USING PLUG-FLOW REACTORS TO DETERMINE THE ROLE OF SOLUBLE FE(III) IN THE CYCLING OF IRON AND SULFUR IN SALT MARSH SEDIMENTS

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Using Plug-Flow Reactors to Determine the Role of Soluble Fe(III) in the Cycling of Iron and Sulfur in Salt Marsh Sediments

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SUMMARY

In marine sediments, iron is found predominantly as a solid in its oxidized form, Fe$^{3+}$, and dissolved in its reduced form, Fe$^{2+}$. Recently, soluble species of Fe$^{3+}$ complexed by natural organic ligands have been detected in coastal marine sediments with Au/Hg microelectrodes (Bull and Taillefert, 2001; Neuhuber, 2003; Taillefert et al., 2000; Taillefert et al., 2002a and b). The existence of soluble Fe$^{3+}$ in salt marsh sediments has already been recognized (Liang et al., 1993a; Luther et al., 1992; Luther et al., 1996), however, the mechanisms by which soluble Fe$^{3+}$ forms are still under investigation. Possible explanations include the rapid chemical oxygenation (Neuhuber, 2003) and microbial oxidation of Fe$^{3+}$ in the presence of organic ligands, or the non-reductive dissolution of solid Fe oxides by organic bidentate ligands containing at least one oxygen atom (Luther et al., 1992). In the latter mechanism, the organic ligands could be produced by iron reducing bacteria in order to dissolve iron oxides prior to reduction (Lovley, 1996).

The exact role of soluble Fe$^{3+}$ complexes in sediment diagenesis is also unknown. In anoxic conditions, soluble Fe$^{3+}$ may effectively oxidize FeS$_2$ (Singer and Stumm, 1970) and thus recycle Fe and S for use as terminal electron acceptors during natural organic matter (NOM) degradation. Alternatively, soluble organic Fe$^{3+}$ complexes may catalyze the formation of FeS and FeS$_2$ (Rickard, 1995; Rickard & Luther, 1997) through the rapid reduction of soluble Fe$^{3+}$ by dissolved sulfide (Taillefert et al., 2000) thus immobilizing Fe and S in the solid phase.

Using small sediment plug-flow reactors, I investigated the cycling of Fe and S in the first few centimeters of salt marsh sediment to better understand the role of soluble
Fe$^{3+}$ in the formation of FeS and FeS$_2$ in marine sediments. Results show that sulfate reduction is minimal under suboxic conditions and that microbial Fe reduction is the dominant pathway for NOM oxidation under these conditions. In the presence of a highly reactive organic metabolite, sulfate reduction accounts for all of the organic matter oxidation and subsequent FeS precipitation results in the total depletion of sulfate from the pore waters. Experiments mimicking the enrichment of soluble organic Fe$^{3+}$ complexes in reduced sediments show that, in fact, soluble organic Fe$^{3+}$ complexes do not reoxidize FeS and FeS$_2$, but rather promote their precipitation by enhancing sulfate reduction via complex bacterial interactions. The rate of pyrite formation in the presence of soluble organic Fe$^{3+}$ is much higher than previously reported in the literature, suggesting that soluble Fe$^{3+}$ may efficiently restrict NOM remineralization by immobilizing Fe and S as solid FeS and FeS$_2$. 

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CHAPTER 1

INTRODUCTION

Continental margins have the highest organic carbon burial on earth and subsequently play an important role in regulating atmospheric chemistry as well as global climate change over geologic time scales (Leithold and Blair, 2001). Organic carbon is produced through weathering of sedimentary rocks and during primary productivity at the ocean surface where it is then transferred to deep waters and either remineralized to CO₂ or buried in sediments (Hedges and Keil, 1995; Liu et al., 2000). Microorganisms obtain their energy from terminal electron acceptors that are required to remineralize NOM (Ehlich, 1996). Primary productivity is defined as the rate of natural organic matter (NOM) synthesis in excess of its respiratory utilization (Stumm & Morgan, 1996). It has been recognized that organic carbon burial in marine sediments acts as a sink for CO₂ (Wallmann, 2001) and ultimately controls the O₂ and CO₂ concentrations in the atmosphere (Berner and Canfield, 1989; Holland, 1984; Ingall and Van Cappellen, 1990).

To predict the relationship between the different carbon reservoirs and CO₂ in the atmosphere, it is necessary to understand the processes controlling organic carbon preservation in coastal marine sediments (Hedges and Keil, 1995).

Salt marshes and estuarine environments comprise 0.35 percent of the world’s surface area and produce an estimated 2 percent of the net world primary productivity (Stumm & Morgan, 1996). Measuring organic carbon directly in these sediments to quantify carbon sequestration in salt marsh sediments is not feasible due to the fact that
90% of sedimentary organic matter in marine depositional environments cannot be physically separated from its mineral matrix (Hedges and Keil, 1995). Also, the numerous forms and various degrees of reactivity of organic carbon in marine sediments make measuring organic carbon outright a difficult endeavor. Consequently, one of the ways to effectively quantify the sequestration of organic carbon in sediments and the significance of salt marsh ecosystems on the global carbon cycle is to measure the changes in concentrations of terminal electron acceptors or their reduced products during heterotrophic microbial processes.

The biogeochemical processes involved in the transformation of NOM have mostly been characterized in deep-sea sediments (Canfield et al., 1993; Hedges and Keil, 1995; Ingall and Jahnke, 1994; Ingall and Van Cappellen, 1990; Jahnke et al., 2000; Marinelli et al., 1998) where the organic carbon flux to the seafloor is relatively small. Only a few studies have attempted to quantify these processes in salt marsh sediments (Boudreau, 1984; Jorgensen, 1977; Kostka and Luther, 1995; Kostka et al., 2002; Lowe et al., 2000; Taillefert et al., 2002; Westrich, 1983) mainly because the flux of organic carbon and physical processes (i.e., tidal forcing and bioturbation) in these environments tend to high biogeochemical complexity. It is generally recognized that sulfate reduction plays a critical role in the oxidation of NOM in salt marsh sediments accounting for up to ninety percent of the organic carbon oxidation (Boudreau, 1984; Jorgensen, 1977; Lowe et al., 2000; Westrich, 1983). Recently, however, the significance of microbial Fe^{3+} reduction on the remineralization of NOM in salt marsh sediments has been demonstrated (King and Greer, 1999; Kostka and Luther, 1995; Lowe et al., 2000; Thamdrup et al., 1994). Kostka et al. (2002) determined that Fe^{3+} reduction was the prevailing mechanism
for organic carbon oxidation in bioturbated, vegetated salt marsh sediments. Hedges and
Keil (1995) also recognized the role of Fe associated with organic matter as the link for
the global carbon, sulfur and oxygen cycles over geologic time.

Table 1.1 illustrates the sequence of microbial redox reactions involved in NOM
remineralization as they should occur with increasing depth in marine sediments
according to their Gibbs free energy yield. Aerobic respiration has the highest free
energy yield for NOM remineralization in sediments and should occur near the sediment-
water interface (SWI) where O₂ concentrations are generally highest. Denitrification and
nitrate reduction are inhibited by dissolved oxygen and should only occur in sediments
depleted in dissolved oxygen. Other important anaerobic carbon oxidation processes
include manganese and iron reduction, sulfate reduction, and methanogenesis. These
microbially mediated reactions should also occur in salt marsh sediments though parallel
chemical reactions and physical processes may alter this ideal sequence.
Table 1.1 Sequence of heterotrophic microbial processes as they occur with depth in deep sea sediments. Formaldehyde is used as a proxy for NOM. $\Delta G^\circ$ (kJ/mol) values taken from Morel and Hering, 1993.

<table>
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<th>Process</th>
<th>Reaction</th>
<th>$\Delta G^\circ$ (kJ/mol)</th>
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<tr>
<td><strong>Respiration:</strong></td>
<td>CH$_2$O + O$_2$ $\leftrightarrow$ CO$_2$ + H$_2$O</td>
<td>-119</td>
</tr>
<tr>
<td><strong>Denitrification:</strong></td>
<td>3CH$_2$O + 4NO$_3^-$ + 4H$^+$ $\leftrightarrow$ 5CO$_2$ + 2N$_2$ + 7H$_2$O</td>
<td>-113</td>
</tr>
<tr>
<td><strong>Mn Reduction:</strong></td>
<td>CH$_2$O + 2MnO$_2$(Pyrolusite) + 4H$^+$ $\leftrightarrow$ CO$_2$ + 2Mn$^{2+}$ + 3H$_2$O</td>
<td>-96.9</td>
</tr>
<tr>
<td><strong>Fe Reduction:</strong></td>
<td>CH$_2$O + 4Fe(OH)$_3$(Ferrhydrite) + 8H$^+$ $\leftrightarrow$ CO$_2$ + 11H$_2$O + 4Fe$^{2+}$</td>
<td>-46.7</td>
</tr>
<tr>
<td><strong>Sulfate Reduction:</strong></td>
<td>2CH$_2$O + SO$_4^{2-}$ + H$^+$ $\leftrightarrow$ HS$^-$ + 2CO$_2$ + 2H$_2$O</td>
<td>-20.5</td>
</tr>
<tr>
<td><strong>Methanogenesis:</strong></td>
<td>2CH$_3$O $\leftrightarrow$ CO$_2$ + CH$_4$</td>
<td>-17.7</td>
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</table>
Figure 1.1 Typical sediment porewater depth profile of (A) $O_2$, $Mn^{2+}$, $Fe^{2+}$, and $H_2S$ in $\mu$M and (B) soluble-organic $Fe^{3+}$ and $FeS_{(aq)}$ in nA obtained in the Skidaway salt marsh (Bull and Tailllefer, 2001). The profiles were obtained by incrementally lowering a single voltammetric Au/Hg microelectrode into a sediment core taken from the salt marsh. Both soluble $Fe^{3+}$ and soluble FeS are reported in Figure 1 in terms of current because it has not yet been possible to quantify their respective concentrations using voltammetry.
Figure 1.1 displays a typical depth profile of the main terminal electron acceptors and/or their reduced products in sediment porewaters of the Skidaway Island salt marsh in Savannah, Georgia (Bull and Taillefert, 2001). These sediments typically show an abrupt decrease in oxygen to non-detectable concentrations at the SWT (Figure 1.1 A) The removal of dissolved oxygen close to the sediment-water interface is assumed to result from a combination of microbial respiration (Reimers et al., 1986), biological oxidation of Mn$^{2+}$ and $\Sigma$H$_2$S (Jorgensen, 1977), and chemical oxidation of Fe$^{2+}$ (Neuhuber, 2003). A few millimeters in the sediment, Mn$^{2+}$ and Fe$^{2+}$ are produced during anaerobic NOM respiration reactions (Table 1.1) (Kostka et al., 2002; Lowe et al., 2000) and/or chemical reduction of Mn and Fe oxides by dissolved sulfide (Eq. 1 - 2) (Bernier, 1970; Bouleque et al., 1983; Canfield, 1989; Jacobson, 1984; Lord and Church, 1983; Luther et al., 1992; Pyszka and Sommer, 1981; Rickard, 1974) or, eventually, Fe$^{2+}$ (Eq. 3) (Postma, 1985):

$$2\text{Fe(OH)}_3 + \text{HS}^- + 5\text{H}^+ \rightarrow 2\text{Fe}^{2+} + \text{S}^{0} + 6\text{H}_2\text{O}$$  

(1)

$$\text{MnO}_2 + \text{HS}^- + 3\text{H}^+ \rightarrow \text{Mn}^{2+} + \text{S}^{0} + 2\text{H}_2\text{O}$$  

(2)

$$\text{MnO}_2 + 2\text{Fe}^{2+} + 4\text{H}_2\text{O} \rightarrow \text{Mn}^{2+} + 2\text{Fe(OH)}_3 + 2\text{H}^+$$  

(3)

Usually, Mn$^{2+}$ and Fe$^{2+}$ reach maximum concentrations just above the onset of dissolved sulfide produced by sulfate reducing bacteria (Taillefert et al., 2000; Taillefert et al., 2002a and b). With the advent of sulfide, the reduced Fe and Mn decrease concurrently to non-detectable limits. While it is uncertain whether Mn$^{2+}$ is removed from porewaters by adsorption onto the solid sediment (Van Cappellen and Wang, 1996), precipitation of authigenic mineral phases such as MnCO$_3$ or MnFe$_2$O$_4$ (Lord and Church, 1983), or simply because microbial manganese reduction is inhibited by sulfate
reducing bacteria (Ehrlich, 1996), it is generally accepted that the main removal of Fe$^{2+}$ from porewaters is by precipitation of insoluble sulfide minerals such as amorphous FeS (Eq. 4) (e.g., Taillefert et al., 2002), mackinawite (Eq. 5) or greigite (Eq. 6) (Canfield, 1989):

\[
\begin{align*}
    \text{Fe}^{2+} + \text{HS}^– &\rightarrow \text{FeS}_{\text{amorph}} + \text{H}^+ \\
    \text{Fe}^{2+} + \text{HS}^– &\rightarrow \text{FeS}_{\text{mack}} + \text{H}^+ \\
    \text{S}^0 + 3\text{Fe}^{2+} + 3\text{HS}^– &\rightarrow \text{Fe}_3\text{S}_4 + 3\text{H}^+ 
\end{align*}
\]

If elemental sulfur (Berner, 1970; Berner, 1982; Boudreau, 1984; Canfield, 1989) and/or polysulfides (Luther, 1991; Rickard, 1975; Rickard et al., 1995; Schoonen and Barnes, 1991) are present, pyrite (FeS$_2$) may form in the presence of FeS (Eq. 7 - 8):

\[
\begin{align*}
    \text{FeS}_{\text{ox}} + \frac{1}{2} \text{S}_{\text{red}} &\rightarrow \text{FeS}_{\text{red}} \\
    \text{FeS}_{\text{ox}} + \text{S}^2_{\text{sulf}} &\rightarrow \text{FeS}_{\text{2ox}} + \text{S}^2_{\text{aq}} 
\end{align*}
\]

Alternatively, FeS may react with aqueous H$_2$S to form pyrite and hydrogen gas (Rickard and Luther, 1997):

\[
\begin{align*}
    \text{FeS} + \text{H}_2\text{S}_{\text{aq}} &\rightarrow \text{FeS}_2 + \text{H}_2\text{S} 
\end{align*}
\]

where FeS acts as the reductant or electron donor and H$_2$S acts as the oxidant or electron acceptor. Reaction (9) proceeds through a complex series of reactions and is fast in slightly acidic conditions when H$_2$S is dominant (i.e., pH < 7).

The porewater profile presented in Figure 1.1 B shows the occurrence of soluble Fe$^{2+}$ and soluble FeS with depth. The detection of both of these species by voltammetry provides fundamental background data in support of this thesis. Soluble organic complexes of Fe$^{2+}$ have been successfully detected in marine porewaters by voltammetric techniques with Au/Hg microelectrodes (Taillefert et al., 2000) and have been found right
below the SWT and in deeper anoxic coastal sediments (Bull and Taillefert, 2001; Neubüser, 2003; Taillefert et al., 2000; Taillefert et al., 2002a and b). The novelty of soluble Fe\(^{3+}\) as an important constituent in salt marsh chemistry has only recently been appreciated (Liang et al., 1993a; Luther et al., 1992; Luther et al., 1996), however, the mechanisms by which soluble Fe\(^{3+}\) forms are still under investigation. Possible explanations include the chemical oxygenation (Neubüser, 2003) and the microbial oxidation of Fe\(^{2+}\) in the presence of organic ligands, or the non-reductive dissolution of solid Fe oxides by organic bidentate ligands containing at least one oxygen atom (Luther et al., 1992). In the latter mechanism, the organic ligands could be produced by iron reducing bacteria in order to dissolve iron oxides prior to reduction (Lovley, 1996).

Profiles such as the two presented in Figure 4.1 are valuable tools for understanding the processes occurring in salt marsh sediments on a sub millimeter scale. They provide a snapshot in time of several of the typical redox species that may be found at varying depths within salt marsh sediments but, more importantly, they impart critical information on the roles that each species, such as soluble Fe\(^{3+}\) and soluble FeS, plays with respect to other dissolved redox species (e.g., Fe\(^{2+}\) and H\(_2\)S). In turn, it is necessary to determine whether these processes are at steady-state or if the dynamics of these geochemical reactions is influenced by physical forcing or seasonal variations.

Soluble organic-Fe\(^{3+}\) is extremely reactive, especially with dissolved sulfide (Taillefert et al., 2000). The reduction of these complexes by dissolved sulfide generates soluble FeS (Eq. 10) (Taillefert et al., 2000; Theberge and Luther, 1997):

\[
\text{Fe}^{3+} + \text{H}_2\text{S} \rightarrow \text{FeS}_{(aq)} + 2\text{H}^+ 
\]

(10)
which is often found close to the appearance of dissolved sulfide in coastal sediments (Figure 1.1 B; Taillefert et al., 2000; Taillefert et al., 2002a and b; Neuhöfer, 2003). It is suggested that FeS$_{0}$ is an intermediate in the formation of amorphous FeS (Rickard, 1995) and eventually pyrite (Rickard & Luther, 1997).

In turn, soluble Fe$^{3+}$ has been found to effectively oxidize pyrite in acidic conditions (Singer and Stumm, 1970) or at circumneutral pH (Eq. 11) if Fe$^{3+}$ is complexed by an organic ligand (Luther et al., 1992):

$$\text{FeS}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+ \tag{11}$$

Therefore, soluble organic-Fe$^{3+}$ could enhance pyrite formation if dissolved sulfide is in excess or oxidize pyrite in the absence of dissolved sulfide. A schematic of the proposed reaction mechanisms is shown in Figure 1.2 below.

There are additional reasons for studying the fate of Fe and sulfur in the natural environment. Iron is the fourth most abundant element in the earth’s crust, comprising an estimated five percent of the crust by weight and thus a constituent of numerous rock forming minerals including pyrite (FeS$_2$). Pyrite is the most common and widespread sulfide mineral found in almost every geologic setting on earth (Gaines et al., 1997). Iron abundance is reflected in the red and brown hues seen in most soils and sedimentary rocks caused by the oxidation of ferrous iron, forming hematite (Fe$_2$O$_3$) and goethite (FeO(OH)). Likewise, the role of sulfur as a vital ingredient in many processes occurring within the atmosphere and biosphere has an effect in many areas of biogeochemistry (Saltzman and Cooper, 1989). Sulfur has been identified as an essential element in microbes by stabilizing protein structures and transferring hydrogen by enzymes during metabolism (Ehrlich, 1996). The production of volatile sulfur compounds such as H$_2$S,
alkylated sulfides and disulfides, COS, CS₂, and SO₂ by microbes has impacted the
global sulfur cycle through the transfer of sulfur from the biosphere to the atmosphere
(Saltzman and Cooper, 1989). The relevance of pyrite burial as an essential process in
controlling oxygen levels in the atmosphere (Garrels and Perry, 1974) and sulfate
concentrations in sea water over geologic time (Holland, 1978) has been well
documented (Berner, 1984 & 2001). Many large-scale sedimentary sulfide deposits have
formed and are still forming as a direct result of sulfate reduction and subsequent
precipitation of metal sulfides. Whether in the form of sulfide, hydroxide, oxide,
carbonate, or silicate, the global pervasiveness of these two elements yields an
inexhaustible reservoir of iron and sulfur that are able to participate in a complex network
of reactions.

Figure 1.2 Illustration showing reaction pathways under investigation in this study.
Iron oxide reduction may occur via H₂S oxidation (reaction 1) and microbial iron reduction (Table 1.1). In turn, dissolved sulfide is exclusively formed during microbial sulfate reduction (Table 1.1). If the solubility product of FeS is exceeded, Fe³⁺ reacts with H₂S to form FeS₀₀ and then FeS₂. If dissolved sulfide or polysulfides are in excess, FeS₂ should form relatively quickly. Alternatively, soluble-organic forms of Fe³⁺ should be reduced by dissolved sulfide (dashed line) much more rapidly than its solid form, leading to an accelerated precipitation of FeS₀₀ and eventually FeS₂ if H₂S is in excess. As a result, the rate of precipitation of pyrite may be limited by sulfate reduction, and soluble organic-Fe³⁺ may prevent efficient recycling of two important terminal electron acceptors in microbial NOM oxidation. In turn, if sulfate reduction is not active, soluble organic-Fe³⁺ may oxidize pyrite efficiently and recycle iron and sulfur for more microbial iron and sulfate reduction.

In this study, small sediment plug-flow reactors were employed to investigate Fe and sulfur cycling in salt marsh sediments in an effort to constrain the reaction mechanisms by which FeS and FeS₂ precipitate. Continuous replenishment of nutrients and organic carbon, mainly derived from the ubiquitous marsh grass Spartina alterniflora, provides for an active environment rich in microbial life that is continually participating in the chemical and microbial reactions highlighted above.

The questions addressed by this investigation are summarized below:

- In what ways can plug-flow reactors be used to quantify biogeochemical reactions occurring in sediment?
- What conditions favor the microbial reduction of Fe oxides and sulfate?
- What role, if any, does soluble-organic Fe³⁺ play in the precipitation of FeS and FeS₂ in salt marsh sediment?
Does soluble Fe^{3+} oxidize FeS/FeS_{2} under anoxic conditions?

It will be shown that under suboxic conditions, SO_{4}^{2-} reduction is minimal and Fe reduction is the dominant pathway for NOM oxidation in these sediments. Conversely, in the presence of highly reactive organic metabolites such as lactate and acetate, SO_{4}^{2-} reduction may account for the most significant fraction of organic matter oxidation leading to the rapid formation of FeS and pyrite. It has recently been shown that, in the presence of soluble organic Fe^{3+}, FeS and FeS_{2} may be reoxidized, thus recycling Fe and sulfur for further NOM degradation. Experiments mimicking such conditions show that, in fact, soluble organic Fe^{3+} complexes do not necessarily reoxidize FeS and FeS_{2}, but rather promote FeS precipitation by enhancing SO_{4}^{2-} reduction via complex bacterial interactions.
CHAPTER 2

EXPERIMENTAL DESIGN

The experimental set-up is important in understanding the way in which samples were collected and analyzed by both voltammetry as well as other methods. Three plug-flow reactors (R1, R2 & R3) were filled with sediment taken from the salt marsh at the Skidaway Institute of Oceanography (SkIO) in Savannah, Georgia in the spring of 2002. The reactors consist of a cylinder approximately 8 cm long with an inside diameter of 3.8 cm (Figure 2.1). On either side of the cylinder are identical covers that screw onto the top and base. Within each cover is an O-ring, a plastic mesh screen, and a 0.45 micron filter (Micro Filtration Systems). The base of the reactor is designed such that the water is funneled through an opening that is surrounded by radial grooves thus directing the solution in a way that encompasses the entire surface area of the base of the reactor. It is then forced upwards through the mesh screen and 0.45 μm filter and into the sediment where it flows through the reactors (Figure 2.1). The porewaters are then filtered in situ through another 0.45 μm membrane before exiting the top of the reactors. The artificial sea water is then pumped through a homemade PEEK™ flow cell approximately 7.4 cm in length, which is fitted for a PEEK™ Au/Hg microelectrode. Given the volume of the reactors (100 ml) and flow rate (~1 ml/hr), the entire volume of the reactors should be replaced in ca. 4.2 days.
Figure 2.1 Illustration of a plug-flow reactor (PFR) and the reactor set-up. Although only one reactor is shown, three reactors were used for the experiment as indicated by the dashed arrows leading from the deaerated artificial sea water (ASW) to the multi-channel pump. The reactors were kept in an anaerobic chamber that was continuously purged with industrial grade N₂ for most of the experiment (thirteen months total). The ASW enters the PFR from the base and is directed through a single opening in the cap with grooves surrounding it so that the ASW fills the entire base of the cap before moving upwards. The ASW then passes through a mesh screen and 0.45 μm filter and, provided there is no channeling or other preferred pathways, should completely replace the entire volume of the reactor in approximately 4.2 days.
Table 2.1 Composition of ASW (modified after Dickson, 1990). Note that all concentrations are reported as millimoles per liter (mM) except NaCl which is reported in moles per liter (M).

<table>
<thead>
<tr>
<th>ASW Composition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl: 0.43</td>
<td>Salinity: 37</td>
</tr>
<tr>
<td>Na₂SO₄: 29</td>
<td>pH: 8.3</td>
</tr>
<tr>
<td>KCl: 11</td>
<td>Ionic Strength: 0.73</td>
</tr>
<tr>
<td>MgCl₂·6H₂O: 55</td>
<td></td>
</tr>
<tr>
<td>NaBr: 10</td>
<td></td>
</tr>
<tr>
<td>CaCl₂·2H₂O: 11</td>
<td></td>
</tr>
<tr>
<td>NaHCO₃: 2.3</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.2 Photograph of the anaerobic chamber showing, from right to left, the multi-channel peristaltic pump, three plug-flow reactors, flow cells, and fraction collector (barely visible). When used, the voltammetric microelectrode is positioned in the flow cell (white arrows) in place of the white plugs. In order to maintain an electrical contact between the counter, reference, and 1 working electrodes, a catchment (5 mL Falcon tube) is positioned immediately adjacent to the flow cell to collect the effluent sea water during the measurements.
Artificial sea water (ASW) was continuously degassed with UHP compressed argon and then fed through the plug-flow reactors via a high precision multi-channel pump (IsmaTec®, Cole-Parmer Instrument Company). The composition of the ASW and other significant parameters has been provided in Table 2.1 and Figure 2.1. Bromide was added to a concentration of 10 mM as a tracer of the transport processes through the reactors. The ASW was always prepared with reagent grade salts and MilliQ water. Nitrate was not included in the ASW in these experiments.

Figure 2.2 shows a photograph of the anaerobic chamber containing the three reactors, peristaltic pump, three flow cells and fraction collector (far left). The white arrows point to the location in the flow cells where the working electrode was positioned during the voltammetric measurements. The counter and reference electrodes were stationed in a catchment at the outlet of the flow cell during measurements to provide the necessary electrical contact between the three electrodes.

Finally, the porewaters went to a universal fraction collector (EldeX) where samples were collected in 15 mL polypropylene Falcon tubes (Fisher) at a rate of two samples per day for each reactor (Figures 2.1 and 2.2). Each day, one of the samples was acidified to a pH of 2 using concentrated trace metal grade HCl (Fisher) and stored at 4°C while the other was immediately frozen until analysis. Acidified samples were analyzed for both Fe$^{2+}$ and total Fe using the ferrozine method modified after Stookey (1979) and Mn using graphite furnace atomic absorption spectroscopy (Varian Spectra AA-600). Unacidified samples were analyzed for pH and salinity as well as sulfate (SO$_4^{2-}$), bromide (Br$^-$), and chloride (Cl$^-$) using ion chromatography (Dionex, DX-300 Series). Solid samples were collected three times over the course of the experiment on days 0, 246 and
379 for the solid phase extractions by opening the reactors in the anaerobic chamber and taking only enough sediment needed for the analyses.
CHAPTER 3

METHODS

3.1 Voltammetric Techniques

Voltammetric analyses (Analytical Instrument Systems) were carried out in line in the flow cell thus providing real-time data. One of the benefits of voltammetry is the ability to analyze for multiple species at one time. Although each procedure described in this section plays an integral role in this research, voltammetry was by far the most important for determination of several dissolved redox species including $\text{O}_2$, $\text{H}_2\text{O}_2$, $\text{Fe}^{2+}$, $\Sigma\text{H}_2\text{S} (= \text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-} + \text{S}^2)$, soluble-organic Fe$^{3+}$, aqueous FeS, and Mn$^{2+}$ (Brendel & Luther, 1995).

All voltammetric measurements were performed using Au/Hg solid-state microelectrodes (Brendel and Luther, 1995). These microelectrodes consist of a 100 $\mu$m diameter Au wire housed in $\frac{1}{16}"$ PEEK tubing connected via a copper conducting wire to a potentiostat. The Au surface is polished with 15, 6, 1 and $\frac{1}{10}$ $\mu$m diamond paste, plated with Hg to provide an electro-active mercury film, and then polarized. Each electrode, once plated, gets polarized at -0.9 V for ninety seconds to form a good amalgam between the Au and Hg (Brendel and Luther, 1995). Finally, electrodes were tested for quality and calibrated. A platinum wire was used as counter electrode and a Ag/AgCl as reference electrode. When using voltammetry, a potential is applied between the working and reference electrodes and varied as a function of time. At a given potential, a species
is reduced or oxidized at the working electrode and the resulting current is measured at the counter electrode (Taillefert and Rozan, 2002).

Table 3.1 Persistent half reactions for this thesis and their respective reduction potentials (V) at the electrode surface (Brendel and Luther, 1995).

<table>
<thead>
<tr>
<th>Electrode Reactions</th>
<th>E° (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ + 2H₂O + 2e⁻ + Hg → H₂O₂(Hg)</td>
<td>-0.30</td>
</tr>
<tr>
<td>H₂O₂(Hg) + 2H₂O + 2e⁻ → 2H₂O(Hg)</td>
<td>-1.30</td>
</tr>
<tr>
<td>HS⁻ + Hg → HgS + H⁺ + 2e⁻</td>
<td>Adsorption onto Hg &lt; -0.6</td>
</tr>
<tr>
<td>HgS + H⁺ + 2e⁻ ↔ HS⁻ + Hg</td>
<td>-0.6</td>
</tr>
<tr>
<td>Fe²⁺ + Hg + 2e⁻ ↔ Fe(Hg)</td>
<td>-1.43</td>
</tr>
<tr>
<td>FeS + 2e⁻ + H⁺ → Fe(Hg) + HS⁻</td>
<td>-1.1</td>
</tr>
<tr>
<td>L-Fe³⁺ + e⁻ + Hg ↔ Fe²⁺ (Hg)</td>
<td>-0.2 to -0.9 (Ligand Dependence)</td>
</tr>
<tr>
<td>Mn²⁺ + Hg + 2e⁻ ↔ Mn(Hg)</td>
<td>-1.55</td>
</tr>
</tbody>
</table>

Table 3.1 illustrates the electrode reactions occurring at the Au/Hg microelectrode surface as well as their reduction potentials. In addition to being able to measure several redox species at once during a potential scan, voltammetry benefits from low detection limits, fast analysis time, in situ capability, high spatial and temporal resolution, and high reproducibility (Brendel & Luther, 1995; Bull and Taillefert, 2001; Luther et al., 1998; Taillefert et al., 2000; Taillefert and Rozan, 2002). Voltammetric techniques employed in this project include linear sweep voltammetry (LSV) for the determination of O₂ and H₂O₂ and anodic (AS WV) and cathodic (CS WV) square wave voltammetry or cyclic voltammetry (CV) for the determination of Mn²⁺, Fe²⁺, soluble-organic Fe³⁺, and ΣH₂S (Brendel and Luther, 1995). Analyses were performed using either a Model DLK-100A
or Model DLK-60 potentiostat manufactured by Analytical Instrument Systems Inc. (AIS). Both systems are computer controlled and may be battery operated, thus allowing for deployments in the field. All calibrations were done in a cell stand (AIS) which provides the necessary set-up for a standard three-electrode voltammetric cell as described in Brendel and Luther (1995).

![Graph showing voltammetry data]

**Figure 3.1** Sample linear sweep voltammograms (LSV) showing several replicate oxygen measurements and the corresponding half reactions occurring at the appropriate reduction potentials for \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \).

Before use, electrode quality was tested by measuring dissolved oxygen in ASW using linear sweep voltammetry (LSV) (Figure 3.1). Generally, the more sensitive the electrode is, the more well defined is the \( \text{O}_2 \) curve, i.e., the higher the current. A poorly defined \( \text{O}_2 \) curve will generally result in a poor Mn\(^{2+}\) calibration and thus it is a useful
test to characterize the quality of the electrode. Following the O₂ measurement, electrodes were calibrated for Mn²⁺ in degassed ASW. Both the O₂ and Mn²⁺ calibrations were run cathodically from a starting potential of -0.1 V to an ending potential of -1.75 V with a scan rate of 200 mV s⁻¹. A pre-conditioning potential of -0.1 V for 10 seconds was applied to all O₂ and Mn²⁺ measurements. The pre-conditioning potential cleans the surface of the working microelectrode between measurements in order to ensure reproducibility (Brendel and Luther, 1995). The Mn²⁺ calibration is obtained using a bulk standard consisting of 0.1 M MnCl₂·4H₂O (Fisher) acidified to a pH of 2 using concentrated trace metal grade HCl (Fisher) and MilliQ water. Aliquots of the bulk MnCl₂ solution are added to ASW in order to generate a calibration ranging from 0 to 400 μM (Figure 3.2).
Figure 3.2  (A) Sample square wave voltammograms (SWV) of a typical Mn²⁺ calibration. The current (nA) at -1.55 V increases with increasing Mn²⁺ concentration. (B) Average current (nA) as a function of Mn²⁺ concentration. Sensitivities usually range between 0.1 to 0.4 nA/µM depending on the condition of the electrode.
Ferrous Fe and soluble FeS were always measured using cathodic square wave voltammetry under the same conditions used for Mn\(^{2+}\). Soluble-organic Fe\(^{3+}\) is measured by CSWV at the electrode by a three-step process. First a cleaning potential is applied at -0.9 V whereby any Fe\(^{3+}\) that has accumulated at the electrode surface is desorbed from the Hg as Fe\(^{2+}\). Following this, a conditioning potential is applied at -0.1 V resulting in the adsorption of Fe\(^{3+}\) onto the Hg. Finally, the Fe\(^{3+}\) is then reduced to Fe\(^{2+}\) and measured at a potential varying between -0.2 and -0.9 V depending on the ligand complexing Fe\(^{3+}\) (Table 3.1). Soluble Fe\(^{3+}\) may only be measured by voltammetry if it is complexed by an organic ligand (Taillefert et al., 2001). Several organic-Fe\(^{3+}\) complexes may be measured at the microelectrode simultaneously and since these complexes have not yet been identified, their concentrations must be reported in terms of current (Taillefert et al., 2001). Sulfide is measured anodically and cathodically depending on the concentration. Since cathodic square wave voltammetry is the more sensitive of the two methods, it was always used for low concentrations of sulfide with a cleaning potential of -0.9 V for 10 seconds followed by a pre-conditioning step at -0.1 V for 10 seconds. When sulfide concentrations were too high, anodic cyclic voltammetry and anodic square wave voltammetry were always used with just the conditioning step of -0.9 V for 10 seconds. The slope from the Mn\(^{2+}\) curve was used to elucidate the concentrations of other species based on the pilot ion method (Brendel & Luther, 1995) in which the slope from the Mn\(^{2+}\) curve is multiplied by a factor that has been pre-established in order to determine the sensitivity of other species, namely HS\(^{-}\) and Fe\(^{3+}\). Although HS\(^{-}\) and Fe\(^{3+}\) may be calibrated separately, the pilot ion method provides an accurate and time efficient means of establishing their respective concentrations.
Figure 3.3 (A) Anodic square wave voltammogram and (B) anodic cyclic voltammogram of the porewaters at the output of one of the reactors (day 294). Both voltammograms show replicate measurements with sulfide being the only species present. Measurements were performed in the flow cell within the anaerobic chamber. Data has been smoothed with an 11 point moving average. A conditioning potential of -0.9 V and scan rate of 200 mV/s were always used when measuring sulfide anodically.
For example, a factor of 0.36 is multiplied by the Mn$^{2+}$ slope to determine the sensitivity for Fe$^{2+}$. This new value is then used to determine the Fe$^{3+}$ concentration.

This method may be used for thiosulfate (S$_2$O$_3^{2-}$), Fe$^{3+}$, and dissolved sulfide. Occasionally, sulfide calibrations were also done to verify the pilot ion ratio between Mn$^{2+}$ and HS$^-$. The sulfide standard was made using reagent grade sodium sulfide (Na$_2$S$\cdot$9H$_2$O, Fisher). It should be noted that the pilot ion method is not applicable to aqueous FeS or soluble-organic Fe$^{3+}$, both of which have yet to be quantified and thus are still reported in current intensities (Taillefert et al., 2001 and 2002).

Figure 3.3 shows a typical (A) anodic square wave and (B) anodic cyclic voltammetry obtained in March, 2003 for one of the reactors. Although only one reactor analysis is shown, each species was consistently measured in each reactor to obtain an average for the three reactors. The voltammetry measurements were generally performed every two to four weeks depending on the variability of the concentrations. All anodic square wave and anodic cyclic voltammetry measurements had a pre-conditioning step at −0.9 V for 10 seconds when measuring ΣH$_2$S. Due to the high sulfide concentrations measured in the reactors and for the sake of consistency, only the anodic square wave measurements were used to determine the sulfide concentrations over time.

3.2 pH and Salinity

The pH and salinity were measured in selected unacidified samples. The pH measurements were obtained using a pHISE meter (ORION, Model 290Aplus). A standard Tris buffer (pH ~ 8) in 0.54 M NaCl (Dickson, 1993) was used as a reference for
all pH measurements. Salinity measurements were obtained using a portable salinity refractometer with automatic temperature compensation (Fisher).

3.3 Ferrozine Method

The colorimetric analyses for ferrous Fe and total Fe were carried out using a spectrophotometer (Milton Roy Spectronic, Model 501). Standards were consistently made in the range of 2 to 20 μM using a 1 mM ferrous ammonium sulfate (Fe(NH₄)₂(SO₄)₂·6H₂O, Fisher) bulk standard made in MilliQ water and acidified to a pH of 2 with trace metal grade HCl. The reagents used in the analyses include 0.01 M ferrozine (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine, Aldrich) and 1 M ammonium acetate (NH₄C₂H₃O₂, Sigma). A 0.2 M hydroxylamine hydrochloride (Aldrich) solution acidified to a pH of 2 was used as a reductant to determine total dissolved Fe (Stokey, 1970).

3.4 Ion Chromatography

Selected unacidified samples were analyzed for sulfate (SO₄²⁻), bromide (Br⁻) and chloride (Cl⁻) via an ion chromatograph (Dionex, DX-300 Series) equipped with a Dionex IonPac® AS4A (4-mm) chromatography column, AG4A (4-mm) guard column and AMMS III (4-mm) suppressor. The Dionex-300 uses a CDM II detector with suppressed conductivity detection. Standards were made in accordance with the concentrations used for the artificial sea water. In each case, the standards were made from the same salt as that used for the ASW. Samples and standards were diluted at 1:100 proportions prior to analysis.
3.5 Graphite Furnace Atomic Absorption Spectroscopy

Selected acidified samples were diluted between 100 and 1000 times and analyzed for total dissolved Mn using GFAAS (Varian Spectra AA-600) with Zeeman background correction. Benefits of this instrument include an auto-sampler, small sample volumes (~2 mL), and auto-dilution capability. A 100 nM bulk Mn standard in MilliQ water and acidified with trace metal grade HNO₃ (Fisher) was used for all calibrations. The standard was made from a 990 µg/mL bulk Mn standard (AA/ICP calibrated, Aldrich).

3.6 Solid Phase Extractions

3.6.1 Total and Reactive Iron Oxides

Amorphous and crystalline Fe oxides were determined in the solid sediment using the ascorbate and dithionite methods (Kostka and Luther, 1994). As outlined in Kostka and Luther (1994), the ascorbate reagent (pH ~ 8) extracts only amorphous forms of Fe³⁺ whereas the dithionite extractant (pH ~ 4.8) dissolves amorphous Fe oxides, acid volatile sulfide (AVS), and crystalline Fe oxides including magnetite (Fe₃O₄/Fe₂O₃/FeO) and goethite/hemaitite (Fe₂O₃·H₂O). The reagents used for the ascorbate extraction include 0.2 M sodium citrate (Fisher), 0.6 M sodium bicarbonate (Fisher), and 0.1 M L-ascorbic acid (Fisher) in MilliQ water. Wet sediment samples were reacted with the ascorbate reagent at 25°C for approximately twenty four hours, after which the samples were filtered, diluted between 20 and 200 times, and then analyzed by the ferrozine method (Stoookey, 1970) described earlier. Reagents used for the dithionite extraction include 0.35 M ammonium acetate (Aldrich), 0.2 M sodium citrate (Fisher), and 0.03 M sodium dithionite (Aldrich). Wet sediment samples were reacted with the dithionite reagent for
approximately four hours in a water bath set at 60°C. Similarly, the samples were filtered and diluted 20 to 200 times prior to analysis via the ferrozine method.

3.6.2 Pyrite and Acid Volatile Sulfide (AVS)

Pyrite (FeS₂) and acid volatile sulfide (AVS) were extracted from the sediment by the methods described by Canfield et al. (1986) and Henneke et al. (1991), respectively. AVS, as described by Henneke et al. (1991) includes amorphous forms of FeS such as mackinawite [(Fe₃Ni)₄S₈], greigite (Fe₃S₄) and pyrrhotite (Fe₁₋ₓS, X = 0-0.7).

For AVS extractions, approximately 0.5 grams of wet sediment was weighed into four reaction vessels. Each vessel was attached to a 15 mL polypropylene Falcon tube containing 10 mL of 1 M NaOH, completely sealed to avoid contamination by oxygen from the atmosphere. Concentrated HCl (3M) was added to each vessel through a nylon septum after the entire apparatus had been degassed with N₂ for at least one hour. The nitrogen gas was continuously stripped of residual oxygen by a copper trap heated to 350°C thus preventing the rapid oxidation of HS⁻ by oxygen (Millero, 1986). The volatile H₂S gas was distilled at normal temperature with N₂ gas as carrier and trapped in a 1 M NaOH solution (Eq. 12) (Henneke et al., 1991):

\[ \text{H}_2\text{S}_{(g)} + \text{NaOH} \rightarrow \text{HS}^- + \text{Na}^+ + \text{H}_2\text{O} \]  \hspace{1cm} (12)

The sediment was reacted with HCl for four hours after which an aliquot of the NaOH solution (~200 – 800 μL) was collected and added to 20 mL of 0.5 M NaCl and analyzed voltammetrically as described earlier. All AVS measurements were done in 0.5 M NaCl to avoid precipitation of CaCO₃ in the presence of NaOH.

The pyrite (FeS₂) extraction involved the reductive dissolution of pyrite by Cr⁶⁺ in acidic conditions which produces H₂S₄. Prior to the FeS₂ extraction, the sediment was
first washed with acetone at room temperature for 24 hours to remove elemental sulfur that could interfere with the pyrite measurements. The sediment was then dried and approximately 0.5 grams of sediment was added to four reaction vessels identical to those used in the AVS extraction. The pyrite extraction required a reduced 1 M Cr$^{3+}$ solution which reduces FeS$_2$ to H$_2$S. Reduced chromium reagent is prepared by a Jones reaction after amalgamating zinc granules with 2% Hg(NO$_3$)$_2$H$_2$O (Aldrich) in 2% trace metal grade HCl. The CrCl$_3$·6H$_2$O (Sigma) is then reduced by the amalgamated zinc granules overnight. The complete reduction of Cr$^{3+}$ to Cr$^{2+}$ is evidenced by a drastic change in the color of the solution from a dark murky green to a bright translucent blue. The reduced Cr$^{2+}$ solution was then added to concentrated HCl (2M) in a ratio of 2:1 for a total volume of 5 mL. Similar to the AVS extraction, 10 mL of 1 M NaOH was attached to each reaction vessel and the system was once again degassed for at least one hour. Following this step, the reduced Cr$^{2+}$/HCl mixture was added to each reaction vessel through its nylon septum. The reaction takes approximately two hours, after which an aliquot of the NaOH solution was collected and added to NaCl for voltammetric analysis. Due to the nature of the experiments and the heterogeneity of the reactor sediment, samples were always extracted in quadruplicate.
CHAPTER 4

RESULTS

To study the role of soluble-organic Fe\(^{2+}\) on the cycling of iron and sulfur in salt marsh sediments, the sediment was manipulated in different ways. First, the sediment was exposed to degassed seawater to determine whether iron reducing conditions could be established. Then, organic compounds that are easily metabolized were injected into the reactors to activate sulfate reduction and precipitate iron and sulfur as FeS\(_{0}\) and pyrite. The reduced sediment was then exposed to ferric citrate to evaluate whether pyrite oxidation by soluble-organic Fe\(^{3+}\) was significant. Finally, a solution of sodium citrate was injected into the reactors to determine if fermentation could provide organic (i.e., acetate) or inorganic (i.e., H\(_2\)) metabolites to sulfate reducing bacteria.

It is important to note that redox conditions within the reactors changed over the course of the experiment in response to experimental manipulations. Three dominant regimes, (micro-aerobic, suboxic and anoxic), have been identified based on the prevailing conditions. Between 0 and 63 days micro-aerobic conditions prevailed. During this time period, the reactors were exposed to the air while deaerated ASW was pumped through them. It is assumed that under these conditions, some oxygen could penetrate the reactors. Suboxic conditions prevailed between 63 and 120 days when the reactors were placed in an anaerobic chamber and dissolved sulfide was below detection limit until day 120. Anoxic conditions began on day 120 and ended on day 366 and have been divided into three sub-sections.
Figure 4.1 Photographs taken of the plug-flow reactors in June, 2002, at the start of the experiment and again six months later in January, 2003. The pictures show the marked difference in the sediment color in June, 2002, when it was more oxidized (lighter in color) as compared to January when it was more reduced and considerably darker.
They include: the time between 120 and 174 days in which an electron donor, 15 mM lactate and 15 mM acetic acid, was continuously injected into the reactors; the period between 290 to 366 days in which 500 μM ferric citrate was continuously pumped through the reactors; and the time between 366 and 396 days, during which 500 μM sodium citrate was injected into the reactors until the reactors were terminated, exactly thirteen months after they were started.

Historically, geochemists have always looked for changes in color as a useful tool in identifying chemical transformations. Color changes were observed in the reactors over time and provided hints to the chemical processes taking place. Figure 4.1 shows the reactors shortly after the experiment was started in June of 2002 when the sediment within the reactors was still oxidized. It is possible to see dark patches within the sediment indicating reduced zones, however the sediment as a whole is fairly light brown in color. This is in contrast to the photograph taken in January, 2003 which shows the same three reactors six months later and indicates a progression towards more reducing conditions as is evidenced by the black color of the sediment.
4.1 Bromide and Chloride as Conservative Tracers

Figure 4.2 illustrates the measured concentrations of chloride (M) and bromide (mM) as a function of time in days. The error bars denote the average and standard deviation for the three reactors. Bromide (10 mM) was injected into the plug-flow reactors as a conservative tracer (Roychoudhury et al., 1998) to determine the dispersion coefficient and advection rate as well as to establish the residence time of artificial sea water in the plug-flow reactors.

Bromide was taken out of solution at 118 days and re-introduced at 136 days to ensure that the hydraulic parameters remained constant with time. The sluggish response of the bromide upon re-introduction may be due to the fact that, during this period of time, a substitute peristaltic pump was being used where the flow rate could not be exactly controlled. Since they are inert, both Cl\(^-\) and Br\(^-\) constrain the flow rate through the reactors (Figure 4.2) so that variations in Cl\(^-\) and Br\(^-\) concentration over time are indicative of changing flow conditions. The slight increase in the Cl\(^-\) concentration at the start of the experiment may be due to an initially higher concentration of Cl\(^-\) in the porewaters than was injected with the artificial sea water.
Figure 4.2 Chloride (M) and Bromide (mM) data obtained via ion chromatography as a function of time in days. Error bars indicate the average and standard deviation for the triplicate plug-flow reactors.
4.2 pH and Salinity

Figure 4.3 illustrates the average and standard deviation for the measured pH and salinity in the three reactors over the course of the experiment. Also provided on each Figure is the average pH and salinity measured for the input artificial sea water. In each case, this value includes the unamended sea water as well as the sea water after it was amended with lactate and ferric citrate. Overall, the salinity measured at the output of the reactors remains relatively constant between 37 and 40 and falls in the range measured for the input ASW in almost all cases. The pH, on the other hand, is not as consistent and shows an obvious decrease prior to 63 days. With placement of the reactors into the anaerobic chamber, the pH increases until day 150 after which it remains fairly constant until the end of the experiment. The response in pH appears to reflect the changing conditions within the reactors. The decrease in pH may be attributed to production of protons during aerobic respiration within sediments (Van Cappellen and Wang, 1996; Table 1.1). In turn, an increase in pH may be due to Mn and/or Fe oxide reduction (2 protons are consumed for each Mn²⁺ or Fe³⁺ produced) and to sulfate reduction (Table 1.1), although to a lesser extent (1 proton consumed for each sulfate reduced).
Figure 4.3 pH and salinity measured as a function of time in days. The error bars denote the average and standard deviation for the triplicate reactors. The average pH (solid disk) and average salinity (solid square) for the input sea water (ASW) are also provided at time 0. The three dominant redox regimes are shown at the top of the Figure.
4.3 Manganese Reduction

Mn reduction appears to be the dominant redox process between 0 and 12 days and declines sharply between 12 and 55 days with a maximum concentration of 84(±15.5) μM on day 2 and a minimum of 0.8(±0.2) μM on day 55 as provided by GFAAS (Figure 4.4). In the μ-aerobic phase of the experiment, the Mn data obtained through voltammetry and GFAAS do not agree although they do indicate similar trends with time. This discrepancy may be due to the fact that GFAAS measures colloidal Mn as well as Mn$^{2+}$ whereas voltammetry is selective for dissolved Mn$^{2+}$ only. The total dissolved Mn concentration remains low at concentrations of 1.7(±0.9) μM and 2.5(±0.6) μM on days 66 and 76 respectively, probably due to placement of the reactors within the anaerobic chamber. After day 80, little to no Mn is produced. Consequently, Mn reduction seems to be the dominant redox process initially under micro-aerobic conditions although the slight increase in Mn with the onset of a nitrogen atmosphere confirms the possibility that both micro-aerobic and suboxic regimes are suitable for Mn reduction.
Figure 4.4 Total dissolved Mn determined by GFAAS (open squares) and Mn$^{2+}$ by voltammetry (solid circles). The error bars denote the average and standard deviation for the three reactors. The three dominant redox regimes are shown at the top of the Figure. Note the break in the x-axis from 120 to 240 days.
4.4 Iron Reduction

The concentrations of ferrous ion, ferric iron and total iron were determined by both the ferrozine (Fe$^{2+}$ and total dissolved Fe) method and voltammetry (Fe$^{2+}$ and soluble-organic Fe$^{3+}$) as a function of time (Figure 4.5). The average concentrations and standard deviations are reported in $\mu$M for the three reactors except for soluble-organic Fe$^{3+}$ determined by voltammetry which is expressed in units of current (nA).

Ferrous Fe, probably present in the residual porewaters, is initially flushed out of the reactors. It starts out as $162\pm4\mu$M on day 1, increases to a concentration of $382\pm95\mu$M on day 3 and then by day 5 decreases to non-detectable values. Iron remains negligible in the porewaters until day 67 when the ferrous iron and total Fe concentrations revealed by both the ferrozine method and voltammetry (Fe$^{2+}$) reach concurrent maximum concentrations of $347\pm32\mu$M and $359\pm40\mu$M, respectively. Similarly, the average maximum current measured for soluble-organic Fe$^{3+}$ occurs in tandem with the maximum concentrations for ferrous and total Fe. The maximum current measured for Fe$^{3+}$ by voltammetry, however, is not supported by a high dissolved Fe$^{3+}$ concentration, obtained as the difference between ferrous Fe and total dissolved Fe measured by ferrozine. These data suggest that the sensitivity of soluble-organic Fe$^{3+}$ at the electrode surface is extremely high. Based on these analyses, the bulk of the iron produced in the effluent during this time is largely in the form of Fe$^{2+}$.

Iron reduction dramatically decreases between day 63 and day 120 (Figure 4.5), yielding minimum mean ferrozine concentrations for ferrous iron and total iron in the three reactors of $62\pm49$ and $64\pm49\mu$M, respectively. The voltammetric signal for Fe$^{2+}$ and Fe$^{3+}$ follows a similar decrease during this period of time. Similar to the first 63
days of the experiment, the total dissolved Fe and Fe$^{2+}$ concentrations are indistinguishable from one another indicating that all of the Fe measured is in a reduced state.

The initial response of the sediment to the lactate solution was a rapid increase in Fe reduction (Inset, Figure 4.5) with maximum average concentrations for Fe$^{2+}$ and total Fe in the three reactors of $116(\pm9)$ $\mu$M and $139(\pm21)$ $\mu$M, respectively. This increase in Fe reduction is further supported by the voltammetry measurements for Fe$^{2+}$ which shows a corresponding increase in concentration up to a maximum of $94$ $\mu$M. This spike in dissolved Fe is then followed by an equally abrupt decrease to non-detectable values for the duration of the lactate experiment.

Overall it is apparent from Figure 4.5 that the conditions most suitable for Fe reduction were those found in the region overlapping the $\mu$-aerobic and suboxic regimes. Had the reactors not been placed in the anaerobic chamber, Fe reduction would have probably continued until all the reactive iron oxides were depleted.
Figure 4.5 Fe speciation by ferrozine and voltammetry as a function of time in days. All points represent the average for the three reactors. Ferrous Fe is indicated by the solid squares (ferrozine) and solid triangles (voltammetry). Total Fe (ferrozine) is indicated by the open circles. Soluble Fe$^{3+}$ as determined by voltammetry is indicated by the open stars. Error bars indicate the average and standard deviation for the three reactors. The inset provided shows the time period defined by the box. The three dominant redox regimes are shown at the top of each Figure.
4.5 The Role of Soluble-Organic Fe$^{3+}$ on Iron and Sulfur Cycling

Degassed artificial sea water was injected into the reactors until day 290 to let the sediment equilibrate after removing lactate from the input solution. At day 290, a solution containing ferric citrate (500 µM) in artificial sea water was introduced into the reactors. Ferric citrate was chosen as a proxy for the soluble-organic Fe$^{3+}$ complexes detected in the porewaters by voltammetry because it contains an organic moiety that is not metabolized by metal or sulfate reducing bacteria.

The voltammetric measurements for the input ferric citrate solution provide Fe$^{3+}$ and soluble-organic Fe$^{3+}$ signals as seen in Figure 4.6. The maximum integrated current for Fe$^{3+}$ and Fe$^{5+}$ obtained from the three scans is approximately 189 nA and 16 nA, respectively. Using the Pilot Ion Method, the current obtained for the Fe$^{3+}$ peak corresponds to a concentration of 139 µM. The Fe$^{5+}$ concentration determined using voltammetry consistently generates values that are not in agreement with the ferrozine measurements and generally not reproducible ($167.7 \pm 56.4$ µM, n = 6). The reason for this discrepancy is not known but may arise from the enhancement of the Fe$^{5+}$ voltammetric signal by the organic ligand potentially leading to erroneous concentrations.
Figure 4.6 Cathodic (CSWV) square wave voltammogram of 500 μM ferric citrate showing the soluble-organic Fe$^{3+}$ and Fe$^{2+}$ peaks at -0.45 V and -1.4 V, respectively. The initial concentration of Fe$^{3+}$ measured in the ferric citrate is 138 μM but is probably overestimated by voltammetry.

Over the course of the ferric citrate injection, the soluble Fe$^{3+}$ was reduced and removed from solution as FeS as no Fe$^{2+}$, soluble Fe$^{3+}$, or FeS$_{aq}$ were detected in the output porewaters (Figure 4.5). These findings suggest that, in contrast to the injection of lactate, which resulted in a spike in Fe$^{2+}$ prior to the onset of sulfate reduction and FeS precipitation as described earlier, the introduction of the ferric citrate solution must have resulted in the very fast reduction of Fe$^{3+}$ to Fe$^{2+}$ and subsequent precipitation of FeS.

This hypothesis was tested in a separate experiment to determine the kinetics of ferric citrate reduction by H$_2$S. In this experiment, a degassed 500 μM ferric citrate
solution was spiked with 20 μM of H₂S. The experiment, which was repeated several times, always yielded the same results shown in Figure 4.7. It was found that the reduction of ferric citrate by H₂S is instantaneous and results in the simultaneous formation of FeS₄(q). The FeS₄(q) signal decreases after 10 seconds, probably due to the rapid precipitation of FeS₄, consistent with the increasingly black color of the solution. This experiment confirms that the reduction of ferric citrate in the presence of dissolved sulfide is extremely fast and suggests that all the ferric citrate in the reactors was removed as solid FeS.
Figure 4.7 Reduction of 500 μM ferric citrate by 20 μM H₂S as a function of time. (A) Evolution of cathodic square wave voltammograms as a function of time. The peaks have been labeled with the appropriate species (Table 3.1). The times at which the voltammograms were collected (t = 0 to 50 seconds) have also been labeled. (B) Time evolution of Fe³⁺ and H₂S quantified by the pilot ion method, and FeS(eq) and soluble-organic Fe³⁺ reported in current (nA). The sulfide and soluble-organic Fe³⁺ peaks were deconvoluted with PeakFit (Jandel Scientific, Inc.).
4.6 Sulfate Reduction

Figure 4.8 shows the average concentration of SO₄²⁻ and H₂S over the course of the experiment. Taken with the Fe speciation data presented in Figure 4.5, these data help to understand the fate of iron and sulfur within the reactors. Most notable is the response in SO₄²⁻ reduction with changing conditions, primarily with respect to the introduction of the lactate solution on day 120 and the ferric citrate solution on day 290, both of which resulted in the rapid and almost instantaneous reduction of SO₄²⁻ and subsequent production of H₂S.

With the addition of the lactate solution (pH ~ 8.3), SO₄²⁻ reduction resulted in an average maximum H₂S concentration of 1.3(±0.6) mM, however, led to an estimated loss of sulfate corresponding to approximately 28 mM. Although the input of the lactate was discontinued after 54 days (day 174), sulfate reduction, as measured by the ∑H₂S concentration, ensued and did not completely subside until day 275 (Figure 4.8), when it decreased to non-detectable values until the input of the ferric citrate solution on day 290. The input of ferric citrate provided some intriguing results, most notably, an increase in dissolved sulfide at the output of the reactors, up to a maximum average H₂S concentration of 334(±44) μM on day 340. The H₂S concentration remained constant until addition of the ferric citrate solution was stopped on day 379 and replaced with a solution of sodium citrate (500 μM). The sulfide concentration then rapidly decreased to a concentration of 154(±47) μM by day 388 and to a final H₂S concentration of 98(±57) μM on day 395.
Figure 4.8 (A) $\text{SO}_4^{2-}$ and (B) $\text{H}_2\text{S}$ concentrations (mM) as a function of time in days. Represented is the average and standard deviation for the triplicate reactors. The three dominant redox regimes are shown at the top of the Figure. Figure B also includes the days corresponding to the injection of lactate, ferric citrate, and sodium citrate into the reactors (black arrows).
4.7 Ascorbate Fe, Dithionite Fe, AVS (FeS) and Cr Reducible Sulfide (CRS, Pyrite) in the Solid Phase

Ascorbate Fe, dithiosite Fe, AVS (FeS) and Cr reducible sulfide (FeS₂) concentrations were determined for the bulk sediment (day 0) and again on days 246 and 379 for the three triplicate reactors (Figure 4.9). Dithionite Fe was not obtained for the three reactors on day 246. It is important to note the break in the y-axis necessitated by the large difference in initial and final concentrations for all measurements.

The initial bulk sediment is characterized by high ascorbate Fe with an average concentration of 131(±19) μmol Fe/g and even higher dithionite Fe with an average concentration of 194(±17) μmol Fe/g. The FeS₂ and AVS are significantly lower in the bulk sediment with average concentrations of 37(±10) μmol S/g and 1.2(±0.4) μmol S/g, respectively. Crystalline Fe, composed of hematite, goethite, magnetite and to a lesser extent, chlorite (Koutka and Luther, 1995), is defined as the difference between dithionite Fe and the sum of ascorbate Fe and AVS. The calculated crystalline Fe fraction in the bulk sediment is approximately 62 μmol Fe/g and indicates that, overall, the crystalline Fe oxide content in the reactors was much less than the concentration of amorphous Fe oxides.

On day 246, the ascorbate Fe oxides were drastically lower in the three reactors with an average concentration of 0.33(±0.09) μmol Fe/g. Relative to the decrease in ascorbate Fe oxidet, the AVS showed a corresponding increase in average concentration in all three reactors to 256(±191) μmol S/g. Interestingly, on day 246, the AVS yielded average concentrations greater than the initial concentration of amorphous Fe oxides in the bulk sediment for two out of the three reactors, 131 μmol/g versus 448 μmol/g and
253 \( \mu \text{mol/g} \), suggesting that reduction of the less reactive, more crystalline iron oxides provided some of the reduced iron in the solid phase. The average pyrite concentration for the three reactors increased slightly to an average concentration of 54(±6) \( \mu \text{mol S/g} \).

On day 379, ascorbate Fe, although still low, increased slightly in all three reactors to reach a final average concentration of 5.2(±1.7) \( \mu \text{mol Fe/g} \). Dithionite Fe showed a marked decrease over the course of the year reaching a final average concentration of 17(±5.6) \( \mu \text{mol Fe/g} \). From these data, crystalline Fe appears to have been totally depleted by the end of the experiment indicating that the crystalline Fe oxides were totally consumed over the course of the experiment. On day 379, the AVS values showed an unexpected decrease from day 246 to reach a final average concentration of 18(±9.5) \( \mu \text{mol S/g} \). However, the decrease in AVS may be attributed, to some degree, to an increase in pyrite between day 246 and day 379 to a final average concentration for the three reactors of 143(±54) \( \mu \text{mol S/g} \).
Figure 4.9 Average ascorbate Fe (solid), dithionite Fe (lined), AVS (dashed) and reducible sulfide (CRS, pyrite) (hatched) extractions as a function of time. Ascorbate Fe and dithionite Fe are reported as μmoles Fe per gram of dry sediment, AVS and pyrite are reported as μmoles S per gram of dry sediment. The days marked on the Figure are the days when the sediment was collected from the reactors for analysis. The error bars denote the average and standard deviation for the triplicate reactors except in the case of the bulk sediment (day 0) which represents the average and standard deviation for triplicate measurements. Note the break in the y-axis. Dithionite Fe was not determined for January, 2003.
CHAPTER 5

DISCUSSION

Plug-flow reactors (PFR) are a useful tool for simulating and understanding chemical reactions occurring in sediments. PFR applications have extended to many different disciplines including hydrology, fluid mechanics, chemical engineering and environmental engineering (Brusheert and Arnost, 2003; Roychoudhury et al., 2003; Roychoudhury et al., 1998). PFR are advantageous because they are relatively easy to use and offer information on the many reactions occurring within a given system. In addition, PFR are one of the best tools available to study complex microbial assemblages present in sediments. As a result, the ability to study and model biogeochemical reactions occurring in PFR provides valuable information that may be applied to reactions occurring in sediments and further broadens the scope of our understanding of these complex and ever changing systems.

The primary goal set of this study is to investigate biogeochemical reactions involving iron and sulfur in the first few centimeters of salt marsh sediments in order to determine the fate of both constituents under given conditions, particularly as they relate to the precipitation of FeS and FeS2.  

5.1 Transport in PFR

Transport within the plug-flow reactors was controlled in order to avoid channelization, which could largely influence the biogeochemical reactions occurring within sediments (Roychoudhury et al., 1998). We used Br− as a tracer and a
mathematical model to calculate transport parameters. Breakthrough curves of Br\(^{-}\) (Figure 4.2) were used in a one-dimensional reactive transport model to determine the dispersion coefficient (D) and advection rate within each reactor (Roychoudhury et al., 1998). The following conservation equation was used to model one-dimensional reactive transport within the reactors:

\[
R \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - u \frac{\partial C}{\partial x}
\]  

(13)

where the first term on the right side of the equation refers to the dispersion term and the second refers to the advection term.

An analytical solution to this differential equation exists (Van Genuchten, 1981) and was used with an optimization procedure to calculate the retardation factor (R), the dispersion coefficient (D), and the advection rate (u). The optimization procedure, written using Matlab\(^{TM}\), minimizes in a least squares fashion, the difference between the data and the analytical solution of the differential equation to determine the three unknown parameters. The boundary conditions consistent with a plug flow reactor design are as follows (Roychoudhury et al., 1998):

\[
C(x,0) = 0 \\
C = Co(t) \\
\frac{\partial C}{\partial x}(L,t) = 0
\]  

(14)

where x is the spatial variable in cm, t is time in seconds, and Co is the input concentration of the bromide in the ASW.

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The model was applied at two different occasions with the bromide data (Figure 4.2) to determine if the hydraulic parameters had changed during the experiment. The modeled average bromide concentrations fit the data well (Figure 5.1), and the model provides similar retardation factors and dispersion coefficients for both breakthrough curves.

![Graph showing bromide concentration versus days](image)

**Figure 5.1** Model of Br⁻ breakthrough in the PFR using an analytical solution to a one-dimensional reactive transport equation (Eq. 13) where R is the retardation factor, D is the dispersion coefficient (cm²/sec) and u is the advection rate (cm/sec).

According to the model, and not surprisingly, retardation is minimum and diffusion dispersive. In turn, the advection coefficient calculated after day 136 is about ten times lower than in the first breakthrough curve. These findings suggest that the
sediment became much more compact over time and are consistent with the changes in sediment morphology due to FeS precipitation.

5.2 Metal Reduction

The first 5 days of the experiment are marked by the production of high concentrations of Mn$^{2+}$ and Fe$^{2+}$. Therefore, it appears that both Mn and Fe oxide reduction were occurring simultaneously within the sediment prior to starting the experiment. Recently, it has been shown that microbial Fe and Mn reduction occur in the suboxic zone of salt marsh sediments (Kostka & Luther, 1995; Kostka et al., 2002). Bacteria use Mn and Fe oxides as terminal electron acceptors in order to grow under anaerobic conditions (Table 1.1), and thus the detection of dissolved Mn as one of the first species to elute from the plug-flow reactors is not unexpected. However, microbial reduction of Mn and Fe oxides is only one mechanism by which Mn and Fe oxides are reduced. Chemical reduction of Mn oxides by ferrous Fe (Postma, 1985) and H$_2$S (Burdige and Myers, 1988), for instance, may result in the rapid and efficient production of reduced Mn in sediments. Myers and Nealson (1988) found that when a Mn reducing strain of bacteria was given ferric Fe and thiosulfate, the rate of Mn oxide reduction increased significantly. In the case of thiosulfate, the bacterial strain was found to disproportionate thiosulfate to H$_2$S and SO$_4^-$, whereby the H$_2$S rapidly reduced the MnO$_2$ to Mn$^{2+}$ as in the following reactions:

\[ \begin{align*}
3\text{S}_2\text{O}_3^{2-} + 2\text{H}^+ & \rightarrow \text{H}_2\text{S} + 2\text{SO}_4^- \\
\text{MnO}_2 + \text{HS}^- + 3\text{H}^+ & \rightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O} + \text{S}^0
\end{align*} \]  (15)\hspace{1cm} (16)
The PFR sediments were oxidized in the first few days of the experiment and since no H₂S nor solid phase reduced sulfides were detected in the bulk sediment, the possibility of Mn reduction by H₂S has been ruled out during that time.

In the same study, Myers and Nealson fed the same strain of bacteria ferric Fe and found that the rate of Mn oxide reduction increased by 25 to 50%. Myers and Nealson (1988) proposed that Fe³⁺ is reduced microbially to Fe²⁺ which, in turn, effectively reduces MnO₂ as is the following reactions:

\[
2\text{Fe}^{3+} + \text{MnO}_2 + 4\text{H}^+ \rightarrow 2\text{Fe}^{2+} + \text{Mn}^{2+} + 2\text{H}_2\text{O} \quad (17)
\]

\[
2\text{Fe}^{3+} + \text{MnO}_2 + 4\text{H}_2\text{O} \rightarrow 2\text{Fe(OH)}_3\text{O}_3 + \text{Mn}^{2+} + 2\text{H}^+ \quad (18)
\]

In this scenario, no ferrous Fe is produced until all the Mn oxides are depleted.

Postma (1985) found that the reaction between Fe²⁺ and Mn oxides is much slower above pH 4 and that the rate increases with increasing Fe²⁺ concentration. Therefore, at the pH measured in the PFR (Figure 4.3), the rate of reduction of Mn oxides by Fe²⁺ should be slow and microbial reduction of Mn and Fe oxides should prevail. Consequently, the immediate spike in the two ions is viewed as a flushing of reduced Mn and Fe that had been collecting in the sediment over time.
Table 5.1 Reaction rate data for Mn, Fe²⁺ (ferrozine and voltammetry), SO₄²⁻, and H₂S over the course of the experiment. The reaction rates represent the concentration change as a function of time and its standard deviation for each set of measurements. Days were separated based on changing conditions within the plug-flow reactors. Positive values indicate production of the species whereas negative values denote consumption of the species. A dash indicates that the species was present but a reaction rate could not be obtained due to insufficient data or poor resolution of the data.

<table>
<thead>
<tr>
<th>Days</th>
<th>GFAAS Mn (µM/day)</th>
<th>Ferrozine Fe²⁺ (µM/day)</th>
<th>Voltammetry Fe³⁺ (µM/day)</th>
<th>IC SO₄²⁻ (µM/day)</th>
<th>Voltammetry H₂S (µM/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 - 40</td>
<td>-1.69 (+/- 0.65)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40 - 63</td>
<td>0</td>
<td>10.74 (+/- 2.73)</td>
<td>10.63 (+/- 0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>63 - 120</td>
<td>0</td>
<td>-4.63 (+/- 0.47)</td>
<td>-4.84 (+/- 0.79)</td>
<td>-86.54 (+/- 19.73)</td>
<td>0</td>
</tr>
<tr>
<td>121 - 125</td>
<td>0</td>
<td>13.54 (+/- 0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 - 174</td>
<td>0</td>
<td>-14.34 (+/- 7.39)</td>
<td>-</td>
<td>-887.74 (+/- 195.35)</td>
<td>19.74 (+/- 3.89)</td>
</tr>
<tr>
<td>174 - 290</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>465.3 (+/- 78.96)</td>
<td>-58.94 (+/- 0)</td>
</tr>
<tr>
<td>290 - 366</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>11.18 (+/- 2.15)</td>
</tr>
<tr>
<td>366 - 396</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-4.41 (+/- 1.09)</td>
</tr>
</tbody>
</table>
Table 5.1 tabulates the production and consumption rates of total dissolved Mn, Fe\(^{2+}\) by ferrozine and voltammetry, SO\(_4^{2-}\), and H\(_2\)S in the PFR (Figures 4.4, 4.5 and 4.8). The rates were calculated as the slope and standard deviation for each set of measurements within a particular interval of time. The positive sign denotes production whereas the negative sign denotes consumption of a particular species. All reaction rates were obtained beginning after day 5, the calculated residence time of the reactors. The rates of Fe\(^{2+}\) production and consumption measured by voltammetry and ferrozine are comparable between days 40 and 120 (Table 5.1). This sediment is characterized by Fe reduction in the suboxic regime but, surprisingly, little Mn reduction. From the data, it is not clear whether the absence of Mn\(^{2+}\) after 40 days is due to a decrease in Mn reduction or removal of Mn\(^{2+}\) by adsorption (Van Cappellen and Wang, 1996).

The bulk of the Fe in the effluent sea water was in the form of Fe\(^{3+}\), suggesting that microbial reduction of Fe oxides within the sediment is the main process of Fe reduction. The fact that no H\(_2\)S was measured by voltammetry in the effluent sea water and that SO\(_4^{2-}\) remained constant between 40 and 63 days (Figure 4.8) provides evidence in support of microbial Fe reduction during this time period. Also, pH increases concomitantly with Fe\(^{3+}\) in the reactors during that time period (compare Figures 4.3 and 4.5) providing another piece of evidence supporting microbial Fe reduction.

5.3 Sulfate Reduction and Precipitation of FeS

Between days 67 and 121, the average sulfate reduction rate for the three reactors was on the order of 85 \(\mu\text{M/day}\). In turn, the decrease in ferrous Fe concentration within the same time interval was on the order of 5 \(\mu\text{M/day}\) (Table 5.1). The advent of sulfate reduction is not corroborated by detection of H\(_2\)S, however, the concomitant decrease in
Fe at the advent of anoxic conditions (Figure 4.5) provides indirect evidence for FeS formation. Since the rate of SO$_4^{2-}$ reduction is much higher than the rate of Fe reduction (Table 5.1), and H$_2$S is never detected between days 67 and 121, the H$_2$S unaccounted for must have reduced the amorphous Fe oxides and precipitated as FeS$_{0}$.

The rate of sulfate reduction is equivalent to the rate of FeS precipitation which, in turn, is equivalent to the total loss of iron in the porewaters and the solid phase. From Table 5.1, we calculate a rate of amorphous Fe oxide reduction by sulfide of about 90 mM Fe/day, which corresponds to about 20% of total amorphous iron oxide reduction in the reactors up to day 246 (Table 5.2).

Poulton et al. (2002), suggest that the initial step in reductive dissolution of Fe oxides by HS$^-$ at a pH of 8.5 occurs via formation of a complex at the Fe oxide surface:

\[ \text{FeOH} + \text{HS}^- \rightarrow \text{Fe}^{III}S^+ + \text{H}_2\text{O} \]  

where the arrow ($\rightarrow$) denotes the oxide surface. An electron is then transferred between the sulfide and Fe$^{3+}$ forming a $S^-$ free radical which is then released during the dissolution of ferrous Fe:

\[ \text{Fe}^{III}S^- \rightarrow \text{Fe}^{II}S^- \]  

<table>
<thead>
<tr>
<th>Days</th>
<th>Ascorbate Fe $\mu $M/day</th>
<th>AVS (FeS) $\mu $M/day</th>
<th>Pyrite (FeS$_2$) $\mu $M/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 246</td>
<td>- 411.0</td>
<td>677.8</td>
<td>27.6</td>
</tr>
<tr>
<td>246 - 379</td>
<td>24.1</td>
<td>-1152.8</td>
<td>258.8</td>
</tr>
</tbody>
</table>
\[ > \text{Fe}^{3+} \text{S}^{-} + \text{H}_{2}\text{O} \leftrightarrow > \text{Fe}^{3+} \text{OH}^{-} + \text{S}^{-} \quad (21) \]

\[ > \text{Fe}^{3+} \text{OH}^{-} + \text{H}^{+} \rightarrow \text{new surface site} + \text{Fe}^{2+} \quad (22) \]

Overall, each S\(^{-}\) free radical may reduce one ferric Fe yielding elemental sulfur and ferrous Fe which reacts with sulfide to form FeS:

\[ 8 \rightarrow > \text{Fe}^{3+} \text{OH}^{-} + 8\text{HS}^{-} \rightarrow \text{S}^{0} + 8\text{Fe}^{2+} \quad (23) \]

\[ \text{Fe}^{2+} \text{(aq)} + \text{HS}^{-} \leftrightarrow \text{FeS}_{\text{aq}} + \text{H}^{+} \quad (24) \]

Lactate/acetate mixtures have been used previously in the cultivation of sulfate reducing bacteria (SRB) by biologists (Koretsky et al., 2002; Lowe et al., 2000; Søs et al., 2002) as shown by the following reaction (Koretsky et al., 2002):

\[ 13\text{SO}_{4}^{2-} \text{(aq)} + 8\text{CH}_{3}\text{CHOHCOO}^{-} \text{(aq)} + 10\text{H}^{+} \rightarrow 13\text{H}_{2}\text{S} \text{(aq)} + 24\text{HCO}_{3}^{-} \text{(aq)} + 4\text{H}_{2}\text{O} \quad (25) \]

Søs et al. (2002) used 20 mM lactate and 10 mM Na\(_2\)SO\(_4\) to cultivate 6 different strains of SRB under anaerobic conditions at a pH of 7.2. Lowe et al. (2000), used 15 mM lactate as a supplement in their SRB as well as in their Fe reducing bacteria (FeRB) liquid growth media. In the PFR, the initial response to the lactate/acetate mixture was a sharp increase in dissolved Fe (Inset – Figure 4.5 and Table 5.1) at a rate of about 15 µM Fe/day between days 121 and 125, followed by an equally sharp removal of Fe\(^{2+}\). The production of dissolved Fe between days 121 and 125 could be due to microbial Fe reduction. This mechanism, presented in equations 19 to 24, could explain the rapid spike in ferrous Fe detected between days 121 and 125. Another factor in supporting this mechanism is that the effluent sea water during this time produced a bright yellow precipitate with the most prominent color occurring in conjunction with the input of the lactate and steadily lessened over the course of the 54 days of lactate injection. The yellow color is attributed to the formation of elemental sulfur (S\(^{0}\)), a product during the
reduction of Fe oxides by sulfide (reaction 9). With the termination of the lactate, the precipitate was no longer visible and the solution turned colorless, remaining that way for the duration of the experiment. The formation of Fe$^{2+}$ and subsequent precipitation of FeS through reduction of Fe oxides by HS$^{-}$ may also occur through the production of polysulfides (S$_{4}^{2-}$ and S$_{6}^{2-}$), thiosulfate (S$_{2}$O$_{3}^{2-}$) and SO$_{4}^{2-}$ (Peiffer et al., 1992). However, thiosulfate was never detected by voltammetry and background sulfate concentrations are too high to observe its anesthetic formation through this process. Although an attempt was made to measure elemental sulfur and polysulfides electrochemically, they were never detected in the sea water during this time.

After 125 days, it is clear that SRB dominate microbial populations in this sediment resulting in the total depletion of Fe from the effluent sea water due to the rapid precipitation of FeS. The input SO$_{4}^{2-}$ decreased to non-detectable values resulting in a maximum loss of approximately 29 mM SO$_{4}^{2-}$ while the H$_{2}$S in solution reached a maximum concentration of approximately 1.1 mM. The difference in SO$_{4}^{2-}$ input and output coupled with the output of H$_{2}$S suggests the remaining 27 or 28 mM of SO$_{4}^{2-}$ input to the system was reduced and precipitated as FeS. The measured AVS on day 246 from the solid phase AVS extraction yielded 215(±136) µmol S/g corresponding to 168(±106) mM FeS when considering an average sediment density of 0.78 g/cm$^{3}$ (Gribsholt, 2003). In order to determine when FeS was precipitated during the incubations, we determined the time evolution of FeS precipitation by taking the difference between the initial input sulfate concentration and resultant H$_{2}$S up to day 250 (Figure 5.2).
Figure 5.2 FeS precipitation calculated from the difference between the input $\text{SO}_4^{2-}$ and the resultant $\text{H}_2\text{S}$ as a function of time. Error bars denote the average and standard deviation for the three reactors. The circle shows the predicted precipitation of FeS coinciding with the decrease in Fe shown in Figure 4.5.
Figure 5.2 shows the average predicted FeS precipitation assuming that all of the 
H₂S produced formed FeS and not FeS₂. This is a valid assumption since the average 
countentration of FeS₂ on day 0 was 37(±10) μmol S/g sediment and on day 246 was 
54(±6) μmol S/g sediment. As a result, very little if any FeS₂ precipitated during this 
interval of time. All FeS₂ concentrations measured fall in the range of pyrite 
concentrations found at the surface of salt marsh sediments (Table 5.3).

<table>
<thead>
<tr>
<th>FeS₂</th>
<th>Sampling Site</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 469</td>
<td>Great Marsh, DE</td>
<td>Lord and Church (1983)</td>
</tr>
<tr>
<td>30 - 230</td>
<td>Great Marsh, DE</td>
<td>Luther and Church (1988)</td>
</tr>
<tr>
<td>14 - 382</td>
<td>Great Marsh, DE</td>
<td>Ferdelman et al. (1991)</td>
</tr>
<tr>
<td>20 - 367</td>
<td>Great Marsh, DE</td>
<td>Luther et al. (1992)</td>
</tr>
<tr>
<td>13 - 141</td>
<td>Great Marsh, DE</td>
<td>King et al. (1985)</td>
</tr>
<tr>
<td>37(±10) – 143(±54)</td>
<td>Skidaway Island, GA</td>
<td>This Study</td>
</tr>
</tbody>
</table>

The pyrite concentrations measured in the plug-flow reactors fall on the low end 
of the ranges found in Great Marsh, DE (Table 5.3). This is probably due to the fact that 
the uppermost portion of salt marsh sediment is oxidized and thus would have low pyrite 
concentrations relative to the deeper, more reduced sediments.

The summed average concentration of FeS determined from Figure 5.2 and the 
solid phase extraction were in close agreement, 136 mM and 168 mM, respectively. 
Based on these data, ca. 91% of the FeS precipitated in response to the injection of the
lactate. Given that FeS precipitation prior to injecting the lactate (highlighted region in Figure 5.2) was minimal, the data suggest that sulfate reduction was insignificant and Fe reduction between days 44 and 63 was bacteriologically driven only. These data are supported by the good correlation between the rate of sulfate reduction determined in Table 5.1 between days 125 and 174 (888±200 μM S/day) and the rate of formation of FeS measured and reported in Table 5.2 (678 μM S/day).

5.4 Microbial Iron Reduction

Had sulfate reduction not been stimulated, Fe reduction would have probably dominated microbial processes in this sediment. The importance of Fe reduction in consistent with the findings of Lowe et al. (2000), Kostka et al. (2002), and Koretsky et al. (2003), who demonstrated the significance of microbial Fe reduction in salt marsh sediments. Lowe et al. (2002) found that Fe reducing bacteria (FeRB) could out-compete sulfate reducing bacteria (SRB) in shallow salt marsh sediments when not limited by the availability of refractory Fe oxides. Similarly, Kostka et al. (2002), in a comparison of salt marsh environments, found biological Fe reduction to account for 100% of organic carbon oxidation in vegetated, bioturbated sediments with a rate of 346.3 mmol/m²/day while sulfate reduction dominated (> 70%) in non-bioturbated, unvegetated sediments. Koretsky et al. (2002) noted seasonal variations in FeRB and SRB in salt marsh sediments and found an order of magnitude decline in FeRB at three separate sampling sites during the summer when sulfate reducers are known to be dominant. Koretsky et al. (2002) also saw the stimulation of FeRB with the additions of amorphous Fe oxides and lactate when sulfate reduction was inhibited by molybdate. Consequently Fe reduction
has been found to be largely inhibited by sulfide produced during sulfate reduction and not necessarily by changes in available Fe oxides or temperature (Koretsky et al., 2003).

By normalizing the Fe$^{2+}$ production rate measured (Table 5.2) to the surface area and volume of the reactors, we calculated a rate of microbial Fe reduction in these sediments to be ca. 0.95 mmol Fe/m$^2$day. This rate is much lower than that determined by Kostka et al. (2002) but it is important to recognize that these authors spiked their samples with amorphous Fe oxides to determine potential microbial Fe reduction rates. Our experiment is more realistic in that it provides actual rates with authigenic minerals in unperturbed sediments. Considering that four iron are required to fully remineralize one organic carbon, this rate suggests that iron oxidizes NOM at a rate of $\sim$0.24 mmol C/m$^2$day. These rates are much smaller than those estimated in salt marsh sediments (22 – 116 mmol C/m$^2$day; Kostka et al., 2002), continental shelf sediments (2.5 – 11 mmol C/m$^2$day; Jahnske and Jahnske, 2000), and even arctic sand sediments (6 – 12 mmol C/m$^2$day; Glat et al., 2000). The experiments presented here were conducted in anaerobic conditions and, therefore, the rate of microbial reduction is limited by the availability of amorphous Fe oxides. Evidence for oxygen penetration in intertidal salt marsh sediments has been found previously (Neuhuber, 2003). This suggests that microbial Fe reduction could play a much more important role than previously thought in coastal marine sediments. Considering that Fe$^{2+}$ oxidation by dissolved oxygen is extremely fast, the end result could be the rapid recycling of Fe for more organic carbon oxidation in real sediments.

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5.5 Precipitation of FeS₂

As mentioned earlier, ferric citrate was introduced into the reactors to determine the role of soluble-organic Fe³⁺ on the iron and sulfur cycling within the PFR (Figure 1.2). Pyrite precipitation was not significant during the first 246 days of the experiment (Figure 4.9). In contrast, pyrite formation tripled during the second part of the experiment when ferric citrate was continuously injected into the reactors. Interestingly, no dissolved Fe was ever detected in the effluent sea water, even with the injection of a total of 3 mmole Fe during this period of time. Since dissolved sulfide concentration was still high during this time interval, the chemical reduction of ferric citrate must have resulted in the precipitation of FeS₂, pyrite or some other mineral phase such as siderite (FeCO₃). Simultaneously, however, a pronounced decrease in the concentration of AVS occurred over this duration. It was suggested previously that the rate of formation of FeS and eventually FeS₂ should increase in the presence of a soluble form of Fe³⁺ if dissolved sulfide is in excess (Yao and MiZero, 1996). These findings suggest that the mechanism of pyrite formation proposed by Rickard and Luther (1997) (reaction 12) may be the main mechanism of pyrite formation. In order to substantiate our findings, the rate of formation of FeS₂ between days 246 and 395 was calculated based on reaction 9 and corresponding rate equation (Eq. 26) after Rickard and Luther (1997):

\[
d\text{FeS}_2/dt = k(\text{FeS})(\text{CH}_{3}\text{S}_2\text{O})
\]

(26)

where \(d\text{FeS}_2/dt\) is the rate of pyrite formation, FeS is the maximum average concentration for the three reactors measured at day 246 and calculated in Figure 5.2, and \(k (1.03 \times 10^4 \text{ mol}^3\text{s}^{-1} \text{ at } 25^\circ\text{C})\) is the rate constant taken from Rickard and Luther (1997). The concentration of hydrogen sulfide was calculated from the maximum total concentration

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of dissolved sulfide measured between days 246 and 395 and equations 2 and 3 with acid base constants and activity coefficients from the literature.

\[ (CH_2SH_3) = S_2/\alpha \]  \hspace{1cm} (27)

\[ \alpha = [1 + (K_{as}/\gamma_{H_2S} / \gamma_{HS})^1/10^{pH}] \]  \hspace{1cm} (28)

where \( K_{as} \) is \( 10^{-5.98} \) and \( \gamma_{HS} \) is 0.667 and \( \gamma_{H_2S} \) is 1.63 (Millero, 1986). The pH used for this calculation was the average pH measured between days 246 and 395 (Figure 4.3).

Table 5.4 tabulates the rate of precipitation of FeS\(_2\) in \( \mu \text{M/day} \) using the maximum FeS concentration measured in the solid phase and the rate constant provided by Rickard (1997) at 25°C.

<table>
<thead>
<tr>
<th>FeS [mM]</th>
<th>H(_2)S [mM]</th>
<th>pH</th>
<th>dFeS(_2)/dt [( \mu \text{M/day} )]</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>1.34 ± 0.6</td>
<td>8.47 ± 0.07</td>
<td>9.7 ± 1.4</td>
</tr>
<tr>
<td>168</td>
<td>0.17 ± 0.09</td>
<td>8.47 ± 0.07</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Rickard</td>
<td>(1997)</td>
<td>9 \times 10^{-7} – 0.09</td>
<td></td>
</tr>
</tbody>
</table>

Rickard (1997) found that the rate of pyrite formation is strongly dependent on FeS but inversely correlated to pH so that as pH increases, the rate decreases. The rates calculated in this study (Table 5.4) are significantly faster than those made available by Rickard (1997) in typical sulfidic sediments at ambient temperatures. Although there are other ways in which to form pyrite, namely through the reaction of FeS with either S\(_{60}\) or S\(^2-\) (Berner, 1984; Luther, 1991; Rickard, 1975; Rickard et al., 1995; Schoonen and
Barnes, 1991). Rickard (1997) found reaction 12 to be the fastest at 25°C. Our results therefore suggest that ferric citrate accelerates the formation of pyrite to levels well beyond those observed in other systems.

5.6 The Role of Soluble-organic Fe³⁺ in FeS/FeS₂ Precipitation

The significance of solid ferric Fe in salt marsh sediments has been well established due to the numerous interactions it can have with other redox species present. Solid ferric Fe may be reduced by iron reducing bacteria, organic reductants, ferrous Fe and sulfide or non-reductively dissolved by organic chelators in the absence of bacteria (Taillefert et al., 2000). Recently, the discovery of organic-Fe³⁺(ox) complexes as an intermediate in the reduction of Fe oxides has led to questions regarding the reactivity of soluble Fe³⁺ versus its solid counterpart. Haas and DiChristina (2002) found that microbial Fe reduction rates vary as a function of the ligand complexing the Fe³⁺. They showed that Shewanella putrefaciens, a strain of Fe reducing bacteria, was able to reduce Fe³⁺ complexed to citrate more rapidly than five other well known ligands at an average rate of 1.23 mM/hour (Haas & DiChristina, 2002). Similarly, Dollhopf et al. (2000) found that the chemical form of an electron acceptor greatly influenced the rate of Fe³⁺ reduction. They too found Shewanella putrefaciens capable of reducing Fe citrate faster than Fe(OH)₂ and α-FeOOH (Dollhopf et al., 2000). However, reduction of metal oxides may be either microbiially or chemically driven. Taillefert et al. (2000) investigated the reactivity of soluble Fe³⁺ complexed to Tris and, similar to ferric citrate, demonstrated the instantaneous reaction between Fe³⁺:Tris with sulfide to form Fe²⁺ and FeS. As a consequence, the occurrence of organically complexed Fe³⁺ should greatly enhance the rate of formation of FeS and potentially FeS₂ over that of solid Fe³⁺, thus accelerating the
rate of FeS and pyrite burial in sediments. Indeed, the H₂S mediated reduction of ferric citrate was found to occur on the order of micromoles per second (Figure 4.7). Based on these data, the reduction of ferric citrate by H₂S and the subsequent precipitation of FeS should occur much faster than microbial reduction of ferric citrate. However, FeS data collected on day 395 show that FeS decreases in the second part of the experiment (i.e., from day 246 to 395), and thus indicate that FeS is simultaneously transformed into pyrite according to the schematic proposed in Figure 1.2. Altogether, the data collected in this study indicate that ferric citrate catalyzed the formation of pyrite instead of oxidizing FeS₂. This process appears to be self sustained as long as soluble ferric citrate is supplied to the sediments because H₂S is produced in response to the addition of ferric citrate.

Two mechanisms can supply sulfate reducing bacteria with electron donors to produce dissolved sulfide. First, ferric citrate could be fermented and eventually produce H₂ for SRB. Second, molecular hydrogen could be produced in sufficient quantities by the pyritization process to sustain sulfate reduction. It is well known from the microbiology literature that SRB oxidize molecular hydrogen to reduce sulfate (Lengeler et al., 1999). Sulfate reducing bacteria generally obtain H₂ from the outside environment or from the degradation of small organic ligands such as citrate, lactate, pyruvate and acetate. These data suggest that in marine sediments, organic Fe³⁺ complexes can promote the precipitation of FeS₂ by providing SRB with the necessary chemicals (i.e., Fe or organic ligands). It is now imperative to determine the abundance of these complexes in sediments and characterize the composition of their organic moieties to assess their importance on a global scale.
CHAPTER 6

CONCLUSIONS

Plug flow reactors (PFR) provide an excellent means for studying complex biogeochemical reaction mechanisms in sediments. They are easily maintained, can be manipulated to isolate specific reactions or biological processes. To accurately determine the biogeochemistry of sediments, it is necessary to continuously monitor the chemistry of the effluent water as well as take intermittent solid phase measurements. Flow cells, complemented with electrochemical techniques offer an innovative and practical way to obtain real-time data while at the same time, preventing risk of contamination or alteration of reduced samples that generally occurs when sampling outside a controlled atmosphere. In this study, small sediment plug-flow reactors were employed to investigate the cycling of Fe and sulfur in the first few centimeters of salt marsh sediments in an effort to better constrain the reaction mechanisms by which FeS and FeS₂ precipitate.

The data provided by the PFR over the thirteen month duration of the experiment cohesively describe the processes involved in the evolution from largely oxidized to dominantly reduced sediments. Taken together, the data confirm previous work that shows the occurrence of microbial Fe reduction in shallow salt marsh sediments. The rate of microbial Fe reduction calculated in these sediments is significantly lower (0.95 mmol Fe/m²-day) than rates determined by Kostka et al. (2002) for the same sediment,

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however these authors amended their samples with amorphous Fe oxides whereas our rates are based on naturally occurring Fe oxides within the sediment.

In the presence of lactate and acetate, sulfate reduction outcompetes microbial Fe reduction and results in the total depletion of Fe from the effluent sea water due to the rapid precipitation of FeS. The rate of sulfate reduction upon injection of lactate and acetate is equivalent to the rate of FeS precipitation which, in turn, corresponds to the total loss of iron in the porewaters and solid phase. Based on these data, it is found ca. 20% of the total amorphous Fe oxides are reduced in the suboxic regime and that ca. 91% of the FeS precipitates in response to the injection of lactate. Had sulfate reduction not been provoked, Fe reduction would have probably dominated microbial processes in this sediment.

The significance of solid Fe$^{3+}$ in salt marsh sediments has been well established due to the variety of interactions it can have with other redox species present. It has been shown that solid ferric Fe may be reduced by iron reducing bacteria, organic reductants, ferrous Fe and sulfide, or non-reductively dissolved by organic chelators in the absence of bacteria. The recent discovery of organic-Fe$^{3+}$ complexes as an intermediate in the microbial reduction of Fe oxides has led to question the role of soluble-organic Fe$^{3+}$ in the cycling of iron and sulfur in shallow salt marsh sediments. The high reactivity of soluble-organic Fe$^{3+}$ may enhance FeS and pyrite precipitation but may also oxidize pyrite because ferric iron is a better oxidant of pyrite than oxygen. To test this hypothesis, ferric citrate was injected into the reactors once sulfate reduction decreased to produce undetectable levels of dissolved sulfide in the porewaters. Interestingly, sulfate reduction is promoted with the addition of ferric citrate. Because sulfate reducing
bacteria (SRB) require H₂ as an electron donor to reduce sulfate, these data suggest that citrate could have been fermented to produce molecular hydrogen or that SRB used H₂ produced exogenically. Pyrite precipitation through reaction of FeS and H₂S is one way to produce H₂ in anoxic conditions. While the concentration of FeS should increase with the injection of ferric citrate, because the chemical reduction of soluble-organic Fe³⁺ by dissolved sulfide is extremely fast, a notable decrease in the concentration of AVS occurs simultaneously with the production of FeS₂. Consequently, the chemical reduction of ferric citrate by H₂S must have resulted in the precipitation of FeS₂ through reaction of FeS and H₂S. A pyritization rate ranging between 1.5±0.2 and 9.7±1.4 μM/day was measured in the PFR. These rates are significantly faster than those made available by Rickard (1997) in typical sulfidic sediments at ambient temperatures, suggesting that the mechanism of FeS₂ formation proposed by Rickard and Luther (1997) may be the main mechanism of FeS₂ formation in these sediments.

Overall, soluble-organic Fe³⁺ promotes FeS₂ formation within shallow salt marsh sediments. If Fe is sequestered in FeS₂, the remineralization of NOM will mainly be achieved through SO₄²⁻ reduction because SO₄²⁻ is not limiting in marine porewaters and because FeS₂ formation generates H₂. Microbial Fe reduction will prevail only if dissolved O₂ is periodically supplied to the sediment. Dissolved O₂ will both inhibit SRB and oxidize Fe very rapidly. In such conditions, the supply of freshly formed Fe oxides will promote microbial Fe reduction and the sediment will remain suboxic. The origin and quantification of soluble-organic Fe³⁺ need to be resolved to elucidate the exact role played by these complexes. Preliminary data indicates that they could be produced microbially as intermediate complexes during the reduction of Fe oxides.
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