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**EXPERIMENTAL ARTICLES**

# The Structure and Biogeochemical Activity of the Phototrophic Communities from the Bol'sherechenskii Alkaline Hot Spring

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**Abstract**—Microbial communities growing in the bed of the alkaline, sulfide hot spring Bol'sherechenskii (the Baikal rift area) were studied over many years (1986–2001). The effluent water temperature ranged from 72 to 74°C, pH was from 9.25 to 9.8, and sulfide content was from 12 to 13.4 mg/ml. Simultaneous effects of several extreme factors restrict the spread of phototrophic microorganisms. Visible microbial mat appears with a decrease in the temperature to 62°C and in sulfide content to 5.9 mg/l. Cyanobacteria predominated in all biological zones of the microbial mat. The filamentous cyanobacteria of the genus *Phormidium* are the major mat-forming organisms, whereas unicellular cyanobacteria and the filamentous green bacterium *Chloroflexus aurantiacus* are minor components of the phototrophic communities. No cyanobacteria of the species *Mastigocladus laminosus*, typical of neutral and subacid springs, were identified. Seventeen species of both anoxygenic phototrophic bacteria and cyanobacteria were isolated from the microbial mats, most of which exhibited optimum growth at 20 to 45°C. The anoxygenic phototrophs were neutrophiles with pH optimum at about 7. The cyanobacteria were the most adapted to the alkaline conditions in the spring. Their optimum growth was observed at pH 8.5–9.0. As determined by the in situ radioisotope method, the optimal growth and decomposition rates were observed at 40–32°C, which is 10–15°C lower than the same parameter in the sulfide-deficient Octopus Spring (Yellowstone, United States). The maximum chlorophyll *a* concentration was 555 mg/m$^2$ at 40°C. The total rate of photosynthesis in the mats reached 1.3 g C/m$^2$ per day. The maximum rate of dark fixation of carbon dioxide in the microbial mats was 0.806 g C/m$^2$ per day. The maximum rate of sulfate reduction comprised 0.367 g S/m$^2$ per day at 40°C. The rate of methanogenesis did not exceed 1.188 μg C/m$^2$ per day. The role of methanogenesis in the terminal decomposition of the organic matter was insignificant. Methane formation consumed 100 times less organic matter than sulfate reduction.

*Key words*: thermal springs, cyanobacterial mats, anoxygenic phototrophs, extremophily, biogeochemical processes.

Most of the terrestrial thermal springs for which the structure and biogeochemical activity of the microbial communities have been examined are either acidic or have neutral pH, which is common in regions of active volcanism or in those of young Quaternary volcanism [1]. The pH of their effluent water, as a rule, is under 8.5. The species composition of the microbial mats developing in the bed of the thermal springs of this type has been studied in detail, as well as their geochemical activity [2–5]. The alkaline thermal springs with pH > 9 and a temperature higher than 45°C are rare and have been studied by microbiologists to a lesser extent.

In the Baikal rift area, a large number of springs are encountered with pH from 6.1 to 9.3 and a temperature reaching 84°C. In this region, most thermal springs belong to those of the nitrogen type and, according to Golubev, their formation depends neither on abyssal nor thermometamorphic processes, which also sets them apart from the hot springs in the regions of active volcanism [6].

The microbiological studies in the Baikal rift thermal springs were started in 1984 [7]. The Bol'sherechenskii Spring with alkaline pH (9.25) a sulfide content of 13.4 mg/l, and a temperature of 72–74°C is of special interest [8]. The presence of several extreme factors suggests that the microbial community of the Bol'sherechenskii Spring is a unique one. Several previously unknown anoxygenic phototrophic bacteria were isolated from the microbial mats of this spring [9–12]. Nevertheless, the effect of extreme environmental factors on the microbial community structure and rates of microbial processes are still insufficiently studied.

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In this work, the structure of the phototrophic communities inherent to the Bol'sherechenskii Springs was studied, as well as the growth and decomposition rates in different temperature zones of the microbial mats.

## MATERIALS AND METHODS

The studies were conducted in September 1986, 1989, and 2001 and in June 1996. To determine sulfide concentration, colorimetric analysis with *para*-phenylenediamine was used [8]. The water temperature was measured with a mercurial maximum thermometer and electron thermometer from Prima (Singapore). The pH and redox potential were determined using an I-102 field pH meter. The content of oxygen in the spring water was determined by the Winkler method [8]. The carbonate titer was determined under field conditions as described earlier [8]. The microbial mat samples for chemical and radioisotope analyses were taken by a 1-cm$^2$ cork borer. The content of chlorophyll *a* in ethanol extracts was determined spectrophotometrically and calculated from the formula $\mu g$ Chl $a = 11.9\ OD_{665} \times (v/l)$, where $OD_{665}$ is the optical density at the wavelength of 665 nm, $v$ is the extract volume in ml, and $l$ is the cuvette length. The presence of bacteriochlorophyll *c* in samples of the microbial mat was determined from the long-wave maximum at 740 nm in the in vivo spectra. The intensity of microbial processes was measured by the radioisotope method [3, 5]. Both light and dark production was estimated with $NaH^{14}CO_3$. Oxygenic photosynthesis was inhibited with diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) applied at a concentration of 7 $\mu$M. The rate of sulfate reduction was determined using $Na_2{}^{35}SO_4$, whereas the intensity of methanogenesis was estimated using [$^{14}$C]-bicarbonate and [$^{14}$C]-acetate. The radiolabel was added immediately after sampling to the penicillin vials containing spring water. After 6- to 24-h incubation in the light or dark at the place of sampling, the radioactivity of the initial and generated products was measured on a Rack-Beta liquid scintillation counter (LKB, Sweden). Cyanobacterial monocultures were grown on the Castenholtz liquid medium [4]; the end-point dilution method and plating the microorganisms grown from the last dilution were used to purify the cultures. Anoxygenic phototrophic bacteria were isolated and grown on modified Pfennig medium [8] containing 300 mg/l $Na_2S \cdot 9H_2O$, 1 g/l thiosulfate, 100 mg/l yeast extract, and 1 g/l sodium acetate. The pH of the medium was 8.0–8.5; the incubation temperatures were 30 and 50°C. The colonies grown were counted on agarized medium of the same composition. The temperature range needed to maintain the growth of the phototrophic bacteria of the microbial mat was determined using a procedure described earlier [15, 19]. The cyanobacterial species were identified from their morphological characteristics in accordance with the manual by Gollerbach *et al.* [14]. Anoxygenic phototrophic bacteria were identified from their phenotypic characteristics (cell morphology, the type of bacteriochlorophyll and carotenoids, the capacity for heterotrophic and autotrophic growth on sulfide and anaerobic growth in the dark, and the growth dependence on temperature and pH of the medium). The temperature and pH growth optima for some bacteria were determined in short-term experiments using [$^{14}$C]-bicarbonate [15]. The vertical structure of the microbial communities and the predominating species were identified by electron scanning microscopy of the fouling slides as described in [13]. Microbial mat samples fixed in 50% glycerol were also examined using direct microscopy. Ultrathin sections were prepared after bacterial cell fixation according to Ritter *et al.* [8] and embedding in a mixture of epoxy resins (Epon). The sections were prepared on a Nova Utratome (LKB). Reinolds-stained specimens [8] were examined under a JEM-100C electron microscope at an accelerating voltage of 80 kV and an instrumental magnification of 8000 to 50000.

## RESULTS

### *Spring Characterization*

The Bol'sherechenskaya group of hot springs is located on the Barguzinskii State Biosphere Reserve (Buryat Republic) in the Bol'shoi River Valley, 25 to 28 km away from Baikal; the geographical coordinates are lat. 54°25' N, long. 109°50' E [8]. The highest water temperature was in the outlet of spring number 6 (according to Martynov's numbering [8]). This is the largest spring, and it was the main subject of our study. The spring belongs to the nitrogen type of thermal waters. The gas released is 88% nitrogen. In addition, it contains 0.9% methane, 0.5% $CO_2$, and 0.116% helium [8]. The outlet water temperature is 74°C. According to our measurements made in different years (July–September of 1986, 1989, 1996, and 2001), the temperature fluctuation was as low as 1–2°C. In terms of the chemical composition, the spring water is of hydrocarbonate–chloride–sulfate–sodium type. The total mineralization of the water is about 2 g/l. The contents of silicon, sulfate, and carbonate comprise 80–100, 225, and 116 mg/l, respectively. The effluent water also contains sulfide (12–13.4 mg/l). The water pH remained within the range from 9.25 to 9.8 in different years.

Spring 6 comes out in two griffons located 3.5 m away from each other (Fig. 1). The temperature, sulfide content, pH, and water debit are similar in both griffons. Their effluent waters are fused 5.5 m away to form a brook, which results in mixing of the sulfide-rich hot water from the short arm with water from the long arm, which contains already cooled water that has lost some dissolved sulfide. Thus, the spring effluent channel consists of zones with different temperatures and sulfide contents, which makes it possible to study the effects of limiting environmental factors on the microbial community. Downstream from the effluent channel, the temperature and sulfide concentration decrease, while the content of dissolved oxygen increases. The scheme (Fig. 1) shows the location of different zones in the bed of the spring. The physicochemical parameters of the environmental conditions of these zones are presented in Table 1.

### *Characterization of Microbial Communities*

Our studies conducted in different years showed that the types of microbial communities in various temperature zones of spring no. 6 remained unchanged. In each temperature zone, five samples were taken from sites differing in their temperature. Microscopic examination of the samples showed that in the effluent zone (zone I) with a temperature range from 74 to 54°C and a sulfide content from 6 to 13.4 mg/l, no visible microbial films developed. Several factors prevent, presumably, the microbial growth in this part of the spring channel: high temperature, pH, and sulfide concentration [8]. In zone II, with a temperature range from 62 to 51°C and a sulfide content of 3–5.9 mg/l, a thin, loose, yellow-green microbial mat 1–2 mm thick developed. In this zone, the water from the short arm of the spring is diluted with the cooled water from the longer arm. Upon mixing of the waters, the environmental conditions become favorable for development of a mat, whose components are the anoxygenic phototrophic bacterium *Chloroflexus aurantiacus*, filamentous cyanobacteria of the genera *Phormidium* and *Oscillatoria*, and unicellular cyanobacteria *Synechococcus elongatus.*

In zone III, at a temperature of 54–37°C and a sulfide content of 2.8 mg/l, a potent microbial mat developed, which was 1–1.5 cm thick and gristly (Fig. 2). This mat is located on the bank of the spring bed, and a significant temperature gradient exists in the mat thickness: from 37°C on the surface to 54°C in the depth. Light and scanning electron microscopy of the fouling slides incubated within the mat thickness (Fig. 3a) showed that the upper microzone was occupied by *Synechococcus elongatus* whereas *Phormidium* sp. grew in deeper layers of the mat (Fig. 3b). *Chloroflexus aurantiacus* was identified in almost the same amounts throughout the entire depth of the mat (Fig. 4). Plating mat samples on selective media showed that they also contained the nonsulfur purple bacteria *Rhodopseudomonas palustris*, *Rubrivivax gelatinosus*, *Blastochloris viridis*, *Blastochloris sulfoviridis*, and *Rhodomicrobium vannielii*, the cyanobacterium *Synechocystis minuscula*, as well as *Chloroflexus aurantiacus*, heliobacteria, and erythrobacteria (Table 2).

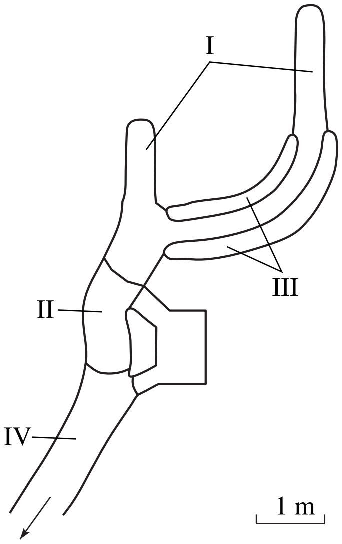


**Fig. 1.** Scheme of the biological zones in the bed of the Bol'sherechenskii Thermal Spring. Zone V, located 15 m away from zone II along the effluent channel, is not shown on the scheme.

In zone IV, at a temperature of 51–39°C and a sulfide content of 2,9 mg/l, a thin microbial mat 3–4 mm thick developed on the bed of the brook. *Phormidium valderiae* f. *medium*, *Synechococcus elongatus*, and *Chloroflexus aurantiacus* predominated. Plating mat samples on selective media also revealed the nonsulfur purple bacteria *Rhodopseudomonas palustris, Rubrivivax gelatinosus*, *Blastochloris viridis*, *Blastochloris sulfoviridis*, and *Rhodomicrobium vannielii*, the cyanobacterium *Synechocystis minuscula*, as well as *Chloroflexus aurantiacus*, heliobacteria, and erythrobacteria (Table 2). As judged from the in vivo absorption spectrum of this mat, the microbial community was dominated by cyanobacteria. This mat covers the maximum effluent area (up to 10–12 m in length) and may be considered typical of the Bol'sherechenskii Spring.

**Table 1.** Physicochemical conditions in the biological zones of the Bol'sherechenskii Thermal Spring (according to data of 1986–2001)

| Biological zone | Microbial mat description | *T*, °C | $H_2S$, mg/l | $O_2$, mg/l | Eh | pH | $S^0$ (in mat), mg/m$^2$ |
|---|---|---|---|---|---|---|---|
| I (outlet zone) | No mat | 74–54 | 12–13.4 | 0.8 | –200 | 9.25–9.8 | |
| II | Thin, yellow-green mat | 62–51 | 5.9 | 1.6 | –90 | 9.8 | |
| III | Gristly mat up to 1–1.5 cm thick | 54–37 | 2.8 | 4 | –57 | 9.7 | 204–685 |
| IV | Green mat up to 3–4 mm thick | 51–39 | 2.9 | 3.7 | –57 | 9.7 | 174–532 |
| V | Thin, loose mat | 39–25 | Under 0.5 | 7.3 | +159 | 9.7 | 1064–1167 |

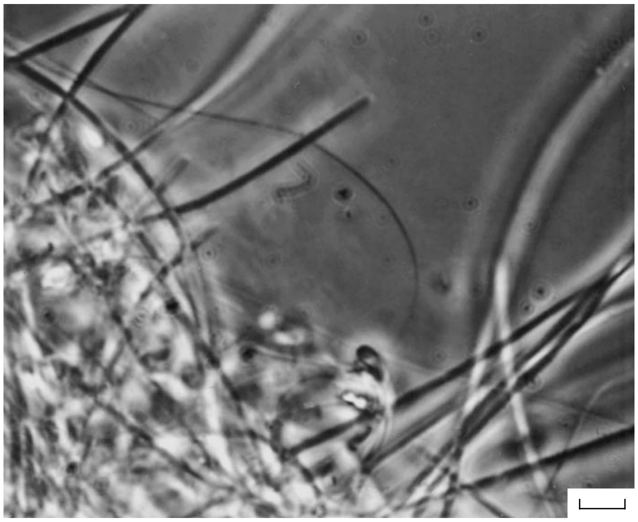


**Fig. 2.** A micrograph of the microbial mat in zone III. Oval cells belong to *Synechococcus elongatus*, and filaments belong to *Phormidium* sp. and *Chloroflexus aurantiacus*. Phase contrast microscopy. Scale bar, 10 µm.

The microbial mats developing in zones III and IV differ in thickness and structure. Nevertheless, the physicochemical conditions in these zones are similar. The species composition of the organisms isolated from mat samples is also the same, suggesting that the microbial communities in these two zones are similar. The two mats differed in their positioning relative to the bed of the brook and, hence, in the temperature and moistening regimes, which may account for their different structures.

In low-temperature zone V (with the temperature ranging from 38 to 25°C), the mat structure is looser and easily disrupted when sampled. *Phormidium valderiae* f. *medium* predominates. The cyanobacteria are also represented by the unicellular *Synechocystis* sp. and mesophilic filamentous *Oscillatoria subcapitata*, which is not encountered in the high-temperature zones. The diatoms are frequent (Fig. 5). A visible microbial biofilm is represented by the phototrophic purple sulfur bacteria *Allochromatium* sp. (Table 2), occurring in the form of separate purple spots, and by the colorless sulfur bacteria *Thiothrix* sp. When the mat samples were plated on selective media, the purple sulfur bacterium *Thiocapsa roseopersicina* was identified, as was the filamentous green bacteria *Oscillochloris* sp. ($10^3$ cells/ml), which were not encountered at higher temperatures. The thermophilic *Chloroflexus aurantiacus* was relatively rare as compared to the high-temperature zones. A number of nonsulfur purple bacteria, heliobacteria, and erythrobacteria similar to those identified in higher-temperature zone IV were also identified in mat samples from zone IV (Table 2).

Seventeen monocultures of cyanobacteria and anoxygenic phototrophic bacteria were isolated from various-temperature zones. The growth optima and the growth-limiting temperatures of the isolates were studied by plating them on the media and incubating them in a chamber with a controlled gradient of temperature (Table 3). Under laboratory conditions, the upper temperature limit for bacterial growth did not exceed 54°C, except for *Synechococcus elongatus*, which grew even at 74°C. The range of the growth temperature for the cultivated microorganisms was narrower than the temperature range at which the same microorganisms were identified in the microbial mats. This also applies to the mesophilic nonsulfur purple bacteria found at a rather high temperature, 54°C (Table 3). Most bacteria (except for *Synechococcus elongatus* and *Chloroflexus aurantiacus*) showed optimal growth at temperatures from 20 to 45°C (Table 3), which correlated with the highest biodiversity of phototrophic bacteria in the mesophilic zone of the thermal spring.

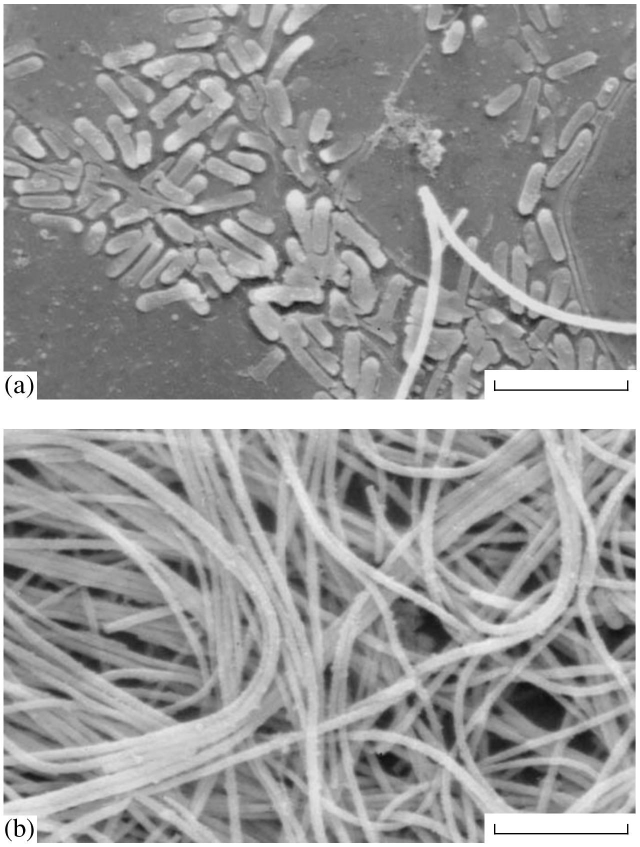


**Fig. 3.** Vertical changes in microbial mat composition as determined using the fouling slides (scanning electron microscopy): (a) upper layer of 0–1 mm. Oval cells belong to *Synechococcus elongatus*; filaments 0.5 µm in diameter, to *Chloroflexus aurantiacus*; filaments of 1.5 µm in diameter, to *Phormidium* sp. (b) Bottom layer of 6–7 mm. Filaments of *Phormidium* sp. Scale bar, 10 µm.

In short-term experiments with $NaH^{14}CO_3$, the optimum pH values were determined for six cultures of phototrophic bacteria (Fig. 6). The anoxygenic phototrophs studied proved to be neurtrophiles with an optimum pH of about 7. The cyanobacteria were adapted to the alkaline conditions characteristic of the thermal spring (pH 9.25–9.8) and exhibited optimum growth at pH 8.5–9.0.

### *The Processes of Production and Decomposition*

Measurements of the chlorophyll *a* content in extracts of the microbial mats showed that it increased gradually with decreasing temperature.

The maximum chlorophyll *a* content was determined at 40°C (555 mg/m$^2$), whereas at higher temperatures, the same parameter comprised 55–244 mg Chl/m$^2$. With a further temperature decrease, the content of the pigment dropped to 240 mg/m$^2$ at 32°C (Fig. 7).

The experiments with [$^{14}$C]-bicarbonate showed that the rate of photosynthesis in mats increased gradually with decreasing temperature in parallel with the content of chlorophyll *a*. In the effluent zone of the spring, no photosynthesis was detected, although the cyanobacterium *Synechococcus elongatus* was present in ground samples. In zone II (62–51°C), the rate of anoxygenic photosynthesis (0.105 g C/m$^2$ per day) was substantially higher than the rate of oxygenic photosynthesis (0.004 g C/m$^2$ per day) (Fig. 7). In the cyanobacterial mats that grow in zones III–IV at a temperature of 54–37°C, the rate of oxygenic photosynthesis (0.737 g C/m$^2$ per day) was higher than that of anoxygenic photosynthesis (0.556 g C/m$^2$ per day), which is, presumably, a result of the predominance of oxygenic cyanobacteria in the phototrophic community. The total rate of photosynthesis was the highest in this zone (1.3 g C/m$^2$ per day). With a further temperature decrease, to 39°C and lower, in zone V, the rate of oxygenic photosynthesis became as low as 0.071 g C/m$^2$ per day, whereas

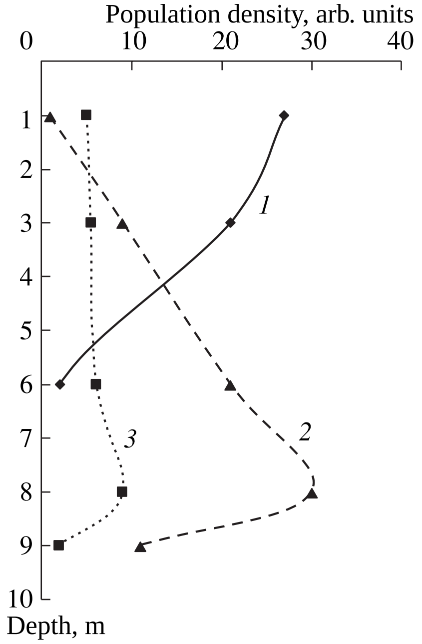


**Fig. 4.** Vertical distribution of phototrophic microorganisms within the microbial mat thickness: (*1*) *Synechococcus elongatus*, (*2*) *Phormidium* sp., and (*3*) *Chloroflexus aurantiacus*.

**Table 2.** Population density of phototrophic bacteria in mats of the Bol'sherechenskii Thermal Spring (log[cells/ml])

| Species | Incubation temperature, °C | Zones III and IV | | | | | | | Zone V | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Temperature in sites of sampling, °C | | | | | | | | | | |
| | | 54 | 53 | 52 | 50 | 46 | 44 | 40 | 33 | 30 | 28 | 24 |
| *Phormidium valderiae* f. *medium* | 50 | 2 | 5 | 4 | 3 | 5 | 5 | 5 | | 3 | | |
| *Synechococcus minuscula* | 30 | | | | | | 3 | | | 3 | 5 | |
| *Synechococcus elongatus* | 50 | 2 | 4 | | 3 | 2 | 5 | 3 | 3 | 1 | | |
| *Chloroflexus aurantiacus* | 50 | 4 | 4 | 3 | 4 | | 3 | | | 1 | | |
| *Oscillohloris* sp. | 30 | | | | | | | | | 3 | 2 | |
| *Allochromatium vinosum* | 30 | | | | | | | | | 3 | | |
| *Thiocapsa roseopersicina* | 30 | | | | | | | | | 3 | 1 | 1 |
| *Rhodomicrobium vannieli* | 30 | | 1 | 4 | 4 | | 2 | 4 | | | | |
| *Rhodopseudomonas palustris* | 30 | 5 | 5 | 5 | 2 | 2 | 5 | 5 | 3 | 7 | 5 | 5 |
| *Rhodopseudomonas blastica* | 30 | 5 | | | | | 5 | | 3 | | | |
| *Rubrivivax gelatinosus* | 30 | 3 | | | | | | | | | | |
| *Blastohloris viridis* | 30 | | 2 | | | | | | | 2 | | |
| *Blastohloris sulfoviridis* | 30 | 2 | | | | 4 | 1 | | | | | |
| *Heliobacterium sulfidophilum* | 30 | | | | 3 | | | | | | | |
| *Roseococcus thiosulfatophilus* | 30 | 3 | | | | | | | | | | |

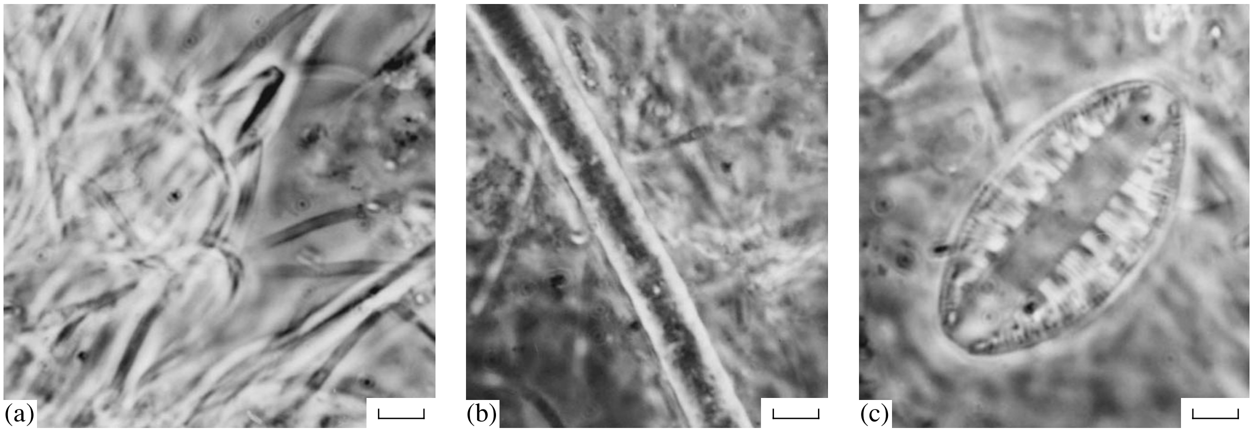


**Fig. 5.** Micrographs of the microbial mat developing in zone V: (a) sheaths of the *Phormidium valderiae* f. *medium*; (b) *Oscillatoria* subcapitata; (c) diatom. Phase contrast. Scale bar, 10 $\mu$m.

anoxygenic photosynthesis is again predominant (0.882 g C/m$^2$ per day).

In the effluent zone, the rate of dark fixation of carbon dioxide was 0.181 g C/m$^2$ per day. At 62°C, this parameter decreased to 0.0775 g C/m$^2$ per day; with decreasing temperature, the rate of carbon dioxide dark fixation increased gradually to reach its maximum at 32°C (0.806 g C/m$^2$ per day) (Fig. 8). This parameter is indicative of the total activity of chemotrophic microorganisms.

The rates of terminal decomposition in the microbial mats and benthic sediments of the spring were studied using radioactive [$^{35}$S]-sulfate, [$^{14}$C]-bicarbonate, and [$^{14}$C]-acetate. The sulfate reduction in the effluent zone leads to hydrogen sulfide formation at a rate of 0.08 g S/m$^2$ per day. At the same site, weak methanogenesis occurs at a rate of 0.726 $\mu$g C/m$^2$ per day. The electron donors supplied with water ($H_2$ and organic matter) presumably support the processes. Along the effluent channel, the rate of sulfate reduction in microbial mats increases gradually (Fig. 9), reaching a maximum at 40°C (0.367 g S/m$^2$ per day). With the temperature decreasing to 32°C, the rate of sulfate reduction becomes as low as 0.053 g S/m$^2$ per day.

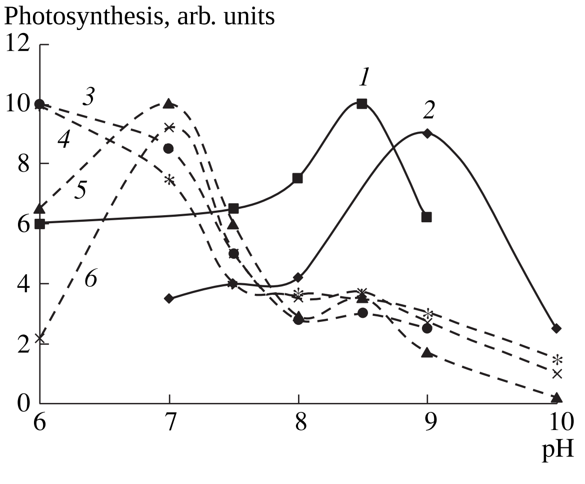


**Fig. 6.** Dependence of the rate of photosynthesis of the phototrophic bacteria isolated from the Bol'sherechenskii Spring on pH of the medium: (*1*) *Synechococcus elongatus*, (*2*) *Synechococcus minuscula*, (*3*) *Blastochloris viridis*, (*4*) *Rubrivivax gelatinosus*, (*5*) *Rhodomicrobium vannielii*, and (*6*) *Rhodopseudomonas palustris*. The spring water pH was 9.25.

The rate of hydrogen-dependent and aceticlastic methanogenesis is insignificant along the effluent channel and reaches a maximum (1.188 $\mu$g C/m$^2$ per day) at 51°C in the thick mat of zone IV. The rate of hydrogen-dependent methanogenesis varies along the effluent channel (Fig. 10). In the effluent stream, it is 0.381 $\mu$g C/m$^2$ per day; afterwards it increases with decreasing temperature, reaching a maximum at 51°C (0.555 $\mu$g C/m$^2$ per day) and then droping to 0.398–0.432 $\mu$g C/m$^2$ per day at 32–39°C. The rate of aceticlastic methanogenesis changes within a broader range (Fig. 10). In the effluent zone, the rate of aceticlastic methanogenesis comprises 0.345 $\mu$g C/m$^2$ per day, then increases up to 0.645 $\mu$g C/m$^2$ per day at 62°C, decreases again within the temperature range 51–39°C (0.151–0.271 $\mu$g C/m$^2$ per day), and then grows up to 0.639 $\mu$g C/m$^2$ per day at a temperature of 32°C.

## DISCUSSION

Observations of the Bol'sherechenskii Spring over many years (the autumn–summer periods of 1986–2001) showed that the physicochemical parameters of the effluent water changed within a narrow range. Note that the microbial community composition remained constant during our observations. The absence of visible microbial fouling in zone I (74–54°C; sulfide content 12 mg/l) is a particular feature of the Bol'sherechenskii Spring, which is most likely caused by the simultaneous effects of the high sulfide content, high temperature, and pH. Note that in the sulfide-deficient springs or in springs with lower pH but similar temperature, visible microbial fouling can be detected. Thus, in the sulfide-deficient, weakly alkaline Octopus Spring, a potent cyanobacterial mat is developed at a temperature lower than 74°C [2], and, conversely, in the sulfide-rich Thermofil’nyi Spring with neutral pH, streamers of the sulfur bacterium *Thermotrix thiopara* developed at a temperature of 70°C [4].

In the effluent channel of the Bol’sherechenskii Spring, the microbial mat appears in zone II after a temperature decrease to 62°C and at a sulfide content of 5.9 mg/l. The mat-forming bacteria are the filamentous cyanobacteria of the genus *Phormidium.* Their heavy growth under these conditions suggests that the spread of cyanobacteria in thermal habitats is actually much broader than previously assumed. At temperatures higher than 55°C and a sulfide content exceeding 1 mg/l, cyanobacteria were considered unable to grow [18]. Thus, in the weakly acid water of the New Peat Spring and Rolland Well of Yellowstone National Park (United States) (pH 6.3, sulfide content, 1–1.3 mg/l) [18], as well as in the Travellodge Spring in New Zealand (pH 5.9; a sulfide content higher than 9 mg/l) [18], the anoxygenic phototrophic bacteria of the genera *Chloroflexus*, *Chlorobium*, and *Allochromatium* are the major producers in the microbial mats, whereas no cyanobacteria were identified. In all microbial mats of the Bol’sherechenskii Spring, cyanobacteria of the genus *Phormidium* predominated. The high pH of the spring water may relieve the hydrogen sulfide toxicity, which allows cyanobacteria to grow at the sulfide concentration incident to the spring water [19]. The enhanced stability of the hydrosulfide ion to the alkaline medium may account for the absence of noticeable deposits of molecular sulfur on the mat surface in the Bol’sherechenskii Spring, as well as for the fact that neither the thermophilic sulfur bacteria *Thermotrix thiopara* nor members of the family *Aquificaceae* showed massive development there at 70°C [4, 20].

The cyanobacteria isolated from the microbial mats are adapted to the alkaline water of the spring. As determined in short-term experiments, the most active fixation of the bicarbonate carbon by cyanobacteria occurred at pH 9–9.5. This is consistent with the data on the microbial mat from the subalkaline (pH 8.2) Octopus Spring obtained by Ward, who showed that, in the day time, pH reached 9.4 on the mat surface [16]. Because of the alkaline pH, the cyanobacterium *Mastigocladus laminosus* does not grow in the microbial mats of the Bol’sherechenskii Spring, although this species frequently occurs in subacid thermal springs. Thus, the thermophilic cyanobacteria of the genera *Phormidium* and *Synechococcus* are tolerant to a high pH. Conversely, all anoxygenic phototrophic bacteria are neutrophiles, although some of them can grow actively at pH 8.0–9.5 [12]. Such a capacity for growth at neutral pH can be explained by the poor buffering of the weakly mineralized spring with carbonates. Note also that anoxygenic phototrophic bacteria grow in the

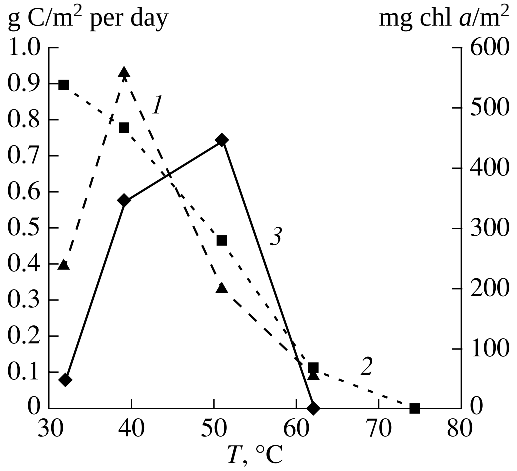


**Fig. 7.** Rate of photosynthesis and content of chlorophyll *a* in the effluent zone of the Bol’sherechenskii Spring: (*1*) oxygenic photosynthesis, (*2*) anoxygenic photosynthesis, and (*3*) content of chlorophyll *a*.

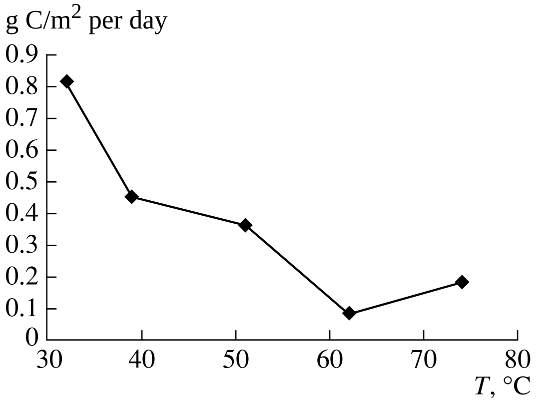


**Fig. 8.** Rate of dark fixation of carbon dioxide along the effluent channel of the Bol’sherechenskii Spring.

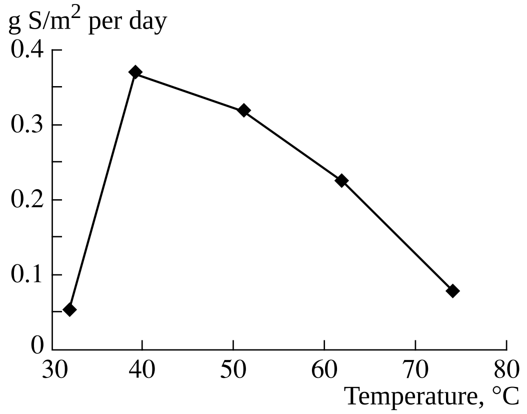


**Fig. 9.** Rate of sulfate reduction along the effluent channel of the Bol’sherechenskii Spring.

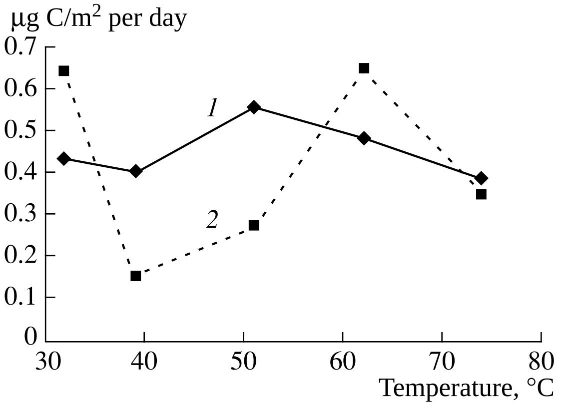


**Fig. 10.** Rate of methanogenesis along the effluent channel of the Bol'sherechenskii Spring: (*1*) hydrogen-dependent and (*2*) aceticlastic.

bottom microzones of the microbial mats in immediate proximity to the zone where active decomposition processes occur and lead to water acidification.

Unicellular cyanobacteria and the filamentous green bacterium *Chloroflexus aurantiacus* are minor components of the phototrophic communities. *Chloroflexus aurantiacus* was identified by cultivation on nutrient medium. This species grew within a temperature range from 54 to 30°C; the optimum growth of the isolates of this *Chloroflexus* ecotype occurred at 50°C. The purple bacteria, aerobic chlorophyll *a*-containing bacteria, heliobacteria, and the filamentous green bacterium *Oscillochloris trichoides* are mesophiles. The isolates of these bacteria exhibited optimum growth at 25–35°C; thus, these bacteria are active only in the low-temperature mats of the spring. The growth temperature of the isolates of mesophilic microorganisms varied within a narrower range than the growth temperature of mesophiles in the microbial mats. This suggests seasonal temperature fluctuation of the spring water, as well as the resistance of the identified mesophilic microorganisms to an increase in temperature. Mixing of the thermal and meteoric water during periods of heavy rain is known to result in a temperature decrease of the effluent thermal water [6].

Because of methodic difficulties, we failed to differentiate bacteriochlorophyll *c* from chlorophyll *a* in ethanol extracts. However, in the spectra of native samples, the bacteriochlorophyll *c* maximum (740 nm) was small as compared to the major peak of chlorophyll *a* (660 nm), suggesting that chlorophyll *a* predominated in the mats. The maximum content of chlorophyll *a* (555 mg chl *a*/m$^2$) in microbial mats of the Bol'sherechenskii Spring was similar to the values reported for other highly productive ecosystems, such as hypersaline marine lagoons and lakes. The primary production rate in the Bol'sherechenskii Spring (1.3 g C/m$^2$ per day) was somewhat lower than that in the Thermofil'nyi and Octopus Springs (2.3 and 2.6 g C/m$^2$ per day, respectively) [4, 16]. The experiments with [$^{14}$C]-bicarbonate and analysis of the chlorophyll *a* content showed that the highest production in mats was at a temperature of 33–39°C.

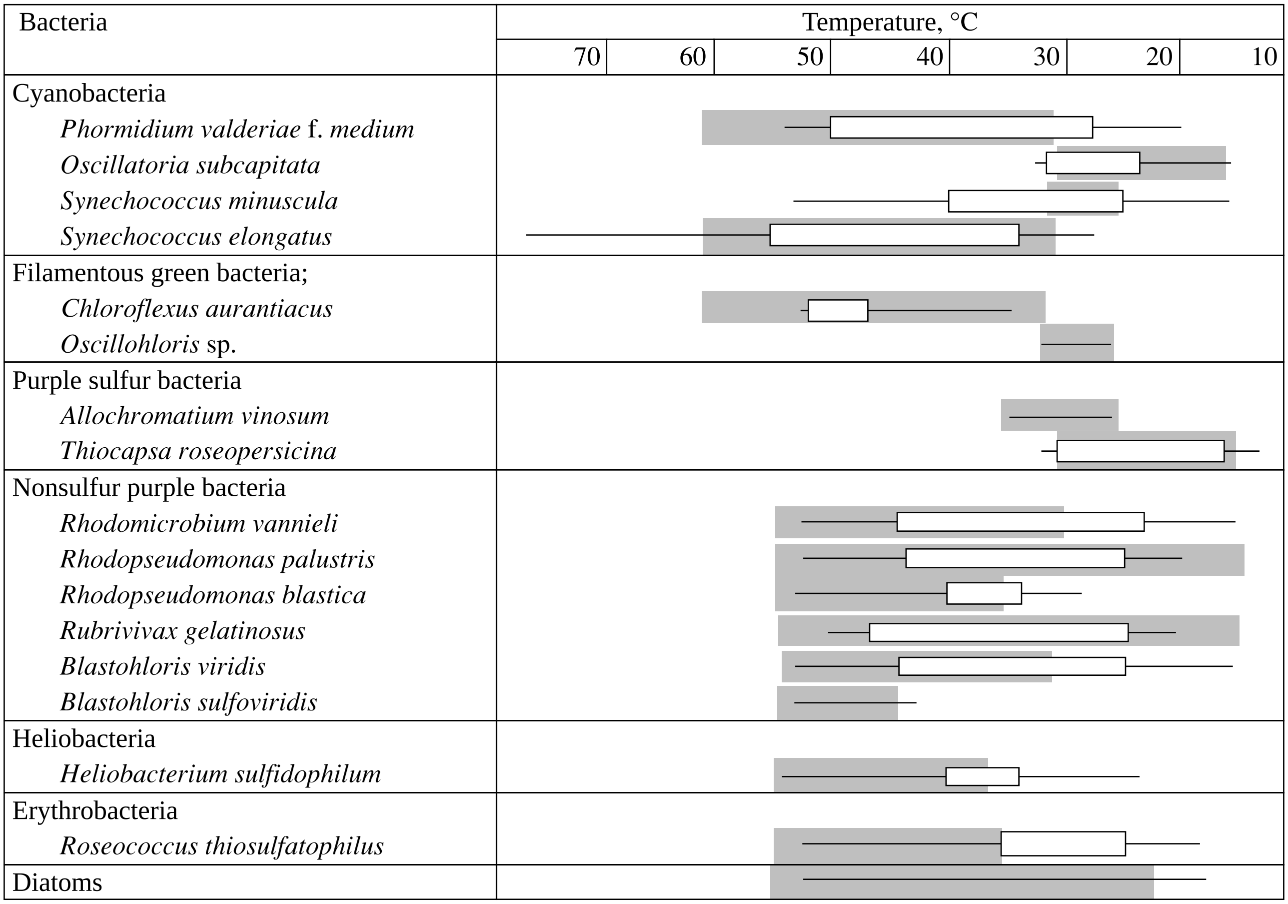
Thus, in the Bol'sherechenskii Spring, active growth of the phototrophic microorganisms is observed at a lower temperature than in the sulfide-deficient Octopus Spring, where the maximum production rate was determined within a temperature range from 50 to 55°C [2].

The ratio of anoxygenic to oxygenic photosynthesis varies along the effluent channel of the Bol'sherechenskii Spring. In zone II, at a temperature of 62°C and a sulfide content of 5.9 mg/l, the high sulfide concentration presumably prevents the most efficient oxygenic photosynthesis; anoxygenic photosynthesis predominates. In this zone, the proportion of oxygenic photosynthesis is as low as 4%, because the cyanobacteria presumably switch to anoxygenic photosynthesis. The proportion of oxygenic photosynthesis increases up to 62% with a decrease in temperature to 51°C and a decrease in sulfide to 2.8 mg/l, which are conditions favorable for cyanobacterial oxygenic photosynthesis. With a further temperature decrease to 39–25°C, anoxygenic photosynthesis again becomes favored over oxygenic photosynthesis (58–93 and 42–7%, respectively). Despite the high water Eh (+159) and minimal sulfide content (under 0.5 mg/l), the growth conditions are favorable for phototrophic sulfur bacteria at the silt and water boundary. This is apparent from numerous bright spots of purple bacteria and the high content of elemental sulfur, which is most likely deposited by purple sulfur bacteria of the genera *Allochromatium* and *Thiocapsa* (Table 2). The high content of elemental sulfur in this mat provides grounds to believe that sulfur reduction may be one of the main sources of sulfide in this zone.

The maximum rate of sulfate reduction in the spring (0.367 g S/m$^2$ per day) was somewhat lower than that determined in other springs (Thermophilic Spring (1.44 g S/m$^2$ per day) and Bas-lake Spring (3.44 g S/m$^2$ per day) [4, 17]. The optimum temperature of sulfate reduction was 39°C, which is much lower than in the Octopus Spring, where optimum decomposition is observed within a temperature range of 52–55°C [2].

The maximum rate of methanogenesis was 1.188 μg C/m$^2$ per day, which is much lower than in the mats of the Thermophilic Spring (from 12.96 to 424800 μg C/m$^2$ per day) [4]. The ratio of hydrogen-dependent methanogenesis to aceticlastic methanogenesis varied along the effluent channel. In the effluent stream, hydrogen-dependent methanogenesis was almost equal to the aceticlastic one (42 and 58%). In zone II at 62°C, aceticlastic methanogenesis exceeded hydrogen-dependent methanogenesis (58 and 42%, respectively). At 51–39°C, the rate of hydrogen-dependent methanogenesis was higher than the rate of aceticlastic methanogenesis (67–72 and 33–28%, respectively). In low-temperature zone V, the rate of hydrogen-dependent methanogenesis was lower than that of

**Table 3.** Temperature range for the presence of phototrophic bacteria in samples of microbial mats from the Bol'sherechenskii Spring and optimum growth temperatures of the isolates



Note:

▨ Temperature range for the presence of bacteria in microbial mats.

— Temperature range for bacterial growth under laboratory conditions.

▭ Bacterial growth optimum determined under laboratory conditions.

the aceticlastic one (40 and 60%, respectively). A strong decrease in both absolute and relative rates of aceticlastic methanogenesis, observed within a temperature range of 51–39°C, may be a consequence of the competition for acetate with the anoxygenic phototrophic bacteria of the species *Chloroflexus aurantiacus*. This bacterium grows in mats of the Bol'sherechenskii Spring within the temperature range of 54–39°C and utilizes acetate during photoheterotrophic growth [17].

The terminal stage of decomposition in mats was previously shown to depend on the sulfate content [4]. In the Bol'sherechenskii Spring, the latter parameter comprised 144 mg/l and sulfate reduction was the predominant process in the terminal decomposition of the microbial mats. The consumption of organic matter via sulfate reduction was as high as 0.2757 g C/m$^2$ per day (at 39°C), whereas consumption via methanogenesis was more than a hundred times lower (0.00159 g C/m$^2$ per day). A similar ratio was determined by Ward in sulfate-rich thermal springs [17].

Along the effluent spring channel, the system productivity increases sharply with a temperature decrease to 39°C. As a consequence, the total decomposition in the corresponding zones is accelerated, although not as rapidly as organic matter production. Hence, the lack of balance between these processes increases with decreasing temperature. In zone IV, where the rates of both processes are the highest, about 21% of the organic matter is mineralized via methanogenesis and sulfate reduction. The remainder of the organic matter of the microbial mat seems to be mineralized mostly via aerobic respiration. Sulfur-reducing microorganisms may also play a noticeable role in mineralization of the organic matter, because elemental sulfur was detected in both ground and mat samples (Table 1). Along the effluent channel, the content of elemental sulfur in mats increases gradually with decreasing temperature from

0.174–0.685 g S/m$^2$ at 54–39°C to 1.064–1.167 g S/m$^2$ at 39–25°C. This is commensurable with the content of sulfate sulfur in the flowing water of the spring.

Thus, in the Bol'sherechenskii Spring, the structure of the microbial communities and the processes of production and decomposition of organic matter depend on the simultaneous effect of several extreme factors.

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