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EXPERIMENTAL ARTICLES

The Phototrophic Community Found in Lake Khilganta (an Alkaline Saline Lake Located in the Southeastern Transbaikal Region)

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Abstract—The structure of the phototrophic community found in Lake Khilganta (the Agin-Buryat Autonomous Area), a shallow saline soda lake (depth, 35–45 cm; water mineralization, 45 g/l; alkalinity, 30 mg-equiv/l; pH 9.5) has been studied. The bottom of the lake is covered with a 10- to 15-mm microbial mat, whose basis is formed by the filamentous cyanobacterium *Microcoleus chthonoplastes.* The mat exhibits pronounced layering and contains a significant amount of minerals. Six zones, which have characteristic colors and consistencies and are composed of intermittent layers, have been identified along the vertical profile. Live phototrophic bacteria have been found in the three upper zones. The bulk of the cyanobacteria is concentrated in the upper zone. In the lower zones, the development of purple bacteria has been observed. The diurnal dynamics of the vertical distribution of phototrophic microorganisms, which results from variations in the physicochemical environmental parameters, is described. *Ectothiorhodospira* sp. are dominant among the anoxyphotobacteria present. Their number, determined according to the inoculation method, is 10^6–10^7 cells/ml. The purple bacteria of the genera *Allochromatium, Thiocapsa*, and *Rhodovulum* are also present. Experiments with isolated pure cultures have shown that the anoxygenic photosynthetic bacteria of Lake Khilganta are halotolerant and alkalitolerant or alkaliphilic. In liquid enrichment cultures, at pH 9.5, the ratio of anoxyphotobacteria species is close to that observed in the lake. When the pH is increased to 10.4, it is *Ectothiorhodospira*, which is the most adapted to life under increased mineralization and alkalinity, that predominantly develops. Photosynthetic activity has been observed in the three upper mat zones and constitutes, on average, 1.5 g C/(m^2 h); the share of anoxygenic photosynthesis accounts for 75–95% of the total productivity. The main role in sulfide oxidation belongs to the phototrophic anoxyphotobacteria and cyanobacteria. In terms of the physicochemical conditions and structure of the phototrophic community, Lake Khilganta is similar to shallow saline water bodies of marine origin. The main differences consist in the increased alkalinity and in the consequent prevalence of alkaliphilic and alkalitolerant microorganisms and in the absence of representatives of the neutrophilic group of green sulfur bacteria.

Key words: saline soda lakes, microbial mat, vertical stratification, anoxyphotobacteria, alkalitolerant and alkaliphilic bacteria.

Recently, soda lakes have attracted the attention of microbiologists in many laboratories around the world. Alkaline water bodies of different types have been investigated, and many new microbial taxa, belonging to virtually all of the physiological groups recognized, have been described [1].

Owing to increased mineralization and alkalinity, the biota of soda lakes predominantly consists of prokaryotic microorganisms whose metabolism is distinguished by a number of specific features determined by the specifics of the conditions of existence. The increasing interest in extremely alkaliphilic microbial communities is also connected with a hypothesis that regards them as a relict analogue of the terrestrial biota of the early Proterozoic era [2].

The subject of this study was Lake Khilganta, a saline alkaline lake situated in the cryoarid zone of the southeastern Transbaikal Region on the territory of the Agin-Buryat Autonomous Area. Lake Khilganta differs from most of the lakes in this region, which are slightly saline, by increased mineralization of its water (45 g/l) and a markedly pronounced development of microbial mats. According to M.G. Valyashko's classification, it belongs to saline lakes of the sodium–sulfate type [3].

The microbiological investigations of Lake Khilganta were started in 1995 by a joint expedition from the Institute of Microbiology, Russian Academy of Sci-

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ences (Moscow), and the Buryat Institute of General and Experimental Biology, Siberian Division, Russian Academy of Sciences (Ulan-Ude). In publications devoted to the soda lakes of the Transbaikal Region (including Lake Khilganta), the physical–geographic and geological characteristics of the region, the chemical composition of the lake water, and the mineralogical composition of the bottom sediments have been presented. The processes of organic matter degradation have also been considered [3, 4]. Gerasimenko *et al.* [5] studied mat samples obtained from Lake Khilganta in 1995. They determined the species affiliation of various cyanobacteria and eukaryotic microalgae and described the macro- and microstructure of the alkaliphilic microbial mat in comparison with the mats of shallow saline water bodies located in the Crimea.

Our work was devoted to further study of the phototrophic community of Lake Khilganta. The main emphasis was placed on anoxyphotobacteria and their adaptability to the conditions of the habitat, on the dynamics of the vertical stratification of the microbial mat, and on the role of the phototrophic microbial community in production processes and sulfide detoxification.

MATERIALS AND METHODS

The subject of this study was the microbial mat covering the whole lake bed of Lake Khilganta. Field investigations were carried out in July 1995 and July 1996. More detailed investigations, including on the diurnal dynamics of the environmental parameters and mat structure, were carried out in 1996.

During the field work, the following environmental physicochemical parameters were determined: the water temperature, with a mercury thermometer; pH, with a conductometer and litmus paper; alkalinity, by titration [6]; and total mineralization of the water, with an areometer and conductometer. The sulfide concentration in the water was determined by iodometric titration [6], and the oxygen content was determined using Winkler's method for sulfide-containing waters, with the addition of mercury nitrate to the sample [6]. The samples used for describing the vertical structure of the microbial mat were obtained from different sites in the lake and at different times of day. The mat layer thickness was measured by means of graph paper.

The immersion slides were incubated in situ for 8–12 h at different times of day. In the laboratory, the slides were stained with crystal violet [7] and examined under a phase-contrast light microscope. Smears were prepared from the sites of mass development of the bacteria (both in the mat and outside) and dried. They were then stained and examined in the same way as the immersion slides. Layer-by-layer mat samples were taken for subsequent microscopy. Each layer was separated with a scalpel, placed in a penicillin vial, and fixed with 30% glycerol. The photosynthesis rate was determined in the isolated samples using the radiocarbon method [8]. In order to inhibit the activity of photosystem II, diuron was used at a concentration of 7 μM.

The enumeration of the phototrophic bacteria was performed by inoculation of columns of an agarized medium with serial tenfold dilutions of homogenized mat samples (1 cm^2). Two variants of medium were used for more exact enumeration of the anoxyphotobacteria belonging to different groups. The basal medium common for both variants contained (g/l) KH_2PO_4, 0.5; Na_4Cl, 0.2; $MgCl_2 \cdot 6H_2O$, 0.2; $CaCl_2$, 0.05; NaCl, 40; $NaHCO_3$, 10; Na_2CO_3, 10; Na acetate, 0.5; microelement solution, 1 ml; and vitamin B_{12}, 20 μg. One medium variant additionally contained 0.5 g of $Na_2SO_3 \cdot 5H_2O$ and 0.5 g of $Na_2S \cdot 9H_2O$, and the other variant contained 0.5 g of sodium malate and 0.5 g of yeast extract. The bacteria were quantified by means of their maximum growth in one of the medium variants. The enumeration and culture isolation of purple bacteria were carried out at pH 9.5. In some experiments, the medium acidity was varied by varying the proportions of sodium carbonate and bicarbonate but without changing their total concentration. The cultures were incubated in a luminostat at 25°C and an illumination intensity of about 2000 lx. The colored colonies grown at different inoculum dilutions were counted and studied by microscopy.

Enrichment cultures were obtained in liquid media of the same composition, with pH 9.5 and 10.4; their dilutions were used to inoculate agar columns.

The reactions of the purple bacteria to the habitat conditions (pH and salinity) were studied using pure cultures of *Ectothiorhodospira* sp. A-20E and *Thiocapsa* sp. A-20T, which were isolated from the microbial mat of Lake Khilganta.

Ultrathin sections of the bacteria, obtained by the standard technique [9], were examined under a JEM 100C electron microscope.

RESULTS

Physicochemical Conditions

Lake Khilganta is nearly round in shape, being slightly elongated from northeast to southwest (its length is 45–50 m). Its maximal size is 0.5 km^2. The water level in the lake depends on the climatic conditions and is subject to significant fluctuations. Consequently, the water mineralization, pH, and alkalinity vary (see table). The structure of the phototrophic community developing in Lake Khilganta was studied in 1995 (July 5) and 1996 (July 14–15). The physicochemical conditions and the water regime of the lake were found to be similar in both of those periods (table). The depth was 35–45 cm, the total mineralization was 40–46 g/l, the alkalinity was 1.0–1.5 g/l (22–30 mg-equiv/l), pH was 9.5–9.8, and the water temperature in the daytime varied between 25 and 35°C.

Physicochemical conditions in Lake Khilganta in different years

Year	Date	Temperature, °C	Mineralization, g/l	pH	Depth, cm
1995	July 7	28	46	9.8	34
1996	July 15	34	45	9.5	35
1997	Aug. 10	32	43	9.7	36
1998	June 16	29	40	9.9	37
1999	July 2	27	38	10.0	40
1999	Aug. 8	24	42	9.8	42
2000	July 25	32	56	9.6	28
2001	June 18	30	102	9.1	8
2001	June 23	43	253	8.9	5
2001	Aug. 20	26	82	9.0	10

The redox conditions in the lake change during the day (Fig. 1). In the daytime, the oxygen content in the water increases and reaches 6–8 mg/l due to intense photosynthesis, which is mainly cyanobacterial. The sulfide formed in the mat and sediment by sulfate reducers is utilized by microorganisms and oxidized chemically by oxygen. With the onset of darkness and termination of both oxygenic and anoxygenic photosynthesis, the O_2 content drastically decreases. The oxygen remaining in the water is consumed by aerobic microflora and reduced chemically by sulfide. As the illumination intensity and oxygen content decrease, hydrogen sulfide accumulates in the sediment and mat and can even be revealed in the water in substantial amounts (up to 1mM). The bicarbonate content slightly decreases in the daytime, whereas the carbonate ion concentration increases insignificantly.

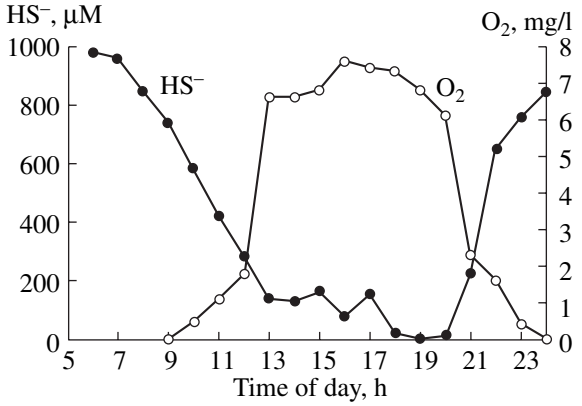

Fig. 1. Diurnal dynamics of the oxygen and sulfide contents in the water of Lake Khilganta.

The Structure of Microbial Mats

The 10- to 15-mm-thick microbial mats completely covered the bottom of the lake. The main part of the mat surface was dark green (sometimes almost black). In some places, spotlike light yellow-green areas occurred.

Over the entire area, the mats exhibited a well-defined thin-layer structure, in which mineralization processes actively occurred. Between the bacterial layers, layers of minerals were present, which, as was earlier shown by Gerasimenko *et al.* [5], mainly consisted of calcium carbonates and phosphates. This afforded the mats a dense, often gristly, consistency and firmness. In the central part of the lake, solid outgrowths of various shapes occurred, which were elevated some centimeters above the mat surface. They consisted of separated mat fragments covered with a mineral crust. Obviously, their formation is related to the emergence of underground mineral waters.

Some of the mat fragments had broken loose and floated upwards. On such fragments, as well as on the decomposing parts of plants that had found their way into the lake from the shore, massive development of purple bacteria, in the form of a pink film, was observed. In places where the mat had sunk or been disrupted, the water was colored red. In 1995, the formation of a pink foam on the water surface was observed.

A pronounced vertical stratification of both the physicochemical parameters and the microbial communities was found to be characteristic of the microbial mats. Despite a certain degree of variability in time and space, general regularities can be revealed in the mat structure and its dynamics.

Because the levels of water and values of the physicochemical parameters in Lake Khilganta were similar during the 1995 and 1996 expeditions, the microbial mat structure in these periods was very much the same, differing mainly in the degree of manifestation of separate zones. The results of a layer-by-layer investigation of a single mat sample obtained in 1995 are presented in the paper by Gerasimenko *et al.* [5]. In 1996, during the expedition, we succeeded in following the diurnal dynamics of the microbial mat's vertical structure, which is determined by fluctuations in physicochemical environmental parameters.

After visual and microscopic examination of the vertical structure of the large number of mat samples obtained from different sites in the lake and at different times of day, six main zones, present in all of the samples, were identified (Fig. 2). Each zone, in turn, had a layered structure.

Zone I, situated near the mat surface, was usually 1–2 mm thick. Its framework was formed by the filamentous cyanobacterium *Microcoleus chthonoplastes* (Fig. 3a). The zone had a layered structure: dark green layers of cyanobacteria were separated by whitish mineral-containing layers. Depending on the time of day, these dark green and whitish layers either interchanged

uniformly or the dark green layers were concentrated in the upper part of the zone while the whitish ones were concentrated in the lower zone. The zone as a whole had a dense gristly consistency; however, the green layers, taken individually, were softer and mucilaginous due to the significant development of glycocalyx surrounding the filament bundles of *M. chthonoplastes.* Microscopy (Fig. 3) of the fixed samples, immersion slides, and dry specimens also revealed the presence of diatoms, the unicellular cyanobacterium *Aphanothece salina*, filaments of *Phormidium molle* and *Oscillatoria* sp., the coccoid microalga identified by Gerasimenko as *Chlorella minutissima* [5], and protozoans in this zone. The purple bacteria *Ectothiorhodospira* sp., *Allochromatium* sp., and *Thiocapsa* sp. were also found in small amounts.

In certain areas of the mat, which appeared as light yellow-green spots against the dark green background, a thin (0.2–0.5 mm) yellow-green layer occurred above the *Microcoleus* layer. This yellow-green layer was formed mainly by the filamentous cyanobacterium *Phormidium molle.* The greatest amount of diatoms was also noted there.

Zone II, situated under zone I, was 1.5–2.5 mm thick on average and had a layered structure and, usually, a gristly or mucilaginous consistency. Some of its layers exhibited various purple tints (varying between pink and dark crimson), whereas the others had a whitish or grayish coloration and consisted mainly of minerals. The layer ratio changed during the day. The purple coloration of the layers of this zone was determined by the massive development of phototrophic bacteria of the genera *Ectothiorhodospira*, *Allochromatium, Thiocapsa*, and *Rhodovulum.* Microscopy revealed the presence of single diatoms and filaments of *Microcoleus* and *Phormidium.* In the lower part of zone II, the green filamentous bacteria *Chloroflexus* sp. occurred (Fig. 3).

Zone III was, on average, 2–3 mm thick. It also had a layered structure but exhibited a less dense (friable) consistency than the other zones. The main color of the layers was brownish green with a purple tint, whose intensity changed during the day.

The brownish green coloration of this zone was due to the presence of detritus particles and *M. chthonoplastes* filaments, both alive and partially destroyed or partially mineralized (Fig. 3). It should be noted that the high photosynthetic productivity recorded in this zone provides evidence for the presence of a significant number of physiologically active cyanobacterial cells. The purple tint was due to anoxyphotobacteria of the genera *Ectothiorhodospira, Allochromatium*, and *Thiocapsa.* Microscopy also revealed the presence of the unicellular cyanobacterium *Aphanothece salina* and the filamentous cyanobacteria *Spirulina* sp.

Zone IV was, on average, 1–3 mm thick. It was characterized by a prevalence of gray coloration, layered structure, and dense gristly consistency. Minerals, among which mineralized fragments of cyanobacteria occurred, formed the basis of this zone.

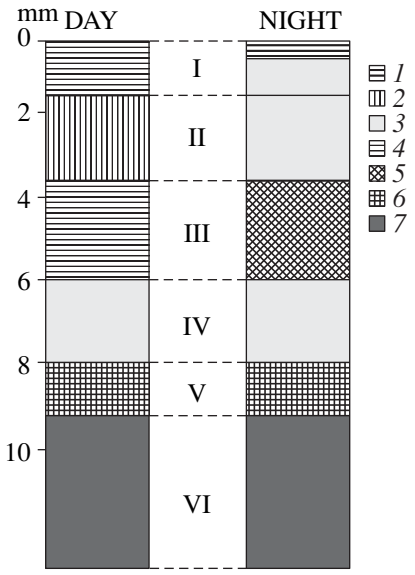

Fig. 2. Scheme of the vertical structure of the microbial mat in Lake Khilganta in the light and dark times of the day: (*1*) dark green layers formed predominantly by *Microcoleus chthonoplastes*; (*2*) purple layers with a predominance of purple bacteria; (*3*) whitish and grayish layers mainly consisting of minerals; (*4*) brownish green layers containing live and partially decomposed or partially mineralized filaments of *M. chthonoplastes*; (*5*) purple–brownish green layers consisting predominantly of *M. chthonoplastes* filaments with different degrees of intactness and purple bacteria; (*6*) light brown layers formed mainly by *M. chthonoplastes* filaments and their fragments at different stages of degradation and mineralization; (*7*) dark gray, almost black, layers with a prevalence of sulfate-reducing bacteria.

Zone V, which was 1–2 mm thick, also had a layered structure. The layers were mainly light brown and dense; however, they were not firm and could easily be disrupted. They were mainly formed by *Microcoleus* filaments and their fragments, at different stages of destruction and mineralization.

Below zone V, dark gray reduced silt was observed. Its upper layer was a structural continuation of the mat: it was mechanically connected with the mat, and was, in fact, its lowest zone (VI). This 3- to 8-mm-thick dark gray silt layer was retained on the mat upon its withdrawal from the lake.

This zone had a friable consistency and a poorly marked layering. Microscopy revealed mineralized filaments of cyanobacteria and their fragments; detritus particles; and unicellular bacteria, among which vibrios, most probably sulfate reducers, predominated. Its dark gray, almost black, color and hydrogen sulfide odor were indicative of the intense process of sulfate reduction in this zone.

In the above description of the zones, we considered the microbial mat structure from the point of view of

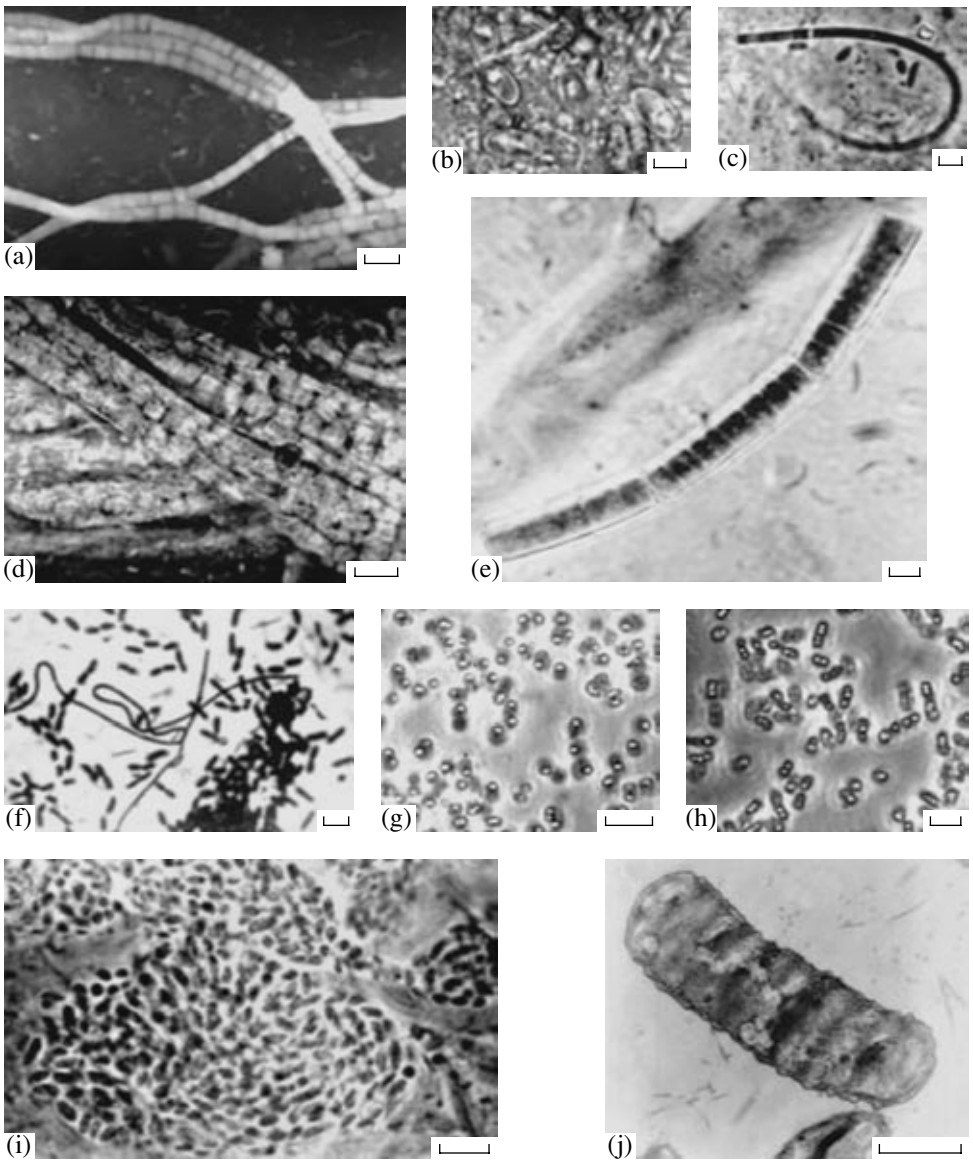

Fig. 3. Microorganisms forming the cyanobacterial mat of Lake Khilganta: (a) *M. chthonoplastes*, bundles of filaments; (b) protozoans; (c) *Phormidium molle*; (d) *M. chthonoplastes*, partially mineralized bundles of filaments; (e) *Oscillatoria* sp.; (f) filaments of *Chloroflexus* sp. and rod-shaped cells of *Ectothiorhodospira* sp.; (g) *Thiocapsa* sp. with intracellular sulfur droplets; (h) *Allochromatium* sp. with intracellular sulfur droplets; (i) "balls" formed by *Ectothiorhodospira* sp. in the zone of contact with oxygen; (j) an ultrathin section of an *Ectothiorhodospira* sp. cell with the membranous apparatus of photosynthesis in the form of stacks of lamellae. The images were obtained by (a)–(i) phase-contrast light microscopy (bars, 5 μm) and (j) transmission electron microscopy (bars, 1 μm).

emphasis on the phototrophic microorganisms. Although we did not mention bacteria of other physiological groups, it should be noted that they were present in various amounts in all of the mat layers. However, it was zones I and II that had the greatest number and diversity of nonphototrophic bacteria.

Diurnal Dynamics of the Microbial Mat Vertical Structure

The microbial mat structure described above was generally retained over the entire area of the lake. The samples obtained from different points differed only in relation to the thickness and nuances of color of individual zones and layers.

As can be seen in Fig. 2, zones IV–VI, which were devoid of live phototrophic microorganisms, retained a constant structure and coloration during the day, whereas the three upper zones underwent significant changes.

In the middle of the day, the dark green layers formed by *M. chthonoplastes* were distributed uniformly throughout zone I, alternating with the whitish

mineral layers. During the early morning and evening hours (no observations were carried out at night), the dark green layers were concentrated in the upper part (0.2–0.5 mm) of the zone, whereas the lower part consisted of the whitish layers only.

In the daytime, the purple bacteria primarily occurred in zone II. The following layers were observed (from top to bottom): lilac-pink, whitish, dark purple, and whitish. During the same period, zone III exhibited brownish green coloration of its layers, determined by the presence of detritus, *Microcoleus*, and a small amount of purple bacteria.

In the early morning and evening hours, zone II assumed a whitish or grayish coloration and consisted primarily of minerals; few purple bacteria remained. At the same time, zone III acquired a marked purple tint caused by an increase in the number of purple bacteria. On the whole, the number of purple bacteria in the mat, as judged from examinations of the immersion slides (Fig. 4), was lower at night than in the day. It is interesting that, when the environmental conditions and the vertical distribution of the microorganisms changed, the mat basis, i.e., its framework, was retained by virtue of the considerable mineralization and dense consistency of most of the layers. Despite the differences between the daytime and nighttime mats, the position of their main zones did not change; even their sizes changed little. Thus, in the nighttime mat, most of the phototrophic microorganisms left the lower part of zone I and zone II. These areas lost their coloration but retained their sizes. As a result, a discolored 3-mm horizon devoid of photosynthetic bacteria was formed between the thin dark green layer of *Microcoleus* and the purple and brownish green zone III.

Diurnal variations in the vertical distribution of the main species of phototrophic microorganisms could also be seen on the immersion slides (the 1996 studies). The slides were exposed for 8 to 12 h at different times of day. Figure 4 shows two examples of the structure of the daytime and nighttime mats. At night, the *M. chthonoplastes* development zone shifted toward the mat surface, while the purple bacterium zone shifted toward the underlying layers.

The concentration of *Microcoleus* at the upper mat boundary occurred at the same time as low-intensity illumination and an increase in the sulfide content of the water, whereas their distribution within zone I coincided with increasing illumination and oxygen concentration (Fig. 1). Evidently, this migration of *Microcoleus* is a response to changes in environmental conditions, mainly in the intensity of the illumination, and testifies to the ability of cyanobacteria to actively move in the direction of more favorable conditions. Similar vertical migrations, depending on the diurnal variations in illumination, have also been described for the filamentous cyanobacterium *Oscillatoria boryana* [10].

The absence of the purple bacteria from zone II of the nighttime mat may be linked to their migration in

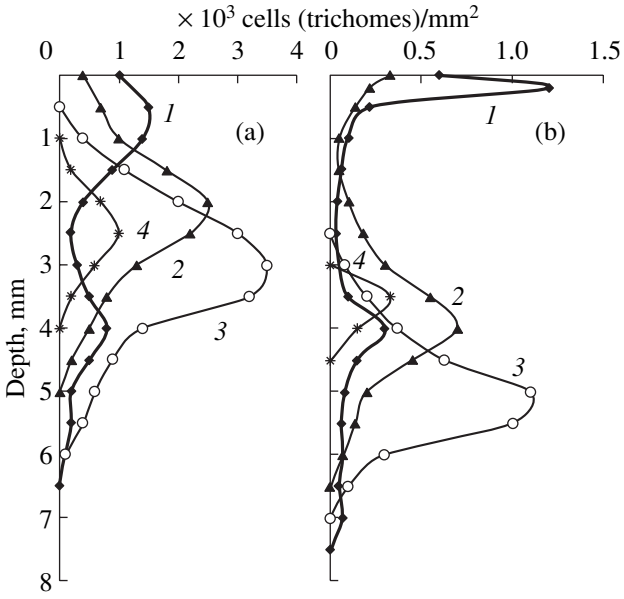

Fig. 4. Vertical distribution of phototrophic microorganisms in the (a) daytime and (b) nighttime as revealed by the immersion slide method: (*1*) *M. chthonoplastes*; (*2*) *Allochromatium* sp.; (*3*) *Ectothiorhodospira* sp.; (*4*) *Thiocapsa* sp.

two directions: upwards into the water and downwards to zone III. Both of these processes most probably take place. Evidence for the former process is provided by the above-described massive accumulation of purple bacteria outside the mat, while an increase in the number of purple bacteria found in zone III at night indicates the latter process.

The illumination intensity and, consequently, the redox conditions in the water and mat change with the day–night interchange. Motile bacteria respond to these changes by migrating in the direction of more favorable conditions. Evidently, in the dark, zone II, the primary daytime localization site of the purple bacteria, becomes unfavorable for most of them. Some of the bacteria, which are less tolerant to sulfide and prefer a less reduced environment, migrate into the water. Others prefer low Eh values and high H_2S concentrations and therefore migrate to the underlying zone III. It is possible that, in the daytime, they have to be present in zone II due to the low illumination intensity in the lower layers, which are more favorable for them in all other respects.

The Cell Number and Ecological Adaptability of Anoxyphotobacteria

As was mentioned above, massive accumulations of purple bacteria also occurred in locations other than the microbial mat. The formation of a whitish pink film was often observed on the surface of floating mat pieces and on decomposing parts of plants, which had found their way into the lake from the shore. In 1996, the primary constituents of this film were *Allochromatium* sp. cells

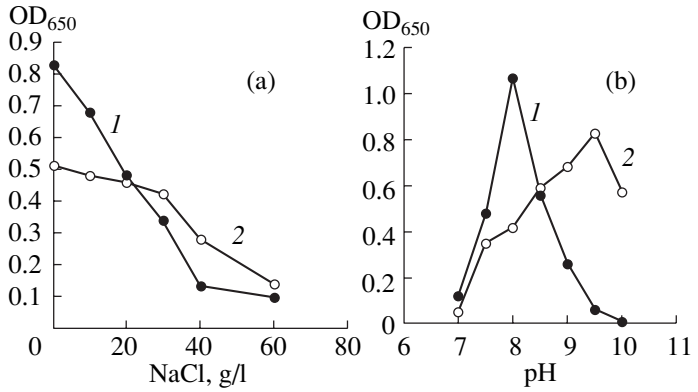

Fig. 5. Growth of pure cultures of (*1*) *Thiocapsa* sp. A-20T and (*2*) *Ectothiorhodospira* sp. A-20E as dependent on the (a) NaCl concentration and (b) pH of the medium.

containing numerous sulfur droplets. The bacteria *Ectothiorhodospira* sp., *Thiocapsa* sp., and *Rhodovulum* sp. were present in smaller numbers. The red coloration of the water in the places where the mat had sunk and/or its surface had been disrupted was accounted for by massive development of the same species of anoxyphotobacteria.

The purple bacteria of the genera *Ectothiorhodospira*, *Allochromatium, Thiocapsa*, and *Rhodovulum* were also revealed in the pink foam observed on the surface of the lake in 1995. They appeared to be quite viable, which was confirmed by the results obtained from inoculating nutrient media with the foam samples.

In these cases, as well as in the upper mat layer during the daytime, i.e., in the zones in contact with oxygen, a specific pattern of growth in the form of microcolonies was characteristic of most of the purple bacteria.

The bacteria *Ectothiorhodospira* sp. formed "balls" in which the cells were tightly pressed against each other and slightly deformed (Fig. 3). We observed the same form of growth with respect to these bacteria in the shallow saline water bodies located in the Crimea. *Allochromatium* sp. formed similar microcolonies. The cells of *Thiocapsa* sp. were united to form tetrads and larger aggregates surrounded by a thick mucilaginous capsule.

In order to determine the number and species composition of the anoxyphotobacteria, they were quantified by inoculating columns of agarized nutrient medium with tenfold dilutions of mat suspensions. Cultures of *Ectothiorhodospira* sp., which is phenotypically similar to *Ect. mobilis*, predominated (10^7 cells/ml). *Allochromatium* sp. and *Thiocapsa* sp., representatives of the family *Chromatiaceae*, ranked second in relation to cell numbers. In 1995, the cell number of *Thiocapsa* sp. was 10^6–10^7 cells/ml, and the cell number of *Allochromatium* sp. was 10^3–10^5 cells/ml. In 1996, the cell number of *Chromatium* sp. attained 10^6–10^7 cells/ml, and that of *Thiocapsa* sp. was 10^4–10^5 cells/ml. The nonsulfur purple bacteria *Rhodovulum* sp., which is similar to *Rdv. euryhalinum* [11] in its morphological characteristics (size, pigments, motility, and deposition of sulfur as the intermediate product of sulfide oxidation), was among the subdominant species (10^5–10^7 cells/ml). *Ectothiorhodospira* representatives with gas vacuoles were also present in significant amounts in the mat samples. Such bacteria, formerly assigned to the recently abolished species *Ect. vacuolata* [12], are currently affiliated to the species *Ect. shaposhnikovii.*

In order to determine the effect of extreme pH values on the species composition of the anoxyphotobacteria in Lake Khilganta, liquid media with pH 9.5 and 10.4 were inoculated with the mat samples. Columns of an agarized medium were inoculated with serial dilutions of the resulting enrichment cultures, with a subsequent count and microscopy of the grown colonies.

At pH 9.5, the proportion of purple bacteria in the enrichment culture was close to that observed in the microbial mat, while, at pH 10.4, *Ectothiorhodospira* sp. representatives were predominant and the presence of other species was insignificant. These results show that an increase in pH to extreme values determines the species composition and limits the species diversity of anoxyphotobacteria, leading to a marked dominance of the most adapted forms; i.e., at its extreme values, pH plays the role of the limiting factor.

Pure cultures of *Ectothiorhodospira* sp. A-20E and *Thiocapsa* sp. A-20T were isolated from the microbial mat of Lake Khilganta. The reaction of these bacteria to environmental factors (pH and salinity) was studied (Fig. 5). *Ectothiorhodospira* sp. A-20E appeared to be alkaliphilic and halotolerant. Optimum growth was observed at pH 9.5, whereas, at pH 7, growth was virtually absent. The bacterium grew equally well in a fresh medium and in media containing up to 40 g/l of NaCl. *Thiocapsa* sp. A-20T developed better at lower pH and salinity values and was alkali- and halotolerant. The best growth was observed in a fresh medium with a pH of about 8, although the bacteria were able to grow in the pH range of 7–9 and at a NaCl concentration of up to 30 g/l.

Functioning of the Phototrophic Community

The main role of phototrophic microorganisms in a community is to produce organic matter at the expense of light energy. As shallow saline water bodies, Lake Khilganta is characterized by high productivity. In 1996, the total photosynthesis rate, determined by the radiocarbon method, was 1.4–1.6 g C/(m^2 h). The use of diuron showed that most of the organic matter was synthesized through anoxygenic photosynthesis, whereas only 3–4% was synthesized via oxygenic photosynthesis. Separate determination of the photosynthetic activity occurring in different mat zones along the vertical profile showed that, in the three upper mat zones, the photosynthesis rates were similar while no photosynthetic activity was recorded in the underlying zones. Oxygenic photosynthesis was only noted in zone I (the upper mat zone), where its share accounted for 25% of the total production. In zones II and III, organic matter production occurred entirely via anoxygenic photosynthesis. The high photosynthesis rate in zone III provides evidence for the presence of a sufficient number of viable *Microcoleus* filaments. Similar results were obtained in 1995: the total production constituted 1.6 g C/(m^2 h), with oxygenic photosynthesis accounting for 15% of this value.

Our results indicate that, in Lake Khilganta, not only anoxyphotobacteria (zone II) but also cyanobacteria (zones I and III) predominantly carry out anoxygenic photosynthesis, using photosystem I. This is connected with the high sulfide content in the water during most of the daytime (Fig. 1). In laboratory experiments [13], the contribution of anoxygenic photosynthesis to the total productivity of an *M. chthonoplastes* culture in a sulfide-free medium has been found to constitute several percent, whereas, in the presence of 0.5 mM Na_2S, it reaches 95%, with the total productivity value changing insignificantly.

The environmental parameters in shallow water bodies are subject to considerable variations (table). In order to assess to what degree the phototrophic community of Lake Khilganta has adapted to the existing habitat conditions, the organic matter production rate in the microbial mat was measured at different salinity values. The mat samples were placed in the lake water, whose mineralization was about 4%, as well as in lake water with the addition of NaCl to adjust its concentration to 6, 8, and 10%. At 6% salinity, the photosynthetic productivity was virtually the same as in the lake water, while, at 8 and 10%, it decreased by 20–30%. Thus, the microbial community of Lake Khilganta is adapted to a salinity of 4–6%, whereas a further increase of salinity to 8–10% has a negative effect on the functioning of the community.

Phototrophic bacteria play the main role in sulfide oxidation in Lake Khilganta. Vials with isolated samples (70 ml of the lake water and 7 cm^2 of the microbial mat) were incubated for 12 h in the lake (1) in daylight, (2) in daylight with the addition of diuron, and (3) in the dark, and the concentration of sulfide in the water was periodically measured (Fig. 6). It was established that sulfide oxidation mainly occurred in the daylight owing to the anoxygenic photosynthesis carried out by the cyanobacteria and anoxyphotobacteria. Apparently, the contribution of nonphototrophic microorganisms to sulfide oxidation was insignificant. In the dark, hydrogen sulfide formation was significantly in excess of its oxidation: the H_2S concentration steadily increased and attained 1.8 mM in the evening. In daylight, the hydrogen sulfide content in the water in the presence of diuron, which inhibits oxygenic photosynthesis, was only slightly higher than in the diuron-free samples, indicating that the contribution of oxygenic photosynthesis to the process of sulfide oxidation is insignificant.

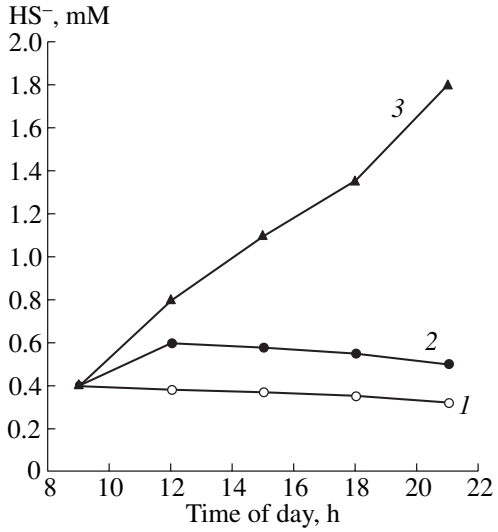

Fig. 6. Dynamics of the sulfide content in the water phase of the water + mat samples from Lake Khilganta incubated in situ (*1*) in daylight, (*2*) in daylight in the presence of diuron, and (*3*) in the dark.

DISCUSSION

With respect to physicochemical conditions, Lake Khilganta is similar to shallow saline water bodies of marine origin [5, 14, 15], from which it differs only in its increased alkalinity and higher pH value. Both types of water bodies are characterized by good illumination, a high content of biogenic elements (and, as a consequence, natural eutrophication), intense sulfate reduction in their sediments, and a pronounced vertical gradient of physicochemical conditions at the water–sediment interface. Thus, the conditions are favorable for the development of photosynthetic microorganisms, including anoxyphotobacteria. The microbial mats developing in shallow saline lakes and saline soda lakes also have much in common [5, 14]. Regarding anoxyphotobacteria, both these types of water bodies are dominated by purple bacteria of the families *Ectothiorhodospiraceae* and *Chromatiaceae*, but nonsulfur pur-

ple bacteria of the genus *Rhodovulum* are also widespread. The anoxyphotobacteria are represented by halotolerant and halophilic forms [14, 15]. However, as distinct from neutral saline water bodies, Lake Khilganta lacks representatives of the neutrophilic group of green sulfur bacteria, and the purple bacteria inhabiting it are represented by alkalitolerant and alkaliphilic forms.

Earlier, we established, using the example of shallow water bodies of marine origin with a wide range of salinity, the regularities of the influence of an increased water mineralization on the structure of communities of anoxyphotobacteria [14]. First and foremost, increased mineralization determines the species composition of the bacteria, which, in contrast to the inhabitants of freshwater lakes, are represented by halophilic forms. A change in salinity from 1 to 20% has very little effect the species diversity of anoxyphotobacteria, which depends on other environmental factors. However, a further increase in salinity results in a reduction in the number of species and in the domination and even monopolistic development of the most adapted forms, namely, representatives of the genus *Ectothiorhodospira.* Thus, salinity exceeding 20–25% plays the role of the limiting factor.

The regularities of the simultaneous influence of increased mineralization, alkalinity, and pH values on communities of anoxyphotobacteria have not yet been studied in detail (although many soda lakes have been investigated in different respects). In Lake Khilganta, with its moderate water mineralization and pH values equal to 9.5–9.8, the species diversity of anoxyphotobacteria is considerable. However, the experiments with enrichment cultures showed that, at the same salinity, a pH increase to 10.4 results in the sharply pronounced domination of *Ectothiorhodospira.* Evidently, with a pH increase to 10, this parameter acquires the role of the main limiting factor for the community of anoxyphotobacteria. It is possible that, at a simultaneous exposure to increased mineralization and alkaline pH, the limitation of the species diversity of anoxygenic phototrophs may well occur, even at a moderate salinity. Interestingly, in both types of limitation (by salinity or alkaline pH), the predominant development of *Ectothiorhodospiraceae* representatives is observed.

The water level in shallow water bodies is liable to considerable fluctuations. It may rise during periods of snow thaw or rainfall or decrease to the point of water body drying (table). As was shown by the experiments with pure and enrichment cultures, *Ectothiorhodospira* are the most competitive microorganisms among the anoxyphotobacteria inhabiting Lake Khilganta, both under the conditions of increased mineralization and a pH increase to an extreme value. Obviously, with the periodic increases in mineralization and pH in Lake Khilganta, the domination of *Ectothiorhodospira* among the anoxygenic photosynthetic bacteria should increase.

Thus, the community of anoxyphotobacteria in the haloalkaliphilic microbial mats of Lake Khilganta is close in its structure to the communities found in saline shallow water bodies of marine origin. The main distinction, determined by the increased alkalinity of the water, consists in the prevalence of alkaliphilic and alkalitolerant forms, as well as in the absence of green sulfur bacteria. In general, the phototrophic community of Lake Khilganta is characterized by high productivity, primarily due to anoxygenic photosynthesis, and by its leading role in sulfide detoxification.

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