Available online at www.sciencedirect.com

SciVerse ScienceDirect

Geochimica et Cosmochimica Acta 83 (2012) 234–251

Geochimica et Cosmochimica Acta

www.elsevier.com/locate/gca

Oxygen and sulfur isotope fractionation during sulfide oxidation by anoxygenic phototrophic bacteria

Michelle Y. Brabec [a], Timothy W. Lyons [b], Kevin W. Mandernack [a,c,*]

[a] Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO 80401, USA
[b] Department of Earth Sciences, University of California, Riverside, CA 92521, USA
[c] Department of Earth Sciences, Indiana University Purdue University at Indianapolis, IN 46202, USA

Received 8 December 2010; accepted in revised form 7 December 2011; available online 14 December 2011

Abstract

Sulfide-mediated anoxygenic photosynthesis (SMAP) carried out by anaerobic phototrophic bacteria may have played an important role in sulfur cycling, formation of sulfate, and, perhaps, primary production in the Earth's early oceans. Determination of $\varepsilon^{34}S_{SO_4\text{-Sulfide-}}$ and $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values for bacterial sulfide oxidation will permit more refined interpretation of the $\delta^{34}S$ and $\delta^{18}O_{SO_4}$ values measured in modern anoxic environments, such as meromictic lakes where sulfide commonly extends into the photic zone, and in the ancient rock record, particularly during periods of the Precambrian when anoxic and sulfidic (euxinic) conditions were believed to be more pervasive than today. Laboratory experiments with anaerobic purple and green sulfur phototrophs, *Allochromatium vinosum* and *Chlorobaculum tepidum*, respectively, were conducted to determine the sulfur and oxygen isotope fractionation during the oxidation of sulfide to sulfate. Replicate experiments were conducted at 25 °C for *A. vinosum* and 45 °C for *C. tepidum*, and in duplicate at three different starting oxygen isotope values for water to determine sulfate-water oxygen isotope fractionations accurately ($\varepsilon^{18}O_{SO_4\text{-}H_2O}$). $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values of $5.6 \pm 0.2‰$ and $5.4 \pm 0.1‰$ were obtained for *A. vinosum* and *C. tepidum*, respectively. Temperature had no apparent effect on the $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values. By combining all data from both cultures, an average $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value of $5.6 \pm 0.3‰$ was obtained for SMAP. This value falls between those previously reported for bacterial oxidation of sphalerite and elemental sulfur (7–9‰) and abiotic and biotic oxidation of pyrite and chalcopyrite (2–4‰). Sulfur isotope fractionation between sulfide and sulfate formed by *A. vinosum* was negligible ($0.1 \pm 0.2‰$) during all experiments. For *C. tepidum* an apparent fractionation of $-2.3 \pm 0.5‰$ was observed during the earlier stages of oxidation based on bulk $\delta^{34}S$ measurements of sulfate and sulfide and became smaller ($-0.7 \pm 0.3‰$) when sulfate concentrations rose above 0.5 mM and sulfide concentrations had became negligible.

1. INTRODUCTION

Before ~2.4 Ga, it is believed that the Earth and its oceans were mostly anoxic (Walker and Brimblecombe, 1985; Farquhar et al., 2000; Canfield, 2005), although others have proposed more oxic conditions during this time (Ohmoto and Felder, 1987; Ohmoto et al., 1993; Watanabe et al., 1997, 2004; Lasaga et al., 2008). Nonetheless, Earth's ecosystems prior to 2.4 Ga and the development of oxygenic photosynthesis were primarily dependent on anaerobic metabolisms with reduced electron donors fueling the dominant metabolic processes. This is also reflected in the phylogenetic tree of life by the early appearance of prokaryotic metabolisms such as iron reduction/oxidation, methanogenesis, and oxidation and/or reduction of sulfide/elemental sulfur (Woese, 1987). These ecosystems likely remained volumetrically important even during the Proterozoic (Canfield, 1998; Johnston et al., 2009; Lyons et al.,

* Corresponding author at: Department of Earth Sciences, Indiana University Purdue University at Indianapolis, 723 W. Michigan St. Indianapolis, IN 46202, USA. Tel.: +1 317 274 8995.
E-mail address: kevinman@iupui.edu (K.W. Mandernack).

0016-7037/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.gca.2011.12.008

2009, and references therein). Many of the earliest prokaryotes were able to metabolize the various forms of sulfur (sulfide, thiosulfate, elemental sulfur, sulfite, sulfate, and polysulfides) as energy sources in the early earth (Woese, 1987; Canfield and Raiswell, 1999).

Prior to the rise in atmospheric oxygen approximately 2.4 billion years ago (Farquhar and Wing, 2003; Bekker et al., 2004; Guo et al., 2009), sulfate concentrations in the Earth's oceans were dominantly low (<200 μM), as compared to concentrations of 28 mM in modern oceans (Canfield and Raiswell, 1999; Habicht et al., 2002; Canfield, 2005; Lyons and Gill, 2010), but may have been much higher in local settings (Buick and Dunlop, 1990; Canfield and Raiswell, 1999). At these globally low sulfate levels, sulfur isotope fractionation by sulfate reducers may not have been expressed due to substrate limitation (Harrison and Thode, 1958; Canfield et al., 2000; Habicht et al., 2002), resulting in generally low $\Delta^{34}S_{SO_4\text{-pyrite}}$ values in the Archean rock record (Shen et al., 2001; Canfield, 2005; Bottrell and Newton, 2006). As atmospheric oxygen levels rose, so did oceanic sulfate concentrations, and, therefore, sulfur isotope fractionation by sulfate reducing prokaryotes was expressed more substantially—leading to greater ranges measured for $\Delta^{34}S_{SO_4\text{-pyrite}}$ in the rock record (Canfield, 1998; Kah et al., 2004, Lyons and Gill, 2010). However, the small range of measured $\Delta^{34}S_{SO_4\text{-pyrite}}$ values prior to the rise in atmospheric oxygen, and shortly after during the early Proterozoic, does not preclude the co-existence of sulfide-oxidizing bacteria, particularly those that carry out sulfide mediated anoxic photosynthesis. Recent reports of free sulfide (euxinia) in the ocean prior to the rise of oxygen (Reinhard et al., 2009; Kendall et al., 2010; Scott et al., 2011) increase this likelihood. Because microbial sulfide oxidation typically exhibits small fractionations of sulfur isotopes, this process might also contribute to these low $\Delta^{34}S_{SO_4\text{-pyrite}}$ values observed during the late Archean.

Although the oxidation of H_2S by *Thiobacillius concretivorus* has been reported to show an isotopic fractionation of −13.2‰ to −10.6‰ between H_2S and sulfate, sulfate produced from sulfide oxidation generally falls within the range of −4‰ to 3.5‰ relative to the sulfide (Nakai and Jensen, 1964; Lewis and Krouse, 1969; Chambers and Trudinger, 1978; McCready and Krouse, 1989; Toran and Harris, 1989; Balci et al., 2007; Pisapia et al., 2007; Brunner et al., 2008). Additionally, $\varepsilon^{34}S_{S^\circ\text{-Sulfide}}$ values of 0–3‰ were reported previously for the anaerobic and phototrophic purple sulfur bacterium, *Allochromatium vinosum* (Fry et al., 1984, 1985, 1986). Better understanding of the role of microbially catalyzed sulfur redox reactions is key to a refined perspective of the biogeochemical cycling of sulfur, past and present. Stable oxygen isotope measurements of sulfate could complement other comparatively new proxy approaches (e.g., $\Delta^{33}S$, $\Delta^{36}S$).

The ability of anoxygenic phototrophs to oxidize reduced sulfur species into sulfate in the absence of oxygen using sulfide mediated anaerobic photosynthesis (SMAP) suggests they could have provided a source of sulfate in the Earth's early oceans prior to and during the early rise of atmospheric oxygen. This anoxygenic pathway may also have played a major role in primary production during the Proterozoic, and, as a positive feedback, any aerobic degradation of the resulting biomass may have perpetuated low oxygen contents in the deeper ocean (Johnston et al., 2009). Widespread anoxic and euxinic (anoxic and H_2S-containing) conditions have been suggested for the Proterozoic ocean (Canfield, 1998). The role of sulfur oxidizing anoxygenic phototrophs is also suggested by biomarkers from purple sulfur bacteria isolated from 1.64 Ga deposits of a marine succession sequence (Brocks et al., 2005; Brocks and Schaeffer, 2008). Quantitative knowledge of the oceanic extent of Proterozoic euxinia awaits further work (Lyons et al., 2009), and euxinic conditions were likely localized along the ocean margins (Planavsky et al., 2011; Poulton and Canfield, 2011). Nevertheless, 16S rRNA analysis reveals that purple sulfur bacteria evolved approximately 3.0–3.5 Ga and further diversified ~0.75–1 Ga (Woese, 1987; Canfield and Teske, 1996; Canfield and Raiswell, 1999; Brocks et al., 2003). Green sulfur bacteria evolved even earlier than purple sulfur bacteria (Woese, 1987) and may have developed by at least 3.5 Ga (Buick and Dunlop, 1990), consistent the recent reports of Archean euxinia.

Most sulfur and oxygen isotopic studies of modern microbial sulfur cycling have focused on prokaryotic sulfate reduction (PSR) and elemental sulfur disproportionation (Habicht et al., 1998; Aharon and Fu, 2000; Böttcher and Thamdrup, 2001; Böttcher et al., 2001; Brunner et al., 2005; Farquhar et al., 2008; and many others) or the oxidation of S^o and metal sulfides by chemolithotrophic bacteria (Taylor et al., 1984; Balci, 2005; Balci et al., 2007, 2012; Pisapia et al., 2007; Brunner et al., 2008; Mazumdar et al., 2008; Thurston et al., 2010). By comparison, only a few sulfur isotope studies of SMAP have been published (Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1978; Fry et al., 1984, 1985), with estimates of $\varepsilon^{34}S_{S^\circ\text{-Sulfide}}$ and $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$ recently reported for *Chlorobaculum tepidum* (Zerkle et al., 2009). A value of 3‰ for $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ during SMAP ($\varepsilon^{34}S_{S^\circ\text{-Sulfide}}$ ~3‰, and $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$ ~0‰) by *A. vinosum* reported by Fry et al. (1984,1986) has been cited as evidence for anoxygenic phototrophic bacteria in ancient environments (Canfield and Raiswell, 1999). However, sulfate reduction under sulfate-limited conditions and chemolithotrophic sulfide oxidation each show similar $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values (Habicht et al., 1998; Brunner et al., 2008).

In order to clarify the ambiguity in sulfur isotope interpretations of the ancient rock record, an additional method to differentiate the metabolic processes is needed. One such method is using the minor sulfur isotopes ($\Delta^{33}S$ and $\Delta^{36}S$), which has been studied for PSR, elemental sulfur disproportionation, and SMAP (Farquhar et al., 2003, 2007, 2008; Johnston et al., 2005, 2007; Zerkle et al., 2009). However, the small changes in $\Delta^{33}S$ and $\Delta^{36}S$ seen in these experiments would be difficult to recognize relative to large $\Delta^{33}S$ and $\Delta^{36}S$ signals seen in the Archean due to the photolysis of SO_2 gas in an oxygen poor atmosphere (Farquhar et al., 2000; Farquhar and Wing, 2003; Ono et al., 2003; Catling and Claire, 2005). Instead, a complementary method may be the oxygen isotopes of sulfate. The microbial processes of sulfate reduction, elemental sulfur disproportionation, and chemolithotrophic sulfide oxidation have all been studied and show unique ranges for oxygen isotope

fractionation between H_2O and sulfate (Böttcher and Thamdrup, 2001; Böttcher et al., 2001; Balci, 2005; Brunner et al., 2005; Balci et al., 2007; Pisapia et al., 2007; Farquhar et al., 2008; Thurston et al., 2010).

The goal of this study was to refine our understanding of the $\delta^{18}O_{SO_4}$ values produced during SMAP. We measured both $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ and $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values during sulfide oxidation by *A. vinosum* and *C. tepidum* and compare the results to previous isotopic studies of microbial sulfur cycling. For these results to have value in studies of the early Earth, the challenge remains to find primary $\delta^{18}O_{SO_4}$ records in very old rocks, perhaps in ancient barite, apatite, anhydrite, or carbonate-associated sulfate—a subject of ongoing interest.

2. METHODS

2.1. Bacterial cultures

Bacterial cultures of a purple sulfur bacterium *A. vinosum*, strain D, and a green sulfur bacterium *C. tepidum*, strain TLS, were obtained from Dr. Michael T. Madigan, Department of Microbiology, Southern Illinois University, Carbondale, IL. *A. vinosum* and *C. tepidum* are well studied examples of purple and green sulfur bacteria that represent the two largest groups of bacteria that use SMAP. Continuous cultures of each bacterium were maintained in a modified Pfennig's media with $Na_2S{\cdot}9H_2O$ and $Na_2S_2O_3$ as sulfur sources and both CO_2 and acetate as carbon sources (Wahlund and Madigan, 1993). *A. vinosum* cultures were grown at 25 °C, pH ~7.0, and re-cultured once a month. *C. tepidum* cultures were grown at 45 °C, pH ~7.0, and re-cultured twice a month. Because both cultures are very pH sensitive, the range was always restricted to pH 6.9–7.1 and was steady throughout the incubations.

2.2. Bacterial experiments

2.2.1. Long term bacterial experiments

Prior to each experiment, all glassware and equipment were acid washed and autoclaved. All experiments were prepared in a Shell Labs Bactron Anaerobic/Environmental Chamber with 5% CO_2, 5% H_2, and 90% N_2 atmosphere using standard sterile techniques. The media used for the experimental cultures was modified for the isotopic experiments by omitting $Na_2S_2O_3$ and replacing $MgSO_4{\cdot}7H_2O$ with $MgCl_2{\cdot}6H_2O$ to minimize the sulfate blank. For each bacterium, we used three different waters that were evaporated to various degrees to change their $\delta^{18}O$ values (unmodified DI tap water, −15.1‰ and −14.5‰; 50% evaporated, −1.0‰ and −4.4‰; and 90% evaporated, 9.2‰ and 0.0‰ for *A. vinosum* and *C. tepidum* experiments, respectively). Only a small sulfate blank (<0.1 mM) was detected in the isotopically lightest and intermediate $\delta^{18}O$ experiments of *A. vinosum*. To minimize this blank, we replaced $Na_2S{\cdot}9H_2O$, which oxidizes readily during storage, with anhydrous Na_2S in the heavy water experiment for *A. vinosum* and for all *C. tepidum* experiments. For all experiments, the initial sulfate blank was insignificant relative to the amount of sulfate produced; therefore, no isotopic corrections were necessary. In fact, switching to anhydrous sulfide resulted in a sulfate blank that was below quantification by ion chromatography (IC) (<0.02 mM) and also minimized an interference during the iodine titrations used for sulfide concentration measurements, presumably due to contamination of the initial sulfide with thiosulfate. The concentration of Na_2S used for each experiment was ~1–2 mM. Experiments were adjusted to pH ~7.0 with 1 M HCl before inoculation with bacteria, and the pH was re-measured at the beginning of the experiments (*T*-0). Prior to inoculation, cells were spun down at 9000 rpm for 20 min into a pellet, decanted, and reconstituted using ~50 mL of experimental media that did not contain any sulfide. Serum bottles (500 mL) were each inoculated with 1 mL of this cell solution and filled completely with the experimental media to avoid any headspace gas.

The cultures were continuously shaken (150 rpm) and incubated in a New Brunswick Scientific Innova 4340 floor environmental shaker at 25.0 ± 0.2 °C for *A. vinosum* and 45.0 ± 0.2 °C for *C. tepidum* under fluorescent light that emitted wavelengths in the orange region. The experiments with *A. vinosum* were run for 25–30 days with duplicate time points taken approximately every 5 days. *C. tepidum* experiments were run for 6 days with duplicate time points taken at approximately daily intervals. Both cultures had abiotic controls (uninoculated media) that were incubated for the same length of time as the biological experiments.

The analytical scheme, as shown in Fig. 1, was carried out in the anaerobic chamber except for the final baking step. At each time point, one of the 500 mL serum vials from each duplicate culture was sacrificed. One milliliter from each vial was added to 4 mL of formaldehyde (2%) and stored for microscopic cells counts, which were made using a Petroff-Hauser counting chamber. The remaining sample was filtered (Millipore membrane, 0.2 μm, 47 mm), and a 50 ml aliquot reserved for iodometric determination of sulfide (Allen et al., 1991). Zinc acetate powder was added in excess to the remainder of this filtrate to precipitate the sulfide as ZnS, filtered, and rinsed several times with DI water before drying the ZnS precipitate. A ~10 mL aliquot was taken from this second filtrate for measurement of sulfate concentration (Fig. 1) using a Dionex-500 IC with an AG-17 guard column, AS-17 analytical column, and KOH as the eluent. IC measurements were made in the laboratory of Dr. Richard Wanty at the USGS in Denver, CO. To the remainder of the second filtrate, ~3 mL of 10% wt/wt $BaCl_2$ solution was added in excess to precipitate the $BaSO_4$, which was subsequently baked for 2 h at 500 °C as described previously (Mandernack et al., 2000).

2.2.2. Short-term bacterial experiments

In order to observe possible non-steady state conditions and any attendant kinetic isotope fractionation effects that occurred during the initial stages of sulfide oxidation by *C. tepidum,* short-term experiments were conducted with *C. tepidum* only, using the same procedure and analyses (cell counts, chemical) as described for the long-term bacterial experiments. However, time points were taken over a limited range of 3 days at approximately 6-h intervals, starting

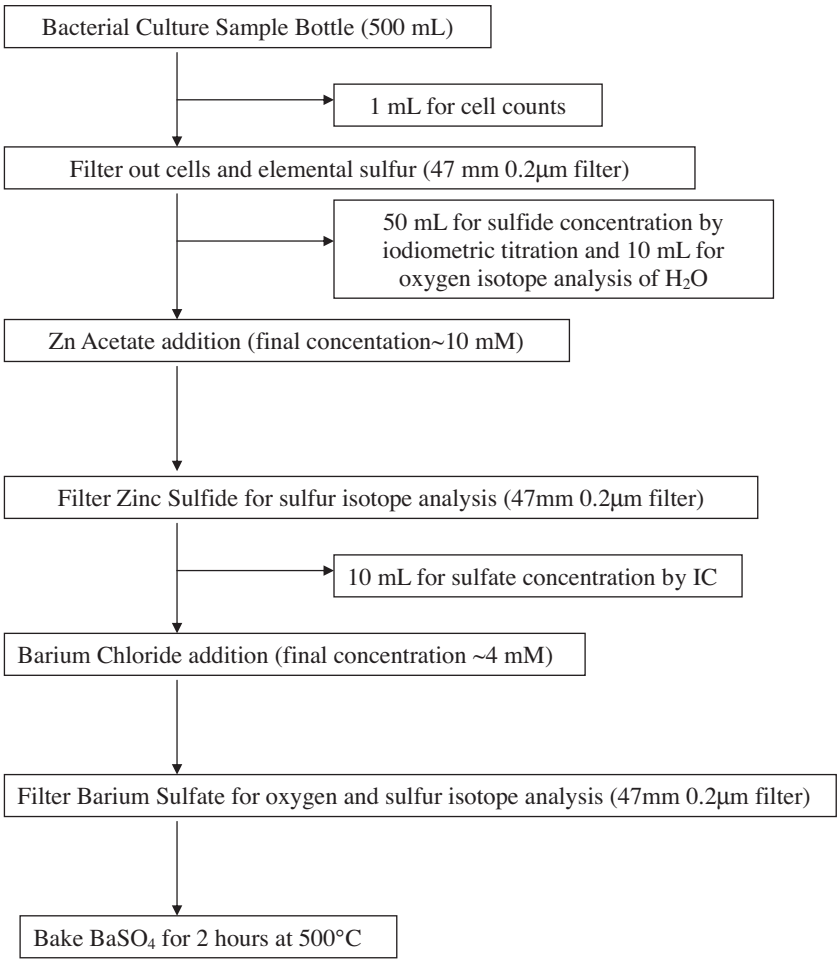

Fig. 1. Flow chart describing the procedure used at each sampling time point.

at 1.25 days. We conducted a second set of short-term experiments to evaluate any possible effects of carbon source on sulfur isotope fractionation by excluding ammonium acetate from the media, with carbonate/CO_2 remaining as the sole carbon source. Additional ammonium chloride was provided to compensate for the lower nitrogen content of the media.

2.3. Isotopic analysis

The zinc sulfide and $BaSO_4$ collected from the experiments was analyzed directly by isotope ratio mass spectrometry. Elemental sulfur was extracted from the filter membranes in the short-term experiments using acetone as described by Rice et al. (1993). The acetone was then evaporated at room temperature to precipitate the elemental sulfur, which was measured directly for $\delta^{34}S$, as described previously (Thurston et al., 2010). A quantitative measure of the elemental sulfur concentration was not possible due to mass loss during the transfer of the dried sulfur from the evaporation vessels during the extraction.

All isotope samples were analyzed at the Colorado School of Mines in Golden, CO, using a GV Instruments Isoprime stable isotope mass spectrometer. Sulfur isotope ratios were determined by continuous flow using a Eurovector Elemental Analyzer (EA). The sulfur isotopes are reported relative to Vienna Canyon Diablo Troilite (V-CDT) and have a standard deviation of $\pm 0.3‰$.

Oxygen isotope ratios for the sulfate were determined via pyrolysis using a Hekatech EA via the continuous flow method (Kornexl et al., 1999). The standard deviation for the $\delta^{18}O_{SO_4}$ analyses was $\pm 0.7‰$. Oxygen isotope ratios for the environmental waters were made by CO_2(g) equilibration with 200 μl aliquots of the media water at 40 °C in septum capped vials. The standard deviation for these samples was $\pm 0.2‰$. Oxygen isotope data are reported with respect to Vienna Standard Mean Ocean Water (VSMOW).

3. RESULTS

Oxidation of sulfide to sulfate by both *A. vinosum* and *C. tepidum* was almost complete by the end of the 30 and 6 day incubation experiments, respectively (Fig. 2a and b and Tables 1 and 2). All abiotic controls showed no evidence of sulfide oxidation, as indicated by the lack of detectable sulfate production or sulfide consumption during the incubation period (Tables 1 and 2). For *A. vinosum*, sulfate production tracked cell growth closely, indicating that both

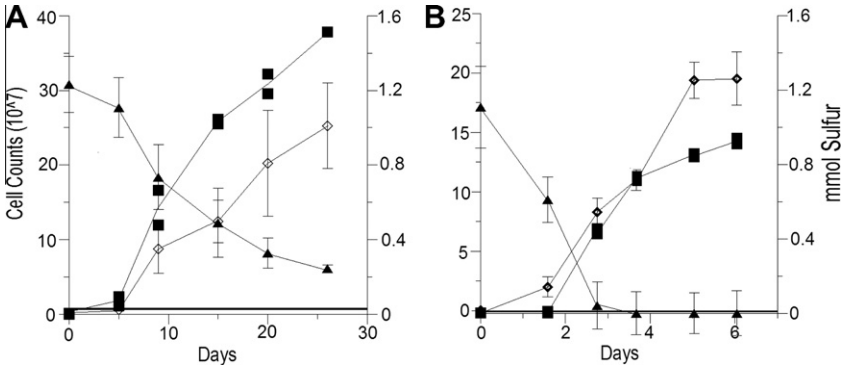

Fig. 2. Solution chemistry for a typical incubation experiment for: A. *A. vinosum* and B. *C. tepidum*. Error on sulfate measurements is ±5%. ▲ Sulfide, ■ sulfate, ◇ cell counts. Black horizontal line indicates ending sulfate concentration in abiotic control experiments.

Table 1
Solution Chemistry for all *A. vinosum* experiments.

Experiment	Day	Sulfide (mmol/L)	Sulfate (mmol/L)[a]	Cell counts (10^6 cells/mL)[b]
Light water	0	0.77 ± 0.14	bql[c]	0.03 ± 0.04
	5	0.77 ± 0.08	0.03	0.31 ± 0.15
	10	0.18 ± 0.08	1.23	17.03 ± 6.21
	15	0.13 ± 0.07	1.39	29.62 ± 4.48
	20	0.12 ± 0.08	1.52	32.30 ± 5.66
	25	0.10 ± 0.09,	1.60	36.69 ± 5.62
Abiotic control	25	0.76 ± 0.12	N.D.	N.A.
Medium water	0	1.2 ± 0.11	bql[c]	0.11 ± 0.04
	6	0.79 ± 0.08	0.13	2.62 ± 0.68
	10	0.27 ± 0.07	1.52	17.92 ± 6.51
	16	0.24 ± 0.05	1.60	33.58 ± 4.28
	20	0.23 ± 0.08	1.72	30.17 ± 14.11
	29	0.21 ± 0.10,	1.92	58.45 ± 3.40
Abiotic control	29	0.97 ± 0.18	N.D.	N.A.
Heavy water	0	1.23 ± 0.15	bql[c]	0.14 ± 0.04
	5	1.11 ± 0.15	0.07	0.59 ± 0.17
	9	0.73 ± 0.17	0.57	8.71 ± 3.24
	15	0.49 ± 0.18	1.03	12.43 ± 2.83
	20	0.32 ± 0.08	1.23	20.23 ± 7.10
	26	0.24 ± 0.02,	1.51	25.26 ± 5.74
Abiotic control	26	1.23 ± 0.14	N.D.	N.A.

N.D. = Not Detectable.
N.A. = Not Applicable.
[a] Average of the two sulfate measurements with instrument error of 5%.
[b] Average of two samples, measured in triplicate, for a total of six measurements.
[c] Below quantitative limit.

processes were coupled (Fig. 2a). Sulfide removal occurred at a rate similar to that of sulfate production on a molar basis, suggesting that intermediates such as elemental sulfur, thiosulfate, and polysulfides did not accumulate in appreciable amounts (Fig. 3). However, a precise mass balance for sulfur is complicated for the *A. vinosum* experiments for two reasons: (1) *A. vinosum* stores elemental sulfur intracellularly, which was trapped during the filtration of the cells, thus removing sulfur from the system, and (2) the difference between sulfide consumed and sulfate produced was significant for *A. vinosum*, particularly for the experiments with water of low and intermediate $\delta^{18}O$, for which the anhydrous Na_2S was not used (Table 1). This difference is likely due in large part to contamination of the $Na_2S{\cdot}9H_2O$ stock with thiosulfate or other partially oxidized sulfur species. Unlike *C. tepidum*, *A. vinosum* can easily oxidize thiosulfate to sulfate, which would result in an excess of sulfate compared to initial sulfide (Avrahami and Golding, 1968; Cline and Richards, 1969; Sorokin, 1970; Chen and Morris, 1972; Fry et al., 1988b; Brune, 1989; Dahl et al., 2005). However, because *A. vinosum* stores elemental sulfur intracellularly, it is also possible that additional oxidation of these stores could have contributed to the excess sulfate, particularly in the experiments with high $\delta^{18}O_{H_2O}$ performed with anhydrous Na_2S, where initial sulfate and thiosulfate was negligible.

Unlike *A. vinosum*, *C. tepidum* showed an obvious lag between sulfide consumption and sulfate production (Fig. 2b). This lag is from the accumulation of elemental sulfur as extracellular globules (Fig. 3, step a), which we observed under a microscope. The steady increase in cell count did not match the sulfide consumption or sulfate production exactly, but growth continued exponentially throughout the experiments. A direct mass balance was also complicated for *C. tepidum* because the extracellular sulfur globules were trapped during filtration of the cells, and, as previously noted, the extracted S^o was not measured quantitatively. Nonetheless, in contrast to *A. vinosum*, the difference between the sulfide consumed and sulfate produced was negligible for *C. tepidum* (Table 2).

Tables 3 and 4 summarize the sulfur isotope data for both *A. vinosum* and *C. tepidum*, respectively. The $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values we report are for the "net fractionation" between the initial sulfide and the final sulfate product and were determined first calculating α from the measured $\delta^{34}S$ values as follows:

$$\alpha = \frac{(\delta^{34}S_{SO_4} + 1000)}{(\delta^{34}S_{Sulfide} + 1000)},$$

from which ε was derived:

$$\varepsilon_{SO_4\text{-Sulfide}} = (\alpha - 1) * 1000.$$

With this notation, negative values correspond to fractionations that enrich the product (sulfate) with the lighter isotope (i.e., ^{32}S) relative to the reactant. The net

Table 2
Solution Chemistry for all *C. tepidum* experiments.

Experiment	Day	Sulfide (mmol/L)	Sulfate (mmol/L)[a]	Cell counts (10^6 cells/mL)[b]
Light water	0	1.11 ± 0.21	bql[c]	0.05 ± 0.02
	1.58	0.61 ± 0.12	0.05	2.01 ± 0.84
	2.75	0.04 ± 0.12	0.44	8.30 ± 1.17
	3.67	0.00 ± 0.11	0.72	10.99 ± 0.86
	5.04	0.00 ± 0.11	0.85	19.38 ± 1.53
	6.04	0.00 ± 0.12,	0.92	19.53 ± 2.23
Abiotic control	6.04	1.05 ± 0.15	N.D.	N.A.
Medium water	0	0.99 ± 0.05	bql[c,d]	0.03 ± 0.04
	1.71	0.59 ± 0.14	bql[c,d]	1.79 ± 0.72
	2.41	0.43 ± 0.08	0.08	6.54 ± 1.05
	3.58	0.08 ± 0.07	0.86	19.28 ± 1.67
	5.38	0.00 ± 0.06	0.97	19.98 ± 1.75
	6.00	0.00 ± 0.08,	1.09	20.53 ± 2.23
Abiotic control	6.00	0.97 ± 0.07	N.D.	N.A.
Heavy water	0	0.96 ± 0.13	bql[c,d]	0.07 ± 0.05
	1.54	0.63 ± 0.08	bql[c,d]	2.59 ± 0.93
	2.54	0.47 ± 0.11	0.75	9.04 ± 1.22
	3.58	0.05 ± 0.06	0.93	19.59 ± 1.47
	5.12	0.00 ± 0.07	0.99	19.87 ± 1.14
	6.08	0.00 ± 0.08,	1.02	20.15 ± 1.97
Abiotic control	6.08	1.03 ± 0.14	N.D.	N.A.

N.D. = Not Detectable.
N.A. = Not Applicable.
[a] Average of the two sulfate measurements with instrument error of 5%.
[b] Average of two samples, measured in triplicate, for a total of six measurements.
[c] Below quantitative limit.
[d] Another instrument was used (<0.05 mmol/L detection limit).

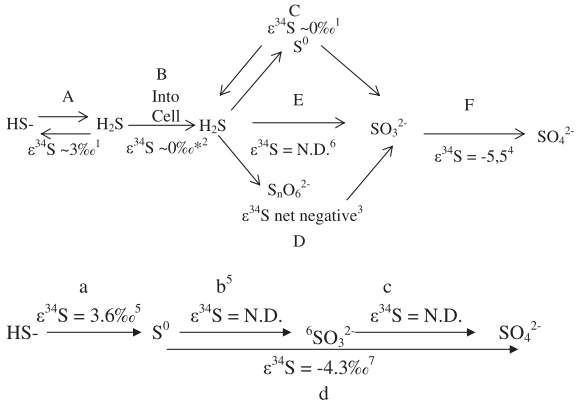

Fig. 3. The proposed pathway of sulfide oxidation by *A. vinosum* (top) and *C. tepidum* (bottom) with the measured and assumed fractionations from each step. Asterisk indicates assumed values. All values are based on a temperature of 30 °C and pH of 7.0 for *A. vinosum* and 45 °C and pH of 7.0 for *C. tepidum*. [1]Fry et al. (1984); [2]Zerkle et al. (2009); [3]Kaplan and Rittenberg (1964); [4]Fry et al. (1985); [5]This step is simplified in this diagram. For a complete discussion of this step, see Frigaard and Dahl (2009) or Brune (1989); [6]Sulfite intermediate is presumed based on known enzymology (see Section 4.2.2 of text); [7]This study; N.D. = Not Determined.

fractionation of sulfur isotopes we report here might result from different mechanisms, such as (1) the fractionation expressed during a rate-limiting step of sulfide oxidation or (2) the fractionation expressed at more than one competing step of oxidation. We specifically used this notation with the intention of distinguishing between microbial processes of sulfur cycling and measured $\Delta^{34}S_{SO_4\text{-Sulfide}}$ values of the sedimentary rock record or in environmental samples, with the assumption that the $\Delta^{34}S_{SO_4\text{-Sulfide}}$ values might reflect $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values for specific microbial processes. Thus, if the intent is to discern prokaryotic sulfate reduction from sulfide oxidation, for which the products and reactants are opposite, use of a consistent nomenclature is essential, such as $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ (see Section 4.3).

There is no sulfur isotope fractionation during sulfide oxidation by *A. vinosum* beyond analytical uncertainty (Fig. 4a). This lack of fractionation was observed at a few discrete time points of each of the three experiments shown in Table 1 (light water, 10 days; medium water, 10 days; and Heavy water, 15 days), where $\delta^{34}S$ values of the co-existing sulfide and sulfate did not vary beyond analytical error (data not shown). In the long-term experiment with *C. tepidum*, using water with the lowest $\delta^{18}O$ value, the first time point for which the sulfate concentration was sufficiently high to recover a measurable quantity of barium sulfate (day 3) yielded a $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ value of $\sim -3.0‰$ (Fig. 4b). The time points following this initial fractionation show only very small sulfur isotope fractionations, with an average $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ of $-0.7 \pm 0.3‰$ ($n = 13$), (Table 3). Because these latter time points generally coincided with the nearly complete consumption of the sulfide, it is possible that the diminished isotopic fractionation resulted from substrate limitation. The short-term experiments for *C. tepidum* showed a fractionation similar to the initial value of the first long-term experiment, with an $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ value of $-2.3 \pm 0.6‰$ ($n = 11$; Table 4). A $\Delta^{34}S_{SO_4\text{-Sulfide}}$ value of $-2.7‰$ was also measured for the co-existing sulfide and sulfate at 2.42 days from a long-term light water experiment (Table 4). This was the only time point from all of the *C. tepidum* experiments where concentrations were high enough to measure the $\delta^{34}S$ of both sulfate and sulfide. Correspondingly, the $\varepsilon^{34}S_{S^\circ\text{-Sulfide}}$ value was estimated to be $3.6 \pm 0.5‰$ ($n = 7$; Table 5) for the short-term experiments of *C. tepidum,* both in the presence and absence of acetate. Values for $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$ could not be calculated directly due to insufficient S° yields at the time points with sufficient sulfate for analysis. However, using the average $\delta^{34}S$ values of the sulfate and elemental sulfur throughout the incubations as an approximation, we estimated $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$ values of $-5.5‰$ and $-4.3‰$ for the short-term and long-term experiments, respectively.

A graph of $\delta^{18}O_{SO_4}$ versus $\delta^{18}O_{H_2O}$ yields a slope of ~1 for both cultures, confirming that all of the oxygen in sulfate is derived from water as expected under anaerobic conditions (Fig. 5). The *y*-intercept of the combined plots for *A. vinosum* and *C. tepidum* is a close approximation of the average enrichment factor ($\varepsilon^{18}O_{SO_4\text{-}H_2O}$) for all experiments (Balci et al., 2007). The linear best fit for these combined data is defined by the equation $y = (0.99 \pm 0.03) + (5.6 \pm 0.3)$,

Table 3
Summary of $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ and $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values measured in this study.

Experiment	$\delta^{18}O_{H_2O}$	*n*	$\delta^{18}O_{SO_4}$	*n*	$\varepsilon^{18}O_{SO_4\text{-}H_2O}$	$\varepsilon^{34}S_{SO_4\text{-Sulfide}}$	*n*
A. vinosum							
Light water	-15.1 ± 0.1	9	-9.1 ± 0.4	8			
Medium water	-1.0 ± 0.1	8	3.5 ± 0.4	8	5.6 ± 0.2[a]	0.1 ± 0.2	12
Heavy water	9.2 ± 0.2	4	15.6 ± 0.4	7			
C. tepidum							
Light water	-14.5 ± 0.1	6	-8.5 ± 0.5	8			
Medium water	-4.4 ± 0.2	4	1.0 ± 0.2	6	5.4 ± 0.1[a]	-0.7 ± 0.3	20
Heavy water	0.0 ± 0.2	6	5.4 ± 0.4	8			

Note: The $\delta^{34}S_{Sulfide}$ was 12.1 ± 0.3‰ for *A. vinosum* light and medium waters, 0.9 ± 0.3‰ for *A. vinosum* heavy water and 0.0 ± 0.4‰ for all *C. tepidum* experiments.
[a] Value is derived from an average of all three experiments using linear regression (see Fig. 5).

Table 4
$\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ and $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values and corresponding sulfate concentrations during early stages of sulfide oxidation by *C. tepidum.*

Metabolism	Sulfate (mmol/L)	$\varepsilon^{34}S_{SO_4\text{-Sulfide}}$	Average $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$	$\varepsilon^{18}O_{SO_4\text{-}H_2O}$[b]
CO_2/acetate[a]	0.33	−2.9	-2.3 ± 0.5	5.3 ± 0.3
CO_2/acetate[a]	0.45	−2.5		
CO_2/acetate[a]	0.47	−2.7		
CO_2/acetate[b]	0.43	−1.6		
CO_2/acetate[b]	0.45	−1.4		
CO_2/acetate[a]	0.42	−3.2		
CO_2/acetate[a]	0.47	−2.8		
CO_2[b]	0.30	−2.1		
CO_2[b]	0.28	−2.0		
CO_2[b]	0.48	−2.2		
CO_2[b]	0.47	−1.8		

[a] Bacterial long term experiments.
[b] Bacterial short term experiments.

$r^2 = 1.00$, thereby providing a robust estimate of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ of 5.6 ± 0.3‰ during sulfide oxidation by SMAP. Despite the 20 °C difference in incubation temperature, individual linear regressions for the *A. vinosum* and *C. tepidum* experiments yield very similar $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values (Fig. 5).

4. DISCUSSION

4.1. Sulfur isotope fractionation

4.1.1. Comparison to other SMAP studies

There have been few studies of sulfur isotope fractionation during sulfide oxidation by SMAP (Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1978), although two studies specifically used *C. tepidum* and *A. vinosum* (Fry et al., 1984; Zerkle et al., 2009). However, none of these studies examined oxygen isotope fractionation during the oxidation of sulfide to sulfate. Zerkle et al. (2009) assessed sulfur isotope fractionation by *C. tepidum* grown at 40 °C with a sulfide concentration of 2–4 mM during the two major steps in sulfide oxidation: (1) sulfide to elemental sulfur and (2) elemental sulfur to sulfate (Fig. 3, steps a and d). The authors observed fractionations of 1.8 ± 0.5‰ and -1.9 ± 0.8 for $\varepsilon^{34}S_{S^\circ\text{-Sulfide}}$ and $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$, respectively, which differ from the corresponding values of 3.6 ± 0.5‰ and −5.5‰ estimated in our study (or the $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$ value of −4.3‰ estimated from our long-term experiments). There is no standard deviation available for $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$ values because sulfur and sulfate were not detectable at the same time, precluding a direct comparison. Instead, $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$ values were estimated using the average $\delta^{34}S$ value of S° and sulfate from the various experiments. Although the values reported here exceed even the largest values reported by Zerkle et al. (2009) (~2.5‰ and −3.0‰ for $\varepsilon^{34}S_{S^\circ\text{-Sulfide}}$ and $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$, respectively), both studies show fractionation effects with similar shifts at both steps of sulfide oxidation by *C. tepidum*, which similarly explain the small net fractionation for both steps combined.

Given that S° is the only known sulfur intermediate of *C. tepidum* (Brune, 1989; Zerkle et al., 2009; Fig. 3), the net fractionation under steady-state conditions should equal the individual fractionations of each reaction step, satisfying the following equation:

$$\varepsilon^{34}S_{SO_4\text{-Sulfide}} = \varepsilon^{34}S_{S^\circ-\text{Sulfide}} + \varepsilon^{34}S_{SO_4-S^\circ} \quad (1)$$

By adding the $\varepsilon^{34}S_{S^\circ-\text{Sulfide}}$ (3.6‰) and $\varepsilon^{34}S_{SO_4\text{-}S^\circ}$ (−4.3‰) values calculated from the short- and long-term experiments, respectively, an overall $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ value of *C. tepidum* is calculated as −0.7‰, which is identical to the value we measured directly under the long-term

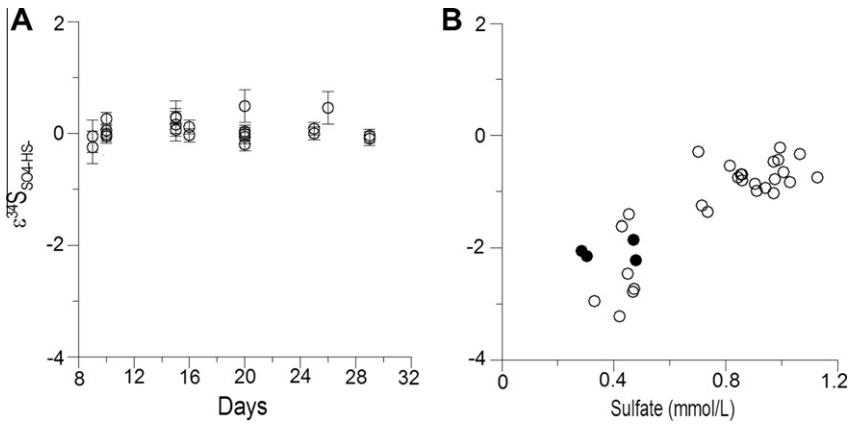

Fig. 4. Plot of $\varepsilon^{34}S_{SO_4\text{-}HS^-}$ for all $\delta^{18}O_{H_2O}$ experiments of: A. *A. vinosum* and B. *C. tepidum*. *A. vinosum* is graphed versus time while *C. tepidum* is graphed versus sulfate concentration. Open symbols for *C. tepidum* indicate long term experiments while closed symbols indicate short term experiments. Error bars were omitted in *C. tepidum* for clarity, but are identical to *A. vinosum*.

Table 5
$\delta^{34}S$ values of elemental sulfur during the short term experiments with *C. tepidum*.

Carbon source	$\delta^{34}S_{HS^-}$	*n*	$\delta^{34}S^{\circ}_{S}$	$\varepsilon^{34}S_{S^{\circ}\text{-Sulfide}}$	Average $\varepsilon^{34}S_{S^{\circ}\text{-Sulfide}}$
CO_2/acetate	0.54 ± 0.4	6	4.70	−4.15	3.6 ± 0.5
CO_2/acetate			4.06	−3.52	
CO_2/acetate			4.18	−3.64	
CO_2			4.72	−4.18	
CO_2			4.58	−4.04	
CO_2			3.39	−2.84	
CO_2			3.57	−3.02	

incubations. This agreement may indicate a steady-state system with minimal net fractionation for the overall oxidation of sulfide to sulfate by *C. tepidum*. However, as previously noted, the smaller $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ value in the longer-term experiments could also have resulted from substrate limitation as the sulfide became more depleted.

The average $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ value of $-2.3 \pm 0.5‰$ observed from the short-term and initial stages of the long-term incubations (Table 4), when sulfate concentrations were low (<0.5 mmol/L) (Fig. 4b), is consistent with the maximum value of $\varepsilon^{34}S_{SO_4\text{-}S^{\circ}}$ (−3.0‰) reported by Zerkle et al. (2009). This fractionation might reflect initial non-steady state conditions, possibly as a result of the lag between the individual steps of oxidation (Fig. 3). This experimental observation is relevant to natural environments, as the extracellular S^{o} may not remain in contact with the cells that produced it, thus expressing the initial fractionation effect if the S^{o} is preserved in the (paleo-)environment, or if it is subsequently oxidized to sulfate independently from the bacteria without isotopic fractionation.

Past workers have proposed that the overall control of sulfur isotope fractionation during SMAP is the equilibrium exchange of sulfur between H_2S and HS^- in solution and subsequent preferential uptake of H_2S by *A. vinosum* (Fry et al., 1984). If the bacterium preferentially uses H_2S, the fractionation at pH 7 and 30 °C might be expected to have a $\varepsilon^{34}S_{H_2S\text{-}HS^-}$ of ~3‰ (Fry et al., 1984, and Fig. 3), barring additional kinetic effects. Fry et al. (1984) and Zerkle et al. (2009) observed maximum fractionations of ~3‰

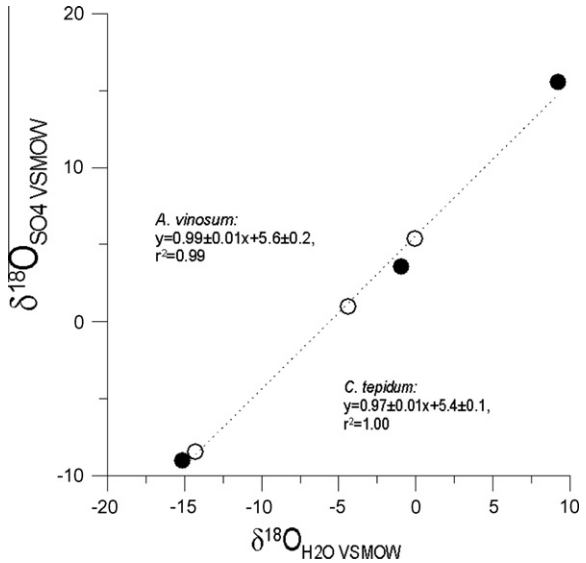

Fig. 5. Plot of $\delta^{18}O_{SO_4}$ versus $\delta^{18}O_{H_2O}$ for both the *A. vinosum* (closed) and *C. tepidum* (open) experiments. Error bars were smaller than the symbols and, therefore, were not shown. The linear regression for all experiments combined, as shown by the dotted line, is $y = 0.99 \pm 0.03x + 5.6 \pm 0.3$, $r^2 = 1.00$. For comparison, regressions for the individual experiments are shown on the figure.

for $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ and $\varepsilon^{34}S_{S^\circ\text{-Sulfide}}$. However, a maximum fractionation for $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ of ~3‰ was not observed for SMAP in other studies (Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1978; and Table 6). Zerkle et al. (2009) attributed this to further equilibrium exchange with other sulfur intermediates inside the cell, resulting in values less than 3‰, but fractionations of more than 3‰ have been observed (Table 6). Furthermore, the incubation experiments of Zerkle et al. (2009) and Fry et al. (1984) were conducted at 40 and 30–35 °C, respectively, and, as noted by Sakai (1968), the equilibrium exchange of H_2S/HS^- is highly dependent on temperature. The inconsistent results among the various studies suggest that preferential uptake of H_2S by the bacteria does not fully explain the net sulfur isotope fractionation seen during SMAP and that kinetic effects may also play a role.

Although the values and differences for $\varepsilon^{34}S_{SO4\text{-Sulfide}}$ reported in this study and by Fry et al. (1984) for *A. vinosum* are small (0.1 ± 0.2‰ versus 1–3‰) and perhaps untraceable in the rock record, they may carry important metabolic implications and thus deserve further discussion. For example, the carbon source can affect sulfur isotope fractionation during prokaryotic sulfate reduction (PSR) when it serves as the electron donor (Detmers et al., 2001). Although organics do not serve as an electron donor in the case of SMAP, we conducted experiments to ascertain whether the carbon source (±acetate) might in anyway influence sulfur isotopic fractionation during SMAP. Fry et al. (1984) used CO_2 as the only carbon source in a defined media designated as NCMS. We grew *C. tepidum* with HCO_3^-, CO_2, and acetate and can provide partial evidence that carbon source has negligible influence on $\varepsilon^{34}S$ values during SMAP—specifically the very similar $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values observed during the short-term experiments with *C. tepidum* (-2.1 ± 0.2‰, $n = 4$ versus -2.3 ± 0.6‰, $n = 7$ for CO_2 only and CO_2 + acetate, respectively). However, because *A. vinosum* uses a pathway of carbon fixation (Calvin-Benson reductive pentose cycle) that differs from that of *C. tepidum* (reductive tricarboxylic acid cycle) (Evans et al., 1966; Brune, 1989), we cannot eliminate the possibility that carbon sources might effect sulfur isotope fractionation by *A. vinosum,* although it is unlikely.

Again, past and present studies of SMAP confirm that $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values are small (0–3‰) (Fry et al., 1984, 1985; Zerkle et al., 2009; this study) but important, reflecting diverse metabolic and environmental controls. The small differences in $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values observed by *A. vinosum* in various studies may be the formation of sulfur intermediates, as previously suggested by Fry et al. (1984, 1985), which are more diverse in *A. vinosum* than in *C. tepidum* (Brune, 1989, 1995). The relative impacts of the various influences on $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ may, however, be difficult to decipher in the geologic record. In contrast, $\delta^{18}O_{SO_4}$ values, controlled primarily by oxygen isotope fractionation with the source water, may serve as a more straightforward paleoenvironmental proxy.

4.1.2. Comparison to prokaryotic sulfate reduction

The $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values for sulfide oxidation are usually distinct from those reported for prokaryotic sulfate reduction (PSR). PSR shows a very wide range of $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ (see Section 3 for definition of nomenclature) values of 0–47‰ (Kaplan and Rittenberg, 1964; Bolliger et al., 2001; Detmers et al., 2001; among many other references), with values typically >10‰, and often much greater. Based on $\varepsilon^{34}S$ estimates alone, however, sulfide oxidation cannot be

Table 6
Summary of sulfur isotope fractionation during oxidation of sulfur species to sulfate.

Substrate	Experiment/Bacteria	$\varepsilon^{34}S_{SO_4\text{-Substrate}}$ (‰)	Reference
Sodium sulfide	*Allochromatium vinosum*	0.1[a]	This study
Sodium sulfide	*Chlorobaculum tepidum*	−0.7[a]	This study
Chalcopyrite	*Acidithiobacillius ferrooxidans*, oxidation by/$Fe(III)_{aq}$	−3.8	Thurston et al. (2010)
Sphalerite	*A. ferrooxidans*, oxidation by $Fe(III)_{aq}$	−2.6	Balci et al. (2012)
Sphalerite	*A. ferrooxidans*, oxidation by O_2	0.0	Balci et al. (2012)
Pyrite	*A. ferrooxidans,* oxidation by O_2	−0.1	Balci et al. (2007)
Pyrite	*A. ferrooxidans*, oxidation by O_2	3.5, −1.7[b]	Brunner et al. (2008)
Pyrite	*A. ferrooxidans,* oxidation by O_2	−1.3, 0.4[c]	Pisapia et al. (2007)
Sodium sulfide	*Chlorobium vibrioforme*	2.4	Fry et al. (1988a)
Sodium sulfide	Abiotic, oxidation by O_2	−5.2	Fry et al. (1988b)
Hydrogen sulfide	Anaerobic, abiotic	−2.6	Lewis and Krouse (1969)
Hydrogen sulfide	*Thiobacillius concretivorus*	−13.2 to −10.6	Kaplan and Rittenberg (1964)
Hydrogen sulfide	*Chromatium* sp.	−2.9	Kaplan and Rittenberg (1964)
Hydrogen sulfide	*Chromatium* sp.	−10	Kaplan and Rittenberg (1964)
Hydrogen sulfide	*Chromatium thiosulfatophilum*	5	Chambers and Trudinger (1978)
Elemental sulfur	*A. ferrooxidans*, oxidation by O_2	−1.8, −1.1[b]	Balci et al. (2012)
Elemental sulfur	*A. vinosum*	0.2	Fry et al. (1984)
Sodium sulfide	*A. vinosum*	1–3	Fry et al. (1984)
Sulfite	*A. vinosum*	−5, 5[b]	Fry et al. (1985)
Thiosulfate	*A. vinosum*	0	Fry et al. (1985)

[a] Represent $\Delta^{34}S_{HS\text{-}SO_4}$ values.
[b] Initial value, final value.
[c] Stoichiometric, non-stoichiometric.

easily distinguished from the net fractionation observed during sulfate reduction under sulfate-limited conditions such as those inferred for the Archean ocean, which average $0.7 \pm 5.2‰$ (Habicht et al., 2002). Therefore, $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ may serve as an additional and useful tool to discern the microbial processes responsible for the isotopic signatures of sulfate preserved in the geological record.

4.2. Oxygen isotope fractionation

The $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ measured in this study ($5.6 \pm 0.3‰$) falls within the range reported from previous studies of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ during anaerobic and aerobic oxidation of reduced sulfur species (Table 7). The similarity of the $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values observed for sulfide oxidation by a purple and green sulfur bacterium is intriguing given the large differences in their phylogenies and the incubation temperatures of 25 and 45 °C, respectively. Most oxygen isotope fractionations in aqueous systems are highly temperature dependent. The apparent lack of temperature dependence observed in this study seems to reflect a reaction that is kinetically/enzymatically controlled. Therefore, for sulfate derived from SMAP, $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values that are largely independent of temperature may tie unambiguously to the $\delta^{18}O$ of the source waters.

Although it may seem counterintuitive that a microbially-mediated reaction could yield a product (sulfate) that is isotopically enriched in ^{18}O relative to a reactant (water), all microbially mediated reactions essentially occur in an open aqueous system, where water is effectively a limitless reservoir. It is not practical for a product, or its precursor, that undergoes oxygen isotope exchange with water to be isotopically lighter than the water itself. In this regard, a kinetic oxygen isotope effect should be regarded as a negative shift in the product from what might be expected purely from equilibrium isotope effects, which for sulfate and water at 25 °C is approximately 25‰ (Brunner et al., 2005; Farquhar et al., 2008).

4.2.1. Comparison of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values from SMAP with other forms of sulfide oxidation

Stable isotopic studies of aerobic metal sulfide oxidation are relevant to the current study as they reveal that most or all of the oxygen in the resulting sulfate was derived from the ambient water (Balci, 2005; Balci et al., 2007; Thurston et al., 2010). The $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values from such studies have ranged from 2.0‰ to 9.5‰ (Taylor et al., 1984; Balci, 2005; Balci et al., 2007, 2012; Pisapia et al., 2007; Brunner et al., 2008; Mazumdar et al., 2008; Thurston et al., 2010). However, most of the measured $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values fall at the upper or lower end of this range. Our values, in contrast, fall in the middle of this range, suggesting that there might be a unique isotopic fingerprint for SMAP.

$\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values of ~2–4‰ are generally observed during oxidation of pyrite (Table 7). Similarly, an $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value of 3.8‰ was measured during anaerobic oxidation of chalcopyrite with $Fe(III)_{aq}$ as the sole oxidant (Thurston et al., 2010). The similarity in oxygen isotope fractionation during aerobic oxidation of pyrite and oxidation of chalcopyrite by $Fe(III)_{aq}$ may result from $Fe(III)_{aq}$ serving as the primary oxidant in each system. Aerobic oxidation of chalcopyrite with O_2 as an oxidant showed higher apparent

Table 7
Summary of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values during oxidation and disproportionation of sulfur species.

Substrate	Experiment/bacteria	$\varepsilon^{18}O_{SO_4\text{-}H_2O}$ (‰)	Reference
	Oxidation		
Sodium Sulfide	A. vinosum/C. tepidum	5.6	This study
Chalcopyrite	*A. ferrooxidans,* oxidation by O_2(aq)	6.5[e]	Thurston et al., (2010)
Chalcopyrite	Abiotic and biological, oxidation by $Fe(III)_{aq}$	3.8	Thurston et al., (2010)
Sphalerite	*A. ferrooxidans*, oxidation by O_2(aq)	9.5[e]	Balci et al. (2012)
Sphalerite	Oxidation by $Fe(III)_{aq}$	8.2	Balci et al. (2012)
Sphalerite (Fe-bearing)	Oxidation by $Fe(III)_{aq}$	7.5	Balci et al. (2012)
Pyrite	*A. ferrooxidans* oxidation by $Fe((III)_{aq}$	3.6	Balci et al. (2007)
Pyrite	Abiotic, oxidation by $Fe((III)_{aq}$	2.9	Balci et al. (2007)
Pyrite	*A. ferrooxidans* oxidation by O_2(aq)	4.1–4.7[e]	Balci et al., (2007)
Pyrite	Abiotic, oxidation by O_2(aq)	2.8	Balci et al., (2007)
Pyrite	*A. ferrooxidans* oxidation by O_2(aq), (200,100 μm) BEM[a], ETM[b]-NS[c]	16.0, 16.3	Pisapia et al., (2007)
Pyrite	*A. ferrooxidans* O_2(aq) oxidation, (100, 200 μm) BEM[a], ETM[b]-S[d]	2.0, 5.6	Pisapia et al., (2007)
Elemental sulfur	*A. ferrooxidans*/oxidation by O_2	8.3	Balci et al. (2012)
	Disproportionation		
Elemental sulfur[e]	*Desulfocapsa thiozymogenes*/Mn oxidation	8–12	Böttcher and Thamdrup (2001)
Elemental sulfur[e]	*Desulfocapsa thiozymogenes*/Fe oxidation	17.4	Böttcher et al., (2001)

[a] BEM – bulk equation model.
[b] ETM – electron transferred model.
[c] NS – non-stoichiometric.
[d] S – stoichiometric.
[e] May reflect incorporation of trace O_2.

values for $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ of 6.3–6.5‰ (Thurston et al., 2010). However, these higher values may have resulted in part from incorporation of trace quantities of atmospheric O_2, which is relatively ^{18}O-enriched (Thurston et al., 2010).

Aerobic and anaerobic oxidation of sphalerite, as well as bacterial oxidation of elemental sulfur by O_2, also showed a relatively large $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value of ~8‰ (Balci, 2005; Balci et al., 2012). Studies of bacterial pyrite oxidation have occasionally shown $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values similar to those reported for sphalerite, with initial $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values of ~10‰ that evolved to 2–3‰ with prolonged incubation (Brunner et al., 2008; Ziegler et al., 2008). Taylor et al. (1984) also reported a large $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value of 18‰ during the aerobic oxidation of pyrite. However, these measurements were made from wet/dry experiments where the solution was periodically drained from the pyrite and may have been subjected to ^{18}O enrichment from evaporation of H_2O or to O_2 incorporation (Taylor et al., 1984). One study looked at the oxygen isotope fractionation at the pyrite surface during bacterial oxidation (Pisapia et al., 2007). Large $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values of ~16‰ were measured if one assumes non-stoichiometric sulfide oxidation (i.e., initial bacterial growth), while $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values of 2–5.6‰ were reported for stoichiometric conditions (late bacterial growth) that were in closer agreement with most other studies of pyrite and chalcopyrite oxidation (Balci et al., 2007; Brunner et al., 2008; Mazumdar et al., 2008; Thurston et al., 2010). We observed no difference in the $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values during the initial non-steady state conditions (i.e., short term incubations) with *C. tepidum*. Furthermore, O_2 incorporation into sulfate is not possible during SMAP, given the anaerobic conditions. Therefore, sulfate preserved in the geological record formed through SMAP can track the source water.

4.2.2. Possible controls of oxygen isotope fractionation during SMAP

The mechanisms by which oxygen isotopes can be fractionated are dominated by either equilibrium or kinetic controls. The $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value of 5.6‰ observed in this study is different from a value of ~25‰ calculated for equilibrium exchange between sulfate and water at 30 °C and neutral pH (Fritz et al., 1989; Brunner et al., 2005), conditions similar to our experiments. Furthermore, the oxygen isotope equilibrium exchange rate between sulfate and water at experimental temperatures of 25–45 °C and neutral pH is on the order of 10^7–10^9 years (Chiba and Sakai, 1985). Our distinct value and the temperature independence, therefore, point to a kinetic effect.

It is generally accepted that most equilibrium exchange between sulfate oxygen and water actually occurs via sulfur intermediates, such as thiosulfate and sulfite, produced by either sulfide oxidation or sulfate reduction. The $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value during PSR can approach the equilibrium value of ~25‰ due to re-oxidation of sulfite to sulfate, which can occur spontaneously even under anaerobic conditions (Fritz et al., 1989). Sulfite is known to exchange oxygen with water at a much faster rate than sulfate at room temperatures (Lloyd, 1968), with $\varepsilon^{18}O_{SO_3\text{-}H_2O}$ values of 8–11‰ depending on temperature and pH (Brunner et al., 2006). Balci et al. (2012) observed $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values of ~8‰ during oxidation of sphalerite and elemental sulfur and suggested that exchange between sulfite and water may be controlling the overall oxygen isotope fractionation. However, equilibrium exchange between sulfite and extracellular water is likely not an important factor during sulfide oxidation by C. *tepidum* due to the impermeability of the cell membrane to sulfite (Brune, 1995). Alternatively, it is possible that intracellular water might exchange isotopically with a sulfite intermediate. Kreuzer-Martin et al. (2005) observed that intracellular water in *E. coli* is isotopically distinct from extracellular water by as much as −8.9‰ to 13.8‰. Given this variability, it is unlikely that exchange between sulfite and intracellular water could have resulted in such a consistent $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value for both of our cultures. Furthermore, *C. tepidum* is only known to form detectable amounts of S°, but not sulfite, as an extracellular intermediate of sulfide oxidation (Brune, 1989, 1995). Therefore oxygen isotope fractionation during SMAP is not likely to be controlled by a sulfoxyl intermediate and is probably kinetically controlled.

A recent study argued that sulfur isotope fractionation during prokaryotic sulfate reduction is controlled by a dissimilatory sulfite reductase (DSR) enzyme, which reduces sulfite to elemental sulfur (Mangalo et al., 2008). This enzyme controls sulfite accumulation, which is also thought to be a controlling factor of oxygen isotope fractionation due to re-oxidation of sulfite to sulfate during PSR (Mitzutani and Rafter, 1973; Brunner et al., 2005; Mangalo et al., 2007). The DSR enzyme is also found in *C. tepidum* and *A. vinosum*, where it is believed to operate in reverse by oxidizing elemental sulfur to sulfite (Fischer, 1984; Trüper, 1984; Brune, 1989; Dahl et al., 2005), though a complete understanding of this pathway is still lacking. Although the evidence does not support sulfite as a controlling intermediate of the $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value, it is nevertheless possible that DSR exerts kinetic control of the oxygen isotope fractionation between sulfate and water during sulfide oxidation by SMAP, perhaps during the final oxidation step when sulfite is oxidized to sulfate. If such enzymatic control exists, this may also explain the apparent lack of any temperature effect.

Other enzymes that may control the oxidation of sulfide to sulfate in *A. vinosum* and *C. tepidum* are APS reductase and ATP sulfurylase, which in tandem are believed to control the oxidation of sulfite to sulfate (Brune, 1989; Kappler and Dahl, 2001; Eisen et al., 2002). However, *A. vinosum* also contains the sulfite: acceptor oxidoreductase enzyme, which oxidizes sulfite to sulfate (Brune, 1989; Kappler and Dahl, 2001). A competition between the two enzyme systems in *A. vinosum* has been invoked to explain the −5‰ to 5‰ range in $\varepsilon^{34}S_{SO_3\text{-Sulfide}}$ values reported by Fry et al. (1985), but the extent to which it might affect oxygen isotope fractionation is not known (Brune, 1989). The APS reductase/ATP sulfurylase system could explain the similarity of the $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values by *A. vinosum* and *C. tepidum*. However, the presence of sulfite: acceptor oxidoreductase in *A. vinosum* and specifically its competition with APS reductase/ATP sulfurylase in controlling sulfur isotope fractionation argues against the APS reductase/ATP sulfurylase

system as the rate-limiting factor in oxygen isotope fractionation during sulfide oxidation by SMAP.

During abiotic and biotic oxidation of acid-insoluble metal sulfides (pyrite), it is thought that the controlling step of oxygen isotope fractionation is the breaking of water molecules by $Fe(III)_{aq}$ (Luther, 1987; Moses et al., 1987; Balci et al., 2007). However, during abiotic and biotic oxidation of acid-soluble metal sulfides (e.g., sphalerite) and elemental sulfur it is thought that the controlling step of oxygen isotope fractionation is the attachment of an oxygen atom to the surface sulfur atoms (Biegler and Swift, 1979; Balci et al., 2007). This attachment may explain the differences in $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ during the oxidation of metal sulfides and elemental sulfur versus sulfide oxidation during SMAP. During chemolithotrophic oxidation of elemental sulfur, the sulfur exists in the solid S_8 form (Balci, 2005; Balci et al., 2012). However, elemental sulfur is stored intracellularly in *A. vinosum* not as solid S_8, but either as polysulfide (S_n, $n \geq 2$), a hydrated form of S_8, or as liquid sulfur (Hageage et al., 1970; Guerrero et al., 1984; Mas and Van Gemerden, 1987; Brune, 1989; Prange et al., 1999). In the case of *C. tepidum*, the extracellular stores of sulfur globules are also not strictly in the form of S_8, rather they exist as S_8 cores with a polysulfide shell (Brune, 1989). The oxygen binding energy of all these forms of sulfur would differ from that of solid S_8 and could affect the fractionation of oxygen isotopes during sulfate formation. This may explain why our $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values are different from those previously reported for the oxidation of acid soluble and acid insoluble metal sulfides (Balci et al., 2007, 2012; Thurston et al., 2010).

4.2.3. Using sulfur and oxygen isotopes to detect sulfide oxidation

As can be seen in Fig. 6, sulfur isotopes are inadequate to differentiate chemolithotrophic sulfide oxidation, sulfide oxidation by SMAP, sulfur disproportionation, and sulfate reduction under sulfate-limited conditions. Oxygen isotopes, on the other hand, can provide meaningful insight into the biological processes that form sulfate. Large $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values normally associated with PSR generally distinguish it easily from sulfide oxidation. However, under extreme sulfate limitation, as existed during the Archean for example, $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ values would predictably be smaller and perhaps indistinguishable from sulfide oxidation—although PSR shows $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values that are generally larger than those observed from sulfide oxidation and can therefore be used to distinguish PSR from sulfate formed by SMAP. Pure culture studies with sulfate reducing prokaryotes have shown $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values averaging from ~9‰ to 15‰ (Brunner et al., 2005); however, experimental and field studies have both shown an oxygen isotope fractionation as large as ~25–30‰ due to recycling (Farquhar et al., 2008). Two additional studies of sulfide oxidation showed $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values that fall within the range reported for sulfate reducing prokaryotes (Taylor et al., 1984; Pisapia et al., 2007). The large $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values reported by Pisapia et al. (2007) were only possible under non-stoichiometric conditions, when fractionation effects are expressed during the initial and transient stages of oxidation, which are not likely to be preserved in the geological record (Fig. 6). Furthermore, as previously noted, the large $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values reported by Taylor et al. (1984) may have resulted from either O_2 incorporation and/or water evaporation and may not be an accurate measure of this fractionation. Therefore, $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values do show promise for distinguishing PSR from sulfide oxidation, particularly oxidation via SMAP (Fig. 6).

During disproportionation of elemental sulfur to sulfate and sulfide, $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ ranges from 8‰ to 17.4‰ as a function of the presence of Mn(IV), which can react with sulfide without readily capturing it, unlike Fe(II) (Böttcher and Thamdrup, 2001; Böttcher et al., 2001). More recently, Böttcher et al. (2005) reported a larger value of ~21‰ for $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ during disproportionation. It has been suggested that the larger $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values for sulfur

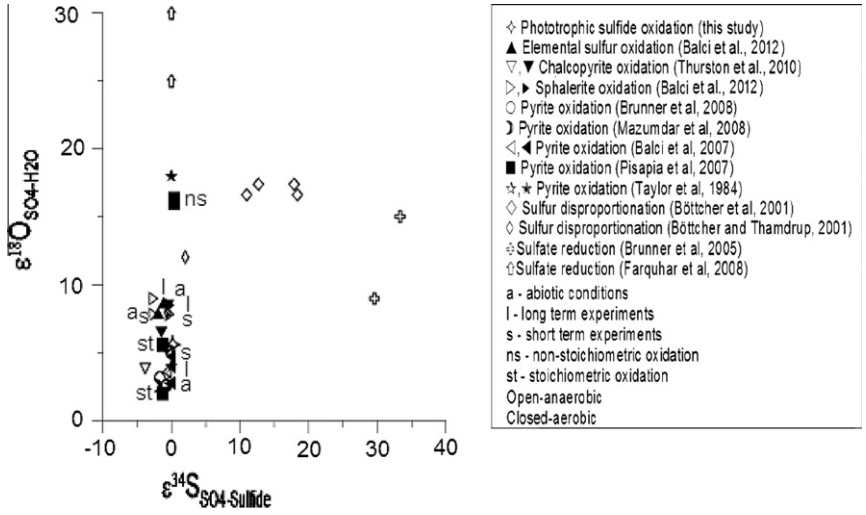

Fig. 6. Plots of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ versus $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$ from various studies of sulfide/sulfur oxidation, sulfur disproportionation, and sulfate reduction in which both sulfur and oxygen isotopes were measured. Note that all fractionations are in the form $\varepsilon^{34}S_{SO_4\text{-Sulfide}}$.

disproportionation and PSR relative to sulfide oxidation are due to re-oxidation of the resultant sulfide and sulfur intermediates, either by microbial or by abiotic processes (Aharon and Fu, 2000; Mandernack et al., 2003; Brunner et al., 2005; Farquhar et al., 2008). The $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values reported during disproportionation overlap with those in the upper-most range reported for sulfide oxidation (~9‰) (Böttcher and Thamdrup, 2001) but are distinctly higher than what we observe here for SMAP (Fig. 6). Therefore, SMAP can be distinguished from PSR and disproportionation based on measured $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values.

4.3. Sulfur and oxygen isotope fractionation in modern and ancient environments

In recent years, a wide range of analytical approaches has been used to explore bacterial sulfur cycling in the ancient rock record, such as measurements of the minor isotopes of sulfur ($\Delta^{33}S$ and $\Delta^{36}S$) in Proterozoic strata (Johnston et al., 2005, 2006, 2008). However, during the Archean when large $\Delta^{33}S$ and $\Delta^{36}S$ values were produced by mass independent fractionations during photolysis of SO_2 in an oxygen-poor atmosphere, the small fingerprints of microbial sulfur cycling should be difficult to detect in the rock record (Farquhar et al., 2000; Ono et al., 2003). From this study, however, we show that when paleo-$\delta^{18}O_{H_2O}$ values can be assessed independently, $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values can help distinguish among sulfide oxidation, sulfur disproportionation, and sulfate reduction pathways and may be useful for assessing microbial sulfur cycling in the rock record. Estimates of ancient $\delta^{18}O_{H_2O}$ are extremely difficult to extract from the rock record. The growing strength of new paleothermometers (e.g., Ghosh et al., 2006; Eiler, 2007), however, when paired with parallel $\delta^{18}O$ measurements of exceptionally well preserved biogenic calcite or apatite, might allow for independent estimates of $\delta^{18}O_{H_2O}$.

Framvaren Fjord and Saanich inlet are examples of modern marine basins that contain relatively shallow euxinic zones where phototrophic sulfur-oxidizing bacteria have been detected (Millero, 1991; Mandernack and Tebo, 1999; Mandernack et al., 2003; Wakeham et al., 2007). Although $\delta^{34}S_{SO_4}$ values have been measured widely in such environments (e.g., Sweeny and Kaplan, 1980; Anderson et al., 1988; Calvert et al., 1996), few $\delta^{18}O_{SO_4}$ measurements have been reported. In the case of Famvaren Fjord, there is a relative shift in both $\delta^{34}S_{SO_4}$ (~−2‰) and $\delta^{18}O_{SO_4}$ (~1‰) near the oxic/anoxic interface (Mandernack et al., 2003). The shift to lower $\delta^{34}S_{SO_4}$ values at the interface may indicate sulfide oxidation. The sulfide at the interface has a $\delta^{34}S$ value of −19‰, while lower in the water column the value is ~−3–4‰. It is possible that phototrophic sulfide oxidizers were responsible for shifting the $\delta^{34}S_{SO_4}$ at the interface due to the ^{34}S-depleted sulfide at that depth, but given the $\delta^{18}O_{H_2O}$ value of −3.6‰ and $\delta^{18}S_{SO_4}$ value of 10.4‰, there is less isotopic leverage for affecting the background $\delta^{18}O_{SO_4}$ value. However, given the $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value of +5.6 ‰ measured in this study and the $\delta^{18}O_{H_2O}$ value of Framvaren fjord, it is difficult to explain only a 1‰ enrichment in $\delta^{18}O_{SO_4}$ at the interface of Framvaren resulting solely from sulfide oxidation by SMAP.

In contrast to the oxic-anoxic interface of Framvaren fjord, sulfate reduction dominates in the deeper waters of this fjord and overrides the $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ signatures. PSR can exert the primary influence on $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ values in modern environments; however, sulfide oxidation may also be detected from depleted $\delta^{34}S_{SO_4}$ and enriched $\delta^{18}O_{SO_4}$ values in modern systems and perhaps in the ancient rock record.

Although $\delta^{34}S_{SO_4}$ values have been used to indicate phototrophic sulfur oxidation in the Archean (Buick and Dunlop, 1990), $\delta^{18}O_{SO_4}$ measurements have never been applied for this purpose to very old rocks. The sulfur isotope data for sulfate in the geologic record are abundant (Holser and Kaplan, 1966; Claypool et al., 1980; Ueda et al., 1991; Bottomley et al., 1992; Shields et al., 1999, 2004; Gorjan et al., 2000; Strauss et al., 2001; Hurtgen et al., 2002; Kah et al., 2004; Kampschulte and Strauss, 2004; Gellatly and Lyons, 2005; Chu et al., 2007). However, dual sulfur and oxygen isotope measurements of sulfate are far less common (Newton et al., 2004; Goldberg et al., 2005; Turchyn et al., 2009; John et al., 2010; Newton et al., 2011). This disparity in part reflects the difficulty in estimating the paleo-$\delta^{18}O_{H_2O}$, which is ultimately necessary for any quantitative interpretation of $\delta^{18}O_{SO_4}$. Another great challenge is the long-term preservation of primary $\delta^{18}O_{SO_4}$ during burial.

Recently, analysis of $\delta^{34}S_{SO_4}$ carbonate associated sulfate (CAS) contained in the lattice structure of carbonates has been developed (e.g., Burdett et al., 1989; Kaiho et al., 2001; Kah et al., 2004) and offers a potentially powerful tool to explore the origins of sulfate in the Archean oceans and during other periods of the geologic past (Newton et al., 2004). Although diagenesis is a concern for the S data, analysis of the $\delta^{18}O$ presents a particular challenge, such as the overprints during shallow and deep burial (e.g., Gill et al., 2008) that haunt many studies of oxygen isotopes in old rocks.

The $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ values of both CAS and evaporite deposits from the Cambrian and Neoproterozoic record prokaryote sulfate reduction, although a few samples suggest otherwise (Goldberg et al., 2005). Of particular interest, CAS of Tommotian age (~530 million years ago) show a small (1–4‰) enrichment in $\delta^{34}S_{SO_4}$, while the $\delta^{18}O_{SO_4}$ values were unusually low (0–9‰). Goldberg et al. (2005) interpreted this relationship to indicate sulfide oxidation. Although the exact $\delta^{18}O_{H_2O}$ value of seawater is unknown during the Tommotian, if the modern value of 0‰ is assumed, a value of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ of 0–9‰ is estimated. This value falls within the range of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ reported for sulfide oxidation by chemolithotrophs and SMAP (this study and elsewhere, Table 7).

Newton et al. (2004) examined $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ in rocks spanning the late Permian to early Triassic when oceanic anoxia is assumed by many to have been widespread and, at least partly, shallow. The CAS showed variations in $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ across the Permian/Triassic boundary, with a distinct shift towards enriched $\delta^{34}S_{SO_4}$ (11.5‰) and $\delta^{18}O_{SO_4}$ (20‰) values at the Permian mass extinction boundary. This shift is followed by a large enrichment in $\delta^{34}S_{SO_4}$ (26.9‰) and $\delta^{18}O_{SO_4}$ values that again show marked

depletion (14‰), perhaps reflecting a return of oxic conditions. The shift at the extinction boundary is interpreted to reflect re-oxidation of sulfide. It is difficult to attribute the re-oxidation of sulfide to a specific process because $\delta^{18}O_{H_2O}$ values are not known, precluding estimates of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$. Also, PSR can severely overprint the $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$, as in the bottom waters of Framvaren fjord (Mandernack et al., 2003). However, extensive sulfide oxidation by green sulfur bacteria is suggested at the P/T boundary (Grice et al., 2005), inviting further investigation of $\delta^{18}O_{SO_4}$.

More recently, Turchyn et al. (2009) compared $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ values of CAS and barite deposits of the middle Cretaceous. It was thought that CAS might provide more temporally continuous record of seawater sulfate. Although the CAS and barite records followed the same general trends, the CAS record showed more variation, pointing to the possibility of diagenetic overprint resulting from subsequent sulfur cycling. Roughly coincident with an anoxic event in the mid-Cretaceous, the $\delta^{18}O_{CAS}$ dropped from ~18–20‰ to ~4–6‰. If ocean water during the mid-Cretaceous had a modern $\delta^{18}O_{H_2O}$ value of 0‰, this drop might then be attributed to sulfide oxidation by SMAP or the biotic/abiotic oxidation of pyrite. During another suspected anoxic event, these same authors observed a shift in $\delta^{18}O_{SO_4}$ in CAS of 16–9‰. Assuming bacterial activity was the main factor, their modeled calculations suggest this shift in $\delta^{18}O_{SO_4}$ might have resulted from a change in $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ of ~18–20‰, during which bacterial sulfide oxidation was dominant, to an $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ of ~5–6‰ when sulfide oxidation was less pronounced. Based on the results of our study and others (Table 7), however, a $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value of ~18–20‰ is consistent with more reducing conditions when bacterial sulfur disproportionation and/or sulfate reduction were more active, whereas a $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value of ~5–6‰ might reflect oxidation of pyrite or SMAP.

Studies of the $\delta^{18}O$ and $\delta^{34}S$ of modern and ancient sulfate show that PSR can overprint the isotopic expressions of other processes in a given environment. However, sulfide oxidation can be detected from depleted $\delta^{34}S_{SO_4}$ and enriched $\delta^{18}O_{SO_4}$ signals, and as previously noted (Turchyn et al., 2009), might remain isotopically intact in some sections of the geological record despite PSR. Assuming primary seawater $\delta^{18}O_{SO_4}$ values can be obtained, SMAP signals might reveal themselves more cleanly during the Archean when a comparatively large proportion of marine sulfate might have derived from this pathway—particularly in light of recent evidence suggesting common euxinic conditions on the margins of the Archean ocean (Reinhard et al., 2009; Kendall et al., 2010; Scott et al., 2011). Furthermore, conditions for SMAP may have been optimized by the likely widespread and possibly shallow euxinia of the Proterozoic (Brocks et al., 2005; Johnston et al., 2009). Finally, delivery of sulfate to the ocean via oxidative weathering of sulfide minerals (e.g., pyrite) on the continents in the presence of strongly ^{18}O-depleted meteoric waters would yield $\delta^{18}O_{SO_4}$ properties very different from those linked to SMAP in seawater. As such, records of exceptional preservation might further constrain the sources and timing of sulfate delivery to the early ocean and their ties to early atmospheric oxygenation—particularly across the Great Oxidation Event ca. 2.4 billion years ago. Many hurdles remain, particularly the difficulty in estimating the isotopic composition of ambient water linked to ancient SMAP, the confounding overprints of PSR, and the challenges in capturing and preserving archives of primary $\delta^{18}O_{SO_4}$. The potential rewards, however, easily eclipse the risks of further study.

5. CONCLUSIONS

Both *A. vinosum* and *C. tepidum* oxidize sulfide stepwise from sulfide to elemental sulfur to sulfite and ultimately to sulfate. However, *A. vinosum* does this as a continuous process, while *C. tepidum* oxidizes almost all the sulfide completely before proceeding to the next step of oxidation. The two different pathways to sulfide oxidation may explain the difference in sulfur isotope fractionation between the two bacteria. The data for *A. vinosum* shows almost no sulfur isotope fractionation effect ($\varepsilon^{34}S_{SO_4\text{-Sulfide}} = 0.1 \pm 0.2$‰). We interpret this as resulting from a short residence time of sulfur intermediates within the cell. In sum, the accumulation of various sulfur intermediates seems to control sulfur isotope fractionation in *A. vinosum*.

Sulfur isotope fractionation by *C. tepidum* was also small ($\varepsilon^{34}S_{SO_4\text{-Sulfide}} = -0.7 \pm 0.3$‰) and reflects its two major steps of sulfide oxidation: sulfide to elemental sulfur and elemental sulfur to sulfate. However, there was an initial fractionation effect ($\varepsilon^{34}S_{SO_4\text{-Sulfide}} = -2.3 \pm 0.6$‰) during the earliest stages of oxidation indicating a kinetic isotope effect under these non-steady state conditions. Both bacteria exhibit sulfur isotope fractionations that fall within the range previously reported for abiotic metal sulfide oxidation, bacterial oxidation of elemental sulfur, and sulfate-limited prokaryotic sulfate reduction.

Oxygen isotope fractionation ($\varepsilon^{18}O_{SO_4\text{-}H_2O}$ of 5.6 ± 0.3‰) was identical for both bacteria despite differences in their phylogeny and experimental incubation temperatures. This $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ value is lower than that observed for bacterial oxidation of elemental sulfur (~8–9‰) and is larger than values of ~3–4‰ reported for biological and abiotic pyrite oxidation. Importantly, the range of $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values reported for sulfide oxidation (2–9‰) is distinct from $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ calculated for sulfur disproportionation and sulfate reduction. These differences in $\varepsilon^{18}O_{SO_4\text{-}H_2O}$ values might assist in interpretation of $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ measurements in modern and ancient systems, especially at times and in places where rates of PSR were inhibited by extremely low sulfate concentrations, such as in the Archean ocean. On the other hand, these lower concentrations and thus the shorter residence times also mean that sulfate is turned over and potentially overprinted more rapidly by bacterial cycling.

While this study has provided insight into the sulfur and oxygen isotope fractionations caused by green and purple sulfur bacteria during experimental anoxic sulfide oxidation, the utility of any paleoenvironmental proxy will also require further study in natural settings—as informed by the experimental work. Additionally, studies of green non-sulfur bacteria would be especially useful as they were

the earliest bacteria to use SMAP for sulfide oxidation and therefore could potentially provide insight into the earliest stages of bacterial sulfur cycling.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Rich Wanty at the USGS in Denver, Colorado for sulfate analyses by ion chromatography and John Humphrey at the Colorado School of Mines for assistance with analysis of water oxygen, sulfate oxygen, and sulfur isotope analyses. Our thanks to Michael Madigan for providing the bacterial cultures and his guidance regarding culturing techniques, and to Simon Botrell, Aubrey Zerkle and an anonymous reviewer for their helpful and constructive comments. A NASA Exobiology grant to TL and KM funded this work.

.

REFERENCES

Aharon P. and Fu B. (2000) Microbial sulfate reduction rates and sulfur and oxygen isotope fractionations at oil and gas seeps in deepwater Gulf of Mexico. *Geochim. Cosmochim. Acta* **64**, 233–246.

Allen H. E., Fu G., Boothman W., DiToro D. M. and Mahony J. D. (1991) Draft analytical method for determination of acid volatile sulfide in sediment. In *EPA-821-R-91–100*. Environmental Protection Agency, pp. 1–22.

Anderson L. G., Dyrssen D. and Hall P. O. J. (1988) On the sulphur chemistry of a super-anoxic fjord, Framvaren, South Norway. *Mar. Chem.* **23**, 283–293.

Avrahami M. and Golding R. M. (1968) The oxidation of sulfide ion at very low concentrations in aqueous solutions. *J. Chem. Soc. A*, 647–651.

Balci N. (2005). Assessment of biogeochemical influence on metal sulfide and Fe(II) oxidation as inferred from oxygen, sulfur and Fe isotopes. Ph. D. thesis, Istanbul Technical University.

Balci N., Mandernack K. W., Shanks, III, W. C., Mayer B. and Gemery-Hill P. (2007) Oxygen and sulfur isotope systematics of sulfate produced by bacterial and abiotic oxidation of pyrite. *Geochim. Cosmochim. Acta* **71**, 3796–3811.

Balci N., Mayer B., Shanks W. C. and Mandernack K. W. (2012) Oxygen and sulfur isotope systematics of sulfate produced during abiotic and bacterial oxidation of sphalerite and elemental sulfur, Geochem. *Cosmochim. Acta.* **77**, 335–351.

Bekker A., Holland H. D., Wang P. L., Rumble, III, D., Stein H. J., Hannah J. L., Coetzee L. L. and Buekes N. J. (2004) Dating the rise of atmospheric oxygen. *Nature* **427**, 117–120.

Biegler T. and Swift D. A. (1979) Anodic behavior of pyrite in acid solutions. *Electrochim. Acta* **24**, 415–420.

Bolliger C., Schroth M. H., Bernasconi S. M., Kleikemper J. and Zeyer J. (2001) Sulfur isotope fractionation during microbial sulfate reduction by toluene-degrading bacteria. *Geochim. Cosmochim. Acta* **65**, 3289–3298.

Böttcher M. E. and Thamdrup B. (2001) Anaerobic sulfide oxidation and stable isotope fractionation associated with bacterial sulfur disproportionation in the presence of MnO_2. *Geochim. Cosmochim. Acta* **65**, 1573–1581.

Böttcher M. E., Thamdrup B. and Vennemann T. W. (2001) Oxygen and sulfur isotope fractionation during anaerobic bacterial disproportionation of elemental sulfur. *Geochim. Cosmochim. Acta* **65**, 1601–1609.

Böttcher M. E., Thamdrup B., Gehre M. and Thuene A. (2005) $^{34}S/^{32}S$ and $^{18}O/^{16}O$ fractionation during sulfur disproportionation by *Desulfobulbus propionicus*. *Geomicrobiol. J.* **22**, 219–226.

Bottomley D. J., Veizer J., Nielsen H. and Moczydlowska M. (1992) Isotopic composition of disseminated sulfur in Precambrian sedimentary rocks. *Geochim. Cosmochim. Acta* **56**, 3311–3322.

Bottrell S. H. and Newton R. J. (2006) Reconstruction of changes in global sulfur cycling from marine sulfate isotopes. *Earth Sci. Rev.* **75**, 59–83.

Brocks J. J. and Schaeffer P. (2008) Okenane, a biomarker for purple sulfur bacteria (Chromatiaceae), and other new carotenoid derivatives from the 1640 Ma Barney Creek Formation. *Geochim. Cosmochim. Acta* **72**, 1396–1414.

Brocks J. J., Buick R., Logan G. A. and Summons R. E. (2003) Composition and syngeneity of molecular fossils from the 2.78 to 2.45 billion-year-old Mount Bruce Supergroup, Pilbara Craton, Western Australia. *Geochim Cosmochim. Acta* **67**, 4289–4319.

Brocks J. J., Love G. D., Summons R. E., Knoll A. H., Logan G. A. and Bowden S. A. (2005) Biomarker evidence for green and purple sulphur bacteria in a stratified Paleoproterozoic sea. *Nature* **437**, 866–870.

Brune D. C. (1989) Sulfur oxidation by phototrophic bacteria. *Biochim. Biophys. Acta* **975**, 189–221.

Brune D. C. (1995) Sulfur compounds as photosynthetic electron donors. In *Anoxygenic Photosynthetic Bacteria* (eds. R. E. Blankenship, M. T. Madigan and C. E. Bauer). Kluwer Academic Publishers, Dordrecht¸The Netherlands, pp. 847–870.

Brunner B., Bernasconi S. M., Kleikemper J. and Schroth M. H. (2005) A model for oxygen and sulfur isotope fractionation in sulfate during microbial sulfate reduction processes. *Geochim. Cosmochim. Acta* **69**, 4773–4785.

Brunner B., Mielke R. E. and Coleman M. (2006) Abiotic oxygen isotope equilibrium fractionation between sulfite and water. *AGU Fall Meeting*. American Geophysical Union, San Francisco. #V11C-0601 (abstr.).

Brunner B., Jae-Young Y., Mielke R. A., MacAskill J. A., Madzunkov S., McGenity T. J. and Coleman M. (2008) Different isotope and chemical patterns of pyrite oxidation related to lag and exponential growth phases of *Acidithiobacillus ferrooxidans* reveal a microbial growth strategy. *Earth Planet. Sci. Lett.* **270**, 63–72.

Buick R. and Dunlop J. S. R. (1990) Evaporitic sediments of Archean age from the Warrawoona Group, North Pole, Western Australia. *Sedimentology* **37**, 247–277.

Burdett J. W., Arthur M. A. and Richardson M. (1989) A neogene seawater sulfur isotope age curve from calcareous pelagic microfossils. *Earth Planet. Sci. Lett.* **94**, 189–198.

Calvert S. E., Thode H. G., Yeung D. and Karlin R. E. (1996) A stable isotope study of pyrite in the Late Pleistocene and Holocene sediments of the Black Sea. *Geochim. Cosmochim. Acta* **60**, 1261–1270.

Canfield D. E. (1998) A new model for Proterozoic ocean chemistry. *Nature* **396**, 450–453.

Canfield D. E. (2005) The early history of atmospheric oxygen: Homage to Robert M. Garrels. *Annu. Rev. Earth Planet. Sci.* **33**, 1–36.

Canfield D. E. and Raiswell R. (1999) The evolution of the sulfur cycle. *Am. J. Sci.* **299**, 697–723.

Canfield D. E. and Teske A. (1996) Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulphur-isotope studies. *Nature* **382**, 127–132.

Canfield D. E., Habicht K. S. and Thamdrup B. (2000) The Archean sulfur cycle and the early history of atmospheric oxygen. *Science* **288**, 658–661.

Catling D. C. and Claire M. W. (2005) How earth's atmosphere evolved to an oxic state: a status report. *Earth Planet. Sci. Lett.* **237**, 1–20.

Chambers L. A. and Trudinger P. A. (1978) Microbiological fractionation of sulfur. *Geomicrobiology* **1**, 249–293.

Chen K. Y. and Morris J. C. (1972) Kinetics of oxidation of aqueous sulfide by O_2. *Environ. Sci. Technol.* **6**, 529–537.

Chiba H. and Sakai H. (1985) Oxygen isotope exchange rate between dissolved sulfate and water at hydrothermal temperatures. *Geochim. Cosmochim. Acta* **45**, 993–1000.

Chu X., Zhang T., Zhang Q. and Lyons T. W. (2007) Sulfur and carbon isotope records from 1700 and 800 Ma carbonates of the Jixian section, northern China: implications for secular isotope variations in Proterozoic seawater and relationships to global supercontinent events. *Geochim. Cosmochim. Acta* **71**, 4668–4692.

Claypool G. E., Holser W. T., Kaplan I. R., Sakai H. and Zak I. (1980) The ages curves of sulfur and oxygen isotopes in marine sulfate and their mutual interpretation. *Chem. Geol.* **28**, 199–260.

Cline J. D. and Richards F. A. (1969) Oxygenation of hydrogen sulfide in seawater at constant salinity, temperature, and pH. *Environ. Sci. Technol.* **3**, 838–843.

Dahl C., Engels S., Pott-Sperling A. S., Schulte A., Sander J., Lübbe Y., Deuster O. and Brune D. C. (2005) Novel genes of the *dsr* gene cluster and evidence for close interactions of dsr proteins during sulfide oxidation in the phototrophic sulfur bacterium *Allochromatium vinosum*. *J. Bacteriol.* **187**, 1392–1404.

Detmers J., Brüchert V., Habicht K. S. and Kuever J. (2001) Diversity of sulfur isotope fractionations by sulfate-reducing prokaryotes. *Appl. Environ. Microbiol.* **67**, 888–894.

Eiler J. M. (2007) "Clumped-isotope" geochemistry – the study of naturally-occurring, multiplysubstituted isotopologues. *Earth Planet. Sci. Lett.* **262**, 309–327.

Eisen J. A., Nelson K. E., Paulsen I. T., Heidelberg J. F., Wu M., Dodson R. J., Gwinn M. L., Nelson W. C., Haft D. H., Hickey E. K., Peterson J. D., Durkin A. S., Kolonay J. L., Yang F., Holt I., Umayam L. A., Mason T., Brenner M., Shea T. P., Parksey D., Nierman W. C., Feldblyum T. V., Hansen C. L., Craven M. B., Radune D., Vamathevan J., Khouri H., White O., Gruber T. M., Ketchum K. A., Venter J. C., Tettelin H., Bryant D. A. and Fraser C. M. (2002) The complete genome sequence of *Chlorobium tepidum TLS*, a photosynthetic, anaerobic, green-sulfur bacterium. *Proc. Nat. Acad. Sci.* **99**, 9509–9514.

Evans M. C. W., Buchanan B. B. and Arnon D. I. (1966) A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium. *Proc. Nat. Acad. Sci.* **55**, 928–934.

Farquhar J. and Wing B. A. (2003) Multiple sulfur isotopes and the evolution of the atmosphere. *Earth Planet. Sci. Lett.* **213**, 1–13.

Farquhar J., Bao H. M. and Thiemens M. (2000) Atmospheric influence of Earth's earliest sulfur cycle. *Science* **289**, 756–758.

Farquhar J., Johnston D. T., Wing B. A., Habicht K. S., Canfield D. E., Airieau S. A. and Thiemens M. H. (2003) Multiple sulfur isotopic interpretations of biosynthetic pathways: implications for biological signatures in the sulfur isotope record. *Geobiology* **1**, 15–27.

Farquhar J., Johnston D. T. and Wing B. A. (2007) Implications of conservation of mass effects on mass-dependent isotope fractionations: influence of network structure on sulfur isotope phase space of dissimilatory sulfate reduction. *Geochim. Cosmochim. Acta* **71**, 5862–5875.

Farquhar J., Canfield D. E., Masterson A., Bao H. and Johnston D. (2008) Sulfur and oxygen isotope study of sulfate reduction in experiments with natural populations from Fællestrand, Denmark. *Geochim. Cosmochim. Acta* **72**, 2805–2821.

Fischer U. (1984) Cytochromes and iron sulfur proteins in sulfur metabolism of phototrophic sulfur bacteria. In *Sulfur, its Significance for Chemistry, for the Geo-, Bio-, and Cosmosphere and Technology, Studies in Inorganic Chemistry*, 5 (eds. A. Müller and B. Krebs). Elsevier, Amsterdam, pp. 83–407.

Frigaard N. and Dahl C. (2009) Sulfur metabolism in phototrophic sulfur bacteria. *Adv. Microbial. Phys.* **54**, 103–200.

Fritz P., Basharmal G. M., Drimmie R. J., Ibsen J. and Qureshi R. M. (1989) Oxygen isotope exchange between sulphate and water during bacterial reduction of sulphate. *Chem. Geol. (Isot. Geosci. Sect.)* **79**, 99–105.

Fry B., Gest H. and Hayes J. M. (1984) Isotope effects associated with the anaerobic oxidation of sulfide by the purple photosynthetic bacterium, *Chromatium vinosum*. *FEMS Microbiol. Lett.* **22**, 283–287.

Fry B., Gest H. and Hayes J. M. (1985) Isotope effects associated with the anaerobic oxidation of sulfite and thiosulfate by the photosynthetic bacterium, *Chromatium vinosum*. *FEMS Microbiol. Lett.* **27**, 227–232.

Fry B., Cox J., Gest H. and Hayes J. M. (1986) Discrimination between ^{34}S and ^{32}S during bacterial metabolism of inorganic sulfur compounds. *J. Bacteriol.* **165**, 328–330.

Fry B., Gest H. and Hayes J. M. (1988a) $^{34}S/^{32}S$ fractionation in sulfur cycles catalyzed by anaerobic bacteria. *Appl. Environ. Microbiol.* **54**, 250–256.

Fry B., Ruf W., Gest H. and Hayes J. M. (1988b) Sulfur isotope effects associated with oxidation of sulfide by O_2 in aqueous solution. *Chem. Geol. (Isot. Geosci. Sect.)* **73**, 205–210.

Gellatly A. M. and Lyons T. W. (2005) Trace sulfate in mid-Proterozoic carbonates and the sulfur isotope record of biospheric evolution. *Geochim. Cosmochim. Acta* **69**, 3813–3829.

Ghosh P., Adkins J., Affek H., Balta B., Guo W., Schauble E. A., Schrag D. and Eiler J. M. (2006) ^{13}C–^{18}O bonds in carbonate minerals: a new kind of paleothermometer. *Geochim. Cosmochim. Acta* **70**, 1439–1456.

Gill B. C., Lyons T. W. and Frank T. D. (2008) Behavior of carbonate-associated sulfate during meteoric diagenesis and implications for the sulfur isotope paleoproxy. *Geochim. Cosmochim. Acta* **72**, 4699–4711.

Goldberg T., Poulton S. W. and Strauss H. (2005) Sulphur and oxygen isotope signatures of late Neoproterozoic to early Cambrian sulphate, Yangtze Platform, China: diagenetic constraints and seawater evolution. *Precambrian Res.* **137**, 223–241.

Gorjan P., Veevers J. J. and Walter M. R. (2000) Neoproterozoic sulfur-isotope variation in Australia and global implications. *Precambrian Res.* **100**, 151–179.

Grice K., Cao C., Love G. D., Böttcher M. E., Twitchett R. J., Grosjean E., Summons R. E., Turgeon S. C., Dunning W. and Jin Y. (2005) Photic zone euxinia during the Permian-Triassic superanoxic event. *Science* **307**, 706–709.

Guerrero R., Mas J. and Pedró-Alió C. (1984) Bouyant density changes due to intracellular content of sulfur in *Chromatium warmingii* and *Chromatium vinosum*. *Arch. Microbiol.* **137**, 350–356.

Guo Q., Strauss H., Kaufman A. J., Schröder S., Gutzmer J., Wing B., Baker M. A., Bekker A., Jin Q., Kim S. T. and Farquhar J. (2009) Reconstructing Earth's surface oxidation across the Archean-Proterozoic transition. *Geology* **37**, 399–402.

Habicht K. S., Canfield D. E. and Rethmeier J. (1998) Sulfur isotope fractionation during bacterial sulfate reduction and disproportionation of thiosulfate and sulfite. *Geochim. Cosmochim. Acta* **62**, 2585–2595.

Habicht K. S., Gade M., Thamdrup B., Berg P. and Canfield D. E. (2002) Calibration of sulfate levels in the Archean Ocean. *Science* **298**, 2372–2374.

Hageage, Jr., G. J., Eanes E. D. and Gherna R. L. (1970) X-ray diffraction studies of the sulfur globules accumulated by *Chromatium* species. *J. Bacteriol.* **101**, 464–469.

Harrison A. G. and Thode H. G. (1958) Mechanism of the bacterial reduction of sulphate from isotope fractionation studies. *Trans. Faraday Soc.* **54**, 84–92.

Holser W. T. and Kaplan I. R. (1966) Isotope geochemistry of sedimentary sulfates. *Chem. Geol.* **1**, 93–135.

Hurtgen M. T., Arthur M. A., Suits N. S. and Kaufman A. J. (2002) The sulfur isotopic composition of Neoproterozoic seawater sulfate: implications for a snowball earth? *Earth Planet. Sci. Lett.* **203**, 413–429.

John E. H., Wignall P. B., Newton R. J. and Botrell S. H. (2010) $\Delta^{34}S_{CAS}$ and $\delta^{18}O_{CAS}$ records during the Frasnian-Fammenian (Late Devonian) transition and their bearing on mass extinction models. *Chem. Geol.* **275**, 221–234.

Johnston D. T., Wing B. A., Farquhar J., Kaufman A. J., Strauss H., Lyons T. W., Kah L. C. and Canfield D. E. (2005) Active microbial sulfur disproportionation in the Mesoproterozoic. *Science* **310**, 1477–1479.

Johnston D. T., Poulton S. W., Fralick P. W., Wing B. A., Canfield D. E. and Farquhar J. (2006) Evolution of the oceanic sulfur cycle at the end of the Paleoproterozoic. *Geochim. Cosmochim. Acta* **70**, 5723–5739.

Johnston D. T., Farquhar J. and Canfield D. E. (2007) Sulfur isotope insights into microbial sulfate reduction: when microbes meet models. *Geochim. Cosmochim. Acta* **71**, 3929–3947.

Johnston D. T., Farquhar J., Summons R. E., Shen Y., Kaufman A. J., Masterson A. L. and Canfield D. E. (2008) Sulfur isotope biogeochemistry of the Proterozoic MacArthur Basin. *Geochim. Cosmochim. Acta* **72**, 4278–4290.

Johnston D. T., Wolfe-Simon F., Pearson A. and Knoll A. H. (2009) Anoxygenic photosynthesis modulated Proterozoic oxygen and sustained Earth's middle age. *PNAS* **106**, 16925–16929.

Kah L. C., Lyons T. and Frank T. D. (2004) Low marine sulphate and protracted oxygenation of the Proterozoic biosphere. *Nature* **431**, 834–838.

Kaiho K., Kajiwara Y., Nakano T., Miura Y., Kawahata H., Tazaki K., Ueshima M., Chen Z. and Shi G. R. (2001) End-Permian catastrophe by a bolide impact: evidence of a gigantic release of sulfur from the mantle. *Geology* **29**, 815–818.

Kampschulte A. and Strauss H. (2004) The sulfur isotopic evolution of Phanerozoic seawater based on the analysis of structurally substituted sulfate in carbonates. *Chem. Geol.* **204**, 255–286.

Kaplan I. R. and Rittenberg S. C. (1964) Microbiological fractionation of sulphur isotopes. *J. Gen. Microbial.* **34**, 195–212.

Kappler U. and Dahl C. (2001) Enzymology and molecular biology of prokaryotic sulfite oxidation. *FEMS Microbial. Lett.* **203**, 1–9.

Kendall B., Reinhard C. T., Lyons T. W., Kaufman A. J., Poulton S. W. and Anbar A. D. (2010) Pervasive oxygenation along late Archaean ocean margins. *Nat. Geosci.* **3**, 647–652.

Kornexl B. E., Gehre M., Höfling R. and Werner R. A. (1999) On-line $\delta^{18}O$ measurements of organic and inorganic substances. *Rapid Commun. Mass Spectrom.* **13**, 1685–1693.

Kreuzer-Martin H. W., Ehleringer J. R. and Hegg E. L. (2005) Oxygen isotopes indicate most intracellular water in log-phase *Escherichia coli* is derived from metabolism. *Proc. Nat. Acad. Sci.* **102**, 17337–17341.

Lasaga A. C., Otake T., Watanabe Y. and Ohmoto H. (2008) Anomalous fractionation of sulfur isotopes during heterogeneous reactions. *Earth planet. Sci. Lett.* **268**, 225–238.

Lewis J. S. and Krouse H. R. (1969) Isotopic composition of sulfur and sulfate produced by oxidation of FeS. *Earth Planet. Sci. Lett.* **5**, 425–428.

Lloyd R. M. (1968) Oxygen isotope behavior in the sulfate water system. *J. Geophys. Res.* **73**, 6099–6110.

Luther, III, G. W. (1987) Pyrite oxidation and reduction: molecular orbital theory considerations. *Geochim. Cosmochim. Acta* **51**, 3193–3199.

Lyons T. W. and Gill B. C. (2010) Ancient sulfur cycling and oxygenation of the early biosphere. *Elements* **6**, 93–99.

Lyons T. W., Reinhard C. T. and Scott C. (2009) Redox Redux. *Geobiology* **7**, 489–494.

Mandernack K. W. and Tebo B. M. (1999) In situ sulfide removal and CO_2 fixation rates at deep sea hydrothermal vents and the oxic/anoxic interface in Framvaren Fjord, Norway. *Mar. Chem.* **66**, 201–213.

Mandernack K. W., Lynch L., Krouse H. R. and Morgan M. D. (2000) Sulfur cycling in wetland peat of the New Jersey Pinelands and its effect on stream water chemistry. *Geochim. Cosmochim. Acta* **64**, 3949–3964.

Mandernack K. W., Krouse H. R. and Skei J. M. (2003) A stable sulfur and oxygen isotopic investigation of sulfur cycling in an anoxic marine basin, Framvaren Fjord, Norway. *Chem. Geol.* **195**, 181–200.

Mangalo M., Meckenstock R. U., Stichler W. and Einsield F. (2007) Stable isotope fractionation during bacterial sulfate reduction is controlled by reoxidation of intermediates. *Geochim. Cosmochim. Acta* **71**, 4161–4171.

Mangalo M., Einsield F., Meckenstock R. U. and Stichler W. (2008) Influence of the enzyme dissimilatory sulfite reductase on stable isotope fractionation during sulfate reduction. *Geochim. Cosmochim. Acta* **72**, 1513–1520.

Mas J. and Van Gemerden H. (1987) Influence of sulfur accumulation and composition of sulfur globule on cell volume and buoyant density of *Chromatium vinosum*. *Arch. Microbiol.* **146**, 362–369.

Mazumdar A., Goldberg T. and Strauss H. (2008) Abiotic oxidation of pyrite by Fe(III) in acidic media and its implications for sulfur isotope measurements of lattice-bound sulfate in sediments. *Chem. Geol.* **253**, 30–37.

McCready R. G. L. and Krouse H. R. (1989) Sulfur isotope fractionation during the oxidation of elemental sulfur by Thiobacilli in a Solonetzic soil. *Can J. Soil Sci.* **62**, 105–110.

Millero F. J. (1991) The oxidation of H_2S in Framvaren Fjord. *Limnol. Oceanogr.* **36**, 1007–1014.

Mitzutani Y. and Rafter T. A. (1973) Isotopic behavior of sulfate oxygen in the bacterial reduction of sulfate. *Geochem. J.* **6**, 183–191.

Moses C. O., Nordstrom D. K., Herman J. S. and Mills A. L. (1987) Aqueous pyrite oxidation by dissolved oxygen and by ferric iron. *Geochim. Cosmochim. Acta* **51**, 1561–1571.

Nakai N. and Jensen M. L. (1964) The kinetic isotope effect in the bacterial reduction and oxidation of sulfur. *Geochim. Cosmochim. Acta* **28**, 1893–1912.

Newton R. J., Pevitt P. B., Wignall P. B. and Bottrell S. H. (2004) Large shifts in the isotopic composition of seawater sulphate across the Permo-Triassic boundary in northern Italy. *Earth Planet. Sci. Lett.* **218**, 331–345.

Newton R. J., Reeves E. P., Kafousia N., Wignall P. B., Bottrell S. H. and Sha J. G. (2011) Low marine sulfate concentrations and the isolation of the European epicontinental sea during the Early Jurassic. *Geology* **39**, 7–10.

Ohmoto H. and Felder R. P. (1987) Bacterial activity in warmer, sulphate-bearing, Archean oceans. *Nature* **238**, 244–246.

Ohmoto H., Kakegawa T. and Lowe D. R. (1993) 3.4-billion-yearold biogenic pyrites from Barberton, South Africa: sulfur isotope evidence. *Science* **262**, 555–557.

Ono S., Eigenbrode J. L., Pavlov A. A., Kharecha P., Rumble, III, D., Kasting J. F. and Freeman K. H. (2003) New insights into Archean sulfur cycle from mass-independent sulfur isotope records from the Hamersley Basin, Australia. *Earth Planet. Sci. Lett.* **213**, 15–30.

Pisapia C., Chaussidon M., Mustin C. and Humbert B. (2007) O and S isotopic composition of dissolved and attached oxidation products of pyrite by *Acidithiobacillius ferrooxidans*: comparison with abiotic oxidation. *Geochim. Cosmochim. Acta* **71**, 2474–2490.

Planavsky N. J., McGoldrick P., Scott C. T., Li C., Reinhard C. T., Kelly A. E., Chu X., Bekker A., Love G. D. and Lyons T. W. (2011) Widespread iron-rich conditions in Mid-Proterozoic oceans. *Nature* **477**, 448–451.

Poulton S. W. and Canfield D. E. (2011) Ferruginous conditions: a dominant feature of the ocean through Earth's history. *Elements* **7**, 107–112.

Prange A., Arzberger I., Engemann C., Modrow H., Schumann O., Trüper H. G., Steudel R., Dahl C. and Hormes J. (1999) In situ analysis of sulfur in the sulfur globules of phototrophic sulfur bacteria by X-ray absorption near edge spectroscopy. *Biochim. Biophys. Acta* **1428**, 446–454.

Reinhard C. T., Raiswell R., Scott C., Anbar A. D. and Lyons T. W. (2009) A late Archean sulfidic sea stimulated by early oxidative weathering of the continents. *Science* **326**, 713–716.

Rice C. A., Tuttle M. L. and Reynolds R. L. (1993) The analysis of forms of sulfur in ancient sediments and sedimentary rocks: comments and cautions. *Chem. Geol.* **107**, 83–95.

Sakai H. (1968) Isotopic properties of sulfur compounds in hydrothermal processes. *Geochem. J.* **2**, 29–49.

Scott C., Bekker A., Reinhard C. T., Schnetger B., Krapež B., Rumble D. and Lyons T. W. (2011) Late Archean euxinic conditions before the rise of atmospheric oxygen. *Geology* **39**, 119–122.

Shen Y., Buick R. and Canfield D. E. (2001) Isotopic evidence for microbial sulphate reduction in the early Archaean era. *Nature* **410**, 77–81.

Shields G. A., Strauss H., Howe S. S. and Siegmund H. (1999) Sulphur isotope compositions of sedimentary phosphorites from the basal Cambrian of China: implications for Neoproterozoic-Cambrian biogeochemical cycling. *J. Geol. Soc. (London)* **156**, 943–955.

Shields G., Kimura H., Yang J. and Gammon P. (2004) Sulphur isotopic evolution of Neoproterozoic-Cambrian seawater: new francolite-bound sulphate $\delta^{34}S$ data and a critical appraisal of the existing record. *Chem. Geol.* **204**, 163–182.

Sorokin Y. I. (1970) Interrelations between sulfur and carbon turnover in leromictic lakes. *Arch. Hydrobiol.* **66**, 391–446.

Strauss H., Banjeree D. M. and Kumar V. (2001) The sulfur isotopic composition of Neoproterozoic to early Cambrian seawater – evidence from the cyclic Hanseran evaporates, NW India. *Chem. Geol.* **175**, 17–28.

Taylor B. E., Wheeler M. C. and Nordstrom D. K. (1984) Isotope composition of sulphate in acid mine drainage as a measure of bacterial oxidation. *Nature* **308**, 538–541.

Thurston R. S., Mandernack K. W. and Shanks, III, W. C. (2010) Laboratory chalcopyrite oxidation by *Acidithiobacillus ferrooxidans*: oxygen and sulfur isotope fractionation. *Chem. Geol.* **269**, 252–261.

Toran L. and Harris R. F. (1989) Interpretation of sulfur and oxygen isotopes in biological and abiological sulfide oxidation. *Geochim. Cosmochim. Acta* **53**, 2341–2348.

Trüper H. G. (1984) Microorganisms in the sulfur cycle. In *Sulfur, its Significance for Chemistry, for the Geo-, Bio-, and Cosmosphere and Technology, Studies in Inorganic Chemistry*, 5 (eds. A. Müller and B. Krebs). Elsevier, Amsterdam, pp. 351–356.

Turchyn A. V., Schrag D. P., Coccioni R. and Montanari A. (2009) Stable isotope analysis of the Cretaceous sulfur cycle. *Earth Planet. Sci. Lett.* **285**, 115–123.

Ueda A., Cameron E. M. and Krouse H. R. (1991) ^{34}S-enriched sulphate in the Belcher Group, N.W.T., Canada: evidence for dissimilatory sulphate reduction in the early Proterozoic ocean. *Precambrian Res.* **49**, 229–233.

Wahlund T. M. and Madigan M. T. (1993) Nitrogen fixation by the thermophilic green sulfur bacterium *Chlorobium tepidum*. *J. Bacteriol.* **175**, 474–478.

Wakeham S. G., Amann R., Freeman K. H., Hopsman E. C., Jørgensen B. B., Putnam I. F., Schouten S., Sinninghe Damsté J. S., Talbot H. M. and Woebken D. (2007) Microbial ecology of the stratified water column of the Black Sea as revealed by a comprehensive biomarker study. *Org. Geochem.* **38**, 2070–2097.

Walker J. C. G. and Brimblecombe P. (1985) Iron and sulfur in the pre-biologic ocean. *Precambrian Res.* **28**, 205–222.

Watanabe Y., Naraoka H., Wronkiewicz D. J., Condie K. C. and Ohmoto H. (1997) Carbon, nitrogen, and sulfur geochemistry of Archean and Proterozoic shales from the Kaapvaal Craton, South Africa. *Geochim. Cosmochim. Acta* **61**, 3441–3459.

Watanabe Y., Stewart B. W. and Ohmoto H. (2004) Organic- and carbonate-rich soil formation 2.6 billion years ago at Schagen, East Transvaal district, South Africa. *Geochim. Cosmochim. Acta* **68**, 2129–2151.

Woese C. R. (1987) Bacterial evolution. *Microbiol. Rev.* **51**, 221–271.

Zerkle A. L., Farquhar J., Johnston D. T., Cox R. P. and Canfield D. E. (2009) Fractionation of multiple sulfur isotopes during phototrophic oxidation of sulfide and elemental sulfur by a green sulfur bacterium. *Geochim. Cosmochim. Acta.* **73**, 291–306.

Ziegler K., Coleman M. L. and Young E. D. (2008) Tracing the source of oxygen during pyrite oxidation with $\Delta^{17}O_{SO_4}$. *Geochim. Cosmochim. Acta* **72**, A1106.

Associate editor: James Farquhar