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# $C_1$-cycle of sulfur compounds

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## Abstract

$C_1$ organic sulfides are part of many ecosystems and play an important role in the global sulfur budget and climate regulation. At this point, fluxes and conversions of these compounds are only superficially understood. Understanding of the regulating mechanisms will be necessary to quantify the role of these compounds in the global sulfur budget at their climatic role. In this review, the current knowledge of fluxes and conversions of $C_1$ organic sulfides in different ecosystems is presented.

*Abbreviations:* CCN - cloud condensation nuclei; COS - carbonylsulfide; DMS - dimethylsulfide; DMDS - dimethyldisulfide; DMSO - dimethylsulfoxide; $DMSO_2$ - dimethylsulfurdioxide; DMSP - dimethylsulfoniopropionate; MA - methylamine; 3-MPA - 3-mercaptopropionate; MPPA - 3-methiolpropionate; MT - methanethiol

## 1. Introduction

It is now 20 years since Lovelock & Maggs (1972) first detected low but significant concentrations of dimethylsulfide (DMS) in ocean waters. This finding changed generally accepted assumptions about the nature of the missing source of atmospheric biogenic sulfur from the ocean in the global sulfur cycle budget. Until 1972 it had been assumed that biogenic sulfur emission from the oceans to the atmosphere consisted mainly of $H_2S$ gas (Bremner & Steele 1978). Accordingly, most research on sulfur compounds in marine systems had concentrated on inorganic sulfur compounds. This concept was maintained for a number of years, even though several factors shed doubt on it. These included the high hydrogen sulfide oxidizing capacity of the top layer of the ocean, and failure to detect significant concentrations of $H_2S$ in the oceanic atmosphere. Since the discovery of DMS as the potential missing link in the atmospheric sulfur budget, research on several aspects of organic biogenic sulfur compounds (e.g. atmospheric chemistry, marine chemistry and (micro) biology, geobiochemistry) has intensified. New analytical techniques to measure trace quantities of volatile sulfur compounds in natural environments (e.g. Andreae & Barnard 1983) have contributed to the quick development of this field of research. Local environmental problems, such as offensive odours from volatile organic sulfur compounds, led to the extension of this research to cover practical applications for removing these compounds (Sivelä & Sundman 1975; Suylen & Kuenen 1986; Kanagawa & Mikami 1989; Zhang et al. 1991a,b; Derikx 1991). From a scientific point of view, the main objectives of this research are a better understanding of the role of the sulfur cycle in various ecosystems, and to answer the question of how dynamic changes in the cycle influence the global sulfur budget. However, re-

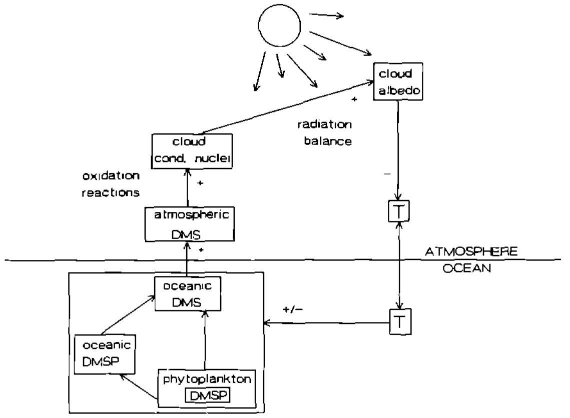


*Fig. 1.* Proposed climatic feedback loop for DMS production and climate. The signs + and − stand for positive (i.e. higher value of previous parameter leads to increase in next parameter) and negative effect respectively. T stands for temperature. After Charlson et al. (1987) and Andreae (1990).

search on the sulfur cycle also has implications for policy making processes in the 'Greenhouse debate'. Charlson et al. (1987) suggested that DMS, in particular, plays an important role in climate control, a view that arose from a 'Gaian' approach to ecosystems (Lovelock 1988; Schneider 1990). DMS originating from dimethylsulfoniopropionate (DMSP), a compound abundant in algae, is emitted from oceans to the atmosphere. In the atmosphere, methane sulfonic acid, a degradation product of DMS, plays a significant role in cloud formation and thus affects the cloud albedo, and as such DMS has a climatic role (Fig. 1). In this context recent research programmes on environmental pollution have emphasized the role of DMS in the global sulfur cycle.

This review aims at integrating some of the available knowledge on the subject from different disciplines.Particular attention will be paid to $C_1$-sulfur compounds that contribute to the transport of oceanic and terrestrial sulfur to the atmosphere. By definition, the sulfur compounds in question must be gases or volatile liquids. The most important $C_1$ sulfur compounds in this category are carbonylsulfide (COS), carbondisulfide ($CS_2$) and the methylated sulfides, dimethylsulfide (DMS), methanethiol (MT) and, to a lesser extent, dimethyldisulfide (DMDS). The occurrence, fluxes and environmental effects of these compounds will be discussed in Section 2. The discussion on sources and sinks of these organic sulfides, as presented in Sections 3 and 4, will show that these compounds are part of a complex network of biological and chemical transformations. Clearly, the subjects of the three sections are intimately linked as the flux of the volatile sulfides from a system is a result of these biological and chemical cycles. Numerous processes and factors determine the actual concentration and the ocean-to-atmosphere flux of these compounds. Determining the controlling mechanisms involved is, in fact, the final object of current research in this field. A better understanding of these mechanisms should identify the factors that determine the total output of gaseous sulfur compounds, thus clarifying the extent of the climatic role of DMS and the role of these sulfur compounds in the global sulfur cycle.

## 2. Occurrences, fluxes and environmental impacts of $C_1$ sulfur compounds

DMS, MT and DMDS in low concentrations are important flavour components of biological origin in beer, cheese, milk and cooked vegetables (Anness 1980; Kadota & Ishida 1972). At somewhat higher concentrations, DMS is partially responsible for 'the smell of the sea'. Industrial applications of these organic sulfur compounds are limited. $CS_2$ is used as a solvent for rubber, waxes etc., as an insecticide, and mainly in the production of viscose rayon (Bhatia 1978). Volatile organic sulfides are mainly waste products. Stack gases from the kraft pulping process in paper industry contain MT, DMS, DMDS, COS and $H_2S$. The methylated compounds are formed from the methoxy groups of lignin during the pulping process. Gas produced during the burning of sulfur containing coal contains significant amounts of COS and $CS_2$. Industrial output of COS and $CS_2$ is relatively low, compared to biogenic output (Khalil & Rasmussen 1984). This can also be expected for MT, DMS and DMDS. Most attention will therefore be paid to natural occurrences and fluxes.

The average natural occurrence of DMS, COS

Table 1. Occurrence of volatile $C_1$-sulfur compounds in the oceans ($10^{-9}$ g S/l) and atmosphere (ppb). Derived from: [1]Khalil & Rasmussen (1984); [2]Brimblecombe (1986); [3]Andreae & Barnard (1984); [4]Cooper & Matrai (1989); [5]Lovelock (1974); [6]Ferek & Andreae (1984).

| Compartment | DMS | COS | $CS_2$ |
|---|---|---|---|
| Ocean | $45^{(1,4)}$ | $3.2–10^{(6)}$ | $0.6^{(5)}$ |
| Atmosphere | $0.001^{(3)}$ | $0.5^{(1)}$ | $0.02^{(1)}$ |

and $CS_2$ in the oceans and atmosphere is presented in Table 1. Average concentrations of MT and DMDS on a global scale are negligible. Oceanic concentrations of DMS were found to be remarkably similar in different regions of the oceans and atmosphere (Andreae et al. 1985; Andreae 1990). In estuaries and coastal waters, however, large variations may occur. Turner et al. (1988;1989) showed that the mean DMS concentration in European coastal waters can be about 3.5 times higher than the world ocean average during spring and summer. As already mentioned, the actual concentrations of organic sulfur compounds are a result of many processes, and may have little significance in themselves. A compound may be rapidly turned over, resulting in its ambient concentration being very low. When a compound is detected in high concentrations, the reverse may be true. However, fluxes of these gases across the ocean-atmosphere boundary are determined by their actual concentrations and by their physical properties, as expressed in the equation derived from Fick's First Law of diffusion (Liss & Slater 1974).

$$F = k.\triangle C \qquad (1)$$

In which F represents the flux along the gradient $\triangle C$. The gradient is determined by the actual concentrations in the atmosphere and the gas phase concentration that would result from equilibrium with the concentration in the ocean, using the Henry coefficient. The exchange velocity along the interface is expressed by the parameter k. Many uncertainties still remain in these calculations, such as the effect of wind speed on the exchange velocity k. Some rough estimates of DMS, COS and $CS_2$ fluxes are presented in Table 2. The flux of sulfur compounds from the atmosphere to the oceans and earth is mainly in the form of sulfate (Brimblecombe et al. 1989). Emission rates of MT and DMDS from terrestrial and coastal areas (marshes) are about 1.3–3.4 Tg S/y (Kelly & Smith 1990). Measurements carried out by Adams et al. (1981) showed that MT was only detected in air samples taken from saline marsh sediments. Emission fluxes varied strongly with location, from $3 \cdot 10^{-4}$ $gS/m^2y$ to 23.5 $gS/m^2y$. DMDS was detected in air samples from saline marshes and peat, with fluxes ranging from $5 \cdot 10^{-3}$ to 1.6 $gS/m^2y$. For comparison, the average oceanic flux of DMS is ca. 0.09 $gS/m^2y$. The obvious magnitude of emissions of MT and DMDS on a local scale indicates that local environmental problems might occur because of the toxic and malodorous properties of these compounds (Table 3). On a global scale, however, MT and DMDS emissions are minor.

### *Environmental impacts of $C_1$ sulfur compounds*

DMS, MT, DMDS, COS and $CS_2$ all have a low boiling point, high toxicity, an offensive odour and low odour threshold (Table 3). The low odour threshold and high toxicity of these compounds make it essential to clean waste gas streams containing them. Most commonly, organosulfur compounds are removed by active carbon adsorption, catalytic conversion to $SO_2$ or $H_2S$, and, more recently, with biofilters (Sivelä & Sundman 1975; Suylen 1988; Wada et al. 1986; Zhang et al.

Table 2. Fluxes of volatile $C_1$-sulfur compounds, with ranges in Tg S/year (1 Tg = $10^{12}$ g). Derived from: [1]Brimblecombe et al. (1989); [2]Khalil & Rasmussen (1984).

| Flux | DMS | COS | $CS_2$ |
|---|---|---|---|
| Ocean-atmosphere | 35 (20–50)[1] | 0.3 (0.2–0.5)[2] | 0.5 (0.2–0.6)[2] |
| Land-atmosphere | 20 (5–35)[1] | 0.4 (0.07–1.5)[2] | 1.4 (0.7–2.5)[2] |

1991a,b). Given their low contribution to the global budget, environmental problems caused by industrial production are local and odour pollution and toxicity are the prime causes of concern.

COS fluxes from terrestrial and oceanic origin to the atmosphere are relatively small (Table 2), but this compound is abundant in the troposphere (Table 1). The explanation is that COS has a lifetime in the troposphere of about two years. This relative stability makes it one of only two sulfur compounds, the other being volcanic $SO_2$, able to reach the stratosphere. COS is therefore one of the main sources of the sulfate aerosol layer in the stratosphere which regulates the earth's radiation balance (Crutzen 1976). An inventory of all sources of COS, and an understanding of the atmospheric processes involved in its transformations, is necessary for the prediction of possible undesirable effects in the stratosphere.

At low altitudes, $CS_2$ has not been detected in significant concentrations in the atmosphere (Khalil & Rasmussen 1984) and at high altitudes (above 6 km), it cannot be detected at all. The atmospheric lifetime of $CS_2$ is about 12 days. $CS_2$ is readily converted to COS, and in this way contributes to the sulfate aerosol layer.

Charlson et al. (1987) suggested that DMS might play an important role in climate control. This hypothesis arose from the following observations: DMS is oxidised in the atmosphere to methanesulfonic acid ($CH_3SO_3H$); cloud condensation nuclei (CCN) contain methanesulfonic acid; the amount of CCN correlates with cloud albedo, as more CCN cause larger numbers of smaller raindrops which reflect the sunlight more effectively (Shaw 1987) and thus influence the radiation balance. These observations were confirmed by Bates et al. (1987), who showed a distinct relationship between the number of CCN and DMS flux; seasonal differences in DMS output and the number of CCN correlated well. Ayers & Gras (1991) indicated a definite relationship between the number of CCN and the concentration of aerosol methanesulfonate throughout the year in marine air, measured over 9 years. It was suggested by Charlson et al. (1987) that DMS emissions might have a negative feedback (i.e. a cooling effect) on the greenhouse warming due to $CO_2$ (Fig. 1). It is generally believed that the average global temperature rises by 2 to 4 degrees Kelvin with a doubling of the $CO_2$ concentration (Houghton et al. 1990). A higher average global temperature would lead to greater primary production of phytoplankton, and therefore to more DMS production and emission. More DMS results in a higher albedo, and this would have a cooling effect on the earth's climate. However, the extra DMS-output is probably not enough to compensate for the predicted $CO_2$ effect. Charlson et al. (1987) estimated that a 30% raise in DMS emission will give cooling of 1.7 degrees Kelvin. However, when all biotic negative feedback systems would be taken into account, this might compensate for the global warming due to the raise in $CO_2$ concentration. Other relevant biological systems with positive or negative feedbacks, e.g. methane production from wetlands and $CO_2$ fertilization (den Elzen et al. 1991), have not yet been incorporated in global climate models and current predictions from these models are necessarily preliminary.

*Table 3.* Properties of some volatile sulfur compounds, derived from Bhatia (1978), Suylen (1988) and the Merck Index & Catalog (1989). Lethal concentrations are expressed as $LD_{50}$. MAC stands for Maximal Accepted Concentration. O.t. stands for odour treshold in gas.

| Compound | $LD_{50}$ (ppm) | MAC (ppm) | O.t. (ppb) |
|---|---|---|---|
| $H_2S$ | 444 | 10 | 8.5–1000 |
| MT | 675 | 0.5 | 0.9–8.5 |
| DMS | 40250 | 20 | 0.6–40 |
| DMDS | 5 | <20 | 0.1–3.6 |

Although strong evidence has been published to support the climate controlling role of DMS, there is, as yet, no explanation for the fact that methanesulfonic acid concentrations are higher in antarctic ice cores from glacial periods than in cores from interglacial periods. Following the Gaian concept, temperature changes due to external causes will be opposed through several mechanisms, of which the feedback mechanism of DMS would be one. Lower temperatures should result in lower primary production, and hence less DMS output and fewer CCN. The cloud albedo would thus diminish and allow more radiation from the sun to reach the earth's surface, resulting in a warming effect (Schneider 1990). This obviously did not happen since more methanesulfonic acid is found in ice cores from glacial periods. A number of possible explanations can be given. For example, colder periods lead to more erosion, resulting in increased nutrient transport to the oceans and hence to increased primary production. Another example arguing against the climatic role of DMS appeared in 1983. Changes in ocean current reduced the upwelling of nutrient-rich cold water, and warm but nutrient-depleted waters remained at the surface. This phenomenon, which recurs regularly, is known as the El Niño southern oscillation (Brock 1984). Primary production of phytoplankton dropped by a factor 5 to 10 (cf. Bates et al. 1987) but DMS emissions stayed roughly the same. These two examples illustrate the difficulty in both predicting future behaviour and explaining the past behaviour of a global ecosystem with currently available knowledge.

MT and DMDS only have an indirect impact on global environmental conditions because they can be converted to DMS.

Summarizing, $C_1$-sulfur compounds, especially DMS, not only have significant roles in many ecosystems, but also in local and global pollution. In the next two sections, sources and sinks of $C_1$-sulfur compounds will be discussed, with the emphasis on DMS. The sources and sinks of these compounds are not independent, because the net fluxes of the different volatile sulfur compounds are the result of their continuous production and consumption, which in turn are controlled by a variety of positive and negative feedback mechanisms. These complex regulating mechanisms result in some form of homeostasis. Clearly, if this was not the case, ecosystems would not display the remarkable resilience to changes in the environment. Understanding the underlying processes, (i.e. the cycles in which these compounds are involved in), and their control mechanisms in terrestrial and oceanic environments will provide more insight into the regulation of the global sulfur cycle and possibly into the mechanisms for climatic control. For convenience, sources and sinks will be discussed separately.

## 3. Sources of $C_1$ sulfur compounds

### *3.1. Sources of DMS*

*DMSP; source of DMS in oceanic environment*

As early as 1935, Haas (cf. Challenger et al. 1957) showed that red algae produce DMS when exposed to air. Challenger et al. showed that dimethylsulfoniopropionate (DMSP) serves as a precursor of DMS. At that time, it was not yet clear that DMS production from DMSP took place on a large scale. However, sensitive analysis has shown that DMS is abundant in marine systems (Lovelock & Maggs 1972; Nguyen et al. 1978; Andreae & Barnard 1984; Turner et al. 1988) and that the main precursor of DMS in marine environment is DMSP. DMSP is produced by many algal species (Table 4). The function of this metabolite is not yet totally clear. The most cited theory is that DMSP is an osmolyte (Dickson et al. 1980). Osmolytes serve as regulators of osmotic balance. Vairavamurthy et al. (1985) showed that the internal DMSP concentration of algae varies with salinity and pH, which strengthens this theory. The osmotic balance cannot be regulated by internal salt concentration because high salt concentrations would inhibit enzymatic activity. Furthermore, the similarity in structure of DMSP with glycine betaine and proline -known plant osmolytes (Dickson et al. 1980)- suggests that these compounds have similar functions. However, there are also theories that DMSP protects against bacterial attack. DMSP can be enzymatically converted to DMS and acrylate (Kadota & Ishida 1972). Sieburth (1961) demonstrated that acrylate is a broad spectrum antibiotic compound. However, Kiene (1990) showed that acrylate can be used by a variety of bacteria as a carbon and energy source. Acrylate might be consumed before it could exert bactericidal effects. It has also been suggested that DMSP is a sulfur storage compound (cf. Dacey et al. 1987) and methylating agent for the production of methionine (Durrell et al. 1957).

Andreae (1990) proposed that high DMSP concentrations in marine organisms in oligotrophic water are related to the scarcity of assimilable nitrogen, i.e. nitrate. Nitrate assimilation into glycine betaine requires less energy than sulfate assimilation in organosulfide compounds. Furthermore, glycine betaine is a more effective osmoregulator than DMSP in high salt concentrations (Dickson et al. 1980), so less must be produced to achieve the same effect. Thus, it may be expected that when nitrate is present in excess, e.g. during eutrophication, glycine betaine will be produced instead of DMSP. It has been shown in laboratory experiments (Turner et al. 1988) that when nitrate is not limiting, production of DMSP diminishes in *Emiliania huxleii* (a Coccolithophore). Dacey et al. (1987) showed that plants such as *Spartina alterniflora* produce less DMSP and more glycine betaine in fertilized soil. Sulfate assimilation requires approximately the same amount of energy as nitrogen fixation (Andreae 1990). This is probably why many cyanobacteria, which can assimilate molecular nitrogen, do not produce a sulfur containing osmolyte (Table 4). It is very difficult to show correlations between oceanic DMSP, DMS, chlorophyll *a* and primary production, because not all algae produce (the same amounts of) DMSP and DMS (Keller et al. 1989; Andreae & Barnard 1984).

*Table 4.* DMSP content of different classes of phytoplankton. Data from Keller et al. (1989). N: number of species tested. $C_{av}$: Average DMSP concentration in $\mu$mol DMSP/cm$^3$ cell volume. N.D.: Not Detectable (> 0.01 $\mu$mol DMSP/cm$^3$ cell volume).

| Class | N | $C_{av}$ (range) |
|---|---|---|
| Centric diatoms | 17 | 23.2 (N.D.–264) |
| Pennate diatoms | 6 | 8.1 (N.D.–41.4) |
| Chloromonads | 2 | N.D. |
| Chlorophytes | 8 | 3.2 (N.D.–25.2) |
| Chrysophytes | 6 | 291 (N.D.–596) |
| Cryptomonads | 8 | 43.1 (N.D.–345) |
| Cyanobacteria | 6 | N.D. |
| Dinoflagellates | 24 | 288 (0.01–2201) |
| Euglenophytes | 1 | N.D. |
| Eustigmatophytes | 1 | 22.8 |
| Prasinophytes | 13 | 108.9 (N.D.–484) |
| Prymnesiophytes | 26 | 145.8 (3.2–412) |
| Rhodophyceae | 2 | 17.8 (N.D.–35.7) |

DMSP is chemically stable in marine systems (Dacey & Blough 1987), having a half life time of about 8 years at 10 °C. Visscher & van Gemerden (1991b) showed that chemical hydrolysis of DMSP can occur in microbial mats at elevated pH, due to depletion of bicarbonate pools by photosynthesis. All conversions to DMS in open waters are, however, biological. DMSP can be broken down enzymatically to DMS and acrylate (Kadota & Ishida 1972):

$$(CH_3)_2S^+CH_2CH_2COO^- \rightarrow \\ (CH_3)_2S + CH_2 = CHCOO^- + H^+ \qquad (2)$$

Challenger et al. (1957) showed that macro algae can enzymatically decompose DMSP to DMS and acrylate. Only a few classes of phytoplankton produce significant amounts of DMS, e.g. Dinophyceae (Dinoflagellates) and Prymnesiophyceae (including Coccolithophores) (Keller et al. 1989). It is known that DMSP can also be converted to DMS outside the algal cells, but it is most unlikely that DMSP passes through intact membranes, because of its size and polarity. DMSP is mainly released when cells are disrupted (Jenkins et al.; cf. Bremner & Steele 1978). This was confirmed by Dacey & Wakeham (1986), who showed that DMS is released when zooplankton grazes on phytoplankton or after algal blooms when cells lose viability. Dacey & Blough (1987) isolated a bacterium from the marine environment which produced DMS and acrylate from DMSP at significant rates. The isolate could grow on acrylic acid, indicating that DMSP is enzymatically cleaved in order to obtain acrylate as a carbon and energy source. Kiene (1990) showed that DMS production from DMSP by bacteria could play an important role in coastal seawater also (Fig. 2). Lyase type enzymes breaking down DMSP in algal cells are clearly also present in a number of bacteria. Lyase activity was also found in filtered water, indicating that soluble lyase enzymes can be present in ocean waters. So far, there have not been any reports on bacteria that are capable of both DMS- and acrylate degradation.

The rates of bacterial DMSP breakdown measured by Kiene (1990) were higher than the DMS production rates measured by Dacey & Wakeham (1986) with zooplankton grazing on phytoplankton. Therefore, Kiene (1990) concluded that this might indicate that grazing zooplankton releases DMSP rather than DMS. Because bacteria are able to break down DMSP at a higher rate than it is normally produced (Dacey & Wakeham 1986), DMSP could not be detected during the measurements. This is a good example of the situation where the actual concentrations of compounds in natural environments do not reflect their ecological significance. DMSP, the key component in this example, cannot be detected because the potential turnover rate into DMS is higher than the rate of production.

*DMSP; source of DMS in sediments*

In the pelagic ocean environment as well as in benthic systems, DMSP is the main precursor of DMS. In 1962 Wagner & Stadtman isolated a *Clostridium* species from river mud. This isolate was capable of anaerobic growth on DMSP, producing DMS, propionic acid, acetic acid and $CO_2$. DMSP in salt marshes originates from macro algae and plants, such as *Spartina* species (de Mello et al. 1987), especially *Spartina alterniflora*. Dacey et al. (1987) argued that only this *Spartina* species contains significant amounts of DMSP, and also produces DMS. Since *Spartina alterniflora* is not present in all salt marshes, emission averages for DMS from salt marshes may have been overestimated.

Kiene & Visscher (1987) and Kiene & Capone (1988) showed that salt marsh sediments have high organo-sulfur decomposition activity. Externally added DMSP was readily converted into DMS, and to a lesser extent into MT, by marsh soil samples. They were not able to show which groups of organisms are active in these conversions. DMSP in salt marshes is not solely degraded to DMS but also to 3-mercaptopropionate (3-MPA) (Kiene & Taylor 1988b). 3-MPA is further metabolized by anaerobic bacteria (Kiene & Taylor 1989). Taylor & Gilchrist (1991) isolated different bacterial species from sediments and algal cultures, able to metabolize DMSP aerobically. They found that DMSP can be cleaved by a lyase or be demethylated. They isolated mainly bacteria capable of demethylating DMSP to 3-methiolpropionate (MPPA). MPPA is further demethylated to 3-MPA or demethiolated to MT. Because numerous bacteria capable of aerobic growth on MPPA were easy to isolate, they concluded that the demethylation of DMSP to MPPA might be a significant process in marine environments. A survey of DMSP conversions in marine systems (sediments and oceanic environment) is presented in Fig. 3. Kiene & Capone (1988) showed that the addition of methionine and S-methylmethionine in marsh sediment samples resulted mainly in the formation of MT. The DMS detected was probably a result of MT methylation. Kiene & Visscher (1987) showed that when MT is added to anoxic salt marsh sediment samples, DMS can be detected after a period of time. Methylation processes as a source of DMS will be discussed at the end of this section.

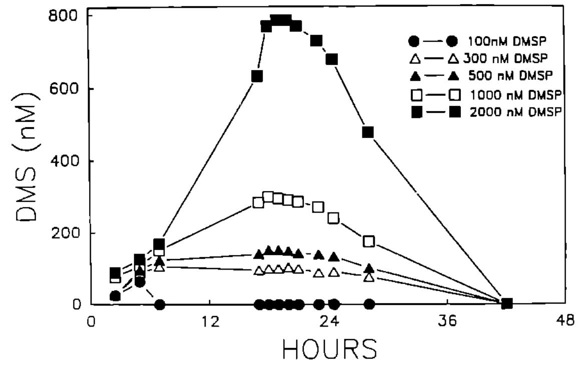


*Fig. 2.* Accumulation of DMS in coastal seawater samples after addition of various concentrations of dissolved DMSP. With permission from Kiene (1990).

*Methoxylated aromatic compounds as a source of DMS*

Bak et al. (1992) isolated bacteria from marine and freshwater mud, which resembled *Pelobacter acidigallici* and produced acetate and DMS from methoxylated aromatic compounds in a sulfide-reduced medium. Methoxylated aromatic compounds are degradation products of lignin, a compound present in both freshwater and estuarine and coastal environments. The isolates were able to cleave the aromatic ring and also to demethoxylate these complex compounds. The release of MT and DMS

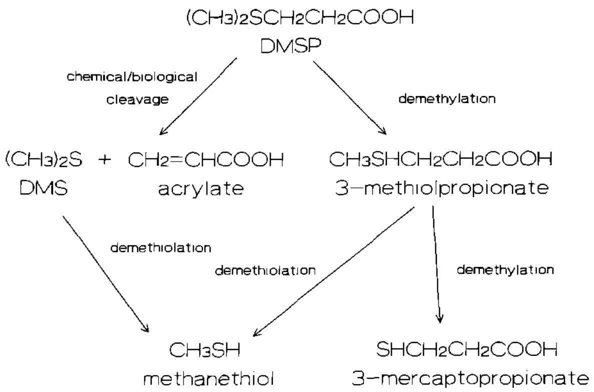


*Fig. 3.* Routes for DMSP degradation in marine environments. Derived from Taylor & Gilchrist (1991) and Visscher & van Gemerden (1991b).

during demethoxylating reactions is a new pathway for volatile organic sulfur compound production in anoxic environments. Its quantative role in natural systems is not clear yet.

### *DMSO reduction*

Another source of DMS production, which has not been defined quantitatively, is DMSO reduction. DMSO is relatively abundant in coastal and oceanic environments, with concentrations ranging from 1 to 200 nmol/l in ocean surface water (Andreae 1980). These values might be slightly overestimated, because, at the time they were measured, detection techniques did not discriminate between DMSO and DMSP (Kiene, in press). Eight axenic cultures of marine phytoplankton (Diatoms, Coccolithophores, Dinoflagellates & Prasinophyceae) were tested in batch and chemostat cultures, and seven were found to produce DMSO, indicating a potential source of DMSO in oceanic waters. Given the argument of Kiene (in press) it is not clear whether the algae were excreting DMSP instead of DMSO. Viability studies on the cultures used for batch and chemostat experiments were not carried out.

Many aerobic and anaerobic Prokaryotes are able to reduce DMSO, e.g. *Escherichia, Proteus, Pseudomonas, Bacillus* and *Clostridium* species (Zinder & Brock 1978a,b). Also Eukaryotes, including higher animals (e.g. cats (Distefano & Borgstedt 1964), and plants, have been shown to reduce DMSO. DMSO is used as a terminal electron acceptor. Valentine-Serano et al. (1991) showed that in *Proteus mirabilis* the reductase is not selective. It reduces DMSO, trimethyl amine N-oxide and nicotinamide N-oxide. Oren (1991) showed that halophilic archaebacteria of the genera *Halobacterium* and *Haloferax* can use DMSO as terminal electron acceptor.

Black et al. (1960) found that enzymes obtained from yeast were able to reduce methionine sulfoxide, a compound resembling DMSO, and that these enzymes were also capable of disulfide reduction to thiols (RSH). These reductive transformations will involve similar enzymes. Rama Rao & Sampath Kumar (1991) reported that they, for the first time, found that baker's yeast catalysed the formation of S-S bonds by the oxidative coupling of thiols. However, it seems likely that this reaction, which is the reverse of the reaction found by Black et al. (1960), involves the same enzymes, and that these enzymes are widespread. Since such enzymes are found in such diverse groups of organisms, it is possible that they have a general role in the control of the redox level. It is not yet clear where DMSO serves as a competitive electron acceptor, and whether DMSO reduction contributes significantly to DMS production. Whether DMSO also originates from the oxidation of DMS, and thus is part of a mini sulfur cycle (Kuenen 1980) driven by light or chemical energy, will be discussed in the section on DMS sinks.

### *DMS production from methylation reactions*

Drotar et al. (1987a,b) found that methyl transferases are widespread among bacteria from different habitats. Methyl transferases are very common in nature; they are found in mammalian and plant cells and in microorganisms. The function of these enzymes is to detoxify sulfide, i.e. to convert it into a volatile compound. In order to test whether bacteria containing methyl transferases might also contribute to the production of methylated sulfides (e.g. MT and DMS), sulfide was added to bacterial cultures. Rapid formation of MT, but no DMS was detected. Methylation of MT in natural habitats cannot be excluded, but the role of this conversion in the total DMS production remains uncertain.

### *DMS production from S-amino acids*

Also in terrestrial areas, DMS and other volatile sulfur compounds are produced during the degradation of sulfur compounds of biological origin, mainly the amino acids methionine and cysteine and the deratives S-methylmethione and S-methylcysteine (Kadota & Ishida 1972; Kiene & Taylor 1988a). DMS may also be produced from MT through methylation reactions, as described by Drotar et al. (1987a,b), and indirectly from DMDS which is chemically, and possibly biochemically, reduced to MT (Kiene & Taylor 1988a). The S-containing amino acids are converted to DMS, MT and $H_2S$ by several species of bacteria.

DMS emissions from terrestrial areas have been reported by Adams et al. (1981), Guenther et al. (1989) and Aneja & Cooper (1989). The total amount of DMS released is strongly dependent on the vegetation. Adams et al. (1981) reported emissions from peat soil in the order of ca $3 \cdot 10^{-3}$ gS/m$^2$y. Crops, such as carrots and corn also generate DMS; ca. 0.1 gS/m$^2$y. Relevant compounds and their decomposition products are presented in Table 5.

In summary, DMSP is the main precursor of DMS in marine systems while in terrestrial areas sulfur containing amino acids give rise to this compound. The contributions of methylation reactions, DMSO reduction and lignin degradation as sources of DMS are still unclear.

## *3.2. MT and DMDS sources*

MT emissions from saline marshes, algal mats, crops and vegetation have been reported by a number of authors (Adams et al. 1981; Zinder et al. 1977; Guenther et al. 1989). MT emissions have also been reported from industrial effluents (Bhatia 1978; Headley 1987). Various microorganisms produce volatile sulfur compounds from methionine and S-methylcysteine (Kadota & Ishida 1972). In terrestrial areas, these mechanisms will be the main source of MT. In saline marsh sediments, methionine and S-methylmethionine are degraded to mainly MT (Kiene & Capone 1988). In these sediments DMDS is quickly reduced to MT (Kiene et al. 1986), and MPPA, a degradation product of DMSP, can be demethiolated to form MT (Taylor & Gilchrist 1991). As mentioned before, Drotar et al. (1987a,b) showed that sulfide is methylated to MT by a number of different species of bacteria.

DMDS emissions have been reported from saline marshes (Adams et al. 1981), soils, crops and vegetation (Guenther et al. 1989), *Spartina* fields (de Mello et al. 1987), municipal waste systems (König et al. 1980) and waste streams from the paper industry (Sivelä & Sundman 1975). Microorganisms isolated from different habitats degrade methionine to DMDS. These include *Pseudomonas, Aspergillus, Clostridium, Alteromonas, Achromobacter, Lactobacillus* and *Corynebacterium* (Tomita et. al. 1987). Mechanisms for DMDS formation have not yet been investigated, but Kelly & Smith (1990) suggested that DMDS formation is, at least partly, a result from oxidation and dimerization of MT to DMDS.

## *3.3. COS sources*

COS has been detected during biomass burning,

*Table 5.* List of the S-containing amino acids and their decomposition products. Derived from Kadota & Ishida (1972), Taylor & Kiene (1989).

| | |
|---|---|
| Methionine<br>$CH_3S(CH_2)_2CH(NH_2)COOH$ | MT, 2-Ketobutyrate, ammonia<br>$CH_3SH$, $CH_3CH_2COCOOH$, $NH_3$ |
| S-methylmethionine<br>$(CH_3)_2S(CH_2)_2CH(NH_2)COOH$ | DMS, homoserine<br>$(CH_3)_2S$, $CH_2OHCH_2CH(NH_2)COOH$ |
| S-methylcysteine<br>$CH_3SCH_2CH(NH_2)COOH$ | MT, pyruvic acid, ammonia<br>$CH_3SH$, $CH_3COCOOH$, $NH_3$ |

fossil fuel combustion, volcanic activity and above salt marshes (cf. Ferek & Andreae 1984), soils and estuaries (Jörgensen & Okholm-Hansen 1985). Total fluxes from these sources are presented in Table 6. Atmospheric production of COS occurs through $CS_2$ oxidation. COS is released by various tree species, such as *Deciduous* and *Coniferous* species (cf. Aneja & Cooper 1989). COS emissions from soil are caused by biological processes (Adams et al. 1981). COS and DMS production have been observed at the same time in a danish estuary, suggesting a biogenic source of COS (Jørgensen & Okholm-Hansen 1985). In the ocean surface layer COS concentrations were found to be independent of salinity, photosynthesis or microbial activity, but dependent on light intensity (Ferek & Andreae 1984). COS can be produced by UV radiation of seawater containing biochemically relevant organosulfur compounds such as methionine, cysteine, glutathione and DMSP. The authors observed COS supersaturation (up to a factor 10) of the ocean top layer, and concluded that earlier emission fluxes might have been underestimated, especially in coastal areas. Andreae (1990) showed that the total COS emission flux is dominated by high productivity coastal and shelf areas. It might be expected that organosulfur compounds are more abundant in eutrophic coastal waters than in the oligotrophic regions of the oceans. As Table 6 shows, COS mainly originates from natural sources. The exact mechanisms of COS production are still unclear.

*Table 6.* Fluxes of COS from different sources (Khalil & Rasmussen, 1984). Values with superscript 1 were derived from Steudler & Peterson (1984).

| Source | Emission (Tg/y) |
|---|---|
| Oceans | 0.60 (0.3–0.9) |
| Soils | 0.40 (0.2–0.6) 1.88[1] |
| Volcanoes | 0.02 (0.01–0.05) |
| Marshes | 0.02 (0.01–0.06) 0.12[1] |
| Industry | 0.20 (0.1–0.5) |
| $CS_2$ oxidation | 0.6 (0–2) |

### *3.4. $CS_2$-sources*

$CS_2$ is primarily produced from oceans and soils (Table 7). $CS_2$ was found in oceans (ca 1 ng/l), especially in the anaerobic mud of the sea floor (Lovelock 1974). Some plants are known to release $CS_2$, including *Spartina Alterniflora* and *Stryphnodendron exculsum* (Aneja & Cooper 1989). The exact mechanisms of formation are still unknown.

## 4. Sinks for $C_1$ sulfur compounds

In Section 3, continuous fluxes of volatile S-compounds from various sources to the atmosphere were discussed. As pointed out, the concentrations of these gases in the atmosphere and marine environment are reasonably constant, and hence sinks also must exist. This section will review atmospheric, marine (pelagic and benthic) and terrestrial sinks for $C_1$-sulfur compounds.

### *4.1. Sinks for DMS*

#### *4.1.1. Atmospheric sinks*

From the 'black box' approach, it is clear that there must be considerable sinks for $C_1$-sulfur compounds in the atmosphere. That is, the sulfur reaching the atmosphere (ca. 35 Tg S/y from the oceans) must also be removed, since atmospheric concentrations are relatively constant. DMS in the atmosphere is readily oxidised by hydroxyl radicals and by $NO_x$, giving several products, including methanesulfonic acid. The average lifetime of DMS in the atmosphere is about 1 day (Brimblecombe 1986). Experiments carried out by Grosjean & Lewis (1982) showed that sulfur dioxide, formaldehyde, ozone, nitric acid, methyl nitrate, inorganic sulfate, methanesulfonic acid and DMSO are formed when DMS is oxidised under atmospheric conditions. DMS reacts with the hydroxyl radical in two ways: an addition reaction from which $CH_3S(OH)CH_3$ is formed and an abstraction reaction, producing $.CH_2SCH_3$. Reaction with oxygen then gives methanesulfonic acid. Methanesulfonic acid can be further oxidised, but it has been shown (cf. Brimblecombe 1986) that, in some cases, as much as 80% may remain. Methanesulfonic acid, as well as other gaseous sulfur compounds, is eventually removed from the atmosphere through both dry and wet deposition. Recently, Baker et al. (1991) isolated several strains of bacteria from soil that can grow on methanesulfonate. In order to help to complete the earth-atmosphere-earth cycle of DMS → methanesulfonic acid → $CO_2$ and $SO_4^{2-}$ → DMS, the authors proposed that these bacteria are ubiquitous in natural systems.

### *4.1.2. Sinks in marine environments*

*Sinks in the open ocean.* As pointed out in the introduction to this section, oceanic sinks for DMS must exist. The DMS flux between the oceans and the atmosphere is not solely controlled by the rate of production of DMS from DMSP. That this is actually the case was shown by Andreae & Barnard (1984). They measured vertical concentration profiles of DMS in the Atlantic Ocean, and showed that there is a steep gradient at the base of the euphotic zone. This gradient is steeper than might be expected if only production, convection and diffusion of DMS are taken into account. From this, together with the observation that DMS was consumed by mixed populations of marine bacteria in sea water, they concluded that DMS is consumed in the euphotic zone. So far three mechanisms have been suggested for DMS consumption in the euphotic zone of the ocean:

- Microbiological degradation (Andreae & Barnard 1984; Kiene & Bates 1990)
- Chemical oxidation, i.e. autooxidation and oxidation with peroxide to DMSO and $DMSO_2$ (Brimblecombe & Shooter 1986; Shooter & Brimblecombe 1989).
- Particle adhesion and transport to deep sea (Shooter & Brimblecome 1989).

This last mechanism was proposed to explain the presence of DMS in the deep sea. Andreae & Barnard (1984) concluded from the presence of DMS in the deep sea ($>100$ m) that DMS is resistant to biotic attack. However, a continuous flow downwards and a continuous consumption in the deep sea is also possible (Shooter & Brimblecombe 1989). It is also possible that DMS in deep sea has a deep sea source, but this has not been fully investigated yet. To compare the contributions of different sinks, the DMS conversion can be expressed in average residence times. Residence time is defined as the volume (water or air) considered, divided by what is removed of the compound in time (Kiene & Bates 1990). Using data presented by Shooter & Brimblecombe (1989), residence times for chemical oxidation using $H_2O_2$, autooxidation and particle adhesion can be calculated as 230, 525 and $8 \cdot 10^5$ days, respectively. It is known that autooxidation in the presence of photosensitizers (e.g. methylene blue, humic acid) takes place at higher rates (Brimblecombe & Shooter 1986), but the nature of the photosensitizers in oceanic environment is unclear. For microbial oxidation Kiene & Bates (1990) determined residence times varying from 0.6 to 4.6 days. Using inhibitors such as chloroform and glutaraldehyde it was possible to show that the conversion in the oceans was biological, and most probably carried out by $C_1$-metabolizing bacteria. To determine the importance of these processes, compared with atmospheric ventilation of DMS, residence times for DMS in the oceanic top layer were determined with respect to atmospheric ventilation. Residence times varying from 14 to 520 days were found. From these figures, it may be concluded that bacterial oxidation takes place at higher rates than atmospheric ventilation, implying that there is a great potential for DMS metabolism in the oceans. The biological cycles in which DMS plays a part thus have a significant impact on the total DMS output. Since DMS conversion will be influenced by environmental factors such as temperature and nutrient availability, these microbiological cycles may have a great impact on

*Table 7.* Sources of $CS_2$. Derived from Khalil & Rasmussen (1984) and Steudler & Peterson (1984) (sup 1).

| Sources | Emission (Tg/y) |
|---|---|
| Oceans | 0.6 (0.2–0.7) |
| Soils | 0.9 (0.5–2) $2.8^1$ |
| Volcanoes | 0.02 ($<0.1$) |
| Marshes | 0.1 (0.05–0.2) |
| Industry | 0.35 ($<0.7$) |

climate control, while the contribution of chemical oxidation and particle adhesion to the conversion of DMS is very small. Microbial sinks will therefore be discussed in further detail.

*Aerobic transformation of DMS in marine environments.* Microbial breakdown of DMS in the open ocean will largely be aerobic, since DMSP, the main precursor of DMS in the oceans, is mainly produced by phototrophic, oxygen producing organisms and DMS is found in oxygen-rich environments. The use of electron acceptors other than oxygen under aerobic conditions has been reported (e.g. Robertson & Kuenen 1984, Fründ & Cohen 1992), but the significance of these processes in oceanic environments is not yet clear.

Bacteria, capable of catabolizing DMS to carbon dioxide and sulfuric acid, have not yet been isolated from the open oceans. These species may be expected to resemble DMS-oxidizing bacteria isolated from soils, sediments, activated sludge, etc. In particular, the chemolithoautotrophic and methylotrophic bacteria are likely to play a significant role. From their studies with inhibitors, Kiene & Bates (1990) concluded that $C_1$-metabolizers are probably responsible for most bacterial DMS removal from oceanic waters. The difficulties that our own laboratory, and that of others have encountered in the enrichment of DMS metabolizing bacteria from marine water bodies may well be related to the general problem of isolating bacteria from oligotrophic environments. The identification of the dominant bacteria, capable of DMS metabolism in any ecosystem, will need special techniques for their detection, enrichment and identification. In addition, in order to explain DMS-cycles in aquatic systems, an understanding of the (eco)physiological and biochemical properties of these bacteria must be acquired.

Isolation of DMS metabolizers from marine sediments has been successful. Visscher et al. (1991) isolated a colourless sulfur bacterium, later identified as *Thiobacillus thioparus* T5, from a microbial mat, using thiosulfate as an energy source. This isolate was able to metabolize DMS aerobically to carbon dioxide and sulfuric acid. Furthermore, they obtained evidence that about $2 \cdot 10^5$ cells/$cm^3$ sediment in the upper 5 mm of the microbial mat were capable of growth on DMS. This estimate was done with a MPN technique in which DMS removed from the headspace was used as a criterium for growth. However, this did not lead to the identification of dominant species.

DMS can be enzymatically oxidised to DMSO (Jocelyn 1972). This reaction has been observed in eukaryotes (higher animals and plants) and in bacteria (cf. Andreae 1980). The oxidation is carried out by monooxygenases, which are widespread among animals, plants, fungi and bacteria (Holland 1988). A function of these enzymes appears to be the detoxification of xenobiotics. Further oxidation of DMSO to $DMSO_2$ is catalysed by haloperoxidases, which are common in marine microorganisms (Neidleman & Geigert 1986). The quantitative role of this oxidation in the natural environment is unknown.

*Anaerobic breakdown of DMS in sediments.* Zinder & Brock (1978c) reported the production of methane and carbon dioxide after the addition of methylated sulfides to anaerobic freshwater lake sediments. Chloroform inhibited all methane production, leading to the conclusion that methanogenic bacteria were mainly responsible for the conversion. A pure culture of a methanogen, able to grow on DMS, was not isolated until 1986 (Kiene et al.), after which research on the anaerobic metabolism of DMS developed. Using selective inhibitors for methanogens and sulfate reducers, Kiene et al. (1986) concluded that sulfate reducers and methanogens were competing for DMS when it was present at low concentrations ($< 10\ \mu M$). At high concentrations, DMS appeared to be a substrate for methanogens only. An obligately methylotrophic methanogen was isolated and shown to metabolize DMS to methane and carbon dioxide. From the $CH_4/CO_2$ ratio of 2.8 , and the ability of the isolate to metabolize MT, the following reactions for DMS degradation were proposed:

$$(CH_3)_2S + 2H^+ + 2e^- \rightarrow CH_4 + CH_3SH$$
$$\Delta G_f = -72.8 \text{ kJ/mol} \qquad (3)$$

$$CH_3SH + H_2O \rightarrow$$
$$1/2CH_4 + 1/2CO_2 + H_2S + 2H^+ + 2e^- \quad \triangle G_f = -1 \text{ kJ/mol} \quad (4)$$

Resulting in:

$$(CH_3)_2S + H_2O \rightarrow 1\ 1/2CH_4 + 1/2CO_2 + H_2S \quad \triangle G_f = -73.8 \text{ kJ/mol} \quad (5)$$

The theoretical value of the methane/carbon dioxide ratio is three, and coincides well with the experimental ratio of 2.8.

It should be realized that reaction (4) is a disproportionation reaction where MT serves both as an electron acceptor and electron donor according to the two following reactions:

$$CH_3SH + 2H^+ + 2e^- \rightarrow CH_4 + H_2S \quad (6)$$

$$CH_3SH + 2H_2O \rightarrow CO_2 + H_2S + 6H^+ + 6e^- \quad (7)$$

Thus a combination of reactions (3), (6) and (7) leads to a closed electron balance. However, if an organism were to grow on MT only, one cannot use reaction (4) only (Kiene et al. 1986), but it must be taken into account that the electron accepting reaction (6) must balance the electron donating reaction (7). As pointed out by Finster et al. (1992), this leads to the following combined reaction:

$$4CH_3SH + 3H_2O \rightarrow$$
$$3CH_4 + HCO_3^- + 4HS^- + 5H^+ \quad \triangle G^{o1} = -36.9 \text{ kJ/mol} \quad (8)$$

Finster et al. (1992) succesfully isolated an anaerobic bacterium growing on MT. They observed a yield of 3 gr/mol MT. In view of the correlation between thermodynamically based data and experimental yields (Heijnen 1991) a yield of 3 gr/mol MT for an energy donating reaction of −37 kJ/mol MT would call for further theoretical explanation.

Activity of methanogenic and sulfate reducing bacteria that metabolize DMS have also been found in anoxic salt marsh sediments (Kiene & Visscher 1987; Kiene & Taylor 1988a). A variety of DMS-metabolizing methanogens have been isolated from different habitats;*Methanohalophilus oregonense* from an alkaline saline lake (Liu et al. 1990), *Methanohalophilus zhilinae* (Mathrani et al. 1988) and *Methanolobus siciliae* HI 350, isolated from an oil well (Ni & Boone 1991). Methanogens examined by Rajogopal & Daniels (1986) were able to use methylated sulfides and carbon disulfide as sulfur sources. Demethylation reactions in anaerobic sediments (freshwater, estuarine or hypersaline) take place not only with the methylated sulfides, but also with methyl mercury, methyl selenide and dimethyl selenide, possibly catalysed by the same bacteria (Oremland & Zehr 1986; Oremland et al. 1991). As already mentioned, acetogenic bacteria that are able to convert methoxylated aromatic compounds to acetate and DMS, using hydrogen sulfide as reducing agent have been isolated (Bak et al. 1992). Acetate is further metabolized by sulfate reducing bacteria and the sulfide is regenerated through DMS breakdown by methanogens (Finster et al. 1990). In this cycle, sulfate reducers are only indirectly involved in DMS metabolism. Sulfate reducers using DMS as carbon and energy source have not been isolated yet and further research is needed before conclusions can be drawn about the role of sulfate reducers in the 'direct' breakdown of DMS.

*DMS oxidation by phototrophic microorganisms.* In anoxic marine sediments, DMS can be used as an electron donor for $CO_2$ fixation by phototrophic bacteria. DMS is converted to DMSO. This may in turn be used as an electron acceptor in anaerobic environments, leading to DMS regeneration. This is an example of a light driven mini sulfur cycle (Kuenen 1980). Zeyer et al. (1987) postulated that some anaerobic phototrophic sulfur bacteria will be able to metabolize DMS as well as $H_2S$. Indeed, they found a *Thiocystis* like isolate in a stratified coastal salt pond, that was able to support growth by DMS oxidation to DMSO. However, growth on DMS as the sole source of carbon was negligible. During mixotrophic growth on DMS and $H_2S$ a yield of 5 g protein/mol DMS was observed. This is close to the theoretically predicted yield if DMS is quantitatively converted to DMSO. A similar yield was found for *Thiocapsa roseopersicina*, a purple sulfur bacterium isolated from a microbial mat

(Visscher & van Gemerden 1991a). This species is able to grow photoautotrophically on DMS, converting it stoichiometrically to DMSO. $H_2S$ can also be used as an electron donor. Both enzyme systems are constitutive in *Thiocapsa*, which shows that the bacterium is well-adapted to the short-term, fluctuating conditions that occur in microbial mats exposed to diurnal variations. It is not clear whether DMS is the major source of DMSO in anaerobic sediments. Zeyer et al. (1987) indicated that the DMSO concentration in the hypolimnion of a coastal salt pond was significantly higher than the DMS concentration, suggesting that DMS is the major source of DMSO. However, as pointed out above, turnover rates of DMS must be known before this suggestion can be substantiated.

### *4.1.3. Terrestrial sinks*

In oceanic environments, it was proved that sinks for DMS must exist. In terrestrial areas, this is more difficult. However, since DMS is produced, microorganisms capable of DMS degradation will be present. Not much research has been done to find DMS metabolizing bacteria from natural terrestrial sources. Most microorganisms that have been found were isolated from industrial sources. It is very likely that similar organisms are found in the natural environment. A number of these isolates and their physiological properties will be discussed in some detail here.

*Physiology of aerobic DMS metabolizing bacteria.* A number of aerobic microorganisms, able to utilize DMS have been isolated from sewage, biofilters and soil. A list of these organisms and their sources is presented in Table 8. Sivelä & Sundman (1975) were the first to describe the isolation of a *Thiobacillus* species, capable of DMS degradation, from a biofilter in a cellulose mill. However, Suylen & Kuenen (1986) showed that the available cultures of *Thiobacillus* MS 1 had lost their capacity for DMS metabolism. With exception of *Hyphomicrobium* VS, all species shown in Table 8 were enriched and isolated on compounds other than DMS. Isolating species using DMS as the sole source of energy in batch system by the MPN method still presents problems because of the toxicity and volatility of DMS. Smith & Kelly (1988a,b) made enrichments for DMS utilizers, but were unable to obtain a pure culture on plates with DMS in the headspace. Using thiosulfate plates, *Thiobacillus thioparus* E6 was isolated from the mixed culture, and in this way they selected for autotrophic organisms. As can be seen in Table 8 also several fungi have been found to oxidize DMS in waste gases (Phae et al. 1991).

Mechanisms for DMS oxidation have been examined for *Hyphomicrobium* S, *Hyphomicrobium* EG and *Thiobacillus thioparus*. Hyphomicrobium S was isolated on DMSO (De Bont et al. 1981). Growth experiments and enzyme studies revealed the pathway for DMSO degradation presented in

*Table 8.* List of species, able to convert DMS, isolated from different terrestrial environments.

| Species | Source | Reference |
|---|---|---|
| *Thiobacillus* MS1 | Biofilter | Sivelä et al. (1975) |
| *Thiobacillus thioparus* TK-m | Sewage | Kanagawa et al. (1982) |
| *Thiobacillus thioparus* E6 | Numerous | Smith & Kelly (1988a) |
| *Thiobacillus thioparus* DW44 | Peat biofilter | Cho et al. (1991b) |
| *Hyphomicrobium* S | Soil | de Bont et al. (1981) |
| *Hyphomicrobium* EG | Peat biofilter | Suylen et al. (1986) |
| *Hyphomicrobium* sp. 155 | Peat biofilter | Zhang et al. (1991a) |
| *Hyphomicrobium* VS | Activated sludge | Pol et al. (1991) |
| *Pseudomonas acidovorans* | Peat filter | Zhang et al. (1991b) |
| *Basidiomycete* (fungi) | Soil | Phae et al. (1991) |
| *Deuteromycotina* (fungi) | – | cf. Phae et al. (1991) |
| *Zygomycotina* (fungi) | – | cf. Phae et al. (1991) |

Fig. 4, later confirmed and supplemented by Suylen et al. (1986). The serine pathway for carbon assimilation was proposed, because high activity of hydroxypyruvate reductase, a key enzyme of the serine pathway, was detected. With this pathway 1 formaldehyde must be fully oxidised to provide NADH for DMSO and DMS conversions, which both require energy. *Hyphomicrobium* S is therefore a heterotrophic organism. *Hyphomicrobium* EG, which was also isolated on DMSO, could be grown at high yield in a DMS-limited chemostat (Suylen & Kuenen 1986). Under these conditions, the $K_s$ for DMS was 3 μM, as compared to 16 μM during growth on DMSO. The enzymes for DMS(O) breakdown appeared to be constitutive. In addition to high hydroxypyruvate reductase activity, strong activity of serine glyoxylate amino transferase, another key enzyme of the serine pathway, was found. *Hyphomicrobium* EG was able to use the sulfur in DMS as extra energy source. Evidence for this capability was obtained from yield studies with methylamine, thiosulfate and sulfide (Table 9) (Suylen et al. 1986), confirming the chemolithoheterotrophic nature of the strain. The enzyme that oxidizes MT to sulfide, peroxide and formaldehyde, MT oxidase, has an affinity constant ($K_s$) of 5–10 μM for MT. It is sensitive to all four reaction components, but especially to MT, with an inhibition constant ($K_i$) of 42 μM (Suylen et al. 1987). This might explain the high MT oxidase and catalase activities found in the cells, to ensure low concentrations of the reaction components. Gould & Kanagawa (1992) purified the MT oxidase of *Thiobacillus thioparus* TK-M, and found a higher $K_s$ (31 μM) for MT, sulfide was not inhibitory. Different enzymes appear to be involved in MT oxidation by *Thiobacillus* and *Hyphomicrobium* species.

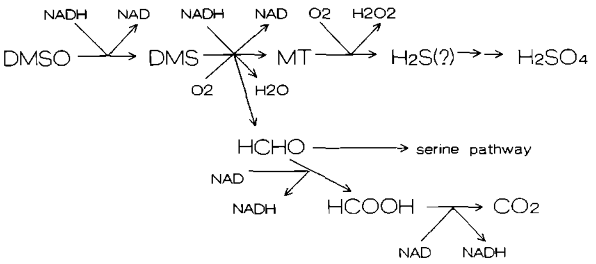


*Fig. 4.* Mechanism for DMSO degradation for *Hyphomicrobium* S. (de Bont et al. 1981).

The exact DMS degradation pathway used by *Thiobacillus thioparus* is still unclear. Following oxidation stoichiometry (Kanagawa & Kelly 1986), DMS should be oxidized to methanol and sulfuric acid. However, methanol was not detected. In analogy with dimethylamine and methylamine oxidation, the following stoichiometry of DMS oxidation was suggested:

$$(CH_3)_2S + 3O_2 \rightarrow 2HCHO + H_2SO_4 \qquad (9)$$

Growth in the presence of $^{14}CO_2$ showed that the $^{14}CO_2$ was diluted with $^{12}CO_2$, indicating that the formaldehyde was further oxidized to carbon dioxide. *Thiobacillus thioparus* was thought to be an obligate chemolithoautotroph, only capable of oxidizing inorganic sulfur compounds, but these experiments showed that some strains have additional abilities. MT, DMDS and $H_2S$ were also shown to be removed from contaminated air by *Thiobacillus thioparus* TK-m (Kanagawa & Mikami 1989), giving cell yields on DMS, $H_2S$, MT and DMDS of 21, 9.2, 20 and 33 g/mol, respectively. These yields agreed with yields found for *Hyphomicrobium* EG of 19 g/mol for DMS and 8 to 10 g/mol for $H_2S$, indicating that *Thiobacillus thioparus* is able to use energy from the methyl groups as well as from sulfide. The higher yields observed with MT and DMDS can be explained on the basis of the path-

*Table 9.* Yield data of Hyphomicrobium EG. Dry weight yields in relation to different amounts of thiosulfate or sulfide added in methylamine (MA) limited chemostat experiments at a dilution rate of $0.035\,h^{-1}$ are given. Derived from Suylen et al. (1986).

| Substrate | Dry wt (mg/l) |
|---|---|
| 10 mM MA | 108 |
| 10 mM MA + 0.84 mM $S_2O_3^{2-}$ | 116 |
| 10 mM MA + 4.4 mM $S_2O_3^{2-}$ | 144 |
| 10 mM MA + 6.9 mM $S_2O_3^{2-}$ | 165 |
| 9.8 mM MA + 0.93 mM $S^{2-}$ | 116 |
| 9.8 mM MA + 5.32 mM $S^{2-}$ | 152 |
| 9.5 mM MA + 10.3 mM $S^{2-}$ | 212 |

way, because conversion of DMS to MT requires energy in the form of NADH (Fig. 4).

Other species capable of metabolizing DMS (Table 8), were isolated from peat biofilters. Research on these bacteria is mainly carried out for practical purposes, i.e., in the construction of biofilters that remove DMS, MT, DMDS and $H_2S$ from contaminated air. In *Pseudomonas acidovorans*, DMS is oxidized to DMSO in an NADH dependent reaction, provided that other organic carbon sources are present. If the DMSO concentration is high, the reverse reaction takes place (Zhang et al. 1991a). The maximum DMS removal rates and the substrate affinities for the different species are listed in Table 10, which also shows data on the marine *Thiobacillus thioparus* T5. General conclusions about maximum rates and efficiency of DMS removal by the different species cannot be drawn from the data in Table 10. It should be remembered that data from biofilter experiments and continuous culture experiments are compared. Furthermore, it is well known that immobilized cells behave differently from suspended cells. However, these figures can serve for a rough comparison of the different species and their DMS oxidizing capabilities. The lowest $K_s$ value (3 μM) was found for *Hyphomicrobium* EG (Suylen & Kuenen 1986). If this value is compared with the actual concentration of DMS in oceanic systems (1 to 3 nM), it becomes clear that the species in natural systems must have different capacities from the bacteria presented in Table 10, or that the $K_s$ value is not a relevant parameter. This problem has been encountered before. Conrad (1983) showed that hydrogen consuming isolates could not be good representatives of the hydrogen consumers in natural environments, because the maximum consumption rate ($v_{max}$) and $K_s$ values of these bacteria were not sufficient to provide enough energy even for maintenance. The existence of 'high-affinity' microorganisms is required to explain the conversion rates observed in natural systems. The discrepancy between isolated strains and natural communities could be due to a number of factors, including bias imposed by isolation techniques or a balance requiring a community rather than a single population. Data on average residence times of DMS with respect to atmospheric ventilation and microbial degradation (Kiene & Bates 1990) showed that the residence times for atmospheric ventilation are an order of magnitude larger than for microbial degradation. A rough estimate is that the amount of DMS metabolized will be 10 times as high as the atmospheric ventilation. This means that about 350 Tg S/year is microbially converted. The average kinetic properties of the strains shown in Table 10 can be used to calculate how many of these bacteria need to be present in the oceans, to account for 350 Tg S removal. Assuming that the cell diameter is 1 μm, the specific cell density 1000 g/l, and that conversion only occurs in the top 50 meters of the ocean, one arrives at $10^6$ DMS oxidizing bacteria. Given that the amount of bacteria in ocean water is $10^6$–$10^8$ cells/ml, it should be possible to isolate

*Table 10.* Maximum specific DMS removal rates and the affinity expressed as $\mu/K_s$ (l-h.mmol). For purposes of comparison, all rates are expressed in gS/g biomass.day. For some data, (*), it was necessary to make the following assumptions: cell diameter 1 μm, specific cell density 1000 g/l, dry weight 15% of total cell mass. For the references, see the text.

| Species | Condition | Removal rate | Affinity |
|---|---|---|---|
| *Thiobacillus thioparus* TK-m | Respiratory experiment | 16 | 1.33 |
| *Thiobacillus thioparus* TK-m | Biofilter | 4* | 1.11 |
| *Thiobacillus thioparus* T5 | Continuous culture | 0.12 | – |
| *Thiobacillus thioparus DW44* | Biofilter | 1.26* | – |
| *Hyphomicrobium* S | Continuous culture | 0.56 | 0.88 |
| *Hyphomicrobium* EG | Continuous culture | 1.4 | 26.7 |
| *Hyphomicrobium* VS | Continuous culture | 32 | – |
| *Hyphomicrobium* sp. 155 | Biofilter | 3.7* | – |
| *Pseudomonas acidovorans* | Biofilter | 0.11* | – |

some of these DMS-metabolizing bacteria. However, as pointed out earlier, isolating these bacteria from the open oceans has proved to be very difficult, and it seems likely that their physiology is very different from the physiology of the bacteria so far isolated and that the isolation techniques used are not suitable.

### *4.2. MT and DMDS sinks*

MT and DMDS emissions from natural systems are relatively low, compared to DMS and $H_2S$. The larger part of MT and DMDS produced in terrestrial and sediment environments is probably converted within these systems. Since MT is found to be an intermediate in aerobic and anaerobic DMS metabolism, all DMS metabolizing bacteria are most probably also capable of degrading MT. Cho et al. (1991a) isolated a *Thiobacillus* species (HA43), capable of MT and $H_2S$ degradation from a peat biofilter. MT removal rates were 26 gS/g biomass.day, which makes this isolate very suitable for biodeodorization systems. The strain was incapable of DMS degradation. Anaerobic MT metabolism was already discussed in the section on anaerobic DMS metabolism.

DMDS-oxidizing bacteria were isolated by Smith & Kelly (1988a,b), using batch cultures on DMDS and DMS. One of those isolates, *Thiobacillus thioparus* strain E6, was further characterized. The proposed mechanism involves a reductive cleavage of DMDS, using NADH, producing MT. MT is oxidized to formaldehyde and $H_2S$, which are further metabolized to $CO_2$ and $SO_4^{2-}$, respectively. Ribulose bisphosphate carboxylase was found in significant amounts, indicating that carbon dioxide assimilation takes place using the Calvin cycle, as might be expected with an autotrophic *Thiobacillus* species. Hydroxypyruvate reductase, present in *Hyphomicrobium* S and *Hyphomicrobium* EG (serine pathway), was not detected. It was concluded that *Thiobacillus* like species play an important role in degradation of methylated sulfides (Smith & Kelly 1988c). However, the method of isolation employed was selective for thiosulfate-using autotrophs, and hence for *Thiobacillus* like species. It should be recalled that Kiene & Bates (1990) obtained indirect evidence with $C_1$-metabolism inhibitors that methylotrophs may play a role in oceanic DMS metabolism too.

### *4.3. COS and $CS_2$ sinks*

The flux of COS to the atmosphere (about 2 to 7 Tg/y according to Khalil & Rasmussen 1984 and Steudler & Peterson 1984) is substantially greater than the 0.3 Tg/y needed to maintain the sulfate aerosol layer (Sze & Ko 1979). The sulfate aerosol layer consists mainly of sulfuric acid and water between 20 and 30 km up in the stratosphere. It is assumed that anthropogenic $SO_2$ (e.g. from fossil fuel burning) does not contribute to the sulfate aerosol layer as it cannot reach the stratosphere because of (acid) precipitation in rain. If some anthropogenic $SO_2$ reached the sulfate aerosol layer, even more COS would be unaccounted for. DMS, MT and $H_2S$ cannot reach this layer due to fast chemical removal in the troposphere. It must therefore be concluded that there must be significant COS sinks. Ultraviolet photolysis converts COS to $SO_2$ and $CO_2$ above 20 km in the atmosphere. This removes about 0.8–1.0 Tg/y (Khalil & Rasmussen 1984), leaving approximately 0.8–4.2 Tg/y unaccounted for. Turco (1981) suggested hydrolysis in the oceans as a significant sink. This seems highly unlikely since COS appears to be supersaturated in ocean water. From the data evaluated by Khalil & Rasmussen (1984) and Brown & Bell (1986) there appears to be at least a missing sink of about 1 Tg/y COS.

Brown & Bell (1986) proposed vegetation as the sink that would close the global COS cycle. COS-uptake by vegetation may account for 2–5 Tg/y. (comparing this with an estimated soil uptake of ca. 0.04 Tg/y). Agricultural crops could take up enough COS to account for the missing sink, and convert it to $H_2S$ and $CO_2$. $H_2S$ is then converted into sulfate and used in amino acid production. Carbonic anhydrase enzymes which hydrolyse COS had already been found in animals and it was assumed that plants would have similar mechanisms. Following the proposed model, COS-uptake should rise with rising COS concentrations. It is unclear whether rising amounts of COS in the atmosphere (caused by anthropogenic sources) will be equally distributed among several sinks. Turco (1980) considered that increasing COS emissions could lead to measurable climatic alterations within the next century. If the only sink for atmospheric COS was the aerosol sulfate layer, this would perhaps be the case. However, regulation of the COS level in the atmosphere is probably more complex.

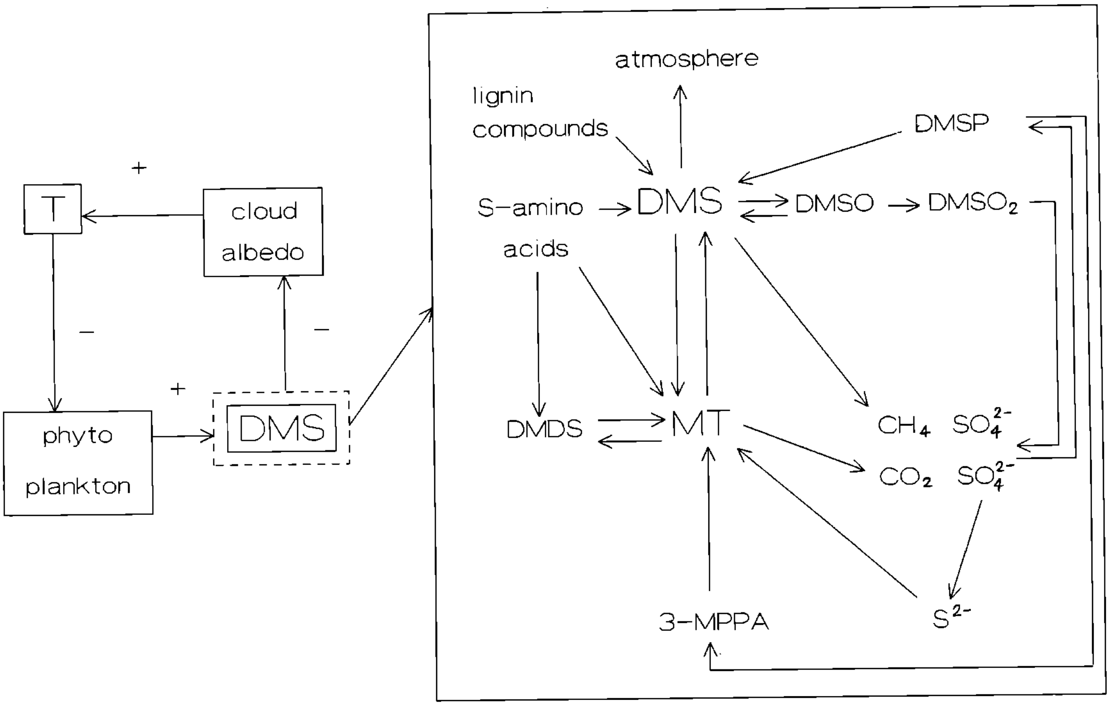
$CS_2$ is readily chemically converted to COS by hydroxyl and oxygen radicals (cf. Turco 1980 and Khalil & Rasmussen 1984). The average lifetime of $CS_2$ in the atmosphere is about 40 days. The calculations carried out by Khalil & Rasmussen show that ca. 1.3 TgS/y is not accounted for, using the accepted reaction constants of the above reactions. However, if the reaction rates are higher, there is even more COS unaccounted for. $CS_2$ uptake by vegetation or soils has not been reported.

Several *Thiobacillus*-like strains, isolated on DMDS, from diverse habitats were capable of growing on COS, but not on $CS_2$ (Smith & Kelly 1988a). It was argued that methylated sulfides are common substrates for many microorganisms, because the organisms can benefit from the sulfur and the methyl group. In the case of $CS_2$ and COS, only autotrophic growth is possible, narrowing the range of bacteria that will use these compounds as sole (carbon) and energy source. These species might have significance in a specific natural environment, but they will probably not be ubiquitous. *Thiobacillus thioparus* TK-m was found to degrade $CS_2$ (Smith & Kelly 1988c) as well as DMS, MT and $H_2S$. These combined capacities make this organism very suitable for waste gas treatment (Tanji et al. 1989).

## 5. Evaluation and conclusions

From Sections 3 and 4, it has become clear that $C_1$-organic sulfides are involved in many marine, terrestrial and atmospheric processes. The volatility of the compounds makes it possible that they can readily be transported and distributed, even on a global scale. In the atmosphere, the organic sulfides undergo mainly chemical and physical transformations. In marine and terrestrial systems, however, they are substrates in ecosystems in which primarily microbial metabolism, but also higher plants, play a role. In these systems, $C_1$ organic compounds are transformed biologically, and, to a lesser extent, chemically.

Interest in $C_1$-organic sulfides has arisen from two directions. Firstly, from the need to identify the missing source in the global budget of biogenic volatile sulfur compounds, and, secondly from the possible role of these compounds in climate control. In 1972, Lovelock & Maggs suggested that DMS was the missing biogenic source of sulfur to the atmosphere. DMS, originating from the oceans, would precipitate in some forms of sulfate in the terrestrial environment and thus provide a net transport of sulfur from the sea to the soil. Lovelock and coworkers also indicated the possible climatic role of DMS (Charlson et al. 1987), which has its origin in the 'Gaia theory'. This concept considers the earth as one big ecosystem which maintains itself in a certain equilibrium (homeostasis) by means of numerous feedback mechanisms. DMS would exert a feedback in climate regulation. From this view, a simple hypothesis indicating the climatic role of DMS, was proposed (Fig. 5a). Research in the last ten years has provided a more detailed view, of which only a part has been discussed in this paper (Fig. 5b). The system presented in Fig. 5b is itself part of a bigger ecosystem, with its own mechanisms for maintaining a certain homeostasis. The very simple scheme in Fig. 5a enables us to look through these complex systems, and may help to ask the right questions, so that the principal goal of this research, an understanding of the mechanisms regulating these processes, can be reached. Bremner & Steele (1978) made an inventory of what was known about the role of microorganisms in the atmospheric sulfur cycle. They pointed out that research was needed to further identify the form and amounts of sulfur compounds emitted from terrestrial and oceanic systems. The present review shows that recent research provided data on the nature and fluxes of volatile sulfur compounds, but it is also clear that many organic sulfur transformations are still only superficially



(a) (b)

*Fig. 5.* Schematic representation of the complexity of 'black boxes' involved in chemical and biological DMS fluxes and conversions. +/− stands for positive and negative effects, respectively. References in text.

understood. The aerobic breakdown of DMS in oceanic environment is an example. As discussed above, the nature of the bacteria involved is still unclear and the fact that all organisms isolated until now seem to have insufficient capacities for DMS-removal in natural ecosystems is a puzzle. Apart from the more traditional attempts to measure conversion processes and to isolate the responsible bacteria, the introduction of molecular techniques in microbial ecology seems promising to explain some of these paradoxa. For example, DNA and RNA probing of DMS metabolizing properties might provide methods for estimating the number and the activity of bacteria involved. Indeed, two different MT oxidases have already been purified, and these enzymes or their genes might yield suitable probes.

At this point, only qualitative information on the global sulfur cycle is available. The quantitative and dynamic behaviour of this system will have to be studied in order to be able to predict its response to environmental changes such as possible temperature rise, increasing $CO_2$ pressure, eutrophication, radiation and other relevant ecological parameters. To study dynamic behaviour, it is necessary to use a model system which has all the characteristics of the natural environment. In the Netherlands, several research teams have now combined to concentrate on processes in microbial mats. In these systems, all of the transformations discussed in this paper can be assumed to take place, although at much higher specific rates and concentrations of intermediates. This should allow a more accurate and detailed analysis of the processes and responsible microorganisms. It is hoped that a better understanding of the dynamic behaviour of these 'concentrated' systems may serve as a basis for the study of similar processes in oceanic environment.

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