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Biomineralization of radioactive sulfide minerals in strong acidic Tamagawa Hot Springs

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Abstract

Bioaccumulation of radioactive sulfide minerals by bacteria in strong acidic hot spring water was found at Tamagawa Hot Springs, Akita prefecture in Japan. The hot spring water produces Hokutolite of radioactive minerals containing high radium and radon. The β-ray measurements of sediments and biofilms indicate 1850-2420 and 5700 cpm, respectively, which are 50-100 times higher than that of the water and the air (50-90 cpm). The characteristics of hot spring water show pH (1.2), Eh (140 mV), EC (29 mS/cm), DO (0.8 mg/l), and water temperature (99.5℃), indicating extremely strong acidic and reducing conditions. The hot spring water contains mainly HCl associated with high concentrations of Ca^{2+}, Al^{3+}, Fe^{2+}, HSO_4^- and SO_4^{2-}. SEM-EDX and TEM demonstrate some insight into how microorganisms affect the chemistry and microbiological characteristics of the strong acidic surroundings with high S, As, Ba, and Ca contents in biofilms. Especially SEM-EDX, ED-XRF, and STEM-EDX elemental content maps illustrate the distribution of sulfur-bearing compounds of barite ($BaSO_4$), gypsum ($CaSO_4 \cdot 2H_2O$), elemental sulfur (S) and orpiment (As_2S_3) in the reddish orange biofilms. The presence of a hydrogen sulfide-rich (H_2S) thermal spring and gypsum deposits suggest the volatilization of H_2S from the spring water, oxidation of the H_2S gas to sulfuric acid, and reaction of the sulfuric acid. TEM micrographs of bacteria in the biofilms reveal in detail the intimate connections between biological and mineralogical processes that the cells are entirely accumulated with spherical grains, 100～200 nm in diameter. The relationship among sulfide minerals, such as barite, gypsum, sulfur, orpiment, and Hokutolite, associated with bacteria implies that heavy metals have been transported from strong acidic hot spring water to sediments through bacterial metabolism. It is possible that the capability of radioactive sulfide biofims for heavy metal immobilization can be used to counteract the disastrous effects of radio nuclide polluted water and radio pharmaceutical medical treatment.

Keywords : Radioactive sulfides, strong acidic hot springs, high β-ray, barite, gypsum, sulfur, orpiment, Hokutolite.

Introduction

Temperature is one of the most important environmental factors controlling the activities and evolution of organisms, and is one of the easiest variables to measure. Many hot springs have significant amounts of hydrogen sulfide which thermal environments are most fit for living organisms. Some hot springs are highly radioactive, whereas others have no more radioactivity than normal ground waters (Brock, 1967). Radioactive materials are located on the Earth's surface, which is closely related to the microorganisms in groundwater and organic materials in soils, and tends to gather at or near lower plants (Markert, 1993 ; Lienert et al., 1994 ; Yoshida et al., 1994). For example, Uranium solute concentration of river, lake, tailings, and natural waters at the Elliot Lake district, Ontario, Canada, shows high Uranium contents. Nordic and Stanrock tailings contain high Ca, Fe, and U, whereas *Euglena algae* enriched these elements more than 7-20 times (Mann and Fyfe, 1985). Filamentous fungi, yeasts, algae, *Actinomycetes*, and bacteria have been evaluated for Uranium removal biosorption (Lovley, 2001). On the other hand, bacteria live under a wide range of physical and chemical conditions and they are quite actively involved in the cycling of heavy metals. Bacteria can oxidize or reduce various heavy metals, such as Fe, Mn, U, As, and Cd in order to gain energy or promote to mineralize (Tazaki and Ishida, 1996 ; Tazaki, 2003). Bacteria are very small, but have the largest surface area to volume ratio of any life form. By providing interfaces for sorption of metal cations, bacteria are efficient scavengers of dilute metals and can concentrate them from the surrounding aqueous environment (Waite et al., 1994 ; Tazaki et al., 2002). It is well established that microbes adsorb and concentrate metals and radionuclide and that the sulfur-cycle impacts radio nuclide solubility. Sulfur-oxidizing bacteria, not aqueous geochemistry, actually control cave growth. The bacteria oxidize H_2S either completely to sulfate for energy or partially to elemental sulfur. But, very little information of radioactive biofilm had been reported.

There are numerous hot springs in Japan, because Japanese Island has abundant active volcanoes with built-in hot spring water (Horiuchi, 2001). However, key developmental processes of radioactive sulfides biomineralization regulating these events are poorly understood. Although the importance of radioactive elements are widely recognized in hydrothermal area and hot springs, there have as yet been remarkably few studies of the interactions by which living microorganisms bind metals. We thus see that bacteria are able to grow in Tamagawa Hot Springs at high temperature and high radioactivity. Unfortunately, biologists are not concerned with geo-chemical and bio-mineralogical significances, but the results of the analyses do show that there are many chemical and mineralogical types in Tamagawa Hot Springs.

In this study, using micro techniques, such as SEM-EDX, TEM, and STEM-EDX, heavy metal ions with high radioactivity and bacterial interactions were demonstrated in strong acidic hot spring water at Tamagawa Hot Springs, Akita, Japan. The purpose of this paper is to provide some insight into how microorganisms interact with the radioactive sulfide minerals.

Area descriptions of Tamagawa Hot Springs

Tamagawa Hot Springs, Akita Prefecture in Japan, is a potentially useful study area of radioactivity in hydrothermal environments, and is the site of Japan's highest production of hot spring water (9,000 l/min) (Fig. 1). The characteristics of hot spring water showed pH (1.2), Eh (140 mV), EC (29 mS/cm), DO (0.8 mg/l), and water temperature (99.5℃) indicating extremely strong acidic and reducing conditions (Table 1). The aqueous chemistry of the hot spring water showed mainly HCl associated with high concentrations of Ca^{2+}, Al^{3+}, Fe^{2+}, HSO_4^-, and SO_4^{2-} (Akita Analytical Center, 2001). Using β-ray survey meter, sediments and biofilms on the surface of hot spring water indicate 1850-2420 and 5700 cpm, respectively (Fig. 1A, C). The β-ray counts of the biofilms are 50-100 times higher than those of the hot spring water and the air at Tamagawa Hot Springs (50-90 cpm) (Fig. 1B, Table 2).

Table 1. Characteristics of Ohbuke hot spring water at Tamagawa Hot Springs, Akita Prefecture, Japan.

		chemistry					
		Traces*		Cations*		Anions*	
pH	1.2	Pb^{2+}	0.37 mg/l	H^+	61.9 mg/kg	F^-	51.9 mg/kg
Eh	140 mV	Cd^{2+}	0.058	Na^+	89.4	Cl^-	2856
EC	29 mS/cm	As	1.5	K^+	52.9	Br^-	5.8
DO	0.8 mg/l	Cr^{6+}	< 0.05	Mg^{2+}	46.9	I^-	1.9
Temperature	99.5 ℃			Ca^{2+}	159.8	$HS_2O_3^-$	0.2
				Sr^{2+}	0.2	HSO_4^-	547.3
				Ba^{2+}	< 0.1	SO_4^{2-}	263.5
				Al^{3+}	111.8	NO_3^-	< 0.1
				Mn^{2+}	3.2	AsO_2^-	-
				Fe^{2+}	97.9	$HSiO_3$	-
				Fe^{3+}	2	SiO_3^{2-}	-
				Cu^{2+}	< 0.1		
				Zn^{2+}	4.6		

-; not detected, *; data from Akita Analytical Center (2001)

Table 2. Measurement of β-ray counts of biofilms, sediments, rocks, hokutolite, the air, and the hot spring water surrounding Ohbuke at Tamagawa Hot Springs, Akita Prefecture, Japan.

Samples	β-ray (cpm)
Reddish orange biofilms	5700
Sediments around the biofilms	4700
Sediments/soils	1850~2420
Hokutolite	1800~3200
Rocks near the sediments	350~420
The air at Tamagawa Hot Spring	80~100
Hot spring water (Source of Ohbuke)	50~90
The air at Morioka City	55

Measured on 22nd Oct., 2003

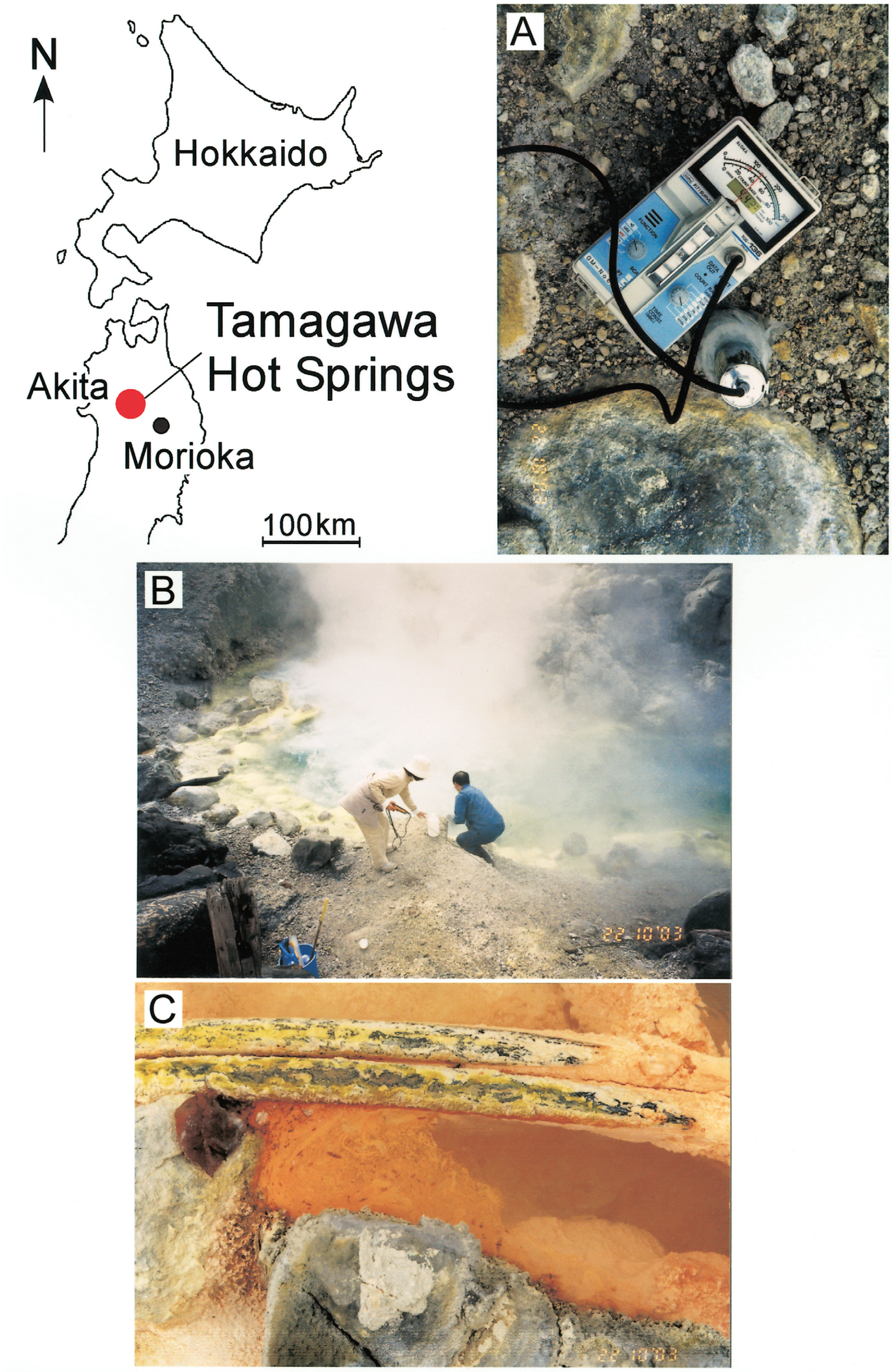


Figure 1. Locality map of Tamagawa Hot Springs, Akita Prefecture, Japan. β-ray survey meter (A), strong acidic hot spring water (pH 1.2) at Ohbuke (B), and reddish orange biofilms with the highest β-ray counts (5700 cpm) (C).

Tamagawa Hot Springs are famous to produce “Plumbian Barite (Hokutolite)” which was first found by K. Sakurai (1898). In the following year, Y. Okamoto independently found similar mineral at the Hokuto Hot Spring in Taiwan. The chemical composition of Hokutolite is (Ba, Pb)SO_4 showed high radioactivity. K. Jimbo(1912) described this mineral as a new radioactive mineral with the composition of (Ba, Pb) SO_4 and gave the name of Hokutolite. The mineral is precipitated in strong acidic hot springs, which was designated as special natural monument of the nation in 1952, and cannot be collected without the permission of the Government (Sugie, 1986).

In this study, reddish orange biofilms with higher β-ray counts than that of hokutolite were found in the field at Tamagawa Hot Springs. The presence of H_2S thermal springs and Hokutolite ((Ba, Pb)SO_4) deposits suggested that volatilization of H_2S from the spring water contribute to radioactive biominerals.

Methods and materials studied

The β-ray and water characteristics were measured on site on 22nd October 2003 at Tamagawa Hot Springs, Akita Prefecture, Japan (Fig. 1). The β-ray of hot spring water, biofilms, sediments, and rocks has been measured by the β-ray GM survey meter (Aloka Company, Model TGS-136 ; Serial No. R04647) (Fig. 1A). The mineralogical identification of the biofilms was performed by X-ray powder diffraction (XRD) with a Rigaku RINT2000 carried out with Cu Kα radiation at 40 kV and 30 mA. The samples were also investigated by using scanning electron microscope (SEM : JEOL JSM-5200 LV) equipped with an energy dispersive X-ray analyzer (EDX : PHILLIPS EDAX PV 9800 EX) operating at 15 kV.

Microbes in the biofilms were examined with a transmission electron microscope (TEM ; JEOL TEM-2000-EX) using wet samples mounted on a micro grid. After air-drying, the samples were observed without coating at an accelerating voltages of 160 kV. Complimentary techniques of Energy-Filtering Transmission Electron microscopy (JEM-2010FEF) were carried out for the semi-quantitative analyses of the biofilms. Mainly mappings for C, O, Al, Si, S, Cl, Ca, Fe, Cu, and As elements were carried out at the accelerating voltages of 200 kV. Hokutolite sample was cut in half and polished surface for elemental content mapping. The analysis was carried out by an ED-XRF with equipment for X-ray probe scanning system (JEOL JSX-3600), using Rh Kα, which operated at an accelerating voltage of 30 kV under the vacuum condition.

Results

The characteristics of radioactive biofilms and sulfide minerals in strong acidic Tamagawa Hot Springs are described below.

1. β-ray measurements

The β-ray counts were measured in the field for comparison of rocks, sediments, the air, and reddish orange biofilms around the fountainhead at Ohbuke (Fig. 1B). The β-ray of reddish orange biofilms (Fig. 1C), sediments, rocks, and the air has counted as 5700, 1850-2420, 350-420, and 80-100 cpm, respectively (Table 2). The β-ray of the biofilms (5700 cpm) is the highest on the site, and that of the biofilms are higher than that of Hokutolite (1800-3200 cpm), suggesting high concentration of radioactive components. The sediments around the reddish orange biofilms also have quite high counts as 4700 cpm. Normally the air should be 55 cpm in Morioka City, whereas 80-100 cpm in Tamagawa Hot Springs area indicates twice much higher than that of normal air value.

2. XRD analysis of biofilms

Wet biofilms were mounted on a glass slide and measured as wet samples. X-ray powder diffraction analysis of the reddish orange biofilms with the highest β-ray counts exhibits strong 3.34, 4.06, and 3.85 Å refrections identified as sulfur (Fig. 2). However, any radioactive crystalline phases could not recognized in the XRD pattern. But, some barite, gypsum, and orpiment can be structurally incorporated in sulfides. The larger arsenate ions seem to be accommodated in the tetrahedral sites by expansion of the unit cell by deficiencies in adjacent Fe-O (OH) octahedral sites (Paktunc and Dutrizac, 2003).

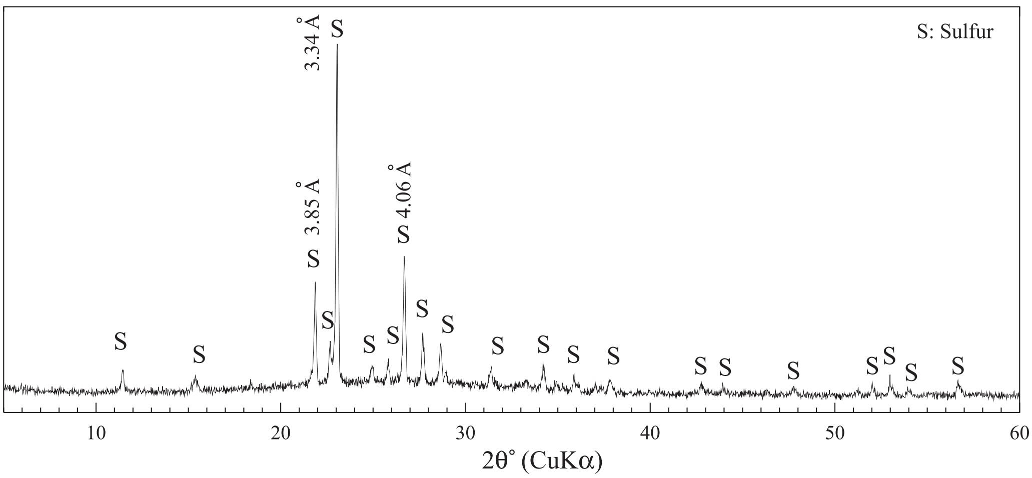


Figure 2. X-ray powder diffraction pattern of the reddish orange biofilms shows the progressive crystallization of sulfur.

3. SEM-EDX observations of biofilms

The SEM observation and the EDX analysis of the reddish orange biofilms with the highest β-ray counts demonstrated the production of sulfides minerals to accumulate high sulfides. In the biofilms, various shapes of sulfur-bearing compounds were found, such as an ellipse shape which was mainly composed of S and Ca (gypsum) (Fig. 3A, B) as well as S and Ba (barite) (Fig. 3A-2). The As-Al-Si-S-Cl compounds were found around gypsum (Fig. 3A-1). The high dense of twig shaped crystals rich in S was commonly found, and that was consistent with mineralogical data by XRD, identified as sulfur (Fig. 2) associated with other sulfide minerals. Transparent microorganisms were covered with thin films scattering the spherical grains identified as orpiment (Fig. 4).

EDX analysis of the spherical grains, < 0.3 μm in diameter, attaching to twig shaped sulfur crystals, exhibited the abundance of S, As, Al, Si, P, Cl, and K (Fig. 4A-1). Closed-up micrograph of the spherical grins suggested the presence of orpiment (As_2S_3) (Fig. 4B-2). The biofilms were composed of aggregation of abundant spherical grains associated with bacteria in agreement with TEM micro morphology shown in Figs. 5-10.

4. Elemental content maps of biofilms

The EF-TEM analysis of the reddish orange biofilms provided more detailed elemental distribution and abundant bacteria associated with spherules than that of SEM-EDX analysis of the sample. The STEM-EDX mapping method had an advantage of distinguishing between sulfide minerals and microorganisms which revealed that abundant bacillus type bacteria (sulfate-reducing bacteria) coexisted with S-As spherical grains (nano crystalline of orpiment), surrounding the cell (Fig. 5). The STEM-EDX analysis of the reddish orange biofilms with the highest β-ray counts, demonstrated accumulation of high S, Cl, and As contents on the spherical grains, suggesting the mixture of orpiment and chlorides. In most cases, these elements (C, O, Si, and N) were detected in the bacterial cell area (Fig. 5). Especially S and As elements were consistent with the spherical grains, whereas other elements such as C, O, Si and N were consistent with the bacterial cells.

5. TEM observations of biofilms

TEM images of the reddish orange biofilms with the highest β-ray counts clearly showed abundant sulfur-bearing minerals associated with various shapes of crystalline. Elongated ellipses were gypsum, 3-5 μm in length and 1-3 μm in wide, with Moire-pattern and stacking fault on the surface (Fig. 6). Some of the tips were sharpened as shown in Fig. 6A. On the other hand, disk shaped barite, 1-3 μm in diameter, were commonly found which also showed Moire-pattern and stacking fault on the surface (Fig. 7). The shape and the size of barite in the natural samples were very similar to the micrographs of $BaSO_4$ particles reported by Yoshiyama (2003). TEM observations clearly showed that some opaque spherical grains were associated with bacterial surface on barite (Fig. 7A, bottom part). Furthermore, intermediated or combined crystals of gypsum and barite showing elongated/

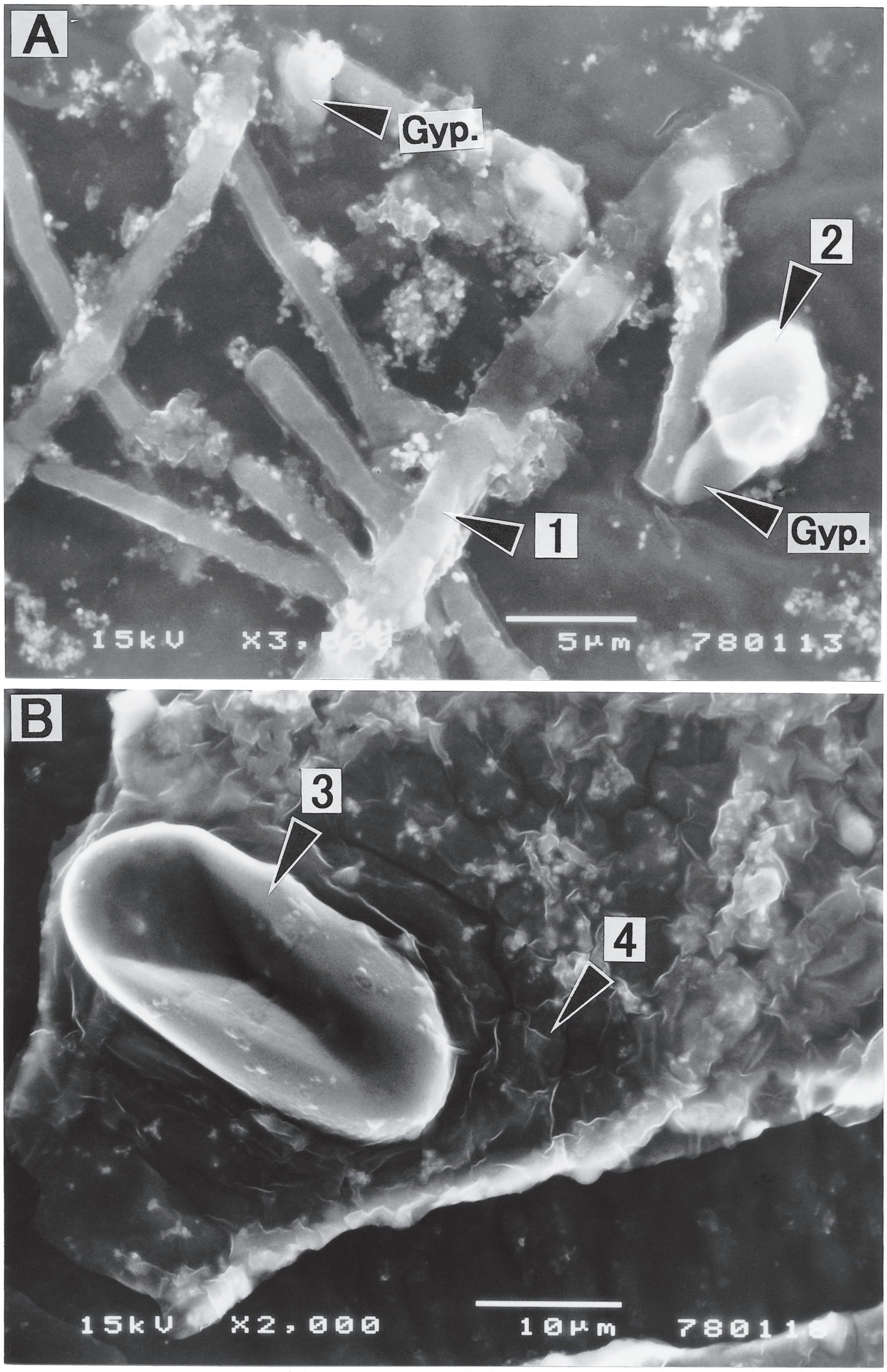
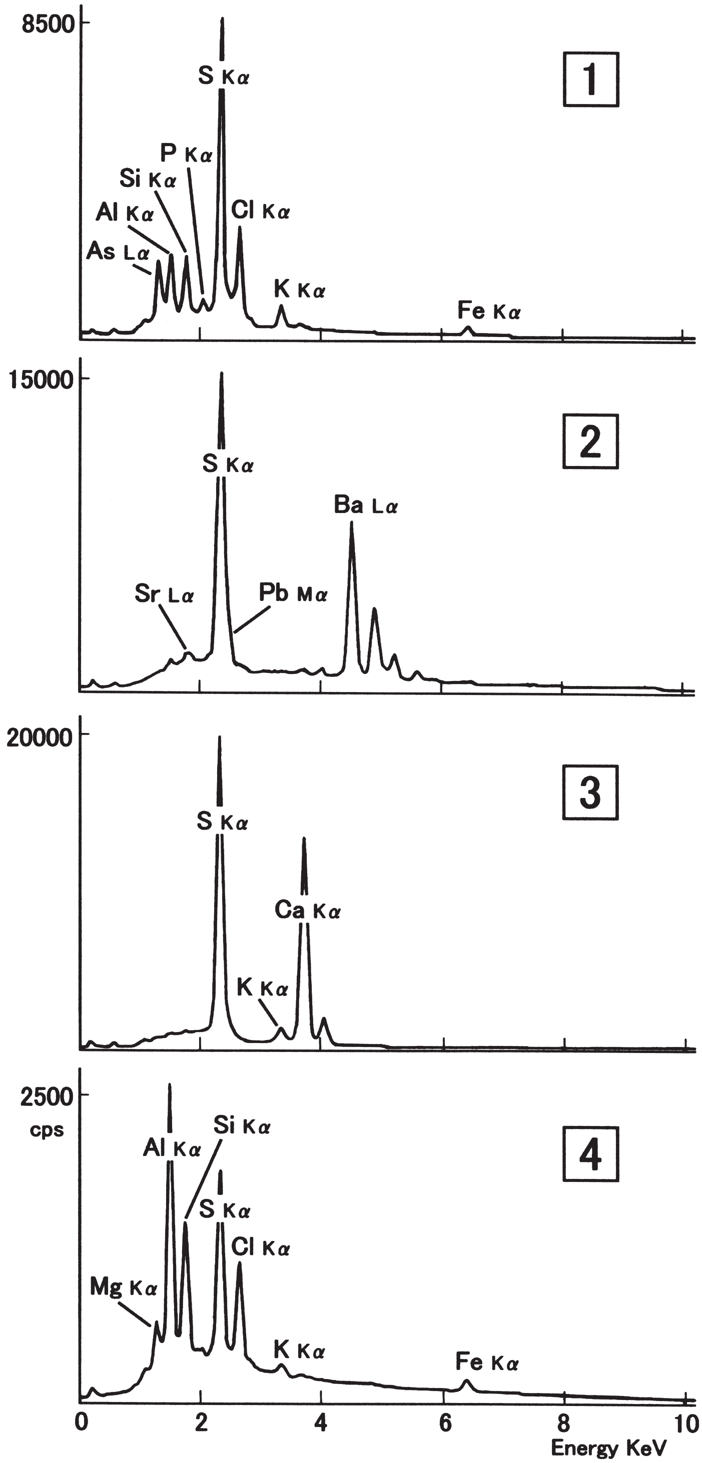


Figure 3. SEM observations and the EDX analyses of the spherical grains of ellipse shaped gypsum and mixture of sulfur-bearing compounds (A-1) and barite (A-2) in the reddish orange biofilms showing various kinds of sulfide minerals produced (B-3, 4). The numbers and arrows in the SEM images coincided with EDX analytical points.


8500
S Kα
1
P Kα
Si Kα
Al Kα
Cl Kα
As Lα
K Kα
Fe Kα
15000
2
S Kα
Ba Lα
Sr Lα
Pb Mα
20000
3
S Kα
Ca Kα
K Kα
2500
cps
Si Kα
Al Kα
4
S Kα
Cl Kα
Mg Kα
K Kα
Fe Kα
0
2
4
6
8
10
Energy KeV

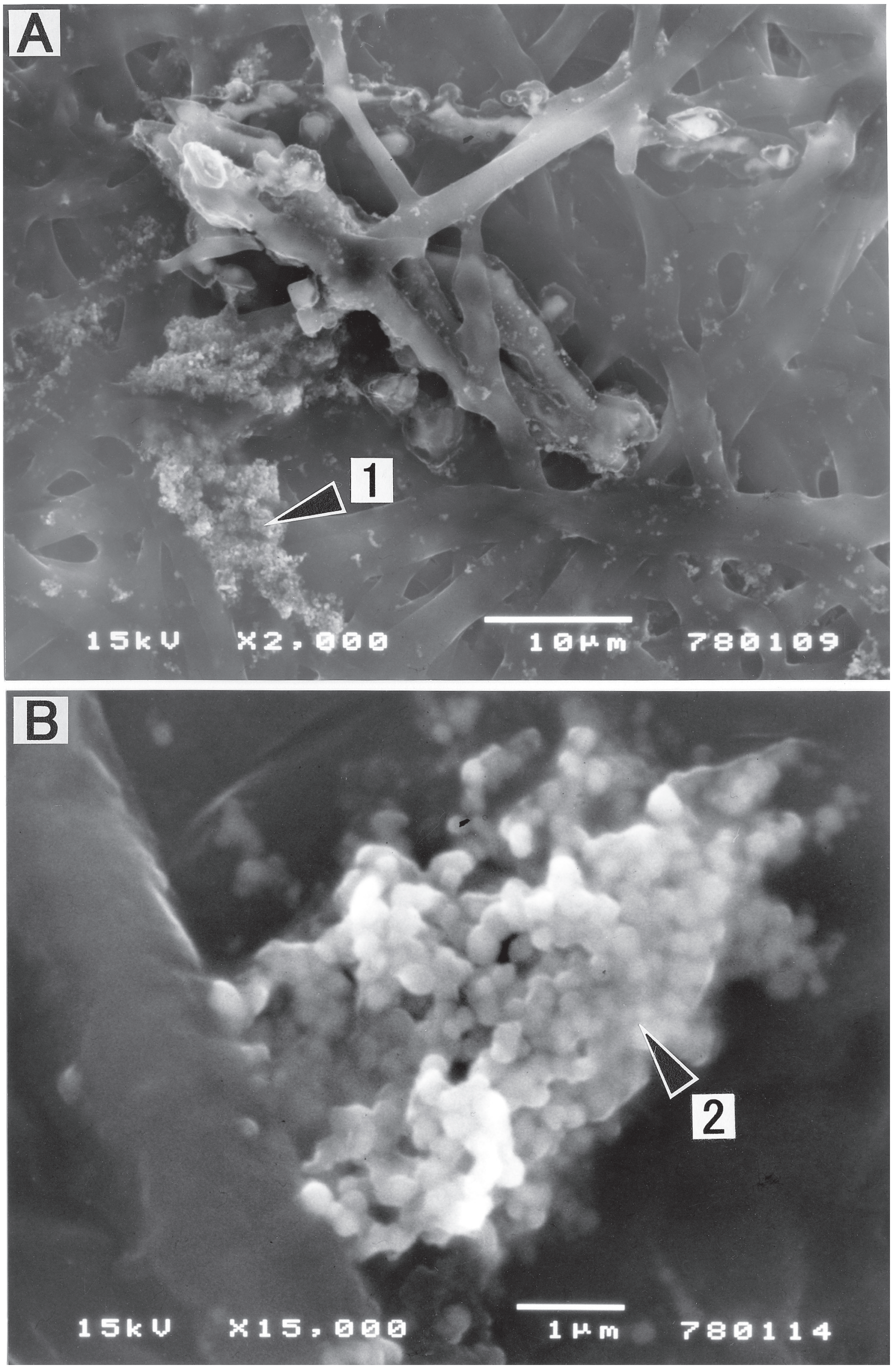
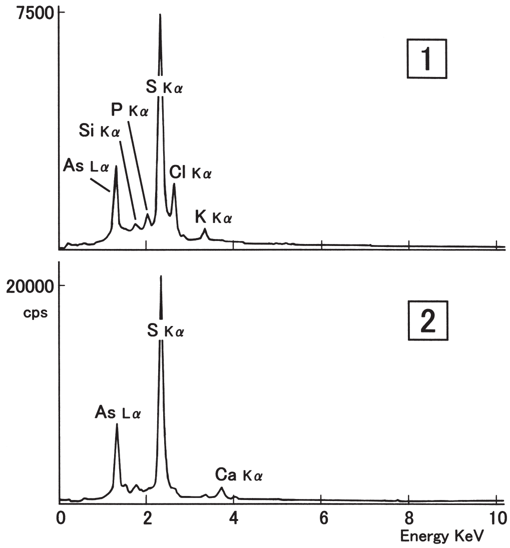


Figure 4. SEM observations and the EDX analyses of spherical grains of barite, gypsum, and orpiment in the reddish orange biofilms. The numbers and arrows in the SEM images are coincided with EDX analytical points.



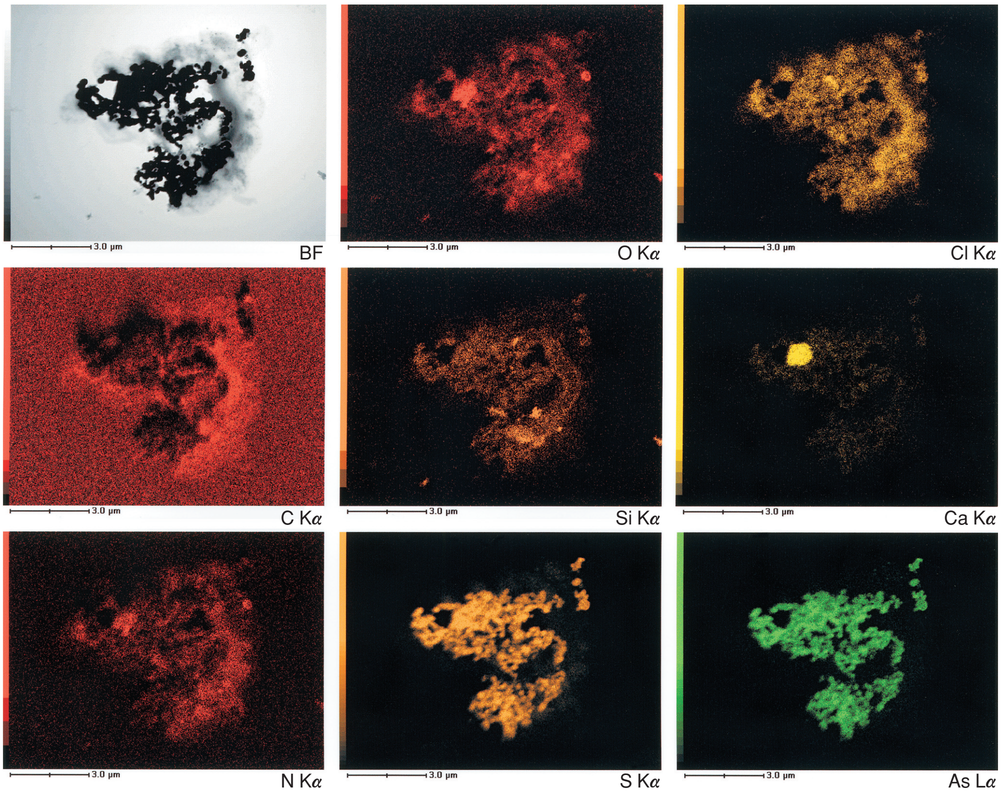


Figure 5. EF-TEM micrograph and the elemental content maps of major compositions of the reddish orange biofilms showing high accumulation of S, Cl and As in the spherical grains of orpiment around bacterial cells.

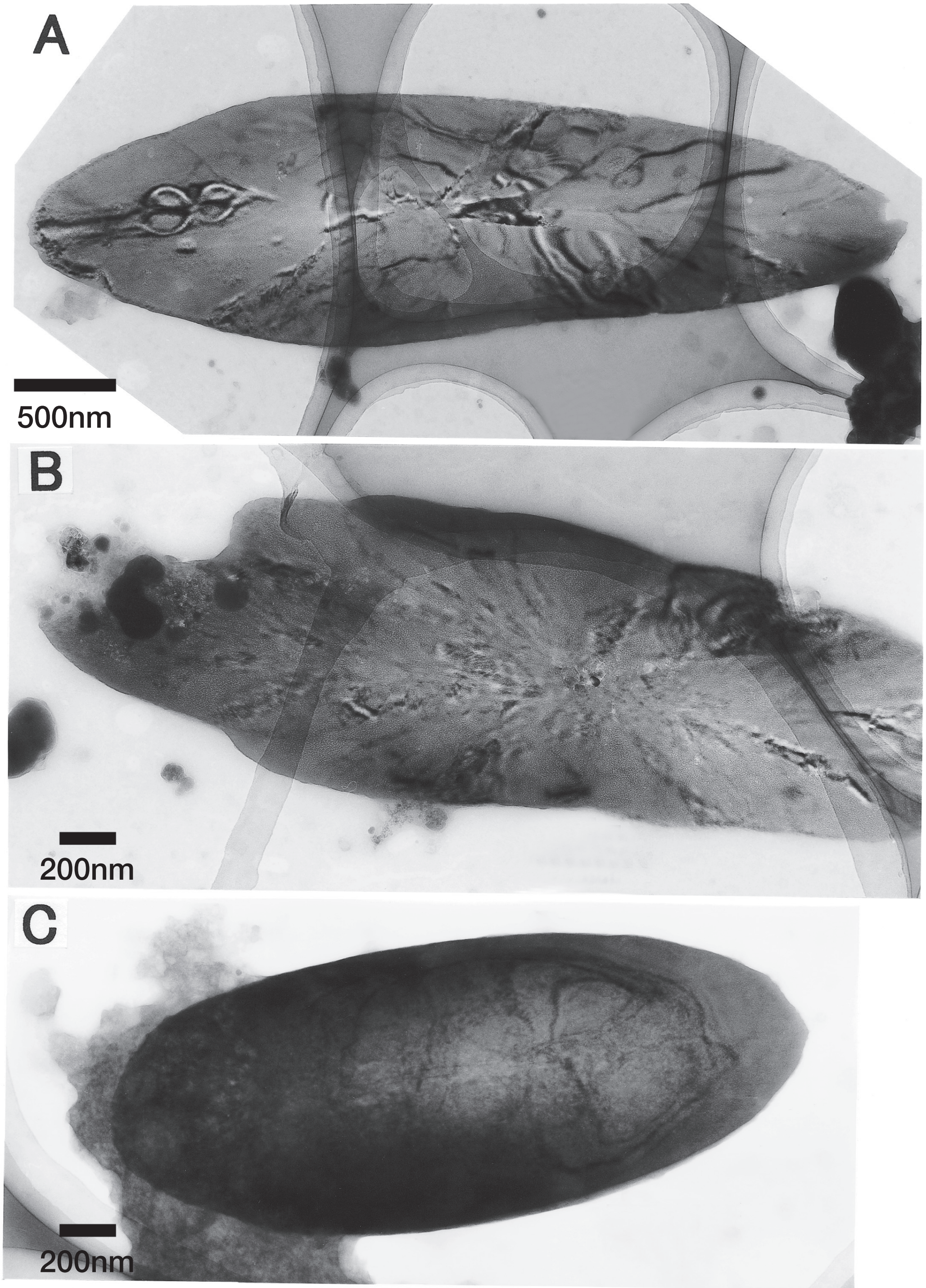


Figure 6. TEM observations of elongated ellipses of gypsum produced in the reddish orange biofilms, showing Moire patterns and stacking faults on the surface of minerals.

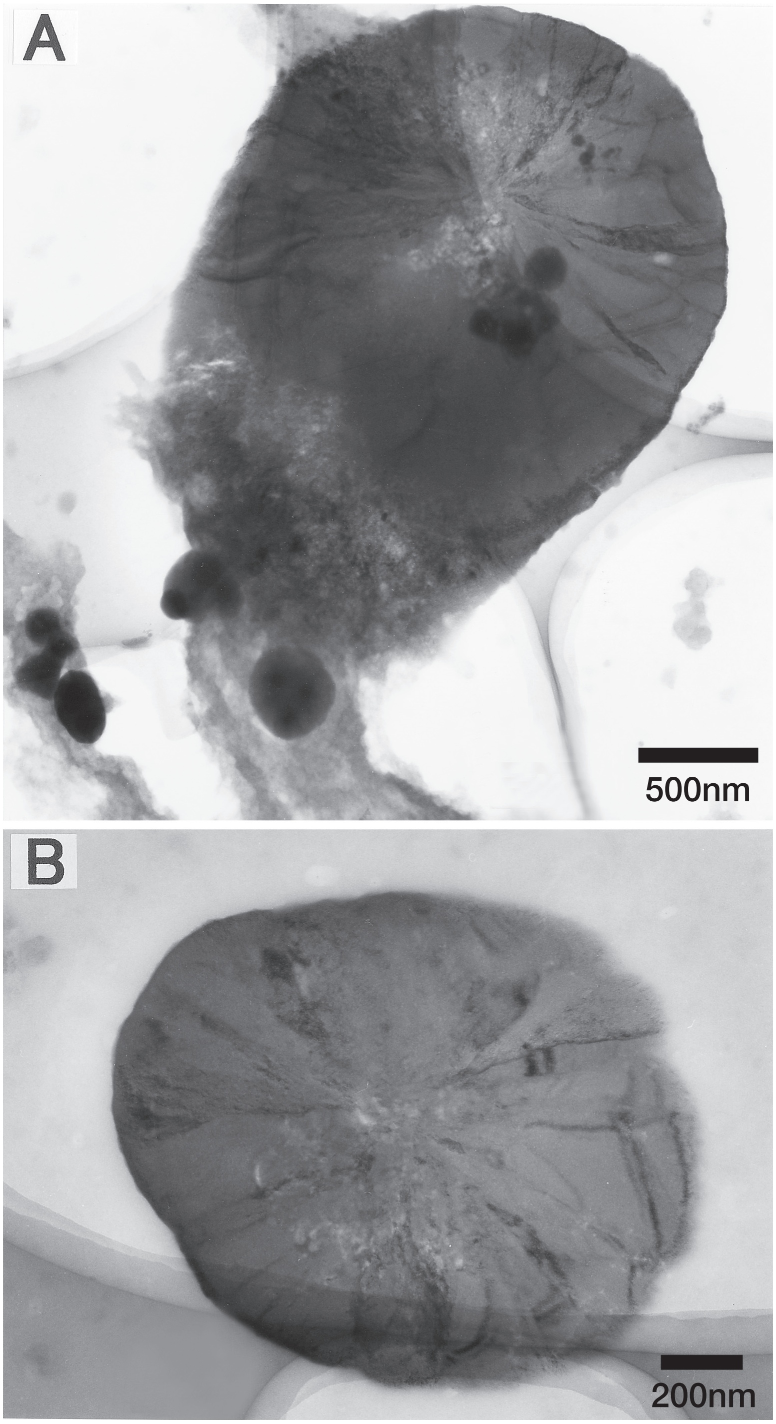


Figure 7. TEM observation of disk shaped barite produced in the reddish orange biofilms, showing Moire pattern and stacking faults on the surface. Note that bacteria and spherical grains of orpiment attached to barite (A). Barite radically grew during aging time (B).

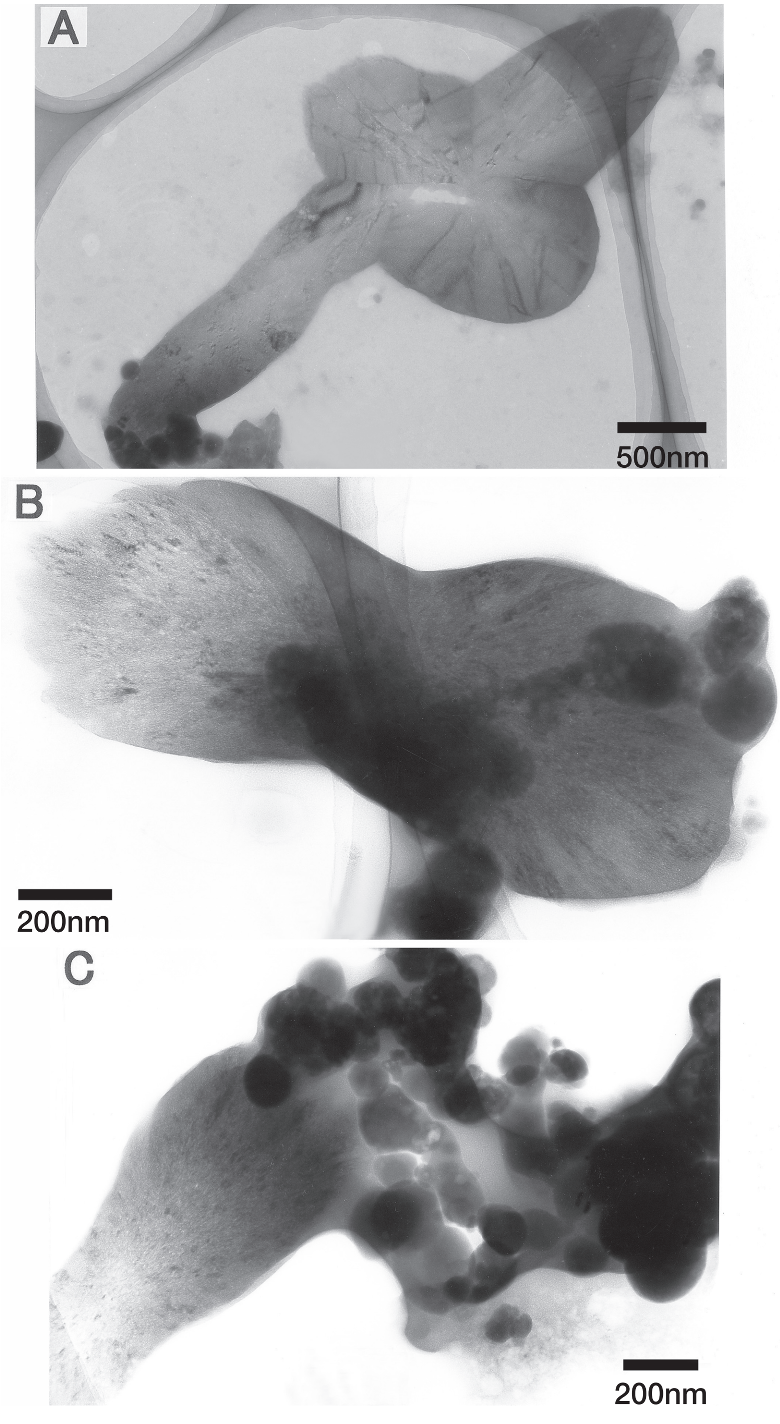


Figure 8. TEM micrographs of combination of the well-developed gypsum and barite produced in the reddish orange biofilms, showing elongated gypsum / rounded barite (A), twins barite (B) and rounded barite / spherical grains of orpiment (C).

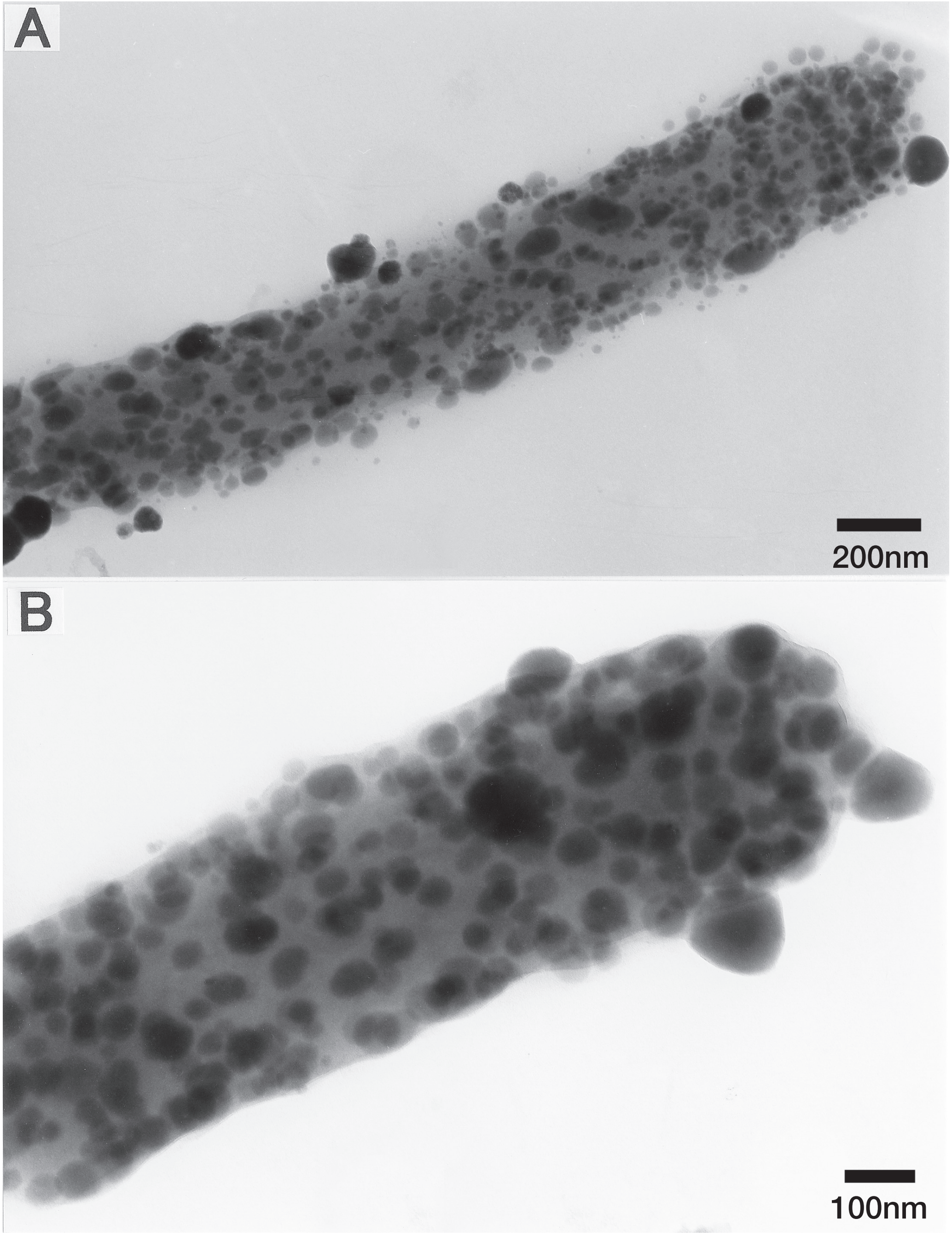


Figure 9. TEM micrographs show spherical grains are stuffed in bacterial cells up. Note that the grain size is relatively free (A) and uniformed (B) suggesting that formation stages are different. The grains developed 200 nm in diameter.

rounded/jointed morphologies are shown in Fig. 8. Some of them appear to be a mixture of gypsum and barite (Fig. 8A), twins of barite (Fig. 8B), and combined crystals of barite and spherical grains of orpiment in the thin films (Fig. 8C). Finally, twins and polytype intergrowths can result if coherencies achieved only in the two dimensions of the interface.

Numerous bacterial cells are entirely coated with high dense of the spherical grains (Fig. 9). The bacterial cells are filled with spherical grains up, <100 nm in diameter, suggesting processes of metabolism. The spherical grains in the bacteria are free sized shown in Fig. 9A, whereas relatively uniform sized grains are shown in Fig. 9B. Few larger sized grains stick to the cell wall. The developed grains with high dense, 200 nm in diameter, appear to be pushed from end of the cell out as shown in Fig. 10A, succeed B and C. Some of spherules are vesicles with cylindrical materials. After all grains were pushed out, the internal cell became empty as shown in Fig. 10-B and -C. It is important to mention that the average size of individual completed grains was approximately 200 nm. Schematic mechanisms of sulfide spherical grains in biofilms with the highest β-ray in strong acidic hot spring, indicate that the accumulation of HSO_4^- or SO_4^{2-} ions in a hot spring water to form particle are hoard in a cell. The particle size is free at the initial stage. Some larger sized particles are jutted from a cell out. The completed particles, approximately 200 nm in diameter, are squeezed from the cell. Note that the bacterium discharge uniform sized particles, < 200 nm in diameter, from the end of the cell. The most of particles are released, and the cell became empty at the vomited stage (Fig. 11).

The interactions between spherical grains of sulfides and bacterial cells could be seen by using TEM microscopy, but the bacteria could not be seen by the SEM-EDX observation, because of aggregations and covering situations. The STEM-EDX clearly showed bacillus type bacteria and spherical grains in the cell, suggesting radioactive sulfides biomineralization during metal immobilization.

6. Biomineralization of radioactive sulfide minerals

Hydrogen sulfide-rich (H_2S) thermal spring and gypsum deposits ($CaSO_4$) were found in Tamagawa Hot Springs suggesting the volatilization of H_2S from the spring water, oxidation of the H_2S gas to sulfuric acid. In this study, the results demonstrated that living bacteria in reddish orange biofilms with the highest β-ray (5700 cpm) under pH 1.2 condition accumulated S, Ba, Ca, and As elements which appeared to be sensitive to the formation of sulfides of barite, gypsum, sulfur, orpiment, and Hokutolite (Fig. 12). Surprisingly, the β-ray value of the biofilms was twice higher value than Hokutorite (1800-3200 cpm).

Brown and white layered Hokutolite was found near Tamagawa Hot Springs (Fig. 13). ED-XRF elemental analyses of layered across section of Hokutolite clearly showed distribution of elements shown in Fig. 14. On the top of surface, Si and Fe were accumulated, whereas Sr, Ba, and Pb were layered in 1-2 mm thick. The layered distributions of Ba and Pb are consistent, but the Sr rich layer is inversely proportional to Ba and Pb rich layers. ED-XRF elemental content maps of layered across section of Hokutolite indicated

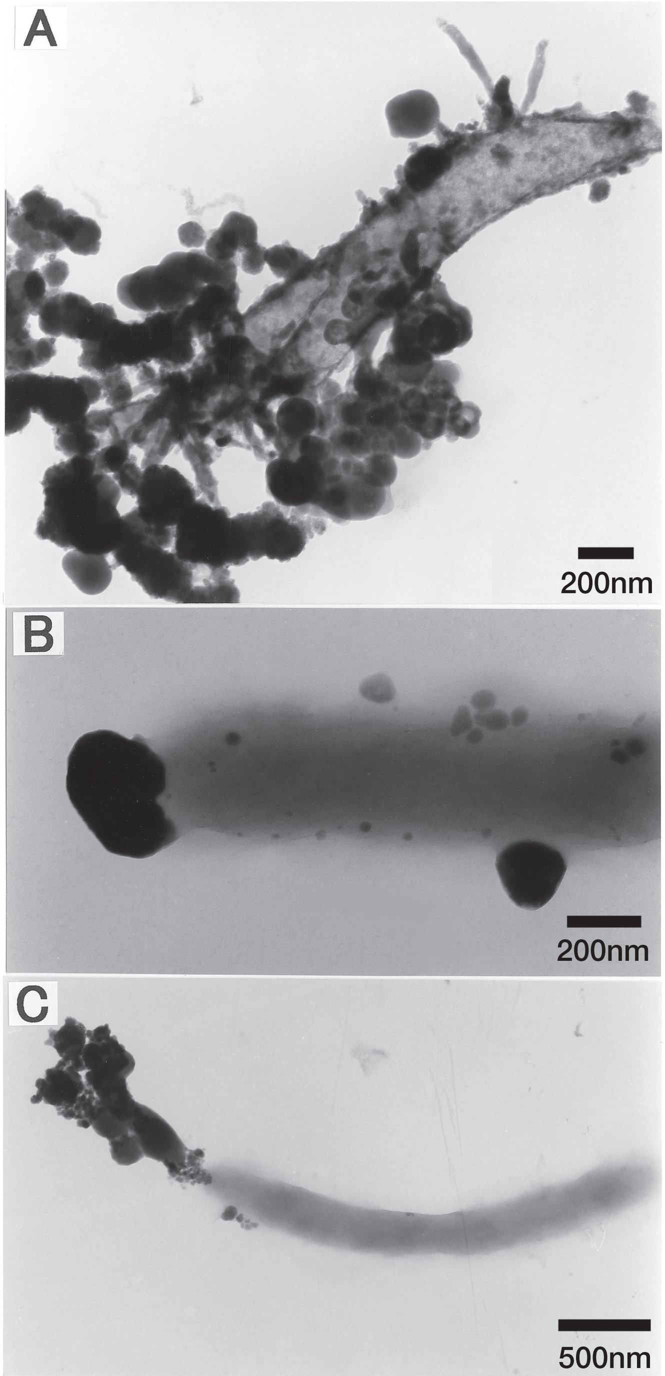


Figure 10. TEM micrographs show that abundant spherical grains of orpiment are pushed from the end of bacterium cell out (A), the most spherical grains are discharged (B), and internal bacterium cell became empty (C). Note that uniform sized spherical grains, approximately 200 nm in diameter, pushed from end of the cell out.

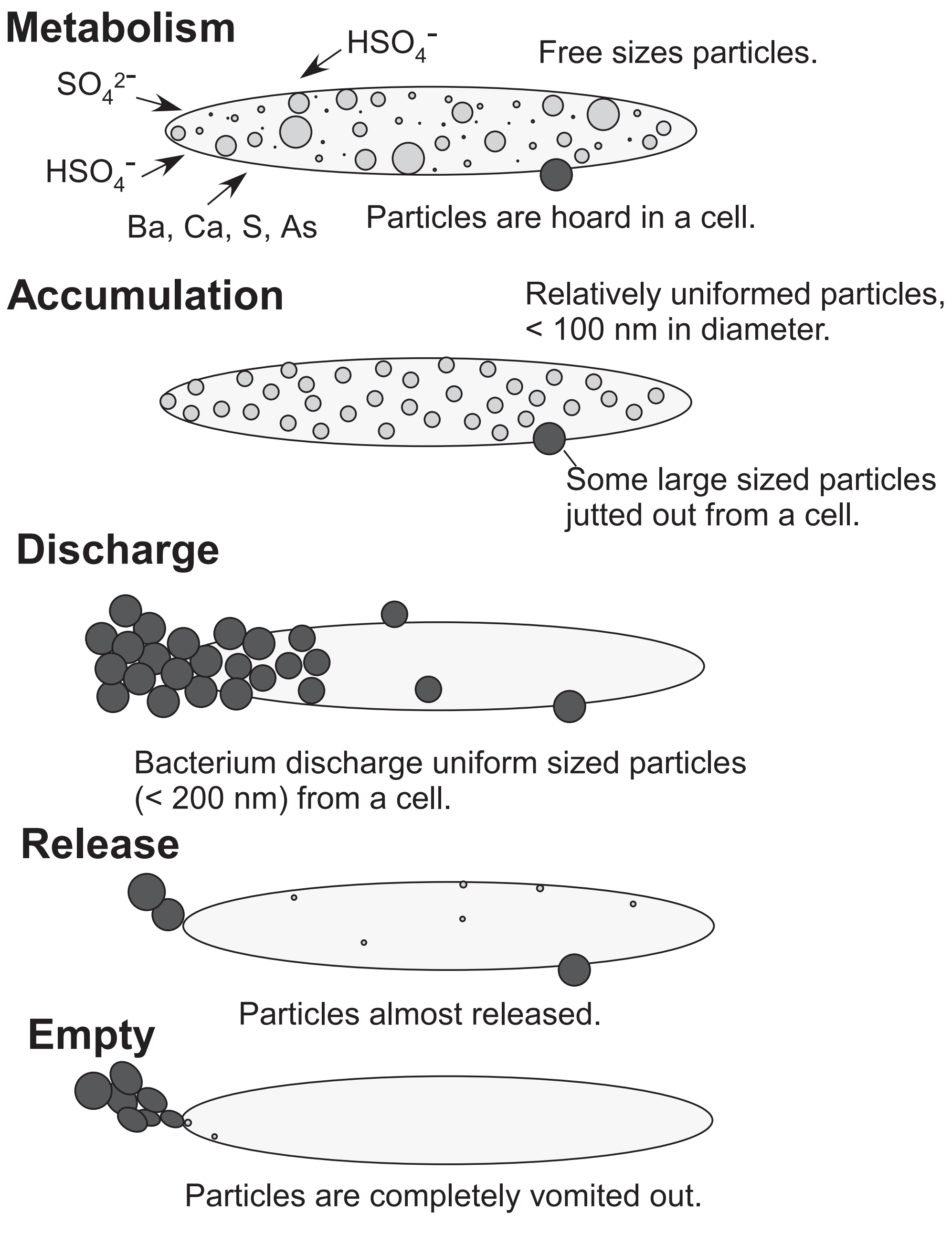


Figure 11. Schematic mechanisms of sulfide spherical grains in biofilms with the highest β-ray in strong acidic hot spring, indicate that the accumulation of HSO_4^- or SO_4^{2-} ions in a hot spring water to form particle are hoard in a cell. The particle size is free at the initial stage. Some larger sized particles are jutted from a cell out. The completed particles, approximately 200 nm in diameter, are squeezed from the cell. Note that the bacterium discharge uniform sized particles, < 200 nm in diameter, from the end of the cell. The most of particles are released, and the cell became empty at the vomited stage.

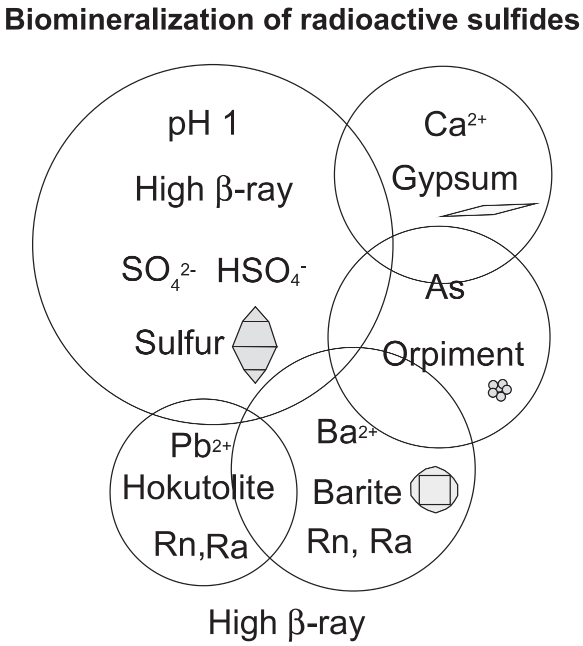


Figure 12. A schematic diagram of the simple system shows formation of sulfur, gypsum, orpiment, barite, and hokutolite minerals under high β-ray radioactive conditions at Tamagawa Hot Springs. The biofilms accumulate heavy metals are mainly present as sulfur in hot spring water at pH 1.

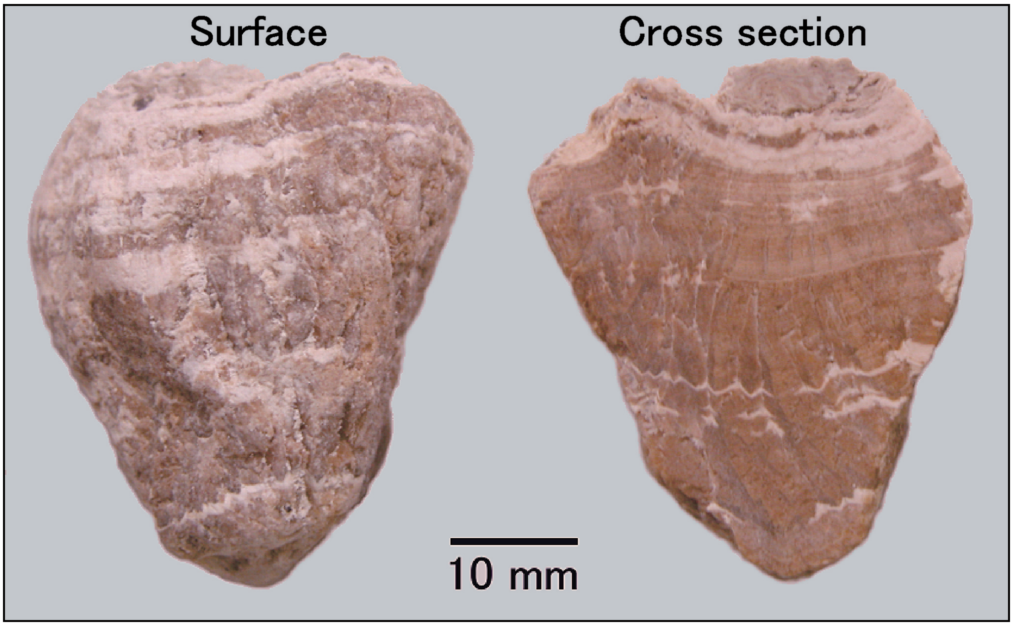


Figure 13. Layered Hokutolite was found at Tamagawa Hot Springs.

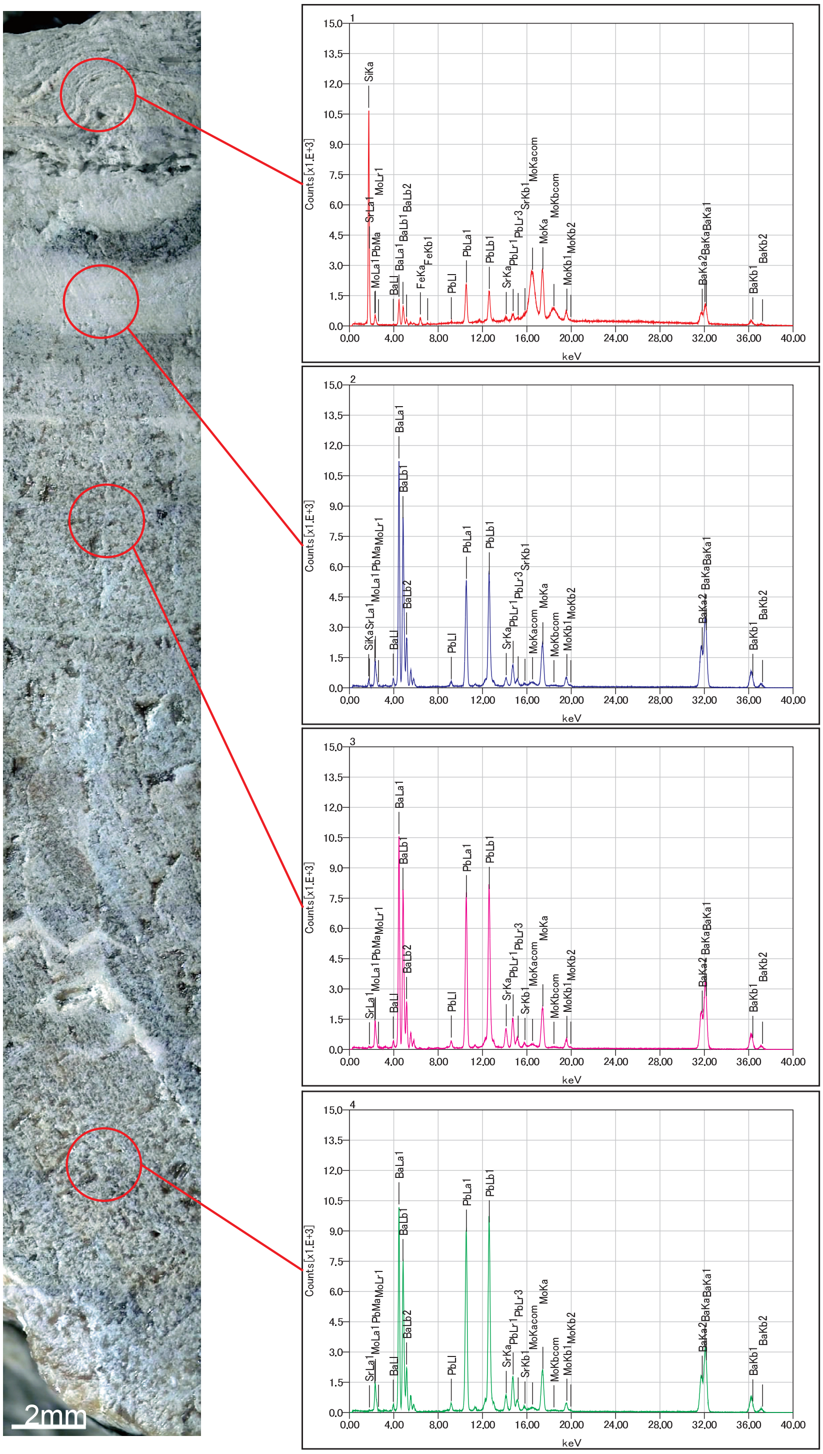


Figure 14. ED-XRF elemental analyses of layered across section of Hokutolite.

Si rich layer on the top surface, Ba and Pb rich layer in white parts with traces of Mo and Sr (Fig. 15 middle). The porous area was concentrated with Pb and Ba in bottom of Fig. 15. In the middle of across section, the white and brown layers appeared to have similar chemistry (Fig. 15), but XRD patterns of white layers and brown layers clearly identified different crystalline as shown in Fig. 16. The white parts contained large amounts of organics or amorphous materials associated with barite, cristobalite, and sulfur minerals. On the other hand, the brown parts contained only high crystalline of barite. The Si rich layer on the top surface contained cristobalite in consistent with ED-XRF analytical data. The high background of the XRD result suggested that abundance of microorganisms was presented to control bio-mineral growth.

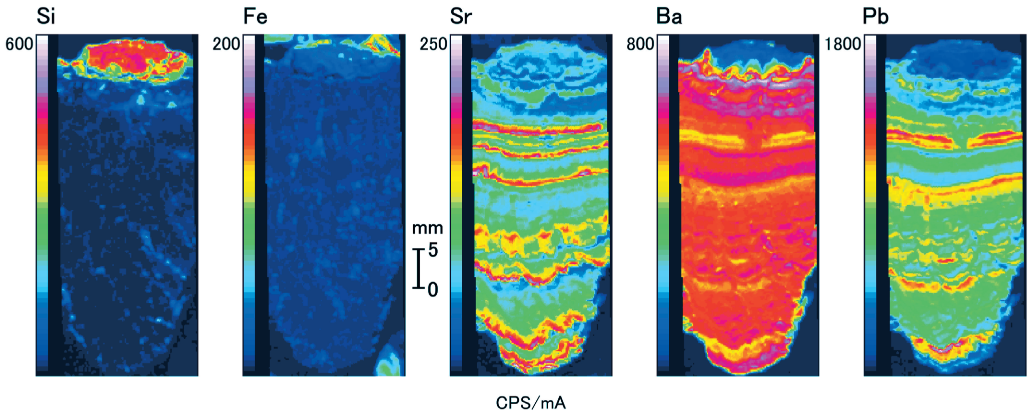


Figure 15. ED-XRF elemental content maps of layered across section of Hokutolite.

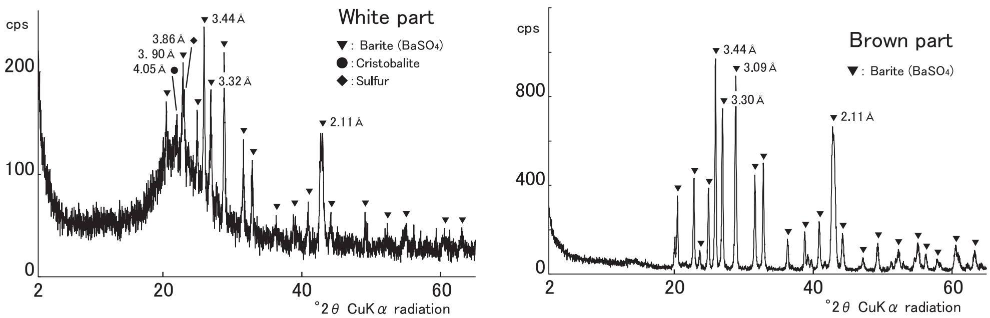


Figure 16. X-ray powder diffraction analysis of the white and brown parts of Hokutolite indicated different mineral formation of crystalline.

Discussions

The metal-loaded cells nucleate the formation of a mixed assemblage of crystalline metal phosphates and metal sulfides (Beveridge et al., 1983). There are many potential intermediate sulfur and sulfoxy compounds generated in the process, including elemental sulfur, thiosulfate, tetrathionate, and sulfite. In addition to water-soluble compounds, special microorganisms are also involved in radioactive mineralization processes (Suzuki et al., 2003). Thus, certain microorganisms can utilize oxidation of some sulfur-bearing compounds (by oxygen) to generate metabolic energy (Banfield and Welch, 2000). The bacteria oxidize H_2S either complexly to sulfate for energy or partially to elemental sulfur that is stored intra cellular for later use. Elemental sulfur interacted with the metal-loaded cells, and formed a variety of crystalline metal sulfides. Several sulfate-reducing microorganisms are able to reduce U (VI). The U (VI) reduction is the likely explanation for the concentration of Uranium in the reduction spot of microbial mats. The ability of bottom sediments of waste treatment ponds to remove dissolved Uranium from Uranium mine wastewater is probably due to reductive precipitation of Uranium (Lovley et al., 1991).

The sulfide spherules were of uniform size and some of them were aggregated, showing a matrix of colonies. The uniformed particles (200 nm in diameter) passed easily through holes at the end of the cell, but larger particles up to 200 nm in diameter failed to go out of an opening. This may lead to preservation of cell-related morphology, which is significance to fossilization and bio-signature preservation (Fig. 11). Interestingly, uniformed particles were able to traverse the blockade by squeezing cells. Leaving the particles emerges out at the only one side of the end. This kind of device might offer a rapid screen for agents that inhibited or reversed the biomechanical effects. A fundamental property of many products of biomineralization reactions, is very small particle size, results from rapid nucleation due to super-saturation following iron oxidation (Banfield and Welch, 2000). Small nucleus size is preserved due to low solubility of ions, which limits diffusion-based crystal growth. All microstructures that arise from nm-crystal aggregation have the potential to subsequently modify the reactivity, and thus the form and fate, of biomineralization products.

In this study, the correlation between the results of β-ray measurements and the microscopic observations of natural evidence of radioactive biominerals are considered to be helpful for further discussion of the nuclide medical treatments. This organism clearly has the potential for use in bioremediation of radioactive areas under strong acidic conditions.

Conclusions

TEM micrographs clearly showed the biological formation of radioactive sulfide minerals in strong acidic hot springs. Bacteria in reddish orange biofilms were able to accumulate S, Ba, Ca, and As elements from strong acidic hot spring water (pH 1.2), indicating the highest β-ray counts (5700 cpm) at Tamagawa Hot Springs, Akita, Japan. Bacterial biomineralization processes were revealed by direct observation of analytical electron microscopy. SEM-EDX and TEM observations provided some insight into how microorganisms affected the chemistry and microbiological characteristics of the strong acidic surroundings. The chemical analysis of the biofilms showed S, Ba, Ca, and As, as the major elemental composition. Especially SEM-EDX, ED-XRF, and STEM-EDX elemental content maps of the reddish orange biofilms illustrated the distribution of sulfur-bearing compounds of barite ($BaSO_4$), gypsum ($CaSO_4 \cdot 2H_2O$), sulfur (S), orpiment (As_2S_3), and Hokutolite ($(Ba, Pb)SO_4$). These minerals were associated with living bacteria. TEM micrographs of bacteria in the biofilms revealed in detail the intimate connections between biological and mineralogical processes that the cells were entirely accumulated with spherical grains, 100～200 nm in diameter. This shows that microbial activity may play a significant role in the radioactive environments, and that this may be a common occurrence. The relationship among radioactive sulfide minerals and bacteria implies that heavy metals had been transported from strong acidic hot spring water to minerals through bacterial metabolism. Sulfide minerals formation in hot spring water may be substantially enhanced by the presence of bacteria. This bacterium clearly has the potential for use in bioremediation of radioactive areas.

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References

Akita analytical center, 2001. Water chemistry of Tamagawa Hot Springs, Akita, pp2.

Banfield, J.F. and Welch, S.A., 2000. Microbial controls on the mineralogy of the environment. EMU Notes in Mineralogy, 2 : 173-196.

Beveridge, T.J., Moloche, J.D., Fyfe, W.S. and Murray, R.G.E., 1983. Diagenesis of metals chemically complex to bacteria : Laboratory formation of initial phosphate, sulfides, and organic condensates in artificial sediments. Applied and Environmental Microbiology, Mar. 1094-1108.

Brock, T.D., 1967. Life at high temperatures. Science, 158 : 1012-1019.

Horiuchi, K., 2001. Hot springs and it radiation in Japan. Jour. Japanese Society of Radiological Technology, 57 : pp. 1462-1468 (in Japanese).

Lienert, C., Short, S., and Gunten, H., 1994. Uranium infiltration from a river to shallow groundwater. Geochimica et Cosmochimica Act, 58 : 24, 5455-5463.

Lovley, D.R.E., 2001. Anaerobes to the rescue. Science, 293 : 1444-1446.

Lovley, D.R.E., Phillips, E.J.P., Gorby, Y.A., and Landa, E.R., 1991. Microbial reduction of uranium.

Nature, 350 : 413-416.

Mann, H. and Fyfe, W.S., 1985. Uranium uptake by algae : Experimental and natural environments. Canadian Journal of Earth Science, 22 : 1899-1903.

Markert, B. (Ed.), 1993. Plants as Biomonitors. VCH Verlagsgesellschaf mbH, D-6940 Weinheim (Federal Republic of Germany), pp 644.

Paktunc, D. and Dutrizac, J.E., 2003. Characterization of arsenate-for-sulfate substitution in synthetic jarosite using X-ray diffraction and X-ray absorption spectroscopy. The Canadian Mineralogist, 41 : 905-913.

Sugie, C., 1986. Guide book for Tamagawa Hot Springs in Akita. 65.

Suzuki, Y., Kelly, S.D., Kemner, K.M. and Banfield, J.F., 2003. Microbial populations stimulated for hexavalent Uranium reduction in Uranium Mine sediment. Applied and Environmental microbiology, Mar. 1337-1346.

Tazaki, K. and Ishida, H., 1996. Bacteria as nucleation sites for authigenic minerals. Jour. Geol. Soc. Japan, 102 : 866-878.

Tazaki, K., Asada, R. and Ikeda, Y., 2002. Quick occurrence of Fe-rich biofilms on the surface of water. Journal of Clay Mineralogical Association of Japan, 42 : 21-36.

Tazaki, K., 2003. Water and Soil Environments ; Microorganisms play an important role. Tazaki, K. (Editor), 21st Century COE Kanazawa University Program, pp 254.

Yoshida, H., Yu, M., and Sibutani, T., 1994. Flow-Path structure in relation to nuclide migration in sedimentary rocks. Journal of nuclear Science and Technology, 31 : 803-812.

Yoshiyama, H., 2003. Elastic light scattering measurement of an ellipsoid aerosol particle. J. Aerosol Res., 18 : 272-277.

Waite, T.D., Davis, J.A., Payne, T.E., Waychunas, G.A. and Xu, N., 1994. Uranium (VI) adsorption to ferrihydrite : Application of a surface complexation model. Geochmica et Cosmochimica Acta. 58 : 5465-5478.