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EXPERIMENTAL ARTICLES

Biogeochemical Processes in the Algal–Bacterial Mats of the Urinskii Alkaline Hot Spring

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Abstract—The structure and production characteristics of microbial communities from the Urinskii alkaline hot spring (Buryat Republic, Russia) have been investigated. A distinctive characteristic of this hot spring is the lack of sulfide in the issuing water. The water temperature near the spring vents ranged from 69 to 38.5°C and pH values ranged from 8.8 to 9.2. The total mineralization of water was less than 0.1 g/liter. Temperature has a profound effect on the species composition and biogeochemical processes occurring in the algal–bacterial mats of the Urinskii hot spring. The maximum diversity of the phototrophic community was observed at the temperatures 40 and 46°C. A total of 12 species of cyanobacteria, 4 species of diatoms, and one species of thermophilic anoxygenic phototrophic bacteria, *Chloroflexus aurantiacus*, have been isolated from mat samples. At temperatures above 40°C, the filamentous cyanobacterium *Phormidium laminosum* was predominant; its cell number and biomass concentration comprised 95.1 and 63.9%, respectively. At lower temperatures, the biomass concentrations of the cyanobacterium *Oscillatoria limosa* and diatoms increased (50.2 and 36.4%, respectively). The cyanobacterium *Mastigocladus laminosus*, which is normally found in neutral or slightly acidic hydrothermal systems, was detected in microbial communities. As the diatom concentration increases, so does the dry matter concentration in mats, while the content of organic matter decreases. The concentrations of proteins and carbohydrates reached their maximum levels at 45–50°C. The maximum average rate of oxygenic photosynthesis [2.1 g C/(m^2 day)], chlorophyll *a* content (343.4 mg/m^2), and cell number of phototrophic microorganisms were observed at temperatures from 45 to 50°C. The peak mass of bacterial mats (56.75 g/m^2) occurred at a temperature of 65–60°C. The maximum biomass concentration of phototrophs (414.63×10^{-6} g/ml) and the peak rate of anoxygenic photosynthesis [0.42 g C/(m^2 day)] were observed at a temperature of 35–40°C.

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Key words: alkaline hydrotherms, microbial mats, cyanobacteria, phototrophic bacteria, diatoms, productivity, chemical composition.

In recent years, the region of the alkaline nitric hydrotherms of the Baikal rift area has been the subject of intensive microbiological investigations. Previously, it was demonstrated that cyanobacterial mats form under the action of high temperatures, high pH, and increased sulfide concentrations in the alkaline hot spring Bol'sherechenskii [1]. The Urinskii Hot Spring, the subject of our present investigations, is of interest because its waters do not contain dissolved hydrogen sulfide. Therefore, the influence of temperature on various properties of algal-bacterial mats can be studied under the smaller effect of other extreme factors.

We investigated the species diversity, production characteristics, and the chemical composition of the biomass of algal–bacterial communities in the various temperature zones of the Urinskii Hot Spring. Scanty data has been published on the chemical composition of microbial mats from hot springs. The majority of these investigations are dedicated to analysis of the content and composition of particular chemical compounds: exopolysaccharides, lipids, fatty acids, hydrocarbons, biomarkers of the photosynthetic apparatus of phototrophic microorganisms, as well as to the formation of low-molecular-weight organic acids [2].

Our intention was to investigate the total content of organic matter (carbohydrates, proteins) and ash elements of microbial mats from the hydrotherms of the Urinskii Hot Spring in the context of the rates of biogeochemical processes and types of microbial communities.

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MATERIALS AND METHODS

Investigations at the Urinskii hot spring were performed in 1997, 2000, and 2002. The water temperature was measured with a sensory electrothermometer, Prima (Portugal), pH was measured potentiometrically with a portable pH meter pHep2 (Portugal). The water mineralization was determined with a portable TDS-4 conductometer (Singapore). The dissolved oxygen concentrations were determined using Winkler's method, the carbonate and hydrocarbonate content was determined by titration, the sulfate concentration was measured by the turbidimetric method, and the sulfide concentration was measured colorimetrically with paraphenylenediamine [1].

Mat samples (1 cm^2) were taken with a cork drill to determine the chlorophyll *a* concentration and to perform radioisotope experiments. In order to perform chemical analyses, a piece of mat (100 cm^2) was cut out; when required, it was cleared of leaves and stones. Then the mat samples were dried and homogenized. The ratios of the investigated compounds were expressed as a percentage of these compounds in the total weight of the air-dry sample. To determine the concentrations of carbonates and hydrocarbonates, the samples were supplemented with 0.02 N HCl. After precipitation, the excess of HCl was titrated with 0.02 N NaOH in the presence of methyl red. The calcium concentration was determined in the acid ash extract by titration with 0.5 N Trilon-B solution with Eriochrome indicator [3]. The total content of carbohydrates in microbial mats was measured photometrically. The air-dry weighed sample of the microbial mat was supplemented with a reagent, which contained a diphenylamine solution in absolute alcohol with glacial acetic acid and concentrated hydrochloric acid. The mixture was then heated for 30 min in a water bath to produce a stable color. The carbohydrate concentrations were defined according to color intensity [4]. The content of organic carbon was determined by Tyurin's method, modified by Nikitin. The air-dry weighed sample of the microbial mat was pre-treated with HCl solution to remove carbonates; then it was dried out and overlaid with chrome mixture. The optical density of the obtained solutions was determined with a photoelectrocolorimeter FEK-46 [3]. Protein concentrations were determined photometrically by use of Coomassie Blue [4, 5]. Chlorophyll *a* concentrations were determined by optical density of ethanol extracts at $\lambda = 655$ nm. We calculated the chlorophyll *a* concentration using the formula 1 μg chlorophyll $a = 11.9\ OD_{665}\ (v/l)$, where OD_{665} is the optical density at $\lambda = 665$ nm, v is the extract volume (ml), and l is the cuvette length.

The rates of microbial production and destruction of organic matter were determined by the radioisotope method. To estimate the rates of light and dark production, $NaH^{14}CO_3$ was used [6]; the rates of sulfate reduction were determined by the addition of $Na_2^{35}SO_4$; to measure the rates of methanogenesis, the samples were supplemented with ^{14}C-containing sodium acetate and sodium bicarbonate [7]. For inhibiting oxygenic photosynthesis, 7 μM of diuron [3-(3,4-dichlorophenyl)]-1,1-dimethylurea] was added. The samples were transferred to 13 ml penicillin bottles and incubated in situ in spring water in the light and dark for 6–24 h. After the end of the exposure, the samples were fixed with 10 N NaOH. The radioactivity of the labeled compounds was determined in a liquid scintillation counter, Rackbeta (Sweden). The rates of microbial processes were calculated according to the formula $A = r\,[C]/Rt$, where A is the rate of the process, C is the substrate concentration, r is the radioactivity of the reaction product, R is the labeled substrate radioactivity, and t is the time of incubation.

The species affiliations of cyanobacteria and diatoms were determined by their morphological properties according to the manuals by Gollerbakh et al. [8] and Zabelina et al. [9], respectively. The cell density and biomass of algae and cyanobacteria were determined in the homogenized mat samples by counting in a Goryaev chamber. The cell density was calculated according to a modified version of the formula used for phytoplankton density calculations [10]:

$$N = rn(A/a)U\ (1000/V), \qquad (1)$$

where N is the number of cells of the investigated species in 1 ml of mat sample; r is the factor demonstrating the ratio of the counting chamber volume to 1 ml; n is the number of cells detected in the microscope fields (squares) investigated; A is the number of microscope fields (squares) on the counting plate; a is the number of microscope fields (squares) in which the counts were performed; V is the volume of cell suspension (ml); and U is the volume of the sampled mat piece (ml).

The biomasses of algae and cyanobacteria were determined with the volume counting method. For this purpose, the average volumes of the cells of each species were calculated by equating them with simple geometrical figures. We calculated the biomass of each species using the following formula: $V = N\nu\rho 10^{-12}$, where V is the biomass (g/ml); N is the number of cells of the given species in the mat sample (cells/ml); ν is the volume of one average cell, μ^3; and ρ is the specific weight of an average cell (arbitrarily taken as 1) (g/ml) [10]. To obtain the data on the total numbers of cells and biomass concentrations of algae and cyanobacteria, we summarized the results. We used integral samples consisting of subsamples collected at various sites of the temperature zone under study to perform all types of assays of the chemical composition, cell numbers, biomass, and species characteristics.

Chloroflexus aurantiacus was cultivated under illumination, on the medium containing (g per liter of distilled water): KH_2PO_4, 0.5; NH_4Cl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; NaCl, 0.5; $CaCl_2 \cdot 2H_2O$, 0.05; $NaHCO_3$, 1.5; vitamin B_{12}, 20 μg; sodium acetate, 1.0; yeast extract, 0.1; $Na_2S_2O_3 \cdot 5H_2O$, 1.0; $Na_2S \cdot 9\ H_2O$, 0.3;

Pfennig microelement solution [11], 1 ml/l. The pH value was adjusted to 8.0–8.5 at 25°C with 0.1 M solutions of HCl and NaOH. The incubation temperature was 50°C. The pure culture was obtained by the method of end-point dilutions in agarized (0.2%) medium. The DNA G+C content and DNA–DNA homology were determined by A.M. Lysenko according to the standard procedures [11].

Determination of the median, upper and lower barriers, and plotting were carried out using MS EXCEL. The values beyond the limits of the upper and lower internal barriers (25 and 75% quartiles) were considered invalid.

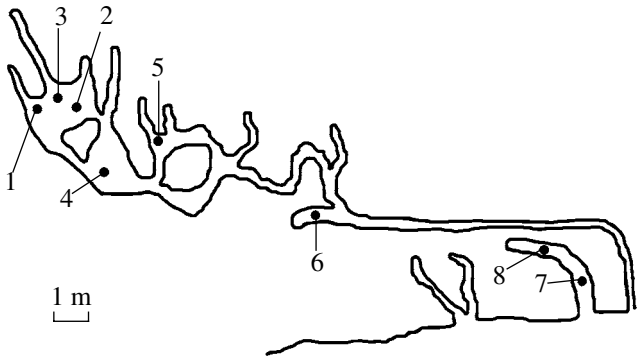

Fig. 1. The disposition of biological zones on the thermal ground of the Urinskii Hot Spring.

RESULTS

Characteristics of the Hot Spring

The Urinskii hot spring is situated on the territory of the Barguzin Region (Buryat Republic, Russia), within the Uro River Basin (the left tributary of the Barguzin River), along the left bank of the Listvennichnyi Spring at a distance of 1.3 kilometers from its mouth. The spring is located at 53°39' N, 110°07' E. Its thermal waters issue from under the mass of biotite granite on an area of 200 m^2. The spring has several vents, randomly distributed (Fig. 1). According to the published data, there are more than 30 low marginal vents (griffins) on the thermal ground. The total discharge of the spring is 1 l/s. The waters are of the hydrocarbonate–sulfate–sodium type. The escaping gas, according to the published data, consists mostly of nitrogen (98%) [12]. The presence of other gaseswas not reported. In the course of investigations, the water temperature near the vents ranged from 69 to 38°C, pH was within the 8.8–9.1 range, the total mineralization of the issuing water was low (less than 0.1 g/l), and sulfide was not detected. It should be emphasized that the sensitivity of the colorimetric method was less than 0.1 mg/liter (Table 1).

Characteristics of Microbial Communities

On the thermal ground, microbial mats of various colors were usually multilayered; their thickness was up to 2.5 cm. Due to the fact that the vents are distributed in a random manner, it is not possible to distinguish any biological zones located in succession along the spring outflow (Fig. 1). Therefore, we isolated 8 types of microbial communities and grouped them into 4 zones according to the water temperature and species compositions of phototrophs (Tables 1, 2). A total of 12 species of cyanobacteria, 4 species of diatoms, and one species of thermophilic anoxygenic phototrophic bacterium, (*Chloroflexus aurantiacus*,) were discovered in mat samples.

Table 1. Physico-chemical conditions in the biological zones of the Urinsky Hotspring

Biological zones	Stations	Microbial mat description	*T*, °C	pH	O_2, mg/l	CO_3^{2-}, mg/l	HCO_3^-, mg/l	SO_4^{2-}, mg/l
I	8	Orange microbial mats	66–69	8.8	–	–	–	55.9
II	5	Two-layer yellow–green mat (1 cm thick)	64	8.8	3.2	15.0	76.3	47.3
	6	White plumes consisting of empty sheaths of *Ph. laminosum*	63	8.8	3.5	–	85.4	47.8
	7	Bright orange mats	61	8.8	–	–	–	–
III	2	Orange three-layer mat (up to 2.5 cm thick)	47	9.7*	5.9	12.0	76.3	67.9
	1	Emerald green two-layer mat (2 mm thick)	46	8.8–9.1*	6.2	9.0	61.0	35.8
IV	4	Two-layer green mat (1 cm thick)	40	9.7*	7.4	6.0	70.2	48.8
	3	Deep-brown wisps	38	9.2	7.7	3.0	76.3	40.2

* On the mat surface; "–" – not identified.

Table 2. Distribution of phototrophic microorganisms in the biological zones of the Urinskii Spring

Group	Zone I, 66–69°C	Zone II, 60–65°C			Zone III, 45–50°C		Zone IV, 35–40°C	
Station, no.	8	5	6	7	2	1	4	3
Temperature, °C	69–66	64	63	61	47	46	40	38
Chloroflexus aurantiacus	+	+		+		+		
Synechococcus lividus		+	+		+	+		
Phormidium laminosum	++	++*	++	++	++**	++	++	+
Phormidium fragile			+		+	+	+	
Oscillatoria sp.		+						
Oscillatoria chalybea							+	
Oscillatoria limosa			+		+	+	+	++
Pleurocapsa sp.		+						
Mastigocladus laminosus		++*	+		++**	+	+	+
Calothrix elenkinii						+	+	
Calothrix parietina							+	
Gloecapsa minor						+	+	
Anabaena sp.						+		
Cocconeis sp.		+	+		+	+	+	+
Epitemia sp.								+
Navicula sp.							+	
Nitzshia sp.							+	
Number of species	2	7	6	2	6	10	11	5

Note: "+" – present, "++" – dominates.

* *Ph. laminosum* dominates in the upper layer, *M. laminosum* dominates in the lower layer.

** *Ph. laminosum* dominates in the upper (orange) and middle (light green) layers, *M. laminosum* dominates in the lower (dark-green) layer.

The diversity of phototrophic microorganisms increases as temperature is reduced, and predominant species were replaced by other microorganisms. At temperatures above 40°C, the filamentous cyanobacterium *Phormidium laminosum* was dominant, while the cyanobacterium *Mastigocladus laminosus* prevailed in the lower layers of the cyanobacterial mat at temperatures of 64 and 47°C. The role of the cyanobacterium *Oscillatoria limosa* and diatoms of the genera *Epitemia, Cocconeis, Navicula*, and *Nitzschia* in microbial communities increased at temperatures below 40°C. At 38.5°C (station 3, zone IV), the rapid development of deep-brown, almost black, plumes 2–3 mm thick was demonstrated. The plumes consisted of clusters of the *O. limosa* filaments (Fig. 2e). At this site, the species diversity was markedly reduced. This fact may be due to the shading of the mat surface by *O. limosa* filaments.

The anoxygenic filamentous green bacteria *Chloroflexus* sp. were found in the mats in lesser amounts than cyanobacteria. They are widespread in all the temperature zones of the Urinskii Spring, except for the low-temperature (35–40°C) zone IV. A culture of moderately thermophilic filamentous green bacteria (strain BG-39) was isolated from microbial mats collected in zone III (station 1). The bacteria developed under photoheterotrophic anaerobic conditions in the light and in the presence of 0.1 mM of sulfide (pH 8.0). DNA–DNA hybridization with the known strain *Chloroflexus aurantiacus* OK-70fl, previously isolated from hydrotherms in Oregon (United States), revealed 87% homology [13]. In addition, the content of G+C base pairs in DNA (55.5%) is close to that of strain OK-70fl (55.6%). Hence, the isolated strain was assigned to *C. aurantiacus*.

Biomass and Cell Numbers of Oxygenic Phototrophic Microorganisms

The average biomass of microbial mats gradually increased from 70.79×10^{-6} g/ml (zone II) up to 414.63×10^{-6} g/ml (zone IV) as the temperature decreased. The peak number of oxygenic phototrophic microorganisms (45.66×10^6 cells/ml) was revealed in zone III at temperatures ranging from 45 to 50°C (Table 3).

In the zones II and III, *Ph. laminosum* dominated, judging from its numbers (91.3–95.1% of the total number) and biomass (59.4–63.91% of the total biom-

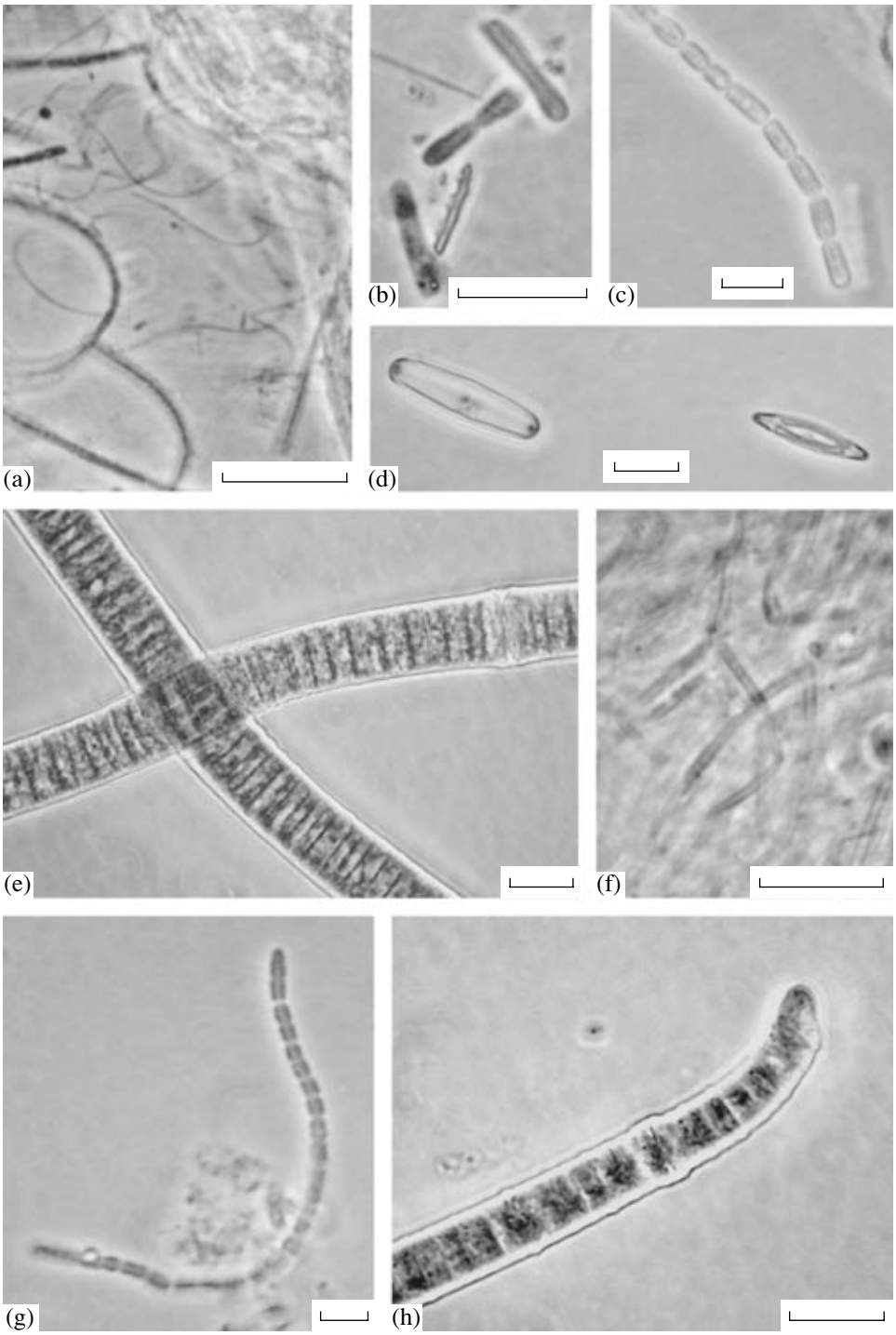

Fig. 2. Key: Algo-bacterial mat: a, phototrophic bacteria, inhabiting the site Uro-5: *Phormidium laminosum* and thin filaments of *Chloroflexus aurantiacus*; b, *Synechococcus lividus*; c, *Mastigocladus laminosus*; d, Diatoms; e, *Oscillatoria limosa*; f, empty sheaths of filamentous cyanobacteria at the Uro-6 Station; g, *Anabaena* sp.; h, *Oscillatoria chalybea*. Phase contrast microscopy. 5 μm scale.

ass) (Table 4). The proportion of *Ph. fragile* did not exceed 7.4% of the total number. Although its number did not exceed 1.3%, *M. laminosus* significantly contributed to biomass production (up to 25.3%) due to its very large cells. The other species in zones II and III constituted less than 1% of the total cell number and biomass of the whole community. In zone IV, *Ph. laminosum* was predominant in terms of numbers (89.4%), although *O. limosa* and diatoms were predominant (50.2 and 36.4%, respectively), in terms of biomass

Table 3. Biomass content and cell numbers of oxygenic phototrophic microorganisms

Parameter		Biological zone		
		Zone II, 60–65°C	Zone III, 45–50°C	Zone IV, 35–40°C
Biomass, 10^{-6} g/ml	Limits	70.65–70.93	108.85–659.14	135.97–693.29
	Median	70.79	383.99	414.63
Cell number, 10^6 cells/ml	Limits	7.63–8.51	13.63–77.68	8.43–4.56
	Median	8.07	45.66	11.49
Average mass of cell, 10^{-12} g/cell	Median	8.77	8.4	36.08

Table 4. Cell numbers and biomass content of the dominant phototrophic microorganisms

	Average cell number (% of total)			Average biomass (% of total)		
	Zone II, 60–65°C	Zone III, 45–50°C	Zone IV, 35–40°C	Zone II, 60–65°C	Zone III, 45–50°C	Zone IV, 35–40°C
Ph. laminosum	91.3	95.1	89.4	63.9	59.4	10.7
M. laminosus	1.3	0.6	1.0	25.3	10.0	2.7
O. limosa	–	0.9	9.6	–	21.7	50.2
Ph. fragile	7.4	3.4	–	3.5	1.8	–
Diatoms	–	–	–	7.3	7.1	36.4

Note: "–" – less than 0.001%.

production, since their numbers were low and their cells were very large. The numbers of diatoms never exceed 0.002% of the total number of other phototrophic microorganisms.

Chemical Composition of Microbial Mats

The maximum average mass of cyanobacterial mats (56.75 g/m^2) was observed at temperatures between 65 and 60°C. In zone III, the average mass decreased to 20.85 g/m^2 as the temperature dropped to 45–50°C, and increased up to 48.6 g/m^2 as the temperature decreased further to 35–40°C (Table 5). The maximum average content of organic matter in microbial mats reached 24.68% at temperatures from 60 to 65°C. As the temperature decreased, the average concentration of C_{org} decreased to 18.02%. The ash content of microbial mats was in inverse proportion to the organic matter content and increased from 35.31% in zone II to 64.32% in zone IV as the temperature decreased. On average, the carbonate concentration increased as the temperature dropped; the calcium content changed insignificantly (Table 5). The maximum average concentrations of carbohydrates and proteins in microbial mats were revealed in zone III (45–50°C), totaling 23.26 and 11.04%, respectively. At lower and higher temperatures, the concentrations of carbohydrates and proteins decreased (Table 5).

Production and Destruction Processes

In vivo spectral analysis of microbial mats demonstrated that chlorophyll *a* predominated. This fact indicates the predominance of oxygenic phototrophic microorganisms. The peak average concentration of chlorophyll *a* was recorded in zone III at temperatures from 45 to 50°C (343.4 mg/m^2) (Table 6).

Experiments with C^{14} bicarbonate have demonstrated that the average rate of oxygenic photosynthesis reached its maximum [2.1 g C/(m^2 day)] in zone III at 45–50°C. The peak average rate of anoxygenic photosynthesis (0.42 g C/(m^2 day)) was detected in zone IV at 35–40°C. The average rate of dark assimilation reached 0.61 and 0.6 g C/(m^2 day) at 65–60 and 45–50°C, respectively.

The average rate of sulfidogenesis in microbial mats reached its maximum (0.99 g S/(m^2 day)) at 45–50°C. The rate of hydrogenotrophic methanogenesis was low. In the outflow zone, it was 131-562.2 μg C/(m^2 day) (Table 6).

DISCUSSION

As compared to the previously investigated alkaline, sulfide-rich hot spring Bol'sherechenskii (74°C, pH 9.8, 13.4 mg/liter sulfide), which is also situated within the Baikal rift area, a peculiarity of the Urinskee Hot Spring is the lack of dissolved sulfide and the lower pH (8.8–9.2) of the thermal waters.

Table 5. Chemical composition of algal–bacterial mats (% of the average mass of an air-dry weighed portion)

Parameter		Biological zone		
		Zone II, 60–65°C	Zone III, 45–50°C	Zone IV, 35–40°C
Mass of cyanobacterial mat, g/m^2	Limits	52.9 – 60.6	15.4–26.3	34.7–62.5
	Median	56.75	20.85	48.6
C_{org}	Limits	24.15–25.2	17.93–25.58	16.35–19.69
	Median	24.68	21.76	18.02
Ash level	Limits	29.73–40.89	42.04–67.54	59.7–68.73
	Median	35.31	54.79	64.22
Hydrocarbons	Limits	16.03–19.49	21.83–24.7	9.72– 7.28
	Median	17.76	23.26	13.5
Protein	Limits	8.25–13.42	9.39–12.7	8.46–9.6
	Median	10.8	11.04	9.03
CO^{+2}	Limits	0.07–0.11	0.06–0.09	0.08–0.09
	Median	0.09	0.08	0.09
$HCO_3^- + CO_3^{-2}$	Limits	8.12–12.1	8.04–10.26	11.8–13.52
	Median	10.11	9.15	12.66

Table 6. Changes in some microbiological parameters along the outflow of the Urinskii Hot Spring

Parameter		Biological zone			
		Outflow zone, 66–69 °C	Zone II, 60–65°C	Zone III, 45–50°C	Zone IV, 35–40°C
Oxygenic photosynthesis, g C/(m^2/day)	Limits	0.018–0.078	0.06–0.12	1.35–2.64	1.06–3.35
	Median	0.048	0.087	2.1	1.19
Anoxygenic photosynthesis, g C/(m^2/day)	Limits	0.053–0.142	0.099–0.3	0.18–0.6	0.2–0.51
	Median	0.097	0.11	0.2	0.42
Oxygenic photosynthesis, %	Limits	26–36	28–36	80–87	67–94
	Median	31	32	81	74
Anoxygenic photosynthesis, %	Limits	64–74	64–72	13–20	6–33
	Median	69	66	19	26
Dark assimilation, g C/(m^2/day)	Limits	0.312–0.318	0.255–0.965	0.417–0.797	0.2–0.457
	Median	0.315	0.61	0.6	0.24
Sulfate reduction, g S/(m^2/day)	Limits	0.1–1.01	0.027–0.64	0.0081–1.97	0.005–0.625
	Median	0.55	0.54	0.99	0.31
Autotrophic methanogenesis, μg C/(m^2/day)	Limits	131–562.2	18–310	19.3–24.2	9.89–18.3
	Median	346	164	21.75	14.06
Chlorophyll *a* concentration, mg/m^2	Limits		1–88.45	94.77–892.38	49.4–463.2
	Median	6.318	56.5	343.4	274.4

During our long-term observations, carried out during the summer and fall sampling periods in 1997–2003, it was revealed that, despite the multitude of small vents (griffins) with different temperatures, the physicochemical parameters of the outflowing water of the Urinskii Hot Spring fluctuated within narrow limits at the same places. During the experiments, the compositions of microbial communities in various temperature zones were stable.

At temperatures above 40°C, the filamentous cyanobacterium *Ph. laminosum* was predominant in

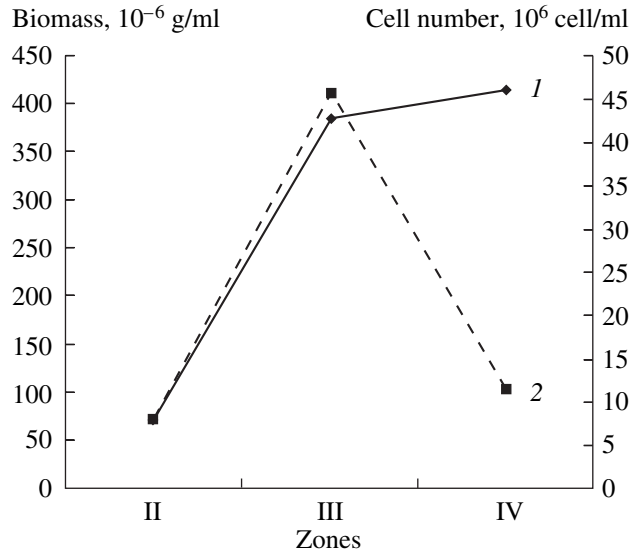

Fig. 3. The ratio between the cell numbers (*2*) and biomass content (*1*) of phototrophic microorganisms.

algal-bacterial mats. This cyanobacterium occurs widely in neutral hydrotherms at temperatures up to 60°C. However, at some stations, the cyanobacterium *M. laminosus*, the presence of which is more characteristic for neutral hydrotherms, was co-dominant. Its presence can be attributed to the relatively low pH level of the Urinskii Spring waters [14]. As the temperature dropped below 40°C, the predominant phototrophic microorganisms were replaced by other species. The role of cyanobacterium *O. limosa* and diatoms became more important. This phenomenon was accompanied by changes in the proportion of cell numbers to the biomass of phototrophs (Fig. 3). When the temperature dropped from 65 to 50°C, both the average cell numbers and average biomass level increased. However, as the temperature decreased further, the cell number decreased as well, while the biomass level continued to increase. This can be attributed to the differences in cell sizes of dominant phototrophic microorganisms. Thin filaments of *Ph. laminosum* (average cell mass 8.4–8.7×10^{-12} g/cell) develop at high temperatures, while the large-cell filamentous cyanobacteria of the genus *Oscillatoria* and diatoms (average cell mass 36.08×10^{-12} g/cell) are predominant at temperatures below 40°C and significantly contribute to biomass production despite low numbers of cells (Table 3).

Unlike in sulfide-containing slightly acidic hydrotherms, anoxygenic phototrophic bacteria are not the main producers in bacterial mats of the sulfide-free alkaline Urinskii Hot Spring, [15]. It was previously demonstrated that the population of *C. aurantiacus* is the most numerous among other microbial populations participating in the aerobic destruction of organic matter in neutral sulfide-free hydrotherms. During the greater part of the day, these microorganisms consume acetate and other products of dark fermentation of cyanobacteria. Photoautotrophic growth of *Ch. aurantiacus* in neutral hydrotherms occurs early in the morning, when the activity of cyanobacteria is low, and the concentration of hydrogen sulfide, produced by sulfur- and sulfate-reducing bacteria, is high [15, 16].

Unlike the type strain of *C. aurantiacus*, a heterotroph, which grows well under aerobic conditions on the surface of agar media, the strain BG-39 is capable only of anaerobic growth [13]. It should be noted that, in the Urinskii Springs, stable anaerobiosis occurred within the mat due to the active sulfate reduction. Hence, these data show that *C. aurantiacus* develops as an anoxygenic photoheterotroph, at least at temperatures ranging from 45 to 50°C.

The peak rate of oxygenic photosynthesis [3.35 g C/(m^2 day)], as well as the maximum concentrations of chlorophyll *a* (892 mg/m^2) in the cyanobacterial mats of the Urinskii Spring considerably exceed these parameters of the mats of the Bol'sherechenskii hot spring [0.737 g C/(m^2 day) and 555 mg/m^2, respectively]. This fact may be due to the lack of inhibition by the relatively high sulfide concentrations and high pH of the Bol'sherechenskii Spring waters [1]. These parameters match the values obtained in the neutral hydrothermal vents of Yellowstone and Kamchatka–Mushroom Springs (873 mg Chl *a*/m^2), Octopus Spring [4.32–5.4 g C/(m^2 day)], and Termofil'nyi, the Uzon caldera [2.3 g C/(m^2 day)] [6, 17, 18]. The rates of anoxygenic photosynthesis [0.6 g C/(m^2 day)] and dark CO_2 assimilation [0.965 g C/ (m^2 day)] in the bacterial mats from the Urinskii Spring were similar to those in the Bol'sherechenskii Spring [0.882 g C/(m^2 day) and 0.8 g/(m^2 day), respectively] [1].

The peak rate of sulfate reduction [1.97 g S/(m^2 day)] was higher than the rates of sulfate reduction in the mats of the Termofil'nyi [1.44 g S/(m^2 day)] and Bol'sherechenskii Springs [0.88 g S/(m^2 day)] [1, 7]. The rate of autotrophic methanogenesis was as low as 562.2 μg/(m^2 day). Hence, sulfate reduction dominates over other processes of terminal destruction in the microbial mats of the Urinskii Spring. The peak value of organic matter consumption via sulfate reduction is 1.48 g C/(m^2 day), while only 0.56 mg C/(m^2 day) is consumed via methanogenesis.

The highest rate of oxygenic photosynthesis and chlorophyll *a* concentration were detected in zone III, with temperature ranging from 45 to 50°C. Similar values were obtained in neutral hydrotherms, where the optimum for oxygenic photosynthesis was observed at 45–55°C, and in the more alkaline Bol'sherechenskii Spring, where the optimum was observed at 50°C [1, 15, 17]. The optimum for anoxygenic photosynthesis in both in the Urinskii and Bol'sherechenskii Springs was observed at 35–40°C. The highest average productivity of phototrophic microorganisms in the Urinskii Spring was revealed at higher temperatures than had previously been observed in the Bol'sherechenskii Spring (45–50 and 33–39°C, respectively). This fact can be

attributed to the lack of inhibition by the high sulfide concentration and pH, as occurred in the Bol'sherechenskii Spring [1].

The ratio of photosynthetic production and biomass production by phototrophic microorganisms varied greatly in different zones of the Urinskii Spring (Fig. 4). While the average biomass production increased relatively gradually with the decrease in temperature from 65 to 35°C, the average photosynthetic production increased markedly as the temperature dropped, peaked at 45–50°C, and then decreased as the temperature fell further. This can be attributed to the differences in cell sizes of dominant phototrophic microorganisms. In the case of the small-celled cyanobacteria, which dominate at 45–50°C, the ratio between the cell area and the cell volume is high. This probably results in the higher growth rates and biomass production in this temperature zone. The growth rates of the large cells of *Oscillatoria* and diatoms are lower. This fact may explain low productivity at 35–40°C.

The optimums of dark assimilation and sulfate reduction in the Urinskii Spring were found at 65–45°C. Similar distribution has been found in the Termofil'nyi Spring; the optimums of these processes were observed in the "green" zone at 40–55°C [6]. In the Bol'sherechenskii Spring, the optimums were observed at 25–40°C; this finding can also be attributed to the lack of inhibition by a high pH level and the presence of sulfide. The optimum of autotrophic methanogenesis in the Urinskii Spring was observed in the outflow zone at 66–69°C. These data agree well with the data obtained from the hydrothermal vents of Kamchatka and Yellowstone, where the peak values were observed at temperatures above 50–60°C [18, 19].

The proportion of organic matter, mineralized via anaerobic processes, decreased from 89 to 12.5% as the temperature decreased from 69 to 35°C. It is obvious that, in low-temperature mats, a considerable portion of organic matter was mineralized in the course of aerobic processes.

The studied algal-bacterial mats were characterized by high ash contents (Table 5). This, evidently, was the result of two factors: the presence of silicates in diatoms (with ash content up to 50–70%) [20], and the carbonates, which can be precipitated by cyanobacteria as calcium salts. It is significant that when the proportion of diatoms increased, the ash level of the Urinskii Spring mats increased as well, while the content of organic matter decreased. The maximal average concentrations of carbohydrates and proteins were observed in the zone III (45–50°C), coinciding with the peak productivity of the mat.

One can infer that the species and chemical composition of algal-bacterial mats in the Urinskii Hot Springs and the biogeochemical processes occurring in them are controlled by the following factors: temperature, slightly alkaline pH, the absence of sulfide, and sulfidogenesis in the mats. The combination of these factors determines the uniqueness of the spring as a habitat for benthic phototrophic communities. The key role of diatoms in silicon accumulation in algal-bacterial communities of hot springs and, obviously, in sinter deposition have been demonstrated.

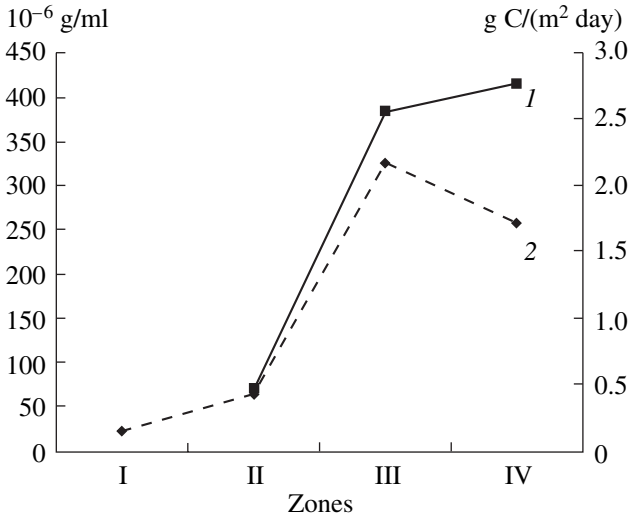

Fig. 4. The ratio between the photosynthetic (2) and biomass (1) production of phototrophic microorganisms.

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