

Microbial formation of lanthanide-substituted magnetites by *Thermoanaerobacter* sp. TOR-39

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Received: 23 May 2007 / Accepted: 18 June 2007 / Published online: 3 August 2007
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Abstract The potentially toxic effects of soluble lanthanide (L) ions, although microbially induced mineralization can facilitate the formation of tractable materials, has been one factor preventing the more widespread use of L-ions in biotechnology. Here, we propose a new mixed-L precursor method as compared to the traditional direct addition technique. L (Nd, Gd, Tb, Ho and Er)-substituted magnetites, $L_yFe_{3-y}O_4$ were microbially produced using L-mixed precursors, $L_xFe_{1-x}OOH$, where $x = 0.01–0.2$. By combining lanthanides into the akaganeite precursor phase, we were able to mitigate some of the toxicity, enabling the microbial formation of L-substituted magnetites using a metal reducing bacterium, *Thermoanaerobacter* sp. TOR-39. The employment of L-mixed precursors enabled the

microbial formation of L-substituted magnetite, nominal composition up to $L_{0.06}Fe_{2.94}O_4$, with at least tenfold higher L-concentration than could be obtained when the lanthanides were added as soluble salts. This mixed-precursor method can be used to extend the application of microbially produced L-substituted magnetite, while also mitigating their toxicity.

Keywords *Thermoanaerobacter* sp. TOR-39 · Mixed-precursor method · Direct addition method · Lanthanide substituted magnetite · Toxicity

Introduction

The lanthanides (Ls) with atomic numbers 57 through 71, i.e., from lanthanum (La) to lutetium (Lu), are of great technological interest because they show particular magnetic, catalytic and optic properties. In nature, lanthanides, also known as rare earth elements, do not exist as pure elements, but rather in sparsely distributed mineral groups such as cerite $[(Ce,Ca)_9(Mg,Fe)Si_7(O,OH,F)_{28}]$, monazite $[(Ce,La,Nd,Th)(PO_4,SiO_4)]$ and euxenite $[(Y,Ca,Ce,U,Th)(Nb,Ta,Ti)_2O_6]$ (Palasz and Czekaj 2000). The antibiotic properties of lanthanides were well known by the end of the nineteenth century (Bokorny 1894; Drossbach 1897). The heavier Ls were found to exhibit bactericidal and fungicidal activities (Karthikeyan et al. 2004) with bacteria being more sensitive than fungi (Evans 1996).

Metal-substituted magnetites (ferrites) are important engineering materials used to manufacture magnetic storage, dry printing, and magnetic devices, all of which need high magnetism and a high Curie temperature (T_c), the temperature above which ferromagnetic material loses its

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characteristic magnetic ability. The type and quantity of substitutions in ferrites are important because they allow for a relative tunability of the magnetic properties. In contrast, the addition of Ls to magnetite has an important role of decreasing T_c (Rezlescu et al. 1996), even though there were some reported exceptions, where increased magnetism (Bhosale et al. 1993) or increased T_c was observed (Kolekar et al. 2002). The L-induced changes in magnetites influence the pyromagnetic coefficient of magnetites ($\partial M/\partial T$) facilitating directed tailoring of nanoscale materials. Enhanced pyromagnetic characteristics may be exploited by demands of new materials for ferrofluids (Cabuil et al. 1996; Love et al. 2005), magnetocaloric pumps (Love et al. 2004) or magnetic particles for Lab-on-a-Chip devices (Holligan et al. 2003).

Traditional synthesis techniques such as chemical coprecipitation and other standard ceramic methods may have certain technical problems, such as producing L_2O_3 or hydrous forms as unexpected secondary precipitates at low substitution fractions such as $Mn_{0.8}Zn_{0.2}Fe_{1.9}Er_{0.1}O_4$ (Yeary 2005). Another undesirable outcome from chemical synthesis is the high-energy consumption during sintering (Rezlescu et al. 1996) or thermal decomposition of organometallic precursors (Hyeon 2003). In contrast to the chemical procedure for manufacturing magnetite particles, microbial formation of magnetites is characterized by their selectivity and precision in magnetite formation (Zhang et al. 1998) and does not consume high energy. Microbes can facilitate magnetite formation even at temperatures as low as 4°C (Roh et al. 2003, 2006).

Metal-reducing bacteria (MRB) have demonstrated an ability to produce magnetite and siderite as a result of Fe(III) reduction (Slobodkin et al. 1995; Slobodkin and Wiegel 1997; Zhang et al. 1997) and they also reduce a number of different metal ions such as Cr, Pb, Hg, U, Se and Tc (Lovley 1995), Co and Cr (Zhang et al. 1996), Cr (Wielinga et al. 2001), and Co, Cr, Ni, Mn and U (Roh et al. 2001, 2002). However, there has been little information on microbial approaches to produce L-substituted magnetite. Many dopants, when added as soluble species to media and when their concentration levels exceed a specific threshold, preclude the nucleation and crystal growth of magnetite (Roh et al. 2001, 2002). Previous work demonstrated that the concentrations of many soluble dopants must be limited to avoid toxicity (Zhang et al. 1996; Roh et al. 2001). Recently, Moon et al. (2007a) showed that using mixed precursors could partially mitigate the toxicity of metals to microbes (presumably by reducing its bioavailability) and could enhance the substitution of dopants into the magnetite structure.

The rates of bacterial dissolution of Mn-, Co-, Al- and Cr-substituted goethite were slower relative to an unsubstituted goethite (Bousserhine et al. 1999). Cr(VI)

consistently showed the slowest bioreduction rate among studied metal ions, although thermodynamically, Cr(VI) reduction should be more favorable (Liu et al. 2002). In these cases, dissolved toxic metals in the media likely suppressed the bacterial growth.

In most studies examining the substitution of metals into magnetites, the Fe(III)-reducing bacteria were concurrently inoculated with electron donor and amorphous Fe(III) oxyhydroxides along with a solution of toxic metal salts (Cooper et al. 2000; Fredrickson et al. 2001; Roh et al. 2001). In the present work, we investigated the feasibility of microbial formation of L-substituted magnetites by adapting the mixed precursor method (Moon et al. 2007a) to reduce lanthanide bioavailability. The objective of this study was to examine the microbial formation of L-substituted magnetites using mixed-L precursors and compare those results to the traditional (direct addition) method using amorphous iron oxyhydroxide while adding soluble L-salts (Table 1).

Materials and methods

Preparation of mixed-L Fe(III) precursors compared to the direct addition method

Pure and mixed-L precursors ($L_xFe_{1-x}OOH$, where L is Nd, neodymium; Gd, gadolinium; Tb, terbium; Ho, holmium; Er, erbium) used for this study were prepared by the neutralization method following that of McLaughlin et al. (1981). It was recognized that intermittently adding small amounts of soluble L salts might hold the soluble L concentration below toxic levels or allow the microbes to adapt. Unfortunately, numerous additions of low concentration L-salts would make it difficult to keep a constant concentration level in the medium, would complicate experimental reproducibility and would likely lead to heterogeneous distribution of substitution elements in the magnetite crystal structure. In this study various forms of mixed-L precursors were prepared by using syringes to add a 10 M NaOH solution dropwise and slowly into a mixture of a total of 0.4 M of $FeCl_3 \cdot 6H_2O$ and lanthanide chloride (hydrate) such as $NdCl_3 \cdot 6H_2O$, $GdCl_3 \cdot 6H_2O$, $TbCl_3 \cdot 6H_2O$, $HoCl_3 \cdot 6H_2O$ and $ErCl_3 \cdot 6H_2O$ according to the target composition (0.01, 0.02, 0.05, 0.1 and 0.2 cation mole fraction, CMF). While the mixture was rapidly stirred throughout the process, a pH of 7 was achieved, and then the solution was allowed to continue oxidizing overnight (Moon et al. 2007a). The precipitates were washed five times with deionized water and then deionized water was added to make the final mixed-L amorphous Fe(III) hydroxide suspension (~ 0.4 M). The final precursors were stored in sealed glass containers under N_2 gas. Due to

Table 1 Evidences of microbial formation of L-substituted magnetites

Properties	Lanthanides				
	Nd	Gd	Tb	Ho	Er
Magnetic attraction to hand magnet	+	+	+	+	+
XRD analysis	M, A + S ^a	M, A + S	M, A + S	M, A + S	M, A + S
Maximum Fe ²⁺ in the medium compared to control (0.47 mM)	ND	0.03	0.03	0.05	0.08 (0.01)
Remaining L ion in the medium compared to 720 μ M input	ND	58.0	84.7	156	39.9
ΔM_S from control (76.6 emu/g)	−38.2	−22.9	−18.8	−28.8	−28.1
ΔT_C with control (slope between 280–390 K, $-0.0709x + 102.94$)	ND	$-0.0412x + 49.842$	ND	$-0.0433x + 53.176$	ND

Results shown are for 0.02 cation mole fraction (CMF), unless a different CMF is identified in parenthesis

A akaganeite; M magnetite; S siderite, ND not determined, + positive response

^a A + S at CMF = 0.05

potential phase transformations that could occur during heating and pressurization, the Fe(III) oxyhydroxides were not autoclaved.

Incubation conditions

The basal medium had the same composition and was prepared according to Moon et al. (2007a). All media contained mineral salts, vitamins and HEPES/MOPS buffers at pH 8.0–8.2. No reducing agents were added and all pressure tubes and serum vials were under a purified N₂ gas phase. Initially, a pre-activated TOR-39 culture (*Thermoanaerobacter* sp. TOR-39, Liu et al. 1997) was prepared by fermenting 10 mM glucose without an electron acceptor at 65°C for 24 h using a 2% (volume) transfer from a stored culture. The resultant cell density was approximately $1.13 \pm 0.58 \times 10^4$ cells/ml. Then, experiments were incubated in duplicate using 10 mM glucose, a 2% inoculum of pre-activated TOR-39 with about 40 mM of mixed-L precursor per 50 ml of medium in serum bottles or per 10 ml of medium in pressure tubes. The TOR-39 tubes were then incubated at 65°C for 30 days. Each control received an equal volume of pure precursor (no lanthanides). To compare with the direct addition method, cultures were also made with pure precursor inoculated with the same target mole fractions of soluble L-salts from either 0.5 or 1 M stock solutions.

Chemical, microbiological and mineralogical study of L-substituted magnetites

To examine the chemical conditions of metal reduction, mineral formation and microbial activity by TOR-39,

subsamples of bacterial cultures and controls were taken from the culture bottles after the termination of incubation. Basic properties of final medium solution such as pH, Eh, acid extractable (0.5 N HCl) ferrous ion and dissolved L concentration were obtained as described previously (Moon et al. 2007a) to monitor lanthanide incorporation into the precipitated phase. Microbial growth was measured using BCATM protein assays (Pierce, Rockford, IL, USA) using bicichoninic acid (BCA) for the colorimetric detection (UV-spectrometer, Hewlett Packard 8453) and quantification of total protein standardized to albumin. Cell numbers were counted by utilizing microscopy and the bac-light live/dead stain.

Solid precipitates were washed with deionized water, stored as aqueous suspensions under N₂ gas. Subsamples for mineralogical analyses were collected by freeze drying and stored under N₂ gas. The slurry of the freeze-dried subsamples was mixed with methanol and dispensed on a silicon zero background plate for acquiring the mineralogical composition of the precipitated phases using an X-ray diffractometer at room temperature (XRD, PAD V, Scintag, Inc., Sunnyvale, CA, USA) equipped with Cu-K α radiation at 45 kV/40 mA, a scan rate of 3° 2 θ /min in continuous step mode in the 10°–70° 2 θ range. Refined lattice parameters were obtained from Rietveld refinement using the EXPGUI graphical user interface (Toby 2001) for the general structure analysis system (GSAS, Larson and von Dreele 2000). The estimated standard deviations are reported as 3 sigma.

A scanning electron microscope (SEM, JSM-35CF, JEOL Ltd., Tokyo, Japan) with energy dispersive X-ray analysis (EDX) was used for the analysis of morphology, mineralogy and chemistry of the precipitated phases by TOR-39. Magnetic measurements were obtained using a super conducting quantum interference device (SQUID,

MPMS-7, Quantum Design, Inc., San Diego, CA, USA). The magnetic properties of the samples were evaluated over the temperature range of 280–390 K in a 10 Tesla field.

Results

Among the $L_xFe_{1-x}OOH$ ($x = 0.01, 0.02, 0.05, 0.1$ and 0.2) samples, TOR-39 could not reduce the highly mixed precursors ($x = 0.05$ – 0.2 samples) during repeated preliminary experiments in pressure tubes. Therefore the CMF of L was limited to no more than 0.05. Inoculated samples made from precursors at 0.01 or 0.02 CMF generally exhibited color change to black and a magnetic response to a hand-held magnet for Er-substituted samples. The other lanthanides examined (Nd, Gd, Tb and Ho) exhibited identical trends indicating that the selected Ls likely had similar toxicity to TOR-39. Employing the direct addition method, the additions of soluble L salts (0.2–2 mM) to the pure magnetite precursor appeared to be more toxic than the corresponding L concentrations of 0.36–1.8 mM when using the mixed precursors. Bacterial growth and magnetic production likely occurred with the new method because the stress imposed by L-ions may have been reduced when they were incorporated into sparingly soluble mixed-phase L-magnetite precursors.

Microbial growth measurements indicated a reduced toxicity when using the new method (Fig. 1). For example, Gd salt at 20 μ M yielded similar growth compared to Gd at 720 μ M (0.02 CMF) using the new precursor method and both produced blackish magnetite. However, tubes containing Gd salts at 100 and 360 μ M remained dark brown even though they showed a weak magnetic response. The new method confirmed the potential to raise the limit of magnetite production more than one order magnitude (720 μ M at 0.02 CMF vs. 20 μ M) above the direct addition method.

Only tubes exhibiting microbial activity contained significantly reduced iron, observed as either L-substituted magnetites or siderite. Akaganeite, assuming a stability field similar to lepidocrocite, was the predominant oxidized iron species at the initiation of all experiments as well as the predominant iron species in killed controls or tubes not exhibiting growth. Figure 2 shows the pH and Eh stability field for the initial medium (pH and Eh were 8.1 and -195 mV, respectively) and end products after 30 days of incubation at 65°C. In all instances the final Eh was more reduced after 30 days of incubation at 65°C.

Interestingly, tubes containing 0.01 and 0.02 CMF L-mixed precursors produced magnetite and decreased the redox potential by about 100 mV, while those prepared at

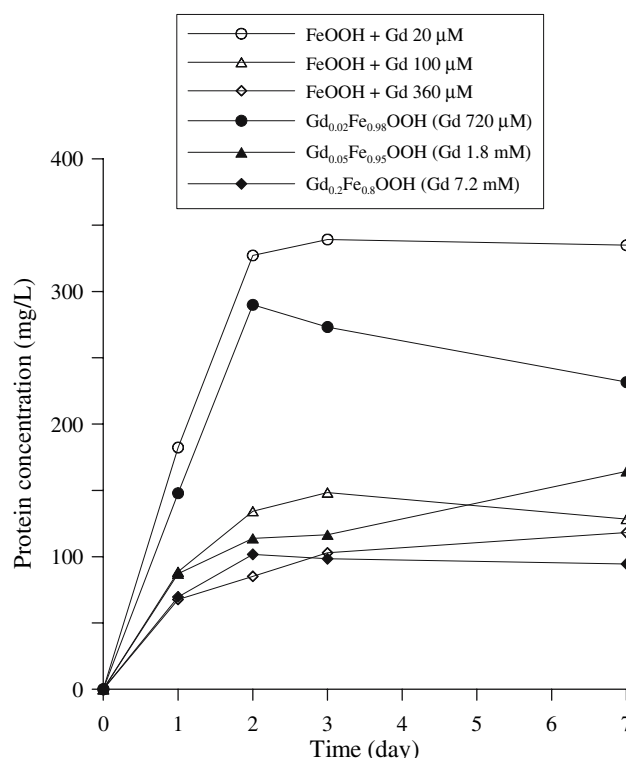


Fig. 1 Growth of strain TOR-39 represented by total protein using the BCATM protein assay. Each value was from triplicates and standardized to an albumin standard. Solid symbols represent the mixed precursor method

0.05 CMF L-mixed precursor decreased the redox potential >150 mV to approximately -350 mV. In agreement with the pH/Eh stability field (Fig. 2), tubes containing the 0.05 CMF L-mixed precursor produced siderite. In contrast, samples prepared by the direct addition of L-salts decreased the redox potential by about 200 mV, but did not produce siderite or magnetite.

Samples inoculated with low mixing amounts at 0.01 and 0.02 CMF generally showed a lower pH (around 7.1) than control samples of pure (non-substituted) magnetite (7.2–7.6). The pH of the medium with the highest amount of 2 mM L-ions and pure precursor fell to about 7.1 without microbial activity. The decrease in pH from 8.1 to 7.1 might have resulted from the inoculation of relatively large amounts of L-salt stock solutions of pH 3.2–4.2. The starting pH of control samples without L-salts was 7.81. The lower pH of the final medium inoculated with mixed-L precursors at 0.01 and 0.02 CMF was attributed to the organic acids and CO₂ produced from the microbial activity (Zhang et al. 1996, 1997).

In our study the microbially synthesized L-substituted magnetite did not require the addition of exogenous electron shuttles such as humic acid or AQDS as reported from experiments using mixed oxides and *S. putrefaciens* (Fredrickson et al. 2001). Eh values of the experiments

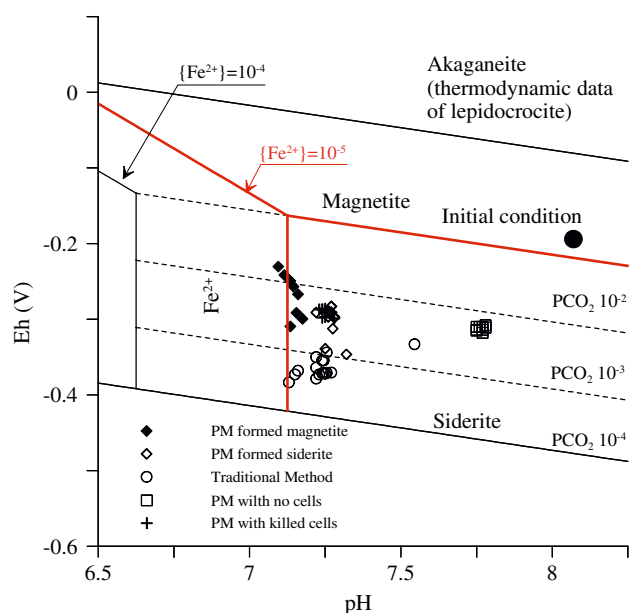


Fig. 2 The pH-Eh stability field diagram for lepidocrocite, magnetite and siderite in the H_2O -Fe- CO_2 systems at 25°C and 1 atm total pressure. The CO_2 partial pressure ranged from 10^{-4} to 10^{-2} atm whereas the activity of Fe^{2+} was allowed to vary between 10^{-4} and 10^{-2} M. Measured pH and Eh values were plotted simultaneously. PM precursor method

using the direct addition method ranged between -400 and -330 mV, which were lower than those obtained using the mixed-L precursors, which ranged between -350 and -230 mV (Fig. 2). This indicated that microbial activity could allow the Eh to be reduced but did not facilitate either magnetite or siderite production.

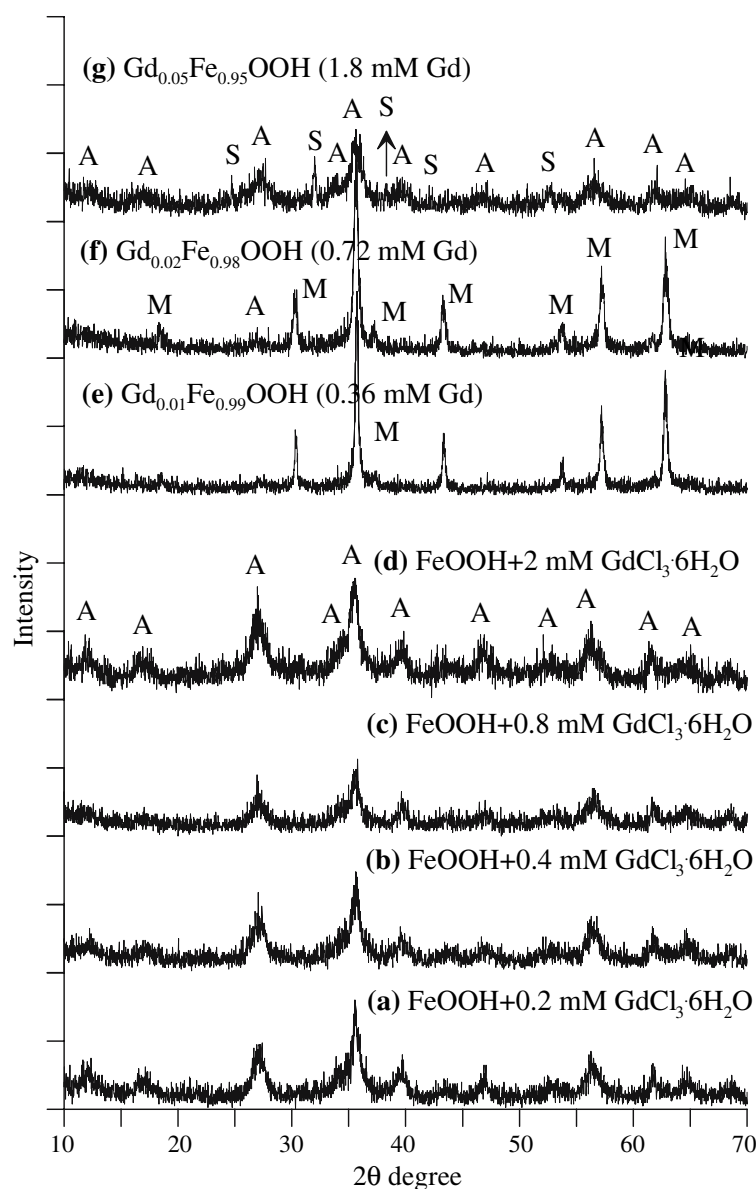
Irrespective of mixed-L ion types, XRD patterns showed that mixed precursors were mostly composed of akaganeite ($\text{L}_x\text{Fe}_{1-x}\text{OOH}$) (Fig. 3). Samples inoculated with mixed precursors, $\text{L}_{0.01}\text{Fe}_{0.99}\text{OOH}$ and $\text{L}_{0.02}\text{Fe}_{0.98}\text{OOH}$ precursors produced L-substituted magnetite, while $\text{L}_{0.05}\text{Fe}_{0.95}\text{OOH}$ inoculated samples produced siderite, as was supported by the low recorded Eh (Fig. 2). Siderite was a predicted product according to the stability field and a logical microbial product from iron reduction in the presence of pCO_2 . Importantly, iron reduction to magnetite or siderite only occurred in the presence of microbial activity with the new precursor method and magnetite was only formed in low L-CMF solutions. Small amounts of lanthanide chloride hydroxides were also present as a minor product impurity with Tb, Ho and Eb (data not shown). The direct addition of L-ions over 0.2 mM or indirect supply using 1.8 mM of $\text{L}_{0.05}\text{Fe}_{0.95}\text{OOH}$ precursor suppressed microbial activity, preventing the nucleation and growth of magnetite. Above 0.1 CMF of lanthanides, even mixed precursors were found to prevent bacterial growth with Nd and Gd (data not shown).

Successful L-incorporation into magnetite was indicated by a change of magnetic susceptibility and saturation magnetization (Moon et al. 2007b) as well as the change of lattice constant values such as 8.367(3) Å for $\text{Gd}_{0.06}\text{Fe}_{2.94}\text{O}_4$, 8.363(6) Å for $\text{Nd}_{0.06}\text{Fe}_{2.94}\text{O}_4$, 8.366(6) Å for $\text{Tb}_{0.06}\text{Fe}_{2.94}\text{O}_4$, 8.370(3) Å for $\text{Er}_{0.06}\text{Fe}_{2.94}\text{O}_4$, and 8.371(3) Å for $\text{Ho}_{0.06}\text{Fe}_{2.94}\text{O}_4$ compared to control samples (8.380 Å) and a magnetite chemical standard (8.396 Å). Unfortunately, because of the detection limits of SEM/EDX (about 0.1%), it was impossible to unequivocally verify that L-ions were substituted into the structure of the samples with the compositions of $\text{L}_{0.03}\text{Fe}_{2.97}\text{O}_4$ and $\text{L}_{0.06}\text{Fe}_{2.94}\text{O}_4$ at 0.01 and 0.02 CMF, where nominal compositions of $\text{L}_y\text{Fe}_{3-y}\text{O}_4$ were used based on the precursor composition.

After 30 days of incubation, the $\text{Tb}_{0.01}\text{Fe}_{0.99}\text{OOH}$ and $\text{Tb}_{0.02}\text{Fe}_{0.98}\text{OOH}$ inoculated samples showed similar ferrous ion concentrations to that of the control sample using pure precursor (Fig. 4a). In contrast, siderite formed and unreacted direct addition samples had 2–4 times higher concentrations of ferrous ion than controls. Most of the added Tb was removed from solution during the incubation. The Tb concentration in the final medium (Fig. 4b), was 740 μM when 2 mM of Tb salt was added with pure precursor, but no growth or magnetite was observed. When the mixed precursor, $\text{Tb}_x\text{Fe}_{1-x}\text{OOH}$ precursors was used, the final medium contained 22–138 μM Tb with approximately 95% of Tb substituted into magnetite or siderite. Considering that the pure precursor with soluble dissolved L-ions did not form L-substituted magnetite and other L-bearing mineral phases were not seen in XRD analysis, L-loss might be attributed to adsorption onto the pure akaganeite as seen in previous research (Ainsworth et al. 1994; Ford et al. 1997; Martínez and McBride 1998a, b). Terbium removal (92–94%) was observed using the mixed precursor method versus 63–84% in the direct addition method. This implied that some portion of lanthanide was incorporated into the L-substituted magnetite at 0.01 and 0.02 CMF and siderite at 0.05 CMF.

Only products formed from mixed precursors with 0.01–0.02 CMF indicated a magnetic response to a hand-held magnet and exhibited a black color change. While only a small amount of L-ions were successfully incorporated, it is significant to note that dramatic changes in magnetization were observed (Fig. 5). Gd- and Ho-substituted magnetite at 0.01 and 0.02 CMF showed decreased magnetization by around 34–42% and 52–55% from 75.4 emu/g of pure, non-substituted magnetite at 390 K. This trend of decreasing magnetization is expected to relate to a decreased T_c for the substituted samples. Less than 3% substitution of L-ions clearly altered saturation magnetization (M_s) and T_c , among magnetic properties.

Fig. 3 X-ray diffraction patterns of the products from different combinations of precursors. *a, b, c* and *d* 36 mM of pure non-substituted precursors with 0.2, 0.4, 0.8 and 2 mM of $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ stock solution; *e, f* and *g* 36 mM of Gd-substituted precursors (0.36, 0.72 and 1.8 mM of Gd, respectively). *A* akaganeite ($\text{Gd}_x\text{Fe}_{1-x}\text{OOH}$); *M* magnetite ($\text{Gd}_y\text{Fe}_{3-y}\text{O}_4$); *S* siderite (FeCO_3)



Discussion

Bioavailability of lanthanides for TOR-39

The suppression of microbial activities was likely attributed to inhibition of bacterial growth at elevated L concentrations such as was seen in an alveolar macrophage culture where concentrations as low as 0.05 mM of Gd were found to be toxic (Lizon and Fritsch 1999). In this study, selected lanthanides (Nd, Gd, Tb, Ho and Er) prohibited microbial transformation from akaganeite to magnetite at concentrations less than 0.1 mM of L-ions by direct addition versus more than 0.72 mM of L-ions using the mixed precursor approach. The direct addition resulted in 0.02–3.3 mM L-ion concentrations in the final media

while the mixed precursor approach resulted in 0.01–0.47 mM in the final media. This implies that the threshold concentration of the direct addition method was close to the 0.05 mM toxic concentration reported by Lizon and Fritsch (1999). In contrast, TOR-39 produced L-substituted magnetite with at least one order of magnitude higher L concentrations using the mixed precursor method, even though there was some variation from one L-ion to another. The actual level of incorporation may be governed by the rate of release of L-ions from the precursor solution, with slow release favoring higher uptake (Cornell and Giovanoli et al. 1989). Therefore, keeping L concentrations in the media low through a slow and constant release of L-ions from precursor was likely the key for microbial formation of L-substituted magnetite.

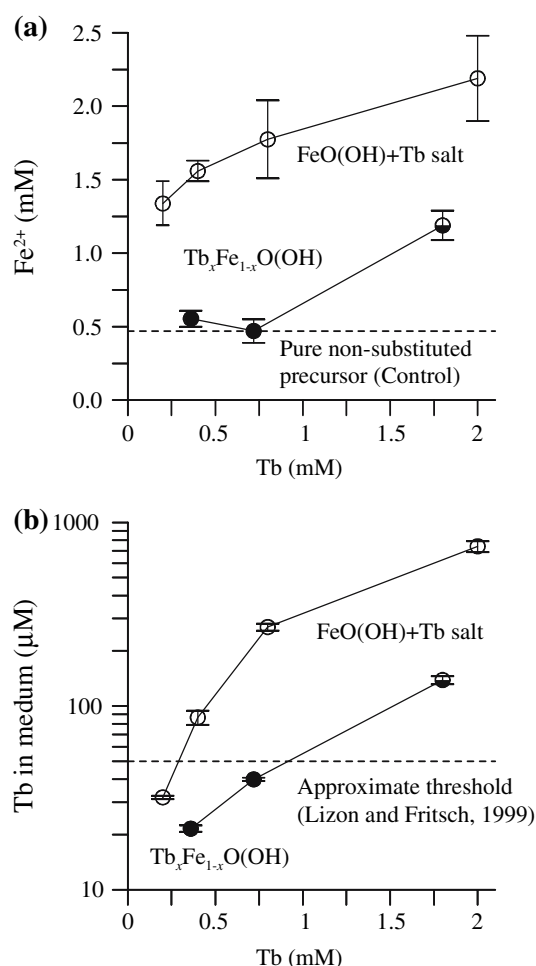


Fig. 4 Acid-extractable (0.5 N HCl) ferrous ion (a) and Tb (b) concentration in the final medium from different combination of precursors, 36 mM of FeO(OH) , pure non-substituted precursor (control) and 0.2, 0.4, 0.8 and 2 mM of $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$ stock solution versus 36 mM of substituted precursors (0.36, 0.72 and 1.8 mM Tb). Symbols: *solid* magnetite; *half-filled* siderite + non-reacted akaganeite; *open* non-reacted akaganeite

Substitution of L-ions in precursors and magnetite

Lanthanide in the final media had about one half to one order lower concentration using the mixed precursor method compared to that of direct addition method which contained similar initial inoculation concentration (Fig. 4) indicating that L-ions released from the mixed precursor adsorbed to newly formed mineral nuclei, and then were incorporated into the crystal structure during microbial mineral production.

The ionic radii of L-ions range from 0.848 Å (Lu) to 1.034 Å (Ce), relatively higher values than other elements with the same oxidation number. In addition, selected L-ions have larger ionic radii [Fe^{2+} (0.74) or Fe^{3+} (0.64) versus Nd^{3+} (1.04), Gd^{3+} (0.97), Tb^{3+} (0.93), Ho^{3+} (0.91) and Er^{3+} (0.89) Å] (Shannon 1976). Even in the formation

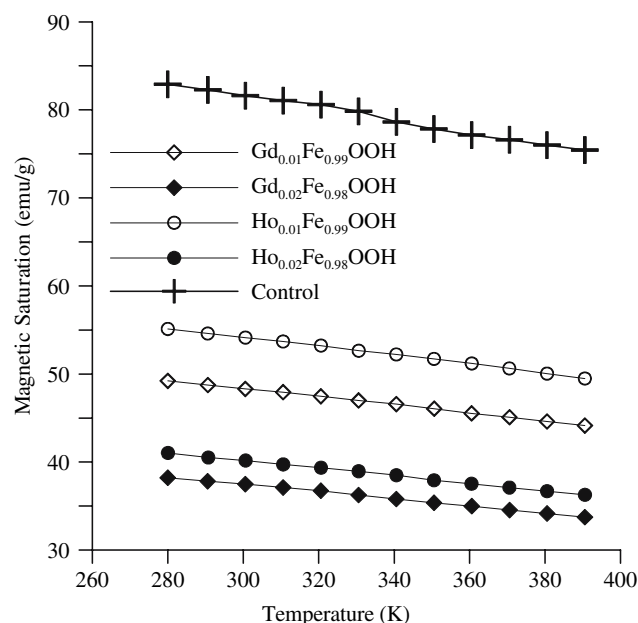


Fig. 5 Saturation magnetization (M_s) versus temperature (K) for microbially formed Gd- and Ho-substituted magnetites compared to pure magnetite as control

of secondary phases in the ferrite during the sintering process, they are influenced by both L-ion type and the amount of L_2O_3 used.

The influence of the L-ion type can be explained as an effect of L-ion size. The ionic radii of Nd and Gd are similar to that of Ca (0.104 nm), an element that plays an essential role in many metabolic processes (Palasz and Czekał 2000). The lack of L-ions in the final medium containing 1.8 mM Tb with the mixed precursor method (Fig. 4b) indicates that microbially precipitated siderite (FeCO_3 similar to calcite structure CaCO_3) likely co-precipitated Nd and Gd from the Nd(or Gd) $_{0.05}\text{Fe}_{0.95}\text{OOH}$ precursor.

The akaganeite and spinel structures impose certain limitations such as the combination of di- and tri-valent elements among precursor and magnetite. Precursors can have some portion of divalent elements in their structure. For example, goethite ($\alpha\text{-FeOOH}$) formation was completely suppressed at Co^{2+} levels >23 mol%, because goethite needs trivalent ions (Cornell and Giovanoli 1989). Ideal magnetite is composed of about 1/3 divalent and 2/3 trivalent cations in its structure and a stoichiometric $\text{Fe}^{2+}:\text{Fe}^{3+}$ ratio (0.5) is not obtained for magnetite or substituted magnetite unless oxidation/reduction conditions are rigorously controlled (Ardizzone et al. 1983). Furthermore, the degree of congruent dissolution and incorporation (Moon et al. 2007b) and the degree of redistribution can affect the final product composition. About 60% of the Co, Ni, Zn and Cu present in the crystal structure of maghemite are ejected from the crystals during the

transformation of maghemite to hematite, whereas the Mn, Cr and Al present in maghemite are redistributed in hematite particles (Sidhu 1988).

Moon et al. (2007a, 2007b) revealed that the mixed precursor method exhibited several advantages especially in the case of toxic metals such as Cr and Ni in increasing the production rate, magnetic properties and crystallinity of the product. This study is in a good agreement with the mixed precursor method mitigating toxicity of lanthanides against microorganisms. The L-ion concentrations that previously precluded magnetite production with the direct addition method now allow microbial formation of L-substituted magnetite using the mixed precursor method.

L-Substitution effect on magnetic properties

Although, some research on L-doped ferrites shows that L-ion substitution increases magnetization (Bhosale et al. 1993), our L-substitution results are in agreement with Kolekar et al. (2002), who showed that the substitution of Gd dramatically decreased the magnetization in a Cd–Cu ferrite. They presented results using $\text{Cd}_x\text{Cu}_{1-x}\text{Fe}_2-y\text{R}_y\text{O}_4$ material (where $x = 0, 0.2, 0.4, 0.6, 0.8$ and 1.0 and $y = 0, 0.1$ and 0.3) that was prepared by the solid state synthesis with a maximum temperature of $1,000^\circ\text{C}$. Upon Gd substitution, M_s decreased, which could be due to the weakening of magnetic interactions inside the crystal lattice, largely in the coupling of the spins of the 3D electrons. It is thought that the Fe–Fe interactions dominate and overshadow the Gd–Fe interactions, thus leading to the changes observed in the M_s and T_c of these samples. Yeary (2005) showed that adding more than 0.033 CMF of L-ions in the magnetite unit cell results in the formation of extra L_2O_3 oxide phases when using a chemical coprecipitation method. Therefore, biomagnetite synthesis using the precursor method which allows up to 0.02 CMF of L may result in better control of the magnetic properties of magnetite than chemical synthesis without producing unexpected secondary phases.

This mixed precursor method provides a means to mitigate the toxic properties of L-ions by incorporating the L species into the colloidal metal oxyhydroxide phase. In addition to reducing the potential toxicity, this has other benefits such as enhancing the uniform substitution of the different metal species in the final product by providing an intimately mixed starting material. Furthermore, it may allow the incorporation of metals, organic/inorganic ions and chelates into the final products whose toxicity is high or whose aqueous solubility in the culture media is limited.

Acknowledgments This research was supported by the Defense Advanced Research Projects Agency (DARPA) Biomagnetics

Program under Contract 1868-HH43-X1 and the US Department of Energy's (DOE) Office of Fossil Energy with student support provided by the DOE Environmental Molecular Science Initiative. ORNL is managed by UT-Battelle, LLC, for the U.S. DOE under Contract DE-AC05-00OR22725. We thank Dr. Scott Brooks for help with thermodynamic software, Ms. Meghan McNeilly for editing, Ms. Shannon Ulrich for protein assays, Ms. Lisa Fagan for cell counting and Mr. Kenneth A. Lowe for ICP-MS analysis. J.-W. Moon is supported by the Post-doctoral Fellowship Program of Korea Science and Engineering Foundation and in part by an appointment to the ORNL Postdoctoral Research Associates Program administered jointly by the Oak Ridge Institute for Science and Education and ORNL.

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