Sulfur speciation monitored in situ with solid state gold amalgam voltammetric microelectrodes: polysulfides as a special case in sediments, microbial mats and hydrothermal vent waters†‡

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Sulfur speciation was determined in real time in salt marsh microbial mats, subtidal sediments and hydrothermal vent diffuse flow waters using solid state gold amalgam voltammetric microelectrodes. Chemical species were measured in situ without any sample manipulation or processing. The partially oxidized sulfur species detected were polysulfides, thiosulfate, elemental sulfur and tetrathionate. Fe(III) oxidation of hydrogen sulfide does not occur within the mats where microbially mediated processes are responsible for oxidation of H2S. In sediments and diffuse flow vent waters, Fe(III) phases are the direct oxidant of H2S. Sulfur speciation determined in this work is due to in situ biogeochemical processes and is not due to artefacts of sample manipulation. The voltammetric data show that polysulfides are the first detectable intermediate during sulfide oxidation which is consistent with previous laboratory studies.

Introduction

In situ measurements are necessary to understand dynamic environments where speciation can change in seconds as a result of chemical, biological and physical processes. A wide range of natural environments produce hydrogen sulfide that can then be oxidized back to sulfate by oxygen, iron(III) and manganese(III,IV) compounds.1–5 The oxidation may be abiotic or bacterially mediated and occur at reasonable rates (minutes to hours).1–3 The oxidation of sulfide frequently leads to intermediate oxidation state compounds, such as elemental sulfur, polysulfides and thiosulfate4,5 which may be used by bacteria6 or may react with metals7 and organic compounds.8,9

Using the mercury electrode and/or the gold amalgam microelectrode it is possible to measure a variety of soluble sulfur compounds and ions including hydrogen sulfide, polysulfides, thiosulfate, aqueous iron monosulfide, elemental sulfur, tetrathionate and organic thiols.10–16 Polysulfides are key intermediates in the oxidation of H2S but could not be easily measured or even identified in situ until recently.16 Previous studies have used a dropping Hg electrode to measure polysulfides discreetly.10,17 In addition, most previous measurements of sulfur species were determined after sample manipulation. Sample manipulation includes: (i) taking a water sample in Niskin or Go-Flo bottles and measuring the speciation back in the lab (either at home or onboard ship); and (ii) taking sediment cores which are then cut into sections (typically > 3 mm), centrifuged for removal, filtration and measurement of the porewater. Sample processing can mix oxidized sediments/waters and reduced sediments/waters which can react to create sulfur species of intermediate oxidation state. Although the measurements are performed well, the sample processing causes artefacts which can complicate the interpretation of what biogeochemical processes occur in the field. Thus, the exact determination of polysulfides, which appear to be the first formed or detected intermediates in sulfide oxidation,4,5 has been hampered.

In this work, we describe the electrode reactions and quantify the presence and/or absence of these species over space and time in microbial mats, sediments and hydrothermal vent fields using a solid state gold amalgam voltammetric microelectrode.18 The electrodes have submillimetre vertical resolution in mats and sediments. The initial survey work that is presented here will show in situ sulfur speciation measurements where: (1) the electrodes and a portable analyzer have been brought into the field for analysis of salt marsh microbial mats; (2) subtidal sediment cores have been collected and returned to the laboratory for electrode insertion; and (3) waters at 2500 m depth have been analyzed with a submersible in situ analyzer from a deep sea submarine (DSV Alvin). Artefacts caused by sample processing are minimized and a correct assessment of the sulfur speciation including the presence of polysulfides can be made.

Experimental

A standard three electrode cell was used in all experiments. The working electrode was a gold amalgam (Au/Hg) electrode of 100 μm diameter. The electrode was made in glass or commercially available PEEK® (polyethyl ether ketone) tubing depending on the application as per Luther et al.19 Glass was used for the bacterial mat and sediment analyses

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because 5 mm glass could be extruded to about 0.2–0.3 mm for ease of insertion into the environmental sample.

Microbial mats from Great Marsh, Delaware, during July 6, 2000, were analyzed in situ with a gold amalgam (Au/Hg) microelectrode which was inserted into the mat with a manually controlled three-axis Narishige micromanipulator. The counter (Pt) and reference (Ag/AgCl in saturated KCl) electrodes were placed in water overlaying the mat so they did not enter the sediment or sulfide zone. A battery operated DLK 100A from Analytical Instrument Systems, Inc. was used for all electrochemical analyses. The overlying water had a salinity of 32 at 29.6 °C and a pH of 8.29 so that O2 saturation is 198.9 µmol dm−3. There was no cloud cover during the experiment, which occurred between 1200 and 1400 h.

For subtidal sediment analyses, cores were obtained at an ambient temperature (15–20 °C), returned to the laboratory and placed in a water bath/incubator for insertion of the gold amalgam (Au/Hg) microelectrode. The reference electrode was a standard Ag/AgCl as above and the counter-electrode was Pt wire. The area chosen for study was northwest Rehoboth Bay of southern Delaware.

For hydrothermal vent research at 9° N East Pacific Rise during May 1999, a Delrin tube was made to hold up to four PEEK6 electrodes and two glass electrodes with a thermo-couple and Teflon tubing to suck water into syringes using the SHIP−1502 and of this tubing a lower end was inserted in the water to hold the working electrodes stationary in the tube. The working electrodes were recessed at the lower end of the wand to protect the tips of the electrodes from losing the Hg film. The lower end also had feet to allow water flow across the electrode surface. The top end of the tube was mated via four stainless steel nuts and bolts to a large diameter tube. Inserted between these lower and upper Delrin tubes was a steel handle for DSV Alvin’s arm to pick up the entire wand assembly for deployment of the simplicities. We refer to this as a sensor package for simplicity. The reference electrode was Ag/AgCl and the counter-electrode was Pt wire, both of which were mounted on the basket of DSV Alvin so that they would not enter sulfidic waters. For hydrothermal vent work, the Ag/AgCl reference was silver wire which was oxidized in seawater at ∼9 V for 10 s to form a AgCl coating; the electrode was used as a solid state electrode in the seawater medium (I = 0.7) so that no pressure effects on filling solutions would hinder electrode performance. Comparison of peak potentials for the analytes measured in situ and onboard ship were the same and were similar to those for a saturated calomel electrode (SCE). The sensor package was held over areas at the base of vent chimneys where the vent tubeworm, Riftia pachyptila, inhabits. These areas are termed diffuse flow because water temperatures are less than 25°C. There was no cloud cover during the experiment, which occurred between 1200 and 1400 h.

Results and discussion

Sulfur species

Table 1 shows many of the redox compounds and ions including some of the sulfur species that can be measured with a Au/Hg electrode. Sulfide oxidation results in formation of polysulfides, elemental sulfur, thiosulfate, sulfite, and polythionates (e.g., tetrathionate) in lab studies but the possible existence of these sulfur intermediates has only been confirmed in a few field studies. 10,13–16 Sulfite can be measured at pH values <6, which are not common in microbial mats and sediments. The sensors gave one peak except for polysulfides. At slow scan speeds, H2S, S0, and polysulfides (S22−) overlap to give one peak at about ~0.60 V and the sum of all their contributions is termed Sred. 10,13–16 However, S2− are unique because they exist in two oxidation states and it is possible to discriminate each oxidation state with fast potential scans. 17 At positive potentials, S2− can react to form a HgS species at the Au/Hg electrode, which is an electrochemical oxidation of the Hg. On scanning negatively, HgS is reduced to Hg and S2− at a more positive potential that overlaps with H2S and S0, then the (x−1)Sx atoms are reduced to sulfide at a more negative potential. Because the reduction of S0 atoms in S2− is an irreversible process, increasing the scan rate shifts to peak to more negative potentials and permits separation of the HgSx reduction from the S0 reduction [Fig. 1; Table 1, eqns. (4a)–(4c)].16,21 The peaks can be fully resolved at scan rates of 1000 mV s−1. On the positive scan in the polysulfide CV, all the S0 that was reduced to sulfide and the S2− in the polysulfide react to reform a HgS film (termed Sx0ag). Lastly, an irreversible peak for the reduction of Fe2+ in soluble FeS, FeSolv, can be observed at ~1.10 V. 12

In situ mat work

Fig. 2 shows representative voltammograms taken from a microbial mat in Great Marsh Delaware. Scan A clearly shows the existence of only oxygen above the mat (4 mm) whereas scan B shows a significant signal due to H2O2 at 0–2 mm above the mat. The H2O2 signal is normally equal to the O2 signal because, at the Hg electrode, O2 reduces to H2O2 [Table 1, eqns. (1a)–(1b)] which in turn reduces to H2O Peroxide has been shown to exist in significant quantities in biofilms. 22 Scan C shows two peaks: one for thiosulfate, S2O32− (Ered = −0.20 V), and Sred (Ered = −0.50 V) at 2 mm below the mat surface. Fast scans of 1000 mV s−1 only show one peak indicating that polysulfides are not present as part of Sred. Thus, Sred in this case is composed of soluble S0 because this upper part of the mat is more oxidized due to the oxygen gradient. Deeper into the mat (5 mm), scan D, shows a clear double peak at Ered = −0.58 V and −0.68 V indicative of polysulfide formation. The more positive signal is due to S2− sulfur from H2S and S2− and the more negative signal is due to S0 sulfur from S2−. Polysulfides persist in the bottom portion
Sulfur oxidation: $2\text{H}_2\text{S} \rightarrow \text{H}_2\text{O} + \text{S}_2$

Oxygenic photosynthesis: $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{O}_2 + \text{CH}_2\text{O} + \text{H}_2\text{O}$

Table 1 Relevant sulfur and iron species electrode reactions at the Au/Hg electrode

<table>
<thead>
<tr>
<th>$E/N$</th>
<th>MDL/µmol dm$^{-3}$</th>
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<tbody>
<tr>
<td>(a) $\text{O}_2 + 2\text{H}_2 = \text{H}_2\text{O}$</td>
<td>0.30</td>
</tr>
<tr>
<td>(b) $\text{H}_2\text{O}_2 + 2\text{H}_2 = 2\text{H}_2\text{O}$</td>
<td>1.3</td>
</tr>
<tr>
<td>(c) $\text{HS}^- + \text{H}_2 = \text{H}_2\text{O} + \text{H}_2\text{S}$</td>
<td>Adsorption onto Hg &lt; -0.60</td>
</tr>
<tr>
<td>(d) $\text{H}_2\text{S} + 2\text{H}_2 = \text{H}_3\text{O}^+ + \text{HS}^- + \text{H}_2\text{O}$</td>
<td>0.60</td>
</tr>
<tr>
<td>(e) $\text{Fe}^{3+} + 2\text{e}^- = \text{Fe}^{2+}$</td>
<td>Typically more positive than Fe/S</td>
</tr>
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</table>

Fig. 1 Separation of the tetrasulfide signal into $\text{S}_0$ and $\text{S}_2^-$ signals as scan rate is increased. At the 1000 mV s$^{-1}$ scan rate, two signals are observed. The transition from reduced sulfide below the mat to more oxidized sulfur species towards the top of the mat can be explained by the biology and chemistry of the mat. Table 2 shows a schematic representation of the chemistry of a microbial mat. Visual inspection indicated that green algae/cyanobacteria and purple sulfur bacteria is the fuel for the mat/water interface as in previous studies. During the day, $\text{O}_2$ increases due to oxygenic photosynthesis and creates the levels of (super)saturation (~200%) shown in Fig. 3. These levels drive the much larger $\text{O}_2$ fluxes from the surface of the mat into the water column, thus forming a maximum at or slightly above the mat/water interface. However, as the mat/water interface was reached, $\text{H}_2\text{O}_2$ an intermediate in $\text{O}_2$ formation via water splitting was observed. Previous microelectrode studies using membrane electrodes rather than solid state microelectrodes used in biofilm work$^{22}$ have shown the presence of peroxide.

These data clearly show that there is a redox transition between the overlying water, the mat and the sediment as expected. The transition from reduced sulfate below the mat to partially oxidized sulfur species ($\text{S}_2^-$) in the center of the mat to more oxidized sulfur species towards the top of the mat can be explained by the biology and chemistry of the mat. Table 2 shows a schematic representation of the chemistry of a microbial mat. Visual inspection indicated that green algae/cyanobacteria (6 mm thick), which produce $\text{O}_2$ and organic matter during photosynthesis, inhabited the top of the mat (7 mm total thickness). Underneath the algae were purple sulfur bacteria (PSB; 1 mm thick) which use $\text{H}_2\text{S}$ to produce $\text{S}_8$ and organic matter. The organic matter from the cyanobacteria and purple sulfur bacteria is the fuel for the dissimilatory sulfate reducing bacteria which lie in the deeper into the sediment (data not shown). In none of these scans is there evidence for dissolved Fe and Mn in the mat.

Fig. 3 shows a profile of oxygen and sulfur species into the mat which is about 7 mm thick. The $\text{O}_2$ profile is typical for microbial mats$^{23,26}$: $\text{O}_2$ in the water column reaches a maximum above the mat/water interface as in previous studies. During the day, $\text{O}_2$ increases due to oxygenic photosynthesis and creates the levels of (super)saturation (~200%) shown in Fig. 3. These levels drive the much larger $\text{O}_2$ fluxes from the surface of the mat into the water column, thus forming a maximum at or slightly above the mat/water interface. However, as the mat/water interface was reached, $\text{H}_2\text{O}_2$ an intermediate in $\text{O}_2$ formation via water splitting was observed. Previous microelectrode studies using membrane electrodes rather than solid state microelectrodes used in biofilm work$^{22}$ have shown the presence of peroxide.

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Table 2 Redox reactions that form chemical gradients within the microbial mat environment

<table>
<thead>
<tr>
<th>Process</th>
<th>Reaction</th>
<th>Pathway</th>
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<tr>
<td>Oxidogenic photosynthesis</td>
<td>$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{O}_2 + \text{CH}_2\text{O} + \text{H}_2\text{O}$</td>
<td>Algal/cyanobacteria</td>
</tr>
<tr>
<td>Anoxogenic photosynthesis</td>
<td>$\text{CO}_2 + 2\text{H}_2\text{S} + \text{H}_2\text{O} + 2\text{S}_2 \rightarrow 2\text{S}_6\text{O}_6^2-$</td>
<td>Purple sulfur bacteria</td>
</tr>
<tr>
<td>Sulfate reduction</td>
<td>$2\text{CH}_2\text{O} + 2\text{H}_2\text{O} + 2\text{H}_2\text{S} \rightarrow 2\text{H}_2\text{O} + 2\text{H}_2\text{S}_2^- + 2\text{H}_2\text{O}$</td>
<td>Sulfate reducing bacteria</td>
</tr>
<tr>
<td>Fe chemistry</td>
<td>$\text{Fe}^{3+} + \text{H}_2\text{S} \rightarrow \text{Fe}^{2+} + \text{H}_2\text{O}$</td>
<td>Chemical reaction</td>
</tr>
<tr>
<td></td>
<td>$\text{Fe}^{2+} + \text{H}_2\text{O} \rightarrow \text{Fe}^{3+} + \text{H}_2$</td>
<td>Chemical reaction and/or sulfur oxidizing bacteria</td>
</tr>
<tr>
<td>Sulfur oxidation</td>
<td>$\text{S}_8 \rightarrow \text{H}_2\text{S}_2^- + \text{S}_6\text{O}_6^2-$</td>
<td>Chemical reaction and/or sulfur oxidizing bacteria</td>
</tr>
</tbody>
</table>
sediments underneath the PSB. Although the sediments contain significant quantities of solid phase Fe(II,III) which react with sulfide to form FeS and FeS$_2$, we did not observe a significant signal for FeS$_{aq}$ in this mat. FeS$_{aq}$ is found readily in the black colored sediments.

The mat surface produces oxidants, O$_2$ and H$_2$O$_2$, which are able to oxidize sulfur compounds whereas the PSB mediate sulfide oxidation directly. The formation of S$_8$ and S$_2$O$_3^{2-}$ at the upper portions of the mat is consistent with the stronger oxidizing characteristics of that section of the mat where oxygenic photosynthesis occurs. Polysulfides form in the interior of the mat and their formation likely occurs from the reaction of HS$^-$ with S$_8$ that PSB produce during anoxygenic organic matter production. The formation of these partially oxidized sulfur species is the result of the oxidation of H$_2$S/HS$^-$ which diffuses from the reducing sediments toward the overlying water. The data set is consistent with laboratory studies of sulfide oxidation$^5$ that show formation of polysulfides early in the oxidation and production of thiosulfate later. In Fig. 3, polysulfides but not thiosulfate are observed deep in the mat whereas thiosulfate but not polysulfides are observed nearer the mat surface.

While colorimetric analyses$^{25,26}$ have been employed to measure sulfur species such as thiosulfate, tetraphionate and polysulfides in lab cultures and processed microbial mat cores on thick films that could be cut and analyzed, this is the first report of $in$ $situ$ sulfur species other than H$_2$S in a microbial mat and their occurrence occurs where predicted. The oxidation appears primarily due to biological processes because no metals

![Fig. 2: Representative LSV and CV scans showing sulfur speciation from a salt marsh microbial mat. A, LSV of 500 mV s$^{-1}$ scan rate showing O$_2$ in overlying water; B, LSV of 500 mV s$^{-1}$ scan rate showing H$_2$O$_2$ at the mat/water interface; C, CV of 1000 mV s$^{-1}$ scan rate showing S$_8$ and S$_2$O$_3^{2-}$ as the electrode penetrates through the green algae section; D, CV of 1000 mV s$^{-1}$ scan rate showing S$_{aq}$ signals from S$^0$ and S$^2$; E, CV of 1000 mV s$^{-1}$ scan rate showing only H$_2$S and a trace of FeS$_{aq}$ as the electrode penetrates the sediment.](image)

![Fig. 3: Profile of all chemical components measured in the microbial mat. When a chemical species is no longer detected, it is not plotted. S$_{red}$ is the peak at $-0.58$ V and is the sum of all sulfur in the 2$^-$ oxidation state from polysulfides and H$_2$S/HS$^-$ and in the 0 oxidation state from S$_8$. Note that the data points for these are connected in the figure to show the transition from S$_8$ to S$^{2-}$ to H$_2$S.](image)

![Fig. 4: Representative CV scans showing sulfur speciation from a subtidal sediment. A, 1000 mV s$^{-1}$ scan rate of the upper zone showing Fe(II), S$_8$, FeS$_{aq}$; B, 1000 mV s$^{-1}$ scan rate of the middle area showing the S$_2$$^{2-}$ signals from S$^0$ and S$^2$; C, 1000 mV s$^{-1}$ scan rate showing only H$_2$S and FeS$_{aq}$ as the major components.](image)
(Fe, Mn), which could oxidize sulfide, were detected and O₂ did not co-exist with H₂S.

Subtidal sediment work

Similar sulfur speciation was also found in subtidal sediments (Fig. 4) from Rehoboth Bay, Delaware. In these submersed sediments, there are no bacterial mats present and O₂ penetrates to only about 2 mm throughout the year. Sulfate reduction does occur and the resulting sulfide reacts with Fe(III) and Mn(IV) phases, which range in concentration from 10 to 100 μmol (g dry wt)⁻¹, to produce partially oxidized sulfur species since O₂ does not penetrate deeply. During Spring, similar speciation was found in these sediments as in the mats except that the sulfur species were found very deep into the core where Fe chemistry dominates. An upper zone consisted primarily of Sₓ, and dissolved Fe phases including Fe²⁺, FeS₉, and Fe(II)–organic complexes. Soluble Fe(III) phases have been found in previous porewater studies.²⁷ A middle zone consisted of S²⁻ and FeS₉, and finally, a lower completely reduced zone composed of H₂S/HS⁻ and FeS₉. The middle zone of polysulfides was only about 5 mm over the 8 cm depth range measured as compared to 4 mm over a 2 cm microbial mat studied. Interestingly a large peak near −0.95 V was also observed in the sediments. We have only been able to reproduce this signal in laboratory solutions of polysulfides that are allowed to oxidize slowly in air. As the oxidation proceeds, white and yellow forms of elemental sulfur are visible in the solution. We hypothesize that the excess elemental sulfur adsorbs to the Hg electrode and interacts with the polysulfide in a donor–acceptor complex (Sₓ⁺ → Sₘ or HS⁻ → Sₘ) that gives rise to the signal.

Although Fe(III) and Mn(IV) solid phases are present in abundance [total of ~100 μmol (g dry wt)⁻¹] in these sediments, solid phases are less reactive than soluble compounds.²³²⁷ Thus, the combination of slower solid phase reactivity and less microbial oxidation relative to microbial mats provides less dramatic sulfur speciation in these subtidal sediments than in the salt marsh mats. The measurement of dissolved Fe compounds in these subtidal porewaters is in contrast to the salt marsh mat work described above and clearly shows that Fe chemistry is not an important component of mat biogeochemistry. Fe²⁺ also reacts quickly with polysulfides and at high micromolar (μmol dm⁻³) concentrations reacts to form FeS and Sₓ.²⁷ Thus, polysulfides should not reach high levels when dissolved metals are present.

In situ hydrothermal vent work

In order to assess sulfur speciation at vents, we placed the sensor package near the plumes of the vent tubeworms Riftia pachyptila, which reside in diffuse flow waters at the base or on the lower walls of the vents. These tubeworms grow up to 2 m tall and harbor bacterial endosymbionts. The primary source of organic carbon for their growth is from the bacterially mediated chemosynthetic reaction of H₂S/HS⁻ with CO₂ by the endosymbionts, and the R. pachyptila move their plumes into diffuse flow waters that contain H₂S/HS⁻. However, the formation of Sₓ from chemosynthesis occurs within the bacteria residing in R. pachyptila not outside Riftia. For partially oxidized sulfur species to form in the vent waters around R. pachyptila, oxygen or Fe(II) formed from Fe(II) oxidation by oxygen must react with H₂S, both H₂S and S²⁻ signals from S⁰ and S²⁻ are observable; C, time course showing the rapid change in sulfur speciation at the tubeworm location.

![Image](https://example.com/image1)

Fig. 5 Representative CV scans showing sulfur speciation from diffuse flow area near the vent tubeworms R. pachyptila. A, Initial 1000 mV s⁻¹ scans showing that only O₂ and H₂S co-exist; B, subsequent 1000 mV s⁻¹ scans showing that O₂, H₂S and S²⁻ signals from S⁰ and S²⁻ are observable; C, time course showing the rapid change in sulfur speciation at the tubeworm location.

![Image](https://example.com/image2)

Fig. 6 Deployment of the sensor package above R. pachyptila. (For a colour version of this figure see Electronic Supplementary Information.)
References

Acknowledgements

Conclusions

The sulfur compounds of intermediate oxidation state (which are typically found in microbial mats, subtidal sediments, and hydrothermal vent waters above the electrode's detection limits) are thiosulfate, polysulfides and aqueous elemental sulfur. Trace amounts of tetrathionate (data not shown) have also been detected. These partially oxidized sulfur species are found at redox transition zones where both an oxidant (e.g., O$_2$) and a reductant (e.g., H$_2$S) can be found or where biological mediation can occur. Fe(n) phases are important in the oxidation of H$_2$S and can form quickly as dissolved components when O$_2$ and Fe$^{2+}$ coexist in waters of pH 6–7. The first detected sulfur species during H$_2$S oxidation in these studies was tetrathionate, 28 which confirms that Fe and Mn chemistry are not significant in the oxidation of H$_2$S and can form in seconds to minutes at circumneutral pH when dissolved Fe and O$_2$ coexist as in hydrothermal vent diffuse flow waters.

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