THE SILICIFICATION OF THERMOPHILIC BIOFILMS: ARE GEOTHERMAL DEPOSITS ANALOGS FOR THE PRESERVATION OF EXTRATERRESTRIAL LIFE?

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SUMMARY – Laboratory experiments have been conducted to study the growth of silica sinters in the presence and absence of thermophilic bacteria at 60°C. In all experiments, a ledge of silica sinter grew off the substrate surface following the air-water interface along the meniscus. In abiotic experiments, this was composed of an aggregate of well-formed silica spherules. In biotic experiments, these spherules were poorly formed due to the inference of organic molecules from the bacterial nutrient. Numerous bacterial cells were observed in the biotic experiments. These form part of thermophilic biofilms. Silicification of these biofilms has a strong influence on the silica sinter textures found.

1. INTRODUCTION

Geothermal deposits of silica sinter display a great variety of complex textures. Microscopic analysis of these deposits shows abundant evidence of microbial activity and sinters often preserve morphological information on the micro-organisms that were present during their formation. The role of microbial surfaces as substrates for silica precipitation suggests that a close connection between microbial communities and silica sinter textures exists (Cady and Farmer, 1996; Mountain *et al*., 2003). However, some have concluded that microbes are not necessary to form some specific silica textures (Walter, 1976; Lowe and Braunstein, 2003), in particular, spicular textures and attribute them to hydrodynamic processes. If certain sinter textures require the presence of micro-organisms and their associated extracellular polysaccharides (EPS) then the presence of these textures in ancient deposits is strong evidence for a biogenic origin. In such deposits mesoscopic textural details are more likely preserved than the microscopic organisms themselves. For example, the presence of stromatolites in many Precambrian terranes is well accepted based on their mesoscopic details. This possibility has profound implications to the interpretation of ancient sinters and possibly to hydrothermal deposits yet to be found on extraterrestrial bodies.

Recent field studies at Champagne Pool, Waiotapu, have shown strong evidence for a microbial origin of the extensive spicular textured sinter around the pool (Handley, 2004). Also present are silica laminae composed of vitreous silica that appear not to have a biogenic origin (Fig. 1). Together, microbe-rich porous layers alternating with vitreous laminae form the "box-girder" texture that comprises the spicular sinter. However, the relationship between the micro-organisms and the silica laminae remains inconclusive. In order to address this problem, laboratory experiments were conducted to simulate the process of sinter formation in the presence and absence of microbes. These experiments are not possible in the field as it is impossible to prevent contamination of sinter by the local thermophilic microbial community.

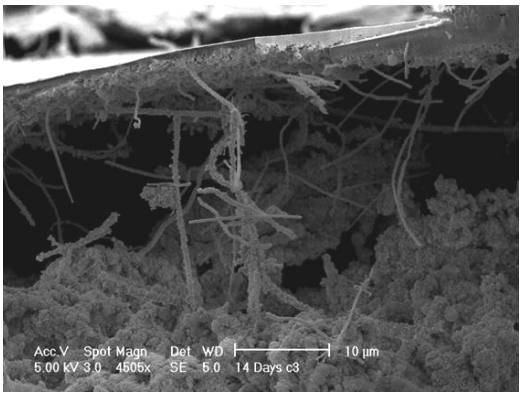

Figure 1. SEM photomicrograph showing a vitreous silica lamina with a smooth upper surface supported by silicified filamentous micro-organisms. The sample is from a field experiment at Champagne Pool, Waiotapu.

2. METHOD

A single pass flow-through set-up was constructed to simulate a geothermal spring. It consists of a HPLC pump, a high temperature oven, an orbital shaker/incubator, and two peristaltic pumps (Fig. 2). A convenient geothermal fluid was supplied by filtered Wairakei Power Station separated bore water collected from the wastewater drain. This fluid contains 570 ppm SiO_2 of which approximately 120 ppm is monomeric at room temperature, the remainder of the silica being polymeric and colloidal. The HPLC pump maintains fluid flow at 0.5 ml min^{-1}. The fluid passes into a 6 metre stainless steel coiled tube contained within the high temperature oven

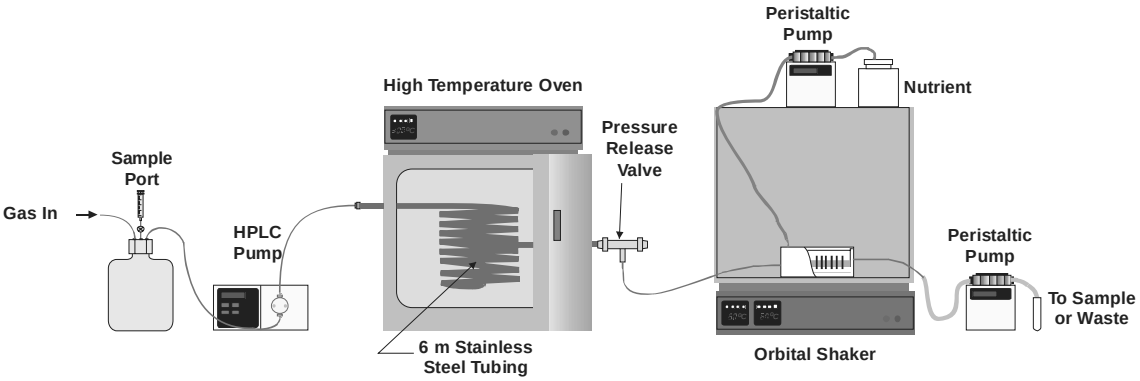

Figure 2. Schematic diagram showing experimental apparatus used to simulate silica sinter growth conditions.

(300°C). During its passage through the tube the solution is sterilised and the polymeric and colloidal silica is depolymerised. This was confirmed by the spectrophotometric molybdate method. After exiting the oven the solution enters a Teflon tray enclosed in the incubator (60°C). The tray contains upright glass microscope slides that protrude above the water surface. Water depth is kept constant by the peristaltic pump that removes excess fluid. A second peristaltic pump can be used to deliver sterilised bacterial nutrient (tryptic soya broth) when required.

3. RESULTS

The first experiment simulated the process of sinter growth in the absence of micro-organisms (no nutrient was delivered to the tray). After 24 hours, a visible line of silica sinter formed on the slide at the air-water interface. Scanning electron microscope (SEM) examination of this line showed it to be a downward-curving, unsupported ledge protruding about 300 m from the slide (Fig. 3A). The shape of this ledge indicates that it is the result of the growth of silica at the air-water interface along the meniscus. Later samples showed continued meniscal growth of silica. Overlapping ledges indicate movement of the meniscus over the experimental period. Also present were rhythmic lineations like strand-lines (Fig. 3B), lobate forms (Fig. 3B) similar to those seen at the mesoscopic scale at some hot springs, and thin discontinuous silica laminae (Fig. 3C) identical to ones in field experiments at Champagne Pool (Handley, 2004). Close SEM examination of the ledge revealed that it is composed of silica spherules that have aggregated to form a perhaps solid, but possibly porous, mass. The steps in the ledge appear to be the result of the coarsening of the silica spherules. The explanation for this is unclear. Transmission electron microscope (TEM) examination of the silica precipitates showed only isolated silica spherules approximately 40 nm in diameter (Fig. 4A). The uniform nature of the silica spherules indicates that they homogeneously nucleated in the fluid and grew during cooling. Consolidated sinter was not observed in the TEM sections possibly due to loss during sample preparation.

During the second experiment, bacterial nutrient was continually added in small quantities. After 48 hours, meniscal growth of the sinter occurred on the slides (Fig. 5A). The sinter was similar to that of the previous experiment, however, it appeared more irregular and porous. Smooth laminae were present that had cracked and curled back revealing a porous granular under surface. This texture is attributed to the growth of silica laminae along the air-water interface and is similar to that observed at Champagne Pool (Fig. 1). A possible explanation for this texture involves the agglomeration of small silica spherules at the air-water interface. These thicken due to the attachment of further spherules to the underside, however, these remain in contact with the solution allowing coarsening and granular growth. TEM examination of the colloidal silica particles in the solution shows that, although they are relatively uniform in size, they are no longer spherical and are quite irregular (Fig. 4B). This is due to the disruption or templating of silica growth by organic molecules from the bacterial nutrient. However, this is not a result of micro-organisms. The 48 hour sample also showed that presence of abundant micro-organisms (Fig. 5B). Both bacilliform and filamentous forms were present. Bacilliform cells occurred aligned in colonies or as disorganised masses. Filamentous forms grew in aligned masses that were encased in silica that subsequently cracked to reveal a honeycomb-like texture (Fig. 5C). Perhaps the most interesting was the presence of isolated colonies of bacilliform bacteria that grew just below the air-water interface (Fig. 5D). These were variably silicified and are interpreted to be the beginning of spicular growths.

Several other experiments were conducted using varying nutrient strengths. All resulted in the formation of a thermophilic biofilm that covered the water surface (Fig. 6A). This biofilm becomes silicified forming a hard, brittle ice-like coating. TEM examination of the biofilm showed an upper

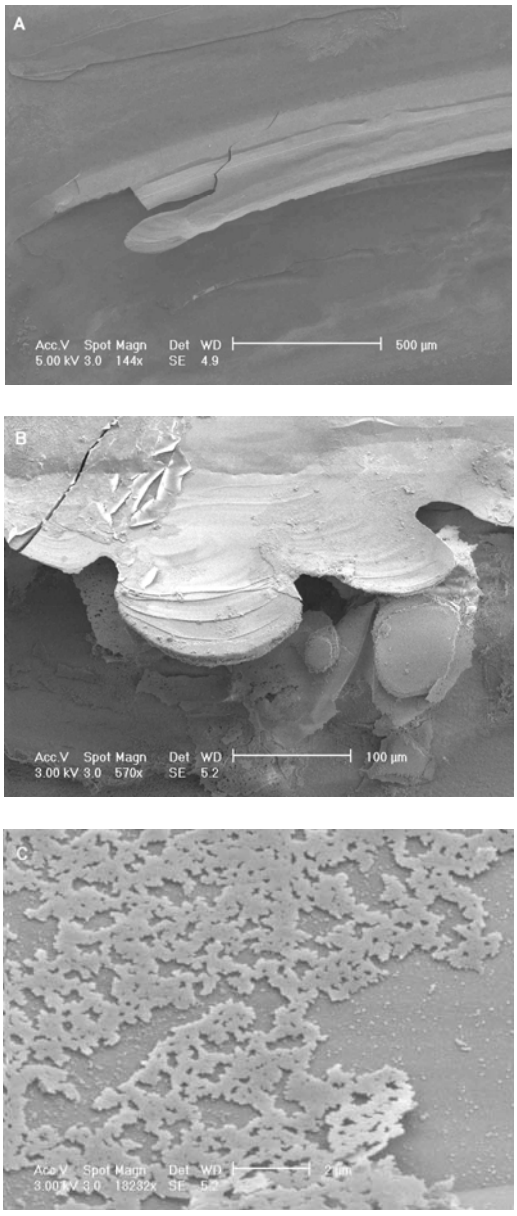

Figure 3. SEM photomicrographs from abiotic experiments. **A.** Sinter ledge formed after 24 hours. **B**. Sinter ledge displaying rhythmic laminations (strand-lines?) and lobate morphology. **C**. A discontinuous sinter lamina.

sharp surface composed of amorphous silica that encompassed countless bacterial cells (Fig. 6B). The continuity of the amorphous silica matrix declines downwards away from the water surface until there is only bacterial cells. It is not possible to prove the existence of continuous EPS mucus between the bacterial cells as this is lost during sample preparation but its presence is suggested by the density and coherence of the bacterial cells. It is also interesting to note that very few cells appear to have intracellular silica suggesting that the cells may still have been viable after encasement in silica.

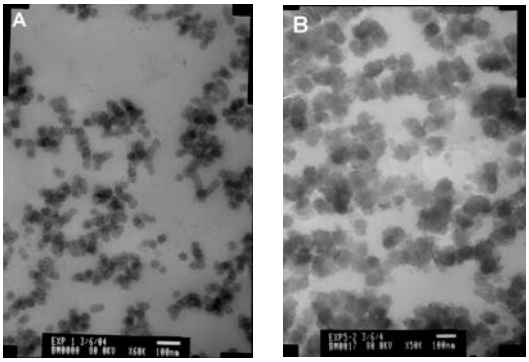

Figure 4. TEM photomicrographs showing colloidal silica particles. **A.** Uniform silica spherules from the abiotic experiment. **B.** Diffuse irregular colloidal particles from an experiment using bacterial nutrient. Scale bar is 100 nm in both photographs.

4. DISCUSSION

In all experiments, we observed the formation of silica laminae and the presence of colloidal silica particles. This indicates that the presence of silica laminae is not diagnostic of microbial activity and that the process of silica polymerisation and colloid growth can occur without bacterial interference. Laminar growth involves the agglomeration of silica spherules at the air-water interface and their cementation by addition of monomeric silica. This process continues on the underside of laminae leading to thickening. In those experiments where nutrient was used, microbial growth occurred and silica sinter was more extensive, eventually covering the entire surface of the tray. This is attributed to either: i) the formation of a biofilm at the water surface that acts as a substrate for silica precipitation. The growth of this silica-biofilm composite continues as more bacteria attach to its bottom; or ii) the abiotic growth of the silica film initially at the air-water interface that is then rapidly colonised by micro-organisms on its underside. The question as to which mechanism dominates requires further study. Rhythmic layering in the silica also occurred in all experiments. This is attributed to detachment and migration of the meniscus down the silica ledge as it grew. Another possibility is that vibrations cause movement of the meniscus. The presence of spaced isolated colonies of bacteria just under the air-water interface is very similar to that observed in field experiments at Champagne pool. In the latter case, these are unquestionably the origin of the spicular growth texture and it is likely that laboratory experiments with longer durations will produce spicular growth textures. This will prove that micro-organisms are necessary for the development of this sinter texture.

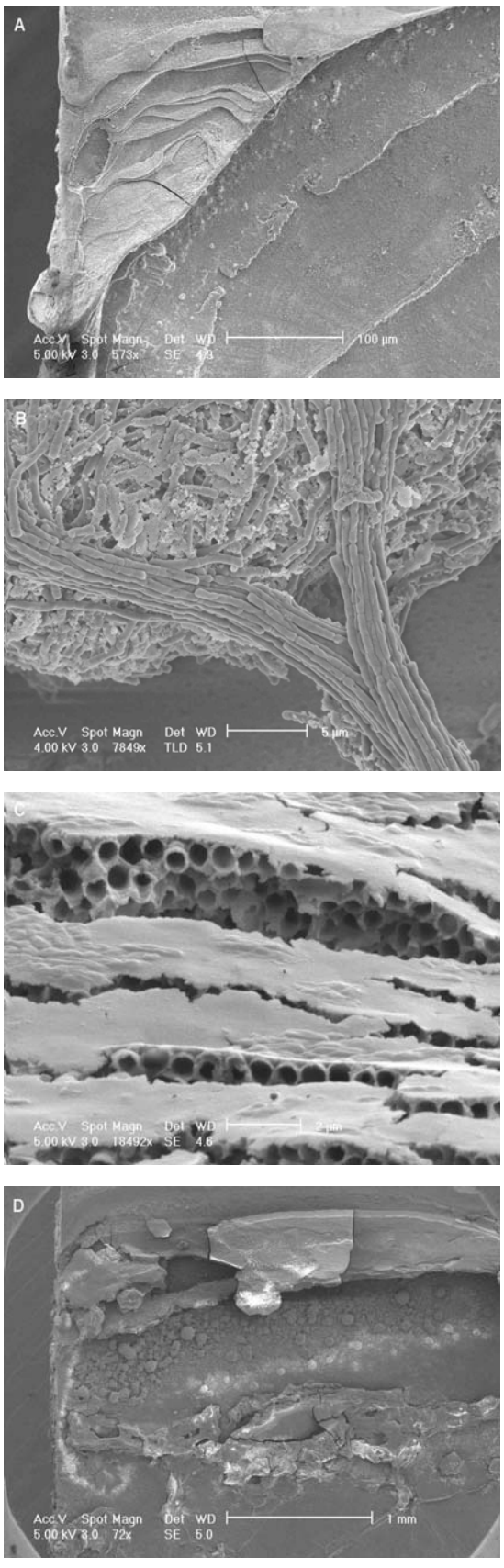

Figure 5. SEM photomicrographs showing textural details from biotic experiments. **A.** Multiple overlapping ridges of meniscal growth at edge of slide. **B.** Bacilliform and filamentous bacterial cells. **C.** Honeycomb texture formed by silicification of filamentous biofilm. **D.** Silica ledge and isolated bacterial colonies.

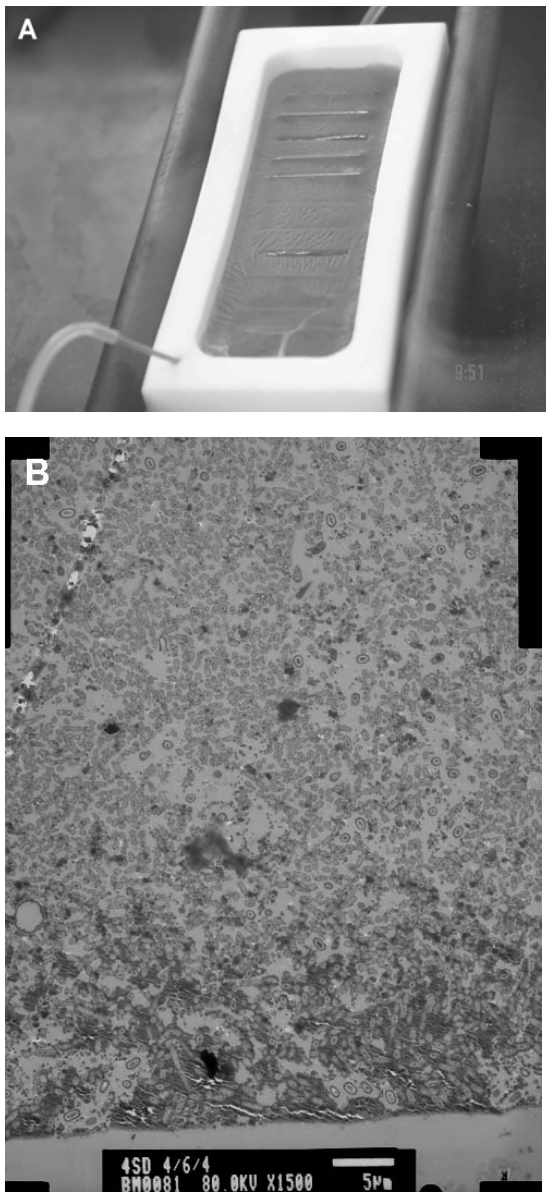

Figure 6. A. Photograph of Teflon tray during a biotic experiment showing the biofilm that has covered the water surface. The tray is 5 cm in width. **B.** TEM photomicrograph showing cross section through the biofilm. The sharp boundary is the air-water interface (sample is inverted). Dark grey material is silica, lighter grey ovoids are bacteria. Scale bar is 5 m.

5. REFERENCES

Cady, S.L. and Farmer, J.D. (1996). Fossilization processes in siliceous thermal springs: trends in preservation along thermal gradients. In: *Evolution of hydrothermal ecosystems on Earth (and Mars?)*, G.R. Brock and J.A. Goode (Eds.), John Wiley, Chichester, pp. 150-173.

Handley, K.M. (2004) In Situ Experiments on the Growth and Textural Development of Subaerial Microstromatolites, Champagne Pool, Waiotapu. Unpublished M.Sc. Thesis, University of Auckland, 111 p.

Lowe, D.R. and Braunstein, D. (2003). Microstructure of high-temperature (>73 degrees C) siliceous sinter deposited around hot springs and geysers, Yellowstone National Park; the role of biological and abiological processes in sedimentation. *Can. J. Earth Sci.* Vol. 40, pp. 1611-1642.

Mountain, B.W., Benning, L.G. and Boerema, J.A. (2003). Experimental studies on New Zealand hot spring sinters: rates of growth and textural development. *Can. J. Earth Sci*., Vol. 40, pp. 1643-1667.

Walter, M.R. (1976). Geyserites of Yellowstone National Park: An example of abiogenic stromatolites. In: *Stromatolites*, M.R. Walter (Ed.), Developments in Sedimentology Vol. 20, pp. 87-112.