Biological Consumption of Dimethyl Sulfide in the Marine Euphotic Zone: Results of Radioisotope Experiments

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ABSTRACT

The flux of dimethyl sulfide (DMS) to the marine atmosphere, hypothesized to have an important climatic effect via modulation of cloud albedo, depends critically on the rapidity of biological cycling of DMS in the water column, which may be much more rapid than its turnover by air–sea exchange. We developed and tested a radioisotope method to measure microbial DMS consumption using ^{14}C-labeled DMS. Results from whole-water incubations in the NE Pacific and Puget Sound, Washington show that CO_2 and particulates (cell material) are the primary products and are typically produced in ratios of about 2 : 1, suggesting methylotrophic use of DMS as a carbon source, although specific DMS consumers have not yet been isolated from ocean surface waters. Because of low specific activity and low in situ [DMS], a quasi-kinetic approach was required, and estimates of in situ DMS consumption ranged from <0.1 nM d^{-1} to 0.5 nM d^{-1}, yielding biological turnover times of 3–70 d. In the NE Pacific, rates were consistently lower than those calculated from a chloroform-inhibition method, and the radioisotope method most likely provides a lower bound on the true consumption rates. However, even the lower estimates of biological DMS consumption rates are faster than typical loss to outgassing, indicating that most DMS produced in the upper water column is consumed within the water column.

Introduction

The hypothesis that biologically produced dimethyl sulfide (DMS) may play an important climatic role via cloud modulation[1] prompted the question of what limits the flux of DMS to the atmosphere. Because this flux is highly dependent on the concentration of DMS in the upper water column, factors controlling seawater DMS concentrations may have a potential climatic impact.

The seawater concentration of DMS has been been the subject of many field investigations (see Ref. 2 for a review). It is the result of the balance between at

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R. S. Oremland (ed.), *Biogeochemistry of Global Change*

least two production pathways, direct and indirect enzymatic conversion of the algal metabolite dimethylsulfoniopropionate (DMSP), and several competing sinks, of which air–sea exchange is only one (Figure 1). Other potentially major sinks include photo-oxidation to dimethyl sulfoxide (DMSO) and biological consumption to cell material, CO_2, and other products.

Evidence suggests that DMSP is usually much more abundant than DMS itself. For example, in the NE Pacific Ocean off the Washington coast, data from three consecutive spring/summer cruises (Wolfe and Bates, manuscript in preparation) have shown that in the upper 20 m, [DMSP] ranged from 10 to 150 nM (particulate fraction) and from 5 to 30 nM (dissolved fraction), whereas [DMS] was typically in the range 0.5–5 nM. These values are similar to those in the North Sea and North Atlantic.[3,4] Thus, a large precursor pool exists relative to the DMS pool. However, our datasets and many others suggest that DMS surface seawater concentrations above even 10 nM are exceedingly rare, and the global average is only about 3 nM.[2]

One likely limit on the concentration of DMS is biological consumption by marine bacteria. Bacteria have been isolated from other environments (soils, sediments, and sewage wastes) which consume DMS.[5–11] DMS is one in a family of C_1 compounds present in the ocean, including methane and methanol, formaldehyde and formate, the methylamines, methyl mercaptan and dimethyl disulfide, and methylated halides and metals. Many of these compounds are known to be microbially degraded in the marine environment by a wide variety of methylotrophs and still-unidentified bacteria.

How important is the biological sink for DMS? Previous measurements of DMS consumption have been made[12,13] using chloroform to selectively inhibit C_1 catabolism. These studies indicated that DMS consumption by bacteria may, in some cases, be far faster than air–sea ventilation, making the biological sink a crucial limit on the flux of DMS to the atmosphere. The chloroform inhibition technique relies on the high sensitivity of the flame-photometric detector (FPD), allowing routine GC analysis at the picomolar to nanomolar level. However, it measures only disappearance of DMS and gives no knowledge of the biochemical fate(s), therefore yielding limited information of the mechanisms of consumption. It also requires the addition of a "specific inhibitor," a technique which invokes some risky assumptions.[14]

An obvious alternative is to use radioisotopically labeled DMS to measure DMS consumption. The isotope method has several potential advantages: It can trace the compound through metabolic products and no inhibitors are needed. This method has been used to study consumption of DMS in anaerobic sediments[15,16] but not in the marine euphotic zone. The main objectives of this study was the development of a ^{14}C–DMS method to quantify the in situ biological consumption of DMS and to identify and quantify the biochemical products.

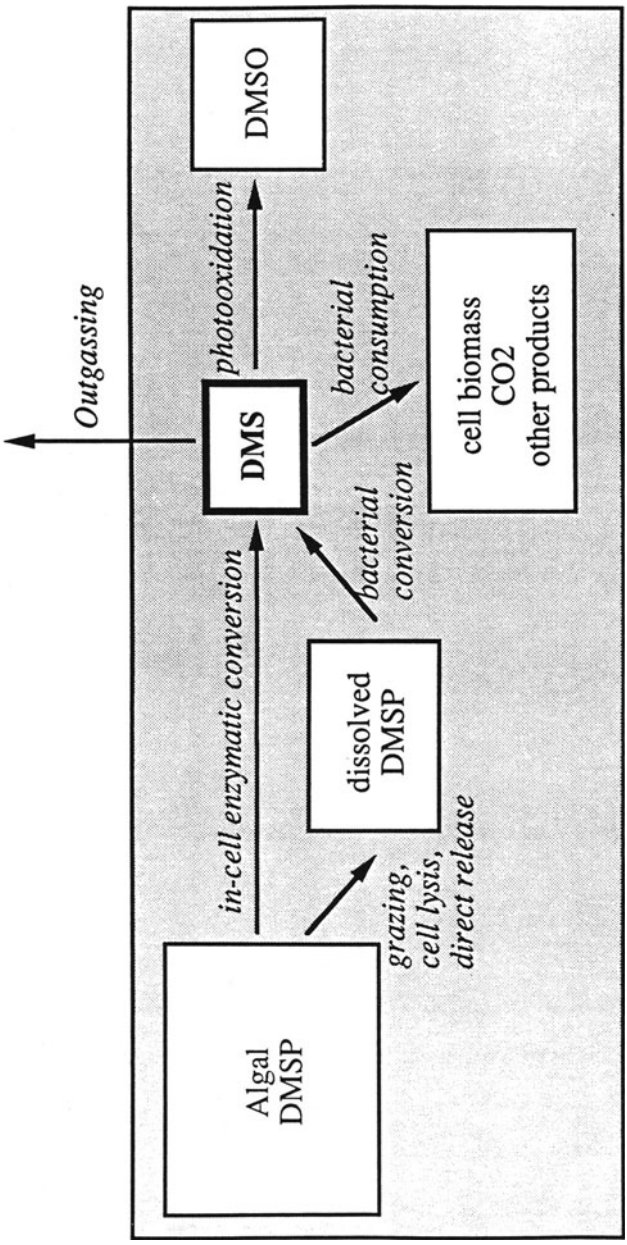

Figure 1. Box schematic of the production and destruction of DMS in the marine photic zone. Boxes denote observables and arrows denote processes.

Materials and Methods

Uniformly labeled ^{14}C–DMS was obtained from Amersham Co. (Arlington Heights, IL). The stock was quoted at 96% purity in 1985 (impurities not specified), with specific activity 22.3 mCi $mmol^{-1}$. This stock was dissolved into 100 ml of sterile deionized water. Subsequent dilutions were made for working stock solutions. Stock solutions were kept cold in the dark and taken out only during additions. Typical additions for experiments were 20–100 μl to 100 ml seawater, for final concentrations of 1–20 nM.

Because of the low specific activity and low concentrations required, true tracer experiments were not possible. Therefore, a quasi-kinetic approach was employed, using two to five concentration additions. Typical experiments ran for 24 h with samples taken every 8 or 12 h. Incubations were conducted in 100-ml serum bottles, capped with Teflon-lined septa. Head space was typically < 10% of the volume, so most of the DMS remained in solution. Experiments were usually conducted in duplicate with a control bottle which was fixed at time zero (t_0) but incubated for the same time period as the others. Numbers reported are the means of duplicates. Runs were also made with 0.2 μm filtered seawater.

Figure 2 outlines the incubation method. To terminate incubations, samples were killed by injection of either NaOH (final pH of 10–11) or formalin (0.5–2% final concentration). The base was used to ensure that any CO_2 produced would stay in solution during the sparging of remaining DMS and because formalin interferes with gas chromatographic analysis for DMS. However, we were concerned that the base might lyse cells and result in loss of cell retention on filters. Therefore, we also conducted experiments using formalin.

Following this, DMS and other volatiles were usually sparged from solution to avoid their contaminating the CO_2 collection. The use of phenethylamine, a standard trap for labeled CO_2, was found to also trap DMS, swamping any CO_2 signal. Increasing additions of labeled DMS into sterile artificial seawater media resulted in approximately 20% recoveries in the CO_2 trap after 16 h static degassing or 2.5 h degassing on a rotary shaker. We found that 0.2 ml of 1 N NaOH was far more selective, trapping CO_2 (> 90% recovery of $H^{14}CO_3^-$ spiked controls; results normalized to CO_2 recovery) but less than 1% of the DMS. (This amount of base was chosen as a minimum to adequately absorb all the CO_2 released from 100-ml seawater samples, but without disruption of the scintillation cocktail which occurred at higher NaOH concentrations). Therefore, sparging the remaining DMS from solution was not strictly necessary but was sometimes performed as a control. In some instances we counted the degassed volatile activity by trapping directly in Omnifluor® on dry ice ($T < -60°C$), which retained about 98% of DMS at flow rates below approximately 100 ml min^{-1}. This allowed recovery of unconverted labeled DMS, which was used as a check on the total recovery.

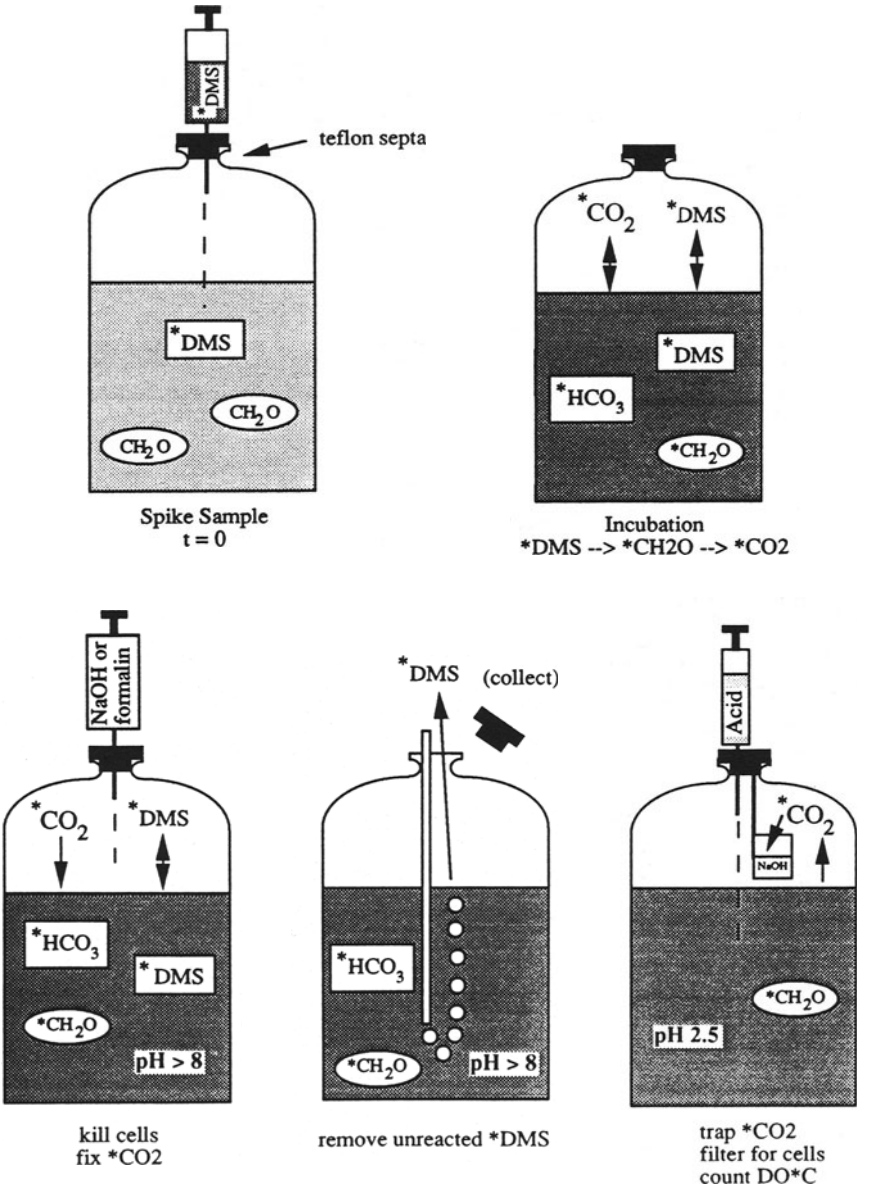

Figure 2. Schematic of ^{14}C–DMS incubation method. "CH_2O" indicates biomass.

Table 1. Fractionations performed for complete recovery of activity.

No.	Fraction	Chemical/Physical Meaning	Use
1	Solution following addition	Total activity—initial	Check activity added
2	Solution at end of incubation	Total activity—final	Check activity left in solution
3	Volatile	Leftover substrate + other volatiles	Fraction unreacted Other S gases (with GC)
4	Nonvolatile	(HCO_3^- + cells + DOC)	Check (3 + 4) = 2
5	Acid-volatile	CO_2	Activity respired
6	Acid-nonvolatile	(cells + DOC)	Check (5 + 6) = 4
7	Filter	Cells	Activity incorporated
8	Filtrate	DOC	Check (7 + 8) = 6

Next the serum bottle was plugged with a cap containing a cup filled with 0.2 ml of 1 N NaOH, and the solution was acidified with 5 N H_2SO_4 to about pH 2.5 to remove CO_2. CO_2 was evolved over 4–24 h on a rotary shaker, and the cup and base were counted in 5 ml of Ecolume® scintillation cocktail (ICN Biomedicals, Costa Mesa, CA).

Following CO_2 degassing, the solution was filtered for cell activity and a subsample of solution counted for nonvolatile activity. Samples were counted on a Packard model 4430 liquid scintillation counter. Counts were converted to DPM using a correction scheme based on an energy spectrum analysis applied to a calibration curve.

Laboratory experiments were also conducted to test the complete recovery of all fractions. The procedure for these was to follow activity per milliliter immediately following the addition of substrate and following termination of incubation, and in all fractions: volatile, nonvolatile, acid-volatile (CO_2), acid-nonvolatile (cells + DOM), filter material (cells), and filtrate (DOC) (Table 1). Gas chromatography was also used in a few cases to look for other volatile sulfur compounds present after incubation and to check the concentration of DMS left in solution after incubations, as well as the initial ambient DMS concentration.

Results

The main field study occurred during the April 1991 PSI-3 (Pacific Sulfur/Stratus Investigation) cruise, in the NE Pacific off the coast of Washington State. Parallel incubations were performed on the same water for both isotope and

chloroform-inhibition methods (done by Dr. Ronald Kiene). Two stations were selected, a near-shore site with relatively high DMS concentrations (3–6 nM at the surface) and high biological activity, and an offshore site, approximately 200 km, with lower [DMS] (less than 1 nM) and activities.

In both instances, isotope experiments were conducted with additions of 1, 5, 10, and 20 nM labeled DMS, and were incubated 24 h in 100-ml serum bottles in a deck incubator at ambient temperature (about 9°C). The bottles were uncovered, so incubations were exposed to a day–night cycle of light intensity.

Results followed a fairly consistent pattern, with roughly 1% of the substrate recovered in the CO_2 fraction after 24 h and less than 0.5% recovered in filter fractions (> 3 μm and 0.2–3 μm). These numbers were calculated from activities normalized against t_0 activities (Figure 3). The fractions generally increased linearly with time, and 0.2-μm filtered samples showed no significant activity. The "DOC" fraction was more variable, with time trends less distinct; up to about 4% of added activity was left in solution even at t_0 due to impurities in the DMS stock (see below).

Rates were highest at the near-shore station and did not show a significant trend with substrate concentration. The in situ rate extrapolated from the ^{14}C–DMS additions was 0.6 nM d^{-1} (Table 2); in contrast, the rates calculated from the chloroform inhibition method were somewhat higher, about 2–3 nM d^{-1} (R. Kiene, personal communication).

At the offshore station, rates were very low (Table 2). Only 0.2-μm filters were used due to the low activities. Less than 0.5% of the substrate was recovered in the CO_2 fraction after 24 h, and less than 0.2% was recovered in filter fraction. These activities were close to the detection limit and sensitivity was poor. The "DOC" fraction showed no significant trend with time. In this instance, the rates of production of CO_2 and cell carbon accounted for a removal of only 0.01–0.1 nM d^{-1}, less than 10% of the rates calculated from the chloroform inhibition method (Table 2) (0.8–0.9 nM d^{-1}, R. Kiene, personal communication).

Following this field study, several laboratory experiments were run using seawater collected off of Shilshole Marina, Puget Sound, Washington in July 1991. These experiments were performed to test two possible sources of reduced activity (impurities in the labeled substrate and the possible breakup of cells from the NaOH used to kill the incubations) and to try to recover the total added activity in all fractions to check for consistency.

The substrate as purchased was found to contain approximately 10% of the activity that did not behave as DMS because it could not be removed from solution by continuous sparging. The stock was purified by sparging DMS from solution and cryotrapping it in a Teflon loop in liquid nitrogen. This was then inserted into sterile deionized water, the trap warmed, and the DMS blown into solution. Tests showed that over 98% of the purified stock's activity could be

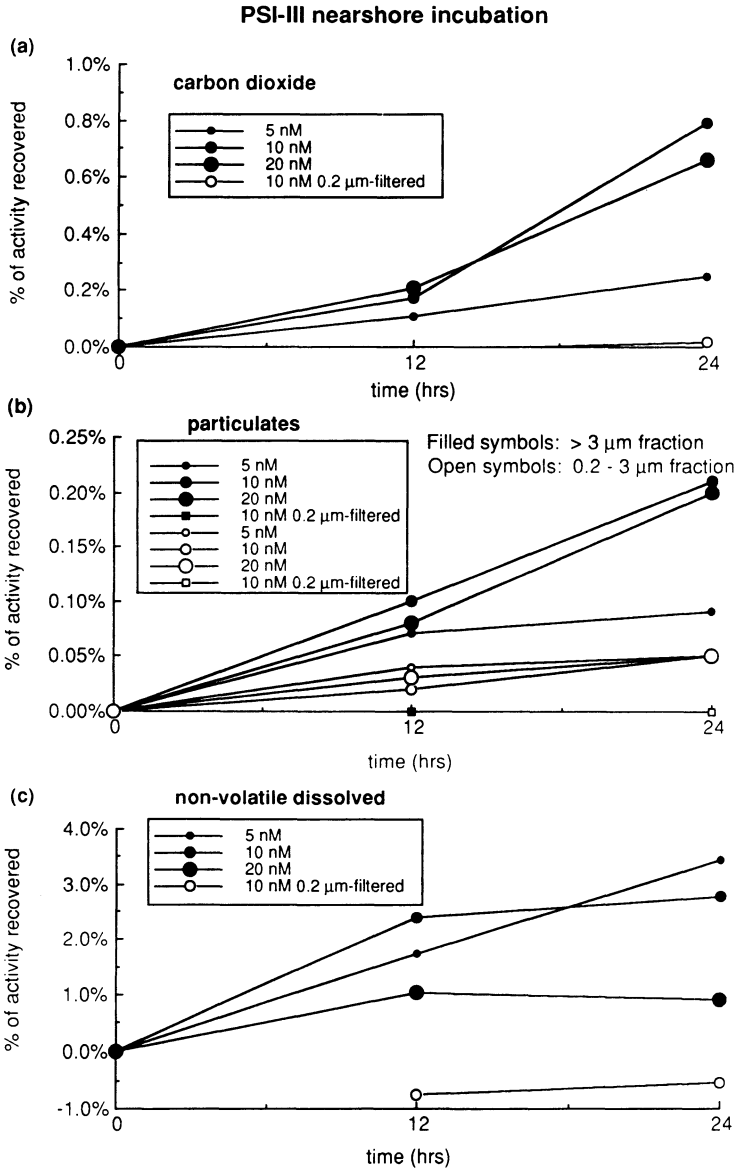

Figure 3. Incubation results from near-shore site of PSI-III study to illustrate typical incubation results. Ambient [DMS] was about 4.5 nM. Shown is relative fraction of added activity recovered as (a) CO_2 (Table 1, fraction #5), (b) > 3-µm and 0.2–3-µm particulates (Table 1, fraction #7), and (c) dissolved material (Table 1, fraction #8). Numbers are normalized to t_0.

Table 2. Comparison of DMS consumption rates during the PSI-III cruise.

Date	$[DMS]_{ambient}$ (nM)	DMS Loss (nM d^{-1})	Turnover Time (d)	Method
		Nearshore Site		
4-23-91	5.8	3.3	1.6	Chloroform[a]
4-23-91	2.0	2.2	1.0	Chloroform[a]
4-28-91	4.5	0.6	8.4	^{14}C–DMS
		Offshore Site		
4-18-91	0.9	0.9	1.0	Chloroform[a]
4-20-91	0.9	0.8	1.0	Chloroform[a]
4-28-91	0.7	<0.01	>100	^{14}C–DMS[b]

[a]Chloroform-inhibition data provided by Dr. R. Kiene.
[b]Recoveries were near limit of detection at this site and consumption rate is very uncertain.

removed after sufficient sparging. Gas chromatography showed that, as with the initial stock, the only sulfur-containing volatile present was DMS, and the specific activity was calculated at 23.8 ± 1.1 mCi $mmol^{-1}$ (n = 6).

In the first experiment, NaOH and formalin were tested against each other as killing agents to see if the base might lyse cells and result in an undercount of cell activity. These incubations, run at 8°C in the dark for 24 h, gave very similar results. Product curves were very similar to the PSI-3 incubations. Analysis of this water by GC found nearly 10 nM ambient DMS, but DMS consumption was very low (roughly 0.1–0.2 nM d^{-1}). This gave assurance that trace impurities in the substrate and using base to terminate the incubation were not causes of low activities.

In the second experiment, the incubation was run at room temperature (21°C) for 72 h, with additions of 5, 10, and 20 nM labeled DMS. Formalin was again used to stop reactions. DMS consumption rates for this experiment were much higher than the previous experiment, consistent with increased biological rates at higher temperatures, and showed increasing rates of production of CO_2 and cell activity with time, suggesting possible adaptation of the culture to the substrate (Figure 4a). Figure 4b shows that rates of conversion of the substrate increased linearly with substrate concentration, indicating no saturation up to 30 nM total [DMS].

For this experiment, a small fraction of the solution was analyzed for complete recovery of added activity. Table 1 details the fractions recovered. Small subsample volumes (7 ml) were analyzed to allow short degassing times (10–12 min). Recoveries were good; about 90% of the added activity was recovered,

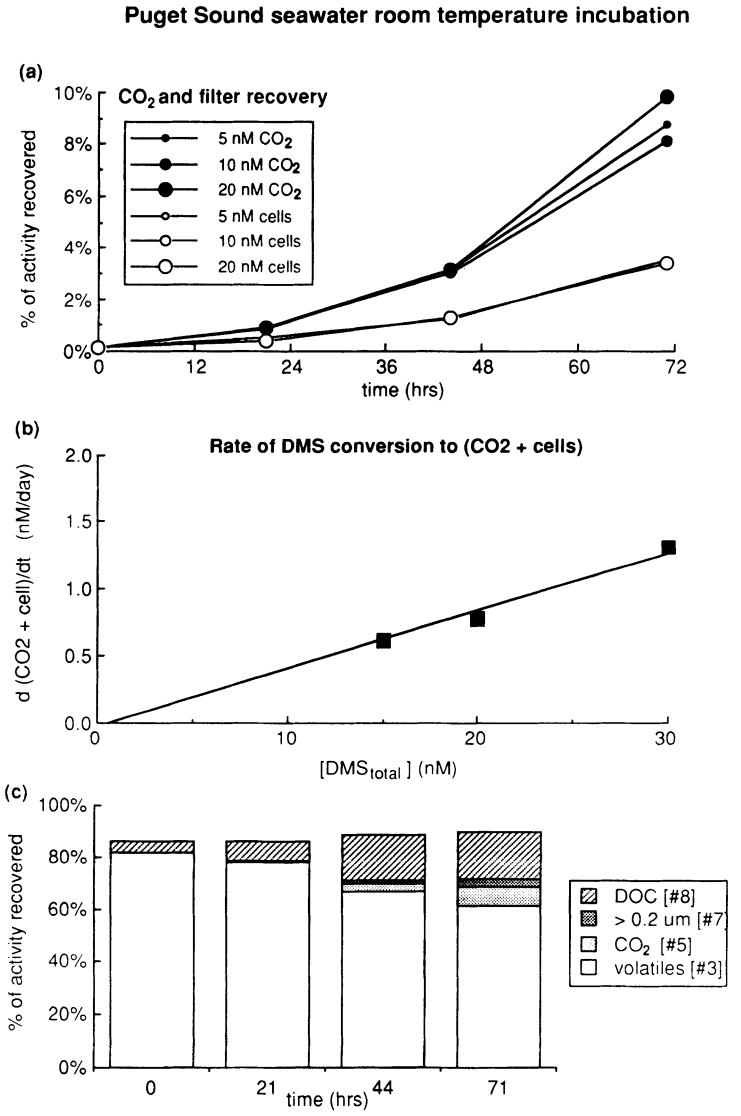

Figure 4. Incubation results from Puget Sound seawater, run at room temperature for 72 h. (a) Activities recovered as CO_2 and > 0.2 μm particulates. (b) Rates of conversion of DMS to CO_2 and > 0.2 μm particulates, averaged for a 71-h incubation period. Rates include ambient [DMS] of approximately 10 nM, plus additions of label at 5, 10, and 20 nM. Line is linear regression. (c) Fractionation results for 10 nM addition of labeled substrate. Shown are fractions recovered as DOC, particulate material > 0.2 μm, CO_2, and volatiles. Numbers in legend refer to fractions in Table 1.

with a concomitant decrease of the volatile fraction and increase in CO_2, particulate material, and DOC fractions over the 72-h time period (Figure 4c). CO_2 fractions from this method were not always identical to those recovered from the standard procedure, probably due to the low total activity of the small volumes used, but by the last time point, there was reasonable agreement.

Discussion

As with the chloroform method, consumption of labeled DMS behaves in a manner consistent with biological uptake: It is removed by 0.2-μm filtering and is inhibited with formalin or alkali. One additional experiment from the PSI-3 cruise (not shown) also demonstrated inhibition of ^{14}C–DMS consumption upon addition of chloroform (500 μM final concentration). The rate of conversion to CO_2 and cell biomass is low but increases with temperature. It appears that a significant fraction of consumed DMS may be released as nonvolatile dissolved material; whether this represents true DOC or cell material which has passed through the 0.2-μm filter is not yet known. Possible soluble compounds which might be produced from DMS include formaldehyde and dimethyl sulfoxide (DMSO). The latter compound might possibly be produced biologically, as has been demonstrated for anaerobes,[10] but could also be produced photochemically.[17] Several measurements of DMSO were taken during PSI-3 and significant concentrations, similar to DMSP, occurred in surface waters (B. Bloomquist, personal communication). Other non-sulfur-containing volatiles, such as formaldehyde or methane, might also be produced which are not currently identified by our method.

There are still several important points which need explanation:

1. Size fractionation of the particulate material results in most activity in the $>$ 3-μm fraction, rather than in the 0.2–3-μm fraction. This is not consistent with bacterial consumption. Possible explanations include: consumption of DMS by eukaryotic organisms; consumption of DMS by bacteria, which are themselves consumed by larger grazers; consumption of DMS by bacteria which are attached to DMSP-containing phytoplankton or which reside in grazer microsites; or filtering artifacts such as flocculation of bacteria or cell breakup/flocculation which results in the bacterial material being retained on the larger-pore filters. The possibility that organisms other than marine bacteria may consume DMS cannot yet be excluded.

2. Consumption of DMS, as measured by CO_2 and cell carbon production, is lower than for the chloroform inhibition method, by as much as an order of magnitude. Because likely by-products of DMS consumption include nonvolatile organics and nonsulfur gases, which have not yet been well quantified, our results should be viewed as a lower bound on the rate of consumption. We hope

to better quantify all fractions in a future study in a direct comparison with the chloroform method.

3. Our attempts to isolate bacteria from seawater which can utilize DMS as a sole carbon or energy source have so far been unsuccessful. We do not yet know what fraction of marine bacteria can consume DMS, or what groups are involved. To our knowledge, no isolates from the marine euphotic zone have been demonstrated to grow on DMS.

4. As of now, the importance of the bacterial sink as a control of DMS outgassing is still largely unknown. It appears that consumption is widespread, but that rates vary considerably and it is likely that the magnitude of this process will be highly dependent on local biological conditions.

Acknowledgments

We wish to acknowledge the generous assistance of Dr. John Baross, University of Washington School of Oceanography. We also wish to thank Dr. James T. Staley, University of Washington Dept. of Microbiology, for his helpful discussions and ideas, and Dr. Ronald Kiene, University of Georgia, for both discussions and his incubation data from the PSI-3 cruise. This work was supported by the NASA Earth Science and Applications Division, Interdisciplinary Research Program.

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