

## Inhibition of Methane Oxidation by *Methylococcus capsulatus* with Hydrochlorofluorocarbons and Fluorinated Methanes

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**The inhibition of methane oxidation by cell suspensions of *Methylococcus capsulatus* (Bath) exposed to hydrochlorofluorocarbon 21 (HCFC-21; difluorochloromethane [CHF<sub>2</sub>Cl]), HCFC-22 (fluorodichloromethane [CHFCl<sub>2</sub>]), and various fluorinated methanes was investigated. HCFC-21 inhibited methane oxidation to a greater extent than HCFC-22, for both the particulate and soluble methane monooxygenases. Among the fluorinated methanes, both methyl fluoride (CH<sub>3</sub>F) and difluoromethane (CH<sub>2</sub>F<sub>2</sub>) were inhibitory while fluoroform (CHF<sub>3</sub>) and carbon tetrafluoride (CF<sub>4</sub>) were not. The inhibition of methane oxidation by HCFC-21 and HCFC-22 was irreversible, while that by methyl fluoride was reversible. The HCFCs also proved inhibitory to methanol dehydrogenase, which suggests that they disrupt other aspects of C<sub>1</sub> catabolism in addition to methane monooxygenase activity.**

Concerns about stratospheric ozone and global warming have focused scientific inquiry upon the microbial degradation of certain atmospheric halocarbons. Hydrochlorofluorocarbons (HCFCs) as well as hydrofluorocarbons (HFCs) may be susceptible to microbial attack under both aerobic and anaerobic conditions (6, 9, 28), but methanotroph-linked degradation is of particular interest because of the widely distributed activities these organisms have in soils (15). Several researchers have reported degradation of HCFCs by methanotrophic soils (28), mixed cultures (6), and pure cultures (9); however, in some cases experiments were conducted at very high concentrations of the gas phases (gas phase mixing ratios of >1,000 ppm) of these substances. An inhibitory effect on methane oxidation in soils with as little as 100 ppm of HCFC 21 (HCFC-21; difluorochloromethane [CHF<sub>2</sub>Cl]) was observed (28), suggesting that these substances may also act as competitive inhibitors of methane monooxygenase (MMO).

Little is known about the interaction of HCFCs and HFCs with methanotrophs. Methanotrophic degradation of HCFCs presumably occurs via MMO, being similar to that observed with chlorinated organics (2), methyl fluoride (22), and other methyl halides (7, 26, 31). Two types of MMOs are made by methanotrophs, the soluble (sMMO) and particulate (pMMO) forms, and both degrade chlorinated solvents (2, 20, 21). While all methanotrophs can make pMMO, some, like *Methylococcus capsulatus*, can synthesize either pMMO or sMMO depending upon the presence or absence of copper in the medium (14a). Competitive inhibition of methane oxidation by chlorinated solvents has been observed (1, 5), but it is not known whether HCFCs inhibit methane oxidation by similar mechanisms. In addition, the oxidation products formed from the attack of HCFCs by MMO may have inhibitory effects of their own, as occurs in the methanotrophic degradation of trichloroethylene (1). Finally, the increased employment of the inhibitor methyl fluoride to quantify methane oxidation in soils and sediments (11, 13, 16, 24, 25, 30) prompted us to examine whether other

fluorinated methanes (HFCs) inhibit methane oxidation by whole cells.

Batch cultures of *M. capsulatus* (Bath) were grown overnight in mineral salts medium without copper or supplemented with 1.2 mg of CuSO<sub>4</sub> per liter under a methane-air (3:5) atmosphere at 37°C with constant vigorous shaking (32). The sMMO subunits were readily detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in cells grown under Cu-free conditions but were not present in cells grown in Cu-supplemented medium. Further confirmation of sMMO in cells was obtained from their ability to oxidize naphthalene, while Cu-grown cells, which expressed only pMMO, were incapable of such oxidation (5a, 32). Cell suspensions were dispensed (~1.4 mg [dry weight]/20 ml) into 57-ml serum bottles and sealed under air with butyl rubber stoppers and aluminum crimp seals (37-ml headspace volume). Methane (5% [vol/vol] in the headspace) was injected after sealing, as were all experimental gases. Halocarbons were procured as follows: HCFC-21 (purity, 99%; PCR Incorp., Gainesville, Fla.), HCFC-22 (fluorodichloromethane [CHFCl<sub>2</sub>]) (purity, 99.9%; Scott Specialty Gases, Plumsteadville, Pa.), methyl chloride (purity, 99.5%; Matheson Gas Co., Lyndhurst, N.J.), methyl fluoride (purity, 99%; Matheson Gas Co.), dimethyl fluoride or HFC-32 (purity, 99.5%; AGA Specialty Gas, Maumee, Ohio), fluoroform or HFC-23 (purity, >98%; Aldrich Chemical Co., Milwaukee, Wis.), and tetrafluoromethane or HFC-14 (purity, 99.97%; Liquid Carbonics Inc., Chicago, Ill.). Acetylene was generated by reaction of calcium carbide with water.

The amounts of HCFCs or HFCs added to the gas phases of the cell suspensions varied from 0.1 to 1.0 ml; however, the broad differences in solubilities of these gases in water prompted us to calculate their dissolved concentrations. Aqueous concentrations were determined with the dimensionless Henry's constant ( $K_H'$ ) for each compound at 37°C in distilled water applied to partitioning equations (27) or with Bunsen coefficients ( $\alpha$ ) applied to the equations of Flett et al. (12). The following  $K_H'$  values were used: 40.26 for methane (33), 2.59 for HCFC-22 (6), 0.522 for methyl chloride (14), and 5.26 for HCFC-21. The value for HCFC-21 was determined empirically by equilibrating HCFC-21 (100  $\mu$ l) with 20 ml of deionized water sealed in a serum bottle (see above). After 3 h of shaking at 37°C, 1 ml of water was withdrawn by syringe and injected

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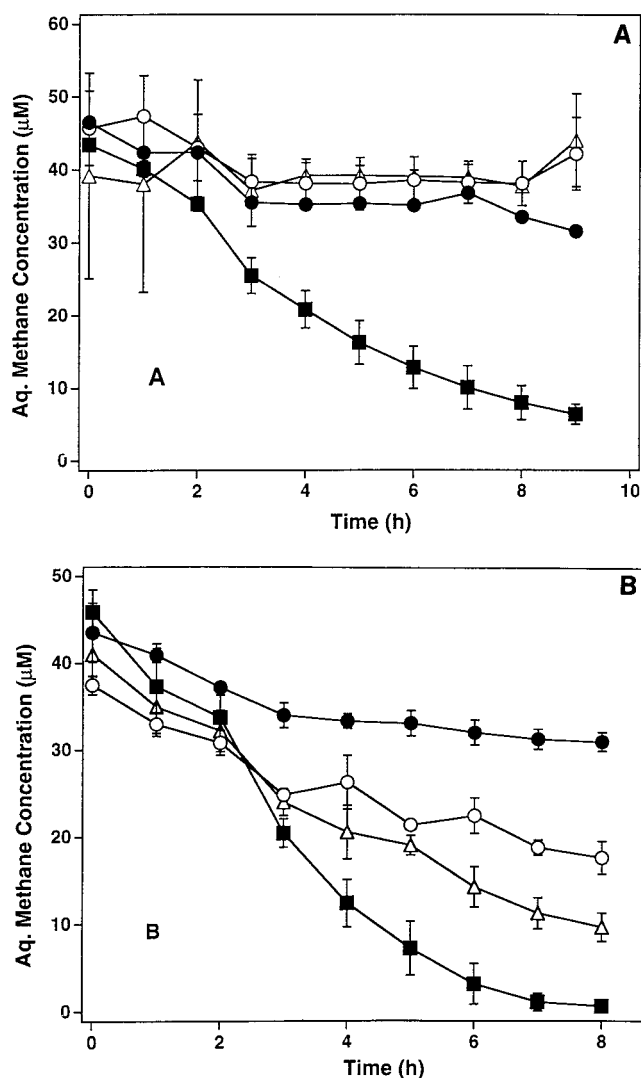


FIG. 1. Inhibition of methane oxidation by HCFC-22 in resting cell suspensions of *M. capsulatus* with sMMO (A) and pMMO (B). Symbols: ■, no HCFC addition;  $\Delta$ , with 16  $\mu\text{M}$  HCFC-22;  $\circ$ , with 32  $\mu\text{M}$  HCFC-22; ●, autoclaved cell suspension without HCFC addition. Symbols represent the means of dissolved methane concentrations in three cell suspensions, and bars are  $\pm 1$  standard deviation. Aq., aqueous.

into a sealed test tube (volume, 25 ml). After 15 min of vigorous shaking, the headspace concentration of HCFC-21 was obtained by syringe sampling followed by gas chromatographic analysis (see below). Values of  $\alpha$  (in milliliter per milliliter) used were 0.7 for acetylene (17), 0.28 for methyl fluoride (13), 1.0 for difluoromethane (4), 0.4 for fluoroform (10), and 0.0046 for carbon tetrafluoride (10). Calculations for concentrations of dissolved gas were not corrected for the effects of dissolved salts or of the cells themselves and therefore represent maximum values. Thus, while initial dissolved methane concentrations were calculated to be 84  $\mu\text{M}$ , the experimental data indicated that starting concentrations were about half this value (e.g., Fig. 1).

Cells were incubated at 37°C with constant shaking (300 rpm). Killed controls consisted of sealed and autoclaved (121°C, 60 min) cell suspensions. Cell weights were determined for each experiment by filtering 10-ml samples of cell suspension through 0.2- $\mu\text{m}$ -pore-size preweighed filters, which were

then dried at 80°C for 12 h and reweighed. The mean cell dry weight ( $n = 25$ ) was  $0.137 \pm 0.008$  mg/ml and did not vary significantly between experiments or in cells with pMMO versus those with sMMO. All experiments were performed in triplicate, and the results are reported as means  $\pm 1$  standard deviation. Methane and halocarbons were sampled by syringe from the gas phases and quantified as described below. Methane consumption curves in uninhibited and inhibited samples were fitted to a first-order kinetic curve fitting program in order to calculate percentage inhibition (19).

To test whether pMMO inhibition by various gases was reversible, the recovery of methane oxidation by cell suspensions exposed for 2 h to headspace additions of 2% methyl fluoride, HCFC-21, HCFC-22, or acetylene or to no addition was investigated. After exposure, the bottles were opened and sparged for 2 h with air, after which time they were resealed and vigorously shaken (300 rpm/10 min) and the headspace was tested for the absence of the halocarbons or acetylene.

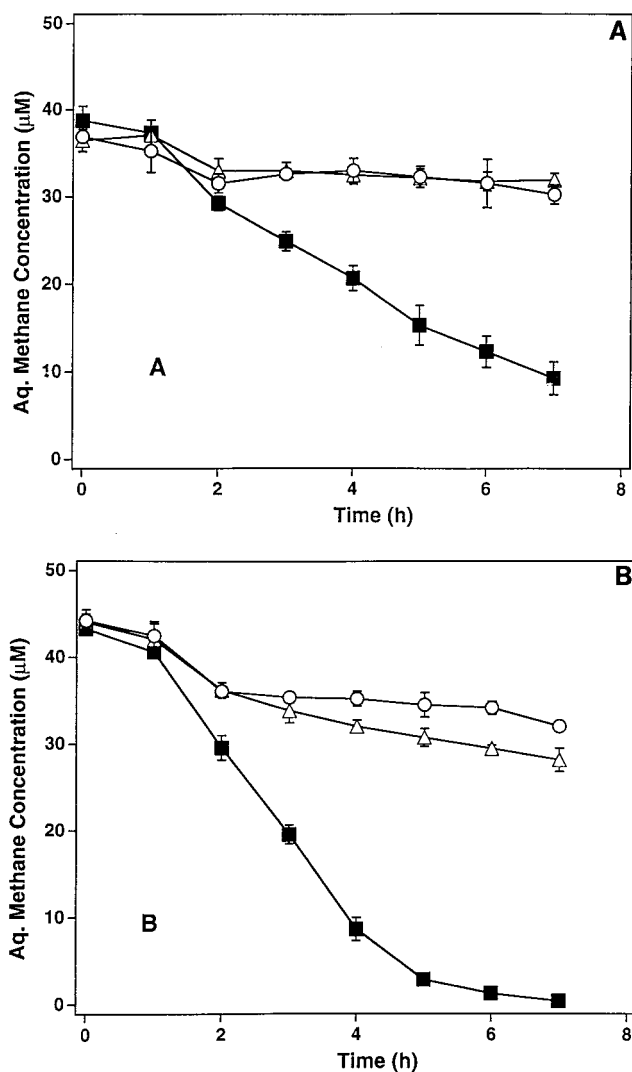


FIG. 2. Inhibition of methane oxidation by HCFC-21 in resting cell suspensions of *M. capsulatus* with sMMO (A) and pMMO (B). Symbols: ■, no HCFC addition;  $\Delta$ , with 11  $\mu\text{M}$  HCFC-21;  $\circ$ , with 23  $\mu\text{M}$  HCFC-21; □, autoclaved cell suspension without HCFC addition. Symbols represent the means of dissolved methane concentrations in three cell suspensions, and bars are  $\pm 1$  standard deviation. Aq., aqueous.

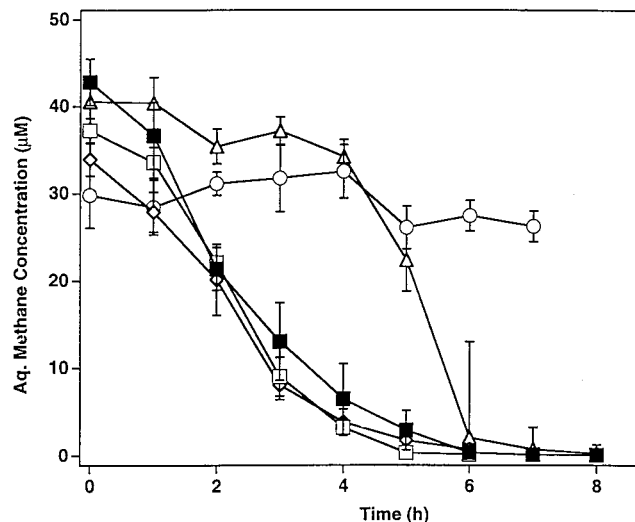


FIG. 3. Inhibition of methane oxidation by various fluoromethanes in resting cell suspensions of *M. capsulatus* expressing pMMO. Symbols: ■, no addition; △, with 26  $\mu\text{M}$  methyl fluoride; ○, with 130  $\mu\text{M}$  difluoromethane; ◇, with 36  $\mu\text{M}$  fluoroform; □, with 3  $\mu\text{M}$  tetrafluoromethane. Symbols represent the means of dissolved methane concentrations in three cell suspensions, and bars are  $\pm 1$  standard deviation. Aq., aqueous.

Once it was verified that the inhibitors were absent from the gas phases, cell suspensions were reinjected with 5% (vol/vol) methane and reincubated as described above.

The effects of HCFCs on the ability of cells to oxidize methanol were investigated with cell suspensions amended with HCFC, 10 mM methanol plus 0.5  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]methanol (specific activity, 59  $\mu\text{Ci}/\mu\text{mol}$ ; Amersham Corp.). At the end of the incubation (9 h), bottles were acidified with 1 ml of 6 N HCl and shaken for 12 h (150 rpm at 20°C) and the headspace was analyzed for  $^{14}\text{CO}_2$ . Controls consisted of cell suspensions incubated without HCFCs or with acetylene.

Methane was quantified by flame ionization gas chromatography (23). Gaseous halocarbons were quantified with the same apparatus but with different oven temperatures. Oven temperatures (in degrees Celsius) and retention times (in minutes), respectively, for each compound were 150 and 3.2 for HCFC-22, 170 and 6.2 for HCFC-21, 150 and 2.1 for methyl fluoride, 150 and 2.5 for HFC-32, 150 and 3 for HFC-23, 170 and 7.5 for HFC-14, 150 and 3.9 for methyl chloride, and 150 and 1.7 for acetylene. HCFC-21 was also determined by electron capture gas chromatography (18).  $^{14}\text{CO}_2$  determinations were made by gas chromatography in series with gas proportional counting (8). Corrections for internal positive pressures of the serum bottles were made by measuring the expansion volume achieved by inserting a wetted glass syringe through the rubber stoppers.

Methane oxidation by *M. capsulatus* expressing sMMO was completely inhibited by the addition of 19 or 39  $\mu\text{M}$  HCFC-22, and the inhibition was essentially as effective as killing the cells by autoclaving (Fig. 1A). Cells with pMMO were only partially inhibited by HCFC-22 (Fig. 1B). HCFC-22 concentrations of 16 and 32  $\mu\text{M}$  inhibited sMMO by 93 and 95%, respectively, while they inhibited pMMO by only 74 and 89%, respectively. At lower concentrations (8  $\mu\text{M}$ ) of HCFC-22, pMMO inhibition was only 55%, while at higher concentrations (83  $\mu\text{M}$ ), pMMO inhibition was essentially complete (97%) (data not shown). HCFC-22 was not consumed by *M. capsulatus* at any of the applied concentrations (data not shown). When the exper-

iment was repeated for HCFC-21, the results were quite similar (Fig. 2). HCFC-21 was a more potent inhibitor of methane oxidation than HCFC-22, because lower aqueous concentrations (10 or 23  $\mu\text{M}$ ) effectively inhibited sMMO (Fig. 2A) as well as pMMO (Fig. 2B), with inhibition at these concentrations being essentially complete ( $\geq 92\%$ ). No consumption of HCFC-21 was observed in these experiments (data not shown). However, cell suspensions of *M. capsulatus* consumed either HCFC-21 or HCFC-22 when they were present at initial concentrations which were approximately 20-fold lower than those employed in the above-described experiments (data not shown).

For cells having pMMO, no inhibition was observed in the presence of fluoroform or carbon tetrafluoride, but inhibition did occur with difluoromethane and methyl fluoride (Fig. 3). Methyl fluoride at an initial aqueous concentration of 26  $\mu\text{M}$  ceased to be inhibitory after  $\sim 4$  h of incubation, because it was oxidized by the cell suspensions and was completely absent from the gas phase by 6 h (data not shown). Rapid oxidation of methyl fluoride by *M. capsulatus* at concentrations which are less than fully inhibitory to methane oxidation has been previously reported (22, 24). At a higher concentration of methyl fluoride (52  $\mu\text{M}$ ), the inhibition of pMMO was complete ( $>99\%$ ) and methyl fluoride was not oxidized (not shown). We also observed complete inhibition (93 to 98%) of pMMO activity with difluoromethane (130 to 650  $\mu\text{M}$ ). However, we did not detect any significant inhibition of methane oxidation for fluoroform at concentrations of 36, 52, and 206  $\mu\text{M}$ , and likewise no inhibition by carbon tetrafluoride was observed at concentrations of 3 or 6  $\mu\text{M}$ . No consumption of difluoromethane, fluoroform, or carbon tetrafluoride was noted during any of these incubations (data not shown). Full inhibition of methane oxidation (pMMO) by methyl chloride occurred only at very high concentrations ( $>3,000$   $\mu\text{M}$ ). At lower concentrations (174 to 1,740  $\mu\text{M}$ ), methyl chloride was oxidized while causing only a partial inhibition of pMMO (not shown).

Methane oxidation (pMMO) by cells exposed to methyl fluoride could be reestablished after the inhibitor was removed,

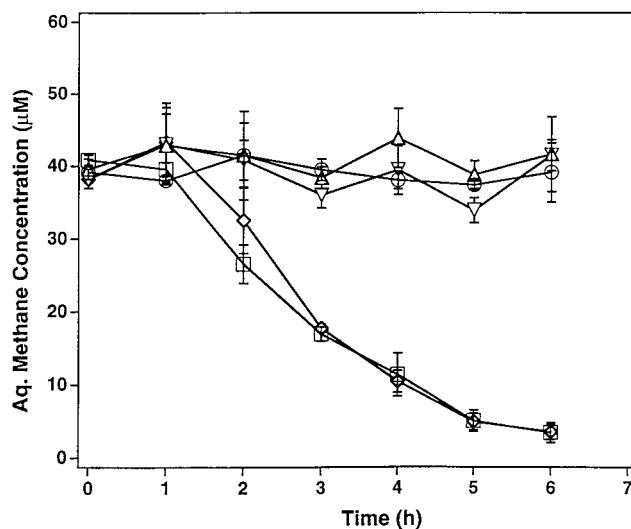


FIG. 4. Reversibility of the inhibition of methane oxidation after 2 h of preexposure to various halomethanes and acetylene in cell suspensions expressing pMMO. Symbols: □, no HCFC addition; ◇, with 192  $\mu\text{M}$  methyl fluoride; ▽, with 252  $\mu\text{M}$  HCFC-22; △, with 138  $\mu\text{M}$  HCFC-21; ○, with 400  $\mu\text{M}$  acetylene. Symbols represent the means of dissolved methane concentrations in three cell suspensions, and bars are  $\pm 1$  standard deviation. Aq., aqueous.

TABLE 1. Inhibition of methanol oxidation by cell suspensions incubated with HCFC-22<sup>a</sup>

Addition	<sup>14</sup> CO <sub>2</sub> formed (nCi) <sup>b</sup>	Methanol oxidized (mM)	% Inhibition
None	471 ± 22	9.4	
68 μM HCFC-22	137 ± 20	2.7	71
378 μM HCFC-22	75 ± 10	1.5	84

<sup>a</sup> Cells were incubated with 10 mM methanol plus 500 nCi of [<sup>14</sup>C]methanol for 9 h.

<sup>b</sup> Data are the means of three samples ± 1 standard deviation.

but exposure to acetylene, HCFC-21, and HCFC-22 caused irreversible inhibition (Fig. 4). Methyl fluoride and acetylene have been used interchangeably as inhibitors of methanotrophy, with the latter preferred because of cost (16), but it should be borne in mind that they differ in their modes of disruption of MMO. Acetylene is a "suicide" substrate for MMO because it not only inactivates the enzyme but also binds irreversibly to the enzyme complex (29). The inhibitory effect has been attributed to an oxidation product rather than to acetylene itself because acetylene is also consumed by MMO. Since the inhibition by the HCFCs was irreversible and these HCFCs can be metabolized (6, 9), the HCFCs and acetylene probably have similarities in their means of inhibiting MMO. Thus, two types of inhibitory effects are likely to be involved here, namely, a competitive inhibition by the HCFCs themselves and an irreversible inhibition caused by the binding of the HCFC oxidation products to MMO. Although we did not observe any consumption of the HCFCs when they were applied at high concentrations, we did observe consumption at much lower concentrations, and we infer that small quantities of the HCFCs were oxidized at the high concentrations and that the oxidation products bound irreversibly with the MMO. Acetylene, however, does not inhibit methanol dehydrogenase (3, 7), and we did not detect any inhibitory effect of acetylene on the ability of *M. capsulatus* to oxidize [<sup>14</sup>C]methanol (data not shown). In contrast, HCFC-22 inhibited methanol oxidation (Table 1), and we also observed inhibition of methanol oxidation with HCFC-21 (data not shown). This suggests that these HCFCs disrupt other facets of the C<sub>1</sub> oxidation pathway besides MMO, such as methanol dehydrogenase, and in this way they differ from acetylene.

A common experimental approach in determining whether methanotrophs constitute a possible global sink for selected atmospheric halocarbons is to screen various soils and cultures for their ability to oxidize these substances (6, 9, 15, 28). Hence, a simple cautionary note needs to be made with regard to the concentrations employed, because some of these substances (or their oxidation products) inhibit both methane oxidation and their own MMO-linked degradation. Additionally, the aqueous concentrations of added halocarbon are important factors to consider, especially in systems in which these halocarbons are used as specific inhibitors. The aqueous concentration of methyl fluoride, a highly soluble gas, should be close to that of methane in order for it to function as an effective MMO inhibitor and not be oxidized by the MMO. One intriguing finding of this work is that difluoromethane also inhibits MMO. Because difluoromethane is soluble as well as inexpensive, it may have some practical advantage over methyl fluoride for use in field studies.

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## REFERENCES

- Alvarez-Cohen, L., and P. L. McCarty. 1991. Product toxicity and cometabolic competitive inhibition modeling of chloroform and trichloroethylene transformation by methanotrophic resting cells. *Appl. Environ. Microbiol.* **57**:1031–1037.
- Alvarez-Cohen, L., P. L. McCarty, E. Boulygina, R. S. Hanson, G. A. Brusseau, and H. C. Tsien. 1992. Characterization of a methane-utilizing bacterium from a bacterial consortium that rapidly degrades trichloroethylene and chloroform. *Appl. Environ. Microbiol.* **58**:1886–1893.
- Bedard, C., and R. Knowles. 1989. Physiology, biochemistry, and specific inhibitors of CH<sub>4</sub>, NH<sub>4</sub><sup>+</sup>, and CO oxidation by methanotrophs and nitrifiers. *Microbiol. Rev.* **53**:68–84.
- Bevins, D. B. (DuPont Fluoroproducts.) 1997. Personal communication.
- Broholm, K., B. K. Jensen, T. H. Christensen, and L. Olsen. 1990. Toxicity of 1,1,1-trichloroethane and trichloroethene on a mixed culture of methane-oxidizing bacteria. *Appl. Environ. Microbiol.* **56**:2488–2493.
- Brusseau, G. A., H.-C. Tsien, R. S. Hanson, and L. P. Wackett. 1990. Optimization of trichloroethylene oxidation by methanotrophs and the use of a colorimetric assay to detect soluble methane monooxygenase activity. *Biodegradation* **1**:19–29.
- Chang, W., and C. S. Criddle. 1995. Biotransformation of HCFC-22, HCFC-142b, HCFC-123, and HFC-134a by methanotrophic mixed culture MM1. *Biodegradation* **6**:1–9.
- Colby, J., D. I. Stirling, and H. Dalton. 1977. The soluble methane monooxygenase of *Methylococcus capsulatus* (Bath). *Biochem. J.* **165**:395–402.
- Culbertson, C. W., A. J. B. Zehnder, and R. S. Oremland. 1981. Anaerobic oxidation of acetylene by estuarine sediments and enrichment cultures. *Appl. Environ. Microbiol.* **41**:396–403.
- DeFlaun, M. F., B. D. Ensley, and R. J. Steffan. 1992. Biological oxidation of hydrofluorocarbons (HCFC's) by a methanotrophic bacterium. *Bio/Technology* **10**:1576–1578.
- Downing, R. C. 1988. Fluorocarbon refrigerants handbook. Prentice Hall, Englewood Cliffs, N.J.
- Epp, M. A., and J. P. Chanton. 1993. Rhizosphere methane oxidation determined via the methyl fluoride inhibition technique. *J. Geophys. Res.* **98**:18413–18422.
- Flett, R. J., R. D. Hamilton, and N. E. R. Campbell. 1976. Aquatic acetylene reduction techniques: solutions to several problems. *Can. J. Microbiol.* **22**: 43–51.
- Frenzel, P., and U. Bosse. 1996. Methyl fluoride, an inhibitor of methane oxidation and methane production. *FEMS Microbiol. Ecol.* **21**:25–36.
- Gossett, J. M. 1987. Measurement of Henry's Law constants for C<sub>1</sub> and C<sub>2</sub> chlorinated hydrocarbons. *Environ. Sci. Technol.* **21**:202–208.
- Hanson, R. S., and T. E. Hanson. 1996. Methanotrophic bacteria. *Microbiol. Rev.* **60**:439–471.
- King, G. M. 1997. Stability of trifluoromethane in forest soils and methanotrophic cultures. *FEMS Microbiol. Ecol.* **22**:103–109.
- King, G. M. 1996. In situ analyses of methane oxidation associated with the roots and rhizomes of a bur reed, *Sparganium eurycarpum*, in a Maine wetland. *Appl. Environ. Microbiol.* **62**:4548–4555.
- Lange, N. A. 1967. Handbook of chemistry, 10th ed. McGraw-Hill, New York, N.Y.
- Lovley, D. R., and J. C. Woodward. 1992. Consumption of freons CFC-11 and CFC-12 by anaerobic sediments and soils. *Environ. Sci. Technol.* **26**: 925–929.
- Matheson, L. J., and P. G. Tratnyek. 1994. Reductive dehalogenation of chlorinated methanes by iron metal. *Environ. Sci. Technol.* **28**:2045–2053.
- Oldenhuis, R., and D. B. Janssen. 1993. Degradation of trichloroethylene by methanotrophic bacteria, p. 121–133. *In* J. C. Murrell and D. P. Kelly (ed.), *Microbial growth on C<sub>1</sub> compounds*. Intercept Ltd., Andover, England.
- Oldenhuis, R., J. Y. Oedzes, J. J. van der Waarde, and D. B. Janssen. 1991. Kinetics of chlorinated hydrocarbon degradation by *Methylosinus trichosporium* OB3b and toxicity of trichloroethylene. *Appl. Environ. Microbiol.* **57**: 7–14.
- Oremland, R. S. 1996. Microbial degradation of atmospheric halocarbons, p. 85–102. *In* J. C. Murrell and D. P. Kelly (ed.), *Microbiology of trace gases: sources, sinks and global change processes*. NATO ASI series 1, vol. 39. Springer, Berlin, Germany.
- Oremland, R. S., and S. P. Polcin. 1982. Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine environments. *Appl. Environ. Microbiol.* **44**:1270–1276.
- Oremland, R. S., and C. W. Culbertson. 1992. Evaluation of methyl fluoride and dimethyl ether as inhibitors of aerobic methane oxidation. *Appl. Environ. Microbiol.* **58**:2983–2992.
- Oremland, R. S., and C. W. Culbertson. 1992. Importance of methane-oxidizing bacteria in the methane budget as revealed by the use of a specific inhibitor. *Nature* **356**:421–423.

26. **Oremland, R. S., L. G. Miller, C. W. Culbertson, T. L. Connell, and L. L. Jahnke.** 1994. Degradation of methyl bromide by methanotrophic bacteria in cell suspensions and soils. *Appl. Environ. Microbiol.* **60**:3640–3646.
27. **Oremland, R. S., L. G. Miller, and F. E. Strohmaier.** 1994. Degradation of methyl bromide in anaerobic sediments. *Environ. Sci. Technol.* **28**:514–520.
28. **Oremland, R. S., D. J. Lonergan, C. W. Culbertson, and D. R. Lovley.** 1996. Microbial degradation of hydrochlorofluorocarbons (CHCl<sub>2</sub>F and CHCl<sub>2</sub>CF<sub>3</sub>) in soils and sediments. *Appl. Environ. Microbiol.* **62**:1818–1821.
29. **Prior, S. D., and H. Dalton.** 1985. Acetylene as a suicide substrate and active site probe for methane monooxygenase from *Methylococcus capsulatus* (Bath). *FEMS Microbiol. Lett.* **29**:105–109.
30. **Schipper, L. A., and K. R. Reddy.** 1996. Determination of methane oxidation in the rhizosphere of *Sagittaria lancifolia* using methyl fluoride. *Soil Sci. Soc. Am. J.* **60**:611–616.
31. **Stirling, D. I., and H. Dalton.** 1979. The fortuitous oxidation and cometabolism of various carbon compounds by whole-cell suspensions of *Methylococcus capsulatus* (Bath). *FEMS Microbiol. Lett.* **5**:315–318.
32. **Summons, R. E., L. L. Jahnke, and Z. Roksandic.** 1994. Carbon isotopic fractionation in lipids from methanotrophic bacteria: relevance for interpretation of the geochemical record of biomarkers. *Geochim. Cosmochim. Acta* **58**:2853–2863.
33. **Thibodeaux, L. J.** 1979. *Chemodynamics*. Wiley Interscience, New York, N.Y.