Pergamon

Geochimica et Cosmochimica Acta, Vol. 66, No. 1, pp. 85–92, 2002
Copyright © 2002 Elsevier Science Ltd
Printed in the USA. All rights reserved
0016-7037/02 $22.00 + .00

PII S0016-7037(01)00745-1

Biogeochemistry of pyrite and iron sulfide oxidation in marine sediments

AXEL SCHIPPERS* and BO BARKER JØRGENSEN

Department of Biogeochemistry, Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359 Bremen, Germany

(*Received February* 26, 2001; *accepted in revised form July* 5, 2001)

Abstract—Pyrite (FeS_2) and iron monosulfide (FeS) play a central role in the sulfur and iron cycles of marine sediments. They may be buried in the sediment or oxidized by O_2 after transport by bioturbation to the sediment surface. FeS_2 and FeS may also be oxidized within the anoxic sediment in which NO_3^-, Fe(III) oxides, or MnO_2 are available as potential electron acceptors. In chemical experiments, FeS_2 and FeS were oxidized by MnO_2 but not with NO_3^- or amorphous Fe(III) oxide (Schippers and Jørgensen, 2001). Here we also show that in experiments with anoxic sediment slurries, a dissolution of tracer-marked $^{55}FeS_2$ occurred with MnO_2 but not with NO_3^- or amorphous Fe(III) oxide as electron acceptor. To study a thermodynamically possible anaerobic microbial FeS_2 and FeS oxidation with NO_3^- or amorphous Fe(III) oxide as electron acceptor, more than 300 assays were inoculated with material from several marine sediments and incubated at different temperatures for $>$ 1 yr. Bacteria could not be enriched with FeS_2 as substrate or with FeS and amorphous Fe(III) oxide. With FeS and NO_3^-, 14 enrichments were obtained. One of these enrichments was further cultivated anaerobically with Fe^{2+} and S^0 as substrates and NO_3^- as electron acceptor, in the presence of $^{55}FeS_2$, to test for co-oxidation of FeS_2, but an anaerobic microbial dissolution of $^{55}FeS_2$ could not been detected. FeS_2 and FeS were not oxidized by amorphous Fe(III) oxide in the presence of Fe-complexing organic compounds in a carbonate-buffered solution at pH 8. Despite many different experiments, an anaerobic microbial dissolution of FeS_2 could not be detected; thus, we conclude that this process does not have a significant role in marine sediments. FeS can be oxidized microbially with NO_3^- as electron acceptor. O_2 and MnO_2, but not NO_3^- or amorphous Fe(III) oxide, are chemical oxidants for both FeS_2 and FeS. *Copyright © 2002 Elsevier Science Ltd*

1. INTRODUCTION

In anoxic marine sediments, pyrite (FeS_2) and iron monosulfide (FeS) are formed as the result of organic matter degradation by sulfate-reducing bacteria. In bioturbated sediments, FeS_2 and FeS can be transported to the sediment surface where O_2 chemically oxidizes the metal sulfides (Luther et al., 1982; Thamdrup et al., 1994; Peterson et al., 1996; Ferdelman et al., 1997). Marine sediments are carbonate buffered and, therefore, neutral pH values prevail. The mechanisms and kinetics of FeS_2 and FeS oxidation by O_2 under these chemical conditions are well described in the literature (Lowson, 1982; Luther, 1987; Moses et al., 1987; Nicholson et al., 1988, 1990; Morse, 1991; Moses and Herman, 1991; Williamson and Rimstidt, 1994; Evangelou et al., 1998; Sand et al., 2001). Sulfur compound intermediates of FeS_2 oxidation might be oxidized to sulfate by aerobic bacteria of the genera *Thiobacillus* or *Thiomicrospira* (Kuenen et al., 1992).

In the deeper anoxic sediment, a variety of sulfur compounds (SO_4^{2-}, $S_2O_3^{2-}$, S^0, and HS^-), but not FeS_2, are oxidized or reduced as revealed with ^{35}S-labeled sulfur compounds (Fossing and Jørgensen, 1990; Elsgaard and Jørgensen, 1992). Anoxic FeS oxidation by MnO_2 and bacteria in marine sediments was shown by Aller and Rude (1988). Recently, we have also shown that FeS_2 is oxidized in anoxic marine sediments (Schippers and Jørgensen, 2001). MnO_2 chemically oxidized FeS_2 via thiosulfate and polythionates to sulfate, whereas FeS was oxidized via polysulfides mainly to elemental sulfur. These intermediate sulfur compounds can be oxidized by bacteria such as sulfur-disproportionating bacteria (Thamdrup et al., 1993; Finster et al., 1998).

Besides MnO_2, NO_3^- or Fe(III) oxides might oxidize FeS_2 and FeS in anoxic marine sediments according to thermodynamic considerations. A purely chemical oxidation of FeS_2 and FeS with NO_3^- or Fe(III) oxides at pH 8 could not be detected (Schippers and Jørgensen, 2001). An FeS-mediated denitrification has been described for a marine sediment (Garcia-Gil and Golterman, 1993). An FeS_2 oxidation by the reduction of NO_3^- has been suggested for aquifers based on geochemical data (Postma et al., 1991; Engesgaard and Kipp, 1992; Cravotta, 1998; van Beek, 2000). A chemical anoxic FeS_2 dissolution in the presence of organic Fe(III) complexes in the pH range of 4 to 6.5 was shown by Luther et al. (1992). Bottrell et al. (2000) provided some isotopic evidence for an anoxic FeS_2 oxidation in marine sediments and proposed Fe(III) to be the oxidant in this process.

The present study explored the role of NO_3^- or Fe(III) oxides for FeS_2 or FeS oxidation in anoxic marine sediments. To reveal the importance of bacteria, more than 300 enrichment assays were inoculated with material from several marine sediments and incubated at different temperatures for $>$ 1 yr. For the most sensitive detection of FeS_2 oxidation, tracer-marked $^{55}FeS_2$ was used in experiments with bacterial cultures and sediment slurries. To study the influence of Fe-complexing organic compounds on anoxic FeS_2 and FeS oxidation, experiments were carried out under conditions relevant for marine sediments using amorphous Fe(III) oxide in a carbonate-buffered solution at pH 8.

* Author to whom correspondence should be addressed (A.Schippers@bgr.de).

2. MATERIAL AND METHODS

2.1. Marine Sediments

To allow us to draw general conclusions about the biogeochemistry of FeS_2 and FeS oxidation, samples for experiments from different marine sediments were taken from the top 10 cm between April and September 1999. The sampling sites were: (1) Skagerrak: Stations S4, S6, and S9 described by Canfield et al. (1993a,b); (2) a salt marsh near Woods Hole, Massachusetts, USA; (3) the estuary of Rio Tinto, near Huelva, Southwest Spain; (4) the Baltic Sea: Bornholm deep, Gotland deep, and a shallow site close to the German coast; (5) the Wadden Sea off the North Sea near Dangast, Germany; (6) the continental margin off central Chile: Stations C7 and C18, described by Thamdrup and Canfield (1996); (7) a fjord near Maarmorilik, Greenland; (8) 10 different sites around Svalbard in the Arctic. The samples were transported under exclusion of air in cooled boxes to Bremen and stored at 4°C in the dark until use.

2.2. Iron Sulfides

Four types of iron sulfides were used for oxidation experiments: (1) a commercially available FeS with troilite (FeS) and pyrrhotite (Fe_7S_8) as the main minerals, (2) a coarse-grained FeS_2 from an ore-processing flotation plant, (3) a fine-grained synthetic FeS_2, (4) a fine-grained synthetic tracer-marked $^{55}FeS_2$ with an activity of 150 kBq/g. The preparation and the mineralogy of the iron sulfides were described previously (Schippers and Jørgensen, 2001). All iron sulfides were stored under dinitrogen until use.

2.3. Chemical Anoxic FeS_2 or FeS Oxidation Experiments With Fe(III) Oxide and Fe-Complexing Organic Compounds

Amorphous Fe(III) oxide was prepared as described by Lovley and Phillips (1986). The suspensions were made to a final concentration of 1-mol L^{-1} Fe. For the experiments, 0.5 g of fine FeS_2 or FeS were weighed into 250-mL flasks. To each assay, 50 mL of a 1 mol/L $NaHCO_3$ solution and 50 mL of a suspension of amorphous Fe(III) oxide were added. To each assay, 10 mM, 1 mM, or 0.1 mM of the following compounds were given: (1) salicylic acid, which forms a Fe(III) salicylate complex oxidizing FeS_2 (Luther et al., 1992); (2) citric acid, which forms a Fe(III) citrate complex accelerating oxic FeS_2 oxidation (Peiffer and Stubert, 1999); (3) oxalic acid, which forms an Fe(II) oxalate complex reducing Fe(III) oxide (Sulzberger et al., 1989; Wehrli et al., 1989); (4) AQDS (2,6-anthraquinone disulfonate), a humic substance analog shown to be able to reduce Fe(III) oxides (Lovley et al., 2000; Zachara et al., 2001). The pH remained at 8 ± 0.5 for all experiments. The flasks were closed with airtight butyl rubber seals, evacuated, and gassed with a mixture of CO_2/N_2 (10/90, v/v) three times. All assays were incubated at 20°C in the dark. Samples were taken after 1 and 3 months by a syringe flushed with a mixture of CO_2/N_2 (10/90, v/v) and analyzed for sulfur compounds as described previously (Schippers and Jørgensen, 2001).

2.4. Attempts to Enrich Anaerobic FeS_2 or FeS Oxidizing Bacteria

To enrich anaerobic FeS_2 and FeS oxidizing bacteria using NO_3^- or amorphous Fe(III) oxide as electron acceptor, more than 300 assays were inoculated with material from the marine sediment sites listed above. The medium for bacterial growth was prepared as described by Widdel and Bak (1992). The artificial saltwater medium at pH 7.0 to 7.3 (containing 1 mM $NaSO_4$ instead of 4 g/L) included nonchelated trace element mixture, selenite-tungstenite solution, bicarbonate solution, vitamin mixture, thiamine solution, and vitamin B_{12} solution. The medium contained 10 mM $NaNO_3$ or 250 mmol L^{-1} amorphous Fe(III) oxide as electron acceptor. As reducing agent, 1 mM $FeCl_2$ was added instead of sulfide to avoid growth of sulfide-oxidizing bacteria or chemical reduction of amorphous Fe(III) oxide by sulfide. Under a stream of CO_2/N_2 (10/90, v/v), ~30 mL of the medium was put into 50-mL serum flasks containing 0.5-g coarse FeS_2 or FeS as electron donor. The medium was inoculated with ~1 g of sediment material. To half of the flasks, 0.1 mM Na-acetate was added as carbon source, enabling heterotrophic growth of bacteria. To several flasks containing amorphous Fe(III) oxide, 0.1-mM AQDS was added to enable the shuttling of electrons from bacteria to amorphous Fe(III) oxide as described previously (Coates et al., 1998; Lovley et al., 2000; Zachara et al., 2001). The flasks were closed with airtight butyl rubber seals, evacuated, and gassed with a mixture of CO_2/N_2 (10/90, v/v) three times. The assays were incubated at 4, 10, 15, or 30°C in the dark for > 1 yr. Samples were taken by a syringe flushed with CO_2/N_2 (10/90, v/v) every 3 months and analyzed for bacteria by microscopy, and for NO_3^- reduction and SO_4^{2-} formation by ion chromatography (Dionex, DX 500). An enrichment was counted as positive if bacteria were visible; < 5-mM NO_3^- and > 2-mM SO_4^{2-} were detected.

2.5. Anoxic Slurry Experiments With $^{55}FeS_2$ and Marine Sediments

To study whether FeS_2, as quantitatively, the most important metal sulfide is oxidized in anoxic marine sediments in the presence of NO_3^- or Fe(III) oxide as electron acceptor, slurry experiments with $^{55}FeS_2$ and sediment from six different sites (site 1: stations S4 and S9, site 4: Bornholm deep and the shallow site, and site 6: stations C7 and C18) were performed. Into small serum bottles, 50-mg $^{55}FeS_2$ (7.5 kBq) was weighed, and added were 9-g sediment plus 1-mL 100 mM $NaNO_3$ in filtered anoxic seawater, 1 mL of 1 mol L^{-1} amorphous Fe(III) oxide plus 30 g/L NaCl, or 1 mL filtered anoxic seawater without electron acceptor. Finally, to each assay, 5-mL filtered anoxic seawater was added. The flasks were closed with airtight butyl rubber seals, evacuated, and subsequently gassed with a mixture of CO_2/N_2 (10/90, v/v) three times. All assays were shaken well and subsequently incubated at 20°C in the dark for 18 d. Samples were taken and radioactivity was measured as described previously (Schippers and Jørgensen, 2001). NO_3^- was measured by ion chromatography (Dionex, DX 500).

3. RESULTS

3.1. Chemical and Microbiological Oxidation of FeS_2 and FeS

To study the biogeochemistry of FeS_2 and FeS oxidation in marine sediments, many different experiments were carried out, which are summarized in Table 1. In anoxic, purely chemical experiments, Schippers and Jørgensen (2001) had found that FeS_2 and FeS were oxidized by MnO_2 but not with NO_3^- or amorphous Fe(III) oxide in a carbonate-buffered solution at pH 8. In the present study, we found also that FeS_2 and FeS were not oxidized by amorphous Fe(III) oxide in the presence of Fe-complexing organic compounds such as salicylic acid, oxalic acid, and citric acid, or in the presence of the electron-transporting compound AQDS (2,6-anthraquinone disulfonate) in a carbonate-buffered solution at pH 8. Accordingly, oxidation products of FeS_2 or FeS oxidation were not detected (data not shown).

To study a possible anaerobic microbial FeS_2 or FeS oxidation with NO_3^- or amorphous Fe(III) oxide as electron acceptor, more than 300 assays were inoculated with material from several marine sediments and incubated at different temperatures for > 1 yr. Bacteria could not be enriched with FeS_2 as substrate. With FeS as substrate and amorphous Fe(III) oxide as electron acceptor, bacterial growth also did not occur. With FeS and NO_3^-, however, seven positive enrichments were obtained at 15°C and seven at 30°C from the following sites: (1) Skagerrak: station S9; (2) the estuary of Rio Tinto, near Huelva, southwest Spain; (3) the Baltic Sea: Bornholm deep and Gotland deep; (4) the Wadden Sea off the North Sea near Dangast, Germany; (5) three different sites around Svalbard in the Arctic.

Table 1. Summary of FeS_2 and FeS oxidation experiments.

Experiment	Oxidant	Result	Reference
Chemical FeS_2 and FeS oxidation	MnO_2	positive	Schippers and Jørgensen (2001)
Chemical FeS_2 and FeS oxidation	Fe(III) oxide	negative	Schippers and Jørgensen (2001)
Chemical FeS_2 and FeS oxidation	NO_3^-	negative	Schippers and Jørgensen (2001)
Chemical FeS_2 and FeS oxidation	Fe(III) oxide and Fe-complexing organic compounds or AQDS	negative	this study
Enrichment of FeS_2 oxidizing bacteria	Fe(III) oxide	negative	this study
Enrichment of FeS_2 oxidizing bacteria	NO_3^-	negative	this study
Enrichment of FeS oxidizing bacteria	Fe(III) oxide (+ AQDS, + acetate)	negative	this study
Enrichment of FeS oxidizing bacteria	NO_3^-	positive	this study
Dissolution of $^{55}FeS_2$ control experiment	O_2	positive	this study
Dissolution of $^{55}FeS_2$ slurry experiment	MnO_2	positive	Schippers and Jørgensen (2001) and this study
Dissolution of $^{55}FeS_2$ slurry experiment	Fe(III) oxide	negative	this study
Dissolution of $^{55}FeS_2$ slurry experiment	NO_3^-	negative	this study
Dissolution of $^{55}FeS_2$ in FeS oxidizing bacterial culture	NO_3^-	negative	this study

An enrichment from the estuary of Rio Tinto was further cultivated anaerobically at 30°C with 2-mM Fe^{2+} and ~10 mg S^0 as substrates and 10-mM NO_3^- as electron acceptor in the presence of 50-mg tracer-marked $^{55}FeS_2$ to test for co-oxidation of FeS_2. Samples were taken from assays inoculated with bacteria or from control assays without bacteria and analyzed for concentrations of SO_4^{2-}, NO_3^-, Fe^{2+}, and ^{55}Fe in the medium. Results from an incubation period of 2.5 months are shown in Figure 1. SO_4^{2-} was formed and NO_3^- and Fe^{2+} were consumed in the assays with bacteria, presumably due to bacterial Fe^{2+} and S^0 oxidation coupled to NO_3^- reduction. Values of ^{55}Fe were not higher in the assays with bacteria than in the controls, which means that an anaerobic microbial dissolution of $^{55}FeS_2$ could not be detected.

3.2. Anoxic Slurry Experiments With $^{55}FeS_2$ and Marine Sediments

^{55}Fe recovery, due to the dissolution of $^{55}FeS_2$ in slurry experiments with samples from different marine sediments and with different electron acceptors, is shown in Figure 2. Irrespective of the addition of electron acceptors, the mean values of ^{55}Fe recovery for five different marine sediments were in the same low range as that of anoxic seawater. Thus, a dissolution of $^{55}FeS_2$ was not detected in these assays, although NO_3^- was consumed in the assays that had been amended with 10-mM NO_3^- twice during the period of incubation (Fig. 3). After each addition, nitrate was consumed presumably due to denitrification. In the three assays with a MnO_2-rich marine sediment, a dissolution of $^{55}FeS_2$ was detected (Fig. 2) due to chemical $^{55}FeS_2$ oxidation by MnO_2 (Schippers and Jørgensen, 2001). The addition of NO_3^- or amorphous Fe(III) did not enhance the degree of $^{55}FeS_2$ dissolution in the assays with the MnO_2-rich marine sediment. The highest amount of dissolved $^{55}FeS_2$ was measured for the assays with oxic seawater due to chemical FeS_2 oxidation by O_2. In summary, FeS_2 can be dissolved by O_2 or MnO_2, but not by NO_3^- or amorphous Fe(III) oxide in marine sediments.

4. DISCUSSION

Thermodynamically, O_2, MnO_2, Fe(III) oxide, and NO_3^- are potential oxidants for FeS_2 and FeS in marine sediments. Possible reactions for complete FeS_2 and FeS oxidation to SO_4^{2-} and the corresponding ΔG_f^0 values are shown in Table 2. In the case of O_2, MnO_2, and NO_3^-, the reactions are exergonic. With Fe(III) oxide as oxidant, the reactions are only exergonic if Fe(II) forms $FeCO_3$ and not Fe_3O_4 (Chaudhuri et al., 2001). Marine sediments contain carbonate; thus, oxidation of FeS_2 or FeS by Fe(III) oxide should be thermodynamically possible there.

4.1. Oxidation of FeS_2

Slurry experiments with tracer-marked $^{55}FeS_2$, as well as chemical and biologic experiments presented in this paper, have shown that FeS_2 is oxidized in marine sediments by O_2 and MnO_2, but not by Fe(III) oxide or NO_3^-.

In the case of FeS_2 oxidation by O_2 or MnO_2, Fe(III) has been shown to be the FeS_2-attacking oxidant, even at neutral pH (Luther, 1987; Moses et al., 1987; Moses and Herman, 1991; Schippers and Jørgensen, 2001). The sulfur moiety of

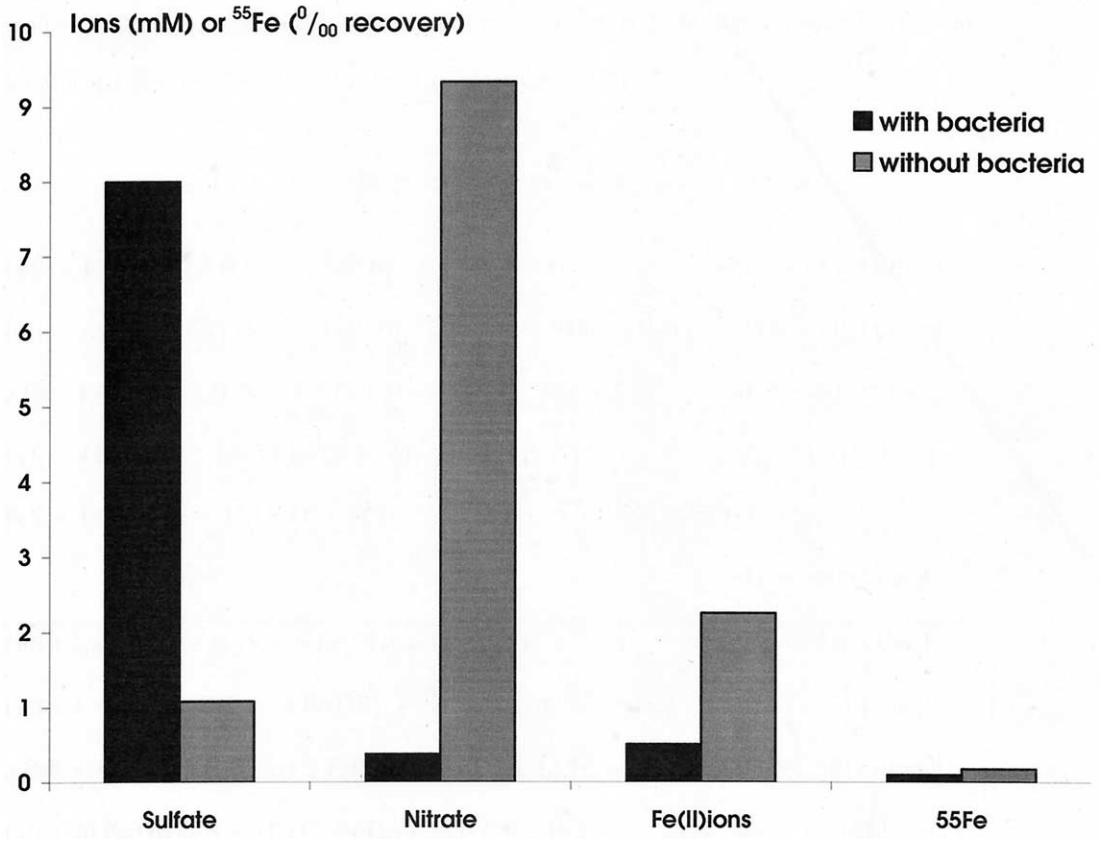

Fig. 1. Experimental study of $^{55}FeS_2$ dissolution in an S^0- and Fe^{2+}-oxidizing and NO_3^--reducing bacterial culture. Mean concentrations of sulfate, nitrate, Fe(II) ions, and extractable ^{55}Fe in the medium are shown for three assays with bacteria and three assays without bacteria after 2.5 months of incubation. The amount of $^{55}FeS_2$ dissolved was measured as HCl-extractable ^{55}Fe and related to total ^{55}Fe. The results show that bacteria do not increase the amount of $^{55}FeS_2$ dissolved above the background value of 0.1 to 0.2‰.

FeS_2 is oxidized via thiosulfate and polythionates to sulfate (Schippers et al., 1996). The Fe(II) is oxidized by O_2 or MnO_2 to Fe(III), thus closing the Fe cycle (Moses and Herman, 1991; Schippers and Jørgensen, 2001). Due to its mineral structure, FeS_2 cannot be dissolved by acid in the environment (Schippers and Sand, 1999; Sand et al., 2001). Based on these findings, we conclude that in the case of a thermodynamically possible FeS_2 oxidation by Fe(III) oxide or by NO_3^-, Fe(III) must be the FeS_2-attacking agent as well. In the following, we discuss why Fe(III) oxide and NO_3^- are not oxidants for FeS_2 in marine sediments.

In seawater at slightly alkaline pH, the solubility of Fe(III) oxides is very low, i.e., close to 10^{-10} M (Liu and Millero, 1999). The concentration of soluble Fe(III) under these conditions is apparently too low to allow for FeS_2 oxidation by Fe(III) oxides (Schippers and Jørgensen, 2001). Dissolved organic Fe(II)/(III) complexes were detected in salt marsh and marine sediment pore waters (Luther et al., 1996; Huettel et al., 1998; Taillefert et al., 2000). Luther et al., (1992) showed a chemical FeS_2 oxidation by 1-mM ferrihydrite and 10-mM salicylic acid at a pH range of 4 to 6.5. Ferrihydrite and salicylic acid form an Fe(III) salicylate complex, which reacts with FeS_2. In our experiments with a carbonate-buffered solution at pH 8 relevant for marine sediments, we were not able to show FeS_2 oxidation by amorphous Fe(III) oxide in the presence of organic Fe complexes or of the electron-transporting compound AQDS. Liu and Millero (1999) showed that the solubility of Fe(III) in the presence of Fe(III)-complexing humic acids is two orders of magnitude higher at pH 4 to 6 than at pH 8. Presumably, the concentration of complexed Fe(III) in our experiments was too low to enable FeS_2 dissolution. Thus, FeS_2 oxidation by soluble organic Fe(III) complexes might be relevant for salt marshes with slightly acidic pH values, but obviously not for carbonate-buffered marine sediments with slightly alkaline pH values. Consequently, $^{55}FeS_2$ was not dissolved in the sediment slurries amended with amorphous Fe(III) oxide, and bacteria could not be enriched using FeS_2 and amorphous Fe(III) oxide.

Precipitation of Fe(III) hydroxide might explain the absence of $^{55}FeS_2$ dissolution in an S^0- and Fe^{2+}-oxidizing and NO_3^--reducing bacterial culture. The bacteria oxidize Fe(II) to Fe(III), which has to diffuse from the Fe-oxidizing enzyme of the bacteria to the FeS_2 surface to serve as an oxidant for FeS_2. Obviously, Fe(III) precipitates immediately and, therefore, cannot serve as an oxidant for FeS_2. Furthermore, NO_3^--amended sediment slurries did not show $^{55}FeS_2$ dissolution, and bacteria could not be enriched with FeS_2 and NO_3^-, which shows that NO_3^- is not an oxidant for FeS_2 in marine sediments. In

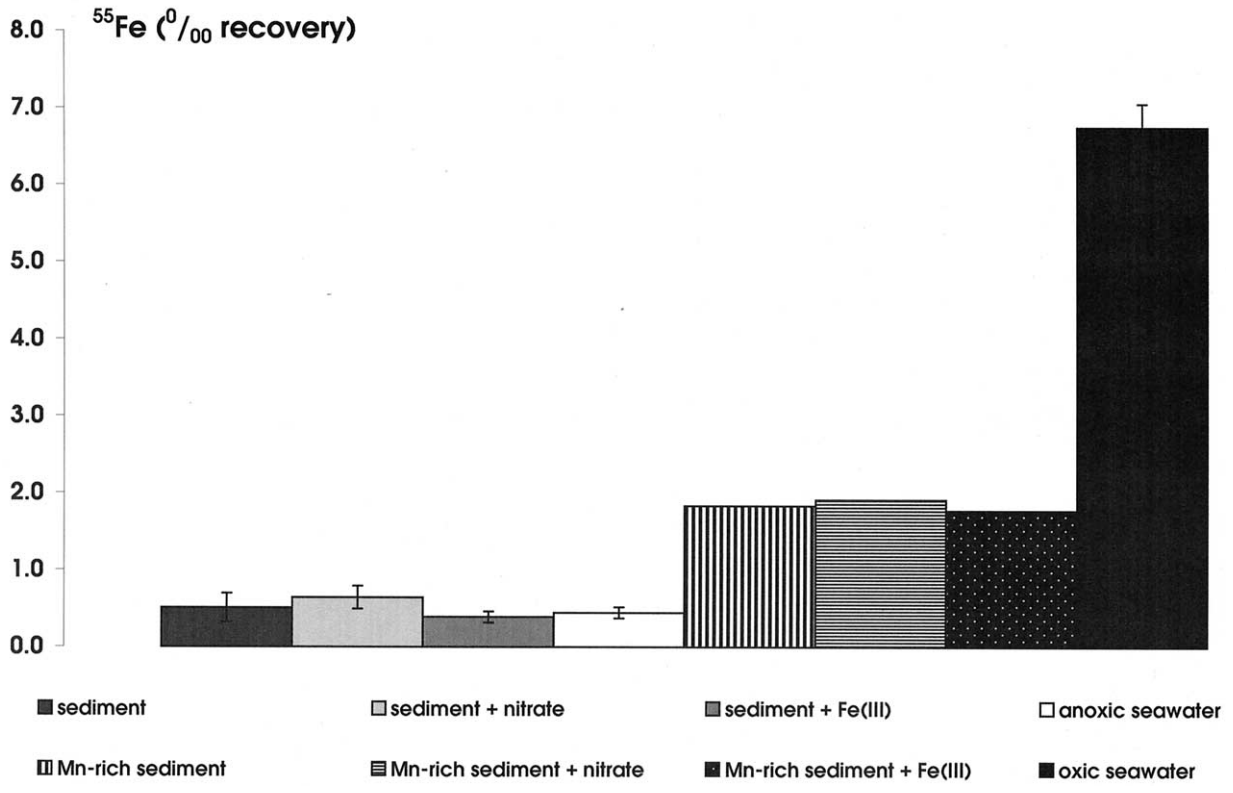

Fig. 2. Dissolution of $^{55}FeS_2$ in slurry experiments with samples from different marine sediments and different electron acceptors. Mean values and standard deviations for five different marine sediments and values for an MnO_2-rich marine sediment are shown. Sediments were incubated anaerobically for 18 d without the addition of an electron acceptor or NO_3^- (see also Fig. 3) or amorphous Fe(III) oxide. For comparison, $^{55}FeS_2$ was incubated in anoxic or oxic seawater. The amount of $^{55}FeS_2$ dissolved was measured as HCl-extractable ^{55}Fe and related to total ^{55}Fe.

aquifers in which slightly acidic pH values were detected, an FeS_2 oxidation by the reduction of NO_3^- has been suggested, based on depth profiles of NO_3^- and SO_4^{2-} (Postma et al., 1991; Engesgaard and Kipp, 1992; Cravotta, 1998; van Beek, 2000). There, Fe^{2+}-oxidizing and NO_3^--reducing bacteria and soluble organic Fe(III) complexes could catalyze an anoxic FeS_2 oxidation with NO_3^- as electron acceptor.

In contrast to Fe(III) oxide and NO_3^-, MnO_2 is an oxidant for FeS_2 in a carbonate-buffered solution at pH 8, presumably because of the direct contact of the two minerals, which allows an electron transport via an Fe(II)/Fe(III) shuttle being adsorbed to the mineral surfaces (Schippers and Jørgensen, 2001). In oxic sediments, O_2 can diffuse to the FeS_2 surface and oxidize adsorbed Fe(II) to Fe(III), which is the direct oxidant for FeS_2 (Moses and Herman, 1991). Consequently, MnO_2 and O_2 chemically oxidize FeS_2 in marine sediments.

4.2. Oxidation of FeS

FeS belongs to the acid-soluble metal sulfides, which are chemically oxidized via polysulfides to mainly elemental sulfur and some sulfate (Thomas et al., 1998, 2001; Schippers and Sand, 1999; Sand et al., 2001; Schippers and Jørgensen, 2001). Similar to FeS_2, FeS is chemically oxidized by O_2 and MnO_2, but not by Fe(III) oxide, neither alone nor in the presence of Fe-complexing organic compounds. In contrast to FeS_2, FeS can be oxidized biologically with NO_3^- as an electron acceptor. This finding is in agreement with results of Garcia-Gil and Golterman (1993) who described an FeS-mediated denitrification for a marine sediment. This biogeochemical coupling of iron, sulfur, and nitrogen cycles may be described by Eqn. 1 to 3 below. Due to its acid solubility, protons dissolve FeS according to:

$$FeS + H^+ \rightarrow Fe^{2+} + HS^- \quad (1)$$

Both products of this reaction may be oxidized by NO_3^--reducing bacteria. The Fe^{2+} can be oxidized according to Straub et al. (1996):

$$10\,FeCO_3 + 2\,NO_3^- + 24\,H_2O \rightarrow 10\,Fe(OH)_3 + N_2 + 10\,HCO_3^- + 8\,H^+ \quad (2)$$

HS^- may be oxidized by, e.g., *Thiobacillus denitrificans* or *Thiomicrospira denitrificans* (Kuenen et al., 1992; Kelly and Wood, 2000):

$$5\,HS^- + 8\,NO_3^- + 3\,H^+ \rightarrow 5\,SO_4^{2-} + 4\,N_2 + 4\,H_2O \quad (3)$$

In Eqn. 2, protons are produced, which continue to dissolve FeS. Bacteria might be attached to the FeS surface embedded in extracellular polymeric substances (EPS). Bacteria produce

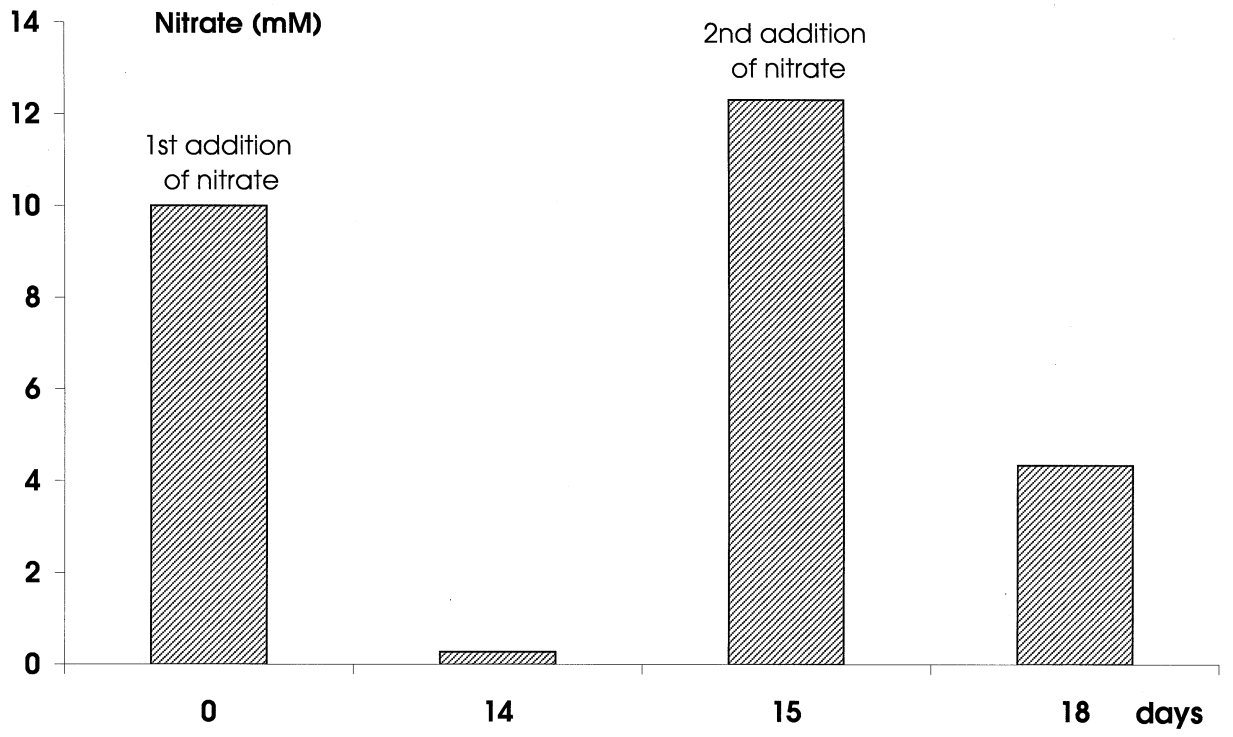

Fig. 3. Mean NO_3^- concentrations for assays with five different marine sediments amended with ~10-mM NO_3^- at day 0 and day 15. After each addition, NO_3^- was consumed, presumably due to denitrification (day 14 and day 18).

EPS to create a microenvironment, which favors their metabolisms (Sand et al., 2001). In such a microenvironment, the pH might be much lower than 8, enabling FeS dissolution. Consequently, Fe^{2+} or HS^--oxidizing and NO_3^--reducing bacteria can grow with FeS as a substrate, and we were able to enrich these bacteria from different marine sediments. With FeS_2 as a substrate, bacteria did not grow, since FeS_2 cannot be dissolved by protons.

5. CONCLUSIONS

The mineralization of particulate organic carbon drives the iron and sulfur cycles in marine sediments. Sulfate reduction is the dominant process of organic carbon oxidation in anoxic marine sediments (Jørgensen, 1982). The product of this process, H_2S, is partly oxidized in the anoxic sediment as documented by depth profiles of S^0, FeS_2, FeS, and porewater SO_4^{2-} concentrations (Schulz et al., 1994; Ferdelmann et al., 1997) or by large differences in the sulfur isotope composition between SO_4^{2-} and FeS_2 (Brüchert et al., 2000, 2001). H_2S oxidation has been confirmed by experimental studies with ^{35}S-labeled sulfur compounds (Jørgensen, 1977; Fossing and Jørgensen, 1990; Elsgaard and Jørgensen, 1992).

In bioturbated sediments, FeS_2 and FeS can be transported to the sediment surface where a chemical oxidation by O_2 occurs (Thamdrup et al., 1994). Aerobic bacteria oxidize intermediates of FeS_2 and FeS oxidation, such as thiosulfate, polythionates, and elemental sulfur, to sulfate. In anoxic sediments, FeS_2 and FeS are oxidized by MnO_2 if the Mn content of the sediment is $> 0.2\%$ w/w (Schippers and Jørgensen, 2001). Sulfur intermediates might be oxidized by sulfur-disproportionating bacteria. Presumably, because of the low solubility of Fe(III) or low concentration of Fe(III) complexes, Fe(III) oxide is not an oxidant for FeS_2 or FeS in marine sediment. FeS can be oxidized biologically in anoxic sediments by Fe^{2+}- or H_2S-

Table 2. Possible reactions and corresponding ΔG_f^0 values for complete FeS_2 and FeS oxidation to SO_4^{2-}. G_f^0 values of compound formation for ΔG_f^0 calculations were taken from Stumm and Morgan (1996) and for FeS from Lowson (1982).

$FeS_2 + 4.25\ O_2 + 2.5\ H_2O \rightarrow Fe(OH)_3 + 2\ SO_4^{2-} + 2\ H^+$	$\Delta G_f^0 = -1435$ kJ/mol
$FeS_2 + 7.5\ MnO_2 + 11\ H^+ \rightarrow Fe(OH)_3 + 2\ SO_4^{2-} + 7.5\ Mn^{2+} + 4\ H_2O$	$\Delta G_f^0 = -1199$ kJ/mol
$2\ FeS_2 + 6\ NO_3^- + 4\ H_2O \rightarrow 2\ Fe(OH)_3 + 4\ SO_4^{2-} + 3\ N_2 + 2\ H^+$	$\Delta G_f^0 = -2439$ kJ/mol
$FeS_2 + 44\ Fe(OH)_3 \rightarrow 15\ Fe_3O_4 + 2\ SO_4^{2-} + 64\ H_2O + 4\ H^+$	$\Delta G_f^0 = +942$ kJ/mol
$FeS_2 + 14\ Fe(OH)_3 + 15\ HCO_3^- + 11\ H^+ \rightarrow 15\ FeCO_2 + 2\ SO_4^{2-} + 34\ H_2O$	$\Delta G_f^0 = -806$ kJ/mol
$FeS + 2.25\ O_2 + 2.5\ H_2O \rightarrow Fe(OH)_3 + SO_4^{2-} + 2\ H^+$	$\Delta G_f^0 = -750$ kJ/mol
$FeS + 4.5\ MnO_2 + 7\ H^+ \rightarrow Fe(OH)_3 + SO_4^{2-} + 4.5\ Mn^{2+} + 2\ H_2O$	$\Delta G_f^0 = -751$ kJ/mol
$5\ FeS + 9\ NO_3^- + 8\ H_2O \rightarrow 5\ Fe(OH)_3 + 5\ SO_4^{2-} + 4.5\ N_2 + H^+$	$\Delta G_f^0 = -3817$ kJ/mol
$FeS + 26\ Fe(OH)_3 \rightarrow 9\ Fe_3O_4 + SO_4^{2-} + 38\ H_2O + 2\ H^+$	$\Delta G_f^0 = +596$ kJ/mol
$FeS + 8\ Fe(OH)_3 + 9\ HCO_3^- + 7\ H^+ \rightarrow 9\ FeCO_2 + SO_4^{2-} + 20\ H_2O$	$\Delta G_f^0 = -515$ kJ/mol

oxidizing and NO_3^--reducing bacteria due to the acid solubility of FeS. Since FeS_2 is resistant against proton attack, these bacteria do not dissolve FeS_2; thus, NO_3^- is not an oxidant for FeS_2. These findings are important for understanding the coupling of sulfur, iron, manganese, and nitrogen cycling in marine sediments.

Acknowledgments—We thank Michael E. Böttcher, Bo Elberling, Heide N. Schulz, and Bo Thamdrup for providing sediment samples. We thank J. Donald Rimstidt and two anonymous reviewers for helpful comments to improve the manuscript. Funding of this work was provided by the Max Planck Society.

Associate editor: J. D. Rimstidt

REFERENCES

Aller R. C. and Rude P. D. (1988) Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. *Geochim. Cosmochim. Acta* **52,** 751–765.

Bottrell S. H., Parkes R. J., Cragg B. A., and Raiswell R. (2000) Isotopic evidence for anoxic pyrite oxidation and stimulation of bacterial sulphate reduction in marine sediments. *J. Geol. Soc.* **157,** 711–714.

Brüchert V., Pérez M. E., and Lange C. B. (2000) Coupled primary production, benthic foraminiferal assemblage, and sulfur diagenesis in organic-rich sediments of the Benguela upwelling system. *Mar. Geol.* **163,** 27–40.

Brüchert V., Knoblauch C., and Jørgensen B. B. (2001) Controls on stable isotope fractionation during bacterial sulfate reduction in Arctic sediments. *Geochim. Cosmochim. Acta* **65,** 753–766.

Canfield D. E., Thamdrup B., and Hansen J. W. (1993a) The anaerobic degradation of organic matter in Danish coastal sediments: Iron reduction, manganese reduction, and sulfate reduction. *Geochim. Cosmochim. Acta* **57,** 3867–3883.

Canfield D. E., Jørgensen B. B., Fossing H., Glud R., Gundersen J., Ramsing N. B., Thamdrup B., Hansen J. W., Nielsen L. P., and Hall P. O. J. (1993b) Pathways of organic carbon oxidation in three continental margin sediments. *Mar. Geol.* **113,** 27–40.

Chaudhuri S. K., Lack J. G., and Coates J. D. (2001) Biogenic magnetite formation through anaerobic biooxidation of Fe(II). *Appl. Environ. Microbiol.* **67,** 2844–2848.

Coates J. D., Ellis D. J., Blunt-Harries E. L., Gaw C. V., Roden E. E. and Lovley D. R. (1998) Recovery of humic-reducing bacteria from diversity of environments. *Appl. Environ. Microbiol.* **64,** 1504–1509.

Cravotta C. A. (1998) Effect of sewage sludge on formation of acidic ground water at a reclaimed coal mine. *Ground Water* **35,** 9–19.

Elsgaard L. and Jørgensen B. B. (1992) Anoxic transformations of radiolabeled hydrogen sulfide in marine and freshwater sediments. *Geochim. Cosmochim. Acta* **56,** 2425–2435.

Engesgaard P. and Kipp K. L. (1992) A geochemical transport model for redox-controlled movement of mineral fronts in groundwater flow systems: A case of nitrate removal by oxidation of pyrite. *Water Resour. Res.* **28,** 2829–2843.

Evangelou V. P., Seta A. K., and Holt A. (1998) Potential role of bicarbonate during pyrite oxidation. *Environ. Sci. Technol.* **32,** 2084–2091.

Ferdelman, T. G., Lee C., Pantoja S., Harder J., Bebout B. M., and Fossing H. (1997) Sulfate reduction and methanogenesis in a *Thioploca*-dominated sediment off the coast of Chile. *Geochim. Cosmochim. Acta* **61,** 3065–3079.

Finster K., Liesack W., and Thamdrup B. (1998) Elemental sulfur and thiosulfate disproportionation by *Desulfocapsa sulfoexigens* sp. nov., a new anaerobic bacterium isolated from marine surface sediment. *Appl. Environ. Microbiol.* **64,** 119–125.

Fossing H. and Jørgensen B. B. (1990) Oxidation and reduction of radiolabeled inorganic sulfur compounds in an estuarine sediment, Kysing Fjord, Denmark. *Geochim. Cosmochim. Acta* **54,** 2731–2742.

Garcia-Gil L. J. and Golterman H. L. (1993) Kinetics of FeS-mediated denitrification in sediments from the Camargue (Rhone delta, southern France). *FEMS Microbiol. Ecol.* **13,** 85–92.

Huettel M., Ziebis W., Forster S., and Luther G. W. III (1998) Advective transport affecting metal and nutrient distributions and interfacial fluxes in permeable sediments. *Geochim. Cosmochim. Acta* **62,** 613–631.

Jørgensen B. B. (1977) The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). *Limnol. Oceanogr.* **22,** 814–832.

Jørgensen B. B. (1982) Mineralization of organic matter in the sea bed—The role of sulphate reduction. *Nature* **296,** 643–645.

Kelly D. P. and Wood A. P. (2000) Confirmation of *Thiobacillus denitrificans* as a species of the genus *Thiobacillus*, in the β-subclass of the *Proteobacteria*, with strain NCIMB 9548 as the type strain. *Int. J. Syst. Evol. Microbiol.* **50,** 547–550.

Kuenen J. G., Robertson L. A., and Tuovinen O. H. (1992) The genera *Thiobacillus*, *Thiomicrospira*, and *Thiosphera*. In *The Prokaryotes* (ed. A. Balows et al.), pp. 2638–2657. Springer-Verlag, New York.

Liu X. and Millero F. J. (1999) The solubility of iron hydroxide in sodium chloride solutions. *Geochim. Cosmochim. Acta* **63,** 3487–3497.

Lovley D. R. and Phillips E. J. P. (1986) Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Environ. Microbiol.* **51,** 683–689.

Lovley D. R., Kashefi K., Vargas M., Tor J. M., and Blunt-Harris E. L. (2000) Reduction of humic substances and Fe(III) by hyperthermophilic microorganisms. *Chem. Geol.* **169,** 289–298.

Lowson R. T. (1982) Aqueous oxidation of pyrite by molecular oxygen. *Chem. Rev.* **82,** 461–497.

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

Luther G. W. III, Giblin A., Howarth R. W., and Ryans R. A. (1982) Pyrite and oxidized iron mineral phases formed from pyrite oxidation in salt marsh and estuarine sediments. *Geochim. Cosmochim. Acta* **46,** 2665–2669.

Luther G. W. III, Kostka J. E., Church T. M., Sulzberger B., and Stumm W. (1992) Seasonal iron cycling in the salt-marsh sedimentary environment: The importance of ligand complexes with Fe(II) and Fe(III) in the dissolution of Fe(III) minerals and pyrite, respectively. *Mar. Chem.* **40,** 81–103.

Luther G. W. III, Shellenbarger P. A., and Brendel P. J. (1996) Dissolved organic Fe(III) and Fe(II) complexes in salt marsh porewaters. *Geochim. Cosmochim. Acta* **60,** 951–960.

Morse J. W. (1991) Oxidation kinetics of sedimentary pyrite in seawater. *Geochim. Cosmochim. Acta* **55,** 3665–3667.

Moses C. O. and Herman J. S. (1991) Pyrite oxidation at circumneutral pH. *Geochim. Cosmochim. Acta* **55,** 471–482.

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.

Nicholson R. V., Gillham R. W., and Reardon E. J. (1988) Pyrite oxidation in carbonate-buffered solution: 1. Experimental kinetics. *Geochim. Cosmochim. Acta* **52,** 1077–1085.

Nicholson R. V., Gillham R. W., and Reardon E. J. (1990) Pyrite oxidation in carbonate-buffered solution: 2. Rate control by oxide coatings. *Geochim. Cosmochim. Acta* **54,** 395–402.

Peiffer S. and Stubert I. (1999) The oxidation of pyrite at pH 7 in the presence of reducing and nonreducing Fe(III)-chelators. *Geochim. Cosmochim. Acta* **63,** 3171–3182.

Peterson G. S., Ankley G. T., and Leonard E. N. (1996) Effect of bioturbation on metal-sulfide oxidation in surficial freshwater sediments. *Environ. Toxicol. Chem.* **15,** 2147–2155.

Postma D., Boesen C., Kristiansen H., and Larsen F. (1991) Nitrate reduction in an unconfined sandy aquifer: Water chemistry, reduction processes, and geochemical modeling. *Water Resour. Res.* **27,** 2027–2045.

Sand W., Gehrke T., Jozsa P. -G., and Schippers A. (2001) (Bio)chemistry of bacterial leaching—Direct vs. indirect bioleaching. *Hydrometallurgy* **59,** 159–175.

Schippers A. and Sand W. (1999) Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. *Appl. Environ. Microbiol.* **65,** 319–321.

Schippers A. and Jørgensen B. B. (2001) Oxidation of pyrite and iron sulfide by manganese dioxide in marine sediment. *Geochim. Cosmochim. Acta* **65,** 915–922.

Schippers A., Jozsa P. -G., and Sand W. (1996) Sulfur chemistry in bacterial leaching of pyrite. *Appl. Environ. Microbiol.* **62,** 3424–3431.

Schulz H. D., Dahmke A., Schinzel U., Wallmann K., and Zabel M. (1994) Early diagenetic processes, fluxes, and reaction rates in sediments of the South Atlantic. *Geochim. Cosmochim. Acta* **58,** 2041–2060.

Straub K. L., Benz M., Schink B., and Widdel F. (1996) Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Appl. Environ. Microbiol.* **62,** 1458–1460.

Stumm W. and Morgan J. J. (1996) *Aquatic Chemistry*, 3rd edition. John Wiley, New York.

Sulzberger B., Suter D., Siffert C., Banwart S., and Stumm W. (1989) Dissolution of Fe(III)(hydr)oxides in natural waters: Laboratory assessment on the kinetics controlled by surface coordination. *Mar. Chem.* **28,** 127–144.

Taillefert M., Bono A. B., and Luther G. W. III (2000) Reactivity of freshly formed Fe(III) in synthetic solutions and (pore)waters: Voltammetric evidence of an aging process. *Environ. Sci. Technol.* **34,** 2169–2177.

Thamdrup B. and Canfield D. E. (1996) Pathways of carbon oxidation in continental margin sediments off central Chile. *Limnol. Oceanogr.* **41,** 1629–1650.

Thamdrup B., Finster K., Hansen J. W., and Bak F. (1993) Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron or manganese. *Appl. Environ. Microbiol.* **59,** 101–108.

Thamdrup B., Fossing H., and Jørgensen B. B. (1994) Manganese, iron, and sulfur cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Geochim. Cosmochim. Acta* **58,** 5115–5129.

Thomas J. E., Jones C. F., Skinner W. M., and Smart R. St. C. (1998) The role of surface sulfur species in the inhibition of pyrrhotite dissolution in acid conditions. *Geochim. Cosmochim. Acta* **62,** 1555–1565.

Thomas J. E., Skinner W. M., and Smart R. St. C. (2001) A mechanism to explain sudden changes in rates and products for pyrrhotite dissolution in acid solution. *Geochim. Cosmochim. Acta* **65,** 1–12.

van Beek C. G. E. M. (2000) Redox processes active in denitrification. In *Redox: Fundamentals, Processes and Applications* (ed. Schüring et al.), pp. 152–160. Springer-Verlag, New York.

Wehrli B., Sulzberger B., and Stumm W. (1989) Redox processes catalyzed by hydrous oxide surfaces. *Chem. Geol.* **78,** 167–179.

Williamson M. A. and Rimstidt J. D. (1994) The kinetics and electrochemical rate-determing step of aqueous pyrite oxidation. *Geochim. Cosmochim. Acta* **58,** 5443–5454.

Widdel F. and Bak. F. (1992) Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes* (ed. Balows et al.), pp. 3352–3378. Springer-Verlag, New York.

Zachara J. M., Fredrickson J. K., Smith S. C., and Gassman P. L. (2001) Solubilization of Fe(III) oxide-bound trace metals by a dissimilatory Fe(III) reducing bacterium. *Geochim. Cosmochim. Acta* **65,** 75–93.