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Oxygen and sulfur isotope systematics of sulfate produced by bacterial and abiotic oxidation of pyrite

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Abstract

To better understand reaction pathways of pyrite oxidation and biogeochemical controls on $\delta^{18}O$ and $\delta^{34}S$ values of the generated sulfate in acid mine drainage (AMD) and other natural environments, we conducted a series of pyrite oxidation experiments in the laboratory. Our biological and abiotic experiments were conducted under aerobic conditions by using O_2 as an oxidizing agent and under anaerobic conditions by using dissolved $Fe(III)_{aq}$ as an oxidant with varying $\delta^{18}O_{H_2O}$ values in the presence and absence of *Acidithiobacillus ferrooxidans*. In addition, aerobic biological experiments were designed as short- and long-term experiments where the final pH was controlled at ~2.7 and 2.2, respectively. Due to the slower kinetics of abiotic sulfide oxidation, the aerobic abiotic experiments were only conducted as long term with a final pH of ~2.7. The $\delta^{34}S_{SO_4}$ values from both the biological and abiotic anaerobic experiments indicated a small but significant sulfur isotope fractionation (~−0.7‰) in contrast to no significant fractionation observed from any of the aerobic experiments. Relative percentages of the incorporation of water-derived oxygen and dissolved oxygen (O_2) to sulfate were estimated, in addition to the oxygen isotope fractionation between sulfate and water, and dissolved oxygen. As expected, during the biological and abiotic anaerobic experiments all of the sulfate oxygen was derived from water. The percentage incorporation of water-derived oxygen into sulfate during the oxidation experiments by O_2 varied with longer incubation and lower pH, but not due to the presence or absence of bacteria. These percentages were estimated as 85%, 92% and 87% from the short-term biological, long-term biological and abiotic control experiments, respectively. An oxygen isotope fractionation effect between sulfate and water ($\varepsilon^{18}O_{SO_4-H_2O}$) of ~3.5‰ was determined for the anaerobic (biological and abiotic) experiments. This measured $\varepsilon^{18}O_{SO_4^{2-}-H_2O}$ value was then used to estimate the oxygen isotope fractionation effects ($\varepsilon^{18}O_{SO_4^{2-}-O_2}$) between sulfate and dissolved oxygen in the aerobic experiments which were −10.0‰, −10.8‰, and −9.8‰ for the short-term biological, long-term biological and abiotic control experiments, respectively. Based on the similarity between $\delta^{18}O_{SO_4}$ values in the biological and abiotic experiments, it is suggested that $\delta^{18}O_{SO_4}$ values cannot be used to distinguish biological and abiotic mechanisms of pyrite oxidation. The results presented here suggest that $Fe(III)_{aq}$ is the primary oxidant for pyrite at pH < 3, even in the presence of dissolved oxygen, and that the main oxygen source of sulfate is water–oxygen under both aerobic and anaerobic conditions.

1. INTRODUCTION

Iron and sulfur are redox active elements that participate in a variety of geochemical and biogeochemical processes. Pyrite, FeS_2, is the most abundant metal sulfide in nature and, therefore, has a major influence on the biogeochemical iron, sulfur and oxygen cycles. In addition, pyrite oxidation has received significant attention because of its environmental impact in the formation of acid mine drainage (AMD). Consequently, the understanding of oxidation pathways of pyrite (biological and abiotic) has the potential

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to elucidate sulfur, iron and oxygen cycling in modern and ancient environments and may help with remediation strategies or predictive modeling of AMD sites.

In AMD systems, oxidation of pyrite to sulfate is described by the following two end-member reactions which utilize either O_2 or $Fe(III)_{aq}$ as oxidants (Garrels and Thompson, 1960; Singer and Stumm, 1970; Taylor et al., 1984a,b; Nordstrom and Alpers, 1999; Nordstrom and Southam, 1999):

$$FeS_2 + 7/2O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (1)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (2)$$

The rate of reaction (1) is enhanced by the bacterium *A. ferrooxidans*. The rate of reaction (1) is limited by the availability of dissolved oxygen and therefore this reaction may represent the common reaction for pyrite oxidation under O_2 saturated conditions. Compared to oxidation by O_2, $Fe(III)_{aq}$ can rapidly oxidize pyrite abiotically and anaerobically via reaction (2). To maintain reaction (2), however, $Fe(III)_{aq}$ must be generated by the following reaction.

$$Fe_{2+} + 1/4O_2 + H^+ \rightarrow Fe^{3+} + 1/2H_2O \quad (3)$$

Under acidic conditions (pH < 3), reaction (3) can be the rate limiting step for reaction (2) and bacterial oxidation of Fe^{2+} at this low pH is several orders of magnitude faster than abiotic oxidation (Singer and Stumm, 1968; Schippers et al., 1996; Nordstrom and Alpers, 1999; Schippers and Sand, 1999). Therefore, generation of $Fe(III)_{aq}$ via reaction (3) is generally mediated by bacteria in AMD sites.

Depending on the reactions (1) or (2), oxygen to produce sulfate may come from either atmospheric oxygen or water during pyrite oxidation. The large contrast in the oxygen isotope composition of molecular oxygen in the atmosphere ($\delta^{18}O = +23.5‰$) and typical meteoric water ($\delta^{18}O < 0‰$) may provide an opportunity to reveal the oxidation pathways for pyrite by determining the relative source of oxygen in sulfate based on its measured $\delta^{18}O_{SO_4}$ value (Taylor et al., 1984a). The $\delta^{18}O$ value of the sulfate produced during abiotic and biological pyrite oxidation may vary depending on reaction pathways and due to differences in the relative amounts of molecular oxygen and water–oxygen that is incorporated into sulfate (Lloyd, 1968; Taylor et al., 1984a,b; van Everdingen and Krouse, 1985; Toran and Harris, 1989). For example, the stoichiometry of reaction (1) implies that the H_2O- to O_2-derived oxygen in sulfate is 1:7 (Taylor et al., 1984a,b; van Everdingen and Krouse, 1985). Sulfate is expected to be the dominant sulfoxyanion product at pH < 3 (Goldhaber, 1983; McKibben and Barnes, 1986; Moses et al., 1987; Schippers et al., 1996; Schippers and Sand, 1999). Therefore, if $\delta^{18}O_{SO_4}$ values preserve the identity of the original source of oxygen (water and/or molecular oxygen), the $\delta^{18}O_{SO_4}$ value can be used to elucidate reaction pathways (Taylor et al., 1984a,b; Van Stempvoort and Krouse, 1994).

The $\delta^{18}O$ value of sulfate is controlled not only by the oxygen sources, but also by isotopic fractionation during uptake of $O_2(\varepsilon_o)$ and water (ε_w):

$$\varepsilon = 1000 \ln \alpha_{(SO_4\text{–}H_2O \text{ or } \text{–}O_2)} \quad (4)$$

The oxygen isotopic enrichment may vary depending on the reaction pathways. According to previous studies ε_o appears to be more negative for bacterial reactions (−11.4‰) than for abiotic oxidation of sulfide (−4.3‰ to −8.7‰) (Lloyd, 1968; Taylor et al., 1984b; van Everdingen and Krouse, 1985). Compared to ε_o, the value for ε_w is less variable and generally falls between 0‰ and 4‰ for both biological and abiotic processes (Lloyd, 1968; Taylor et al., 1984a,b; van Everdingen and Krouse, 1985; Van Stempvoort and Krouse, 1994).

With respect to the sulfur isotope composition of sulfate, the oxidation of sulfide to sulfate produces small or negligible sulfur isotope fractionation at low pH (<3) (Taylor et al., 1984b). Values of ε_s are generally considered to be negligible during bacterial and abiotic oxidation of solid metal sulfides as the mineral surface becomes oxidized and dissolves "layer-by-layer" (Nakai and Jensen, 1964; McCready and Krouse, 1982; Taylor et al., 1984a,b). Therefore, the $\delta^{34}S$ values of sulfate formed via sulfide oxidation are generally indistinguishable from those of the parent sulfide minerals (Taylor et al., 1984b; Seal and Wandless, 1997). However, sulfur isotope enrichment factors (ε_s) ranging from +2‰ to −5‰ have been reported for abiotic and biological oxidation of dissolved HS^- (Fry et al., 1984; Fry et al., 1988). Experiments that involve aqueous sulfide oxidation to sulfate under neutral and alkaline conditions produced a negative kinetic isotopic effect (0–5‰) between sulfate and sulfide (Toran and Harris, 1989). Consequently, interpretation of oxygen and sulfur isotopes of sulfate generated via sulfide oxidation is not straightforward, but may yield insights into reaction pathways and environmental conditions during the oxidation reactions.

In order to distinguish the reaction pathways of sulfide oxidation based on the $\delta^{34}S$ and $\delta^{18}O$ composition of sulfate requires carefully controlled laboratory investigations with a variety of different experimental conditions where the $\delta^{18}O$ values of H_2O, O_2, and sulfate, and the $\delta^{34}S$ values of sulfide and sulfate are all measured. In this study, we build upon the results of Taylor et al. (1984a,b) and present a more detailed and systematic study of oxygen and sulfur isotope ratios of sulfate produced abiotically and by a pure culture of *A. ferrooxidans* during O_2 and $Fe(III)_{aq}$ oxidation of pyrite. The experiments were designed to mimic two common oxidation pathways (reactions (1) and (2)) and to determine other environmental effects influencing the oxygen isotope composition of sulfate. A goal of these experimental studies was to re-assess $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ as indicators of the mechanisms of pyrite oxidation (biological vs. abiotic) and/or oxidation environments (aerobic vs. anaerobic).

2. EXPERIMENTAL METHODS

2.1. Pyrite preparation

Natural pyrite was obtained from the Geology Museum of the Colorado School of Mines (Golden, USA). Purity was verified (~99%) by ICP chemical analysis at the United States Geological Survey (USGS), in Denver, Colorado.

Prior to use, the pyrite was ground and sieved to a grain size of <63 μm. Iron and sulfur coatings on the pyrite surface were removed by boiling in 6 M HCl for approximately 15 min. The pyrite was subsequently rinsed three times with deionized water, followed by rinses with acetone, and then dried under a nitrogen atmosphere at room temperature (Moses et al., 1987). For sterilization, the pyrite samples were soaked with 70% ethanol and spread in a thin even layer under UV radiation (germicidal) in a sterile hood for ~30 min. Following these treatments, the pyrite was immediately placed in sterile experimental containers. The specific surface area of the pyrite grains was determined in triplicate by BET measurements as 0.53 ± 0.08 m^2 g at the Colorado School of Mines.

2.2. Bacterial culture preparation

The acidophilic Fe(II)- and sulfur-oxidizing bacterium, *A. ferrooxidans* (23270; formerly, *Thiobacillus ferrooxidans*) was obtained from the American Type Culture Collection (ATCC). Bacteria were maintained in a modified 2039-ATCC medium which contains the following per liter: 0.6 g NH_4Cl, 0.2 g $MgCl_2{\cdot}6H_2O$, 0.1 g K_2HPO_4; 7.1 g $FeCl_2$ and 1.5 g $FeSO_4$ and 5 ml modified Wolfe's mineral solution (1.5 g nitrilotriacetic acid, 3 g $MgSO_4{\cdot}7H_2O$, 0.5 g $MnSO_4{\cdot}7H_2O$, 1 g NaCl, 100 mg $FeSO_4{\cdot}7H_2O$, 100 mg $CoCl_2{\cdot}6H_2O$, 100 mg $CaCl_2$, 100 mg $ZnSO_4{\cdot}7H_2O$, 100 mg $CuSO_4{\cdot}7H_2O$, 10 mg $AlK(SO_4)_2{\cdot}12H_2O$, 10 mg H_3BO_3, 10 mg $Na_2MoO_4{\cdot}2H_2O$ per liter) (ATCC web site). The medium was prepared by adding the above to 800 ml of de-ionized (DI) water that contained 5 ml of the modified (low sulfate) Wolfe's mineral solution. The medium used for growing bacteria was adjusted to pH 2.3 with trace-metal grade HCl and autoclaved for 25 min. To the medium used only for maintenance of the culture, $FeCl_2$ (7.1 g) and $FeSO_4$ (1.35 g) salts were both added as energy substrates to 200 ml acidified DI water and immediately filter sterilized. It is not feasible to completely substitute $FeSO_4$ with $FeCl_2$ because *A. ferrooxidans* requires some sulfate for growth and chloride concentrations, if too high, become inhibitory. The salt medium and the $Fe(II)_{aq}$ solution were aseptically combined. *A. ferrooxidans* was subcultured three times before being used in the final biological pyrite experiments. Cell cultures for the experiments were prepared according to methods described in Yu et al. (2001). The actual cell densities in the biological experiments were estimated by the Most Probable Number Method (MPN) using the ferrous sulfate medium previously described. Five milliliters of the cell suspension, which contained $\sim 2.7 \times 10^7$ cells/ml, was added to 250 ml of the medium in the biological experiments.

2.3. Biological and abiotic oxidation of pyrite by Fe(III)

For the biological and abiotic experiments, 100 ml of the microbiological medium was placed into 200 ml serum bottles and the pH was adjusted to ~2 with trace metal grade HCl to stabilize aqueous $Fe(III)_{aq}$. The serum bottles were then autoclaved for 20 min and purged with nitrogen for 30 min by using pre-sterilized gas filters (0.2 μm) at both the inlet and outlet in order to keep the medium sterile. After purging, serum bottles were equilibrated overnight in the anaerobic microbiological glove box before starting the experiments. The atmosphere in the box was 5% CO_2, 5% H_2, and 90% N_2. For the anaerobic experiments, ~200 mg of pyrite was cleaned and sterilized according to the methods described above.

The $Fe(III)_{aq}$ used in the anaerobic experiments was prepared by dissolving $FeCl_3$ in acidified DI water (pH 2), then filter sterilized (0.2 μm) and aseptically purged with nitrogen for ~25 min. The $Fe(III)_{aq}$ stock solution (1 M) was kept inside the anaerobic chamber until used. According to the mass balance of reaction (2), 10 mM $Fe(III)_{aq}$ was necessary to oxidize the 200 mg of pyrite added for the anaerobic experiments. Pyrite (200 mg), 1 ml of the cell culture (2.7×10^7 cells/ml) and the $FeCl_3$ solution (10 mM final) were added to 100 ml of microbiological medium in each 200 ml vial and then sealed with a gas headspace inside the anaerobic chamber. As a final step, the serum bottles were covered with aluminum foil to prevent iron photo oxidation/reduction, and placed in an environmentally controlled room at 25 °C. The vials were shaken continuously at 150 rpm during the entire incubation period of 35 days. Abiotic experiments were prepared and conducted under identical conditions except that no bacterial culture was added. Biological and abiotic experiments were performed in duplicate and concentrations of $Fe(II)_{aq}$ and sulfate were monitored during the course of the experiments. To determine the kinetic oxygen isotope fractionation effect between sulfate and water ($\varepsilon^{18}O_{SO_4-H_2O}$), the anaerobic experiments were also performed in duplicate for each water with distinct isotopic composition.

2.4. Biological and abiotic oxidation of pyrite by O_2

For the biological experiments, low sulfate- and Fe(II)-medium was used as the experimental solution (NH_4Cl, 0.6 g; $MgCl_2{\cdot}6H_2O$, 0.2 g; K_2HPO_4, 0.1 g; modified Wolfe's mineral solution, 5 ml) and pH was adjusted to 3 with HCl at 25 °C. Unlike the anaerobic experiments with 10 mM $Fe(III)_{aq}$, no ferric salt was added to the aerobic experiments. All experiments were performed under O_2-saturated conditions with continuous sparging with air. Furthermore, the biological experiments were designed as short and long term to assess if changes in the $\delta^{18}O_{SO_4}$ might occur with increasing incubation time and decreasing pH. Short term biological experiments were ended at pH ~2.7 after circa 20 days, while long term experiments were ended at pH 2.2 after circa 44 days. These pH values mimic the range observed in many AMD sites.

The following modified Wolfe's mineral solution was prepared with chloride salts instead of sulfate salt to minimize sulfate carry over to the final experimental solution: 1.5 g nitrilotriacetic acid, 3 g $MgCl_2{\cdot}6H_2O$, 0.5 g $MnCl_2{\cdot}4H_2O$, 1 g NaCl, 100 mg $FeCl_2$ 100 mg $CoCl_2{\cdot}6H_2O$, 100 mg $CaCl_2$, 50 mg $ZnCl_2$, 100 mg $CuCl_2{\cdot}6H_2O$, 10 mg $AlK(SO_4)_2{\cdot}12H_2O$, 10 mg H_3BO_3, 10 mg $Na_2MoO_4{\cdot}2H_2O$ per liter. The salts and 5 ml of sterile modified Wolfe's mineral solution were added to DI water. Trace metal grade HCl was subsequently added to the medium to an adjusted

pH of 3. The 250 ml of medium was placed into acid washed 500 ml Erlenmeyer flasks and autoclaved at 121 °C for 20 min. After autoclaving the flasks were kept in the sterile hood under UV light for 25 min to decontaminate the surface. Five hundred milligram of pyrite sample was sterilized and cleaned by the methods described previously and 5 ml ($\sim 2.7 \times 10^7$ cell/ml) of the *A. ferrooxidans* cell suspension was added to 250 ml of medium.

To determine the kinetic oxygen isotope fractionation effect (ε_o) and the relative contribution of water and molecular oxygen to the sulfate oxygen, four different bacterial incubations were made with waters having different $\delta^{18}O$ values of −15.3‰, −2.5‰, 1.8‰ and 13.0‰, respectively. The experiments were performed in duplicate for each water with distinct isotopic composition. The flasks were covered with rubber stoppers and fitted with inflow and outflow tubes for continuous aeration. Sterile filters (0.2 μm) were connected to the compressed air inflow and exit ports to prevent microbial contamination and to minimize evaporation. During each set of incubations, additional biological and abiotic laboratory experiments with normal laboratory water (−15.3‰) were also performed under identical experimental conditions in order to monitor pH and solution chemistry. Aliquots from these experiments were taken periodically to measure the concentration of sulfate, $Fe(III)_{aq}$, $Fe(II)_{aq}$ and pH using standard techniques.

At the end of the short and long term pyrite oxidation experiments, the solutions were filtered (0.1 μm, Supor polyethersulfone cellulose) to remove trace amounts of iron oxides that may interfere with oxygen isotope measurements of sulfate. The water samples were kept tightly sealed and frozen until oxygen isotope measurements. The remainder of the filtrate (~200 ml) was reserved for addition of $BaCl_2$ to precipitate $BaSO_4$ for subsequent sulfur and oxygen isotope ratio measurements. Air samples from both biological and abiotic experiments were collected in an evacuated 200 ml collection vessel at the end of the experiments and analyzed for the oxygen isotope ratio ($^{18}O/^{16}O$) of O_2 in the air. The air sample was collected with a gas-tight syringe which was flushed twice before taking the final sample.

Abiotic control experiments were performed under identical conditions as the biological experiments. In order to obtain enough SO_4^{2-} for isotope analysis, abiotic experiments were carried out in 500 ml of medium in 1 liter Erlenmeyer flasks. All experimental conditions and surface cleaning methods were identical to the biological experiments, except that 1 g of pyrite was used for 500 ml medium in order to keep the ratio of mineral mass to solution volume (1 g/500 ml) the same as in the biological experiments (500 mg/250 ml).

2.5. Isotopic analysis of sulfate

For isotope measurements, sulfate from all incubation experiments was precipitated as $BaSO_4$ by addition of a few drops of concentrated HCl immediately followed by the addition of 10 ml of a 10% (wt/wt) $BaCl_2$ solution. The precipitate was allowed to settle overnight. The barium sulfate precipitate was filtered and collected on a 0.2 μm Millipore filter, washed first with 100 ml of 1 N HCl, then rinsed 3 times with a total of 500 ml of DI water. The $BaSO_4$ samples were dried and homogenized by grinding. As a final purification, prior to $\delta^{34}S$ and $\delta^{18}O$ analysis the samples were baked at 500 °C for two hours to remove possible organic contaminants as described elsewhere (Mandernack et al., 2000). Sulfur and oxygen isotope ratios of samples were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) using an elemental analyzer ($\delta^{34}S$) or a Thermo-Finnigan TC/EA at 1450 °C ($\delta^{18}O$) coupled to a gas source mass spectrometer. The oxygen and sulfur isotope results of this study are expressed relative to the Vienna Standard Mean Ocean Water (V-SMOW), and Canyon Diablo Troilite (V-CDT) standards using the standard δ notation:

$$\delta^{34}S \text{ or } \delta^{18}O\ [‰] = [(R_{sample}/R_{standard}) - 1] \times 10^3 \quad (5)$$

where R are $^{34}S/^{32}S$ and $^{18}O/^{16}O$ of sample and reference, respectively. For sulfur isotope measurements, IAEA S1 (−0.3‰), S2 (+21.7‰), SO-5 (+0.49‰), and SO-6 (−34.05‰) were repeatedly analyzed for calibration and normalization purposes. Oxygen isotope ratios of sulfate were normalized to NBS 127 ($\delta^{18}O = +9.3‰$), SO-5 (+12.0‰), and SO-6 (−11.0‰). Reproducibility of the $\delta^{34}S$ and $\delta^{18}O$ values for sulfate were generally better than ±0.2‰ and ±0.5‰, respectively.

The $\delta^{18}O$ value of water was determined by analyzing CO_2 gas that had equilibrated with 200 μl aliquots at 40 °C in septum-capped vials. Raw data were corrected for the H_2O–CO_2 isotopic fractionation, and then adjusted for small instrumental effects using results obtained for water standards that had been previously calibrated against VSMOW and SLAP. Duplicate analyses normally agreed to within ±0.1‰.

The $\delta^{18}O$ value of molecular oxygen was determined by one of two methods. In the first, gas samples were admitted to a vacuum line and exposed to hot graphite to convert O_2 to CO_2. The isotopic composition of the CO_2 was measured by comparison to a reference gas that had been previously calibrated against CO_2 from NBS 19 calcite (28.65‰, Coplen et al., 1983). In the second method, gas samples were exposed to a liquid nitrogen trap to remove trace H_2O and CO_2. The resulting N_2–O_2–Ar mixtures were then admitted directly to the mass spectrometer where the isotopic composition of the O_2 was determined by comparison to atmospheric O_2 (23.5‰, Kroopnick and Craig, 1972) that had been prepared identically. Reproducibility is estimated to have been ±0.5‰.

2.6. Calculation of oxygen source and isotopic fractionation in sulfate

The $\delta^{18}O$ values of SO_4^{2-} produced during oxidation of pyrite by O_2 indicate a mechanism that incorporates oxygen from both H_2O and O_2. In a dual-source mechanism, the resulting $\delta^{18}O_{SO_4^{2-}}$ value is a function of five variables: the $\delta^{18}O$ values of both oxygen sources ($\delta^{18}O_{H_2O}$ and $\delta^{18}O_{O_2}$), any kinetic oxygen isotopic fractionation effects associated with the incorporation from either oxygen

source, and the fraction of oxygen derived from each source. Assuming that any kinetic isotope fractionation effects are relatively small, the $\delta^{18}O_{SO_4^{2-}}$ value can be represented by the mass balance equation

$$\delta^{18}O_{SO_4^{2-}} = X\left(\delta^{18}O_{H_2O} + \varepsilon^{18}O_{SO_4-H_2O}\right) + (1-X)\left(\delta^{18}O_{O_2} + \varepsilon^{18}O_{SO_4-O_2}\right) \quad (6)$$

where X is the fraction of oxygen derived from H_2O, $(1-X)$ is the remaining fraction from O_2, and $\varepsilon^{18}O_{SO_4^{2-}-H_2O}$ and $\varepsilon^{18}O_{SO_4^{2-}-O_2}$ are the kinetic oxygen isotope fractionation effects between SO_4^{2-}–H_2O and SO_4^{2-}–O_2, respectively (Mandernack et al., 1995). The fraction of oxygen derived from water can be determined by replicate experiments with variable $\delta^{18}O$ values of water. A rearrangement of Eq. (6) yields

$$\delta^{18}O_{SO_4^{2-}} = X\left(\delta^{18}O_{H_2O}\right) + \left[(1-X)\left(\delta^{18}O_{O_2} + \varepsilon^{18}O_{SO_4-O_2}\right) + X\left(\varepsilon^{18}O_{SO_4-H_2O}\right)\right] \quad (7)$$

A linear least squares regression for $\delta^{18}O_{SO_4^{2-}}$ vs. $\delta^{18}O_{H_2O}$ has a slope, X, equal to the fraction of oxygen derived from water. For these plots, average $\delta^{18}O_{H_2O}$ values from the initial T_0 and T_{final} time points were used. The y-intercept value, b, can be used to describe a relationship between $\varepsilon^{18}O_{SO_4^{2-}-O_2}$ and the other variables

$$\varepsilon^{18}O_{SO_4-O_2} = \left[\frac{b - X(\varepsilon^{18}O_{SO_4-H_2O})}{1-X}\right] - \delta^{18}O_{O_2} \quad (8)$$

If the $\varepsilon^{18}O_{SO_4^{2-}-H_2O}$ value can be estimated independently, as we did using the measured $\varepsilon^{18}O_{SO_4^{2-}-H_2O}$ values from the anaerobic experiments, an estimate for $\varepsilon^{18}O_{SO_4^{2-}-O_2}$ can be calculated. Linear regressions for estimating X and b with their associated 95% confidence intervals were calculated using the statistical package in Microsoft Excel.

3. RESULTS

3.1. Chemical compositions of experimental solutions

For this study, calculated pyrite oxidation rates (R_{FeS2}) are based on linear regression of the accumulated amount of sulfate with time as presented in Figs. 1 and 2. To convert these rates from mol day^{-1} to surface area-based rates in mol/m^2/s, which can more easily and directly be compared with previous rate estimates of pyrite oxidation, we used the following equation from Gleisner et al. (2006):

$$R_{FeS2} = \frac{a}{\text{BET}\ mc}\ (\text{mol m}^{-2}\ \text{s}^{-1})$$

where, a is the slope (mol day^{-1}) from the linear regressions provided in Figs. 1 and 2, BET is the surface area of the pyrite grains (m^2 g^{-1}) which was measured to be 0.53 m^2 g^{-1}, m is the mass of the pyrite grains and c is a stoichiometric factor (2 for S). These surface area based rates are provided in Tables 1 and 2.

For the biological anaerobic experiments with $Fe(III)_{aq}$ as the oxidant, similar rates of $Fe(II)_{aq}$ production and sulfide oxidation were observed in both the biological and abiotic experiments during the 35 day incubations, suggesting

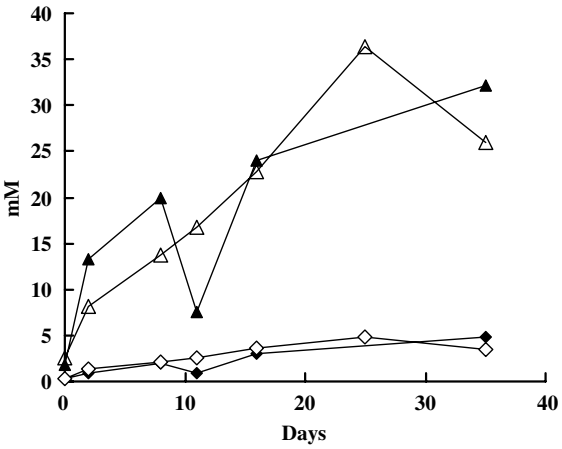

Fig. 1. Change in solution chemistry with time during the oxidation of pyrite by $Fe(III)_{aq}$ in the anaerobic experiments in the presence and absence of *A. ferrooxidans*. (♦) sulfate in biological experiments; (◇) sulfate in abiotic control experiments; (▲) Fe(II) in biological experiments; (△) Fe(II) in abiotic control experiments.

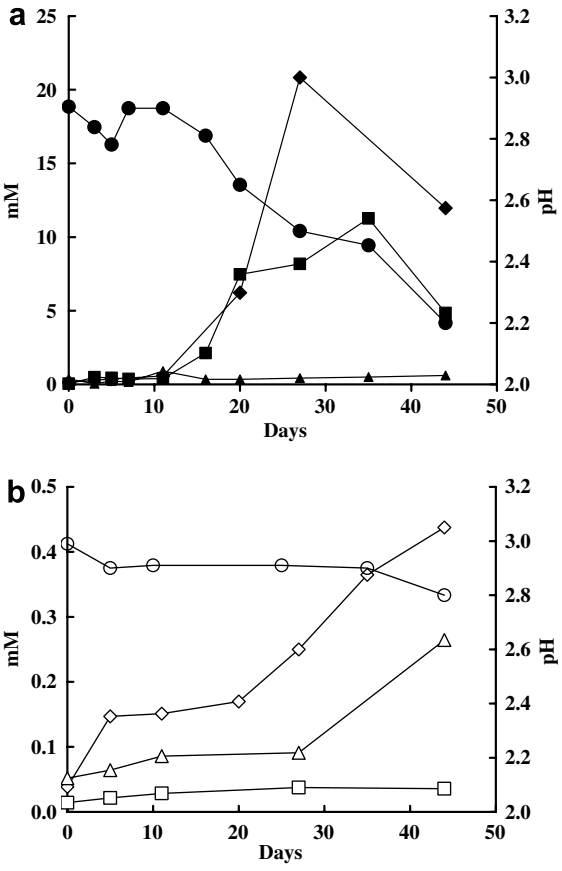

Fig. 2. (a) Change in solution chemistry with time during the oxidation of pyrite by O_2 in the biological experiments in the presence of *A. ferrooxidans*. (♦) sulfate; (●) pH; (■) Fe(III); (▲) Fe(II). (b) Change in solution chemistry with time during the oxidation of pyrite by O_2 in the abiotic control experiments without *A. ferrooxidans*. (◇) Sulfate; (○) pH; (□) Fe(III); (△) Fe(II).

Table 1
Chemical composition of experimental solution during oxidation of pyrite by $Fe(III)_{aq}$

Biological						Abiotic				
Days	pH	Fe(II) (mM)	SO_4[a] (mM)	SO_4[b] (mM)	Oxidation rate ($mol/m^2/s$)	pH	Fe(II) (mM)	SO_4[a] (mM)	SO_4[b] (mM)	Oxidation rate ($mol/m^2/s$)
0	1.90	1.79	0.23	0.18		1.98	2.50	0.33	0.25	
2		13.21	0.87	1.00			8.21	1.30	1.13	
8	1.85	20.00	1.89	2.23		1.83	13.75	2.15	1.62	
11[c]		7.50	0.86	1.00			16.79	2.59	2.20	
16	1.87	23.93	3.08	3.18		1.86	22.86	3.56	3.35	
25		n.d.	n.d.	n.d.			36.43	4.85	4.18	
35	1.88	32.14	4.81	4.28	7.09×10^{-9}	1.87	25.89	3.45	4.29	9.28×10^{-9}

n.d., not determined.
[a] Sulfate concentration measured directly by IC.
[b] Sulfate concentration based on mass balance calculation.
[c] Lower sulfate and Fe(II) concentrations for biological experiments may have resulted from analytical error.

Table 2
Chemical composition of experimental solution during oxidation of pyrite by O_2

Biological								Abiotic					
	Days	pH	SO_4 (mM)	Fe_t (mM)	Fe(II) (mM)	Fe(III) (mM)	Oxidation rate ($mol/m^2/s$)	pH	SO_4 (mM)	Fet (mM)	Fe(II) (mM)	Fe(III) (mM)	Oxidation rate ($mol/m^2/s$)
	0	2.91	0.15	0.0	0.36	0.04		3.0	0.04	0.06	0.05	0.01	
	3	2.84	n.d.	0.53	0.04	0.49		n.d.	n.d.	n.d.	n.d.	n.d.	
	5	2.78	0.36	0.66	0.23	0.43		2.9	0.15	0.08	0.06	0.02	
	7	2.90	n.d.	0.53	0.16	0.37		n.d.	n.d.	n.d.	n.d.	n.d.	
	11	2.90	0.57	1.30	0.91	0.39		2.9	0.15	0.12	0.09	0.03	
	16	2.81	n.d.	2.47	0.34	2.13		n.d.	n.d.	n.d.	n.d.	n.d.	
Short-term	20	2.65	6.22	7.83	0.34	7.49		2.9	0.17	n.d.	n.d.	n.d.	
	27	2.50	20.83	8.61	0.43	8.18	1.32×10^{-8}	n.d.	0.25	0.13	0.09	0.04	
	35	2.45	n.d.	11.77	0.50	11.27		2.8	0.36	n.d.	n.d.	n.d.	
Long-term	44	2.20	11.97	5.45	0.61	4.84		2.8	0.44	0.30	0.26	0.04	1.8×10^{-10}

n.d., not determined.

that abiotic oxidation may have been dominant in each (Table 1, Fig. 1). Lower sulfate and $Fe(II)_{aq}$ concentrations measured for the biological experiments on day 11 is probably the result of analytical error (Table 1, Fig. 1).

For the biological aerobic experiments with O_2 as the oxidant the rates were only calculated for days 0 to 27 since there was sulfate lost from the solution after 27 days. Similar rate determinations were made for the abiotic control aerobic experiments (Table 2). Pyrite oxidation with O_2 by *A. ferrooxidans* proceeded much faster than in the corresponding abiotic experiments (Table 2, Fig. 2a and b). The sulfate concentration in the biological experiments at 44 days was much higher (11.97 mM) than in the abiotic incubations (0.44 mM). In the biological experiments, sulfate concentration consistently increased until day 27 when the pH had decreased to 2.5 (Fig. 2a). Although pH continued to drop to 2.2 at day 44, the sulfate concentration decreased relative to that on day 27. There was also a corresponding decrease in the $Fe(III)_{aq}$ concentration between days 34 and 44 (Fig. 2) which suggests that the decrease in sulfate could be due to the precipitation of ferric iron sulfate minerals such as jarosite (Blowes et al., 2003; Jambor, 2003). After filtration the filter contained red iron oxides, which have previously been shown in other similar culture experiments with *A. ferrooxidans* to contain sulfate-bearing schwertmannite and jarosite (Kawano and Tomita, 2001; Balci et al., 2006; Lefticariu et al., 2006). Precipitation of sulfate-bearing minerals has very little effect on the isotopic composition of the residual aqueous sulfate as recently reported (Prietzel and Mayer, 2005). Therefore, we are confident that the isotopic composition of aqueous sulfate measured in our experiments primarily reflects the oxidation process.

The short term biological experiments were terminated after 20 days when pH, sulfate and $Fe(III)_{aq}$ reached ~2.7, 6.22 mM and 7.49 mM, respectively (Table 2). The long term biological experiments, which were part of the same experimental set up as the short term, were ended at pH 2.2 after 44 days. Although the abiotic experiments were run for the same time as the long term biological experiments (44 days), the pH dropped only slightly to 2.8 which approximated the final pH of the short term biological experiments (Fig. 2b). Nonetheless, SO_4^{2-} and $Fe(III)_{aq}$ were higher in the biological experiments than in the long term abiotic experiment (Fig. 2a and b).

3.2. Oxygen and sulfur isotopic compositions of experimental solutions

The $\delta^{18}O_{SO_4}$ vs. $\delta^{18}O_{H_2O}$ plots for both the biological and abiotic oxidation of pyrite with $Fe(III)_{aq}$ yields slopes of 0.94 ± 0.12 and 0.95 ± 0.09, respectively, suggesting that essentially all of the sulfate oxygen was derived from H_2O (Fig. 3). These results are not surprising because the exclusion of oxygen from the experimental solutions would be expected to suppress reaction (1). Because all oxygen atoms in the generated sulfate are derived from water, Eq. (8) reduces to $b = \varepsilon^{18}O_{SO_4-H_2O}$ and the kinetic fractionation can be calculated explicitly. The estimates for $\varepsilon^{18}O_{SO_4-H_2O}$ are not significantly different at 3.6‰ and 2.9‰ for the biological and abiotic experiments, respectively (Fig. 3). The $\delta^{34}S_{SO_4}$ values from the anaerobic experiments indicated a sulfur isotope fractionation of about −0.7‰. This minor fractionation was observed in both the biological and abiotic control experiments (Table 3).

The $\delta^{18}O_{SO_4^{2-}}$ vs. $\delta^{18}O_{H_2O}$ plots for the biological and abiotic aerobic pyrite oxidation experiments show strong positive linear correlations ($r^2 \geqslant 0.98$) (Fig. 4). In the short-term biological experiments, the slope from the linear regression indicates that $85 \pm 0.04\%$ (at 95% confidence interval, slope ±0.07 at the 99% confidence interval) of the sulfate-oxygen was derived from H_2O and the remaining 15% from O_2 (Fig. 4). The incorporation of water–oxygen into sulfate increased with the extent of the reaction as inferred from a greater slope of 92 ± 0.07 in the long-term biological experiments (Fig. 4). The abiotic-control experiments produced sulfate that had similar $\delta^{18}O_{SO_4^{2-}}$ values to the short-term biological experiments, with a slope suggesting that 13% of the oxygen atoms in sulfate are derived from O_2. In the biological experiments there were small increases in $\delta^{18}O_{H_2O}$ during the incubation experiments, which were attributed to evaporation (Table 4).

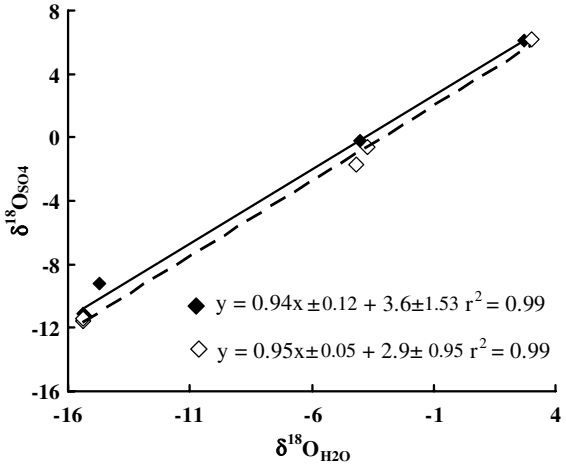

Fig. 3. Plot of the $\delta^{18}O$ value of SO_4 produced during oxidation of pyrite by $Fe(III)_{aq}$ (pH ~2, 35 days) vs. the $\delta^{18}O$ value of the ambient H_2O. (♦) Biological experiments; (◇) abiotic experiments.

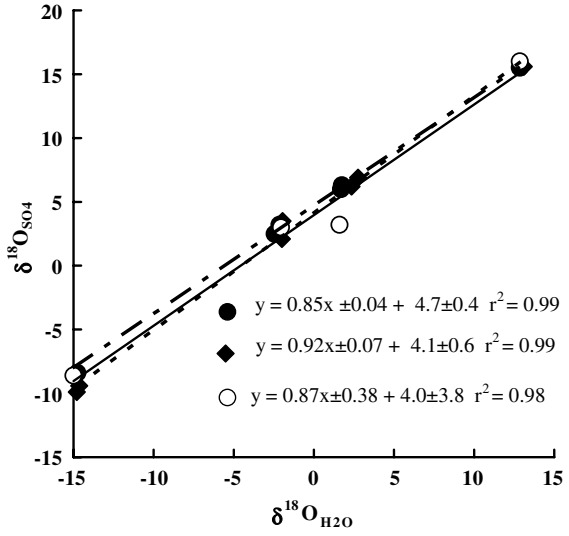

Fig. 4. Plot of the $\delta^{18}O$ value of SO_4 produced during oxidation of pyrite by O_2 vs. the $\delta^{18}O$ value of the ambient H_2O. (○), Abiotic-control experiments (pH 2.8, 44 days); (●), short term biological experiments (pH 2.8, 20 days); (♦), long term biological experiments (pH 2.1, 44 days). *Avg $\delta^{18}O_{H_2O}$ values were used for linear regression analysis (see Table 4).

Table 3
Oxygen and sulfur isotope composition of sulfate from oxidation of pyrite by $Fe(III)_{aq}$

	$\delta^{18}O_{H_2Oinitial}$ (‰)	$\delta^{18}O_{H_2Ofinal}$ (‰)	Avg $\delta^{18}O_{H_2O}$ (‰)	$\delta^{18}O_{SO_4}$ (‰)	$\varepsilon_{SO_4-FeS_2}$ [a] (‰)
Biological 35 days	−15.4	−14.0	−14.7	−9.2	−0.7
	−15.4	−15.3	−15.3	−11.3	−0.6
	−15.4	−15.4	−15.4	−11.1	−0.7
	−3.2	−4.9	−4.0	−0.2	−0.7
	3.2	2.2	2.7	6.1	−1.3
Abiotic 35 days	−15.4	−15.4	−15.4	−11.6	−0.7
	−15.4	−15.4	−15.4	−11.4	−0.6
	−3.4	−5.1	−4.2	−1.7	−0.8
	−3.2	−4.2	−3.7	−0.6	−0.6
	3.4	2.7	3.0	6.2	−0.8
Average					-0.75 ± 0.20

[a] $\delta^{34}S$ of pyrite used in experiments was 2.3 ± 0.5‰ ($n = 3$).

Table 4
Oxygen and sulfur isotope composition of sulfate from oxidation of pyrite by O_2

	$\delta^{18}O_{H_2Oinitial}$ (‰)	$\delta^{18}O_{H_2Ofinal}$ (‰)	Avg $\delta^{18}O_{H_2O}$ (‰)	$\delta^{18}O_{SO_4}$ (‰)	$\varepsilon_{SO_4-FeS_2}$[a] (‰)
Biological					
Short-term	−15.3	−14.8	−15.0	−7.5	0.0
	−15.3	−14.2	−14.7	−8.4	0.1
	−2.5	−1.8	−2.1	3.2	0.0
	−2.5	−2.4	−2.4	2.5	0.1
	1.8	1.7	1.7	6.3	0.1
	1.8	1.6	1.7	6.0	0.1
	13.2	12.5	12.8	15.5	0.0
Long-term	−15.3	−14.3	−14.8	−9.9	−0.1
	−15.3	−14.0	−14.7	−9.4	0.2
	−2.5	−1.4	−1.9	3.5	0.2
	−2.5	−1.5	−2.0	2.1	0.1
	1.8	3.7[a]	2.8	6.9	0.0
	1.8	2.9	2.4	6.2	0.1
	13.2	13.0	13.1	15.6	0.1
Average					0.1 ± 0.08
Abiotic					
Long-term	−15.3	−14.7	−15.0	−8.6	−0.2
	−2.5	−1.6	−2.0	3.0	−0.2
	1.8	1.4	1.6	3.2	0.2
	13.2	12.5	12.8	16.0	−0.2
Average					−0.1 ± 0.20

[a] $\delta^{34}S$ of pyrite used in experiments was 2.3 ± 0.5‰ ($n = 3$).

Eq. (8) demonstrates that the y-intercept of the $\delta^{18}O_{SO_4^{2-}}$ vs. $\delta^{18}O_{H_2O}$ regressions does not equal $\varepsilon^{18}O_{SO_4^{2-}-H_2O}$ for the aerobic experiments as it did in the anaerobic pyrite oxidation experiments with $Fe(III)_{aq}$. Sessions and Hayes (2005) have discussed the inherent difficulties with the determination of fractionation effects associated with dual-source systems, and which has similarly been described for the formation of manganese oxides (Mandernack et al., 1995). However, if we assume that $\varepsilon^{18}O_{SO_4^{2-}-H_2O}$ for oxidation of pyrite with O_2 is similar to that for anaerobic pyrite oxidation with $Fe(III)_{aq}$, $\varepsilon^{18}O_{SO_4^{2-}-O_2}$ can be estimated using Eq. (8). For these calculations, average $\delta^{18}O_{SO_4}$ and $\delta^{18}O_{H_2O}$ values of the respective experiments were used. The $\varepsilon^{18}O_{SO_4^{2-}-O_2}$ results are −10.0‰ and −10.8‰ for the short and long term biological experiments, respectively, and −9.8‰ for the abiotic control experiments. Because the measured $\delta^{18}O_2$ value of +29.7‰ from the abiotic experiment was so different from all five of the measured values from the biological experiments, and from three other measurements of another study using the same tank of air and experimental design (Balci Celik, 2005), we believe this value is suspect. Therefore, in order to calculate the $\varepsilon^{18}O_{SO_4^{2-}-O_2}$ value for the abiotic aerobic experiments we instead used the average value of 21.0 ± 0.5 ‰ ($n = 5$) measured for O_2 from the biological experiments. Consistent with previous studies (Nakai and Jensen, 1964; McCready and Krouse, 1982; Taylor et al., 1984a,b), the $\delta^{34}S_{SO_4}$ values of the newly formed sulfate indicated no significant sulfur isotope fractionation relative to the pyrite substrate (Table 4).

4. DISCUSSION

4.1. Rates of pyrite oxidation

Sulfate production rates from our experiments can be used to estimate the rates of pyrite oxidation by O_2 and $Fe(III)_{aq}$. These estimates carry some uncertainty and may underestimate the actual rates because other sulfur species, such as elemental sulfur, could also form as oxidation products (Schippers et al., 1996). However, Schippers et al. (1996) reported less than 1% of the total oxidized sulfur was elemental sulfur with pure cultures of *A. ferrooxidans* grown under aerobic conditions, so sulfate provides a close approximation of the oxidation rate. Our measured pyrite oxidation rate(s) of 1.32×10^{-8} mol/m^2/s and 1.80×10^{-10} mol/m^2/s for the aerobic biological and abiotic experiments, respectively, compares well with previous rate measurements of pyrite oxidation, which varied from 8.80×10^{-8} to 6.96×10^{-10} mol/m^2/s and 1.70 to 3.50×10^{-10} mol/m^2/s for the biological and abiotic oxidations, respectively (Olson, 1991; Williamson and Rimstidt, 1994; Gleisner et al., 2006).

From comparisons of abiotic control and biological experiments, oxidation of pyrite by O_2 is accelerated ~100x by *A. ferrooxidans* (Fig. 2a and b, Table 2). Under aerobic and low pH conditions, when bacteria can also catalyze the oxidation of Fe(II) to $Fe(III)_{aq}$, pyrite might be oxidized by both $Fe(III)_{aq}$ and O_2. Although $Fe(III)_{aq}$ and O_2 may also contribute to pyrite oxidation in the abiotic experiments, oxidation by $Fe(III)_{aq}$ was probably minimal

since abiotic oxidation of Fe(II) is very slow at pH < 3 (Schwertmann and Fitzpatrick, 1992; Morgan and Stumm, 1998). Therefore, abiotic oxidation of pyrite by O_2 showed the lowest oxidation rate (44 days, 1.8×10^{-10} mol/m^2/s) of all the experiments and may represent the lower limit at pH < 3 (Fig. 2, Table 2). Thus at low pH, pyrite oxidation appears to be primarily controlled by biological reactions.

The oxidation rates were generally similar in the biological and abiotic experiments during oxidation of pyrite by $Fe(III)_{aq}$ (Fig. 1, Table 1), suggesting that abiotic oxidation of pyrite by $Fe(III)_{aq}$ dominated in both. The oxidation rates with $Fe(III)_{aq}$ were lower than that observed during biological oxidation with O_2, but higher than that during abiotic oxidation of pyrite by O_2 (Tables 1 and 2). Because the solubility of Fe(III)-oxides and the $Fe(III)_{aq}$ concentrations are very low at higher pH values, pyrite oxidation by $Fe(III)_{aq}$ may be dominant only in acidic environments (pH < 3). In abiotic experiments, it has been reported that FeS_2 and FeS were oxidized in anoxic marine sediments by MnO_2 but not with NO_3 and Fe(III) oxide (Schippers and Jørgensen, 2001). In a different study of the same sedimentary system, these authors carried out tracer experiments with labeled $^{55}FeS_2$ by using NO_3^-, Fe(III) oxide and MnO_2 as electron acceptors of FeS_2 and FeS oxidation (Schippers and Jørgensen, 2002). They showed that dissolution of tracer–marked $^{55}FeS_2$ occurred only with MnO_2, and concluded that MnO_2 and O_2 oxidize FeS_2 and FeS at neutral pH, but NO_3^- and Fe(III) oxide do not. Therefore, pyrite oxidation by $Fe(III)_{aq}$ may dominate only at low pH.

4.2. Kinetic oxygen isotope fractionation between SO_4, H_2O and O_2

The $\varepsilon^{18}O_{SO_4-H_2O}$ values of 3.6‰ and 2.9‰ for the biological and abiotic anaerobic pyrite oxidation experiments, respectively, are consistent with the range measured by previous studies (0–4.1‰) (Tables 3 and 5) (Lloyd, 1968; Taylor et al., 1984b; van Everdingen and Krouse, 1985). Although it is reported that *A. ferrooxidans* is able to grow under anaerobic conditions by using $Fe(III)_{aq}$ as an electron acceptor and elemental sulfur as a substrate (Sugio et al., 1985; Pronk et al., 1992; Suzuki et al., 1990), the similar rates of oxidation and $\delta^{18}O_{SO_4}$ values from the biological and abiotic experiments suggest that abiotic oxidation of pyrite by $Fe(III)_{aq}$ was rate controlling (Figs. 1 and 3). Therefore, bacteria may not be able to compete with Fe(III)-driven abiotic oxidation. The similar kinetic oxygen isotope fractionation observed in the biological and abiotic control experiments are consistent with this conclusion and may closely approximate the value expected for sulfate formed from pyrite oxidation by $Fe(III)_{aq}$ with ~100% of the sulfate-oxygen derived from water. Future studies should examine whether this similarity holds true for oxidants such as MnO_2 or NO_3^-.

Table 5
Summary of oxygen isotope fractionation during oxidation of pyrite by O_2 and $Fe(III)_{aq}$

Days	Sample	% Oxygen from H_2O[a]	$\delta^{18}O_{O_2}$ (‰)	Oxygen isotopic Fractionation		References
				$\varepsilon^{18}O_{SO_4-O_2}$[b]	$\varepsilon^{18}O_{SO_4-H_2O}$	
Oxidation of pyrite by O_2						
22	Short term biological	85	21	−10.0[c]	3.5[d]	
				−11.4[e]		[e]Taylor et al. (1984b)
44	Long term biological	92	21	−10.8[c]	4.0[d]	
44	Abiotic	87	21 ± 0.5[i]	−9.8[c]	2.8[d]	
				−4.3[e]	0.0[e]	[e]Taylor et al. (1984b)
				−8.7[f]		[f]Lloyd (1968)
Oxidation of pyrite by Fe(III)						
35	Biological	94		NA	3.6[h]	
35	Abiotic	95		NA	2.9[h]	
					2.6[g]	[g]van Everdingen and Krouse (1985)
					4.1[e]	[e]Taylor et al. (1984b)

NA, not applicable.
[a] % Estimated from linear regressions between SO_4 and H_2O, see Figs. 3 and 4.
[b] $\Delta_{SO_4-O_2} = (\delta^{18}O_{SO_4-O_2}) - (\text{measured } \delta^{18}O_{O_2})$; $\delta^{18}O_{O_2} = \sim 21‰$ for short and long term biotic and abiotic experiments.
[c] Calculated using % water oxygen and % molecular O_2 from linear regression, see Fig. 4, and anaerobic [h]$\Delta_{SO_4-H_2O}$ values.
[d] Calculated using % water oxygen and % molecular O_2 from linear regression, see Fig. 4, and aerobic [c]$\Delta_{SO_4-O_2}$ values.
[e] From Taylor et al. (1984b).
[f] From Lloyd (1968).
[g] From van Everdingen and Krouse (1985).
[h] Obtained from the intercept calculated from linear regressions, see Fig. 3.
[i] A $\delta^{18}O_2$ value of +29.7‰ for O_2 measured from this experiment was omitted as it appeared to be an outlier when compared to five other replicate measurements, the average from which is used here, see the text.

The $\varepsilon^{18}O_{SO_4-H_2O}$ values of 4.0‰ and 3.5‰ for the biological long and short term experiments of pyrite oxidation by O_2 are also consistent with the previous studies and are similar to our anaerobic experiments (Lloyd, 1968; van Everdingen and Krouse, 1985; Taylor et al., 1984b). This similarity suggests that oxygen isotopes may have been fractionated by comparable mechanisms during oxidation of pyrite with either O_2 or $Fe(III)_{aq}$. Water–oxygen incorporation into sulfate occurs during oxidation of pyrite by aqua-Fe(III) complexes (Luther, 1987; Moses et al., 1987). Three primary steps are involved in the oxidation process (Luther, 1987; Moses et al., 1987). The first step is the formation of aqua Fe(III) complexes through the oxidation of adsorbed Fe(II) by O_2 (Singer and Stumm, 1970; Wiersma and Rimstidt, 1984; McKibben and Barnes, 1986; Moses et al., 1987). The next step involves the binding of $Fe(III)(H_2O)_6$ complexes to the pyrite surface. The remaining step is the electron transfer between the sulfur side of pyrite and the aqua Fe(III) complexes, which involves removing an oxygen atom from water to the sulfur side of pyrite (Luther, 1987; Rimstidt and Vanguah, 2003). Based on this reaction sequence, the most likely step to cause oxygen isotope fractionation is during transfer of an oxygen atom from the hydroxyl radical of the aqua-Fe(III) complexes to the sulfur side of pyrite. The latter scenario is consistent with the results of Biegler and Swift (1979) who reported that pyrite oxidation is controlled by the attachment of an oxygen atom from water to a sulfur atom at the mineral surface. These reaction sequences could participate in the oxidation of pyrite by either $Fe(III)_{aq}$ or O_2 and thus could account for the similar $\varepsilon^{18}O_{SO_4-H_2O}$ values observed in these experiments.

Using the fractional contribution of oxygen into sulfate from O_2 vs. water (Fig. 4), the oxygen isotope fractionation during incorporation of O_2 oxygen into sulfate was determined for biological and abiotic oxidation of pyrite by O_2 (Table 5). The kinetic oxygen isotope fractionation ($\varepsilon^{18}O_{SO_4-O_2}$) was estimated to range between −10.0‰ and −10.8‰ for the short and long term biological experiments, respectively. These compare to an estimate of −9.8‰ for the abiotic experiment, −11.4‰ previously measured for bacterial oxidation of pyrite (Taylor et al., 1984b), and values between −10 and −29‰ for biological respiration with O_2 (Lane and Dole, 1956; Kroopnick and Craig, 1976; Guy et al., 1987). Abiotic and bacterial Mn(II) oxidation also produce Mn oxides whose $\delta^{18}O$ values indicate that oxygen is derived from O_2 and H_2O (Mandernack et al., 1995). All of these previous studies and our current one indicate that ^{16}O is favored when O_2 oxidizes organics, sulfide, and Mn(II).

Pyrite oxidation is generally considered to be initiated by reaction with molecular oxygen (Moses and Herman, 1991; Sato, 1992; Nesbitt and Muir, 1994; Sasaki, 1994; Sasaki et al., 1995). Molecular oxygen is reduced through the oxidation of $Fe(II)(H_2O)_6$ complexes on the pyrite surface forming $Fe(III)_{aq}$ which in turn oxidizes pyrite. Pyrite oxidation by $Fe(III)_{aq}$ is considered to be much faster relative to oxidation by O_2 due to the unlikelihood of oxidation between paramagnetic O_2 and diamagnetic FeS_2 (Luther, 1987; Moses et al., 1987). Luther (1987) argued that the faster rate of pyrite oxidation by $Fe(III)_{aq}$ was the result of $Fe(III)_{aq}$ binding directly to the pyrite surface via formation of a transition intermediate oxidant (i.e., persulfido bridge), which facilitates the electron transfer from highest occupied molecular orbital of S_2^{-2} to the lowest unoccupied molecular orbital of Fe(III). Oxidation of pyrite by $Fe(III)_{aq}$ produces thiosulfate (Luther, 1987; Moses et al., 1987; Moses and Herman, 1991; Descostes et al., 2001; Rimstidt and Vanguah, 2003). Because there is not a direct attack of sulfur atoms by O_2 during formation of thiosulfate, it is unlikely that oxygen atoms from O_2 are incorporated into thiosulfate. Therefore, molecular oxygen incorporation into sulfate may only occur during subsequent oxidation of thiosulfate to sulfate. Because thiosulfate is commonly oxidized to sulfite prior to oxidation to sulfate by both biological and abiotic pathways (Williamson and Rimstidt, 1992; Suzuki et al., 1994), O_2 incorporation into sulfate may only occur during sulfite oxidation as suggested in the proposed mechanism of pyrite oxidation that follows in Section 4.4 (reaction (12); Fig. 5).

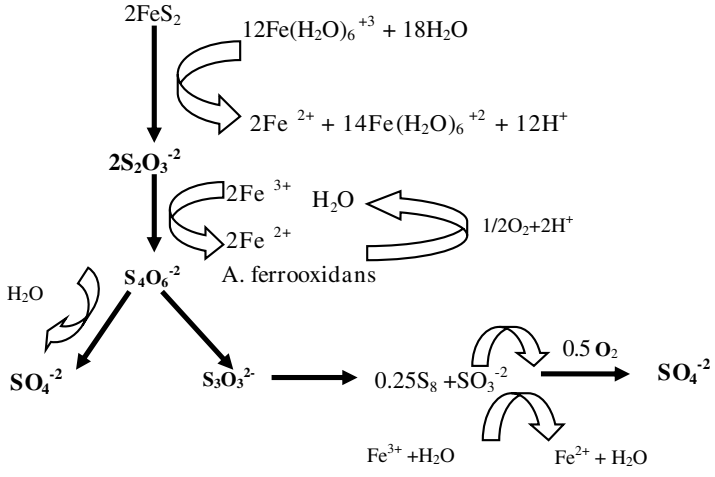

Fig. 5. Proposed pyrite oxidation pathways for the short term biological (pH 2.7) experiments under O_2 saturated conditions, as originally presented by Schippers et al. (1996).

4.3. Kinetic sulfur isotope fractionation between SO_4^{2-} and pyrite

In general, sulfur isotope effects associated with solid phase metal sulfide oxidation is insignificant compared to oxidation of aqueous sulfide (Sakai, 1957; Nakai and Jensen, 1964; McCready and Krouse, 1982; Taylor et al., 1984b; Fry et al., 1986). Oxidation of sulfide minerals to sulfate at earth surface temperatures seems to be a quantitative and unidirectional process that produces negligible sulfur-isotope fractionation. This often results in $\delta^{34}S_{SO_4}$ values being indistinguishable from those of the parent sulfide minerals (Gavelin et al., 1960; Nakai and Jensen, 1964; Field, 1966; Rye et al., 1992). However, sulfur isotope fractionation during bacterial sulfide oxidation has been observed at low (Taylor et al., 1984b; Seal and Wandless, 1997) and high pH (Toran and Harris, 1989). The largest fractionation (−15‰) was reported by Kaplan and Rittenberg (1964) during oxidation of dissolved Na_2S in experiments inoculated with *Acidithiobacillus thiooxidans* (formerly *Thiobacillus thiooxidans*). They measured enrichment in ^{34}S in polythionates, which accumulated during oxidation, and attributed this fractionation to sulfur isotope exchange between these intermediates and sulfate. Other studies report sulfur isotope fractionations of 2‰ to −5.5‰ for FeS_2 and ZnS oxidation with a mixture of Thiobacillus species (Toran and Harris, 1989), −4.7‰ during oxidation of thiosulfate by *Thiomonas intermedia* (formerly *Thiobacillus intermedius*) (Chambers and Trudinger, 1978), and −5.2‰ during abiotic oxidation of Na_2S in aqueous solution at pH 11 (Fry et al., 1988). Sulfur isotope fractionation associated with sulfide oxidation at high pH seems to be related to the enhanced formation of intermediate sulfoxyanions (Goldhaber, 1983).

The $\delta^{34}S_{SO_4}$ values from biological and abiotic-control aerobic experiments in this study closely reflect the $\delta^{34}S$ value of pyrite, and this lack of significant sulfur isotope fractionation did not vary much with time (Table 4). This lack of fractionation in the aerobic experiments is consistent with previous studies and indicates the complete oxidation of sulfur on the pyrite surface to sulfate under the experimental conditions (Nakai and Jensen, 1964; McCready and Krouse, 1982; Taylor et al., 1984a,b). The $\delta^{34}S_{SO_4}$ values from the pyrite oxidation experiments with $Fe(III)_{aq}$ were circa 0.7‰ lower compared to $\delta^{34}S_{FeS_2}$ (Table 3), indicating a small but significant sulfur isotope fractionation. Abiotic oxidation of pyrite by $Fe(III)_{aq}$ can result in intermediate sulfur species between pyritic sulfur and sulfate (Goldhaber, 1983; Moses et al., 1987; Schippers et al., 1996; Schippers and Sand, 1999; Borda et al., 2003; Druschel et al., 2003). Elemental sulfur and polysulfides have been suggested as intermediates of pyrite oxidation (Buckley and Riley, 1991; Sasaki et al., 1995; Kamei and Ohmoto, 1999). The incomplete oxidation of these intermediates to sulfate at low pH in the presence of excess $Fe(III)_{aq}$ has been reported elsewhere (Schippers and Sand, 1999; McGuirre et al., 2001; Druschel et al., 2003; Descostes et al., 2004) and this may have caused the sulfur isotope fractionation observed in the pyrite oxidation experiments with $Fe(III)_{aq}$.

4.4. Mechanisms of pyrite oxidation

The results of the pyrite oxidation experiments with O_2 provide insight into the role and relative contribution of O_2 to sulfate during pyrite oxidation. Although reactions (1) and (2) are commonly used to describe the oxidation of pyrite, the actual mechanisms are much more complex. Most studies suggest that it is the Fe–S bond in pyrite that preferentially ruptures during oxidation rather than the S–S bond (Moses and Herman, 1991; Sato, 1992; Rimstidt and Vanguah, 2003; Seal, 2003). According to this pathway, the first step in the oxidation of pyrite is release of Fe(II) to solution, which leaves a S rich surface. Under acidic conditions, surface studies have confirmed a sulfur rich layer on pyrite containing disulfides, monosulfides and polysulfides (Nesbitt and Muir, 1994; Sasaki, 1994; Sasaki et al., 1995), which gradually are oxidized to thiosulfate and sulfate (Nesbitt and Muir, 1994; Schippers et al., 1996; Guevremont et al., 1998). Although molecular oxygen is needed for the release of iron from the pyrite lattice to initiate the reaction, most studies indicated that $Fe(III)_{aq}$ is the primary oxidant for pyrite in both biological and abiotic systems (Wiersma and Rimstidt, 1984; McKibben and Barnes, 1986; Moses et al., 1987; Brown and Jurinak, 1989; Moses and Herman, 1991). Rimstidt and Vanguah (2003) suggested that molecular oxygen probably interacts with Fe^{2+} sites on the pyrite surface, but not directly with sulfur atoms. According to their model, pyrite oxidation is electrochemical in nature and consists of three different steps: (1) a cathodic reaction, (2) electron transport, and (3) an anodic reaction. Although the cathodic reaction is not well understood, there is evidence that electrons are transferred from the metal rather than from sulfur in the mineral to the oxidant at the cathodic sites. At the anodic sites, the oxygen atom of a water molecule interacts with a sulfur atom to produce a sulfoxy species. As a result, oxidation occurs at the anodic site. However, to initiate this oxidation, it is necessary to remove electrons from the cathodic sites. Therefore, the cathodic reaction is thought to be the rate-limiting step for pyrite oxidation. According to these reaction mechanisms, water molecules seem to be the only source of oxygen for sulfate during pyrite oxidation. Therefore, Rimstidt and Vanguah (2003) indicated that reaction (1) does not accurately describe the mechanistic role of molecular oxygen in the formation of sulfate from pyrite even though the reaction can describe the overall net transformation. In reaction (2), all oxygen atoms of sulfate are derived from water and the oxygen source is consistent with the electron transfer pathways proposed in the literature (Moses et al., 1987).

All of the of pyrite oxidation experiments with O_2 showed that SO_4 contains a large percentage (85–92%) of water-derived oxygen (Table 4, Fig. 4), but also significant incorporation of O_2, consistent with previous studies (Taylor et al., 1984a,b; Krouse et al., 1991). Taylor et al. (1984b) investigated the $\delta^{18}O_{SO_4}$ and $\delta^{34}S_{SO_4}$ values produced during biological and abiotic pyrite oxidation at pH 2 under submerged and wet/dry conditions. From these experiments, they estimated the percent contribution of reactions (1) and (2) during abiotic and bacterially-mediated pyrite

oxidation based on their measured oxygen isotope fractionation effects between sulfate and water ($\varepsilon^{18}O_{SO_4-H_2O}$) and molecular oxygen ($\varepsilon^{18}O_{SO_4-O_2}$). In their submerged abiotic control experiments, they estimated that 94% of pyrite was oxidized via reaction (2) with $Fe(III)_{aq}$ as the oxidant and 6% was via reaction (1) with O_2 as an oxidant. In the submerged biological experiments, they reported that 65% of pyrite oxidation was mediated by reaction (2). The same approach was applied to their wet-dry experiments, whereby 72% and 23% of pyrite oxidation was mediated by reaction (2) in the sterile and biological experiments, respectively. From these results, Taylor et al. (1984b) estimated the percentage of water-derived and O_2-derived oxygen into sulfate based on reaction types and stoichiometry. However, they were not able to statistically determine the exact percentage of O_2- and H_2O-derived oxygen in sulfate as we did here.

During the short (20 days) and long term (45 days) experiments, the percentages of oxygen atoms incorporated into sulfate from water and O_2 indicate that neither reaction (1) nor (2) represent the true stoichiometry of pyrite oxidation under O_2 saturated conditions (Fig. 4). Also, greater incorporation of water-derived oxygen into sulfate was observed with increasing extent of the reaction as inferred from the long-term experiments (Fig. 4, Table 4). The reaction pathway proposed by Schippers et al. (1996) for pyrite oxidation under O_2 saturated conditions at pH 2.7 (Fig. 5) is consistent with the percentage of molecular O_2 incorporation of 85% to 87% during our short term biological and abiotic aerobic experiments (Fig. 4). According to this pathway, two steps (11 and 13) produce sulfate (Schippers et al., 1996).

$$2FeS_2 + 12Fe(H_2O)_6^{+3} + 18H_2O \rightarrow 2S_2O_3^{-2} + 14Fe(H_2O)_6^{+2} + 12H^+ \quad (9)$$

$$2S_2O_3^{-2} + 2Fe^{3+} \rightarrow S_4O_6^{-2} + 2Fe^{2+} \quad (10)$$

$$S_4O_6^{-2} + H_2O \rightarrow SO_4^{-2} + S_3O_3^{-2} + 2H^+ \quad (11)$$

$$S_3O_3^{-2} \rightarrow 0.25S_8 + SO_3^{-} \quad (12)$$

$$SO_3^{-2} + 1/2O^*_2 \rightarrow SO^*O_3^{-2} \quad (13)$$

*Denotes oxygen incorporation from O_2

Overall reaction:

$$2FeS_2 + 12Fe(H_2O)_6^{+3} + 19H_2O + 2Fe^{3+} + 1/2O_2 \rightarrow 14Fe(H_2O)_6^{+3} + 2Fe^{2+} + 14H^+ + 2SO_4^{-2} + 0.25S_8 \quad (14)$$

In the proposed pathway, the first intermediate oxidation product is thiosulfate ($S_2O_3^{2-}$) (reaction (9)) (Moses et al., 1987; Xu and Schoonen, 1995; Schippers and Sand, 1999). The pyrite surface catalyzes the oxidation of thiosulfate to tetrathionate (reaction (10)) (Xu and Schoonen, 1995; Schippers and Sand, 1999). Tetrathionate is then hydrolyzed to form sulfate and disulfane-monosulfonic acid (reaction (11)). Different reactions can be initiated from disulfane-monosulfonic acid but ultimately this gives rise to elemental sulfur, sulfite and other sulfoxyanions at pH < 3 (Schippers et al., 1996; Schippers and Sand, 1999; Descostes et al., 2004). Sulfite is unstable in acidic solutions and it may be chemically oxidized to sulfate by incorporating O_2 as previously suggested by Krouse et al. (1991, see reaction 13). Sulfate produced by reaction (11) derives all oxygen atoms from water (100%) whereas sulfate from reaction (13) derives 75% of oxygen atoms from water and 25% from O_2. When the net stoichiometry (reaction (14)) is taken into account, 87.5% of sulfate oxygen comes from water and correspondingly 12.5% from O_2. Therefore, this reaction scheme is in good agreement with the results of our short term aerobic experiments (Fig. 4) and suggest that pyrite may have been oxidized according to the net stoichiometry of this proposed pathway (reaction (14)).

Because $Fe(III)_{aq}$ is still the main oxidizing agent for pyrite in this proposed pathway (Fig. 5), and the abiotic oxidation of $Fe(II)_{aq}$ to $Fe(III)_{aq}$ is very slow at pH < 3, the dominant bacterial role is likely the oxidation of Fe(II) to $Fe(III)_{aq}$ which catalyzes reaction (3). The similar kinetic oxygen isotope fractionations ($\varepsilon^{18}O_{SO_4-H_2O}$ and $\varepsilon^{18}O_{SO_4-O_2}$) and $\delta^{18}O_{SO_4}$ values observed in our abiotic and biological aerobic pyrite oxidation experiments may suggest that the proposed reaction mechanism (reactions (9)–(13)) also applies to abiotic pyrite oxidation (Table 2, Fig. 4). This is in agreement with previous studies that indicate that $Fe(III)_{aq}$ is the dominant oxidizing agent for abiotic and biological experiments at pH < 3 (Taylor et al., 1984b; Moses et al., 1987; Schippers et al., 1996; Schippers and Sand, 1999). Elemental sulfur formed during reaction (14) may further oxidize to sulfate because *A. ferrooxidans* is capable of oxidizing elemental sulfur to sulfate with O_2 during which all of the oxygen sulfate is derived from H_2O (McCready and Krouse, 1982; Pronk et al., 1992; Balci Celik, 2005). This may have important implications for interpreting the results from our biological long term experiments.

4.5. Mechanisms of pyrite oxidation with prolonged incubation and/or lower pH

Compared to the short term experiments, the $\delta^{18}O_{SO_4}$ values from the long term experiments showed larger oxygen incorporation from water into sulfate (92%) and an estimated $\varepsilon^{18}O_{SO_4-H_2O}$ value of 4.0‰ that closely compares with $\varepsilon^{18}O_{SO_4-H_2O}$ values we measured from the oxidation of pyrite by $Fe(III)_{aq}$ (Figs. 2 and 4, Table 5). This comparable $\varepsilon^{18}O_{SO_4-H_2O}$ value may have resulted from less O_2 incorporation during sulfite oxidation (Eq. (12)) and is in general agreement with a previous study that reported higher O_2 incorporation into sulfate during sulfide oxidation at increasing and more neutral pH (Lloyd, 1968).

The results of the long-term experiment do not exactly follow the stoichiometry of the proposed pathway by Schippers et al. (1996) (Fig. 5). Although the difference in the estimated slopes from the short and long term experiments is not statistically significant (Fig. 4), the 15% percent incorporation of O_2 into sulfate during the short term biological

experiment is significant, even at the 99% confidence interval (i.e., incorporation of H_2O-derived oxygen is $85 \pm 7\%$). There are several plausible explanations for increased water–oxygen incorporation into sulfate with prolonged incubation: (1) Subsequent oxidation of the S° product (Eq. (14)) during which no O_2 incorporation occurs, (2) slower rate of reaction (13) relative to reaction (11) with decreasing pH, (3) increased oxygen isotopic exchange between a sulfite intermediate and H_2O, and (4) oxidation by $Fe(III)_{aq}$ became relatively more important with increasing extent of the reaction due to increasing $Fe(III)_{aq}$ concentration which activated reaction (2). It has been reported that oxygen in sulfate formed during biological oxidation of elemental sulfur at pH < 3 is derived completely from water oxygen (McCready and Krouse, 1982; Balci, 2005). Therefore, the longer incubation may have permitted subsequent bacterial oxidation of S° to sulfate and an apparently higher incorporation of H_2O-derived oxygen.

Assuming that pyrite oxidation occurred in our experiments according to Eqs. (9)–(13), the lower pH of the long term experiments may have augmented the hydrolysis of tetrathionate to sulfate (reaction (11)) relative to reaction (13). Furthermore, disulfane-monosulfonic acid ($S_3O_3^{2-}$) is also produced during reaction (11) and reacts to elemental sulfur and sulfite (reaction (12)). Because intermediate sulfoxyanions and sulfite are more common at higher pH (Goldhaber, 1983), this might similarly slow the oxidation of sulfite to sulfate (reaction (13)) at lower pH. Therefore, any relative decrease in reaction (13) would result in less O_2 incorporation into sulfate and correspondingly lower $\delta^{18}O_{SO_4}$ values would be expected with decreasing pH.

Alternatively, variations in contributions of water-derived oxygen to sulfate may also occur because of several different pathways of sulfite oxidation, which may involve disulfate ($S_2O_7^{2-}$) or other intermediates (Chang et al., 1987). These sulfoxyanion intermediates could exchange oxygen isotopes with H_2O and thus increase the percentage of water–oxygen incorporated into sulfate. The oxygen isotope exchange between sulfite and water was demonstrated to be on the order of nanoseconds at pH < 3 by previous studies (Lloyd, 1968; Pearson and Rightmire, 1980; Holt et al., 1981). Therefore, if sulfite is present in the system, oxygen isotope exchange between sulfite and water may occur very rapidly and be reflected in the sulfate product.

Lloyd (1968) estimated the time required for isotopic equilibration of ocean water and sulfate–oxygen exchange (pH 8.2, 4 °C) to be on the order of 250,000 years based on laboratory experiments at pH 7 and 25 °C. Further, Chiba and Sakai (1985) extrapolated their laboratory studies for conditions of 100–300 °C and pH 2–7 to estimate a half-life of 10^9 years for ocean water–sulfate oxygen isotope exchange. Chiba and Sakai's results are in agreement with earlier experimental studies of water–sulfate oxygen isotope equilibration at pH < 0 by Hoering and Kennedy (1957). Moreover, Seal et al. (2000, 2003) compared the kinetics of oxygen isotope exchange between sulfate and water with respect to pH and $\log t_{1/2}$ using data from these earlier studies for which they demonstrated good overall agreement (Hoering and Kennedy, 1957; Lloyd, 1968; Chiba and Sakai, 1985). Based on these studies, it appears that at most Earth surface conditions, the oxygen isotope exchange between water and sulfate is extremely slow. Therefore, under the long term experimental conditions of this study, oxygen isotopic exchange between sulfate and H_2O is not likely the reason for greater water–oxygen incorporation into sulfate. However, Mizutani and Rafter (1969, 1973) and Fritz et al. (1973) indicated oxygen isotope exchange between sulfate and water proceeds through enzyme-bound intermediates during the bacterial reduction of sulfate. Similar oxygen isotope exchange processes may also occur during sulfide oxidation and might be enhanced with prolonged incubation times.

The incorporation of oxygen into sulfate exclusively from water is generally attributed to the $Fe(III)_{aq}$ oxidation pathway by previous studies (Taylor et al., 1984a,b). Reaction (2) represents the stoichiometry of this reaction and indicates 100% water–oxygen incorporation into sulfate. Since the long term experiments showed ~92% water–oxygen incorporation into sulfate, pyrite oxidation by $Fe(III)_{aq}$ may have become increasingly important during the long term pyrite experiments as the $Fe(II)_{aq}$ was continuously leached and oxidized by the bacteria. Almost identical oxygen isotope fractionation between sulfate and water ($\varepsilon^{18}O_{(SO_4-H_2O)}$) was observed for the aerobic long term (4.0‰) and anaerobic experiments (3.6‰), which would be consistent with $Fe(III)_{aq}$ as an oxidant.

5. CONCLUSIONS

We have investigated the isotopic composition (S, O) of sulfate formed during biological and abiotic pyrite oxidation under aerobic and anaerobic conditions by using O_2 and $Fe(III)_{aq}$ as oxidizing agents, respectively. Water is the sole oxygen source to sulfate during anaerobic oxidation of pyrite by $Fe(III)_{aq}$, with indistinguishable $\varepsilon^{18}O_{(SO_4-H_2O)}$ values for the abiotic and biological experiments. $Fe(III)_{aq}$ also appears to be the main oxidizing agent under aerobic conditions, and consequently water is the main oxygen source for sulfate. However, the short-term aerobic experiments indicate that ~12.5% of the oxygen in sulfate is derived from O_2, which is consistent with the reaction scheme proposed by Schippers et al. (1996). Although $\delta^{18}O_{SO_4}$ values may reflect the mechanisms and environmental conditions (aerobic vs. anaerobic) of pyrite oxidation, sulfate produced during aerobic biological and abiotic pyrite oxidation has similar percent contributions of water–oxygen, comparable $\varepsilon^{18}O_{(SO_4-H_2O)}$ and $\varepsilon^{18}O_{(SO_4-O_2)}$ values and thus similar $\delta^{18}O$ values. Therefore, $\delta^{18}O_{SO_4}$ values do not clearly reflect biological vs. abiotic origins. Previous work on pyrite oxidation (biological and abiotic) suggested that $\delta^{18}O_{SO_4}$ values produced by biological reactions were enriched in ^{18}O relative to abiotic reactions due to greater incorporation of atmospheric oxygen. However, we observed no evidence of this under our experimental conditions. Therefore, elevated $\delta^{18}O_{SO_4}$ values may not conclusively indicate biological activity during the oxidation process.

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