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Microbial Synthesis of Cobalt-Substituted Magnetite Nanoparticles by Iron Reducing Bacteria

미생물을 이용한 나노입자의 코발트로 치환된 자철석의 합성

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ABSTRACT : The use of bacteria as a novel biotechnology to facilitate the production of nanoparticles is in its infancy. Cobalt-substituted magnetite nanoparticles were synthesized by a thermophilic iron(III) -reducing bacterium, TOR-39, under anaerobic conditions using amorphous Fe(III) oxyhydroxides plus cobalt (Co^{2+} and Co^{3+}) as an electron acceptor and organic carbon as an electron donor. Microbial processes produced copious amounts of nm-sized cobalt substituted magnetites. Chemical analysis and X-ray powder diffraction analysis showed that cobalt was substituted into biologically facilitated magnetites. Microbially facilitated synthesis of the cobalt-substituted magnetites may expand the possible use of the specialized ferromagnetic particles.

Keywords : magnetite nanoparticles; microbial synthesis; thermophilic Fe(III)-reducing bacteria, cobalt substitution

요약 : 미생물을 이용한 광물 합성은 현재 초기 연구 단계에 있으나 신소재 개발 측면에서 다양한 활용 가능성을 보이고 있다. 이 연구의 목적은 철 환원 박테리아를 이용한 코발트로 치환된 자철석의 합성 및 이의 광물학적 특성을 알아보는데 있다. 호열성 철 환원 박테리아인 TOR-39는 65에서 비정질 철 수화물과 코발트 이온 (Co^{2+} 와 Co^{3+})을 환원 및 침전시켜 자철석을 합성하였다. EPMA 분석과 X-선회절분석 결과에 의하면 호열성 박테리아가 철수화물을 환원시켜 자철석을 합성시킬 때, 코발트 이온도 동시에 환원 및 침전시켜 코발트로 치환된 자철석을 형성시킨다. 미생물에 의한 코발트로 치환된 자철석의 합성은 나노미터 크기로 생성되기 때문에 산업적으로 많은 이용 가치가 있을 것으로 본다.

주요어 : 나노입자의 자철석, 호열성 Fe(III)-환원 박테리아, Co-치환

Introduction

Synthetic or substituted powder magnetites are generally prepared either conventionally by solid phase reaction or by wet methods (Sidhu *et al.*,1978; Schwertmann and Murad, 1990; Diamandescu *et al.*, 1998; Petrovsky *et al.*, 2000). Solution synthesis techniques can produce fine, high purity, stoichiometric particles of single and multi-component metal oxides (Diamandescu *et al.*, 1998). Mechanical processing such as high-energy milling, has been used to produce various glassy and metastable materials (Ding *et al.*, 1998; Petrovsky *et al.*, 1996). The incor-

poration of Co(II) and Ni(II), and Cr(III) into magnetite, Fe_3O_4, with the inverse spinel-related structure has been of interest because of the magnetic, electric, and physical properties of the substituted magnetites (Sidhu *et al.*, 1978; Diammandescu *et al.*, 1998; Berry *et al*, 1999).

Chemical preparation of magnetite in the laboratory typically relies on experimental regimes utilizing high temperature, high pressure, and high pH (Diamandescu et al., 1998; Berry *et al.*, 1999; Ishikawa *et al.*, 1998). 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. Wustite and maghemite are also expected to be formed during the milling of mixtures of Fe and Fe_2O_3(Ding *et al.*, 1998). Ball milling has been attributed to decreasing grain size of the final product (Ding *et al.*, 1998).

In contrast to purely chemical procedure for the manufacture of magnetite particles, microbial reactions are characterized by their selectivity and precision for magnetite formation (Roh and Moon, 2000). Although the inorganic synthesis of magnetite and metal-substituted magnetite have been extensively examined (Sidhu *et al.*, 1978; Schwertmann and Murad, 1990; Diamandescu *et al.*, 1998), little is known about the microbial processes facilitating the formation of metal-substituted magnetite (Fredrickson *et al.*, 2001). The objectives of this study were to biologically faciliate the synthesis of cobalt-substituted magnetite using Fe(III)-reducing bacteria and to mineralogically characterize the synthesized magnetite nanoparticles.

Materials and Methods

The procedure for biologically facilitated production of cobalt-substituted magnetite is schematically shown in Figure 1. Magnetite precursors, amorphous iron oxyhydroxides (FeOOH), were prepared by slowly adding a 10 M NaOH solution into a 0.4 M $FeCl_2 \cdot 6H_2O$ solution with rapid stirring (Roh and Moon, 2000). The final pH of the precipitated slurry was 7.0. The precipitated precursors were washed three times with deionized water using a centrifuge to clean residual ions such as sodium and chlorine. The supernatant was decanted after washing and deionized water added to the wet precursors to make the final amorphous iron hydroxide solution (~0.7 M). The final precursors were flushed with N_2 gas, autoclaved, and stored aseptically under N_2 gas for use in experiments.

The thermophilic iron-reducing bacteria (TOR-39), *Thermoanaerobacter ethanolicus*, was isolated from a shale formation at about 3 km depth from the Taylorsville Triassic Basin in Virginia, USA (Liu *et al.*, 1997). The thermophilic iron-reducing bacteria (TOR-39) was grown at 65℃ under a N_2 atmosphere and then inoculated into a basal medium. Microbial synthesis of the metal-substituted magnetite was performed using a basal medium with large culture vessels (16-L of medium in a 20-L bottle or 8-L medium in a 13.25-L glass bottle) at 65℃. The medium had

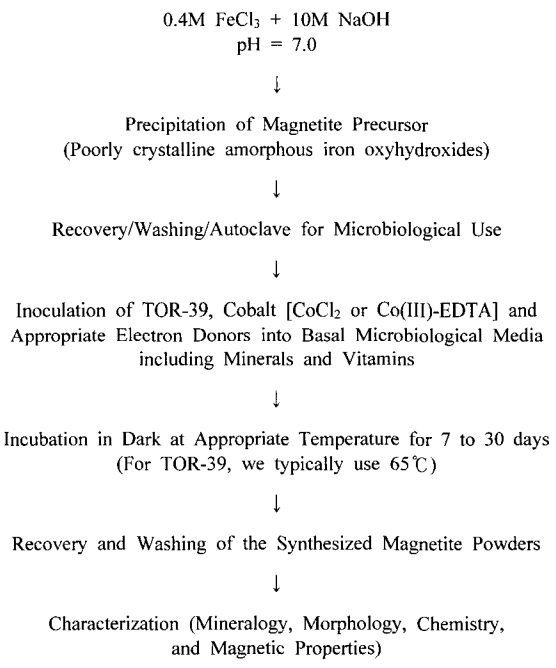

Fig. 1. Procedure for the microbial synthesis of the trace-metal incorporated magnetite.

the following ingredients (g/L): 2.5 $NaHCO_3$, 0.08 $CaCl_2 \cdot 2H_2O$, 1.0 NH_4Cl, 0.2 $MgCl_2 \cdot 6H_2O$, 10.0 NaCl, 7.2 HEPES (hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 0.5 yeast extract, 1.0 ml of rasarzurine (0.01%), and 10 ml of Oak Ridge National Laboratory (ORNL) trace minerals and 1 ml of ORNL vitamin solutions (Phelps *et al.*, 1989). No reducing agents were added to the medium. The medium was prepared under N_2 gas and had a final pH of 8.0~8.5.

Sterile magnetite precursors, amorphous Fe(III) oxyhydroxide (final concentration=70 mM), was added as an electron acceptor and sterile glucose (10 mM) or lactate (10 mM) was added as an electron donor. Substituting metal included reducible Co^{3+} and non-reducible Co^{2+}species. Sterile substituting cobalt such as cobalt(III)-EDTA (final concentration=1, 4, 6, 10 mM) and cobalt chloride (concentration=1, 4, 6, 10 mM) were added together with the amorphous iron oxyhydroxides (final concentration=70 mM) (Table 1). Experiments were performed at 65℃ and terminated after a minimum of 7 days to a maximum of 30 days of incubation. During the incubation, subsamples were taken at different times using sterile syringes and needles to monitor medium pH and mineralogical changes. Strong pH buffers, such as HEPES and NaOH, were used to maintain the solution pH of 8.0~8.5.

The final synthesized products were washed three times by repeated cycles of centrifugation and redispersion in deionized water and once with acetone as a final step, and then stored in vacuum before characterization. The mineralogical and morphological characteristics of the precipitates were investigated using a Scintag XDS 2000 X-ray diffractometer (XRD) and a Hiatchi S4500A scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDX). Microbially-synthesized magnetite with Co (Co:Fe ratio=1:70) was mounted in epoxy and polished in order to analyze the interior of several particles by electron microprobe analysis (EMPA) (JEOL, Tokyo). This analysis confirmed that the metals were incorporated into the crystallites, and not merely adsorbed onto their surface.

Table 1. Cobalt to iron mole ratios used for the microbial synthesis of cobalt-substituted magnetite.*

Metals	Metal concentration	Mole ratios†
Co(III), Co(III)-EDTA	1 mM	0.014
	4 mM	0.054
	6 mM	0.079
	10 mM	0.125
Co(II), $CoCl_2$	1 mM	0.014
	4 mM	0.054
	6 mM	0.079
	10 mM	0.125

* FeOOH (amorphous iron oxyhydroxide) concentration =70 mM

† (metal)/(metal + FeOOH)

Synchrotron X-ray powder diffraction analysis was also used to investigate the metal substitution into microbially synthesized magnetite. Synchrotron X-ray powder diffraction data were collected on beamline X14A at the National Synchrotron Light Source at Brookhaven National Laboratory, USA. To reduce the systematic errors attributed to preferred orientation and inadequate powder averaging, the samples were loaded into 0.1 mm thin walled glass capillaries and continually rotated. One magnetite sample synthesized with Co (Co:Fe ratio=6:70)) were examined. X-ray powder diffraction data at 7.092 KeV and 15 KeV were collected. The program GSAS (General Structure Analysis System) (Larsen and Von Dreele, 1994) was used for Rietveld refinements and calculations of the powder patterns using magnetite structure (Wechsler *et al.*, 1984).

Results and Discussion

Figure 2 shows the changes in Eh and pH after 7~14 days of incubation at 65℃. The pH and Eh of the medium was 8.6 and 40 mV, respectively. The pH typically decreased to 7.5 during 2-week incubation. The Eh of the initial medium decreased from ~40 mV to between -200 mV and -300 mV upon incubation with the metal reducing microorganisms. Measured

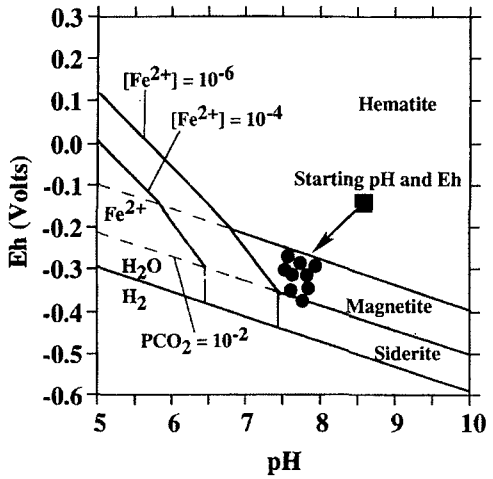

Fig. 2. Eh-pH stability fields for hematite, magnetite, and siderite in the water-iron-CO_2 system at 25℃ and 1 atm pressure (modified from Roh and Moon, 2000).

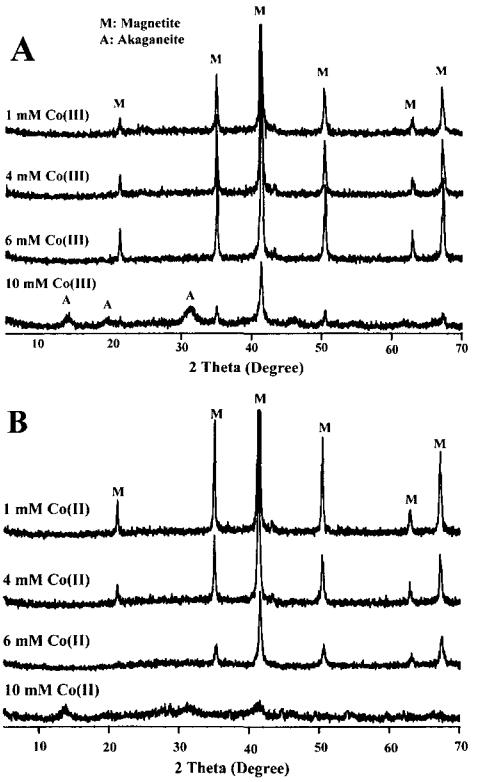

Fig. 3. X-ray diffraction patterns of the products at different quantities of metals with amorphous iron oxyhydroxides: (a) Co(III)-EDTA (1, 4, 6, 10 mM) and (b) $CoCl_2$ (1, 4, 6, 10 mM).

pH and Eh are consistent with the thermodynamic stability of magnetite formation (Roh and Moon, 2000). The decrease in pH is due to glucose fermentation that producing organic acids and CO_2 (Zhang *et al.*, 1997, 1998).

Figure 3 shows XRD patterns of the materials precipitated by microbial cultures with differing quantities of cobalt ($Co^{3+}$and Co^{2+}) plus amorphous Fe(III) oxyhydroxides. The culture medium also included the Fe(III)-reducing bacteria (TOR-39) and lactate (10 mM) or glucose (10 mM) at 65℃. Biologically facilitated magnetite was observed to incorporate reducible cobalt (Co(III)-EDTA) (Fig. 3a) and non-reducible cobalt ($CoCl_2$) (Fig. 3b) with amorphous iron oxyhydroxides. The addition of certain concentration (10 mM) of $CoCl_2$ and Co(III)-EDTA with the magnetite precursor suppressed the nucleation and crystal growth of magnetite. Some of the magnetite precursors at the elevated concentrations remained as a poorly crystalline iron hydroxide, akaganeite (FeOOH) (Fig. 3). The suppression of magnetite formation (Fig. 3) at the concen--trations of 10.0 mM Co may have been due to inhibition of bacterial growth at these trace metal concentrations (Zhang *et al.*, 1996). Magnetite did not form in control bottles containing magnetite precursor and substituting metals without cells.

Scanning electron micrographs of the co-substituted magnetites showed that octahedral shaped magnetite crystals coexisted with poorly crystalline magnetite crystals (Fig. 4a). EDX analysis of the magnetites incorporation with Co (Co:Fe=1:70) showed that Co was not differentiate from Fe peaks throughout the magnetite particles (Fig. 4b). EMPA analysis of the microbially-synthesized magnetites with Co (Co:Fe= 1:70) correlated with EDX analysis of that sample and showed that ~0.6 % (wt.%) of Co was present throughout the particle interiors, representing substitution for about 1% of the Fe in the magnetite structure (Table 2). The Co:Fe ratio of 1:70 is in good agreement with the chemical composition of the synthesized material

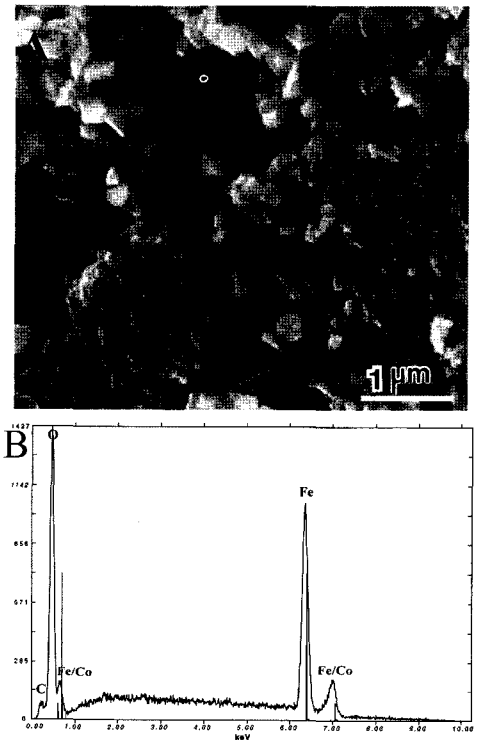

Fig. 4. Scanning electron micrograph (a) and the energy dispersive X-ray analyses (b) of Ni-incorporated magnetite. Residual salt from microbial media coexisted with magnetite crystals.

(Co/Fe=1.4%). The total Fe content of the magnetites was lower than the Fe content of magnetite standards (Table 2). This discrepancy could be due to the presence of strongly adsorbed water because a small quantity of water is retained by the synthetic magnetite. The samples prepared in this study were dried at room temperature under anaerobic conditions because drying at higher temperatures (> 100℃) in air resulted in the oxidation of magnetite to maghemite or hematite (Sidhu *et al.*, 1978). X-ray powder diffraction patterns collected at 7.092 KeV and 15 KeV showed that the unit cell parameter (Table 3) of the Co-substituted magnetite is slightly smaller than that of magnetite (Fe_3O_4) (a=8.394 Å) (Fleet, 1981), which is consistent with the smaller octahedral ionic radii of Co^{2+} (0.72 Å) compared with Fe^{2+} (0.78 Å) (Shannon, 1976).

Table 2. Electron microprobe analysis of cobalt substituted magnetite

Sample	Fe	Mn	Co	O	Total	No. of analysis
	Element Wt.%					
Magnetite with Co (Co:Fe=1:70)	65.32 (2.49)	0.06 (0.05)	0.66 (0.11)	25.9 (1.47)	91.94 (3.93)	5
Magnetite Standard	72.63 (0.23)	0.1 (0)	0.1 (0)	27.16 (0.55)	99.99 (0.78)	3

Values quoted are the mean and standard deviation for the number of individual spot analysis shown

Table 3. Unit-cell parameters of the magnetite synthesized with Co

	Sample	
	7.092 keV	15 keV
Magnetite with Co (Co:Fe=6:70)	8.3880(2)	8.3868(2)

Maintaining media pH between 6.9 and 8.5 by using pH buffers such as HEPES and NaOH during bacterial growth and magnetite production is important for the biologically facilitated magnetite production using TOR-39. The Fe(III)-reducing bacterium produces organic acids during glucose fermentation lowering the pH substantially if the medium is not well buffered (Roh and Moon, 2000). If the pH becomes too low (i.e., < 6.0) magnetite will not form because the high proton concentrations may favor the dissociation of magnetite to $Fe(OH)_3$.

X-ray diffraction (XRD) and scanning electron microscopic (SEM) analysis showed that the iron-reducing bacterium formed magnetite and that the inclusion of cobalt did not change the phase morphology. The details of the biological reduction and mineralization process are not yet fully understood (Roh and Moon, 2000; Lovley, 1991, 1993). The Eh-pH diagram shows that cobalt-substituted magnetites are precipitated and mineralized by alternation of the Eh and pH conditions, thereby creating conditions of supersaturation with respect to a mineral phase. Magnetite formation is the result of biologically-mediated mineralization; that is, the organisms

may alter the local Eh and pH conditions which, in turn, shifts local mineral solubility equilibria, potentially facilitating magnetite nucleation and formation of magnetite particles on or near the exterior surface of the cell (Roh and Moon, 2000; Siering, 1998). Despite the existence of appropriate conditions for magnetite formation (e.g., Eh and pH), magnetite did not formed in control samples without bacteria. This means that the formation of extracellular deposits of magnetite seems to be controlled by both solution chemistry and bacterial nucleation action (Roh and Moon, 2000). Direct contact between cell and oxide surfaces may be necessary for microbial respiration and reduction (Lovley and Phillips, 1988).

Chemical and X-ray powder diffraction analyses support that the cobalt could be directly incorporated into the magnetite crystal structure. It is unclear how magnetite crystals nucleated and grew, and how trace metals are incorporated into magnetite during the biomineralization by the TOR-39 culture. Although the exact mechanism is currently unknown, TOR-39 appears to have caused the formation of trace metal-incorporated magnetite by enhancing the reduction of Fe^{3+} and cobalt via electron transfer from organic carbon oxidation. TOR-39 isolated from the deep subsurface has the ability to reduce Fe(III) and other heavy metals such as Cr(VI), Mn(IV), and Co(III) from solution (Roh and Moon, 2000; Zhang *et al.*, 1996). The solid phase conversion of amorphous iron hydroxides is in accordance with the strong sorption of metals to amorphous iron hydroxide. The reduction of cobalt (Co^{3+}) by iron-reducing bacteria, and the incorporation of other metals (Co^{2+}) added as reduced forms may be adsorbed on iron oxides in alkaline media and may also be incorporated into iron oxide structure during their crystallization (Cornell and Schwertmann, 1996). These adsorbed trace metals may be incorporated into magnetite and become a part of the crystals as magnetite crystal growth continues. The cobalt metal may be directly incorporated into the magnetite crystal structure when the thermophilic iron-reducing bacteria formed magnetite because the ionic radii, electronegativities, and solubility products of Co^{2+} is very similar to those of Fe^{2+}(Goldschmidt, 1954).

In this manner, biosolid-state reactions can establish the formation of metal-suibstituted magnetite under conditions of low temperature (< 70℃), ambient pressure, and at pH values near to neutral to slightly basic (pH <9). Furthermore, precise biological control over the activation and regulation of the solid-state processes can result in magnetite particles of well-defined size and crystallographic morphology. The advantages of biologically-faciliatated production of metal-substituted magnetites may include: (1) particles can in principle be grown to a size that would not be feasible if the particles formed inside the cell; (2) the bacteria do not need to die in order for the product to be harvested; and (3) agitation, fluid flow, or magnetic forces may be capable of dislodging the particles when they reached a desired size. Knowledge concerning such novel bio-solid processes may be important in the development and design of submicron-sized ferromagnetic materials.

Conclusions

Cobalt-substituted magnetite powder was synthesized by microbiologically mediated iron mineralization under low temperature conditions (< 75℃). Microbial synthesis of cobalt-substituted magnetite formation was influenced by chemical milieu and metal concentrations. The biological process rapidly produces copious amounts of nm-sized cobalt-substituted magnetite particles which may expand the possible roles and utilities of nm-sized magnetic minerals. It is unclear how the magnetite crystals grow or precise mechanisms by which the trace metals were incorporated into magnetite during the biomineralization processes. These biosolid-state reactions may represent novel solid-state processes and potentially useful to the development

and design of submicron-sized ferromagnetic materials.

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References

Berry, F. J., Greaves, C., Helgason, ö., and McManus, J. (1999) Synthesis and characterization of tin-doped iron oxides. J. Mat. Chem. 9, 223-226.

Cornell, R. M. and Schwertmann, U. (1996) The iron oxides: structure, properties, reactions, occurrence and uses. VCH, New York, USA. 573p.

Diamandescu, L., Mihaila-Tarabasanu, D., Teodorescu, V., and Popescu-Pogrion, N. (1998) Hydrothermal synthesis and structural characterization of some substituted magnetites. Mat. Let. 37, 340-348.

Ding, J., Miao, M. F., Pirault, E., Street, R., and McCormick, P. G. (1998) Structural evolution of $Fe+Fe_2O_3$ during mechanical milling. J. Magnetism. Magnetic Mat. 177-181, 933-934.

Fedrickson, J., Zachara, J. M., Kukkadapu, R. K., Gorby, Y., Smith, S. C., and Brown, C. F. (2001). Biotransformation of Ni-substituted hydrous ferric oxide by an Fe(III)-reducing bacterium. Environ Sci.Technol. 35, 703-712

Fleet, M. E. (1981) The structure of magnetite. Acta Cryst. B37, 917-920.

Goldschmidt, V. M. (1954) Geochemistry, Clarendon, Oxford, 730p.

Ishikawa, T., Nakazaki,H., Yasukawa, A., Kandori, K., and Seto, M. (1998) Structure and properties of magnetite formed in the presence of nickel (II) ions. Mat. Res. Bullet. 33, 1609-1619.

Larson, A. C. and Von Dreele, R. B. (1994) General Structure Analysis System, Los Alamos National Laboratory Report LAUR 86-748, 179p.

Liu, S. V., Zhou, J., Zhang, C., Cole, D. R., Gajdarziska-Josifovska, M. and Phelps, T. J. (1997) Thermophilic Fe(III)-reducing bacteria from the deep subsurface: The evolutionary implications. Science, 277, 1106-1109.

Lovley, D. R. (1991) Dissimilatory Fe(III) and Mn(VI) reduction. Microbial Rev. 55, 259-287.

Lovley, D. R. (1993) Dissimilatory metal reduction. Ann. Rev. Microbiol. 47, 263-290.

Lovley, D. R. and Phillips, E. J. P. (1998) Novel mode of microbial energy-metabolism-organic-carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl. Environ. Microbiol. 54, 1472-1480.

Petrovsky, E., Kropacek, V., Dekkers, M. J., deBoer, C., Hoffmann, V., and Ambattiello, A. (1996) Transformation of hematite to maghemite as observed by changes in magnetic parameters: Effects of mechanical activiation? Geophys.Res. Let. 23, 1477-1480.

Petrovsky, E., Alcala, M. D., Criado, J. M., Grygar, T., Kapicka, A., and Subrt, J. (2000) Magnetic properties of magnetite prepared by ball milling of hematite with iron. J Magnetism and Magnetic Mat. 210, 257-273.

Phelps, T. J., Raione, E. G, White, D. C., and Fliermans, C. B. (1989) Microbial activity in deep subsurface environments. Geomicrobiological J. 7, 79-91.

Roh, Y. and H.-S. Moon (2000) Microbial synthesis of magnetite powder by iron reducing bacteria. J. Miner. Soc. Korea, 13, 65-72.

Schwertmann, U. and Murad, E. (1990) The influence of aluminum on iron oxides. 16. Al-substituted magnetite synthesized at ambient-temperatures. Clays Clay Min. 38, 196-202.

Shannon, R. D. (1976) Revised effective ionic-radii and systematic studies of interatomic distances in halides and chalcogenises. Acta Cryst. A32, 751-767.

Sidhu, P. S., Gilkes, R. J., and Posner, A. M. (1978) The synthesis and some properties of Co, Ni, Zn, Cu, Mn and Cd substituted magnetites. J. Inorg. Nucl. Chem. 40, 429-435.

Siering, P. L. (1998) The double helix meets the crystal lattice: The powder and pitfalls of nucleic acid approaches for biomineralogical investigations. Am. Min. 83, 1593-1607.

Wechsler, B. A., Lindsay, D. H., and Prewitt, C. T. (1984) Crystal-structure and cation distribution in titanomagnetites ($Fe_{3-x}Ti_xO_4$). Am. Min. 69, 754-770.

Zhang, C. L., Liu, S., Logan, J., Mazumder, R., and Phleps, T. J. (1996) Enhancement of Fe(III), Co(III), and Cr(VI) reduction at elevated

temperatures and by a thermophilic bacterium. Appl. Biochem. Biotechnol. 57-58, 923-932.

Zhang, C., Liu, S., Phelps, T. J., Cole, D. R., Horita, J., Fortier, S. M., Elless, M., and Valley, J. W. (1997) Physiochemical, mineralogical, and isotopic characterization of magnetic iron oxides formed by thermophilic Fe(III)-reducing bacteria. Geochim. Cosmichim. Acta, 61, 4621-4632.

Zhang, C., Vali, H., Romanek, C. S., Phelps, T. J., and Liu, S. (1998). Formation of single-domain magnetite by a thermophilic bacterium. Am. Min. 83, 1409-1418.

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