ABSTRACT

Title of Document: ISOTOPE GEOCHEMISTRY OF ORGANIC SULFUR COMPOUNDS WITH LINKS TO BIOGEOCHEMICAL SULFUR CYCLING AND RADICAL CHEMISTRY

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Research on volatile organic sulfur compounds (VOSCs) such as dimethylsulfide (DMS), methanethiol (MT), carbonylsulfide (OCS), dimethyl disulfide (DMDS), and carbon disulfide (CS_2) from aquatic environments has focused on the production and flux of DMS from the oceans into the atmosphere. In contrast, the biogeochemical connections between the atmosphere and the major reservoirs of VOSC species in freshwater, estuarine, wetlands and coastal marine environments are poorly understood. This thesis reports one of the first sulfur isotope constraints on the factors that control the expression on the S-isotope effects of VOSCs and their natural precursors. It describes ties to their formation, connections with inorganic and microbial processes, and chemical reactions that link the various productions of VOSCs in natural environments. Results from the four field sites studied in this

research – Two Pacific Northwest Islands in the Washington State, the York River Estuary in Virginia, Fayetteville Green Lake in New York, and the Delaware Great Marsh – have demonstrated several strikingly different pathways for VOSCs production. In the Pacific Northwest Islands and York River Estuary, DMSP produced by marine algae and phytoplankton have $\delta^{34}S$ values of +18.5 ‰ to +19.2 ‰, and $\Delta^{33}S$ and $\Delta^{36}S$ nearly similar to seawater sulfate. These values are slightly ^{34}S-depleted relative to seawater sulfate. This observation is consistent with the origin of sulfur in DMSP being related to assimilatory pathways of sulfate. Analyses of VOSCs from Fayetteville Green Lake, a stratified freshwater system and the Delaware Great Marsh yield different $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$ values of total VOSCs (consisting of MT, DMS, CS_2, and DMDS) that are similar to but slightly ^{34}S-enriched relative to the compositions of coexisting sulfide produced via bacterial sulfate reduction (negative $\delta^{34}S$ and $\Delta^{36}S$, and positive $\Delta^{33}S$) and reflect organic matter sulfurization pathways in addition to assimilatory sulfate pathways. Extension of chemical protocols to thermochemical sulfate reduction (TSR) process using a simple amino yielded sulfur radical adducts with uncompensated electron spins and ^{33}S isotope enrichment of up to 13‰. These enrichments are hypothesized to originate from reactions involving sulfur radicals generated by thiyl-mediated thermolysis reaction via sulfur ion-radical pair mechanisms leading to the manifestation of magnetic isotope effect (MIE).

ISOTOPE GEOCHEMISTRY OF ORGANIC SULFUR COMPOUNDS WITH LINKS TO BIOGEOCHEMICAL SULFUR CYCLING AND RADICAL CHEMISTRY

By

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Dissertation submitted to the Faculty of the Graduate School of the
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Preface

The practical work and writing of this thesis was carried out at the Department of Geology and Earth System Science Interdisciplinary Center (ESSIC) at the University of Maryland, College Park. This Ph.D. thesis is based on 4 research papers. Two of them (chapters 2 and 5) is already published in peer reviewed journal; chapters 3 and 4 are accepted for minor revision at the time this thesis was submitted.

Chapter 1 - Gives a general introduction to factors that control the production, biogeochemical transformation of VOSCs in natural environments and also highlights the influence of sulfur radical and spin chemistries in thermochemical sulfate reductions. The chapter also highlights the use of multiple sulfur isotope measurements as an efficient tool to trace the reaction mechanisms and pathways of sulfur in reaction networks.

Chapter 2 - Presents an overview of the experimental approach, methods of extraction, and techniques for measurements of four sulfur isotope compositions of VOSCs, and their major inorganic and organic sulfur species present in an aquatic natural environment. **Harry Oduro, Alexey Kamyshny Jr., Weifu Guo, & James Farquhar** - Multiple sulfur isotope analysis of volatile organic sulfur compounds and their sulfonium precursors in coastal marine environments**.** Published in ***Marine Chemistry*** *(2011) 124:78-89*.

Chapter 3 – Presents comprehensive reaction mechanisms and pathways of VOSCs formation and cycling in freshwater systems via biotic and abiotic processes using multi-sulfur isotope approach and concentration measurements. **Harry Oduro, Alexey Kamyshny Jr., Aubrey L. Zerkle, Yue Li, & James Farquhar** - Quadruple sulfur isotope constraints on the origin and cycling of volatile organic sulfur compounds in a stratified sulfidic lake. Accepted with revision in ***Geochimica et Cosmochimica Acta.***

Chapter 4 – Documents one of the first sulfur isotope measurements for oceanic production of DMS and its cellular precursor DMSP from marine algae and phytoplankton to constrain marine biogenic sulfur cycle that can be used in future studies to trace ocean-atmosphere interactions involving DMSP/DMS. **Harry Oduro, Kathryn L. Van Alstyne, & James Farquhar** - Sulfur isotope variability of oceanic DMSP: Implications for DMSP generation and its contributions to biogenic sulfur emissions. Accepted with revision in the ***Proceedings of the National Academy of Sciences of the United States of America.***

Chapter 5 – Provides a framework of sulfur radical chemistry produced in high temperature reactions leading to a unique sulfur-33 isotope effect as a result of ion-radical pair polymerization of organic sulfur radicals. **Harry Oduro, Brian Harms, Herman O. Sintim, Alan J. Kaufman, George Cody, & James Farquhar** - Evidence of magnetic isotope effects during thermochemical sulfate reduction. Published in the ***Proceedings of the National Academy of Sciences of the United States of America*** *(2011)108 :(43)17635-17638.*

Dedication

I dedicate this work in memory of my late
father, Peter Oduro, beloved Mother, Mary
and all friends and family members who
emphasized the importance of
education and supported me
through their actions and
words to pursue my
Ph.D. degree.

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There are many people who deserve my thanks and gratitude, and without whom this work would have been significantly more difficult to complete. First of all, I would like to thank my advisor, James Farquhar for his support and guidance during those years in working in his lab. Always enthusiastic about new ideas, his generous leadership style allowed me to believe that I would get a reasonable result every time I was doubting. James was always honest, and never pretended work was better or worse than it actually was. He never limited my imagination and let me wander off to explore the possibilities of new research projects and new collaborations. Sometimes it was not easy to live up to one's expectations when working with a well-reputable scientist/supervisor desperately wishing to be involved in more than one project at a time. But his mentorship style and free spirit attitude motivated me to pursue my interest freely and finish this thesis with the feeling that I grew immensely in our interactions.

I also acknowledged the work and support of my committee members, Richard Walker, Mike Evans, Jay Kaufman, George Helz, and Russell Dickerson for their guidance and help to shape this project, and the rest of the faculty at the University of Maryland, Geology Department for maintaining a challenging and collegial working environment. I would like to acknowledge the primary financial support of the NASA Astrobiology Institute (NAI) - Carnegie Institution of Washington and National Science Foundation (NSF).

I am also grateful to all members of the Farquhar's group, past, present who set-up the stage for this work and helped me in my studies. I especially want to thank Alexey Kamyshny Jr. from whom I benefited directly through my collaboration for his helpful discussions and for pointing out errors in the some of my manuscripts. Other collaborators I would like to thank are: Dr. Kathy Van Alstyne who provided marine algal samples; Dr. Kam Tang who assisted me to directly sample phytoplankton; Dr. George Luther (III) for his assistance in taking sediment cores from the Delaware Great marsh; Dr. George Helz for discussions on sulfur speciation;

Dr. Yue Li for access to ESI-MS for this work – it made all the difference; and Dr. Weifu Guo who helped me in the modeling of DMS/DMSP isotope fractionations.

Finally, I am exceeding grateful to my partner and children Cheryl, Wilbert, and Gilbert Oduro, whose love and encouragement has helped to keep me on track over the past four years. Your support has been invaluable, and you all provided a safe haven and a pool of calm during my PhD work.

Table of Contents

List of Tables

List of Figures

Common Abbreviations

AVS……Acid Volatile Sulfur

CS_2 ……....Carbon disulfide

CLAW……Charlson, Lovelock, Andreae, Warren

CCN……Cloud Condensation Nuclei

CRS……Chromium Reducible Sulfur

CVOSC……..Combined Volatile Organic Sulfur Compound

DMS……..Dimethylsulfide

DMDS……Dimethyldisulfide

DMSO……Dimethylsulfoxide

DMSP…….Dimethylsulfoniopropionate

$DMSO_2$……..Dimethylsulfone

DMSHB……...4-dimethylsufonio-2-hydroxybutyrate

KIE……Kinetic Isotope Effect

MIE……Magnetic isotope Effect

MT……..Methanethiol

MET……..Methionine

MSA…….Methanesulfonic Acid

MSIA…… ….Methanesulfinic acid

MTHB……....Methylthio-2- hydroxybutyrate

MTOB……..4-methylthio-2-oxobutyrate

VOSC……..Volatile Organic Sulfur Compound

ZVS……Zero Valent Sulfur

Chapter 1

Introduction

1.0 Background and Scientific Motivations

There is a long, rich history of studies of atmospheric sulfur compounds. Sulfur-containing species were first recognized in air and rain by several English scholars, including Robert Boyle in the 17th century and Robert A. Smith in the 19th century (Wang, 2008). More recent investigations have focused on the impacts of anthropogenic and natural sulfur gases emitted across a wide range of spatial scales (Popovics et al., 1987; Charlson et al., 1987; Spiro et al., 1992; Charlson et al., 1992; Pham et al., 1995). Anthropogenic sulfur-containing gases (particularly SO_2, and H_2S) are readily converted to acidic sulfate aerosols, which are removed from the atmosphere by wet and dry deposition (Figure 1.1).

Acidic deposition (acid rain) can cause damage to terrestrial and aquatic ecosystems, and can also lead to potential consequences for human health (Cowling, 1982; Bernard et al., 2001). The realization that acid deposition was linked to anthropogenic emissions of sulfur-containing gases guided research into the sources, emissions, and atmospheric chemistry of gaseous sulfur compounds (Eriksson, 1963; Granat et al., 1976). More recently, revisiting speculations by Lovelock and co-workers (1972), lead to the realization that elevated SO_2 concentration above the sea surface is connected to biogenic dimethylsulfide (DMS) production from marine macro- and microalgae.

Biogenic processes in natural environments emit reduced forms of organic sulfur compounds particularly dimethylsulfide, with lesser amounts of other sulfur-containing

gases such as carbon disulfide (CS_2), carbonyl sulfide (OCS), methanethiol (MT, CH_3SH), and dimethyl disulfide (DMDS, CH_3SSCH_3) into the atmosphere (Figure 1.1) (Lovelock et al., 1972; Andreae, 1986).

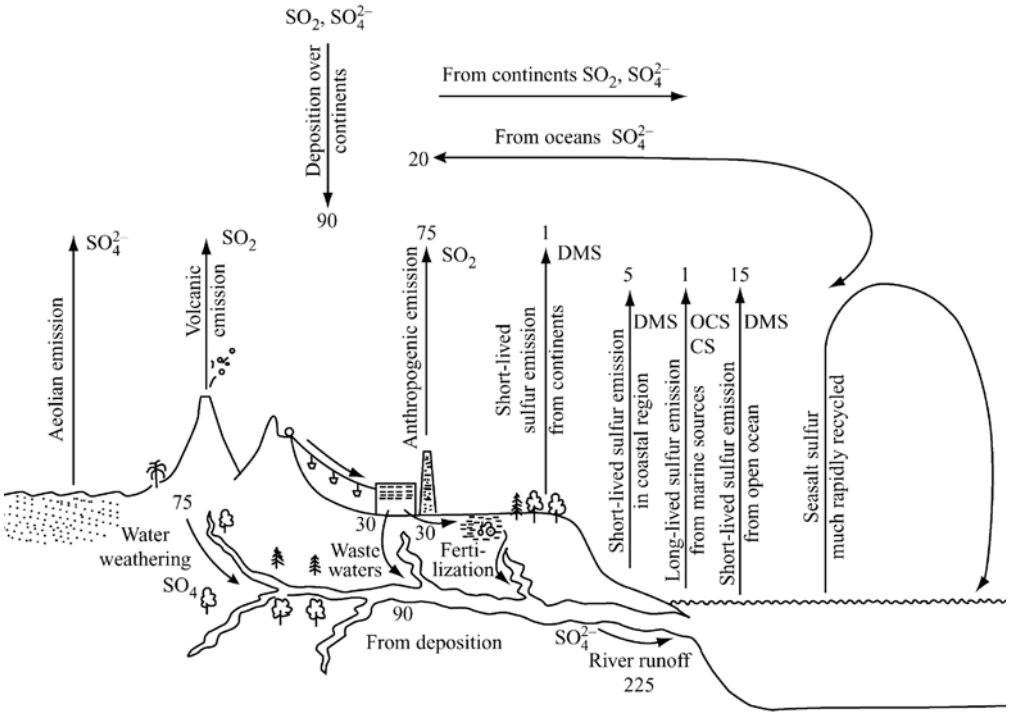

Figure 1.1: Global sulfur emissions showing key fluxes at Tg(S) yr^{-1}. Fluxes to and from different reservoirs (atmosphere, ocean and land) are characterized by black arrows and numbers indicate their contribution to the total sulfur budget. (After Brimblecombe, 2007).

Dimethylsulfide (DMS, CH_3SCH_3) is a major biogenic sulfur gas that is produced from its precursor dimethylsulfoniopropionate (DMSP) by phytoplankton in ocean surface environments where it is released into the atmosphere and oxidized by hydroxyl ($^{\bullet}OH$) and nitrate ($NO_3^{\bullet}$) radicals to form a variety of sulfur-containing compounds (Yin et al., 1999; Bates et al., 1987). The oxidation of DMS to dimethylsulfoxide (DMSO) and subsequent oxidation of this to methanesulfonic acid (MSA) and non-seasalt sulfate (NSS) are considered important sources of cloud condensation nuclei (CCN) in the marine troposphere with the potential to drive changes in cloud cover (Andreae, 1990;

Andreae and Crutzen, 1997) and cloud albedo (Twomey, 1977; Nguyen et al., 1978; Bates et al., 1987; Barnes et al., 2006; Ayers and Cainey, 2007). DMS is an important first step in a climate feedback, known as the CLAW (**C**harlson, **L**ovelock, **A**ndreae, **W**arren) hypothesis, which argues for a feedback between biological DMS production, radiation, and regulation of global climate (Charlson et al., 1987). While DMS is known to play an important role in the earth's radiation budget, the specific connections between it biology, ocean chemistry, and atmospheric chemistry remain to be better understood (Andreae and Crutzen, 1997). Other VOSC species (such as CS_2, OCS, MT, and DMDS) produced by biological and abiological processes in surface waters of marine (Andreae, 1986; Andreae and Ferek, 1992) and terrestrial ecosystems (Adams et al., 1981; Lamb et al., 1987; Staubes et al., 1989) have also been identified as important players in oceanic and atmospheric cycling of sulfur. While progress has been made in describing the chemistry, concentrations, and emission strengths of these compounds, there are still no reliable methods to directly measure the source and flux of VOSC emissions to the atmosphere (Andreae, 1985).

Numerous studies have calculated the fluxes of VOSCs from seawater (particularly DMS) (Kettle et al., 1999), and global climate models have included estimates (see table 1.1) of DMS from aquatic and terrestrial sources (Kettle and Andreae, 2000; Aumont et al 2002; Simo and Dachs, 2002; Bopp et al., 2004; Kloster et al., 2006). These estimates vary from one model to another, because only small portion of dimethylsulfoniopropionate (DMSP) is degraded and converted to DMS by healthy algal cells in the ocean, and only a small percentage of DMS in surface seawaters ever enters the atmosphere. The large amount of contradictory data on DMS, DMSP, and other

VOSC species calls for an additional research effort to improve our understanding on their sources of production, their relation to cellular processes affecting sulfur metabolism of DMSP biosynthesis, and the role of marine alga contributions to the emission of atmospheric sulfur at a large scale and on the biogeochemical S-cycle of VOSCs in general (IPCC, 1995; SOLAS, 2004).

Global Sulfur Emission Estimates (TgS /yr)		
Sources	**Compound**	**Emissions**
Oceans (without sea-salt)	DMS (CS_2, OCS)	15-25
Volcanoes	SO_2, SO_4^{2-} (H_2S, OCS)	9-12
Plants + Soils	SO_4^{2-}, H_2S (DMS, CS_2)	0.2-1
Fossil Fuel Combustion (+industry)	SO_2 (SO_4^{2-}, H_2S, CS_2)	70-80
Biomass Burning	SO_2 (SO_4^{2-}, OCS)	2-3
	Total	98-120
Sinks		
Dry Deposition	SO_2, SO_4^{2-}	50-60
Wet Deposition	SO_2, SO_4^{2-}	50-60

Table 1.1: Variability in global sulfur emissions estimates Tg(S) yr^{-1}. (Source – Seinfeld and Pandis, 2006)

2.0 Aims of Thesis

2.1 General Sources of Volatile Sulfur Compounds

The first aim of this thesis will be the use of specific sulfur isotope fingerprints to gain a more quantitative understanding of the sources and sinks of VOSCs in different natural systems. VOSCs are produced in marine (Dacey and Wakeham 1986; Malin et al., 1998; Steinke et al., 2002; Stefels et al., 2007), wetland (Kiene and Visscher, 1987; Kiene and Taylor, 1988; Lomans et al., 2002), and freshwater ecosystems (Richards et al., 1991, 1994; Fritz and Bachofen, 2000).

In marine environments, production of VOSC (mainly DMS and MT) proceeds by enzymatic cleavage of dimethylsulfoniopropionate (DMSP - $(CH_3)_2S^+CH_2CH_2COO^-$), a compound found in high concentrations in a variety of marine algae (Karsten et al., 1990; Kirst et al., 1991; Kiene et al., 1996; Malin and Kirst, 1997; Stefels, 2000; Van Alstyne et al., 2003). This algal compatible β-sulfonium compound serves several physiological roles, including as an osmoprotective agent (osmolyte), as an antioxidant, and as a cryoprotectant (Sunda et al., 2002; Stefels et al., 2007). A fourth role for DMSP may be as a deterrent to grazing by zooplankton or protozoa, possibly by formation of DMS (Welsh, 2000). DMSP can be present in relatively high concentrations (e.g., 100 – 400 mmol L^{-1}) in marine macro- and microalgae (Keller et al., 1989; Sunda et al., 2002). The quantity of DMSP released into the water column by phytoplankton depends on the species composition and the species abundance (Nguyen et al., 1988; Andreae, 1990), the presence or absence of viral infection (Malin et al 1992; Bratbak et al., 1995), and the amount of grazing by zooplankton (Dacey and Wakeham, 1986). DMSP released in oceanic water column contributes a significant proportion of the organic matter that flows through the microbial food web, and on a global scale produces 38-40 TgS/year in form of DMS that is estimated to represent approximately 3–10 % of the global marine primary production (Kiene et al., 2000; Lomans et al., 2002; Simó et al., 2002).

In nonmarine settings, production of VOSCs such as DMS and MT has been observed in isolates from salt marshes, swamps, and wetlands when amended with DMSP (Yoch, 2002). In anaerobic freshwater and wetland sediments, formation of VOSCs has been ascribed to methanogenic activity (via methylation of sulfide) and degradation of sulfur containing amino acids (Hayward et al., 1977; Finster et al., 1990; Bak et al., 1992;

Higgins et al., 2006). Sulfate-reducing bacteria have also been implicated in VOSC formation and degradation (Lomans et al., 1999; Yoch, 2002). Although several bacteria and Archaea involved in the cycling of VOSC (mainly MT, DMS, and DMDS) have been isolated and characterized from various habitats, little is known about their source composition, production pathways, and their fluxes into the atmosphere, which depend on their steady-state concentrations (Bouillon and Miller, 2005).

2.2 Chemical Principles of VOSC production in Freshwater and Wetlands

A second goal of this dissertation will be to examine the link between the distribution of VOSCs produced by biotic and abiotic means to understand mechanisms that control their production on a seasonal time frame in freshwater and salt marsh sediments. A number of reaction mechanisms, both biological and abiological, have been described for the formation and degradation of VOSC, particularly DMS, MT, and DMDS (Kodata and Ishida, 1972; Kiene and Visscher, 1987; Lomans et al., 2001; Higgins et al., 2006). For instance, the degradation of sulfur containing amino acids such as cysteine and methionine is catalyzed by S-alkylcysteinase and L-methionine-γ-lyase enzymes respectively to produce MT, pyruvate, and ammonia (Hayward et al., 1977; Warneck, 1988). Amino acid monomers derived from proteins in anaerobic sediments have been demonstrated to contain cysteine and methionine (Mayer et al., 1986; Lawrence et al., 1995; Drennan and DiStefano, 2010). These mechanisms include the sequential breakdown of proteins to form peptides, and subsequent degradation of peptides to form a variety of biochemical precursors listed in Table 1.2, which are further broken down to form a number species such H_2S, MT, DMS, DMDS, CS_2, and OCS in

coastal wetland, and freshwater environments. Methylation of H_2S and MT (See reactions (R_1) and (R_2)) are another important mechanism for VOSC formation. This biotransformation is performed by aerobic and anaerobic bacteria found in a variety of environments. These organisms utilized syringate - a methyl donor compound to methylate hydrogen sulfide to produce MT, and then methylate MT to produce DMS (Drotar et al., 1987; Lomans et al., 2001). The source of methyl groups is often methoxylated aromatic compounds from lignins and biopolymers (Bak et al., 1992).

Volatile Species	**Biochemical precursors**
H_2S	Proteins, Polypeptides, Cystine, Cysteine, Glutathionine
CH_3SH	Methionine, Methionine sulfoxide, Methionine sulfone, S-methylcysteine
CH_3SCH_3	Methionine, Methionine sulfoxide, Methionine sulfone, S-methylcysteine, Homocysteine
CH_3SSCH_3	Methionine, Methionine sulfoxide, Methionine sulfone, S-methylcysteine, Cysteine
CS_2	Cysteine, Cystine, Homocysteine, Lanthionine, Djekolic acid
OCS	Lanthionine, Djekolic acid

Table 1.2: Biochemical origin of volatile sulfur compounds produced in wetlands and freshwater systems by microbial degradation of organic matter under aerobic and anaerobic conditions (Source - Warneck, 1988)

$$R\text{-}O\text{-}CH_3 + H_2S \longrightarrow R\text{-}OH + CH_3SH \qquad (R_1)$$

$$2R\text{-}O\text{-}CH_3 + H_2S \longrightarrow 2R\text{-}OH + CH_3SCH_3 \qquad (R_2)$$

In natural settings, the methanethiol that is formed can be chemically oxidized through abiotic reactions to form DMDS, reaction (R_3) (Kelly and Smith, 1991; Lomans et al., 1999), as well as CS_2, and OCS, which are usually detected together with DMS and MT in sulfidic freshwater and sediments (Finster et al., 1990; Richards et al., 1991; Fritz and Bachofen, 2000; Hu et al., 2007).

$$CH_3SH + CH_3SH + O_2 \longrightarrow CH_3SSCH_3 + H_2O \qquad (R_3)$$

2.3 The Biogeochemical Cycling of DMS and its Precursors

The third aim of this thesis will be to study the metabolic processes and pathways used by marine macro- and microalgae to biosynthesize DMSP from seawater sulfate. The biosynthesis of DMSP is an energy-requiring process and starts with assimilation of marine seawater sulfate into the cytoplasm of the algal cells, where sulfate is reduced to sulfide through a network of biochemical reactions (Brunold, 1990; Leustek and Saito, 1999) in the chloroplasts (Figure 1.2). Inside the cell, assimilated sulfur is chemically transformed into cysteine and methionine (Giovanelli, 1990).

From methionine, there several key biochemical pathways through different intermediates towards the synthesis of DMSP, one of which is preferred by marine algae (Gage et al., 1997; Summers et al., 1998). Bacterioplankton are one of the main mediators of the fate of DMSP in seawater. When DMSP is used as a sulfur and carbon source, it undergo demethylation to form methylmercaptopropionate (MMPA), which can be further demethylated to methanethiol (MT/MeSH) and used in amino acid synthesis by marine algae (Figure 1.3). Alternatively, DMSP can also be cleaved by enzyme to produce DMS and a C_3 compound (acrylate) (Kiene and Linn, 2000; Todd et al., 2007).

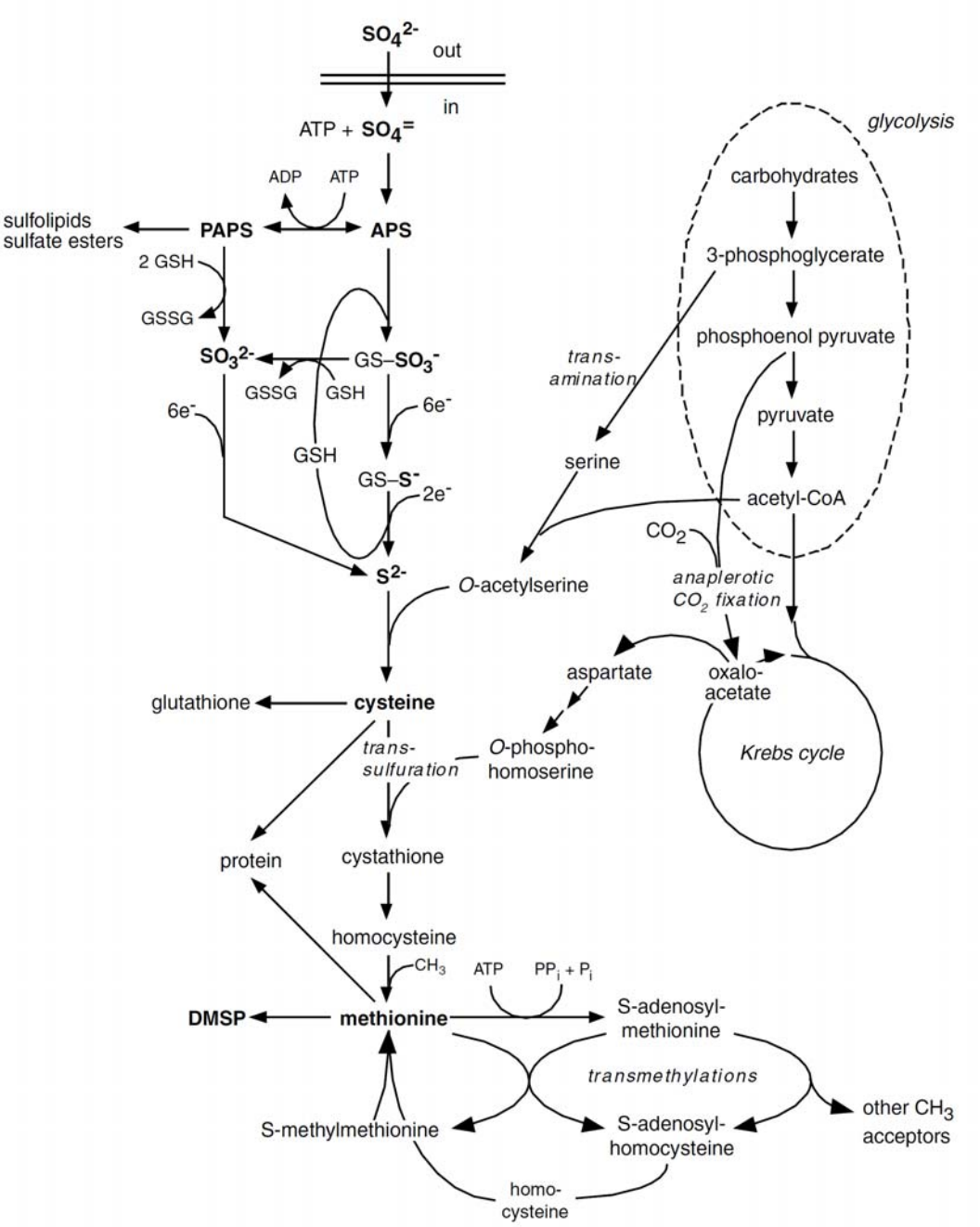

Figure 1.2: Biosynthetic pathway of DMSP through assimilatory sulfate reduction. (Taken with permission from Stefels, 2000).

In open ocean microbial transformation (Kiene and Service, 1991; Ledyard and Dacey, 1994; González et al., 1999; Malmstrom et al., 2004) and turbulent diffusion

(eddy diffusion) processes (Suhre and Rosset, 1994 and Nightingale et al., 2000) released DMS into surface waters and marine boundary layer. Where it undergoes photochemical oxidation (Brimblecombe and Shooter, 1986; Kieber et al., 1996, Toole et al., 2004;

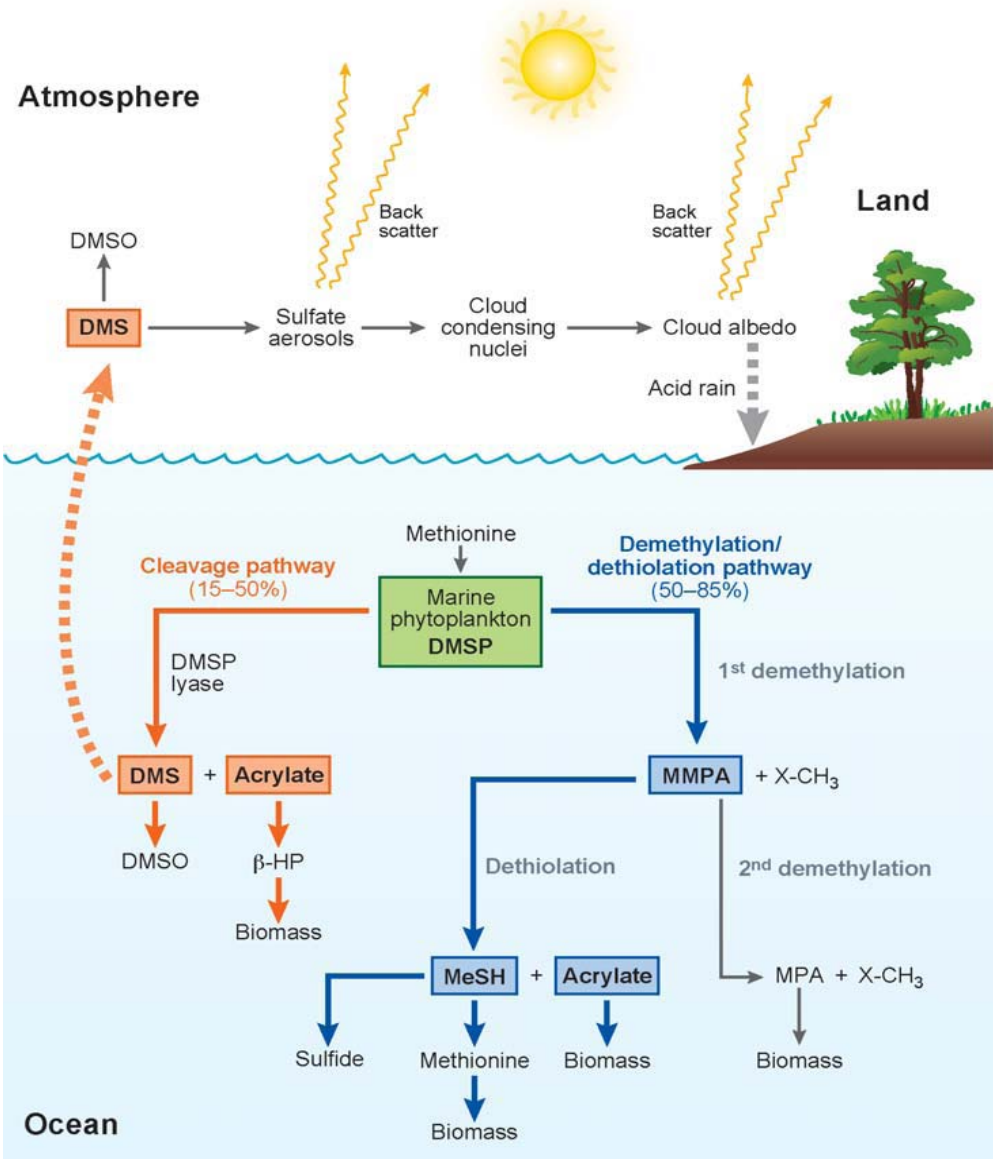

Figure 1.3: A simplified DMS cycling from the ocean to atmospheric marine boundary layer. (After Wagner-Döbler and Biebl, 2006).

Bouillon and Miller, 2004) with HOx and NOx species (Bates et al., 1987; Yin et al., 1990; Barnes et al., 2006). The gas-phase products of these reactions include, but are not limited to SO_2, H_2SO_4, dimethylsulfoxide ($CH_3S(O)CH_3$, DMSO), dimethylsulfone ($CH_3S(O)_2CH_3$, $DMSO_2$) and methanesulfonic acid (CH_3SO_3H, MSA) (Ayers and Gillet., 2000; Baboukas et al., 2002; Lucas and Prinn, 2002). The products of these oxidation reactions are extremely hygroscopic, and they condense on existing aerosols or form new particles through particle nucleation processes. These freshly nucleated particles, through coagulation and condensation process in the atmosphere, grow into cloud condensation nuclei (CCN) to influence the Earth's radiation balance (Charlson et al., 1987; Falkowski et al., 1992) and the acid-base chemistry of the atmosphere (Charlson and Rhode, 1982).

While DMS-related research has recognized the importance of oxidation pathways since Charlson et al. (1987), the complex connections between phytoplankton and microalgae DMS/DMSP production, environmental stresses, and the non-linear production of gas-phase MSA, and NSS-SO_4^{2-} from oceanic DMS require a more comprehensive understanding (Andreae and Crutzen, 1997). The use of sulfur isotope measurements to study DMSP produced by marine algae can provide much information and better insight on the 1) assimilation and degradation pathways, 2) factors regulating the levels of the important amino acids cysteine and methionine, 3) and factors controlling the biological switch and conversion capacity of cellular DMSP/DMS production as well as the oxidation pathways to MSA and NSS-SO_4^{2-} in marine air.

2.4 Sulfur-centered radical chemistry

A fourth part of this thesis work focuses on the chemistry of sulfur-centered radicals produced during thermochemical sulfate reduction. Sulfur-centered radicals represent a very interesting class of radicals since they exhibit very interesting redox chemistry, whose reactions are important in many atmospheric, biological, and radiochemical processes. The importance of these radicals in biogeochemical reactions stem from the fact that the lone electron pairs present in the sulfur atom can affect the overall electronic structure of the molecule and can serve as convenient models for evaluating the mechanisms and characteristic features of sulfur compounds in chemical reactions. This is particularly true for sulfides, thiols and their radical species-thiyl ($RS^{\bullet}$), which have been implicated to play a major role in acid rain chemistry (Tyndall and Ravishankara, 1991) and the mechanisms whereby biological thiols are used to repair free-radical damaged sites in living organisms (Halliwell and Gutteridge, 1990).

In the past few years, unprecedented progress has been made in the recognition and understanding of the structure and reactivity of sulfur-centered radicals. Research on these transients flourished particularly in biochemical systems that use Electron Paramagnetic Resonance (EPR) spectroscopy via spin trapping process to quantify sulfur-centered radicals (Harley and Gordy, 1975; Zhao et al., 2001; Barriga et al., 2010). More recently, research focus has shifted to sulfur radical formation in geochemical applications that involve thermochemical sulfate reduction (TSR) process and its importance in petroleum maturation (Goldstein and Aizenshtat, 1994; Lewan, 1998; Watanabe et al., 2009). Inspite of much research and great body of factual knowledge of TSR, the nature and forms of the organic-inorganic sulfur compounds and their radical

species produced in such thermal reactions are not known in detail. Experimental studies (Turro et al., 1983; Step et al., 1990; Buchachenko, 2001; 2009), however, have shown that sulfur radicals can be generated from a variety of organic sulfur compounds via photochemical reaction to produce magnetic S-33 electron-spin nuclei for the paired sulfur radical. In other geochemical systems, production of sulfur radicals (e.g., thiyl ($RS^{\bullet}$) and their disulfides cations ($RSSR^{+\bullet}$)) are difficult to identify. Since these radical intermediate species possess a fast spin orbit coupling (Autrey et al., 1995) and can undergo dimerization via self-annihilation radical-radical interactions to form a disulfide and their corresponding radical-anions (Bonifacic et al., 1985; Coates et al., 1992).

At ambient conditions, this radical-radical interaction is thermodynamically favorarable (ΔH= -73Kcal/mol) and may lead to the rapid disappearance of thiyl radicals in solutions, and generate an alkyl disulfide or polysulfide as the major product according the reaction below:

$$RS^{\bullet} + RS^{\bullet} \longrightarrow \quad R\text{-}S\text{-}S\text{-}R \longrightarrow R\text{-}S_n\text{-}R$$

The rate of this process, however, has been shown to be diffusion–limited, and thiyl radical can react with other reactive species or it may undergo a radical recombination reaction, provided that the relatively long (micro seconds) lifetime of the radical pair, can generate hyperfine coupling interactions to produce electron-spin nuclei for the paired sulfur radical (Turro et al., 1983; Step et al., 1990; Buchachenko, 2001; 2009). Recently, it has been argued that thermochemical sulfate reduction leaves a unique isotopic signature as an anomalous S-33 abundance that can be detected by high-

precision multiple sulfur isotope analysis (Watanabe et al., 2009). But the source of this anomalous isotope signature is unknown.

The development of sequential methods for isolating and extracting the various sulfur compounds (both organic and their inorganic forms) for isotopic analysis provides an opportunity to examine the nature and functionalities of sulfur-centered radicals and their dimeric products. In this work, special attention will be focused on the isotopic effects of sulfur species produced by the thermal decomposition of sulfur compounds like those in Watanabe et al. (2009) to isolate the major elementary reactions and mechanisms that lead to the observed isotope effects. To help clarify the situation better, thermolytic decomposition of selected pure inorganic sulfur compounds in the presence of other pure organic compounds, which typify the kinds of natural compounds that may be found in geochemical environments, will be used as a starting point for more detailed study of TSR.

3.0 Stable Sulfur Isotopes and Notation

Different isotopes of an element have different numbers of neutrons and hence, a different atomic mass. For example, the most abundant sulfur isotopes are ^{32}S - containing 16 protons, 16 electrons and 16 neutrons; ^{33}S-containing 16 protons, 16 electrons and 17 neutrons; and ^{34}S-containing 16 protons, 16 electrons and 18 neutrons; and ^{36}S-containing 16 protons, 16 electrons and 20 neutrons. Chemical processes in earth systems can cause some of the isotopes to be unstable (e.g., ^{35}S), and these ultimately form stable products by radioactive decay from cosmic ray spallation of ^{40}Ar. Other

isotopes, which have stable combinations of neutrons and protons (e.g. ^{32}S, ^{33}S, ^{34}S, and ^{36}S) do not decay, and are referred to as stable isotopes.

Sulfur has four stable isotopes (^{32}S, ^{33}S, ^{34}S, and ^{36}S) with fractional abundances of approximately: ^{32}S = 95.04, ^{33}S = 0.75, ^{34}S = 4.20 and ^{36}S = 0.02 % (Ding et al., 2001; Coplen et al., 2002), and variations in the relative abundances of these isotopic compositions are commonly reported using delta notation ($\delta^{33}S$, $\delta^{34}S$, and $\delta^{36}S$)* and capital delta notation ($\Delta^{33}S$ and $\Delta^{36}S$)†. Early studies of stable sulfur isotope geochemistry (e.g., Kaplan and Rittenberg, 1964; Kaplan and Hulston, 1966) use the $\delta^{34}S$ (or $^{34}S/^{32}S$) to report the sulfur isotope distribution. Recent high-precision measurements of all four isotopes of sulfur isotopes (^{32}S, ^{33}S, ^{34}S, and ^{36}S) allow us to overcome a number uncertainty in natural systems and also help us differentiate conventional mass-dependent isotope effects from anomalous processes (Hulston and Thode, 1965; Farquhar et al., 2000). These measurements have revealed features of the sulfur isotope system that can be used to evaluate a variety of physical, chemical, and biological transformations

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* Isotopic composition of sulfur species (in permil, ‰) is presented using the standard delta (δ) notation:

$$\delta^{33}S = [(^{33}S/^{32}S)_{sample}/(^{33}S/^{32}S)_{reference} - 1]$$

$$\delta^{34}S = [(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{reference} - 1]$$

$$\delta^{36}S = [(^{36}S/^{32}S)_{sample}/(^{36}S/^{32}S)_{reference} - 1],$$

which are given in units of permil (‰).

† The less abundant isotopes (^{33}S and ^{36}S) are reported using capital delta notation (Δ);

$$\Delta^{33}S = (^{33}S/^{32}S)_{sample}/(^{33}S/^{32}S)_{reference} - [(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{reference}]^{0.515}$$

$$\Delta^{36}S = (^{36}S/^{32}S)_{sample}/(^{36}S/^{32}S)_{reference} - [(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{reference}]^{1.9},$$

which are given in units of permil (‰). The exponents in these relationships (0.515 and 1.90) define the reference fractionation line (RFL) and approximate single-step thermodynamic equilibrium isotope exchange effects (Hulston and Thode, 1965) and therefore covariation between $\delta^{34}S$ and $\Delta^{33}S$ (or $\Delta^{36}S$) can be used to provide information in addition to $\delta^{34}S$.

(Farquhar et al., 2000; 2003; Johnston et al., 2007; Ono et al., 2007). For example, studies of isotope fractionations produced by sulfate reducers have demonstrated different $\Delta^{33}S$ and $\Delta^{36}S$ than those produced by abiological exchange processes even when the magnitude of fractionations for $\delta^{34}S$ are similar (Johnston et al., 2005, 2007; Farquhar et al., 2007; Zerkle et al., 2009; 2010). Similarly enzymatic effects associated with metabolic activity of sulfur disproportionation of sulfur intermediates also appear to generate similar diagnostic effects (Johnston et al., 2005). This implies that, the metabolic processes that discriminate between $\delta^{34}S$ do not discriminate between $\delta^{34}S$ and $\Delta^{33}S$ (or $\Delta^{36}S$) in exactly the same way during enzymatic sulfur transformations. These differences reflect both primary (differences in the relationship for single-step processes) and secondary (differences resulting from mass conservation in multiple step processes) isotope effects[‡] that occur at the cellular level.

The observed differences in minor isotopic effects provide a framework that may be used to cross-examine mixing and chemical reaction processes that occur in ecosystems where biological and abiological effects have similar $\delta^{34}S$ variations. In practical terms, this means that new information from the production and cycling of VOSCs as well as the spin chemistry of sulfur-centered radicals in systems targeted in this dissertation maybe accomplished by combined measurements of $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$.

[‡]The term *isotope effect* is used to describe a change in isotope ratios that is produced by a physical or a chemical process. We use the term *fractionation factor* (α) to quantify the change in isotope ratios produced by an isotope effect. We define the fractionation factor between two substance A and B for $^{34}S/^{32}S$ using the following equation:

$$^{34}\alpha_{\text{substance-A – substance-B}} = [(^{34}S/^{32}S)_{\text{substance-A}}/(^{34}S/^{32}S)_{\text{substance-B}}].$$

Sulfur isotope effects influenced by factors other than the mass of the isotopes can produce large changes in $\Delta^{33}S$ and $\Delta^{36}S$ for small changes in $\delta^{34}S$ resulting from isotope selection process other than mass. Evidence of mass-independent isotopic fractionation mechanisms is reported in gas-phase photochemical experiments of sulfur–bearing molecules, including SO_2, H_2S, and CS_2 (Zmolek et al., 1999; Farquhar et al., 2001), modern sulfur aerosol samples (Romero and Thiemens, 2003), sulfate-rich horizons (Savarino et al., 2003; Baroni et al., 2007) and large number of samples of sedimentary and metasedimentary rocks older than ~2.4 Gya (Farquhar et al., 2000; Ono et al., 2003; Papineau et al., 2007; Kaufman et al., 2007). To date, the geological literature has focused on processes that produce mass-independent fractionation, and evidence strongly favors an atmospheric origin of SO_2 with very strong connection of atmospheric UV photochemistry.

Magnetic isotope effect (MIE) separate nuclei according to their spin state and magnetic moments (Turro et al., 1995; Buchachenko, 2001). These effects originate from hyperfine coupling of magnetic S-33 isotopes through radicals intermediate reactions and have been studied extensively in liquid and solid phase reactions through photo- and thermochemistry. Among four stable isotopes of sulfur, only S-33 has a nuclear magnetic moment due to its quadrupolar nucleus (with spin multiplicity 3/2). The hyperfine coupling (coupling of nuclei magnetic moment and electron spin moment) is generally very weak so that the magnetic isotope effect is only expressed during radical-radical interactions when a change of spin multiplicity occurs via hyperfine coupling during otherwise spin forbidden processes (spin-allow process). These processes produce magnetic isotope effects (MIE) that can be detected by high precision S-33 isotope

measurements. However their measurement in geochemical applications has been hampered by their faster spin-orbit coupling and their large g-factor anisotropy that arises from the near degeneracy of the two π-type orbital's at the sulfur center. This thesis seeks to measure this isotope effect in geochemical systems using S-33 as a radical indicator.

4.0 Overview and Research Objectives

There are numerous active questions related to organic sulfur compounds biogeochemical cycling. However, their isolation and analysis in natural systems is not a trivial matter. The main goal of this dissertation is to develop methods to measure the four sulfur isotope compositions of volatile sulfur compounds and their organic and inorganic precursors, which will be used:

1. to explore the distribution of VOSCs in marine, estuarian, wetland, and freshwater settings;
2. to identify sulfur isotope compositions of VOSCs to gain a more quantitative understanding of the sources, sinks and the various reaction routes and cycling in these natural systems;
3. to examine the metabolic role and mechanisms of seawater sulfate assimilation and DMSP production by marine algae species as well as studying the isotope effects associated with gas and aqueous phase DMS generation;
4. to study sulfur isotope effects of specific sulfur compounds on the rate of thermal decomposition to produce radical species; and

5. to identify the mechanistic routes that leads to the concentration of magnetic or non-magnetic nuclei in thermochemically sulfur reduction products.

This work will draw on both laboratory and field techniques and will seek to establish relationships that extend beyond either medium.

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Chapter 2

Multiple Sulfur Isotope Analysis of Volatile Organic Sulfur Compounds and their Sulfonium Precursors in Coastal Marine Environments*

Abstract

Volatile methylated sulfur compounds emitted from terrestrial and aquatic ecosystems play a significant role in the global sulfur cycle, yet no satisfactory methods are available to trace their source and transformation in natural systems. Here we present a method for quantification and multiple sulfur isotopic analysis of a variety of volatile sulfur species as well as their natural precursors via hydrodesulfurization with a Raney nickel catalyst. The detection limit of this method for methanethiol (MT), dimethylsulfide (DMS), dimethyldisulfide (DMDS), and carbon disulfide (CS_2) is 0.2 milligrams of sulfur per sample. Average recovery of ~95% was attained for samples containing more than 1.3 mg of these sulfur compounds. Triplicate to quadruplicate sulfur isotopic analyses of reduced standard materials yield average standard deviations of 0.3 ‰, 0.02 ‰, and 0.1 ‰ respectively for $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$. The method developed here was used for determination of sulfur isotopic compositions of volatile organic sulfur compounds (VOSCs) and their precursor dimethylsulfoniopropionoate (DMSP) in sediment cores and a C_4 plant *Spartina alterniflora* collected from the Delaware Great Marsh. Application of the method to these natural samples indicates that the S-isotope compositions of VOSCs and DMSP-S are similar to, but slightly ^{34}S-depleted (~0.6 - 0.9‰), relative to porewater sulfide. These compounds are ^{34}S-enriched (~1.7 - 2.0‰) relative to the compositions of the coexisting sulfide. Both suggest a relationship between source sulfide and these organic sulfur compounds.

Keywords: Sulfur; volatile organic sulfur compounds; dimethylsulfoniopropionoate; Raney nickel catalyst; multiple sulfur isotopes; hydrodesulfurization; *Spartina alterniflora*

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1.0 Introduction

Considerable effort has been dedicated to field and laboratory studies seeking to understand biogenic emissions of volatile sulfur gases from the ocean and their role in the atmosphere. Of particular interest are volatile organic sulfur compounds (VOSCs) such as dimethyl sulfide (DMS - CH_3SCH_3), methanethiol (MT - CH_3SH), dimethyl disulfide (DMDS - CH_3SSCH_3), carbon disulfide (CS_2) and carbonyl sulfide (OCS) (Steudler and Peterson, 1984; Kiene and Taylor, 1988; Finster et al., 1990; De Zwart and Kuenen, 1992; Luther and Church, 1992; Lomans et al., 2002; Bentley and Chasteen, 2004; Stefels et al., 2007; Schäfer et al., 2010).

The volatile organic sulfur compound DMS has been postulated to play a role in atmospheric chemistry and cloud microphysics (Lovelock et al., 1972; Charlson et al., 1987; Andrea, 1990). Gas–phase photo-oxidation of DMS to SO_2 to sulfuric acid (H_2SO_4) is a biogenic source of sulfate aerosols. These sulfate aerosols alter radiation through scattering, reflection, absorption, and contribute to acidity of precipitation leading to the formation of cloud condensation nuclei, changing the number density and size distribution of cloud droplets and in turn influencing the Earth's radiation budget (Charlson et al., 1987).

Dimethyl sulfide, and methanethiol largely originate from degradation of a common precursor, dimethylsulfoniopropionoate (DMSP- $(CH_3)_2S^+CH_2CH_2COO^-$) a product of bacterio-plankton (Taylor and Visccher, 1996; Turner et al., 1998; Kiene et al., 2000; Yoch, 2002; Van Alstyne and Puglisi, 2007). This β-sulfonium compound serves several physiological roles in marine algae (Karsten et al., 1996; Stefels, 2000; Sunda et al., 2002) and in certain halophytic plants, including salt marsh grasses of the genus

Spartina (Kocsis et al., 1998; Kocsis and Hanson 2000; Otte et al., 2004). In addition to DMSP, other natural precursors of VOSCs include methionine (MET), cysteine (CYS), glutathionine (GSH), dimethyl sulfoxide (DMSO), dimethylsulfoniopentanoate (DMSPent), dimethylsulfonioacetate (DMSAcet), homocystiene, and mercaptopropionate (Kodata and Ishida, 1972; Calhoun and Bates, (1989); Howard and Russell, (1997); Stefels et al., 2000).

Natural settings such as salt marsh ecosystems – the focus here – are a source for these compounds. High production rates occur for VOSCs in salt marsh ecosystems because high levels of biological activity cycle carbon, nitrogen, and sulfur that vary on seasonal timescales and occur at the terrestrial-marine interface (Sorensen, 1988; Kiene, 1988; Kiene and Capone, 1988; Lomans et al., 2002). Sulfur cycling in these systems involves a number of biogeochemical processes such as microbial sulfate reduction, redox cycling of metals, pyrite formation, energy transport, and biogenic sulfur gas emissions into the atmosphere (Howarth et al., 1983; Steudler and Peterson, 1984; Luther et al., 1986; Kiene and Taylor, 1987; Luther and Church, 1992; DeLaune et al., 2002; Cozic-Houly et al., 2009). The presence of short cord grass *Spartina alterniflora* plants and high bacterial sulfate reduction rates in the Delaware Great Marsh (DGM), located at the edge of the Delaware Bay near Lewes provides an excellent opportunity to understand the role and distribution of VOSCs and their major precursor, DMSP in coastal marine environments.

Isotopic measurements of VOSCs provide a way to fingerprint sulfur sources and to trace transformations associated with biological, physical and chemical processes. Isotopic measurements of VOSCs have been hampered because these compounds exist in

low concentration in ambient air and natural waters, because these compounds can be challenging to separate and characterize, and because general methods of preparation of these compounds are lacking. To date, most constraints on the sulfur isotopic composition of biogenic volatile sulfur compounds are made on the basis of inferences from submicron marine sulfate aerosols and measurements of aerosol sulfate and methanesulfonic acid (MSA) in ice cores (Calhoun et al., 1991; Patris et al., 2000; 2002; Jonsell et al., 2005; Sanusi et al., 2006). A few preliminary measurements of DMS (and DMSP) are reported by Calhoun (1990) ($\delta^{34}S_{DMS}$ value of +17 ‰); Calhoun and Bates (1989) report a $\delta^{34}S_{DMSP}$ value of +19.8 ‰ as personal communication in that study as well as estimates from non-sea salt sulfate (nss-sulfate) by Calhoun et al., (1991) ($\delta^{34}S_{DMS}$ value of +17 ± 1.9 ‰), but follow up measurements have not been made. A recent study (Amrani et al., 2009), describes a method for measuring only $\delta^{34}S$ for relatively small concentrations of volatile sulfur species involving a GC coupled with a multicollector ICP-MS. This technique is in its early stages, and its applications look promising. Herein, we describe another method to sample and convert the sulfur in a variety of VOSCs and precursors to a form that can be analyzed for their four sulfur isotope distributions, which is complementary to the ICP-MS techniques.

The method presented in this paper utilize a modification of techniques by Granatelli (1959) for reduction of VOSC species (including their biological precursors, major oxidants and intermediate species) through a Raney nickel hydrodesulfurization reaction to quantitatively yield corresponding alkane and hydrogen sulfide that is captured as ZnS or Ag_2S, which is subsequently used for determination of concentration and four isotope compositions of sulfur. The technique described here is used to

determine the major ($\delta^{34}S$) and minor isotopes ($\Delta^{33}S$, and $\Delta^{36}S$) of combined volatile organic sulfur compounds (CVOSCs), natural precursor (DMSP), and other organosulfur species in a coastal wetland system.

2.0 Materials and Methods

2.1. Site description and sampling procedures

In April 2010, during a high tidal inundation of the Great Delaware Marsh (Lat. 38°48 'N and Long. 75°12'W), located on the southern shore of Delaware Bay in Lewes (Figure 2.1). Three sediment cores (about 12 cm in length) were collected (~ 2 meters apart) by pushing a plastic coring device into the marsh sediments. The cores,

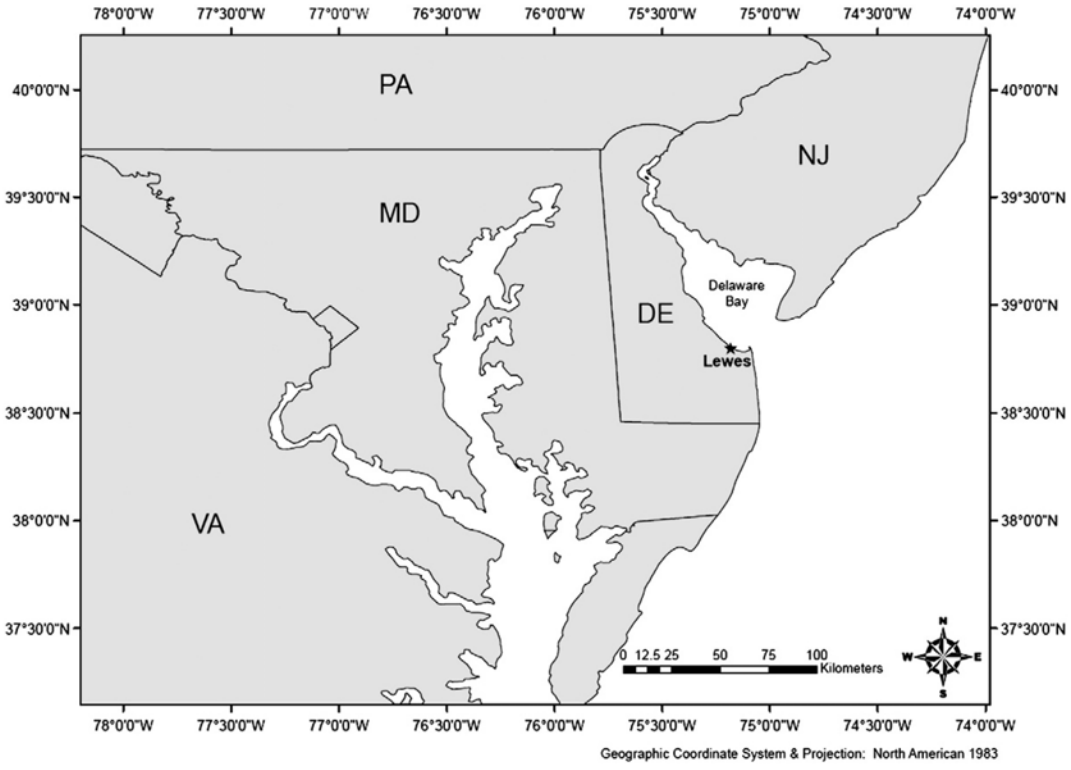

Figure 2.1: Geographic location of the Delaware Great Marsh sampling site (★) located on the Southern shore of Delaware Bay, near Lewes**,** DE.

which contained *Spartina alterniflora* roots were located approximately 3 meters from a tidal creek. The sediment cores were capped with rubber stoppers secured at both ends with duct tape. The core samples were immediately transported to the laboratory at the University of Maryland for further processing on the same day. One core was used for chemical analysis and the two cores were used for sulfur isotope analysis. Wet sediments were extruded inside a nitrogen filled glove bag, and sectioned at 4 cm intervals for extraction of organic and inorganic sulfur fractions.

2.2. Extraction of Organic and Inorganic Sulfur Fractions

Extracts of organosulfur products sampled from DGM sediments are the combined volatile organic sulfur compounds (CVOSCs – comprising of MT, DMS, DMDS, and CS_2), DMSP sulfur (DMSP-S) from *Spartina* plants, and humic sulfur from sediments. Figure 2.2 illustrates the scheme for a sequential extraction for organic and inorganic sulfur fractions from wet sediment (described detail in appendix 2A).

CVOSCs sulfur fraction – VOSCs concentrations were measured by a procedure described elsewhere (Kiene and Capone, 1988) using gas chromatography (Shimadzu model GC-14A equipped with a flame photometric detector). The VOSC species that were detected, namely MT, DMS DMDS, and CS_2 were extracted from ~20 g of wet sediment with n-hexane cooled to ethyl acetate-liquid nitrogen temperature (-84^{o}C) to prevent volatilization. The hexane extract was later washed with 40% aqueous diethanolamine in a separatory funnel to remove traces of hydrogen sulfide (Sidi-Boumedine et al., 2004). CVOSCs present in the hexane were finally precipitated with 5% $HgCl_2$ as mercury complexes (e.g., $HgMT_2$, 3DMS-2Hg, 3DMDS-2Hg) (Nguyen et

al., 1978; Yang et al., 1996; 1999; 2006). The stabilized VOSC complexes were degraded with 6 $molL^{-1}HCl$ and redissolved in cold n-hexane followed by Raney nickel hydrodesufurization (a method described in section 2.3.2.) to convert CVOSCs to Ag_2S.

DMSP sulfur - *Spartina* roots and leaves were washed threefold with cold DI water followed by freezing and crushing of plant material in liquid nitrogen. DMSP was extracted in dark conditions by the method described by Zhang et al., (2005). A mixture of cold methanol, chloroform and water (12:5:3 v/v) was used to extract DMSP. Organic solvents were removed by evaporation using a rotary evaporator under vacuum at 30 ^oC. The extract pH was adjusted to 5.5 to keep DMSP stable before final purification using a cation-exchange resin, Dowex-50W (H^+) (James et al., 1994; Kocsis et al., 1998). The aqueous extract was characterized by Electrospray Ionization Mass Spectrometry (ESI-MS) in a positive mode before and after purification to confirm the identity of a DMSP peak at m/z=135, a DMSP-Na^+ adduct at m/z=158, and other C_5 DMSP homologs (Figure2.3). Purity of protonated DMSP was determined by thin layer chromatography (TLC) to be ≥ 97%. DMSP was converted to Ag_2S using the Raney nickel desulfurization method.

Humic sulfur fraction – Sedimentary humic sulfur was sequentially extracted after the removal of elemental sulfur (S_8) with 0.1 N NaOH according to the method described by Ferdelman et al. (1991). Acidification of the base extract to a pH = 2, precipitates humic acid fractions from fulvic acid. Humic fraction was finally isolated by centrifugation and dried in an oven overnight at temperature of 60^oC. A portion of the dried sample was subsequently reduced to Ag_2S by Raney nickel catalyst. For comparison of isotopic ratios of other sulfur species present in the salt marsh, we extracted the following inorganic

sulfur species: porewater sulfate and sulfide; and a sequential extraction of wet sediment acid volatile sulfide (AVS - consisting mainly of free sulfides and iron monosulfides), elemental sulfur, and pyritic sulfur. The free sulfides and sulfate from pore water were extracted by centrifugation of sediments under anoxic condition. Porewater sulfide (PWS) and H_2S was precipitated as ZnS (from Zn-acetate) that was acid distilled with 6 $molL^{-1}$ HCl (Canfield et al., 2006), while porewater sulfate was precipitated as $BaSO_4$ (from $BaCl_2$ solution) and was reduced into H_2S gas by boiling with 25 mL solution mixture consisting of 320 mL HI, 524 mL HCl, and 156 mL H_2PO_4 (Thode solution - Forrest and Newman, 1977). AVS was extracted by distillation with 3 $molL^{-1}$ HCl (Cutter and Oatts, 1987); elemental sulfur from residual sediment was extracted by methanol-chloroform mixture (1:1) and reduced by chromium acid distillation in an ethanol solution (Gröger et al., 2009); pyritic sulfur was reduced with chromium acid distillation, following methods described in Canfield et al. (1986). In all distillation-reduction reactions, evolved H_2S gas was quantitatively trapped in a silver nitrate buffer solution (0.3 mol L^{-1} $AgNO_3$ in 1.55 mol L^{-1} HNO_3) precipitating as Ag_2S.

2.3. Experimental Methods for Raney Nickel Hydrodesulfurization

2.3.1. Reagents and Standards

The Raney nickel desulfurization method described in section 2.3.2 was validated using a variety of laboratory standards (described detailed in appendixes 2B and 2C). Reagent grade chemicals were used throughout the experiments. DMS, MT (in the form CH_3SNa), DMDS, CS_2, DMSO, MSA, $DMSO_2$, CYS, GSH, and MET were obtained from Sigma-Aldrich.

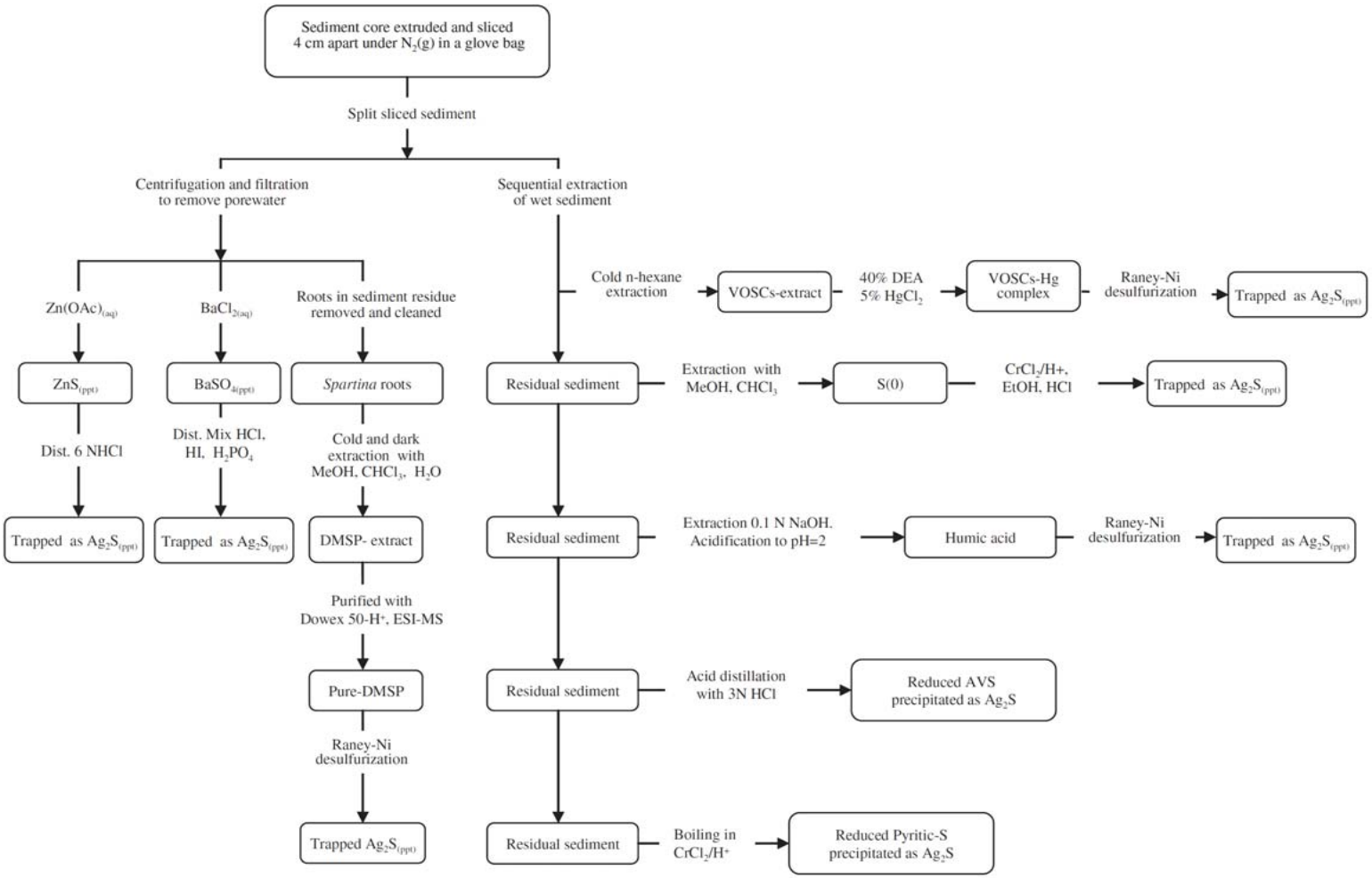

Figure 2.2: Schematic diagram showing extraction procedure for organic and inorganic sulfur fractions, and their method of conversion to Ag_2S for S-isotope analysis as SF_6 gas.

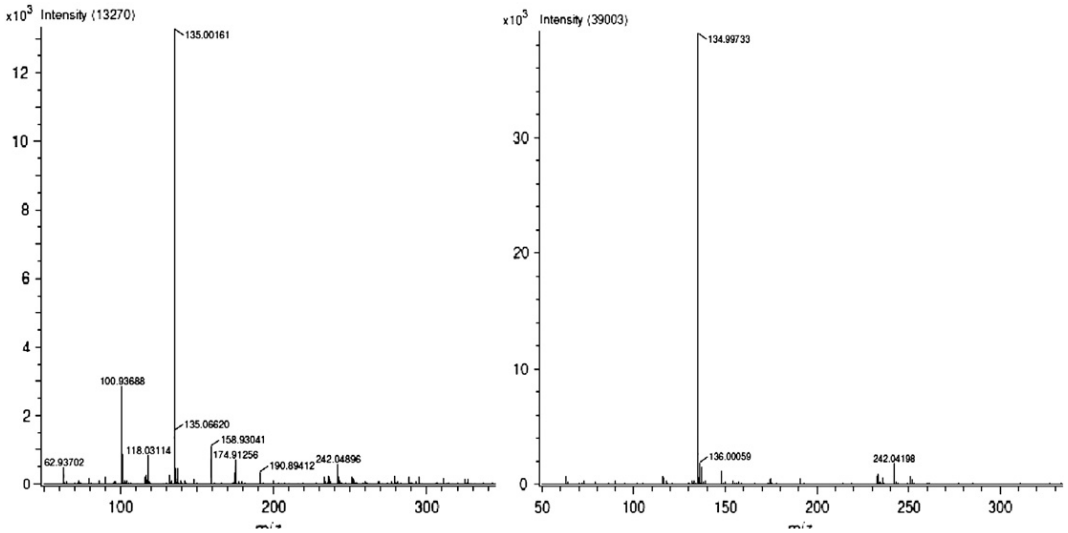

Figure 2.3: ESI-MS (+) mode spectra for DMSP (m/z=135), DMSP-Na^+ adduct (m/z=158), and C_5 homologs of DMSP extracted from *Spartina alterniflora* plant in Delaware Great Marsh. The left panel is the mass spectrum obtained from concentrated crude extract. The right panel is a singly charged ion mass spectrum of purified DMSP peak from the crude extract.

A nickel-aluminum, Raney-type non-activated alloy was purchased from Alfa Aesar. As international standards do not exist for most VOSC species, relevant in-house standards were prepared from a recognized DMS (Sigma Aldrich; Catalog no. 274380) standard.

Dimethylsulfoniopropionate Chloride (DMSP-HCl) was prepared according to the method described by Chambers et al., 1987. Briefly, an aliquot of 5 ml of DMS (Sigma-Aldrich; 4.2g, 67.6 mmol) was dissolved in a flask containing 20 mL of methylene chloride, followed by addition of acrylic acid (Alfa Aesar: 4 mL, 4.2 g, and 58.3 mmol) while stirring. The mixture was bubbled slowly with hydrogen chloride gas (Sigma-Aldrich) to yield a white precipitate, which was isolated by filtration, crystallized in a cold ethanol (-50°C) solution, and freeze dried to yield the protonated zwitterionic DMSP (6.77 g, 50.15 mmol, and 74.2%). Purity of DMSP was ≥ 98% as confirmed by

electrospray ionization mass spectrometry (ESI-MS/MS) in (+) mode at M/Z 135 and Thin Layer Chromatography (TLC).

Dimethylsulfonioacetate (DMSAcet) was prepared by addition of DMS (Sigma-Aldrich; 5 mL, 4.2g, 67.6 mmol) to reagentPlus® grade bromoacetic acid (Sigma-Aldrich; 7 g, 50.83 mmol) in an ice-water bath (Howard and Russell (1997). The reaction mixture was stirred for 2 minutes, and heated to 45^{o}C for 5 minutes to yield a white crystalline material. Final purification and characterization was performed using methods described by Howard and Russell (1997). The yield was 7.1 g, 60.1 mmol, and 87.9%. 100 $\mu molL^{-1}$ aqueous stock methanol solutions of DMS, CS_2, DMDS, DMSO, and $DMSO_2$ were prepared using a set of adjustable positive displacement microsyringes (calibrated against a primary standard). Aqueous stock solutions (100 $\mu molL^{-1}$) were also prepared for the following organic salts; sodium methanethiolate, sodium methanesulfinate, methanesulfonic acid, methionine, cysteine, glutathionine, DMSP-HCl and DMSAcet by dissolving appreciable weight of each organic reagent in deionized water (18 MΩ) purified with a Milli-Q system (Millipore, Bedford, MA). All stock solutions were prepared in 100 mL serum vials with no headspace and sealed with butyl rubber septum stoppers. The solutions were stored in a refrigerator at 0ºC. All glassware was cleaned prior to use by soaking in 10% HCl overnight, rinsed thoroughly with ultra-purified water and dried overnight at 100 ^{o}C.

2.3.2. Activation of Raney Nickel-Aluminum Alloy

Pure Raney nickel was prepared in a fume hood using the method described by Granatelli (1959). Briefly, 1g of nickel-aluminum alloy was weighed in 100 mL polyethylene beaker. Ten milliliters of 2.5 $molL^{-1}$ NaOH solution was slowly added. The

beaker was gently swirled to generate a violent reaction of sodium aluminate, which is accompanied by the rapid evolution of hydrogen gas. The mixture was left overnight in a desiccator to undergo further hydrolysis of sodium aluminate to complete the activation of the Raney alloy. Excess supernatant sodium hydroxide was slowly decanted from the activated Raney nickel with care to minimize loss of the catalyst. Before using the activated catalyst, it was washed with a series of successive 10 mL volumes of deoxygenated Milli-Q water until the washing solutions were no longer basic as indicated with litmus paper.

2.3.3. Acidified zinc acetate trapping solution

A 0.4% (w/v) zinc acetate buffer solution was prepared from 40 g zinc acetate dihydrate in ca. 200 mL of Milli-Q water, and 30 mL glacial acetic acid solution. The mixture was made up to 1.0 L by addition of Milli-Q water and was used for trapping hydrogen sulfide derived from the desulfurization of organic sulfur compounds.

2.3.4. Analytical Procedure for hydrodesulfurization

A flow diagram of the analytical procedure is presented in figure 2.4. Approximately 0.8 g freshly activated Raney nickel was added to a modified 100 mL one-neck round bottom flask. The flask was modified by adding a two glass blown threaded, air-tight, bushing glass sealed joints. For reduction of VOSC species, ethanol (20 mL) was added to the flask and it was chilled to -90^{o}C in an ether/dry ice bath.

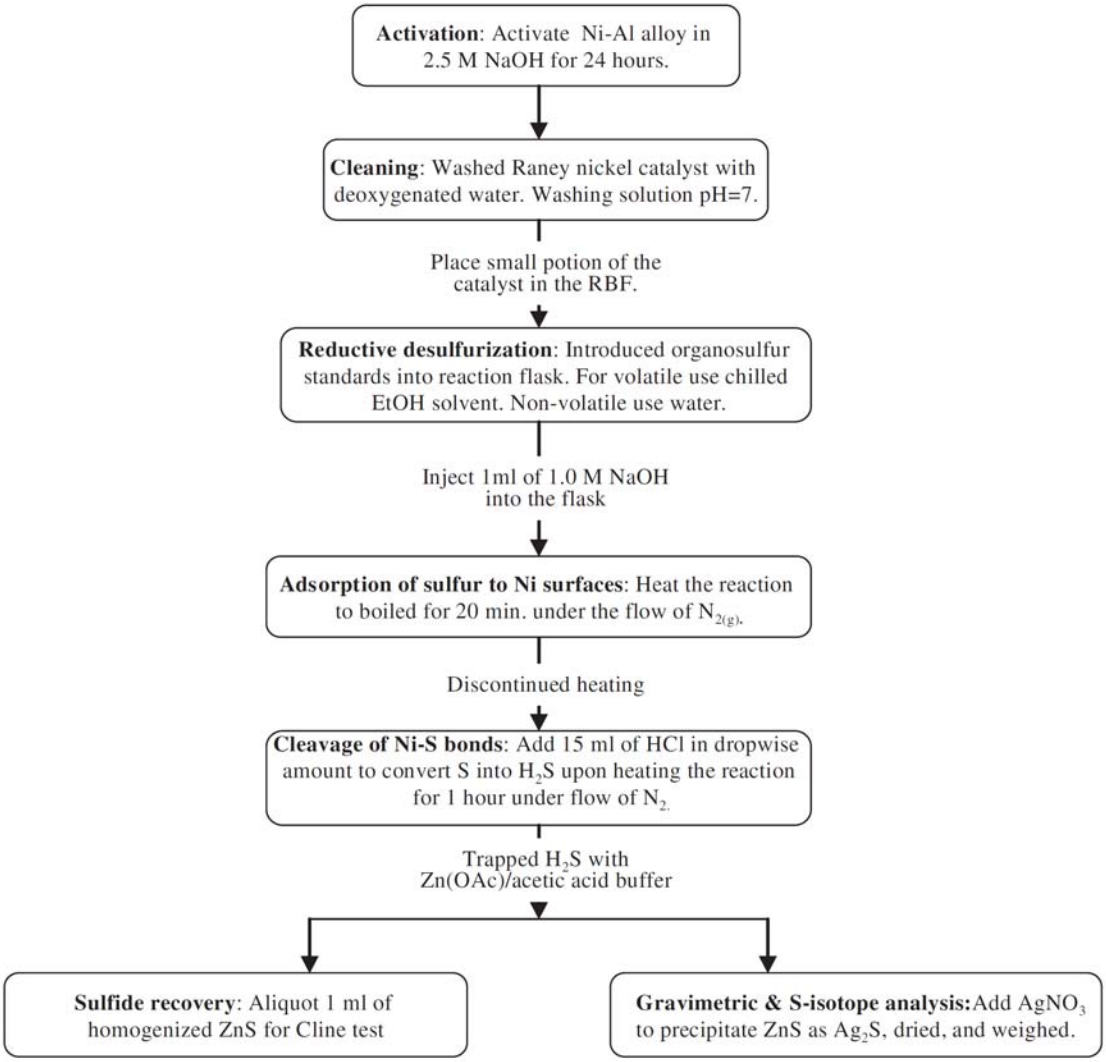

Figure 2.4: Analytical flow diagram for Raney nickel reduction.

The flask was immediately attached to an Allihn style reflux condenser equipped at the top with a short length of Tygon tubing connected to a semi-enclosed test tube containing 15 mL of trapping solution. The set-up (Figure 2.5) was purged with ultra high purity nitrogen (UHP-N_2) gas through one of the modified threaded glass septum-sealed joints for 5 min to remove molecular oxygen arising from dissolved air. An air-tight micro syringe capable of dispensing micro liter volumes was used to aliquot stock solution into the reaction flask via the second threaded glass septum sealed adapter. One milliliter of

1.0 $molL^{-1}$ NaOH solution was injected into the chilled flask to initiate the catalytic activity of the reaction. The flask was set on a heating mantle to boil under constant flow of nitrogen (bubble rate -1 bubble per second). After ~20 min of boiling, the flask content was gradually cooled to room temperature.

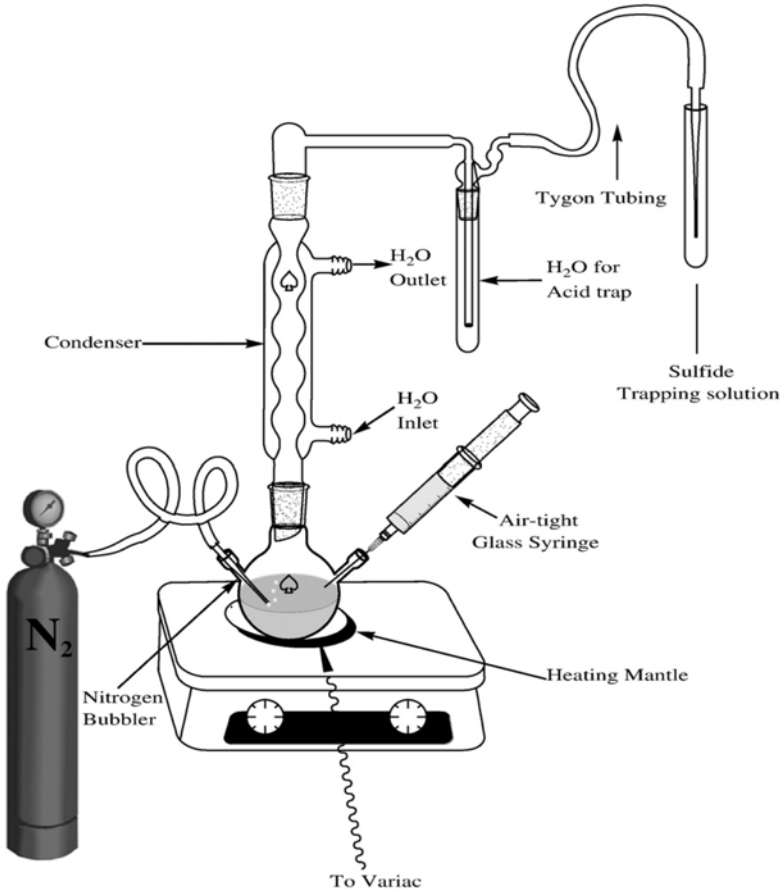

Figure 2.5: Experimental set-up for organic sulfur hydrodesulfurization.

A clean glass syringe was used to inject 15 mL of treated hydrochloric acid (Kijowski and Steudler, 1982) in dropwise increments into the flask while maintaining a constant nitrogen flow. After all the acid solution was added, heating was continued for 1 hour to completely convert all the sulfur in the reaction mixture into H_2S. Hydrogen sulfide evolved from the reaction was captured by a Zn-acetate buffer, yielding a white crystalline ZnS precipitate.

The ZnS precipitate was homogenized after the reaction and tested for sulfide concentration by the method of Cline (1969) using a UV-VIS double beam (model UVD-3200) scanning spectrophotometer (Labomed Inc., CA, USA). Triplicate absorbance measurements of reactive sulfide captured by zinc acetate solution were immediately measured at λ_{max}(670 nm) in 1.0 mL aqueous sample. The response of recovered sulfide species was compared to a range of 1.0 $\mu molL^{-1}$ – 10.0 $\mu molL^{-1}$ calibrations made using standard concentrations of Na_2S, and in all cases a linear increase in absorbance with increasing concentration of sulfide (H_2S/HS^-) was observed.

The remaining ZnS was converted to Ag_2S through dropwise addition of 0.3 $molL^{-1}$ $AgNO_3$. Precipitated Ag_2S was collected by centrifugation, rinsed with 15 mL 1.0 $molL^{-1}$ NH_4OH solution and then twice with 15 mL of Milli-Q water. Samples were dried in an oven (100^oC) for gravimetric and isotopic analyses. Activated Raney nickel blank samples were tested for the presence of sulfide in the catalyst using this reduction procedure. In all the blank tests, no sulfide was detected in zinc acetate trapping solutions.

2.4. Multiple Sulfur ($\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$) Isotope Ratio Measurement

Samples of Ag_2S were reacted in Ni bombs with ten-fold excess F_2 gas at 320^oC for approximately 8-12 hours. SF_6 product gas was cryogenically frozen and separated from unreacted F_2 gas in a liquid-nitrogen trap cooled to -196^oC. Excess F_2 gas was passivated by reaction with hot KBr. The SF_6 product was purified through cryogenic distillation (at -110^oC) to condense traces of HF contaminants, before transferring it into an injection loop of a gas chromatograph (GC) cooled to -196^oC. Final purification of SF_6

by GC-TCD was accomplished using a composite column made up of 1/8 in. diameter 6 ft long packed column containing type 5A molecular sieve, followed by another 1/8 in. diameter, 12 foot long Hayesp-Q^{TM} column. A carrier flow of He set at 20 mL min^{-1} was utilized with a GC temperature of 50^{o}C to elute SF_6 peaks between 12 and 18 minutes. The SF_6 gas eluting from the column was captured by diverting it together with He carrier gas into a glass spiral trap chilled at liquid nitrogen temperature (-196^{o}C), where the He gas was slowly pumped off from the trap. The GC column temperature was ramped to 150^{o}C at 5 ^{o}C min^{-1} to flush the column for 5 mins and cooled 50^{o}C between samples. The purified SF_6 was transferred to a Finnigan MAT 253 mass spectrometer where its isotopic composition was measured in dual-inlet mode. Four collectors were arranged to measure the intensity of SF_5^+ ion beams at m/e values of 127, 128, 129, and 131 ($^{32}SF_5^+$, $^{33}SF_5^+$, $^{34}SF_5^+$, and $^{36}SF_5^+$). Isotopic analysis of each sample consisted of 3-5 data acquisitions, with each acquisition consisting of 8 sample-to-reference cycles (~ 13 minutes per acquisition).

Sulfur isotopic compositions of measured samples are presented using the standard delta (δ) notation ($\delta^{33}S$, $\delta^{34}S$, and $\delta^{36}S$):

$$\delta^{34}S = [(^{34}S/^{32}S)_{samp}/(^{34}S/^{32}S)_{ref} - 1]$$

$$\delta^{33}S = [(^{33}S/^{32}S)_{samp}/(^{33}S/^{32}S)_{ref} - 1]$$

and are reported relative to the international reference standard Vienna Canyon Diablo Troilite (V-CDT) in units of permil (‰). Where $_{'samp'}$ and $_{'ref'}$ represent the measured sample and reference standard, respectively. Note that this convention drops the factor of 1000 included in some other studies due to small variation in fractionation coefficient between working standards and a common reference material (Mook and Grotes, 1973;

Gonfiantini, 1983; Coplen, 2011). The less abundant isotopes (^{33}S and ^{36}S) are also reported using capital delta notation (Δ);

$$\Delta^{33}S = \delta^{33}S - [(1 + \delta^{34}S)^{0.515} - 1]$$

$$\Delta^{36}S = \delta^{36}S - [(1 + \delta^{34}S)^{1.90} - 1],$$

which are also given in units of permil (‰). The $\Delta^{33}S$ (or $\Delta^{\delta 36}S$) describes the difference between a measured $\delta^{33}S$ (or $\delta^{36}S$) of a given sample and the point with the same $\delta^{34}S$ on a reference fractionation line (RFL) that approximates single step, low-temperature equilibrium isotope effects between sulfide and sulfate (Hulston and Thode, 1965; Farquhar et al., 2007).

3.0 Results and discussion

3.1. Sulfur Transformation in DGM influenced by Bacterial Sulfate Reduction

Experimental results (see Table, 2.1) presented here support the notion that different organic and inorganic sulfur species are formed in sedimentary environments as a result of electron transfer reactions. These reactions involved bacterial sulfate reduction under reduced conditions, uptake and assimilation of mixed sulfate and sulfide sulfur in plants, as well as diagenetic formation of pyritic sulfur. The relative abundance of these sulfur fractions depends on physico-chemical parameters, including pH, redox potential, and concentrations of dissolved sulfide and Fe, as well as biological factors, such as the activities of microorganisms whose metabolism depends on the oxidation or reduction of either S or Fe.

In DGM, the average sulfur isotopic composition of organic and inorganic sulfide products within the investigated upper 12 cm are depleted in the heavier ^{34}S isotope

relative to the mean porewater sulfate ($\delta^{34}S \sim 21.6‰$). This is an indication that microbial dissimilatory reduction of dissolved sulfate to sulfide is the dominant process at depth in this system. This process is carried out by strictly anaerobic bacteria in anoxic environments and is accompanied by a large $\delta^{34}S$ isotopic fractionation between dissolved porewater sulfate and sulfidic-sulfur (e.g., Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Canfield, 2001a; 2001b). The process has been shown to discriminate $\Delta^{33}S$ and $\Delta^{36}S$ minor isotopes in bacterial cultures (Johnston et al., 2008; Zerkle et al., 2010). A majority of bacterial species involved in dissimilatory sulfate reduction in sedimentary systems are chemoorganoheterotrophic using organic carbon compounds as electron donors and carbon source to biologically transform sulfate-sulfur (terminal electron acceptor) to hydrogen sulfide according to the idealized chemical reaction below.

$$2CH_2O + SO_4^{2-} \longrightarrow 2HCO_3^- + H_2S \qquad (1)$$

The observed changes in $\delta^{34}S\text{-}SO_4^{2-}$ with an average close to the marine sulfate value of +20‰ and a single porewater sulfate value at 8-12 cm of +27‰, suggest that bacterial sulfate reduction fractionated the $\delta^{34}S$ of dissolved sulfate in these marsh sediments at depth. Depth variations of the sulfur isotopic fractionations of organic and inorganic sulfur species may reflect changes in overall isotope effects that may due to superimposed physico-chemical parameters (such as changes in tidal inundation), biological and abiotic reactions. For instance, the reoxidation H_2S to sulfate in the depth resolved cycling of sulfur. The profiles of AVS, pyrite, pore water sulfide, combinedVOSC, DMSP, and Humic sulfur have more negative $\delta^{34}S$ at depth. This is

interpreted to reflect short term (seasonal, tidal cycle) changes in the cycling of sulfur in the sediments.

3.2. VOSCs, DMSP, Humic-Sulfur Formation in DGM

Table 2.2 shows the various detectable levels of VOSCs (in units of mol g^{-1} of sediment of wet sediments) found only within the upper 12 cm of the core sediment. The average yield of H_2S recovered from the Raney nickel extractions within the upper 12 cm core sediment was 95.2%. The reproducibility of Raney nickel extraction was determined by analysis of three replicates of representative samples at different locations. The resulting standard deviations at each depth are shown in table 2.2. The fraction unrecovered may reflect volatilization during (1) precipitation with mercuric chloride and (2) heating of the reaction during the hyrodesulfurization step of the reduction, or (3) a decrease in the activity of the Raney nickel catalyst by oxygen and possibly extracted sulfonate compounds. The highest concentrations of all the four VOSC species (MT, DMS, DMDS, and CS_2) were observed within the 8-12 cm sediment depth. The accumulation of high concentration of VOSCs, particularly MT and DMS in the upper 12 cm are most likely associated with sulfidation of decomposing fragments of macro-algae and rapid transformation of the DMSP being released from damaged roots of *Spartina alterniflora*, which were buried in the sediment during early summer. In addition to sulfidation and decomposition of dead organic matter, tidal pumping may be responsible for transporting some VOSCs upwards from subsurface layer by faunal activity (bioturbation) (Jorgensen & Okholm-Hansen 1985).

Results of multiple sulfur isotope measurements for extracted inorganic and organic sulfur species in salt marsh are summarized in Table 2.1. We found from the

Inorganic sulfur fractions	Abbr. sulfur species	Depth (cm)	$\delta^{34}S$ (‰)	$\Delta^{33}S$ (‰)	$\Delta^{36}S$ (‰)	N
Combined volatile organic sulfur compounds	CVOSCs	0–4	-3.30	0.076	-0.87	2
		4–8	-3.50	0.113	-1.03	2
		8–12	-4.55	0.128	-1.15	2
		Mean/S.D.	-3.78 ± 0.7	0.106 ± 0.03	-1.02 ± 0.1	
Spartina DMSP sulfur	DMSP-S	0–4	-1.82	0.083	0.45	2
		4–8	-5.55	0.113	-1.07	2
		8–12	-5.01	0.150	-1.20	1
		Mean/S.D.	-4.12 ± 2.0	0.115 ± 0.03	-0.61 ± 0.9	
Humic sulfur	Humic-S	0–4	-2.83	0.108	-0.92	2
		4–8	-6.59	0.123	-1.14	2
		8–12	-7.53	0.129	-1.36	2
		Mean/S.D.	-5.65 ± 2.5	0.120 ± 0.01	-1.14 ± 0.2	
Inorganic Sulfur						
Porewater sulfide	PWS	0–4	-2.27	0.131	-0.97	2
		4–8	-4.26	0.129	-1.20	2
		8–12	ND	ND	ND	ND
		Mean/S.D.	-3.21 ± 0.9	0.133 ± 0.01	-1.16 ± 0.2	
Acid volatile sulfur	AVS	0–4	-4.62	0.132	-1.15	2
		4–8	-5.67	0.135	-1.23	2
		8–12	-7.23	0.122	-0.94	2
		Mean/S.D.	-5.84 ± 1.3	0.129 ± 0.01	-1.10 ± 0.2	
Porewater sulfate	SO_4^{2-}	0–4	19.25, 13.35*	0.037	-0.17	1
		4–8	20.86	0.040	-0.20	2
		8–12	27.57	0.005	0.14	2
		Mean/S.D.	21.60 ± 5.6	0.027 ± 0.02	-0.08 ± 0.2	
Elemental sulfur	S(0)	0–4	-9.02	0.132	-0.78	2
		4–8	-8.79	0.156	-1.25	2
		8–12	-8.09	0.133	-1.29	2
		Mean/S.D.	-8.63 ± 0.5	0.140 ± 0.01	-1.11 ± 0.3	
Pyritic sulfur	FeS_2	0–4	-9.22	0.141	-1.27	2
		4–8	-11.47	0.162	-1.42	2
		8–12	ND	ND	ND	ND
		Mean/S.D.	-10.34 ± 1.6	0.152 ± 0.02	-1.34 ± 0.1	

Table 2.1: Sulfur isotope ($\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$) distribution, their mean and standard deviation of AVS, CVOSCs, *Spartina* DMSP-S, PWS, and other sulfur fractions extracted from the uppermost 12cm layer of the salt marsh sediment. N – denotes repeated analysis from different sediment core. ND – denotes core sections where sulfur species was not extracted for S-isotope analysis. (*) repeated analysis of a core section which yielded a low ^{34}S-SO_4^{2-} value and might result from mixing or from an unknown source.

marsh that VOSCs and DMSP from *Spartina* have negative $\delta^{34}S$ and $\Delta^{36}S$ values and positive $\Delta^{33}S$ values, with average $\delta^{34}S$ values of VOSCs (-3.8 ± 0.7‰) and DMSP in plant roots (-4.1 ± 2.0‰). These isotopic values are similar to average $\delta^{34}S$ values of acid volatile sulfide (-5.8 ± 1.3‰) and pore water sulfide (-3.2 ± 0.9‰). This implies that inorganic and organic reduced sulfur species are produced predominantly from sulfide precursors produced by sulfate reduction (Luther et al., 1986; 1991; Luther and Church, 1988; Ferdelman et al., 1991).

Concentration VOSCs extracted from sediment core	0 - 4 cm	4 - 8 cm	8 - 12 cm
MT (μmol/g)	0.12	0.24	0.28
DMS (μmol/g)	0.01	0.02	0.03
DMDS (μmol/g)	0.02	0.03	0.04
CS_2 (μmol/g)	0.01	0.01	0.01
CVOSCs (μmol/g)	0.19	0.34	0.41
Yield of H_2S after RN (μmols)	3.80	6.74	8.23
Percentage Yield (%)	93.4	96.7	95.9
Reproducibility (% SD)	18 (n=3)	16 (n=3)	n.d.

Table 2.2: VOSCs sediment concentrations (μmol/g of wet sediments) and sum of molar percentage yields of sulfur from total VOSC after Raney nickel extraction.

The minor isotope ratios of CVOSC and DMSP sulfur exhibits a very narrow range in values ($\Delta^{33}S$= +0.11±0.03‰ to +0.12±0.03‰, and $\Delta^{36}S$= -1.0±0.1‰ to -0.6±0.9‰) that are relatively close to range AVS and pore water sulfide $\Delta^{33}S$ and $\Delta^{36}S$ ratios (0.13±0.01‰ to 0.13±0.01‰ and -1.2±0.2‰ to -1.1 ±0.2‰, respectively). The small shift to negative $\Delta^{33}S$ and positive $\Delta^{36}S$ with more positive $\delta^{34}S$ values of VOSCs in the shallowest sediments suggests a small contribution from organic sulfur compounds (e.g., methionine, cysteine) ultimately derived from assimilatory reduction of sulfate.

The $\Delta^{33}S$ and $\Delta^{36}S$ of sedimentary sulfur species reflects the composition of sulfur pools from which they form and also any isotopic fractionation in those reactions.

Isotopic data suggests that organic sulfur species are produced predominantly from the sulfide nucleophiles, H_2S/HS^- and possibly polysulfides by one of the following two reactions:

1) Uptake and incorporation of cellular sulfide by the process of assimilatory sulfate reduction through the rhizomes of *Spartina* plants.
2) Reaction with buried or decomposed organic matter to form carbon-bonded organic sulfur compounds.

To put our inferences in proper perspective, the mean $\delta^{34}S$ values for CVOSC, DMSP, AVS, PWS are -3.8‰, -4.1‰, -5.8‰, and -3.2‰ respectively, with their respective standard deviations of ±0.7, ±2.0, ±1.3 and ±0.9 are to some extent genetically related. The low $\delta^{34}S$ values of CVOSCs, DMSP from *Spartina* plant tissues suggests that pore water sulfide sulfur (H_2S/HS^-) or dissolved species from AVS was taken up by plant roots for biosynthesis of DMSP through assimilatory sulfide processes (King et al., 1982; Fry et al., 1982; Fry and Trust, 1992), which undergo cleavage and methylation/ demethylation pathway reactions to form DMS, MT and other VOSCs product in salt marsh (Kiene and Visscher, 1987; Lomans et al., 2002; Schäfer et al., 2010). The small differences between $\delta^{34}S$ fractionations might result from the processes in which dissolved sulfide species are: (a) assimilated to form DMSP through the rhizomes of *Spartina* plant) and (b) incorporated to methyl groups from dead organic matter to form VOSCs in the wet sediment. These two processes have been investigated to be associated by small $\delta^{34}S$ values, and depends on the starting sulfur substrate involved the reaction

(Kaplan and Rittenberg, 1964; Goldhaber and Kaplan, 1974; Fry et al., 1986; 1988; Sinninghe Damsté et al 1988; Trust and Fry, 1992; Amrani and Aizenshtat, 2004). For example, Calhoun and Bates (1989) reported +19.8‰ $\delta^{34}S$ values of DMSP, only slightly depleted in ^{34}S relative to surrounding seawater sulfate of +21.0 %. Whether the sulfides taken up by the plant roots are incorporated in the reduced form to synthesize DMSP directly through sulfur amino acid pathways, or are first converted to a less toxic form (SO_4^{2-}) in the plant tissues via reoxidation by molecular oxygen still remains a question that needs be addressed. If the latter transformation is prevalent in *Spartina alterniflora* tissues, then the DMSP sulfur produced from the re-oxidized SO_4^{2-} would have the same $\delta^{34}S$ value as the original sulfide. Although relatively few samples were analyzed for *Spartina* DMSP, the average $\delta^{34}S$ value in DGM is consistent with the range of total *Spartina* sulfur isotope measurements ($\delta^{34}S$ = -2.4 ± 4.4) taken from the Great Sippewissett Marsh in Falmouth, Massachussetts (Peterson et al., 1985) and Port Marsh Salt marshes in North Carolina (Carlson and Forrest, 1982; Currin et al., 1995). Sulfide uptake by wet plants has been identified to serve as a potent phytoxin (Howarth and Teal, 1979; Koch and Mendelssohn, 1989; Koch et al., 1990). But studies in flood-tolerant salt marsh macrophyte such as *Spartina* have adaptations for minimizing their exposure to sediment sulfide accumulation in anoxic marine sediments (King et al., 1982). The overall ranges in minor isotope ratios ($\Delta^{33}S$ and $\Delta^{36}S$) are consistent with the suggesting that pore water sulfide and AVS are used for VOSCs, and DMSP formation in DGM

Organic matter sulfurization has also been identified as an important mechanism for the preservation of functionalized organic compounds during early diagenesis (Sinninghe Damsté and deLeeuw, 1990; Kohnen et al., 1991) and may be important for

explaining S-isotope data of humic sulfur (Aizenshtat and Amrani, 2004). Incorporation of sulfur to organic matter is thought to be isotopically similar to the inorganic sulfur species from which it is derived (Amrani and Aizenshtat, 2004). Bisulfide (HS^-) is the most abundant sulfur nucleophile in typical salt marsh systems. This species appears to play a crucial role in formation of VOSCs. The formation of humics may also be controlled by pathways involving polysulfide nucleophiles (Amrani et al., 2006). Because oxidation at the uppermost layer of the sediment where sulfide is maximum generates polysulfide and elemental sulfur through an equilibrium reaction (in reaction 2). Our isotopic results for various sulfur pools including elemental sulfur, AVS, porewater sulfide, and pyrite reveal some variations that are interpreted to reflect seasonal variations and variations induced by chemical sink reactions preventing interpretations of the formation pathways using isotopes.

$$HS^- + (n-1)/8S_8 \longrightarrow Sn^{2-} + H^+ \qquad (2)$$

4.0 Conclusions

Raney nickel's selectivity and its ability to quantitatively remove sulfur from organic compounds for isotopic measurements make it a valuable reagent for determining the sources of organic sulfur in many environmental systems. The methodology presented here enables the extraction of VOSCs in sediments. The complexity and the abundance of other volatile organic S-compounds in salt marsh sediments may be complicated by the presence of other VOSC species that are not reported in table 2.2 (eg.,carbonylsulfide (COS) and dimethyltrisulfide (DMTS)), but are present at concentrations too low (<nmolar) to be detected by GC-FPD. In principle, it not possible to avoid potential

conflicts between compounds using these methods, especially those with the same volatile-sulfur characteristics. These factors may contribute to the reproducibility for sulfur recovery and isotopic ratio measurements of VOSC from sediments and should be considered in future studies that undertake identification, quantification, and subsequent S-isotope analysis in complex natural systems.

It has been found that if sulfur in the original material is bound to oxygen atoms, or the reaction solution is saturated with oxygen, desulfurization is inhibited. This is because the quantitative recovery of hydrogen sulfide is dependent on the efficiency and stability of the Raney catalyst. The chemical conversion of organic volatile sulfur, their precursors, and oxidized species into SF_6 enables the measurement of all stable sulfur isotopes. The repeatability and precision of this method for S-isotopic analysis has been assessed with synthetic in-house standards as well as with standard reference materials. Application of the above method for measuring the isotopic composition of sulfonium compounds in natural systems may be crucial to understand the role of biogenic sulfur in the global sulfur cycle.

Marsh plants such as *Spartina*, which metabolize DMSP via their roots in suboxic and anoxic sediments, has been shown assimilate/incorporate sulfide into their membranes for different metabolic processes. Given that pore water sulfide is depleted in ^{34}S and ^{33}S, and enriched in ^{36}S, organic sulfur produced by *Spartina* (e.g., DMSP) and sediments (e.g., CVOSCs – DMS, MT, CS_2, and DMDS) is also depleted in ^{34}S and ^{33}S – and similarly enriched in ^{36}S. Although we have demonstrated that a significant amount of pore water sulfide enters *Spartina* plant roots, it is not certain whether such sulfide

uptake is a passive or active process. Therefore, additional work is needed to investigate the impact of the salt marsh grass metabolisms on pore water sulfide chemistry.

In this study we have presented data for $\delta^{34}S$ and $\Delta^{33}S$, and $\Delta^{36}S$ (Table 2.1), which provides three separate isotopic compositional parameters that can be used to make inferences about the processes leading to production of different VOSCs. In addition, the minor isotopes of sulfur (^{33}S and ^{36}S) are subject to inorganic and organic sulfur isotope fractionation mechanisms that complement the information provided by ^{34}S fractionations and can be used to study redistribution of sulfur within biogeochemical systems (at both the cellular and ecosystem level). The chemical methods can still be used when only $\delta^{34}S$ is measured, such as by IRMS using SO_2 or SO as the analyte and significant information can be obtained. Wet sediments of the marsh are highly enriched in VOSCs species (especially MT and DMS) that result from the methylation by interaction of putrefying lignin components of the *Spartina* plants with reduced sulfur species. Results of these investigations indicate that enrichment of organic compounds with sulfide nucleophiles may be a common phenomenon for organic sulfur production in coastal salt marsh sediments.

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Appendix – 2A. Sedimentary Solid Phase Extraction of Sulfur Species

A sequential extraction procedure was applied to wet sediments subsamples to isolate organic and inorganic sulfur species for measurement of four sulfur isotope compositions. A typical extraction experiment was conducted in the following way. A 20 g wet sediment samples were sliced from the wet core that was purged under N_2 atmosphere in a glove bag. The sliced core was first extracted with 100 mL cold hexane solution (-84^{o}C) for an hour in brown Niskin bottle using a laboratory shaker to isolate volatile organic sulfur compounds (VOSCs). To prevent volatilization of VOSC species, the temperature was kept -20^{o}C while extracting by placing the bottles in a 1:3 ratio NaCl/ice-water bath. After extraction, portions of hexane layer were analyzed for volatile organic sulfur species (such as DMS, MT, DMDS, CS_2, etc.) using Gas Chromatography – Flame Photometric Detector (GC-FPD). Residual sediment was treated with 50 mL

mixture of methanol and chloroform (1:1) to remove elemental sulfur after hexane extraction. Subsequently, the residue of the methanol and chloroform extract was treated with 0.1 $molL^{-1}$ NaOH to remove humic sulfur. NaOH extracts containing humic sulfur was treated with HCl to remove humic acid. We finally employed procedures very similar to those established by Cutter and Oatts, 1987 and Canfield et al., 1986 to sequentially extract AVS-Acid volatile sulfur and CRS-Chromium reducible sulfur from the remaining sediments for sulfur isotope analysis as SF_6 gas.

Appendix-2B. Validation of Raney Nickel Method

Precision and Accuracy of Isotopic Analyses of Organosulfur Compounds

The recovery of sulfide following reduction from organic sulfur standards was determined from samples sizes of 5-10 $\mu molL^{-1}$ sulfur based on both Cline's (1969) spectrophotometric procedure and the gravimetric yield of Ag_2S (Table 2A). Yields were greater than 95% which we regard as satisfactory, given the volatility and reactivity of the compounds as well as the technical difficulties involved in the reduction. When the same reduction protocols were tested on mixtures of known quantities of oxidized sulfonium compounds (such as DMSO, MSIA, $DMSO_2$, and MSA), yields were lower (sometimes zero), indicating the method is not suitable for these compounds. These low recoveries are suspected to result from the accumulation of conjugated oxygen bonds in the reaction mixture, which reduces the activity and selectivity of coordinated nickel making it chemically unreactive to reduced organic sulfur bonds.

Table 2A presents the results of multiple sulfur isotopic analyses ($\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$) of various organic sulfur compounds prepared with this desulfurization method.

The analytical protocols utilized in this study were validated using commercially prepared compounds and two compounds ($DMSPH^+$ and DMSAcet) prepared from the DMS standard. Reported values for the organic standards and our in-house synthetic standards were normalized to a working gas calibrated against IAEA-S1 (Ag_2S), which has a consensus value of -0.3 ‰ for $\delta^{34}S$ (Coplen and Krouse, 1997; Ding et al., 2001) on the V-CDT scale and we assume has values of 0.94 ‰ and -0.7 ‰ for $\Delta^{33}S$, and $\Delta^{36}S$, respectively from our measurements of IAEA-S1 and CDT. The isotopic composition of our in-house standards with their mean standard deviations presented in table 2A1 shows a slight depletion of ^{34}S (~1.61‰ for $DMSPH^+$ and 0.67‰ for DMSAcet) with relatively similar $\Delta^{33}S$, and $\Delta^{36}S$ values compared to the starting DMS composition. We interpret these differences to be consistent with the incomplete yields associated with synthesis of DMSP and DMSAcet (74% and 88%) respectively, and the calculated equilibrium isotope effects (free energy difference) associated with isotopic substitution in these compounds. The general agreement with synthesized compounds with the DMS used for synthesis is therefore taken as an indication that these Raney nickel techniques do not introduce significant biases in the measured isotopic compositions.

Appendix-2C- Reaction Mechanisms for Desulfurization

The use of Raney nickel as a desulfurization agent for homogeneous mixtures of organic sulfides, thiols, and their amino acid analogs offers clear advantages due to its ease of use and reactivity. The reaction permits a near-quantitative cleavage of sulfide adduct in the form of hydrogen sulfide gas. This has a number of potential applications in aquatic natural systems. One of which is a salt marsh ecosystem. Although the exact

mechanism is unclear, it has been suggested that Raney nickel desulfurization of organic sulfur probably proceeds through a free radical intermediate reaction (Cope and Engelhart, 1969) followed by adsorption of sulfur to metallic Ni surfaces.

$$\text{R-S-R} \xrightarrow{\text{Ni -///-\{H\}}} \text{R-S}^{\bullet} + \text{R-H} \qquad (3)$$

$$\text{R-S}^{\bullet} \xrightarrow{\text{Ni -///-\{H\}}} \text{R-H} + \text{Ni-S} \qquad (4)$$

Nickel-based catalysts have a strong affinity for electronegative atoms such as sulfur (Hauptmann and Wladislaw, 1950a; 1950b; Nagai et al., 1989; Rufael et al.1998). In solution, the π-electron density on a sulfur atom during desulfurization could explain its tendency to adsorb to nickel. Based on Hückel theory, Nagai and co-workers Nagai and co-workers (1988; 1989) postulated that the rate-determining step in this reaction is not breakage of C-S bonds, but rather adsorption of sulfur onto a Raney-nickel surface. Hydrogenolysis with hydrochloric acid weakens the Ni-S bond and releases hydrogen sulfide. Reaction (4) illustrates the overall desulfurization reaction:

$$\text{R-S-R}_{(aq)} + \text{Ni-Al}_{2(s)} \xrightarrow[\text{3.Conc. HCl, } \Delta\text{ -Heat}]{\text{1.ETOH} \quad \text{2. NaOH}} \text{H}_2\text{S}_{(g)} + \text{R-H} + \text{NiCl}_{2(aq)} + \text{H}_{2(g)} + \text{NaAlO}_{2(aq)} \qquad (5)$$

Organic sulfur compound	Abbr. symbol	Coordinated sulfur bonds	H_2S recovery (%)	Ag_2S recovery (%)	$\delta^{34}S$ (‰)	$\Delta^{33}S$ (‰)	$\Delta^{36}S$ (‰)	N
Common VOSCs								
Dimethyl sulfide	DMS	R-S-R	95.5 ± 1.5	93.5 ± 2.6	-7.79 ± 0.27	-0.003 ± 0.006	-0.60 ± 0.07	5
Methanethiol	MT	R-S-H	94.8 ± 0.3	93.0 ± 0.5	-4.59 ± 0.11	-0.010 ± 0.010	-0.30 ± 0.13	3
Carbon disulfide	CS_2	S = C = S	94.0 ± 0.2	92.4 ± 1.1	12.92 ± 0.15	0.031 ± 0.010	-0.23 ± 0.22	3
Dimethyl disulfide	DMDS	R-S-S-R	96.6 ± 0.8	94.9 ± 1.5	14.34 ± 0.36	0.031 ± 0.007	-0.17 ± 0.12	4
Some VOSCs precursors								
Dimethylsulfoniopropionate	DMSP.HCl	$(R)_2$-S^+-C	94.2 ± 0.2	93.1 ± 0.9	-6.18 ± 0.45	-0.009 ± 0.005	-0.24 ± 0.12	4
Dimethylsulfonioacetate	DMSAcet	$(R)_2$-S^+-C	95.4 ± 0.5	93.7 ± 0.8	-7.12 ± 0.41	-0.001 ± 0.034	-0.24 ± 0.23	3
Methionine	MET	-C-S-R	97.1 ± 0.9	94.0 ± 1.9	-6.22 ± 0.31	0.077 ± 0.052	-0.54 ± 0.00	3
Cysteine	CYS	-C-S-H	97.8 ± 1.1	95.8 ± 0.6	-3.53 ± 0.01	0.014 ± 0.020	-0.32 ± 0.07	3
Glutathionine	GSH	-C-S-H	96.3 ± 1.2	94.3 ± 1.5	-2.36 ± 0.47	0.014 ± 0.065	-0.12 ± 0.08	3
Oxidation products								
Dimethyl sulfoxide	DMSO	$(R)_2$-S = O	92.5 ± 2.9	90.2 ± 2.0	-6.90 ± 0.28	0.030 ± 0.007	-0.41 ± 0.19	3
Dimethyl sulfone	$DMSO_2$	$(R)_2$-S-$(O)_2$	0	0	ND	ND	ND	2
Methanesulfonic acid	MSA	R-S-$(O)_2$-OH	0	0	ND	ND	ND	2
Methanesulfinic acid	MSIA	R-S-O-OH	90.6 ± 2.7	89.4 ± 0.8	7.55 ± 0.33	0.010 ± 0.016	-0.11 ± 0.03	3
Int. reference Std.								
IAEA-S1	Ag_2S	Ag-S-Ag	ND	ND	22.16 ± 0.06	0.039 ± 0.004	-0.23 ± 0.07	6
IAEA-S2	Ag_2S	Ag-S-Ag	ND	ND	-0.36 ± 0.05	0.099 ± 0.008	-0.88 ± 0.06	7
IAEA-S3	Ag_2S	Ag-S-Ag	ND	ND	-32.62 ± 0.15	0.072 ± 0.012	-1.31 ± 0.13	5

Table 2A: Reported percentage yields and standard deviations for sulfide recoveries by methylene blue (Cline, 1969) and gravimetric analysis (in form Ag_2S) for the selected organic sulfur compounds. Multiple sulfur isotope results of VOSCs, their precursors, synthetic in-house standards, and standard reference materials are normalized to the V-CDT scale. Uncertainties in S- isotope measurements are derived from repeated analysis (N), and are consistent with the long-term reproducibility of 0.08‰, 0.14‰ and 0.2 ‰ (1σ) for $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$, respectively. ND-denotes sulfur species that was not reduced by Raney nickel.

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Chapter 3

Quadruple Sulfur Isotope Constraints on the Origin and Cycling of Volatile Organic Sulfur Compounds in a Stratified Sulfidic Lake*

Abstract

We have quantified the major forms of volatile organic sulfur compounds (VOSCs) distributed in the water column of stratified freshwater Fayetteville Green Lake (FGL), to evaluate the biogeochemical pathways involved in their production. The lake's anoxic deep waters contain high concentrations of sulfate (12 to 16 mmol L^{-1}) and sulfide (0.12 μmol L^{-1} to 1.5 mmol L^{-1}) with relatively low VOSC concentrations, ranging from 0.1 nmol L^{-1} to 2.8 μmol L^{-1}. Sulfur isotope measurements of combined volatile organic sulfur compounds (CVOSCs) demonstrate that VOSC species are formed primarily from reduced sulfur (H_2S/HS^-) and zero-valent sulfur (ZVS), with little input from sulfate. The data support a role for both biological and abiotic reaction routes that incorporate reactive sulfur species into methylated groups (e.g., CH_3-) from lignin components. These processes are responsible for very fast turnover of VOSC species, maintaining their low levels in FGL. No dimethylsufoniopropionate (DMSP) was detected by Electrospray Ionization Mass Spectrometry (ESI-MS) in the lake water column or in planktonic extracts. These observations indicate a pathway distinct from oceanic and coastal marine environments, where dimethylsulfide (DMS) and other VOSC species are principally produced via the breakdown of DMSP by plankton species.

Keywords: Volatile Organic Sulfur Compounds (VOSCs); Methylation; Sulfidation; Nucleophile; Methoxylated aromatic compounds; Monimolimnion; Mixolimnion; Chemocline; Zero-valent sulfur; Polysulfide.

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1.0 Introduction

The use of stable isotope studies to understand the biogeochemical cycling of sulfur in oceanic (Rees et al., 1970; Jørgensen et al., 2004; Böttcher et al., 2006), freshwater (Fry et al., 1995; Canfield et al., 2010; Zerkle et al., 2010), and terrestrial systems (Goldhaber and Kaplan, 1980; Habicht and Canfield, 2001) has focused mostly on the dynamics of inorganic sulfate, sulfide and their intermediate species. Few studies (e.g., Amrani et al., 2009; Oduro et al., 2011) have examined organic sulfur compounds, such as dimethylsulfide (DMS; CH_3SCH_3), methanethiol (MT; CH_3SH), dimethyldisulfide (DMDS; CH_3SSCH_3), carbon disulfide (CS_2), and carbonylsulfide (OCS). These compounds are highly reactive and are found at pico- to micromolar concentrations in oxic and anoxic natural waters (Radford Knoery and Cutter, 1993; Gun et al., 2000). Most studies of volatile organic sulfur compounds (VOSCs) in the past few decades have been focused on the marine environment due to its role in climate regulation in the atmosphere (Charlson et al., 1987; Calhoun et al., 1991; Andreae and Crutzen, 1997).

Three major biotic and abiotic processes have been suggested to be responsible for the production of VOSCs in aquatic natural environments:1) methylation of free sulfide ($H_2S_{(aq)}$, HS^-, and S^{2-}) (Kreft and Schink, 1993; Lomans et al., 2002), zero-valent sulfur (ZVS) and polysulfide (Gun et al., 2000); 2) degradation of sulfur containing amino acids (Kodata and Ishida, 1972; Kiene and Capone, 1988); and 3) enzymatic cleavage of β-dimethylsulfoniopropionate (DMSP; $(CH_3)_2S^+CH_2CH_2COO^-$) by marine algae (Ginzburg et al., 1998; Kiene, 2000; Yoch, 2002). The latter process is believed to be the dominant route for production of VOSCs (particularly DMS) in freshwater

(Ginzburg et al., 1998) and marine environments (Kiene and Taylor, 1989; Stefels, 2000; Simo et al., 2002).

In freshwater systems, a combination of the above processes may produce VOSCs, depending on water chemistry, density stratification, and the type of bacterioplankton community that is present. For instance, Gun et al. (2000) argued that nucleophilic polysulfides are the direct precursors for DMDS and probably other volatiles, such as OCS, in Lake Kinneret (Israel). In the same freshwater system, Ginzburg et al. (1998) found the DMS precursor, DMSP (up to 5.5 pg/cell) was produced by a freshwater dinoflagellate *Peridinium gatunense*. This organism dominates the phytoplankton population in Lake Kinneret. Yoch et al. (2001) also observed the production of DMS in freshwater sediment slurries upon addition of DMSP, and suggested that DMS-producing Gram-positive bacteria were present in non-marine environments.

Here we report the abundance of a number of VOSC species (including DMS, MT, DMDS, and CS_2) in the anoxic and sulfidic deep waters of density-stratified Fayetteville Green Lake (FGL). We have coupled a sulfur isotope approach, using variations in the relative abundances of the four stable sulfur isotopes, with concentration analysis to examine the mechanisms and pathways responsible for VOSC formation in this freshwater system. This contribution concentrates on the relative role of abiotic and biogenic pathways between organic and inorganic sulfur species in the lake water column using sulfur isotope measurements.

2.0 Geographic settings and geochemical parameters

Fayetteville Green Lake (FGL) is a stratified (meromictic) freshwater lake located near the town of Fayetteville, New York, USA (Fig. 3.1). The lake is permanently stratified, with oxic waters (the mixolimnion) occupying the uppermost ~20 m depth, a redox interface (chemocline) at around 20-21 m depth, and sulfidic anoxic waters (the monimolimnion) extending to the deepest part of the lake, at ~52 m depth.

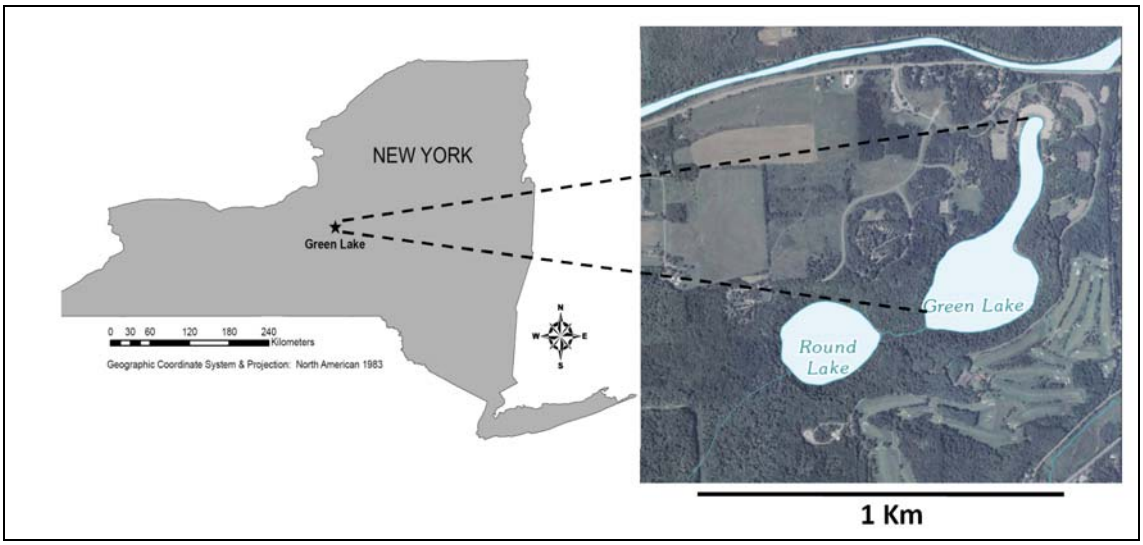

Figure 3.1: Geographic and aerial photo map of Green Lake showing a sister Round Lake in Fayetteville, New York. Aerial imagery extracted from manilius NY 2010 quadrangle of USGS topographic map.

The lake's small size (~0.26 km^2), bathymetry, and density profile stabilize lake stratification by inhibiting mixing and overturn. Stratification of FGL is maintained in large part by an inflow of calcium and sulfate-rich saline groundwater at ~18 m water depth (Brunskill and Ludlam, 1969; Hilfinger and Mullins 1997). Sulfate, which occurs at high concentrations throughout the water column, is the ultimate source of reduced inorganic and organic forms of sulfur in FGL (Takahashi et al., 1968; Brunskill and Harris, 1969; Thompson et al., 1997). One striking feature of the chemocline in this lake is the presence of a large population of phototrophic

Depth (m)	SO_4^{2-} (mmol L^{-1})	HS^-/H_2S (mmol L^{-1})	DO (%)	pH	Eh (mV)	Chl a (µg L^{-1})	TDS (NTU)
0-20 (Mixol.)	13.19 ± 1.6	0.0	91.59 ± 16.5	6.67 ± 0.4	92.34 ± 14.6	5.62 ± 2.6	-2.32 ± 0.8
20-23 (Chemo.)	15.84 ± 0.1	0.26 ± 0.2	3.30 ± 2.6	6.78 ± 0.1	-281.86 ± 30.8	50.96 ± 24.9	22.01 ± 3.1
23-50 (Monim.)	15.72 ± 0.3	1.06 ± 0.4	0.08 ± 0.1	6.73 ± 0.0	-360.22 ± 16.3	11.15 ± 1.5	1.27 ± 0.4

Table 3.1: Laboratory and field measurements of chemical parameters and their standard deviations (SD) arranged according to the lake's mixing regimes - Mixolimnion (Mixol.), Chemocline (Chemo.) and Monimolimnion (Monim.). Data compiled from the profiles in Zerkle

sulfur oxidizers at the chemocline. This bacterial community oxidizes and recycles reduced sulfur compounds produced via sulfate reduction in the water column and in the sediments (Fry, 1986; Zerkle et al., 2010).

A recent study (Zerkle et al., 2010) during the same sample campaign has reported the profiles of the lake's geochemical and physical parameters, summarized in table 3.1. The table contains the mean values and standard deviations for dissolved sulfate, sulfide, dissolved oxygen (DO) concentration, pH, redox potential (Eh), Chlorophyll a (*Chl a)* concentration, and turbidity (total dispersed solid – TDS) content. These parameters are arranged by depth according to the lake's mixing regimes. The lake's mixolimnion (0-20 m) has no observable sulfide and does not show any significant changes in DO, pH, and Eh measurements. The chemocline is characterized by relatively high sulfate (15.8 ± 0.1mmol L^{-1}) and sulfide (0.26 ± 0.2 mmol L^{-1}) concentrations with high TDS and a *Chl a* that peak at 22.01 ± 3.13 NTU and 50.96 µg L^{-1}, respectively. TDS in aquatic environments has been shown to correspond to *Chl a*, implying the involvement of bacterioplankton in TDS production in the lake (Luther and Tsamakis,

1989; Andreae, 1990; Walsh et al., 1994). These maximum values of TDS and *Chl a* are attributed to the abundance of anoxygenic phototrophic bacteria (presumably purple sulfur bacteria, given the purple color of the chemocline water).

Dissolved oxygen was absent from the monimolimnion of the lake, but was near saturation (91.59 ± 16.5%) in the mixolimnion. The significant increase in sulfide concentration between the chemocline (0.26 ± 0.2 mM) and the monimolimnion (1.04 ± 0.4 mM) reflects microbially-mediated dissimilatory sulfate reduction (Fry et al., 1986; Zerkle et al 2010). Sulfide formation in this zone is consistent with the lake's redox potential (Eh = -281.9 to -360.2 mV) measured in table 3.1, and the values fall within the range of experimentally determined Eh measurements (-95 to -450 mV) where sulfate is used as an electron acceptor to produce sulfide at circumneutral pH conditions (Knaff and Buchanan, 1975; Zinder and Brock, 1978).

In general, the FGL exhibits the following characteristics: (i) a well-mixed, oxygenated, low-salinity, upper water mass (mixolimnion), (ii) an intermediate water mass (chemocline) where salinity increases and dissolved oxygen usually decreases rapidly with depth, and (iii) a lower anoxic water mass (monimolimnion) which has an approximately constant temperature and a higher salinity than the mixolimnion.

3.0 Materials and Method

3.1. Sample Collection and Fixation

Water samples for concentration and isotopic measurements were taken from approximately the middle of the lake (N 43.0395 - W 75.9663) between 3-5 m horizontal intervals, using a General Masterflex® E/S™ portable sampler together with a horizontal

water sampler. Samples were obtained during the Fall of 2008 (October – November) and the Spring of 2009 (April–May). Samples were preserved on site by sorption, precipitation, and freezing before being transported back to the lab for chemical analysis. Sulfur fractions were obtained for two groups of samples: (i) volatile organic sulfur compounds (VOSCs) fraction; and (ii) water and cellular planktonic sample collected by filtration for DMSP analysis. This sampling was completed in parallel with analyses of inorganic sulfur species (sulfide, sulfate, and ZVS), as reported in Zerkle et al. (2010).

(i) Water samples for VOSCs concentration measurements were pumped through Tygon tubing directly into a 1.0 liter clean, acid washed Erlenmeyer flask. To avoid contact with atmosphere or contamination due to headspace, samples were allowed to overflow for 15 seconds before purging on-board for 5 minutes with Ultra High Purity Nitrogen (UHP-N_2). The volatile sulfur gases were flowed through a mixture of $FeCl_3$/$CaCl_2$ to remove H_2S and naffion tubing to remove water. The final VOSC products were trapped by cryoadsorption (using liquid nitrogen) onto a 30-50 mesh Tenax GC polymer packed in a cold-finger, which was kept cold and saved for later concentration analyses by Gas Chromatography (GC).

Approximately 18-24 liters of water was processed at each depth for combined-VOSC (CVOSCs) isotope analysis by concentrating and extracting CVOSCs with 600 mL of cold n-hexane (-84ºC), washed with 40% diethanolamine to remove trace sulfide and polysulfane sulfur (Jou and Mather, 2000; Sidi-Boumedine et al., 2004). The hexane layer was re-extracted with 100 mL of 5% $HgCl_2$ to precipitate VOSC species as mercury complexes (e.g., $HgMT_2$, 3DMS-2Hg, 3DMDS-2Hg)

(Wagner et al.,1967 Nguyen et al., 1978; Yang et al., 1996; 2006; Oduro et al., 2011). Precipitated CVOSC products were stored at 4^{o}C in a dark-brown Niskin bottle until analysis.

(ii) Planktonic samples were collected by successively filtering lake water using vacuum filtration with 0.45 μm disposable polyamide membranes. To control the process of lysing algal cells that would degrade any DMSP present (Kiene and Slezak, 2006), a lower flow rate (15mL/min) was employed during the filtration process. Filters coated with cellular planktonic materials were stored in the dark at -80^{o}C until analysis.

3.2. Analytical Techniques

3.2.1. VOSCs Concentration Analyses

Concentrations of VOSCs trapped on Tenax polymer were measured using a GC equipped with a Pulsed Flame Photometric Detector (PFPD - model 5380 from O.I. Analytical). Prior to chromatographic separation, a sample concentrator device (model O.I. Analytical 4560) was used to preconcentrate VOSCs via a chemical trap at 190^{o}C. A flow of helium carrier gas was used to elute VOSC species from the chemical trap onto a GAS-PRO capillary PLOT column (32 mm x 30 m, J/W Scientific Inc.). Separation of VOSCs was achieved with a He flow rate of 1.5 ml min^{-1} and GC oven temperature initially held at 60^{o}C for 2 min, followed by temperature increase to 260^{o}C with 20^{o}C min^{-1} gradients. All VOSCs were detected with a PFPD with an operating temperature set at 350^{o}C. Retention times were 4.2, 6.3, 9.8 and 11.7 min for MT, CS_2, DMS, and

DMDS, respectively. The relative precision, based on four consecutive (n=4) replicate standard measurements of 20 nM VOSCs, was 11%, 10%, 4%, and 8% (RSD) for MT, CS_2, DMS, and DMDS, respectively. At described conditions, the linear calibration range was 1 - 1000 nM of VOSCs.

3.2.2. Sample Preparation for Sulfur Isotope Analyses

The stored VOSC-Hg complex samples were decomposed by addition of 6 N HCl solution in an ice-water bath followed by addition of 50 mL cold n-hexane (-84 ^{o}C) to re-dissolve the VOSC species in the hexane layer by extraction by subsequent. Fractions of the hexane-containing CVOSCs were reduced to Ag_2S by a modified Raney nickel hydrodesulfurization method described by Oduro et al. (2011). Precipitated ZnS and $BaSO_4$ were reduced to H_2S by boiling in 25 mL of 5 N HCl and Thode reducible solution (consisting of a mixture 320 mL HI, 524 mL HCl, and 156 mL of H_2PO_3), respectively. ZVS was extracted with chloroform, concentrated by rotary evaporation under vacuum, purified by HPLC (as in Kamyshny et al., 2009; Zerkle et al., 2010) and reduced to H_2S according to methods described by Gröger et al. (2010) and Oduro et al. (2011). In all distillation-reduction reactions, evolved H_2S was captured by an $AgNO_3/HNO_3$ buffer solution to convert into Ag_2S for S-isotope analyses as SF_6 gas.

Multiple sulfur isotope measurements were performed using a Finnigan MAT 253 - Dual Inlet Isotope Ratio Mass Spectrometer (DI-IRMS). Milligram samples of Ag_2S were reacted in Ni bombs with ten-fold excess fluorine gas at 320^{o}C for approximately 8-12 hours. Product SF_6 was cryogenically separated from F_2 (at -196^{o}C) and then distilled from HF and other trace contaminants (at -115^{o}C). Final purification of SF_6 by GC-TCD

was performed on a composite column comprised of a 1/8 in. diameter, 6 ft. long packed column containing type 5A molecular sieve, followed by another 1/8 in. diameter, 12 ft. long Hayesp-Q^{TM} column. Sulfur hexafluoride eluted between 12 and 18 minutes at a He flow rate of 20 mL min^{-1} and a 50^{o}C column temperature. Sulfur hexafluoride eluting from the column was captured in a spiral glass trap frozen in liquid nitrogen. The sulfur isotope composition of purified SF_6 was measured in dual inlet mode of the gas-source isotope ratio mass spectrometer with four collectors arranged to measure the intensity of SF_5^+ ion beams at m/z values of 127, 128, 129, and 131 ($^{32}SF_5^+$, $^{33}SF_5^+$, $^{34}SF_5^+$, and $^{36}SF_5^+$). We report sulfur isotope ratios using the delta (δ) notation, reflecting the permil (‰) deviation of the sample composition from that of an international reference standard, in the Vienna Canyon Diablo Troilite (V-CDT):

In equation (1), x = 33, 34 or 36, and 'samp' and 'ref' represent the measured sample and reference standard, respectively. The less abundant isotopes (^{33}S and ^{36}S) are reported using capital delta notation (Δ), which are also given in units of permil (‰):

$$\delta^{x}S = [(^{x}S/^{32}S)_{samp}/(^{x}S/^{32}S)_{ref} - 1] \quad (1)$$

$$\delta^{x}S = [(^{x}S/^{32}S)_{samp}/(^{x}S/^{32}S)_{ref} - [(^{34}S/^{32}S)_{samp}/(^{34}S/^{32}S)_{ref}]^{\lambda} - 1]$$

$$^{33}\lambda=0.515; \; ^{33}\lambda=1.90 \quad (2)$$

Equation (2), $\Delta^{33}S$ (or $\Delta^{36}S$) describes the difference between the measured $^{33}S/^{32}S$ (or $^{36}S/^{32}S$) abundance of a given sample and that of the point with the same $\delta^{34}S$ on a reference fractionation line (RFL). The RFL approximates single step, low-temperature equilibrium mass isotope exchange fractionation (Hulston and Thode, 1965; Farquhar et al., 2007).

3.2.3. Characterization and identification of β-DMSP

Residues of cellular planktonic materials sampled from the chemocline and anoxic zones were extracted with a (12:5:3 v/v) mixture of MeOH:$CHCl_3$:H_2O within 24 hours of collection, and measured in both positive and negative ion modes for DMSP using an AccuTOF (JEOL USA, Inc., Peabody, MA) time-of-flight mass spectrometer (TOF-MS). The mass spectrometer uses an electrospray ionization source (ESI) and has a mass resolving power (Δm/m) of 6000 Full Width at Half Maximum (fwhm). The spray voltage was set to 2.3 kV, and the capillary and orifice temperatures were maintained at 250 °C and 80 °C, respectively. The instrument was typically operated at the following potentials: orifice 1 = 30 V, orifice 2 = 5 V, ring lens = 10 V. The RF ion guide voltage was generally set to 1000 V to allow detection of ions greater than m/z =100.

4.0 Results

4.1. VOSCs Concentration in Green Lake

The concentrations of MT, DMS, DMDS, and CS_2 in FGL waters are shown in Fig. 3.2 along with the depth profile of water at the sample site. Four types of VOSC species (DMS, MT, DMDS, and CS_2) dominate the chemocline and monimolimnion section of the lake where reactive sulfide species exist. Profiles from individual VOSC species display no significant differences between the two sampling seasons, implying seasonally invariant rates of formation, degradation, and cycling of VOSCs in anoxic waters of the lake.

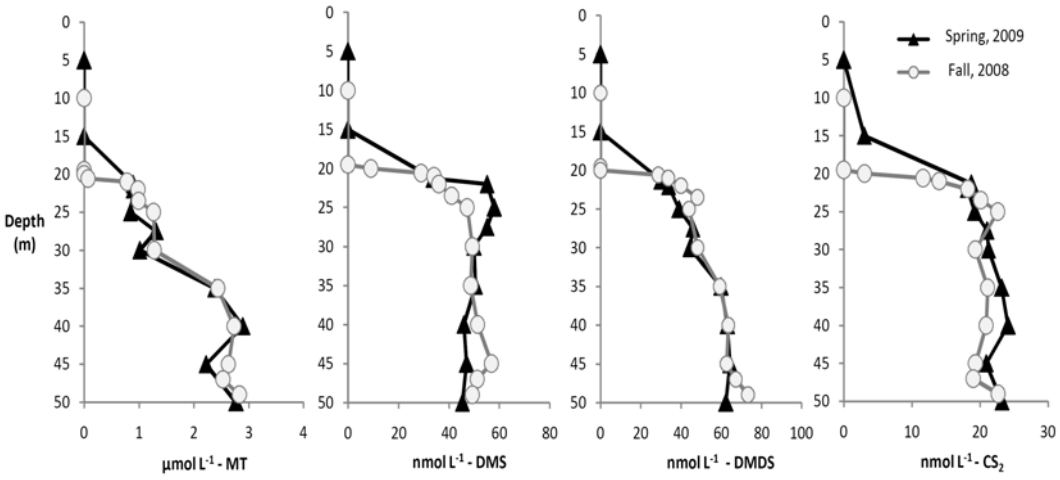

Figure 3.2: Concentration profiles of volatile organic sulfur compounds (VOSCs) measured with GC-PFPD. Dark triangles and open circles represents measurements made in Spring, 2009 and all, 2008, respectively.

Concentrations of DMS, DMDS, and CS_2 (ranging from ~ 3 to 80 nmol L^{-1}) were mostly detected at the chemocline and increased steadily to the bottom of the lake, with the exception of CS_2, which showed a slight decrease in concentration from the lower 40 m to the sediment interface. Also, CS_2 and DMDS were detected in the mixolimnion during the spring sampling along with traces of OCS that were occasionally detected in the oxic (between ~ 2.4 – 6.1 nmol L^{-1}) and anoxic (between ~ 3.2 – 5.8 nmol L^{-1}) portions of the water column. Although we detected OCS concentrations in the lake, its profile was not shown in fig. 3.2 due to chromatographic interference with H_2S that may underestimate our measurements (Radford-Knoery and Cutter, 1993). Based on the concentrations, we estimate a minimum concentration of ~ 2.8 nmol L^{-1} to exist in the lake oxic and anoxic layers.

Depth (m)	MT ($\mu molL^{-1}$)	DMS ($nmolL^{-1}$)	Spring,2009 DMDS ($nmolL^{-1}$)	CS_2 ($nmolL^{-1}$)	Depth (m)	HPLC MT ($\mu molL^{-1}$)
5	0	0	0	0	10	0
15	0	0	0	2.98	19.5	0
21.3	0.89	33.81	30.12	18.69	20	0
22	0.53	55.11	33.71	18.17	20.6	0
25	0.84	57.98	39.01	19.23	21	0
27.5	1.31	55.09	45.86	21.01	22	0.56
30	1.01	49.98	44.33	21.23	23.5	0.77
35	2.38	50.52	59.93	23.17	30	1.25
40	2.89	45.91	63.02	24.11	45	2.93
45	2.22	47.01	64.35	20.91	47	2.58
50	2.77	45.31	62.34	23.21	49	2.76

Depth (m)	MT ($\mu molL^{-1}$)	DMS ($nmolL^{-1}$)	Fall, 2008 DMDS ($nmolL^{-1}$)	CS_2 ($nmolL^{-1}$)	Depth (m)	HPLC MT ($\mu molL^{-1}$)
10	0	0	0	0	10	N/D
19.5	0	0	0	0	19.5	N/D
20	0	9.10	0	3.03	20	N/D
20.6	0.07	28.97	28.68	11.63	20.6	N/D
21	0.78	34.11	33.69	14.01	21	N/D
22	0.98	36.02	40.01	18.22	22	N/D
23.5	0.10	41.10	48.17	20.11	23.5	N/D
25	1.26	47.19	43.97	22.61	25	N/D
30	1.28	49.31	48.32	19.32	30	N/D
35	2.44	48.60	59.19	21.11	35	N/D
40	2.73	51.38	63.47	20.89	40	N/D
45	2.64	56.89	62.67	19.33	45	N/D
47	2.53	51.24	67.22	19.01	47	N/D
49	2.83	49.32	73.31	22.66	49	N/D

Table 3.2: Methanethiol (MT), Dimethylsulfide (DMS), Dimethyldisulfide (DMDS), and Carbon disulfide (CS_2) concentration data measured by GC-PFPD and HPLC derivatization by monobromobimane. N/D – denotes no data taken for this sample season.

The MT concentration profile in the lake was first detected at the chemocline at a concentration of 0.18 μmol L^{-1}, and increased sharply to 2.8 μmol L^{-1} throughout the monimolimnion. The MT concentration was higher than that measured in the Canadian Shield lakes (Richards and Kelly, 1991) and meromictic Alpine Lake of Cadagno in Switzerland (Fritz and Bachofen, 2000). Further analysis of MT concentrations by the HPLC derivatization method using monobromobimane showed an increase in concentration, from 0.56 μmol L^{-1} in the redox transition zone to 2.93 μmol L^{-1} at the sediment interface (See table 3.2). This unusual concentration of MT relative to that seen in other lakes might be attributed to the high sulfide concentration built up in the anoxic section of the lake, since sulfide and MT serve as competing substrates for methyltransferases that are responsible for the demethoxylation processes. The MT concentrations detected by HPLC analysis is consistent with GC analysis only at higher concentrations.

4.2. S-isotopes

The sulfur isotope compositions ($\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$) for CVOSCs, sulfate, sulfide, and ZVS are represented in the depth profile plotted in fig. 3.3. Correlation of the $\delta^{34}S$ values of CVOSCs, sulfide, and sulfate were similar for the two sampling seasons. The $\delta^{34}S$ of sulfate showed a slight increase in ^{34}S composition with depth starting at 25 m. The $\delta^{34}S$ value for ZVS extracted from the chemocline was higher by ~8 ‰ in the spring compared to the fall season. Changes in these profiles reflect the way that sulfur is transformed between sulfate, sulfide, and CVOSCs (Zerkle et al., 2010; and this study).

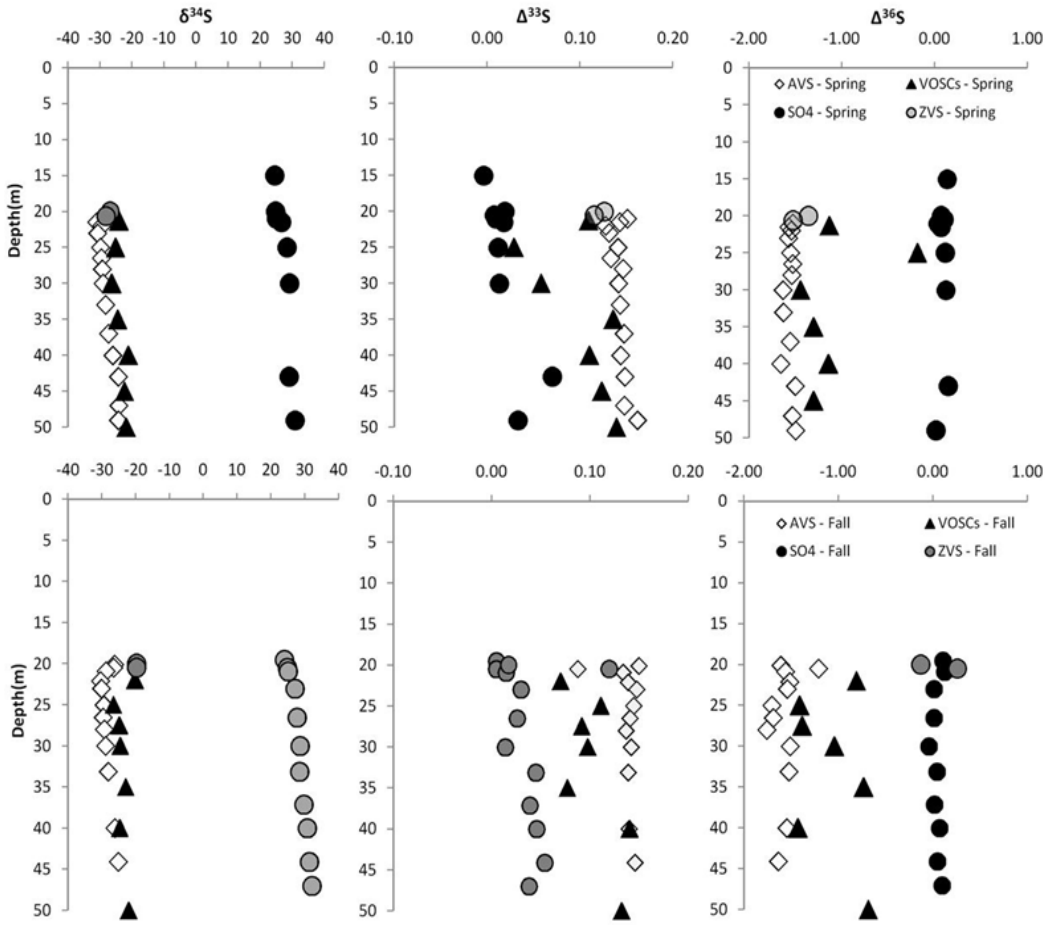

Figure 3.3: Depth profiles for $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$ isotope composition of different sulfur species (Sulfate - SO_4^{2-}, Acid Volatile Sulfur - AVS, Combined Volatile Organic Sulfur Compounds - CVOSCs, and Zero-Valent Sulfur – (ZVS) in Fayetteville Green Lake, (FGL) determined for Spring, and Fall, 2008**.** Data for SO_4^{2-}, AVS, and ZVS taken from Zerkle et al. (2010) from the same field expedition.

The average isotopic differences between sulfur fractions observed during early Spring 2009 and Fall 2008 seasonal sampling periods were: sulfate and sulfide ($\Delta^{34}S_{(SO_4^{2-} - H_2S)}$ = +53 to +56 ‰); CVOSC-sulfur and sulfide ($\Delta^{34}S_{(CVOSCs - AVS)}$ = +4 to +5 ‰); and CVOSC-sulfur and ZVS ($\Delta^{34}S_{(CVOSCs - ZVS)}$ = +0 to +3 ‰). The large isotopic fractionation between sulfate and sulfide is consistent with previous studies made by Deevey (1963) and Fry (1986). Sulfides are typically depleted in ^{34}S relative to sulfate, due to biogeochemical sulfur cycling via bacterial sulfate reduction, sulfide oxidation,

and disproportionation of sulfur intermediates (Deevey 1963; Fry, 1986; Zerkle et al., 2010). The sulfur isotope compositions of CVOSCs in table 3.3 (inferred to be mostly methanethiol – based upon concentration) are closer to the compositions of sulfide and ZVS than to that of sulfate. Values of $\Delta^{33}S$ and $\Delta^{36}S$ for the three sulfur species increase linearly (with some scatter) downward in the water column, with a maximum $\Delta^{33}S = 0.16$ ‰ and minimum $\Delta^{36}S = -1.64$ ‰. The depth profiles for $\Delta^{33}S$ and $\Delta^{36}S$ for the measured sulfur species shown in fig. 3.3 consistent with $\delta^{34}S$ isotope values and the trends compare to multi-sulfur isotope studies in meromictic alpine lake (Canfield et al., 2010).

Spring - April, 2009				**Fall - October, 2008**			
Depths (m)	**$\delta^{34}S$(‰)**	**$\Delta^{33}S$ (‰)**	**$\Delta^{36}S$ (‰)**	**Depths (m)**	**$\delta^{34}S$ (‰)**	**$\Delta^{33}S$ (‰)**	**$\Delta^{36}S$ (‰)**
21.3	-24.09	0.109	-1.133	22	-20.10	0.070	-0.811
25	-25.20	0.029	-0.184	25	-26.59	0.111	-1.411
30	-26.31	0.058	-1.441	28	-24.72	0.092	-1.384
35	-24.53	0.136	-1.302	30	-24.56	0.098	-1.041
40	-21.16	0.110	-1.141	35	-22.91	0.077	-0.732
45	-22.41	0.124	-1.300	40	-24.71	0.141	-1.428
50	-21.80	0.139	1.405	50	-21.98	0.132	-0.685

Table 3.3: Sulfur isotope values of combined volatile sulfur compounds (CVOSCs), measured from the FGL water column. All the data are normalized to VCDT and plotted in Figure 3. Analytical uncertainties of sulfur isotope measurements, estimated from long-term reproducibility of Ag_2S fluorinations, are 0.02, 0.008, and 0.20 (1σ) for $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$, respectively.

4.3. ESI-MS Analyses

Characteristic fragmentation patterns of intracellular DMSP and their sodium adducts ($DMSP\text{-}Na^+$) have been detected using ESI-MS in positive ion mode at m/z=135 and 158, respectively (Oduro et al., 2010). Such structural characterization in the water

samples and planktonic extracts measured in positive ion mode did not indicate the presence of DMSP in FGL bacterioplankton species. Further analysis performed in negative ion mode (Gun et al., 2004) of fresh planktonic extracts from the chemocline buffered with ammonium acetate at pH=9.0 shows the characteristic spectra of $HS_2O_3^-$ (m/z =113), deprotonated polysulfide, S_5^{2-} product (m/z = 160), and NaS_7^- (m/z= 247) (Fig. 3.4). The deprotonated form of pentasulfide (S_5^{2-}) is stable at environmental conditions as pK_{a2} of H_2S_5 is 5.7 (Schwarzenbach and Fischer, 1960).

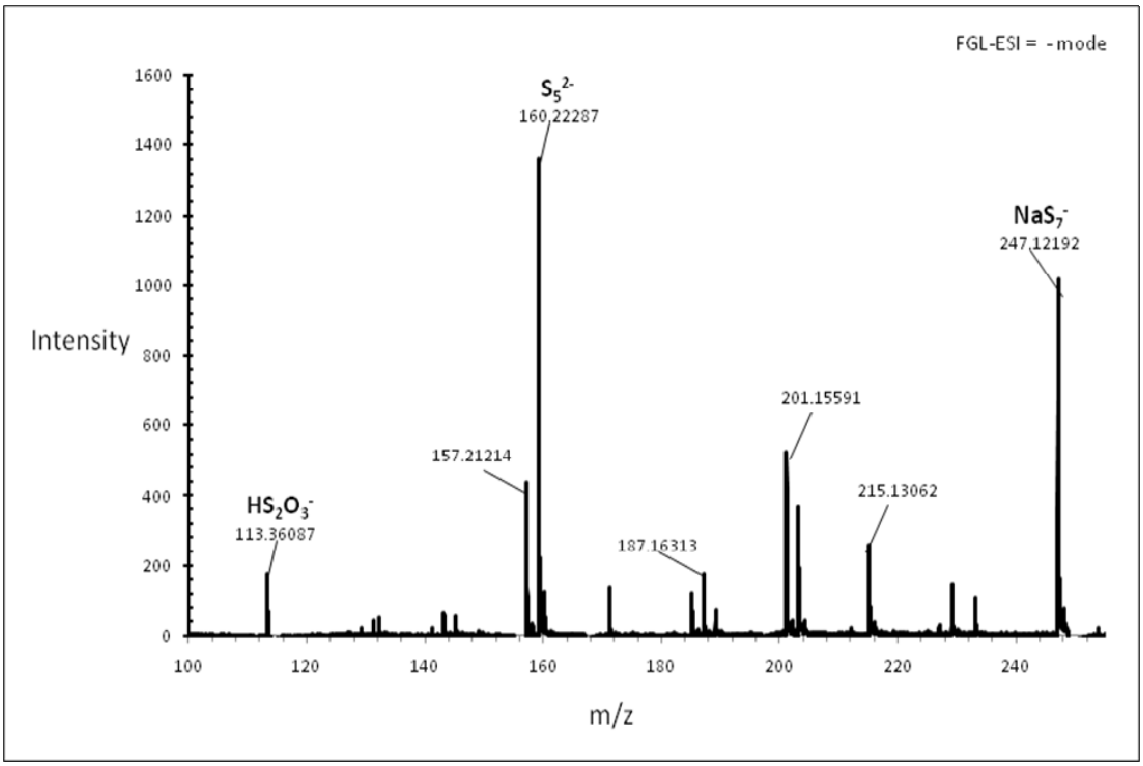

Figure 3.4: ESI-MS (-) mode spectra of polysulfidic species (S_5^{2-} and S_7^{2-}) and their disproportionation product ($HS_2O_3^-$) identified in the extracts of bacterioplankton cells in FGL. No observable DMSP and its sodium adducts, DMSP-Na^+ were detected at m/z = 135, and 158, respectively in (+) mode.

5.0 Discussion

5.1 Implications of VOSCs cycling in stratified sulfidic lakes

The results of sulfur isotope composition and concentration measurements of VOSC species presented herein suggest that the formation of MT, DMS, DMDS, and CS_2 in anoxic regimes of FGL occur by one or more abiotic and biotic processes, as discussed in more detail below.

5.1.1. Incorporation of reactive sulfur species to demethylated groups

Similarities in the $\delta^{34}S$, $\Delta^{33}S$ and $\Delta^{36}S$ of CVOSCs, AVS, and ZVS suggest that VOSC levels in FGL are linked to the chemistry and processes involving sulfide and ZVS formational pathways. This interpretation is consistent with the absence of DMSP in the lake's oxic and anoxic water columns. The isotope profiles in Fig. 3.3 show that $^{34}S/^{32}S$ compositions between CVOSCs, H_2S/HS^- and ZVS vary from +4 to +5 ‰ and 0 to +3 ‰, respectively, for the two sampling seasons. The values are consistent with the range of fractionations produced in experimental work with sulfide and polysulfide (Amrani et al., 2006), and with pathways proposed for incorporation of reactive sulfur nucleophiles into organic compounds (Amrani et al., 2004; 2006). We envision that similar reactions (R_1) and (R_2) may likely occur in FGL sulfidic waters to form MT and subsequently DMS, if anaerobic decomposition of organic matter and lignin components generates syringic acid and other methoxylated compounds in the water column.

$$[C_6H_2OH(OCH_3)_2COO^-] + HS^- + H_2O \longrightarrow [C_6H_2(OH)_2(OCH_3)COO^-] + CH_3SH + OH^-$$
$$\longrightarrow 3CH_3COO^- + CH_3SCH_3 + HCO_3^- + 2H^+ \qquad (R_1)$$

$$[C_6H_2OH(OCH_3)_2COO^-] + S_n^{2-} + H^+ \longrightarrow [C_6H_2(OH)_2(OCH_3)COO^-] + CH_3S_n^- + H^+$$
$$\longrightarrow CH_3SCH_3 + S_8 + [C_6H_2(OH)_3COO^-] \qquad (R_2)$$

Methylated groups from aromatic methoxylated compounds can also be metabolized by carbonylation reaction to acetate via the acetyl-CoA pathway, which involves CO dehydrogenase (in reaction R_3) (Kreft and Schink, 1993). Reactions (R_1) and (R_3) have been shown by Brune and Schink (1992) to degrade to acetate through the phloroglucinol pathway.

$$[C_6H_2OH(OCH_3)_2COO^-] + 4H_2O \longrightarrow 4.5CH_3COO^- + 3.5H^+ \qquad (R_3)$$

Common sources of methylated groups that combine with reactive sulfur nucleophiles to form MT or DMS according to these reactions include methoxylated aromatic compounds from decaying lignin, such as gallic acid trimethyl ester (3,4,5-trimethoxybenzoate), and syringic acid esters (4-hydroxy-3,5-dimethobenzoate) (Bak et al., 1992; Lomans et al., 2001; 2002; Higgins et al., 2006; Lin et al., 2010). Some of these lignin-containing methoxylated compounds may result from deforestation activity in the areas around the lake. Also, the breakdown of fallen trees and other woody debris in FGL may have perhaps stimulated higher input of organic materials in the form of lignin biopolymers, which undergo microbial degradation by homoacetogenic bacteria in the lake. Previous studies by Bak and co-workers (1992) proposed that methylation proceeds in a stepwise fashion according to reaction (R_1) with MT as an intermediate compound. Incubation experiments with freshwater sediment slurries amended with DMDS provide evidence that MT and DMS can be formed from DMDS under anoxic conditions (Kiene et al., 1986; Kiene and Capone, 1988). This implies that biological processes in anoxic sediments may be responsible for VOSC formation, an observation that may explain the

increase in concentration of MT at the bottom of the lake sediment interface (Lomans et al., 1997; 1999).

5.1.2. Oxidation and thiolation of methylated sulfur compounds

Redox sensitive species such as O_2, or Fe^{3+} in the water column can drive the oxidation of MT to produce DMDS in the chemocline and oxic layer, as shown in R_4. This abiotic formation of methylated sulfur species has been demonstrated by Higgins et al. (2006) to proceed via two methanethiol molecules.

$$CH_3SH + CH_3SH + ½O_2 \longrightarrow CH_3S\text{-}SCH_3 + H_2O \qquad (R_4)$$

Alternatively, stronger nucleophiles (e.g., methylated thiolates (RS^-)) that might exist in anoxic sediment interface of FGL may potentially form DMDS and H_2S, shown in R_5, if sulfur is present in limited concentrations (Jocelyn et al., 1972).

$$2CH_3S^- + H^+ + S^0 \rightleftharpoons CH_3S\text{-}SCH_3 + HS^- \qquad (R_5)$$

5.1.3. Degradation of sulfur-containing amino acids

The breakdown of organic sulfur compounds by various bacterial species has been demonstrated by a number of studies in anaerobic environments (Kodata and Ishida 1972; Bak et al., 1992; Lomans et al., 2002). However, in anaerobic sulfidic aqueous systems like FGL, simultaneous mechanisms for VOSC production and subsequent biotransformation in both sediment and water columns are anticipated to occur at different rates. A typical example of organic sulfur biotransformation to VOSCS species is the degradation of protein-derived amino acids (such as methionine;

$HOOCCH(NH)CH_2CH_2\text{-}SCH_3$ and cysteine; $HOOCCH(NH_2)CH_2\text{-}SH$), which is another likely source of MT/DMS, and H_2S respectively in FGL, as written in R_6.

$$HOOCCH(NH_2)\text{-}CH_2CH_2\text{-}SCH_3 \xrightarrow[\text{Deamination (CO)}]{\text{Oxidative}} NH_3 + HOOC\text{-}CO\text{-}CH_2CH_2\text{-}SCH_3$$
$$\xrightarrow{\text{Demethiolation } (H_2)} HOOC\text{-}CO\text{-}CH_2CH_3 + CH_3SH \qquad (R_6)$$

Cysteine and methionine amino acids are often derived from proteins in anaerobic sediments (Mayer et al., 1986; Morgan et al., 1991; Lawrence et al., 1995). Processes of anaerobic degradation of sulfur-containing amino acids are catalyzed by S-alkylcysteinase and L-methionine-γ-lyase enzymes, respectively, and result in the formation of MT, pyruvate, and ammonia (Hayward et al., 1977; Kiene and Capone, 1988). These processes have been suggested to occur by sequential breakdown of proteins to form peptides and subsequent degradation of peptides to form cysteine and methionine, which are further degraded to predominantly MT, with lower levels of DMS (Kiene and Capone, 1988; Higgins et al., 2006).

5.1.4. Possible sources of non-methylated VOSCs production in FGL

A number of studies have demonstrated the formation of CS_2 and OCS in both oxic and anoxic freshwater systems (Henatsch and Juttner, 1990; Richards et al. 1994; Roberts and Burton, 1994; Fritz and Bachofen, 2000). Their presence in FGL waters suggests that both biological and abiotic processes are responsible for their formation. CS_2 and OCS are known to have a number of biogenic sources, including the degradation of cysteine, thiocyanates and other sulfur-containing compounds in aquatic and marine environments (Bremner and Steele, 1978; Conrad, 1996). Oxidative growth of OCS and CS_2 has been obtained with *Thiobacillus thioparus* cultures (Smith and Kelly, 1988;

Hartikainen et al., 2000). Another potential metabolite for OCS was identified as carbonic anhydrase of cyanobacteria, which is able to react with OCS as a structural analog of CO_2, catalyzing the hydrolysis of OCS to CO_2 and H_2S (Miller et al., 1989; Badger and Price, 1990). Carbonic anhydrase may be widespread among several autotrophic microorganisms in FGL, and could be potentially responsible for OCS consumption to facilitate the equilibration between CO_2 and bicarbonate in many freshwater systems. Although significant work on the microbial breakdown and metabolism of CS_2 and OCS has been reported (Prontoschill-Krebs et al., 1995; 1996), a systematic approach for their formation and cycling in natural systems remains unclear.

On the basis of nanomolar concentrations of CS_2 and OCS observed in the FGL, we propose that the biotransformation and cycling of CS_2 in freshwater systems proceeds through DMDS oxidation according to R_7, which sequentially undergoes a hydrolytic cleavage to produce traces of OCS and H_2S (reaction R_8).

$$2CH_3S\text{-}SCH_3 + 2O_2 \longrightarrow CS_2 + 2CH_3SH + 2H_2O + CO_2 \qquad (R_7)$$

$$CS_2 + H_2O \longrightarrow OCS + H_2S \qquad (R_8)$$

$$OCS + H_2O \longrightarrow H_2S + CO_2 \qquad (R_9)$$

Further hydrolysis of dissolved OCS (reaction R_9) may yield H_2S and CO_2 as an energy source that is utilized by autotrophic bacteria in the form of carbon. From the above discussions, we propose the following scheme (Fig. 3.5) for the formation and cycling of VOSC species in FGL. The scheme shows the various biotic and abiotic pathway processes for methylated and non-methylated VOSCs production in sulfidic freshwater environments if appropriate methylated groups and sulfur amino acids are present

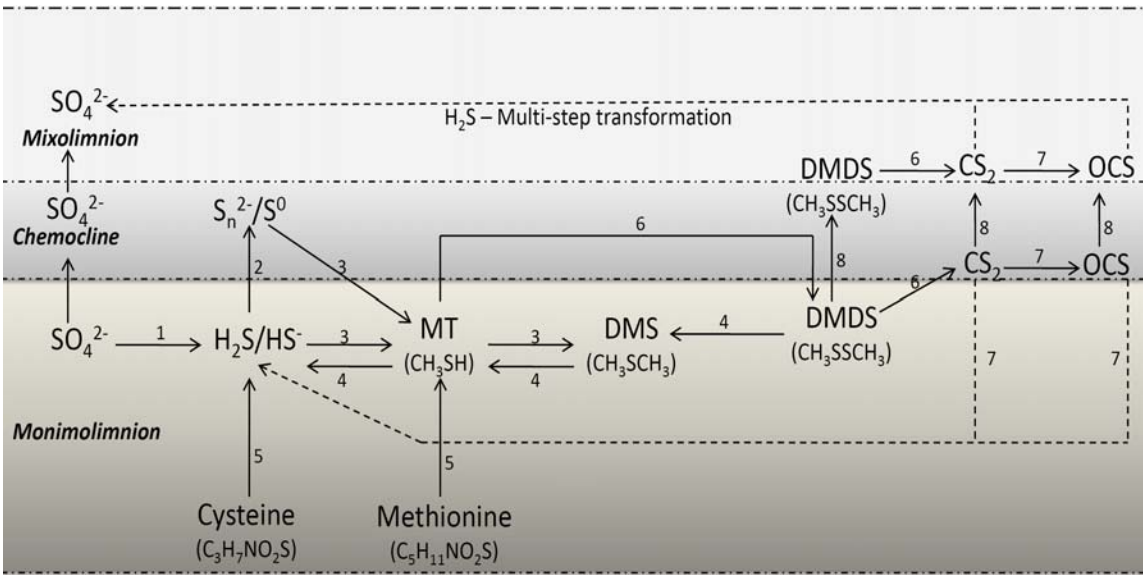

Figure 3.5: Proposed reaction scheme for VOSCs production and cycling in FGL. The various processes that lead to their production and cycling in the oxic and anoxic water column are: 1) Bacteria sulfate reduction (BSR); 2) Sulfide oxidation/reduction; 3) Methylation; 4) Demethylation; 5) Enzymatic biodegradation; 6)Volatilization and abiotic oxidation; 7) Hydrolysis; 8) Volatilization. Scheme adopted from Lomans et al., (2002) and Higgins et al., (2006).

5.2. Energetic consideration of VOSC formation in FGL

Thermodynamic properties of chemical reactions, such as the change in Gibbs free energy of a reaction (ΔG°_R), have been employed to investigate the sulfidogenic and methanogenic anaerobic degradation of methylated sulfur compounds in a wide variety of environments to predict the minimum amount of energy needed to sustain growth or biotransformation processes (Bak et al., 1992; Tanimoto and Bak, 1994; Kreft and Schink, 1997; Scholten et al., 2003). One example is the methanogenic anaerobic degradation of MT (in R_{10}) and DMS by *Methanosarcina barkeri (*Finster et al., 1992), which proceed through MT intermediate in R_{11}.

$$4CH_3SH + 3H_2O \longrightarrow 3CH_4 + HCO_3^- + 4HS^- + 5H^+$$
$$\Delta G_R^{\circ} = -39.6 \text{ KJ/mol/MT} \qquad (R_{10})$$

$$2CH_3SCH_3 + H_2O \longrightarrow 3CH_4 + HCO_3^- + H_2S + 5H^+$$
$$\Delta G_R^{\circ} = -110.9 \text{ KJ/mol/DMS} \qquad (R_{11})$$

Although abiotic reactions can often superimpose with microbial reactions in natural environments. We used thermodynamic Gibbs free energy data (ΔG°_{f}) (in Appendix 3A - Table 3A) from Thauer et al. (1977) and Dean (1979) to estimate the ΔG°_{R} for VOSCs formation in sulfidic water column. Measured ΔG°_{f} values for MT, DMS, and DMDS estimated by Scholten et al. (2003) and Mavrovouniotis (1991) in Table 3A, along with geochemical constraints imposed by the environment were used to calculate the energetic driving force, ΔG°_{R} for some of the proposed reactions that may occur in FGL.

Type of Reaction	Reaction	Equation for the reaction	ΔG°_{R} (kJmol^{-1} of reaction)
Oxidation MT	R_4	$CH_3SH + CH_3SH + ½O_2 \longrightarrow CH_3S\text{-}SCH_3 + H_2O$	-185.6
Oxidation DMDS	R_7	$2CH_3S\text{-}SCH_3 + 2O_2 \longrightarrow CS_2 + 2CH_3SH + 2H_2O + CO_2$	-965.9
Hydrolysis of CS_2	R_8	$CS_2 + H_2O \longrightarrow OCS + H_2S$	-21.6
Hydrolysis of OCS	R_9	$OCS + H_2O \longrightarrow H_2S + CO_2$	-11.1

Table 3.4: Estimated Gibbs free energy of reaction (ΔG°_{R}) values for some proposed chemical reactions in FGL calculated from standard thermodynamic conditions.

The overall ΔG°_{R} of the various mechanisms for VOSC formation and degradation in sulfidic freshwater environments are given in Table 3.4, and all the calculated ΔG°_{R} were exergonic values. The calculated Gibbs free energy of formation indicated that abiotic hydrolysis of VOSCs yields similar energy, ranging from approximately -11 to -22 KJ mol^{-1} of CS_2 and OCS, respectively. The ΔG°_{R} for OCS and CS_2 hydrolysis suggests that both transformations are comparable. Based on these observations, we suggest that within narrow energetic limits, hydrolysis of CS_2 and OCS is allowed by

thermodynamics and can proceed simultaneously in the water column to cycle VOSCs. The ΔG°_{R} for oxidation reactions involving MT and DMDS are more negative than the hydrolysis reactions. Two lines of reasoning can be used to describe the mechanistic bases for these observations: (i) the products, DMDS, CS_2, and MT, may be formed as a side product of the two oxidation reactions, or (ii) the products may originate as intermediate compounds during the oxidation process. This thermodynamic-based argument, combined with the observation of nanomolar concentrations of CS_2 (and OCS), suggests that oxidation of MT and DMDS may likely occur in the FGL water column to produce organic CS_2 and OCS species.

These observed values suggest that methylation process involves multiple intermediate steps in order to undergo such biotransformation. These thermodynamic results together with the constant sulfur isotopic composition of ^{34}S-VOSCs up to +4‰ and +3‰ relative to AVS and ZVS, respectively indicate that in anoxic freshwater environments where HS^- and ZVS are present together with decomposing organic matter, organic matter sulfurization through biotic and abiotic processes maybe the dorminate production of VOSC species (such as MT and DMS) in the absence of their major precursor, DMSP.

6.0 Conclusions

Investigation of chemical, isotopic and molecular compositions of volatile organic sulfur species and their inorganic sulfur products reveals a systematic variation of VOSC production in FGL sulfidic waters. This study demonstrates for the first time the potential of multiple sulfur isotopes of VOSCs to discriminate between their syngenetic

(depositional) sulfate and their reduced sulfur sources, leading to an enhanced understanding of the processes by which volatile methylated sulfur species are formed in freshwater environments. Isotopic data clearly illuminates the pathways of VOSC formation through abiotic sulfide and their intermediates (such as polysulfides or elemental sulfur) or incorporation into lignin residues or dead organic matter. Our isotope results support the hypothesis that organic matter sulfurization occurs via a mixture of sulfur sources (such as reduced sulfides and their reactive intermediates) in the water column.

Trends in sulfur isotope values of inorganic sulfur species in the lake suggest complex microbial sulfur cycling through sulfate reduction, S-oxidation, and the disproportionation of intermediate S-compounds (Zerkle et al., 2010). The importance of sulfide oxidation, methylation activity, and hydrolysis processes in the oxic and anoxic section of the lake are contributing to VOSCs cycling. Furthermore, our inability to detect DMSP in both surface water and from planktonic cells likely indicates that DMSP is not a major player for VOSCs formation in this lake system, but it could be generated by microbial degradation of sulfur-containing cell constituents (e.g., amino acids). Although DMSP was not detected, it should be pointed out here that it might perhaps produced at lower concentrations but decomposes rapidly by algal species to DMS, or the majority of it may under a rapid turnover and metabolised into dissolved non-volatile products (Zubkov et al., 2002).

Finally, our study provides multiple lines of evidence that within a productive freshwater sulfidic system, simultaneous biological and abiotic processes will promote the formation of VOSCs that will ultimately alter the redox and diffusive cycling of some

these volatile compounds into the atmosphere. A detailed understanding of the role of VOSCs emissions from freshwater environments into the atmosphere will improve our estimates on global sulfur budget.

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Table 3A: Standard Gibbs free energy state (ΔG_f^o) for compounds in aqueous and gaseous state

Compound	State	ΔG_f^o ($kJmol^{-1}$)	Reference
CH_3SH	Aqueous	-9.6	Scholten et al. (2003)
CH_3SCH_3	Aqueous	8	Mavrovouniotis (1991)
CH_3SSCH_3	Aqueous	46.9	Scholten et al. (2003)
CO_2	Aqueous	-386	Thauer et al. (1977)
CO	Gaseous	-137.2	Thauer et al. (1977)
CS_2	Gaseous	65.3	Dean. 1979
HCO_3^-	Aqueous	-586.9	Thauer et al. (1977)
H_2O	Aqueous	-237.2	Thauer et al. (1977)
HS^-	Aqueous	12.1	Thauer et al. (1977)
H_2S	Aqueous	-27.9	Thauer et al. (1977)
H_2	Gaseous	0.0	Thauer et al. (1977)
H^+ (pH=7)	Aqueous	-39.9	Thauer et al. (1977)
OH^-	Aqueous	-157.3	Thauer et al. (1977)
OCS	Gaseous	-165.6	Dean. 1979
O_2	Aqueous	28.9	Thauer et al. (1977)
Acetate	Aqueous	-369.4	Thauer et al. (1977)

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Chapter 4

Sulfur Isotope Variability of Oceanic DMSP: Implications for DMSP Generation and its Contributions to Biogenic Sulfur Emissions*

Abstract

Oceanic dimethylsulfoniopropionate (DMSP) is the precursor to dimethylsulfide (DMS), which plays a role in climate regulation through transformation to methanesulfonic acid (MSA) and non-sea-salt sulfate (NSS-SO_4^{2-}) aerosols. Here we report measurements of the abundance and sulfur isotope compositions of DMSP from one phytoplankton species (*Prorocentrum minimum*) and five intertidal macroalgal species (*Ulva lactuca*, *Ulva linza, Ulvaria obscura, Ulva prolifera* and *Polysiphonia hendryi)* in marine waters. We show that the sulfur isotope composition ($\delta^{34}S$) of DMSP are depleted in ^{34}S relative to the source seawater sulfate by ~1–3‰ and are correlated with intracellular methionine concentrations, suggesting a link to metabolic pathways of methionine production. We suggest that this variability of $\delta^{34}S$ is transferred to atmospheric geochemical products of DMSP degradation (DMS, MSA, and NSS-SO_4^{2-}), carrying implications for interpretation of variability in $\delta^{34}S$ of MSA and NSS-SO_4^{2-} that links them to changes in growth conditions and populations of DMSP producers in addition to the contributions of DMS and non DMS sources.

Keywords: Assimilation, Cloud Condensation Nuclei, Dimethylsulfoniopropionate, Marine algae, Methionine, Sulfur Isotopes, Methanesulfonic acid.

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1.0 Introduction

Dimethylsulfoniopropionate (DMSP; $(CH_3)_2S^+CH_2CH_2COO^-$) is a secondary metabolite that is produced and stored in large amounts by marine macroalgae (Van Alstyne 2008) and microalgae (Malin and Kirst, 1997). This β-sulfonium compound is widespread among marine taxa but is particularly abundant within specific groups of phytoplankton, zooplankton, macroalgae, halophytic plants, macroinvertebrates, and fishes (Stefels, 2000; Van Alstyne and Puglisi, 2007). DMSP plays important ecophysiological functions in marine algae by acting as an antioxidant (Sunda et al., 2002) a cryoproctectant, an osmolyte, and a precursor to an activated defense system (Stefels, 2000). It is also an important carbon and sulfur source for marine bacterioplankton (Kiene et al., 2000).

The synthesis of DMSP by algae has been reviewed (Stefels, 2000**;** Bentley and Chasteen 2004) previously. It starts with the assimilation of seawater sulfate into the cytoplasm. The sulfate is subsequently transported into the chloroplasts, where it is reduced to sulfide in the presence of glutathionine and then transformed into cysteine. Cysteine is used to synthesize methionine, which is then transformed into DMSP via one of three pathways that differ among taxonomic groups of plants and algae (Hanson and Gage, 1996; Gage et al., 1997; Kocsis et al., 1998; Summers et al., 1998). Thus, the biosynthesis of DMSP ultimately depends upon the activity of the sulfate assimilation pathway; however, little is known about how DMSP synthesis differs among algae from diverse origins, except that the whole molecule is derived from sulfur amino acids.

DMSP and its cleavage product dimethyl sulfide (DMS; $(CH_3)_2S$) have attracted much research interest because of their possible role in climate regulation (Bates et al.,

1987; Charlson et al., 1987). Since the introduction of the CLAW (**C**harlson, **L**ovelock, **A**ndreae, **W**arren) hypothesis, which argues for a feedback between biological DMS production, Earth's solar radiation, and the regulation of global climate (Ayers and Cainey, 2007), there has been an increasing emphasis by environmental scientists on determining the strength of the sea-to-air biogeochemical sources of DMS. This sea-to-air exchange of DMS is mediated through turbulent diffusive processes in marine environments. Once released into the atmosphere, DMS is oxidized by NOx and HOx compounds through addition and abstraction reactions (Yin et al., 2006) to form dimethyl sulfoxide (DMSO), dimethyl sulfone ($DMSO_2$), sulfur dioxide (SO_2), non seasalt sulfate ($NSS\text{-}SO_4^{2-}$), and methanesulfonic acid (MSA). These products serve as sources for sulfuric acid, which has the potential to create new aerosols that can act as cloud condensation nuclei (CCN) (Barnes et al., 2006). These CCN are thought to regulate cloud formation in the remote atmosphere and may have a significant impact on the Earth's cloud cover and albedo (Charlson et al., 1987; Yin et al., 1990; Ayers and Cainey, 2007); however, many details of the connections between the biology, ocean chemistry, and atmospheric chemistry remain to be better understood (Andreae and Crutzen, 1997).

The use of sulfur isotopes provides a powerful method for elucidating the mechanisms underlying the transformation of sulfur present in seawater sulfate into biogenic DMSP and the subsequent transfer of this sulfur, via DMS, into the atmosphere. The proportion of $NSS\text{-}SO_4^{2-}$ and MSA derived from DMS and DMSP has previously been explored using sulfur isotopes (Calhoun et al., 1991; Patris et al., 2000; Sanusi et al., 2006); however, the sulfur isotope compositions of these organic sulfur compounds and their atmospheric oxidation products were estimated from aerosol sulfate composition

(McArdle et al., 1998; Calhoun et al., 1991; Patris et al., 2000) and MSA in ice cores (Patris et al., 2002;Sanusi et al., 2006). These constraints have been used in turn by other studies to constrain the fraction of NSS-SO_4^{2-} in atmospheric aerosols.

Direct measurements of the sulfur isotope composition of DMS and DMSP precursors are needed to establish whether these molecules have a singular sulfur isotope composition, or instead preserve a level of isotopic variability that they may then pass on to their oxidation products, which may complicate interpretations made on the basis of their inferred composition. Recent advances in analyses of methylated sulfur compounds by gas chromatography coupled with multicollector inductively couple plasma mass spectrometry (GC-MC-ICPMS) (Amrani et al., 2009) and Raney-Ni desulfurization (Oduro et al., 2011) provide a unique opportunity to investigate organosulfur biochemical processes from the ocean into the atmosphere.

2.0 Results and Discussion

DMSP concentrations were measured and shown to differ in 5 species of intertidal macroalgae and a planktonic dinoflagellates (Table 4.1). These differences reflect genetic and environmental factors known to influence the synthesis and degradation of DMSP, and its loss from cells (Stefels et al., 2000; Van Alstyne, 2007). DMSP was measured relatively in high concentrations in all members of the Order Ulvales (ranging from 69 ± 13 to 102 ± 34 μmol g^{-1}FM) and the concentrations are comparable to previous measurements from ulvoid algae in this region (Van Alstyne et al., 2007). We also observed relatively low DMSP concentrations (21 ± 3 μmol g^{-1}FM) in *Polysiphonia hendryi* (Table 4.1) even though red algae in this region have also been reported to have

high DMSP concentrations (Van Alstyne and Houser, 2003). These low concentrations may reflect DMSP losses due to sample handling and shipping; *P. hendryi* has been reported to break down DMSP as a result of minor physical damage (Van Alstyne and Houser, 2003). Cellular levels of DMSP were measured for only one phytoplankter, *Prorocentrum minimum,* and were found to have a value of 16 ± 4 μmol g^{-1} FM.

Electrospray ionization mass spectrometry (ESI-MS) was used to characterize intracellular extracts from both the macroalgae and *Prorocentrum*. We demonstrated the presence of the protonated DMSP molecule ($M+H^+$) at *m/z* =135, and its corresponding sodium adduct ($M+Na^+$) at *m/z* =157 in all of species examined (Fig.4.S1, see SI). Fragmentation product suspected to be glycine betaine sulfur-bound amino acid derivative gave N,N-dimethylated sulfur product in the *Prorocentrum* extract with a well-pronounced peak at *m/z* =107; this fragment was not detectable in the macroalgal extracts. The *Prorocentrum* extract produced other fragments in the spectrum at *m/z* 149 (methionine) and at *m/z* 163 (a C_5-DMSP homolog of dimethylsulfoniopropionate) (Fig. 4.S1–panel A) that were not detected in the macroalgal extracts. These differences in peaks between the macroalgal and *Prorocentrum* spectra support differences in operation of the pathways by which DMSP is biosynthesized by macroalgae and *Prorocentrum*. Methionine has been implicated to be an intermediate compound in the synthesis of DMSP through the competitive reaction sequence reviewed by Stefels, 2000, Bentley and Chasteen, 2004 in Fig. 4.1. The lack of methionine peaks in the ESI-MS spectra of macroalgae (Fig. 4.S1–panel B) and their presence in the *Prorocentrum* spectra imply differences between macroalgae and *Prorocentrum* in the relative strengths of either the methionine source or sink fluxes.

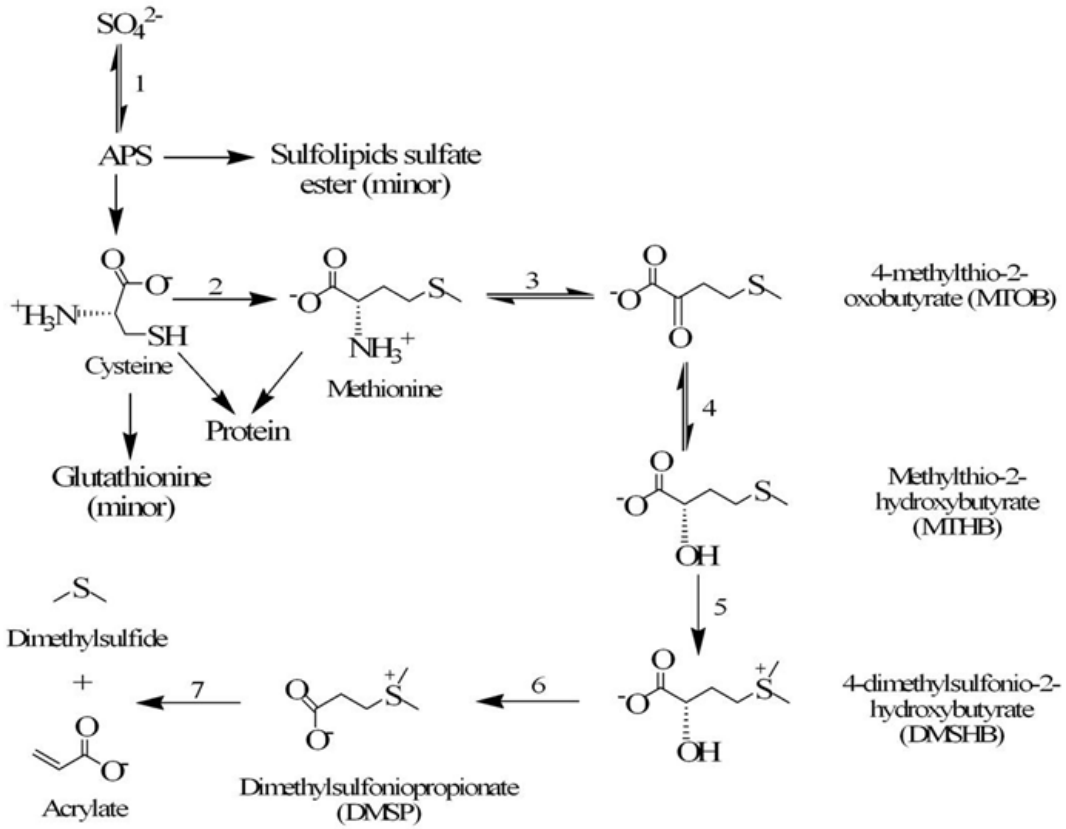

Figure 4.1: Biosynthetic pathway of DMSP/DMS by marine algae through assimilatory sulfate reduction via methionine enzymatic biotransformation. The reaction processes involve in seawater sulfate assimilation by marine algae species are: 1-Carrier-bound sulfate reduction; 2 – Trans-sulfuration to methionine biosynthesis; 3- Transamination; 4- Reduction; 5- Methylation; 6- Oxidative decarboxylation; 7- Cleavage/degradation. (Scheme modified from Stefels, 2000). See SI calculated fractionation factors for S-bonding ($\alpha^{34}S_{compound\text{-}methionine}$) in metabolic intermediates.

Sulfur isotopes (^{32}S, ^{33}S, ^{34}S, and ^{36}S) were measured in macroalgal and *Prorocentrum* extracts, in seawater sulfate, and in gaseous and aqueous DMS that was generated from macroalgal DMSP. The mean $\delta^{34}S_{DMSP}$ signatures of the 6 primary producers ranged narrowly from approximately +18.0 to +19.9‰, with the macroalgal species being the least positive (+18.2 ± 0.6) and the phytoplankton being the most positive (+19.6 ± 0.3‰) (Fig. 4.2, Table 4.2). The $\delta^{34}S$ values obtained for phytoplankton DMSP are consistent with reported values of +19.8‰ (personal communication by Fry to

Calhoun and Bates, 1989). Pair-wise comparisons of seawater sulfate $\delta^{34}S_{SO4}$ (+21 ± 0.3‰) and the $\delta^{34}S$ from the algal DMSP yielded values between 1 and 3‰ that differed among algal species (Fig. 4.3). The differences between seawater sulfate and DMSp from macroalgae were generally larger than the differences between seawater sulfate and DMSP from *Prorocentrum*.

The ^{34}S enrichment of *Prorocentrum* spp. is interpreted to reflect a more strongly bound sulfur in methionine (C-S-C bonds) relative to that in protein (some C-S-S-C bonds – see SOM) as confirmed using relatively low-level molecular orbital calculations in Table 4.5. Steps downstream of methionine to methylthio-2-hydroxybutyrate (MTHB) are reversible which also allows expression of potentially large isotope effects associated with methylation of MTHB to 4-dimethylsulfonio-2-hydroxybutyrate (DMSHB), due to changes in the bonding for S in this biotransformation (S bound to two or three C atoms – see Fig. 4.1). The relationship between methionine concentrations and DMSP $\delta^{34}S$ values does not, however, support this as an explanation for these changes because of higher flow of sulfur from methionine to protein, which might be implied by lower methionine concentrations.

This would essentially yield ^{34}S enrichments in the products rather than the observed depletions. The critical step is interpreted to be competition between methionine and protein production from cysteine in the reaction network. The correspondence between smaller sulfur isotope fractionations and cellular methionine concentrations reflects a higher demand for protein synthesis from cysteine and methionine by algae compared with phytoplankton.

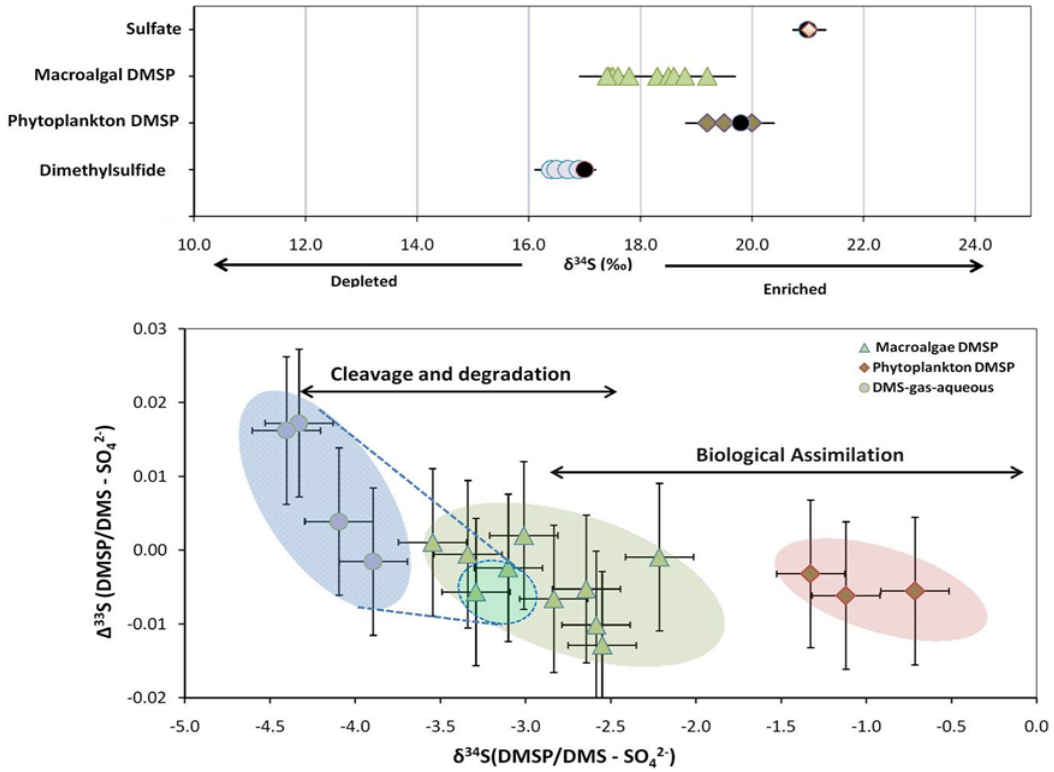

Figure 4.2: Panel A - above shows a summary plot of $\delta^{34}S$ enrichment and depletion of sulfate, macroalgal DMSP, planktonic DMSP, and aqueous/gas phase experimental data for DMS. Panel B - below shows S-isotope plot of $\Delta^{33}S$ versus $\delta^{34}S$ for biological assimilatory process of seawater sulfate assimilation by macroalgal/phytoplankton to form cellular DMSP, and subsequent degradation experiments of ulvoid DMSP yielded aqueous and gas phase DMS . All the data are normalized to starting seawater sulfate compositions.

The difference between $\Delta^{33}S_{SO4}$ and $\Delta^{33}S_{DMSP}$ was within analytical uncertainties, consistent with the assimilation of sulfate being a mass-dependent process without significant variability being introduced by the mixing of the highly fractionated metabolite sulfur pools (Farquhar et al., 2007). The differences in transfer of sulfur through the pathways for the production of DMSP (mixing between metabolite pools) are inferred on the basis of the differences among the $\delta^{34}S_{DMSP}$ values for macroalgae and *Prorocentrum*.

Sulfur isotope compositions were determined for DMS generated by the cleavage of DMSP obtained from *Ulva lactuca* and *Ulva linza*. The $\delta^{34}S_{DMS}$ values were lower

relative to $\delta^{34}S_{DMSP}$ values by 1.2‰ for both green algae (*Ulva lactuca* and *Ulva linza*) (see Fig. 4.3). The measured $\Delta^{33}S_{DMS}$ values were enriched by 0.013 ‰ (Fig. 4.1), which is indistinguishable from various analyses at the level of estimates for 2σ analytical uncertainty. In all cases, the proportion of the aqueous DMS to the initial DMSP was less than 1%, so the measured fractionations are assumed to be representative of the fractionations associated with the process of producing aqueous DMS. It is not known whether the sulfur isotope fractionation rates associated with cleavage of DMSP to form DMS will differ among taxonomic groups of organisms. The branching biogeochemical pathways associated with the loss of DMS to the atmosphere and the recycling of DMS back to the biota via assimilation could also result in additional variability in the sulfur isotope composition of dissolved oceanic and outgassed DMS.

3.0 Conclusions and Implications to Marine Atmosphere

In the remote atmosphere, MSA, and NSS-SO_4^{2-} aerosols are the principal oxidation products (~80%) of DMS (MSA/NSS-SO_4^{2-} is between ~0.1 and 0.4) (Legrand et al., 2001). These products are produced through reaction chains involving few branches and predominantly unidirectional radical abstraction and addition reactions **(16)**. Given the high proportion of the ultimate sulfate product (NSS-SO_4^{2-}) and the general similarity in the molecular structure of the reaction intermediates, it is inferred that the sulfur isotope composition of NSS-SO_4^{2-} will approximate that of oceanic DMS emissions. Direct measurements of MSA collected over the Pacific Northwest Ocean yielded $\delta^{34}S$ values of 17.7 ± 0.7‰ (Sanusi et al., 2006), which is within the range of $\delta^{34}S_{DMSP}$ reported here (Fig. 4.3), taking into account fractionations associated with

degradation of DMSP to DMS. Marine biogenic sulfate $\delta^{34}S_{NSS\text{-}SO4}$ values have been estimated to range from +14 to + 22‰ (Patris et al, 2000), with measurements of Pacific aerosols being +15.6 ± 3.1‰ (Calhoun et al., 1991), North Atlantic coastal aerosols being +22‰ (McArdle et al., 1998), and Greenland ice cores being +18.6 ± 0.9‰ (Patris et al., 2002). These are similar to the DMS sulfur isotope compositions predicted on the basis of DMSP measurements. These measurements support the hypothesis that variations in the sulfur isotope composition of NSS-SO_4^{2-} can be tied to variations in the sulfur isotope composition of regional oceanic or coastal DMS emissions.

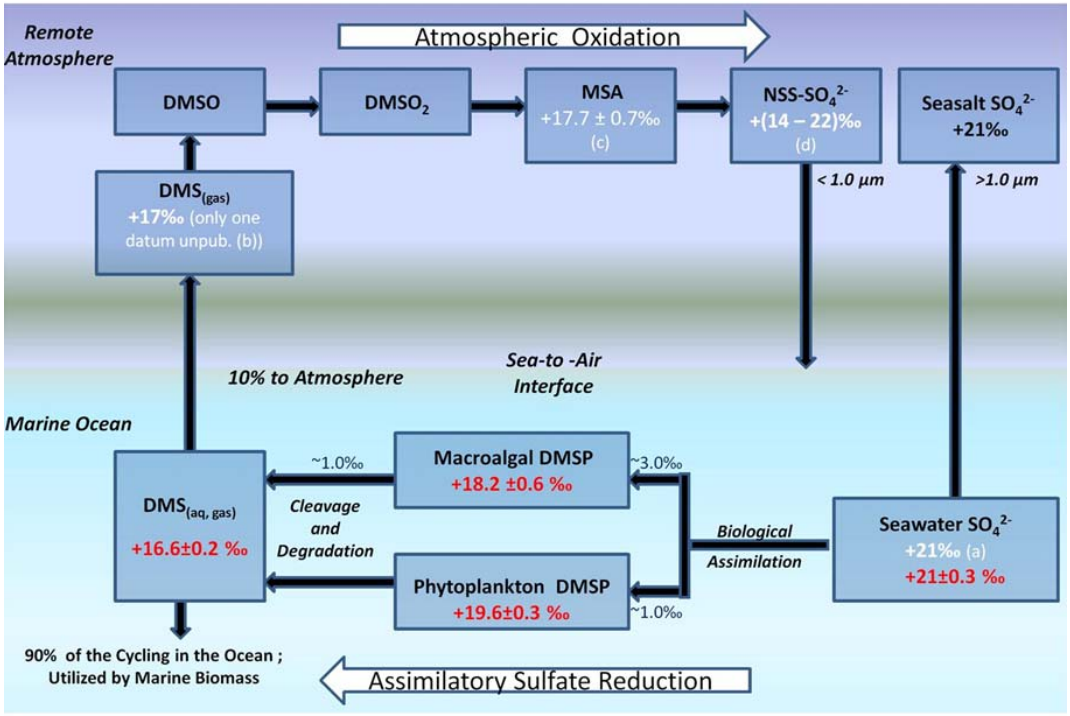

Figure 4.3: Sulfur isotope compositions of the major biogenic sulfur products formation and transformations in the ocean by marine algae and emissions of DMS to the atmosphere produces the two major oxidation products, MSA and NSS-SO_4^{2-}. The $\delta^{34}S$ compositions written in red are from this study, whereas $\delta^{34}S$ values written in white are compiled data ((a- and b- (Calhoun and Bates, 1989); C-(Sanusi et al., 2006); and d-(Patris et al., 2000) from different independent measurements in different geographic regions.

These regional DMS sulfur isotope compositions are, in turn, ultimately derived from the sulfur isotope compositions of the DMSP that is produced by different types of organisms that may be growing under different environmental conditions or at different life-cycle or bloom stages. Studies seeking to use sulfur isotopes to constrain the fractional contribution of sulfate resulting from the oxidation of biogenic DMS/DMSP to NSS-SO_4^{2-} aerosols will need to take into account the resulting levels of heterogeneity of ~1-10 percent that are introduced by variations in $\delta^{34}S_{DMSP}$. However, this heterogeneity also provides an opportunity to track changes in source DMS/DMSP that reflect changes in ecological or environmental conditions in different geographical regions.

4.0 Materials and Experimental Methods

Algal Sampling **-** Five macroalgal species (*Ulva lactuca*, *Ulva linza, Ulvaria obscura, Ulva prolifera* and *Polysiphonia hendryi)* were collected by hand from intertidal or shallow subtidal habitats at Ship Harbor, Anacortes, WA (48^o 30 N, 122^o 40' W) and Penn Cove, Coupeville, WA (48^o 14 N, 122^o 44' W). The algae were brought back to the Shannon Point Marine Center in Anacortes, WA, where the green algae were identified by examining microscopic sections. All algae were cleaned of visible epiphytes and then shipped on ice on the day of collection to the Stable Isotope Laboratory at the University of Maryland, College Park for intracellular DMSP analysis.

DMSP from marine phytoplankton was sampled in April 2009, from an extensive bloom of *Prorocentrum minimum* in the York River, a tidal estuary that is a tributary of Chesapeake Bay in Virginia. To select sites for further sampling of DMSP, 1.0 L sub-surface seawater samples were analyzed for chlorophyll a (an indicator of high

phytoplankton productivity). On the same day, at the selected sites, samples of 50 L of seawater containing planktons and particulate DMSP ($DMSP_p$) were taken from different transects and, within 5 hours of collection, the samples were filtered through a Whatman GF/F filter under vacuum (<5 mm Hg) in a dark room. Residues from filtrates were lysed in liquid nitrogen before DMSP analysis. At each of the sampling sites, seawater sulfate samples were also collected. They were processed for sulfate by first acidifying with 0.5 mol L^{-1} HCl, and then precipitating the sulfate as $BaSO_4$ with a 1.0 mol L^{-1} $BaCl_2$ solution.

4.1 Experimental design for analysis of macroalgal DMSP to DMS S-isotope composition

The production of DMS from macroalgae was investigated to elucidate the sulfur isotope composition of the aqueous and gas phase DMS in ocean-atmosphere interactions. In these experiments, two macroalgal species (*Ulva lactuca and Ulva linza*) from Washington state were tested for DMSP production and conversion into DMS. Fresh algal samples were placed in clean, 1.0 L silanized Erlenmayer flasks containing 1.0 L deoxygenated filtered seawater. The flasks were immediately sealed with gastight seals, leaving no headspace, and incubated at 2^{o}C for 48 hours in a dark room. The DMS generated by the breakdown of the algal DMSP was sampled with an aqueous phase extraction to recover the DMS dissolved in the seawater and by purge and trap followed by the precipitation of DMS to recover gaseous DMS. In the aqueous phase extraction, DMS was extracted with carbon tetrachloride at -10^{o}C then re-extracted with 30 mL of 5% $HgCl_2$ to precipitate the DMS into a white crystalline mercury complexes (e.g., 3DMS-2Hg) (Yang et al., 2006; Oduro et al., 2011). The precipitated DMS-complexes

were stored at 4°C in a dark-brown Niskin bottles for later S-isotope analysis. The gaseous DMS produced by the cleavage of DMSP was stripped out with ultra high purity nitrogen (UHP-N_2), dried through a glass tube containing K_2CO_3 and a naffion tube, and trapped with 5% $HgCl_2$ to precipitate DMS as mercury complexes.

Purified algal DMSP samples and 3DMS-2Hg were reduced to Ag_2S with a modified Raney nickel hydrodesulfurization method described by Oduro et al. 2011. Precipitated $BaSO_4$ was reduced to H_2S by boiling in 25 mL of 5 N HCl and Thode solution (a mixture 320 mL HI, 524 mL HCl, and 156 mL of H_2PO_3). In all distillation-reduction reactions, the evolved H_2S was captured with an $AgNO_3/HNO_3$ buffer solution as Ag_2S for S-isotope analyses as an SF_6 gas.

5.0 Supporting Information (SI)

5.1 DMSP concentration measurements

DMSP was analyzed after cold alkaline cleavage to DMS in 1:1 stoichiometry Dacey and Blough, 1987) from known amounts of algae. Algal samples were placed directly into 20 ml silanized Hungate glass vials containing 20 ml of 0.5 mol L^{-1} NaOH solution. Then the vials were immediately sealed with butyl rubber gastight seals, leaving no headspace. DMSP was fully transformed to DMS after incubation in the dark at 2°C for at least 24 hours. The DMS generated by the breakdown of DMSP was analyzed with a cryo-purge and trap technique (Kiene and Service, 1991) using gas chromatography (Shimadzu model GC-14A equipped with a flame photometric detector - FPD). DMS measurements were carried out in duplicates and triplicate. Calibration was performed with DMS (Sigma Aldrich; Catalog no. 274380) standards. The average precision of the

DMS measurements, which was determined from 6 injections of fixed DMS concentrations, was 12.1%.

5.2 Characterization of DMSP from algal samples

Macroalgae and microalgae DMSP and other cellular constituents were extracted in cold and dark conditions with the method described by Zhang et al**.** 2005. Briefly, the algae were extracted in a mixture of cold methanol, chloroform and water (12:5:3 v/v) and the organic solvents were then removed with a rotary evaporator under vacuum at 30 °C. The extract pH was adjusted to 5.5 to keep the DMSP stable before the final purification of DMSP using cation-exchange resin, Dowex-50W (H+). The aqueous extract was analyzed before and after purification in with positive ion modes for DMSP using an AccuTOF (JEOL USA, Inc., Peabody, MA) time-of-flight mass spectrometer (TOF-MS). The mass spectrometer used an electrospray ionization source (ESI) and had a mass resolving power (Δm/m) of 6000 full width at half maximum (fwhm). The spray voltage was set to 2.3 kV, and the capillary and orifice temperatures were maintained at 250°C and 80°C, respectively. The instrument was typically operated at the following potentials: orifice 1 = 30 V, orifice 2 = 5 V, ring lens = 10 V. The RF ion guide voltage was generally set to 1000 V to allow detection of ions greater than m/z =100. The protonated DMSP was determined by thin layer chromatography after purification to be ≥ 97%.

5.3 Multiple sulfur isotope measurements

Four sulfur isotope measurements were performed using a Finnigan MAT 253 - dual inlet isotope ratio mass spectrometer (DI-IRMS). One to three mg samples of Ag_2S

wrapped in aluminium foil were reacted in Ni bombs with ten-fold excesses of fluorine gas at 320°C for approximately 8-12 hours. The SF_6 product was cryogenically separated from F_2 at -196°C and then distilled from HF and other trace contaminants at -115°C. The final purification of SF_6 by GC-TCD was performed on a composite column comprised of a 1/8 in. diameter, 1.8 m packed column containing type 5A molecular sieve, followed by a 1/8 in. diameter, 3.7 m Hayesp-Q™ column. SF_6 eluted between 12 and 18 min at a He flow rate of 20 mL min^{-1} and a 50°C column temperature. SF_6 eluting from the column was captured in a spiral glass trap frozen in liquid nitrogen. The sulfur isotope composition of purified SF_6 was measured in dual inlet mode of the gas-source isotope ratio mass spectrometer with four collectors arranged to measure the intensity of SF_5^+ ion beams at m/z values of 127, 128, 129, and 131 ($^{32}SF_5^+$, $^{33}SF_5^+$, $^{34}SF_5^+$, and $^{36}SF_5^+$). Estimates of analytical uncertainties of the sulfur isotope measurements were deduced from the long-term reproducibility of Ag_2S fluorinations to be 0.02, 0.008, and 0.20 (1σ) for $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$, respectively.

The sulfur isotope results ($^{34}S/^{32}S$) are expressed in permil (‰) relative to Vienna Cañyon Diablo Troilite (VCDT) using the standard delta notation (δ):

$$\delta^{34}S_{sample} = \left[\frac{^{34}S/^{32}S_{sample}}{^{34}S/^{32}S_{VCDT}} - 1\right] \quad (1)$$

The less abundant isotopes ($^{33}S/^{32}S$ and $^{36}S/^{32}S$) also given in units of permil (‰) are reported using capital delta notation (Δ), where

$$\Delta^{33}S = \left[\left(\frac{^{33}S/^{32}S_{sample}}{^{33}S/^{32}S_{VCDT}}\right) - \left(\frac{^{34}S/^{32}S_{sample}}{^{34}S/^{32}S_{VCDT}}\right)^{0.515}\right] \quad (2)$$

$$\Delta^{36}S = \left[\left(\frac{^{36}S/^{32}S_{sample}}{^{36}S/^{32}S_{VCDT}} \right) - \left(\frac{^{34}S/^{32}S_{sample}}{^{34}S/^{32}S_{VCDT}} \right)^{1.90} \right] \quad (3)$$

The exponents in these relationships (0.515 and 1.90) define the reference fractionation line (RFL) and approximate single-step thermodynamic equilibrium isotope exchange effects (Houlston and Thode, 1965).

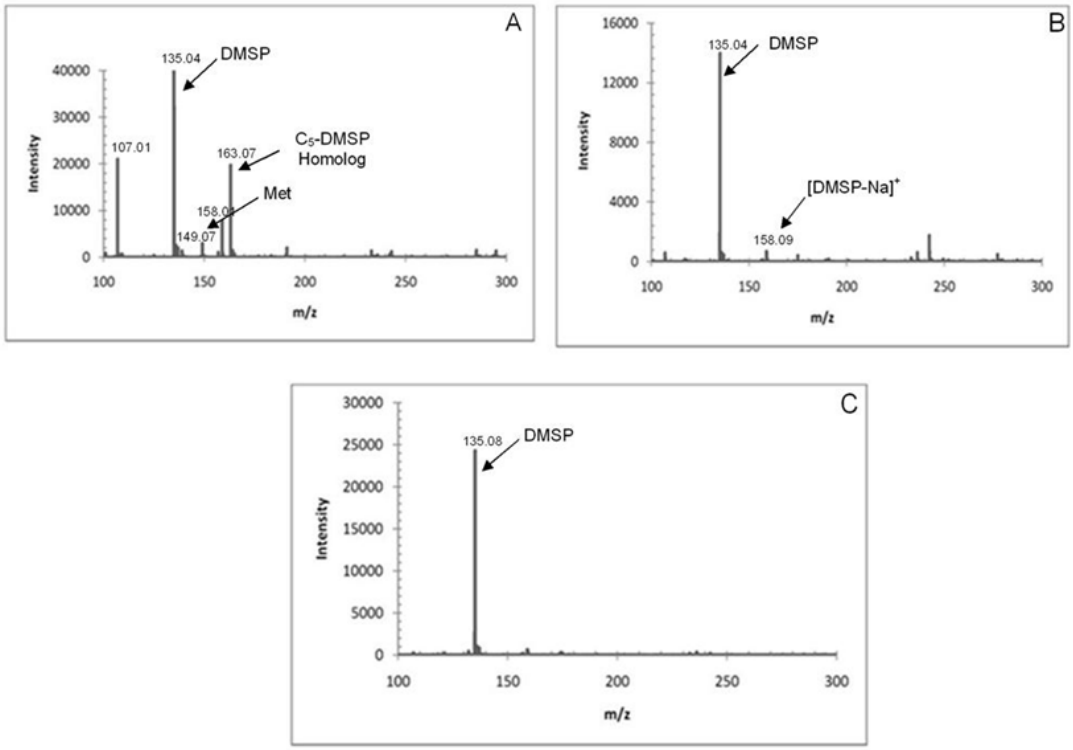

Figure 4.S1: ESI-MS positive mode spectra of cellular marine algae extracts. Panel A – Cellular phytoplankton extract showing fragments of $(CH_3)_2N\text{-}SO_2$ moiety (*m/z* =107.01); a ($DMSPH^+$; $(CH_3)_2S^+CH_2CH_2COOH$) peak (*m/z* =135.04); a methionine peak (*m/z* =149.07); $[DMSP\text{-}Na]^+$ adduct (*m/z* =158.01); and C_5-homolog of $DMSP_{pent}$; $(CH_3)_2S^+(CH_2)_2COO^-$) at (*m/z* =163.07). Panel B - Cellular macroalgal species extract with DMSP and their sodium adduct peaks at (*m/z* =135.04) and (*m/z* =158.09) respectively. Panel C- Purified extracts with cation-exchange resin showing parent ion peak of DMSP (*m/z* =135.08).

Table 4.1: Variation in concentrations of algal DMSP

Marine Macroalgae		**Location**	**Conc. DMSP (µmol g^{-1}FM)**
Phylum Chlorophyta	*Ulva lactuca*	WA: Penn Cove, Ship Harbor	102 ± 34
	Ulva linza	WA: Penn Cove, Ship Harbor	78 ± 17
	Ulvaria obscura	WA: Ship Harbor	69 ± 13
	Ulva prolifera	WA: York River Estuary in Virginia	77 ± 15
Phylum Rhodophyta	*Polysiphonia hendryi*	WA: Ship Harbor	21 ± 3
Marine Phytoplankton			
Phylum Dinoflagellata	*Prorocentrum spp.*	VA: York River	16 ± 4

Concentration measurements are reported as µmol per gram fresh mass of algal species and are the results of duplicate and triplicate analysis ± SD.

Table 4.1: Variation in concentrations of algal DMSP

Marine Macroalgae		**Location**	**Conc. DMSP (µmol g^{-1}FM)**
Phylum Chlorophyta	*Ulva lactuca*	WA: Penn Cove, Ship Harbor	102 ± 34
	Ulva linza	WA: Penn Cove, Ship Harbor	78 ± 17
	Ulvaria obscura	WA: Ship Harbor	69 ± 13
	Ulva prolifera	WA: York River Estuary in Virginia	77 ± 15
Phylum Rhodophyta	*Polysiphonia hendryi*	WA: Ship Harbor	21 ± 3
Marine Phytoplankton			
Phylum Dinoflagellata	*Prorocentrum spp.*	VA: York River	16 ± 4

Concentration measurements are reported as µmol per gram fresh mass of algal species and are the results of duplicate and triplicate analysis ± SD.

Table 4.2: $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$ of DMSP in marine macro- and microalgae.

DMSP - Macroalgae **DMSP - Penn Cove, Coupeville, WA**	**$\delta^{33}S$**	**$\delta^{34}S$**	**$\delta^{36}S$**	**$\Delta^{33}S$**	**$\Delta^{36}S$**
(Fall, 2010)					
Ulva lactuca	9.405	18.282	34.95	0.031	-0.07
Ulva linza	9.856	19.169	36.95	0.029	0.21
DMSP- Ship Harbor, Anacortes, WA					
Ulva lactuca	9.149	17.772	34.09	0.035	0.05
Ulva linza	9.013	17.492	33.52	0.043	0.02
Ulvaria Obscura	9.684	18.747	36.10	0.048	0.09
Polysiphonia hendryi	8.984	17.444	33.42	0.038	0.02
DMSP - Penn Cove, Coupeville, WA (Spring, 2010)					
Ulva lactuca	9.533	18.518	35.16	0.039	-0.31
Ulva prolifera	9.066	17.616	33.49	0.032	-0.25
DMSP- Ship Harbor, Anacortes, WA					
Ulva linza	9.094	17.667	33.90	0.034	0.06
Ulvaria Obscura	9.548	18.584	35.46	0.020	-0.14
Polysiphonia hendryi	9.392	18.291	34.99	0.014	-0.05
DMSP - Phytoplankton Bloom Chesapeake Bay York River, VA (Apr, 2009)					
Prorocentrum spp.	9.881	19.192	36.42	0.043	-0.36
	10.324	20.005	38.28	0.040	-0.19
	10.035	19.500	37.38	0.040	0.00

One additional digit is retained for $\delta^{33}S$, $\delta^{34}S$, and $\delta^{36}S$ (beyond the significant ones), to allow calculation of $\Delta^{33}S$ and $\Delta^{36}S$ to the appropriate number of significant digits.

Table 4.3: $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$ of sulfate in collection localities

Seawater Sulfate		**$\delta^{33}S$**	**$\delta^{34}S$**	**$\delta^{36}S$**	**$\Delta^{33}S$**	**$\Delta^{36}S$**
(August, 2010)	**Abbr. Symbol.**					
	SO_4^{2-} -					
Sulfate	PC	10.985	21.384	41.38	0.029	0.36
	SO_4^{2-} -					
Sulfate	SH	10.853	21.144	40.92	0.019	0.36
(Apr, 2010)						
	SO_4^{2-} -					
Sulfate	PC	10.876	21.162	40.78	0.033	0.20
	SO_4^{2-} -					
Sulfate	SH	10.836	21.134	40.86	0.007	0.33
Chesapeake Bay York River VA (Apr,2009)						
	SO_4^{2-} -					
Sulfate	CYR	10.675	20.747	39.46	0.043	-0.33
	SO_4^{2-} -					
Sulfate	CYR	10.612	20.619	39.28	0.046	-0.26

One additional digit is retained for $\delta^{33}S$, $\delta^{34}S$, and $\delta^{36}S$ (beyond the significant ones), to allow calculation of $\Delta^{33}S$ and $\Delta^{36}S$ to the appropriate number of significant digits.

Table 4.4: $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$ of DMS extraction experiment tests

DMS(gas)	**$\delta^{33}S$**	**$\delta^{34}S$**	**$\delta^{36}S$**	**$\Delta^{33}S$**	**$\Delta^{36}S$**
Ulva lactuca	8.494	16.453	31.49	0.055	-0.01
Ulva linza	8.456	16.379	31.48	0.054	0.13
DMS(aqueous)					
Ulva lactuca	8.601	16.687	32.34	0.041	0.39
Ulva linza	8.698	16.888	32.30	0.036	-0.04

One additional digit is retained for $\delta^{33}S$, $\delta^{34}S$, and $\delta^{36}S$ (beyond the significant ones), to allow calculation of $\Delta^{33}S$ and $\Delta^{36}S$ to the appropriate number of significant digits.

5.4 Inferences for fractionations of different sulfur bonding in metabolic intermediates

Table 4.5 presents calculations of reduced partition function ratios (and inferred equilibrium fractionations) made using molecular orbital calculations (Gaussian 09W – Frisch et al. 2009 using Hartree Fock level theory, 6-31G+ (d,p) basis sets, and the integral equation formalism variant of the Polarizable Continuum Model (IEFPCM) for solvation in water. These calculations are presented using these relatively low-level molecular orbital calculations to provide preliminary insight into the way that changes bonding of sulfur atoms in metabolic intermediates (C-S-H, C-S-C, C-S-S-C, or S bound to three C atoms) influences the vibrational and zero point energy shifts associated with isotopic substitution. These constraints are used as a basis for interpreting changes in the isotopic composition of product DMSP resulting from changes in metabolic fluxes. Higher-level kinetic treatments and more detailed knowledge of reaction paths will be needed to correctly model isotope effects associated with these transformations. Note for instance, the disagreement in magnitude between experimentally measured fractionations between DMSP and DMS with those predicted from equilibrium considerations (Table 4.5). See Figure 4.1 of the text for illustrations of the compounds. Cystine ($C_6H_{12}N_2O_4S_2$) and DMDS ($C_2H_6S_2$) were used as analogs for cross-linking sulfur in proteins with C-S-S-C bonds.

Table 4.5: Calculated fractionation factors for intermediate compounds

Compound	**$^{34}\alpha_{compound\text{-}methionine}$**	**$1000*\ln(^{34}\alpha_{compound\text{-}methionine})$**	Bond type
Cysteine	0.9946	-5.4	C-S-H
Methionine	1.0000	0.0	C-S-C
MTOB	1.0002	0.2	C-S-C
MTHB	1.0001	0.1	C-S-C
DMSHB	1.0133	13.2	$C - S < \begin{matrix} C \\ C \end{matrix}$
DMSP	1.0133	13.3	
DMS	1.0000	0.0	C-S-C
Cystine	0.9977	-2.3	C-S-S-C
DMDS	0.9975	-2.5	C-S-S-C

Calc. w/ Hartree Fock 6-31G/+d,p IEFPCM solv. model, solvent =water, T = 25°C

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Chapter 5

Evidence of Magnetic Isotope Effects during Thermochemical Sulfate Reduction*

Abstract

New thermochemical sulfate reduction experiments with simple amino acid and dilute concentrations of sulfate reveal significant degrees of mass-independent sulfur isotope fractionation. Enrichments of up to 13‰ for ^{33}S are attributed to a magnetic isotope effect (MIE) associated with the formation of thiol-disulfide ion-radical pairs. Observed ^{36}S depletions in products are explained here by classical (mass-dependent) isotope effects and mixing processes. The experimental data contrasts strongly with multiple sulfur isotope trends in Archean samples, which exhibit significant ^{36}S anomalies. These results support an origin other than thermochemical sulfate reduction for the mass-independent signals observed for early Earth samples.

Keywords: Anomalous, Sulfur radical, Thermolysis, Spin-selective, Hyperfine coupling.

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1.0 Introduction

Since the report by Farquhar et al., 2000 that significant deviations from the terrestrial fractionation line (TFL) are observed in samples older than ~2.32-2.45 Ga (Bekker et al., 2004; Guo et al., 2009), considerable effort has been dedicated to identifying the origin and significance of the mass-independent sulfur isotope signal (Farquhar et al., 2001; Pavlov and Kasting, 2002; Zahnle et al., 2006; Lyons, 2007; Domagal-Goldman et al., 2008; Watanabe et al., 2009; Halevy et al., 2010). The sulfur isotope MIF-signal in these ancient samples is expressed as variations in both $\Delta^{33}S$ and $\Delta^{36}S$[‡§] (Farquhar et al., 2000). Given the observations that gas-phase reactions can produce mass-independent signals for both $\Delta^{33}S$ and $\Delta^{36}S$, the first studies on this subject attributed this ancient signal to photolytic reactions in the early atmosphere. Subsequent studies also pointed out that the mass-independent reactions may also be produced by variations in the spectrum of light that drives atmospheric photolytic reactions (Lyons et al., 2007; 2009), and other studies speculated that liquid phase reactions involving weakly bound transition states may account for these variations (Lasaga et al., 2008; Watanabe et al., 2009).

In a recent report (Watanabe et al., 2009), demonstrated that high temperature reduction of sulfate using alanine and glycine as organic substrates caused moderate mass-independent sulfur isotope fractionations. These authors did not identify the origin of the effect, but suggested that it was either a Magnetic Isotope Effect (MIE) (Buchachenko et al., 2001) or another type of isotope effect accompanying heterogeneous

[‡]Here $\Delta^{33}S = (^{33}S/^{32}S)_{sample}/(^{33}S/^{32}S)_{cdt} - [(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{cdt}]^{0.515}$ and $\Delta^{36}S = (^{36}S/^{32}S)_{sample}/(^{36}S/^{32}S)_{cdt} - [(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{cdt}]^{1.90}$. Note this is a different definition than that used in [(7)]. The definition used here is consistent with definitions that normalize to a reference array defined by single-step equilibrium isotope exchange reactions and does not impact the conclusions of this study.

reactions such as adsorption of S-bearing compounds on surfaces of solids (Lasaga et al., 2008). Magnetic isotope effects are expressed in rare cases for isotopes with nuclear magnetic moments, like ^{13}C, ^{17}O, ^{29}Si,^{199}Hg, ^{201}Hg, ^{73}Ge, ^{235}U, and ^{33}S (Buchachenko et al., 2001; Bergquist and Blum, 2007; Ghosh et al., 2008). The effect is expressed when the lifetime of a radical pair is sufficient for hyperfine coupling between magnetic nuclei and unpaired electrons to influence inter-conversions between singlet and triplet states. This coupling in turn changes the proportion of reactive intermediates that can participate in spin selective reactions. The ^{33}S nucleus has a spin of 3/2 and a magnetic moment of 0.643 nuclear magnetons and has been implicated in at least one well-characterized example of a ^{33}S MIE (Turro, 1983; Step et al., 1990; Buchachenko et al., 2001; 2009). The alternative suggestion, related to a proposal that anomalous isotope effects may be associated with heterogeneous reactions as a result of possible missing of vibrational levels involving very weakly-bound transition states (Lasaga et al., 2008). This proposal has been contested by Balan et al. 2009, who argue that no effect exists when a more complete treatment of the reaction mechanisms is undertaken. Uncertainty in ascribing the origin of the effect to an MIE arose because two of their samples possessed $\Delta^{36}S$ that was different from that of the starting materials. Uncertainty, ruling out an MIE (and demonstrating a different type of anomalous isotope effect) also arose because other processes relevant to the complex reaction pathways of TSR in their system involve mixing and can generate mass conservation effects (Farquhar et al., 2007) that have been shown to produce small variations in $\Delta^{33}S$ and more significant variations in $\Delta^{36}S$ (e.g. $\geq$2 ‰ in biological and biogeochemical systems (Ono et al., 2006; Johnston et al., 2008).

Here we report results from two sets of high temperature experiments (i.e., a flow-reactor and Carius tube experiments) that suggest the observation of ^{33}S anomalies in these reactions are related to a magnetic isotope effect in the reaction products. The mechanistic aspects for the origin of ^{33}S anomalies via disulfide ion-radical pair reaction routes have been proposed through multiple sulfur isotope measurements and ^{33}S electron spin resonance (ESR) spectroscopic evidence (Shine and Sullivan, 1967; Hadley and Gordon, 1975).

2.0 Results and Discussion

The flow through reactor experiments yielded two distinct sulfur products as seen in Table 5.1 (gaseous H_2S and chromium-reducible sulfur, CRS) with anomalous ^{33}S enrichments ($\Delta^{33}S$ from +0.25 to +13.1‰), but no significant change in ^{36}S composition from starting sulfate (Fig. 5.1, panel A, B). In Carius tube experiments, ^{33}S enrichments in AVS and CRS products were small to undetectable except when S_8 or $Na_2S_2O_4$ was added (see Table 5.2) to catalyze reduced sulfur production in the reaction products with no effect to the S-radical chemistry.

We speculate that the observed isotope effect originates from ion-radical pair ($RS^{\bullet}H^{+}/RS^{\bullet}SH$) intermediates (Hadley and Gordon, 1975; Buchachenko et al., 2004) through the following sequence of reactions;

1. Prolonged heating of glycine (mp= 262^{o}C) affords three major classes of compounds: (a) small neutral molecules; (b) carbon and other carbon-based polymers, and (c) radical intermediates (both heteroatom and carbon centered

radicals) as indicated in reaction scheme 5.2 below (Johnson and Wang, 1971; Simmonds et al., 1972).

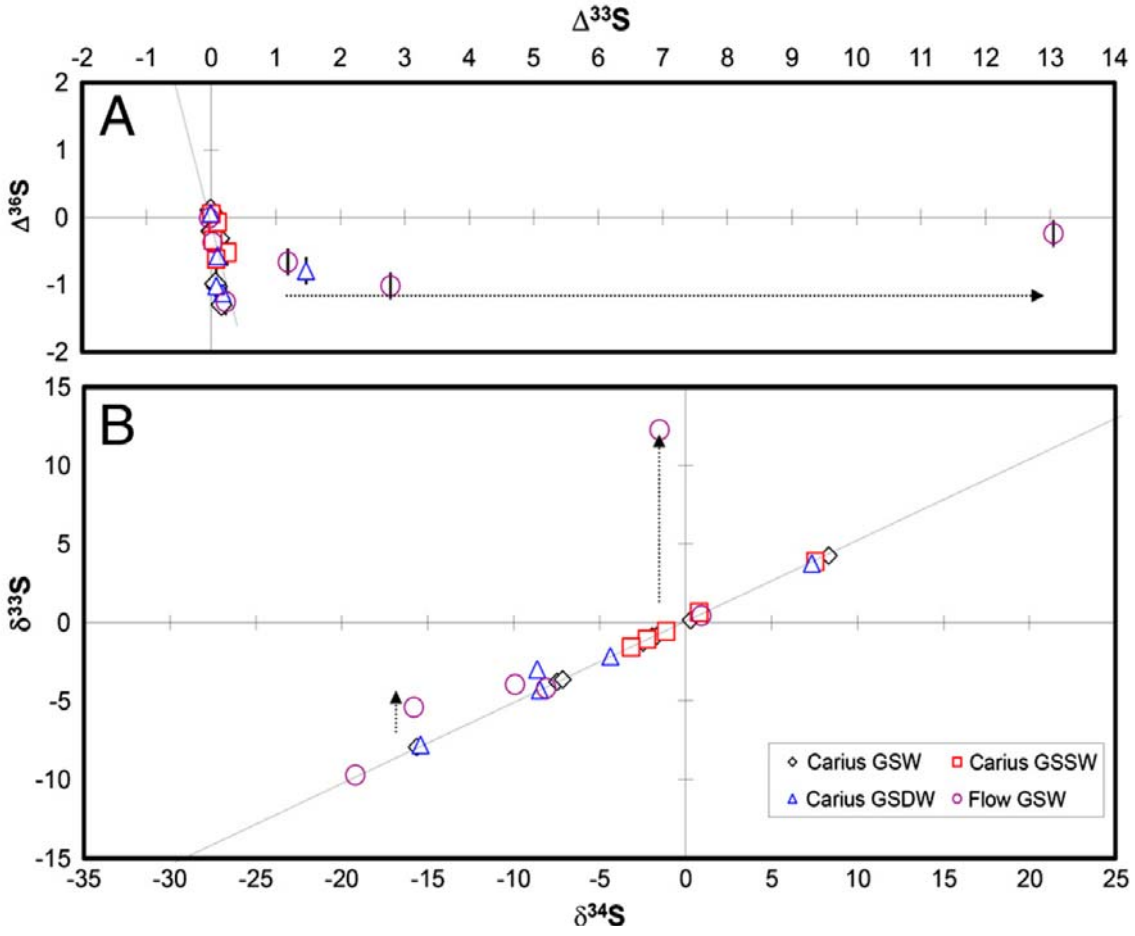


Fig. 5.1: S-isotope plots of $\Delta^{36}S$ versus $\Delta^{33}S$ (Panel A) and $\delta^{33}S$ versus $\delta^{34}S$ (Panel B) for Carius tube and flow reactor experiments, abbreviated as GSW (Gly-SO_4^{2-}-H_2O), GSSW (Gly-SO_4^{2-}-S^0-H_2O), and GSDW (Gly-SO_4^{2-}-$S_2O_6^{2-}$-H_2O). Typical mass-dependent arrays are plotted in both panels. Most data follow a tightly constrained, mass-dependent relationship of $\delta^{33}S = 0.515\ (\pm 0.008) \times \delta^{34}S$ (Panel B). Deviations from this array and the $\Delta^{36}S$ versus $\Delta^{33}S$ array are interpreted as magnetic isotope effects. The MIE trends are distinct from sulfur photoexcitation experiments and are not a likely explanation for the Archean sulfur isotope record. Error bars represent 1σ analytical uncertainties of 0.02 and 0.2 for $\Delta^{33}S$ and $\Delta^{36}S$, respectively.

2. Sodium sulfate can then be reduced by some of the reducing gases produced in scheme 5.2 (eg., H_2, CO etc) to give sodium sulfide (R_1, R_2). Trace metal

impurities in sodium sulfate can catalyze this reaction. Solid carbon or carbon-based polymers, generated during pyrolysis can also reduce sodium sulfate to sodium sulfide (R_3) (Cameron and Grace, 1982; 1983).

$$Na_2SO_4 + 4CO \longrightarrow Na_2S + 4CO_2 \qquad (R_1)$$

$$Na_2SO_4 + 4H_2 \longrightarrow Na_2S + 4H_2O \qquad (R_2)$$

$$Na_2SO_4 + nC \longrightarrow Na_2S + CO + CO_2 \qquad (R_3)$$

3. Sodium sulfide in the presence of trace acid and/or water and heat will generate hydrogen sulfide (H_2S) (R_4). The acid/water would come from the H_2O, HCN, and COOH generated during glycine pyrolysis (see Scheme 5.2) or even from the glycine starting material,

$$Na_2S + 2H\text{-}Y \longrightarrow 2NaY + H_2S \qquad (R_4)$$

(Y = CN or OH or $H_2NCH_2COO^-$)

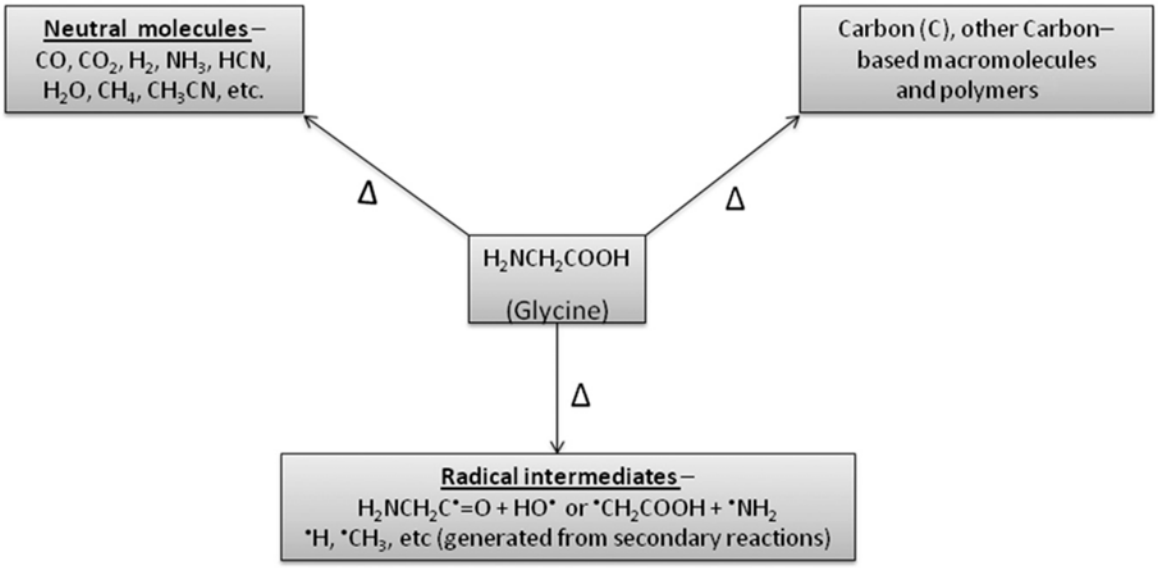
4. Hydrogen sulfide can undergo thermolysis (Chivers and Lau, 1985; Adesina et al., 1995), which can react with radical intermediates generated during glycine pyrolysis (see Scheme 5.2) to give thiyl radicals as in R_5 and R_6 (Beare and Coote, 2004). Note that these reactions are not spin-selective so no ^{33}S anomaly will result.

$$HS\text{-}H + R^{\bullet} \longrightarrow HS^{\bullet} + RH \qquad (R_5)$$

R= carbon-centered radical

$$HS\text{-}H + Y^{\bullet} \longrightarrow HS^{\bullet} + HY \qquad (R_6)$$

Y= heteroatom-centered radical



Scheme 5.2: Pyrolytic decomposition products of glycine. [(a)]Solid-state NMR and High resolution ESI-MS (in positive mode) confirm the presence of polymers in the reaction mixture.

5a. The thiyl radical ($HS^{\bullet}$) can recombine with other radicals to give neutral, sulfur containing molecules. Reactions between free radicals (e.g., R_7, R_8, and R_9) can be spin-selective (Buchachenko, 1995). The absence of measureable sulfur-33 enrichment in the carbon-bound sulfur (Raney Ni fraction) suggests, however, that this reaction is not the origin of the isotope effect in these experiments.

$$HS^{\bullet} + H^{\bullet} \rightleftharpoons [HS^{\bullet}\ {}^{\bullet}H]^{T,S} \longrightarrow HSH \qquad (R_7)$$

$$HS^{\bullet} + R^{\bullet} \rightleftharpoons [HS^{\bullet}\ {}^{\bullet}R]^{T,S} \longrightarrow RSH \qquad (R_8)$$

$$HS^{\bullet} + {}^{\bullet}Y^{\bullet} \rightleftharpoons [HS^{\bullet}\ {}^{\bullet}Y]^{T,S} \longrightarrow YSH \qquad (R_9)$$

Y= heteroatom, R= carbon-centered radical

5b. The products of R_7, R_8, and R_9 can undergo further homolytic cleavage of S-H bonds shown in reaction scheme 5.3 to give thiyl radical moieties ($^{\bullet}SH$, $^{\bullet}SY$, and $^{\bullet}SR$). These thiyl radicals have strong reactivity and can also react with other radicals to

form polysulfide products via sulfur polymerization (R_{10}). Such reactions will not produce sulfur-33 enrichments due to strong spin-orbit coupling (see below).

$$RS^{\bullet} + HS^{\bullet} \longrightarrow [RS^{\bullet}\ {}^{\bullet}SH]^{T,S} \longrightarrow RS_nH \qquad (R_{10})$$

6. The thiyl radical can also abstract hydrogen from C-H bonds to give carbon-centered radicals. These reactions will not be spin-selective.

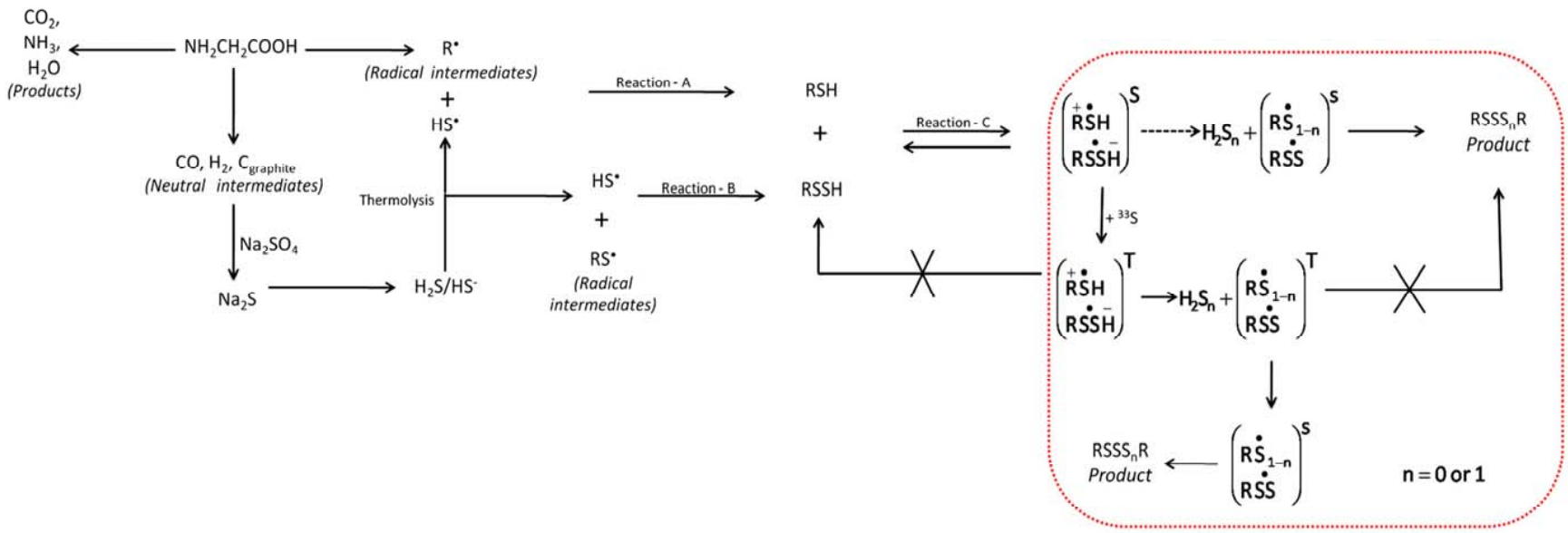
$$HS^{\bullet} + H\text{-}R \longrightarrow H_2S + R^{\bullet} \qquad (R_{11})$$

$$HS^{\bullet} + H\text{-}R \longrightarrow RSH + R^{\bullet} \qquad (R_{12})$$

Based on the above sequence of reactions, we proposed scheme 5.2, which describes the pathway envisioned for production of the observed sulfur-33 enrichments in Cr-reducible sulfur and hydrogen sulfide. We have generalized this sequence of reaction by writing it for only R (and not Y and H sulfur bonded radicals). During accidental encounter of free radicals (Reactions A and B) in scheme 5.3, the statistical distribution of radical pair spin states will be ¼ singlet and ¾ triplet states. MIE may occur if this 1:3 proportion is altered by the rapid formation of singlet products upon initial encounter, leaving an excess (> ¾) of triplet radical pairs that undergo triplet to singlet conversion. However, these reactions are an unlikely source of MIE because it has been shown that thiyl radicals exhibit strong spin-orbit coupling (Khudyakov et al., 1993; Autrey et al., 1995) and therefore are expected to experience rapid spin flipping independent of hyperfine coupling.

We instead suggest that the observation of ^{33}S enrichments in the CRS fraction implicates MIE associated with the formation of polysulfide species (Reaction C – in

scheme 5.3) and the subsequent network highlighted in the red box). We suggest this polymerization reaction of sulfur product is mediated by an ion-radical mechanism similar to that proposed by Buchachenko (Buchachenko et al., 2004). Here RSH acts as an electron donor to RSSH, forming an ion-radical pair intermediate that is initially in a singlet state. Coulombic attraction inhibits dissociation of the radical pair. Here, the radical pair may either i) reform the original reactants by back electron transfer, ii) slowly lose H_2S_n (where n = 0 or 1) in a non spin-selective manner or iii) undergo singlet to triplet conversion via ^{33}S hyperfine coupling. Back electron transfer from the triplet state is spin forbidden, and therefore the radical pair must lose H_2S_n to form a $RS^{\bullet}_{1-n}/RSS^{\bullet}$ radical pair. Subsequent triplet-singlet conversion allows for the radical pair to combine and thus form a polysulfide ($RSSS_nR$) product. Such products that are formed through the triplet pathway are therefore enriched in ^{33}S. Our mechanism is supported by prior electron spin resonance (ESR) measurements (Shine and Sullivan, 1967; Hadley and Gordon, 1975), which show that disulfide radicals have ^{33}S hyperfine structure values (~ 10 gauss) similar to sulfur nuclei that exhibit MIE. The experimental products also preserve evidence for significant mixing and classical isotope effects that influence $\Delta^{36}S$ in a mass-dependent manner (Ono et al., 2006; Farquhar et al., 2007), supporting this as the cause of ^{36}S variation reported in Watanabe et al., 2007. The Eschka sulfur and the Cr-reducible sulfur appear to be isotopically fractionated relative to the residual sulfate by two distinct processes. The Eschka sulfur has a mass-dependent ^{34}S enrichment with respect to the starting composition, and the Thode fraction (which includes residual sulfate), which is, in turn, ^{34}S-depleted relative to the starting composition.



Scheme 5.3: Proposed ion radical pair (RP) mechanism showing spin evolution between triplet and singlet states during thermochemical sulfate reduction.

We infer that these observations indicate the principal loss pathway for sulfate is the mass-dependent formation of product sulfur in the Eschka fraction. The Cr-reducible sulfur fraction is ^{33}S-enriched and ^{34}S-depleted, consistent with an MIE following the mechanism described that would yield a smaller secondary product fraction, and a residue with a small ^{33}S-depletion. We interpreted the absence of a measurable fractionation in the Eschka and Raney Ni fractions to reflect dilution of R-SH that formed from non spin-selective reactions.

3.0 Conclusions

We conclude that the origin of MIE is related sulfur radicals generated by thiyl-mediated thermolysis of H_2S. Which undergo a rapid ion-radical pair polymerization reaction to form the chromium (II) reducible sulfide product. Since the MIE captured in these TSR experiments principally affects $\Delta^{33}S$ without significantly affecting $\Delta^{36}S$, the relevance of these reactions as an explanation for mass-independent sulfur isotope effects reported from Earth's most ancient rocks (where deviations from mass-dependent arrays are noted for both ^{33}S and ^{36}S) is limited. Moreover, the absence of sulfur isotope MIF in post-Archean organic-rich rocks suggests that that TSR is not a widespread source of fractionations in typical sediments and further supports the assertion that the early record does not reflect this chemistry. Thermal reactions have, however, been proposed as a mechanism for formation of sulfur-containing compounds as well as their radical species in a variety of natural systems where organic matter and sulfur radicals are present (e.g. where sulfur radicals control petroleum maturation (Lewan, 1998)). It is possible

therefore that sulfur MIE are generated in some settings, and evidence for this should be sought.

4.0 Experimental Procedure and Methods

Two sets of experiments were undertaken to monitor the products of TSR: (i) flow-reactor experiments and (ii) Carius tube experiments. Reagent grade sodium sulfate (~0.5 mol/L) and powdered glycine were used in both experiments.

$$SO_4^{2-} + 2H_2N\text{-}CH_2\text{-}COOH + H_3O^+_{(W)} \xrightarrow[\Delta\text{ -Heat}]{} H_2S + 3CO_2 + [CH_2]^{\bullet} + 2NH_3 + 3H_2O \qquad (1)$$

For the flow reactor experiments, glycine and 1.0 mL sodium sulfate solution were added to a reaction flask, matching the stoichiometry of reaction (1) which was heated continuously at ~300^{o}C for 340 h hours under 15 bubbles/min nitrogen flow. Water lost to evaporation was replenished by injecting 0.5 mL of Milli-Q water through a septum in the reaction flask (3 or 4 times per experiment-Table 5.1). Product hydrogen sulfide carried by the nitrogen flow was isolated by trapping with a Zn-acetate buffer, yielding a white crystalline ZnS precipitate. Solid and liquid residues in the reaction flask were treated by procedures outlined for the Carius tube experiments.

High-purity Pyrex glass Carius tubes (dimension 35.5 cm long, 12 mm outer diameter, wall thickness ~1.2 mm) were loaded with 0.5 mL sodium sulfate solution and glycine to match the stoichiometry of reaction (1). Two experiments also included sulfur intermediate species (S_8 and $Na_2S_2O_4$ – amounts listed in Table 5.3) to catalyze the production of sulfide in reaction products. Sample tubes were placed in a stainless steel jacket before being heated in a muffle furnace at temperatures listed in Table 5.1. After

heating, the Carius tubes were chilled with liquid-nitrogen, crack-opened, and zinc acetate added to fix sulfide.

Solid and liquid fractions were isolated from the Carius tubes and placed into a flask for sequential extraction. The sulfur from reaction products were extracted using sequential reaction with 5 N HCl for acid volatile sulfide (AVS); Cr(II) acid distillation in ethanol for S-S_n (where $n \geq 1$) fractions (CRS); Raney nickel desulfurization for carbon bonded sulfur; Thode reducible sulfur for sulfate; and Eschka oxidized sulfur for total organic sulfur (methods described in Oduro et al., 2011). Recovery was incomplete because some material adhered to the Carius tube walls, but upper estimates of the fraction of product Cr-reducible sulfur and Raney Ni reducible sulfur are provided (Table 5.2). The proportion of Cr-reducible and Raney–Ni reducible sulfur relative to Eschka- and Thode-sulfur was determined by Cline method (Cline 1969) using a UV-VIS double beam (model UVD-3200) scanning spectrophotometer (Labomed Inc., CA, USA) before converting sulfur into Ag_2S for fluorination in Ni bombs, conversion to SF_6 by heated reaction with F_2, and subsequent S-isotopic analysis in a dual inlet ThermoFinnigan-253 mass spectrometer.

A solid state Nuclear Magnetic Resonance spectra (NMR) was acquired for residual solid fractions after the experiments using a Varian/Chemagnetics Infinity 300 Solid State NMR Spectroscopy. High resolution Electrospray Ionization Mass Spectrum (ESI-MS – Resolving power 6000 fwhm) were also taken for liquid fractions in both a positive ion mode using an AccuTOF (JEOL USA, Inc., Peabody, MA) time-of-flight mass spectrometer (TOF-MS). The spray voltage was set to 2.3 kV, and the capillary and orifice temperatures were maintained at 250 °C and 80 °C, respectively. The instrument

was typically operated at the following potentials: orifice 1 = 30 V, orifice 2 = 5 V, ring lens = 10 V. The RF ion guide voltage was generally set to 1000 V to allow detection of ions greater than m/z =100. Both Solid State NMR and ESI-MS analyses confirm the presence of neutral molecules, complex carbon-based macromolecules, and polymers that were formed through radical condensation reactions.

5.0 Supporting Information (SI)

5.1 Analysis of Multiple Sulfur ($\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$) Isotopes

Samples of Ag_2S were reacted in Ni bombs with ten-fold excess fluorine gas at 320^{o}C to convert sulfur into sulfur hexafluoride (SF_6) gas. The SF_6 was cryogenically separated from F_2 (at -196 ^{o}C) and then distilled from HF and other trace contaminants at -115^{o}C. Final purification of SF_6 by GC-TCD was performed on a composite column made up of a 1/8 in. diameter, 6 ft. long packed column containing type 5A molecular sieve, followed by another 1/8 in. diameter, 12 ft. long Hayesp-QTM column. Sulfur hexafluoride eluted between 12 and 18 minutes at He flow rate of 20 mLmin^{-1} and 50^{o}C column temperature. Sulfur hexafluoride eluting from the column was captured in a spiral glass trap cooled with liquid nitrogen. Sulfur isotope composition of purified SF_6 were measured using a ThermoFinnigan MAT 253 - Dual Inlet Isotope Ratio Mass Spectrometer with four collectors arranged to measure the intensity of SF_5^+ ion beams at m/e values of 127, 128, 129, and 131 ($^{32}SF_5^+$, $^{33}SF_5^+$, $^{34}SF_5^+$, and $^{36}SF_5^+$). Analytical uncertainties of sulfur isotope measurements, estimated from long-term reproducibility of Ag_2S fluorinations are 0.008, 0.02, and 0.20 (1σ) for $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$, respectively. Sulfur isotope ratios are reported using delta (δ) notation (2) as a deviation of an isotope

$$\delta^{x}S = [(^{x}S/^{32}S)_{samp}/(^{x}S/^{32}S)_{ref} - 1] \quad (2)$$

ratio in a sample $_{(samp)}$ relative to that for V-CDT with an assumed composition of S-1 of $\Delta^{34}S = -0.30$, $\Delta^{33}S = 0.094$, and $\Delta^{36}S = -0.7$, where x = 33, 34 or 36 reported in units of permil (‰). We do not include the factor of 1000 included in some other studies.

Equilibrium isotope effects are described by $(\delta^{33}S/1000 + 1) \approx (\delta^{34}S/1000 + 1)^{0.515}$ and $(\delta^{36}S/1000 + 1) \approx (\delta^{34}S/1000 + 1)^{1.9}$ and are used to define reference fractionation arrays. Deviations from the mass-dependent fractionation array are given using capital delta notation $\Delta^{33}S$, and $\Delta^{36}S$, which are defined as:

$$\Delta^{33}S = [(1 + \delta^{34}S)^{0.515} - 1] \quad (3)$$

$$\Delta^{36}S = [(1 + \delta^{34}S)^{1.90} - 1] \, . \quad (4)$$

Note this is a different definition than that used in Watanabe et al., 2007. The definition used here consistent with definitions that normalize to a reference array defined by single-step equilibrium isotope exchange reactions and does not impact the conclusions of this study and only result in small modifications to the calculated $\Delta^{33}S$ and $\Delta^{36}S$.

We used only glycine in our experiment to simplify the experiment to one reaction, and because both glycine (Gly, H_2N-CH_2-COOH) and alanine (Ala, H_2N-$CH(CH_3)$-COOH) are classified as simple amino acids with similar pk_a values for the α-carboxy (COOH-Gly =2.4; Ala = 2.3) and α-amino (NH_3^+ Gly =9.8; Ala = 9.9) ionizable groups. We expect that hydrolysis and subsequent pyrolytic decomposition of both amino acids may yield similar product that show comparable $\Delta^{33}S$ anomalies at same experimental conditions, but have not demonstrated this. The isotopic compositions of

the flow reactor experiment products have highly variable $\delta^{34}S$ and point to the presence of significant isotope effects operating during the reactions. These experiments are potentially subject to loss of fractionated material that is not trapped by the trapping solutions, but were done as a preliminary effort to reproduce the Watanabe et al., 2009 results. These were broadly consistent, but with one experiment yielding a large positive $\Delta^{33}S$ (13‰) with little variation for $\Delta^{36}S$. Variations for $\Delta^{36}S$ are interpreted to reflect conservation of mass-effects related to mixing of pools in the reaction network rather than interpretating as primary anomalous effects. Variations in isotopic composition of sulfur extracts in different experiments are interpreted to result from differences in the carrier flow rate, addition rate for water, and variation in temperature conditions. Because of their design, these experiments did not allow for the capture of possible volatile organic species escape during the reaction and closure of mass balance was not attained. Experiments with carius tubes (sealed glass tubes) were used as a way to address this issue. These experiments yielded products with smaller, but still significant $\Delta^{33}S$, variability in $\Delta^{36}S$ that is associated with significant $\delta^{34}S$ fractionations and is also interpreted to reflect mass conservation effects. Closure of mass balance was not attain in the carius tube experiments due to the difficulty in recovering all the reaction products from the tube, which adhere to walls of the glass after the reaction.

Table 5.1: Experiments with continuously flowing nitrogen, very low flow rate normalized to S-isotopic composition.

Experimental conditions/comments	Sample identification	$\delta^{33}S$	$\delta^{34}S$	$\delta^{36}S$	$\Delta^{33}S$	$\Delta^{36}S$
Temperature = 258 ± 10 ºC	H_2S (product)	-9.71	-19.21	-37.44	0.23	-1.26
Add 0.5 ml - water - 3 times						
Reaction run time =14 days -336- 340 hrs.	Cr(II) reduction of residue	-5.39	-15.81	-30.84	2.78	-1.02
	Sulfate in residue	-4.17	-8.12	-15.74	0.02	-0.37
Temperature = 298 ± 10 ºC	H_2S (product)	-3.94	-9.93	-19.44	1.19	-0.66
Add 0.5 ml-water - 4 times						
Reaction run time =14 days -336- 340 hrs.	Cr(II) reduction of residue	12.27	-1.52	-3.12	13.05	-0.24
	Sulfate in residue	0.44	0.91	1.72	-0.03	-0.01

Table 5.2: Experiments with Carius tube products normalized to starting S-isotopic composition.

Experimental conditions/comments	Sample identification	$\delta^{33}S$	$\delta^{34}S$	$\delta^{36}S$	$\Delta^{33}S$	$\Delta^{36}S$	% of total sulfur
Glycine-sulfate-water Temperature =175- 200ºC Reaction run time = 14 days -336- 340 hrs	Acid Volatile Sulfur	-1.05	-2.09	-4.20	0.03	-0.22	
	Cr(II) reduction	-1.27	-2.47	-4.61	0.01	0.08	2.2
	Raney Nickel reducible sulfur	NP	NP	NP	NP	NP	0.0
	Thode solution reducible sulfur	-3.77	-7.48	-15.19	0.09	-1.03	
	Eschka-oxidized sulfur	0.15	0.29	0.34	0.00	-0.21	
Glycine-sulfate-water Temperature =250- 298ºC Reaction run time =14 days -336- 340 hrs.	Acid Volatile Sulfur	-7.92	-15.63	-30.79	0.16	-1.30	
	Cr(II) reduction	-0.89	-1.95	-4.02	0.12	-0.31	2.0
	Raney Nickel reducible sulfur	-0.87	-1.71	-3.46	0.01	-0.21	1.0
	Thode solution reducible sulfur	-3.62	-7.15	-14.53	0.07	-0.99	
	Eschka-oxidized sulfur	4.26	8.31	15.96	-0.01	0.10	
Glycine-sulfate-elemental sulfur-water Temperature =250- 298ºC Reaction run time =14 days -336- 340 hrs.	Acid Volatile Sulfur	-1.06	-2.24	-4.33	0.10	-0.07	
	Cr(II) reduction	0.65	0.78	0.96	0.25	-0.52	6.9
	Raney Nickel reducible sulfur	-0.56	-1.14	-2.50	0.02	-0.34	2.5
	Thode solution reducible sulfur	-1.56	-3.18	-6.65	0.08	-0.62	
	Eschka-oxidized sulfur	3.88	7.54	14.42	0.00	0.05	
Glycine-sulfate-sodium dithionate-water Temperature =250- 298ºC Reaction run time =14 days -336- 340 hrs.	Acid Volatile Sulfur	-7.80	-15.42	-30.22	0.17	-1.12	
	Cr(II) reduction	-2.99	-8.64	-17.14	1.47	-0.79	6.2
	Raney Nickel reducible sulfur	-2.16	-4.38	-8.88	0.10	-0.57	3.2
	Thode solution reducible sulfur	-4.30	-8.48	-17.08	0.08	-1.02	
	Eschka-oxidized sulfur	3.76	7.32	14.02	-0.01	0.06	

Table 5.3: Starting sulfur species compositions

Description	Amount	$\delta^{33}S$	$\delta^{34}S$	$\delta^{36}S$	$\Delta^{33}S$	$\Delta^{36}S$
$NaSO_4$ – J.T. Baker CAS # - 7757-82-6	0.5M (1.6%)	1.51	2.91	5.49	0.01	-0.05
S_8 - Alfa Aesar Stock # - 10343	2-3 mg	1.48	2.87	5.18	0.00	-0.28
$Na_2S_2O_4$ - Sigma Aldrich Batch # - 20425MA	2-3 mg	-3.67	-7.18	-14.40	0.03	-0.79

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Chapter 6

Summary and Conclusions

1.0 General Summary

In this dissertation, I have shown that high precision multiple sulfur isotope measurements of organic sulfur compounds by SF_6 methods can be used provide source, sink and the transformation information for specific organosulfur compounds in a variety of natural systems. Various isotope laboratories employ different methods to measure isotope ratios, $^{34}S/^{32}S$, $^{33}S/^{32}S$, and $^{36}S/^{32}S$ by means of mass spectrometry. These existing methods including inductively-coupled plasma mass spectrometry (ICP-MS) (Mason et al., 1999) secondary ion mass spectrometry (SIMS) (Winterholler et al., 2006; Kozdon et al., 2010; Kita et al., 2011), thermal ionization mass spectrometry (TIMS) (Mann and Kelly, 2005), and laser ablation multiple-collector inductively coupled plasma mass spectrometry (LA-MC-ICP-MS) (Santamaria-Fernandez et al., 2009) are not ideally suited for volatile methylated sulfur isotope analysis particularly DMS and other VOSC species in natural environments, with only one exception that involves the measurement $^{34}S/^{32}S$ ratio of DMS and organosulfur species in petroleum oil using gas chromatography (GC) coupled with multicollector inductively plasma mass spectrometry (MC-ICPMS) (Amrani et al., 2009).

The direct isolation and subsequent measurements of four sulfur isotope composition by SF_6 method of VOSCs, including their natural precursors and related inorganic sulfur species in different environmental

systems, was the main objective of this research. A secondary objective was to use these isotopic variations in $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{36}S$ to understand the source, sink and the distribution these compounds. Most early studies of sulfur isotope composition measurements of microbial and geochemical systems principally make use only $^{34}S/^{32}S$ ratios to interpret physicochemical and biological processes that cause variations in the abundances of isotopes that are dependent of their masses.

Here I demonstrate that $^{33}S/^{32}S$ and $^{36}S/^{32}S$ ratios can be used in conjunction with conventional $^{34}S/^{32}S$ to differentiate the signatures of a) biological sulfate assimilation and reduction processes, b) abiological and biological sulfurization reactions of functionalized organic matter, and c) magnetic isotope effects generated by sulfur-centered radical polymerization reaction under thermal conditions. The systematic correlations among the major $\delta^{34}S$ and minor (e.g., $\Delta^{33}S$) sulfur isotopes indicate that these isotopic variations can be distinguished from those produced by biological and non-biological processes, and hence provide new constraints on VOSCs and sulfur-centered radical generation, as well as their subsequent transformation mechanisms in natural and perhaps other geothermal environments. Variations in the stable S-isotope compositions in this research can result from a variety of equilibrium and kinetically controlled processes, which were concluded, and categorized into 1) mass-dependent, 2) mass-independent fractionation, and 3) magnetic isotope effect processes. This dissertation has focused on the general questions of:

1. Chemical methods and analytical techniques for measurement of four sulfur isotope compositions of organic sulfur compounds;
2. Application of these methods to constrain the sources of VOSCs and their precursor's in oceanic, estuarine, coastal wetland, and freshwater systems and their impact on marine biogenic sulfur aerosol formation; and
3. Utilization of some of these methods to explain the complex radical chemistries of organosulfur compound production during thermochemical sulfate reduction leading to enrichment of the magnetic ^{33}S nuclei.

The fundamental problem with direct sulfur isotope measurements of VOSCs species are sample loss from volatilization, low concentrations in ambient air and natural waters, and the lack of effective methods of identification, separation, and pre-concentration techniques in natural environments.

In chapter 2, I have shown that it is possible to sample and pre-concentrate various VOSCs by the precipitation with 5% $HgCl_2$ as mercury complexes (e.g., $HgMT_2$, 3DMS-2Hg, 3DMDS-2Hg) and subsequent reduction using the Raney nickel hydrodesulfurization to quantitatively convert the various organosulfur species including their biochemical precursors into the corresponding alkane and hydrogen sulfide. The hydrogen sulfide evolved from the reaction is captured as ZnS or Ag_2S, which is subsequently, used for the determination of concentration and for

measurement of the major ($\delta^{34}S$) and minor sulfur isotopes ($\Delta^{33}S$, and $\Delta^{36}S$) by SF_6 method. These protocols were then used as a practical and reliable chemical method to extract VOSC species from coastal wetland, sulfidic freshwater and algal DMSP/DMS produced in marine water systems. The method also uses various strengths of acids and metal chlorides as a selective reducing/oxidizing agent to convert the various forms of organic and inorganic sulfur into Ag_2S for their four sulfur isotope measurements. In an addition to these chemical methods, analytical techniques such as Gas Chromatography equipped with Pulse Flame Photometmetric Detector (PFPD) for simultaneously analysis different VOSCs and Electrospray Ionization Tandem Mass Spectrometer (ESI-MS/MS) for cellular measurements DMSP and other sulfonium products were employed to quantify and identify various methylated sulfur compounds. This work demonstrates for the first time that sulfur present as VOSCs, as other organically bound sulfur, and as inorganic sulfur can be distinguished to trace the sulfur sources and the biogeochemical transformation in coastal salt marsh environments.

In chapter 3, I employed the same chemical and isotopic measurement techniques to study VOSCs formation and cycling in sulfidic freshwater system at the Fayetteville Green Lake (FGL), New York. I demonstrated that VOSCs (such as MT, DMS, DMDS, CS_2, and OCS) are generated by a network of chemical reactions in both oxic and anoxic water columns. The general conclusions for VOSCs formation apparently involve incorporation of

reduced inorganic sulfur and their intermediate species (produced as a result of bacteria sulfate reduction) into dead organic matter. Also, sulfur transformations in FGL were sensitive to both pH and redox conditions. Overall, VOSCs concentrations and their combined isotopic compositions were the two most common parameters used to interpret sulfur geochemistry in the ancient lake.

Extension of these techniques in chapter 4, to measure sulfur isotope fractionations between DMS and DMSP in phytoplankton and marine algal cells, reveals a range of $\delta^{34}S$ values, which were depleted relative to the source seawater sulfate by ~ 1– 3‰. These variations are asserted to provide information on how marine algae metabolized seawater sulfate into DMSP through assimilatory sulfate reduction, summarized in six transformational steps as – 1) a multi-step carrier-bound sulfate reduction; 2) trans-sulfurization to methionine biosynthesis; 3) transamination; 4) reductive elimination; 5) methylation; and the final 6) oxidative decarboxylation processes. The observed differences in cellular DMSP concentrations reflect genetic and environmental factors known to influence the synthesis and degradation of DMSP, and its loss from the cells. Given the first time measurements of intracellular DMSP and a basic understanding of the key controls on sulfur isotope fractionation of DMSP conversion into DMS, we can test the CLAW hypothesis (Charlson et al., 1987) by tracking the changes and variability in source DMS/DMSP formation of sulfate aerosols in marine air on regional or temporal scales.

In Chapter 5, the variety of sequential extraction protocols for organic sulfur isolation and techniques developed in this dissertation enables us to examine the S-isotope chemistry of thermochemical sulfate reduction (TSR), which has been discovered to produce anomalous $\Delta^{33}S$ signatures (Watanabe et al. 2009). Such anomalies can only be understood by isolation, characterization, and identification of the various sulfur compounds in order to describe the sulfur species generated during the thermal process. Results in the TSR experiments display the sulfur isotopic distribution of various organic and inorganic sulfur forms in the reaction products. The analyses suggest triplet-to-singlet conversion, which allow ion-radical pair interaction products to combine and thus form a disulfide/polysulfide ($RSSS_nR$) product through a triplet pathway to preserve $\Delta^{33}S$ signature in chromium (II) reducible products. These effects also involved significant mixing and classical isotope effects that perturb $\Delta^{36}S$ in some of the reduced sulfur products. Enrichments of ^{33}S are attributed to a magnetic isotope effect (MIE) associated with only odd isotopes via the formation of thiyl-disulfide ion-radical pairs. The findings in this TSR experiments are not consistent with multiple sulfur isotope trends in Archean samples (Farquhar et al., 2000), which exhibit significant ^{36}S anomalies, and further, the assertion that the Archean record does not reflect this thermally induced radical sulfur chemistry.

2.0 Conclusion Remarks and Future Recommendations

The geochemistry and biogeochemistry of organosulfur in natural system is complex and does not readily lend itself to the interpretation by traditional methods based on marine or freshwater lake studies. The problem is magnified when working in modern sedimentary and ancient freshwater systems (such as FGL), as diagenetic overprinting and production of different sulfur intermediate species may erase any record of formation, transformation, and depositional processes. However, a more fruitful approach to understand the organosulfur bio-(geochemistry) natural environments was accomplished in this thesis work by isolation, identification of different organosulfur compounds, and analyzing their individual isotopic compositions.

The results presented in this dissertation represent one the first sulfur isotope investigations that make ties to the formation and cycling of VOSCs, connections with metabolic and microbial processes, and chemical reactions that interconvert inorganic and organic sulfur species in aquatic and sedimentary environments. The S-isotope results in chapters 2, 3, and 4 support the fact that the formation, transformation, and pathway processes of VOSCs were similar. But their distribution varies considerably from sample to sample in a particular system. This shows that incorporation reactions of inorganic sulfur with organic matter can be selective. Therefore conclusions of organosulfur isotopic data should be drawn cautiously or interpreted well by analyzing together their predominant inorganic sulfur products in a

particular system. In addition, there were notable precursors of DMS product, DMSP, detected exclusively by ESI-MS/MS in the *Spartina* roots and algal samples in coastal salt marsh and oceanic water respectively. This compound was absent in cellular extracts in the freshwater system, implying an additional pathway of DMS formation in freshwater systems in addition to cleavage and demethylation processes in coastal salt marsh and marine systems. On the other hand, the sulfur isotope chemistries of CVOSCs formation in coastal wetland and freshwater systems revealed the same conclusion as products derived from reduced sulfide forms and their intermediate species. With the exception of estuarine and oceanic DMS and DMSP products that were derived exclusively from assimilated sulfate sulfur products.

Uncertainty exists in the contribution of biogenic DMS sources to the atmosphere. Reasons for the uncertainty regarding biogenic sulfur estimates from continents and oceans are: 1) difficulty in accurately determining the various biogenic sulfur species; 2) technical problems involved in measuring the emission fluxes of these compounds in different ecosystems; 3) inadequate geographical coverage of existing data. The methods employed in these studies will potentially provide a new tool to estimate the relative contribution of these VOSC gases from oceanic, continental, and freshwater environments into the atmosphere. The approach employed in chapter 4, aimed to measure directly the $\delta^{34}S$, $\Delta^{33}S$, and $\Delta^{33}S$ compositions of seawater sulfate, DMSP, DMS, and other volatile species in different aquatic environments. An

extension that includes a coordinated measurement of sulfur isotope compositions of various VOSCs in the ocean, along with simultaneous measurements of atmospheric species such as DMS, MSA, SO_2, and NSS-SO_4^{2-} are still needed to overcome the uncertainties associated with biogenic sulfur gas emissions and their current and future contributions to climate warming.

Finally in chapter 5, it is evident that detectable enrichments of sulfur-33 are observed in TSR experiments. The source of these anomalies results from thiyl radical production, which appeared to be enhanced by heating, water, and the molecular structure of sulfur compound present. Chemical reactions of sulfur-centered radicals are quite variable in nature and often constitute redox processes (Schöneich et al., 1989). The thiyl radical ($RS^{\bullet}$) and their disulfide radical cation ($RSSR^{\bullet+}$) for example, has been found to serve as an oxidant and capable for H-abstraction for organic compounds in biochemical and petrochemical systems (Chen et al., 2009; Riyad et al., 2005). It has also been argued that the rate of petroleum formation depends critically on the concentration of sulfur radicals generated during the initial stages of thermal maturation (Lewan, 1998; Chang et al., 2001). However, the fates of sulfur-centered radical under oxidizing conditions in these systems are poorly understood. A further multiple sulfur isotope studies of organic sulfur species in these systems may provide new insight into the processes and mechanism on organic sulfur radical formation in petroleum and geothermal systems. Information gained from these systems will also complement our observed S-

33 effects and the abundance of saturated hydrocarbon formations as a result of carbon-sulfur and sulfur-sulfur cleavage in a thermally maturated organic rich sulfur petroleum source rock system.

The oxidation state and the evolution of the Earth's atmosphere is an important issue in geological and earth system sciences, and is linked the sedimentary distribution of carbon, sulfur, ferric and ferrous iron. The records of these elements depend greatly upon ambient oxygen pressure and should reflect any major preservational process and postdepositional processes of Archean sedimentary rocks. Future investigations of Precambrian sedimentary rocks containing kerogen or graphitic sulfur as remnants of ancient organic matter may provide additional information to study the evolution of life at the early stage of Earth history. The lack of reported Archean organic sulfur isotope data is attributed to the general lack of methods to extract the organosulfur and differentiate them from their inorganic counterparts. The next logical step after this thesis is to apply some of these methodologies to understand the digenetic history and redox chemistries of organic sulfur and carbon cycles in Archean sedimentary rocks.

3.0 Speculations and Ideas to Constrain Global Biogenic-S Fluxes

A key pathway in the sulfur cycle is the transfer of DMS from the sea to the land via the atmosphere. DMS may influence both the hydrologic cycle and the global heat budget through its role in cloud formation, which may alter patterns of precipitation and influence sea surface and land surface

temperatures. Planktonic production of DMS and its escape to the atmosphere is believed to be one of the mechanisms by which the biota can regulate the climate.

Evidence in this thesis suggests that S-isotopic composition of DMS in remote ocean areas far from terrestrial sulfur sources may be different from previously inferred. A better constraint on the $\delta^{34}S$ for marine algal DMSP/DMS provided by future measurements may offer a new way to determine the global fluxes of DMS. This approach can also be used to better constrain the biological and chemical reactions as well as ocean-atmosphere interactions DMSP/DMS cycle. To do this, we need to know the following about what affects its production in the ocean and escape to the atmosphere: 1) which phytoplankton species are high in DMSP and which have DMSP-lyase, 2) the species composition of the phytoplankton community and its succession in an area, 3) their global distribution and population density, and 4) other biotic interactions that effect DMS concentrations (e.g. zooplankton and bacteria populations). Abiotic factors, such as sea surface temperature and mixed layer depth, also have a direct impact on DMS production. Results of this type of analysis will help us to improve our future emission flux estimates of biogenic sulfur gas and its impact on the modeling of cloud physics, which will eventually improve climate models that will help to provide a more credible climate change projections.

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