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Influence of Microorganisms on the Formation of Imogolite in Daisen Kurayoshi Pumice — Occurrence of Bio-Imogolite —

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Abstract - Numerous microorganisms have been found on the surface of imogolite films collected from Daisen Kurayoshi Pumice in Daisen tephras located in Syuki, Kurayoshi, Tottori, Japan. The imogolite contains relatively high concentration of nitrogen, carbon and sulfur suggesting presence of microorganisms and adsorption of organics. Electron microscope observation showed imogolite-like network structure existing abundant microorganisms on the surface of imogolite. The microorganisms were covered with poorly-ordered fine particles. Occurrence of bio-imogolite was described here.

I Introduction

Microorganisms play an important role in the concentration, crystallization, transportation and sedimentation of almost all elements in the Earth [1]. Recently, it is reported that microorganisms affect the formation of clay minerals. For example, bio-mineralization of poorly-ordered Al-Si-Fe minerals has been observed in weathered pyroclastic deposits [2]. In laboratory experiment, kaolinite has been formed on bio-film [3].

Imogolite of clay minerals is paracrystalline mineral and commonly found in volcanic ash. It often formed gelatinous film in weathered pumice [4]. Fine particles like amorphous and paracrystalline mineral play an important role for the various geological phenomenon on the earth because they have high surface activity [5].

Gelatinous film of imogolite is found in Daisen Kurayoshi Pumice located in Kurayoshi, Tottori, Japan. The imogolite have been studied chemically, physically and mineralogically [6][7]. But, there are few reports about relation between the formation of imogolite and microorganisms.

In this study, the surface of imogolite films in Daisen Kurayoshi Pumice was observed by electron microscope, showing numerous microorganisms inhabit on imogolite films.

II. Materials

Imogolite was collected from Daisen Kurayoshi Pumice in Daisen tephras, which erupted about 50,000 years ago.

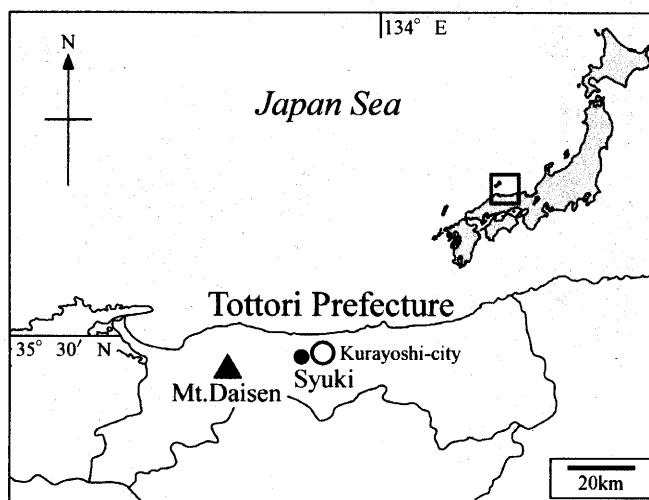


Fig. 1. Location map of Mt. Daisen and sampling point in Syuki near Kurayoshi city.

Sampling point locates in Syuki, Kurayoshi, Tottori, Japan (Fig. 1). It is reported that film-like and tubular imogolite, 1 ~ 10 mm in size, was developed in the pumices [7]. The pumices were collected about 10 cm below the surface of the outcrop, and immediately sealed in a plastic bag to avoid biological contamination and water loss by evaporation. After sampling, film-like and tubular imogolite was collected from the pumices by hand picking in laboratory, then gently rinsed with distilled water to remove adhering fragments.

III. Experimental Methods

X-ray diffraction (XRD) analysis was conducted using a RIGAKU RINT 2000 diffractometer (CuK α radiation, 40 kV, 30 mA). Imogolite was lightly crushed in a mortar and pestle under the wet condition, then the slurry was suspended in distilled water and deposited as oriented mounts onto glass slide. Specimen treated with ethylene glycol (E.G.) was also analyzed in order to identify 14 Å peak.

Imogolite was air dried and ground to fine powder in order to determine chemical composition. The powder sample mounted on the Mylar film was analyzed by an

energy dispersive X-ray fluorescence spectrometer (ED-XRF: JEOL JSX 3201 using Rh K α), which operated at an accelerating voltage of 30 kV under a vacuum condition. For comparison, ED-XRF analysis was also conducted for pumice.

Contents of nitrogen, carbon and sulfur in imogolite were determined by an automatic gas chromatographic elemental analyzer (CE Instruments NA 2500-NCS) at 1000 °C with 20 ml oxygen. For comparison, NCS concentration of pumice was also analyzed.

Imogolite was inspected using optical microscope to identify the presence of microorganisms. The sample was lightly crushed in a mortar and pestle under the wet condition, then the slurry was suspended in distilled water and a drop was mounted on glass slide. The sample was stained with 4',6-diamidino-2-phenylindole (DAPI, 50 μ g/ml) to observe under epifluorescent microscope (Nikon EFD 3). A filter UV-1 A (wavelength of exposed light: 360-370 nm) was used for the epifluorescent microscopic observation.

A scanning electron microscope equipped with an energy dispersive X-ray spectroscopy (SEM-EDS: JEOL JSM-5200 LV and PHILLIPS EDAX PV 9800EX) was used in order to observe the micro-morphological surface of imogolite and analyze its chemical composition. Especially, distribution of microorganisms was observed. Freeze dried imogolite was mounted on brazen stub and then carbon coated.

Bacterial mineralization was examined by transmission electron microscopy (TEM) using a JEOL JEM 2000EX microscope operated at an accelerating voltage of 120 kV. The moist sample was lightly crushed in a mortar and pestle, then the slurry was dispersed ultrasonically in distilled water and a drop was mounted on a Cu grid.

IV. Results and Discussion

A. X-ray powder diffraction analysis

The XRD profile included broad diffraction peaks at 14.0, 8.2, 5.5, 3.3 and 2.25 Å (Fig. 2). The 14.0 Å peak didn't shift by E.G. treatment. Therefore, the sample was confirmed as imogolite. The peaks of feldspars (3.89, 3.75, 3.18 and 3.13 Å) and amphibole (8.4 Å) were also found, adhering fragments which were not removed by rinse with distilled water.

B. Energy dispersive X-ray fluorescence spectrometer analysis

ED-XRF analysis showed the presence of Al₂O₃, SiO₂ and Fe₂O₃ with traces of P₂O₅, K₂O, CaO, TiO₂ and MnO in both imogolite and pumice (TABLE I). Trace Sr was detected only in pumice. Although generally SiO₂/Al₂O₃ molar ratio of imogolite is about 1, that of imogolite in this study showed 1.9 because of adhering fragments, such as feldspars, amphibole and volcanic glass.

C. Nitrogen, carbon and sulfur elemental analysis

NCS elementary analysis revealed that imogolite contains higher concentration of nitrogen, carbon and sulfur than that of pumice (TABLE II). Carbon concentration of imogolite is about twelve times as many as that of pumice. Moreover sulfur is about four times in imogolite. Nitrogen is detected only in imogolite. The results indicate that imogolite has relatively high amount of organic matter.

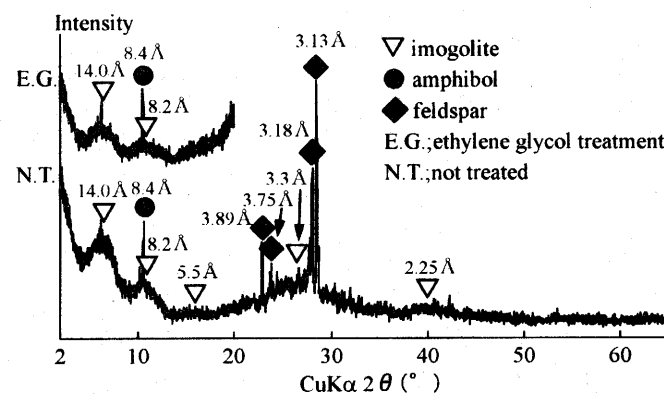


Fig. 2. XRD patterns of imogolite collected from Daisen Kurayoshi Pumice located in Syuki, Kurayoshi, Tottori, showing existence of imogolite with adhering feldspars and amphibole.

TABLE I
Chemical compositions of imogolite and pumice collected from Daisen Kurayoshi Pumice located in Syuki, Kurayoshi, Tottori.

	imogolite	pumice
Al ₂ O ₃	39.0	30.8
SiO ₂	42.7	44.2
P ₂ O ₅	1.0	0.7
K ₂ O	0.2	0.2
CaO	2.9	7.3
TiO ₂	1.2	1.6
MnO	0.2	0.3
Fe ₂ O ₃	12.8	14.9
SrO	n.d.	0.1
total	100.0	100.0
n.d.; not detected (wt%)		

TABLE II
Nitrogen, carbon, sulfur contents of imogolite and pumice collected from Daisen Kurayoshi Pumice located in Syuki, Kurayoshi, Tottori.

	Nitrogen	Carbon	Sulfur
imogolite	0.08	2.20	0.11
pumice	n.d.	0.18	0.03
n.d.; not detected (wt%)			

D. Optical microscope observation

Imogolite films were consisted of aggregates of yellowish brown granules. Observation of DAPI stained imogolite revealed that many microorganisms, 1 ~ 2 μm in size, inhabit imogolite surface. In some cases, they made a colony, 10 ~ 40 μm in diameter.

E. Scanning electron microscope (SEM) observation

SEM observation of imogolite revealed that three kinds of microorganisms made a colony on imogolite films (Fig. 3). A colony was composed of coccus and bacillus typed bacteria, 1 ~ 2 μm in size (Fig. 3A). The colony was partly covered with thin films. The other colony was composed of only rod shaped bacteria (Fig. 3B). The bacteria spread enough to cover surface of imogolite films. EDS analyses showed presence of Al, Si and Fe, due to imogolite behind microorganisms, with traces of P, S, Cl, K, Ca, Ti (Fig. 3 inset). The detection of P and S, which most organisms have in their cell, proves presence of microorganisms. Whereas,

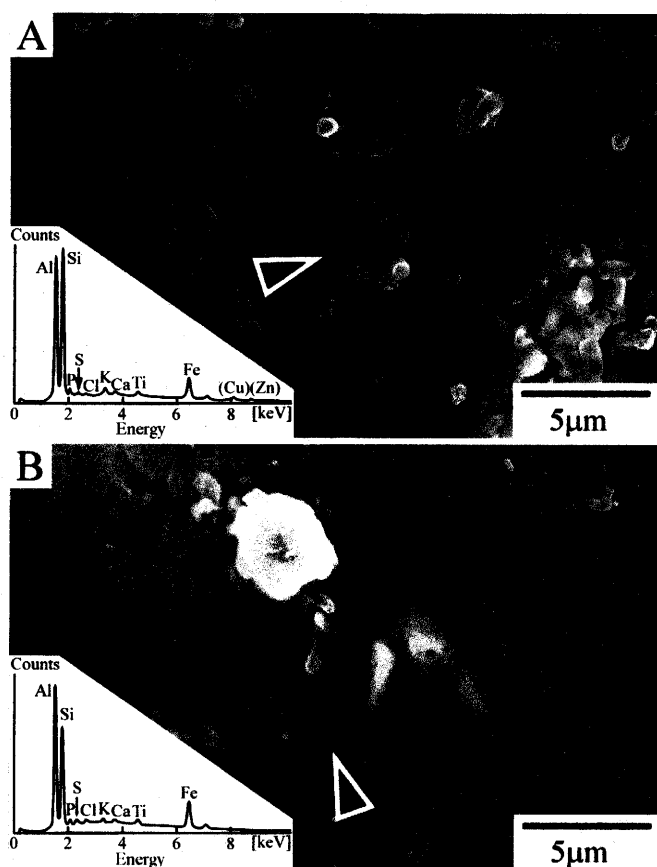


Fig. 3. SEM micrographs of microorganisms on the surface of imogolite films. Coccus and bacillus typed bacteria made colony together. Some of them were covered with thin film (A). Rod shaped bacteria made a colony on the surface of imogolite films (B). EDS analyses indicated Al, Si and Fe composition suggesting imogolite (arrows; analytical points). The P and S elements are originated in microorganisms.

imogolite-like network structures existing abundant microorganisms were formed on the surface (Fig. 4A). Around the network structure, there are some crude films (Fig. 4B), which suggest developmental stage from films to network structure.

F. Transmission electron microscope (TEM) observation

TEM observation of microorganisms on the imogolite showed that the most microorganisms were encrusted by nm-order fine particles on thin thread network structure identified imogolite-like minerals (Fig. 5). The electron diffraction pattern showed a diffuse halo with no conspicuous spots or rings, suggesting a poorly-ordered

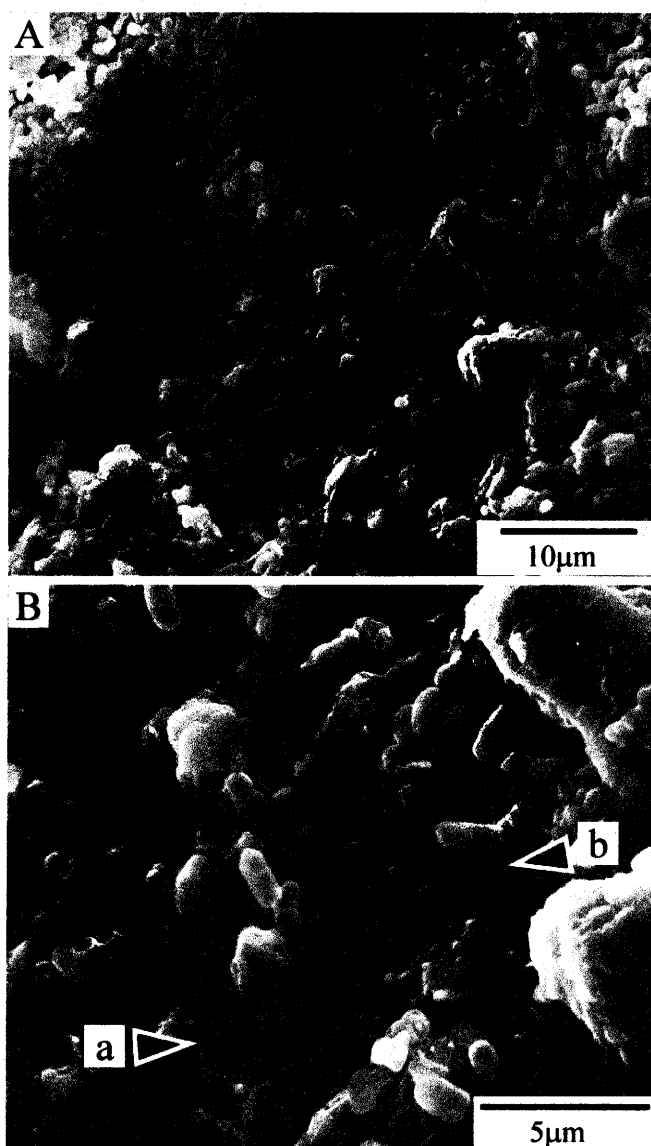


Fig. 4. SEM micrographs of imogolite-like network structure existing abundant microorganisms on the surface of imogolite films (A). Closeup photograph shows that around the network structure (arrow a in B), there are some crude films (arrow b in B), which suggest formation processes from film to network structure.

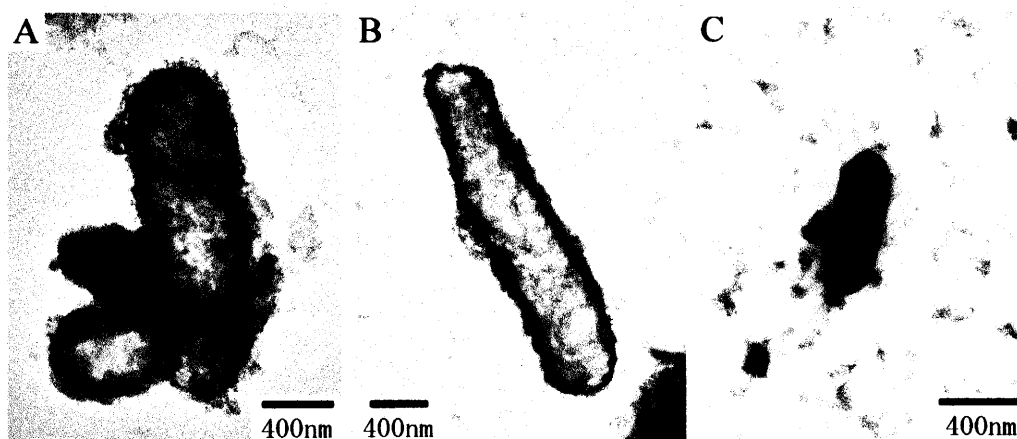


Fig. 5. TEM micrographs showing microorganisms inhabit on imogolite films. Most microorganisms were covered with poorly-ordered materials (A, B). Furthermore the microorganisms often inhabit network structure of imogolite (B, C). Bacillus typed bacteria partly accumulate amorphous materials internal cell (C).

structure. The fine particles might be mainly composed of Al and Si. Moreover some bacillus typed bacteria accumulated amorphous materials internal cell (Fig. 5C).

The microorganisms exhibit a remarkable ability to concentrate Si, Ca, Mn and Fe [8]. They synthesize extracellular polymeric layers that are complex mixture composed primarily of extracellular polysaccharides (EPS). EPS material can bind ambient primary minerals together and act as nucleation sites for authigenic clay mineral formation [9][10]. Therefore, the results suggest that many microorganisms inhabit on imogolite films which accumulate mainly Al and Si with Fe to form precursors of imogolite.

But in some cases, microorganisms were not observed on imogolite films, suggesting completed biomineralization.

V. Conclusions

Gelatinous imogolite in Daisen Kurayoshi Pumice was investigated about the influence of microorganisms on the formation of imogolite. The imogolite contained relatively high concentration of nitrogen, carbon and sulfur suggesting presence of microorganisms and adsorption of organics. Electron microscope observation showed that many microorganisms inhabited on the surface of the imogolite with forming colony. Whereas imogolite-like network structure existing abundant microorganisms was observed on the surface of imogolite. The microorganisms were encrusted by poorly-ordered fine particles. The results suggest many microorganisms inhabiting imogolite formed precursors of imogolite.

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