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EXPERIMENTAL ARTICLES

Laboratory Simulations of Cyanobacterial Mats of the Alkaline Geochemical Barrier

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Abstract—The goal of this work was to illustrate a possible interaction between the “soda continent” and the ocean. A laboratory simulation was undertaken of the development of alkaliphilic mat with calcium carbonate and calcium phosphate interlayers in the zone where ocean waters, containing calcium and manganese, come into contact with carbonate- and phosphate-rich alkaline waters. The macrostructure of the layered cyanobacterial mat turned out to be little dependent on the chemical conditions causing sediment formation. The chemical composition of freshly formed mineral interlayers of the mat was found to vary with the medium composition. The mineralogical composition of the sediment is determined by diagenesis conditions in its depth, which can cause mineral phase conversions.

Key words: cyanobacteria, cyanobacterial mats, modeling, mineralization, calcium carbonates and phosphates, dolomite.

On the geological time scale, the removal of carbon dioxide from the atmosphere occurred through its binding into carbonates. The scale of carbonate rock formation makes it a major geochemical process determining the overall redox conditions on the earth. The first biogenic carbonate rock is represented by stromatolites, which are the metabolic products of benthic cyanobacterial communities. The sedimentation of carbonate is believed to proceed within cyanobacterial mats due to the occurrence of an alkaline barrier created in the mat by photosynthesis and absorption of CO_2 during autotrophic assimilation. Although such a barrier does indeed arise at the depth of 1–2 mm from the mat surface, as readily detected by microelectrode probes, the sedimentation of calcium carbonate occurs on a very limited scale, apparently because of the contradictory diffusion requirements: a change in the pH value requires restricted mass exchange, while precipitation of carbonate requires a steady inflow of Ca^{2+}. This mechanism, therefore, is to be recognized as insufficient [1].

Stromatolites are formed in warm shallow waters in the zone of ocean and land interaction. The precipitation of carbonate occurs in the zone of water mixing. Two variants of such interaction are possible: (a) bicarbonate-rich land waters serve as the source of Ca^{2+} and Mg^{2+} and the “soda ocean” serves as the alkaline pool [2] and (b) weakly acid marine waters are the pool of Ca^{2+} and Mg^{2+} and alkaline waters are drained from the land, where, as a result of carbonic acid weathering, the “soda continent” is formed [3]. Both variants are similar in that carbonates are subject to chemical precipitation in the mixing zone, which, at the same time, is an ecotone populated by cyanobacterial communities.

The formation of layered structures similar to stromatolites in the course of sedimentogenesis can be readily simulated under laboratory conditions [4]. The objective of our experiments was to simulate the process of carbonate sedimentation under conditions where calcium-containing seawater medium with pH values close to neutral comes into contact with simulated alkaline waters of the “soda continent.” Reproduction of the overall mat structure with the participation of organic matter degraders and microorganisms of the sulfur cycle was not the goal of this work, and so we limited ourselves to a single edifying organism responsible for the creation of the structure.

MATERIALS AND METHODS

A strain of *Microcoleus chthonoplastes* isolated from the Khilganta steppe lake (Chita oblast, Russia) and exhibiting adaptability to the pH and mineralization of the medium [5] was used in the experiments. The culture was grown in a soda medium (pH 9.2) containing (g/l) $NaHCO_3$, 3; Na_2CO_3, 17; NaCl, 30; $K_2HPO_4 \cdot 3H_2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; KNO_3, 2.5; $CaCl_2 \cdot 6H_2O$, 0.04; $FeSO_4 \cdot 7H_2O$, 0.01; and 1 ml of trace-element solution A. This medium was designed to simulate continental soda waters; the content of biogenic elements was elevated to sustain the development of *Microcoleus.* The cyanobacterium showed abundant growth on this medium, forming a thick film.

The mineral medium (pH 7.0) imitating the composition of seawater with an increased content of biogenic elements was composed of (g/l) NaCl, 23.5; $MgCl_2 \cdot 6H_2O$, 5.0; $CaCl_2 \cdot 6H_2O$, 1.1; KCl, 0.7; Na_2SO_4, 4.0; $NaHCO_3$, 0.2; KBr, 0.1; H_3BO_3, 0.03; $FeSO_4 \cdot 7H_2O$, 0.01; $Na_2SiO_3 \cdot 9H_2O$, 0.3; $K_2HPO_4 \cdot 3H_2O$, 0.01; and NH_4Cl, 0.01.

To attain the formation of mineral interlayers in the mat, the concentrations of magnesium, phosphorous, and iron salts in the seawater medium were increased to 4 g/l, 600 mg/l, and 18 mg/l with respect to the corresponding element.

Calcium was determined by trilonometry [6]. Growth of cyanobacteria was judged from the content of chlorophyll *a* extracted with 80% alcohol [7]. Crystallographic analysis of the obtained sediment was performed using a Rigaku D max 3C powder diffractometer. The elemental composition of mineral interlayers was determined with a Link-860 X-ray microanalyzer.

The experiments were run in three variants simulating (1) counterdiffusion of seawater and soda water, (2) seawater flooding of a benthic cyanobacterial mat in tidal areas, and (3) percolation of alkaline carbonate-rich waters through the seabed (Figs. 1–3).

In the first variant (Figs. 1, 4), a slope of clay was formed in the cultivation vessel. Its surface was strengthened with liquid agar to reduce turbidity. The higher part of the slope had a depression containing soda medium with phosphate (pH 10.2). The lower part was submerged in seawater medium with 20 mM Ca^{2+} (initial pH 6.5). *Microcoleus* developed between the “soda lake” and the “sea” in the zone of carbonate sedimentation caused by counterdiffusion. It should be noted that we were not concerned with obtaining a layered stromatolite-like structure.

In the second variant (Fig. 2), cyanobacteria were cultured in two vials, one inside another. The inner vial was a turned over plastic bottle with the bottom cut off; the bottle was filled with washed sand. The outer vial was used to control the level of liquid soda medium. The vials were wrapped in black paper and illuminated from above with incandescent lamps at 1500 lx. *Microcoleus* developed on the sand surface in the form of a thick film. When the surface was flooded with seawater medium, a white layer of carbonate sediment immediately started to form on the film.

In the third variant (Fig. 3), the apparatus consisted of two communicating vials filled with an alkaline solution containing (g/l) $NaHCO_3$, 7.5; Na_2CO_3, 42.5; and $K_2HPO_4 \cdot 3H_2O$, 0.5 (pH 9.6). A perforated plastic disk covered with capron tissue was put in the first vial, and on top of this disk a *Microcoleus* film was placed. The second vial was used to adjust the liquid level. The liquid covering the *Microcoleus* film in the first vial was seawater medium.

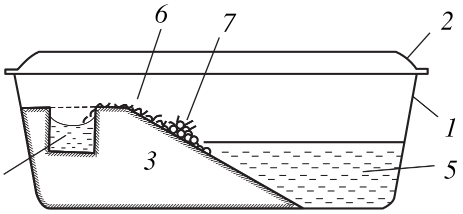

Fig. 1. A variant of a laboratory setup to simulate the interaction between oceanic and continental waters: (*1*) cultivation chamber; (*2*) lid; (*3*) clay slope; (*4*) depression containing alkaline medium; (*5*) seawater medium; (*6*) growth zone of cyanobacteria; and (*7*) zone of carbonate formation.

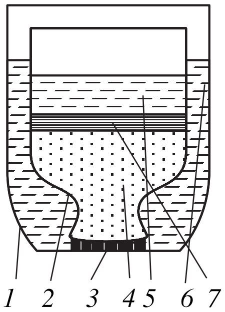

Fig. 2. A variant of the apparatus to simulate seawater flooding of the region of alkaline vadose waters: (*1*) external vial containing alkaline medium; (*2*) internal vial with alkaliphilic mat; (*3*) stopper with holes for media communication; (*4*) river sand; (*5*) seawater medium; (*6*) alkaline medium; and (*7*) cyanobacterial mat.

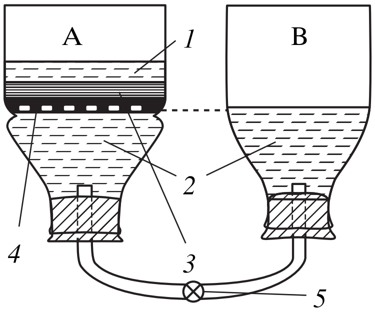

Fig. 3. A variant of the apparatus to simulate an upward drain of alkaline medium into a marine body of water: (*1*) seawater medium; (*2*) alkaline medium; (*3*) cyanobacterial mat; (*4*) filter; and (*5*) clamp. A is the work vial and B is the auxiliary vial.

RESULTS AND DISCUSSION

In the first experimental variant, a film of *Microcoleus* developed in the zone between the “soda lake” rich in phosphate and the reservoir of calcium-rich water (Figs. 4a–4c). The precipitation of carbonate took place in the same zone. Because of the variation of precipitation conditions along the slope, the sediment formed in a variety of shapes, mostly poorly crystallized spheroid

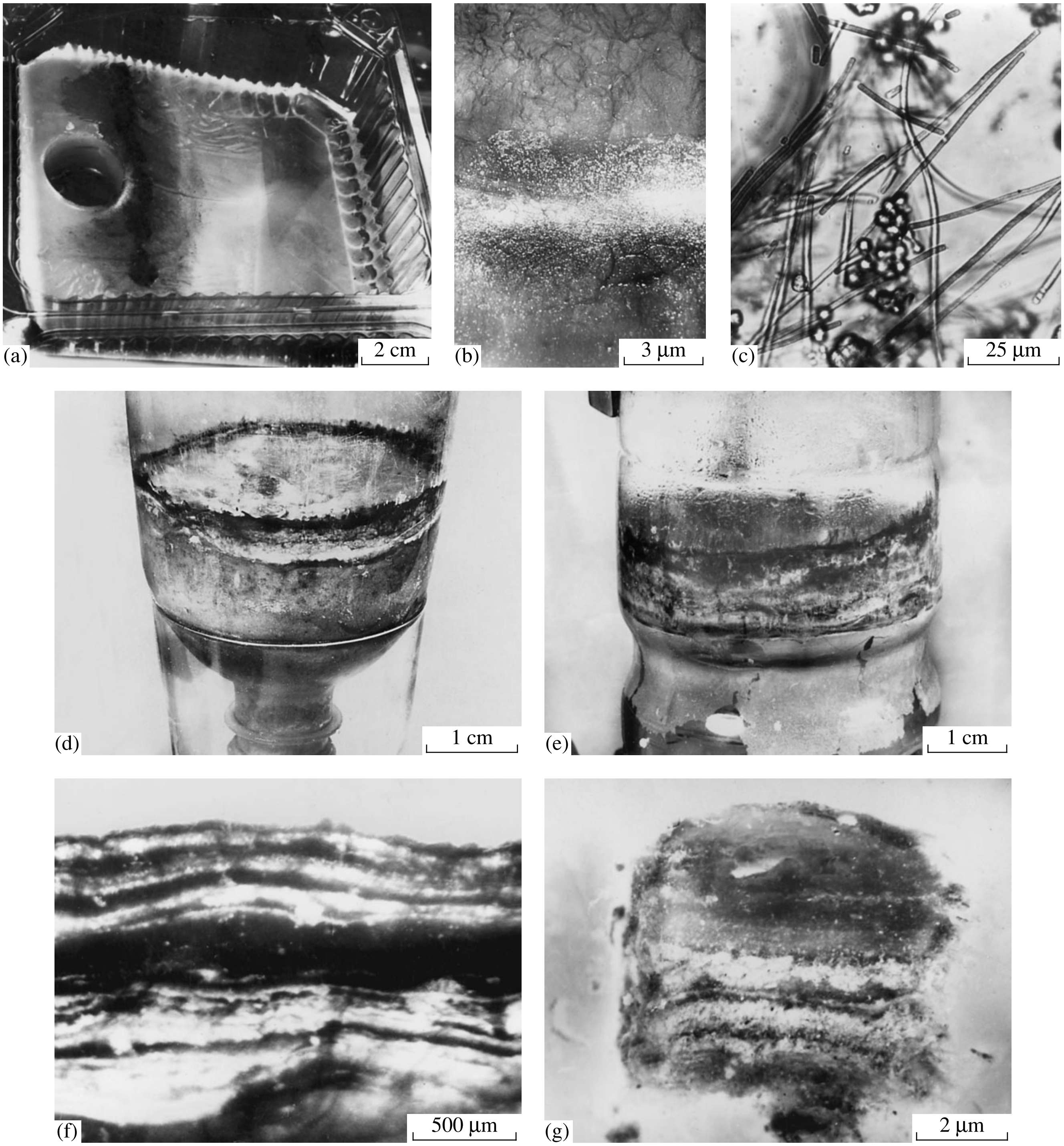

Fig. 4. (a) A general view of the simulation setup (see Fig. 1); (b) the carbonate zone formed as a result of the interaction between the seawater medium and the alkaline medium; (c) crystals of chemically formed carbonate amid filaments of cyanobacteria; (d) alkaline cyanobacterial mat residing on sand (Fig. 2); (e) alkaline cyanobacterial mat under the conditions of alkaline medium upflow (Fig. 3); (f) a vertical section of alkaline cyanobacterial mat; and (g) a natural mat from Lake Khilganta (Buryatiya, Russia).

lumps, which indicated fast chemical precipitation. Trichomes of *Microcoleus* were located amid these lumps, but there was no sediment deposition on live motile filaments. It was firmly established that the crystallizing nuclei were dead filaments or slimy polysaccharide sheaths arising on trichomes in older cultures or in response to increasing concentration of certain elements in the medium. At the same time, actively photosynthesizing motile trichomes remained free of sediment. This is at odds with the notion of the leading role of photosynthesis in carbonate precipitation and points to the presence of a physiological mechanism that precludes deposition of crystals on the surface of *Microcoleus* trichomes while sedimentation is in progress in the space between the trichomes. Photosynthesis in this case was not the driving force of calcite formation. The precipitation of calcite also occurred in the zone free of *Microcoleus* growth. It was particularly rapid in the evaporation area, with conditions favorable for CO_2 release into the atmosphere. In the surface film, trichomes occurred along with precipitated calcite. A high concentration of phosphate in the soda solution had a stimulating effect on the development of cyanobacteria. In this experimental variant, the conditions favorable for the development of a *Microcoleus* film matched those for chemical precipitation of calcite. The hypothesis of the development of a cyanobacterial community in the zone of the alkaline geochemical barrier is, therefore, quite plausible.

The layered structure of the sediment was more pronounced in the second and third experimental variants (Figs. 4d, 4e). The second variant simulated the development of mat in the tidal area subjected to cyclic alteration of seawater and subaerial conditions. The third experimental variant simulated upward flow of alkaline medium in a marine body of water. When the mineral sediment settled on the surface of the film formed by grown cyanobacteria, it immediately started to be penetrated by actively motile trichomes. Within an hour, the surface of the sediment was covered by a layer of cyanobacteria, and the next layer arose within a day. The process of layer generation depended on two biological mechanisms: the first was the spread of cyanobacteria over the sediment surface by virtue of their motility and the second was their multiplication on the illuminated surface. *Microcoleus* developed in alkaline medium, whereas the addition of calcium-rich seawater medium gave rise to precipitation. The layered structure was created as a result of reiteration of these processes (Fig. 4f).

The mat obtained in the second experimental variant over a period of 3 months (Fig. 4g) was similar in its morphology and other characteristics (chlorophyll content, 3.6 mg/cm^2; calcium content, 6 mg/cm^2) to natural *Microcoleus* mats, both to those formed under thalassic conditions of Sivash hypersaline lagoons [8] and those formed under the athalassic conditions of Lake Khilganta [9].

In our simulation experiments, the carbonate sediment was observed to undergo diagenesis in the course of mineral layer formation. The freshly formed calcium carbonate lacked crystalline structure. Within a day, however, the crystallization process became effective and started to produce crystals that shined in the polarizing microscope, in agreement with evidence obtained in geochemical studies [9]. Crystallographic analysis showed the presence of monohydrocalcite.

The elemental and mineralogical makeup of the three-month-old mat showed variation across the layers. The topmost layer, with live cyanobacteria (Fig. 5b), exhibited a remarkably large variety of constituent elements (Fig. 5a). With time, however, this layer underwent diagenesis resulting in the predominance of calcite (85%) and aragonite (15%) (Figs. 5c, 5d). The layer located under that of live cyanobacteria consisted of hydroxylapatite (35%) (Fig. 5e, 5f) and monohydrocalcite (65%) (Figs. 5g, 5h). The bottommost layer was entirely composed of monohydrocalcite. An elevated content of phosphates was observed to cause sedimentation of monohydrophosphate. In all of the cases studied, the obtained mats had the same macrostructure.

With larger quantities of magnesium added, precipitation of magnesite was followed by the formation of an amorphous sediment with the gross formula $CaMg(CO_3)_2$, which corresponds to that of dolomite.

Dolomite is a carbonate that formed huge depositions, mostly in the Precambrian. Modern formation of primary dolomite is known to occur under extraordinary conditions of highly mineralized lakes and marine lagoons. The “dolomite epoch of carbonate sediments” was followed in geological time by the limestone epoch. In the laboratory, dolomite does not form except under very special conditions: pH 9.5, high sulfate concentration, high ratio of Mg/Ca, and elevated temperature. Under ordinary conditions, a mixture of calcite and magnesite is formed. Dolomite may originate not as a primary sediment but via a geologically slow interaction between dissolved Mg^{2+} and a $CaCO_3$ sediment [10]. Magnesium can come from seawater. The conversion of $CaCO_3$ to dolomite normally occurs during diagenesis a short time after sediment deposition, as suggested by complete dolomitization of the sediment and the lack of any influence on the dolomite rock of subsequent geological conversions. In Precambrian stromatolites, dolomitization is quite pronounced [11].

The relatively fast diagenesis of carbonate sediment does not agree with the concept of a very slow subsequent conversion of limestone to dolomite and the corresponding vanishing of ancient limestone. Calcium carbonates have been preserved in skeletons for several hundred million years. As noted above, high degree of mineralization, high pH, and low concentration of Ca^{2+} (resulting in a low Ca/Mg ratio) in the interstitial solution in our simulation experiments led to the formation of an amorphous $CaMg(CO_3)_2$ sediment. The first to be formed were complex amorphous carbonates of Ca and Mg. The conversion of the amorphous sediments to the crystal state was likely to proceed during the postbiogenic phase of diagenesis. However, no crystalline structures typical of dolomite were detected in our simulation experiments.

Our simulations suggest that the zones of development of cyanobacterial mats (stromatolite precursors) could coincide with the large-scale alkaline geochemical barrier occurring at the boundary between the land

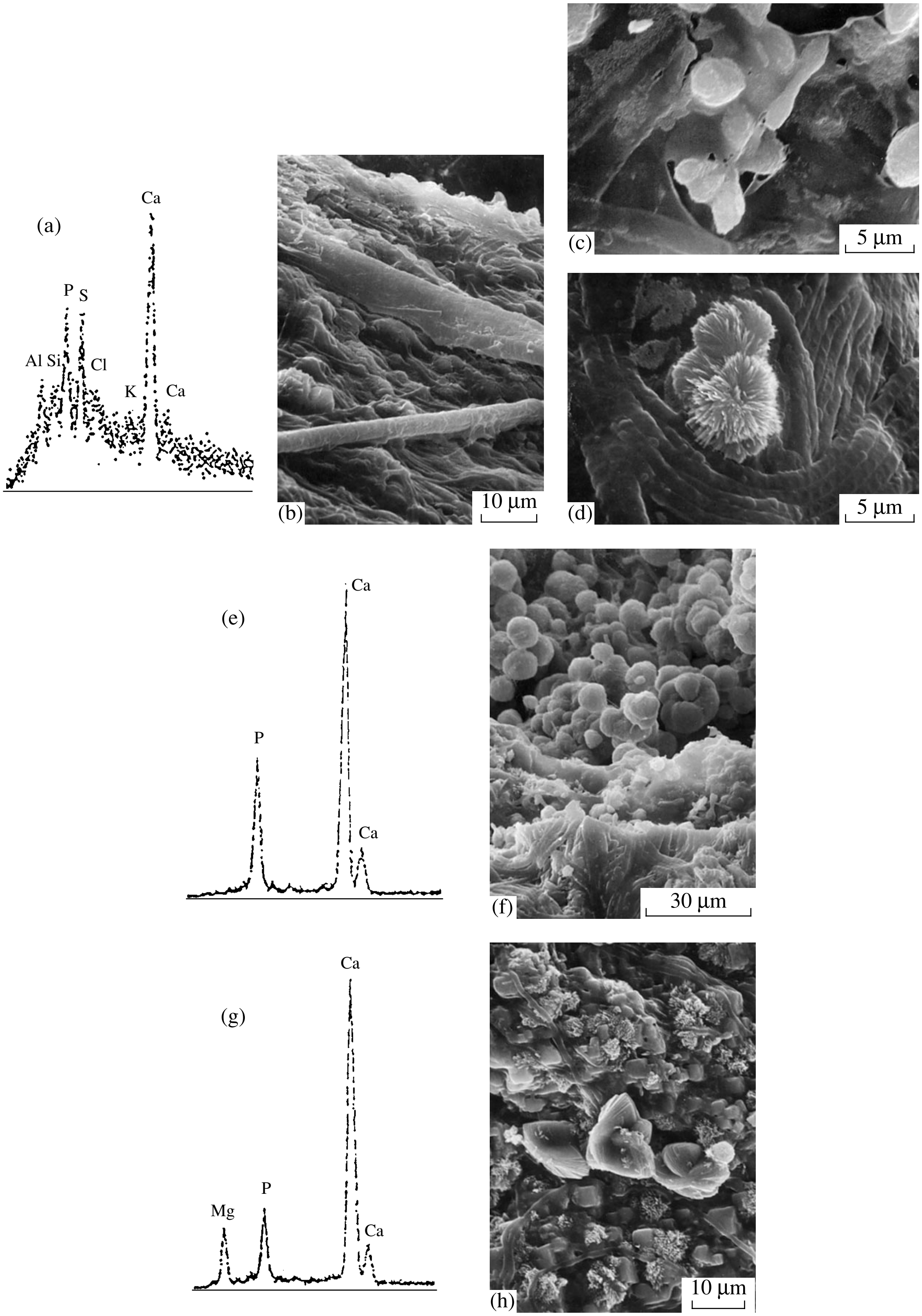

Fig. 5. (a) Elemental composition of the topmost cyanobacterial layer in the third experimental variant; (b) the surface layer of a growing mat; (c) calcite crystals; (d) aragonite crystals; (e) elemental composition of the intermediate mineral layer; (f) a view of hydroxylapatite crystals; (g) elemental composition of the bottommost mineral layer; and (h) a view of the bottommost mineral layer.

and the ocean. The culture of *Microcoleus* chosen for our simulations is an ordinary cyanobacterium, lacking, unlike limestone or drilling cyanobacteria, any specific properties with regard to carbonates. The benefit of this choice is that it provides the possibility of focusing on the underlying universal mechanism.

The macrostructures of layered cyanobacterial mats turn out to be little dependent on the chemical conditions causing sediment formation. The chemical composition of freshly formed mineral interlayers in the mat corresponds to the state of the body of water at the moment of sediment formation. The mineralogical composition, however, is determined by diagenesis processes in the depth of the sediment, and this may cause mineral phase conversions.

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