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KAZUE TAZAKI, TOSHIKAZU MORIKAWA, HIROAKI WATANABE, RYUJI ASADA and MASAYUKI OKUNO

Department of Earth Sciences, Faculty of Science, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192 Japan

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ABSTRACT

The interaction between clays and microbes was investigated from field on pumice and imogolite in volcanic ash soils collected from Kurayoshi Pumice in Tottori, Japan. In comparison to laboratory studies on microbial films of cultures derived from fresh water system were examined. Observation of DAPI stained films revealed that numerous microbes have found in the system. Optical and electron microscopic observations of microbes showed that the imogolite films encrusted with areas of the bacterial cells. XRD of the films showed a 1.4, 0.82, 0.55, and 0.33 nm d-spacing consistent with a imogolite $(OH)_3Al_2O_3SiOH$. EDX analysis revealed that the films were mainly composed of Al, Si, and Fe elements. FT-IR analysis exhibited the characteristic adsorption bands of clays for O-H (3400 cm^{-1}), H-O-H (1650 cm^{-1}) and Si-O-Al (1000 and 950 cm^{-1}) and organic materials for C-H-COOH (3000 cm^{-1}) and CNH (1200 and 1500 cm^{-1}). The imogolite films contain higher concentration of nitrogen (0.08 wt%), carbon (2.20 wt%), and sulfur (0.11 wt%) than that of pumice in the same volcanic ash soils, suggesting that the imogolite was closely associated with adhesive organics. Scanning electron microscopy (SEM) showed a variety of bacterial- like forms both in the natural volcanic ash soils and cultured water system. Transmission electron microscopy (TEM) revealed extensively mineralized bacterial cells with fibrous network structure of imogolite formed on both coccus and bacillus type bacterial cells, showing electron diffraction pattern of a diffuse halo at the initial stage. The well-developed bio-films show lattice images of dehydrated imogolite with 1.0 - 1.1 nm d-spacings. The present investigation strongly suggests that imogolite was interacted with bacterial cohesion as a bio- organic clay product. The identity of the bacteria responsible for bio- imogolite formation is unknown, but microorganisms living in the films make an important role as a nucleation site for the formation of imogolite in volcanic ash soils. The bio-imogolite may have been formed from biochemical weathering products of cohesive materials, which subsequently controlled the formation of clay-organic complexes.

Key words: Microbial formation, Bio-imogolite, Fibrous network structure, Bacterial cell wall, Cohesion, Electron microscope

INTRODUCTION

These are instructions for the preparation of camera-ready imogolite was the first described by Yoshinaga and Aomine [1] in a soil derived from glassy volcanic ash known as "imogo". The name of imogolite was approved as a new mineral species by the AIPEA Nomenclature Committee. It appears as threads and has an ideal composition of$SiO_2/Al_2O_3/2.5H_2O(+)$. The thread consists of assemblies of a tube unit (paracrystalline) with inner and outer diameters of 1.0 and 2.0 nm, respectively. Soil is an integral compartment of environment whereas minerals, organic components, and microorganisms are three major solid components of the soil. Fundamental understanding of these interactions at the microscopic levels is essential for restoring on analytical and physical chemical of environmental ecosystems. Upper layers of a soil have higher organic carbon concentrations and organic content decreases with increasing depth in the soil. This gradient is usually thought to be due to the dominance of above- ground organic input to the carbon profile and much lower activity of organisms in the soil itself [2]. The diversity of bacterial populations developed in the surface layer (0-0.25 m depth) of volcanic mudflow deposits from the Mt. Pinatubo volcano. The bacterial group (Actinobacteria) is expected to predominate in the land [3]. However, the major formation processes for clay minerals are thought to be biochemical, largely depending on high microbial activity in soils [4), 5].

Bacteria are known to have reactive surfaces that can effect the cycling of dissolved elements through sorption reactions. Bacteria (Pseudomonas) adhering to sand surface, located within or at the boundary of a water meniscus between two sand grains [6]. Various microorganisms have the ability to accumulate metallic ions (Fe, Mn, Cd, Pb etc.) from their external aquatic environments. Laboratory and field studies have provided evidence that the Fe bacteria are found

commonly in Fe-rich seep under neutral-pH conditions, and are primarily responsible for most of the Mn and Fe oxidation[7]. Bacteria have adapted to almost every conceivable environment, and researchers have realized that bacteria undoubtedly play a dominant role in the environments. Numerous papers have been published on the formation of clays in volcanic soils. Imogolite commonly has been found in association with allophane, and is similar to allophane in chemical properties. However involvement of bacteria in mineralization of imogolite has not been well characterized using electron microscopy.

In this study, natural occurrences of imogolite both in volcanic ash soils and in laboratory studies of freshwater systems have been observed at nano-meter to micro- meter scales. Little work has been carried out on the biogeochemical behavior of imogolite using electron micro-analytical techniques. Laboratory cultivation experiments clarified the role of microbes in clay biomineralization. The fibrous thin films of a 1.4 nm imogolite phase, here after termed bio-imogolite, produced by bacteria in volcanic ash soils, and conduct laboratory simulation experiments to understand the role of bacteria in natural freshwater systems at room temperature. We also discuss the mechanism of formation of this phase on bacterial cell surface. Essential features of allophane and imogolite are emphasized, along with their effect on the micro organisms properties, and the principles governing their behavior in the volcanic soils.

MATERIAL AND METHODS

Mt. Daisen in Tottori Prefecture in Japan, is located at Japan Sea coast, and the volcanic ash soils of Daisen Kurayoshi Pumice (DKP) are distributed in a NE direction. The pumice and imogolite films were collected from Kurayoshi Pumice layer in Kurayoshi, Tottori, Japan. The reddish brown Kurayoshi pumice, composed mainly of allophane, imogolite, vermiculite, mica clay minerals, and kaolin minerals with quartz, cristobalite, and feldspars [8), 9), 10)]. Imogolite films are commonly found in the pumice are comparatively coarse and large inter-granular space. Parts of pumice samples were rinsed with distilled water ten times to remove fine particles. The washed sediments were incubated at room temperature under distilled water in a covered beaker with a glass slide oriented (Fig. 7A). The incubation periods ranged from a few months to 1 year, during which time pH, Eh, and DO (Dissolved Oxygen) of the solution in the beaker were measured using a HORIBA portable inspection meter.

Microbes in the wet films were examined with a polarizing optical and fluorescence microscope (Nikon OPTIPHOT-2 EFD3). The samples stained with 4',6-diamidino-2-phenylindole (DAPI) were observed through an episcopic fluorescence microscope. DAPI method stains the DNA in bacterial cells, and blue fluorescence under ultraviolet light (365 nm) indicates metabolically active bacteria. These samples were incubated for microscopic observation.

A low-vacuum scanning electron microscope (LV-SEM, JEOL JSM-5200LV) equipped with an energy dispersive X-ray (EDX) spectrometer (Philips EDAX PV9800EX) was used to observe the micromorphology of the bacterial surface and its chemical composition. Microbial films were mounted on stubs using carbon tape. Wet samples were without carbon coating and examined at accelerating voltages of 15-25 kV.

Transmission electron microscopy (TEM) has the highest resolving power and is an essential tool for visualizing the association between clays and bacteria. The extracellular and intracellular condition of the bacteria were observed using TEM (JEOL JEM-2000 EX). Electron diffraction analysis was used to identify phases present in the thin films. One drop of the suspension was taken by pipette and mounted on the micro-grid for observation. The accelerating voltage ranged between 120 and 200 kV. Crystalline phases can be determined by selected area electron diffraction (SAED). Nano-scale observations and elemental distribution analyses (STEM-EDX) of imogolite and bacteria were performed with a transmission electron microscope (JEOL JEM-2010FEF), equipped with a scanning TEM image generator (EM-Z90471) and an energy dispersive X-ray spectrometer (EX-34025JGT).

Mineralogical investigations of clay-bio-film aggregates were performed by X-ray powder diffraction (XRD). A Rigaku Rinto 1200 X-ray diffractometer (CuK α radiation) operating at 40 kV and 30 mA, and a scanning speed of 1 - 20 sec./0.02 degree, was used. Chemical investigations of the bio-films were carried out by a scanning electron microscope (SEM) equipped with an energy dispersive X-ray analysis (JEOL JSX-3201).

The organic compounds associated with minerals and organometallic complexes in the biofilm were analyzed by Fourier-Transform Infrared absorbance spectroscopy (FT-IR; Jasco FT/IR-610, MICRO-20). The biofilm was air-dried and ground to a fine powder, 3 μ g of powdered microbial films and 10 mg of amorphous potassium bromide (KBr) were placed in a mortar, mixed thoroughly, then made into tablets using an MP-1 micro tablet maker and an MT-1 model mini-press. FT-IR analysis was then carried out on the tablets using IR frequencies between 400 and 4000cm^{-1}.

Chemical composition of imogolite and pumice was analyzed using the energy-dispersive X-ray fluorescence (ED-XRF) analyzer (JEOL JSX 3201) applying Rh radiation at 30 kV. The chemical composition of imogolite was determined the quantity of N (nitrogen), C (carbon), and S (sulfur) using an automatic gas chromatographic elemental analyzer (CE Instruments NA 2500-NCS) at 1000℃ with 20 ml oxygen, in order to determine the biological origin.

GEOLOGY AND CLAY MINERALOGY IN DAISEN VOLCANIC ASH SOILS

Mt. Daisen volcanic ash soil and pyroclastic sediments are dated to the Quaternary, which are widely distributed throughout the Sanin district, the southwestern coast of Japan Sea, are the famous Quaternary strata- volcanoes Daisenn Kurayoshi Pumice (DKP)(Fig. 1). The rocks are composed of calc-alkaline two-pyroxene andesite and biotite-hornblende dacite. Thick terrestrial sediments of middle Daisen volcanic ashes with 4 strata of pumice layers (D1, 2, 3, and 4) and pyroclastic rocks in DKP of D2 (55 Ka) are distributed in Tottori Prefecture, usually weathered into secondary minerals such as clay minerals and various hydroxides [10] (Fig. 2). The clay minerals are mainly of 1.4 nm minerals (chlorite,

Al-vermiculite), 1.0 nm minerals (mica-like clay minerals, halloysite), 0.7 nm minerals (kaolin minerals, 0.7 nm chlorite), allophane and imogolite. Imogolite is developed with allophane and a small amount of gibbsite in the DKP pumice layer but can't be found with halloysite, showing formation of imogolite from plagioclase by SEM, EDX, and electron probe microanalysis [8]. This is the first report on the micro morphology of imogolite from plagioclase directly. The high magnification replica of weathered plagioclase showed a complex network structure of imogolite fibers.

The criteria for imogolite were given based on the relative intensities of XRD, chemical compositions, and the thermal reactions [8), 11), 12), 13)]. Imogolite presents several X-ray diffraction peaks. The most outstanding peak is notice ably broad with maximum at about 1.4 nm, with minor beaks clearly seen also at about 0.8 nm and about 0.33 nm. The differential thermal analyses of imogolite show acharacteristic absorb peak at 410-450 ℃ . Imogolite networks bridged over the hollows in plagioclase are well demonstrated in Figs. 4-6. On the other hand, formation of primitive clay precursors on K-feldspar under extreme leaching conditions had reported [14), 15)]. Microbial formation of imogolite has not been reported before. In this paper proof of bio-imogolite have shown in the volcanic ash soils.

RESULTS

X-ray diffraction analyses (XRD) of imogolite and pumice in DKP

Imogolite in DKP indicated crystalline clay minerals at 1.4, 0.82, 0.55, 0.33, and 0.23 nm peaks (Fig. 3 upper). The two strong intensities of 1.4 and 0.82 nm have not shifted by E.G.-treatment. The XRD analyses of pumice in DKP clearly showed different patterns from imogolite, showing a hilly peak at 0.34 and 0.22 nm, suggesting the presence of low crystalline of allophane, associated with amphibole, feldspars, pyroxene, magnetite, and cristobalite (Fig. 3 middle).

On the other hand, in laboratory studies XRD analysis of the initial sediments showed broad peaks at 0.34 and 0.22 nm with hilly background in rinsed pumice to remove fine particles. Wet biofilms from the 2-year old culture in a fresh water system with DKP-pumice only showed small weak peaks at 1.4 and 0.8 nm. XRD analysis of the initial sediments showed that 1.4, 0.82, 0.55, and 0.33 nm consistent with a imogolite whereas broad peaks at 0.34 and 0.22 nm with hilly background in rinsed pumice to remove fine particles. After the 2 year aging of culture the morphological characteristics are consistent with biofilms of imogolite phase to call it "bio-imogolite" which has no evidence for feldspar was seen. The d-spacing of the newly formed clay was the same as that of imogolite clay minerals.

Energy dispersive X-ray fluorescence analysis (ED-XRF)

The untreated samples (dispersed on Mylar film) of imogolite and pumice in DKP by ED-XRF showed that they contained large amounts of Al_2O_3, SiO_2, and Fe_2O_3, with traces of P_2O_5, K_2O, CaO, TiO_2, and MnO (Table 1). The SiO_2 /Al_2O_3 ratio are 1.2 or 1.3 [8), 11)]. The fiber of imogolite is a short of para-crystal composed of the 1:2 type chain structure units, and that the ideal structural formula of the unit cell can be represented by$(Al_8O_4/(OH)_{20}/4H_2O)(Si_8O_{10}/(OH)_4)(A_{18}O_4/(OH)(OH)_{20}/4$ $H_2O)$. Nitrogen, carbon and sulfer contents in pumice in DKP are different from imogolite due to organics of the bio-imogolite (Table 1). The high carbon contents in imogolite (2.20 wt%) agree with the result of XRD analysis. High background in the XRD pattern of imogolite suggest that the presence of organic materials.

FT-IR spectra of bio-imogolite

The chemical and mineralogical components of the bacteria show chemical bonds with Si-O, Al-OH and organics, based on ED-XRF and FT-IR data (Fig. 3 bottom). Major infrared (IR) absorption bands of imogolite appear in four regions. The FT-IR spectrum of bio-imogolite showed a wide range of bands at 3400cm-1 (O-H stretch) and 1500 cm^{-1} (CNH combination) associated with prominent absorption bands at 1650 cm^{-1} (H2O, C=O). The C-H-COOH stretch (3000 cm^{-1}), Si-O stretch (1000 cm^{-1}), and Al-OH stretch (950 cm^{-1}) were identified as bio-imogolite (Fig. 3 bottom). Imogolite shows two absorption maxim at about 940 and 1000-1 in this region of Si(Al)O vibrations [13)]. The other peaks were mainly from the organic matter of microorganisms, such as N-H, C-H, C=O, and C-N-H stretches derived from nucleic acids, fatty acids, and polypeptides[16)], whereas the Si-O and Al-OH bands are derived from imogolite. The FT-IR spectrum of bio-films in a beaker (Fig. 7A) showed bands characteristic of organics in contrast to the pumice samples. The absorption band at 1100 cm-1 was likely due to organic P-O. Either nucleic acids or cell wall and capsular lipids containing polysaccharides, could be responsible for these features. The cell proteins were typically indicated by a number of amide bands[16)]. Bio-imogolite has therefore been shown to be a mixture of a 1.4 nm- imogolite phase and bioorganic products through the mineralogical, chemical, and biological measurements described above.

Scanning electron microscopy (SEM) of imogolite in DKP

SEM showed a variety of bacterial-like forms in DKP. Bacilli and cocci typed microorganisms in imogolite from DKP inhabited on the surface of feldspars (Fig. 4A, B) whereas the surfaces are encased in exo-polymeric substance films knitted of fine yarn (Figs. 4C, D). Elongated yarn connected each other to make larger films. Imogolite films are formed into bud on the surface of feldspars at the initial stage (Fig. 5A). The buds are developed to spread outside of spaces middle stages (Fig. 5B). The surface was covered completely by thin films (Fig. 5C). The SEM morphological observation suggested that imogolite films get thicker and thicker to spread over the whole surface, by weatheringreactions, with the advance of high crystallization.Low-magnification electron micrographs of fine clays particles differing in the relative contents of fibrous imogolite, granular allophane and bacteria. The cosed-up micrographs show that the threads are composed of bundles of fibers with network structure (Fig. 6). The small amounts of mineral particles with smooth surface were also observed. The presence of bacteria associated with imogolite has not been reported before.

Optical and fluorescence microscopic observations

Thin films of imogolite were observed both in natural volcanic ash soils and in laboratory studies of the glass surface in the system after a few months, and grew to 0.01 to 0.1 mm thick after 1-2 years. Thin films formed not only on the glass slide in the beaker, and top surface of the sediments, but also were apparent as a fibrous film on the surface of the pumice grains (Fig. 7A). The films are encrusted with newly formed clays after a few months aging at room temperature. The slightly reduced redox state of the solution was confirmed by Eh values of –26mV and dissolved oxygen levels of 1.3 mg/l at pH 7.4.

The thin films were mainly composed of colonies of right brown or transparent microbes associated with filamentous microbes (Fig. 7B, C). Optical micrographs showed abundant microbes associated with brown pumice grains. The microbes fluoresced red when exposed to light of 510-560 nm wavelength, indicating that the microbes contain chlorophyll-a, which has absorption bands at 409 and 665 nm (Fig. 7D, E, and F). The various microbes were tentatively identified on the basis of their morphologies as filamentous algae (15 – 20 nm in width), filamentous algae (10 – 100 nm in diameter), Cyanobacteria (20 nm in width), and bacteria having coccoid or bacillus morphology (<10 nm in width) [17]. The size of spherical cells ranged from < 10 to 100 nm in diameter. Filamentous algae and the coccoid / bacilloid bacteria (possibly sulfate-reducing bacteria) were the primary producers on the algae. Filamentous algae produced pieces of thin films (Fig. 7E) whereas the coccoid / bacilloid bacteria make clusters (Fig. 7C), for which thin films around cell could only be seen by TEM magnification.

Low vacuum scanning electron microscope (LV-SEM) observations

In laboratory studies bio films were hand picked to observe using optical micrographs as shown in Fig. 7. The same bio-films clearly showed abundant filamentous, coccoid / bacilloid bacteria using LV-SEM micrographs of the wet bio-film without coating revealed filamentous bacteria with clay pieces of 1-5 μ m in diameter (Fig. 8). Abundant clay pieces were also observed on the surface of the cell wall. In most cases three elements (Al, Si, and Fe) were detected in the cell wall, whereas additional S was detected in some bacterial cell. In that case the EDX spectra of bacteria indicated that the most abundant elements were Al, Si, S, and Fe and the high background suggested the presence of organic materials.

Transmission electron microscope (TEM) observations

Important information about the morphology and internal structures of imogolite is obtained from the use of a TEM. Imogolite appears in the electron microscope as threads, smooth and curved, varying in diameter from 10 to 30 nm and extending up to several 10 nm in length. No fibrous particle other than imogolite was found at the higher magnifications. All the threads found at the low magnification were therefore identified as imogolite. TEM images of thin films, about 10 nm in diameter, show different stages of growth, with darker marginal areas (indicating higher electron density than in the center) that strongly suggest fibrous imogolite (Fig. 9). The inner parts appear amorphous, nearly transparent in the TEM images (Fig. 9A and B). The thin outer skin of Figs. 5B and C seems to thicken, and to eventually produce a dense mass of imogolite-like material as in Figs 9C and D. Electron diffraction spots are not clear on the surface of thin films. The EDX data showed high Al, Si, and Fe contents. The mineral assemblage probably contained small amounts of ferrihydrite.

The observations revealed that the thin films thicken to eventually form bio-imogolite film in weathered volcanic ash soils in Japan [13), 18), 19), 20), 21)]. The micro morphology and the size of imogolite in their study are very similar to the bio-imogolite in this study. TEM observations clearly showed that network structure of bio-imogolite is associated with bacterial cell (Figs. 10 and 11). The formation processes of imogolite from thin films to thick-walled sheets are seemed to be form by bacterial interaction. Lattice images of de-hydrated imogolite range from 1.06 to 1.14 nm, parallel oriented structure on the bacterial cell wall suggesting "biologically induced mineralization" (Fig. 11). Using STEM-EDX, formation of imogolite is found as associations of fibrous minerals with bacterial cell (Fig. 12). The EDX analyses and elemental maps of O, Al, Si, P, and Fe revealed that Si/Al ratio between bacteria and background has different distribution, and not associated with P and Fe suggesting organic materials. The formation mechanisms based on TEM observation showed in Fig. 13, showing cohesive material around cell wall reacted with Si-O-Al ions to form clays-organics complexes.

DISCUSSION

Almost all living organisms are generally relevant to soil sciences and clay mineral formation. Organic compounds have a major role in the physical structure of soils. Various organic molecules are able to adsorb ions to form soils. Electron micro techniques have been used to demonstrate the formation processes. The occurrence of imogolite associated with microbes in Daisen Kurayoshi pumice (DKP) was reported by SEM and TEM micrographs evidences presented in this study, indicating that bacteria are major components of imogolite formation in volcanic ash soils.

Bio-clay formations

Examples of clay-biochemical interactions reported that the clay montmorillonite catalyzes the polymerization of RNA from activated ribosome nucleotide and that montmorillonite accelerates the spontaneous conversion of fatty acid micelles into vesicles[22]. The physical and chemical interaction between microorganisms and soils in the ecosystems have discussed [23]. The occurrence of mineralized bacteria with kaolinite, nontronite, and bentonite on living cells of microorganismF in natural and cultivated systems have been well documented [4), 5), 7), 24), 25)]. The bacterial precipitate association was shown by SEM and TEM. The degree of mineralization in these examples ranged from poorly crystalline granules to well oriented crystalline solids.

Several studies have been focused on polysaccharides, because these are, among the organic constituents, particularly active in soil aggregation. In soils, polysaccharides are either inherited from plants or produced in situ by microorganisms and roots as extracellular

polysaccharides[6]. Polymeric substances secreted by microbial cells including the crystalline surface (i.e., SF) layer proteins,polysaccharides, and capsules often provide nucleation sites and possibly a favorable chemical microenvironment for bio-mineral formation. Bacterial cells can act as a nucleation site for minerals. Formation of such bacterial mineral composites has been shown to enhance the immobilization of dissolved metals [26]. The layer of extra cellular polymeric substances surrounding microbial cells can act as a template in the formation of iron hydroxides[27]. Urrutia and Beveridge [28] suggested a cation bridging mechanism in which multivalent metal cations complex with a functional group (e.g. COO-) that in turn bridges with ionic silicates to form large aggregates. Multivalent cations might have served as cation bridges in the interaction between clays and microbial extracellular polymeric substances[23].

Biomineralization of imogolite

Imogolite and allophane are common constituent in medium degrees of weathered volcanic ash soils and pumice throughout Japan. The clay of very young volcanic ash soils is mainly composed of allophan, without containing imogolite. On the other hand, old volcanic ash soils do not contain imogolite, either, but they have halloysite and gibbsite. Consequently, the chances are that imogolite is produced from allophane, has morphology of aggregates composed of fine hollow spherules with diameter of 3.5 to 5.0 nm. Allophane as a poorly ordered aluminum silicate is known to have lower tolerance to alkaline condition than layer silicates have, and easily dissolve completely under higher alkali concentrations [29]. The chemical analysis of the fine clays (< 2 nm) showed that allophane has a SiO_2/Al_2O_3 ratio (mole) ranging at least from 1 to 2, while coexisting imogolite ("proto-imogolite") has a SiO_2/Al_2O_3 ratio close to 1.0 [12), 19)]. Imogolite has a 1:1 aluminum / silicon ratio, and exhibits fibrous morphology with network structure covering on the surfaces of pumice and feldspar grains. Most commonly, imogolite has a 1.4 nm basal spacing, with two interlayer water molecules per formula unit. However, imogolite is susceptible to dehydration, which leads to a 1.0 nm basal spacing. Imogolite is made from the same (aluminum and silicic acid) units as kaolinite but arranged somewhat differently: the alminum hydroxide sheet is rolled into a seamless tube with silicic acid units attached separately on the inside [30]. In both Al- and Si-rich allophanes, octahedral Al (3 ppm by ^{27}Al NMR) and imogolite-like Si (Q33AlVI, -78 ppm by ^{29}Si NMR) were the major components in four Japanese volcanic glasses [31].

The implication is that the bacterial mineralization in the volcanic ash soils and water systems both fosters and augments the solid phase partitioning of metals from solution. In this study, bio-imogolite was successfully formed from the dissolution of allophane or feldspars in a cultivated biofilm after a few months and 2 years of aging at room temperature. SEM and TEM electron microscopic observations revealed that bio-imogolite grew as a thin film on the cell wall of filamentous, coccus, and bacillus type bacteria. The thin films of bio-imogolite are inflated at the cell wall to form a network structure form similar to that found in volcanic ash soils. While the silica and alumina in the soil solution surrounding pumice settled, in a definite successive order, on the end of the cells. When scleroglucan is adsorbed onto clay, it appears as fibrils about 10 nm in thickness and 50-350 nm long, which are attached to the clay surfaces or to other fibrils. The polysaccharides were then visualized as thin mesh of fibrils 1-5 nm thick and 20-100 nm long [6].

Imogolite exhibits a wide range of structural disorders due to random stacking of structural layers. A poorly crystalline form of halloysite termed embryonic halloysite[32]. These materials showed no X-ray diffraction peaks but exhibited infrared absorption bands characteristic of halloysite. In this study, FT-IR data of bio-imogolite showed spectral bands at 3400 and 3000 cm^{-1} suggesting the characteristic O-H and C-H-COOH bonds of structural water (Fig. 3). The absorption bands at 1500 and 1200 cm^{-1} were CNH bonds due to abundant organic materials in imogolite[16), 33)]. Silicic acids are adsorbed rapidly to the metal ions by the formation of Si-O-Al (or Fe) bonds. Both metal ions and microbes are essential for precipitation of silicic acid. The surface of microbe combining aluminum and/or ferric ions may serve as active adsorption sites of silicic acid to accelerate the silica precipitation kinetics. The adsorbed silicic acid may act as nucleation site of silica deposition on the surface of microbe [34].

Various types of Gram-positive or –negative bacterial surfaces can affect the cycling of dissolved elements through sorption reactions. Bacterial cells are known to be potent sorbents of dissolved metals [37]. The cell wall of a microbe is covered with organic adhesive material, includingpolysaccharides that might organize the formation of oriented clusters associated with clay particles[35]. Organic carbon controls the orientation of mineral surfaces in black shale[36]. Therefore, clay mineral formation by bacteria is linked with the adhesive nature of surface materials that leads to inflated fibrous structures.

In the present study, a fibrous imogolite phase having high carbon and sulfur contents formed with the mediation of microorganisms under reducing conditions. The biomineralization of imogolite correlated with allophane under aerobic conditions are necessary for the initial breakdown of hydrocarbons and in subsequent steps, nitrate may serve as a terminal electron acceptor [38]. The low Eh and dissolved oxygen values observed in this experiment suggest that reducing conditions were present, and that sulfate-reducing bacteria may have played a role in the formation of bio-imogolite. The Al of the fundamental allophane structure, imogolite sheet, is susceptible to hydroxyl attack, and in KnP structure, accessorily attached Si prevented hydroxyls to approach the site [29]. In this study, we demonstrate that the adsorption of bio-imogolite forming Al-Si-ions onto bacterial surface can lead to enhanced clay mineral interactions.

Formation mechanism of bio-imogolite

Knowledge of the structure of clay minerals has great value for predicting and understanding their behavior in soils. The use of electron microscopy has enabled us to deduce the structure of imogolite and bacteria on a more solid basis than before. The results have wider implications for the mechanisms by which organisms control mineral formation. Based on the current evidence, one can only speculate as to the formation mechanism of the bio-imogolite (Fig. 13). One

such mechanism is proposed suggest that bacterial cohesive material in capsule or external cell wall accumulated Al and Si from pumice in volcanic ash soils and in the culture fresh water system. If only coccoid / bacilloidal bacteria are associated with the bio-imogolite, cohesive materials could be involved to form clay-organic complexes. The pH changed during the course of experiment, from pH 6 at the starting point to pH of 7.4 after 2 years of aging. The cell walls of microbes generally include proteins, polysaccharides, proteolipids, and proteoglycans,, and have an affinity for clay minerals. A specific set of macromolecules regulatesnucleation, growth, size, and orientation of the materials. The model was investigated using a native microbial consortium and a variety of Al- and Fe-bearing silicates and oxides to determine if other controls, such as mineral composition, also influence the interaction between cells and surfaces [39]. Surface adherent cells demonstrate that electrostatic effects dominante microbial colonization on positively charged oxide surfaces regardless of mineral composition, in an anaerobic groundwater at pH 6.8. The fibrous films consist of outer membrane and periplasmic materials and make good nucleation sites for mineral development.

In this study, the laboratory experiments indicated that bio-imogolite formation in fresh water with pumice after a few months to 2 years of aging can be ascribed to an initial crystallization of biological origin, rather than inorganic origin. Clay films may have initially produced by bacterial film formation. With time the thin films thicken, becoming a dense wall as shown in Figs. 9-11. Finally, the well-crystallized imogolite might have been released from bacterial surface to the external environment, cutting off all contact with the bacterial cell. Microorganisms preferentially colonize those silicate surfaces that beneficial nutrients and avoid those that contain potentially toxic elements. These bio-clays might serve as a precursor for network imogolitecrystallization. However, we argue that the major imogolitenucleation processes are biochemical, largely depending on high microbial activity in volcanic ash soils. The observation that microbial cells could mediate the formation of imogolite phase is a significant one that should have important consequences for the soil sciences. In addition to the adsorption of biomonomers, it has been suggested that clay could have been important in the subsequent condensation and production of bio-oligomers from these monomers. The clay surface and specific sites therin are proposed to act as direct templates for the production of a repeated, select sequence of biomolecules in the formed bio-oligomers [40].

CONCLUSION

SEM and TEM micrographs clearly showed the biological formation of primitive clay films and the growth of a transitional product of fibrous imogolite. The volcanic ash sediments contain abundant highly mineralized bacteria with coatings of poorly crystalline biofilms. The clayey thin films occurred on the external surface of the bacterial cell wall or capule. The chemical analysis of the clay films showed Al, Si, and Fe with traces of P, S, Cl and Ca as the major elemental composition. X-ray diffraction, FT-IR spectroscopy, ED-XRF, SEM-EDX, and TEM analytical methods used in this study suggested that the clay films did not resemble hisingerite or other clay minerals, but represented a new ordered aluminum silicate phase, hereafter called bio-imogolite. This shows that microbial activity may play a significant role in thenucleation of clays, and that this may be a common occurrence. It is likely that the clay films on the surface of bacterial cells are the bioorganic product of bacterial formation activity. Imogolite formation in a volcanic ash soils may be substantially enhanced by the presence of bacteria. The bio-imogolite in volcanic ash soils may have been formed from biochemical weathering products of cohesive materials, whichsubsequently controlled the formation of clay-organic complex.

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REFERENCES

1) Yoshinaga, N., and Aomine, S. (1962) *Soil Sci. Plant Nutr.*, **8**, 6-13.
2) Shoji, S., Nanzyo, M., and Dahlgren, R.A. (1993) *Volcanic ash soils: Genesis, properties, and utilization*, Elsevier, Netherlands. 288pp.
3) Ohta, H., Ogawa, K., and Murakami, E. (2003) *Soil Biology & Biochemistry*, **35**, 1155-1158.
4) Ueshima, M., and Tazaki, K. (1998) *Clay Science Japan*, **38**, 68-82.
5) Ueshima, U., Mogi, K., and Tazaki, K. (2000) *Clay Science Japan*, **39**, 171-183.
6) Chenu, C., and Tessier, D. (1995) *Scanning Microscopy*, **9**, 989-1010.
7) Tazaki, K. (1997) *Clays and Clay Minerals*, **45**, 203-212.
8) Tazaki, K. (1971) *Geological Journal of Japan*, **77**, 407-414.
9) Tazaki, K. (1975) *Journal of Clay Science Society of Japan*, **15**, 3-8.
10) Tazaki, K. (1986) *Contri. Mineral. Petrol.*, **92**, 86-88.
11) Yoshinaga, N., and Yamaguchi, M. (1970) *Soil Sci. Planet Nutr.*, **16**, 215-223.
12) Henmi (1985) *5th meeting of the European clay groups, Prague*, 459-464.
13) Wada, K. (1989) *Allophane and imogolite. In:Minerals in soil environments (2nd Edition)*, SSSA Book Series, no.1. 1051-1097.
14) Tazaki, K., and Fyfe, W.S. (1987) *Proceedings of the International Clay Conference, Denver*, 1985, 53-58.
15) Tazaki, K., and Fyfe, W.S. (1987) *Canadian Journal Earth Science*, **24**, 506-527.
16) Filip, Z., and Hermann, S. (2001) *European Journal of Soil Biology*, **37**, 137-143.
17) Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., and Williams, S.T. (1994) *Bergey's manual of determinative bacteriology. Ninth Edition*, Williams & Wilkins, USA, pp787.
18) Cradwick, P.D.G., Farmer, V.C., Russell, J.D., Masson,

C.R., Wada, K., and Yoshinaga, N. (1972) *Nature Phys. Sci.*, **240**, 187-189.
19) Henmi, T., and Wada, K. (1976) *American Mineralogist*, **61**, 379-390.
20) Farmer, V.C., Smith, B.F. L., and Tait, J.M (1977) *Clay Minerals*, **12**, 195-198.
21) Sudo, T., and Shimoda, S. (1978) *Clays and clay minerals of Japan: Developments in Sedimentlogy 26*, Elsevier. 300pp.
22) Hanczyc, M.M., Fujikawa, S.M., and Szostak, J.W. (2003) *Science*, **302**, 618-622.
23) Theng, B.K.G., and Orchard, V.A. (1995) *Interactions of clays with microorganisms and bacterial survival in soil: A physicochemical perspective. Environmental impact of soil component interactions*. P.M. Huang, J. Berthelin, J.M. Bollag. W.B. McGill and A.L. Page (eds.), CRC press, Florida, 3, 123-139.
24) Asada, R., and Tazaki, K. (2000) *Clay Science Japan*, **40**, 24-37.
25) Tazaki, K. (2000) *Clays and Clay Minerals*, **48**, 511-520.
26) Sara, M., and Sleytr, U.B. (2000) *J. of Bacteriology*, **182**, 859-868.
27) Tashiro, Y. and Tazaki, K. (1999) *Earth Science*, **53**, 27-35.
28) Urrutia, M.M., and Beveridge, T.J. (1993) *Journal of Bacteriology*, **175**, 1936-1945.
29) Abidin, Z., Matsue, N., and Henmi, T. (2004) *Clay Science*, **12**, 213-222.
30) Cairns-Smith, A.G., and Hartman, H. (1986) *Clay minerals and the origin of life*, Cambrige University Press 193pp.
31) Hiradate, S., and Wada, S. (2005) *Clays and Clay Minerals*, in press.
32) Wada, K., and Kakuto, Y. (1985) *Soil Science Soc. American J.*, **49**, 1309-1318.
33) Fialips, C-I., Petit, S., Decarreau, A., and Beaufort, D. (2000) *Clays and Clay Minerals*, **48**, 173-184.
34) Aramaki, Y., Yokoyama, T., Okaue, Y., and Watanabe, K. (2004) *Chemical Geology*, **212**, 339-349.
35) Ueshima, M., and Tazaki, K. (2001) *Clays and Clay Minerals*, **49**, 292-299.
36) Kennedy, M.J., Pevear, D.R., and Hill, R.J. (2002) *Science*, **295**, 657-660.
37) Beveridge, T.J. (1999) *J. Bacteriology*, **181**, 4725-4733.
38) Bartha, R.(1986) *Microbial. Ecol.*, **12**, 155-172.
39) Roberts, J.A. (2004) *Chemical Geology*, **212**, 313-327.
40) Lawless, J.G. (1985) *Clay-organic interaction and the origin of life. In: Clay minerals and the origin of life*. A.G. Cairns-Smith & H. Hartman (eds.), Cambrige University Press, 135-137.

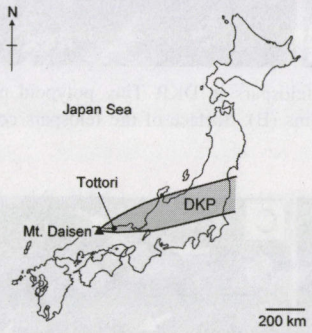


Fig. 1. Locality map of Mt. Daisen, Tottori Prefecture, Japan, indicate distribution of Daisen Kurayoshi Pumice (DKP) in a NE

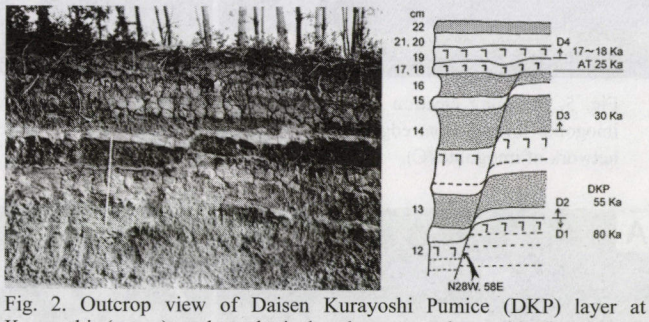
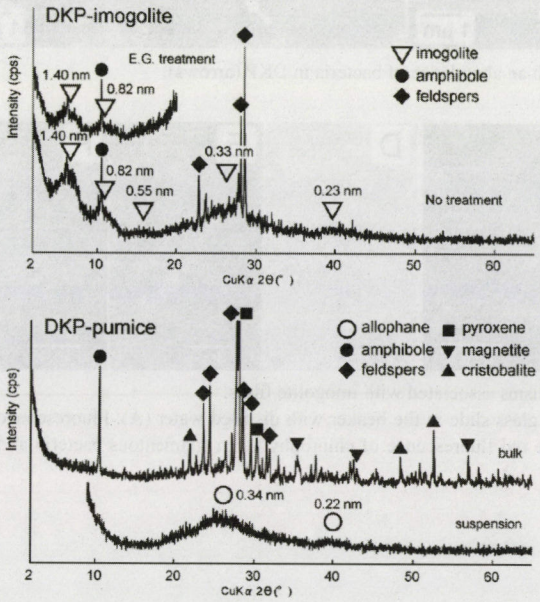


Fig. 2. Outcrop view of Daisen Kurayoshi Pumice (DKP) layer at Kurayoshi (upper) and geological column correlate with the outcrop indicating geological dating and fult.



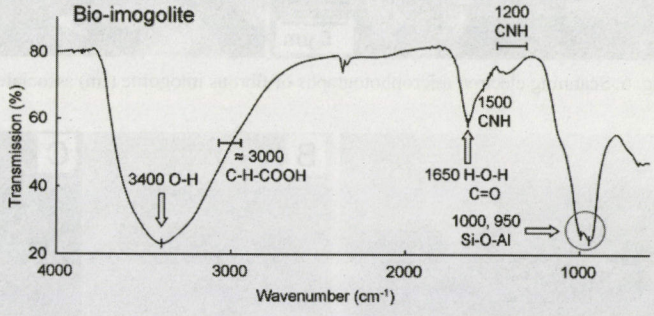


Fig. 3. X-ray powder diffraction patterns of imogolite (no treatment and E.G. treatment) and pumice (bulk sample and suspension) were collected from Daisen Kurayoshi Pumice (DKP)(left). The FT-IR spectroscopy of imogolite in DKP, showing not only Si-O-Al bands due to clay minerals, but also various kinds of organic bands, such as C-H-COOH, CNH and C=O due to fatty acids, nucleic acids, and polypeptides of bacterial cells (right)(according to Filip and Hermann, 2001).

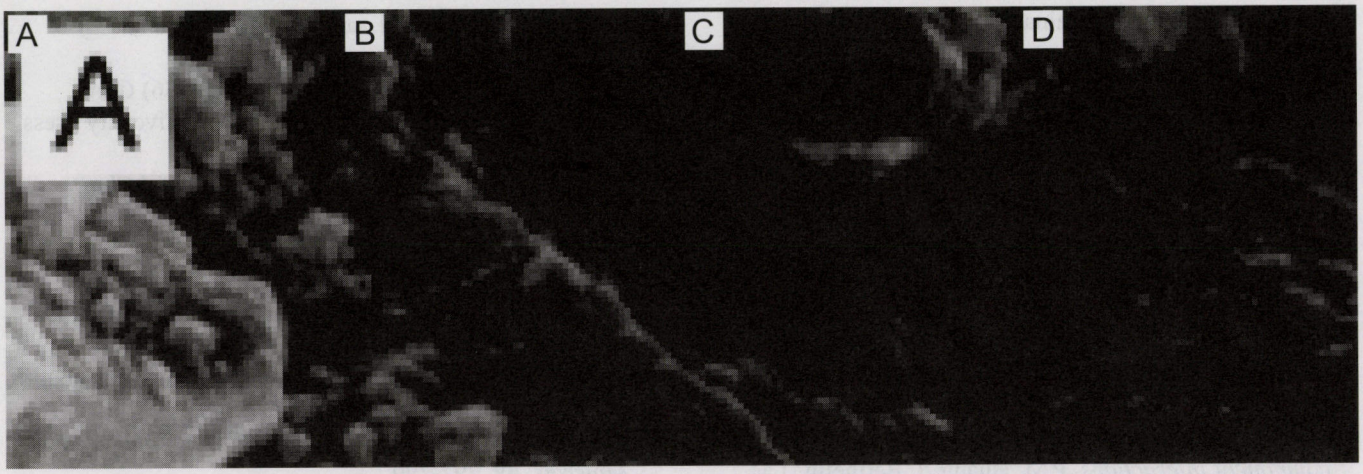


Fig. 4. Scanning electron microphotographs of imogolite formed on feldspars in DKP. Tiny spherules on the surface of feldspars suggest that microorganisms inhabited (A and B), and filamentous bio-films covering on the surface of pumice grains (C and D). Tiny polypoid projections of imogolite (C) develop to longer fibroid imogolite (D).

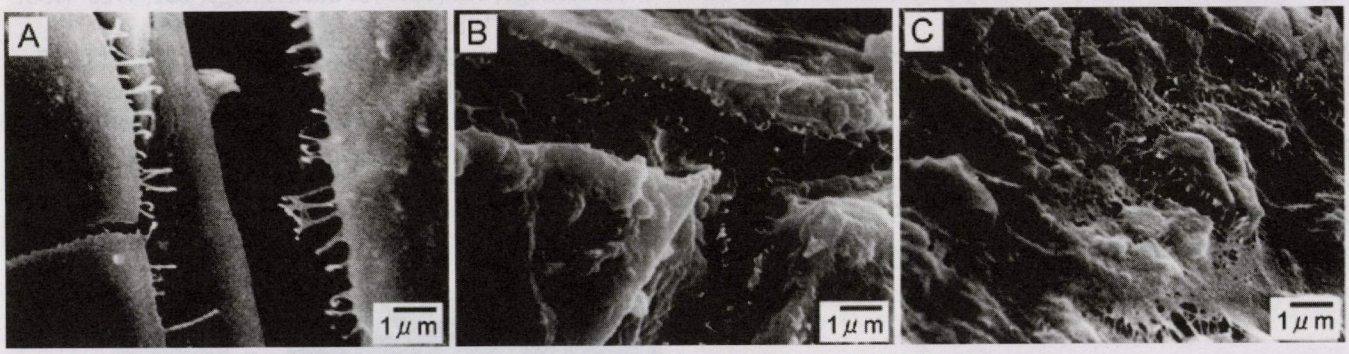


Fig. 5. Scanning electron microphotographs of formation processes of fibrous imogolite on feldspars in DKP. Tiny polypoid projections of imogolite formed from edges of thin layers (A). Imogolite fibers develop into a network or films (B). Surface of the feldspars covered with a network of imogolite (C).

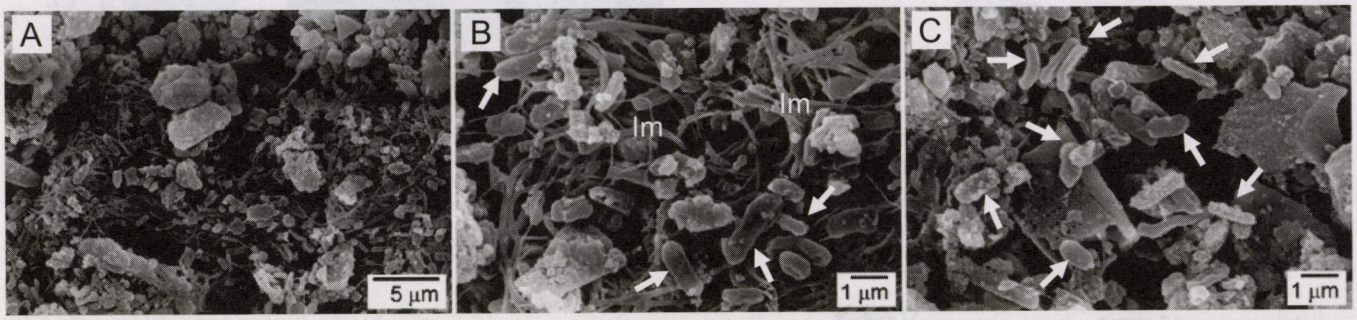


Fig. 6. Scanning electron microphotographs of fibrous imogolite (Im) associated with an abundance of bacteria in DKP (arrows).

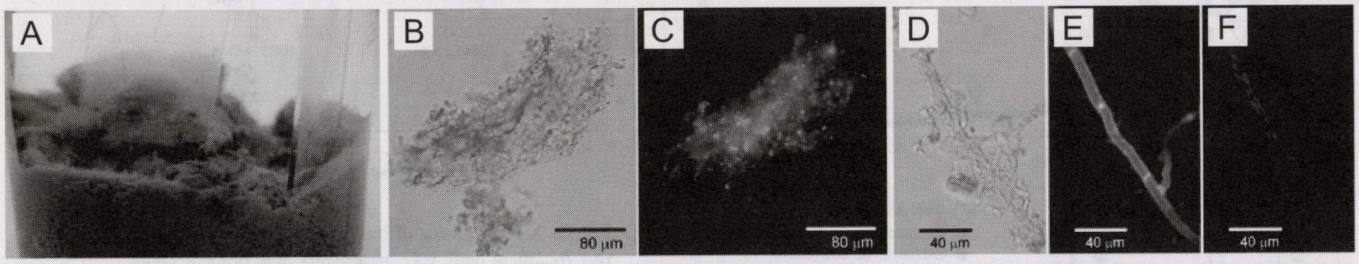
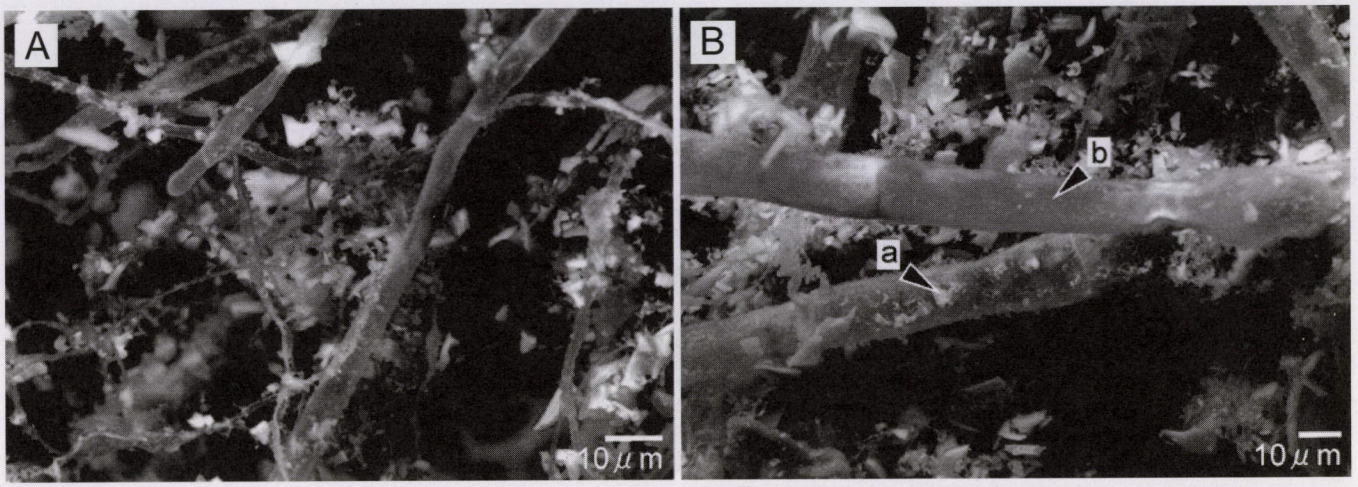


Fig. 7. Optical micrographs of thin films with pumice showing abundant microorganisms associated with imogolite films.
Optical micrograph of formation of thin films on the surface of pumice grains and glass slide in the beaker with distilled water (A). Fluorescence micrograph of a DAPI-stained sample shows the blue spots of bacteria (C) and the red fluorescence of chlorophyll-a in filamentous bacteria and algae (E and F).



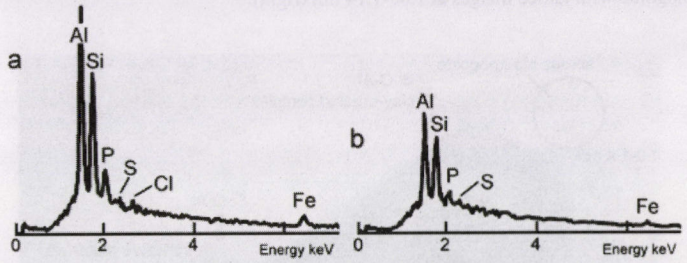


Fig. 8. Low vacuum scanning electron micrographs of bio-film witout coating show clayey particles on the surface of abundant fibrous microorganisms (A and B). The EDX spectrum of living filamentous bacteria (a and b in B) showed major elements of Al, Si, S, and Fe (left, a, b).

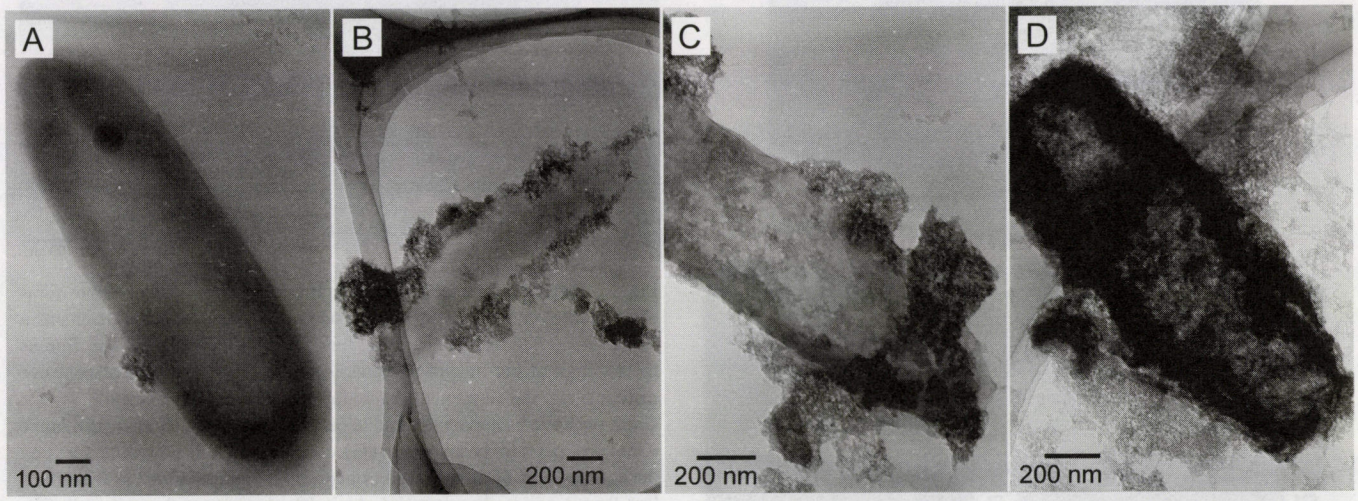


Fig. 9. Transmission electron micrographs of formation processes for imogolite films on bacterial cell wall. Coccus and bacillus type bacteria offer a nucleation site for imogolite films (A and B). Bacterial network structure is encrusted with thin films on the external face of the cell wall (C and D). Thickened bio-films occurred at a tip parts (D).

Fig. 10. Transmission electron micrographs of the well-developed bio-films of imogolite mineralized filamentous (A), bacillus (B), and coccus (C) type bacteria showing electric high density, from cultural water systems. These included clay mineral accretions both on the external face and the extra cellular polymeric matrix.

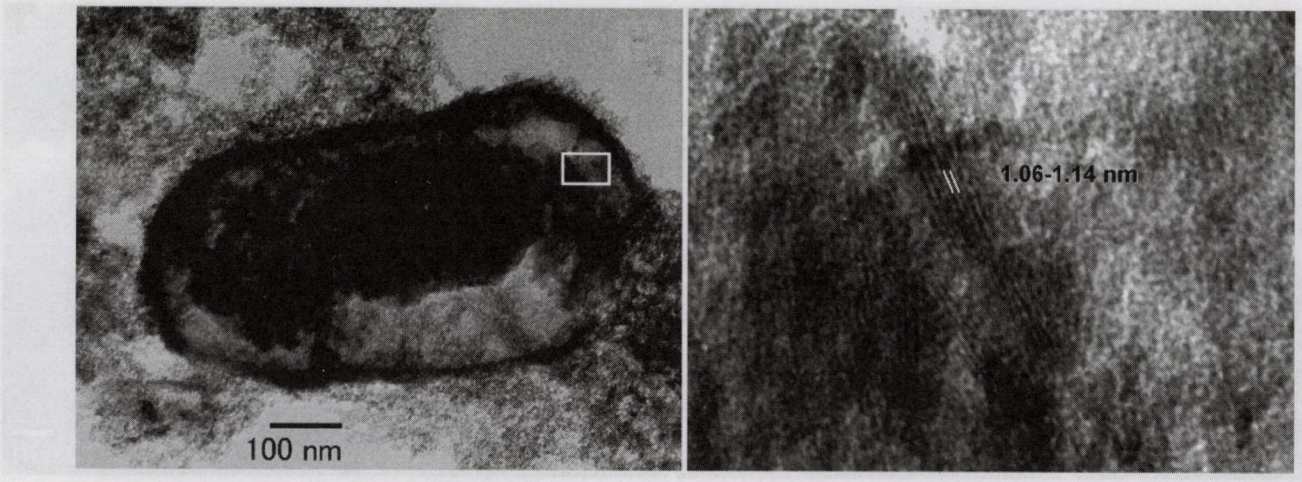
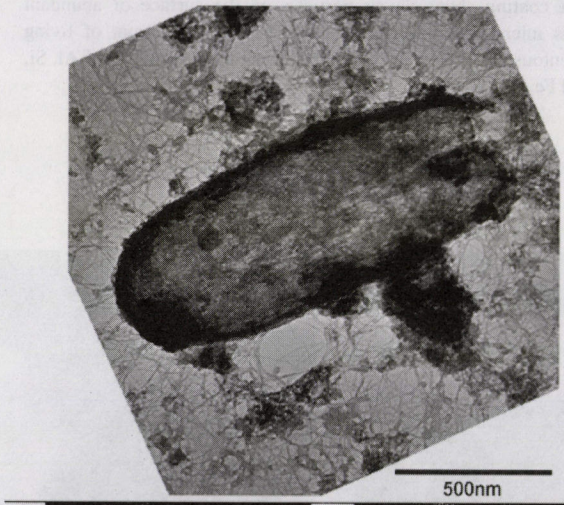
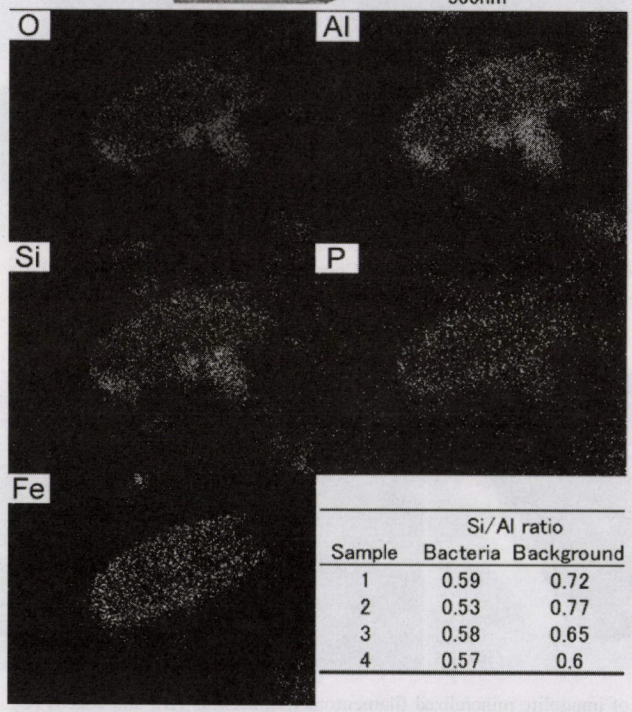


Fig. 11. Transmission electron micrographs of imogolite with lattice images at 1.06-1.14 nm (right).





Sample	Si/Al ratio Bacteria	Background
1	0.59	0.72
2	0.53	0.77
3	0.58	0.65
4	0.57	0.6

Fig. 12. Transmission electron microscopy equipped with a scanning TEM image generator and energy dispersive X-ray spectrometer (STEM-EDX) maps of O, Al, Si, P, and Fe contents in bacterial cell and the background showing different Si/Al ratio. TEM image shows bright field image (upper part).

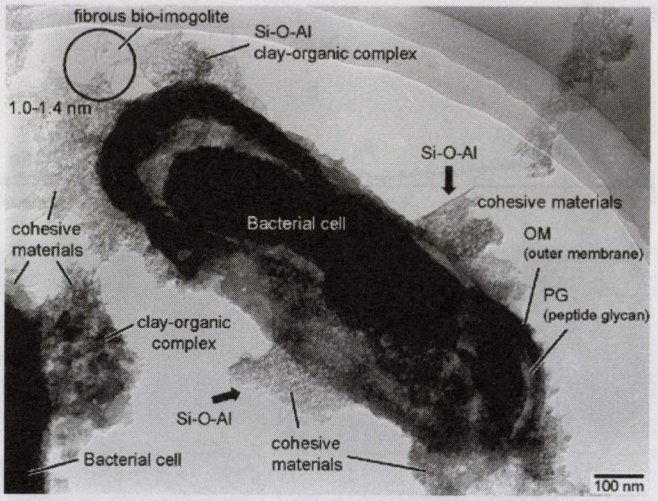


Fig. 13. Transmission electron micrograph of the well-developed bio-films of imogolite with fine network structures suggesting possible mechanism of bio-imogolite formation. The formation mechanism of the fibrous morphology of bio-imogolite seems to be the spider's thread of cohesive materials into the clays on the bacterial cell wall.
Gram-negative bacteria with double cell wall showing the outer membrane (OM) and peptidoglycan layer (PG) covered by thick imogolite films on the external face of the cell wall or capsule. Imogolite occurs as thin fibers less than 1 nm in diameter and about 10 nm long on the cell wall. The fibers branch out and grow into wide spread networks or thin films which finally cover the entire surface of the cell.

Table 1. Chemical composition of imogolite and pumice collected from DKP. Note that SiO_2/Al_2O_3 ratio is slightly different between them and high carbon content in imogolite.

	Bio-imogolite	Pumice
Al_2O_3	39.0	30.8
SiO_2	42.7	44.2
P_2O_5	1.0	0.7
K_2O	0.2	0.2
CaO	2.9	7.3
TiO_2	1.2	1.6
MnO	0.2	0.3
Fe_2O_3	12.8	14.9
SrO	n.d.	0.1
total	100.0	100.0
SiO_2/Al_2O_3	1.9	2.4

n.d.; not detected (wt%)

	Bio-imogolite	Pumice
N	0.08	n.d.
C	2.20	0.18
S	0.11	0.03

n.d.; not detected (wt%)

Imogolite	Yoshinaga (1970)	Tazaki (1971)
Na_2O	1.7	0.3
MgO	0.2	0.8
Al_2O_3	47.1	30.8
SiO_2	32.2	23.3
P_2O_5	0.1	0.0
K_2O	0.1	0.6
CaO	0.2	1.5
TiO_2	tr.	0.4
MnO	tr.	0.1
Fe_2O_3	0.5	3.2
H_2O^+	18.6	18.8
H_2O^-		19.7
total	100.5	99.5
SiO_2/Al_2O_3	1.2	1.3

tr.; trace (wt%)