Patterns of biomediated CaCO₃ crystal bushes in hot spring deposits

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A B S T R A C T

In the Eryuan hot spring, located in south China, the vent pool is covered with “crystal bushes”, up to 2 cm high, 1 cm in diameter, that grew in the biofilms that thrive in the spring waters that have a pH of 7.5 and a temperature of 88 °C. The biofilms are formed largely of phototrophic purple bacteria and green bacteria. Growth of the crystal bushes, which are formed of aragonite crystals (wheat-sheaves, radiating clusters), rhombohedral and dodecahedral calcite crystals, amorphous CaCO₃ (ACC), and opal-A, is attributed to precipitation in the micro-domains of the biofilms where physiochemical conditions can vary on the sub-micron scale. There is no evidence that the calcite was formed through recrystallization of the metastable aragonite and most of the calcite crystals developed as mesocrystals that are characterized by incomplete growth and porous crystal faces. With the onset of diagenesis, there is a high probability that the crystal bushes will lose much of their identity as the (1) biofilm is lost through decay, (2) ACC and aragonite change to calcite, (3) identities of the mesocrystals and incompletely formed crystals are lost through continued precipitation and/or recrystallization, and (4) porous crystal faces are converted to solid crystal faces. This means that most of the features considered indicative of biomediated calcite precipitation have a low preservation potential and that the recognition of biomediated precipitates in old spring deposits may remain problematical.

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1. Introduction

Precipitates found in and around vent pools of hot springs, with water temperature > 75 °C, are commonly varicolored and highly ornate. Formed as hot geothermal waters come to the surface and start to equilibrate with local climatic conditions, variability of these precipitates is manifested in terms of their (1) composition (e.g., SiO₂, CaCO₃), (2) large-scale architecture (e.g., laminae, shrubs and crystal bushes), and (3) crystal morphology (e.g., dendrite crystals) (Walter, 1976; Jones and Renaut, 1995; Cady and Farmer, 1996; Jones et al., 1997, 2004; Guidry and Chaletz, 2003b; Peng and Jones, 2012).

Interpretations of precipitates found in spring pools are commonly linked to the geochemistry of the spring water by using various parameters, including calculated saturation indices. Substrates in many spring pools, however, are covered with biofilms that are formed of diverse microbial communities held in extracellular polymer substances (EPS). These biofilms effectively isolate the substrates from the spring waters (Decho and Lopez, 1993; Decho, 2000, 2010). In these situations, mineral precipitation may take place in the micron-scale microdomains that exist in the biofilm hydrogels (Decho, 2010). Critically, the geochemical conditions (e.g., saturation levels) that exist in neighboring microdomains may differ significantly from each other and may also be significantly different from those in the overlying spring water. Such considerations raise the possibility that precipitates that develop in spring pools may be dictated largely the micro-scale processes that operate in the microdomains of the biofilms rather than pool-scale processes that evolve as the geothermal waters are modified by local climatic conditions. Thus, the challenge becomes one of developing criteria by which these biomediated precipitates can be recognized. This is especially true for the CaCO₃ producing systems where the preservation potential of the biofilms and their formative microbes are very low.

In Eryuan hot spring, located in the western part of Yunnan Province, China (Figs. 1, 2), the vent pool is covered with highly ornate “crystal bushes” that are covered with biofilms (Fig. 2B–D). Herein, the “crystal bushes” are described with the view of establishing (1) their architectural development, (2) the mineralogy of the precipitates, (3) the morphology of the constituent crystals, and (4) the spatial relationships between the precipitates and the biofilms. Collectively, these data reveal the wide diversity of precipitates that can develop within the biofilm microcosms. With diagenesis, however, many of the features that point to biomediated precipitation will be lost as the biofilm is lost through decay and as various CaCO₃ minerals diagenetically change to their more stable polymorphs.

2. Geological setting

This study is based on samples collected from Eryuan hot spring, which is located in the in the Red River Fault Zone (RRFZ) in Yunnan Province, China (Fig. 1). The Red River Fault Zone is a large strike-slip
fault, which became the primary displacement zone between the Indochina Block and the Yangtze Block during the collision of the Indian and Eurasian plates. This zone is divided into the southern and northern segments based on seismo-geological features (Allen et al., 1984). Many low and high temperature springs are located along the fault in the northern segments of the RRFZ. Lin (1994), based on

Fig. 1. Location of Eryuan Spring. (A) Map of China showing location of Eryuan. (B) Tectonic sketch map of study area (after Li et al., 2008). (C) Location of Eryuan hot spring in northern segment of Red River Fault Zone (after Lin, 1994).

Fig. 2. Hot spring at Eryuan. (A) General view of spring pool enclosed by concrete wall. Pool is about 20 m in diameter with water up to 1 m deep. (B) Substrate in pool covered with “calcite bushes”. Water depth to the base of the calcite bushes shown in this image is about 1 m. (C) Samples from pool wall showing “crystal bushes”. (D) Complex and irregular branching patterns in a crystal bush.
the geochemical characteristics and spatial distribution of springs, divided the northern segment of RRFZ into the Jianchuan, Eryuan, Dali and Midu sub-segments.

Eryuan hot spring is located to the east of Dali City in Eryuan county in the Eryuan sub-segment of the northern segment of the RRFZ (Fig. 1B). The spring water (Fig. 2A), with water up to 1 m deep, has a temperature of 88 °C at its vent. Around the edge of the pool there are numerous boulders coated with “crystal bushes”, formed of various CaCO₃ precipitates (Fig. 2B–D), which appear to have developed in biofilms that coat the substrate.

3. Methodology

Small fracture samples (~1 cm³) extracted from hand samples were mounted on SEM stubs using double-side tape and/or by conductive glue and spatter coated with gold (coating – 10 nm thick) before being examined on a JOEL 6400FE scanning electron microscope (SEM) with an accelerating voltage of 5 kV. The elemental compositions of selected spots were determined by energy dispersive X-ray (EDX) analysis on the SEM with an accelerating voltage of 20 kV. EDX analyses were critical for distinguishing between the amorphous calcium carbonate (ACC) and the opal-A because they are similar in appearance.

The morphological attributes of the spring deposits were derived largely from 978 SEM photomicrographs acquired during this study. The contrast and grey levels of the photomicrographs were balanced and modified using Photoshop CS4 ©. For some figures, a black background was introduced in order to highlight the features being shown.

A Leica M125 stereoscopic microscope was used to obtain the close-up views of the samples. Fluorescence micrographs of the biofilms were also imaged on a LEICA DMI 4000B fluorescence microscope.

The water temperature, pH, and Eh were measured in the field on September 12, 2012. Water samples were passed through a syringe filter with a 0.22 μm filtration membrane before being stored in polypropylene bottles. Those water samples were analyzed for major cations and anions at the Saskatchewan Research Council (Saskatoon, Canada). δ¹⁸O(water) isotope analyses, done by Isotope Tracer Technologies Inc. (Ontario, Canada), are reported in the VDPB notation.

4. Terminology and criteria for recognition of biofilms, mesocrystals, and ACC

4.1. Biofilm

Biofilms are “...heterogeneous matrices of micro-organisms held together and tightly bound to the underlying surfaces by extracellular polymeric substances (exopolysaccharides)...” (Rosenberg, 1989). The biofilms are attached to substrates by the extracellular polymeric substances (EPS) that is secreted by the microbes (e.g., Decho, 1990; Riding, 2000). The EPS also insulates the formative microbes from the surrounding spring waters (Decho, 1990, 2010).

The biofilms in the samples from Eryuan hot spring are largely nonmineralized and only a few microbes have been mineralized. When viewed on the SEM, however, most substrates are coated with thin, smooth films that are considered to be EPS because of their similarity to EPS illustrated by Decho and Lopez (1993, their Figs. 2 and 3), Allen et al. (2000, their Figs. 3, 6, 9 and 11), Westall et al. (2000, their Figs. 6 and 8), McKenzie et al. (2001, their Fig. 3E), Guidry and Chafetz (2003a, their Fig. 7C), Hassan et al. (2003, their Fig. 3), Handley et al. (2008, their Figs. 3 and 4), and Pedley and Rogerson (2010, their Figs. 3A, 4A and 5C). The presence of diverse arrays of filamentous and coccoid microbes in these films further substantiates their identification as biofilms/EPS.

4.2. Monocrystal and mesocrystal

A monocrystal, or single crystal, “…is a crystalline solid in which the crystal lattice of the entire sample is continuous and unbroken to the edge of the sample with no grain boundaries” (Meldrum and Colfen, 2008, p. 4336). A mesocrystal, which is an abbreviation for a “…mesoscopically structured crystal…” , is built up of nanocrystals that “…are aligned in a common crystallographic register” (Meldrum and Colfen, 2008, p. 4343). Mesocrystals, with the consistent alignment of their constituent nanocrystals, will yield scatter X-rays or electrons like a single crystal and show birefringence properties like those of a single crystal (Meldrum and Colfen, 2008). With high magnification SEM imaging, however, mesocrystals can be recognized by virtue of the numerous smaller crystals, all in crystallographic alignment, that form the crystal (e.g., Colfen and Antonietti, 2005, their Figs. 9, 10 17; Meldrum and Colfen, 2008, their Fig. 19).

A mesocrystal is equivalent to aggregate crystals (Brinkley et al., 1980; Chafetz et al., 1985; Jones, 1989), composite crystals (Given and Wilkinson, 1985; Sandberg, 1985; Jones and Renaut, 1996), and poly-crystalline crystals (Towe, 1967). The subcrystals that collectively form the large crystals (Sandberg, 1985; Jones, 1985; Jones and Renaut, 1996; Jones et al., 2005) are equivalent to the nanocrystals of Meldrum and Colfen (2008).

4.3. Amorphous calcium carbonate

By definition, amorphous calcium carbonate (ACC) is non-crystalline CaCO₃ that is formed largely of nanoparticles that are <1 μm long (e.g., Brečević and Nielson, 1989; Aizenberg et al., 1996a; Beniash et al., 1997; Aizenberg et al., 2002, 2003; Loste et al., 2003; Han and Aizenberg, 2008; Meldrum and Colfen, 2008; Obst et al., 2009; Jones and Peng, 2012a). X-ray diffraction (XRD) cannot be used to identify the ACC in the samples from Eryuan spring because it is intimately associated with crystalline calcite and/or aragonite (cf., Aizenberg et al., 1996b). Following Jones and Peng (2012a), recognition of ACC is based on the presence of nanoparticles that are (1) morphologically akin to experimentally produced ACC nanoparticles (Ogino et al., 1987, their Fig. 5E; Aizenberg et al., 2002, their Fig. 7B; Ajikumar et al., 2007, their Figs. 1–6; Faatz et al., 2005, their Figs. 5, 6; Xu et al., 2005, their Figs. 1, 2; Han and Aizenberg, 2008, their Fig. 1B; Xu et al., 2008, their Figs. 1, 2; Meiron et al., 2010, their Fig. 1B, C; Njiegi-Džakula et al., 2010, their Fig. 8), (2) (sub)spherical particles with no evidence of crystal faces and/or edges, and (3) variable size up to a maximum of 1 μm. Identification in the Eryuan samples is complicated by the fact that opal-A, which is also present in some of the samples, is morphologically alike ACC. Separation of the ACC from the opal-A is based on EDX analyses that show if Si is present.

5. Results

5.1. Spring water

The spring pool which is ~20 m in diameter with a water depth of ~1 m, is surrounded by a concrete wall that rises ~4 m above ground level (Fig. 2A). An ornate concrete fence, up to 1.4 m high (Fig. 2A), surrounds the spring pool and separates it from the walkway that is on top of the concrete wall. The spring vent, surrounded by large boulders that rise up to ~1 m above water level, is located in the centre of the pool (Fig. 2A). At the vent, the spring water has a temperature of 88 °C and pH of 7.5 whereas the water in the marginal areas of the pool has a temperature of 68 °C and a pH of 7.3. The spring water contains 120 ppm Ca, 20 ppm Mg, 115 ppm Na, 9 ppm K, 7 ppm Cl and 48 ppm SiO₂. The Cl level of 225.2 ppm recorded in Jones and Peng (2012a,b, their Table 1) was erroneous. Calculations using the PHREEQC software (Parkhurst and Appelo, 1999) yielded saturation index (SI) values greater than 0 for aragonite (0.08), calcite (0.92),
dolomite (1.49), quartz (0.33), and talc (3.80). These values suggest that there is the potential for the precipitation of aragonite, calcite, dolomite, quartz, and talc from the Eryuan spring water.

The $\delta^{18}$O$_{\text{water}}$ values determined from samples collected in 2011 and 2012 were $-119.90$ and $-113.50\%$ whereas the $\delta^{18}$O$_{\text{water}}$ values were $-15.77$ and $-13.79\%$, respectively.

5.2. General attributes of precipitates

The wall around the spring pool is built of large boulders that are now covered with “crystal bushes” (Fig. 2B–D). Below water level, bushes appear dark brown with individual bushes, up to 2 cm high and 1 cm wide, that are characterized by randomly arranged branches of variable lengths (Figs. 2D, 3). Although all the bushes are approximately the same size, they are all different from one another because of the variations in branching patterns.

Small crystal bushes are also evident on the concrete wall around the margins of the spring pool that is above the present water level. These bushes, which probably formed when water level was higher than it is today, are cone-shaped, less than 0.4 cm high and 0.6 cm wide, that are characterized by randomly arranged branches of variable lengths (Figs. 2B, C). Although all the bushes are approximately the same size, they are all different from one another because of the variations in branching patterns.

5.3. Biofilms

The crystal bushes are covered with biofilms that, on the SEM, seem to be smooth and formed largely of EPS (Fig. 4A), or formed of arrays of filamentous and coccioid microbes with variable amounts of EPS (Fig. 4B–H). The filamentous microbes, with diameters of 0.25 to 1.25 $\mu$m, include septate and non-septate forms (Fig. 4E). In some areas, the filamentous microbes are entwined around ovate coccioid microbes that are up to 7 $\mu$m long and 5 $\mu$m wide (Fig. 4F, G). Elsewhere, colonies of bicellular coccioid microbes up to 2.5 $\mu$m long and 1.0 $\mu$m wide are found (Fig. 4H). Cross-sections through the biofilms show that some are 0.25 $\mu$m thick and not laminated (Fig. 4I) whereas others are up to 5 $\mu$m thick and internally laminated (Fig. 4J). Locally, some surfaces are covered with various types of filaments and coccioid microbes, but no EPS (Fig. 4K).

Phototrophic purple and/or green bacteria dominate the purple or green biofilms that cover the crystal bushes (Fig. 5A). There is no obvious pattern to the distribution of the different coloured biofilms with one commonly grading into the other on individual branches. Cocoid and rod-shaped microbes dominate the biota in the purple biofilms, whereas filamentous microbes are dominant in the green biofilms (Fig. 5B). The coccioid and rod-shaped microbes from the purple biofilms emit yellow light when excited by blue fluorescence light (Fig. 5C). These microbes are tentatively identified as the member of Rhodospirillaceae, which is a family of purple, non-sulfur bacteria. The filamentous microbes from the green biofilms, which are not autofluorescent, may belong to Chromatiaceae and Chloroscus. These observations are consistent with the results from DNA analysis of the biofilms (data not shown here).

Most biofilms and their formative microbes show little or no evidence of mineralization (Fig. 4). Locally, however, some filamentous microbes have been partly calcified (Fig. 6A–C) or replaced and encrusted with opal-A (Fig. 6D–J). The notion that some of the filamentous microbes were calcified is open to debate because aligned strings of CaCO$_3$ nanoparticles may have simply formed around strands of EPS (Fig. 6A–C).

Some biofilms are well preserved because they were replaced and encrusted by opal-A (Fig. 6D–J). Similarly, aggregations of opal-A microspheres are apparent in the EPS (Fig. 6E). The three-dimensional preservation of the microbes, including many with open lumens (Fig. 6H), shows that silification took place before the microbes underwent any decay. Although dominated by straight filamentous microbes up to 1 $\mu$m in diameter, there are also some spiral filaments (Fig. 6I). In many areas, the opal-A microspheres and silicified filaments have one flat side that seems to have grown against the surface of a biofilm (Fig. 6J).

5.4. Aragonite

Elongate aragonite crystals form radiating and “wheat-sheaf” arrays that are usually associated with biofilms (Fig. 7). Clusters of radiating crystals are up to 10 $\mu$m long (Fig. 7A) whereas wheat-sheaves are up to 20 $\mu$m long and 10 $\mu$m in diameter (Fig. 7B, C). The formative crystals are typically <15 $\mu$m long and ~0.5 $\mu$m wide at their distal ends (Fig. 7D, E). Flat bases covered by biofilms characterized many of the clusters and wheat-sheaves (Fig. 7B, F) that typically have hollow cores (Fig. 7A, F, G). No microbes or other precipitates are present in the hollow cores. Although most crystals appear to be formed of a single unit (Fig. 7E), some may have formed through the merger of numerous subcrystals (Fig. 7H).

5.5. Calcite

Calcite, the dominant component of the crystal bushes, is characterized by a morphologically diverse array of completely- and incompletely-formed crystals (Fig. 8). Rhombic crystals up to 20 $\mu$m long, which are common throughout the deposits, occur as discrete crystals (Fig. 8A), as intergrown pairs (Fig. 8B), or more rarely as intergrown clusters of three or more crystals. Associated

![Fig. 3. SEM photomicrographs of branching structures that characterize the Eryuan spring deposits. Black backgrounds added in Photoshop. (A, B) Irregular branching pattern in three-dimensional “crystal bushes” (see Fig. 2D). (C) Top part of a branch formed of numerous calcite crystals and covered by a biofilm.](image-url)
with these crystals are dodecahedral crystals with pentagonal faces that are up to 20 μm long (Fig. 8C). Although most crystal faces are smooth, others are characterized by overgrowths or a porous texture (Fig. 8D). Rhombic crystals with beveled edges and corners are found in some crystal bushes (Fig. 8E, F).

Incompletely formed calcite crystals (Fig. 8G–L) are invariably enmeshed in biofilms and commonly occur side-by-side with calcite crystals characterized by smooth faces and sharp edges. These crystals display various combinations of (1) beveled edges in which subcrystals are clearly evident (Fig. 8G, H), (2) porous crystal faces (Fig. 8G–J), (3) incompletely formed faces and edges (Fig. 8I–J), and (4) incompletely formed overgrowth zones (Fig. 8K, L).

Fortuitous cross-sections through some of the calcite crystals (Fig. 9A) and many of the incompletely formed crystals (Fig. 9B)
show clear evidence of growth zoning. Multiple, well-formed zones are evident in some crystals (Fig. 9A) whereas others include zones that are incomplete and allow views of the inner parts of the crystals (Fig. 9B). In rare examples, the subcrystals in the growth zones are readily apparent (Fig. 9C). Numerous growth zones that commonly appear to envelop more than one crystal are found in some crystal bushes (Fig. 9D). Many growth layers are highly porous and seem to be formed of short, sinuous ridges that merge to form complex patterns on faces of the growth zones (Fig. 9E, F). In most cases, however, the outer rims of these growth zones are dense and show little or no evidence of their complex internal architecture (Fig. 9F).

Some calcite crystals have a “Swiss-cheese” appearance with numerous round to slightly ovate pits up to 10 μm in diameter (Fig. 9G–I). Growth laminae are readily apparent in the walls of these pits (Fig. 9H, I). The fact that nonmineralized filamentous microbes can commonly be traced from the crystal surface and the walls of the pits shows that the pits are natural features. Given that large ovate coccoid microbes are commonly present on the floor of the pit (Fig. 9I), they are herein informally referred to as “bug pits”.

5.6. ACC

Clusters of ACC, formed of nanoparticles <1 μm long, lie under, inside, or on top of the biofilm. In some areas, the ACC lies close to and on top of calcite crystals that are only partly submerged in the biofilm (Fig. 9K). ACC is also associated with the bundles of aragonite crystals that are found throughout the crystal bushes. Locally, the ACC is located between the distal ends of the aragonite crystals (Fig. 9L).

5.7. Spatial relationships between aragonite, calcite, and ACC

There is no recognizible pattern to the distribution of the aragonite, calcite, and ACC in the crystal bushes (Figs. 9J–L, 10). All, however, are intimately associated with the biofilms that cover the crystal bushes. Most aragonite clusters and wheat-sheaves are wedged between the calcite crystals (Fig. 10A–C). Many aragonite crystal bundles and some of the small clusters of calcite crystals have flat bases (Fig. 10D) that probably reflect growth against a biofilm (Fig. 7B). Many calcite crystals have partly (Fig. 10E) to totally enveloped clusters of aragonite crystals (Fig. 10F). The boundaries between the aragonite and calcite are always sharp with no indication that the aragonite is changing to calcite (Fig. 10E, F).

The ACC is associated with the biofilms that cover the crystal bushes (Fig. 9J–L). In these settings, the ACC is found (1) on the surfaces of calcite crystals (Fig. 8K), (2) filling spaces between the aragonite crystals (Fig. 9L), and (3) in a thin zone that surrounds the hollow cores of the aragonite crystal bundles. ACC is not found in areas where there are no biofilms.

6. Discussion

Precipitates in Eryuan Spring are variable in terms of their composition (CaCO3 vs. opal-A — Fig. 6), the polymorphs of CaCO3 (aragonite, calcite and ACC — Figs. 7–10), the crystal form of the aragonite (radial arrays, wheat sheaves — Fig. 7) and calcite (rhombohedra, dodecahedra — Fig. 8A–C), crystal type (monocrystals, mesocrystals, incomplete crystals — Figs. 8A–C, 9A–C), and the appearance of the crystal faces (solid, porous — Fig. 9D–F). From an interpretative perspective it is important to determine if these features can be considered indicative of precipitation in biofilms as opposed to abiogenic precipitation that was governed solely by the chemistry of the spring water.

The precipitation of calcite as opposed to aragonite from water has been attributed to various abiogenic parameters, including sulfate poisoning (Siegel, 1965; Rowling, 2004), high concentrations of Mg, Fe, Sr, and/or Mn (Fischbeck and Müller, 1971; Seemann, 1985; Hill and Forti, 1997; Urbani, 1997; Bosák et al., 2002; Rowling, 2004), saturation levels and precipitation rates (Rowling, 2004), water temperature (Kitano, 1962b; Folk, 1994), and ion delivery rates (Buczynski and Chafo, 1991), or CO2 degassing rates (Kitano, 1962a; Kitano et al., 1962). It is, however, commonly difficult to pinpoint the exact cause because these variables commonly operate together and the separation of one from the other becomes impossible.

Precipitation in Eryuan Spring is intimately associated with the microbial biofilms that cover the substrates (Figs. 3–5). The hydrogels in these biofilms are akin to gels that have been used as growth media for CaCO3 precipitation in the laboratory (e.g., Nickl and Henisch, 1969; McCauley and Roy, 1974; Garcia-Ruíz, 1985; Domínguez Bella and Garcia-Ruíz, 1986, 1987; Grassmann et al., 2002, 2003). SEM photomicrographs of freeze-dried gels (e.g., Nickl and Henisch, 1969, their Fig. 5; Hellbig, 2008, her Fig. 1), for example, look remarkably similar to many SEM images of biofilms from spring pools and marine stromatolites (Défarge et al., 1994, their Fig. 4A, B; Défarge et al., 1996, their Figs. 1, 2). In most laboratory experiments, crystal growth is achieved by allowing solutions of soluble salts to diffuse through a gel with nucleation and CaCO3 precipitation taking place in that gel (e.g., McCauley and Roy, 1974; Meldrum, 2003; Fernández-Díaz et al., 2006; Meldrum and Colfen, 2008). Such experiments can lead to the precipitation of all three polymorphs of CaCO3 within microns of each other (Nickl and Henisch, 1969; McCauley and Roy, 1974), mesocrystals (Colfen and Antonietti, 2005), aragonite and calcite crystals of many different
crystal morphologies (Dominguez Bella and Garcia-Ruiz, 1987, their Fig. 2; Grassmann et al., 2002, their Figs. 6, 7; Grassmann et al., 2003, their Fig. 1; Cölfen and Antonietti, 2005, their Figs. 9, 10, 17), crystals with curved faces (e.g., Prieto et al., 1981, their Figs. 2, 4), and incompletely formed crystals (e.g., Fernández-Díaz et al., 1996). In essence, the parameters deemed to control precipitation in gels are as varied as those invoked to explain precipitation from natural fluids. With gels, however, it is perhaps easier to explain the microscale variations in the precipitates because they can be linked to the microscale growth networks that develop in the gels (Falini et al., 2000; Grassmann et al., 2002).

Based on spring precipitates from the Kenya Rift Valley, Jones and Renaut (1995, their Fig. 14) suggested that as a disequilibrium factor increased, the morphology of the crystals progressively changed from skeletal crystals to dendrite crystals, to wheat sheaves, to spherulites (Fig. 11A). Their “disequilibrium factor” denoted the collective effect that all the variables (e.g., rate of CO2 degassing, water flow rates and water temperature) had on the saturation levels that ultimately triggered precipitation. Oaki and Imai (2004, their Fig. 1) depicted similar relationships based on their experimental growth of CaCO3 crystals in silica gels (Fig. 11B). They argued that crystal morphologies reflected the correlation between the driving force of crystallization and the diffusion of atoms, ions, molecules, or heat. According to their scheme, polyhedral crystals form when the driving force is low and dendritic crystals grow when the driving force is high (Fig. 11B). For crystal growth in a
gel, however, other factors related to the nature of the gel may also be important. Nickl and Henisch (1969), for example, argued that crystal growth in gels is controlled by (1) diffusion that dictates the solute concentrations at the growth boundaries, and (2) suppression of nucleation sites because the gel is an efficient filtering agent that leads to the isolation of solute in microdomains that are too small to permit effective homogeneous nucleation. Oaki and Imai (2004) supported this viewpoint by noting that increases in the saturation levels in the parent fluids did not lead to a change in crystal form whereas an increase in gel density caused different crystal forms to develop. This along with information from other experimental studies (e.g., Dennis and Henisch, 1967; Robert and Lefaucheux, 1988; Cecal et al., 1996) has led to the notion that diffusion-limited crystal growth dominates in gels (Oaki and Imai, 2004).

In the Eryuan Spring deposits, the presence of silicified microbes (Fig. 6D–J) alongside CaCO₃ precipitates provides more evidence of the microscale variance in the processes that are operative in the biofilms. There is, however, little evidence of microbe calcification apart from rare strings of calcite nanoparticles that may have formed on filamentous microbes (Fig. 6A–C). Comparison of hot-springs throughout the world shows that microbes are commonly preserved in opal-A precipitating systems (e.g., Cady and Farmer, 1996; Jones et al., 2003, 2004) but rarely if ever preserved in systems where calcite and/or aragonite are the primary precipitates (e.g., Jones and Renaut, 1995; Renaut et al., 2002; Jones and Peng, 2012b). Mineralization must take place rapidly because microbes will decay and disappear very rapidly (Krumbein, 1979; Buczynski and Chafetz, 1991; Chafetz and Buczynski, 1992). Thus, the 3-dimensional preservation of the silicified microbes in the Eryuan deposits implies rapid mineralization, as is known from other settings (e.g., Oehler and Schopf, 1971; Francis et al., 1978; Westall et al., 1995; Renaut et al., 1998; Konhauser et al., 1999). It seems, however, that the microbes are far less susceptible to calcification and are therefore lost to decay before they can be preserved.

In the Eryuan Spring pool, the diversity of the precipitates evident from their composition and their precipitation styles is indicative of growth that is strongly influenced by biofilms. Similarly, precipitates in Gongxiaoshe hot spring and Zhuyuan hot spring (located in the village of La Xin, Ruidian geothermal area, western Yunnan Province) that developed in association with biofilms (Jones and Peng, in press) are characterized by (1) aragonite, calcite, and ACC; (2) incompletely
formed calcite crystals, (3) dodecahedral and rhombohedral calcite crystals, and (4) calcite mesocrystals. Unlike the precipitates from Eryuan hot spring, those precipitates are characterized by (1) alternating aragonite and calcite laminae, (2) pseudodendrites constructed of calcite crystals, (3) Si–Mg reticulate coatings, (4) scattered crystals of barite and gypsum, and (5) the lack of any opal-A precipitates. The deposits in these three springs are linked by (1) their intimate association with microbial biofilms, (2) the mineralogical and morphological diversities of the precipitates that are commonly evident at the microscale, (3) the presence of incompletely grown crystals, and (4) mesocrystals.

If the precipitates from Eryuan Spring and other springs are considered from a theoretical perspective, then most of the features that are indicative of biomediated precipitation will probably vanish.

Fig. 8. SEM photomicrographs showing calcite crystals in Eryuan Spring deposits. (A) Euhedral rhombic calcite crystal. (B) Interlocking rhombic calcite crystals. (C) Euhedral dodecahedron with pentagonal faces associated with aragonite (A). Arrow labeled D indicates crystal corner shown in panel D. (D) Contrasting morphologies of crystal faces associated with corner of crystal. (E, F) Interlocking rhombic crystals with each crystal having beveled edges. Arrow labeled F indicates corner shown in panel F. (G) Incompletely formed rhombic calcite crystal associated with biofilm. Arrow labeled H indicates corner shown in panel H. (H) Corner of crystal shown in panel G, showing incompletely formed crystal faces and microstructure on bevel edges. (I) Incompletely formed dodecahedron (?) crystal associated with biofilm. Box labeled J indicates position of panel J. (J) Enlarged view of crystal shown in panel I, highlighting partly formed crystal faces and crystal edge. (K) Incompletely formed calcite crystals associated with biofilm. Note the hole in upper crystal and irregular overgrowths on some crystal faces. Arrow labeled L indicates position of panel L. (L) Incomplete calcite overgrowth.
during early diagenesis. Specifically, these early diagenetic changes may include (1) breakdown of crystal bushes as the biofilm is lost through decay with the constituent crystals settling to the pool floor, (2) loss of the extremely unstable ACC (e.g., Nebel and Epple, 2008; Xu et al., 2008; Njegić-Džakula et al., 2010; Radha et al., 2010), which will transform into a crystalline CaCO₃ phase, (3) loss of the aragonite, which will be dissolved or transformed into calcite, (4) loss of identity of the mesocrystals, which will probably be transformed into monocrystals as precipitates fill the gaps between the constituent nanocrystals, and/or (5) the loss of porous and incompletely formed crystal faces as continued precipitation takes place. These processes would effectively disguise most of the features that could be considered indicative of precipitation that was mediated in the microcosms of the biofilms.
**Fig. 10.** SEM photomicrographs showing spatial relationships between the CaCO₃ precipitates in the deposits from Eryuan Spring. A = aragonite, C = calcite, ACC = amorphous CaCO₃, BF = biofilm. (A) Clusters of aragonite crystals lying between euhedral calcite crystals. Patches of biofilm are evident between and on top of some of the aragonite bundles. (B) Biofilm overlying substrate formed of euhedral calcite crystals and bundles of aragonite crystals. (C) Wheat-sheaf bundles of aragonite crystals lying between euhedral calcite crystals. (D) Radiating cluster of aragonite crystals with flat base, lying next to euhedral calcite crystal. (E) Calcite crystal growing around a wheat-sheaf bundle of aragonite crystals that is partly covered by a biofilm. (F) Partly formed calcite dodecahedron that grew around bundles of aragonite crystals. Note associated biofilm.

**Fig. 11.** Comparison of crystal morphologies relative to crystal growth factors as given by (A) Jones and Renaut (1995, their Fig. 14) based on interpretation of crystals found in various Kenyan hot springs, and (B) Oaki and Imai (2004, their Fig. 1) based on interpretations of crystals grown in gels.
7. Conclusions

Analysis of crystal bushes that grow on the floor of the Eryuan hot spring has led to the following conclusions:

• The crystal bushes develop beneath biofilms that are dominated by phototrophic purple bacteria and green bacteria.
• The crystal bushes are formed of aragonite, calcite, ACC and, in some areas, opal-A.
• The aragonite was precipitated as clusters of radiating crystals and wheat–sheaves.
• The rhombohedral and dodecadal calcite crystals are intimately associated with the aragonite.
• Many of the calcite mesocrystals are incompletely formed and charac-
terized by porous crystal faces.
• There is no indication that the calcite formed through recrystalliza-
tion of the aragonite or ACC.
• The fact that some microbes were preserved through silicification whereas none were preserved by calcification stresses the notion that microbes have a low preservation potential in CaCO₃ precipi-
tating systems.

The diversity of precipitates and variations in the style of precipita-
tion of CaCO₃ polymorphs are indicative of precipitation that took place in the microcosms of the biofilms where physiochemical condi-
tions can vary on the submicron scale. Early diagenetic changes, how-
ever, quickly destroy many of these features and thereby mask the fact that the constituents formed through biomediated precipitation.

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