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Microbial Synthesis of Magnetite Powder by Iron Reducing Bacteria

철 환원 박테리아를 이용한 자철석 합성

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ABSTRACT : The use of bacteria as a novel means of producing mineral phases is in its infancy. The objective of this study is to explore conditions that influence the formation of magnetite nanocrystals formed by a thermophilic iron reducing bacterial strain, TOR-39. The bacterium was isolated from the deep subsurface shales (about 3-km depth) in Taylorsville Triassic Basin, Virginia, USA. Microbiologically- mediated magnetite formation initially began within 12 hours incubation at 65℃ using amorphous iron oxyhydroxides as a precursor (~70 mM). Magnetite particles formed at 25 days of incubation had diamond shapes and sharp edges with lengths of 50 to 100 nm and were similar to intracellular magnetite. Chemical milieu (pH = 7.9 - 8.5 and Eh < -200 mV), incubation time, and incubation temperature (45℃ - 75℃) are main factors in affecting the magnetic iron mineral formation during bacterial iron reduction. Microbial synthesis of magnetite nanocrystals may play an important role in possible use of the materials for many industrial purposes such as radar absorbent materials, transformer cores, magnetic memories and heterogeneous catalysts.

Keywords : magnetite, iron reducing bacteria, mineral synthesis, biomineralization, thermophilic bacteria

요약 : 미생물을 이용한 광물 합성은 현재 초기 연구단계에 있으나 신소재 개발측면에서 다양한 활용성을 보인다. 본 연구의 목적은 철환원 박테리아를 이용한 자철석 합성에 있어 미치는 환경조건들을 알아보는데 있다. 본 연구를 위해 지하 3-km 코아 시료에서 분리한 호열성 철 환원 박테리아인 TOR-39을 이용하였다. TOR-39은 65℃ 에서 12시간이내에 비정질 철수화물을 환원시켜 자철석을 형성한다. 25일 동안 배양하여 형성된 자철석은 정육각형 모양으로 입자 크기는 50 - 100 나노미터이다. TOR-39을 이용한 자철석 합성시 적절한 조건은 pH 는 7.9 - 8.5, Eh 는 -200 mV 이하, 배양기간는 3 - 25일 그리고 온도는 45 - 75℃ 이다. 미생물에 의한 자철석 합성은 나노미터 크기의 광물을 직접 합성하므로, 산업적으로 많은 이용 가치를 가질 것으로 본다.

주요어 : 자철석, 철환원 박테리아, 광물합성, 호열성 철환원박테리아

Introduction

Recently, there has been an increasing interest in the synthesis of non-dispersed metal oxides. Solution synthesis techniques have the potential to meet the increasing demand for the direct preparation of crystalline ceramic powders in the production of anhydrous oxide pow-

ders (Hirano, 1987; Bae *et al.*, 1998). Solution synthesis techniques can produce fine high purity, stoichiometric particles of single and multi-component metal oxides (Dawson, 1988). Hydrothermal synthesis meets the increasing demand for the direct preparation of crystalline ceramic powders in the production of anhydrous oxide powders. However, preparation of magnetite in the laboratory requires an experimental regime that often requires high temperature, high pressure, and high pH (Cornell and Schwertmann, 1996). A further aspect of inorganic synthesis is the experimental difficulty involved in the preparation of particles of homogeneous shape and size.

The biological synthesis of the mixed-valence iron oxide, magnetite (Fe_3O_4), poses several fascinating and intriguing questions for the solid state and materials scientist. Biological reactions are characterized in their selectivity and precision for functional design. In this way, biosolid-state reactions can establish the formation of magnetite under conditions of low temperature (<100℃), ambient pressure, and at pH values close 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 (Mann, 1985). These aspects of chemical and crystallographic specificity in biosolid-state reactions are important criteria which need to be investigated and elucidated. Knowledge concerning such novel solid processes may be important in the development and design of new material for technological use.

Bacteria mediate the biomineralization of several iron minerals that include the iron oxide, magnetite (Fe_3O_4), and the iron sulfides, greigite (Fe_3S_4) and pyrrhoite (Fe_7S_8). These minerals can be formed in one of two fundamentally different ways or modes of biomineralization. The first is an indirect means of mineral formation called "biologically-induced mineralization" (Lowenstam, 1981) whereas the second is considered to be a direct or indirect mechanism of mineralization termed "biologically-controlled mineralization" (Lowenstam, 1981). The mineral particles are usually formed extracellularly and have broad size distributions and no defined morphology (Bazylinski and Moskowitz, 1997). This study examined biologically-induced mineralization because the bacteria do not need to die for the product to be harvested.

Although the inorganic synthesis of various iron oxides such as hematite, maghemite, magnetite, and goethite have been studied in detail (Taylor and Schwertsmann, 1974; Sidhu *et al.*, 1978; Schwertsmann and Murad, 1983), little is known about the microbial formation of synthetic magnetite. The objective of this study is to examine the conditions of magnetite synthesis by thermophilic iron reducing bacteria. Direct bacterial production of optimized, single domain-magnetite particles would eliminate time-consuming milling operations.

Materials and Methods

Source of organism

The thermophilic iron reducing bacteria (TOR-39) were isolated from the shale, at about 3000 m in depth in Triassic Taylorsville Basin in Virginia, USA and were cultured under anaerobic atmosphere of N_2.

Process of the microbial synthesis of magnetic powder

Process for preparing magnetic powder microbially is shown in Fig. 1. A magnetite precursor, amorphous iron oxyhydroxide, was prepared by adding 10 M NaOH into 0.4 M $FeCl_3 \cdot 6H_2O$ solution by gravity only with rapid stirring. The final pH of the precipitated slurry was 7.0. The precipitated precursors were washed three times in deionized water using a centrifuge. The supernatant was decanted after the final washing and deionized water was added to the precursor to make up the final

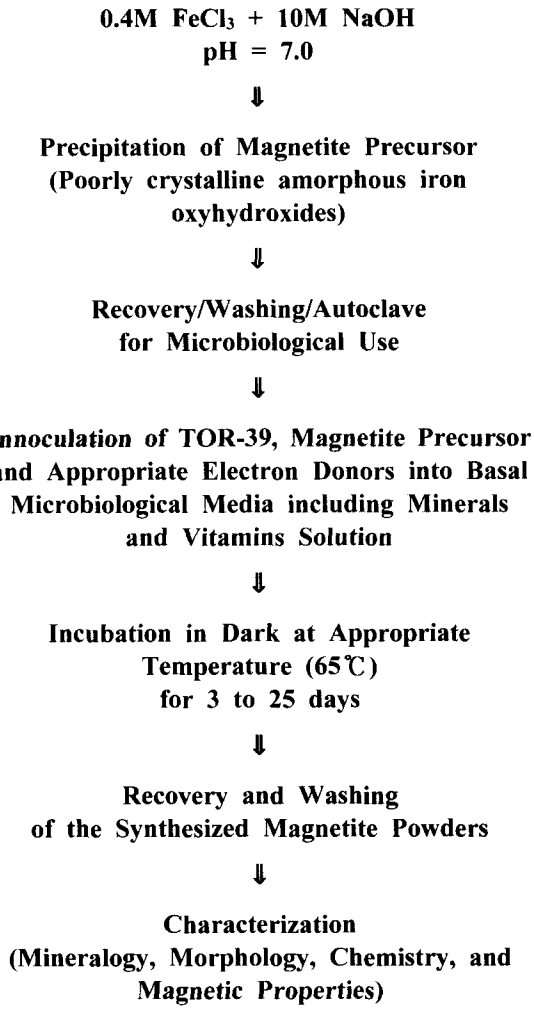

Fig. 1. Procedure for the microbial synthesis of magnetic powder.

concentration of precursor (~0.7 M). In addition to amorphous iron oxyhydroxides, goethite, hematite, and ferric chloride were used as precursors to examine the effect of ferric iron species on the microbial production of magnetic powder. These precursors were flushed with N_2 gas and autoclaved for microbiological use.

Microbial synthesis of the magnetite was performed using large culture vessels (100 mL medium in 160 mL and 8-L medium in a 13.25-L glass bottle). Each vessel contained an aqueous medium in which anaerobic bacteria were grown. The medium had the following ingredients (g/L): 2.5 $NaHCO_3$, 0.08 $CaCl_2 2H_2O$, 1.0 NH_4Cl, 0.2 $MgCl_2 6H_2O$, 10.0 NaCl, 7.2 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 1.0 Rasarzurine (0.1%), 0.5 yeast extract, and trace mineral and vitamin solutions (Phelps *et al.*, 1989). The medium was prepared anaerobically under N_2 gas.

Examination of the effect of ferric iron species, electron donors, pH, time, and temperature on magnetic end product formation

A systematic study was undertaken in which many experimental parameters were adjusted to determine the influence of different growth conditions and electron donors on the products formed, and on the overall efficiency of production. Ferric iron species included amorphous iron oxyhydroxides (FeOOH), goethite (FeOOH), hematite (Fe_2O_3), and ferric chloride ($FeCl_3$). Incubation temperatures ranged from 25℃ to 85℃ to examine the effect of temperature on microbial synthesis of magnetite. To obtain more information on the influence of pH on the magnetic powder production while using amorphous iron oxyhydroxide (70 mM) as an electron acceptor and glucose (10 mM) as an electron donor, the medium was adjusted to pH values of 7.6, 7.9, 8.5, and 9.6 with HCl or NaOH and kept at an incubation temperature of 65℃. Incubation time ranged from 0 hour to 25 days at 65℃ to observe mineralogical changes during bacterial respiration. An electron donor such as hydrogen (80% balance with CO_2), acetate (10 mM), lactate (10 mM), pyruvate (10 mM), formate (10 mM), or glucose (10 mM) was added to the culture medium. Abiotic controls accompanied each bacterial magnetite formation experiment.

Chemical and mineralogical characterization

To examine the chemical conditions of magnetite formation by microbes, subsamples of bacterial cultures and abiotic controls were

taken from the culture bottles at different incubation times and measured for Eh and pH at room temperature in an anaerobic chamber. The Eh was measured using a platinum microelectrodes (Microelectrodes. Inc., Londonderry, N.H.) connected to a ORION pH meter. The pH was also measured.

The final synthesized products after 25 days of incubation were washed at least three times by repeated cycles of centrifugation and redispersion in deionized water and once with acetone as a final step. The mineralogical and morphological characteristics of precipitates were investigated by X-ray diffraction (XRD) and transmission electron microscopy (TEM). All XRD analyses were performed on a Scintag (Scintag Inc., Sunnyvale, CA, USA) XDS 2000 diffractometer (45 KV, 40 MA) equipped with CoK-alpha radiation with a scan rate of 2°/min. Culture media containing bacterial cells, organic matter, inorganic solids (magnetite and amorphous iron oxyhydroxides) were fixed with 2.5% glutaraldehyde in 0.1 M cacodelate. After washing with a HEPES buffer and an alcohol/water solution (1:1 ratio), samples were dehydrated with propylene oxide and embedded in a low-viscosity, thermally curing epoxy resin. Ultrathin section (70 - 80 nm) was cut from resin blocks with a diamond knife and transferred to 30 mesh formvar-coated Cu TEM grid for image analysis on a JEOL FZ 2000 TEM equipped with an energy dispersive X-ray detector.

Results and Discussion

Examination of the effect of ferric iron species, electron donors, pH, time, and temperature on magnetic end product formation

Effect of ferric iron species: Thermophilic iron reducing bacteria reduced Fe(III) in amorphous iron oxyhydroxides and produced magnetic powder; however, these bacteria could not reduce Fe(III) in crystalline iron oxides such as goethite and hematite. Although these bacteria also reduced Fe(III) in ferric chlorides (data not shown), magnetic powder did not form as a respiration byproduct.

Effect of incubation temperature and pH of the medium: Temperature profile of the microbial Fe(III)-reducing activity of the TOR-39 culture showed that brown nonmagnetic amorphous iron oxyhydroxides in incubation tubes at 45℃ to 75℃ became black and magnetic after incubation for 5 days. Precipitates at <25℃ and >85℃ remained brown and non-magnetic after 5 days of incubation.

Phase transformation of amorphous iron oxyhydroxides to magnetite can occur at pH 7.9 to 8.8 (Fig. 2) provided the Eh of the reaction environment is established in the range of <-200 mV. These results suggest that the iron-reducing bacteria crystallized magnetite at a high pH (>7.6) and a low Eh value (<-200 mV). Magnetite formation requires a source of Fe(III) and living metal reducing cultures, and is favored by high pH (Lovley, 1990; Bell *et al.*, 1987):

$$2OH^- + Fe^{2+} + 2Fe(OH)_3 \Rightarrow Fe_3O_4 + 4H_2O \quad (1)$$

Microbial formation of magnetite by TOR-39 requires a high starting pH (>6.9) and a pH buffer such as HEPES because this bacterium produces large amounts of organic acids during

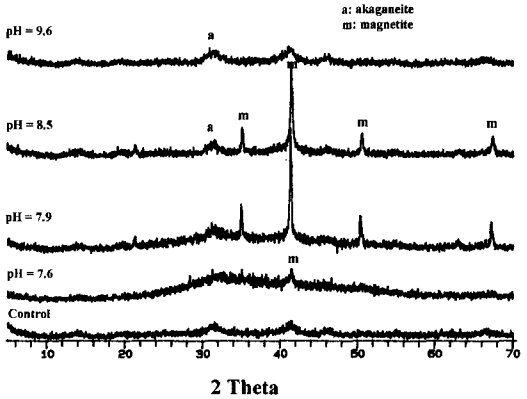

Fig. 2. X-ray diffractograms showing the effect of pH on the microbial magnetite formation.

glucose fermentation and lowers pH substantially (Zhang *et al.*, 1997). If the pH becomes too low (i.e., <6.0), magnetite will not form because high proton concentrations favor the reverse direction of reaction 1. Microbial magnetite formation did not occur at high pH (i.e., >9.6) because the iron reducing bacteria did not grow at that pH.

Effect of electron donors: Thermophilic iron reducing bacteria, TOR-39, used glucose, lactate and formate as electron donors for Fe(III) reduction; however, this bacteria did not utilize hydrogen, pyruvate, and acetate as electron donors for Fe(III) reduction. Despite the existence of appropriate conditions (Eh and pH) for iron mineral formation, magnetite did not form in control tubes containing magnetite nuclei. Previous studies have shown these acids are potentially available in terrestrial subsurface environments (Liu *et al.*, 1997). The oxidation of organic compounds coupled to reduction of Fe(III) oxides can be expected to release Fe(II) ions from iron hydroxide (Lovley, 1993):

$$\text{formate}^- + 2Fe(OH)_3 = 2Fe^{2+} + HCO_3^- + 2H^+ \quad \text{(formate oxidation)} \quad (2)$$

$$4Fe(OH)_3 + \text{lactate}^- = 7H^+ \Rightarrow 4Fe^{2+} + \text{acetate}^- = HCO_3^- + 10\ H_2O \quad \text{(lactate oxidation)} \quad (3)$$

Effect of incubation time : X-ray diffraction analyses of iron minerals formed in TOR-39 cultures at different incubation times at 65℃ showed that the initial iron was predominantly amorphous iron ohyhydroxides (Fig. 3). After a day of incubation, a magnetite peak appeared at about 42° in 2θ using Co K-alpha radiation. This first evidence of magnetite peak agreed with the visual observation of the enrichment culture in which the reddish precursor turned into black. After 3 days of incubation, the precipitates became black, and concurrently magnetite/maghemite peaks were dominant in the diffraction pattern. At the end of the experiments (25 days), major magnetite peaks became prevalent. Because magnetite and maghemite have similar crystal structures, it is difficult to distinguish these mineral based on XRD pattern alone. No magnetite/maghemite was detected in the abiotic control at 65℃.

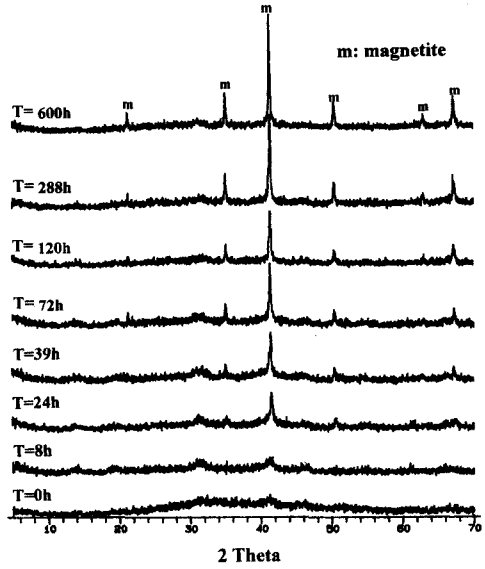

Fig. 3. X-ray diffractograms showing the effect of incubation time on the development of crystalline magnetite peaks.

This experiment indicates that magnetite could not be synthesized by a purely inorganic mechanism under the conditions examined, even with the presence of highly reactive seed material (precursor). These results further suggest that bacterial activity played a governing role in biogenic magnetite synthesis under the experimental conditions.

Chemical characterization: Solution pH and Eh were measured at 65℃ during the incubation. The Eh of the medium decreased from an initial electrode potential of 50±20 mV to -300±20 mV by the first measurement after 11 hour incubation. Within the first 11 hour, pH decreased from initial values of 8.5 to 8.3. The pH continued to decreased slowly thereafter, but it remained above 7.5 during the subsequent incubation. The decrease in pH is due to glucose fermentation that produces

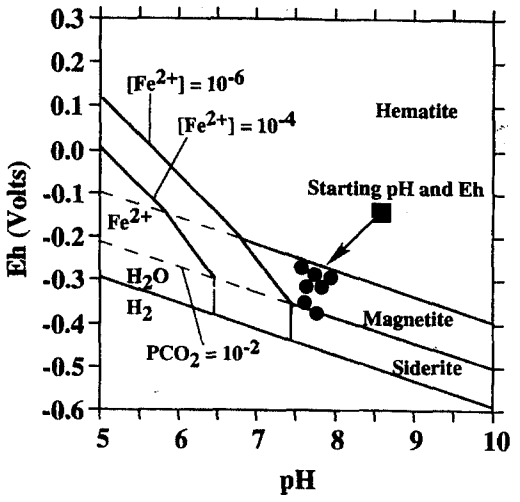

Fig. 4. Eh-pH stability for hematite, magnetite, and siderite in the water-iron-CO_2 system at 25℃ and 1 atm total pressure (modified from Zhang *et al.*, 1997).

organic acids and HCO_3^- (Zhang *et al.*, 1997). Measured pH and Eh values are consistent with the thermodynamic stability of magnetite formation (Zhang *et al.*, 1997) (Fig. 4). Despite the existence of appropriate conditions of magnetite formation on a thermodynamic basis, magnetite did not form at 65℃ in control tubes containing magnetite precursor.

In case of fermentative strain, pH is kept between 7.5 and 9.5 using HEPES and NaOH innoculation during bacterial magnetite growth. The formation of magnetite by dissimilatory iron-reducing bacteria, such as thermophilic bacterium TOR-39, represents biologically-induced mineralization in which the particles are formed extracellualrly as a byproduct of the bacterial respiration. Microbial magnetite formation is the result of biologically-mediated mineralization; that is, the organism alters the local Eh and pH conditions which, in turn, shift local mineral solubility equilibria (Zhang *et al.*, 1998). The mineral formation is mediated by bacteria altering, either the Eh or pH condition or both, and creating conditions of supersaturation with respect to a mineral phase.

Fig. 5. Transmission electron micrographs showing (a) microbial magnetite formation at 12 hour of incubation at 65℃ using magnetite precursors and (b) magnetite formation by bacteria after 25 days of incubation at 65℃.

Mineralogical characterization: Growth of magnetite was at the expense of amorphous iron (Fig. 5a). Magnetite was diamond-shaped and had a sharp edge during early formation. TEM images of magnetite formation in TOR-39 cultures for 25 days incubation at 65℃ showed that well formed ultrafine (50 - 100 nm diameter) magnetic particles were formed (Fig. 5b). Until now, it has been generally agreed that magnetotactic bacteria produce single domain magnetite with unique octahedral shapes, whereas dissimilatory iron-reducing bacteria such as GS-15

form irregular and poorly crystalline magnetite particles that are mostly superparamagnetic (Bazylinski and Moskowitz, 1997).

However, this study showed that thermophilic-iron reducing bacteria, TOR-39, can form well-crystallized magnetite crystals that are single domain. The boundary between superparamagnetic and single domain magnetite particles is 30 nm in diameter, which is based on theoretical calculation (Butler and Banerjee, 1975; Dunlop and Ozdemer, 1997). These magnetite crystals have similar morphology to some particles produced by magnetotactic bacteria. The mechanism of biomineralization of magnetite is presently unknown; however, its study has implications in the practical applications of material science.

Thermophilic bacteria from the deep subsurface produced extracellular single-domain magnetite. Compared to conventional processing techniques such as calcining process, microbial synthesis has several advantages: (1) crystalline products can be obtained directly from precursor at low temperature (65℃) by iron reducing bacteria, (2) the equipment and processing required are simple and the control of the reaction conditions are easy, and (3) direct bacterial production of optimized, single domain particles would eliminate time-consuming milling operations and improve yields, making these materials affordable for such widespread application such as automative shock absorbers, power transmissions, and clutches.

Conclusion

Ultrafine, nearly cubic, and high purity magnetite powder was prepared by microbiologically mediated iron mineralization under low temperature conditions (at 65℃). The biological process rapidly produces copious amounts of nanomater-sized (50 - 100 nm) magnetite that may expand the possible role and utility of such minerals in commercial processes and scientific disciplines. Both the uniformity and the small size of the magnetite crystals pose several fascinating and intriguing questions for the solid state and material scientist because grinding or use of larger crystals is no longer necessary.

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