Thermodynamics and kinetics of sulfide oxidation by oxygen: a look at inorganically controlled reactions and biologically mediated processes in the environment

George W. Luther III1*, Alyssa J. Findlay1, Daniel J. MacDonald1, Shannon M. Owings1, Thomas E. Hanson1, Roxanne A. Beinart2 and Peter R. Girguis2

1 School of Marine Science and Policy, College of Earth, Ocean and Environment, University of Delaware, Lewes, DE, USA
2 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA

INTRODUCTION
The oxidation of hydrogen sulfide is arguably one of the most important processes in the environment as the oceans have been suboxic or anoxic and euxinic (i.e., sulfidic) for long spans of geologic time (Canfield and Raiswell, 1999; Turchyn and Schrag, 2006). These are the oceans in which eukaryotes evolved and sulfur metabolism may have helped shape initial symbiotic events leading to the euarchontic lineage (Overmann and van Gemerden, 2000; Theissen et al., 2003; Mentel and Martin, 2008). Modern analogs of these euxinic environments can be found in anoxic marine basins like the deep Black Sea (Wakeham et al., 2007), near the Cariaco Trench (Lin et al., 2008), and highly sulfidic marine sediments like those in the Santa Barbara Basin that have been characterized as symbiosis oases (Bernhard et al., 2000). Other oceanic regions experience periodic anoxia and sulfidic water columns due to the disturbance of sulfide laden sediments underlying areas of high primary productivity, like the upwelling zones off the coasts of Chile (Canfield et al., 2010) and Namibia (Bruchert et al., 2003). In addition, hydrothermal vents are a key source of H2S to the ocean and for vent associated ecosystems supported by sulfide driven chemolithotrophic primary production (Jannasch and Wirsen, 1979).

The thermodynamics for the first electron transfer step for sulfide and oxygen indicates that the reaction is unfavorable as unstable superoxide and bisulfide radical ions would need to be produced. However, a two-electron transfer is favorable as stable S(0) and peroxide would be formed, but the partially filled orbitals in oxygen that accept electrons prevent rapid kinetics. Abiotic sulfide oxidation kinetics improve when reduced iron and/or manganese are oxidized by oxygen to form oxidized metals which in turn oxidize sulfide. Biological sulfur oxidation relies on enzymes that have evolved to overcome these kinetic constraints to affect rapid sulfide oxidation. Here we review the available thermodynamic and kinetic data for H2S and HS− as well as O2, reactive oxygen species, nitrate, nitrite, and NOx species. We also present new kinetic data for abiotic sulfide oxidation with oxygen in trace metal clean solutions that constrain abiotic rates of sulfide oxidation in metal free solution and agree with the kinetic and thermodynamic calculations. Moreover, we present experimental data that give insight on rates of chemolithotrophic and photolithotrophic sulfide oxidation in the environment. We demonstrate that both anaerobic photolithotrophic and aerobic chemolithotrophic sulfide oxidation rates are three or more orders of magnitude higher than abiotic rates suggesting that in most environments biotic sulfide oxidation rates will far exceed abiotic rates due to the thermodynamic and kinetic constraints discussed in the first section of the paper. Such data reshape our thinking about the biotic and abiotic contributions to sulfide oxidation in the environment.

Keywords: sulfide, oxidation, abiotic, biotic, Chlorobaculum tepidum, chemolithotrophy, photolithotrophy

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oxygen in anoxic basins, dissolved Mn and Fe are present and help to overcome the kinetic barrier to sulfide oxidation with oxygen as Mn(II) and Fe(II) can be oxidized abiotically or by microbial activity (Trouwborst et al., 2006; Clement et al., 2009). Here the oxidation of Fe(II) and Mn(II) to Fe(III) and Mn(III, IV) chemical species permit the oxidation of sulfide, which is a trace metal catalyzed process as Fe(II) and Mn(II) are regenerated (Konovalov et al., 2003; Ma et al., 2006; Yakshevs et al., 2009).

While the importance of microbial biochemistry to sulfide oxidation in many systems is recognized (e.g., Lavik et al., 2009), this paper seeks to provide a concise demonstration of the underlying chemical principles for this observation. In this paper, we summarize the thermodynamics of one and two-electron transfers in sulfide oxidation showing that one-electron transfers are unfavorable whereas two-electron transfers are favorable. However, two-electron transfers have a kinetic barrier for the direct reaction of sulfide and oxygen, which is a paramagnetic species, or of sulfide with nitrate. To demonstrate this point clearly, we compare abiotic rates of sulfide oxidation with O2 under trace metal clean conditions as well as with biotic rates for the anaerobic photolithothrophic, Chlorobaculum tepidum, and aerobic chemolithothrophic bacterial symbionts from Lau Basin and free-living microbial communities from the Juan de Fuca Ridge. These data reveal that microbes enhance sulfide oxidation by three or more orders of magnitude and indicate that chemolithothrophic organisms are able to overcome the kinetic barrier for the reaction of sulfide with oxygen. By comparison with existing literature data, we demonstrate that biological sulfide oxidation rates will exceed trace metal catalyzed abiotic sulfide oxidation rates under many conditions.

**MATERIALS AND METHODS**

**ABIOTIC REACTIONS**

All abiotic sulfide oxidation experiments were performed in a class 100 clean bench to prevent metal contamination. Plastic falcon tubes and other materials were cleaned of trace metal contamination by the following procedure. First the plastic materials are cleaned with a detergent in deionized (DI) water; then for a day each in three consecutive acid baths of (1) 10% trace metal clean HCl acid followed by DI water rinsing; (2) another 10% trace metal clean HCl acid followed by DI water rinsing; (3) 1% ultra pure HCl acid followed by DI water rinsing. After the last step, the plasticware is used for reaction or stored in plastic freezer bags until needed. Pipette tips were left to soak in 10% trace metal clean HCl until needed and were rinsed with DI water before they were used.

The sulfide reactions with oxygen were performed in trace metal clean base, ~15 mM NaOH. The trace metal clean base was prepared by adding NaOH to air saturated DI water and then 0.01 M MgCl2•6H2O was added to precipitate any oxidized Fe and other trace metal contaminants. The solution is then centrifuged and decanted into an acid washed bottle. The pH of the resulting solution is about 12. A stock solution of sulfide was made by dissolving solid Na2S•9H2O in trace metal clean base to give a concentration of 24 mg mL−1 (0.01 M). The solid Na2S•9H2O was first rinsed with DI water and dried with a kimwipe before it was weighed and dissolved. An aliquot of a Na2S•9H2O solution is then added to the oxygen saturated (~250 μM) base solution so that the total sulfide in the solution is 50 or 100 μM. The loss of HS− is monitored over time by UV–Vis spectrophotometry using the 230-nm peak (Ellis and Golding, 1959). When an aliquot is taken for analysis, it is discarded and not returned to the reaction vessel. The vessel is shaken to insure air saturation after each aliquot is removed.

**BIOTIC REACTIONS**

Chlorobaculum tepidum is a phototrophic, anoxic sulfide-oxidizing bacterium. Electrochemical methods were employed in order to measure the rate of sulfide loss in the presence of these bacteria under different light intensities.

**Culture growth**

Wild type C. tepidum (strain TLS 1, Wahlund et al., 1991) were grown at 42°C under 20 μmol photons m−2 s−1 provided by a full spectrum 60 W incandescent bulb for 3 days in Pf-7 medium containing 2.71 mM sulfide. Cultures were started by transferring 1 mL of a previously grown culture into a 100-mL septum vial containing anoxic media, which was then pressurized with argon gas to 10 psi. The vial was placed in the dark for 45 min, then transferred to a water bath for growth under the conditions specified above. After 3 days, 50 mL of cells were centrifuged at 3000 rpm for 45 min and rinsed in anoxic HEPES buffer (0.1 M, pH 7.4) to remove salts from the cells. After three rinses, the cells were stored in 5 mL anoxic HEPES buffer in a sealed 20 mL septum vial. Conditions were kept anoxic by using a glove bag purged with ultra high purity argon gas for transferring the cells. To determine the number of cells in the culture, 90 μL of cells were fixed with 10 μL of paraformaldehyde and diluted 1000-fold in 9 mL HEPES and 1 mL Triton X-100. Five milliliter of this solution were filtered through a 0.20-μm polycarbonate filter, and biomass was found through direct counts done by fluorescence microscopy using 4′,6-diamidino-2-phenylindole (DAPI) stain and a UV light (Cottrell and Kirchman, 2003). Concentrations were calculated from the average of 10 fields of view with a 250-s exposure time. The concentration of cells after the final rinse was 3.3 × 109 cells mL−1 ± 0.4 × 109 cells mL−1.

**ANALYTICAL METHODS**

Voltammetry with solid state electrodes was used to monitor sulfide loss, and the details of the method are described in Brendel and Luther (1995) and Luther et al. (2008). Briefly, a typical three-electrode cell for the placement of electrodes with ports for stirring, the introduction of materials, and the purging with argon was used to measure sulfide loss in situ and in the absence of oxygen. The cell consists of a 100-μm gold amalgam (Au–Hg) working electrode, a platinum counter electrode, and a Ag/AgCl reference electrode, used in conjunction with a DLK-60 electrochemical analyzer [Analytical Instrument Systems (AIS), Inc.]. AIS software was used to run the equipment, and data were analyzed using a program written in Python. Peak heights were converted to concentrations using the method of standard additions in HEPES buffer with no cells. Cyclic voltammetric scans were run from −0.1 to −1.8 V and back to −0.1 V at a scan rate of 2 V s−1. Two conditioning steps preceded each scan: one at −0.9 V for 5 s, which served as an electrochemical cleaning step to prevent sulfide from plating onto the electrode, and another at −0.1 V for 2 s (Eq. 1). Under these experimental conditions, sulfide reacts at the Au–Hg
electrode to form a HgS surface via the following equations and the
peak for the removal of sulfide from the HgS surface near −0.7 V
from Eq. 2 is used for analysis:
\[
\text{HS}^- + \text{Hg} \rightarrow \text{HgS} + 2e^- \quad (1)
\]
\[
\text{HgS} + \text{H}^+ + 2e^- \rightarrow \text{HS}^- + \text{Hg} \quad (2)
\]
The solution was stirred with a mechanical stirrer prior to each scan.
The detection limit for sulfide using this method is about 0.2 μM.

Stock solutions of HEPES and sulfide were made from salts dissolved
in DI water that was purged in a pressure equalizing dropping funnel for 45 min with ultra high purity argon gas prior
to use. The sulfide solution was prepared as above by first rinsing
the solid Na2S•9H2O with DI water and drying it with kimwipes
before being weighed and dissolved in degassed DI water.

**EXPERIMENTAL PROCEDURE FOR PHOTOTROPHIC SULFIDE OXIDATION**

Experiments were run in sterile, anoxic HEPES buffer (0.1 M, pH 7.4) at 44°C. Temperature was held constant using a water jacketed
electrochemical cell mated to a thermostatically controlled water
bath, and an argon purge was used to keep oxygen from entering
the cell. Once anoxic conditions were established, an appropriate
volume of cells was added to the cell and were diluted to the
desired concentration. The number of cells used varied between
experiments, and ranged from 3.0 × 10^9 to 1.4 × 10^10. Replicate
experiments were conducted with different cultures to determine
sulfide oxidation rate reproducibility. After a short period was
allowed for the cells and solution to reach thermal equilibrium,
up to 100 μM sulfide was added. The electrochemical cell was then
capped to eliminate headspace and minimize volatile sulfide loss.

Electrochemical measurements were taken every 9 s throughout
the course of the experiment. Light was provided by a full spec-
trum 60 W incandescent bulb and the intensity was measured using
a LI-COR Biosciences LI-1400 data logger light meter inside the
electrochemical cell and ambient light was measured to be 5 μmol
photons m^{-2} s^{-1}. For dark conditions (0 μmol photons m^{-2} s^{-1})
the electrochemical cell was covered, and measurements with the light
meter ensured that no light was infiltrating the cell. For each set
of conditions, experiments were also run without cells in order
to establish sulfide loss due to abiotic factors with voltammetric
measurements taken every 30 s for an hour. Rates were calculated
from the linear range of a plot of sulfide concentration (μM) versus
time for each experiment.

**DETERMINING RATES OF CHEMOLITHOTROPHIC SULFIDE OXIDATION BY SYMBIOTICS**

All experiments were conducted on board the R/V Thomas G.
Thompson during an expedition in June and July 2009 at Lau Basin.
*Ifremeria nautili* (Bouchet and Warén, 1991; Windoffer and Gierie,
1997) snails were collected by the ROV JASON from the ABE vent
field (20 45.794323°S, 176 11.466148°W) during dive J2-423 from
a depth of 2152 m. Snails were brought to the surface in a thermally
insulated container (Mickel and Childress, 1982). After arrival on
board ship, the snails most responsive to touch were immediately
placed into titanium flow-through, high-pressure respirometer
aquaria (as in Henry et al., 2008), where they were maintained in
0.2 micron filter-sterilized flowing seawater for approximately 24 h
at 15°C and 27.5 Mpa prior to experimentation.

To simulate the seawater chemistry found in situ, the 0.2–micron
filter-sterilized flowing seawater was pumped into an acrylic gas equilibra-

tion column and bubbled with carbon dioxide, hydrogen sulfide,
oxogen, and nitrogen to achieve the desired dissolved gas concentra-

tions (Girguis and Childress, 2006). Seawater from the equilibra-
tion column was delivered to the three aquaria by high-pressure
pumps (American Lewa, Inc. Holliston, MA, USA). High-pressure
aquaria were maintained in our climate-controlled laboratory,
while aquaria pressures were maintained at 27.5 Mpa via diaphragm
back pressure valves (StraVal, Inc.). Vessel effluents were directed
through a computer-controlled stream-selection valve that diverted
one stream to the analytical instrumentation every 30 min so that
either the initial water or a chamber with live animals could be
analyzed for chemical components. The analytical system consisted
of a membrane-inlet quadrupole mass spectrometer to determine
dissolved gas concentrations, an inline oxygen optode (Golden
Scientific Inc.), and an inline pH electrode (Radiometer Inc.). In
addition to the mass spectrometric analyses, hydrogen sulfide concen-
trations were determined by a quantitative colorimetric assay
(Cline, 1969). For this study, a voltammetric flow cell was also added
to the inline analytical instrumentation (Luther et al., 2002) so that
hydrogen sulfide, polysulfides, thiosulfate, and oxygen could be
determined. All carbon dioxide and hydrogen sulfide measurements
were confirmed and calibrated using discrete samples analyzed back
using a Hewlett-Packard 5890 Series II gas chromatograph (as in
Childress et al., 1991).

For these experiments, *I. nautili* were placed in the respirom-
eur aquaria and were maintained in conditions typical of those
in situ, namely total dissolved inorganic carbon (i.e., ΣCO2) = 5.5
to 6 mM, total dissolved sulfide (i.e., ΣH2S) from 312 to 650 μM,
dissolved O2 from 110 to 180 μM, and dissolved NO3 = 40–50 μM,
pH = 6.1 to 6.6, temperature = 15–30°C, pressure = 27.5 Mpa,
flow rate = 15 mL min\(^{-1}\). *I. nautili* were maintained in these
conditions until “autotrophy,” during which they exhibited a net
uptake of dissolved inorganic carbon, oxygen, and sulfide, as
well as net elimination of proton equivalents. All dissolved sub-
strate concentrations, as well as pH and temperature, were held
at these “typical” conditions for the duration of the experiment.
At the end of each experiment, snails were promptly removed,
weighed on a motion-compensated shipboard balance, dissected,
dissected, and frozen in liquid nitrogen for other analyses. Once removed,
“free-living” microbes remained in the chambers and the aquaria
were re-pressurized so experiments with only microbes could be
performed.

**DETERMINING RATES OF CHEMOLITHOTROPHIC SULFIDE OXIDATION BY FREE-LIVING ASSEMBLAGES**

All experiments were conducted on board the *R/V Atlantis* during
an expedition in August 2008 to the Juan de Fuca ridge. Active solid
sulfide samples were collected by the DSV Alvin using a sampling
scop, and were brought to the surface in a thermally insulated
container (Mickel and Childress, 1982). After arrival on board
ship, sulfide samples were immediately placed into titanium flow-
through, high-pressure respirometer aquaria (as in Henry et al.,
2008), where they were maintained in 0.2 μm filter-sterilized flowing
seawater for approximately 24 h at 15°C and 27.5 Mpa prior to experimentation.
In situ seawater chemistry was simulated as described above. We incubated the sulfide samples at the following conditions: 5 mM inorganic carbon, 650 μM hydrogen sulfide, 110 μM oxygen, pH 5.0, temperature of 30°C, and pressure of 27.5 Mpa (ca. 4000 psi). All fluids were directed through a computer-controlled stream-selection valve that diverted one stream to the analytical instrumentation every 30 min as described above. All dissolved substrate concentrations, as well as pH and temperature, were held at the aforementioned conditions for the duration of the experiment. At the end of each experiment, sulfide samples were promptly removed, weighed on a motion-compensated shipboard balance, dissected, and frozen in liquid nitrogen for other analyses.

RESULTS AND DISCUSSION

THERMODYNAMICS OF SULFIDE OXIDATION

The thermodynamic equations and calculations for the reactions of sulfide with various oxidants have been demonstrated in Luther (2010), who used the thermodynamic data (at 25°C and 1 atm) tabulated by Maloy (1985), Stumm and Morgan (1996) and Stanbury (1989). Briefly a reduction half reaction and an oxidation half reaction are combined to provide a ΔG or Δlog K, which indicates the favorability of a given reaction over pH. For example, the one-electron transfer reaction (Eq. 3) of O2 with HS- leads to two thermodynamically unfavorable products (superoxide ion and bisulfide radical).

\[ \text{O}_2(aq) + \text{HS}^- \rightarrow \text{O}_2(aq) + \text{HS}^- \] 

(3)

The thermodynamic functions for the reduction half reactions are Eqs 4 and 5. The function \( \log K \) is the log K value at the standard state for all reactants and products. The complete reaction for Eq. 3 is the sum of Eq. 4 minus Eq. 5. An extensive list of half reactions are given in Luther (2010).

\[ \text{O}_2(aq) + \text{e}^- \rightarrow \text{O}_2(aq) \quad \log K = -2.72 \] 

(4)

\[ \text{HS}^- + \text{H}^+ + \text{e}^- \rightarrow \text{H}_2\text{S} \quad \log K = 25.21 - \text{pH} \] 

(5)

Similarly, the two-electron transfer reaction of \( \text{O}_2 \) with \( \text{H}_2\text{S} \) leads to stable products (Eq. 6).

\[ \text{O}_2(aq) + \text{H}_2\text{S} \rightarrow \text{S}^0 + \text{H}_2\text{O}_2 \] 

(6)

The thermodynamic functions for the reduction half reactions describing Eq. 6 are given in Eqs 7 and 8. Equation 6 is an accurate representation for the energy driving chemolithotrophy by an aerobic sulfide oxidizer using \( \text{O}_2 \) as the terminal electron acceptor.

\[ \text{O}_2(aq) + \text{H}_2\text{S} \rightarrow \text{S}^0 + \text{H}_2\text{O}_2 \] 

(7)

\[ \text{H}_2\text{S}^- + \text{H}^+ + \text{e}^- \rightarrow \frac{1}{2} \text{H}_2\text{O}_2 \quad \log K = -13.18 + \text{pH} \] 

(8)

Figure 1 shows plots over pH for one and two-electron transfer reactions for the reaction of sulfide with oxygen, reactive oxygen species and oxidized nitrogen species. In some instances there is no pH dependence for a given reaction, but in most instances there is a significant pH dependence. Figure 1A describes the thermodynamics of sulfide oxidation in one-electron transfers of oxygen in four steps from \( \text{O}_2 \) to \( \text{O}_2 \) to \( \text{H}_2\text{O}_2 \) to OH• to produce \( \text{H}_2\text{O} \). Only OH• can lead to formation of HS•. Even the oxidation of sulfide by \( \text{O}_2 \) and \( \cdot\text{O}_2 \) are unfavorable. However, the two-electron transfer reactions (e.g., Eq. 6) for \( \text{O}_2 \) to \( \text{H}_2\text{O}_2 \), \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \), \( \cdot\text{O}_2 \) to \( \text{H}_2\text{O}_2 \), and \( \text{O}_2 \) to \( \text{H}_2\text{O} \) and \( \text{H}_2\text{O} \) are thermodynamically favorable over all pH.

Figures 1C,D are the data for the one-electron and two-electron transfer reactions of oxidized nitrogen species with sulfide. Again one-electron transfer reactions are not thermodynamically favorable over all pH as HS• results whereas two-electron transfer reactions are favorable. The one-electron thermodynamic calculations show that the oxidation of sulfide by other oxidants has similar problems as \( \cdot\text{O}_2 \). Also, the reaction of sulfide at near neutral or basic pH has a kinetic constraint as nitrate and nitrite are anions as is HS•; thus, two negative species repel each other as they come near each other in the transition state.

A major problem for the reaction of \( \text{O}_2 \) with any reactant that can donate a pair of electrons is the partial occupancy of the highest occupied π antibonding orbitals, which are similar in energy (Figure 2). A direct two-electron transfer is not possible and a one-electron transfer requires the unpairing of electrons in a donor such as sulfide. Thus, there is a kinetic constraint on \( \text{O}_2 \) reactivity and this constraint needs to be overcome as it is in chemolithotrophic sulfide-oxidizing microbes. On the other hand, these kinetic constraints allow sulfide to persist for long enough periods in oxic and nitrate dominated environments thereby defining niches that can be exploited by sulfide-oxidizing microbes.

Figure 3 shows the ΔG for three reaction sequences. First, the one-electron transfer sequential reactions of \( \text{O}_2 \) with \( \text{H}_2\text{S} \) to form \( \text{O}_3 \) and \( \text{HS}^- \) and of \( \text{O}_2 \) with \( \text{HS}^- \) to form \( \text{H}_2\text{O}_2 \) and S(0) (blue). In this case, the first step is thermodynamically unfavorable. A second reaction possibility is the reaction of \( \text{O}_2 \) with \( \text{H}_2\text{S} \) to form \( \text{O}_2 \) and \( \text{HS}^- \) followed by the reaction of another \( \text{O}_2 \) with \( \text{HS}^- \) to form \( \text{O}_3 \) and S(0). Again, the first step is thermodynamically unfavorable. The direct two-electron transfer reaction of \( \text{O}_2 \) with \( \text{H}_2\text{S} \) described in Eq. 6 (red) is favorable.

ABIOTIC REACTION OF SULFIDE WITH OXYGEN

A concrete demonstration of kinetic limitations on the direct oxidation of sulfide by \( \text{O}_2 \) can be found by examining the reaction of bisulfide ion (HS•−) with excess \( \text{O}_2 \) in basic solutions. Over the pH range 8–12, Millero et al. (1987) showed that the kinetics of oxidation do not change at constant temperature (T) and ionic strength (I) where HS•− is the dominant sulfide species. Figure 4 shows a plot for the abiotic oxidation of sulfide in air saturated solutions at a pH of 12 and 25°C using trace metal clean base solution. The slope of the ln (sulfide) versus time shows pseudo first order behavior and yields the pseudo first order rate constant, \( k \). For three replicates, \( k \) is 7.12 × 10−3 day−1 (±1.96 × 10−4). The half-life is 55 ± 1 day and the loss of sulfide is 0.91 μM day−1.

We compare our data with those of Millero et al. (1987). In their work, they found the rate expression

\[ -d[\text{H}_2\text{S}] / dt = k_1 [\text{HS}^-][\text{O}_2] \] 

(9)

where \( k_1 \) is the second order rate constant (M−1 h−1) and has the following form for \( \text{pH} = 4–8 \).

\[ \log k_1 = 10.5 + 0.16 \text{pH} - (3 \times 10^3) \text{T}^{-1} + 0.49 \text{I}^{1/2} \] 

(10)

From our concentration and rate data as in Figure 4 and using Eq. (9), we calculate using initial rate theory a \( k_1 \) of 6.99 M−1 h−1 or \( k_1 \) of 0.845 assuming first order behavior for HS•− and \( \text{O}_2 \). Our
of sulfide with oxygen is truly slow. As indicated by the thermodynamics, one-electron reactions are unfavorable whereas two-electron reactions are favorable, but have a kinetic problem due to the spin pairing problem with O$_2$ (Figure 2). Ma et al. (2006) showed that a catalytic Fe, O$_2$, H$_2$S cycle occurs at the oxic–anoxic interface, and this cycle results in sulfide oxidation to elemental sulfur.

**Figure 1** | One-electron transfer reactions of H$_2$S with oxygen species; the $\Delta \log K$ on the $y$-axis indicates a favorable complete reaction and $-\Delta \log K$ indicates an unfavorable reaction as $\Delta G = -RT \ln K = -2.303 RT \log K$. HS$^-$ reactions are not included but are similar in reactivity (note pK of H$_2$S ∼7 and depends on salinity and temperature). (A) one-electron transfer reactions of oxygen species coupled with Eq. 5. (B) two-electron transfer reactions of oxygen species coupled with Eq. 8. (C) one-electron transfer reactions of oxidized nitrogen species coupled with Eq. 5. (D) two-electron transfer reactions of oxidized nitrogen species coupled with Eq. 8.

$k_2$ value is significantly smaller than that found by Millero et al. (1987). Because Millero et al. (1987) found a faster half-life of about 1–2 days for reactions at pH values similar to ours, we conclude that trace metal clean conditions hinder the oxidation of sulfide with O$_2$. In their work, their plexi glass reaction vessel was rinsed with acid prior to (re)use whereas each experiment here was performed in a separate trace metal clean plastic tube. The trace metal clean condition shows clearly that the reaction of O$_2$ and sulfide is not a favorable reaction.

In subsequent work, Vazquez et al. (1989) showed that the addition of trace metals in particular Fe(II) to the reaction vessel increased the rate of oxidation such that the half-life is on the order of minutes. Vazquez et al. (1989) showed that the rate doubled at the concentrations of Mn(II) found in the Black Sea, but increased 20-fold at the Fe(II) concentrations found at the Black Sea interface. The metal data indicate that the reaction of sulfide with oxygen is truly slow. As indicated by the thermodynamics, one-electron reactions are unfavorable whereas two-electron reactions are favorable, but have a kinetic problem due to the spin pairing problem with O$_2$ (Figure 2). Ma et al. (2006) showed that a catalytic Fe, O$_2$, H$_2$S cycle occurs at the oxic–anoxic interface, and this cycle results in sulfide oxidation to elemental sulfur.

**SULFIDE OXIDATION KINETICS OF ANAEROBIC PHOTOLITHOTROPHIC BACTERIA**

Figure 5 shows representative data for sulfide oxidation in the presence and absence of *C. tepidum* under laboratory light of 5 μmol photons m$^{-2}$ s$^{-1}$. The rate of sulfide oxidation in the absence of cells is negligible as O$_2$ or other dissolved oxidants are not present; thus, Eq. 9 is not operative. Any minor sulfide loss is due to volatilization. The loss of sulfide over time with *C. tepidum* (3.3 × 10$^9$ cells mL$^{-1}$)
et al., 2007). The Fe(II) oxidation is external to the cyanobacterial cells whereas sulfide oxidation with *C. tepidum* occurs internally. Genetic experiments have shown that SQR is required for *C. tepidum* to grow with sulfide as the electron donor (Chan et al., 2009). SQR’s are flavin containing enzymes that pass two-electrons from sulfide via the flavin cofactor to the quinone pool. The quinone pool ultimately is reoxidized by the activity of the photosynthetic reaction center. This model predicts the observed light dependence of sulfide oxidation as observed by the electrochemical experiments here.

In the presence of cells in stationary growth phase, the sulfide oxidation rate is $26,200 \pm 1200 \mu M \text{ day}^{-1}$ at $44^\circ C$ and a pH = 7.4. The data in Figures 4 and 5 can be compared to give relative rates for abiotic oxidation under trace metal clean conditions to biotic oxidation with *C. tepidum* cells of 0.91: 26,200. If we assume that the abiotic rate and light is linear with $r^2 = 0.949$ versus a first order plot for the ln (sulfide) versus time giving $r^2 = 0.930$. The linear plot of sulfide with time indicates that the reaction is zeroth order in sulfide and that sulfide oxidation depends on light as the external stimulus and not the concentration of sulfide as a reactant. The rate law for a zeroth order reaction is given as

$$-d[H_2S]/dt = k$$

which on integration gives

$$kt = [H_2S]_0 - [H_2S]$$

where $[H_2S]_0$ is the initial sulfide concentration. In our experiments, the value of $k$ may include constants for the intensity of light and the number of cells.

This zeroth order kinetic pattern has been shown for Fe(II) oxidation in hot springs when cyanobacteria produce $O_2$, which then reacts with Fe(II) and removes it as Fe(III) solid phases (Trouwborst et al., 2007). The Fe(II) oxidation is external to the cyanobacterial cells whereas sulfide oxidation with *C. tepidum* occurs internally. Genetic experiments have shown that SQR is required for *C. tepidum* to grow with sulfide as the electron donor (Chan et al., 2009). SQR’s are flavin containing enzymes that pass two-electrons from sulfide via the flavin cofactor to the quinone pool. The quinone pool ultimately is reoxidized by the activity of the photosynthetic reaction center. This model predicts the observed light dependence of sulfide oxidation as observed by the electrochemical experiments here.

In the presence of cells in stationary growth phase, the sulfide oxidation rate is $26,200 \pm 1200 \mu M \text{ day}^{-1}$ at $44^\circ C$ and a pH = 7.4. The data in Figures 4 and 5 can be compared to give relative rates for abiotic oxidation under trace metal clean conditions to biotic oxidation with *C. tepidum* cells of 0.91: 26,200. If we assume that the abiotic rate
doubles for every 10°C increase, then the ratio becomes 1:7,200 at 25°C. Figure 6 shows the variation of sulfide oxidation rate versus cell counts when independent cultures of C. tepidum were in exponential phase growth. The average sulfide oxidation rate is 6,500 ± 2900 μM day$^{-1}$ (10$^6$ cells)$^{-1}$ at 44°C and a pH = 7.4. The rate of sulfide loss varies only eightfold over the two orders of magnitude range in cell count. Replicates rates for the same cell count and culture have a SD of 300 μM day$^{-1}$ normalized to cell count. These data suggest that under most conditions, phototrophic sulfide oxidizers are not limited by the abiotic oxidation rate of sulfide under anoxic conditions. Furthermore, these data allow one to conclude that in anoxic systems dominated by anoxicogenic phototrophs, biological sulfide oxidation rates will exceed abiotic oxidation by several orders of magnitude as no oxygen is present to oxidize sulfide. While the cell concentrations used here may seem high, in the New Zealand mats where C. tepidum was isolated and in other microbial mat systems, cell densities of 10$^9$–10$^{10}$ g$^{-1}$ of sediment are not uncommon (Wahlund et al., 1991).

**SULFIDE OXIDATION KINETICS OF AEROBIC CHEMOLITHOTROPHIC SYMBIONTS AND FREE-LIVING ASSEMBLAGES**

As chemolithotrophic symbionts cannot be cultured in the lab, we relied on shipboard high-pressure incubations with freshly collected samples to make inferences about the nature and capacity for sulfide oxidation. Table 1 shows data for the chemolithotrophic oxidation of sulfide by chemolithotrophic symbionts of the vent snail I. nautilei in high-pressure incubation chambers (geochemical conditions are described in the Materials and Methods). These incubations were conducted in the presence of sulfide (H$_2$S), and there is substantial uptake of H$_2$S. Notably, the effluent from these vessels after exposure to I. nautilei showed substantial enrichment in polysulfide S(−2) and S(0) (Rozan et al., 2000) The sulfide oxidation rate from these data is 27.83 ± 1.89 μM min$^{-1}$ (40,100 μM day$^{-1}$) compared to the calculated abiotic rate of 0.0304 μM min$^{-1}$ (43.8 μM day$^{-1}$) for these conditions using Eqs 9 and 10. Previous studies (Girguis and Childress, 2006) have also shown that the majority of sulfide oxidation is attributable to the symbiont. However, it is apparent that non-symbiotic animals, e.g., Urechis sp. worms, also have a native sulfide oxidation capacity though at substantially lower rates (Girguis et al., 2002). The data presented here are the first to characterize the different species of soluble oxidized sulfur compounds produced by intact symbioses, and future studies should aim to better characterize this response in other symbioses, as well as non-symbiotic animals.

Moreover, Table 2 also shows data for the chemolithotrophic oxidation of sulfide by free-living chemolithotrophic microbes in high-pressure incubation chambers (geochemical conditions are described in the Materials and Methods). The actual sulfide oxidation rate is 34.6 μM min$^{-1}$ (49,800 μM day$^{-1}$) compared to the calculated abiotic rate of 0.0105 μM min$^{-1}$ (15.1 μM day$^{-1}$) Eqs 9 and 10.

These data clearly illustrate that biologically mediated oxidation of H$_2$S by O$_2$ occurs rapidly, and indicates that chemolithotrophic microbes have found an efficient way to perform a two-electron transfer as in Figure 1B while overcoming any kinetic constraints based on the electron orbital diagram in Figure 2. These chemolithotrophic rates are over 10$^4$ times that of the abiotic rate from Eqs 9 and 10 (Table 3). It is important to note that these data cannot resolve the relative contributions of sulfide oxidation by the mineralogical assemblage versus the biological community, but iron (oxy)hydroxide phases have rates on par with the trace metal catalyzed reactions (Vazquez et al., 1989). Again, these data provide a first glimpse at rates of sulfide oxidation by free-living microbes associated with sulfides, and future experiments should be aimed at differentiating the relative contributions of biotic and abiotic sulfide oxidation, as well as the relative differences in oxidation by different microbial communities (e.g., diffuse flow communities versus high temperature communities) and mineralogical assemblages.

<table>
<thead>
<tr>
<th>Organisms initial time (h)</th>
<th>Incurrent sulfide (μM)</th>
<th>Excurrent H$_2$S (μM)</th>
<th>Excurrent S(0) (μM)</th>
<th>H$_2$S uptake rate (μM min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. nautilei 12.43</td>
<td>312.5</td>
<td>20.69</td>
<td>25.04</td>
<td>26.3</td>
</tr>
<tr>
<td>I. nautilei 14.43</td>
<td>280.5</td>
<td>24.39</td>
<td>24.41</td>
<td>30.0</td>
</tr>
<tr>
<td>I. nautilei 16.93</td>
<td>271.6</td>
<td>22.03</td>
<td>20.91</td>
<td>27.3</td>
</tr>
</tbody>
</table>

The H$_2$S data indicate the substantial loss of sulfide on exiting the reaction chamber, and the S(0) data indicate an oxidized product as polysulfide. Sulfide uptake and oxidation rate calculations are based on the mass of the animals in the chamber.
Table 2 | Data for the chemolithotrophic oxidation of H₂S with O₂ in high-pressure incubation chambers by free-living microbial communities (40 mL volume) recovered from hydrothermal diffuse flows.

<table>
<thead>
<tr>
<th>Organisms initial time (h)</th>
<th>Incurrent H₂S (μM)</th>
<th>Excurrent H₂S (μM)</th>
<th>H₂S uptake rate (μM min⁻¹; N = number of scans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free-living community, 12</td>
<td>154.7</td>
<td>21.2</td>
<td>33.3</td>
</tr>
<tr>
<td>Free-living community, 22</td>
<td>137.2</td>
<td>16.9</td>
<td>30.3</td>
</tr>
<tr>
<td>Free-living community, 36</td>
<td>184.5</td>
<td>23.7</td>
<td>40.2</td>
</tr>
</tbody>
</table>

The H₂S data indicate the substantial loss of sulfide on exiting the reaction chamber.

Table 3 | Comparative rates for the oxidation of sulfide under abiotic and biotic conditions.

<table>
<thead>
<tr>
<th>Sulfide oxidation</th>
<th>relative rate (μM day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SULFIDE OXIDATION WITH O₂</strong></td>
<td></td>
</tr>
<tr>
<td>Trace metal clean (this work)</td>
<td>1</td>
</tr>
<tr>
<td>Vazquez et al. (1989) – 8 μM Mn(II)</td>
<td>43.8</td>
</tr>
<tr>
<td>Vazquez et al. (1989) – 0.3 μM Fe(II)</td>
<td>876</td>
</tr>
<tr>
<td>I. nautilei with chemolithotrophic microbes</td>
<td>832</td>
</tr>
<tr>
<td>Chemolithotrophic microbes alone</td>
<td>40,100</td>
</tr>
<tr>
<td><strong>SULFIDE OXIDATION WITHOUT O₂</strong></td>
<td></td>
</tr>
<tr>
<td>C. tepidum (stationary phase)</td>
<td>49,800</td>
</tr>
<tr>
<td>C. tepidum (exponential phase)</td>
<td>1,625</td>
</tr>
</tbody>
</table>

The first six entries oxygen is the oxidant. The last two entries are for the anaerobic photolithothrophic. The relative rates are corrected to a temperature of 25°C as the C. tepidum experiments were performed at 44–45°C.

CONCLUSION

The goal for this work was to integrate theoretical chemical considerations with newly collected data to provide a concise rationale for why biological sulfide oxidation rates far exceed abiotic sulfide oxidation rates under both aerobic and anaerobic conditions. Direct comparison of the rates from our experiments with those from previous work (Table 3) indicate that chemolithotrophic sulfide oxidizers using O₂ as the oxidant display sulfide oxidation rates over three orders of magnitude greater than the newly established abiotic oxidation rate of bisulfide. This new rate was determined in strict trace metal clean conditions and suggests that prior estimates of the direct reactivity of HS⁻ with O₂ are too high, and that biologically mediated sulfide oxidation may be responsible for the majority of oxidation observed in the field. The data from photo-lithotrophic organisms further indicates that biological processes in anaerobic environments are also likely to exceed abiotic rates of sulfide oxidation in aerobic environments, even when the presence of metal oxidation is considered. The implication of these findings is that flux of sulfide from anaerobic environments will largely be determined by the population size and activity of microbial communities in anaerobic–aerobic transition zones rather than by chemical oxidants alone. This implication is consistent with many other observations and is explained by the thermodynamic and kinetic constraints of 1 versus 2-electron transfer reactions from HS⁻ to O₂.

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REFERENCES


Decho, A. W., Buckley, D. H., and Spear, J. R. (2006). Sulfate reducing bacteria in anaerobic–aerobic transition zones rather than in anaerobic environments are also likely to exceed abiotic rates of sulfide oxidation in aerobic environments, even when the presence of metal oxidation is considered. The implication of these findings is that flux of sulfide from anaerobic environments will largely be determined by the population size and activity of microbial communities in anaerobic–aerobic transition zones rather than by chemical oxidants alone. This implication is consistent with many other observations and is explained by the thermodynamic and kinetic constraints of 1 versus 2-electron transfer reactions from HS⁻ to O₂.


