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Metal reduction and biomineralization by an alkaliphilic metal-reducing bacterium, *Alkaliphilus metalliredigens* (QYMF)

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ABSTRACT: Microbial metal reduction has the potential for immobilizing toxic metals and radionuclides in diverse environments. Little is known about metal reduction and immobilization under extreme conditions, and only recently bacterial reduction of metals has been demonstrated under extremely alkaline conditions. The objective of this study was to examine metal reduction and mineral formation using an alkaliphilic bacterium, *Alkaliphilus metalliredigens* (QYMF), isolated from a leachate-pond containing high levels of salt (Na concentration = 440–12,100 mg/L) and boron (2,000–3,000 mg/L) at pH 9.0-10.0. The bacterium was able to use lactate, acetate, and hydrogen as alternative electron donors and Fe(III)-citrate, Fe(III)- ethylenediaminetetraacetate (EDTA), selenate (SeO_4^{2-}), chromate (CrO_4^{2-}), and Co(III)-EDTA as electron acceptors at medium pH=9.5. The reduction of Fe(III)-citrate and Fe(III)-EDTA in the presence of K_2HPO_4 and boron resulted in the precipitation of vivianite [$Fe_3(PO_4)_2{\cdot}8H_2O$]. Formation of sparingly soluble iron phosphates, mediated by the alkaliphilic Fe(III)-reducing bacterium, sequestered iron, phosphate, and other metals into more stable and less toxic forms. These results suggest that bioremediation of metal-contaminated alkaline environments may be feasible, and that the process of metal-reduction may occur in alkaline habitats.

Key words: bacteria, biomineralization, metal reduction, alkaliphile, bioremediation

1. INTRODUCTION

Bacterial metal reduction has widened the realm of life-supporting biological reactions (Nealson and Cox, 2002), and has been implicated as an important biogeochemical process on early Earth (Liu et al., 1997; Straub et al., 2001; Weigel and Hanel, 2002). Microorganisms are known to mediate the reduction and immobilization of radionuclides and toxic metals, including the metal-reducing *Geobacter* sp. (Lovely et al., 1991), *Shewanella* sp. (Fredrickson et al., 2001; Stapleton et al., 2005; Roh et al., 2006), *Clostridium* sp. (Francis et al., 2002), and *Thermoanaerobacter* sp. (Zhang et al., 1996; Liu et al., 1997; Roh et al., 2001, 2002). They exhibited the ability to reduce Co(III), Cr(VI), Fe(III), Mn(IV), Tc(VII), Se(VI), and U(VI) as electron acceptors to Co(II), Cr(III), Fe(II), Mn(II), Tc(IV), Se(0), and U(IV), respectively (Zhang et al., 1996; Liu et al.,1997; Roh et al., 2001, 2002, 2006). Microbial reduction of toxic metals and radionuclides as well as precipitation of reduced toxic metals [i.e., Se(0)] and radionuclides [i.e., UO_2] has the potential to restrict the risk and the transport of radionuclides and toxic metals in aqueous environments by producing sparingly soluble phases.

Microorganisms participate in a variety of geochemical processes such as weathering and formation of minerals (Lovley, 1991; Zhang et al., 1997: Fredrickson et al., 1998; Roh et al., 2003), formation of iron ore deposits and cycling of organic matter (Lovley, 1991, 1993; Roh et al., 2003). Many species of microorganisms, mainly anaerobic bacteria, are capable of reducing Fe(III) in amorphous Fe(III) oxides (Zhang et al., 1997; Fredrickson et al., 1998) and crystalline iron oxides (Lovley, 1993). Anaerobic Fe(III)-reducing bacteria precipitate or transform these iron oxides into crystalline Fe phases such as magnetite (Fe_3O_4), siderite ($FeCO_3$), vivianite [$Fe_3(PO_4)_2{\cdot}8H_2O$], and maghemite (Fe_2O_3) (Zhang et al., 1997; Fredrickson et al., 1998). Fe(III)-reducing bacteria were used to examine the reduction of a poorly crystalline iron oxide, akaganeite (β-FeOOH), without a soluble electron shuttle, anthraquinone disulfuonate (AQDS), in the presence of N_2, N_2-CO_2(80:20, V:V), H_2, and H_2-CO_2 (80:20, V:V) headspace gases as well as in HCO_3^- buffered medium (30–120 mM) under a N_2 atmosphere (Roh et al., 2003). Iron biomineralization was also examined under different growth conditions such as salinity, pH, incubation time, incubation temperature, and electron donors (Roh et al., 2003). Magnetite formation was dominant under a N_2 and a H_2 atmosphere. Siderite formation was dominant under a H_2-CO_2 atmosphere. A mixture of magnetite and siderite was formed in the presence of a N_2-CO_2 headspace. Akaganeite was reduced and transformed to siderite and magnetite in HCO_3^- buffered medium (>120 mM) with lactate as an electron donor in the presence of a N_2 atmosphere.

Recent studies have shown that pure cultures and consortia of iron reducer have shown to reduce amorphous iron oxyhydroxide or poorly crystalline iron oxides including akaganeite (β-FeOOH) and ferrihydrite ($Fe_5HO_8{\cdot}4H_2O$) as

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an electron acceptor and formed magnetite, siderite, green rust, and vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$] crystals (Zhang et al., 1997; Roh et al., 2001, 2002, 2003, 2006; Fredrickson et al., 2001; Zachara et al., 2002). Magnetite, siderite, and vivianite are common mineral products and ubiquitous in natural environments (Emerson, 1976; Emerson and Widmer, 1978; Pye et al., 1990). Although a direct link can not be established between these laboratory-produced biogenic minerals and those observed in nature, several lines of evidence suggest that at least some proportion of these minerals present in natural environments are biogenic (Pye et al., 1990).

Little is known about metal reduction and iron biomineralization under extreme conditions, and only recently has bacterial Fe^{3+} reduction been demonstrated under thermophilic (Liu et al., 1997; Roh et al., 2002), psychrophilic (Zhang et al., 1999; Roh et al., 2002), or acidic conditions (Kusel et al., 1999). Sites contaminated with toxic metals can have drastically different environmental conditions, and the biological reduction of most metals has commonly been studied at circum neutral pH values (Strab et al., 2001). Microbial metal reduction and iron biomineralization under alkaliphilic growth conditions has not been demonstrated well.

A novel metal-reducing, alkaliphilic species named as *Alkaliphilus metalliredigenes* sp. nov. was proposed by Ye et al. (2004) and they studied genomic characteristics and physiology of the microorganism in detail. Therefore, the objectives of this study were to further examine metal reduction and iron mineral formation in alkaline conditions as well as to perform mineralogical characterization of the precipitates formed via microbial reduction of Fe(III)-citrate and Fe(III)-EDTA using an alkaliphilic bacterium, *Alkaliphilus metalliredigens* (QYMF), isolated from a boron containing pond.

2. MATERIALS AND METHODS

2.1. Source of Microorganisms

In this study, we examined the microbial formation of iron minerals and metal reductions using an alkaliphilic bacterium (QYMF, *Alkaliphilus metalliredigens* sp. nov.) isolated from boron leachate ponds at the U.S. Borax Mines in Boron, CA (Ye et al., 2004). The hydrogeochemical analysis of water samples from the boron leachate ponds indicated that Na, Si, and K were the major elements. Sodium concentration is 11,800 mg/L. Boron and Fe concentrations were about 2000 mg/L and 4.9 mg/L, respectively. Cr and Ba concentrations were 0.2 mg/L and 0.4 mg/L, respectively. U concentrations ranged from 0.2 mg/L to 0.4 mg/L, and arsenic concentration was 126 mg/L. Phylogenetic analysis of the Small subunit rDNA indicated the bacterium, QYMF, was a low G+C Gram-positive microorganism in the *Clostridiaceae*, and had 96% and 92% nucleotide identity with *Alkaliphilus transvaalensis* and *Alkaliphilus crotonoxidans,* respectively (Ye et al., 2004). The isolate might represent a novel metal-reducing, alkaliphilic species and the name *Alkaliphilus metalliredigenes* sp. nov. was proposed (Ye et al., 2004).

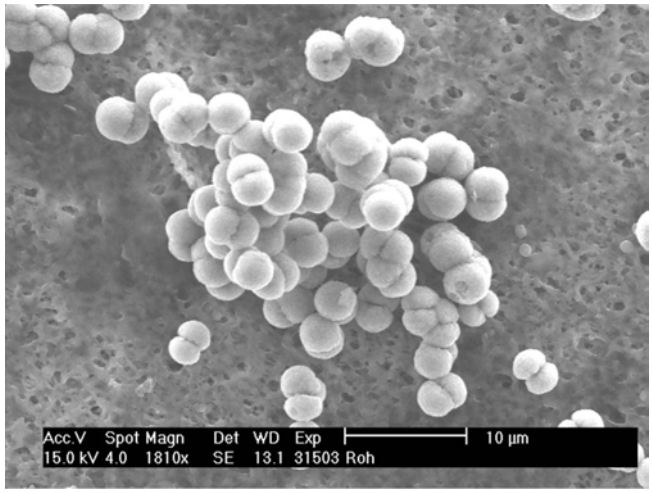

Fig. 1. An SEM image of endspores produced by isolate QYMF grown with Fe^{3+}-EDTA as an electron acceptor and lactate as an electron donor.

Bacterial cell morphology examined using scanning electron microscopy (SEM) showed that QYMF cells were straight with some cells slightly curved, and a mean length of 3 to 6 mm and an approximate width of 0.5 mm (Ye al., 2004). As the cells entered stationary phase growth, terminal endospores were observed (Fig. 1). The temperature range for growth was between 4 °C (lowest temperature attempted) and 45 °C (Ye et al., 2004), The maximum Fe(III)-citrate reduction rates and bacterial growth rates were observed at 35 °C. When the *Alkaliphilus metalliredigens* sp. (QYMF) was grown in the presence of Fe(III)-citrate at 25 °C, the pH range for growth was between 7.0 and 11.0, growth below pH 7.5 or above pH 11.0 was negligible, and the pH optimum was approximately 9.5. By definition, true alkaliphiles should have an optimum pH above 9.0, and display little growth at or below pH 7.0. Microbial Fe(III) reduction by *Alkaliphilus metalliredigens* sp. (QYMF) occurred at a salinity range of 0–80 g/L NaCl at 25 °C using lactate (10 mM) as an electron donor and Fe(III)-citrate as an electron acceptor. Sodium chloride was not essential for growth, but the optimal concentration appeared to be approximately 20 g/l. The isolate could grow and reduce Fe(III) in the presence of up to 1.5% borax ($Na_2B_4O_7$).

2.2. Growth Conditions

Two types of growth media including sodium carbonate ($NaHCO_3$) buffer medium and tris(hydroxymethyl)amino

Table 1. Media composition for microbial reduction of metals

Component	$NaCO_3$-buffer medium	TRIS-buffer medium
	g/L	
$CaCl_2$	-	5.545
$MgCl_2$	-	9.5
$(NH_4)_2SO_4$	1.2	1.2
100X Trace Mineral Solution	10 ml	10 ml
NaCl	20	20
Na_2CO_3	3.0	-
tris(hydroxymethyl)amino methane (TRIS)	-	6.15
Yeast extract	0.25	0.25
$Na_2B_4O_7$	2	2
K_2HPO_4	1	1
Headspace	N_2 (100%) or N_2-CO_2 (80%:20% v:v)	N_2 (100%) or N_2-CO_2 (80%:20% v:v)

methane (TRIS) buffer medium were prepared anaerobically and used for microbial metal reduction and biomineralization (Table 1). The trace mineral solution contained 1.5 g of nitrilotriacetic acid, 3 g of $MgSO_4$, 0.5 g of $MnSO_4$, 1.0 g of NaCl, 0.1 g of $FeSO_4$, 0.1 g of $CaCl_2$, 0.1 g of $CoCl_2$, 0.154 g of $ZnSO_4$, 0.01 g of $CuSO_4$, 0.01 g of $AlK(SO_4)$, 0.01 g of H_3BO_3, 0.025 g of Na_2MoO_4, 0.1 g of $NiCl_2$ per liter of deionized water. The medium was prepared under N_2 or N_2-CO_2 (80% N_2-20% CO_2) and had a final medium pH of 9.5.

To assess the capability of metal reduction and mineral formation by QYMF (*Alkaliphilus metalliredigenes* sp. nov.), various electron acceptors such as Fe(III)-citrate (10-20 mM), Fe(III)-EDTA (10 mM), cobalt(III)-EDTA (5 mM), potassium chromate (100 μM), Mn(IV)-oxide (10 mM), iron oxyhydroxide [akaganeite, β-FeOOH, ~70 mM]], selenate (5 mM), and uranyl carbonate (1 mM) were examined at 25 °C with lactate (20 mM) or acetate (20 mM) as an electron donor. Iron reduction and biomineralization were also examined by QYMF with Fe(III)-citrate (15 mM) in the presence of arsenate (5 mM) using lactate (10 mM) or acetate (10 mM) as an electron donor.

The Mn(IV) oxide was prepared as described by Roh et al. (2002) and uranyl carbonate [$UO_2(CO_3)_2^{2-}$] was prepared by mixing uranyl-nitrate [UO_2 $(NO_3)_2^{2-}$] solution with sodium bicarbonate (Roh et al., 2000). Analysis of X-ray diffraction showed that the Mn oxide was amorphous. The iron oxyhydroxide was prepared by neutralizing a solution of 0.4 M $FeCl_3$ with 10M NaOH as described previously (Roh et al., 2003). Analysis of X-ray diffraction showed that the iron oxyhydroxide was poorly crystalline akaganeite (β-FeOOH).

2.3. Metal Reduction Studies

To examine the chemical conditions of metal reduction and mineral formation by the alkaliphilic Fe(III)-reducing bacteria, subsamples (1 ml) of bacterial cultures and abiotic controls were taken from the culture bottles at different times. Fe(II) and Cr(VI) concentrations (0.5 N HCl soluble) were determined by measuring the absorbance at 562 nm (for Fe^{2+}) and at 540 nm (for Cr^{6+}) on a Hewlett Packard 8453 spectrophotometer using the ferrozine method with anaerobic water for sample dilution (Stookey, 1970; Zhang et al., 1996).

2.4. Mineralogical Characterization

Precipitates in each sample were collected on a 0.22 μm Millipore filter (Millipore Corporation, Bedford, MA) and washed three times with deionized water (Roh et al., 2003). The filtered precipitates were dried under an N_2 atmosphere to prevent oxidation of the precipitated minerals. The filtered and dried precipitates were carbon coated with a Ted Pella carbon sputter coater and immediately examined. The morphology, mineralogy, and chemistry of precipitated phases during microbial metal precipitation of iron were determined with scanning electron microscope (SEM) (XL30FEG, Phillips, Netherlands) with energy dispersive X-ray (EDX) analysis. X-ray diffraction analysis (a Scintag XDS 2000, Scintag, Sunnyvale, CA) was used to monitor mineralogical characteristics with operating at a scan rate of 2° 2θ min^{-1}. Cobalt K-α radiation (40 kV, 35 mA) was used to minimize fluorescence of Fe-rich minerals. Laser induced plasma spectroscopy (LIPS) was used to detect boron in the precipitate formed by the bacterium (Martin et al., 2002).

3. RESULTS AND DISCUSSION

3.1. Metal Reduction

Fe(II) concentrations were monitored at 25 °C in a time course experiments using Na_2CO_3 buffered medium containing Fe(III)-citrate (20 mM) as an electron acceptor and acetate (20 mM) as an electron donor. A dramatic increase in Fe(II) concentration was observed within four days of

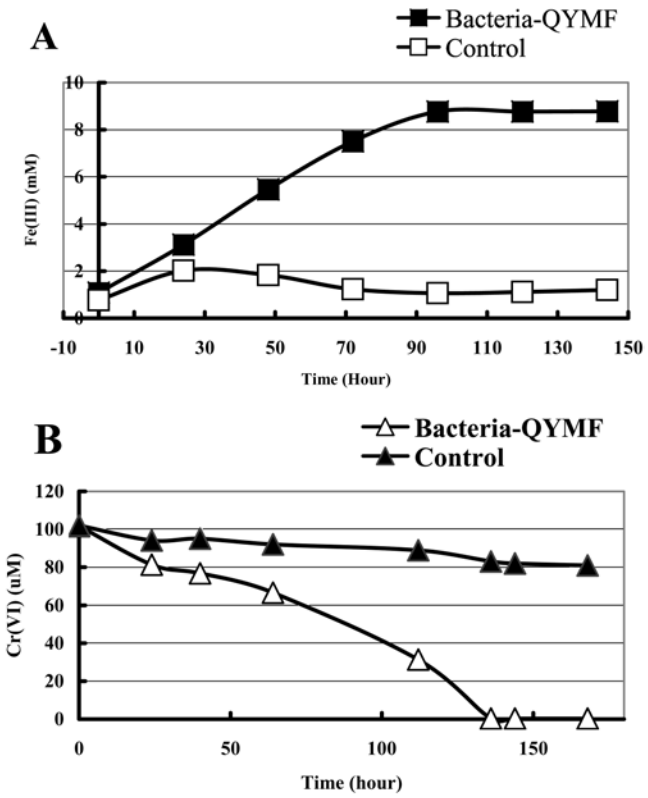

Fig. 2. (A) Time course experiments of Fe (III) reduction using the Na_2CO_3 buffered medium. (B) Time course experiments of K_2CrO_4 (100 μM) reduction. The results are the means of duplicate experiments.

incubation at 25 °C. The bacteria reduced the Fe(III)-citrate and changed Fe(II) concentration from 1 mM to 8.8 mM Fe(II) within 100 hours of bacterial incubation (Fig. 2a). The color of the culture solution also changed from yellowish brown (ferric citrate) to colorless at 25 °C. Fe(II) concentration ranged from 0.7 to 2.2 mM in the abiotic controls, which may result from abiotic reduction of Fe(III) by organic nutrients such as yeast extract in the medium. However, the abiotic controls did not show any color change in the time course experiment. The bacterium was also able to reduce Fe(III)-EDTA (10 mM) as an electron acceptors using acetate or lactate as an electron donor.

Cr(VI) concentrations with time were also examined using Na_2CO_3 buffered medium containing ~100 μM K_2CrO_4 and 20 mM acetate at 25 °C. A dramatic decrease in Cr(VI) concentration was observed within 5 days of incubation at 25 °C and reached 30 μM Cr(VI) (Fig. 2b) and showed the color change of the culture solution from yellow (K_2CrO_4) to colorless at 25 °C. The bacteria reduced the Cr(VI) and changed Cr(VI) concentration from 100 μM up to below detection limit within 150 hours of bacterial incubation (Fig. 2b). Cr(VI) concentration decreased slightly from 102 to 83 μM in the abiotic controls, which may result from abiotic reduction of Cr(VI) by organic nutrients such as yeast extract in the medium. However, the abiotic controls did not show any color change in the time course experiment.

The ability of the *Alkaliphilus metalliredigens* sp (QYMF) to reduce metals besides Fe(III) and Cr(VI) was also examined using Na_2CO_3- or TRIS-buffered medium. The bacterium was able to reduce Co(III) [Co(III)-EDTA, 5 mM] to Co(II) and U(VI) ($UO_2CO_3^{2-}$, 1 mM) to U(IV) with lactate (10 mM) as an electron donor, as indicated by the color change of the culture solution from purple [Co(III)-EDTA] and yellow (uranyl carbonate) to colorless at 25 °C. The reduced cobalt, Co(II), is colorless and the reduced U(VI) is precipitated as UO_2 in the media (Roh et al., 2002). The bacterium was also able to reduce selenate [Se(VI), 5 mM] and precipitated reddish brown phases. An SEM analysis showed that the bacterium precipitated trigonal crystals or ball-like selenium (0) particles during selenate reduction using lactate as an electron donor at pH=10 (Fig. 3). The precipitated phases mainly consisted of Se, thereby demonstrating the reduction of Se(VI) to Se(0) (Fig. 3). The reduction of the oxyanions, arsenate and selenate, is a common biogeochemical process in other alkaliphiles such as *Bacillus selenitrireducens* and *Bacillus arsenicoselenatis* (Stoltz and Oremland, 1999). These results indicated that the *Alkaliphilus metalliredigens* sp. (QYMF) can reduce several other transition metals beside iron as long as their concentrations are held below the toxic levels.

The bacterium was also able to reduce Mn(IV)-oxide (10 mM) in Na_2CO_3 buffer medium and to produce white precipitates using lactate (10 mM) as an electron donor. The XRD analysis showed that the precipitated phase formed by reduction of Mn(IV)-oxide has 1.3 nm and 0.7 nm peaks (Fig. 4d). It is an unknown mineral and further study is required for proper identification. The SEM with EDX analyses showed that this crystalline phase contained significant quantities of Mn, Ca, and P (Fig. 4b) and had a leaf-shaped morphology (Fig. 4a). But the *Alkaliphilus metalliredigens* sp (QYMF) could not reduce akaganeite (~70 mM) as an electron acceptor. The *Alkaliphilus metalliredigens* sp (QYMF) used acetate (10 mM), glucose (10 mM), lactate (10 mM), pyruvate (10 mM), and H_2 (20% H_2-80% N_2) as an electron donor. The *Alkaliphilus metalliredigens* sp (QYMF) could not use fumurate (10 mM) as an electron donor to reduce Fe(III)-citrate.

This study showed that metal reduction by microbial Fe(III) reduction can be an important process for organic matter oxidation in anaerobic alkaline subsurface environments. Previous studies have shown that organic compounds such as lactate, formate, acetate, and pyruvate are potentially available in terrestrial subsurface environments (Lovley, 1991). The oxidation of organic compounds coupled with the reduction of Fe(III) oxides can be expected to release Fe(II) ions in subsurface environments (Lovley, 1993; Fredrickson et al., 1998). Hydrogen gas is generated from anaerobic decomposition of organic matter as well as from

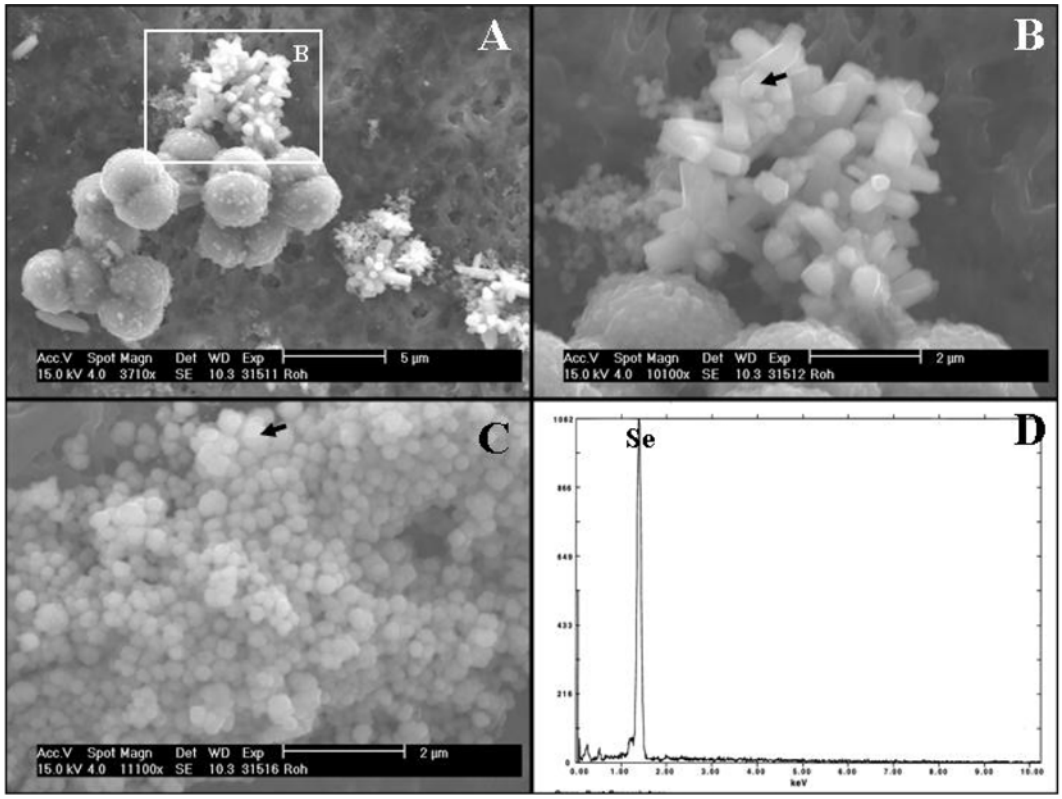

Fig. 3. Scanning electron photomicrographs of precipitates formed by the selenate reduction with QYMF. Extracellular trigonal (A and B) or ball-like (C) selenium particles are elemental selenium as determined by the EDX analysis (D).

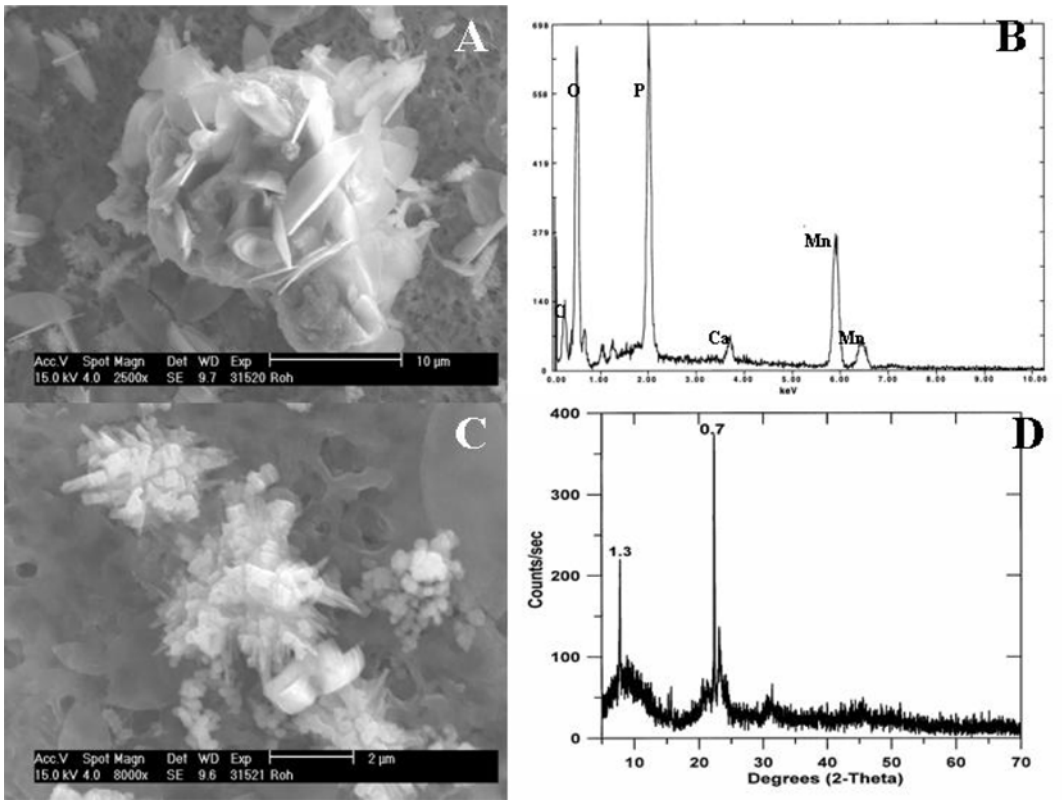

Fig. 4. SEM photomicrographs together with EDX and XRD analyses of minerals formed by the microbial reduction of manganese oxide using alkaliphilic Fe(III)-reducing bacteria, QYMF.

geochemical processes in subsurface environments and probably constitutes a sustainable source of energy for subsurface biosphere ecosystems.

3.2. Biomineralization of Vivianite

No magnetite was formed with the microbial reduction of Fe(III)-citrate and Fe(III)-EDTA. During the course of Fe(III) reduction, color of Fe(III)-citrate and Fe(III)-EDTA changed from amber to green. Approximately 3 days after reduction of the Fe(III)-citrate and Fe(III)-EDTA, a white or brown mineral precipitates were produced when QYMF cells were grown in bicarbonate or TRIS buffered medium in the presence of lactate or acetate as an electron donor at pH 7.5-11.0. Detailed mineralogical characterization of the white precipitate or greenish brown precipitates formed during microbial reduction of Fe(III)-citrate and Fe(III)-EDTA were performed. No precipitates were formed in control tubes without cells. Analysis with SEM and EDX analysis showed that the precipitated phase mainly contained Fe and P with keg- or barrel-shape morphology and a pronounced platy habit (Fig. 5). The XRD analysis of the precipitate formed by reduction of Fe(III)-citrate displayed sharp reflection patterns and all peaks were attributable to vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$]. The precipitate formed by reduction of Fe(III)-citrate displayed a broader background than that of precipitate formed by Fe(III)-EDTA reduction (Fig. 6). The broadness may be attributable to the poor crystallinity of the former vivianite crystals. On the other hand, the latter vivianite crystals showed two sharp and intense XRD peaks, attesting the highly crystalline nature of the precipitate. The presence of two vivianite peaks only could be attributed to flattened {010} growth of the precipitates (Fig. 6). The crystals can be found in encrusting masses with a divergent bladed structure. They occur as relatively well-crystallized secondary minerals in natural environment (Deer et al., 1966). Vivianite can be found in sedimentary deposits where it is often associated with bone, decayed wood, and other remains. Microbes play a role in the authigenic or diagenetic formation of the phosphate minerals such as vivianite, strengite ($FePO_4 \cdot 2H_2O$), and variscite ($AlPO_4 \cdot 2H_2O$) (Ehrlich, 1990). Vivianite has been observed in the upper iron reduction zone in anaerobic lake sediments (Emerson and Widmer, 1978), freshwater swamps (Postma, 1981) and in river sediments (Hearn et al., 1983). Vivianite also forms in iron rich sediments within or below the sulfate reduction zone or the methanogenic zone. Vivianite has been detected in anoxic river sediments receiving the treated sewage, although little

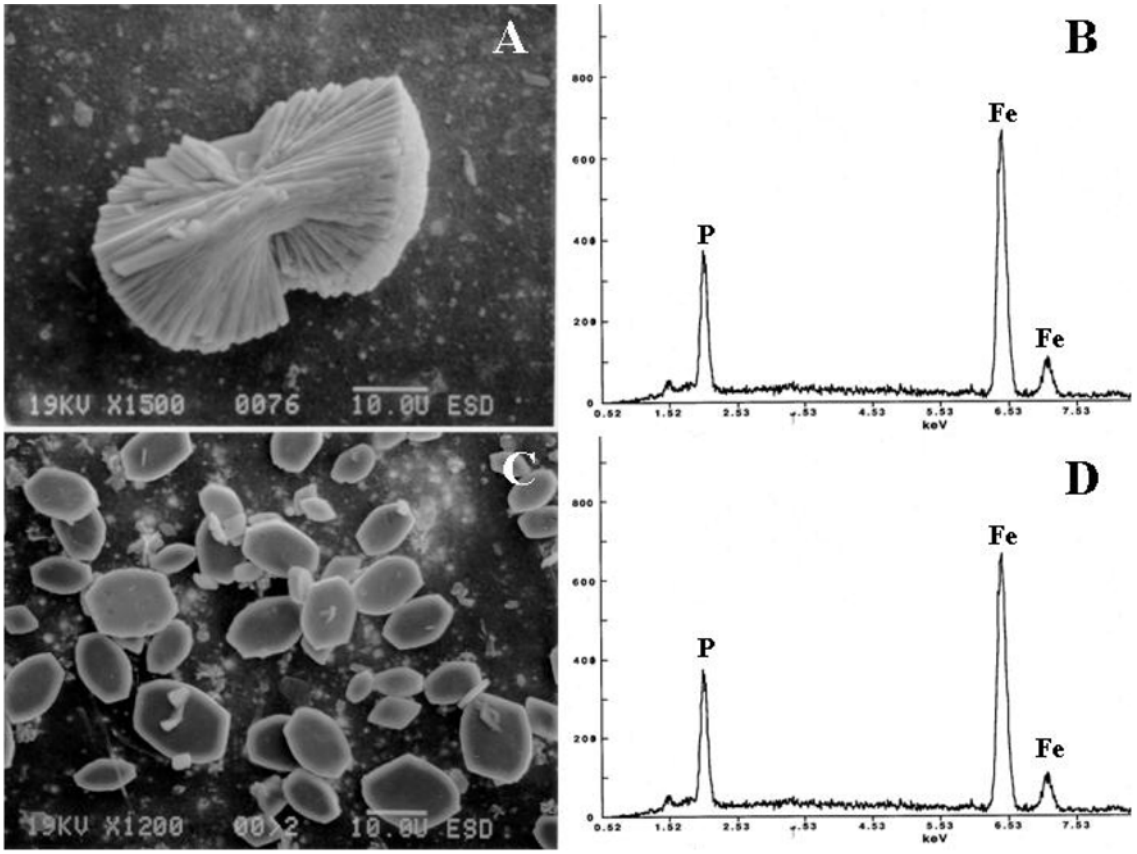

Fig. 5. SEM photomicrographs and EDX results of microbially precipitated phases using Fe(III)-citrate and acetate (A, C) and for those using Fe(III)-EDTA and lactate(C, D).

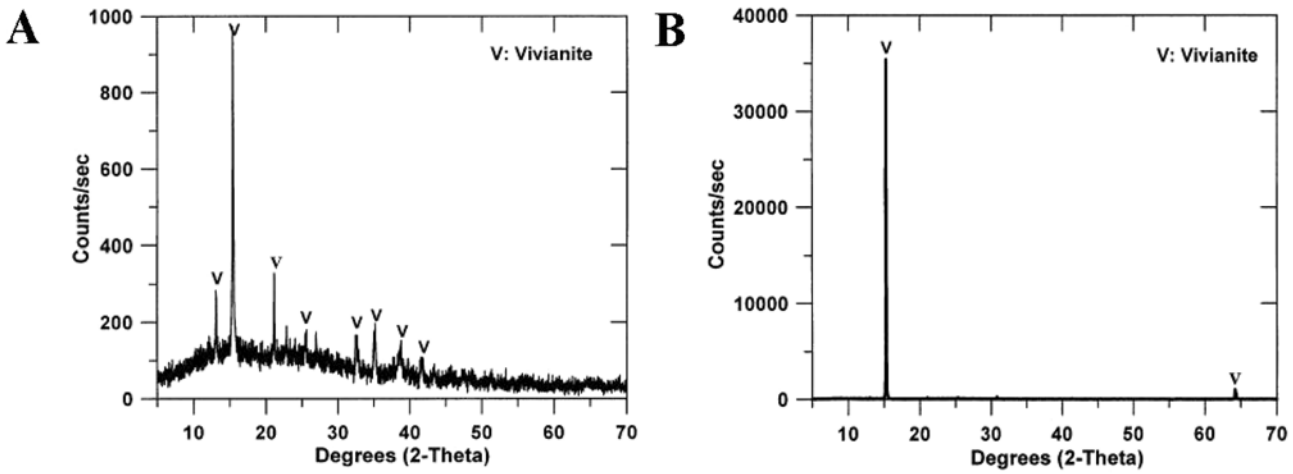

Fig. 6. X-ray diffraction patterns of precipitates from the reduction of (A) Fe(III)-citrate and (B) Fe(III)-EDTA.

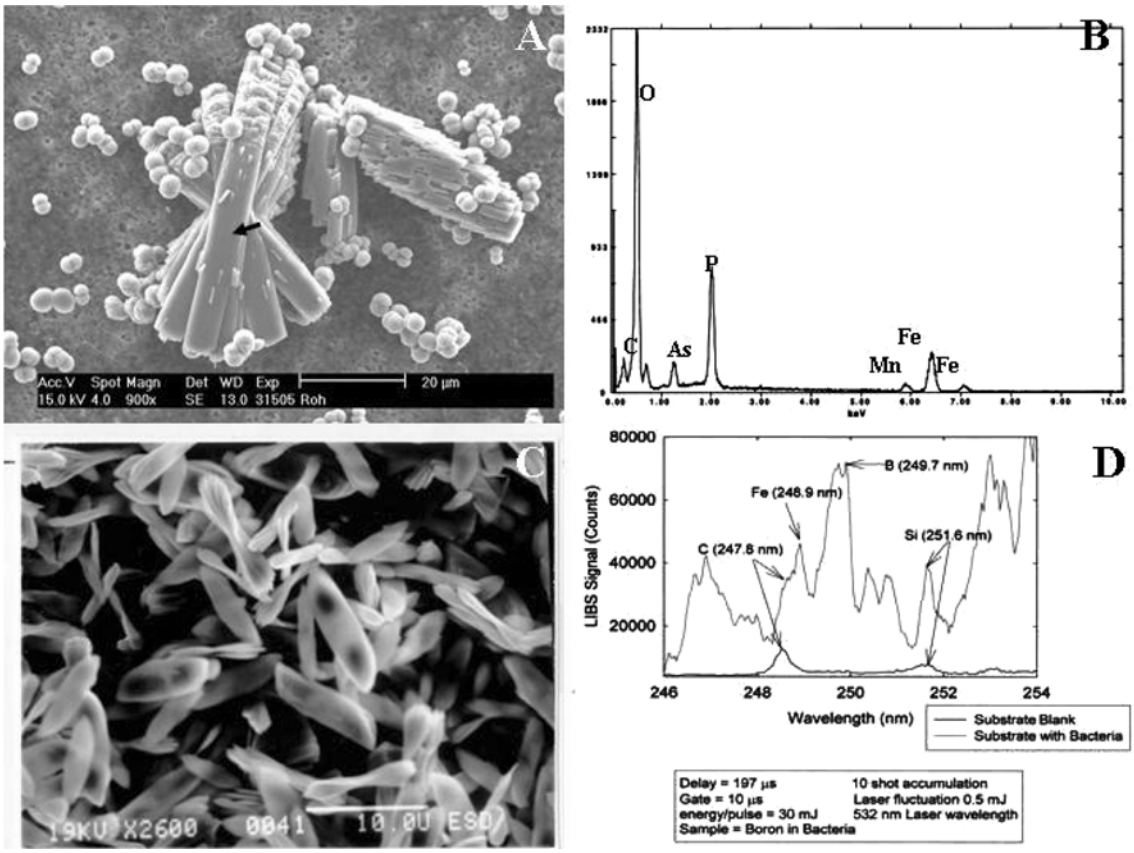

Fig. 7. An SEM photomicrograph (A) and EDX result (B) of microbially precipitated phases using Fe(III)-citrate (15 mM) with arsenate (5 mM) and lactate (10 mM) and an SEM photomicrograph (C) and laser induced spectra (D) of the precipitated phases using Fe(III)-citrate and acetate.

is known about the condition of formation and the frequency of occurrence for vivianite in sediments (House, 2003).

A previous study by Zhang et al. (1996) showed that no crystalline minerals precipitated when Fe(III)-citrate or Co(III)-EDTA were used as the electron acceptor, which is probably due to chelation of Fe(II) with dissolved citrate or EDTA in solution. Citrate acts as a chelating agent in decontamination operations, forming highly recalcitrant and mobile metal-citrate (Jardine et al., 2002). An industrial chelating agent EDTA also forms very strong complexes with divalent and trivalent metal ion (Jardine et al., 2002). Newly isolated cultures of *Alkaliphilus metalliredigens* sp (QYMF) was, however, able to grow using Fe(III)-citrate and Fe(III)-

EDTA as an electron acceptor, while iron mineral precipitation was promoted by the addition of inorganic phosphate.

Borate was present in the culture medium (2.0 g/l), and the SEM and LIPS results (Fig. 7c,d) indicated that boron was incorporated into the crystalline iron-precipitate, vivianite. Naturally occurring bacteria from boron-containing sites may prove beneficial in minimizing the effects of boron toxicity from fly ash ponds. The alkaliphilic Fe(III)-reducing bacteria (QYMF) reduced Fe(III)-citrate and precipitated vivianite in the presence of up to 5 mM arsenate. The SEM-EDX analysis suggested that arsenic can be reduced and co-precipitated with vivianite. The formation of sparingly-soluble vivianite precipitates, mediated by the alkaliphilic Fe(III)-reducing bacterium, may sequester iron, phosphate, and arsenic into more stable and less toxic forms of iron phosphate minerals.

Naturally-occurring bacteria from boron-containing sites (e.g. the U.S. Borax mine, Boron, California) may not only prove beneficial in reducing the effects of boron toxicity of fly ash ponds but also potentially assist the remediation of boron contaminated soils and sediments through bacterially-facilitated precipitation; i.e., by complexing boron into mineral forms that are both sparingly soluble and unavailable for bio-uptake. These results indicate that metal-reduction can occur in alkaline habitats, and iron mineral precipitation may vary according to the dynamics of chemical environments. Future work is needed to fully understand the role of metal-reducing microorganisms in alkaline environments and to assess the potential applicability for immobilization of boron via iron biomineralization.

4. CONCLUSIONS

Alkaliphilus metalliredigens sp (QYMF), isolated from a leachate-pond containing high levels of salt (Na concentration = 440–12,100 mg/L) and boron (2000–3000 mg/L) at pH 9.0-10.0, was able to use lactate, acetate and hydrogen as alternative electron donors and Fe(III)-citrate, Fe(III)-EDTA, Cr(VI), Co(III)-EDTA, and Mn(IV) as electron acceptors. The reduction of Fe(III)-citrate and Fe(III)-EDTA in the presence of K_2HPO_4 and boron resulted in the precipitation of vivianite [$Fe_3(PO_4)_2{\cdot}8H_2O$]. The variation in chemical milieu altered the size and morphology of vivianite precipitates. The formation of sparingly-soluble iron phosphate, mediated by the alkaliphilic Fe(III)-reducing bacterium, may sequester iron, phosphate, boron and other metals (e.g. As and Se) into more stable and less toxic forms. These results suggest that bioremediation of metal-contaminated alkaline environments should be feasible, and that the process of metal-reduction occurs in alkaline habitats. Natural bacteria from boron-containing sites may prove beneficial for immobilizing boron in subsurface environments.

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