APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1994, p. 3640–3646
0099-2240/94/$04.00+0

Vol. 60, No. 10

Degradation of Methyl Bromide by Methanotrophic Bacteria in Cell Suspensions and Soils

RONALD S. OREMLAND,[1]* LAURENCE G. MILLER,[1] CHARLES W. CULBERTSON,[1] TRACY L. CONNELL,[1] AND LINDA JAHNKE[2]

U.S. Geological Survey, Menlo Park, California 94025,[1] and NASA Ames Research Center, Moffett Field, California 94035[2]

Received 23 May 1994/Accepted 3 August 1994

Cell suspensions of *Methylococcus capsulatus* mineralized methyl bromide (MeBr), as evidenced by its removal from the gas phase, the quantitative recovery of Br^- in the spent medium, and the production of $^{14}CO_2$ from [^{14}C]MeBr. Methyl fluoride (MeF) inhibited oxidation of methane as well as that of [^{14}C]MeBr. The rate of MeBr consumption by cells varied inversely with the supply of methane, which suggested a competitive relationship between these two substrates. However, MeBr did not support growth of the methanotroph. In soils exposed to high levels (10,000 ppm) of MeBr, methane oxidation was completely inhibited. At this concentration, MeBr removal rates were equivalent in killed and live controls, which indicated a chemical rather than biological removal reaction. At lower concentrations (1,000 ppm) of MeBr, methanotrophs were active and MeBr consumption rates were 10-fold higher in live controls than in killed controls. Soils exposed to trace levels (10 ppm) of MeBr demonstrated complete consumption within 5 h of incubation, while controls inhibited with MeF or incubated without O_2 had 50% lower removal rates. Aerobic soils oxidized [^{14}C]MeBr to $^{14}CO_2$, and MeF inhibited oxidation by 72%. Field experiments demonstrated slightly lower MeBr removal rates in chambers containing MeF than in chambers lacking MeF. Collectively, these results show that soil methanotrophic bacteria, as well as other microbes, can degrade MeBr present in the environment.

The release of methyl bromide (MeBr) to the atmosphere is of environmental concern because of its destructive potential with regard to stratospheric ozone (3, 30, 37). It is currently believed that anthropogenic MeBr sources, such as from soil fumigation, account for about one-third of its annual flux of 200×10^6 kg, with the rest derived from natural sources (2, 15, 17, 18, 33). The atmospheric residence time of MeBr is about 2 years, based on its reaction kinetics with hydroxyl radicals (15, 33). However, the importance of chemical and biological degradation of MeBr in soils and natural waters is not well understood, either in limiting flux from localized soil application (12, 37) or as a global sink for MeBr relative to hydroxyl attack.

The chemical reactivity of MeBr includes its slow hydrolysis to methanol and its nucleophilic substitution with other halides and sulfide (10, 11, 13, 28, 35, 38). The latter reaction results in the formation of methylated sulfur gases which are in turn metabolized by methanogens in anoxic sediments (28). With regard to aerobic degradation of MeBr, cell extracts of *Methylomonas methanica* and *Methylococcus capsulatus* removed MeBr from the gas phases of assay flasks (5, 6), and both whole cells and extracts of *M. capsulatus* demonstrated measurable respiration (O_2 consumption) when MeBr was a substrate in lieu of methane (21). Collectively, these results suggested that methane monooxygenase was responsible for the observed MeBr consumption. Similarly, three species of ammonium-oxidizing nitrifiers, *Nitrosomonas europaea*, *Nitrosolobus multiformis*, and *Nitrosococcus oceanus*, consumed MeBr, and inhibition patterns suggested involvement of ammonia monooxygenase (31). However, all of these investigations were of very limited scope, in that they did not pursue broader aspects of MeBr metabolism by cells, its biochemical constraints, or its environmental significance. We now report on the ability of methanotrophs in culture, soils, and field incubations to mineralize MeBr.

* Corresponding author. Mailing address: U.S. Geological Survey, 345 Middlefield Rd., Menlo Park, CA 94025. Phone: (415) 329-4482. Fax: (415) 329-4463.

MATERIALS AND METHODS

Experiments with cell suspensions. Batch cultures (500 ml) of *M. capsulatus* were grown overnight ($A_{680} = 0.42$) in mineral salts medium under a CH_4-air (1:1) atmosphere at 37°C with constant vigorous shaking (26). After each culture had been allowed to grow overnight, each flask was opened and residual CH_4 was removed by sparging with N_2 for 0.5 h. The cells were dispensed (~3.0 mg [dry weight] in 20 ml) into serum bottles (59 ml) and sealed with thick black butyl rubber stoppers. In the initial experiments, we sealed cells under a starting partial O_2 pressure equivalent to that of the growth conditions. Suspensions were first sealed under N_2, and the headspace was subsequently altered by flushing with a mixture of 10% O_2, 70% N_2, and 20% CH_4 gases, which was prepared in large syringes (total volume injected and vented, 180 ml). Smaller quantities of MeBr were added with syringes, and the bottles were incubated at 37°C with shaking (300 rpm). Under these conditions, solubility calculations estimated that 36% of the MeBr was partitioned into the gas phase, with the remaining 64% dissolved in the liquid phase (1, 28, 32). Oxygen was added to the samples by syringe injection as the need became evident by the presence of negative pressures. Subsamples of the gas phase were taken with syringes during the incubation and immediately analyzed by gas chromatography for consumption of CH_4 and MeBr (see below). In another experiment, the effect of increased CH_4 concentrations upon the consumption of MeBr was studied. Cell suspensions were sealed with 1% MeBr and a range of CH_4 concentrations (1, 2.5, 5, 10, 25, and 40%) and incubated as described above.

Oxygen was injected periodically into the bottles containing >5% CH_4 as the need arose.

To determine the products of MeBr metabolism by cell suspensions, we conducted an experiment with [^{14}C]MeBr (New England Nuclear, Boston, Mass.) (specific activity, 29.7 mCi/mmol; purity determined by flame ionization gas chromatography, 100%). Cell suspensions (35 ml) were sealed in serum bottles under air (gas volume, 80 ml), because they were found to be capable of methane oxidation at this partial O_2 pressure. Suspensions were otherwise treated as outlined above. Sealed suspensions were injected with 1.44 μCi of [^{14}C]MeBr. Cells were incubated with or without 3.5% CH_4, and controls consisted of boiled cells incubated with [^{14}C]MeBr and 3.5% CH_4 or cells inhibited with 1.25% methyl fluoride (MeF) and incubated with [^{14}C]MeBr with or without 3.5% CH_4. MeF inhibits methane monooxygenase (26). For comparative purposes, we also incubated cells with 1.46 μCi of $^{14}CH_4$ (Amersham Corp., Arlington Heights, Ill.) (specific activity, 56 mCi/mmol; purity, 97.5%) in lieu of the [^{14}C]MeBr. These suspensions were incubated with or without 3.5% CH_4. The gas and liquid phases were subsampled during the experiment for analyses of CH_4 and CO_2 and their radioactive counterparts as well as for soluble products (see below). After 6.25-h incubation, the experiment was terminated by injecting 4 ml of 5 N HCl. The bottles were shaken continuously overnight before analysis of the headspace for ΣCO_2 and $\Sigma^{14}CO_2$.

To determine if *M. capsulatus* was capable of growth on MeBr, crimp-sealed tubes containing 10 ml of medium were inoculated with 0.5 ml of fresh cell suspension. Tubes were sealed under an atmosphere of 10% O_2 and 90% N_2 and injected with 0.3, 0.6, and 1.2 ml of either MeBr or CH_4. Tubes were incubated overnight at 37°C with reciprocal shaking (200 rpm). Methane and MeBr levels were determined at the start and end of the experiment, and growth was measured by monitoring A_{680} with a Spectronic 21 colorimeter.

Experiments with methanotrophic soils. Methanotrophic soils were taken from an agricultural field located in the Sacramento River Delta and from a compost pile at the Palo Alto Municipal Waste Treatment Center, as reported previously (26). Agricultural soils were dispensed (10 g; volume, ~7 ml) into 59-ml serum bottles and crimp sealed under air with thick black butyl rubber stoppers. Methane and small quantities of MeBr were added with syringes (see below and figure legends for concentrations). Controls consisted of heat-killed soils (autoclaved at 121°C and 250 kPa for 1 h), live soils incubated without O_2 (N_2 flushed), or aerobic live soils without CH_4. Controls inhibited by 1% MeF (vol/vol) were incubated under aerobic or anaerobic conditions. Compost material (200 g) was incubated in sealed mason jars fitted with septa for gas sampling (26). Soils were incubated statically at 20°C. To determine the products of MeBr consumption in soils, [^{14}C]MeBr (1.29 μCi) was added to agricultural soils (10 g in 59-ml serum bottles) and incubated with the same experimental variables and controls as listed above. After 4-h incubation, 5 ml of 1 N HCl was injected into each bottle to generate a slurry, and samples were shaken overnight before the headspace was analyzed for $\Sigma^{14}CO_2$. Subsequently, the bottles were opened, and 15 ml of deionized water was added to each bottle to make a fluid slurry. The bottles were resealed and shaken for 1 h to achieve homogeneity before being reopened, and the contents were poured into centrifuge tubes. Slurries were centifuged at 5,000 × *g* for 30 min, and the supernatant was saved and stored at −20°C for analysis of soluble intermediates (see below).

Field incubations with static chambers. Triplicate sets of circular plexiglass flux chambers (gas phase volume, 4 liters; area, 284 cm^2) were deployed on the moist sediment surface of an exposed freshwater lake bed at Searsville Lake, Calif. (27) and injected with traces of MeBr (3 ppm). Dichlorofluoromethane (HCFC-21) and dichlorodifluoromethane (CFC-12) were also injected at final concentrations of 3 and 1 ppm, respectively. Sulfur hexafluoride (2 ppm) was added to serve as a biologically and chemically inert internal standard to detect against the possible loss of trace gases due to leakage. Experimental manipulation consisted of the addition of 1% MeF to inhibit methanotrophic bacteria (26, 27). Details on the deployment and operation of the chambers have been published elsewhere (25, 27).

Analytical methods. Gas chromatographic analyses were carried out with flame ionization and/or electron capture detectors as described previously (23, 26, 28, 29). A column (4.6 m by 0.42 cm) consisting of Krytox 143 A (60/80 mesh) on a Graphpak support (Alltech Associates, San Jose, Calif.) was used for separation and quantification of MeBr. Ion chromatography was employed to measure the quantity of bromide ions present in medium exposed to MeBr with and without live cells (26). Separation and detection of possible ^{14}C-labelled soluble intermediates from the soil experiments was achieved by collecting fractions eluting from a high-performance liquid chromatograph (HPLC), followed by liquid scintillation counting of the fractions (8). Retention intervals for potential ^{14}C-labelled intermediates, including formate (4.5 to 8.0 min), methanol (8.5 to 11.0 min), and acetate (11.5 to 13.5 min), were made by injection of authentic radioisotopes into the HPLC which was equipped with an in-line radiation detector (7). However, because the detection limit of this method was relatively high (~5 nCi/100-μl injectant), we could not employ it for sample analyses. Radioactive gases were separated and quantified by gas chromatography coupled with gas proportional counting (8).

RESULTS

Experiments with cell suspensions. Cells were able to consume 1% MeBr while oxidizing CH_4, but CH_4 was not required for cells to achieve MeBr consumption (Fig. 1). In contrast, neither gas was consumed in an autoclaved control. The rate of consumption of 1% MeBr varied inversely with the supply of CH_4 (Fig. 2). At high concentrations of MeBr (≥5% [vol/vol] initial gas phase), cell suspensions neither oxidized methane nor consumed MeBr (not shown).

Cells which metabolized MeBr accumulated Br^- in the spent fluid, while autoclaved controls did not. We quantified Br^- accumulation in an experiment by using a larger cell volume (40 ml of fluid under 60 ml of gas phase) to which we added 28 μmol of MeBr plus 120 μmol of CH_4. After 4 h, all the MeBr and 82% of the CH_4 were consumed. The final Br^- concentration was 0.7 mM, which was equivalent to 28 μmol of Br^- present in the 40 ml of spent fluid. This was in stoichiometric balance with the amount of MeBr injected. The Br^- concentration in the sterile medium was 0.06 mM, and deionized water incubated under air plus MeBr did not consume MeBr or accumulate Br^- after a 24-h incubation.

Cells incubated with [^{14}C]MeBr formed $^{14}CO_2$ (Fig. 3). Killed controls or cells inhibited with MeF did not form $^{14}CO_2$ (Fig. 3A) or consume CH_4 (Fig. 3B). Production of $^{14}CO_2$ slowed after 1 to 3 h of incubation, but injection of additional [^{14}C]MeBr after 3.5 h to one of the cell suspensions increased the rate of $^{14}CO_2$ production (Fig. 3A). The recovery of $^{14}CO_2$ from cells incubated with CH_4 and 2.88 μCi of [^{14}C]MeBr was 1.98 μCi, which represented 69% conversion. This value would

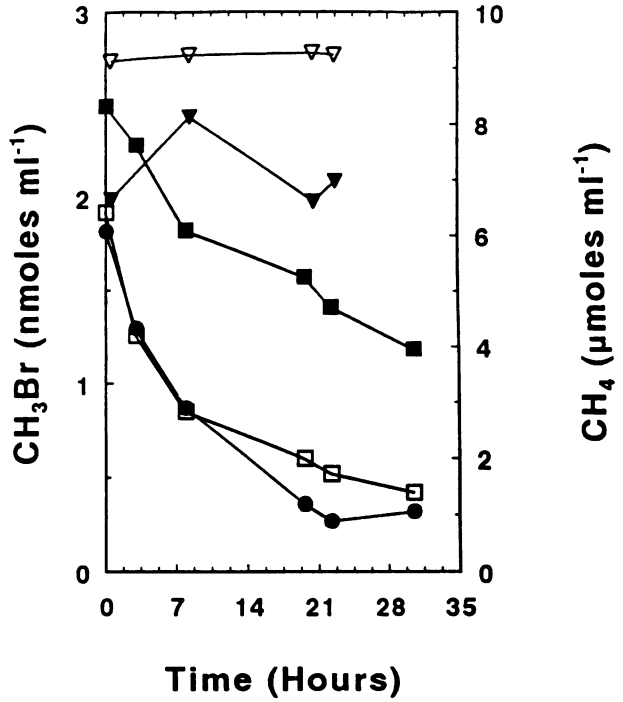

FIG. 1. Headspace methane (CH_4) and MeBr (CH_3Br) concentrations during incubation of cell suspensions of *M. capsulatus*. The levels of CH_4 (■) and MeBr (□) in a live cell suspension, the levels of CH_4 (▽) and MeBr (▼) in a heat-killed control, and the level of MeBr (●) in a live cell suspension incubated without CH_4 are shown. A total of 20 µmol of MeBr was initially enclosed (gas phase volume, 37 ml).

likely have been higher had the experiment been allowed to continue after 6 h. Cells incubated without CH_4 and with only 1.44 µCi of [^{14}C]MeBr formed 0.69 µCi of $^{14}CO_2$, which represented 48% conversion. Cells incubated with $^{14}CH_4$ consumed this gas (not shown) and produced $^{14}CO_2$ (Fig. 3A); however, consumption of $^{14}CH_4$ was more extensive and produced more $^{14}CO_2$ in the cells incubated with CH_4. Cells

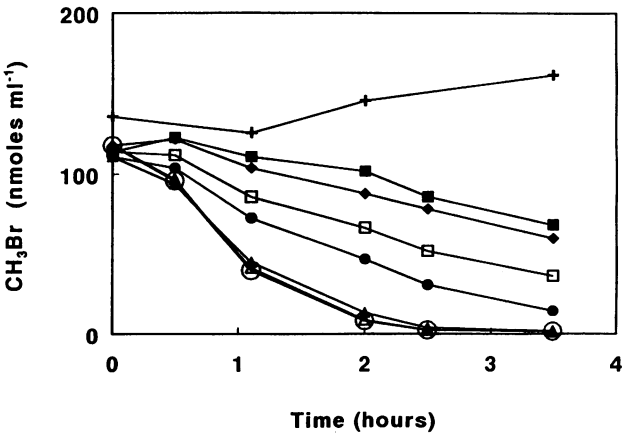

FIG. 2. Effect of CH_4 on the rate of MeBr (CH_3Br) consumption from the headspaces of cell suspensions of *M. capsulatus*. The cells were incubated with the following percentages of CH_4: 1.0 (△), 2.5 (○), 5.0 (▲), 10 (●), 15 (□), 25 (◆), and 40 (■). A heat-killed control (+) is also shown. A total of 20 µmol of MeBr was initially enclosed (gas phase volume, 37 ml).

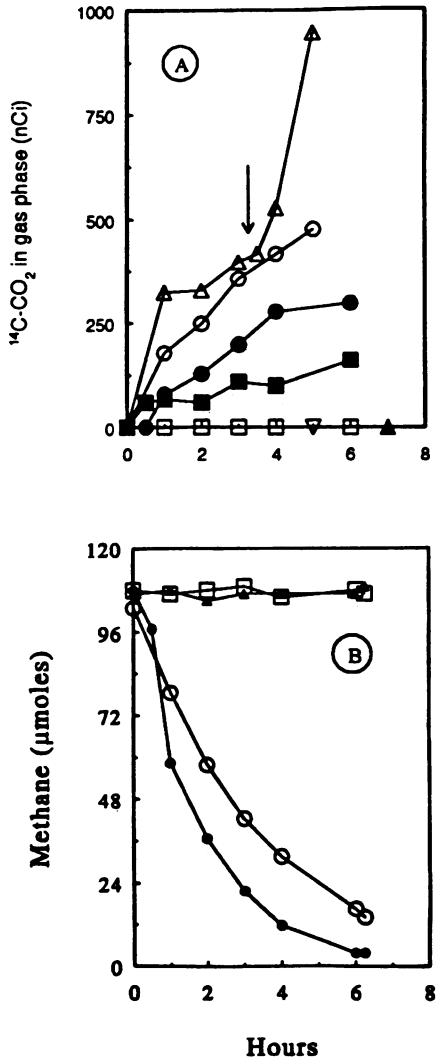

FIG. 3. Production of $^{14}CO_2$ (A) and consumption of CH_4 (B) from the headspaces of cell suspensions of *M. capsulatus*. The cells were incubated with [^{14}C]MeBr (△), [^{14}C]MeBr plus CH_4 (○), [^{14}C]MeBr plus MeF (▽), [^{14}C]MeBr plus MeF plus CH_4 (▲), $^{14}CH_4$ (■), or $^{14}CH_4$ plus CH_4 (●). Heat-killed cells were incubated with [^{14}C]MeBr plus CH_4 (□). The arrow indicates the injection of additional [^{14}C]MeBr only to cells incubated with [^{14}C]MeBr (△). Because of overlapping symbols, we have displayed ▲ and ▼ at one offset time point.

incubated with CH_4 consumed 94% of the $^{14}CH_4$ by 6 h, while cells incubated without CH_4 consumed only 52% of the $^{14}CH_4$. The percents recovery of $^{14}CO_2$ from $^{14}CH_4$ were 54 and 46, for cells incubated with and without CH_4, respectively. No loss of MeF was observed over the incubation period (data not shown).

Medium inoculated with *M. capsulatus* did not demonstrate any growth of the methanotroph on MeBr, although significant amounts of MeBr were consumed. After 25 h of incubation, sealed tubes with 12, 24, and 48 µmol of MeBr had consumed 6, 7, and 29 µmol of the gas, respectively, but had demonstrated no increase in absorbance. In contrast, inocula incubated with similar amounts of CH_4 in lieu of MeBr consumed all of the enclosed gas and had A_{680} increases of 0.02, 0.04, and 0.09, respectively.

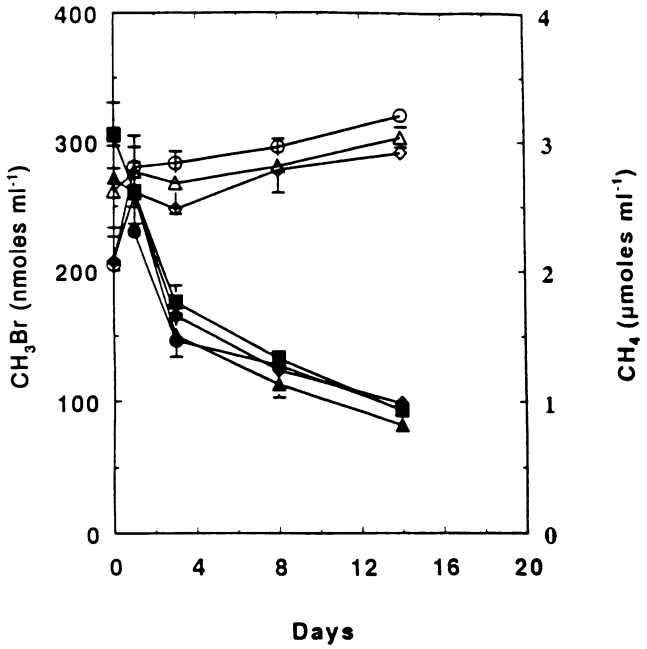

FIG. 4. Consumption of gases by soils incubated with MeBr (CH_3Br) (10,000 ppm) (closed symbols) plus CH_4 (open symbols). Aerobic live soils (▲, △), anaerobic live soils (◆, ◇), autoclaved aerobic soils (●, ○), and aerobic soils without CH_4 (■) were tested. Each point represents the mean of three soil samples, and each error bar represents ±1 SD. The absence of bars indicates that the error bar was smaller than the symbol.

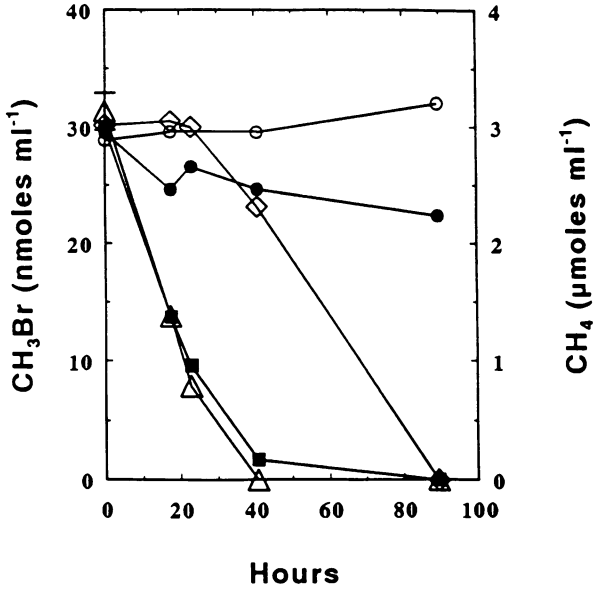

FIG. 5. Consumption of gases by soils incubated with MeBr (CH_3Br) (1,000 ppm) (closed symbols) and CH_4 (open symbols). Aerobic soils incubated with CH_4 (■, ◇), aerobic soils without CH_4 (△), and autoclaved soils (●, ○) were used. Each point represents the mean of three soil samples, and each error bar represents ±1 SD. The absence of bars indicates that the error bar was smaller than the symbol.

Experiments with soils. Soils incubated with 10,000 ppm of MeBr did not oxidize CH_4 but did demonstrate a slow consumption of MeBr over a 2-week period (Fig. 4). However, equivalent rates of MeBr consumption were displayed in all of the experimental variables, which included autoclaved controls, anaerobic soils, and aerobic soils with and without CH_4. Soils incubated with 10-fold-less MeBr (1,000 ppm) clearly demonstrated microbial activity (Fig. 5). In live samples, removal of MeBr preceded that of CH_4 and was nearly complete before methane consumption was apparent. In addition, consumption of MeBr did not require the presence of CH_4. In contrast, an autoclaved control did not consume methane and demonstrated a loss of only 17% of the MeBr by 90 h. A live anaerobic control did not demonstrate any loss of methane but did exhibit a 57% decline in MeBr concentration by 90 h (not shown).

Soils incubated with trace levels of MeBr (10 ppm) demonstrated a rapid consumption of the gas (Fig. 6). Aerobic soils consumed MeBr at an initial rate of ~0.28 nmol g^{-1} h^{-1}, while aerobic soils inhibited with MeF had a 50% lower rate (~0.14 nmol g^{-1} h^{-1}). The aerobic controls inhibited with MeF had MeBr removal rates comparable to those of the anaerobic controls incubated with or without MeF. Autoclaved controls, after the first 2 h of incubation, had a markedly lower rate of MeBr consumption than did the live soils.

The inhibitory effect of MeF on MeBr (10 ppm) consumption was not observed with compost material, although nearly all the MeBr was consumed by 5-h incubation (not shown). Compost not inhibited with MeF had a rate of consumption of 0.099 ± 0.073 nmol g^{-1} h^{-1}, while compost inhibited with MeF had a rate of 0.090 ± 0.015 nmol g^{-1} h^{-1} (n = 3; ±1 standard deviation [SD]). The consumption rate in the anoxic samples was 0.063 ± 33 nmol g^{-1} h^{-1}.

Soils incubated with [^{14}C]MeBr demonstrated the oxidation of [^{14}C]MeBr to $^{14}CO_2$ (Table 1). MeF addition to aerobic soils decreased $^{14}CO_2$ production by 72% from that in soils without MeF. Anaerobic soils also produced $^{14}CO_2$ but achieved only 15% of the oxidation attained under aerobic conditions. MeF did not affect the amount of $^{14}CO_2$ produced by anaerobic soils. No $^{14}CH_4$ was detected in any of the samples. We were unable to detect the presence of ^{14}C-labelled products with the HPLC and in-line radiation detector, but we did detect small quantities of formate, methanol, and acetate when we collected and counted fractions eluting

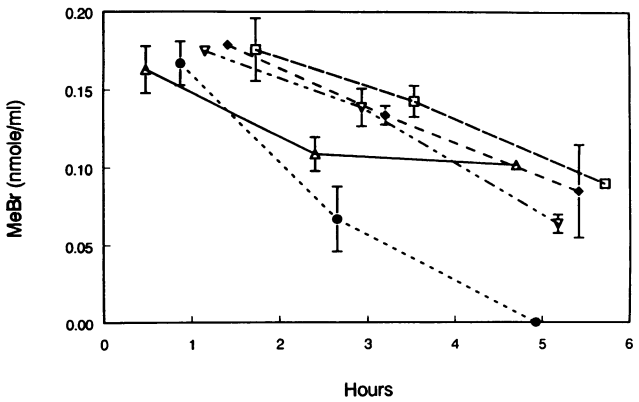

FIG. 6. Consumption of MeBr by soils incubated with 10 ppm MeBr. Symbols: ●, aerobic; △, autoclaved; ▽, aerobic and with MeF; ◆, anaerobic; □, anaerobic and with MeF. Each point represents the mean of three soil samples, and each error bar represents ±1 SD. The absence of bars indicates that the error bar was smaller than the symbol.

TABLE 1. Oxidation of [^{14}C]MeBr by soils

Condition	$^{14}CO_2$ (nCi) (mean ± SD)	% Oxidation[a] (mean ± SD)
Aerobic	558 ± 30	43.3 ± 2.3
Aerobic + 1% MeF	158 ± 41	12.3 ± 3.2
Anaerobic	85 ± 15	6.6 ± 1.2
Anaerobic + 1% MeF	73 ± 1.6	5.7 ± 0.1
Autoclaved	0 ± 0	0 ± 0

[a] Based on a 4-h incubation period with 1.29 μCi of MeBr (n = three soil samples).

from the HPLC. Percent conversions of [^{14}C]MeBr to formate, methanol, and acetate in the different soils were, respectively, 1.5, 2.3, and 0.26 (aerobic soils), 0.7, 4.4, and 0.5 (anaerobic soils), 0.7, 2.2, and 0.04 (autoclaved soils), and 0.9, 3.5, and 0.2 (aerobic soils with MeF).

Field experiments with flux chambers. MeBr concentrations in the flux chambers declined steadily over the 2-day incubation, and consumption was slightly inhibited by the presence of MeF (not shown). After 22-h incubation, differences in MeBr concentrations between control chambers and chambers containing MeF were the most pronounced, being 11 ± 4 nmol liter^{-1} in control chambers and 23 ± 4 nmol liter^{-1} in the chambers containing MeF (mean of three chambers ± 1 SD). By the end of the incubation (46 h), most of the MeBr (>95%) was removed from chambers with and without MeF. In contrast, there was significantly less loss of HCFC-21 over the same period, and a slight inhibitory effect of MeF was discernible in that by 46 h chambers without MeF consumed 39% of the HCFC-21 while those containing MeF consumed 28%. There was no observable loss of CFC-12 during this experiment and concentrations in chambers with and without MeF were virtually identical after 46 h (31 ± 3 nmol liter^{-1}). Likewise, the internal standard sulfur hexafluoride levels did not vary, being 72 ± 1 and 67 ± 6 nmol liter^{-1} at the start in the chambers without and with MeF, respectively. After 46 h, these values were the same (71 ± 3 nmol liter^{-1}) in both sets of chambers. The quantity of methane which accumulated in the chambers increased during the incubation, but there was much variability from chamber to chamber. At the start, methane concentrations were 13.6 ± 0.4 (chambers without MeF) and 46.5 ± 63.3 (chambers with MeF) μmol liter^{-1}. After 46 h, the methane levels were 1,965 ± 1,230 (uninhibited) and 1,501 ± 1,179 (MeF inhibited) μmol liter^{-1}.

DISCUSSION

MeBr can be readily metabolized by methane-oxidizing bacteria (Fig. 1), and the stoichiometric recovery of Br^- (see Results) along with the oxidation of [^{14}C]MeBr to $^{14}CO_2$ (Fig. 3) demonstrates that cell suspensions achieve mineralization by complete oxidation. DeFlaun et al. (9) reported the stoichiometric recovery of Cl^- and F^- ions from metabolism of selected hydrofluorocarbons and hydrochlorofluorocarbons by *Methylosinus trichosporium* as evidence of oxidation. Presumably, MeBr undergoes a pattern of oxidation identical to that of methane, in which methanol, formaldehyde, and formate are sequential metabolites. Alternatively, hydroxylation of MeBr would ultimately result in the formation of formaldehyde and HBr. Formaldehyde was detected when *N. europaea* metabolized MeBr (31).

The fact that high levels of MeBr inhibited methane oxidation as well as consumption of the MeBr itself suggests that it is a competitive inhibitor of methane monooxygenase, much like MeF (26). This conclusion is reinforced by the observations of the antagonistic effect of CH_4 upon the rates of MeBr consumption by cell suspensions (Fig. 2) and of the inhibition of $^{14}CO_2$ production by MeF (Fig. 3). MeBr is unlike MeF, in that MeBr is highly chemically reactive and can form methanol from its hydrolysis with water (35). However, hydrolysis is a relatively slow reaction compared with nucleophilic substitution with other halides (10, 11). Indeed, the fact that we did not observe a significant loss of MeBr in any of the autoclaved controls (Fig. 1 to 3), even after 24-h incubation (Fig. 1), shows the potential for the rates of biological oxidation far exceeding those of chemical reactivity. This observation is important when considering the possible sinks for MeBr in soils (see below).

Cell suspensions were able to oxidize MeBr in the absence of CH_4 (Fig. 1), but they could not utilize it as a growth substrate (see Results). The inability of *M. capsulatus* to grow on 30% MeBr was observed previously (20), although at that high a level, MeBr would have certainly inhibited its own oxidation. Since we did not observe growth but did observe the partial consumption of MeBr at much lower concentrations (2, 4, and 8%), perhaps metabolism of MeBr occurs via a fortuitous oxidation (34).

Soils responded somewhat differently to MeBr than did the cell suspensions, and being composed of a diversity of microbes rather than a monoculture of methanotrophs, this is to be expected. In soils, the slow consumption of 1% (10,000 ppm) MeBr over a 2-week period was entirely due to chemical reactions (Fig. 4), whereas 1% MeBr was oxidized rapidly by cell suspensions and chemical degradation was not observed (Fig. 2). Hence, methanotrophs in these soils were inhibited from oxidizing both methane and MeBr when the latter was present at too high a concentration. When the MeBr concentration was lowered by an order of magnitude, the biological consumption of MeBr was readily apparent and complete within about 40 h (Fig. 5). Trace levels (10 ppm) of MeBr were consumed within a few hours (Fig. 6), and the addition of MeF to aerobic samples caused about 50% inhibition and slowed the rate of consumption to that of the live, anaerobic soils. This result implies that methanotrophs were responsible for about half of the initial MeBr oxidation but that other processes, including perhaps anaerobic reactions involving methanogens (28) or acetogens (19, 36) may have contributed to the biological degradation of MeBr. However, since we did not detect $^{14}CH_4$ or significant amounts of [^{14}C]acetate in the radioisotope experiments (see Results), these microbes did not appear to play a major role, at least during the 4-h duration of the incubation. Hence, the activity of other microbes is inferred. This conclusion was reinforced by our experiments with compost material. Here the contribution of methanotrophs to the rapid consumption of MeBr was not obvious from the MeF inhibition data (see Results) and the significant involvement of other microbes to the degradation of MeBr was implied.

The soil radioisotope experiment supported the observations we made in the gas metabolism studies. Methanotrophs appear to have accounted for as much as 72% of the activity detected (Table 1). However, significant $^{14}CO_2$ was also found in the aerobic MeF-inhibited samples as well as in the anaerobic samples. This result reinforces the conclusion that methanotrophs and nitrifiers, both of which are inhibited by MeF (23, 26), are not the only microbes in soils which can attack MeBr. We did detect small quantities of [^{14}C]methanol (2.2 to 4.4%) in all the samples, including the autoclaved controls. It is possible that some of the $^{14}CO_2$ recovered in the anaerobic samples was due to oxidation of [^{14}C]methanol, possibly by denitrifying bacteria.

Our results have implications for the fate of MeBr in soils. Clearly, we have observed that MeBr can be easily oxidized by various soil bacteria at rates which greatly exceed any chemical consumption. Chemical kinetic experiments with soils or natural waters should consider microbial consumption as a potentially important "sink." Thus, work with sterilized samples should be undertaken or at least the specific products of the anticipated reactions should be measured (e.g., methanol, methyl chloride, and carbon dioxide) rather than assuming that all observed loss of MeBr can be attributed solely to chemical processes. Quantitative recovery of products from various MeBr nucleophilic reactions has been reported for seawater (10, 11), but attempts to model MeBr hydrolysis to methanol in soils may have been clouded by unanticipated microbial degradation (12).

Although our results show that methanotrophs can oxidize MeBr and presumably this activity is conferred upon ammonia monooxygenase-containing nitrifiers as well (31), it is not clear if significant microbial consumption occurs during agricultural fumigation operations. In practice, nearly 0.3 mol of MeBr m^{-2} is injected into the upper 0.3 m of topsoil and retained therein for several days by immediately covering the soil with an impermeable plastic tarp (37). At these high levels of MeBr, it is likely that the biochemical mechanisms for its oxidation are strongly impaired. High levels of MeBr inhibit methane oxidation in cultures (see Results) and in soils (Fig. 4), as well as ammonia oxidation by *N. europaea* (24). In addition, MeBr acts as a competitive inhibitor of methane oxidation (Fig. 2). Therefore, it is likely that fumigated soils are exposed to a high enough quantity of MeBr to preclude its consumption by soil microbes. Indeed, field flux data indicate that most of the MeBr injected into dry soils is released after the tarps are removed (37). In addition, we have observed a marked increase in subsurface soil methane levels from a prefumigation value of 0.27 mmol m^{-2} to a postfumigation value of 13.9 mmol m^{-2}, which suggests that MeBr inhibited soil methanotrophy (22). Collectively, these results imply that microbial consumption of MeBr will be confined to the spatial or temporal "margins" of fumigation operations, where MeBr concentrations are at low enough levels to be metabolized. This could be at the periphery of the fields or within the fields themselves after the tarps are removed and a significant amount of the MeBr has escaped to the atmosphere.

The results from our field deployment of flux chambers show that consumption of trace quantities of MeBr from the entrapped atmosphere is possible (see Results). However, on the basis of MeF data, we did not observe a major contribution from methane-oxidizing bacteria to MeBr removal. Indeed, the methane data from the chambers did not suggest a particularly active methanotrophic community, because there was no enhanced methane accumulation with MeF as was observed previously at this site (27). Nonetheless, the rate of MeBr consumption greatly exceeded that of HCFC-21, and there was no observable loss of CFC-12. This is a significant observation, because although oxidation of HCFC-21 has been reported for *M. trichosporium* (9), and CFC-12 is consumed by anaerobic soils and by *Clostridium pasteurianum* (16), presently there are no "guideposts" which reconcile how significant these reactions are under field conditions or how rapidly they proceed in relation to one another. Clearly, the order of degradation is MeBr > HCFC-21 > CFC-12, a sequence which fits with the chemical reactivity of these gases. Whether microbial oxidation in soils can constitute a significant global sink for atmospheric MeBr as it does for atmospheric methane (4) was not addressed in our studies. Atmospheric methane mixing ratios are about 1.7 ppm, while they are about 5 to 6 orders of magnitude lower for MeBr (15, 18, 33). However, field deployments of flux chambers over termite mounds yielded some detectable consumption of atmospheric mixing ratio quantities of CFC-11 and CFC-12 (14). It would be of interest if similar studies can be extended to MeBr.

ACKNOWLEDGMENTS

We are grateful to R. J. Cicerone and A. J. Meyers for helpful discussions and to G. M. King, B. B. Ward, P. Visscher, and M. A. K. Khalil for their constructive reviews of earlier drafts of this manuscript.

This work was supported by the NASA Earth Sciences Division Upper Atmosphere Research Program and by the Water Resources Division of the USGS.

REFERENCES

1. **Braker, W., and A. L. Mossman.** 1980. Matheson gas data book, 6th ed. Matheson Co., Lindhurst, N.J.
2. **Butler, J. H.** 1994. The potential role of the ocean in regulating atmospheric CH_3Br. Geophys. Res. Lett. **21**:185–188.
3. **Cicerone, R. J., L. E. Heidt, and W. H. Pollock.** 1988. Measurements of atmospheric methyl bromide and bromoform. J. Geophys. Res. **93**:3745–3749.
4. **Cicerone, R. J., and R. S. Oremland.** 1988. Biogeochemical aspects of atmospheric methane. Global Biogeochem. Cycles **4**:299–328.
5. **Colby, J., H. Dalton, and R. Whittenbury.** 1975. An improved assay for bacterial methane mono-oxygenase: some properties of the enzyme from *Methylomonas methanica*. Biochem. J. **151**:459–462.
6. **Colby, J., D. J. Stirling, and H. Dalton.** 1977. The soluble methane mono-oxygenase of *Methylococcus capsulatus* (Bath). Biochem. J. **165**:395–402.
7. **Culbertson, C. W., F. E. Strohmaier, and R. S. Oremland.** 1988. Acetylene as a substrate in the development of primordial microbial communities. Origins Life Evol. Biosphere **18**:397–407.
8. **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.
9. **DeFlaun, M., B. D. Ensley, and R. J. Steffan.** 1992. Biological oxidation of hydrochlorofluorocarbons (HCFCs) by a methanotrophic bacterium. Bio/Technology **10**:1576–1578.
10. **Elliott, S., and F. S. Rowland.** 1993. Nucleophilic substitution rates and solubilities for methyl halides in seawater. Geophys. Res. Lett. **20**:1043–1046.
11. **Elliott, S., and F. S. Rowland.** Methyl halide hydrolysis rates in natural waters. J. Atmos. Chem., in press.
12. **Gentile, I. A., L. Ferrais, and S. Crespi.** 1989. The degradation of methyl bromide in some natural freshwaters. Pestic. Sci. **25**:261–272.
13. **Haag, W. R., and T. Mill.** 1988. Some reactions of naturally occurring nucleophiles with haloalkanes in water. Environ. Toxicol. Chem. **7**:917–924.
14. **Khalil, M. A. K., and R. A. Rasmussen.** 1989. The potential of soils as a sink of chlorofluorocarbons and other man-made chlorocarbons. Geophys. Res. Lett. **16**:679–682.
15. **Khalil, M. A. K., R. A. Rasmussen, and R. J. Gunawardena.** 1993. Atmospheric methyl bromide: trends and global mass balance. J. Geophys. Res. **98**:2887–2896.
16. **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.
17. **Mano, S., and M. O. Andreae.** 1994. Emission of methyl bromide from biomass burning. Science **263**:1255–1258.
18. **Mellouki, A., R. K. Talukdar, A. Schmoltner, T. Gierczak, M. J. Mills, S. Solomon, and A. R. Ravishankara.** 1992. Atmospheric lifetimes and ozone depletion potentials of methyl bromide (CH_3Br) and dibromomethane (CH_2Br_2). Geophys. Res. Lett. **19**:2059–2062.
19. **Meßmer, M., G. Wohlfarth, and G. Diekert.** 1993. Methyl chloride metabolism of the strictly anaerobic, methyl chloride-utilizing homoacetogen strain MC. Arch. Microbiol. **160**:383–387.
20. **Meyers, A. J.** 1978. Evaluation of the "dimethylether pathway" for methane metabolism. Ph.D. dissertation, University of Miami, Miami.

21. **Meyers, A. J.** 1980. Evaluation of bromomethane as a suitable analogue in methane oxidation studies. FEMS Microbiol. Lett. **9:**297–300.
22. **Miller, L. G., T. L. Connell, J. R. Guidetti, and R. S. Oremland.** Unpublished data.
23. **Miller, L. G., D. M. Coutlakis, R. S. Oremland, and B. B. Ward.** 1993. Selective inhibition of ammonium oxidation and nitrification-linked N_2O formation by methyl fluoride and dimethyl ether. Appl. Environ. Microbiol. **59:**2457–2464.
24. **Miller, L. G., B. B. Ward, and R. S. Oremland.** Unpublished data.
25. **Oremland, R. S.** 1983. Hydrogen metabolism by decomposing cyanobacterial aggregates in Big Soda Lake, Nevada. Appl. Environ. Microbiol. **45:**1519–1525.
26. **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.
27. **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 (London) **356:**421–423.
28. **Oremland, R. S., L. G. Miller, and F. E. Strohmaier.** 1994. Degradation of methyl bromide in anaerobic sediments. Environ. Sci. Technol. **28:**514–520.
29. **Oremland, R. S., C. Umberger, C. W. Culbertson, and R. L. Smith.** 1984. Denitrification in San Francisco Bay intertidal sediments. Appl. Environ. Microbiol. **47:**1106–1112.
30. **Prather, M. J., and R. T. Watson.** 1990. Stratospheric ozone depletion and future levels of atmospheric chlorine and bromine. Nature (London) **344:**729–733.
31. **Rasche, M. E., H. R. Hyman, and D. J. Arp.** 1990. Biodegradation of halogenated hydrocarbon fumigants by nitrifying bacteria. Appl. Environ. Microbiol. **56:**2568–2571.
32. **Schwarzenbach, R. P., P. M. Gschwend, and D. M. Imboden.** 1993. Environmental organic chemistry. John Wiley & Sons, Inc., New York.
33. **Singh, H. B., and M. Kanakidou.** 1993. An investigation of the atmospheric sources and sinks of methyl bromide. Geophys. Res. Lett. **20:**133–136.
34. **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.
35. **Swain, C. G., and C. B. Scott.** 1953. Quantitative correlation of relative rates. Comparison of hydroxide ion with other nucleophilic reagents toward alkyl halides, esters, epoxides, and acyl halides. J. Am. Chem. Soc. **75:**141–147.
36. **Traunecker, J., A. Preuß, and G. Diekert.** 1991. Isolation and characterization of a methyl chloride utilizing, strictly anaerobic bacterium. Arch. Microbiol. **156:**416–421.
37. **Yagi, K., N. Y. Williams, and R. J. Cicerone.** 1993. Agricultural soil fumigation as a source of atmospheric methyl bromide. Proc. Natl. Acad. Sci. USA **90:**8420–8423.
38. **Zafiriou, O. C.** 1975. Reaction of methyl halides with seawater and marine aerosols. J. Mar. Res. **33:**75–81.