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Removal of methyl bromide in coastal seawater: Chemical and biological rates

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Abstract. A stable isotope tracer technique was used to investigate the loss rate of methyl bromide in surface ocean waters. Unfiltered and 0.2 μm-filtered or autoclaved aliquants of Biscayne Bay seawater samples were spiked with $^{13}CH_3Br$ at roughly 10-100 times ambient concentrations (50-800 p*M*) and incubated for 10-30 hours. The concentration of $^{13}CH_3Br$ was monitored using gas chromatography with isotope dilution mass spectrometry, with CD_3Br as the isotope spike. Removal rates in unfiltered aliquants were significantly faster than in the 0.2 μm-filtered or autoclaved aliquants, indicating that some of the loss of methyl bromide was associated with particulate matter. Filtration experiments indicate that the particulate material responsible for methyl bromide loss is between 0.2 and 1.2 μm in diameter, suggesting that bacteria are likely to be responsible. The particulate-related removal of methyl bromide was inhibited by autoclaving, supporting a biological mechanism.

Introduction

Recent interest has been focused on the atmospheric chemistry of bromine due to its role in the destruction of stratospheric ozone. Methyl bromide is believed to be the primary source of bromine to the stratosphere [*World Meteorological Organization*, 1995]. The use of methyl bromide as an agricultural fumigant has led to its inclusion in the Montreal Protocol on Substances that Deplete the Ozone Layer [*United Nations*, 1994] and the U.S. Clean Air Act [*U.S. Environmental Protection Agency*, 1993]. However, there remains considerable uncertainty in the factors controlling its atmospheric budget and lifetime [*Butler*, 1995].

The oceans are thought to be the single largest source of methyl bromide to the environment, estimated at approximately 60 Gg/yr [*Butler*, 1995]. Most of this is recycled within the oceans as a result of chemical reactions and possibly biological uptake. The oceans also act as a sink for atmospheric methyl bromide. Roughly one-third of the atmospheric methyl bromide burden is ultimately lost to the oceans [*Yvon and Butler*, 1996]. Field measurements suggest that the oceans act as a net sink for atmospheric methyl bromide in oligotrophic regions and a net source in coastal and upwelling regions [*Singh et al.*, 1983; *Lobert et al.*, 1995]. Recent measurements have demonstrated that coastal waters in high-latitude regions also act as a net sink for atmospheric methyl bromide [*Moore and Webb*, 1996; *Lobert et al.*, 1997]. Due to the unusual role of the oceans as both a source and a sink of methyl bromide, *Butler* [1994] proposed that air-sea exchange may "buffer" the atmosphere against changes in methyl bromide resulting from emission controls on anthropogenic sources.

In current models of methyl bromide cycling in seawater, the lifetime of methyl bromide is estimated solely on the basis of its chemical reaction rate [*Anbar et al.*, 1996; *Pilinis et al.*, 1996; *Yvon and Butler*, 1996]. The primary components of the chemical removal are chloride substitution and hydrolysis:

$$CH_3Br + Cl^- \rightarrow CH_3Cl + Br^- \quad (1)$$

$$CH_3Br + H_2O \rightarrow CH_3OH + HBr \quad (2)$$

with (1) the primary removal mechanism [*Elliott and Rowland*, 1993; *Jeffers and Wolfe*, 1996]. Biological removal of methyl bromide has been observed in soil, sediments, and cell suspensions [*Stirling and Dalton*, 1980; *Rasche et al.*, 1990; *Bartnicki and Castro*, 1994; *Oremland et al.*, 1994a, b; *Shorter et al.*, 1995]. These studies have demonstrated that microbial pathways exist for the metabolism of methyl bromide in both aerobic and anaerobic systems. However, no such studies have been conducted in marine systems.

In this study a stable isotope tracer ($^{13}CH_3Br$) technique was used to determine the loss rate of methyl bromide in surface seawater samples. Use of the isotopically labeled molecule enabled work at near-ambient concentrations without interference from the natural production of methyl bromide. We present measurements of the chemical loss rate of methyl bromide in seawater at near-ambient concentrations and evidence for biological removal of methyl bromide in surface seawater.

Experimental Approach, Procedure, and Data Treatment

The approach used in this study was to compare the removal rate of $^{13}CH_3Br$ in unfiltered seawater with the removal rate in 0.2 μm-filtered or autoclaved seawater. The loss rate in 0.2 μm-filtered or autoclaved seawater should be due primarily to hydrolysis and nucleophilic substitution by chloride ion [*Zafiriou*, 1975; *Elliott and Rowland*, 1993]. If biological uptake also exists, it should provide an additional removal mechanism in the untreated sample.

Incubation of a seawater sample in a closed container creates a system that is potentially very different from the

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oceanic environment due to nutrient depletion [*Goldman et al.*, 1981], chemical toxicity [*Fitzwater et al.*, 1982], and alteration in species composition [*Venrick et al.*, 1977]. Such changes can occur on timescales of several hours [*Gieskes et al.*, 1979]. Because of the variability among samples and properties, it has not been possible to define a maximum incubation time before confinement begins to affect plankton activity [*Marra*, 1980]. To minimize the potential problems of confinement, we have kept the incubation as short as experimentally possible.

The basic experiment consisted of inoculating a seawater sample with $^{13}CH_3Br$ at a level of about 10-100 times ambient concentration (5-10 p*M* $^{12}CH_3Br$) and subsequently monitoring its removal over the course of 10-30 hours. Coastal seawater samples were collected off the dock at the Rosenstiel School of Marine and Atmospheric Science campus, Virginia Key, Florida, between December 1995 and August 1996. Incubations were started within 1 hour of sample collection. For each experiment, the seawater was divided into two to four aliquants. One aliquant was gently vacuum-filtered (<1 in. Hg pressure differential) through a 0.2 μm nylon filter (Cole Parmer). For each experiment, all aliquants were spiked with a $^{13}CH_3Br$ gas standard in helium (Scott Specialty Gases) to approximately the same concentration. The concentrations used in this study ranged from 50-800 p*M*. These concentrations are well below the saturation solubility, which is estimated to be 0.29 *M* at 25°C [*Glew and Moelwyn-Hughes*, 1953; *De Bruyn and Saltzman*, 1997]. The aliquants were stored in 100 or 250 cm^3 glass syringes in a circulating water bath in the dark at a temperature within 1-2°C of the sample upon collection. Samples were stored with no headspace in the syringe to eliminate any loss of methyl bromide due to gas exchange. Subsamples were removed from the syringe and analyzed at 2 to 10 hour intervals.

The concentration of methyl bromide was determined over the course of the incubation by purge and trap with gas chromatography/mass spectrometry. Isotope dilution was employed to improve the precision of the technique. Aliquants of incubated seawater (15 cm^3) were filtered through a GF/F filter (0.7 μm pore size; Whatman) during transfer to a glass stripper. Methyl bromide was stripped from the seawater with an 80 cm^3/min flow of helium for 7 min. The gas was dried with $Mg(ClO_4)_2$, trapped on a stainless steel loop packed with Chromosil B (80/100 mesh) or Unibeads 1S (80/100 mesh) at -65°C, and then refocused onto a stainless steel loop (0.05 cm i.d.) immersed in liquid nitrogen. During stripping, 8 pmol of CD_3Br (99.3% D; Isotech, Inc.) was injected from a 450 μL gas loop (400 ppb) into the helium flow upstream of the seawater sample. Methyl bromide was separated on a capillary column (DB1, 100 m, film thickness 0.2 μm), held at 25°C for the duration of the run.

Methyl bromide isotopes were detected with a quadrupole mass spectrometer (Model C-50, Extrel Corp.) in single ion monitoring mode (m/z 94, 95, 96, 97, 99). The concentration of $^{13}CH_3Br$ was determined from the ratio of peak areas of m/z 99/95. $^{12}CD_3{}^{81}Br$ was the sole contributor to the signal at m/z 99. The signal at m/z 95 was primarily due to $^{13}CH_3{}^{79}Br$, with minor contributions from $^{12}CD^{81}Br$, $^{12}CD_2{}^{79}Br$, $^{13}CH^{81}Br$, and $^{12}CH_2{}^{81}Br$. In experiments used to determine the kinetic carbon isotope effect, the concentrations of both $^{13}CH_3Br$ and $^{12}CH_3Br$ were determined from the m/z 99/97 and 99/96 ratios, respectively. The signal at m/z 97 was mainly $^{13}CH_3{}^{81}Br$, with a small contribution from $^{12}CD_3{}^{79}Br$, and the signal at m/z 96 was due primarily to $^{12}CH_3{}^{81}Br$, with a minor contribution from $^{13}CH_2{}^{81}Br$. The isotope dilution technique compensated for variations in sample recovery and drift in the detector response. Using this technique, the concentration of $^{13}CH_3Br$ could be determined with a precision of better than ±1% (1σ). Details of the analytical procedure will be published elsewhere (D.B. King and E.S. Saltzman, manuscript in preparation, 1997). The isotope dilution spike was calibrated with a $^{13}CH_3Br$ gas standard in helium (Scott Specialty Gases). The uncertainty in the absolute calibration is estimated to be ±1.3% (1σ).

Figure 1 shows the results of one incubation experiment (July 15) as the natural log of the $^{13}CH_3Br$ concentrations as a function of time. In this figure the $^{13}CH_3Br$ concentrations of each aliquant were first normalized to their respective initial concentrations before the natural log was taken ($^{13}CH_3Br_{time=t}/^{13}CH_3Br_{time=0}$). The first-order loss rate constants are determined using a least squares linear regression of ln ($^{13}CH_3Br$) against time. The loss rate constant is equal to the slope of the linear regression. Between 3 and 5 time points are used for the calculation of each loss rate constant. The uncertainty in a single rate constant measurement is estimated using the variance in the slope of the linear regression [*Natrella*, 1966]. In this paper we report the uncertainty in each measured rate constant as the 90% confidence interval for the corresponding rate constant, according to the following equation:

$$\text{confidence interval} = t_{1-\alpha/2} \times s_{b1} \quad (3)$$

where $t_{1-\alpha/2}$ is determined from the degrees of freedom and the t distribution and s_{b1} is the square root of the estimated variance in the slope of the regression line [*Natrella*, 1966]. In all of the tables presented in this paper, the loss rate constants reported represent single measurements.

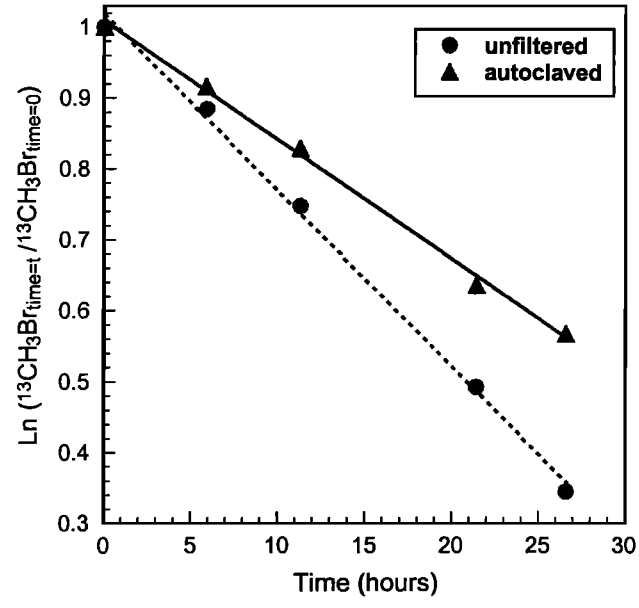

Figure 1. $^{13}CH_3Br$ removal with time in two samples: unfiltered seawater and autoclaved seawater. The data are plotted as the natural log of the concentration normalized to the initial value ($^{13}CH_3Br_{time=t}/^{13}CH_3Br_{time=0}$). The estimated uncertainty (1σ) in a given measurement is approximately equal to the height of each symbol.

Table 1. Comparison of 0.2 μm-Filtered and Unfiltered, Autoclaved Seawater Samples

Temperature, °C	Initial Concentration, p*M*	k_{chem}, % d^{-1}
	0.2 μm-Filtered	
21.3	689	9 (±2)
29.3	70	39 (±3)
35.2	630	80 (±8)
	Autoclaved	
21.4	683	12 (±6)
29.4	109	40 (±3)
35.2	605	83 (±7)

Uncertainties are reported as the 90% confidence interval for each single measurement.

Determination of the Chemical Loss Rate Constant

The chemical loss rate of methyl bromide in seawater was determined from experiments with both 0.2 μm-filtered seawater samples and unfiltered, autoclaved seawater samples. Similar results were observed at three different temperatures (21°, 29°, and 35°C) for these two treatments, suggesting that removal of methyl bromide does not occur on non living particulate matter (Table 1). At all three temperatures, the difference between the rate constants obtained for the 0.2 μm-filtered and autoclaved samples was less than the associated uncertainties in the measurements. Hereinafter, the observed first-order loss rate constant of methyl bromide in 0.2 μm-filtered and autoclaved seawater samples will be referred to as k_{chem}, where $d(CH_3Br)/dt = -k_{chem}\,CH_3Br$.

The observed values for k_{chem} in 16 coastal seawater samples ranged from 9% per day at 21°C to 94% per day at 35°C (Table 2). To directly compare the rates measured in this study to previously published estimates of k_{chem} in seawater, we must correct for the kinetic carbon isotope effect associated with the use of $^{13}CH_3Br$ instead of $^{12}CH_3Br$. The fractionation factor was determined by measuring the loss rate of $^{13}CH_3Br$ and $^{12}CH_3Br$ in a 0.2 μm-filtered seawater sample. These experiments were carried out at relatively high concentrations (1.75-2.4 n*M*) to minimize any potential effect caused by production of $^{12}CH_3Br$ in the sample. The chemical loss rate constants for $^{13}CH_3Br$ ($^{13}k_{chem}$) and $^{12}CH_3Br$ ($^{12}k_{chem}$) were determined at 21.2° and 29.3°C (Table 3). At both temperatures $^{13}k_{chem}$ was slower than $^{12}k_{chem}$. This indicates that the presence of ^{13}C in the methyl bromide molecule acts to decrease the removal rate. It is not known if the presence of ^{13}C inhibits the attack of the nucleophile or decreases the leaving ability of the Br^- ion, or a combination of the two. A more detailed mechanistic study would be necessary to determine the cause of this isotopic effect. These two experiments also indicated that there might be a positive temperature dependence associated with the kinetic carbon isotope effect. However, since the uncertainty in each fractionation factor was larger than the difference between the two values, this difference was not considered to be statistically significant. Consequently, a mean value for $^{13}k_{chem}/^{12}k_{chem}$ of 0.931±0.008 (1σ) was used to correct the values for k_{chem} measured in this study. This value seemed large for a fractionation factor; however, a similar fractionation factor was reported by *Lynn and Yankwich* [1961] for the reaction of methyl bromide with cyanide ion (CN^-). They observed a $^{13}k_{chem}/^{12}k_{chem}$ of 0.92-0.93 over a temperature range of 11-55°C.

In order to compare the values of k_{chem} measured in 0.2 μm-filtered and autoclaved seawater in this study to k_{chem} determined in other studies, it was also necessary to normalize all of the rate constants to a salinity of 35. To facilitate this normalization, k_{chem} was assumed to be equal to the sum of the chloride substitution (k_{Cl}) and hydrolysis (k_{H_2O}) rate constants, as shown in the following equation [*Elliott and Rowland*, 1993; *Jeffers and Wolfe*, 1996]:

Table 2. First-Order Rate Constant for the Chemical Loss (k_{chem}) of Methyl Bromide in Coastal Seawater Samples Measured Using $^{13}CH_3Br$

Date	Temperature, °C	Initial Concentration, p*M*	k_{chem} (Measured), % d^{-1}	$k_{chem\text{-}35}$ (Calculated), % d^{-1}
Dec. 28	21.3	689	9 (±2)	10 (±2)
Dec. 30	21.3	752	10 (±8)	11 (±8)
Jan. 23	21.4	683	12 (±6)*	13 (±6)*
Jun. 25	28.1	130	31 (±14)*	33 (±14)*
Jul. 15	29.4	109	40 (±3)*	46 (±3)*
Jul. 17	29.3	63	34 (±3)	41 (±3)
Jul. 22	29.3	62	37 (±3)	49 (±3)
Jul. 24	29.4	59	37 (±1)	43 (±1)
Jul. 29	29.3	70	39 (±3)	42 (±3)
Jul. 31	29.3	53	38 (±2)	41 (±2)
Aug. 4	29.3	61	38 (±2)	42 (±2)
Jan. 21	34.9	678	82 (±5)	83 (±5)
Jan. 22	35.0	657	91 (±10)	91 (±10)
Jan. 22	35.0	659	94 (±5)	95 (±5)
Mar. 12	35.2	631	80 (±8)	81 (±8)
Mar. 12	35.2	606	83 (±7)*	80 (±7)*

First-order rate constants have also been corrected for the kinetic carbon isotope effect and normalized to a salinity of 35 ($k_{chem\text{-}35}$) (see text for further explanation). Uncertainties are given as the 90% confidence interval for each single measurement.

* Measurements made with unfiltered, autoclaved seawater. All other measurements made with 0.2 μm-filtered seawater.

Table 3. Kinetic Carbon Isotope Effect in 0.2 μm-Filtered Seawater

Temperature, °C	$^{13}CH_3Br$ Initial Concentration, p*M*	$^{13}k_{chem}$, % d^{-1}	$^{12}CH_3Br$ Initial Concentration, p*M*	$^{12}k_{chem}$, % d^{-1}	$^{13}k_{chem}/^{12}k_{chem}$
21.2	1750	11.1 (±0.3)	2400	12.0 (±0.4)	0.925 (±0.043)
29.3	1750	34.3 (±1.1)	1780	36.6 (±1.7)	0.937 (±0.056)

The chemical loss rate constants are given for $^{13}CH_3Br$ and $^{12}CH_3Br$, $^{13}k_{chem}$ and $^{12}k_{chem}$, respectively. Uncertainties are given as the 90% confidence interval for each single measurement.

$$k_{chem} = k_{Cl}[Cl^-] + k_{H_2O} \quad (4)$$

where k_{Cl} (L mol^{-1} d^{-1}) and k_{H_2O} (d^{-1}) are determined for (1) and (2), respectively. The first step involved subtracting k_{H_2O} [*Moelwyn-Hughes*, 1938] from the measured k_{chem}. The resulting values were normalized to a salinity of 35, and the corresponding values for k_{H_2O} were added to obtain the new values of k_{chem}. The values of k_{chem} were then corrected for the kinetic carbon isotope effect to give the appropriate value for $^{12}CH_3Br$. The final values corrected for both salinity and isotope effect are expressed as $k_{chem-35}$. This correction procedure is illustrated in the following equation:

$$k_{chem-35‰} = \left[(k_{chem} - k_{H_2O}) \times \frac{Cl_{35‰}}{Cl} + k_{H_2O}\right] \times 0.931 \quad (5)$$

The values for $k_{chem-35}$ are listed in Table 2 and plotted in Figure 2. In Figure 2b, the natural log of $k_{chem-35}$ is plotted as a function of temperature.

Previous estimates of the chemical loss rate constant (k_{chem}) were determined using both seawater and sodium chloride solutions [*Elliott and Rowland*, 1993; *Jeffers and Wolfe*, 1996]. *Elliott and Rowland* [1993] reported the rate constant of the removal of CH_3Br by chloride substitution (k_{Cl}) in seawater and in 0.5 *M* NaCl solution at 0° and 22°C. Those experiments were carried out at 10^{-3} *M* CH_3Br concentrations and lasted 4 months. *Jeffers and Wolfe* [1996] determined k_{chem} of CH_3Br in several NaCl solutions and in artificial seawater over a temperature range of 20°-60°C. Those experiments were done using a CH_3Br concentration of 10^{-4} *M* over a period of 1-14 days. For comparison, we converted both sets of data to an overall rate constant for salinity 35 seawater ($k_{chem-35}$). In the case of *Elliott and Rowland* [1993], we multiplied their values of k_{Cl} by 0.546 *M* Cl^- (salinity 35) and added k_{H_2O} [*Moelwyn-Hughes*, 1938] to yield values for $k_{chem-35}$. For *Jeffers and Wolfe* [1996] the data were normalized according to (5), with two differences: (1) the values of k_{H_2O} were calculated from *Jeffers and Wolfe* [1997] at the appropriate temperature and (2) no correction for the kinetic carbon isotope effect was needed. The values of $k_{chem-35}$ determined in this study agree with the values of $k_{chem-35}$ calculated from the data of *Elliott and Rowland* [1993] and *Jeffers and Wolfe* [1996] within the experimental uncertainty

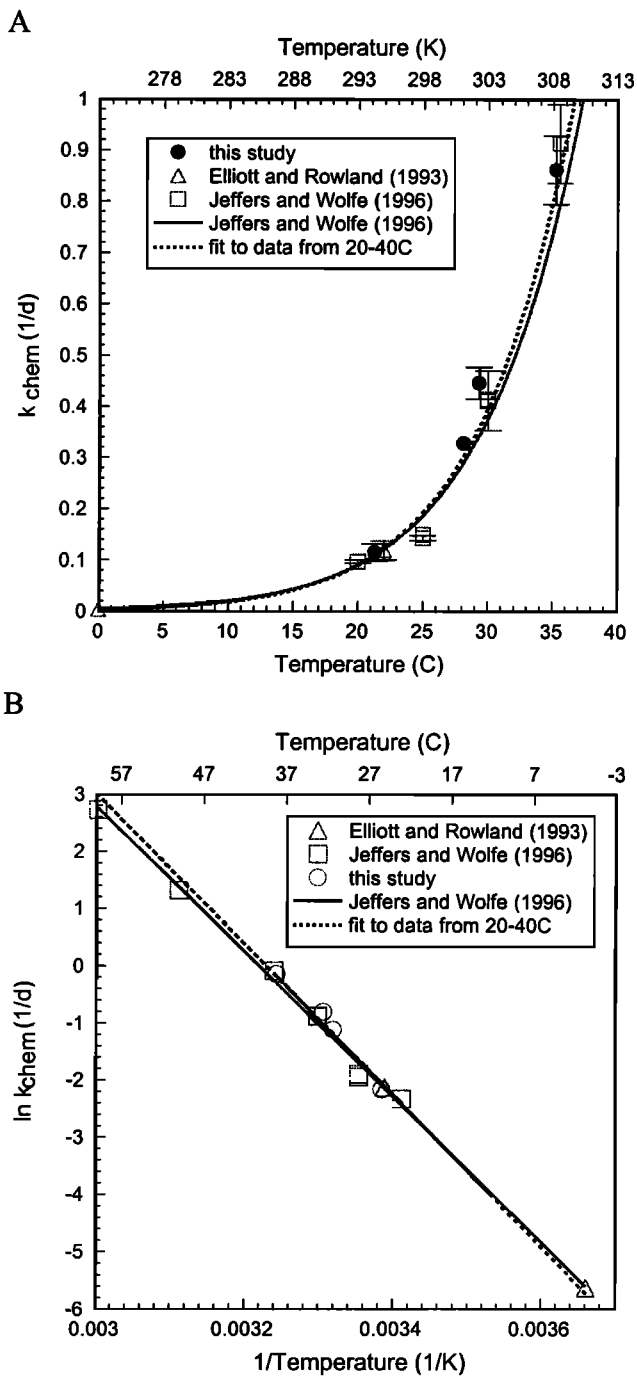

Figure 2. a) Chemical loss rate constants normalized to a salinity of 35 ($k_{chem-35}$) from this study, *Elliott and Rowland* [1993], and *Jeffers and Wolfe* [1996]. Data from this study were also corrected for the kinetic carbon isotope effect. Symbols represent mean values of $k_{chem-35}$ at a given temperature. The solid line represents the rate expression reported by *Jeffers and Wolfe* [1996]. The dotted line represents a rate expression derived from all three data sets. Error bars show the estimated uncertainty in the reported rate constants for *Elliott and Rowland* [1993] and the standard deviation about the mean (1σ) for the two remaining studies. (b) The same data from Figure 2a plotted as the natural log of $k_{chem-35}$. Also included are measurements by *Jeffers and Wolfe* [1996] at temperatures above 40°C which were not shown in Figure 2a.

of the various studies (Figure 2). The agreement among the data sets implies that the chemical removal rate constant in seawater is essentially the same at picomolar concentrations and at millimolar concentrations.

Also plotted in Figure 2 is the rate expression obtained by *Jeffers and Wolfe* [1996] by fitting an Arrhenius equation to their data. Most of the data below 40°C lie above this line. As evident from Figure 2b, the slope of the *Jeffers and Wolfe* [1996] line is largely controlled by the two measurements above 40°C. This suggests that the chemical removal of methyl bromide in seawater may exhibit non-Arrhenius behavior at temperatures above 40°C. *Moelwyn-Hughes* [1938] observed non-Arrhenius behavior in the hydrolysis of methyl bromide at temperatures above 60°C. We propose that an Arrhenius equation fit to only the measurements below 40°C is more appropriate for the temperature range of oceanic interest. The measured rate constant at 0°C was also omitted from the expression because (1) there are no measurements between 0° and 20°C, (2) the uncertainty in that measurement is large, and (3) an Arrhenius fit was observed to be strongly influenced by the measured rate constant at 0°C. We have developed an expression for k_{Cl} using the data from this study, *Elliott and Rowland* [1993], and *Jeffers and Wolfe* [1996] for use in (4). Values of k_{Cl} between 20° and 40°C from all studies were calculated by subtracting k_{H_2O} [*Moelwyn-Hughes*, 1938], from $k_{chem-35}$. The values of k_{Cl} were then fit with an Arrhenius equation. The resulting expression can be used in (4) to provide an overall equation for k_{chem}:

$$k_{chem}\ (d^{-1}) = (5.083 \times 10^{18} e^{-13207/T}) \times [Cl^-] + k_{H_2O} \quad (6)$$

where [*Moelwyn-Hughes*, 1938]

$$k_{H_2O}\ (d^{-1}) = \left(8.64 \times 10^4\right) 10^{(112.656 - 10{,}236/T - 34.259 \log T)} \quad (7)$$

and T is temperature in Kelvin. This expression is plotted in both Figures 2a and 2b. The resulting rate constant is about 13% lower at 0°C than that predicted by the *Jeffers and Wolfe* [1996] expression and about 12% higher at 35°C. Measurements of k_{chem} between 0° and 20°C would further improve this expression.

Determination of the Biological Loss Rate Constant

Single measurements of the loss rate constants in pairs of unfiltered and 0.2 μm-filtered or autoclaved aliquants from 12 coastal seawater samples are listed in Table 4. The loss rate constants (k_{chem}) in the 0.2 μm-filtered and autoclaved samples ranged from 9 to 12% per day at 21°C, while the total loss rate constants (k_{total}) in the unfiltered aliquants ranged from 14 to 19% per day at the same temperature. At 29°C, k_{chem} ranged from 34 to 40% per day in the 0.2 μm-filtered and autoclaved samples, while k_{total} ranged from 40 to 55% per day. In all cases, the loss rate constant in the unfiltered aliquant was greater than that in the corresponding filtered or autoclaved aliquant. A student's t test was used to test the hypothesis that k_{total} and k_{chem} are significantly different [*Natrella*, 1966; *Snedecor and Cochran*, 1989]. The loss rate constants were determined to be different at the 95% confidence level for all of the experiments, with three exceptions. The rate constants measured on January 23 and June 25 were only different at the 90% confidence level, while the rate constants determined on December 30 were only different at the 80% confidence level. The lower confidence levels associated with these experiments are due to the large uncertainties associated with the measurement of k_{chem} during these experiments. Since no value for k_{chem} was obtained on January 5, the t test could not be run for that experiment. The magnitude of the difference between the unfiltered and 0.2 μm-filtered or autoclaved aliquants is expressed as a percent enhancement, defined as $((k_{total} - k_{chem})/k_{chem}) \times 100$. This enhancement ranged from 10 to 110%, with a mean of 41 (±25) % (1σ). There is clearly loss of methyl bromide associated with particulates in the coastal seawater samples. This activity is presumed to be biological since it is not observed in unfiltered, autoclaved seawater samples.

The rate constants listed in Table 4 were neither normalized for salinity nor corrected for the kinetic isotope effect associated with $^{13}CH_3Br$ loss due to chemical processes. Any corrections would affect both k_{total} and k_{chem} equally, which would not change the observed enhancement. Isotopic discrimination against ^{13}C during biological removal of

Table 4. Single Measurements of Loss Rate Constants in Unfiltered (k_{total}) and 0.2 μm-Filtered or Autoclaved (k_{chem}) Seawater Samples

Date	Temperature, °C	Unfiltered Initial Concentration, pM	k_{total}, % d^{-1}	k_{chem}, % d^{-1}	0.2 μm-Filtered or Autoclaved, (F or A)	$\frac{k_{total} - k_{chem}}{k_{chem}}$, %
Dec. 28	21.3	578	19 (±3)	9 (±2)	F	111 (±4)
Dec. 30	21.3	816	14 (±2)	10 (±8)	F	40 (±8)
Jan. 5	21.3	513	15 (±3)	10*	F	50
Jan. 23	21.4	664	17 (±4)	12 (±6)	A	42 (±7)
Jun. 25	28.1	61	43 (±3)	31 (±14)	A	39 (±14)
Jul. 15	29.4	90	55 (±3)	40 (±3)	A	38 (±4)
Jul. 17	29.3	67	40 (±1)	34 (±3)	F	18 (±3)
Jul. 22	29.3	70	50 (±2)	37 (±3)	F	35 (±4)
Jul. 24	29.4	69	47 (±2)	37 (±1)	F	27 (±2)
Jul. 29	29.3	300	43 (±1)	39 (±3)	F	10 (±3)
Jul. 31	29.3	66	52 (±3)	38 (±2)	F	37 (±4)
Aug. 4	29.3	75	53 (±6)	38 (±2)	F	39 (±6)

Uncertainties are given as the 90% confidence interval for each single measurement.

* Chemical loss rate constant was not measured in this experiment. The mean chemical loss rate constant at that temperature is reported here.

methyl bromide in the unfiltered aliquants could affect the measured difference in the rate constants. Experimental uncertainty associated with the measurement of $^{12}CH_3Br$ in unfiltered seawater samples was too large to enable the determination of the carbon isotopic fractionation during biological removal. Published estimates of carbon isotopic fractionation by biological processes vary widely. *Kaplan and Rittenberg* [1964] measured the carbon isotopic fractionation factor during metabolism of lactate by a sulfur reducing bacterium. In that study, $^{13}k/^{12}k$ generally ranged from 0.99 to 1.00. Other investigations have yielded fractionation factors ranging from 0.96 to 0.99 for a variety of processes involving the biological incorporation of ^{13}C [*Degens*, 1969; *Peters et al.*, 1978]. Since the magnitude of the carbon isotopic fractionation factor due to biological removal is unknown, no correction for this effect was made to the observed enhancements. However, the biological fractionation would only be present in the unfiltered sample, so its effect would be to increase the observed enhancement, indicating that biological removal might be faster than estimated in this study.

Experiments were conducted to ensure that the enhanced removal was not a function of the storage container. Aliquants of unfiltered seawater were stored in both a 100 cm^3 glass syringe and a 4 L polyethylene cubitainer (Hedwin). At both temperatures, k_{total} agreed within the experimental uncertainties associated with the measurements (Table 5). This demonstrates that the enhanced removal is not a function of either material (both glass and polyethylene yielded the same removal rate) or surface area to volume ratio (a large difference in this factor did not affect the loss rate constant), unless one compensated for the other, which is unlikely.

The concentration dependence of the total loss rate constant for methyl bromide in unfiltered aliquants (k_{total}) was tested over the range of concentrations used in this study. An unfiltered seawater sample was separated into three aliquants, which were spiked to 26, 297, and 1242 p*M* of $^{13}CH_3Br$. The total loss rate constants in the aliquants were identical, within the precision of the analysis, demonstrating that k_{total} is not a function of concentration over the concentration range used in this study (Table 6).

Filtration experiments were conducted to classify the size of the particulates responsible for methyl bromide removal. These experiments involved the filtration of aliquants of a seawater sample through filters with pore sizes of 1.2, 0.7, and 0.45 μm to compare the removal by different fractions of the biological population. Figure 3 shows the results of one such experiment, in which aliquants of a seawater sample were treated in four different ways: unfiltered, gravity-filtered through a GF/C glass fiber filter (1.2 μm pore size; Whatman), gently vacuum-filtered (<1 in. Hg pressure differential) through an HA nylon membrane (0.45 μm pore size; Millipore), and gently vacuum-filtered (<1 in. Hg pressure differential) through a 0.2 μm nylon membrane (Cole Parmer). Concentrations were normalized to the respective initial value for each sample, and the natural log of the normalized data was plotted as a function of time. Both the unfiltered and 1.2 μm-filtered aliquants yielded the same loss rate constant, within the experimental uncertainty: 52 (±3) % d^{-1} and 53 (±3) % d^{-1}, respectively. The loss rate constant determined from the 0.2 μm-filtered sample was 38 (±2) % d^{-1}. The loss rate constant observed in the 0.45 μm-filtered aliquant, 46 (±3) % d^{-1}, was intermediate between the unfiltered and 0.2 μm-filtered values. These results suggest that bacteria are responsible for the removal of methyl bromide above the chemical removal processes. According to *Sieburth et al.* [1978], the only plankton which exist in the 0.2-1.2 μm size range are bacterioplankton, which comprise both free-living cells and bacteria associated with colonies. If phytoplankton were responsible for the biological removal, the biological enhancement would have been reduced in the

Table 5. Loss Rate Constants in Unfiltered Seawater (k_{total}) Stored in Two Different Containers

Temperature, °C	k_{total} in Glass Syringe, % d^{-1}	k_{total} in Polyethylene Cubitainer, % d^{-1}
28.1	43 (±3)	42 (±3)
29.4	60 (±4)	55 (±3)

Uncertainties are given as the 90% confidence interval for each single measurement.

Table 6. Concentration Dependence of Total Loss Rate Constant in Unfiltered Seawater at 29°C

$^{13}CH_3Br$ Initial Concentration, p*M*	k_{total}, % d^{-1}
26	43 (±1)
297	43 (±3)
1242	40 (±2)

The uncertainties are given as the 90% confidence interval for each single measurement.

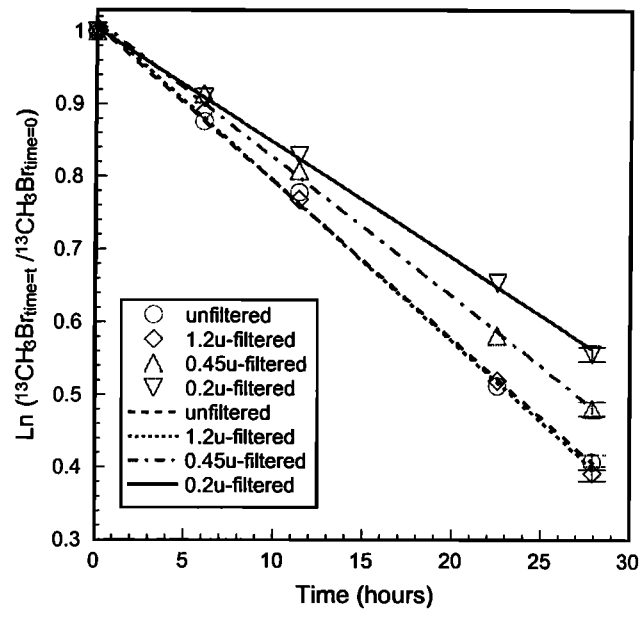

Figure 3. $^{13}CH_3Br$ removal with time in four seawater samples: unfiltered, 1.2 μm-filtered, 0.45 μm-filtered, and 0.2 μm-filtered seawater. The data are plotted as the natural log of the concentration normalized to the initial value ($^{13}CH_3Br_{time=t}$/$^{13}CH_3Br_{time=0}$). Error bars indicate estimated uncertainty (1σ) in a given measurement.

1.2 μm-filtered aliquant. The intermediate loss rate constant observed in the 0.45 μm-filtered aliquant is an indication that only some of the bacteria were removed during the filtration. However, confirmation of organism type might best be assessed in experiments with specific inhibitors and/or antibiotics.

Conclusions

This study demonstrates that biological pathways exist for the removal of methyl bromide in coastal subtropical seawater. The results suggest that global models of the oceanic methyl bromide cycle need to incorporate biological removal processes if they are to adequately describe regional and seasonal variations in the lifetime of methyl bromide. Further field measurements of the type presented here are needed to examine the spatial and temporal variability in biological removal in a variety of water mass types and seasonal conditions.

The rates of biological removal observed are significant relative to the chemical loss rates even in these warm waters, where the chemical loss rates are high. It is possible that biological destruction dominates methyl bromide removal in highly productive polar waters, where low temperatures cause the chemical loss rate to be extremely slow. This may help explain recent observations that the high-latitude oceans are not highly supersaturated with methyl bromide, as had been predicted from model calculations, but rather are undersaturated [*Moore and Webb*, 1996; *Lobert et al.*, 1997]. Microbiological studies are needed to identify the nature of the organisms and the types of metabolism involved.

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