BIOMINERALISATION IN NEW ZEALAND GEOTHERMAL AREAS: A PROGRESS REPORT

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SUMMARY – Field biomineralisation experiments, initiated in March/April **2001**, continue to be monitored and sampled. At Wairakei main drain, growth rate experiments confirmed that the fibres comprising silica precipitates are cored by filamentous microorganisms. Growth rate is **–10** mm/day. At Waiotapu, glass slides placed in August 2001, continue to grow and are now terminated by silica spicules up to 7 mm in length. Growth rate is **–0.023** mm/day. At Rotokawa, growth rates are extremely slow (<0.001 mm/day) probably due to the lack of wave action and low pH values. At Tokaanu, sinter growth rate is –0.002 mm/day and encompasses appreciable amounts of green cyanobacteria and other microorganisms. At Waikite, mineralisation at the higher temperature end of the artificial terrace is dominated by calcite that appears to have no biotic characteristics (Growth rate -0.027 mm/day). Further along the terrace at Waikite, the decrease in temperature allows growth of considerable cyanobacteria, as well as, thick biomats in the side drain. Results from Ngatamariki and Orakei Korako are hard to interpret because of variable sinter growth due to improper positioning of the slides. A new experiment has been initiated at Ngatamariki.

1. INTRODUCTION

The presence of thermophilic organisms in hot springs is not a recent discovery. Von Hochstetter, as early as **1864**, mentions the presence **of** green "plants" in hot pools of the TVZ. Since then several researchers have studied thermophilic microorganisms from hot springs (Skey, **1878; ** Weed, **1889;** Kaplan, **1956;** Brock and Brock, 1971). Several studies on sinter morphology are also available in the more recent literature (c.f. Jones et al., 2001; Campbell et al., **2001).** There are few studies, however, that have investigated the relationship between community succession, growth rate, sinter morphology, and trace metal distribution.

In March/April **2001** several biomineralisation experiments were initiated in seven geothermal areas in the Taupo Volcanic Zone. The purpose of these experiments is to determine the contribution of microorganisms to the textural development of sinter structures and whether these same organisms biomediate metal distributions in silica sinters. The initial objective during the first year was to determine the type of biomineralisation and the rate of growth of this mineralisation at each locality. Preliminary results from Wairakei, Waiotapu, and Rotokawa were presented at the 23rd New Zealand Geothermal Workshop (Mountain et al., **2001).** Based on these results, further experiments were initiated using modified methods between May **2001** and July **2002.** Many of these, and earlier, experiments continue to the present.

This contribution comprises a progress report on developments since November **2001**. Included are updates on sinter growth experiments, trace metal measurement results, and preliminary molecular biology results on species diversity.

2. METHODS

Seven geothermal systems were chosen for this study. These included Wairakei, Rotokawa, Waiotapu, Waikite, Ngatamariki, Orakei Korako, and Tokaanu (Table 1). In each of these areas, glass microscrope slides were placed to allow bacterial growth and biomineralisation to form.

Table 1. Location, pH, and Temperature of Experiments*

System	Location	pH	T(°C)
Wairakei	Main Drain	8.5	62
Rotokawa	North Springs	3.0	60 - 85
Waiotapu	Champagne Pool	5.5	71 - 75
Waikite	Artificial Terrace	7.6	55 - 100
Ngatamariki	Pavlova Spring	7.2	74 - 83
Orakei Korako	Tim & Terry Geyser	7.4	76-91
Tokaanu	Old Bore	7.5	56-62

*Temperatures are variable. Approximate range is shown.

Slides are taken from the pools at various intervals that depend on growth rate estimates. Samples for SEM and TEM are preserved in **2.5%** glutaraldehyde and prepared by sequential ethanol or acetone exchange and critical point drying. Samples for DNA extraction were preserved in doubly-distilled water or 0.85% saline solution. LA-ICP-MS analyses were done at Macquarie University, Sydney, using a New Wave/ Merchantek laser microprobe attached to an Agilent 7500 ICP-MS. Metal counts were normalized using SiO_2 concentrations determined on a Cameca **SX-50** electron microprobe.

3. RESULTS

3.1 Wairakei

The main drain at the Wairakei Power Station contains the combined wastewater from the western borefield. The chemistry of the water is uniform throughout the drain with pH ~8.5, 1900 Cl^- and 570 ppm SiO_2. Between March and November 2001, the temperature of the right drain (facing downstream) was typically 62°C but can vary periodically due to surface runoff. Mineralisation in the drain consists of fan-shaped accumulations of amorphous silica with a white to pink colouration (Fig. 1a). A dark brown colouration occurs periodically after heavy rain due to the introduction of fine silt (Fig. 1b). These episodes provide markers for estimating growth rates of the silica mineralisation. SEM and TEM studies show that the mineralisation is composed of narrow amorphous silica fibres <1 μm in width. Further silica precipitation causes the older fibres to thicken and form a porous mass. It is estimated that the growth rate of individual fibres or groups of fibres is approximately 10 mm/day.

In our first experiment, sampling intervals were too infrequent to allow close examination of the initial stages of silicification due to the rapid growth rate. It was originally assumed that the fibres were the result of silica precipitation on the surface of filamentous microorganisms, however, this may not have been a valid assumption. Another possibility is that it is the result of silica growth on polypeptide or polysaccharide chains excreted by microorganisms further upstream. These could be caught on the glass slides as the exudates are carried past. In order to test this hypothesis, a further experiment was carried out in which samples were collected at 2 - 4 hour intervals over a 29 hour period. This allowed closer examination of the initiation and growth of the mineralisation. After about 17 hours, the slides show only a few isolated filaments (Fig. 1c). Over the next 12 hours, the number of filaments increased rapidly and on the final slide (at 29 hours) the whole surface is covered with extensive silica fibre growth (Fig. 1d). These results indicate that the rate of growth is more exponential than linear with time, strong evidence that microorganisms are indeed coring the silica fibres.

Significant microbial diversity was revealed in samples collected from the Wairakei drains using culture-based methods employing a range of media and temperatures (30-60°C). Following isolation of genomic DNA from each pure culture 16S rDNA was amplified by PCR with bacterial primers. Twenty-seven phylogenetically distinct bacterial sequence types were determined by partial (~600bp) or near complete (~1350bp) 16S rRNA gene sequence analysis. Isolates sharing >97% partial or near complete 16S rRNA gene sequence homologies with sequences in GenBank were affiliated with ***Thermus, Meiothermus, Bacillus, Tepidomonas, Thermomonas, Porphyrobacter, Thermonema, Hydrogenophilus*** spp. and previously uncultured bacteria.

3.2 Waiotapu, Champagne Pool

A progression of biomediated silicification features is present around the margin of Champagne Pool (T = 71-75°C, pH 5.5, 1926 ppm Cl^-, 430 ppm SiO_2). Below the pool surface, silica mineralisation is covered with a layer of orange filamentous material composed of sulphur coatings on filamentous microorganisms (Fig. 1e). The sulphur is rich in Sb (1.1%) and As (0.6%) explaining the bright orange colour. Immediately at and slightly above the pool surface, a rim of yellow native sulphur is present. This is regularly washed with pool water causing orange particulates floating of the pool surface to accumulate. Spicular microstromatolites grow on this sulphur layer and become progressively larger with distance from the pool. Periodic exposure, the result of wave action, of the sulphur layer and rapid cooling of the pool water allows minute quantities of silica to accumulate eventually building up a layer that remains above the water during more quiescent periods. Once a relatively persistent subaerial "island" is formed, spicular microstromatolites can proceed to grow.

In order to determine the textural/biological succession and growth rate of these structures, glass slides were placed in the pool in August 2001 with their ends protruding approximately 5 mm above the surface. Several have been collected over the last year to preserve time shots of mineralisation development (Fig. 1f). By June 2002, considerable silica mineralization had grown on the slides (Fig. 1g). Individual spicules are up to 7 mm in height translating to an average growth rate of 0.023 mm/day. In cross section, the microstromatolites consist of alternating thin layers of organism-free silica and thicker layers of siiicified filamentous microorganisms (Fig. 1h). The laminations are clearly not the result of seasonal changes as they are too numerous for a 10 month period. On the other hand, they cannot be due to diurnal cycles as they are too few in number. One possibility is that they are the result of varying wave intensity due to changing wind conditions. During calm periods, temperature sensitive organisms would be able to grow on the tips of the spicules unharmed. Periodically, more intense wave activity would result in hot pool water topping the spicules, arresting microbe growth and forming a layer of abiotic silica. The origin of the laminations is the subject of further experimentation with 80 new slides being placed in the pool in July 2002.

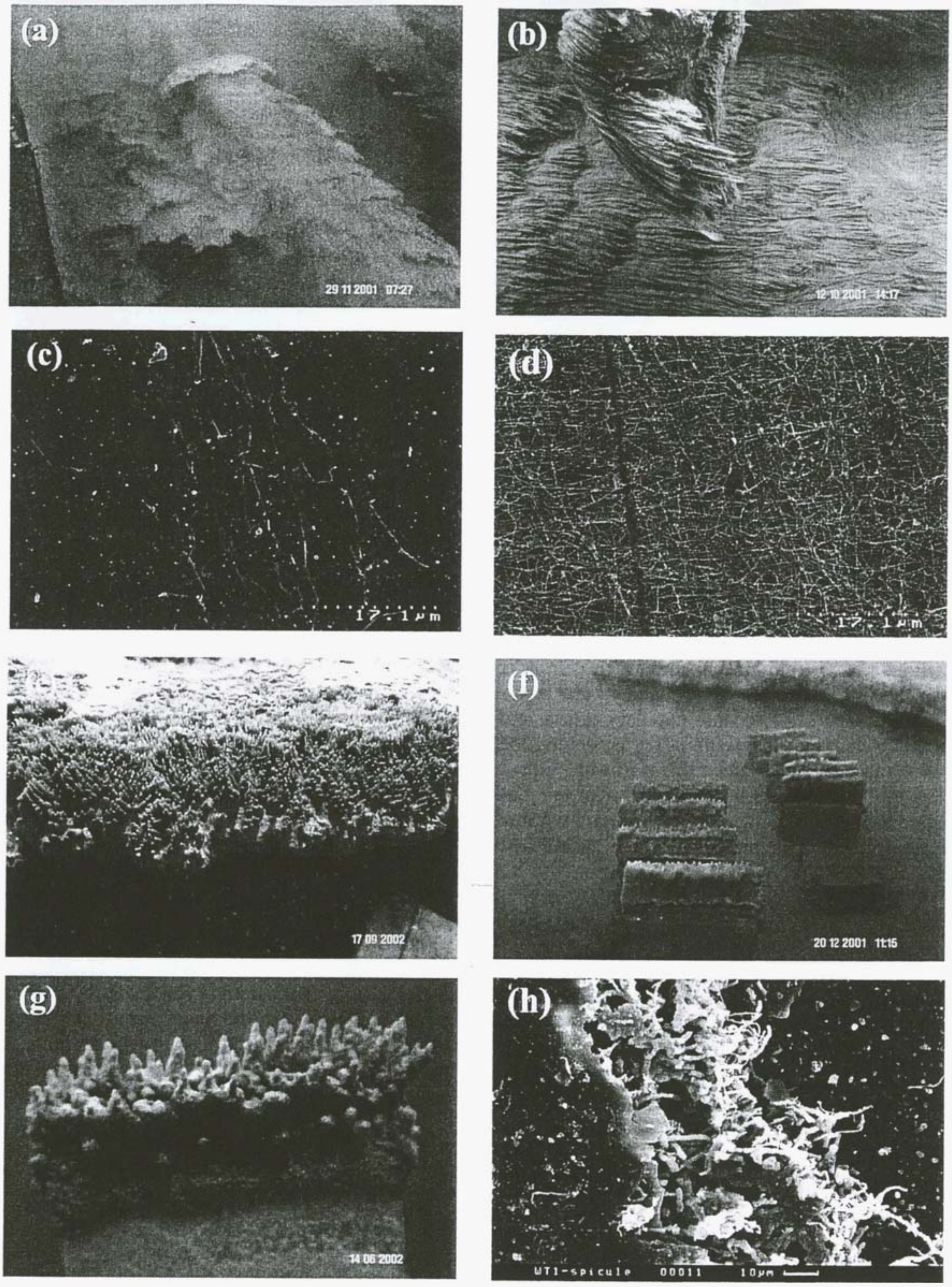

Figure 1. a) Main drain at Wairakei Power Station. Large fan-shaped growths of amorphous silica cover the concrete walls and internal divider. Drain is approximately **3** metres in width. b) Underwater photograph in Wairakei main drain showing amorphous silica growths. Brown colour is due to trapping of fine silt washed into the drain during a heavy rainstorm three days earlier. White tips are the growth that has occurred since that time. The large mass in the centre is a plastic rack that has been suspended in the drain for seven months. Field of view approximately 1 metre. c) SEM photograph of glass from drain after 17 hours of exposure. Only a few filamentous microorganisms are evident. Scale bar is 17.1 pm. d) SEM photograph of glass slide from drain after 29 hours exposure. Scale bar is 17.1 μm. e) Edge of Champagne Pool. Note orange particulates floating in the water (bottom). Water edge is defined by the sharp transition from deep orange subaqueous precipitates to yellow sulphur. The sulphur layer is continuously washed by wave action. Silica spicules grow in height and density with distance from the pool. f) Glass slides protruding from Champagne Pool. These slides show four months growth (August – December 2001). Note small spicules on the edge of each slide. g) Glass slide recovered from Champagne Pool in June 2002 (ten months growth). This is the same slide shown in the foreground of previous photograph. h) SEM photograph of spicule from Champagne Pool showing abiotic and biota-rich layering. Scale bar is 10 pm.

3.3 Rotokawa

The North Springs at Rotokawa sinter flat are two ebullient hot pools with temperatures greater than 85°C. Outflow from the springs (pH 2.7 - 3.2, 400 - 1200 ppm Cl^-, 600 - 1000 ppm SO_4^{2-}) flows as a shallow sheet (<0.5 cm) with temperatures ranging from 60 to 85°C. The mudflat in this outflow is populated by microstromatolites (Fig. 2a). These structures start as finely-laminated mineralisation on pumice rocks and wood fragments that originally protruded from the water. These laminations continue build upwards and outwards, creating coral-shaped structures that keep pace with the thickening mud deposits (Fig. 2b). The presence of hard sinter layers within the mud indicates that periodically the spring has been flooded by thermal waters from the larger hot lake to the south.

A series of glass slides were inserted in the mud in May 2001. Samples collected since that time show that spicule growth occurs just above the water line. A sharp boundary defines the water line above which an irregular dark gray coating of silica mineralisation is present (Fig. 2c). The shallow water depth means that wave action is insufficient to explain the coatings and their concave shape suggests that capillary action and steam condensation are responsible for wetting the slides above the water line growth. Silica mineralisation consists of numerous small spicules with a maximum height of 1 mm. The growth rate of the spicules is estimated to be less than 0.001 mm/day.

Rotokawa is well-known for its high concentrations of heavy metals, including As, Sb, Au, Tl, Hg and W, in the sinter (Hedenquist, 1986). Because the microstromatolites are exposed to these fluids through wave and capillary action, it is possible that they may also play a role in concentrating heavy metals. In order to test this hypothesis, a single microstromatolite was sampled and sectioned. Metal concentrations were measured by LA-ICP-MS across a traverse encompassing a 2 cm section of the sample from its pumice rock core outwards at 0.2 – 0.4 mm spacings. Highly elevated concentrations of As, Sb, W, Tl, Au, and Tl were found (Fig. 2d). These metals also correlated with each other quite well, especially in the darker, presumably organic-rich layers. A strong correlation between Fe and Mn is also present and these metals are enriched in the layers where the other metals are lower in concentration. These results indicate that the stromatolite is recording the redox state of the fluid with time. Laminations rich in heavy metals probably represent periods when H_2S concentrations were high while Fe/Mn-rich laminations represent more oxidized solutions.

3.4 Tokaanu

A set of glass slides, held in a plastic rack, was placed in the shallow outflow (pH 7.5, Cl^- 2100 ppm, SiO_2 290 ppm) of the artificial geyser located south of the Tokaanu thermal area in April 2002 (Fig. 2e). The purpose was to examine the silicification process of microorganisms growing in the outflow from a geyser, albeit a man-made one. The flatness of the terrace allowed a constant water depth over the rack of approximately 10 mm. Temperature decreases from 62 to 56°C with distance from the geyser over the span of the samples. The samples also encompass the transition from pink-white granular sinter to slightly greenish cemented sinter (Fig. 2e). This represents the position at which temperature decreases low enough to allow the growth of cyanobacteria.

Samples collected over the next several months showed a slow but continuous growth of cloudy silica on the slides. Preserved in the silica were green cyanobacteria as well as abundant orange-brown microorganisms. By December 2001, all slides showed a continuous coating of white to orange-brown silica (Fig. 2f). This coating was approximately 0.5 mm thick representing an approximate growth rate of 0.002 mm/day. Also of interest was the increased growth of microorganisms below the cable ties holding the slides to the rack (Fig. 2f) and the prodigious amounts of green cyanobacteria growing underneath the rack. One possibility is that the plastic rack retarded fluid flow over the terrace thus allowing a decrease in temperature and an increase in cyanobacterial growth. This, however, is not supported by the slides as no increase in cyanobacterial growth was present on them. A more likely explanation is that the plastic rack reduced light flux creating conditions more amenable to cyanobacterial growth. This is supported by the almost exact replica of the rack's grid structure by the cyanobacteria.

3.5 Waikite

The artificial terrace at Waikite consists of a 30 metre long concrete construction over which thermal water from a single bore flows (pH 7.6, Cl^- 146 ppm, SiO_2 149 ppm). Water exits the bore at 99°C and initially flows over a wide (~5 metre) series of terraces cover in small rocks. Mineralisation on these terraces consists mainly of light brown calcite. Slides placed on the terrace have been recovered over the last 18 months.

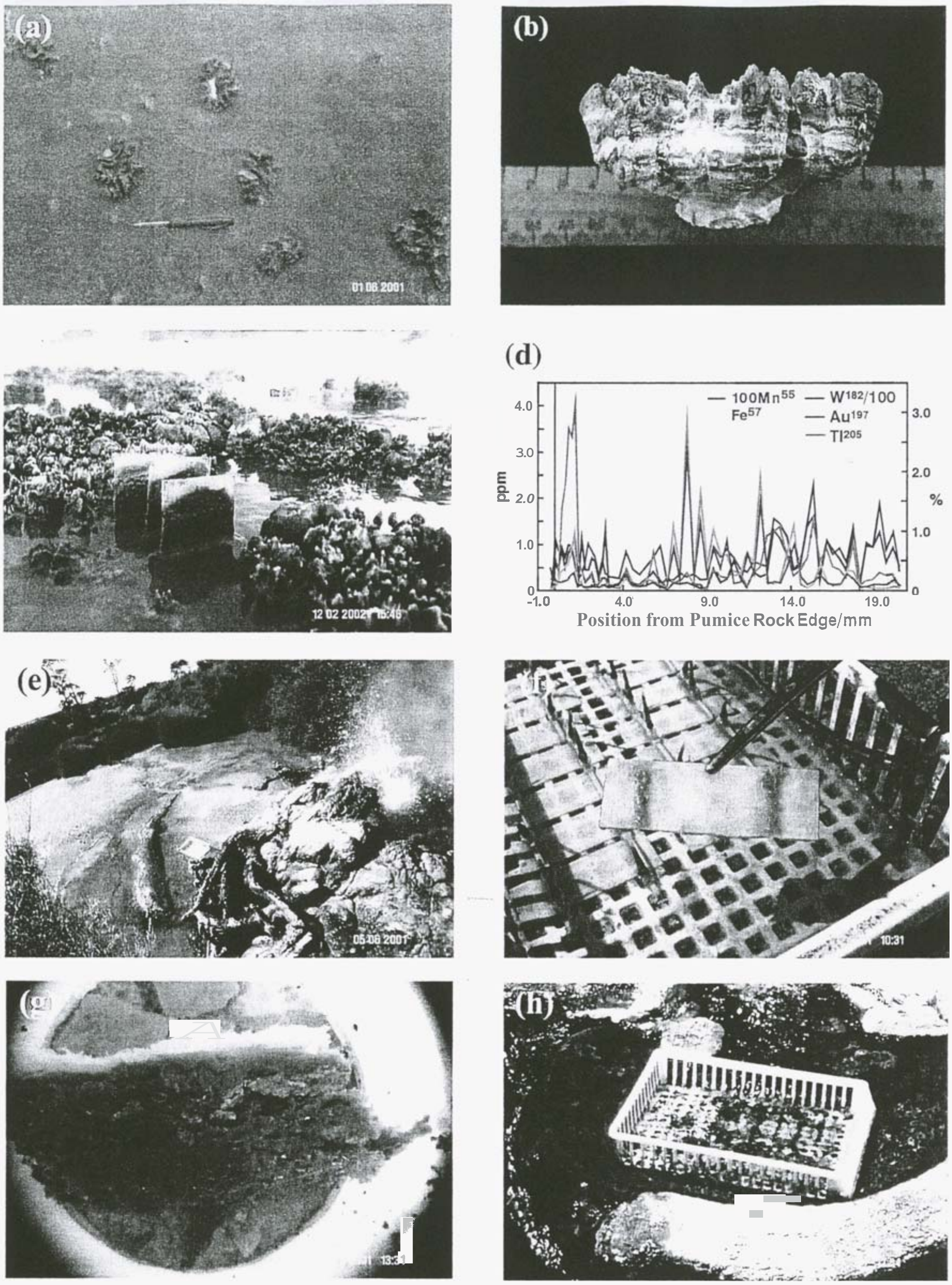

Figure **2.** a) Microstromatolites growing in muddy outflow deposits from one of the North Springs at Rotokawa. Note that only the tips of the stromatolites are exposed above the mud. b) One of the stromatolites from the location shown in Figure 2a. c) Three glass slides placed in the stromatolite field next to one of the North Springs. Slides show a dark gray coating of amorphous silica. Note the natural stromatolites surrounding the slide. Slide is 25 mm in width. d) LA-ICP-MS profiles for heavy metals, Fe and Mn across 20 mm of a sectioned microstromatolite from Rotokawa. The units for Fe and 100xMn are percent. The units for the remaining elements are ppm. Note how Fe and Mn peak in areas where the heavy metals are lower in concentration. e) Silica terrace next to the artificial geyser at Tokaanu showing the location of the plastic rack containing the glass slides. Geyser is spouting on the right. f) Glass slides from silicification experiment at Tokaanu (rack shown in Figure 2e). Note how orange-brown organisms are thicker where they were protected by cable ties. g) Biomat from Waikite artificial terrace. Round circle is bottom of glass-bottomed bucket. Brown fragments at top are pieces of the calcite layer that was originally located on top of the biomat. h) Plastic rack in plunge pool at Waikite. Rack is covered with thick growths of cyanobacteria.

The growth rate estimate for the calcite is 0.027 mm/day, however, this rate must be a maximum as calcite in other areas accumulates at a much slower rate. SEM examination of the calcite shows euhedral crystals with no apparent biota or biomediated textures. Small star-shaped crystals coating the calcite are comprised of amorphous silica.

An interesting feature of the Waikite terrace is the presence of thick biomats (up to 50 mm thick) growing in the small side drain next to the main terrace. The biomats consist of laminated organic material topped with a layer of calcite (Fig. 2g). Temperature at the top of the biomat can reach 60°C and drops to about 50°C at the bottom next to the concrete drain. Below the calcite, a gelatinous white layer is present under which the organic material grades from orange-brown to green (remarkably similar to the colour gradation found at Tokaanu). The mat is also contains abundant vesicles originally filled with the gaseous by-products of microbial activity. The laminations in the mat are probably the result of a combination of decreasing temperature and light flux with depth. Similar mats have been observed at Orakei Korako.

Further down the artificial terrace, temperature drops to below 60°C and the thermal water flows through a series of plunge pools. These pools are coated with thick masses of cyanobacteria (Fig. 2h). Slides placed in one of the plunge pools in March 2001 show a relatively steady growth of green cyanobacteria and other orange-brown microorganisms. Although the plunge pools appear to be accumulating silica sinter, the growth rate is extremely slow and no significant silicification has formed on the slides to date. This can be explained by the relatively low concentration of silica in the bore water.

3.6 Ngatamariki

Both these thermal areas are of interest because they are precipitating sinter composed of both silica and calcite (Campbell et al., 2002). Two slide racks were placed in the outflow of Pavlova Spring in April 2001 (pH 7.22, Cl^- 567 ppm, SiO_2 234 ppm). Slides placed at the high temperature end of the spring (T = 83°C) were subaqueous and showed no silica growth, however, considerable organic slime covered the slides. This did not remain on the slides when they were sampled. Slides placed further downstream from the spring (T = 74°C) showed considerable growth of flaky sinter similar to the natural material surrounding the spring. Unfortunately, it was not possible to keep the rack level and mineralisation was very discontinuous. We have set up a modified experiment in July 2002 in an attempt to obtain more representative growth.

3.7 Orakei Korako

At Orakei Korako, we were prevented from placing plastic racks in the thermal features because of their proximity to the tourist walks. Glass slides were placed in three features but because conditions are quite variable from position to position, silica mineralisation is not comparable between slides and estimated growth rates have limited meaning. No new experiments have been initiated at Orakei Korako.

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