APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2001, p. 2844–2848 Vol. 67, No. 6
0099-2240/01/$04.00+0 DOI: 10.1128/AEM.67.6.2844–2848.2001

Biogenic Magnetite Formation through Anaerobic Biooxidation of Fe(II)

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Received 29 November 2000/Accepted 28 March 2001

The presence of isotopically light carbonates in association with fine-grained magnetite is considered to be primarily due to the reduction of Fe(III) by Fe(III)-reducing bacteria in the environment. Here, we report on magnetite formation by biooxidation of Fe(II) coupled to denitrification. This metabolism offers an alternative environmental source of biogenic magnetite.

The contribution of biogenic magnetite produced through reduction of Fe(III) by both dissimilatory Fe(III)-reducing bacteria (DIRB) and magnetotactic bacteria (MB) to natural remanent magnetization of deep-sea and other sediments is well recognized (4, 15, 25). Although the relative contribution of the two metabolic processes to natural remanent magnetization is controversial (4), the former process is thought to have resulted in the accumulation of large amounts of extracellular magnetite (Fe_3O_4) through the reduction of Fe(III) oxides as the terminal electron acceptor for organic matter oxidation (2). Magnetite is a mixed Fe(II)-Fe(III) mineral with magnetic properties. The discovery of biogenic magnetite at depths of ~6.7 km in the subsurface (16) has been used as a marker of activity of DIRB in the deep subsurface. In addition, the presence of magnetite in association with isotopically light carbon in carbonate minerals in the Precambrian banded iron formations (BIFs), the world's oldest and largest iron deposits (26), suggests that these organisms played a role in the Precambrian biosphere (1). However, a satisfactory explanation for the oxidation of soluble Fe(II) to Fe(III) required for DIRB to produce magnetite in the anoxic Precambrian hydrosphere before the evolution of oxygenic photosynthesis is still not available (7, 8, 12, 22).

It is unanimously agreed that in Precambrian times, both the hydrosphere and the atmosphere were anoxic, life was restricted to prokaryotes, and deep ocean waters contained significant amounts of soluble Fe(II) that resulted from the chemical weathering of the continental crust and/or subseafloor hydrothermal convection processes (1, 7). According to the accepted reductive mechanisms of biogenic magnetite formation by both DIRB (15, 25, 31) and MB (4), iron would have to be present as Fe(III) or oxidized from existing Fe(II). The most widely accepted theory for the presence of Fe(III) is the interaction of Fe(II) with oxygen produced as a result of microbial oxygenic photosynthesis (9, 31). However Canfield (7) recently demonstrated that deep-ocean water did not become oxic until the Neoproterozoic era (1.0 to ~0.54 Gyr.). In contrast, the isolation of Fe(II)-oxidizing anoxygenic phototrophs (14, 34) suggested that oxygen-independent biological oxidation of Fe(II) was possible before the evolution of oxygenic photosynthesis. Nonetheless, this type of microbial metabolism could be operative only in shallow seas that received unrestricted sunlight (2). Alternatively, the possibility of direct photooxidation of Fe(II) by UV radiation from sunlight near the surface of Precambrian oceans, prior to the formation of the ozone layer that today reduces UV radiation, has been suggested (8). However, the concentration of Fe(II) in ocean waters and its velocity of upwelling to the upper water column, where photooxidation occurred, are two factors that would have controlled the rate of photooxidation. The existence of upwelling velocities for transferring soluble Fe(II) to the upper oceans along specific basins for geologically extended periods is controversial (22). Moreover, the required high upwelling eddy velocities would hinder the formation of the finely laminated layered structure of BIFs.

The last massive deposition of BIFs around 1.8 Gyr (20) suggests that there would have been sufficient time for slow microbial oxidative precipitation of significant quantities of dissolved Fe(II) from ocean water. Moreover, it has been documented that in the primitive era before the development of oxygenic atmosphere, the net effect of lightning converted atmospheric N_2 mainly to dissolved NO_3, which remained in the ocean until the evolution of organisms capable of using it as a resource (28). If so, microorganisms that are capable of light-independent direct oxidation of soluble Fe(II) in the anoxic environs of the deep sea through nitrate reduction may offer an alternative explanation of Fe(III) formation (17). This microbial metabolism was only recently identified (5, 17, 32), but magnetite formation as a result of biooxidation of Fe(II) was never demonstrated.

As part of a study of the metabolic diversity of organisms capable of growth by the anaerobic respiration of perchlorate, we isolated a novel organism, *Dechlorosoma suillum* strain PS, from a swine waste lagoon (10, 29). Physiological characterization revealed that *D. suillum* rapidly oxidized (10 mM) Fe(II) in the form of $FeCl_2$ with nitrate as the electron acceptor under strict anaerobic conditions (21) (Fig. 1). With 10 mM acetate as a cosubstrate, more than 70% of the added iron was oxidized within 7 days. No Fe(II) was oxidized in the absence of cells or if the nitrate was omitted (data not shown). Fe(II) oxidation was initiated after complete mineralization of acetate to CO_2, and growth was not associated with this metabolism (Fig. 1). Nitrate reduction was concomitant with Fe(II)

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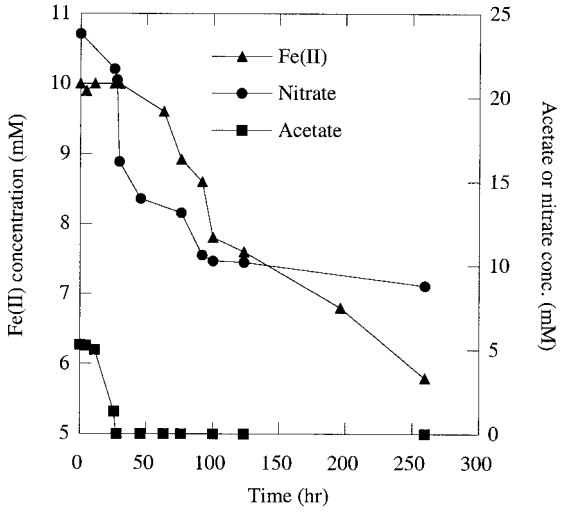

FIG. 1. Oxidation of acetate and $FeCl_2$ by *D. suillum* in bicarbonate-buffered anaerobic medium with 5 mM acetate as a cosubstrate and nitrate as the sole electron acceptor.

oxidation throughout the incubation (Fig. 1), and the oxidation of 4.2 mM Fe(II) resulted in the reduction of 0.8 mM nitrate, which is 95% of the theoretical stoichiometry of nitrate reduction coupled to Fe(II) oxidation according to the equation

$$10Fe^{2+} + 12H^{+} + 2NO_3^{-} \rightarrow 10Fe^{3+} + N_2 + 6H_2O$$

Difference spectrum studies performed as previously described (6, 10) on anaerobic washed whole cells in the presence of Fe(II) demonstrated that the oxidized *c*-type cytochrome content of *D. suillum* was reduced after 1 h of incubation in the presence of Fe(II) (Fig. 2), indicating that electrons from Fe(II) are transferred to the electron transport chain of *D. suillum*. In addition, the main product of nitrate reduction was N_2. Chromatographic analysis (ion chromatography and gas chromatography [6, 10]) of headspace gases and culture broths throughout incubation revealed no detectable quantities of nitrite or N_2O formed by *D. suillum* (29). These results suggest that Fe(II) oxidation by *D. suillum* is enzymatic and is not the result of an abiotic reaction with highly oxidized intermediates potentially formed transiently during the reductive metabolism of nitrate.

Immediately after the addition of Fe(II), in the form of $FeCl_2$, to freshly prepared anoxic basal medium (6) inoculated with an active culture of *D. suillum*, Fe(II) ions reacted, probably with carbonate in the medium, to form a white fluffy precipitate similar to that observed in previous studies with anoxygenic phototrophs (34). The white precipitate was transformed into a greenish-gray substance within a week after the start of incubation. Previously reported enrichment and pure cultures of anaerobic Fe(II)-oxidizing organisms oxidized added Fe(II) directly to yellow-brown precipitates resembling amorphous Fe(III) oxides and hydroxides (5, 32). No greenish-gray mixed Fe(II)-Fe(III) hydroxides were formed in the course of those incubations (5, 32). In contrast, *D. suillum* formed greenish-gray mixed Fe(II)-Fe(III) hydroxides, known as carbonate-containing green rusts (13), as major metabolic products within a week after the start of incubation. Green rusts are generally unstable in the environment (11), and further slow oxidation can lead to the formation of magnetite (13, 30). On prolonged incubation (14 days), the green rusts gradually transformed into blackish brown-green. Previous studies have demonstrated that green rust will chemically react with nitrate to form magnetite and ammonia as the sole end products (18, 19). Since green rust is one of the precursors of magnetite formed by *D. suillum* with nitrate as the electron acceptor, it is possible that the biogenic green rusts are abiotically reacting with the remaining nitrate to form magnetite. In

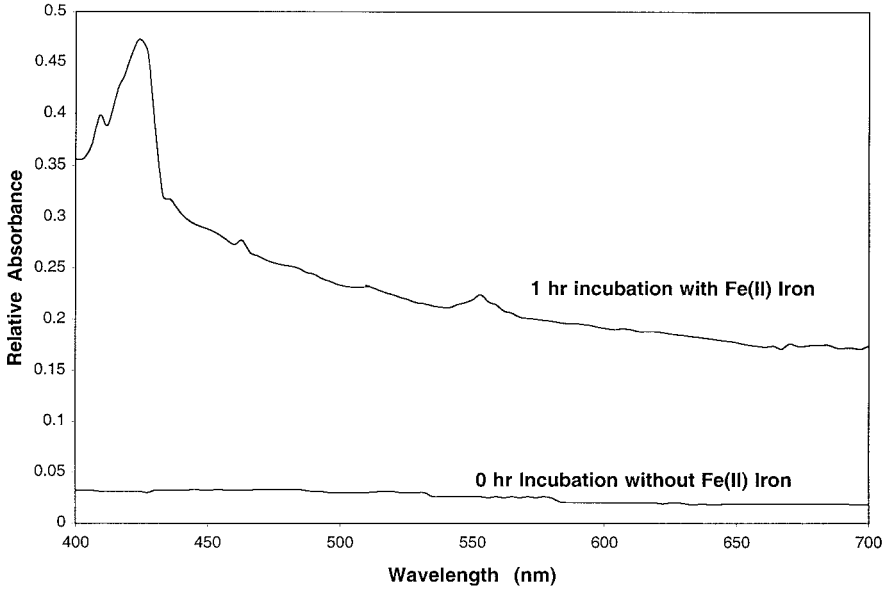

FIG. 2. Difference spectra of the *c*-type cytrochrome content of an anoxic washed whole-cell suspension of *D. suillum* initially and after 1 h of incubation in the presence of Fe(II) at 30°C.

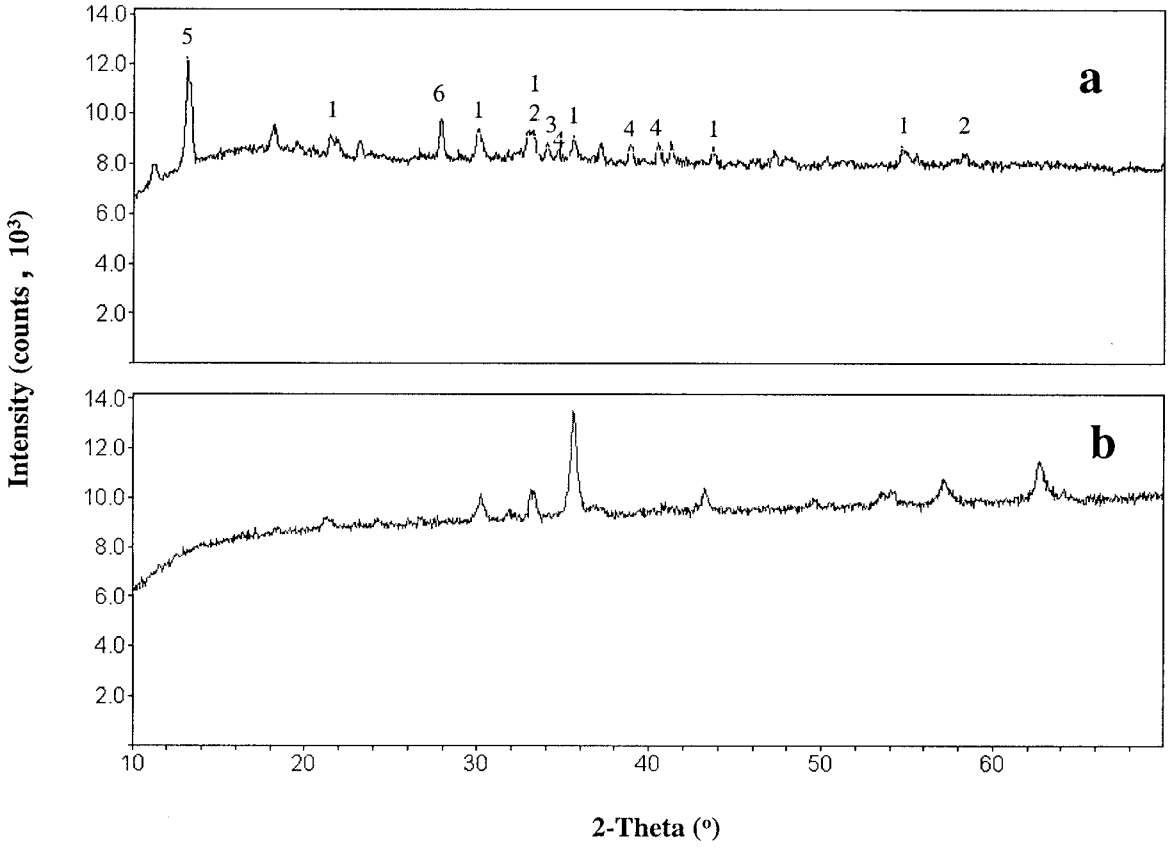

FIG. 3. X-ray diffractogram of biologically produced Fe(III) oxides (a) and magnetite mineral (b). 1, magnetite; 2, hematite; 3, iron hydrogen carbonate; 4, green rust; 5, vivianite; 6, maghemite.

contrast, no transformation of the original white precipitate was observed in abiotic controls.

X-ray diffraction (XRD) analysis of the biologically produced Fe(III) oxides 1 week after precipitation showed initial development of various crystalline phases. Samples for XRD analysis were collected and centrifuged under an N_2 gas phase and washed twice before being dried overnight in an anoxic glove bag containing a headspace of N_2-H_2 (95:5). In abiotic controls in which the $FeCl_2$ was oxidized with air, crystalline phases did not develop for several weeks (data not shown). The peak intensities of the biogenic crystalline phases gradually increased with time as the precipitates aged. Figure 3a shows the XRD pattern of biogenic Fe(III) oxides that were 4 months old. The presence of peaks indicative of magnetite in Fig. 3a was confirmed by comparison with the XRD peaks of a known magnetite mineral (Fig. 3b). The dissolution of the precipitate using solutions of dithionite-citrate buffered with bicarbonate (DCB) resulted in a soft, insoluble, black residue. Aqueous solutions of DCB dissolve most common magnetic minerals except magnetite, including hematite, maghemite, goethite, and phyrrhotite (24). Assuming that the black residue was 100% magnetite, total iron analysis of the aged precipitate (4 months old) in DCB gave a magnetite yield of ~215 to 255 g per kg of dry precipitate produced from biooxidation of soluble Fe(II).

In most reduced environments, soluble Fe(II) represents only a small proportion of the total Fe(II) available (27). Most of the Fe(II) is present as insoluble carbonate or siliceous mineral phases such as siderite ($FeCO_3$), almandine [$Fe_3Al_2(SiO_4)_3$], or glauconite {[$(Fe_{1.097}Al_{0.849}Mg_{0.442}Ti_{0.003}Mn_{0.001})(Si_{3.611}Al_{0.389})O_{10}(OH)_2]K_{0.725}Ca_{0.096}$}. If this iron is not bioavailable, abiotic oxidative reactions are more likely to have been the first step in the biogenic formation of magnetite,

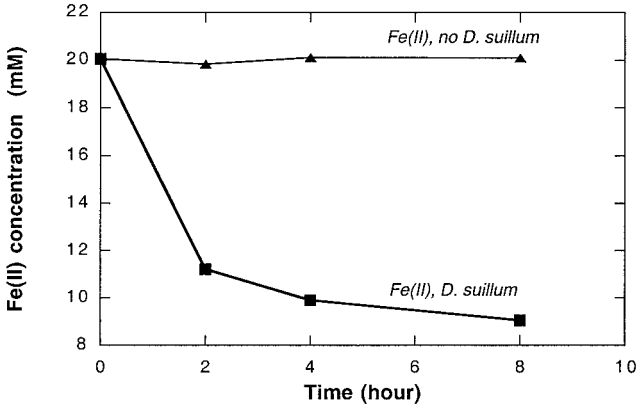

FIG. 4. Anaerobic oxidation of Fe(II) in almandine, an insoluble crystalline Fe(II) mineral, by anoxic washed whole-cell suspensions of *D. suillum* strain PS in the presence of nitrate as the electron acceptor.

TABLE 1. Microbial oxidation of Fe(II) present in different natural iron minerals by anoxic washed whole-cell suspensions of *D. suillum* coupled to the reduction of nitrate

Mineral	Chemical formula	Amt of Fe(II) oxidized[a] (mmol kg^{-1})	% of total Fe(II) in starting mineral that was oxidized
Almandine	$Fe_3Al_2(SiO_4)_3$	10.32	52.00
Arsenopyrite	FeAsS	18.27	31.00
Chromite	$FeCr_2O_4$	9.42	95.00
Siderite	$FeCO_3$	288.91	30.42
Staurolite	$(Fe,Mg,Zn)_2Al_9(Si,Al)_4O_{22}(OH)_2$	0.96	16.67

[a] The Fe(II) content was determined by a ferrozine assay after dissolution of the insoluble mineral in 6 N HCl over 24 h.

as previously suggested (9, 31). However, washed anaerobic whole-cell suspensions of *D. suillum* rapidly oxidized the Fe(II) content in various natural iron minerals including both siderite and almandine (Fig. 4; Table 1). Both the rate and extent of Fe(II) oxidation were different for the various minerals, probably due to differences in bioavailability of the Fe(II) in the mineral matrices. No oxidation of Fe(II) was observed in abiotic controls or in the absence of a suitable electron acceptor. These results demonstrate that Fe(II) oxidation is not limited to soluble Fe^{2+} ions and that direct oxidation of Fe(II) in insoluble minerals may also potentially result in the formation of magnetite.

The results presented here demonstrate that the presence of biogenic magnetite is not necessarily indicative of the activity of DIRB or other similar reductive metabolisms. Here we show that anaerobic microbial oxidation may also account for the geological evidence. The present model of magnetite formation by nitrate-reducing bacteria through biooxidation of Fe(II) can also account for the presence of isotopically light carbon observed in carbonate minerals (3) as well as in BIFs (33), since these organisms can cometabolize and completely oxidize organic substrates such as acetate. Such heterotrophic metabolisms would result in the isotopic fractionation of carbon, leading to the formation of CO_2 and ultimately carbonates with a lighter isotope signature. Although the evolutionary timescale of microbial nitrate reduction is unknown, it is hypothesized that microbial nitrate reduction arose prior to the end of the Precambrian era. The presence of nitrate and Fe(II) in Precambrian ocean waters could have driven organisms capable of Fe(II) oxidation to form magnetite in anoxic sediments. The confinement of the process of bacterial magnetite formation to a zone between the levels of nitrate reduction and Fe(III) reduction in neoteric aquatic sediments (23) supports the above mechanism.

We thank L. A. Achenbach for critical review of the manuscript.

Support for this research was provided by grant DE-FG02-98ER62689 from the Department of Energy.

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