Marine Bacterial Degradation of Brominated Methanes

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · OCTOBER 1997

Impact Factor: 5.33 · DOI: 10.1021/es970165g

2 AUTHORS, INCLUDING:

Kelly D Goodwin
National Oceanic and Atmospheric Administr…

46 PUBLICATIONS **835** CITATIONS

SEE PROFILE

Environ. Sci. Technol. **1997**, *31*, 3188−3192

Marine Bacterial Degradation of Brominated Methanes

KELLY D. GOODWIN* AND
MARY E. LIDSTROM†

Environmental Engineering Science 138-78, California Institute of Technology, Pasadena, California 91125

RONALD S. OREMLAND

National Research Program, United States Geological Survey, 345 Middlefield Road MS 480, Menlo Park, California 94025

Brominated methanes are ozone-depleting compounds whose natural sources include marine algae such as kelp. Brominated methane degradation by bacteria was investigated to address whether bacterial processes might effect net emission of these compounds to the atmosphere. Bacteria in seawater collected from California kelp beds degraded CH_2Br_2 but not $CHBr_3$. Specific inhibitors showed that methanotrophs and nitrifiers did not significantly contribute to CH_2Br_2 removal. A seawater enrichment culture oxidized $^{14}CH_2Br_2$ to $^{14}CO_2$ as well as $^{14}CH_3Br$ to $^{14}CO_2$. The rates of CH_2Br_2 degradation in laboratory experiments suggest that bacterial degradation of CH_2Br_2 in a kelp bed accounts for $<1\%$ of the CH_2Br_2 produced by the kelp. However, the half-life of CH_2Br_2 due to bacterial removal appears faster than hydrolysis and within an order of magnitude of volatilization to the atmosphere.

Introduction

Methyl bromide (CH_3Br), dibromomethane (CH_2Br_2), and bromoform ($CHBr_3$) are produced by marine macroalgae (e.g., refs 1 and 2) and phytoplankton (*3*, *4*). These compounds are volatile, and their destruction in the atmosphere releases reactive, free bromine. Bromine involvement in the depletion of stratospheric ozone was first recognized in the 1970s (*5*) and is now widely acknowledged (e.g., ref 6). Even a relatively minor redistribution of ozone in the troposphere and stratosphere may have significant impacts on climate (*7*, *8*).

Little is known about the biological sinks of brominated methanes. However, an accurate biogeochemical picture is needed to understand and predict how brominated methane emissions affect atmospheric chemistry. For example, bacterial sinks in soils may influence the atmospheric burden and lifetime of CH_3Br, thus affecting its ozone depletion potential (*9*). In addition, biological consumption of CH_3Br is suggested to occur in high-latitude seas (*10*, *11*).

A number of studies have quantified production rates of brominated methanes from algae (e.g., ref 12). Typically, production is estimated assuming there is no biological consumption during algal incubations. Algal production is then used for estimates of emission, assuming no biological consumption in the water column. However, microbial degradation of brominated methanes has not been fully assessed.

* Corresponding author presently at the U.S. Geological Survey, 345 Middlefield Road MS 480, Menlo Park, CA 94025. E-mail: kgoodwin@usgs.gov. Fax: (415) 329-4463.

† Presently at the Department of Chemical Engineering Box 351750, University of Washington, Seattle, WA 98195-1750. E-mail: lidstrom@chemeng.cheme.washington.edu.

This study investigated the existence, character, and extent of marine microbial degradation of CH_2Br_2 and $CHBr_3$ in order to better understand the biogeochemistry of these compounds and to determine whether microbial degradation could alter estimates of brominated methane production or emission by algae. To this end, we studied microbial degradation of CH_2Br_2 and $CHBr_3$ in sites of significant brominated methane production, namely *Macrocystis pyrifera* kelp beds. We describe for the first time degradation of CH_2Br_2 by bacteria from a marine system and show that CH_2Br_2 is oxidized to CO_2 by these microbes.

Materials and Methods

Marine microbial degradation of brominated methanes was investigated using surface seawater in or near beds of the Giant Kelp, *M. pyrifera*. Seawater was collected from southern California (Corona del Mar and Dana Point) from July, 1993, to September, 1995, and from central (Dog Beach, Monterey Bay) and northern (Van Damme Beach, Mendocino County) California during April and August, 1996. Seawater temperatures ranged from 10.3 to 21.1 °C at the time of collection. Bacteria and other particles were concentrated using a cell concentrator (0.22 μm tangential flow filtration; Pellicon) or serial filtration through 10, 1, and 0.2 μm filters and suspension of material captured on the 1 and 0.2 μm filters (142 mm polycarbonate; Poretics). Concentration was 6−213-fold depending on filtration apparatus and water turbidity. Live samples and formaldehyde-killed controls (3.4% v/v) were amended with CH_2Br_2, $CHBr_3$, or CH_2Br_2 plus $CHBr_3$ administered from a double-distilled water (ddH_2O) solution ($\approx$10 μL to a 10 mL sample) made by serial dilution of the pure compounds. Samples were incubated in darkness at room temperature (23 °C) in 38 mL glass vials crimp sealed with teflon/butyl septa and shaken at 200 rpm.

Enrichment cultures were established by repeated additions of CH_2Br_2 to seawater samples and by successive transfer of 1 mL aliquots of seawater samples to 10 mL autoclaved seawater amended only with CH_2Br_2. The ability to degrade CH_2Br_2 was maintained in enrichments for several months by weekly addition of up to 62 μM CH_2Br_2. To maintain aerobic conditions in long-term enrichments, vials were opened and swirled in a sterile hood and then resealed prior to injection of additional CH_2Br_2.

Enrichments of CH_2Br_2-degrading bacteria were tested for their ability to degrade various halogenated methanes by amending samples with CH_2Br_2 (10 μM), $CHBr_3$ (10 μM), CH_3Br (29 μM), dichloromethane (CH_2Cl_2, 11 μM), $^{14}CH_2Br_2$ (2.4 μM; 1.3 μCi total), or $^{14}CH_3Br$ (0.32 μM; 0.17 μCi total). Live samples were monitored for loss relative to formaldehyde-killed or 0.2-μm-filtered controls. Samples amended with $^{14}CH_2Br_2$ (49.7 mCi/mmol stock in water) or $^{14}CH_3Br$ (29.7 mCi/mmol stock in ethanol) were additionally monitored for production of $^{14}CO_2$. $^{14}CH_3Br$ stock was diluted 1:5 $\times$ 10^4 for a final ethanol concentration of $\sim$0.5 mM.

Inhibitors were used to investigate the type(s) of microorganisms mediating CH_2Br_2 degradation in enrichment cultures. Samples were monitored for CH_2Br_2 loss relative to paired samples not incubated with an inhibitor and relative to formaldehyde-killed controls. The inhibitors used were acetylene (2% v/v) to inhibit monooxygenase enzymes, cycloheximide (350 μg/mL) to inhibit eukaryotic protein synthesis, chloramphenicol (260 μg/mL) to inhibit prokaryotic protein synthesis, or tetracycline/carbenicillin (50 μg/mL each) to inhibit prokaryotic protein and cell wall synthesis. All enrichments were kept in darkness to preclude phytoplankton activity.

Brominated methane concentrations were monitored by syringe injection of 100 μL of vial headspace into an electron-

S0013-936X(97)00165-X CCC: $14.00

capture gas chromatograph (Shimadzu 14-A, Restek 502.1 column or Hewlett-Packard 5890, Supelco Carbopack B column). Calibration standards of CH_2Br_2, $CHBr_3$, and CH_2Cl_2 were made by serial dilution of pure compound (Chem Service) in CH_3OH. Calibration standards of CH_3Br were made by serial dilution of pure compound (Matheson) in air. ^{14}C-labeled compounds were monitored by syringe injection of 200 μL of vial headspace into a thermal conductivity detector gas chromatograph (Hewlett-Packard 5730A, Supelco Carbopack B column) connected to a gas proportional counter (Packard 894). Calibration standards of $^{14}CH_2Br_2$ and $^{14}CH_3Br$ (Dupont, NEN) were made from dilution of stock in 0.2 μm filter-sterilized seawater. $^{14}CO_2$ standards were made from serial dilution of [^{14}C]bicarbonate stock (specific activity 57 mCi/mmol; Dupont NEN) in nitrogen. Standards were made in a range appropriate for each study.

Salting-out coefficients were determined by the method of Gossett (*13*) to assess the effect of salinity on gas solubility. Values (CH_2Br_2, 1.18 ± 0.03; $CHBr_3$, 1.23 ± 0.08) were consistent with the salting-out coefficient of Singh et al. (*14*) for methyl halides. Multiplying 1.2 by freshwater values interpolated from Tse et al. (*15*) for CH_2Br_2 and $CHBr_3$ and from Gossett (*13*) for CH_2Cl_2 gave the following dimensionless equilibrium partition coefficients (concd gas/concd liquid) for seawater at 23 °C: $CH_2Br_2 = 0.040$; $CHBr_3 = 0.023$; $CH_2Cl_2 = 0.097$. For CH_3Br, 0.28 was used (*14*). The fraction of total mass in the headspace (28 mL of headspace, 10 mL of seawater) was 10% for CH_2Br_2 and 6.1% for $CHBr_3$ at 23 °C.

Dibromomethane degradation rates were calculated by regression over the linear portion of the curve (i.e., after lag). Loss in control samples during the same period (typically <20%) were calculated by linear regression and subtracted from the degradation rate measured for live samples.

Protein concentrations were measured at the end of an incubation. Because of low protein concentrations, samples were concentrated an additional 10-fold by successive centrifugation in a microcentrifuge (14 min at 14 000 rpm), removing the supernatant after each centrifugation. The final pellet was resuspended in 1 mL of ddH_2O. Half of the final suspension was diluted 2-fold, and both parts of the sample were assayed for protein concentration (BioRad DC Microassay). Only samples showing 25% agreement or better between the diluted and nondiluted fractions were included here. Cell density was determined by acridine orange direct counting (AODC) (*16*).

Microbial degradation of CH_2Br_2 was compared to algal release. Brominated methane production rates by *M. pyrifera* given per unit kelp weight (*2*) were converted to per unit seawater volume using an estimate of kelp density. Detailed methods and results are given in Goodwin (*17*). Briefly, the kelp bed was assumed vertically mixed and a kelp density averaged over the water column was used. This value (0.4 kg/m^3) was obtained by dividing the area kelp density (6 kg/m^2) by the average kelp-bed water depth (15 m). The area kelp density was estimated by multiplying the frond density (fronds per meter^2) by the average frond mass (~1 kg/frond). Frond density was determined by field measurements of the number of stipes on 100 tagged *M. pyrifera* plants in a 362 m^2 area. Average frond mass was calculated using a frond length distribution. This distribution was obtained by dividing the number of fronds of a particular length by the total number of fronds. That information was determined by field measurements of the number of fronds in 2 m increments for all fronds (n = 264) on 10 tagged plants.

Results

Live samples degraded CH_2Br_2 to nondetectable levels (≤2 nM) relative to formaldehyde-killed (Figure 1) or sterile-filtered controls (not shown). Degradation of CH_2Br_2 typically occurred after a few days lag period, whereas subsequent additions of CH_2Br_2 were degraded within 24 h (Figure 1). In contrast, significant degradation of $CHBr_3$ was not observed. Even samples incubated with both CH_2Br_2 and $CHBr_3$ did not remove $CHBr_3$ after CH_2Br_2 was depleted. After 39 days, no biological loss of $CHBr_3$ was observed although CH_2Br_2 had been depleted for up to a month in some sample vials (Figure 2).

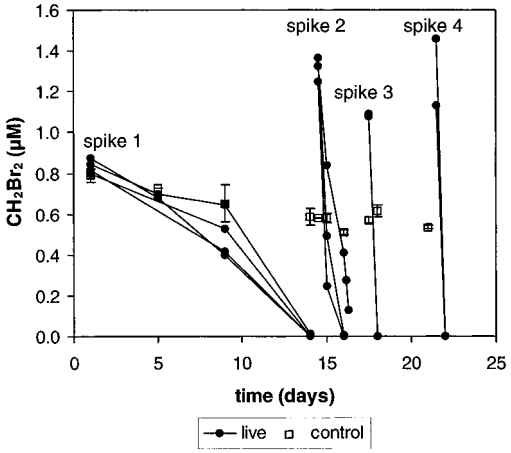

FIGURE 1. Microbial removal of CH_2Br_2 in seawater. CH_2Br_2 was added (spike) after it was degraded by samples of seawater collected from a southern California kelp bed and passed through a cell concentrator. Data for replicate live samples are shown individually. Controls were formaldehyde killed and error bars are ±1 standard deviation (SD) of three replicate samples.

Dibromomethane degradation rates appeared linear over the tested concentration range (0.1−9.2 μM) for a seawater sample collected in northern California (Figure 3A). AODC measurements gave 8.9×10^5 cells/mL; protein concentration was not measured. Degradation rates (calculated after lag) were normalized to protein concentration (285−2148 μg/L) for seawater samples collected in southern California from July, 1993, to September, 1994. Rates ranged from 0.027 to 2.33 nmol of CH_2Br_2/(day μg of protein) and showed a linear trend with concentration (Figure 3B). Bacteria and particles were concentrated by filtration (transverse or serial) for all samples.

Several enrichment cultures were established from seawater collected in southern California (see Materials and Methods). Aliquots of a culture could degrade CH_2Br_2 whether or not CH_3OH (0.05% v/v) or CH_4 (20% v/v) was added. Samples could also degrade CH_2Cl_2, but not $CHBr_3$. Removal of CH_2Br_2 and CH_2Cl_2 could be halted by addition of formaldehyde.

An enrichment culture was also established from Monterey Bay seawater. An aliquot of that culture quickly oxidized $^{14}CH_2Br_2$ to $^{14}CO_2$. Within 8 h, the $^{14}CH_2Br_2$ was consumed with concomitant production of $^{14}CO_2$ (Figure 4A). Upon acidification at the end of the experiment (0.5 mL of 6 N HCl), 49% of the $^{14}CH_2Br_2$ was recovered as $^{14}CO_2$. No $^{14}CO_2$ was measured in filter-sterilized controls even after acidification. In addition, an aliquot of this enrichment oxidized $^{14}CH_3Br$ to $^{14}CO_2$ (Figure 4B). Upon acidification, 73% of the $^{14}CH_3Br$ was recovered as $^{14}CO_2$ while no $^{14}CO_2$ was measured in controls. Degradation of $^{14}CH_2Br_2$ was substantially faster (2.7 nmol of CH_2Br_2/h) than degradation of $^{14}CH_3Br$ (0.05 nmol of CH_3Br/h).

We tested four methanotrophic strains for the ability to degrade brominated methanes under conditions allowing expression of only the particulate form of the methane monooxygenase enzyme (pMMO). Certain methanotrophic bacteria expressing the soluble form of the methane monooxygenase (sMMO) are known to degrade brominated methanes (*18*). However, the pMMO enzyme is more likely to be expressed in marine environments (*19*), but it is unknown whether methanotrophs expressing pMMO can

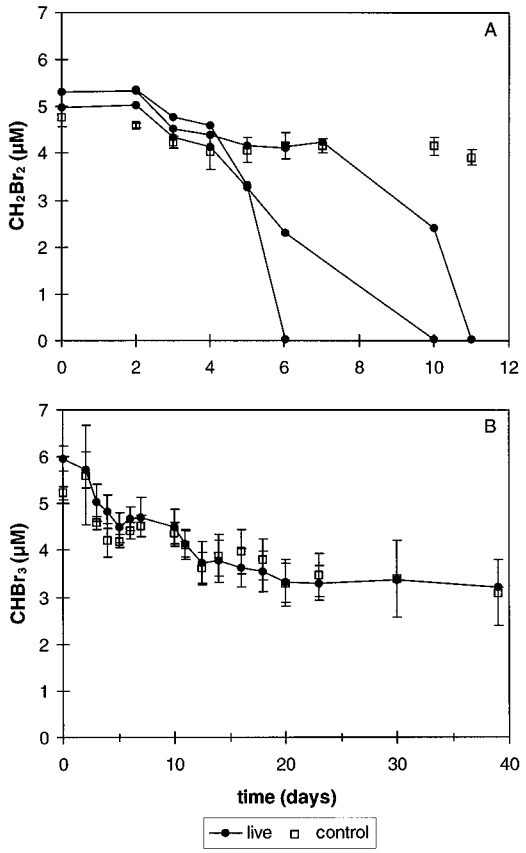

FIGURE 2. Microbial removal of (A) CH_2Br_2 but not (B) $CHBr_3$ in seawater samples incubated with both CH_2Br_2 and $CHBr_3$. Microbial degradation of $CHBr_3$ was not observed after 39 days of incubation and up to 1 month after the disappearance of CH_2Br_2. Seawater was collected from a southern California kelp bed and passed through a cell concentrator. Controls were formaldehyde killed. Data for replicate live samples are shown individually for CH_2Br_2, otherwise error bars are ±1 SD of three replicate samples.

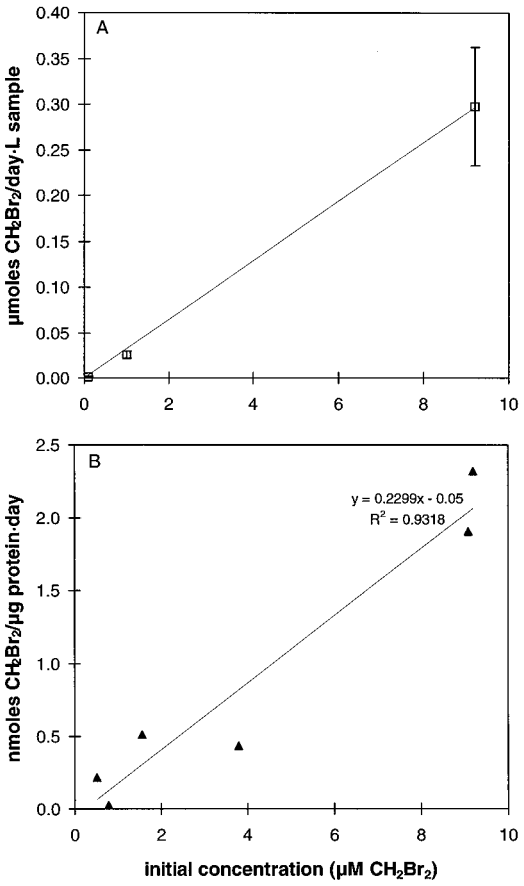

FIGURE 3. CH_2Br_2 degradation rate versus initial concentration. Three replicates were used for each concentration. Bacteria and particles were concentrated by filtration. (A) Degradation rates for varied amounts of CH_2Br_2 applied to one seawater sample collected from a northern California kelp bed in August 1996. Error bars are ±1 standard error (SE) of the regression calculation for degradation rate. (B) Degradation rates normalized to protein concentration for varied amounts of CH_2Br_2 applied to six separate seawater samples collected from southern California kelp beds during July, 1993, to September, 1994. The error in rates is dominated by the error in protein concentrations which was ≤25%.

degrade brominated methanes. The strains tested were the marine methanotroph *Methylobacter marinus* A45, the soil methanotroph *Methylomicrobium albus* BG8, and two new Type I marine methanotrophs designated strains KML E-1 and KML E-2, isolated from a submerged eel grass bed (*20*). *M. marinus* A45 degraded CH_2Br_2 and CH_3Br but not $CHBr_3$. Strain KML E-2 degraded $CHBr_3$ but not CH_2Br_2. *M. albus* BG8 and strain KML E-1 did not degrade any of the brominated methanes tested (Table 1).

Methanotrophic cultures were able to degrade brominated methanes in the laboratory. However, degradation of CH_2Br_2 in seawater samples was not inhibited by acetylene, an inhibitor of methanotrophic bacteria. Acetylene did not affect CH_2Br_2 degradation whether added at the start of an incubation of southern California seawater (Figure 5) or to actively degrading samples of an enrichment culture (not shown). However, chloramphenicol, an inhibitor of bacterial protein synthesis, completely blocked CH_2Br_2 degradation when added at the start of an incubation (Figure 5). Conversely, neither chloramphenicol nor tetracycline/carbenicillin blocked CH_2Br_2 degradation when added to actively degrading samples. Cycloheximide, a eukaryotic inhibitor, also did not block degradation of CH_2Br_2.

Discussion

CH_2Br_2 was degraded in seawater samples, but the lag prior to detectable degradation suggests that bacterial consumption should not significantly interfere with short-term algal incubations used to measure production of CH_2Br_2. Established populations of CH_2Br_2-degrading bacteria may be attached to kelp and could encounter elevated concentrations of CH_2Br_2; thus, significant degradation might occur at kelp surfaces. If this were the case, macroalgal production measurements would record net production, but the value of CH_2Br_2 release would remain unchanged.

$CHBr_3$ appeared resistant to microbial attack even though both CH_2Br_2 and $CHBr_3$ are produced by marine algae (*2,12*). Microbial degradation thus should not interfere with algal incubations used to measure production of $CHBr_3$. Higher concentrations of $CHBr_3$ measured in kelp beds and surrounding waters may reflect not only enhanced macroalgal production of $CHBr_3$ relative to CH_2Br_2 (*12*), but perhaps microbial degradation of CH_2Br_2 but not $CHBr_3$. More pronounced consumption of CH_2Br_2 relative to $CHBr_3$ has been inferred for deep waters in the northwest Atlantic Ocean (*21*). Investigations of open-ocean waters could indicate whether microbial CH_2Br_2 degradation contributes to such profiles.

Although certain eukaryotic microorganisms can degrade brominated organic compounds (*22*), cycloheximide did not inhibit CH_2Br_2 degradation. This result suggests that bacteria, not eukaryotes, were responsible for removal of CH_2Br_2 from southern California seawater samples. Furthermore, microscopic examination revealed only bacteria in enrichment cultures, and CH_2Br_2 degradation occurred in samples filtered through 1.2 or 11 μm filters. Degradation did proceed after

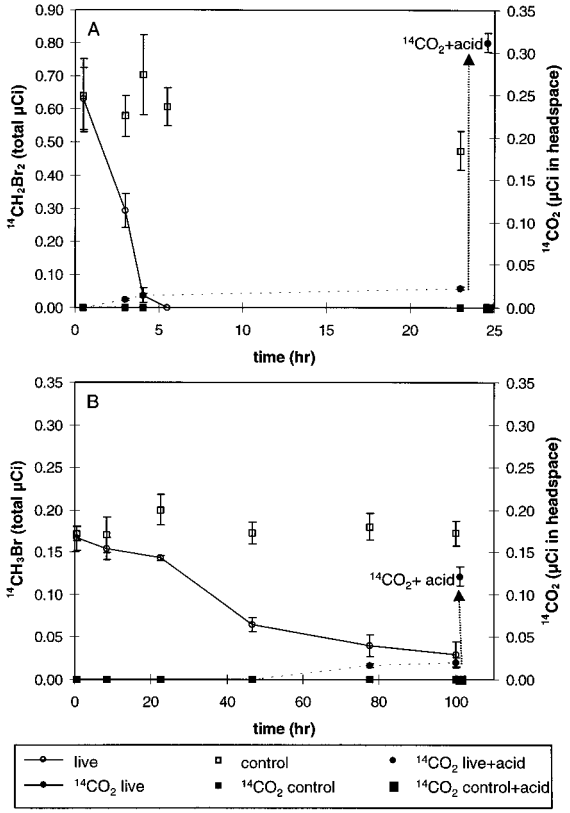

FIGURE 4. Oxidation of (A) $^{14}CH_2Br_2$ and (B) $^{14}CH_3Br$ to $^{14}CO_2$ by a CH_2Br_2 seawater enrichment established from Monterey Bay. Open symbols represent total µCi of $^{14}CH_2Br_2$ or $^{14}CH_3Br$. Closed symbols represent µCi of $^{14}CO_2$ measured in the vial headspace for live samples before (dotted line) and after (arrow) acidification as compared to controls (square symbols). Controls were 0.2 µm-filtered seawater. Error bars are ±1 SD of three replicate samples.

TABLE 1. Brominated Methane Removal by Methanotroph Isolates[a]

strain name; environment; MMO type	CH_2Br_2	$CHBr_3$	CH_3Br
Methylobacter marinus A45; marine; pMMO	+	−	+
Methylomicrobium albus BG8; soil; pMMO	−	−	−
KML E-1; marine; pMMO	−	−	NT
KML E-2; marine; pMMO	−	+	NT

[a] Organism description and enzyme characteristics are noted. + represents compound removed below level of detection; − represents insignificant loss of compound relative to controls. NT = not tested. Initial concentrations were as follows: CH_2Br_2 = 6 µM, $CHBr_3$ = 5 µM, CH_3Br = 18 µM.

a lag relative to unfiltered samples (not shown) which indicates that some activity was associated with particles. However, AODC results suggest that cell concentration could increase clumping of bacteria onto particles, so it is unclear how much activity is particle-associated in the environment. In addition, the results of chloramphenicol and tetracycline/carbenicillin treatments demonstrated that bacterial protein synthesis was necessary for the development of CH_2Br_2 degradation activity. Once activity was developed, however, protein synthesis and cell growth were not needed to sustain it over the short term. In addition, phytoplankton activity was precluded by incubation in darkness.

Inhibitors were used to identify or rule out the types of bacteria involved in the degradation of CH_2Br_2. Acetylene acts on broad spectrum monooxygenases such as methane and ammonia monooxygenases (*23*). It is known to inhibit

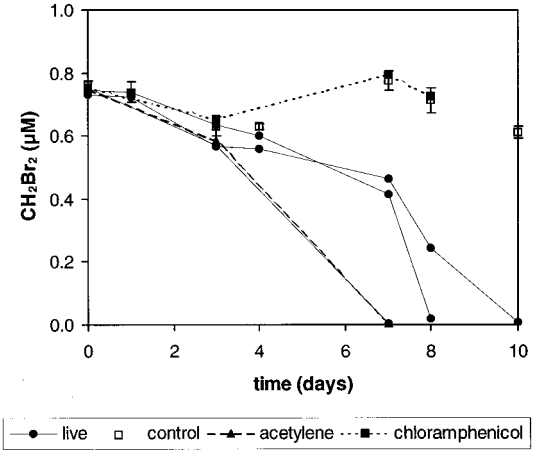

FIGURE 5. Microbial removal of CH_2Br_2 in seawater incubated in the presence and absence of microbial inhibitors. Acetylene, an inhibitor of monooxygenases, and chloramphenicol, a prokaryotic protein synthesis inhibitor, were added at the start of the incubation. Seawater samples were collected from a southern California kelp bed and passed through a cell concentrator. Data for live samples are shown individually. Controls were formaldehyde killed; error bars are ±1 SD of three replicate samples.

CH_2Br_2 degradation for the methanotroph *M. trichosporium* OB3b (*18*). Although we identified marine methanotrophs capable of degrading brominated methanes in the laboratory (Table 1), acetylene did not inhibit CH_2Br_2 degradation in kelp bed seawater. Therefore, neither methanotrophs nor nitrifiers contributed significantly to the removal of CH_2Br_2 in these samples. Acetylene does not affect the activity of methanol-utilizing bacteria, and certain nonmarine methylotrophs are known to degrade CH_2Br_2 (*24*). Methylotrophic bacteria thus remain possible candidates for CH_2Br_2 degradation.

A Monterey Bay seawater enrichment degraded both $^{14}CH_2Br_2$ and $^{14}CH_3Br$, producing $^{14}CO_2$. About half of the $^{14}CH_2Br_2$ was recovered as $^{14}CO_2$ while the remaining $^{14}CH_2Br_2$ may have been incorporated into cell material. Although methanotrophs can degrade CH_2Br_2 and CH_3Br (Table 1), addition of the methanotrophic inhibitor methyl fluoride to Monterey Bay seawater did not inhibit CH_2Br_2 degradation (*25*), consistent with results using unlabeled CH_2Br_2 and acetylene (Figure 5). Several studies have pointed to the possibility of biological consumption of CH_3Br in the ocean (*10, 11, 26*); oxidation of $^{14}CH_3Br$ in this enrichment culture supports that suggestion.

Bacterial degradation of brominated methanes in the environment is of interest, but *in situ* concentrations are lower than those used in these studies. The apparently linear relationships between micromolar concentrations and degradation rates (Figure 3) suggest that linear extrapolation could give an order of magnitude estimate for rates at nanomolar concentrations. For this exercise, a specific rate of 0.034 ± 0.005 day^{-1} was calculated from the slope of Figure 3B applied to a protein density of 150 µg of protein/L for a kelp bed region. Protein density was derived from cell density assuming sample protein to be predominately bacterial, 1×10^{-12} g/wet cell (*28*) and dry weight as 30% wet weight and 50% protein (29). Cell density (1×10^6 cells/mL) was taken as the average AODC measurements for a California kelp bed (8.9×10^5 cells/ml; Mendocino County) and a South African kelp bed [1.5×10^6 cells/mL; (*27*)]. A potential degradation rate of 6.6×10^{-4} nmol of CH_2Br_2/day L was obtained by multiplying the specific rate (0.0345 day^{-1}) by the average CH_2Br_2 concentration measured in a kelp canopy [1.9×10^{-2} µM (*12*), recalculated using ref 15)]. For comparison, release of CH_2Br_2 on a unit kelp weight basis [48 ng of CH_2Br_2/(day g of fresh kelp)] (*2*) equals about 0.11 nmol of CH_2Br_2/day L

TABLE 2. Lifetimes of CH_2Br_2 and $CHBr_3$ Due to Selected Loss Mechanisms

process	CH_2Br_2	$CHBr_3$
atmospheric lifetime	~5 months[a]	~2 weeks[b]
half-life aquatic hydrolysis[c]	183 y	686 y
half-life aquatic halide substitution	NF[d]	1.3−18.5 y[e]
half-life volatilization (5−15 m mixed layer)[f]	~4−11 days	~4−11 days
half-life bacterial degradation in kelp area[g]	~20 days	not detected

[a] Ref 36. [b] Ref 37. [c] Ref 33. [d] NF = literature value not found. [e] Ref 38. [f] Ref 30. Half-life = $0.69LK^{-1}$ where L = mixed-layer depth and K = piston velocity = 4.1 cm/h. [g] Half-life = 0.69(specific degradation rate)$^{-1}$ assuming ~1×10^6 cells/ml in kelp area (see text).

for a 15 m deep kelp bed (see Materials and Methods). Therefore, potential bacterial degradation of CH_2Br_2 in a vertically mixed kelp bed would account for <1% of the CH_2Br_2 released by the kelp.

Estimates of macroalgal emission assume that all the halomethane released eventually reaches the atmosphere because water column sinks are slow relative to volatilization. However, bacterial degradation has not been previously considered. The half-life due to bacterial degradation for waters near a kelp bed is estimated at 20 days based on the potential degradation rate (Table 2). The half-life of CH_2Br_2 due to volatilization is on the order of 4−11 days, depending on mixed-layer depth (Table 2). Winds in southern California kelp beds were typically calm (<1 mph), and the piston velocity of CH_2Br_2 should be similar to $CHBr_3$ (*30*) based on similar diffusivities (equations in ref 31) and mass transfer being limited by liquid phase resistance (Henry's law constant $>1.6 \times 10^{-4}$ atm m^3/mol) (*32*). Bacterial degradation of CH_2Br_2 in waters near a kelp area thus acts faster than hydrolysis (*33*) but is within an order or magnitude of volatilization (see above) (Table 2).

California coastal waters, including kelp beds, seasonally stratify and thus isolate bottom waters from the atmosphere. The fate of CH_2Br_2 will depend on a balance between microbial degradation and mixing into offshore waters, although longshore currents predominate off the California coast (*34*, *35*). Isolation of water by vertical stratification would act to enhance loss of CH_2Br_2 due to microbial degradation relative to volatilization.

Acknowledgments

We thank Dr. Margo Haygood and Dr. Brad Tebo from the Scripps Institute of Oceanography for use of polycarbonate filter holders. We thank Dr. Wheeler North from the California Institute of Technology for guidance and assistance with kelp biomass work and Penny Sherman for laboratory assistance. This research was funded by a grant from the Mellon Foundation and a grant from NSF (EID 9023500). K.G. was partially supported by an NIH training grant (T32 GM08346).

Literature Cited

(1) Manley, S. L.; Dastoor, M. N. *Limnol. Oceanogr.* **1987**, *32*, 709−715.
(2) Goodwin, K. D.; North, W. J.; Lidstrom, M. E. *Limnol. Oceanogr.* **1997**, in press.
(3) Moore, R. M.; Webb. M.; Tokarczyk, R.; Wever, R. *J. Geophys. Res.* **1996**, *101*, 20899−20908.
(4) Scarratt, M. G.; Moore, R. M. *Mar. Chem.* **1996**, *54*, 263−272.
(5) Wofsy, S. C.; McElroy, M. B.; Yung, Y. L. *Geophys. Res. Lett.* **1975**, *2*, 215−218.
(6) Penkett, S. A. *Scientific Assessment of Ozone Depletion: 1994*; Rep. No. 37, World Meteorological Organization Global Ozone Research and Monitoring Project: Geneva, Switzerland, 1995; Chapter 10.
(7) Ramanathan, V.; Cicerone, R. J.; Singh, H. B.; Kiehl, J. T. *J. Geophys. Res.* **1985**, *90*, 5547−5566.
(8) Lacis, A. A.; Wuebbles, D. J.; Logan, J. A. *J. Geophys. Res.* **1990**, *95*, 9971−9981.
(9) Shorter, J. H.; Kolb, C. E.; Crill, P. M.; Kerwin, R. A.; Talbot, R. W.; Hines, M. E.; Harriss, R. C. *Nature* **1995**, *377*, 717−719.
(10) Lobert, J. M.; Yvon-Lewis, S. A.; Butler, J. H.; Montzka, S. A.; Myers, R. C.; *Geophys. Res. Lett.* **1997**, *24*, 171−172.
(11) Moore, R. M.; Webb, M. *Geophys. Res. Lett.* **1996**, *23*, 2951−2954.
(12) Manley, S. L.; Goodwin, K.; North, W. J. *Limnol. Oceanogr.* **1992**, *37*, 1652−1659.
(13) Gossett, J. M. *Environ. Sci. Technol.* **1987**, *21*, 202−208.
(14) Singh, H. B.; Salas, L. J.; Stiles, R. E. *J. Geophys. Res.* **1983**, *88*, 3684−3690.
(15) Tse, G.; Orbey, H.; Sandler, S. I. *Environ. Sci. Technol.* **1992**, *26*, 2017−2022.
(16) Hobbie, J. E.; Daley, R. L.; Jaspar, S. *Appl. Environ. Microbiol.* **1977**, *33*, 1225−1228.
(17) Goodwin, K. D. Ph.D. thesis, California Institute of Technology, Pasadena, California, 1996.
(18) Bartnicki, E. W.; Castro, C. E. *Environ. Toxicol. Chem.* **1994**, *13*, 241−245.
(19) Lidstrom, M. E. In *Microbiology of Atmospheric Trace Gases, Sources Sinks and Global Change Processes, NATO ASI Series 1: Global Environmental Change*; Murrell J. C., Kelly, D. P., Eds.; Springer-Verlag: Berlin, 1996; Vol. 39; pp 121−134.
(20) Smith, K. S. Ph.D. Thesis, California Institute of Technology, Pasadena, California, 1996.
(21) Moore, R. M.; Tokarczyk, R. *Glob. Biogeochem. Cycles* **1993**, *7*, 195−210.
(22) Kaska, D. D.; Polne-Fuller, M.; Gibor, A. *Appl. Microbiol. Biotechnol.* **1991**, *34*, 814−817.
(23) Oremland, R. S.; Capone, D. G. In *Adv. Microbial Ecology 10;* Marshall, K. C., Ed.; Plenum Publish. Co.: NY 1988; pp 285−383.
(24) Doronina, N. V.; Trotsenko, Y. A. *Microbiology* **1994**, *63*, 298−302.
(25) Goodwin, K. D.; Schaefer, J. K.; Oremland, R. S. American Society of Limnology and Oceanography Meeting Abstracts, 1997; p 172.
(26) King, D. B.; Saltzman, E. S. *J. Geophys. Res.* **1997**, in press.
(27) Linley, E. A. S.; Newell, R. C.; Bosma, S. A. *Mar. Ecol. Prog. Ser.* **1981**, *4*, 31−41.
(28) Bailey, J. E.; Ollis, D. F. *Biochemical Engineering Fundamentals*, 2nd ed.; McGraw-Hill: San Francisco, 1986; pp 5.
(29) Schlegel, H. G. *General Microbiology*; Cambridge University Press: Cambridge, U.K., 1993, pp 22−23.
(30) Helz, G. R.; Hsu, R. Y. *Limnol. Oceanogr.* **1978**, *23*, 858−869.
(31) Hayduk, W.; Laudie, H. *AIChE J.* **1974**, *20*, 611−615.
(32) Mackay, D.; Leinonen, P. J. *Environ. Sci. Technol.* **1975**, *9*, 1178−1180.
(33) Mabey, W.; Mill, T. *J. Phys. Chem. Ref. Data* **1978**, *7*, 383−409.
(34) Winant, C. D.; Bratkovich, A. W. *J. Phys. Oceanogr.* **1981**, *11*, 71−86.
(35) Jackson, G. A.; Winant, C. D. *Cont. Shelf Res.* **1983**, *2*, 75−80.
(36) Mellouki, A.; Talukdar, R. K.; Schmoltner, A.; Gierczak, T.; Mills, M. J.; Solomon, S.; Ravishankara, A. R. *Geophys. Res. Lett.* **1994**, *19*, 2059−2062.
(37) Penkett, S. A.; Jones, B. M. R.; Rycroft, M. J.; Simmons, D. A. *Nature* **1985**, *318*, 550−553.
(38) Geen, C. E. M.S. Thesis, Dalhousie University Halifax, Nova Scotia, 1992.

Received for review February 25, 1997. Revised manuscript received August 1, 1997. Accepted August 13, 1997.[⊗]

ES970165G

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.