SULFATE REDUCTION AND ORGANIC SULFUR FORMATION IN LAKE SEDIMENTS

BY

JOHN A. AMARAL

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology
University of Manitoba
Winnipeg, Manitoba

ISBN 0-315-76963-7

SULFATE REDUCTION AND ORGANIC SULFUR FORMATION IN LAKE SEDIMENTS

BY

JOHN A. AMARAL

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

To my Mother and Father,

for their continuous support

and encouragement.

ABSTRACT

The formation and chemical characterization of organic sulfur compounds (S_O) from SO_4^{2-} reduction in lake sediments were studied using radiolabelled $^{35}SO_4^{2-}$ and a suite of wet chemical analyses. Using a variety of extraction and chromatographic methods, analytical interference by inorganic-S (S_I) was removed, and positive evidence for formation of S_O from short term SO_4^{2-} reduction obtained. Prior to this, S_O was measured only by difference between total S and S_I. The S_O was characterized as being i) small (<1000 Mw) ii) polar and iii) non-volatile, and consisted of carbon-bonded S (68%), ester sulfate (6%), and organic polysulfide (10-20%), which had not previously been measured in freshwater sediment.

Commonly used methods for estimation of S_O were shown to yield substantial underestimates because of problems associated with i) drying of sediment, ii) use of strong acid in sequential analyses and iii) presence of organic polysulfides.

The verified methods were used to monitor disruption of the S cycle in severely acidified lakes (pH<5). Metaphytic algal proliferation changed sediment redox conditions, enhancing S oxidation at some times and reduction at others, apparently contributing to greater swings in $[SO_4^{2-}]$ over the annual cycle.

PREFACE

Most chapters in this thesis are written in the format of scientific papers or notes. Chapter 1 is a review of pertinent literature and is intended as a general introduction to the thesis. Chapters 2 and 3 deal with methodology of sedimentary sulfur analysis and are both written as notes. Chapter 4 is written in the format of a paper and describes the extraction and characterization of radiolabelled organic sulfur compounds from sulfate reduction in sediment. In Chapter 5, also written as a paper, the disruption of the sulfur cycle in an acidified lake is investigated. Chapter 6 has been included to provide supplementary information on the end-products of sulfate reduction, and is written as a note for consistency with the rest of the thesis. The final chapter (7) is a brief summary of the main points of the thesis. An appendix is included at the end.

It is intended that Chapters 3, 4, 5 and 6 be submitted to scientific journals for publication. Chapter 2 has already been published (Amaral, J.A., R.H. Hesslien, J.W.M. Rudd, and D.E. Fox. 1989. Loss of total sulfur and changes in sulfur isotopic ratios due to drying of lacustrine sediments. Limnol. Oceanogr. 34: 1351-1358).

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr. C. Kelly and Dr. R. J. Flett, for their guidance and support throughout the period of my research. I am grateful for their encouragement and insights which helped me greatly in my work, and no doubt improved the quality of this thesis. R. Flett was "instrumental" in reviving long dead analytical equipment, without which much of this work would not have been possible. My many discussions with C. Kelly throughout the thesis writing helped in both the organization and clarity of data presentation. Her knowledge of limnology was important in placing my experiemntal data and results in a larger "lake context".

I am also indebted to Drs. J. W. M. Rudd, G. J. Brunskill, and I. Suzuki, members of my committee, who voluntarily reviewed preliminary versions of this thesis, and made many valuable suggestions for its improvement. Their advice during several long and arduous committee meetings helped to shape the course of my research. I am especially indebted to J. Rudd for suggesting the work on total sulfur underestimation by current methodology (Chapter 2), and for discussions of my work. Dr. R. Hesslein and D. E. Fox kindly provided stable isotope data included in Chapter 2. Drs. Rudd and Kelly provided data from Lake Hovvatn, included in Chapter 5.

I would like to thank M. Turner for providing invaluable help and expertise in the sampling and study of metaphytic algae and pore-water and sediment sampling of Lake 302 South (Chapter 5). B. Townsend has my eternal gratitude for teaching me how to SCUBA dive, but still agreeing to "take the plunge" himself into 3°C water for that all important winter data point.

Many others in both the Department of Microbiology (U. of Manitoba) and at the Freshwater Institute were of great help through the years. I would like to thank Drs. P. Y. Maeba, H. Halvorson, D. Burton, and M. Stainton for generously lending laboratory equipment; B. Miskimmin and M. Holoka for collecting and delivering sediments; M. Amaral, a summer student, for analysing sediment samples; the staff of the Freshwater Institute chemistry section for chemical analyses of water and sediment samples; D. Findlay for algal speciation (Chapter 5); and S. Berg for making sure all the paper work was done.

Akira Furutani introduced me to the analysis of sulfur in sediments, and showed me how to function in a biogeochemistry laboratory.

I also wish to express my deepest gratitude to my wife, Shirley Richards, who, although working on a thesis of her own, was a constant source of support to me. She provided valued advice on the organization of my thesis and devoted many long evenings in helping to shape it into a printable

form. Throughout the period of my research she provided much help in field sampling, and withstood the slings and arrows of sediment retrieval, by Ekman dredge, during cold and windy days with not a word of protest. My association with her made the period of my work a thoroughly enjoyable one even at the most difficult of times. I am also grateful to my family for their moral and financial support. They were always a source of encouragement.

Finally, I acknowledge the financial support from the Natural Science and Engineering Research Council (Government of Canada).

Table of Contents

List of Figures

List of Tables

List of Appendices

1. General Introduction:

Review of pertinent literature

1.0 An overview of sulfate reduction and organic sulfur production in the environment

Bacterial sulfate reduction is recognized to be of major importance in the cycling of sulfur (S), helping to transport the element between the litho-, hydro-, bio- and atmospheres (Feely and Kulp 1957; Kaplan et al. 1962; Zobell 1963; Ivanov 1968; Ivanov 1983; Ehrlich 1990). The reduction of sulfate to sulfide is an energy-expensive process, requiring nearly 200 Kcal per mole (Zobell 1963), and, for this reason, does not occur by purely chemical means at the pressures and temperatures normal to the Earth's surface (Feely and Kulp 1957; Zobell 1963; Ehrlich 1990). Therefore, biological mediation is a crucial step in the transformation of sulfates into other, more reactive, forms of S. Biological sulfate reduction occurs via two biochemically distinct pathways, assimilatory and dissimilatory reduction (e.g. Roy and Trudinger 1970; Postgate 1959, 1968).

Both plants and microbes carry out assimilatory reduction of sulfate as a reaction that supplies S for biosynthesis. In most sulfate assimilation, the sulfate is reduced to the sulfide level (S^{2-}) and incorporated into S-containing enzyme co-factors, and amino acids. Sulfate can also be "activated", by adenosine triphosphate, and assimilated directly into organic compounds to form ester

sulfates (Gregory and Robbins 1960; Freney 1967; Postgate 1968; Roy and Trudinger 1970). These compounds often function as structural components, and are produced by microbes as well as higher organisms (Freney 1967; Fitzgerald 1976; Zehnder and Zinder 1980).

Dissimilatory sulfate reduction is carried out by a ubiquitous group of microbes, the sulfate reducing bacteria, which use sulfate as a terminal electron acceptor during the metabolic oxidation of certain organic compounds. In the process, they reduce much more sulfate (to sulfide) than is required for biosynthesis (e.g. Postgate 1959, 1968; Kuznetsov 1970; Roy and Trudinger 1970; Nedwell 1982; Ehrlich 1990). The overall process can be summarized as (eq. 1):

$$2\ CH_2O + SO_4^{2-} + 2\ H^+ \rightarrow H_2S + 2\ CO_2 + 2\ H_2O \qquad (1)$$

Sulfate reducers are obligate anaerobes that are active in environments where anoxic and reducing conditions (-100 to -200 mV; Nedwell 1982; Kuznetsov 1970) exist. These environments include submerged muds and sediments and the anoxic bottom waters of lakes, coastal bays and reservoirs. The activity of these organisms has several important economic and ecological consequences (Postgate 1959, 1968). For example, the hydrogen sulfide produced (1) can cause the anaerobic corrosion of iron and steel, spoilage of fuels,

lubricants and canned foods, as well as massive fish deaths in bodies of water where it periodically accumulates. Hydrogen sulfide can also undergo several different reactions to produce other forms of S compounds. Oxidation of biogenically derived H_2S is considered primarily responsible for major sedimentary deposits of native S (elemental S or S^o; Ivanov 1968; Erlich 1990). At least some pyrite and other iron sulfide deposits are also believed to be of a similar biogenic origin (Ivanov 1983; Ehrlich 1990).

One important ecological consequence of sulfate reduction in lakes is the consumption of H^+ by production of reduced S compounds (eq. 1; Stumm and Morgan 1970; Hongve 1978; Kelly et al. 1982; Rudd et al. 1986; Anderson and Schiff 1987). This process provides lakes with an internal buffering mechanism (ie. alkalinity generation; Cook et al. 1986) against acidification due to acid precipitation. Sedimentary reactions leading to the formation of FeS and pyrite (FeS_2) provide routes through which net H^+ consumption can occur (Kelly et al. 1982; Rudd et al. 1986; Anderson and Schiff 1987). For example, formation of FeS, like that of FeS_2, removes $2H^+$ from the water column for every sulfate reduced (1986; eq. 2):

$$9\ CH_2O + 4\ FeOOH + 4\ SO_4^{2-} + 8\ H^+ \rightarrow 9\ CO_2 + 4\ FeS + 15\ H_2O \qquad (2)$$

The production of organic S (S_O) compounds by assimilatory reduction or by reaction of H_2S with organic matter also consumes H^+ with the same stoichiometry (Rudd et al. 1986; Baker et al. 1989). Formation of ester sulfates, which does not involve sulfate reduction, may consume one H^+ per sulfate molecule incorporated (Rudd et al. 1986; Baker et al. 1989), but this is debatable (Urban and Baker 1989; Kelly and Rudd 1989).

The formation of iron sulfides in sediments from secondary reactions of bacterially produced sulfide with iron, is well known (e.g. Galliher 1933; Cook and Schindler 1983; Berner 1984; Howarth and Merkel 1984; Giblin 1988; Erlich 1990) and was once assumed to be the major end-product of dissimilatory sulfate reduction. The production and occurence of other intermediate forms of inorganic S compounds (S°, thiosulfate, sulfite, polythionates, polysulfides) has also been studied, usually in marine and estuarine systems where their concentrations are high (e.g. Boulegue et al. 1982; Howarth and Jorgensen 1984; Luther et al. 1986a, b).

Early models of the aquatic sulfur cycle incorporated the production of inorganic S (S_I) compounds since these are well studied. However, S_O formation was generally ignored in these models (e.g. Galliher 1933). When sedimentary S_O was incorporated into S cycle models it was generally assumed to originate from sedimentation of seston, and

served as a source of H_2S due to the activity of putrefying bacteria (e.g. King and Klug 1980; David and Mitchell 1985).

This emphasis on inorganic S was probably due, at least in part, to the large body of work on iron-sulfur interactions in marine sediments (e.g. Berner 1970; 1982; 1984) where the reduced S pool is dominated by pyrite (90%), S_0 being only about 10% of the pool (Ivanov 1983; Ostroumov 1953). In lake sediments, however, S_0 is an important constituent making up 60-80% of total S, which has been measured as 0.05-0.9% on a dry weight basis (David and Mitchell 1985, Landers and Mitchell 1988, Losher and Kelts 1989). The origin of this S_0 in sediments is not completely clear. A portion of this pool must clearly originate from sedimenting biota and from organic matter washed in from the watershed (David and Mitchell 1985). Recently, however, it was found that S_0 is produced *in situ* and was the most important product of sulfate reduction in sediments of several freshwater lakes (Nriagu and Soon 1985; Rudd et al. 1986). As much as 30% of sulfate supplied to the sediment has been estimated to be stored annually by this process (Rudd et al. 1986). Several workers have hypothesized that S_0 compounds in sediments can, like iron sulfides, be produced from secondary reactions of biogenic H_2S with organic matter in sediments (Nriagu and Soon 1985; Rudd et al. 1986a; Francois 1987b). The exact mechanism of S_0 formation in lake sediments, as well as the nature of these

compounds, needs to be understood to better elucidate the aquatic S cycle and to better assess the ability of lakes to store reduced S and generate net internal alkalinity.

1.1 The determination of organic sulfur in sediments

One reason that little is known about the nature and identity of sedimentary S_O is the lack of adequate methods of analysis. Current methodology is based on reactivity with certain chemical reagents (e.g. Landers et al. 1985; Wieder et al. 1985). Consequently, since many S compounds share similar chemical characteristics, these compounds are measured as a group and are only operationally defined, rather than positively identified.

The largest grouping of organic S compounds is the simple differentiation of sedimentary S into S_I and S_O forms. As described above, S_I in sediments and peat includes such compounds as sulfate, polythionates, polysulfides, elemental S, and iron sulfides (FeS, FeS_2, Fe_3S_4). Total reduced S_I (i.e. non-sulfate) is determined by a chromium reduction method (Zhabina and Volkov 1978) that, based on analysis of various S-containing compounds, is considered to be specific for S_I (eg. Zhabina and Volkov 1978; Wieder et al. 1985; Canfield et al. 1986; Cutter and Oatts 1987). However, this method has never been fully tested because the true composition of sedimentary S is

largely unknown. One report has shown that this method may also measure a part of the S_o in peat samples (Brown 1986). No direct and specific method is presently available for S_o, so that this fraction is assumed to be all of the remaining S after chromium reducible S (CRS) and sulfate are determined (Zhabina and Volkov 1978). Therefore, accurate measurements of both total S and S_I are required for accurate estimates of S_o.

Sedimentary S_o is often separated into two pools, carbon-bonded S (C-S) and sulfate esters (C-O-S), based on the type of linkage between the C and S atoms. Sulfate esters are operationally defined as those organic compounds which are reducible by hydriodic acid (Freney 1961; Landers and Mitchell 1983) or that yield sulfate with acidification (King and Klug 1980). The remaining S_o is considered to be C-S. In some cases, the C-S fraction is divided into two further pools, based on susceptibility to desulfuration by Raney nickle (Bonner and Grimm 1966). It is believed that Raney nickle reduction measures all amino acid S, while the C-S resistant to this reagent is thought to be the very stable sulfonate compounds (Fitzgerald 1976). However, all of these analyses still give only an operationally defined sulfur pool, since cross reactivity between the different forms of S makes it difficult to measure them accurately and specifically when present in a complex mixture such as is found in sediments. Knowledge of the identity of the

compounds in this sulfur pool would be usefull in devising methods for its analysis.

1.2 Characterization and identity of some organic sulfur compounds in sediments

Relatively few attempts have been made to identify S_o compounds in sediments, although some work has been done on marine and marsh pore waters. Organic sulfhydryl compounds or thiols (3-mercaptopropionate, methanethiol (MSH), glutathione, cysteine) have been detected or inferred by chromatographic and polarographic methods at the micro and millimolar level (Boulegue et al. 1982; Luther et al. 1986a, b; Mopper and Taylor 1986; Kiene and Taylor 1988). Richards et al. (1991, and pers. comm.) have measured nanomolar quantities of volatile sulfur compounds (MSH, dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and carbonyl sulfide (COS)) in pore-water and overlying water of freshwater lakes. However, these soluble components make up only a small fraction of the total S_o in sediments, much of which is incorporated into humic compounds (e.g. Casagrande et al. 1979; Brown 1986; Francois 1987a, b; Ferdelman et al. 1991). Consequently, the actual identities of the majority of S_o compounds in the sediment are unknown. Since a wide variety of compounds having different chemical

characteristics are possible, their analysis is difficult.

Sulfoxides, isoprenoid thiophenes and thiolanes (Brassel et al. 1986; Kohnen et al. 1990) and cyclic di- and tri-sulfides (Kohnen et al. 1989) have been found in the hydrocarbon fraction of ancient and recent marine sediments and identified by gas chromatography-mass spectrometry. Sinninghe Damste et al. (1987, 1989a, b), after studying S_o in oils, shales and marine kerogen and sediments also described hundreds of similar compounds.

Identification or characterization of organic sulfur present in forms not amenable to GC-analysis (e.g. humic S), is more difficult. Ferdelman et al. (1991), using X-ray photoelectron spectroscopy to detect S functional groups, showed that humic sulfur of salt marsh sediments consists of organic sulfides and polysulfides as well as sulfoxides and sulfones. Marine humic acid S content has also been studied (Nissenbaum and Kaplan 1972; Francois 1987a, b). Studies of the solid phase S_o fraction of freshwater sediments however, have generally been restricted to "bulk" analysis.

1.3 Reactions leading to S_o formation

Clearly, one pathway of production of S_o in sediments is via the assimilatory pathway by biota. However, addition of S to organic matter by chemical reactions is also possible. The idea that organosulfur compounds may form in

nature from abiotic reactions between H_2S and organic compounds is not new. For example, Shorey (1913) hypothesized that trithiobenzaldehyde $(SCHC_6H_5)_3$ isolated from soil arose from a reaction of H_2S with benzaldehyde from the decomposition of lignin. Sinninghe Damste et al. (1987; 1989a, b) also concluded that the organic S in oils and ancient organic matter was incorporated in early diagenesis in organic-rich sediments by reaction of dissolved sulfide with low molecular weight organic compounds. Supporting evidence for this conclusion was the fact that organic S compounds had carbon skeletons the same as well known hydrocarbons from geological materials. Sulfurization of small hydrocarbon compounds accelerates the formation of large molecular weight compounds such as are present in oil (e.g. Zobell 1963).

Studies measuring isotope fractionation have provided support for this pathway of S_0 formation in both marine and freshwater systems. Sulfate reduction by bacteria preferentially selects the lighter isotope (^{32}S), so that the resulting H_2S has a higher $^{32}S/^{34}S$ ratio than the starting sulfate (see Brock et al. 1984). Furthermore, dissimilatory sulfate reduction shows a much larger isotopic discrimination than assimilatory reduction (Brock et al. 1982). Therefore this type of isotopic discrimination can be used to determine the origin of S in a particular compound. Several studies have shown that S in S_0 of both

marine and freshwater sources is isotopically light, indicating the S_0 originated from incorporation of H_2S, from dissimilatory sulfate reduction, into organic compounds (Kaplan et al. 1963; Nissenbaum and Kaplan 1971; Aizenshtat et al. 1981; Nriagu and Soon 1985; Francois 1987a). This pathway of S_0 formation has also been suggested as the most likely explanation for the observation that humic acids in salt marsh and marine sediments become S enriched with depth, since the S:C ratio of humic acids was higher than that of organic matter delivered to the sediment (Ferdelman et al. 1991; Francois 1987a).

Organic chemical evidence also suggests that possible pathways exist for formation of S_0 in sediments and fossil fuels. The abiotic formation of organosulfur has been observed by the addition of H_2S and S° to different organic materials, such as carbohydrates, alkanes, and carbonyl compounds (e.g. Campaigne 1961; Pryor 1962; Mango 1983). Martin and Hodgson (1973, 1977) observed the formation of a complex mixture of organosulfur compounds, including thiophenes, thionaphthenes, phenyl-substituted thiazines and thiatidenes, such as occur in crude petroleum, from the reaction of elemental S with benzylamine and the amino acid phenylalanine at geochemically plausible temperatures (135°C-190°C). Similarly, organosulfur compounds formed from the reaction of elemental S (at 130°C) with ethylbenzene, a mixed hydrocarbon/aromatic compound (De Roo and Hodgson

1978).

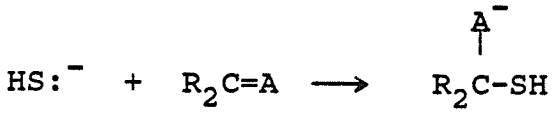
Evidence that similar pathways of S_0 formation occur under natural or near natural conditions has also been obtained. Experiments with peat and humic compounds from peat also showed that addition of $H_2{}^{35}S$ resulted in the incorporation of ^{35}S into this material (Casagrande et al. 1979, Brown 1986). Similarly, S° can also be involved in the abiotic formation of organosulfur. Casagrande and Ng (1979) found that refluxing $^{35}S^\circ$ at low temperature (boiling chloroform) with humic compounds extracted from peat resulted in the incorporation of ^{35}S into humic and fulvic acid compounds. Similarly, incubation of lake sediments and peat with radiolabelled ^{35}S-sulfate have shown that organic-^{35}S makes up a large percentage (often >50%) of the initial endproducts of sulfate reduction (Landers et al. 1983, Rudd et al. 1986a, Brown 1986, Landers and Mitchell 1988, Baker et al. 1989).

1.4 Mechanisms and control of formation

The formation of S_0 in sediments by the reaction of reduced inorganic S compounds with organic matter can occur via several possible mechanisms (Fig. 1.1) (e.g. Boulegue et al. 1982; Luther et al. 1986; Francois 1987a, b). Hydrogen sulfide, as the bisulfide ion (HS^-), may be incorporated into organic matter by nucleophilic substitution with labile

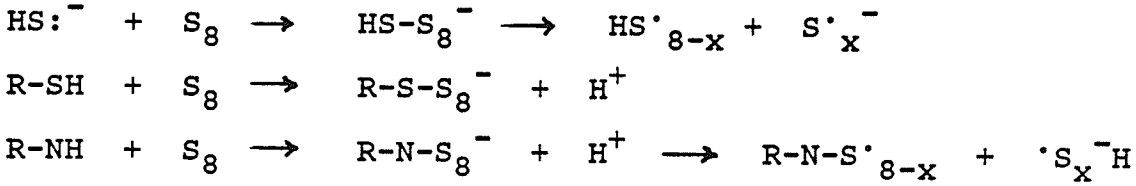
Fig. 1.1. Possible mechanisms for the incorporation of sulfur into organic matter. Where, R and R′ are organic carbon groups, A is an electron withdrawing group, Z is a leaving group (organic or inorganic), and "=" refers to a double bond. Modified from Francois (1987b).

Nucleophilic addition:

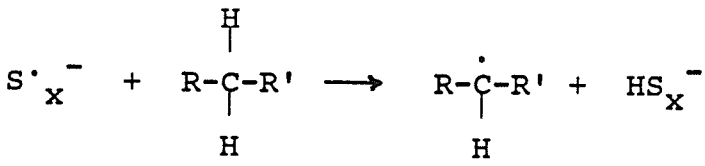


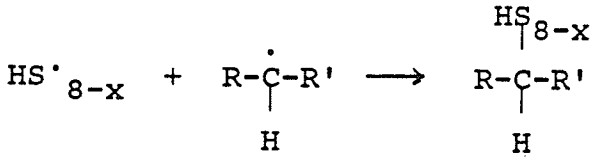
Nucleophilic substitution:

Nucleophilic attack on S^o:



Reactions with S radicals:





groups (alcohol, methoxy and halide; Luther et al. 1986; Schwarzenbach et al. 1985). Similarly, HS^- can react with organic matter via nucleophilic addition across a double bond (Luther et al. 1986; Francois 1987a, b). For example, the formation of of 3-mercaptopropionic acid occurs, under conditions found in marine systems, from a reaction of HS^- and the activated double bond of acrylic acid (Vairavamurthy and Mopper 1987; eq. 3):

$$HS^- + CH_2{=}CHCOO^- \rightarrow HS{-}CH_2CH_2COO^- + OH^- \qquad (3)$$

Similar experiments showed the formation of the corresponding organosulfur compounds by reacting H_2S with other compounds containing an activated double bond, analogous to acrylic acid, that is, crotonic, methacrylic, fumaric and maleic acids (Vairavamurthy and Mopper 1987). These types of reactions result in the formation of thiol compounds (RSH), one of the forms of S_o generally described as carbon-bonded S (C-S)(Fig. 1.1).

Other forms of S_I can also react abiotically with organic matter to produce organosulfur compounds. Polysulfides (HS_x^-, or S_x^{2-}), for example, undergo the same types of reactions as the HS^- ion to form organic polysulfides (Fig. 1.1). Tetrasulfide has been shown to react with acrylic acid to form 3-tetrasulfidopropionic acid (Vairavamurthy and Mopper 1989; eq. 4):

$$^{-}SSSS^{-} + CH_2{=}CHCOO^{-} + H_2O \rightarrow {}^{-}SSSS{-}CH_2CH_2COO^{-} + OH^{-} \quad (4)$$

Reactions where the nucleophile is a polysulfide occur more rapidly than with HS^- as the nucleophile (Vairavamurthy and Mopper 1989; Francois 1987). The higher reactivity of S_n^{2-} has been explained by molecular orbital considerations (Vairavamurthy and Mopper 1989). Nucleophilic addition reactions (Fig. 1.1) also occur more rapidly when the unsaturated carbon bond is adjacent to electron withdrawing groups (e.g. -COOH, -CHO, -CN) which can make the unsaturated carbon more electronegative and susceptible to nucleophilic attack. Consequently, conditions that affect the electrophilicity of the unsaturated center by influencing the polarity and ionization of the adjacent group, such as pH and salinity, will also affect the reaction rate (Vairavamurthy and Mopper 1989).

Elemental S in its cyclical form (S_8) has been found to be unreactive towards some organic compounds (Mango 1983, Francois 1987a). A ring opening reaction is required to make the molecule more reactive. The cyclical S_8 molecule can be opened by several nucleophilic groups, including OH^-, H_2S, RSH, CN^- and amine groups, to form reactive polysulfide(s) (Pryor 1962; Roy and Trudinger 1970; Francois 1987a,b; Senning 1972). The presence of these types of ring opening groups could lead directly to the formation of organic polysulfides, or to inorganic polysulfides that

could then react with organic matter to form organic polysulfides (Fig. 1.1).

Polysulfide molecules of small chain lengths (3-5 atoms) are more stable than longer chain polysulfides so that the latter tend to hydrolyse to more stable forms. Polysulfides can also undergo homolytic cleavage to form highly reactive radicals (Pryor 1962; Francois 1987a, b) which may then react with organic matter to form organic polysulfides and thiols (Fig. 1.1; Francois 1987a, b).

Luther and co-workers (1986) have also proposed a likely route for the formation of organosulfur in a salt marsh sediment as occurring via the reaction of thiosulfate with organic matter by a nucleophilic addition mechanism (Eq. 5; Roy and Trudinger, 1970):

$$ROH + S_2O_3^{2-} \rightarrow RS^- + HSO_4^{1-} \qquad (5)$$

Thiosulfate is present in very low levels in marine sediments and freshwater sediment although it has been suggested to be a rapidly utilized intermediate in the S cycle of these environments (Jorgensen 1990) and so may react with organics before being reduced to sulfide. Thiosulfate could also arise from the partial oxidation of H_2S, organic sulfides or sulfide minerals such as pyrite (Luther et al. 1986a). It has also been proposed that pyrite can be used directly for chemosynthesis by salt marsh

organisms during its enzymatic oxidation, and the incomplete reaction is given by Luther et al. (1986) as:

$$FeS_2 \rightarrow RSH + SO_4^{2-} + Fe^{2+} \quad (6)$$

The parameters causing the production of the different forms of reduced S_I that may react with organic matter will determine which of the above reactions are more important in a particular environment. For example, conditions encountered at redox boundaries in sediments can cause the production of polysulfides and so increase the potential for these types of reactions. For example, the partial oxidation of H_2S by oxygen or Fe^{3+} can produce polysulfides (Chen and Morris 1972; Chen and Gupta 1973). Most of the information on these reactions in nature has been obtained for marine or estuarine systems due to the large sulfate content. Information on these processes in freshwater lakes is small, in part due to the lower concentration of these compounds in the sediment.

Formation of organic ^{35}S varies greatly between (Rudd et al. 1986) and within lakes (Kelly and Rudd, unpub. data). The factors controlling the relative amounts of organic and inorganic S formed from reaction of H_2S and sediment components are not all clear. Iron concentration is expected to affect the distribution of reduced S into these two fractions, since H_2S can react with Fe to form iron

sulfide. The quality and quantity of organic C may also be important in determining organic S formation (Cook and Kelly in press). Baker et al. (1989) have shown that additions of Fe^{2+} to sediment decreased the proportion of organic ^{35}S produced from the reduction of $^{35}SO_4^{2-}$. Low pH has been shown to have the same effect (Baker et al 1989). However, no clear trends are observed by examination of data from a variety of lakes (Rudd et al. 1986a; Giblin et al. 1990; Cook and Kelly in press). Thus, while it is established that reduced S, from sulfate reduction and/or other origins, is incorporated into S_0 compounds, the identities of these compounds and factors controlling their formation are largely unknown.

1.5 Objectives of this study

The general purpose of this work was to study the formation of S_0 from sulfate reduction in lake sediments. An ancillary purpose was to study changes in the S cylce of an experimentally acidified lake. Within this context, there were three main ojectives;

(i) to critically examine the reliability of current methodology for measuring S_0, and to determine the best available method of analysis.

(ii) to isolate from the sediment matrix and to

characterize the S_0 fraction that is formed from sulfate reduction.

(iii) to investigate how the S cycle of a lake may be affected by metaphytic algal mats that proliferate under very low pH conditions (<5).

Sediments used in this research were obtained from several Canadian lakes at the Experimental Lakes Area, in northwestern Ontario, and from two naturally acidified Norwegian lakes. Experimentally acidified, Lake 302 South, at the ELA, was used to monitor the effects of metaphytic algae on the aquatic S cycle. Radiolabelled sulfate ($^{35}SO_4^{2-}$) was used to detect the endproducts of sulfate reduction and to distinguish them from reduced S compounds already present in the sediments.

1.6 References

Aizenshtat, Z., A. Stoler, Y. Cohen, and H. Nielsen. 1981. The geochemical sulphur enrichment of recent organic matter by polysulfides in the Solar-Lake, p. 279-288. In M. Bjory [ed.], Advances in Organic Geochemistry. John Wiley and Sons.

Anderson, R.F., and S.L. Schiff. 1987. Alkalinity generation and the fate of sulfur in lake sediments. Can. J. Fish. Aquat. Sci. 44: 188-193.

Baker, L.A., N.R. Urban, P.L. Brezonik, and L.A. Sherman. 1989. Sulfur cycling in an experimentally acidified seepage lake, p. 79-100. In E. Saltzman and W.J. Cooper [eds.], Biogenic sulfur in the environment. ACS 393.

Boulegue, J., C.J. Lord, and T.M. Church. 1982. Sulfur speciation and associated trace metals (Fe, Cu) in the pore waters of Great Marsh, Delaware. Geochim. Cosmochim. Acta 46: 453-464.

Berner, R.A. 1984. Sedimentary pyrite formation: An update. Geochim. Cosmochim. Acta 48: 605-615.

Berner, R. A. 1982. Burial of organic carbon and pyrite sulfur in the modern ocean: its geochemical and environmental significance. Amer. J. Sci. 282: 451-473.

Berner, R. A. 1970. Sedimentary pyrite formation. Amer. J. Sci. 268: 1-23.

Bonner, W.A. and R.A. Grimm. 1966. Mechanisms of Raney nickle desulfuration, p. 35-71. In N. Kharasch and C.Y. Meyers [eds.], The chemistry of organic sulfur compounds. Pergamon Press.

Brassell, S.C., C.A. Lewis, J.W. de Leeuw, F. de Lange and J.S. Sinninghe Damste. 1986. Isoprenoid thiophenes: novel products of sediment diagenesis. Nature 320: 160-162.

Brock, T.D., D.W. Smith, and M.T. Madigan. 1984. Biology of microorganisms, 4th ed. Prentice-Hall, Inc.

Brown, K.A. 1986. Formation of organic sulphur in anaerobic peat. Soil Biol. Biochem. 18: 131-140.

Campaigne, E. 1961. Addition of thiols or hydrogen sulfide to carbonyl compounds, p. 134-144. In N. Kharasch [ed.], Organic sulfur compounds, Vol. 1. 134-144. Pergammon Press.

Canfield, D.E., R. Raiswell, J.T. Westrich, C.M. Reaves, and R.A. Berner. 1986. The use of chromium reduction in the analysis of reduced inorganic sulfur in sediments and shales. Chem. Geol. 54: 149-155.

Casagrande, D.J., G. Idowu, A. Friedman, P. Rickert, K. Siefert, and D. Schlenz. 1979. H_2S incorporation in coal precursors: origin of organic sulfur in coal. Nature 282: 599-600.

Casagrande, D.J., and L. Ng. 1979. Incorporation of elemental sulfur in coal as organic sulfur. Nature

282: 598-599.

Chen, K.Y., and S.K. Gupta. 1973. Formation of polysulfides in aqueous solution. Environ. Lett. 4: 187-200.

Chen, K.Y., and J.C. Morris. 1972. Kinetics of oxidation of aqueous sulfide by O_2. Environ. Sci. Technol. 6: 529-537.

Cutter, G.A., and T.J. Oatts. 1987. Determination of dissolved sulfide and sedimentary sulfur speciation using gas chromatography-photoionization detection. Anal. Chem. 59: 717-721.

David, M.B., and M.J. Mitchell. 1985. Sulfur constituents and cycling in water, seston, and sediments of an oligotrophic lake. Limnol. Oceanogr. 30: 1196-1207.

De Roo, J., and G.W. Hodgson. 1978. Geochemical origin of organic sulfur compounds: thiophene derivatives from ethylbenzene and sulfur. Chem. Geol. 22: 71-78.

Ehrlich, H.L. 1990. Geomicrobiology. 2nd Ed. Marcel Dekker, Inc. New York.

Feely, H.W., and J.L. Kulp. 1957. Origin of Gulf Coast salt-dome sulphur deposits. Amer. Assoc. Petr. Geol. Bull. 41: 1802-1853.

Ferdelman, T.G., T.M. Church, and G.W. Luther III. 1991. Sulfur enrichment of humic substances in a Delaware salt marsh sediment core. Geochim. Cosmochim. Acta 55: 979-988.

Fitzgerald, J.W. 1976. Sulfate ester formation and

hydrolysis: a potentially importabnt yet often ignored aspect of the sulfur cycle of aerobic soils. Bacteriol. Rev. 40: 698-721.

Francois, R. 1987a. A study of sulphur enrichment in the humic fraction of marine sediments during early diagenesis. Geochim. Cosmochim. Acta 51: 17-27.

Francois, R. 1987b. A study of the extraction conditions of sedimentary humic acids to estimate their true *in situ* sulfur content. Limnol. Oceanogr. 34: 964-972.

Freney, J.R. 1961. Some observations on the nature of organic sulfur compounds in soil. Aust. J. Agric. Res. 12: 424-432.

Freney, J.R. 1967. Sulfur-containing organics, p. 229-259. In McLaren and Peterson [eds.], Soil Biochemistry, Vol. 1. Marcel Dekker Inc.

Froelich, P.N., G.P. Klinkhammer, M.L. Bender, N.A. Luedtke, G.R. Heath, D. Cullen, P. Dauphin, D. Hammond, B. Hartman, and V. Maynard. 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: Suboxic diagenesis. Geochim. Cosmochim. Acta 43: 1075-1090.

Galliher, E.W. 1933. The sulfur cycle in sediments. J. Sed. Petrology 3: 51-63.

Giblin, A.E. 1988. Pyrite formation in marshes during early diagenesis. Geomicrobiol. J. 6: 77-97.

Gregory, J. D., and P. W. Robbins. 1960. Metabolism of

sulfur compounds (sulfate metabolism). Ann. Rev. Biochem. 29: 347-364.

Howarth, R.W., and B.B. Jorgensen. 1984. Formation of ^{35}S-labelled elemental sulfur and pyrite in coastal marine sediments (Limfjorden and Kysing Fjord, Denmark) during short-term $^{35}SO_4^{2-}$ reduction measurements. Geochim. Cosmochim. Acta 48: 1807-1818.

Ivanov, M.V. 1968. Microbiological processes in the formation of sulfur deposits. Israel program for scientific translations. U.S. Department of Agriculture and National Science Foundation, Washington, D.C.

Ivanov, M.V. 1983. Major fluxes of global biogeochemical cycle of sulphur, p. 449-463. In M.V. Ivanov and J.R. Freney [ed.], The global biogeochemical cycle. SCOPE 19.

Jorgensen, B.B. 1990. A thiosulfate shunt in the sulfur cycle of marine sediments. Science 249: 152-154.

Kaplan, I.R., K.O. Emery, and S.C. Rittenberg. 1963. The distribution and isotopic abundance of sulphur in recent marine sediments off southern California. Geochim. Cosmochim. Acta 27: 297-331.

Kelly, C.A., and J. W. M. Rudd. 1989. Reply to comment by Urban and Baker. Limnol. Oceanogr. 34: 1146-1148.

Kelly, C.A., R.B. Cook, and D.W. Schindler. 1982. The potential importance of bacterial processes in

regulating the rate of lake acidification. Limnol. Oceanogr. 27: 868-882.

Kiene, R.P., and B.F. Taylor. 1988. Biotransformations of organosulfur compounds in sediments via 3-mercaptopropionate. Nature 332: 148-150.

King, G.M. and M.J. Klug. 1980. Sulfhydrolase activity in sediments of Wintergreen Lake, Kalamazoo County, Michigan. Appl. Environ. Microbiol. 39: 950-956.

Kohnen, M.E.L., J.S. Sinninghe Damste, H.L. ten Haven, and J.W. de-Leeuw. 1989. Early incorporation of polysulphides in sedimentary organic matter. Nature 341: 640-641.

Kohnen, M.E.L., J.S. Sinninghe Damste, A.C. Kock-van Dalen, H.L. ten Haven, J. Rullkotter, and J.W. de Leeuw. 1990. Origin and diagenetic transformations of C_{25} and C_{30} highly branched isoprenoid sulphur compounds: Further evidence for the formation of organically bound sulphur during early diagenesis. Geochim. Cosmochim. Acta 54: 3053-3063.

Kuznetsov, S.I. 1970. The microflora of lakes and its geochemical activity. [C.H. Oppenheimer, ed.]. University of Texas Press.

Landers, D.H., M.B. David, and M.J. Mitchell. 1983. Analysis of organic and inorganic sulfur constituents in sediments, soils and water. Intern. J. Environ. Anal. Chem. 14: 245-256.

Landers, D.H., and M.J. Mitchell. 1988. Incorporation of $^{35}SO_4^{2-}$ into sediments of three New York lakes. Hydrobiologia 160: 85-95.

Losher, A.J., and K.R. Kelts. 1989. Organic sulphur fixation in freshwater lake sediments and the implication of C/S ratios. Terra Nova 1: 253-261.

Luther, G.W., T.M. Church, A.E. Giblin, and R.W. Howarth. 1986. Speciation of dissolved sulfur in salt marshes by polarographic methods, p. 340-355. In M.L. Sohn [ed.], Organic Marine Geochemistry. ACS 305.

Luther, G.W., T.M. Church, J.R. Scudlark, and M. Cosman. 1986. Inorganic and organic sulfur cycling in salt-marsh pore waters. Science 232: 746-749.

Mango, F.D. 1983. The diagenesis of carbohydrates by hydrogen sulfide. Geochim. Cosmochim. Acta 47: 1433-1441.

Martin, T.H., and G.W. Hodgson. 1973. Geochemical origin of organic sulfur compounds: reaction of phenylalanine with elemental sulfur. Chem Geol. 12: 189-208.

Martin, T.H., and G.W. Hodgson. 1978. Geochemical origin of organic sulfur compounds: precursor products in the reactions of phenylalanine and benzylamine with elemental sulfur. Chem. Geol. 20: 9-25.

Mopper, K., and B.F. Taylor. 1986. Biogeochemical cycling of sulfur, p. 324-339. In M.L. Sohn [ed.], Organic Marine geochemistry. ACS 305.

Nedwell, D.B. 1982. The cycling of sulphur in marine and freshwater sediments, p. 73-106. In D.B. Nedwell and C.M. Brown [eds.], Sediment microbiology. Academic.

Nissenbaum, A., and I.R. Kaplan. 1972. Chemical and isotopic evidence for the *in situ* origin of marine humic substances. Limnol. Oceanogr. 17: 570-582.

Nriagu, J.O., and Y.K. Soon. 1985. Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. Geochim. Cosmochim. Acta 49: 823-834.

Postgate, J. 1959. Sulphate reduction by bacteria. Ann. Rev. Microbiol. 13: 505-520.

Pryor, W.A. 1962. Mechanisms of sulfur reactions. McGraw-Hill, New York.

Richards, S.R., C.A. Kelly, and J.W.M. Rudd. (1991). Organic volatile sulfur in lakes of the Canadian Shield and its loss to the atmosphere. Limnol. Oceanogr. 36: 468-482.

Roy, A.B., and P.A. Trudinger. 1970. The biochemistry of inorganic compounds of sulfur. Cambridge University Press.

Rudd, J.W.M., C.A. Kelly, and A. Furutani. 1986. The role of sulfate reduction in long-term accumulation of organic and inorganic sulfur in lake sediments. Limnol. Oceanogr. 31: 1281-1291.

Schwarzenbach, R.P., W. Giger, C. Schaffner, and O. Wanner.

1985. Groundwater contamination by volatile halogenated alkanes: abiotic formation of volatile sulfur compounds under anaerobic conditions. Environ. Sci. Technol. 19: 322-327.

Sinninghe Damste, J.S., J.W. de Leeuw, A.C. Kock-van Dalen, M.A. de Zeeuw, F. de Lange, W. Irene C. Rijpstra, and P.A. Schenck. 1987. The occurance and identification of series of organic sulphur compounds in oils and sediment extracts. I. A study of Rozel Point oil (U.S.A.). Geochem. Cosmochim. Acta 51: 2369-2391.

Sinninghe Damste, J.S., T.I. Eglinton, J.W. de Leeuw, and P.A. Schenck. 1989a. Organic sulphur in macromolecular sedimentary organic matter: I. Structure and origin of sulphur-containing moieties in kerogen, asphaltenes and coal as revealed by flash pyrolisis. Geochim. Cosmochim. Acta 53: 873-889.

Sinninghe Damste, J.S., W.I. Rijpstra, A.C. Kock-van Dalen, J.W. de Leeuw, and P.A. Schenck. 1989b. Quenching of labile functinalised lipids by inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis. Geochim. Cosmochim. Acta 53: 1343-1355.

Stumm, W., and J.J. Morgan. 1970. Aquatic Chemistry. An introduction emphasizing chemical equilibria in natural waters. John Wiley and Sons, Inc.

Urban, N.R., and L.A. Baker. 1989. Alkalinity generation

associated with the formation of ester sulfates. Limnol. Oceanogr. 34: 1144-1146.

Vairavamurthy, A and K. Mopper. 1987. Geochemical formation of organosulphur compounds (thiols) by addition of H_2S to sedimentary organic matter. Nature 329: 623-625.

Vairavamurthy, A and K. Mopper. 1989. Mechanistic studies of organosulfur (thiol) formation in coastal marine sediments, p. 231-241. In E.S. Saltzman and W.J. Cooper [eds.], Biogenic sulfur in the environment. ACS 393.

Wieder, R.K., G.E. Lang, and V.A. Granus. 1985. An evaluation of wet chemical methods for quantifying sulfur fractions in freshwater wetland peat. Limnol. Oceanogr. 30: 1109-1114.

Zehnder, A.J.B., and S.H. Zinder. 1980. The sulfur cycle, p. 105-145. In O. Hutzinger [ed.], The handbook of environmental chemistry. V. 1A, The natural environment and biogeochemical cycles. Springer-Verlag.

Zhabina, N.N. and I.I. Volkov. 1978. A method of determination of various sulfur compounds in sea sediments and rocks, p. 735-745. In W.E. Krumbein [ed.], Environmental biogeochemistry and geomicrobiology , V. 3. Methods materials and assessment. Ann Arbor Science.

Zobell, O.E. 1963. Organic geochemistry of sulfur, p. 543-578. In I.A. Breger [ed.], Organic Geochemistry. Pergamon Press.

2. Loss of total sulfur and changes in sulfur isotopic ratios due to drying of lacustrine sediments

2.0 Acknowledgements.

This work was supported by NSERC grant no. A2671 and by the Department of Fisheries and Oceans, Canada. We thank D. W. Schindler, G. Brunskill, G. Koshinsky and C. Anema for providing criticism of this manuscript. We are also grateful to C. A. Kelly for advice and help in the experimental work as well as in manuscript preparation, and to S. Kasian for suggestions with regard to statistical analyses.

2.1 Abstract

Two independent methods for measuring total sulfur were used to show that underestimates of sulfur content of lacustrine sediments can occur when sediments are dried before total sulfur analysis. Different types of sediments were oven-dried at 60°C or 100°C or lyophilized to assess the effect of the drying method on the amount of sulfur lost. Losses from 0-86% were observed. Common losses were 6-22% and dependent on the sample and drying method used. Lyophilization caused greater sulfur losses (1.5-fold) than the two oven-drying methods. These sulfur losses caused changes in the sulfur isotopic content of the sediments, and could underestimate rates of sulfur burial in sediments, organic-S formation in sediments, and internal alkalinity production in lakes.

2.2 Body of note

Current methods for the estimation of total sulfur in sediments usually involve a preliminary drying step. Little attention has been paid to the possibility that certain volatile sulfur compounds may be lost during this treatment. Some studies have concluded that no loss of reduced inorganic sulfur species occurs from different types of sediments under various drying conditions (Canfield et al. 1986; Carignan and Tessier 1988; Morse and Cornwell 1987). Others have found that drying of peat and soil samples causes no loss of organic sulfur (Wieder et al. 1985, David et al. 1982). In our experience, oven-drying of lake sediments results in the loss of total sulfur. It is our purpose to caution those studying sulfur accumulation in sediments that drying sediments before analysis leads to underestimates of the total sulfur content. This bias will affect the measurement of stable isotope content and estimates of sulfur burial in lake sediments, both of which require a quantitative knowledge of total sulfur concentration. Sediment cores were obtained from Lake 302S at the Experimental Lakes Area (ELA) in northwestern Ontario. Lake 302S has been artificially acidified with sulfuric acid since 1982 (Rudd et al., submitted). At the time of sampling, the pH of the surface water was 4.9. Cores A, B and F were taken from a depth of 10 m. At this

site, the sediment is high in organic content and has a porosity in excess of 0.98 (Table 2.1). Core C was obtained from a site 3 m deep with sandy sediment of much lower porosity.

Cores were also taken from Lake 239 at ELA. This is an unacidified lake that typically has an epilimnetic pH near 7.0. Stable isotopic analyses were done on sediment from both lakes (Table 2.2) and included cores from 1 and 4 m sites in Lake 302S. All shallow cores were taken by diver. The 10 m cores were obtained with a KB gravity corer (Wildco).

Sediment was extruded from the plexiglass core tube at 1 cm intervals and was sliced with a thin metal plate. Sulfur was analyzed on both wet and dry subsamples. The wet subsamples were analyzed immediately, while the other subsamples were analyzed as soon as drying was complete. Three methods of drying were tested: hot-air drying at 60°C and at 100°C, and lyophilization after freezing at -65°C (Table 2.1). The duration was, in all cases, sufficient to dry the sediment samples to a constant weight. The sulfur content of wet sediment is presented on a dry-weight basis. It was calculated by dividing the sulfur content of wet sediment by the ratio of dried to wet sediment weights.

The total sulfur content of wet and dry sediment samples was determined for cores A, C, E and F by a modification of the alkaline oxidation method of Tabatabai

Table 2.1. Properties and analyses of the sediments studied.

	Sediment type					Methods	
Core	Porosity*	LOI+ (%)	Date	Lake	Depth (m)	S analysis	Drying≠
A	0.983-0.986	--	Au 87	302S	10	NaOBr/HI	60°C
B	0.981-0.993	36.2-37.7	Au 87	302S	10	HNO_3/dry ash	lyoph.
C§	0.524-0.595	--	Au 87	302S	3	NaOBr/HI	60°C
D	0.924-0.955	29.5-35.9	Se 86	239	2	HNO_3/dry ash	lyoph.
E	0.834-0.916	--	Se 87	239	3	NaOBr/HI	60°C,100°C
F	0.982-0.988	--	Oc 87	302S	10	NaOBr/HI	60°C,100°C
G	0.911-0.928	11.6-16.1	Ma 87	302S	4	HNO_3/dry ash	lyoph.
H	0.927-0.946	17.4-16.2	Ma 87	302S	4	HNO_3/dry ash	lyoph.
I	0.483-0.557	1.6-2.5	Ma 87	302S	1	HNO_3/dry ash	lyoph.
J	0.506-0.583	2.1-2.5	Ma 87	302S	1	HNO_3/dry ash	lyoph.

* Calculated with 2.6 g ml^{-1} as the average density of dried sediment. The range of values for the top 4 or 6 cm of each core is given.

+ Loss-on-ignition; organic-C content is 47% of this value (Hesslein et al. unpubl. data).

≠ Oven dried at 60°C or 100°C or lyophilized.

§ LOI values were not determined but are expected to be similar to cores I and J.

Table 2.2. Changes in the sulfur isotopic composition in sediments before and after lyophilization. For sediments with very low sulfur content (cores I and J), recovery of sulfur is more difficult and isotopic analyses tend to be more variable than the typical 0.3 °/₀₀.

Core	Depth (cm)	LOI (g g^{-1})	Sulfur (mg g^{-1})			$\sigma^{34}S$ (°/₀₀)		
			Dry	Wet	Dry/wet	Dry	Wet	Diff
B	0-1	0.377	5.82	7.47	0.78	7.06	8.37	-1.31
	1-2	0.371	5.98	8.06	0.74	6.45	7.56	-1.11
	2-3	0.369	5.68	9.79	0.58	6.26	7.89	-1.63
	3-4	0.373	6.21	7.94	0.78	7.03	7.86	-0.83
	4-5	0.372	7.19	7.21	1.00	6.85	7.59	-0.74
	5-6	0.362	6.58	8.56	0.77	6.25	6.57	-0.32
	10-15	0.392	5.82	9.06	0.64	4.55	4.59	-0.04
	25-30	0.387	3.78	7.37	0.51	3.49	3.81	-0.32
D	0-1	0.313	1.45	5.86	0.25	2.20	2.52	-0.32
	2-3	0.330	1.13	2.98	0.38	2.22	2.63	-0.41
	3-4	0.359	1.22	1.36	0.90	2.25	2.09	0.16
G	0-1	0.148	1.12	2.08	0.54	2.94	2.57	0.37
	1-2	0.154	1.13	1.74	0.65	3.63	2.92	0.71
	2-3	0.161	1.33	2.26	0.59	4.62	4.32	0.30
	3-4	0.116	1.09	1.12	0.97	4.87	4.51	0.36
H	0-1	0.162	1.12	1.01	1.11	2.97	2.20	0.77
	1-2	0.178	1.58	2.00	0.79	2.80	2.34	0.46
	2-3	0.178	1.56	3.54	0.44	3.19	2.97	0.22
	3-4	0.174	1.73	2.50	0.69	5.06	2.20	1.86
I	0-1	0.020	0.03	0.18	0.14	3.91	-5.09	9.00
	1-2	0.025	0.05	0.07	0.68	1.88	-3.46	5.34
	2-3	0.021	0.08	0.12	0.64	5.48	-5.53	0.05
	3-4	0.016	0.03	0.15	0.17	1.24	2.94	-1.70
J	0-1	0.021	0.03	0.18	0.16	1.32	3.10	-1.78
	1-2	0.025	0.04	0.01	3.94	0.92	6.69	-5.77
	2-3	0.021	0.04	0.09	0.46	0.69	8.44	-7.75
	3-4	0.022	0.05	0.02	2.63	0.59	2.87	-2.28

and Bremner (1970). Reduced sulfur in the sediment was oxidized to sulfate by a sodium hypobromite solution (prepared immediately before use; 3% Br_2 in 2 M NaOH). Two to three grams of sediment plus 5 ml of the hypobromite solution were added to a 3-port, 250-ml boiling flask. The contents of the flask were swirled, allowed to sit for 5 min., and then heated at 250-260°C until dry; 2-3 ml more NaOBr solution were added and the sample was again heated to dryness. Heating was continued for a further 30 min. to ensure complete oxidation of organic sulfur to sulfate (Tabatabai and Bremner 1970). After cooling, 3 ml of 90% formic acid were added. The contents were swirled and allowed to sit for 25 min. Addition of the acid destroyed any remaining hypobromite and acidified the sample in preparation for the reduction of the sulfate to H_2S. The sulfate was reduced to H_2S with a modification of the reduction mixture of Johnson and Nishita (1952) and Johnson and Ulrich (1959). This reducing agent was prepared by mixing hydriodic acid (Fisher Scientific Co., S.G. 1.7), 90% formic acid (Anachemia) and 50% hypophosphorous acid (Anachemia), respectively, in a 4:2:1 volumetric ratio, and refluxing for 1.5-2 h under nitrogen. Approximately 8 ml of this solution was added to each sample. The flasks were boiled for 1 h while the liberated H_2S was removed by flushing the headspace with oxygen-free nitrogen. To minimize carried over acid fumes and water vapor the flasks

were fitted with a condenser maintained at -10 to -20°C. The H_2S was trapped in a solution of zinc hydroxide (Howarth and Teal 1979) and determined iodometrically using 0.0025 N thiosulfate titrant.

The method was standardized using known quantitites of K_2SO_4, Na_2SO_4, $Na_2S_2O_3$, $(NH_4)_2S_2O_8$, methionine, and phenylthiourea. The average efficiency of sulfur recovery was 94%. The standard deviation for 14 determinations was 2%. The presence of sediment did not significantly decrease this efficiency. Replicate analysis of mixed sediment (n=11) gave an analytical variance of about $\pm$ 7% (SD). Controls were run periodically to check for sulfur contaminated reagents.

A second method was tested for its capability to accurately determine total S content of wet sediments (cores B and D). For this method, the sulfur in one-half of a core slice was oxidized with 50-100 ml of HNO_3. After oxidation, the sample was heated to dryness, mixed with approximately 0.5 g $NaNO_3$ or wetted with 7% $Mg(NO_3)_2$ (Gorsuch 1970) as an ashing agent, and left at 380°C for 16 h. Loss on ignition (LOI) was determined by weight. The resulting sulfate was extracted by a dilute solution of HCl and particles were removed by filtration (GF/C filter). Barium chloride (2 ml, 1 N) was added to the filtrate which was then heated to just below boiling for 1 h and then held at 80°C for 16 h. The $BaSO_4$ was recovered on a Whatman ashless filter which was

then ignited for 2 h at 800°C. Sulfur recovery was determined by weighing the residue.

A modification of the above method was used to ash the dried sediments (Table 2.1). In this procedure, the dried sediments were ashed directly in a porcelain crucible using 7% $Mg(NO_3)_2$ as an ashing agent. Further processing and gravimetric determination of S as $BaSO_4$ was done as described above. Citrus leaves (National Bureau of Standards) were used to standardize this method. Recovery of total sulfur for five determinations was found to be 95%-100%. Unless otherwise stated, the results were statistically analyzed using two-way ANOVA and least significant difference tests (Steel and Torrie 1980).

Stable isotopic analyses were performed on the sulfur extracted by the HNO_3 and dry ashing methods. Barium sulfate was thermally decomposed to sulfur dioxide following the method of Halas and Wolacewicz (1981). All isotopic ratio measurements were made using a VG Micromass 602E mass spectrometer with changeover valve, capillaries, and inlet system at 110°C. A tank of SO_2 was used as a machine reference. The tank was checked frequently against an ocean sulfate sample calibrated against Pacific seawater sulfate of +21 $^{\circ}/_{\infty}$ (Rees et al. 1978). Standard deviations of replicate analyses were routinely 0.1 $^{\circ}/_{\infty}$. Overall precision is estimated to be 0.3 $^{\circ}/_{\infty}$ (SD).

Lake 302S, from which five of the cores tested were

taken, has been acidified by addition of sulfuric acid since 1982. The sulfur in the sulfuric acid has an isotopic value of +18 $^{o}/_{oo}$, compared to natural sulfate of +5 - +7 $^{o}/_{oo}$ in lake waters in this area (Hesslein et al. 1988).

Large losses of sulfur occurred when lacustrine sediments were dried prior to total sulfur determination (Fig. 2.1, Table 2.2). A total of 10 cores, representing different types of sediment (Table 2.1), were analyzed for total sulfur content. Loss of sulfur upon drying was observed for each type of sediment regardless of the analytical or drying method used. Of the 69 dried samples analysed, only 9 did not show a decrease in sulfur content as compared to wet sediment analysis (Fig. 2.1, Table 2.2). It is unlikely that these results are due to chance alone (Chi-square, $P<0.001$). The magnitude of the sulfur deficiencies observed varied widely and was found to be related not to gross sediment type but to the individual core and method of drying.

The two methods used to determine total sulfur (NaOBr-HI and HNO_3-dry ashing) were compared for 6 slices each from duplicate cores A and B. There was no significant difference ($P>0.2$) in the total sulfur estimates for wet sediments in these cores, and it was concluded that we could directly compare results obtained by the two methods. The significantly higher losses observed for core B ($P<0.001$), therefore, were a result of lyophilization as the drying

Fig. 2.1. Frequency distribution of sulfur losses for cores A-F. Note that the negative values represent an apparent gain of sulfur after drying.

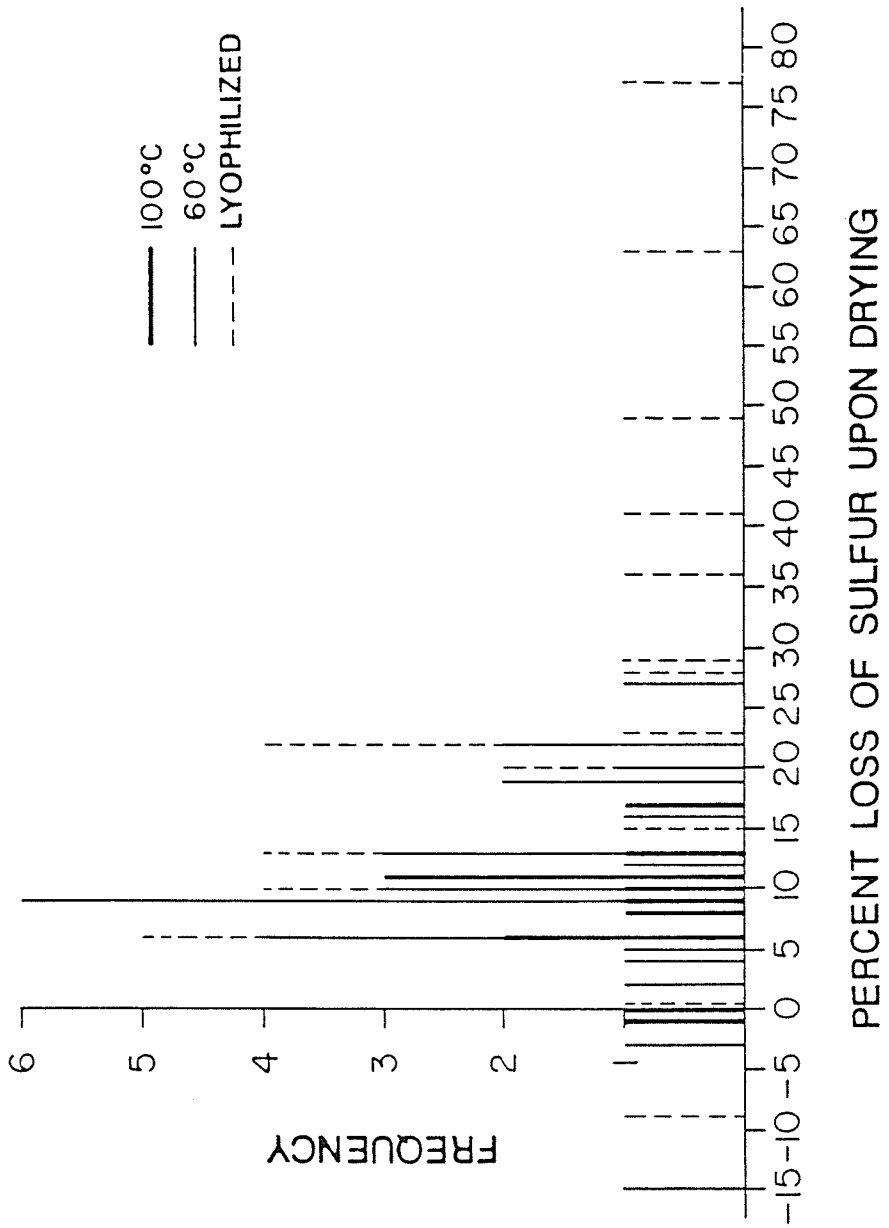
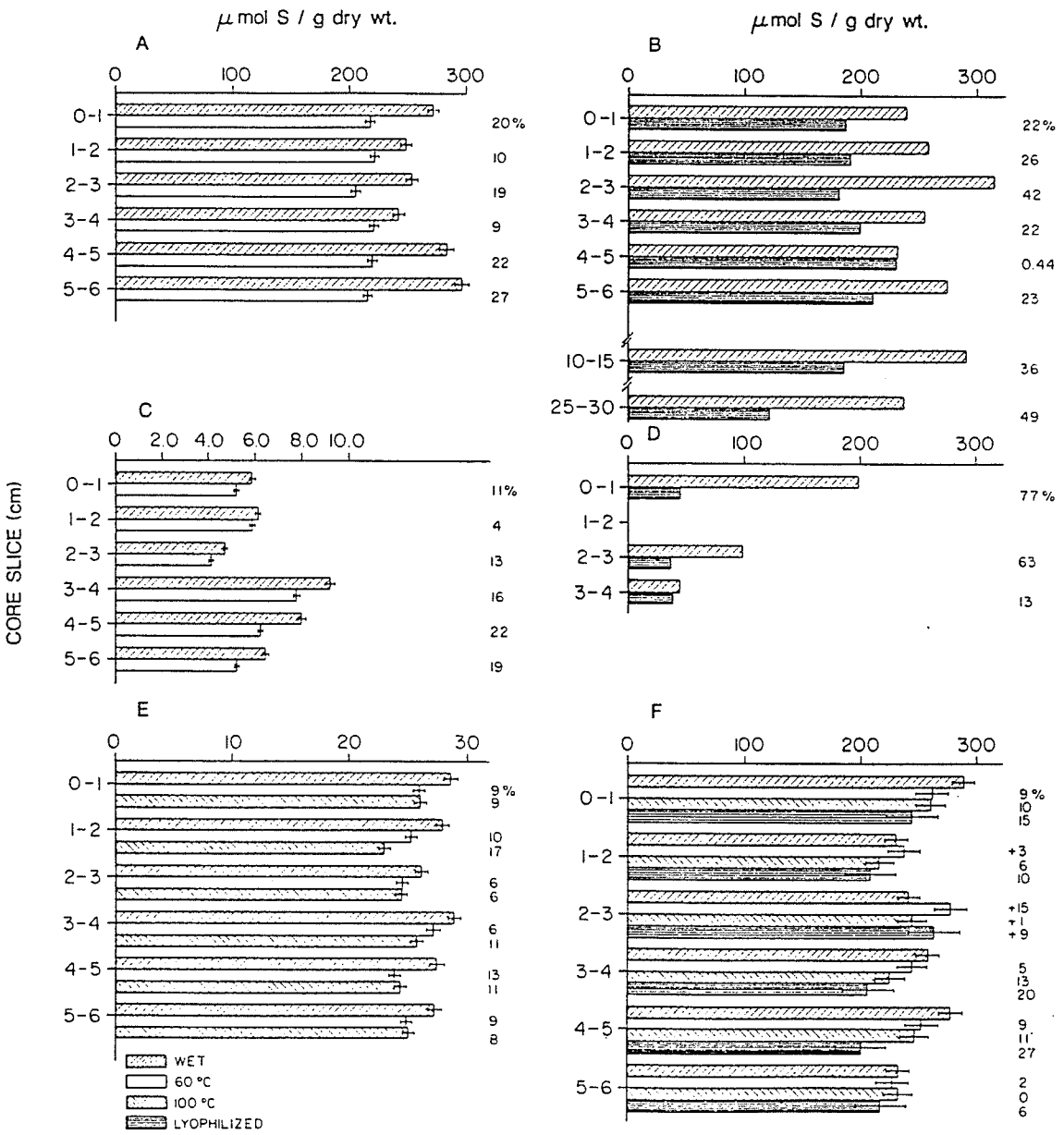

100°C
60°C
LYOPHILIZED
FREQUENCY
1
2
3
4
5
6
-15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
PERCENT LOSS OF SULFUR UPON DRYING

Fig. 2.2. Total sulfur profiles obtained by wet and dry digestion. A-F correspond to cores A to F, respectively. The numbers in front of the bars represent a percent loss of sulfur with respect to wet digestion values. Positive signs indicate an increase in sulfur content. Error bars in F represent the standard deviation between duplicates.


μmol S / g dry wt.
A
0
100
200
300
0-1
1-2
2-3
3-4
4-5
5-6
20%
10
19
9
22
27
μmol S / g dry wt.
B
0
100
200
300
0-1
1-2
2-3
3-4
4-5
5-6
10-15
25-30
22%
26
42
22
0.44
23
36
49
C
0
2.0
4.0
6.0
8.0
10.0
0-1
1-2
2-3
3-4
4-5
5-6
11%
4
13
16
22
19
D
0
100
200
300
0-1
1-2
2-3
3-4
77%
63
13
CORE SLICE (cm)
E
0
10
20
30
0-1
1-2
2-3
3-4
4-5
5-6
9%
9
10
17
6
6
6
11
13
11
9
8
WET
60 °C
100 °C
LYOPHILIZED
F
0
100
200
300
0-1
1-2
2-3
3-4
4-5
5-6
9%
10
15
+3
6
10
+15
+1
+9
5
13
20
9
11
27
2
0
6

method used (Table 2.1, Fig. 2.2a and b).

The greatest losses occurred in core D (Fig. 2.2d), a section of which lost 77% of its sulfur content following lyophilization, and in cores I and J which, when also lyophilized, lost up to 86% and 84% of their total sulfur, respectively (Table 2.2). More common losses, however, were between 6% and 22% for other cores which were not lyophilized (Fig. 2.1). For example, core E (Fig. 2.2e), containing sediment of a similar type to core D but obtained from a different site of Lake 239 (Table 2.1), showed sulfur losses from 6% to 17% when oven dried. The total sulfur content per gram dry weight of core E sediment was also considerably lower than that for core D (Fig. 2.2d and e).

The effects of the two oven drying methods on sulfur losses were variable. For example, oven drying of core E sediments at 60 and 100°C showed no statistical difference in the mean sulfur loss for the two treatments (paired difference t test, $P>0.3$; Fig. 2.2e). The same treatments for core F slices resulted, however, in a significant loss of sulfur at 100°C (avg. 7.8%; $P<0.05$) but no loss at 60°C relative to the wet sediment analysis (Fig. 2.2f). In contrast, the sediment of core A, which was similar to that of core F (Table 2.1), lost an average of 17.8% sulfur when oven dried at 60°C (Fig. 2.2a).

All three drying methods were compared for core F (Fig. 2.2f). This core was obtained from the same site as cores A

and B, but at a later date (Table 2.1). Total sulfur estimates on wet sediment were typical for this type of sediment (Figs. 2.2a,b and f). As expected, all dried samples showed a loss in sulfur content, with the exception of the 2-3 cm slice samples (Fig. 2.2f). For this core, the difference in sulfur content between wet and 60°C-dried sediment was not significant. Drying at 100°C, however, caused significant losses ($P<0.05$). Lyophilization caused the highest average loss (11.5% or 1.5-fold) of all the treatments ($P<0.01$). Our conclusion that lyophilization causes the greatest sulfur loss is supported by a comparison of cores A and B, which showed statistically significant differences, and by the lyophilized cores used for isotopic analysis (Table 2.2), which generally showed sulfur losses of from 40% to 60%. Why lyophilization should cause greater sulfur losses than oven drying was not studied. It is possible that subjecting the sediment to a vacuum may facilitate the volatilization of certain sulfur compounds.

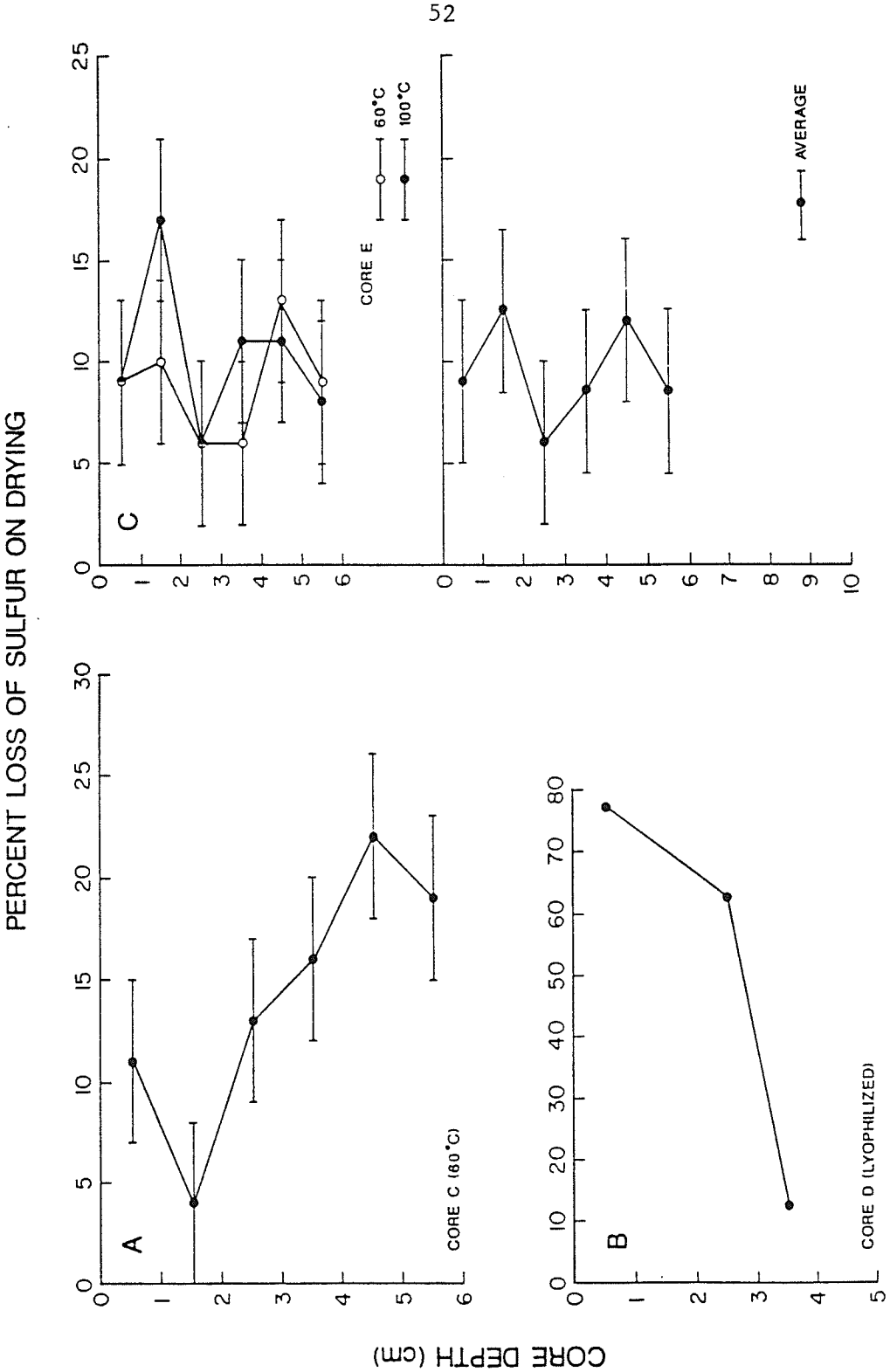
Samples from core F were processed in duplicate to assess sediment variability. The variance between duplicates was higher for the oven-dried sediments than for the wet sediments, and was greatest for the lyophilized sediments (Fig. 2.2f).

There was no discernible pattern relating sulfur loss and organic content. The low-organic, sandy sediment of core C (Fig. 2.2c) showed losses of sulfur not significantly

different from those observed for the highly organic sediment of core A (Fig. 2.2a). In another comparison, sediment with a higher organic content (core E) had a significantly lower average sulfur loss (9.6%) than did the sandier sediment of core C (14.2%)($P<0.001$). No correlations between sulfur loss and porosity or depth in the lake were observed in the cores studied. Sulfur loss below the sediment-water interface was also variable (Figs. 2.3a, b and c). Sulfur deficiency peaks occurred from near the top to near the 6-cm depth. In one case, the loss of sulfur was constant throughout this interval (Fig. 2.3c). Core B was further analyzed at 10-15 cm and 25-30 cm and showed large losses in these slices (Fig. 2.2b). Overall, there was much variability between similar types of sediments (cores A and B vs core F) and even within cores. Thus it does not appear that a single correction factor can be determined for any particular sediment to compensate for sulfur lost on drying.

An observation similar to ours was made by Kaplan et al. (1963), who used a Leco apparatus to analyze dried marine sediments. They obtained sulfur losses comparable to ours but did not show that the losses were due to drying. Instead, they suggested that the Leco apparatus, which analyzes only dried sediments, gave less reliable results than comparative analyses with wet digestion by a bromine-aqua regia treatment. At present, most researchers continue

Fig. 2.3. Percentage loss of sulfur upon drying versus depth in the sediments.


PERCENT LOSS OF SULFUR ON DRYING
CORE DEPTH (cm)
A
CORE C (60°C)
B
CORE D (LYOPHILIZED)
C
CORE E
60°C
100°C
AVERAGE

to use dried sediment methods to estimate total sulfur.

The identity of the sulfur fraction that is lost upon drying was not extensively studied. Preliminary gas chromatographic analyses showed easily detectable losses of dimethylsulfide (DMS) and methane thiol (MSH), possibly explaining in part our observations. Other compounds may also have been lost.

It is likely that sulfur was lost from both the pore water and solid phase of the sediment samples. Volatile sulfur compounds have been measured in the pore waters of other ELA lakes, and their concentrations are in the nanomolar range (DMS, 11.0 - 37.7 nM; MSH, 38.2 - 86.5 nM; S. Richards, U. of Manitoba, pers. comm.). If the losses observed occurred solely from pore water, they would require concentrations of volatilizable, dissolved organic-S in the millimolar range. Thus, most of the observed loss must have been from the particulate phase.

Others have shown that lyophilization and air drying do not underestimate reduced inorganic S content of marsh, lake and marine sediments (Canfield et al. 1986; Carignan and Tessier 1988; Morse and Cornwell 1987). The suggestion is that the sulfur losses we observed were due to losses of organic sulfur compounds. In peat samples, Wieder et al. (1985) found no difference in total sulfur content between wet and dry analysis at 50°C. They observed, however, that conversion of carbon-bonded S to sulfate or ester sulfate S

appeared to occur. A similar observation was made by David et al. (1982) for mineral soils.

For several cores the effect of drying on stable isotopic composition was tested. Variable changes in stable isotopic ratios upon drying of the sediments were shown to occur (Table 2.2). In some cases, we observed large changes while in other cases the changes were within the precision of the method (0.3 $^{o}/_{oo}$ SD). The core from lake 239, while showing large sulfur losses during drying, had only small isotopic shifts. Therefore, changes in isotopic ratios are not always related to the amount of sulfur lost during drying. Generally, the changes were in a consistent direction within a core. Cores with low loss on ignition, a surrogate for low organic carbon content (47% of weight lost on ignition is organic carbon, Hesslein et al. unpub. data), had lower sulfur content by dry weight and showed larger changes in isotopic signals between wet and dry analysis.

The sizes of isotopic changes that we observed are commonly attributed to isotopic fractionation by biological or chemical reactions (eg. Nriagu and Coker 1983; Fry et al. 1987). Thus, analyses of dried sediments could lead to erroneous conclusions because the study of sulfur diagenesis through changes in stable isotopic composition requires quantitative measurement of the sulfur pool in the sediment.

We have shown that the current practice of drying sediments prior to analysis can underestimate total sulfur

content. We suspect, based on the data of Kaplan et al. (1963), that the same bias may occur in marine sediments. This phenomenon can seriously affect the determination of sulfur species in sediments, as well as the interpretation of results from studies on the end products of sulfate reduction. Organic sulfur (especially C-bonded S) is most frequently defined as the difference between total and inorganic sulfur. Therefore, an underestimate of total sulfur would cause an underestimate of organic sulfur content, and diminish the importance of organic sulfur as an end product of sulfate reduction.

Studies on the rate of sulfur burial in sediments may also be compromised if total sulfur content is not determined prior to drying. Lower total sulfur values will lead to underestimates of the rate of sulfur burial and result in the underestimation of internal alkalinity production in lakes.

2.3 References

Canfield, D. E., R. Raiswell, J. T. Westrich, C. M. Reaves, and R. A. Berner. 1986. The use of chromium reduction in the analysis of reduced inorganic sulfur in sediments and shales. Chem. Geol. 54: 149-155.

Carignan, R., and A. Tessier. 1988. The co-diagenesis of sulfur and iron in acid lake sediments of southwestern Quebec. Geochim. Cosmochim. Acta 52: 1179-1188.

David, M. B., M. J. Mitchell, and J. P. Nakas. 1982. Organic and inorganic sulfur constituents of a forest soil and their relationship to microbial activity. Soil Sci. Soc. Am. J. 46: 847-852.

Fry, B., H. Gest, and J. M. Hayes. 1987. $^{34}S/^{32}S$ fractionation in sulfur cycles catalyzed by anaerobic bacteria. Appl. Environ Microbiol. 54: 250-256.

Gorsuch, T. T. 1970. The destruction of organic matter. 1st ed. Pergamon.

Halas, S., and W. P. Wolacewicz. 1981. Direct extraction of sulfur dioxide from sulfates for isotopic analysis. Anal. Chem. 53: 686-689.

Hesslein, R. H., M. J. Capel, and D. E. Fox. 1988. Sulfur isotopes in the inputs and outputs of a Canadian Shield watershed. Biogeochemistry 5: 263-273.

Howarth, R. W.,and J. M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnol. Oceanogr. 24:

999-1013.

Johnson, C. M., and H. Nishita. 1952. Microestimation of sulfur in plant materials, soils, and irrigation waters. Anal. Chem. 24: 736-742.

Johnson, C. M., and A. Ulrich. 1959. Analytical methods for use in plant analysis. California Agric. Exp. Sta. Bull. 766, 55.

Kaplan, I. R., K. O. Emery, and S. C. Rittenberg. 1963. The distribution and isotopic abundance of sulphur in recent marine sediments off southern California. Geochim. Cosmochim. Acta 27: 297-331.

Morse, J. W., and J. C. Cornwell. 1987. Analysis and distribution of iron sufide minerals in recent anoxic marine sediments. Mar. Chem. 22:55-69.

Nriagu, J. O., and R. D. Coker. 1983. Sulphur in sediments chronicles past changes in lake acidification. Nature 303: 692-694.

Rees, C. E., W. J. Jenkins, and Jan Monster. 1978. The sulphur isotopic composition of ocean water sulfate. Geochim. Comochim. Acta 42: 377-381.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. 2nd ed. McGraw-Hill.

Tabatabai, M. A., and J. M. Bremner. 1970. An alkaline oxidation method for the determination of total sulfur in soils. Soil Sci. Soc. Am. Proc. 34: 62-65.

Wieder, R. K., G. E. Lang, and V. A. Granus. 1985. An evaluation of wet chemical methods for quantifying sulfur fractions in freshwater wetland peat. Limnol. Oceanogr. 30: 1109-1114.

3. Measurement of organic-^{35}S and organic-S in sediments from chemically dilute lakes: Methodological considerations

3.0. Abstract

Three protocols for the determination of various S fractions were tested for their suitability to estimate total indigenous organic sulfur (S_o) and $^{35}S_o$ formed from added $^{35}SO_4^{2-}$ in sediments of chemically dilute lakes in the ELA. The protocols tested have all been reported in the literature. It was found that two protocols involving sequential analyses for S fractions following acid treatment gave estimates of both S_o and $^{35}S_o$ up to 87% lower than a non-sequential protocol. The low estimates were largely due to hydrolysis and solubilization of solid phase S which was then removed in a rinsing step. The non-sequential protocol, in which total reduced inorganic sulfur and total sulfur were determined on separate aliquots, is recommended as the most reliable of the three. Individual analyses in this protocol were verified for these lake sediments using a variety of S standards.

3.1. Body of note.

Interest in sedimentary organic sulfur (S_o) has increased because recent findings show that it is an important end-product of dissimilatory sulfate reduction in freshwater peat (Behr 1985; Brown 1986) and sediments (e.g. Rudd et al. 1986a; Landers et al. 1983: Landers & Mitchell 1988; Baker et al. 1989). These findings indicate a mechanism for S storage in sediments other than the formation of insoluble iron sulfide minerals from the reaction of H_2S and Fe (e.g. Kelly et al. 1982; Cook and Schindler 1983; Berner 1984). Generally, S_o makes up the majority of sedimentary S (often >80%) in lakes, and is thought to consist of C-bonded (C-S) and sulfate ester forms (C-O-S) (Mitchell et al. 1984; David and Mitchell 1985; Nriagu and Soon 1985). Although organic matter carried in from the watershed and produced by biota in the water column can be major sources of S_o in sediments, stable and radioisotope studies have shown that it is also formed *in situ* from sulfate reduction, through reactions of H_2S with organic compounds (Nriagu and Soon 1985; Rudd et al. 1986a). Formation of S_o in this way is of particular importance in freshwater systems because it provides a pathway for long term alkalinity production from sulfate reduction (Rudd et al. 1986a).

Our understanding of the importance of S_o in the lacustrine S cycle depends greatly on the ability to

quantify it accurately. Currently, S_0 in sediments is estimated as the difference between inorganic sulfur (S_I) and S_T contents. Thus, any loss in S_T would cause an underestimation of the importance of S_0. Correct measurement of S_I is also necessary to obtain accurate values for S_0. The chromium (CrII) reduction method of Zhabina and Volkov (1978) is commonly used to determine all S_I forms except sulfate. However, there have been suggestions that CrII treatment also reduces some S_0 forms (Brown 1986), leading to overestimation of S_I and underestimation of S_0. To date, there is no reliable direct method for determining total S_0 in sediments.

The same methods used for S_0, S_I, and S_T in sediments are used in sulfate reduction studies to track incorporation of radiolabelled sulfate-S into organic and inorganic sedimentary sulfur pools. Newly formed ^{35}S fractions have a different distribution than pre-existing cold S (Rudd et al. 1986a; Appendix II) fractions and may have different chemical characteristics. These differences could mean that the analytical methods commonly used for cold S may not be suitable for the measurement of short-term end-products of $^{35}SO_4^{2-}$ reduction. This hypothesis is supported by the fact that negative values for $^{35}S_0$ are sometimes obtained with some analytical methods (J. Rudd, pers. comm.). Thus, it is important to know the accuracy of these methods for both ^{35}S and cold S in order to further our understanding of the

short-term, as well as long-term, fate of sulfur in lake sediments.

Two important questions must be considered in order to determine the suitability of a particular analysis method for measuring S_o and $^{35}S_o$ in sediments. First, what are the specificities and efficiencies of the different analytical steps involved? Second, what, if any, is the effect of the order in which the analytical steps are done? Both of these questions are addressed in this work.

Epilimnetic sediment was obtained from several lakes at the Experimental Lakes Area (ELA) located in northwestern Ontario at 93°30′-94°00′W and 49°30′-49°45′N (Brunskill and Schindler 1971). Details of the area's geology, and vegetation are given in Brunskill and Schindler (1971). The ELA is situated on the Precambrian Shield, which is overlain in areas by a thin overburden of glacial deposits of quartz, plagioclase and K-feldspar.

The lakes (114, 223, 224, 239, 240 and 302 South) are small, with surface areas of 10.9-56.1 x 10^4 m^2 and mean depths of 1.7-10.5 m (Brunskill and Schindler 1971). Details of light, temperature and O_2 for several ELA lakes are described by Schindler (1971). These lakes are among the most chemically dilute in the world and have overlying water sulfate concentrations in the order of 31 μmol L^{-1} (Armstrong and Schindler 1971). Lake 302 South has been experimentally acidified with sulfuric acid (Rudd et al.

1990) and at the time of sampling had a sulfate concentration of 104-125 μmol L^{-1}. ELA lakes have low pore-water sulfate concentrations (Brunskill et al. 1971) which have been reported as 3-37 μmol L^{-1} for Lakes 114 and 302 South (Rudd et al. 1986a). Lake 5-0, a small acid lake in the Florida panhandle, also served as a source of sediment.

Cores were retrieved by diver or by means of a gravity corer from depths of 1.5 to 2m. The majority of the work presented here was done on sediment from Lakes 114 and 302 South. The other sediments were used to test the efficiency of chemical methods and to verify the results obtained. Lake 114 sediment was flocculent, of high porosity (>0.95) and relatively high organic content (loss on igniton (L.O.I.) = 58.5% by weight; Brunskill et al. 1971). In Lake 302 South, the sediment at the sampling site was of low porosity (0.5-0.64) and can be visually described as sandy. This type of sediment contains little organic matter, with values of organic C and N <2.0% on a dry weight basis (Sweerts et al. 1986). More details of sedimentary chemical characteristics are given in Rudd et al. (1986b). The sediment obtained from Lakes 223, 224, 239, and 240 was visually similar to that from Lake 302 South, and was of comparable porosity (0.55- 0.63). Sand from lake 5-0 was very fine and compact (porosity <0.5; where porosity = volume of water/ volume of water + solid sediment) and had very low S content (<1 μmol S g^{-1}). This low S content was

usefull in experiments testing the effect of sediment on recovery of S from different compounds.

Indigenous S_0 and recently formed $^{35}S_0$ were measured in intact cores from Lake 302 South by three analytical protocols (see below). Radiolabelled ^{35}S-sulfate was added to the overlying water of undisturbed cores from Lake 302 South within 30 hours of their removal from the lake. The cores were incubated at *in situ* temperature (21°C) for 24-48 hours and then sliced at 1 cm intervals. The slices were immediately frozen in whirlpack bags under a N_2 atmosphere until analysis.

Several wet chemical methods were used to measure operationally defined sedimentary S fractions. A summary of the common S fractions and the compounds contained in each is given in Table 3.1. Acid volatile sulfur (AVS = mostly H_2S and amorphous FeS) was determined by sparging sediment with deoxygenated 6N HCl (25°C) under N_2 gas (Rudd et al. 1986a). This analysis results in an acid slurry which can contain several non-volatile, soluble S components (Table 3.1). The AVS content may be underestimated in sediments with high Fe^{3+}, due to oxidation of S^{2-} to S° (Chanton and Martens 1985; Cornwell and Morse 1987), however, this is expected to be of minor importance with the reducing sediments used here, due to low Fe^{3+} content.

Total reduced S_I (primarily FeS, FeS_2 and S°) was determined by conversion to H_2S by 1M $CrCl_2$ in strong acid

Table 3.1. Sulfur species contained in some S fractions described in the literature. Details of the treatments used to obtain each S fraction are given in the text.

Sulfur fraction	Main sulfur species	Example references
HCl volatile (AVS)	Dissolved H_2S and HS^-, FeS	a
HCl soluble (pre-water rinse)	Pore-water SO_4^{2-}, $S_2O_3^{2-}$, polythionates, polysulfides, soluble organic-S, HCl-hydrolyzable organic-S (eg. sulfated polysaccharides and amino acids)	b
CrII reducible	FeS, FeS_2, S^o, $S_2O_3^{2-}$, poly-sulfides (organic/inorganic), polythionates	c,d e,f
Org-S (TS-CrII reducible)	C-O-S (ester sulfates), C-N-S (sulfamic acid), C-S (eg. amino acids, petides, protein, sulfolipids, sulfonic acids, heterocyclics.	b,f, g
water soluble	Pore-water SO_4^{2-}	h

Modified from Krairapanond et al. (1991).

[a] Jorgensen and Fenchel (1974)

[b] Nriagu and Soon (1985)

[c] Zhabina and Volkov (1978)

[d] Wieder et al. (1985)

[e] Canfield et al. (1986)

[f] Chapter 4

[g] Fitzgerald (1976)

[h] for ELA sediment; this work

solution (Howarth and Jorgensen 1984; Rudd et al. 1986a). This S fraction is commonly referred to as chromium (CrII) reducible S (CRS) although for some compounds (e.g. FeS, FeS_2) conversion of S to H_2S is via reductive dissolution and not a reduction of S which is already in its most reduced state (S^{2-}).

Total sulfur was determined by preliminary oxidation with NaOBr (250°C) followed by hydriodic acid (HI) reduction of the resulting sulfate to H_2S as described by Tabatabai and Bremner (1970) and Amaral et al. (1989). The analysis was done on wet sediment to avoid S losses due to drying (Amaral et al. 1989). The reducing solution of HI, hypophosphorous and formic acids of Johnson and Ulrich (1959) was prepared as described in Amaral et al. (1989).

The H_2S and $H_2{}^{35}S$ produced by CrII and HI reduction and acid volatilization was sparged from the sediment by N_2, trapped in zinc acetate-NaOH traps (Howarth and Teal 1979), and quantified iodometrically. Radiolabel was measured by liquid scintillation counting of aliquots of the traps.

Unreacted $^{35}SO_4^{2-}$ was determined by rinsing the sediment once with deoxygenated 2.5 M $MgSO_4.7H_2O$ and distilled water (Rudd et al. 1986) followed by 3 more rinses with deoxygenated, distilled water alone. Rinse water and sediment were separated by centrifugation (20 min. at 3000 rpm). Aliquots of the supernatants were counted in a Rackbeta (LKB) liquid scintillation counter to measure ^{35}S

isotope.

Specificities and efficiencies of S recovery by CrII reduction and NaOBr-HI analyses have been determined with known standards (Tabatabai and Bremner 1970; Wieder et al. 1985; Canfield et al. 1986; Rudd et al. 1986a; Chapter 2). In the present work, these parameters were tested in the presence of lake sediment (Tables 3.2 and 3.3). Previous determinations have usually been done on standards alone, although CrII reduction and NaOBr-HI analyses have been verified in the presence of peat (Wieder et al. 1985) and soil (Tabatabai and Bremner 1970).

This study showed that, in the presence of lake sediment, the recovery efficiencies of S from Fe-S compounds and S^o were >90%, while those for S_o and sulfate were very low (<1%) (Table 3.2). The results confirm the currently accepted conclusion that chromium reduction is specific for non-sulfate S_I, as has been verified by different authors using a variety of inorganic and organic S standards including Fe-S minerals, protein and thiophene (e.g. Wieder et al. 1985; Canfield et al. 1986). The presence of sediment did not appear to inhibit the action of the reagent since recoveries of non-sulfate S_I were high. However, based on further work (see Chapter 4 and below) the conclusion that CrII reduction is specific only for S_I and never S_o is inaccurate.

Because the nature of sedimentary S_o is largely unknown

Table 3.2. Specificity and efficiency of the chromium reduction method for the measurement of S in different chemical compounds. Analyses were carried out in the presence of 5g of sediment from Lake 114 (see text for sediment description).

Chemical species	% of S recovered (n= 2-5)
$FeS_{1.8}$*	100 ± 10
$Na_2S_2O_3$	68 ± 3
Na_2SO_4	<0.1
Pyrite ore	95 ± 3
S^o	91 ± 9
Methionine	0.0
Phenyl-thiourea	5.9 ± 0.5
SDS^+	1.2 ± 0.3

* Prepared as described in Wada (1977)

\+ Sodium dodecyl sulfate

Table 3.3. Efficiency of the total sulfur method for several S compounds in the absence and presence of sediment.

System Analyzed	Mean S Content μmol/g	μmol S added	% Recovery Range	Mean	SD	n
S Standards (alone)*	-	9.2-19.7	90.2-98.4	94.5	3.0	6
Sediment (L114, ELA)	7.77	9.2-19.7	89.6-94.0	91.3	1.9	4
Sediment (L50, Florida)	0.26	9.2-19.7	80.7-90.5	84.5	3.2	6

* The sulfur standards used were; phenylthiourea, 2-methyl-2-thiopseudourea sulfate, L-methionine and K_2SO_4. Approximately 1.5 g and 5 g of floccy and sandy sediments were tested (wet weight). See text for description of sediments.

and may differ chemically from tested standards, it is impossible to be sure that none of these compounds are chromium reducible. Indeed, recent work on the extraction and characterization of sedimentary S_O (Chapter 4) has shown that a portion of the S in some organic compounds is released by chromium reduction (Table 3.4). A similar observation was also made by Brown (1986) who measured chromium reducible S in peat from which all S_I had been removed and obtained greater than 14% of S_O. Similarly, I have found that 10-20% of $^{35}S_O$ was obtained by this analysis. Therefore, chromium reduction may overestimate the amount of S_I in sediments.

The alkaline oxidation-reduction method for S_T measures all S present, both organic and inorganic. Tabatabai and Bremner (1970) observed quantitative recoveries of S from several different standards in the presence and absence of soil with this method. In this study, the efficiency of the NaOBr-HI method was tested in the presence and absence of lake sediments, and was found to be affected differently by

Table 3.4. Chemical characterization of $^{35}S_o$ components isolated from 2.5 N HCl extracts of $^{35}SO_4^{2-}$-incubated sediment from Lake 303 at the ELA. The components were separated by Sephadex G-10 and LH-20 chromatography.

Component name	% of total 35S in extract	% $CR^{35}S$* in component	% of total $^{35}S_o$ produced
HCl i	40	40	10.5
HCl ii	16	25	5.1
HCl iii	11	15	4.0
HCl iv	10	6	4.4
HCl v	11	nd+	nd
HCl vi	12	33	3.4

Modified from Chapter 4.

* The absence of $^{35}S_I$ in components HCl i to vi was verified by electrophoretic and chromatographic techniques (see Chapter 4), so that all $CR^{35}S$ is considered to be derived from $^{35}S_o$. Soluble S_I was removed by extraction with pyrophosphate buffer prior to HCl extraction.

\+ nd= not determined

different sediments (Table 3.3). Recoveries of 85-95% were obtained for several S standards, depending on whether or not the standard was analyzed alone or with sediment (Table 3.3). A similar efficiency (94%) has previously been reported for this method in the presence of sediment from Lake 114 (Amaral et al. 1989). Sand from Lake 5-0 interfered with the method and S recovery was significantly lower than for S standards alone (Table 3.3). Interestingly, Freney et al. (1970) noted that the presence of silicates in soil protected some S from reaction with HI since efficiencies increased slightly if the soil was pre-treated with hot hydrofluoric acid. However, this was not tested here and it is not clear why a decrease in S recovery efficiency occured with this sediment. The results in Table 3.3 show that it is important to determine the efficiency of the method on each sediment to be analyzed since efficiency of recovery may vary. Estimates of S_T should then be corrected for efficiency of recovery to avoid underestimating S_O. Also, analysis must be done on wet sediment since drying causes loss of S from lake sediments (Amaral et al. 1989). It should be noted, however, that the identity of this S is unknown and volatile organic S compounds present in the porewater are not an important component (Chapter 2; Richards et al. 1991).

The efficiency of the rinsing step was tested by mixing $^{35}SO_4^{2-}$ with sediment for 30 min., at 4°C, and under room

atmosphere to minimize sulfate reduction, and rinsing with magnesium sulfate solution and distilled water to displace any adsorbed $^{35}SO_4^{2-}$ (Rudd et al. 1986a). The majority of the radiolabelled sulfate (80-95%) was recovered in the first rinse for two different sediments (Table 3.5). The remainder of the label was recovered by subsequent rinses with distilled H_2O (Table 3.5). Therefore, it is clear that the rinsing procedure presented here removes all of the unreacted $^{35}SO_4^{2-}$ from lake sediments. Any free $H_2{}^{35}S$ present would be removed in this step.

Unlabelled sulfate and H_2S are negligible in ELA porewaters (<1% of total S) and concentrations of dissolved volatile S_0 are also very low (Richards et al. 1991). Therefore, these fractions were not analyzed. This may not be the case in other systems, such as in salt marsh sediments, where the concentration of soluble reduced S compounds is high (Luther et al. 1986) and where cold and radiolabelled H_2S may accumulate.

The efficiency of the AVS method was not tested here but has been reported to be 95% with a standard of CdS (Rudd et al. 1986a).

As mentioned above, two major questions concerning S methodology were addressed in this study. In addition to testing the specificity and efficiency of the chemical analyses with freshwater lake sediments, the effect of order of analysis was also examined. Three different protocols

Table 3.5. The removal of unreacted $^{35}SO_4^{2-}$ from two sediments by rinsing with $MgSO_4$ and distilled water. Duplicate determinations were carried out on ≈30 g of sediment from each of Lake 302 South and Lake 114.

Rinse number*	Cumulative recovery of added $^{35}SO_4^{2-}$ (%)	
	L. 302 South	L. 114
1	95.6 ± 9.5	80.0 ± 1.0
2	101.0 ± 9.6	91.5 ± 1.1
3	102.2 ± 9.3	99.0 ± 1.0
4	102.5 ± 9.2	101.1 ± 1.5

* Rinse 1 consisted of shaking sediment for 5 min. with 25 mL of 2.5 M $MgSO_4.7H_2O$, before adding 150 mL of distilled water and centrifuging. Rinses 2-4 were done using 180-200 mL of distilled water. Further details are given in the text.

(i.e. the order in which chemical analyses are done; Fig. 3.1), all of which have been described in the literature, were examined for the ability to accurately estimate S_O and $^{35}S_O$ in these low S sediments (0.03-0.5% S by weight; Amaral unpub. data).

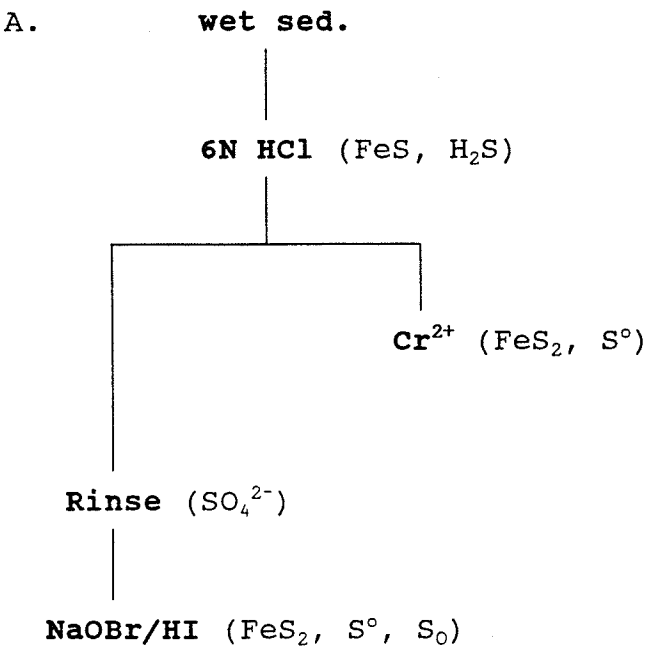
In the first protocol (A), AVS was determined first on wet sediment. One half of the resulting sediment-acid slurry was then analyzed by CrII reduction, while the other half was first rinsed, to remove unreacted $^{35}SO_4^{2-}$, and then analyzed for total remaining S (Rudd et al. 1986a). This protocol has generally been used to measure the distribution of $^{35}SO_4^{2-}$ due to sulfate reduction and an aqua regia oxidation is used to determine total ^{35}S (but not unlabelled S). For this study, the aqua regia step was replaced with the alkaline oxidation-reduction method (NaOBr-HI) in order to quantify both labelled and unlabelled S pools. Estimates of labelled and unlabelled S_O were obtained as the difference between the CrII and NaOBr-HI measurements.

In the second protocol (B), CrII reducible S (all non-sulfate S_I) was determined first on wet sediment. The sediment was then rinsed to remove unreacted $^{35}SO_4^{2-}$, and analyzed (by NaOBr-HI) for total remaining S which, at this stage, should be only S_O of the carbon bonded S and ester sulfate forms (Wieder and Lang 1988).

In the third protocol (C), S_T (i.e. NaOBr-HI analysis) and CRS were determined on separate sediment aliquots after

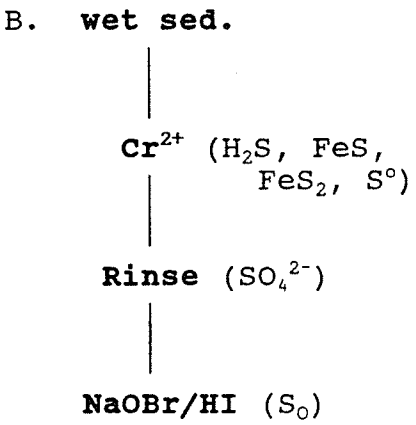
Fig. 3.1. Three protocols used in the analysis of S and ^{35}S fractions in sediments and peats. All three protocols have been described in the literature (A, (Rudd et al. 1986a); B and C, (Wieder and Lang 1988), and the major S compounds assumed to be measured at each step are given in brackets. More detailed descriptions of the compounds obtained by different analyses are given in Table 3.1.

A.

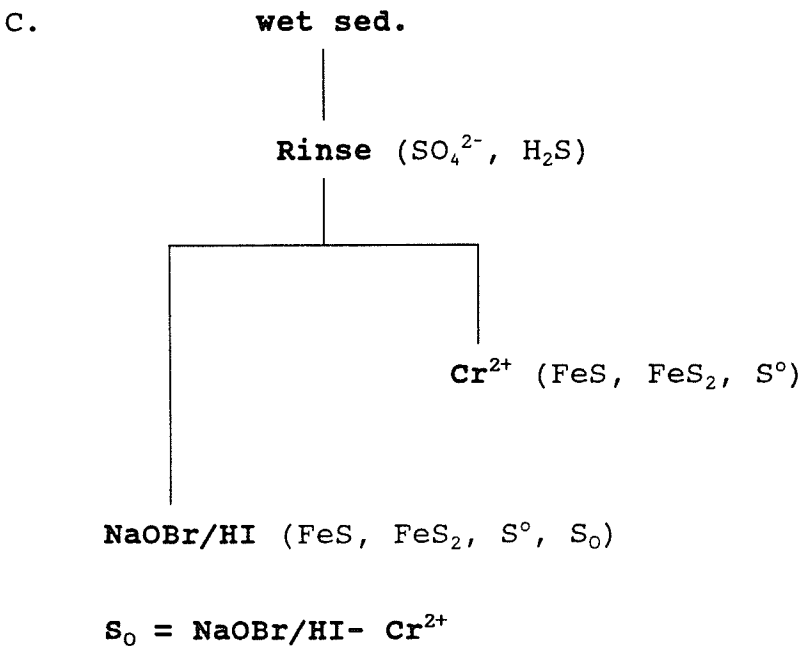


S_o = NaOBr/HI-Cr^{2+}

B.



C.



S_o = NaOBr/HI- Cr^{2+}

the sediment was rinsed to remove soluble sulfur (e.g. H_2S and SO_4^{2-}). S_O was estimated as the difference between the CRS and S_T measurements (e.g. Carignan and Tessier 1988, Wieder and Lang 1988). One important difference between these and the current study is the fact that in the latter, sediments were not dried prior to determination of S_T, to avoid large losses of S (Amaral et al. 1989).

The results showed that the measurement of total unlabelled S_O and $^{35}S_O$ in lake sediments was severely affected by the order in which individual analyses were done. When protocol A was used on sediment from two Lake 302 South cores, 7 out of 12 core slices analyzed gave negative estimates of $^{35}S_O$ (Table 3.6). These are clearly impossible values indicating that there are problems associated with this protocol. These results can be explained on the basis of the sequential determination of different S species and the arithmetic determination of $^{35}S_O$ (Fig. 3.1). One important assumption of this protocol is that after strong acid is added and AVS is sparged away, the only dissolved radioactive component is unreacted $^{35}SO_4^{2-}$. This $^{35}SO_4^{2-}$ is rinsed away to avoid overestimation, by the NaOBr-HI or aqua regia digestion, of reduced ^{35}S compounds remaining in the sediment after AVS analysis. However, it is likely that strong acid solubilizes some organic ^{35}S-labelled compounds, as has been suggested by Nriagu and Soon (1985; Table 3.1). Proof of this was obtained by

Table 3.6. Estimates of S_o and $^{35}S_o$ in two sediment cores from Lake 302 South (1.5 m) as determined by protocol A.

Core	Sediment depth (cm)	unlabelled S_o	$^{35}S_o$
		% of total reduced S(^{35}S)	
1	0-1	70.2	-2.2
	1-2	51.8	-5.8
	2-3	57.0	4.1
	3-4	54.1	-2.4
	4-5	49.4	17.0
	5-6	16.9	72.6
2	0-1	82.9	-11.5
	1-2	77.2	3.2
	2-3	71.0	4.1
	3-4	83.4	-0.7
	4-5	53.0	-1.8
	5-6	37.7	-9.6

chromatography of ^{35}S extracted from sediment with 2.5 N HCl, which showed that the radiolabel was associated with $^{35}S_O$ compounds (Table 3.4; see also Chapter 4). Similarly, many ester sulfates, a labile group of S_O compounds, release free sulfate by acid hydrolysis (King and Klug 1980). Clearly, then, removal of solubilized $^{35}S_o$ by the rinsing step must lead to decreased $^{35}S_O$ estimates.

If this solubilization and rinsing away of $^{35}S_O$ were the only problem, this protocol (A) would not lead to negative $^{35}S_O$ estimates, because the solid phase ^{35}S left for $^{35}S_T$ determination would still contain all the $CR^{35}S$, and $^{35}S_O$ = $^{35}S_T$ - $CR^{35}S$. For example, if all of the $^{35}S_O$ were solubilized and rinsed away then $CR^{35}S$ should equal $^{35}S_T$ and result in a calculated $^{35}S_O$ value of zero. However, sulfur in some S_O compounds is recovered by CrII reduction and these compounds have been found in acid extracts of lake sediments (Table 3.4). Characterization of these compounds has indicated that they are organic polysulfides (Chapter 4). If these compounds are present as soluble components in the acid supernatant, they will be measured by CrII reduction but not by NaOBr-HI (ie. S_T) analysis, since protocol A involves a rinse before this step (Fig. 3.1). This situation can lead arithmetically to negative estimates of $^{35}S_O$. Therefore, ^{35}S-organic polysulfides in the acid supernatant cause a decrease in the estimate of $^{35}S_O$ in two ways; i) by increasing the apparent size of the S_I fraction, and ii) by

being rinsed away before NaOBr-HI analysis.

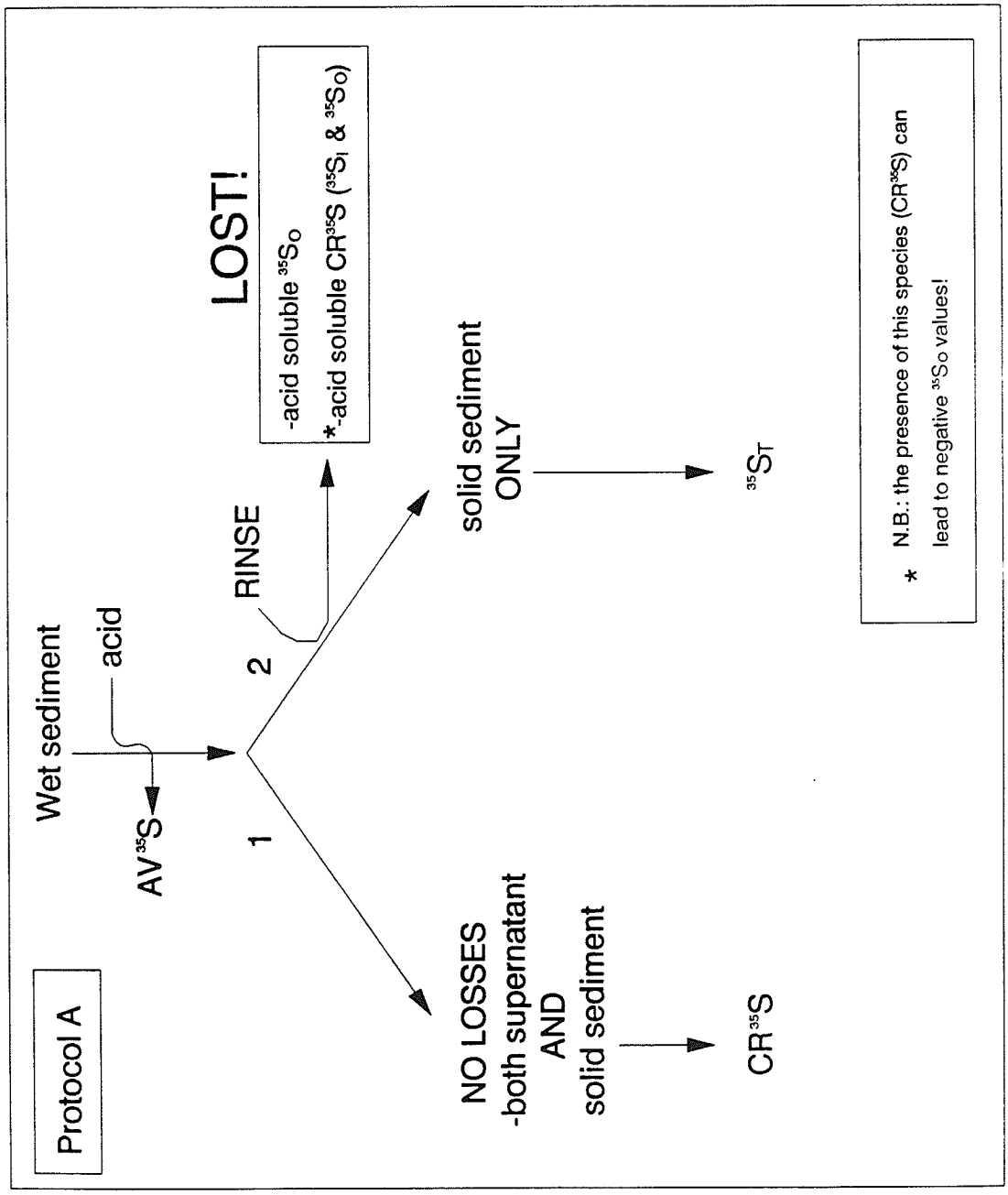
Similarly, inorganic compounds such $S_2O_3^{2-}$, polythionates and polysulfides (Table 3.1) could also lead to apparently negative values for $^{35}S_0$ because they are soluble and CrII reducible, and could be rinsed away prior to NaOBr-HI analysis.

Therefore, protocol A gives minimum estimates of $^{35}S_0$ in sediments. The degree of underestimation will depend on how much $^{35}S_0$ is acid-solubilized and rinsed away and the amount of soluble ^{35}S (organic and inorganic) that is CrII reducible. The potential problems associated with this protocol are summarized in Fig. 3.2.

Interestingly, unlabelled S_0 measured by protocol A in the same sediment samples were never negative and were generally greater than 50% of total S (Table 3.6). These results suggest that indigenous S_0 is chemically different from S_0 produced from recent sulfate reduction. This difference may be due to the fact that S_0 already present in the sediment originated from algal and terrestrial plant and animal sources and not only from sulfate reduction. Furthermore, long-term diagenetic changes in S_0 produced from sulfate reduction could lead to very acid resistant types of S compounds, such as sulfonic acids (Ferdleman et al. 1991).

Negative estimates of $^{35}S_0$ are avoided using protocol B, which does not involve an arithmetic determination (Fig.

Fig. 3.2. Summary outline of the possible problems leading to underestimates and negative estimates by protocol A (see Fig. 3.1 and text)


Protocol A
Wet sediment
acid
AV ^{35}S
1
2
RINSE
LOST!
-acid soluble $^{35}S_O$
*-acid soluble CR^{35}S ($^{35}S_I$ & $^{35}S_O$)
NO LOSSES
-both supernatant
AND
solid sediment
CR ^{35}S
solid sediment
ONLY
$^{35}S_T$
* N.B.: the presence of this species (CR^{35}S) can lead to negative $^{35}S_O$ values!

3.1). Protocol B is also a sequential method and has been used to measure the endproducts of $^{35}SO_4^{2-}$ reduction in peat, where it has some verification (Wieder and Lang 1988). However, as previously discussed, because the procedure involves an initial contact of the sediment with hot, concentrated HCl, during the CrII reduction step, it is expected that sedimentary $^{35}S_0$ will be underestimated due to its solubilization and subsequent removal by the rinsing step (Table 3.3). Interestingly, ester sulfate compounds in peat from Big Run Bog, where protocol B has been used (Wieder and Lang 1988; Yavitt and Lang 1990), are resistant to acid hydrolysis (Jarvis et al. 1987), and in this respect differ from their lacustrine counterparts (King and Klug 1980). Therefore, this protocol is not expected to be suitable for estimating S_0 and $^{35}S_0$ in freshwater sediments.

The third alternative, protocol C, has been used to determine unlabelled S fractions in peat (Wieder and Lang 1988). The same general procedure with only minor modifications is often used in freshwater sediments (e.g. Carignan and Tessier 1987; Landers et al. 1983). This protocol is non-sequential and does not involve acid treatment preceding either CrII or NaOBr-HI analyses (Fig. 3.1). Therefore, underestimates of $^{35}S_0$ due to acid hydrolysis do not occur. However, if S_T is determined on dried, instead of wet, sediment underestimates of S_0 may occur (Amaral et al. 1989).

Analysis of sediments done by protocols B and C showed that the latter gave much higher estimates of $^{35}S_o$ (75-87%; Table 3.7). The large percentages of soluble ^{35}S obtained with protocol B as compared to protocol C (Table 3.7) substantiate the hypothesis that acid addition solubilizes more than just unreacted $^{35}SO_4^{2-}$.

Similar results were obtained for estimates of cold S_o in sediments from different lakes (Table 3.8). Protocol B resulted in significantly lower S_o estimates than protocol C in 4 out of 5 sediments analyzed (Table 3.8), indicating much of the indigenous S_o was solubilized or hydrolyzed by hot acid. The high S_o estimates obtained with protocol A suggest that strong acid alone (at 20-25°C) is not sufficient to solubilize a large percentage of this material without heat (Table 3.8).

Although protocol C gave higher estimates of S_o and $^{35}S_o$, the question still remains as to whether or not it is accurate. The accuracy of protocol C in estimating these fractions depends on the specificity and efficiency of the three steps: rinse, NaOBr-HI analysis and CrII reduction (Fig. 3.1).

Experiments showed the rinsing procedure was very efficient in removing unreacted $^{35}SO_4^{2-}$ from the sediment (Table 3.5), but it is also important to know if other components were also solubilized in this way. In the freshwater sediments used here, soluble radiolabel recovered

Table 3.7. Comparison of ^{35}S-species estimates using two different analysis schemes on each of three slices from a core from Lake 302S. Refer to Fig. 3.1 for details of protocols used.

Protocol	Core slice (cm)	Reduced $^{35}S_I$	Soluble $^{35}S^*$	$^{35}S_O$
		(% of total ^{35}S recovered)		
B	0-1	64.7	32.3	3.0
	1-2	55.4	37.4	13.0
	2-3	44.9	46.6	8.5
C	0-1	75.0	1.9	23.0
	1-2	47.4	0.5	52.0
	2-3	64.6	5.0	35.4

* Soluble ^{35}S refers to the rinse step (see Fig. 3.1).

Table 3.8. Estimates of S_0 in sediment as determined by two different protocols (B and C). Single determinations by each protocol were done on subsamples from core slices of the top 2 cm of sediment from several lakes.

Sediment source	S_0 (μmol S g^{-1} wet weight)		
	Protocol B	Protocol C	B/C
L 223	0.9	1.7	0.52
L 224	1.7	1.7	1.00
L 239	0.9	6.2	0.15
L 240	1.1	2.5	0.44
L 302S	1.4	2.7	0.52

in the rinse fraction (protocol C) travelled with a $^{35}SO_4^{2-}$ standard under paper electrophoresis (data not shown). It was also found that 95% of the radiolabel in rinse fractions was precipitated by the addition of $BaCl_2$ (J. Amaral unpub. data). Both of these results indicate that $^{35}SO_4^{2-}$ is the only important radiolabelled soluble component in these water rinses, unlike the acidic rinses in protocols A and B (Fig. 3.1).

Similarly, as previously discussed, the NaOBr method is reliable provided the efficiency is tested with each sediment used (Table 3.8). However, evidence that some S_o is CrII reducible (Table 3.4) means that protocol C can underestimate S_o if this type of compound is present in significant quantities. Underestimation of S_o and $^{35}S_o$ in the order of 10-20% have been reported for peat and epilnmetic lake sediments (Brown 1986; Chapter 4). Nevertheless, this protocol is the preferred of the three since the degree of S_o underestimation is minimized. This is evident from the results obtained (Tables 3.6, 3.7 and 3.8).

The nature of solid phase S_o in sediment is not well understood. Most studies have focussed on identification of soluble S compounds in high sulfide environments, such as salt marshes and coastal marine sediments (Ferdelman et al. 1991), and not in freshwater (low sulfide) systems where ther is little soluble S_o. The identity of the $^{35}S_o$

solubilized by acid in protocols A and B is not known, although the major portion was likely C-S (Chapter 4). Compounds such as peptides, proteins and individual amino acids could be extracted/hydrolyzed by acid treatment, although these compounds were found to be of minor importance in the ^{35}S-labelled fraction of acid extracts from an ELA sediment (Chapter 4). S bound to hydrolyzed humic compounds may make up a large fraction of the acid-solubilized material.

In conclusion, it was found that sequential analysis of S fractions in several ELA sediments lead to underestimates of S_o and $^{35}S_o$ if preliminary analyses involved strong acid. A scheme which determines total reduced S_I and S_T on separate aliquots of a sediment gave the most reliable estimates of both S_o and $^{35}S_o$. The use of protocols A and B can lead to an underestimation of the importance of S_o in the sulfur cycle as a mechanism of sulfur storage. As a consequence, the amount of alkalinity generated by sulfate reduction in lake sediments may also be severely underestimated if the S_o produced by this process is not quantitatively measured.

3.2. References

Amaral, J.A., R.H. Hesslein, J.W.M. Rudd, and D.E. Fox. 1989. Loss of total sulfur and changes in sulfur isotopic ratios due to drying of lacustrine sediments. Limnol. Oceanogr. 34: 1351-1358.

Armstrong, F.A.J., and D.W. Schindler. 1971. Preliminary chemical characterization of waters in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28: 171-187.

Baker, L.A., N.R. Urban, P.L. Brezonik, and L.A. Sherman. 1989. Sulfur cycling in an experimentally acidified lake, p 79-100. In: Biogenic sulfur cycling in the environment. E. Saltzman and W. Cooper [eds.]. ACS.

Behr, R.S. 1985. Sulfur dynamics in an experimentally acidified mire in northwestern Ontario. M.S. Thesis. University of Manitoba.

Berner, R.A. 1984. Sedimentary pyrite formation: An update. Geochimica et Cosmochimica Acta 48: 605-615.

Brown, K. 1986. Formation of organic sulphur in anaerobic peat. Soil Biology and Biochemistry 18: 131-140.

Brunskill, G.J., D. Povoledo, B.W. Graham and M.P. Stainton. 1971. Chemistry of surface sediments of sixteen lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28: 277-294.

Brunskill, G.J., and D.W. Schindler. 1971. Geography and

bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28: 139-155.

Canfield, D.E.. R. Raiswell, J.T. Westrich, C.M. Reaves, and R.A. Berner. 1986. The use of chromium reduction in the analysis of reduced inorganic sulfur in sediments and shales. Chem. Geol. 54: 149-155.

Carignan, R., and A. Tessier. 1988. The co-diagenesis of sulfur and iron in acid lake sediments of southwestern Quebec. Geochim. Cosmochim. Acta 52: 1179-1188.

Chanton, J.P., and C.S. Martens. 1985. The effects of heat and stannous chloride addition on the active distillation of acid-volatile sulfide from pyrite-rich marine sediment samples. Biogeochemistry 1: 375-383.

Cook, R.B., and D.W. Schindler. 1983. The biogeochemistry of sulfur in an experimentally acidified lake. Ecol. Bull. 35: 115-127.

David, M.B., and M.J. Mitchell. 1985. Sulfur constituents and cycling in waters, seston, and sediments of an oligotrophic lake. 1985. Limnol. Oceanogr. 30: 1196-1207.

Ferdelman, T.G., T.M. Church, and G.W. Luther III. 1991. Sulfur enrichment of humic substances in a Delaware salt marsh sediment core. Geochim. Cosmochim. Acta 55: 979-988.

Fitzgerald, J.W. 1976. Sulfate ester formation and

hydrolysis: a potentially important yet often ignored aspect of the sulfur cycle of aerobic soils. Bacteriol. Rev. 40: 698-721.

Freney, J.R., G.E. Melvile, and C.H. Williams. 1970. The determination of carbon bonded sulfur in soil. Soil Sci. 109: 310-318.

Howarth, R.W. and B.B. Jorgensen. 1984. Formation of ^{35}S-labelled elemental sulfur and pyrite in coastal marine sediments (Limfjorden and Kysing Fjord, Denmark) during short-term $^{35}SO_4^{2-}$ reduction measurements. Geochim. Cosmochim. Acta 48: 1807-1818.

Howarth, R.W. and J.M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnol. Oceanogr. 24: 999-1013.

Johnson, C.M., and A. Ulrich. 1959. Analytical methods for use in plant analysis. Cal. Agric. Exp. Sta. Bull. 766.

Jarvis,B.J., G.E. Lang & R.K. Wieder. 1987. Arylsulphatase activity in peat exposed to acid precipitation. Soil Biol. Biochem. 19: 107-109.

Jorgensen, B.B., and T. Fenchel. 1974. The sulfur cycle of a marine sediment model system. Mar. Biol. 24: 189- 201.

Kelly, C.A., J.W.M. Rudd, R.B. Cook, and D.W. Schindler. 1982. The importance of bacterial processes in regulating the rate of lake acidification. Limnol.

Oceanogr. 27: 868-882.

King, G. M., and M.J. Klug. 1980. Sulfhydrolase activity in sediments of Wintergreen Lake, Kalamazoo County, Michigan. Appl. Environ. Microbiol. 39: 950-956.

Krairapanond, N., R.D. Delaune, and W.H. Patrick, Jr. 1991. Sulfur dynamics in Louisiana coastal freshwater marsh soils. Soil Sci. 151: 261-273.

Landers, D.H., M.B. David, and M.J. Mitchell. 1983. Analysis of organic and inorganic sulfur constituents in sediments, soils and water. Int. J. Environ. Chem. 14: 245-256.

Landers, D.H., and M.J. Mitchell. 1988. Incorporation of $^{35}SO_4^{2-}$ into sediments of three New York lakes. Hydrobiologia 160: 85-95.

Mitchell, M.J., D.H. Landers, D.F. Brodowski, G.B. Lawrence, and M.B. David. 1984. Organic and inorganic sulfur constituents of the sediments in three New York lakes: Effect of site, sediment depth and season. Water Air Soil Poll. 21: 231-245.

Morse, J.W., and J.C. Cornwell. 1987. Analysis and distribution of iron sulfide minerals in recent anoxic marine sediments. Mar. Chem. 22: 55-69.

Nriagu, J.O., and Y.K. Soon. 1985. Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. Geochimica et Cosmochimica Acta 49: 823-834.

Richards, S.R., C.A. Kelly, and J.W.M. Rudd. 1991. Organic volatile sulfur in lakes of the Canadian shield and its loss to the atmosphere. Limnol. Oceanogr. 36: 468-482.

Rudd, J.W.M., C.A. Kelly and A. Furutani. 1986a. The role of sulfate reduction in long term accumulation of organic and inorganic sulfur in lake sediments. Limnol. Oceanogr. 31: 1281- 1291.

Rudd, J.W.M., C.A. Kelly, V. St. Louis, R.H. Hesslein, A. Furutani, and M.H. Holoka. 1986b. Microbial consumption of nitric and sulfuric acids in acidified north temperate lakes. Limnol. Oceanogr. 31: 1267-1280.

Rudd, J.W.M., C.A. Kelly, D.W. Schindler, and M.A. Turner. 1990. A comparison of the acidification efficiencies of nitric and sulfuric acids by two whole-lake addition experiments. Limnol. Oceanogr. 35: 663-679.

Schindler, D.W. 1971. Light, temperature and oxygen regimes of selected lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28: 157-169.

Sweerts, J.P., J.W.M. Rudd, and C.A. Kelly. 1986. Metabolic activities in flocculent surface sediments and underlying sandy littoral sediments. Limnol. Oceanogr. 31: 330-338.

Tabatabai, M.A., and J.M. Bremner. 1970. An alkaline

oxidation method for the determination of total sulfur in soils. Soil Sci. Soc. Am. Proc. 34: 62-65.

Wada, H. 1977. The synthesis of greigite from a polysulfide solution at about 100°C. Bull. Chem. Soc. Jpn. 50: 2615-2617.

Wieder, R.K., and G.E. Lang. 1988. Cycling of inorganic and organic sulfur in peat from Big Run Bog, West Virginia. Biogeochem. 5: 221-242.

Wieder, R.K., G.E. Lang, and V.A. Granus. 1985. An evaluation of wet chemical methods for quantifying sulfur fractions in freshwater wetland peat. Limnol. Oceanogr. 30: 1109-1114.

Zhabina, N.N., and I.I. Volkov. 1978. A method for determination of various sulfur compounds in sea sediments and rocks, p. 735-745. In W.E. Krumbein [ed.]. Environmental biogeochemistry and geomicrobiology. Ann Arbor Science.

4. The Isolation and Characterization of ^{35}S-Organosulfur from $^{35}SO_4^{2-}$ Reduction in a Freshwater Sediment

4.0 Abstract

The chemical characteristics of radiolabelled ^{35}S-organic sulfur compounds ($^{35}S_o$) formed from the reduction of $^{35}SO_4^{2-}$ in freshwater sediment were studied. Previously, S_o has been generally categorized as either C-bonded S or ester sulfate, and C-bonded S has been measured by difference rather than directly. With a variety of extraction and chromatographic methods I eliminated possible chemical interference from S_o and other inorganic sulfur (S_I) forms and found positive evidence for the formation of S_o from sulfate reduction. Carbon bonded S made up the greatest fraction of $^{35}S_o$ (68%) while ester sulfate was a minor product (6%). In addition, evidence for a third type of S_o, containing polysulfur moieties bound to organic molecules, was obtained. This type of compound may be important as a mechanism of S storage that is not linked to iron. A part of this third type of S was chromium reducible and it may cause overestimation of S_I (normally considered to be FeS^o, FeS_2 and S^o) by the chromium reduction assay, and so, overestimate the importance of iron in alkalinity generation by the production of iron sulfides. Therefore, inhibition of alkalinity generation in lakes by iron limitation may not occur. Most of the extracted $^{35}S_o$ compounds were relatively small (<1000 Mw), water soluble, non-volatile molecules.

4.1 Introduction

Bacterial sulfate reduction is an important process in neutralization of acid precipitation in lakes and in storage of sulfur in freshwater and marine sediments. Production of hydrogen sulfide (H_2S) from sulfate consumes hydrogen ions (Hongve 1978), and net alkalinity generation results if sulfur is stored in a reduced form. The reaction of reactive iron with H_2S to form insoluble iron sulfide (iron monosulfide, FeS; greigite, Fe_3S_4; pyrite, FeS_2) is well known (e.g. Cook and Schindler 1983; Berner 1984) and, for some time, was believed to be the only important mechanism in storage of sulfide from bacterially reduced sulfate.

Recently, however, organic matter has also been found to be an important sink for this sulfide. Stable isotope analyses have shown that much organic sulfur in marine and freshwater sediments contains isotopically light sulfur, indicating a pathway of formation via dissimilatory sulfate reduction (e.g. Nissenbaum and Kaplan 1972; Nriagu and Soon 1985; Francois 1987b). Also $^{35}SO_4^{2-}$ incubated in sediments produced both inorganic and organic labelled products (Rudd et al. 1986; Landers and Mitchell 1988; Baker et al. 1989). These findings suggest that a significant part of S_0 found in sediments is formed by the reaction of H_2S, the product of dissimilatory sulfate reduction by bacteria, with organic matter. Sulfur enrichment of marine humic acids in the

upper layers of sediment has been ascribed to this mechanism (Ferdelman et al. 1991; Francois 1987a & b). The other possible mechanism of formation, assimilatory sulfate reduction by biota, is relatively minor in sediments (Nedwell 1982). Therefore, organic material, like iron, can act as an important sulfur sink in sediments.

However, for reasons that are not yet completely clear, large variations in $^{35}S_0$ production can occur between different sediments (Carignan 1988; Herlihy et al. 1988; Giblin et al. 1990). It is important, therefore, to understand how this process of S_0 formation occurs in order to explain and understand these differences in the biogeochemistry of S between study sites. In order to do this, it would be helpful to know what kind of compounds make up this S_0 fraction. Little is known about the nature or character of the S_0 formed from SO_4^{2-} reduction in sediments, and the lack of a direct and specific analytical method has been one of the main factors impeding the study of this type of compound. The present work addresses both of these points.

In this study, the partial characterization of radiolabelled S_0 formed from SO_4^{2-} reduction in lake sediments is described. $^{35}SO_4^{2-}$ was used as a tag for newly formed $^{35}S_0$ to distinguish it from S_0 already present from a variety of sources. Previously, this $^{35}S_0$ was measured by difference, between the total ($^{35}S_T$) and $^{35}S_I$ fractions. In

this study, the $^{35}S_o$ formed was measured specifically rather than simply as the "non-inorganic" fraction. Size exclusion chromatography, dialysis, paper chromatography and electrophoresis, as well as wet chemical methods, were used to isolate and characterize the ^{35}S-organosulfur compounds extracted with aqueous solvents. Several labelled organosulfur components were isolated in this way.

4.2 Materials and Methods

4.2.0. Sample collection and treatment. Sediment from Lake 303, a small (9.93 X 10^4 m^2), shallow (mean depth = 1.5m) Canadian Shield lake at the Experimental Lakes Area (Brunskill and Schindler 1971), was collected by Ekman grab in the summer of 1990. The sediment was a high porosity (0.98), highly organic flocc (C = 28.2% dry weight; N = 3.51 % dry weight; Brunskill et al. 1971) visually described as gyttja. The interstitial water had a pH of 6.5 and has been determined to be low in Fe (30 μmol L^{-1}; Brunskill et al. 1971) and very low sulfate (<3 μmol L^{-1}; J. Amaral, unpub. data). Solid phase S in this sediment consisted of about 60% S_I and 40% S_O (Appendix II). A glass bottle (500 mL) was filled to the top with the upper 4 cm of sediment from several collections, and tightly capped for transport to the laboratory. The data presented were obtained from one incubation. The sediment was homogenized by shaking under N_2 and 50 cc was delivered into a culture tube (53cc) that had been flushed with N_2. The tube was then spiked with ≈10 X 10^6 Bq of carrier-free $^{35}SO_4^{2-}$ and 0.5 μmoles of unlabelled SO_4^{2-}, sealed with an O_2-impermeable butyl rubber stopper, and shaken. Incubation was carried out in the dark (at *in situ* temperature; 20-25°C) for 3 days to allow for complete reduction of the added sulfate. The incubated sediment was then extracted, as described below, to recover the $^{35}S_O$

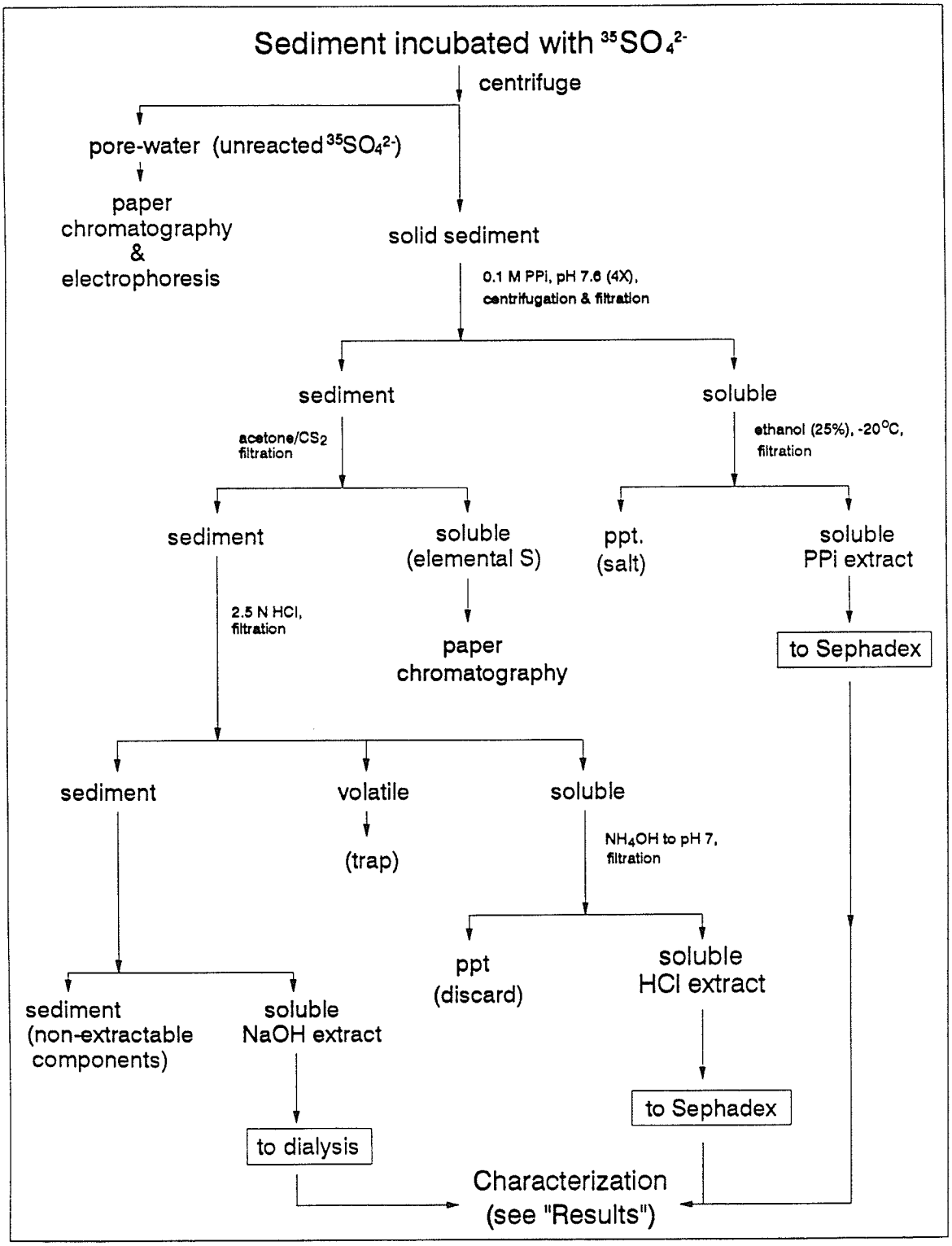
formed from sulfate reduction.

Total $^{35}S_O$ was measured as the difference between total-^{35}S ($^{35}S_T$) and $^{35}S_I$. $^{35}S_T$ was determined on 0.5 g of wet sediment before extraction, by the method of Tabatabai and Bremner (1970), and as described in Amaral et al. (1989). Chromium (CrII) reduction (Howarth and Merkel 1984) was used to measure $^{35}S_I$ in another 0.5 g of sediment before extraction. This method is believed to be specific for non-sulfate, S_I, and has been shown ineffective for various S_O compounds (Wieder et al. 1985; Canfield et al. 1986). Extracted fractions, and remaining non-extractable sediment components, were analyzed in the same way, except that the $^{35}S_T$ in the former was determined directly by scintillation counting of 10-50 μL of the solution. Carbon-bonded ^{35}S in extracts was estimated as the difference between $^{35}S_T$ and hydriodic acid reducible ^{35}S ($HIR^{35}S$)(Casagrande et al. 1979). HI acid mixture (Johnson and Ulrich 1959), prepared and used as described in Amaral et al. (1989), reduces all non-pyritic S_I as well as sulfate and ester sulfates (Wieder et al. 1985). Therefore, the $^{35}S_T$ minus $HIR^{35}S$ fraction may contain both $Fe^{35}S_2$ and $C-^{35}S$. Chromium reduction was performed on a sediment aliquot which had previously been analyzed by HI to determine if $Fe^{35}S_2$ was present in significant quantities. $H_2{}^{35}S$ released by these methods was trapped in zinc acetate-NaOH (Howarth and Teal 1979), and the radioactivity quantified by scintillation counting of

trap aliquots.

4.2.1. Extraction procedure. The general extraction procedure used is summarized in Fig. 4.1. Pore-water was first partly removed (≈60%) from incubated sediment by centrifugation (20 min. at 3000 rpm). The sediment pellet was then extracted 4 times with 4 volumes of deoxygenated 0.1 M sodium pyrophosphate-NaOH buffer (PPi), adjusted to pH 7.6 with KH_2PO_4 (Strickland and Fitzgerald 1985). This relatively mild procedure for extracting humic compounds was used because the strongly basic extractants commonly employed (0.1-0.5 N NaOH) have been shown to yield falsely high estimates of the S content of marine humic acids (Francois 1987a). Extractions with PPi were carried out under a N_2 atmosphere with occasional shaking at 4°C for 8, 8, and 1 hours. One last extraction was done with high speed blending (Waring blender) for a total of 10 min. (10 min. on ice after 2 min. of blending). Supernatant was separated from the remaining solid sediment by centrifugation. Extracts were pooled and filtered under N_2 through GF/D and Nucleopore (0.45 μm) filters to remove particulates. Ethanol (25%) was added to the filtrate and the solution placed at -20°C to precipitate buffer salt. The salt was removed by filtration (GF/D) and the filtrate concentrated by rotoevaporation at 50°C. The labelled components in the concentrated filtrate were then separated

Fig. 4.1 . The extraction procedure used to solubilize ^{35}S-labelled organosulfur compounds produced in the sediment.


Sediment incubated with $^{35}SO_4^{2-}$
centrifuge
pore-water (unreacted $^{35}SO_4^{2-}$)
paper chromatography & electrophoresis
solid sediment
0.1 M PPi, pH 7.6 (4X), centrifugation & filtration
sediment
soluble
acetone/CS_2 filtration
ethanol (25%), -20°C, filtration
sediment
soluble (elemental S)
ppt. (salt)
soluble PPi extract
2.5 N HCl, filtration
paper chromatography
to Sephadex
sediment
volatile
soluble
(trap)
NH_4OH to pH 7, filtration
ppt (discard)
soluble HCl extract
sediment (non-extractable components)
soluble NaOH extract
to Sephadex
to dialysis
Characterization (see "Results")

by Sephadex gel chromatography.

S^o was removed from the remaining solid sediment by first rinsing with acetone (2 volumes) and then extracting with carbon disulfide (4 volumes; room temperature) until the radioactivity solubilized decreased to 5% of the total extracted. The solvents were removed from the solid sediment by filtration (GF/D). The filtrate was concentrated under vacuum and chromatographed on paper. The sediment (retentate) was then hydrolyzed with 100 mL of 2.5 N HCl (3 hours at 20°C and 15 min. at 100°C) to extract compounds resistant to PPi. The headspace of the extraction vessel was constantly flushed with oxygen-free N_2, and acid volatile sulfide (AVS= mostly FeS; e.g. Chanton and Martens 1985) was trapped in a zinc acetate-NaOH trap. A trap aliquot was counted in a scintillation counter. The non-volatile acid extract was adjusted to pH 7 with NH_4OH and filtered through GF/D and Nucleopore (0.45 μm) filters before concentration by rotoevaporation at 50°C, in preparation for Sephadex chromatography. ^{35}S not solubilized by either PPi buffer or strong acid was extracted with 2 N NaOH (12 hrs., room temp) (Strickland and Fitzgerald 1984). After filtation as above, the extract was dialysed against distilled water (see below). Any ^{35}S remaining after this step was considered to be non-extractable.

4.2.2. Separation of labelled components. Sephadex gel

columns were used to separate the different ^{35}S labelled components in filtered and concentrated PPi and HCl extracts (Fig.4.1). These extracts were first applied to a Sephadex G-10 column (bed volume 200mL) and eluted with distilled water. Volumes of sample not exceeding 5% of the bed volume were loaded on the gels. Column eluates were collected in test tubes (LKB Ultrorac fraction collector) in 3-5 mL volumes and monitored for radioctivity by scintillation counting (LKB Rackbeta). Test tubes corresponding to each radioactivity peak were pooled and the eluate concentrated by rotoevaporation. Peaks recovered in this way were in turn run through a Sephadex LH-20 column (bed volume 480 mL) with 50% methanol to further resolve labelled components. In cases where high salt concentration precluded Sephadex chromatography (eg., part of the PPi fraction; Fig. 4.1), liquid-liquid extractions with different solvents were used first to separate labelled components before separation with Sephadex was done. The NaOH-extracted material was separated into high and low molecular weight (HMW, >1000 and LMW, < 1000) fractions by dialysis (see below). All isolated components were stored at -20°C in vials flushed with N_2 gas until further analysis.

4.2.3. Characterization of isolated components.

Several methods were used to determine some chemical properties of isolated ^{35}S-labelled components and to

determine their nature (ie., organic or inorganic). The techniques used are listed below.

a) Electrophoresis and chromatography.

Electrophoresis of separated ^{35}S-labelled components was carried out on Whatman no. 1 paper strips (4.5 X 46 cm wide) in 0.1 M sodium acetate-acetic acid buffer (pH 4.5) (Fitzgerald et al. 1982). A water-cooled, flat plate electrophoresis unit with a high voltage source (Savant Instruments Inc.) was used. Samples were applied (25 to 150 μL) to the midpoint of the paper strips, which were then subjected to 2,500 V for 30-45 min. Electrophoretic mobilities are reported with reference to the mobility of SO_4^{2-} (R_{SO4}) which travels about 13-16 cm towards the anode under these conditions.

Ascending paper chromatography was done on Whatman no. 1 paper strips (4.5 X 22 cm). Samples (25 to 150 μL) were applied in a line to one end of the strips and chromatographed with ethanol (95%): water: butanol (5:2.5:1), or hexane, for 3 to 4 hours. Chromatographic mobilities were expressed relative to the solvent front (R_f).

Radiolabelled components on strips were located by a radiochromatogram scanner (Nuclear Chicago). Positional accuracy of scanning was determined by scintillation counting of 1 cm sections of the scanned strip. Known S

compounds ($Na_2{}^{35}SO_4$, $Na_2S_2O_3$, Na_2SO_3, $^{35}S^o$, methionine, cysteine, cystine, ß-mercaptopropionate and polysulfides) were used to compare their electrophoretic and chromatographic mobilities with isolated radiolabeled components.

b) Wet Chemical methods.

The types of sulfur linkages in isolated ^{35}S-components were determined by chromium (CrII) reduction and hydriodic acid (HI) reduction, as described above, as well as reactivity with KCN. Cyanide was used to determine the presence of polysulfur components by the formation of thiocyanate ion (SCN^-). Potassium cyanide powder was added to a small volume of sample to a final concentration of 0.5% and the solution was kept at 45°C for 30 min. SCN^- was separated from bulk material by chromatography with ethanol-water-butanol solvent (R_f = 0.74) and visualized as a pink band by spraying the strip with 10% ferric nitrate (Brown 1986).

PPi and NaOH extracts were also subjected to strong acid (HCl, 2.5 N final concentration) at 20°C, 100°C, and 120°C (autoclave) for 20 min to 4 hrs, to further elucidate the nature of ^{35}S-compounds present. For example, release of S^o from high molecular weight humic material upon acidification is considered to originate from organic polysulfides (Francois 1987a & b), while release of SO_4^{2-} indicates the presence of sulfate esters (eg. King and Klug

1980). The presence or absence of $^{35}S^{o}$ and $^{35}SO_4^{2-}$ before and after acidification was determined by paper chromatography and electrophoresis.

Chromatographic and electrophoretic strips were sprayed with different reagents after scanning for radiolabel. Ninhydrin spray (Sigma) was used to detect the presence of amino acids, peptides, proteins or other amines. Reducing sugars were detected with ρ-anisidine hydrochloride (3% in n-butanol; developed at 100°C for 2-10 min.; Macek 1963). 2,2'-Dithio-bis(5-nitro-pyridine) (DNTP) was used to visualize reduced S compounds, such as thiols , and oxy-sulfur compounds more reduced than sulfate, such as sulfite and thiosulfate (Grassetti and Murray 1969; Vairavamurthy and Mopper 1990). Toluidine blue (0.3% in acetone-water) was used to visualize acidic compounds such as acidic polysaccharides (Macek 1963). These reagents were used when their color development was not masked by the natural color of extracted material.

c) Molecular weight determination.

Spectrapor 2 (MWCO 12000-14000) and 6 (MWCO 1000) dialysis tubings were used to estimate the size of radiolabelled components. Dialysis was carried out at 4°C with 20 volumes of deionized water for 12 hours. The dialysis bag was removed and the solvent evaporated by blowing hot air from a hair blower, 0.5 m distant, until the

volume approached that of pre-dialysis or less. The sample was then re-dialyzed for 6 hours with 20 volumes of fresh deionized water. The radioactivity in the dialyzed solution and dialysates was determined by scintillation counting.

d) Gas chromatography

Gas chromatography was used to isolate and purify volatilizable components for possible identification by mass spectrometry (GC/ms). A Hewlet Packard 5072A GC equipped with a FID detector and a 6 foot glass column (0.24" I.D.) packed with 20% SE-30 on Chromsorb P was used. A proportional counter (Nuclear Chicago) was used to detect radioactivity in volatile components. Column effluent was split so that 80% was channeled to the proportional counter.

4.2.4. Isotope exchange considerations. Recently, it has been shown that the use of ^{35}S radioisotope to measure the rate of formation of different S fractions in sediments can lead to erroneous results due to isotopic exchange processes (Fossing and Jorgensen 1990a, b). This process is not expected to significantly affect the results presented here since it occurs readily with some S_I compounds, but not S_O, the subject of this work. Isotope exchange between between S° and H_2S is mediated by polysulfides which form from their reaction. Other reactive forms of sulfur (sulfite, thiosulfate, etc) have also been shown to undergo

isotope exchange (Voge 1939). However, more stable forms of S compounds do not undergo appreciable isotope exchange. For example, no isotope exchange was observed between S° and carbon disulfide (Cooley et al. 1939). Furthermore, the author observed no isotope exchange with cysteine in a system containing cysteine, H_2S and $^{35}S°$, over a 28 hr period (Appendix III). Similarly, isotope exchange between several S_I compounds and sulfate has also been found to be negligible (Voge 1939; Fossing and Jorgensen 1990a). Therefore, neither carbon bonded S nor ester sulfates are expected to become labelled by isotope exchange processes. In support of this conclusion, longterm incubation with unlabelled SO_4^{2-} added to sediment distributed into S_O and S_I fractions in in similar proportions as did a single pulse of $^{35}SO_4^{2-}$ (Chapter 6). The organic polysulfides demonstrated in this freshwater sediment (see Results) could possibly participate in isotope exchange processes. Thus, estimates of their rates of formation could be either over or underestimated.

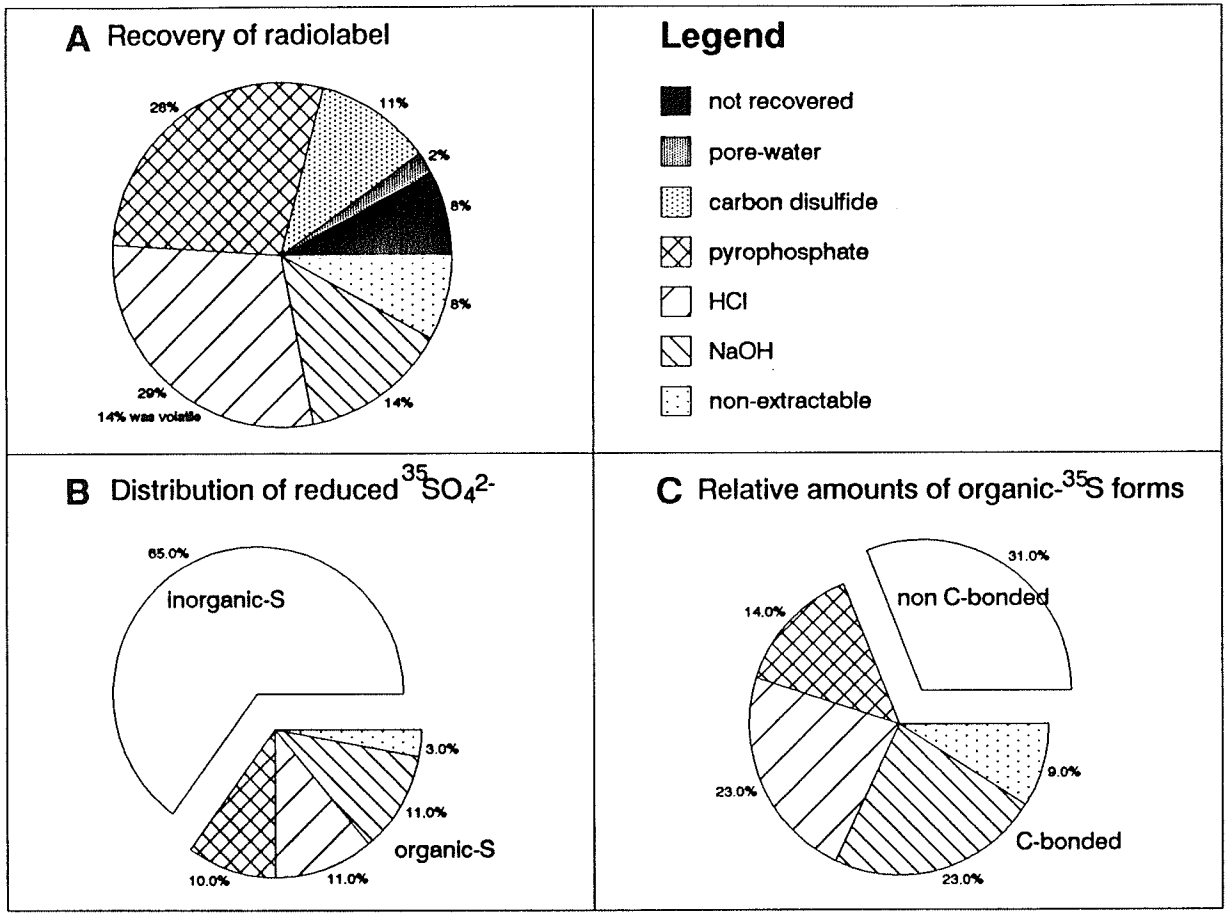
4.3 Results

4.3.0. Extraction of ^{35}S-labelled material.

The extraction procedures (Fig. 4.1) removed 84% of the radiolabel added to the sediment (Fig. 4.2a). The organosulfur compounds produced were polar in nature. $^{35}S^{o}$ was the only significant labelled component in sediment extractions with non-polar solvents, such as dichloromethane (data not shown). About 8% of the ^{35}S remained tightly associated with the solid sediment matrix even after treatment with strong acid and base (Fig. 4.2a). Together, these fractions accounted for 92% of the total radiolabel added. A small amount (8%; Fig. 4.2a) of the label added was not recovered probably due to loss to vessel walls during the various manipulations. It was assumed that their "missing" ^{35}S was reduced since only 2% of the label was recovered in the pore-water as unreacted $^{35}SO_4^{2-}$.

The $^{35}S_o$ contents of the individual extracts and the non-extractable fraction were estimated (total ^{35}S minus chromium reducible ^{35}S ($CR^{35}S$)) and the sum of these was 35% of the total reduced $^{35}SO_4^{2-}$ (Fig. 4.2b). This value was similar to the $^{35}S_o$ content of the sediment measured on an aliquot before extraction (30%)(Fig 4.2b). Essentially all of the $^{35}S_o$ produced was removed by the extraction scheme described (Fig. 4.1), with only 3% remaining with the solid sediment (Fig. 4.2b). Most of the $^{35}S_o$ formed (68%) was

Fig. 4.2. The recovery and distribution of ^{35}S-radiolabel added to the sediment. Part A shows the fraction of ^{35}S recovered in different extracts and residual sediment. Part B shows the distribution of $^{35}SO_4^{2-}$-S into $^{35}S_O$ (35%) and $^{35}S_I$ (65%) as a percentage of the total $^{35}SO_4^{2-}$ reduced. Measurement of these fractions on whole sediment, before extraction, gave a distribution of 30% $^{35}S_O$ and 70% $^{35}S_I$. Part C, shows the proportion of $^{35}S_O$ that is carbon-bonded. The fraction which is not carbon-bonded is usually considered to be organic sulfate (ester sulfate). All results are from single determinations.


A Recovery of radiolabel
26%
11%
2%
8%
8%
29%
14% was volatile
14%
Legend
not recovered
pore-water
carbon disulfide
pyrophosphate
HCl
NaOH
non-extractable
B Distribution of reduced $^{35}SO_4^{2-}$
65.0%
inorganic-S
3.0%
11.0%
organic-S
10.0%
11.0%
C Relative amounts of organic-^{35}S forms
31.0%
non C-bonded
14.0%
9.0%
23.0%
C-bonded
23.0%

composed of carbon-bonded ^{35}S ($C-^{35}S$), as determined by hydriodic acid analysis (total ^{35}S minus $HIR^{35}S$)(Fig. 4.2c). HI reduced all but 4% of the $CR^{35}S$ in the sediment (Table 4.1) which suggests little $Fe^{35}S_2$ (non-HI reducible) was present in the sediment.

Electrophoretic and chromatographic analysis showed that radiolabel in porewater and acetone-carbon disulfide fractions was not S_0 but SO_4^{2-} and S°, respectively (data not shown). Only PPi, HCl and NaOH extractions removed significant amounts of labelled organosulfur compounds (Fig 4.2b). Much of the label recovered using HCl (about 50%) was acid volatile (Fig. 4.2a).

4.3.1. Separation of extracted radiolabeled components

Several labelled components were resolved from each crude extract. Compounds in both pyrophosphate (PPi) and HCl hydrolysis extracts were first separated using a Sephadex G-10 column (Fig. 4.3). PPi gave two major peaks of radioactivity (P-1 and P-2), as indicated in Figs. 4.3 and 4.4. The first peak (P-1), which contained larger sized compounds, was further separated into three peaks (P-1-1, P-1-2, and P-1-3) by a Sephadex LH-20 gel with 50% methanol (Fig. 4.4). A fraction of P-1 (25%) was insoluble in 50% methanol (Fig. 4.3) and the resulting precipitate, designated P-1-H_2O, was removed by filtration before loading the LH-20 column (Fig. 4.3). The second peak (P-2) was

Table 4.1. The similar reactivity of $CR^{35}S$, $HIR^{35}S$ and cyanide-reactive fractions in sediment from Lake 303 (incubated with $^{35}SO_4^{2-}$). Single determinations were done.

Chemical treatment	whole sediment	whole HCl extract
	% of the ^{35}S reduced by treatment	
CrII reduction	70%	44%
CrII red. (post HI reduction)	4%	nd*
CrII red. (post cyanide)+	nd	9%

* nd = not determined

\+ Cyanide reacted with $^{35}S^{o}$ and organic polysulfides to produce $^{35}SCN^{-}$. The $^{35}SCN^{-}$ was removed by paper chromatography before CrII reduction.

Fig. 4.3. The procedure used for the separation of ^{35}S-labelled components in each of the sediment extracts described in Fig. 4.1. The components in bold type were characterized by physical and chemical methods. Numbers in brackets are the % of total $^{35}S_o$ contained in each component.

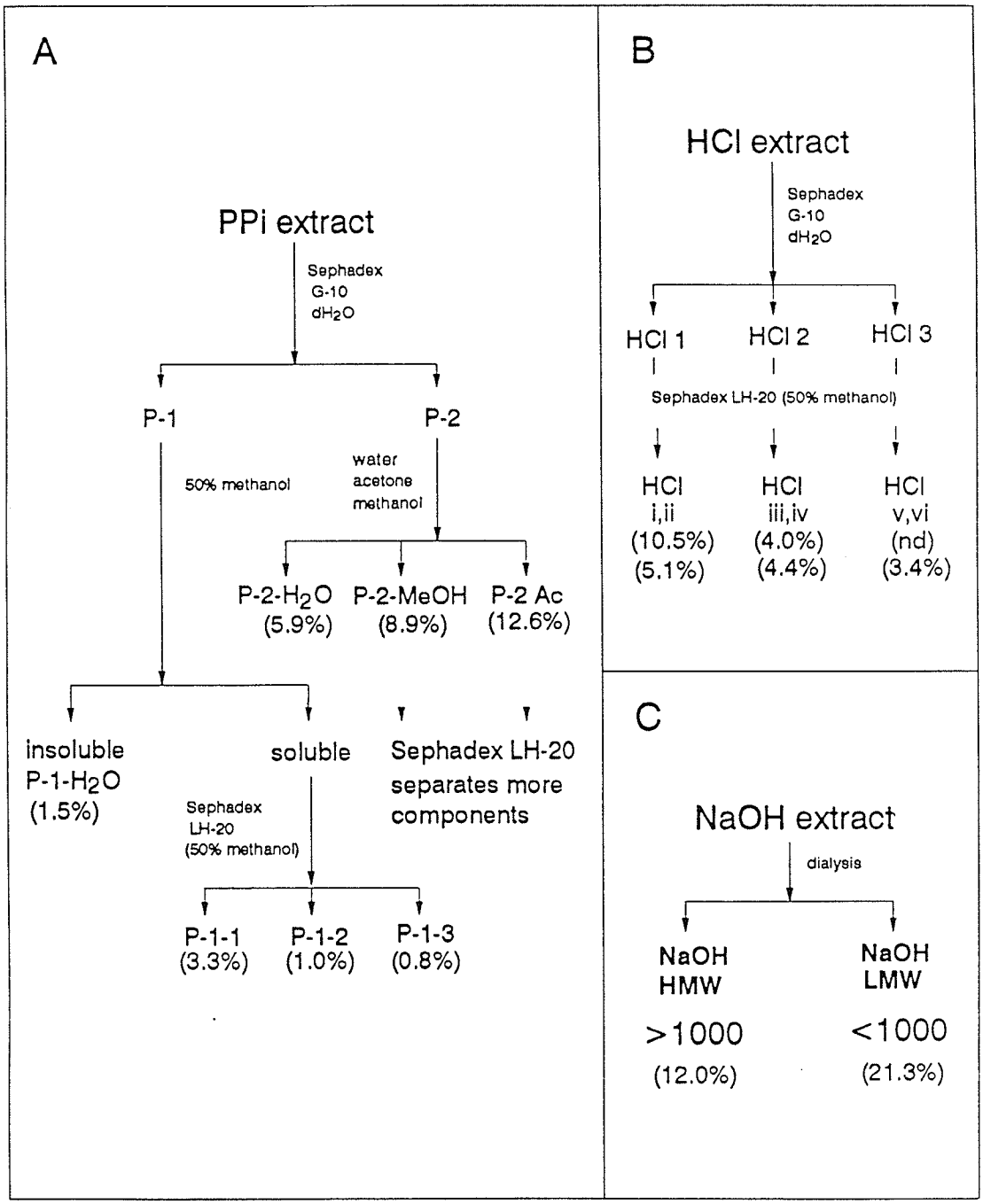
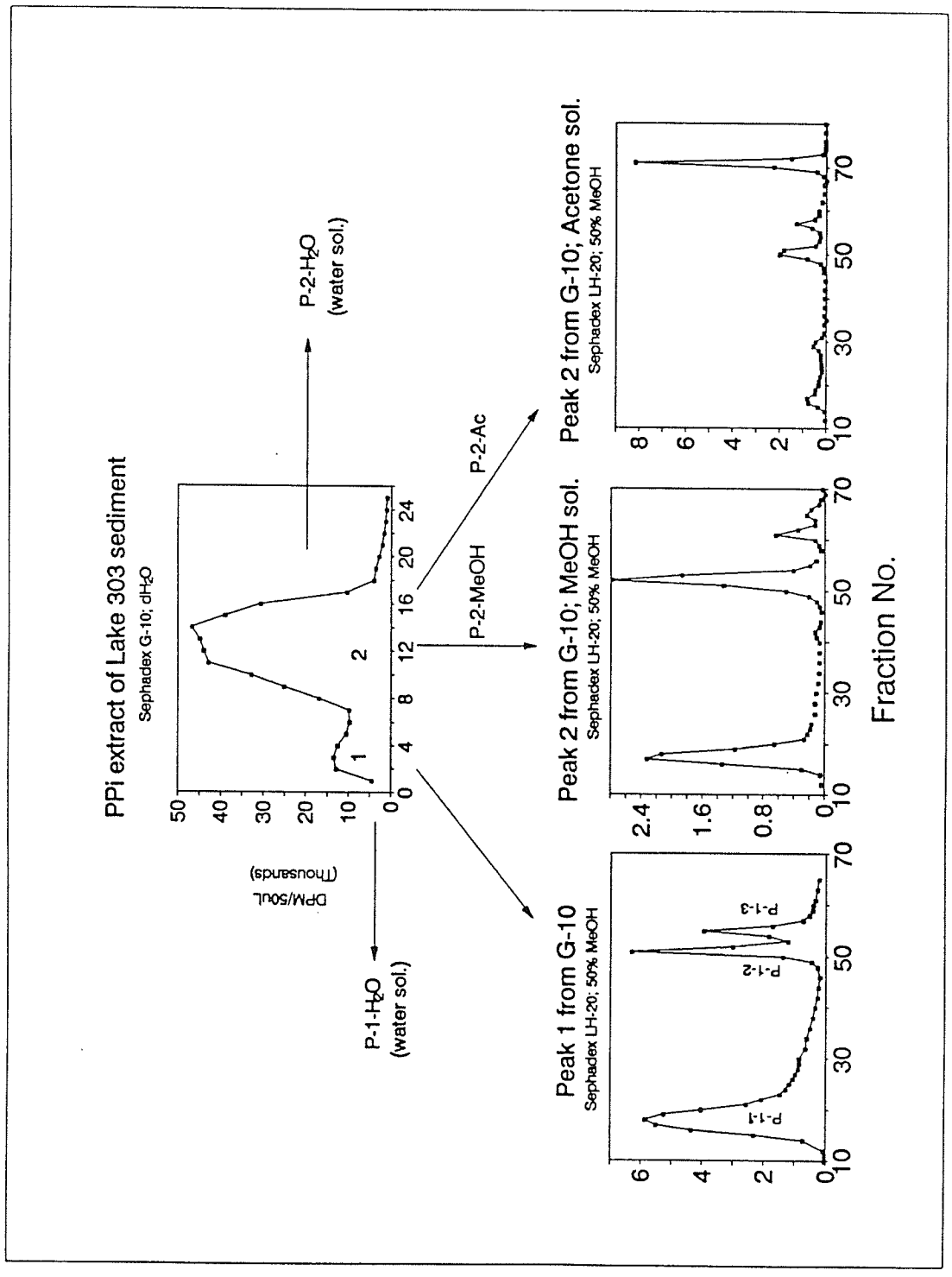

A
PPi extract
Sephadex
G-10
dH2O
P-1
P-2
50% methanol
water
acetone
methanol
P-2-H2O
(5.9%)
P-2-MeOH
(8.9%)
P-2 Ac
(12.6%)
insoluble
P-1-H2O
(1.5%)
soluble
Sephadex LH-20
separates more
components
Sephadex
LH-20
(50% methanol)
P-1-1
(3.3%)
P-1-2
(1.0%)
P-1-3
(0.8%)
B
HCl extract
Sephadex
G-10
dH2O
HCl 1
HCl 2
HCl 3
Sephadex LH-20 (50% methanol)
HCl
i,ii
(10.5%)
(5.1%)
HCl
iii,iv
(4.0%)
(4.4%)
HCl
v,vi
(nd)
(3.4%)
C
NaOH extract
dialysis
NaOH
HMW
>1000
(12.0%)
NaOH
LMW
<1000
(21.3%)

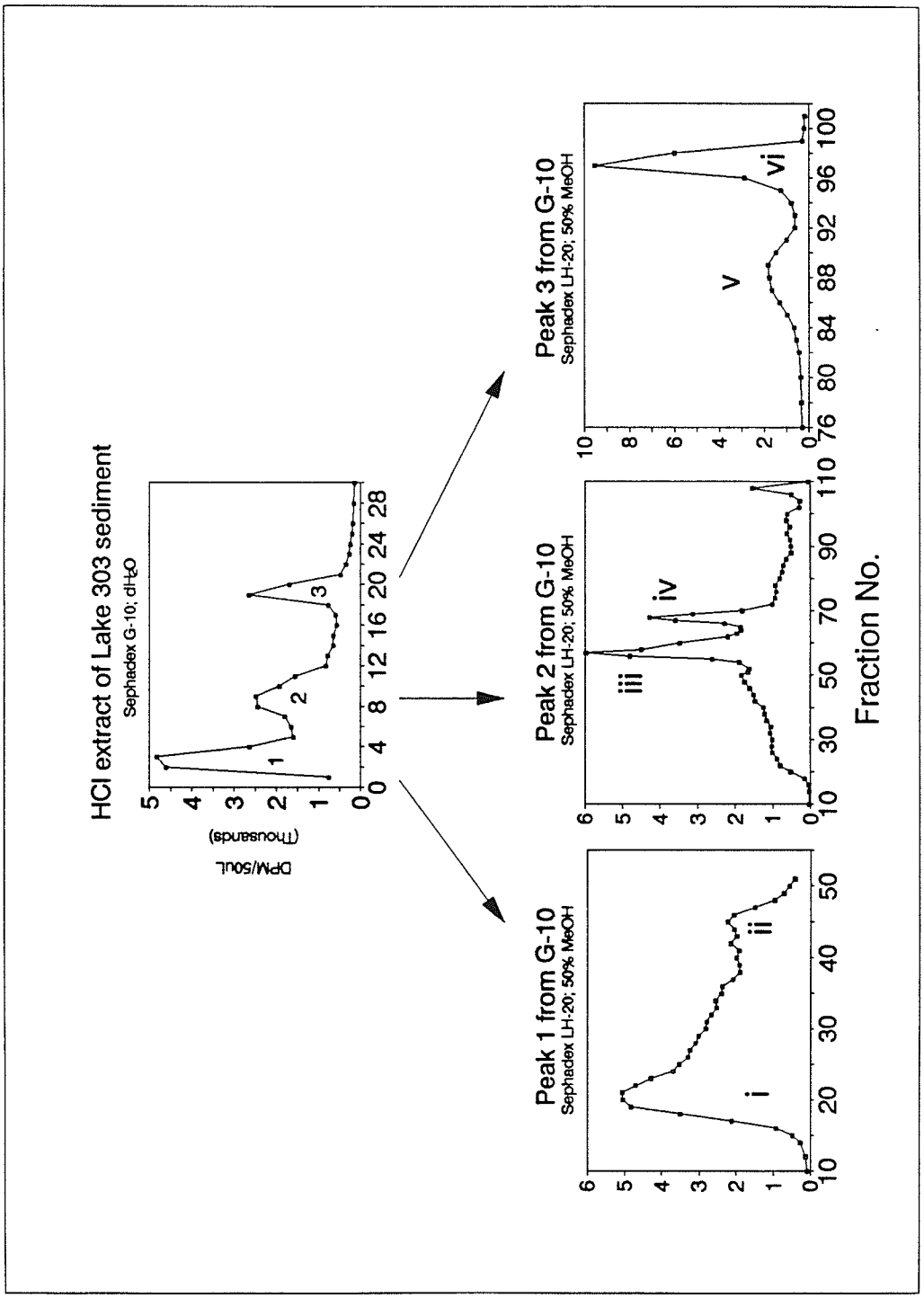
Fig. 4.4. The resolution, by Sephadex gel chromatography, of different ^{35}S-labelled components in the PPi extract. The second peak (P-2), obtained with G-10 gel, was separated into water, methanol and acetone soluble fractions. These fractions were chemically characterized at this stage, although methanol and acetone soluble material was amenable to chromatography with LH-20 gel.


PPi extract of Lake 303 sediment
Sephadex G-10; dH2O
DPM/50uL (Thousands)
P-1-H2O (water sol.)
P-2-H2O (water sol.)
P-2-MeOH
P-2-Ac
Peak 1 from G-10
Sephadex LH-20; 50% MeOH
P-1-1
P-1-2
P-1-3
Peak 2 from G-10; MeOH sol.
Sephadex LH-20; 50% MeOH
Peak 2 from G-10; Acetone sol.
Sephadex LH-20; 50% MeOH
Fraction No.

associated with high salt concentration and attempts to run it through LH-20 gel were unsuccessful due to crystallization of salt which impeded flow through the column. Therefore, radiolabelled components in this fraction were separated based on their solubility in water (P-2-H_2O), methanol (P-2-MeOH) and acetone (P-2-Ac). Components of the methanol and acetone extracts could be further resolved with LH-20 gel (Fig. 4.4), although only the components separated based on solubility in the three solvents were further characterized (Fig. 4.3). The amount of radiolabel extracted by PPi did not decrease to low levels with increasing numbers of extractions, as would be expected. Sephadex chromatography showed that the majority of P-1 activity was obtained in the first two extractions and subsequent extractions contained mostly P2 activity (data not shown). Furthermore, radioactivity in P-2 had high R_{SO4} values, as might be expected for S_I compounds (see below). The later extractions were probably causing hydrolysis of esters or other compounds and so the number of PPi extractions was confined to four.

The HCl extract was resolved into three main radioactive peaks with G-10 gel (HCl-1 to HCl-3; Figs. 4.3 and 4.5). A total of six different peaks of activity (HCl i to vi) were resolved when each of these peaks was subsequently run on LH-20 gel (Fig. 4.5). All of the radiolabel in the acid extract was soluble in 50% methanol.

Fig. 4.5. The resolution, by Sephadex gel chromatography, of different ^{35}S-labelled components in the HCl extract.


HCl extract of Lake 303 sediment
Sephadex G-10; dH2O
DPM/50uL
(Thousands)
1
2
3
Peak 1 from G-10
Sephadex LH-20; 50% MeOH
i
ii
Peak 2 from G-10
Sephadex LH-20; 50% MeOH
iii
iv
Peak 3 from G-10
Sephadex LH-20; 50% MeOH
v
vi
Fraction No.

This fraction contains hydrolysis products and fulvic acids not extracted with PPi (Strickland and Fitzgerald 1984). The labelled components resolved were named HCl i-vi (Figs. 4.3 and 4.5).

The 2 N NaOH was used to remove radiolabel associated with humin, a fraction of organic matter in sediment and soil which is not solubilized by dilute acid or base (Schnitzer 1978). Sephadex chromatography was not practical with this fraction due to high salt concentration and so components were separated by dialysis into a high molecular weight fraction (HMW; largely >1000 MW), and a low molecular weight fraction (LMW; <1000 MW). The LMW fraction was alcohol soluble while HMW was not.

4.3.2. Characterization of isolated ^{35}S-labelled compounds

a) Molecular weight estimation

Approximately 90% of the radiolabel extracted by all the different methods passed through a dialysis bag with a molecular weight cut off of 1000. Only components P-1-H_2O, HCl i and HMW (NaOH) contained radiolabelled compounds of >1000 MW (Tables 4.2-4.4). Molecular weight estimates were based only on dialysis because the high salt content and nature of extracted compounds prevented accurate determination of molecular weights by Sephadex gel elution (Pharmacia 1987).

Table 4.2. Chemical characterization of radiolabelled components in pyrophosphate (PPi) buffer (pH 7.6) extract of Lake 303 sediment (incubated with $^{35}SO_4^{2-}$). The components were separated by Sephadex G-10 and LH-20 chromatography.

Component name	Mw	%CRS	%HIRS	%SCN^-	% of total org-^{35}S	No. of sub-components*
P-1-H_2O	>12000	32	4	nd+	1.5	1
P-1-1	<1000	27	42	42	3.3	2
P-1-2	<1000	16	56	50	1.0	4
P-1-3	<1000	23	50	24	0.8	6
P-2-H_2O	<1000	21	97	0	5.9	1
P-2-MeOH	<1000	28	68	14	8.9	2
P-2-Ac	<1000	58	62	28	12.6	6

* Subcomponents determined by paper electrophoresis and chromatography.

\+ nd= not determined

Table 4.3. Chemical characterization of radiolabelled components in hydrochloric acid extract of Lake 303 sediment (incubated with $^{35}SO_4^{2-}$). The components were separated by Sephadex G-10 and LH-20 chromatography.

Component name	Mw	%CRS	%HIRS	%SCN^-	% of total org-^{35}S	No. of sub-components*
HCl i	25% >1000	40	33	35	10.5	2
	75% <1000					
HCl ii	<1000	25	60	26	5.1	3
HCl iii	<1000	15	32	29	4.0	3
HCl iv	<1000	6	23	+[+]	4.4	3
HCl v	<1000	nd[#]	nd	0	--	1
HCl vi	<1000	33	37	+	3.4	1

* Subcomponents determined by paper electrophoresis and chromatography

[+] SCN^- was not quantified due to co-migration with other labelled component(s).

[#] nd= not determined

Table 4.4. Chemical characterization of radiolabelled components in the NaOH extract of Lake 303 sediment (incubatedwith $^{35}SO_4^{2-}$). The components were separated by dialysis.

Component name	Mw	%CRS	%HIRS	%SCN^-	% of total org-^{35}S	No. of sub-components*
HMW	>1000	20	25	14	12.0	2
LMW	<1000	16	49	19	21.3	2

* Subcomponents determined by paper electrophoresis and chromatography.

b) Paper chromatographic analysis

Paper chromatography separated several more radiolabelled contituents (often associated with a brown or yellow color) from most components isolated by Sephadex chromatography (Tables 4.2-4.4). Some of the resolved compounds were present in very small amounts (Tables 4.5-4.7), and in general, each Sephadex-isolated mixture was dominated by one or two different paper chromatographically-separated components. The R_f values obtained were usually much lower than that expected for S^o (Table 4.8; 0.89) in the solvent system used. Components which had R_f values similar to S^o were chromatographed with hexanes in which S^o travels with the solvent front. The author found no extracted radiolabelled component that showed an R_f similar to that of S^o with hexanes as the solvent system. However, several of the components had R_f values similar to some of the common oxy-sulfur compounds (Table 4.8; sulfate and thiosulfate, R_f= 0.26 and 0.33), and so could not be positively considered to be organic molecules.

c) Paper electrophoretic analysis

Paper electrophoresis resolved several radiolabelled constituents in Sephadex-isolated components. Each Sephadex peak was generally dominated by one or two constituents (Tables 4.5-4.7). The majority of the ^{35}S label in larger sized components (P-1-1, HCl i, HMW) was associated with

Table 4.5. R_f and R_{SO4} values (as determined by paper chromatography and electrophoresis) of labelled sub-components of different radioactive fractions separated from PPi (pH 7.6) extract by Sephadex chromatography. Numbers in brackets refer to the relative amount of the subcomponents as a % of the total radioactivity on the paper strip.

Component name	Fraction of total ^{35}S added (%)	Electrophoretic mobility (R_{SO4}) pH 4.5	Chromatographic mobility (R_f) EtOH:H_2O:BuOH (5:2.5:1)
P-1-H_2O	0.8	0.11	0.0
P-1-1	1.5	0.0 0.8	0.0
P-1-2	0.4	0.0 0.07 0.21 1.00 (5%)	0.0 (24%) 0.26 (48%) 0.64 (27%)
P-1-3	0.3	0.0 0.08 0.17 0.33 0.48 1.00 (5%)	0.0 (45%) 0.13 (28%) 0.50 (16%) 0.62 (11%)
P-2-H_2O	2.5	0.0	0.0 (100%)
P-2-MeOH	4.2	0.02 (22%) 0.14 (38%) 0.72 (19%) 0.95 (20%)	0.0 (64%) 0.11 (28%)
P-2-Ac	10.1	0.02 0.17 0.41 0.73 (43%) 1.05	0.0 (27%) 0.15 (10%) 0.38 (41%) 0.57 (5%) 0.77 (12%) 0.95 (5%)

Table 4.6. R_f and R_{SO4} values (as determined by paper chromatography and electrophoresis) of labelled sub-components of different radioactive fractions separated from HCl extract by Sephadex chromatography. Numbers in brackets refer to the relative amounts of the subcomponents as a % of the total radioactivity on the paper strip.

Component name	Fraction of total ^{35}S added (%)	Electrophoretic mobility (R_{SO4}) pH 4.5	Chromatographic mobility (R_f) EtOH:H_2O:BuOH (5:2.5:1)
HCl i	5.9	0.17 (95%) 0.76	0.0 (79%) 0.37 (21%)
HCl ii	2.3	0.05 0.14	0.0 (21%) 0.07 (53%) 0.42 (15%)
HCl iii	1.6	0.0	0.07 (68%) 0.41 (21%) 0.68 (11%)
HCl iv	1.6	0.0	0.23 (24%) 0.50 (39%) 0.67 (37%)
HCl v	1.2	0.20	0.82
HCl vi	1.7	0.05	0.64

Table 4.7. R_f and R_{SO4} values (as determined by paper chromatography and electrophoresis) of labelled sub-component of different radioactive fractions separated from NaOH extract by dialysis. Numbers in brackets refer to the relative amount of the subcomponents as a % of the toal radioactivity on the paper strip.

Component name	Fraction of total ^{35}S added (%)	Electrophoretic mobility (R_{SO4}) pH 4.5	Chromatographic mobiltiy (R_f) EtOH:H_2O:BuOH(5:2.5:1)
HMW	5.1	0.0 0.14	0.0 (65%) 0.26 (35%)
LMW	8.6	0.12 0.37	0.19 (79%) 0.58 (21%)

Table 4.8. R_f and R_{SO4} values of some common S compounds.

Chemical species	Chromatography R_f EtOH:H_2O:BuOH 5:2.5:1	Electrophoresis R_{SO4} pH 4.5
Na_2SO_4	0.26	1.00
$Na_2S_2O_3$	0.33	1.09
Na_2SO_3	0.31	0.85
S^o	0.89	nd*
KSCN	0.74	nd
Polysulfides+	0.56	0.31
Methionine	0.67	0.20
Cysteine	0.55	0.22
Cystine	0.17	0.19
3-MPA∓	0.81	nd

* nd= not done

\+ Mixture prepared as described in Wada (1977).

∓ MPA = mercaptopyruvic acid

dark brown material of very low electrophoretic mobility (R_{SO4} <0.2; Tables 4.5-4.7). The low R_{SO4} value suggests that the component is organic in nature, since water soluble S_I compounds have high electrophoretic mobilities (Table 4.8; Roy and Trudinger 1970). The components not so highly colored (HCl ii-vi, NaOH LMW) also showed low R_{SO4} values. The exceptions were those fractions of the PPi extract designated as P-2-MeOH and P-2-Ac, which had major constituents with high R_{SO4} values (0.73, 0.95 and 1.05) (Table 4.5). Some of the activity in the P-2-Ac material was also distributed as a smear from the origin to the high mobility peak (R_{SO4}= 0.73), indicating the presence of several other unresolved, labelled constituents. The component in P-2-Ac with the R_{SO4} of 0.73 was purified by separation with LH-20 Sephadex (Fig. 4.4; major peak) followed by electrophoresis. It was a colorless compound that developed a yellow color with DNTP, a reagent used to locate reduced S compounds on paper strips (Grassetti and Murray 1969). Soluble S_I molecules have high electrophoretic mobilities (Table 4.8; Roy and Trudinger 1970). Therefore, extracted labelled material with low electrophoretic mobilities was assumed to be free of $^{35}S_I$ compounds.

d) Chemical characterization of S bonds

Components isolated from the PPi extract all showed a

small amount of acid volatile sulfide ($AV^{35}S$) which was always <10% of the total radioactivity present (data not shown). A significant fraction of ^{35}S in most isolated components was CrII reducible (ie. >20%; Tables 4.1-4.3), even though $^{35}S^{\circ}$ and CrII reducible, water soluble, $^{35}S_I$ species (eg. thiosulfate) were shown to be absent (Tables 4.2-4.7). A large percentage of ^{35}S in each component was also HI reducible. In almost every case, the HI reducible fraction was larger than the CrII reducible fraction, often being greater than 50% of the total ^{35}S present. In fact, the $CR^{35}S$ fraction was itself reduced by HI (Table 4.1). In the case of component P-2-H_2O, essentially all of the ^{35}S was HI reducible (Table 4.2) with little $CR^{35}S$, indicating that this component probably contained an ester sulfate.

A possible explanation for the high CRS and HIRS content of these extracted "organics" is that they contained easily reducible polysulfur groups attached to organic moities. To look for this type of compound, KCN was added to extracted radiolabeled material. Cyanide reacts readily with sulfur chains (S°, polysulfides) and degrades them to form SCN^- (Bartlett and Skoog 1954; Roy and Trudinger 1970). The percentage of $^{35}SCN^-$ formed in each of the extracted components was high and almost always similar in magnitude to the $CR^{35}S$ fraction (Tables 4.2-4.4). Indeed, for the HCl extract, it was found that by first reacting the sample with cyanide and then removing (by chromatography) the SCN^- which

formed, the chromium reducible fraction was also removed (Table 4.1). All but one of the isolated components tested (P-2-H_2O) reacted with cyanide to produce thiocyanate ion (Tables 4.2-4.4). In some cases (HCl iv and vi), the amount of $^{35}SCN^-$ was not quantified because of interference from other radiolabelled components with a similar R_f (Table 4.6).

Cyanide was also useful in detecting whether iron was chemically bound to the ^{35}S atoms in different components. Addition of KCN to some of the extracted organic material formed iron-cyanide complexes ($[Fe^{II}(CN)_6]^{4-}$). These complexes were evidenced by the formation of Prussian blue dye ($Fe_4^{III}[Fe^{II}(CN)_6]_3 \cdot xH_2O$) when chromatographic strips were sprayed with ferric nitrate. However, the formation of this dye could be eliminated if the samples were first electrophoresed before being reacted with cyanide. Electrophoresis, therefore, removed the iron in the extracts which formed the complex with cyanide. The removal of this iron was not accompanied by loss of ^{35}S (data not shown), showing that the former was free and not bound to sulfur, which is further evidence that the ^{35}S present in the extract was $^{35}S_o$.

f) Effect of acid

The addition of acid to humic extracts has been used as a method of determining organic polysulfide content by

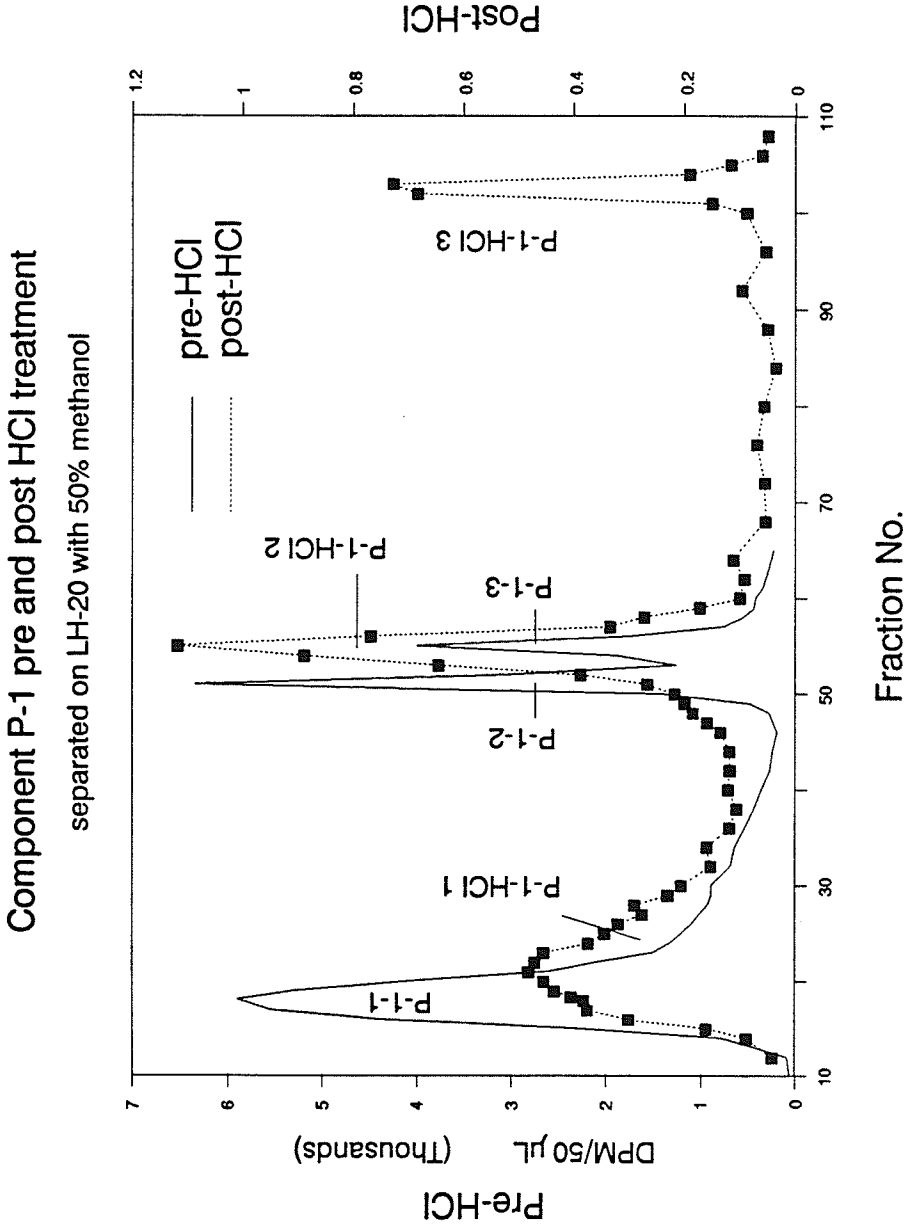
quantifying the S° formed (Francois 1987a & b). Acidification (2.5 N HCl) at 20°C of humic compounds in component P-1 of the PPi extract, released a labelled component with an R_f (0.84) similar to that of $^{35}S°$. However, paper chromatography with hexane showed this component was not S°. The component was highly chromium reducible and produced SCN^- when KCN was added, indicating it did not contain SO_4^{2-}. When the acidified solution was heated (100°C), a compound with an R_f (0.24) similar to SO_4^{2-} was formed (Table 4.9). The changes in the distribution of activity in this component due to acid hydrolysis (under N_2) were also determined by Sephadex chromatography (Fig. 4.6). The peak with the highest elution volume (P-1-HCl-3) corresponded to the high R_f component first produced by acidification at 20°C. The large center peak in the hydrolyzed sample (P-1-HCl-2) corresponded to the component with the R_f of SO_4^{2-} formed after heating. This peak contained little $CR^{35}S$ (8%) but a large amount of $HIR^{35}S$ (79%), indicating that it likely was $^{35}SO_4^{2-}$. Paper chromatographic analysis showed that heating the acidified extract converted the P1-HCl-3 peak to P1-HCl-2. Both of these peaks were produced at the expense of the peak with the lowest elution volume (P-1-HCl-1; Fig. 4.6). Similar results were obtained for the HMW fraction of the NaOH extract (Table 4.9). The failure of acid hydrolysis to directly release either $^{35}S°$ or $^{35}SO_4^{2-}$ suggested that PPi

Table 4.9. Effect of acidification on R_f and R_{SO4} values of some labelled components isolated from crude extracts of Lake 303 sediment (incubated with $^{35}SO_4^{2-}$). Formation of $^{35}S^{\circ}$ and $^{35}SO_4^{2-}$ due to acidification was determined by comparing mobilities with those of standards in Table 4.8. Numbers in brackets refer to the relative amount of the different components resolved as a % of the total activity on the paper strip.

Component ID	Pre-HCl treatment		Post-HCl treatment*	
	R_f	R_{SO4}	R_f	R_{SO4}
P-1	0.0	0.0	0.0 (18%)	0.0
	0.24 (13%)	0.08	0.24 (40%)	0.24
		1.00 (10%)	0.84 (36%)	0.76
				1.00 (40%)
P-2-H_2O	0.0	0.0		0.0 (63%)
				1.00 (37%)
P-2-MeOH	0.0 (64%)	0.05		0.0 (24%)
	0.11 (36%)	0.95		0.07 (28%)
			0.39 (13%)	
P-2-Ac	0.0 (27%)	0.02		0.0 (23%)
	0.15 (10%)	0.17		0.10 (14%)
	0.38 (41%)	0.41		0.95
(54%)	0.57 (5%)	0.73		
	0.77 (12%)	1.05		
	0.95 (5%)			
NaOH HMW	0.0 (65%)		0.0 (20%)	
	0.26 (35%)		0.27 (11%)	
			0.92 (69%)	
NaOH LMW	0.19 (79%)		0.25 (42%)	
	0.58 (21%)		0.88 (41%)	

* HCl treatment carried out at 100°C, except for P-2-H_2O which was autoclaved for 20 min.

Fig. 4.6. The effect of acidification (2.5 N HCl at 100°C for 3 hrs; under N_2) on the P-1 component resolved by Sephadex gel G-10 from the PPi extract (Fig. 4.4).


Component P-1 pre and post HCl treatment
separated on LH-20 with 50% methanol
pre-HCl
post-HCl
P-1-1
P-1-HCl 1
P-1-2
P-1-3
P-1-HCl 2
P-1-HCl 3
Pre-HCl
DPM/50 µL (Thousands)
Post-HCl
Fraction No.

and NaOH extracts did not contain acid-sensitive sulfate esters and organic polysulfides of the type previously described (Francois 1987a, b). It is possible that more acid-resistant forms of organic polysulfides were present since heating was required to release ^{35}S bound in component P-1-1 (Fig. 4.6).

Table 4.9 summarizes the effect of acidification on other labelled components. All of the components showed some change in their chromatographic and electrophoretic mobilities when acidified, indicating they were not acid stable. Of particular interest is component P-2-H_2O, which released a labelled component with an R_{SO4} similar to sulfate, as might be expected from acid hydrolysis of a sulfate ester. Other samples did not directly release significant quantities of this component.

g) Spray reagents

Only three labelled components (P-2-Ac, R_f=0.15 and 0.77 and HCl iii, R_f=0.41) gave positive reactions with ninhydrin, an indicator of amino groups (Table 4.10). These compounds could have been amino acids, or their derivatives, based on the similarity of their R_f values with known standards (Tables 4.8 and 4.9). One component from P-2-Ac (R_f=0.15 and R_{SO4}=0.17) may have been the amino acid cystine, which has similar R_f and R_{SO4} values (Tables 4.5, 4.8 and 4.10). These components made up a small percentage

Table 4.10. Reactions of selected radiolabelled components from sediment extracts with a variety of detecting reagents for specific chemical groups (sprayed onto paper strips from chromatographic and electrophoretic analysis).

Component name	Reaction with			
	ninhydrin	DNTP	tol. blue	ρ-anisidine
P-1	-	-	-	-
P-2-H_2O	-	-	+	-
P-2-MeOH	-	-		
P-2-Ac R_f= 0.15 (R_{SO4}=0.17)	+			
0.38 (R_{SO4}=0.73)	-	+		-
0.77	+			
HCl i	-			
HCl ii	-			
HCl iii R_f=0.41	+			
HCl iv	-			
HCl v	±			
HCl vi	-			
NaOH HMW	-			
NaOH LMW	-	-		

of the total $^{35}S_o$ (Tables 4.5 and 4.6). One labelled compound from the P-2-Ac component (R_f=0.38; R_{SO4}=0.73) gave a positive reaction with DNTP, an indicator of partly reduced S such as thiols, and oxy-sulfur compounds (Table 4.10). Only P-2-H_2O showed a positive reaction with toluidine blue, indicating that the material was acidic. No labelled reducing sugars were detected with ρ-anisidine hydrochloride for those samples analyzed.

h) Gas chromatography

No volatile ^{35}S-labelled components were observed by GC-proportional counter analysis. Therefore, possible identification of isolated ^{35}S-components by GC-mass spectrometry was not possible.

4.4. Discussion

Based upon chromatographic and electrophoretic evidence (Tables 4.5-4.8), most of the soluble ^{35}S isolated from the PPi, HCl and NaOH sediment extracts was associated with organic material. The possible presence of ^{35}S from i) $^{35}S^{\circ}$, ii) soluble $^{35}S_I$ (eg. $^{35}SO_4^{2-}$, $^{35}S_2O_3^{2-}$, etc.) and iii) co-extracted, finely dispersed, iron sulfides ($Fe^{35}S$, $Fe^{35}S_2$, $Fe_3{}^{35}S_4$) in the extracts was investigated and determined to be minimal by the following analyses.

First, paper chromatography showed that $^{35}S^{\circ}$ was not coextracted with humic compounds since none of the subcomponents in the extracts migrated with an R_f value expected for this compound. Removal of sedimentary $^{35}S^{\circ}$ with acetone-CS_2 prior to acid and base extractions precluded its presence in these fractions. It is possible that some $^{35}S^{\circ}$ was co-extracted with organics in the PPi fraction, but was removed during the clean-up and separation stages, since none was found in the components isolated by gel chromatography.

Second, electrophoresis showed that soluble $^{35}S_I$ was not present in the majority of the isolated components. This indicates that $^{35}S_I$ was present largely as acid volatile and insoluble Fe-S compounds as well as elemental S. There were only two components (P-2-MeOH and P-2-Ac) that contained large amounts of ^{35}S-compounds sufficiently mobile to be

considered soluble $^{35}S_I$ compounds (R_{SO4} >0.5)(Tables 4.5 and 4.8; Roy and Trudinger 1970).

Third, iron present in the Sephadex-isolated components behaved electrophoretically as free, ionic iron, and not as covalently bound iron such as in iron sulfides (see Results). In addition, all solutions were passed through filters of 0.45 μm pore size, which have been used by others to remove iron sulfide particles from slurries (Doyle 1968), making contamination by these compounds unlikely.

All of these factors indicate that, with two exceptions, the radiolabel present in aqueous extracts was largely associated with organic material (Tables 4.5-4.7). Therefore, the techniques used allowed for positive identification of $^{35}S_O$ by showing that $^{35}S_I$ was largely absent from the extracts.

The extraction procedure neither produced nor destroyed $^{35}S_O$, since the amount of $^{35}S_O$ (= Total ^{35}S minus $CR^{35}S$) measured in whole sediment (pre-extraction) was essentially the same as the sum of $^{35}S_O$ in the individual extracts and non-extractable fraction (Fig. 4.2). However, a compound which is found in the PPi extract, but not the porewater, may be a cleavage or oxidation product of reduced $^{35}S_I$ (ie. $CR^{35}S$). The compound (a sub-component of P-2-Ac (Fig. 4.4; major peak) is tentatively identified as a thionate, based on its positive reaction with DNTP (Table 4.10), and R_f and R_{SO4} values which showed it was not sulfite

or thiosulfate, (Tables 4.5 and 4.8). The compound also gave a positive reaction with cyanide (Table 4.2), further supporting this conclusion. The increase in magnitude of component P-2 (Fig. 4.4) of the PPi extract with increasing numbers of extractions could be explained by such a mechanism. For example sulfoxy anions, including polythionates, are known intermediates of pyrite oxidation involving abiotic reactions with oxygen and Fe(III) (Moses et al. 1987).

The results suggest that the measurement of S_0 formation, from SO_4^{2-} reduction, by measuring specific compounds of known identity, may be impractical in sediments since a large number of ^{35}S-labelled compounds was resolved (Tables 4.2-4.7). The present strategy of measuring groups of compounds (ie. carbon-bonded S and ester sulfate) is more appropriate since some isolated compounds shared similar chemical and physical characteristics.

The majority (about 90%) of organosulfur components labelled with newly reacted ^{35}S were polar, non-volatile molecules of relatively small molecular weight (<1000 MW; Tables 4.2-4.4), as determined by dialysis. Other studies (eg. Ferdelman et al. 1991; Francois 1987a, b) have concentrated mainly on S content of and incorporation into the high molecular weight (>12000-14000) humic acid fraction of sedimentary organic matter. The large molecular size of this latter fraction is a good indication that S is present

as S_o, since inorganic S compounds are of much smaller size. The results from the present study, however, suggest that incorporation of S into organic matter is greatly underestimated if only very large molecular weight compounds are studied.

At least one radiolabelled compound, making up about 6% of the total $^{35}S_o$ (P-2-H_2O; Fig. 4.4 and Table 4.2) is believed to be (or contain) an ester sulfate, based on its chemical characteristics, such as, its low $CR^{35}S$ content relative to $HIR^{35}S$ (Table 4.2). More importantly, $^{35}SO_4^{2-}$ was directly released by acid hydrolysis at 120°C (Table 4.9), a common test for detecting this type of compound (eg. King and Klug 1980; Fitzgerald et al. 1985). The formation of ester sulfate must be a biosynthetic process since, unlike H_2S, abiotic reactions between sulfate and organic matter are unlikely at low temperatures (see Chapter 1). Many examples of ester sulfate synthesis (often a sulfated carbohydrate) by animals, plants and bacteria have been reported (e.g. Fitzgerald 1976). Fitzgerald et al. (1982) tentatively identified a ^{35}S-labelled sulfated polysaccharide, as well as sulfated sugars, in soil incubated with $^{35}SO_4^{2-}$. In this study, no labelled reducing sugars were detected in P-2-H_2O with ρ-anisidine hydrochloride (Table 4.10), although it is still possible that this component contains acidic polysaccharide. The acidity of this material, as shown by the positive toluidine

blue reaction (Table 4.10), may simply be due to sulfate groups present. This material is probably internally compensated (ie. neutral charge) since it has no electrophoretic mobility (Table 4.5). Since $^{35}SO_4^{2-}$ was not initially released from component P-1 (PPi extract; Fig. 4.2) by acid, it is not considered to have originated from an ester sulfate (Fig. 4.6; Table 4.9; see Results).

Carbon bonded-^{35}S (C-^{35}S) (ie. Total ^{35}S minus HIR^{35}S) was the dominant form of $^{35}S_O$ in the extracts (68%; Fig. 4.2c). An example of this type of S_O, ^{35}S-cystine, was tentatively identified as one of the sub-components in the P-2-Ac fraction of the PPi extract (see Results and Table 4.10). Cystine, an oxidation product of the amino acid cysteine, most likely originated via the assimilatory reduction of $^{35}SO_4^{2-}$ by biota. However, assimilatory sulfate reduction is a minor process compared to dissimilatory reduction in sediments (Nedwell 1982), and so can account for only a small fraction of the C-^{35}S. Therefore, the majority of this type of compound must have formed from the reaction of reduced S_I (eg. H_2S) with reactive sites on sedimentary organic material, such as unsaturated carbons, alcohol groups and amines (Luther et al. 1986; Vairavamurthy and Mopper 1987; Francois 1987b; see also Chapter 1). Luther et al. (1986) have also proposed the formation of thiols from reaction of thiosulfate (and pyrite) with organic matter in salt marsh sediments. C-S is considered to be resistant to

both chromium and hydriodic reduction (eg. Wieder et al. 1985). None of the Sephadex-isolated components contained only C-^{35}S, since each had some large portion of either CrII or HI reducible ^{35}S (Tables 4.2-4.4). These results suggest that some C-^{35}S compounds may either be partially reduced by these reagents, or may contain more than one type of S linkages, some of which are easily reduced. It is also possible that the isolated components contained more than one chemically different ^{35}S-compound.

Evidence for a third group of $^{35}S_O$, organic polysulfides, in the sediment extracts was also obtained (10-20% of $^{35}S_O$). For example, the high degree of CR^{35}S and HIR^{35}S in almost every isolated component (Tables 4.2-4.4) would normally be interpreted as indicating the presence of $^{35}S_I$ or ester-$^{35}SO_4^{2-}$. However, it was shown, by paper chromatography and paper electrophoresis, that contamination by $^{35}S_I$ could not be the source of CR^{35}S. Furthermore, acid hydrolysis (Table 4.9) showed the presence of ester-$^{35}SO_4^{2-}$ (by direct release of $^{35}SO_4^{2-}$) in only one component. Therefore, the most likely explanation for these results is the presence of polysulfur moieties (containing S-S which bonds can be reduced by both CrII and HI) bound to an organic core. Because of the highly reducing conditions in the sediment used (ie. the sediment was methanogenic) it is assumed that these polysulfur moieties are polysulfides.

The formation of $^{35}SCN^-$ upon addition of cyanide to the

extracts supports the conclusion that labelled organic polysulfur moeities were present. Indeed, the cyanide reactive and $CR^{35}S$ made up the same ^{35}S pool (Table 4.1). Cyanide readily reacts with chains of S atoms, such as S^o, polysulfides and polythionates, to form thiocyanate ion, which is easily detected as a pink-red complex with ferric iron (eg. Bartlett and Skoog 1954; Hanley and Czech 1970; Roy and Trudinger 1970). When these inorganic polysulfur compounds were shown to be absent (eg. components P-1-1, HCl i-vi, NaOH-HMW), the $^{35}SCN^-$ likely originated from an organic poly-S compound. Cyanolysis of polythionates is incomplete and leads to the formation of SCN^- as well as thiosulfate and sulfate (eg. Roy and Trudinger 1970; Blasius et al. 1968). However, paper chromatography resolved only $^{35}SCN^-$ in most of the cyanolysed extracts again suggesting the presence of labelled organic polysulfides. The results may explain a previous observation that, in the absence of S_I, chromium reduction still reduced a significant amount of S in peat (Brown 1986).

The ^{35}S-polysulfides found in this study may be chemically different from those that have been previously measured in sediments, since they are more resistant to breakdown by strongly acidic conditions. Little or no $^{35}S^o$ was formed by acidification of various extracts in this study, unlike previous reports for marine samples (Ferdelman et al. 1991; Francois 1987a, b). For example, component P-1

of the PPi extract released a compound which, although chromium reducible and cyanide-reactive, was not $^{35}S^{o}$ (Fig. 4.6 and Results), and because of its association with a brown color in both the Sephadex and paper chromatographic separations likely consisted of organically bound reduced ^{35}S (e.g. polysulfide). Similarly, evolution of H_2S by acidification is also used in detection of polysulfide S (Hanley and Czech 1970). However, except for the large amount of $H_2^{35}S$ (assumed to be mostly from $Fe^{35}S$) released by the sediment during extraction with HCl (Fig. 4.3a), little $AV^{35}S$ (<10% of activity; data not shown) was found in the aqueous extracts, while a large percentage of cyanide-reactive ^{35}S was present (Tables 4.2-4.4). This suggests the presence of an acid stable form of polysulfide.

Acid resistant polysulfides are known to exist. For example, bis-(2-amino-2-carboxyethyl)trisulfide (thiocystine), and the tetrasulfide analogue, are stable in 2.5-5N HCl (Fletcher and Robson 1963). These compounds can be synthesized from an acidified solution of a thiol (cysteine) and S^{o} at room temperature. Based on chromatographic and electrophoretic mobilities, no significant amounts of a compound behaving like thiocystine was present in the labelled extracts (data not shown). It is possible that organic polysulfides formed in this sediment are stabilized against electrophilic attack by H^{+} by an electron reducing group adjacent to the polysulfide

portion (e.g. Cardone 1972). Another possibility is that organic polysulfides were chemically changed during extraction to forms which are acid stable (produce no S^o) but still cyanide-reactive.

The detection of organic polysulfides in sediment extracts indicates a potential for common analytical methods to underestimate the importance of organic matter as a sink for sulfide. The polysulfur moiety of these compounds is partially inorganic in nature (eg. C-S-S_n, C-S-S_n-S-C, R-N-S-S_n), and is, therefore, partly chromium reducible. Therefore, since chromium reduction is used to measure sedimentary S_I (mostly iron sulfides) some organic polysulfide S will be measured as S_I (^{35}S), and will be assumed to belong to the iron sulfide fraction. However, organic material, and not iron, is involved in trapping this sulfur in sediments. The significance of binding of reduced S by organic matter is that in acidified lakes storage of S from dissimilatory sulfate reduction does not cease if iron becomes limiting in sediments, and consequently, in-lake alkalinity generation can continue.

Alkalinity generation by sulfate reduction in lakes requires sulfur in a reduced form (see Chapter 1). Both Fe and organic matter are known to act as sinks for reduced S by forming S_I (iron sulfide) and S_O compounds (e.g., Nriagu and Soon 1985; Rudd et al. 1986a; Giblin et al. 1990), but their relative importance in different sediments is not

clear. In some sediments, storage of iron sulfides resulting from increased sulfate loading predominates (Carignan and Tessier 1988; Giblin et al. 1990). In other cases (Rudd et al. 1986; Nriagu and Soon 1985) S_o is important. An overemphasis of the importance of the Fe-S fraction may lead to the prediction that iron limitation will limit alkalinity generation in lakes. The underestimation of S_o formation by current methods, as shown in this study, suggests that rapid acidification may not occur due to a depletion of reactive iron.

The existence of organic polysulfides in marine pore-waters has been determined by polarographic methods (e.g. Boulegue et al. 1982) and evidence for polysulfide reactions with organic matter has been obtained in the laboratory. For example, Varaivamurthy and Mopper (1987) found that tetrasulfide reacts with acrylic acid to form an organic polysulfide. Others have identified a cyclic trisulfide in sedimentary organic matter from the Quaternary to Pliocene age (Kohnen et al. 1989), which indicates an origin of S_o from the reaction of polysulfides with organic matter. The results from the present study provide further evidence for this mechanism of S storage using radioisotope methods. It is likely that organic polysulfides that form and persist in sediment contain small chains (e.g. trisulfides) since larger polysulfide chains are unstable (Kohnen et al. 1989).

4.5 Summary

$^{35}S_o$ produced from $^{35}SO_4^{2-}$ reduction was extracted from lake sediment with a variety of aqueous solvents. Organic solvents obtained mostly $^{35}S^o$. Only 1/3 of the total org-^{35}S was easily removed with PPi buffer. The remaining 2/3 was resistant to extraction and was solubilized only with strong acid and base treatments. The extraction procedure neither created nor destroyed $^{35}S_o$, as determined by a commonly used analytical method (total ^{35}S minus $CR^{35}S$). Several different labelled components were resolved with Sephadex gel chromatography. Paper chromatography and electrophoresis showed that these isolates consisted mainly of ^{35}S bound to organic matter. The majority of the label was associated with compounds of relatively small molecular weight (about 90% <1000) and not large humic complexes.

Chemical characterization showed three forms of $^{35}S_o$; i) carbon-bonded S, ii) ester sulfate and iii) organic polysulfides. Carbon-bonded ^{35}S was the greatest fraction of $^{35}S_o$ (68%). Only about 6% was identified as ester sulfate. Organic polysulfide was estimated at 10-20% of the $^{35}S_o$, barring significant isotope exchange reactions with this fraction. Therefore, for this sediment the current method for estimating $^{35}S_o$ (ie. S_T minus CrII reducible S) would underestimate it by a significant amount (ie. 10-20%). The degree of this underestimation may differ for other

sediments where conditions may be more, or less, amenable to organic polysulfide formation. For example, at oxic-anoxic interfaces, or in sediments which undergo cycles of oxic-anoxic conditions (e.g. tidal marshes), large amounts of polysulfides may be produced from the partial oxidation of sulfide (Chen and Morris 1972; Losher and Kelts 1989). These polysulfides may then react with organic matter to form elevated levels of organic polysulfides.

The methods used allowed positive measurement of $^{35}S_O$ (ie. in the absence of $^{35}S_I$) and showed, in a more rigorous way than has been previously described, that S_O is produced from sulfate reduction. Furthermore, observed formation of organic polysulfides indicates that the importance of organic matter as a sink for S in sediments is underestimated by current analytical methods, since this fraction is measured as S_I.

4.6 References

Amaral, J.A., R.H. Hesslein, J.W.M. Rudd, and D.E. Fox. 1989. Loss of total sulfur and changes in sulfur isotopic ratios due to drying of lacustrine sediments. Limnol. Oceanogr. 34: 1351-1358.

Baker, L.A., N.R. Urban, P.L. Brezonick, and L.A. Sherman 1989. Sulfur cycling in an experimentally acidified seepage lake, p.79-100. In E. Saltzman & W.J. Cooper [eds], Biogenic sulfur in the environment. ACS 393.

Bartlett, J.K., and D.A. Skoog. 1954. Colorimetric determination of elemental S in hydrocarbons. Anal. Chem. 26: 1008-1011.

Berner, R.A. 1984. Sedimentary pyrite formation: an update. Geochim. Cosmochim. Acta 48: 605-615.

Blasius, E., G. Horn, A. Knochel, J. Munch, and H. Wagner. 1968. Analytical chemistry of sulphur compounds in Inorganic sulphur chemistry, p. 199-240. In G. Nickless [ed.], Inorganic sulphur chemistry. Elsevier.

Boulegue, J., C.J. Lord III, and T.M. Church. 1982. Sulfur speciation and associated trace metals (Fe,Cu) in the porewaters of Great Marsh, Delaware. Geochim. Cosmochim. Acta 46: 453-464.

Brown, K.A. 1986. Formation of organic sulphur in anaerobic peat. Soil Biol. Biochem. 18: 131-140.

Brunskill, G.J., and D.W. Schindler. 1971. Geography and

bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28: 139-155.

Brunskill, G.J., D. Povoledo, B.W. Graham, and M.P. Stainton. 1971. Chemistry of surface sediments of sixteen lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28: 277-294.

Canfield, D.E., R. Raiswell, J.T. Westrich, C.M. Reaves, and R.A. Berner. 1986. The use of chromium reduction in the analysis of reduced inorganic sulfur in sediments and shales. Chem. Geol. 54: 149-155.

Casagrande, D.J., G. Idowa, A Friedman, P. Rickert, K. Siefert, and D. Schlenz. 1979. H_2S incorporation into coal precursors: origins of organic sulphur in coal. Nature 282: 599-600.

Cardone, M.J. 1972. Di- and polysulphides, p. 89-431. In J.H. Karchmer [ed.], The analytical chemistry of sulfur and its compounds, Vol II. Wiley-Interscience.

Chanton, J.P. and C. S. Martens. 1985. The effect of heat and stannous chloride addition on the active distillation of acid volatile sulfide from pyrite-rich marine sediment samples. Biogeochem. 1: 375-383.

Chen, K.Y., and J.C. Morris. 1972. Kinetics of oxidation of aqueous sulfide by O_2. Environ. Sci. Technol. 6: 529-537.

Cooley, R.A., D.M. Yost, and E. McMillan. 1939. The non-interchange of elementary radiosulfur with carbon disulfide. J. Am. Chem. Soc. 61: 2970-2971.

DeRoo, J., and G.W. Hodgson. 1978. Geochemical origin of organic sulfur compounds: Thiophene derivatives from ethylbenzene and sulfur. Chem. Geol. 22: 71-78.

Doyle, R.W. 1968. Identification and solubility of iron sulfide in anaerobic lake sediment. Am. J. Sci. 266: 980-994.

Ferdleman, T.G., T.M. Church, and G.W. Luther III. 1991. Sulfur enrichment of humic substances in a Delaware salt marsh sediment core. Geochim. Cosmochim. Acta 55: 979-988.

Fitzgerald, J.W. 1978. Naturally occuring organosulfur compounds in soils, p.391-443. In J.O.Nriagu [ed.], Sulfur in the environment. Wiley-Interscience.

Fitzgerald, J.W., T.C. Stricland, and W.T. Swank. 1982. Metabolic fate of inorganic sulphate in soil samples from undisturbed and managed forest ecosystems. Soil Biol. Biochem. 14: 529-536.

Fletcher, J.C., and A. Robson. 1963. The occurrance of bis-(2-amino-2-carboxyethyl) trisulphide in hydrolysates of Wool and other proteins. Biochem. J. 87: 553-559.

Fossing, H., and B.B. Jorgensen. 1990. Oxidation and reduction of radiolabelled inorganic sulfur compounds

in an estuarine sediment, Kysing Fjord, Denmark. Geochim. Cosmochim. Acta 54: 2731-2742.

Fossing, H., and B.B. Jorgensen. 1990. Isotope exchange reactions of radiolabelled sulfur compounds in anoxic seawater. Biogeochem. 9: 223-245.

Francois, R. 1987a. A study of the extraction conditions of sedimentary humic acids to estimate their true *in situ* sulphur content. Limnol. Oceanogr. 32: 964-972.

Francois, R. 1987b. A study of sulphur enrichment in the humic acid fraction of marine sediments during early diagenesis. Geochim. Cosmochim. Acta 51: 17-27.

Giblin, A.E., G.E. Likens, D. White, and R.W. Howarth. 1990. Sulfur storage and alkalinity generation in New England lake sediments. Limnol. Oceanogr. 35: 852-869.

Grassetti, D.R., and J.F. Murray Jr. 1969. The use of 2,2′-dithiobis-(5-nitropyridine) as a selective reagent for the detection of thiols. J. Chromatog. 41: 121-123.

Hanley, A.V., and F.W. Czech. 1970. Nonoxygen-containing inorganic sulfur compounds, p. 285-464. In J.H. Karchmer [ed.], The Analytical chemistry of sulfur and its compounds, Part II. Wiley-Interscience.

Herlihy, A.T., A.L. Mills, and J.S. Herman. 1988. Distribution of reduced inorganic sulfur compounds in lake sediments receiving acid mine drainage. Appl.

Geochem. 3: 333-344.

Hongve, D. 1978. Buffering of acid lakes by sediments. Int. Ver. Theor. Angew. Limnol. Verh. 20: 743-748.

Howarth, R.W., and S. Merkel. 1984. Pyrite formation and the measurement of sulfate reduction in salt marsh sediments. Limnol. Oceanogr. 29: 598-608.

Howarth, R.W., and J.M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnol. Oceanogr. 24: 999-1013.

Johnson, C.M., and A. Ulrich. 1959. Analytical methods for use in plant analysis. Cal. Agric. Exp. Sta. Bull. 766.

King, G.M., and M.J. Klug. 1980. Sulfhydrolase activity in sediments of Wintergreen Lake, Kalamazoo County, Michigan. Appl. Env. Microbiol. 39: 950-956.

Kohnen, M.E.L., J.S. Sinnighe Dampste, H.L. ten Haven and J.W. de Leew. 1989. Early incorporation of polysulphides in sedimentary organic matter. Nature 341: 640-641.

Landers, D.H., and M.J. Mitchell. 1988. Incorporation of $^{35}SO_4^{2-}$ into sediments of three New York lakes. Hydrobiol. 160: 85-95.

Losher, A.J., and K.R. Kelts. 1989. Organic sulphur fixation in freshwater lake sediments and the implication of C/S ratios. Terra Nova 1: 253-261.

Luther, G.W., T.M. Church, J.R. Scudlark, and M. Cosman.

1986. Inorganic and organic sulfur cycling in salt-marsh pore-waters. Science 232: 746-748.

Macek, K. 1963. Detection reagents, p.782-874. In I.H. Hais and K. Macek [eds.], Paper chromatography: a comprehensive treatise. Czechoslovak Academy of Sciences.

Martin, T.H., and G.W. Hodgson. 1977. Geochemical origin of organic sulfur compounds: Precursor products in the reactions of phenylalanine and benzylamine with elemental sulfur. Chem. Geol. 20: 9-25.

Moses, Co., D.K. Nordstrom, J.S. Herman, and A.L. Mills. 1987. Aqueous pyrite oxidation by dissolved oxygen and by ferric iron. Geochim. Cosmochim. Acta 51: 1561-1571.

Nedwell, D.B. 1982. The cycling of sulphur in marine and freshwater sediments, p. 73-106. In D.B. Nedwell and C.M. Brown [eds.], Sediment microbiology. Academic.

Nissenbaum, A., and I.R. Kaplan. 1972. Chemical and isotopic evidence for the *in situ* origin of marine humic substances. Limnol. Oceanogr. 17: 570-582.

Nriagu, J., and Y.K. Soon. 1985. Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. Geochim. Cosmochim. Acta 49: 823-834.

Pharmacia. 1987. Gel filtration theory and practice. Rahms i Lund, Sweden.

Roy, A.B., and P.A. Trudinger. 1970. The biochemistry of inorganic compounds of Sulfur. Cambridge University

Press.

Rudd, J.W.M., C.A. Kelly, and A. Furutani. 1986. The role of sulfate reduction in long term accumulation of organic and inorganic sulfur in lake sediments. Limnol. Oceanogr. 31: 1281-1291.

Schnitzer, M. 1978. Reactions of humic substances with minerals in the soil environment, p. 639-647. In W.E. Krumbein [ed.], Environmental biogeochemistry and geomicrobiology, Vol. 2: The terrestrial environment. Ann Arbor Science.

Sorbo, B. 1957. A colorimetric method for the determination of thiosulfate. Biochim. Biophys. Acta 23: 412-416.

Strickland, T.C., and J.W. Fitzgerald. 1985. Incorporation of sulphate-sulphur into organic matter extracts of litter and soil; involvement of ATP sulphurylase. Soil Biol. Biochem. 17: 779-784.

Strickland, T.C., and J.W. Fitzgerald. 1984. Formation and mineralization of organic sulfur in forest soils. Biogeochem. 1: 999-1013.

Vairavamurthy, A., and K. Mopper. 1987. Geochemical formation of organosulphur compounds (thiols) by addition of H_2S to sedimentary organic matter. Nature 329: 623-625.

Vairavamurthy, A., and K. Mopper. 1989. Mechanistic studies of organosulfur (thiol) formation in coastal

marine sediments, p. 231-242. In E.Saltzman and W.J. Cooper [eds.], Biogenic sulfur in the environment. ACS 393.

Vairavamurthy, A., and K. Mopper. 1990. Determination of sulfite and thiosulfate in aqueous samples including anoxic seawater by liquid chromatography after derivatization with 2,2'-dithio-bis(5-nitropyridine). Environ. Sci. Technol. 24: 333-337.

Voge, H.H. 1939. Exchange reactions with radiosulfur. J. Am. Chem. Soc. 61: 1032-1035.

Wada, H. 1977. The synthesis of greigite from a polysulfide solution at about 100°C. Bull. Chem. Soc. Jpn. 50: 2615-2617.

Wieder, R.K., G.E. Lang, and V.A. Granus. 1985. An evaluation of wet chemical methods for quantifying sulfur fractions in freshwater wetland peat. Limnol. Oceanogr. 30: 1109-1114.

5. The influence of benthic algal development on S cycling in lakes acidified below pH 5.

5.0. Abstract

The effects of proliferation of benthic algae, which are characteristic of very acidic lakes (pH<5), on the sulfur cycle of two lakes were studied. In experimentally acidified Lake 302 South, photosynthetically active algae on the surface of littoral sediments caused the oxidation of solid phase sulfides in late summer and early fall, making the algal-covered sediments a source of sulfate and H^+ to the water column. Bare sediments were sites of sulfate reduction. In winter the situations were reversed. The algae decayed and sulfate reduction was enhanced at algal-covered sites, as evidenced by accumulation of reduced sulfur compounds within the decaying algal mats and in surface sediments just below the algae. In contrast bare sites showed S oxidation in the winter. It is suggested that changes in S cycling induced by the development of these benthic algae are linked to large excursions in the over-winter epilimnetic sulfate concentration observed since the lake was acidified to pH 4.5 three years ago.

In Lake Hovvatn, a chronic-acidified lake, production of sulfate and H^+ in the sediment was induced by light, suggesting photosynthesis controlled sediment redox conditions. Also in this lake, which has been acidified for decades, sulfate and H^+ profiles showed less net sulfate reduction, frequent peaks of S oxidation and generally more

acidic conditions within the sediment, suggesting that the effects of benthic photosynthesis may become pronounced if a lake remains at very low pH.

5.1. Introduction

The enhanced development of benthic filamentous green algae (FGA) in the littoral zone is a common characteristic of acidified lakes (e.g. Grahn et al. 1974; Muller 1980; Schindler and Turner 1982; Stokes 1986; Turner et al. 1987; Howell et al. 1990). Large amounts of both attached and unattached (metaphytic) algae are produced which can develope into extensive mats overlying the sediment (e.g. Schindler et al. 1985; Howell et al. 1990). The reasons for this increase in benthic algal biomass are not clear. Turner et al. (1987) have suggested that the FGA are acid tolerant species adapted to low dissolved inorganic carbon (DIC) concentration in the water column. Increased water clarity in acid lakes (Yan 1983), although not directly responsible (Stokes 1986), may contribute to increased benthic algal biomass because of greater light penetration.

The consequences of algal mat development in acid lakes have yet to be fully investigated. A decrease in the recreational value of the lakes has been cited (Howell et al. 1990), and there are indications that fish spawning may be disrupted (Schindler et al. 1985). Furthermore, because of their location near the sediment-water interface, increased benthic O_2 production in the light may change the redox conditions in the underlying sediments. Many element cycling processes are affected by redox.

Sulfate reduction in lake sediments leads to the

production and storage of a variety of organic and inorganic reduced S compounds (e.g. Cook and Schindler 1983; Rudd et al. 1986a; Landers and Mitchell 1988; Baker et al. 1989). As long as these compounds remain reduced in the sediment there is a net consumption of H^+ (Kelly et al. 1982; Cook and Schindler 1983; Kelly and Rudd 1984), providing some buffering in lakes acidified by sulfuric acid in acid rain. However, these S compounds may be re-oxidized and returned to the water column as dissolved sulfate when the redox potential becomes oxidizing (Rudd et al. 1986a, b; Rudd et al. 1990; Kling et al. 1991). The re-oxidation of reduced S also leads to the regeneration of H^+, and so it is important to study the processes affecting this part of the S-cycle.

Lake 302 South, in the Experimental Lakes Area of northwestern Ontario, has been experimentally acidified with reagent grade sulfuric acid since 1982 (Rudd et al. 1986a, b; Rudd et al. 1990). Large mats of metaphytic algae (*Zygogonium spp.*) developed in the lake and covered extensive areas of the littoral sediment when the pH was decreased below 5 (M. Turner, pers. comm.). This work investigates the effect of these algal mats on sulfate reduction and also how their growth and decay may be changing the annual S-cycle. The effect of benthic algae on sulfate reduction in an anthropogenically acidified lake, Lake Hovvatn, in southern Norway, is also examined for comparison. Lake Hovvatn has been acidified for several

decades, and at the time of sampling was at pH 4.8.

5.2. Materials and methods

5.2.0. Site description.

Lake 302 South, located at the Experimental Lakes Area (ELA) in northwestern Ontario, has been experimentally acidified with sulfuric acid (Rudd et al. 1990). At the time of sampling the epilimnetic water pH was approximately 4.5, and sulfate concentration was >170 μmol L^{-1}. Average values for ELA lakes are pH 5.6-6.7 and 32 μmol sulfate L^{-1} (Armstrong and Schindler 1971). The lake (10.9 ha, mean depth 5.1m) is located on the Canadian Shield and has a watershed of Precambrian granodierite covered by a thin overburden (<1m) of quartz, plagioclase and K-feldspar (Brunskill and Schindler 1971). Watershed vegetation includes native jackpine, black spruce, white pine, white birch, trembling aspen, and a forest floor cover of *Sphagnum* moss. More details of the hydrology, vegetation, and limnology of Lake 302 South are given elsewhere (Brunskill and Schindler 1971; Rudd et al. 1990).

Two epilimnetic sites (2 and 3m) in this lake were chosen for study. At each site, an area of $2m^2$ was marked on the sediment by four stakes, and sediment cores and porewater samples were retrieved from within these areas only. The lake bottom at both sites was generally composed of "sandy", low porosity (<0.6), low organic content (C and N <2% by dry weight; Sweerts et al.1986) sediment with

little organic floc on the surface. Organic floc in this lake reportedly contains C and N each in the amount of 10% on a dry weight basis (Sweerts et al. 1986). At the 3m site, the bottom consisted of a patchwork of fist-sized hummocks and hollows. The hollows were about 1 cm deep and often contained accumulations of a black floc, presumed to be decaying algae (see Results).

Lake Hovvatn, located in southernmost Norway (Wright and Skogheim 1983), is atmospherically acidified and had an epilimnetic pH of about 4.8 at the time of sampling. This is a chronic-acidified lake which pH (4.4) was temporarily raised to >6 by liming in 1981, before re-acidification (Wright and Skogheim 1983). Pore-water samples and sediment cores for pH measurement were obtained from a site at 2m depth. The sediment at this site was highly organic (13.6-26.8% C by weight) and of high porosity (>0.96)(Rudd et al. 1986b).

5.2.1. Sediment sampling.

Sediment cores were obtained by diver using acrylic tubes 5 cm in diameter. Samples were obtained monthly from the 2m site of Lake 302 South during June to October, 1990 and in January, 1991. The 3m site was sampled only in June and July. Two to six cores were obtained from each site. The intact sediment cores were transported to the laboratory immediately after collection. Metaphytic algae, if present,

were removed with tweezers and frozen with dry ice under a N_2 atmosphere. The sediment was then extruded 1.5 cm at a time and sliced. The slices were transferred to plastic (Whirlpac) bags, under N_2, and frozen within 2 min. by dry ice. The frozen slices and algae were stored at -20°C for 1 to 2 weeks before being analyzed for S species. This method has been shown to preserve acid volatile S (AVS)(Rudd et al. 1986a).

Algal samples were also collected by hand, or with a 60cc plastic syringe with an enlarged inlet tip. These samples were retrieved from rocks and sediment outside the sample quadrat at depths of 0.5 to 2m, since large amounts of algal material only appeared on the designated sampling sites later in the summer. Samples were transferred into plastic bags under water and stored refrigerated until analysis.

Qualitative information on the presence or absence of algal mats on the littoral sediment and on their apparent conditon was obtained through visual inspection by diver.

5.2.2. Determination of pore-water sulfate and pH profiles.

Pore-water equilibrating devices (Hesslein, 1976b) were filled with deoxygenated (equilibrated with N_2), deionized water and inserted into the sediment. The devices were left to equilibrate with the pore-water for a period of 1-2 weeks. Samples were removed from the individual cells by

syringe (Kelly and Rudd 1984), transferred into deionized water-rinsed glass vials and refrigerated. Sulfate concentrations were determined by Dionex ion chromatography. In one case, pore-water for sulfate analysis was obtained by slicing a core and centrifuging the slices to separate the water from the sediment. Slicing and centrifugation of sediment was done under N_2. The pore-water was filtered through 0.22μ filters (pre-rinsed with deionized water) before Dionex analysis.

Because pore-water samples usually contain CO_2(aq) above atmospheric equilibrium, samples for pH measurement were handled at all stages in such a way as to prevent loss of CO_2 or oxidation of reduced species, both of which affect pH. Samples were collected from the pore-water sampler in glass or plastic syringes equipped with 20G needles. The dead space was filled with boiled, and cooled, deionized water. The pH was determined potentiometrically using a Ag/AgCl Microprobe glass combination electrode (6.4 mm dia.; Fisher Scientific). In the laboratory, samples were transferred with minimum agitation into a vessel that fit onto a rubber stopper around the pH electrode. The stopper was pierced with a needle to allow air exclusion while seating, and to prevent pressure build-up around the electrode tip. A pH reading was taken when the electronic drift was less than 0.01 unit per 30 s.

A second method for measuring pH was sometimes used in

sediment cores from Lake Hovvatn. Profiles were obtained at 1 mm intervals using microelectrodes mounted on a motor-driven threaded-rod assembly (Kelly and Rudd 1984). A flexible micro-pH glass probe (2 mm dia.; Microelectrodes, Inc., Londonberry, New Hampshire) was used in conjunction with a Ag/AgCl reference electrode, held just above the sediment surface. Oxygen in the overlying water in cores was measured by a micro Winkler method. Oxygen at depth in the sediment was measured with a platinum microelectrode (Flett Research, Winnipeg, Manitoba), and reported as a percent of the surface water oxygen.

5.2.3. Determination of sulfur in sediment and algae.

Total S (S_T), chromium reducible S (CRS) and acid volatile S (AVS) were measured in portions of sediment core slices (3-5 g wet weight) and algal material (0.5-1 g wet weight). All analyses were done on wet samples to avoid S losses during the drying step (Amaral et al. 1989). The three procedures were carried out on separate sample aliquots. S_T was determined by the method of Tabatabai and Bremner (1970), as modified by Amaral et al. (1989). Reduced S in the sample was oxidized, by NaOBr and heat, to sulfate, which was then reduced to H_2S by HI acid mixture (Johnson and Ulrich 1954; Amaral et al. 1989). A chromium reduction method (Zhabina and Volkov 1978; Howarth and Merkle 1984) was used to quantify what is operationally

defined as the non-sulfate, inorganic-S (S_I) pool (e.g. FeS, FeS_2 and S°). The method is generally believed to be specific for S_I (Canfield et al. 1986), although the sulfur in some organic-S compounds (e.g. organic polysulfides) may be measured as well (Brown 1986; see also Chapter 4). Acid volatile S (AVS; FeS and H_2S), was determined by acidifying a sample with deoxygenated, 6N HCl (Rudd et al. 1986a). The H_2S produced by the three procedures above was flushed by N_2, into a zinc acetate-NaOH trap, and quantified by iodometric titration (Howarth and Teal 1979). Any free H_2Spresent will be measured by all three analytical steps. By subtracting the AVS from the CRS fraction, an estimate of what is normally assumed to be FeS_2 and S° was obtained. However, it should be noted that this CRS-AVS fraction could also contain other compounds which are chromium reducible but not acid volatile. An estimate of the S fraction operationally defined as organic sulfur (S_O) was obtained by subtracting total reduced S_I (CRS) from S_T. Dissolved inorganic sulfate, if not removed, is included with S_O with this procedure. This was not a problem in these sediments because the amount of sulfate present in the pore-water was small compared to the solid phase S fractions (<5% of S_T; see Results), and so no correction was done for the presence of this compound. Algal material was squeezed and rinsed once with distilled water, to remove adsorbed sulfate before analysis. The standard deviation in S_T between cores collected on the same

date was from 7 to 33% (n=2 to 5).

S content of the sediment is reported on a per volume basis. The water content of the sediment was determined for parallel portions of each slice (drying oven, 60°C until no further weight change). The average density of crustal material (2.6 g per cc; Rudd et al. 1986b) and water (1 g per cc) was used to calculate the volume of each sediment slice. This approach was used since it is difficult to measure 1.5 cm precisely when slicing cores.

5.2.4. Incubation experiments.

Algal material, collected from Lake 302 South in August, was distributed into two core tubes, one containing Lake 302 South sediment and lake water and the other lake water only. The core tubes were incubated uncapped in the laboratory (21^0C), away from direct sunlight, for 3 weeks. At the end of the incubation period, changes in the visual appearance of the algal material were noted and analysis of different S species was done.

5.3. Results

5.3.0. S content of algal material collected from 0.5 to 2 m (Lake 302 South).

Three types of algal material were sampled: green filamentous masses of apparently viable algae, and both brown and black floc, or particulates, containing recognizable algal material that was apparently decaying. Several genera of algae were identified in this material including *Zygogonium* and its zygospores, *Oedogonium*, *Frustrulia*, *Peridinium inconspicuum*, *Tabellaria quadriseptata* and *Eunotia pectinalis*. All three types of algal material could be found in separate isolated patches on the lake bottom throughout the sampling period. However, after large algal mats developed (greater than 20 cm in thickness) at the end of August, all types of material could be found together at one site. In general, it was found that depressed areas of the green mats were underlain by brown floc, which in turn was underlain by black floc.

This algal material, collected over the sampling period from rocks and sediment (0.5-2 m depth) surrounding the designated sampling sites, showed variable S content and S speciation (Table 5.1). Green algae (assumed to be viable) had a S content of about 0.5% by wt., except in October when it was only about one half the value (Table 5.1). As expected, the majority of S was organic in form. Algae in

Table 5.1. Sulfur speciation of algal material collected from 0.5 - 2m depths in Lake 302 South from June, 1990 to January, 1991.

Date	Sample Description		AVS	CRS	Org-S	TS	% S by wt.
			μmol S g^{-1} dry weight*				
Ju 7	black floc	1	271	291	387	678	2.2
		2	218	258	221	479	1.5
Jl 5	green fil.‡	1	nd+	18	79	97	0.3
		2	nd	32	20	52	0.2
		3	nd	nd	nd	146	0.5
	algal puff§		nd	nd	nd	267	0.9
Au 30	green fil.		0	8	150	158	0.5
	brown floc		2	44	133	177	0.6
	black floc		41	118	69	187	0.6
Se 28	green fil.		1	13	157	170	0.6
	brown floc		1	23	187	210	0.7
	black floc		43	49	241	290	0.9
Oc 25	green fil.		0	8	50	58	0.2
	black fil.‡		0	73	37	110	0.4
	black floc		58	74	156	230	0.7
Ja 25	green fil.		2	32	99	131	0.4
	black floc		40	144	16	160	0.5

* Sample variability was <5% (1 S.D.).

\+ nd = not determined

‡ green/black fil. = algal matter with distinctive filamentous structure

§ algal puff = small (10 cm dia.) floating filamentous algal aggregate spherical in shape

this state contained little CRS and almost no AVS (Table 5.1). High levels of S_I (AVS and other CRS) were associated with algal material that appeared to be in a state of decay. Brown and black particulates had higher S contents (0.5-2.2% by wt.) than green algal material. The brown material had elevated amounts of CRS but this did not include AVS (Table 5.1). In the black material, however, about half the S was inorganic S, most of which was in the form of AVS (Table 5.1), indicating that dissimilatory sulfate reduction was occurring in the floc.

Laboratory incubations confirmed the black floc originated from algal material (Table 5.2). An increase in S concentration occurred in both incubated samples, but was more pronounced for algal material incubated in absence of sediment (Table 5.2). A loss in non-CrII reducible S may be due to mineralization of algal S_O. No bioturbating organisms were noted.

5.3.1. Speciation of S in algal material and sediment at the 2 and 3m sampling sites (Lake 302 South).

Significant amounts of algae first appeared at the shallower (2m) site in late August. The areal coverage, as well as the thickness, of the algal mats increased in September (15-25 cm), although some deterioration of the mats could be seen in October. Isolated pools of green-black algae were still present in late January, and harbored

Table 5.2. Laboratory incubation (3 weeks, 21°C) of filamentous green algae (FGA) from Lake 302 South in open core tubes with and without sediment. The (viable) algae were collected from the lake in Aug. (see Table 5.1).

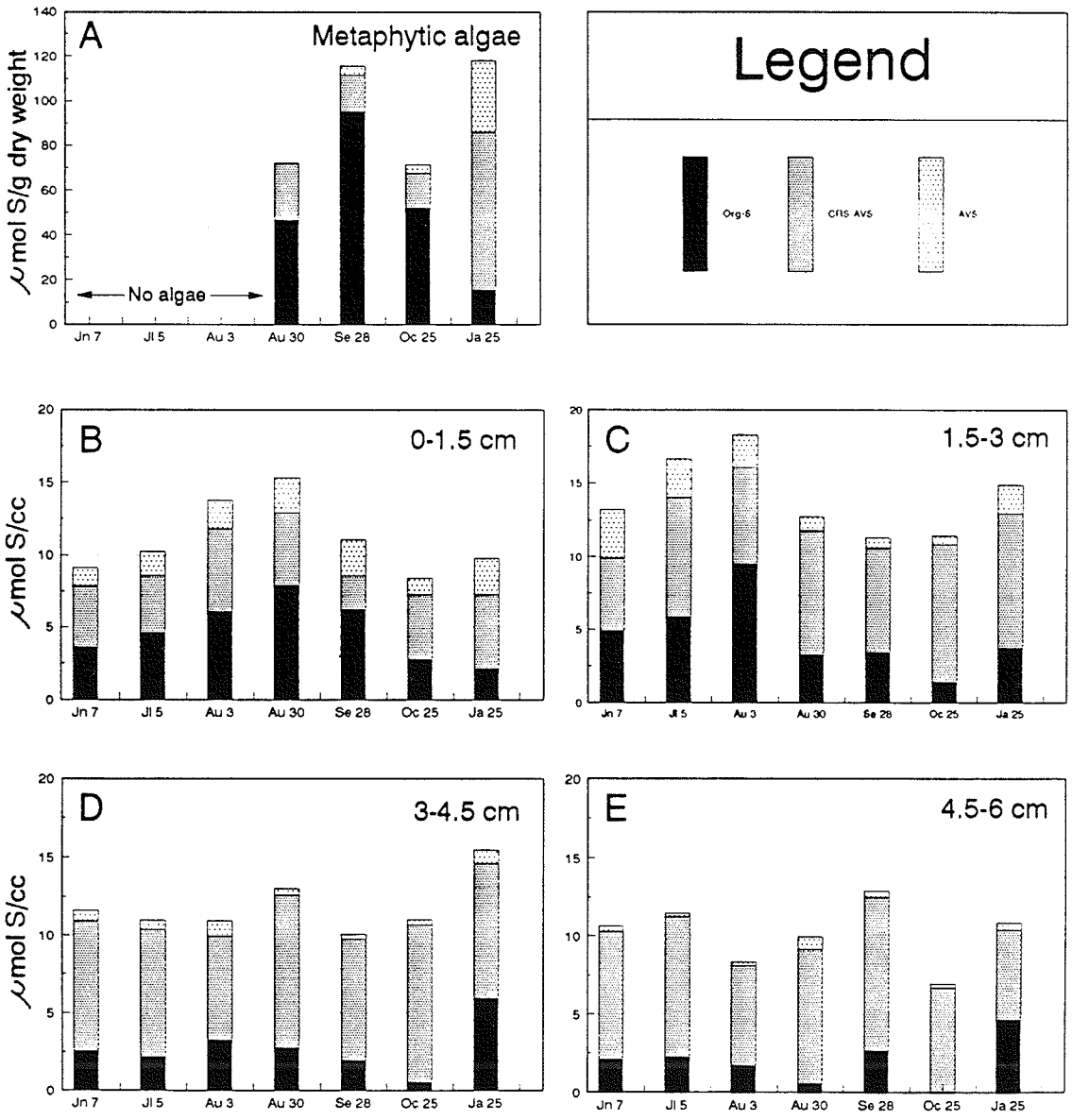
Incubated algae	AVS	CRS	TS	Description of algal material
	μmol S g^{-1} dry weight			
pre inc.	0	8	158	- green filaments
inc. + sed.	25	59	190	- brown-black floc
inc. - sed.	350	369	459	- black floc

a large number of caddis flies (B. Townsend, pers. comm.), which appeared to be grazing on it. Algal growth at the 3m site was sparse and algal mats were only a few cm thick. The same trends in algal S speciation (Fig. 5.1A) were observed as for other sites (Table 5.1).

The sediment at the 3m sampling site showed very large differences in S_T and AVS among triplicate cores, even when the cores were obtained no more than a few cm apart (data not shown). The high, and unpredictable, variability in S content made this site unsuitable for monitoring changes in S content and speciation which might occur with time.

The 2m site showed much less variation between replicate cores (7 to 33% S.D.; n= 2 to 6), and so, was sampled throughout the summer of 1990 and into the winter of 1991, to monitor any changes in S content and forms. The total S content in the top 1.5 cm of the sediment nearly doubled from early June (9.3 $\pm$ 1.1 (S.D.) μmol cc^{-1}) to late August, when it reached a maximium value of 15.3 $\pm$ 1.4 (S.D.) μmol cc^{-1} (Fig. 5.1B). By late October, however, the S gained over the summer had all been lost (8.42 $\pm$ 2.2 (S.D.); Fig. 5.1B). This significant loss of S from the sediment coincided with the appearance of algal mats at this site (Fig. 5.1A and B) and cannot be explained by sample variability alone. No further loss in total S from this sediment horizon was observed by January (Fig. 5.1B). A similar pattern was also seen in the next lower layer (1.5-3

Fig. 5.1. Temporal changes in sulfur speciation and content of algal material (A) and different depths in the sediment (B, C, D and E) of cores taken at the 2m sampling site in Lake 302 South from June, 1990 to January, 1991. Each bar represents the average of 2 to 6 samples. Total solid phase S equals the sum of S_0 + CRS-AVS + AVS. Algal mats first appeared at this site in late August. Standard deviation between S_T in cores from the same date varied from 7 to 33%.


A
Metaphytic algae
No algae
μmol S/g dry weight
Jn 7
Jl 5
Au 3
Au 30
Se 28
Oc 25
Ja 25
Legend
Org-S
CRS AVS
AVS
B
0-1.5 cm
C
1.5-3 cm
D
3-4.5 cm
E
4.5-6 cm
μmol S/cc

cm) of sediment (Fig. 5.1C). In this layer the S maximum occured earlier in the summer (July), reached a minimum in September, and partly recovered by January. The next two layers (3-4.5 and 4.5-6 cm) showed no obvious patterns in S_T content with time except for an increase in S_T in winter due mostly to an increase in S_O (Fig. 5.1D, E).

In the top 1.5 cm of the sediment, the proportion of S_O relative to S_I increased over the summer months to a maximum of 1.28:1 in late Sept. (Fig. 5.1B; Appendix V). Since losses in S_T had occured at this time, the results indicate that S_O was more resistant to oxidation than S_I, an observation previously made by others (Rudd et al. 1986a). In January S_T increased due to an increase in the inorganic fraction and a slight decrease in the organic fraction (Fig. 5.1 B). The pattern of S_O content with time closely followed that of the S_T, while CRS (i.e. reduced S_I, including AVS) quantities varied little during the summer (Fig. 5.1 B).

A similar, but less obvious, pattern was observed in the lower layers of sediment. S_O was always lowest in October, but recovered in January, although a large amount of CRS was also present (Fig. 5.1C-E). The ratio of S_O to S_I remained the same in the lower sediment layers over the summer, but quadrupled in January, reflecting an increase in S_O production (Fig. 5.1 D, E).

It should be noted that changes in S_O over time are the

net result of several processes. For example, S_0 present in algal cells (produced by assimilatory sulfate reduction) may be mineralized during decomposition of these same cells. At the same time, fine detrital S_0 could become incorporated in the top layers of sediment. Furthermore, S_0 could also be produced by abiotic reactions of sulfide (produced by dissimilatory sulfate reduction) with organic matter in the sediment.

It is also instructive to examine the shapes of the profiles of S_T and S species using the data in Fig. 5.1. AVS profiles in early summer showed maxima in the 1.5-3 cm slices. With time, however, AVS was lost from this horizon and a new AVS peak developed in the top most sediment layer (0-1.5 cm), suggesting recent sulfate reduction had occured. Other forms of chromium reducible S (CRS-AVS = FeS_2, S°, some organics?) were highest in the middle sediment horizons throughout the sampling period. S_0 was always highest in the top of the sediment, except in January when the highest S_0 value was in the 3-4.5 cm horizon. Depth profiles of S_T varied over the sampling period but the general trend showed a maximum in the 1.5-3 cm and 3-4.5 cm core slices (see Appendix for profile graphs).

5.3.2. Pore-water chemistry.

In Lake 302 South, sulfate concentration with depth in the sediment showed the expected pattern in the summer (e.g.

Carignan 1987; Rudd et al. 1986a), when algal mats had not yet developed. In the early summer, sulfate concentrations decreased from 150-160 μmol L^{-1} in the overlying water to <10 μmol L^{-1} 6 cm below the sediment water interface in sediments at the 2m sampling site (Fig. 5.2A). The gradient was steeper later in the summer (Fig. 5.2A). On all dates, the sulfate gradient was steeper at the 3m site than at the 2m site (Fig. 5.3A), suggesting faster rates of sulfate reduction.

In early fall (Sept. 28), the 2m site had 50-60% algal cover and the 3m site had only 25% algal cover. At 2m, two pore-water profiles in areas overlain by algal mat showed an increase in sulfate just above the sediment-water interface with one profile continuing to increase into the solid sediment (Fig. 5.2B), indicating that oxidation of solid phase sulfides was occurring. In contrast, in an area without algal cover pore-water sulfate decreased quickly with depth (Fig. 5.2B).

One month later (Oct. 25), the algal material appeared to be dead or dying. At this time, the presence of algal mat at the 2m site was linked to a decrease in sulfate just below the sediment-water interface, leaving a sub-surface sulfate maximum at about 4 cm depth (Fig. 5.2C). The profile of sulfate concentration in the algal mat showed a steep gradient as well (Fig. 5.2C), indicating that sulfate reduction was occuring in the mat itself. Sediment not

Fig. 5.2. Pore-water sulfate profiles at the 2m site of Lake 302 South in 1990. A, July 5 to August; B, September 28; C, October 25. Dates refer to the day that pore-water equilibrators were retrieved.

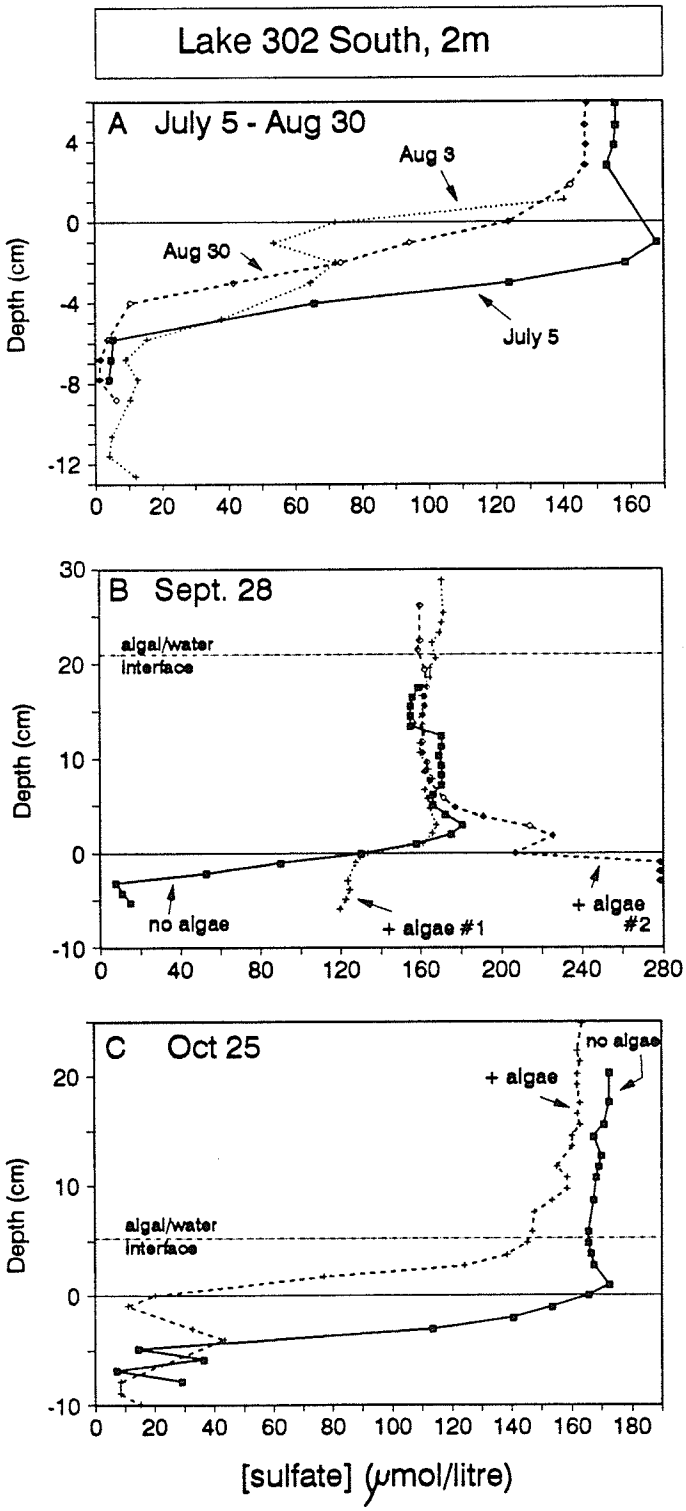
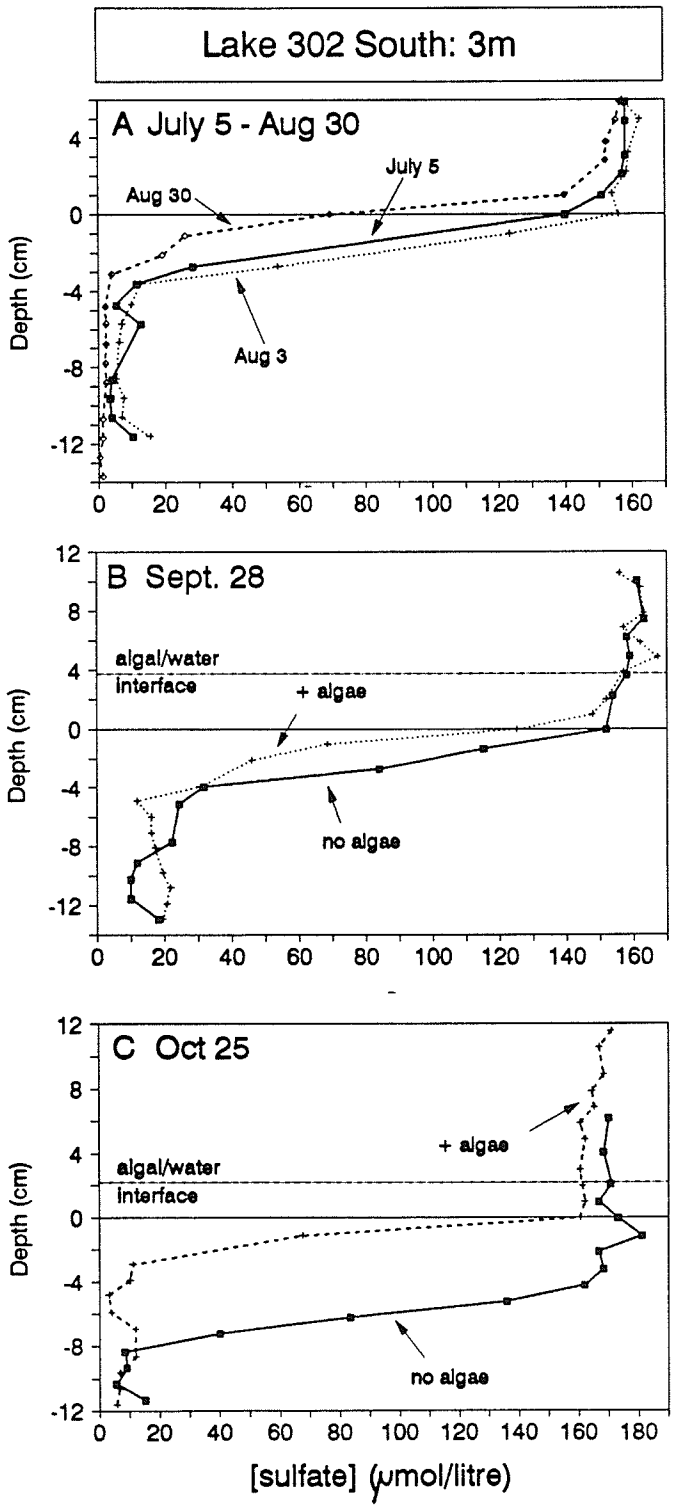

Lake 302 South, 2m
A July 5 - Aug 30
Aug 3
Aug 30
July 5
Depth (cm)
4
0
-4
-8
-12
0
20
40
60
80
100
120
140
160
B Sept. 28
algal/water interface
no algae
+ algae #1
+ algae #2
30
20
10
0
-10
0
40
80
120
160
200
240
280
C Oct 25
no algae
+ algae
algal/water interface
20
10
0
-10
0
20
40
60
80
100
120
140
160
180
[sulfate] (μmol/litre)

Fig. 5.3. Pore-water sulfate profiles at the 3m site of Lake 302 South in 1990. As described for Fig. 5.2.


Lake 302 South: 3m
A July 5 - Aug 30
July 5
Aug 30
Aug 3
B Sept. 28
algal/water interface
+ algae
no algae
C Oct 25
+ algae
algal/water interface
no algae
Depth (cm)
[sulfate] (μmol/litre)

overlain by algal mat showed much deeper sulfate penetration in the 2m site (Fig. 5.2C).

In January the effect of algal cover was opposite. Under ice (Jan 25), sulfate reduction was occurring at the 2m site in areas overlain by small pools of decaying algal remains, as shown by the sulfate profile (Fig. 5.4). The sulfate concentration of the overlying water was halved in the relatively thin (<0.5 cm) algal covering alone, indicating high microbial activity at these sites. This was in contrast to the profiles earlier in the year which did not show sulfate consumption within the mat (Fig. 5.2B).

At the 3m site, a small layer of algae (<5 cm) developed in September. However, measurements in early fall (Sept. 28; Fig. 5.3B), showed little effect of algal material on sulfate profiles compared to a site without algae (Fig. 5.3B) or to profiles from earlier in the summer (Fig. 5.3A). The only noticeable effect occurred in October when the site with algae (now decaying) had a steeper sulfate gradient than the site without algae (Fig. 5.3C).

Profiles of pH in October (Fig. 5.5) complemented the sulfate profiles at both sampling sites (Fig. 5.2C and 5.3C). At this time, algal-covered sites showed steeper sulfate profiles and this was presumably responsible for steeper H^+ gradients at the same sites.

Fig. 5.4. Pore-water sulfate profile at 2m site on January 25, 1991. Determined from core slices and collection of overlying water by syringe.

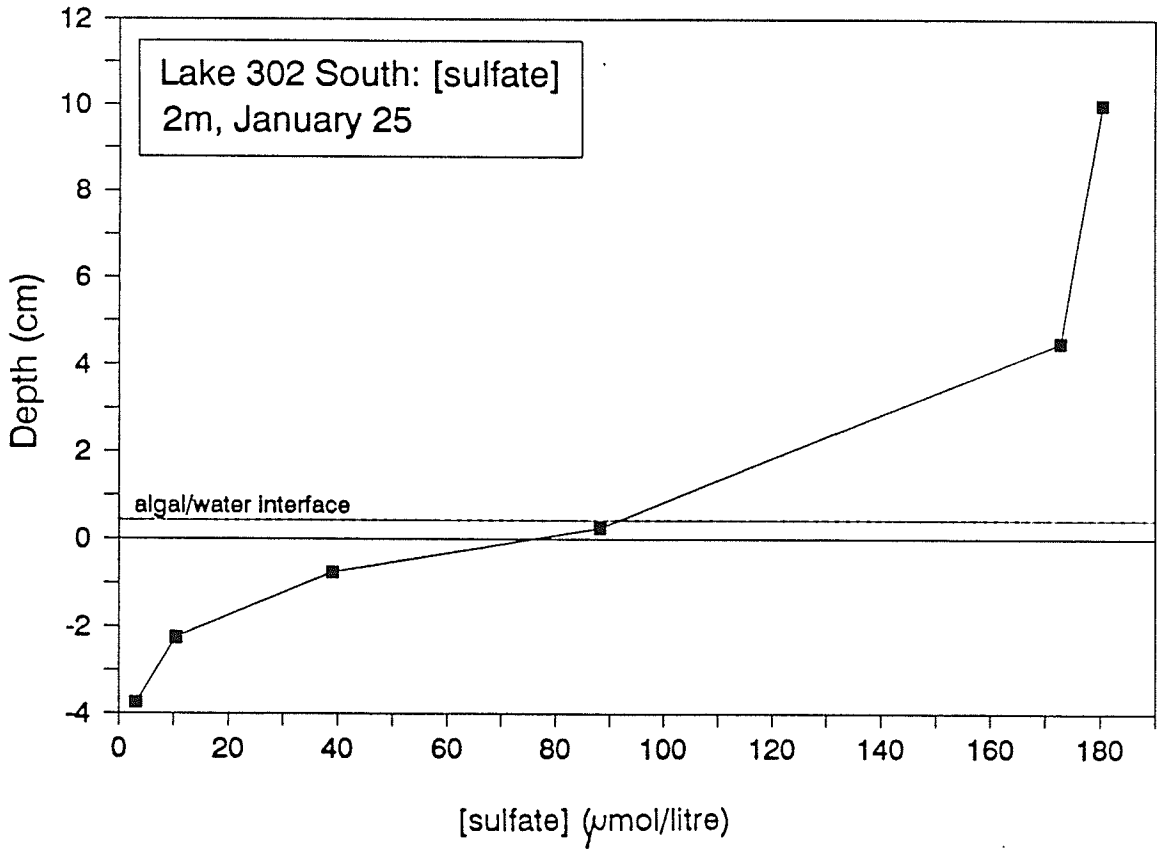
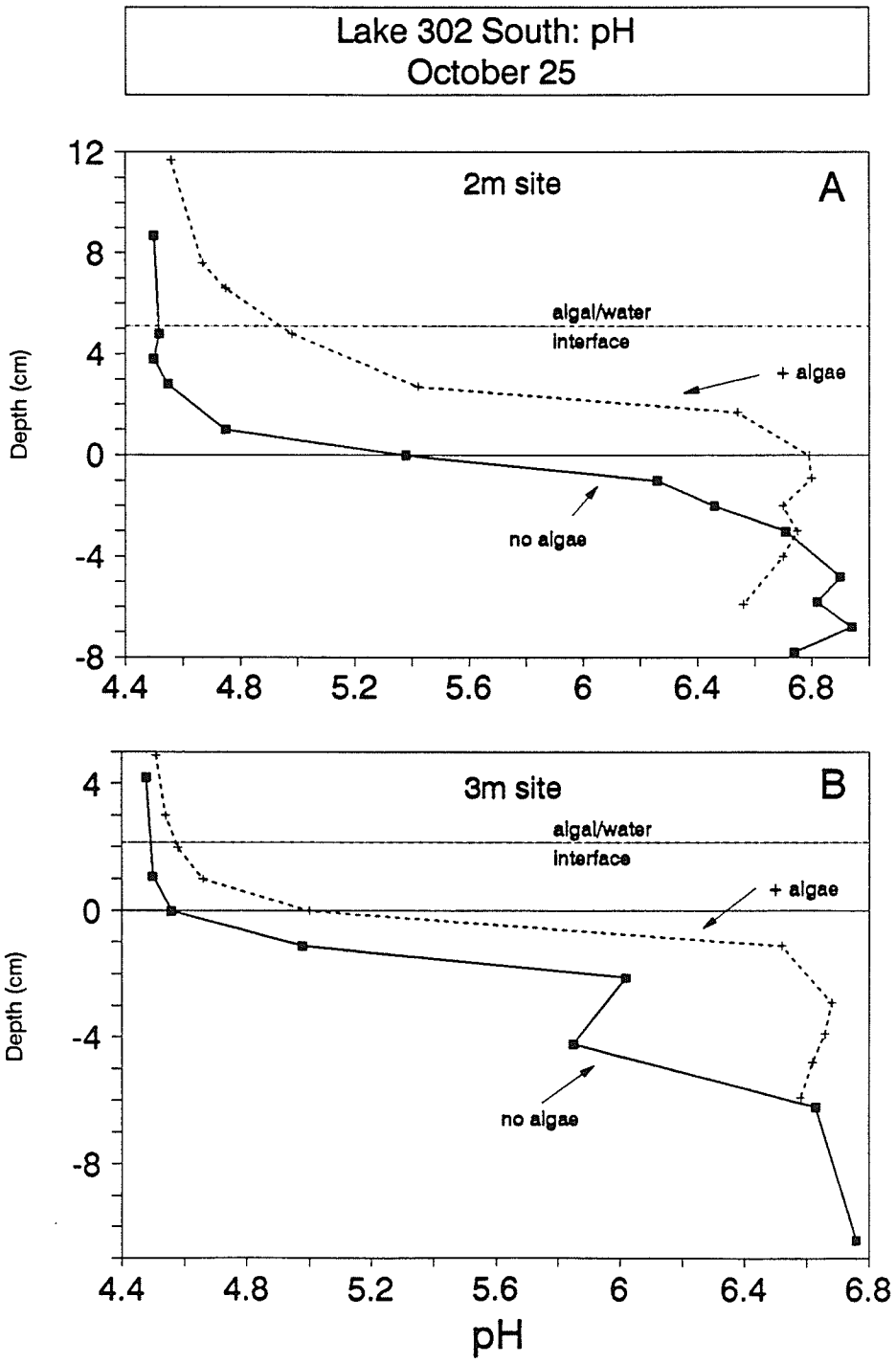

Lake 302 South: [sulfate]
2m, January 25
algal/water interface
Depth (cm)
12
10
8
6
4
2
0
-2
-4
0
20
40
60
80
100
120
140
160
180
[sulfate] (μmol/litre)

Fig. 5.5. Depth profiles of pore-water pH at the 2m (A) and 3m (B) sites of Lake 302 South. Measurements were made on the same pore-water samples as for sulfate on Oct. 25 (see Figs. 5.2C and 5.3C).


Lake 302 South: pH
October 25
2m site
A
algal/water
interface
+ algae
no algae
Depth (cm)
12
8
4
0
-4
-8
4.4
4.8
5.2
5.6
6
6.4
6.8
3m site
B
algal/water
interface
+ algae
no algae
Depth (cm)
4
0
-4
-8
4.4
4.8
5.2
5.6
6
6.4
6.8
pH

5.4. Discussion

Before describing conditions in Lake 302 South at pH 4.5, it is important to review conditions as they were when the pH was greater than 5. In the first stages of acidification (1981-1986) pore-water chemistry profiles showed that there was always a net flux of sulfate from the overlying water into the sediment (Kelly and Rudd 1984; Rudd et al. 1986b; C. Kelly, pers. comm.). This was also observed in other neutral to mildly acid lakes (Carignan 1987). The mechanisms of sulfate consumption in sediments include biological assimilation and, most importantly, bacterial sulfate reduction, leading to the formation of a variety of sulfide compounds as well as sulfate esters (Rudd et al. 1986a; Chapter 4). Microbial sulfate reduction consumes H^+ (e.g. Kelly et al. 1982) and this process is partly responsible for maintaining pore-water pH at near neutral levels. Thus, in Lake 302 South, pore-water pH varied between 6.2 to 6.9 with depth and was maintained even when the overlying water pH decreased to below 6 (Rudd et al. 1986b; Rudd et al. 1990). Winter pore-water profiles showed that sulfate penetrated more deeply into the sediment than at other times of the year in Lake 302 South above pH 5 (Rudd et al. 1990), and in other lakes (Carignan 1987; Kling et al. 1991) due to the invasion of oxygenated water and lowered microbial activity. Sulfate reduction continued as

a general trend during the winter but at low rates, as judged by the decreased flux of sulfate into sediments (Rudd et al. 1990). *In situ* experiments with radiolabelled sulfate also showed that some reduced S was returned, as sulfate, to the water column over winter (Rudd et al. 1986a). However, no significant net sulfate loss occurred from the sediments since the water sulfate concentration did not increase appreciably (J. Rudd, pers. comm.; see Discussion).

When Lake 302 South was lowered below pH 5, one of the most obvious changes in S cycling was the appearance of profiles with large sub-surface maxima in pore-water sulfate concentration, such as described in the present work (Fig. 5.2B). Some of the observations made in this study suggest that changes in the biological regime of Lake 302 South due to acidification (to pH 4.5) have changed the S cycle of the lake. Results from Lake Hovvatn, in southern Norway, indicate that the S cycle may be similarly disrupted in other lakes acidified to very low pH's.

The proliferation of benthic filamentous green algae (FGA), is a well known consequence of lake acidification (Ghran et al. 1978; Muller 1980; Schindler and Turner 1982; Stokes 1986; Turner et al. 1987; Howell et al. 1990). Lake 302 South, where the pH was lowered by the addition of reagent grade H_2SO_4 (Rudd et al. 1990), showed an increase in epilithic and metaphytic FGA biomass soon after

acidification began (Turner et al. 1987; Howell 1990). Very large quantities of thick (0.5-1 m) *Zygogonium spp.* mats, covering large areas of the littoral sediment, first occurred when the pH decreased to 4.5 in 1988 and continued in the years following (M. Turner, pers. comm.).

The effect of the algae on S cycling was very different when they were viable than when decaying. For example, the S content of the algal material when viable was 0.5-0.6% S by weight, (an average value for algae; Healy 1973), almost all being S_O, as expected (Table 5.1). When senescent, however, high amounts of reduced S_I were measured in some of the FGA samples (Table 5.1, Fig. 5.1A) indicative of sulfate reduction. Other forms of S_I (CRS-AVS) in algal material preceded the appearance of AVS and could have included S^o, an oxidation product of H_2S. The incubation experiment (Table 5.2) confirmed that the brown and black particulates associated with the algal mats did arise as the algae senesced.

Within the period of study (June 1990 to January 1991) the algal mats had two quite different effects on the sedimentary S pool: i) accelerating the oxidation of solid phase S to sulfate in the early fall, and ii) retarding the release of S (as sulfate) from sediments to the water column in the late fall and winter.

In the early fall, a large subsurface sulfate peak was observed (Fig. 5.2B) in those sites overlain by algae but

not in algal-free areas, suggesting that perfusion of the sediment by algal-generated oxygen was occurring. The pore-water sulfate increase coincided with the loss of solid phase S from the 2m site (Fig. 5.1), confirming that this was the source of the extra sulfate. The decrease in total sedimentary sulfur also coincided with the appearance of algal mat (Fig. 5.1A) on the sediment. Similar subsurface sulfate maxima were seen by Rudd et al. (1986b) in naturally acidified (pH<5) Norwegian lakes (also see below). It is unlikely that this increased sulfate was due to invasion of oxygenated epilimnetic water into the sediment caused by a sudden temperature decrease (Hesslein 1976a), since the non-algal site at 2m (Fig. 5.2B) was unaffected.

The lack of algal effect on the sulfate profiles at the 3m site at this time (Fig. 5.3B) could be a result of lower photosynthetic activity leading to lower quantities of O_2 that were easily consumed by sediment microflora. For example, measurements in this lake showed that photosynthetic activity at 3m depth was less than half that at 1.5m (Sweerts et al. 1986).

In contrast, later in the fall and in winter, the algal mat appeared to enhance sulfate reduction in the sediment immediately below. Pore-water sulfate profiles showed steeper gradients within the decaying algal mat than in bare sediment (Fig. 5.2C and 5.4). The high S_I (particularly AVS) content of algal material at this stage (Table 5.1;

Fig. 5.1A), and the increase in S_T content from October 1990 to January 1991 in the top 6 cm of sediment (Figs. 5.1 B-E), also indicate increased sulfate uptake from the water column.

The resumption of sulfate reduction in October and January (Figs. 5.2C and 5.4) was accompanied by a loss of S_0 and a preferential increase in S_I (CRS) in the top layers of sediment but not the lower layers (Figs. 5.1B-E). The reason for these results is not clear, but they are consistent with observations of large variations in the production of sedimentary organic and inorganic S with site (Kelly and Rudd, unpub. data) and with redox conditions (Chapter 6).

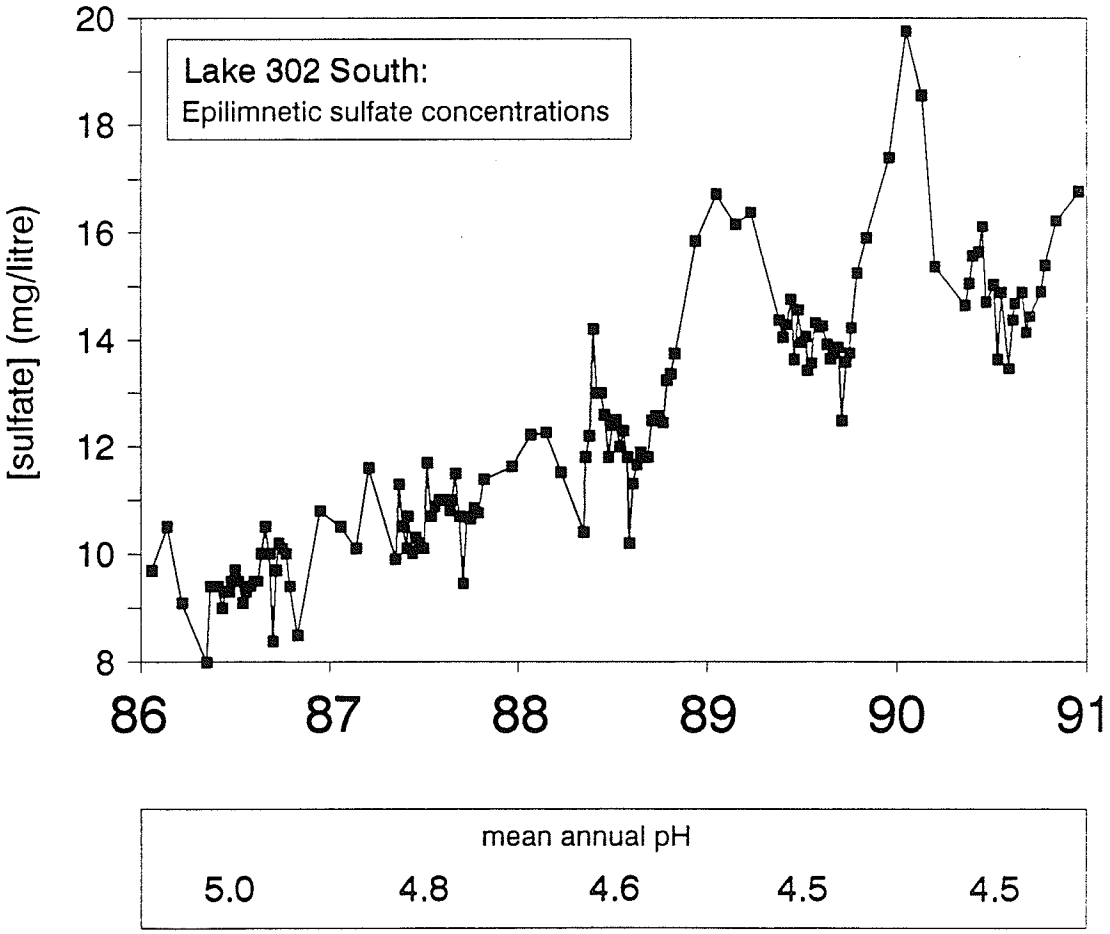
In addition to affecting the rate of sulfate reduction, the establishment of algal mat at the 2m site also changed the vertical position of the AVS maximium, from a depth of 2-3 cm, to the top of the sediment column (Fig. 5.1; Appendix VI). The concentration of AVS in the top 1.5 cm of sediment in January 1991 was twice that in early summer ($\approx$2.5 μmol cc^{-1}). It is possible that this layer of reduced S at the top of the sediment could serve as a source of sulfate after all of the easily degradable carbon from the decaying algae is used up (or removed by water currents after "ice-out") and reducing conditions cannot be maintained. Sweerts et al. (1986) found that such a mechanism was indeed occurring with organic flocculent

material (mm in thickness) on the sandy sediment of Lake 302 South. Because most over-winter S oxidation is limited to the top few cm of sediment (Rudd et al. 1986a) it is reasonable to conclude that S deposited closer to the surface would be more likely to be oxidized. This should lead to greater swings in sulfate concentration and, in fact, larger than usual seasonal fluctuations (late fall-winter increases) in the epilimnetic sulfate concentration have occurred in Lake 302 South since the winter of 1988-89 (Fig. 5.6). This has coincided with the increased coverage of littoral sediments by the thick mats of metaphytic algae (M. Turner, pers comm.). Therefore, the question is raised: were the algal mats responsible for these fluctuations?

A direct role of the algal mats in the epilimnetic sulfate concentration increase is minor. Given an average 4.4g dry weight of algae per m^2 of epilimnetic sediment (M. Turner, pers. comm.), and an average S content of about 170 μmol S g^{-1} dry algae (Table 5.1), even if all the algal S were converted to sulfate (3.4 Kg) it would account for <1% of the total increase in epilimnetic sulfate mass between the summer and winter ($\approx$1 X 10^3 Kg; calculation not shown).

The role of the algae must be indirect and, given the masses involved, be related to effects on S buildup and oxidation in the sediments themselves. The mass of S in the upper 1.5 cm is enough to support concentration swings of the order observed. For example, if the midsummer S_T at the

Fig. 5.6. Changes in the epilimnetic sulfate concentration in Lake 302 South (acidified with sulfuric acid) over 5 years. Average epilimnetic pH values for the summer of each year are given. The epilimnetic sulfate concentration on January 25, 1991 was 18.5 mg/L. (data obtained from M. Turner, FWI, Winnipeg, MB)


Lake 302 South:
Epilimnetic sulfate concentrations
[sulfate] (mg/litre)
20
18
16
14
12
10
8
86
87
88
89
90
91
mean annual pH
5.0
4.8
4.6
4.5
4.5

2m site (Fig. 5.1B) is typical of epilimnetic sediment, and one half of the S is lost to the water column as sulfate during the winter, it could account for >50% of the observed increase in epilimnetic sulfate concentration.

Therefore, the algal mats may contibute to increased epilimnetic sulfate swings by, i) extending the period of oxidation of sedimentary sulfides so that it occurs earlier in the year, and ii) causing the accumulation of reduced S near the top of the sediment where it may later be oxidized with ease.

Verification of these mechanisms, however, requires that many sites in the lake be intensively sampled, since S content and dynamics differ between sites. For example, sulfate was quickly consumed in October and January at the 2m site where decaying algal mat was present (Fig. 5.2C), but sulfate was being released at the 3 m site where there was no algal cover. The epilimnetic sulfate concentration increased continuously from early fall to January (Fig. 5.6). Thus, sites where S oxidation was occuring must have predominated and sulfate reduction closer to the surface at some sites and times must be contributing to the larger changes in water concentrations after 1986.

Some of the unusual sulfate profiles sometimes seen at the 2m site of Lake 302 South have also been observed in epilimnetic sediments of at least two other acidic lakes. The Hovvatn lakes of southern Norway, in which the

epilimnetic pH has been below 5 for much longer (decades) than in Lake 302 South (3 years), also contain mobile mats and clouds of FGA (C.A Kelly, pers. comm.). These lakes also showed large sulfate maxima below the sediment-water interface unrelated to the normal periodic oxidation events during winter and overturn (Fig. 5.7A, B; Rudd et al. 1986b). The sub-surface sulfate peak in the sediment coincided with a peak in H^+ concentration at the same depth (Fig. 5.7A), as would be expected from the oxidation of sulfides to sulfate. Rudd et al. (1986b) previously reported this phenomenon in another acid Norwegian lake.

These observations were made during a period when conditions were sunny (J. Rudd, pers. comm.). Involvement of benthic algal communities in this process was indicated by the sediment pH profiles under high and low light conditions (Fig. 5.8). It appeared that under high light conditions (sunny), photosynthesis at the sediment-water interface enhanced diffusion of O_2 in the sediment causing a decrease in pH due to the oxidation of sulfides and other reduced compounds. Under low light conditions (cloudy) the sedimentary pH was high, indicating that microbial activity in the sediment was sufficient to consume this lower rate of O_2 production, preventing oxidation of solid phase sulfides. The limited capacity for O_2 consumption in these sediments could have been due to inhibition of decomposition activity, which has been shown to occur at pH 5 and below (Kelly et

Fig. 5.7. A, pore-water sulfate and H^+ profiles in Lake Hovvatn sediment (2m) in August of 1984; B, pore-water sulfate profiles in Lake Hovvatn sediment (2, 4 and 5m depths) in July 1985. Horizontal dotted lines in (A) refer to O_2 as a % of the concentration in the overlying water. (data obtained from J. Rudd and C. Kelly, FWI and U. of Manitoba, Winnipeg, MB)

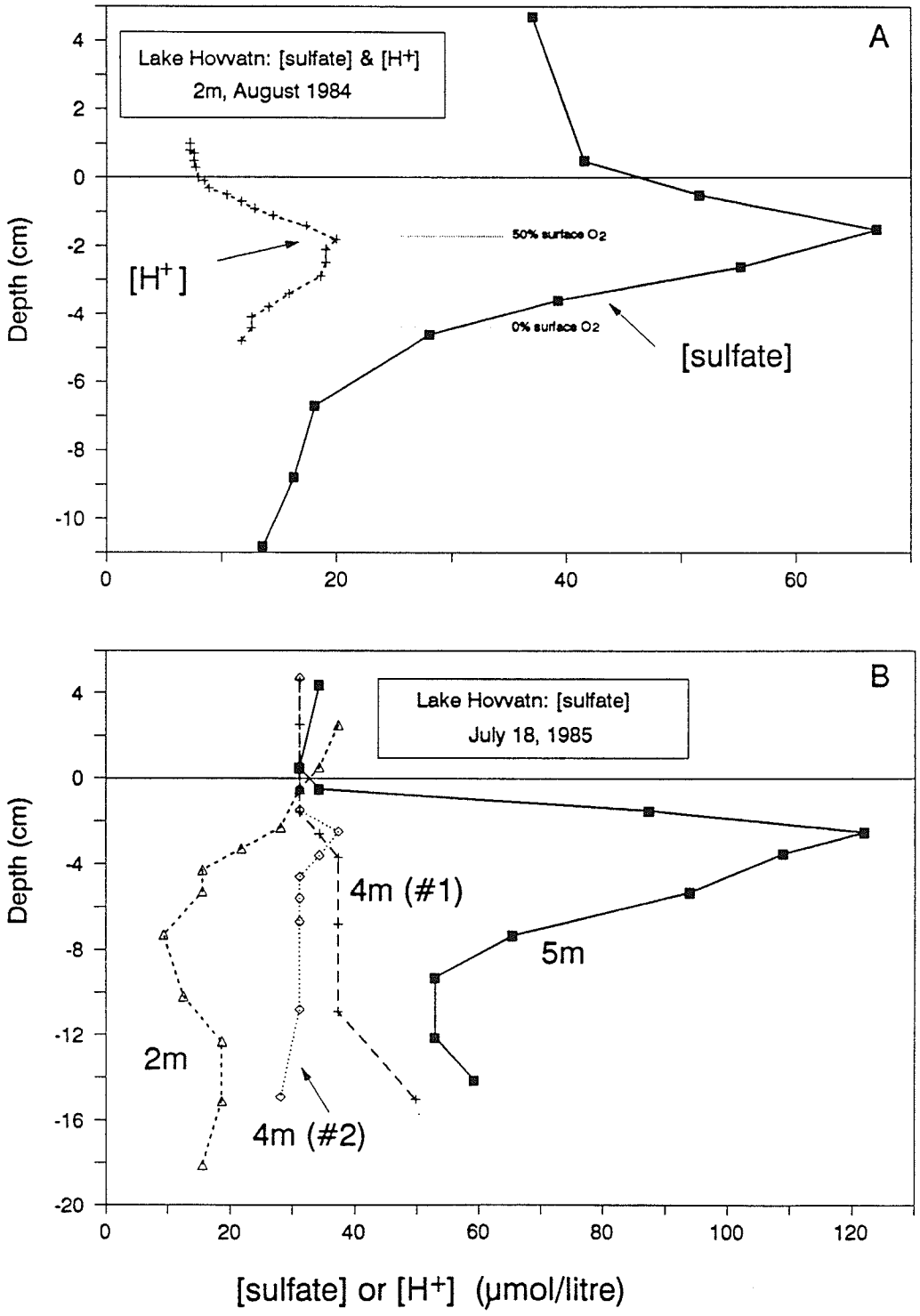
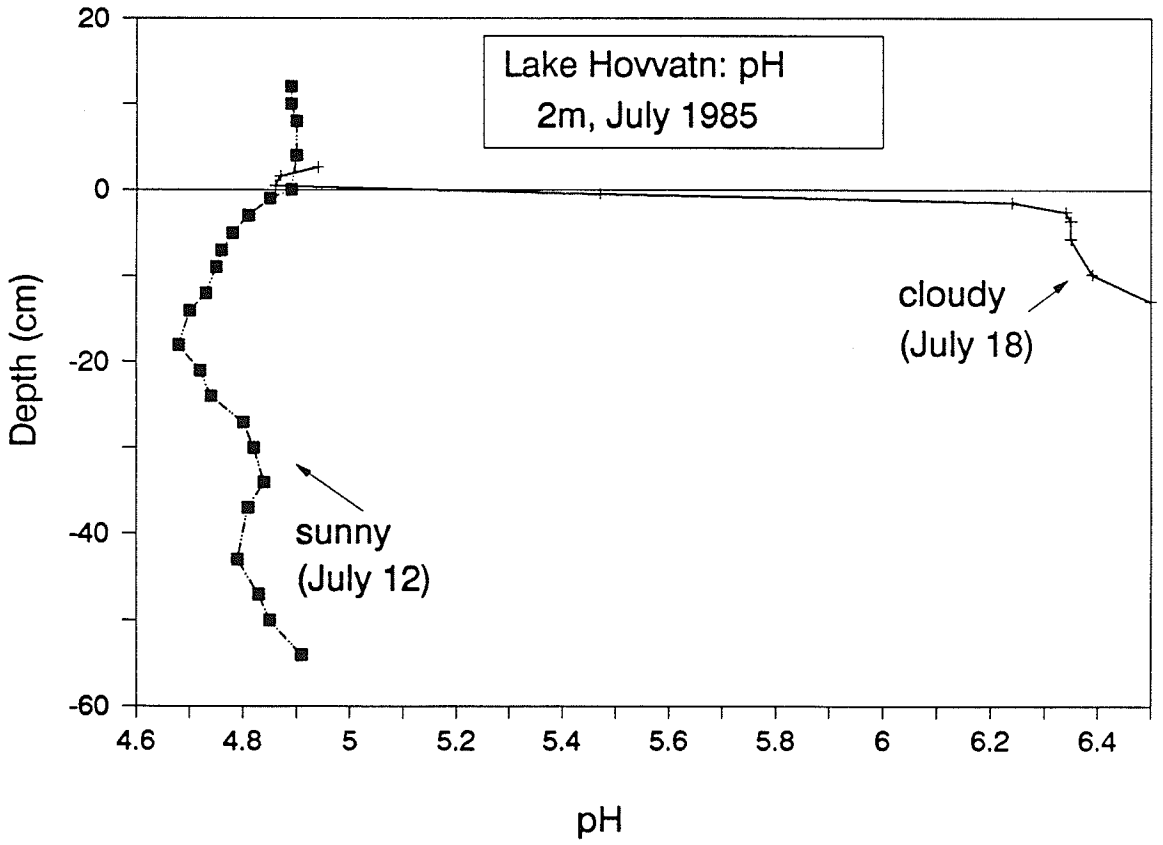

A
Lake Hovvatn: [sulfate] & [H+]
2m, August 1984
50% surface O2
0% surface O2
[H+]
[sulfate]
Depth (cm)
4
2
0
-2
-4
-6
-8
-10
0
20
40
60
B
Lake Hovvatn: [sulfate]
July 18, 1985
4m (#1)
5m
2m
4m (#2)
Depth (cm)
4
0
-4
-8
-12
-16
-20
0
20
40
60
80
100
120
[sulfate] or [H+] (µmol/litre)

Fig. 5.8. Effect of light on the pore-water pH of Lake Hovvatn. Profiles of pH were determined on a sunny day (probe measurement) and during an extended overcast period (equilibrator measurement) at the same site (2m depth). (data obtained from J. Rudd and C. Kelly, FWI and U. of Manitoba, Winnipeg, MB)


Lake Hovvatn: pH
2m, July 1985
cloudy
(July 18)
sunny
(July 12)
Depth (cm)
20
0
-20
-40
-60
4.6
4.8
5
5.2
5.4
5.6
5.8
6
6.2
6.4
pH

al. 1984). This is indicated by the little or no CH_4 production in these sediments (C. Kelly, pers. comm.).

The data show that the S cycle in both 302 South and the Hovvatn lakes is directly affected by the benthic algal community. It appears that the increased algal productivity on the sediments of acidified lakes may affect the S cycle by changing the O_2 regime when viable, and by moving the zone of sulfate reduction close to the surface when decaying. It is possible that the unstable conditions observed in the Hovvatn lakes is a result of the long history of low pH in these systems. It is possible that Lake 302 South will also become an unstable system, with respect to sediment redox conditions, if it remains at pH <5 as long as the Norwegian lakes, and benthic algal communities become more important in controlling sediment redox conditons.

5.5. References

Amaral, J.A., R.H. Hesslein, J.W.M. Rudd, and D.E. Fox. 1989. Loss of total sulfur and changes in sulfur isotopic ratios due to drying of lacustrine sediments. Limnol. Oceanogr. 34: 1351-1358.

ArmstrongF.A.J., and D.W. Schindler. 1971. Preliminary chemical characterization of waters in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28: 171-187.

Baker, L.A., N.R. Urban, P.L. Brezonik, and L.A. Sherman. 1989. Sulfur cycling in an experimentally acidified seepage lake, p. 79-100. In E.S. Saltzman and W.J. Cooper [eds.], Biogenic sulfur in the environment. Am. Chem. Soc.

Brown, K. 1986. Formation of organic sulphur in anaerobic peat. Soil Biol. Biochem. 18: 131-140.

Brunskill, G.J., and D.W. Schindler. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada. 28:139-155.

Canfield, D.E., R. Raiswell, J.T. Westrich, C.M. Reaves, and R.A. Berner. 1986. The use of chromium reduction in the analysis of reduced inorganic sulfur in sediments and shales. Chem. Geol. 54: 149-155.

Carignan, R. 1987. Seasonal dynamics of sulfate reduction

and hydrogen sulfide near the sediment-water interface of an oligotrophic acid lake. Verh. Internat. Limnol. 14: 1-10.

Cook, R.B. and D.W. Schindler. 1983. The biogeochemistry of sulfur in an experimentally acidified lake. Ecol. Bull. 35: 115-127.

Grahn, O., H. Hultberg, and L. Landner. 1974. Oligotrophication - a self-accelerating process in lakes subjected to excessive supply of acid substances. Ambio 3: 93-94.

Healey, F.P. 1973. Inorganic nutrient uptake and deficiency in algae. CRC Critical Rev. Micobiol. 3: 69-113.

Hesslein, R.H. 1976a. The fluxes of CH_4, CO_2, and NH_3-N from sediments and their consequent distribution in a small lake. PhD. thesis, Columbia Univ. 187 p.

Hesslein, R.H. 1976b. An in situ sampler for close interval porewater studies. Limnol. Oceanogr. 21: 912-914.

Howarth, R.W., and S. Merkle. 1984. Pyrite formation and the measurement of sulfate reduction in salt marsh sediments. Limnol. Oceanogr. 29: 598-608.

Howarth, R.W. and J.M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnol. Oceanogr. 28: 70-82.

Howell, E.T., M.A. Turner, R.L. France, M.B. Jackson, and P.M. Stokes. 1990. Comparison of Zygnematacean (*Chlorophyta*) algae in the metaphyton of two acidic

lakes. Can J. Fish. Aquat. Sci. 47: 1085-1092.

Johnson, C.M., and A. Ulrich. 1959. Analytical methods for use in plant analysis. Cal. Agric. Exp. Sta. Bull. 766.

Kelly, C.A. and J.W.M. Rudd. 1984. Epilimnetic sulfate reduction and its relationship to lake acidification. Biogeochemistry 1: 63-77.

Kelly, C.A., J.W.M. Rudd, R.B. Cook, and D.W. Schindler. 1982. The potential importance of bacterial processes in regulating rate of lake acidification. Limnol. Oceanogr. 27: 868-882.

Kelly, C.A., J.W.M. Rudd, A. Furutani, and D.W. Schindler. 1984. Effects of lake acidification rates of organic matter decomposition in sediments. Limnol. Oceanogr. 29: 687-694.

Kling, G.W., A.E. Giblin, B. Fry, and B. J. Peterson. 1991. The role of seasonal turnover in lake alkalinity dynamics. Limnol. Oceanogr. 36: 106-122.

Landers, D.H. and M.J. Mitchell. 1988. Incorporation of $^{35}SO_4^{2-}$ into sediments of three New York Lakes. Hydrobiologia 160: 85-95.

Muller, P. 1980. Effects of artificial acidification on the growth of periphyton. Can. J. Fish. Aquat. Sci. 37: 355-363.

Rudd, J.W.M., C.A. Kelly, and A. Furutani. 1986a. The role of sulfate reduction in long term accumulation of

organic and inorganic sulfur in lake sediments. Limnol. Oceanogr. 31: 1281-1291.

Rudd, J.W.M., C.A. Kelly, V. St. Louis, R.H. Hesslein, A Furutani, and M. Holoka. 1986b. Microbial consumption of nitric and sulfuric acids in acidified north temperate lakes. Limnol. Oceanogr. 31: 1267-1280.

Rudd, J.W.M., C.A. Kelly, D.W. Schindler, and M.A. Turner. 1990. A comparison of the acidification efficiencies of nitric and sulfuric acids by two whole-lake experiments. Limnol. Oceanogr. 35: 663-679.

Schindler, D.W., K.H. Mills, D.F. Malley, D.L. Findlay, J.A. Shearer, I.J. Davies, M.A. Turner, G.A. Lindsey, and D.R. Cruikshank. 1985. Long-term ecosystem stress: The effects of years of experimental acidification on a small lake. Science 228: 1395-1401.

Schindler, D.W. and M.A. Turner. 1982. Biological, chemical and physical responses of lakes to experimental acidification. Water, Air, Soil Pollut. 18: 259-271.

Stokes, P.M. 1986. Ecological effects of acidification on primary producers in aquatic systems. Water, Air, Soil Pollut. 30: 421-438.

Sweerts, J.P., J.W.M. Rudd, C.A Kelly. 1986. Metabolic activities in flocculent surface sediments and underlying sandy littoral sediments. Limnol. Oceanogr. 31: 330-338.

Tabatabai, M.A., and J.M. Bremner. 1970. An alkaline

oxidation method for the determination of total sulfur in soils. Soil Sci. Soc. Am. Proc. 34: 62-65.

Turner, M.A., M.B. Jackson, D.L. Findlay, R.W. Graham, E.R. DeBruyn, and E. M. Vandermeer. 1987. Early responses of periphyton to experimental lake acidification. Can. J. Fish. Aquat. Sci. 44: 135-149.

Wright, R.F., and O.K. Skogheim. 1983. Aluminum speciation at the interface of an acid stream and a limed lake. Vatten 39: 301-304.

Yan, N.B. 1983. Effects of changes in pH and thermal regimes of Lohi Lake, near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. 40: 621-626.

Zhabina, N.N., and I.I. Volkov. (1978). A quick method of determination of various sulfur compounds in sea sediments and rocks, p. 735-745. In W.E. Krumbein [ed.], Environmental biogeochemistry and geomicrobiology: Methods, metals and assessment. Ann Arbor Sci.

6. Effect of organic carbon and sediment redox conditions on the end-products of sulfate reduction.

6.0. Abstract

The effects of organic C additions and sediment redox conditions on the distribution of $^{35}SO_4^{2-}$-S into organic ^{35}S ($^{35}S_O$) and inorganic ^{35}S ($^{35}S_I$) fractions in lake sediments were studied in long and short-term incubations. Addition of organic C (sediment-trap material) to sediments stimulated sulfate reduction and increased $^{35}S_O$ formation by 2-3 fold. Glucose and acetate additions stimulated sulfate reduction to the same extent but $^{35}S_O$ production was less affected. Diagenesis in sediment that was kept anoxic (275 d) favored an increase in $^{35}S_I$ with time. In contrast, in sediment incubated open to the atmosphere, no net change in relative amounts of $^{35}S_I$ and $^{35}S_O$ was observed between initial and final (275 d) distributions.

6.1. Body of note.

Dissimilatory reduction of sulfate to hydrogen sulfide in anoxic sediments leads to the formation of a variety of sulfur containing compounds. This process is an important step in the sulfur cycle and also contributes to internal alkalinity generation in lakes (Kelly et al. 1982; Cook et al 1986). Persistence of these compounds in sediment determines net alkalinity produced by sulfate reduction. Therefore, it is important to know the relative amounts and stabilities of the S compounds produced in this way.

Inorganic sulfur (S_I) compounds (FeS, FeS_2, Fe_3S_4 and S^o), produced from the reactions of H_2S with sedimentary iron (Berner et al. 1979; Cook & Schindler 1983; Berner 1984) initially were considered to be the only important end-products of sulfate reduction, based on studies of marine systems (e.g. Howarth & Merkle 1979; Howarth & Jorgensen 1984). However, it is now recognized that organic sulfur (S_O) can also form from a variety of reactions between organic matter and reduced S_I (see General Introduction). Although it appears clear that S_O formation from sulfate reduction in lakes does occur, the controlling factors which regulate its formation and persistance in sediment relative to S_I are not well understood.

In sulfate reduction experiments where $^{35}SO_4^{2-}$ was added, the relative amounts of $^{35}S_O$ and $^{35}S_I$ produced varied greatly between (Rudd et al. 1986a) and within lakes (Kelly and

Rudd, unpub. data) and with season (Landers and Mitchell 1988). Similarly, in some lakes, increased S storage from increased sulfate supply rates (Holdren et al. 1984; Kelly and Rudd 1984) occurs primarily as S_I (Carignan and Tessier 1988; Herlihy et al. 1989; Giblin et al. 1990), while in other cases, S_O is an important part of the increase (Rudd et al. 1986a; White et al. 1989). The reasons for this variability in type of S stored are not clear. However, it is logical that conditions that affect both initial production as well as stability of these two sulfur fractions will determine their relative importance in sediment.

In the present study, two possible factors affecting the distribution of $^{35}SO_4^{2-}$-S into organic and inorganic forms in sediment were studied. These were, i) amount and form of organic C available, and ii) different sedimentary redox conditions. Short (2.5 d) and longer-term (275 d) incubations were used to measure the effects of these parameters. The results suggest two possible ways in which lake-to-lake and site-to-site variations in S_O and S_I formation and retention may occur.

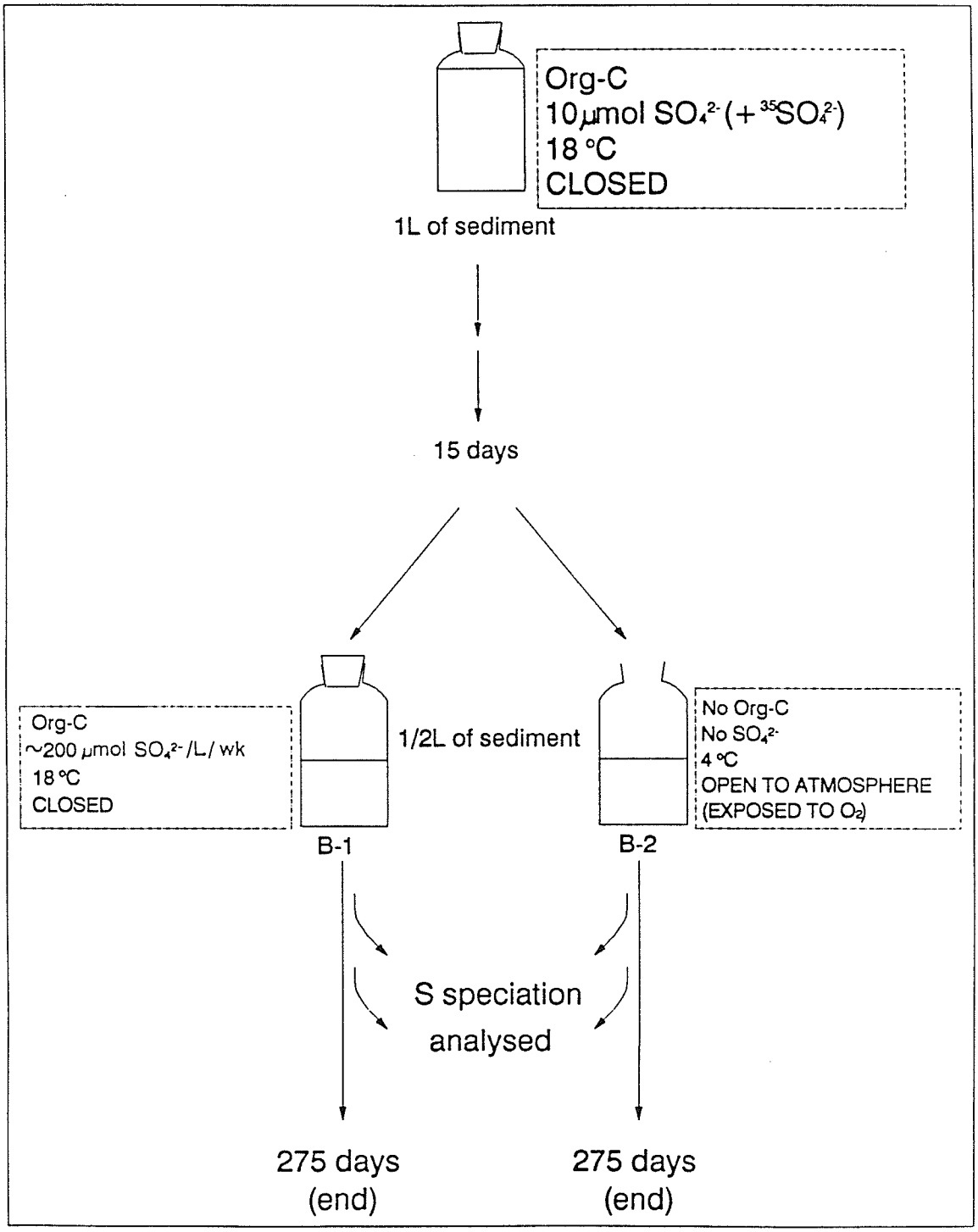
Sediment was obtained by Ekman grab from Lakes 239 (2m) and 303 (2m) at the Experimental Lakes Area (ELA) in northwestern Ontario (Brunskill and Schindler 1972). The top 4 cm of sediment were collected and transported to the laboratory in capped bottles with no headspace. Lake 303

sediment was high in organic content (loss on ignition (LOI) ≈58.4%; Brunskill et al. 1972) and water (porosity >0.95), and is visually described as gyttja. Lake 239 sediment had lower water (porosity 0.9) and organic content (LOI ≈30%; Amaral et al. 1989).

Sediment from Lake 303 was homogenized by shaking under N_2 before distribution into N_2-flushed Erlenmeyer flasks (100 mL), capped with butyl rubber stoppers. Incubations with carrier-free $^{35}SO_4^{2-}$ were carried out at *in situ* temperature (21°C) for 48 h in the dark. Determination of ^{35}S species was done immediately after incubation. Five of six flasks were supplemented with different carbon sources. Dried sediment trap material (1.5, 15 and 30 mg of C), glucose (5 mg of C), and sodium acetate (3 mg of C) were added to 50 mL of sediment to determine their effect on the distribution of ^{35}S. The sediment trap material was collected in acrylic tubes suspended at the thermocline of Lake 240 at the ELA. The material was recovered bi-weekly, dried at 60°C, ground and stored in a dessicator.

Mixed epilimnetic sediment from Lake 239 was used in a "pulse-chase" experiment (275 d), in which redox conditions were varied (Fig. 6.1). Briefly, approximately 1.03L of homogenized sediment was incubated in a butyl rubber-stoppered reagent bottle with $^{35}SO_4^{2-}$ and 10μmol L^{-1} (final concentration) of cold sulfate. The sediment was kept anaerobic by supplying it weekly with 200-240 μmol SO_4^{2-} L^{-1}

Fig. 6.1. Summary of the longer-term, pulse-chase incubation of sediment from Lake 239 with $^{35}SO_4^{2-}$.

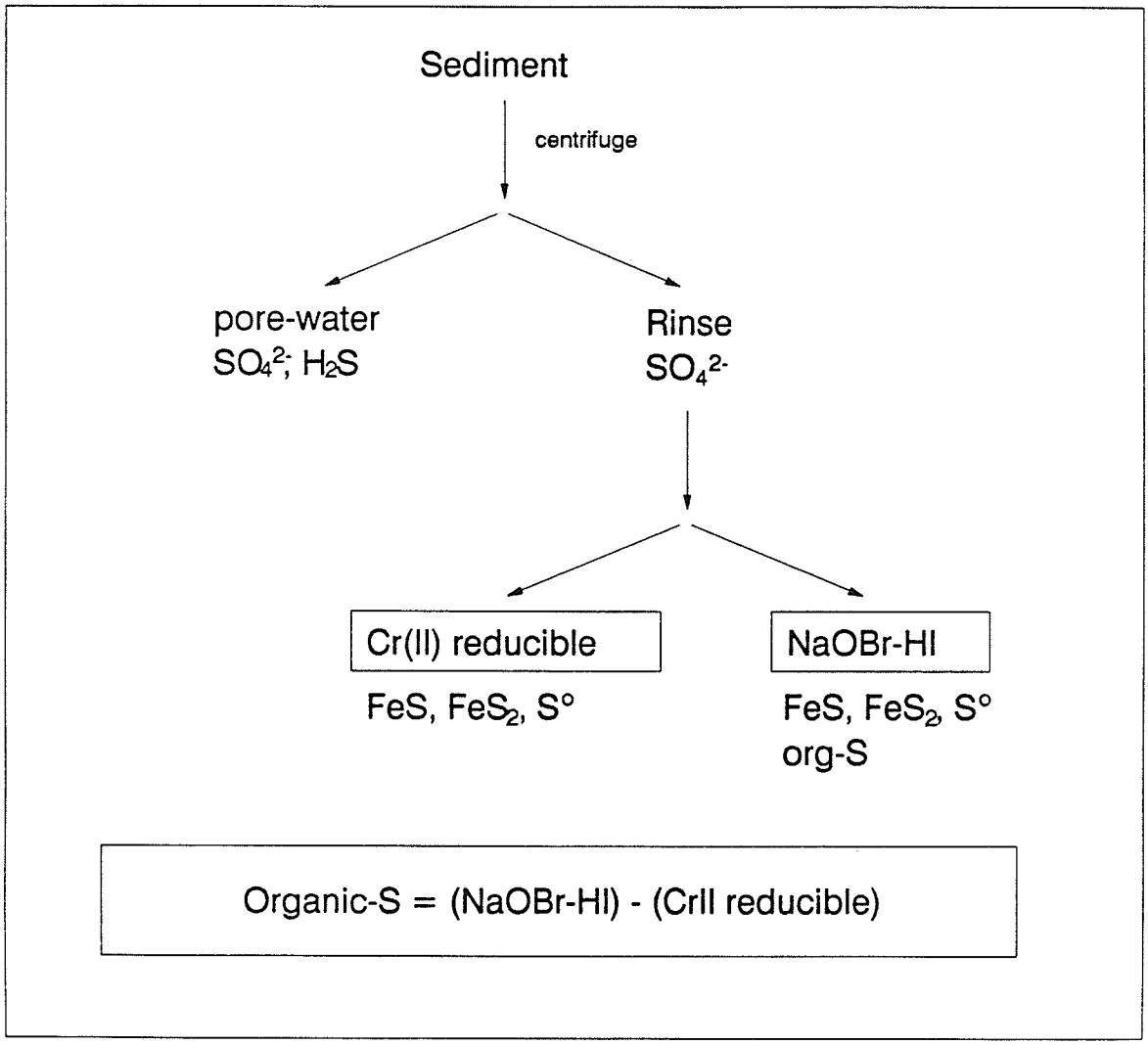

Org-C
10 µmol SO_4^{2-} (+ $^{35}SO_4^{2-}$)
18 °C
CLOSED
1L of sediment
15 days
Org-C
~200 µmol SO_4^{2-}/L/ wk
18 °C
CLOSED
1/2L of sediment
No Org-C
No SO_4^{2-}
4 °C
OPEN TO ATMOSPHERE
(EXPOSED TO O_2)
B-1
B-2
S speciation
analysed
275 days
(end)
275 days
(end)

and fresh carbon in the form of sediment trap material (50 mg L^{-1} of sediment). Samples (15 mL) for S and ^{35}S analysis, were removed periodically (2, 15, 48, 87, 117 and 275 days) after shaking the bottle to ensure homogeneity. The system of Furutani et al. (1984) was used to maintain anoxia during sampling. At 15 days after addition of radiolabel, one half of the sediment in the bottle (B-1) was transferred to a second bottle (B-2). This second bottle was incubated unstoppered, in the dark, and at 4°C, with frequent shaking to ensure exposure of the sediment to oxygen. No sulfate or carbon supplements were added. The bottle was shaken at least once a week, and sampled at the same times as the anoxic bottle (B-1). These manipulations were done to approximate conditions encountered in sediments of anoxic hypolimnia and in the top few cm of epilimnetic sediments perfused with oxygenated water over winter.

Both indigenous and radiolabelled organic and inorganic sulfur species in sediment samples were determined using an analysis protocol that minimizes underestimation of S_0 and $^{35}S_0$ (Fig. 6.2; see also Chapter 3). The protocol consists of a suite of wet chemical procedures, which operationally define the nature of the sulfur fraction measured (e.g. Wieder et al. 1985).

Pore-water (≈40-50%) was first removed from the sediment by centrifugation (3000 rpm, 20 min.), and then filtered through 0.22 μ Millipore filters to remove

Fig. 6.2. Protocol used to measure ^{35}S-labelled and non-labelled S_I and S_O fractions in sediment.


Sediment
centrifuge
pore-water
SO_4^{2-}; H_2S
Rinse
SO_4^{2-}
Cr(II) reducible
FeS, FeS_2, S°
NaOBr-HI
FeS, FeS_2, S°
org-S
Organic-S = (NaOBr-HI) - (CrII reducible)

particulates. Sulfate in the pore-water was measured by hydriodic acid (HI) reduction after concentration of samples by rotoevaporation. The reducing mixture of HI-hypophosphorous-formic acids of Johnson and Ulrich (1959) was prepared as described in Amaral et al. (1989). Sulfate values were corrected for that portion of the pore-water which was not removed by centrifugation. Soluble H_2S is also removed at this step but this fraction is usually negligible for these sediments.

The sediment was then rinsed (see Chapter 3) to remove unreacted, adsorbed $^{35}SO_4^{2-}$. Radiolabel in the porewater and rinse fractions was determined by scintillation counting of aliquots. It was previously determined that only labelled sulfate is removed by this step (Chapter 3).

Separate aliquots of rinsed sediment were then analyzed for S_I and total sulfur (S_T). Chromium (CrII) reduction was used to measure all non-sulfate S_I (Zhabina & Volkov 1978). The procedure involves reductive dissolution of iron sulfides, and reduction of S^o to H_2S with 1M $CrCl_2$ in strong acid, and was carried out as described by Howarth & Merkel (1984). The sulfur released was trapped in basic Zn-acetate traps and titrated iodometrically (Howarth and Teal 1979). Radioactivity was measured in aliquots of the trap by scintillation counting. CrII can also reduce S from organic polysulfides, if these are present, and so may overestimate S_I and $^{35}S_I$ content (Chapter 4).

S_T (labelled and unlabelled) was determined on wet sediment by a modification of the method of Tabatabai and Bremner (1970), as described in Amaral et al. (1989). Briefly, sediment sulfur was oxidized to sulfate by alkaline bromine solution and heat. The resulting sulfate was then reduced to H_2S, by HI solution. S_O, including carbon bonded (C-S) and ester sulfate, was determined as the difference between S_T and chromium reducible S (CRS). This fraction of S is not expected to undergo isotope exchange reactions, as discussed in Chapter 4 (Materials and Methods).

Sulfate reduction rates were determined by the radioisotope method described in Jorgensen (1978).

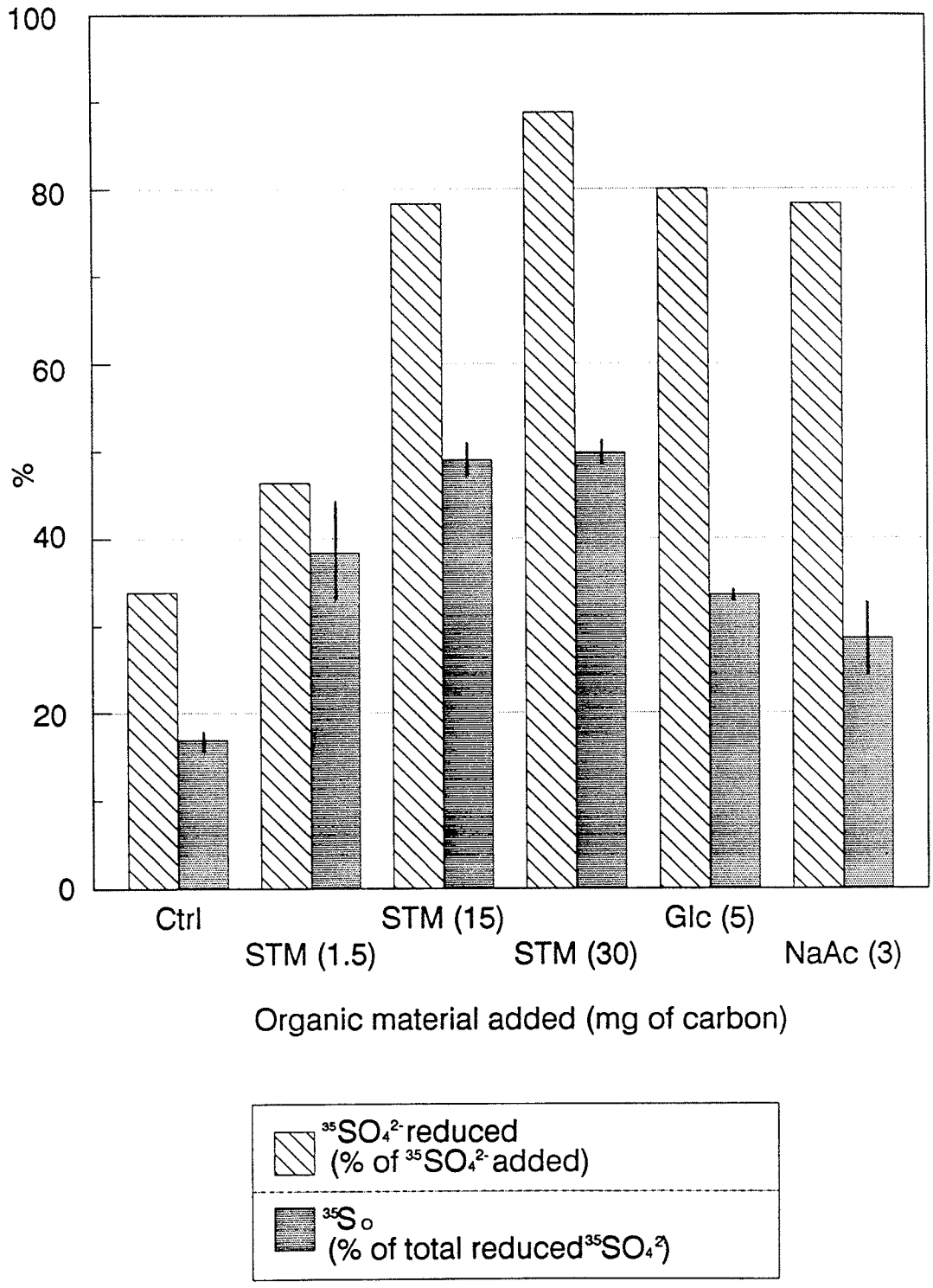
The main purpose of this work was to examine some of the factors which may regulate the formation and retention of products from the reaction of bacterially produced sulfide and sedimentary components. It follows logically that the concentrations of reactants should affect the production of the different forms of S in sediments. For example, Fe concentrations should have a positive effect on production of S_I (Fe-S minerals). This has been verified in the laboratory by Baker et al. (1989) who showed that increased S_I formation resulted from the addition of Fe^{2+} to sediment incubated with $^{35}SO_4^{2-}$. It is expected that organic C should have a similar effect on the production of S_O (e.g. Anderson and Schiff 1987), and this hypothesis was tested here. Previous studies have looked at the effect of C

additions on rates of sulfate reduction (e.g. Westrich and Berner 1984). However, to the author's knowledge, the effect of organic C on the speciation of sulfur has not before been tested in this way.

Addition of organic matter to Lake 303 sediment (2m depth) increased the amount of $^{35}SO_4^{2-}$ reduced in the 2.5 day incubation period (Fig. 6.3). In the control vessel, which received no C supplement, only 34% of the added $^{35}SO_4^{2-}$ was reduced (5.8 μmol SO_4^{2-} reduced L^{-1} day^{-1}). Addition of 1.5 mg C as sediment trap material (STM) increased the amount of reduced $^{35}SO_4^{2-}$ only slightly (46%), while 15 and 30 mg of STM-carbon caused reduction of 78 and 89% of added $^{35}SO_4^{2-}$ (13.8-15.7 μmol SO_4^{2-} L^{-1} day^{-1}). Glucose, and acetate additions also increased considerably the amount of sulfate reduced in 2.5 d (78 to 80%; $\approx$13.5 μmol SO_4^{2-} L^{-1} day^{-1}). The observed increases in sulfate reduction were not unexpected, for even though the sediment was itself high in organic content ($\approx$27% C by weight (i.e. 47% of LOI; R. Hesslein, unpub data), quality and not just quantity of C is important in stimulating this process (Westrich et al. 1984).

The results suggested that the quality of carbon was also important in the formation of S_o from reactions with H_2S. This conclusion is supported by the fact that even though all of the organic C supplements stimulated sulfate reduction to the same extent (Fig. 6.3), the percentage of reduced $^{35}SO_4^{2-}$ going into $^{35}S_o$ with STM addition (38-50%), was

Fig. 6.3. The effect of different forms of organic C on the reduction of $^{35}SO_4^{2-}$ and production of $^{35}S_0$ in sediment from Lake 303. Incubations lasted 2.5 days. Vertical lines represent 1 SD. STM= sediment-trap material, Glc= glucose, NaAc= sodium acetate, Ctrl= control. Numbers in brackets refer to the amount of C (mg) added.


100
80
60
40
20
0
%
Ctrl
STM (1.5)
STM (15)
STM (30)
Glc (5)
NaAc (3)
Organic material added (mg of carbon)
$^{35}SO_4^{2-}$ reduced
(% of $^{35}SO_4^{2-}$ added)
$^{35}S_o$
(% of total reduced $^{35}SO_4^{2-}$)

2-3 fold higher than in the control sediment (Fig 6.3), while smaller increases in $^{35}S_O$ production (29-34%) were observed with glucose and acetate (Fig. 6.3). Thus, the added organic C appeared to act both as a fuel for sulfate reduction as well as a sink for $H_2{}^{35}S$.

Differences in the quality (i.e. chemical structure) of the organic C, then, may partly account for differences in S speciation between sediments and sites. It is possible that "old" organic matter in the sediment may have lost the reactive groups which commonly react with H_2S and S^o (Francois 1987). Further evidence for this was obtained in laboratory experiments by Bestougeff and Combaz (1974) who found that H_2S was more reactive toward fresh organic matter (algal cells) than with organics in rocks and kerogen.

It is unlikely that the parameter of "quality" of organic C alone can be used to predict the relative formation of S_O versus S_I. This is also true for iron content, since examination of data from several lakes showed no clear correlation between pore-water $[Fe^{2+}]$ and relative formation of $^{35}S_O$ and $^{35}S_I$ (Rudd et al 1986a, b). However, organic C quality should be considered as a possible controlling factor in the initial distribution of S between chemical groups in the sediment.

Possible factors that might change the relative amounts of S_I and S_O initially produced from sulfate reduction were also examined in this work. A longer-term "pulse chase"

incubation was used to follow an initial pulse of $^{35}SO_4^{2-}$ into different S fractions with time and under different redox conditions. In this experiment, $^{35}SO_4^{2-}$ added to sediment (Lake 239 2m) was almost totaly reduced (97%) after two days ($CR^{35}S$ + $^{35}S_O$, Table 6.1). Approximately 30% of the reduced ^{35}S label was initially partitioned into the organic fraction, so that, $^{35}S_I$ ($CR^{35}S$) dominated (70%) (Table. 6.1). The $^{35}S_O$, in the bottle that was kept closed and supplemented with C and SO_4^{2-} (B-1; Fig. 6.1), decreased with time to one half of its original amount, while $^{35}S_I$ increased concomitantly (Table 6.1; Fig. 6.4). For this sediment, the ratio of $^{35}S_I$:$^{35}S_O$ doubled from an initial 2.3:1, to 5.3:1, after 275 days of incubation (Table 6.2).

The water soluble fraction persisted throughout the 275 day incubation, although at very low levels (1-2% of total ^{35}S)(Table 6.1). The radiolabel was assumed to be mostly free $^{35}SO_4^{2-}$, with 10% of the activity (at 275 days) being due to $H_2^{35}S$ (detected by GC-proportional counter analysis; Richards, in prep.) (data not shown). This fraction could represent low level turnover of reduced ^{35}S compounds possibly due to hydrolysis of ester sulfates, anaerobic oxidation of sulfides or desulfurization of $^{35}S_O$ compounds (King and Klug 1980); Luther et al. 1988; Freney 1967). If ^{35}S released in this manner reacts once again with sedimentary components (favoring $^{35}S_I$ production), this may

Table 6.1 Distribution of $^{35}SO_4^{2-}$-S in Lake 239 epilimnetic sediment with time and incubated open and closed to the atmosphere.

	Incubation time (d)	Distribution of ^{35}S (dpm g^{-1} wet weight of sed.)*			
		Soluble	CRS	S_o	S_{T+}
B-1 & B-2	2	26,170	721,962 (70)	316,455 (30)	1,064,587
	15	15,042	734,738 (72)	281,566 (28)	1,031,346
B-1	48	22,347	738,689 (71)	296,609 (29)	1,057,645
	87	11,408	833,842 (83)	176,834 (17)	1,022,084
	117	11,849	854,253 (85)	152,689 (15)	1,018,791
	275	9,639	858,440 (84)	162,423 (16)	1,030,502
B-2	48	231,424	558,163 (91)	57,181 (9)	846,768
	87	461,522	316,436 (67)	118,824 (33)	896,782
	117	523,899	315,965 (67)	152,535 (33)	992,399
	275	378,829	316,333 (76)	99,318 (24)	794,480

* number in parantheses = % of total reduced ^{35}S

\+ $^{35}S_T$ = solid ^{35}S + soluble ^{35}S

B-1 = closed to atmosphere throughout incubation period

B-2 = open to atmosphere (after 15 days of incubation)

Fig. 6.4. Changes in ^{35}S distribution between $^{35}S_I$ and $^{35}S_O$ in sediment incubated open (B-2) and closed (B-1) to the atmosphere. (see Fig. 6.1). The arrow indicates when B-2 was opened to the atmosphere.

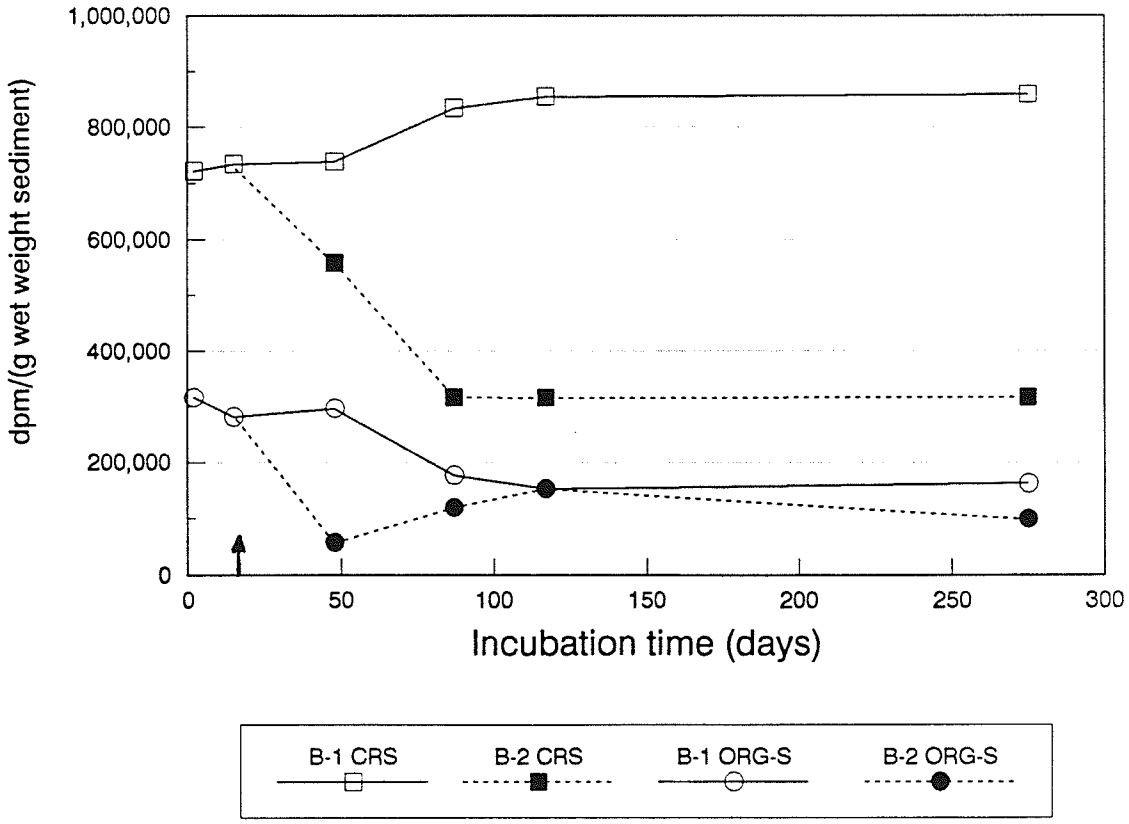

1,000,000
800,000
600,000
400,000
200,000
0
dpm/(g wet weight sediment)
0
50
100
150
200
250
300
Incubation time (days)
B-1 CRS
B-2 CRS
B-1 ORG-S
B-2 ORG-S

Table 6.2 Summary of results for the 275 day incubation of Lake 239 sediment.

Bottle name	$^{35}S_I:^{35}S_O$		$S_I:S_O$		pH	
	initial	final	initial	final	initial	final
B-1* (closed)	2.3:1	5.3:1	0.3:1	1:1	6.11	6.43
B-2 (open)	2.6:1	2.6:1	0.3:1	0.2:1		5.23

* the "cold" sulfate added to B-1 over the incubation period was distributed at a ratio of 3:1, $S_I:S_O$.

be a mechanism by which $^{35}S_I$ can increase at the expense of $^{35}S_O$. Soluble cold SO_4^{2-} in the porewater was always low at sampling times (Table 6.3), indicating that the added sulfate was continuously reduced.

By measuring the increase in cold S_I and S_O produced from the weekly additions of cold SO_4^{2-} (Table 6.3), one can calculate the distribution of this S into these two S pools. At the end of the incubation the cold SO_4^{2-} added throughout the experiment distributed into S_I and S_O fractions in a ratio (3:1) intermediate to the initial and final ratios for $^{35}SO_4^{2-}$ (Table 6.2). This is the expected result if the changes in the ^{35}S distribution were due to diagenesis and not due to isotope exchange reactions, confirming the assumption made earlier.

In the bottle that was opened to the atmosphere (B-2) and incubated in the cold without C or SO_4^{2-} supplements, the radioactivity in the water soluble fraction increased 10-20 fold (Table 6.1). Co-electrophoresis (on paper) with a $^{35}SO_4^{2-}$ standard and precipitation with $BaCl_2$ confirmed that the radiolabel was $^{35}SO_4^{2-}$ (data not shown). Unlabelled sulfate was also released from the solid phase CRS (S_I), but not the S_O fraction, after oxygenation of the sediment (Table 6.3). At the same time, large decreases in solid phase $^{35}S_O$ and $^{35}S_I$ (Table 6.3) occurred. These events, as well as a decrease in sedimentary pH (Table 6.2), indicated that oxidizing redox conditions were established

Table 6.3 Distribution of S in Lake 239 epilimnetic sediment incubated with and without SO_4^{2-} over time and incubated open and closed to the atmosphere.

Incubation time (d)		Distribution of S (umol g^{-1} wet weight of sed.)			
		SO_4^{2-}	CRS	S_O	S_T
B-1 & B-2	2	ND*	1.56	5.91	7.47
	15	ND	1.88	5.95	7.83
B-1	48	0.13	2.50	6.11	8.74
	87	0.20	3.63	--+	--
	117	0.13	--	--	--
	275	0.10	7.94	8.03	16.07
B-2	48	0.56	1.92	5.50	7.98
	87	0.61	1.82	--	--
	275	0.67	1.26	6.11	8.04

* ND = not detected

\+ dashes = analysis not done

B-1 = closed to atmosphere throughout incubation period

B-2 = open to atmosphere (after 15 days of incubation)

in the sediment, even though direct measurements of O_2 and Eh were not done.

The $^{35}S_O$ fraction increased with time, after an initial decrease of 80%, and both this and the $^{35}S_I$ fraction stabilized at a ratio equal to the pre-oxidation value, although at only one half of the initial quantity (Tables 6.2 and 6.3; Fig. 6.4). The increase of the $^{35}S_O$ fraction may indicate production of compounds more resistant to oxidation, such as sulfonates (Ferdelman et al. 1991).

Measurements of ^{35}S in bottle B-2 showed that about 15% of the radioactivity added was lost (Table 6.1), possibly due to volatilization of some sulfides due to the decrease in pH. However, no losses of unlabelled S occurred from this bottle. The ratio of reduced S_I:S_O decreased slightly, indicative of the preferential oxidation of CRS (Tables 6.2 and 6.3). Kling et al (1991), also found that 30-70% of S oxidized in test cores originated from S_I. It would appear from the results that indigenous S_O was more resistant to oxidation than the newly formed $^{35}S_O$.

The results showed that changes in ^{35}S distribution can occur over time and under different redox conditions (Fig. 6.3). The environment maintained in bottle B-1 was analogous to that in hypolimnetic sediments overlain by anoxic bottom waters. Under these conditions, reduced $^{35}S_I$ became more important with time at the expense of $^{35}S_O$ (Table 6.1; Fig. 6.4). It is interesting to note that studies of

accumulation of S from sulfate reduction in hypolimnetic sediments (e.g. Carignan & Tessier 1988; Herlihy et al 1989: Giblin et al. 1990) have found S_I to be the main storage product. The results also agree with the observation that in the deeper sections of freshwater peat and sediment cores there is a relative decrease of ester-sulfate and carbon-bonded S_O and a concomitant increase in S_I (Altschuler et al. 1983; Losher and Kelts 1989).

In contrast, in bottle B-2, where conditions were manipulated to cause oxidation of solid phase sulfides, the relative amount of $^{35}S_O$ versus $^{35}S_I$ remaining in the sediment at the end of the incubation period was much higher (Table 6.2). The conditions in this bottle could be considered analogous to the top layers of epilimnetic sediments which are perfused with oxygenated water during winter. These bottle incubations suggest that in sediments where there is more fluctuation of redox potential (e.g. epilimnetic), storage of S_O produced from dissimilatory reduction of SO_4^{2-} may be more important than in sediments that remain anoxic in the long term (eg. hypolimnetic). If these results can truly be applied to the *in situ* behaviour of S compounds in lake sediments, they may partly explain some of the site-to-site variation seen in the prefered form of S that is stored in sediments. It is significant to note that Rudd et al. (1986a), in an *in situ* experiment conducted in epilimnetic sediment, concluded that $^{35}S_O$ was more resistant than $^{35}S_I$ to

oxidation over the winter.

In summary, both organic C (quantity and quality) and sedimentary redox conditons affected the formation and retention of newly formed S_o and S_I. Because, these factors may differ from site-to-site within and between lakes, they may explain at least part of the variability commonly observed in the production and storage of S compounds in lake sediments.

6.2. References

Altschuler, Z.S., M.M. Schnepfe, C.C. Silber, and F.O. Simon. 1983. Sulfur diagenesis in Everglades peat and origin of pyrite in coal. Science 221: 221-227.

Amaral, J.A., R.H. Hesslein, J.W.M. Rudd, and D.E. Fox. 1989. An evaluation of the effects of drying on the total sulfur content and sulfur isotopic composition of lacustrine sediments. Limnol. Oceanogr. 34: 1351-1358.

Anderson, R.F., and S. L. Schiff. 1987. Alkalinity generation and fate of sulfur in lake sediments. Can. J. Fish. Aquat. Sci. 44:188-193.

Baker, L.A., N.R. Urban, P.L. Brezonik, & L.A. Sherman. 1989. Sulfur cycling in an experimentally acidified lake, p. 79-100. In E. Saltzman & W. Cooper [eds.], Biogenic sulfur in the environment. ACS Books.

Berner, R.A., T. Baldwin, G.R. Holdren, Jr. 1979. Authigenic iron sulfides as paleosalinity indicators. J. Sed. Petrol. 49: 1345-1350.

Berner, R.A. 1984. Sedimentary pyrite formation: an update. Geochim. Cosmochim. Acta 48: 605-615.

Bestougeff, M. and A Combaz. 1973. Action de H_2S et de S sur quelques substances organique actuelle et fossiles, p. 747-759. In B.T. Tissot and F. Bienner [eds.], Advances in organic geochemistry. Pergamon Press.

Brown, K.A.. 1986. Formation of organic sulphur in anaerobic peat. Soil Biol. Biochem. 18: 131-140.

Brunskill, G.J., and D.W. Schindler. 1971. Geography and bathymetry of lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28: 139-155.

Brunskill, G.J., D. Povoledo, B.W. Graham, and M.P. Stainton. 1971. Chemistry of surface sediments of sixteen lakes in the Experimental Lakes Area, nothwestern Ontario. J. Fish. Res. Bd. Canada 28: 277-294.

Carignan, R., and A. Tessier. 1988. The co-diagenesis of sulfur and iron in acid lake sediments of southwestern Quebec. Geochim. Cosmochim. Acta 52: 1179-1188.

Cook, R.B. and D.W. Schindler. 1983. The biogeochemistry of sulfur in an experimentally acidified lake. Ecol. Bull. 35: 115-127.

Cook, R.B., C.A. Kelly, D.W. Schindler, and M.A. Turner. 1986. Mechanisms of hydrogen ion neutralization in an experimentally acidified lake. Limnol. Oceanogr. 31: 134-148.

Ferdelman, T.G., T.M. Church, and G.W. Luther III. 1991. Sulfur enrichment of humic substances in a Delaware salt marsh sediment core. Geochim. Cosmochim. Acta 55: 979-988.

Francois, R. 1987. A study of sulfur enrichment in the

humic acid fraction of marine sediments during early diagenesis. Geochim. Cosmochim. Acta 51: 17-27.

Freney, J.R. 1967. Sulfur-containing organics, p. 229-259. In A.D. Mclaren and G.H. Petersen [eds.]. Soil Biochemistry. Marcel Dekker.

Furutani, A., J.W.M. Rudd, and C.A. Kelly. 1984. A method for measuring the response of sediment microbial communities to environmental perturbations. Can. J. Microbiol. 30: 1408-1414.

Giblin, A.E., G.E. Likens, D. White, and R.W. Howarth. 1990. Sulfur storage and alkalinity generation in New England lake systems. Limnol. Oceanogr. 35: 852-869.

Herlihy, A.T., A.L. Mills and J.S. Herman. 1988. Distribution of reduced inorganic sulfur compounds in lake sediments receiving acid mine drainage. Appl. Geochem. 3: 333-344.

Holdren, G.R. Jr, T.M. Brunelle, G. Matisoff, and M. Wahlen. 1984. Timing the increase in atmospheric sulphur deposition in the Adirondack Mountains. Nature 311: 245-247.

Howarth, R.W., and S. Merkel. 1984. Pyrite formation and the measurement of sulfate reduction in salt marsh sediments. Limnol. Oceanogr. 29: 598-608.

Howarth, R.K., and B.B. Jorgensen. 1984. Formation of ^{35}S-labelled elemental sulfur and pyrite in coastal marine sediments (Limfjorden and Kysing Fjord, Denmark) during

short-term $^{35}SO_4^{2-}$ reduction measurements. Geochim. Cosmochim. Acta 48: 1807-1818.

Howarth, R.W., and J.M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnol. Oceanogr. 28: 70-82.

Johnson, C.M., and A. Ulrich. 1959. Analytical methods for use in plant analysis. California Agr. Exp. Sta. Bull. 766, 55.

Jorgensen, B.B. 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurement with radiotracer techniques. Geomicrobiology J. 1: 11-27.

Kelly, C.A., R.B. Cook, and D.W. Schindler. 1982. The potential importance of bacterial processes in regulating the rate of lake acidification. Limnol. Oceanogr. 27: 868-882.

Kelly, C.A., and J.W.M. Rudd. 1984. Epilimnetic sulfate reduction and its relationship to lake acidification. Biogeochem. 1: 63-77.

King, G.M., and M.J. Klug. 1980. Sufhydrolase activity in sediments of Wintergreen Lake, Kalamazoo County, Michigan. Appl. Environ. Microbiol. 39: 62-65.

Kling, G.W., A.E. Giblin, B. Fry, and B. Peterson. 1991. The role of seasonal turnover in lake alkalinity dynamics. Limnol. Oceanogr. 36: 106-122.

Landers, D.H., and M.J. Mitchell. 1988. Incorporation of $^{35}SO_4^{2-}$ into sediments of three New York lakes.

Hydrobiologia 160: 85-95.

Losher, A.J., and K.R. Kelts. 1989. Organic sulphur fixation in freshwater lake sediments and the implication for C/S ratios. Terra Nova 1: 253-261.

Luther, G. W., T.M. Church, J.R. Scudlark, and M. Cosman. 1985. Inorganic and organic sulfur cycling in Salt Marsh pore-waters. Science 232: 746-749.

Luther, G. W., T. Ferdelman, and E. Tsamakis. 1988. Evidence suggesting anaerobic oxidation of the bisulfide ion in Chesapeake Bay. Estuaries 11: 281-285.

Nriagu, J.O., and Y.K. Soon. 1985. Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. Geochim. Cosmochim. Acta 49: 823-834.

Rudd, J.W.M., C.A. Kelly, and A. Furutani. 1986a. The role of sulfate reduction in long term accumulation of organic and inorganic sulfur in lake sediments. Limnol. Oceanogr. 31: 1281-1291.

Rudd, J.W.M., C.A. Kelly, V. St. Louis, R.H. Hesslein, A. Furutani, and M. Holoka. 1986b. Microbial consumption of nitric and sulfuric acids in acidified north temperate lakes. Limnol. Oceanogr. 31: 1267-1280.

Tabatabai, M.A., and J.M. Bremner. 1970. An alkaline oxidation method for the determination of total sulfur in soils. Soil Sci. Soc. Amer. Proc. 34: 62-65.

Westrich, J.T., and R.A. Berner. 1984. The role of

sedimentary organic matter in bacterial sulfate reduction: The G model tested. Limnol. Oceanogr. 29: 236-249.

White, J.R., C.P. Gubala, B. Fry, J. Owen, and M.J. Mitchell. 1989. Sediment biogeochemistry of iron and sulfur in an acidic lake. Geochim. Cosmochim. Acta. 53: 2547-2559.

Wieder, R.K., G.E. Lang, and V.A. Granus. 1985. An evaluation of wet chemical methods for quantifying sulfur fractions in freshwater wetland peat. Limnol. Oceanogr. 30: 1109-1114.

Zhabina, N.N. & I.I. Volkov. 1978. A method of determination of various sulfur compounds in sea sediments and rocks, p. 735-746. in W.E. Krumbein [ed.], Environmental biogeochemistry and geomicrobiology. Vol. 3. Ann Arbor Sci.

7. Thesis Summary

The preceding chapters have dealt largely with the measurement of organic sulfur (S_O) and $^{35}S_O$ (from sulfate reduction) in lake sediments. The importance of S_O in the lacustrine S cycle may have previously been underestimated due to analytical difficulties. Three basic problems were identified, each of which can lead to falsely low estimates of S_O and $^{35}S_O$.

First, the common practice of drying sediment before determination of total S (S_T), usually by Leco analyzer, resulted in large and variable losses of S. Because S_o is determined as the difference between S_T and inorganic-S (S_I), the error in the S_T values would lead to S_O values that are too low. The S lost by drying was derived from the solid phase of the sediment and was hypothesized to be S_O in nature. Significant changes in stable isotope ratios due to drying supported the view that S loss did not occur equally from sedimentary S species. Therefore, methods suitable for measurement of S_T on wet sediment must be used to determine accurately both S_T values and S_O values.

Second, analytical protocols in which strong acid was followed by sequential analyses of the same sediment sample underestimated S_O and $^{35}S_O$ content. The acid teatment caused loss of S_O and $^{35}S_O$. There are two possible mechanisms for this: (i) ester sulfate linkage hydrolysis under acidic conditions, resulting in the release of free sulfate, and (ii) acid solubilization or hydrolysis of other S_O compounds

from the solid phase of the sediment and their subsequent removal by washing before further analysis of the remaining solid phase S. A non-sequential analytical protocol that did not use acid prior to S_T and S_I determination was found to give higher and more accurate estimates of S_0 and $^{35}S_0$ than two sequential methods tested.

Third, compounds behaving as organic polysulfides were identified in sediment from the Experimental Lakes area (Lake 303). In sediments where these compounds are present in significant amounts, large underestimates of S_0 (or $^{35}S_0$) will occur since chemical methods in current use (CrII reduction) measure these compounds as part of the S_I pool. Therefore, in order to avoid this type of underestimate organic polysulfides must be measured separately. Practical methods for this S fraction in sediments need to be developed.

Reliable estimates of S_0 and $^{35}S_0$ are important for several reasons. Accurate rates of sulfate reduction, as determined by incubation with $^{35}SO_4^{2-}$, require that the amount of reduced radiolabel (both $^{35}S_I$ and $^{35}S_0$) be quantitatively known. Underestimation of S_0 production would underestimate not only total sulfate reducing activity, but also the importance of S_0 as an end-product of sulfate reduction. This in turn would underestimate the importance of S_0 formation to internal alkalinity production in lakes. The use of appropriate and standardized methods for the

measurement of S_0 would allow for direct intercomparison of results from different studies and would lead to a better understanding of the relative importance of S_0 formation and storage in sediments. The present uncertainty about whether or not S_0 or S_I is the most important form of S storage in lakes polluted by increased sulfate loading may be due in part to analytical differences.

Differing conclusions concerning the importance of S_I or S_0 in S storage may also arise due to other factors. For example, it was found that addition of organic carbon to sediments increased the relative amount of $^{35}S_0$ formed from the reduction of $^{35}SO_4^{2-}$, and this increase was related to the type of C added. Similarly, a longer-term (275d) bottle experiment also suggested an effect of sediment redox potential since the relative amount of $^{35}S_0$ retained was greater in sediment exposed to the atmosphere, than in sediment that was not. These two factors may be partly responsible for the variation observed in the type of end-product of sulfate reduction that is stored in sediments. The results may also in part explain why short term end-products of $^{35}S_04^{2-}$ reduction differ in distribution from pre-existing sedimentary S_0. Some of this difference in distribution is also due to the fact that not all of the pre-existing sedimentary S_0 was formed from dissimilatory sulfate reduction, but may have originated from plant and animal matter from the watershed and water column of the

lake.

Due to the lack of direct and specific analytical methods for S_O in sediment, this fraction is usually measured by difference ($S_O = S_T - S_I$). This means that there has been no positive evidence that this difference is indeed due to S_O compounds and there exists the possibility that some or all of the difference is due to S_I compounds not recoverable by chromium reduction. A variety of extraction, chromatographic and electrophoretic techniques, coupled with wet chemical methods, were used to measure $^{35}S_O$ directly. The procedure could distinguish between $^{35}S_I$ and $^{35}S_O$ so that chemical interference by S_I could be removed. In this way, I was able to measure directly the S_O formed from sulfate reduction in lake sediment. The results show, under conditions more stringent than have previously been used, that S_O is an important endproduct of sulfate reduction in lake sediments. As well as confirming the production of carbon bonded S (C-S) and ester sulfate (C-O-S), the procedure allowed for the identification of a third type of S_O, organic polysulfide. As previously discussed this type of compound is measured as S_I, even though the S is bound to an organic core, and may be an important form of S storage in sediments that is not usually considered. If this type of S storage is not measured, the importance of Fe as a sink for S in sediments will be overestimated.

The last part of this work dealt with the broader topic

of the lacustrine S cycle and how it might be affected by acidification. The S cycle of experimentally acidified Lake 302 South was disrupted when water column pH decreased below 5.0. Since the pH dropped below this point, metaphytic algal mats (*Zygogonium spp.*) have covered extensive areas of the littoral sediments, and have enhanced the oxidation of sedimentary S when they were photosynthetically active, thereby increasing the period in which the sediment acted as a source of sulfate to the water column. Upon senescence, the algae had an opposite effect, enhancing reduction of sulfate in the winter in areas immediately below and within the algal mat. This enhancement of sulfate reduction was also seen by addition of fresh carbon (sediment-trap material) to sediment (Chapter 6). The resulting accumulation of reduced S in the top of the sediment column likely makes it more susceptible to reoxidation once algal C is used up, or if the decaying mat is removed by water currents. The algal effects may be the cause of large annual fluctations of sulfate concentration which also began when the pH of the lake dropped below 5. A similar disruption in the S cycle was observed in a chronically acid lake where the degradation of C was apparently inhibited by low sedimentary pH, and it was frequently observed that sediments acted as a source of of sulfate rather than a sink. Also, as in Lake 302 South, the direction of sulfate flux was linked to benthic algal O_2 production. The

inability of the sediment to act as a net sulfate sink may decrease in lakes that remain severely acidified for prolonged times.

Appendix I.

Specificity and efficiency of some analytical methods for different sulfur compounds. Determinations done in the presence of lake sediment (≈5 g) from Lake 114 at ELA.

Chemical species	% of S recovered (n= 2-5) AVS (6N HCl)*		HI reduction+	
	25°C	boiling	25°C	boiling
$FeS_{1.8}$ ∓	0.0	0.5±0.06	0.0	68±4
$Na_2S_2O_3$	8.7±3.8	-	-	101±0
Na_2SO_4	-	-	0.16±0.17	93±3
Pyrite ore	<0.01	-	-	8.7±1.3
S°	-	-	-	59±6
Methionine	-	-	-	<0.01
Phenyl-thiourea	-	-	-	4.6±1.0
SDS§	-	-	-	92.4±1.6

* AVS = Acid volatile sulfide

\+ HI = Hydriodic acid

∓ Synthesized in lab according to Wada (1987)

§ Sodium dodecyl sulfate.

Appendix II.

Indigenous distribution of S and the distribution of ^{35}S after short term (24-48 hrs) incubation with $^{35}SO_4^{2-}$ in mixed, anoxic sediments.

Sediment ID	Porosity	^{35}S Distribution		S Distribution	
		%CRS*	$\%S_0$	%CRS	$\%S_0$
L 239 epi	0.88	79.8	20.2	17.3	82.7
L 303 epi	0.98	77.5	22.5	39.0	61.0
L 302N epi	0.65	56.9	43.1	36.4	63.6
L 302N hypo	0.98	70.2	29.8	62.3	37.7

* CRS = chromium reducible sulfur (inorganic sulfur)

Appendix III.

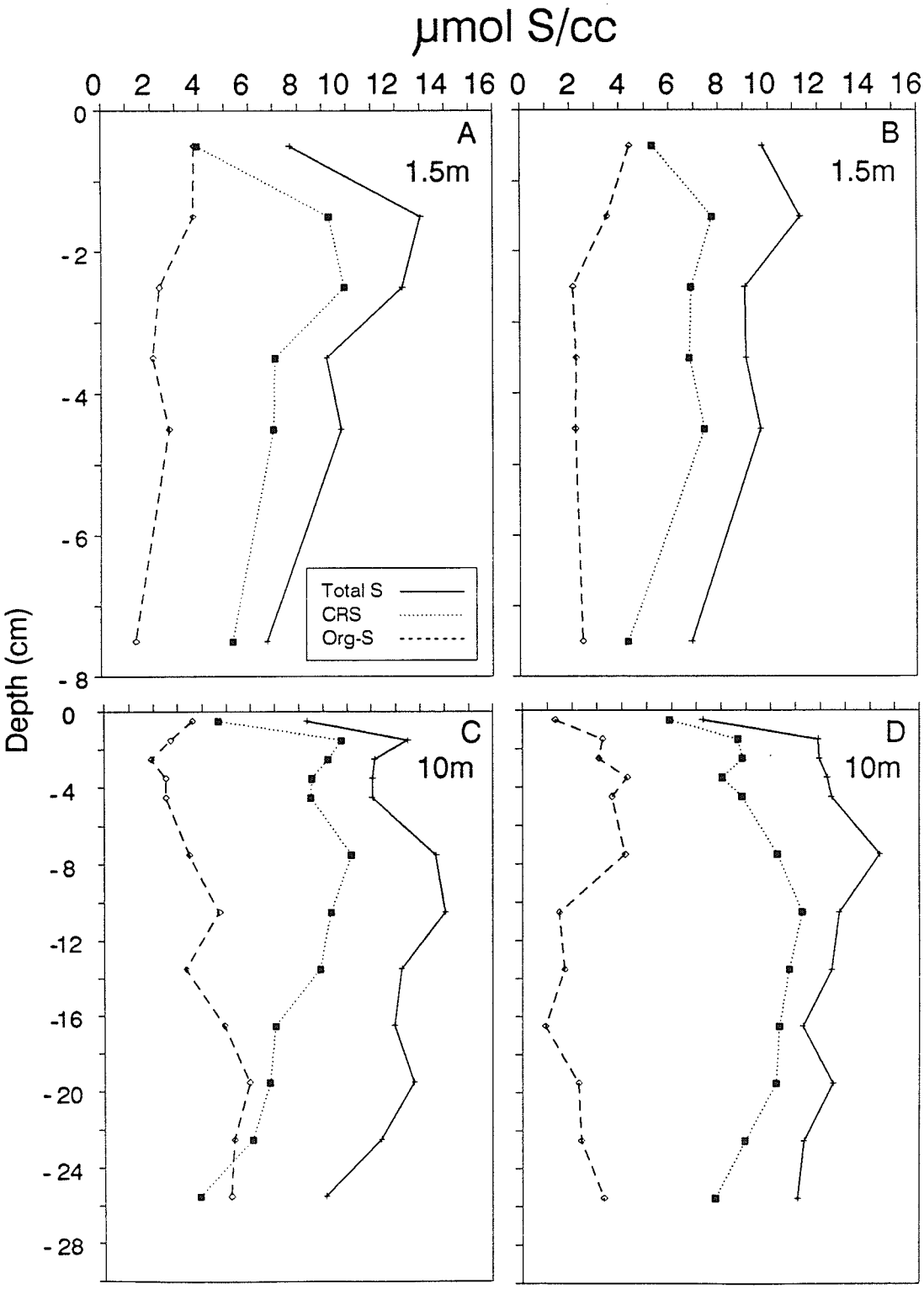
Summary of results of an experiment to determine if isotope exchange occurs between elemental S and cysteine in the presence and absence of NaS. Approximately 1 mM (final concentration) of cysteine and NaS were added to duplicate vials with deoxygenated, deionized water (about 5 mL). A saturated solution of elemental S ,including a small amount of radiolabelled S° (Amersham), in acetone (degassed with He) was added to the vials until there was no headspace volume (about 3mL). The same procedure was followed for two other vials to which no NaS was added. All vials were capped and incubated without a headspace at 22°C, in the dark, for 28 hours. Aliquots (25 μL) from each vial were paper chromatographed (ethanol (5):butanol (2.5):water (1)) and scanned for radioactivity. Ninhydrin spray was used to visualize the cysteine. In this system S° and cysteine have R_f values of approximately 0.89 and 0.55, respectively.

	Cysteine + S° (^{35}S°) + NaS	Cysteine + S° (35S°)
Ninhydrin positive (R_f)	0.52 ± 0.4	0.53 ± 0.3
Radioactivity detected (R_f)	0, 0.78 ± 0.7	0, 0.84 ± 0.3

Values given are means of duplicates ± 1 SD.

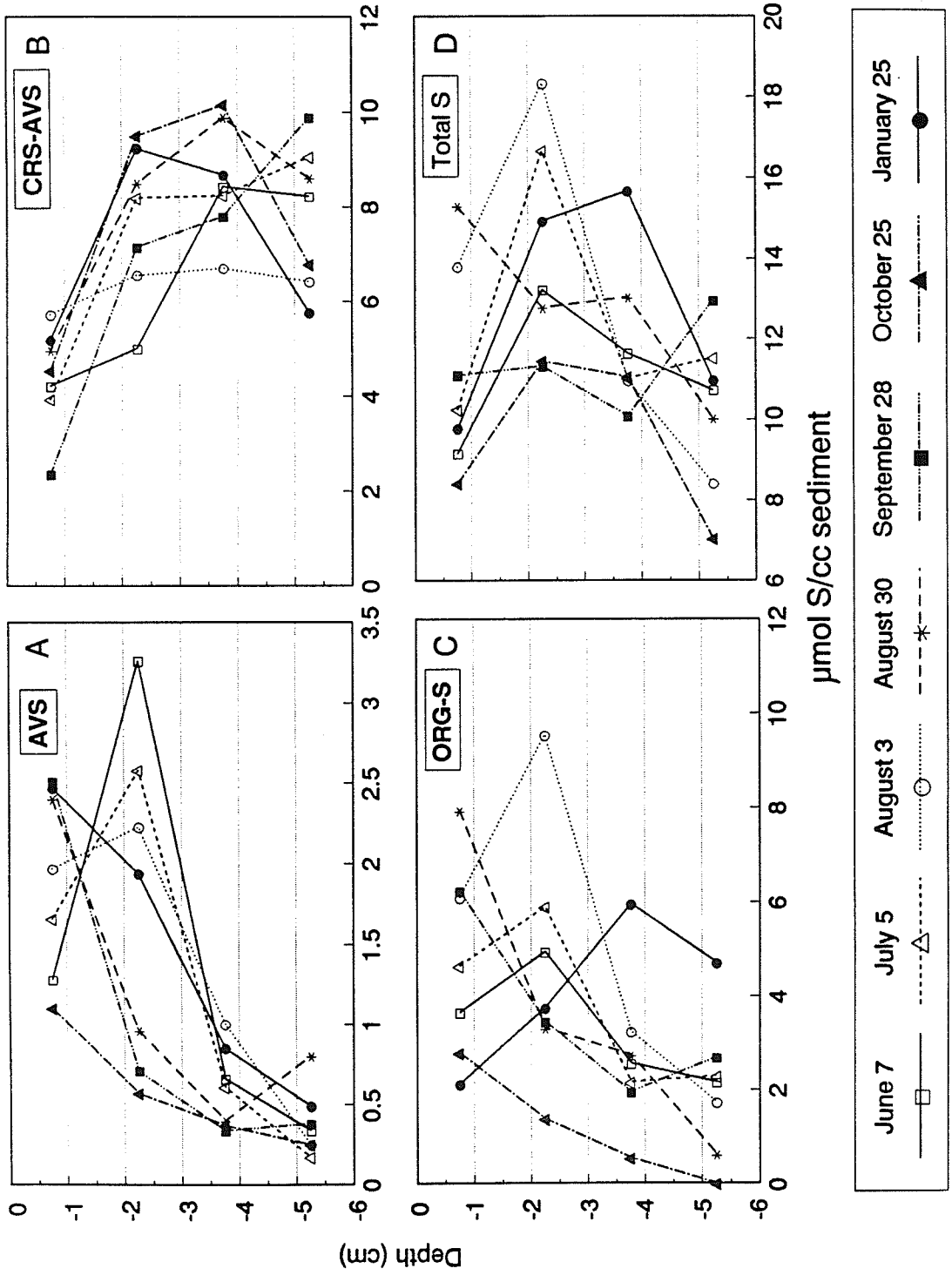
Appendix IV.

Concentrations of organic sulfur, inorganic sulfur (CRS = chromium reducible sulfur) and total sulfur in sediments from 1.5 and 10m depths in Lake 302 South. Sediment cores were taken in May, 1989.


µmol S/cc
0 2 4 6 8 10 12 14 16
Depth (cm)
0
-2
-4
-6
-8
A
1.5m
B
1.5m
C
10m
D
10m
Total S
CRS
Org-S
-12
-16
-20
-24
-28

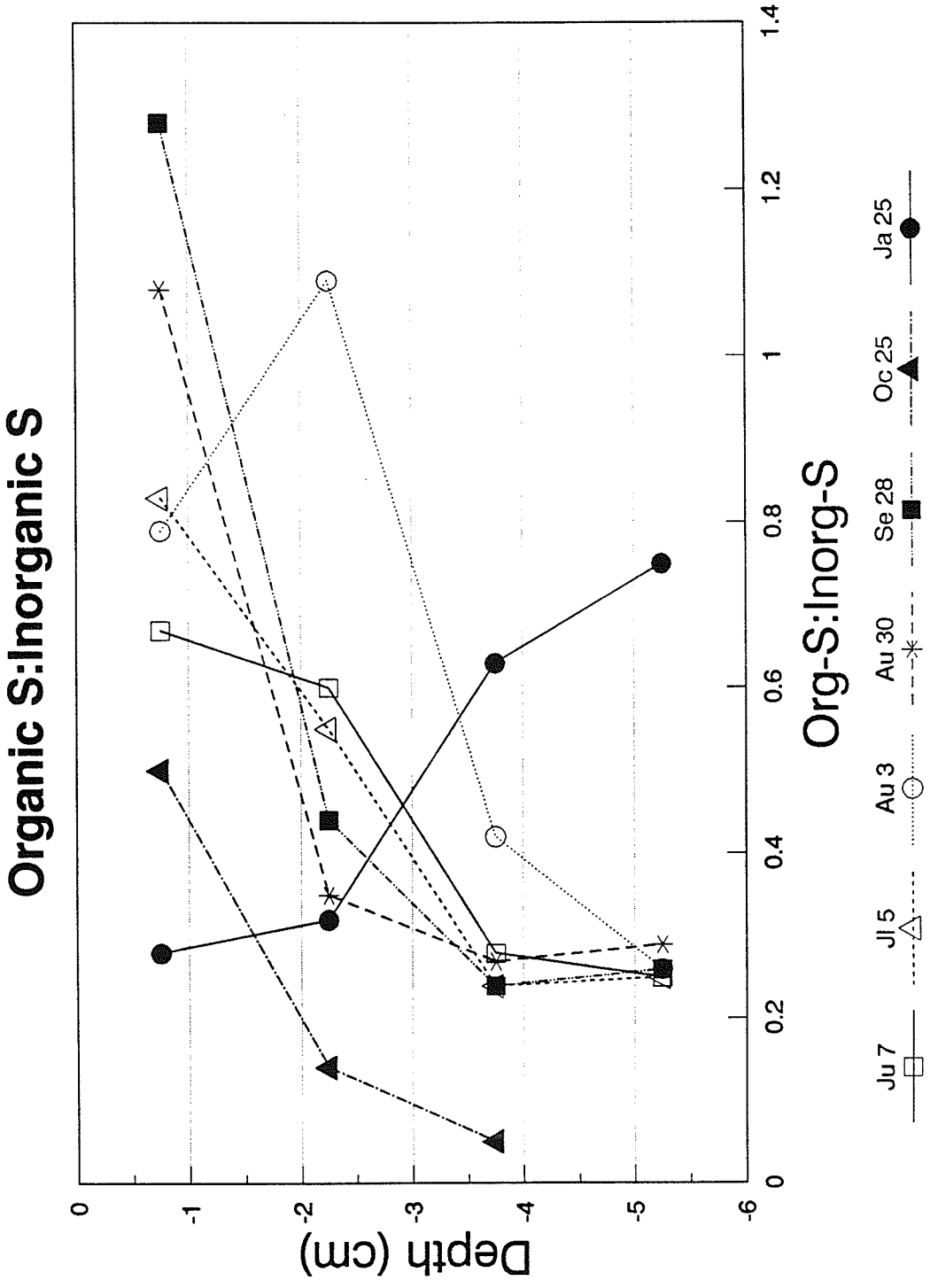
Appendix V.

Concentrations of different sulfur species in sediment from Lake 302 South (2m) from June 7, 1990 to January 25, 1991. AVS = acid volatile sulfur; CRS = chromium reducible sulfur (including AVS); Org-S = organic sulfur.


A
AVS
B
CRS-AVS
C
ORG-S
D
Total S
Depth (cm)
µmol S/cc sediment
June 7
July 5
August 3
August 30
September 28
October 25
January 25

Appendix VI.

Changes in the ratio of organic sulfur to inorganic sulfur in sediment from Lake 302 South from June 7, 1990 to January 25, 1991.


Organic S:Inorganic S
Depth (cm)
0
-1
-2
-3
-4
-5
-6
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Org-S:Inorg-S
Ju 7
Jl 5
Au 3
Au 30
Se 28
Oc 25
Ja 25