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Formation of single-domain magnetite by a thermophilic bacterium

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ABSTRACT

Magnetite is a common product of bacterial iron reduction and may serve as a potential physical indicator of biological activity in geological settings. Here we report the formation of single-domain magnetite under laboratory conditions by a thermophilic fermentative bacterial strain TOR-39 that was isolated from the deep subsurface. Time-course analyses were performed at 65 °C to study the effect of bacterial activity on solution chemistry and magnetite formation during the growth of TOR-39. Run products were examined by transmission electron microscopy. Magnetite particles formed exclusively outside of bacterial cells and exhibited octahedral shapes having relatively equal length and width (<15% difference). Tiny magnetite particles (<12 nm) nucleated between 10 and 11 h of incubation and increased to average lengths of 55.4 ± 26.8 nm after 24 h of incubation. Between 24 h and 22 d of incubation, magnetite particles maintained average lengths of 56.2 ± 24.8 nm. Based on size constraints, greater than 85% of the particles observed fell within the magnetic single domain. Little to no magnetite was detected in abiotic controls at 65 or 95 °C, or in TOR-39 cultures whose activity was suppressed. Unlike mesophilic iron-reducing bacteria (e.g., GS-15), TOR-39 produced crystals having shapes and sizes similar to some particles produced intracellularly by magnetotactic bacteria. Thus the single-domain magnetite produced by thermophiles such as TOR-39 may represent a heretofore unrecognized biological contribution to natural remanent magnetization in sedimentary basins and other geothermal environments.

INTRODUCTION

The geosciences community has increasingly recognized the importance of bacteria in geological processes (e.g., Banfield and Nealson 1997; Skinner and Banfield 1997). It is now known that microbes inhabit nearly every portion of the Earth's crust, including the most extreme environments such as permafrost in Antarctica (e.g., Gilichinsky 1997), hot geothermal vents (e.g., Brock 1978; Stetter 1995), and the deep subsurface (Phelps et al. 1989; Boone et al. 1995; Stevens and McKinley 1995; Fredrickson and Onstott 1996; Liu et al. 1997; Pedersen et al. 1997). Microbes participate in a variety of geochemical processes such as weathering of rocks (Ferris et al. 1994; Sillitoe et al. 1996), formation of mineral ores (Juniper et al. 1995; Vasconceios et al. 1995; Tebo et al. 1997), and cycling of organic matter (Ehrlich 1990; Lovley 1991; Nealson and Saffarini 1994).

Iron-reducing bacteria are widespread in the Bacteria domain (Coates et al. 1996; Lonergan et al. 1996), and have been found in various natural environments including freshwater and marine sediments (Lovley and Phillips 1986; Caccavo et al. 1992; Nealson and Saffarini 1994), pristine or contaminated aquifers (Lovley et al. 1990; Coates et al. 1996), geothermal vents (Slobodkin and Wiegel 1997; Slobodkin et al. 1997), and the deep subsurface (Boone et al. 1995; Liu et al. 1997; Pedersen et al. 1997; Onstott et al. 1997). Because of their wide occurrence, iron-reducing bacteria may have global significance in the geochemical cycling of mineral-forming elements such as carbon, oxygen, sulfur, and iron. Nealson and Myers (1990) suggest that bacterial iron reduction may have played an important role in the genesis of Precambrian banded iron-formations, and that the enzymes responsible for iron reduction may have preceded many of the more common enzymes present in modern oxic environments. Lovley (1990) suggests that at the pH, temperature, and pressure of most sedimentary environments, Fe^{3+} reduction is mainly the result of microbial enzymatic activity, and that Fe^{3+} reduction may have been the first globally important mechanism for microbial oxidation of organic matter in the Archaean biosphere.

Magnetite is a common end product of bacterial iron

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reduction, and may potentially serve as a physical indicator for biological activity in modern or ancient geological settings (Vali et al. 1987; Chang and Kirschvink 1989; Frankel and Blakemore 1990; Zhang et al. 1997a, 1997b). Furthermore, extraterrestrial magnetite in the Martian meteorite ALH84001, which in some respects resembles biogenic magnetite formed on Earth, has been used as evidence supporting past biologic activity on Mars (McKay et al. 1996). Biogenic magnetite also contributes significantly to the natural remanent magnetization of sediments (Peterson et al. 1986; Chang and Kirschvink 1989; Stolz et al. 1990; Moskowitz et al. 1993).

Studies in microbial Fe^{3+} reduction have mostly been directed toward understanding the physiology and metabolic capabilities of the bacteria; few have looked closely at the processes of magnetite mineralization during bacterial iron reduction (Zhang et al. 1997a, 1997b). Here we characterize the size and morphological changes that result from magnetite growth during Fe^{3+} reduction by a thermophilic fermentative bacterial strain TOR-39.

Material and methods

Source of bacterium and experimental conditions

Strain TOR-39 was isolated from a sedimentary rock consisting of sand, silt, and clay at about 2.8 km depth in the Triassic Taylorsville Basin in Virginia. Modern temperature at the sampling depth is about 75 °C. Geological and microbiological evidence suggests that viable microorganisms may have inhabited the deep subsurface of the Taylorsville Basin for millions of years (Phelps et al. 1994; Tseng et al. 1996).

TOR-39 is an anaerobic, Gram-negative, rod-shaped bacterium that can ferment glucose and other carbohydrates (Zhang et al. 1996). It grows at temperatures from 50 to 70 °C and at NaCl concentrations up to 0.86 M. For this study, TOR-39 was grown in a basal medium that contained the following reagents (g/L): NaCl (10), $MgCl_2 \cdot 6H_2O$ (0.2), $CaCl_2 \cdot 2H_2O$ (0.1), NH_4Cl (1.0), yeast extract (0.5), trace minerals, and vitamins (Phelps et al. 1989). HEPES (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid], final concentration 30 m*M*) or sodium bicarbonate (final concentration 90 m*M*) was used as a pH buffer. The media were prepared anaerobically under a nitrogen gas atmosphere. Sterile glucose (final concentration 10 m*M*) was added as an organic substrate for growing TOR-39. Sterile Fe^{3+} oxyhydroxide (final concentration 70 m*M*) was added as a starting material for magnetite formation during bacterial growth. Analysis of X-ray diffraction showed that most of the oxyhydroxide was amorphous and a small portion was poorly crystalline akaganeite (Zhang et al. 1997a). In this paper we refer to this material as Fe^{3+} oxyhydroxide. Resazurin was added to media as colorimetric indicator of redox potential. Final pH of the media ranged from 8.5 to 8.8 prior to inoculation. For all bacterial experiments, 10 vol% inocula from a pregrown culture were used.

Experiments were performed in 26 mL pressure tubes containing 10 mL medium under a N_2 atmosphere. Suites of identical tubes were used to perform time-course analyses. Thirty-six tubes were inoculated at the beginning of an experiment and incubated at 65 ± 3 °C; individual tubes were withdrawn periodically for chemical and biological analysis. An additional 29 tubes were incubated at the same temperature range for transmission electron microscopic analysis of solids, among which 22 tubes were inoculated with TOR-39 and 7 tubes served as abiotic controls (amended with the same chemical constituents but not inoculated with TOR-39). During incubation, a single tube was removed from the incubator at 3 h intervals for the first 9 h of incubation, and at about 17 min intervals from 10.33 to 12.17 h of incubation, during which period precipitates became magnetic. Subsequent samples were collected at 14, 16, 24, 48, and 528 h. Abiotic control tubes were removed from the incubator at 6, 10, 10.83, 11.50, 14, 24, and 528 h of incubation. We performed an additional experiment in which control tubes were incubated at 95 °C for the observation of abiotic magnetite formation at elevated temperatures.

To examine inorganic growth of magnetite after biological induction of magnetite nuclei, anaerobic gluderaldehyde (final concentration 2.5%) was added to TOR-39 tubes at two time points: when magnetite was first detected (10.33 h) and at 1.2 h after magnetite was first detected (11.5 h). These tubes were continuously incubated until the end of experiments (22 d).

In a separate experiment, two tubes were inoculated and incubated continuously for over 70 days to examine the effect of extended incubation on magnetite formation. A horse-shoe magnet (Al-Ni-Co alloy, leg size 1 $inch^2$) was used to detect the presence of magnetic precipitates through out the course of all experiments.

Bacterial cell counts

Bacterial cell numbers were determined by acridine-orange direct counts (Zhang et al. 1996). Subsample aliquots (approximately 2 mL) from individual pressure tubes were sonicated in a water bath for five minutes to facilitate the separation of bacterial cells from mineral particles and then diluted 20- to 200-fold in filter-sterilized distilled water. One milliliter of the diluted sample was mixed with 1 mL of a particle-free and sterile solution of acridine-orange (1 g per liter distilled water), and after 3 min the solution was filtered onto a black Nuclepore filter (0.2 μm pore diameter). The filter was then mounted onto a slide and viewed under an epifluorescence microscope for cell counts. At least 10 fields of view were counted to determine cell abundance.

Eh and pH measurements

Eh and pH of bacterial cultures and abiotic controls were measured at room temperature in an anaerobic chamber. The Eh was measured using a platinum microelectrode (Microeletrodes, Inc., Londonderry, N.H.) connected to a pH meter. The probe was placed directly into

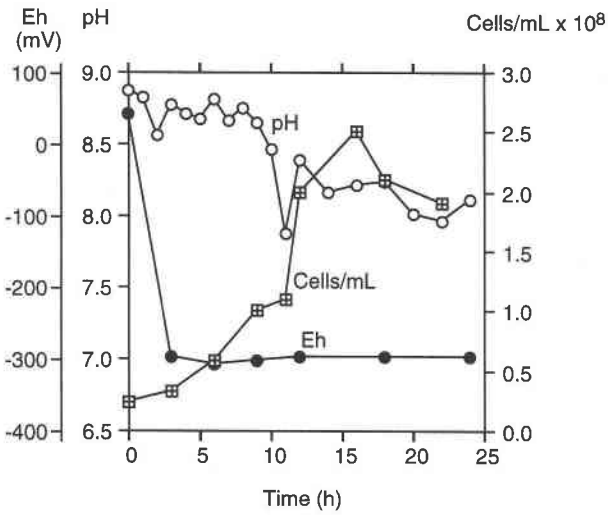

FIGURE 1. Time-course analysis of pH, Eh, and bacterial abundance during Fe^{3+} reduction by thermophilic bacterium TOR-39 at 65 °C. The medium contained 90 mM sodium bicarbonate under a N_2/CO_2 (80:20) atmosphere. Magnetite formation was first detected between 10 and 11 h.

the sample tube and equilibrated for at least 5 min before recording the value. The pH was then measured by transferring approximately 5 mL of the liquid portion into a 10 mL beaker into which a pH probe was placed.

TEM analysis

The morphological characteristics of magnetite were examined with transmission electron microscopy (TEM). Culture medium containing bacterial cells, organic matter, and inorganic solids, was fixed with 2.5% glutaraldehyde in 0.1 *M* cacodelylate. After washing with buffer and an alcohol/water solution, samples were dehydrated with propylene oxide and embedded in a low-viscosity, thermally curing epoxy resin. Ultrathin sections (70–80 nm) were cut from resin blocks with a diamond knife and transferred to 300 mesh, formvar-coated Cu TEM grids for image analysis on a JEOL FX 2000 equipped with an energy-dispersive X-ray detector. To enhance TEM images of bacterial cells, selected samples were stained with lead citrate and uranium acetate.

Observation of natural magnetite

Three samples from the Taylorsville Basin were processed for the examination of natural magnetite associated with the sediments from which thermophilic iron-reducers were obtained. Two samples were sandstones obtained at depths of 2793 and 2798 m below land surface (bls), and the third was a siltstone/claystone obtained at 2799 m. These samples were taken from a depth close to where TOR-39 was isolated (~2770 m).

During processing, samples were gently broken into fine particles using non-metallic tools. A non-destructive technique was used to separate single-domain magnetite particles by applying a high-magnetic-field gradient to the sample while maintaining relatively low absolute field intensity. This technique resulted in the attraction of particles having a permanent magnetic moment. Magnetite particles obtained by this technique were examined by TEM in the same manner as experimental magnetite from TOR-39 cultures.

RESULTS

Effect of microbial activity on solution chemistry and mineralogy

Bacterial abundance and solution pH and Eh were monitored at 65 °C in a time-course experiment using bicarbonate-buffered medium containing 70 m*M* Fe^{3+} oxyhydroxide and 10 m*M* glucose. Cell density increased from a starting concentration of 2.4×10^7 cells/mL to 1.1×10^8 cells/mL within the first 11 h of incubation (Fig. 1). Cell density increased almost twofold within the next hour, reaching 2.0×10^8 cells/mL after 12 h of incubation, and a maximum value at 16 h (2.5×10^8 cells/mL), after which cell density began to decrease with time (Fig. 1).

The Eh of the medium decreased from an initial potential of 44 mV to about −300 mV by the first measurement after 3 h of incubation. Further growth of TOR-39, however, did not lower Eh any further (Fig. 1). Within the first 10 h of incubation, pH decreased slowly from an initial value of 8.8 to 8.5 at 10 h. By 11 h, pH dropped sharply to 7.9 followed by a rebound to 8.4 at 12 h. The pH continued to decrease slowly thereafter, but it remained above 7.9 during the subsequent incubation (Fig. 1). The general decrease in pH can be explained by the metabolic activity of TOR-39, which produces organic acids and carbon dioxide during glucose fermentation (Zhang et al. 1996). The excursion to a pH of 7.9 at 11 h occurred just prior to the largest increase in bacterial abundance (11 to 12 h) of the entire course of experiment. The most likely explanation for the abrupt change in pH between 11 and 12 h is that the buffering capacity of bicarbonate could not keep up with the production of organic acids and carbon dioxide caused by bacterial metabolism over this brief time interval.

Magnetite formation was first detected in TOR-39 cultures between 10 and 11 h in both bicarbonate- and HEPES-buffered media. No magnetite was detected in abiotic controls at 65 °C and only trace amounts of magnetic material were detected in abiotic controls at 95 °C using the magnet. More importantly, magnetite was not observed to precipitate in TOR-39 cultures in which bacterial cells were killed by gluderaldehyde after crystal nucleation. These experiments indicate that a significant amount of magnetite could not be precipitated by a purely inorganic mechanism under the conditions examined, even with the presence of highly reactive seed material. These results further suggest that bacterial activity played a governing role in biogenic magnetite formation under the applied experimental conditions.

Measured pH and Eh values in TOR-39 cultures after

12 h of incubation fall within the stability field of magnetite on an Eh-pH plot (Fig. 2; see Zhang et al. 1997a). This is consistent with the detection of magnetite after 10 h of incubation. Despite the existence of appropriate conditions for inorganic growth on a thermodynamic ground, magnetite did not form at 65 °C in control tubes or tubes containing magnetite nuclei but not living cells. If gluderaldehyde does not inhibit the inorganic growth of magnetite, it may be concluded that living TOR-39 cells are required to catalyze the reaction that produces magnetite in these experiments.

TEM analysis of magnetite particles formed by TOR-39

Magnetite particles were observed in TEM images of all TOR-39 samples after 10.33 h (Fig. 3a) but not in any abiotic controls (Fig. 3b) at 65 °C. Magnetite particles greater than 20 nm had octahedral shapes, and in all instances, they were formed outside of the cell wall (Fig. 3a). Tiny (<12 nm) magnetite particles were first observed at 10.33 h and they were associated with Fe^{3+}oxyhydroxide (Fig. 4a). By 22 d, most of the Fe^{3+}oxyhydroxide had disappeared, and large magnetite particles were observed in great abundance (Fig. 4d).

Table 1 shows that the maximum and average grain sizes of the magnetite particles increased with incubation times. The size distributions of magnetite particles formed at 10.33, 11.5, 16, 24, and 528 h are plotted in histograms (Fig. 5). Crystal sizes ranged from 3 nm to 12 nm at 10.33 h, with most grains being less than 10 nm in length (Fig. 5a). The size of the largest particles increased to 40 nm at 11.5 h (Fig. 5b), to 80 nm at 16 h (Fig. 5c), to 110 nm at 24 h (Fig. 5d), and to 130 nm at 22 d. During this time, the number of small particles (<15 nm) in a given area decreased. For example, in an area of 1.3 × 1.9 μm^2, the number of magnetite particles in the size range of 5 to 10 nm decreased from 18 at 11.5 h to 9 at 24 h. At 22 d, no particles were observed in the 5 to 10 nm size range, with the smallest grains being greater than 15 nm in length (Fig. 5e).

An extended incubation of 72 d in an independent experiment resulted in degraded textures of magnetite such as irregular shapes and rounded margins (Fig. 6a). Although this may be due to the acidic conditions caused by glucose fermentation during the growth of TOR-39, intact magnetite crystals were still present.

Observation of magnetite in natural samples

Natural magnetite particles were observed in the clay and organic-rich matrices of the siltstone/claystone sample but not in sandstones from the Taylorsville Basin (Fig. 6b). Some particles were rounded whereas others had relatively sharp edges; both of these textures were observed in magnetite grains in laboratory experiments from the 72 d incubation experiment (Fig. 6a). The sizes of natural particles with relatively sharp edges were about 50 nm in length (Fig. 6b).

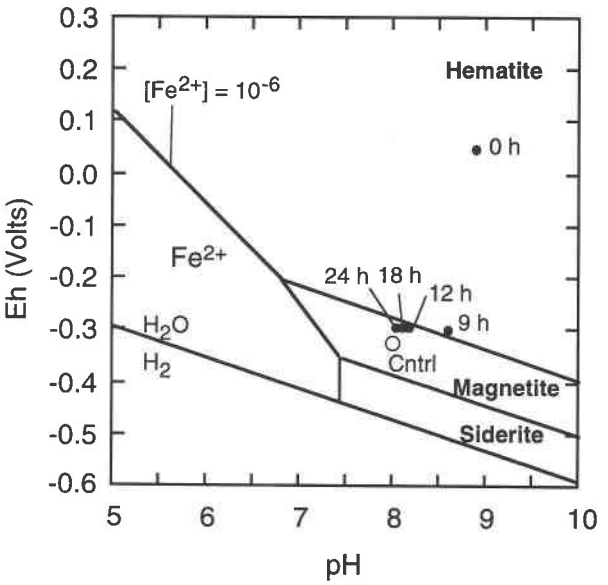

FIGURE 2. Eh-pH stability fields for hematite, magnetite, and siderite in the water-iron-CO_2 system at 25 °C and 1 atm total pressure (modified from Zhang et al. 1997a). A study by Machel and Burton (1991) suggests that stability fields of iron minerals at 50–70 °C do not differ significantly from those at 25 °C. Measured Eh and pH values for TOR-39 cultures (solid circles) and the control containing spent medium were also plotted. Note that the 0 and 9 h samples plot in the hematite stability field whereas samples at 12, 18, and 24 h plot in the magnetite stability fields.

Magnetic domain of magnetite particles formed by TOR-39 and in the natural sample

Based on size constraints, magnetite nuclei produced by TOR-39 at 10.33 h were superparamagnetic but grew rapidly to single domain (see Fig. 5). For example, some magnetite crystals grew to 40 nm in length within 1.5 h of nucleation (Fig. 5b). The abundance of single-domain particles increased to more than 60% of total grains after 16 h of incubation (Fig. 5c) and to about 90% of total grains after 22 d of incubation (Fig. 5e). Most of the magnetite grains that remained after 70 d of incubation appeared to be still single domain (Fig. 6a), and so were the natural magnetite particles from the Taylorsville Basin (Fig. 6b).

DISCUSSION

Formation of magnetite by bacteria

Iron biomineralization has been commonly divided into two modes: biologically controlled mineralization in which bacteria genetically control the mineralization process (Frankel and Blakemore 1990), and biologically induced mineralization in which bacteria facilitate magnetite formation by creating external chemical environments suitable for precipitation. The formation of magnetite by magnetotactic bacteria represents biologically controlled mineralization in which the particles are formed inside bacterial cells (e.g., Blakemore 1982; Bazylinski et al.

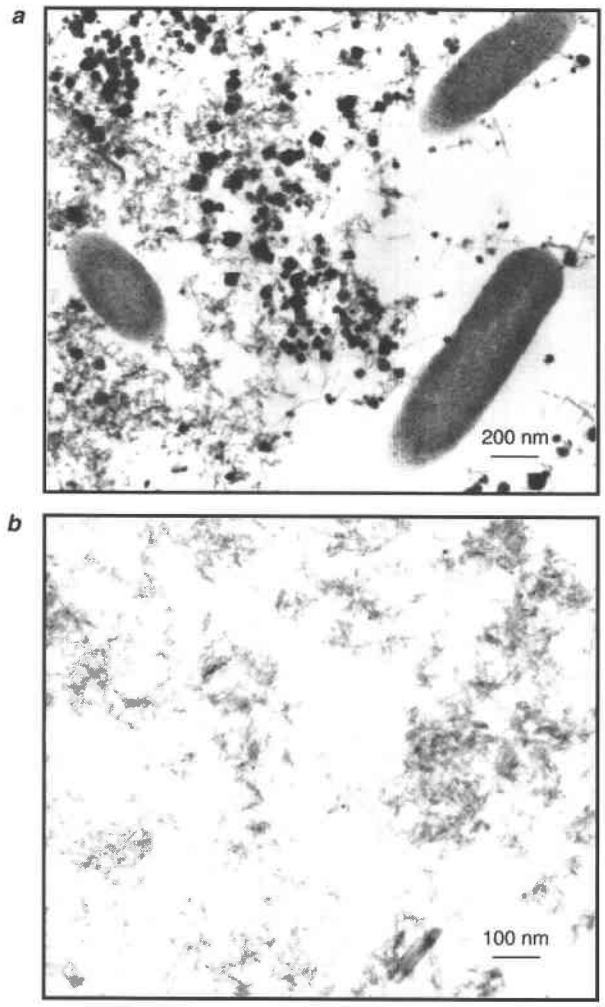

FIGURE 3. (a) TEM images of magnetite particles (black, octahedral shapes), Fe^{3+} oxyhydroxide (thin needle like material), and bacterial cells (large rods) in TOR-39 cultures after 24 h of incubation at 65 °C. (b) Only Fe^{3+} oxyhydroxide was observed in the abiotic control after 24 h of incubation at 65 °C.

1988; Frankel and Blakemore 1990). In contrast, the formation of magnetite by dissimilatory iron-reducing bacteria, such as thermophilic bacterium TOR-39 or mesophilic bacterium GS-15, represents biologically induced mineralization in which the particles are formed extracellularly as a byproduct of bacterial iron reduction.

The formation of intra- and extra-cellular magnetite requires precise regulation of redox, pH, and iron chemistry (Mann et al. 1990; Schwertmann and Fitzpatrick 1992). Schwertmann and Fitzpatrick (1992) suggest that magnetite formation is the result of a suitable pH (and Eh), a favorable rate of Fe^{2+} and Fe^{3+} supply to the growing crystal, and a buffering capacity of the system. Further, for any mineral to precipitate, the solution must be supersaturated with respect to the mineral.

The chemistry of the media in TOR-39 experiments indicated that the Eh and pH conditions were appropriate for magnetite formation. Although calculations of saturation state for the chemical meliu showed that the solution was supersaturated with respect to magnetite (see Zhang et al. 1997a), it was the bacterium that induced magnetite mineralization. During the time-course experiments, magnetite formed in the presence of living cells when pH and Eh conditions fell into the magnetite stability field (see Fig. 2) while in the absence of living cells magnetite did not form even when suitable seed material (magnetite nuclei), Fe^{3+} oxyhydroxide, or dead cells were present for magnetite growth. This suggests that TOR-39 accelerated the precipitation kinetics of magnetite formation under the conditions examined.

It is unclear how magnetite grains in TOR-39 cultures nucleated and grew. Banfield and Hamers (1997) suggest that surface free energy of a precipitating phase is an important factor controlling the size to which a nucleus must grow before it becomes stable. Organic molecules are also important in mediating mineral crystallization (e.g., Belcher et al. 1996). In this study, the growth of larger crystals and the disappearance of magnetite nuclei (<15 nm) from 24 h to 22 d may be related to the changes in (1) solution chemistry such as the availability of Fe^{2+} or Fe^{3+}, (2) the greater reactivity of smaller grains, (3) the proximity of Fe^{3+} oxyhydroxide and magnetite to the bacterial cell wall, or (4) bacterial cell surface properties such as types of protein or lipids. Determining the importance of each factor in magnetite formation by TOR-39, however, requires additional work on various aspects of organic and inorganic chemistry, mineralogy, and bacterial physiology, which are beyond the scope of this study.

Although the exact mechanism is currently unknown, fermentative TOR-39 might have caused magnetite formation by enhancing Fe^{3+} reduction at thermophilic temperatures. Zhang et al. (1996) reported that when fermenting glucose, TOR-39 enhanced Fe^{3+} reduction by fourfold at 55 °C relative to the abiotic control. On the other hand, mesophilic fermentative iron-reducing bacteria transfer less than 5% of reducing equivalents from the substrates they metabolize to the ferric iron (Lovley 1991). Quantitative analysis of electron transfer between organic substrate and Fe^{3+} by TOR-39, however, has yet to be determined. The bacterial cells may also serve as nucleation sites for mineral formation. For example, Schultze-Lam et al. (1992) demonstrate that a cyanobacterial S layer participates in the formation of fine-grained gypsum and calcite by providing discrete, regularly arranged nucleation sites for initial mineral formation. Fortin et al. (1997) report that metal-sulfide precipitates are commonly found on the external surfaces of bacterial cells collected from natural environments, and Slobodkin et al. (1997) show that bacterial cell walls from thermophilic iron-reducing bacterium, *Thermoterrabacterium ferrireducens,* are sites for the precipitation of electron dense material.

The bacterial cell walls of TOR-39 appeared to be clean and smooth through out the time-course experi-

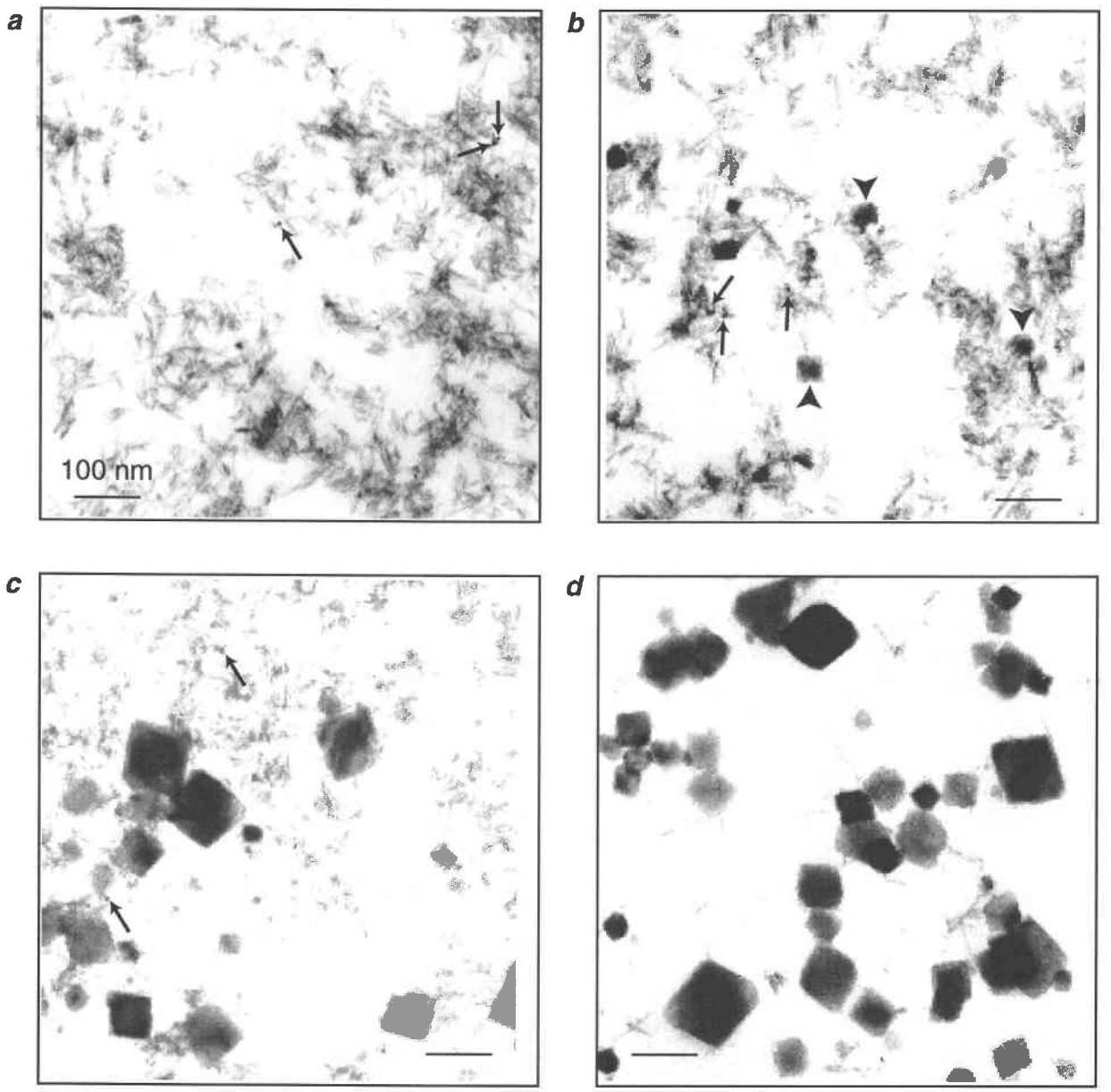

FIGURE 4. TEM images of magnetite formation in TOR-39 cultures at different times of incubation at 65 °C. (**a**) 10.33 h, (**b**) 11.5 h, (**c**) 24 h, (**d**) 22 d (528 h). Thin arrows in *a, b,* and *c* point to magnetite nuclei; large arrow heads in *b* point to growing magnetite crystals. Scale is 100 nm for all images. Note changes in individual particle size, the range of sizes, and the decrease of Fe^{3+} oxyhydroxide with time.

ments, and only a few particles were observed to be attached to them (e.g., Fig. 3a). This suggests that cells of TOR-39 were not sites for the heterogenous growth of magnetite. The potential exists, however, that sample preparation might have detached particles from the cell wall. More likely, the cells were not directly used as nucleation sites but rather they facilitated magnetite precipitation by catalyzing chemical reactions that lowered the activation energy barrier for crystal nucleation (Fortin et al. 1997).

Biogenic magnetite in natural environments

Based on the results of magnetite mineralization by TOR-39 in this study, the origin of magnetite particles in the sedimentary sample retrieved from the Taylorsville Basin is still unclear. Although we cannot prove these magnetite particles are biogenic, their presence in siltstone/claystone but not sandstones is intriguing. In natural environments, silt and clay usually contain more organic matter than coarse material such as sand (McMahon and Chapelle 1991; Krumholz et al. 1997). Snowball (1994)

TABLE 1. Maximum (Max.) and average (Avg.) sizes of magnetite particles formed by TOR-39

Time (h)	No. of particles*	Max. length (nm)	Max. width (nm)	Avg. length (nm)	Avg. width (nm)	Magnetic domain†
10.33	10	12	12	6.9 ± 3.0	6.9 ± 3.0	SPM
11.5	41	40	40	18.6 ± 13.5	16.2 ± 11.2	SPM
16	52	80	70	37.9 ± 21.7	33.1 ± 19.2	SD
24	85	110	110	55.4 ± 26.8	50.3 ± 25.0	SD
528	113	130	130	56.2 ± 24.8	52.2 ± 23.7	SD

* Numbers of magnetite particles were counted on TEM grids for an area of about 1.3 × 1.9 μm^2 at each time point.

† Based on average sizes from columns 5 and 6. The boundary between superparamagnetic (SPM) and single domain (SD) is 30 nm according to Dunlop and Özdemir (1997).

documented a biogenic magnetite in organic-rich lake sediments, and Elmore et al. (1987) and McCabe et al. (1987) found authigenic magnetite associated with biodegraded oil in petroleum reservoirs, which might be a result of bacterial activity. Because iron-reducing bacteria play an important role in the cycling of organic matter, biogenic magnetite should be a common product in organic rich environments. For example, Lovley and Reynolds (1987) suggest that the increase in magnetic susceptibility over time in freshwater sediments from the Potomac River may have been caused by magnetite production during dissimilatory Fe^{3+} reduction coupled to organic matter oxidation. Thus, the occurrence of magnetite in siltstone/claystone of the Taylorsville Basin and the similarities in morphology with biologically induced magnetite produced under our laboratory conditions by TOR-39 lends support to a biogenic origin for magnetite from the deep subsurface.

Identification of biogenic magnetite in natural environments

The morphology and size of fine-grained magnetites have been used as important criteria for identifying magnetite formed by magnetotactic bacteria. Until now it has been generally agreed that magnetotactic bacteria produce single-domain magnetite with unique octahedral, prismatic, or bullet shapes, whereas dissimilatory iron-reducing bacteria such as GS-15 form irregular and poorly crystalline magnetite particles that are mostly superparamagnetic (Fig. 7). Results of this study show that thermophilic iron-reducing bacteria such as TOR-39 can form well-crystallized magnetite particles that are single domain (Fig. 7), and these magnetite grains have similar morphologies to some particles produced by magnetotactic bacteria. Although the mechanism(s) of mineralization for mesophiles and thermophiles is presently unknown, formation temperature may be an important factor governing the character of the precipitating phase. Magnetite grains produced by dissimilatory iron-reducing bacteria are within the size range where surface free energy is an important component of the total free energy of the system (Banfield and Hamers 1997). The solubility of mag-

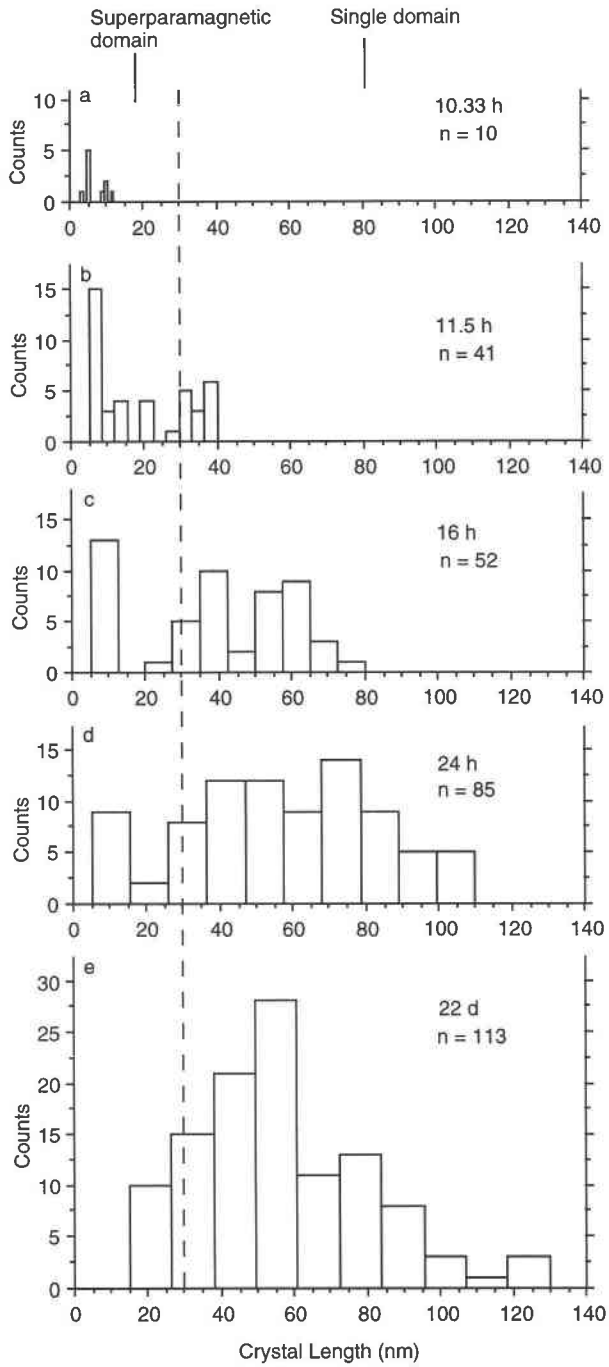

FIGURE 5. Histograms of magnetite crystal lengths at different time points of incubation in TOR-39 cultures at 65 °C. Numbers (n) of magnetite particles were counted for an area of about 1.3 × 1.9 μm^2 at each time point. The boundary length (30 nm) between superparamagnetic and single-domain magnetite particles is based on theoretical calculations of Butler and Banerjee (1975), and Dunlop and Özdemir (1997).

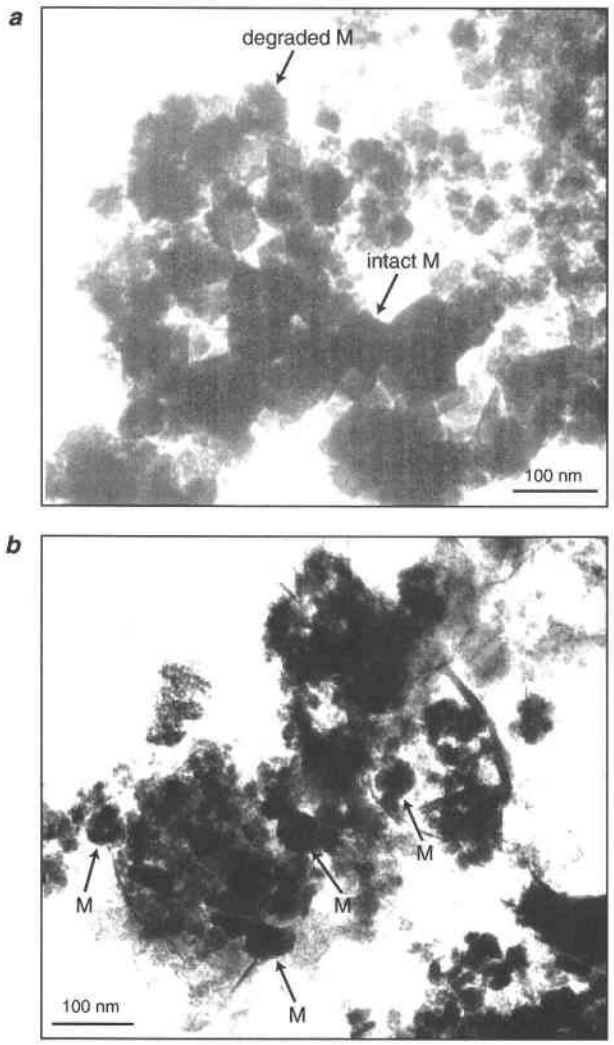

FIGURE 6. (a) TEM images of magnetite particles in TOR-39 cultures incubated for over 70 days at 65 °C showing degraded magnetite (M) as well as intact crystals. (b) Magnetite (M) particles observed in a siltstone/claystone sample collected from the deep Taylorsville Basin in Virginia.

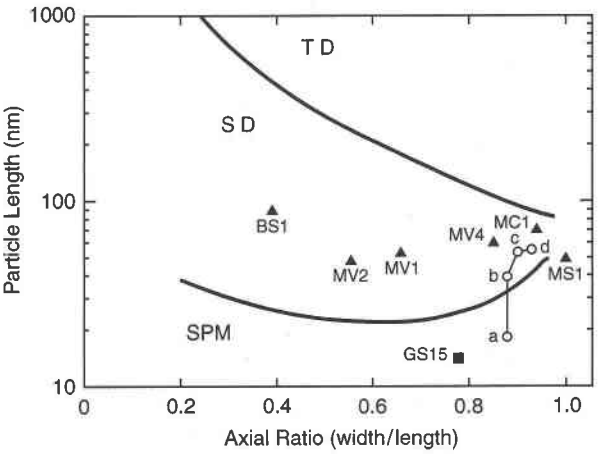

FIGURE 7. Modified diagram from Bazylinski and Moskowitz (1997) showing theoretical domain states for magnetite. SPM = superparamagnetic, SD = single domain, TD = two domain. Symbols represent average sizes of magnetite particles formed by bacteria. Solid triangles represent particles formed by magnetotactic bacterial strains BS-1, MV-1, MV-2, MV-4, MC-1, and MS-1 (Bazylinski and Moskowitz 1997), and the solid square represents particles formed by dissimilatory iron-reducing bacterium GS-15 (Sparks et al. 1990). Open circles represent particles formed by TOR-39 in this study; a = 11.5 h incubation, b = 16 h incubation, c = 24 h incubation, and d = 22 d incubation.

netite increases dramatically as gain sizes decrease below 200 nm but the effect is dampened with increasing temperature (for a discussion of free energy effects, see Stumm 1992). Thus the activation barriers associated with the formation of single-domain magnetite may be exceeded above some critical point that is within the range of temperatures that thermophiles thrive. Undoubtedly, other factors such as solution chemistry and metabolic capability also contribute to the size and character of biogenic magnetite produced by these organisms.

Geological implications of iron biomineralization

Recognition of biogenic magnetite in terrestrial environments will help us understand the origin of iron minerals here on Earth and on other planets such as Mars (Boston et al. 1992; McKay et al. 1996). As a byproduct of bacterial iron-reduction, biogenic magnetite in geological settings may indicate microbial cycling of carbon and iron. This cycling may have been expressed as magnetite in the Precambrian banded iron-formations (Perry et al. 1973; Walker 1984; Baur et al. 1985). The association of authigenic magnetite with petroleum hydrocarbons in oil reservoirs (Elmore et al. 1987; McCabe et al. 1987) suggests that iron-reducing bacteria may be involved in oil genesis and/or degradation. Thermophiles may be critical consortia involved in oil generation and degradation since temperatures at which crude oil is formed and biodegraded correspond closely to temperatures at which the thermophiles grow (Philippi 1977).

A stable primary natural remanent magnetization (NRM) is usually associated with fine-grained magnetite (e.g., Moskowitz et al. 1993). It is widely held that biogenic magnetite produced by magnetotactic bacteria contributes significantly to the NRM of carbonates and limestones, and hemipelagic and deep sea marine sediments (Peterson et al. 1987; Vali et al. 1987; Chang and Kirschvink 1989; Stolz et al. 1990; Moskowitz et al. 1993). Knowledge of magnetic contributions from dissimilatory iron-reducing bacteria, however, is limited to the study of mesophilic bacteria such as GS-15 that produces less than 5% of single-domain particles (Moskowitz et al. 1989). While the low percentage and poor crystalinity of single-domain magnetite formed by GS-15 casts doubt on its contribution to sediment NRM, Lovley (1990) proposes that on a per cell basis, GS-15 can generate more single-domain magnetite than a typical magnetotatic bacterium

because GS-15 readily produces 5000-fold more magnetite than an equivalent biomass of magnetotactic bacteria (Frankel 1987). Compared to GS-15, TOR-39 produces magnetite particles that predominantly fall within the single domain (Figs. 5 and 7). Thus thermophilic iron-reducing bacteria may contribute significantly to NRM in sedimentary basins and other natural environments that may have experienced temperatures conducive to thermophiles (e.g., 45 to 85 °C).

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