METAL SULFIDES IN OXIDIZING FRESHWATER SYSTEMS

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ABSTRACT

The overall goal of this research project was to examine the occurrence and fate of S(II-) and associated trace metals in aqueous systems under oxidizing conditions, specifically freshwater sediments and surface waters. Three studies were conducted that examined the following: (a) the fate of Ag(I) sorbed to FeS(s) and the stabilization of S(II-) by Ni(II), Mn(II), Cu(II) and Zn(II) upon oxidation of an FeS(s) suspension, (b) the stabilization of S(II-) in oxic solution by Zn(II), Fe(II), Fe(III), Cu(II) and Ag(I) in association with Suwannee River fulvic acid (SRFA), and (c) the applicability of the colourimetric methylene blue-sulfide (MBS) method to the determination of trace S(II-) in oxic freshwaters.

Silver(I) added to an aqueous FeS(s) suspension was scavenged from solution by the solid phase. Upon oxidation of the Ag-FeS(s) suspension, S(II-) concentration in solution rapidly decreased while Ag(I) was released back into solution. Similar experiments were repeated with added amounts of Ni(II), Mn(II), Cu(II) and Zn(II) to examine the effect of these metals on S(II-) in solution during FeS(s) oxidation. Zinc(II) in the presence of FeS(s) retarded the loss of S(II-) from solution, suggesting the stabilization of S(II-) by Zn(II) under oxic conditions. Sulfide did not appear to be stabilized by the other metals as concentrations decreased below detectable levels. In the case of Cu(II) and Ni(II) however, stable metal sulfide species may have formed and persisted under oxic conditions, but were not reacted and detected in the MBS analysis.

Sulfide in aqueous solution in the absence of metals, with and without SRFA, decreased over time under oxic conditions due to reaction with oxygen and/or NOM. Zinc(II) was able to bind and stabilize S(II-) in the presence of NOM under oxic conditions, and this effect was attained even when other S(II-) binding metals, Fe(III) or Ag(I), were added to the sample. It was not, however, clear whether the association of Zn(II) with SRFA offered more stability to S(II-) than the Zn(II) alone. Iron in either oxidation state (II, III) did not stabilize S(II-) under oxic conditions regardless of whether NOM was present. Although Ag(I) and Cu(II) were both able to bind S(II-), even in the presence of NOM, it was not possible to assess whether these metals stabilized S(II-) under oxic conditions. In the presence of Ag(I) and Cu(II), MBS formation was inhibited in the acidic colourimetric reagent (MDR) and S(II-) could not be determined.

Using the methylene blue method of S(II-) determination (MBS), S(II-) was measured at low nanomolar concentrations in a number of natural freshwater samples of varying organic carbon content. This study revealed a number of limitations in the application of the MBS method to the measurement of S(II-) in natural samples. Most significantly, total S(II-) concentration was underestimated by this method, as S(II-) bound to Ag(I) and Cu(II) and possibly other metals, was not readily reactive in the colourimetric reagent to form the methylene blue complex. Pretreatment of the sample with Cr(II) to reduce such metal sulfide species, resulted in higher S(II-) recoveries relative to MBS. Particulates and NOM contained in samples interfered with absorbance measurements, while adsorption of metal sulfide species to container surfaces caused S(II-) concentrations to be underestimated.

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The course of my Ph.D. has taken me down a long and winding road and as much as it saddens me to end this chapter of my life, I'm happy to have finally found the end. Finding the way wasn't always easy nor was it always clear, and I never would have succeeded without the help and support of my colleagues, friends and family.

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LIST OF ABBREVIATIONS

APHA	American Public Health Association
ASV	Anodic Stripping Voltammetry
AVS	Acid Volatile Sulfide
CLS	Chromium Labile Sulfide
CSSWV	Cathodic Stripping Square Wave Voltammetry
DMS	Dimethyl Sulfide
DOC	Dissolved Organic Carbon
EXAFS	Extended X-ray Absorption Fine Structure
GC-FPD	Gas Chromatography - Flame Photometric Detection
GC-PID	Gas Chromatography - Photoionization Detection
HEPA	High Efficiency Particulate Air
HMDE	Hanging Mercury Drop Electrode
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IHSS	International Humic Substances Society
LM	Luther Marsh
MBS	Methylene Blue-Sulfide
MDR	Mixed Diamine Reagent
NOM	Natural Organic Matter
POC	Particulate Organic Carbon
POTW	Publicly Owned Treatment Works
PP	Polypropylene
SRFA	Suwannee River Fulvic Acid
STP	Sewage Treatment Plant
TOC	Total Organic Carbon
WTP	Wastewater Treatment Plant
UV/VIS	Ultraviolet/Visible
XAS	X-ray Absorption Spectroscopy
XANES	X-ray Absorption Near-Edge Spectroscopy
XRD	X-ray Diffraction

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CHAPTER 1

OVERVIEW

Reduced sulfur (S(II-)) forms under anoxic conditions predominantly in sedimentary systems. Sediments are generally presumed to be a sink for S(II-) where it is precipitated as an insoluble metal sulfide phase or alternatively becomes incorporated into organic matter. Changes in redox conditions however, resulting from activities such as sediment dredging or bioturbation, may induce S(II-) oxidation. The oxidation of metal sulfide phases could release associated metals into solution, thereby increasing their potential bioavailability. In reducing waters, inorganic and organic S(II-) species form soluble metal complexes and clusters. This speciation explains the higher than expected concentrations of metals (predicted from the solubility of mineral phases) often observed in natural waters, especially in sediment pore waters.

Within the last decade, the presence of S(II-) at pico- to nanomolar concentrations has been confirmed in oxic surface waters. This represents a very significant finding as S(II-) at these concentrations could play an important role in the speciation, and consequently the bioavailability of ultra-trace, Class B metals (e.g. Ag(I), Hg(II)). It has been suggested that S(II-) stabilization in oxic waters is attained through the formation of kinetically inert clusters with metals such as Zn, Cu and Fe. An association of these species with natural organic matter (NOM) is also likely to occur. Such an association could increase the stability of metal sulfide species as well as enhance or hinder metal bioavailability depending on the nature of the interaction. Consequently, the ability to determine S(II-) and how it occurs in surface waters is vital to our understanding of trace metal biogeochemistry and toxicity.

1.1 OBJECTIVES

The overall goal of this research project has been to examine the occurrence and fate of S(II-) and associated trace metals in aqueous systems under oxidizing conditions, specifically freshwater sediments and surface waters. The following questions were asked in order to achieve this goal:

(a) Are trace metals associated with FeS(s) released into the water column upon exposure to oxygen? Can metals stabilize S(II-) in solution during FeS(s) oxidation?

(b) Is S(II-) stabilized in solution in the presence of trace metals and/or NOM under oxic conditions?

(c) Is S(II-) present in oxic surface waters and, if so, is it associated with trace metals and NOM?

(d) Can colourimetric analysis (methylene blue-sulfide method, MBS) be applied to the trace level determination of S(II-) in natural waters, particularly in routine field measurements?

1.2 SUMMARY OF RESEARCH FINDINGS

A summary of the studies conducted and the respective findings is presented below:

Oxidation of Iron Monosulfide in the Presence of Sulfide Binding Metals

Amorphous iron monosulfide (FeS(s)) is formed in high proportions in anoxic sediments due to the abundance of Fe(II) compared to other metals. The instability of synthetic FeS(s) in the presence of molecular oxygen was demonstrated in some laboratory experiments. A suspension of synthetic FeS(s) was mixed with air and its oxidation examined over time by monitoring changes in solution pH, dissolved oxygen and S(II-) concentration. Upon exposure to oxygen, FeS(s) was rapidly oxidized to form an Fe(III) hydroxide while S(II-) concentration simultaneously decreased.

Silver(I) added to an aqueous FeS(s) suspension was scavenged from solution by the solid phase. Upon oxidation of the Ag-FeS(s) suspension, S(II-) concentration in solution rapidly decreased while Ag(I) was released back into solution. Similar experiments were repeated with added amounts of Ni(II), Mn(II), Cu(II) and Zn(II) to examine the effect of these metals on S(II-) in solution during FeS(s) oxidation. Zinc(II) in the presence of FeS(s) retarded the loss of S(II-) from solution, suggesting that Zn(II) stabilized S(II-) under oxic conditions. Sulfide in solution did not appear to be stabilized by the other metals as concentrations decreased below detectable levels. In the case of Cu(II) and Ni(II) however, stable metal sulfide species may have formed and persisted under oxic conditions but were not detected in the MBS analysis.

Stabilization of Sulfide in the Presence of Trace Metals and/or Natural Organic Matter

A series of experiments were conducted in the laboratory to assess the stabilization of S(II-) under oxic conditions by trace metals and NOM. Sulfide was added to aqueous solutions containing a metal [Zn(II), Fe(II), Fe(III), Ag(I) or Cu(II)] or a metal pair [Zn(II) & Fe(III), Zn(II) & Ag(I) or Zn(II) & Cu(II)] at concentrations ranging from 0 to 25 μM. Samples were prepared in either water or aqueous NOM (Suwannee River fulvic acid, SRFA) solution with an organic carbon content of ~ 5 mg C/L. Sulfide concentration was monitored over time as methylene blue-sulfide (MBS) during an anoxic (1 – 3 days) and an oxic period (3 – 7 days).

Sulfide in aqueous solution with and without NOM, decreased over time during the oxic period due to reaction with oxygen and/or SRFA. Zinc(II) was able to bind and stabilize S(II-) in the presence of SRFA under oxic conditions, and this effect was attained even when other S(II-) binding metals, Fe(III) or Ag(I), were added to the sample. It was not clear, however, whether the association of Zn(II) with SRFA offered more stability to S(II-) than Zn(II) alone. Iron in either oxidation state (II, III) did not appear to stabilize S(II-) under oxic conditions regardless of whether NOM was present, while the ability of Ag(I) and Cu(II) to stabilize S(II-) in these experiments could not be assessed. In the presence of these metals, MBS formation was inhibited in the acidic colourimetric reagent (MDR) and S(II-) could not be determined. Sulfide was, however, found to preferentially bind Ag(I) or Cu(II) to Zn(II).

Sulfide in Natural Surface Waters and its Determination Using Colourimetry

Using the methylene blue method of S(II-) determination (MBS), S(II-) was measured at low nanomolar concentrations in a number of natural freshwater samples. These samples originated from a variety of freshwater systems with a wide range in organic carbon content. This study provided further evidence for the presence and persistence of S(II-) in oxic waters, but revealed a number of limitations in the application of the MBS method to the measurement of S(II-) in natural samples. Most significantly, total S(II-) concentration was found to be underestimated by this method, as S(II-) bound to Ag(I) and Cu(II) was not readily reactive in the colourimetric reagent to form the methylene blue complex. Pretreatment of the sample with Cr(II) to reduce such metal sulfide species, resulted in higher S(II-) recoveries relative to MBS. Particulates and NOM in samples interfered with absorbance measurements, while adsorption of metal sulfide species to container surfaces caused S(II-) concentrations to be underestimated.

CHAPTER 2

INTRODUCTION

This review provides some background information on the chemistry and geochemistry of S(II-) species and NOM to aid in the interpretation of the research findings presented later in this thesis.

2.1 FORMS OF SULFUR

Sulfur is classified in Group VI in the periodic table. Relative to oxygen, sulfur is much larger and less electronegative and forms bonds with greater covalent character. It also exists in a wider range of formal oxidation states (-2 to +6) and exhibits higher coordination and a great propensity for catenation. Unlike oxygen, sulfur uses its empty *d* orbitals to form octahedral hybrid sp^3d^2-orbitals. It is the formation of such orbitals that allow sulfur to exist in oxidation states as high as +4 and +6 and to coordinate as many as 4, 5 or 6 atoms (Cotton, 1972; Nelson, 1978).

Sulfur forms a variety of mononuclear and polynuclear organic and inorganic species that are commonly found in the environment (see Table 2.1). Sulfide, S(II-), is the most reduced form of sulfur and contains sulfur in the lowest oxidation state, –2, and a number of different S(II-) containing species exist. The occurrence and fate of S(II-) species in oxygenated aqueous systems constitutes the main focus of this research thesis.

Table 2.1: Common sulfur ions and molecules (Nelson, 1978; Luther and Church, 1992; Luther *et al.*, 1996; Kramer *et al.*, 2000).

Sulfur Ion/Molecule	Formula	S Oxidation State
Inorganic Forms		
Elemental sulfur	S_8	0
Polysulfides	$^{-}S\text{-}S_n\text{-}S^{-}$, S_nS^{2-}	-1 (terminal S), 0
Hydrogen sulfide	H_2S	-2
Bisulfide	HS^-	
Sulfide	S^{2-}	
Carbonyl sulfide	COS	-2
Carbon disulfide	CS_2	-2
Thiosulfate	$S_2O_3^{2-}$	0 (terminal S), +4
Dithionite	$S_2O_4^{-2}$	+3
Sulfur dioxide	SO_2	+4
Sulfite	SO_3^{2-}	+4
Bisulfite	HSO_3^-	
Polythionates	$S_nO_6^{2-}$	0 (central S), +5 (SO_3)
Sulfur trioxide	SO_3	+6
Sulfate	SO_4^{2-}	+6
Sulfuric acid	H_2SO_4	
Metal Sulfide Forms		
Metal sulfides	e.g. FeS(s)	-2
Metal mono(bi)sulfide complexes M = Fe(II), Ni(II), Co(II), Mn(II)	M(SH) $M_2(SH)^{3+}$ $M_3(SH)^{5+}$	-2
Metal sulfide complexes, M = Zn(II), Cu(II)	M_nS_n $M_{2n}S_{3n}^{2-}$	-2
Organic Forms (R = organic group)		
Organic polysulfides	$R\text{-}S_n\text{-}S^-$	-1 (terminal S), 0
Thiols (e.g. cysteine, 3-MPA, glutathione)	R-SH	-1
Dimethyl sulfide (DMS)	$S(CH_3)_2$	0
Sulfoxide	R_2SO	+2
Sulfone	R_2SO_2	+4

Table 2.2: Metal ion classification (Pearson, 1966; Nieboer *et al.*, 1999).

CLASS A	INTERMEDIATE	CLASS B
Li^{+}, Na^{+}, K^{+}, Cs^{+}, Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Sc^{3+}, Y^{3+}, La^{3+}, Gd^{3+}, Lu^{3+}, Al^{3+}	Ti^{2+}, V^{2+}, Cr^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Sn^{2+}, Pb^{2+}, Fe^{3+}, Ga^{3+}, As^{3+}, In^{3+}, Sb^{3+}, Sn^{4+}	Cu^{+}, Ag^{+}, Au^{+}, Tl^{+}, Pd^{2+}, Pt^{2+}, Hg^{2+}, Bi^{3+}, Tl^{3+}, Pb^{4+}

The classification of metal ions as Class A ("hard sphere"), Intermediate or Class B ("soft sphere") is based on their ability to form covalent bonds (Table 2.2). Class A metal ions form bonds of greater ionic character and preferentially bind with oxygen (O) donor-atoms. In contrast, Class B ions form bonds of greater covalent character and preferentially bind with nitrogen (N) and sulfur (S) donor-atoms. Intermediate ions display ambivalent affinity for all three donor-atom types (Pearson, 1966; Stumm and Morgan, 1996; Nieboer *et al.*, 1999). Metal toxicity generally increases with covalent character (Stumm and Morgan, 1996; Nieboer *et al.*, 1999). Reduced sulfur, S(II-), binds with Class B and Intermediate metals to form insoluble metal sulfide compounds and soluble metal sulfide complexes and clusters.

Based on their respective sulfide solubility products listed in Stumm and Morgan (1996), Class B and Intermediate metal cations show a decrease in their affinity for S(II-), in the following order:

$$\mathrm{Hg(II)} > \mathrm{Ag(I)} > \mathrm{Cu(I)} > \mathrm{Cu(II)} \gg \mathrm{Cd(II)} > \mathrm{Pb(II)} > \mathrm{Ni(II)}, \mathrm{Co(II)} > \mathrm{Fe(II)} > \mathrm{Mn(II)}$$

2.2 CYCLING OF REDUCED SULFUR IN AQUEOUS SYSTEMS

The main sources and sinks of S(II-) species in aqueous systems are described in this section.

In anoxic sediments and waters, microbial dissimilatory reduction of sulfate (SO_4^{2-}) results in the production of hydrogen sulfide (H_2S) and the decomposition of organic matter. Most of the reduced sulfur, S(II-), produced finds a sink in such environments, as it reacts with metals to precipitate insoluble metal sulfide compounds or becomes incorporated into organic matter. Iron sulfides are particularly abundant in anoxic sediments due to the high concentration of Fe(II) relative to other metals. A variety of metastable iron sulfide compounds occur (e.g. amorphous iron monosulfide (FeS), mackinawite (FeS_{1-x}), greigite (Fe_3S_4) and pyrrhotite ($Fe_{1-x}S$)) as well as thermodynamically stable pyrite (FeS_2) (Pankow and Morgan, 1980; Morse and Cornwell, 1987; Gagnon *et al.*, 1995). Reduced sulfur also forms in the interstitial waters of sediments as dissolved inorganic sulfide and organosulfur species. Interactions of these species with metals results in the formation of soluble metal sulfide complexes and clusters (Boulegue *et al.*, 1982; Theberge *et al.*, 1997).

An increase in redox potential induces S(II-) oxidation resulting in the formation of a number of intermediate sulfur species such as elemental sulfur (S_8), polysulfides (S_nS^{2-}) and oxysulfur compounds (e.g. $S_2O_3^{2-}$). Ultimately, sulfate (SO_4^{2-}) is produced which is precipitated in the form of salts, reduced back to S(II-), or taken up by plants (Grinenko and Ivanov, 1983; Volkov and Rozanov, 1983; Luther and Church, 1992).

Dimethyl sulfide (CH_3SCH_3 or DMS) is the most abundant volatile organosulfur compound in open ocean waters, while smaller amounts of carbonyl sulfide (OCS), carbon disulfide (CS_2), and hydrogen sulfide (H_2S) also occur (Andreae and Jaeschke, 1992; Caron and Kramer, 1994). In freshwaters, methanethiol (CH_3SH) is the predominant volatile organosulfur compound, but dimethyl disulfide (CH_3SSCH_3), H_2S, OCS and CS_2 are also found (Caron and Kramer, 1994).

Dimethyl sulfide is produced by phytoplankton excretion and elimination from dimethylsulfonium propionate (DMSP), while consumption by bacterioplankton, photo-oxidation, volatilization across the air-sea interface and downward mixing into deeper waters, all serve to remove DMS from surface waters (Andreae and Jaeschke, 1992). Carbonyl sulfide is produced during photochemical reactions involving organosulfur compounds and is mainly lost from the ocean by diffusion to the atmosphere (Andreae and Jaeschke, 1992). Processes resulting in the production and removal of CS_2 and H_2S from surface waters are not well understood. Production of CS_2 in coastal surface waters is believed to occur in the underlying sediments from where the gas diffuses upwards to accumulate in the water surface. Carbon disulfide is either lost directly from surface waters by diffusion into the atmosphere, or is first photooxidized to SO_2 and OCS (Andreae and Jaeschke, 1992).

Proposed sources of H_2S in surface waters include, OCS hydrolysis (Elliot *et al.*, 1987), dissimilatory sulfate reduction within microenvironments throughout the water column (Cutter and Krahforst, 1988), release by phytoplankton that produce H_2S through biochemical pathways involving assimilatory sulfate reduction (Andreae and Jaeschke, 1992; Walsh *et al.* 1994; Radford-Knoery and Cutter, 1994; Ciglenečki and Ćosović, 1996) and atmospheric deposition (Andreae *et al.*, 1991). Although some H_2S diffuses into the atmosphere, removal predominantly occurs through oxidation of S(II-) by molecular oxygen (O_2) (Millero *et al.*; 1987), hydrogen peroxide (H_2O_2) (Millero *et al.*,

1989) and iodate (IO_3^-) (Zhang and Whitfield, 1986; Luther and Tsamakis, 1989), as well as the formation of insoluble metal sulfide compounds that become associated with sinking particles (Cutter and Krahforst, 1988; Luther and Tsamakis, 1989). Despite the instability of S(II-) under oxic conditions, pico- and nanomolar concentrations of S(II-) have recently been measured in air saturated marine waters and freshwaters (refer to *Section 2.5*). The stabilization of S(II-) in oxic waters is an unexpected finding and this phenomenon is examined in this thesis.

2.3 SULFUR SPECIATION IN NATURAL WATERS

Hydrogen sulfide is a weak diprotic acid that dissociates to form bisulfide (HS^-) and sulfide (S^{2-}) ions depending on the pH:

$$H_2S \rightleftharpoons H^+ + HS^- \qquad {}^{a}pK_1 = 6.98$$

$$HS^- \rightleftharpoons H^+ + S^{2-} \qquad {}^{b}pK_2 = 17 \text{ to } 19$$

[a] Berner, 1967; Goldhaber and Kaplan, 1975
[b] Stumm and Morgan, 1996

Although pH is the main variable determining the distribution of S(II-) species in solution, ionic strength and temperature may also have important effects. The estimation of pK_1 and pK_2 values show that HS^- would be the predominant species in most surface waters (pH = 6 – 9).

H_2S, HS^- and S^{2-} represent the main species of sulfur in reducing waters, but the incomplete oxidation of S(II-) can lead to the formation of elemental sulfur (S_8) and thiosulfate ($S_2O_3^{2-}$). Slow diffusion of oxygen, or the presence of Fe(III) minerals or organic matter may accomplish this in a reducing environment. In addition, polysulfide species (S_nS^{2-}) may form from the reaction of zero-valent sulfur (S(0)) with HS^-. Polysulfides become more important as pH and total sulfur concentration (S(II-), S(0)) increase (Bell and Kramer, 1999). The number of zero-valent sulfur atoms, *n*, varies from one to five and no clear agreement exists on this value in natural waters (Bell and Kramer, 1999). Hydrogen sulfide and polysulfides react with organic matter to produce a variety of organosulfur compounds (e.g. thiols, organic polysulfides) (Boulegue *et al.*, 1982; Aizenshtat *et al.*, 1995).

The distribution of stable sulfur species in aqueous solution as a function of Eh (redox potential) and pH is approximated in Figure 2.1 for total sulfur, $\Sigma S = 10^{-3}$ M (25 °C, 1 bar) (Brookins, 1988). Also plotted are the approximate Eh-pH zones of oxidizing, transitional and reducing environments, as well as the Eh-pH conditions of specific water types (1-10) (Brookins, 1988). Oxidizing environments fall within the range of highest Eh values (0.2 – 0.6) and vary in pH from 4 to 10. Reducing environments occur at redox potentials < 0 and range in pH from 3 to 9, and transitional environments are mildly reducing (Eh = -0.1 – 0.1).

Thermodynamically stable species of sulfur occurring within the entire pH range are shown in Figure 2.1 and consist of, sulfide (H_2S, HS^-), elemental sulfur (S_8) and sulfate (HSO_4^-, SO_4^{2-}). Although other sulfur species have been found to exist in solution for extended periods of time, they occur only as metastable intermediates (e.g. $S_2O_3^{2-}$). It is also noted that the entire zone of S(II-) stability lies in the field of low redox potentials (< 0), within the zone of reducing environments. Thus, in oxygenated waters, S(II-) is

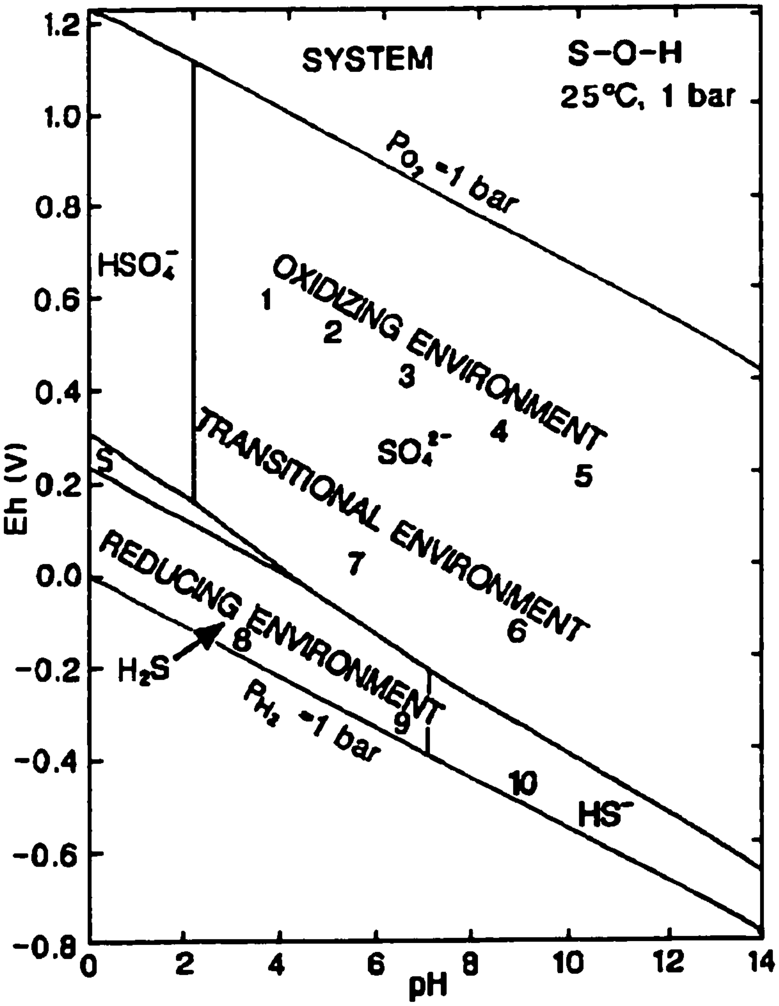


Figure 2.1: Stability field diagram for sulfur species in aqueous solution for $\Sigma S = 10^{-3}$ M (25 °C and 1 bar). Also plotted are the approximate Eh-pH zones of oxidizing, transitional and reducing environments, as well as the Eh-pH conditions of specific water types (1-10): *1* mine waters; *2* rain; *3* streams; *4* normal ocean water; *5* aerated saline water residues; *6* ground waters; *7* bog waters; *8* water-logged soils; *9* euxenic marine waters, *10* organic-rich, saline waters (modified from Brookins, 1988).

unstable and should be oxidized to SO_4^{2-} and various intermediate oxidation products.

2.4 FATE OF SULFIDE UNDER OXIC CONDITIONS

As was discussed in the previous section, H_2S, HS^- or S^{2-} are stable only in reducing waters and the presence and persistence of S(II-) in oxic solutions is unexpected. This section reviews the stability of reduced sulfur in oxic waters and describes its expected behaviour and fate in such environments.

A number of researchers have studied the oxidation of S(II-) by dissolved oxygen in natural waters in both laboratory and field studies (Skopintsev *et al.*, 1964; Cline and Richards, 1969; Östlund and Alexander, 1969; Snavely and Blount, 1969; Sorokin, 1970; Chen and Morris, 1972; Chen *et al.*, 1972; Almgren and Hagström, 1974; O'Brien and Birkner, 1977; Hoffmann and Lim, 1979; Millero *et al.*, 1987; Vazquez *et al.*, 1989; Millero 1991a; 1991b; 1991c; Zhang and Millero, 1993). Although other oxidants such as hydrogen peroxide (H_2O_2), iodate (IO_3^-) and Fe(III) and Mn(IV) (hydr)oxides may also oxidize S(II-) (Zhang and Whitfield, 1986; Millero *et al.*, 1989; Yao and Millero, 1993; 1995; Zhang and Millero, 1993), in natural waters dissolved oxygen represents the most important and abundant S(II-) oxidant. In laboratory studies, Millero *et al.* (1987) examined the oxidative effects of molecular oxygen on S(II-) and determined half-lives for S(II-) of 25 ± 9 h and 50 ± 16 h in seawater and freshwater, respectively. These findings are in good agreement with numbers determined by Chen and Morris (1972) and O'Brien and Birkner (1977), but half-lives ranging from 20 min to 65 h have been reported in the literature (Millero, 1986).

The oxidation kinetics of S(II-) have been found to be very complicated and the results of many workers are not in good agreement possibly due to the use of different experimental methods and conditions. In general, the reaction rate, extent of reaction and consequent product formation and distribution appear to be dependent on, the starting reactants (O_2, H_2S vs. HS^-), the absolute and relative concentrations of the starting reactants, pH, temperature, ionic strength, bacterial mediation and the presence of trace metals and organic compounds. Most commonly, a first-order dependence with respect to total S(II-) ([ΣS(II-)]) as well as oxygen concentration ($[O_2]$) has been reported for the initial rate of S(II-) disappearance, i.e.,

$$-d[\Sigma S(II-)]/dt = k\,[\Sigma S(II-)]\,[O_2]$$

where the rate constant, *k*, is pH dependent (Kuhn *et al.*, 1983; Millero, 1986; Morse *et al.*, 1987; Eary and Schramke, 1990; Zhang and Millero, 1993).

The end product of S(II-) oxidation is sulfate (SO_4^{2-}), the most thermodynamically stable sulfur compound under oxic conditions. However, the oxidative process involves many reaction steps and several metastable intermediate products such as elemental sulfur (S_8), polysulfides (S_nS^{2-}), sulfite (SO_3^{2-}) and thiosulfate ($S_2O_3^{2-}$) are formed depending on the reaction conditions. Mechanisms for the formation of these products are not well understood. The formation of S_8 and subsequently S_nS^{2-} is favoured at high $[\Sigma S(II-)]/[O_{2(aq)}]$ ratios, in neutral to low alkaline solutions when [ΣS(II-)] is ~ 10^{-3} M. At low $[\Sigma S(II-)]/[O_{2(aq)}]$ ratios, when oxygen is in excess, the formation of oxysulfur species is observed and SO_3^{2-}, $S_2O_3^{2-}$ and SO_4^{2-}, become the main products of S(II-) oxidation. Sulfite is formed first and then reacts to form other products (Chen and Morris, 1972; O'Brien and Birkner, 1977; Eary and Schramke, 1990; Kotronarou and Hoffmann, 1991; Zhang and Millero, 1993).

At low pH, H_2S is slowly oxidized to SO_3^{2-} that in turn is rapidly oxidized to $S_2O_3^{2-}$ and SO_4^{2-}. Thiosulfate however is unstable in acidic solution and consequently, both SO_3^{2-} and $S_2O_3^{2-}$ rapidly disappear from solution while SO_4^{2-} is accumulated. At more basic pH values, S(II-) oxidation is more rapid due to the greater reactivity of HS^- relative to H_2S, while SO_3^{2-} and $S_2O_3^{2-}$ are more stable and thus persist in solution. Over time however, these species are oxidized to SO_4^{2-}, unless bacterial mediation is absent in which case $S_2O_3^{2-}$ remains stable (Zhang and Millero, 1993).

Several studies have examined the catalytic effects of transition metals on the rate of S(II-) oxidation (Chen *et al.*, 1972; Cline and Richards, 1969; Snavely and Blount, 1969; Hoffmann and Lim, 1979; Vazquez *et al.*, 1989). Unlike other workers, Vazquez *et al.* (1989) studied this effect in seawater at low metal concentrations that precluded the formation of solid phases and more realistically reflected the occurrence of metals in natural waters. Of the metals studied, only Fe^{2+}, Cu^{2+} and Pb^{2+} affected the reaction rate at concentrations below 300 nM, while at higher concentrations, an increase in the rates followed the order:

$$Fe(II) > Pb(II) > Cu(II) > Fe(III) > Cd(II) > Ni(II) > Co(II) > Mn(II)$$

when the initial sulfide concentration was 25 μM. Zinc(II) did not exhibit a catalytic effect on the oxidation of S(II-) and actually suppressed the rate of oxidation at metal concentrations above 2 μM. At equal concentrations of Zn(II) and S(II-) ($pH = 8.1$, $T = 25\ ^\circ C$), the rate decreased by a factor of 30.

Metal ions and metal complexes tend to be effective catalysts of S(II-) oxidation because they are able to alter the electronic structure of either S(II-) or oxygen to lower the activation energy of the reaction (Kotronarou and Hoffmann, 1991). Apart from catalyzing the rate of S(II-) oxidation, metal ions may also influence the distribution of oxidation products (Zhang and Millero, 1993). Finally, catalytic effects on S(II-) oxidation have also been observed by some organic compounds such as phenols, aldehydes, aniline, urea, and vanillin, while others such as ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), cyanide, citrate, peptone and glycerol exhibit inhibitory effects (Chen *et al.*, 1972; Cline and Richards, 1969).

2.5 PRESENCE OF SULFIDE IN OXIC WATERS

Elliot *et al.* (1987) first postulated that hydrogen sulfide is released into ocean surface waters from the hydrolysis of carbonyl sulfide (OCS). Calculations involving the rates of OCS hydrolysis and oxidative removal of S(II-) by molecular oxygen, indicated that residual S(II-) would be expected to occur in ocean surface waters at pico- to nanomolar concentrations. The report of a single S(II-) measurement by Cutter and Oatts (1987) determined in the oxic surface waters of the western North Atlantic Ocean, verified this hypothesis. Using a newly developed, highly sensitive technique that employed gas chromatography coupled with a photoionization detector, Cutter and Oatts (1987) successfully measured S(II-) at a concentration of 0.51 ± 0.07 nM. Using the same method, Cutter and Krahforst (1988) subsequently determined spatial and temporal distributions of S(II-) throughout the western North Atlantic Ocean and reported concentrations in the range of < 0.1 to 1.1 nM.

Sulfide concentrations were also measured throughout the oxic water columns of the Mediterranean Sea and the western North Atlantic Ocean (Luther and Tsamakis, 1989). Luther and Tsamakis (1989) used cathodic stripping square wave voltammetry (CSSWV) with a hanging mercury drop electrode (HMDE) to make these S(II-) measurements. They found S(II-) to be constant at approximately 2 nM throughout the water column of the Mediterranean Sea (4000 m), while concentrations determined at several locations in the Atlantic Ocean, to a depth of 1500 m, ranged from 0.24 – 2.26 nM.

Prior to the first measurement made by Cutter and Oatts (1987), sampling protocols and analytical techniques had not been sensitive enough to determine S(II-) at such trace levels. Since then, improvements in both areas have allowed several researchers to verify the presence of S(II-) in oxic marine waters, and more recently freshwaters, by three different methods: gas chromatography, electrochemistry and colourimetry (methylene blue-sulfide method). Reported S(II-) concentrations in surface waters are compiled in Table 2.3 for marine waters, and Table 2.4 for freshwaters. The analytical method employed in each determination is also indicated.

Sulfide has been found to persist in oxic waters for periods much longer than expected from oxidation studies of S(II-) (see *Section 2.4*). Rozan *et al.* (1999) calculated half-lives ranging between 5 and 21 days for S(II-) occurring in freshwater samples taken from the Naugatuck River (CT, USA), while Luther and Tsamakis (1989)

Table 2.3: Sulfide concentrations measured in surface marine waters.

Sampling Location	Sulfide Concentration	Detection Limit	Analytical Method	Reference
Western North Atlantic Ocean	0.51 ± 0.07 nM	0.13 nM	*GC-PID	1
Western North Atlantic Ocean	< 0.1 – 1.1 nM	0.13 nM	*GC-PID	2
Mediterranean Sea	~ 2 nM	0.1 nM	CSSWV	3
Western North Atlantic Ocean	0.24 – 2.26 nM	0.1 nM	CSSWV	
Western North Atlantic Ocean	33 – 930 pM	0.5 pM	GC-PID	4
Black Sea	2.25 – 5.5 nM	na	CSSWV	5
Pacific Ocean	46 ± 2.7 pM 39.1 ± 1.5 pM	0.2 pM	GC-FPD	6
San Francisco Bay	< 1 – 162 nM	na	CSSWV	7
Western North Atlantic Ocean	< 1 – 550 pM	0.5 pM	GC-FPD	8
Northern Adriatic Sea	10 – 50 nM	na	CSSWV	9
North Atlantic Ocean	57.8 ± 23.7 pM	0.2 pM	GC-FPD	10
Arabian Sea	2 nM	na	CSSWV	11
Santa Barbara Basin	3 ± 1 nM	na	CSSWV	12
Galveston Bay	2.1 – 4.2 nM	na	*Colourimetry-HPLC-UV/VIS	13

1. Cutter and Oatts, 1987
2. Cutter and Krahforst, 1988
3. Luther and Tsamakis, 1989
4. Andreae *et al.*, 1991
5. Luther *et al.*, 1991
6. Radford-Knoery and Cutter, 1993
7. Kuwabara and Luther, 1993
8. Radford-Knoery and Cutter, 1994
9. Ciglenečki and Ćosović, 1996
10. Cutter *et al.*, 1999
11. Theberge *et al.*, 1997
12. Kuwabara *et al.*, 1999
13. Tang and Santschi, 2000

GC: gas chromatography
FPD: flame photometric detection
PID: photoionization detection
CSSWV: cathodic stripping square wave voltammetry
Colourimetry: methylene blue-sulfide method

*sulfide was preconcentrated
na: not available

Table 2.4: Sulfide concentrations measured in surface freshwaters.

Sampling Location	Sulfide Concentration	Detection Limit	Analytical Method	Reference
Pattaquamscutt River, RI, USA	615 ± 27 pM	0.2 pM	GC-FPD	1
Cobalt Mining Camp, ON, Canada	<1 – 570 nM	1 nM	Colourimetry-UV/VIS	2
Rivers, USA:		5 nM	CSSWV	3
Hammonasset, CT	~4.2 – ~5.8 nM			
Pawcatuck, RI	4.9 – 25 nM			
Quinnipiac, CT	31.5 – 64.5 nM			
Naugatuck, CT	18.6 – 54.8 nM			
ON, Canada:		1 – 2 nM	Colourimetry-UV/VIS	4
Dundas WTP	223 – 256 nM			
Burlington WTP	280 nM			
Desjardins Canal	184 – 206 nM			
Rivers, USA:		1 nM	CSSWV	5
Broadkill, DE	22 nM			
Bransdywine, DE	390 nM			
Christina, DE	480 nM			
Patuxent, MD	360 nM			
Raritan, NJ	250 nM			
Schuykill, PA	200 nM			
Potomac, VI	580 nM			
STPs, USA:				
Wil., DE	3250 nM			
Lewes, DE	1690 nM			

1. Radford-Knoery and Cutter, 1993
2. Kramer *et al.*, 1999
3. Rozan *et al.*, 1999
4. Adams and Kramer, 1999b
5. Rozan *et al.*, 2000a

GC: gas chromatography
FPD: flame photometric detection
PID: photoionization detection
CSSWV: cathodic stripping square wave voltammetry
Colourimetry: methylene blue-sulfide method

STP: sewage treatment plant
WTP: wastewater treatment plant

measured S(II-) in seawater samples from the Mediterranean Sea and Atlantic Ocean ten months after their collection. This finding is particularly surprising in seawater where S(II-) oxidation by iodate (IO_3^-) represents a significant sink for S(II-) and occurs at a much faster rate than with oxygen (Zhang and Whitfield, 1986; Zhang and Millero, 1993; Radford-Knoery and Cutter, 1994). It has been suggested, that IO_3^- reacts only with free, uncomplexed S(II-) (Luther and Tsamakis, 1989; Cutter *et al.*, 1999). This indicates that S(II-) would have to be stabilized in seawater, presumably by binding to Class B metals, otherwise rapid removal by IO_3^- would preclude its existence and measurement (Cutter *et al.*, 1999).

Nanomolar concentrations of S(II-) in surface waters are sufficient to control the speciation and bioavailability of S(II-) binding metals present at trace concentrations in such waters. Typical concentrations of S(II-) binding metals in surface marine and freshwaters are summarized in Table 2.5.

2.6 SULFIDE STABILIZATION UNDER OXIC CONDITIONS

The stabilization of S(II-) in surface waters has been attributed predominantly to the formation of stable, metal sulfide clusters, particularly of Cu, Zn and Fe (Luther and Tsamakis, 1989; Luther *et al.*, 1996; Rozan *et al.*, 1999; Rozan *et al.*, 2000a). Luther and Tsamakis (1989) suggested that sulfide bound to metals with inert gas electron

Table 2.5: Metal ion concentrations in surface marine waters and freshwaters. Mean values are shown in brackets following each concentration range.

Metal Ion	Marine Waters (M)	Freshwaters (M)
Mn[a]	$3\times10^{-10} - 4\times10^{-9}$ (6×10^{-10})	$8\times10^{-10} - 5\times10^{-6}$ (2×10^{-7})
Fe[a]	$2\times10^{-10} - 6\times10^{-9}$ (2×10^{-9})	$3\times10^{-7} - 5\times10^{-5}$ (1×10^{-5})
Ni[a]	$4\times10^{-9} - 3\times10^{-8}$ (2×10^{-8})	$6\times10^{-10} - 8\times10^{-7}$ (2×10^{-8})
Co[a]	$2\times10^{-11} - 2\times10^{-10}$ (4×10^{-11})	$1\times10^{-9} - 2\times10^{-7}$ (6×10^{-9})
Cu[a]	$8\times10^{-10} - 8\times10^{-9}$ (8×10^{-9})	$6\times10^{-9} - 8\times10^{-7}$ (1×10^{-7})
Zn[a]	$8\times10^{-11} - 2\times10^{-8}$ (1×10^{-8})	$5\times10^{-9} - 3\times10^{-6}$ (3×10^{-7})
Cd[a]	$8\times10^{-13} - 1\times10^{-9}$ (8×10^{-8})	$1\times10^{-10} - 4\times10^{-8}$ (2×10^{-9})
Pb[a]	$3\times10^{-11} - 2\times10^{-10}$ (1×10^{-11})	$1\times10^{-9} - 1\times10^{-6}$ (3×10^{-8})
Ag[b,c]	$1\times10^{-12} - 2\times10^{-11}$	$<1\times10^{-13} - 1.4\times10^{-9}$
Hg[d,e]	$4\times10^{-12} - 1\times10^{-11}$	1×10^{-11}

[a] Buffle and De Vitre, 1994
[b] Miller and Bruland, 1995
[c] Kramer *et al.*, 2000
[d] Gill and Fitzgerald, 1988
[e] Fitzgerald, 1989

configurations form stronger than expected metal-sulfide bonds due to the involvement of high ligand field stabilization energies. Zinc(II), for instance, may form tetrahedral, as opposed to octahedral, complexes with S(II-) allowing for the formation of shorter bonds of greater covalent character (Luther *et al.*, 1999).

The formation of kinetically inert metal sulfide clusters of Cu(II) and Zn(II) in the laboratory (Daskalakis and Helz, 1993; Helz *et al.*, 1993; Luther *et al.*, 1996; Luther *et al.*, 1999), and the very recent identification and measurement of Cu, Zn and Fe sulfide clusters in a variety of oxic river waters (Rozan *et al.*, 1999; Rozan *et al.*, 2000a), have supported this theory.

2.7 METAL SULFIDES IN SOLUTION

Soluble metal sulfide species form as precursors (intermediates) in the formation, and as products in the dissolution, of sulfide minerals (e.g. ZnS, CdS, CuS, Ag_2S, FeS, FeS_2) (Gammons and Barnes, 1989; Daskalakis and Helz, 1992; Daskalakis and Helz, 1993; Theberge *et al.*, 1997; Luther *et al.*, 1999). Consequently, the presence of such species in natural waters would be expected and a brief review follows to help determine the role of metal sulfide clusters in the biogeochemical cycling of metals and the stabilization of S(II-) in oxic waters.

2.7.1 Metal Sulfide Clusters

Metal nanoclusters (clusters) are generally defined as quantum sized "particles" that consist of an indefinite number of atomic or molecular units with a defined stoichiometry (Nedeljković *et al.*, 1993; Luther *et al.*, 1999). They are multinuclear with respect to the metal and may incorporate more than one metal type. Clusters range in size from 1 to almost 10 nm (in diameter), or have agglomeration numbers (number of individual atoms or molecular units in a given cluster) of less than a few hundred (Nedeljković *et al.*, 1993). Metal clusters exhibit unique electronic and optical properties that are size dependent, and differ considerably from those of either the individual molecules comprising the cluster, or the bulk materials (Hao *et al.*, 1998). Decreasing the particle to small sizes (< 5 nm in diameter) induces a quantum (spatial) confinement of the photogenerated electron-hole pair, and a blue shift (higher energy, shorter wavelength) in the absorption edge relative to the bulk material, is observed. This phenomenon is referred to as the "quantum size effect" (Hao *et al.*, 1998) and allows for the identification and characterization of these clusters.

Metal sulfides in solution were thought to form as mononuclear metal (bi)sulfide and polysulfide species. In an EXAFS (extended X-ray absorption fine structure) study however, examining sulfide complexes of Zn(II) and Cu(II) at elevated concentrations, Helz *et al.* (1993) provided evidence for the occurrence of multinuclear sulfide clusters. Studies on the dissolution of sphalerite (ZnS) have indicated that zinc sulfide occurs in solution as a neutral six-membered ring cluster, $Zn_3S_3(H_2O)_6$ and an anionic tetrameric cluster, $[Zn_4S_6(H_2O)_6]^{-4}$ (Daskalakis and Helz, 1993; Luther *et al.*, 1999). Furthermore, titrations of (bi)sulfide with Cu(II) and Zn(II) have revealed the formation of stable clusters with sulfide ion (S^{2-}) and similar empirical stoichiometries (Cu, Zn: S^{2-}) of 1:1 and 2:3 (Luther *et al.*, 1996; Rozan *et al.*, 1999; Kramer *et al.*, 2000). Finally, Cu_3S_3, Cu_4S_4, Zn_3S_4 and $Fe_2S_4OH_5$ clusters have very recently been identified in oxic river waters (Rozan *et al.*, 2000a).

2.7.2 Characterization of Metal Sulfide Clusters

Metal sulfide clusters formed in solution at low concentrations (< 20 μM) have been examined in a number of laboratory and field studies combining the use of voltammetric, spectroscopic, filtration, electrophoresis, kinetic and molecular modelling approaches (Luther *et al.*, 1996; Luther *et al.*, 1999; Rozan *et al.*, 1999; Kramer *et al.*, 2000; Rozan *et al.*, 2000a). The findings of these studies are summarized below.

In the laboratory (Luther *et al.*, 1996), Mn(II), Fe(II), Ni(II) and Co(II) formed weak, kinetically labile (exchangeable ligands) clusters with bisulfide (HS^-) ion at seawater pH (8.1). Proposed structures and empirical stoichiometries for these species were, MSH^+, $M_2(SH)^{3+}$ and $M_3(SH)^{5+}$. Dissociation of these clusters to release H_2S occurred when the solution was purged with N_2 or Ar gas, or the pH was lowered below 7. Under the same conditions, Cu(II) and Zn(II) formed more stable and kinetically inert (resisted dissociation at the electrode) clusters with sulfide (S^{2-}) ion (i.e. MS, $M_2S_3^{2-}$). In the formation of Cu(II) sulfide cluster, a variable amount of Cu(II) is reduced to Cu(I), while sulfide is oxidized to polysulfide species (Radford-Knoery and Cutter, 1993; Luther *et al.*, 1996; Rozan *et al.*, 2000a). Copper(II) and Zn(II) sulfide clusters were not dissociated upon purging of the solution at seawater pH. At $pH < 6.7$, Zn(II) clusters dissociated to release H_2S, but dissociation of Cu(II) clusters did not begin until the pH was lowered below 5, and was not complete even at pH values below 2.

The formation of Cu(II) and Zn(II) sulfide clusters occurred with very large thermodynamic stability constants (log *K*) [11.20 ± 0.78 (CuS), 38.29 ± 0.89 ($Cu_2S_3^{2-}$), 11.74 ± 014 (ZnS); 41.09 ± 0.59 ($Zn_2S_3^{2-}$)] (Luther *et al.*, 1996). These species are stable and would be expected to resist oxidation by molecular oxygen.

Zinc sulfide clusters synthesized in the laboratory absorbed in the UV region at 200 – 290 nm (Luther *et al.*, 1999). Diameters for these clusters were calculated based on the quantum size effect and ranged from 1.6 to 2.8 nm.

As a Class B metal, Ag(I) binds S(II-) very strongly and consequently, the formation of Ag(I) sulfide clusters has also been examined. Furthermore, Ag(I) is extremely toxic to aquatic organisms (Andren and Bober, 2000) and its occurrence and behaviour in oxic waters has generated much interest within the last decade.

Electrochemical and mass spectrometric measurements revealed the formation of multinuclear Ag(I) sulfide clusters with empirical stoichiometries (Ag:S) of 1:1 and 2:1 upon titration of the solution with (bi)sulfide ion. The high thermodynamic stability constants (log $K_{AgS} = 22.3$), and a resistance to acid dissociation above pH of 2, indicated that these clusters are very stable (Rozan and Luther, 2000) and should resist oxidation. Silver(I) has was also shown to rapidly replace Zn(II) and Cu(II) to become incorporated in their respective sulfide cluster species (Rozan and Luther, 2000). This finding is very important as it suggests that in natural waters, more abundant trace metals may provide a pool of S(II-) for Ag complexation as well as for other less abundant, S(II-) binding metals. This in turn may affect the bioavailability and toxicity of such metals.

Adams and Kramer (1999a) measured Ag(I) and S(II-) in oxic wastewaters and found that Ag(I) bound to colloidal metal sulfide phases with high binding constants in the range of 10^{11} to 10^{12}. The findings of Adams and Kramer (1999b) further indicate that Ag(I) sulfide cluster species may occur and persist in oxic waters.

2.8 NATURAL ORGANIC MATTER (NOM)

Natural organic matter (NOM) is a ubiquitous component of natural waters that interacts with metals and affects their mobility and bioavailability. Consequently, an association between NOM and metal sulfide species may occur in surface waters and NOM may play a role in the stabilization of S(II-) in oxic waters. This concept is explored in this research project and thus some background information on the geochemistry of NOM is provided in this section.

2.8.1 Description of Aquatic Humic Substances

Natural organic matter (NOM) occurring in natural waters can be divided into dissolved (DOC) and particulate (POC) organic carbon. Operationally defined as that portion of the NOM that passes through a 0.45 μm filter, DOC represents the chemically reactive fraction of the NOM and includes primarily the following organic compound groups: humic substances (50%), hydrophilic acids (30%), carbohydrates (10%), carboxylic acids (7%), amino acids (3%), and hydrocarbons (< 1%) (Aiken, 1985; Thurman, 1985).

Humic substances which consist of fulvic acids (80%) and humic acids (20%), comprise half of the DOC in natural waters (Thurman, 1985). They originate in plant and soil systems and are leached by interstitial waters into streams and rivers, while algal productivity also contributes to their formation in lakes and marine waters (Thurman, 1985). DOC concentration measured in visually uncoloured surface waters of the United States was found to range between 1.5 and 10 mg C/L with an average value of 5 mg C/L (Malcolm, 1985). The concentration was extremely variable in organically coloured stream waters, and ranged from approximately 5 mg C/L to greater than 50 mg C/L (Malcolm, 1985). Groundwater and marine waters typically contain the lowest DOC concentrations ranging from 0.05 to 0.25 mg C/L (Thurman, 1985).

Table 2.6: Summary of metal binding functional groups occurring in aquatic natural organic matter (Thurman, 1985; Stevenson, 1994).

Functional Group	**Structure**
Amino	$-NH_2$
Amine	$R-CH_2-NH_2$
Amide	$R-C(NH_2)=O$
Imino	$=NH$
Alcohol	$R-CH_2-OH$
Phenol	$Ar-OH$
Enol	$R-CH=CH-OH$
Aldehyde	$R-C(H)=O$
Ketone	$RC(R')=O$
Ester	$R-C(OR')=O$
Carboxyl	$R-C(OH)=O$
Keto acid	$R-C(COOH)=O$
Sulfhydryl	$R-SH$

R: organic group
Ar: benzene ring

Humic substances are coloured, nonvolatile, polyelectrolytic acids that range in molecular weight from 500 to 5000 Da. They exhibit an approximate elemental composition of 50% C, 35 – 40% O, 4 – 5% H, 1 – 2% N and < 1% S and P. They contain predominantly, carboxylic acid (COOH), phenolic hydroxyl (ArOH), carbonyl (C=O), and hydroxyl (OH) functional groups (Thurman, 1985). Table 2.6 summarizes the most important metal binding sites occurring in aquatic humic substances. Aquatic humic substances are defined as the fraction of organic matter isolated from water by sorption onto XAD resins, weak–base anion exchange resins, or any other comparable procedure. Humic acids represent the fraction that precipitates out of aqueous solution at pH below 2.0 while fulvic acids are soluble at all pH values. This forms the basis for the separation of humic acids from fulvic acids (Thurman, 1985).

Variability in composition encountered between different samples of humic substances is related to the biomass precursors involved in their formation and thus their environment of origin, and the methods employed in their isolation and concentration. Changes in organic matter caused by the processing procedure is a serious problem as the sample may no longer be truly representative of the original material (Aiken, 1988; Xia *et al.*, 1998). This has severe implications in interpreting the geochemical and environmental behaviour of aquatic humic substances (Aiken, 1988).

2.8.2 Interactions of Metals with Humic Substances

Organic matter binds metals through the numerous functional groups present within its complex structure. Interaction of metallic ions with organic matter may be in the form of purely electrostatic bonds (e.g. metal adsorption and ion exchange reactions), or strong, covalent bonds (e.g. formation of complexes and chelates) (Francois, 1990). Monovalent cations generally interact electrostatically with oxygen-containing functional groups (e.g. $RCOO^-$) and are easily exchanged with H^+, NH_4^+ as well as other metals. Covalent bonds are formed when metals possessing empty orbitals in their valence shell interact with electron donating groups (O, N, S, P) contained in the organic matter (Francois, 1990). Class B transition metal ions containing 8 or 10 electrons in their valence shells – e.g. Cu(I), Ag(I), Hg(II) – preferentially coordinate with S containing groups in this manner. Covalently bound metals are strongly held in place and can not be completely displaced by neutral salt extraction or acidification (Francois, 1990).

Metals interacting with organic matter can coordinate with more than one organic ligand and with more than one binding site on a single ligand. Metals may also become physically entrapped within condensed organic structures which are released only upon degradation of the organic molecule (Francois, 1990).

2.8.3 Sulfur Incorporation into Organic Matter

Organic matter represents an important sink for sulfur in sedimentary systems. Incorporation of sulfur into sedimentary organic matter to form organosulfur compounds, occurs predominantly through geochemical pathways during the stages of early diagenesis. The process involves the reaction of organic matter mainly with H_2S formed during the dissimilatory reduction of sulfate, or polysulfide species (HS_n^-, S_nS^{2-}) resulting from the partial oxidation of sulfide. Other oxidation intermediates such as sulfite (SO_3^{2-}) or thiosulfate ($S_2O_3^{2-}$) may also be involved while the direct reaction of elemental sulfur (S_8) with organic matter is usually not observed under the mild conditions of early diagenesis (Francois, 1987; Aizenshtat *et al.*, 1995; Adam *et al.*, 1998).

Formation of low molecular weight or macromolecular organosulfur compounds from the reaction of organic matter with reduced sulfur species has been demonstrated in laboratory experiments. Suggested reaction pathways include, addition of elemental sulfur on unsaturated aldehydes in the presence of an amine, addition of anionic sulfur species (HS^-, HS_n^-, S_nS^{2-}) on carbonyl functionalities, conjugated carbonyls (Michael addition) or an isolated double bond and, photo-induced and other radical mechanisms involving H_2S or elemental sulfur (Adam *et al.*, 1998). Under the slightly basic conditions usually encountered in sediments, the reaction most likely proceeds via a nucleophilic pathway (Michael addition) where a reduced sulfur species is added on an aldehyde, ketone or α,β-unsaturated carbonyl group contained in the organic matter (Aizenshtat *et al.*, 1995).

2.8.4 Sulfur Speciation in NOM

Although the incidence of sulfur containing functional groups in humic substances is much lower than that of oxygen, they also represent important metal binding sites. Sulfur donor atoms bind metals strongly in covalent bonds and consequently may affect the biogeochemical cycling of toxic trace metals (Xia *et al.*, 1998). Reduced sulfur groups such as thiols (R-SH) are particularly suitable complexing functionalities for Intermediate and Class B metals, and their presence in humic substances may account for the metal enrichment observed in sediments and sediment pore waters (Boulegue *et al.*, 1982; Francois, 1987). An understanding of the speciation of sulfur in humic substances may help elucidate the role of organosulfur in the complexation of metals.

Using X-ray absorption near-edge structure spectroscopy (XANES), Xia *et al.* (1998) found that sulfur contained in Suwannee River reference humic and fulvic acids exists in four major oxidation groups similar to: thiol and sulfide (R-SH, R-S-R), sulfoxide (R_2SO), sulfonate (RSO_3H) and sulfate ($ROSO_3H$). Thiol and sulfide sulfur represented approximately 46% and 35% of the total sulfur fraction in the humic and fulvic acid, respectively. Furthermore, great variation was reported in reduced sulfur composition between humic substances of different origins suggesting that the observed speciation is dependent on the environmental origins of the organic matter. In a more qualitative study using XANES analysis, Morra *et al.* (1997) reported the occurrence of similar sulfur oxidation states in Suwannee River humic and fulvic acids.

In a study employing XAS (X-ray absorption spectroscopy) (Xia *et al.*, 1997), Zn(II) was shown to preferentially bind with thiol functional groups when complexed with a Chelex-extracted soil humic substance. When complexed with Suwannee River humic or fulvic acid however, coordination occurred exclusively with oxygen containing functional groups indicating that the average binding sites for Zn(II) differed between the various types of humic matter. Cobalt(II), Ni(II) and Cu(II) interacted only with oxygen containing groups regardless of the type of humic substance.

2.9 METHODS OF SULFIDE DETERMINATION IN NATURAL SURFACE WATERS

Three analytical methods are most commonly applied to the determination of S(II-) in natural surface waters: electrochemistry, gas chromatography, and colourimetry (methylene blue-sulfide method). Other methods are also available for the quantitative determination of sulfide and include: (a) iodometric titration (volumetric), (b) oxidation to sulfate followed by precipitation of barium sulfate (gravimetric) and, (c) precipitation with bismuth or lead in a supporting medium (turbidimetric) (Siegel, 1965). These methods however are too insensitive for the analysis of S(II-) in natural waters.

Electrochemical, gas chromotographic and colourimetric methods of S(II-) determination in natural waters are described and compared in the following sections.

The methylene blue-sulfide method was used for all S(II-) measurements conducted in this research project.

2.9.1 Electrochemical Determination

Electroanalysis is a powerful technique for the study of trace element speciation in natural waters. Anodic stripping voltammetry (ASV) is the most widely used electrochemical technique for trace metal speciation in solution due to its extremely high sensitivity (Florence, 1986). Cathodic stripping (square wave) voltammetry (CSSWV) is commonly applied to the determination of S(II-) in solution, and has been used to identify and measure free and metal complexed S(II-) in oxic waters at nanomolar concentrations (Luther and Tsamakis, 1989; Luther *et al.*, 1991; Kuwabara and Luther, 1993; Ciglenečki and Ćosović, 1996; Kuwabara *et al.*, 1999; Rozan *et al.*, 1999; Rozan *et al.*, 2000a).

Under basic conditions, (bi)sulfide ion (HS^-) electrochemically oxidizes the Hg(s) electrode in a reversible reaction thus allowing S(II-) to be detected by cathodic stripping voltammetry (CSV) (Luther *et al.*, 1996; Al-Farawati and van den Berg, 1999):

$$HS^- + Hg \rightleftharpoons HgS(ads) + H^+ + 2e^-$$

Initially, at a potential more positive than that of S(II-) reduction, the reaction is driven to the right and sulfide is concentrated on the electrode. During the stripping step (CSV scan), the Hg^{2+} contained in the precipitated (or adsorbed) S(II-) species is reduced, and the above reaction is driven to the left to release S(II-) back into solution. The height of the peak obtained is directly dependent on the amount of S(II-) reacting at the electrode (Al-Farawati and van den Berg, 1999).

Free S(II-) reacting at the electrode may be contributed from S(II-) dissociating in the electrode diffusion layer from metal sulfide species. Metal sulfide species that dissociate in this manner to release S(II-) under diffusion control conditions, are termed labile (reactive). In contrast, metal sulfide species that are not dissociated in this manner are termed nonlabile, and are considered to be kinetically inert (Luther *et al.*, 1996).

As was noted previously, the reaction between HS^- and Hg(s) at the electrode is reversible and thus can be expressed in Nernstian form:

$$E = E^o - \left(\frac{RT}{nF}\right) \ln \frac{[HS^-]}{[H^+]}$$

where E is the experimentally determined peak potential, E^o is the standard electrode potential, R is the gas constant, T is the temperature, F is the Faraday constant and n is the number of electrons transferred in the reaction.

The Nernst equation indicates that shifts in the peak potential may be linked to changes in the solution pH allowing for the stoichiometry of a given sulfur species with H^+ to be determined (Luther *et al.*, 1996; Rozan *et al.*, 1999). Shifts in the peak potential may also occur in response to changes in the concentration of the electroactive species (HS^-), or when S(II-) is complexed by cations (Luther *et al.*, 1996). Addition of free S(II-) to the solution would induce a negative shift in the peak potential, while S(II-) complexation to a metal would induce a positive shift in the peak potential (Luther and Ferdelman, 1993).

Voltammetric methods have thus been used to identify and measure free sulfide and complexed sulfide in natural waters (detection limit < 0.1 nM) (Luther and Tsamakis, 1989), discriminate between labile (reactive) and inert forms of soluble metal complexes (Luther *et al.*, 1996), and provide information on the stoichiometry and thermodynamic stability of metal sulfide species (Zhang and Millero, 1994; Luther *et al.*, 1996; Al-Farawati and van den Berg, 1999). The development of these methods to measure S(II-) and metal sulfide species directly and simultaneously in natural water samples, and to identify and characterize metal sulfide clusters in surface waters, has been very recent and has coincided with the time of the present project.

Although voltammetric methods can identify individual metal sulfide species, even those of Cu(II) and other metals that are not affected in acid digestions, limitations to the use of CSSWV have been observed. For instance, peaks indiscernible from that of free S(II-) have been identified and ascribed to thiol compounds (Ciglen cki and Ćosović, 1996; Al-Farawati and van den Berg, 1997) which would overestimate the amount of free S(II-) in surface waters. Furthermore, Rozan *et al.* (2000b) reported that voltammetric signals produced by S(0) in S_8 and polysulfides occur at identical potentials as that of HS^-. These authors suggested an alternative electrochemical method using cyclic and linear sweep voltammetry to simultaneously identify and quantify elemental sulfur (S_8), polysulfides (S_nS^{2-}) and sulfide (S^{2-}) in natural waters (Rozan *et al.*, 2000b).

2.9.2 Chromatographic Determination

Gas chromatography was the first analytical method to be applied to the measurement of S(II-) in surface waters (Cutter and Oatts, 1987). In a closed loop method, Cutter and Oatts (1987) first acid digested the collected sample to release H_2S, which was then purged from solution and cryogenically trapped onto a chromatographic column. Upon heating, the H_2S was mobilized and passed through the column into a photoionic detector (PID) for S(II-) quantification. Only acid labile sulfide (e.g. H_2S, FeS, ZnS, CdS, MnS), which releases S(II-) as H_2S under acidic conditions, is measured by this method. The S(II-) detection limit for this gas chromatographic method was improved from 12.7 nM to 0.13 nM when S(II-) was preconcentrated in the sample as zinc sulfide upon zinc acetate addition.

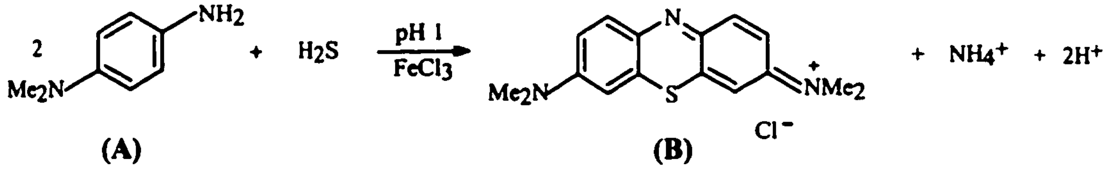
Radford-Knoery and Cutter (1993) modified the procedure of Cutter and Oatts (1987) to simultaneously determine H_2S and carbonyl sulfide (OCS) in a given sample. In order to be able to measure OCS, a more sensitive detector was necessary, and consequently flame photometric detection (FPD) was used. In using FPD, detection limits for S(II-) as low as 0.2 pM were obtained when the sample was preconcentrated as zinc sulfide. Furthermore, using their procedure, Radford-Knoery and Cutter (1993) were able to discriminate between free and complexed forms of S(II-). Finally, these authors noted that treatment of the sample with Cr(II) and HCl was necessary to release S(II-) from copper sulfide species for measurement.

2.9.3 Colourimetric Determination (Methylene Blue-Sulfide Method)

The colourimetric method for S(II-) determination involves the formation of methylene blue (Cline, 1969). In this method, S(II-) reacts under highly acidic conditions to form hydrogen sulfide (H_2S):

$$S^{2-} + 2H_3O^+ \rightleftharpoons H_2S + 2H_2O$$

In the presence of HCl, N,N-dimethyl-*p*-phenylenediamine (A) undergoes oxidation first by Fe^{3+} and then reaction with H_2S to form the methylene blue chloride complex (B) (Pomeroy, 1936). This reaction will only proceed when S(II-) is in the protonated form of H_2S:



The concentration of S(II-) is then determined photometrically by measuring the absorbance of the methylene blue complex at 670 nm. Sulfide was determined as methylene blue in this work and is referred to as methylene blue-sulfide (MBS).

Since H_2S evolution is necessary for the methylene blue complex to form, this method determines only acid labile sulfide (e.g. H_2S, FeS, ZnS, CdS, MnS) in solution. Sulfide from organosulfur (thiol) compounds (e.g. glutathione, 3-mercaptopropanoic acid, cysteine) is not reactive in the MDR reagent (Siegel, 1965; Wu and Kramer, 1997) while only terminal sulfide in polysulfides (S_nS^{2-}) can be measured (Wu and Kramer, 1997).

When absorbance can not be measured within a reasonable period following sample collection, S(II-) is often preserved in the form of zinc sulfide precipitate by adding zinc acetate/NaOH to the solution (A.P.H.A, 1992). The zinc sulfide is later acid digested to release H_2S which is then reacted with the colourimetric reagent to form MB. Preconcentrating sulfide in this manner may also serve to improve detection limits. In an attempt to minimize interferences in absorbance measurements from NOM contained in natural water samples, Tang and Santschi (2000) concentrated MB on a reversed phase chromatographic column (Sep-Pak plus tC_{18} cartridge, Waters, USA). Once concentrated, high performance liquid chromatography (HPLC) was used to separate MB from other solution components and to detect it by retention time and calibration.

2.10 METHODS OF SULFIDE DETERMINATION IN SEDIMENTS

2.10.1 Acid Volatile Sulfide (AVS)

Acid extraction of sulfide has traditionally been used to determine sulfide in sediments. Acid volatile sulfide (AVS) is a colourimetric technique similar to the methylene blue method for sulfide determination in water samples (*Section 2.9.3*), but involves a purge and trap method (Allen *et al.*, 1993). The sediment sample is digested in 1 N HCl to extract the sulfide and convert it to H_2S. The H_2S is then purged from the sample with N_2 and trapped in a NaOH solution. Mixed diamine reagent (MDR) is added directly to the basic solution to form MBS, and S(II-) is determined spectrophotometrically at 670 nm. Sulfide concentrations as low as 0.01 μmol/g of dry weight sediment can be determined by this method.

This method determines only the acid labile fraction of sulfide in sediments that forms H_2S in acid to react with MDR to form the methylene blue complex (refer to *Section 2.9.3*). Such sulfides are collectively termed acid volatile sulfide (AVS) and include metastable iron sulfide compounds - amorphous iron monosulfide (FeS), mackinawite (FeS_{1-x}), greigite (Fe_3S_4) and pyrrhotite ($Fe_{1-x}S$) – and less abundant metal sulfides such as ZnS, PbS, CdS, and MnS (Morse *et al.*, 1987; Di Toro *et al.*, 1990; Allen *et al.*, 1993; Rickard *et al.*, 1995).

Wu and Kramer (1997) assessed the validity of the AVS method as established by Allen *et al.* (1993), and found that sulfide from precipitated $Ag_2S(s)$, NiS(s) and CuS(s) was not readily recovered. The lack of reactivity of Cu, Ni and Hg sulfide phases to form MBS has been noted by other researchers as well (Allen *et al.*, 1993; Cooper and Morse, 1998). Thiol compounds (glutathione, 3-mercaptopropanoic acid, L-cysteine, ethanethiol and dimethyl sulfide) were also found to be unreactive in the AVS method which is in agreement with previous work conducted by Siegel (1965) on MBS. Finally, polysulfides showed an 80% recovery of sulfide (terminal sulfide only) while more oxidized forms of sulfur, thiosulfate and sulfite, did not produce MBS.

2.10.2 Chromium(II) Labile Sulfide (CLS)

Zhabina and Volkov (1978) developed a method for the specific determination of reduced inorganic sulfur forms occurring in sediments. This method involves reductive dissolution of sediment components with $CrCl_2$ in a HCl solution. The chromium labile sulfide (CLS) method collectively measures sulfur from acid insoluble sulfide (e.g. FeS_2, CuS, Ag_2S), acid volatile sulfides (see *Section 2.10.1*), elemental sulfur (S_8), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}) (Zhabina and Volkov, 1978; Canfield *et al.*, 1986; Bowles, 2000), but does not recover S(II-) from organosulfur compounds and sulfate (Canfield *et al.*, 1986).

In the CLS method, reduced sulfur species are first decomposed to H_2S in a hot acidic $CrCl_2$ solution. The H_2S is then precipitated as ZnS and the sulfide is determined by iodometric titration (Zhabina and Volkov, 1978; Canfield *et al.*, 1986). Photometric determination may also be applied to increase the method's sensitivity, by forming MBS.

2.11 IRON MONOSULFIDE (FeS) AND TRACE METALS

Due to the high abundance of iron sulfides (see *Section 2.2*) in sediments and their high specific area, trace metals are most commonly associated with these sulfide compounds in the sediment zone. Di Toro *et al.* (1990) and Di Toro *et al.* (1992) studied the interactions of Cd(II) and Ni(II) with AVS (see *Section 2.10.1*) to describe the formation of insoluble metal sulfide phases in anoxic sediments. It was generally found that as metal concentrations in sediments increased, metal sulfides formed that were less soluble than FeS(s). Free sulfide used in the precipitation of these compounds was provided by FeS(s) that dissolved in response to the depletion of pore water sulfide that had been in equilibrium with the solid. The overall process was summarized by the following displacement reaction:

$$Me^{2+} + FeS(s) \rightarrow MeS(s) + Fe^{2+}$$

This process occurred rapidly (minutes to hours) and applied to any trace metal that forms a sulfide less soluble than FeS (Di Toro *et al.*, 1990, 1996a, 1996b; Casas and Crecelius, 1994).

Iron monosulfide (FeS(s)) is particularly unstable in the presence of molecular oxygen. Oxidation of FeS(s) leads to the formation of Fe(III) hydroxides, sulfate (SO_4^{2-}) and a number of intermediate sulfur products. Protons are released in the process causing a decrease in pH. The oxidation of FeS(s) to SO_4^{2-} by molecular oxygen is described by the following reaction (Nelson, 1978):

$$FeS(s) + {}^{9}/{}_{4}O_2 + {}^{3}/{}_{2}H_2O \rightarrow FeOOH(s) + SO_4^{2-} + 2\ H^+$$

Oxidation of FeS(s) in anoxic sediments induced by activities such as sediment dredging, bottom trawling or bioturbation by benthic organisms, may result in the release of associated toxic, trace metals into solution (Nelson, 1978; Calmano *et al.*, 1994) making them available for uptake by organisms. This concept is briefly explored in this project with respect to Ag(I).

2.12 SUMMARY

In recent years, reduced sulfur, S(II-), has been measured in oxic waters at pico- to nanomolar concentrations. The first measurement was made by Cutter and Oatts in 1987 and since then, S(II-) has been measured in a variety of surface marine waters and freshwaters employing three different analytical methods (voltammetry, gas chromatography and colourimetry). Given the instability of S(II-) in the presence of oxidants such as molecular oxygen, this finding has been surprising. More surprising however has been the fact that S(II-) persists in such waters for periods much longer than those predicted from its thermodynamic stability.

Pico- to nanomolar concentrations of S(II-) represent a sufficient amount to speciate trace metals occurring in surface waters that exhibit a high affinity for S(II-) (Class B and some Intermediate). Although the complexation of metals with dissolved inorganic and organic S(II-) ligands may help maintain metals in solution, the bioavailibility of metals may become enhanced or hindered depending on the nature of the metal ligand association and the ligand itself. Consequently, since S(II-) may play an important role in the biogeochemical cycling of trace metals, it is necessary to understand how S(II-) occurs, as well as its behaviour and fate in surface waters.

The stabilization of S(II-) in oxic waters has been attributed to the formation of extremely stable clusters with metals, particularly Cu(II) and Zn(II). Sulfide clusters of these metals have recently been identified and measured in oxic river waters which further corroborates the role of these metal sulfide species in the stabilization of S(II-). In laboratory studies, extremely stable sulfide clusters with Ag(I) have also been formed in solution. More importantly however, Ag(I) has been shown to rapidly replace Cu(II) and Zn(II) in sulfide species of these metals. Similar processes are likely to occur in nature suggesting that metal sulfide species in solution may provide a pool of S(II-) ligand to less abundant, toxic trace metals such as Ag. This of course has implications for the bioavailability and toxicity of trace metals.

Natural organic matter is a ubiquitous component of natural waters and is able to bind and interact with metals through the numerous functionalities (including -S) contained within its structure. Metal sulfide species in oxic waters have been found to associate with size fractions > 3000 MW, suggesting an interaction between metal sulfides and NOM. Given the high adhesiveness of metal sulfide species, such interactions are very possible. Consequently, NOM may also play an important role in the stabilization of S(II-) in surface waters and the bioavailability of associated trace metals.

CHAPTER 3

EXPERIMENTAL METHODS

3.1 REAGENTS AND MATERIALS

3.1.1 General Considerations

Water used for rinsing of materials and for sample or reagent preparation was purified using a Milli-Q (Millipore, USA) system. All reagents utilized were of ACS reagent grade or of equivalent analytical purity unless otherwise specified.

3.1.2 Oxygen Scrubber

Gases used for degassing of aqueous solutions or filling of the glove bag or glove box were purified of oxygen using a vanadium(II) chloride scrubbing solution. The scrubber was prepared by mixing the following two components: a) 2.5 g of ammonium metavanadate (NH_4VO_3, Baker, USA) dissolved in 30 mL of boiling, concentrated HCl and diluted to 250 mL with water, and b) ~15 g of granulated zinc metal that was amalgamated with Hg^0 and covered with water containing a few drops of HCl. After mixing of the two components, the solution colour became purple as the amalgamated zinc reduced V(V) to V(II). The oxygen scrubber was followed by an in-line drying column consisting of Drierite (Waltammond Drierite, USA) to help remove water, acid or metal aerosols carried over from the scrubber.

3.1.3 Metal Stock Solutions

Iron(III), copper(II), manganese(II), nickel(II), zinc(II) and silver(I) stock solutions were prepared in 2% high-purity HNO_3 (Ultrex II, Baker, USA) from the following salts: ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$, BDH, Canada), cupric nitrate ($Cu(NO_3)_2 \cdot \frac{1}{2}H_2O$, BDH, Canada), manganous sulfate ($MnSO_4 \cdot H_2O$, J.T Baker, USA), nickel nitrate ($Ni(NO_3)_2 \cdot 6H_2O$, BDH, Canada), zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$, Baker, USA), and silver nitrate ($AgNO_3$, Baker & Adamson, Canada). Iron(II) stock solutions were prepared from ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, BDH, Canada) in deoxygenated water immediately prior to use.

3.1.4 Mixed Diamine Reagent (MDR)

Mixed Diamine Reagent (MDR) was prepared by carefully mixing the following two solutions (Adams and Kramer, 1999b): a) 2.25 g of N,N-dimethyl-*p*-phenylenediamine oxalate ($[C_8N_2H_{12}]_2 \cdot C_2O_4H_2$, Baker, USA) dissolved in 660 mL of concentrated H_2SO_4 and 340 mL of water, and b) 5.4 g of ferric chloride ($FeCl_3 \cdot 6H_2O$, BDH, Canada) dissolved in 100 mL of concentrated HCl and diluted to 200 mL with water. The reagent was stored in an amber polyethylene bottle.

3.1.5 Sulfide Stock Solutions and Standards

Sulfide stock solutions (10^{-3} and 10^{-4} M) were prepared from sodium sulfide ($Na_2S \cdot 9H_2O$, BDH, Canada) dissolved in deoxygenated water (degassed with ultra pure He). The pH of the water was adjusted to > 10 with crystalline sodium carbonate (Na_2CO_3, Fisher Scientific, Canada) prior to the addition of S(II-). Sulfide stock solutions were standardized iodometrically against a 0.025 M thiosulfate standard prepared daily from crystalline sodium thiosulfate ($Na_2S_2O_3$, Fisher Scientific, USA). Tri-iodide (I_3^-) solution (0.0125 M) was made by first dissolving 20 – 25 g of potassium iodide (KI, McArthur Chemical, Canada) in water and then dissolving 3.2 g of solid iodine (I_2, McArthur Chemical, Canada) in the KI solution and diluting to 1 L with water. The tri-iodide solution was stored in an amber polyethylene bottle and was standardized weekly against a 0.025 M thiosulfate standard solution.

Spectrophotometric calibration standards were prepared by diluting a S(II-) stock solution into deoxygenated 10^{-4} N NaOH solution. New standards were prepared prior to each calibration.

3.1.6 Natural Organic Matter (NOM) Stock Solutions

Experiments requiring natural organic matter (NOM) were conducted with NOM originating from either the Suwannee River (GA, USA) or the Luther Marsh (ON, Canada).

3.1.6.1 Suwannee River Fulvic Acid (SRFA)

Suwannee River fulvic acid (SRFA) stock solutions were prepared from the International Humic Substances Society (IHSS) reference material 1R101F. This material has been studied extensively and is well characterized (Averett *et al.*, 1994). Procedures used to isolate and concentrate the reference fulvic acid are described elsewhere (Thurman and Malcolm, 1981; Aiken, 1985; Aiken, 1988; Malcolm *et al.*, 1994). Suwannee River fulvic acid stock solutions were prepared in water at a concentration of 10 mg/L and contained ~ 5 mg C/L of organic carbon. Stock solutions were allowed to sit overnight prior to use.

3.1.6.2 Luther Marsh Natural Organic Matter (LM NOM)

A highly concentrated slurry of Luther Marsh NOM was provided by the National Water Research Institute (Burlington, ON, Canada). This material was collected with physical isolation methods in the following manner: (a) centrifugation of the water sample (10,000 g), (b) filtration of the supernatant through a glass-fiber filter (1.0 μm pore size), (c) concentration of the material from the filtrate using a reverse osmosis apparatus with a 400 Da Nanofilm^{TM} membrane (NF40-40, FilmTec, USA), and (d) passage through a hydrogen cation-exchange resin (AG5OW-X8) to reduce metal concentrations (Burnison, 2000). The slurry was diluted in pure water to obtain stock solutions of various organic carbon concentrations (0.5 – 13.4 mg C/L). Stock solutions were allowed to sit overnight prior to use.

3.1.7 Preparation of Iron Monosulfide (FeS) and Copper Sulfide (CuS)

Iron monosulfide (FeS(s)) was precipitated under an N_2 atmosphere in a glove bag. Separate solutions of S(II-) ($Na_2S{\bullet}9H_2O$, BDH, Canada) and Fe(II) ($Fe(NH_4)_2(SO_4)_2{\bullet}6H_2O$, BDH, Canada) were initially prepared in 100 mL of deoxygenated water at a concentration of 0.5 M. The pH of the water used for S(II-) solution was adjusted to 10 with sodium carbonate (Na_2CO_3). Both solutions were simultaneously added to 300 mL of deoxygenated water while stirring. A fine black precipitate was immediately formed. The precipitate was collected on a 0.45 μm cellulose acetate filter (Millipore, USA) and washed several times with water. Batches of FeS(s) were stored at 5 ^{0}C. The precipitate was characterized by powder X-ray diffraction (XRD) using Cu K_α radiation and was found to be amorphous.

Copper sulfide (CuS) was precipitated according to the same procedure used for FeS(s) by mixing solutions of S(II-) ($Na_2S\bullet9H_2O$, BDH, Canada) and Cu(II) ($Cu(NO_3)_2\bullet\frac{1}{2}H_2O$, BDH, Canada). A green precipitate was formed.

3.2 ANALYTICAL PROCEDURES

3.2.1 Organic Carbon Determination

The organic carbon concentration of NOM stock solutions and natural samples was determined by IR detection of CO_2 following persulfate-UV digestion with a Dorhmann DC-180 Carbon Analyzer (Tekmar-Dohrmann, USA). The sample was acidified to pH ~ 2 with HNO_3 and then purged with He to eliminate inorganic carbon as CO_2. Detection limits were calculated based on three times the standard deviation of replicate blanks ($3 \times \sigma$) and the blank consisted of acidified water. Analytical precision at 1.8 mg C/L contained in a SRFA stock solution was 1.8% RSD (n = 4).

3.2.2 Methylene Blue-Sulfide (MBS) Determination

Sulfide concentration was determined colourimetrically by the methylene blue method (Cline, 1969) and is referred to as methylene blue-sulfide (MBS). Colourimetric reagent (MDR) was added to a standard or sample at a ratio of 1:10 (v/v) and the colour was allowed to develop for at least 0.5 h prior to measuring the absorbance of the methylene blue complex. Sulfide concentrations were determined from a standard calibration curve of absorbance versus concentration. Samples exceeding the calibration range for S(II-) were diluted 10 or 100 fold in water prior to the addition of MDR. In calculating S(II-) concentration, the absorbance contributed by coloured NOM components was subtracted from the measured absorbance of the sample.

Absorbance was measured at 670 nm using a Beckman Model B (Beckman Instruments, USA) or a Spectronic 20 (Bausch & Lomb, USA) spectrophotometer with a 2 cm path length cell. Transmittance (%T) readings for a given sample were found to be similar from the two instruments when measurements were made using the same path length. Detection limits were calculated based on three times the standard deviation of replicate blanks ($3 \times \sigma$) and the blank consisted of water with MDR added at the appropriate ratio. On average, the absorbance of the blank was 0.0052 that was calculated to be equivalent to 0.07 μM S(II-) on the Beckman. Analytical precision at 1.0 μM of S(II-) was 2.0% RSD (n = 5).

The absorbance of natural samples requiring higher sensitivity was measured on a Cary 50 (Varian, USA) spectrophotometer with a 10 cm path length quartz cell.

3.2.3 Chromium(II) Labile Sulfide (CLS) Determination

Reduction of copper and silver sulfides by Cr(II) was required in order for such S(II-) to be included in the MBS measurement. The procedure followed is a modification of one used for pyrite in sediments (Canfield *et al.*, 1986). An aliquot of 10 mL of 1 M $CrCl_2$ was added to a 40 mL sample contained in a distillation flask. The sample was heated to reflux and the derived H_2S was purged with a low flow of N_2 gas into a trapping flask with a non-fritted tube. The trapping solution was 20 mL of 0.05 M NaOH. Mixed diamine reagent was added to the sulfide solution to form MBS as per the MBS method (refer to *Section 3.2.2*).

3.2.4 Metal Determination

Metal concentrations were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on either a PE-Sciex Elan-250 coupled with an ultrasonic nebulizer, or a PE-Sciex Elan-6100. Instrument settings were optimized for ultra trace measurements. Detection limits for each metal were calculated based on the standard deviation of replicate blanks consisting of acidified Milli-Q water (1% HNO_3 (v/v), Ultrex II, Baker, USA).

3.3 ACCOUNTING FOR LOST SULFIDE: OXIDATION AND ADSORPTION TO CONTAINER SURFACES

Unexpected decreases in S(II-) concentration under anoxic conditions were noted over time in a number of experiments. Despite efforts to eliminate oxygen from each sample during preparation, storage and analysis, it is possible that residual amounts of oxygen remained or leaked into the samples. Information on S(II-) oxidation under the specific conditions of these experiments involving trace amounts of oxygen and sulfide is not available. Based on estimates however from existing data and kinetic relationships, it was concluded that oxidation could explain the observed decreases in S(II-) in some samples (Chen and Morris, 1972; O'Brien and Birkner, 1977; Millero *et al.*, 1987).

Upon further investigation of the problem, it was hypothesized that S(II-) was also being lost from solution by adsorbing to container surfaces. A few supplementary studies addressing this problem are presented here as these findings are pertinent to experimental observations and discussions appearing in later chapters. They should also serve to improve experimental methods involving S(II-) analysis.

3.3.1 Sulfide Adsorption to Polypropylene (PP)

An attempt was made to recover S(II-) from polypropylene (PP) containers that held samples containing Zn(II) and/or S(II-) in water or aqueous SRFA solution. Each PP tube was rinsed and then filled with water to the original sample volume of 50 mL. Acidic MDR (5 mL) was added to each container to react with S(II-) bound to the PP surface and to subsequently form MBS. The initial S(II-) concentration in each sample was 2.5 μM and Zn(II) concentrations ranged from 0 to 25 μM.

Sulfide was only recovered from PP tubes that contained samples prepared in water with at least 2.5 μM of Zn(II) (Figure 3.1) and the amount of sulfide generally increased with the Zn(II) concentration in the sample. The greatest amount of recovered sulfide, normalized to the initial sample volume, was ~ 1.0 μM which represents ~ 40% of the initial concentration. Smaller amounts of sulfide (4 – 16 %) were recovered from Teflon stir bars that had been immersed in water samples containing at least 2.5 μM of Zn(II). Sulfide was not recovered from container walls that had housed samples that either lacked Zn(II) or were prepared in SRFA solution regardless of the amount of Zn(II) present. Although analogous recoveries of Zn(II) were not examined, it may be inferred from these results that S(II-) is removed from solution by adsorbing to the PP surface in the form of a zinc sulfide species.

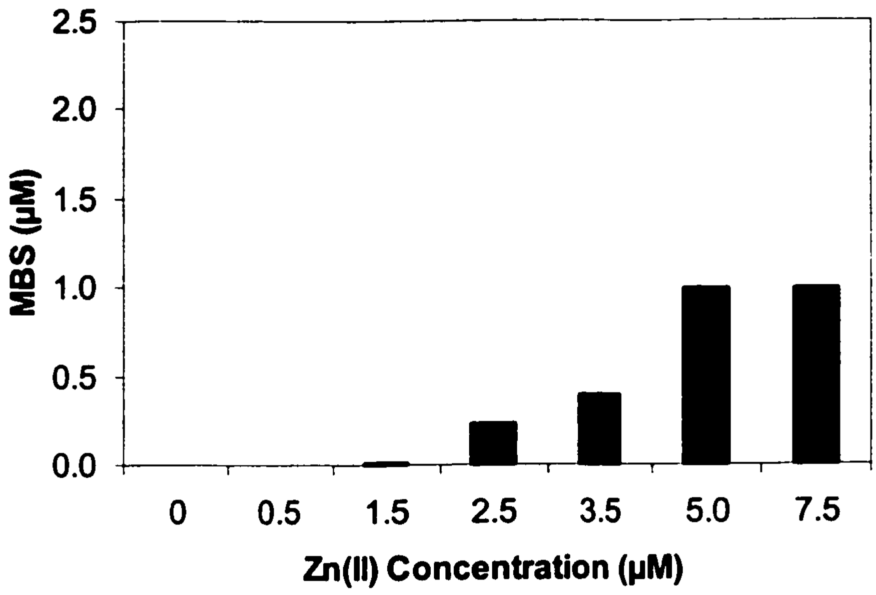


Figure 3.1: Sulfide recovered from PP tubes that contained samples prepared in water with 2.5 µM of S(II-) and various concentrations of Zn(II).

Polypropylene, as well as other pure hydrocarbon polymers such as polyethylene and polystyrene, are known to acquire a negative charge when exposed to a polar solvent. It has been suggested that these surface charges arise from molecules containing negatively charged functional groups that have remained from the polymerization process (MacFarlane *et al.*, 1986). Plastics may also develop carboxyl ($-COO^-$) and carbonyl ($C=O$) sites with adsorptive properties as they become degraded under the influence of oxygen, heat or light (Robertson, 1968). Metal cations would be attracted to such negatively charged surfaces.

The strong attraction of Zn(II) for negative oxygen sites on the PP surface in this experiment, would result in the adsorption of zinc sulfide species to the container by overcoming any electrostatic repulsive forces. In the presence of SRFA, Zn(II) competition from functional groups on the NOM (mainly $-COO^-$ and $-O^-$) would cause Zn(II) to remain in solution. This explains why decreases in S(II-) were only observed for samples that were prepared in water with added Zn(II).

3.3.2 Sulfide Adsorption to Borosilicate Glass

Adsorption of S(II-) to borosilicate glass was demonstrated in another experiment. Duplicate 20 mL aqueous S(II-) samples were prepared in borosilicate vials with Zn(II) ranging in concentration from 0 to 100 µM. Following the addition of Zn(II), the pH of each sample was adjusted to ~ 8.5 with 1 N NaOH and the samples were degassed and stored under nitrogen where S(II-) was added at a concentration of ~ 2.5 µM. After 2.5 h, 10 mL aliquots were taken from each sample and fixed with MDR (1 mL) to determine the amount of S(II-) in solution. After 48 h, 1 mL of MDR was added to the remaining 10 mL sample in each vial. Addition of the acidic reagent to the sample container allowed for the measurement of S(II-) occurring in solution as well as adsorbed to the container walls.

Measured MBS concentrations are shown in Figure 3.2 where the bar plots indicate the range in MBS concentrations (n = 2). The white series represents S(II-) measured in solution and the dark series represents S(II-) measured both in solution and from the container walls. As Zn(II) concentration in each sample increased up to 5.0 µM, the amount of S(II-) measured in solution decreased (white series). Sulfide was being removed from solution by adsorbing to the container surface in the form of a zinc sulfide

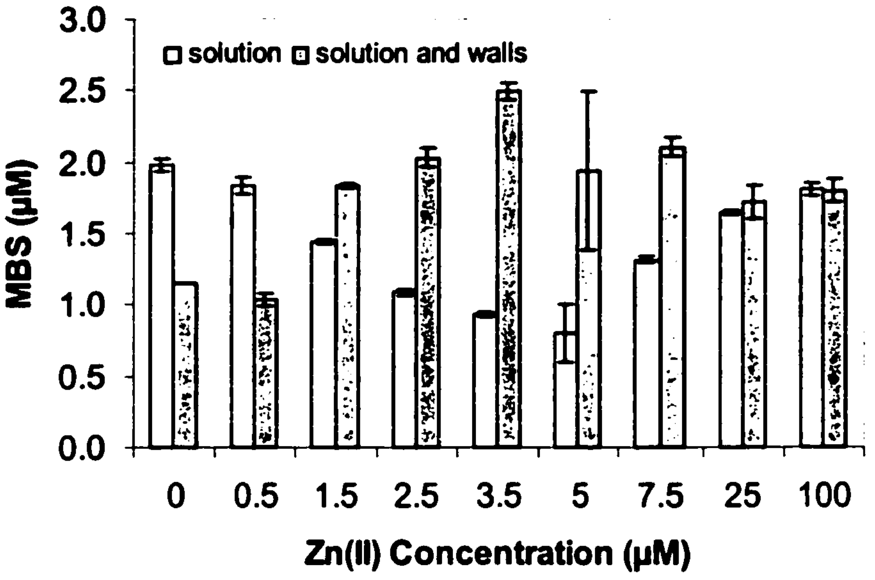


Figure 3.2: Sulfide measured in anoxic samples prepared in borosilicate vials containing 2.5 µM of S(II-) and various concentrations of Zn(II). The white series represents S(II-) in solution while the dark series represents S(II-) in solution and from the container walls. The error bars represent the range in measured MBS concentrations (n = 2).

species. Sulfide adsorbed to the container in each sample was recovered in the next MBS analysis when MDR was added to the sample while in the vial. The combined concentration of S(II-) in solution and that desorbing from the container walls increased with Zn(II) concentration (dark series) as greater amounts of S(II-) were desorbed.

Negatively charged silanol sites (-SiO$^-$) are created on the surface of sodium borosilicate due to proton loss by hydroxide or leaching of Na$^+$ from the glass upon exposure to water. Metals such as silver (Struempler, 1968; Sato, 1989) and zinc (Struempler, 1968; Tait and Jensen, 1982) are known to adsorb to glass surfaces through cation exchange reactions at these negative sites. The strong attraction of Zn(II) for negative oxygen sites on the borosilicate surface in this experiment would result in the adsorption of hydrated zinc sulfide species to the container through the exchange of water groups.

At Zn(II) concentrations greater than ~ 5.0 μM, either enough metal remained in solution to pull S(II-) from the container back into solution, or enough metal was present to compete with zinc sulfide species for the container surface. Thus the amount of MBS measured in solution (white series) started to increase with Zn(II) concentration. At the highest metal concentrations, S(II-) was no longer adsorbing/desorbing to or from the container walls and the MBS measured in each series became equal.

For samples containing zero or very low Zn(II) concentrations, the amount of S(II-) lost in 2.5 h was not recovered in the direct measurement 48 hours later. Consequently, some S(II-) was being lost by another process, probably oxidation by trace amounts of oxygen.

3.3.3 Conclusions

The results of these experiments show that zinc sulfide phases adsorb to PP and borosilicate glass. As a result, the amount of S(II-) being measured is underestimated as some of it is removed from solution. Adsorption to other types of container surfaces is also possible. Furthermore, other metal sulfides may exhibit similar adsorptive behaviour. In a study conducted by Simpson *et al.* (1998b), lower than expected concentrations of copper were measured in unacidified as well as acidified sulfidic water and seawater samples. Samples were stored in high density polyethylene or Teflon containers. Losses in copper were attributed to the formation of copper sulfide phases that were thought to have a high adsorptive affinity for container surfaces.

This problem of S(II-) adsorption to container surfaces is of particular concern when S(II-) is determined in oxic waters. Sulfide occurs at trace concentrations in such waters and in the form of metal sulfide complexes and clusters. It is thus recommended that precautions be taken in every S(II-) analysis, regardless of the method employed, to ensure that adsorbed S(II-) is recovered from containers and included in the measurement. Furthermore, as will be discussed in later chapters, treatment of the sample with Cr(II) is necessary in many cases to measure and account for S(II-) bound in acid insoluble sulfides (e.g. Ag, Cu sulfides). Finally, measures should be taken to keep samples anoxic to avoid S(II-) losses due to oxidation.

CHAPTER 4

SYNTHETIC IRON MONOSULFIDE AND METALS UNDER OXIC CONDITIONS

4.1 OBJECTIVES

The work presented in this chapter provided a starting point for this project by assessing the oxidation of simple FeS(s) systems. These experiments resulted in some pertinent and significant findings. The objectives of these experiments were as follows:

(a) To demonstrate the instability of FeS(s) upon exposure to air (oxic conditions) and examine the fate of Ag(I) sorbed to FeS(s) under such conditions.

(b) To examine the effect of S(II-) binding metals, Ni(II), Mn(II), Cu(II) and Zn(II), on S(II-) during FeS(s) oxidation solution.

4.2 EXPERIMENTAL METHODS

4.2.1 General Considerations

The reagents used in these experiments are described in *Section 3.1*. All materials contacting metal solutions were rigorously washed sequentially with 20% and 1% HNO_3 (Trace Metal Grade, Baker, USA) and dried in a class 100 HEPA filtered laminar flow hood. Materials re-used in FeS(s) oxidation experiments were first soaked in 20% H_2SO_4 to remove iron hydroxide phases.

4.2.2 Reactive Sulfide Determination

Sulfide was determined as methylene blue-sulfide (MBS) on a Spec20 (Bausch & Lomb, USA) according to the procedure outlined in *Section 3.2.2*. Detection limits were typically in the range of 0.1 μM. Samples collected for MBS analysis were dispensed into 50 mL amber glass bottles and immediately fixed with MDR and stored at 5 °C until their analysis. Analysis usually occurred within 24 to 36 hours of sample collection. Dissolved samples were filtered through a 0.45 μm polysulfone in-line filter (Acrodisc, Gelman Laboratory, USA).

Changes in MBS concentration for each experiment are depicted graphically as a function of time starting at negative hours. The "negative" hours represent the anoxic period and zero hours marks the beginning of the oxic period.

4.2.3 Silver Determination

Silver concentration was determined by ICP-MS on a PE-Sciex Elan-250 according to the procedure outlined in *Section 3.2.4*. The detection limit for Ag(I) was 0.18 nM (3 × σ). Samples collected for Ag analysis were collected in 10 mL polypropylene tubes and acidified to 2% (v/v) with concentrated, high purity HNO_3 (Ultrex II, Baker, USA) and stored in the dark until their analysis. Analysis usually occurred within a month of sample collection. Dissolved samples were filtered through a 0.45 μm polysulfone in-line filter (Acrodisc, Gelman Laboratory, USA).

4.2.4 Experimental Design

The stability of synthetic amorphous iron monosulfide (FeS(s)) suspended in aqueous solution was examined upon exposure to oxic conditions by monitoring changes over time in the solution pH, dissolved oxygen (DO) and S(II-) concentration at constant temperature. Iron monosulfide was studied because it is a common constituent of anoxic sediments that is known to scavenge trace (see *Sections 2.2 & 2.10.1*) and ultra trace metals such as Ag(I) (Adams and Kramer, 1998), from solution. Dissociation of FeS(s) upon oxidation could release associated metals back into solution making them available to organisms for uptake.

The fate of Ag(I) sorbed to FeS(s) was examined by oxidizing an FeS(s) suspension spiked with Ag(I), and monitoring changes over time in the solution pH, dissolved oxygen (DO) and dissolved (< 0.45 μm) Ag(I) and S(II-) concentration at constant temperature. The behaviour of Ag(I) was studied because although it is known to bind strongly to FeS(s) (Adams and Kramer, 1998), limited information is available on the fate of Ag(I) in sediments under oxic conditions despite its severe toxicity to aquatic organisms (Andren and Bober, 2000).

The effect of Ni(II), Mn(II), Cu(II) and Zn(II), on S(II-) concentration during FeS(s) oxidation was examined by monitoring changes over time in the solution pH, dissolved oxygen (DO) and dissolved (< 0.45 μm) S(II-) concentration at constant temperature. These metals were chosen because they exhibit a high affinity for S(II-) and commonly occur in sediments. Furthermore, Cu(II) and Zn(II) have been implicated in the stabilization of S(II-) in natural oxic waters.

4.2.5 Experimental Procedures

A 2.2 L Teflon container placed in a water bath (23 – 24 °C) in a dark room (UV filtered light) was filled with 1800 mL of water. The container was capped tightly with a Teflon lid containing inlet ports for a propeller stirrer (Model 102 T-Line Laboratory Stirrer, Talboys Engineering, USA), an oxygen probe (Model 600 Oxan oxygen probe, Engineered Systems & Design, USA), a pH electrode (Ross combination glass electrode, Orion Scientific Systems, USA), and a thermometer. The electrodes and the thermometer were each fitted into silicone stoppers to keep them steady, while the stoppers sealed the ports to prevent oxygen penetration during the anoxic period. Furthermore, the stopper holding the thermometer had an extra hole which contained a glass tube through which N_2 was streamed. This way an N_2 atmosphere could be maintained in the headspace of the container throughout the anoxic period.

The stirrer was left running at the same setting (795 rpm) for the duration of the experiment starting at the time of degassing. Dissolved oxygen (mV) and pH were monitored with a titrimeter (Model 8901, Tanager Scientific Systems, Canada) that was interfaced with a computer to record the data over time. Temperature was recorded manually at each sampling time.

The thermometer port also served as a port for degassing as well as sampling. A coarse glass frit was placed through the port into the water and the water was degassed for at least 0.5 h with N_2 purified of oxygen (refer to *Section 3.1.2*). Once degassing was complete, an appropriate amount of wet FeS(s) (0.5 – 4.0 g) was weighed into a polypropylene syringe flushed with N_2. Another portion was weighed onto a petri dish and placed in an oven overnight at 50 °C to determine the approximate water content of the FeS(s) slurry. A Teflon tube was attached to the tip of the syringe and the FeS(s) was released into the water through the sampling port.

Once in solution, the FeS(s) suspension was allowed to homogenize upon stirring for ~ 0.5 h at which time a sample was taken to determine the initial S(II-) concentration in the system. In experiments incorporating metals, the metal was added prior to the FeS(s) and a sample was taken after ~ 15 min to determine the initial metal concentration. Samples were extracted from the solution through the thermometer port using a polypropylene syringe fitted with a Teflon tube.

The length of the anoxic and oxic periods varied depending on the experiment. The anoxic period lasted anywhere between 16.5 and 42 h, while the length of the oxic period ranged from 8 to 104 h. At the end of the anoxic period, the lid was removed from the container and air was allowed to freely penetrate the solution and oxidize the FeS(s). The number of samples taken for S(II-) analysis depended on the particular experiment. All S(II-) and metal (Ag(I)) data are summarized in the Appendix.

4.3 RESULTS

4.3.1 Experiment 1a: Stability of Synthetic FeS(s) in Oxic Solution

This experiment examined the stability of synthetic amorphous FeS(s) in solution upon exposure to air. Changes in solution pH, dissolved oxygen (DO) and total S(II-) concentration were monitored for 16.5 h under anoxic conditions and 56 h under oxic conditions. The pH and DO data for the first 50 h of the experiment are shown in Figure 4.1 and the S(II-) data in Figure 4.2. The initial concentration of FeS(s) was ~ 0.04 g/L (0.45 mM).

An examination of Figure 4.1 shows that an increase in DO concentration occurred which provides evidence for oxygen penetration into the solution. The concentration increased sharply from 0 to ~ 8.4 mg/L within 3 h where it remained

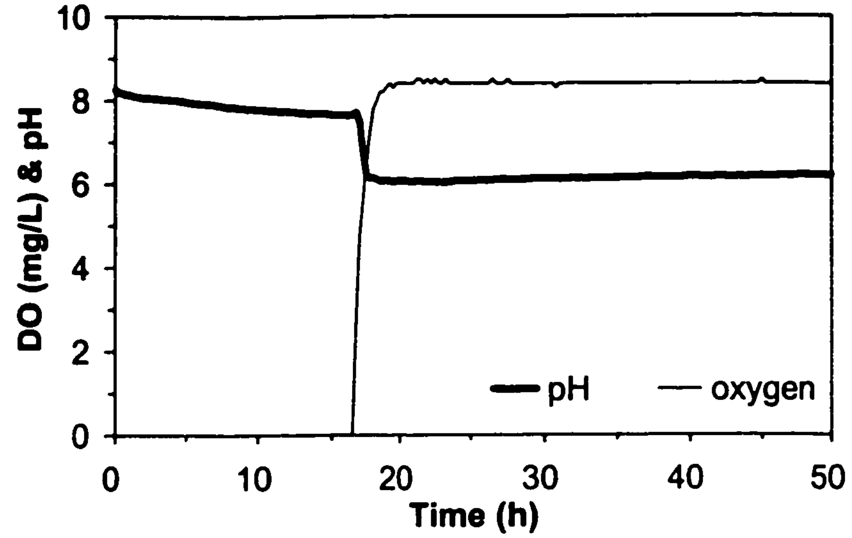


Figure 4.1: Solution pH and dissolved oxygen measured during the oxidation of a FeS(s) suspension (0.04 g/L). Oxidation began at 16.5 h.

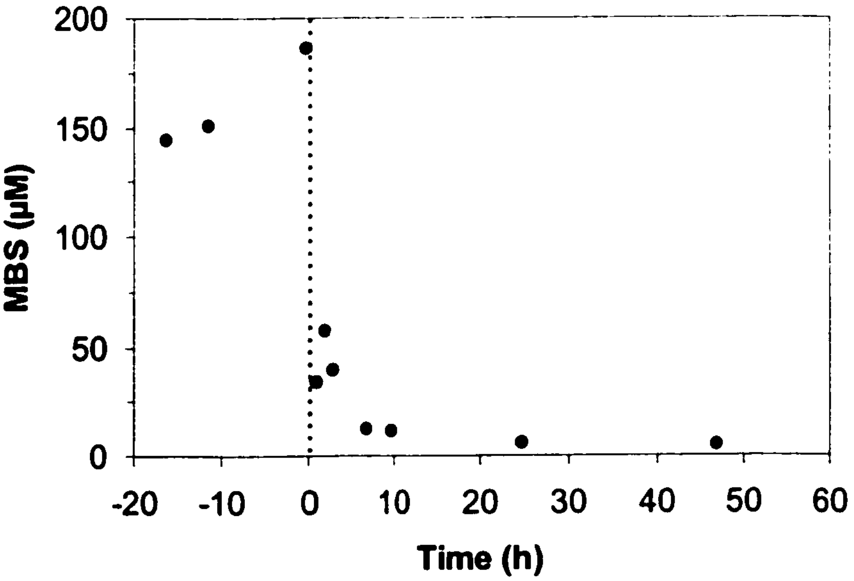


Figure 4.2: Sulfide measured during the oxidation of a FeS(s) suspension (0.04 g/L). Sulfide was monitored under anoxic (< 0 h) and oxic (> 0 h) conditions.

constant for the rest of the oxidation period. This indicates that in the FeS(s) system, oxygen was quickly being replenished in the solution during FeS(s) oxidation. Simultaneous to the increase in DO, a sharp drop in the pH of about 2 units (from 7.8 to 6.0) occurred within a period of ~ 2 h starting at time 16.5 h when the FeS(s) suspension was exposed to air. The release of protons into solution as a result of FeS(s) oxidation explains the observed decrease in pH (refer to *Section 2.11*). Attainment of a constant pH after 2 h indicated completion of the FeS(s) oxidation, or at least for the bulk of the FeS(s).

The course of the FeS(s) oxidation was also monitored qualitatively. After 1 h of oxidation, the colour of the suspension changed from black to light grey, and within 2 h it became orange. The orange powder was characterized by X-ray diffraction analysis and was identified as lepidocrocite which is an Fe(III) hydroxide (γ-FeOOH). Similar qualitative observations and changes in pH and DO were noted in a later experiment (*Experiment 2*, Figure 4.9) where ~ 0.45 g (5.1 mM) of FeS(s) originating from a different batch was oxidized.

Total S(II-) concentration in the FeS(s) suspension was also monitored over time as MBS (Figure 4.2). An increase in S(II-) was noted over time under anoxic conditions which could be due to FeS(s) particles breaking up into finer particles resulting in a more concentrated suspension.

Figure 4.2 shows that upon exposure to air, the amount of total S(II-) in the suspension decreased as indicated by the decrease in measured MBS concentration. Within 3 h of oxidation, MBS concentration decreased from 185 to 33 μM and by 7 h, only about 5% of the initial MBS concentration was detected. This experiment clearly demonstrated the instability of synthetic FeS(s) in oxic solution. In a similar manner, FeS(s) occurring in sediments may be oxidized when sediments are suspended into overlying oxic waters. As FeS(s) is dissociated upon oxidation, metals associated with FeS(s) could be released into solution where they are more accessible and potentially toxic to organisms.

Copper is commonly found in anoxic sediments where it is precipitated in the form of copper sulfide compounds (e.g. CuS) due to its high affinity for S(II-). Oxidation of CuS could release Cu(II) into solution. For this reason, an experiment similar to the one described above for FeS(s) was also conducted with synthetic CuS(s). Unlike the case with FeS(s) however, a decrease in the pH was not observed during the oxic period and no visible signs of oxidation were apparent. Furthermore, although CuS(s) was visibly present in collected samples, MBS was not detected in any of the samples. These observations indicate that CuS(s) remained stable under oxic conditions and S(II-) bound in this solid phase could not be determined as MBS. The recovery of S(II-) from CuS(s) by acid extraction has also proven to be problematic in the colourimetric AVS method for S(II-) determination in sediments (Wu and Kramer, 1997; Cooper and Morse, 1998) (see *Section 2.10.1)*.

4.3.2 Experiment 1b: Fate of Ag(I) Sorbed to Synthetic FeS(s) in Oxic Solution

This experiment examined the fate of Ag sorbed to FeS(s) upon FeS(s) oxidation. Changes in solution pH, dissolved oxygen (DO), dissolved (< 0.45 μm) S(II-) and Ag(I) concentrations were monitored under anoxia for 42 h and oxic conditions for 105 h.

Dissolved oxygen and pH data are summarized in Figure 4.3, while data for dissolved S(II-) and Ag(I) concentration are shown in Figures 4.4 and 4.5, respectively. The initial concentration of colloidal (> 0.45 μm) FeS(s) was ~ 0.06 g/L (0.63 mM). Silver(I) was added to the FeS(s) suspension at a concentration of 4 μg/L (37 nM) or 0.007% by weight of the FeS(s).

A gradual decrease in pH and increase in DO were noted over time during the anoxic period. It appears as though oxygen was leaking into the system and causing the oxidation of some FeS(s). Oxidation of FeS(s) releases protons into solution which would explain the decrease in pH. Upon exposure of the Ag-FeS(s) suspension to air at 42 h, the pH started to decrease more rapidly and a drop from 8.2 to 7.3 occurred over a 6 h period. Stabilization of the pH indicated the end of the FeS(s) oxidation which was also confirmed by qualitative observation as the colour of the suspension changed from black to orange. Within the same 6 h period that the pH dropped, an increase in DO from < 1 mg/L to 8.8 mg/L also occurred indicating that oxygen had actually penetrated the solution to oxidize the FeS(s). The rate of oxygen penetration into the solution however, was not as rapid as with the FeS(s) system alone (Figure 4.1). The slope of the DO line for the Ag-FeS system was not as steep and two changes in the slope occurred, indicating that saturation of the solution with oxygen was being inhibited at times. Furthermore, the oxidation in this system was completed over a longer time period. These differences could be related to variations that may have occurred in the stirring rate.

Although particulate FeS(s) (> 0.45 μm) was added to the solution, ~ 5% (33 μM) of the S(II-) concentration was measured as MBS in the dissolved (< 0.45 μm) phase

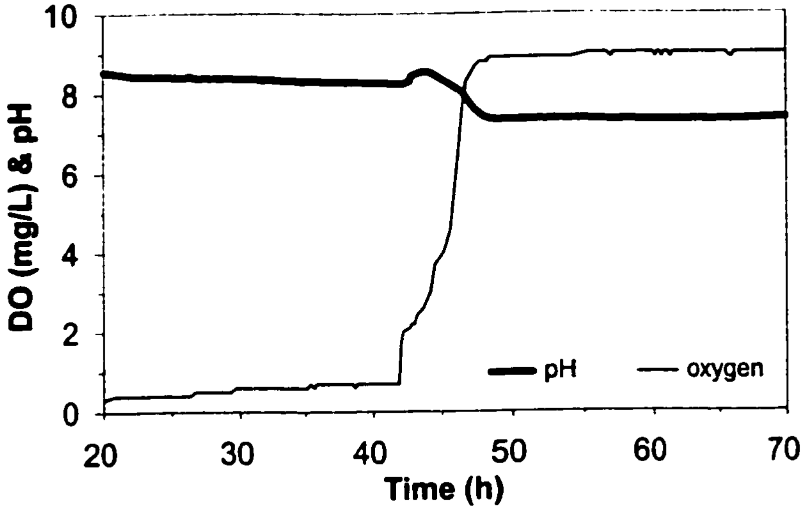


Figure 4.3: Solution pH and dissolved oxygen measured during the oxidation of an Ag-FeS(s) suspension (0.06 g/L). Oxidation began at 42 h.

(Figure 4.4). Finer particles that would pass through the filter may not have been have been completely washed out of the collected FeS(s) fraction during its preparation. Also, once in solution, the FeS(s) may have been broken into finer particles as a consequence of the stirring motion. An increase in MBS concentration during the first part of the anoxic period suggests that this process probably occurred as the finer particles would be more reactive in MDR to yield more MBS. After 24 h of anoxia however, a great drop in the MBS concentration occurred and by the end of the anoxic period, S(II-) was no longer detected in solution. It was not understood why this decrease occurred.

Upon exposure to air, the dissolved S(II-) concentration increased to a high of 46 μM within the first 2.5 h, and then decreased to below detectable levels (< 0.1 μM) by the sixth hour of oxidation. It appears as though interaction with oxygen breaks the FeS(s) into to finer particles resulting in an initial increase in S(II-) concentration in solution during the early stages of the oxidation. The time of S(II-) disappearance from solution coincided with the stabilization of pH which indicated the completion of the FeS(s) oxidation.

The changes in pH, DO and S(II-) described here are in general accordance with observations made previously for the FeS(s) system and confirm that oxidation of FeS(s) in the Ag-FeS system occurred upon exposure to air.

Although 37 nM (4 µg/L) of Ag(I) were added to the solution, only 13.5 nM was detected in the dissolved phase (< 0.45 µm) prior to the addition of FeS(s). Since retention of Ag(I) on the filter was not found to be a problem, adsorption of Ag(I) to the Teflon may account for lower Ag(I) values. Upon addition of FeS(s) to the solution, Ag(I) concentration decreased approximately by half as the solid phase scavenged it from solution. As was the case with dissolved S(II-), the amount of Ag(I) in solution decreased below detectable levels (< 0.18 nM) by the end of the anoxic period. Sorption of Ag(I) over time to larger FeS(s) particles would explain this decrease in Ag(I) concentration.

As shown in Figure 4.5, Ag(I) appeared in solution during the oxic period. Although difficult to see on the graph, starting at ~ 3.5 h, high picomolar concentrations of Ag(I) (< 0.50 nM) were detected by the end of the FeS(s) oxidation (6 h). The concentration continued to increase to a maximum of 1.50 nM at 24 h of oxidation and after 104 h of oxidation, 1.10 nM was detected in solution. If Ag(I) is associated with

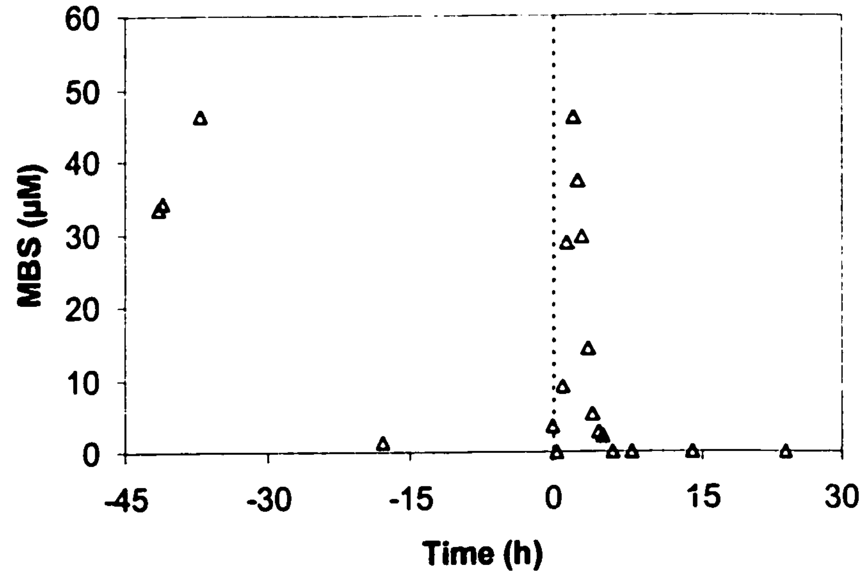


Figure 4.4: Dissolved sulfide (< 0.45 µm) measured during the oxidation of an Ag-FeS(s) suspension (0.06 g/L). Sulfide was monitored under anoxic (< 0 h) and oxic (> 0 h) conditions.

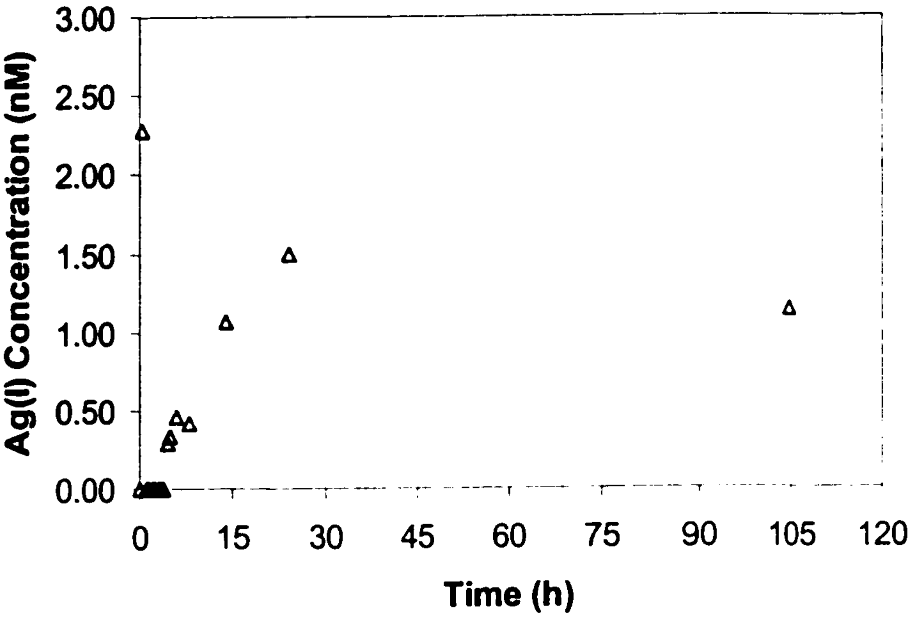


Figure 4.5: Dissolved Ag(I) (< 0.45 µm) measured during the oxidation of an Ag-FeS(s) suspension (0.06 g/L).

larger particles which may require more time to oxidize, then this would explain why Ag(I) is not detected in solution (in the dissolved phase) during the first part of the oxidation. Silver may also be forming soluble, stable clusters with S(II-) in solution that are resistant to oxidation by molecular oxygen. Such clusters have been observed in the laboratory (see *Section 2.7.2*).

The results of this experiment once again demonstrate the instability of FeS(s) in the presence of oxygen and suggest that Ag(I) associated with this phase is released into solution upon its oxidation. Consequently, although Ag(I) is thought to find a sink in anoxic sediments by forming a highly insoluble sulfide (Ag_2S), resuspension of sediments into overlying oxic waters may result in the release of Ag(I) into solution upon sediment oxidation.

4.3.3 Oxidation of Synthetic FeS(s) in the Presence of Sulfide Binding Metals

These experiments examined the effect of S(II-) binding metals (Ni(II), Mn(II), Cu(II) and Zn(II)) on S(II-) during FeS(s) oxidation. Synthetic FeS(s) was first oxidized alone and then in the presence of each metal. Changes in solution pH, dissolved oxygen (DO) and dissolved S(II-) ($< 0.45\ \mu m$) concentration were monitored over time under both anoxic and oxic conditions. The concentration of the colloidal FeS(s) suspension was ~ 0.3 g/L (3.2 mM). Metals were added to the suspension at ~ 0.2% of the FeS(s) mass to reflect the relative abundance of these metals relative to Fe(II) in FeS(s) (Rankama and Sahama, 1950). This resulted in a range of metal concentrations between 8 and 10 μM.

Duplicate samples for S(II-) analysis were collected at ~ 1 and 18 h of anoxia and ~ 1 and 8 h of oxidation. Given the presumed role of Zn(II) in the stabilization of S(II-) in solution under oxic conditions, its effect was examined in more detail and a greater number of samples were collected in those experiments. In order to avoid significant changes in solution volume however, single samples were collected at each sampling time.

4.3.3.1 Experiment 2a: Oxidation of Synthetic FeS(s)

Changes in solution pH and DO during the oxidation of FeS(s) alone are presented in Figure 4.6 while data on dissolved S(II-) (< 0.45 μm) are shown in Figure 4.7. Once again, the pH showed a characteristic drop when the FeS(s) suspension was exposed to air. The pH decreased from 7.3 to 6.0 within 2 h while the DO simultaneously increased from ~ 0.2 to 8.5 mg/L. The colour of the suspension changed from black, to light grey and finally to orange by the end of the oxidation.

Reactive S(II-) concentration (MBS) increased in the dissolved fraction (< 0.45 μm) during the anoxic period. This was probably the result of FeS(s) breaking into finer particles over time due to the stirring motion. The S(II-) concentration increased from ~ 3 to 8 μM. Upon exposure to air, the concentration decreased to ~ 2 μM and was no longer detected in solution after 8 h of oxidation (< 0.1 μM). This experiment demonstrated the instability of synthetic FeS(s) upon exposure to air and showed that S(II-) in the dissolved fraction (< 0.45 μm) is removed from solution during

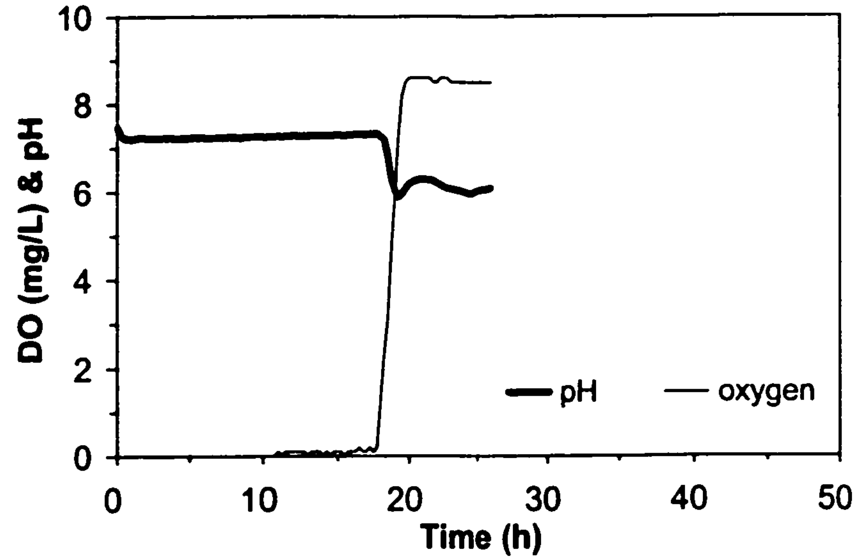


Figure 4.6: Solution pH and dissolved oxygen measured during the oxidation of a FeS(s) suspension (~ 0.3 g/L). Oxidation began at 18 h.

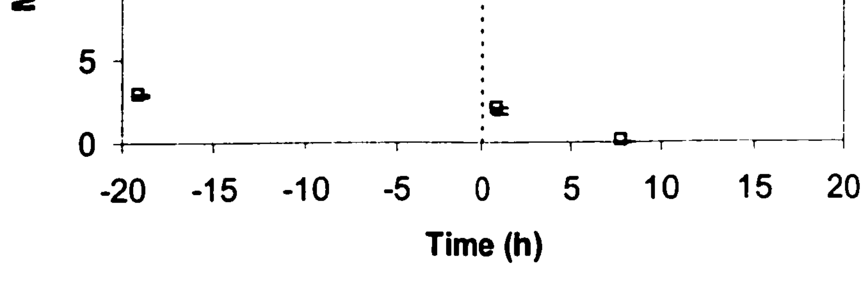


Figure 4.7: Dissolved S(II-) (< 0.45 µm) measured during the oxidation of a FeS(s) suspension (0.3 g/L). Sulfide was monitored under anoxic (< 0 h) and oxic (> 0 h) conditions. The bars represent the range in measured values (n = 2).

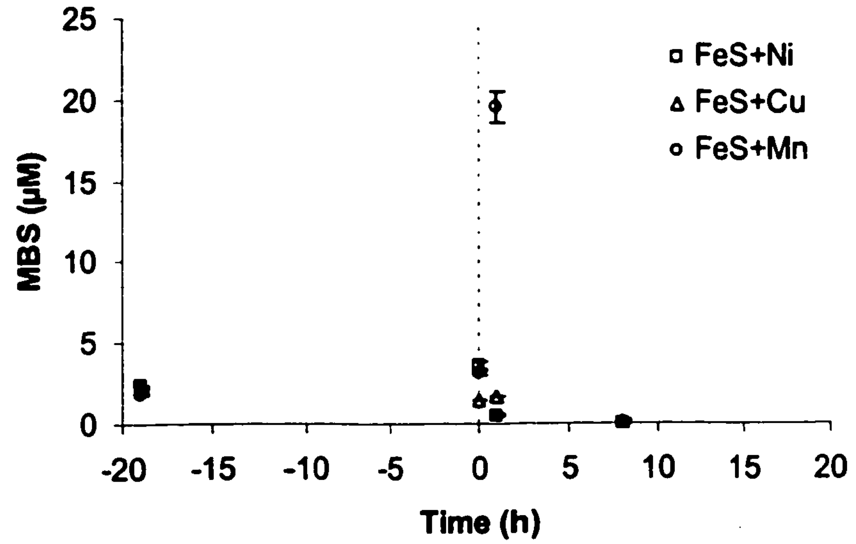


Figure 4.8: Dissolved S(II-) (< 0.45 μm) measured during the oxidation of a colloidal FeS(s) suspension (0.3 g/L) with added Ni(II), Cu(II) or Mn(II) (0.2 % wt/wt). Sulfide was monitored under anoxic (< 0 h) and oxic (> 0 h) conditions. The bars represent the range in measured values (n = 2).

FeS(s) oxidation.

4.3.3.2 Experiment 2b: FeS(s) Oxidation in the Presence of Ni(II), Mn(II), or Cu(II)

In experiments involving the addition of S(II-) binding metals (Ni(II), Mn(II) or Cu(II)) to the FeS(s) suspension, dissolved oxygen and pH showed the same general trends that were observed in the oxidation of FeS(s) alone (see Figure 4.6). Consequently, these data are not repeated here to avoid redundancy. Changes in dissolved S(II-) (< 0.45 μm) data obtained for each metal-FeS(s) system are summarized in Figure 4.8. As was the case with FeS(s) alone, S(II-) concentration decreased to or below detectable levels (< 0.1 µM) after 8 h of oxidation when Ni(II), Mn(II) or Cu(II) were added to the suspension. Relative to the concentrations of dissolved S(II-)

measured in the FeS(s) system alone (Figure 4.7), metal concentrations were high enough (~ 10 μM) to bind a significant portion of the S(II-).

The results of this experiment indicate that Ni(II), Mn(II), and Cu(II) do not stabilize S(II-) from FeS(s) in solution under oxic conditions, at least not at concentrations > 0.1 μM. Metal sulfide species thus formed, either in the solid phase or in solution, were not stable enough to resist oxidation. In the case of Cu(II) and Ni(II) however, stable species may have formed that allowed S(II-) to persist in oxic solution, but the S(II-) was not detected through the MBS analysis. The inability to measure S(II-) bound in CuS(s) by the MBS method was previously noted in *Section 4.3.1,* and is noted and discussed again in later experiments involving the formation of copper sulfide species in solution (see *Sections 5.3.3.3, 5.3.4.3 & 5.3.5.2*). Other researchers have also noted the acid insoluble nature of Cu(II) and Ni(II) sulfide phases in studies involving the AVS method of sulfide determination in sediments (Allen *et al.*, 1993; Wu and Kramer, 1997; Cooper and Morse, 1998). Alternatively, Ni(II), Mn(II) or Cu(II) might be stabilizing S(II-) under oxic conditions but in association with particulates (> 0.45 μm), i.e. iron hydroxide phases.

4.3.3.3 Experiment 2c: FeS(s) Oxidation in the Presence of Zn(II)

Changes in the solution pH and DO over time during the oxidation of FeS(s) with added Zn(II) (0.2% wt/wt, 8.4 μM), are shown in Figure 4.9. Similar changes in the pH were observed as with the oxidation of FeS(s) alone. The pH decreased from 8.3 to 6.8 within a period of ~ 2 h from the onset of oxidation. Approximately 5 h were required, however, for the DO to stabilize as it increased from 0 to 8.3 mg/L. Furthermore, the shape of the DO graph was different than previously observed for the oxidation of FeS alone, as well as with other metals in *Experiment 2a*. Instead of being straight, small plateaus occurred at ~ 1 and 2 h of oxidation (19 and 20 h on graph). This indicates a delay in oxygen saturation of the solution and suggests that oxygen reacting with the FeS(s) was not being replenished as quickly as it had been in the FeS(s) system alone. The dissolved (< 0.45 μm) S(II-) data for this experiment are presented in Figure 4.10. It is noted that in the Zn-FeS(s) system, substantially greater amounts of dissolved S(II-) were detected in solution than in the FeS(s) system or metals added other than Zn(II) (*Experiments 2a & 2b*). Although comparable amounts of FeS(s) were added in each case, ~ 3 μM of dissolved S(II-) were measured in solution in previous experiments at ~ 1 h of anoxia, compared to 167 μM in this system. The FeS(s) used in the previous experiments had aged over a few months, while the FeS(s) used in the Zn(II) experiments originated from a new, fresh batch. The finer particles in the new batch may not have been thoroughly washed out during filtration of the precipitate (refer to *Section 3.1.7*), or alternatively, FeS(s) may have nucleated over time in the aged batch to form larger particles.

In the FeS(s) system alone and with added Ni(II), Mn(II) or Cu(II), dissolved S(II-) concentrations decreased below detectable levels as the suspension was exposed to air (Figures 4.7 & 4.8). By the end of the 8 h oxidation period, S(II-) in each system had been removed from solution. In the Zn-FeS(s) system however, ~ 1.3 μM of S(II-) were still detected in solution after 18 h of oxidation. These observations suggest that Zn(II) stabilized S(II-) in solution during FeS(s) oxidation. The stabilization of S(II-) through

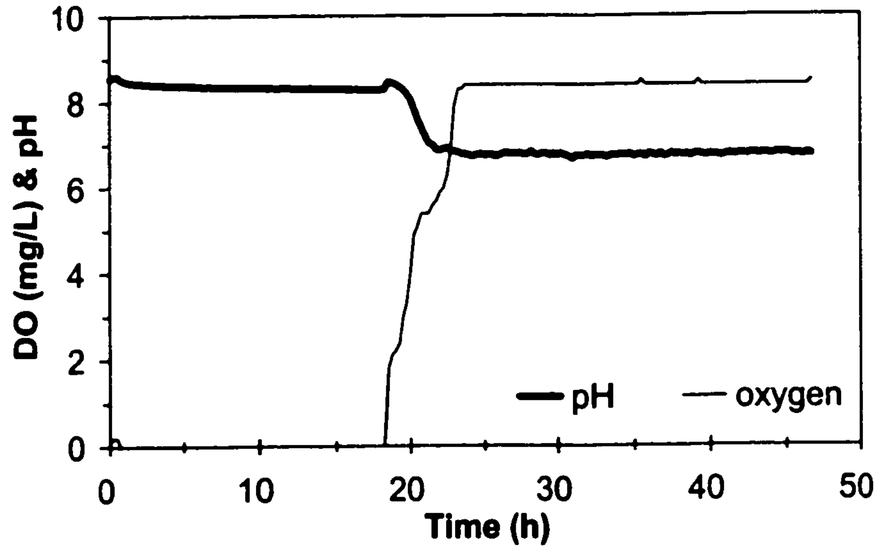


Figure 4.9: Solution pH and dissolved oxygen measured during the oxidation of a FeS(s) suspension (0.3 g/L) with added Zn(II) (0.2 % wt/wt). Oxidation began at 18 h.

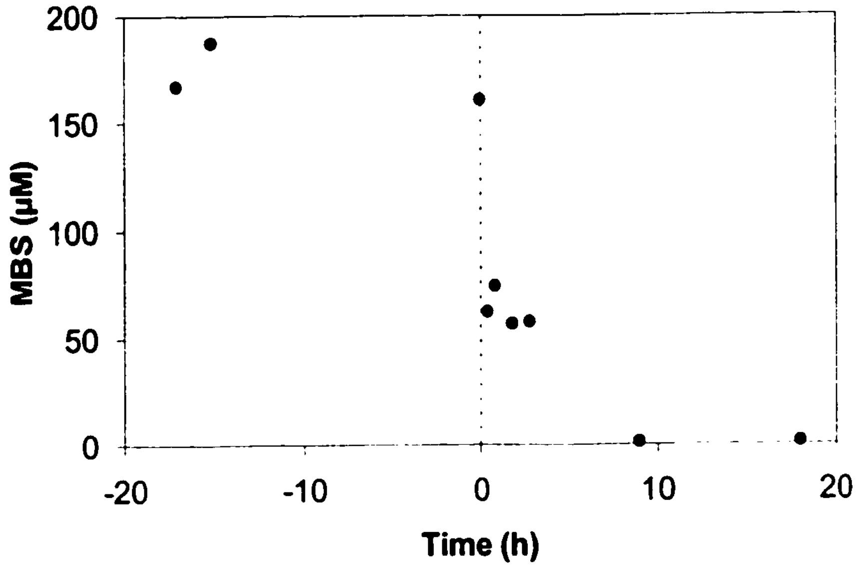


Figure 4.10: Dissolved S(II-) (< 0.45 μm) measured in a FeS(s) suspension (0.3 g/L) with added Zn(II) (0.2 % wt/wt). Sulfide was monitored under anoxic (< 0 h) and oxic (> 0 h) conditions.

the formation of zinc sulfide species is also observed in later experiments (see *Sections 5.3.3.1 & 5.3.4.1*), while ZnS(s) suspended in oxic solution has been shown to resist oxidation (Simpson *et al.*, 1998a; Simpson *et al.*, 2000).

The effect of Zn(II) on FeS(s) oxidation was re-examined by repeating the experiment with an extended oxidation period, 70 h, and with more Zn(II) (2% wt/wt, 84 μM). The S(II-) data are shown in Figure 4.11 for the oxidation period only. As the same fresh FeS(s) was used in this experiment, relatively high concentrations of dissolved S(II-) (246 μM) were once again measured in solution at the end of the anoxic period. At 6 h of oxidation, the S(II-) concentration had decreased to 1.8 μM and by 9 h to ~ 1.3 μM where it remained constant for the remainder of the oxic period. The fact that MBS concentration became constant suggests that S(II-) measured under oxic conditions in this system, relative to the other FeS(s) systems, was stabilized by Zn(II). Furthermore, pH and DO showed the same deviations from the FeS(s) system as those observed in the Zn-FeS(s) system 0.2% (wt/wt) Zn(II). These data are shown in Figure 4.12 for the first part of the oxidation period.

These experiments strongly suggest that Zn(II) stabilizes S(II-) from FeS(s) in oxic solution. This implies that a similar process may occur in sedimentary systems upon sediment resuspension into overlying oxic waters. Oxidation of FeS(s) may be induced in this manner. The presence of Zn(II) in solution may stabilize S(II-) which, as will be discussed in later chapters, could provide a S(II-) pool to less abundant, but more toxic metals in solution.

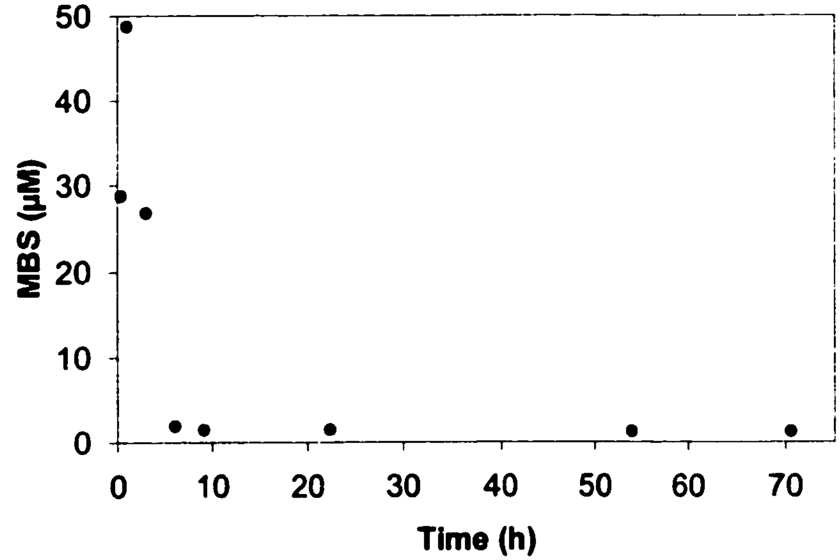


Figure 4.11: Dissolved S(II-) (< 0.45 μm) measured in a FeS(s) suspension (0.3 g/L) with added Zn(II) (2% wt/wt). Sulfide was monitored under anoxic (< 0 h) and oxic (> 0 h) conditions.

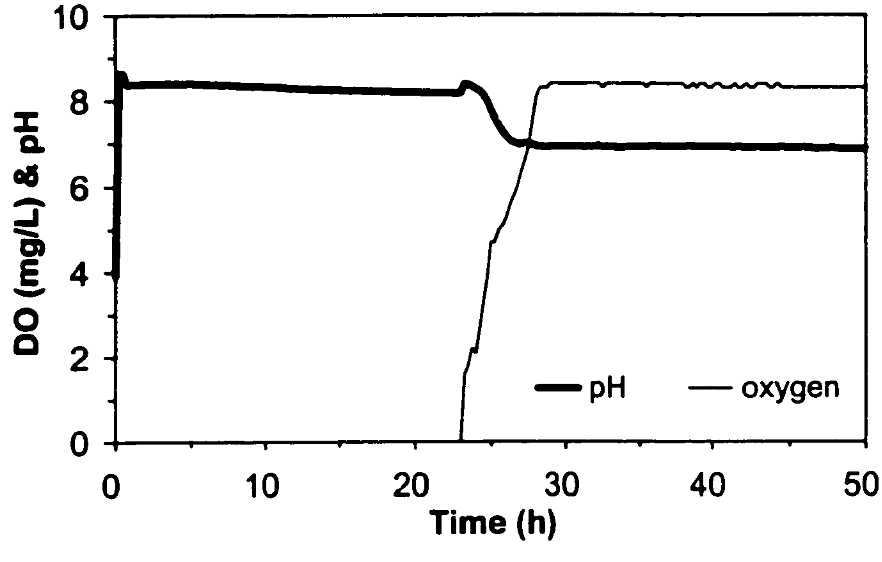


Figure 4.12: Solution pH and dissolved oxygen measured during the oxidation of a FeS(s) suspension (0.3 g/L) with added Zn(II) (2.0% wt/wt). Oxidation began at 23 h.

4.4 CONCLUSIONS

The following conclusions were drawn from the experiments discussed in this chapter:

(1) Synthetic amorphous iron monosulfide (FeS(s)) suspended in oxic solution rapidly oxidizes to iron hydroxide (lepidocrocite), while S(II-) is simultaneously removed from solution.

(2) Silver sorbed to synthetic FeS(s) is released into solution upon FeS(s) oxidation.

(3) Nickel(II), Mn(II) and Cu(II) sorbed to synthetic FeS(s) do not appear to stabilize S(II-) in solution upon oxidation of FeS(s).

(4) Zinc(II) sorbed to synthetic FeS(s) exerts a stabilizing effect on S(II-) in solution upon FeS(s) oxidation .

CHAPTER 5

STABILIZATION OF SULFIDE BY METALS AND NATURAL ORGANIC MATTER UNDER OXIC CONDITIONS

5.1 OBJECTIVES

The experiments discussed in this chapter were designed to fulfil the following objectives:

(a) Determine whether certain S(II-) binding metals alone and in conjunction with NOM, have a stabilizing effect on S(II-) in oxic solution.

(b) Determine whether Zn(II) alone and in conjunction with NOM maintains a stabilizing effect on S(II-) in oxic solution when other metals are present that exhibit higher or lower affinities for S(II-).

(c) Determine the approximate binding stoichiometry of Ag(I) and Cu(II) with S(II-).

5.2 EXPERIMENTAL METHODS

5.2.1 General Considerations

The reagents used in these experiments are described in *Section 3.1.* All plasticware and glassware was washed in 20% HNO_3 (Trace Metal Grade, Baker, USA), rinsed with Milli-Q water and dried prior to use.

5.2.2 Sulfide Determination

Sulfide was determined as methylene blue-sulfide (MBS) on a Beckman Model B (Beckman Instruments, USA) or a Spectronic 20 (Bausch & Lomb, USA) according to the procedure outlined is *Section 3.2.2*. Detection limits for S(II-) were calculated at 0.03 μM and 0.1 μM on the Beckman and Spectronic 20, respectively.

5.2.3 Experimental Design

These experiments examined the persistence and stability of S(II-) in solution over time under anoxic and oxic conditions, with and without the effect of S(II-) binding metals and NOM. In the first set of experiments, the effects of the following metals were studied in the presence and absence of Suwannee River fulvic acid (SRFA): Zn(II), Fe(II), Fe(III), Ag(I) and Cu(II). These metals were chosen due to their presumed role in stabilizing S(II-) in oxic waters by forming stable metal sulfide clusters. A wide range of metal concentrations was used resulting in different metal to S(II-) ratios in each sample. Similarly, the effects of the following mixed metal systems were studied in another set of experiments: Zn(II) & Fe(III), Zn(II) & Ag(I) and Zn(II) & Cu(II). Mixed metal systems were examined because they more closely simulate the occurrence of metals in natural waters. Summaries of the samples prepared for each experiment along with their respective metal concentrations and metal to sulfide ratios are presented in Tables 5.1, 5.2 and 5.3. Higher concentrations of Ag(I) were used because S(II-) can bind more than one monovalent Ag(I) atom (Ag_2S).

Table 5.1: Summary of metal concentrations and metal to S(II-) ratios used to examine the stability of S(II-) in oxic solution in the presence of metals. The initial concentration of S(II-) in each sample was 2.5 μM.

Zn(II)		Fe(II)		Fe(III)		Ag(I)		Cu(II)	
Zn:S	**Zn (μM)**	**Fe:S**	**Fe (μM)**	**Fe:S**	**Fe (μM)**	**Ag:S**	**Ag (μM)**	**Cu:S**	**Cu (μM)**
0:2.5	0	0:2.5	0	0:2.5	0	0:2.5	0	0:2.5	0
0.6:1.0	1.5	0.6:1.0	1.5	0.6:1.0	1.5	0.6:1.0	1.0	0.6:1.0	1.5
1.0:1.0	2.5	1.0:1.0	2.5	1.0:1.0	2.5	1.2:1.0	3.0	1.0:1.0	2.5
1.4:1.0	3.5					2.0:1.0	5.0		
2.0:1.0	5.0								
3.0:1.0	7.5								

Table 5.2: Summary of metal concentrations and metal to S(II-) ratios used to examine the stability of S(II-) in oxic SRFA solution (5 mg C/L) with added metals. The initial concentration of S(II-) in each sample was 2.5 μM.

Zn(II)		Fe(II)		Fe(III)		Ag(I)		Cu(II)	
Zn:S	**Zn (μM)**	**Fe:S**	**Fe (μM)**	**Fe:S**	**Fe (μM)**	**Ag:S**	**Ag (μM)**	**Cu:S**	**Cu (μM)**
0:2.5	0	0:2.5	0	0:2.5	0	0:2.5	0	0:2.5	0
0.2:1.0	0.5	0.2:1.0	0.5	0.2:1.0	0.5	1.2:1.0	3.0	0.2:1.0	0.5
0.6:1.0	1.5	0.6:1.0	1.5	0.6:1.0	1.5	2.0:1.0	5.0	0.6:1.0	1.5
1.0:1.0	2.5	1.0:1.0	2.5	1.0:1.0	2.5	2.8:1.0	7.0	1.0:1.0	2.5
1.4:1.0	3.5	1.4:1.0	3.5	1.4:1.0	3.5			1.4:1.0	3.5
2.0:1.0	5.0								
3.0:1.0	7.5								
10:1.0	25								

Table 5.3: Summary of metal concentrations used in mixed metal experiments examining the stability of S(II-) in oxic solution. The initial S(II-) concentration in each sample was 2.5 μM. Samples were prepared in either water or SRFA solution (5 mg C/L).

	Zn(II) & Fe(III)		Zn(II) & Ag(I)		Zn(II) & Cu(II)	
Sample Matrix	**Zn (μM)**	**Fe (μM)**	**Zn (μM)**	**Ag (μM)**	**Zn (μM)**	**Cu (μM)**
WATER	2.5	0	2.5	0	2.5	0
	2.5	1.5	2.5	2.5	2.5	1.5
	2.5	2.5	2.5	5.0	2.5	2.5
			2.5	7.5		
SRFA	2.5	0	2.5	0	2.5	0
	2.5	0.5	2.5	0.5	2.5	0.5
	2.5	1.5	2.5	1.5	2.5	1.5
	2.5	2.5	2.5	2.5	2.5	2.5
	0	2.5	2.5	5.0	0	2.5
			2.5	7.5		
			0	5.0		

5.2.4 Preparation of Samples

Samples were prepared in either water or aqueous Suwannee River fulvic acid (SRFA) solution (~ 5 mg C/L) with an initial S(II-) concentration of 2.5 μM and various metal concentrations.

Approximately 47 mL of water or SRFA solution were poured into pre-weighed 50 mL polypropylene (PP) centrifuge tubes that contained Teflon covered stir bars. An appropriate volume of 0.01 M metal stock solution (Zn(II), Fe(III), Ag(I) or Cu(II), see *Section 3.1.3* for preparation), was added to each sample to attain the desired concentrations shown in Tables 5.1, 5.2 and 5.3. Once the metal was added, the pH of each sample was adjusted to ~ 8.5 with 0.01 M NaOH and the sample volume was brought to 50 or 52 mL by weight. All samples were degassed with He for 3 – 5 minutes and S(II-) was added under an Ar blanket or a N_2 atmosphere inside a glove bag or glove box. Sulfide addition increased the pH to ~ 9.5. Following their preparation, samples were stored under N_2 for the duration of the anoxic period.

Metals were allowed to equilibrate with SRFA for ~ 24 h prior to the addition of S(II-). In the preparation of mixed metal samples, the second metal (Fe(III), Ag(I) or Cu(II)) was added ~ 24 h after the S(II-) to provide enough time for S(II-) equilibration with the system. The second metal was always added from a non-acidified stock solution to avoid changing the pH. Samples testing the effect of Fe(II) on S(II-) stability were prepared in a slightly different manner to minimize oxidation to Fe(III). The metal in this case was added from a non-acidified stock solution after the pH and volume had already been adjusted and the sample degassed.

Changes in the volume of the sample resulting from the addition of S(II-) or metal stock solution were always < 1%, and < 5% resulting from evaporation over the course of the experiment.

5.2.5 Experimental Procedures

Sulfide concentration was monitored under anoxic conditions for 45 – 71 h. Measurements for S(II-) were made 1 – 2 times during this period and the first measurement usually occurred within the first 24 h. Aliquots for S(II-) analysis were extracted from the sample while inside the glove bag or glove box, or the sample was taken out, sampled under an Ar blanket, recapped and stored under N_2. After the last anoxic measurement was made, the samples were left uncapped and placed into large beakers covered with perforated parafilm paper to allow for exposure to air. The beakers were seated on magnetic stirrers and the samples were stirred for the entire oxidation period. Depending on the length of the oxidation period (65 – 167 h), between 2 and 4 S(II-) measurements were made. One measurement always occurred within the first 26 h. The aliquot volume for S(II-) analysis was 8 or 10 mL.

The pH was measured (Ross combination glass electrode, Orion, USA) at the end of the anoxic period and at every sampling time during the oxic period of the first few experiments. The pH was found to remain constant at ~ 9.5 throughout the anoxic period. Within the first 24 h of oxidation, the pH dropped to and stabilized between 7.1 – 7.9. Since the pH was always found to behave in the same manner in all samples, rigorous monitoring of the pH was abandoned in later experiments. Sulfide concentrations, available pH data, and sampling times for each experiment are summarized in the Appendix.

Changes in MBS concentration for each experiment are depicted graphically as a function of time starting at negative hours. The "negative" time period represents the anoxic period, while zero hours marks the end of anoxia and the beginning of the oxic period.

5.3 RESULTS

5.3.1 Experiment 1: Sulfide Reaction with Natural Organic Matter (NOM)

An experiment was conducted to test the stability of S(II-) in the presence of

NOM under anoxic conditions. Aqueous solutions of NOM from the Luther Marsh (LM) were prepared in PP tubes with various concentrations of organic carbon (1.5 – 13.4 mg C/L). The pH of each sample was adjusted to ~ 8.5 with NaOH and all solutions were degassed with He and spiked to a concentration of 2.0 μM of S(II-). Samples were stored in a glove bag under a N_2 atmosphere for ~ 24 h and then analyzed for MBS. Following MBS analysis, each sample was degassed and enough S(II-) was added to attain an overall concentration of 4.5 μM. The samples were placed back in the glove bag for another ~ 24 h and then reanalyzed for MBS. This procedure was repeated one more time where enough S(II-) was added to each sample to attain an overall concentration of 7.0 μM. In summary, S(II-) was added to samples at ~ 24 h intervals to attain S(II-) concentrations of 2.0, 4.5 and 7.0 μM. Methylene blue-sulfide was measured ~ 24 h after each S(II-) addition. The results are shown in Figure 5.1.

The expected concentrations of S(II-) (2.0, 4.5 and 7.0 μM) at each sampling time were not recovered. Although some S(II-) may have been lost from each sample during the degassing steps, the amount of S(II-) measured was clearly dependent on the concentration of the NOM. As the NOM concentration in the sample increased, the amount of measured S(II-) decreased. After the first S(II-) addition of 2.0 μM (at ~ 24 h), S(II-) was detected in only one sample, the sample with the lowest organic carbon content (1.5 mg C/L) (white series) and only 22% of the initial S(II-) was recovered.

The direct dependence of the amount of S(II-) removed from solution on the NOM concentration suggests that S(II-) was being lost by reacting with a component of

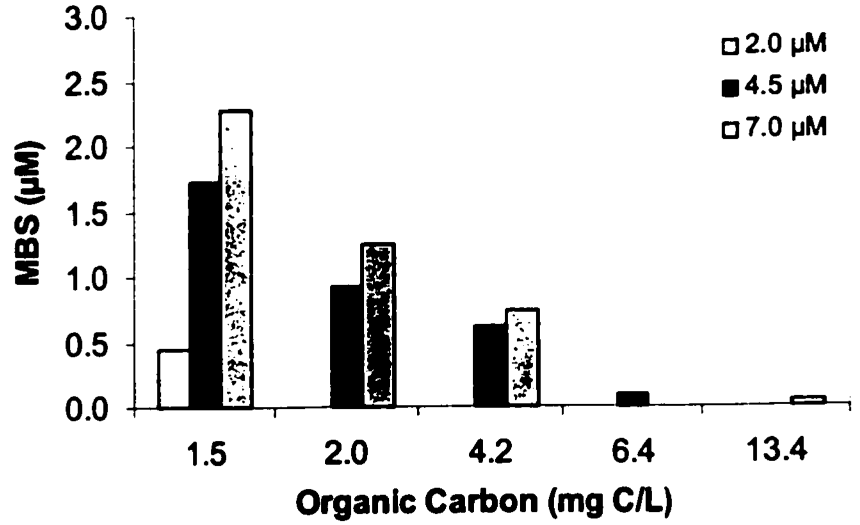


Figure 5.1: Sulfide measured in anoxic Luther Marsh NOM solutions of various organic carbon concentrations. Sulfide was added in doses at ~ 24 h intervals and the MBS was measured after ~ 24 h. The expected cumulative S(II-) concentrations at each sampling time were 2.0, 4.5 and 7.0 µM.

the NOM. Reaction of organic matter with reduced sulfur species to form low molecular weight or macromolecular organosulfur compounds has been demonstrated in laboratory experiments and occurs in anoxic sediments (refer to *Section 2.8.5*). Natural organic matter typically contains conjugated carbonyls and other functional groups that may react with S(II-) (refer to *Section 2.8.1*).

5.3.2 Experiment 2: Experimental Variability

Changes in S(II-) concentration were monitored over time in water or aqueous SRFA solution with 2.5 µM of S(II-). Metals were not added to any of these samples. The results are plotted in Figures 5.2 and 5.3 and indicate the extent of variability

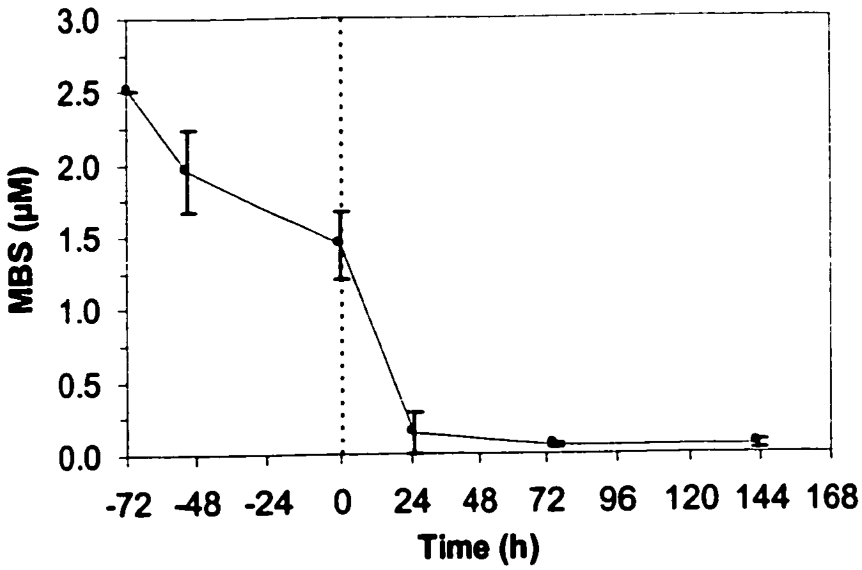


Figure 5.2: Sulfide measured over time in solution under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM. The bars represent the standard error on replicate samples (n = 5).

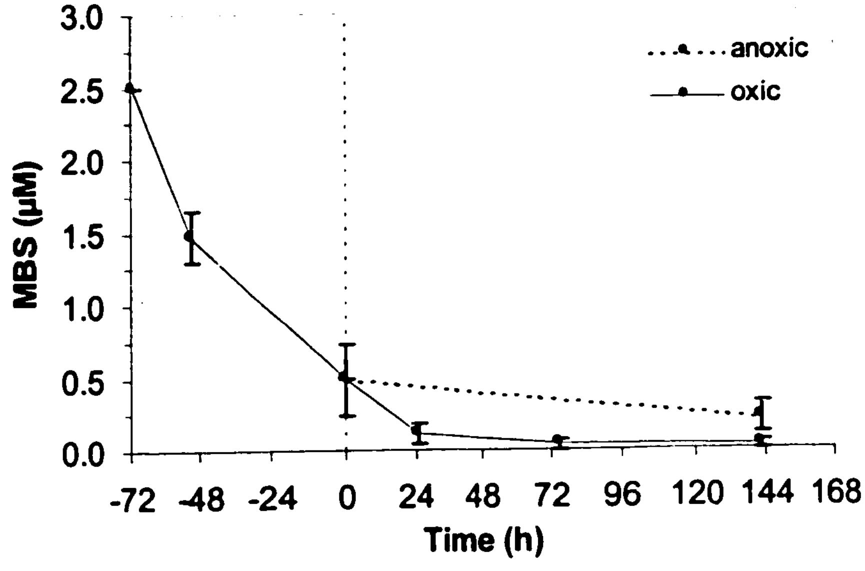


Figure 5.3: Sulfide measured over time in SRFA solution (5 mg C/L) under anoxic (< 0 h) and oxic (> 0 h) conditions. The dashed line depicts changes in MBS for samples that were kept anoxic beyond zero hours. The initial S(II-) concentration was 2.5 µM. The bars represent, the standard error on four replicate samples for measurements made up to and including zero hours, and the range in MBS measured in duplicate samples for measurements made beyond zero hours.

encountered in a single S(II-) measurement at a given time throughout the course of a typical experiment.

The mean S(II-) concentrations of five replicate samples prepared in water are plotted in Figure 5.2 where the bars represent their standard error (n = 5). Concentrations of S(II-) in SRFA samples (n = 4) are summarized in Figure 5.3. At zero hours, two of these samples were exposed to oxic conditions as per usual, but the other two were kept anoxic (dashed line). The MBS concentrations plotted up to and including zero hours are the mean of all four replicate samples, and the bars indicate their standard error (n = 4). After zero hours, the average of only two replicates is plotted for anoxic and oxic samples respectively, and the bars show the range in the measured concentrations (n = 2).

As indicated by the large error bars, great variability in S(II-) concentrations was observed for all measurements made during the anoxic period. Furthermore, unexpected decreases in S(II-) were noted over time and were most pronounced in samples prepared in SRFA solution. These decreases resulted from S(II-) oxidation by residual amounts of oxygen remaining in or leaking into the samples, as well as reaction with SRFA. As was the case with LM NOM (*Experiment 1, Section 5.3.1*), SRFA contains appropriate functional groups for reaction with S(II-) (refer to *Section 2.8.1*). The extent of S(II-) removal by these processes would vary between samples which explains the high variabilities observed.

Under oxic conditions, S(II-) concentration in all samples decreased over time and became constant at 0.03 – 0.06 μM sometime between 24 and 72 h of oxidation. Measurements made during the oxic period displayed little variability. In Figure 5.3, it can be seen that the amount of S(II-) remaining in SRFA solution under anoxic conditions was more than that remaining under oxic conditions. Consequently, both oxidation and reaction with SRFA removed S(II-) from solution under oxic conditions.

These experiments clearly illustrate the instability of S(II-) under oxic conditions in both water and SRFA solution.

5.3.3 Sulfide and Metals in Aqueous Solution

An unexpected decrease in S(II-) concentration was observed throughout the anoxic period in all samples prepared in water. Similar losses in S(II-) were previously observed in experiments described in *Section 3.3* and were attributed to the adsorption of zinc sulfide species to PP container surfaces. In addition to zinc sulfide species, adsorption of iron, copper and silver sulfides probably occurred in the experiments presented in this section. Sulfide oxidation by trace amounts of oxygen remaining in or leaking into samples may also have contributed to these losses.

5.3.3.1 Experiment 3: Sulfide in the Presence of Zn(II)

Methylene blue-sulfide concentration was monitored over time under anoxic and oxic conditions in samples prepared in water with various amounts of Zn(II) (Table 5.1) and an initial S(II-) concentration of 2.5 μM. The results are summarized in Figures 5.4 and 5.5. The second experiment (Figure 5.5) repeated the first one with an extended oxidation period and additional samples prepared with higher Zn(II) concentrations. Unexpected increases in S(II-) concentration were observed in some samples during the

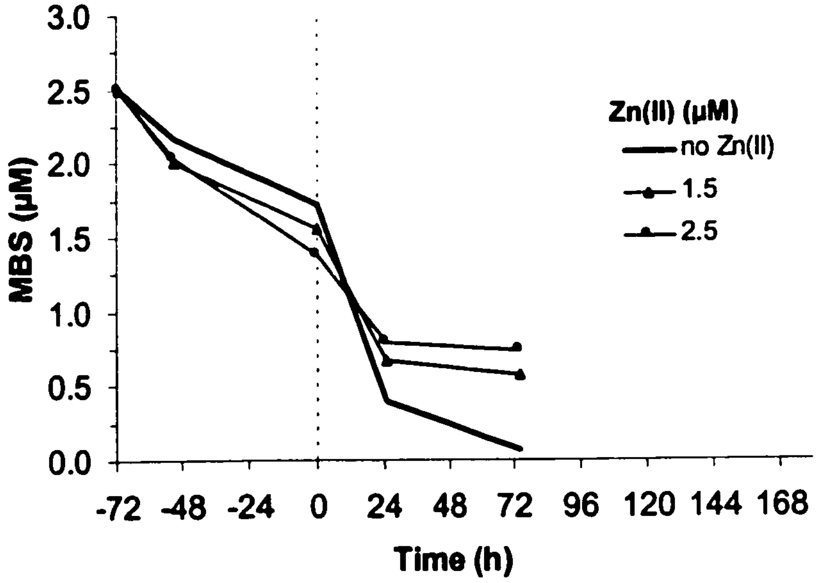


Figure 5.4: Sulfide measured over time in solution with Zn(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

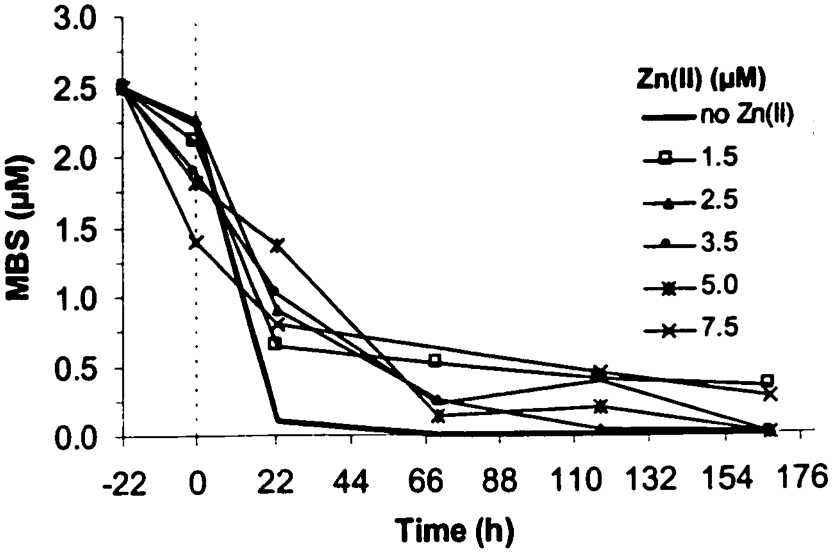


Figure 5.5: Sulfide measured over time in solution with Zn(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

oxic period. Zinc sulfide species desorbing from the PP container would explain such erratic increases and changes in MBS concentrations.

The results of the first experiment (Figure 5.4) showed that after ~ 75 h of oxidation, 23% and 30% of the initial S(II-) remained in samples containing 1.5 and 2.5 μM of Zn(II), respectively. Furthermore, at 118 and 167 h of oxidation (Figure 5.5) up to 16% of S(II-) could still be measured in some of the samples in the second experiment. In contrast, MBS in samples lacking Zn(II) decreased close to or below detectable levels (< 0.03 μM) in both experiments during the oxidation period.

These results indicate that Zn(II) stabilizes S(II-) under oxic conditions. Sulfide persisted in oxic solution for at least 7 days at which time the experiment was terminated. The amount of S(II-) stabilized appeared to be independent of the Zn(II) concentration but a dependence probably exists. Since zinc sulfide is lost from solution by adsorbing to the PP container, such a dependence is not apparent. Consequently, the amount of S(II-) measured and stabilized by Zn(II) under oxic conditions in these experiments is underestimated.

5.3.3.2 Experiment 4: Sulfide in the Presence of Fe(II) or Fe(III)

Once again, MBS concentration was monitored over time in samples prepared in water, but with various amounts of Fe(II) or Fe(III) (Table 5.1). The initial S(II-) concentration was 2.5 μM. The results are presented in Figures 5.6 and 5.7 for Fe(II) and Fe(III), respectively.

During the oxidation period, S(II-) concentrations decreased close to or below

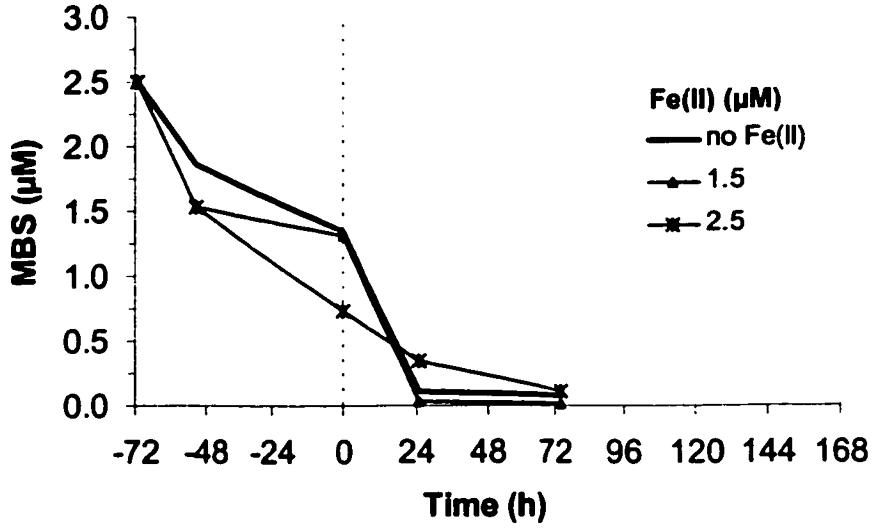


Figure 5.6: Sulfide measured over time in solution in the presence of Fe(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

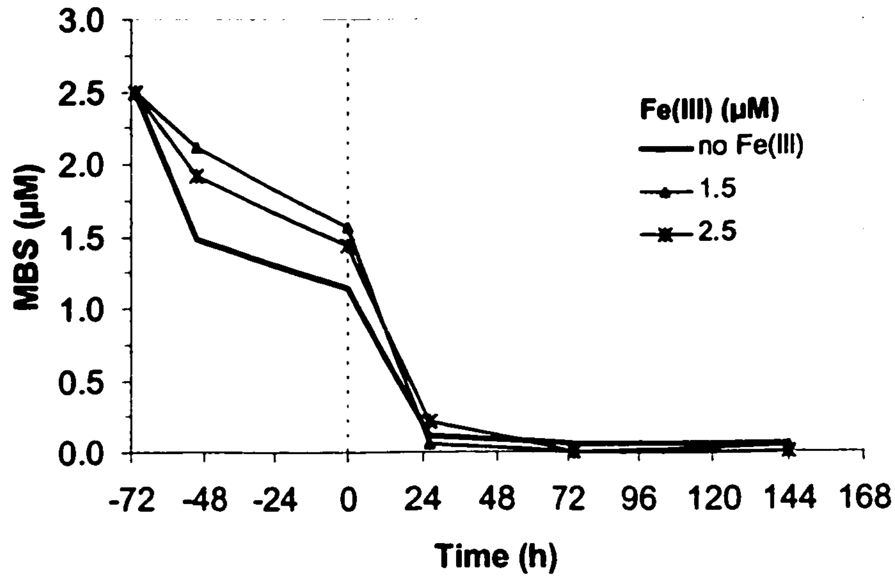


Figure 5.7: Sulfide measured over time in solution with Fe(III) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

detectable levels (< 0.03 μM) in all samples, regardless of whether they contained Fe in either oxidation state. It appears from these results that Fe(II) and Fe(III) do not stabilize S(II-) in oxic solution. The lack of FeS(s) precipitation and Fe(III) (oxy)hydroxide formation however, may suggest that acid insoluble and MDR unreactive iron sulfide species are formed in solution. This idea is further supported by the fact that under anoxic conditions, S(II-) concentration in samples containing Fe(II) are lower than with S(II-) alone.

5.3.3.3 Experiment 5: Sulfide in the Presence of Cu(II) or Ag(I)

Sulfide samples containing either Cu(II) or Ag(I) at various concentrations (Table 5.1) were prepared in water with an initial S(II-) concentration of 2.5 μM. Once again, S(II-) was monitored over time by measuring MBS under anoxic and oxic conditions. The results are shown in Figures 5.8 and 5.9 for Cu(II) and Ag(I), respectively.

When Cu(II) containing samples were analyzed for MBS during the anoxic period, S(II-) was not detected in any sample except for the one lacking Cu(II). Under anoxic conditions, even with some loss of S(II-) to oxidation or adsorption of copper sulfide phases to the PP container, a significant portion of S(II-) should have remained in solution.

The inability to detect S(II-) in these samples suggested that somehow the formation of MBS was being inhibited. One possibility is that S(II-) was not dissociated from Cu in the MDR to form H_2S and subsequently MBS. The acid insoluble nature of CuS(s) was noted in a previous experiment (*Experiment 1, Section 4.3.1*) and has also

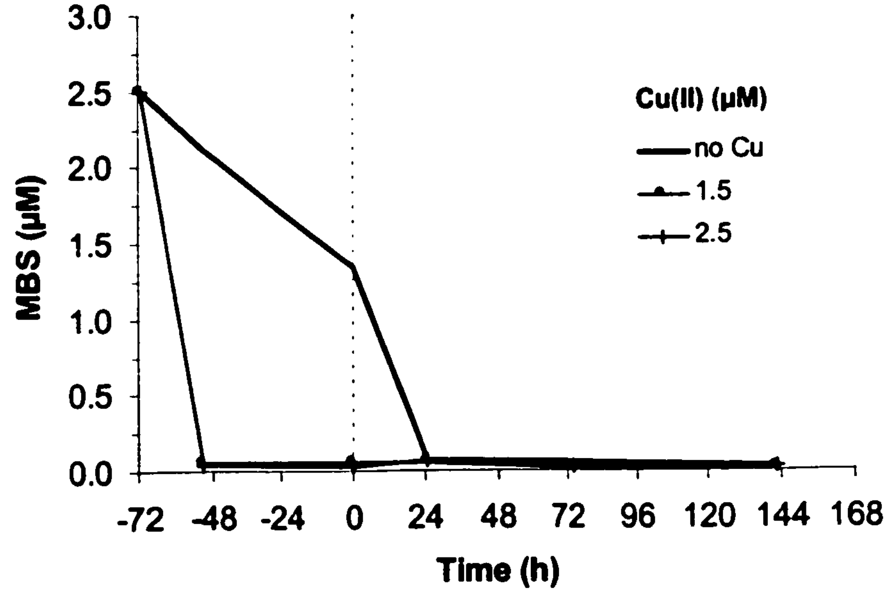


Figure 5.8: Methylene blue-sulfide measured in solution in the presence of Cu(II). The initial S(II-) concentration was 2.5 µM. Measurements were made under anoxic (< 0 h) and oxic (> 0 h) conditions.

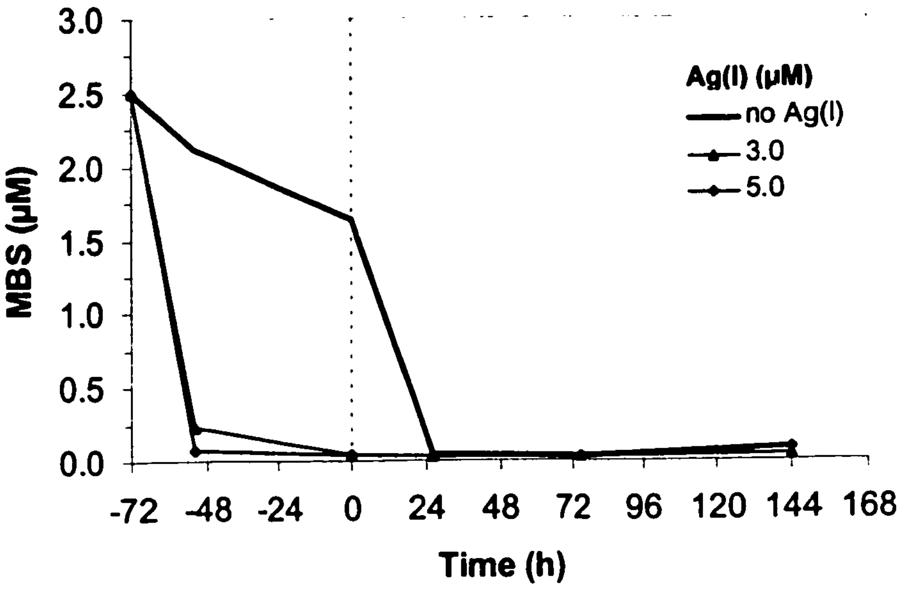


Figure 5.9: Methylene blue-sulfide measured in solution in the presence of Ag(I). The initial S(II-) concentration was 2.5 µM. Measurements were made under anoxic (< 0 h) and oxic (> 0 h) conditions.

been reported by other researchers both as a solid (Wu and Kramer, 1997) and in solution (Radford-Knoery and Cutter, 1993; Luther *et al.,* 1996). Complete dissociation of copper sulfide complexes does not occur even at pH values below 2, and treatment with an acidic Cr(II) reducing solution is necessary to recover S(II-). Another possibility is that the presence of Cu(II) itself might interfere in the reaction of H_2S with dimethylphenylenediamine in MDR to form MBS. Finally, the reduction of Cu(II) to Cu(I) upon complexation with S(II-) and simultaneous formation of polysulfide species has also been reported (Radford- Knoery and Cutter, 1993; Luther *et al.*, 1996; Rozan *et al.*, 2000). Polysulfide species are not completely reactive in MDR as only the terminal S(II-) atoms form MBS.

The formation of MBS also seemed to be inhibited in the presence of Ag(I) (Figure 5.9). Unlike the case with Cu(II), however, some S(II-) (< 10%) was measured in samples containing Ag(I) (3.0 and 5.0 μM). This MBS formed either from unbound S(II-), or S(II-) that partially dissociated from the silver sulfide phase in the acidic MDR.

These experiments were continued into the oxic period to determine whether S(II-) is liberated from Cu(II) or Ag(I) by oxidation. Sulfide however, was not detected in either case starting at ~ 24 h of oxidation when the first analysis was conducted.

The stabilization of S(II-) by Cu(II) or Ag(I) under oxic conditions could not be verified in these experiments due to the inability of the MBS method to measure S(II-) in the presence of these metals.

5.3.3.4 Experiment 6: Sulfide in the Presence of Zn(II) & Fe(III)

This experiment examined the effect of Zn(II) on S(II-) in aqueous solution in the presence of Fe(III) under anoxic and oxic conditions. Sulfide concentration was monitored over time in samples prepared in water with 2.5 μM of Zn(II), various concentrations of Fe(III) (Table 5.3), and an initial S(II-) concentration of 2.5 μM. The results are shown in Figure 5.10. After 75 h of oxidation, significant amounts of S(II-) were measured in all samples. Approximately 30, 18 and 9% of the initial S(II-) remained in samples containing 2.5 μM of Zn(II) and 0, 1.5 and 2.5 μM of Fe(III), respectively. According to these results, S(II-) is stabilized by Zn(II) under oxic conditions even in the presence of Fe(III).

In previous experiments, Zn(II) alone was shown to stabilize S(II-) under the same oxic conditions (*Experiment 3, Section 5.3.5.1*), while Fe(III) alone did not attain the same effect and the S(II-) concentration decreased over time (*Experiment 4, Section 5.3.5.2*). Iron(III) along with other metals has been shown to catalyze the oxidation of S(II-) by oxygen (Vazquez *et al.*, 1989). In samples containing Fe(III), the two metals might be competing to react with the S(II-) and thus less S(II-) is stabilized by the Zn(II). This however does not rule out the possibility that Zn(II) might stabilize S(II-) in conjunction with Fe(III), perhaps in the form of a mixed metal sulfide species.

By the end of the oxidation period (~ 6 days), S(II-) concentrations decreased close to or below detectable levels (< 0.03 μM) in two of the samples. Although the S(II-) appears to have oxidized in these samples, it is possible that adsorption of the metal sulfide species to the container occurred over time.

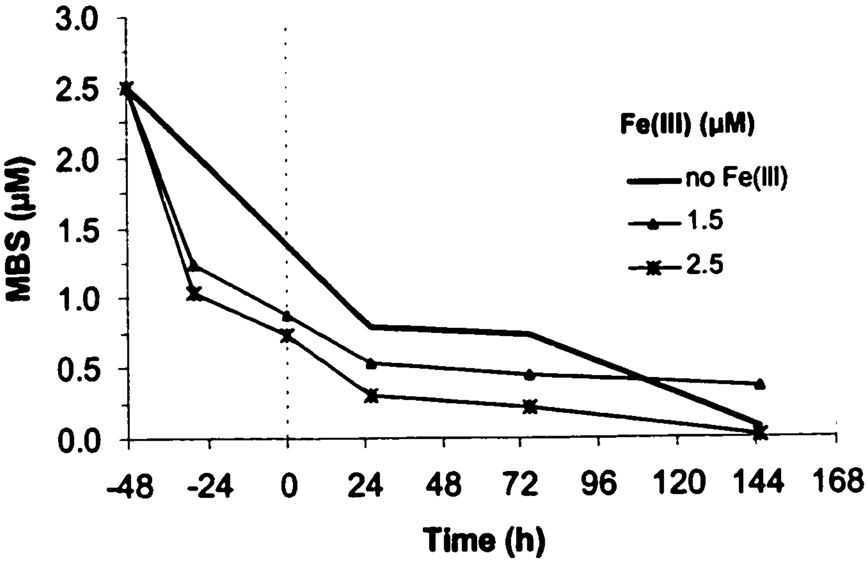


Figure 5.10: Sulfide measured over time in solution with 2.5 µM of Zn(II) and various concentrations of Fe(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

According to these results, Zn(II) appears to stabilize S(II-) in oxic solution in the presence of Fe(III).

5.3.3.5 Experiment 7: Sulfide in the Presence of Zn(II) & Cu(II) or Zn(II) & Ag(I)

Experiments were also conducted to examine the effect of Zn(II) on S(II-) in aqueous solution in the presence of Cu(II) or Ag(I) under anoxic and oxic conditions. Sulfide concentration was monitored over time in samples prepared in water with 2.5 µM Zn(II), various concentrations of Cu(II) or Ag(I) (Table 5.3), and an initial S(II-) concentration of 2.5 µM. The results are shown in Figures 5.11 and 5.12 for Cu(II) and Ag(I), respectively. In both experiments, S(II-) was generally not measured in any samples containing Cu(II) or Ag(I) regardless of the experimental conditions (anoxic or

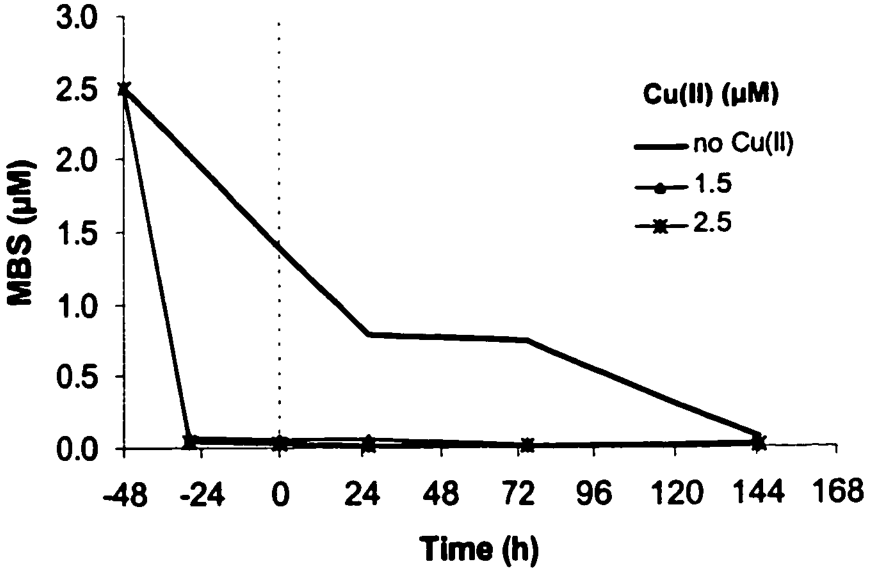


Figure 5.11: Sulfide measured over time in solution with 2.5 µM of Zn(II) and various concentrations of Cu(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

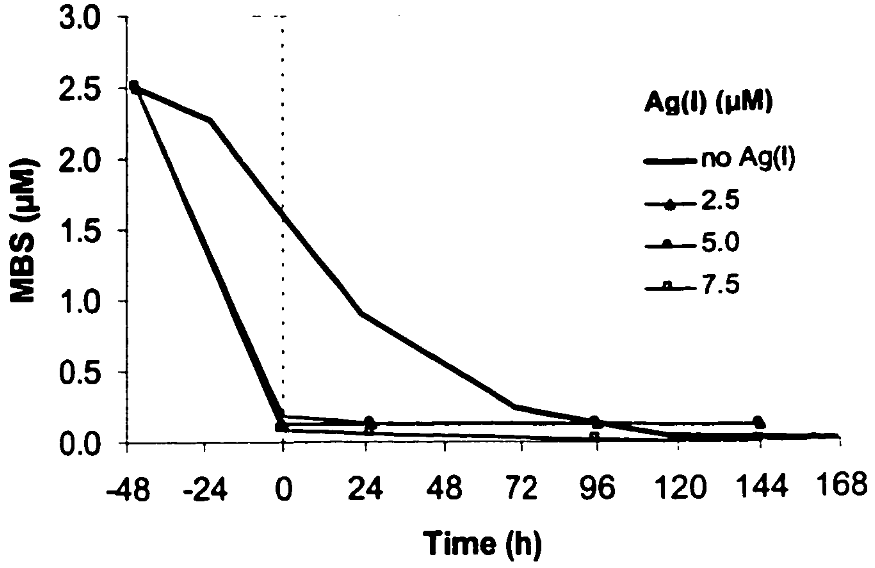


Figure 5.12: Sulfide measured over time in solution with 2.5 µM of Zn(II) and various concentrations of Ag(I) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

oxic). Sulfide should have been detected under anoxic conditions, even if some was lost by adsorption to the container or oxidation. As was discussed in *Experiment 5*, this apparent lack of S(II-) is due to the inability of S(II-) to react in MDR in the presence of Cu(II) or Ag(I). As a result, MBS formation is not achieved. The results of this experiment indicate that this inhibition of MBS formation is also accomplished in the presence of Zn(II), despite the ability of Zn(II) to bind and stabilize S(II-). Providing that the lack of MBS detection is due to the acid insolubility of copper and silver sulfides, these experiments also suggest that S(II-) preferentially binds Cu(II) or Ag(I) to Zn(II). This does not however preclude the possibility that mixed metal sulfide species incorporating Zn(II) are formed.

In samples containing only Zn(II), significant amounts of S(II-) were measured in the early stages of the oxic period. At ~ 24 of oxidation, ~ 30 – 36% of the initial S(II-) remained in these samples. By the end of each oxic period however (144 – 168 h), S(II-) concentrations had decreased close to or below detectable levels (< 0.03 μM). Although the decrease in MBS suggests that S(II-) was lost by oxidation, adsorption of zinc sulfide species to the container probably occurred as well.

5.3.4 Sulfide and Metals in Aqueous Suwannee River Fulvic Acid (SRFA) Solution

The stabilization of S(II-) by Zn(II) in pure water was shown in the previous section (*Experiment 1, Section 5.3.3*). In the experiment described here the effect of Zn(II) on S(II-) was also examined but in the presence of NOM. A decrease in S(II-) concentration under anoxic conditions occurred over time in all samples prepared in

SRFA solution. Similar losses were observed in previous experiments (*Experiments 1 & 2, Sections 5.3.1 & 5.3.2*) and attributed to the reaction of S(II-) with NOM (SRFA). Oxidation by trace amounts of oxygen remaining in or leaking into samples probably contributed to these losses.

5.3.4.1 Experiment 8: Sulfide in the Presence of Zn(II) and SRFA

Experiments were conducted with samples containing various concentrations of Zn(II) in aqueous SRFA solution and an initial S(II-) concentration of 2.5 μM (Table 5.2). The organic carbon content of each sample was ~ 5 mg C/L. Sulfide concentration was monitored over time, and the results are presented in Figures 5.13 and 5.14. The second experiment (Figure 5.14) repeated the first one with an extended oxidation period and additional samples prepared with higher Zn(II) concentrations.

Similar observations were made in both experiments. Once the unstable S(II-) was oxidized (initial 24 h), the MBS concentration remained constant for the remainder of the oxic period in the majority of the samples. The oxic period was as long as 7 days in the second experiment. In contrast, S(II-) concentrations in samples lacking Zn(II) decreased close to or below detectable levels (< 0.03 μM) under the same conditions.

Although no obvious stoichiometric relationship between Zn(II) and S(II-) could be discerned, the amount of S(II-) stabilized in each sample was dependent on the Zn(II) concentration. As the amount of Zn(II) available to bind S(II-) increased, so did the amount of measured MBS. In the last 24 h of the experiment (Figure 5.14), the following fractions of the initial S(II-) concentration were measured in samples containing 0, 0.5, 1.5, 2.5, 5.0, 7.5 and 25 μM of Zn(II), respectively: ~ 0, 5, 16, 35, 66, 56, 130 and 100%.

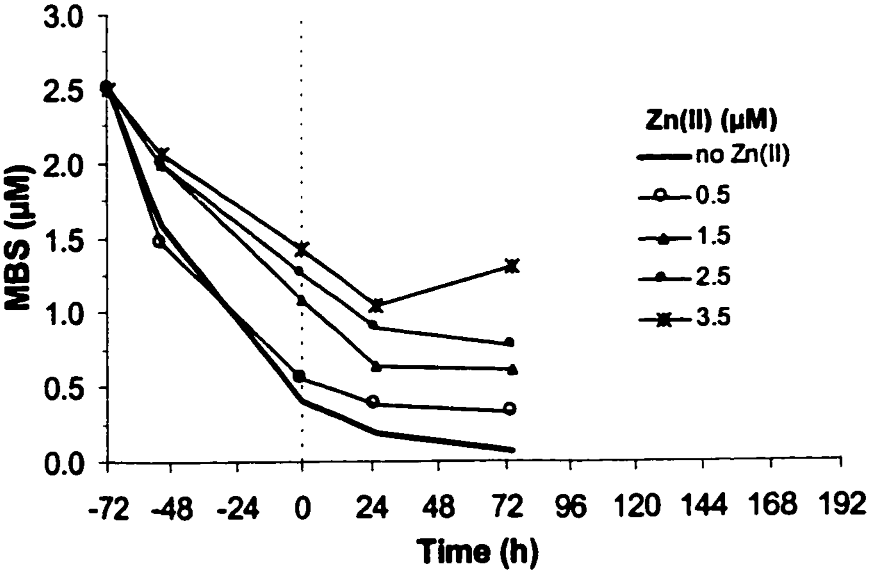


Figure 5.13: Sulfide measured over time in SRFA (5 mg C/L) solution with Zn(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

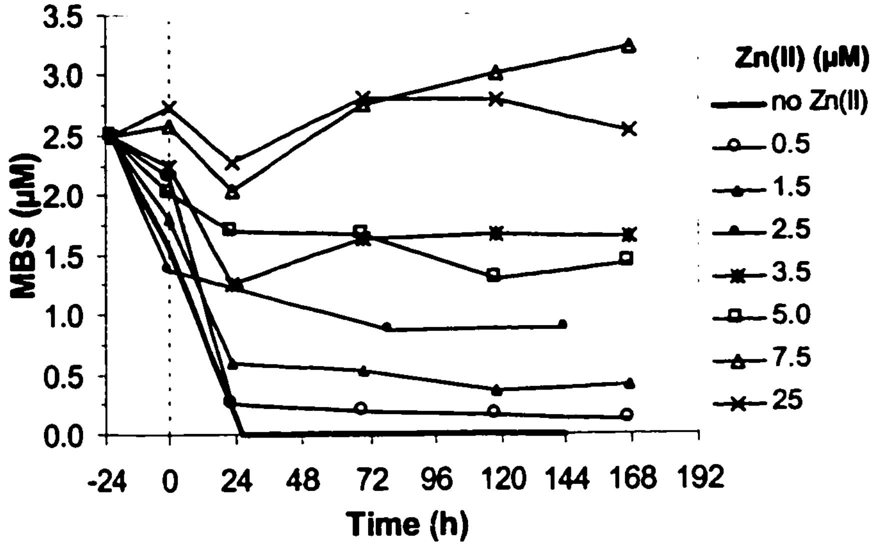


Figure 5.14: Sulfide measured over time in SRFA solution (5 mg C/L) with Zn(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

The results of these experiments clearly show that Zn(II) stabilizes S(II-) under oxic conditions and that this stabilizing effect is attained in the presence of SRFA. Furthermore, an interaction between zinc sulfide species and organic matter is suggested because the stabilizing effect of Zn(II) observed in the presence of SRFA was much more pronounced than in solutions lacking SRFA (*Experiment 3, Section 5.3.3.1*). Also, it was previously shown that in the presence of SRFA, Zn(II) sulfide adsorption to container surfaces is suppressed (*Section 3.3.1*). This suggests that the oxygen donor groups (e.g. $-\mathrm{COO}^-$, $-\mathrm{O}^-$) contained in NOM successfully compete with the container for Zn(II).

These experiments provide clear evidence for the stabilization of S(II-) by Zn(II) in the presence of SRFA. It is not clear, however, whether an interaction of the zinc sulfide species with NOM provides greater stability to S(II-). In a previous experiment conducted without NOM (*Experiment 3, Section 5.3.3.1*), although Zn(II) stabilized S(II-) under oxic conditions, a dependence on the Zn(II) concentration was not observed, and the MBS decreased throughout the oxic period. The decrease in MBS may have resulted from the adsorption of zinc sulfide phases to the container as opposed to oxidation.

Under anoxic conditions, S(II-) decreased over time and this decrease was more pronounced in samples containing no Zn(II) or low concentrations of Zn(II). As was mentioned before, decreases in S(II-) were due to reaction of S(II-) with SRFA and probably some oxidation from trace amounts of dissolved oxygen. This observation indicates that Zn(II) also stabilizes S(II-) against reaction with SRFA.

A few unusual observations were also made during the course of these experiments. In some samples, the MBS concentration often showed unexpected increases between sampling times during the oxic period. Erratic changes in MBS concentrations were also noted previously (*Experiment 3, Section 5.3.3.1*) in water samples containing S(II-) and Zn(II). These observations were explained by an increase in zinc sulfide phases in solution due to desorption from container surfaces. In the presence of SRFA however, S(II-) was not found to adsorb to PP containers (*Section 3.3*). Furthermore, MBS in a sample containing one of the highest Zn(II) concentrations (7.5 μM) was found to increase beyond the initial amount of S(II-) (2.5 μM).

On average, NOM contains < 1 % (wt/wt) of S and some of it occurs in the form of S(II-) functional groups (refer to *Section 2.8.5*). An experiment was thus conducted to explore the possibility that S(II-) might be originating from the NOM itself to react and bind with the Zn(II). In brief, samples containing various concentrations of Zn(II) (0 – 100 μM) were prepared in SRFA solution without added S(II-). The pH of each sample was adjusted to ~ 9.5 and the samples were left stirring in air for ~ 6 days. At ~ 45 and ~ 141 h of oxidation, MBS was measured in each sample. Sulfide was not detected in any of the samples at either sampling time indicating that the surplus S(II-) was not originating from the NOM. The origin of this excess S(II-) could not be determined.

5.3.4.2 Experiment 9: Sulfide in the Presence of Fe(II) or Fe(III) and SRFA

Sulfide concentration was monitored over time in samples prepared in aqueous SRFA solution (~ 5 mg C/L). The samples contained various amounts of Fe(II) or Fe(III) (Table 5.2) and an initial S(II-) concentration of 2.5 μM. The results are summarized in

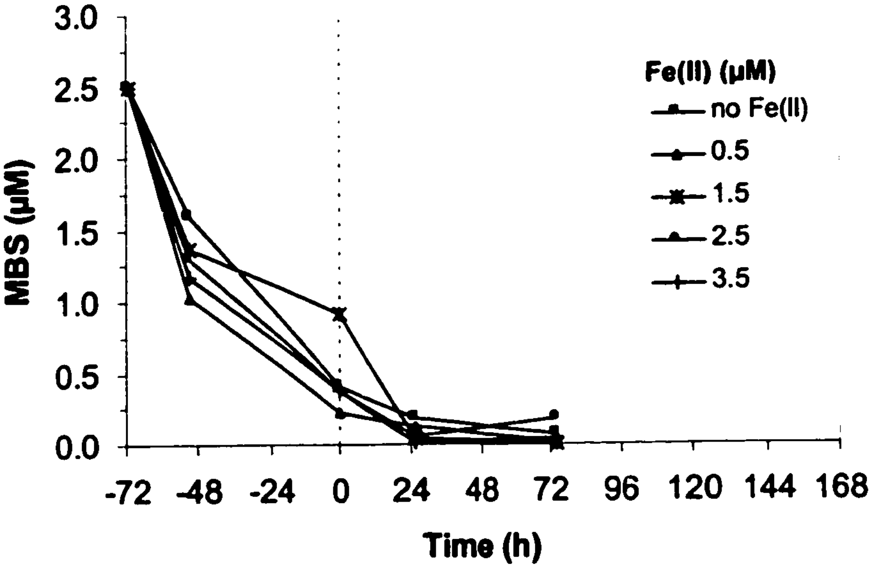


Figure 5.15: Sulfide measured over time in SRFA solution (5mg C/L) with Fe(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

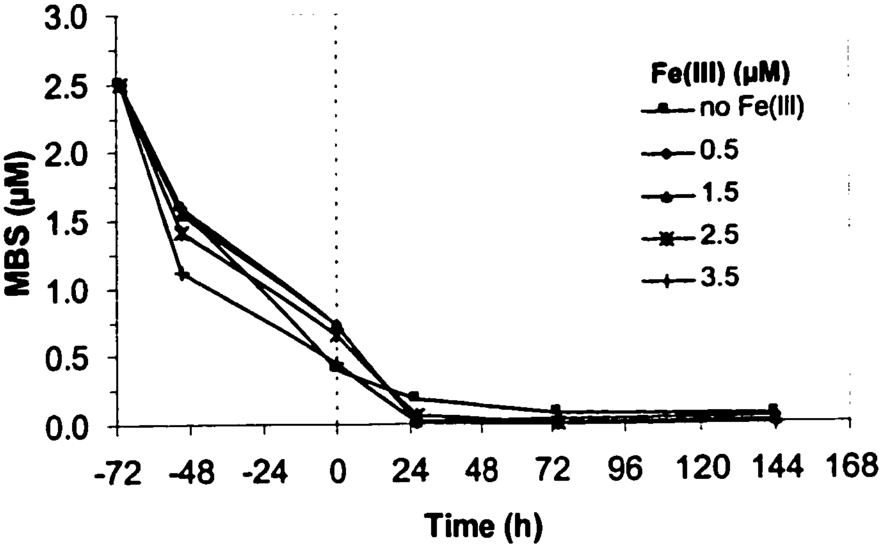


Figure 5.16: Sulfide measured over time in SRFA solution (5 mg C/L) with Fe(III) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

Figures 5.15 and 5.16. Similar trends were observed in both experiments. Within the first 24 h of oxidation, S(II-) concentrations decreased close to or below-detectable levels (< 0.03 μM) due to the compounded effect of oxidation and reaction with SRFA. This was observed in all samples regardless of whether they contained Fe(II) or Fe(III).

The results of these experiments show that iron does not stabilize S(II-) in the presence of SRFA against oxidation or reaction with SRFA. The same observations were made for Fe(II) and Fe(III).

5.3.4.3 Experiment 10: Sulfide in the Presence of Cu(II) or Ag(I) and SRFA

Sulfide samples containing either Cu(II) or Ag(I) at various concentrations (Table 5.2) were prepared in aqueous SRFA solution (~ 5 mg C/L) with an initial S(II-) concentration of 2.5 μM. Once again, S(II-) was monitored over time by measuring MBS under anoxic and oxic conditions. The results are shown in Figures 5.17 and 5.18 for Cu(II) and Ag(I), respectively.

As was the case in *Experiment 5* (*Section 5.3.3.3*) which examined analogous samples prepared in the absence of NOM, S(II-) was not detected in any sample containing Cu(II) under anoxic as well as oxic conditions. This finding is particularly surprising for anoxic samples where S(II-) should have been present even if some had been lost to oxidation by oxygen or reaction with NOM. Similar observations were made for Ag(I) except that some S(II-) was detected in samples containing < 3 μM of Ag(I). As was previously explained (*Section 5.3.3.3*), MBS formation is inhibited in the presence of Cu(II) and Ag(I) sulfide phases. The detection of some S(II-) in the presence

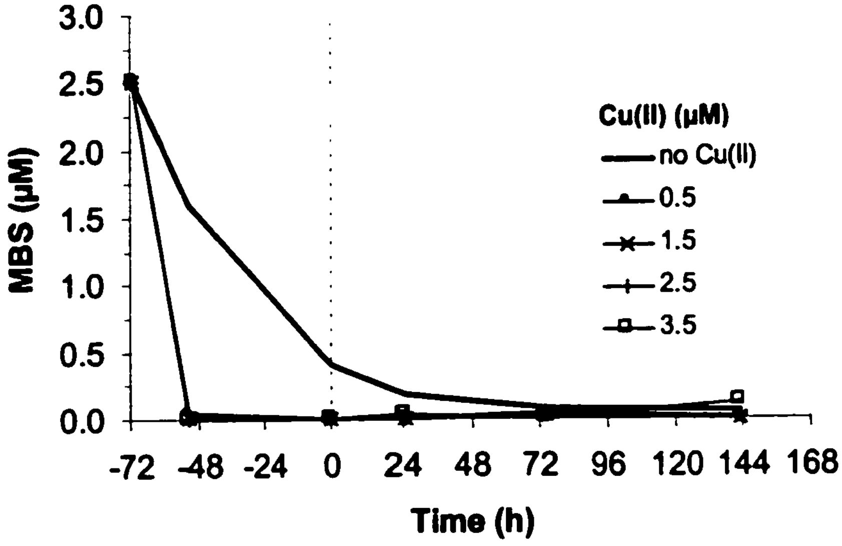


Figure 5.17: Methylene blue-sulfide measured in SRFA solution (5 mg C/L) in the presence of Cu(II). The initial S(II-) concentration was 2.5 µM. Measurements were made under anoxic (< 0 h) and oxic (> 0 h) conditions.

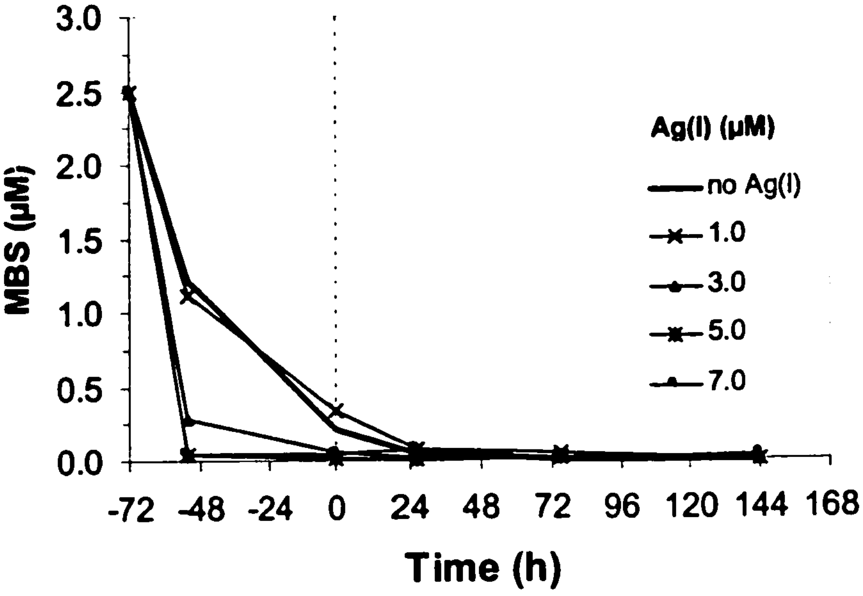


Figure 5.18: Methylene blue-sulfide measured in SRFA solution (~ 5 mg C/L) in the presence of Ag(I). The initial S(II-) concentration was 2.5 µM. Measurements were made under anoxic (< 0 h) and oxic (> 0 h) conditions.

of Ag(I) is probably a result of silver sulfides being more acid labile to form MBS, or because Ag(I) does not bind S(II-) as efficiently as Cu(II).

Since MBS gave no signal in these experiments, it was not possible to assess whether Cu(II) and Ag(I) have a stabilizing effect on S(II-) under oxic conditions in the presence of SRFA.

5.3.4.4 Experiment 11: Sulfide in the Presence Zn(II) & Fe(III) and SRFA

This experiment examined the effect of Zn(II) on S(II-) in the presence of SRFA, as well as Fe(III). Sulfide concentration was monitored over time in samples prepared in aqueous SRFA solution (~ 5 mg C/L) with 2.5 μM of Zn(II), various concentrations of Fe(III) (Table 5.3), and an initial S(II-) concentration of 2.5 μM. The results are shown in Figure 5.19.

After 144 h of oxidation, ~ 10 to 40% of the initial S(II-) concentration was measured in all samples containing Zn(II) (2.5 μM) with various concentrations of Fe(III) (0 – 2.5 μM). In the sample containing Fe(III) but lacking Zn(II), S(II-) decreased below detectable levels (< 0.03 μM) within the first 24 h of oxidation. These results are in agreement with those from *Experiment 8* (*Section 5.3.5.1*) where Zn(II) alone stabilized S(II-) in the presence of SRFA under oxic conditions, and *Experiment 9* (*Section 5.3.5.2*) where Fe(III) alone did not attain the same effect under similar conditions.

The results of this experiment show that Zn(II) can stabilize S(II-) under oxic conditions in the presence of SRFA when Fe(III) is also present. When the analogous experiment was conducted without SRFA (*Experiment 6, Section 5.3.3.4*), Zn(II) exerted

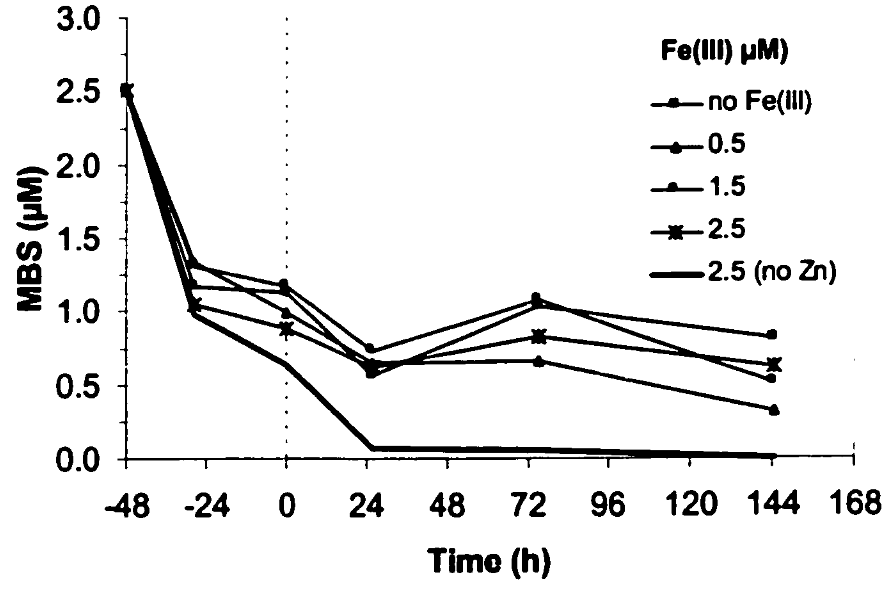


Figure 5.19: Sulfide measured over time in SRFA solution (5 mg C/L) with 2.5 μM of Zn(II) and various concentrations of Fe(III) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 μM.

the same stabilizing effect on S(II-) but there appeared to be a dependence on the Fe(III) concentration. The amount of S(II-) stabilized decreased with an increase in Fe(III). Such a trend is not obvious in the presence of SRFA suggesting that an interaction of Zn(II) with NOM might be enhancing the stabilization of S(II-). It is not known however whether Zn(II) stabilizes S(II-) with NOM alone or in conjunction with Fe(III), i.e. by formation of a mixed metal sulfide species.

Unexpected increases in S(II-) concentration were noted during the oxic period. Such increases had been noted in a previous experiment (*Experiment 8, Section 5.3.5.1*), but a satisfactory explanation for this phenomenon could not be provided.

5.3.4.5 Experiment 12: Sulfide in the Presence of Zn(II) & Cu(II) or Zn(II) & Ag(I) and SRFA

Experiments were also conducted to examine the effect of Zn(II) on S(II-) in aqueous SRFA solution in the presence of Cu(II) or Ag(I). Samples were prepared in aqueous SRFA solution (~ 5 mg C/L) with 2.5 μM of Zn(II), various concentrations of Cu(II) or Ag(I) (Table 5.3), and an initial S(II-) concentration of 2.5 μM. Sulfide was monitored over time and the results are shown in Figures 5.20 and 5.21 for Cu(II) and Ag(I), respectively.

Methylene blue-sulfide was not detected in any samples containing Cu(II) regardless of the experimental conditions (anoxic or oxic) (Figure 5.20). Sulfide should have been detected in anoxic samples even if some had reacted with SRFA or was lost to oxidation. As was discussed in *Experiment 5* (*Section 5.3.3.3*), this apparent lack of S(II-) is due to the acid insoluble nature of copper sulfide and the inability of H_2S to react with MDR in the presence of Cu(II). This inhibitory effect is also attained in the presence of Zn(II), despite the ability of Zn(II) to stabilize S(II-). Since S(II-) could not be measured in these experiments using the MBS method, it was not possible to assess whether Zn(II) stabilizes S(II-) with SRFA under oxic conditions when Cu(II) is also present.

Similar observations to Cu(II) were made for samples containing high concentrations of Ag(I) exceeding that of S(II-) (> 2.5 μM) (Figure 5.21). At lower Ag(I) concentrations however, MBS was detected. In the sample containing 0.5 μM of Ag(I), ~ 20% of the initial S(II-) was measured at 144 h of oxidation, while < 10% was

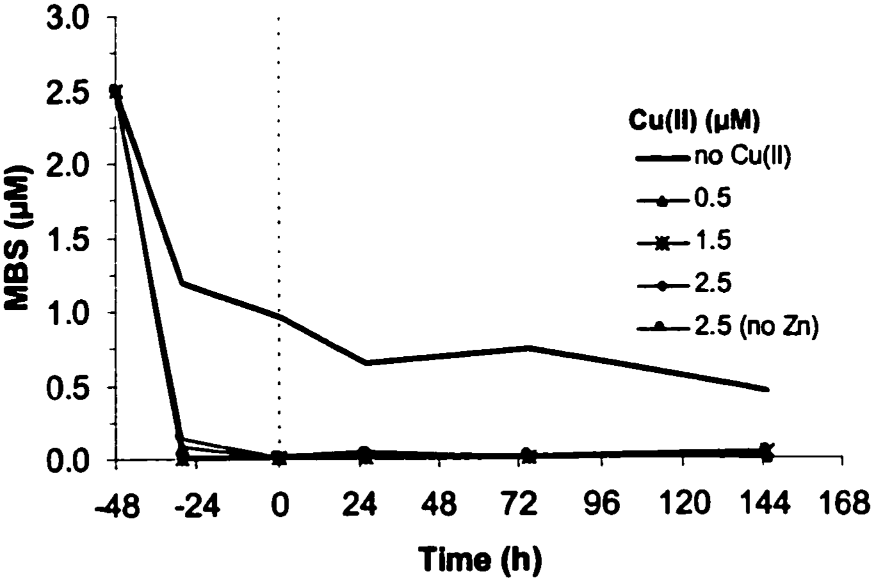


Figure 5.20: Sulfide measured over time in SRFA solution (5 mg C/L) with 2.5 µM of Zn(II) and various concentrations of Cu(II) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

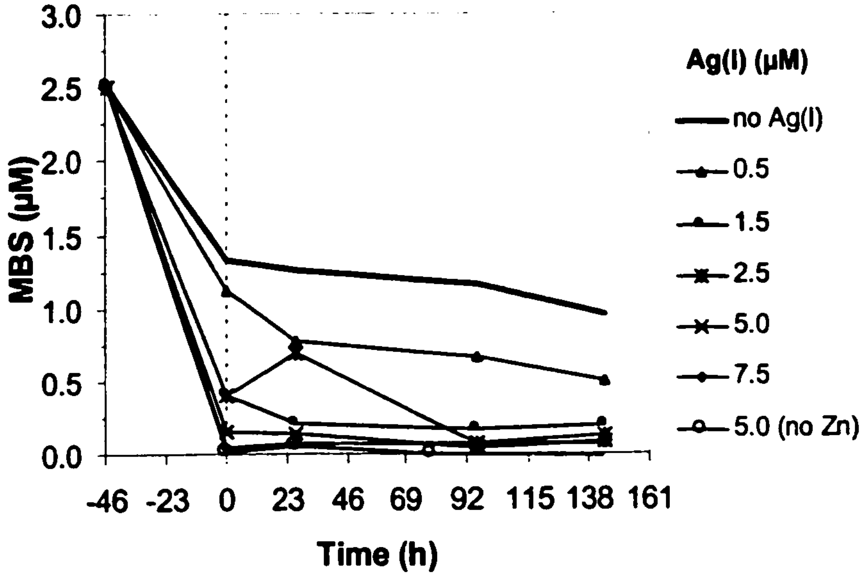


Figure 5.21: Sulfide measured over time in SRFA solution (5 mg C/L) with 2.5 µM of Zn(II) and various concentrations of Ag(I) under anoxic (< 0 h) and oxic (> 0 h) conditions. The initial S(II-) concentration was 2.5 µM.

measured in all other samples with Ag(I). At these lower concentrations, the amount of Ag(I) might not be sufficient to bind all of the S(II-). Silver sulfide species might also be more reactive in acid than those of copper allowing for the formation of some MBS. Working at nanomolar concentrations, Bowles (2000) reported the recovery of S(II-) from silver sulfide species using the MBS method.

In samples containing only Zn(II), significant amounts of S(II-) were measured throughout the oxic period. After ~ 144 h of oxidation, ~ 20 to 40% of the initial S(II-) was still present in these samples. These results enforce the point that Zn(II) stabilizes S(II-) under oxic conditions in the presence of SRFA. Furthermore, these experiments demonstrated that this stabilizing effect exerted by Zn(II) may be attained in the presence of another S(II-) binding metal, Ag(I). It is not known though whether Zn(II) attains this stabilizing effect in conjunction with Ag(I), i.e. in a mixed metal sulfide species. In the presence of Ag(I) at high concentrations or Cu(II), S(II-) appeared to preferentially bind these metals to Zn(II) as was indicated by the suppression in MBS formation. This is in accordance with the fact that both Cu(II) and Ag(I) exhibit a higher affinity for S(II-) based on the solubility of their sulfide compounds (refer to *Section 2.1*).

5.3.5 Sulfide Binding to Ag(I) and Cu(II)

Experiments were conducted to determine the approximate binding capacities of Ag(I) and Cu(II) for S(II-). Anoxic samples contained in PP tubes were prepared in water with 2.5 μM of S(II-) and various amounts of added Ag(I) or Cu(II). Since silver and copper sulfide complexes are not readily soluble in acid to form MBS, the amount of unbound (MDR reactive) S(II-) was measured at various metal to sulfide ratios.

5.3.5.1 Experiment 13: Ag(I)

An initial experiment using Ag(I) to S(II-) ratios (Ag:S) spanning a few orders of magnitude was conducted to determine the approximate ratio at which Ag(I) would bind up all the S(II-). All samples, contained in PP tubes or borosilicate vials, were prepared and sampled under anoxic conditions in the dark. First, Milli-Q water was added to each container followed by an appropriate volume of Ag(I) stock solution prior to adjusting the pH to ~ 8.5 with 0.01 M NaOH. Each sample was then degassed with He for 5 minutes and S(II-) was added under an Ar blanket or a N_2 atmosphere contained in a glove box. Approximately 22 h following sample preparation in the first experiment, and 2 h in the second experiment, MDR was added to each sample and the concentration of MBS determined.

The results are shown in Figure 5.22 where the error bars indicate the range in MBS measurements in duplicate samples. Methylene blue-sulfide measured in samples where S(II-) was 1000, 100 and 10 times in excess of Ag(I) (Figure 5.22a), were similar to the amount of MBS measured in the sample lacking Ag(I). In these samples, either silver sulfide formed in concentrations too low to be detected in each measurement, or at low enough concentrations to be reactive in MDR to form MBS along with the free S(II-). Measurement of silver bound S(II-) in the MBS method has been observed at nanomolar concentrations (Bowles, 2000).

At equimolar concentrations (1:1), the amount of measured MBS sharply declined

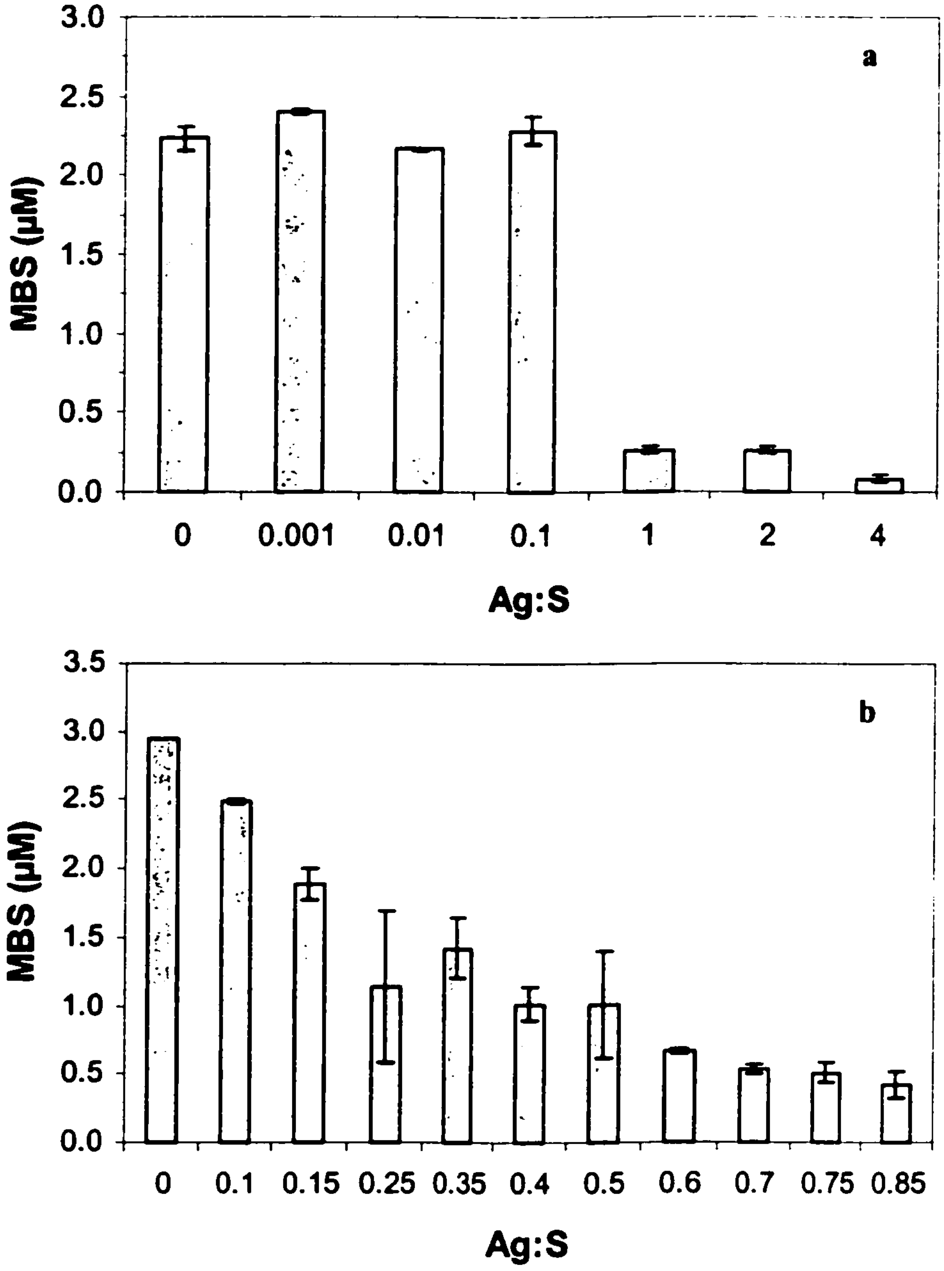


Figure 5.22: Free S(II-) measured in solution in the presence of Ag(I). Samples contained 2.5 μM of S(II-) and various concentrations of Ag(I). Figure (**b**) examines free S(II-) in samples containing Ag:S ratios ranging between 0.1 and 1 where an obvious decrease in S(II-) concentration was noted in Figure (**a**).

to less than 0.3 µM, indicating that ~ 90% of the S(II-) was bound to Ag(I). The amount of bound S(II-) might be slightly underestimated if silver sulfide is marginally reactive in the MDR reagent. Regardless however, the results of this experiment show that complete binding of S(II-) occurs at an Ag:S ratio between 0.1 and 1, and that every one Ag(I) atom may bind between 1 and 10 S(II-) atoms. A similar experiment was also conducted with Ag:S ratios ranging between 0.1 and 1 to determine more closely the binding stoichiometry of S(II-) to Ag(I) (Figure 5.22b). The results are summarized in Figure 5.22b. The MBS concentration decreased as the amount of Ag(I) increased up to a ratio of 0.7. At higher ratios, the MBS concentration remained constant despite increases in the Ag(I) concentration. According to these results, every Ag(I) atom binds between 1.5 and 1.7 S atoms, but as was mentioned before the amount of bound S(II-) might be underestimated if silver sulfide is marginally reactive in MDR.

5.3.5.2 Experiment 14: Copper

An experiment using Cu(II) to S(II-) ratios (Cu:S) spanning a few orders of magnitude was conducted to determine the approximate ratio at which Cu(II) would bind up all the S(II-). The results are shown in Figure 5.23. The MBS concentration decreased as the amount of Cu(II) increased. Complete binding of the S(II-) occurred at a ratio of 0.1 and 0.6. At a ratio of 0.6, MBS was no longer detected (< 0.03 µM). According to these ratios, every Cu(II) atom may bind between 1.7 and 10 S atoms.

The amount of unbound S(II-) appeared to be depressed relative to the blank (no Cu(II)) even when S(II-) greatly exceeded the amount of Cu(II) in the sample (100 and 1000 times in excess). At a Cu:S ratio of 0.001, 0.2 µM of S(II-) was bound to the

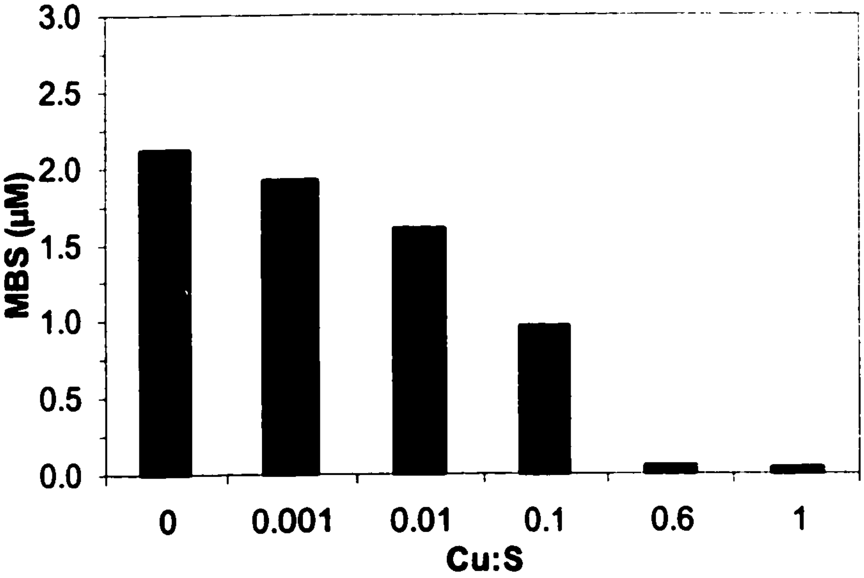


Figure 5.23: Free S(II-) measured in solution in the presence of Cu(II). Samples were contained 2.5 µM of S(II-) and various concentrations of Cu(II).

Cu(II). According to this result, every Cu(II) atom binds ~ 80 S atoms. At a Cu:S ratio of 0.01, every Cu(II) atom binds ~ 20 S atoms. These binding capacities are high and not in accordance with estimated stoichiometries for soluble copper sulfide clusters by other researchers (Luther *et al.*, 1996; Rozan *et al.*, 2000). These suspiciously high binding capacities indicate that Cu(II) at these low concentrations is probably inhibiting the reaction of free S(II-) with MDR to form MBS, as opposed to actually binding all of the S(II-). To clarify whether Cu(II) actually inhibits this reaction, the experiment should be repeated using a purge and trap method where H_2S is collected and reacted with MDR in a separate container in the absence of Cu(II). Alternatively, the application of voltammetry to determine S(II-) in these experiments might help elucidate this problem.

5.4 CONCLUSIONS

The main conclusions from these experiments are summarized below:

(1) Zinc(II) in association with NOM stabilizes S(II-) in oxic solution and allows it to persist for periods of at least 7 days. The interaction with NOM appears to enhance this stabilizing effect.

(2) Zinc(II) stabilizes S(II-) in oxic solution in the presence of other S(II-) binding metals exhibiting a higher or lower affinity for S(II-). This was observed with Ag(I) as well as Fe(III), with and without an association with NOM. Stabilization of S(II-) in the presence of more than one S(II-) binding metal raises the possibility that heterometal sulfide clusters occur.

(3) Sulfide in the presence of Zn(II) with Ag(I) or Cu(II) preferentially binds to Ag(I) and Cu(II) that exhibit a higher affinity for S(II-) than Zn(II). If these metals displace Zn(II) from zinc sulfide species, then heterometal sulfide clusters may be formed.

(4) Sulfide is removed from solution due to reaction with NOM and Zn(II) is able to stabilize S(II-) against this reaction.

(5) Zinc sulfide species exhibit a high propensity for adsorption to container surfaces. Adsorption to containers removes S(II-) from solution resulting in an underestimate of S(II-) concentration.

(6) Stabilization of S(II-) under oxic conditions is not attained by Fe(II) or Fe(III) regardless of whether NOM is present.

(7) Silver(I) and Cu(II) sulfide species are not reactive in acidic MDR as they inhibit the formation of MBS. Consequently, S(II-) bound to Ag(I) or Cu(II) is generally not measured by the methylene blue-sulfide method. This inhibitory effect is attributed predominantly to the resistance of these species to dissociation under acid conditions to release H_2S. In the case of Cu(II), an additional inhibitory effect on the methylene blue forming reaction may occur, which prevents the determination of free S(II-) as well.

(8) Silver(I) was found to bind between 1.5 and 1.7 S atoms.

CHAPTER 6

COLOURIMETRIC DETERMINATION OF SULFIDE IN SURFACE FRESHWATERS

6.1 OBJECTIVES

This chapter considers the following objectives:

(a) To test the applicability of the methylene blue colourimetric method to the measurement of S(II-) in surface freshwaters.

(b) To determine background concentrations of reactive S(II-) in a variety of oxic freshwaters, and to relate these concentrations to metal and organic carbon concentrations.

6.2 EXPERIMENTAL METHODS

6.2.1 Field Sampling

All sampling and processing of samples was conducted according to clean metal protocols. Oxic freshwater samples were collected on July 12 & 14, 2000, at 8 locations throughout Southern Ontario, Canada (Table 6.1). The chosen locations were meant to represent a variety of freshwater systems with a wide range in organic matter content.

At each location, a bulk water sample was collected in a 1 L polyethylene bottle at 10 cm depth. Duplicate sub-samples for reactive S(II-), organic carbon and metal analyses were collected immediately (within 15 min) from the bulk sample and prepared (acidified or fixed with MDR depending on the analyte) while in the field. Samples were kept on ice in the dark until analyzed.

6.2.2 Analyte Determination

6.2.2.1 Determination of Organic Carbon

Sub-samples for total and dissolved (< 0.7 μm) organic carbon analysis were collected in 10 mL polypropylene (PP) tubes and acidified with concentrated HNO_3 (Ultrex II, Baker, USA) to pH < 2. Dissolved samples were filtered in-line through an acid washed GF/F filter (Whatman, UK) using a 20 mL PP syringe. Samples were analyzed according to the procedure described in *Section 3.2.1*. The detection limit was 0.2 mg C/L ($3 \times \sigma$ of blanks, n = 8).

Filtered blanks were on average 0.2 ± 0.3 mg C/L indicating that contamination from the filtration procedure was negligible.

6.2.2.2 Determination of Reactive Sulfide

Sulfide was measured as both methylene blue-sulfide (MBS) and chromium labile sulfide (CLS). The MBS method was used because it is relatively simple and requires minimal sample manipulation. CLS was measured to compare with the amount of S(II-) measured by the MBS method. The MBS and CLS methods are described in *Section 3.2.2* and *Section 3.2.3*, respectively.

Sub-samples for total and dissolved (< 0.45 μm) MBS analysis were collected in 40 mL borosilicate vials and fixed with MDR. Addition of acidic MDR to the sample in the original sampling container avoided losses of S(II-) due to adsorption of metal sulfide phases to the container surface. Dissolved samples were filtered in-line through a 0.45 μm polysulfone filter (Acrodisc, Gelman Laboratory, USA) using a 10 mL PP syringe. Similar samples without MDR added were collected at each location to determine the background absorbance of each water type. Absorbance was measured within 24 – 36 h of sample collection on a Cary 50 spectrophotometer (Varian, USA) using a 10 cm path length quartz cell. The detection limit on different analysis days varied between 10 and 30 nM (3 × σ of blanks, n = 3 – 5).

Sub-samples for CLS analyses were collected in 40 mL borosilicate vials and processed in the laboratory for S(II-) determination within a week. Absorbance was measured on a Cary 50 spectrophotometer (Varian, USA) using a 10 cm path length quartz cell. The analytical detection limit was 5 nM (3 × σ of blanks).

6.2.2.3 Determination of Metals

All materials contacting metal samples were washed by sequentially soaking in 10% and 1% HNO_3 (Trace Metal Grade, Baker, USA) and dried in a class 100 HEPA filtered laminar flow hood.

Sub-samples for total and dissolved (< 0.45 μm) metal analysis were collected in 10 mL PP tubes and acidified to 2% (v/v) with concentrated, high purity HNO_3 (Ultrex II, Baker, USA). Dissolved samples were filtered in-line through a 0.45 μm polysulfone filter (Acrodisc, Gelman Laboratory, USA) using a 10 mL PP syringe. Concentrations of S(II-) binding metals were determined by ICP-MS on a PE-Sciex-Elan-6100 (see *Section 3.2.4*). Detection limits for each element are included in Table 6.2 (3 × σ of blanks).

Samples collected for metal determination were analyzed 5 months after their collection. To recover metals adsorbed to container surfaces, the samples were treated with hydrogen peroxide (H_2O_2, BDH, Canada) added at 2% (wt/wt).

6.3 RESULTS

Total and dissolved organic carbon, MBS and CLS data are summarized in Table 6.1 along with the sampling locations. Concentrations of S(II-) binding metals are presented in Table 6.2. Waters at each sampling location ranged in temperature from 15 to 21 °C and were assumed to be oxygenated as they were collected near the water surface.

TOC concentrations varied considerably between sampling locations and ranged from 1.95 mg C/L in Lake Ontario (Oakville) to 67.3 mg C/L at the Luther Marsh. DOC concentrations accounted for virtually all of the total organic carbon in all cases.

MBS was only detected at the Beverly Swamp (50 nM) and Spring Creek although CLS was not detected at Spring Creek. MBS concentrations were too low to discern differences between total and filtered concentrations except for the Beverly Swamp where filtered MBS accounted for 60% of the total.

In applying the MBS method to the determination of S(II-) in freshwater samples, some problems became apparent. Suspended particulates in unfiltered samples resulted in positive interferences and caused fluctuations in the absorbance signal. A stable measurement was obtained after ~ 20 – 30 min which made the analysis of large sample batches impractical. NOM colour contributed to the absorbance of the sample, resulting in overestimated MBS concentrations. A correction for this background absorbance was applied to each measurement. In samples containing very high concentrations of organic carbon though, i.e. Luther Marsh, background correction was not possible and MBS

Table 6.1: Organic carbon, methylene blue-sulfide (MBS), chromium(II) labile sulfide (CLS) and pH data for samples collected at various locations throughout Southern Ontario, Canada.

General Location	Code	pH	DOC/TOC (mg C/L)		MBS (nM)		CLS (nM)
			< 0.7 μm (n = 2)	Total (n = 2)	< 0.45 μm (n = 2)	Total (n = 2)	Total (n = 1)
Lake Ontario, Oakville	LO	8.4	2.0 ± 0.1	1.9 ± 0.1	< 10	< 10	20
Lake Ontario, Niagara-on-the-Lake	NOTL	8.5	2.1 ± 0.1	2.2 ± 0.0	< 10	< 10	<5
Spring Creek, Dundas	SC	8.2	2.6 ± 0.0	2.7 ± 0.0	< 20	30	<5
Lake Ontario, Hamilton Harbour	HH	8.3	3.9 ± 0.0	4.2 ± 0.1	10	< 10	25
Sixteen Mile Creek, Oakville	SMC	8.5	4.4 ± 0.1	4.5 ± 0.0	< 10	< 10	28
Desjardins Canal (below POTW), Dundas	DC	7.9	4.7 ± 0.0	4.7 ± 0.1	< 10	< 10	215
Beverly Swamp	BS	8.2	17.9 ± 0.0	18.4 ± 0.2	30	50	138
Luther Marsh	LM	7.9	66.0	67.3	nd	nd	86

POTW: Publicly Owned Treatment Works
nd: no data

Table 6.2: Summary of metal data for samples collected throughout Southern Ontario, Canada (n = 2 for each measurement).

Code	Ag (nM) (dl: 0.074 nM)	Cd (nM) (dl: 0.018 nM)	Cu (nM) (dl: 0.6 nM)	Hg (nM) (dl: 0.065 nM)	Mn (nM) (dl: 0.5 nM)	Ni (nM) (dl: 0.3 nM)	Pb (nM) (dl: 0.251 nM)
LO							
< 0.45 μm	< 0.074	0.049 ± 0.002	9 ± 1	< 0.065	9 ± 1	16 ± 0	< 0.251
Total	< 0.074	0.079 ± 0.020	10 ± 1	< 0.065	18 ± 1	16 ± 1	< 0.251
NOTL							
< 0.45 μm	< 0.074	0.100 ± 0.003	14 ± 1	0.174 ± 0.115	39 ± 1	22 ± 1	< 0.251
Total	< 0.074	0.160 ± 0.058	14 ± 2	< 0.065	117 ± 15	20 ± 2	0.475 ± 0.013
SC							
< 0.45 μm	< 0.074	0.072 ± 0.003	20 ± 1	< 0.065	725 ± 110	33 ± 3	0.249 ± 0.008
Total	< 0.074	0.108 ± 0.010	22 ± 0	< 0.065	1100 ± 130	41 ± 4	2.440 ± 0.170
HH							
< 0.45 μm	< 0.074	0.126 ± 0.006	21 ± 1	< 0.065	34 ± 1	35 ± 1	0.340 ± 0.345
Total	< 0.074	0.150 ± 0.002	22 ± 1	< 0.065	136 ± 1	34 ± 1	0.930 ± 0.136
SMC							
< 0.45 μm	< 0.074	0.389 ± 0.031	20 ± 1	< 0.065	39 ± 1	26 ± 1	< 0.251
Total	< 0.074	0.412 ± 0.017	22 ± 0	< 0.065	72 ± 3	27 ± 1	0.400 ± 0.051
DC							
< 0.45 μm	< 0.074	0.254 ± 0.003	41 ± 1	< 0.065	1150 ± 34	71 ± 4	0.450 ± 0.000
Total	< 0.074	0.361 ± 0.002	58 ± 9	0.074 ± 0.105	1640 ± 270	88 ± 14	3.550 ± 0.310
BS							
< 0.45 μm	< 0.074	< 0.018	1 ± 0	< 0.065	498 ± 29	35 ± 2	< 0.251
Total	< 0.074	0.037 ± 0.003	2 ± 0	< 0.065	499 ± 6	34 ± 0	0.422 ± 0.012
LM							
< 0.45 μm	< 0.074	0.150 ± 0.012	7 ± 1	0.192 ± 27	2680 ± 90	23 ± 1	2.720 ± 0.050
Total	< 0.074	0.122 ± 0.012	9 ± 2	0.232 ± 57	3100 ± 580	24 ± 5	3.450 ± 0.200

nd: no data

concentration could not be determined. Background correction in samples containing NOM is problematic because the acid in the MDR alters the sample colour by inducing coagulation of the humic acids ($pH < 2$).

Chromium(II) labile sulfide (CLS) was detected at nanomolar concentrations in the majority of the oxic freshwaters that were sampled. The highest CLS concentration was measured at the Desjardins Canal below the POTW at 215 nM. This was much greater than was measured in the other samples (< 5 – 138 nM) and suggests that POTWs are a source of S(II-) to aquatic systems. Also, the fact that substantially higher concentrations of CLS were detected than MBS, suggests that S(II-) in the sampled waters generally occurs bound to metals such as Cu that form acid insoluble metal sulfides. Other possible explanations are discussed below.

As was mentioned in *Section 2.10.2*, treatment of the sample with Cr(II) in an acidic solution results in the dissociation of acid volatile metal sulfides (e.g. FeS, ZnS, MnS, CdS), and the reduction of acid insoluble metal sulfides (e.g. CuS, Ag_2S, FeS_2) to release bound S(II-). In addition, the CLS method also determines S(II-) from inorganic sulfur species with lone pairs of electrons such as elemental sulfur (S_8), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}) (Canfield *et al.*, 1986; Bowles, 2000). In comparison, the MBS method only recovers S(II-) from acid volatile metal sulfides and consequently underestimates S(II-) concentrations relative to the CLS method.

One final problem was noted with the MBS method. Reaction of MDR with Cu or possibly other species contained in the natural sample, possibly interfere with the reaction between H_2S and MDR to form MBS. Such an interference would suppress

MBS formation and cause an underestimate of S(II-) concentration. This problem was identified in a previous experiment which examined the binding stoichiometry of Cu and S (*Experiment 14, Section 5.3.7.2*). In this experiment, when S(II-) was 1000 times in excess of Cu(II), a substantial decrease in free S(II-) concentration occurred suggesting binding of the S(II-) to Cu(II). At this observed ratio of Cu to bound S(II-) (1:80), however, every one Cu(II) atom would bind 80 S(II-) atoms. This binding capacity is very high and not in accordance with estimated empirical stoichiometries for soluble copper sulfide clusters by other researchers, e.g. 1:1 and 2:3 (Luther *et al.*, 1996; Rozan *et al.*, 2000a). This result indicated that Cu probably inhibited the reaction of free S(II-) with MDR to form MBS, as opposed to actually binding all of the S(II-). The CLS method avoids this problem by purging the generated H_2S from the sample and trapping it in a separate basic solution where it is reacted with MDR away from the sample matrix.

Concentrations of Class B and Intermediate metals that would bind and possibly stabilize S(II-) under oxic conditions were determined in each freshwater system. Of particular interest were Zn, Cu and Fe because they form stable clusters with S(II-) in solution and are thought to play a role in S(II-) stabilization (see *Section 2.6*). Unfortunately, however, reliable data for Zn and Fe was not obtained. Consequently, as these metals probably represent the dominant S(II-) binding and stabilizing metals in these waters, no attempt was made to statistically correlate metal, S(II-) and organic carbon concentrations.

Other researchers have also used the MBS method to measure S(II-) in surface freshwaters. Adams and Kramer (1999b) used this method to measure mid to high nanomolar concentrations of S(II-) in wastewater effluents as well as the surface waters of the Desjardins Canal. In contrast, we were not able to detect MBS in the waters of the Desjardins Canal (Table 6.1). Furthermore, in a follow-up study conducted by the same authors (Adams and Kramer, 1999a), concentrations of inorganic S(II-) ligands determined by the competitive ligand exchange/solvent extraction (CLS/SE) technique were found to be 70 – 90% lower than the previously measured MBS concentrations in the Desjardins Canal (Adams and Kramer, 1999b). Although explanations relating to the CLE/SE technique were suggested for this discrepancy, it is possible that these inconsistencies were the result of problems with the MBS method that overestimated S(II-).

An examination of Table 6.2 indicates that Ag was not detected (dl: 0.07 nM) in any of the sampled waters, while measurable concentrations of Hg (dl: 0.07 nM) only occurred in the organically enriched waters of the Luther Marsh. Samples from the Luther Marsh were also enriched in Mn and Pb relative to the other water types, and despite the high organic carbon content, showed little variation in metal concentrations between filtered and unfiltered fractions. This suggests that metals resided in the dissolved fraction (< 0.45 μm) and were probably associated with relatively small organic molecules. This is not surprising as metal complexation with soluble organic molecules is often responsible for higher concentrations of metals in solution than predicted from sulfide mineral equilibria (Francois, 1990).

Considerably higher concentrations of all S(II-) binding metals relative to the other locations, were observed in the waters of the Desjardins Canal, where a relatively high concentration of CLS was also detected. This suggests an association of S(II-) with metals in these oxic waters which is in accordance with the findings of Rozan *et al.* (2000a) where metal sulfide clusters of Zn, Cu and Fe were identified in oxic river waters.

6.4 SUMMARY

In summary, this study revealed the following findings:

(1) Sulfide was detected at nanomolar concentrations in a variety of oxic freshwaters with varying organic carbon content. Sulfide was measured colourimetrically either as MBS or CLS.

(2) A number of limitations were identified in the application of the MBS method to the determination of S(II-) in natural freshwater samples:

- Sulfide concentrations may be seriously underestimated if Cu, and possibly other constituents of natural water, are present which inhibit the reaction of free S(II-) with MDR resulting in suppression of the MBS signal. Such an excessive interference could make the determination of S(II-) impossible using the MBS method.
- Sulfide concentrations may be underestimated if acid insoluble metal sulfides (e.g. CuS and Ag_2S) are present in the sample because these sulfides fail to dissociate in acidic MDR to form MBS.
- Sulfide concentrations may be overestimated if the colour of organic components contained in the sample contributes to the sample absorbance. At low organic carbon concentrations this can be corrected. At very high organic

carbon concentrations, however, background correction is not possible, and MBS can not be determined accurately.

- Lowering the sample pH below 2 with MDR addition induces coagulation of humic acids in the sample which alters the sample colour and produces suspended particles which may interfere with absorbance measurement.
- Sulfide concentrations may be overestimated if particulates are present in the sample which interfere with the absorbance measurement. Waiting for particulates to settle out of solution results in lengthly measurement times making the analysis of large sample batches impractical.

(3) The CLS method resulted in reactive S(II-) concentrations that were significantly higher than values determined by the MBS method. The CLS method measures more S(II-) because it recovers S(II-) bound in acid insoluble metal sulfides (e.g. CuS, Ag_2S, FeS_2), as well as S(II-) reduced from inorganic sulfur species with lone pairs of electrons (e.g. S_8, $S_2O_3^{2-}$, SO_3^{2-}).

In conclusion, the MBS method is not recommended for the determination of S(II-) in oxic freshwaters due to the problems observed in this study. The CLS method is more promising for this application, but is limited by its lack of specificity to reduced sulfur only, which may result in an overestimate in S(II-). Based on these findings, recommendations for the development of an improved colourimetric method for S(II-) determination in oxic freshwaters are given in Chapter 7.

CHAPTER 7

DISCUSSION AND RECOMMENDATIONS FOR FUTURE WORK

7.1 SULFIDE OXIDATION STUDIES

Evidence suggesting that the stabilization of S(II-) in oxic waters is attained through the formation of very stable, kinetically inert Zn, Cu and Fe sulfide clusters, has emerged within the last few years. The formation of metal sulfide clusters in laboratory experiments (Daskalakis and Helz, 1993; Luther *et al.*, 1996; Luther *et al.*, 1999), and their very recent identification in surface freshwaters (Rozan *et al.*, 2000a), have corroborated this theory.

Despite the ubiquitous occurrence of NOM in natural waters and its tendency to complex metals, the association between metal sulfide species and NOM has been ignored. The potential effects of such an association on S(II-) stabilization and metal bioavailability in surface waters were recognized and investigated in this project resulting in some interesting findings. Most significantly, the ability of Zn(II) to stabilize S(II-) in oxic solution when associated with NOM (see *Section 5.3.4.1*) was demonstrated through this work. Zinc(II) was able to stabilize S(II-) against oxidation by molecular oxygen but also against reaction with NOM which in the absence of Zn(II) removed S(II-) from solution. This finding implies that the stabilization of S(II-) in surface freshwaters containing substantial concentrations of DOC, probably involves NOM. Depending on the nature of the metal sulfide-NOM association, this arrangement may enhance or hinder metal bioavailability.

The experiments presented in Chapter 5 showed that zinc sulfide species interacting with SRFA stabilized S(II-) against oxidation. Sulfide was stabilized over the entire period of measurement (7 days) but probably persists for longer periods. An approximate lifetime for S(II-) in oxic solution under these conditions would be worthwhile determining as S(II-) occurring at low nanomolar concentrations may control the speciation of ultra trace, toxic metals (e.g. Ag(I), Hg(II)).

Although the persistence of S(II-) and its stabilization by Zn(II) under oxic conditions was clearly demonstrated in the presence of SRFA, it was not conclusively shown that the association of Zn(II) with NOM offered S(II-) more stability than Zn(II) alone. In the absence of SRFA, S(II-) was removed from solution upon exposure to air despite complexation with Zn(II) (see *Section 5.3.3.1*). The concentration of S(II-) in oxic solution did not remain constant as was previously observed with SRFA and furthermore, a dependence on the Zn(II) concentration was not apparent. These observations suggest that SRFA does contribute the stabilization of S(II-) against oxidation. However, problems that became apparent later involving the adsorption of zinc sulfides to PP container surfaces in the absence of SRFA may have been responsible for the removal of S(II-) from solution. This discovery made it difficult to assess the role of SRFA in S(II-) stabilization. To determine this conclusively, these experiments would have to be repeated using sampling procedures that circumvent the problem of metal sulfide adsorption to container surfaces.

As was previously mentioned, the interaction between NOM and metal sulfide clusters could serve to enhance or hinder metal bioavailability depending on the nature of the interaction. The strength of the interaction will determine the capacity for ligand exchange, while the type of interaction will determine whether the exchange is physically possible. Consequently, an understanding of such interactions would be useful in predicting metal toxicity. In its association with SRFA, Zn(II) would interact predominantly with oxygen containing functional groups (e.g. $-\mathrm{COO}^-$, $-\mathrm{O}^-$) due to their relative abundance within the NOM macromolecule (see *Section 2.8.1*). Although zinc sulfide species are often negatively charged, the interaction between Zn(II) and oxygen would be strong enough for an association to occur. The high adsorptive capacity of these species was previously demonstrated through their tendency to adsorb to negatively charged container surfaces (see *Section 3.3*). The nature and the strength of the Zn-NOM interaction was not determined through these experiments and should be examined.

It is important to realize that the findings of these experiments involving SRFA may vary with other NOM types (e.g. humic acid, NOM from different locations) depending on the inherent characteristics of the NOM, or characteristics (alterations) acquired through processing procedures. This needs to be investigated to determine whether the stabilization of S(II-) is generally attained in all freshwater systems of various DOC contents.

The ability of Zn(II) to stabilize S(II-) in solution under oxic conditions was also demonstrated in a different system that simulated the oxidation of anoxic sediments. Sulfide from FeS(s) persisted in solution during the oxidation of an FeS(s) suspension when Zn(II) was added to the suspension (see *Section 4.3.3.3*), presumably due to the formation of ZnS(s) or soluble zinc sulfide species. This is consistent with observations on soluble zinc sulfide species in this work (see *Sections 5.3.3.1 & 5.3.4.1*), as well as the results of other studies examining the stability of synthetic ZnS(s), CdS(s) and PbS(s) suspended in oxic water (Simpson *et al.*, 2000a; Simpson *et al.*, 2000b). The solid metal sulfide phases resisted oxidation and persisted in solution for periods as long as 24 h.

The stabilization of S(II-) during the oxidation of sediments implies that anoxic sediments may release S(II-) to overlying oxic waters when perturbed through bioturbation or anthropogenic activities such as dredging. Metals such as Zn(II) already occurring in the water column or released into the water column during the perturbation of sediments, could stabilize S(II-) in oxic waters and provide a pool of S(II-) to more toxic, less abundant sulfide binding metals.

Due to the abundance of Fe in natural waters and its potential to dominate S(II-) speciation in surface waters, the effects of Fe(II) and Fe(III) on S(II-) were also examined. Iron, in either oxidation state, did not exhibit a stabilizing effect on S(II-) under oxic conditions regardless of whether SRFA was present (*Sections 5.3.3.2 and 5.3.4.2*). Sulfide concentration decreased close to or below detectable levels under oxic conditions. In accordance with these results, both forms of Fe have been found to catalyze S(II-) oxidation by oxygen in laboratory studies (Vazquez *et al.*, 1989) and field studies (Millero 1991a; 1991b; 1991c; Zhang and Millero, 1993). Although stable iron sulfide species are known to form in solution (Luther and Ferdelman, 1993; Beinert *et al.*, 1997) and $Fe_2S_4OH_5$ species have very recently been identified in oxic waters (Rozan *et al.*, 2000a), the results of this study indicate that such species do not successfully stabilize

S(II-) against oxidation. Furthermore, they show that NOM does not provide a substrate for iron sulfide stabilization.

Given the simultaneous occurrence of different metals in natural waters, interactions between metals would be expected. Experiments (*Sections 5.3.3.4*, *5.3.3.5*, *5.3.4.4* and *5.3.4.5*) were conducted to examine the effect of such interactions on S(II-) stabilization. These studies showed that the stabilizing effect of Zn(II) on S(II-) is still attained when other S(II-) binding metals are present. This was accomplished in the presence of Ag(I) and Fe(III), with and without an association with NOM. The main implication of this finding is that in a natural setting where NOM and various metals are present, S(II-) can still persist in oxic waters.

Whether the stabilization of S(II-) described above involved the formation of pure Zn(II) or heterometal sulfide species of Zn/Fe and Zn/Ag, was not verified. Silver(I) exhibits a higher affinity for S(II-) than Zn(II), and consequently, S(II-) should preferentially bind Ag(I). In these experiments, suppression of the methylene blue signal at Ag(I) concentrations equal to or greater than that of S(II-), indicated that S(II-) binding was dominated by Ag(I). At lower Ag(I) concentrations, S(II-) was measured in solution indicating that some was being stabilized through binding with Zn(II). Laboratory studies have demonstrated that Ag(I) not only forms very stable sulfide clusters in solution (Kramer *et al.*, 2000; Rozan and Luther, 2000), but also rapidly replaces Zn(II) and Cu(II) in their respective S(II-) clusters. Incorporation of Ag(I) into zinc sulfide species is thus very likely. In the presence of NOM, the binding of Ag(I) to zinc sulfide species is probably stabilized by the NOM, but this stability has not been shown experimentally.

Metal incorporation into cuboidal iron sulfide clusters has also been demonstrated in the laboratory. Zhou *et al.* (1997) showed that Ag(I) and Cu(I) may be incorporated into the cuboidal cluster $[Fe_3S_4(LS_3)]^{3-}$ (L = CN^-, Ph_3P). The potential of forming stable mixed metal sulfide species has important implications for the bioavailability of metals. Due to their abundance, Zn, Cu and Fe may dominate the speciation of S(II-) in surface water. Furthermore, by stabilizing S(II-) and allowing it to persist in surface waters, a pool of S(II-) is made available to less abundant, toxic S(II-) binding metals such as Ag(I) and Hg(II). These metals exhibit a higher affinity for S(II-) than Zn, Cu or Fe, and may displace them in a sulfide species.

Recommendations for Future Work

Conducting experiments in a very new research area often lead to the attainment of inexplicable results. As more information became available throughout this research work and other research efforts, a better understanding of our results was provided. Time restraints however, did not always permit for further investigation of these findings, as well as the investigation of new research ideas. Some of these research pursuits should be addressed and are discussed in this section.

Although the stabilization of S(II-) by Zn(II) in association with SRFA was demonstrated, it was not conclusively shown that the NOM offered further stability to the zinc sulfide species to prolong the persistence of S(II-) in oxic solution. The oxidation of zinc sulfide clusters in solution with and without NOM should be reexamined in parallel experiments incorporating measures to eliminate interferences resulting from the adsorption of sulfide phases to container surfaces. These experiments should also be run with longer oxidation periods (> 7 days) to determine an approximate lifetime for S(II-) in oxic solution under these conditions, and to see whether zinc sulfide species persist for longer periods in the presence of NOM.

Similar experiments should also be conducted with other NOM types. This would help determine whether the stabilization of S(II-) is generally attained in the presence of all or only certain NOM types, and consequently in different freshwater systems varying in NOM content. Furthermore, a comparison between more natural (e.g. Luther Marsh NOM) and processed NOM (e.g. Aldrich humic acid) types can provide information on whether isolation and purification procedures significantly alter NOM, at least with respect to its reactivity with S(II-) and metal sulfide species.

The stabilization of S(II-) under oxic conditions by Cu(II) or Ag(I) in the presence of NOM should be re-examined. Copper occurs at high enough concentrations in freshwaters to play an important role in S(II-) speciation, while S(II-) would control the speciation of Ag(I) which is a very toxic to aquatic organisms. Consequently, the role of these metals in S(II-) stabilization needs to be assessed. Problems relating to the measurement of S(II-) bound to Cu(II) or Ag(I) by the MBS method made it impossible to assess this role in our work. A method such as the one recommended in *Section 7.3* would analyze Cu(II) and Ag(I) sulfides and would provide more meaningful results in these experiments.

As was discussed previously, an understanding of the metal sulfide-NOM interaction could be useful in predicting metal toxicity. Fluorescence spectroscopy could provide useful qualitative as well as quantitative (binding strength) information on this interaction. The fluorescence of humic substances is quenched by bound metal ions and this property allows for differentiation between free and bound sites in metal ion binding sites (Stevenson, 1994). If this change in fluorescence is assumed to be proportional to the amount of metal bound to the humic substance, then stability constants and total ligand concentrations may be determined (Senesi, 1990; Smith and Kramer, 1998). It is recommended that fluorescence studies complimentary to the experiments presented in Chapter 5 be conducted to obtain information on the binding of Zn(II), Fe(II), Fe(III), Cu(II) and Ag(I) sulfides to SRFA.

7.2 ANALYTICAL METHODS

Another important finding of these experiments was the fact that Ag(I) and Cu(II) sulfide species in solution are generally not reactive in MDR to form MBS. This inhibition was attributed predominantly to the acid insolubility of Ag(I) and Cu(II) sulfide species and an inability to dissociate to release H_2S, although Cu(II) may also interfere in the reaction between H_2S and MDR. The acid insoluble nature of these as well as Ni and Hg sulfide species, has been previously noted in the AVS method of S(II-) determination in sediments involving solid phases (Wu and Kramer, 1997; Cooper and Morse, 1998). The main implication of this finding is that S(II-) from Ag(I) and Cu(II) and possibly other metal sulfide species, is not included in S(II-) measurements conducted on natural waters using the colourimetric MBS method. Consequently, the total amount of reactive S(II-) is underestimated. Another implication is that, metal sulfide species that are resistant to oxidation and stable in acid solution, may allow for persist S(II-) in acidic mining waters.

The importance of determining S(II-) in natural waters has been emphasized in this work due to its effect on metal speciation, bioavailability and toxicity. This work showed that the MBS method alone suffers from a number of serious limitations in its application to natural freshwater samples. These limitations may lead to an inaccurate determination of S(II-) where concentrations are over- or underestimated. Some recommendations and guidelines are provided in the next section to develop a more appropriate method for the colourimetric determination of S(II-) in both laboratory and natural freshwater samples.

Voltammetry may provide a more promising, alternative method to colourimetry for the measurement of S(II-) in natural waters. Very recent improvements in voltammetric methods have overcome previously reported problems to successfully discriminate between sulfur species in solution and to accurately measure S(II-) (Rozan *et al.*, 2000b). Improved methods involve the use of cyclic or linear sweep voltammetry. Furthermore, a combination of acid titrations and electrochemical measurements has been used to provide information on metal sulfide speciation in the sample (Luther *et al.*, 1996; Rozan *et al.*, 2000a).

7.3 RECOMMENDATIONS FOR THE COLOURIMETRIC DETERMINATION OF TRACE SULFIDE IN NATURAL FRESHWATERS

(a) The sample is best analyzed in the container in which it is collected. Metal sulfides adsorbed to container surfaces are thus recovered and included in the S(II-) measurement. Substantial losses in S(II-) could otherwise be suffered.

(b) Reaction of S(II-) with MDR is best not conducted in the sample matrix. Rather, the sample is acidified in the original sampling container and the generated H_2S purged from the sample and trapped in a basic solution where reaction with MDR occurs. This procedure is used in the AVS method of S(II-) determination in sediments (Allen *et al.*, 1993), but needs to be adapted to the measurement of low nanomolar S(II-) in water samples. The purge and trap method eliminates interferences in absorbance measurements from NOM colour and particulates that are usually present in freshwater samples. Interferences in the reaction between H_2S and MDR from Cu, and possibly other components in the sample, are avoided by the purge and trap method.

Alternatively, Tang and Santschi (2000) have reacted natural samples with MDR to form MBS, and then separated the methylene blue complex from the sample matrix by HPLC. Although this method eliminates interferences from NOM and particulates, it does not eliminate interferences from Cu. It also underestimates S(II-) as acid insoluble metal sulfides are omitted from the analysis.

(c) In order to obtain information on S(II-) speciation, S(II-) can be recovered from the sample in a two stage process:

- Step (1): Acid digestion to recover S(II-) from acid soluble metal sulfides (e.g. FeS, ZnS, CdS, MnS, PbS).
- Step (2): Treatment with an acidic Cr(II) solution to reduce and release S(II-) from acid insoluble metal sulfides (e.g. CuS, Ag_2S, HgS, NiS).

Reduction of the sample with Cr(II) allows S(II-) bound in acid insoluble metal sulfides to be included in the S(II-) determination. These recalcitrant S(II-) species represent a substantial portion of S(II-) in natural waters, and omitting them from measurements can result in a serious underestimation of S(II-). Treatment of a natural sample with Cr(II) however, may also complicate interpretation of the data since sulfur species such as pyrite (FeS_2), elemental sulfur (S_8), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}) are reduced to S(II-) and included in the analysis resulting in an overestimate of labile S(II-). These other species need to be identified, and an adjustment made for them in the labile S(II-) measurement.

(d) Interferences to the MBS analysis, occurring in a natural sample, do not allow detection limits for reactive S(II-) at pico- to nanomolar concentrations. Detection limits may be improved however, by preconcentrating the S(II-) in the sample. A standard method used to precipitate and concentrate S(II-) from solution involves the addition of zinc acetate/NaOH to the sample (A.P.H.A, 1992). This removes S(II-) from solution in the form of a zinc sulfide precipitate that can be acid digested to release and measure the S(II-) as described previously.

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APPENDIX: DATA SUMMARY

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CHAPTER 3 DATA

Table A.1: Sulfide data for Figure 3.1 (p. 51) (detection limit: 0.1 μM).

Sample Zn(II) (μM)	Recovered Sulfide (μM)	% S(II-) Recovered (initial S(II-): 2.5 μM)
0	< 0.1	0%
0.5	< 0.1	0%
1.5	0.2	10%
2.5	0.4	16%
3.5	1.0	40%
7.5	1.0	40%

Table A.2: Sulfide data for Figure 3.2 (p. 53) (detection limit: 0.03 μM).

Sample	S(II-) in Solution			S(II-) in Solution and from Container Walls		
Zn(II) (μM)	#1 S(II-) (μM)	#2 S(II-) (μM)	Mean (μM)	#1 S(II-) (μM)	#2 S(II-) (μM)	Mean (μM)
0	2.03	1.94	1.98 ± 0.05	1.15	1.15	1.15 ± 0
0.5	1.89	1.77	1.83 ± 0.06	1.08	0.99	1.03 ± 0.04
1.5	1.45	1.43	1.44 ± 0.01	1.82	1.85	1.84 ± 0.02
2.5	1.10	1.07	1.09 ± 0.02	1.96	2.10	2.03 ± 0.07
3.5	0.91	0.94	0.93 ± 0.01	2.44	2.54	2.49 ± 0.05
5.0	1.00	0.60	0.80 ± 0.20	2.50	1.38	1.94 ± 0.56
7.5	1.29	1.34	1.31 ± 0.02	2.17	2.04	2.10 ± 0.07
25	1.66	1.64	1.65 ± 0.01	1.60	1.84	1.72 ± 0.12
100	1.76	1.85	1.81 ± 0.05	1.88	1.71	1.80 ± 0.08

CHAPTER 4 DATA

Table A.3: Sulfide data for Figure 4.2 (p. 61) (detection limit: 0.1 μM).

Time (h)	S(II-) (μM)
-16	143
-11	150
0	185
1	32.8
2	56.1
3	38.7
7	11.0
10	9.8
25	4.8
47	3.8

Table A.4: Sulfide and Ag(I) data for Figures 4.4 & 4.5 (p. 67).

Time (h)	S(II-) (μM) (dl: 0.1 μM)	Ag(I) (nM) (dl: 0.18 nM)
-41.5	33.5	nd
-41	34.3	nd
-37	46.2	nd
-18	1.3	nd
0	3.6	< 0.18
0.5	0	0.23
1	9.0	< 0.18
1.5	28.7	< 0.18
2	46.0	< 0.18
2.5	37.1	< 0.18
3	29.6	< 0.18
3.5	14.3	< 0.18
4	5.2	< 0.18
4.5	2.8	0.29
5	2.3	0.33
6	< 0.1	0.46
8	< 0.1	0.42
14	< 0.1	1.07
24	< 0.1	1.49
48	< 0.1	< 0.18
72	< 0.1	< 0.18
104.5	< 0.1	1.13

nd: no data
dl: detection limit

Table A.5: Sulfide data for Figure 4.7 (p. 70) (detection limit: 0.1 μM).

Experiment	**Time (h)**	**#1 S(II-) (μM)**	**#2 S(II-) (μM)**	**Mean S(II-) (μM)**
FeS	-19	3.0	2.8	2.9 ± 0.1
	0	5.8	14.6	10.2 ± 4.4
	1	1.8	2.1	1.9 ± 0.2
	8	< 0.1	< 0.1	< 0.1

Table A.6: Sulfide data for Figure 4.8 (p. 71) (detection limit: 0.1 μM).

Experiment	**Time (h)**	**#1 S(II-) (μM)**	**#2 S(II-) (μM)**	**Mean S(II-) (μM)**
FeS + Ni (0.2%)	-19	2.4	2.4	2.4 ± 0.0
	0	3.3	3.8	3.57 ± 0.2
	1	0.5	0.5	0.5 ± 0.0
	8	< 0.1	<0.1	< 0.1
FeS + Cu (0.2%)	-19	2.2	2.3	2.2 ± 0.1
	0	1.5	1.5	1.5 ± 0.0
	1	1.6	1.6	1.6 ± 0.0
	8	0.1	0.2	0.2 ± 0.0
FeS + Mn (0.2%)	-19	1.9	1.8	1.8 ± 0.1
	0	3.0	3.2	3.1 ± 0.1
	1	18.5	20.4	19.5 ± 1.0
	8	< 0.1	< 0.1	< 0.1

Table A.7: Sulfide data for Figure 4.10 (p. 74) (detection limit: 0.1 μM).

Experiment	Time (h)	S(II-) (μM)
FeS + Zn (0.2%)	-17	167
	-15	187
	0	160
	0.5	62.1
	1	73.9
	2	56.6
	3	57.2
	9	1.3
	18	1.3

Table A.8: Sulfide data for Figure 4.11 (p. 76) (detection limit: 0.1 μM).

Experiment	Time (h)	S(II-) (μM)
FeS + Zn (2%)	-22	123
	-20	186
	0	246
	0.5	5.0
	1	10.0
	3	30.0
	6	1.8
	9	1.3
	22.5	1.3
	54	1.2
	70.5	1.2

Table A.11: Sulfide data for Figure 5.3 (p. 86) (detection limit: 0.03 μM, 0.1 μM at -51 h). Available pH data appears in brackets. The initial nominal S(II-) concentration was 2.5 μM.

Sample	-71 h S(II-) μM	-51 h S(II-) μM	0 h S(II-) μM	25 h S(II-) μM	75 h S(II-) μM	143 h S(II-) μM
1	2.5	1.6	0.41 (9.4)	0.18 (7.5)	0.07 (7.6)	0.06 (7.6)
2	2.5	1.2	0.20 (9.5)	0.04 (7.6)	<0.03 (7.6)	<0.03 (7.7)
3	2.5	1.5	0.78	nd	nd	_*0.32 (9.4)_
4	2.5	1.5	0.56 (9.5)	nd	nd	_*0.11 (9.4)_
Mean	2.5	1.5 ± 0.2	0.49 ± 0.24	0.11 ± 0.07	0.04 ± 0.04	0.03 ± 0.03
% S(II-) remaining	100%	59%	20%	4%	2%	1%

*samples kept anoxic
nd: no data

Table A.12: Sulfide data for Figure 5.4 (p. 89) (detection limit: 0.03 μM, 0.1 μM at -51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) concentration (2.5 μM) remaining in solution.

Sample	-71 h	-51 h		-0 h		25 h		75 h	
Zn(II) (μM)	S(II-) (μM)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)
0	2.5	2.17	87%	1.72 (9.6)	69%	0.39 (7.3)	16%	0.07 (7.5)	3%
1.5	2.5	2.01	80%	1.55 (9.5)	62%	0.67 (7.4)	27%	0.57 (7.5)	23%
2.5	2.5	2.04	82%	1.38 (9.5)	55%	0.79 (7.5)	32%	0.74 (7.4)	30%

Table A.13: Sulfide data for Figure 5.5 (p. 89) (detection limit: 0.03 µM). Per cent S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	-22 h	0 h		23 h		70 h		119 h		167 h	
Zn(II) (µM)	S(II-) (µM)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)
0	2.5	2.23	89%	0.10	4%	<0.03	0%	<0.03	0%	<0.03	0%
1.5	2.5	2.10	84%	0.64	26%	0.51	20%	0.40	16%	0.33	13%
2.5	2.5	2.27	91%	0.90	36%	0.24	10%	0.04	2%	<0.03	0%
3.5	2.5	1.88	75%	1.02	41%	0.22	88%	0.37	15%	<0.03	0%
5.0	2.5	1.81	72%	1.37	55%	0.13	5%	0.18	7%	<0.03	0%
7.5	2.5	1.39	56%	0.80	32%	nd	nd	0.43	17%	0.26	10%

nd: no data

Table A.14: Sulfide data for Figure 5.6 (p. 91) (detection limit: 0.03 µM, 0.1 µM at -51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	-71 h	-51 h		0 h		25 h		74 h	
Fe(II) (µM)	S(II-) (µM)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)
0	2.5	1.9	75%	1.35 (9.6)	54%	0.11 (7.4)	4%	0.08 (7.5)	3%
1.5	2.5	1.5	62%	1.31 (9.4)	52%	0.03 (7.4)	1%	<0.03 (7.5)	0%
2.5	2.5	1.5	61%	0.73 (9.5)	29%	0.34 (7.5)	14%	0.11 (7.5)	4%

Table A.15: Sulfide data for Figure 5.7 (p. 91) (detection limit: 0.03 µM, 0.1 µM at -51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-71 h**	**-51 h**		**0 h**		**26 h**		**74 h**		**144 h**	
Fe(III) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
0	2.5	1.5	59%	1.15	46%	0.11	4%	0.05	2%	0.06	2%
				(9.4)		(7.1)		(7.4)		(7.6)	
1.5	2.5	2.1	84%	1.56	62%	0.05	2%	<0.03	0%	0.05	2%
				(9.6)		(7.4)		(7.7)		(7.8)	
2.5	2.5	1.9	77%	1.44	58%	0.21	8%	<0.03	0%	<0.03	0%
				(9.6)		(7.6)		(7.7)		(7.9)	

Table A.16: Sulfide data for Figure 5.8 (p. 93) (detection limit: 0.03 µM, 0.1 µM at -51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-71 h**	**-51 h**		**0 h**		**26 h**		**74 h**		**143 h**	
Cu(III) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
0	2.5	2.1	84%	1.34	54%	0.06	2%	0.06	2%	0.03	1%
				(9.6)		(7.4)		(7.5)		(7.6)	
1.5	2.5	<0.1	2%	0.06	2%	0.05	2%	<0.03	0%	<0.03	0%
				(9.6)		(7.5)		(7.7)		(7.6)	
2.5	2.5	<0.1	2%	0.03	1%	0.06	2%	<0.03	0%	<0.03	0%
				(9.5)		(7.6)		(7.7)		(7.8)	

Table A.17: Sulfide data for Figure 5.9 (p. 93) (detection limit: 0.03 µM, 0.1 µM at -52 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-72 h**	**-52 h**		**0 h**		**26 h**		**75 h**		**144 h**	
Ag(II) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
0	2.5	2.1	84%	1.63 (9.7)	65%	0.05 (7.6)	2%	0.03 (7.5)	1%	0.09 (7.7)	4%
3.0	2.5	0.2	9%	0.05 (9.5)	2%	0.04 (7.6)	2%	0.03 (7.7)	1%	0.05 (7.8)	2%
5.0	2.5	0.1	3%	0.05 (9.5)	2%	0.04 (7.7)	2%	0.04 (7.7)	2%	0.09 (7.8)	4%

Table A.18: Sulfide data for Figure 5.10 (p. 96) (detection limit: 0.03 µM). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-48 h**	**-28 h**		**0 h**		**26 h**		**75 h**		**145 h**	
Zn(II) Fe(III) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
Zn 2.5 Fe 0	2.5	2.04	82%	1.38 (9.5)	55%	0.79 (7.5)	32%	0.74 (7.4)	30%	0.07 (7.6)	3%
Zn 2.5 Fe 1.5	2.5	1.23	49%	0.88 (9.6)	35%	0.53 (7.8)	21%	0.45 (7.7)	18%	0.36 (7.8)	14%
Zn 2.5 Fe 2.5	2.5	1.03	41%	0.74 (9.5)	30%	0.04 (7.6)	2%	0.22 (7.7)	9%	<0.03 (7.8)	0%

Table A.19: Sulfide data for Figure 5.11 (p. 97) (detection limit: 0.03 μM). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 μM) concentration remaining in solution.

Sample	**-48 h**	**-28 h**		**0 h**		**26 h**		**75 h**		**145 h**	
Zn(II) Cu(II) (μM)	**S(II-) (μM)**	**S(II-) (μM)**	**% S(II-)**	**S(II-) (μM)**	**% S(II-)**	**S(II-) (μM)**	**% S(II-)**	**S(II-) (μM)**	**% S(II-)**	**S(II-) (μM)**	**% S(II-)**
Zn 2.5 Cu 0	2.5	2.04	82%	1.38 (9.5)	55%	0.79 (7.5)	32%	0.74 (7.4)	30%	0.07 (7.6)	3%
Zn 2.5 Cu 1.5	2.5	0.07	3%	0.06 (9.5)	2%	0.05 (7.7)	2%	<0.03 (7.6)	0%	0.04 (7.7)	2%
Zn 2.5 Cu 2.5	2.5	0.05	2%	0.03 (9.4)	1%	<0.03 (7.6)	0%	<0.03 (7.5)	0%	<0.03 (7.6)	0%

Table A.20: Sulfide data for Figure 5.12 (p. 97) (dl: 0.03 μM). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 μM) concentration remaining in solution.

Sample	**45 h**	**0 h**		**26 h**		**96 h**		**144 h**	
Zn(II) Ag(I) (μM)	**S(II-) (μM)**	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)
*Zn 2.5 Ag 0	2.5	2.27	91%	0.90	36%	0.24	10%	0.04	2%
Zn 2.5 Ag 3.0	2.5	0.13	5%	0.13	5%	0.13	5%	0.11	4%
Zn 2.5 Ag 5.0	2.5	0.19	8%	0.13	5%	0.13	5%	0.11	4%
Zn 2.5 Ag 7.5	2.5	0.09	4%	0.06	2%	<0.03	0%	<0.03	0%

*sampled at -23, 23, 70 and 118 h

Table A.21: Sulfide data for Figure 5.13 (p. 100) (detection limit: 0.03 μM, 0.1 μM at –51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 μM) concentration remaining in solution.

Sample	-71 h	-51 h		0 h		25 h		75 h	
Zn(II) (μM)	S(II-) (μM)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)
0	2.5	1.6	64%	0.41	16%	0.18	7%	0.07	3%
				(9.4)		(7.4)		(7.5)	
0.5	2.5	1.5	59%	0.55	22%	0.38	15%	0.33	13%
				(9.5)		(7.4)		(7.5)	
1.5	2.5	2.0	80%	1.08	43%	0.64	26%	0.61	24%
				(9.5)		(7.6)		(7.5)	
2.5	2.5	2.0	80%	1.27	51%	0.89	36%	0.77	31%
				(9.5)		(7.6)		(7.6)	
3.5	2.5	2.1	82%	1.43	57%	1.04	42%	1.30	52%
				(9.5)		(7.4)		(7.7)	

Table A.22: Sulfide data for Figure 5.14 (p. 100) (detection limit: 0.03 μM). Per cent S(II-) refers to the fraction of the initial nominal S(II-) (2.5 μM) concentration remaining in solution.

Sample	-22 h	0 h		23 h		70 h		118 h		167 h	
Zn(II) (μM)	S(II-) (μM)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)	S(II-) (μM)	% S(II-)
*0	2.5	1.57	63%	<0.03	0%	<0.03	0%	<0.03	0%	nd	nd
0.5	2.5	2.16	86%	0.26	10%	0.18	7%	0.16	6%	0.12	5%
1.5	2.5	1.81	72%	0.59	24%	0.52	21%	0.36	14%	0.40	16%
*2.5	2.5	1.38	55%	1.20	48%	0.86	34%	0.89	36%	nd	nd
3.5	2.5	2.24	90%	1.26	50%	1.64	66%	1.67	67%	1.65	66%
5.0	2.5	2.02	81%	1.71	68%	1.67	67%	1.29	52%	1.42	57%
7.5	2.5	2.58	103%	2.03	81%	2.75	110%	3.00	120%	3.24	130%
25.0	2.5	2.74	110%	2.28	91%	2.80	112%	2.78	111%	2.53	101%

*sampled at 26, 79 and 143.5 h during oxic period
nd: no data

Table A.23: Sulfide data for Figure 5.15 (p. 103) (detection limit: 0.03 µM, 0.1 µM at -51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-71 h**	**-51 h**		**0 h**		**25 h**		**74 h**	
Fe(II) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
0	2.5	1.6	64%	0.41 (8.4)	16%	0.18 (7.3)	7%	0.07 (7.4)	3%
0.5	2.5	1.0	41%	0.22 (9.5)	9%	0.12 (7.6)	5%	<0.03 (7.6)	0%
1.5	2.5	1.4	54%	0.92 (9.5)	37%	0.04 (7.5)	2%	<0.03 (7.6)	0%
2.5	2.5	1.3	52%	0.37 (9.4)	15%	0.06 (7.5)	2%	0.18 (7.5)	0%
3.5		1.2	46%	0.37 (9.4)	15%	<0.03 (7.6)	0%	0.04 (7.5)	0%

Table A.24: Sulfide data for Figure 5.16 (p. 103) (detection limit: 0.03 µM, 0.1 µM at -51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-71 h**	**-51 h**		**0 h**		**26 h**		**74 h**		**144 h**	
Fe(III) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
0	2.5	1.6	64%	0.41 (9.4)	16%	0.18 (7.4)	7%	0.07 (7.5)	3%	0.06 (7.6)	2%
0.5	2.5	1.6	64%	0.72 (9.5)	29%	<0.03 (7.5)	0%	0.04 (7.6)	2%	0.04 (7.7)	2%
1.5	2.5	1.5	62%	0.73 (9.5)	30%	<0.03 (7.5)	0%	<0.03 (7.6)	0%	<0.03 (7.8)	0%
2.5	2.5	1.4	57%	0.65 (9.4)	26%	0.07 (7.7)	3%	<0.03 (7.7)	0%	<0.03 nd	0%
3.5	2.5	1.1	45%	0.45 (9.3)	18%	<0.03 (7.7)	0%	<0.03 (7.7)	0%	<0.03 (7.8)	0%

Table A.25: Sulfide data for Figure 5.17 (p. 105) (detection limit: 0.03 µM, 0.1 µM at -51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	-71 h	-51 h		0 h		26 h		74 h		144 h	
Cu(II) (µM)	S(II-) (µM)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)
0	2.5	1.6	64%	0.41	16%	0.18	7%	0.07	3%	0.06	2%
				(9.4)		(7.4)		(7.5)		(7.6)	
0.5	2.5	0.0	2%	<0.03	0%	<0.03	0%	<0.03	0%	<0.03	0%
				(9.5)		(7.6)		(3.8)		nd	
1.5	2.5	<0.1	0%	<0.03	0%	<0.03	0%	0.03	1%	<0.03	0%
				(9.6)		(7.6)		(7.5)		(7.7)	
2.5	2.5	<0.1	0%	<0.03	0%	<0.03	0%	0.04	2%	<0.03	0%
				(9.6)		(7.7)		(7.6)		(7.6)	
3.5	2.5	<0.1	0%	<0.03	0%	0.03	1%	<0.03	0%	0.12	5%
				(9.5)		(7.7)		(7.6)		(7.6)	

Table A.26: Sulfide data for Figure 5.18 (p. 105) (detection limit: 0.03 µM, 0.1 µM at -51 h). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	-71 h	-51 h		0 h		26 h		74 h		144 h	
Ag(I) (µM)	S(II-) (µM)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)	S(II-) (µM)	% S(II-)
0	2.5	1.2	48%	0.20	8%	0.04	2%	<0.03	0%	<0.03	0%
				(9.5)		(7.6)		(7.5)		(7.7)	
1.0	2.5	1.1	44%	0.33	13%	0.08	3%	0.04	2%	<0.03	0%
				(9.5)		(7.4)		(7.6)		(7.7)	
3.0	2.5	0.3	11%	0.06	2%	<0.03	0%	<0.03	0%	<0.03	0%
				(9.5)		(7.7)		(7.7)		(7.8)	
5.0	2.5	0.0	0%	<0.03	0%	<0.03	0%	<0.03	0%	<0.03	0%
				(9.4)		(7.6)		(7.7)		(7.8)	
7.0	2.5	0.0	0%	0.04	2%	0.07	3%	<0.03	0%	<0.03	0%
				(9.4)		(7.8)		(7.8)		(7.9)	

Table A.27: Sulfide data for Figure 5.19 (p. 107) (detection limit: 0.03 µM). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-48 h**	**-28 h**		**0 h**		**26 h**		**75 h**		**145 h**	
Zn(II) Fe(III) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
Zn 2.5 Fe 0	2.5	1.16	46%	1.13 (9.5)	45%	0.56 (7.7)	22%	1.03 (7.7)	41%	0.81 (7.8)	32%
Zn 2.5 Fe 0.5	2.5	1.34	54%	0.99 (9.5)	40%	0.65 (7.7)	26%	0.66 (7.7)	26%	0.32 (7.7)	13%
Zn 2.5 Fe 1.5	2.5	1.30	52%	1.18 (9.5)	47%	0.73 (7.6)	29%	1.07 (7.7)	43%	0.51 (7.8)	20%
Zn 2.5 Fe 2.5	2.5	1.04	42%	0.88 (9.5)	35%	0.62 (7.7)	25%	0.83 (7.7)	33%	0.62 (7.8)	25%
Zn 0 Fe 2.5	2.5	0.98	39%	0.64 (9.5)	26%	0.07 (7.4)	3%	0.05 (7.6)	2%	<0.03 (7.7)	0%

Table A.28: Sulfide data for Figure 5.20 (p. 109) (detection limit: 0.03 µM). Available pH data appears in brackets, and %S(II-) refers to the fraction of the initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-48 h**	**-28 h**		**0 h**		**26 h**		**75 h**		**145 h**	
Zn(II) Cu(II) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
Zn 2.5 Cu 0	2.5	1.19	48%	0.96 (9.5)	38%	0.65 (7.6)	26%	0.74 (7.5)	30%	0.45 (7.7)	18%
Zn 2.5 Cu 0.5	2.5	0.09	4%	<0.03 (9.5)	0%	0.03 (7.6)	1%	<0.03 (7.6)	0%	<0.03 (7.7)	0%
Zn 2.5 Cu 1.5	2.5	<0.03	0%	<0.03 (9.4)	0%	<0.03 (7.6)	0%	<0.03 (7.6)	0%	0.04 (7.7)	2%
Zn 2.5 Cu 2.5	2.5	<0.03	0%	<0.03 (9.5)	0%	0.04 (7.7)	2%	<0.03 (7.4)	0%	<0.03 (7.7)	0%
Zn 0 Cu 2.5	2.5	0.14	6%	<0.03 (9.3)	0%	0.03 (7.4)	1%	<0.03 (7.5)	0%	0.04 (7.6)	2%

Table A.29: Sulfide data for Figure 5.21 (p. 109) (dl: 0.03 µM). Available pH data appears in brackets, and %S(II-) refers to the fraction of initial nominal S(II-) (2.5 µM) concentration remaining in solution.

Sample	**-45 h**	**0 h**		**26 h**		**96 h**		**144 h**	
Zn(II) Ag(I) (µM)	**S(II-) (µM)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**	**S(II-) (µM)**	**% S(II-)**
Zn 2.5 Ag 0	2.5	1.32	53%	1.26	50%	1.16	46%	0.95	38%
Zn 2.5 Ag 0.5	2.5	1.12	45%	0.78	31%	0.66	26%	0.50	20%
Zn 2.5 Ag 1.5	2.5	0.40	16%	0.21	8%	0.16	6%	0.19	8%
Zn 2.5 Ag 2.5	2.5	0.40	16%	0.69	28%	0.07	3%	0.13	5%
Zn 2.5 Ag 5.0	2.5	0.15	6%	0.13	5%	0.04	2%	0.08	3%
Zn 2.5 Ag 7.5	2.5	0.05	2%	0.08	3.2%	nd	nd	0.07	3%
Zn 0 Ag 5.0	2.5	<0.03	0%	0.06	2%	*<0.03	0%	<0.03	0%

*sampled at 79 h
nd: no data

Table A.30: Sulfide data for Figure 5.22a (p. 112) (detection limit: 0.03 µM). The nominal S(II-) concentration added to each sample was 2.5 µM.

Sample Ag:S	**Mean (n = 2) S(II-) (µM)**
0	2.23 ± 0.07
0.001	2.40 ± 0.02
0.01	2.17 ± 0.00
0.1	2.28 ± 0.09
1	0.27 ± 0.03
2	0.27 ± 0.03
4	0.09 ± 0.02

Table A.31: Sulfide data for Figure 5.22b (p. 112) (detection limit: 0.03 µM). The nominal S(II-) concentration added to each sample was 2.5 µM.

Sample Ag:S	**Mean (n = 2) S(II-) (µM)**
0	2.95 ± 0.00
0.1	2.49 ± 0.01
0.15	1.89 ± 0.12
0.25	1.14 ± 0.55
0.35	1.42 ± 0.22
0.4	1.01 ± 0.12
0.5	1.01 ± 0.39
0.6	0.66 ± 0.02
0.7	0.54 ± 0.03
0.75	0.51 ± 0.07
0.85	0.42 ± 0.10

Table A.32: Sulfide data for Figure 5.23 (p. 114) (detection limit: 0.03 μM). The nominal S(II-) concentration added to each sample was 2.5 μM.

Cu:S	S(II-) (μM)
0	2.11
0.001	1.91
0.01	1.60
0.1	0.97
0.6	0.05
1	0.04