# EPISODIC FOSSILIZATION OF MICROORGANISMS ON AN ANNUAL TIMESCALE IN AN ANTHROPOGENICALLY MODIFIED NATURAL ENVIRONMENT: GEOCHEMICAL CONTROLS AND IMPLICATIONS FOR ASTROBIOLOGY.

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**Introduction:** Morphological features suggestive of microbial origin have been observed by scanning electron microscopy (SEM) in several categories of meteorites. These including meteorites thought to have originated on Mars (including ALH84001 and Nakhla) [1,2] and some carbonaceous chondrites [3]. In ALH84001, the putative fossil microbes occur in carbonate globules distributed along fractures [1]. Contentious issues include (1) the origin of the morphological features (microbial or inorganic) [1,4-6]; (2) whether the carbonate with which they are associated formed at temperatures conducive to life [7-10]; (3) whether the putative microbes were indigenous to the rock or were introduced post-mortem.

Observations relevant to some of these questions were made during scanning electron microscope (SEM) investigation of a microbiologically active terrestrial aquifer [11]. This paper reports an instance of carbonate mineralization of microbes in this well-characterized terrestrial environment. The distribution of the mineralogically preserved forms in space and in time is described. Geochemical conditions in the system that were conducive to morphological preservation of the microbes are explored, along with implications for physical biomarkers in the terrestrial and possible extraterrestrial microbial fossil records.

**Methods:** Mineralogical and textural consequences of mineral exposure to an anoxic contaminant plume in a glacial aeolian/outwash-sand aquifer were examined experimentally using in-situ microcosms. Grains of garnet (an iron silicate mineral known to exhibit varying surface textures in different low-temperature alteration environments [12-14]) were placed into the aquifer at a single multi-level well at depths of 25, 28, 32 and 37 feet below land surface and removed after a period of eleven months. Prior to and during the eleven-month incubation period, the geochemical conditions of the plume were evaluated. The microcosms placed at depths 25 and 28 feet experienced more contaminated reduced water than those placed at the 32 and 37-foot depths. Both crushed and euhedral garnet grains were considered in the experiment; some were kept as controls and observed without exposure to the aquifer. The crushed grains are nearly end-member almandine in composition and their fresh conchoidally fractured surfaces were not previously exposed to a weathering environment. Euhedral grains are intermediate almandine-pyrope, and were naturally exposed prior to acquisition for this study. Organic matter was removed from experimentally exposed garnets by standard soil-chemical techniques. Garnets removed from the plume and control garnets were imaged with (SEM) and chemically analyzed with Energy Dispersive Spectrometry (EDS).

**Results:** Precipitates, typically individual rugose rodlike forms or clusters of ellipsoidal bodies 2-10 microns in long dimension, were discovered on both crushed and euhedral grains from depths corresponding to the most reduced groundwater, but not at greater depths. Precipitates are most likely calcite. EDS indicates the precipitates contain Ca, C, and O; sand above the water table is depleted of sand-size carbonate fragments, whereas sand below the water table is up to ~6% carbonate; and groundwater is near equilibrium with respect to calcite. Precipitates strongly resemble encrusted microorganisms; many have filamentous protrusions morphologically resembling extracellular polysaccharides that connect to the mineral surfaces. Some cell-shaped precipitates are demonstrably hollow, consistent with mineralization of a sheath around microbes, followed by removal of organic material. Precipitates on crushed garnets are randomly distributed, whereas on euhedral grain surfaces the precipitates occur along striations (growth steps) and within pits. On smooth dodecahedral surfaces of euhedral grains, no micron-scale morphological features occur, but a biofilm of submicron thickness is widespread. Micron-scale microbe-shaped structures did not appear on garnets incubated at the deeper 32 to 37 ft levels, or on the control garnets. Cell counts and 16S rRNA indicate that a diverse but variable microbial assemblage was present in the aquifer before and during the experiment [15], but no precipitates were observed on native aquifer sand pre-experiment. We conclude that these

features were formed by reactions that occurred exclusively within the contaminant plume.

During the bulk of the eleven-month incubation period, the well experienced very unique biogeochemical conditions as a result of a test flow-through bioreactor system located approximately 270 feet upgradient from the test well. Aquifer mineralogy and groundwater geochemistry favored carbonate stability over a much longer period of time than the duration of the microcosm experiments. However, mineralization of microbes occurred only during a brief time interval, probably in response to anthropogenic groundwater alkalinity fluctuations during attempted bioremediation upgradient of the microcosms. This combination of subtle increases in calcite saturation coupled with enhanced microbial activity may have lead to the observed biofossilization.

**Discussion:** We interpret the larger elongate and elliptical features found on the surface of crushed garnet grains in the shallow chemically reduced plume to be carbonate-mineral encrustation of microorganisms. We base this interpretation in part on the hollow elongate forms (Fig. 1), which appear to be encrustations or sheaths of carbonate around microbes that are themselves no longer present (decayed during incubation, microcosm recovery, and/or sample preparation for SEM). Such hollow mineralized forms are known from other microbiologically active aquifer systems [16] as well as from terrestrial thermal springs [17]. Furthermore, the entire ensemble of features satisfies a number of morphological criteria identified by Westall [18] for identifying fossil microorganisms. Finally, the rapidly formed features on aquifer-exposed garnet compare favorably with numerous published SEM images of modern microorganisms, naturally fossilized microbes, and experimentally fossilized microbes. The aquifer carbonate encrustations of microbes are morphologically very unlike rapidly formed terrestrial carbonates produced by Antarctic weathering of stony meteorites [19, 20].

**Implications:** Microbes colonized the surfaces of experimentally introduced garnet, and were sheathed in carbonate, during an eleven-month exposure. The microbial morphological features in the plume did not form on native aquifer material over the ~10ka existence of the aquifer; they only formed during a unique biogeochemical episode in the history of the aquifer. During this episode, the system was likely a predominantly methanogenic system. The short timescale for preservation is consistent with previous work on fossilization in chemically reducing environments. The observed biomineralization preserves a "snapshot" of the overall microbial history of the aquifer. Microbial species preserved may not be representative of the complete microbial assemblage at the time of garnet colonization, let alone represent the microbial assemblage characteristic of any longer lived interval of the aquifer's history. The apparent requirement that geochemical conditions must be unusual and fluctuating in order to facilitate preservation is similar to the necessary conditions for terrestrial instances of exceptional preservation (e.g. Mazon Creek type).

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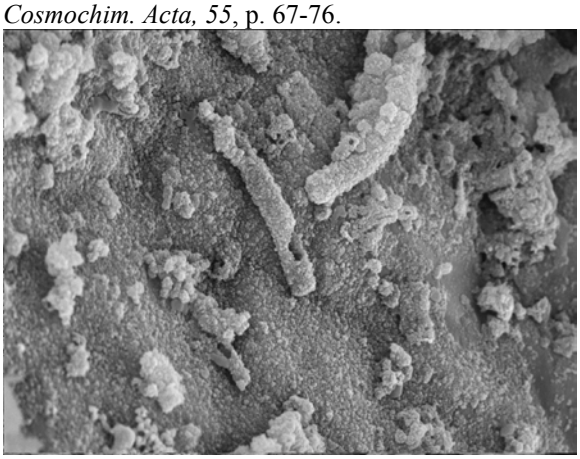

Fig. 1. SEM image of microcosm-exposed garnet from 25ft depth showing hollow elongate forms. Field of view is 20 microns wide.