Journal of
Nanoscience and Nanotechnology
Vol. 6, 3517–3520, 2006

Extracellular Synthesis of Magnetite and Metal-Substituted Magnetite Nanoparticles

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We have developed a novel microbial process that exploits the ability of Fe(III)-reducing microorganisms to produce copious amounts of extracellular magentites and metal-substituted magnetite nanoparticles. The Fe(III)-reducing bacteria (*Theroanaerobacter ethanolicus* and *Shewanella* sp.) have the ability to reduce Fe(III) and various metals in aqueous media and form various sized magnetite and metal-substituted magnetite nano-crystals. The Fe(III)-reducing bacteria formed metal-substituted magnetites using iron oxide plus metals (e.g., Co, Cr, Mn, Ni) under conditions of relatively low temperature (<70 °C), ambient pressure, and pH values near neutral to slightly basic (pH = 6.5 to 9). Precise biological control over activation and regulation of the biosolid-state processes can produce magnetite particles of well-defined size (typically tens of nanometers) and crystallographic morphology, containing selected dopant metals into the magnetite ($Fe_{3-y}X_yO_4$) structure (where X = Co, Cr, Mn, Ni). Magnetite yields of up to 20 g/L per day have been observed in 20-L vessels. Water-based ferrofluids were formed with the nanometer sized, magnetite, and metal-substituted biomagnetite particles.

Keywords: Nanoparticle, Magnetite, Metal-Substitution, Microorganism, Ferrite.

1. INTRODUCTION

Magnetite, $Fe^{3+}(Fe^{2+}, Fe^{3+})O_4$, is an "inverse" spinel and the unique electronic and magnetic properties of magnetite are directly associated with the extremely rapid exchange of electrons among the octahedrally-coordinated iron ions. Other divalent and trivalent metal ions readily substitute for the iron atoms in both site types. Magnetite formed naturally inevitably contains impuritiy cations, the most frequent ones being Ti, Al, Mg, and Mn. The effect of metal substitution in magnetite produces systematic variation in magnetic and physical properties: saturation magnetization, curie temperature change; coercivity; magnetocrystalline anisotrophy, cell parameter, and electrical resistivity changes. There are many approaches to the synthesis of magnetic nanoparticles such as size reduction through ball milling, chemical precipitation, and microbial synthesis.[1–3] Mechanical processing such as high-energy milling, has been used to produce various glassy and metastable materials. Chemical preparation of magnetite in a laboratory typically relies on experimental regimes utilizing high temperature, high pressure, and high pH. A further difficulty of inorganic synthesis is the preparation of particles exhibiting homogeneous shape and size. Mechanical milling requires high energy for the synthesis of amorphous and other non-equilibrium materials such as wustite and maghemite. A new approach is based upon microbial processes that reduce iron oxyhydroxides with metals to nanometer-sized iron oxides. The objective of this study was to further examine mineralogical characteristics, magnetic properties, and applications of extracellularly synthesized magnetites and metal-substituted magnetite nanoparticles.

2. EXPERIMENTAL DETAILS

2.1. Microorganisms

We examined the extracellular synthesis of magnetite and metal-substituted magnetite nanoparticles using thermophilic (*Thermoanaerobacter ethanolicus*, TOR-39) and psychrotolerant (*Shewanella sp.*, PV-4) Fe(III)-reducing bacteria isolated from subsurface and oceanic environments (Table I).[4,5] Two bacteria, *Thermoanaerobacter ethanolicus* (TOR-39) and *Shewanella sp.* (PV-4), reduce various metals including Fe(III), Mn(IV), Co(III), Cr(VI), and other metals using organic carbon or hydrogen as an electron donor.[5,6] The two anaerobic Fe(III)-reducing bacteria precipitate or transform the poorly crystalline

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Table I. Fe(III)-reducing bacteria investigated to synthesize magnetite and metal-substituted magnetite nanoparticles.

Bacteria	Growth condition	Site description	Geology/ Sample type	Genus & species	Ref.
TOR-39	Thermophilic (40–75 °C)	Taylorsville Triassic Basin, Virginia, USA	Shale, Siltstone, and Sandstone	*Thermo-anerobacter ethanolicus*	[4]
PV-4	Psychrotolerant (0–37 °C)	Hydrothermal Naha Vent, the South Rift of Loihi Seamount, Hawaii, USA	Microbial mat near hydrothermal vents	*Shewanella sp.*	[5]

iron oxides into crystalline Fe phases such as magnetite (Fe_3O_4), siderite ($FeCO_3$), vivianite [$Fe_3(PO_4) \cdot 2H_2O$], iron sulfide (FeS), and green rust [$Fe^{2+}Fe^{3+}(OH)_{16}CO_3 \cdot 4H_2O$].[5, 7, 8]

2.2. Medium, Growth Condition, and Microbial Synthesis

Extracellular synthesis of magnetite and metal-substituted magnetite nanoparticles was performed using akaganeite (β-FeOOH) as a magnetite precursor with and without metals (i.e., Co, Cr, Mn). The magnetite precursor, akaganeite, was prepared as follows: NaOH solution (10 M) was slowly added to a $FeCl_2 \cdot 6H_2O$ solution (0.4 M) to precipitate FeOOH by gravity only and with rapid stirring at pH 7.0.[1] Detailed experimental conditions for extracellular synthesis of metal-substituted magnetite were shown by Roh and others.[1] No exogenous electron carrier substance (i.e., anthraquinone disulfonate) or reducing agent (i.e., cystene) was added to the medium. Lactate (10 mM) or glucose (10 mM) was used as the electron donor and Fe(III) oxyhydroxide (~70 mM akaganeite) or Fe(III) oxyhydroxide (~70 mM akaganeite) with Co [6 mM Co(III)-EDTA], Cr (0.6 mM $K_2CrO_4^{2-}$), Mn (Mn-FeOOH), or Ni (1 mM $NiCl_2$). Experiments were performed at 25 °C for pyschrotolerant culture (PV-4) and at 60 °C for thermophilic culture (TOR-39). Experiments were terminated after 21 days of incubation for both psychrotolerant Fe(III)-reducing bacterium (PV-4) and for thermophilic Fe(III)-reducing bacterium (TOR-39). The initial medium pH was ranged from 8.0 to 8.5.

2.3. Characterization

The mineralogical composition, morphology, and magnetic properties of the precipitated or transformed phases was determined using X-ray diffraction (XRD) analysis, synchrotron X-ray powder diffraction analysis, transmission electron microscopy (TEM), and ferromgnetic resonance (FMR) spectra.[7, 9, 10]

3. RESULTS AND DISCUSSION

Extracellular synthesis of magnetite nanoparticles is based on microbial synthesis that Fe(III)-reducing bacteria reduced iron oxyhydroxides and produced magnetite nano-crystals. Both PV-4 and TOR-39 produced magnetite using serum bottles with size range of 160 ml to 20-L vessels. Magnetite yields of up to 20 g/L per day have been observed in 20-L vessels. X-ray diffraction analyses of iron minerals formed by PV-4 at different incubation times at 25 °C showed that the magnetite precursor, iron oxyhydroxide (akaganeite), showed the first evidence of magnetite peak within 6 days (data not shown). No magnetite formation occurred in control tubes. After 3 days of incubation of TOR-39, the precipitates became completely black, and concurrently magnetite peaks were dominant in the diffraction pattern (data not shown). Major magnetite peaks became prevalent at the end of the experiments

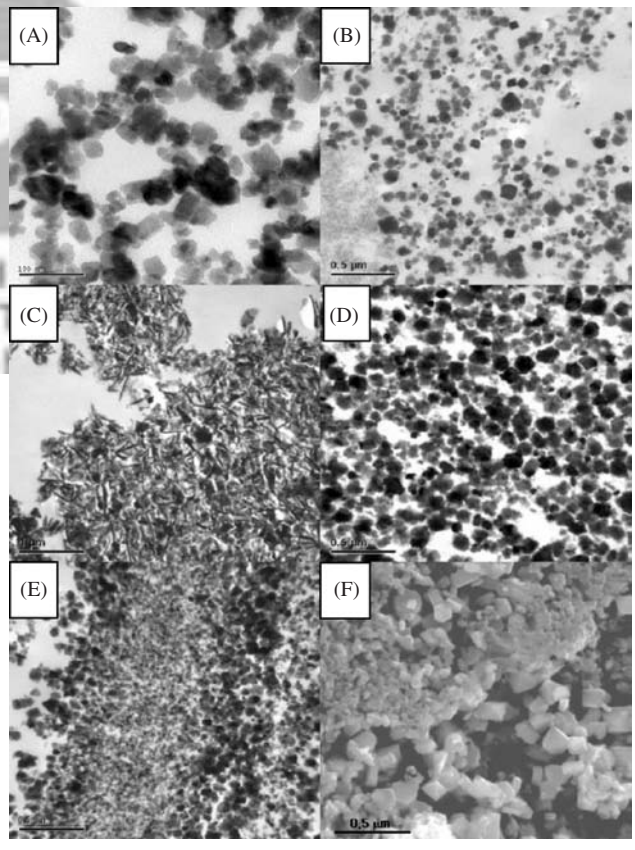

Fig. 1. TEM images of magnetite nanocrystals produced by PV-4 at 25 °C (A) and by TOR-39 at 60 °C (B). TEM and SEM images of metal-substituted magnetite nanocrystals produced by TOR-39 at 65 °C using akaganeite with Co (C), Cr (D), Mn (E), and Ni (F).

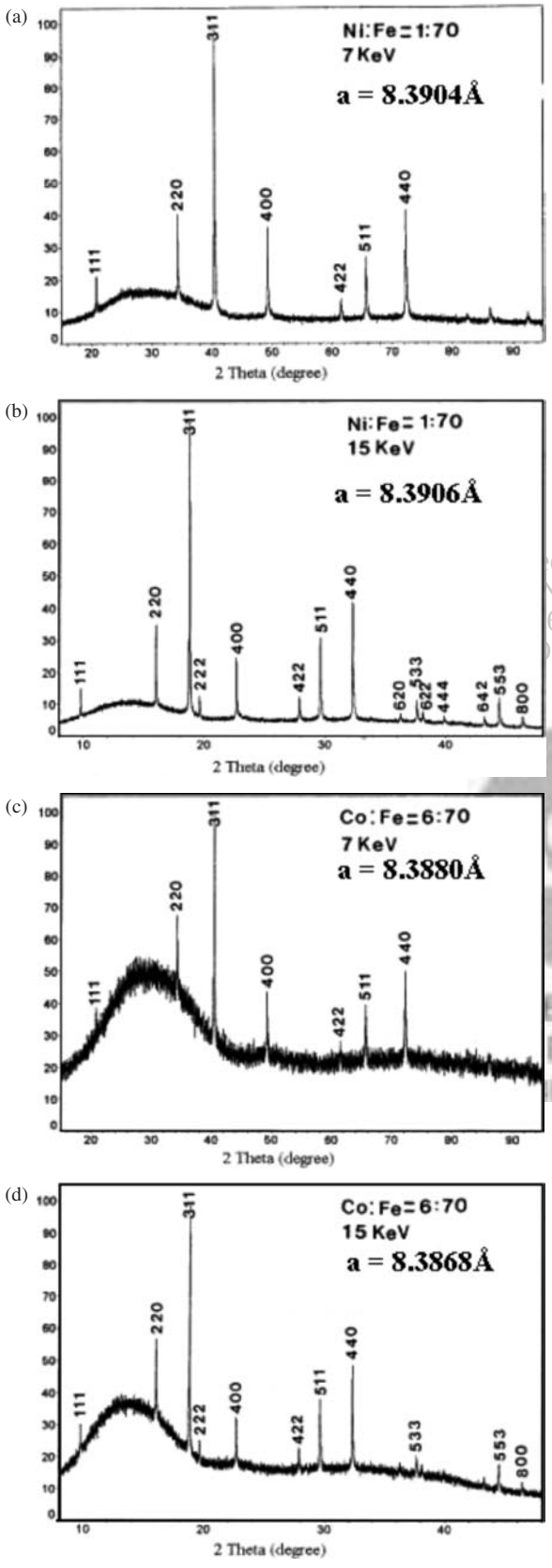

Fig. 2. Synchrotron X-ray powder diffraction analysis of Ni-substituted (a, b) and Co-substituted (c, d) magnetite nanocrystals produced by TOR-39.

(21 days) with PV-4 and TOR-39 using akaganeite as a magnetite precursor. After incubation for 21 days at 25 °C and 60 °C, transformed phases in the serum bottle became black and magnetic, and were attracted to the magnetic bar.

Thermophilic Fe(III)-reducing bacteria, TOR-39, reduced iron oxyhydroxides in the presence of metals (i.e., Co, Cr, Mn, Ni) and produced metal-substituted magnetite nanoparticles. Even though both the thermophilic (TOR-39) and psychrotolerant (PV-4) Fe(III)-reducing bacteria have demonstrated an ability to reduce Fe(III) and a number of metals,[5, 6, 8] the psychrotolerant did not form magnetite in the presence of metals such as Co, Cr, and Ni. The psychrotolerant Fe(III)-reducing bacteria formed siderite ($FeCO_3$) using akaganetite with manganese as a magnetite precursor. As a chemical process, it is possible to incorporate other metals such as Co, Cr, Mn, and Ni into magnetite structure by biomineralization with TOR-39 to control magnetic, electric, and physical properties.

Transmission electron microscopy (TEM) of magnetite crystals formed by PV-4 (Fig. 1A) showed aggregates of small magnetite crystals ranging in size from 20 to 40 nm. Most of the particles are single-domain size range (>35 nm) and different from particles formed by *Geobacter metallireducens* (GS-15).[11] And TOR-39 formed sharp, well-formed octahedral crystals (Fig. 1B). These crystals are in the single-domain size range with size range of 30–100 nm.[9] TEM analysis of the magnetites formed using akaganeite (~7 mM) with Co showed tabular shaped crystals. TEM and SEM analyses of the magnetites formed using akaganeite with Cr, Mn, and Ni showed well crystalline magnetite crystals (Fig. 1C, D, E, F).

Synchrotron X-ray powder diffraction analysis (Fig. 2) of Ni- and Co-substituted magnetites showed that the unit cell parameter of the Co-substituted ($a = 8.397$ Å–8.388 Å) and Ni-substituted ($a = 8.390$ Å–8.391 Å) magnetite is slightly smaller than that of magnetite ($a =$ 8.394 Å), which is consistent with the smaller ionic radii of Co^{2+} (0.72 Å) and Ni^{2+}(0.69 Å) compared with Fe^{2+} (0.78 Å). Unit cell parameter of magnetite is $a = 8.394$ Å and that of maghemite is $a = 8.34$ Å. Ferromgnetic resonance (FMR) spectra of Co-doped magnetite shows greater magnetism ($\Delta H = 2{,}242$) than the magnetism ($\Delta H =$ 1,737) of magnetite nanoparticles synthesized by TOR-39 without metal doping (Table II).

Table II. Ferromgnetic resonance (FMR) spectra of biosynthesized magnetite by TOR-39 (295 °K).

Samples	G′ Value	Line-width, ΔH (Gauss*)
Biosynthesized magnetite	3.452	1,737
Biosynthesized Co-doped magnetite (Co:Fe=6:70)	2.879	2,242

*Gauss = the centimeter-gram-second unit of magnetic flux of magnetic flux density that is equal to 1×10^{-4} tesla.

Water-based ferrofluids were formed with nm-sized magnetite particles produced by PV-4 using 25% aqueous ($(CH_3)_4NOH$) solution (Aldrich, Milwaukee, WI).[12] The water based ferrofluid forms spikes in the presence of moderate field when we hold a magnet (data not shown).

Magnetite and metal (Co, Cr, Mn, Ni)-substituted magnetite nanopartilces were formed by microbiologically mediated iron biomineralization under low temperature conditions (<60 °C). The biologically facilitated formation of metal-substituted magnetite does not require the reducing agents and the addition of exogenous electron carrier substances such as humic acids. Microbially facilitated formation of the metal-substituted magnetite nanoparticles at near ambient temperatures may influence the biogeochemical cycles of carbon and metals in subsurface environments. The biologically mediated reactions for mineral synthesis may represent a novel way to make a number of inorganic nanomaterials and are potentially useful for the synthesis for nm-sized ferromagnetic materials. Magnetic nanoparticles synthesized by microorganisms may be useful for improved magnetorheological (MR) fluids and ferrofluids for applications in active damping and for advanced power transmission devices such as fluid clutches.

4. CONCLUSION

It has developed a novel biosolid-state process that exploits the ability of iron-reducing microorganisms to produce copious amounts of extracellular magnetite and metal-substituted magnetite nanoparticles. The iron-reducing bacteria have the ability to reduce iron oxides and various metals in aqueous media forming various sized magnetite and metal-substituted magnetite nano-crystals. The iron-reducing bacteria formed magnetites and metal-substituted magnetites using iron oxyhydroxide plus metals under conditions of relatively low temperature (<70 °C), ambient pressure, and pH values near neutral to slightly basic. Precise biological control over activation and regulation of the biosolid-state processes can produce magnetite particles of well-defined size (typically tens of nanometers) and crystallographic morphology, containing selected dopant metals into the magnetite structure. This novel microbial approach to make engineered inorganic nanoparticles is potentially attractive because fermentation and microbial respiration is a well-understood, highly scalable, and environmentally benign industrial process.

Acknowledgments: We are grateful to Dr. B. Weiss (MIT) for FMR spectra and Dr. J. Bai (Oak Ridge National Laboratory) for Synchrotron X-ray diffraction analysis. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2005-070-C00137).

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Received: 14 November 2005. Revised/Accepted: 9 January 2006