Досліджено ефективність послідовно розташованих анаеробних, аноксидних, аеробних процесів біологічного очищення стічних вод міста від сполук нітрогену та фосфору з використанням іммобілізованих мікроорганізмів. В аеробній зоні для аерування іммобілізованих мікроорганізмів досліджено напрям руху струменів повітря перпендикулярно напряму руху води, при якому досягається окисна потужність за нітрогеном амонійним до 120–130 г/(м^3·доб)

Ключові слова: анаеробні, аноксидні і аеробні біореактори, іммобілізовані мікроорганізми, біотехнологія, очищення від сполук нітрогену і фосфору

Исследовано эффективность последовательно расположенных анаэробных, аноксидных, аэробных процессов биологической очистки сточных вод города от соединений нитрогена и фосфора с использованием иммобилизованных микроорганизмов. В аэробной зоне для аэрации иммобилизованных микроорганизмов исследовано направление движения струй воздуха перпендикулярно направлению движения воды, при котором достигается окислительная мощность за азотом аммонийным до 120–130 г/(м^3·сут)

Ключевые слова: анаэробные, аноксидных и аэробные биореакторы, иммобилизованные микроорганизмы, биотехнология, очистка от соединений нитрогена и фосфора

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PROCESSES OF BIOLOGICAL WASTEWATER TREATMENT FOR NITROGEN, PHOSPHORUS REMOVAL BY IMMOBILIZED MICROORGANISMS

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1. Introduction

One of the major challenges of today is the preservation of natural resources and ensuring environmental safety. The discharge of non-treated and poorly treated municipal wastewater contaminates water bodies, soils with organic matter, including nitrogen-containing, – human metabolism products, phosphorus compounds. In water bodies, there is a transformation of these compounds with the participation of hydrobionts. Anaerobic processes, fermentation, rotting, gas emissions, formation of decomposition products of high molecular weight organic matter are observed.

Anaerobic wastewater treatment processes have not yet been widely used, despite a number of obvious advantages over aerobic biological and chemical processes. The main advantage is the high degree of conversion of carbon of the organic matter, contained in the water inflow, into gases – methane, carbon dioxide, etc. The reduction of the number of dissolved carbon compounds is accompanied by a decrease in the energy the bacterial population spends generating biomass, and therefore, the amount of excess sludge to be removed is less than in the aerobic biotreatment process. In addition, biogas, which is a valuable fuel is formed.

The majority of earlier constructed wastewater treatment facilities did not provide for removal of nutrients, the concentration of which in wastewater was insignificant. At the moment nutrients removal from wastewater is a worldwide problem and a priority task in the municipal wastewater treatment. Therefore, research aimed at intensifying existing treatment methods and developing new technologies, facilities for nutrients removal should be considered relevant.

2. Literature review and problem statement

Wastewater treatment in the vast majority of existing treatment plants in the world is carried out by biological methods. Treatment facilities built in the 60–80ies of the 20th century were not intended for nitrogen removal by today's standards since they provided only complete biological wastewater treatment [1]. The efficiency of total nitrogen removal when applying various wastewater treatment methods is: for mechanical – 15 %, physicochemical (addition of reagents) – 25–35 %, traditional biological – 30 %, biological-chemical (addition of reagents) – 35 %, deep nitrogen removal in the biological treatment process – 60–85 %.

The content of various forms of nitrogen compounds in the treated water depends mainly on technological parameters of treatment facilities (organic load, oxidation rate, hydraulic load, oxidation capacity, aeration period, sludge dose). Accepted regimes provide complete biological treatment and partial nitrification. At organic load on activated sludge of 0.2–0.3 g BOD_5/(g·day), there is a decrease in ammonium nitrogen by no more than 40 %, and a small amount of nitrates (3–4 mg/dm^3) is formed [2]. Such results indicate insufficient nitrogen removal.

Anaerobic processes can be used as the first stage of biological wastewater treatment for nitrogen removal, which is used in the world, and there is a trend to expand this practice [2]. There are several advantages of the anaerobic process over aerobic: much lower electric power consumption (no aeration required), the formation of several times less amount of excess biomass. In addition, gas formation, the removal degree of organic matter exceed the result obtained in aerobic processes dozens of times, and the process efficiency in terms of COD reduction reaches 80 % [3]. As is known [4], after anaerobic wastewater treatment, it is necessary to apply aerobic conditions to achieve standard values of wastewater discharge into natural water bodies.

Nitrifying bacteria multiply much more slowly than more numerous heterotrophic microorganisms. Removing excess sludge containing some amount of nitrifying autotrophs from the system can cause community reduction. Therefore, the age of sludge in aeration tanks is taken on the basis of preservation and accumulation conditions of nitrifying bacteria. The maximum growth rate of nitrifying bacteria is determined experimentally and makes 0.45–0.52 $days^{-1}$. For aeration tanks [2], this value varies within 0.25–0.35 $days^{-1}$. The pH value should be 7–8. In production conditions, under the action of heavy metal salts, synthetic surfactants and petroleum products on activated sludge, the growth rate of nitrifying bacteria decreases.

An important factor is effective consumption of dissolved oxygen by microorganisms, which in the reactions of biochemical oxidation of ammonium nitrogen is an acceptor of electrons, the transfer of which produces the energy spent for construction of cellular matter. The authors [5] have investigated that the concentration of dissolved oxygen in wastewater, the COD removal efficiency of organic matter, nitrogen compounds depend on the aeration rate in the system with free-floating activated sludge. It is found that the optimum concentration of dissolved oxygen is 2 mg/dm^3.

In the world, perforated-membrane disk aerators [6], which form a moving up air column of certain diameter gained wide popularity. Using such air flow in a facility with immobilized microorganisms leads to damage, separation of the biofilm from the carriers located closest to the disk aerator.

Biological wastewater treatment in conventional aeration tanks, which is widely used in existing treatment facilities of Ukraine and the world, is intended for organic matter removal, while the degree of wastewater treatment for nitrogen removal is 30–85 %, phosphorus – 50–80 %.

The disadvantage of the denitrification scheme, where return activated sludge is used as a substrate, is that the sludge contains only a small amount of nitrified nitrogen, and therefore the denitrification efficiency is low [6].

To achieve standard values of concentrations of nitrogen compounds, post-treatment is used in the conventional technology. More often, complete biological treatment is complemented by biological oxidation in biological ponds, biological plateau [7]. Disadvantages of biological ponds are low hydraulic loads and efficiency of 70–98 % at the initial of ammonium nitrogen concentration of 5–7 mg/dm^3.

The A2/O (Anaerobic/Anoxic/Oxic), UCT process (University of Cape Town), MUCT technologies with alternating anaerobic, anoxic and aerobic conditions allow ammonium nitrogen and phosphorus removal from wastewater by 80–98 % [8]. These technologies are characterized by high operating costs since all processes use external and several internal recirculations as wastewater, which increases the size of facilities, energy costs.

Biological processes of deep wastewater treatment for nitrogen, phosphorus removal can be carried out in two ways: using activated sludge or immobilized microorganisms.

The technologies with activated sludge are considered above. But today experts in Ukraine and abroad pay more and more attention to the possibility of using immobilized biomass in wastewater treatment [9]. Immobilized-cell bioreactors provide deep removal of both organic matter and nitrogen, phosphorus compounds.

Immobilized microorganisms are those for which artificial restrictions of mobility in the external environment are created, and the intermediate material providing these restrictions is called the immobilized biomass carrier.

Some of the applications of the immobilized biofilm are MBBR (moving bed biological reactor), FBBR (fixing bed biological reactor), IFAS (integrated fixed-film activated sludge).

For the implementation of the MBBR technology, corridors of facilities are divided into cells to retain biomass carriers. A number of carriers are loaded into each cell, which will circulate in the water column by means of the bottom aeration system mounted parallel to wastewater flow [10]. Such technology creates operation difficulties due to the possibility of pipeline contamination by free-floating carriers.

For the implementation of the FBBR technology, stationary biomass carriers, mounted perpendicular over the aeration system are installed in corridors of facilities [11]. Due to stationary carriers, the concentration of free-floating activated sludge is reduced. Also, the direction of wastewater flow changes: in addition to the flow along the corridors, the wastewater moves vertically with respect to the carriers, thereby increasing the time of contact with the biofilm [12]. Perpendicular, relative to stationary carriers, air flow creates turbulent streams along the carriers, which causes biofilm separation and damage.

For the implementation of the IFAS technology, in addition to wastewater, return activated sludge is fed to the reactor from secondary settling tanks [13]. IFAS can be implemented jointly with both MBBR and FBBR.

The authors [14] have investigated the anaerobic-aerobic treatment of municipal wastewater using polyurethane carriers and found that the COD removal efficiency for 36 h was 90 % at the initial concentration of 642 mg/dm^3. The disadvantage of this technology is the significant process duration, which can be explained by the insufficient biomass concentration in the facility.

The influence of the air flow direction on the effective use of dissolved oxygen by immobilized microorganisms is poorly studied. There is a reason to believe that insufficient knowledge of the consistent combination of anaerobic, anoxic and aerobic processes, together with the practical implementation of trophic bonds through the use of immobilized microorganisms, determines the necessity and prospects for research in this area.

3. The aim and objectives of the study

The aim of the study is to investigate the removal of pollutants (organic matter, nitrogen compounds and phosphorus) in the anaerobic and aerobic treatment of municipal wastewater using immobilized on synthetic fibers microorganisms.

To achieve this aim, the following objectives are defined:

– to study the aerobic process of municipal wastewater treatment with immobilized microorganisms with the perpendicular air distribution relative to water flow;

– to determine the sequence of setting oxygen conditions using immobilized microorganisms for effective biological treatment of municipal wastewater.

4. Materials and methods of the study of the technology of biological treatment of municipal wastewater

4. 1. Experimental materials and equipment

The pilot study of anaerobic-aerobic processes was carried out at treatment facilities of the city of Rivne, Ukraine. Wastewater characteristics: suspended solids 150–400 mg/dm^3, ammonium nitrogen 30–50 mg/dm^3, COD 400-900 mg/dm^3, BOD$_5$150–250 mg/dm^3, phosphates 7–18 mg/dm^3.

Wastewater flow rate was measured by a volumetric method. On the basis of standard techniques, photocolorimetric (KFK-2, Russia), spectrocolorimetric (PV1251B, Belarus), potentiometric (I160-MI, Belarus) methods of determination of nitrogen compounds were used. Determination of the ammonium nitrogen concentration is based on the interaction of ammonium ions with the Nessler's reagent to form a brown, water-insoluble iodide, which transforms into a colloidal state at a low concentration of ammonium ions (λ=425 nm). Determination of nitrates is based on the interaction of nitrate ions and salicylate ions in the sulfuric acid medium to form a mixture of 3-nitrosalicylic and 5-nitrosalicylic acids. Salts of these acids in the alkaline medium are yellow (λ=410 nm). Determination of nitrite ions in wastewater is based on the diazotation of sulfanilic acid with nitrites and the interaction of the resulting salt with α-naphthylamine to form a red-violet azo dye (λ=510 nm). To determine the COD value, the bichromatic method based on oxidation with potassium bichromate in the acidic medium during boiling was used. To increase the completeness of organic matter oxidation, silver sulfate is added to the test sample as a catalyst. Determination of the orthophosphate concentration in wastewater is based on the reaction with molybdate in the sulfuric acid medium, in the presence of trivalent antimony ions, and reduction by ascorbic acid.

The gravimetric method was used to determine the concentration of suspended solids, dry matter concentration of the biomass on the AXIS AD200 analytical scales (Poland). Dissolved oxygen, BOD$_5$ were determined by the iodometric titration (Winkler's method).

The dissolved oxygen concentration in wastewater was determined using the HQ30D oximeter (USA), ANKAT 7645-01 analyzer (Russia).

Immobilized biomass was characterized by the following parameters: dry matter concentration of the biofilm (gravimetric method), ash content (gravimetric method). The hydrobiological analysis of biomass was carried out by indicator microorganisms using a "Biolam-R15" microscope (Russia); photo- and video-shooting were carried out with the CANON EOS550D camera. For the study, low-power (x8) and high-power (x20, x40, x60) lenses, an eyepiece with x10 magnification were used.

4. 2. Method of the experimental study of technology parameters of wastewater treatment for nitrogen and phosphorus removal

For the study of wastewater treatment for organic pollutants, nitrogen and phosphorus removal, real municipal wastewater fed to the biological wastewater treatment plant was used. The study was carried out at the installation located at treatment facilities of Rivne regional municipal enterprise of water supply and wastewater services "Rivneoblvodokanal" (Rivne, Ukraine).

The study of the processes of biological municipal wastewater treatment was carried out on a pilot plant – anaerobic-aerobic bioreactor using immobilized on VIYA carriers microorganisms. The technology of biological treatment in the bioreactor was implemented in four consecutively located zones with anaerobic, anoxic, anaerobic and aerobic conditions using immobilized on synthetic fibers microorganisms (TU 6-06-S116-87) in each zone. In the anaerobic zones, wastewater was mixed with pumps. In the anoxic and aerobic zones, the aeration system was arranged to maintain the desired oxygen concentration (0.1–0.2 and 1.5–2 mg/dm^3, respectively). Aerators in the aerobic zone were placed so as to form a perpendicular air distribution relative to water flow (Fig. 1) [15].

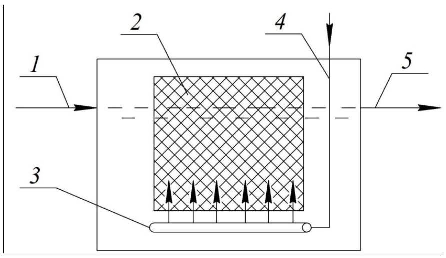

Fig. 1. Layout of aerators and carrier in the bioreactor aerobic zone: 1 – wastewater inflow to the bioreactor aerobic zone; 2 – carriers of immobilized microorganisms; 3 – aerator; 4 – air feed from the compressor; 5 – wastewater outflow from the bioreactor aerobic zone

One of the inhibitors of nitrogen and phosphorus removal processes is the high organic matter concentration characterized by COD, BOD indicators. The anaerobic zone at the beginning of the technology is arranged to reduce COD and to further implement nitrogen and phosphorus removal processes.

In experimental studies of the bioreactor, water quality indicators were determined at the bioreactor inlet, after each zone (anaerobic, anoxic, aerobic), at the bioreactor outlet. To characterize the efficiency of biological wastewater treatment, the following indices were determined: pH, temperature, suspended solids, COD, BOD_{total}, BOD_5, nitrogen group (nitrates, nitrites, ammonium), phosphates. And also the following parameters: process duration, activated sludge composition, ash content and humidity of the biomass. These indicators were determined by standard methods and using appropriate equipment.

The hydrobiological analysis complements the technological control of water treatment quality and bioreactor operation. The results of microscopy and determination of the quantitative ratio and physiological condition of biocenosis allowed making conclusions about the condition of sludge and its pollutant-transforming ability.

Samples for analysis were taken from each zone of the anaerobic-aerobic bioreactor. The hydrobiological analysis was carried out for both free-floating and immobilized biocenosis. The sludge sample after settling was applied with a pipette on a glass, covered with a cover glass and microscoped. Thus, the species, physiological condition, sludge composition, presence of zoogloea, algae, fungi, bacteria, chromaticity, etc. were determined.

To determine the periphyton, samples of fibers from each zone of the anaerobic-aerobic bioreactor were cut off and examined under a microscope. The hydrobiological analysis was carried out every day the first month after the plant commissioning, the analysis of sludge composition was carried out once a week.

The points on the graphs correspond to the average values of the studied samples at a certain frequency (at least three values). The error is ±3.2 % (Fig. 2), ±10 mg/dm^3 (Fig. 3).

5. Results of the study of biological wastewater treatment processes in different oxygen conditions

At perpendicular air flow relative to water flow (Fig. 1), a boundary layer with the laminar air flow is formed near the surface of the carriers. This allows microorganisms, which are fixed on the carrier, to capture air microbubbles as efficiently as possible, thereby increasing the efficiency of aerobic degradation of organic matter contained in wastewater. It was found that the oxidation capacity of ammonium nitrogen in the aerobic zone was 120–130 $g/(m^3/day)$ with the biomass concentration of 18–20 g/dm^3.

The organic pollution removal efficiency is influenced by the pollution concentration in the inlet water and time of stay in the facility. As determined by the results of studies (Fig. 2), the critical point is the treatment time of 4 hours, after which the efficiency curve approaches a straight line. Based on this, the estimated time of wastewater treatment can be taken 4 hours.

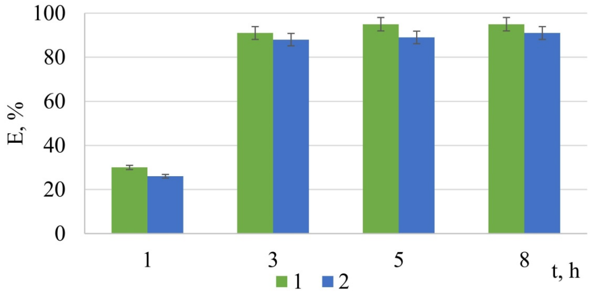

Fig. 2. Dependence of COD treatment efficiency E on the time of stay in the facility t at initial COD values, mg/dm^3: 1 — 400; 2 — 900

The rate of decomposition of organic pollutants by immobilized microorganisms was investigated. It was found (Fig. 3) that the specific oxidation rate of organic matter in the municipal wastewater treatment reaches 25 mg COD/(g/h), providing COD treatment efficiency of up to 90 %.

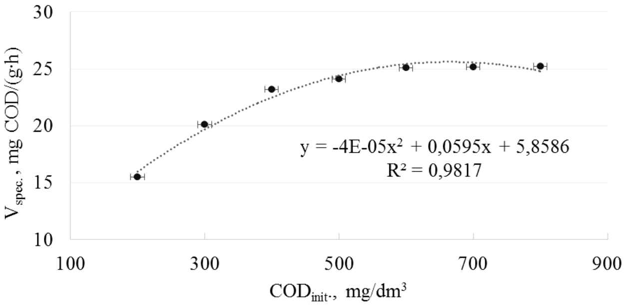

Fig. 3. Dependence of specific decomposition rate of organic matter $V_{spec.}$ on the inlet wastewater COD concentration $COD_{init.}$

The high COD treatment efficiency – 90 % (Fig. 2) with the time of stay in the facility of 4 hours indicates the expediency of using anaerobic processes at the initial stage of municipal wastewater treatment.

The wastewater treatment efficiency is affected by both hydraulic load and organic load (COD, BOD). As wastewater is treated, there is a reduction in the organic load q, which is determined by wastewater BOD_5 per 1 g of ashless biomass matter per day.

When determining the efficiency of the work, the values of BOD_5 oxidation capacity (OC) were determined. It was revealed that with the oxidation capacity of more than 800 $g/(m^3/day)$ and a hydraulic load of 4.04 $m^3/(m^3/day)$, the BOD_5 removal efficiency of organic pollutants sharply decreases (Fig. 4). The OC is determined by the difference of BOD_5 at the bioreactor inlet and outlet per 1 m^3 a day.

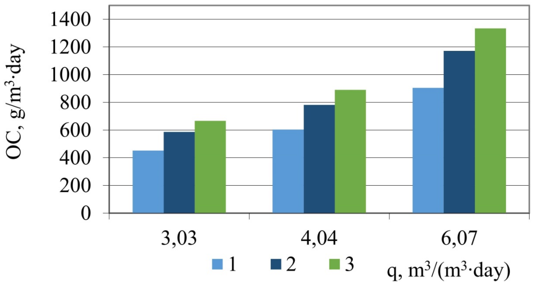

Fig. 4. Dependence of oxidation capacity OC on hydraulic load q on the facility at different initial BOD_5 concentrations, mg/dm^3: 1 — 150; 2 — 200; 3 — 250

The value of wastewater treatment efficiency, depending on the initial ammonium nitrogen concentration (30–50 mg/dm^3), is about 97.3–99 %. The ammonium nitrogen concentration at the outlet is 0.76 mg/dm^3, the nitrate nitrogen concentration does not exceed 9.8 mg/dm^3, and the nitrite concentration is 0.25 mg/dm^3, which does not exceed the norms of discharge to a fishery surface water body.

The efficiency of phosphate removal due to the creation of anaerobic conditions at the initial stage is 86 % with the outlet concentration of 2 mg/dm^3. The process of phosphorus removal from wastewater is based on consumption of orthophosphates by microorganisms for energy and construction of biomass cells. In the aerobic stage, phosphorus-accumulating bacteria actively absorb phosphates in the form of polyphosphates and orthophosphates from wastewater. Free-floating activated sludge with phosphorus-accumulating bacteria is separated after the bioreactor aerobic zone, and part of it is returned to the second bioreactor anaerobic zone.

Due to the immobilization of microorganisms, high biomass concentrations have been achieved, which in aerobic conditions are 7–8 g/dm^3 with the ash content of 50–60 % and in anaerobic 20 g/dm^3 with the ash content of 30–40 %, which allows increasing the load on the facility and providing high oxidation capacity.

As a result of the hydrobiological analysis (Fig. 5) of the carrier fibers, it was revealed that in the aerobic zone, ciliates dominated in the protozoan group, sarcodines were represented by the *Arcella* species.

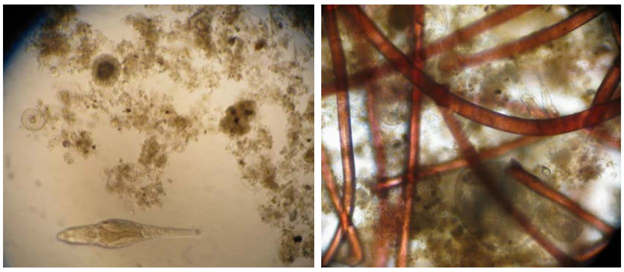
a *b*

Fig. 5. Microphotos of the aerobic zone of the pilot plant: *a* – free-floating biomass (×200); *b* – immobilized on carriers microorganisms (×600)

Compared to the anoxic zone, the number of rotifers has increased, which indicates the passage of both nitrification stages. The presence of oligochaetes indicates a high biomass mineralization and the formation of the highest-level trophic chain. The active participation in aerobic wastewater treatment and biomass processing is taken by rotifers, oligochaetes and several ciliate varieties.

6. Discussion of the results of the study of the processes of biological treatment of municipal wastewater

The application of perpendicular aeration relative to water flow and parallel aeration relative to the carrier of immobilized microorganisms leads to the intensification of processes of oxidation of ammonia nitrogen and provides its removal to the norms of discharge into a water body and low nitrate concentrations (8–9.8 mg/dm^3). The main thing is that such an air flow direction does not damage the biofilm and provides the formation of the boundary layer with the air laminar flow near the surface of the carriers. These factors ensure the integrity of the hydraulic biofilm, the main layer of immobilized microorganisms. Perpendicular aeration allows more efficient saturation of immobilized on carriers microorganisms with oxygen and creates conditions for effective oxidation of organic matter, ammonium nitrogen, nitrites. Therefore, it provides the ammonium nitrogen oxidation capacity in the aerobic zone of up to 120–130 g/(m^3/day), compared with values in aeration tanks with parallel arrangement of the aeration system – up to 50 g/(m^3/day), and high biomass concentrations – 18–20 g/dm^3, in aeration tanks 2.5–4.5 g/dm^3, respectively. An increase in oxidation capacity can be explained by the fact that immobilized microorganisms have greater access to dissolved oxygen due to the laminar flow of air bubbles to the surface of the biofilm on carriers when applying the proposed air flow direction.

It is known that the intensity of conversion of nitrogen compounds is limited by the rather low growth rate of nitrifying bacteria, sensitivity to pH fluctuations, and competitive relationships with heterotrophic bacteria. The efficiency of ammonium nitrogen removal reaches 97.3–99 %, which is due to an increase in the concentration of nitrifying bacteria and creation of favorable conditions for the biomass development using immobilized microorganisms.

High COD concentrations in the studied wastewater are due to the discharge of dye-containing wastewater of non-woven fabric factories to wastewater facilities in the city of Rivne. Treatment of wastewater with the COD concentration of organic matter of more than 700 mg/dm^3 in biological treatment facilities without carriers causes swelling of activated sludge and violates normal operation of these facilities. Therefore, the anaerobic bioreactor with immobilized on carriers microorganisms was used in wastewater treatment for removal of high organic matter concentrations. At the bioreactor outlet, the required COD concentration of organic matter for the phosphorus-accumulating bacteria, which is up to 300 mg/dm^3 is maintained. Phosphorus-accumulating bacteria are mainly represented by bacteria of the genera *Acinetobacter*, *Acetobacter*, *Nocardia*, *Citrobacter frendii*, which are capable of competing for substrate with other microorganisms in anaerobic conditions because their metabolism is more energy-efficient, and in the subsequent aerobic conditions, phosphorus-accumulating bacteria can assimilate organic matter contained in wastewater coming from the bioreactor anoxic zone.

The highest values of BOD$_5$ oxidation capacity – 1300 g/(m^3/day), COD – 1800 g/(m^3/day) due to the anaerobic conditions in the initial stage and the fiber carrier in each bioreactor zone were obtained.

Based on the results, it is logical to increase the load to 380–400 mg of BOD$_5$ per 1 g of ashless biomass matter a day to achieve BOD$_5$ efficiency of up to 95 %.

It was established that an increase in hydraulic load up to 6 m^3/(m^2·day) leads to a decrease in the efficiency of wastewater treatment for organic pollutants removal by up to 60 % by COD and 88 % by BOD$_5$, and with the load of 2–4 m^3/(m^2·day) lasting 6–10 hours the effect is 90 % by COD and 95 % by BOD$_5$.

The use of immobilized microorganisms in the studied technology has allowed:

– passing from the cyclic scheme to more efficient direct-flow technology with simultaneous reduction of the sizes of the applied facilities;

– reducing energy consumption up to 40 % due to the absence of wastewater recirculation;

– increasing the biooxidant properties of bacterial cells in the immobilized state, in contrast to the use of free-floating activated sludge;

– increasing the efficiency of substrate transformations into biomass by immobilized microorganisms due to increasing the biomass concentration in the unit volume of the bioreactor;

– ensuring cell resistance to various unfavorable inactivating external factors (temperature, acidity, presence of electrolytes or toxic substances, etc.).

The application of the developed technology in the anoxic and aerobic zones provides the creation of hydrobiont groups in wastewater of different conditions and composition at different stages of treatment and removal of excess biomass by the trophic chain of microorganisms up to 50–70 g/m^3.

According to the results of studies, for the efficient removal of organic pollutants, nitrogen, phosphorus compounds from wastewater, the technology using the flow-type facility with anaerobic, anoxic, anaerobic zones and aerobic conditions was developed [16]. Due to the successive anaerobic-anoxic-anaerobic-aerobic method of treatment without wastewater recirculation, concentrations of organic pollutants, nitrogen and phosphorus compounds in the treated wastewater do not exceed the norms of discharge into natural water bodies. The costs of wastewater recycling and pumping are reduced, a mineralized sediment is formed. Upon reaching the high biomass concentration of immobilized microorganisms, the load on the facility increases, providing high oxidation capacity.

In the first anaerobic zone, intensive processes occur: degradation of organic compounds due to hydrolysis, ammonification, acid formation, acetogenesis, denitrification, phosphatation (phosphorus release in wastewater by organisms) and gas formation.

In the second anoxic zone, the first stage of nitrification – nitritation, which reduces the flow rate of air to oxidize ammonium nitrogen, is provided.

In the third anaerobic zone, in the presence of easily oxidized organic matter, the process of denitrification proceeds intensively.

In the fourth aerobic zone, in the presence of air, processes of nitrification and dephosphatation occur intensively.

At all stages, due to the immobilization of microorganisms in the fouling layer, there is anaerobic ammonium oxidation – anammox, and biomass gain is observed.

The processes occurring in certain oxygen conditions are as follows. In anaerobic conditions, the degradation of organic compounds occurs as a result of hydrolysis, ammonification of proteins by acid bacteria with an increase in NH_4^+ and release of N_2. Enzymatic hydrolysis under the action of extracellular proteases leads to splitting of complex protein molecules, first to peptides, and then to amino acids. In anaerobic conditions, ammonification is carried out by hybrid cultures of bacteria of the genera *Clostridium*, *Micrococcus*, etc. The main way of transformation of amino acids in anaerobic conditions is the Stickland reaction – a combination of oxidation and reduction of two amino acids, one of which plays the role of an oxidizer, another – a reducer. The ammonification process does not change the alkalinity of water, but reduces its pH due to CO_2 accumulation. Ammonium nitrogen, formed during ammonification, can be a source of nitrogen for microorganisms or oxidized in the course of nitrification to nitrites and nitrates.

Heterotrophs carry out denitrification, for which the organic matter oxidant is nitrate (contained in a small amount in the inlet water), and for the effective conversion of nitrate to molecular nitrogen or nitrite, an electron donor is required, which may be methanol. The denitrification process is based on enzymatic reduction of nitrates to molecular nitrogen in anaerobic conditions. Denitrifying bacteria, which are chemoorganoheterotrophs, include bacteria of the genera *Pseudomonas*, *Bacillus*, *Micrococcus*, *Proteus*, *Alcaligenes* (*Bacterium denitrificans*, *Pseudomonas flourescens*), which use bound oxygen of nitrites and nitrates for respiration. The process proceeds in stages:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2. \qquad (1)$$

Gibbs free energy is calculated for the general denitrification equation:

$$\frac{1}{70}C_{18}H_{19}O_9N + \frac{1}{5}NO_3^- + \frac{1}{5}H^+ \rightarrow$$
$$\rightarrow \frac{1}{10}N_2 + \frac{17}{70}CO_2 + \frac{1}{70}HCO_3^- + \frac{1}{70}NH_4^+ + \frac{1}{5}H_2O,$$

$$\Delta G^o(W) = -103 \text{ kJ/el.eqv.} \qquad (2)$$

During nitrogen reduction reactions, there is a mass gain of activated sludge and increase in the amount of bicarbonate HCO_3^-. The denitrification process restores alkalinity (at a rate of 3.6 mg per $CaCO_3$/mg N–NO_3^-). Also, the increase of carbonate alkalinity has a positive effect on the course of nitrification, when in the technological scheme the denitrifier is placed in front of the nitrifier. In terms of oxidation capacity, 1 g of nitrate nitrogen is equivalent to 2.86 g of molecular oxygen. The consumption of the external oxidant of organic matter in the anoxic zones is 0.6–0.8 g of molecular oxygen per 1 g of the removed organic matter by BOD_5, which corresponds to the nitrate nitrogen consumption of 0.2-0.3 g per 1 g of BOD_5. That is, removal of 1 g of nitrate nitrogen requires 3–5 g of BOD_5 [2].

In the layer of carriers with immobilized microorganisms, heterotrophic acid-forming bacteria carry out the hydrolysis of complex organic matter to form monomers and utilize them to form acids, CO_2, NH_4^+, and H_2O. As a result of these processes, the biomass concentration increases, which can reach up to 40 g/dm^3.

In anaerobic conditions, in the presence of fermentation products and stressful conditions, the cell releases orthophosphate using the energy for accumulating simple organic matter.

In anoxic conditions where a low oxygen concentration of 0.2 mg/dm^3 is maintained, the first stage of nitrification – nitritation with Nitrosomonas bacteria with NO_2^- formation and biomass gain proceeds, the anammox process with N_2 release and NO_3^- increase in the biomass layer may also occur. The general equation of the ammonium anaerobic oxidation reaction:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O,$$

$$\Delta G^o(W) = -357 \text{ kJ/el.eqv.} \qquad (3)$$

The process takes place in two stages. The first stage is nitritation – reduction of ammonium to nitrite, i.e. the first stage of nitrification. The second stage is the anammox process itself. As a result, the total process is accompanied by the formation of free nitrogen and water, and in a more detailed study – the formation of intermediate products [17, 18]:

$$NH_4^+ + 1{,}32\,NO_2^- + 0{,}066\,HCO_3^- + 0{,}13\,H^+ \rightarrow$$
$$\rightarrow 1{,}02\,N_2 + 0{,}26\,NO_3^- + 0{,}066\,CH_2O_{0.5}N_{0.15} + 2{,}03\,H_2O. \qquad (4)$$

Anaerobic ammonium oxidation is the only biological process in which an intermediate product, hydrazine, is formed. Anaerobic ammonium oxidation occurs at temperatures ranging from 6 to 43 °C and pH of 6.7 to 8.3 with an optimum of 8.0. There are at least three genera of autotrophic bacteria that provide ANAMMOX: *Brocadia*, *Kuenenia* and *Scalindua*. The growth rate of these organisms is quite low: biomass doubling takes about two weeks, which is solved by using immobilized microorganisms.

In the third zone with anaerobic conditions, phosphatation occurs – release of phosphorus in wastewater by microorganisms in the process of metabolism and biogas formation; in the presence of easily oxidized organic matter, the denitrification process proceeds intensively.

In aerobic conditions, there are complete nitrification with the first and second stages and additional oxidation of substances that were not decomposed in anaerobic conditions.

Nitration occurs in two stages. The first stage of conversion of ammonium to nitrite is carried out by nitrifying microorganisms of the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, *Nitrosovibrio* by the reaction:

$$\mathrm{NH_4^+ + 1{,}5\,O_2 \rightarrow NO_2^- + H_2O + 2H^+},$$

$$\Delta G^O(W) = -270\,kJ/mol\,\mathrm{NH_4^+ - N}. \qquad (5)$$

With the help of *Nitrobacter*, *Nitrospira*, *Nitrococcus* microorganisms, the second stage of nitrification– conversion of nitrite to nitrate is carried out:

$$\mathrm{NO_2^- + 0{,}5O_2 \rightarrow NO_3^-},$$

$$\Delta G^O(W) = -80\,kJ/mol\,\mathrm{NO_2^- - N}. \qquad (6)$$

The metabolism of these microorganisms is very sensitive to environmental factors. Optimum oxygen concentration – 2.0–3.5 mg/dm^3, at values of 0.5–1.5 mg/dm^3 the nitrification process is inhibited, 5–7 mg/dm^3 – do not affect the nitrification rate. Oxygen demand to oxidize 1 mg of ammonium nitrogen to nitrate nitrogen is 4.57 mg O_2, with the biomass gain of activated sludge of 0.2 mg [19].

The nitrification process, which occurs in aerobic conditions, is influenced by:

– temperature (optimum 30–35 °C), decrease from 20 °C to 10 °C inhibits the process by 30 %;

– dissolved oxygen concentration (4.57 mg O_2/mg of oxidized $N–NH_4^+$);

– sludge age (10–12 days for systems with recirculation);

– alkalinity (7.14 mg of alkalinity as $CaCO_3$ per 1 mg of $N–NH_4^+$, alkalinity reduction decreases pH and inhibits nitrification);

– pH within 6.5–8.0 (pH below 6 inhibits the process);

– concentration of inhibitors (some heavy metals and organic compounds) [12].

In aerobic conditions, organisms accumulate orthophosphates as polyphosphates. Free-floating activated sludge with phosphate-accumulating bacteria is separated after the bioreactor aerobic zone, and part of it is returned to the second bioreactor anaerobic zone.

At all stages, due to immobilization of microorganisms, anaerobic ammonium oxidation – anammox occurs in the fouling layer.

Such a sequence of the above processes with immobilized microorganisms allows deep removal of organic matter, nitrogen and phosphorus compounds, providing the norms of discharge of pollutants into fishery water bodies. This reduces the cost of aeration due to anaerobic, anoxic conditions at initial stages.

The investigated technology can be used in municipal wastewater treatment for nitrogen and phosphorus removal with a high concentration of organic matter at wastewater treatment plants of cities, settlements both in Ukraine and in the world. It will be effective if high COD concentrations in wastewater are due to discharges of dye-containing wastewater of light industry enterprises to treatment facilities in the city or settlement.

It should be noted that biological treatment plants can be constructed as separate capacities and in the combined version in one facility by the arrangement of partitions with holes. The latter option can be used for the re-equipment of operating treatment facilities according to the proposed biotechnology.

Therefore, the studied technology of biological wastewater treatment allows increasing the treatment efficiency and bringing the quality to existing norms of discharge to natural water bodies; provides the reduction of sediment volumes by 25–35 %, reduction of energy costs up to 40 %.

7. Conclusions

1. It was determined that the use of perpendicular air flow in relation to wastewater flow and parallel – carrier of immobilized microorganisms – provides the efficiency of ammonium nitrogen removal of up to 99 % compared with the parallel arrangement of the aeration system.

2. It was found that the use of successively arranged anaerobic, anoxic, anaerobic and aerobic conditions with immobilized on carriers microorganisms allows increasing the efficiency of wastewater treatment for organic pollutants, nitrogen and phosphorus removal.

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