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WATER QUALITY AND PROTECTION: ENVIRONMENTAL ASPECTS

Distribution and Activity of Bacteriobenthos in the Upper Volga Reservoirs

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Abstract—Regularities of bacteria distribution in the bottom sediments of the Upper Volga reservoirs are studied. Human-induced changes in the structure and activity of bacteriobenthos communities occurring in water body areas adjacent to towns and settlements are described. Reservoir zones subject to long-term human impact are distinguished.

INTRODUCTION

Bacteriobenthos is studied to a lesser extent than marine and freshwater bacterioplankton, for which a large body of data on its structure and functional activity has been accumulated. This is explained by the underdevelopment of methodology and concepts of investigating complicated-structure pore matrix of bottom sediments [22]. Sediment is extremely heterogeneous habitat, comprising water, soluble and insoluble organic compounds, and inorganic substances, whose physical and chemical properties vary within a wide range. Counting of bacteria in bottom sediments is still a complicated problem due to the small size of bacterial cells and the masking effect of sediment particles [29]. At the same time, the concentration and activity of bacteriobenthos are two to four orders of magnitude higher than those of bacterioplankton, which testifies to tremendous metabolic and ecological potential of the former. Benthic microorganisms play the most important role in organic matter degradation, elements turnover, and self-purification processes in water bodies. They are important components of food networks. These microorganisms have a considerable effect on the metabolism of benthic animals and plants and form the chemical composition and physical properties of bottom sediments [18, 22, 24, 28]. The structural and functional characteristics of bacteriobenthos adequately represent the state of water bodies and can be successfully used for bioindication and biomonitoring purposes.

Information on the structure and functioning of bacterial communities in the bottom sediments of the Upper Volga reservoirs that can be found in scientific literature is scanty and reduced, primarily, to the description of local zones singled out in areas affected by large industrial centers [4, 15]. This work is aimed at establishing the regularities of bacteriobenthos distribution in the Upper Volga reservoirs, singling out persistently contaminated areas of water bodies and studying human-induced changes in the structure and functional activity of bacteriobenthos communities in the zones of water bodies that are adjacent to towns and settlements.

MATERIALS AND METHODS

The studies were carried out in 1992 and in 1995–1999 during the time periods when water was free of ice. Bottom sediment samples were taken in individual areas of the Uglich, Rybinsk, and Gorki reservoirs located, mainly within the territory of Yaroslavl province. Four areas were singled out according to their hydrological regime: the near-dam area of the Uglich Reservoir (all in all, 8 sampling sites), the channel area of the Volzhskii Pool of the Rybinsk Reservoir (12 sites), the open part of the Rybinsk Reservoir (23 sites), and the channel area of the Gorki Reservoir (31 sites). Water and sediment samples were taken near towns, settlements, and mouth areas of rivers. Near-bottom water was sampled using a Ruttner bathometer, the surface layer of bottom sediment (0–3 cm) was sampled with a DAK-250 dredge or with a rod dredge.

Hydrochemical and microbiological analyses were carried out using the methods, most of which are described in manuals on aquatic microbiology [9, 16]. The concentration of oxygen dissolved in the bottom water layer was calculated using the Winkler technique; pH and Eh of bottom sediments were measured using an *I-102* portable ion meter. The density and moisture content of sediments were determined using the thermostatic–weight method. The concentration of C_{org} was determined from losses on ignition of dry sediment at the temperature of 550°C during four hours. The concentration of carbonates in silt solutions obtained using centrifuging was analyzed by gas chromatography method.

The number of aerobic saprophytes was counted according to the amount of colonies, growing on fishpeptone agar in a Petri dish after seeding of the respective ten-fold dilutions of the sediment. The rate of oxygen absorption by bottom sediments of water bodies was determined using the method of isolated cylinders. The intensity of dark assimilation of CO_2 was measured using the radiocarbon method: 0.2 ml of $NaH^{14}CO_3$ solution with an activity of 40 kBq was added to vials containing sediment suspension. These vials were incubated in the dark at *in situ* temperature. In 12–20 hours, the samples were fixed with formalin to the final concentration of 4%, after which they were diluted with tap water filtered through a 0.2 μm filter and mixed by a magnetic stirrer. After that, sodium pyrophosphate was added to the sample thus processed and it was treated by ultrasound using UZDN-1 low-frequency ultrasonic dispersant. Then the aliquot of the bottom sediment suspension was filtered through membrane filters with the pore diameter of 0.2 μm, which were washed by hydrochloric acid and then in distilled water, dried, and put into vials with ZhS-106 scintillation liquid. The radioactivity of bacterial cells settled on the filters was determined using Mark II liquid scintillation counter (USA).

The total number and size of bacteriobenthos were determined by epifluorescent microscopy with the use of fluorochromes of orange acridine or 4',6-diamidine 2-phenilindole (DAFI) [21, 25]. Sodium pyrophosphate used as detergent was added to the bottom sediment samples fixed with glutaraldehyde (to the final concentration of 2%). Then these samples were subjected to the impact of ultrasound and 1000 to 2000-fold dilution (depending on the type of sediment) with the tap water filtered through membrane filters with a pore diameter of 0.2 μm [32]. After that, 2 to 5 ml of the sediment suspension was colored with fluorochrome and filtered through black nucleopore filters with the pore diameter of 0.2 μm. The bacteria were studied using Lumam-I1 microscope under 1000-fold magnification. The size of bacterial cells was measured using a linear ocular-micrometer; their volumes were calculated according to the formulae of a sphere, ellipsoid, or cylinder. On each filter, not less than 400 cells in ten fields of vision were counted, and not less than 100 cells were measured. A coefficient equal to 0.11 was used when transferring from wet bacterial biomass to C [20].

RESULTS AND DISCUSSION

The following types of bottom sediments were sampled within the investigated areas of the Upper Volga reservoirs: sand, silty sand, sandy silt, gray silt, and peaty silt, sometimes with an admixture of gravel, shell rock, clay, and plant fragments. The concentration of oxygen dissolved in the bottom water layers varied from 5.6 to 12.7 mg/l. The values of Eh (redox potential) of the surface layer of most of the investigated sediments did not exceed 200 mV. Lower Eh values were recorded in the silt; and high values, in sand and peaty sediments. The most pronounced oxidation conditions (Eh varying from 385 to 445 mV) were typical of sandy gravel sediments found in the channel area of the Gorki Reservoir, sampled downstream of the town of Rybinsk. The values of sediment pH, which are usually close to neutral, varied from 6.7 to 7.4. It was only peaty sediment, sampled in the central part of the Rybinsk Reservoir, that was characterized by a lower pH. The density and moisture content of the bottom sediments varied from 1.05 to 1.88 g/ml and from 23.5 to 82.3%, respectively. The temperature of the sediment surface varied from 5.6 to 24°C. The concentration of C_{org} was minimum in the sand (1.3%) and maximum in peaty silt (7.7%). The total concentration of carbonates in silt solutions made 55 to 82 mg C/l.

The reservoir bottom sediments were characterized by a high concentration and uneven distribution of bacteria inhabiting them over the water area. The mosaic pattern of bacteria distribution is caused by the complicated hydrological regime of the water bodies, the heterogeneity and microzonality of their sediments, as well as by human impact. The total number of bacteriobenthos varied from 0.76×10^9 to 21.44×10^9 cells per 1 ml of wet sediment, depending on the type of sediment, sampling type and anthropogenic load (Table 1). The minimum value of this parameter was recorded in sandy–gravel sediment of the upstream flow-through part of the Gorki Reservoir. The maximum values were observed in silty sediments, sampled near the mouths of rivers (Cheryomukha, Kotorosl', and Solonitsa), as well as near towns and settlements (Uglich, Rybinsk, Yaroslavl, and Perebory), i.e., where allochthonous organic substances of both natural and anthropogenic origin found their way to the water body. Most probably, the main factor governing the development of bacteriobenthos in the Upper Volga reservoirs during the vegetation period is the concentration of organic substrates. The studies have shown that the distribution and activity of bacteria in the bottom sediments of water bodies are determined by a complicated and dynamic set of constantly interacting and changing factors, including water temperature, the concentration of dissolved oxygen and other electron acceptors, redox conditions, and the activity of bacteriotrophic organisms, in addition to the concentration and composition of organic substances [20, 26, 28].

It should be emphasized that the number of bacteria in the bottom sediments of the Upper Volga reservoirs was determined by fluorescent microscopy with the use of fluorochromes, with preliminary treatment of samples with detergents and ultrasound. Therefore, our data on the total population of bacteriobenthos largely exceed the ones obtained with the help of the previously used method of optical microscopy, involving dying of bacteria with erythrosine (adsorption dye). Thus, in 1972, the number of bacteria in the bottom sediments sampled within the whole Volga River course from Tver to Astrakhan, averaged over the vegetation period, did not exceed 1.35×10^9 cells/g. The number of bacteria was minimum in the sand and max-

Table 1. Total abundance of bacteria, 10^9 cells/ml, in the bottom sediments of the Upper Volga reservoirs (here and in Tables 2 and 4, the to numbers are the limits of parameter values fluctuations; the bottom numbers are the mean value ± standard deviation; the dash here and in Tables 2–5 denotes the absence of data)

Observation period	Uglich Reservoir	Rybinsk Reservoir		Gorki Reservoir
		channel area	open par	
1992 (August)	–	–	5.96–12.31 9.30 ± 2.88	11.11–5.41 2.76 ± 2.00
1995 (May–June)	2.35–4.90 3.42 ± 1.33	2.22–4.55 3.33 ± 0.95	2.00–9.96 4.15 ± 2.07	1.81–5.01 3.17 ± 0.95
1995 (August–September)	4.77–12.6 7.98 ± 2.77	6.26–6.91 6.59 ± 0.46	9.62	0.76–10.15 3.93 ± 3.17
1996 (June)	14.04	4.44–14.21 10.11 ± 4.12	8.12–16.07 12.12 ± 3.96	1.13–14.76 4.33 ± 4.00
1996 (September)	6.87–10.15 8.57 ± 1.64	3.39–10.26 7.27 ± 12.85	10.93	1.82–12.56 4.89 ± 2.90
1997 (June–July)	9.16	6.78–10.07 8.46 ± 1.42	7.94–10.39 9.08 ± 1.23	2.6–12.94 6.54 ± 3.34
1997 (August)	8.37–13.66 10.85 ± 2.02	13.85	10.21–13.38 11.60 ± 11.01	4.88–10.36 7.93 ± 2.31
1997 (September)	20.93–21.44 21.19 ± 0.36	5.74–20.01 11.53 ± 5.51	9.55–18.84 14.40 ± 4.66	3.06–18.34 8.55 ± 5.60
1998 (October)	17.55–19.51 18.53 ± 1.39	8.19–14.07 11.15 ± 2.52	3.76–20.92 9.29 ± 4.56	3.76–17.66 9.65 ± 4.06
1999 (July)	3.78–3.84 3.81 ± 0.04	1.27–8.47 4.75 ± 2.26	2.80–6.22 4.23 ± 1.58	3.20–9.48 5.94 ± 1.61
For the whole observation period	2.35–21.44 9.60 ± 5.05	1.27–20.01 8.26 ± 4.31	2.00–20.92 8.56 ± 4.30	0.76–18.34 5.93 ± 3.99

imum in the gray silt [13]. The average value of this parameter for the investigated section of the Upper Volga turned out to be 5.4 times higher and amounted to $(7.35 \pm 4.47) \times 10^9$ cells/ml. The incomplete account of the bacteria colored with erythrosine, is explained by the masking effect of organic–mineral sediment particles. It is a well-known fact that only a small part of bacteria occur in pore solutions of the bottom sediments, whereas the greater part of bacteria is attached to sediment particles with extracellular polymers [14, 23]. In addition, the method of fluorescent microscopy allows for counting small bacterial cells (their diameter being approximately 0.1 μm), which are invisible under the optical microscope. It is very difficult to compare the amount of bacteria counted by optical microscopy to their amount counted by epifluorescent microscopy. Even the number of bacterioplankton counted in one and the same water sample using the erythrosine method and the method of epifluorescent microscopy at a time can hardly be interpreted [7].

Where the procedure of preliminary treatment of the bottom sediment samples was modified (using dilution, processing with ultrasound, and centrifuging), the completeness of accounting the bacteria using the erythrosine method increased by approximately one order of magnitude. The amount of bacteria in the Rybinsk Reservoir bottom sediments, determined using the modified erythrosine method, varied from 11.05×10^9 to 19.75×10^9 cells/ml [2]. However, such a modification of the erythrosine method is labor-consuming and is not widely used in practical microbiology because methods of epifluorescent microscopy are now widely used [26].

The greatest number of bacteria, counted in bottom sediments of different reservoirs, was found in silt sediment of the near-dam area of the Uglich Reservoir, whereas the least amount was detected in the sediment of the flow-through channel section of the Gorki Reservoir. The population of bacteriobenthos, averaged over all the years of observation, amounted to 9.60×10^9 cells/ml for the Uglich Reservoir; 8.26×10^9 cells/ml, for the channel part of the Volzhskii Pool of the Rybinsk Reservoir; 8.56×10^9 cells/ml for the open parts of the Volga Pool and the Main Pool of the Rybinsk Reservoir; and 5.93×10^9 cells/ml, for the channel part of the

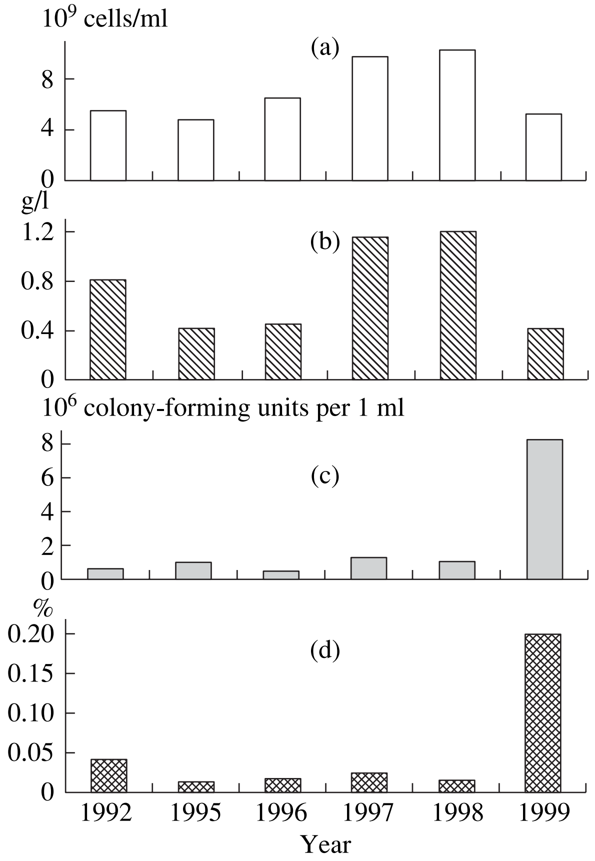

Fig. 1. Total number (a) and biomass (b) of bacteria, the number of saprophytes (c), and the ratio of the number of saprophytes to the total number of bacteria (d) in the bottom sediments of the Upper Volga reservoirs during different years of observations.

Gorki Reservoir. In the latter, the amount of bacteriobenthos grew to 18.34 × 10^9 cells/ml (in the Solonitsa River) with the distance from the Rybinsk Hydroelectric Power Plant, which is explained by a decrease in the current velocity, more intense sedimentation, and input of organic substrates and biogenic elements of anthropogenic origin. The bottom sediments in the channel section of the Gorki Reservoir (downstream of Yaroslavl) were characterized by an appreciable number of bacteria, whereas in the areas affected by Yaroslavl and Kostroma, as well as by the Kostromskaya State District Power Plant (the village of Volgorechensk), the number of benthic bacteria was maximum (18.50 × 10^9 cells/ml) [3].

In the Upper Volga Reservoirs, the greatest amount of bacteriobenthos was found late in summer and early in autumn. Seasonal variations in this parameter are related, primarily, to the input of allochthonous organic matter and microorganisms with flood water, death and settlement of dead phytoplankton onto the bottom, and water temperature fluctuations. Round-the-year observations over the dynamics of bacteria population in the bottom sediments of the Volga reservoirs revealed the maximum number of bacteria in May and June, as well as in August and September, whereas the minimum number was recorded late in autumn and in winter. Water temperature was assumed to be the main natural factor governing bacteriobenthos development in reservoir bottom sediments [6, 17].

Results of studies carried out prior to 1998 demonstrated a gradual increase in the population of bacteriobenthos. In 1998, its average value amounted to $(10.25 \pm 4.52) \times 10^9$ cells/ml. In 1999, the amount of bacteriobenthos sharply fell to $(5.96 \pm 1.84) \times 10^9$ cells/ml (Fig. 1). In the Upper Volga reservoirs, year-to-year variations in this parameter are determined by the amount of precipitation, as well as by water level and temperature regimes in the given year. Human factors, such as the input of industrial and domestic wastewaters from cities and towns, the intensity of navigation, etc. also have a considerable impact on the development of bacteriobenthos.

In general, in spite of appreciable variances in physical and chemical characteristics, the concentration of bacteria in the surface layers of bottom sediments of different water bodies can be regarded as stable and varying from 10^9 to 10^{10} cells/ml [30]. After recalculation of the number of bacteria per sediment dry weight, the volume of the pore solution, the average size of sediment particles, their surface area, or organic matter concentration, the parameter in question demonstrated a reliable correlation with different physical and chemical characteristics of bottom sediments (e.g., moisture content, organic matter concentration, size of silt particles, etc.). The values of the amount and activity of bacteria are usually maximum in the surface layers of bottom sediments and decrease with the depth due to the exhaustion of readily-oxidizable organic matter fraction. For instance, in silty sediments of an eutrophic lake, at the depth of 5 to 10 cm, the biomass of microorganisms and the activity of their electron-transport system amounted to 25–60% and 40–75% of the biomass and activity of microorganisms in the surface (0 to 1 cm) sediment layer, respectively [19]. The amount of bacteriobenthos depends on the character and degree of the respective water body contamination. In the places of industrial and domestic waste water inflow in the Elba River, the concentration of bacteria varied from 10^{10} to 10^{11} cells per 1 g of dry sediment, which largely exceeded the amount of bacteria in less contaminated river sections [31]. Analysis of the available information reveals a tendency toward an increase in the amount of bacteriobenthos with increasing trophic level of the water body. However, it is not always possible to establish a reliable correlation between these parameters, because other factors (such as the type of mixing, flow-through character of the water body, its catchment area, and geographical position), as well as some other morphological and hydrological parameters of the water body have a considerable impact on the correlation in question [5].

Table 2. Biomass of bacteria, mg/l, in the bottom sediments of reservoirs

Observation period	Uglich Reservoir	Rybinsk Reservoir		Gorki Reservoir
		channel area	open part	
1992 (August)	–	–	843–1566 1190 ± 295	233–942 534 ± 296
1995 (May–June)	291–500 380 ± 108	246–819 474 ± 246	206–777 448 ± 192	120–817 375 ± 206
1995 (August–September)	305–668 452 ± 135	282–338 310 ± 40	702	87–764 385 ± 213
1996 (June)	421	195–861 531 ± 272	260–675 486 ± 210	93–487 231 ± 130
1996 (September)	739–1066 857 ± 182	285–811 661 ± 252	940	164–1017 423 ± 258
1997 (June–July)	617	392–1074 620 ± 269	476–1047 761 ± 286	323–884 547 ± 201
1997 (August)	578–1311 929 ± 245	1149	737–1577 1164 ± 271	596–1009 823 ± 190
1997 (September)	1586–1842 1714 ± 181	801–2301 1430 ± 618	1403–3448 2309 ± 1042	440–5629 1527 ± 1226
1998 (October)	1307–1737 1522 ± 304	1023–1561 1338 ± 252	729–2698 1332 ± 515	436–1413 933 ± 332
1999 (July)	246–284 265 ± 27	244–754 466 ± 200	224–367 275 ± 63	243–754 448 ± 132
For the whole observation period	246–1842 711 ± 441	195–2301 810 ± 530	206–3448 970 ± 624	87–5629 661 ± 671

Diversity of morphological forms was not typical of bacteriobenthos of the Upper Volga water bodies. Their bacteriobenthos was represented, mainly, by bacilli and cocci with the predominance of the latter. The values of microbial cell volume, averaged for different types of bottom sediments, differed by one order of magnitude, varying from 0.03 to 0.342 μm^3. The volume of bacterial cells, averaged over the whole observational period, turned out to be equal to 0.108 ± 0.053 μm^3. As a rule, larger bacteria were found in the sand. The smallest size of benthic bacteria cells, found in bottom sediments of different reservoirs, was typical of the open part of the Rybinsk Reservoir, where the average cell volume was 0.119 μm^3. The lowest values of bacterial cell volume were typical of the Uglich Reservoir silt (the average value being 0.076 μm^3). Mind that in this part of the reservoir, the amount of bacteriobenthos was maximum.

For four zones of reservoirs, the values of wet bacteriobenthos biomass, averaged for the whole observation period, made 711, 810, 970, and 661 mg/l, respectively (Table 2). The maximum value of microbial biomass (5629 mg/l) was recorded in September 1997 in the silt sampled in the Kotorosl River mouth, flowing within the limits of Yaroslavl. The amplitude of bacteriobenthos biomass fluctuation was larger than that of bacteriobenthos amount: the ratios of the maximum to minimum values of these parameters were 65 and 28, respectively. Permanently high values of the number and biomass of bacteria were observed in the bottom sediments sampled near Yaroslavl and Uglich and near the villages of Perebory, Krasnyi Profintern, and Priluki, where these values, averaged for the whole observation period, amounted to 10^{10} cells/ml and 1 g/l, respectively (Table 3). The maximum average values of bacteriobenthos biomass were recorded in 1997 and 1998, when they turned out to be equal to 1158 ± 831 and 1204 ± 452 mg/l, respectively (Fig. 1). In the summer of 1999, microbial biomass was almost three times lower, making only 416 ± 155 mg/l.

The number of saprophyte microorganisms in the bottom sediments of the Upper Volga reservoirs varied within a wide range: from 3×10^3 to 119×10^6 colony-forming units per 1 ml of wet sediment (Table 4). The highest number of these microorganisms was typical of the silt in the near-dam part of the Uglich Reservoir. In the Rybinsk and Gorki reservoirs, their number turned

Table 3. Total number *N*, 10^9 cells/ml; and biomass *B*, mg/l, of bacteriobenthos near towns and settlements during different years of observations (here and in Table 5, the mean values of the parameters are presented)

Sampling site	1995		1996		1997		1998		1999		Mean	
	N	*B*	*N*	*B*	*N*	*B*	*N*	*B*	*N*	*B*	*N*	*B*
Priluki	4.90	500	11.37	593	14.50	992	17.55	1737	3.78	246	10.42	814
Uglich	6.59	310	10.26	811	10.76	1080	14.07	1520	5.48	359	9.43	816
Myshkin	4.55	819	7.72	774	10.54	1001	10.21	1561	8.47	754	8.30	982
Perebory	5.01	777	10.93	940	12.24	1203	13.32	1869	4.74	265	9.25	1011
Rybinsk, water intake	2.05	228	–	–	–	–	–	–	9.48	502	7.13	553
Rybinsk	2.63	301	4.79	433	12.76	1620	6.34	1394	6.48	479	6.60	845
Rybinsk, downstream of the water treatment facility	1.81	120	2.61	185	3.29	537	5.83	711	3.20	243	3.35	359
Tutaev, water intake	5.01	817	1.82	164	4.97	485	10.32	588	–	–	5.53	514
Downstream of Tutaev	–	–	2.48	181	8.42	852	5.44	560	5.35	444	5.42	509
Yaroslavl, the central water intake:												
the central water intake	3.88	435	2.21	166	–	–	–	–	5.05	384	3.71	328
within the city	4.93	558	9.43	418	13.66	3025	11.27	1138	9.08	491	9.67	1126
downstream of the water treatment facility	2.95	286	6.34	710	6.22	818	11.66	980	5.41	417	6.52	642
Krasnyi Profintern, water intake	–	–	–	–	15.49	1921	9.59	873	5.16	356	10.08	1050
Krasnyi Profintern	2.33	221	3.15	291	6.2	17.38	17.66	1413	5.8	449	7.03	478

out to be less. Permanently high amount of saprophytes was recorded in the vicinity of the towns of Uglich, Myshkin, and Rybinsk, near the city of Yaroslavl and near the villages of Perebory and Priluki, as well as in the Solonitsa River mouth (Table 5).

The ratio of the number of saprophytes to the total number of bacteria in the water is used to characterize the trophic state and saprobility index of water bodies, as well as to estimate their water quality [10, 12]. Distinct criteria of estimating the state of aquatic ecosystems according to bacteriobenthos parameters have not been developed till present. Usually, the per cent of saprophytes in bacteriobenthos is one to three orders of magnitude lower, than in bacterioplankton. In the bottom sediments of the Upper Volga reservoirs, the number of saprophytes amounted to 0.0002 to 3.132% of the total number of bacteria (on the average, 0.045 ± 0.232%) (Tables 4 and 5). Such broad range of fluctuations in the ratio of the number of saprophytes to the total number of bacteria is caused, mainly, by the different intensity and character of human impact. The maximum value of this parameter was recorded near the village of Priluki in the Uglich Reservoir in the summer of 1999, when the number of saprophytes constituted 3.13% of the total amount of bacteriobenthos, which is about one order of magnitude higher than the percentage of saprophytes in bacterioplankton in this part of the reservoir. The increase in the share of quickly growing saprophyte bacteria, which occur under high concentration of the substrates in the medium, testifies to the input of allochthonous microorganisms, as well as biogenic compounds and organic substances, which do not have enough time to degrade in the water column and settle onto the bottom, where they are intensely mineralized by sediment microbiocenosis.

In the process of analyzing year-to-year fluctuations in the number of saprophytes in the bottom sediment, the maximum value ($(8.26 \pm 22.36) \times 10^6$ colony-forming units per 1 ml) was recorded in 1999 (Fig. 1). This value largely exceeds the average ones obtained during other years of the research. In the same year, not only the number of saprophytes was maximum, but their relative concentration in the bacteriobenthos as well, which amounted to 0.199%. This is approximately one order of magnitude higher than during other years (figure). The minimum number of saprophytes was recorded in 1996, when it made, on the average, $(498 \pm 621) \times 10^3$ colony-forming units per 1 ml. In order to answer the question about the causes of the sharp increase in the number of saprophyte microorganisms in 1999 against the background of a decrease in the total amount of bacteriobenthos, it is necessary to continue microbiological monitoring and compare the obtained data with those on the scale and character of the Upper Volga water bodies contamination.

Table 4. Number of saprophyte microorganisms N_S, 10^3 colony-forming units per 1 ml, in the bottom sediments of the reservoirs

Observation period	Uglich Reservoir	Rybinsk Reservoir		Gorki Reservoir
		channel area	open part	
1992 (August)	–	–	$\frac{280\text{–}2700}{884 \pm 1038}$	$\frac{3\text{–}2100}{460 \pm 777}$
1995 (August–September)	$\frac{250\text{–}4500}{904 \pm 1163}$	$\frac{400\text{–}500}{450 \pm 71}$	3200	$\frac{20\text{–}4800}{1012 \pm 1532}$
1996 (June)	200	$\frac{200\text{–}800}{500 \pm 258}$	$\frac{10\text{–}1200}{533 \pm 586}$	$\frac{1200\text{–}2600}{1760 \pm 541}$
1996 (September)	$\frac{160\text{–}400}{287 \pm 121}$	$\frac{20\text{–}480}{250 \pm 325}$	70	$\frac{20\text{–}1600}{258 \pm 371}$
1997 (June–July)	400	$\frac{150\text{–}2600}{1270 \pm 1102}$	$\frac{500\text{–}1100}{833 \pm 306}$	$\frac{10\text{–}9300}{1069 \pm 2737}$
1997 (August)	$\frac{200\text{–}4000}{1960 \pm 1532}$	11900	$\frac{300\text{–}3000}{1222 \pm 818}$	$\frac{80\text{–}6300}{2120 \pm 2827}$
1997 (September)	$\frac{1400\text{–}2900}{2150 \pm 1061}$	$\frac{200\text{–}1600}{818 \pm 548}$	$\frac{500\text{–}1100}{800 \pm 300}$	$\frac{20\text{–}4300}{768 \pm 1169}$
1998 (October)	$\frac{150\text{–}500}{325 \pm 247}$	$\frac{30\text{–}1150}{316 \pm 556}$	$\frac{300\text{–}9700}{1785 \pm 2496}$	$\frac{50\text{–}1150}{554 \pm 361}$
1999 (July)	$\frac{22800\text{–}118550}{70675 \pm 67705}$	$\frac{420\text{–}27250}{7038 \pm 9310}$	$\frac{2200\text{–}6600}{3888 \pm 2085}$	$\frac{17\text{–}20330}{2088 \pm 586}$
For the whole observation period	$\frac{150\text{–}118550}{5875 \pm 22074}$	$\frac{20\text{–}27250}{2385 \pm 5136}$	$\frac{70\text{–}9700}{1529 \pm 1837}$	$\frac{3\text{–}20330}{992 \pm 2386}$

The intensity of dark assimilation of CO_2 is traditionally used as an integral index of plankton and benthos microorganisms activity [9]. In the bottom sediments of water bodies, CO_2 is fixed by aerobic and anaerobic, autotrophic and heterotrophic microorganisms. Among them, hemolithoautotrophic microorganisms, such as colorless, nitrifying, iron-oxidizing, manganese-oxidizing, knellgasbacteria, and other bacteria, use the energy of oxidation of reduced inorganic compounds (NH_4^+, NO_2^-, H_2, H_2S, $S_2O_3^-$, Fe^{2+}, Mn^{2+}) for assimilating CO_2 via reductive hexose monophosphate way or reductive cycle of tricarboxylic acids. Anaerobic methanogenic, homoacetogenic, and sulfate-reducing bacteria consume the energy released in the process of hydrogen oxidation by carbonic acid for assimilating CO_2 via the non-cyclic acetyl-CoA pathway. Finally, heterotrophic organisms involve CO_2 in the constructive exchange by means of the Wood–Verkman reaction in the cycle of tricarboxylic acids [27].

The intensity of dark assimilation of CO_2 in the upper layer of the Upper Volga reservoir bottom sediments varied within a broad range: from 31 to 2250 μg C/(l day). The most active benthic microorganisms fixed CO_2 in the Rybinsk Reservoir. In the open part of this reservoir, the average rate of this process amounted to 512 ± 482 μg C/(l day), whereas in the channel part of the Volga Reach, this value made 454 ± 18 μg C/(l day). The maximum intensity of the process under consideration was recorded in the gray silt, sampled in the near-dam area of the Rybinsk Reservoir near the village of Perebory. The rate of CO_2 fixation by microorganisms turned out to be lowest in the coarse-grained sand of the channel area of the Gorki Reservoir, where it averaged 231 ± 198 μg C/(l day). The specific activity of bacteria, calculated as the ratio of the intensity of CO_2 dark assimilation to the bacteria biomass, varied from 0.0008 to 0.0208 day^{-1} (on the average, 0.0079 ± 0.0051 day^{-1}). The activity of benthic microorganisms grew near the places of domestic effluents disposal and in the mouth areas of rivers flowing into the investigated water bodies, which can be explained by additional input of organic substrates and biogenic element compounds. Our data on the rate of CO_2 inclusion in the biomass of benthic organisms in the bottom sediments of the Upper Volga reservoirs was similar to the data obtained earlier by other researchers. Thus, in the Rybinsk Reservoir, the value of the parameter under consideration, averaged over the whole vegetation period, turned out to be 370 μg C/(l day). Depending on the sediment type, this

Table 5. Number of saprophyte microorganisms N_S, 10^3 colony-forming units per 1 ml, and the ratio of the number of saprophytes to the total number of bacteria N_S/N, %, in the bottom sediments of reservoirs near towns and settlements during different years of observations

Sampling site	1995		1996		1997		1998		1999		Mean	
	N_S	N_S/N	N_S	N_S/N	N_S	N_S/N	N_S	N_S/N	N_S	N_S/N	N_S	N_S/N
Priluki	800	0.0110	300	0.0030	1667	0.0103	150	0.0009	118550	3.1320	24293	0.6314
Uglich	500	0.0080	480	0.0047	1500	0.0139	30	0.0002	17410	0.3030	3984	0.0660
Myshkin	–	–	800	0.0078	4913	0.2963	1150	0.0141	1578	0.0480	2110	0.0916
Perebory	–	–	1200	0.0148	1033	0.0085	750	0.0036	2300	0.0048	1321	0.0079
Rybinsk, water intake	60	0.0029	360	0.0036	–	–	–	–	11750	0.0018	723	0.0029
Rybinsk	777	0.0116	680	0.0587	1833	0.0124	500	0.0079	4450	0.0690	1648	0.0319
Rybinsk, downstream of water treatment facility	–	–	110	0.0053	100	0.0038	–	–	–	–	105	0.0046
Tutaev, water intake	80	0.0063	110	0.0060	30	0.0007	400	0.0039	–	–	155	0.0043
Downstream of Tutaev	–	–	230	0.0085	45	0.0008	50	0.0009	63	0.0010	97	0.0028
Yaroslavl:												
central water intake	–	–	50	0.0023	40	0.0012	60	0.0015	36	0.0010	47	0.0015
within the city	300	0.0046	500	0.0122	1050	0.0068	950	0.0084	479	0.0050	656	0.0074
downstream of water treatment facility	2900	0.0497	650	0.0250	3185	0.0347	600	0.0051	20330	0.3760	5533	0.0981
Krasnyi Profintern, water intake	–	–	–	–	600	0.0039	450	0.0047	302	0.0060	451	0.0049
Krasnyi Profintern	90	0.0039	–	–	600	0.0102	1050	0.0059	472	0.0081	553	0.0070

value was maximum in the surface layer of bottom sediments and correlated with organic matter concentration in the sediment under consideration [11].

Consumption of CO_2 by bottom sediments is an important factor, largely determining the oxygen regime of the water body. Oxygen is consumed in the processes of benthic bacteria breathing, microbial and chemical oxidation of reduced compounds of Fe, Mn, S, and N, as well as CH_4 and H_2. In the bottom sediments of the Uglich, Rybinsk, and Gorki reservoirs, the rate of oxygen consumption, characterizing the intensity of organic matter mineralization, varied from 43 mg/(m^2 day) in coarse-grained sand in the channel sections to 970 mg/(m^2 day) in gray silt. Silty sediments with the prevalence of zebra mussel (sampled near Uglich, downstream of the Cheryomukha and Solonitsa rivers mouths), can be singled out as a special group in respect of the parameter under consideration. The rate of oxygen consumption by these sediments was very high, varying from 2444 to 2962 mg/(m^2 day). Here, the greater part of oxygen was consumed by breathing zebra mussel, which considerably intensified the oxygen consumption not only in the silt, but in the sand as well. Thus, the rate of O_2 consumption by sandy sediment, sampled downstream of the Nora River mouth and containing much zebra mussel, amounted to 703 mg/(m^2 day), which is comparable with the rate of this process in gray silt not inhabited by zebra mussel.

Results of studying numerous lakes and reservoirs in the European part of Russia, in the Ukraine, and in the Baltic countries have shown that, depending on the type of mixing and the trophic level of water bodies, the intensity of oxygen consumption by their bottom sediments varies within a broad range. This value is maximum (940 mg/m^2 day) in holomictic lakes and in the littoral zone of stratified mesotrophic and eutrophic lakes. During the period of stratification, aerobic processes cease in the lake profundal. Seasonal dynamics of oxygen consumption has one or two peaks. The first peak is related to warming of silt in spring and input of allochthonous organic matter with flood water. The second peak is related to the sedimentation of dead phytoplankton late in summer and early in autumn. In autumn, as the water temperature falls, the rate of destruction processes decreases. The main factors determining oxygen consumption intensity are water temperature and the concentration of readily-oxidizable fraction of organic substances. In the investigated water bodies, 60% to 100% of oxygen is consumed by benthic microorganisms [1]. In other bottom sediments, chemical consumption of oxygen can prevail [33].

CONCLUSION

In the 1990s, Russia faced a sharp decline in industrial and agricultural production, caused by an economic crisis. As a result, the volume of industrial effluents, atmospheric discharge of pollutants, and application of chemical fertilizers, weed- and pest-killers in agriculture largely reduced. However, due to the lack of financial resources in possession of industrial enterprises and municipal services, which could be used to maintain the normal operation of water treatment facilities (moreover, to introduce up-to-date nature conservation technologies), the input of insufficiently treated wastewater increased. Such effluents contain numerous microorganisms (including pathogenic ones) and various pollutants, which find their way in natural water bodies. The greater part of inflowing pollutants settle onto the bottom, thus intensifying oxygen consumption by benthic microorganisms and aerobic processes, entailing H_2S, NH_3, and CH_4 formation, in addition to the release of toxic reduced products of decay in the water column. These processes, in turn, lead to the formation of anaerobic zones, violation of links and changes in the structural and functional organization of benthos and plankton organism communities.

Analysis of bacterial communities found in the bottom sediments of the Uglich, Rybinsk, and Gorki reservoirs shows that microorganisms actively participate in the mineralization of pollutants, finding their way to water bodies with industrial and domestic effluents and overland flow. At the same time, zones of persistent human impact can be singled out near Uglich, Rybinsk, Tutaev, and Yaroslavl, the villages of Perebory and Krasnyi Profintern. In these zones, the concentration and activity of bacteriobenthos is much higher than in the reservoir areas, located far from the above towns and villages. Here, increased biomass and activity of bacterioplankton are also recorded [8]. All this suggests a persistent negative effect of the settlements mentioned above on the adjacent areas of the Upper Volga reservoirs. The fact that in some cases, such negative effect, revealed as a result of the analysis of benthic microorganism communities, also manifests itself near water intake facilities of cities and towns, causes special concern.

We are forced to state that microorganism communities found in the bottom sediments of the Upper Volga reservoirs and rivers flowing therein remain insufficiently studied. Owing to the fact that the functions of benthic microorganisms are diverse and important, the necessity of comprehensive analysis of their activities is self-evident. For practical purposes, it should be done to use the bacteriobenthos potential for *in-situ* bioremediation of persistently contaminated areas of water bodies. The high sensitivity of microorganisms to any changes in their habitat, the high growth rate, and abundance constitute prerequisites for their successful use in bioassaying and bioindication. However, till present, no clear-cut criteria of estimating the state of aquatic ecosystems according to bacteriobenthos inhabiting them have been developed. Unification of microbiological monitoring techniques is also a very important problem. It is also relevant to measure the rate of biogeochemical turnover of C, S, N, P, and metals in different water bodies (taking into account their seasonal and year-to-year dynamics), and study microorganisms implementing this circulation.

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