Sr-rich microbial mats at Zhemchug hot springs, southwest Lake Baikal, Russia

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Abstract : At Zhemchug hot springs, southwest Lake Baikal, Russia, the biomats contain high concentration of Ca and Sr, which microbiological, chemical and mineralogical analyses were carried. Optical and scanning electron microscopic observation revealed that carbonate minerals were associated with cyanobacteria. The biomats have colorful layered structure, such as green, white, brown and black. Chemical analysis of the biomats revealed high concentration of CaO (85-96 wt%) and SrO (1.1-2.2 wt%) in the four layers. The mineralogical composition of the layered structure is mainly aragonite associated with calcite. Elemental layered patterns of the biomats are shown in EPMA elemental content maps indicating the coincidence of Ca and Sr. By increasing pH and the oxygen concentration on the surface of biomats, the cyanobacteria could potentially increase the rate of metal accumulation. The cyanobacteria in hot spring biomats may carry important role to accumulate Ca with Sr.

Key words : hot springs, biomats, cyanobacteria, Sr, Ca, aragonite

1. Introduction

Hot springs are one of the main important and interesting objects in studying of processes of metal accumulation. In the hot springs, biomineralization has progressed, because of high temperature and large amount of nutrients. Biomats in hot springs often have banded structure and consist of calcium carbonate with high concentration of heavy metals (Yasuda et al., 2000 ; Ohno and Tazaki, 2000 ; Akai, 2000). Chemical and biological diversity of biomats influence water ecosystem (Tazaki, 2000). Microbiological variety in hot springs also influenced water chemistry and formed different biominerals (Ohno and Tazaki, 2000). Microorganisms selectively accumulated heavy metals in mining waste water system (Tazaki, 2000). In the accumulation processes, various groups of living organisms are usually involved. Diatoms can precipitate silicon on their cells walls, whereas bacteria can accumulate Fe, Mn, Sr, and some other elements in/on the cells

(Beveridge, 1989).

In this study, Sr-rich microbial mats with cyanobacteria were found at Zhemchug hot springs, southwest Lake Baikal, Russia. Observation of the biomats by using optical and scanning electron microscope, ED-XRF, XRD, NCS and EPMA micro techniques clearly showed layered Ca-Sr biomineral formation.

2. Materials and Methods

Biomats and hot spring water were collected from Zhemchug hot springs in July 2001, southwest of Irkutsk, Russia (Fig. 1 A, B). The characteristics (pH, Eh, EC, DO and WT) of hot spring water were measured at the field (Table 1) (Eh : Electrode potential versus the standard hydrogen electrode, EC : Electrical conductivity, DO : Dissolved oxygen,

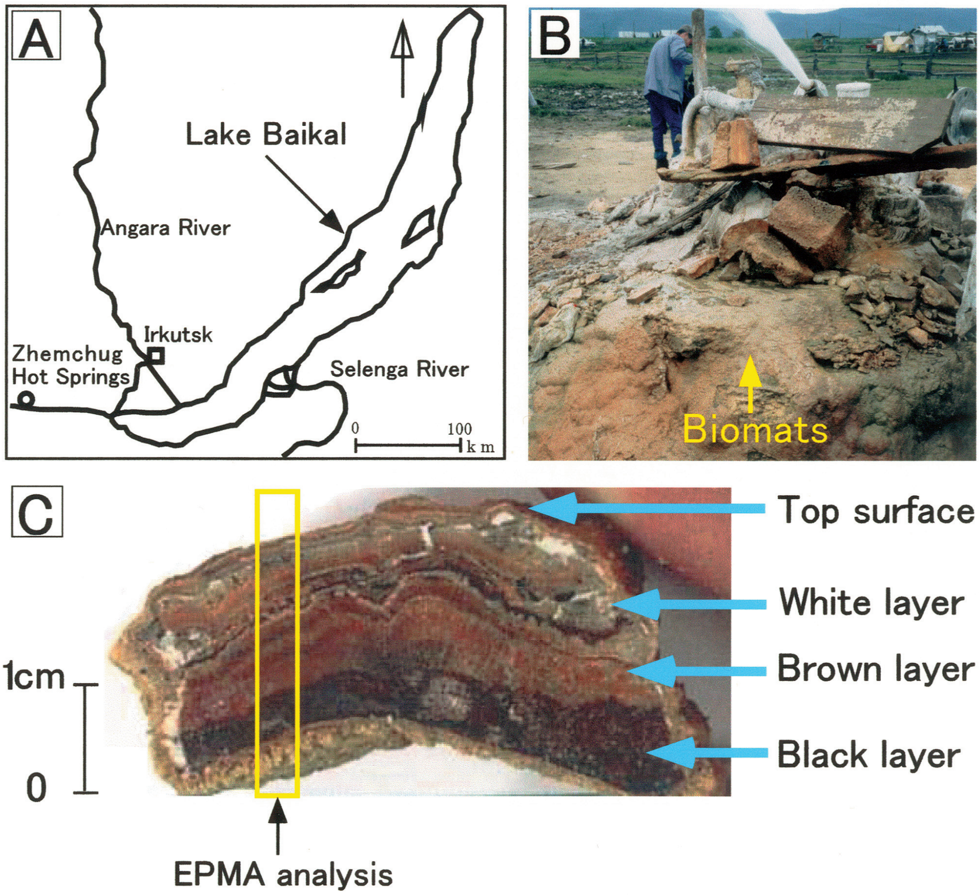

Fig. 1. Location map of sampling points at Zhemchug hot springs, southwest Lake Baikal, Russia (A). Field view of biomats at Zhemchug hot springs (B). Cross section of the biomats (C). In biomats 4 layers can be clearly distinguished according to different colors, such as green, white, brown and black layers from the top surface in order.

Table 1. Water characteristics at Zhemchug hot springs, southwest Lake Baikal, Russia.

Measurements	pH	Eh(mV)	EC(mS/cm)	DO(mg/l)	WT(℃)
Field	7.0	36	0.67	3.5	52.0
4 months later	9.3	139	5.31	6.8	26.0

WT : Water temperature). One liter of hot spring water was filtrated through bacterial 0.22 μm filter (Millipore) with peristaltic pump to determine bacteria in hot spring water. The biomat samples were incubated in hot spring water at room temperature during 4 months at light conditions. The measurements of water characteristics were also carried after 4 months storage (Table 1).

Biomats have layered structure, which were separated into 4 layers due to differentiation in color (Fig. 1 C). The chemical composition in each layer and mineralogy were analyzed. The polished thin section of biomats was prepared for optical microscopic observation and EPMA analysis. Furthermore, optical microscopy and SEM-EDX techniques were used for observation of green biomats on top surface.

2.1. Optical microscopy observation of biomats and bacteria

Green biomats on top surface and microorganisms collected on the filter (pore size : 0.22 μm) were stained with 4', 6-diamidino-2-phenilindole (DAPI, 50μg/ml) for 3 minutes to observe under epifluorescent microscope (Nikon EFD 3, Digital camera : COOLPIX 995). For epifluorescent microscopy, a filter UV-1 A (wavelength of exposed light : 360-370 nm) was used for DAPI-staining observation and a filter G-2 A (wavelength of exposed light : 510-560 nm) for observation of chlorophyll in cyanobacterial cells. The surface of biomats was observed twice, within 1 week after collection of samples and after 4 months storage. The polished thin section of biomats were examined using a polarizing optical microscope (Nikon UFX-Ⅱ A) and EPMA.

2.2. Energy dispersive X-ray fluorescence spectrometer analysis of biomats

After air-drying, biomat samples were ground to fine powder for ED-XRF analysis. The powder samples mounted on the Mylar film measured by an energy dispersive X-ray fluorescence spectrometer (JEOL JSX 3201 using Rh Kα), which operated at an accelerating voltage of 30 kV under a vacuum condition.

2.3. X-ray powder diffraction analysis of biomats

The mineralogical properties of biomats were analyzed by X-ray powder diffractometer (Rigaku RINT 2000) with Cu Kα generated at 40 kV and 30 mA using 2 θ/θ method and a scan speed 2°/min. The powder of each sample was mounted onto slide glasses to fit the diffractometer sample-holder.

2.4. Nitrogen, carbon and sulfur elemental analyses of biomats

By an automatic gas chromatographic elemental analyzer (CE Instruments NA 2500-NCS) at 1000℃ with 20 ml oxygen contents of carbon, sulfur and nitrogen of biomats were determined. All powder samples were analyzed after treatment with HCl.

2.5. Electron probe microanalysis of biomats

Polished thin section of biomats was examined by electron probe microanalysis (EPMA : JEOL JXA 8800 R Super Probe) at an accelerating voltage of 15 kV for elemental content maps and quantitative analysis of major elements.

2.6. Scanning electron microscope (SEM) observation of biomats

Green biomats were micromorphologicaly investigated using a scanning electron microscope (SEM : JEOL-JSM-5200 LV), equipped with an energy dispersive X-ray spectrometer (Philips-EDAX PV 9800 STD).

3. Results

Water chemistry

Field measurements of hot spring water revealed anaerobic condition : pH 7.0, Eh 36 mV, EC 0.67 mS/cm, and DO 3.5 mg/l. After 4 months storage, pH increased till 9.3 and DO also increased twice as (6.8 mg/l) indicating microbial oxygenic activity (Table 1).

3.1. Optical microscopic observation

The observation of green top surface of biomats revealed that different types of bacteria live in aggregates, and in free-living forms (Fig. 2 A, B). Coccus typed bacterial cells, 5 μm in size, similar to *Anabaena* species cells, aggregated together with bacterial cells and mineral particles were clearly distinguished. In the hot spring water, only small sized bacterial cells (filtrated through 0.22 μm) aggregates in free-living form were found (Fig. 2 C, D). Numerous community of chains of *Anabaena*-like cells, filaments, other species of bacteria and mineral particles (Fig. 3 A, B and C) were shown by optical microscopy of green biomats after 4 months storage.

Observation of thin sectioned biomats showed that the layered structures were separated into four sections due to not only by color, but also by structure of minerals (Fig. 4). The top surface showed colloform structure. White layer was reach in chink, whereas brown and black layers compacted with radial growth structure were found. Black layer has well-crystallized banded structure. Underneath the black layer, porous carbonate grains are present.

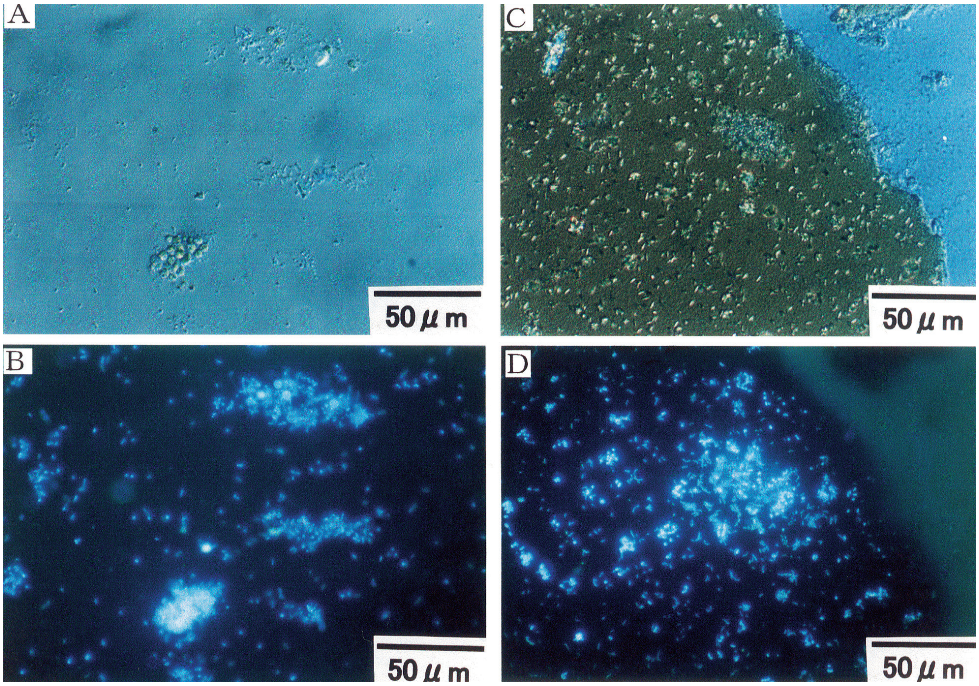

Fig. 2. Optical micrographs of green top surface of biomats (A ; light, B ; epifluorescent) and bacteria, collected on 0.22 μm filter (C ; light, D ; epifluorescent) from hot spring water. All micrographs were taken within 1 week after sampling.

3.2. ED-XRF analyses of biomats and water

The ED-XRF analysis of biomats showed the presence of CaO, Fe_2O_3, SiO_2, and SrO with the traces of MgO, PO_4, SO_4, Al_2O_3, and MnO in biomats (Table 2). The content of CaO (88.88 wt%) in biomats is very high, especially top surface in green and brown layers contain more than 90 wt% of CaO. Analysis of hot spring water showed large amount of CaO (30.42 wt%) and Fe_2O_3 (23.33 wt%). Such elements as Na_2O, MgO and SiO_2 in the hot spring water were also found in higher concentration than in biomats. Although the elements of K_2O and SrO were found as trace elements in the water, but other elements of PO_4, Al_2O_3, SO_4, MnO_2 and As were not detected. Concentration of SrO on top surface of green biomats (1.11 wt%) was five times as high as in hot spring water (0.27 wt%) suggesting selective accumulation of SrO in biomats.

3.3. XRD analysis

The XRD patterns of all sections in biomats showed the same tendency. The strong aragonite peaks were found at 3.4Å, 2.7Å, 2.0Å in all layers (Fig. 5). The small peaks at 3.0Å, 2.3Å, and 1.9Å in white layer of biomats also showed the presence of calcite. Any SrO minerals were not detected at whole biomats.

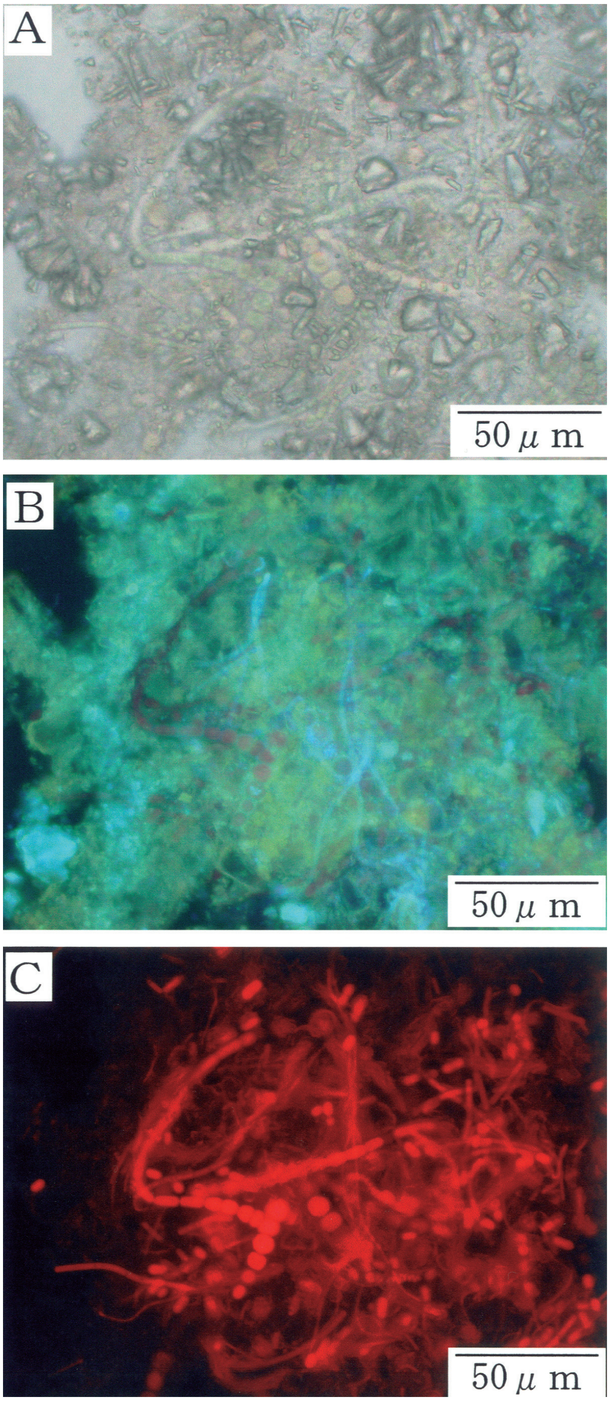

Fig. 3. Micrographs of green biomats on top surface (A ; light, B ; epifluorescent with UV-1 A filter set, C ; epifluorescent with G-2 A filter set) showing cyanobacterial colony with carbonate grains. The observations were carried after 4 months storage at room temperature.

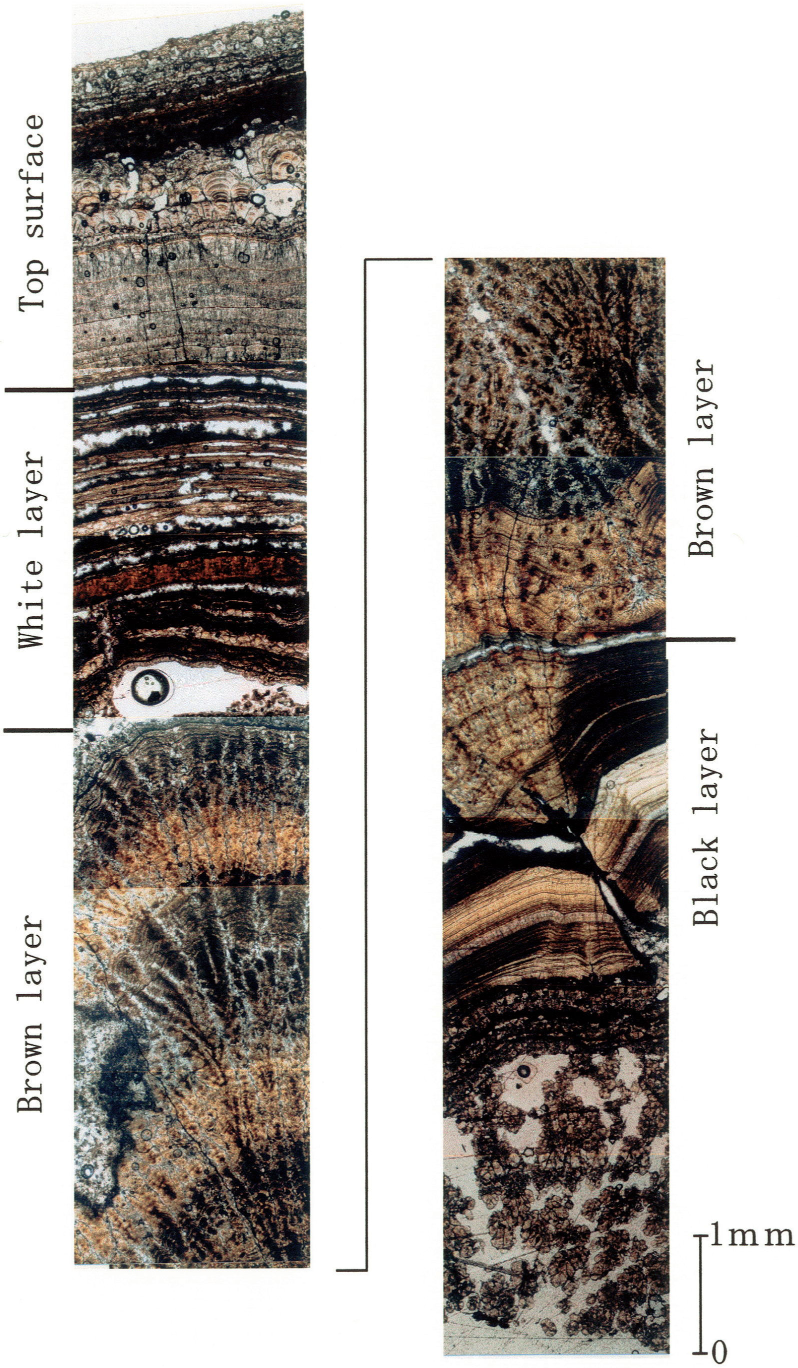

Fig. 4. Optical micrographs of polished thin section of biomats at Zhemchug hot springs. The layered structures were separated into four sections due to not only by color, but also by structure of minerals.

Table 2. Energy dispersive X-ray fluorescence spectrometer analyses of biomats and water at Zhemchug hot springs.

	Biomats (wt %)					Water
	Bulk	Top surface	White layer	Brown layer	Black layer	
Na_2O	nd	nd	nd	nd	nd	9.59
MgO	0.13	0.18	0.33	0.12	0.05	9.05
Al_2O_3	0.10	0.06	0.15	0.15	0.01	nd
SiO_2	3.59	0.76	4.12	2.00	2.33	25.73
PO_4	0.30	0.25	0.31	0.34	0.37	nd
SO_4	0.18	0.13	0.17	0.10	0.15	nd
K_2O	nd	nd	nd	nd	nd	1.60
CaO	88.88	96.10	87.44	90.03	85.44	30.42
MnO	0.09	0.03	0.07	0.07	0.11	nd
Fe_2O_3	5.10	1.38	5.20	4.98	9.92	23.33
As	nd	0.004	0.005	0.010	0.005	nd
SrO	1.64	1.11	2.21	2.21	1.61	0.27

nd: not detected.

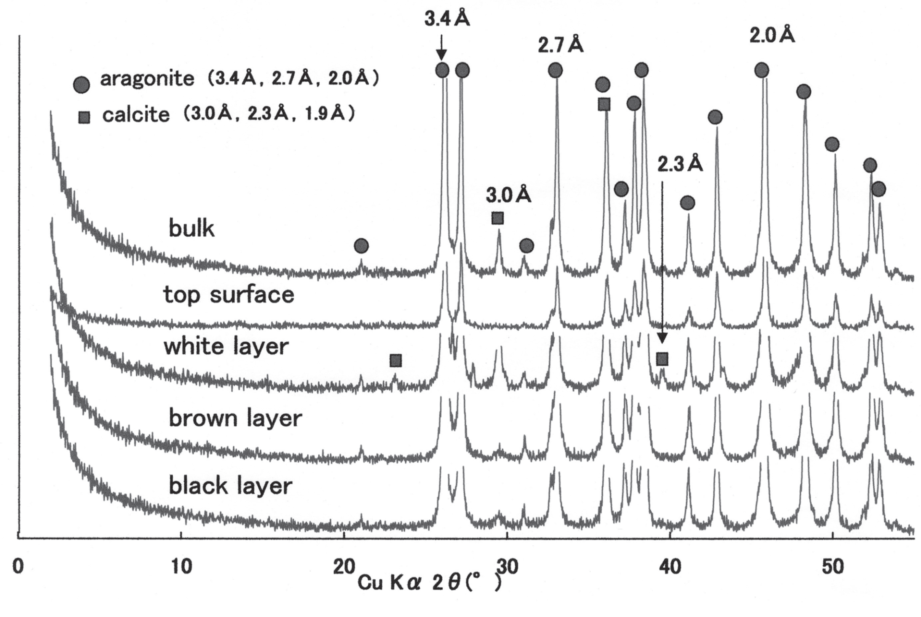

Fig. 5. X-ray powder diffraction of biomats at Zhemchug hot springs, indicating mainly aragonite with calcite.

3.4. NCS analysis

NCS elementary analysis of four layered biomats revealed that the tendency is similar in carbon, nitrogen and sulfur contents (Table 3). The concentration of elements ranged as carbon from 0.55 to 0.75 wt%, nitrogen from 0.06 to 0.14 wt% and sulfur from 0.14 to 0.17 wt%. In white layer, amount of carbon and sulfur were relatively low concentration suggesting less organic materials, according to association of calcite with aragonite.

Table 3. Elemental analysis of carbon, nitrogen and sulfur in biomats at Zhemchug hot springs.

	C (wt %)	N (wt %)	S (wt %)
Top surface	0.75	0.06	0.17
White layer	0.55	0.08	0.14
Brown layer	0.68	0.07	0.15
Black layer	0.68	0.14	0.14

3.5. EPMA analysis

EPMA analysis of biomats shows compositional banded structure of Ca, Sr, Fe and Mg (Fig. 6). Although Sr and Fe are associated with Ca, the concentration of Mg shows

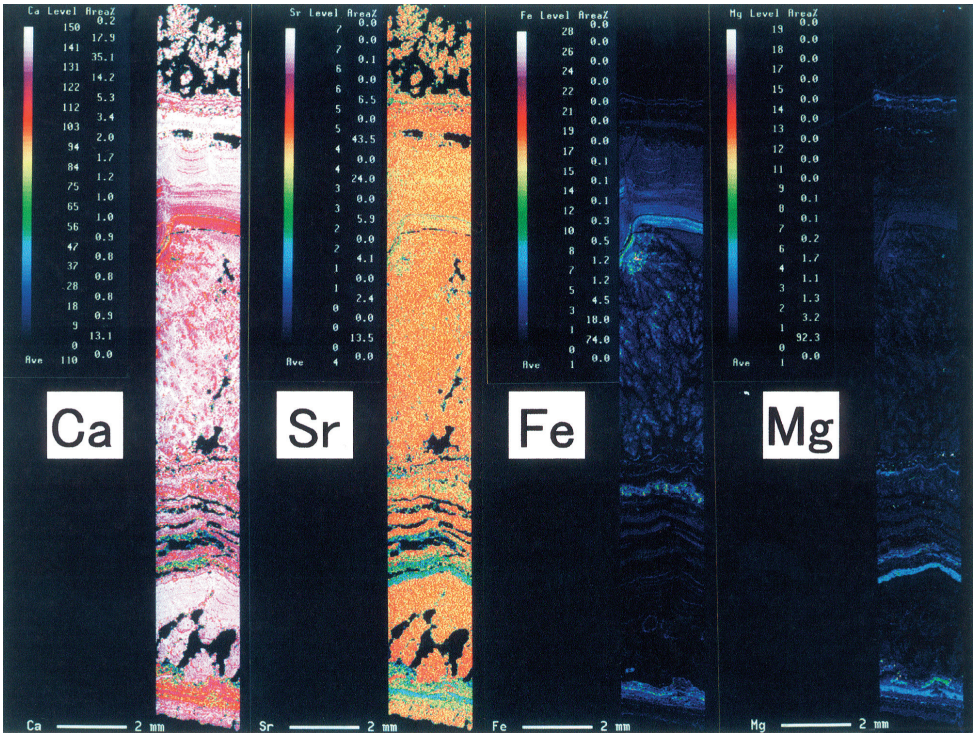

Fig. 6. Electron microprobe elemental content maps of thin sectioned biomats at Zhemchug hot springs. Elemental distribution maps of Ca, Sr, Fe and Mg indicated chemical layered structures. The Sr-rich layers are consistent with Ca-rich layers.

Table 4. Electron microprobe quantitative analysis of 4 different layers of biomats at Zhemchug hot springs, southwest Lake Baikal, Russia.

(wt %)	Layer 1	Layer 2	Layer 3	Layer 4
SrO	1.36	1.55	1.39	0.64
CaO	52.46	52.34	52.25	49.59
Fe_2O_3	1.64	1.71	1.48	0.99
MgO	0.17	0.16	0.13	3.34
MnO	0.00	0.07	0.02	0.00

reverse pattern of Ca. Quantitative analysis of the layered biomats produces a profile of elemental concentrations (Table 4). Layer 1, 2 and 3 of biomats showed the similar chemical composition of SrO, CaO, Fe_2O_3, MgO, and MnO. The concentration of CaO ranged from 52.25 to 52.46 wt%, and SrO from 1.36-1.55 wt%. While, in the layer 4 the concentrations of SrO, CaO and Fe_2O_3 were lower than in the other layers. Only the concentration of MgO was relatively high suggesting mixture of dolomite. The results of EPMA analysis are consistent with that of ED-XRF (Table 2), showing SrO concentration ranges from 1.11 to 2.21 wt%.

3.6. SEM observation

SEM observation of green biomats on top surface revealed that the biomats were composed of Ca grains, Si grains and filamentous bacteria (Fig.7 A, B and C). Ca grains (arrow A in Fig. 7) is about 5 μm in size, Si grains (arrow B in Fig. 7) is about 2 μm in size whereas filamentous bacteria are about 1 μm in diameter (arrow C in Fig. 7). The EDX analysis revealed that bacteria contain high concentration of Si, Cl and Ca with traces of P and S characteristically (analytical point: arrow C in Fig. 7). The Si grain contains relatively high Fe, whereas the bacteria have no Fe at all. The concentration of Sr was not detected because of detection limits of EDX.

4. Discussion

Formation of periodic alternations of iron, manganese and silica-rich strata are well known from the Archean, as represent by Banded Iron Formation (Fortin et al., 1998). In this study, accumulation of SrO and CaO in layered biomats were found in Zhemchug hot springs, southwest Lake Baikal, Russia, essentially result from bacterial activity. The biomats at Zhemchug hot springs composed of mainly aragonite. Observations of Sr-rich microbial mats described here suggest that cyanobacteria produce structure by periodic biomineralization of aragonite. Calcite is also formed by fibrous bacterial activity in hot springs has reported (Yasuda et al., 2000 ; Ohno and Tazaki, 2000). The specially defined pattern of these elements showed layer structure in different color, such as white, brown and black. Various bacteria, including coccoid, rod and filamentous forms, concentrate Si, Cl and Ca on the cell walls during their lives, and accumulate additional hydroxides to

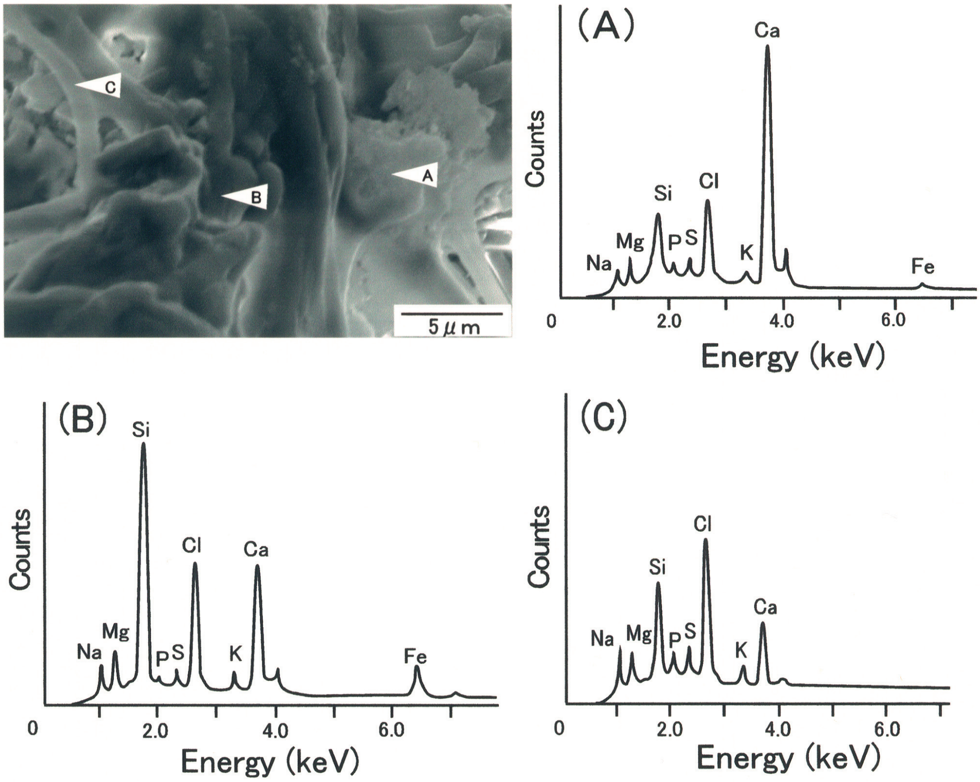

Fig.7. SEM micrograph and the EDX analysis of green biomats on top surface at Zhemchug hot springs. (arrow A : carbonate grain, B : silicate grain, C : bacteria, (A) : EDX spectrum of carbonate grain, (B) : silicate grain, (C) : bacteria).

form aragonite. Aragonite in hot springs contains higher concentration of Sr in comparison with calcite (Ichikuni, 1980). In this study, it was revealed that the Zhemchug hot spring biomats was composed of aragonite with high concentration of Sr. The EPMA content maps and electron micrographs of layers suggested that cyanobacteria grew rapidly and accumulated Sr from hot spring water at the initial stages of the microbial process. Bacteria in the biomats absorb abundant Sr in high crystalline aragonite to form layered structure within short period. By increasing the pH and the oxygen concentration in the surface sediments, the cyanobacteria could potentially increase the rate of iron oxidation *in situ* (Pierson et al., 1999). The Zhemchug hot spring biomats are suitable models for studying the interactions among photosynthesis microorganisms, metal accumulation and the cycle of microbial Sr.

The results indicate that biomineralization can be applied to bioremediation of radioactive elements. Furthermore, the biomats can be used for 3 R purposes, as follows ; Recovery of clean water, Resource of useful bacteria and Recycle of Sr-rich aragonite.

5. Conclusion

The biomats at Zhemchug hot springs, southwest Lake Baikal, Russia was composed of mainly high crystallin aragonite with high concentration of Sr. The EPMA maps show the elemental layered structures. The Sr-rich biomas was formed by cyanobacterial activity.

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