A new world in the science of biomineralization
-Environmental Biomineralization in Microbial Mats in Japan-

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Abstract : Bacteria play an important role in the concentration, crystallization, transportation and sedimentation of almost all elements in the Earth's environment. In the geo-, aqua-, and eco-systems, microbial mats of bacterial colonies with biominerals are an exciting area of study. Bacterial biomineralization plays a key role in the remediation of toxic heavy metals in polluted soils and water systems at abandoned mining sites. Colorful microbial mats can be seen in hot springs and geothermal areas. The biomineral assemblages can include carbonates, silicates, Fe-Mn oxides, hydrated phosphates, sulfides and clay minerals formed through bacterial activities. The nature of the assemblage is dependent on temperature, pH, Eh, DO and EC conditions. Electron microscopic techniques in geo-microbiology have opened a new world of observation in the geosciences. The objective of this study is to introduce the natural occurrence of biominerals in microbial mats in Japan to better understand the role of bacteria in their nucleation and crystallization.

Key words **:** Biomineralization, Microbial mats, Bacteria, Environmental pollution, Bioremediation, Electron microscopy, Water chemistry.

1. Introduction

In the Earth's surface environment bacteria, biofilms and microbial mats (*biomats* ; bacterial colonies with biominerals) are universal, and a host of minerals is synthesized in complex bio-mediated processes. Various microorganisms have the ability to accumulate metallic ions from their external aquatic environments of ground water, hot springs or wetlands. Dissolution and precipitation of minerals are primarily mediated by microorganisms under natural surficial conditions. All species can exist in the presence of a wide range of major and trace elements and a variety of environmental conditions.

Disposal of toxic metal wastes to surface and subsurface waters poses unacceptable health risks. Pollution of soils and water supplies and concentration of heavy metals in mining areas are of particular concern. Very little is known about microbial mobilization of metals under environmentally realistic conditions.

Understanding micro-biomineral synthesis is essential to understanding our environment.

For example, in tailings ponds, river sediments, hot springs, sea water and on the deep sea floor, we have found varied biominerals, such as carbonates, silicates, Fe-Mn oxides, hydrate phosphates, sulphides, sulphates and clay minerals, all formed by bacterial activity.

Microorganisms can be used for bioremediation in environmental pollution problem areas. Such organisms not only reduce heavy metal concentrations, but may also be utilized to reuse and recycle metals and biominerals from mine wastes.

Colorful microbial mats can be seen in hot springs and geothermal areas. These mats contain varied biomineral assemblages dependent on water temperature, hydrogen ion concentration (pH), oxidation reduction potential (Eh), dissolved oxygen (DO) and quantity of dissolved ion present (measured by electrical conductivity [EC]). Even in the high temperature, strongly acid conditions of hot springs, microorganisms produce various minerals outside and/or inside the living cell. The recent discoveries of clear smokers on the sea floor add to the possibilities of such biomineralization. Understanding the mechanisms of bacterial biomineralization is essential to our understanding of Paleo-Earth and the origin of life.

Electron microscopic techniques in geo-microbiology have opened a wonderful new world of observation in the geosciences. Using scanning electron microscope-energy dispersive X-ray spectrometer (SEM-EDX) and transmission electron microscope (TEM) techniques, the interactions between microorganisms and metals in biomats in the geo-, aqua-, and eco-systems can be studied.

The objective of this study is to introduce the natural occurrence of biominerals in biomats in Japan as a basis to understanding the role of bacteria in their nucleation and crystallization. This paper describes current information on biomats at 24 localities and the environmental factors that affect the cycle, transport, and transformation of metals.

2. Study Methods

In this section, the methods used to study biomats and specifically the methods used to investigate those biomats occurrences covered by this paper are briefly introduced. The study of microbial mats is, of necessity, multi-disciplinary. Geological, mineralogical, microbiological and chemical approaches are necessary components of interpretive synthesis.

< *Field work* >

1. Geology and Sampling

The geology of an area where biomats are formed is fundamental to their investigation. The geological environment affects both underground and thermal waters. Since microorganisms living in biomats metabolize using materials from the air, water, sediments and rocks, it is essential to record details not only of the microbial mats, but also of the surrounding geological environment, such as terrain, basement lithology soils and weathering conditions. Depending on the situation, samples are taken of sediments, rocks, water, and other materials. Where biomats themselves are collected, appropriate sample handling of the study material is critical.

2. Water Chemistry

Since microorganisms absorb materials into cells as ions, water conditions affect the microorganisms living in biomats. Water chemistry data are thus important in identifying the species and properties of the microorganisms present.

Water temperature, pH, Eh, DO, EC are measured in the field to obtain data on water conditions.

- pH is an index of concentration of hydrogen ion,
- Eh is the oxidation-reduction potential of an aqueous solution,
- DO indicates the quantity of dissolved oxygen,
- EC is the electrical conductivity and relates to the quantity of dissolved ion.

Seasonal changes in these indexes should be taken into consideration. The chemical compositions of water in the field are also essential data.

3. Observations of Biomats

During field observations, the following information is recorded in detail.

1. Color (s) of biomats,

   The color of biomats reflects the species of microorganisms and biominerals present. Depending on the situation, the spatial distribution of mat colors is also recorded. Spectroscopic methods for analyzing the color differences should also be used where appropriate.
2. Exposure of biomats to solar radiation,
3. Volume and flow rate of surrounding water,
4. Sediment and rock type on which the biomats aggregate,
5. Thickness, hardness and any other physical characteristics of the biomats,
6. It is desirable to take photographs of field occurrence to record information for further analyses in the laboratory.

< *Laboratory work* >

1. Sample preparation (thin section and ultra thin section)

1-1. Thin section preparation using resin.

Optical microscopic observations of thin sections are essential to determine the spatial relationship between the microorganisms and the authigenic minerals in the biomats. As biomats contain water and are often fragile, thin section preparation is difficult. Accordingly, to prevent breakage, it is often necessary to coat samples with resin. Thin sections are made by the following procedure.

1. Drying ; to eliminate water,
2. Embedding ; embed the inner microtexture with cyanobond,

3. Resin coating ; coat the biomat samples with epoxy or polyester resin,
4. Thin section preparation ; the preparation is completed in the same way as for ordinary preparation.

However, extreme care must be taken as the microorganisms and minerals in the biomats are fragile so are easily abraded or broken.

1-2. Ultra thin section preparation

Ultra thin sectioning produces a section about 0.1 μm thick which is used to observe the inner microtexture of the cells by TEM. Bacteria in biomats produce authigenic minerals both within and outside their cells. Ultra thin sectioning is, therefore, useful for observing the distribution of minute mineral grains. The procedures for preparation of ultra thin sections are as follows.

1. Fixing; to preserve the microtexture of the samples with glutaraldehyde or similar fixative,
2. Dehydration; to substitute for water by means of a water soluble synthetic resin,
3. Resin coating ; to coat the samples with a synthetic resin,
4. Cutting ; to cut the samples using an ultra microtome,
5. Dyeing or coating ; to increase the contrast of TEM image.

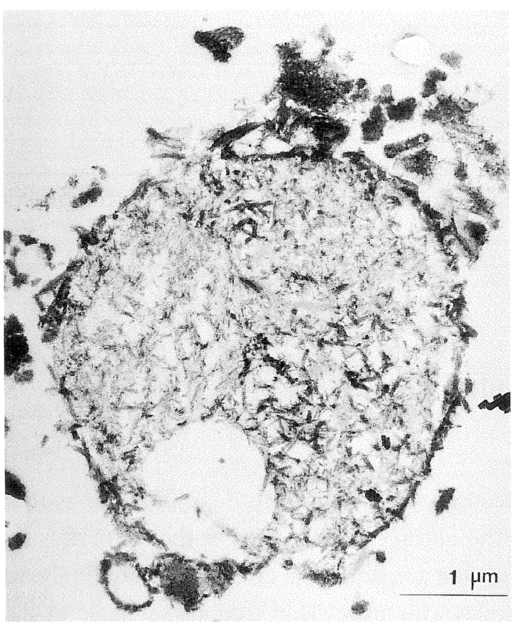

Fig. 1 Transmission electron micrograph of ultra thin-sectioned *Euglena* sp. (unicelluar algae). Intracellular development of lepidocrocite (γ-FeOOH) can be observed.

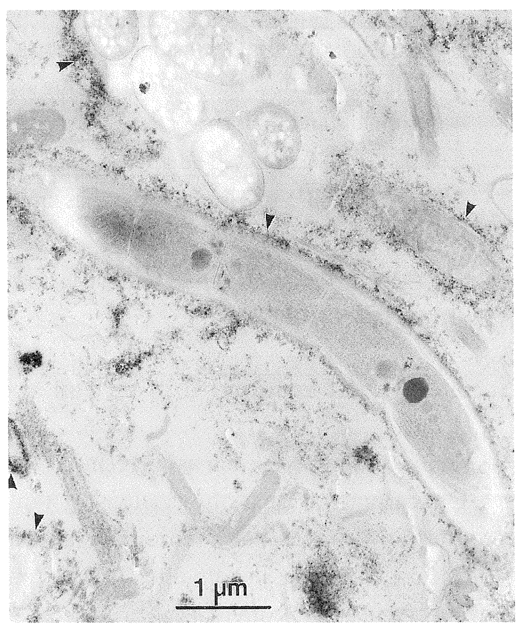

Fig. 2 Transmission electron micrograph of an ultra thin-sectioned microorganism precipitating hydrated iron within the cell.

2. Optical microscope

Observation methods using optical microscopy are as follows.

2-1. Bright field image

Observation by a bright field image is a standard observation method in transmitted light. As a first step in laboratory studies of biomats, this observation method is usually used in conjunction with stereoscopic and differential interference imagery. Distribution, form, color and texture of microorganisms and minerals ranging in size from a few to several hundred microns can be observed at the level of magnification of an optical microscope.

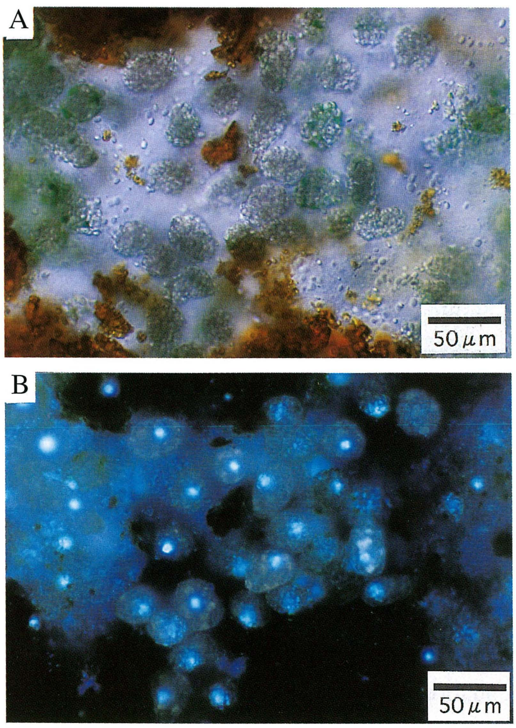

Fig. 3 Differential interference micrograph showing protists in biomats at Ogoya Mine (A) and fluorescence micrograph showing DAPI-stained protists (B)

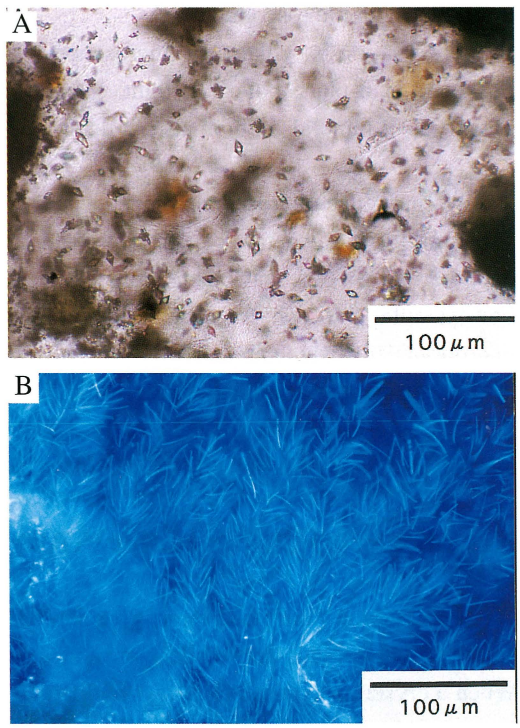

Fig. 4 Differential interference micrograph showing sulfur-turf and α-sulfur crystals found at Hirayu Hot Springs (A) and fluorescence micrograph showing the DAPI-stained sulfur-turf and α-sulfur crystals (B).

2-2. Differential interference image

A differential interference microscope is frequently used for purposes similar to observations using a bright field image. While contrast in a bright field image results from the differences in intensity of transmitted light, the interference color of a differential interference image represents differences in optical path. It is often difficult to observe transparent microorganisms in a bright field image but in the case of an interference image, the shapes of such microorganisms in three dimensions can be obtained in clear outline. This is because of the small differences in refractive index between microorganisms and water, and the differences in the thickness of the microorganisms themselves. Caution needs to be exercised, however, to determine if the contrasts observed by this method are real or are artifacts of optical effects.

2-3. Polarizing microscopic image

Observation by polarizing microscope is essential to identify the minerals present through observation in plane polarized light, crossed nicols and conoscopic illumination. The optical properties of minerals in biomats can determine the minerals identified.

2-4. Fluorescence microscopic image

Sample fluorescence can be observed by means of a fluorescence microscope. When exposed to ultraviolet rays excitation, some minerals and microorganisms in biomats fluoresce. For example, chlorophyll or bacteriochlorophyll exhibits red fluorescence. In the case of some microorganisms which are hard to distinguish from other materials, fluorescence before and after dyeing can be a determining factor. Nucleic acids such as DNA and RNA singularly combine with a fluorescing stain to change into a fluorescent material. For example when exposed to ultraviolet rays, DAPI-stained DNA displays blue fluorescence.

3. X-ray powder diffractometer (XRD)

X-ray powder diffractometry is a relatively simple method of identifying minerals. As the atoms within crystals are regularly arranged, the crystals have numerous crystal lattice planes. When a crystal is exposed to X-rays and Bragg's equation for reflection is satisfied, the reflected X-ray has the same angle as the incident X-ray. Bragg's equation for reflection is :

$$2d \sin\theta = n\lambda$$

where d is a lattice spacing, θ is an angle of diffraction, n is an integral number and λ is the wavelength of the incident X-ray.

For practical analysis, a chart with the horizontal axis showing 2θ and the vertical axis showing the intensity of diffraction is used. Every mineral has a characteristic chemical composition and crystal structure which affect the intensity and the angle of diffraction. So according to the type of mineral, the diffraction pattern in the chart is different, and the mineral can be identified by its characteristic diffraction pattern. XRD is also used for analyzing lattice constants, and to quantify mixtures, crystallinity and other parameters. For XRD analysis of biomats, powdered samples or the biomats themselves are set on a non-refracting surface.

4. X-ray fluorescence spectroscopy (XRF)

When X-rays excite an atom, characteristic X-rays are emitted, the wave length of which is dependent on the element. The elements present can be determined qualitatively and quantitatively by this method.

5. Scanning electron microscope (SEM)

When a focused electron beam scans the surface of a sample, secondary electrons are emitted from the sample. Observations by SEM are made by detecting the secondary electrons which are used to produce a magnified image on the monitor. Stereoscopic vision is a feature of SEM images. Biomat samples must be dried before observation under high-vacuum conditions. Extreme care should be taken to avoid deformation of the sample from dehydration. Where this is a problem, methods such as freeze drying (Suzuki *et al.*, 1995), or observation under low-vacuum conditions can be used.

6. Energy dispersive X-ray spectrometer (EDX)

When an electron beam strikes against an atom, characteristic X-rays are emitted. By detecting the characteristic X-ray, the elements contained in the sample are be identified. The use of EDX in combination with SEM and TEM allows qualitative and quantitative analysis of specific sites within the image of the sample under observation. There are two X-ray detection methods. Energy dispersive X-ray spectrometry (EDX) and wavelength dispersive X-ray spectrometry (WDX). The speed of EDX analysis makes it very useful for biomat studies.

7. Transmission electron microscope (TEM)

When an electron beam strikes a very thin sample, part of the electron beam is transmitted.

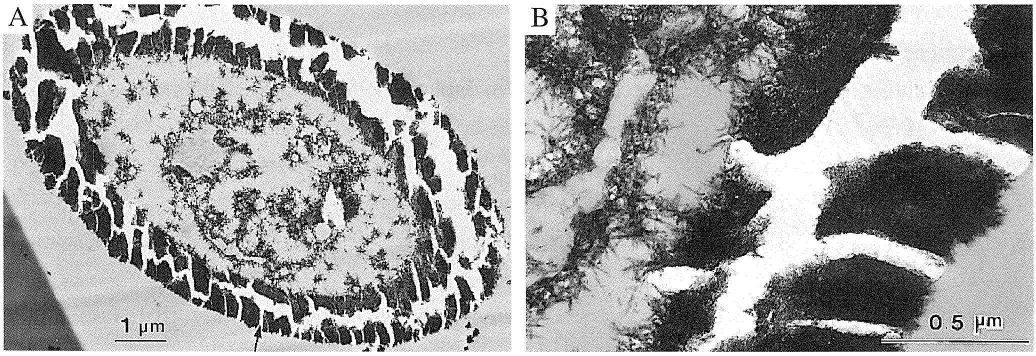

Fig. 5 Transmission electron micrographs of ultra thin-sectioned microorganism forming goethite and maghemite within the cell.

TEM obtains a magnified image by taking advantage of the transmitted electrons. TEM images can be divided into bright field images (high-resolution images, crystal lattice images), dark field images and electron diffraction patterns. Basically, the bright field image on a TEM is produced made by the same process as an optical microscope image. While the contrast in an optical microscopic image is derived mainly from differential absorption of light by the sample, that of a TEM image is mainly obtained by the differences in the scattering of electrons in the sample.

In the study of biomats, the TEM bright field image is used mainly to investigate the shape of very fine materials which are barely observed under the SEM, and the inner microtexture of the substances such as microorganisms.

Ordinarily, an electron diffraction pattern means a selected-area diffraction pattern which can be obtained only from a selected mineral within the TEM image. As biomats contain very fine mineral grains, which can scarcely be seen under an optical microscope, TEM is very useful in the study of biomats.

8. Microorganism culture methods

By supplying water and sediments collected from the field to living biomat samples, the field environmental conditions can be replicated. This natural means of culture retains the properties of the mats. Other environmental parameters such as pH, temperature, light, and partial pressure of oxygen are controlled and altered during culture in the laboratory to determine which factors are essential in controlling microorganism behavior.

These culture methods are effective, but difficulties can be encountered, when culturing biomats by such methods over a long period. It should also be taken into account that the phenomena observed in the biomats containing plural species of microorganisms result from interactions between the microorganisms. An alternative method is to culture a particular species of microorganism separated from the biomats in an artificial medium. This method is useful for studying the properties of individual types of microorganisms, but the range of microorganisms which can adjust to an artificial medium is very limited.

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3. Microbial mat map

Microbial mats from 24 localities in Japan, such as hot-springs, river sediments, seashore, deep seafloor, mining areas and ponds, are described in the following section. Characteristics of color, geology, water chemistry, microbiology, biomineralogy and chemical composition are listed for each occurrence as shown on the map and in the tables. A number has been assigned to each site. These are ordered from north to south on the map and in the tables and the numbers correspond to those of each catalog entry in the next chapter.

Observation and the information available suggest that microorganisms occur in a wide variety of eco-systems and that the colored mats contain a wide range of biominerals and metals.

4. Catalog of Biomats in Japan

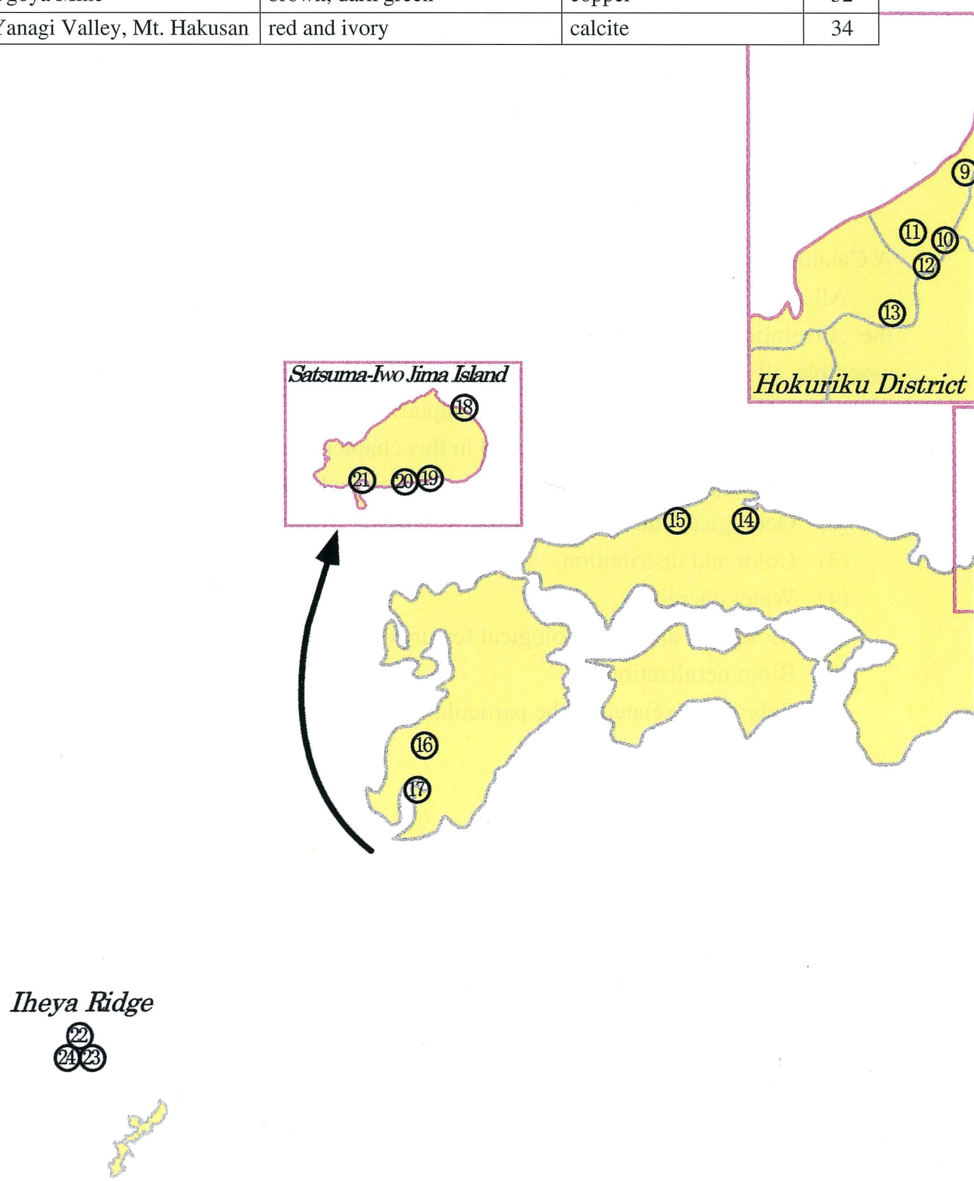
A Catalog of Microbial mats in Japan

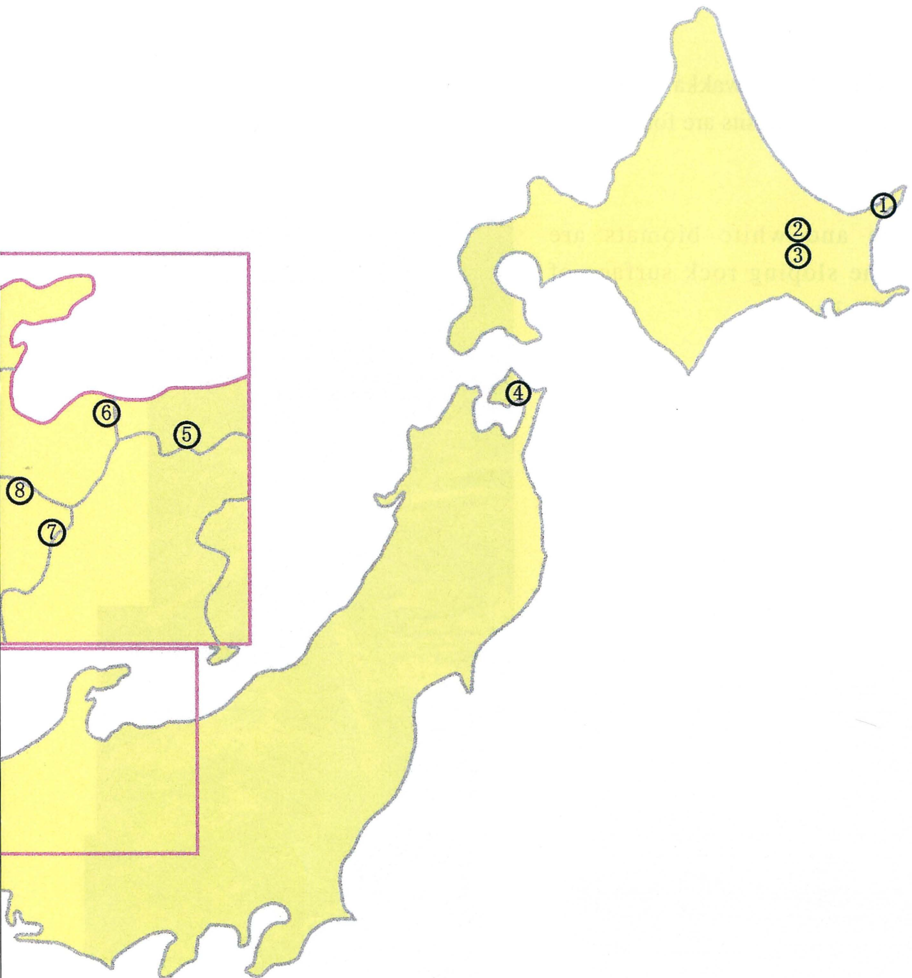
All data obtained from field and laboratory work are invaluable to understand collectively the correlation between biomats and the natural environment. It is therefore essential to assemble all field information. Characteristics of biomats and their natural environments at various localities are introduced in this chapter.

Data from each locality introduced in this chapter are as follows;

(1) Locality and photograph showing the immediate area,
(2) Geological and mineralogical information in and around the biomats,
(3) Color and distribution,
(4) Water chemistry,
(5) Biological and morphological features of microorganisms present,
(6) Biomineralization,
(7) References related to the particular biomat occurrence.

number	localities	color	principal biominerals	page
4-1	Kamuiwakka Falls	green and white	silicate minerals	12
4-2	Onneto Nishiki-Numa Pond	black, red and brown	ferrihydrite	14
4-3	Onneto Yunotaki Fall	black	buserite	16
4-4	Mount Osore-Zan	black and gray	pyrite	18
4-5	Seki Hot Springs	brown	amorphous iron minerals	20
4-6	Ogawa Hot Springs	reddish brown, black and green	quarts, calcite	22
4-7	Hirayu Hot Springs	white, brown and green	sulphur	24
4-8	Kamioka Mine	red-brown	lepidocrocite	26
4-9	Kanazawa University Pond	red-brown	amorphous iron minerals	28
4-10	Chugu Hot Springs	reddish brown	calcite	30
4-11	Ogoya Mine	brown, dark green	copper	32
4-12	Yanagi Valley, Mt. Hakusan	red and ivory	calcite	34





number	localities	color	principal biominerals	page
4-13	Nakatatsu Mine	black	illite	36
4-14	Hohman-Zan Mine	dark-red or orange-brown	hematite	38
4-15	Ohmori Mine (Iwami Silver Mine)	orange or black, dark-brown	illite	40
4-16	Hishikari Mine	brown	smectite	42
4-17	Kirishima, Sakura-Jima District	green	silicate minerals	44
4-18	Kitabirashita Beach, Satsuma-Iwo Jima Island	yellow-brown	jarosite	46
4-19	Higashi Hot Springs, Satsuma-Iwo Jima Island	yellow, green	jarosit	48
4-20	Akayu Hot Springs, Satsuma-Iwo Jima Island	reddish brown	ferrihydrite	50
4-21	Nagahama Port, Satsuma-Iwo Jima Island	reddish brown	ferryhydrit	52
4-22	Iheya Basin, Okinawa Trough	yellow	nontronite	54
4-23	Seafloor Hydrothermal Systems (1), Iheya Ridge	(hydrothermal fluid)	barite	56
4-24	Seafloor Hydrothermal Systems (2), Iheya Ridge	white	quartz	60

4-1 Kamuiwakka Falls

Locality

Shari Cho, Shari Gun, Hokkaido < N 44° 09′, E 145° 08′ >

Geology

Basement around the Kamuiwakka River consists of Quaternary Mt. Ioh-Zan andesites. Sublimate and fusion sulfur deposits are found at Mt. Ioh-Zan (Doi *et al.*, 1970).

Occurrence

Belt-like green and white biomats are distributed along the sloping rock surface of Kamuiwakka Falls (Fig. 1).

Water Chemistry

WT	32.6 °C	Eh	590 mV
pH	1.6	DO	5.3 mg/l

(measured by Koiwasaki, Nov. 1995)
(Koiwasaki *et al.*, 1996).

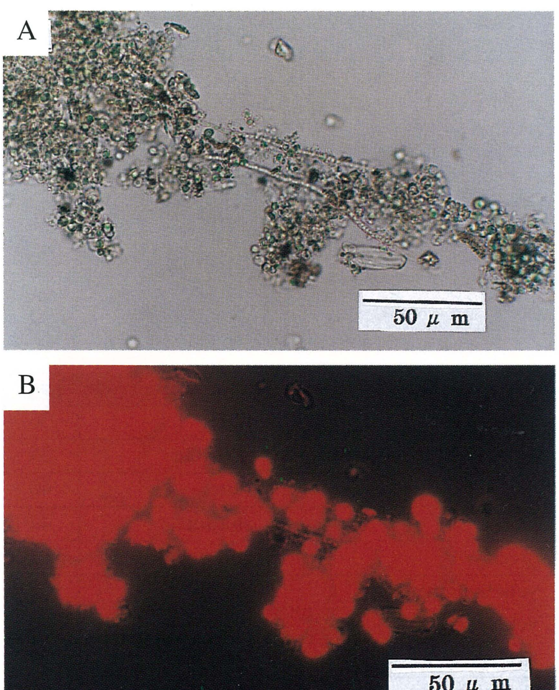


Fig. 2 Optical micrograph (A) and fluorescence micrograph (B) of the green biomats.

Fig. 1 Green and white biomats distributed over the sloping surface of Kamuiwakka Falls.

Microbiology and Biomineralization

Optical microscopic observations revealed spherical and filiform microorganisms and diatoms (Fig. 2A). Some microorganisms are greenish, while others are transparent. The greenish species affect the color of the biomats.

Fluorescence microscopy showed that the green microorganisms contained chlorophyll, while those exhibiting a transparent appearance were dead and had lost chlorophyll (Fig. 2B).

The diameter of the spherical microorganisms was about 4 μm. The apical axes of the diatoms ranged from 5 to 70 μm. The length of filiform microorganisms was about 20 μm, and the diameter about 4 μm. SEM observation of the spherical microorganisms revealed fine particles adhering to the surface (Fig. 3A, B). The result of EDX analysis indicated that the fine particles contained mainly Si (Fig. 3C).

XRD analyses were made of focusing on the green biomats cultured in the laboratory and of the mineral and rock substrates. The results indicated that the green biomats contained amorphous material and cristobalite. The relationship between the microorganisms and the formation of silicate minerals is interesting and requires further investigation.

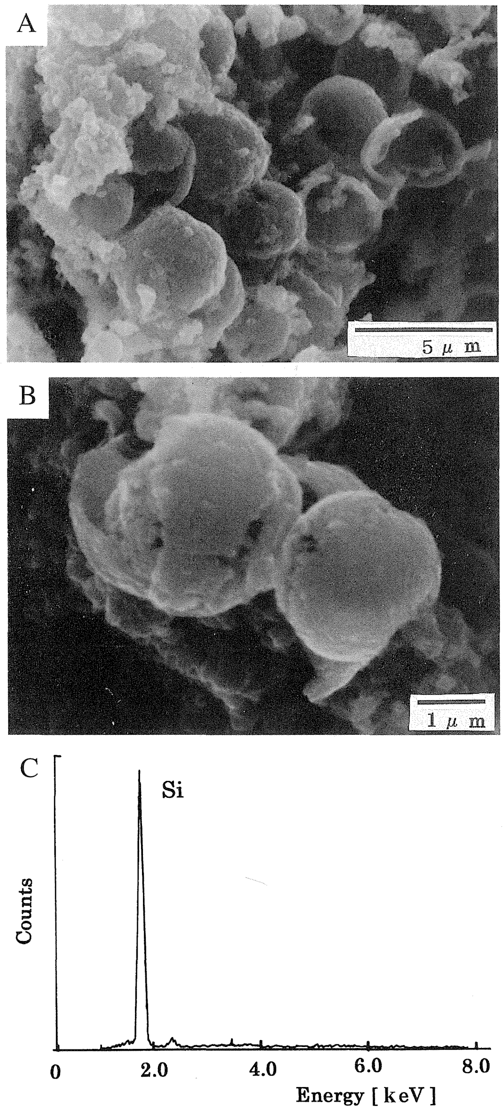


Fig. 3 SEM images (A, B) and EDX spectrum (C) of green biomats.

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4-2 Onneto Nishiki-Numa Pond

Locality

Ashoro Cho, Ashoro Gun, Hokkaido
< N 43° 23´, E 143° 58´ >

Geology

Alluvium composed of Me-Akan hypersthene andesites and the platy shale member of the Miocene Honbetsuzawa formation are distributed in this area. The Me-Akan volcanic products were formed by volcanoes built along the south-western margin of Akan caldera. The platy shale member of the Honbetsuzawa Formation consists of hard shale, mudstone and tuffaceous sandstone.

Lake Onneto is located in the eastern part of the distribution of the Me-Akan volcanic products. Hot springs flow from cracks in the Me-Akan volcanics about 3 km northeast of the lake. Nishiki-Numa Pond is located about 500 m southwest of the lake. Limonite occurring in the pond is an organic-sedimentary ore deposit resulting from metasomatism of plants, such as reeds (Mitani *et al.*, 1964).

Occurrence

Black, red and brownish biomats occur around Nishiki-Numa Pond and on the pond floor (Fig. 1).

Water Chemistry

Cold mineral springs flow out from multiple sites adjacent to and within the pond. The chemical composition of the water flowing into the pond is:

Fe 20 ppm, Mn 2 ppm, Ca 91 ppm, Mg 24 ppm, Na 2 ppm, K 2 ppm, SO_4^{2-} 540 ppm, Cl^- 14 ppm (Mita *et al.*, 1997).

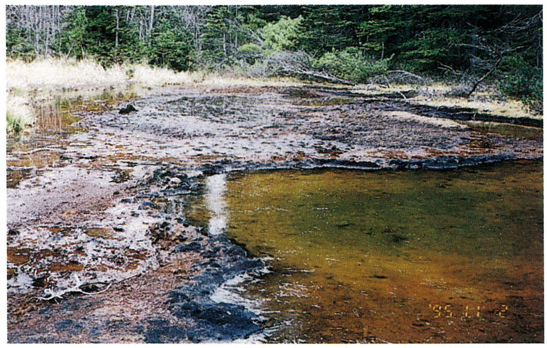

Fig. 1 Black and brownish biomats around Nishiki-Numa Pond (photographed by Tazaki, Nov. 1995).

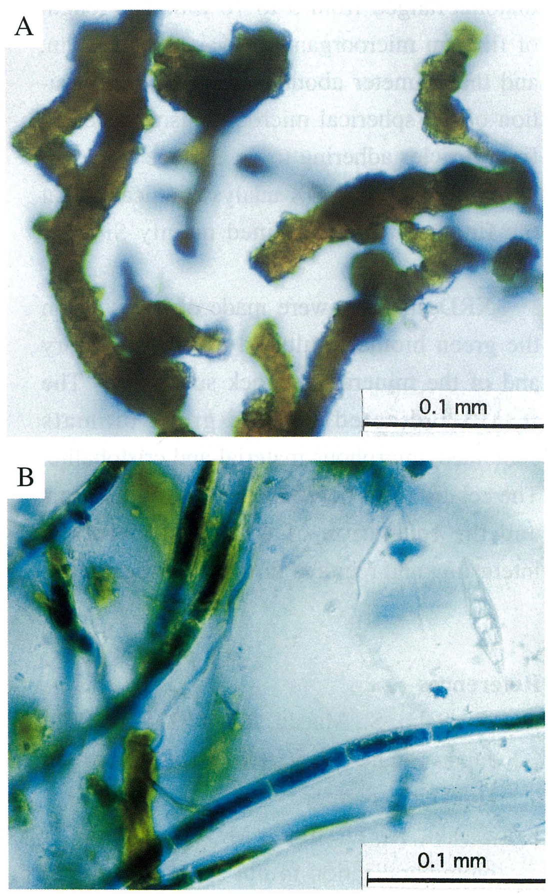

Fig. 2 Optical micrograph of brownish biomats (A) and black biomats (B).

Characteristics of the water over the brown biomats was:

WT 6.1 °C pH 4.1

Eh 425 mV DO 15 mg/l

(measured by Koiwasaki, Nov. 1995) (Koiwasaki *et al.*, 1996).

Microbiology and Biomineralization

Optical microscopic observations showed short filamentous materials in the brownish biomats (Fig. 2A). The black biomats contained filamentous materials and algae. Algae were precipitating black materials into the cell wall (Fig. 2B). SEM-EDX observations and analyses indicated the presence of Fe and Si in the brownish biomats (Fig. 3A, B).

The 2.50 Å reflection corresponding to ferrihydrite was recognized by XRD obtained by Cu Kα radiation (Fig. 3C). The result of the studies suggests that iron minerals were formed and precipitated onto the bacterial cell wall. These biomats show the first stage of formation of iron ore by microbial biomineralization.

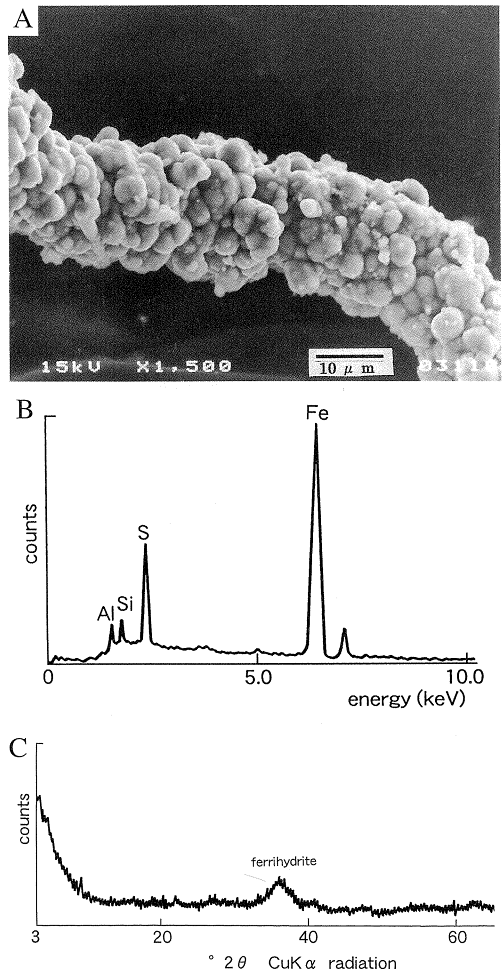

Fig. 3 SEM image (A), EDX spectrum (B) and XRD pattern (C) of brownish biomats.

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4-3 Onneto Yunotaki Fall

Locality

Ashoro Cho, Ashoro Gun, Hokkaido
< N 43° 22′, E 143° 58′ >

Geology

Alluvium consisting of Me-Akan hypersthene andesite debris is distributed in this area. The Me-Akan volcanics were erupted from volcanoes constructed along the south-western margin of the Akan caldera.

Lake Onneto is located in the eastern part of the distribution of the Me-Akan volcanic products. Hot springs containing manganese flow from the mountainside about 3 km east of the lake. Black lutaceous manganese ore is found around the hot springs, resting on a basement of Me-Akan lava flow (Mitani *et al.*, 1964).

Occurrence

Black biomats have precipitated around the spring where it flows down the lava cliff, and along the spring channel (Fig.1 A, B).

Water Chemistry

Hot springs containing manganese flow from the cliff at the foot of a lava flow (Mitani *et al.*, 1964). The composition of the headspring water was :

Mn 3 ppm, Fe < 0.01 ppm, Ca 102 ppm, Mg 125 ppm, Na 127 ppm, K 32 ppm, SO_4^{2-} 690 ppm, Cl^- 132 ppm, HCO_3^- 237 ppm
(Mita *et al.*, 1994).

The characteristics of the water on the various biomats were as follows:

WT 16~36 ℃ pH 6.1~8.3
Eh 167~398 mV DO 2~12 mg/l
(measured by Koiwasaki., Nov. 1995)

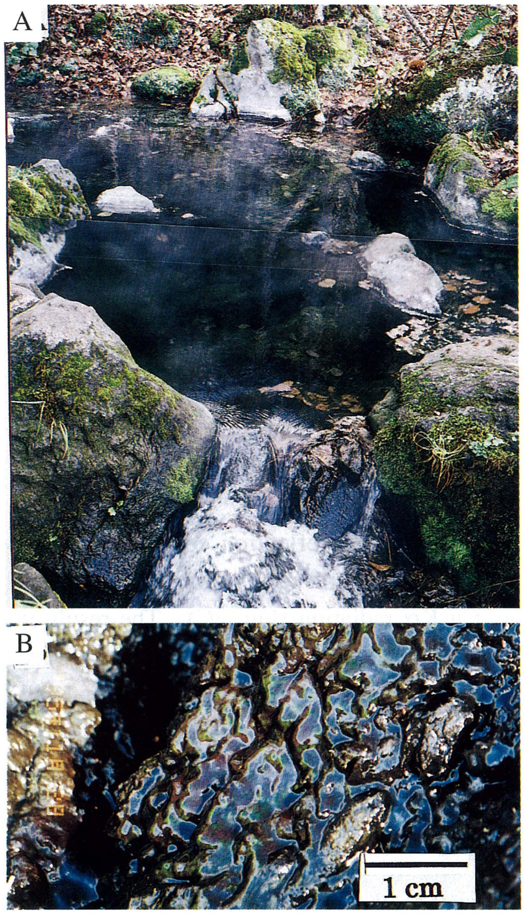

Fig. 1 Biomats on the surface of Onneto Yunotaki Falls (A, B).

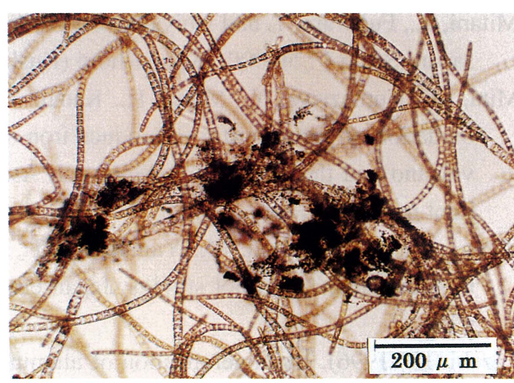

Fig. 2 Optical micrograph of cultured black biomats.

Microbiology and Biomineralization

Black materials surrounding filamentous microbes were observed by optical microscope in natural culture using water collected in the field (Fig. 2). SEM observations revealed filamentous minerals on spherical bacteria. These minerals had a morphology typical of buserite (Fig. 3A).

The Mn Kα peak was recognized by EDX (Fig. 3B). XRD analysis identified 10.1, 5.0 and 2.4 Å reflections and backgrounds which suggest the presence of organic materials (Fig. 3C). These black biomats show the formative process of manganese ore by bacterial biomineralization.

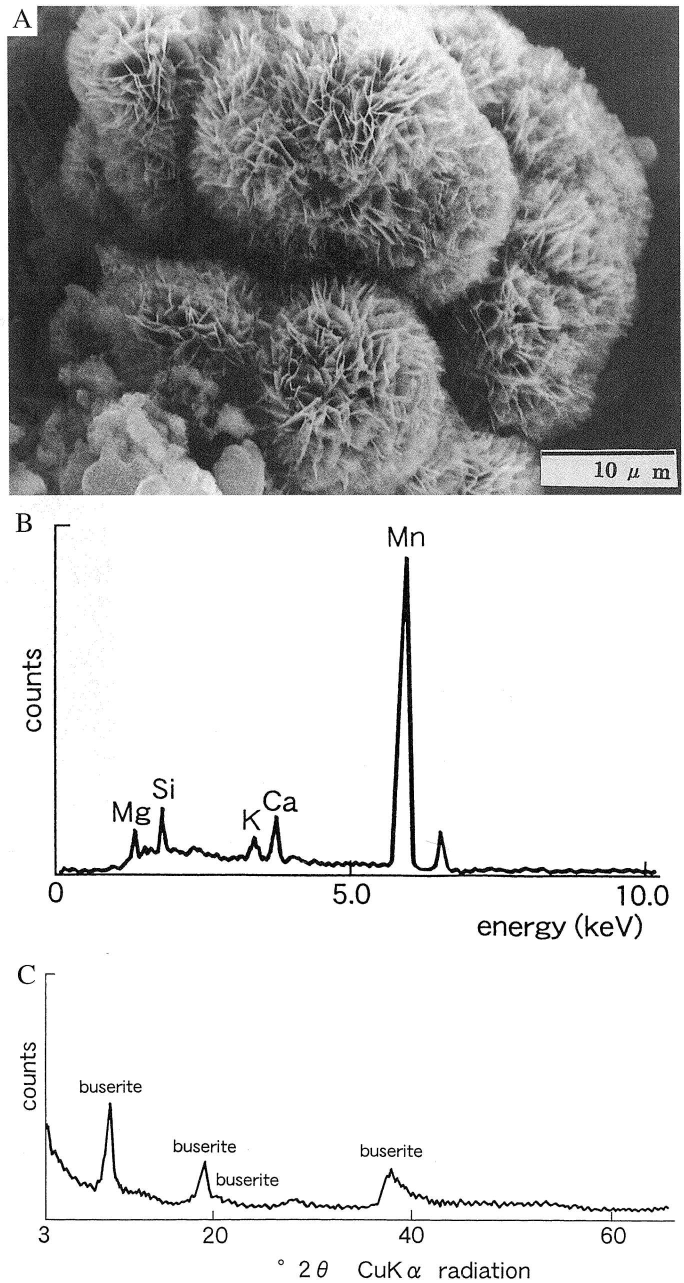

Fig. 3 SEM image (A), EDX spectrum (B) and XRD pattern (C) of black biomats.

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4-4 Mount Osore-Zan

Locality

Mutsu City, Aomori Prefecture (Osore-Zan geothermal area)
< N 41° 20´, E 141° 05´ >

Geology

Pleistocene Osore-Zan volcanics and Neogene dacite comprise basement in this area. The Osore-Zan volcanic products consist of the Shozugawa Tuff-breccia originating from a caldera, and Tsurugi-Yama agglomerate and lava originating from Mt. Tsurugi-Yama. Shozugawa tuff-breccia is a complex of andesitic tuff-breccia, tuff, pumice and mud flow deposits, including sand, gravel and wood fragments. Tsurugi-Yama agglomerate and lava are augite-hypersthene-hornblende dacite. Solfataras and alteration zones are scattered through the area. (Uemura *et al.*, 1957).

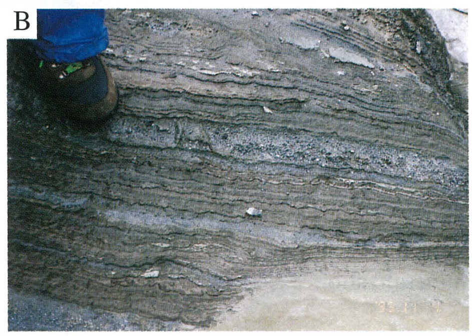

Fig. 1 Margins of the river near Lake Usorisan (A), and the banded clay sediments observed at that site (B).

Occurrence

Black biomats have formed on the bed of a small river flowing from a hot spring chimney into Lake Usorisan in and around the Osore-Zan Hot Spring (Fig. 1 A).

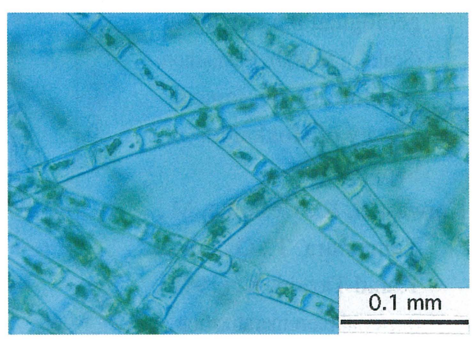

Fig. 2 Optical micrograph of algae breeding on the riverbed.

Water Chemistry

Osore-Zan Hot Spring is an acid hot spring containing ferrous sulphate (Shirozu, 1994).

The water conditions around the biomats were measured as

WT 11 °C pH 3.0 Eh 102 mV DO 8.7 mg/l

(measured by Koiwasaki., Nov. 1995) (Koiwasaki *et al.*, 1996).

Microbiology and Biomineralization

A black and gray banded clay formation can be seen along the river at Mt. Osore-Zan (Fig. 1B) and diatoms breed on the riverbed. Filamentous algae were recognized by optical microscope (Fig. 2). Microorganisms were cultured by adding distilled water to clay samples. Algae and bacilli breeding on the clays, and sulphur concentrated in and on the microbes were recognized (Fig. 3A, B).

XRD analyses revealed that the black bands in the clay formation included iron sulphide minerals, such as pyrite and marcasite. The gray bands consisted mostly of clay minerals, such as kaolin and smectite. SEM image shows idiomorphic pyrite particles in the black clays (Fig. 4A) which contain iron, sulphur and arsenic (Fig. 4B). It is suggested that this banding was caused by microbial breeding conditions and that such a formation may reflect the environmental conditions at the time of clay precipitation.

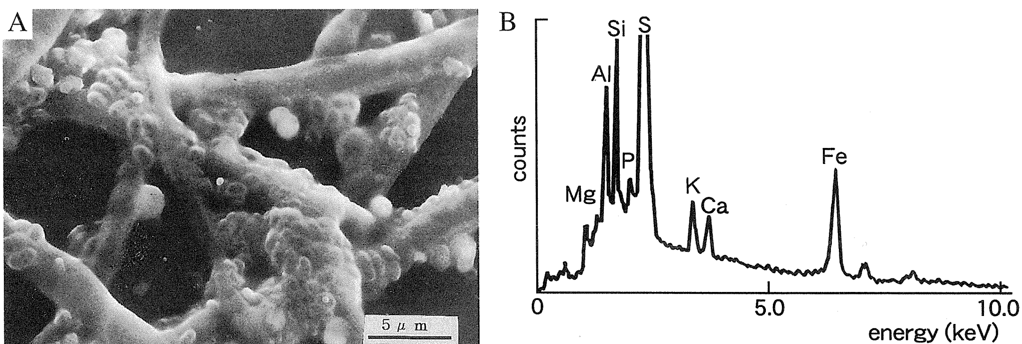

Fig. 3 SEM image (A) and EDX spectrum (B) of cultured microbes inhabiting the banded clays.

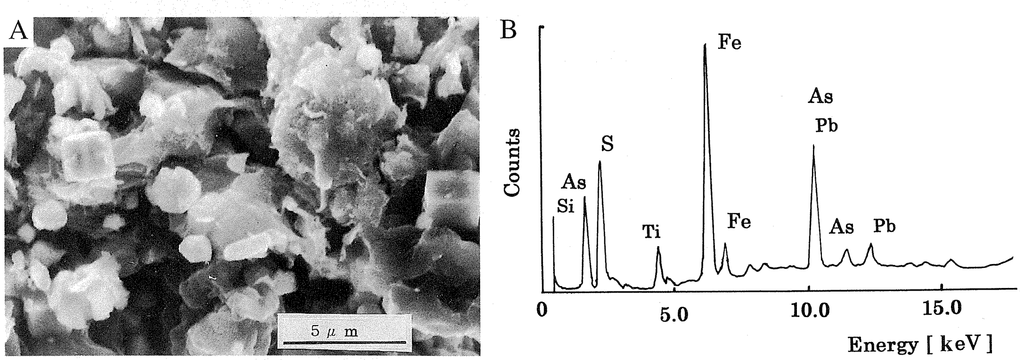

Fig. 4 SEM image of the black clay (A) and XRF spectrum of the banded clays (B).

References

Uemura, F. and Saito, M. (1957). Explanatory text of the geological map of Japan (Scale 1 : 50,000); Ohata (Aomori-3) Geological survey of Japan, Tsukuba.

Koiwasaki, K., Ueshima, M., Yamamura, T., Okaniwa, T. and Tazaki, K. (1996). Bacterial biomineralization in hot springs. Japan earth and planetary science joint meeting (Osaka), p256.

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4-5 Seki Hot Springs

Locality

Seki-Onsen, Myoko-Kogen Cho, Naka-Kubiki Gun, Niigata Prefecture
< N 36° 54′, E 138° 10′ >

Geology

Seki Hot Spring is located in the southwest of Niigata Prefecture, and is one of a number of hot springs scattered around Mt. Myoko (2446 m above sea level). Mt. Myoko is a stratovolcano covered with Neogene sediments. Mt. Myoko is a double volcano composed of the central crater cone and an encircling somma. The rock around Mt. Myoko is generally andesite, but ranges from augite and olivine basalt to hypersthene and hornblende quartz andesite. (Yokoyama *et al.*, 1975), (The Association for the Geological Collaboration in Japan, Takada Branch Office, 1978).

Occurrence

Brownish biomats are observed in a cave situated 5 m above the headspring, and along a spring flowing through the cliff 50 m from the cave (Fig. 1A). Numerous stalactite-like formations covered with biomats hang from the ceiling of the cave (Fig. 1B). Gelatinous biomats also occur on the cave floor.

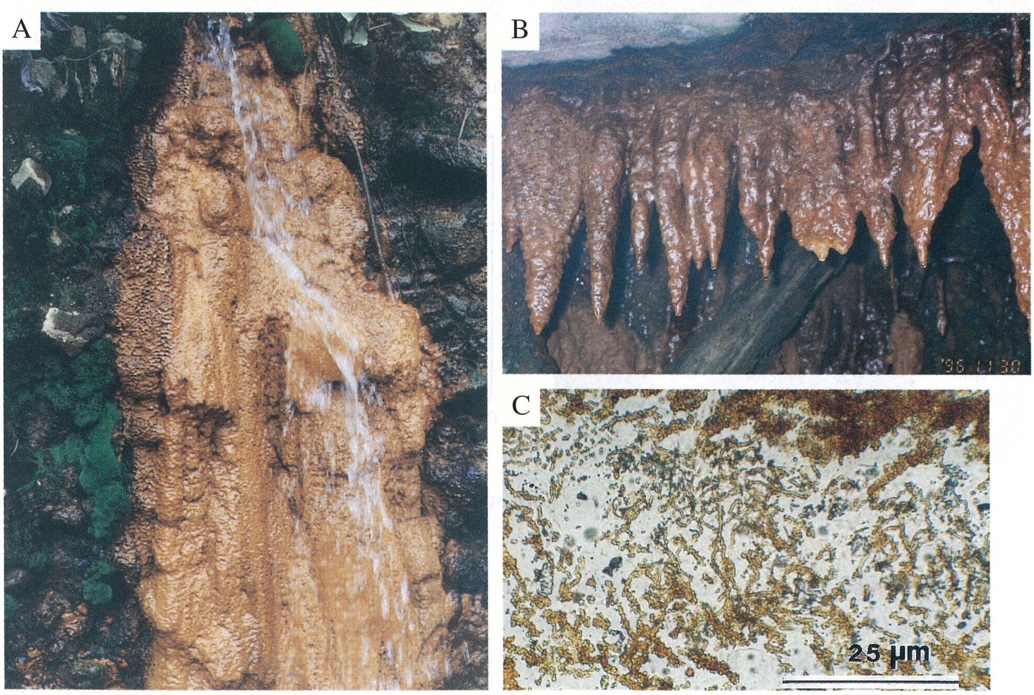

Fig. 1 Lamellate biomats are observed along the spring flowing through the cliff (A). The stalactite-like formations covered with biomats are found in the cave situated 5 m up from the headspring (B). Optical micrograph of the stalactites showing filamentous materials on the surface (C).

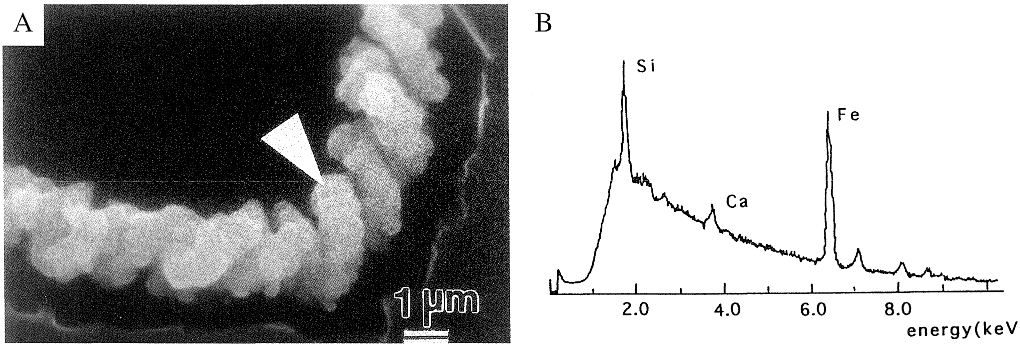

Fig. 2 SEM image (A) and EDX spectrum (B) of lamellate biomats indicates that the surfaces of spiral materials mainly contain Si and Fe.

Water Chemistry

The characteristics of the water flowing through the cave are:

WT	29.3 °C	pH	6.2,
Eh	212 mV	DO	1.4 mg/l

(measured by Yoneyama, 1996~1997). Refer also to Table 1.

Table 1 Water Conditions in and around the cave.

	Temperture (°C)	pH	DO (mg/l)	Eh (mV)
Botom of the cave	29.3	6.2	1.4	191
River	8.6	7.4	3.8	216
Spring	18.9	6.4	2.7	253

Microbiology and Biomineralization

Although the "stalactites" covered with biomats seem solid, they are in fact very fragile. Optical microscopic observations revealed that microorganisms, such as *Toxothrix* sp. and *Siderococcus* sp. propagated on the surface of the stalactites, and filamentous materials and numerous diatoms were also present (Fig. 1C). SEM observations showed granular aggregations of spiral materials on the surface of lamellate biomats (Fig. 2A). EDX analysis identified that the spiral materials were rich in Fe, Si and organic materials.

No strong reflection was obtained by XRD analysis of the stalactites, and it is suggested that the stalactites are composed of amorphous iron minerals or organic materials.

References

Yokoyama, J., Morishita, A. and Itoigawa, J. (1975). *Geological Journal of Central Japan*. Asakura bookstore, Tokyo, p389-395.

The Association for the Geological Collaboration in Japan, Takada Branch Office (1978). *Geological hiking in Niigata*. Niigata daily reports enterprise, Niigata.

4-6 Ogawa Hot Springs

Locality

Ogawa-Onsen, Asahi Machi, Shimoniikawa Gun, Toyama Prefecture
< N 36° 50′, E 137° 40′ >

Geology

Conglomerates, sandstones and shales of the Kurobishi-Yama and Mizukami-Dani Formations occur in and around Ogawa Hot Spring (Harayama *et al.*, 1996).

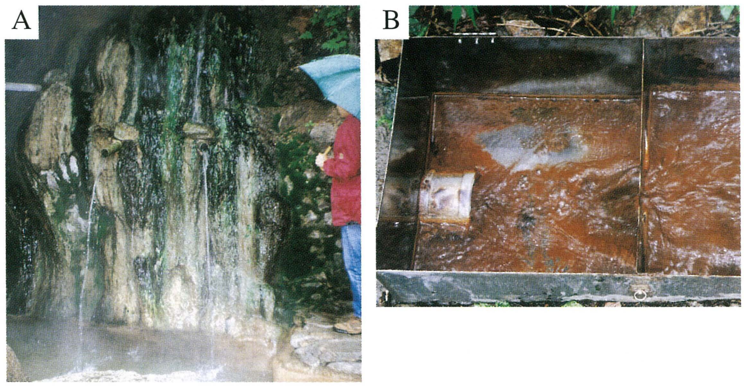

Fig. 1 Belt-like black and green biomats (A) and reddish brown biomats (B) in and around Ogawa Hot Spring.

Occurrence

Reddish brown biomats have accumulated at the joints of pipes supplying hot spring water to the "*onsen*", and belt-like black and green biomats are observed on the surfaces of the walls over which the hot spring water flows.

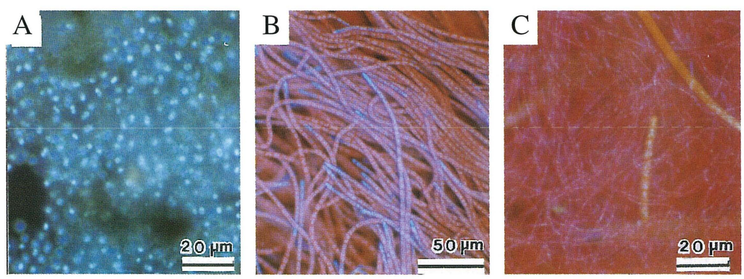

Fig. 2 Fluorescence micrographs of biomats; reddish brown (A), black (B) and green (C).

Water Chemistry

The color of biomats reflects water chemistry (Table 1) (measured by Kato, 20 July 1997).

Table 1 Results from testing of hot spring water on differently colored biomats.

Color of biomat	pH	Eh (mV)	DO (mg/l)	EC (mS/cm)	WT (°C)
reddish brown	7.0	53	4.5	2.0	51
black and green	8.3	70~100	9.0	1.5	41

Microbiology and Biomineralization

Black and green biomats form at places where hot spring water is discharged (Fig. 1A), and reddish brown biomats occur in the supply reservoir feeding the "*onsen* " (Fig. 1B). Fluorescence microscopy on reddish brown biomats colored with DAPI dye confirmed the presence of DNA. Examination of the black and green biomats revealed tangles of numerous filamentous microorganisms. In the green biomats these contained chlorophyll (Fig. 2A, B, C). The diameter of the filamentous microorganisms is 3~4 μm in the black mats and 1~2 μm in the green mats. SEM-EDX observations and analyses showed that the reddish brown biomats contained a large quantity of granular materials around the tube-like materials. These granules contain mainly Fe and Si (Fig. 3A). EDX analyses further revealed that the filamentous microorganisms propagating in the black and green biomats contained S, P, Ca and Fe, and S, P, and Ca respectively. They also contained abundant spherical material, high in Ca, around which filamentous bacteria were entangled, forming "*Itodemari*" (a handball made of twine)-structure (Fig. 3B, C, D).

As a result of the XRD analysis, the reddish brown biomats were found to contain quartz (3.3 Å reflection) and amorphous iron minerals (2.5 Å reflection). The black and green biomats contained calcite (3.0, 2.3, and 2.1 Å reflections).

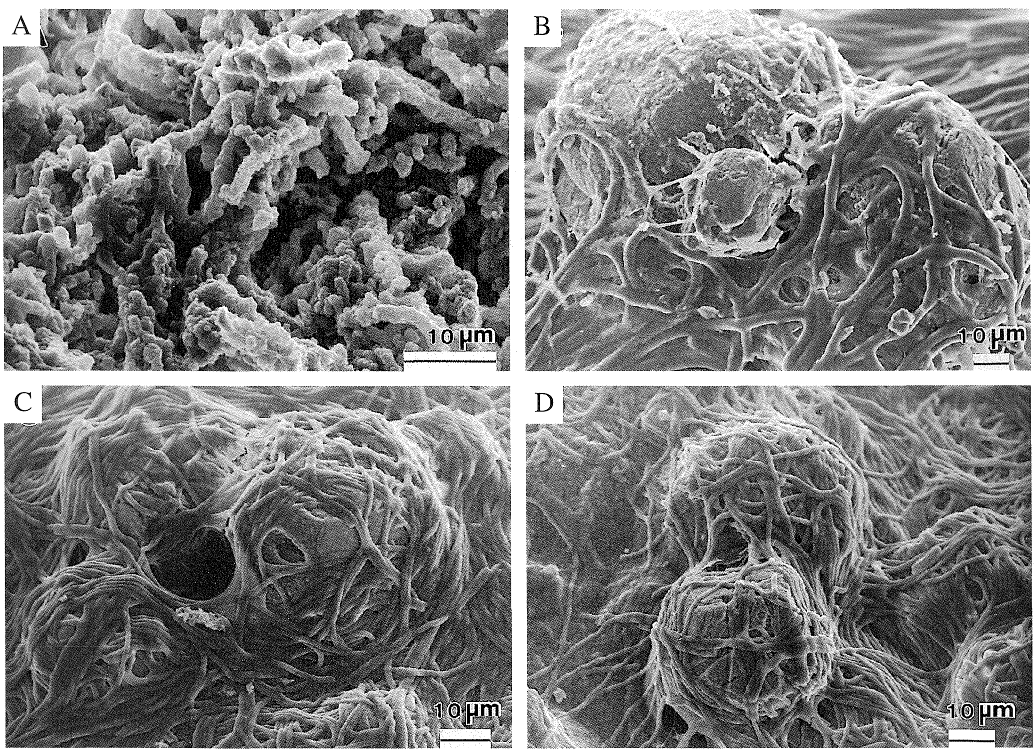

Fig. 3 SEM image of biomats; brown (A), black (B) and green (C, D).

Reference

Harayama, S., Takizawa, F., Komazawa, M., Hiroshima, T. and Sudo, S. (1996). Explanatory text of the geological map of Japan (Scale 1 : 200,000); Toyama. Geological survey of Japan, Tsukuba.

4-7 Hirayu Hot Springs

Locality

Hirayu-Onsen, Kamitakara Mura, Yoshiki Gun, Gifu Prefecture < N 36° 11´, E 137° 32´ >

Geology

The geology around the headspring at Hirayu Hot Springs is dominated by cherts from an allochthonous block in the Jurassic Mino Belt system (Yamada *et al.*, 1989).

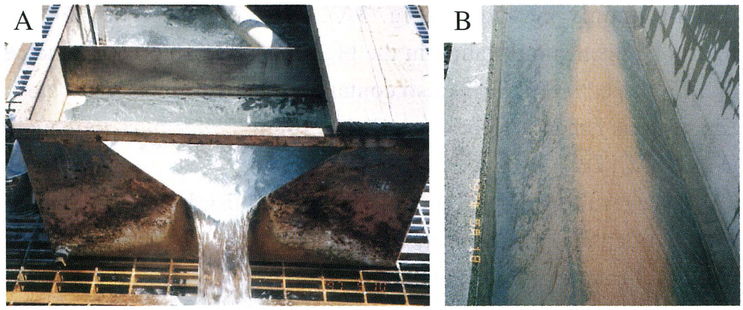

Fig. 1 White biomats (A) and belt-like brown and green biomats (B) in and around Hirayu Hot Springs.

Occurrence

White biomats adhere to the walls of tubs in the "*onsen*" and belt-like brown and green biomats are distributed in the lower reaches of drainage ditches.

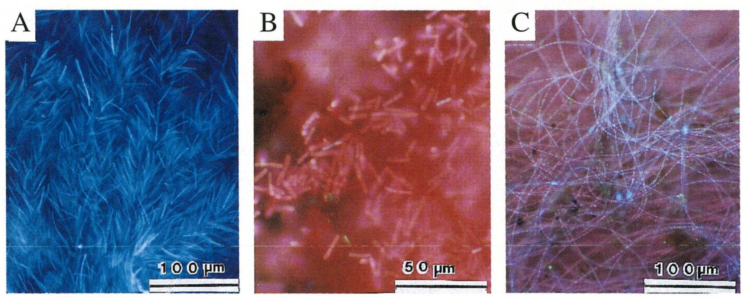

Fig. 2 Fluorescence micrographs of white biomats composed of sausage shaped bacteria (A), brown biomats composed of bacilli (B) and green biomats composed of filamentous bacteria (C).

Water Chemistry

The chemistry of the water in drainage ditches in and around Hirayu Hot Springs varies, and the biomat color tends to reflect this phenomenon (Table 1). The pH-Eh phase diagram shown in Fig. 4 indicates that the environments of green, brown and white biomats are high pH, neutral pH and pH 6 respectively. The green biomats occur in oxidizing conditions, brown biomats in a wide range from oxidizing to reducing conditions and white biomats in reducing conditions. This result suggests that the colors of biomats reflect the environmental conditions.

Table 1 Results of water examinations of the hot spring water around differently colored biomats (Measured by Ohno, April~June 1997).

Color of biomat	White	Brown	Green
pH	6.1~6.6	6.8~7.8	7.9~8.4
Eh (mV)	-221~-150	-89~109	70~100
DO (mg/l)	0.8~2.9	1.9~6.5	4.7~7.5
EC (mS/cm)	1.31~1.69	1.13~1.50	1.11~1.57
WT (°C)	57~66	43~60	34~42

Microbiology and Biomineralization

White sulphur-turf occurs in the areas of high temperature, such as the headspring (Fig. 1A), and belt-like brown and green biomats are found in the lower reaches of drainage ditches (Fig. 1B). Fluorescence microscopic observation revealed numerous sausage shaped bacteria in the white biomats, most of which are 30~40 μm in size (Fig. 2A). Filamentous bacteria and bacilli were recognized in the brown biomats. The filamentous bacteria have a diameter of 0.5 μm, and the bacilli were 7 μm long and 2 μm wide. Observations under fluorescence microscope detected chlorophyll in the bacilli (Fig. 2B). The 2 μm diameter filamentous bacteria were recognized in the green biomats by fluorescence microscope with DAPI staining (Fig. 2C) and SEM observations (Fig. 3C).

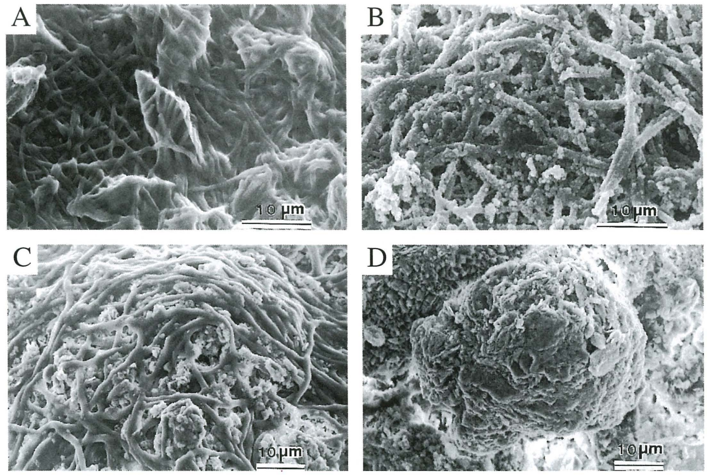

Fig. 3 SEM image of white biomats composed of orthorhombic sulphur (A), brown biomats composed of granular materials congregating on the surface of filamentous bacteria (B), green biomats composed of filamentous bacteria (C) and calcite (D).

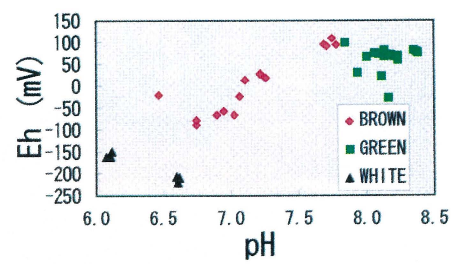

Fig. 4 pH-Eh phase diagram of spring water at Hirayu Hot Springs, showing that the habitats of green, brown and white biomats are high pH, neutral pH and pH 6 respectively. Green biomats occur in oxidizing conditions, brown biomats in a wide range from oxidizing to reducing conditions and white biomats in reducing conditions.

SEM observations and XRD analyses revealed that the white biomats contained abundant sulphur with rhombic and indeterminate form (Fig. 3A), which had a 3.9 Å reflection. The presence of clay minerals with a 7 Å reflection was also recognized in white biomats.

SEM-EDX observations and analyses of the brown biomats identified granular material high in Fe and Si and contained a little Ca (Fig. 3B). XRD analyses identified a significant 3.3 Å reflection corresponding to quartz. Additionally, feldspar (2.8 Å), cristobalite (4.0 Å) and clay minerals (7.0 Å) reflections were recognized. Other SEM observations revealed abundant spherical calcite in the green biomats (Fig. 3D), which gave a strong 3.3 Å XRD reflection. XRD analysis also identified weak 3.3 and 7.0 Å reflections corresponding to quartz and clay minerals respectively.

References

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4-8 Kamioka Mine

Locality

Kamioka Cho, Yoshiki Gun, Gifu Prefecture (Jabara Valley in Kamioka Mine)
< N 36° 22´, E 137° 19´ >

Geology

Jabara Valley within the Kamioka Mine is underlain by Hida metamorphic rocks, including metabasites. The valley is an abandoned mining area with wastes and slag containing sphalerite, galena and chalcopyrite (Kano and Terayama, 1995 ; Hirokawa *et al.*, 1995). Jabara Valley is a source of heavy metal pollution along the Jinzu River.

Occurrence

Red-brown biomats occur in both solid and colloidal states on the stream bed in Jabara Valley (Fig. 1A, B).

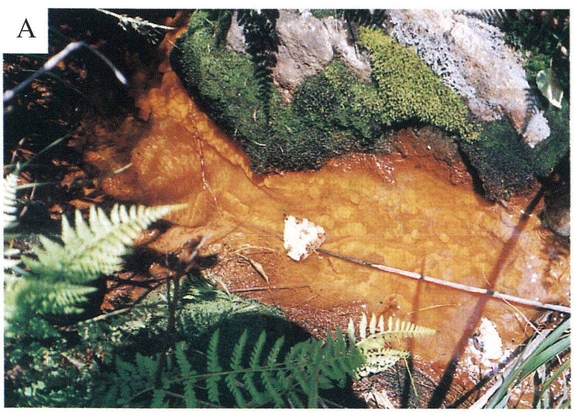

Fig. 1A The upper reaches of the stream in Jabara Valley. Orange colloidal balls of biomats are observed.

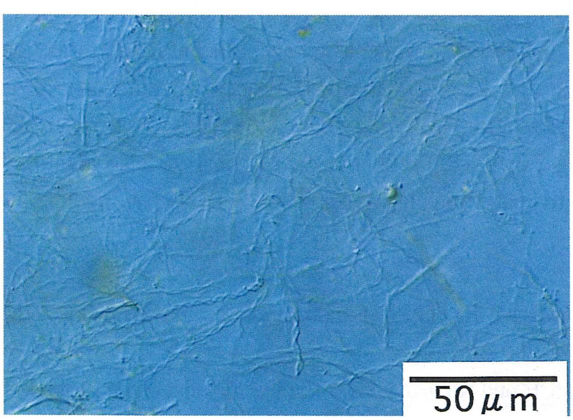

Fig. 2 Optical micrograph of colloidal biomats. The ribbon-like fibers are *Gallionella ferruginea*.

Fig. 1B The upper part of Jabara Valley. The stream bed is covered with red-brown biomats.

Water Chemistry

While Eh and EC vary, the pH and DO of the stream waters are constant (Table 1).

Table 1 Stream water characteristics, Jabara Valley (measured by Aoki *et al.*, 1996~1997).

	pH	DO (mg/l)	Eh (mV)	EC (μS/cm)	WT (°C)
1996/10/13	7.5	5.4	320	111	12
1997/4/28	6.8	7.4	190	37	11
1997/8/3	6.2	4.3	10		25

Microbiology and Biomineralization

Gallionella ferruginea, an iron bacterium was observed by optical and scanning electron microscopy (Fig. 2, 3A). EDX analysis of the bacteria detected Fe, Si and Ca. It appears that *Gallionella ferruginea* concentrate elemental Fe (Fig. 3B). XRD analysis by means of Cr Kα radiation revealed that the biomats consisted mostly of amorphous materials. Significant peaks corresponding to lepidocrocite (6.1, 3.3, 2.6 and 1.9 Å) and goethite (4.2, 2.8 and 2.2 Å) were also detected (Fig. 4).

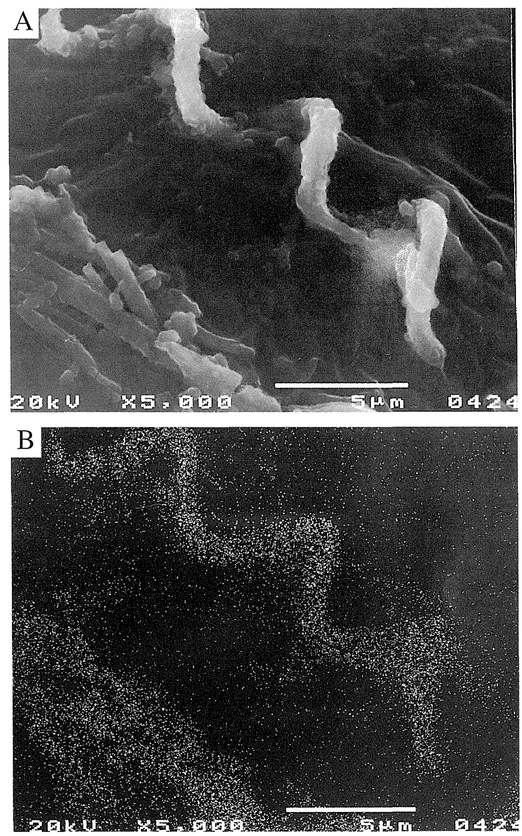

Fig. 3 SEM image of *Gallionella ferruginea* (A) and elemental image of Fe (B).

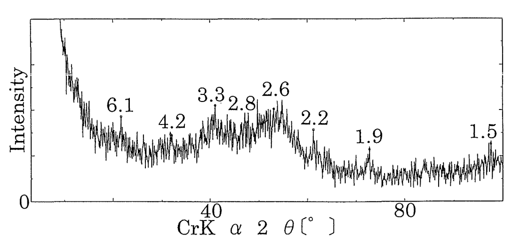

Fig. 4 XRD patterns of biomats. Lepidocrocite (6.1, 3.3, 2.6 and 1.9 Å) and goethite (4.2, 2.8 and 2.2 Å) were identified.

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Aoki, A., Matsumoto, K., Nishida, S. and Tazaki, K. (1997). Earth environmental biomineralization associated with concentration of heavy metals. Japan Earth and Planetary Science Joint Meeting (Nagoya), p225.

Aoki, A., Matsumoto, K., Nishida, S. and Tazaki, K. (1997). Environmental investigations of heavy metals pollution along Jinzu River in Toyama prefecture, Japan. 11th I.C.C. (Ottawa), A4.

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Kano, T. and Terayama, S. (1995). Metadioritic and gabbroic rocks in the Kamioka mining area, Hida metamorphic region, Japan. *RESOURCE GEOLOGY*, **45**,25-40.

4-9 Kanazawa University Pond

Locality

Kakuma Machi, Kanazawa City, Ishikawa Prefecture < N 36° 33´, E 136° 43´ >

Geology

Shallow marine sandstones of the Pleistocene Omma Formation and placer alternations of the overlying Utatsu-Yama Formation are widely distributed throughout Kanazawa City (Nakagawa *et al.*, 1996).

Fig. 1
Red-brownish biomats around a settling pond at Kanazawa University.

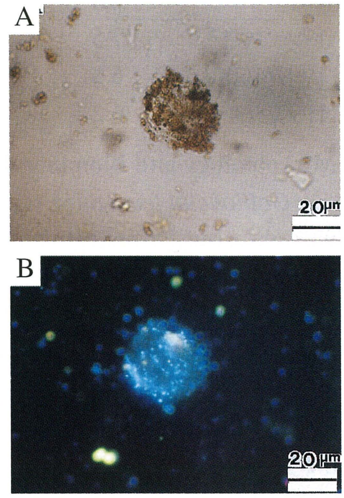


Fig. 2
Optical micrograph of an aggregation of bacillus and brownish grains (A). DAPI-stained bacilli fluoresce blue (B).

Occurrence

Red-brownish biomats occur in solid or colloidal states at various locations, such as a cut cliff in the Omma Formation and drains and settling ponds in and around Kakuma Campus of Kanazawa University, (Fig. 1). In contrast, there are almost no biomats formed along the river running through the Utatsu-Yama Formation (Kitado and Tazaki, 1996).

Water Chemistry

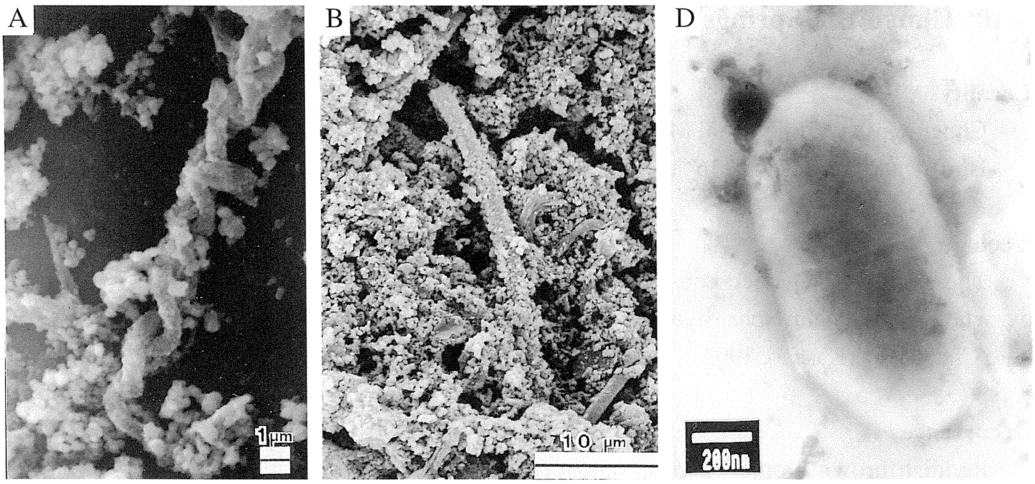
Water flowing through the Omma Formation into settling ponds tends to range from weakly acidic to neutral. The dispersion of Eh and DO data show seasonal variations (Table 1). Comparison between the spring water flowing through the Omma Formation and that flowing through the Utatsu-Yama Formation shows that they have quite different water chemistry (Kitado and Tazaki, 1996).

Table 1
Result of water examinationat a settling pond. (measured by Tashiro and Ito, 1997).

WT	12~15 °C
pH	6.4~7.1
Eh	-40~80 mV
EC	0.26~0.40 mS/cm
DO	6.3~9.7 mg/l

Microbiology and Biomineralization

Optical and fluorescence microscopic observations revealed spiral and filamentous microbes in red-brownish biomats were sampled at a settling pond. Colonies of bacilli containing brownish grains were also observed (Fig. 2A , B). SEM observations revealed that micro grains



are absorbed on the surface of the spiral microbe cell walls (Fig. 3A, B). EDX analysis identified that such grains contained mainly Fe, Si and Ca (Fig. 3C). TEM observations also revealed that micro grains were absorbed on the cell wall of bacillus (Fig. 3D). The biology and morphology of the microbes are suggestive of iron bacteria. XRD patterns showed that the mats mainly contained amorphous iron minerals. Reflections corresponding to feldspar were also recognized (Fig. 4). Natural culture revealed the production of ringed buserite (Yoshizu and Tazaki , 1996, 1997).

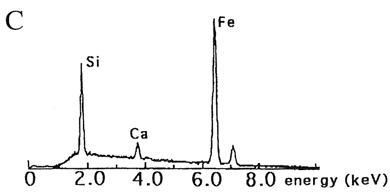


Fig. 3 SEM image of spiral bacterium (A) and micro grains (B) in the biomats. EDX spectrum of the micro grains (C). TEM image of bacillus in the mats (D).

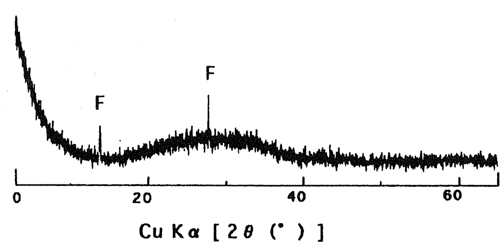


Fig. 4 XRD pattern of the biomats (F; Feldspar).

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4-10 Chugu Hot Springs

Locality

Chugu-Onsen Yoshinodani Mura, Ishikawa Gun, Ishikawa Prefecture < N 36° 15´, E 136° 46´ >

Geology

The geologic sequence consists of volcanic and pyroclastic rocks, and Nohi rhyolites of Paleogene to Late Cretaceous (Kaseno, 1993).

Occurrence

Reddish brown biomats can be observed on the walls around pipes carrying water from the spring, and green biomats occur along drainage ways (Fig. 1).

Fig. 1 Solidified reddish brown biomats on the walls around spring pipes and soft reddish brown biomats 1~2 cm in thickness and green biomats occur along drainage ways.

Water Chemistry

WT 42 ℃ pH 8.1 Eh 25 mV
EC 4.5 mS/cm DO 9.4 mg/l
(measured by Yasuda, 19 May 1997).

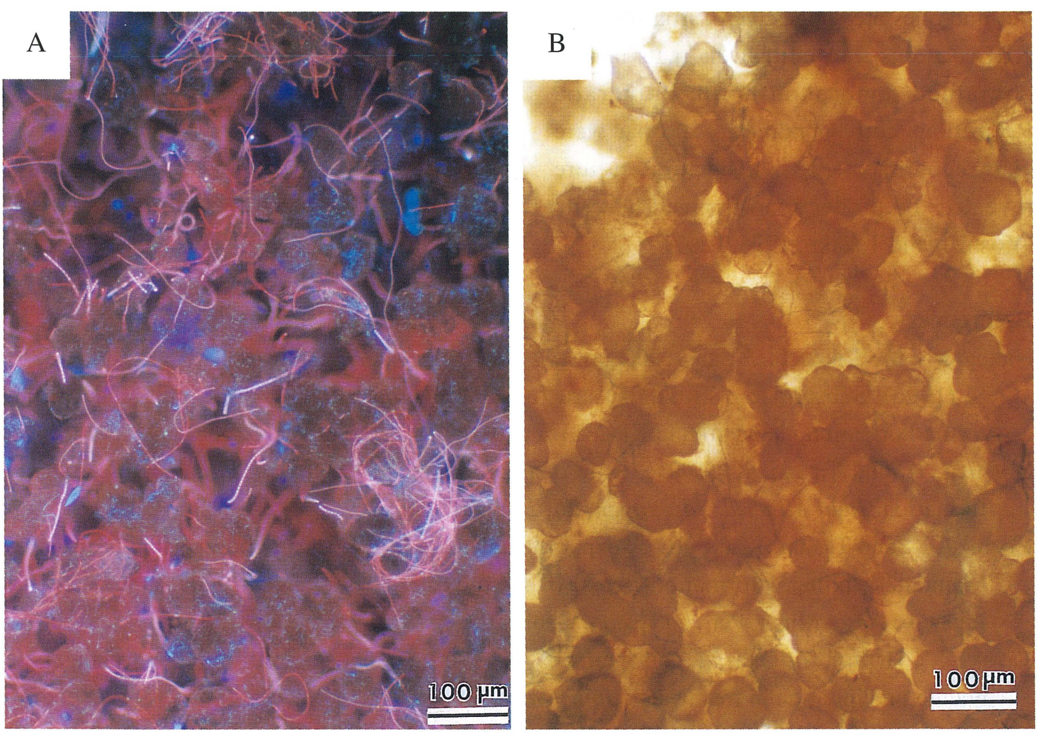


Fig. 2 Fluorescence micrograph (A) and optical micrograph (B) of reddish brown biomats.

Microbiology and Biomineralization

Optical and fluorescence microscopy revealed filamentous chlorophyll-bearing microbes twined around crystals which ranged in size from 40 μm to 70 μm. On the surface of the crystals, cocci without chlorophyll were also observed (Fig. 2A, B). XRD analysis recognized a strong 3.0 Å reflection corresponding to calcite. SEM observations also revealed filamentous microbes 1 μm in width and cocci on the surface of rhombohedral crystals (Fig. 3). The cocci contained mainly Si, Ca and Fe (Fig. 4A), and filamentous microbes contained Ca (Fig. 4B) . The rhombohedral crystals also contained significant Ca (Fig. 4C). Symbiotic colonies of cocci and filamentous microbes thus accumulate Si, Ca and Fe, and Ca respectively on the surface of crystals in the reddish brown biomats.

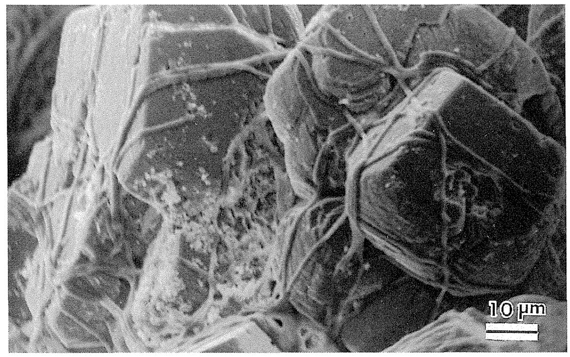
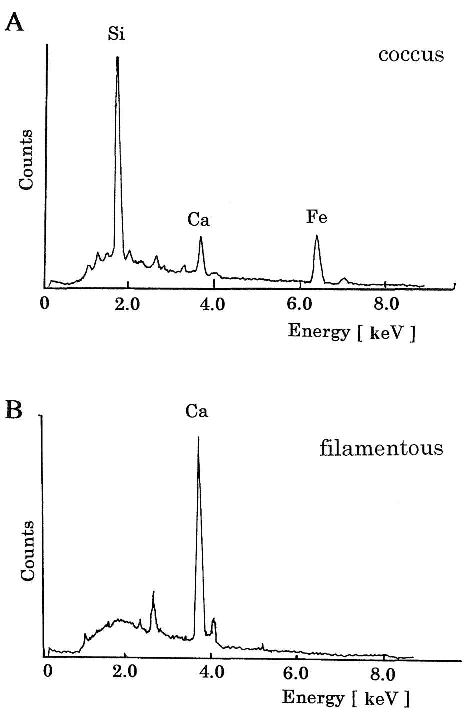


Fig. 3 SEM image of rhombohedral crystals in reddish brown biomats.



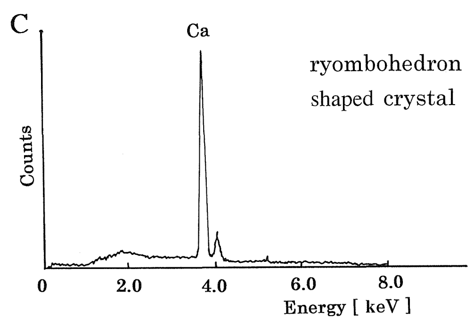


Fig. 4 EDX analysis of cocci (A), filamentous microbes (B), and rhombohedral crystals (C) in reddish brown biomats.

Reference

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4-11 Ogoya Mine

Locality

Ogoya Mine, Ogoya Cho, Komatsu City, Ishikawa Prefecture < N 36° 03´, E 136° 06´ >

Geology

Ogoya Mine is underlain by tuffs and rhyolitic lava flows. Rhyolitic and andesitic dikes are also common (Kaseno, 1993). Until its closure in 1971, Ogoya Mine produced pyrite, chalcopyrite, galena and sphalerite (Hokuriku Mining Co., Ltd., 1996).

Occurrence

Abundant brown deposits have accumulated on the bed of a stream draining out from the No. 6 Pit of Ogoya Mine (Fig. 1). The surface of the deposits is covered with green biomats (Fig. 2A). Additionally, dark green biomats characterized by a partially sticky consistency occur around the pithead.

Fig. 1 Acid mine water flowing into the stream. Neutralization treatment is being carried out in the lower parts of this river.

Water Chemistry

Examination of water draining from the mine gave the following:

WT 15 °C pH 3.5 Eh 370 mV EC 0.9 mS/cm

DO 1.4 mg/l (measured by Sakurayama and Kishigami, 16 May 1997).

The water contains Fe (26.85 mg/l), Cu (3.97 mg/l), Zn (23.94 mg/l) and Cd (0.09 mg/l) (Hokuriku Mining, 1996).

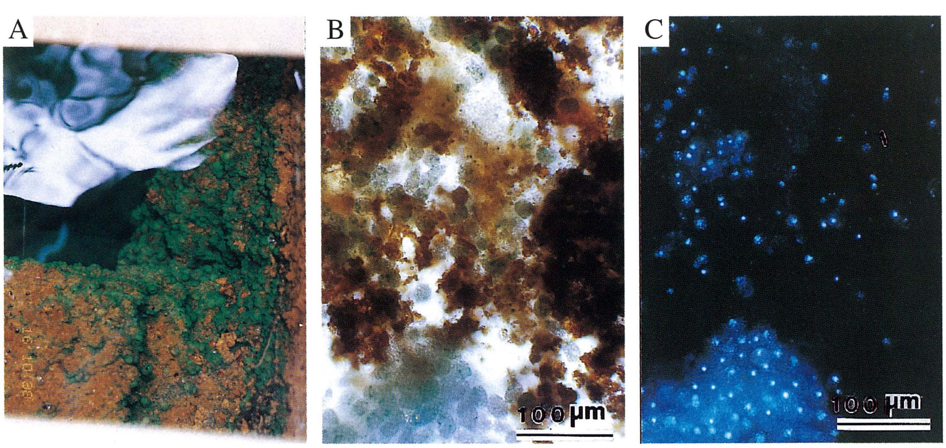

Fig. 2 Green biomats on the surface of brown deposits (A), optical micrograph (B) and fluorescence micrograph (C) of the biomats.

Microbiology and Biomineralization

The partially sticky dark green biomats contain diatoms and green algae which have accumulated abundant Cu (Fig. 3A).

Optical and fluorescence microscopy revealed numerous protozoa in the green biomats covering the brown deposits (Fig. 2B, C). SEM observation of the protozoa colonies revealed that some had retained their form, while others were losing their original structure (Fig. 4A). EDX analysis identified conspicuous accumulations of Al, Si, S, Ca and Fe (Fig. 4B), and XRD confirmed the presence of copper and quartz (Fig. 3B).

In conclusion, the green biomats at this site contain microorganisms which accumulate Fe and Cu selectively from mine drainage containing Fe, Cu, Zn and other metals. This phenomenon suggests the green biomats play a key role for bioremediation in environmental pollution by concentrating toxic heavy metals.

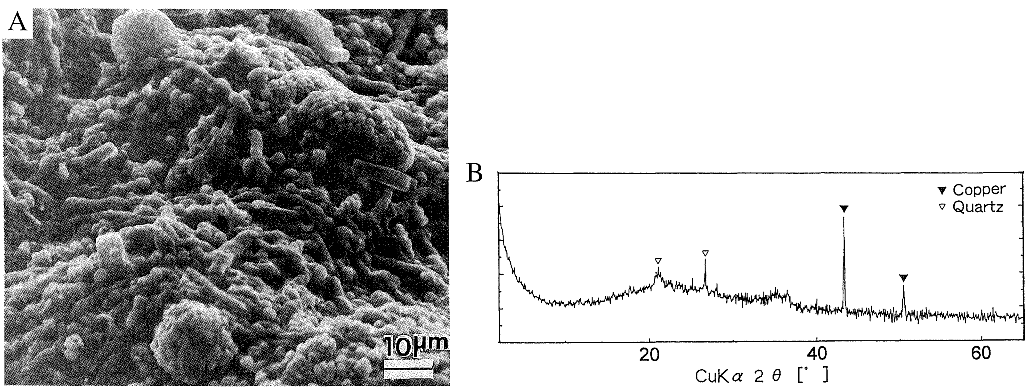

Fig. 3 SEM image of dark green biomats composed of diatoms and green algae (A). XRD pattern of bulk sample of dark green biomats (B).

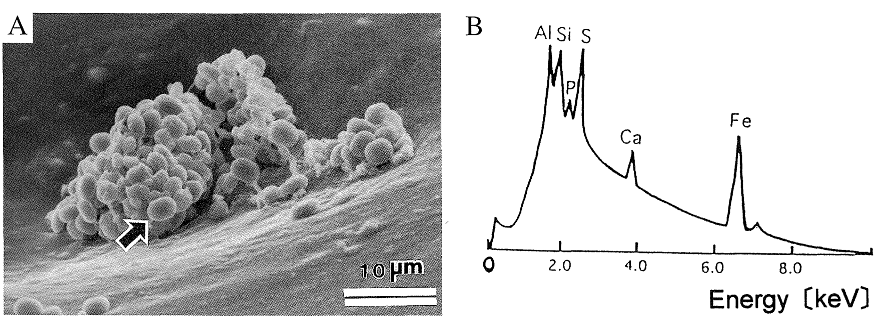

Fig. 4 SEM image (A) and EDX spectrum (B) of elliptical protozoa. An arrow shows analytical point.

References

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4-12 Yanagi Valley, Mt. Hakusan

Locality

Shiramine Mura, Ishikawa Gun Ishikawa Prefecture (Yanagi Valley)
< N 36° 07′, E 136° 45′ >

Geology

The Yanagi Valley is located on the west-facing slope of Mt. Hakusan in the south-east of Kaga Region in Ishikawa Prefecture. Sandstones, shales and conglomerates of the Tetori Group occur in this region (Kaseno, 1976, 1993).

Occurrence

Red or ivory biomats can be observed around the headsprings and for 40~50 m along the river. They are located on terraces or curtain-like form coverings on the dam walls (Fig. 1A, B).

Water Chemistry

1996 ; pH 6.2~6.4 EC 1.6 mS/cm
(measured by Ueshima, Oct. 1996)
1997 ; pH 6.5 EC 1.4 mS/cm
Eh 130 mV DO 3.4 mg/l
(measured by Yasuda, Aug. 1997).

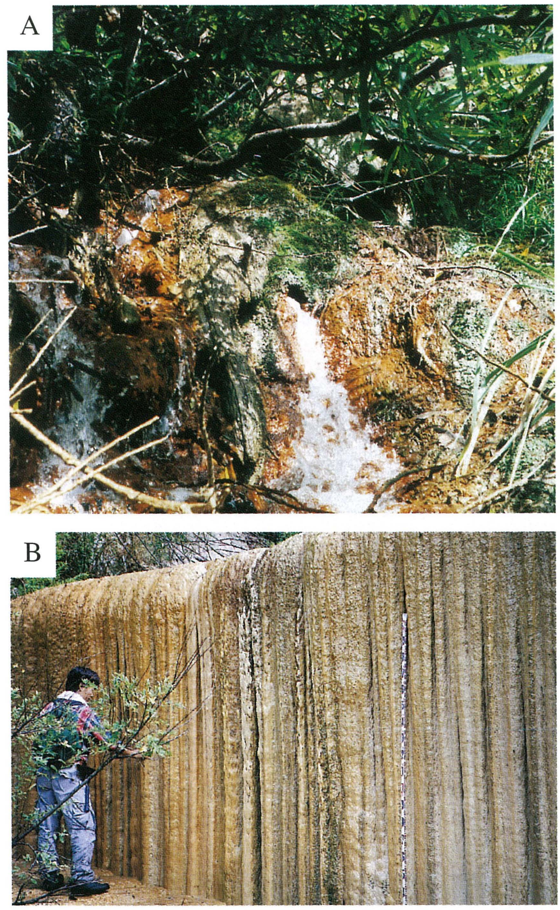

Fig. 1 Red biomats around the source (A) and ivory microbial mats in the lower reaches of the stream (B).

Microbiology and Biomineralization

Iron precipitation associated with green algae was recognized in the red biomats by means of optical microscopy (Fig. 2). Numerous twisted microorganisms consisting of Si, Ca and Fe were detected by SEM-EDX observation and analysis (Fig. 3A, B). TEM observations revealed three different appearrances of precipitate material; filamentous, spherical and film-like (Fig. 4A, B).

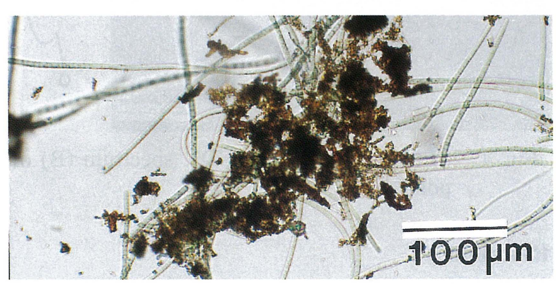

Fig. 2 Optical micrograph of red biomats.

XRD analysis recognized calcite in the ivory biomats. SEM observations revealed entwined algae, around which spherical calcite particles were formed (Fig. 5). The ivory biomats contained abundant Ca and the algae themselves, elements such as Si and Fe.

The curtain-like biomats were soft and ivory colored mats in October 1996. However the most recent field inspection in August 1997 recognized that the biomats had solidified and changed color to red. This phenomenon suggests that the microbe population had changed, due to breeding of iron bacteria in summer. A cross section of the congealed biomats revealed a banded formation. This formation pattern indicates a cyclical process of alternate calcite and iron mineral formation. The concentrated elements and the quantity of precipitation depend on the microbe population in the biomats.

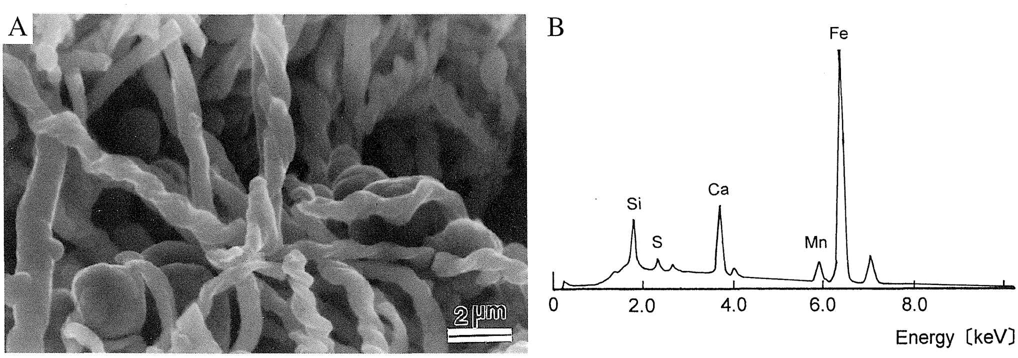

Fig. 3 SEM image (A) and EDX analysis (B) of the red biomats.

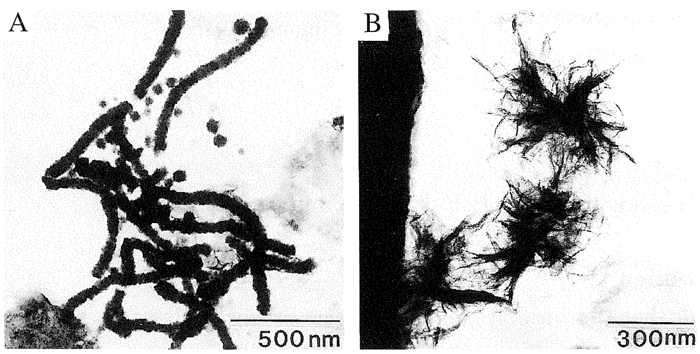

Fig. 4 TEM image of the red biomats. Filamentous, spherical (A) and film-like (B) materials are shown.

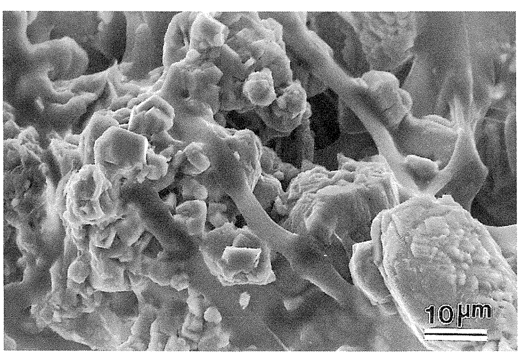

Fig. 5 SEM image of ivory biomats.

References

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4-13 Nakatatsu Mine

Locality

Izumi Mura, Ohno Gun, Fukui Prefecture
< N 35° 56´, E 136° 40´ >

Geology

Nakatatsu Mine is located in the circum-Hida tectonic zone. In this area, the stratigraphic sequence consists of Palaeozoic Fujikura-Tani and Akiu Formations and Mesozoic Motodo and Tetori Formations. Quartz porphyry, the host to the mineralization, is intruded into these formations. Numerous ore deposits are found in this area. The major deposits are Pb-Zn ore bodies in skarns formed by metasomatism between zinc-rich fluid and limestones of the Fujikura-Tani Formation. The ore minerals from this mine are mainly sphalerite and galena with minor chalcopyrite, molybdenite and scheelite (Fukahori *et al.*, 1983; Shimizu and Iiyama, 1982; Nishikawa and Tochimoto, 1985; Nakamura and Shimazaki, 1987).

Fig. 1 Soft black biomats on the surface of garnet skarn rocks in the Nakatatsu skarn mining gallery.

Occurrence

Black biomats are observed coating the garnet rich skarn in a mining gallery (Fig. 1).

Microbiology and Biomineralization

SEM observations showed that the biomats are composed of algae characterized by filamentous, rod-shaped and teardrop-shaped micro textures with flaky materials coating the surface (Fig. 2A, B) (Tazaki *et al.*, 1994; Tazaki, 1995). EDX analysis showed that the flaky materials were rich in Mn and Ca (Fig. 2C).

The XRD pattern (Fig. 3) of bulk biomats from the mining gallery gave strong reflections for illite (9.9 Å), minute or poorly crystalline Mn-rich calcite (2.9 Å), iron oxide minerals (2.5 Å) and manganese oxides (Tazaki *et al.*, 1994). XRF analysis detected CaO (10.31 %), MnO (83.72 %) and ZnO (2.06 %) (Table 1) (Tazaki, 1996).

Table 1 Results of XRF analysis of biomats from mining drainage within Nakatatsu Mine.

Elements	Na_2O	SiO_2	CaO	MnO	Fe_2O_3
wt.%	0.61	1.21	10.31	83.72	0.10
Elements	ZnO	As_2O_3	SrO	MoO_3	BaO
wt.%	2.06	0.19	0.22	0.49	0.21

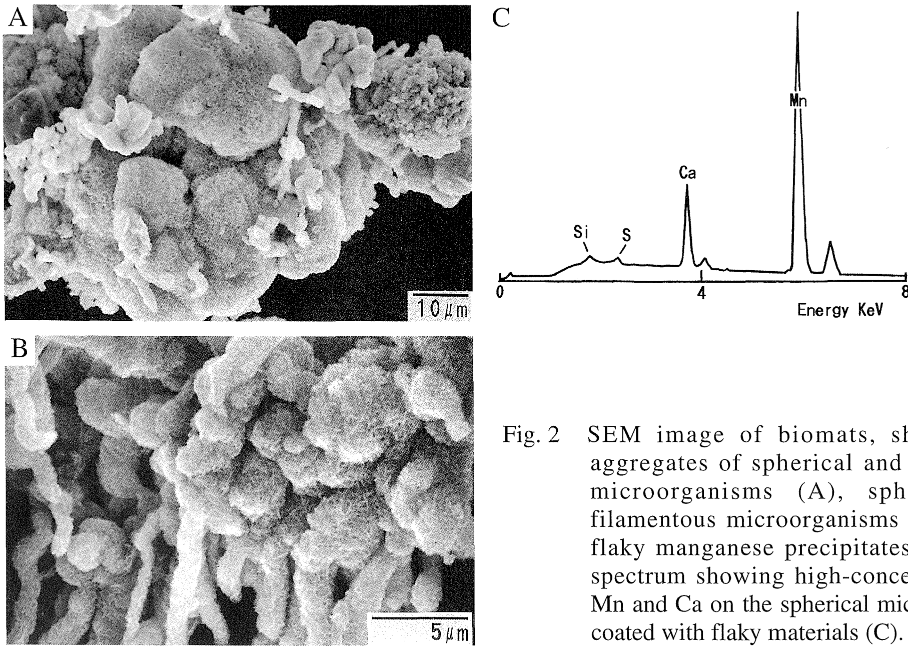

Fig. 2 SEM image of biomats, showing the aggregates of spherical and rod-shaped microorganisms (A), spherical and filamentous microorganisms coated with flaky manganese precipitates (B). EDX spectrum showing high-concentrations of Mn and Ca on the spherical microorganisms coated with flaky materials (C).

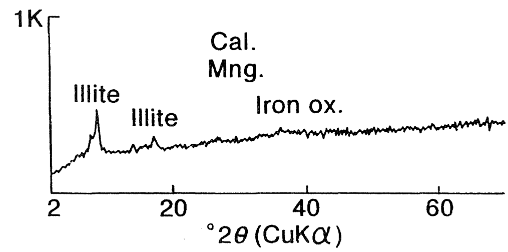

Fig. 3 XRD pattern of bulk sample of the biomats.
Cal.: calcite, Mng.: manganese minerals, Iron ox.: iron hydroxide minerals.

References

Fukahori, Y., Nishikawa, Y., Nakamura, K. and Machida, M. (1983). Recent exploration of the Kurotodo deposits in Nakatatsu Mine. *Mining Geol.*, **33**, 73-86.

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Nishikawa, Y. and Tochimoto, K. (1985). Pb-Zn mineralization controlled by geological features in the Nakatatsu skarn-type ore deposit, Fukui Prefecture. *Mining Geol.* **35**, 161-177.

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4-14 Hohman-Zan Mine

Locality

Higashi Izumo Cho, Yatsuka Gun, Shimane Prefecture
< N 35° 30′, E 132° 50′ >

Geology

The stratigraphic sequence consists of the Tertiary Kuri, Ohmori and Fujina Formations, overlying granite and diorite basement rocks (Inoue *et al.*, 1982; Toyao *et al.*, 1985). Hohman-Zan Mine is a fissure-filling vein type copper deposit in tuff and rhyolite host rocks. Metallic minerals from this mine are mainly chalcopyrite and pyrite (Toyao *et al.*, 1985).

Occurrence

Dark-reddish or orange-brownish biomats cover the bottom of drainage ways and tailings ponds (Fig. 1A). The gravels in the drainage channels are also covered with dark-reddish or orange-brownish crusts. While red-brownish hematite forms the outer surface of these gravels, yellow-brownish goethite comprises the inner part of the coating (Tazaki, 1993, 1995; Tazaki *et al.*, 1994).

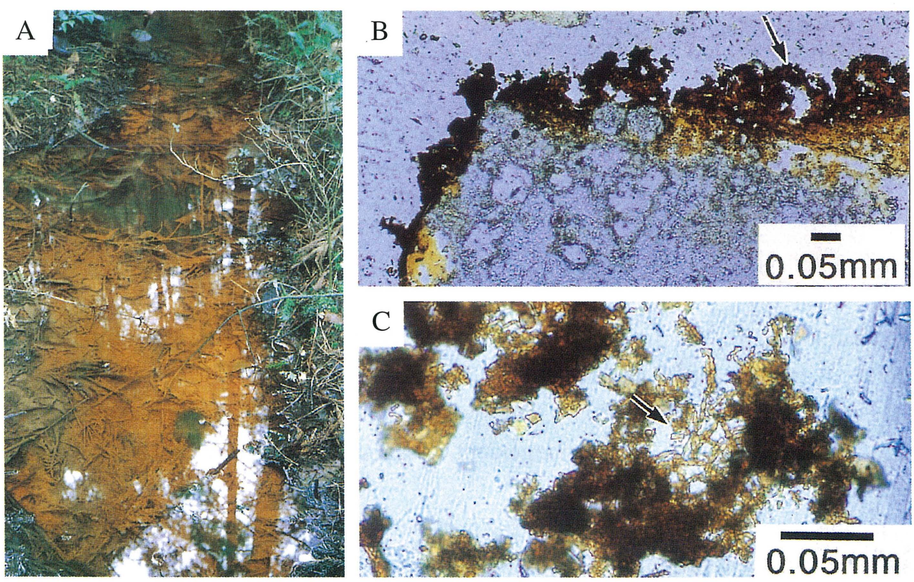

Fig. 1 General view of an iron seep in mine drainage ways within Hohman-Zan Mine (A). Optical micrograph of a thin section of the orange-brownish or dark-reddish crusts covering the surface of gravels collected from the bottom of a drainage way (B, an arrow). Optical micrograph of a thin section revealed the chain-like microbes from the crust of the gravels (C, an arrow).

Water Chemistry

The pH of the water from mine-tailing ponds, streams and rivers where the biomats occur averages 3.6 (3.0~4.0). Chemical composition of stream water was:

Al (8.12 ppm), S (20.42 ppm), Ti (0.01 ppm), Cr (0.05 ppm), Mn (0.22 ppm), Fe (4.49 ppm), Ni (0.03 ppm) and Cu (0.05 ppm) (Tazaki *et al.*, 1994).

Microbiology and Biomineralization

Optical microscopic observations showed that the biomats were predominantly composed of filamentous algae, twisted algae and diatoms (*Aulacoseira* sp. and *Navicula oblonga*). Gravels collected from the bottom of a drainage way were covered with iron oxide or hydroxide. Filamentous microorganisms occurred on the surface of the crust on the gravels (Fig. 1B, C) (Tazaki, 1993; Tazaki *et al.*, 1994).

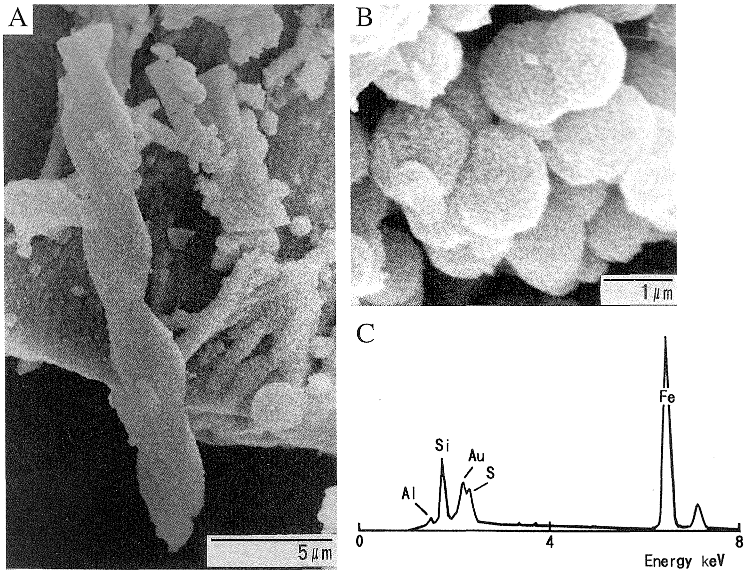

Fig. 2 SEM images of biomats composed of iron-precipitating microorganisms show twisted algae (A) and congregations of spherical algae covered with fine granular materials (B). The EDX spectrum of a bulk sample of twisted algae shows high Fe and trace Al, Si and S (C).

Most of the filamentous algae were characterized by a twisted morphology, the surface of which was coated with granular materials (Fig. 2A, B). These granular materials were composed of a large amount of Fe with traces of S, Al, Si and K (Fig. 2C). This suggests the presence of filamentous iron bacteria (Tazaki, 1993, 1995; Tazaki *et al.*, 1994).

The XRD pattern showed predominant quartz with traces of poorly crystalline hematite, magnetite and akaganeite (Tazaki *et al.*, 1994).

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Toyao, S., Shimada, I. and Togitani, M. (1985). The Shimane Pref. Geol. Mapping Committee (eds.), Shimane Prefecture, Matsue, p439-442.

4-15 Ohmori Mine (Iwami Silver Mine)

Locality

Ohmori Cho, Ohda City, Shimane Prefecture < N 35° 00´, E 132° 40´ >

Geology

Ohmori Mine was opened in the 14th century to extract silver from outcrops. Ohmori Mine was gradually developed on the large scale as a silver mine from the 16th century until the Edo era, and as a copper mine from the Meiji to the Taisyo eras. Ohmori Mine has two different ore types. Disseminated ore deposit (Fukuishi) and epithermal vein-type deposits (Eikyu). Both are hosted in the Quaternary Oetakayama (Oh-e-takayama) volcanic rocks of Mt. Sen-Yama. The Fukuishi ore deposit was renowned, due to the high production of the Iwami Silver Mine.

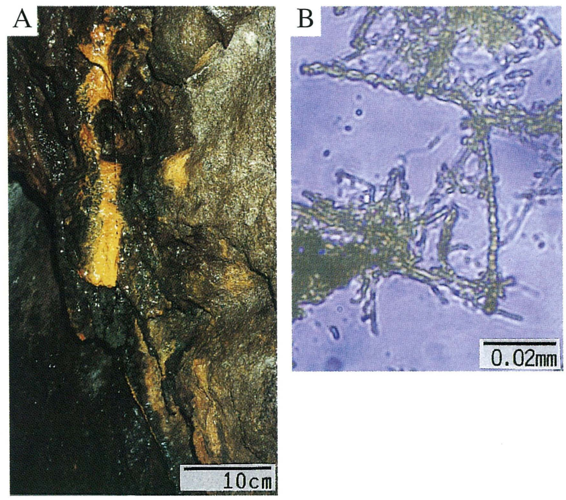

Fig. 1 Biomats on pit walls in Fukuishi ore (A). Optical micrograph of biomats composed of coccoidal and ellipsoidal algae (B).

Around this area, the stratigraphic sequence consists of Neogene Kuri Formation, Quaternary Tsunozu Formation and Oetakayama volcanic rocks. Ohetakayama volcanic rocks consist of dacite lava, breccia and dacite tuff. Fukuishi ore was hosted in the latter.

The Fukuishi ore assemblage includes silver, argentite, galena, sphalerite and hematite, with small amounts of jalpaite, mackinstryite, stromeyerite, covellite, chalcopyrite and pyrite. The gangue minerals are siderite-rhodochrosite system minerals and minor quartz (Torigoe and Akasaka, 1994; Izawa, 1997; Torigoe, unpublished data).

Occurrence

Biomats are observed in places where ground water seeps from the pit walls in the Fukuishi ore. Two different occurrences can be recognized; orange or black biomats and black biofilms on the walls (Fig. 1A) (Tazaki *et al.*, 1994); and orange biomats on the surfaces of dark-brown fan-shaped mound deposits formed under the drifthead.

Water Chemistry

Drainage seeping from the drift:

WT 10.8 °C pH 6.8 Eh 161 mV EC 155 μS/cm DO 4.64 mg/l

(measured by Watanabe, 13 Aug. 1997).

Microbiology and Biomineralization

Optical microscopic observations revealed that the biomats were predominantly composed of chains of cocci (Fig. 1B). Single units of the cocci have coccoidal or ellipsoidal morphology. SEM observations revealed that the microbes characterized by filamentous, rod-shaped or twisted structure were covered with fine flaky materials and spherules (Fig. 2A, B). Flaky minerals and aggregates of granular or coccoidal materials were also observed. XRF analysis (Table 1) detected high Zn concentrations in the biomats (Tazaki *et al.*, 1994; Tazaki, 1995).

XRD analysis (Table 2) of the biomats and films gave a mineral assemblage of illite, interstratified illite/smectite, smectite, kaolinite, quartz, feldspar and poorly crystalline materials (Tazaki *et al.*, 1994).

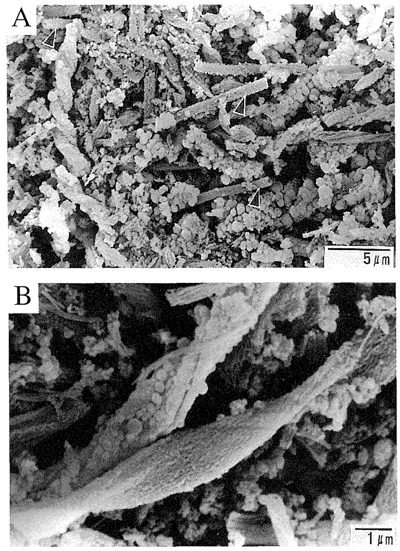

Fig. 2 SEM image of biomats showing twisted microbes and tubular materials (A, arrows), the surface of which are covered with spherules or flaky materials (B).

Table 1 Results of XRF analysis of bulk sample of biomats. The biomats were characterized by enrichment of zinc.

Elements	Mo	Nb	Zr	Y	Sr	Rb	Pb
	-	1	1	-	91	11	-
Elements	Zn	Cu	Ni	Cr	V	Ba	
	>11000	45	12	-	-	-	(cps)

Total counts are over 30,000 cps. - : not detected.

Table 2 Biomat mineral assemblage.

	I/S,S	Ill	Ka	Qtz	Feld
Orange	+		+		
Black	+	+++		++++	++

I/S: illite/smectite interstratified minerals, S: smectite, Ill: illite, Ka: kaolinite, Q : quartz, Feld: feldspar.++++: abundant, +++: common, ++: small amounts, +: trace.

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4-16 Hishikari Mine

Locality

Hishikari Cho, Isa Gun, Kagoshima Prefecture
< N 32° 00′, E 130° 40′ >

Geology

The basement in this area is the Tertiary Shimanto Group which is composed of alternating shales and sandstones. This is unconformably overlain by Quaternary volcanics and alluvium.

Mineralization within the Hishikari Mine is epithermal gold-silver bearing quartz-adularia vein type, developed in Quaternary volcanic rocks. The ore body is composed of several major veins and numerous veinlets. Metallic minerals are mainly electrum, naumannite and chalcopyrite, with minor miargyrite, freibergite, pyrargyrite, galena, sphalerite, stibnite and pyrite. Gangue mineralogy is characterized by a large amount of adularia, along with quartz and clay minerals (montmorillonite) (Metal Mining Agency of Japan and Sumitomo Metal Mining Co., Ltd., 1987; Sumitomo Metal Mining Co., Ltd., 1992).

Occurrence

Brown biomats occur in mine-tailings ponds and drainage ways (Tazaki *et al.*, 1994).

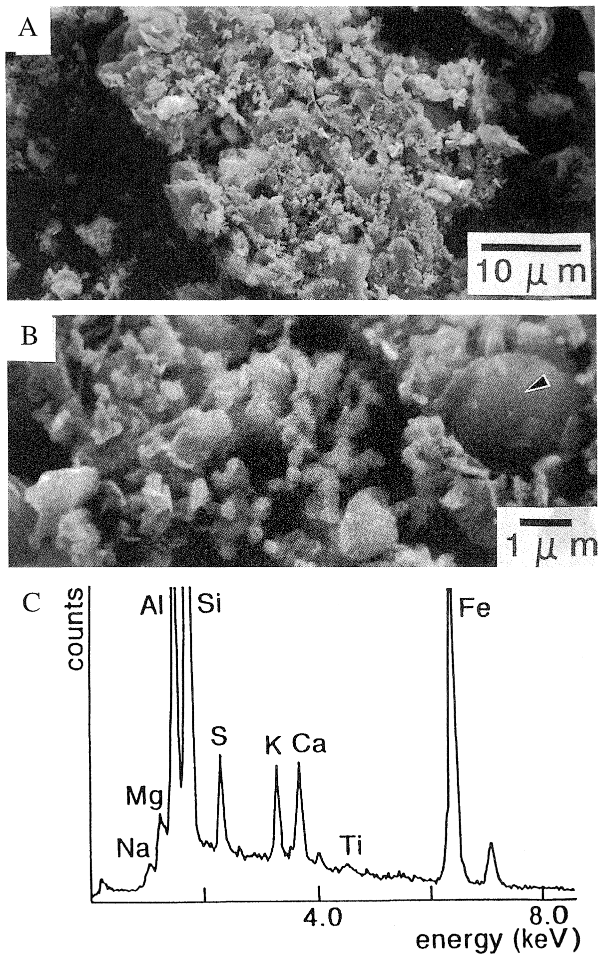

Fig. 1 SEM image and EDX spectrum showing the structure of spherical and granular microbes with abundant Fe (A, C). It is noted that the large spherical materials have granules (B, an arrow). Polygonal grains are clay minerals (B is a magnified photograph of A).

Microbiology and Biomineralization

SEM-EDX observations and analyses showed that the biomats consisted of spherical or granular shaped microbes enriched in Fe and S (Fig. 1 A, B, C). High concentrations of Al, Si, K and Ca are suggestive of clay minerals and quartz with organic matter. Traces of Na, Mg and Ti were also detected (Tazaki *et al.*, 1994; Tazaki, 1995, 1996).

XRF analysis (Table 1) revealed that the biomats contained large amounts of Al_2O_3 (18.14 mg/l), SiO_2 (57.98 mg/l) and Fe_2O_3 (14.23 mg/l). Various trace elements were also detected, such as As, Y, Rb, Sr and Zr (Tazaki, 1996).

An XRD pattern (Fig. 2) obtained by Cu Kα radiation confirmed the presence of smectite (15.3 Å), kaolinite (7.1 Å), quartz (3.3 Å) and amorphous materials as major components with small amounts of iron oxide minerals (2.5 Å), feldspar (3.2 Å), cristobalite (4.0 Å) and poorly crystalline manganese oxide (rancieite ; 7.8 Å) (Tazaki *et al.*, 1994).

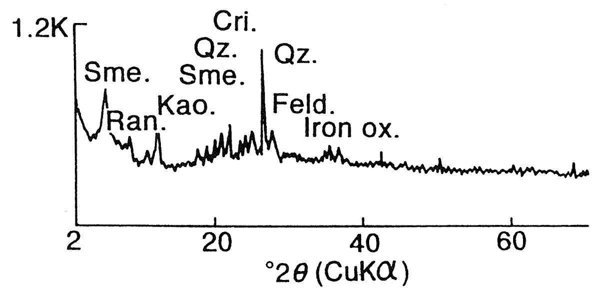

Fig. 2 XRD pattern of biomats.
Sme.: smectite, Ran.: rancieite, Kao.: kaolinite, Qz.: quartz, Cry.: cristobalite, Feld.: feldspar.

Table 1 XRF analysis of biomats from drainage way.

Elements	Na_2O	MgO	Al_2O_3	SiO_2	P_2O_5	SO_3	K_2O	CaO	TiO_2
wt.%	0.51	2.46	18.14	57.98	0.11	0.41	3.26	1.96	0.61
Elements	MnO	Fe_2O_3	CuO	ZnO	As_2O_3	Rb_2O	SrO	Y_2O_3	ZrO_2
wt.%	0.09	14.23	0.01	0.03	0.11	0.03	0.03	0.01	0.03

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4-17 Kirishima, Sakura-Jima District

Locality

Maruo Hot Springs: Makizono Cho, Aira Gun, Kagoshima Prefecture
Tarumizu Hot Springs, Sarugajoh Hot Springs, Kaigata Hot Springs : Tarumizu City, Kagoshima Prefecture
Furusato Hot Springs, Magma Hot Springs: Kagoshima City, Kagoshima Prefecture
< Sakura-Jima volcano : N31° 36´, E130° 40´ >

Geology

The Maruo Hot Springs form the nucleus of the Kirishima Hot Springs community of the Kirishima volcano group situated 600 m above sea level. The basement in the area is composed of relatively hard altered andesite, overlain by clays. This structure causes landslides all over the area. Gravel and tuffaceous sand strata 5 m in thickness are extensively distributed in the lower part of the Shirasu plateau in Tarumizu City located on the southern and western base of Sakura-Jima volcano. The basement rocks of the southeastern part of Sakura-Jima volcano are pyroxene andesite and dacite. These are overlain by a thick covering of lava and pyroclastic flow deposits originating from Mt. Minami, itself a part of Sakura-Jima volcano (Karakida *et al.*, 1980).

Occurrence

Green biomats are observed around a boiling spa within Maruo Hot Springs (Fig. 1). Green biomats also occur around the sources of springs at Tarumizu, Kaigata, Furusato, and Magma Hot Springs.

Fig. 1 Green biomats around a boiling spa within Maruo Hot Springs, Kirishima volcano group.

Water Chemistry

The water temperature of Maruo Hot Springs is over 60 ℃. The water is weakly alkaline chloride (saline spring), due to heated ground water (Tsuyuki *et al.*, 1990). Measurements of pH, Eh, EC and DO at hot springs around Sakura-jima volcano show wide variations (Table 1).

Table 1 Results of water testing of the major hot springs around Sakura-Jima volcano (Measured by Tawara, 9 July, 1997)

Hot Springs	WT(℃)	pH	Eh(mV)	EC(mS/cm)	DO(mg/l)
Tarumizu	47.1	9.0	-366	242.0	1.2
Sarugajo	40.0	7.5	41	131.6	6.8
Kaigata	45.4	9.7	-341	273.0	1.4
Furusato	35.4	7.2	24	50.9	6.8
Magma	41.6	6.6	-121	22.6	6.0

Microbiology and Biomineralization

SEM observations of green biomats around the boiling spa at Maruo Hot Springs showed bacillus- or coccus-like particles 1~3 μm in size covering the surface (Fig. 2A, an arrow). These particles contained abundant Si (Fig. 2B). TEM observations detected bacilli accumulating amorphous materials containing mainly SiO_2 (XRD / EDX) on the cell walls (Fig. 2C).

More investigation of this biomat-rich locality is planned.

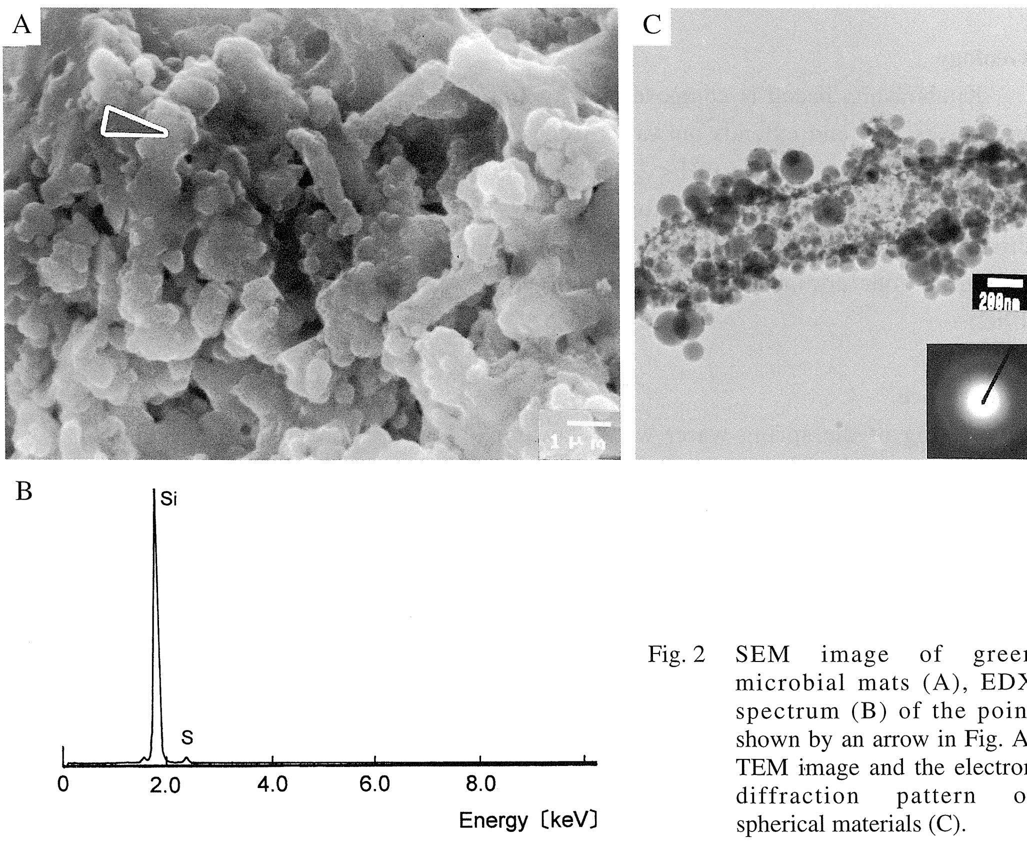

Fig. 2 SEM image of green microbial mats (A), EDX spectrum (B) of the point shown by an arrow in Fig. A, TEM image and the electron diffraction pattern of spherical materials (C).

References

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4-18 Kitabirashita Beach, Satsuma-Iwo Jima Island

Locality

Satsuma-Iwo Jima Island, Mishima Mura, Kagoshima Gun, Kagoshima Prefecture (Hot-spring at Kitabirashita beach)

< N30° 48´, E130° 19´ >

Geology

Kitabirashita beach is composed of rhyolite breccia gravels and extends outwards from tall cliffs. Strongly acidic water (pH < 2) gushes from sites in and around the beach. Native sulphur, α-cristobalite and alunite have been found in the alteration zone around the source (Ono *et al.*, 1982).

Occurrence

Mixing of the spring water with seawater precipitates amorphous hydroaluminum silicate, which gives the seawater a milky cloudiness (Fig. 1A). White sediments containing aluminum silicate are precipitated in the tidal pools of the onshore reef. Biomats are found on the sides of the tidal pool (Fig. 1B). These biomats develop into yellow-brownish solid materials which accumulate on the rock surfaces around the coast (Fig. 1C, arrows).

Water Chemistry

Cloudy seawater gave the following results :

WT	27.2~30.1 ℃	pH	1.9~5.6
Eh	167~465 mV	EC	47.5 mS/cm
DO	49.5 mg/l		

(measured by Shikaura, 12 July 1997).

The differences are due to degree of mixing between seawater and hot spring water.

SEM-EDX observation and analysis of seawater (after evaporation to dryness) identified Al, Si, Fe and $CaSO_4$.

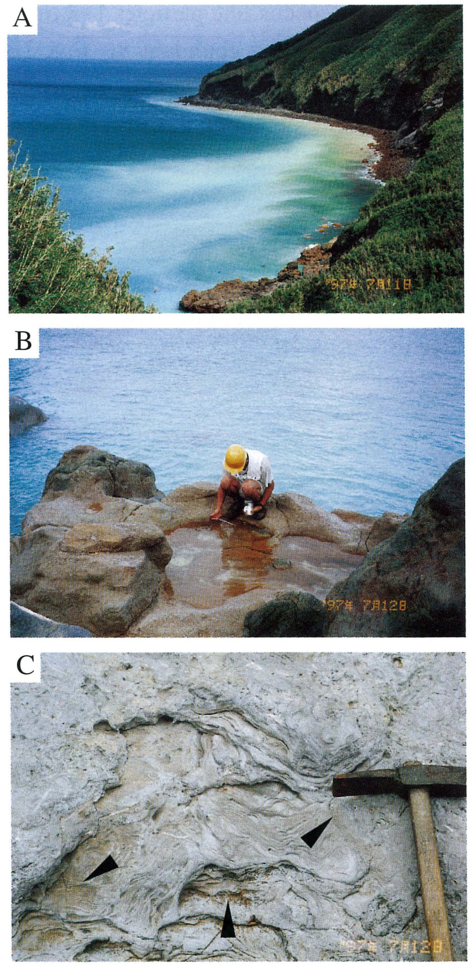

Fig. 1 A general scene of Kitabirashita beach. The cloudy nature of seawater can be clearly distinguished (A). White sediments are observed in the tidal pools of the onshore reef. Biomats are found on the sides of tidal pools (B). Accumulations of altered mats on rock surfaces around coast. Solid yellow brownish material (C, arrows).

Microbiology and Biomineralization

Optical and electron microscopy showed that the biomats were predominantly composed of filamentous algae and diatoms (Fig. 2A, B). Observations under a fluorescence microscope detected chlorophyll in the filamentous algae. Biomats accumulated Al, Si and Fe (Fig. 2C).

SEM observations of the yellow-brownish solid deposited on the rock surfaces revealed numerous diatoms. The elemental composition of the solid is similar to that of the biomats. According to analysis by XRD, the solid material gives 3.1 and 5.1 Å reflections corresponding to jarosite, and a 1.9 Å reflection corresponding to alunite (Fig. 2D).

The rock on which the mats occur did not show the presence of diatoms in thin section, and XRD analyses failed to recognize minerals similar to those occurring on the rock surface.

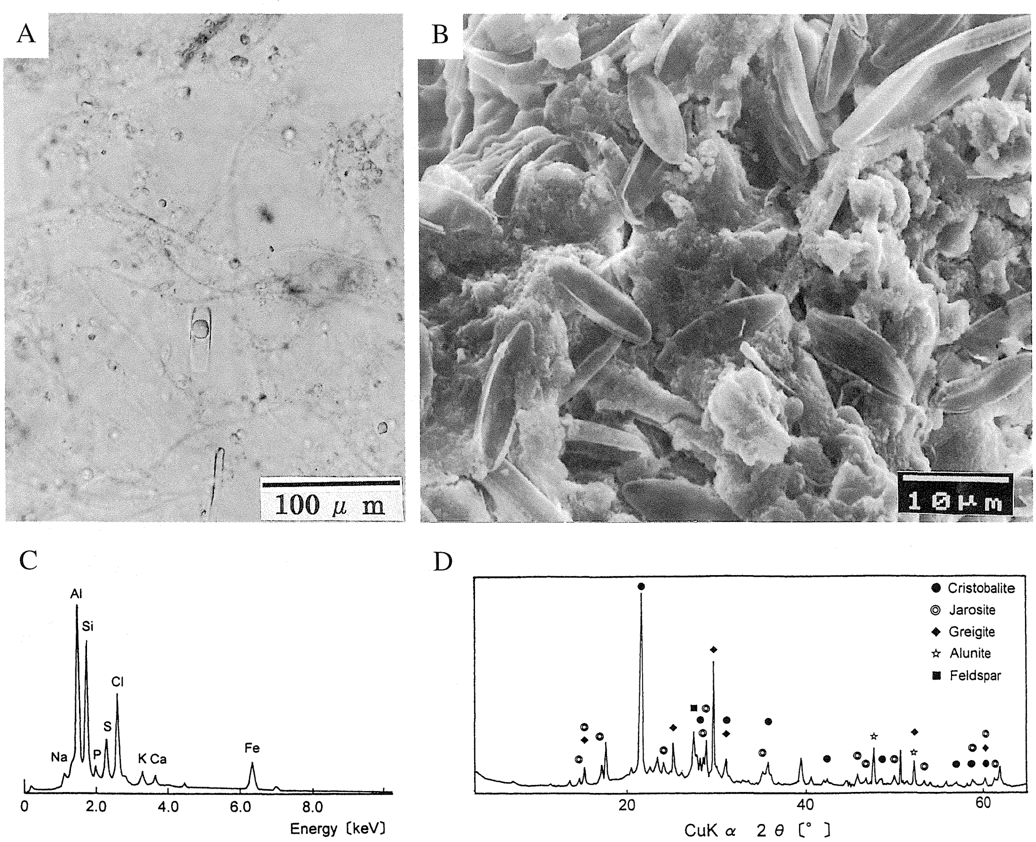

Fig. 2 Optical microscope photograph of biomats (A). SEM image of biomats from the sides of tidal pool (B). EDX spectrum of biomats (C). XRD pattern of biomats accumulated on the surface of the rock around the coast after alteration into a yellow brownish solid (D).

References

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4-19 Higashi Hot Springs, Satsuma-Iwo Jima Island

Locality

Higashi-Onsen, Satsuma-Iwo Jima Island, Mishima Mura, Kagoshima Gun, Kagoshima Prefecture < N30° 47′, E130° 18′ >

Geology

Higashi Hot Springs are located on the southwest foot of Iwo-Dake volcano (703 m elevation), on the southern coast of the island. The bed rock is pyroxene olivine basalt which forms Inamura-Dake volcano (236 m elevation). In the upper part, the stratigraphic sequence consists of volcanic breccia and thick lavas originating from Iwo-Dake volcano (Ono *et al.*, 1982).

Occurrence and Water Chemistry

The water of Higashi Hot Springs is strongly acidic, and is rich in aluminum and iron ions (Ono *et al.*, 1982). The area has been developed into a series of pools. Maximum water temperature is 50.6 °C at the discharge of the spring around which yellow biomats can be observed (Fig. 1A). In contrast, the temperature of the lower baths is less than 45 °C, and green biomats have been formed on the pool margins (Fig. 1B). Water examination indicates that both biomats occur in strongly acid conditions. However the widely different DO measurements indicate that yellow biomats are formed in anoxic conditions (Table 1).

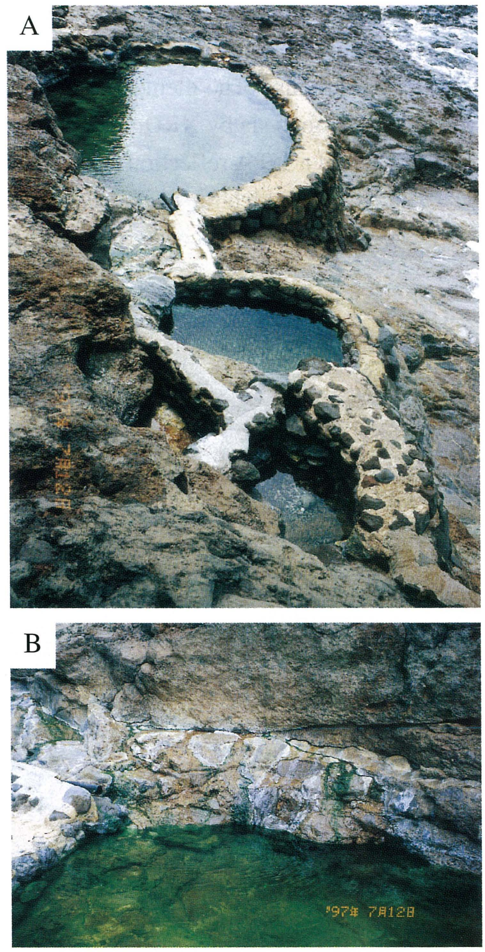

Fig. 1 An overall view of Higashi Hot Springs. The water source is located in the lowest bath in the photograph, around which yellow biomats can be observed (A). Green microbial mats (B) which can be observed on the wall of the upper bath shown in photograph A.

Table 1 Results of water testing of the hot spring water around the differently colored biomats (measured by Tawara, 12 July 1997).

	WT(°C)	pH	Eh(mV)	EC(mS/cm)	DO(mg/l)
Yellow Biomats	50.6	1.3	491	12.3	0.09
Green Biomats	43.4	1.2	504	13.5	3.98

Microbiology and Biomineralization

TEM observations of the hot spring water at source revealed bacilli which had precipitated jarosite on the cell walls (Fig. 2). SEM-EDX observations and analyses of the yellow biomats at this point showed bacillus- or coccus-like particles containing abundant Si, S and Fe, and traces of Al, P and K (Fig. 3A, B). XRD of the yellow biomat samples identified 5.09, 3.11 and 3.08 Å reflections corresponding to jarosite, and a 4.05 Å reflection corresponding to cristobalite.

These observations contrast with the results from the green biomats which form at lower temperatures. These show colonies of cocci and mainly contain amorphous materials.

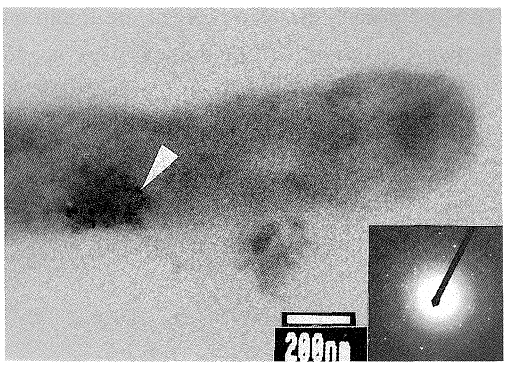

Fig. 2 TEM image of bacillus in the hot spring water and electron diffraction pattern. The fine materials on the cell walls (an arrow) were identified as jarosite from the electron diffraction pattern.

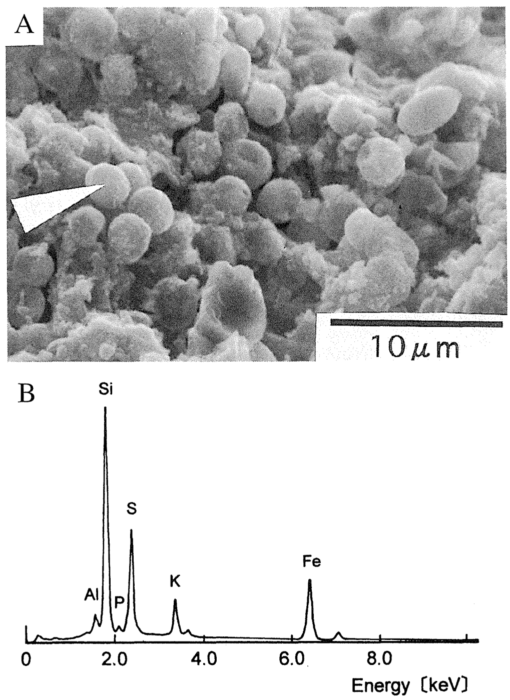

Fig. 3 SEM image of yellow microbial mats (A) and the EDX spectrum (B). The analytical point is shown by an arrow in the photograph A.

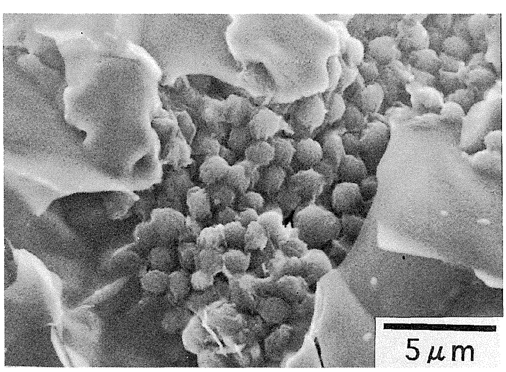

Fig. 4 SEM image of a microbe colony in the green biomats.

Reference

Ono, K., Soya, R. and Hosono, T. (1982). Geology of Satsuma-Iwo Jima district. Geological Survey of Japan, Tsukuba, p45-64.

4-20 Akayu Hot Springs, Satsuma-Iwo Jima Island

Locality

Akayu-Onsen, Satsuma-Iwo Jima Island, Mishima Mura, Kagoshima Prefecture
< N30° 47´, E130° 17´ >

Geology and Occurrence

There are many active hot springs in and around Satsuma-Iwo Jima Island. The hot spring water is generally derived from the seafloor. Reddish brown precipitates derived from hot spring water can be seen on the sand / rock beach at Akayu Hot Springs. Banded biomats are found on the surfaces of basalt rock. The basalt was erupted from the foothills of Inamura-Dake volcano in the Holocene (Ono *et al*., 1982).

The rocky bay and sand beach are stained with reddish seawater, which has given the area its name, "Akayu", (derived from "*aka*" meaning red). Reddish brown biomats infill vesicles in porous basalt near the shore. Colloidal materials in the reddish brown biomats hardened into striped structures (Fig. 1A). Each banded layer is very thin, typically < 0.1 mm (Fig. 1B).

Chemistry of Microbial Mats

The reddish seawater gave the following results:

WT	27.6~29.3 °C	pH	6.7~7.1
Eh	81~281 mV	EC	49.5 mS/cm
DO	5.9~7.3 mg/l		

CO_2 gas is constantly discharged from the beach sand.

Table 1 gives chemical compositions of reddish brown biomats from Akayu Hot Springs as derived from EDX and XRF analysis. The mats were found to be rich in Fe_2O_3.

Table 1

Na_2O	3.41 (wt. %)	K_2O	0.20 (wt. %)
MgO	2.66	CaO	1.19
Al_2O_3	3.48	MnO	3.13
SiO_2	7.66	Fe_2O_3	72.90
P_2O_5	0.14	ZnO	0.08
SO_3	1.22	As_2O_3	0.10
Cl	3.76	SrO	0.06

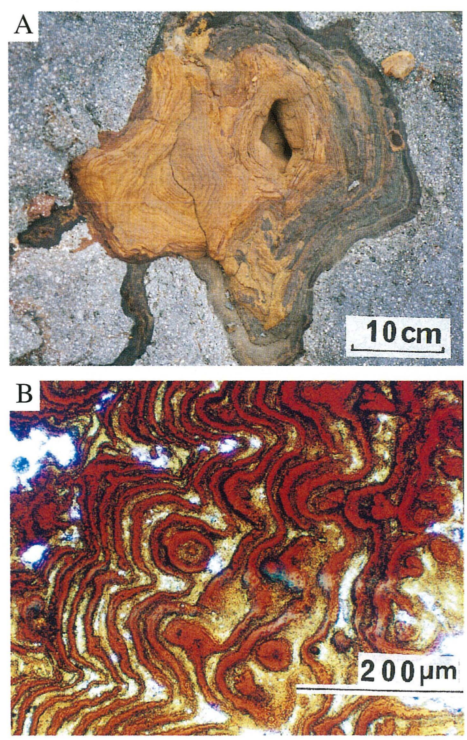

Fig. 1 Reddish brown mats formed on basaltic rock (A) and the optical microphotograph of a thin section (B).

Microbiology and Biomineralization

Under an optical microscope, numerous cocci with aggregating iron minerals could be seen in the banded mats. The cocci size ranged from 1 to 5 μm. In thin section the banded mats revealed a micron-sized layered structure (Fig. 1B). Two different types of layers were observed ; a red-brownish Fe rich layer and a black Mn rich layer. Abundant bacterial colonies can be seen at the margin of each band. Aggregates of cocci together with fibrous bacterial cells and homogeneous granular iron particles were revealed by SEM-EDX (Fig. 2A, C). The granular particles contain abundant Fe and Si, with traces of Mg, Al, P, S, Cl, Ca and Mn. Some of the bacterial cells had concentrated both Fe and Mn with Si.

TEM observations confirmed the presence of coccus bacteria and fibrous or bacillus bacteria covered with granular particles (Fig. 2D). The fibrous bacteria are covered with granular particles of ferrihydrite and amorphous hydroxides or needle-shaped goethite. The particles present an appearance of nearly perfect spheroids covered with flaky Mn microcrystals (Fig. 2D upper). Dead bacterial cells are completely filled and fixed with Fe-Mn minerals giving high density images. Electron diffraction analysis revealed that ferrihydrite on the cell wall at an early stage of the deposition crystallized as goethite during diagenesis. The mineral phases present in the biomats were mainly amorphous iron oxides including goethite (4.8 and 4.18 Å) and ferrihydrite (2.54 Å) (Fig. 2B).

Consideration of the processes of iron mineralization in this environment shows that banded iron formations can be formed under intermittent oxygenated and anoxic conditions.

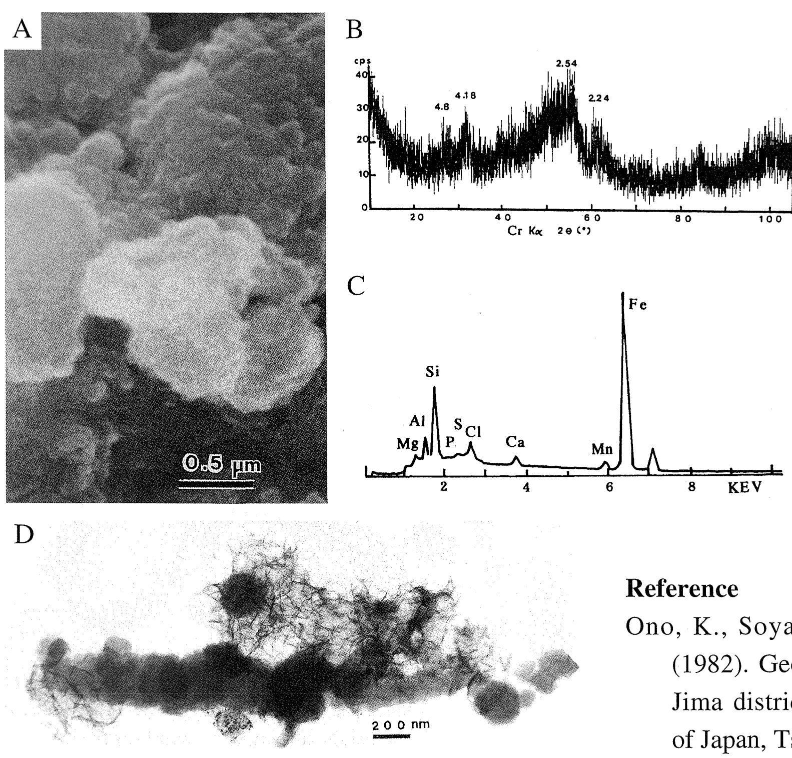

Fig. 2
SEM image (A), XRD pattern (B), EDX spectrum (C) and TEM image (D) of reddish brown biomats from Akayu Hot Springs.

Reference

Ono, K., Soya, R. and Hosono, T. (1982). Geology of Satsuma-Iwo Jima district. Geological Survey of Japan, Tsukuba, p45-64.

4-21 Nagahama Port, Satsuma-Iwo Jima Island

Locality

Satsuma-Iwo Jima Island, Mishima Mura, Kagoshima Gun, Kagoshima Prefecture (Nagahama Port) < N30° 47′, E130° 18′ >

Fig. 1 An overall view of the breakwaters at Nagahama Port. The arrow shows the location of the terrace-like sediments .

Geology

Satsuma-Iwo Jima Island is a volcanic island located in the northernmost of the Tokara Islands and on the northwesternmost margin of the Kikai caldera. The volcanic forming the island consist of basalt-andesite (SiO_2 50~57 %) through to dacite-rhyolite (SiO_2 68~72 %). At Nagahama Port, a sandy beach derived from Nagahama lava has formed (Ono *et al.*, 1982).

Occurrence and Water Chemistry

In and around Nagahama Port, hot spring water containing abundant iron and carbonic ion gushes from the seafloor. The hot spring water reacts with seawater to produce ferric colloid (Ono *et al.*, 1982). This causes reddish brown turbidity (Fig. 1). Terrace-like sediments are exposed at low tide (Fig. 2).

Examination of the water at this site gave a water chemistry of :

WT 28.1 °C pH 7.0 Eh - 45 mV EC 48.6 mS/cm DO 7.6 mg/l

(measured by Tawara, 11 July, 1997). These results indicate reducing conditions.

Fig. 2 Terrace-like sediments appear exposed at low tide.

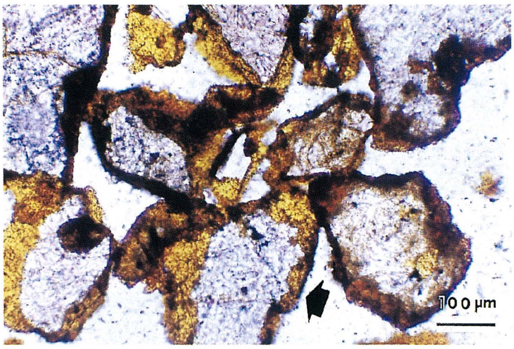

Fig. 3 Optical micrograph of the sediments shown in Fig. 2. The arrow shows ferric materials surrounding sand particles.

Microbiology and Biomineralization

Optical microscopic observations of a thin section taken from the terrace-like sediment revealed bacteria-like particles around sand grains coated with ferric materials (Fig. 3). SEM-EDX observations and analyses found the particles mainly contained Al, Si and Fe, with traces of P, S, Ca and Mn (Fig. 4A, B). The XRD analysis with Cr Kα radiation of the reddish brown sediments observed in thin section, identified amorphous materials with a broad 2.5 Å reflection, suggesting the presence of ferrihydrite (Fig. 6).

TEM observations of oil slicks floating around the terrace-like sediments revealed numerous colonizing microorganisms 1.5~2.0 μm long and 0.2~0.4 μm wide (Fig. 5).

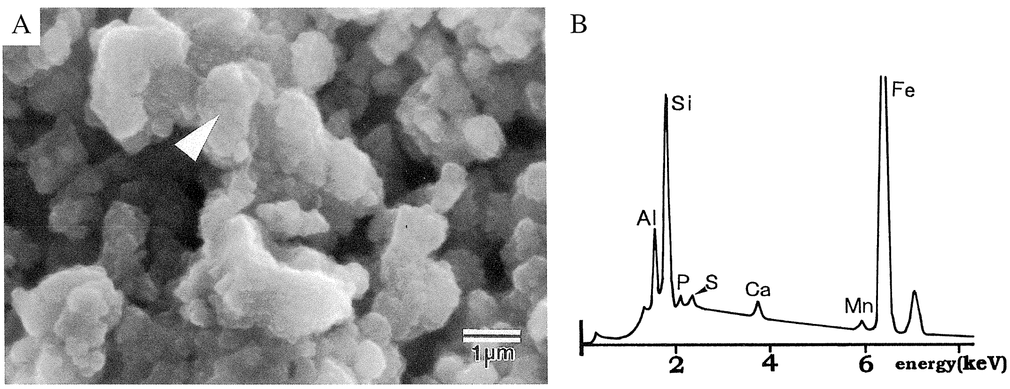

Fig. 4 SEM image of the surface of red brown terrace-like sediments (A), and the spectrum of EDX analysis (B).

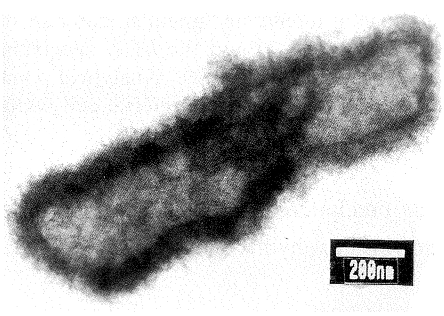

Fig. 5 TEM image of a microorganism recognized in the oil slick floating around terrace-like sediments.

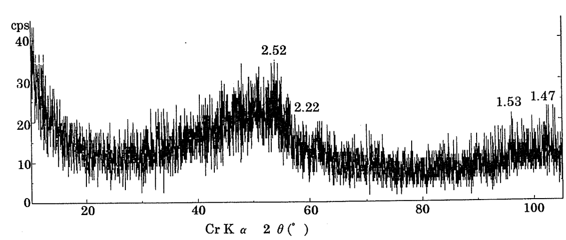

Fig. 6 XRD pattern of terrace-like sediments.

Reference

Ono, K., Soya, R. and Hosono, T. (1982). Geology of Satsuma-Iwo Jima district. Geological Survey of Japan, Tsukuba, p7-20.

4-22 Iheya Basin, Okinawa Trough

Locality

Okinawa Trough, Northwest of Iheya-Jima Island, Okinawa Prefecture
< N 27° 34.5´, E 127° 8.5´ >

Geology

Okinawa Trough is one of the back arc basins developed along the western part of the Eurasian Plate associated with subduction of the Philippine Sea Plate along the Ryukyu Trench. The Trough is thought to be in the initial rifting and spreading stage (Letouzey and Kimura, 1985).

Occurrence

Hydrothermal venting was observed at the Natsushima Seamound in the Iheya Basin of the Middle Okinawa Trough. The mound runs 25 m EW and 12 m NS, and rises about 5 m above the seafloor. It was located during a research dive of the "Shinkai 2000" (Uyeda, 1987; Kimura *et al.*, 1988). Black manganese oxide covers the mound and yellowish sediments are distributed along the ridge, suggesting that iron oxide precipitation has occurred along the venting fissure. The yellowish sediments are composed of iron oxyhydroxide, amorphous silica and nontronite (Masuda *et al.*, 1987).

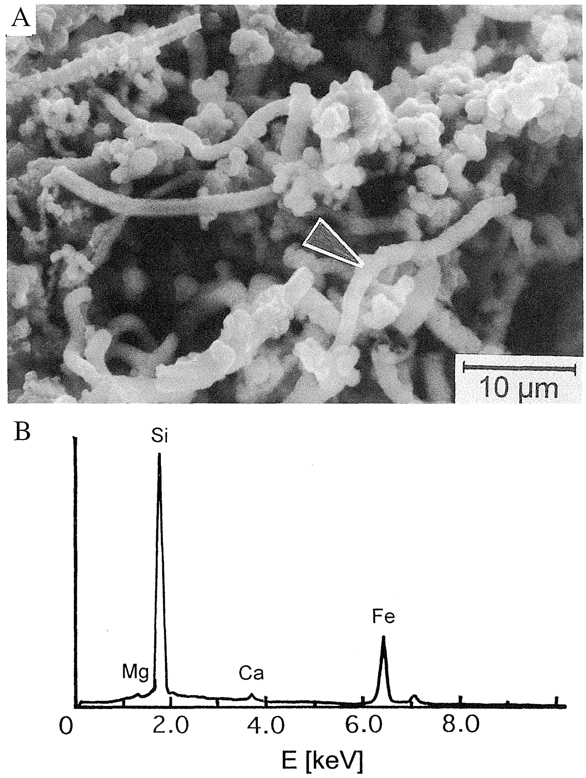

Fig. 1 SEM image of tubular and granular materials in Iheya sediments (A) and the EDX spectrum showing Si and Fe peaks (B). Analytical point is indicated by an arrow. Bacterial cell walls are covered with nontronite.

Water Chemistry

The temperature of the discharge water is 2~3 °C higher than that of ambient seawater. A 40 cm long thermometer inserted into the mound recorded temperatures ranging from 20 to 50 °C. Analysis of water showed a methane content of about 200 ml/kg (Kimura *et al.*, 1988).

Microbiology and Biomineralization

The yellowish sediments were analyzed by XRD and observed by SEM-EDX and TEM. Tubular and granular nontronite was identified (Fig. 1A). The sediments contained mainly Si and Fe (Fig. 1B). Tubular nontronite has also been collected from the vicinity of other deep sea smokers (Kohler *et al.*, 1994). Flake-like nontronite which presented bacterial morphologies was recognized from a sample of the sediments subjected to ultrasonic treatment for three minutes (Fig. 2).

TEM observations revealed tube-like materials uniformly coated with a film nontronite (Fig. 3). After ultrasonic treatment, bacterial colonies were observed (Fig. 4). The bacterial morphologies suggest that nontronite is formed due to biomineralization around bacterial cell walls in the deep sea sediments.

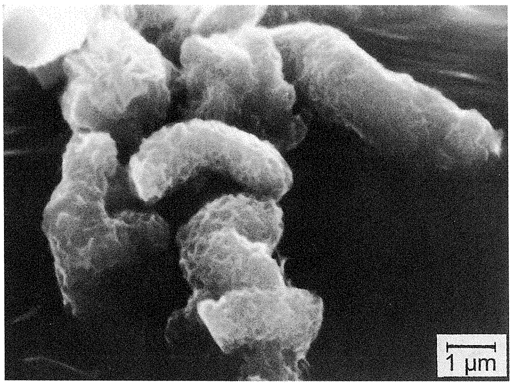

Fig. 2 Scanning electron micrograph of bacteria covered with nontronite in the Iheya sediments.

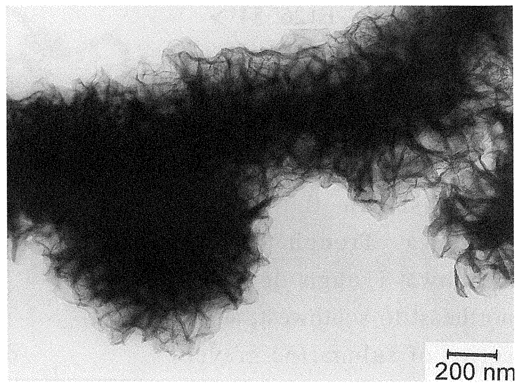

Fig. 3 Transmission electron micrograph showing filamentous bacteria covered with nontronite, Iheya sediments.

Fig. 4 Transmission electron micrograph of bacilli colony in the Iheya sediment after a 3 minutes ultrasonic treatment.

References

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Masuda, H. (1995). Iron-rich smectite formation in the hydrothermal sediment of Iheya basin, Okinawa trough. Biochemical Process and Ocean Flux in the Western Pacific, (eds.). Terra Scientific Publishing, Tokyo, p509-521.

Kohler, B., Singer, A. and Stoffers, P. (1994). Biogenic nontronite from marine white smoker chimneys. *Clays and Clay Minerals*, **42**, 689-701.

4-23 Seafloor Hydrothermal Systems (1), Iheya Ridge

Locality

North Knoll of Iheya Ridge, Okinawa Trough, Northwest of Iheya-Jima Island, Okinawa Prefecture

< N27° 47´, E126° 54´ >

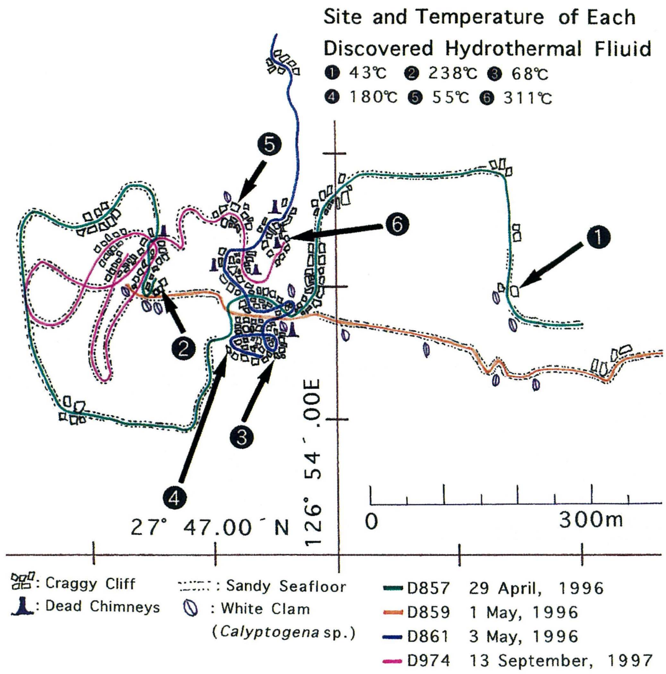

Fig. 1 Track lines and the results of observations of 1996 Dives (D 857, D 859 and D861) and 1997 Dive (D 974) by the "Shinkai 2000" (After Chiba *et al.*, 1996 and 1997).

Geology

Iheya Ridge is the most active area of submarine volcanism in the central Okinawa Trough. The Okinawa Trough develops northeast to southwest, and a series of submarine graven belts extend in a east-northeast to west-southwest direction from the axis of the trough. Intrusion of igneous rock ranging from basic to acid compositions has been recognized in the center of the trough. Iheya Ridge has the greatest volume of intrusion in the area. Research dives by the "Shinkai 2000", a Japanese submarine for scientific research, have revealed hydrothermal phenomena, distribution of new lava, and tectonic relief produced by active submarine volcanic activity at a depth of 950~1400 m on the axis of the Iheya Small Ridge. Hydrothermal deposits at Iheya Ridge are mainly manganese carbonate or iron-manganese oxide, and a small amount of sulphide has also been recognized (Tanaka *et al.*, 1990).

Conditions of Hydrothermal Venting

The hydrothermal emissions around Iheya Ridge are characterized by the clear color of the fluids, rather than black or white as observed elsewhere. Hydrothermal fluids have sometimes been observed to vent not from chimneys, but directly from fractures in surface deposits solidified by plantation, or from gentle mounds 1 m in height. In addition, the area of strong hydrothermal veining is very restricted (Tanaka *et al.*, 1990).

Water Chemistry

Until quite recently, the submarine hydrothermal systems at the CLAM site of the Small Iheya Ridge were the only sites known in the Iheya Sea area. Measured maximum temperatures of the fluids were 220 °C. However, during towing researches in 1995, large scale hydrothermal biological communities and hydrothermal fluid flows were recognized at North Knoll (950~1050 m depth), 30 km north of the CLAM site (Chiba *et al.*, 1996). Subsequently, three research dives by the "Shinkai 2000" were undertaken at North Knoll in April-May 1996, and another dive was made in September 1997. Six active "*clear smoker*" sites were discovered (Fig. 1). The maximum fluid temperature measured during the 1996 research dives was 238 °C (D 857, D 859 and D 861) (Fig. 2). Chemical compositions of the fluids indicate that they are similar to the hydrothermal fluids of the Izena and South-Ensei seafloor hydrothermal systems (Table 1). Accordingly hydrothermal systems at the North Knoll have characteristic chemical compositions of the Okinawa Trough (Chiba et at., 1996). Other hydrothermal fluids were sampled by the 1997 research dive (D 974). Measured maximum fluid temperature, pH, Eh and EC measurements are shown in Table 2 (measured by Ohmori, Chiba and Tawara, Sept. 1997). It is notable that the maximum fluid temperature of 311 °C is nearly equal to boiling point at the depth of the Iheya seafloor hydrothermal systems (Chiba *et al.*, 1997).

Chemical compositions of the fluids will be reported in the future.

Table 1 Chemical compositions of vent and ambient waters sampled by D 857 on 28 April, 1996 (After Chiba *et al.*, 1996).

Sample	Temp.	pH	Mg mM/Kg	Ca mM/Kg	Na mM/Kg	K mM/Kg	NH_4 mM/Kg	Ba μM/Kg	Mn μM/Kg	Cl mM/Kg	SO_4 mM/Kg	Si mM/Kg
D857 RV2	43	5.35	43.9	11	473	16.8	0.45	0	85	523	22.2	1.54
D857 RV3	43	5.29	45.9	11.6	493	17.3	0.57	0	89	538	22.7	1.46
D857 RV5	238		8.2	15.3	377	50.1	2.18	11.6	447	441	3.6	7.68
D857 RV6	238	4.61	8.5	13.8	377	49.9	1.87	10	440	453	0.5	7.53
D857 RV7	238	4.59	8.8	13.9	385	49.2	2.58	11	441	448	2	7.76

Table 2 Water analyses of vent and surface waters sampled by D 974 on 13 September 1997.

Sample	pH	WT(°C)	Eh(mV)	EC(mS/cm)
D974-3	5.2	55	-117	43
D974-4	5.0	55	-110	49
D974-5	5.0	55	-98	48
D974-6	5.0	55	-101	47
D974-7	4.9	311	-98	46
D974-8	4.9	311	-84	45
Surface			-109	41

Microbiology and Biomineralization

Both research dives in 1996 and 1997 recognized numerous dead chimneys at the North Knoll, which was the first discovery of chimneys on the Iheya Ridge. XRD results identify that these chimneys consist of sulphides, such as sphalerite, pyrite, chalcopyrite and also sulphur and barite. SEM observations recognized microbial colonies on the surface of sulphide minerals, which were found to contain significant sulphur (Fig. 3A, B).

TEM observations also recognized bacilli 1.0~1.5 μm long and 0.3 μm wide, absorbing numerous particles around the cell wall. Electron diffraction pattern obtained from the particles gave reflections corresponding to barite (Fig. 4). Further TEM observations of the 238 °C hydrothermal fluids also recognized bacilli 1.5~2.0 μm long and 0.2 μm wide, on the cell walls of which particles had congregated in a radiate growth pattern. Electron diffraction analysis of these particles also corresponded to barite (Fig. 5). Similar barite particles were also recognized in association with spherical or elliptical amorphous materials of nearly equal size during TEM observations of the 55 °C hydrothermal fluid (Fig. 6 A, B). However, TEM observations of the 311 °C hydrothermal fluid did not reveal any microorganism, and also identified barite in another crystal growth pattern (Fig. 7). These results suggest that the barite observed in the 311 °C fluid is the product of inorganic crystallization.

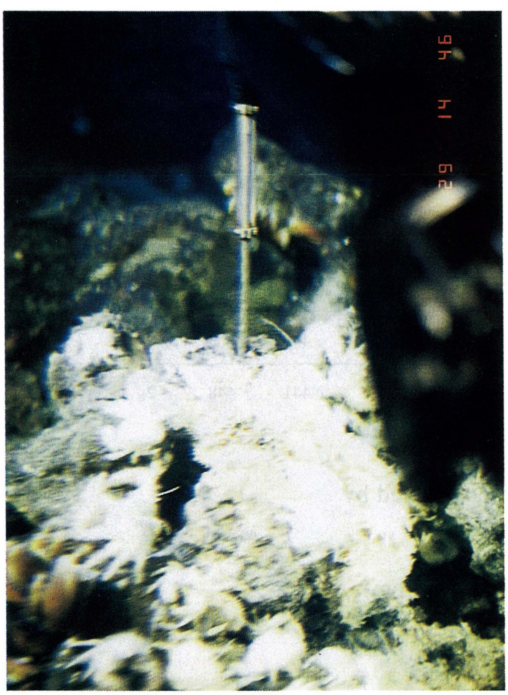

Fig. 2 Current clear smoker activity. Measured maximum fluid temperature was 238 °C, and the venting pattern of the hydrothermal fluid was diffuse flow (Photographed by Hitoshi Chiba, 29 April, 1996).

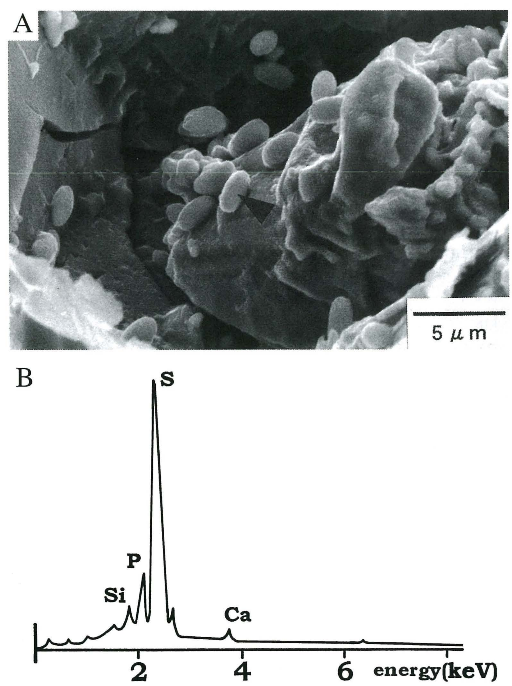

Fig. 3 SEM image of a microbial colony on the surface of a dead chimney (A). EDX spectrum of the microorganism (B).

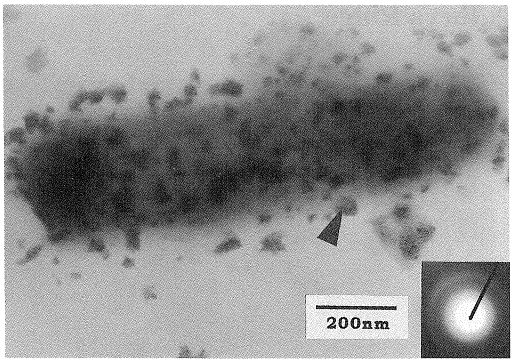

Fig. 4 TEM image of bacillus absorbing numerous particles around the cell wall, and particle electron diffraction pattern. The analytical point is shown by an arrow. The particles around the cell wall were identified as barite by the electron diffraction pattern.

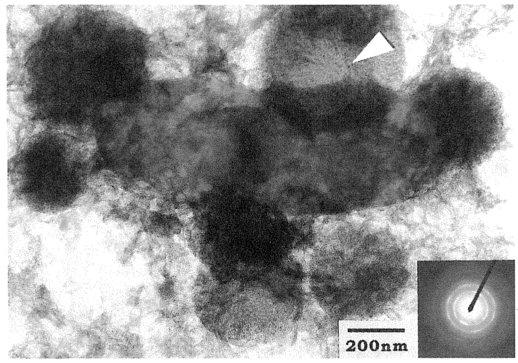

Fig. 5 TEM image of bacillus in the 238 °C hydrothermal fluid, and electron diffraction pattern of a radiate crystal. The analytical point is arrowed. Electron diffraction patterns show these particles are barite.

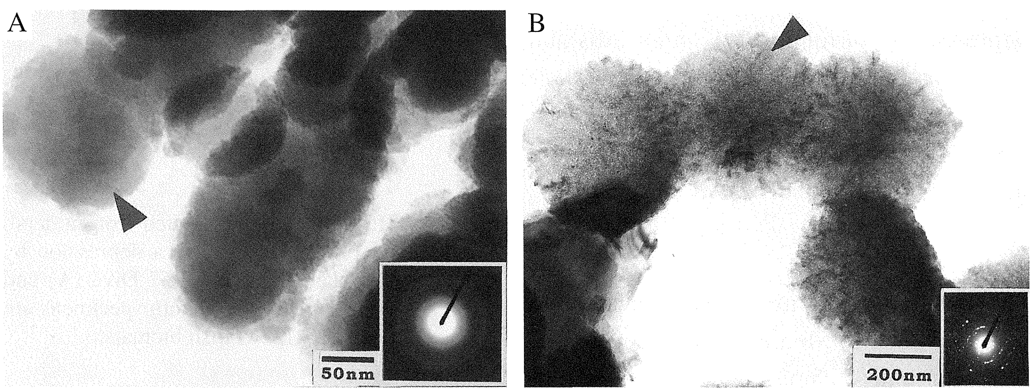

Fig. 6 TEM image of amorphous materials in the 55 °C hydrothermal fluid and electron diffraction pattern of the material (A). The analytical point is shown by an arrow. The size of the material is almost the same as that of the radiate crystal growth recognized as barite in the 238 °C fluid. The amorphous particles illustrated were also recognized as barite by electron diffraction patterns (B).

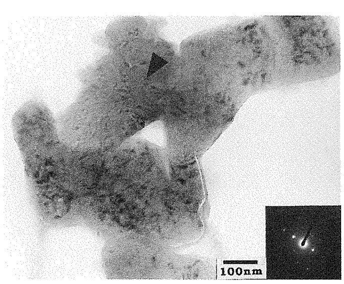

Fig. 7 TEM image and electron diffraction pattern of barite crystallizing inorganically in the hydrothermal fluid of 311 °C.

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4-24 Seafloor Hydrothermal Systems (2), Iheya Ridge

Locality

North Knoll of Iheya Ridge, Okinawa Trough, Northwest of Iheya-Jima Islands, Okinawa Prefecture

< N 27° 47′, E126° 54′ >

Geology

Okinawa Trough is located in the back arc side of the Ryukyu Trench. The Okinawa Trough is in the first stage of back arc spreading and features active volcanism. At Iheya depression in the middle Okinawa Trough, many hydrothermal vents have been reported. The sea floor in the Iheya depression is surrounded by small hills and pillow basalt and breccias crop out on their surface (Izawa *et al.*, 1991). Hydrothermal carbonate chimneys in the Iheya depression have also been reported.

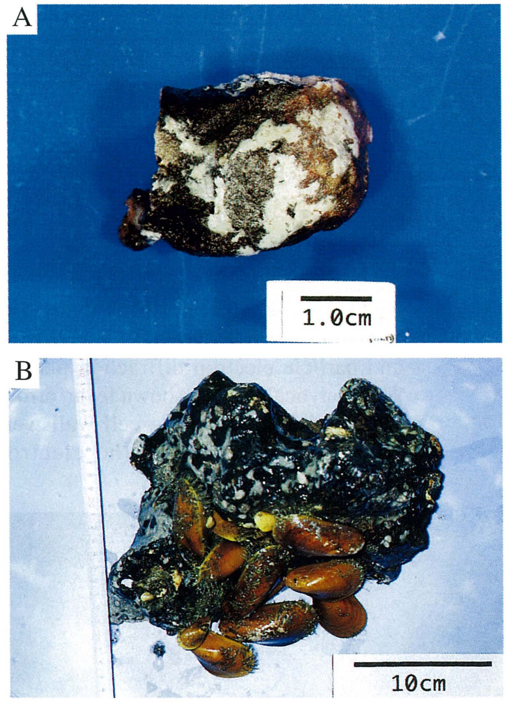

Fig. 1 Rock specimen collected from a depth of 1000 m in the Iheya depression by "Shinkai 2000" No. 857 Dive (A) and No. 974 Dive (B). Both specimens are covered with whitish biomats.

Occurrence

Rocks covered with whitish biomats were collected by the "Shinkai 2000" submersible from near a dead chimney site that vented the 43 °C hydrothermal fluid during No. 857 Dive and the 311 °C fluid from the No. 974 Dive (Fig. 1A, B). The No. 857 Dive reported whitish biomats spread over the seafloor near the hydrothermal vents (Chiba *et al.*, 1996).

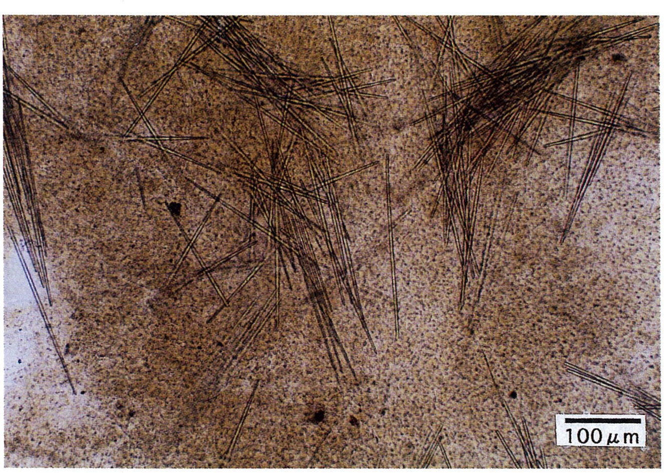

Fig. 2
Optical micrograph of whitish biomats. Numerous needle-like materials occur.

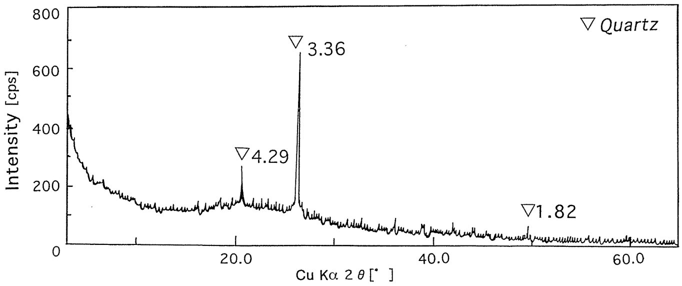

Fig. 3
XRD pattern of whitish biomats, strong reflections are characteristic of quartz.

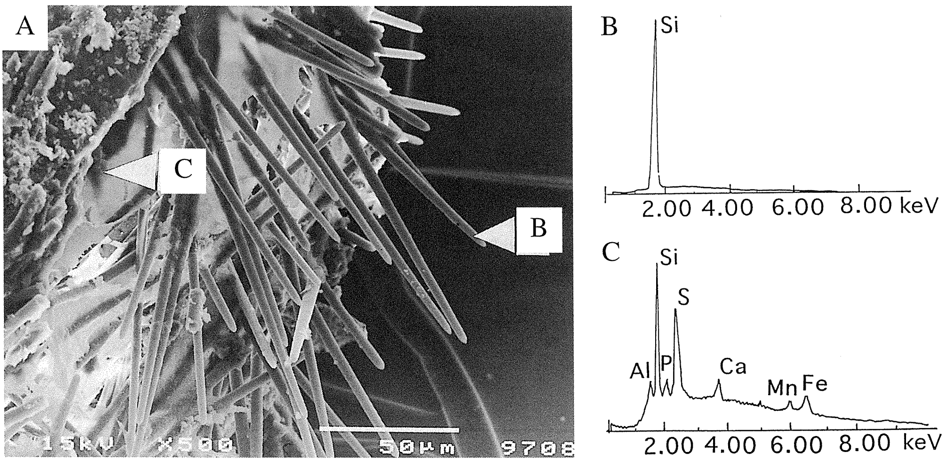

Fig. 4 SEM image (A) and EDX spectrum (B, C) of the needle like quartz crystals covered with thin organic films.

Microbiology and Biomineralization

Optical microscope observations found numerous needle-like materials and microbes both on the surface and within the whitish biomats (Fig. 2). The biomat XRD pattern showed strong 4.29, 3.36 and 1.82 Å reflections (Fig. 3). These results suggest that the needle-like materials are quartz crystals. SEM observations revealed that needle-like quartz crystals were covered with thin film microbial colonies (Fig. 4A, B, C).

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5. Conclusions

This introduction to biomats through 24 case studies has described the natural occurrence of biominerals in biomats in differing geological, chemical, or biological conditions, in order to better understand the role of bacteria in mineral nucleation and crystallization.

Biominerals are synthesized in complex bio-mediated processes. Amorphous iron oxides plus goethite, lepidocrocite and ferrihydrite are synthesized simultaneously. Such phenomena are commonly observed in reddish brown mats existing in river water and in mining areas, associated with *Gallionella ferruginea*. Green cyanobacterial mats produce various calcite morphologies at warm temperatures under photosynthetic conditions. Mn oxides are produced in black mats in hot springs. Sulfides and sulfur in white or yellow mats are readily formed through bacterial activity in hot springs. In the case of Hirayu Hot Springs, the color of biomats clearly exhibit a specific pH-Eh-Temp. dependent association.

In the geo-, aqua-, and eco-systems, biomats play a key role in remediation of concentrations of toxic heavy metals such as Cu, Ni, Pb, Zn and Cd, in polluted mining sites.

Bacteria play an important role in the various processes of concentration, crystallization, transportation and sedimentation of almost all elements in the environment.

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