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Authors: Andrew L. Neal, Somkiet Techkarnjanaruk, Alice Dohnalkova, D. McMready, Brent M. Peyton, & Gill G. Geesey.

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Iron sulfide and sulfur species produced at hematite surfaces in the presence of sulfate-reducing bacteria

ANDREW L. NEAL, SOMKRIET TECHKARNJANARUK, ALICE DOHNALKOVA, DAVID MCCREADY, BRENT M. PEYTON, and GILL G. GEESEY

In the presence of sulfate-reducing bacteria (Desulfovibrio desulfuricans) hematite (α-Fe₂O₃) dissolution is affected potentially by a combination of enzymatic (hydrogenase) reduction and hydrogen sulfide oxidation. As a consequence, ferrous ions are free to react with excess H₂S to form insoluble ferrous sulfides. X-ray photoelectron spectra indicate binding energies similar to ferrous sulfide having pyrrhotite-like structures (Fe₂p₃/2 708.4 eV; S₂p₃/2 161.5 eV). Other sulfur species identified at the surface include sulfate, sulfité and polysulfides. Thin film X-ray diffraction identifies a limited number of peaks, the principal one of which may be assigned to the hexagonal pyrrhotite (102) peak (d = 2.09 Å; 2θ = 43.22°), at the hematite surface within 3 months exposure to sulfate-reducing bacteria (SRB). High-resolution transmission electron microscopy identifies the presence of a hexagonal structure associated with observed crystallites. Although none of the analytical techniques employed provide unequivocal evidence as to the nature of the ferrous sulfide formed in the presence of SRB at hematite surfaces, we conclude from the available evidence that a pyrrhotite stoichiometry and structure is the best description of the sulfide we observe. Such ferrous sulfide production is inconsistent with previous reports in which mackinawite and greigite were products of biological sulfate reduction (Rickard 1969a; Herbert et al., 1998; Benning et al., 1999). The apparent differences in stoichiometry may be related to sulfidé activity at the mineral surface, controlled in part by H₂S autooxidation in the presence of iron oxides. Due to the relative stability of pyrrhotite at low temperatures, ferrous sulfide dissolution is likely to be reduced compared to the more commonly observed products of SRB activity. Additionally, biogenic pyrrhotite formation will also have implications for geomagnetic field behavior of sediments.

Any sulfur compound with an oxidation state greater than S²⁻ (i.e., S⁰, S⁴⁺ and S⁶⁺) has the potential to act as terminal electron acceptor in biologic oxidation of organic compounds (Goldhaber and Kaplan, 1974). Whilst assimilatory sulfate-reduction is commonplace, dissimilatory sulfate-reduction is carried out by a specialized group of nutritionally diverse organisms known collectively as anaerobic sulfate-reducing bacteria (SRB), including the genus Desulfovibrio. The observed activity of SRB in diverse habitats, including freshwater (Smith and Klug, 1981) and marine (Jørgensen, 1977) sediments, subsurface aquifers (Olson et al., 1981) and hydrothermal vent systems (Baross and Deming, 1983) points to their environmental significance.

The geological significance of SRB activity derives from the production of H₂S, resultant from sulfate-reduction, and its subsequent reaction with Fe²⁺ (the most abundant sulfidé binding ion in typical reducing sediments) to form ferrous sulfides.

Such sulfide are common components of both recent and ancient sediments and include the tetragonal, sulfur-deficient Fe²⁺-sulfide mackinawite (Fe₅SₓFe₁₋ₓS, Lennie et al., 1995a), the mixed-valence thiospinel, greigite (Fe⁺, Fe²⁺, S₄, Vaughan and Ridout, 1971) and the Fe²⁺-polysulfide pyrite (FeS₂). Iron sulfide mineral formation can be significant annually; an estimated 3.9 × 10¹³ g of pyrite-S are deposited in deltaic and anoxic continental shelf sediments (Berner, 1982). Sulfidé production by SRB also has implications for steel corrosion (Hamilton, 1991) and bioremediation of heavy metal pollution in anoxic environments (Miller, 1950; Bacon et al., 1980; Webb et al., 1998).

Ferrous sulfide resulting from SRB activity have been studied previously and described as mackinawite and/or greigite (Rickard, 1969a; Herbert et al., 1998; Benning et al., 1999). In the aforementioned studies, ferrous sulfide were produced by free-living bacteria in liquid culture with added ferrous ions. However, most subsurface bacterial activity is likely to be associated with surfaces (Ghirose and Wilson, 1988; Costerton et al., 1995), for example, the majority (98%) of bacteria in a Cape Cod aquifer were found to be attached (Harvey et al., 1984). We have therefore chosen to study ferrous sulfide production by bacteria associated with hematite surfaces.

In this study we identify ferrous sulfide produced by Desulfovibrio desulfuricans associated with hematite (α-Fe₂O₃) surfaces. The iron oxides goethite, lepidocrocite, ferrihydrite...
and hematite are important Fe(III)-containing constituents of soils and sediments (Appelo and Postma 1996). The specular hematite used for these experiments lends itself to manipulation both during bacterial culture and subsequent chemical analysis. Dissimilatory bacterial sulfate reduction, using lactate as the electron donor, is described in Eqp. 1 (Thauer et al., 1977),

$$2 \text{Lactate} + \text{SO}_4^{2-} + \text{H}^+ \rightarrow 2 \text{Acetate} + 2\text{CO}_2 + 2\text{H}_2\text{O} + \text{HS}^-.$$  

(1)

With no ferrous salts added to the growth medium, aqu. $\text{Fe}_2\text{O}_3$ could be assessed.

2.1. Organisms and Culture

Two Desulfovibrio desulfuricans strains were used in the experiments described here, G20 and Essex 6 (ATCC 29577, NCIMB 8307, Postgate and Campbell, 1966). G20 was derived from D. desulfuricans G100A (Wall et al., 1993). Both strains were a gift of Dr. J. Wall, University of Missouri. A green fluoroscein protein (GFP) reporter gene construct was used to visualize cells of D. desulfuricans strain G20. In our laboratory, the IncQ plasmid pds519 encoding for GFP::mut2 (Matthysse et al., 1996) was modifie for chloramphenicol resistance and mobilized into G20 with GFP expression under control of the constitutive npt2 promoter. The mut2 derivative of the wild type GFP gene confers a 30-fold increase in chlorophore fluorescenc intensity (Cormack et al., 1996). Use of such recombinant organisms allows in situ, non-destructive visualization of bacteria at surfaces avoiding artifacts such as changes in surface chemistry caused by more traditional staining methods (e.g., DAPI, see review by Errampalli et al., 1999).

D. desulfuricans, a facultative anaerobe, was grown in batch culture in 25 mL anaerobic serum bottles with butyl rubber septa and aluminum crimp caps (Wheaton, Millville, NJ) in Lactate Medium C (Butlin et al., 1949; Postgate, 1963). Lactate Medium C contains 8 ml$^{-1}$ 60% sodium lactate syrup, 4.5 g$^{-1}$ MgSO$_4$, 2 g$^{-1}$ MgSO$_4$, 1 g$^{-1}$ yeast extract, 1 g$^{-1}$ NH$_4$Cl, 0.5 g$^{-1}$ K$_2$HPO$_4$ and 0.06 g$^{-1}$ CaCl$_2$, (ZiSOF$^-$] = 40 mM) in distilled, deionized water and adjusted to pH 7 with 6N NaOH. Na-thioglycollate (C$_2$H$_3$O$_2$SNa) and ascorbic acid were added at a concentration of 0.01 g$^{-1}$ to poise E$_m$ of the medium at circa. −100 mV. All media was sterilized by autoclave at 121°C for 20 min. The medium was supplemented with chloramphenicol (20 µg ml$^{-1}$) to maintain selective pressure for plasmid retention by the cells.

D. desulfuricans Essex 6 was also grown in the absence of SO$_4^{2-}$ employing pyruvate fermentation with fumarate as the electron acceptor (Postgate and Campbell, 1966; Magee et al., 1978). The medium contains 4.5 g$^{-1}$ Na-fumarate (35 mM), 3.5 g$^{-1}$ Na-pyruvate (34 mM), 1.6 g$^{-1}$ MgCl$_2$, 1 g$^{-1}$ NH$_4$Cl, 1 g$^{-1}$ yeast extract, 0.5 g$^{-1}$ KH$_2$PO$_4$ and 0.1 g$^{-1}$ CaCl$_2$ (pH 7). Reductants were again added to poise medium E$_m$. Preliminary experiments have demonstrated that Essex 6 is unable to couple the oxidation of pyruvate to thioglycollate reduction. Thus the effect of D. desulfuricans presence at the hematite surface in the absence of sulfate (and therefore H$_2$S), could be assessed.

2.2. Materials and Experimentation

Natural specular hematite ($\alpha$-Fe$_2$O$_3$) from Bahia, Brazil was used for this study, a gift of Dr. K. Rosso (Pacific Northwest National Laboratory, Richland, WA). Hematite samples (dimensions approximately $5 \times 3 \times 0.2$ mm) were washed before use in distilled, deionised water to remove particulate surface contamination.

The potential reductive effect of Na-thioglycollate and ascorbic acid as well as any possible photoreduction of hematite in the medium was evaluated by incubating a hematite sample in Lactate Medium C without the addition of bacteria for seventeen days at room temperature (23–26°C). Additionally samples were exposed to Lactate Medium C in the presence of G20 or Essex 6 for 17 d and to Essex 6 for three months. Other treatments included Essex 6 in sulfate-free medium and Lactate medium C in the absence of bacteria but with the addition of approximately 150 µM H$_2$S. Hematite samples were added to serum bottles before autoclaving. Following incubation, hematite samples with associated precipitates were removed from the culture medium and observed using epifluorescenc microscope to evaluate the presence/absence of SRB, after which they were washed in O$_2$-free distilled, deionised water and dried under a stream of N$_2$ before being mounted and placed in the XPS vacuum chamber.

2.3. Instrumentation

2.3.1. Optical microscopy

Observation of bacteria attached to the hematite surface was made using an Olympus BX60 microscope equipped with an infinity-cor rected, long working distance water immersion objective lens (40×, NA = 0.55, Nikon Inc., Torrence, CA) and 100 W Hg-vapor discharge lamp. Reflecte differential interference constrast (DIC) images were captured using a U-DICR polarizer (Olympus America Inc., Lake Success, NY), fluorescenc images with a WIBA filte block (460–490 nm excitation; 505 nm dichroic mirror; 515–550 nm emission; Olympus America Inc.). Video capture was performed using an ImagePoint monochrome, Peltier cooled (+10°C) CCD camera (Photometrics Ltd., Tuscon, AZ) and Image-Pro Plus software (Media Cybernetics, Silver Springs, MD).

2.3.2. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was performed on a Model 5600ci spectrometer (Perkin Elmer Inc., Eden Prairie, MN). The instrument was calibrated employing the Au L$_2$3 photopeaks with binding energies of 83.99, 932.66 and 368.27 eV. The instrument was calibrated employing the Au4f$^7$/2, Cu2p$^3$/2, and Ag3d$^5$/2 photopeaks with binding energies of 85.39, 932.66 and 388.27 eV, respectively. A 5 eV floor gun was used to offset charge accumulation on the samples. A consistent 800 µm diameter area was analyzed on all surfaces using a monochomatous AlK$_x$ X-ray source (1486.6 eV) at 300 W and a pass energy of 93.9 eV for broad scans, 29.35 eV for high-resolution scans (nominal resolution = 0.3 eV). The system was operated at a base pressure of 10$^{-8}$–10$^{-9}$ Torr. To correct for sample charging, all reported literature-based binding energies have been referenced to the adventitious C1s peak observed on hematite exposed to Lactate Medium C in the absence of bacteria at 285.1 eV.

Fe2p$_{3/2}$ spectra were analyzed using a multiplet splitting model derived from consideration of electrostatic and spin-orbit interactions (Gupta and Sen, 1974; Gupta and Sen, 1975). We have however followed the practice of McIntyre and Zetaruk (1977), Pratt et al. (1994a) and Pratt et al. (1994b) in fitting only three major peaks to the Fe2p$_{3/2}$ spectrum, ignoring two minor peaks at elevated binding ener (E$_b$). Fe2p$_{3/2}$ spectra have been fitte using four peaks, consistent with the aforementioned authors. S2p spectra have been fitte employing asymmetric doublets ($\Delta E_b$ 1.18 eV) reflect the spin-orbit split-ting of S2p$_{3/2}$ and S2p$_{1/2}$ photopeaks. Following baseline subtraction (Shirley, 1972), curves were fit employing combinations of Lorentzi
and Gaussian line shapes. Table 1 contains Fe\(^{2+}\) and S\(^2-\) binding energies from previous studies, referenced to the adventitious C\(^1s\) of 285.1 eV.

### 2.3.3. X-ray Diffraction

Thin film X-ray diffraction was carried out on precipitates at the hematite surface using a Philips X'Pert MPD (Phillips Analytical, Natick, MA) incorporating a vertical theta-theta goniometer (220 mm radius) and a long focus ceramic X-ray tube with a Cu anode. The instrument was operated at a power of 40 kV, 50 mA, using Cu K\(^\text{a}\) radiation (\(\lambda = 1.541\) Å). Parallel beam optics were employed with a Gutmann mirror providing a high intensity, parallel collimated incident beam. The receiving optics comprised a 0.09 radian parallel plate collimator and proportional counter detector. The sample was mounted at room temperature on an Anton-Paar TTK 450 thin-fil stage under vacuum (<10\(^{-2}\) Torr). Diffractograms were run over the 2\(\theta\) range 5°–75° with a (constant) angle of incidence (\(\psi\)) of 2.5°. Diffraction pattern analysis was performed using Jade 5\(^\text{TM}\) software (Materials Data Inc., Livermore, CA) with comparison to the Joint Committee on Powder Diffraction Standards (JCPDS) database.

### 2.3.4. High resolution TEM (HR-TEM)

Hematite samples with associated precipitates were anaerobically embedded in hard grade LR White\(^\text{TM}\) resin, and cured for six hours at 60°C. Hardened blocks were sectioned in an anaerobic glove box using a Leica UltraCut R microtome (Leica Microsystems Inc., Deerfield IL). In an attempt to minimize sample compression and hematite fracture during sectioning, a Diatome Ultra 35° knife was used in favor of the more common 45° knife (see Jesior 1986). The lower angle edge resulted in sections without hematite fracture of sufficient area to investigate the attached biofilm precipitates. Fifty nanometer thick sections were collected at room temperature on copper grids supported with lacey carbon film and observed at 200 kV using a JEOL 2010 high-resolution analytical electron microscope (JEOL USA Inc., Peabody, MA). High-resolution images were collected and analyzed by DigitalMicrograph\(^\text{TM}\) software (Gatan Inc., Pleasanton, CA).

### 3. RESULTS

#### 3.1. Evaluation of Culture Medium and Bacteria Attached to Hematite

Throughout incubation, culture media were visually evaluated for the formation of ferrous sulfide in suspension as a black discoloration of the normally straw-colored (due to the inclusion of yeast extract in the medium) solution. In no treatment was such discoloration observed, suggesting that if any ferrous sulfid production had taken place it was confined to the hematite surfaces. Epifluorescence microscopic observation con-
Fig. 1. Reflecte DIC (above) and corresponding epifluorescence (below) photomicrographs of Desulfovibrio desulfuricans G20 (pNpt2CmGFP) attached to a hematite surface after 17 d in culture. The scale bar associated with the DIC image is representative of both images. 40× long working distance, water immersion lens. DIC image, 32 msec exposure, 4 dB gain, Epifluorescence image, 10 sec exposure, 4 dB gain.
crystal (Junta-Rosso and Hochella, 1996). The Fe\(^{2+}\) between adjacent hematite platelets in the original hematite growth medium but also potentially, from fluid infiltratio

3.2.1. Sterile hematite surface

formed at the mineral surfaces (Fig. 1). No bacteria were observed on sterile mineral surfaces.

3.2.2. Hydrogen sulfid exposed hematite surface

The Fe\(^{2+}\) region exhibited a contribution at low \(E_s\) indicative of Fe\(^{2+}\) (Fig. 3). Curve fitting employed the hematite model established from the unexposed surface (Fe\(^{3+}\)-O, see Fig. 2), with the addition of a principal photopeak at 708.7 eV (FWHM = 1.4 eV) and two multiplets 0.9 eV either side of the major peak (\(\chi^2 = 1.24\), see Fig. 3). The \(E_s\) of the principal peak is in good agreement with the Fe\(^{2+}\)-S photopeak collected on pyrrhotite by Buckley and Woods (1985, see Table 1). Since the Fe\(^{3+}\)-S component of the putative pyrrhotite (Pratt et al., 1994a,b) is likely to be confounded with the Fe\(^{3+}\)-O component of hematite, whilst accepting the lack of adherence to the pyrrhotite model, no attempt was made to include these photopeaks in the curve fitting procedure. Instead, corroborating evidence was sought in the S2p core region for the identificatio of the ferrous sulfide

The S2p region is potentially complicated by contributions from monosulfid (S\(^{2-}\)), disulfid (S\(^{2-}\)) and polysulfid (S\(^{n-}\)) species (Hyland and Bancroft, 1989). S\(^{2-}\) and S\(^{n-}\) peaks originate from S-Fe bonds whilst S\(^{2-}\) peaks originate from S-S bonds. To obtain a good fit to the data (\(\chi^2 = 1.33\)), we have adopted the approach of previous authors (Pratt et al., 1994a; Herbert et al., 1998) in fitting doublets (\(\Delta E_d\) 1.18 eV) for S\(^{2-}\) and S\(^{n-}\) to the high \(E_s\) tail in the S2p core region. The principal S2p\(_{1/2}\) photopeak was identified at 167.9 eV (FWHM = 1.3 eV) corresponding to SO\(^{2-}\), undoubtedly likely due to adsorption of sulfate from the growth medium, accompanied by a second peak corresponding to SO\(^{2-}\) at 166 eV (FWHM = 1.3 eV) (Wagner et al., 1992). Minor photopeaks at 161.4 (FWHM = 1.3 eV) and 163.2 eV (FWHM = 1.2 eV) were ascribed to S\(^{2-}\) and S\(^{3-}\) respectively (Mycroft et al., 1990; Pratt et al., 1994a; Pratt et al., 1994b). Whilst the S\(^{2-}\) and S\(^{3-}\) may potentially be due to interactions with other cations in the growth medium (for example Mg\(^{2+}\) and Na\(^{2+}\)) the position of the monosulfid peak is again similar to pyrrhotite-like structures (Buckley and Woods, 1985; Jones et al., 1992; Pratt et al., 1994a; Pratt et al., 1994b).
3.2.3. SRB exposed hematite surface

Broad scan spectra (not shown) suggested C1s and N1s peak intensities were increased (relative to O1s) on all hematite surfaces exposed to D. desulfuricans (compared to unexposed surfaces) and a prominent photopeak was present in the C1s core region at 288 eV. Such surface differences can be accounted for, among other things, organic species associated with bacteria (i.e., proteins, lipids, polysaccharides etc., see Rouxhet and Genet, 1991).

The XPS spectra of the hematite surface exposed to Essex 6 in the presence of sulfate demonstrated that the sulfid layer was of sufficient thickness to mask any hematite-related signal (see Fig. 4). The Fe2p region at 708.4 eV (FWHM = 1.4 eV, \( \chi^2 = 1.72 \)). In the S2p region, the monosulfid \( \text{S}_2p_{3/2} \) peak is at 161.5 eV (FWHM = 1.3 eV), the \( \text{S}_2p_{3/2} \) \( \text{S}_2p_{1/2} \) peak at 162.2 eV (FWHM = 1.3 eV) and the \( \text{S}_2p_{1/2} \) \( \text{S}_2p_{3/2} \) peak at 163.6 eV (FWHM = 1.9 eV). The high \( E_B \) region was resolved into two pairs of multiplets, the \( \text{S}_2p_{3/2} \) peaks being at 166.7 eV (FWHM = 1.7 eV) and 168 eV (FWHM = 1.8 eV). These peak positions were suggestive of \( \text{SO}_3^{2-} \) and \( \text{SO}_4^{2-} \) respectively.

Interestingly, the hematite surface exposed to Essex 6 in the absence of medium-SO_{4}^{2-} (but containing sodium thioglycolate as a reductant and hence a potential source of sulfur) indicated not only a lack of metal sulfid species but the presence of \( \text{S}_0^{2-}/\text{S}_2^{2-} \) and \( \text{S}_n^{2-} \) (peak maxima \( \sim 164 \) and \( \sim 170 \) eV) and \( \text{SO}_{4}^{2-}/\text{SO}_{3}^{2-} \) (peak maximum \( \sim 169 \) eV, Fig. 5).

The Fe2p spectrum of G20 exposed hematite exhibited a shoulder at low \( E_B \), the position of which was determined at 708.4 eV (FWHM = 1.4 eV, \( \chi^2 = 1.72 \)). In the S2p region, the monosulfid \( \text{S}_2p_{3/2} \) peak is at 161.5 eV (FWHM = 1.3 eV), the \( \text{S}_2p_{3/2} \) \( \text{S}_2p_{1/2} \) peak at 162.2 eV (FWHM = 1.3 eV) and the \( \text{S}_2p_{1/2} \) \( \text{S}_2p_{3/2} \) peak at 163.6 eV (FWHM = 1.9 eV). The high \( E_B \) region was resolved into two pairs of multiplets, the \( \text{S}_2p_{3/2} \) peaks being at 166.7 eV (FWHM = 1.7 eV) and 168 eV (FWHM = 1.8 eV). These peak positions were suggestive of \( \text{SO}_3^{2-} \) and \( \text{SO}_4^{2-} \) respectively.

The XPS spectra of the hematite surface exposed to the sulfate-reducing bacterium Desulfovibrio desulfuricans Essex 6 for 17 d. Fe2p region, the monosulfid \( \text{S}_2p_{3/2} \) peak is at 161.5 eV (FWHM = 1.3 eV), the \( \text{S}_2p_{3/2} \) \( \text{S}_2p_{1/2} \) peak at 162.2 eV (FWHM = 1.3 eV) and the \( \text{S}_2p_{1/2} \) \( \text{S}_2p_{3/2} \) peak at 163.6 eV (FWHM = 1.9 eV). The high \( E_B \) region was resolved into two pairs of multiplets, the \( \text{S}_2p_{3/2} \) peaks being at 166.7 eV (FWHM = 1.7 eV) and 168 eV (FWHM = 1.8 eV). These peak positions were suggestive of \( \text{SO}_3^{2-} \) and \( \text{SO}_4^{2-} \) respectively.

Interestingly, the hematite surface exposed to Essex 6 in the absence of medium-SO_{4}^{2-} (but containing sodium thioglycolate as a reductant and hence a potential source of sulfur) indicated not only a lack of metal sulfid species but the presence of \( \text{S}_0^{2-}/\text{S}_2^{2-} \) and \( \text{S}_n^{2-} \) (peak maxima \( \sim 164 \) and \( \sim 170 \) eV) and \( \text{SO}_{4}^{2-}/\text{SO}_{3}^{2-} \) (peak maximum \( \sim 169 \) eV, Fig. 5).
3.3. X-ray Diffraction of Precipitated Iron Sulfide

Thin film X-ray diffraction of precipitates on the hematite sample exposed to bacteria for 17 d indicated a broad peak at low angle suggestive of amorphous material and an absence of crystal structure associated with the precipitate. Following 3 months incubation in bacterial culture peaks were identified associated with precipitates on the hematite surface with \( d \)-spacings of 1.28, 1.81, 2.04 and 2.09 Å (20, 74.04°, 50.32°, 44.46° and 43.22° respectively, see Figure 6). The low number of peaks identified may result from a lack of heterogeneous orientation of the crystals at the hematite surface as well as the presence of small unobservable crystallites. With so few lines assignment of an unequivocal crystal structure is difficult however the principal 2.09 Å (43.22° 2\( \theta \)) peak may be assigned to the hexagonal pyrrhotite (102) peak (Lennie et al., 1995b). We remain cautious concerning this assignment however because minor peaks at such angles are a common feature of ferrous sulfide as well as \( S_n^2 \). Even so, XRD suggests that a crystal phase forms at the hematite surface in under three months.

3.4. HR-TEM of Precipitated Iron Sulfide

High magnification HR-TEM images of the hematite surface incubated for 3 months illustrate the presence of numerous crystals with regions of overgrowth (Fig. 7). Lattice spacings of 2.6, 3.8 and 5.1 Å were consistently measured for the observed crystals however, again unequivocal assignment to a particular phase was not possible. However, Fourier transformation of the 5.1 Å lattice spacing, the most commonly observed, yielded a hexagonal crystal structure (Fig. 8) adding weight to the identification of the ferrous sulfide phase as a putative pyrrhotite.

4. DISCUSSION

4.1. Ferrous Sulfid Production by Sulfate-Reducing Bacteria at Hematite Surfaces

The production of ferrous sulfide by SRB is dependent upon \( Fe^{2+} \) release from the hematite surface, affected potentially both by direct (i.e., hydrogenase) and indirect (i.e., \( H_2S \) oxidation) processes. Reaction between ferrous ions and excess \( H_2S \) subsequently results in ferrous sulfid production. Although none of the analytical techniques employed here (XPS, thin film XRD and HR-TEM) provide unequivocal evidence (either on their own or in combination) as to the nature of the ferrous sulfid formed in the presence of SRB at hematite surfaces, we conclude from the available evidence that a pyrrhotite stoichiometry and structure is the best description of the sulfide we observe. This observation is at variance with previous studies of SRB ferrous sulfid production, although it is not without precedent; pyrrhotite is observed in anoxic marine sediments (Kobayashi and Nomura, 1972; Roberts and Turner, 1993; Horng et al., 1998) and at the surface of corroding iron and steel in \( H_2S \)-rich environments (Meyer et al., 1958; Berner, 1964; Ringas and Robinson, 1988). Moreover, low temperature pyrrhotite formation has been observed in laboratory studies (Berner, 1964; Sweeney and Kaplan, 1973).

The production of ferrous sulfide has long been used to identify SRB in mixed and single species culture (Butlin et al., 1949). Rickard (1969a) described the initial production of mackinawite and subsequently, greigite by \textit{Desulfovibrio desulfuricans} Canet 41 in medium containing 182 mM \( Fe(aq.)^{2+} \). More recently, using a mixed bacterial culture and 14 \( \mu \)M \( Fe(aq.)^{2+} \), Herbert et al. (1998) have again identified mackinawite and greigite and Benning et al. (1999) have described mackinawite formation by \textit{D. desulfuricans} ATCC 29578 (ferrous iron content unspecified). Magnetotactic bacteria, with close affiliation to SRB (DeLong et al., 1993), have been shown to produce intracellular mackinawite and greigite (Pósfai et al., 1998; Frankel et al., 1998; Schüler and Frankel, 1999).

Pyrite is understood to replace mackinawite by reaction between FeS and \( S_n^2 \) or \( S_n^2^- \) (Berner 1964; Berner, 1970; Rickard 1969b; Taylor et al., 1979; Luther, 1991; Schoonen and Barnes, 1991b; Wilkin and Barnes, 1996; Benning et al., 2000) following the amorphous FeS \( \rightarrow \) mackinawite \( \rightarrow \) greigite \( \rightarrow \) pyrite sequence. Previous studies have described little or no pyrite formation in SRB batch cultures despite significant accumulation of precursor ferrous sulfide (Rickard, 1969a; Herbert et al., 1998; Benning et al., 1999). An exception has been described by Donald and Southam (1999) who report that pyrite formation from FeS is accelerated in the presence of
SRB, compared to abiotic processes, an apparent consequence of nucleation of pyrite on inner and outer surfaces of the cell envelope. However, despite the presence of oxidized sulfur species, in no instance was pyrite observed at the hematite surfaces in this study (FeS$_2$; Fe$_{2p3/2}$ 707.5 eV, S$_{2p3/2}$ 162.8 eV, Buckley and Woods, 1987). This fact may corroborate our identification of the ferrous sulfid as pyrrhotite since in the presence of oxidized sulfur species mackinawite and/or greigite, were they present, would be expected to transform to pyrite relatively quickly.

One significant difference between previous experiments and this study is the amount of Fe(aq.) in the growth media. Whilst SO$_4$(aq.) concentrations (and therefore likely subsequent H$_2$S concentrations) in the four studies range from 32 mM (Herbert et al., 1998) to 50 mM (Rickard, 1969a), Fe$_{\text{aq.}}$ concentrations vary greatly between 182 mM (Rickard, 1969a) and an unknown but potentially low concentration in this study arising from the low dissolution rate of hematite (Byrne and Kester, 1976; dos Santos and Stumm, 1992). Fe$_{\text{aq.}}$ supply could potentially play a role in determining the resultant ferrous sulfid stoichiometry. Thermodynamic calculations suggest the greigite stability field is particularly sensitive to changes in the H$_2$S$_{\text{aq.}}$:Fe$_{\text{aq.}}^{2+}$ ratio (Anderko and Shuler, 1997). Specifically at a fixed Fe$_{\text{aq.}}^{2+}$ molality of 10 mM kg$^{-1}$, greigite is not formed below 40 mM kg$^{-1}$ H$_2$S (Anderko and Shuler, 1997). Berner (1971) also indicates that with sulfid activity ($p$S$_2^-$) as the controlling factor the pyrrhotite stability field exists at lower $p$S$_2^-$ relative to pyrite (at equivalent $E_h$, see also Lord and Church, 1983). These sources place emphasis on $p$S$_2^-$ in determining the nature of the ferrous sulfid formed under particular circumstances, rather than the availability of ferrous ions. Since autooxidation of H$_2$S is likely to occur at the hematite surface (see dos Santos Afonso and Stumm, 1992; Herszage and dos Santos Afonso, 2000) the potential exists for $p$S$_2^-$ to be reduced in close proximity to the mineral surface. Visual inspection of the culture media indicates that ferrous sulfid formation is limited to the mineral surface—the very place where we might expect the most reduced $p$S$_2^-$. Alternatively the greigite stability field lies above the H$^+/H_2$ redox equilibrium, suggesting the mackinawite → greigite → pyrite sequence is thermodynamically unfeasible in strong reducing environments (Anderko and Shuler, 1997), indeed there is now considerable empirical evidence suggesting the conversion of mackinawite to pyrite requires an oxidant, i.e., S$_8^0$ or

SRB, compared to abiotic processes, an apparent consequence of nucleation of pyrite on inner and outer surfaces of the cell envelope. However, despite the presence of oxidized sulfur species, in no instance was pyrite observed at the hematite surfaces in this study (FeS$_2$; Fe$_{2p3/2}$ 707.5 eV, S$_{2p3/2}$ 162.8 eV, Buckley and Woods, 1987). This fact may corroborate our identification of the ferrous sulfid as pyrrhotite since in the presence of oxidized sulfur species mackinawite and/or greigite, were they present, would be expected to transform to pyrite relatively quickly.

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polysulfide (for example Berner, 1970; Schoonen and Barnes, 1991a,b; Wilkin and Barnes, 1996; Benning et al., 2000). Thus, the apparent stoichiometric differences in ferrous sulfide produced could arise either from reduced $pS^{2-}$, or from the generation of an extremely reducing environment at the mineral surface in the presence of SRB. Since XPS indicates that precipitates formed at the hematite surface upon addition of $H_2S$ in the absence of bacteria had equivalent $E_h$ to those formed in the presence of SRB we conclude that $pS^{2-}$ may exert more influence over formation of a particular phase than $E_h$, although we should not discount the potential control of $E_h$ completely. Assessment of $pFe^{2+}$ is not trivial. Metal ions are likely to interact with bacterial membranes (Fein et al., 1997), and extracellular polymers (Geesey and Jang, 1989; Barker and Banfield 1996), both phenomena are likely to reduce the availability of free ferrous ions within bacterial biofilms. With regard to $pS^{2-}$, we are currently attempting to assess sulfid concentrations at mineral surfaces employing microelectrodes.

Fig. 7. HR-TEM micrograph of crystal phases present at a hematite surface in the presence of sulfate-reducing bacteria following 3 months of incubation. Several crystals are visible with regions of overgrowth in between. D-spacings of 2.6, 3.8 and 5.1 Å were consistently measured for the crystal phases at the surface.
4.2. Sulfur Species other than Fe-Sulfid Present at the Hematite Surface

Reductive dissolution of hematite by H$_2$S (Eqn. 2) is complex and although the products are often described as Fe-sulfid and elemental sulfur there are in fact many intermediates. Studies show these to include S$^{2-}$, SO$_3^{2-}$, SO$_4^{2-}$ and S$_2$O$_3^{2-}$ (Pyzik and Sommer, 1981; dos Santos Afonso and Stumm, 1992; Davydov et al., 1998; Herszage and dos Santos Afonso, 2000) as well as S$_8^0$ (dos Santos Afonso and Stumm, 1992). Tetra- and pentasulfide are likely to be the only stable polysulfide in the culture medium (Giggenbach, 1972). Hematite dissolution is surface-controlled, the rate dependent upon the concentration of reductant(s) at the surface. Surface complexation models (Yates et al., 1974; Sulzberger et al., 1989) point to the significance of surface functional groups in mineral dissolution. Specifically, for hematite, dos Santos Afonso and Stumm (1992) postulate the formation of FeS$^-$ and FeSH surface complexes by exchange of O$^{2-}$ for S$^{2-}$ and SH$^-$, these new surface groups would then undergo electron transfer. The existence of SH$^-$ groups at the hematite surface has been confirmed using FTIR spectroscopy (Davydov et al., 1998). Since Fe$^{2+}$-O$^{2-}$ bonds in the hematite lattice are weakened in the process, Fe$^{2+}$$_{\text{aq.}}$ is released from the surface.

XPS spectra of all the hematite surfaces exposed to H$_2$S-practicing SRB revealed the presence of SO$_3^{2-}$ and SO$_4^{2-}$ as well as S$^{2-}$, at no time were peaks indicative of S$_8^0$ or thiosulfate observed. However, on the two hematite surfaces not exposed to H$_2$S, i.e., the surface exposed only to Lactate medium C in the absence of SRB and the surface exposed to Essex 6 growing in the absence of medium-SO$_4^{2-}$, peaks consistent with S$_8^0$ and S$_2$O$_3^{2-}$ were observed coincident with surface associated SO$_4^{2-}$. Reduction of hematite by H$_2$S (or Fe-sulfide Tiller and Booth, 1962) precludes the accumulation of elemental sulfur and thiosulfate at the surface. A likely explanation is the reaction between elemental sulfur and sulfhydryl ions (HS$^-$) forming polysulfide (Teder 1971). At the same time both S$_2$O$_3^{2-}$ and SO$_4^{2-}$ may disproportionate to SO$_4^{2-}$ (Jørgensen, 1990; Canfield and Thamdrup, 1994; Habicht et al., 1998).

4.3. Implications of Pyrrhotite and Polysulfid Formation at Iron Oxide Surfaces

Iron sulfide have an important role to play in both the sulfur and iron cycles (Lovley, 1993; Nealson and Saffarini, 1994). The final product of iron sulfid formation, pyrite (FeS$_2$) (Rickard, 1969b; Berner, 1970; Berner, 1984), is stable (Lennie and...
sediments, of significance not only to the S and Fe cycles but also S, Fe and other trace metal concentrations in anoxic soils and polysulfid formation by SRB has the potential to greatly affect principally as scales upon other minerals in sediments. Sulfide by Morse and Cornwell (1987) that iron sulfide may exist production. Such a mechanism is consistent with observations (Morse, 1991; Holmes, 1999) are likely to result in dissolution of sulfid precipitates. Such potential dissolution attains greater significance when one considers that other, potentially toxic, elements are often co-precipitated with Fe (Miller, 1950). The formation of pyrrhotite, a species considered stable at low temperatures (Kissin and Scott, 1982), will greatly limit the dissolution of Fe, S and other co-precipitates caused by environmental fluctuation in Fe, S and O2. Polysulfides being reduced species, represent a reactive form of elemental sulfur and altogether with monosulfide are responsible for maintaining trace metal concentrations in anoxic sediments at relatively high concentrations (Brooks et al., 1968; Presley et al., 1972).

Potential pyrrhotite formation by SRB also has implications for paleomagnetic studies of geomagnetic field behavior (Laj et al., 1991; Tauxe, 1993). Ferrous sulfid formation results in part from the dissolution of iron oxide minerals, thus the relative amounts of diagenetic ferrimagnetic minerals (i.e., pyrrhotite and greigite) compared to authogenic ferrimagnetic minerals (i.e., magnetite) are important considerations for the interpretation of paleomagnetic records. Roberts and Turner (1993) have identified pyrrhotite, together with greigite, associated with recent fine-grained sediments. They conclude that these phases exist as a result of interrupted sulfidation of precursor ferrous sulfide (i.e., arrested pyritization) due to low permeability of fine-grained sediments. Our results suggest that low sediment permeability need not be required for pyrrhotite formation but that low sulfid activity due to sulfid autooxidation at iron oxide surfaces may also result in pyrrhotite formation.

Thus, the presence of SRB at a hematite surface results in mineral dissolution and the formation of the stable iron sulfid pyrrhotite and reactive polysulfide resultant from excess H2S production. Such a mechanism is consistent with observations by Morse and Cornwell (1987) that iron sulfide may exist principally as scales upon other minerals in sediments. Sulfide polysulfid formation by SRB has the potential to greatly affect S, Fe and other trace metal concentrations in anoxic soils and sediments, of significance not only to the S and Fe cycles but also to the bioavailability of toxic trace metals and paleomagnetism.

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