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Development and application of a headspace solid-phase microextraction and gas chromatography/mass spectrometry method for the determination of dimethylsulfide emitted by eight marine phytoplankton species

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Abstract

Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) has been developed, optimized, and applied to investigate the dimethylsulfide (DMS) emissions from eight marine phytoplankton species, namely, *Calcidiscus leptoporus, Emiliania huxleyi, Phaeodactylum tricornutum, Chaetoceros neogracilis, Dunaliella tertiolecta, Synechococcus*, *Prochlorococcus*, and *Trichodesmium*. Four SPME fiber coatings (PDMS, PDMS-DVB, PDMS-CAR, and CW-DVB) were tested for linearity and limit of detection. Key parameters such as equilibrium and extraction times, desorption temperature and time, and headspace volume were optimized to make extraction as efficient as possible using the PDMS-DVB fiber coating. This fiber enabled the characterization of DMS in seawater under 0.005 nM levels and within 1 min exposure time. Among the different algae groups, the two coccolithophorids, *C. leptoporus* and *E. huxleyi,* were the strongest emitters of DMS. Within the cyanobacteria*, Prochlorococcus* and *Trichodesmium* expressed no DMS emission, whereas *Synechococcus* showed very low DMS emission. The DMS emission of *C. leptoporus,* however, was several orders of magnitude higher than all other algae, including *E. huxleyi,* which is known to be a prolific emitter of DMS.

Dimethylsulfide (DMS, CH_3SCH_3) is an important volatile organosulfur present in the atmospheric marine boundary layer and has a strong marine phytoplankton source (Keller et al. 1989; Liss et al. 1997). DMS is known to be produced by enzymatic cleavage of β-dimethylsulfoniopropionate (DMSP), which is abundant in marine plankton (Challenger 1951; Ackman et al. 1966). Oxidation of DMS in the atmosphere, primarily by the hydroxyl radical (OH^-), leads to sulfur dioxide (SO_2) and, subsequently, sulfuric acid (H_2SO_4). This acid profoundly affects particle nucleation and growth over the oceans, and, therefore, has the potential to impact the global radiative balance and climate (Lovelock et al. 1972; Charlson et al. 1987; Andreae 1990). Approximately 15-25 Tg sulfur are estimated to be emitted from the global oceans each year, mostly in the form of DMS (Seinfeld and Pandis 1998).

To investigate the role of DMS at the ocean-atmosphere interface, in detail, it is necessary to develop reliable analytical techniques. The ideal technique would permit measurement in both phases with minimum disturbance of biology and surface layer physics, so that production in the aqueous phase and oxidation in the gas phase can be investigated in parallel. Recently, measurements of DMS have been made in seawater and air using two PTR-MS systems (Williams et al. 2004). Although this method offers fast responses in the gas phase, it is not as sensitive as gas chromatographic methods, and thus, large volumes of seawater needed to be purged and the gases preconcentrated. Such procedures, which involve sparging water samples with helium, also common in gas chromatography (GC) sample preparation, may provoke artificially high emissions for the biological system.

In this study, we have tested the potential of solid-phase microextraction (SPME) for DMS measurement in plankton characterization experiments. Because the SPME method concentrates chemicals on the fiber in the headspace with minimal disturbance to the sample, this method may have consid-

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erable potential for marine research. Invented in 1989 by Pawliszyn and coworkers (Belardi and Pawliszyn 1989; Arthur and Pawliszyn 1990), SPME is an innovative, solvent-free technology that is fast, economical, sensitive, and versatile. The fiber coating removes the compounds from the sample by absorption in the case of liquid coatings or adsorption in the case of solid coatings. After the sample has been acquired, the SPME fiber is then inserted directly into the GC for desorption and analysis.

Two basic types of extractions can be performed using SPME: direct and headspace extraction (Lord and Pawliszyn 2000). In the direct extraction mode, the coated fiber is inserted into the sample medium and the analytes are transported directly to the extraction phase. To facilitate rapid extraction, some level of agitation is required to enhance transport of the analytes from the bulk of the solution to the vicinity of the fiber. For gaseous samples, natural convection and diffusion in the medium is sufficient to facilitate rapid equilibration. For aqueous matrices, more efficient agitation techniques, such as fast sample flow, rapid fiber or vial movement, stirring, or sonication are required. These actions are undertaken to reduce the effect caused by the depletion zone, which occurs close to the fiber as a result of fluid shielding and slow diffusion of analytes in liquid media.

In the headspace mode, the analytes need to be transported through a layer of air before they can reach the coating. This approach also has the advantage of protecting the fiber coating from damage by high molecular-weight species and other nonvolatile contaminants present in the liquid sample matrix, such as humic materials or proteins. This headspace mode also allows modification of the matrix, such as a change of the pH, without damaging the fiber. Concentrations derived from the same vial at equilibrium using direct and headspace sampling are identical, as long as sample and gaseous headspace volumes are the same and that calibration is performed in the same mode. This is a result of the fact that the equilibrium concentration in each phase is independent of fiber location in the sample/headspace system. The choice of sampling mode has a significant impact on extraction kinetics. In fact, the equilibration times for volatile components are shorter in the headspace SPME mode than for direct extraction under similar agitation conditions. When the fiber coating is in the headspace, the analytes are removed from the headspace first, followed by indirect extraction from the matrix. Therefore, volatile analytes are extracted faster than semi-volatile components since they are at a higher concentration in the headspace, which contributes to faster mass transport rates through the headspace.

In this work, headspace-SPME has been optimized and applied to the determination of DMS emission from eight phytoplankton species, namely, *Calcidiscus leptoporus* (strain AC365), *Emiliania huxleyi* CCMP 371*, Phaeodactylum tricornutum, Chaetoceros neogracilis* CCMP1318, *Dunaliella tertiolecta, Synechococcus* RCC 40, *Prochlorococcus* RCC 158, and *Trichodesmium* IMS 101. Four SPME fiber coatings, 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB), 65 μm carbowax-divinylbenzene (CW-DVB), and 75 μm PDMS-Carboxen (PDMS-CAR), were tested by comparing the linearity and limit of detection. Key parameters such as equilibrium and extraction times, desorption temperature and time, and headspace volumes were all optimized in this work.

Table 1. Compounds and their mixing ratios containing in the calibration gas bottle

Compounds	Mixing ratios (ppb)
Methanol	509
Acetonitrile	508
Acetaldehyde	501
cis-2-Butene	504
Acetone	510
Dimethylsulfide (DMS)	510
Isoprene	473
Methylvinylketone	484
Methylethylketone	509
Hydroxyacetone	506
Benzene	503
2-Methylfuran	505
2-Pentanone	506
Toluene	507
Dimethyldisulfide	506
trans-2-Hexenal	506
Hexanal	510
o-Xylene	507
1,3,5-Trimethylbenzene	498
1,2,4,5-Tetramethylbenzene	530
α-Pinene	472

Materials and procedures

Chemicals and standard solutions—DMS (≥99% purity) was purchased from Sigma-Aldrich (Taufkirchen, Germany). A DMS solution of 50 μM was prepared in chromatography-grade deionized water (Merck), and dilutions were made immediately afterward. Aliquots of 20 mL of each DMS solution (ranging from 0.005 nM to 50 μM) were dispensed and sealed in 40 mL vial with PTFE/Silicone septum (Supelco) and immediately subjected to SPME extraction.

Calibration of SPME fiber with gaseous DMS—To determine how much DMS is uptaken by an SPME fiber, calibrations need to be performed with representatively small amounts of DMS. A practical way to achieve both this and facilitate multiple dilutions was to make the calibration in gas phase. Therefore, a gravimetrically prepared standard gas bottle (Apel-Riemer Environmental) containing the compounds listed in Table 1 has been used for the calibration of SPME fiber. Three mass flow controllers were used to dilute the calibration gas with purified synthetic air in the range of 0.1 to 510 ppbv. The

diluted standard gas with 0.3 ppbv of DMS and several other compounds in similar concentration ranges were introduced into 2.4 L electropolished stainless steel canister. Thus a static gas volume with a particular mixing ratio was made. Insertion of the SPME needle was enabled by fitting a PTFE-coated septum to the bottle fill port. Note that the frequency of these calibrations in field measurements will depend on the stability of the detector response with time.

Algal species and culture conditions—All emission analysis experiments were carried out with batch cultures of algae provided by IFM-GEOMAR Kiel. Prior to the experiments, all cultures were kept at room temperature between 20°C and 25°C and adapted to a 12:12 hour light:dark cycle. The two coccolithophorids, *Calcidiscus leptoporus* (strain AC365, from South Atlantic off South Africa, CODENET culture collection, ALGOBANK http://www.unicaen.fr/algobank) and *Emiliania huxleyi* CCMP 371, the two diatoms *Chaetoceros neogracilis* CCMP1318 and *Phaeodactylum tricornutum* (Phaeo, originating from the Falkowski laboratory), and the chlorophyte *Dunaliella tertiolecta* (DUN, originating from the Falkowski laboratory) were grown in f/2-medium (Guillard 1975; Guillard and Ryther 1962). These last four cultures were kept axenic (bacteria-free) prior to the experiment. These cultures are constantly used in IFM-GEOMAR Kiel for various purposes and checked regularly with "Marine Broth" for contamination of bacteria. *Calcidiscus leptoporus* was not axenic; however, all CCMP1318 and CCMP 371 are axenic according to the CCMP culture collection list; and *Phaeodactylum tricornutum* and the chlorophyte *Dunaliella tertiolecta* are our home-grown axenic cultures. However, it cannot be ruled out that, in the course of the experiment, they became contaminated with ambient bacteria because the equipment used for the experiment could not be sterilized. The two cyanobacteria *Prochlorococcus* RCC 158 and *Synechococcus* RCC 40 were grown in PCR-Tu_2-medium (Rippka et al. 2000), and the nitrogen-fixing cyanobacterium *Trichodesmium* IMS 101 was kept in YBCII medium with no dissolved nitrogen source for growth (Chen et al. 1996). All cyanobacteria were not axenic. All algae were in the transition from the exponential to the stationary phase of growth. This was measured using a PhytoPAM (WALZ).

The light intensity was approximately 250 $\mu E\ s^{-1}\ m^{-2}$, and the samplings of DMS emissions were performed in the middle of the light cycle (between 10:00-14:00). Chlorophyll *a* was measured according to the following procedure: 10 mL of the cultures were filtered through GF/F filters and frozen at –20°C. Chlorophyll was extracted with 10 mL 90%-acetone, centrifuged, and the supernatant was measured fluorometrically with a Turner fluorometer according to Welschmeyer (1994).

SPME sampling procedure—A manually operated SPME holder was used throughout. Four different fiber coatings were tested: 100 μm PDMS, 65 μm PDMS-DVB, 65 μm CW-DVB, 75 μm PDMS-CAR, all from Supelco. Each new fiber was thermally conditioned before use, and its life time is a hundred extractions according to the manufacturer specifications.

DMS was extracted from standard solution or culture sample by centrally piercing the septum of the vial containing 20 mL of a given sample with the protected needle and exposing the fiber to the headspace gas 2 mm above the water for a given time. Before retracting the SPME fiber holder, the fiber was withdrawn into the protecting needle, thus preventing further gas diffusion to the fiber. All experiments were performed at 20°C and without prefiltering or agitation, so that no modification in the growing conditions of algae took place.

Blank tests—Prior to the transfer of algae to the vials, blank tests were performed with empty vials. Further blank tests were performed with vials containing only the f/2 medium, which was used to cultivate the phytoplankton species, and with filtered seawater (filtered to 0.2 μm with cellulose-acetate filter) with the same sampling conditions. The filtered water used for blanks was not sterile.

Chromatographic analysis—By following the sample collection procedure described above, DMS in the headspace of standard solutions, algae cultures, and in blank samples was examined by adsorbtion onto an SPME fiber. Immediately after extraction, the needle was introduced into the split/splitless injector of the gas chromatograph. A glass inlet liner with a narrow internal diameter (0.75 mm i.d., Supelco) was used to improve the GC resolution and the peak shape. Desorption was achieved in splitless mode at 250°C for 2 min.

Analyses were conducted using a gas chromatograph (Agilent Technologies a GC 6890A) coupled to a mass selective detector (MSD 5973 *inert*) from the same company. The MSD with an electron impact source running in SIM mode (monitored m/z was 62 for DMS) was operated with the following conditions: potential ionization 70 eV and source temperature 230°C. The method developed here can, however, also be applied to other GC detector systems (e.g., GC-FPD or GC-PFPD). The DMS peak was separated using a β-cylodextrin capillary column (CYCLODEX-B, 30 m long, 0.256 mm ID, 0.25 μm film thickness) supplied by J & W Scientific, initially installed for the separation of enantiomeric monoterpenes emitted by plants collected by SPME (Yassaa and Williams 2005). Following the introduction of the SPME needle to the GC injector and the analytes entering the column, the column temperature was maintained at 40°C for 5 min, then increased to 200°C at 1.5°C per minute, and finally held at this temperature for 5 min. With a helium (Messer Griesheim 6.0) gas carrier flow rate of 1 mL min^{-1}, the retention of DMS in the chromatogram was 1.458 min.

Results and discussion

Comparison of different fiber coatings—Four widely used SPME fibers, PDMS (100 μm), PDMS-DVB (65 μm), PDMS-CAR (75 μm), and CW-DVB (65 μm), were tested in this work. The limit of detection (LOD) for each fiber was determined by exposing it to the headspace above water spiked with a known amount of DMS and sequentially decreasing this amount until a signal-to-noise ratio of 3 was reached. Extraction was performed for 1 min in several 40 mL vials containing 20 mL of

Table 2. Linearity and limits of detection (LOD) of DMS in four SPME fiber coatings

Fiber coatings	r^2	LOD (nM)
PDMS	0.994 (from 50 nM to 5 μM)	5
CW-DVB	0.992 (from 50 nM to 5 μM)	3
PDMS-DVB	0.996 (from 0.05 nM to 5 μM)	0.005
PDMS-CAR	0.997 (from 0.05 nM to 5 μM)	0.003

each DMS solution. Triplicate injections were performed for all experiments. Table 2 reports the LOD, the linear ranges, and square of the correlation coefficients (r^2) obtained for each fiber. The limit of detection of SPME used to determine DMS in the headspace above the water heavily relies on the amount of analyte adsorbed by coating on the fiber and the sensitivity of the GC-MS. The PDMS fiber was found to be the least efficient, leading to an average detection limit of 5 nM. This fiber has already been shown to extract sulfur compounds poorly (see, for example, Haberhauer-Troyer et al. 1999). It is, therefore, not particularly suitable for the analysis of sulfur compounds at trace levels. The same is also true for CW-DVB, where the average of LOD was in the same range (3 nM). As has been demonstrated in previous studies (Lestremau et al. 2004 and references therein), the use of the PDMS-CAR fiber achieved better sensitivity than the DVB-PDMS fiber, and the detection limit of DMS using PDMS-DVB fiber (0.005 nM) was close to that of PDMS-CAR fiber (Table 1). Lestremau et al. (2003) reported similar LODs for DMS in the gas phase using both SPME fibers in conjuction with gas chromatography–pulsed flame photometric detection (0.09 μg m^{-3}). However, several limitations were observed with PDMS-CAR fiber concerning the decomposition or reaction of analytes in the GC injection port (Lestremau et al. 2004 and references therein). For example, oxidation of dimethylsulfide to dimethylsulfoxide was observed (Lestremau et al. 2004). It was also found that, in contrast to PDMS-CAR, the PDMS-DVB generates no significant artefact formation during air analysis of volatile amines (Lestremau et al. 2001). Therefore, based on its good limit of detection and low propensity for artefact formation, the PDMS-DVB was chosen for the rest of experiments. No evidence of dimethylsulfoxide was observed in these analyses. The linearity of the method for a wide range of concentrations (from 0.05 nM to 5 μM) was sufficient to conduct quantitative evaluation ($r^2 \sim 0.996$ for PDMS-DVB).

Optimization of equilibrium and sampling time—To determine the partition equilibrium time of DMS between the water and the headspace, SPME extractions were taken at different times (0 to 30 min) immediately after the preparation of DMS solutions at different concentrations (0.5, 5, 50, and 500 nM). The PDMS-DVB fiber was exposed for 5 min to the headspace above each 20 mL DMS solution dispensed in a 40 mL vial and for each equilibrium period.

As shown in Fig. 1, the recovery was greater than 90% at an equilibrium time longer than 10 min for the range of concentrations studied (0.5 to 500 nM), suggesting that 10 min of equilibration is a practical condition for analysis. This behavior is due to the high volatility of DMS, which is quickly transferred from the liquid to the gas phase. The same equilibrium time was found by Niki et al. (2004). Further studies were performed using this 10 min equilibration time.

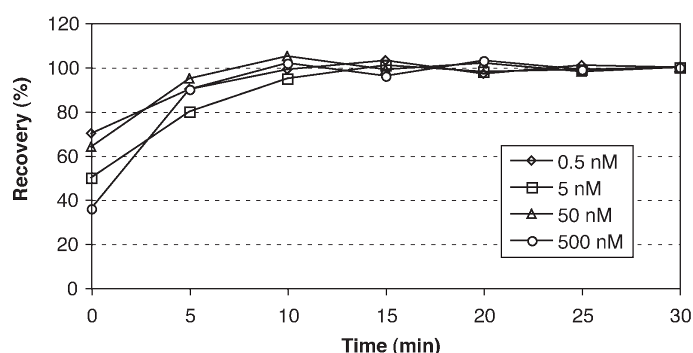

Fig. 1. Percentage recoveries of DMS versus partition equilibrium time (from 0 to 30 min). The peak area counts for 30 min were set as 100%.

The second step was to evaluate the fraction of DMS, which is extracted from the headspace by the SPME fiber versus sampling time. Note the “extraction equilibrium time” refers to the time of equilibration on the fiber. This is distinct from the previously determined “equilibrium time,” which is the time for the DMS partition equilibrium between the gas and liquid phases. This has been achieved using a gas phase DMS standard contained in a canister and the headspace of a DMS/water solution. (1) PDMS-DVB fiber was inserted via a septum through a 2.4 L stainless steel electropolished canister filled with calibration gas containing 0.3 ppbv of DMS. The fiber was exposed for various periods ranging from 1 to 60 min. The maximum peak area count of DMS was obtained for a 5 min exposure time, and this was assumed to represent 100% recovery (see Fig. 2). (2) PDMS-DVB fiber was exposed to the headspace of the DMS/water solution (5 nM) for different periods of time (5 s to 90 min). For each

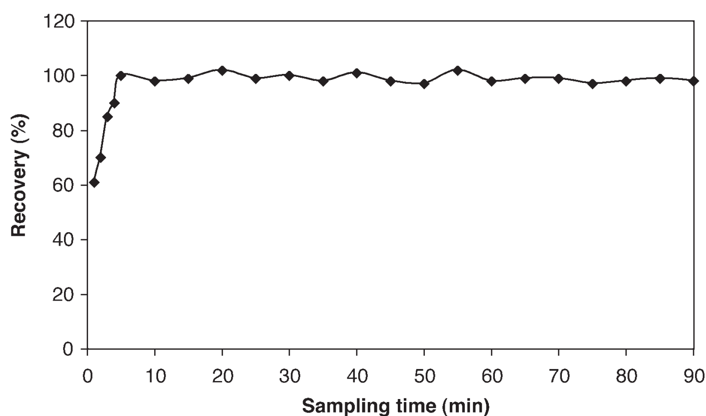

Fig. 2. Percentage recoveries of DMS in gas phase versus extraction time (from 1 to 60 min). The peak area count for 5 min was set as 100%.

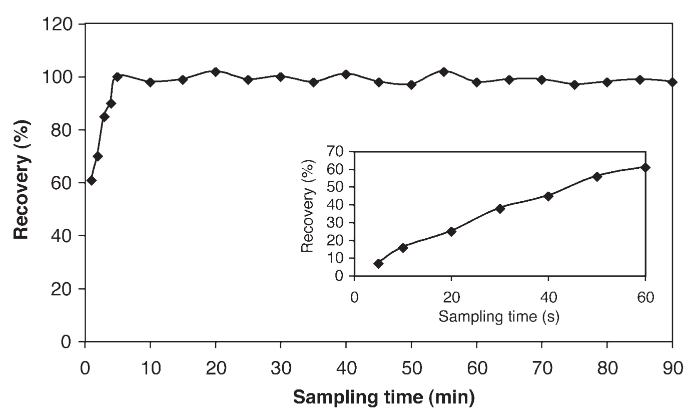

Fig. 3. Percentage recoveries of DMS in the headspace above water versus extraction time (from 5 s to 90 min). The peak area count for 5 min was set as 100%.

exposure time, triplicate SPME extractions were performed on a given mixture. As the maximum peak area count of DMS was also obtained for a 5 min exposure time (the extraction equilibrium time), it was set as 100% of recovery (see Fig. 3). The extraction equilibrium process in both cases is similar (ca. 5 min) and fast. This result confirms also that in 10 min equilibration, DMS has partitioned completely between the phases.

Due to the small volume of the SPME coating, the limited number of adsorption sites on the fiber can, under certain circumstances, become saturated. This leads to competitive adsorption between low-molecular-mass sulfur compounds and other compounds with high affinity for the sorbent. Under these conditions, accurate quantification is difficult, particularly when complex matrices are studied. To prevent this phenomenon, the amount of adsorbed molecules needs to be maintained at a low level. This can be easily achieved by decreasing the exposure time. The use of a mixed gas standard containing 21 components having different physical properties listed in Table 1 has shown that under 1 min SPME fiber exposure time, the competition for active sites was negligible. Therefore, the extraction time for subsequent analyses was fixed under non-equilibrium conditions using a 1 min exposure time. Competitive adsorption was thus avoided in the subsequent culture samples.

Effect of headspace volume—According to SPME theory, the equilibration time can be reduced by using a smaller volume headspace because the analytes will take less time to diffuse throughout the headspace (Yang and Peppard 1994). In this study, the effect of the water sample and the headspace volumes were studied to optimize the extraction procedure of DMS. This was performed using 1, 5, 10, 15, and 20 mL of 5 nM DMS solution in 40 mL vials. The extraction time was 1 min at 20°C. The results clearly showed that the sample volume affects the recovery of DMS (Fig. 4). The recovery increases as the headspace volume decreases. No more than 20 mL of sample was used so that the fiber did not touch the liquid. Further experiments were performed using a 20 mL water sample.

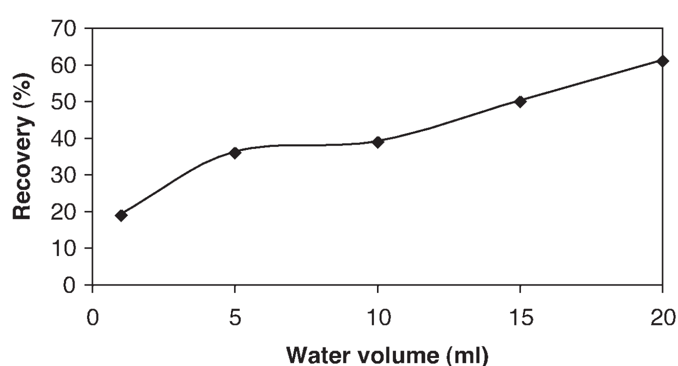

Fig. 4. Effect of solution volume on percentage recoveries of DMS in the headspace.

Desorption time and desorption temperature—Desorption time and desorption temperature determine the amount of analytes desorbed from the fiber coating. For this experimental part, a PDMS-DVB fiber was exposed to the headspace of 5 nM DMS solution for 1 min. Desorption time was investigated within a range of 0.3 min to 5 min by leaving the fiber in the injection port for an increased period and maintaining the temperature of the injector at 250°C. The desorption of DMS increased with desorption time and reached maximum after 2 min. The desorption temperature ranged from 200°C to 270°C. According to the results, the peak area of DMS increased slightly with the desorption temperature. DMS exhibited complete desorption at a temperature of 250°C without any carryover. This was confirmed by heating the same fiber for a second time after the initial desorption. To avoid any artefact formation at high temperatures, a 250°C desorption temperature and a 2 min desorption time were used in all experiments.

Repeatability and reproducibility—Repeatability was evaluated for one PDMS-DVB fiber through five independent extractions (1 min exposure time) of a model DMS concentration (5 nM). Relative standard deviation (RSD) determined from the peak area was generally within an acceptable level (8%). Day-to-day repeatability ranged from 5% to 12%.

To assess the reproducibility of the measurement with different SPME fibers, three new PDMS-DVB fibers were compared. Fig. 5 shows the comparison between the three fibers for the extraction of DMS in the headspace above the water at 5 nM concentration and during 1 min exposure time. Considerable differences were observed in the DMS relative peak area for the three fibers. As a result, the overall experiments should be performed with a single fiber and, if more than one is used, the calibration graphs must be recalculated for each fiber. This is especially important for field sampling. The manufacturer of the SPME fibers recommends that they are suitable for circa 100 injections. Thus, it is likely that several be used in the course of an intensive measurement period. Regular calibration is essential. It should be noted that contact with seawater does not damage the fiber. Indeed, as stated in the introduction, the compound of interest can be determined in either

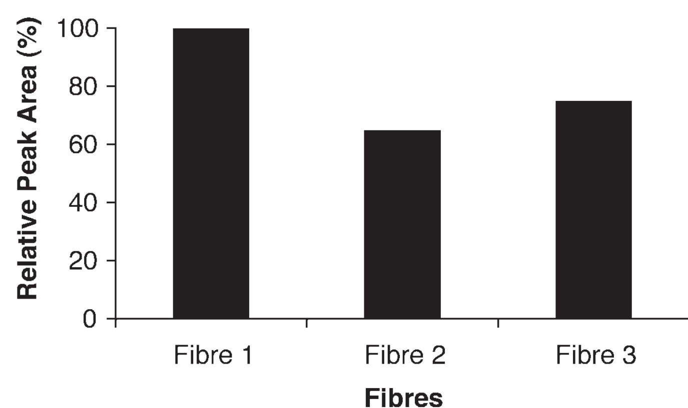

Fig. 5. Comparison of the performance of three new PDMS-DVB fibers. The peak area count of fiber 1 was set as 100%.

the aqueous (direct) or in the gas phase (indirect). However, if headspace SPME were to be employed at sea then a gimbled sample stand would be required to hold the vial for the 1 min extraction time. Since the extraction time is 1 min and the GC/MS run including desorption is 2 min (the DMS peak appears at 1.458 min), and the SPME fiber does not need to be conditioned after the GC run, a measurement frequency of about 1 sample per 5 min is quite feasible. Storage of SPME samples for DMS measurements has been reported (Sakamoto et al. 2006), however, it is recommended that analyses occur immediately to avoid any potential storage artefacts.

HS-SPME-GC-MS of phytoplankton emissions

Blank tests. The GC/MS analysis of the blank samples in (1) empty glass bottle, (2) plankton-free media, and (3) filtered seawater resulted in no detection of DMS. These experiments indicate that glass materials, the media, and the seawater are not a source of artefacts that can affect our results.

Comparison of DMS emission from eight phytoplankton species. The effectiveness of the proposed method in determining DMS in real samples was tested by analyzing the emissions of eight phytoplankton species: *C. leptoporus*, *E. huxleyi, P. tricornutum, C. neogracilis*, *D. tertiolecta, Synechococcus*, *Prochlorococcus*, and *Trichodesmium.* The HS-SPME was operated under the optimum determined conditions using PDMS-DVB fiber coating and 1 min exposure time. Triplicate analyses were performed for each single culture. A comparison of DMS emission by the eight algae species tested and the different biomass per unit volume of each sample measured through chlorophyll are given in Table 3. The *C. leptoporus* was by far the strongest emitter of DMS followed by *E. huxleyi*. Two species, namely, *Prochlorococcus* and *Trichodesmium* do not emit DMS significantly. These results suggest that the enzyme activity leading to DMS production is more significant in *C. leptoporus* and, to a lesser extent, in *E. huxleyi* than in the other phytoplankton species studied here.

The DMS emissions reported here are generally in agreement with previous studies. The two cyanobacteria *Prochlorococcus* and *Trichodesmium* measured show no detectable DMS emission while *Synechococcus* revealed very low DMS emission. Within the group of haptophytes, besides *Phaeocystis sp.* (Stefels and Van Boekel 1993), coccolithophorids are generally known to have a high intercellular DMSP content and a high DMSP lyase activity (Steinke et al. 2000; Steinke et al. 1998). This is corroborated by the current results, where coccolithophorids have, from one to several orders of magnitude higher DMS emission compared with all other investigated algae. The extremely high DMS emission of *C. leptoporus* found here has, to our knowledge, not been described earlier. *C. leptoporus* is a cosmopolitan species (Mcintyre and Be 1967) with a fossil record dating back to the Early Miocene (Young 1998). Although *C. leptoporus* never reaches high abundances in surface waters, it can dominate coccolith assemblages in sediments since it is resistant to dissolution (Mcintyre and Mcintyre 1970). Although sometimes low in abundance, *C. leptoporus* can have a high contribution (up to 50%) to the coccolithophorid carbon biomass (Steinke et al. 2002). In the aforementioned paper, Steinke et al. (2002) found a mismatch between high DMSP lyase activity and the abundance of *Emiliania huxleyi*, assumed to be the extreme DMS producer. They concluded that dinoflagellates must be responsible for the high DMS production. Here we argue that possibly the extremely high DMS production of *C. leptoporus* might explain the mismatch of the DMS emission. Although we cannot

Table 3. Comparison of DMS emission by eight phytoplankton species

Phytoplankton species	Chlorophyll *a* (μg L^{-1})	Concentrations (nM)	Concentrations/Chl *a*
Calcidiscus leptoporus	87	2134	24.528
Emiliania huxleyi	87	67	0.770
Phaeodactylum tricornutum	683	12	0.018
Chaetoceros neogracilis	134	1.58	0.012
Dunaliella tertiolecta	432	3.15	0.007
Synechococcus	164	4.73	0.028
Prochlorococcus	30	n.d.*	
Trichodesmium	50	n.d.*	

*Not detected.

exclude that bacteria might have affected our results, and moreover, we did not measure intercellular DMSP content and potential DMSP lyase activity, we conclude that *C. leptoporus* must have a high DMSP content per cell and a high DMSP lyase activity to produce the high concentrations of DMS observed during this experiment. Because an acrylate is produced with each DMS that might have a protection role in the microlayer surrounding a cell wall (Sieburth 1960), the high DMS concentrations produced by *C. leptoporus* might also explain the high preservation rate observed in sediments (McIntyre and McIntyre 1970). Future experiments, based on the experimental techniques developed here, should verify the current results and aim to quantify the emissions as a function of environmental parameters (light and nutrients) temperature, in particular for the apparently prodigious DMS producer *C. leptoporus*.

Conclusion

The suitability of SPME-GC-MS for the extraction of DMS in the headspace above seawater has been studied in this work. Using 65 µm PDMS-DVB fiber coating, the limit of detection was 0.005 nM, the optimum water volume was 20 mL in 40 mL vial, the equilibrium time between the water and gas phase was 10 min, the extraction equilibrium time was 5 min, and the optimum desorption temperature and time were 250°C and 2 min, respectively. In order to avoid competitive adsorption, the extraction time for subsequent analyses was fixed under nonequilibrium conditions using a 1 min exposure time.

The headspace SPME-GC-MS, combined with PDMS-DVB fiber, is a suitable technique for qualitative and quantitative determination of DMS emitted from phytoplankton species, with minimum disturbance to the investigated biology. Among the eight phytoplankton species studied, namely, *C. leptoporus*, *E. huxleyi*, *P. tricornutum, C. neogracilis*, *D. tertiolecta, Synechococcus*, *Prochlorococcus,* and *Trichodesmiu, C. leptoporus*, was by far the strongest emitter of DMS. Further experiments using this method with multiple cultures of *C. leptoporus* are needed to fully understand the emission profile of this species as a function of temperature, growth curves, cell counts, cell integrity, DMSP content, and DMSP lyase activity.

Compared to the commonly used techniques, HS-SPME coupled to GC/MS appears to be simple, fast, sensitive, and nondestructive technique, with potential for laboratory-based studies of plankton emissions as well as for shipboard measurements of DMS. For the assessment of DMS fluxes in the field the water concentrations determined by the above method would have to be combined with ambient air measurements. This could be achieved by using a portable dynamic SPME sampler (as described by Yassaa and Williams 2005). Furthermore, the applicability of SPME to DMS measurements suggests that the technique may be also used to measure other less abundant organic species present in the marine boundary layer.

References

Ackman, R. G., C. S. Tocher, and J. Mac Lachlan. 1996. Occurence of dimethyl-β-propiothetin in marine phytoplankton. J. Fish Res. Board Can. 23:357-364.

Andreae, M. O. 1990. Ocean-atmosphere interactions in the global biogeochemical sulfur cycle. Mar. Chem. 30:1-29.

Arthur, C. L., and J. Pawliszyn. 1990. Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal. Chem. 62:2145-2148.

Belardi, R. P., and J. Pawliszyn. 1989. The application of chemically modified fused silica fibers in the extraction of organics from water matrix samples and their rapid transfer to capillary columns. Water Poll. Res. J. 24:179-191.

Challenger, F. 1951. Biological methylation. Adv. Enzymol. 12:429-491.

Charlson, R. J., J. E. Lovelock, M. O. Andrea, and S. G. Warren. 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326:655-661.

Chen, Y. B., J. P. Zehr, and M. Mellon. 1996. Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp IMS 101 in defined media: Evidence for a circadian rhythm. J. Phycol. 32: 916-923.

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 26-60. *In* W. L. Smith and M. H. Chanley, [eds.] Culture of marine invertebrate animals. Plenum Press.

Guillard, R. R. L., and J. H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Can. J. Microbiol. 8:229-239.

Habehauer-Troyer, C., E. Rosenberg, and M. Grasserbauer. 1999. Evaluation of solid-phase microextraction fro sampling of volatile organic sulfur compounds in air for subsequent gas chromatographic analysis with atomic emission detection. J. Chromatogr. A 848:305-315.

Keller, M. D., W. K. Bellows, and R. R. L. Guillard. 1989. Dimethyl sulfide production in marine-phytoplankton, p. 167-182. *In* E. S. Saltzmann and W. J. Cooper [eds.], Biogenic sulfur in the environment. American Chemical Society.

Lestremau, F., V. Desauziers, and J. -L Fanlo. 2001. Formation of artefacts during analysis of volatile amines by solid-phase microextraction. Analyst 126:1969-1973.

Lestremau, F., V. Desauziers, J. -C. Roux, and J. -L. Fanlo. 2003. Development of a quantification method for the analysis of malodorous sulphur compounds in gaseous industrial effluents by solid-phase microextraction and gas chromatography-pulsed flame photometric detection. J. Chromatogr. A 999:71-80.

Lestremau, F., F. A. T. Andersson, and V. Desauziers. 2004. Investigation of artefact formation during analysis of volatile sulphur compounds using solid phase mircoextraction (SPME). Chromatographia 59:607-613.

Liss, P. S., A. D. Hatton, G. Malin, P. D. Nightingale, and S. M. Turner. 1997. Marine sulphur emissions. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 352:159-168.

Lord, H., and J. Pawliszyn. 2000. Evolution of solid-phase microextraction technology. J. Chromatogr. A 885:153-193.

Lovelock, J. E., R. J. Maggs, and R. A. Rasmussen. 1972. Atmospheric dimethylsulfide and the natural sulphur cycle. Nature 237:452-453.

McIntyre, A., and A. W. H. Be. 1967. Modern coccolithophoridae of Atlantic Ocean, I. Placolith and Cyrcoliths. Deep-Sea Res. 14:561-597.

McIntyre, A., and R. McIntyre. 1970. Coccolith concentration and differential solution in ocenanic sediments, p. 253-261. *In* B. M. Funnel and W. R. Riedel [eds.], Micropaleontology of the oceans. Cambridge Univ. Press.

Niki, T., T. Fujinaga, M. F. Watanabe, and J. Kinoshita. 2004. Simple determination of dimethylsulfide (DMS) and dimethylsulfoniopropionate (DMSP) using solid-phase microextraction and gas chromatography-mass spectrometry. J. Oceanogr. 60:913-917.

Rippka, R., et al. 2000. *Prochlorococcus marinus* subsp pastoris subsp nov strain PCC 9511, the first axenic chlorophyll *a* (2)/*b* (2)-containing cyanobacterium (*Oxyphotobacteria*). Inter. J. System. Evol. Microbiol. 50:1833-1847.

Sakamoto, A., T. Niki, and Y. W. Watanabe. 2006. Establishment of lon-term preservation for dimethyl sulfide by the solid-phase microextraction method. Anal. Chem. 78: 4593-4597.

Seinfeld, J. H., and S. N. Pandis. 1998. Atmospheric chemistry and physics: from air pollution to climate change. Wiley.

Sieburth, J. M. 1960. Acrilic acid, an "antibiotic" principle in *Phaeocystis* blooms in Antarctic waters. Science 132: 676-677.

Stefels, J., and W.H.M. Van Boekel. 1993. Production of DMS from Dissolved DMSP in Axenic Cultures of the Marine-Phytoplankton Species *Phaeocystis* sp. Mar. Ecol.-Progr. Ser. 97:11-18.

Steinke, M., G. Malin, S. D. Archer, P. H. Burkill, and P. S. Liss. 2002. DMS production in a coccolithophorid bloom: evidence for the importance of dinoflagellate DMSP lyases. Aquat. Microb. Ecol. 26:259-270.

Steinke, M., G. Malin, S. M. Turner, and P. S. Liss. 2000. Determinations of dimethylsulphoniopropionate (DMSP) lyase activity using headspace analysis of dimethylsulphide (DMS). J. Sea Res. 43:233-244.

Steinke, M., G. V. Wolfe, and G. O. Kirst. 1998. Partial characterisation of dimethylsulfoniopropionate (DMSP) lyase isozymes in 6 strains of *Emiliania huxleyi*. Mar. Ecol. Prog. Ser.175:215-225.

Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. 39:1985-1992.

Williams, J., R. Holzinger, V. Gros, X. Xu, E. Atlas, and D. W. R. Wallace. 2004. Measurements of organic species in air and seawater from the tropical Atlantic. Geophys. Res. Let. 31, L23S06, doi:10.1029/2004GL020012.

Yang, X., and T. Preppard. 1994. Solid-phase microextraction for flavor analysis. J. Agr. Food Chem. 42:1925-1930.

Yassaa, N., and J. Williams. 2005. Analysis of the enantiomeric composition of monoterpenes in plant emissions using portable dynamic sample/SPME and chiral gas chromatography/mass spectrometry. Atmos. Environ. 39: 4875-4884.

Young, J. R. 1998. Neogene, p. 225-265. *In* P. R. Brown [ed.], Calcareous nanofossils bioistratigraphy. Chapmann & Hall.

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