PRODUCTION OF VOLATILE SULFIDES FROM FRESHWATER ALGAE AND IMPLICATIONS TO THE ENVIRONMENT

By

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ABSTRACT:

Dimethyl sulfide (DMS) is the major volatile sulfide (VS) species of biogenic origin released to the atmosphere. Large amounts of DMS can originate from the decomposition of the metabolic precursor Dimethylsulfoniopropionate (DMSP) in marine algae. Quantitatively, the emissions of DMS and other VS to the atmosphere are in the same order of magnitude as atmospheric sulfur from man-made pollution. Therefore the study of DMS and other VS has a significant relevance to atmospheric chemistry.

Early studies on DMS focused on the marine environment and coastal waters. The presence of other biogenic species such as H_2S, COS, CS_2, CH_3SH (MeSH), CH_3SSCH_3 (DMDS) was also detected in various types of environments including salt ponds, meromictic lakes, soils, freshwaters.

The present work is a laboratory-oriented study on the processes of formation of VS from freshwater algae. The approach undertaken is threefold: 1) Establish the presence and/or production of DMS and possibly other VS from algae cultures and surrounding lake waters. 2) Identify some key parameters influencing the formation of VS from freshwater algae. 3) Estimate the atmospheric contribution of VS from some freshwater algae and lakes.

This study showed that all species of algae and all natural waters contain DMS in amounts comparable to marine waters. However, in contrast to marine waters, DMS generally constitutes only 5 to 25% of all the sulfides, whereas MeSH usually predominates (40-80 %). H_2S+COS, CS_2 and DMDS are

commonly present, whereas other species are rarely detected.

Changes in sulfate concentration had no significant effect on VS production by algae, but there was a threshold level of about 2 mg/L $SO_4^=$ for VS production. Cysteine also caused no significant change in VS production, but a small influence was observed with the addition of methyl donors. Methionine holds a key part in formation of methylated sulfides: additions provoked an increase of MeSH and DMDS production by up to 2 or 3 orders of magnitude.

Calculations suggest that VS from freshwater algae contribute about 115 tons S/year in Lake Ontario. This estimate is quite variable, but it is small compared to other sulfur inputs, including pollution.

RESUME:

Le sulfure diméthylé (DMS) est un sulfure volatil (SV) biogénique majeur émis vers l'atmosphère. Une partie importante du DMS provient de la décomposition d'un précurseur métabolique présent chez les algues marines, le Diméthyle sulfoniopropionate (DMSP). Quantitativement, les émissions du DMS et d'autres SV vers l'atmosphère sont dans un même ordre de grandeur que celles provenant de pollution anthropogénique. L'étude de ces composés a donc une implication particulière sur la chimie atmosphérique.

De nombreuses études précédentes ont porté sur la présence du DMS surtout dans les milieux marins et côtiers. Cependant, d'autres espèces d'origine biologique comme H_2S, COS, CS_2, CH_3SH (MeSH), CH_3SSCH_3 (DMDS) ont été détectées dans d'autres types d'environnements dont, par example, marais salins, lacs méromictiques, sols, eaux douces.

Le présent travail est une étude de laboratoire orientée sur les processus de formation des SV dans les algues d'eaux douces. L'approche se divise selon trois grandes lignes: 1) Etablir la présence et/ou la production de DMS et d'autres SV dans les algues et les cours d'eau avoisinants. 2) Identifier certains paramètres clés qui influencent la formation de SV dans les algues d'eaux douces. 3) Estimer la contribution atmosphérique de ces SV provenant d'algues et de cours d'eaux douces.

Les résultats de cette étude ont démontré que toutes les espèces d'algues d'eaux douces et tous les cours d'eaux analysés contiennent du DMS

à des niveaux comparables à ceux des eaux marines. Cependant, le DMS n'est pas le SV le plus abondant dans nos échantillons. Le DMS constitue généralement entre 5 et 25 % des SV, tandis que le MeSH prédomine habituellement avec une contribution allant de 40 à 80 % des sulfures. Les espèces H_2S+COS, CS_2 and DMDS sont habituellement présentes, tandis que d'autres espèces sont rarement détectées.

Des changements en teneur d'ions sulfate n'ont pas démontré une influence sur la production de SV dans les cultures d'algues, mais un seuil limite à environ 2 mg/L $SO_4^=$ a été observé. La cystéine n'a pas changé significativement la production de SV, et une influence mineure a été observée par des donneurs de groupe méthyle. La méthionine tient un rôle clé dans la formation de sulfures méthylés, car la production de MeSH et DMDS a augmenté de 2 à 3 ordres de grandeur après l'addition de cet acide aminé.

Nos calculs suggèrent que les algues d'eaux douces transfèrent environ 115 tonnes de soufre par année, du Lac Ontario vers l'atmosphère. Cet estimé ne donne qu'un ordre de grandeur de ce flux, et cette valeur est négligeable par rapport à d'autres sources, incluant la pollution atmosphérique.

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1. INTRODUCTION

1.1 Sulfur in nature

Sulfur constitutes approximately 1.9% of the earth's mass (Press and Siever 1982) and 0.05% of the earth's crust (Meyer 1977). It occurs widely in nature under several oxidation states: elemental sulfur (oxidation state 0); H_2S and metal sulfides (-II); SO_2 (+IV), sulfate in natural waters and precipitated anhydrite (+VI). Reduced sulfur (-II) is present in coal, crude oil and petroleum as carbon-bonded mercaptans and sulfides, and mostly as H_2S in natural gas.

Sulfur has a unique chemistry among its periodic table analogues, namely O, Se, Te and Po. Sulfur has a lower electronegativity than oxygen, and therefore has a weaker ionic character in its chemical bonds. This also lessens hydrogen bonding, although weak interactions still exist. Sulfur has the particularity to hybridize its empty d orbitals with its filled s and partially filled p orbitals to form σ bonds with other atoms. This characteristic is unique and makes the element like none other of the same period. The sulfate ion is an example of this behaviour, where the empty $d\pi$ orbitals of the sulfur atom accept electrons from filled $p\pi$ orbitals of the oxygen atoms. This means that the coordination number of sulfur is not limited to 6 nor its valence confined to 2.

Sulfur is present in nature in many allotropes. S_8 is the most stable form of the element under ambient pressure and temperature. Other less stable

allotropes containing 6 atoms and 7, 9, 10, 11, 12, 18 and 20 membered rings also exist.

Sulfur in its (-II) oxidation state is found deep in the crust in the absence of oxygen. Meyer (1977) lists the most important reservoirs of reduced (exploitable) sulfur in the earth's crust. Most of the world's exploitable sulfur is in coal and bitumen as pyrite, organic sulfur and some sulfate. Petroleum and natural gas, the next category, contain large amounts of low molecular weight sulfides and mercaptans (some are listed in Rall 1972, op. cit. in Meyer 1977). Sulfide ores and pyrite deposits constitute the third largest reservoir of exploitable reduced sulfur.

Reduced sulfides, namely pyrite or hydrogen sulfide, are readily oxidized to sulfate in the presence of oxygen. Sulfide minerals are usually very insoluble in water. Only alkalis and alkaline earths can form water soluble sulfides because of their dominant ionic character. The ion S^{2-} is a strong to weak base, and forms a weak acid upon reaction with water.

Sulfur in the +VI oxidation state is found almost exclusively as sulfate. This is the most stable form of sulfur in nature. The acid, sulfuric acid (H_2SO_4) is one of the most widely used chemicals by man. It is completely dissociated as the sulfate ion in water at almost all pH ranges. Sulfate ion is present in all natural waters around the world. The main reservoirs of sulfate are oceanic waters and evaporite beds (gypsum or anhydrite).

Sulfur in its (+IV) state is produced naturally as sulfur dioxide in flue

gases from volcanic emissions. SO_2 is very hygroscopic, it has a short lifetime in the atmosphere and readily oxidizes to sulfuric acid, producing some acidity to rainwaters ("acid rain"). It is absorbed by plants from the air through dry deposition. SO_2 is also an intermediate in the oxidation of reduced volatile sulfur species in the atmosphere.

Other forms of inorganic sulfur also exist, but many are not stable thermodynamically. Further discussion is beyond the scope of the present work. A detailed discussion on sulfur chemistry is available in textbooks such as Cotton and Wilkinson (1980) or the Gmelin Inorganic Chemistry series.

1.2 Man's uses of sulfur

Sulfur is a polyvalent element and its properties are used in various industrial and commercial applications. Sulfuric acid is used primarily as a processing agent. It is involved in the production of phosphate fertilizers, in various chemical industries (synthetic detergents, catalysts, pharmaceuticals, etc.), in titanium pigments and paints, in iron and steel industries, in textiles, petroleum and in many other industries. Other sulfur compounds are used in pulp and paper industries for the making of fine papers, carbon disulfide (rubber processing chemical), insecticides and pesticides, and in such diverse industrial processes as bleaching, leather processing, and photography (Meyer 1977, and references therein). Sulfate contained in evaporites (e.g., gypsum), is used mostly untransformed in building material for household and industry.

Sulfur contributes to an important fraction of the United States GNP (Meyer 1977). The same author also showed that there is a direct relationship between the energy and sulfur consumptions per capita, and the GNP per capita for some countries. Prosperous countries (US, UK, W. Germany) are large energy and sulfur consumers, implying that the wealth of a nation is related to its use of sulfur.

1.3 Biological need for sulfur.

Sulfur is an essential element for life. It constitutes approximately 0.2 to 0.7% of the total mass of most living organisms (Anderson 1978). Sulfur is present in organisms mostly as the reduced form in amino acids (Methionine, Cysteine and cystine) and proteins, in peptides (Glutathione), in essential metabolites (Coenzyme A, ferredoxins, biotin, thiamin or vitamin B) and in many other important cellular compounds (Anderson 1978; Lehninger 1975; Stryer 1981). The sulfur atoms are responsible for the cross-linkages in proteins and protoplasms. Sulfur-containing compounds are involved in the synthesis of essential enzymes, glucosides and many other compounds.

The sulfur atoms contained in amino acids are in the (-II) oxidation state. Therefore some organisms in the food chain must have the ability to reduce sulfate, the natural precursor, to a lower oxidation state in order to synthesize carbon-bonded sulfur chains. Table 1.1 shows a general classification of the types of organisms capable of reducing sulfur from sulfate. The first column

Table 1.1: Some general examples of the types of organisms performing sulfate reduction (after Anderson 1978; Lehninger 1975).

Classification of organisms	Assimilatory sulfate reducers	Dissimilatory sulfate reducers
Autotrophic (photosynthetic) [energy source: light]	Green and higher plants Blue-green algae Photosynthetic bacteria	No known example
Heterotrophic (chemotrophic) [energy source: oxidation of substrate]	Most aerobic bacteria Most fungi	Sulfur bacteria: *Desulfovibrio* *Desulfotomaculum*

outlines the types of organisms that perform sulfate reduction according to the energy source. The autotroph (photosynthetic) organisms use light as an energy source to reduce sulfur from the sulfate ion. The heterotrophs (chemotrophs) use a complex molecule (e.g., glucose) to supply energy to the organism for sulfate reduction. The other two columns summarize the mechanisms of sulfate reduction: assimilatory sulfate reduction (ASR), and dissimilatory sulfate reduction (DSR). The two mechanisms will be discussed further in section 3.

Assimilatory sulfate reducers provide a link between inorganic sulfate and sulfur-containing compounds via the synthesis of cysteine and methionine. These amino acids in turn have many metabolic functions such as protein building, etc. Animal cells do not produce sulfur amino acids, hence their diet must contain cysteine and especially methionine produced by primary producers.

1.4 Environmental effects: man-made pollution

Mankind has added a massive quantity of sulfur to the natural cycle with the extraction of sulfides (Figure 1.1). Most of this sulfur mobilized by anthropogenic activities originates from the upper crust. The residence time of sulfur in the lithosphere is quite long, and as a result, this new input has induced an imbalance in the natural sulfur cycle. Figure 1.1 shows that this imbalance creates a net accumulation of sulfur in the pedosphere (soils).

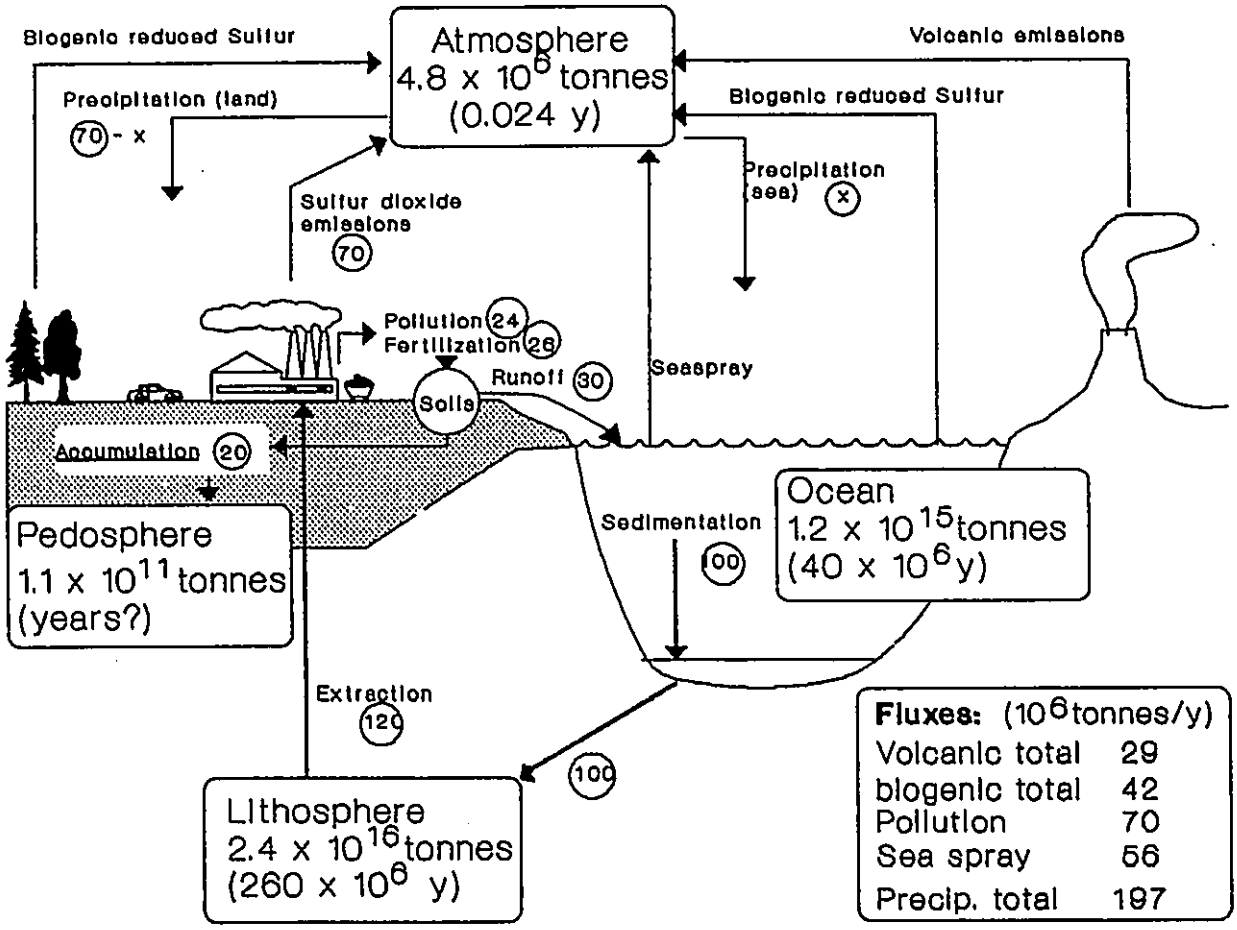

Figure 1.1: Diagram showing the main fluxes of sulfur in the atmosphere (Modified from Devai et al 1985). The circled numbers represent the fate of sulfur mobilized by man's activities. Numbers in boxes represent the principal reservoirs affected by these fluxes.

This additional sulfur is extracted from the lithosphere via the mining of metal sulfides and the extraction of crude oil and coal, in which sulfur has a reduced state (0 or -II oxidation number) (120 Tg S/year; Tg = 10^6 tonnes). Conversion of the sulfur to a form more serviceable by man involves oxidation and purification through various processes (Meyer 1977). Such an operation often involves emission of SO_2. For example, in the metal processing industry, the metal ore is roasted in the presence of oxygen for the recovery of the metal, hence producing SO_2. The latter can be recovered to minimize pollution, and converted to sulfuric acid for further use. Fossil fuels also contain an appreciable amount of sulfur (0.05 to 14%; Meyer 1977) which is also converted to SO_2 and adds to the total atmospheric sulfur burden (70 Tg S/y). Increases of sulfur emissions to the atmosphere have been an environmental concern especially in the last few decades, because of the associated problem of acidic precipitation (Cullis and Hirschler 1980; Moller 1984a,b; Ryaboshapko 1983; Warneck 1988).

Sulfuric acid, obtained either from elemental sulfur or from SO_2 recovery, is used in many sectors of the industry as a processing agent. It eventually reaches surface waters and soils, and runs off to the ocean (24 Tg/y). Sulfuric acid used in fertilizers (26 Tg/y) eventually reaches soils and surface waters. This scheme predicts a net accumulation of 20 Tg S/y in soils (Devai et al. 1985). Finally, all sulfur reaching the ocean (100 Tg S/y) is buried in the sediments and is considered immobilized in the lithosphere. These numbers

are valid assuming that sulfur is extracted from the crust at a constant rate. However, it is known (Cullis and Hirschler 1980; Ryaboshapko 1983) that sulfur consumption increases year after year. This short time global cycle does not allow thorough mixing within the pedosphere, thus local accumulation can be very important.

On a worldwide basis, man-made atmospheric emissions are now in the same order of magnitude or higher than natural emissions (Table 1.2). The atmosphere is particularly sensitive to this because of its small sulfur content, its fast rate of mixing and the nature of the reaction controlled by sulfur oxidation.

1.5 Environmental effects: natural causes.

The natural sources of atmospheric sulfur can be simplified into four major categories (Table 1.2). Sea spray plays a major role in the atmospheric sulfur cycle, but since sulfur is already emitted as sulfate, further redox transformations do not occur on sulfate. Hence, this does not change the chemistry of other elements. The same statement can be made for dust, because they are predominantly sulfates. Volcanic gases are emitted mostly as SO_2. The estimates (Table 1.2) do not reach a consensus, which is largely due to the estimation method. These numbers should be taken lightly because some volcanic eruptions can blast many thousands of tonnes of sulfur into the atmosphere within only a few days (Krueger 1983). Profound effects can occur

Table 1.2: The global atmospheric sulfur cycle. Fluxes are in Tg S per year worldwide (1 Tg S = 10^{12} g S = 10^{6} tonnes S)

Source of sulfur	Fluxes (Tg S/year) References					
	1	2	3	4	5	6
Biogenic reduced sulfur	41	70	42	48	43	65
Salts from sea spray	140	175	54	44	150	144
Dust and particles	20	-	2	8*	?	20
Volcanic emissions	28	2	29	2	7	20
Man-made ("pollution")	113	77	70	70	106**	93
TOTAL:	342	324	197	172	306	342

*Includes 1 Tg S for dust, 7 Tg S for forest fires.
**Includes 3 Tg S of dust and salts.

References: 1 Ryaboshapko (1983); 2 Moller (1984a, b); 3 Devai et al. (1985); 4 Berner and Berner (1987); 5 Warneck (1988); 6 Brimblecrombe et al. (1989).

locally without a corresponding large number on the global figure.

Biogenic sulfur emissions to the atmosphere are in the same order of magnitude as man-made pollution. They include emissions of H_2S and other volatile sulfides from the decomposition of organic matter and bacterial reduction of sulfate in anoxic sediments. Several reduced volatile sulfur compounds such as dimethyl sulfide, methanethiol, dimethyl disulfide, other mercaptans and sulfides are also produced by microorganisms (see Table 1.3). Their mode of production is not well characterized. The production of these sulfides occurs in both anaerobic (sediments, decomposition, bacterial sulfate reduction) and aerobic environments (marine plankton metabolism, decomposition of organic matter, land plants).

Biogenic sulfur is the least well characterized component of natural sulfur emissions to the atmosphere. The importance of these sulfides towards the global sulfur cycle has been established only since Lovelock and colleagues (1972) proposed that DMS emissions from marine algae can balance the overall global atmospheric sulfur cycle. His work and the importance of DMS in the marine area were substantiated with the work of Andreae and colleagues (Andreae 1980; Andreae et al. 1983; Andreae and Raemdonck 1983; Andreae and Barnard 1984; Andreae et al. 1985; Barnard et al. 1982). Further investigations showed that DMS was produced by algae and microorganisms in all types of marine waters around the world (Dacey et al. 1987; Franzmann et al. 1987; Holligan et al. 1987; Iverson et al. 1989; Jorgenson and Okholm-

Table 1.3: List of some natural sulfur compounds commonly found in the environment (Caron and Kramer 1989; this work). DMSP is a non-volatile species.

Acronym	Name (long)	Formula
	Hydrogen sulfide	H_2S
	Carbonyl sulfide	COS
	Carbon disulfide	CS_2
MeSH	Methanethiol	CH_3SH
PrSH	1-Propanethiol	$CH_3CH_2CH_2SH$
DMS	Dimethyl sulfide	CH_3SCH_3
DMDS	Dimethyl disulfide	CH_3SSCH_3
Other:		
DES*	Diethyl sulfide	$C_2H_5SC_2H_5$
DMSP (or DMPT)	Dimethylsulfoniopropionate (Dimethyl sulfoniopropiothetin)	$(CH_3)_2S^+CH_2CH_2COOH$

*Used as internal standard (Caron and Kramer 1989; this work)

Hansen 1985; Nguyen et al. 1983, 1988; Turner and Liss 1985; Turner et al. 1988; Wakeham et al. 1984). Furthermore, micro-organisms in soils as well as land plants also produce an appreciable amount of DMS, along with other volatile sulfides (VS) (Adams et al. 1981a, b; Aneja 1986; Aneja et al. 1981, 1982; Lamb et al. 1987; Steudler and Peterson 1985; Westberg et al. 1987). Finally, volatile sulfides were also found in freshwaters (Bechard and Rayburn 1979; Caron and Kramer 1989; Nriagu and Holdway 1989; Richards et al. 1990). It becomes evident from these studies that DMS and other VS are present in all types of environments.

It is now established that DMS and other VS from microorganisms and higher plants play an important role in the atmospheric sulfur cycle. The oxidation of these compounds in the atmosphere produces SO_2 (Sze and Ko 1980), thus some potential acidity in both dry and wet deposition. Their influence can explain the low pH of rain events in some areas remote from human influence (Charlson and Rodhe 1982; Delmas and Gravenhorst 1983; Galloway et al. 1982). Their importance in the budget of continental atmosphere is noticeable (Nriagu et al. 1987). Bates et al. (1987), and Charlson et al. (1987) showed the implications that DMS can have over the climate. All these points show that DMS and possibly other VS have a marked influence on the atmosphere over a regional/continental environment.

1.6 Summary

All living organisms use sulfur in their metabolism. They also play an important part in the sulfur cycle because they can transform the element from an inorganic form (sulfate) to amino acids, and further to proteins, and many other metabolites. Microorganisms can mediate the reoxidation of sulfur to semi-organic compounds or back to inorganic sulfate. This dynamic aspect of sulfur involves the change to different oxidation states and chemical forms in various environments. Living organisms play an active role in the cycling, the release and the mobilization of sulfur from the lithosphere, pedosphere, hydrosphere and atmosphere. With the advent of the industrialized era, more sulfur is mobilized in nearly all spheres of the environment. Given the impact that sulfur has on the environment, especially when one considers its acidifying capabilities on softwaters, some fundamental questions now arise. Do higher emission and deposition rates of sulfur enhance or hinder the biological (natural) sulfur cycle? Is sulfur recycled faster (i.e. are more VS produced) by microorganisms since this element is more available for their metabolic needs?

It is crucial at this point to understand the metabolism of sulfur and the chemical parameter(s) controlling or influencing it. The influence that increased sulfur emission/deposition has on the metabolism of some organisms in some areas of the ecosystem probably has a vital importance on the production of VS and the recycling of sulfur.

2. DEFINITION OF THE RESEARCH:

2.1 Statement of the problem

DMS and other Volatile Sulfides (VS) from marine, inland, and terrestrial environments are potentially important sources of sulfur to the atmosphere. As a consequence, this natural sulfur input produces acidic precipitation in remote areas from the oxidation of Volatile Sulfides (VS) to sulfuric acid. There is also the possibility that the "natural" recycling of sulfur is enhanced by increased levels of sulfur from anthropogenic activities. Volatile Sulfides (VS) are probably responsible in a large part for the variations in background concentrations of sulfate in rainwater.

The production of VS was shown to be important especially when Lovelock et al. (1972) postulated that DMS, emitted from marine algae, could balance the global atmospheric sulfur cycle. DMS and other VS can be produced in softwater environments via different processes. Decomposition of organic matter, dissimilation (reduction) of sulfate in waterlogged soils and sediments, and finally algae metabolism all produce volatile sulfides. VS are ubiquitous in natural waters.

The chemical process(es) leading to DMS formation in marine algae is(are) fairly well defined. The factors controlling the production rates of DMS, however, are not well understood. The equivalent processes in softwater algae

are not well characterized. Moreover, is DMS the only significant volatile sulfide emitted by biogenic activity? Can the analytical method create an artifact as to the presence/absence of other sulfides?

With the large surface of softwaters and the vast pristine areas on this continent, an account of sulfur emission from these ecosystems is needed. In addition, since there is considerable emission of sulfur from industrial sources in the Great Lakes area, it is important to evaluate the extent of biological recycling of sulfur. The intensity of sulfur reemission would also give the contribution of some biological sources of sulfur to a continental/regional atmospheric budget. It is suspected that freshwater algae plays a key role in this sulfur recycling.

The broad objectives of this study are: to identify (or characterize) the major processes leading to and controlling the formation of volatile sulfides from freshwater algae; and to estimate the atmospheric contribution of volatile sulfides from some freshwater algae and lakes.

2.2 Specific objectives:

1. To develop an analytical technique which will determine qualitatively and quantitatively several species of volatile sulfides in aqueous samples. The desired features are: routine analysis of freshwaters; low detection limits for all sulfides; a good selectivity for multispecies determination.

2. To ascertain the production of volatile sulfides from algae using several unialgal cultures grown in axenic conditions.

3. To evaluate how various chemical compounds can stimulate or influence the production of volatile sulfides.

4. To confirm the pathway leading to the production of volatile sulfides from freshwater algae.

5. To outline the main difference(s) in the mechanism(s) of volatile sulfide production between seawater algae and freshwater algae.

6. To verify whether the amounts of volatile sulfides produced by freshwater algae can be normalized to a biological crop indicator (e.g., Chlorophyll a).

7. To conceive and calibrate a VS emission model for lakes, and to incorporate the results in an atmospheric budget. Such an estimate will show whether volatile sulfides from freshwater algae can account for a significant part of a regional/continental sulfur cycle.

8. To determine if an increase in sulfate deposition from acidic precipitation has an influence on the cycling rate of sulfur and the production of volatile sulfides.

2.3 General procedure

The general and specific objectives are contained under these categories of information and procedures:

1. Sulfur biochemistry: sulfur is essential for life. It is readily abundant for all organisms and sulfur transformations precede emissions of volatile sulfides. It is therefore important to use what is known about sulfur biochemistry as a tool for designing experiments.

2. Laboratory algal cultures: softwater algae produce volatile sulfides. The study of algae cultures in controlled conditions will facilitate the determination of the key factors influencing volatile sulfide production.

3. Mass transfer model: a mass transfer model will be applied to the lakes for which measurements will have been made. When no such VS data are available, a crop biomass factor determined from laboratory cultures (e.g. amount of VS/Chlorophyll a) will be used. The purpose of this factor is to use available data on Chlorophyll a as an indirect measurement of VS. A critique of this approach is applied to the Great Lakes region.

3. SULFUR BIOGEOCHEMISTRY

3.1 The biochemical need for sulfur

The metabolic requirements of sulfur in organisms arise predominantly from the presence of the amino acids cysteine and methionine (Anderson 1978). The metabolic functions of these amino acids are various: they constitute a part of virtually all proteins in living organisms. They are also the building material of other numerous metabolites. Cysteine is the starting material for some peptides (Glutathione) and essential metabolites (Coenzyme A, ferredoxins). Methionine (activated) is a methyl transfer agent used in the synthesis of many other metabolites and substrates. Biotin and thiamin (vitamin B) are other miscellaneous cofactors essential in cell metabolism. Table 3.1 summarizes some of the most important sulfur-containing metabolites and their principal functions.

The sulfur contained in these amino acids and metabolites originates from diverse forms of inorganic sulfur, e.g., sulfate ion present in natural waters, elemental sulfur, hydrogen sulfide (Lehninger 1975). For plants, plankton and microorganisms living in oxygenated environments, their source of sulfur is the sulfate ion. Since almost all sulfur present in these organisms is in the reduced form, they spend a considerable amount of energy to reduce sulfate (at +VI oxidation state) to its -II form. This type of biological reduction is achieved via

Table 3.1: Some uses of sulfur in organisms (From Anderson 1978; Lehninger 1975)

Compound	Comments; Metabolic uses
Cysteine	First sulfur-containing molecule synthesized from the reduction of sulfate; intermediate in the synthesis of other metabolites; protein constituent; responsible for cross-linkages in proteins.
Methionine	Intermediate in the synthesis of other metabolites; protein constituent.
S-Adenosylmethionine	Methyl transfer agent.
Co-enzyme A	Metabolic acetate transfer (as Acetyl-CoA); oxidation and synthesis of fatty acids; synthesis of amino acids; oxidation of pyruvate and intermediates in the TCA (Krebs) cycle.
Ferredoxins	Reduction of chemical metabolic species: photosynthesis, nitrogen fixation, sulfate reduction; electron transport in mitochondria (respiration).
Glutathione	Reducing agent; transport of amino acids.
Lipoic acid	Oxidative cofactor in the TCA cycle.
Biotin (vitamin B)	Cofactor for carboxylation reactions.
Sulfolipids	A constituent of chloroplast membranes.
Thiamine pyrophosphate	Cofactor in decarboxylation (plus some other reactions).

two major mechanisms: the Dissimilatory sulfate reduction (DSR), and the Assimilatory sulfate reduction (ASR). DSR is an energy-yielding mechanism analogous to respiration whereby H_2S is released as one of the products. ASR uses sulfate solely to satisfy the sulfur metabolic needs of the cell (or organism).

3.2 Assimilatory and dissimilatory Sulfate reduction

Dissimilatory sulfate reduction (DSR) is the microbial process by which sulfur (from the sulfate ion) is used as the electron acceptor and H_2S is the product. The process is carried on principally by bacteria of the genera *Desulfovibrio* and *Desulfotomaculum* in the absence of oxygen (i.e., in reductive environments). This process is functionally analogous to respiration for aerobes and fermentation for some anaerobes. The electrons required for sulfate reduction are obtained from the oxidation of a substrate (e.g. a carbohydrate such as glucose) in the tricarboxylic acid cycle (TCA or Krebs cycle). Two molecules of CO_2 are formed and 8 electrons are transferred to 3 NAD^+ (as $NADH_2$) and 1 FAD^+ (as $FADH_2$) (Stryer 1981). These electron carriers produce ATP (hence metabolic energy) upon oxidation with oxygen from O_2 (aerobes) or $SO_4^=$ (DSR) from the appropriate electron transfer chain (Anderson 1978):

Aerobes: $2\ NADH_2 + O_2 \rightarrow 2\ NAD^+ + 2\ H_2O$ [3.1]

DSR: $4\ NADH_2 + SO_4^= \rightarrow 4\ NAD^+ + S^{2-} + 4\ H_2O$ [3.2]

This example illustrates how the production of sulfide (H_2S) is a

consequence of the respiration of the dissimilatory sulfate reducers. Reaction [3.2] shows why reduced sulfides are so abundant in reducing environments. Bacteria use some of this reduced sulfur in cysteine synthesis by enzymatic addition to o-acetylserine (see, for example, Michal 1974; Tsang and Schiff 1975).

Assimilatory sulfate reduction (ASR) involves the reduction of sulfate to satisfy the sulfur metabolic needs of the organism. Higher plants, green plants, algae and most fungi are capable of operating in this mode of sulfate reduction. A simplified picture of the ASR is shown in Figure 3.1 for a typical Assimilatory Sulfate reducer (*Chlorella*). A more complete review of the ASR is described in Schiff and Frankhauser (1981).

Uptake and transport of the sulfate ion through the cell wall is the first step in ASR. ATP "activates" the sulfate ion prior to entering the reduction cycle. The "activated sulfate" (APS, figure 3.1) is bound to a carrier, which is analogous to glutathione in *Chlorella* (Tsang and Schiff 1978). Enzymes help carry out the reduction of the sulfate-carrier with ferredoxin. The electrons on the reduced ferredoxin are transferred using the energy gained by photosynthesis. Once reduced to sulfide, o-acetylserine substitutes its acetate moiety with sulfide and cysteine is produced. Cysteine is thus the first truly carbon bonded sulfur compound arising from sulfate reduction (as in DSR) and it is the start of many metabolic functions.

Cofactors other than o-acetylserine can accept the S^{-2} group to produce

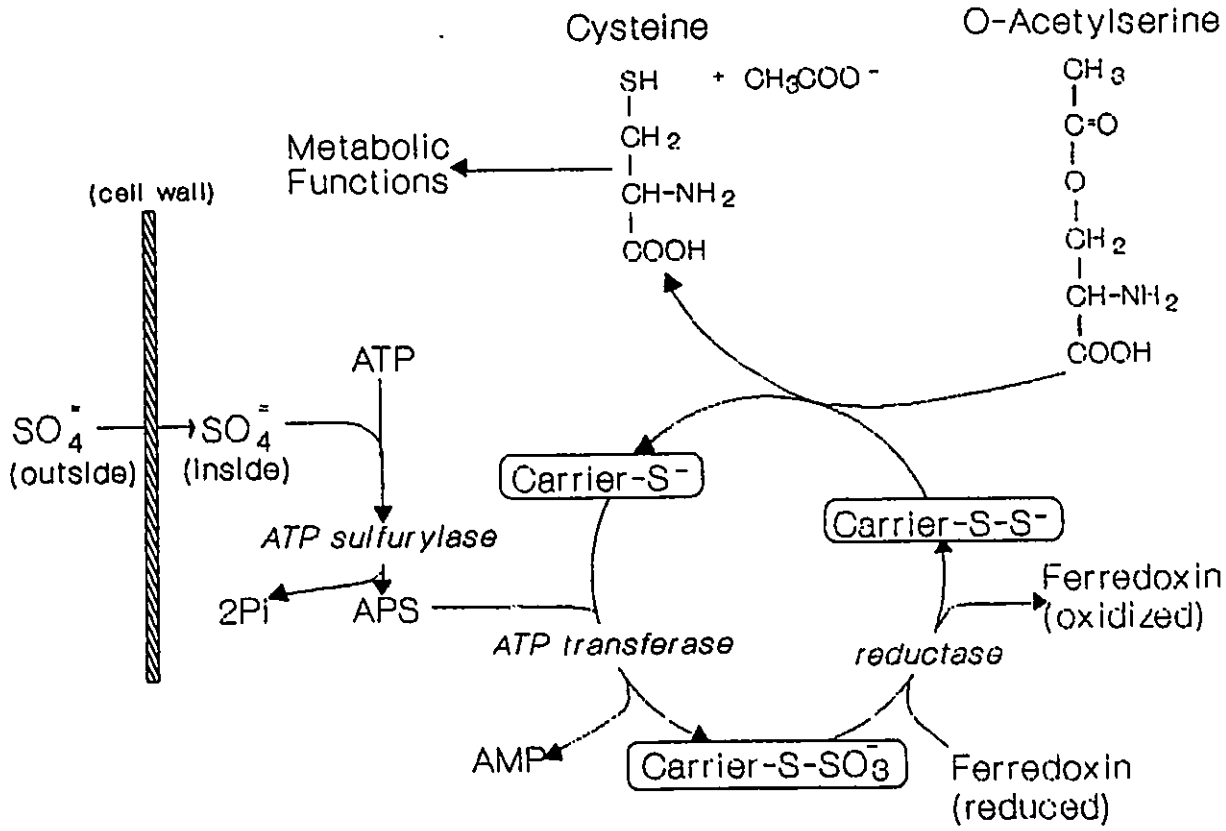

Figure 3.1: Schematic representation of Assimilatory Sulfate Reduction in *Chlorella* (Modified from Tsang and Schiff 1975).

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Cofactors other than o-acetylserine can accept the SH^- group to produce

processes leading to volatile sulfide production must be examined in more detail.

Decomposition and putrefaction of organic matter produces predominantly H_2S, and this is well documented (e.g., Zinder and Brock 1978; Dunnette 1989). MeSH, DMS and DMDS are found as well (Zinder et al. 1977) during the decomposition of algal mats under anaerobic conditions. The mechanism of production of these sulfides, however, is not necessarily caused entirely by a methyl transfer. Kiene and Visscher (1987), Zinder and Brock (1978) reported that inhibition of the one-carbon metabolism (i.e., affecting methyl transfer) still produced methylated volatile sulfides in anoxic sediments, but the overall yield and number of methylated sulfides were affected. This suggests that methyl transfers cannot account for all volatile sulfides produced. The final product of anaerobic decomposition is predominantly H_2S (Zinder and Brock 1978), in part because methanogens further assimilate the carbon on methylated sulfides. The end products of this second-hand sulfide production are CH_4, CO_2 and H_2S. The use of methylated sulfides as a source of carbon by methanogens is documented (Kiene et al. 1986; Kiene and Visscher 1987; Suylen et al. 1986; Wakeham et al. 1987). This process is a net sink of alkylated sulfides from the water column.

Similarities occur between DSR and the situation above. In this sulfate reduction mechanism, H_2S is the major product released but other volatile sulfides can also be formed. Kiene and Visscher (1987) mention that a sulfate

reducing bacterium also performs demethiolation (i.e. loss of terminal -S-CH_3) of methionine to produce methyl sulfur compounds. However, the final overall product is H_2S because the bacteria re-uses the methiol compounds produced as a new substrate.

The picture appears to be clearer for the production of DMS from marine algae. Sulfate reduction via the ASR pathway produces cysteine, and then methionine (Figure 3.2). DMS in marine algae was shown to be generated by the decomposition of a thetin, DMSP (Challenger and Simpson 1948; Table 1.2), which is present in many marine algae (White 1982; Keller et al. 1989). Greene (1962) revealed the direct synthesis of DMSP from methionine. Therefore, a metabolic pathway can be drawn for the reduction of sulfate up to the formation of DMSP in marine algae (e.g. Figure 3.2). The factors controlling the decomposition of DMSP to DMS and acrylic acid are not well understood, and this is marked by question marks in Figure 3.2. DMSP has an influence on osmoregulation (Dickson et al. 1980; Vairavamurthy et al. 1985; Iverson et al. 1989), thus variations in salinity of seawater can influence DMS release (Vairavamurthy et al. 1985). Alkaline degradation (Hofmann degradation) releases DMS but this factor is negligible at oceanic pH (Dacey and Blough 1987). Its release may be enzymatic (Challenger 1951; Cantoni and Anderson 1956; Barnard et al. 1984) or mediated by bacteria (White 1982). Other factors such as phytoplankton grazing (Dacey et al. 1986) and senescence (Nguyen et al. 1988) play a key role in the decomposition of DMSP

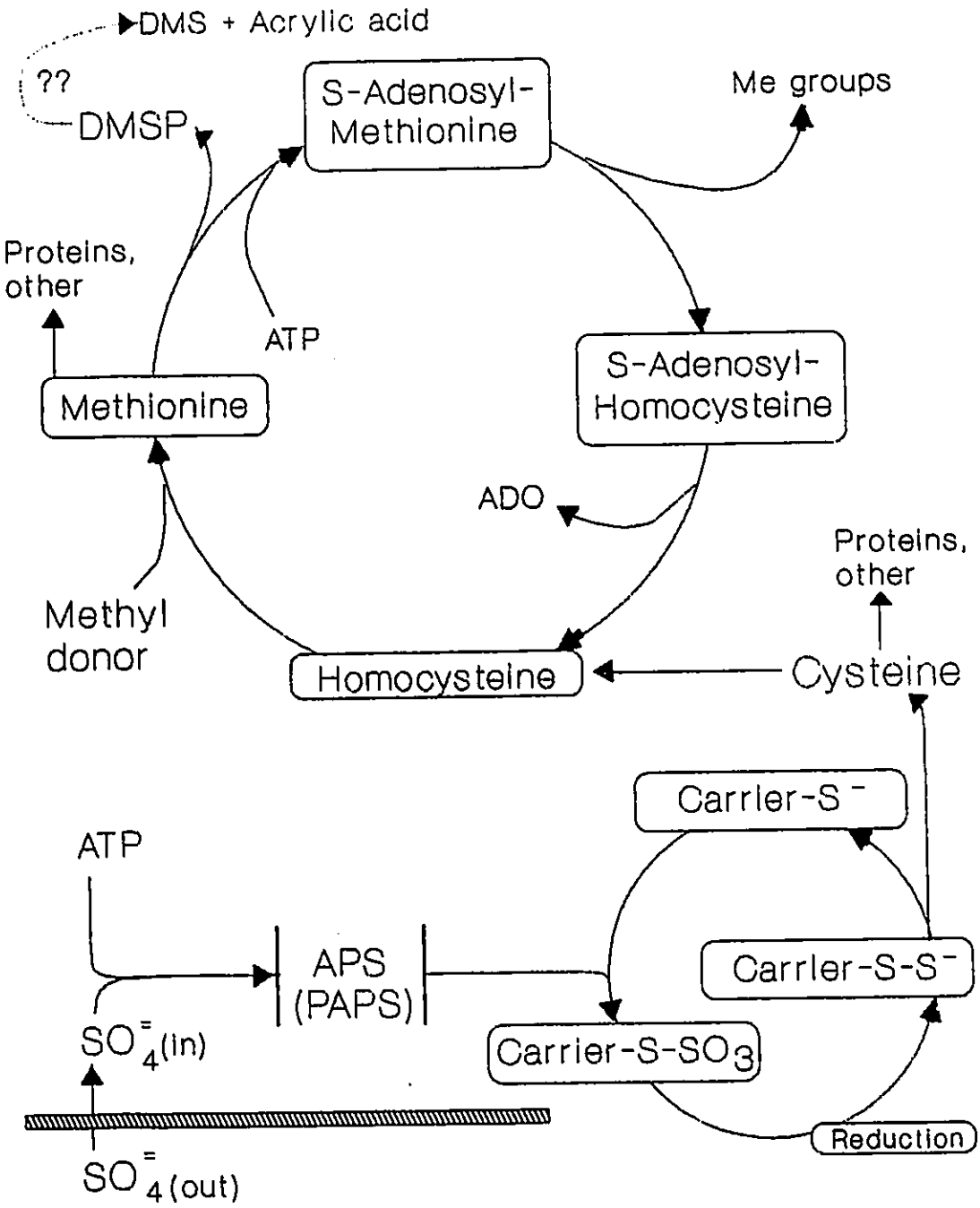

Figure 3.2: Simplified overall representation of the ASR featuring the production of DMS from DMSP in marine algae.

to DMS and acrylic acid.

DMS is the only sulfide released from DMSP decomposition in seawater. Other volatile sulfides are also present in this environment even though algae producing these volatile sulfides also contain DMSP. There is no explanation so far for the occurrence of these other volatile sulfides produced by the metabolism of these algae. DMSP can account only for the production of DMS.

Softwater algae produce several volatile sulfides species (Bechard and Rayburn 1979; Caron and Kramer 1989). DMS does not always predominate over the other volatile sulfides, and often MeSH is the most abundant one. Furthermore, the presence of DMSP is not significant in softwater algae (White 1982), thus decomposition of the thetin does not explain the presence of DMS, nor does any known major metabolism.

Therefore, no known metabolic path for sulfur seems to explain satisfactorily the production of most volatile sulfides from normal, healthy seawater and softwater algae.

ASR metabolism alone does not seem to be a discriminatory factor for explaining the formation of VS. Klein and Cronquist (1967) mentioned that the pathways of sulfur metabolism used by photosynthetic bacteria, algae and higher plants are quite uniform, hence a relationship between the type of algae and the production of VS is difficult to predict. An exception was observed when Bechard and Rayburn (1979) found high levels of VS (H_2S, MeSH, DMS, DMDS) produced by many species of algae belonging to the division

Cyanophyta (Blue-green algae). They reported a clear trend of VS emission from these algae as opposed to specimens of other divisions (*Chlorophyta, Xanthophyta, Bacillariophyta*). Blue-green algae and photosynthetic bacteria (including *Desulfovibrio* and many other species producing VS) have many similarities. Like bacteria, *Cyanophyta* lack a membrane-bound nucleus. Many bacteria and Blue-green algae are able to perform nitrogen fixation (Klein and Cronquist 1967; Lehninger 1975; Bold and Wynne 1978), and there is a possibility that nitrogen fixation is related to the mode of sulfate reduction (Klein and Cronquist 1967). The hypothesis that Blue-Green algae are good VS producers was not observed by Keller et al. (1989) in marine algae, who observed no or little DMSP or DMS production from cultures of marine *Cyanophyta*. Other generalities pertaining to the differences among divisions of algae (Bold and Wynne 1978) do not hint at differences which can affect VS production.

In summary, the general biochemistry of transsulfuration, or the cellular organization do not seem to provide answers for predicting the amounts and the species of VS formed. On a (bio)chemical basis, the evidence outlined above suggests that VS production is not the sole result of methyl transfer onto a substrate. Biochemical processes other than the latter are responsible for at least some of the VS. The nature of these mechanisms needs further characterization. Perhaps chemical and/or environmental factors are able to control or influence the VS production/emission. Selective additions of

chemicals on the one-carbon mechanism (methyl transfer, and the influence on C-S bond formation) may provide an answer to some of these concerns.

3.4 Sulfur and "classical" methylation.

According to the concepts outlined in Challenger (1959), the methylation of a substrate implies either (1) the biological transfer of a methyl group from a donor A to a substrate B, or (2) the biological elimination of a small fragment C containing one carbon (not necessarily a methyl group; for example formate, HCO^-) which is later assimilated by a molecule D, then reduced to methyl. One-carbon groups transferred by tetrahydrofolate illustrate the latter mechanism. "True" methylation, or transmethylation, is depicted in case (1), whereas case (2) has no clear designation. Transmethylation induced biologically (hence called biomethylation) is known to occur with some elements such as arsenic and mercury (Craig and Brinckman 1986). There is also substantial evidence that biomethylation occurs on Se, Te, Sb and Ge (see Craig 1986 for a general discussion). Methyltin and methyllead compounds have been found in the environment, but their presence does not necessarily result from endogenous processes or biological mediation. Nevertheless, studies showed that their formation is possible under environmental conditions (Craig and Rapsomanikis 1985; Rapsomanikis et al. 1987).

Three types of one-carbon groups can be transferred onto a substrate. Methyl carbanions (CH_3^-) from methylcobalamin (the only known CH_3^- donor),

methyl radicals ($CH_3^{\cdot}$) and methyl carbocation (CH_3^{+}). A short list of some methyl donor groups occurring naturally is shown in Table 3.2.

The general mechanisms of classical biomethylation are known. Four general types of biomethylation mechanisms are described in Craig and Brinckman (1986, and references therein), and they are summarized here. They are:

Type 1: Direct substitution

$$CH_3CoB_{12} + Hg^{2+} \text{ --------> } (H_2O)\ CH_3Hg^{+} + H_2OCoB_{12} \qquad [3.5]$$

(CH_3CoB_{12} = methylcobalamin, or vitamin B_{12})

The transfer of ($CH_3^{\cdot}$) is direct and the substrate's oxidation state is unchanged. However, in some exceptions, the oxidation state of the substrate with a low oxidation state can change as a function of the type of methyl group transferred ($CH_3^{\cdot}$ or CH_3^{+}), when the methyl group is more electrophilic than (CH_3^{-}).

Type 2: transfer with facile oxidation (1 unit)

$$CH_3CoB_{12} + Sn(II) \text{ ---> } CH_3Sn(III)^{\cdot} + CoB_{12}$$

$$CH_3Sn(III)^{\cdot} + O_2 \text{ ---> } CH_3Sn(IV)^{+} + O_2^{-} \qquad \text{(aerobic), or}$$

$$CH_3Sn(III). + H_2OCoB_{12}^{+} \text{ ---> } CH_3Sn(IV)^{+} + H_2O + CoB_{12}^{\cdot} \qquad [3.6]$$

(anaerobic)

The example above features the transfer of a radical group. The initial transfer is accompanied with oxidation of the metal one unit higher, towards a more stable state. The second step, if present, is a stabilization of the

Table 3.2: Methyl donor groups found in nature (from Craig and Brinckman 1986; Lehninger 1975; Stryer 1981).

Name and structure (if necessary)	Nature of the one-carbon group transferred
Methyl Cobalamin	$CH_3^{\cdot}$ CH_3^{-}, CH_3^{+}
Tetrahydrofolate	
Methyl	CH_3^{+}
Methylene	$-CH_2-$
Formyl	-CHO
Formimino	-CHNH
Methenyl	-CH=
Methyl halides CH_3X (X = I, Br, Cl)	CH_3^{+}
Choline $(CH_3)_3N^{+}-CH_2-COOH$	CH_3^{+}
Betaine $(CH_3)_3N^{+}-CH_2-CH_2OH$ (Glycine betaine)	CH_3^{+}
Dimethyl thetin $(CH_3)_2S^{+}-COOH$	CH_3^{+}
Trimethyl sulfonium $(CH_3)S^{+}X^{-}$ (X = I, Br, Cl)	CH_3^{+}

products.

Type 3: transfer with facile oxidation (2 units)

$$CH_3I + Sn(II)(Acac)_2 \text{ ---> } CH_3Sn(IV)(Acac)_2I \qquad [3.7]$$

(acac = bidentate acetylacetonate, $CH_3COCHCOCH_3$)

This process is common in abiotic transformations. It is related to type 2. The oxidation of the substrate is easy, and the major difference is the change of 2 in the degree of oxidation of the metal. Note that both components of the oxidant form bonds with the metal.

Type 4: is similar to type 3, except that only a (CH_3^+) or equivalent is transferred. This mechanism is known to occur for arsenic.

Mechanisms 2 to 4 result in oxidation of the metal acceptor. The methylated metal is not necessarily stable nor the amount of methyl groups accepted limited to 1. Chau et al. (1987) demonstrated the possibility of transmethylation between methylated Sn and Pb, as well as disproportionation of some organometallic compounds. These are not major methylation mechanisms, but they do exist under environmental conditions.

The fundamental concept of biomethylation sought by Challenger, and as shown in the examples above, is that a methyl group has to be transferred directly onto the substrate by the action of bacteria (or molds, fungi). The reduced sulfur atom shares a few characteristics with some of the substrates above: it is in a low oxidation state, it has lone pairs of electrons which can be shared. Sulfur biomethylation would proceed according to mechanism (1)

above. However, many different substrates showed no production of volatile sulfides when methylation was expected to take place with bacteria and fungi (Challenger 1951 and references therein). Only specific substrates (most of them containing -SH and S-CH_3 groups; Taylor and Kiene 1989) seem to have a tendency to promote DMS production, whether it is due to methyl transfer or degradation of the substrate. Methylation of sulfur does occur in cells, both in ASR or DSR, because the presence of methionine requires a methylation step in its synthesis. The presence of tertiary sulfonium compounds (dimethylthetin, DMSP) in marine algae is another example. Methyl transfer inhibition (Kiene and Visscher 1987) induced lower amounts of volatile sulfides produced by methanogens, but the production of alkylated sulfides still occurred. This implies that methyl transfer alone cannot account for all volatile sulfides produced.

Methylation of sulfur producing volatile sulfides is perhaps not entirely a "true" methylation (Challenger 1959; also see above). Nevertheless, evidence suggests that methyl transfer on a sulfur substrate produced methiol compounds, but this factor cannot account for all the volatile sulfides found.

3.5 Volatile sulfide production: the rationale for a biological indicator

The extensive work done in various types of environments shows the abundance of volatile sulfides other than DMS. Additional sulfur species (DMDS, MeSH, COS, CS_2, H_2S) are also important, especially in freshwaters

and in gases emitted from soils. An example (Table 3.3) features the volatile sulfides found in waters from Luther Marsh, located NE of Guelph, Ont. DMS is present, but in lower amounts than MeSH and CS_2. The concentrations of DMS are nevertheless in the same range as in seawater. The two major differences in chemical composition between these two types of waters are: (1) the ionic strength of the water, and (2) the sulfate level in seawater (2700 mg/L $SO_4^=$; average taken from Stumm and Morgan 1981) and in Luther Lake (4.5 to 6.8 mg/L $SO_4^=$). In addition, the biological productivity and the standing crop may be different from freshwaters. DMSP is present in most marine algae, in both free and particulate forms in seawater (Turner et al. 1988), but it was not present in Luther lake nor has it been reported in other softwaters. White (1982) did not find it in softwater algae.

The few observations outlined above tend to suggest that the mechanism of production of volatile sulfides is different in seawater algae compared to freshwater algae. The major evidence is the presence of DMSP in marine waters and algae, leading to DMS as the predominating volatile sulfide in seawaters.

Sulfate availability in softwaters can be a factor in the rate of sulfate reduction, especially when the concentrations are as low as 1-2 mg/L found in many pristine lakes on the Canadian Shield. In addition, in areas where heavy sulfur deposition occurred, e.g. in the Sudbury area, sulfate has become more available. It is uncertain whether algae assimilate sulfate at a faster rate due to

Table 3.3: Volatile sulfides from Luther Lake. Also shown in this table: average DMS concentration from Pacific Ocean cruises (Bates et al. 1987b).

Sample	VS concentration in water (ng S/L)				
	H_2S+COS	CS_2	MeSH	DMS	DMDS
Luther Soil	132.6	381.3	188.9	52.3	16.2
Luther Lake	62.5	117.6	36.4	25.9	20.4
Luther Bog #1	219.4	364.2	221.7	69.5	32.4
Luther Bog #2	119.8	N.D.	334.7	23.8	32.7
Pacific Ocean (>1000 values)				92.6	

N.D.: Not detected.

sulfur pollution, possibly implying greater volatile sulfide production from algal metabolism. This can also hold for bacteria. Hence, it is imperative to consider the influence of microorganisms and softwater algae on sulfur recycling. One can also expect that if sulfate stimulates growth (i.e. if sulfate is a limiting nutrient), enhanced sulfur deposition caused by transportation of atmospheric pollutants allows sulfur to be recycled faster.

The magnitude of sulfur recycling by algae can be followed using two approaches: 1) direct measurement of the flux of volatile sulfides; and 2) the use of a biomass factor that correlates with the production of volatile sulfides. The first method gives direct results but it necessitates a fair amount of equipment to be moved to the field sampling sites. The second approach involves mostly laboratory work, so a proper parameter which correlates with VS production can be formulated.

The rationale behind the second approach is to normalize the VS concentrations per unit of biomass. The use of this biomass factor gives an indirect measurement of VS present in a lake. This calculated VS amount can be incorporated into a mass transfer model to estimate how much sulfur is transferred to the atmosphere. The major obstacle is to find a satisfactory biological indicator, readily available and easily measured.

Many studies have attempted to correlate DMS levels with Chlorophyll a. For example, Barnard et al. (1982, 1984), Turner et al. (1988) showed that DMS concentrations are not well correlated with Chlorophyll a. In these

studies, a better relationship was found between DMS concentrations and Chlorophyll a when the sample was dominated by one algal species.

No attempt has yet been made to relate VS levels with a field indicator in freshwater environments. This work will investigate if Chlorophyll a measurements can be used as an alternative for the determination of VS concentrations in the field. The premise for its use are the following: (1) similarly to studies on marine waters, one will attempt to find a correlation with primary productivity; (2) this parameter is easy to measure and readily available for the Great Lakes; (3) this biological indicator is more valid when algae are grown in well characterized conditions. Such use of Chlorophyll a, however, is severely limited and the matter will be discussed in a subsequent section.

3.6 Summary

Volatile sulfides provide an important link in the cycling of atmospheric sulfur. The physiological need or metabolic pathway leading to emission of these volatile sulfides is not clear. It is believed that environmental, chemical and physiological stresses combine to produce these sulfides. The aim of this study is to find how some of these factors influence the production of volatile sulfides from freshwater environments. The extent of sulfur recycling will also be evaluated using a mass transfer model based on some parameters readily available.

4. MATERIALS AND METHODS.

Axenic algae cultures were grown under laboratory conditions so factors (physical and chemical) influencing the production of volatile sulfides (VS) could be controlled. Table 4.1 outlines the algae used in this study, the main physical and chemical parameters applied on the cultures, and the major analytical instruments used in this study.

4.1 Gas chromatography

4.1.1 Distillation line for sulfur analysis.

The main system for VS analysis has been published (Caron and Kramer 1989) and the reader should refer to this paper for additional details on the construction of the distillation line. The following section describes the system and updates the procedures and internal standard recoveries.

The system consists predominantly of a HP 5890 Gas Chromatograph, equipped with a Hall Electrolytic Conductivity Detector (HECD) operated in the sulfur mode. The column used is a treated silica gel (Chromosil 330). The conditions and analytical parameters of the analysis system are outlined in Table 4.2. A typical chromatogram is shown in Caron and Kramer (1989).

The samples were analyzed either in a gaseous form (headspace), or as a liquid phase. The samples analyzed for the Hofmann degradation products

Table 4.1: Summary of the experimental conditions, algae and apparatus for chemical analysis.

Parameter	Comments; method
1. Apparatus for:	
Volatile sulfides	Gas chromatograph with Hall detector (sulfur selective); purge and trap distillation line (Caron and Kramer 1989)
Chlorophyll a	Fluorometry (Environment Canada 1978; Strickland and Parsons 1972)
Major ions	Ion chromatography
2. Algae species	
Softwater	*Anabaena* sp.; *Anacystis nidulans; Ankistrodesmus* sp.; *Oscillatoria* sp; *Scenedesmus starodub; Scenedesmus quadricauda*
Growth medium	Bold's Basic medium (Bold and Wynne 1978; see appendix A for medium and taxonomic information)
Marine	*Amphidinium carterae; Coccolithophora; Spirulina major*
Growth medium	Ott's enriched with Van Stosch microelements seawater medium (Bold and Wynne 1978; see appendix A for medium and taxonomic information)
3. Physical parameters	
Temperature	Laboratory (18-22 °C)
Photoperiod	16:8 day:night
Light intensity	1400 lux

Table 4.2: Detailed description of the gas chromatograph and the detector settings used for sulfur detection.

Apparatus:	Hewlett-Packard 5890 gas chromatograph.
Detector:	Hall Electrolytic Conductivity Detector, O.I. Corporation model #4420, operated on sulfur mode.
Recorder:	Hewlett-Packard 3390A peak integrator.
Column:	Chromosil 330, 8' (2.4 m), packed on 6' (1.8 m) x 1/8" (0.32 cm) O.D. in FEP Teflon tube (Supelco inc., Bellefonte, CA).
Carrier gas:	Helium Ultra High Purity (Canadian Liquid Air), with on-line filters: Molecular sieve 5A (Supelco) and Oxytrap (Chromatographic Specialties, Brockville, Ont.). Flow rate: 12.5 mL/min.
Detector gas:	Air Ultra Zero grade (Canadian Liquid Air), with Glass Moisture Trap (Chromatographic Specialties). Flow rate: 90 mL/min.
Detector solvent:	Methanol HPLC grade, added to Millipore deionized water (200 mL water diluted to 1 L with methanol). Solvent flow rate: 50-55 µL/min.
Detector output:	0-10 mV.
Injector temperature:	200 °C.
Detector temperature:	125 °C (base), 860 or 950 °C (reactor).
Oven temperature:	6 minutes at 40 °C, temperature program: 30 °C/min for 1 min and 9 min hold at 70 °C.

(see below) were introduced in a Mininert vial (9 mL glass vial closed with a Mininert valve; Chromatographic Specialties, Brockville, ON). The gaseous samples were sampled from the headspace directly above the solution. A 1 mL syringe was used to extract the gas which was then injected directly into the sample port. For the liquid phase samples, a custom-made distillation line was built at the McMaster glassblowing shop (Figure 4.1).

The distillation line is made of pyrex glass treated with a solution of dimethyldichlorosilane in toluene (5% w/vol; Sylon CT, Supelco; Caron and Kramer 1989). The principal components of the line are as follows: The sample flask (B); a condenser (C) followed by a drying trap (D) containing anhydrous $CaCl_2$; a coiled glass tubing used as a cold trap (F); finally, a cold trap/injection loop (I), custom made with medical stopcocks and FEP teflon tubing. Additional essential manipulation devices are the heating mantle (A), the cooling water for the condenser (2), the helium gas inlet (1), a vacuum pump (3). The general procedure for the operation of the distillation line is described in detail in appendix E.

4.1.2 Internal standard procedure

Quality assurance was obtained using an internal standard (diethyl sulfide, DES) added to the solution prior to analysis. The use of an internal standard (ISTD) is useful to normalize the sample for irregularities in the preparative work and for varying chromatographic conditions. This sulfide has not been detected in this work as a product from algae or microorganisms and

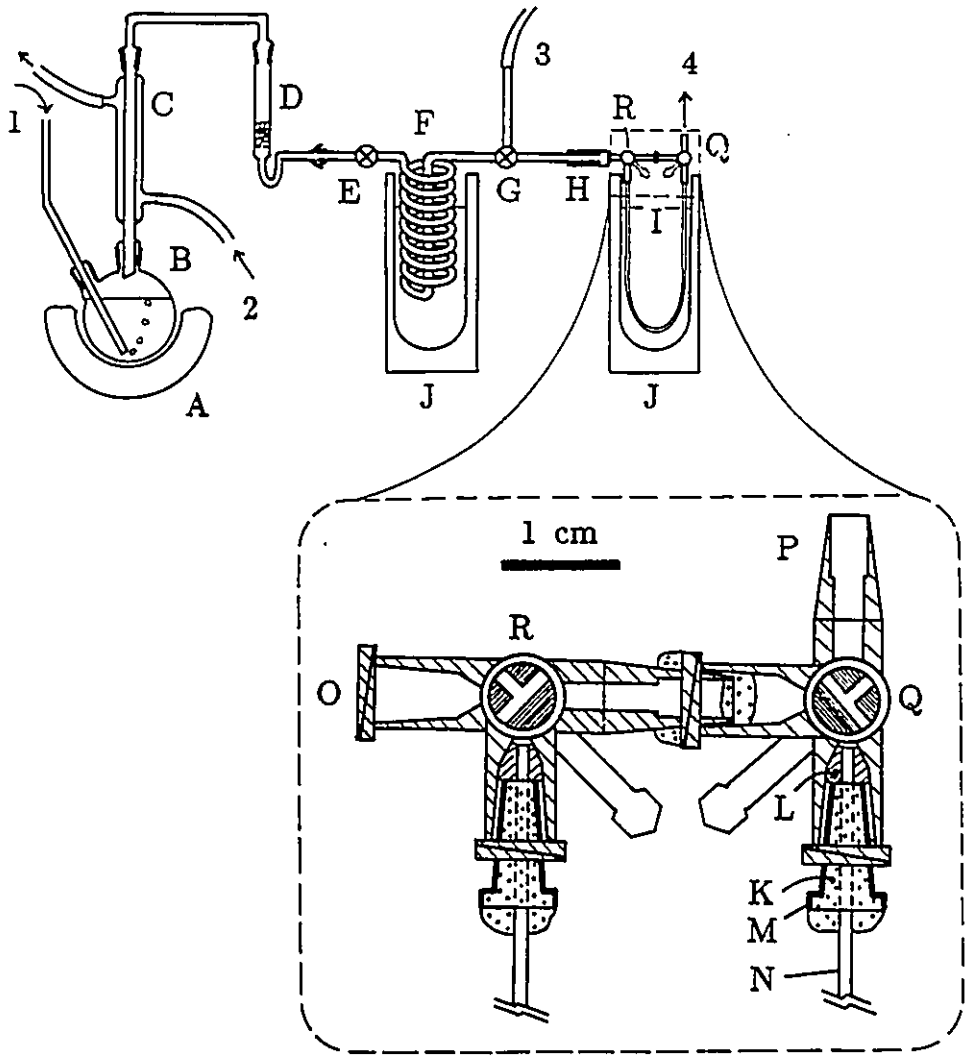

Figure 4.1: Schematic representation of the distillation line. Inlets and outlets: 1. Helium carrier gas; 2. Cold water inlet (condenser); 3. Vacuum outlet; 4. Line outlet. Line components: A. Heating mantle; B. 2-necked sample flask (100 mL capacity); C. Condenser; D. Drying tube; E. 2-way ground glass stopcock; F. Coiled cold trap; G. 3-way ground glass stopcock; H. Luer tip line junction; I. Removable teflon sample loop/cold trap; J. Liquid Nitrogen dewars; K. Bostik glue; L. Teflon tape; M. Luer tip (end of a disposable syringe cut off; N. FEP Teflon tubing (1/16" O.D.); O. Female connector of the sample loop; P. Male tip connector of the sample loop; Q, R. Sample loop valves.

it has been successfully used elsewhere (Andreae and Barnard 1983; Holdway and Nriagu 1987).

DES (liquid at room temperature) was diluted to approximately 0.05 µg/mL in ethylene glycol (the ethylene glycol had previously been degassed with helium for 3 hours at 170 °C). Ethylene glycol was chosen because it is neutral, it is miscible with both water and the volatile sulfides, and it has a low vapour pressure at the boiling point of water. The use of the ISTD is justified only if its efficiency of recovery varies linearly with that of the other sulfides. This hypothesis was addressed using blank runs of artificial lake water (Bold's Basic Medium; see appendix A). The other spikes were CS_2, DMS and DMDS, all diluted to about 0.01 to 0.05 µg/mL in ethylene glycol.

A good linear relationship was found between these sulfides and DES (Table 4.3). The slope is close to 1 and the intercept (in 0-100 scale) is reasonably close to 0, so the hypothesis is confirmed. The slope for DMDS is about 0.7, which is probably due to the low volatility of the compound. The extraction efficiency of the sulfides which are gaseous at room temperature could not be determined, and a slope of 1 was assumed.

Figure 4.2 shows that the use of the ISTD was justified. The recovery efficiency of DES in spiked standards, natural samples and algae cultures was quite variable. No recovery was poorer than 10%, and 50% of the values were within 30-60% efficiency. This variable recovery of the sulfides with the distillation line is still a significant concern at the moment. During the calibration

Table 4.3: Recoveries of volatile sulfides as a function of the internal standard.

	CS_2	DMS	DMDS
Slope (A):	0.976	1.001	0.684
Intercept (B):	4.500	2.729	5.660
Linear correlation (r):	0.876	0.941	0.884
Number of runs (n):	25	25	25

Equation: (%)y = Ax(%) + B, where (%)y and (%)x are the recovery efficiencies expressed as percent. Variable y is the dependent variable, namely CS_2, DMS or DMDS, and x is for DES.

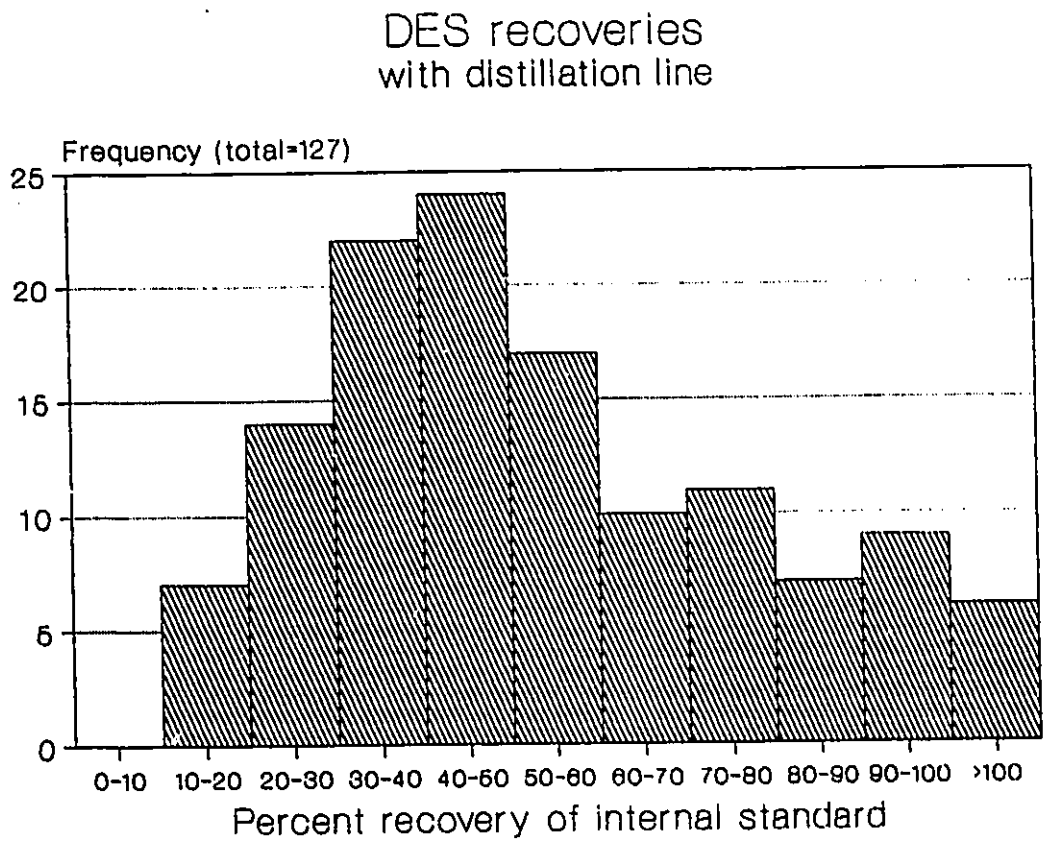

Figure 4.2: Distribution of DES recoveries for cultures and natural waters.

from variations in ionic strength, quantity of standard (5 to 500 µL of ethylene glycol solution), pH (4 to 10), or the time of heating. The purge rate was optimized at 100-120 mL/min. Incomplete purging of the aqueous solution is likely to be an important part of the problem. In fact, successive runs of the same sample (without opening the sample flask or changing its content) showed residual sulfides and ISTD. Up to three successive purges are necessary to remove all sulfides (>97%) from the solution. The design of the solution bubbler could be modified to improve the internal standard recovery. Perhaps a fritted glass sparger producing fine bubbles of helium would improve the recovery of volatile sulfides from the solution.

4.1.3 The cross-check line

The concentration of ISTD in ethylene glycol had to be verified at times using an independent method. A small purge line was built to accommodate the ISTD and small volumes of concentrated samples (e.g. samples of algae spiked with methionine). A cross-check line was built for such use (Figure 4.3). The operation of the line is similar to the other line (see Appendix E). The result obtained with this line is the reference value for the ISTD. Duplicates generally agreed within 10 to 15%. The ISTD was stored in a freezer (Temperature approx. -5 °C), and checked periodically.

The cross-check line allowed a better recovery of the ISTD and produced less variability than the standard distillation line for samples containing high amounts of VS (e.g., cultures of algae spiked with methionine). The ISTD recovery was complete as evidenced by successive purges of the same

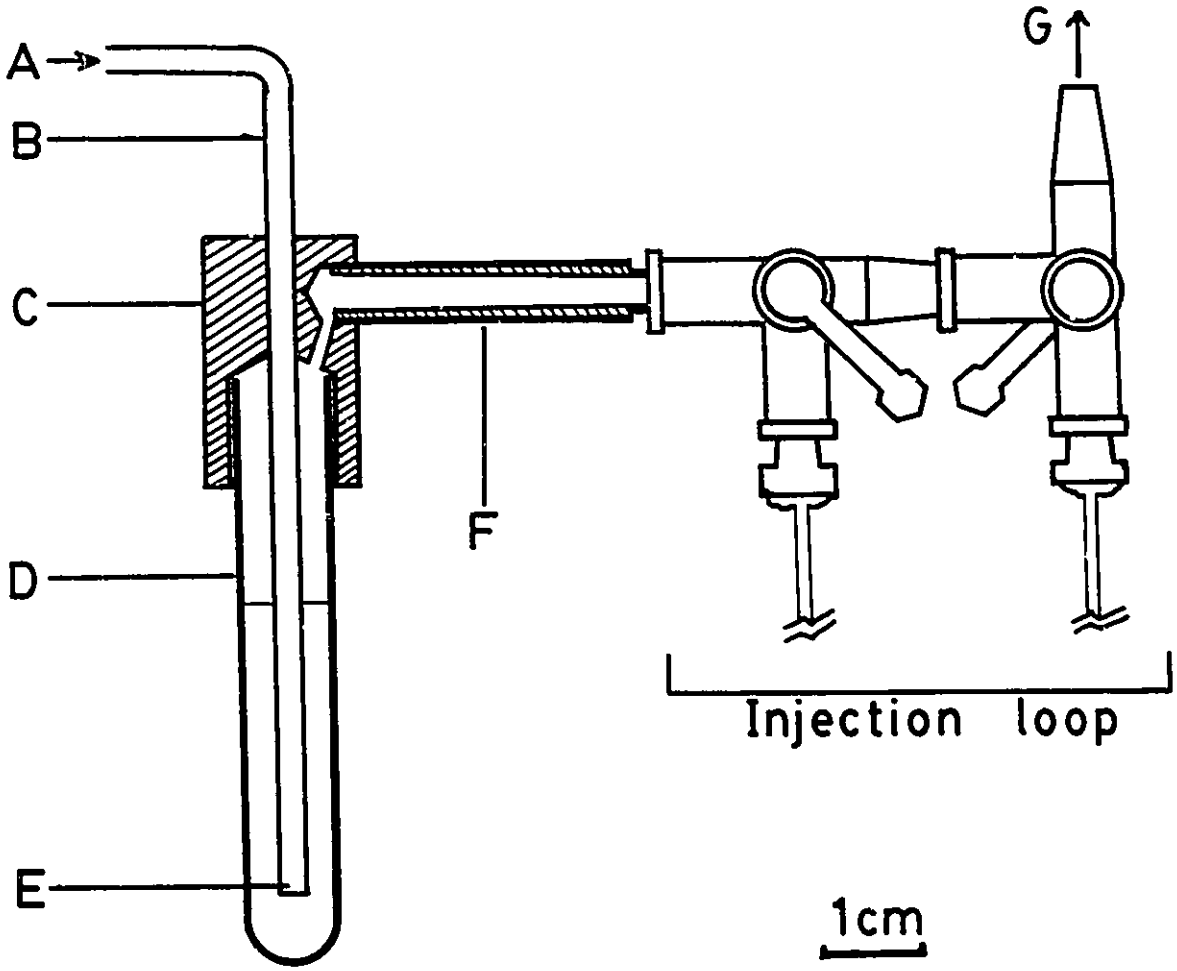

Figure 4.3: Schematic representation of the Cross-check line. A. Gas (Helium) inlet; B. Pyrex tubing (3 mm O.D.); C. Polyethylene support; D. Sample tube (9 mm O.D.); E. Tip of the bubbler; F. Polypropylene junction; G. Outlet.

sample.

4.1.4 Gas chromatograph for ^{35}S analysis

The gas chromatographic system used for radioactive assay was a Varian Aerograph (model 1800) with a splitter separating a flame ionization detector (FID) (10%) and a Nuclear Chicago gas proportional detector (90%). The column used was also a Chromosil 330. Detailed conditions are outlined in Table 4.4. The results were plotted on two separate chart recorders, one for each detector, and the peak areas were measured manually.

Headspace gas (with a 2.5 mL syringe) or purge/trap with the cross-check line were used to collect volatile sulfides. In the latter situation, a syringe equipped with a 12" needle was used to withdraw some algal medium through the foam plug. The sample was poured into the test tube of the cross-check line. Sparging was done manually using a 50 mL syringe filled with helium. Manual purge of this small sample was adequate.

4.1.5 Gas chromatograph calibration

Accurate calibration was achieved with permeation tubes. Tubes of the gaseous species (H_2S, COS, MeSH) were obtained from Vici Metronics (Santa Clara, CA). These tubes were certified and did not require further calibration. The tubes of the other sulfides, which are liquid at room temperature (CS_2, DMS, PrSH, DES and DMDS), were custom made in our laboratory. The principle is described in O'Keefe and Ortman (1966) and Scan.:gelli et al. (1970). The high vapour pressure of the liquid allows the gas phase to

Table 4.4: Description of the gas chromatograph used for ^{35}S assay.

Apparatus	Varian Aerograph model 1800.
Column	Chromosil 330 (Same as previously)
Carrier gas	Helium (ultra high purity). Flow rate: 12 ml/min.
Temperatures	Oven: 70 °C (isothermal). Injector: 200 °C. Detector (FID only) and splitter: 240 °C.
Splitter	10% FID, 90% GRC
Detector system 1:	
Detector	Flame ionization detector (FID)
Gases	Air (ultra zero grade), flow rate: 120 mL/min. Hydrogen, flow rate: 20 mL/min
Recorder	6.5" chart recorder.
Detector system 2:	
Detector	Gas Radio Chromatograph (GRC), Nuclear Chicago Model 4998 system (#8733 ratemeter, #8765 amplifier-discriminator, #461 gas proportional detector (85 mL internal volume)).
Settings	Voltage applied: 2800 V, 3400 V. Count range: C/M 300, 1000. Time constant: 20 seconds (for C/M 300), 10 seconds (for C/M 1000). Sensitivity for #8765 discriminator: 1 mV.
Gas	Counting gas: Methane (Matheson); 60 mL/min.
Temperature	Inlet tubing (from Varian GC): 95 °C. Counting chamber: 115 °C.
Recorder	6" chart recorder.

permeate through the teflon membrane at a constant rate with time, when the tube is maintained at a constant temperature. The tubes were weighed periodically on an analytical balance to obtain a weight loss as a function of time, thus an emission rate, or permeation rate. Figure 4.4 shows a typical gravimetric calibration obtained with a DES tube monitored for close to 2 years.

The home-made permeation tube is made of a FEP teflon sleeve connected to a reservoir filled with the appropriate liquid sulfide at one end. The other end is hermetically sealed with a glass rod (Figure 4.5). The permeation devices were inserted into a mixing line flushed with helium (Scaringelli et al. 1970; Figure 4.5). The flow of helium is adjustable, so syringe injections using the mixing line and the permeation devices can easily achieve concentrations ranging from 0.01 to 10 ng/mL of compound.

The permeation devices and the mixing line were always maintained at 25 °C (± 0.1 °C) to ensure a constant emission rate. However, some home made tubes showed important variations in their permeation rate. The reason for this is unknown and solving this problem was not pursued. This did not affect the detector calibration because the tube weights were carefully monitored, so their permeation rate was constantly updated.

4.1.6 Hall detector (HECD)

The information published about the detector performance in the original method (Caron and Kramer 1989) was valid for the first year of use of the HECD. All the calibration curves were fairly similar for all gases and their

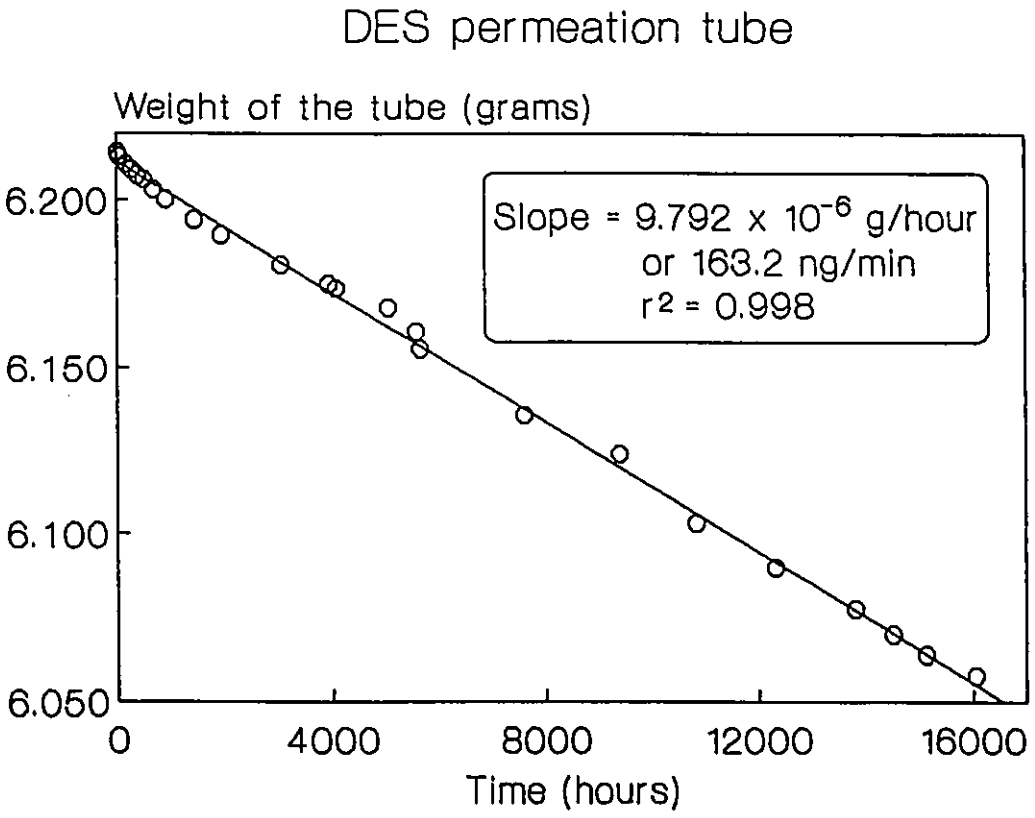

Figure 4.4: Weight of the home-made DES permeation tube as a function of time.

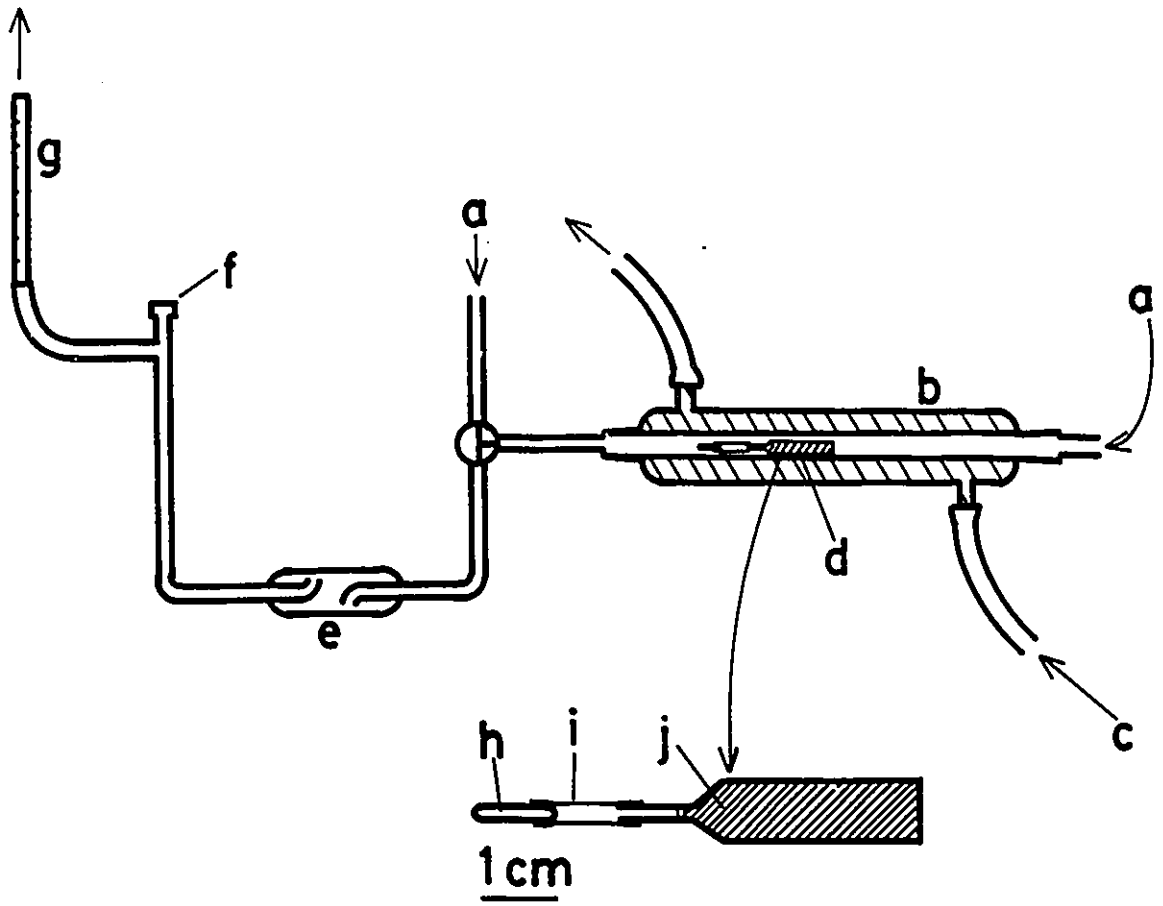

Figure 4.5: Mixing line and permeation tube used for calibration. a. Helium gas inlets; b. Condenser; c. Thermostated water; d. Permeation tube; e. Mixing chamber; f. Septa; g. Rotameter; Enlarged section: h. Glass rod; i. Teflon tubing; j. Reservoir of volatile liquid.

working ranges. Figure 4.6 shows the calibration curves obtained at that time with the detector for 9 sulfur species frequently found. Note that the two symbols at the extreme ends of each slope represent the working ranges attained with the calibration system. Each single curve is composed of at least 7 points. The slope of the curve associated with each sulfide is close to unity, thus the signal is directly proportional to the amount of sulfur found in the analyte.

In October 1987, the original element in the furnace responsible for high temperature pyrolysis on the HECD failed. The element was then rebuilt on-site with Nichrome wire (4Ω/ft, gauge number unknown). Many designs were attempted offering only a limited working time (about 1-2 months of normal work before failing again). The working temperature was also lowered to extend the element life. Since the reaction temperature is critical for attaining slopes of 1 and intercepts of 6 for all sulfides (Caron and Kramer 1989), a lower temperature of 860-900 °C meant different slopes and intercepts for the calibration curves of individual sulfides. This affected the sensitivity of the detector and the detection limit. Runs made with the new element gave slopes ranging from 1.6 to 1.9 and intercepts from 4.7 to 4.9, for COS, CS_2, MeSH, DMS, and DMDS. DES had an intercept of 5.6. The background noise was low, and the detection limits were virtually unchanged from previous calculations (Caron and Kramer 1989). The new element, operating at a lower temperature, did not affect the stability of the calibration curves, which remained reproducible within a few weeks. Calibration curves were done frequently so

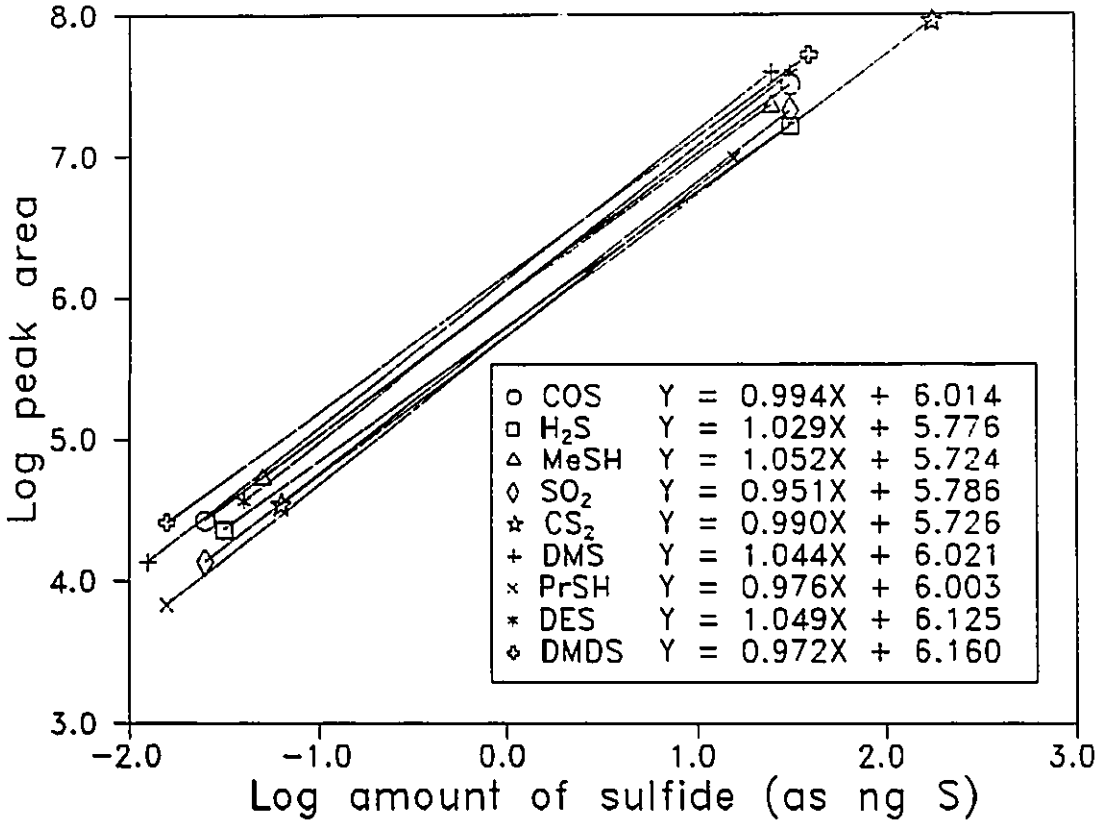

Figure 4.6: Calibration curves of nine selected sulfides. the two symbols indicate the extremes of the range attained with the calibration line. Each line has at least 7 points ($r > 0.98$ each). HECD conditions: reactor 950 °C, attenuation 5.

uncertainty was minimized.

4.1.7 Flame ionization detector (FID) and gas radio chromatograph detector (GRC)

The mixing line and the permeation tubes were also used for calibration of the GC-FID system. Tubes with higher emission rates were built to compensate for the anticipated lower sensitivity of the FID for the volatile sulfides. Peak areas were calculated manually from the chart strip. The detection limits and sensitivities of DMS and DMDS on the FID are shown on Table 4.5.

Peak areas from the radioactive tracers were calculated manually from the chart recorder. Corrections for the residence time of the compound in the counting chamber, the gas flow rate at the detection temperature, etc. were done according to the Nuclear Chicago instruction manual. Corrections for ^{35}S decay were also done whenever applicable.

Accurate quantitative determination of the radioactive signal could not be achieved. Possible reasons are: firstly, losses of MeSH, DMS and DMDS are suspected on metal surfaces. The splitter was made of steel, and metal tubing was connecting the GC to the GRC. Some labelled DMS, originating from the decomposition of labelled methionine in water at room temperature, was detected on the FID, but not on the GRC. It is not known how the metallic surface affected MeSH and DMDS, but radioactive signals from the latter were detected. Secondly, it was not possible to get an internal standard of ^{35}S-MeSH

Table 4.5 Detection limits for selected volatile sulfides.

1. HECD

Species	Detectability (D=2N/S) (pgS/sec)	$W_{0.5}$ (sec)	L_D (L_D=DxW$_{0.5}$) pg compound	Method* limit (ng S/L)
H_2S	0.45	6.0	2.9	0.03
COS	0.27	6.0	3.0	0.02
MeSH	0.50	10.8	8.0	0.06
CS_2	0.52	15.0	9.2	0.09
DMS	0.30	14.3	8.2	0.05
PrSH	0.37	15.2	13.4	0.07
DES	0.26	21.3	15.8	N/A
DMDS	0.26	24.4	9.3	0.10

* The method limit is for the distillation line with the original element. It is calculated with each sulfide detection limit, a sample volume of 0.1 L and a 90% efficiency extraction as estimated from the DES spike.
N/A: not applicable

2. FID (on Varian/Aerograph)

Species	Detectability (D=2N/S) (pgS/sec)	$W_{0.5}$ (sec)	L_D (L_D=DxW$_{0.5}$) pg compound	Method** limit (ng S/L)
DMS	11.7	19.7	0.230	2.30
DMDS	14.0	31.9	0.446	4.46

** The method limit is the detector's limit divided by 10 to account for the 10:90 splitter.

or ^{35}S-DMDS. The counting efficiency of the detector could not be determined. For these reasons, the radioactive results can be used for qualitative information only.

4.1.8 Flame Photometric Detector (FPD)

A Varian 3400 GC equipped with a FPD was used for an interlaboratory comparison of the standard calibration curves and the detection limits of selected sulfides. Table 4.6 gives a description of the system. Figure 4.7 shows a comparison of two sets of calibration curves obtained with FPD along with one set with HECD. Figure 4.7d shows the detection limits of the two systems. The purpose of this comparison is two-fold: (1) to verify whether DMS has a different sensitivity (intercept on the log-log plot) from the other sulfides, and (2) to compare the detection limits.

The slopes of the calibration curves obtained with the FPD are somewhat variable among sulfides, which is not observed with the HECD. Their values also changed after stabilizing overnight as evidenced in Figure 4.7 a and b. The DMS curve does not show major differences compared to those of other sulfides. The FPD has poor detection limits, which are caused primarily because of a high background noise.

To summarize the aim of this comparison, DMS does not have a preferential high sensitivity, which would mislead FPD users to report only DMS in natural waters. But the high detection limit of the FPD can wrongfully dismiss the presence of other sulfides which are in significant amounts in

Table 4.6: Description of the GC/FPD.

Apparatus	Varian model 3400
Column	Chromosil 330 (same as previously)
Carrier gas	Helium (12.5 mL/min)
Detector	Varian dual flame photometric detector. Photomultiplier: 500 V, autozero off.
Detector gases	Air 1: 80 mL/min; Air 2: 172 mL/min; H_2: 140 mL/min.
Recorder	HP 3390A integrator
Temperatures	Oven: temperature program (same as Table 4.2); Injector: 160 °C; Detector: 220 °C.

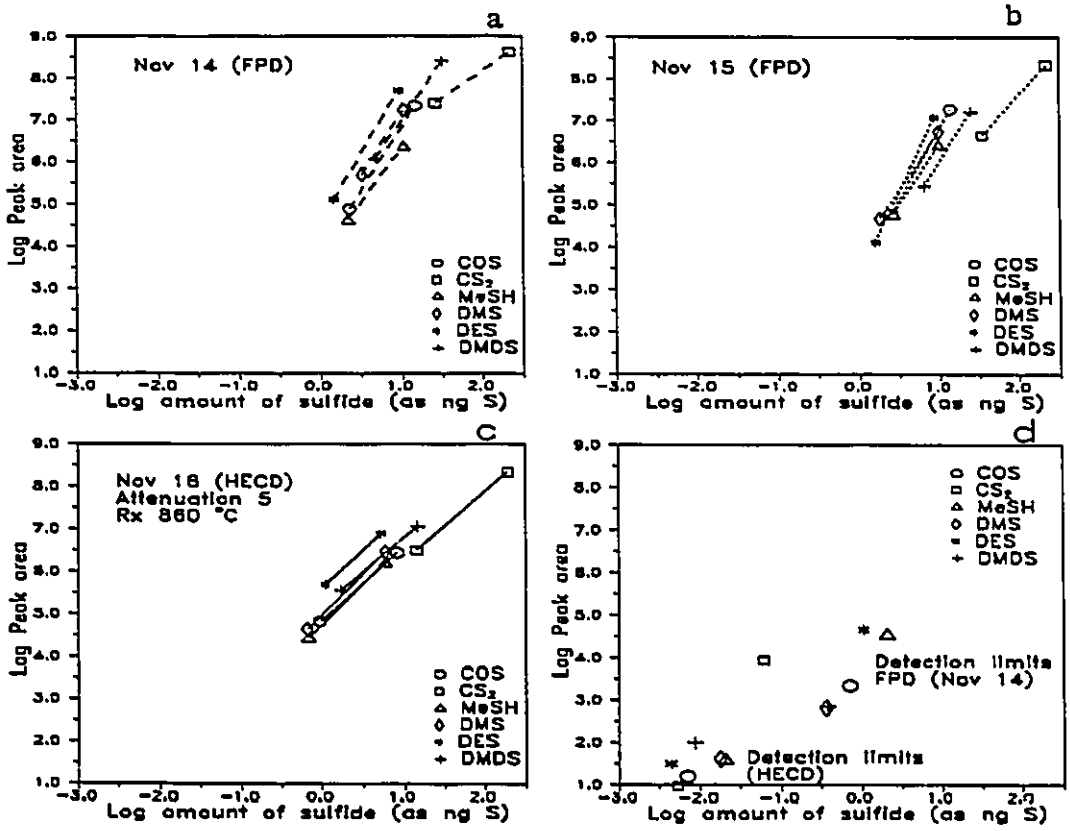

Figure 4.7: Comparison of calibration curves and detection limits obtained with FPD and HECD. a and b: FPD. c: HECD. d: Detection limits for both detectors. The symbols represent the upper and lower ranges of the calibraton curves. Each curve has a minimum of 6 points.

natural waters. The FPD does not create an artificial bias towards DMS detection, but it is important to have a sample volume adequate for the detection of all sulfides with this type of detector.

4.2 Algae cultures

4.2.1 General settings

Some freshwater algae found in the Great Lakes basin, specifically *Oscillatoria* sp., *Anacystis nidulans*, *Scenedesmus starodub*, *Ankistrodesmus* sp., *Scenedesmus quadricauda*, *Anabaena* sp., were grown in Bold's Basic Medium (BBM). The pH of all BBM solutions was adjusted to 7.10-7.20.

The algae were grown in the laboratory at room temperature (19-21 °C) in Erlenmeyer flasks. The volume of solution was usually 100 mL in a 250 mL flask, and a foam plug was inserted at the mouth of the flask to avoid bacterial contamination. Occasionally other flask sizes were used (500 mL, 100 mL, 50 mL or 25 mL) and the same ratio liquid:flask volume was kept. The flasks were laid approximately 60 cm under fluorescent lights (GRO & SHO Wide Spectrum Plant Light, 40W, type F40/GS/GS, Canadian General Electric). The photoperiod was 16:8 hours (light:dark). The cultures were static, and hand shaken occasionally (3-6 times/week). The BBM solutions were autoclaved at 15 psi of hot vapour pressure (121 °C) for 20 minutes in Barnstead autoclaves. The unused BBM and algae were stored in a cold room suitable for storage of biological specimens maintained in the dark at 4 °C. Transfers of inocula were performed aseptically following standard procedures over a flame with aseptic

pipettes. Despite these precautions, it is possible that bacterial contamination occurred. Experiments were usually carried out on algae approximately 15-18 days after introduction of the inocula. The algae were usually 3 weeks old at the time of transfer, which corresponds to the end of the exponential phase.

Seawater algae were grown in artificial Von Stosch Enriched Seawater Medium (appendix A2) with pH adjusted to 8.10-8.20. Sterile Millipore filter units (Type E, 0.45 µm and 0.22 µm) were used to sterilize the medium. The sterile media were transferred aseptically (over a flame) in Erlenmeyer flasks pre-sterilized at 125 °C in oven. Unused solutions and leftover algae were stored in a cold room.

4.2.2 Addition of nutrients to cultures

Cultures were generally grown for approximately 2 to 3 weeks (corresponding to the middle of the exponential phase). Addition of extra nutrients was done at this stage using sterile material to ensure aseptic conditions inside the flask. For this purpose, sterile disposable needles (2") and syringes (medical grade), on-line 0.22 µM Millex filter (Millipore) were used. The solution was injected into the culture with the syringe/filter assembly through the foam plug. The 0.22 µm filter ensured the sterility of the spike.

The amounts of sulfur-containing spikes were designed to replace the sulfate in equimolar amounts, i.e. 0.3 mM in BBM. The amount of methyl donor was chosen as 4 times the methylated substrate (Rapsomanikis and Craig 1985), thus 1.2 mM in normal BBM. Sulfur-containing spikes were added to solutions of "no sulfate BBM" usually containing no more than 0.5 mg/L $SO_4^{=}$

(0.005 mM).

4.3 DMSP

4.3.1 Synthesis of DMSP

Dimethylsulfoniopropionate, DMSP (bromide salt), was synthesized following the procedure outlined in Challenger and Simpson (1948) and Dickson et al. (1980). The detailed procedure is in Appendix C. Proton NMR, IR spectroscopy and melting point determination ensured the identity of the compound.

4.3.2 Analysis of DMSP in algae

Alkali degradation of DMSP, also called Hofmann degradation (Morrison and Boyd 1973; Windholz 1983), is quantitative (Dacey and Blough 1987; White 1982) and produces an olefin (acrylic acid) and a sulfide (DMS). Quantification of DMS provides a direct account of DMSP. This method has been used in other studies on marine algae (Ackmann et al. 1962; 1966; Vairavamurthy et al. 1985), and most of the DMS produced originates from DMSP decomposition (White 1982). The latter also noted that DMSP decomposition to DMS and acrylic acid is complete after a 20 minute reaction with 1M NaOH at 23 °C, based on experiments done with marine macrophytes.

The algae analyzed for their DMSP content were filtered on GF/C fibreglass filter (pre-weighed) and stored in a desiccator. After the dry weight determination, the whole filter was introduced into a 9 mL Mininert vial with 1

mL of 1M NaOH. The bottle was closed and incubated at 70 °C for 1 to 2 hours. 1 mL of headspace was withdrawn for analysis. The DMS measured was converted to the quantity of DMSP present in algae tissue (dry weight) according to the relationship:

$$DMSP_A = DMS/(W_R * W_A) \qquad [4.1]$$

Where:

$DMSP_A$: Experimental result: DMSP present in algae (ng comp/mg algae)

DMS: Amount DMS (as ng of compound)

W_R: Weight ratio and conversion factor, which includes molecular conversion from DMS/DMSP, etc. Standard runs with pure DMSP gave $W_R = 0.0217 \pm 0.0038$ (dimensionless)

W_A: Dry weight of algae (mg)

4.4 Water analysis

4.4.1 Volatile sulfides in cultures

The cultures, unless otherwise specified, were analyzed in the distillation line as is. Comparisons of filtered vs unfiltered samples did not show an appreciable bias among each other (vide infra). Small samples were diluted to 100 mL with Milli-Q water for analysis in the distillation line. The culture medium (BBM), fresh or autoclaved, did not contribute any volatile sulfide.

4.4.2 Volatile sulfides in field samples

All field samples were analyzed unfiltered unless otherwise specified. Some samples of freshwaters from the Hamilton area were taken in assorted

types of environments. The Desjardins Canal, Dundas, Ontario, was sampled. The canal is connected to the Royal Botanical Gardens (RBG), at the western point of Lake Ontario. It receives partially treated domestic wastewater of the town of Dundas. A eutrophic pond from Cootes Paradise (also part of the RBG), behind McMaster University, was sampled. Another site was Luther Marsh Conservation area (on the Grand River Watershed, North of Guelph, Ont.), which is a natural lake surrounded by bogs. Three different sites of the bog water and lake water were sampled. The collection was carried out with stainless steel containers closed hermetically after sampling. The samples were stored in a cold room (5 °C) until analysis (within 48 hours). The sediment water from Harp Lake (near Huntsville, Ont.) is a suspension of bottom sediments mixed with filtered (100 μM) surface water of the same lake. The sampling was done when the lake was covered by a 40 cm ice cover. A few weeks elapsed prior to analysis, but the water was maintained in the dark at 5 °C, close to the lake conditions. This sample is not expected to simulate the lake's conditions at the moment of sampling, but rather to demonstrate that volatile sulfides are present. Similarly, Lake Ontario surface water was collected offshore from Burlington, Ont., and analysis was done the following day.

4.4.3 Chlorophyll a measurements

The standardization curve was done empirically using Environment Canada NAQUADAT Method manual No 06716 (Environment Canada 1978). A spectrophotometer (Hewlett-Packard 8541A) was used to obtain absorbance

readings at 663, 645 and 630 nm. These absorbance numbers were converted to obtain the concentration of pigments in the acetone extracts (Environment Canada 1978). The same extracts were then used to calibrate a Turner 110 fluorometer. Subsequent analyses were done on the fluorometer in a dark room (Strickland and Parsons 1968).

4.4.4 Anion analyses

The anion concentrations were determined on a Wescan 262-100 ion analyzer equipped with a 213A electrolytic conductivity detector. The column used was a Vydac 300IC405 ion exchange resin. The results were recorded on a chart and the concentrations calculated manually from the chart strip.

5. RESULTS

The sulfides are identified on the basis of their retention times (R_t) in GC. A possible R_t overlap could occur for CO_2, COS and H_2S, which all eluted between 0.9 and 1.5 minute. CO_2 (R_t = 0.95 min.) was usually not a strong interference because the detector is not sensitive to this compound. It was difficult to separate COS (R_t = 1.15 min.) and H_2S (R_t = 1.35 min.). Moreover, the injection with the disposable loop causes the peaks to shift to shorter retention times (by 0.1 to 0.15 min.) which further complicates separation and identification of the individual peaks. Therefore, in this study, the designation of "H_2S+COS" represents the presence of H_2S or COS or both when the identification is ambiguous. Otherwise each peak is appropriately labelled.

The ethylene glycol solvent peak eluting at about 9.0 to 9.5 min. did not interfere with the sulfide analyses. False identification is unlikely because the peak is easily recognized by its characteristic tailing. In addition, no sulfide has ever been found near the ethylene glycol peak. The above are the only potential interferences encountered with the GC/HECD system.

5.1 Natural samples

The results of the analysis of volatile sulfides in some freshwaters from the Hamilton area are shown in Figures 5.1, 5.2 and 5.3.

Some of the sample types reported in these figures are not necessarily

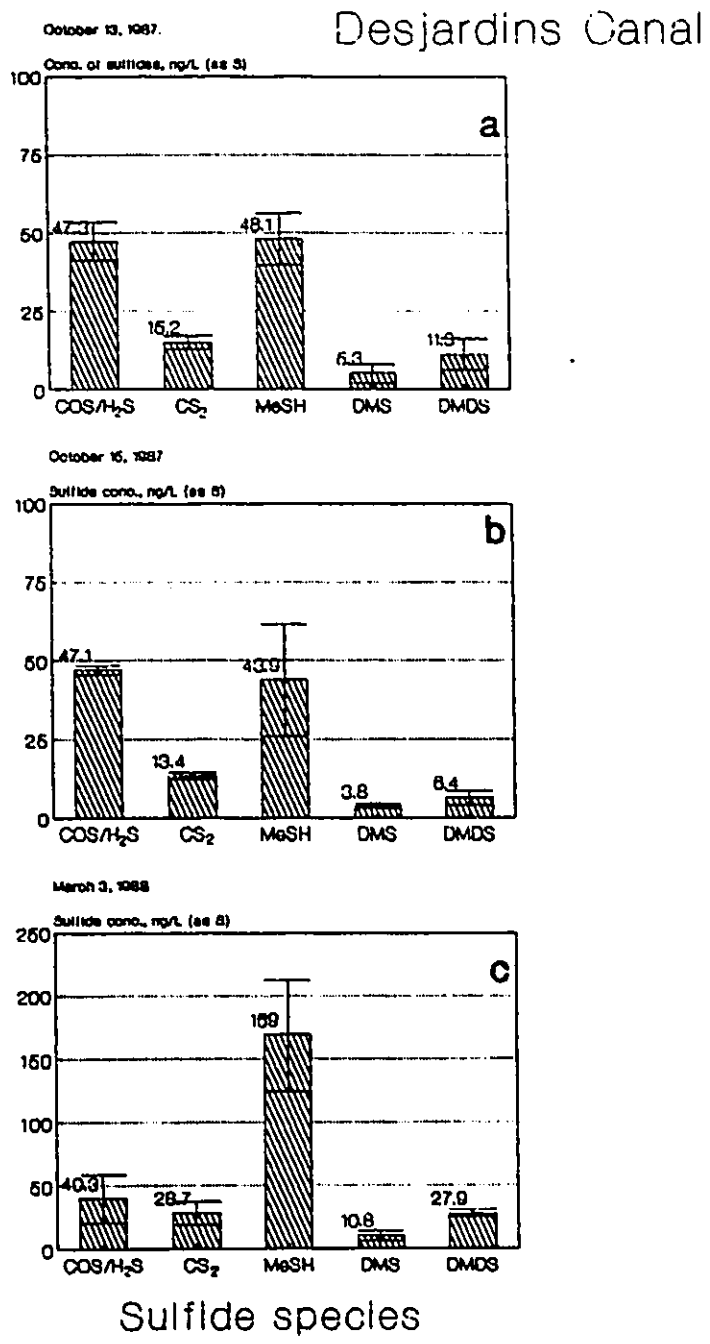

Figure 5.1: Samples from the Hamilton area: Desjardins Canal (Dundas, Ont.). a: October 13, 1987. b: October 15, 1987. c: March 3, 1988. Note the scale difference between a, b, and c.

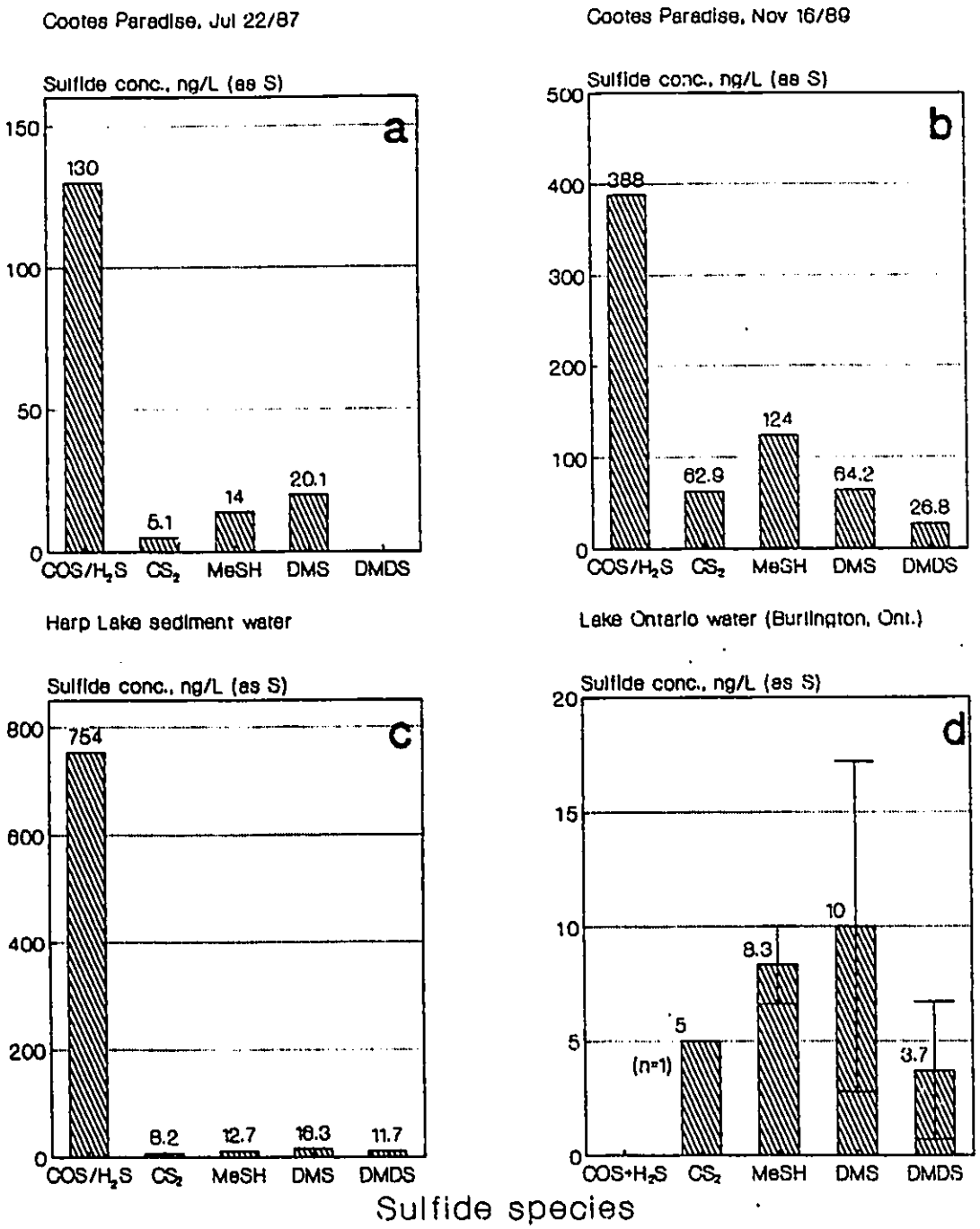

Figure 5.2: Samples from the Hamilton area. a: Cootes Paradise (Hamilton, Ont.), July 22, 1987. b: Cootes Paradise at Valley Inn Road, November 16, 1989. c: Harp Lake (near Huntsville, Ont.), sediment suspension. c: Lake Ontario (in front of Burlington, Ont.), sediment suspension.

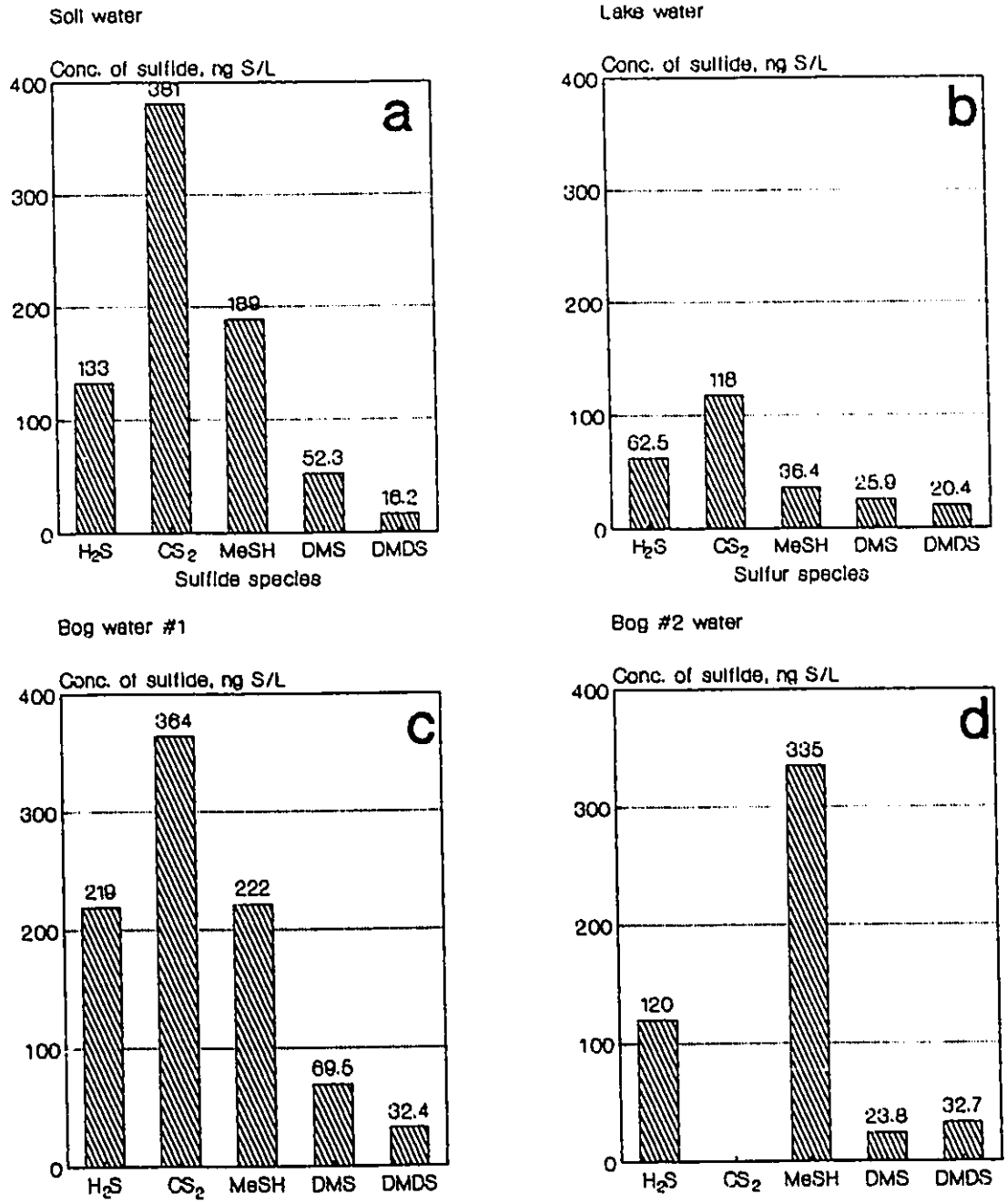

Figure 5.3: Samples from Luther Lake (North of Guelph, Ont.). a: Soil water (0.3 M deep). b: Lake water. c: Bog water, shade. d: Bog water, sunlight.

representative of the status of natural freshwaters. For example, the waters from Desjardins Canal contain some domestic wastewater from the city of Dundas. Sediment water from Harp Lake and Lake Ontario are a suspension of sediment mixed with surface water from their respective lakes. These samples are shown here to demonstrate the omnipresence of VS in freshwaters. They do not necessarily represent the status of these waters at the time of sampling. Several types of waters are represented: these samples were taken in oligotrophic and eutrophic waters, with low to high pH, and they contain various amounts of Dissolved Organic Carbon (DOC or DOM).

The results of all samples show the presence of 4 or more species of volatile sulfides. H_2S and/or COS can be quite important in softwaters, and often MeSH is the dominating species. CS_2 is present in most samples (except for example Fig. 5.3d). DMS is also omnipresent, but only in moderate amounts. DMDS is usually found everywhere, also in small quantities. In all the freshwater samples analyzed (lake water, algae cultures), MeSH usually predominates, making up 40 to 80 % of the volatile sulfur species. Then, COS+H_2S makes up 10 to 40 %, CS_2 10 -30 %, DMS 5-25 % and usually DMDS constitutes less than 10 % of all the volatile sulfides. Sulfides other than the ones above were found in less than 10 % of the samples, making a negligible contribution (<5 %) in all instances.

5.2 Volatile sulfides from algae cultures

Figure 5.4 shows that some representative freshwater algae, found in softwaters and also in the Great Lakes, produce some volatile sulfides in various amounts. These algae were grown in axenic conditions in duplicate flasks under the same conditions, except for the sulfate concentrations (see later). Other species which were studied to a lesser extent in this work are featured in Figure 5.5. Note that *A. carterae* is a marine variety.

All of these species of algae produce volatile sulfides. The absolute amounts and relative distribution of all volatile sulfides differ from one species to another. There is no predominant species of volatile sulfides. H_2S+COS is almost always present, and CS_2 is often important. The methylated reduced sulfur species are also present: MeSH is often the major species, then DMS and DMDS in decreasing amounts. The situation is different for *A. carterae* (fig. 5.5a): DMS is the dominant sulfur species in solution, and its level is far higher than what is found in softwater algae by about two orders of magnitude. DMS also strongly predominates over all other sulfide species, although MeSH, DMDS, CS_2 and H_2S+COS are still present. This is probably evidence of the presence of the DMS precursor DMSP (Vairavamurthy et al. 1985).

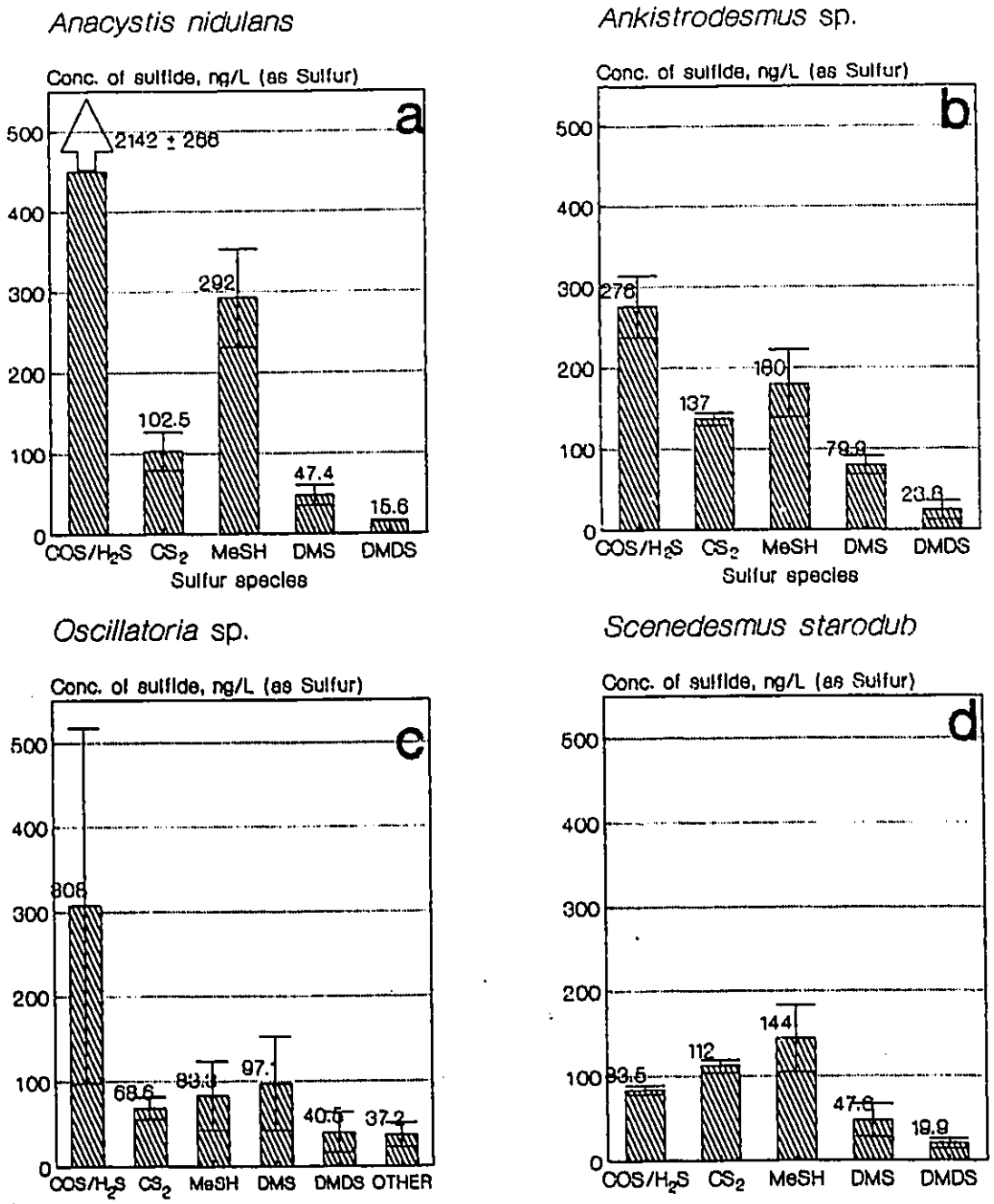

Figure 5.4: VS found in algae cultures. a: *Anacystis nidulans* (n=4). b: *Ankistrodesmus* sp (n=4). c: *Oscillatoria* sp. (n=3). d: *Scenedesmus starodub* (n=3).

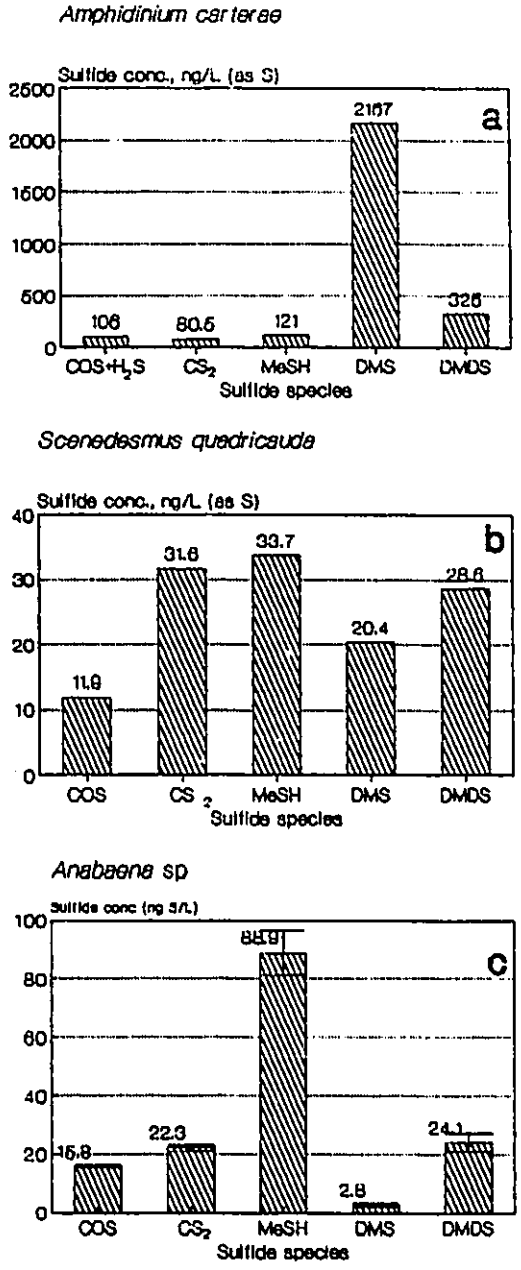

Figure 5.5: Volatile sulfides present in aqueous cultures of algae. a: *Scenedesmus quadricauda*. b: *Anabaena* sp (n=2). c: *Amphidinium carterae* (seawater). Note the scale difference between *A. carterae* and the other two.

5.3 Hofmann degradation

Results of DMSP analyses are reported in Table 5.1. All samples of seawater algae contained DMSP as evidenced by the DMS detected from the Hofmann degradation. The amounts found are somewhat lower than what is reported in Dacey et al. (1987), and White (1982), and they are quite variable among seawater species, as well as for the two runs using *A. carterae*. The softwater algae did not consistently show the presence of DMSP. Two samples of *Ankistrodesmus* gave a DMS signal, but the latter was not detected in three other runs. One sample of *Oscillatoria* incubated with CH_3I also gave a DMS signal. This is possible evidence of the formation of a dimethyl sulfonium compound (vide infra).

5.4 Influence of filtration

The need to filter and the effect of filtration on samples had to be tested because: (1) the physical strain applied on the algae cells can disrupt the membranes and release some intracellular volatile sulfides into solution (if present); (2) the filtered samples can show lower volatile sulfide concentration due to solution degassing during vacuum filtration; (3) the samples analyzed in this study, especially the algae cultures, were analyzed without being filtered. For this purpose, filtration partitioning was tested on some algae cultures. A Millipore ground glass filtering funnel assembly was used along with Whatman GF/C fibreglass filters (1.2 μM retention size). It is assumed that the findings

Table 5.1: DMSP in some algae (obtained from Hofmann degradation)

Algae	Amount of DMS found (ng comp.)	DMSP in algae Dry weight (ng DMSP/mg Algae)
SEAWATER ALGAE:		
Amphidinium carterae (young)	6.62	450.8
Amphidinium carterae (old)	2.14	33.5
Coccolithophora (old)	5.88	116.9
Spirulina major (old)	5.28	25.7
Dried Sea dulse	1.14	18.2
SOFTWATER ALGAE:		
Anacystis nidulans (mature to old)	N.D.*	
Ankistrodesmus (Mature)	0.54	8.5
Ankistrodesmus (old)	0.47	5.1
Ankistrodesmus sp.	N.D.	
Oscillatoria (young to mature)	N.D.	
Oscillatoria (with betaine)	N.D.	
Oscillatoria (with CH_3I)	3.44	200.4
Oscillatoria (with choline)	N.D.	
Scenedesmus starodub	N.D.	

* N.D.: Not detected. Each "not detected" sample was done in duplicate or triplicate for confirmation.

will be similar for all types of samples (algae and natural waters).

The results are shown in Figures 5.6 to 5.9 for cultures of *A. carterae, Anabaena* sp, *A. nidulans,* and finally *Ankistrodesmus.* Each culture is fractionated in three partitions: (1) raw or unfiltered solution; (2) GF/C filter solution (particulates (>1.2 μm) retained on the filter), and (3) Filtrate (filtered solution including small particles <1.2 μm). All samples were analyzed with the distillation line immediately after partitioning. The filtrate was analyzed first to avoid further losses due to solution degassing. The filter (particles >1.2 μm or retentate) was set aside in the dark and used for the next run. The latter was introduced into the distillation flask filled with 100 mL Milli-Q water for analysis. The concentration units expressed in this fraction denote the amounts of volatile sulfides present in the whole sample contributed by particulate algae when a raw sample is analyzed. They do not necessarily reflect the volatile sulfide concentrations within the cells. In Figure 5.6, the result of a *A. carterae* culture is reported. In Figures 5.7 to 5.9, boxes a and b are the fractions obtained from one culture flask, whereas c and d are runs of a different flask.

In all samples of softwater algae analyzed in this way, an important H_2S peak (not COS!) was recorded in the GF/C filter fraction, and only small amounts of the other volatile sulfides were observed. The H_2S peak was generally small in the filtrate fraction and the other volatile sulfides were generally higher than in the particulate algae fraction. The raw (unfiltered) algae and the solution filtrate generally show volatile sulfide patterns similar in relative distribution and in concentration, except perhaps for *Anabaena* (Fig.

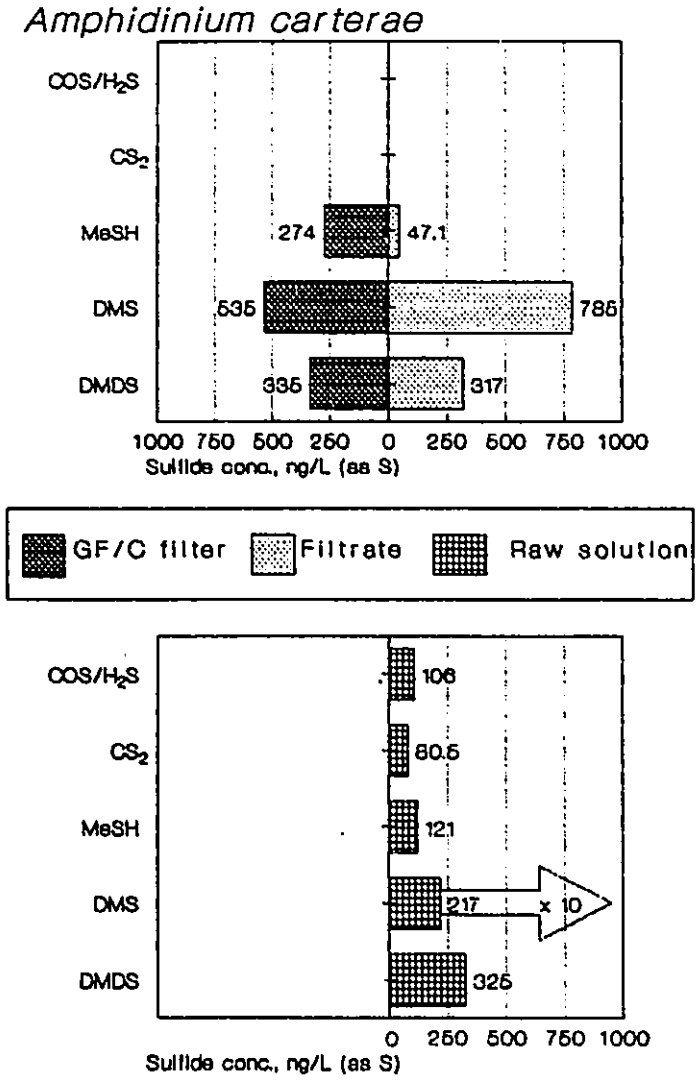

Figure 5.6: Filtration: unfiltered and filtered fractions in a culture of *A. carterae.*

Anabaena sp.

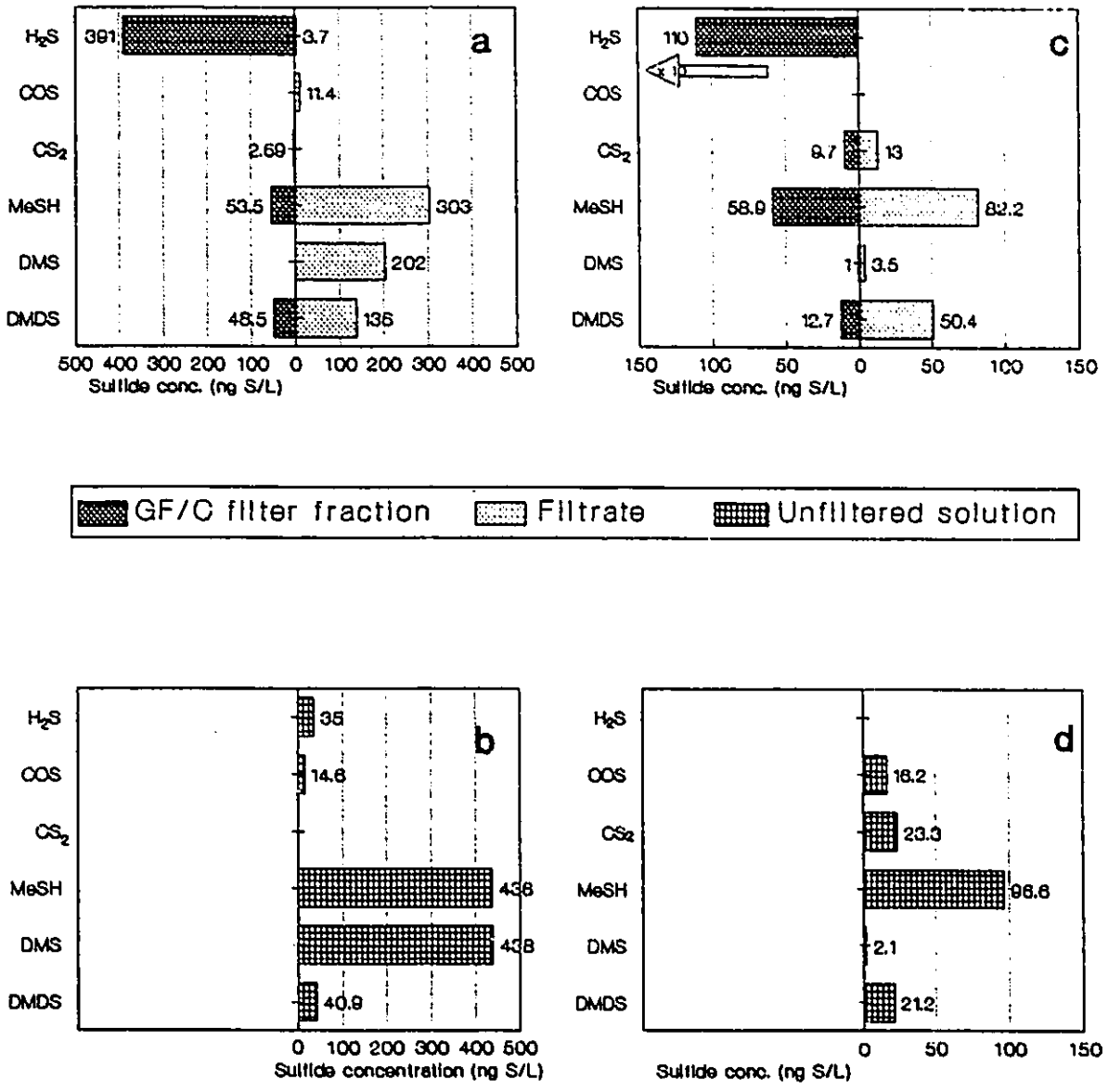

Figure 5.7: Filtration: unfiltered and filtered fractions of *Anabaena* sp cultures. Culture #1: a, b; culture #2: c, d.

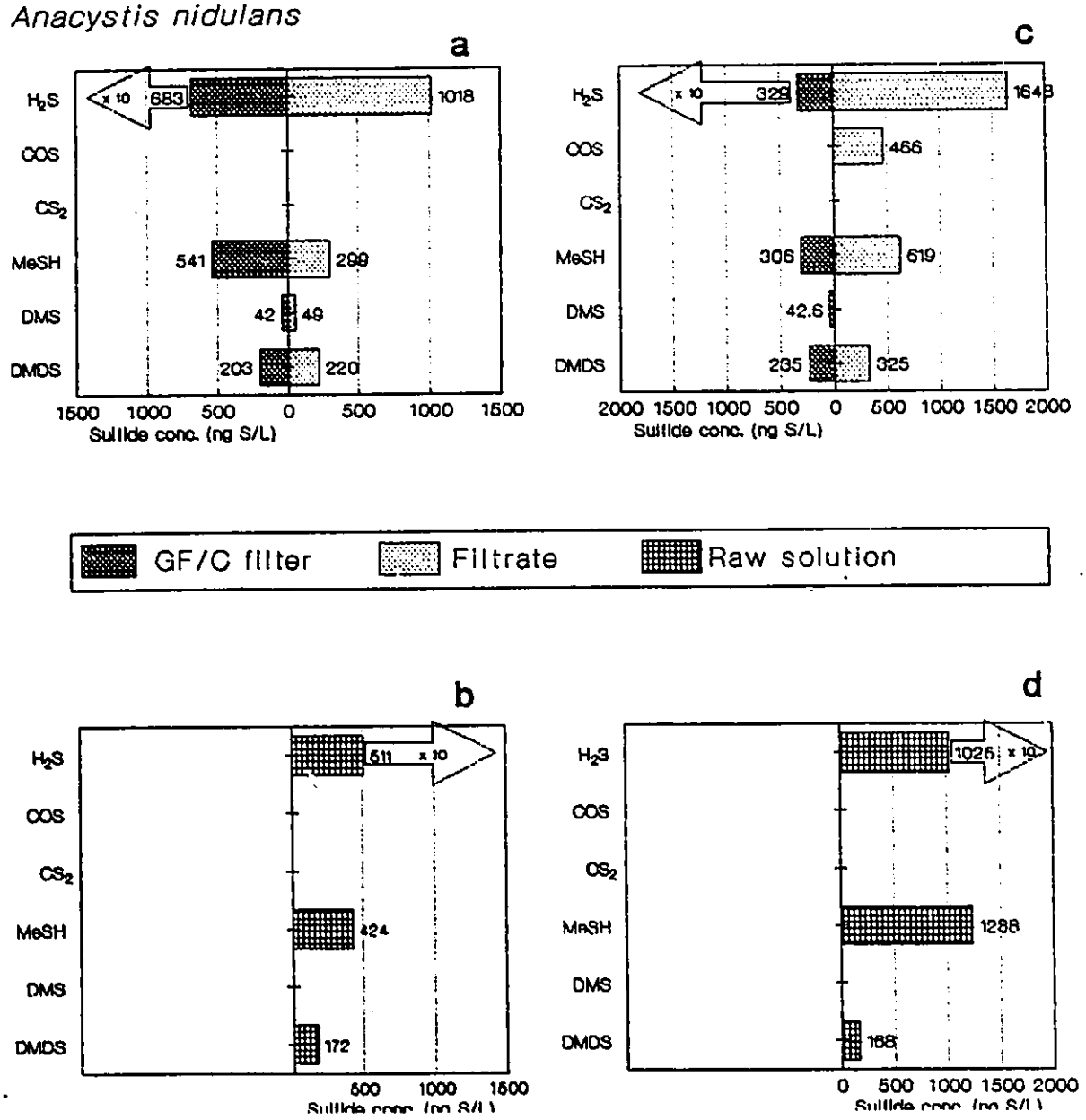

Figure 5.8: Filtration: unfiltered and filtered fractions of *A. nidulans* cultures. Culture #1: a, b; culture #2: c, d.

Ankistrodesmus sp.

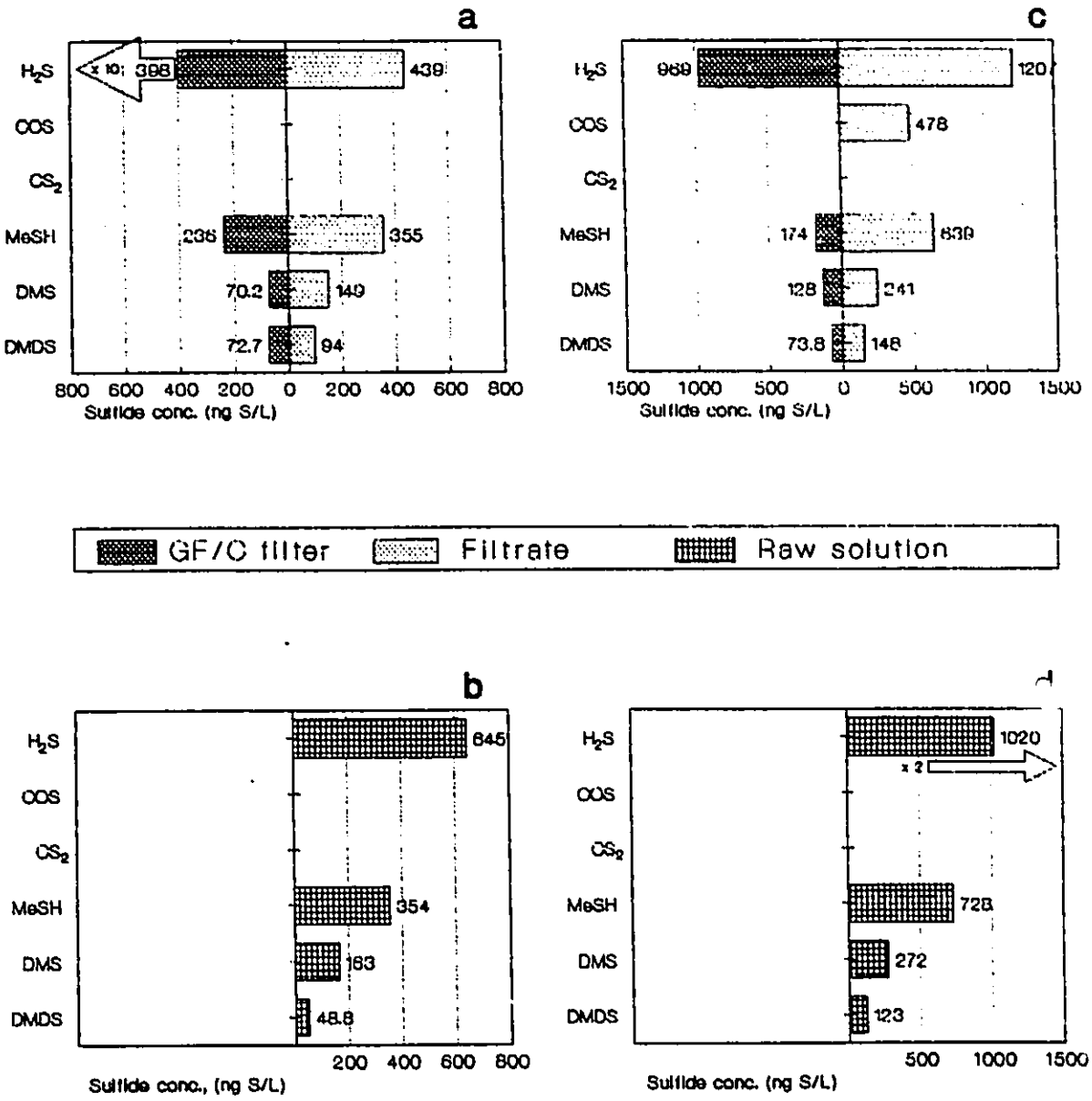

Figure 5.9: Filtration: unfiltered and filtered fractions of *Ankistrodesmus* sp cultures. Culture #1: a, b; culture #2: c, d.

5.7). There are, however, inconsistencies in the cultures analyzed.

A. carterae (Figure 5.6) shows that five (or six) species were found in the raw solution, whereas "H_2S+COS" and "CS_2" were not observed in the fractionated sample. There was less MeSH in the filtrate than in the particulate fraction, but DMS was more concentrated in the filtrate as opposed to the particulate phase. The raw solution, however, showed a high concentration of DMS, but MeSH and DMDS concentrations were in the same range as in the separate fractions. Filtration partitioning is critical because DMSP was found in this algae (Table 5.1). DMS can be produced as a result of thermal decomposition of DMSP from the distillation procedure. Although DMSP is more likely to be found in the particulate algae fraction (Turner et al. 1988), results do not show an important DMS bias from DMSP decomposition. This suggests that DMSP is fairly stable under the conditions of analysis. The matter will be discussed in detail in a subsequent section.

Anabaena sp. (Figure 5.7) shows MeSH and DMS levels to be comparable between filtered and raw solutions. DMDS has lower levels in the raw solution as opposed to the filtrate. H_2S gives a strong signal in the particulate fraction, which is not found in any other fraction. This compound may be produced by a catabolic degradation of organic constituents within the cells. This is known for cysteine in bacteria (Michal 1974).

Anacystis nidulans (Figure 5.8) shows a general agreement between filtered and raw solutions. However, there is a big disparity in H_2S evolution

between these samples. It is possible that the high H_2S is caused by catabolysis of organic constituents in cell debris of sizes between 0.45 μm and 1.2 μm. Additional filtration showed that particulates of this size were abundant for *A. nidulans*. There is no satisfactory explanation on this at the moment.

Ankistrodesmus sp. (Figure 5.9) shows a general agreement between the raw solution and the filtrate. Here again, high H_2S peaks were observed in the particulate fraction.

In most of these filtered/unfiltered samples, the raw solution and the filtered sample of the cultures generally show similar patterns and values. The lower levels of volatile sulfides in the filtrate fraction are likely to be caused by degassing effects during vacuum filtration. Breakdown of a volatile sulfide precursor in the raw solution (e.g., DMSP decomposition to DMS) does not seem to contribute an appreciable amount to the concentration of the raw (unfiltered) sample, including *A. carterae*.

Hence, it will be assumed that the raw (unfiltered) samples will be representative of the solution, and that the particulates will not have an appreciable influence on the total amount of volatile sulfides in aqueous samples.

5.5 Volatile sulfides from algae grown with different sulfate concentrations.

Figure 5.10 gives the amounts of the most abundant sulfides in cultures of *Anabaena* sp. at six concentrations of sulfate in the culture solution. Figures 5.11, 5.12 and 5.13 show the same sulfides in cultures of *Ankistrodesmus* sp,

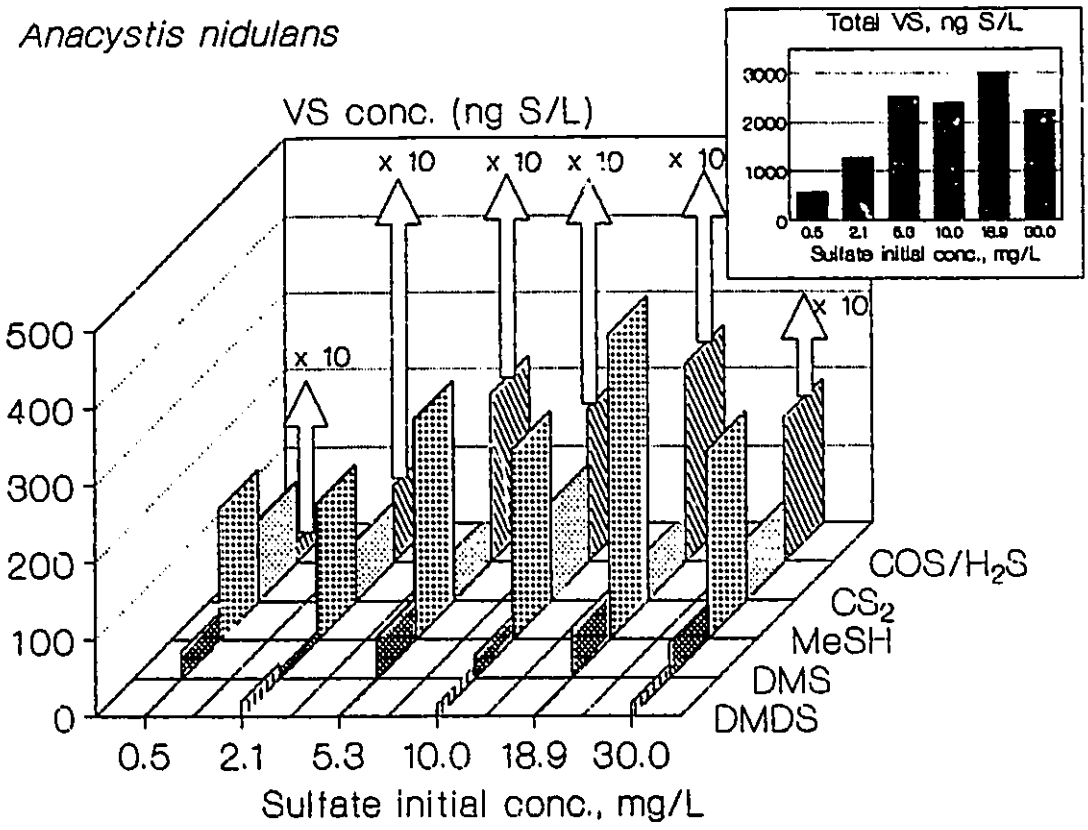

Figure 5.10: VS produced from different algae cultures grown as a function of a sulfate gradient: *A. nidulans.*

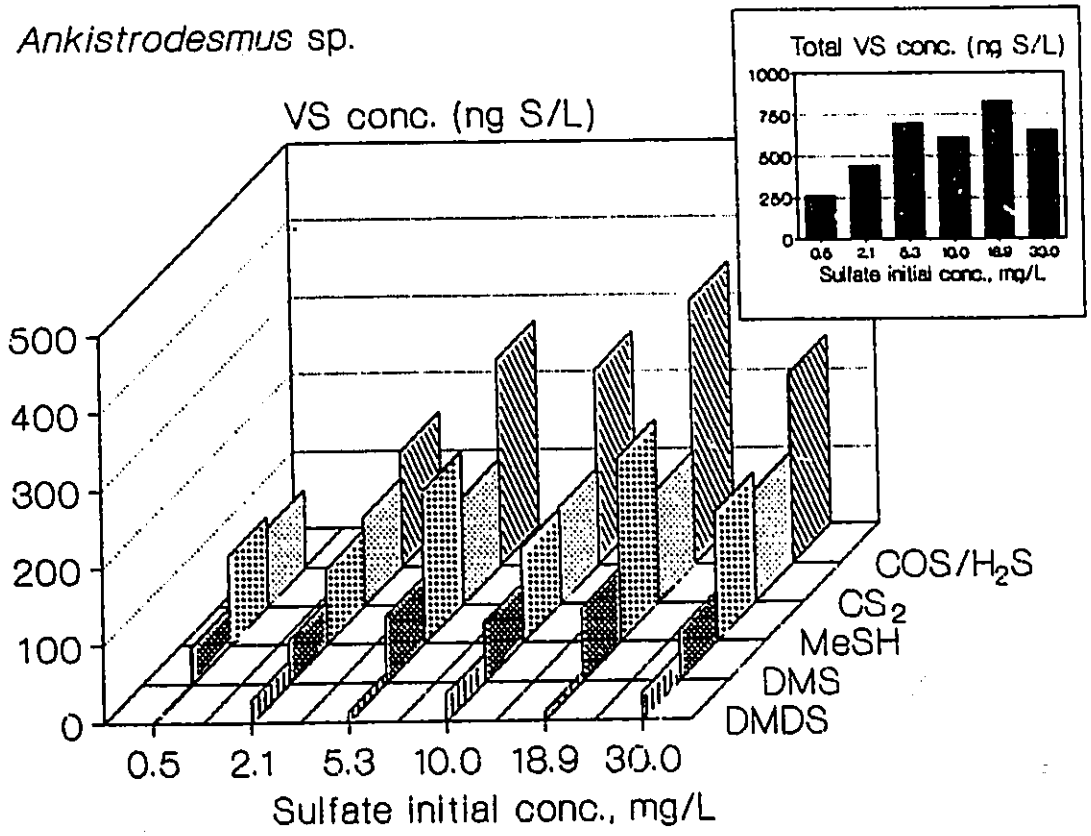

Figure 5.11: VS produced from different algae cultures grown as a function of a sulfate gradient: *Ankistrodesmus* sp.

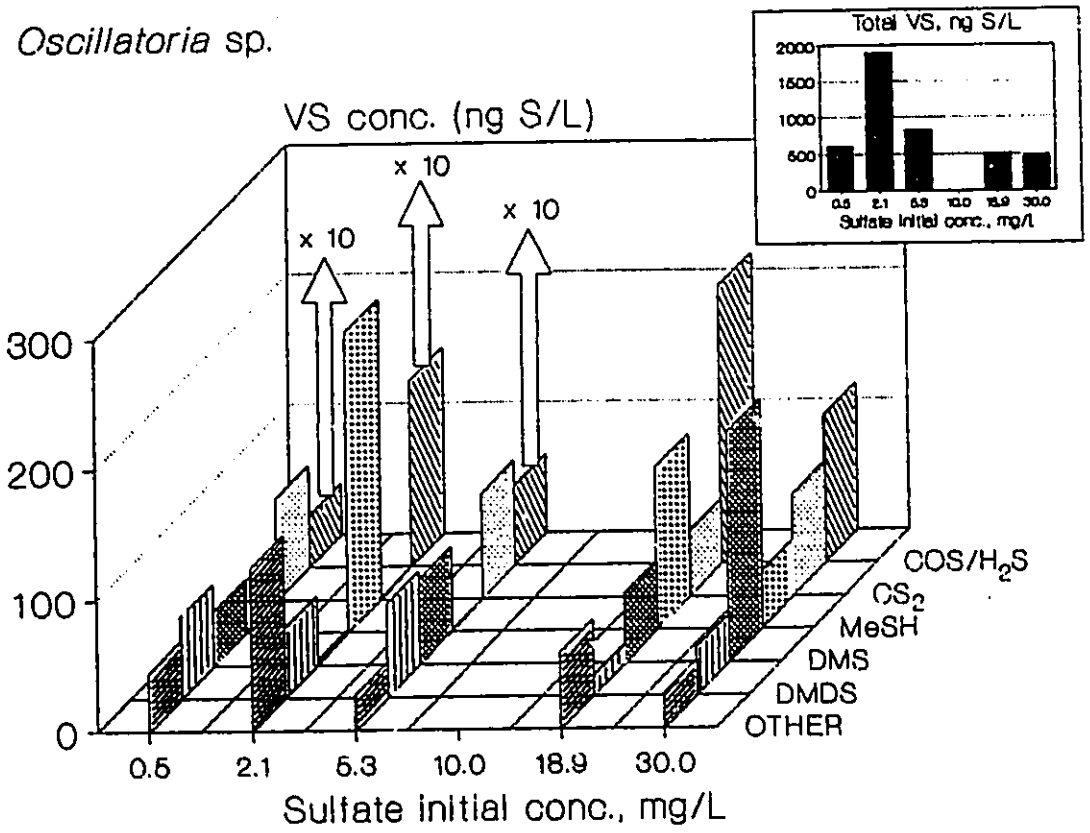

Figure 5.12: VS produced from different algae cultures grown as a function of a sulfate gradient: *Oscillatoria* sp.

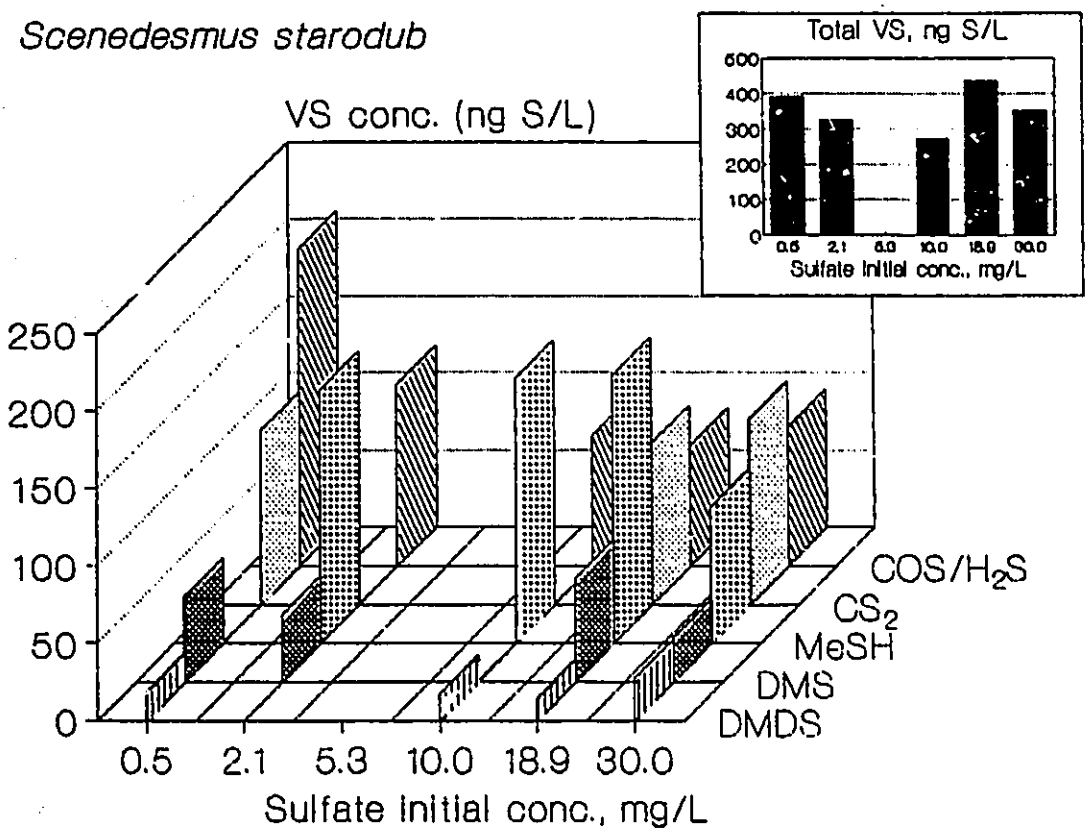

Figure 5.13: VS produced from different algae cultures grown as a function of a sulfate gradient: *S. starodub*.

Oscillatoria sp. and *Scenedesmus starodub*, respectively. In each one of these figures, the upper right graph (boxed in) shows the total amount of sulfides measured, also as a function of sulfate concentration. These results represent the algae growing in middle to late exponential phase. The amounts of sulfides vary throughout the growth stage, but the relative distribution of volatile sulfides shown in these figures is similar throughout most of the algal growth.

These results portray the algae growing in normal Bold's Basic Medium (BBM) containing all nutrients in excess amounts except for sulfate. The initial sulfate concentrations were varied from 0.5 to 30 mg/L (5 to 310 µMolar) in six flasks, which represent a wide range of levels attainable in freshwaters. In addition to volatile sulfides, chlorophyll a and the major anions were determined in each of these flasks at the end of the incubation period (Table 5.2). The initial sulfate concentration was calculated from weighed amounts of salts and the amount of solution added to the cultures.

Visual inspection at the time of collection suggested that sulfate was limiting algal growth in the 0.5 and 2.1 mg/L $SO_4^=$ flasks, but to a lesser extent in the latter. The specimens in these flasks were pale and had a reduced biomass compared to the ones in the 5 mg/L $SO_4^=$ and greater. Chlorophyll a, used as an estimate for biomass, confirmed this, especially for the 0.5 mg/L sample (Table 5.2). The algae specimens grown in the 0.5 and 2.1 mg/L $SO_4^=$ will be referred to as "unhealthy", whereas the other ones will be called "healthy".

Table 5.2: Sulfate concentrations before and after incubation for the algae grown in a sulfate gradient.

Algae	Sulfate concentration (mg/L) Initial	Final	change	Chlorophyll a (µg/mL)
Anacystis nidulans	0.50	0.19	-0.31	0.269
	2.10	0.28	-1.82	1.543
	5.30	4.29	-1.01	1.739
	10.00	9.89	-0.11	1.574
	18.90	19.85	0.95	1.625
	30.00	35.60	5.60	1.176
Ankistrodesmus sp.	0.50	0.08	-0.42	0.659
	2.10	0.05	-2.05	4.256
	5.30	3.57	-1.73	4.255
	10.00	9.22	-0.78	4.451
	18.90	18.77	-0.13	2.435
	30.00	33.00	3.00	2.405
Oscillatoria sp.	0.50	0.06	-0.44	0.038
	2.10	0.05	-2.05	0.158
	5.30	5.73	0.43	0.156
	10.00	7.73	-2.27	0.869
	30.00	32.75	2.75	1.068
Scenedesmus starodub	0.50	0.17	-0.33	0.014
	2.10	0.28	-1.82	0.019
	5.30	9.45	4.15	0.025
	10.00	9.27	-0.73	0.020
	18.90	18.98	0.08	0.018
	30.00	33.50	3.50	0.023

The levels of sulfate at the end of the incubation period were close to nil in the 0.5 and 2.1 mg/L $SO_4^=$ samples (Table 5.2), suggesting again that sulfate was a limiting nutrient to the growth of algae at those levels. Beyond the 2.1 mg/L level, the concentrations of sulfate did not appreciably change throughout the experiment.

The volatile sulfide production associated with these samples does not seem to be related to sulfate levels beyond 2.1 mg/L in *A. nidulans* and *Ankistrodesmus*. For both these species, the total amount of sulfides in solution was comparatively low in the first flask (0.5 mg/L initial sulfate), higher in the second one (2.1 mg/L sulfate), but seems to reach a plateau in the flasks containing increased sulfate. Conversely, the volatile sulfide levels remained similar for *Scenedesmus* along the sulfate gradient. *Oscillatoria* seems to show a decrease of total volatile sulfides, especially when the 2 mg/L sample is included. The culture in this flask was unhealthy, and perhaps bacterial contamination was responsible for the high levels of VS. In all four species, the trends of total volatile sulfides with sulfate concentration do not change appreciably when the amount of volatile sulfides is normalized per unit of chlorophyll a.

The relative distribution and the total amount of each individual sulfide do not show appreciable variations for *A. nidulans* and *Ankistrodesmus*, with the exception of the two flasks containing low sulfate. The variability of volatile sulfide production with the sulfate gradient is moderately marked for *Oscillatoria*

and *S. starodub*, but nothing shows a definite influence of sulfate concentration.

In short, low sulfate levels seem to influence both the amounts of sulfides and their relative distribution in the aqueous phase. However, at higher sulfate levels (5 mg/L and above), no clear trend of volatile sulfide production was observed with any of the four algae species. The test on these four species of algae was designed to evaluate their ability to produce volatile sulfides under variable sulfate concentrations. Sulfate was probably the limiting nutrient in some samples, as evidenced by a quasi-absence of the ion after incubation and a low algae biomass. Sulfate was in apparent excess in the 5 mg/L and above. There is no correlation between the production of volatile sulfides in these samples and the concentration of sulfate. Once sulfate is present in sufficient amounts to provide growth (here it corresponds to around 2 mg/L), it does not hinder nor stimulate the production of volatile sulfides because of its increased availability.

Production of volatile sulfides by freshwater algae as a function of sulfate levels has not been previously reported, to the author's knowledge. In another study, a correlation was found between the production of DMS from the marine algae *Hymenomonas carterae*, and sulfate concentration (Vairavamurthy et al. 1985). The algae were grown in artificial seawater maintained at constant ionic strength, with sulfate level varied from 0.1 mM to 28 mM (10 to 2700 mg $SO_4^{=}$/L). DMS production was correlated with sulfate levels up to 0.7 mM (67 mg $SO_4^{=}$/L). After this plateau, the DMS production was independent of the

sulfate concentration, including the normal seawater level. In the present work, no dependence of VS was linked with sulfate concentrations, even in the high softwater samples (5 to 30 mg/L sulfate). These levels are within the limit for which DMS was linked to sulfate concentrations in the marine algae experiment. It is not clear, however, what causes this difference. Perhaps the ionic strength of the solution, or the assimilation rate of sulfate are important. In both cases, DMSP found in marine algae may have a role to play, whether it is in osmoregulation or as an end product of sulfur metabolism.

5.6 The use of Chlorophyll *a* as a substitute for volatile sulfides.

The cultures previously featured in Table 5.2 were clones of the same age. Replicates did not necessarily contain the same concentration of algae in solution, and consequently VS results were normalized for biomass. Chlorophyll a, as a biomass estimator, showed that variations occurred in algal growth. Nevertheless, the general pattern showed in Figures 5.10 to 5.13 did not change appreciably. The normalized data is shown in Table 5.3 for 4 species of algae. This data was taken from the "healthy" clones only.

The ratios given for each algae have a reasonably small standard deviation (4-55%, except for *Oscillatoria*). The normalized ratios of VS per unit of chlorophyll a obtained here are probably characteristic of each species of algae. *Oscillatoria* sp is a filamentous algae growing in clumps and this can explain the reproductibility problem. The problem was twofold: firstly, it was

Table 5.3: Ratios of volatile sulfides normalized per unit of chlorophyll a for four algal species. The ratios were obtained from replicates of cultures grown in the laboratory. Chlorophyll a is corrected for Phaeophytin.

	R (ng S/mg Chlorophyll *a*) for					
	H_2S+COS	CS_2	MeSH	DMS	DMDS	OTHER
Anacystis nidulans						
Ave*	3950	204.1	538	86.2	30.1	
Std** (n=4)	578	65.4	118	20.7	0.8	
Ankistrodesmus sp.						
Ave	210.7	104.9	137.9	60.4	18.7	
Std (n=4)	43.4	19.7	38.9	9.5	10.4	
Oscillatoria sp.						
Ave	6598	972	281	927	794	378
Std (n=3)	8554	1070	133	781	1001	334
Scenedesmus starodub						
Ave	4124	5480	7324	2470	961	
Std (n=3)	174	276	2515	1230	170	

* Average; ** Standard deviation

difficult to transfer equal amounts of inocula to replicate flasks. Secondly, the clumps had to be mechanically disrupted to homogenize the solution prior to chlorophyll a measurement.

Some of the algal species used in this work have previously been used by Bechard and Rayburn (1979), who also analyzed VS in algal cultures. They found significant amounts of VS only in Cyanophyta (blue-green algae). MeSH and DMDS were detected in some of these cultures, whereas H_2S and DMS were detected in all of them. VS were detected in specimens from other divisions (Chlorophyta (green) and Xanthophyta (golden-green)), but the amounts were smaller and on an inconsistent basis. Triplicate analysis gave a 10-60% standard deviation for Cyanophyta, which is comparable with the ranges obtained in Table 5.3 (4-55%). The ratios in Table 5.3 show that, in contrast with the study of Bechard and Rayburn (1979), Chlorophyta specimens produce about the same amounts of VS as those belonging to Cyanophyta. The implications of this observation are not known yet. It is probably an indication that VS production is widespread in natural waters, and blue-green algae are not the sole responsible organisms.

The ratios (Table 5.3) will be used in section 8 to substitute for VS when this data is not available. Direct measurement of Chlorophyll a or existing values in data bases can provide an indirect measurement for VS in waters.

5.7 Addition of Sulfur amino acids

Other attempts to find some of the chemical parameters controlling the production of volatile sulfides from algae included the introduction of sulfur-containing precursors into the culture medium. The Assimilatory Sulfate Reduction (ASR) path suggests that cysteine (and its dimer cystine) and methionine are likely to be involved in VS production.

Cysteine and cystine additions to algal cultures induced no or little effect on volatile sulfide production. However, methionine enhanced the production of methylated sulfides by 2 to 4 orders of magnitude on the algae tested (Figure 5.14). In three of these algae tested, MeSH was the most important sulfide formed, whereas DMDS predominated in *Oscillatoria*. Interestingly, the production of all three major methylated sulfides (MeSH, DMS and DMDS) increased upon additions of methionine, but in different proportions for each algae. CS_2 was virtually absent, and $COS+H_2S$ was not detected in two occasions. This suggests that methionine plays a key role in the formation of methylated sulfides. These observations are consistent with results of Kiene and Visscher (1987), and Segal and Starkey (1969), with bacteria, and of Ruiz-Herrera and Starkey (1969) with fungi.

Experiments with methionine were carried further with *Oscillatoria*. Cultures of the algae were spiked with ^{35}S D,L-methionine and the volatile sulfides produced (MeSH, but mostly DMDS) were assayed with a gas-phase proportional gas radio chromatograph detector. Results are shown in Figure

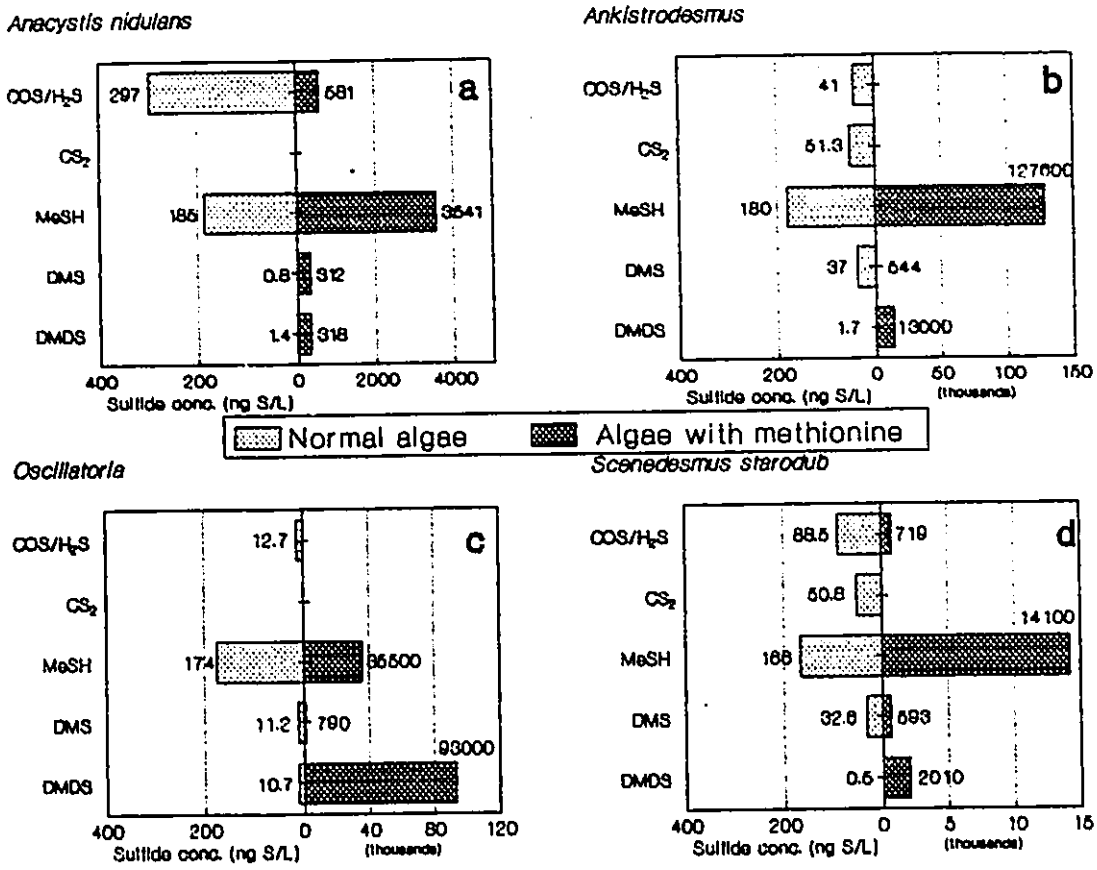

Figure 5.14: Increase in production of methylated sulfides upon addition of methionine in four cultures. a: *A. nidulans*. b: *Ankistrodesmus* sp. c: *Oscillatoria* sp. d: *S. starodub*.

5.15. The detection limit of this system (Table 4.5) is poorer than the GC/HECD detector and consequently only the DMDS peak could be recorded on the two detectors of the radio chromatograph system. The arrows at t = 6 hours indicate that the specific radioactivity counts (per unit of DMDS) are higher than the points show. In these two runs, DMDS was detected on the GRC but not on the FID. The DMDS detection limit on the FID was assumed to be the maximum amount present in the sample for that number of counts. Four sets of experiments were needed to get these data because of the difficulty to obtain data. The count efficiency could not be determined because no ^{35}S DMDS of known activity was available. Furthermore, the GRC detector required some fine tuning during the course of the experiments. Therefore the results obtained with the radiotracer can only be used qualitatively.

Radioactive DMDS (and possibly MeSH) was detected only a few hours after the addition of methionine (Figure 5.15, expt. #3, 4). The volatile sulfides in solution analyzed in experiment #6 showed that radioactive DMDS is produced for at least 1 week after inoculation of the methionine. The strength of the signal given by DMDS was quite variable for an unknown reason (e.g. experiment #6), but it seemed to increase with time. Therefore methionine is the direct precursor of DMDS and possibly the other methylated sulfides in *Oscillatoria* sp., and its release is fast.

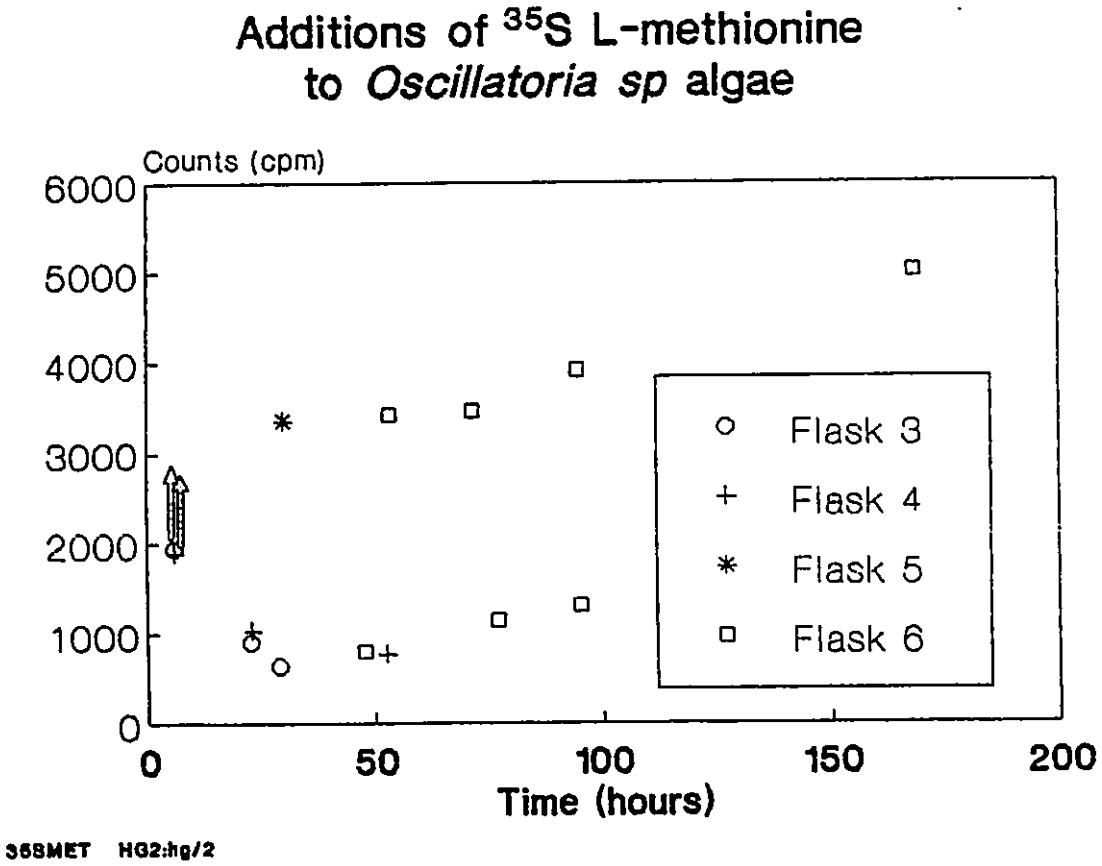

Figure 5.15: Presence of labelled DMDS upon addition of ^{35}S L-methionine in cultures of *Oscillatoria* sp.

5.8 Addition of methyl donor groups

The assimilatory sulfate reduction (ASR) pathway (Figure 2.2) showed that biosynthesis of methionine involves the transfer of a methyl group onto the sulfur atom of homocysteine. Methyl transfers can have an influence on methionine synthesis, and perhaps on VS formation.

Simple methyl transfer molecules, choline ($(CH_3)_3N^+CH_2CH_2OH\cdot OH$), (glycine) betaine ($(CH_3)_3N^+CH_2COO^-$), and methyl iodide (iodomethane, CH_3I) were added to cultures of *Oscillatoria*. Analysis of VS present in the aqueous culture (Figure 5.16) show a limited influence of methyl donors on the algae. Choline and betaine do not seem to have an appreciable influence on volatile sulfide production compared to the control. However, the methylated species are found in greater concentrations in the sample spiked with CH_3I. Neither H_2S+COS nor CS_2 seem to have been affected by the addition of the spike.

Various combinations of amino acids, amino acids with methyl donors, and methyl donors were tested on *Oscillatoria*. Methionine dominated the effects of VS production in all flasks, regardless of the presence of the other donors. Only two exceptions occurred in flasks containing methionine as a spike: an addition of CH_3I dramatically increased the DMS production; an addition of cysteine stopped the production of MeSH and DMDS. The production of these sulfides was restored to the normal "methionine levels" approximately 48 hours later. These two experiments were subsequently repeated but the results were not conclusive.

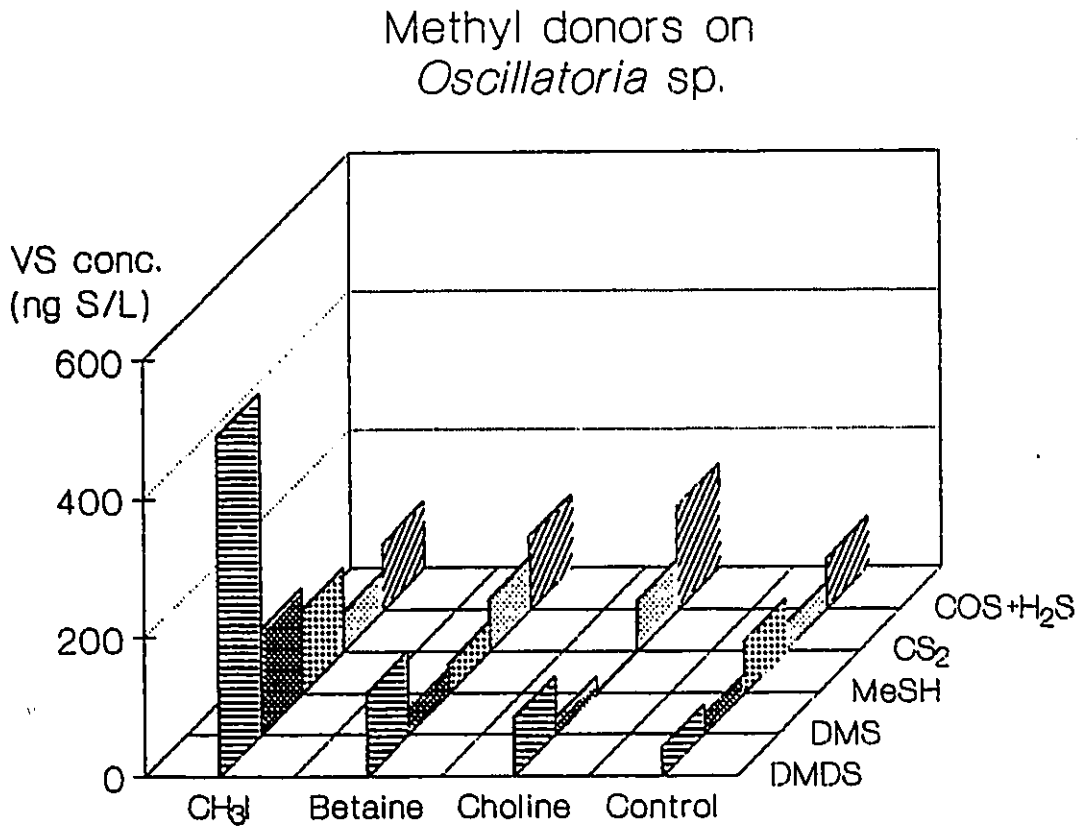

Figure 5.16: Influence of some selected methyl donors on VS production in *Oscillatoria* sp.

None of the other combinations, which did not include methionine, showed any significant enhancement of VS levels in *Oscillatoria* cultures. DMSP, which is structurally close to dimethylthetin (Lehinger 1975) and can be used as a methyl donor (Vairavamurthy et al. 1985), was added to *Ankistrodesmus* and *S. starodub.* No major effect was observed, except that MeSH was absent in the sample after incubation.

5.9 Summary

Volatile sulfides were present in all the natural samples analyzed in this study. Sulfate concentrations did not affect the production of volatile sulfides, but a threshold of about 2 mg/L sulfate was required. Each algae species produces a characteristic distribution of VS which did not vary appreciably with sulfate concentrations in the culture. Additions of cysteine did not produce more VS, but methionine seems to play a key role in the production of methylated sulfides. Some of the methyl donors added to cultures had a small influence on the production of methylated sulfides. It is important to note that other species, COS, H_2S and CS_2 do not seem to be affected by additions of any substrate.

6. DISCUSSION

6.1 Volatile sulfide analysis

Previous work showed that volatile sulfides are found in all types of natural waters. All the samples of waters analyzed in this study showed the presence of more than one species of volatile sulfides (Figures 5.1 to 5.3). The DMS results obtained in this study are in the same range as those found in the Great Lakes (Nriagu and Holdway 1989) and in bogs in Southern Ontario (Nriagu et al. 1987). The DMS values found for Luther Marsh in this study, however, are slightly lower than in the latter case. The DMS concentrations in the freshwaters analyzed in this study are also in the same range as in seawaters (Table 6.1).

Documentation is very scarce as to the presence and analysis of volatile sulfides in freshwaters. Most analysis methods applied to waters, including those of marine waters, report only DMS results. Thus the results presented here are unique.

At least 5 different species of sulfides were simultaneously detected and measured, and the sulfur content of unknown sulfides can be determined on the basis of their sulfur content. The detection limits in the present study range from 0.02 ng S/L (COS) to 0.1 ng S/L (DMDS) (Table 4.5) for a 100 mL sample are superior to many other works.

Table 6.1: Concentrations of DMS and other sulfides in marine water. Also shown for comparison: a sample from this study, and a Northern Ontario lake from a different project.

	Sample type, location	Concentration (ng S/L)	Reference
DMS	Gulf of Mexico (range)	55-60	Andreae et al. (1983)
DMS	Oceanic waters (averages):		
	Oligotrophic areas	67.1	Andreae and
	Upwelling areas	177.1	Raemdonck (1983)
	Coastal and shelf zones	136.6	
DMS	Atlantic cruise (average)	84.4	Andreae and Barnard (1984)
DMS	Pacific Ocean (average 1982-1985 cruises)	92.6	Bates et al. (1987b)
H_2S	Baltic sea sample	11.4	Leck and Bagander
CS_2		1.9	(1988)
MeSH		8.6	
DMS		95.3	
DMDS		1.8	
H_2S+COS		388	This work (Figure
CS_2	Cootes Paradise at Valley	62.9	5.2b)
MeSH	Inn Road (Nov. 16, 1989)	124	
DMS		64.2	
DMDS		26.8	
COS	ELA 226S (Experimental Lake	11.5	Richards et al.
MeSH	Acidification Project,	0.6	(1990)
DMS	Ontario)	41.6	
DMDS		4.2	

Other investigators showed the presence of species other than DMS, but further quantification was pursued for DMS only (Holdway and Nriagu 1988 for Lake Ontario; Andreae et al. 1983 for water from the Gulf of Mexico). The detection and quantification of species other than DMS is new. Deprez et al. (1986) and Franzmann et al. (1987) reported high concentrations of DMS, some COS and H_2S in waters from a hypersaline meromictic lake in Antarctica. Kim and Andreae (1987) developed a method for CS_2 analysis and reported some results for seawater. Leck and Bagander (1988) reported a method used for the simultaneous analysis of 5 (and possibly more) species of volatile sulfides from aqueous samples. Their method features detection limits and a reproducibility comparable to the ones outlined in this work (chap. 2; also see Caron and Kramer 1989). The selectivity is good, particularly between COS and H_2S, because a cryogenic unit is used. The stripping efficiency of their aqueous sample is more reliable than in our work. A sample taken from the Baltic sea (Table 6.1) features the presence of all common sulfides also encountered in our work. Recently, Richards et al. (1990) reported various sulfides detected in the waters of the Experimental Lake Acidification project (ELA) in Northern Ontario. The sulfur species found are the same as in this study (Table 6.1), and the concentrations are in the same range as in Lake Ontario (Figure 5.2) but lower than Cootes Paradise or Luther Lake.

The recent improvement in volatile sulfide analysis demonstrates that DMS is not the only sulfide present in significant amounts in natural samples.

Perhaps the detector used in this work (HECD) gives a better detection limit than the Flame Photometric Detector (FPD), hence a higher possibility of detecting minor species.

The (marine) sample from Leck and Bagander (1988; Table 6.1) is dominated by DMS (80 %), and shows that other volatile sulfides are present at a measurable level. In most samples of natural waters analyzed in this work, DMS constitutes only a small percentage of all sulfides found in the sample. This shows that only an analysis of DMS is not sufficient to account for the total production of volatile sulfides.

Freshwater systems (waters and algae cultures) are characterized by the presence of many major species of volatile sulfides (Figures 5.1 to 5.4, 5.5a, b). The situation is therefore more complex in freshwaters. This is in contrast with seawater algae (Figure 5.5c) and in marine waters, where one species, DMS, predominates over the other sulfide species. DMSP alone can explain the production of DMS, although the reason and/or its mechanism of release is unclear. There is a precursor-product relationship among these two compounds.

The production of DMS from freshwater algae is unlikely to be mediated by DMSP since the latter has not been detected. Precursor(s) other than DMSP can probably explain the origin and the multiplicity of the sulfides found in freshwaters. Methionine and some methyl donors influence VS production, but only the methylated species MeSH, DMS and DMDS are affected. In

addition, the constancy of VS distribution is probably controlled by a metabolic or enzymatic pathway which is characteristic for each algal species. Therefore, in addition to the substrate, other factors (i.e. age, physiological state, etc.) may affect the production of VS.

It is important to emphasize that only the methylated species MeSH, DMS and DMDS will be discussed in the remaining part of this work. Two major species, COS and CS_2, have an unknown metabolic origin. Their presence in the environment as a biogenic sulfide has been confirmed elsewhere and in this study, but so far no one has found any metabolic precursor nor any metabolic path explaining their formation.

6.2 Chemical controls

Figure 6.1 is a simplified scheme of the Assimilatory Sulfate Reduction (ASR) showing a few steps which are believed to be critical in the sulfur metabolism: Stage 1, sulfate assimilation, followed by reduction. Stage 2, cysteine synthesis and transsulfuration to homocysteine. Stage 3, methionine synthesis and metabolism. Finally, in stage 4, prior to methionine metabolism, the methylation step of homocysteine.

6.2.1 Variations in sulfate levels.

Algae grown in different sulfate concentrations (Figures 5.10 to 5.13) showed that excess sulfate did not have a major effect on volatile sulfide production. This suggests that uptake and diffusion of the sulfate ion (step 1,

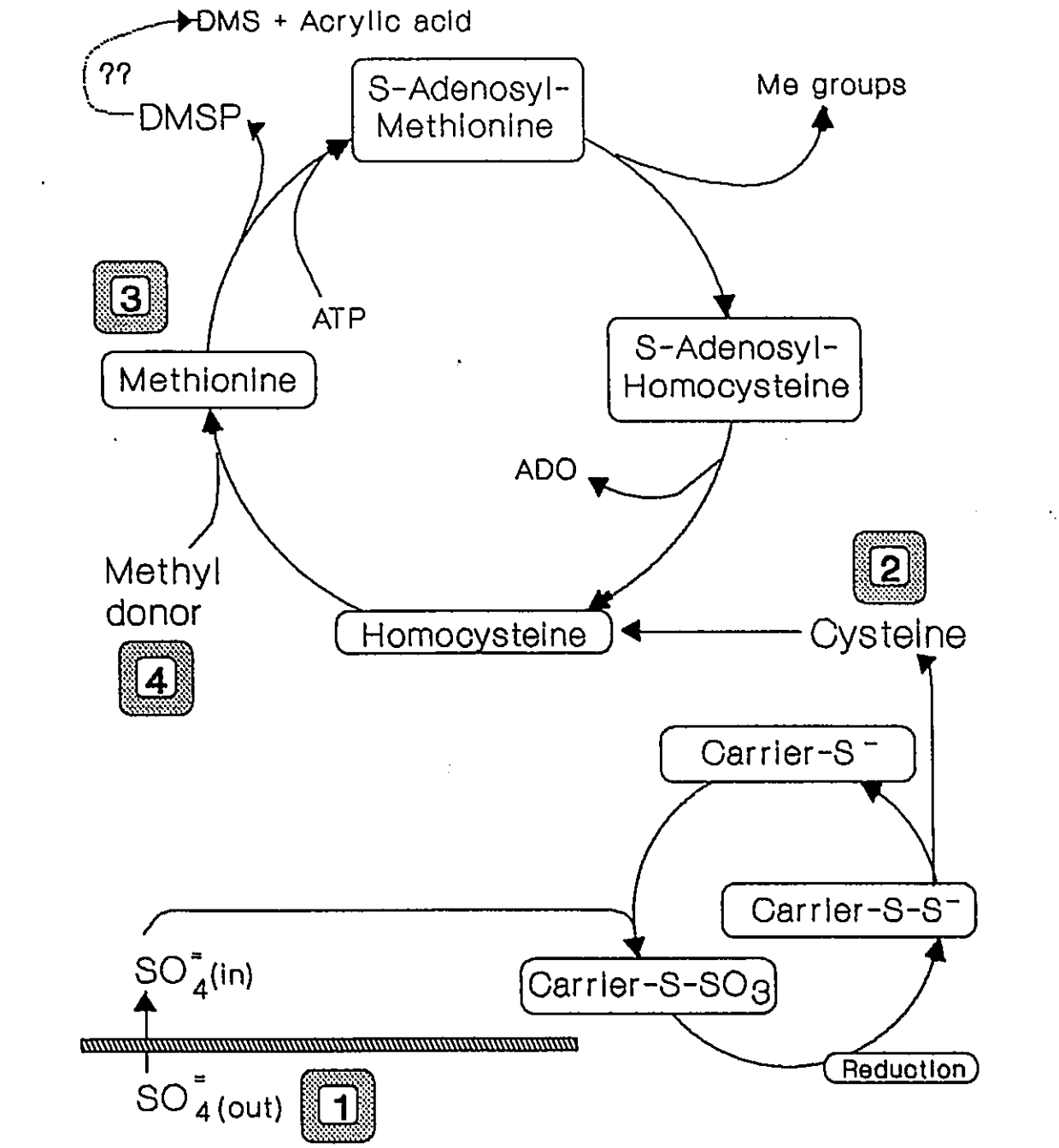

Figure 6.1: Simplified scheme of the ASR (in context with chemical additions).

Figure 6.1) are not limiting volatile sulfide production in algae or the environment. Therefore, when excess sulfate is present, no enhancement nor inhibition of VS production should be observed.

Cuhel and Lean (1986) showed that only a small fraction of the sulfate assimilated is transformed into volatile sulfides. This indicates that the sulfate assimilated is used to satisfy almost exclusively the metabolic need for sulfur in the algae. Volatile sulfide synthesis (or excretion) would then be a minor path in the whole ASR scheme.

Richards et al. (1990) mentioned that increased levels of sulfate did not seem to generate more volatile sulfides in artificially acidified lakes (Experimental Lakes Area, Ontario). Sulfuric acid was used to acidify the lakes. DMDS levels, however, were high in one acidified lake and the authors suggest that it may be due to an effect of acidification.

The observations on algae cultures also showed that there was an apparent growth inhibition below a value (about 2 mg/L sulfate). This means that sulfate was probably a limiting nutrient. The value of 2 mg/L sulfate represents the lower limit found in pristine freshwaters, and therefore lower sulfate concentrations in natural waters are unlikely to be found.

Consequently, in areas of high sulfur deposition or areas subjected to acidification caused by atmospheric sulfur deposition, higher levels of sulfate do not induce higher volatile sulfide emission and recycling by algae. Recycling of sulfur, if this is significant, would probably be under the realm of other

(micro)organisms, not algae.

6.2.2 Addition of cysteine

The detailed ASR (Figure 3.1) shows cysteine as the first carbon-bonded sulfur compound after reduction of sulfate. Cysteine addition would provide a test to determine whether sulfate reduction is rate-limiting in sulfur assimilation and VS production.

Cysteine addition (and cystine, the cysteine dimer) however, induced no significant change in volatile sulfide production from algae. This appears to be in an opposite situation with the marine species *H. carterae*, in which Vairavamurthy et al. (1985) reported that cysteine can serve as a sulfur source. Some fungi (Ruiz-Herrera and Starkey 1969) showed no growth with addition of cysteine as sole source of sulfur. This is not necessarily representative of primary producers, but fungi are also assimilatory sulfate reducers.

6.2.3 Addition of methionine

Addition of methionine produced an increase of MeSH and DMDS, and a more moderate increase of DMS in some algae culture flasks (Figure 5.14). The production of these compounds in the headspace is quite rapid, i.e. within hours (Figure 6.2), and sometimes it occurred in less than 20 minutes. The figure shows the production of MeSH, DMS and DMDS in headspace of an *Oscillatoria* culture as a function of time after addition of methionine. Even if the pattern of VS production with time in headspace cannot be duplicated, the equivalent in liquid phase is reproducible. Unlike the other algae species,

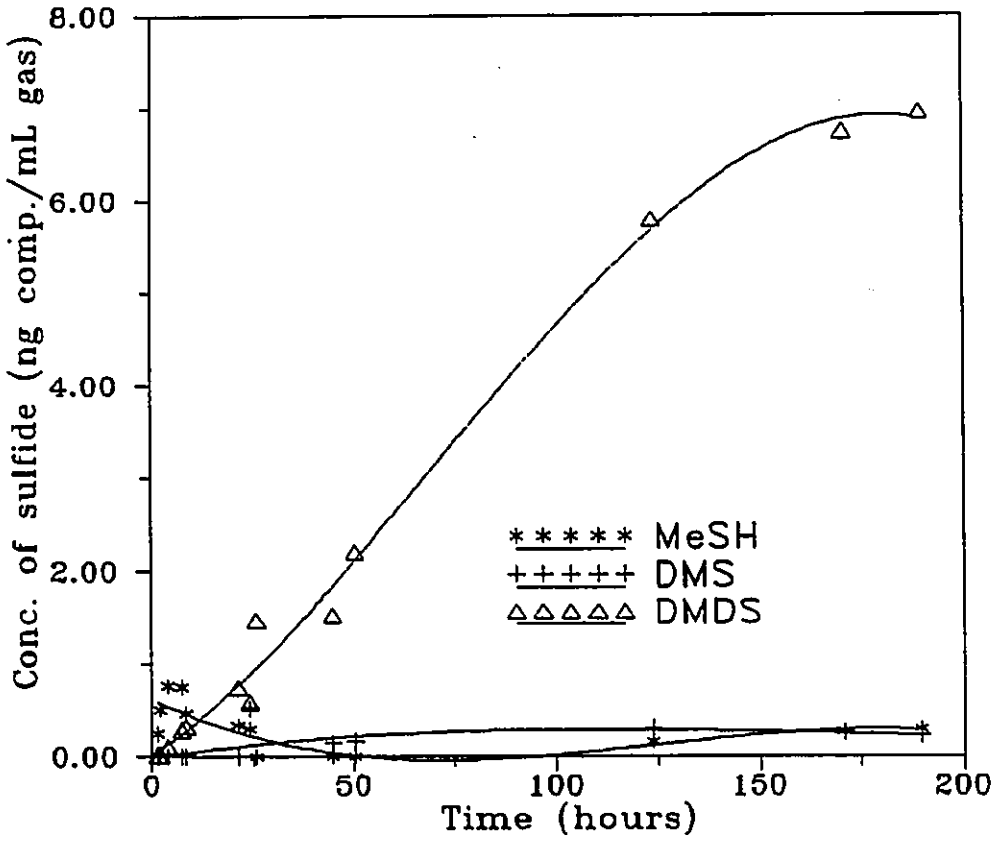

Figure 6.2: Production of MeSH, DMS and DMDS in the headspace of an *Oscillatoria* culture spiked with methionine.

Oscillatoria sp. produced predominantly DMDS with smaller amounts of MeSH and DMS.

The dissimilation of methionine by algae (freshwater or seawater) producing methylated sulfides appears to be a new observation. Methionine dissimilation was observed from bacteria living in anaerobic sediments (Kiene and Visscher 1987) and fungi (Challenger 1951 and references therein; Ruiz-Herrera and Starkey 1969). Conversely, Vairavamurthy et al. (1985) reported that methionine did not sustain growth of *H. carterae* in their cultures. This again shows that the mode of production of methylated sulfides (DMS in this situation) is different between marine algae (*H. carterae*) and the softwater algae under investigation in this study.

However, Segal and Starkey (1969) did not observe DMS, ethanethiol (EtSH), cysteine, sulfide, thiosulfate, sulfite or sulfate upon methionine dissimilation by bacteria. In our study, DMS and sulfate have been measured during and at the end of the incubation period. Methionine converts to sulfate, also producing MeSH, DMDS and small amounts of DMS during methionine dissimilation by algae.

DMDS, MeSH and DMS losses through volatilization are significant throughout the experiment. The loss rate, or the yield of methionine conversion to methylated species can be estimated using the data shown in Figure 6.2 for *Oscillatoria* (10 mL culture solution in 25 mL flask). The sulfur budget for the

experiment is:

$$\text{Total } \underline{\text{Initial}} \text{ S} = (\text{initial } SO_4^{=}) + (\text{methionine added})$$

$$\text{Total } \underline{\text{Measured}} \text{ S} = (\text{final } SO_4^{=}) + (VS_{emitted}) + (VS_{solution})$$

where:

Initial $SO_4^{=}$ = 36.5 nmoles (average for algae cultures in "no sulfate BBM")

Methionine added = 1650 nmoles (measured)

Final $SO_4^{=}$ = 1100-1400 nmoles (measured; the range indicates variations among duplicates)

$VS_{emitted}$ = 236 nmoles (VS in headspace is assumed to have left the culture and the flask. This number is obtained by integrating the polynomial equations linking the points in Figure 6.2)

$VS_{solution}$ = 54 nmoles (measured)

Comparison between the <u>total measured</u> (1390-1690 nmoles) and the <u>total initial</u> (1686 nmoles) sulfur contents in the flask show that sulfur is almost all accounted for. This calculation does not consider the sulfur present in algae (initial and final), nor does it account for methionine and dissolved sulfur species other than those measured above. The calculation above suggests that other sulfur species and losses of VS through oxidation are minimal. Sulfur from methionine is converted into volatile species (about 14% escaped, 3.2% was left in solution), whereas conversion to sulfate ranges from 65 to 83% of the initial sulfur. At the end of the incubation period, the numbers indicate that virtually all methionine was converted to some other sulfur species, most of it

as the sulfate ion. The intermediates or the process of this conversion to sulfate are not known. It is not known either if bacterial contamination producing sulfate can occur or if this is significant.

Methionine seems to play a key role in the production of volatile sulfides from algae. It can explain the presence of MeSH and DMDS, and DMS to some extent. Experiments with ^{35}S labelled methionine showed that volatile sulfide production occurs mostly through methionine dissimilation. The transfer of a methyl group from S-adenosylmethionine onto a substrate other than methionine is possible, but the extent of this mechanism needs to be verified. The use of ^{14}C or ^{3}H methionine on the terminal carbon group would likely bring an answer to this.

6.2.4 Methyl donors

Three of the methyl donors described in Table 3.2 were added to cultures of *Oscillatoria*. Figure 5.16 shows the effects of addition of choline, betaine and methyl iodide on the culture.

Betaine and choline induced a small change in the DMDS production, but this is probably not significant. A major change is observed for CH_3I, which induces an increase of 1.6x, 5.6x and 11.7x in MeSH, DMS and DMDS concentrations respectively, with respect to the control.

The methyl donors tested here are not necessarily specific to methionine synthesis. They can act in two ways: (1) in the synthesis of methionine from homocysteine in the ASR. This would produce larger amounts of methionine

which can undergo dissimilation to produce MeSH, DMS or DMDS. (2) The transfer of a methyl group on a substrate containing a reduced sulfur or sulfhydryl (other than homocysteine) followed by the release of a small molecule. This substrate requires a terminal -SH or -S-CH_3 group, because only methylated sulfide production is enhanced. Evidence points to the second possibility, because Hofmann degradation of the algae *Oscillatoria* spiked with CH_3I gave DMS (Table 5.1). The substrate is unknown and must have a terminal -S-CH_3, because DMS is detected. Methionine is a possible substrate (Figure 6.3, path 2a). If such a mechanism occurred, it was probably mediated biologically because no DMS was detected from the blank containing both methionine and CH_3I. The abiotic methylation of methionine with CH_3X (X=Br, Cl) is possible (Baldwin et al. 1988), but such conditions are unlikely to occur with the cultures or the blank.

DMSP, which can also transfer methyl groups (Vairavamurthy et al. 1985), has a structure similar to dimethylthetin, which is another widespread methyl donor (Lehninger 1975). DMSP additions were carried out on *S. starodub* and *Ankistrodesmus* without any positive conclusion. In both cases, DMS production was enhanced but within normal limits (*S. starodub*) or explained solely by DMSP decomposition (*Ankistrodesmus*; section 7). With both these algae, however, MeSH production was inhibited.

Nevertheless, the methyl donors do not generally show a strong influence on the production of volatile sulfides. These methyl donors were

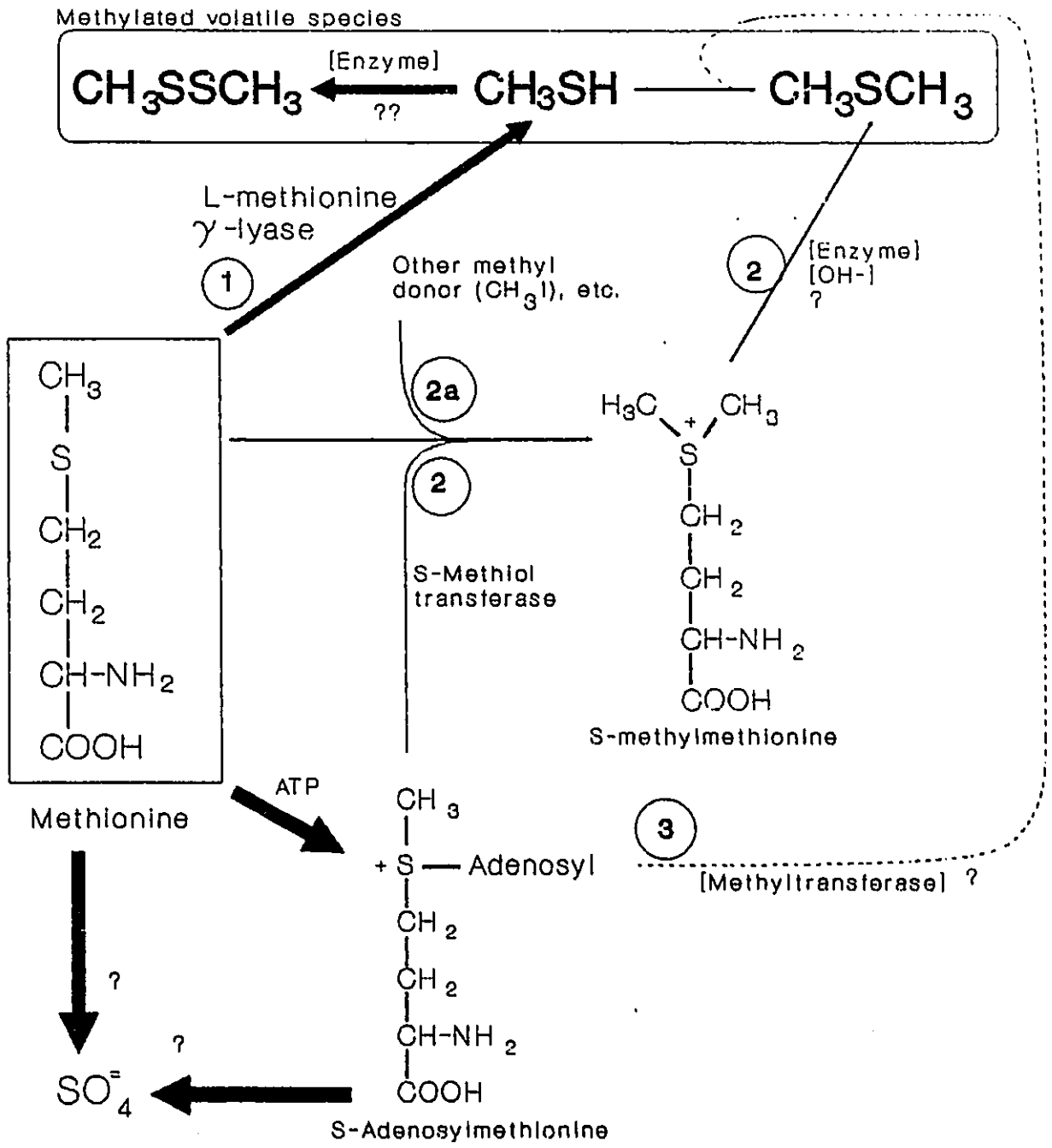

Figure 6.3: Summary of the methionine (bio)chemistry in *Oscillatoria* sp. with regard to methylated sulfide production. The width of the arrows is proportional to the rate of reaction of the compound(s) (arbitrary scale).

chosen because they were simple, and perhaps other donors such as methylcobalamin or methyltetrahydrofolate have more influence on volatile sulfide production.

6.2.5 Review on chemical controls

Discussion has focused on selected sulfur-containing compounds and some methyl transfer substrates which can have some influence on the amino acid synthesis in the ASR. The experiments carried out in this study showed that methionine has a critical importance on the production of methylated sulfur compounds. The information gathered on methylated sulfides using *Oscillatoria* sp. is summarized in Figure 6.3, along with information from the works of Segal and Starkey (1969) and Drotar et al. (1987a, b).

Methionine (bio)chemistry can explain the presence of methanethiol with enzymatic reactions. The methionine ß-γ-C-S bond can be cleaved upon the action of an enzyme similar to L-methionine-γ-lyase (Segal and Starkey 1969; Drotar et al. 1987a), hence releasing a terminal methiol group. This is illustrated by path #1 in Figure 6.3.

DMDS production can be explained through oxidation and formation of S-S bond from MeSH. Such a phenomenon has been observed widely, especially when MeSH is produced. The different MeSH:DMDS ratio between *Oscillatoria* and the other algae suggests that this oxidation may be catalyzed by an enzyme.

DMS production can be explained in two ways: (1) the transfer of a

methyl group from S-adenosylmethionine onto the sulfur atom of methionine, followed by elimination of DMS (Drotar et al. 1987a, b; path #2). The methyl transfer is catalyzed by a S-methioltransferase. This is where CH_3I can influence the production of methylated sulfides (path #2a). Hofmann degradation of the algae spiked with CH_3I produced DMS, which corroborates the likelihood of this mechanism. The other way (2), is the transfer of a methyl group, probably again from S-adenosylmethionine, onto MeSH (path #3; Drotar et al. 1987b). However, no experimental evidence supported the latter in this study.

Evidence discussed in section 6.2.3 suggests that the major path of sulfur from methionine addition is towards the reoxidation to sulfate. The most likely intermediate would be through S-adenosylmethionine, or perhaps methionine which was not assimilated. Oxidation would involve several steps and intermediates. Oxidation of the volatile species would probably not be important due to their stability in water (Adewuyi 1989).

6.3 Summary

A simple method for the analysis of volatile sulfides in freshwaters was developed. The Hall Electrolytic Conductivity Detector (HECD) proved to be a key part of the instrument for achieving low detection limits with only 100 mL of sample or less. The multiple analyses of cultures and waters showed that at least 5 sulfides are almost always present. MeSH was usually the

predominating species.

Chemical controls and additions on cultures showed that algae are not influenced by the concentrations of sulfate. A minimum of about 2 mg/L (sulfate) is required, which corresponds to concentrations in pristine lakes commonly found on the Canadian Shield. At this level of sulfate in natural waters, it is doubtful that sulfate would be a limiting nutrient. Cysteine additions did not influence the formation of volatile sulfides. Methionine proved to be a key factor for explaining the production of methylated sulfides. Only methyliodide had a significant influence on VS production among the methyl donors tested.

The status of VS production in freshwaters deviates from the situation in marine algae. The levels of DMS in both environments are in the same range but the diversity is higher in softwaters. Moreover, marine DMS originates from DMSP, which is not found in freshwaters. Methionine, the precursor of DMSP, did not sustain algal growth in *H. carterae* (Vairavamurthy et al. 1985) as opposed to all freshwater species in this study.

The essential difference between freshwater and seawater algae is the presence of DMSP. The stability of this compound in waters must be investigated because its possible thermal and abiotic degradation can bias sample analysis.

7. FRESHWATER AND SEAWATER ALGAE: THE CHEMISTRY OF DMSP

7.1 DMSP in marine algae

Dimethylsulfoniopropionate (DMSP; Table 1.3) is a tertiary sulfonium compound whose decomposition releases DMS and acrylic acid. DMSP is present in a wide variety of marine algae (White 1982; Keller et al. 1989). Consequently, marine algae cultures and seawaters are characterized by the predominance of DMS over other reduced sulfur species. The mode of DMS release and production, however, is still unclear. Dacey and Blough (1987) dismissed the importance of alkaline degradation of the compound under natural seawater conditions. There is strong evidence that it is enzymatic (Cantoni and Anderson 1956) or mediated by bacteria (Barnard and Andreae 1984; White 1982). The analysis performed in this laboratory on filtered marine algae confirmed the presence of DMSP (Table 5.1). Cultures of *A. carterae* produced a series of volatile sulfides, with a marked dominance of DMS over the other species (Figure 5.5c). Partitioning using GF/C filters on *A. carterae* (figure 5.6) suggests that DMSP decomposition is exocellular as evidenced by higher DMS amounts in the filtrate. Thermal degradation of DMSP from the distillation line may have influenced the levels of DMS, and for this reason, chemical degradation of DMSP has to be investigated.

7.2 Stability of DMSP with temperature and pH

Very little is known on the bias generated by DMSP decomposition during the distillation procedure used for liquid samples. It is important to know whether an artifact is present.

Dacey and Blough (1987) investigated the kinetics of abiotic decomposition of DMSP (Hofmann degradation) as a function of pOH (14 - pH) and temperature. The reaction is of second order between DMSP and OH^-. The kinetics become ("pseudo") first order with respect to DMSP in alkaline solutions because OH^- is in excess. Arrhenius plots of the decomposition of DMSP were drawn as a function of temperature at a given pH. The plot gave the reaction constant from which the half-life of the reaction is calculated.

The dependency of the reaction of DMSP with OH^- was calculated graphically from the work of Dacey and Blough (1987). The half-life of the reaction, t(sec) = ln 2/k(sec^{-1}) at 20 °C was found to be:

$$\text{Log } k = 1.028 \text{ Log}[OH^-] - 2.4 \qquad [7.1]$$

or

$$\text{Log } k = 1.028 \text{ (pH - 14)} - 2.4 \quad (20\ ^\circ C) \qquad [7.2]$$

The Arrhenius equation is:

$$k = Ae^{-(\frac{E_a}{RT})} \qquad [7.3]$$

or

$$Log k = Log A - (E_a/2.303RT) \qquad [7.4]$$

where k: constant of reaction (ln 2/k gives the half-life of the reaction).

pH: ambient pH of the solution (measured)

A: Arrhenius constant (unknown; to be calculated).

E_a: Energy of activation of the reaction (14.4 Kcal/mole; Dacey and Blough 1987).

R: Gas constant (1.987 cal/mole K)

T: Absolute Temperature (K)

This approach requires the pH (equation 7.2), from which Log k (20 °C) is obtained. Log k is used in equation 7.4 to get log A at 20 °C (293 K). For example, equation 7.4 gave Log A = 7.32 (for NaOH 0.1 M, pH = 13) and Log A = 8.35 (for NaOH 1 M, pH = 14). The value of log A thus obtained is used in equation 7.4, but at the specified temperature.

This sequence is adapted for all pH and temperatures. Similar calculations suggest that the half-life of the pure chemical degradation of DMSP at pH 8.3 and T = 10°C (typical of oceanic waters) is about 8 years (Dacey and Blough 1987) or 9.5 years (as obtained with equations 7.2 and 7.4). Therefore pure chemical degradation of DMSP is too slow to be an important process in natural waters. Enzymatic and/or bacterial degradation of DMSP is the most likely route, and the process is exocellular.

This approach also confirmed that the Hofmann degradation of algae tissue for DMSP analysis (section 4.3.2) is complete. Reaction half-lives give 2.9 minutes (20 °C) and 0.08 minute (70 °C) with NaOH 1.0 M.

7.3 Bias from the distillation procedure

The conditions (temperature, heating time) in the distillation procedure

have been simplified to estimate a bias caused by a possible degradation of DMSP. The assumptions are: heating time of the sample, 20 minutes at 100 °C; the degradation is fitted according to first order kinetics. The pH of all the water samples analyzed was approximately 7.2 (measured). Calculations give a half-life of 97 days for the reaction under these conditions.

Two concentration levels of DMSP were used in Bold's Basic Medium (BBM) control solutions. The first one represents the levels of free DMSP found in marine waters around Mainland Britain (0.2 µMolar; Turner et al. 1988), henceforth called "low DMSP". In the second one ("high DMSP"), DMSP was added in amounts similar to sulfate in original BBM (300 µMolar). Calculations suggests that DMSP chemical degradation would produce 0.06 ng S (DMS) in the "low DMSP" BBM run, and 95 ng S (DMS) in the "high DMSP" sample.

The results obtained for blank BBM "low DMSP" (Table 7.1) gave a small DMS peak close to the detection limit, and it amounts to 1.7 ng S (DMS) when normalized for 100% internal standard (ISTD) recovery. This is somewhat higher than the calculation suggests. The "high DMSP" BBM run gave a fairly strong DMS peak (about 12 ng as S), which is far below the calculated amount. This suggests that the distillation procedure does not significantly enhance the chemical degradation of DMSP. The peak reported in the "low DMSP" run is probably evidence of DMS residue in the pure DMSP, because the latter always has a slight DMS smell. The DMS peak obtained in the "high DMSP" run can be explained solely on the basis of abiotic degradation.

Table 7.1 Amounts of DMS found in flasks after DMSP addition. (Results obtained with the distillation line.)

Sample	Amount DMS found (ng S)	recovery of ISTD*(%)	Corrected DMS (as S) for 100 mL
BBM Low DMSP	0.4[e]	23.3	1.7
BBM High DMSP	6.8	56.5	12.1
S. starodub Low DMSP	1.8	36.2	5.1
Ankistrodesmus High DMSP	16.9	71.8	23.6
A. carterae (raw)	34.5	78.0	44.2
Amounts expected from calculation (chemical/thermal degradation)			
Low DMSP (pH 7.2)			0.06
High DMSP (pH 7.2)			95

* ISTD: Internal standard

e: estimated. A trace amount of DMS was detected, but it was too small to be reported on the integrator.

The levels of DMSP in the *A. carterae* filtration experiments (Figure 5.6) were representative of the DMSP (particulate) found by Turner et al. (1988) around Mainland Britain. This *A. carterae* run had a value of 33.5 ng DMSP/mg algae (measured; Table 5.1), and with the concentration of algae in the distillation flask, this corresponds to 0.25 μMolar DMSP. The chemical degradation of DMSP at this level would explain only a negligible fraction of the peak obtained, namely less than 4 % of the total. Here again, evidence suggests that the analytical procedure does not significantly bias the levels of DMS obtained from DMSP, especially for levels found in oceanic waters.

7.4 DMSP: freshwater vs seawater

DMSP has not been reported in freshwaters, either in algae (White 1982; table 5.1) or free. Therefore the presence of DMS cannot be explained from the decomposition of the DMSP precursor in freshwaters. In addition, DMS is most often not the dominating species. Five or more species are usually found in freshwater algae and natural softwaters, and often MeSH predominates. This suggests that the mechanism of production of volatile sulfides in freshwater environments is quite different from those of seawaters, where algal DMSP precedes DMS emission.

DMSP has not been found in freshwater algae. Biotic degradation of DMSP is catalyzed by an enzyme (Cantoni and Anderson 1956), and freshwater algae should possess this enzyme if DMSP is a substrate in their

sulfur metabolism. The freshwater algae *S. starodub* and *Ankistrodesmus* were used to test this hypothesis. DMSP was added to *S. starodub* to obtain a 0.2 μMolar concentration in experiment A, and experiment B used 300 μMolar DMSP with *Ankistrodesmus*.

The results of analysis (Table 7.1) of *S. starodub* show a DMS peak stronger than the blank alone, part of which (up to 33%) is explained by chemical degradation of DMSP. Nevertheless, this peak is still within the range of DMS found for this algae. Seawater varieties (e.g. *A. carterae*) give a much stronger DMS peak for this level of DMSP. On the other hand, the *Ankistrodesmus* grown in "high DMSP" medium also showed stronger DMS signal with respect to the control. This peak, however, can be explained solely by chemical and thermal degradation.

Both these algae do not seem to decompose DMSP to DMS. These levels of DMSP do produce a DMS peak much stronger with varieties of seawater algae. This is added evidence that the mode of production of VS in freshwater algae is different from seawater algae, and DMSP is not likely to be involved in freshwaters.

There is however some evidence that DMSP is metabolized in a different way in these two freshwater algae. In all such analyses, MeSH was distinctly absent, and DMDS was present in amounts slightly higher (up to 2x) than a typical run of the normal algae. One does not know if the sulfur in DMSP would sustain the growth of these algae, but nevertheless it appears that it has

a small influence on VS production.

The evidence shown above suggests that there are differences in the mode of production of VS between freshwater and seawater algae. The key factor is the presence or the absence of DMSP in these different varieties of algae.

8. TRANSFER OF VOLATILE SULFIDES FROM THE LIQUID PHASE

Volatile sulfide production from algae and other various sources has a significant influence on sulfur cycling in the environment. The main sink of VS produced in oxygenated surface waters is the atmosphere. This section summarizes a few approaches to quantify the amounts of the various sulfides transferred to the atmosphere from the aqueous phase.

8.1 Mass transfer coefficient

The principle of Fick's law can apply to this mass transfer situation. Liss and Slater (1974) give the basic relationship for the transfer of volatile substances across the water/air interface. Their equation is:

$$F = -D\left(\frac{\partial C}{\partial z}\right) = K(C_l - C_g) \qquad [8.1]$$

Where: F: flux of gas across the water/air interface [amount/area*time]

C_l, C_g: concentration of solute B in liquid (subscript "l") and gas ("g"), respectively [concentration units]

K: is the overall gas transfer coefficient; it is referred to as a piston velocity [velocity units]

The overall gas transfer coefficient can be separated into two distinct coefficients, the gas-phase transfer coefficient, and the liquid phase transfer coefficient:

$$\frac{1}{K} = \frac{1}{k_l} + \frac{1}{Hk_g} \qquad [8.2]$$

Where: k_l: gas transfer coefficient in liquid phase [velocity units]

k_g: gas transfer coefficient in gas phase [same units]

H: Henry's law constant [dimensionless: equilibrium concentration of solute in gas/concentration in liquid]

The major difficulty arises in the determination of the two gas transfer coefficients (k_l and k_g). Gas phase coefficients (k_g) are in the range of 1000-3000 cm/h, whereas the liquid phase coefficients k_l have values around 10-20 cm/h (Liss and Slater 1974). Field measurements usually give the overall mass transfer coefficient (K). The numbers above show that usually only one phase controls the mass transfer, thus K usually indicates the gas phase coefficient (k_g) for volatile solutes, or the liquid phase coefficient (k_l) for non volatile solutes. The temperature, the wind speed, and the nature of the solute and solvents influence these transfer coefficients.

The model used in this study follows the approach used by Mackay and Yeun (1983). They calculated empirical relationships for k_l and k_g as a function

of the wind velocity (U), and the Schmidt (Sc) numbers of different organic compounds in water. Two sets of equations are suggested, one for laminar, and the other for turbulent wind velocities:

$$k_g = 10^{-3} + (46.2 \times 10^{-3})\, U^* Sc_g^{-0.67} \qquad [8.3]$$

$$k_l = 10^{-6} + (34.1 \times 10^{-4})\, U^* Sc_L^{-0.5} \qquad [8.4]$$

(for U^* > 0.3 m/sec; turbulent air flow)

$$k_l = 10^{-6} + (144 \times 10^{-4})\, U^{*2.2} Sc_L^{-0.5} \qquad [8.5]$$

(for U^* < 0.3 m/sec; laminar air flow)

where: U^*: friction wind velocity [m/sec]

U_{10}: wind velocity, 10 m above the surface [m/sec]

Sc_g, Sc_l: Schmidt number of the solute in gas phase and liquid phase, respectively [dimensionless]

The wind velocity (U_{10}) and the friction wind velocity over the water surface (U^*) are related by the following equation (derived from figures in Mackay and Yeun 1983):

$$U^* = 0.01884\, U_{10}^{1.274} \qquad [8.6]$$

The Schmidt numbers (Sc_g, Sc_l) are defined as (Mackay and Yeun 1983; Reynolds 1974):

$$Sc_{(l,g)} = \frac{\eta}{\rho D_{(l,g)}} \qquad [8.7]$$

where η is the viscosity of the compound

ρ is the density of the compound

D_l, D_g are the molecular diffusivities of the volatile compound in liquid and gas phase, respectively.

The Schmidt numbers (Sc_g, Sc_l) are not available for the sulfides. D_l and D_g can be calculated for all sulfides (Table 8.1; see Appendix D for calculations), but the viscosities, and the temperature dependence of the viscosity and the density are not available. The Schmidt numbers can be substituted in equations [8.3] to [8.5] using an alternate approach. A list of Sc and D was given for 11 compounds (Mackay and Yeun 1983), and the product (Sc x D) gives a constant ratio (viscosity/density):

$$Sc_l \times D_l = 9.78 \times 10^{-3} \ (\pm 1\% \text{ std. dev.}) \qquad [8.8]$$

(n=10, n-butanol omitted)

$$Sc_g \times D_g = 0.175 \ (\pm 0.15\% \text{ std. dev., n=11}) \qquad [8.9]$$

The ratios [8.8] and [8.9] hold if the volatile compounds present in the liquid phase have a constant ratio (viscosity/density). Compounds such as alcohols or glycols are viscous (because of H-bonding) and their ratio can deviate from the one obtained in [8.8]. The ratios [8.8] and [8.9] are subject to

Table 8.1: Some physical and transport properties of selected sulfides at 25 °C used in the calculation of the mass transfer coefficient.

Compound	H [dimensionless]	D_g [cm^2/sec]	D_l [cm^2/sec] ($\times 10^6$)
H_2S	0.40[a]	0.139	20.32
COS	6.17[b]	0.105	16.27
CS_2	0.67[c]	0.090	13.41
MeSH	9.88[d]	0.107	14.93
DMS	13.70[de]	0.089	12.19
DMDS	23.45[d]	0.075	10.27

a: Critical Tables (Smith and Martell 1978; op. cit. in Stumm and Morgan 1981)
b: Johnson and Harrison (1986)
c: Elliott (1989)
d: Przyjazny et al. (1983)
e: Dacey et al. (1984)

changes with temperature. Estimates using equations (Reid et al. 1987) suggest that this ratio would vary by about 10% between 0 and 25 °C.

Equations [8.3] to [8.5] can be rewritten using the relationship of Sc with D_g and D_l from equations [8.8] and [8.9], and equation [8.6] is substituted for U^*:

$$k_g = 10^{-3} + (2.798 \times 10^{-3}) U_{10}^{1.274} D_g^{0.67} \qquad [8.10]$$

$$k_l = 10^{-6} + (2.335 \times 10^{-5}) U_{10}^{2.803} D_l^{0.5} \qquad [8.11]$$

(for $U^* > 0.3$ m/sec, or $U_{10} > 8.8$ m/sec)

$$k_l = 10^{-6} + (6.5 \times 10^{-4}) U_{10}^{1.274} D_l^{0.5} \qquad [8.12]$$

(for $U^* < 0.3$ m/sec, or $U_{10} < 8.8$ m/sec)

Equations [8.10] to [8.12] are used in equation [8.2] to determine the mass transfer coefficient (K) as a function of the field wind velocity (U_{10}) and the water temperature. The plots are shown in Figure 8.1: a, K vs wind velocity (Temperature 15 °C) and b, K vs temperature (wind velocity 8 m/s) for six sulfides.

The main feature obtained from this series of calculations is the determination of individual mass transfer coefficients for the six most abundant sulfides found in this work. The calculation of the liquid diffusivity (D_l; Appendix D) includes a term for the volume of the molecule. As a consequence, a compound like CS_2, whose molecule is smaller than DMS but is heavier and has a higher boiling point than DMS, has a mass transfer coefficient close to the lighter MeSH (Figure 8.1a). The mass of the solute is also included in the

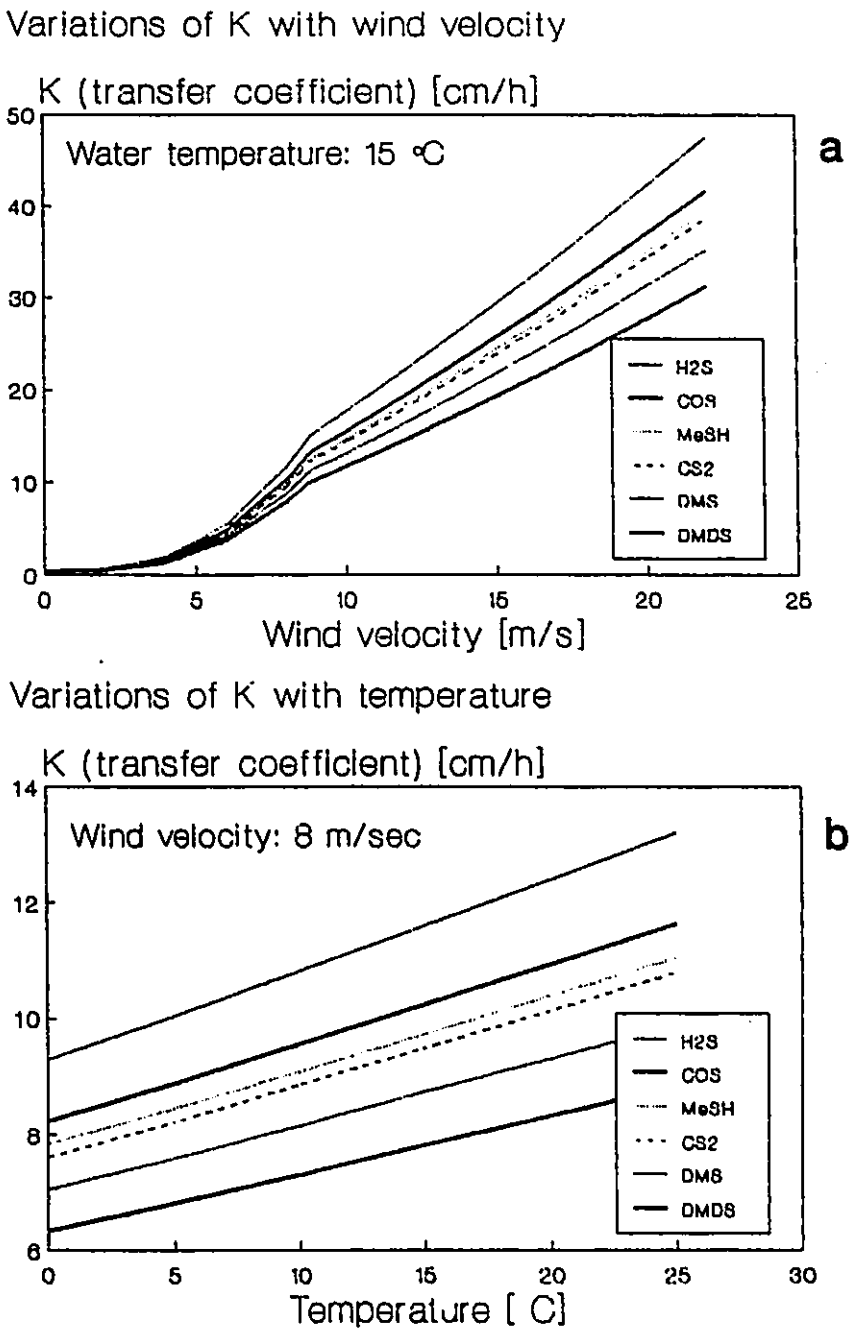

Figure 8.1 Variations of the mass transfer coefficient (K) for the most abundant sulfides as a function of: a. wind velocity; b. temperature.

calculation, but it has a small effect on the overall mass transfer coefficient.

The mass transfer coefficient of DMS and/or other sulfides has often been calculated on the basis of results obtained from SF_6 or Rn transfer (Barnard et al. 1982; Bates et al. 1987; Nriagu et al. 1987; Nriagu and Holdway 1989). Such approach often does not have a temperature relationship (Nriagu et al. 1987), which can be appreciable if the survey is spread throughout a season or more. The dependence of K with temperature (a) and wind velocities (b) are shown in Figure 8.2 for DMS.

The values of the mass transfer coefficient determined in this study compare well with other published data, although the values at low wind velocities seem underestimated (Figure 8.3). The DMS points from Bates et al. (1987) are values obtained from radon data in seawater, corrected for mass effect. The values for Crowley, Mono and Rockland Lakes were measured with SF_6 (Wanninkhof et al. 1985; 1987). The value from Liss and Slater (1974) is shown as a line, because the wind velocity and temperature were not specified.

The mass transfer coefficient determined in this section was applied to Lake Ontario as a test field site. The average monthly surface water temperature (Environment Canada 1989) and wind velocities (10 m above the surface; Environment Canada 1985) from six surrounding stations were used. Calculations of K (overall mass transfer coefficient) gave the results in Figure 8.4. Also plotted are the monthly average wind speed and surface water temperature, averaged for the whole lake.

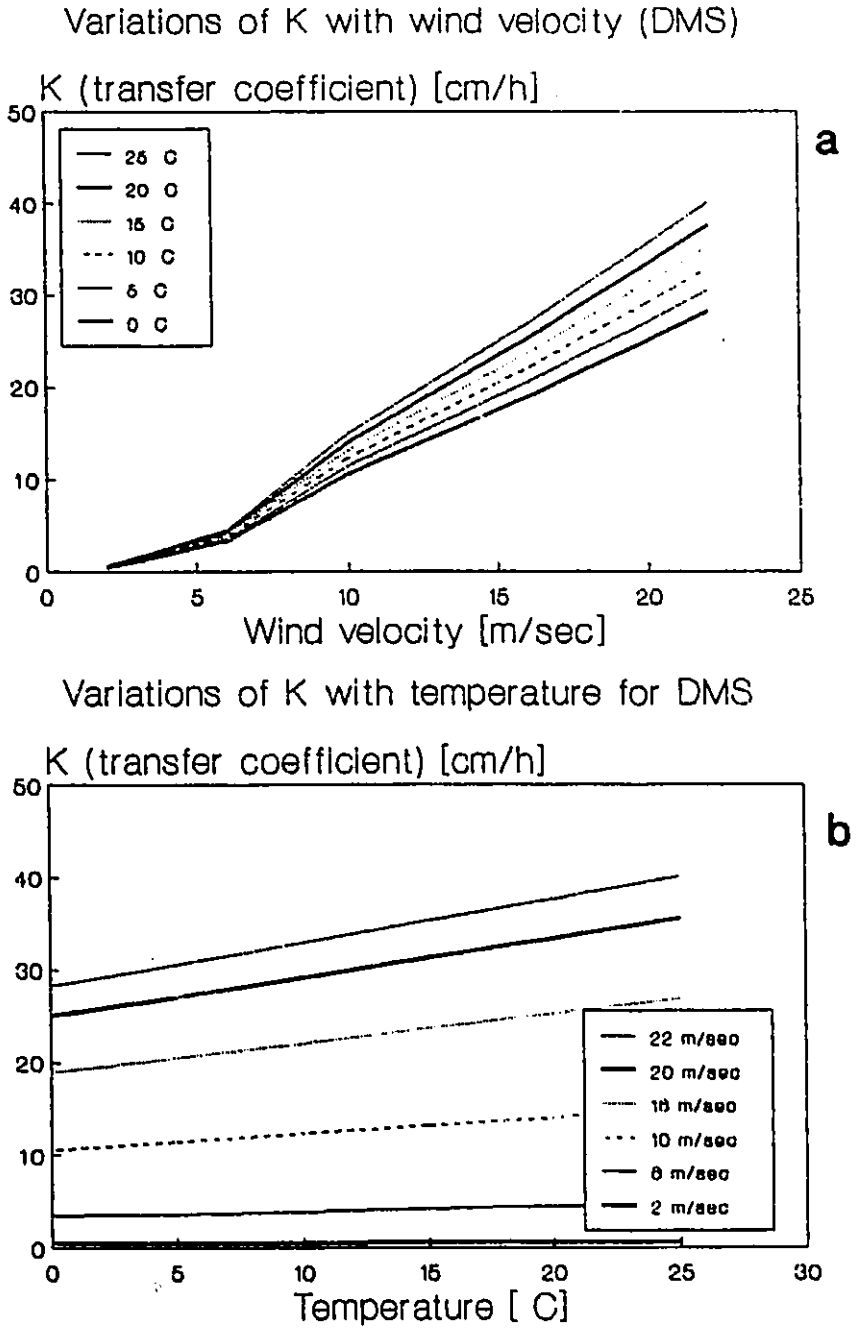

Figure 8.2: Variations of the mass transfer coefficient (K) for DMS as a function of: a. wind velocity; b. temperature.

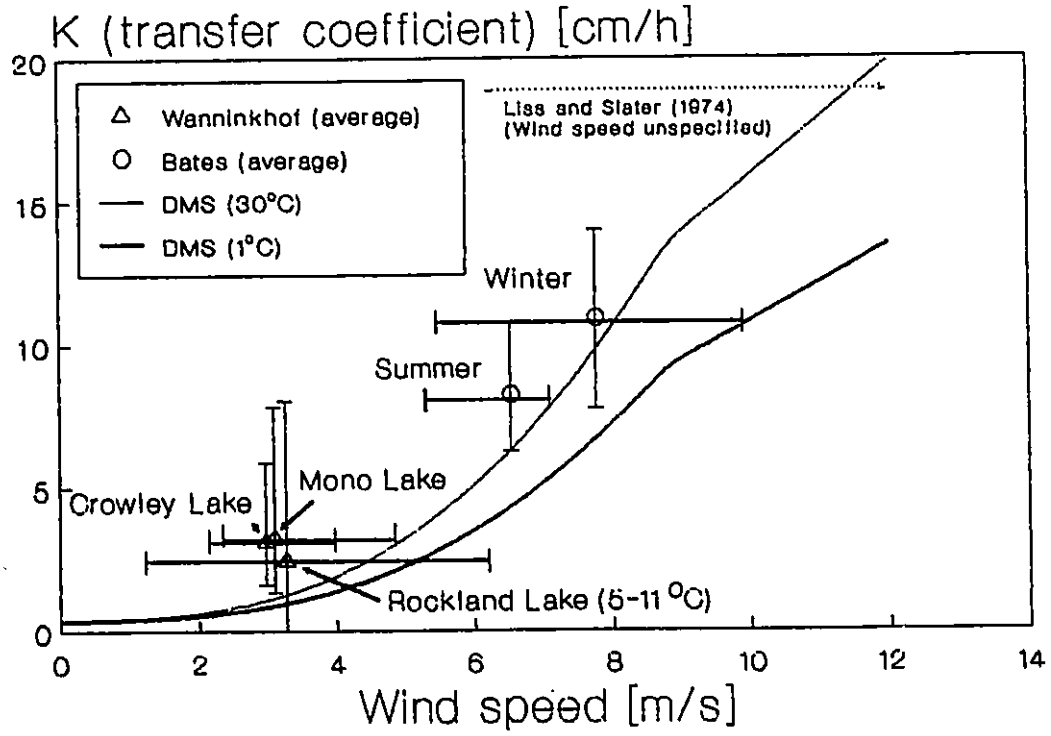

Figure 8.3: Mass transfer coefficient: comparison with other studies. The DMS values are the solid lines for 0 °C (thin line) and 30 °C (dark line). The error bars represent the ranges in wind velocities and K taken from the original papers. The symbol at their intersection is the average.

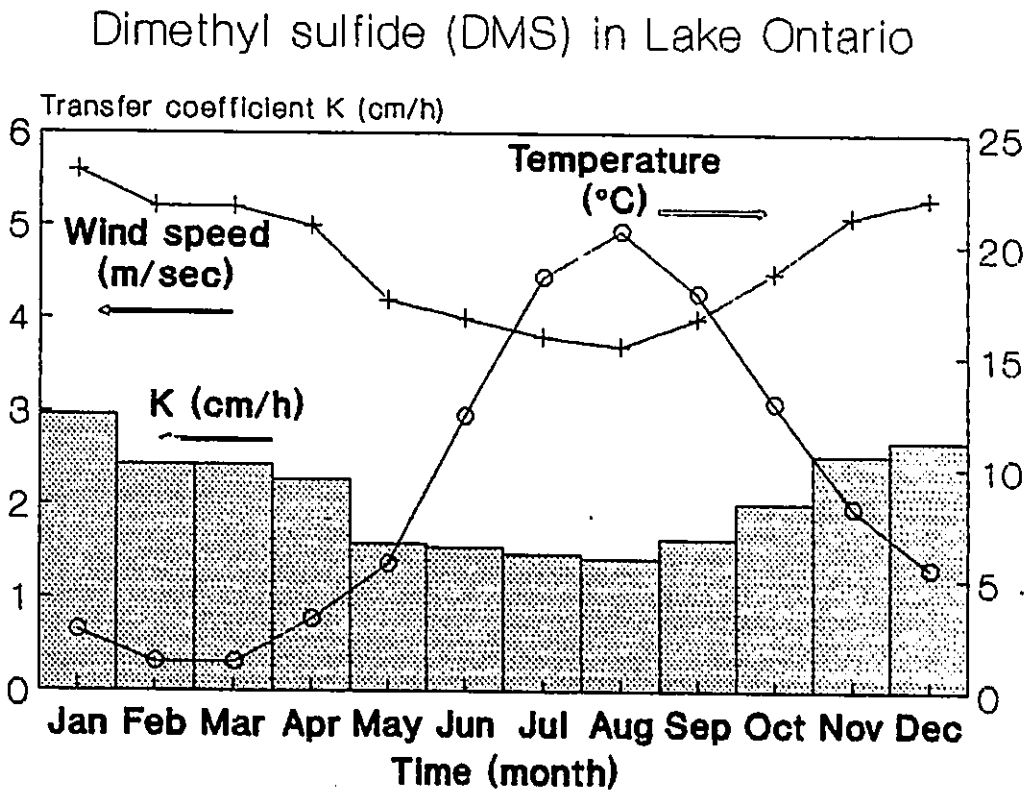

Figure 8.4: Monthly variations of the mass transfer coefficient for Lake Ontario.

The figure shows higher mass transfer coefficients in the cold months (November to April), which is caused by a greater wind velocity. The wind velocity controls the value of the mass transfer coefficient, whereas temperature has a small influence that is noticeable in the summer months (June to September). This is important, because the phytoplankton blooms in the summer producing high volatile sulfide concentrations would show a smaller mass transfer to the atmosphere compared to the winter, due to lower wind velocity.

The annual average of K values for DMS calculated for Lake Ontario is 2.12 cm/hr, which is very close to that chosen by Nriagu and Holdway (1989) of 2.2 cm/hr. This assumes that the wind speed and the water temperatures are uniform throughout the lake. Calculated values for the other sulfides are 1.97 (DMDS), 2.20 (CS_2), 2.31 (MeSH), 2.39 (COS) and 2.63 cm/hr (H_2S). A small correction factor is needed for H_2S where the ambient water pH is 6 or more, to compensate for the ionization of the species ($pK_1 = 7$).

8.2 Chlorophyll *a* as a substitute for volatile sulfides

Values of VS concentrations are very scarce, and very often only DMS values are available for softwater lakes, for example, Lake Ontario. The use of Chlorophyll a as a biological indicator of VS production is suggested in this section to substitute for volatile sulfide concentrations whenever they are not available.

The normalization of VS per unit of biomass (or Chlorophyll a) must be used cautiously. Andreae and Barnard (1984) found that the levels of DMS vary as a function of the mean primary productivity of phytoplankton in different types of oceanic waters. Many researchers have attempted to normalize the production of DMS to Chlorophyll a in marine waters (Barnard et al. 1984; Holligan et al. 1987; Turner et al. 1988; Iverson et al. 1989). All of them had limited success because DMS production is also a function of the algae species, the physiological state of the algae, and other miscellaneous factors (senescence, phytoplankton grazing, etc.). A better correlation was achieved when the sample was dominated by one species of DMS producers (Barnard et al. 1984; Turner et al. 1988).

The use of cultures in this study can minimize some of these variables. Algae grown in axenic conditions, under similar physical settings have a better chance to give a steady volatile sulfide:Chlorophyll a ratio. Table 5.3 is a compilation of VS ratios found in algal cultures normalized per unit of Chlorophyll a. Despite the growth of algae in controlled conditions, the ratios (Table 5.3) still show a wide variability. This set of data therefore must be considered cautiously, and one must bear in mind that it is used only as an alternative to direct measurements.

Data available for the Great Lakes, particularly Lake Ontario (Munawar and Munawar 1986), give a relative abundance of algae species and taxa present, and their seasonal variations. Chlorophyll a measurements from Lake

Ontario (Environment Canada 1990) are used in the following equation to estimate the amount of VS produced by algae:

$$C_l = R\,[\text{Chl } \underline{a}] \qquad [8.13]$$

Where: C_l: concentration of sulfides in liquid phase (to substitute in equation [8.1])

R: ratio **Volatile sulfide/Chlorophyll** a, taken from Table 5.3 (ng S/mg Chlorophyll a)

[Chl a]: measured concentration of Chlorophyll a, corrected for phaeophytin.

The following limitations must be understood while using equation [8.13]:

1. Only 4 species of algae are used in this model, and one has to assume that they are representative of what is found in Lake Ontario and the Great Lakes at any time of the year.

2. The numbers in this calculation show only a potential estimate of the contribution of VS from softwater algae. The contribution by other sources of volatile sulfides, e.g., bacteria in the water column and sediments, other aquatic plants and organisms, etc., is not included.

3. The chlorophyll and VS data are obtained for static cultures in the laboratory. The ratio is calculated with healthy algae growing in ideal conditions. The field ratio (VS/Chlorophyll a) can be drastically different from the *in vitro* ratio. Field production rates certainly vary as a function of some

environmental factors, such as age, zooplankton grazing, etc.

4. The *in vitro* growth temperature was ambient (20 +/- 2°C) thus significantly different from lake environment, which varies substantially between winter and summer. Furthermore, light was always constant in the laboratory (intensity, photoperiod), whereas it varies with depth (turbidity, etc.) and time (seasonal variations in the photoperiod).

5. External stresses enhancing the production of volatile sulfides, namely grazing and senescence were not estimated for the cultures. The ratio R can be underestimated in the cultures because of the enhancement of volatile sulfide production expected from these stresses.

8.3 Emission estimates for Lake Ontario

Nriagu and Holdway (1989) published the only known estimate of DMS emission from Lake Ontario: 69 tons S/year. The estimate was based on samples taken from a cruise.

The present work gives ranges of total release of VS from Lake Ontario and its surrounding catchment using different approaches (Table 8.2). The first approach uses chlorophyll a data and equation 8.13 to estimate the amounts of volatile sulfides present in the lake waters. The relative abundance of the algae differs with the seasons (Munawar and Munawar 1986) and the range shown considers the upper and lower limits of VS emissions using the VS/Chlorophyll a ratio (equation [8.13]). The different scenarios are: lower limit assumes

Table 8.2: Different emission estimates of total volatile sulfides from the Lake Ontario waters and surrounding basin. The explanations for the numbers are in the text.

	Tonnes S/year
Lake Ontario:	
Chlorophyll a substitute (eq. 8.13):	
calculated range	7.0 - 260
mean	116.1
Lake Ontario (June 1988)	96.3 - 130.9
Lake Ontario basin (excluding lake):	
Adams* model (uncorrected for temperature):	1053
Adams model (with 14% wetlands):	2525
Adams model (with 33% wetlands):	4523
Toronto-Hamilton corridor** (SO_2 pollution)	265000

* Adams et al. (1981b).
** Katz (1978)

Ankistrodesmus as the only algae producing VS; the high limit assumes the same for *S. starodub*; the average assumes that each of the four algae contributes 25 % of the VS. The total number is in agreement with the 69 Tons S/year obtained by Nriagu and Holdway (1989), who obtained their number for DMS only. Our estimate gives only approximately 10 to 12 % of the total sulfur attributed to DMS.

A sample of Lake Ontario waters, taken offshore from Burlington (Ont.) in June 1988 was analyzed shortly after sampling. Assuming that these samples are representative of the Lake and the conditions all year long, the total flux estimated is within the same range as the estimation with equation [8.13]. However, the November 16 sample (Figure 5.2b) taken in Cootes Paradise would give a flux of 2540 Tonnes S/year, but this is highly unlikely to be representative of the lake. This number is used only as an upper limit for comparison. In this sample, chlorophyll a was very low as estimated by visual inspection. The algae content was low, and the ratio (equation [8.13]) would give only a small value for VS. Similarly, the samples from Desjardins Canal (Figure 5.1) and Luther Lake (Figure 5.3) had low Chlorophyll a levels (visual inspection) along with moderate VS concentrations. If all algae in natural waters (e.g., Figures 5.1, 5.2a, b, and 5.3) have VS/Chlorophyll a ratios in the same range as those in Table 5.3, the VS produced by algae in these samples would be very low. Sources and/or processes other than algae have to produce VS in these waters. The use of chlorophyll a as a biomass indicator

does not seem to be adequate to estimate the production of VS.

The numbers for Lake Ontario basin were determined with the model of Adams et al. (1981b), using three different scenarios. The first scenario does not include any wetlands. The second scenario includes 14 % of the basin as wetlands (average for Canada), whereas the third one uses Ontario's average for wetlands. Luther Lake (Figure 5.3c) was assumed to be representative of wetlands for these calculations, and K values for Lake Ontario were used for the sulfides detected in Figure 5.3c.

The lake and the catchment give VS values ranging from about 1060 to 4650 tons of sulfur/year. Only a small fraction of this estimate comes from freshwater algae (as estimated with equation 8.13). When this picture is compared to the heavily polluted Great Lakes basin, the numbers of naturally produced sulfur compounds fall short of being significant. However, if the study site was remote from pollution, the local endogenous sulfur emitted by natural sources would likely release VS in a proportion stronger than in the present situation.

8.4 Summary

A mass transfer coefficient was calculated for the most common sulfides found in this study. The calculation involves factors correcting for mass and size effects of the solutes in a water/air system. Variations of the mass transfer coefficient with temperature and wind velocity are also included in the

calculations.

Estimates for Lake Ontario suggest that volatile sulfide emissions from algae make a negligible contribution to a regional sulfur budget. The Great Lakes basin is heavily polluted, and consequently the release of natural sulfur compounds is negligible compared to man-made emissions.

Finally, the use of a VS/Chlorophyll a ratio to substitute for VS did not seem to be adequate. More data are needed from algae and more measurements from lakes should complement this study. Wetlands and shallow waters probably also make a significant contribution to natural sulfur emissions.

9. SUMMARY AND CONCLUSIONS

This study deals with the analysis, the production and the fate of biogenic volatile sulfides in predominantly freshwater systems. New knowledge has been brought because the dynamic aspect of biogenic VS in this type of environment is not well documented. Most of the work previously published in the literature feature different systems: marine waters, coastal waters, salt marshes, ponds, soils, etc.

An analytical method for the detection of these sulfides had to be developed for this work. The concentration levels of sulfides was anticipated to be low, therefore a low detection limit was required. The Hall Electrolytic Conductivity Detector used in this study proved to be a key factor for achieving this important task. The method was used to establish the omnipresence of many volatile sulfides (5 species or more) in waters around the Hamilton area, and in axenic cultures of algae.

Experimental evidence suggested that the process of formation of volatile sulfides, particularly the methylated species (MeSH, DMS, DMDS), was different between softwater and marine algae. The intermediate compound DMSP was the focus of this discrepancy, and the distinction between the sulfur metabolism producing VS had to start from there. Chemical controls and direct analysis on tissues failed to demonstrate the presence of DMSP in freshwater

algae. However, the influence of sulfate availability, methionine and some methyl donors was assessed.

The fate of the VS was also evaluated with the development of a mass transfer model. Estimates indicate that emissions of VS from Lake Ontario are a small fraction of all the sulfur pollution from the surrounding cities.

Some specific conclusions:

1. Volatile sulfide analysis: gas chromatography with a HECD was a vital instrument for achieving the low detection limit required for freshwater samples. The analysis was based on a purge/trap method applied to an aqueous sample. Recovery of the analytes (estimated with an internal standard) was not ideal and more work should be done to improve the stripping efficiency.

Sample conservation was also a problem. There are two ways this step can be improved. First, selected sample preservatives and special containers can be used to minimize microbial activity, oxidation and sample losses. The second and perhaps the best approach is the use of a solid sorbent trap in the field followed by desorption/analysis in the laboratory. The field step would feature degassing of the sample and adsorption on the sorbent. This solid trap can be stored for a longer period of time prior to analysis. Literature studies support this approach and it would be beneficial to modify our system for this type of analysis.

2. Marine and freshwater algae: All the species of softwater algae analyzed in this work produced volatile sulfides. My results suggest that VS

production is not limited to blue-green algae, but also occurs in green algae. The work was not extended further to other divisions of algae or bacteria.

I have found that there is a set distribution of each of the VS species for a given species of algae. In many situations, MeSH was the most abundant sulfide. The combined H_2S+COS species also constitutes an important part of the VS present in algal cultures. DMS was always present but represented rarely more than 25% of all sulfides in a sample. The fact that DMS was not the dominant sulfide was traced back to the absence of DMSP, supported by experimental evidence.

Sulfides in marine algae were dominated by DMS, which constituted more than 80% of the sulfides in the culture. A few marine varieties analyzed in this study showed the presence of DMSP.

Sulfate is low in freshwaters (2-30 mg/L) when compared to marine waters (2700 mg/L), yet VS levels are comparable. Sulfate is likely to be assimilated in a similar way by algae (marine or freshwater), thus the main difference in VS production mechanism must be further in the transsulfuration chain.

3. Chemical controls: Sulfate levels did not seem to have an influence on the relative distribution and the levels of VS produced in algae. However, a lower threshold was observed at about 2 mg/L of sulfate, which corresponds to low sulfate, pristine waters.

Using the Assimilatory Sulfate Reduction scheme as a guide, cysteine

additions did not induce VS production. Methyl donors had a small influence, but methionine had a prime importance. Enzymatic dissimilation of methionine can explain the formation of methylated sulfides. The species H_2S, COS and CS_2 were not significantly influenced by the methionine (bio)chemistry.

4. Fate of the sulfides: The atmosphere was considered as the main sink of VS transfer from the water column. A mass transfer coefficient was calculated for the most common sulfides. The wind velocity and temperature relationships were also determined for each mass transfer coefficient. Calculations suggested that VS from biogenic origin are only a small source of sulfur within the Lake Ontario basin.

5. Future research: The data base of VS production by freshwater algae should be increased. In the present study, only a few species were used, which belong to only two taxonomic divisions. Other species, most commonly found in the Great Lakes, should be analyzed for VS content, the relative VS distribution, and VS production as a function of time (and age) of the algae.

It is unclear as yet if a suitable and simple biological factor can be found to relate to the production of VS. Methionine proved to be a key factor. Perhaps an enzyme (methyltransferase, methionine lyase, etc.) affecting the dissimilation of methionine is a good biological indicator capable of predicting VS production.

The total flux of sulfides from the water column does not seem to show a clear picture of what is happening at the present time. More data, along with

seasonal variations are needed for a better estimation of VS contribution from lakewaters. Evidence suggested that wetlands and shallow waters produce high amounts of VS, regardless of primary productivity. Bacterial activity, especially in wetlands, sediments and soils, seems to play an important role in VS production. Their influence in the water column should also be investigated. Freshwater algae are probably not a major contributor of VS in the environment.

Finally, the present work did not find any parameter that would affect the production of H_2S, COS and CS_2. Those species were present and they were accounted for in the mass transfer, but no mechanism could predict their formation. They shall deserve more attention in the future.

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APPENDIX

APPENDIX A: Composition and recipes of algae growth media.

A.1 SOFTWATER MEDIUM

A.1.1. Normal Bold's Basic Medium (BBM)

Two sets of concentrated solutions are first prepared:

SALT SOLUTIONS: each one of these salts is dissolved in 400 mL of glass-distilled water or Milli-Q (low organic) water.

Salt	amount/400 mL (g)	Salt	amount/400 mL (g)
$NaNO_3$	10.0	KH_2PO_4	6.0
$MgSO_4 \cdot 7H_2O$	2.0	$CaCl_2 \cdot 2H_2O$	1.0
K_2HPO_4	4.0	NaCl	1.0

TRACE ELEMENT SOLUTIONS: each solution (A, B, C, D) shall be composed of the following salts (per 100 mL):

Solution	Salt	amount/100 mL (g)
A: EDTA stock	EDTA, disodium salt	5.0
	KOH	3.1
B: Fe stock*	$FeSO_4 \cdot 7H_2O$	0.5
C: Boron stock	H_3BO_3	1.14
D: H-H5 stock*	$ZnSO_4$	0.88
	$Na_2MoO_4 \cdot 2H_2O$**	0.12
	$Co(NO_3)_2 \cdot 6H_2O$	0.05
	$MnCl_2$	0.14
	$CuSO_4 \cdot 5H_2O$	0.16

* Stock solutions B and D are dissolved in 100 mL acidified water (0.1 mL conc. sulfuric acid in 100 mL water).
**This replaced MoO_3 which was not available.

Add 10 mL of each one of the SALT SOLUTIONS, and 1 mL of each of TRACE ELEMENT SOLUTIONS to distilled water. Complete the volume to 1000 mL. Add HCl or NaOH to adjust the pH to the desired value.

A.1.2. No sulfate BBM:

Each of the sulfate-containing salt of the normal BBM was replaced by the following:

Salt, compound	Amount	Replacement for
$MgCl_2 \cdot 6H_2O$	1.04 g/250 mL	$MgSO_4$
$FeCl_3 \cdot 6H_2O$	0.5 g/100 mL	$FeSO_4$
$ZnCl_2$	0.76 g/100 mL	$ZnSO_4$
$Cu(NO_3) \cdot 3H_2O$	0.15 g/100 mL	$CuSO_4$
HCl	0.1 mL/100 mL	H_2SO_4

A.1.3. Low sulfate BBM:

Only 1 mL of the $MgSO_4$ and 9 mL of the $MgCl_2$ solutions were used along with the normal ingredients.

The calculated concentrations of the major ions in normal BBM (mg/L) are:

Na^+	77.3	SO_4^{--*}	28.7
Mg^{++}	5.0	Cl^-	32.1
Ca^{++}	9.0	NO_3^-	183.2
K^+	88.0	$\Sigma\ PO_4^{3-}$	159.2

* Calculated concentrations of sulfate: Low sulfate BBM: 11.2 mg/L; No sulfate BBM: 0.5 mg/L. Not calculated for other species.

A.2. ARTIFICIAL SEAWATER: Ott's medium enriched with Von Stosch's trace elements.

Salt	amount (g)	Salt	amount (g)
NaCl	21.0	H_3BO_3	0.06
$MgSO_4 \cdot 7H_2O$	6.0	$Na_2SiO_3 \cdot 9H_2O$	0.01
$MgCl_2 \cdot 6H_2O$	5.0	$Sr(NO_3)_2$	0.03
$NaCl_2 \cdot 2H_2O$	1.0	Na_2HPO_4	0.023
KCl	0.8	$FeSO_4 \cdot 7H_2O$	0.00013
NaBr	0.1	$MnCl_2$	0.0002
$NaNO_3$	0.25	EDTA (Na salt)	0.0037
$NaHCO_3$	0.2		

Add all the salts above plus 1 mL of each of the TRACE ELEMENT SOLUTIONS above from the BBM, and dilute to 1000 mL. Adjust pH to desired value with NaOH or HCl.

APPENDIX B: Source and information on algae.

All algae cultures were obtained either from Carolina Biological Supply (CBS) (Burlington NC; marine species), or Dr. P.T. Wong (CCIW).

Name	type*	Division, other information
Anacystis nidulans	S	Cyanophyta (blue-green)
Anabaena sp.	S	Cyanophyta (blue-green)
Ankistrodesmus sp.	S	Chlorophycophyta (green)
Amphidinium carterae	M	Pyrrhophycophyta (dinoflagellate)
Coccolithophora	M	Coccolithophore. No additional specific information is available.
Oscillatoria sp.	S	Cyanochloronta (blue-green)
Scenedesmus quadricauda	S	Chlorophyta (green)
Scenedesmus starodub	S	Chlorophyta (green)
Sea dulse	M	Dried "dulse" (type of algae unknown). Atlantic mariculture, Grand Manan, N.B., Canada (available in supermarkets).
Spirulina major	M	Cyanochloronta (blue-green)

* Type: S softwater; M marine

APPENDIX C: Synthesis of Dimethylsulfoniopropionate.

The synthesis of Dimethylsulfoniopropionate (DMSP), the precursor of DMS, is based on the procedure outlined in Dickson et al (1980), and Challenger and Simpson (1948). A mixture of 3-Bromopropionic acid (15-16 g. recrystallized from absolute ethanol) and excess DMS (25 mL), was poured into a 2-necked, 100 round bottomed flask. A thermometer was inserted in the side arm, and a condenser affixed vertically completed the reflux distillation apparatus. The reaction mixture was held at 40-45 °C overnight with a heating mantle. The mixture was allowed to settle for an additional 24 hours after which the solid was washed with anhydrous diethylether. The solid, DMSP (bromide salt), was obtained as white needle-like crystals upon 4 successive recrystallizations in absolute ethanol. The purified solid was stored in a dry place (desiccator or a freezer (-5 °C)).

Characterization:

The identity of DMSP was confirmed with these tests: melting point: 114.5-117 °C (112-114 °C; Challenger and Simpson 1948; Dickson et al 1980); IR spectrum (Perkin-Elmer 283 on a KBr pellet); Proton NMR (Bruker spectrometer, DMSP dissolved in CD_3OD). Both spectra matched very well those in the Stadtler tables (Stadtler Reference Index). The NMR signals matched the structure of the compound, and peaks shifts given in Blunden et al. (1986).

APPENDIX D: Calculations of molecular diffusivities (D_l and D_g) of sulfides.

1. The molecular diffusivity of solute A in solvent B (D_l) is calculated using the Wilke-Chang method (Reid et al. 1987):

$$D_l = \frac{7.4\times10^{-8}(\varphi M_B)^{0.5}T}{\eta_B V_A^{0.6}} \qquad (1)$$

φ : association factor for solvent B [φ = 2.6 for water; dimensionless]

M_B: molar weight of solvent B [18 g/mole for H_2O]

T: absolute temperature [K]

η_B : viscosity of solvent B [cP]

V_A: molar volume of solute A at its normal boiling temperature (see table below).

Species	V_A (cm^3/mol)	Species	V_A (cm^3/mol)
H_2S	33.0	MeSH	55.2
COS	47.8	DMS	77.4
CS_2	66.0	DMDS	102.6

The Wilke-Chang method is valid for compound A infinitely diluted in solvent B. They found an average error of 10% between the calculated and experimental results for 251 solvents.

V_A was estimated with LeBas' method (Reid et al. 1987). This is an additive method to estimate the molar volume of compounds at their normal boiling point. It is given as:

$$V_A = \Sigma n_i I_i$$

where: n: number of atoms of species i

I: atomic increment for species i (cm^3/mol)

Atomic increments:

Atom	Increment
Carbon	14.8
Hydrogen	3.7
Oxygen	7.4
Sulfur	25.6

2. D_g (diffusivity of compound A in gas B) is calculated using the Fuller method (Reid et al. 1987). The overall error from experimental data is 4%. D_g is obtained from:

$$D_g = \frac{0.00143 T^{1.75}}{P M_{AB}^{0.5} [(\Sigma_v)_A^{0.33} + (\Sigma_v)_B^{0.33}]^2}$$

where T: absolute temperature [K]

P: pressure [bar; P=1]

$M_{AB} = 2 (\frac{1}{M_A} + \frac{1}{M_B})^{-1}$, $M_{A, B}$ = molecular weight of A and B (air = 29)

Σ_v : sum of atomic diffusion volumes for a component (subscript A: air; B: component)

These molecular diffusion volumes were calculated using the sum of individual atomic diffusion volumes as for Le Bas' method.

Species	Σ_v	Species	Σ_v
H_2S	27.52	MeSH	48.04
COS	44.91	DMS	68.56
CS_2	61.7	DMDS	91.46
Air	19.7		

The individual atomic diffusion volumes are:

Atom	Diff. volume
Carbon	15.9
Hydrogen	2.31
Oxygen	6.11
Sulfur	22.9

APPENDIX E: General procedure for water analysis using the distillation line.

1. The main distillation line (Figure 4.1)

The water sample is poured into a 2-necked 100 mL flask (B) along with an internal standard (ISTD; DES in ethylene glycol). The volume of the solution is diluted to 100 mL with Milli-Q water if necessary. Stopcocks (E) and (Q) are closed, and an injection loop is affixed on the luer-lok connector (H). Stopcock (R) is opened and (G) is switched to vacuum to evacuate residual gases in the right-hand part of the line. Trap (F) and loop (I) are immersed in liquid nitrogen dewars (J) after approximately 2 minutes of vacuum purge. Stopcock (G) is switched to isolate the line from the vacuum pump. The next operation is done simultaneously: helium is turned on (100-120 ml/min), the heating mantle (A) is turned on, and valve (E) is opened. Valve (Q) is opened a few seconds later, so the internal pressure inside the line is slightly higher than the room pressure. This is important because liquid nitrogen can draw air and block the loop (I) if valve (Q) is opened too early. The sample is heated just below the water boiling point (95-100 °C) in 8-10 minutes and held at this temperature for an additional 10-12 minutes, for a total heating time of 20 minutes. After this time, helium and the heating mantle are turned off, stopcocks (E) and (Q) are closed. (G) is opened to vacuum for 30 seconds and closed again. The liquid nitrogen dewar under the coiled trap (F) is replaced by hot water (approx. 90-100 °C) for 20 minutes to transfer the sample from trap (F) to (I). The vacuum pump is turned on the sample loop only (for 30 seconds) by rotating stopcock (G) the

appropriate way, then valve (R) is closed.

The injection is performed when loop (I) is removed from the line and a syringe (previously filled with 2.5 mL of air) is attached to the female luer tip (O). A needle is attached at the other end (P). The loop is immersed in hot water (90 °C) for 30 seconds to volatilize the sample. The needle of the assembly is then inserted into the injection port. Stopcocks (Q) and (R) are opened simultaneously and the user pushes the plunger of the syringe, as a normal injection. The internal volume of the loop (0.4 mL) is completely flushed by the volume of gas contained in the syringe, so the injection is complete.

2. The cross-check line (Figure 4.3)

This cross-check line is operated in a procedure analogous to the standard distillation line. A liquid sample is poured into the test tube (D; capacity of the tube 1 mL). A sample/injection loop is attached to the luer tip outlet (F) and the first valve of the loop is opened. The carrier gas helium is turned on (20-30 mL/min), then the last stopcock of the loop is opened. Again, extreme care must be taken when opening the loop valves to avoid air to condense and block the loop. Similarly, a burst of helium from the inlet can cause the sample to enter and clog the injection loop. After the helium gas is turned on, heat is provided to the test tube with a hot water dewar (90-100 °C). The sample is purged for a maximum of 3 minutes. The sample is injected into the gas chromatograph as with the other line.