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Minerals Associated with Biofilms Occurring on Exposed Rock in a Granitic Underground Research Laboratory

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The concept of disposal of nuclear fuel waste in crystalline rock requires the effects of microbial action to be investigated. The Underground Research Laboratory excavated in a pluton of the Canadian Shield provides a unique opportunity to study these effects. Three biofilms kept moist by seepage through fractures in granitic rock faces of the Underground Research Laboratory have been examined. The biofilms contained a variety of gram-negative and gram-positive morphotypes held together by an organic extracellular matrix. Nutrient levels in the groundwater were low, but energy-dispersive X-ray spectroscopy has shown biogeochemical immobilization of several elements in the biofilms; some of these elements were concentrated from extremely dilute environmental concentrations, and all elements were chemically complexed together to form amorphous or crystalline fine-grained minerals. These were seen by transmission electron microscopy to be both associated with the surfaces of the bacteria and scattered throughout the extracellular matrix, suggesting their de novo development through bacterial surface-mediated nucleation. The biofilm consortia are thought to concentrate elements both by passive sorption and by energy metabolism. By Mössbauer spectroscopy and X-ray diffraction, one of the biofilms showed that iron was both oxidized and precipitated as ferrihydrite or hematite aerobically and reduced and precipitated as siderite anaerobically. We believe that some Archean banded-iron formations could have been formed in a manner similar to this, as it would explain the deposition of hematite and siderite in close proximity. This biogeochemical development of minerals may also affect the transport of material in waste disposal sites.

Over the last decade there has been considerable interest in deep subsurface microbiology (24, 27). Bacteria have now been found in deep aquifers (1), at the bottom of oil wells (3), and in deep-sea thermal vents (20). So far, the only reports on bacteria in the groundwaters of crystalline rocks are either Swedish (26–29) or preliminary Canadian observations (7, 34). AECL Research (Atomic Energy of Canada Ltd.) has excavated an underground research laboratory (URL) in a granitic pluton of the Canadian Shield to investigate a vault in such rock for the disposal of nuclear fuel waste (12). Although crystalline rocks in the natural environment are anoxic, when excavated, air penetrates, so there will be both aerobic and anaerobic conditions in the vault. The URL provides a unique opportunity to investigate bacterial activity in these environments.

The groundwaters of the Canadian Shield are oligotrophic. Their main source of carbon is from organic matter leached from plant biomass at the surface, so few nutrients reach the deep subsurface. Although these groundwaters have a general tendency to increase in salinity with depth, which is possibly a function of groundwater residence time (15, 16), only inorganic bicarbonate can be obtained during the leaching of the crystalline host rock. In such natural oligotrophic environments the formation of biofilms is a survival strategy for bacterial communities. Bacteria preferentially adhere to solid surfaces, where nutrients tend to concentrate, and these surfaces therefore provide a more abundant food source than the bulk fluid (8).

As a biofilm develops after colonization of a surface by microorganisms from the fluid phase, an ecological succession leads to a climax or mature community, conceptually similar to that found in other ecosystems. Internal concentration gradients of nutrients and wastes within the biofilm can influence the diversity and spatial distribution of the microbial population as well as the metabolic activity, producing structured physiological relationships between organisms. Exopolysaccharides excreted by bacteria accumulate to form a gel, and ultimately, because of transfer limitations, the inner layer of the biofilm often becomes anoxic, stimulating the development of an anaerobic population. Finally, protozoa may graze on the bacterial population to maintain a climax community (11).

Bacteria need to control the level of metal ions to which they are exposed, since although some are essential for growth, others can be toxic. In soil, the occurrence of free ions is rare, as most are complexed to either inorganic (such as clays and metal oxides) or organic (such as humates, microbial exudates, or microbial surfaces) substituents (14, 17, 33). However, in crystalline rocks there may be little to complex the ions. Bacterial cell walls and their exopolymers are usually electronegative, enabling bacteria to sorb and bind metal cations from the surrounding media to form minerals (5, 33). Clay can also develop on bacterial surfaces, initially in the amorphous state but eventually becoming dehydrated to form a more highly ordered crystalline, platy phase (14, 35, 36). The reactions of bacteria found in rocks and groundwater include passive

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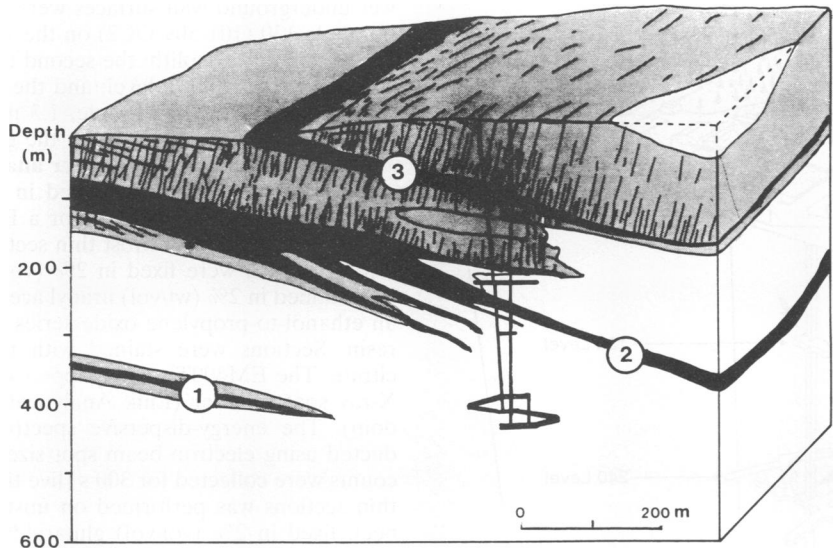

FIG. 1. Block diagram of the geology and fault zones of the URL. Thrust faults are numbered 1 to 3. White areas show grey granite, grey areas show pink granite, and black areas show fracture zones.

accumulation by sorption of cations (e.g., metals) and anions (e.g., silicates), utilization of metal ions as an inorganic source of energy, and incorporation of metal elements into cell components, notably as metalloenzymes.

Some metals, such as iron, can be an energy source to bacteria since the metal has a multiple-valence state and can undergo either oxidation or reduction by donating or accepting electrons; these electrons are then utilized by the energy-producing mechanisms of the cell (37). Small quantities of metal ions are normally associated with bacterial surfaces, but in some cases the metal sorption is so great that precipitates are formed (33). Over time, these can become mineralized and can be important both in removing heavy metals from natural waters and in the genesis of some sedimentary formations (5). Because of the growing scientific awareness of the role of groundwater in the transport of contaminating toxic heavy metals, it is important to understand how microorganisms can impact the transport process, especially in subterranean environments. Several studies have addressed this issue in soils, sediments, mine tailings, and freshwater environments and their simulations (5, 14, 32, 36, 38), but it has not been addressed in the deep subsurface. This is especially important now that deep containment facilities are being designed for radioactive waste.

Clearly, the identification of deep subsurface bacteria and the understanding, at least, of some of their metabolic processes have increased dramatically over the last decade (24, 27), but their biofilm mode of growth and their ability to interact with the variable concentrations of electrolytes in groundwater are poorly understood. This understanding should help in the rational design of underground radioactive-waste facilities. The URL presents a unique opportunity to investigate the bacteriology of the deep subsurface and the effect that the formation of biofilms may have in a near-pristine deep geologic environment. We report here a study of three different biofilms that have spontaneously formed on excavated granite walls at various depths in the AECL Research URL.

TABLE 1. Analysis of three biofilms from the URL

Mineral	Composition of:								
	Borehole 130			Vent raise			Room 405		
	Rock (%)	Water (mg liter^{-1})	Biofilm (%)	Rock (%)	Water (mg liter^{-1})	Biofilm (%)	Rock (%)	Water (mg liter^{-1})	Biofilm (%)
Na	2.00	37.53	3.46	4.38	70.65		4.38	152.00	
K	0.92	2.07	8.95	4.80	1.63		4.80	71.30	
Ca	9.63	33.70	9.81	1.55	31.10		1.55	71.40	
Cl		12.50	2.12		48.00			245.00	
SO_4		8.90	3.70		39.00			48.00	
NO_3		1.28			0.93			14.50	
HCO_3		2.00			1.70			2.20	
Si	49.40	11.10	11.00	71.60	5.47	2.39	71.60	10.70	10.58
Al	41.25	1.48		14.20	0.04		14.20	0.04	
Fe	12.00	6.89	44.16	2.10	0.02	95.13	2.10	0.02	82.22
Mn	0.35	5.47	13.83	0.03	0.01		0.03	0.02	
Mg	7.53	14.10	2.81	0.50	9.18		0.50	6.97	

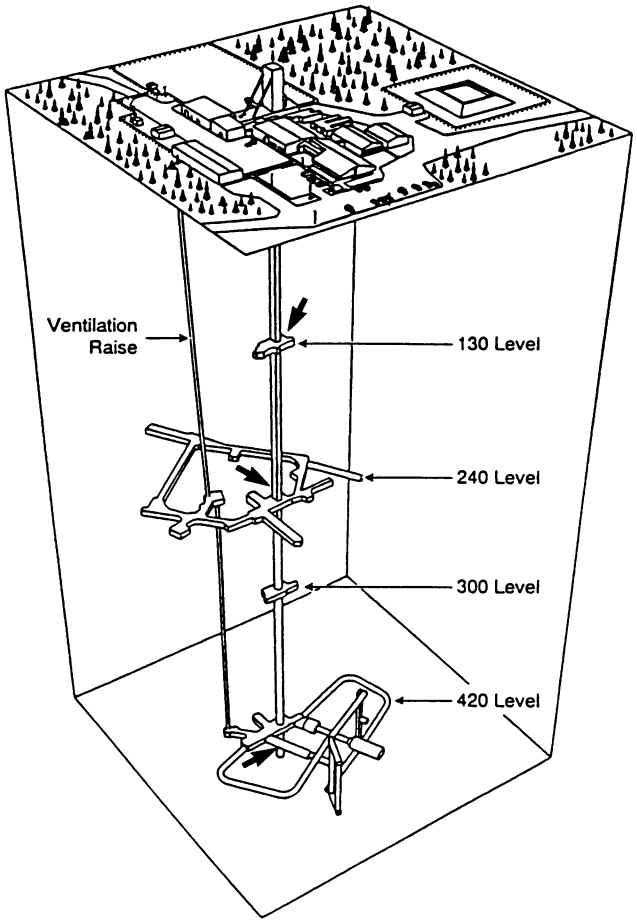

FIG. 2. Diagram of the URL. Arrows show positions of the biofilms examined.

MATERIALS AND METHODS

Geology. The URL is situated in the Archean Lac du Bonnet batholith in southeastern Manitoba, Canada, within the tectonic Superior Province. The geology has been described by Everitt and Brown (13) and Brown et al. (6). The granite, which is now at the surface, crystallized at a depth close to 12 km some 2.7 billion years ago. The URL shaft intersects a variety of lithological domains, the major units of which are gneissic pink granite near the surface, massive to weakly gneissic grey granite at deeper depths, and xenolithic granite distributed throughout the batholith (Fig. 1). The pluton has two low- to intermediate-dip water-bearing thrust faults (labelled 1 and 2 in Fig. 1) with some splays and several subvertical joints that provide groundwater pathways to a depth of at least 250 m. The URL groundwater is recharged from high ground around the head frame; the water then flows through small vertical surface fractures into the two main fracture zones, from whence it is discharged back to the surface.

The shaft is 443 m deep, and underground facilities have been excavated at depths of 240 and 420 m; there are also shaft stations at 130 and 300 m (Fig. 2). Ventilation raises, parallel to the main shaft, are used for air circulation and the removal of waste products from excavation blasting. Three biofilms (shown by arrows in Fig. 2) that had formed on permanently wet underground wall surfaces were examined: the first from borehole 130 (101-509-OC2) on the 130-m level, which drains an amphibolite xenolith; the second from the ventilation raise walls above the 240-m level; and the third from room 405 on the 420-m level, where a hole, 1.3 m in diameter by 5 m in depth, has been drilled through the grey granite floor.

Electron microscopy and other analyses. Biofilm specimens obtained aseptically were viewed in either a Philips EM300 electron microscope at 60 kV or a Philips EM400T electron microscope at 100 kV. Most thin sections were obtained from biofilms which were fixed in 2% (vol/vol) glutaraldehyde, en bloc stained in 2% (wt/vol) uranyl acetate, dehydrated through an ethanol-to-propylene oxide series, and embedded in Epon resin. Sections were stained with uranyl acetate and lead citrate. The EM400T was equipped with an energy-dispersive X-ray spectrometer (Link Analytical, London, United Kingdom). The energy-dispersive spectroscopy (EDS) was conducted using electron beam spot sizes of 2.0 μm or less, and counts were collected for 300 s (live time) (32, 35, 36). EDS of thin sections was performed on unstained material that had been fixed in 2% (vol/vol) glutaraldehyde and embedded in Epon (see reference 14 for more details). Powder X-ray diffraction patterns were taken using CuK_α graphite-monochromated X rays from a Rigaku rotation anode X-ray generator. Mössbauer transmission spectroscopy was performed at temperatures of 295, 77, and 4 K with a 30-mCi $^{57}CoRh$ source. The spectra were fitted by the least-squares method to form Lorentzian-shaped adsorption lines, and the results were compared with those of various reference compounds.

Laboratory biofilm reactors. Laboratory biofilm reactors were set up to simulate underground biofilms for further investigation. Glass vessels were filled with chipped granite and irrigated initially with synthetic URL water (7). The water of the aerobic reactor was oxygenated and circulated by air piped into the reactor at a pressure of 2 to 3 atm (ca. 200 to 300 kPa), and that of the anaerobic reactor was circulated by gravity. Both reactors were run at room temperature (ca. 20°C) under dim lighting conditions to discourage the growth of photosynthetic bacteria and to mimic URL conditions (see reference 7 for more details). Inoculants were obtained by homogenizing a portion of the biofilm from room 405 in the URL. Since it was difficult to obtain reasonable growth over short periods of time, the water was augmented with 2 g of ferric ammonium citrate $liter^{-1}$ to provide a carbon and energy source (7). The same cell morphotypes seen in the URL were obtained from the aerobic and anaerobic reactors. No attempt was made to isolate and purify either the cells in the simulated biofilms or those collected from the URL.

Geochemical modelling. To determine what compounds could be chemically precipitated (rather than biologically formed) from oversaturation in oxidizing (E_h = +150 mV) and reducing (E_h = −50 mV) environments, calculations were made with the WATEQ4F program (2), which assumes FeII-FeIII equilibrium at a temperature of 20°C. The computed saturation index (SI) for minerals is expressed as SI = $\log_{10}(IAP/K_{sp})$, where IAP is the ion activity product and K_{sp} is the solubility product.

RESULTS

Although the URL shaft cuts the two major fracture zones, there is only one small splay fracture within the main excavation, and below 300 m no fractures have been encountered (Fig. 1). The underground environment is thus unusually dry, and the biofilms described here were the only major ones found (Fig. 2).

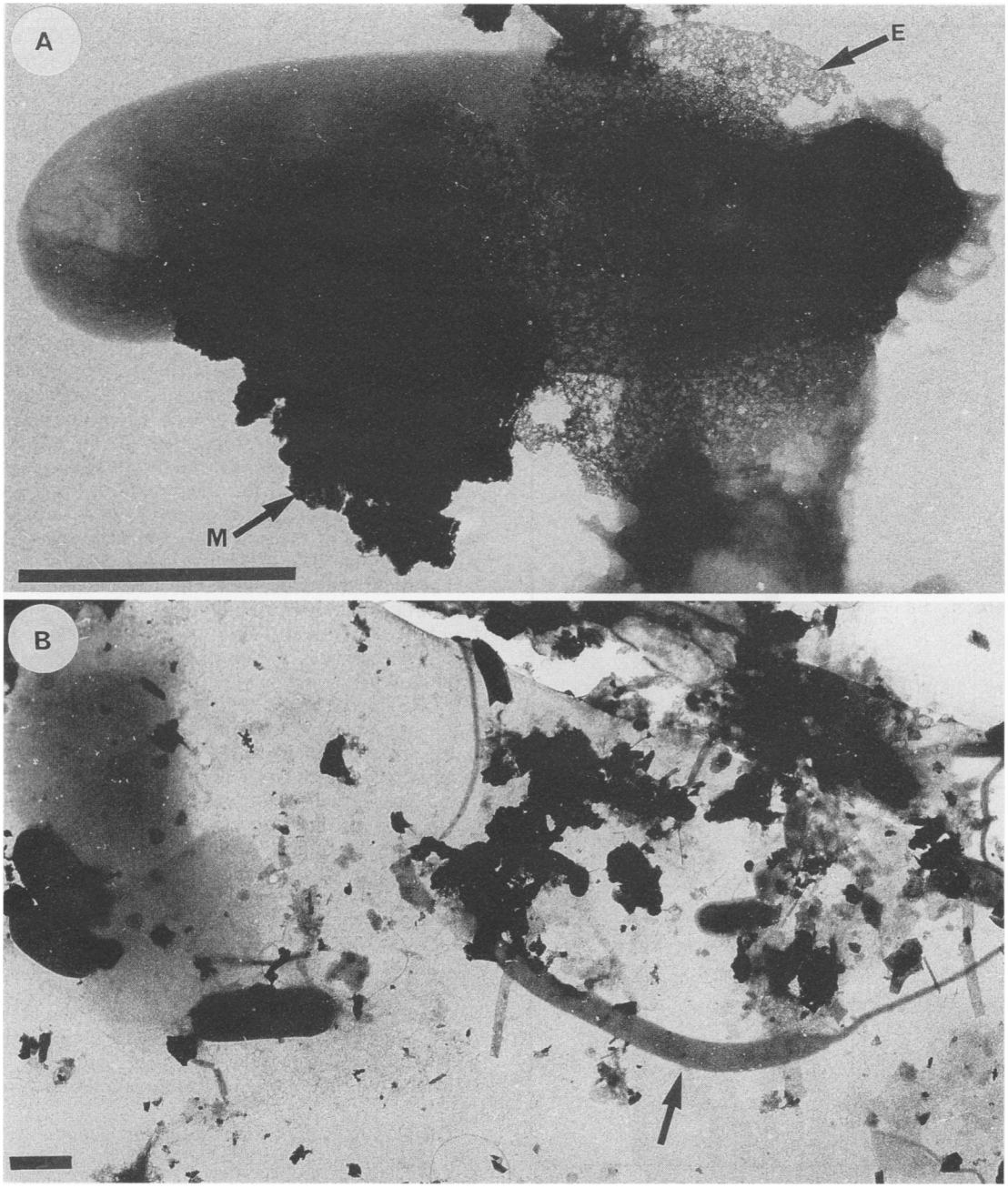

FIG. 3. (A) Negative stain of a flagellated gram-negative rod from borehole 130; E, exopolymer matrix; M, fine-grained minerals; bar, 0.5 μm. (B) A lower-magnification negative stain from the vent raise which contains both gram-negative bacteria, including caulobacters (arrow), and gram-positive bacteria; bar, 0.5 μm.

Borehole 130. A dark-colored biofilm formed on the 130-m level at the base of the drill hole draining an iron-rich xenolith. The biofilm varied in size, depending on the amount of water draining through the borehole. This biofilm consisted of a single cell morphotype, a flagellated gram-negative bacterial rod possessing a paracrystalline array (S layer) surrounding the bacterium but underlying an exopolymer matrix.

By transmission electron microscopy, the cell surface and associated extracellular matrix (Fig. 3A) showed extensive mineralization of <5-nm particles containing Fe, Mn, Si, Ca, K, and, in a few samples, Ti. The groundwater contained mainly Na, Ca, Mg, Cl, and Si but less Fe and Mn. The minerals associated with the biofilm thus have considerably more Fe, Mn, and K but rather less Na, Ca, and Mg than the groundwater (Table 1; Fig. 4A). The source of Fe, Mn, Si, Ca, and K could be from hornblende in the amphibolite since water releases these ions, and although Mg and Al are also solubilized, little was found in the biofilm. Oxygen was the only counterion detected in the mineral phase, and the elements are therefore probably present either as oxides or hydroxides, both of which are commonly nucleated by bacteria (5).

Ventilation raise. An extensive biofilm, light grey to light

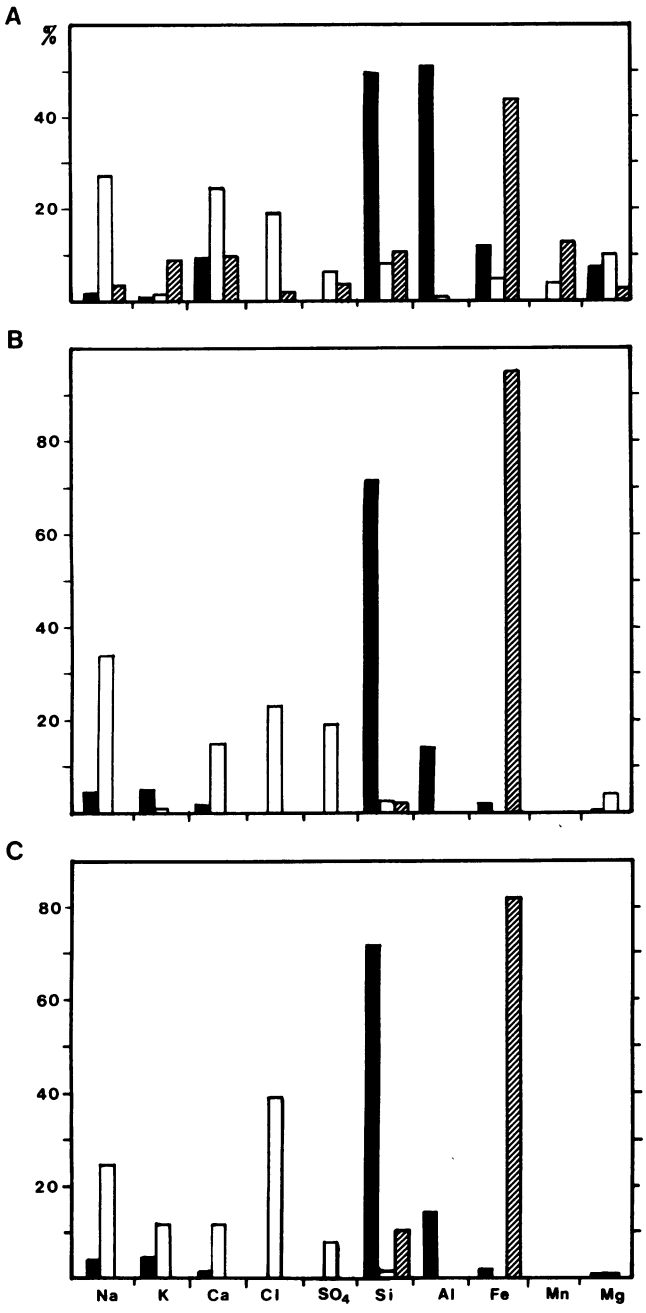

FIG. 4. Comparison of the percent components of the rock (■), water (□), and biofilm (▨). (A) Borehole 130; (B) ventilation raise; (C) room 405.

brown, completely covered the surface of the ventilation raise walls above the 240-m level. The walls were kept wet by groundwater flowing from the fractures and were also exposed to gaseous carbon and nitrogen endproducts of explosives used during the excavation. There was a wide range of gram-negative and gram-positive bacteria present, including prosthecate morphotypes resembling caulobacters (Fig. 3B). Bacteria possessing an S layer similar to those found in borehole 130 were also seen. Within this biofilm there was extensive mineralization dominated by Fe with some Si. In one sample, the EDS of a platy mineral contiguous to a bacterial cell showed entrapment of Si and Al (unpublished data), and both hydrated amorphous and dehydrated platy interstratified phases were identified.

The groundwater at this level contained increased amounts of Na, Cl, SO_4 and Ca ions. The main element in the grey granite of the vent raise walls is Si, with a lesser amount of Al and some K, Na, and Fe (Table 1; Fig. 4B). The minerals most susceptible to dissolution under low-temperature conditions here are plagioclase and biotite and are the probable sources of Na, Ca, and Mg (18).

Room 405. Room 405 was excavated in the grey granite on the 420-m level. The rock walls were extensively fractured by strain release to a depth of about 30 cm as a result of the excavation. The water irrigating this biofilm had originally been used for drilling and construction activities and was taken from a local creek at the surface, since there are no known groundwater sources at this level. The water apparently accumulated in the damage zone, beneath the concrete floor, so when the borehole was excavated it provided a route for the water to flow through the fractures to a lower level (Fig. 5). The biofilm developed rapidly (4 to 6 weeks) along the southeast wall of the hole and eventually covered all the wet surface (about 20% of the borehole surface).

This biofilm has been the most thoroughly investigated of the three, since its formation was witnessed shortly after the borehole was drilled. The water initially flowed at 10 liters day^{-1}, decreasing to approximately 1 liter day^{-1} after 3 months. This irrigating water was originally from the surface and contained a range of bacteria, including sporeformers, as well as protozoans and nematodes. It had also been contaminated by ammonium nitrate from blasting materials and a hexanol possibly from a floor-cleaning fluid, both of which could have provided nutrients. In some places the biofilm was up to 10 mm thick and initially appeared greyish with light-brown streaks running through it similar to that in the vent raise; darker brown markings developed later. Runnels were formed by water flowing down the wall within the biofilm; when breached, they had a positive water pressure, smelled of sulfide, and were stained black. The surfaces and exopolysaccharide of the bacteria were highly mineralized, sheaths were often present surrounding chains of cells (Fig. 6), and frequent accumulations of polyphosphate (EDS showed high P) and β-polyhydroxybutyrate (identified by large size and by C and O peaks by EDS) were seen within the cytoplasm, all of which indicate nutrient limitation or some other growth-related stress (4).

The granite in this room has the same composition as that of the vent raise, but at this depth the groundwater has much higher concentrations of Cl, Na, and SO_4 ions (Table 1), as well as the contaminants nitrate and hexanol. The main elements concentrated within this biofilm were Fe and Si, although the concentration of iron in the water is less than 20 μg $liter^{-1}$. This is the same concentration as that in the vent raise but 2 orders of magnitude less than in borehole 130; furthermore, the amount of iron in the grey granite is six times less than that in the amphibolite (Table 1; Fig. 4C).

Although no attempts to isolate pure cultures were made, we believe that both iron-oxidizing and sulfate-reducing bacteria were present in room 405 biofilms. More than 60 groundwater samples from the URL have all shown considerable populations of iron-oxidizing and iron-reducing bacteria, while only a quarter of the samples was found to contain sulfate reducers (7). A more recent study also confirms the presence of both types of iron bacteria (34). The first attempts to culture these bacteria in the laboratory were unsuccessful because of the

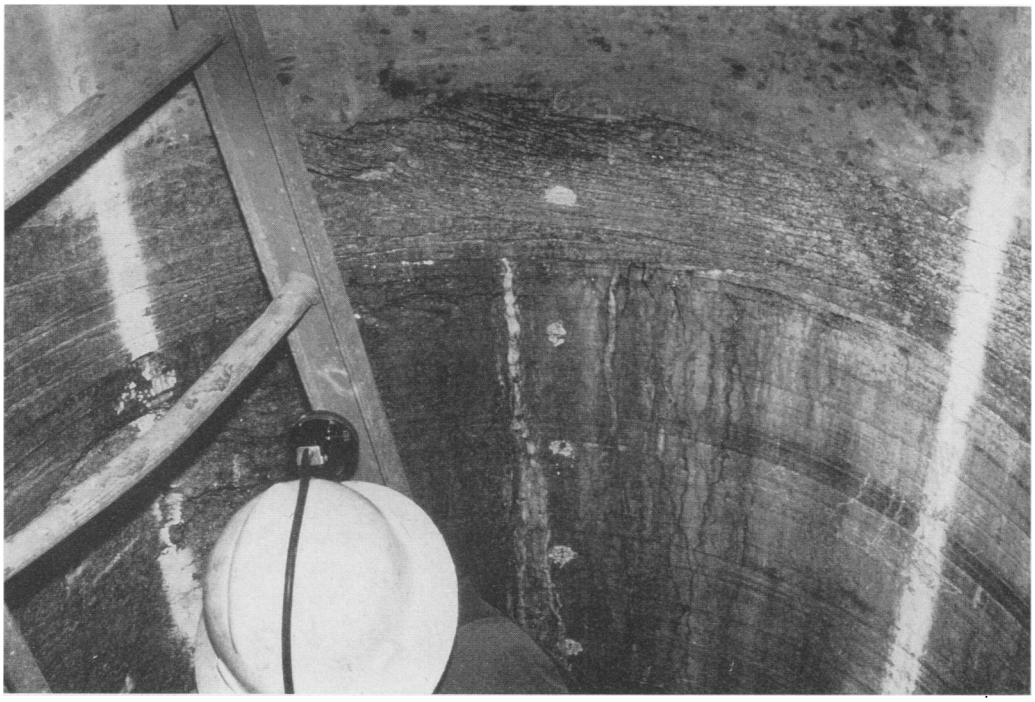

FIG. 5. View of the room 405 biofilm.

large number of protozoans that grazed on them when they were freed from the biofilm. Treatment with cycloheximide reduced the protozoan numbers; over time protozoan grazing diminished and was not a problem.

Samples for analysis by Mössbauer spectroscopy and X-ray diffraction were obtained aseptically both from the biofilm in room 405 and from the laboratory biofilms described above; these were rapidly freeze-dried to prevent alteration of the iron minerals. The aerobic culture produced a brown sludge, while the anaerobic culture formed a black precipitate. There was no sludge or coloration in sterile abiotic reactors, confirming that these were biologically mediated reactions between microbes and metal ions.

The Mössbauer spectra of the brown samples at room temperature indicated ferric iron [Fe(III)] as small particles of protoferrihydrite (Fig. 7A). The spectrum taken at 4.2 K showed a broadened sextet, similar to that observed both in deep-sea iron-manganese nodules and in ferric gels deposited near freshwater springs (9). The loss of water molecules over time causes small ferrihydrite particles to coalesce into larger hematite grains, and we have observed a small signal, similar to that of fine-grained hematite, in several specimens left open to the air.

The Mössbauer spectra of the black fragments of the biofilm and sludge from the anaerobic bioreactor showed two broad absorption lines at 295 K, with a large isomer shift and large quadrupole splitting, characteristic of ferrous iron [Fe(II)] (31) (Fig. 7B). The best fit was to two quadrupole doublets with almost equal intensities, the first of which matched those of siderite [$FeCO_3$] (25) (Fig. 7C and D), and this has been confirmed by X-ray diffraction (Fig. 8). The second doublet could not be attributed to any known Fe(II) compound, but since studies on other bacterial systems have shown that initiation of mineral formation is due to ion adsorption to a bacterial surface (32), we tentatively believe this component to be Fe(II) held in loose attachment by negatively charged microbial polymers. Further Mössbauer measurements at 77 and 4 K of air-protected black sludge strongly indicated that 55% of the Fe(II) was present as siderite. From the broadening of the X-ray diffraction peaks, the size of the crystallites was estimated to be between 2 and 3 nm, comparable to the size of the iron precipitated around the bacteria as seen by electron microscopy.

The WATEQ4F modelling program (2) was used to investigate the possibility of chemical precipitation in the biofilm by using the concentrations of ions as analyzed from the irrigating water of the biofilm. Two different oxidation potentials (+150 and −50 mV) were modelled, since biological activity in microenvironments determines the redox state. Hematite was shown by this program (Table 2) to be oversaturated (positive numbers) in both aerobic and anaerobic conditions for all biofilms. Pyrite was oversaturated in all six conditions modelled, even when only a minimum of HS^- was present, whereas siderite showed undersaturation.

White streaks formed over the water runnels in the biofilm after 4 months and were shown by EDS to be gypsum ($CaSO_4$). The results from the modelling program (Table 2) also suggested that gypsum should not be chemically precipitated. It seems likely that gypsum is present only where, possibly due to evaporation of water from the surface, there is sufficient saturation. However, this effect could be selective entrapment of Ca followed by $CaSO_4$ precipitation by the biofilm, similar to that recently seen with a cyanobacterium in a freshwater lake (32), for although gypsum is often found within fractures, it is rare in the Lac du Bonnet batholith.

DISCUSSION

Elements concentrated in the URL biofilms must have been present in either the host rock or the groundwater or migrated from the surface waters after excavation. However, we could find no discernible correlation between those elements con-

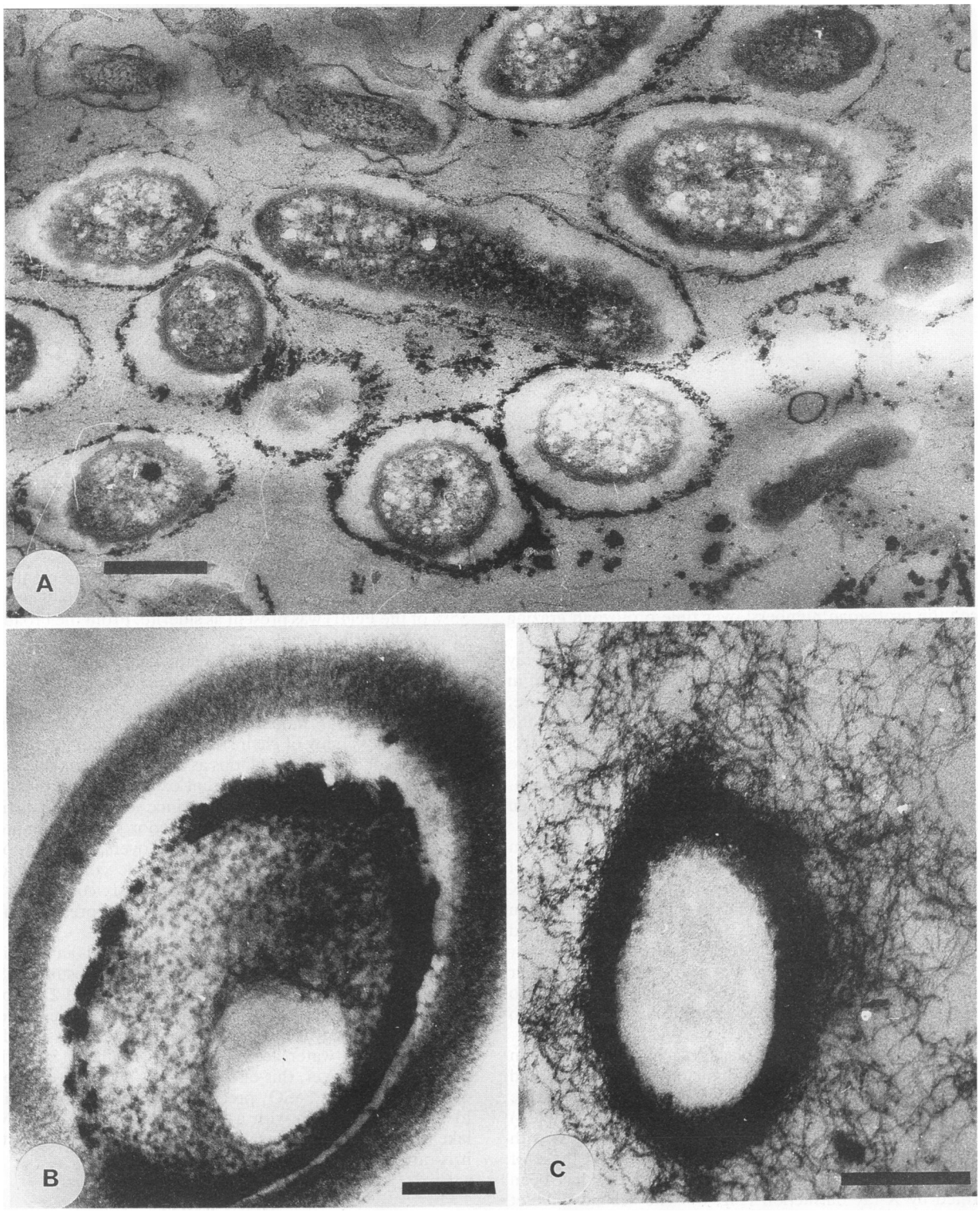

FIG. 6. Thin sections of various bacteria from the room 405 biofilm. (A) Low-level-magnification image showing the cellular morphotypes; bar, 0.5 μm. (B and C) Higher magnifications showing extensive exopolymers and their mineralization; bars, 0.1 μm (B) and 0.5 μm (C).

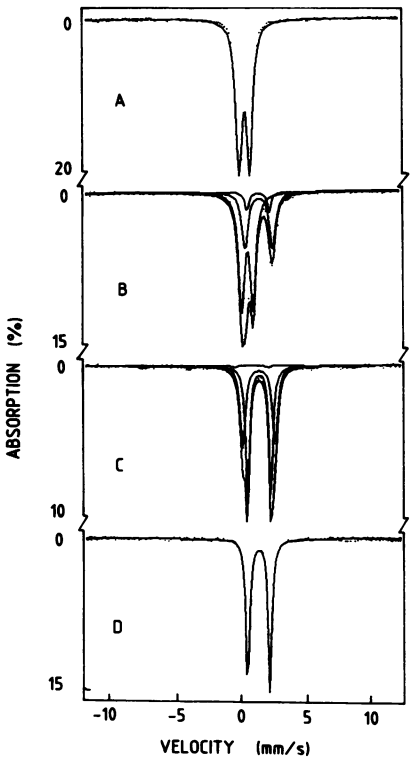

FIG. 7. Mössbauer transmission spectra of a biofilm with brown coloration (A), biofilm with black coloration (B), cultured specimen protected from oxidation (C), and siderite (for reference) (D). The spectra were obtained at 275 K.

centrated in the biofilms and the composition of either the water or the surrounding rock. Therefore, it appears that selective sorption by the bacterial consortia must have taken place and that this is unique for each URL environment. The fine-grained minerals associated with the surfaces of the bacteria and scattered throughout the matrix are thus thought to have developed de novo through bacterial surface-mediated nucleation.

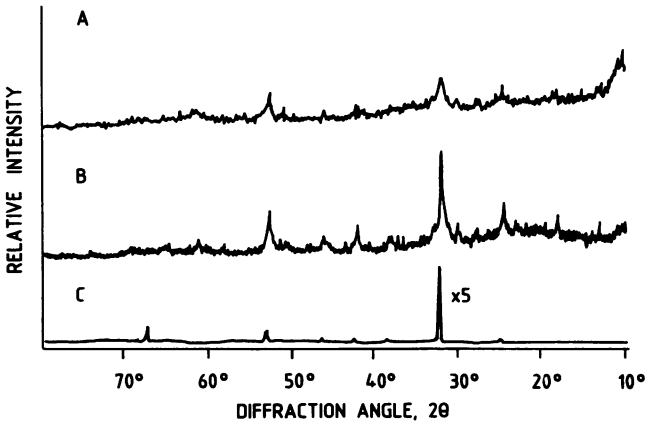

FIG. 8. X-ray diffractograms of a biofilm with black coloration (A), black precipitate from a laboratory bioreactor (B), and reference siderite (C). The scale of relative intensity in panel C is five times greater than that in panels A and B.

TABLE 2. SI (log IAP/K_{sp}) of some minerals from the irrigating biofilm water in both aerobic (+150-mV) and anaerobic (−50-mV) environments at 20°C, calculated by WATEQ4F

Mineral	E_h (mV)	SI in:		
		Borehole 130	Vent raise 240	Room 405
Ferrihydrite	150	3.97	1.13	1.12
	− 50	2.25	− 0.77	− 0.83
Goethite	150	9.86	7.02	7.01
	− 50	8.14	5.12	5.06
Gypsum	150	− 2.85	− 2.26	− 1.95
	− 50	− 2.86	− 2.26	− 1.95
Hematite	150	21.34	16.04	16.04
	− 50	17.90	12.24	12.12
Pyrite	150	16.90	14.60	16.56
	− 50	12.22	9.32	9.22
Quartz	150	− 0.64	0.26	0.55
	− 50	0.64	0.26	0.55
Siderite	150		− 4.53	− 4.49
	− 50		− 3.05	− 3.07

Presumably, the bacterial consortia in the biofilms are able to influence both pH and E_h so that each cell is enshrouded in a microenvironment (32). This in turn determines the local chemical stability fields, allowing different minerals to be precipitated. The nature of the local conditions could be determined by whether the elements are passively accumulated or metabolized for energy.

All three of the URL biofilms have specifically accumulated Fe and Si, which should have originated from the grey granite. Biofilms associated with more complex granites had a wider spectrum of elements; e.g., Mn, Ca, K, and Ti were present in the biofilm attached to the xenolithic granite of borehole 130. This suggests that biofilms can accumulate only dissolved species that are in equilibrium with elements present in the rock, and it is the biofilm microorganisms that ultimately determine which elements are sorbed. The URL is an extremely dry environment, but when water is introduced for drilling purposes, there is considerable staining of the rock by iron, apparently mediated by biofilm formation. When these drill holes are sealed, the water dries up and there is no further staining, confirming that the presence of water is necessary for iron mobilization in the URL.

In all cases, the concentration of Si was much greater than that of Fe in the groundwater samples, even though Fe was the predominant element found in the biofilms. It seems likely, therefore, that while Si might be passively absorbed by bacterial surfaces, it is not as interactive as Fe and is not actively accumulated within cells (36). This would result in an extremely low concentration of Fe in the water. While energy-producing Fe reactions are known to be widespread in natural environments (22), no metabolism of Si has been reported for bacteria.

Growth in laboratory biofilm reactors has confirmed that both the aerobic oxidation of Fe(II) to Fe(III) (with the formation of ferrihydrite or hematite) and the anaerobic reduction of Fe(III) to Fe(II) (with the precipitation of siderite) were taking place. The formation of siderite in the biofilm was unexpected. Until recently, most black iron precipitates in natural environments were thought to be iron sulfides, which are produced by the reaction of HS^- (from bacterial sulfate reduction) with reduced iron to form pyrite (FeS_2). However, it has now been shown (10, 30) that bacteria can enzymatically reduce Fe(III) to Fe(II), which may in turn form siderite.

Although the concentration of sulfate in the URL groundwater is considerable (48 mg liter^{-1}), it was only when the biofilm was disrupted that there was any smell of sulfide, and none could be detected in the water. Biofilms on sewage trickle filters have a zone of oxic respiration at the outer surface, while sulfate reduction occurs in the inner, more anaerobic zone (21). The H_2S produced in this zone was then reoxidized as a narrow reaction band, preventing escape of the H_2S to the overlying water. We suggest that the room 405 biofilm also has this narrow band of sulfide oxidization, since it was not until the biofilm was breached that there was any indication of sulfide.

Recently, sulfate-reducing bacteria in some marine environments have been shown to preferentially reduce Fe(III) and possibly nitrate (10, 30). The reduction of sulfate should therefore take place only in the absence of Fe(III) or when iron-reducing bacteria are not available. The Fe(II), produced by the dissimilatory Fe(III) reduction, may then form siderite by reacting with carbon dioxide (as HCO_3^-) formed during the fermentation of organic carbon. Because of reaction stoichiometry, there would be insufficient carbonate in the URL water to react with all the Fe(II) produced, leaving a considerable portion in solution or sorbed onto the biofilm.

We suggest that in room 405 iron is leached from the biotite in the granitic host rock into the groundwater as Fe(II) and, at the outer face of the biofilm in contact with the air, it is bacterially oxidized to Fe(III), forming ferrihydrite or hematite. In this region there is also precipitation of gypsum, generally over areas where iron has already been precipitated. The long time required for the iron to be precipitated (about 3 months) is probably explained by the low concentration of iron in the groundwater (less than 20 μg liter^{-1}). At the inner anaerobic face of the biofilm, next to the rock wall, Fe(III) is reduced back to Fe(II), which is then either sorbed onto the biofilm itself or reacted with HCO_3^- to form siderite. It is this mineral which stains the rock and the inside of the water runnels black. The structure of the room 405 mineralized biofilm after these various reactions is shown diagrammatically in Fig. 9, where the major energy reactions appear to be oxidation of Fe(II) and reduction of Fe(III).

The banded-iron formations of the Archean contain sedimentary layers of iron in both oxidized and reduced forms that are intercalated with silicon bands which may or may not contain iron silicates. These iron bands, which spread over large expanses, may be only millimeters thick; however, their formation has not yet been satisfactorily explained (19). The URL biofilms could indicate how Fe and Si were accumulated, since they cannot be accounted for by physical processes alone. Furthermore, our results show that narrow bands of oxidized and reduced iron can form in close proximity to one another so long as a microbial biofilm mediates their deposition, allowing even hematite and siderite, which have very different stability fields, to exist only millimeters apart. Microbial mats are known to have been present in shallow Archean seas, and these could have been responsible for the formation of large-scale banded-iron formations.

Biofilms are important regions of microbial activity in both aerobic and anaerobic oligotrophic groundwaters, but their reactions vary depending on the local conditions. Bacteria are known to form clay complexes (35, 36) and to immobilize heavy metals (5, 14, 38). These systems could therefore have the potential to provide a natural barrier to diffusible toxic and radioactive metallic wastes. Furthermore, some iron-reducing bacteria have been shown to be capable or reducing U(VI) to U(IV) (23). Since uranium can be a constituent of plutonic rock as well as a major component in nuclear fuel waste, this reaction could be important in the transport of uranium in the natural environment, since U(VI) is soluble while U(IV) is not. This may also be a factor in the natural deposition of some uranium ores.

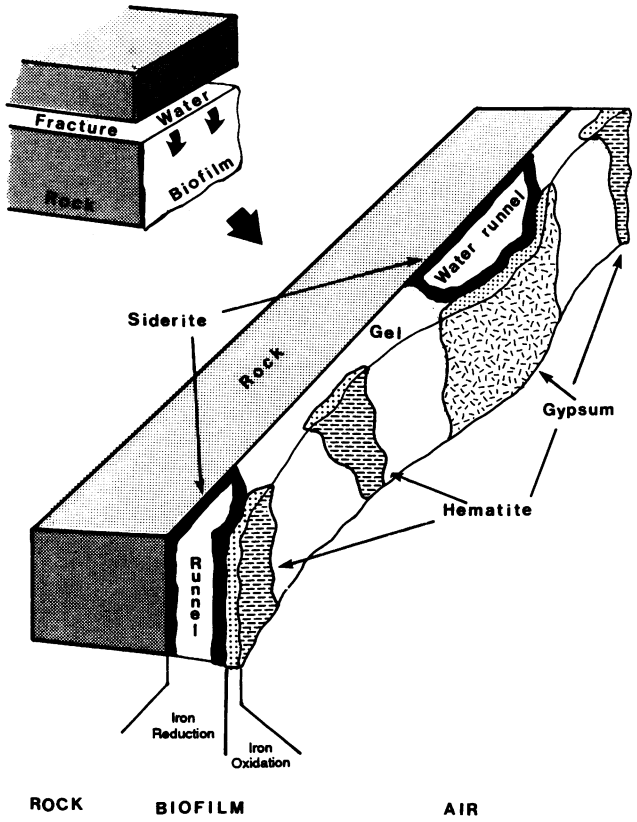

FIG. 9. Schematic representation of proposed structure of the biofilm from room 405.

In summary, biofilms on deep subsurface mineral surfaces are able to selectively accumulate elements dissolved in natural waters. This accumulation may be considerable, provided that there is a substantial source to maintain the concentration in the water. Presumably, in crystalline rock, biofilms can occur only where the rock is fractured. Although the disposal of nuclear fuel waste in crystalline rock should take into account the effects of microbial activity, it seems from these initial results that the formation of biofilms could be beneficial by sorbing possible contaminants.

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