

Studying the Effect of Mineral Fertilizers on the Development of the Eutrophication Process in the Water Bodies

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ABSTRACT

The article is devoted to the disclosure of the effect on the vital activity of cyanobacteria by changes in the nutrients in the chemical composition of artificial aquatic biotopes. The research results allowed us to establish that the dissolution of complex mineral fertilizer (nitrogen, phosphorus and potassium) in the range of up to 10 g/L contributes to the active development of cyanobacteria, which is accompanied by complete binding of mineral additives and absorption of oxygen. The addition of fertilizers in the amount of more than 1 g/L contributes to a sharp increase in the growth rate of the cyanobacteria population, which provokes the conditions for the blooming of the solutions. Enrichment of the nutritive base of biotopes ensures rapid growth of biocenosis and accelerated consumption of dissolved substances. The recorded average absorption rate of phosphorus reached more than 17 g/(L·day), and nitrogen – 30 g/(L·day). The formation of sediment and foam in the test vessels was associated with the depletion of nutrients and the death of hydrobionts. This phenomenon can serve as an indicator of oxygen decrease in the water. The results of the experiment confirmed the ability of cyanobacteria to transform nitrogen and phosphorus dissolved in water into bound organic forms with high intensity. This opens up the prospect of creating biological methods and means of wastewater demineralization.

Keywords: mineral fertilizers, cyanobacteria, biocenosis, artificial biotope, eutrophication.

INTRODUCTION

Anthropogenic activity is always accompanied by the occurrence of negative changes in the environment. The intensification of agriculture and the uncontrolled use of mineral fertilizers led to the ingress of nitrogen and phosphorus compounds into the water bodies along with the surface runoff (up to 75% of all biogenic elements entering water sources are from agriculture) (Marmen et al., 2020; Michalak et al., 2013; Padedda et al., 2017). Although nitrogen and phosphorus are considered

important biogenic elements in the aquatic ecosystem, their excess causes the active development of cyanobacteria and a decrease in the number of green algae that produce oxygen. This leads to the occurrence of oxygen deficiency, and as a result, there is a violation of the ecological balance and deterioration of the conditions for the existence of hydrobionts in small rivers and water bodies (Kostenko et al., 2018). Therefore, the issue of studying the parameters of effect of mineral fertilizers on the changes in hydrophysical and hydrobiological indicators of the water bodies remains relevant.

Cyanobacterial blooming is a widespread phenomenon throughout the world, which is conditioned by the interaction of geographically and ecologically diverse environmental variables. The long evolutionary history of blooming types of cyanobacteria has dictated a high degree of tolerance and adaptability to both short-term (i.e., seasonal, decadal) and long-term (geological) environmental changes.

The main biomass of the pathogenic microflora of the water bodies is concentrated in the 'blooming spots'. Therefore, in addition to disturbing the environmental balance of the water body, the blooming of some algae has many medical, economic and social consequences (Malyovanyy et al., 2015, a).

Several common genera of cyanobacteria have the ability to produce toxins called cyanotoxins that affect people, livestock, domestic animals, and wildlife. Algae density, genetic potential and environmental factors have an impact on the process of toxin production: water temperature, light intensity, water hydrogen index (pH), wind characteristics (Matthews et al., 2015; Stumpf et al., 2012). Interactions between aquatic organisms, such as predation and competition for nutrients, are also important. Toxin production is more common in summer, but can occur at any time of the year (Beck et al., 2016). These toxins are hepatotoxic, neurotoxic, dermatotoxic, cytotoxic, inflammatory and irritating (Akedrin et al., 2017). Animals can be exposed to cyanotoxins through drinking or contact with water in contaminated lakes and ponds. People are most often exposed to cyanotoxins while swimming and boating in contaminated waters.

Furthermore, the increased development of blue-green algae, which causes the 'blooming' of water with the accumulation of excess biomass, creates technical difficulties when supplying water to the municipal water supply network, worsens its chemical composition and sanitary indicators. As a result, it becomes impossible to treat river water to the drinking water standards at the primary treatment stations of water intakes of coastal cities (Malyovanyy et al., 2015, b).

Despite the fact that eutrophication disrupts the environmental conditions of water systems and reduces biological diversity, coincidentally, the total amount of phytoplankton in the water bodies is a good biological indicator of the trophic state, since algae are ubiquitous, numerous and quickly respond to changes in the environment and in ecosystem.

Under natural conditions in aquatic ecosystems, there is a balance between cyanobacteria and other groups of phytoplankton (Matthews et al., 2013). However, specific factors contribute to the spread of cyanobacteria. They are defined by a number of peculiarities, including cell physiology (gas bubbles in cells allow buoyancy regulation), physiological response (e.g., light and nutrient utilization), cell size, cell structure, and overall morphology. The predominance of cyanobacteria over other species occurs under certain environmental conditions, including optimal light intensity and water temperature, availability of nutrients, and stability in the water column (Celikkol et al., 2021). Anthropogenic activity, in the form of intensive industrial, domestic and agricultural and industrial runoff, leads, primarily, to significant changes in the chemical composition of the aquatic environment as a biotope for hydrobionts.

From this perspective, it is important to determine how a change in the parameters of aquatic ecosystems affects the nature of the existence of cyanobacteria. In this regard, there are good reasons to investigate how the development of phytoplankton, in particular cyanobacteria, is affected by changes in the concentration of nitrogen and phosphorus, the main nutrient compounds for cyanobacteria, as well as the concentration of oxygen dissolved in water.

The purpose of the work is to disclose the effect on the vital activity of cyanobacteria by changes in the nutrients in the chemical composition of artificial aquatic biotopes.

The subject of the research is to establish the regularities of the process of reproduction of cyanobacteria depending on the presence of the main types of nutrient substances using the example of complex mineral fertilizer.

The object of the research is the effect of the concentration of nutrients in an artificial biotope on the reproduction of cyanobacteria at a constant temperature and limited natural lighting and gas exchange.

MATERIALS AND METHODS

The methods used in the research generally included experimental methods based on laboratory microscopic studies of indicators of the number of cyanobacteria, and test methods to study the effect of nitrogen and phosphorus concentration in the water environment. Furthermore,

visual observations of the colour grade of the solutions and the presence of suspended matter and sediment in the vessels were carried out.

To conduct the experiment under stable weather conditions, water with a natural concentration of hydrobionts and the following parameters was taken from the Nuliovka pond, Pokrovsk: temperature – 24 °C, concentration of dissolved oxygen – 5 mg/L, background concentration of phosphates – 0.8 mg/L, that of nitrates – 5 mg/L. Given that the reproduction and activity of cyanobacteria directly depends on the water temperature, the research was conducted in the summer, when the temperature in the laboratory was in the range of 24 to 28 °C in order to ensure stable results. The lighting of the room was natural through the windows, which limited the process of photosynthesis. Gas exchange took place through the free surface of the water in the vessels.

As a chemical additive, one of the most popular brands of fertilizers currently used in the agricultural and industrial complex was chosen, namely “Master Agro”, which contains 21% nitrogen, 12% phosphorus and 21% potassium.

At the first stage of research, the ‘critical’ level of fertilizers was determined, i.e., the concentration at which the death of all representatives of oxygen-consuming hydrobionts is observed. For this reason, seven samples of the solutions with a volume of one litre were prepared from the selected water, with the addition of the mineral fertilizers in a concentration of up to 100 g/L.

Taking into account the obtained primary data, at the second stage of the experiment, seven one-litre samples of the solutions with the concentration of added fertilizers, g/L, were prepared from the natural water selected for the research: No. 1 – 0 (control); No. 2 – 0.1; No. 3 – 0.5; No. 4 – 1.0; No. 5 – 3.0; No. 6 – 5.0; No. 7 – 8.0.

With the help of a binocular microscope XSP-128B LED with a Levenhuk M1000 PLUS attachment and a standard counting chamber, the number of representatives of algae was measured.

Counting the number of cyanobacteria was carried out according to Yehorov method, which is described below. The solution was stirred with a glass rod, carefully so as not to damage the algae, and a sample of the test water was placed in the counting chamber. Next, those cyanobacteria located in the upper and left part of the cell were counted. Cyanobacteria located on the right and lower border of the cell were disregarded when counting. To reduce the statistical error, the number

of algae in the water was counted 10 times; the water samples from the vessel were taken using a pipette from different parts of the beaker.

The water temperature in the test samples during the experiment was in the range of 23 to 25 °C.

The concentration of oxygen in the test samples, as well as the concentration of phosphates and nitrates, was determined by the laboratory test method using the “Rikka test PO₄” and “Rikka test NO₃”.

RESULTS AND DISCUSSION

As a result of the first stage of the experiment on the assessment of the so-called ‘critical’ level of fertilizers, it was established that in the samples of the solutions with a concentration of fertilizers up to 10 g/L, the death of hydrobionts almost did not occur; at 10 to 20 g/L, a decrease in the number of hydrobionts was observed up to 50 to 70%, while the level of fertilizers over 30 g/L turned out to be ‘critical’. Thus, for further research, it was accepted to use initial solutions where the total level of nutrients does not exceed 10 g/L, i.e., the initial concentration in the vessels is up to 10%.

At the beginning of the experiment, the background amount of cyanobacteria in the natural water samples was determined, which was about $2.3 \cdot 10^6$ units/L.

The growth of both representatives of cyanobacteria (Fig. 1) and green algae was observed in the test samples.

Counting of the number of cyanobacteria in the test solutions was carried out on the second, fifth and eighth week of observations. As an example, images, at a tenfold magnification, of the evolution of algae in sample No. 6 with an initial fertilizer concentration of 5 g/L over a period of eight weeks are presented in Figure 2.

The observations carried out during the specified period showed that the presence of nutritional additives in general has a positive effect on the reproduction of cyanobacteria, and when the nutritional resources are depleted, their number decreases (Table 1). Thus, in the control vessel, without fertilization doses, cyanobacteria began to disappear, and at the end of the observations, they were four times less. In the presence of small fertilization doses (0.1 to 0.5 g/L), the process of population reduction slowed down in the second to fifth weeks (samples No. 2 and No. 3).

The addition of fertilizers in the concentration of about 1 g/L (sample No. 4) ensured the stable existence of cyanobacteria at an almost constant level during the observation period. However, the addition of fertilizers in the range of 3 to 5 g/L (samples No. 5 and No. 6) contributed to a

significant increase in the number of bacteria relative to the initial level by approximately 3.5 to 4.5 times in the second week of observations, and by 1.3 to 3.2 – in the eighth week. The addition of higher doses of fertilizers close to the ‘critical’ level (sample No. 7) led to a slight decrease in

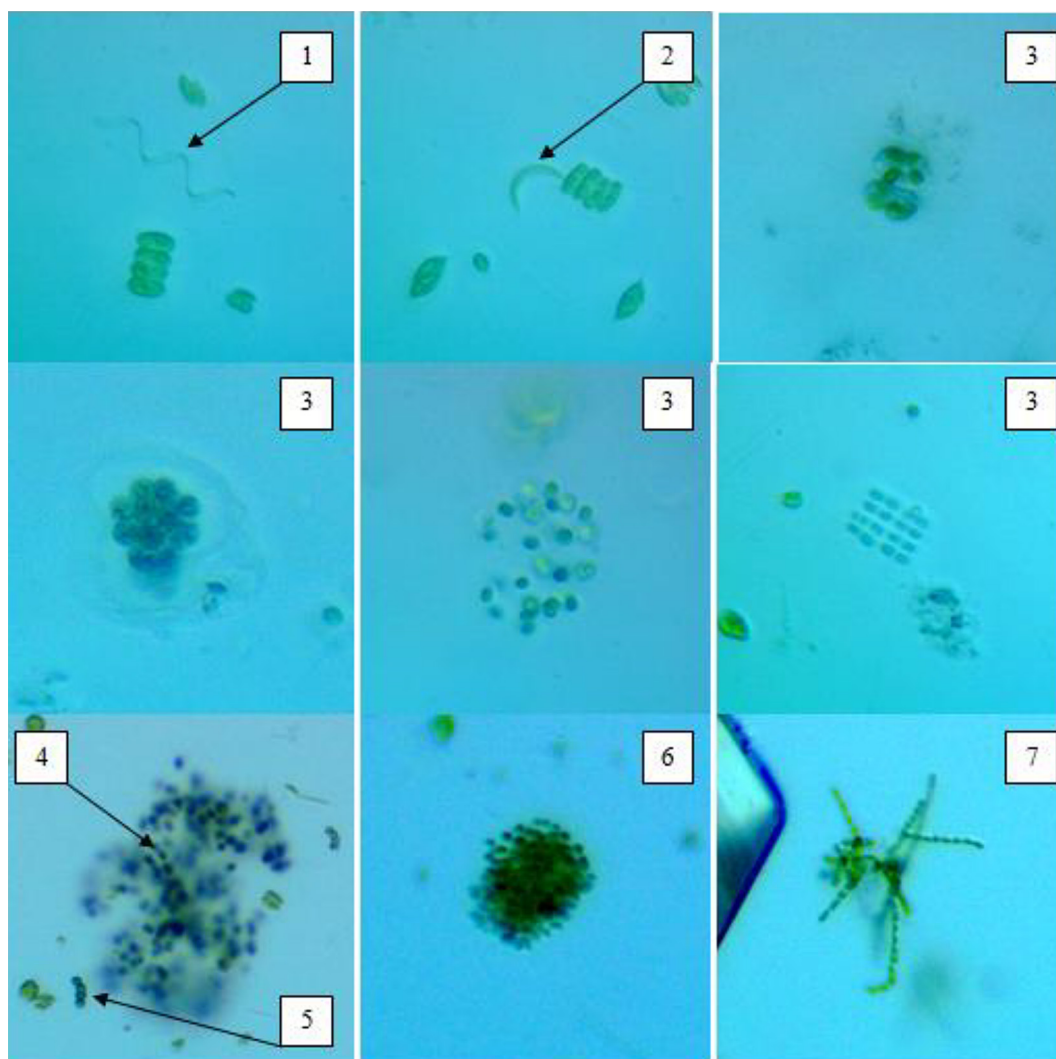


Figure 1. Cyanobacteria of the genus *Oscillatoriales* (1), *Dactylococcopsis raphides* (2), *Chroococcales* (3), *Microcystis aeruginosa* (4), *Nostoc* (5), *Pleurocapsales* (6), *Stigonematales* (7) in the original water

Table 1. Effect of added fertilizers on the dynamics of the number of cyanobacteria

Sample No.	Added fertilizers, g/L	Number of cyanobacteria, units/L·10 ⁶ % (prior to background)			
		Background	Week of observations		
			Second	Fifth	Eighth
1	-	2.3/100	2.5/108	1.5/65	0.6/26
2	0.1		2.7/117	2.8/122	0.4/17
3	0.5		2.9/126	1.1/48	0.5/22
4	1.0		2.4/104	2.9/126	2.7/117
5	3.0		2.6/113	8.4/365	3.1/134
6	5.0		3.1/135	10.3/448	7.5/326
7	8.0		1.9/83	7.6/330	1.3/56

the number of cyanobacteria in the second week, then, in the fifth week, to a short-term threefold increase in the number, and in the subsequent decrease to about two times relative to the background level.

In the eighth week of research, the concentration of phosphates and nitrates in the experimental samples, as well as the concentration of oxygen, were measured by the test method using the “Rikka test PO₄” and “Rikka test NO₃” (Table 2).

During the entire period of the experiment, visual control of changes in the colour of the experimental solutions, the presence of sediment, as well as the appearance of films or bubbles on the surface was carried out. The results of this kind of processes are shown in Table 3.

The idea of the project was to study the development of the cyanobacterial part of the biocenosis in artificially created biotopes with a dosed addition of nutrients, namely nitrogen and phosphorus, at a constant temperature and limited lighting and gas exchange.

The first stage of research was devoted to establishing the ‘critical’ level of nitrogen and phosphorus in the water environment, where the existence of cyanobacteria is impossible. It was established that the presence of complex mineral fertilizer additives in concentrations of more than 10 g/L accelerates the death of cyanobacteria through intensive consumption of oxygen dissolved in the liquid and the formation of a toxic anaerobic environment. A concentration of more

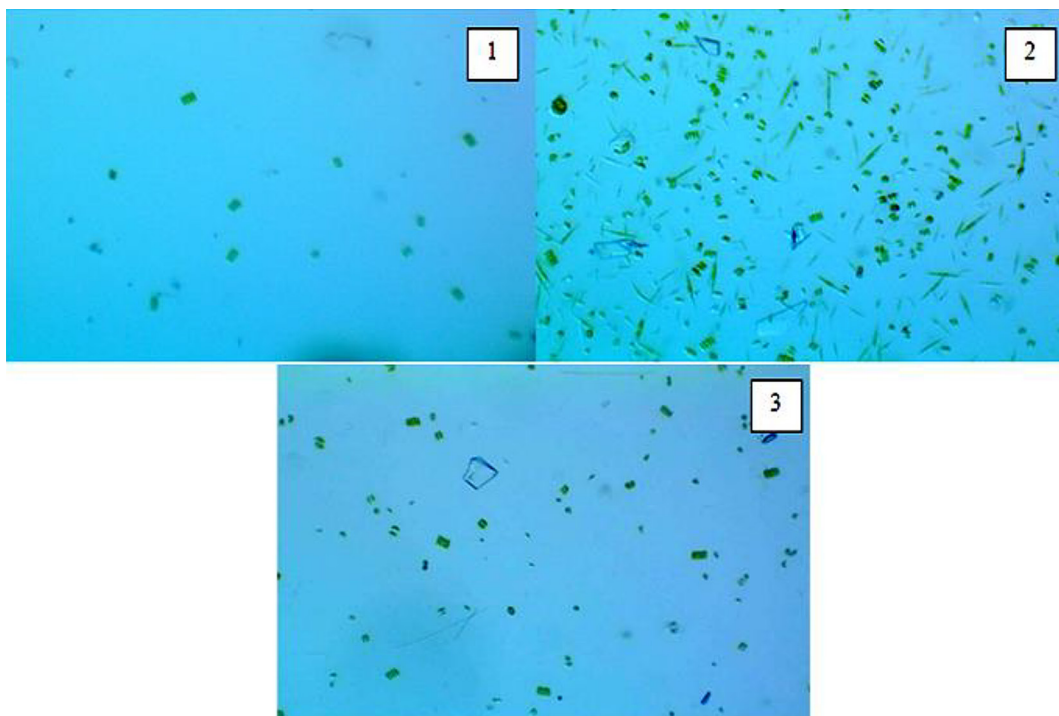


Figure 2. Presence of hydrobionts in sample No. 6 during the second (1), fifth (2) and eighth (3) weeks of the experiment

Table 2. Results of measurement of concentrations of phosphates, nitrates and dissolved oxygen in the samples

Sample No.	Concentrations, mg/L								
	Background			After adding fertilizers			Eighth week		
	PO ₄	NO ₃	O ₂	PO ₄	NO ₃	O ₂	PO ₄	NO ₃	O ₂
1	0.8	5	5	0.8	5	5	>2	5	8
2				12.8	26		2	10	8
3				60.8	110		1.5	100	<2
4				120.8	215		0.8	>50	2
5				360.8	635		0.1	20	<1
6				600.8	1055		<0.05	2	0
7				960.8	1685		<0.05	0	0

than 30 g/L determines the conditions for an avalanche-like complete disappearance of cyanobacteria, lasting several days. This made it possible to choose for further research artificial biotopes where there are initial conditions for the life of

aerobes, i.e., those containing no more than 10 g/L of mineral fertilizers.

Based on the results of microscopic studies (see Table 1), a graph of the dynamics of the presence of cyanobacteria was plotted depending on

Table 3. Visual changes in the solutions and sediments in the test vessels

Sample No.	Fertilizer concentration, g/L	Week of observations								
		Second			Fifth			Eighth		
		Colour	Sediment	Surface condition	Colour	Sediment	Surface condition	Colour	Sediment	Surface condition
1	-	Transparent, green shade	-	-	Transparent	Traces	-	Transparent	Thin, brown	-
2	0.1	Same	-	-	Same	Thin, brown	-	Same	Same	White film
3	0.5	-«»-	-	-	-«»-	Thin, green	-	-«»-	Thin, green	Same
4	1	-«»-	-	-	Transparent, green shade	Same	-	-«»-	Same	Green bubbles
5	3	-«»-	-	-	Same	-«»-	-	-«»-	Same	Same
6	5	-«»-	-	-	Turbid, green shade	-«»-	-	Turbid.	Green	Fine green with bubbles
7	8	-«»-	-	-	Same	-«»-	Thin film	Same	Thick green	Green with bubbles

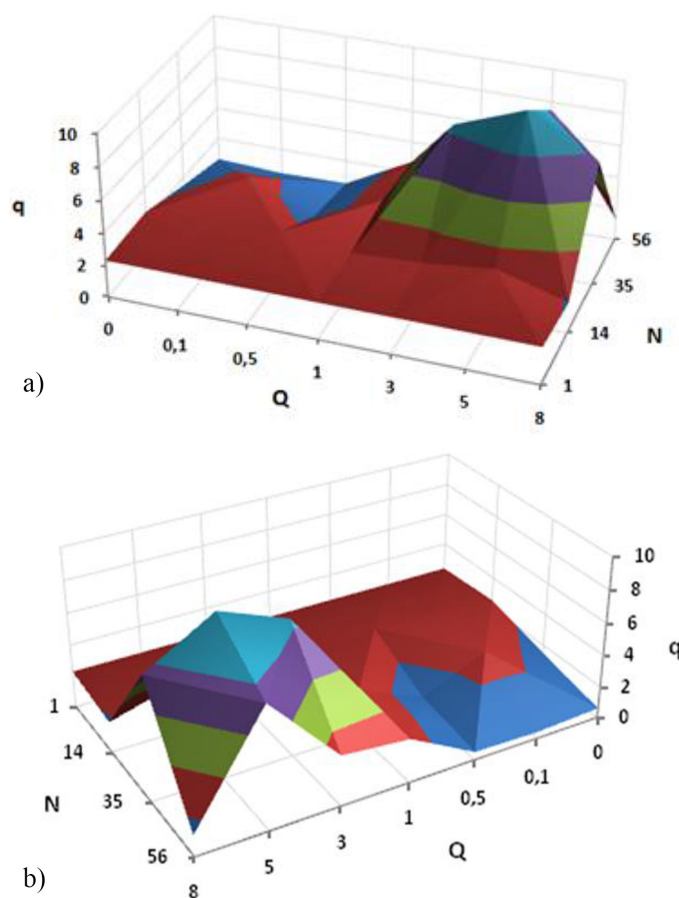


Figure 3. Effect of the addition of mineral fertilizers on the dynamics of the number of cyanobacteria (a) projection of the graph with an abundance of nutrient substances; (b) in the depletion conditions, q – number of cyanobacteria, unit/L·10⁶; Q – initial fertilizer concentration, g/L; N – duration of observations, days

the amount of added mineral fertilizers (Fig. 3). It clearly shows that the life process of cyanobacteria is not simple; its main components, according to logic, are the consumption of nutrient substances and oxygen, as well as the competition with other types of hydrobionts. Upon depletion of nutrient solutions, hydrobionts die and sediment is formed, followed by decomposition of organic residues with the formation of gases and flotation of foam-like particles.

At the beginning of the experiment, a uniform growth of algae was observed in all samples, but in the fifth week of observations, a significant acceleration occurred in samples No. 5, No. 6, and No. 7 with the concentration of fertilizers, g/L: 3, 5 and 8, respectively.

In samples No. 1 and No. 2, where the concentration of nutrients for cyanobacteria is minimal, the oxygen concentration increases during the research period from 5 mg/L to 8 mg/L (see Table 2); this is due to the growth of mainly green algae that photosynthesize oxygen in the process of vital activity. The limitation of the phosphorus and nitrogen nutritive base led to a decrease in the number of cyanobacteria at the end of the experiment by 4 to 6 times relative to the background. However, thanks to the availability of light and nutrients sufficient for them, algae begin to develop rapidly, as evidenced by the appearance of brown sediment in vessels No. 1, No. 2. The remains of diatom algae prevail in the composition of the dark sediment. Decomposition of dead algae led to the formation of white foam in the eighth week (see Table 3).

Addition of fertilizers in the concentration of more than 1 g/L contributes to a sharp increase in the growth rate of the cyanobacterium population, which provokes the conditions for the blooming of the solutions. A richer biotope provides a rapid increase in the number of bacteria several times, e.g., No. 5 and No. 6. However, their vigorous activity requires significant amounts of fertilizers and oxygen, and after the biotope resources are depleted, after the fifth week, the number of cyanobacteria decreases sharply. This is confirmed by the practical absence of phosphorus, nitrogen and oxygen in the solutions in the eighth week (see Table 2). Dissolved elements were spent on the construction of bacteria and algae cells, i.e., they went from a liquid state to a bound (colloidal or quasi-solid) state. The presence of a significant mass of dead hydrobionts when nutrients were depleted led to the formation of a rather significant green sediment and turbidity of the solutions.

Oxygen supply from the free surface of the solutions and photosynthesis were limited. Oxygen dissolved in water was used mainly for the nutrition of cyanobacteria. In samples No. 3 and No. 4, the final oxygen concentration decreases to a level of no more than 2 mg/L, and in sample No. 5 – <1 mg/L. In samples No. 6 and No. 7, oxygen is not observed at all; this is explained by the fact that representatives of cyanobacteria actively spread and consumed all the oxygen and most of the fertilizers.

At first glance, it seems that in sample No. 7 the development of cyanobacteria occurred less intensively than in No. 5 and No. 6, but the absence of fertilizers and oxygen in the solution in the eighth week, and also earlier: turbidity of the solution, the formation of a large green sediment and foam with bubbles indicate the presence of a violent process of consumption of fertilizers and oxygen in a period shorter than the interval of observations.

The average absorption rate of fertilizers and nitrogen in the test vessels according to the results of the experiments varied significantly as the concentration increased from 0.19 to 17.14 g/(L·day) for phosphorus, and from 0.28 to 30.0 g/(L·day) for nitrogen, i.e., it was proportional to the number of biocenosis. In those samples (No. 5 - No. 7) where the life process of the cyanobacterium was approaching the end, i.e., the oxygen concentration in the solutions was approaching zero, the ratio of absorbed phosphorus and nitrogen was approximately the same as that of the added dose of fertilizer. It further follows that the biocenosis consumed almost all the nutrient resources of the biotope. Unfortunately, the interval of observations was short, which should be noted as a drawback of the methodology. This conditioned the fact that numerical and time indicators were obtained only in the first approximation.

The final solutions were devoid of nitrogen and phosphorus, i.e., a certain demineralization of water took place. This means that there is an opportunity to provide biological treatment, e.g., of runoff from agricultural fields that contain fertilizers. To effectively prevent the development of anaerobes, there are good reasons to enrich the demineralized water with oxygen, e.g., with the help of aerators for shallow water bodies (Tavrel et al., 2022; Kostenko et al., 2022) The sediment and foam formed during this process contain concentrated phosphorus and nitrogen compounds that can serve as a semi-finished product for the manufacture of fertilizers.

CONCLUSIONS

The conducted experiment to assess the vital activity of the cyanobacterial biocenosis in an artificial biotope with a dosed addition of nutrient substances made it possible to obtain the following conclusions.

The dissolution of complex mineral fertilizer (nitrogen, phosphorus and potassium) in the range of 0 to 30 g/L in water allows the cyanobacterial biocenosis to exist in it.

In the range of up to 10 g/L, the addition of fertilizer contributes to the active development of cyanobacteria, which is accompanied by complete binding of mineral additives and absorption of oxygen. Depletion of nutrient resources leads to the impoverishment of the biocenosis and the formation of an anaerobic environment.

Addition of fertilizers in the concentration of more than 1 g/L contributes to a sharp increase in the growth rate of the cyanobacterium population, which provokes the conditions for the blooming of the solutions.

Enrichment of the nutritive base of biotopes ensures rapid growth of biocenosis and accelerated consumption of dissolved substances. The recorded average absorption rate of phosphorus reached more than 17 g/(L·day), and nitrogen – 30 g/(L·day).

The formation of sediment and foam in the test vessels was associated with the depletion of nutrients and the death of hydrobionts. This phenomenon can serve as an indicator of oxygen decrease in the water.

The results of the experiment confirmed the ability of cyanobacteria to transform nitrogen and phosphorus dissolved in water into bound organic forms with high intensity. This opens up the prospect of creating biological methods and means of wastewater demineralization.

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