5.3 mg/liter; temperature, 12.6 to 15.3°C; pH, 5.7 to 6.0; TCE, 87 to 230 μg/liter; total chlorinated alkenes, 1,579 to 2,286 μg/liter (C. W. Kimbrough, personal communication).

Mixed methane-oxidizing cultures. Mixed bacterial cultures were grown by combining well water with mineral salts concentrate (total liquid volume, 100 ml) in 250-ml septum bottles. Mineral salts concentrate was adapted from that of Whittenbury et al. (19) and was diluted with well water to obtain the following concentrations per liter: 1 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of $CaCl_2$, 1 g of KNO_3, 0.1 g of NH_4Cl, 50 μg of $CuSO_4 \cdot 5H_2O$, 70 μg of $Zn(NO_3)_2 \cdot 6H_2O$, and 10 μg each of H_3BO_3, $MnSO_4 \cdot H_2O$, MoO_3, and $CoCl_2 \cdot 6H_2O$. Twenty milliliters of 5% phosphate buffer (pH 6.8) and 10 ml of a 0.27-g/liter $FeCl_3$ solution were added per liter of heat-sterilized medium when cool. Filter-sterilized methane

* Corresponding author.

† Publication no. 3075 of the Environmental Sciences Division, Oak Ridge National Laboratory.

gas (15 ml, 0.67 mmol) was injected to obtain a 10% (vol/vol) methane atmosphere in the headspace of the bottle. Bottles were incubated at 22°C on a shaker table. Bacterial growth was generally visible 1 to 3 weeks after such cultures were initiated.

Pure methanotrophic strains. Pure strains of methane-oxidizing bacteria were isolated from mixed cultures on mineral salts-agarose plates incubated at 22°C in a 10% methane atmosphere. Criteria used to determine strain purity included the following: (i) repeated colony isolation; (ii) constant, homogeneous colony morphologies; (iii) homogeneous cell morphology (determined by transmission electron microscopy); and (iv) the complete absence of growth on carbon substrates other than methane or methanol. Strain purity was checked periodically throughout these experiments by streaking methanotroph cultures on dilute yeast extract or nutrient agar plates and was confirmed at the end of several TCE degradation assays that showed substantial TCE biodegradation.

Enzyme assays. Strains 46-1 and 68-1 were tested for enzyme activities characteristic of methanotrophic bacteria. Assays included tests for methanol dehydrogenase (15), hydroxypyruvate reductase (10), ribulose bisphosphate carboxylase (17), and hexulose phosphate synthase (ribulose monophosphate-dependent formaldehyde disappearance; M. E. Lidstrom, unpublished method).

TCE degradation assays. TCE degradation assays were conducted in 250-ml septum bottles containing 100 ml of liquid culture. TCE biodegradation was quantified by headspace gas chromatography (GC) and ^{14}C radiolabel analysis. Teflon-lined septum-cap bottles containing pure or mixed methane-oxidizing cultures were dosed with [1,2-^{14}C]trichloroethylene (3.0 mCi/mmol [111 MBq/mmol], Pathfinder Laboratories, St. Louis, Mo.) supplemented with reagent-grade TCE to a total nominal concentration of 400 μg/liter. This level was chosen as typical of groundwater contamination levels at the collection site. Each bottle received 12 ml of filter-sterilized methane (0.536 mmol, 8% of headspace volume) by injection at the start of incubation. Radiolabeled TCE was shown by GC to be 98% radiochemically pure as measured with a gas-proportional radiation detector and by quantifying ^{14}C in trapped fractions of GC column effluent by liquid scintillation spectrometry. Bottles containing liquid culture and [^{14}C]TCE were inverted and incubated on a shaker table at 22°C. Duplicate culture bottles were sacrificed at periodic intervals for cell counting, GC, and radiolabel analysis. Autoclaved cultures were incubated concurrently and sacrificed at intervals to serve as controls.

TCE concentrations in culture bottle headspace samples were determined with a 3920 gas chromatograph (The Perkin-Elmer Corp., Norwalk, Conn.) equipped with an electron capture detector and an SP-1000 column (6 mm [inside diameter] by 1.8 m) held at 120°C with nitrogen (50 ml/min) as the carrier gas. Headspace TCE levels were shown to be directly related to water concentrations over the concentration range employed in experiments.

Radiolabel accumulation was measured in washed cells, CO_2 traps, and cell-free, hexane-extracted culture liquid. After incubation, culture liquid pH was adjusted to between pH 9 and pH 10 with 1 N NaOH, and bottles were shaken overnight to trap CO_2 in the medium. Culture samples (50 ml) were then centrifuged on a tabletop centrifuge to sediment cells. Cells were washed with 40 ml of medium, centrifuged, and resuspended in 10 ml of medium. The cell suspension was adjusted to pH 8.5 with 1 N HCl, and ^{14}C accumulation was determined by liquid scintillation spectrometry. Supernatant was extracted with 20 ml of hexane to remove TCE and acidified in a closed container, and $^{14}CO_2$ was trapped overnight in a 40-ml vial containing 9 ml of 0.1 N NaOH. Tests with $NaH^{14}CO_3$ demonstrated that trapping efficiency was greater than 99%. Trap liquid pH was adjusted to 8.5, and radiolabel accumulations in CO_2 traps and extracted supernatant were determined by liquid scintillation spectrometry.

Ion chromatography of TCE breakdown products. To investigate the mechanism of TCE biodegradation by strain 46-1, we attempted to isolate and identify the water-soluble TCE breakdown products by ion chromatography. Cell-free, hexane-extracted culture liquid was concentrated by vacuum rotary evaporation at 55°C. The concentrate was injected on a 4000I ion chromatograph (Dionex Corp., Sunnyvale, Calif.) equipped with an AS1 micromembrane column. The column effluent was collected at 1-min intervals, and the amount of ^{14}C in each fraction was determined by liquid scintillation spectrometry. A variety of short-chain organic acids considered to be likely TCE breakdown products were assayed by ion chromatography for comparison of retention times.

RESULTS

Characterization of isolates. Methanotrophic bacteria were readily isolated from methane-enriched groundwater cultures. Many of the initial colonies isolated on mineral salts-agarose plates were heterotrophic bacteria that grew in association with methane-oxidizing bacteria. These organisms grew on nutrient agar or yeast extract and did not require methane. By repeated streaking and isolation, we obtained several isolates (including strains 46-1 and 68-1) that grew only in the presence of methane and did not require other carbon sources for growth on mineral salts-agarose.

Strains 46-1 and 68-1 met the criteria for strain purity described above. Both strains underwent at least eight single-colony transfers in the initial phases of isolation. The cultures thus obtained displayed constant, homogeneous colony morphologies over a 1-year period, possessed homogeneous cell morphologies (determined by transmission electron microscopy of over 1,000 cells per strain), and showed complete absence of growth on a variety of other carbon substrates including glucose, acetate, glycolate, yeast extract, glutamate, dilute nutrient agar, and Casamino Acids. Strain 68-1 exhibited better growth in the presence of trace quantities of Casamino Acids or yeast extract, although it could not grow on these substrates without methane. Strain 46-1 exhibited no dependence on carbon nutrients other than methane or methanol and hence was chosen for further TCE degradation studies.

Strains 46-1 and 68-1 both appear to be type I methanotrophs, possessing both enzyme activities for the ribulose monophosphate pathway of formaldehyde fixation and a stacked arrangement of internal membranes (Fig. 1). Hexulose phosphate synthase activities (characteristic of type I methanotrophs) for strains 46-1 and 68-1 were 0.022 and 0.030 μmol/min per mg of protein, respectively, and methanol dehydrogenase activities were 0.052 and 1.08 μmol/min per mg of protein, respectively. No hydroxypyruvate reductase activity or ribulose bisphosphate carboxylase activity was detected. Hydroxypyruvate reductase activity is characteristic of type II methanotrophs (1).

TCE biodegradation. When incubated in pure culture, strain 46-1 converted up to 40% of added TCE to biodegra-

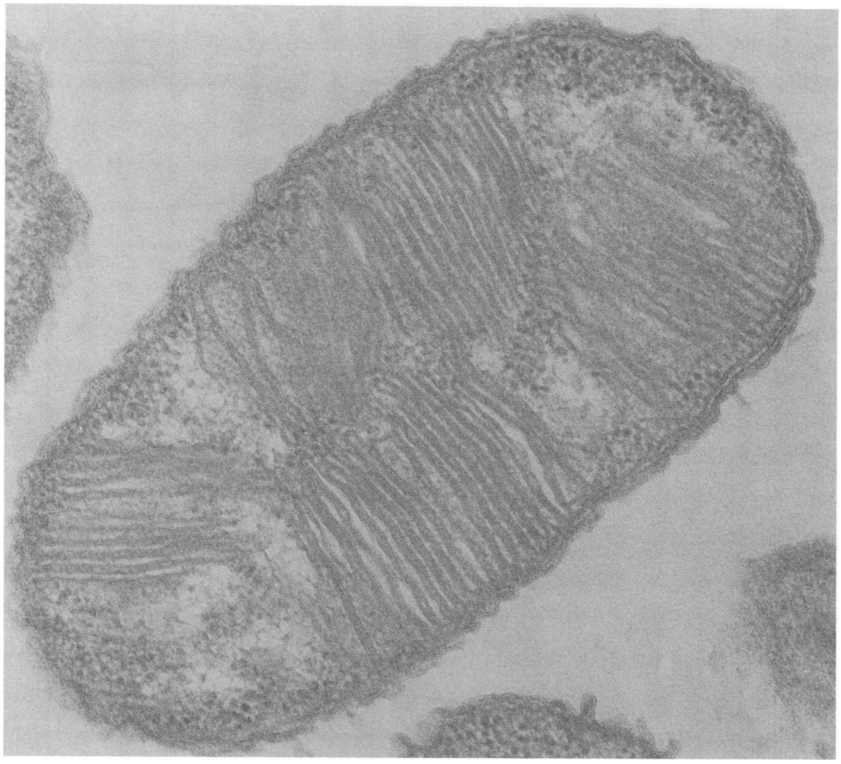

FIG. 1. Transmission electron micrograph of strain 46-1 methanotrophic bacterium showing stacked arrangement of internal membranes characteristic of type I methanotrophs. Cells were fixed with osmium tetroxide and stained with uranyl acetate and lead citrate.

dation products in methane-limited batch cultures incubated for 20 days (Fig. 2). Most of the converted TCE (15.1% of initial TCE) appeared as water-soluble breakdown products, but a substantial fraction (11.4% of initial TCE) was completely converted to CO_2. A corresponding depletion of TCE in culture bottles was observed. Methane depletion, cell growth, and TCE biodegradation were essentially complete after 10 days of incubation. Sterile and live (active cultures of non-TCE-degrading bacteria) control cultures showed less than 2% TCE loss by GC and no significant radiolabel accumulation in cells, CO_2 traps, or culture liquid.

The maximum rate of TCE degradation occurred during the active phase of cell growth (days 2 to 6) and dropped to zero after methane was depleted (Fig. 2). Addition of methane at this time stimulated further TCE biodegradation. Duplicate culture flasks that received an additional 0.27 mmol of methane (4% of headspace volume) on day 11 converted a total of 16.1% of added TCE to CO_2 and 19.9% to water-soluble products by day 13. The corresponding mean values observed in flasks that did not receive additional methane were 11.1 and 13.9%, respectively. A similar pattern was observed in flasks sacrificed on day 20; cultures that received additional methane converted 16.1% of TCE to CO_2 and 21.3% to water-soluble products, while cultures that did not receive additional methane converted 11.6 and 16.3%, respectively. TCE degradation in these bottles ceased when the added methane was consumed. Active metabolism of a suitable growth substrate such as methane thus seems to be necessary for TCE biodegradation to occur.

TCE loss (as measured by GC) corresponded fairly closely with the appearance of ^{14}C-labeled breakdown products. By day 13, a 34% loss of TCE was measured by GC and 26% of initial [^{14}C]TCE had appeared as breakdown products. By day 20, a 44% TCE loss was measured and 29% of initial [^{14}C]TCE was accounted for as breakdown products. Incomplete recovery of TCE breakdown products may account for the discrepancies in these percentages. Hexane-soluble breakdown products (such as dichloroethylene) would not be measured by our radiolabel analysis; however, volatile chlorinated products were never observed by GC analysis.

Proportions of converted radiolabel appearing in washed cells, CO_2 traps, and culture liquid differed substantially in pure and mixed cultures (Fig. 3). In methane-enriched mixed cultures collected from five wells, 2.7 to 14.9% of the converted TCE appeared as water-soluble breakdown products and 65.1 to 78.6% appeared as CO_2 after 2 to 4 weeks incubation. In contrast, in strain 46-1 cultures, 53.5 to 56.2% of converted TCE appeared as water-soluble breakdown products and 40.1 to 42.7% appeared as CO_2. These proportions did not change significantly in pure cultures of strain 46-1 during the 20-day incubation period of this experiment (Fig. 2). The high levels of water-soluble degradation products in strain 46-1 cultures apparently reflected an inability of this strain to metabolize TCE fully.

Methanol was also shown to stimulate TCE biodegradation by strain 46-1. In one experiment, strain 46-1 cultures that received 200 mg of methanol per liter instead of methane and 400 μg of TCE per liter converted 10.3% of TCE to water-soluble products, 6.6% of TCE to CO_2, and 0.64% of TCE to cell-bound radiolabel in 30 days. Differences in culture density precluded quantitative comparisons of methane-grown cultures with methanol-grown ones.

Analysis of TCE breakdown products. The water-soluble TCE breakdown products produced in pure cultures of strain 46-1 were nonvolatile at pH 7 and were not hexane extractable. Ion chromatography of the water-soluble TCE breakdown products revealed two radiolabeled products, one

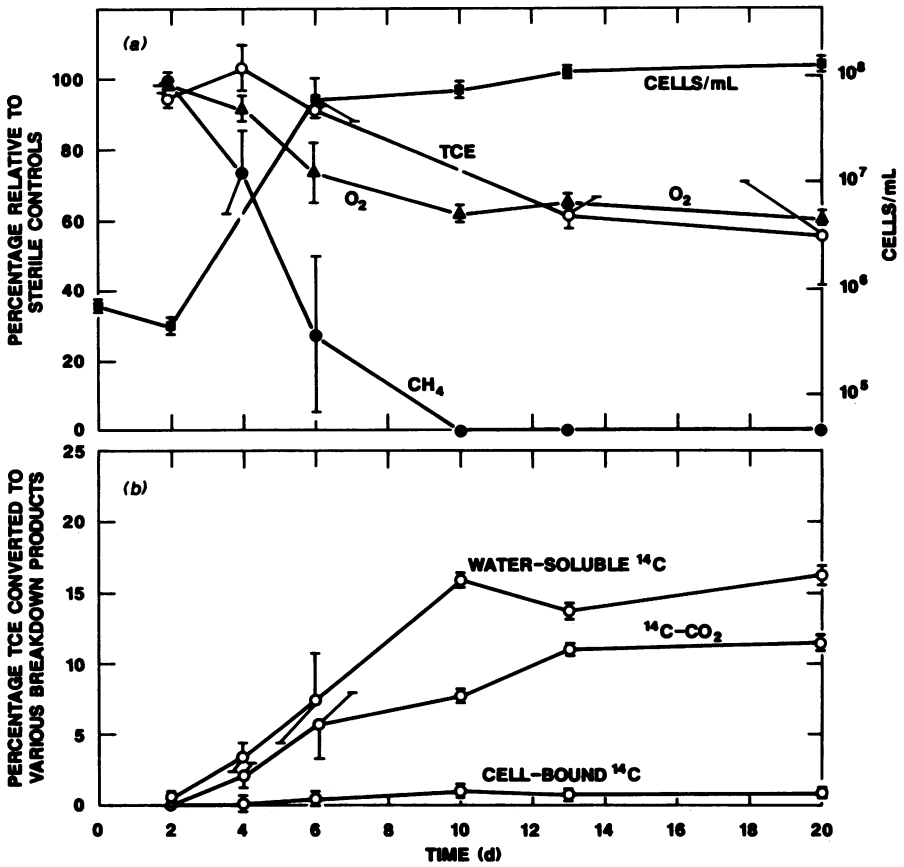

FIG. 2. (a) Cell growth and corresponding depletion of TCE, O_2, and CH_4 in methane-limited batch cultures of strain 46-1. TCE, O_2, and CH_4 data are expressed as percentages relative to corresponding average values in sterile controls, which showed <3% loss in any of these parameters during the course of the experiment. Error bars indicate ranges of values observed in duplicate cultures. An instrument malfunction on day 10 led to anomalous values for TCE concentration, which were omitted from this figure. (b) Appearance of ^{14}C-labeled TCE breakdown products in methane-limited batch cultures of strain 46-1.

eluting at 6 to 7 min and the other eluting at 10 to 11 min. No other effluent fractions showed a detectable elevation of radiolabel. A variety of short-chain acids considered to be possible TCE breakdown products were assayed by ion chromatography as standards. These included acetic, formic, oxalic, glyoxylic, and mono-, di-, and trichloroacetic acids. The retention times of glyoxylic acid (CHOCOOH) and dichloroacetic acid (CCl_2HCOOH) matched those of peaks 1 and 2, respectively. These products have been shown to accumulate when TCE epoxide decomposes under aqueous conditions (6, 11, 12).

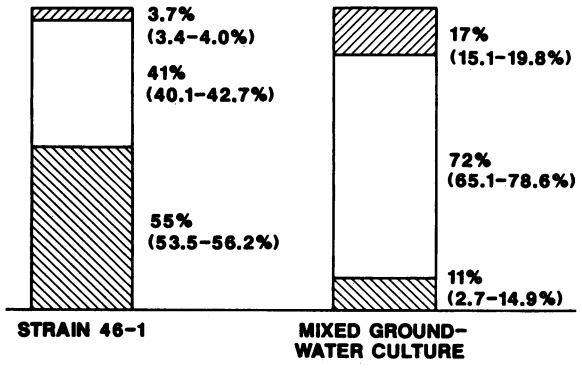

FIG. 3. Mean proportions of TCE breakdown products in pure and mixed cultures of methane-oxidizing bacteria. Numbers in parentheses represent ranges of values, expressed as percentages of total converted [^{14}C]TCE, observed in 4 pure and 14 mixed methane-enriched cultures. Symbols: ▨, Cell-bound ^{14}C; □, $^{14}CO_2$; ▧, water-soluble ^{14}C.

DISCUSSION

In pure cultures of strain 46-1, TCE is converted to stable water-soluble degradation products and CO_2. This result strongly suggests that methanotrophic bacteria are responsible for the TCE biodegradation observed by other researchers in methane-enriched soil columns (21) and mixed cultures (5). In these previous studies, TCE was converted either completely to CO_2 (21) or to CO_2, cell-bound material, and water-soluble products (5). In complex biological systems, such as the soil microcosms of Wilson and Wilson (21) and our mixed groundwater cultures, water-soluble TCE breakdown products produced by methanotrophs appear to be further metabolized to CO_2 by heterotrophic bacteria. In pure cultures, and in well-defined mixed cultures such as those investigated by Fogel et al. (5), limited bacterial metabolic abilities may preclude complete biodegradation of TCE.

The accumulation of stable TCE breakdown products in pure cultures of strain 46-1 suggests that methanotrophic

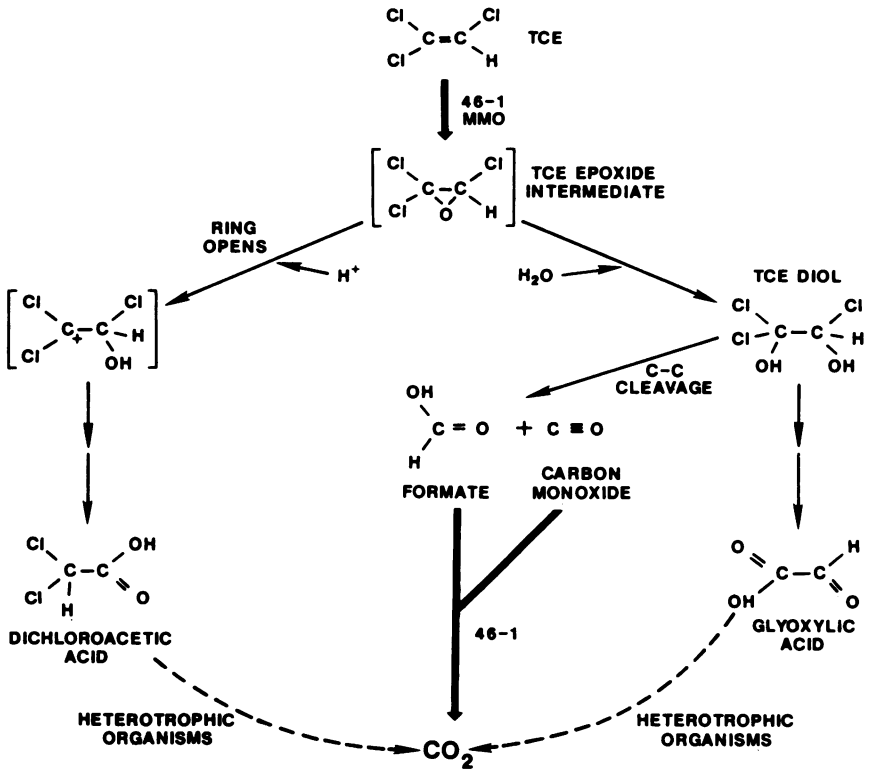

FIG. 4. Proposed mechanism of TCE breakdown by methanotrophic bacteria. Strain 46-1 methane monooxygenase (MMO) epoxidates TCE to form TCE epoxide, which then breaks down spontaneously in water to form dichloroacetic acid, glyoxylic acid, or one-carbon compounds such as carbon monoxide and formate. Steps were mediated by strain 46-1 (—), proposed to occur spontaneously in H_2O (——), or apparently mediated by heterotrophic bacteria in mixed cultures (-----).

bacteria in isolation may not be able to metabolize chlorinated alkenes such as TCE completely. Although methanotrophic organisms may initiate oxidation of a wide variety of organic molecules, type I strains generally cannot completely metabolize molecules containing more than one carbon (1), with one known exception (22). In our mixed methane-enriched cultures, heterotrophic bacteria apparently metabolized most of the water-soluble TCE breakdown products, decreasing levels of water-soluble radiolabel and increasing production of $^{14}CO_2$ (Fig. 3).

The inability of strain 46-1 to degrade TCE in the absence of methane or methanol suggests that TCE biodegradation by methanotrophs is a cometabolic process which provides little or no metabolic benefit to the organism. Cometabolism of an alternate substance occurs only when an organism is actively metabolizing a suitable growth substrate (4). In the case of strain 46-1, TCE biodegradation may represent nonspecific enzyme activity of methane monooxygenase. After initial oxidation of the TCE molecule, further metabolism by methanotrophic organisms such as strain 46-1 may not be possible.

The apparent accumulation of glyoxylic acid, dichloroacetic acid, and CO_2 in TCE-degrading cultures of strain 46-1 suggests a possible mechanism of TCE biodegradation (Fig. 4). We propose that methane-oxidizing bacteria such as strain 46-1 convert TCE to its epoxide, which then breaks down spontaneously in water to form dichloroacetic acid, glyoxylic acid, or one-carbon compounds such as formate or carbon monoxide (CO). The two-carbon acids accumulate in the water phase, while formate and CO are further oxidized by methanotrophic bacteria to CO_2. The spontaneous hydrolysis of TCE epoxide to form one-carbon products explains the unexpected production of CO_2 from TCE by this methanotrophic organism. This mechanism is similar to one proposed by Henry and Grbic-Galic (S. M. Henry and D. Grbic-Galic, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, Q64, p. 294; S. M. Henry, unpublished report) but differs in that glyoxylic acid is postulated as a substantial breakdown product. Epoxidation mechanisms are well known among the methanotrophic bacteria (8). In addition, studies with chemically produced TCE epoxide have revealed the formation of dichloroacetic and glyoxylic acids and one-carbon products in aqueous systems (6, 11, 12). Miller and Guengerich (12) found competing pathways for the breakdown of TCE epoxide in aqueous systems. Under acidic conditions, TCE epoxide was converted to glyoxylic and dichloroacetic acids, while under basic conditions, carbon monoxide and formate were formed. In phosphate-buffered systems at pH 7.7, all four products were formed, with formate predominating.

The ability to degrade TCE and other chlorinated alkenes may be a general characteristic of methanotrophic bacteria. Several researchers have observed biodegradation of chlorinated alkenes in methane-enriched mixed cultures from a variety of environmental sources (5, 21). These studies and our work with strain 46-1 confirm the potential for treatment of sites contaminated by chlorinated alkenes with stimulation of methanotrophic bacteria.

ACKNOWLEDGMENTS

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 with Martin Marietta Energy Systems, Inc.; and by U.S. Department of Energy grant DE-FG02-85ER13386 to M. E. Lidstrom.

We thank D. P. Allison, M. C. Rorvik, and T. Fassel for electron micrography and D. M. Genung, K. S. Ironsides, N. M. Ferguson, A. R. Herndon, and T. J. Phelps for technical assistance. H. I. Adler, L. W. Barnthouse, T. L. Donaldson, S. E. Lindberg, G. S. Sayler, G.W. Strandberg, L. E. Toran, V. Tripathi, B. T. Walton, and D. C. White reviewed this manuscript and provided helpful suggestions. John Bartmess of The University of Tennessee and Susan Henry of Stanford University provided thoughtful discussion concerning the mechanism of TCE breakdown.

LITERATURE CITED

1. **Anthony, C.** Biochemistry of methylotrophs. 1982. Academic Press, Inc. (London), Ltd., London.
2. **Bouwer, E. J., B. E. Rittman, and P. L. McCarty.** 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. Environ. Sci. Technol. **15**:596–599.
3. **Dalton, H., S. D. Prior, D. J. Leak, and S. H. Stanley.** 1984. Regulation and control of methane monooxygenase, p. 75–82. *In* R. L. Crawford and R. S. Hanson (ed.), Microbial growth on C_1 compounds. Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, D.C.
4. **Dalton, H., and D. I. Stirling.** 1982. Co-metabolism. Philos. Trans. R. Soc. Lond. B Biol. Sci. **297**:481–496.
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. **Henschler, D., W. R. Hoos, H. Fetz, E. Dallmeier, and M. Metzler.** 1979. Reactions of trichloroethylene epoxide in aqueous systems. Biochem. Pharmacol. **28**:543–548.
7. **Higgins, I. J., D. J. Best, and R. C. Hammond.** 1980. New findings in methane-utilizing bacteria highlight their importance in the biosphere and their commercial potential. Nature (London) **286**:561–564.
8. **Hou, C. T., R. N. Patel, and A. I. Laskin.** 1980. Epoxidation and ketone formation by C1-utilizing microbes. Adv. Appl. Microbiol. **26**:41–69.
9. **Infante, P. F., and T. A. Tsongas.** 1982. Mutagenic and oncogenic effects of chloromethanes, chloroethanes, and halogenated analogs of vinyl chloride. Environ. Sci. Res. **25**:301–327.
10. **Large, P. J., and J. R. Quayle.** 1963. Enzyme activities in extracts of Pseudomonas AM 1. Biochem. J. **87**:386–396.
11. **McKinney, L. L., E. H. Uhing, J. L. White, and J. C. Picken.** 1955. Autoxidation products of trichloroethylene. J. Agric. Food Chem. **3**:413–419.
12. **Miller, R. E., and F. P. Guengerich.** 1982. Oxidation of trichloroethylene by liver microsomal cytochrome P-450: evidence for chlorine migration in a transition state not involving trichloroethylene oxide. Biochemistry **21**:1090–1097.
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. **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. Toxicol. Chem. **6**:89–97.
15. **O'Connor, M. L., and R. S. Hanson.** 1977. Enzyme regulation in *Methylobacterium organophilum*. J. Gen. Microbiol. **101**:327–332.
16. **Parsons, F., P. R. Wood, and J. DeMarco.** 1984. Transformations of tetrachloroethylene and trichloroethylene in microcosms and groundwater. J. Am. Water Works Assoc. **76**:56–59.
17. **Tabita, F. R., P. Caruso, and W. Whitman.** 1978. Facile assay of enzymes unique to the Calvin cycle in intact cells, with special reference to ribulose 1,5-bisphosphate carboxylase. Anal. Biochem. **84**:462–472.
18. **U.S. Environmental Protection Agency.** 1985. Substances found at proposed and final NPL sites through update number three. Document NPL-U3-6-3. U.S. Environmental Protection Agency, Washington, D.C.
19. **Whittenbury, R., K. C. Phillips, and J. F. Wilkinson.** 1970. Enrichment, isolation, and some properties of methane-utilizing bacteria. J. Gen. Microbiol. **61**:205–218.
20. **Wilson, J. T., J. F. McNabb, B. H. Wilson, and M. J. Noonan.** 1983. Biotransformation of selected organic pollutants in ground water. Dev. Ind. Microbiol. **24**:225–233.
21. **Wilson, J. T., and B. H. Wilson.** 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. **49**:242–243.
22. **Zhao, S.-J., and R. S. Hanson.** 1984. Isolate 761M: a new type I methanotroph that possesses a complete tricarboxylic acid cycle. Appl. Environ. Microbiol. **48**:1237–1242.