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# Formation of magnetite and iron-rich carbonates by thermophilic iron-reducing bacteria

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## 1. ABSTRACT

Laboratory experiments were performed to study the formation of iron minerals by a thermophilic (45–75°C) fermentative iron-reducing bacterial culture (TOR39) obtained from the deep subsurface. Using amorphous Fe(III) oxyhydroxide as an electron acceptor and glucose as an electron donor, TOR39 produced magnetite and iron-rich carbonates at conditions consistent, on a thermodynamic basis, with Eh (-200 mV to -415 mV) and pH (6.2 to 7.7) values determined for these experiments. Analyses of the precipitating solid phases by X-ray diffraction showed that the starting amorphous Fe(III) oxyhydroxide was nearly completely converted to magnetite and Fe-rich carbonate after 20 days of incubation. Increasing bicarbonate concentration in the chemical milieu resulted in increased proportions of siderite relative to magnetite and the addition of MgCl<sub>2</sub> caused the formation of magnesium-rich carbonate in addition to siderite. The results suggest that the TOR39 bacterial culture may have the capacity to form magnetite and iron-rich carbonates in a variety of geochemical conditions. These results may have significant implications for studying the past biogenic activities in the Martian meteorite ALH84001.

**Keywords:** Thermophilic iron-reducing bacteria, biogenic magnetite and iron-rich carbonate, Martian meteorite

## 2. INTRODUCTION

It has long been suggested that life may have existed on Mars because its warm and wet conditions were similar to those of primordial Earth when terrestrial life began<sup>1</sup>. A recent report<sup>2</sup> on possible relic biogenic activity in a Martian meteorite ALH84001 provides indirect evidence supporting this hypothesis. One of the lines of evidence is the formation of single domain magnetite and iron sulfides in ALH84001 carbonates, similar to those formed by terrestrial microorganisms<sup>3–7</sup>. However, interpretations of this evidence are inconclusive because the individual mineral phases can be explained by either inorganic or biological processes<sup>2</sup>. In addition, our interpretation of potential biogenic activity on Mars is based largely on what we know about life or the record of life on Earth. Recent studies in molecular phylogeny indicate that the last common ancestor of all living things was thermophilic<sup>8,9</sup>. Thus the study of iron biomineralization by thermophiles should enhance our understanding of how possible biogenic activities would result in the formation of iron-rich carbonates and magnetite in the Martian meteorite ALH84001.

We have obtained thermophilic iron-reducing bacteria from deep subsurface sediments deposited millions of years ago; these bacteria are phylogenetically unique, exhibit primitive features, and reduce amorphous Fe(III) oxyhydroxide at temperatures of 50–75°C under anaerobic conditions<sup>10</sup>. The discovery of

these thermophilic iron-reducing bacteria adds a significant dimension to the study of Martian biogenic activity using terrestrial analogs. Early studies revealed that some of the thermophiles are dissimilatory iron-reducers and form magnetite and iron-rich carbonates when growing on short-chain fatty acids or hydrogen as an energy source<sup>10,11</sup>. In this study, we examined the formation of magnetite and iron-rich carbonate formed by a fermentative thermophilic bacterial culture obtained from the deep subsurface sediment in Taylorsville Triassic Basin (TTB) in USA.

### 3. MATERIAL AND METHODS

#### 3.1. Bacterial Sources and growth conditions

The bacterial culture (TOR39) used in this study was obtained from core samples at 2.65- to 2.80-km depths in TTB in Virginia, USA. Modern temperature at the sampling depths is about 75°C. Geological evidence suggests that viable microorganisms detected in the deep subsurface in TTB may have survived in situ for millions of years<sup>12,13</sup>.

TOR39 is a Gram-negative rod-shaped bacterial culture that can ferment glucose and other carbohydrates but can not use short-chain fatty acids or hydrogen as an energy source<sup>14</sup>. It grows at temperatures from 50 to 70°C and at NaCl concentrations from 0.1 to 5% (wt/v).

A primary medium for growing TOR39 contains the following ingredients (g/l): NaCl, 10; MgCl<sub>2</sub> · 6H<sub>2</sub>O, 0.2; CaCl<sub>2</sub> · 2H<sub>2</sub>O, 0.1; NH<sub>4</sub>Cl, 1; 3-(*N*-morpholino)-propanesulfonic acid (MOPS), 1.0; NaHCO<sub>3</sub>, 2.5; glucose, 10; yeast extract, 0.5; peptone, 0.25; and trace mineral and vitamin solutions<sup>15</sup>. The medium was prepared anaerobically under a N<sub>2</sub> gas atmosphere and transferred into 15-mL pressure tubes. Additional sterile NaHCO<sub>3</sub> (50–260 mM, final concentration) and MgCl<sub>2</sub> · 6H<sub>2</sub>O (40–80 mM, final concentration) were added as required after autoclave of the medium. Sterile amorphous Fe(III) oxyhydroxide (final concentration 70 mM) was added as the starting iron for the formation of magnetite and iron-rich carbonate. The final pH in the medium was 7.8–8.5.

To begin an experiment, freshly grown TOR39 culture was transferred into the medium and incubated at 50–70°C for 20 days to three months. Abiotic controls accompanied each bacterial experiment. Precipitation of magnetic minerals during bacterial growth was monitored by using a magnet. The growth of bacteria was monitored by taking a subsample and observing it under a microscope. For a time-course experiment, two replicate tubes were used for each time point; the incubation was stopped at a given time point and tubes were stored in a refrigerator. Measurements of pH and Eh and the identification of mineral phases were performed after we completed the experiments.

#### 3.2. Eh and pH

The Eh values in the bacterial culture were determined at the room temperature in an anaerobic chamber. To perform the analysis, the tip of a platinum micro-electrode (Microelectrodes, Inc., Londonderry, N.H.) connected to an Orion-611 pH meter was placed in the solid phase in the culture tube; the Eh value (in millivolts) was recorded after equilibration for 5–10 min. Before and between sample measurements, the probe was checked for proper operation by using freshly made quinhydrone-saturated pH 4 (Eh 263 ± 20 mV) and pH 7 (Eh 86 ± 20 mV) buffers. Measurements of pH were performed at room temperature after Eh measurement.

Measured Eh and pH values for magnetite and iron-carbonate samples were plotted on a Eh-pH diagram for the iron-water-CO<sub>2</sub> system at 25°C; a previous study reported that stability diagrams of iron minerals at 50–70°C did not differ significantly from those at 25°C<sup>16</sup>.

#### 3.3. X-Ray Diffraction

Analysis of powder X-ray diffraction (XRD) of the iron minerals was accomplished with a Scintag automated diffractometer by using cobalt K $\alpha$  radiation. The XRD samples were prepared by spreading

acetone slurries on glass slides. The scan range for all samples was 10–85° (2θ) with a scanning rate of 2°/min. The mineralogical compositions of the sample were determined by comparing sample diffraction patterns to mineral standards provided by the International Center for Diffraction Data.

#### 4. RESULTS

The activity of TOR39 had a dramatic effect on solution chemistry during early incubation. Figure 1 shows a time-course analysis of pH and Eh changes in the bacterial culture at 65°C. At time zero, the pH was 7.73 and Eh was -46 mV. During the first five hours incubation, the pH increased slightly to 7.80; at the same time, however, the Eh value dropped to -377 mV (Fig. 1). As pH decreased to 6.93 after 2 days incubation, the Eh increased to -260 mV. Between 2 and 6 days of incubation, changes in pH and Eh were small. However, extended incubation up to 23 days showed further decreases in pH and also a decrease in Eh (Fig. 1). Active growth of TOR39 requires an appropriate starting pH; bacterial cell numbers increased little with time if the pH was less than 7.0 or greater than 9.0.

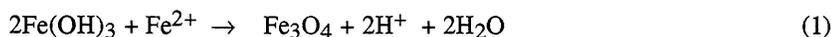
Changes in mineralogy reflected changes in solution chemistry mediated by the bacterial activity. Figure 2 shows the time course analysis of mineral phases by XRD for the same experiment as in Figure 1. The initial iron (t<sub>0</sub>) was reddish amorphous Fe(III) oxyhydroxide with a small portion of poorly crystallized akaganeite (Fig. 2A). A siderite peak (37°, 2θ) also appeared in the XRD pattern at time zero; this peak increased in intensity after 12 hours of incubation (Fig. 2B). After 12 hours incubation, the magnetite peak (41°, 2θ) appeared where akaganeite would otherwise be the dominant peak (Fig. 2B). After 24 hours incubation, a dark layer of magnetic material was formed at the interface between the liquid and the bulk phase of the amorphous iron. After 2 days of incubation, the bulk phase of solids became black and magnetic; the XRD pattern showed sharp magnetite peaks (i.e., 35° and 41°, 2θ) and the siderite peak became relatively small (Fig. 2C). Continued incubation increased the proportion of magnetite relative to siderite and diminished the peak of akaganeite (Fig. 2D).

In other bacterial experiments, increasing the concentration of NaHCO<sub>3</sub> (50–260 mM) resulted in higher final pH (up to 7.7) of the solution and the formation of greater abundance of siderite (data not shown). At the highest bicarbonate concentration (260 mM), the volume of the black precipitate was significantly less than that formed at lower bicarbonate concentrations (30–150 mM). On the other hand, additions of MgCl<sub>2</sub> (40–80 mM) resulted in the formation of sergeevite [Ca<sub>2</sub>Mg<sub>11</sub>(CO<sub>3</sub>)<sub>9</sub>(HCO<sub>3</sub>)<sub>4</sub>(OH)<sub>4</sub>], a magnesium-rich hydrated carbonate, but did not change the pH significantly (6.76–6.87).

In contrast to the magnetite formation in bacterial cultures containing TOR39, no magnetic material was detected in abiotic controls or when the pH was not suitable for bacterial growth (<7.0 or >9.0). The XRD patterns in abiotic controls were similar to those at the beginning of the experiment (Fig. 2A). These results suggest that the formation of magnetite in the bacterial culture was caused by the activity of TOR39 culture.

#### 5. DISCUSSION

Results of this study reveal that biogenic magnetite formation strongly depends on microbial activity, solution chemistry, and duration of the experiments. The exact mechanisms for the formation of magnetite by TOR39 are not known. It has been hypothesized that the high crystallinity of the biogenic magnetite crystals is the result of a favorable rate of Fe supply to the growing crystal at a suitable pH mediated by bacterial activity, Fe adsorption on cell structures, and the buffering capacity of the system<sup>17</sup>. Our experience appears to support this hypothesis. For example, the formation of magnetite in this study requires a high starting pH (7.8–8.5) and a strong pH buffer (>5 mM MOPS). This is because TOR39 produces a large amount of organic acids during glucose fermentation and lowers the pH substantially (Fig. 1). If the pH becomes too low (i.e., <6.0) magnetite will not form because high proton concentrations favor the reaction in the reverse direction:



Schwertmann and Fitzpatrick<sup>17</sup> suggested that the pH has to be around 7–8 or higher for magnetite to form through ferrihydrite (amorphous iron) reaction with Fe(II). In this study, the pH values at which initial magnetite formation was observed were around 7.2, even though the final pH could be as low as 6.1. However, magnetite, once formed, appears to be thermodynamically stable in a wide range of pH and Eh conditions (Fig. 3).

The activity of TOR39 culture appears to provide an adequate supply of Fe(II) by reducing Fe(III) from the amorphous iron and to create an appropriate Eh-pH condition for magnetite formation (Fig. 3). However, an sufficient amount of Fe(III) should also remain in solution to form the Fe(II) and Fe(III) mixture in magnetite. It is also possible that cells of TOR39 serve as nucleation sites for iron minerals to form. This biological function is well known for mineral precipitation by other bacteria in a variety of environments<sup>18,19</sup>.

The formation of iron-rich carbonate may be an equilibrium or kinetic process controlled by the concentrations of Fe(II) and CO<sub>3</sub><sup>=</sup> in solution. The identification of trace amounts of siderite at the beginning of incubation (Fig. 2A) and in the abiotic control indicates that siderite can form without bacterial mediation under the conditions examined. However, formation of significant amounts of siderite requires high concentrations of Fe(II) which can be obtained by bacterial reduction of Fe(III). This suggests that deposition of large quantities of siderite and magnetite in low temperature (<100°C) environments may be complemented by bacterial iron reduction.

In summary, the TOR39 bacterial culture can form magnetite and iron-rich carbonates at conditions similar to the geochemical environments of the deep subsurface from where the bacterial culture was obtained. This information may help us better understand the biogeochemical conditions for early life to begin on Earth and possibly on other planets such as Mars.

## 6. ACKNOWLEDGMENTS

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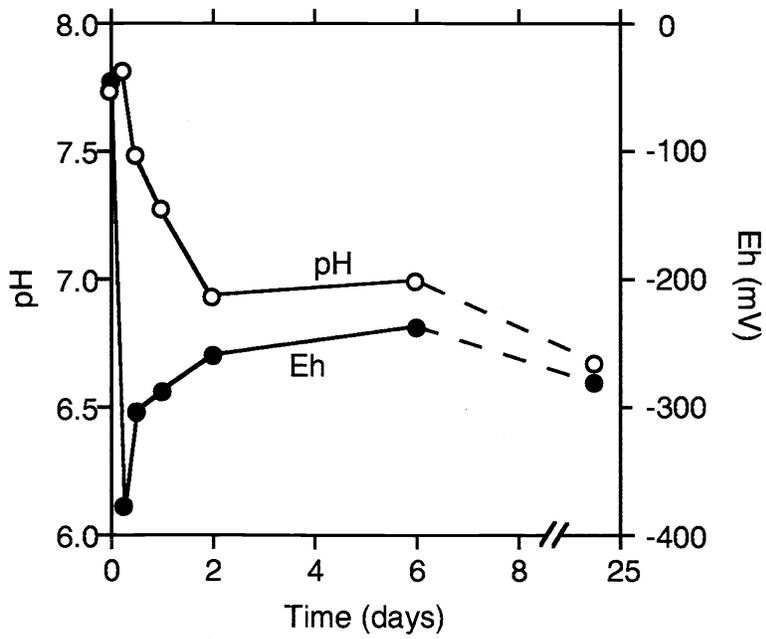


Fig. 1. Measurements of pH and redox potentials (Eh) as a function of time during magnetite formation by TOR39 culture.

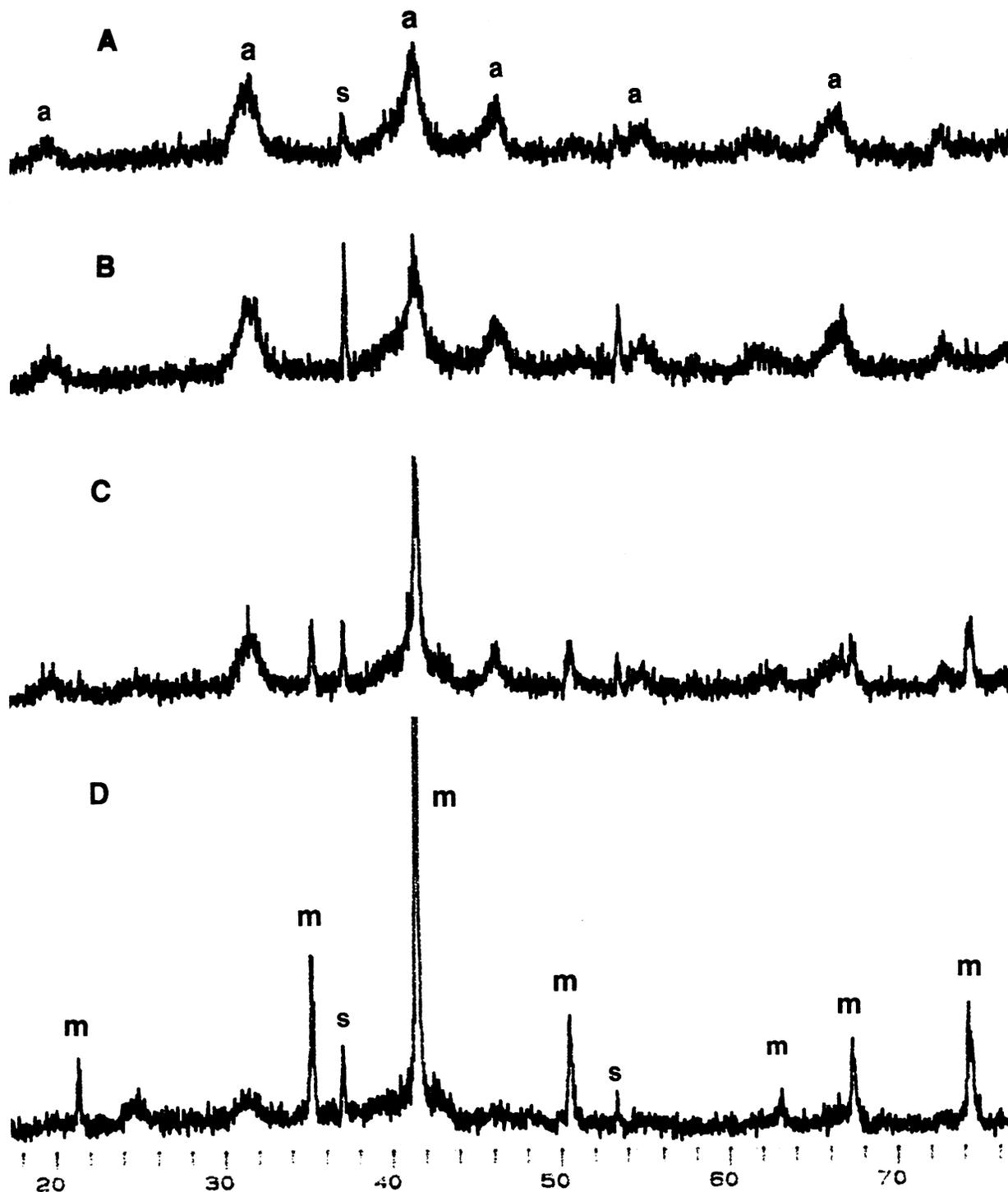


Fig. 2. Time-course analysis of X-ray diffraction patterns for iron minerals precipitated at 65°C in a bacterial experiment. (A) XRD at time zero. (B) XRD at 0.5 days. (C) XRD at 2 days. (D) XRD at 23 days. In the XRD profile, a = akaganeite, m = magnetite, and s = siderite.

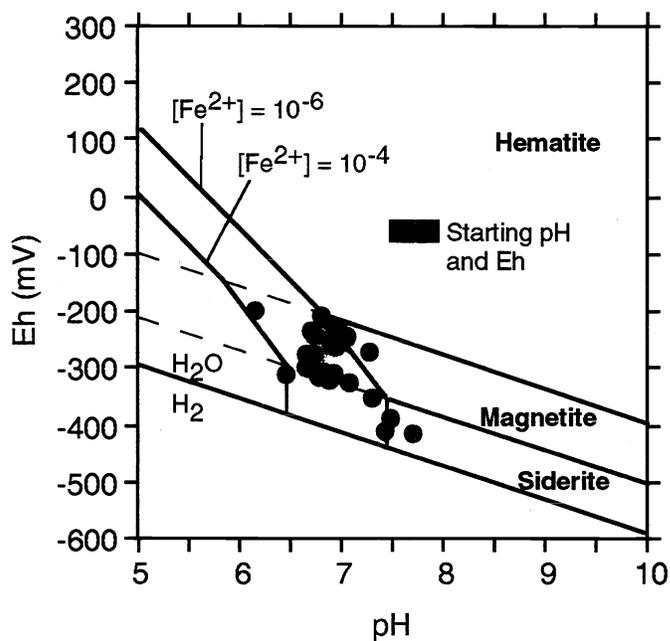


Fig. 3. Eh-pH stability fields for hematite, magnetite, and siderite in the water-iron-CO<sub>2</sub> system at 25°C and 1-atm total pressure. Measured Eh and pH values were also plotted for magnetite and siderite samples formed by TOR39.