Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA

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Abstract

Calcium carbonate precipitation and microbialite formation at highly supersaturated mixing zones of thermal spring waters and alkaline lake water have been investigated at Pyramid Lake, Nevada. Without precipitation, pure mixing should lead to a nearly 100-fold supersaturation at 40°C. Physicochemical precipitation is modified or even inhibited by the properties of biofilms, dependent on the extent of biofilm development and the current precipitation rate. Mucus substances (extracellular polymeric substances, EPS, e.g., of cyanobacteria) serve as effective Ca²⁺-buffers, thus preventing seed crystal nucleation even in a highly supersaturated macroenvironment. Carbonate is then preferentially precipitated in mucus-free areas such as empty diatom tests or voids. After the buffer capacity of the EPS is surpassed, precipitation is observed at the margins of mucus areas. Hydrocarbon biomarkers extracted from (1) a calcifying Phormidium-biofilm, (2) the stromatolitic carbonate below, and (3) a fossil ‘tufa’ of the Pleistocene pinnacles, indicate that the cyanobacterial primary producers have been subject to significant temporal changes in their species distribution. Accordingly, the species composition of cyanobacterial biofilms does not appear to be relevant for the formation of microbial carbonates in Pyramid Lake. The results demonstrate the crucial influence of mucus substances on carbonate precipitation in highly supersaturated natural environments. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: extracellular polymeric substances; microbialites; biomarkers; calcification; lacustrine; thermal springs; Pyramid Lake

1. Common views on carbonate precipitation at thermal springs

CaCO₃ precipitation at thermal springs has been considered as an essentially inorganic–physicochemical process since its recognition in the last century (e.g., Buch, 1809, p. 29). Such subaerial deposits are regarded as a result of CO₂ degassing, causing a raise of pH and shift in the carbonate equilibrium (Kitano, 1963; Friedman, 1970; Bargar, 1978). Since 1862 (Cohn, 1862) several investigators focused on photosynthetic micro-organisms which should promote CaCO₃ precipitation by CO₂ removal (Cohn, 1864; Weed, 1889), although the efficiency of this process has been questioned (Allen, 1934; Pentecost, 1990). Distinctive patterns, such as ‘streamers’, demonstrate the influence of filamentous bacteria on fabrics. Based on the observation of numerous enclosed bacterial remains, Chafetz and Folk (1984)
proposed that calcitic shrubs and clumps form by bacterial precipitation. However, the precipitation process itself is generally considered as being devoid of ‘organic’ parameters, such as physiological modification of the microenvironment. This is because supersaturation is very high and any substrate may serve as crystallisation nucleus. Pente- 
cost (1990) demonstrated that travertine shrubs form inorganically at Mammoth Hot Spring, Yellowstone National Park. Generally, the porous deposits of thermal springs (‘tufa’, for discussion of terminology see Ford, 1989) are considered to be a result of external superficial precipitation upon micro-organisms or algae (Golubic, 1973; Riding, 1991). If bacteria play any role in tufa or travertine formation, they may serve as low-energy surface sites for nucleation.

At first glance, an unequivocally inorganic system is given, when hot, Ca\(^{2+}\)-rich spring waters mix with highly alkaline lake water. At Pyramid Lake, this is apparently sustained by the presence of large pinnacles composed of decimetre-sized skeletal crystals. These structures originated at sublacustrine thermal springs during the Pleistocene (Benson, 1994). A temporal participation of micro-organisms in the formation of the pinnacles is only indicated by intercalated stromatolitic crusts, that show erect filament traces.

2. Geographical and geological setting

Pyramid Lake is the largest, athalassic saline lake in the Western Hemisphere (Galat and Jacobsen, 1985). It is located within the Pyramid Lake Paiute Indian Reservation in western Nevada, approximately 50 km northwest of Reno (Fig. 1). Pyramid Lake is an alkaline mesotrophic lake, 40 km long and up to 16 km wide, with a maximum depth of 103 m (Galat and Jacobsen, 1985). The lake basin is a NNW–SSE-trending graben structure along the western margin of the North American Basin and Range Province, which originated by extensional tectonics during the Tertiary. Andesitic to basaltic volcanics, 17 to 6 million years in age, form the surrounding hills and mountains of Pyramid Lake. In addition, rhyolites are exposed southeast of Pyramid Lake. During Pleistocene times, the basins of Nevada were flooded, forming the continuous ‘Lahontan Lake’, which reached its maximum extension at about 14,500 years B.P. (Benson et al., 1995). As a consequence of the post-glacial climatic change to arid conditions, only remnants of the Lahontan Lake exist in Nevada today, the largest of them being Pyramid Lake. During the 1930s, irrigation for agricultural projects drastically reduced the water influx by the only permanent inflow to Pyramid Lake, the Truckee River. Salinity and alkalinity of the lake rose to alarming values, so that Pyramid Lake fishery was almost ruined. Today, water inflow of the Truckee River is controlled and pH values of the lake have decreased to 9.3.

Pyramid Lake is famous for its impressive, up to 100 m high, tufa pinnacles, composed of barrel-shaped crystal pseudomorphs of dm-sized ikaite (CaCO\(_3\)·6H\(_2\)O). These pinnacles formed within the last 35,000 years, when thermal spring water discharged into the cold, deep Pyramid Lake of the last glacial. Recent ikaite formation is only observed in cold environments, like fjords of Greenland, deep submarine fans, or the highly alkaline Mono Lake during winter times (Council and Bennett, 1993). The bulk of carbonate rocks of the Pyramid Lake pinnacles reveals radiocarbon ages between 23,500 and 12,000 years B.P. (Benson et al., 1995). The youngest tufa encrustations and beachrocks formed between 3500 to 1350 years B.P. (Benson et al., 1995). Recent CaCO\(_3\) precipitation is restricted to the last remaining thermal springs at the north end of the lake, a place called ‘The Needle Rocks’ (Fig. 1). Here, numerous small warm and hot seepage sites are found at the base of tufa pinnacles, and two wells have been drilled which eject hot water. Pyramid Lake is also known for its whiting events during late summer, which are presumably triggered by nanoplanktonic cyanobacteria (Galat and Jacobsen, 1985).

Our paper focuses on calcification and microbialite formation immediately at, and in the vicinity of, these thermal springs. Where spring waters rich in calcium enter the lake, enhanced carbonate saturation and precipitation are likely to occur. In the mixing zones of spring and lake water, local whitings are commonly observed throughout the year. We selected a hot pool (Fig. 5) and a profile along the rocky shore (Fig. 2), from the immediate spring water influx to the open lake, to study the differences...
in precipitation with respect to temperature, supersaturation, and biofilm composition. As a control, water samples and non-calcifying biofilms from the shore at Pelican Point, 15 km south of The Needle Rocks, were analysed.

3. Material and methods

Electrical conductivity (EC), temperature, pH, and redox potential of water samples were measured immediately in the field. Temperature compensated conductivity (20°C) was determined with a LF 323 instrument (WTW Co.). Redox potential and pH were measured with a HI 9025 pH-meter (Hanna Instr.) and a pH 91 pH-meter (WTW Co.) equipped with pH combination electrodes (Ross), respectively. Standardisation of pH-measurements was made against NBS buffers pH 7.413 (at 25°C) and 9.180 (at 25°C). Total alkalinity was analysed in the field using a hand-held titrator and 1.6 n sulphuric acid cartridges (Hach Co.). Dissolved oxygen was determined according to the Winkler method employing the same titration instrument and 0.2 n
sodium thiosulphate cartridges. For the analysis of main cations, samples were filtered through glass fibre filters (Whatman GF/F, nominal pore size 0.7 μm) and preserved with HNO₃. In the laboratory cations were analysed by flame atomic absorption (Philips PU 9200X). Parameters of the carbonate system, partial pressure of CO₂ (pCO₂) and the saturation with respect to calcite and aragonite, were calculated with the program PHREEQE (Parkhurst et al., 1990).

Samples of microbialites and adherent biofilms were fixed with 4% formol in buffered lake water for 4–12 h, rinsed with filtered lake water, and partly dehydrated in a graded alcohol series (15–30–50–70%) prior to transport. Subsamples were stained in bulk with fluorochrome calcein to enhance contrast for fluorescence microscopy. After final dehydration (85–95–100%) in the home lab the samples were embedded in LR-White resin. Thin sections were cut with a Leica hardpart microtome. After mounting on glass slides, the sections were cut down to 5–10 μm thickness and covered. The investigation and photographic documentation was carried out using a Zeiss Axiolab microscope with phase contrast and epifluorescence equipment.

Biofilm samples for TEM studies were fixed with 4% glutaraldehyde in filtered lake water (buffered with 0.4 M cacodylic acid) for 4 h on ice. They were intensively rinsed and transported to the home lab in filtered cacodylic acid-buffered lake water. Post-fixation with 2% OsO₄ was done in artificial lake water for 1–2 h at 4°C. Following rinsing and dehydration in a graded alcohol series (15–30–50–70–85–95–100%) and propyleneoxide (2 × 100%), the samples were Epon-embedded and sectioned with a Sorvall MT2-B Ultramicrotome. Ultrathin sections were poststained in Na-acetate-buffered uranyl acetate (1%, pH 5.6, 20 min, 30°C) and lead citrate according to Reynolds (1963) (pH 12, 5 min, 20°C). The sections were examined at a Jeol 100 B transmission electron microscope at 80 kV. For SEM studies, glutaraldehyde-fixed samples were dried with Peldri II (Pelco) according to the manufacturer’s instructions. Investigations were performed with a Hitachi SEM model S-2300.

Samples for biomarker analyses were air-dried on aluminium foil and sealed for transport in plastic bags with a large amount of drying agent (silica gel). Organic extracts were obtained from (1) the active mat of a recent stromatolite (Fig. 2, ‘green calcifying Phormidium-biofilm’, sample PL96/B6; 0–1 mm), (2) the inner core (30–40 mm) of the same stromatolite consisting of a dense micritic carbonate, and (3) a Pleistocene stromatolite sample obtained from the prominent carbonate buildups exposed subaerially at the lake shore. Carbonate was removed by treatment with diluted hydrochloric acid. The residues were washed neutral with
distilled water, centrifuged, and ultrasonically extracted with dichloromethane/methanol (3:1; v:v). The resulting extracts were fractionated by silica gel column chromatography. Hydrocarbons were eluted with n-hexane and analysed by gas chromatography and combined gas chromatography–mass spectrometry (GC–MS). Analytical details are described elsewhere (Thiel et al., 1997).

4. Water chemistry

The water chemistry of Pyramid Lake is generally dominated by sodium, chloride and alkalinity (Na–Cl–HCO₃ type) with 1600 mg l⁻¹ Na⁺, 2000 mg l⁻¹ Cl⁻, and 23 meq l⁻¹ total alkalinity (resp. 1400 mg l⁻¹ as HCO₃⁻). Magnesium content is moderate (110 mg l⁻¹), calcium content is low (8 to 9 mg l⁻¹), and alkalinity (i.e. HCO₃⁻ and CO₃²⁻) is mainly balanced by sodium (Benson, 1994). Therefore, Pyramid Lake represents a typical soda lake, exhibiting high pH-values of 9.2 to 9.3.

In May 1996 and August 1997 water samples were collected in the vicinity of Needle Rocks. The lake level was at approximately 1160 m above sea level. Samples were taken from the lake, springs, the well flow-off, whiting zones, and a hot pool. Selected chemical data are given in Table 1. For reference, a lake water sample from Pelican Point at the western shoreline is added, where no influence of spring water was expected. Our data are compared to analyses of lake, spring, and well water at Needle Rocks available from the literature (Galat and Jacobsen, 1985; Benson et al., 1995).

Calculation of saturation state (program PHREEQE; Parkhurst et al., 1990) show the lake water to be already highly supersaturated with respect to aragonite and calcite. A more than 10-fold supersaturation in Pyramid Lake waters has also been reported by Galat and Jacobsen (1985). Inhibition of carbonate precipitation has been found in several field and laboratory experiments. Kinetic reasons for this effect are only partly understood; however, a SI >0.8 has been suggested to be a critical factor necessary to induce inorganic carbonate precipitation (e.g., Herman and Lorah, 1987; Svensson, 1992). Supersaturation in the alkaline Pyramid Lake clearly exceeds this value which is comparable to observations from other high alkaline environments (e.g., Walker Lake, USA: Domagalski et al., 1990; Lake Van, Turkey: Reimer, 1996).

Spring waters can be characterised as low oxygenated Na–Cl waters with considerable high calcium content. They exhibit low pH-values corresponding with high partial pressures of CO₂, and they are undersaturated or moderately supersaturated with respect to aragonite and calcite.

Water from the hot well at Needle Rocks shows a higher pH-value, moderately high calcium content, but is nearly bare of alkalinity (Benson et al., 1995). In 1997, water flowing off the well was observed to mix with the high alkaline lake water producing extensive whittings within a small bay. In the whiting zone, pH was distinctively lower (8.08) than the pH-values of the mixing endmembers (well flow-off 8.37; lake 9.33). Mixing was modelled by PHREEQE using a mixture of 40% lake water at 18°C and 60% well water at 60°C to result at the measured temperature of 43°C of the whiting zone. Simple mixing results in extremely high, nearly 100-fold supersaturation, and a pH of 8.8. However, using the programs ability to extract (i.e., precipitate) mineral phases it is possible to reproduce the natural findings by the model. If a threshold is set for aragonite at 10-fold supersaturation (SIₐᵣ = 1.0) the resulting water shows a pH-value of 8.2 and an alkalinity of 8 meq l⁻¹. Using a slightly lower SIₐᵣ of 0.9 leads to a pH of 8.1 and an alkalinity of 7 meq l⁻¹ which closely reproduces measured conditions in the whiting zone. Therefore, thermodynamics clearly favour carbonate precipitation in the whiting zone and in mixed water of hot pools. Striking differences occur in nucleation, crystal growth, and fabric formation at the various sites, indicating a severe influence of biological processes on CaCO₃ precipitation. These are described below.

5. Calcifying biofilms and ‘free’ precipitation at The Needle Rocks, Pyramid Lake

5.1. Substrate carbonate rocks of the biofilms

The biofilm samples were taken from the surface of dendroid to smooth stromatolitic crusts, which form the cm-thick veneer of large, rounded tufa
Table 1
Water chemistry data of Pyramid Lake spring and lake water (May 1996, August 1997; Galat and Jacobsen, 1985; Benson et al., 1995)

<table>
<thead>
<tr>
<th>Sample</th>
<th>T (°C)</th>
<th>pH</th>
<th>EC (mS cm⁻¹)</th>
<th>Eh (mV)</th>
<th>O₂ (mmol l⁻¹)</th>
<th>Tot. alk (meq l⁻¹)</th>
<th>Ca (mmol l⁻¹)</th>
<th>Mg (mmol l⁻¹)</th>
<th>SIₐr</th>
<th>SIₖc</th>
<th>pCO₂ (µatm)</th>
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<tr>
<td>Sampling period May 1996</td>
<td></td>
<td></td>
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<tr>
<td>Lake water at Needle Rocks</td>
<td>15.8</td>
<td>9.30</td>
<td>7.27</td>
<td>390</td>
<td>0.254</td>
<td>22.08</td>
<td>0.26</td>
<td>3.29</td>
<td>1.04</td>
<td>1.19</td>
<td>333</td>
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<td>Spring at Needle Rocks</td>
<td>65.5</td>
<td>7.14</td>
<td>5.97</td>
<td>102</td>
<td>0.063</td>
<td>1.96</td>
<td>3.99</td>
<td>1.23</td>
<td>0.04</td>
<td>0.15</td>
<td>13760</td>
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<tr>
<td>Sampling period August 1997</td>
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<td>Lake water at Pelican Point</td>
<td>25.4</td>
<td>9.33</td>
<td>6.84</td>
<td>351</td>
<td>0.233</td>
<td>20.38</td>
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<td>8.37</td>
<td>5.48</td>
<td>248</td>
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<td>1.84</td>
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<td>Bay (whiting) at Needle Rocks</td>
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<td>8.08</td>
<td>5.79</td>
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<td>64.3</td>
<td>7.48</td>
<td>6.11</td>
<td>83</td>
<td>–</td>
<td>–</td>
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<td>Hot pool at Needle Rocks, edge</td>
<td>59.6</td>
<td>7.79</td>
<td>6.13</td>
<td>101</td>
<td>0.067</td>
<td>2.14</td>
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<td>Hot pool at Needle Rocks, centre</td>
<td>59.7</td>
<td>8.26</td>
<td>6.17</td>
<td>103</td>
<td>0.098</td>
<td>3.20</td>
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<td>Galat and Jacobsen (1985)</td>
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<td>Lake water (January 1979)</td>
<td>6</td>
<td>9.32</td>
<td>8.4</td>
<td>–</td>
<td>–</td>
<td>24.51</td>
<td>0.21</td>
<td>4.90</td>
<td>0.89</td>
<td>1.05</td>
<td>339</td>
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<td>62</td>
<td>7.36</td>
<td>7.1</td>
<td>–</td>
<td>–</td>
<td>0.38</td>
<td>3.24</td>
<td>1.44</td>
<td>−0.60</td>
<td>−0.48</td>
<td>1470</td>
</tr>
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<td>Spring 2 at Needle Rocks (1979)</td>
<td>60</td>
<td>7.56</td>
<td>5.7</td>
<td>–</td>
<td>–</td>
<td>4.54</td>
<td>2.94</td>
<td>0.58</td>
<td>0.62</td>
<td>0.74</td>
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</tr>
<tr>
<td>Spring at Needle Rocks (1992)</td>
<td>57.8</td>
<td>7.5</td>
<td>5.51</td>
<td>–</td>
<td>–</td>
<td>2.04</td>
<td>4.3</td>
<td>1.4</td>
<td>0.32</td>
<td>0.44</td>
<td>5250</td>
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<tr>
<td>Well at Needle Rocks (1972)</td>
<td>85.3</td>
<td>8.3</td>
<td>4.47</td>
<td>–</td>
<td>–</td>
<td>0.24</td>
<td>5.7</td>
<td>0.002</td>
<td>−0.23</td>
<td>−0.12</td>
<td>21</td>
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EC: electrical conductivity. Saturation index SI is given on logarithmic scale, that means saturation is reached at SI = 0, and SI = 1 denotes a 10-fold supersaturation (Ω). Ar: aragonite; Cc: calcite.
structures or boulders. These crusts occur from below the present lake level to several metres above it.

The smooth stromatolites are composed of wavy, radial-fibrous aragonite layers (50–200 μm), rich in inclusions and organic particles. The lamination is caused by thin (10–50 μm) micritic interlayers. A younger, columnar generation of stromatolites is composed of microcrystalline (250–550 μm) and microsparitic (50–500 μm) carbonate layers (Fig. 3A). Erect filament traces suggest that filamentous cyanobacteria were involved in their formation (Fig. 3B). Occasionally, diatoms of the genus *Stephanodiscus* are enclosed in the carbonate. Pockets between the stromatolitic columns are filled with dark micrite, abundant ostracod and gastropod shells, and a few ooids. The sharp discontinuity between calcifying biofilms and the carbonate substrate is often associated with partial erosion or truncation of

Fig. 3. Subfossil stromatolites of Pyramid Lake shore. (A) Subfossil, columnar stromatolites form the terminal veneer on large tufa boulders and pinnacles near the lake shore at The Needle Rocks. Sample PL94/2. Plane light. Scale 500 μm. (B) Erect filament traces (arrow) within the stromatolitic columns of (A) suggest a participation of filamentous cyanobacteria in their formation. Sample PL94/2. Plane polarized light. Scale 100 μm. (C) *Nostoc–Calothrix*-biofilm on subfossil stromatolitic crusts apart from thermal spring influence. Despite of the supersaturated macroenvironment the stromatolite is superficially corroded (scalloped surface, arrow) by the biofilm. Crossed nicols. Sample PL97/A1. Scale 100 μm. (D) Epifluorescence micrographs of the same view showing filamentous cyanobacteria (LPP group, *Nostoc* sp., *Calothrix* sp.) upon the corroded stromatolite surface. Calcein-contrasted hardpart section. Excitation 460–490 nm, Emission >510 nm. For scale see (C).
stromatolitic columns and their micritic sediment between them (Fig. 4B). This pattern indicates that the stromatolites are of subfossil origin, thus unrelated to the living biofilms. The stromatolitic crust probably corresponds to the ‘porous postbeach encrusting tufa’ of Benson (1994), which yields radiocarbon ages of 2100–5080 years B.P. (Benson et al., 1995).

5.2. Carbonates precipitation along the littoral: from whiting zones to soft biofilms

Five littoral sites near The Needle Rocks were selected for sampling and measurements. Samples were taken at (1) the conspicuous whiting zone at the mouth of a warm creek, (2) at a warm seepage site at the rocky shore line (Fig. 2), and (3) from three submerged rock surfaces and boulders a few metres from these sites.

5.2.1. Whiting zones

The mixing zone at the creek mouth was characterized by a cloudy trail of precipitates in the lake water and white friable veneers on substrates (macrophytes, wood, bird feathers, etc.). Sections of green algal filaments show a 40 to 100 μm thick, dense coating of microcrystalline to microsparitic carbonate. This coating is composed of 5–30 μm large rhombs and bladed crystals roughly perpendicular to the substrate surface. Remains of macrophytes are marginally impregnated and veneered with microcrystalline aragonite and calcite rhombs (Fig. 4A). Bacteria and filamentous cyanobacteria (*Phormidium*) are present but rather scarce. No true biofilm is developed. The precipitates remain rather friable, so that wave agitation easily destroys the veneers to form detrital carbonate silt and mud.

5.2.2. Thermal springs

Small thermal springs and seepage sites at the foot of the tufa pinnacle have strikingly green-coloured biofilms. Growing right within the mixing zone, they are rapidly mineralized. The biofilm is up to 2 mm thick and displays a distinct vertical zonation. A dense edifice of prostate *Phormidium* filaments forms the 700–1100 μm thick top layer (Fig. 4B,C). Numerous small diatoms are dispersed throughout this layer, especially in dense domains of *Phormidium* filaments. In the interior of the tests, lysed diatom cells serve as a nucleation site for calcium carbonate, resulting in vast numbers of 5–10-μm-large, spindle-shaped crystals or crystal aggregates (Fig. 4D). By further crystal growth, however, the small spindle-shaped precipitates coalesce to microcrystalline, wavy laminae (Fig. 4B,C). In contrast, living diatoms as well as cyanobacteria and their extracellular polymeric substances (EPS) remain, at first, uncalcified. At the border of lenticular voids of the EPS mucus, initial precipitates tend to form microsparitic to botryoidal aggregates. TEM sections reveal that the crystal traces within the biofilm are surrounded by accumulations of osmiophilic mucus substances (Fig. 4E). It appears that most macromolecular polysaccharides are not enclosed within the crystal lattice. Instead, they remain outside the growing crystals, forming a dense accumulation. However, intracrystalline inclusion of...
the polysaccharides cannot be ruled out because the samples were decalcified with EDTA prior to embedding. In any case, they seem to be rare and loosely dispersed within the carbonate crystals. Below the Phormidium-diatom top layer, coccoid phototrophic bacteria of 2 μm size are locally abundant. They form a discontinuous, 10–20-μm-thick interlayer. The lower biofilm part, which is up to 1 mm thick, shows only scattered coccoid phototrophs and is essentially free of mucus substances. This layer is characterized by dendritic aggregates of large bladed crystals, which support the wavy filamentous top layer (Fig. 4B). These aggregates form upon a succession of subfossil carbonate layers. The interfingering of the Phormidium-diatom layer and the dendritic base layer indicates that precipitation occurs synchronously in both zones.

5.2.3. Littoral biofilms adjacent to hot springs

Samples taken several decimetres to metres from thermal springs show highly inhomogeneous biofilms of 50 to 200 μm in thickness (Fig. 4F). They are dominated by diatoms and colonies of the heterocystous cyanobacteria Nostoc and Calothrix. The biofilm is disrupted by patchy carbonate crystal aggregates, which precipitated between the cyanobacterial colonies or diatoms. The precipitates are very irregular, microcrystalline to microsparitic aggregates, and locally botryoidal crystal fans. Cyanobacterial colonies remain free of calcium carbonate. Since no continuous biofilm is developed, a patchy irregular precipitation leads to the formation of a highly inhomogeneous crust.

A rough lamination is displayed in the just previously formed crust by the alternation of predominantly fibrous/botryoidal or microcrystalline layers. Dead benthic and trapped planktonic diatoms (Stephanodiscus) are enclosed in the carbonate as well as detrital particles (quartz, insect remains, plant debris). filamentous cyanobacteria are increasingly abundant only near the thermal springs. As a result, well developed microcrystalline laminae covering irregular crusts may form in this transition zone near thermal springs.

Since the whole section of the littoral zone at The Needle Rocks is influenced by thermal spring water, we sampled a dark-green biofilm at Pelican Point (Fig. 3C,D). Local input of Ca²⁺ by thermal springs is not known from this place. The dark-green biofilm was taken from the surface of a subfossil, partly eroded stromatolitic crust, which veneers the ikaite mounds. Diatoms, filamentous cyanobacteria (LPP-group, Nostoc, Calothrix) and green algae (Cladophora, Ulothrix) are the main components of the 10–50-μm-thick biofilm (Fig. 3D). The fibrous stromatolitic substrate rock is superficially corroded or etched (Fig. 3C,D), resulting in a secondary microporosity (dissolution voids, etched crystals). Some microcrystalline dissolution remnants occur within the biofilm, and no precipitation is observed.

5.3. Calcifying biofilms in a hot pool open to lake water

Due to flooding in January 1997, the lake level of late summer (August) was still about 2 m higher compared to May 1996. Our former sampling sites were completely submerged and hardly accessible. Hot seepage sites moved upwards together with the lake level, forming new springs and pools at previously dry and inactive sites. One of these recently flooded pools was investigated in detail (Fig. 5). Calcifying biofilms were present from the bottom of the pool up to 15 cm above the water level and displayed a colour zonation. A recent, porous carbonate veneer was found up to 140 cm above the lake water level, indicating the maximum highstand of January 1997. The pool was fed by 64°C hot water discharging from a fissure at the pool rim. Lake water is provided by two channels and mixing and exchange occurs. Three types of biofilms were recognized:

1. Brown, mucilaginous biofilms that cover the pool walls from 8 cm depth down to the bottom. Approximately 4 cm below water level, they are most extensively developed at 40–55°C. Below that they remain patchy. Any substrate at the bottom of the pool is veneered by white, friable carbonate precipitates.

2. Intensively green calcified biofilms that cover the pool rim from 8 cm depth up to 2 cm above the pool water level. They form at 55–60°C. The biofilm is mineralized with white porous and friable carbonate.

3. From 2 cm to 15 cm above the water level (warm spray water and vapour) an orange-yellow biofilm of tough or leathery consistency developed.
Fig. 5. Field photograph of the investigated hot pool and its calcifying biofilms. Narrow connections to the open lake water exist to the right and to the left (not shown). The lower left-hand side shows a schematic topview of the pool. The lower right-hand side shows a cross-section with a projection of an adjacent large tufa boulder. Grey-shaded areas represent tufa rocks.

with a green underlayer. 3–5 cm above water level the biofilm shows a striking purple-red band. Above that, the recent friable carbonate veneer of the tufa boulders is essentially free of living biofilms. Only scattered, black, dried remains of former biofilms occur in depressions of the white crusts.

Thin sections of the brown biofilms show an intensively developed, 1–3 mm thick plexus of prostrate Oscillatoria filaments upon the subfossil substrate rock (Fig. 6A). Internal parts of the mucilaginous biofilm show numerous rod-shaped, phototrophic bacteria, 4 to 8 μm in length and 1 μm in diameter. The presence of sulphate in pool waters suggests that these bacteria are sulphur purple bacteria. Additionally, 0.5-μm-thin filamentous bacteria occur. The marginal parts of mucilaginous Oscillatoria areas are characterized by euhedral rhombs 5–20 μm in size, which concentrate in a 30–80 μm thick zone below the mucus surface (Fig. 6A,B). The crystals remain separated and do not form a framework. Additionally, dark microcrystalline clots occur in mucilaginous areas with abundant detritus (insect and plant debris, quartz). These clots are arranged in string-like aggregates and do not show any relation to cyanobacterial filaments. The central biofilm parts with dense bundles of Oscillatoria remain nearly free of precipitates.

Intensively green biofilms at the water level (Fig. 6C) show a top layer of 50–100 μm in thickness, formed by rod-shaped phototrophic bacteria,
Fig. 6. Hardpart sections of calcifying hot pool biofilms. Scale bar in (A). All samples from The Needle Rocks, Pyramid Lake. (A) Mucilaginous *Oscillatoria*-biofilm showing a marginal precipitation zone, 50–100 µm thick (arrows) due to the excessive diffusion of Ca\(^{2+}\). Sample PL97/D4. Scale 500 µm. (B) Detail of (A) showing polygonal and rhombic microsparite precipitates. *Oscillatoria* filaments remain uncalcified due to continuously secreted, Ca\(^{2+}\)-buffering EPS. Scale 125 µm. (C) Biofilm of coccolid phototrophs (arrow) on a microsparite framework. Initial crystals coalesce to a porous framework after degradation of Ca\(^{2+}\)-buffering mucus substances. Sample PL97/D3. Epifluorescence micrograph of a calcein-contrasted hardpart section. Excitation 460–490 nm, emission >510 nm. Scale 125 µm. (D) Highly porous carbonate crust on subfossil ‘tufa’. The superficial *Oscillatoria* biofilm remains uncalcified in this highly supersaturated environment, but immediately below it microcrystalline to microsparitic precipitation runs (arrow), causing a further lamina of the ‘tufa crust’. Sample PL96/D1. Plane light. Scale 500 µm.
4–8 μm long and 1 μm in diameter. The biofilm contains a few detrital crystals and a few newly precipitated rhombs at its base. The biofilm grew on a terminal microsparite lamina of a reticular framework of microsparite bridges (Fig. 6C). These are 50–100 μm wide and are composed of euhedral rhombs, 10–40 μm in size. The polygonal voids between that reach 300 μm in diameter. Dark patches in the centre line of microsparite bridges are rich in inclusions. Further rods and cocci of bacteria are distributed throughout the porous framework.

Orange-yellow biofilms are dominated by a second Oscillatoria species, forming 150–400-μm-thick, coherent films on top of a reticular, porous microsparite framework (Fig. 6D). The filamentous top layer is essentially free of precipitates but has trapped quartz and pollen grains and insect and plant debris. Numerous coccoid purple bacteria, 2 μm in diameter, are concentrated at the biofilm’s base, causing the purple-red macroscopic band. Microcrystalline precipitates form a lamina of 50–150 μm thick, restricted to the coccoid layer, which grades downwards into microsparite rhombs. Microporite rhombs, 5–40 μm in size, also form a 2-mm-thick, highly porous, irregular framework. Occasionally discontinuous microcrystalline laminae are developed within the reticular, porous framework. Below 2 mm a porous meshwork is formed by small irregular botyroids, spherulitic aggregates, and micron-sized needles of aragonite. Wavy microcrystalline to fibrous laminae (100–300 μm thick) occur within and at the top of this layer.

6. Biomarker investigations

Organic geochemical techniques can be used to trace a former presence of micro-organisms by the recognition of organic molecular fossils, the so-called biomarkers (e.g., Peters and Moldowan, 1993). We analyzed the lipid inventory of an active layer from a massive stromatolite from Pyramid Lake and compared it to the one obtained from the core of the respective structure. Variations in the occurrences and the relative abundances of distinctive molecular markers signify the role of different sources of organic matter contributing to the respective facies types (Fig. 7).

Gas chromatograms of the total hydrocarbon fractions of the active mat and the inner part of the stromatolite are shown in Fig. 8. The hydrocarbon pattern of the active mat shows \( n \)-heptadecane \( (n-C_{17}) \), and \( n \)-pentadecane \( (n-C_{15}) \) as the most abundant compounds. Further significant constituents of the hydrocarbon fraction are the isoprenoids pristene, phytene, two phytadienes and several \( n \)-heptadecenes isomers. In addition, an isomeric mixture of proposedly bicyclic compounds of unknown structure is present in minor concentrations. Whereas the origin of \( n \)-pentadecane is yet unclear, a predominance of \( n \)-heptadecane \( (n-C_{17}) \) and/or \( n \)-heptadecenes is typically observed in sediments receiving a significant contribution of organic matter derived from phototrophic micro-organisms. They are particularly abundant in pure cultured cyanobacteria and in nat-

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Fig. 7. Distinctive cyanobacterial markers according to their abundance in the Pyramid Lake samples.
Fig. 8. Gas chromatograms of the total hydrocarbon fractions from the active mat and the inner, micritic core of the microbialite studied.

Urally grown cyanobacterial mats (e.g., Han et al., 1968; Dobson et al., 1988; Shiea et al., 1991; Miranda de Castro, 1994; Thiel et al., 1997). An organic matter input derived from cyanobacteria is further indicated by the co-occurrence of individual mid-chain branched monomethylalkanes which are regarded as characteristic biomarkers for these organisms (Shiea et al., 1990). The ‘phototrophic’ character of the active mat is also reflected by the regular C₁₉ and C₂₀ isoprenoids pristene, phytene, and several phytadienes which are derived from the degradation of the chlorophyll-a side chain (Rontani et al., 1990). The nature and significance of the bicyclic compounds found is yet unclear, although their prominent occurrence in a diatom-dominated benthic community at Walker Lake (Nevada; unpublished material) may point to an origin from this group of microalgae. In the inner, subfossil part of the stromatolite, typical biomarkers for phototrophic micro-organisms are present, but not prevalent (Fig. 8, bottom). Instead, the hydrocarbon pattern shows prominent long-chain n-alkanes with odd
carbon numbers (maximum at \(n\)-C\(_{29}\)) and hopanoid-type hydrocarbons. Long-chain \(n\)-alkanes are typical constituents of higher plant waxes (Eglinton and Hamilton, 1967). They reflect an incorporation of allochthonous organic matter into the growing microbialite. Hopanoids are generally regarded to derive from bacterial lipids (Ouirisson et al., 1979). By far the most prominent hydrocarbon present in the subfossil sample is the hop-17(21)-ene. This compound has not been found in living organisms, but has been shown to originate from the diageneric rearrangement of hop-22(29)-ene (diploptene), a ‘primitive’ C\(_{30}\) triterpenoid which is broadly distributed in prokaryotic lipids (Rohmer et al., 1984; Ageta et al., 1987). In the sample studied, diploptene co-occurs with its diageneric product. Apparently, the diageneric transformation reaction leading to hop-17(21)-ene has not yet been completed. The exact bacterial source of the hopanoids is difficult to determine. Diploptene is very abundant in many cyanobacteria and may reach up to 86% of their lipid fraction (De Rosa et al., 1971), but it has also been observed in various heterotrophs (Rohmer et al., 1984). It should be stressed that this compound shows only trace concentrations in the active mat. Therefore, the source organisms of the subfossil diploptene do not contribute significantly to the current mat-forming community. In general, biologically produced organic compounds are subjected to chemical changes like defunctionalization or isomerization as well as to microbial reworking on their way from the living system into the ancient rock (Peters and Moldowan, 1993). These processes generally obscure the original biomarker pattern to a varying degree and must be considered when comparing samples of different age and diageneric history. For instance, a low abundance of \(n\)-heptadecane is observed in the micritic core with respect to the active mat (see Fig. 8). This observation does not necessarily prove an originally low source input, but may also be due to preferential degradation of lower \(n\)-alkane homologues compared to their long-chain counterparts. This was, for example, shown for different horizons of microbial mats (‘kopara’) from Polynesia (Miranda de Castro, 1994). Nevertheless, distinctive molecular fossils signify differences in the source inputs during the formation of the Pyramid Lake microbialites. With respect to cyanobacterial molecular fossils, this holds true not only for the more recent carbonate precipitates, but is also evident from a comparison with hydrocarbons from a microbialite of Pleistocene age. It is interesting to see that the fossil material contains distinctive dimethylalkanes of proposed cyanobacterial origin (Fig. 9; Kenig et al., 1995). These are neither present in the young samples nor can they be regarded as degradation products of other non-specific lipid components.

Fig. 9. Partial gas chromatogram of the total hydrocarbon fractions extracted from a subaerially exposed, Pleistocene microbialite (Pyramid Lake). Black dots = \(n\)-alkanes, numbers indicate number of carbon atoms. Peaks are labelled according to shorthand notations given in Fig. 7. Structures of dimethylalkanes are tentatively assigned based on GC–MS measurements.

7. Discussion: supersaturation vs biofilm control

Mixing zones of Ca\(^{2+}\)-supplying thermal spring and HCO\(_3^-\)-rich lake water are very suitable to evaluate the potential influence of micro-organisms and their extracellular polymeric substances on carbonate formation. In this system, several parameters which are important in other microbially mediated precipitation processes can be considered as negligible. Autotrophic CO\(_2\) removal (e.g., by photosynthesis), HCO\(_3^-\)-production by sulphate reduction, and ammonification (summarized in Krumbein, 1979) are unlikely to affect substantially the calcium carbon-
ate system in biofilms of highly alkaline, highly supersaturated macroenvironments. In other words, carbonate precipitation is running in these mixing zones in any case.

At Pyramid Lake almost pure physicochemical precipitation is observed within the whiting zone at the warm creek mouth. Heterogeneous nucleation and crystal growth is nearly devoid of biofilm influence, although the surfaces of green algae and macrophytes, which serve as nucleation sites, occasionally show a monolayer of scattered bacterial cells. Unfortunately we have no data of dissolved organics, which might promote or inhibit nucleation and crystallization.

In contrast, mm-thick biofilms are developed on hard substrates in hot pools or at warm spring sites. Thin sections show that precipitation in and upon these biofilms is inhibited at first, although supersaturation is as high as in the creek mouth whiting zone. Acidic mucus substances, mainly carboxylated and sulphated polysaccharides, are known to bind Ca$^{2+}$ from the liquid phase, thus preventing CaCO$_3$ precipitation at first (Addadi and Weiner, 1989; Reitner, 1993). Additionally, diffusion is generally slowed down by the highly hydrated polymers of the biofilms (Decho, 1990; Costerton et al., 1995). The EPS is therefore considered as a Ca$^{2+}$-buffer, whose capacity has to be surpassed before nucleation is possible. Once precipitation starts within the mucus because of Ca$^{2+}$-excess, the macromolecular polysaccharides are kept outside the growing crystals. Crystal traces in ultrathin sections are free of osmiophilic substances, but their immediate vicinity shows an accumulation of polymers (Fig. 4E). They possibly limit crystal growth to microsparite size by a further decrease in diffusion, until they are enzymatically decomposed. Low molecular weight components, such as amino acids or certain biomarkers, may be locked in or even preferentially adsorbed by the growing crystals, but are generally not osmiophilic, thus not detectable by standard TEM. Tests of lysed diatoms are rapidly filled by carbonate crystals, whereas the surrounding mucus remains free of CaCO$_3$. These diatom moulds form the most significant part of the initial precipitates in warm spring biofilms of Pyramid Lake.

A comparable setting is given at spring mounds of cold sublacustrine springs of soda lakes, although temperatures are lower and primary production of EPS relative to their subsequent degradation may be lower. Enzymatic breakdown, gas bubble formation, and shrinkage due to dehydration are of crucial importance in precipitation and fabric formation in these spring mounds (Arp et al., 1998). In contrast, organic influence in Pyramid Lake hot spring biofilms is limited to the Ca$^{2+}$-buffer effect and the slow-down of diffusion by the mucus.

Apart from the immediate vicinity of thermal springs, Pyramid Lake shore biofilms are only poorly to moderately developed, less mucilaginous, and often discontinuous. Consequently, Ca$^{2+}$-buffering is less effective, except for the mucilaginous cyanobacterial or diatom colonies. In this moderately supersaturated macroenvironment, the sheaths and envelopes of Nostoc and Calothrix remain uncalcified, whereas growth of aragonite fans is observed at ‘free’ sites, side by side with the colonies. Completely away from thermal Ca$^{2+}$-influx, the acidity of the EPS even causes a slight, superficial corrosion of the carbonate substrate rock, although the macroenvironment is still slightly supersaturated with respect to calcium carbonate.

The biomarker analyses support our interpretation, that precipitation is not determined by the species composition of the biofilms involved. All three analysed samples, a recent Phormidium mat, the subfossil stromatolitic substrate rock, and a fossil tufa of the pinnacles, indicate the presence of cyanobacteria.

8. Conclusions

(1) Stromatolitic substrate rocks show no relation to recent calcifying biofilms. They were constructed by cyanobacterial communities of different species composition (confirmed by biomarker) within a different environmental setting than today.

(2) Calcifying biofilms at Pyramid Lake are restricted to mixing zones of Ca$^{2+}$-supplying thermal spring and alkaline lake water. Lake shore sections with moderate CaCO$_3$ supersaturation but without sufficient Ca$^{2+}$-supply reveal only soft, slightly corrosive biofilms.

(3) Despite of high supersaturation, carbonate precipitation is inhibited or slowed in thermal spring
biofilms by the large amount of acidic mucus substances, which serve as Ca\(^{2+}\)-buffer. A distinct precipitation zone near the biofilm–water interface results from the exceeding of Ca\(^{2+}\)-binding capacity within the continuously produced EPS. Initial precipitates remain as separate crystals in the mucilaginous substances.

(4) After mucus substances are decomposed, initial microsparite precipitates coalesce to form a porous, still friable framework (‘tufa’). The reticular fabric shows no relation to filamentous organisms, but may reflect the structure of the EPS.

(5) Dead diatoms within the mucilaginous biofilms calcify at first, because within the lysed cells no inhibition of precipitation by mucus substances occurs.

(6) Biofilms composed of preferentially prostrate filaments cause episodic laminae in porous tufa crusts. Bladed crystals may form in mucus-free cavities below that, due to the filter effect of the overlaying biofilm and lack of buffering.

(7) The Ca\(^{2+}\)-supply and the extent of biofilm development, which is dependent on the temperature gradient, determine the extent of benthic precipitation. Thick filamentous biofilms on hard substrates at spring sites or pools produce highly porous tufa crusts. Thin biofilms with patchy diatom/cyanobacterial colonies apart from immediate spring water influx lead to thin, irregular, but massive crusts. Friable precipitates, which are easily destroyed, are found on any substrate at a whiting zone of a warm creek mouth. Here, no real biofilms are developed upon the encrusted green algae and macrophytes.

(8) The biomarker compositions demonstrate on a molecular level that the cyanobacterial primary producers have been subject to significant temporal changes in their species distribution. Accordingly, the nature of the cyanobacterial communities does not appear to be critical for the formation of microbial carbonates in Pyramid Lake.

(9) Organic extracts of recent precipitates contain dicyclic components which possibly reflect the participation of diatoms. These dicyclic components were not found in the subfossil and fossil carbonate crusts.

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