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# DMSP-lyase activity in a spring phytoplankton bloom off the Dutch coast, related to *Phaeocystis* sp. abundance

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ABSTRACT: An enzyme assay for measuring dimethylsulfoniopropionate (DMSP)-lyase activity was applied to natural sea water samples taken during the 1993 spring bloom off the Dutch coast. When relating the measured DMSP-lyase activity to the most abundant algal species found during the cruise, a significant correlation with *Phaeocystis* sp. numbers ($r^2 = 0.9660$, $n = 23$) was observed, but not with any of the other species present, or with total diatom numbers, total diatom biovolume or total protein. The calculated dimethyl sulfide (DMS) production by *Phaeocystis* sp. in a typical spring bloom off the Dutch coast appeared to be 1.5 to 4.5 times higher than the DMS loss due to flux to the atmosphere and photochemical oxidation. The results indicate that the alga *Phaeocystis* sp. has a very active DMSP-lyase, specific for this species, that was potentially responsible for the conversion of DMSP to DMS in the area investigated, leaving 35 to 75% of DMS for bacterial consumption.

KEY WORDS: Dimethylsulfide · Dimethylsulfoniopropionate · DMSP-lyase · *Phaeocystis* sp.

## INTRODUCTION

The volatile organosulfur compound dimethylsulfide (DMS) has generated much interest in the past decades because of its possible role in the biological regulation of the climate (Charlson et al. 1987, Andreae 1990, Fitzgerald 1991, Malin et al. 1992, Lawrence 1993, Charlson & Wigley 1994, Malin et al. 1994). Oceans are the main source of DMS to the atmosphere. In the atmosphere, DMS is oxidized to form aerosols, thereby influencing cloud albedo and subsequently climate. In sea water, DMS is produced from dimethylsulfoniopropionate (DMSP), a compound that is found in several marine micro- and macroalgae (Reed 1983, Keller et al. 1989, Blunden et al. 1992), and is suggested to serve mainly as an osmoprotectant (Vairavamurthy et al. 1985, Dickson & Kirst 1987a, b). Conversion of DMSP into DMS and acrylic acid is thought to take place after its release from the cells, when part of the dissolved DMSP is cleaved through enzymatic activity. DMSP-lyase activity has been found in crude extracts of the macrophyte *Polysiphonia lanosa* (Cantoni & Anderson 1956) and of the heterotrophic dinoflagellate *Gyrodinium cohnii* (Ishida 1968), and more recently also in whole cells of axenic *Phaeocystis* sp. cultures (Stefels & van Boekel 1993). The bulk enzymatic conversion of DMSP to DMS in natural sea water samples has, until now, been attributed to bacterial lyase activity (Kiene 1990, 1992, Kiene & Service 1991). Zooplankton has also been suggested to be directly or indirectly involved in the conversion of DMSP to DMS and acrylate (Dacey & Wakeham 1986, Leck et al. 1990). In the latter 2 studies, the possibility could not be excluded that zooplankton only played an intermediate role by releasing DMSP from the algae grazed upon due to sloppy feeding or digestion, making DMSP available for bacterial or algal enzymes. Wolfe et al. (1994) reported on the impact of grazing by a herbivorous dinoflagellate on the fate of intracellular DMSP of *Emiliania huxleyi*. They found that 30 to 70% of the lost algal DMSP could not be traced back in the form of dissolved DMSP or DMS. An alternative pathway for DMSP decomposition has been postulated by Kiene & Service (1991). In samples from estuarine waters off Georgia, USA, they found that only 28% of the DMSP consumed could be traced back as DMS; demethyla-

tion was suggested to be an alternative route for DMSP metabolism. These authors also suggested a close coupling between the production and consumption of DMS. Kiene & Bates (1990) found that microbial DMS consumption was generally 10 times faster than the flux of DMS to the atmosphere.

Although attempts to quantify the processes mentioned above have been scarce, with today's knowledge it is tempting to believe that, on a global scale, only a small part of the DMSP-sulfur will ever reach the atmosphere (Matrai & Keller 1993, Bates et al. 1994). On the other hand, the relative magnitude of the processes involved may differ greatly, depending on the composition of the plankton assemblage (including bacteria, phytoplankton and zooplankton), which, in its turn, differs from season to season and from place to place. This seasonality and locality of the processes involved can temporarily result in relatively large fluxes of DMS compared to the DMSP concentration in the water. Therefore, the possibility that blooms of specific algal species provide the main part of the DMS flux to the atmosphere in a short time span is a phenomenon worth investigating.

An interesting species in this respect is *Phaeocystis* sp. This colony-forming alga contains large amounts of DMSP (Keller et al. 1989, Stefels & van Boekel 1993), is known to form excessive blooms in temperate as well as polar regions (Cadée & Hegeman 1986, Smith et al. 1991, Davidson & Marchant 1992), and has gained interest in DMS studies (Barnard et al. 1984, Gibson et al. 1990, Baumann et al. 1994, Liss et al. 1994). *Phaeocystis* sp. is one of the species which benefits strongly from eutrophication in the North Sea (Lancelot et al. 1987, Riegman et al. 1992). Stefels & van Boekel (1993) showed that *Phaeocystis* sp. is able to convert dissolved DMSP enzymatically, and that this enzyme activity is approximately in the same range as bacterial production of DMS from DMSP, reported in the literature. In their study the possibility that DMSP conversion was a secondary effect of an enzyme not specific to DMSP nor specific for *Phaeocystis* sp. could not be excluded, and the actual contribution of *Phaeocystis* sp. to DMS production in the field therefore remained to be investigated.

The objective of the present study was to investigate whether there is a relationship between the potential enzymatic conversion of DMSP to DMS in natural waters and the presence of *Phaeocystis* sp. or any other algal species. To this end, a DMSP-lyase enzyme assay was applied to natural sea water samples off the Dutch coast, taken during the spring bloom of 1993. The potential contribution of *Phaeocystis* sp. to DMS production was calculated and compared to the main abiotic loss factors, fluxes of DMS to the atmosphere and photochemical oxidation.

## MATERIALS AND METHODS

**Sample collection.** During the spring phytoplankton bloom of 1993, surface water samples were taken with a bucket off the Dutch coast from aboard the RV 'Pelagia' (Fig. 1). In order to relate DMSP-lyase activity to specific groups in the plankton, the plankton assemblage was separated into size fractions. The particulate matter of 350 to 1000 ml was fractionated and concentrated qualitatively. To prevent disruption of cells the >100 µm fraction was gently separated and concentrated using a 100 µm mesh sieve in the bottom of a plastic beaker. The <100 µm fraction was run — under gravity only — through a cascade of tangential reversed-flow filters using polycarbonate filters with pore diameters of 10, 2 and 0.2 µm respectively (Fig. 2). Flow rates were 10 to 15 ml $min^{-1}$. The total filtration time never exceeded 1 h. After filtration, the different fractions were gently shaken, to resuspend part of the cells that had stuck to the filter, and tapped from the chambers underneath the filters; volumes of the fractions were 25 to 30 ml. Of all fractions 2 ml subsamples were taken for cell counts, fixed with 1% buffered formaldehyde and stored at 4°C. To the remaining fraction 1 ml 100 mM Tris/HCl buffer (pH 8) with 125 mM dithiothreitol (DTT) was added and stored at −80°C for further analyses in the laboratory. Storage always resulted in loss of activity, but experiments showed that storage at −80°C gave 2 times higher DMSP-lyase activity than storage at −20°C. An aliquot of the <0.2 µm fraction was taken for analyses

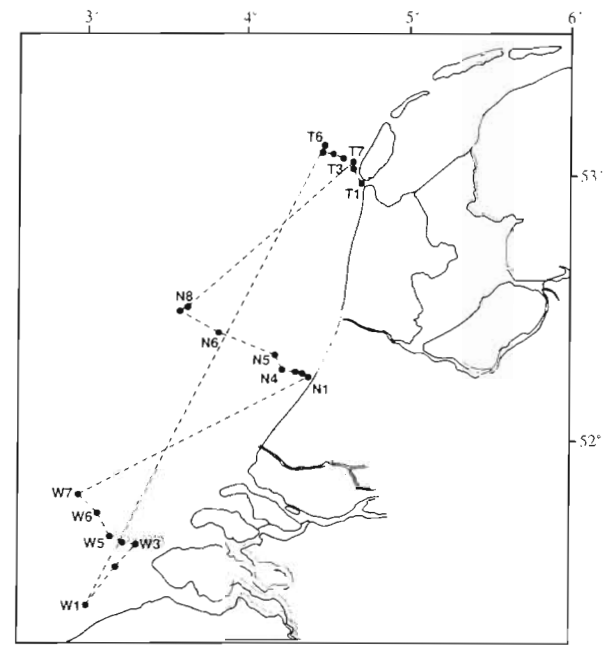


Fig. 1. Locations of the sampling stations along the cruise track of RV 'Pelagia', 13 to 16 April 1993

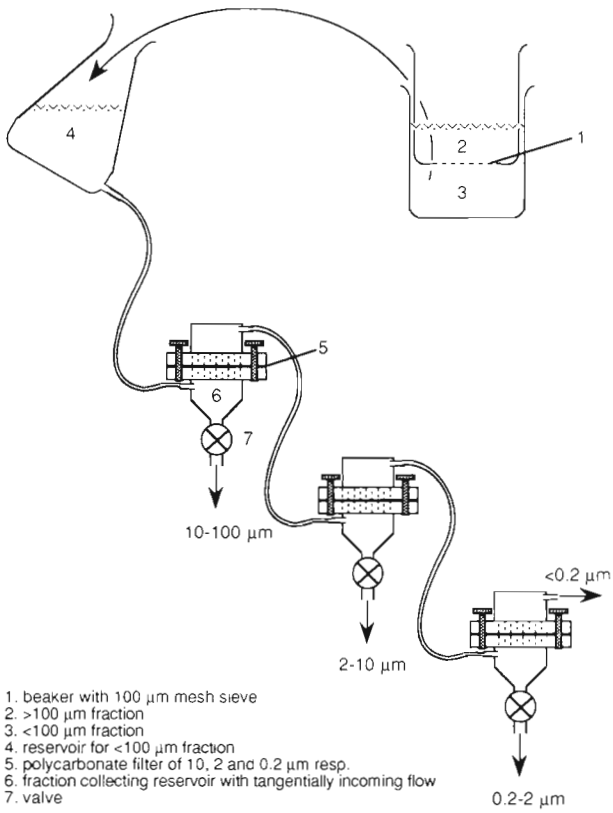


Fig. 2. Fractionation and concentration device for sea water samples, showing a cascade of tangential, reversed flow filters

of the dissolved DMSP + DMS [DMS(P)] concentration. To this end, a 4 ml sample was stored in a 14 ml vial, 0.4 ml 10 N NaOH was added, the vial was quickly closed with teflon-lined crimp-cap seals and stored at 4°C. Headspace analysis of the evolved DMS was performed in the laboratory within 1 wk. No loss was detected from standards stored for more than 1 wk.

**DMSP-lyase assay.** A DMSP-lyase assay was applied to the particulate matter of samples taken on board. In the laboratory the deeply frozen fractions were thawed and kept on ice as much as possible during extract preparation. The buffer used for the preparation of the extract and the assay was a 50 mM Tris buffer (pH 8.5) with 5 mM DTT. Particulate matter was harvested by centrifugation (15 min at 3300 × *g*), resuspended in 1 ml Tris/DTT buffer, and disrupted with a French pressure cell at 130 MPa. Depending on the expected DMSP-lyase activity during the assay, 50 to 340 µl of the crude extract was diluted with Tris/DTT-buffer. The enzyme substrate DMSP·HCl was added from a 10 mM standard, that was accurately 'preneutralised' with 5 mM NaOH, giving an end concentration of 250 µM. Total volumes were 4.5 ml in 14 ml vials. DMS production was measured in the headspace (see below). After the assay, samples were checked for pH. In each series control samples for abiotic DMS production were included, containing the same buffer/substrate system but replacing the extract for buffer. Samples were regularly subjected to dilution series, to check for undersaturation or inhibition; DMS production was always linearly related with extract volume. The DMSP.HCl was prepared according to Chambers et al. (1987), and checked for purity with nuclear magnetic resonance (NMR) and by comparison to DMS standards after alkaline conversion to DMS.

**DMS analysis.** All assays were performed in 14 ml vials stoppered with a teflon Mininert valve. Vials were placed in the dark at 20°C in a constant-temperature water bath. During 60 min, with 15 min intervals, DMS evolution was measured in the headspace using a Varian 3600 gas chromatograph equipped with a sulfur-specific Hall Electrolytic Conductivity Detector (Stefels & van Boekel 1993). DMS production proved to be linear during this time period. A calibration curve was prepared from DMSP standards in seawater to which 10 N NaOH was added (final concentration 1 M), giving equimolar conversion of DMSP into DMS and acrylic acid (White 1982, Dacey & Blough 1987). Working standards were stored under the same conditions as assay samples.

**Protein analysis.** Protein concentrations were measured in 200 to 400 µl aliquots of the crude extracts according to the Bio-Rad protein assay, after treatment with boiling NaOH (0.5 M) for 15 min and subsequent centrifugation, using bovine serum albumin (BSA) as standard.

**Cell counts.** Cell numbers in the different fractions were counted with an inverted microscope after sedimentation of aliquots in micro plates, with magnifications up to 600×. Biovolumes of the different species were computed with specially developed software, in which for each species an approximation of the form and mean size is given. Sizes are adjustable for each sample. For diatoms an approximation of the biovolume was calculated by assuming a mean plasma layer of 1 µm between the cell membrane and the vacuole (Edler 1979), giving a mean biovolume of 30% of the total cell volume.

## RESULTS

During the cruise the northern and offshore stations were dominated by *Phaeocystis* sp., whereas mainly diatom species were found in the southern and central coastal regions. This reflects a typical spring phytoplankton bloom off the Dutch coast, where an early diatom bloom is always followed by a *Phaeocystis* sp. bloom (Gieskes & Kraay 1975).

From the samples taken, the >100 µm fraction mainly retained *Phaeocystis* sp. colonies and the larger and part of the medium-sized diatom species. Although no fluorescent staining was performed on the samples, microscopy showed that this fraction was without large numbers of bacteria. The 10 to 100 µm fraction was much more complex and contained medium-sized and smaller diatoms, individual *Phaeocystis* sp. cells, as well as most of the detritus. Only few algae passed the 10 µm filter. As a biomass measure the calculated biovolume was taken to compare groups of algae within and between the >100 µm and the 10 to 100 µm fractions (Table 1); the 2 to 10 µm and 0.2 to 2 µm fractions did not contain significant numbers of algae and are not included in this table. Protein measurements of the 2 largest size fractions are also given. The large range in protein to biovolume values probably reflects the combined effect of a change in species composition and variation in the detritus content of the samples. *Phaeocystis* sp. numbers in the original samples were calculated from the >100 µm fractions which retained >99% of total *Phaeocystis* sp. numbers in all but the 2 samples with lowest *Phaeocystis* sp. numbers (Table 2). The results suggested that the peak of the bloom had not been reached yet. This was confirmed by data from R. L. J. Kwint & K. J. M. Kramer (unpubl.), who reported maximum *Phaeocystis* sp. numbers at the near shore station 10 d later.

In the DMSP-lyase assay a substrate concentration of 250 µM DMSP was used. Kinetic experiments with some of the samples, using DMSP concentrations up to 6 mM, showed that 250 µM did not saturate the enzyme. However, during the 1 h assay the amount of substrate used was at most 2% of the initial concentration, resulting in activities linear in time (with typical $r^2$ of 0.998) and with enzyme concentrations. Thus, by taking care that all conditions were constant during the assay, there was no problem in comparing the samples for their activity in relation to species composition. DMSP-lyase activity proved to be highly variable in the different samples. In the 0.2 to 2 µm fraction we had hoped to concentrate part of the bacterial population so that a distinction could be made between algal and bacterial DMSP-lyase activity. However, in this fraction as well as in the 2 to 10 µm fractions no activity could be detected. The 10 to 100 µm fractions gave low activities; highest activities were found in the >100 µm fractions. Relating DMSP-lyase activity in the crude extracts with the most abundant species found in the samples with highest activity showed a highly significant correlation with *Phaeocystis* sp. numbers ($r^2$ = 0.966, n = 23) (Fig. 3A). There was no correlation with

Table 1. Total biovolume, total protein content and the ratios of these parameters observed for algae in the >100 µm and 10 to 100 µm fractions of surface water samples taken at various stations off the Dutch coast (see Fig. 1 for locations). From Stns W3, W5 and W6 no >100 µm fractions were taken; the T1 and T7 10 to 100 µm fractions were lost

| Stn | Fraction (µm) | Biovolume ($10^8$ µm$^3$) *Phaeocystis* sp. | Non-*Phaeocystis* | Total | Total protein (µg) | Ratio protein biovolume (µg µl$^{-1}$) |
|---|---|---|---|---|---|---|
| T1 | >100 | 8.42 | 12.96 | 21.38 | 97.53 | 45.62 |
| T3 | >100 | 2.95 | 1.42 | 4.38 | 41.12 | 93.92 |
| | 10–100 | 0.028 | 0.872 | 0.90 | 17.72 | 196.72 |
| T6 | >100 | 5.24 | 0.687 | 5.92 | 29.76 | 50.25 |
| | 10–100 | 0.035 | 0.427 | 0.46 | 10.66 | 230.75 |
| T7 | >100 | 12.46 | 0.93 | 13.39 | 43.93 | 32.81 |
| N1 | >100 | 0.244 | 10.6 | 10.79 | 28.01 | 25.95 |
| | 10–100 | 0.001 | 2.11 | 2.11 | 50.02 | 236.68 |
| N4 | >100 | 0.114 | 1.92 | 2.03 | 13.68 | 67.35 |
| | 10–100 | 0.0004 | 0.715 | 0.72 | 16.13 | 225.48 |
| N5 | >100 | 0.064 | 0.587 | 0.65 | 9.23 | 141.87 |
| | 10–100 | 0.003 | 0.158 | 0.16 | 15.91 | 989.57 |
| N6 | >100 | 1.32 | 0.036 | 1.36 | 5.73 | 42.17 |
| | 10–100 | 0.015 | 0.091 | 0.11 | 11.51 | 1089.50 |
| N8 | >100 | 2.01 | 0.015 | 2.02 | 23.87 | 118.0 |
| | 10–100 | 0.012 | 0.013 | 0.025 | 12.62 | 5060.84 |
| W1 | >100 | 0.063 | 6.24 | 6.3 | 20.26 | 32.15 |
| | 10–100 | 0.005 | 4.5 | 4.5 | 29.55 | 65.61 |
| W3 | 10–100 | 0 | 1.72 | 1.72 | 35.49 | 206.54 |
| W5 | 10–100 | 0 | 1.26 | 1.26 | 22.49 | 178.01 |
| W6 | 10–100 | 0 | 0.017 | 0.017 | 4.24 | 2495.88 |
| W7 | >100 | 0.281 | 0.523 | 0.8 | 10.34 | 128.82 |
| | 10–100 | 0.003 | 0.477 | 0.48 | 10.18 | 212.36 |

Table 2. *Phaeocystis* sp. cell numbers at the various sampling stations as calculated from the >100 µm fraction and dissolved DMSP + DMS concentrations in the corresponding sample. nd = not detectable

| Stn | *Phaeocystis* sp. cell numbers ($10^6$ $l^{-1}$) | Dissolved DMSP + DMS (nM) |
|---|---|---|
| T1 | 15.442 | 25 |
| T3 | 8.018 | 31 |
| T6 | 10.153 | 38 |
| T7 | 26.576 | 26 |
| N1 | 0.237 | 1 |
| N4 | 0.118 | 1 |
| N5 | 0.063 | 1 |
| N6 | 2.861 | 17 |
| N8 | 4.089 | 9 |
| W1 | 0.063 | 6 |
| W3 | nd | nd |
| W5 | nd | nd |
| W6 | nd | nd |
| W7 | 0.590 | 11 |

any of the other species, nor with total diatom numbers or total diatom biovolume (Fig. 3B to F). The relation with total protein content gave a slightly higher correlation (Fig. 3G), reflecting the presence of *Phaeocystis* sp. cells in some of the samples with high protein content. On the other hand, the sample with second highest protein content exhibited hardly measurable lyase activity, and proved to be mainly composed of detritus.

Dissolved DMS(P) concentrations were measured in the <0.2 µm fraction. Because head space analysis was used, the detection limit was rather high (1 nM). To avoid high pressure on cells during filtration and subsequent disruption and release of intracellular DMSP, a reversed filtration technique was choosen (Fig. 2). Although no direct check on leakage of DMSP from cells was done, the very low DMS(P) values in the <0.2 µm fraction compared to total DMS(P) values from samples taken directly from the bucket (data not shown) indicated that no lysis of cells had occurred during the filtration procedure. Checks on loss of dissolved DMSP due to adsorption in the filtration unit gave similar concentrations for all compartments. Ratios of total DMS(P) to dissolved DMS(P) were on the order of 8 to 20. This is in good agreement with Kwint & Kramer (unpubl.), who measured ratios of 6 to 10 in surface water samples from the same area. Dissolved DMS(P) was detected in samples with *Phaeocystis* sp. cells only (Table 2).

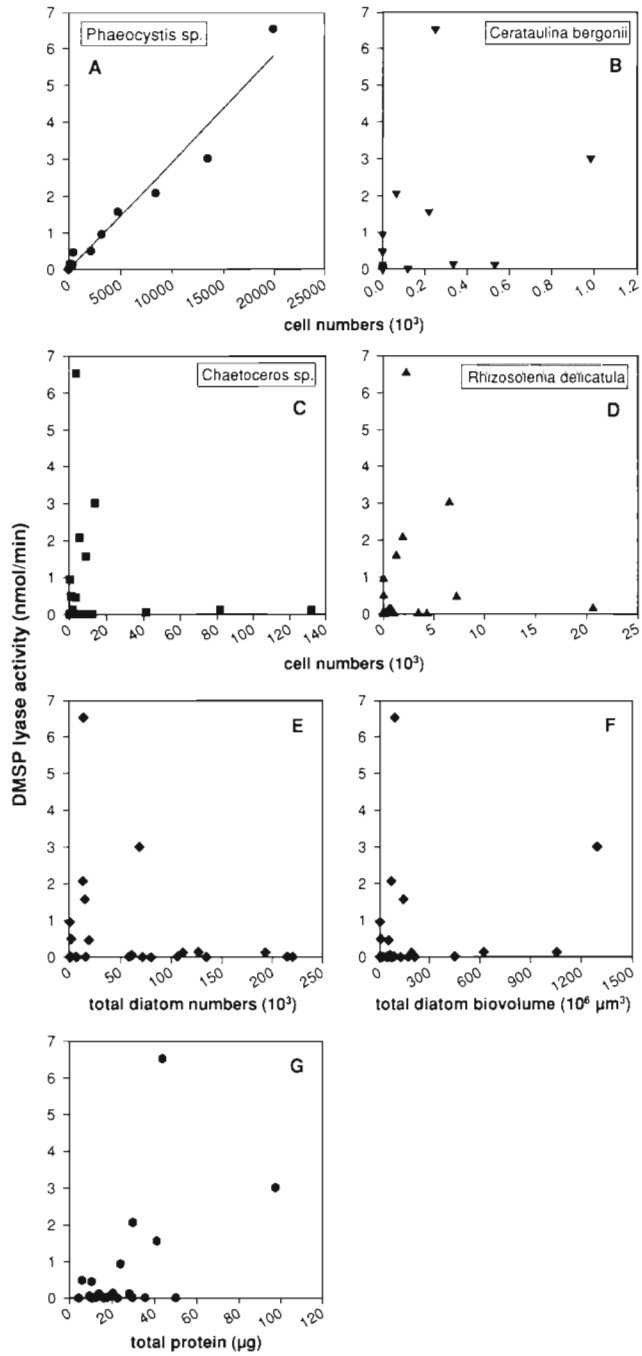


Fig. 3. DMSP-lyase activity in 1 ml crude extract, using a substrate concentration of 250 µM DMSP, in relation to numbers of (A) *Phaeocystis* sp., (B) *Cerataulina bergonii*, (C) *Chaetoceros* sp., (D) *Rhizosolenia delicatula*, (E) total diatom numbers, (F) total diatom biovolume and (G) total protein content. The equation of the regression line for *Phaeocystis* sp. is $y = 0.2905 \times 10^{-6} x + 0.0105$, $r^2 = 0.9660$, n = 23

## DISCUSSION

A DMSP-lyase assay was applied to natural sea water samples. The large variety in species composition in these samples provided us with the opportunity to test natural plankton assemblages for their DMSP-lyase activity in relation to species composition. A highly significant correlation between DMSP-lyase activity

and *Phaeocystis* sp. cell numbers was found, whereas other species failed to show any such correlation.

Two points require further consideration. First, the assay was developed with crude extracts of *Phaeocystis* sp. cells (Stefels unpubl.), and one can argue that this might obscure the possible activity of other DMSP-lyases present either in other algal species or in bacteria. To avoid this problem the assay was performed at pH 8.5: this pH is far from optimal for the *Phaeocystis* DMSP-lyase, which has a more alkaline optimum, but high enough to give a 50% activity of this enzyme (Stefels unpubl.). When comparing the assays used by Ishida (1968) and Cantoni & Anderson (1956) for other algal species with the assay used in this study, it appears unlikely that the latter would cause strong inactivation of all other DMSP-lyases present. Second, highest activities were found in the >100 µm fractions, in which *Phaeocystis* sp. colonies were trapped. The possibility that these colonies were associated with large numbers of DMSP-converting bacteria containing the DMSP-lyase activity could not be excluded unambiguously due to lack of bacterial counts, but was rejected on the basis of the following arguments. (1) If bacteria exhibit the main part of the enzyme activity, the highly significant correlation between *Phaeocystis* sp. numbers and DMSP-lyase activity, and the *y*-axis intercept not being significantly different from zero (Fig. 3A), would imply a constant ratio of *Phaeocystis* sp. cells and these bacteria. However, healthy cells produce little extracellular DMSP (Stefels & van Boekel 1993), thus being a poor substrate to select bacteria on. A more prominent role for bacteria is to be expected during the senescent phase of the bloom, when colonies disintegrate with subsequent release of large amounts of organic matter. Large numbers of bacteria indeed are only found during late stages of *Phaeocystis* sp. blooms (Verity et al. 1988, Billen et al. 1990, van Boekel et al 1992, Thingstad & Billen 1994). The actual contribution of DMSP as a substrate for bacteria within the bulk of released organic matter is unknown. A constant ratio between *Phaeocystis* sp. cells and DMS-producing bacteria therefore appears unlikely. (2) If bacteria were active in cleaving DMSP in our samples, one would also expect to find bacterial DMSP-lyase activity associated with the detritus that is found in samples with large numbers of *Phaeocystis* sp. and which is retained in the 10 to 100 µm fraction. In this fraction, however, activities were near the limit of detection. Therefore, it was concluded that the DMSP-lyase activity measured can be attributed solely to *Phaeocystis* sp. cells.

With respect to the possible role of *Phaeocystis* sp. in the release of DMS to the atmosphere, it is interesting to compare the potential DMS production rates by *Phaeocystis* sp. cells with the main abiotic loss factors such as fluxes of DMS to the atmosphere and photochemical oxidation. Calculations of DMS production in the water column were done according to the following equation.

$$P_{wc} = P_{ph} \times N \times D \times 1440 \quad (1)$$

where $P_{wc}$ is the DMS production rate over the water column in µmol $m^{-2}$ $d^{-1}$; $P_{ph}$ is the DMS production by *Phaeocystis* sp. cells in nmol $min^{-1}$ $cell^{-1}$, $N$ is the number of *Phaeocystis* sp. cells $l^{-1}$ (Table 2), and $D$ is the depth of the mixing layer in m, over which a homogenious distribution of the plankton can be assumed. $P_{ph}$ was calculated from experiments with exponentially growing cells, in which effects of pH and temperature were measured on the DMSP-lyase activity in whole cells (Stefels unpubl.). Assuming linear lyase activity in the lower substrate concentration ranges, these experiments yielded activities of $3.5 \times 10^{-11} \times$ [DMSP] at the *in situ* temperature of 8°C, an estimate which is confirmed in several other experiments with whole cells (unpubl.), and which is in good agreement with activities published earlier (Stefels & van Boekel 1993). The dissolved DMSP concentration is expressed in nM and assumed to be 75% of the total DMS(P) concentration measured in the <0.2 µm fraction (Table 2). This percentage was estimated in more detailed chemical studies performed in the same area during the same period (Kwint & Kramer unpubl.), and is in accordance with data from around the British mainland (Turner et al. 1988). Turner et al. (1989) monitored a *Phaeocystis* sp. bloom in the Southern Bight of the North Sea and measured dissolved DMSP concentrations approximately 10 times higher than DMS concentrations. Therefore the 75% used here may be a slight underestimation. For $D$ a mean depth of 5 m was taken, although fluorescence and transmittance measurements made on board, sometimes showed homogeneity of the water column over 15 m. Homogeneous distributions of *Phaeocystis* sp. over the water column were confirmed by studies of R. Riegman (pers. comm.).

The resulting potential DMS production rates are given in Fig. 4. Highest values were calculated for the Texel track (all 'T' stations in Fig. 1), in the northern part of the study area, where the *Phaeocystis* sp. bloom was most abundant, ranging from 47 to 131 µmol $m^{-2}$ $d^{-1}$. We compared these production rates with the main abiotic loss factors, flux to the atmosphere and photochemical oxidation. The mean flux of DMS to the atmosphere in this northern area can be calculated following Liss & Merlivat (1986): flux = $k_w \times \Delta C$. During the cruise the mean wind speed was 5 m $s^{-1}$, giving a corresponding transfer velocity $k_w$ of 4.6 cm $h^{-1}$ (Liss & Merlivat 1986). Because we were not able to measure DMS concentrations accurately, an average of 15 nM was assumed, as measured at that time near our

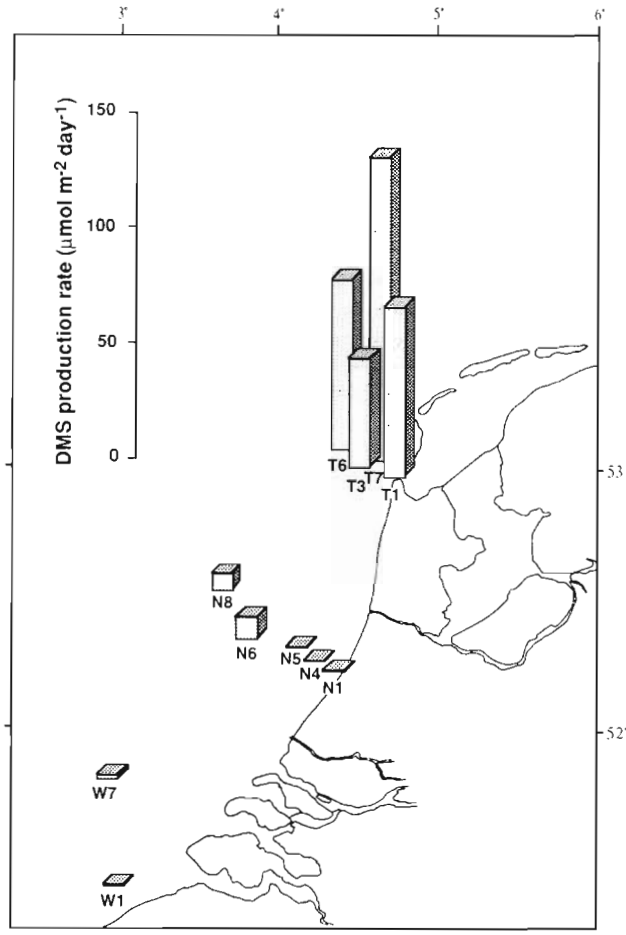


Fig. 4. *Phaeocystis* sp. Potential DMS production rates in Dutch coastal waters during the spring bloom of 1993, calculated using extrapolated activities found in exponentially growing cultures; a mixing depth of 5 m; dissolved DMSP concentrations equalling 75% of the DMSP + DMS measurements in the <0.2 µm fractions of the corresponding samples

inshore station by Kwint & Kramer (unpubl.). Thus a flux of 16.6 µmol $m^{-2}$ $d^{-1}$ was calculated. Concerning photochemical oxidation of DMS to DMSO, Brimblecombe & Shooter (1986) calculated a half life time for DMS in coastal waters of 8 h, with total loss rates presumably comparable with losses by air-sea exchange. Total abiotic loss rates can therefore be estimated to be approximately 30 µmol $m^{-2}$ $d^{-1}$. Compared with the potential DMS production by *Phaeocystis* sp. this is in the same range; even a 1.5 to 4.5 times overproduction of DMS can be calculated, potentially available for bacterial consumption. Considering the conservative estimations of the parameters used, production may even be higher. Previously, rates of production and consumption of DMS have been studied with the chloroform inhibition technique (Kiene & Bates 1990, Kiene & Service 1991, Bates et al. 1994). These studies also suggest that loss by air-sea exchange is minor when compared with microbial production and consumption rates of DMS. The chloroform inhibition technique does not distinguish between bacterial and algal production of DMS.

In field situations, where low dissolved DMSP concentrations prevail, the affinity of the enzyme system for DMSP has to be taken into account, and it is hard to judge whether bacterial or algal DMSP-lyases will dominate, since conversion rates at low substrate concentrations have not been measured yet. Recently, Ledyard & Dacey (1994) isolated a bacterium from the Sargasso Sea, in which uptake of DMSP took place prior to cleavage. They found that the rate of DMS production showed a sigmoidal dependence on extracellular DMSP at low concentrations. At 50 nM DMSP this resulted in a DMS production rate of ca 0.05 fmol $cell^{-1}$ $h^{-1}$. Experiments with whole cells and crude extracts of axenic *Phaeocystis* sp. cultures gave indications for an extracellular location of the DMSP-lyase (Stefels unpubl.), making it unlikely that membrane transport interferes with DMSP conversion. There were no indications of a deviation from linearity of lyase activity in the nonsaturating substrate range. Therefore, we assumed linearity, resulting in a production rate at 50 nM DMSP of 0.105 fmol $cell^{-1}$ $h^{-1}$ for cells from the exponential growth phase at 8°C. If the kinetic parameters of the Sargasso strain are the same as those of bacteria from more eutrophied areas, a population density of $10^7$ to $10^8$ cells $l^{-1}$ of these bacteria will give DMS production rates comparable with those from *Phaeocystis* sp., as found in our samples. Although the results of our study do not exclude bacterial activity, it appears reasonable to assume that during the early stages of a *Phaeocystis* sp. bloom, this alga is the main DMS-producing species. In the literature, a close correlation is found between *Phaeocystis* sp. abundance and high DMS concentrations in the sea water (Barnard et al. 1984, Holligan et al. 1987, Gibson et al. 1990). Liss et al. (1993) showed large seasonal variations of DMS in the southern North Sea, with a maximum in front of the Dutch coast during the *Phaeocystis* sp. bloom. Our study has made it plausible that *Phaeocystis* sp. itself plays an important role in this production of DMS.

In summary, this study shows that, during an early spring bloom in the North Sea, *Phaeocystis* sp. exhibits a very active DMSP-lyase, specific for this species, causing a potential DMS production in excess of abiotic loss factors such as flux to the atmosphere and photochemical oxidation, leaving 35 to 75% of DMS for bacterial consumption. It was only possible to give a rough estimate of the importance of algal DMS production. Environmental conditions may change rapidly, leading to deviations in the abiotic as well as biotic production and consumption terms of DMS, including the possible demethylation of DMSP (Kiene

& Service 1991, Bates et al. 1994). For a better understanding of the fate of the total DMSP-sulfur pool in a *Phaeocystis* sp. bloom, the relative importance of all processes involved should be investigated simultaneously and on a daily basis.

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