

Review

## Metal Reduction and Mineral Formation by Fe(III)-Reducing Bacteria Isolated from Extreme Environments

### 철환원 박테리아에 의한 금속 환원 및 광물 형성

Yul Roh (노 열)<sup>1</sup> · Hi-Soo Moon (문희수)<sup>2</sup> · Yungoo Song (송윤구)<sup>2</sup>

<sup>1</sup>Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6038, USA  
E-mail: rohy@ornl.gov

(미국 오크리지 국립연구소, 환경과학실)

<sup>2</sup>Department of Earth System Sciences, Yonsei University, Seoul 120-749, Korea  
(연세대학교 지구시스템과학과)

**ABSTRACT** : Microbial metal reduction influences the biogeochemical cycles of carbon and metals as well as plays an important role in the bioremediation of metals, radionuclides, and organic contaminants. The use of bacteria to facilitate the production of magnetite nanoparticles and the formation of carbonate minerals may provide new biotechnological processes for material synthesis and carbon sequestration. Metal-reducing bacteria were isolated from a variety of extreme environments, such as deep terrestrial subsurface, deep marine sediments, water near hydrothermal vents, and alkaline ponds. Metal-reducing bacteria isolated from diverse extreme environments were able to reduce Fe(III), Mn(IV), Cr(VI), Co(III), and U(VI) using short chain fatty acids and/or hydrogen as the electron donors. These bacteria exhibited diverse mineral precipitation capabilities including the formation of magnetite (Fe<sub>3</sub>O<sub>4</sub>), siderite (FeCO<sub>3</sub>), calcite (CaCO<sub>3</sub>), rhodochrosite (MnCO<sub>3</sub>), vivianite [Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> · 8H<sub>2</sub>O], and uraninite (UO<sub>2</sub>). Geochemical and environmental factors such as atmospheres, chemical milieu, and species of bacteria affected the extent of Fe(III)-reduction as well as the mineralogy and morphology of the crystalline iron mineral phases. Thermophilic bacteria use amorphous Fe(III)-oxyhydroxide plus metals (Co, Cr, Ni) as an electron acceptor and organic carbon as an electron donor to synthesize metal-substituted magnetite. Metal reducing bacteria were capable of CO<sub>2</sub> conversion into sparingly soluble carbonate minerals, such as siderite and calcite using amorphous Fe(III)-oxyhydroxide or metal-rich fly ash. These results indicate that microbial Fe(III)-reduction may not only play important roles in iron and carbon biogeochemistry in natural environments, but also be potentially useful for the synthesis of submicron-sized ferromagnetic materials.

**Key words** : extremophile, Fe(III)-reducing bacteria, metal-reduction, biomineralization

요약 : 미생물에 의한 금속이온의 환원은 탄소와 금속의 생지화학적 순환에 영향을 줄 뿐만 아니라 또한 금속, 방사성원소, 그리고 유기물로 오염된 지하수와 토양의 정화에 있어서 중요한 역할 가능성을 시사하고 있다. 지구의 극한 환경(예: 심해저 퇴적, 알칼리성 호수 등)에서 서식하는 철환원 박테리아를 분리하여 금속이온의 환원과 광물 형성 등의 실험에 이용하여 본 결과에 의하면, 이들 철환원 박테리아는 Fe(III), Mn(IV), Cr(VI), Co(III), U(VI) 이온 등을 환원시킬 뿐만 아니라, 자철석

( $\text{Fe}_3\text{O}_4$ ), 능철석( $\text{FeCO}_3$ ), 방해석( $\text{CaCO}_3$ ), 능망간석( $\text{MnCO}_3$ ), 비비아나이트 [ $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ], 우라니나이트( $\text{UO}_2$ ) 등의 광물을 형성한다. 철 환원 박테리아에 의한 광물 형성과 금속이온의 환원에 영향을 미치는 주요소는 대기의 조성, 화학 조성, 및 박테리아의 종이다. 호열성 철환원 박테리아는 철수화물과 금속이온(Co, Cr, Ni) 등을 동시에 환원시켜 금속 치환된 자철석을 합성하며, 또한 석탄 회 등을 이용하여 탄산염 광물을 형성하여 대기 중의 이산화탄소를 고정하는 역할을 하기도 한다. 따라서 미생물에 의한 금속이온이 환원은 자연계에서 철과 탄소의 지화화학적 순환에 영향 미치며, 또한 미생물에 의한 자철석의 합성은 산업적으로 많은 이용가치가 있을 것으로 본다.

주요어 : 극한환경, 철환원 박테리아, 금속이온 환원, 생광화작용

## Introduction

Microbial Fe(III) reduction plays an important role in the cycling of iron(III) and organic matter in natural environments (Canfield *et al.*, 1993; Lovley, 1991, 1993, 1995; Nealson and Saffarini, 1994). Many Fe(III)-reducing bacteria can also reduce a variety of other metals and radionuclides including uranium(VI) (Lovley, 1995; Lovley and Phillips, 1992), technetium(VII) (Lloyd and Macaskie, 1996), cobalt(III) (Zhang *et al.*, 1996; Gorby *et al.*, 1998), chromium(VI) (Zhang *et al.*, 1996; Lovley, 1993), arsenic(V) (Laverman *et al.*, 1995), and selenium(VI) (Oremland, 1994) as well as degrade petroleum hydrocarbons (Lovley *et al.*, 1989; Lovley and Lonergan., 1990).

Fe(III)-reducing bacteria participate various geochemical processes, such as weathering and formation of minerals (Lovley *et al.*, 1987; Bazylinski *et al.*, 1988; Ferris *et al.*, 1994; Zhang *et al.*, 1997, 1998; Fredrickson *et al.*, 1998), formation of ore deposits (Nealson and Myers, 1990), and cycling of organic matter (Lovley, 1991; Nealson and Saffarini, 1994). Fe(III)-reducing bacteria can precipitate or transform amorphous or crystalline Fe(III) oxides into other crystalline iron phases, such as magnetite ( $\text{Fe}_3\text{O}_4$ ), siderite ( $\text{FeCO}_3$ ), vivianite [ $\text{Fe}_3(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ ], and maghemite ( $\text{Fe}_2\text{O}_3$ ) (Zhang *et al.*, 1997, 1998; Fredrickson *et al.*, 1998; Roh and Moon, 2000, 2001a, 2001b).

Microbial Fe(III) reduction is considered to be an early form of respiration and may have implications in the evolution of life on Earth (Nealson and Myers, 1990; Lovley, 1991;

Nealson and Saffarini, 1994; Liu *et al.*, 1997). Most of the oxidation of organic matter coupled to Fe(III) reduction in Precambrian sedimentary environments was probably the results of dissimilatory Fe(III)-reducing microorganisms which could completely oxidize organic compounds to carbon dioxide with Fe(III) as the sole electron acceptor (Lovley, 1991, 1993; Nealson and Myers, 1990; Baur *et al.*, 1985; Walker, 1984, 1987). Deposition of large quantities of siderite and magnetite in low temperature ( $<100^\circ\text{C}$ ) environments may be complemented by bacterial iron reduction (Nealson and Myers, 1990).

Extremophiles, microorganisms that grow under extreme conditions, prefer such environments as very hot (thermophiles), very cold (psychrophiles), increased salt concentrations (halophiles), very acidic (acidophiles), or highly alkaline (alkaliphiles) environments (Bowman *et al.*, 1997; Colwell *et al.*, 1997; Liu *et al.*, 1997; Slobodkin *et al.*, 1997). Their habitat may be cold polar seas, deep-sea sediments, deep subsurface sedimentary rock, high saline lakes, carbonate springs, or soda lakes. Extremophiles are of great interest to researchers trying to develop improved ways for the synthesis of useful materials, radionuclide immobilization at contaminated filed sites, or hydrogen production for energy. Information about microbial metal reduction under thermophilic, psychrophilic, and alkaliphilic conditions is limited (Liu *et al.*, 1997; Stapleton *et al.*, 2002; Roh *et al.*, 2002; Zhang *et al.*, 1999; Ye *et al.*, 2002). Previous studies reported isolation of phylogenetically different thermophilic and psychrophilic enrich-

ment cultures capable of reducing Fe(III) and forming Fe(II)-containing iron minerals (Liu *et al.*, 1997; Stapleton *et al.*, 2002; Roh and Moon, 2001a; Roh *et al.*, 2002). As an extension of this study, we continue to examine metal reduction and biomineralization by thermophilic, psychrophilic, and alkaliphilic bacteria isolated from extreme environments.

The objective of this paper summarizes recent research results on metal reduction and biomineralization by psychrophilic, thermophilic, and alkaliphilic Fe(III)-reducing bacteria as well as the utilization of biomineralization processes for magnetite nanoparticle synthesis and carbon sequestration.

### Fe(III) Reducing Bacteria Isolated From Extreme Environments

Table 1 shows thermophilic, psychrophilic,

and alkaliphilic Fe(III) reducing bacteria isolated by Oak Ridge National Laboratory (ORNL) researchers from a variety of cold, hot, and alkaline environments, such as deep marine sediments, sea water near hydrothermal vent, deep subsurface environments, and a leachate-pond containing high levels of salt and boric acid.

Thermophilic Fe(III)-reducing bacteria were isolated from deep subsurface sedimentary rocks including Taylorsville Triassic Basin and Piceance Cretaceous Basin (Table 1). Geological and hydrological evidences suggested that the deep basins were hydrogeologically separated from the surface for millions of years. The current temperature of sampling depth (860~2800 m below land surface) ranged from 42~85°C and fluid pressure ranged from 30 to 35 Mpa (Coldwell *et al.*, 1997; Boone *et al.*, 1995; Tseng *et al.*, 1996). The sampling processes and

**Table 1.** Microbial isolates investigated at Oak Ridge National Laboratory

Isolates	Growth condition	Genus and species	Site Description	Geology/Sample Type	References
TOR-39	Thermophilic (40~75°C)	<i>Thermoanaerobacter ethanolicus</i>	Taylorsville Triassic Basin, Northern Virginia	shale, siltstone, and sandstone	Liu <i>et al.</i> , 1997
C1	Thermophilic (40~75°C)	<i>Thermoanaerobacter ethanolicus</i>	Piceance Basin Wasatch Formation, Western Colorado	cemented sandstone; cross-bedded siltstones and shales	Liu <i>et al.</i> , 1997
X513 X514 X561	Thermophilic (40~75°C)	<i>Thermoanaerobacter ethanolicus</i>	Piceance Basin Wasatch Formation, Western Colorado	cemented sandstone; cross-bedded siltstones and shales	Roh <i>et al.</i> , 2002
NV-1	Psychrotolerant (0~37°C)	<i>Shewanella alga</i>	Naha vents, Coast of Hawaii	Iron-rich microbial material associated with a hydrothermal vent	Stapleton <i>et al.</i> , 2002
W3-6-1 W3-7-1	Psychrotolerant (0~37°C)	<i>Shewanella pealeana</i>	Deep Pacific Ocean Marine Sediments	Marine sediment	Stapleton <i>et al.</i> , 2002
CBS-011	Alkaliphilic pH = 8.0~11	<i>Alkaliphilus transvaalensis</i>	Boron-rich sites at the U.S. Borax mine in Borax, CA	Leachate-pond containing high level of salt (~12 % NaCl) and boric acid (2~8 g/L B) at pH 9~10.	Ye <i>et al.</i> , 2002

quality controls were described elsewhere (Liu *et al.*, 1997; Roh *et al.*, 2002). Phylogenetic analysis indicate that the thermophilic Fe(III)-reducing bacteria were closely related to *Thermoanaerobacter ethanolicus* (Liu *et al.*, 1997; Roh *et al.*, 2002). Psychrotolerant Fe(III)-reducing bacteria were isolated from deep marine sediments in the Pacific Ocean off of the Washington state, and sea water near hydrothermal vents off the coast of Hawaii (Table 1). Phylogenetic analysis indicated that the psychrotolerant Fe(III)-reducing bacteria were closely related to the members of *Shewanella* genus, *Shewanella alga* and *Shewanella pealeana* (Roh and Moon, 2001a; Roh *et al.*, 2001c; Stapleton *et al.*, 2002). Alkaliphilic Fe(III)-reducing bacteria were isolated from a leachate pond containing high levels of salt (~12% NaCl) and boric acid (2~8 g/L B) at pH 9.0~10.0 (Ye *et al.*, 2002). Phylogenetic analysis indicate that the alkaliphilic Fe(III)-reducing bacteria were closely related to *Alkaliphilus transvaalensis*, an alkaliphilic bacterium isolated from a deep South African gold mine. These results suggest that the microbial population responsible for Fe(III) reduction were

different among these sampling locations, in-situ temperatures, and salinity. The results also indicated that microbially mediated iron reduction is likely widespread in cold (<10°C), hot (>45°C), and alkaline (>3M salt concentration) natural environments.

## Metal Reduction and Biomineralization

Fe(III)-reducing microorganisms isolated from extreme environments have the capability of reducing various contaminant metals and radionuclides (Table 2), which can lead to immobilization of these contaminants in subsurface environments. Thermophilic microorganisms isolated from Taylorsville and Piceance Basin were found to reduce Co(III), Cr(VI), Fe(III), Mn(IV), and U(VI) to reduced species, such as Co(II), Cr(III), Fe(II), Mn(II), and U(IV) at temperature of up to 75°C. Psychrotolerant and alkaliphilic Fe(III)-reducing bacteria were also able to reduce Co(III), Cr(VI), Fe(III), and Mn(IV) to reduced species, such as Co(II), Cr(III), Fe(II), and Mn(II).

The thermophilic bacteria were able to reduce several Fe(III)-species including Fe(III)-citrate,

**Table 2.** Metal reduction and mineral formation by thermophilic, psychrotolerant, and alkaliphilic Fe(III)-reducing bacteria

Isolates	Growth temperature range	Electron donors	Electron acceptors	Minerals formed
TOR-39 X513 X561	40~75°C	Lactate Pyruvate Acetate Formate Glucose	Fe(III) Mn(VI) U(VI) Cr(VI) Co(III)	Magnetite (Fe <sub>3</sub> O <sub>4</sub> ) Siderite (FeCO <sub>3</sub> ) Maghemite (Fe <sub>2</sub> O <sub>3</sub> ) Uraninite (UO <sub>2</sub> ) Rhodochrosite (MnCO <sub>3</sub> )
C1 X514	40~75°C	Lactate Pyruvate Acetate Formate Glucose Hydrogen	Fe(III) Mn(VI) U(VI) Cr(VI) Co(III)	Magnetite (Fe <sub>3</sub> O <sub>4</sub> ) Siderite (FeCO <sub>3</sub> ) Rhodochrosite (MnCO <sub>3</sub> )
NV-1 W3-6-1 W3-7-1	0~37°C	Lactate Formate Pyruvate Hydrogen	Fe(III) Co(III)	Magnetite (Fe <sub>3</sub> O <sub>4</sub> ) Siderite (FeCO <sub>3</sub> ) Vivianite [Fe <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> · 8H <sub>2</sub> O]
CBS-011	25°C, pH up to 11	Lactate Acetate Hydrogen	Fe(III) Co(III) Cr(VI)	Magnetite (Fe <sub>3</sub> O <sub>4</sub> ) Siderite (FeCO <sub>3</sub> ) Vivianite [Fe <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> · 8H <sub>2</sub> O]

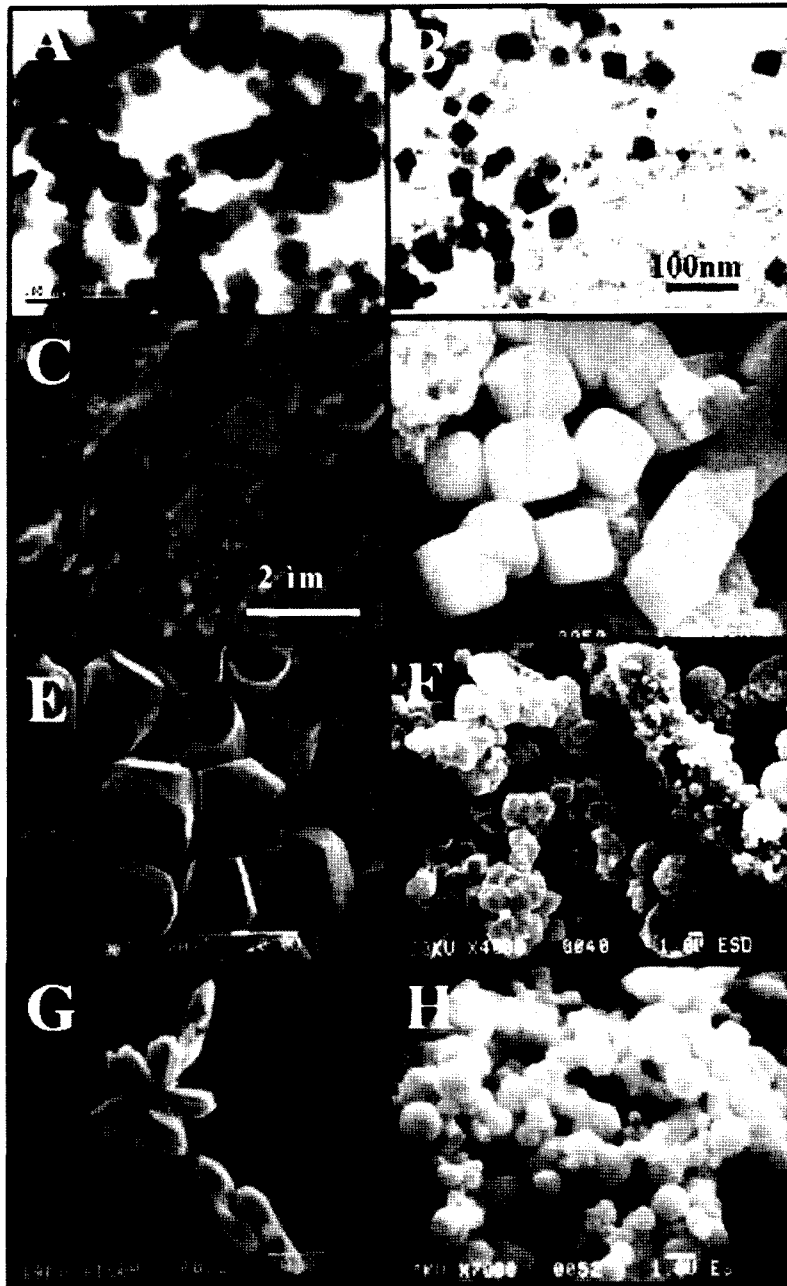
Fe(III)-EDTA, and amorphous Fe(III)-oxides using acetate, lactate, formate, glucose, pyruvate, and hydrogen as the electron donors. The psychrotolerant and alkaliphilic Fe(III)-reducing bacteria were also able to reduce the Fe(III)-species using lactate, formate, pyruvate, and hydrogen as the electron donors. However, the psychrotolerant cultures (NV-1, W3-6-1, W3-7-1) could not use acetate as an electron donor to reduce the Fe(III)-species.

Table 2 and Fig. 1 show X-ray diffraction (XRD) analyses and electron micrographs of crystalline minerals formed by the Fe(III)-reducing bacteria, respectively. The thermophilic and psychrotolerant Fe(III)-reducing bacteria were capable of reducing amorphous Fe(III) oxides and transform them into Fe(II) containing minerals, such as magnetite ( $\text{Fe}_3\text{O}_4$ ) and siderite ( $\text{FeCO}_3$ ) (Table 2; Fig. 1A, B, and C). The thermophilic bacteria reduced uranyl carbonate [ $\text{UO}_2(\text{CO}_3)_3^{4-}$ ] and  $\text{MnO}_2$  and formed sparingly soluble minerals including uraninite ( $\text{UO}_2$ ) (Fig. 1H) and rhodochrosite ( $\text{MnCO}_3$ ) (Fig. 1E). Psychrotolerant and alkaliphilic Fe(III)-reducing bacteria precipitated vivianite [ $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ] from  $\text{PO}_4$  (25 mM) containing media when soluble Fe(III), Fe(III)-citrate and Fe(III)-EDTA were used as the electron acceptor. Vivianite formation was only observed when soluble Fe(III), Fe(III)-citrate and Fe(III)-EDTA, were used as the electron acceptor.

Geochemical and environmental factors, such as atmospheres, chemical milieu, salinity, pH, and incubation temperature affected the extent of Fe(III) reduction as well as the mineralogy of the crystalline iron mineral phases. Magnetite was dominantly formed under  $\text{N}_2$  and  $\text{H}_2$  atmospheres (Fig. 1A and B). Siderite formation was dominant under an  $\text{H}_2$ - $\text{CO}_2$  atmosphere ( $p\text{CO}_2 = 10^{-2}$ ) (Fig. 1D). A mixture of magnetite and siderite was formed in the presence of a  $\text{N}_2$ - $\text{CO}_2$  headspace ( $p\text{CO}_2 = 10^{-2}$ ). Predominant mineral formed in  $\text{HCO}_3^-$  buffered medium (>120 mM) with lactate was siderite (Fig. 1D). Temperature profile of microbial Fe(III) reduction showed that the thermophilic Fe(III)-

reducing bacteria reduced amorphous Fe(III) oxyhydroxide to magnetite in the temperature range of 40 to 75 °C (Liu *et al.*, 1997; Roh and Moon, 2000) and psychrotolerant Fe(III)-reducing bacteria formed magnetite at temperature ranging from 0 to 37 °C using amorphous Fe(III) oxyhydroxide as an electron acceptor (Roh and Moon, 2001a, Stapleton *et al.*, 2002). Thermophilic and psychrotolerant Fe(III)-reducing bacteria reduced amorphous Fe(III) oxyhydroxide to magnetite at pH range between 6.9 and 8.5 (Roh and Moon, 2000). But alkaliphilic Fe(III)-reducing bacteria, CBS-011, reduced Fe(III)-species, Fe(III)-citrate and Fe(III)-EDTA, at pH up to 10.5 and formed vivianite (Fig. 1G) in phosphate buffered media (>25 mM  $\text{PO}_4$ ).

Thermophilic Fe(III)-reducing bacteria formed Ni-substituted magnetite using amorphous Fe(III) oxyhydroxide plus metals ( $\text{Ni}^{2+}$ ) as an electron acceptor and organic carbon as an electron donor without a soluble electron shuttle, anthraquinone disulfonate (AQDS) (Fig. 1C). The microbial processes produced copious amounts of nm-sized, metal-substituted magnetite crystals. Chemical and X-ray powder diffraction analyses showed that these metals, such as Co, Cr, Ni substituted for Fe in biologically facilitated magnetite (Roh and Moon, 2001b; Roh *et al.*, 2001a). Psychrotolerant and thermophilic Fe(III)-reducing bacteria formed calcite ( $\text{CaCO}_3$ ) (Fig. 1F) and siderite ( $\text{FeCO}_3$ ) using metal-rich fly ash and lime in the presence of  $\text{CO}_2$  headspace gases ( $p\text{CO}_2 = 10^{-2}$ ) as well as in  $\text{HCO}_3^-$  buffered media (30~210 mM). Chemical analysis of the culture media after the incubation revealed that the leaching of Ca and Fe from fly ash was significantly reduced in the presence of  $\text{CO}_2$  atmosphere and in  $\text{HCO}_3^-$  buffered media (140~210 mM) (Roh *et al.*, 2001b). This effect was a consequence of microbial metal reduction and the precipitation of siderite in the presence of appropriate electron donors, such as lactate and glucose. The precipitation of carbonate minerals is likely facilitated by the organisms altering local Eh, pH, and nucleation conditions (Roh *et al.*,



**Fig. 1.** Scanning and transmission electron micrographs of minerals formed by the Fe(III)-reducing bacteria: (A) Magnetite ( $\text{Fe}_3\text{O}_4$ ) formed by NV-1 at 28°C (B) Magnetite ( $\text{Fe}_3\text{O}_4$ ) formed by TOR-39 at 65°C; (C) Ni-substituted magnetite formed by TOR-39 at 65°C; (D) Siderite ( $\text{FeCO}_3$ ) formed by NV-1 at 14°C; (E) Rhodochrosite ( $\text{MnCO}_3$ ) formed by X514 at 60°C; (F) Calcite ( $\text{CaCO}_3$ ) formed by TOR-39 at 65°C; (G) Vivianite [ $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ] formed by CBS-011 at 25°C; (H) Uraninite ( $\text{UO}_2$ ) formed by X514 at 60°C.

2001b). Biological carbonate mineral formation using fly ash and lime materials indicated that bacteria may complement the capture of carbon dioxide from fossil fuel plants while potentially stabilizing fly ash wastes.

### Geochemical and Biotechnological Implications

In extreme environments, microbial oxidation of organic matter or hydrogen coupled to the reduction of Fe(III) and other metals is an important process for the bioremediation and natural attenuation strategy for waters and sediments contaminated with heavy metals and radionuclides. The biotechnological applications of Fe(III)-reducing bacteria isolated from extreme environments include (1) remediation of subsurface water and sediments contaminated with metals, radionuclide and organics, (2) synthesis of magnetite nanoparticles, and (3) utilization for carbon sequestration.

#### Geochemical Implications

Fe(III) reduction and mineral formation by Fe(III)-reducing bacteria isolated from extreme environments support the hypothesis that metal-reducing bacteria, such as *Thermoanaerobacter* and *Shewanella* species are important components in the overall biogeochemical cycling of iron, manganese, and other elements in subsurface sediments. Microbial Fe(III) respiration influences not only the speciation of iron in anoxic marine sedimentary environments but also the carbon cycles and the fate of a variety trace metals and nutrients.

The data suggested that the Fe(III)-reducing bacteria in subsurface environments may have the capability of forming magnetite (Fe<sub>3</sub>O<sub>4</sub>) and siderite (FeCO<sub>3</sub>) by growing on short chain fatty acids or hydrogen as an energy source various geochemical conditions. The biomineralization of Fe(II)-containing minerals is a complex process influenced by multiple biological and chemical factors as they are reduced by

Fe(III)-reducing bacteria. The major factors controlling iron biomineralization is the compositions and concentrations of cations and anions, medium pH, atmosphere, and other conditions, such as incubation temperature and time. Increasing bicarbonate concentration and pCO<sub>2</sub> in the chemical milieu resulted in increased proportions of siderite relative to magnetite. In fact, the presence of the inorganic ligand, CO<sub>3</sub><sup>2-</sup>, facilitated the reduction of amorphous Fe(III) oxyhydroxide, probably by creating conditions which thermodynamically favors reduction. This study showed that microbial magnetite formation is favored by neutral to slightly alkaline and reducing conditions. If the pH becomes too low (i.e., <6.0) magnetite will not form because the high proton concentrations may incline the decomposition of magnetite to Fe(OH)<sub>3</sub> (Roh *et al.*, 2001a).

This study showed that the biologically facilitated formation of magnetite and siderite using amorphous Fe(III) oxyhydroxide or amorphous Fe(III) oxyhydroxide added heavy metals (Co, Cr, Ni) as the electron acceptor does not require the addition of exogenous electron carrier substances, such as humic acids. The ability of Fe(III)-reducing bacteria to reduce amorphous Fe(III) oxide plus heavy metals and to form magnetite and siderite has far reaching implications for microbial processes in subsurface sediments where Fe(III) oxides associated with heavy metals may represent the largest mass of electron acceptor. Microbial formation of carbonate mineral and iron oxides may play an important role in trace metal immobilization because metals (Co, Cr, Ni) are readily incorporated into the magnetite and siderite crystal structure when the Fe(III) reducing bacteria formed magnetite (Fredrickson *et al.*, 2001; Roh *et al.*, 2001a; Roh and Moon 2001b).

Anaerobic extremophiles including thermophiles, psychrophiles, and alkaliphiles from isolated diverse subsurface environments exhibited the ability of reducing Co(III), Cr(VI), Fe(III), Mn(IV), and U(VI) using as an electron acceptor to Co(II), Cr(III), Fe(II), Mn(II), and

U(IV). Microbial reduction of uranyl carbonate and precipitation of uraninite ( $\text{UO}_2$ ) has the potential to restrict the risk and the transport of uranyl-carbonate in aqueous environments by producing sparingly soluble phases. The use of Fe(III) and other metals by certain microbial groups as terminal electron acceptors for anaerobic respiration is of particular relevance to bioremediation and natural attenuation of heavy metals and radionuclides (Lovley, 1995; Zhang *et al.*, 1996). In addition to direct precipitation of redox-sensitive metals, the utilization of organic matter to produce carbonates and crystalline magnetite may also contribute to indirect sequestration of metals and radionuclides by facilitating precipitation of metal-carbonates or magnetic co-precipitation of non redox-sensitive radionuclides. Nitroaromatics and chlorinated solvents can be abiotically reduced by microbially generated Fe(II) ions and formed Fe(II) containing minerals (Heijman *et al.*, 1993, 1995; Lovley and Lonergan, 1990).

#### *Biotechnological Implications*

Given the abundance of Fe in anaerobic sedimentary systems, the capacity of Fe(III)-reducing bacteria to precipitate siderite using iron oxides and dissolved Fe ion species could have a significant impact on carbon sequestration. In addition to precipitation of iron carbonate mineral, the microbial utilization of organic matter and hydrogen may also contribute to direct or indirect precipitation of redox sensitive metals in subsurface environments.

The capability of iron reducing bacteria to precipitate carbonate minerals using fly ash creates the possibility of more effective  $\text{CO}_2$  sequestration than would be possible with photosynthetic systems in alkaline ponds. In the environments with high bicarbonate concentrations, the microbial production of Fe(II) from Fe-rich fly ash may stimulate siderite formation. Ca-rich fly ash or lime facilitated calcium carbonate crystallization by the organisms alter-

ing local Eh, pH, and nucleation conditions. Biological carbonate mineral formation using fly ash and lime materials indicated that bacteria may complement the capture of carbon dioxide from fossil fuel plants while potentially stabilizing fly ash wastes.

The use of bacteria to facilitate the production of magnetite nanoparticles may provide new biotechnological procedures for material synthesis (Roh and Moon 2000, 2001b; Roh *et al.*, 2001a). The microbial processes produced copious amounts of nm-sized, metal-substituted magnetite crystals. These biosolid-state reactions may describe novel solid-state processes and maybe potentially useful to the development and design of submicron-sized ferromagnetic materials. 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-facilitated production of metal-substituted magnetites may include: (1) the biologically-facilitated production of magnetite does not require the addition of exogenous electron carrier substances such as humic acids (Fredrickson *et al.*, 2001); (2) particles can in principle be grown to a size that would not be feasible if the particles formed inside the cell; (3) the bacteria are not sacrificed in order for the product to be harvested; and (4) agitation, fluid flow, or magnetic forces may be capable of dislodging the particles when a desired size is attained. Knowledge concerning such novel bio-solid processes may be important in the development and design of submicron-sized ferromagnetic materials.

#### **Acknowledgments**

This research was sponsored by Oak Ridge National Laboratory which is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725.



## References

- Baur, M.E., Hayes, J.M., Studley, S.A., and Walter, M.R. (1985) Millimeter-scale variations of stable isotope abundances in carbonates from banded iron-formations in the Hamersley Group of Western Australia. *Economic Geol.*, 80, 270-282.
- Bazylnski, D.A., Frankel, R.B., and Jannasch, H.W. (1988) Anaerobic magnetite production by a marine magnetotactic bacterium. *Nature*, 334, 518-519.
- Boone, D.R., Liu, Y., Zhao, Z.J., Balkwill, D.L., Drake, G.R., Stevens, T.O., and Aldrich, H.C. (1995) *Bacillus infernus* sp. nov., an Fe(III)- and Mn(IV)-reducing anaerobe from the deep terrestrial subsurface. *Intern. J. Sys. Bacteriol.*, 45, 441-448.
- Bowman, J.P., McCammon, S.A., Brown, M.V., Nichols, D.S., and McMeekin, T.A. (1997) Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl. Environ. Microbiol.*, 63, 3068-3078.
- Canfield, D.E., Jorgensen, B.B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N.B., Thamdrup, B., Hansen, J.W., Nielsen, L.P., and Hall, P.O.J. (1993) Pathways of organic carbon oxidation in three continental margin sediments. *Marine Geol.*, 113, 27-40.
- Colwell, F.S., Onstott, T.C., Delwiche, M.E., Chandler, D., Fredrickson, J.K., Yao, Q.J., McKinley, J.P., Bone, D.R., Griffiths, R., Phelps, T.J., Ringelberg, D., White, D.C., LaFreniere, L., Balkwill, D., Lehman, R.M., Konisky, J., and Long, P.E. (1997) Microorganisms from deep, high temperature sandstones: Constraints on microbial colonization. *FEMS Microbiol. Rev.*, 20, 425-435.
- Fredrickson, J.K., Zachara, J.M., Kennedy, D.W., Dong, H., Onstott, T.C., Hinman, N.W., and Li, S. (1998) Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. *Geochim. Cosmochim. Acta*, 62, 3239-3257.
- Fredrickson, J.K., Zachara, J.M., Kukkadapu, R.K., Gorby, Y.A., 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.
- Ferris, F.G., Wiese, R.G., and Fyfe, W.S. (1994) Precipitation of carbonate minerals by microorganisms: Implications for silicate weathering and the global carbon dioxide budget. *Geomicrobiol. J.*, 12, 1-13.
- Gorby, Y.A., Caccavo, F.Jr., and Bolton, H.Jr. (1998) Microbial reduction of cobalt(III) EDTA<sup>-</sup> in the presence and absence of manganese(IV) oxide. *Environ. Sci. Technol.*, 32, 244-250.
- Heijman, C.G., Holliger, C., Glaus, M.A., and Schwarzenbach, R.P. (1993) Abiotic reduction of 4-chlorobenzene to 4-chloroaniline in a dissimilatory iron-reducing enrichment culture. *Appl. Environ. Microbiol.*, 59, 4350-4353.
- Heijman, C.G., Grieder, E., Holliger, C., and Schwarzenbach, R.P. (1995) Reduction of nitroaromatic compounds coupled to microbial iron reduction in laboratory aquifer columns. *Environ. Sci. Technol.*, 29, 775-783.
- Laverman, A.M., Switzer Blum, J., Schaefer, J.K., Phillips, E.J.P., Lovley, D.R., and Oremland, R.S. (1995) Growth of strain SES-3 with arsenate and other diverse electron acceptors. *Appl. Environ. Microbiol.*, 61, 3556-3561.
- Liu, S., Zhou, J., Zhang, C.L., 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.
- Lloyd, J.R. and Macaskie, L.E. (1997) Microbially-mediated reduction and removal of technetium from solution. *Res. Microbiol.*, 148, 530-532.
- Lovley, D.R. (1991) Dissimilatory Fe(III) and Mn(III) reduction. *Microbiol. Rev.*, 55, 259-287.
- Lovley, D.R. (1993) Dissimilatory metal reduction. *Ann. Rev. Microbiol.*, 47, 263-290.
- Lovley, D.R. (1995) Bioremediation of organic and metal contaminants with dissimilatory metal reduction. *J. Ind. Microbiol.*, 14, 85-93.
- Lovley, D.R., Stolz, J.F., Nord, G.L.Jr., and Phillips, E.J.P. (1987) Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. *Nature*, 330, 252-254.
- Lovley, D.R., Phillips, E.J.P., and Lonergan, D.J. (1989) Hydrogen formation and formate oxidation coupled to dissimilatory reduction of iron or manganese by *Alteromonas putrefaciens*. *Appl. Environ. Microbiol.*, 55, 700-706.
- Lovley, D.R. and Lonergan, D.J. (1990) Anaerobic oxidation of toluene, phenol, and p-cresol by the dissimilatory iron-reducing organism, GS-15. *Appl. Environ. Microbiol.*, 56, 1858-1864.
- Lovley, D.R. and Phillips, E.J.P. (1992) Bioreme-

- diation of uranium contamination with enzymatic uranium reduction. *Environ. Sci. Technol.*, 26, 2228-2234.
- Nealson, K.H. and Myers, C.R. (1990) Iron reduction by bacteria: A potential role in the genesis of banded iron formation. *Am. J. Sci.*, 290A, 35-45.
- Nealson, K.H. and Saffarini, D. (1994) Iron and manganese in anaerobic respiration: Environmental significance, physiology, and regulation. *Ann. Rev. Microbiol.*, 48, 311-343.
- Oremland, R.S. (1994) Biogeochemical transformation of selenium in anoxic environments. In: Frankenberger, W.T.J. and Benson S.N. (eds.), *Selenium in the Environments*, Marcel Dekker, Inc. New York. pp. 389-419.
- Roh, Y. and Moon, H.-S. (2000) Microbial synthesis of magnetite powder by iron reducing bacteria. *J. Min. Soc. Korea.*, 13, 65-72.
- Roh, Y. and Moon, H.-S. (2001a) Iron reduction by a Psychrotolerant Fe(III)-reducing bacteria. *Geosci. J.*, 5, 183-190.
- Roh, Y. and Moon, H.-S. (2001b) Microbial synthesis of cobalt-substituted magnetite nanoparticles by iron reducing bacteria. *J. Min. Soc. Korea.*, 14, 111-118.
- Roh, Y., Lauf, R.J., McMillan, A.D., Zhang, C., Rawn, C.J., Bai, J., and Phelps, T.J. (2001a) Microbial synthesis and the characterization of metal-substituted magnetite. *Solid State Com.*, 118, 529-534.
- Roh, Y., Phelps, T.J., McMillan, A.D., and Lauf, R.J. (2001b) Utilization of biomineralization processes with fly ash for carbon sequestration. In: *Proceeding of U.S. DOE-First National Conference on Carbon Sequestration*, U.S. Department of Energy National Energy Technology Laboratory, Pittsburgh, PA.
- Roh, Y., Vali, H., Stapleton, R.D., Phelps, T.J., and Zhou, J. (2001c) Single-domain magnetite formation by a psychrotolerant iron reducing bacterium. American Society for Microbiology 101th General Meeting (Abstract), American Society for Microbiology, Washington, DC, pp. 517.
- Roh, Y., Liu, S., Li, G., Huang, H., Phelps, T.J., and Zhou, J. (2002) Isolation and characterization of metal-reducing *Thermoanaerobacter* strains from deep subsurface environments, *Appl. Environ. Microbiol.*, (in review).
- Slobodkin, A., Reysenbach, A.L., Strutz, N., Dreier, M., and Wiegel, J. (1997) *Thermoterrabacterium ferrireducens* gen. nov., sp. nov., a thermophilic anaerobic dissimilatory Fe(III)-reducing bacterium from a continental hot spring. *Int. J. Sys. Bacteriol.*, 47, 541-547.
- Stapleton, R.D.Jr., Sabree, Z.L., Palumbo, A.V., Moyer, C., Devol, A., Roh, Y., and Jhou, J. (2002) Metabolic capabilities and distribution of *Shewanella* isolates from diverse marine environments. *Limnol. Ocean.* (in review).
- Tseng, H., Onstott, T.C., Burruss, R.C., and Miller, D.S. (1996) Constraints on the thermal history of Taylorsville Basin, Virginia, U.S.A., from fluid-inclusion and fission-track analysis: Implications for subsurface geomicrobiology experiments. *Chem. Geol.*, 127, 297-311.
- Walker, J.C.G. (1984) Subtoxic diagenesis in banded iron formation. *Nature*, 309, 340-342.
- Walker, J.C.G. (1987) Was the Archean biosphere upside down. *Nature*, 329, 710-712.
- Ye, Q., Roh, Y., Phelps, T.J., Zhou, J., Zhang, C., and Fields, M.W. (2002) Ferric iron-reducing bacterium from alkaline leachate ponds containing salt and boric acid. American Society for Microbiology 102th General Meeting (Abstract), American Society for Microbiology, Washington, DC, pp 248.
- Zhang, C., Liu, S., Logan, J., Mazumer, R., and Phelps, 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) Physicochemical, mineralogical, and isotopic characterization of magnetite-rich iron oxides formed by thermophilic iron-reducing bacteria. *Geochim. Cosmochim. 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. Mineral.*, 83, 1409-1418.
- Zhang, C., Stapleton, R.D., Zhou, J., Palumbo, A.V., and Phelps, T. J. (1999) Iron reduction by psychrotrophic enrichment cultures, *FEMS Microbiol. Ecol.*, 30, 367-371.

2002년 9월 2일 원고접수, 2002년 9월 11일 게재승인.