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The Influence of Abiotic Factors on Organisms-Hydrobionts of Activated Sludge

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ABSTRACT

Rational use of water resources is one of the urgent problems for arid regions of Kazakhstan. The biocenosis of activated sludge of urban wastewater treatment plants in Zhanaozen periodically encounters stressful situations associated with violation of the operating mode of equipment or emergency discharges of toxicants into the wastewater treatment plant system. It has been established that with sharp fluctuations in the physicochemical parameters of aqueous solutions, protozoal organisms are primarily eliminated from the composition of activated sludge. This pattern was noted with an increase in the content of ammonium nitrogen, phosphorus, monoethanolamine and pH fluctuations in wastewater. Under the conditions of a 10-month period of active insolation in Western Kazakhstan, the species diversity of the algoflora of activated sludge correlates with the duration of daylight hours; it was found that diatoms predominate in the autumn-winter period of the year, whereas blue-green and green algae predominate in the spring-summer period.

Keywords: activated sludge, hydrobiont organisms, pH, light mode, ammonium nitrogen, phosphorus, monoethanolamine.

INTRODUCTION

Serious attention of specialists around the world is attracted by the problem of quantitative and qualitative depletion of water resources, determined by the continuous growth of water consumption in industry, agriculture and everyday life (quantitative depletion) as well as water pollution (qualitative depletion). The analysis of the information showed that most of the inventions are related to the improvement of existing technological purification schemes, where innovations are introduced in the form of more productive structures or devices (Raouf et al., 2019), such as nanofiltration methods (Shon et al., 2013), nanocompositions (Deshmukh et al., 2023) or the use of various gels (Zhao, He, 2022). It is known that the efficiency of physico-chemical wastewater treatment increases significantly when using the biological treatment stage. For biological wastewater treatment, aerotanks, biotanks, biological ponds,biocoagulants (Issayeva et al., 2022) etc. are used. Biological or biochemical purification is one of the main methods of wastewater treatment of industrial enterprises, both before their discharge them into a reservoir and before reuse in recycling water supply systems (Rashed et al., 2022). This process is essentially natural, and its nature is the same for processes occurring in a natural reservoir and in wastewater treatment plants. Biological oxidation is carried out by a community of microorganisms (biocenosis),

including many different bacteria, protozoa and a number of more highly organized organisms – algae, fungi, etc., interconnected by complex interactions (Bouras et al., 2019; Mahesh et al., 2021). The main role in this community belongs to bacteria, the following ecological groups of microorganisms have been identified in the microflora of treatment facilities: obligate, oxytolerant, facultative aerobes; obligate and facultative halophiles; mesophiles, tolerant thermophiles (Bian et al., 2015; Islas-García et al., 2017; Msanne et al., 2020). Microorganisms are able to use hydrocarbons of different classes of simple and complex structure (Gao et al., 2023). The parameters of the operation of aerotanks (the load of waste fluid, air supply, the dose of activated sludge, etc.) can be adjusted, thereby providing conditions for cleaning. The active sludge is an amphoteric colloidal system with a negative charge at pH = 4–9.The aeration tank is home to many algae that positively affect the sanitary condition of reservoirs and secrete biologically active metabolites (Bhandari et al., 2023; Raj et al., 2023). In biological wastewater treatment, the algae species that can multiply rapidly in polluted water and cause water blooming in a short time are of great importance (Gil-Izquierdo et al., 2021; Vladić et al., 2023). During photosynthesis of algae, a very large amount of oxygen is released, and aerobic conditions are created in ponds in which oxidative processes occur rapidly (Mathias et al., 2023, Chitthaluri et al., 2023). Algae are also important as indicators of the state of water resources (Pérez-Morales et al., 2023). Quantitative patterns of formation of the activated sludge ecosystem are dictated mainly by the technological mode of operation of aerotanks. As a rule, the total number of microorganisms is directly proportional to their oxidizing capacities, which is important for low-waste industries, especially for rural areas (Ibarruri, Hernández, 2021; López-Sánchez et al., 2022; Vishwakarma et al., 2022), where various devices can be used to collect algae biomass (Wang et al., 2022). The absence or insufficiently complete averaging of wastewater before it enters the aeration tank causes a complex of disturbing factors that negatively affect the efficiency of oxidation of organic pollutants in the aeration tanks. Salvo discharges of toxic substances lead to a complete degeneration of activated sludge and changes in its morphological and physical properties, as under natural conditions (Wang et al., 2023). With prolonged disturbing effects, an overload of activated sludge occurs, the inertial capacity of the ecosystem is exhausted, which manifests itself in a sharp violation of the oxidizing ability of activated sludge, a change in its physical and morphological properties, the destruction of zooglue structures, and a sharp deterioration in sanitary and chemical indicators. The composition of activated sludge also changes, protozoa and rotifers disappear, a mass of free-floating bacteria appears, zooglues of activated sludge acquire a loose structure. Under the influence of abiotic factors, the morphogenesis of individual algae species changes (Stablein et al., 2021; Kumari et al., 2022).

In general, according to a number of morpho-physiological characteristics of activated sludge, it is possible to judge the level of efficiency of biological wastewater treatment. The growth and development of activated sludge organisms are significantly influenced by factors such as the concentration of chemical compounds, the content of dissolved oxygen, the active acidity of the medium and temperature. Exceeding the MPC for these indicators can lead to the inhibition or death of activated sludge organisms and, as a result, to a decrease in the efficiency of biological wastewater treatment. In this regard, the aim of the study was to study the reaction of activated sludge from biological wastewater treatment plants to stressful situations occurring at the treatment facilities of Zhanaozen.

MATERIAL AND METHODS

The material was the active sludge of the biological treatment plant of the municipal wastewater treatment plants of the city of Zhanaozen in Western Kazakhstan. Optimal conditions for the vital activity of activated sludge are as follows: dissolved oxygen at least 4.0 g/l, temperature +10 +25°C, pH of the active reaction 6.5–7.0, the dose of sludge on the dry residue from 0.5 to 1.0 g/l. Urban wastewater treatment plants have an estimated capacity of 21,500 ^{m3}/day and were inaugurated in 1982, but today the equipment is worn out in 60–70%. Currently, 5700 m^3 is received per day, the average volume of wastewater after the purification process is 5160 m^3.

The determination of water temperature and pH was carried out according to State standard (SS) 24902-81, chloride content according to SS 26449.1-85, nitrite and nitrate content according

to SS 33045-2014, dry residue content according to SS 18164-72, sulfate content according to SS 31940-2012, phosphate content according to SS 18309-2014, ammonia content according to SS 33045-2014, petroleum products content according to GSI RK 07.00.01667-2017, surfactants – according to GSI RK 07.00.02007-2014, COD –according to ST RK 1322-2005.

Statistical analysis of the research results was carried out by calculating the arithmetic mean and the value of the standard deviation according to the Student's table. All definitions were carried out in 3-fold repetition. The data was processed using an IBM Pentium personal computer based on Excel application software packages.

RESULTS AND DISCUSSION

Statistical analysis of the average monthly data on the chemical composition of wastewater in general, the degree of wastewater treatment ranges from 85.0–92.2%. A high degree of purification was noted for synthetic surfactants –98.0–99.5%. The operation of treatment facilities is considered normal and efficient if the quantity and quality of wastewater meets the requirements of temporary permitted discharge (TPD).

Microbiological examination of samples showed that the qualitative composition of the wastewater microflora is represented by heterotrophic lactose-positive microorganisms. Autotrophic microflora consists of sulfate-reducing microorganisms. The degree of nitrification varies depending on the state of operation of treatment facilities. The averaged data of wastewater discharge into the storage tank show that, the average annual degree of purification for all constituent ingredients corresponds to the TPD parameters, despite slight annual fluctuations in the main indicators.

However, a more detailed analysis of the quality of wastewater, both quarterly and monthly, shows that there are cases of exceeding the MPC and TPD for such indicators as ammonium nitrogen, nitrates, petroleum products, suspended solids. The reasons for this include both salvo, emergency discharges of enterprises, and the unstable flow of wastewater from a number of other enterprises, the wastewater composition of which stabilizes the overall chemical composition of municipal wastewater. Both the first and the second reason can almost equally affect the wastewater treatment process, due to the fact that in the first case, there is an excess concentration of toxic ingredients, in the second case, there is an imbalance in the ratio of biogenic elements K: N: P. The consequence of such changes may be a violation of trophic bonds in the biocenosis of activated sludge at the stage of biological purification.

Microscopy of activated sludge during the period of a well-functioning aeration tank showed the presence of a complex biocenosis of various organisms, colorless flagellate organisms prevailed, amoebas and infusoria were singly noted. The following representatives belong to the *Ciliophora class: Litonotuscignus, L. fasciola, Colpodacolpidium, Epistilis, Vorticella convalaria, V. microstoma, V. alba, V.companulla, Vaginicotastriata, Thuricolasp, Chaetospirasp, Oxytrichafallax, Aspidiscaturrita, Tokophryalemnarum*. The group of amoeba *Amoebaradiosa, Arcelladiscoedes* was found only with good performance of aerotanks with adjusted nitrification. Algoflora is represented by diatoms: *Naviculagracilis, Navicula sp., Nitschiasp, Pinnulariaviridis, P. appendiculata*; green algae species: *Spirogyraporticalis, Ulothrixtenerrima* as well as euglenic algae *Phacuspleuronectes*, *Euglena intermedia* and *E. acus* are found in large numbers. The presence of rotifers, small-scale worms, coelenterates and copepods in significant quantities in the biocenosis is an indicator of the effectiveness of purification processes and the presence in the water of a large amount of dissolved oxygen necessary for the bio-oxidation of petroleum products by hydrobionts. The species diversity of bacterial flora is also directly dependent on the chemical composition of wastewater. Microbiological examination of wastewater samples showed that the leading place in the composition of microbial cenosis is occupied by bacteria pp. *Pseudomonas sp., Micrococcus roseus, M. varians, M.luteus, Rhodococcus sp*., the number of which ranges from 10 kl/ml at the entrance to the treatment facilities to 10^4-10^5CFU /ml in the aeration tank, reaching a maximum at the exit from the aeration tanks into the radial settling tanks – 10^7-10^8CFU/ ml. The degree of wastewater treatment in such an aerotank meets the design requirements and is at the level of 85.0 ± 8.0%.

However, this level of wastewater treatment is not always maintained. Very often, due to various errors in the operating mode of the biological treatment plant, changes occur in the composition of the biocenosis of activated sludge in aerotanks

and, as a result, fluctuations in the chemical composition of the treated wastewater. The reasons for deviations from the optimal mode of biological purification can be divided into two groups:

1. Influent wastewater is unfavorable for biological treatment, i.e. it can be toxic, unbalanced in terms of nutrition elements, unevenly distributed among biogenic elements.
2. Violations in the operation of wastewater treatment plants as errors in the aeration regime (which was especially common in 1995-2005, due to problems with the power supply of cities in Kazakhstan) or untimely unloading of settled sludge in aerotanks.

When studying the influence of abiotic factors on the hydrobiological composition of activated sludge in the wastewater of the aerotanks of urban wastewater treatment plants in Zhanaozen, the main attention was paid to the facts that create a stressful situation under real production conditions: the influence of light, aeration, changes in the concentration of phosphates and ammonium, fluctuations in pH, changes in the concentration of monoethanolamine on the vital activity of the biocenosis of activated sludge in the aerotanks of the wastewater treatment plant structures. Due to the fact that methods of wastewater treatment by ozonation and ultraviolet irradiation were tested at the enterprise to solve the problem of recycled water use, studies were conducted on the influence of these factors on hydrobiont organisms.

Problems for the normal operation of aerotanks were created by power outages, as a result of which factors such as light and aeration could not correspond to the parameters necessary for the normal functioning of the biocenosis of activated sludge. An experiment was carried out in the laboratory, where situations were simulated: natural lighting (during daylight hours), artificial lighting

Table 1. Results of laboratory experiments to study the effect of light on the hydrobiological composition of activated sludge of aerotanks

Types of organisms	Natural lighting	Round-the-clock lighting	No light
Aspidiscacostata	+	+	–
Glaucoma scintillans	+	–	–
Colepshirtus	+	+	–
Litonotuscignus	+	–	–
Pleuronemacoronatum	+	–	–
Tachisomapelionella	+	–	–
Podophriafixa	+	+	–
Vorticella convalaria	+	+	–
Vorticella microstoma	+	–	–
Vaginicotastriata	+	+	–
Vaginicotacristalina	+	+	–
Campanellaumbelaria	+	+	–
Chilodonellauncinata	+	+	–
Eugliphacilliata	+	+	+
Bodo lens	+	–	–
Eusphoranajas	+	–	–
Habrotricha recluse	+	–	–
Notommata ansata	+	–	–
Aelosomatenebrarum	+	–	–
Haetonotus maximus	+	+	–
Scenedesmusquadricauda	+	+	+
Scenedesmusbijugatus	+	+	+
Stigeoclonium tenue	+	–	–
Chlorella vulgaris	+	+	–
Ankistrodesmusfalcatus	+	+	–

Note : + detected; – not detected.

(around the clock), lack of light (the flasks were closed with light-proof paper). For aeration of aqueous solutions, the flasks were placed on a shaker. As a result of the study, it was found that representatives of protozoofauna primarily react to changes in the level of illumination, as can be seen from Table 1 and in accordance with Figure 1. Complete death or encysting in the absence of light can lead to their loss as an ecological niche in the food pyramid of the biocenosis of activated sludge, which as a consequence can cause disruption of the normal operation of the aeration tank. In Zhanaozen, the active solar period lasts 9-10 months a year. The study of the seasonal dynamics of the number of individual groups of algae in wastewater that has passed the stage of biological purification has shown that their species diversity is closely dependent on the duration of daylight. It was found that in the autumn-winter period of the year, mainly diatoms predominate, whereas blue-green and green algae are predominant in the spring-summer period, in accordance with Figure 2. The influence of errors in the aeration mode on the efficiency of biological wastewater treatment was established during a visual inspection of the aeration tanks and the secondary radial sump. It was noted that the water in the aeration tank is greenish in color, purple flakes are small with a greenish tint, foam areas are marked on the surface of the water; the smell of petroleum products is sharp. In the secondary radial sump, which receives wastewater from the aeration tank, dark-colored water with a pungent smell of hydrogen sulfide, dark-colored swollen silt is marked on the surface of the water. Hydrobiological and microbiological analysis of water samples taken from the secondary radial sump showed an almost complete absence of viable organisms in the water. Single specimens of green and diatom algae were in a state of plasmolysis.

The high titer of sulfate–reducing anaerobic bacteria *Desulfovibriodesulfuricans* – 10^{10}-10^{12}CFU/g in the sludge from the bottom of the sump was the evidence of a violation in the

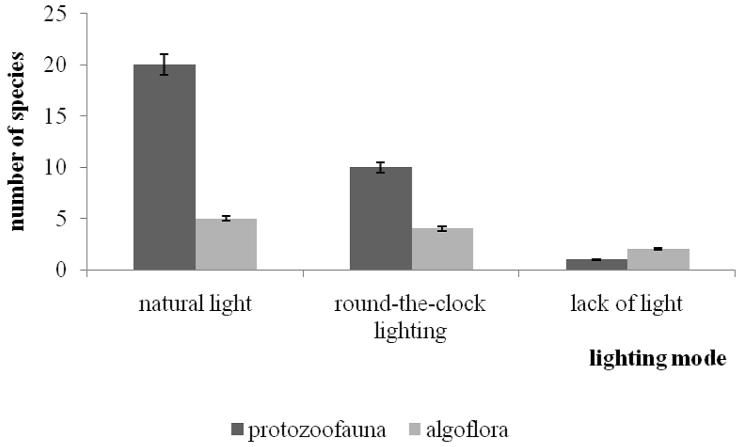

Figure 1. The influence of the illumination mode on the number of species of organisms-hydrobionts

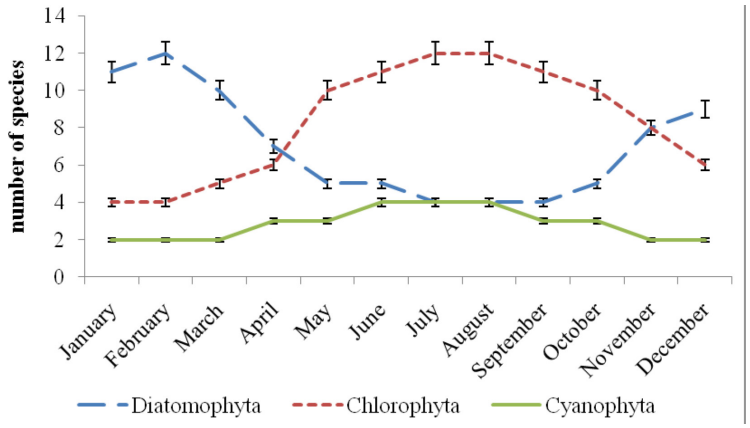

Figure 2. Seasonal dynamics of algoflora abundance in wastewater treatment plants

aeration regime. When pumping water from this sump, the occurrence of activated sludge on the bottom was revealed, i.e. a dark dense homogeneous mass with a pungent smell of hydrogen sulfide. As a result of the conducted research, it was found that there are two reasons for the current situation: power outages (due to which there was a down of the aeration system from several hours to a day) and the outdated aeration system (which caused silting in the corners of the aerotanks), recommendations were given to eliminate the causes of the current situation. Both causes were eliminated within the next 6 months by switching to an autonomous power supply and installing an aeration system that meets international standards. An important factor negatively affecting the formation of activated sludge in aeration tanks was an imbalance in the ratio of biogenic elements in wastewater (Kokina et al., 2022). A source of "salvo" discharges was installed – the "Thermal power station", the wastewater of which discharged into the treatment facilities contains a large amount of polyphosphates and ammonium salts. These reagents are used at the enterprise for washing boilers and flushing pipes. During the research period, sharp fluctuations in the content of phosphates up to 15.0–20.5 mg/l at a maximum permissible concentration (MPC) of 1.8 mg/l, and ammonium nitrogen 15.5–17.0 mg/l at a MPC of 8.2 mg/l were revealed.

There are known studies related to the study of the effect of ammonium nitrogen on the activity of activated sludge (Puigagut et al., 2005, Zhang et al., 2018). It was found that short-term exposure to approximately twice the higher concentration of NH_4 led to significant changes in the properties of activated sludge due to the inhibition of bacteria oxidizing NH_4, a decrease in the diversity of the microfauna of sludge and deterioration in the ability of sludge to precipitate (Golovko et al., 2019). As a result of the hydrobiological analyses carried out, it was found that after a period of a sharp increase in ammonium nitrogen in aqueous solutions, the absence of infusoria *Vaginicotacrystallina, V. ctriata, Stentor polymorphus, Vorticella alba* is observed in the wastewater of aerotanks, and the species *Aspidiscaturrita, A. lynceus, A. costata, Colepshirtus, Stylonychiamuscurum* decrease to a minimum in their numbers.

However, protozoa *Chilodonellauncinata* and *Astasiaquartana* are found in large numbers; in all likelihood, they are more resistant to high nitrogen concentrations. It is also interesting to note that ammonium nitrogen has a great influence on the vital activity of amoeba, small flagellates and rotifers, which die at its peak concentrations. Perhaps the reason for this is the imbalance in nitrification of phase 1, which, in turn, is confirmed by microbiological analyses (the number of nitrifiers is 10^6–10^8 CFU/ml). The increased concentration of nitrogen has a stimulating effect on the growth and development of algoflora, especially green algae, which gave the appropriate color to the water in the aeration tanks. Microscopy of water samples marked the massive development of *Chlorella vulgaris.* Due to a sharp increase in the concentration of phosphates up to 20.5 mg/l, the composition of the activated sludge shows a complete absence of such roundworm infusoria as *Vaginicotacrystallina, V. ctriata, Vorticella alba, V.convalaria, Campanellaumbelaria;* gastropods - *Stentor polymorphus, Aspidiscaturrita, A. lynceus, Colepshirtus;* suckers - *Tokophryalemnarum*. There was an increase in the number of gastropod infusoria *Euplotes patella* and rotifers *Caridinasp,* which occur only once in a normally functioning sludge. Changes in the concentration of phosphates also affect the composition of phytoplankton, the number of green and diatom algae decreases sharply; at the same time, fouling of the walls and pipes of the aerating plant with filamentous blue-green algae *Oscillatoriachlorina* is observed, which, with prolonged excess of the MPC for phosphates and pH, can develop up to 0.2–0.5 m.

On the basis of the results obtained, it can be stated that with a significant excess of the MPC for nitrogen and phosphorus (by 4, 6, 8 times), the total number of microorganisms sharply decreases, species diversity decreases, and some especially sensitive species are eliminated from the composition of activated sludge. The effect of nitrogen and phosphorus on a group of sedentary infusoria should be considered as a complex effect; increased concentrations of both elements are equally detrimental to them, but the total number of organisms depends on the quantitative ratio between these elements. Free-floating infusoria are more resistant to the influence of these factors, in response to an increase in nitrogen content, they reduce their numbers, and an increase in the phosphorus content only slightly inhibits their development. As a result of the "stress" of activated sludge, the ratio between sedentary and free-floating infusoria changes dramatically, which serves as an indicative sign when evaluating the work of aerotanks. A group of green algae reacts

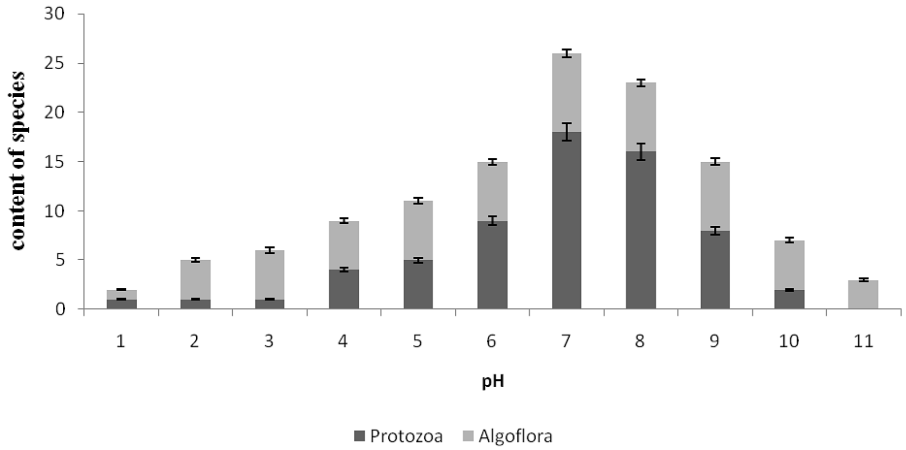

Figure 3. The effect of pH values on the change in the number of species of organisms-hydrobionts of activated sludge

sharply and negatively to an increase in phosphorus concentration, while an increase in nitrogen content has a positive effect on the growth of their biomass, especially *Chlorella vulgaris* and some other species are developing rapidly.

The study of changes in the pH of wastewater in aeration tanks for a long time has shown that in a number of emergency situations, pH deviations from the normative indicators take place. A model experiment was set up in the laboratory, where conditions with different pH values were modeled on the basis of wastewater. The dependence of the qualitative composition of activated sludge on different pH values was revealed (Figure 3). In studies by Baldwin et al. (2001) the acute toxic effect of low pH values was established. In the research conducted in this paper, the most sensitive to pH changes are the species *Bursaria truncatella, Euplotes patella, Carchesiumpolypinum* and *Stylonychiamytilus*. Algae, in general, were more resistant to pH fluctuations than representatives of the protozoofauna of activated sludge. The most resistant among them are *Spirogyra porticalis, Chlorella vulgaris, Chlorococcumtridium*. Among zooplankton, the organisms of the amoeba group are quite stable.

A number of reagents used in the technological scheme of oil refining can be attributed to abiotic factors that can create a stressful situation for activated sludge organisms (Dubovik et al., 2022). One of these reagents is monoethanolamine, which is used to absorb acid gases and sulfur-containing organic compounds (Kim et al., 2008). The MPC for monoethanolamine in water and in air is -0.5 mg/l. As a result of laboratory studies, it was found that monoethanolamine is a factor that has a sufficiently strong effect on the hydrobiological composition of activated sludge, even with a slight excess of MPC. At a concentration of monoethanolamine equal to 1.0 mg/l, the most sensitive organisms of activated sludge die: *Amphyleptusclaparedei, Litonotuscignus, Scepanotricharubra, Euplotes patella* and small-scale worms of the *Aelosomatenebratum*. Free-floating infusoria slow down the movement, there is a slight lightening of plant cells. With an increase in the concentration of monoethanolamine to 2,0 mg/l, many infusoria lose motor activity, cells become encysted, algae become colorless, many organisms die: *Embatacammensa, Carchesiumpolypinum, Vorticella campanulla, V. microstoma.* A further increase in the content of the toxicant in the water leads to the total death of all organisms-hydrobionts.

CONCLUSIONS

The study of the influence of abiotic factors on the biocenosis of activated sludge of urban wastewater treatment plants in Zhanaozen showed its large inertial capacity capable of "extinguishing" short-term toxic effects. However, prolonged stress is a serious burden for hydrobiont organisms, as a result of which the quality and intensity of biological wastewater treatment sharply deteriorates. In the conditions of a long period of active insolation of Western Kazakhstanit is established that the species diversity of algoflora of activated sludge depends on the duration of daylight. It was found that diatoms predominate in the autumn-winter period of the year, whereas blue-green and green algae predominate in the spring-summer

period. It was observed that short-term exposure to high concentrations of NH_4 led to a decrease in the diversity of the microfauna of silt and deterioration in the ability of silt to precipitate. With a short-term excess of ammonium nitrogen content in wastewater, *Vaginicola crystalline, V. striata, Stentor polymorphus, Vorticella alba* disappear, and the number of species A*spidiscaturrita, A. lynceus, A. costata, Colepshirtus, Stylonychiamuscurum*decreases to a minimum. Algae are more resistant to changes in the pH of the medium, the species Spirogyra porticalis, Chlorella vulgaris, Chlorococcumtridium were stable in the pH range 6-9. The species most sensitive to the content of monoethanolamine in water are: *Amphileptusclaparedei, Litonotuscygnus, Scepanotricharubra, Euplotes patella* and *Aelosomatenebratum* worms, but the 3% content of the toxicant in the water causes the total death of all organisms.

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