Production of dimethylsulfonionpropionate and dimethylsulfide in intertidal sediment ecosystems.
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Concluding remarks

Production of DMS(P)

The research presented in this thesis focused on the contribution of oxygenic phototrophs to DMSP metabolism in intertidal sediment ecosystems. It was found that diatoms are a major source of DMSP in these systems (Chapters 2 and 5). The range of specific DMSP contents found in benthic diatoms was similar to the range reported in literature for planktonic diatoms (Keller et al. 1989). This confirms the general assumption that diatoms are modest producers of DMSP compared to prymnesiophytes and dinophytes, which are considered high producers (Keller et al. 1989). However, since diatoms can reach high biomasses in intertidal sediments, high concentrations of DMSP were observed correspondingly (Chapters 4 and 5). Apart from diatoms, certain free-living green algae (Chapter 5), as well as green algae living in symbiosis with a flatworm (Chapter 6), might also account for considerable production of DMSP.

Another important result from this research is the observation that cyanobacteria do not produce significant amounts of DMSP in intertidal sediments and perhaps do not produce it at all (Chapters 2 and 5). This is in agreement with several published reports (White 1982, Keller et al. 1989, Corn et al. 1996, Vogt et al. 1998), but it contradicts the publications by Visscher & Van Gemerden (1991a) and Vogt (1997) who reported high concentrations of DMSP in a number of cyanobacteria. Also, Wilson et al. (1998) found very high concentrations of DMSP associated with *Synechococcus* spp, corresponding to an intracellular concentrations of >1 M. These different results may be partly explained by differences in culture conditions or physiological status of the organisms. In chapter 2 it was shown that some cyanobacteria obtained from culture collections contained some DMSP, which was lost after cultivation in our laboratory, and that a few cyanobacteria contained some DMSP after nitrogen starvation. The amounts of DMSP detected in these strains were, however, still very small. Up to now it remains uncertain if cyanobacteria are actually capable of producing DMSP. Vogt (1997) found that DMS continued to increase for days after addition of alkali to cell extracts of cyanobacteria, which contradicts the presence of DMSP since this is very rapidly cleaved to DMS at high NaOH concentrations. The question whether cyanobacteria really produce DMSP should be resolved because cyanobacteria contribute significantly to primary production in some environments. Moreover, it would be interesting to know if freshwater cyanobacteria produce DMSP. It is usually assumed that DMSP production is restricted to marine species. However,
production of DMS has been associated with freshwater cyanobacteria (Jenkins et al. 1967, Bechard & Rayburn 1979), though DMSP was not determined in these studies. Thus, a variety of freshwater and marine strains of cyanobacteria should be tested on their DMSP content using methods available at present that specifically determine DMSP, such as HPLC-FPD (Howard and Russell 1995), GC-MS (Gage & Hanson 1996) and NMR spectroscopy (Macdonald et al. 1996).

Many marine algae possess DMSP-lyase (Stefels & Van Boekel 1993, Nishigushi & Goff 1995, De Souza et al. 1996, Steinke et al. 1996). It is being recognized that DMSP-lyase activity by algae can have a strong impact on DMS production and fluxes to the atmosphere. The prymnesiophyte Phaeocystis is a well-know example (Stefels et al. 1995, Van den Berg et al. 1996). Van Duyl et al. (1998) suggested that a high DMS concentration measured in coastal seawater during the exponential phase of a Phaeocystis globosa bloom was the result of DMSP-lyase activity of this alga. Simó & Pedrós-Alió (1999) pointed to the striking resemblance between the dependencies of photosynthetic activity and DMS yield from DMSP on the mixing layer depth of oceans, indicating an important role of phytoplankton in DMSP to DMS conversion.

During the study presented in this thesis DMSP-lyase activity was tested in crude extracts of a number of benthic diatoms, but DMSP-lyase activity could not be detected (Chapter 2). This was the first time that DMSP-lyase activity was directly tested in diatoms and the results confirm other studies suggesting that diatoms do not possess DMSP-lyase (Stefels et al. 1995, Kwint et al. 1996). The number of species that was tested, however, was quite small and the assay was performed in buffers without addition of NaCl or other salts. Recently, it was observed that some DMSP-lyases have a salt dependency (Steinke et al. 1998). Because of the potential impact of DMSP-lyase activity of diatoms on DMS fluxes a larger number of strains (benthic as well as planktonic) should be tested for DMSP-lyase activity, taking into account the different physiological optima of different DMSP-lyases.

In chapter 3 it was shown that DMSP had an osmoprotective function in the benthic diatom Cylindrotheca closterium. This was concluded from the observation that uptake of externally supplied DMSP by C. closterium was stimulated by salinity. This is the first report of uptake of DMSP by a eukaryotic alga. In bacteria this phenomenon is quite common and the protective effect of DMSP on bacteria under high salinity has been well-established (Pichereau et al. 1998, Cosquer et al. 1999, Gage and Rathinasabapathi 1999). Although DMSP synthesis was stimulated at high salinities in C. closterium, DMSP synthesis after a salinity upshock was slow and changes in intracellular DMSP concentration could not compensate rapid changes in extracellular osmotic pressure (Chapter 3). Moreover, in several benthic diatoms DMSP content was found to increase during growth at constant sa
Other researchers also drew this conclusion from their results (Colmer et al. 1996, Stefels et al. 1996).

Considering the fact that it has been known for almost half a century that algae synthesize DMSP in high amounts, it is surprising that the regulation of the synthesis of this compound is still largely unknown. Already in 1966, Ackman & Tocher (1966) suggested that DMSP might be a secondary metabolite in phytoplankton. Much later this opinion was repeated by Kirst (1996), who suggested that DMSP may be a sink for excess reducing power under photosynthetically favorable conditions. Gröne & Kirst (1992) proposed that DMSP production reflects the availability of methionine, which may increase under conditions of stress when protein degradation exceeds protein production. Stefels (2000) postulated an elaborate hypothesis, which explains DMSP production as an overflow mechanism for excess reduced sulfur and excess energy and embraces the theories of Kirst (1996) and Gröne & Kirst (1992). The hypothesis of Stefels (2000) suggests that the regulatory coupling between assimilatory sulfate and nitrate reduction, which is common in higher plants, does not occur in DMSP-producing algae. This assumption lacks experimental evidence, because the regulation of sulfate reduction in marine algae has not been an object of study. The reason for this is that sulfate is very abundant in marine systems and would not be a limiting growth factor for marine algae.

It is a challenge for future research to obtain a better insight in the regulation of DMSP synthesis. Not only should the pathway of DMSP synthesis from methionine be studied, but also the regulation of assimilatory sulfate reduction in algae deserves attention since this is a key process in DMSP synthesis.

Fluxes of DMS from intertidal sediment ecosystems

It was shown that production of DMSP by marine diatoms in intertidal sediment ecosystems can result in high total concentrations of DMSP in the sediment surface layer. But do these high concentrations of DMSP also lead to high fluxes of DMS and do intertidal sediments in that way contribute significantly to the total flux of DMS to the atmosphere? Fluxes of DMS were not measured in this study, but a mathematical model was constructed that predicts fluxes of DMS from intertidal sediments over a diel cycle (Chapter 7). As is often the case with ecological models, several assumptions were made during the process and some of the guesstimated parameter values may not have been realistic (Chapter 7). However, the degradation rate constants (\( \lambda \)) calculated by the model were similar to the constants measured in sediment slurries presented in Chapter 4. (Because the sediment was diluted 6-fold in the sediment slurries, the values of \( \lambda \) in Table 4.1, Chapter 4, were multiplied with a factor 6). For example, the degradation constant of DMSP under oxic conditions, \( \lambda_{ox}^{D} \), was 158 d\(^{-1} \) in the model and about 117 d\(^{-1} \) in sediment slurries. The degradation constant of DMS under oxic conditions, \( \lambda_{ox}^{D} \), was
2.4 to 5.1 d\(^{-1}\) in the model and 19 to 27 d\(^{-1}\) in sediment slurries (Chapter 4). Although in the model fluxes of DMS were sensitive to \(\lambda_{\text{ox}}^D\), an increase in \(\lambda_{\text{ox}}^D\) from 2.5 to 25 d\(^{-1}\) would cause a less than 50 % decrease in DMS emission (Fig. 7.6, Chapter 7). Despite the fairly high similarity between these calculated and measured degradation constants, the model predicted a DMS flux of 0.61 nmol m\(^{-2}\) h\(^{-1}\), which is 1 to 2 orders of magnitude lower than values measured in the field (Table 8.1). In retrospect, a protein content of the oxygenic phototrophs of 3.75 \(\mu\)g protein cm\(^{-3}\) sediment as assumed in the model, is probably 1 to 2 orders of magnitude too low. A realistic estimate of biomass concentration in intertidal sediment is 5 - 10 \(\mu\)g chlorophyll a cm\(^{-3}\) (Chapters 4 and 5, Visscher & Van Gemerden 1991a, Visscher et al. 1994). Assuming a protein:chlorophyll a ratio of 50 in oxygenic phototrophs, this would give a more realistic biomass estimate of 250 - 500 \(\mu\)g protein cm\(^{-3}\). An increase in oxygenic phototrophic biomass in the model yields a proportional increase in DMSP exudation rate. Linear extrapolation to DMS emission gives a flux of DMS between \((250/3.75) \times 0.61 = 40.6\) and \((500/3.75) \times 0.61 = 81.2\) nmol m\(^{-2}\) h\(^{-1}\). This falls in the range of the local DMS fluxes measured in intertidal sediments (Table 8.1). This calculation only holds if oxygenic phototrophic biomass is kept unaltered in the DMS exudation and DMSP cleavage terms for oxygenic phototrophs used in the model. This may not be unrealistic because the results in this thesis do not support DMS exudation or DMSP cleavage by diatoms or cyanobacteria (Chapter 2).

Table 8.1 shows that, at a global scale, intertidal sediment ecosystems do not emit significant quantities of DMS. But not only do intertidal sediments cover a relatively small amount of the Earth’s surface, also absolute fluxes of DMS from intertidal sediments are not high compared to fluxes from marine pelagic systems. A conspicuous exception was the extremely high flux of DMS from sediments harbouring dense populations of the marine flatworm C. roscogenensis (Chapter 6). The value presented in Table 8.1 gives only a very rough estimate of this flux, but it shows that despite its restricted distribution this organism might be responsible for as much as 10% of the global DMS emission from intertidal sediments. Apart from this special case, it seems that intertidal sediment systems, despite their high productivity, do not emit large amounts of DMS. What could be the reason for this? First, the degradation of dissolved DMSP and DMS under oxic conditions in intertidal sediments is faster than in seawater: 105 - 117 d\(^{-1}\) for DMSP and 19 - 27 d\(^{-1}\) for DMS in sediment (Chapter 4), compared to an average of about 10 d\(^{-1}\) for DMSP (Kiene 1996a) and 0.3 - 2 d\(^{-1}\) for DMS (Simó & Pedrós-Alió 1999a) in seawater. Second, the release of dissolved DMSP from the pool of particulate DMSP seems slow compared to DMSP release in pelagic systems. A porewater concentration of DMS(P) of around 100 nM was measured in the surface layer of an estuarine intertidal sediment (Chapter 4). With a turnover of dissolved DMSP of about 110 d\(^{-1}\) the maximal degradation rate of dissolved DMSP (in the case that only DMSP was
Table 8.1. Fluxes of DMS from oceans and intertidal sediment ecosystems.

<table>
<thead>
<tr>
<th>Ecosystem</th>
<th>Local flux (nmol DMS m(^{-2}) h(^{-1}))</th>
<th>Area(^{#}) (km(^{2}))</th>
<th>Total flux (mol DMS d(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seas and oceans</td>
<td>20 - 1200 (300)</td>
<td>3.6x10(^{6})</td>
<td>2.6x10(^{7})</td>
<td>1</td>
</tr>
<tr>
<td>Intertidal mudflats</td>
<td>30 - 100(^{h})</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Saltmarsh pan and tidal creek</td>
<td>15 - 150(^{i})</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Intertidal sand- and mudflats</td>
<td>3 - 70(^{d})</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Intertidal sediment ecosystem</td>
<td>40 - 80(^{p})</td>
<td>3.0x10(^{4})</td>
<td>2.9x10(^{4}) - 5.8x10(^{4})</td>
<td>this thesis</td>
</tr>
<tr>
<td>C. roscoffensis*</td>
<td>200000(^{f})</td>
<td>1</td>
<td>4.8x10(^{4})</td>
<td>this thesis</td>
</tr>
</tbody>
</table>

* Intertidal sediment containing colonies of the marine flatworm C. roscoffensis (Chapter 6)

\(^{a}\) minimum and maximum values are given with average in parentheses; fluxes were calculated from surface water DMS concentrations, using transfer velocities.

\(^{b,d}\) ranges of average values from different seasons are given; fluxes were measured directly, using dynamic flux chambers.

\(^{c}\) corrected value predicted by mathematical model (see text).

\(^{f}\) value calculated by multiplying DMS concentration in field samples (55 \(\mu\)mol l\(^{-1}\), Chapter 6) with mass transfer coefficient \(\kappa_{D}\) (0.1 m d\(^{-1}\), Chapter 7).

\(^{\#}\) Areas are taken from Watts (2000).

\(^{g}\) Calculated using average value.

1 Cerqueira & Pio (1999) and references therein.
2 Harrison et al. (1992).
3 Bodenbender et al. (1999).

present in the porewater) would be 100 nM \(\times\) 110 d\(^{-1}\) = 11 \(\mu\)M DMSP d\(^{-1}\). The concentration of DMS(P) in the porewater was constant over a diel cycle (Chapter 4), indicating that production and consumption were in balance. Hence, release of dissolved DMSP from particulate DMSP would also be about 11\(\mu\)M d\(^{-1}\). Since the total DMSP content of the sediment was about 100 \(\mu\)M, this implies a turnover rate of particulate DMSP of about 10% d\(^{-1}\). Using data from pelagic systems (Ledyard & Dacey 1996, Kiene 1996\(^{a}\), Van Duyl et al. 1998) turnover rates of particulate DMSP of 14 - 200% d\(^{-1}\) were calculated. It was shown that the balance of production and consumption of DMS(P) in the sediment could be disturbed by the rapid excretion of DMSP by diatoms in response to a salinity downshock (Chapters 3 and 4), which could lead to a transient increase in DMS emission (Chapter 7). Third, ventilation of DMS from intertidal sediments may be much lower than from seas and open oceans. Fluxes of DMS from seawater are usually calculated as \(F = K_{w} C_{w}\) (Liss & Slater 1974, Putaud & Nguyen 1996), where \(K_{w}\) is the transfer velocity and \(C_{w}\) is the concentration of DMS in the water. The transfer velocity \((K_{w})\) depends on wind speed in a non-linear way, with \(K_{w}\) progressively increasing with wind speed. Cerqueira & Pio (1999) summarized some data on DMS concentrations, wind speeds, transfer velocities and DMS fluxes from marine pelagic systems. To calculate fluxes of DMS from a sheltered estuary these authors used transfer velocities of 0.05 - 0.18 m d\(^{-1}\) and our estimate of the transfer coefficient \((\kappa_{D})\) of 0.1 m d\(^{-1}\) that was used...
in the model to calculate fluxes of DMS from intertidal sediments therefore seems a realistic value. Cerqueira & Pio (1999) showed that DMS emissions from sheltered areas, with low wind speeds, can be substantially lower than from open aquatic systems, where wind speeds are usually much higher, even if concentrations of DMS are similar.

In conclusion, intertidal sediments are highly productive ecosystems, which can support high total concentrations of DMSP. These high concentrations do not necessarily lead to high fluxes of DMS to the atmosphere, which is explained by a relatively slow release of particulate DMSP present in diatoms, a relatively high degradation of dissolved DMSP and DMS and a relatively low sediment-air exchange of DMS.