

Chapter 7

**Production of DMS from dissolved DMSP in axenic cultures  
of the marine phytoplankton species Phaeocystis sp.**

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## PRODUCTION OF DMS FROM DISSOLVED DMSP IN AXENIC CULTURES OF THE MARINE PHYTOPLANKTON SPECIES PHAEOCYSTIS SP.

### ABSTRACT

*In the marine environment, production of dimethylsulfide (DMS) from dissolved dimethylsulfoniopropionate (DMSP<sub>d</sub>) - an algal osmolyte - is thought to occur mainly through bacterial activity. We have investigated the possibility that phytoplankton itself converts DMSP<sub>d</sub> into DMS, using axenic batch cultures of Phaeocystis sp. at different growth stages. DMSP<sub>d</sub> added to the medium was converted enzymatically to DMS by Phaeocystis. A culture in the exponential growth phase displayed Michaelis-Menten type kinetics for DMSP<sub>d</sub> conversion, yielding an apparent  $K_m$  value of  $11.7 \pm 3.1 \mu\text{M DMSP}_d$  and a  $V_{max}$  value of  $3.05 \pm 0.48 \text{ nmol DMS (min } 10^6 \text{ cells)}^{-1}$ . DMS production rates declined during the transition from exponential to stationary phase. This decrease was not caused by accumulation of inhibiting substances in the medium or the undersaturation of the enzyme. Intracellular DMSP concentrations in Phaeocystis batch cultures increased from 71 mM in exponential phase cells to approx. 150 mM in stationary phase cells. DMS and DMSP<sub>d</sub> concentrations in the culture remained very low during the exponential growth phase. DMS production started in early stationary phase. In a senescent culture DMSP<sub>d</sub> appeared when cell number started to decline. DMSP production in this culture continued even when cell number declined. In completely lysed batch cultures approx. 25% of the particulate DMSP produced in the culture was left as DMSP<sub>d</sub>. The results indicate that Phaeocystis may contribute significantly to DMS production from DMSP<sub>d</sub> during bloom situations in the field.*

### INTRODUCTION

In recent years, dimethylsulfide (DMS) production and consumption processes in marine environments as well as the flux of DMS to the atmosphere have been studied intensively (see reviews by Cooper & Matrai 1989, Taylor & Kiene 1989, Andreae 1990, Kelly & Smith 1990, Fitzgerald 1991). This interest is caused by the realization that DMS may be involved in the biological regulation of the climate: 90 - 95 % of the aerosols found above remote oceans exist of non-seasalt sulfate (nss sulfate) that is formed by gas-to-particle conversion of the oxidation products of organosulfur gases (principally DMS). Aerosols serve as cloud condensation nuclei (CCN). The amount of DMS released into the atmosphere influences the number of CCN and thereby cloud droplet size, cloud albedo and, consequently, climate. Charlson et al. (1987) presented a theoretical model in which they suggested that in this way oceanic phytoplankton could counteract greenhouse warming. The strenght of this feedback mechanism is still under discussion (Schwartz

1988, Foley et al. 1991). Recently, evidence has been presented of a correlation between concentration of DMS and both concentration of nss sulfate and number of CCN in the atmosphere (Ayers & Gras 1991, Ayers et al. 1991, Bürgermeister & Georgii 1991, Prospero et al. 1991). The flux of DMS from the ocean into the atmosphere is determined by its concentration in the water, which is the result of several production and removal processes. In sea water DMS is produced from dimethylsulfoniopropionate (DMSP), a compound that is found in marine macroalgae (Reed 1983, Karsten et al. 1990) and a number of phytoplankton species from different taxonomical groups (Keller et al. 1989). The function of DMSP in the cell is not fully known, but it has been suggested to act as an osmolyte (Vairavamurthy et al. 1985, Dickson & Kirst 1987a and b), cryoprotectant (Kirst et al. 1991) or methylgroup donor (Ishida 1968). Conversion of DMSP into DMS and acrylic acid is thought to occur mainly after its release from the cells. In seawater, chemical conversion through hydroxide decomposition is negligible: at pH 8.2 and 10 °C dissolved DMSP (DMSP<sub>d</sub>) has a chemical half-life of 8 years (Dacey & Blough 1987). Most DMSP<sub>d</sub> is cleaved through enzymatic activity. Until now, little is known about the mechanisms involved. A specific enzyme, DMSP-lyase, has been found in crude extracts from the macroalga Polysiphonia lanosa (Cantoni & Anderson 1956). Ishida (1968) isolated a crude enzyme from extracts of the heterotrophic dinoflagellate Gyrodinium cohnii. Enzymatic DMSP conversion has also been found in cultures and natural populations of bacteria (Dacey & Blough 1987, Kiene 1990, Kiene & Service 1991, Kiene 1992). Grazing of zooplankton on algae stimulated DMS production in the water. It is not clear whether this was due to enzymatic conversion of DMSP in the guts of the zooplankton or to increased release of DMSP<sub>d</sub> in the water (Dacey & Wakeham 1986). Removal processes acting on the DMS concentration in the water are the flux to the atmosphere (Liss & Slater 1974) as well as the consumption of DMS by bacteria (Taylor & Kiene 1989, Kiene & Bates 1990, Kiene 1992). Until now, attempts to quantify the production and consumption of DMS have been scarce. Kiene (1990, 1992) and Kiene & Service (1991) suggested that bacteria are most important in DMS production from DMSP<sub>d</sub>. Kiene & Bates (1990) found that in the eastern Pacific Ocean microbial DMS consumption was more than ten times faster than the flux of DMS to the atmosphere. Evidence on the role of phytoplankton in the actual conversion of DMSP to DMS is lacking. DMS production by phytoplankton may occur through intracellular conversion of DMSP or extracellularly after DMSP release in the water. Wakeham & Dacey (1989) estimated the turnover of intracellular DMSP in microalgae to be 1 % per day. The possibility that phytoplankton is able to produce DMS from extracellular DMSP has not been investigated. Still, this may be an important route of DMS production during algal

blooms. Most DMSP will be released in the water at the end of the bloom when algal biomass is at its top (Nguyen et al. 1988). Bacterial biomass often is still low at that time (Billen et al. 1990, van Boekel et al. 1992) so bacterial DMSP<sub>d</sub> conversion into DMS and subsequent consumption of DMS might be relatively unimportant. In that case, when algal enzymes are able to convert DMSP<sub>d</sub> into DMS, a large proportion of the produced DMS might escape into the atmosphere.

The possible algal conversion of DMSP<sub>d</sub> into DMS was investigated using *Phaeocystis* sp. as model organism. This bloom-forming phytoplankton species occurs in Arctic and Antarctic oceans and in some temperate coastal waters and is one of the most important DMSP producers (Barnard et al. 1984, Turner et al. 1989, Gibson et al. 1990). The objective of this study was to establish a relationship between DMSP<sub>d</sub> concentration and DMS production by *Phaeocystis* cells of different physiological conditions. We also followed DMS production in the senescence phase of a *Phaeocystis* batch culture, in order to get an indication of the processes involved in DMSP breakdown during the decline of a natural *Phaeocystis* bloom.

## **MATERIALS AND METHODS**

### **Algal strain**

An axenic strain of *Phaeocystis* sp. (strain K) isolated from the North Sea was used in all experiments.

### **Culturing conditions**

The culture medium was as described by Veldhuis & Admiraal (1987) with the exception that nitrate was the only nitrogen source. *Phaeocystis* cultures were incubated in 1 l. serum bottles placed on a rolling device (3 rpm) at 10 °C and light intensity of 85 μE m<sup>-2</sup> s<sup>-1</sup> in a 14:10 L:D cycle.

### **Experiments**

#### *Conversion of DMSP<sub>d</sub> by Phaeocystis*

This experiment was done in order to examine whether or not *Phaeocystis* cells were able to convert DMSP<sub>d</sub> to DMS. The conversion of DMSP<sub>d</sub> was determined as the increase of DMS concentration in the medium. Samples for determination of DMSP<sub>d</sub> conversion rate were taken from a batchculture at the end of the exponential phase. Activity was measured in an untreated sample, a sample filtered over a GF/F Whatman filter and in a heated sample (45 min., 60 °C). Abiotic conversion of DMSP<sub>d</sub> was examined in fresh culture medium adjusted to the pH of the *Phaeocystis* culture (pH 9.1). To all samples DMSP was

added from a 2 mM stock in HCl (pH 2.5) to a final concentration of 10  $\mu$ M. DMS production in these samples and in a culture sample without added DMSP was followed in time. Exact procedures for DMS analysis are described below.

#### *Effect of DMSP<sub>d</sub> concentration and Phaeocystis growth phase on DMSP conversion*

The relation between DMSP<sub>d</sub> concentration and DMS production by Phaeocystis was determined by measuring the DMSP<sub>d</sub> conversion rate in culture samples at DMSP<sub>d</sub> concentrations between 0 - 100  $\mu$ M. Exact procedures are described below. Preliminary experiments showed that DMSP<sub>d</sub> conversion rates changed during growth of Phaeocystis in batch culture. Therefore, the experiment was performed at three moments during the growth of a Phaeocystis batch culture. Simultaneously, samples were taken for cell counts, bacterial contamination control, and concentrations of DMS, DMSP<sub>d</sub> and particulate DMSP (DMSP<sub>p</sub>) in the culture. Abiotic conversion of DMSP<sub>d</sub> at the concentrations used in the experiment was measured using fresh medium.

#### *Causes for changes in DMSP<sub>d</sub> conversion rate*

A possible cause for the changes in DMSP<sub>d</sub> conversion rate found in batch cultures could be the accumulation of inhibiting substances in the medium. This possibility was tested by filtering part of an end-exponential phase Phaeocystis culture over a 0.45  $\mu$ m cellulose acetate filter and resuspending the cells retained on the filter in fresh medium. From this culture and the untreated culture samples were taken for determination of activity at 10 and 100  $\mu$ M DMSP<sub>d</sub>. Cell counts were done to correct for cell loss during filtration. Another possible cause for the changes in DMSP<sub>d</sub> conversion rate could be the increasing cell density in the culture. At very high enzyme concentrations and low substrate concentrations part of the enzymes present may remain inactivated and deviations from normal Michaelis-Menten enzyme kinetics may occur (Dixon & Webb 1979). We tested this possibility by diluting part of an end-exponential phase culture fivefold with filtered medium from the same culture and measuring DMSP<sub>d</sub> conversion rate in both diluted and untreated culture at different DMSP<sub>d</sub> concentrations.

#### *Fate of DMSP in a decaying Phaeocystis culture*

In senescent cultures, intracellular DMSP will be released in the medium upon cell lysis. Since cell number is decreasing and cells are almost inactive the DMSP conversion rate may drop. It is of interest to know if the remaining algal activity is able to convert the DMSP released by the cells to DMS. We followed the concentrations of DMS, DMSP<sub>d</sub>, DMSP<sub>p</sub> and Phaeocystis cell number in a senescent batch culture.

#### **DMS and DMSP analysis**

All DMS measurements were done using 20 ml samples stored in 60 ml glass vials stoppered with teflon Mininert valves. Vials were placed in the dark at 20 °C in a constant

temperature waterbath. DMS was allowed to equilibrate with the headspace for 30 minutes. For DMSP analysis 0.52 ml 10 M NaOH was added to a 20 ml sample in a 60 ml vial (final pH 13), and quickly stoppered with a Mininert valve. The sample was allowed to react for at least 5 hours in the dark at 20 °C. At pH 13, DMSP is decomposed quantitatively to DMS and acrylate (White 1982, Dacey & Blough 1987). DMS and total DMSP (DMSP<sub>t</sub>) were measured in unfiltered samples. DMSP<sub>d</sub> was measured in samples that were gently filtered over GF/F Whatman filters. Only gravity filtration was used to prevent cell rupture. The filtration procedure caused loss of DMS but not of DMSP<sub>d</sub>. DMS loss proved to be large but reproducible (37.5% ± 4.4%, n=4), so correction factors could be applied. DMSP<sub>t</sub> and DMSP<sub>d</sub> were corrected for the DMS present in the samples. DMSP<sub>p</sub> was calculated as the difference between DMSP<sub>t</sub> and DMSP<sub>d</sub>. For measurement of the DMS production rate at different concentrations of DMSP<sub>d</sub> 20 ml culture samples were transferred to vials after which DMSP was added from stock solutions and the vials stoppered with Mininert valves. Two stock solutions in HCl (pH 2.5) were used: 2mM for final DMSP concentrations up to 10 µM and 20 mM for final DMSP concentrations up to 100 µM; addition volumes never exceeded 100 µl. Vials were stored dark at 20 °C. At regular intervals samples for DMS analysis were taken from the vials.

DMS analysis was done on a Varian 3600 gas chromatograph equipped with a sulfur specific Hall Electrolytic Conductivity Detector (ELCD) and fitted with a widebore DB5 column. Methanol was used as detector solvent. Analyses were performed isotherm at 40 °C, with a He carrier gas flow of 10 ml min<sup>-1</sup>. Under these conditions DMS resolves at 1 min.; detection limit is 5 pg DMS-S. Due to the very low detection limit of the ELCD it was possible to measure DMS concentration in the culture samples by injecting 100 µl headspace samples from the vials directly on column. For calculation of DMS concentrations a calibration curve was made, using standard stocks in the range of 0 - 10 µM DMS. Since preparing DMS stocks gravimetrically is subject to errors due to the volatile nature of DMS, standards were made using DMSP (obtained from Research Plus Inc., USA). A primary standard of 2 mM DMSP was prepared in HCl (pH 2.5) and stored at 4 °C. A secondary standard of 20 µM DMSP and the DMSP working standards were prepared in sterilized artificial seawater. 20 ml of working standards were brought in 60 ml vials and converted to DMS through addition of 0.52 ml 10 N NAOH. Working standards were stored under the same conditions as culture samples. Working standards were found to be stable for two weeks.

### **Biological analysis**

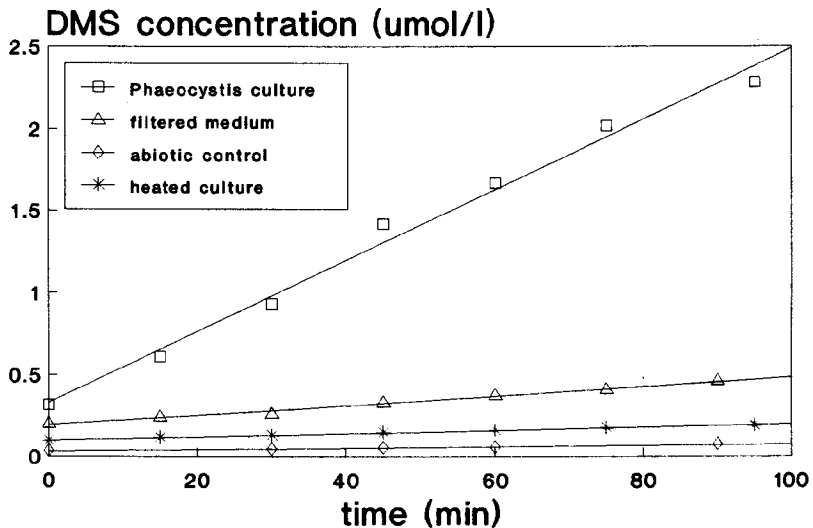
Phaeocystis cells were counted with the Utermöhl sedimentation technique (Utermöhl 1958) after fixation of samples with buffered Lugol solution.

Cultures were examined regularly for bacterial contamination with Hoechst dye no. 33258, using fluorescence microscopy (Paul, 1982). No bacterial contamination was detected during the experiments.

## **RESULTS**

### *DMSP conversion by Phaeocystis*

DMS was produced after addition of 10  $\mu$ M DMSP to a sample from an end-exponential phase Phaeocystis culture (Fig. 1). Some DMS production (13 % of total) was found in the medium, but most activity was associated with the cells. No significant DMS production was found in a culture sample without added DMSP (not shown) or in a culture sample that had been heated prior to DMSP addition. No abiotic DMS production occurred. Other experiments showed that in early-exponential phase cultures of Phaeocystis the activity in the filtered fraction was negligible (not shown).



*Figure 1: DMS concentration as a function of time after addition of 10 $\mu$ M DMSP to a Phaeocystis culture, the filtrated medium, fresh medium (abiotic control), and a heated culture*

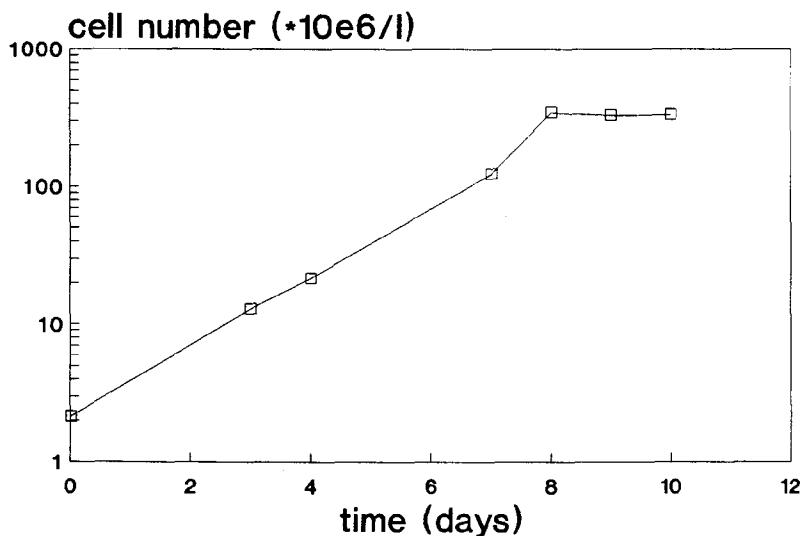


Figure 2: Time course of Phaeocystis cell number during growth in the batch culture used for determining the effect of DMSP concentration and of growth phase on the DMS production rate (Figure 3)

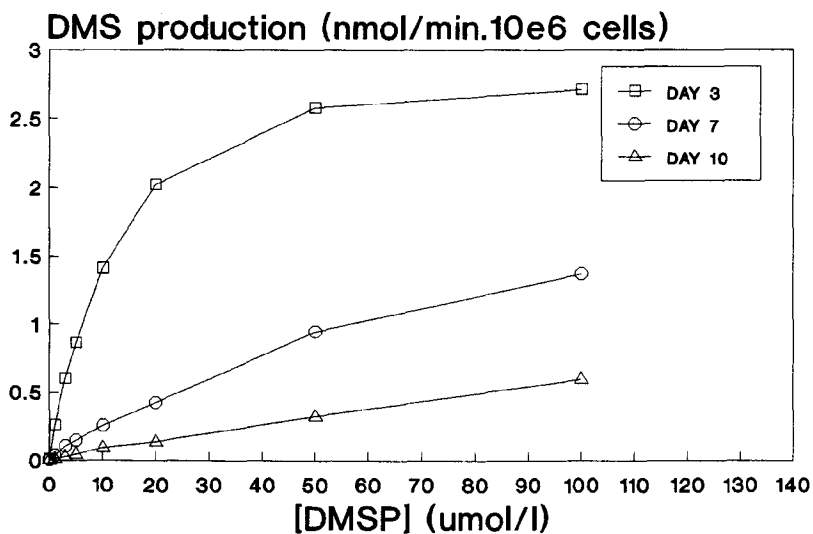


Figure 3: Relation between dissolved DMSP concentration in the medium and DMS production rate at day 3, 7, and 10 during growth of Phaeocystis in the batch culture shown in Figure 2

### *Effect of DMSP concentration and Phaeocystis growth phase on DMSP conversion*

In the batchculture used for this experiment the cellnumber increased exponentially until day 8 (Fig. 2). Thereafter cellnumber remained constant. DMS production at DMSP<sub>d</sub> concentrations between 0 and 100 µM was measured in samples taken from the batch culture at day 3, 7 and 10 (Fig. 3). During the early-exponential phase (day 3) the DMS production rate showed a Michaelis-Menten type relation with the DMSP<sub>d</sub> concentration in the medium. Using the direct-linear plot method, a K<sub>m</sub> value of 11.7 ± 3.1 µM DMSP<sub>d</sub> and a V<sub>max</sub> value of 3.05 ± 0.48 nmol DMS (min 10<sup>6</sup> cells)<sup>-1</sup> (n=21 for both) were calculated from these results. At the end of the exponential phase (day 7) and in the stationary phase (day 10) DMS production rates (normalized to cell number) declined and the relation with DMSP<sub>d</sub> concentration became more or less linear over the range of DMSP<sub>d</sub> concentrations used. Abiotic DMS production was negligible for all DMSP<sub>d</sub> concentrations used (not shown). In the batch culture itself DMS and DMSP<sub>d</sub> concentrations were low during the exponential phase. In the stationary phase DMS accumulated in the medium, while DMSP<sub>d</sub> remained at low concentrations (Table 1). DMSP<sub>p</sub> increased with Phaeocystis cell number. Intracellular DMSP concentrations were calculated using an average cell volume of 113 µm<sup>3</sup>. Cell volume did not change during growth. Intracellular DMSP concentration increased more than twofold from day 3 to day 7 and remained apr. the same thereafter (Table 1).

### *Causes for changes in DMSP<sub>d</sub> conversion rate*

Stationary phase Phaeocystis cells (cell number in culture 329 10<sup>6</sup> l<sup>-1</sup>) were transferred to fresh medium and DMS production measured at two DMSP<sub>d</sub> concentrations. The cells in fresh medium showed approximately the same DMS production rates as non-refreshed cells from the same culture (Table 2). Fivefold dilution with fresh medium of an end-exponential phase Phaeocystis culture (cell number in culture 93 10<sup>6</sup> l<sup>-1</sup>) also did not result in increased DMS production rates compared with the undiluted control (Fig. 4).

TABLE 1

Concentrations of DMS and of dissolved and particulate DMSP in the medium and the intracellular DMSP concentration in the Phaeocystis batchculture used in the experiment shown in figure 2 and 3

| daynumber | DMS     | dissolved DMSP | particulate DMSP | cellular DMSP |
|-----------|---------|----------------|------------------|---------------|
| 3         | 0.06 µM | 0.02 µM        | 0.10 µM          | 71 mM         |
| 7         | 0.22    | 0              | 2.22             | 169           |
| 10        | 0.59    | 0.03           | 5.31             | 141           |

TABLE 2

Rate of production of DMS from added dissolved DMSP in a refreshed Phaeocystis culture and in the not-treated control at two DMSP concentrations

| DMSP concentration ( $\mu\text{M}$ ) | DMS production rate in $\text{nmol min}^{-1}$ ( $10^6 \text{ cells}^{-1}$ ) |           |
|--------------------------------------|---|-----------|
|                                      | not treated   | refreshed |
| 10                                   | 0.062   | 0.068     |
| 100                                  | 0.373   | 0.426     |

TABLE 3

Cellular DMSP concentration of Phaeocystis cells during the senescence phase of the batch culture experiment shown in figure 5

| daynumber | DMSP $\text{cell}^{-1}$ in mM |
|-----------|-------------------------------|
| 11        | 116                           |
| 14        | 160                           |
| 15        | 133                           |
| 16        | 103                           |
| 18        | 134                           |
| 21        | 163                           |

#### *Fate of DMSP in a decaying Phaeocystis culture*

The concentrations of DMS,  $\text{DMSP}_d$ ,  $\text{DMSP}_p$  and Phaeocystis cell number were followed from day 11 on in the stationary phase of a batch culture. The number of (in visual appearance) normal cells increased until day 16 and declined sharply thereafter (Fig. 5a). Large numbers of bleached, deformed or broken cells were present on day 18 and 21. DMS accumulated in the culture from day 14 on (Fig. 5b).  $\text{DMSP}_d$  concentration was below  $0.17 \mu\text{M}$  until day 15 and then increased to  $\pm 0.4 \mu\text{M}$ . Concentration of  $\text{DMSP}_p$  increased until day 15 and then declined. Intracellular DMSP concentration (calculated using numbers of normal cells only) remained apr. constant (Table 3). The intracellular

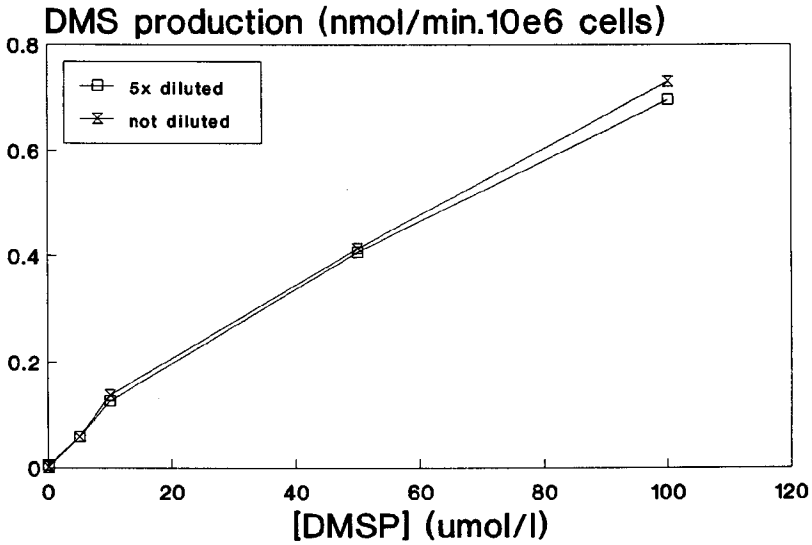


Figure 4: Relation between dissolved DMSP concentration and DMS production rate in a not diluted and in a fivefold diluted *Phaeocystis* batch culture

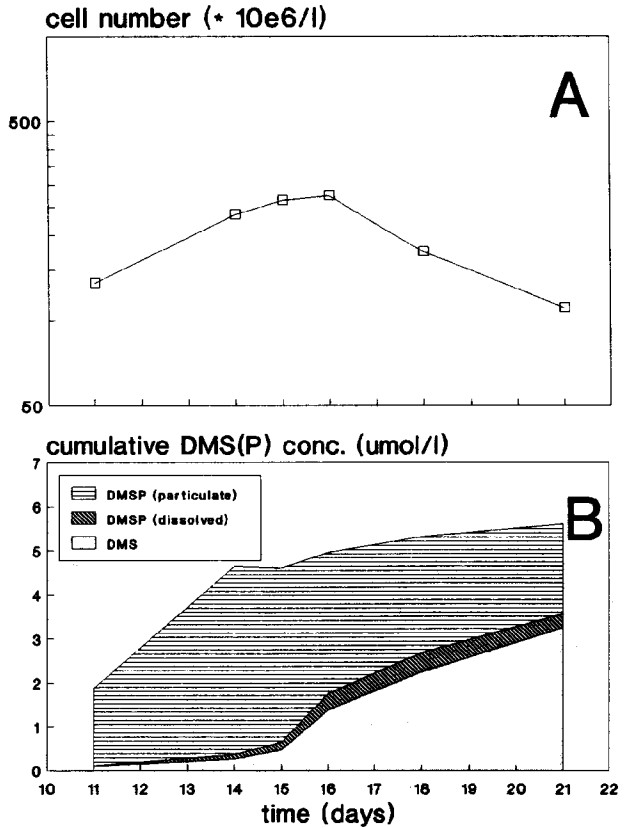


Figure 5: Time course of *Phaeocystis* cell number (a) and of concentrations of DMS, dissolved DMSP and particulate DMSP in the medium (b) during the senescence phase of a batch culture

DMSP concentration may, however, have declined if deformed cells still contributed to DMSP<sub>p</sub>. The total of DMS and DMSP continued to increase even in the period after day 16 when cell number was declining, indicating that intact cells still produced DMSP. Measurements in Phaeocystis cultures that had completely died (all cells lysed) showed that 73 % of total DMSP had been converted to DMS during the senescence phase (not shown).

## DISCUSSION

The results clearly show that Phaeocystis was able to produce DMS from DMSP<sub>a</sub> added to the medium. The conversion of DMSP<sub>a</sub> was the result of an enzymatic reaction showing Michaelis-Menten type kinetics. The activity was associated with the cell, although some activity occurred in the medium in older cultures. DMSP-lyases have been found in the macroalga Polysiphonia lanosa (Cantoni & Anderson 1956) and the heterotrophic dinoflagellate Gyrodinium cohnii (Ishida 1968). The enzyme produced by Phaeocystis might also be a DMSP-lyase, but the possibility that DMSP<sub>a</sub> conversion is a secondary reaction of an enzyme not specific for DMSP cannot be excluded. This possibility is especially realistic if we assume that the enzyme is located extracellularly, since there is no clear function for an extracellular enzyme specific for DMSP. The production of acrylic acid, which would serve as a bactericide, has been mentioned as an important function of extracellular DMSP conversion by Phaeocystis (Sieburth 1960, Barnard et al. 1984). The antibiotic properties of acrylic acid are, however, rather questionable at the pH of seawater (Sieburth 1961). Ishida (1968) found that cells of G. cohnii incubated with labelled DMSP did not incorporate radioactivity. In the case this also holds for Phaeocystis this would provide an argument for the extracellular location of the enzyme. If, however, the enzyme produced by Phaeocystis has an intracellular location it could be involved in the regulation of the DMSP concentration in the cell. Conversion of DMSP<sub>a</sub> would then include transport of DMSP<sub>a</sub> through the cell membrane into the cell and of produced DMS out. This would mean that DMSP<sub>a</sub> is taken up by the cell against a strong concentration gradient (thousandfold increase in concentration). Although this seems unlikely it cannot be excluded. Intracellular location of the enzyme might give an explanation for the decrease in cellular activity found during the end-exponential phase of Phaeocystis batch cultures (Fig. 3). This decrease was not caused by accumulation of inhibiting substances in the medium or by partial inactivity of the enzyme present (Table 2, Fig. 4). Possibly, changing cell physiology during the transition from the exponential phase to stationary phase led to

a decreased transport of DMSP<sub>d</sub> through the cell membrane or to changes in the regulation mechanism of intracellular DMSP concentrations, resulting in decreased conversion of the DMSP<sub>d</sub> added to the culture. If the enzyme is located extracellularly the decreasing activity can only be explained by assuming changes in both enzyme concentration per cell and affinity of the enzyme for DMSP<sub>d</sub>.

The apparent  $K_m$  value of 11.7  $\mu\text{M}$  found for DMSP<sub>d</sub> conversion of Phaeocystis cells is high compared with DMSP<sub>d</sub> concentrations normally encountered in the field ( $[\text{DMSP}_d] < 1.2 \mu\text{M}$ , Turner et al. 1989). The DMSP-lyase found in Gyrodinium cohnii by Ishida (1968) had a  $K_m$  value for DMSP of 1.5 mM. Clearly, no statements about the location of the DMSP converting enzyme of Phaeocystis can be made on the basis of its  $K_m$  value for DMSP<sub>d</sub>.

The intracellular DMSP concentration of Phaeocystis increased more than twofold during the transition from exponential phase to stationary phase (Table 1). Gröne & Kirst (1992) found a 75 % increase in intracellular DMSP concentration in N-limited Tetraselmis subcordiformis compared with a non-limited control. Turner et al. (1988) concluded from experimental and field work that DMSP contents increased in Emiliania huxleyi populations due to N-limitation. In both cases the increase of DMSP content was assumed to be a reaction to nitrogen depletion in the cell. Nitrogen-free DMSP was thought to substitute for the nitrogen-containing glycine betaine (a structural analog) that serves as a compatible solute in the osmotic regulation of the cell (Kirst 1989). Unfortunately, Gröne and Kirst (1992) and Turner et al. (1988) did not include P-limited cultures in their experiments. In our opinion the increase in DMSP content might very well be a response to changes in growth rate and physiological state of the cell independently of the kind of limitation the cell experiences. In our experiments the N:P ratio in the medium used was 18, and, although not measured, phosphate was most probably limiting Phaeocystis biomass development. More research is needed on the relation between DMSP and glycine betaine inside the cell in connection with growth rate and the kind of limitation.

In the senescent Phaeocystis culture DMSP<sub>d</sub> concentration increased after day 15 (Fig. 5). Also, in totally lysed cultures DMSP<sub>d</sub> was still present, indicating that the DMSP liberated from cells through cell death was only partly converted to DMS; approx. 25% remained present as DMSP<sub>d</sub>. The total of DMS and DMSP in the senescent culture increased slightly even though cell number was decreasing. This indicates that even in declining populations of Phaeocystis DMSP production can occur. Translation of these results to field situations is difficult, since the processes involving DMS(P) that take place in natural Phaeocystis blooms are poorly understood. Still, in field situations, Phaeocystis may be a producer of DMS at all stages of a bloom. We tried to estimate the maximal DMS

production by Phaeocystis during natural blooms in the Southern Bight of the North Sea using the data from our Fig. 3, a maximal cell number of  $50 \cdot 10^6 \text{ l}^{-1}$  (Cadée & Hegeman 1986) and a maximal DMSP<sub>d</sub> concentration of  $1.2 \mu\text{M}$  (Turner et al. 1988). These values are most likely to be found during the end-exponential or stationary phase of the Phaeocystis bloom although cell number can reach values over  $100 \cdot 10^6 \text{ l}^{-1}$  (Cadée & Hegeman 1986). Depending on growth phase of the Phaeocystis cells DMS production rates from DMSP<sub>d</sub> would range between  $0.65 - 14.2 \text{ nmol l}^{-1} \text{ min}^{-1}$  with the higher value found for early-exponential phase cells and the lower value for stationary phase cells. DMS production rate calculated for the senescent culture after day 16 (Fig 5A) was  $0.31 \text{ nmol l}^{-1} \text{ min}^{-1}$ . These calculated values are in the same range as values found by others in natural systems. Kiene (1990) measured DMS accumulation rates of  $0.37 - 0.6 \text{ nmol l}^{-1} \text{ min}^{-1}$  after addition of  $0.5 \mu\text{M}$  DMSP to samples from coastal waters off Georgia, USA. Dacey & Wakeham (1986) found a DMS accumulation rate of  $0.14 \text{ nmol l}^{-1} \text{ min}^{-1}$  resulting from zooplankton grazing on DMSP-containing algae.

Next to production from DMSP, the DMS concentration in the water is subject to consumption processes. Kiene & Bates (1990) and Kiene (1992) estimated bacterial DMS consumption rates in Pacific Ocean surface waters to be  $0.001 - 0.013 \text{ nmol l}^{-1} \text{ min}^{-1}$ . In estuarine waters off Georgia, USA, Kiene & Service (1991) found a bacterial DMS consumption rate of approx.  $0.002 \text{ nmol l}^{-1} \text{ min}^{-1}$ . It should be mentioned that this was not a bloom situation and DMS concentrations were low, approx.  $2 \text{ nM}$ . At a DMS concentration of  $60 \text{ nM}$ , as found during Phaeocystis blooms (Turner et al. 1989), the DMS consumption rate will probably be higher. However, at the top of a Phaeocystis bloom bacterial biomass is still low while DMS production by algal enzymes probably is maximal. This imbalance of production and consumption might result in relatively high DMS concentrations in the water and consequently in high DMS fluxes to the atmosphere. The role of Phaeocystis in DMS fluxes in natural systems is probably not restricted to the production of DMSP but includes direct production of DMS as well. The actual contribution of Phaeocystis to total DMS production during bloom situations will however have to be measured in the field.