Influence of Deep-Treated Wastewater Discharge on Nitrification Activity in a Natural Reservoirs

Valentyna Iurchenko[1], Mykyta Radionov[2*], Pavlo Ivanin[1], Oksana Melnikova[1]

[1] Faculty of Sanitary Engineering, Kharkiv National University of Civil Engineering and Architecture, Sumska Str. 40, Kharkiv, Ukraine

[2] Ukrainian Research Institute of Environmental Problems, Bakulina Str. 6, Kharkiv, Ukraine

* Corresponding author's e-mail: radionov.nikita@ukr.net

ABSTRACT

The data from regular monitoring of the concentration of nitrogen-containing compounds and nitrification indicators in the Udy river (at sites 500 m before the discharge of treated wastewater in Kharkiv city and 500 m after discharge downstream) and in the wastewater being treated at urban wastewater treatment facilities were analyzed. Chemical and biological kinetic constants of nitrification in the water layer and biochemical characteristics of nitrification in the bottom deposits in these areas were determined in special laboratory experiments. It was stated that the rate of nitrification in the water layer increases twice in the area after the discharge of treated wastewater compared to the area before discharge. Biochemical and microbiological indicators of nitrification in the active sludge of wastewater treatment facilities were established. It was shown that the discharge of wastewater (and hence nitrifying bacteria) from wastewater treatment facilities that perform biological treatment with nitrification (without denitrification) increases the activity of nitrification processes in the water layer of a natural reservoir downstream.

Keywords: nitrification, natural reservoirs, discharge of treated wastewater, chemical kinetic constants, biokinetic constants, concentration of nitrifying bacteria, bottom deposits, active sludge.

INTRODUCTION

Wastewater treatment aimed at purification of water from the ammonium nitrogen is an important environmental problem, which is prevented by the introduction of closed water circulation systems (Shmandiy et al., 2017), application of adsorption (Malyovanyy et al., 2013) and reagent (Tulaydan et al., 2017) purification technologies, the use of biological methods of nitrification. Nitrification – two unique reactions of the nitrogen cycle in the biosphere carried out by chemolithotrophic nitrifying bacteria (Ward et al., 2011, Stahl et al., 2012). The ammonia oxidizing bacteria (I-phase nitrifying agents) carry out the first phase of nitrification by oxidizing ammonia to nitrite, whereas the nitrite oxidizing bacteria (II-phase nitrifying agents) carry out the second phase of nitrification by oxidizing nitrite to nitrate (Lancaster et al., 2018). The nitrification activity determines the "self-purification"

activity of natural reservoirs from nitrogen compounds, and at the same time it is more environmentally safe if the rates of the first and second phases of nitrification are equal (Zlyvko et al., 2014, Shiozaki et al., 2016). In some areas of reservoirs, the rate of phase II of nitrification is lower than of phase I, which causes the accumulation of nitrites. Maintaining low concentrations of nitrites in aquatic systems is a serious problem, as nitrite is a very toxic substance for biota (Veuger et al., 2013, Ryzhakov, 2012).

The ability of nitrifying microorganisms to oxidize $N-NH_4$ and $N-NO_2$ has been widely used in biological wastewater treatment (Semenova et al., 2012, Gnida et al., 2016). Biological nitrification as a large-scale biotechnology is used as the final stage of wastewater treatment, when the treated water containing nitrates can be discharged into the reservoir, and in modern technologies for deep purification of nitrogen compounds – as an

intermediate stage when it is combined with subsequent biological denitrification (Haandel et al., 2012, How et al., 2018). Modern urban wastewater treatment schemes use sludge liquid recycling in two-, three-, four- and five-stage processes that allow deep purification of not only nitrogen compounds but also other biogenic elements. In the practice of urban wastewater treatment, single-sludge systems, which must carry out both chemoorganotrophic and chemoautotrophic (mainly chemosynthesis of nitrifying bacteria) microbiological processes, are used as a rule (Semenova et al., 2012, Nezdoyminov, 2013).

Biological treatment facilities are a potential source of biogenic elements as well as microorganisms (including nitrifying ones) for river watercourses, where the treated wastewater is discharged. The species and activity of microorganisms present in the wastewater of biological treatment facilities may differ from those found in the river upstream, and change the ecological functioning of the river downstream (Nozhevnikova et al., 2017). Therefore, the wastewater from treatment facilities can change the nitrification and concentration of nitrifying bacteria in a natural reservoir. Particularly dangerous in this sense is the discharge of insufficiently treated landfill filtrates into natural reservoirs, the concentration of ammonium ions in which is ten times higher than this in urban wastewater (*Malovanyy* et al., 2018). Although the wastewater treatment processes have been significantly improved over the past decade, the discharge of treated wastewater can lead to an increase in the concentration of ammonium in river systems, an increase in the concentration of nitrifying bacteria, and as a potential consequence – to a change in the kinetics of nitrification and nitrite dynamics within the water system (Raimonet et al., 2015).

The aim of the work was to determine the activity of nitrification processes in the Udy river before and after discharge of treated wastewater from the complex of municipal treatment facilities in Kharkiv.

OBJECTS AND METHODS OF EXPERIMENTAL INVESTIGATIONS

The object of experimental research were:
- aquatic environment and bottom deposits in the Udy river at sites 500 m before and 500 m after discharge of treated urban wastewater;
- wastewater and activated sludge of aeration tanks of the municipal treatment facilities complex No. 2, which discharges the treated wastewater into the Udy river.

Preliminary studies on the presence of nitrification processes in the Udy river at the site of discharge of treated urban wastewater were performed based on the analysis of long-term data pertaining to the daily control of the concentration of nitrogen-containing compounds in the studied areas. Determination of the chemical and biological constants of nitrification in a natural reservoir was performed on the basis of the results of a laboratory experiment conducted according to the method described in (Ryzhakov, 2012). For the laboratory experiment, the water samples were taken from the Udy river at sites 500 m before and 500 m after wastewater discharge. On the day of sampling, the content of mineral forms of nitrogen ($N-NH_4$, $N-NO_2$, $N-NO_3$) and organic nitrogen was determined in water. Each variant of water sample (2.5 dm^3) was incubated without the addition of reagents for 31 days in a dark place at a temperature of 19°C in a tightly closed container to ensure access to oxygen. At certain time intervals, the samples were taken from each variant and the concentration of nitrogen compounds was determined.

On the basis of the obtained experimental data, the chemical kinetic constants of ammonification and nitrification of phases I and II were calculated according to the formulas given in (Ryzhakov, 2012) using a mathematical program developed in the C++ programming language using the Qt framework. The values of biokinetic constants (Michaelis constants – K_m, and the maximum rate of biochemical reaction – V_{max}) were determined by linearization of the obtained experimental data using the Walker-Schmidt method (Vinnov et al., 2013).

The nitrifying capacity and rate of nitrification in bottom deposits sampled from the Udy river at sites 500 m before and 500 m after wastewater discharge, and in activated sludge of treatment facilities that discharge treated wastewater into the Udy river, was determined using the biochemical method (Iurchenko, 2007) on the basis of the activity of the enzyme catalyzing the reaction of hemolitotrophic oxidation of ammonium – hydroxylamine oxidoreductase. The concentration of nitrifying bacteria of the first nitrification phase in activated sludge was

determined using the microbiological method of limiting dilutions (Vinogradova et al., 2012).

Hydrochemical analysis of aqueous media ($N-NH_4$ – colorimetric with Nessler's reagent, $N-NO_2$ – colorimetric with α-naphthylamine, $N-NO_3$ – colorimetric with sodium salicylate, N_{org} after wet mineralization titrometrically, pH – electrometrically, CSA – using the arbitration method with potassium dichromate) was carried out using the standard methods, according to the requirements of regulative documents of Ukraine (List, 2007). Statistical data processing was performed in a Microsoft Excel computer program.

RESULTS OF EXPERIMENTAL INVESTIGATIONS

Assessment of nitrification activity in the water environment of the Udy river

The concentrations of the nitrogen-containing compounds in the Udy river (average daily water consumption is 950 thousand m^3/day) according to regular monitoring at sites 500 m before and 500 m after discharge of treated wastewater are presented in Figure 1. As can be seen from this figure, in the Udy river at sites 500 m before and 500 m after discharge of
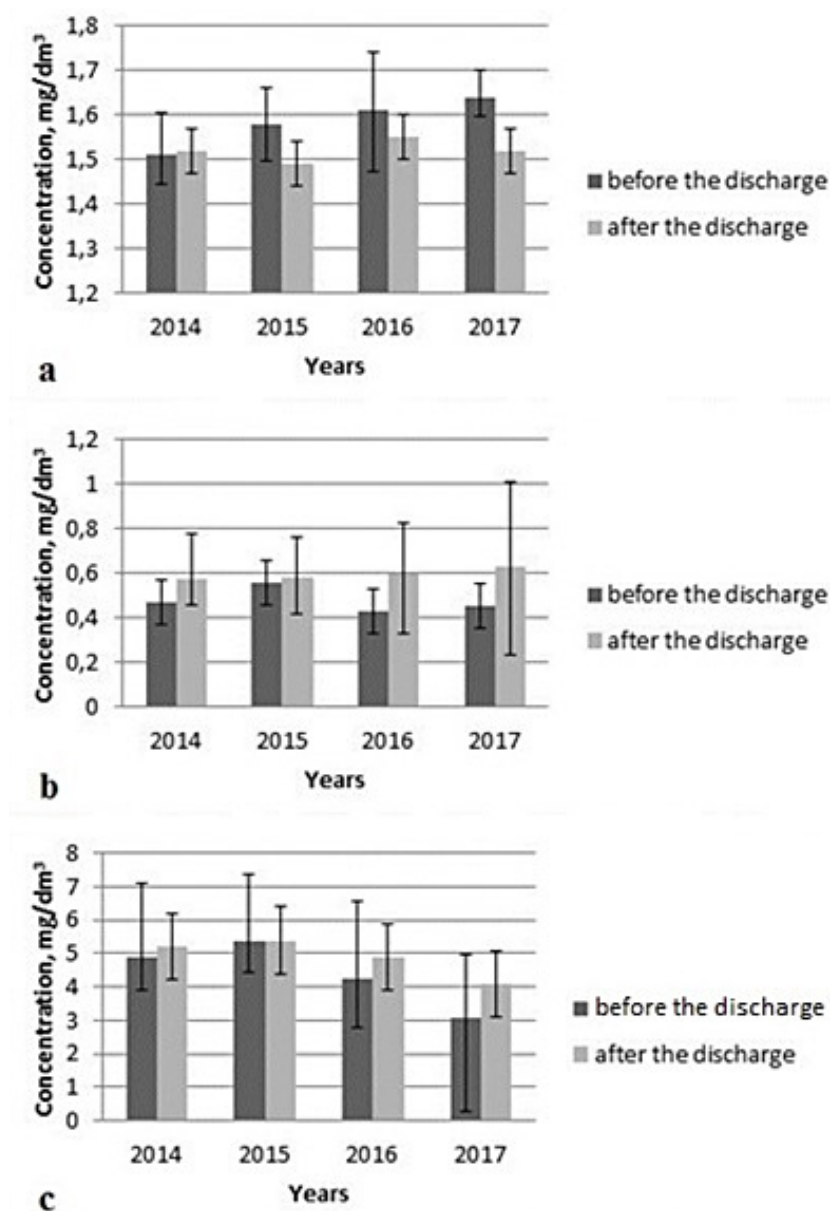

Figure 1. Dynamics of the average annual concentration of ammonium nitrogen (a), nitrite nitrogen (b) and nitrate nitrogen (C) in the water of the Udy river

treated wastewater, the concentration of N-NH$_4$ in the dynamics of the 5-year period mainly decreases, and N-NO$_2$ and N-NO$_3$ – constantly increases indicating an enhancement of the nitrification activity in the Ury river after the discharge of treated wastewater.

This conclusion was confirmed by the calculation of the nitrification index (I_{nitr}) for the period of observations (2014–2017) at the sites before and after the discharge of wastewater (Fig. 2). The index of nitrification ($I_{nitr.}$) of water was determined using the formula recommended in the scientific literature (Zlyvko et al., 2014):

$$I_{нimp} = C_{NO_3}/(C_{NO_3} + C_{NH_4} + C_{NO_2}) \qquad (1)$$

where: C_{NO3}, C_{NH4}, C_{NO2} are the nitrogen concentration of nitrates, ammonium, and nitrites, respectively.

As can be seen, the nitrification index from 2015 to 2017 at the site after the discharge of wastewater exceeded this figure at the site before the discharge by 5–13%.

The pH dynamics of the aquatic environment at the sites 500 m before and 500 m after the discharge of treated wastewater (Fig. 3) demonstrates stable acidification of water in the area after the discharge of treated wastewater. This also indicates an increase in the nitrification activity in this area compared to the area before the discharge of wastewater.

Determination of the kinetic characteristics of nitrification

Determination of the kinetic characteristics of nitrification in the water environment of the Udy river before and after the wastewater discharge was performed in October, i.e. during the period of extinction and destruction of biomass of aquatic organisms and aquatic vegetation after summer vegetation, and hence the increased concentration of organic compounds and nitrogen compounds in water. According to the results of the experiment (Fig. 4a, d), the concentration of N-NH$_4$ during exposure in both versions of the experiment initially increased due to the presence of free ammonia and ammonification reactions (Shiozaki et al., 2016, Ryzhakov, 2012), but then fell steadily, and, in the version with water in the
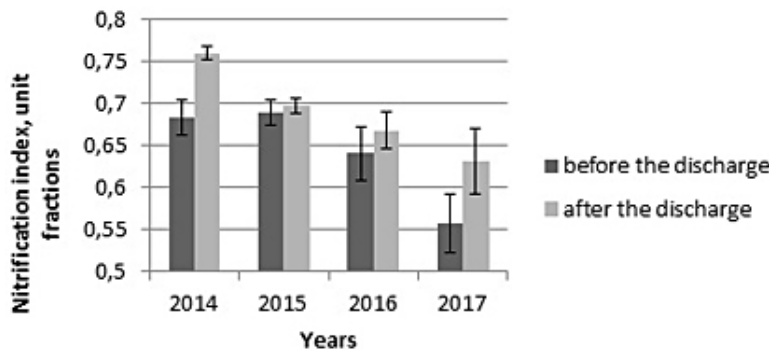

Figure 2. Dynamics of the average annual nitrification index (2014–2017) in the Udy river before and after the discharge of treated wastewater
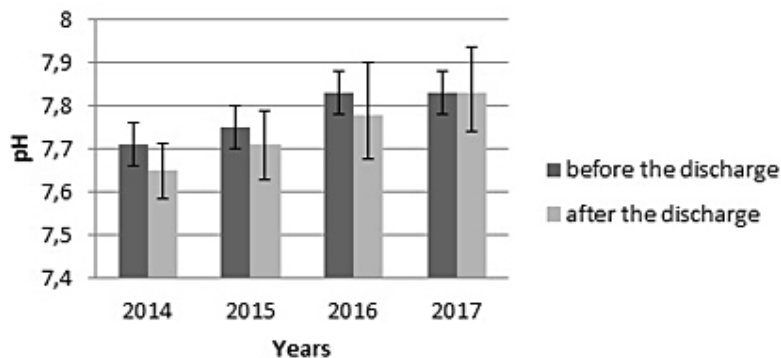

Figure 3. Dynamics of the average annual pH value in the area of the Udy river before and after the discharge of treated wastewater
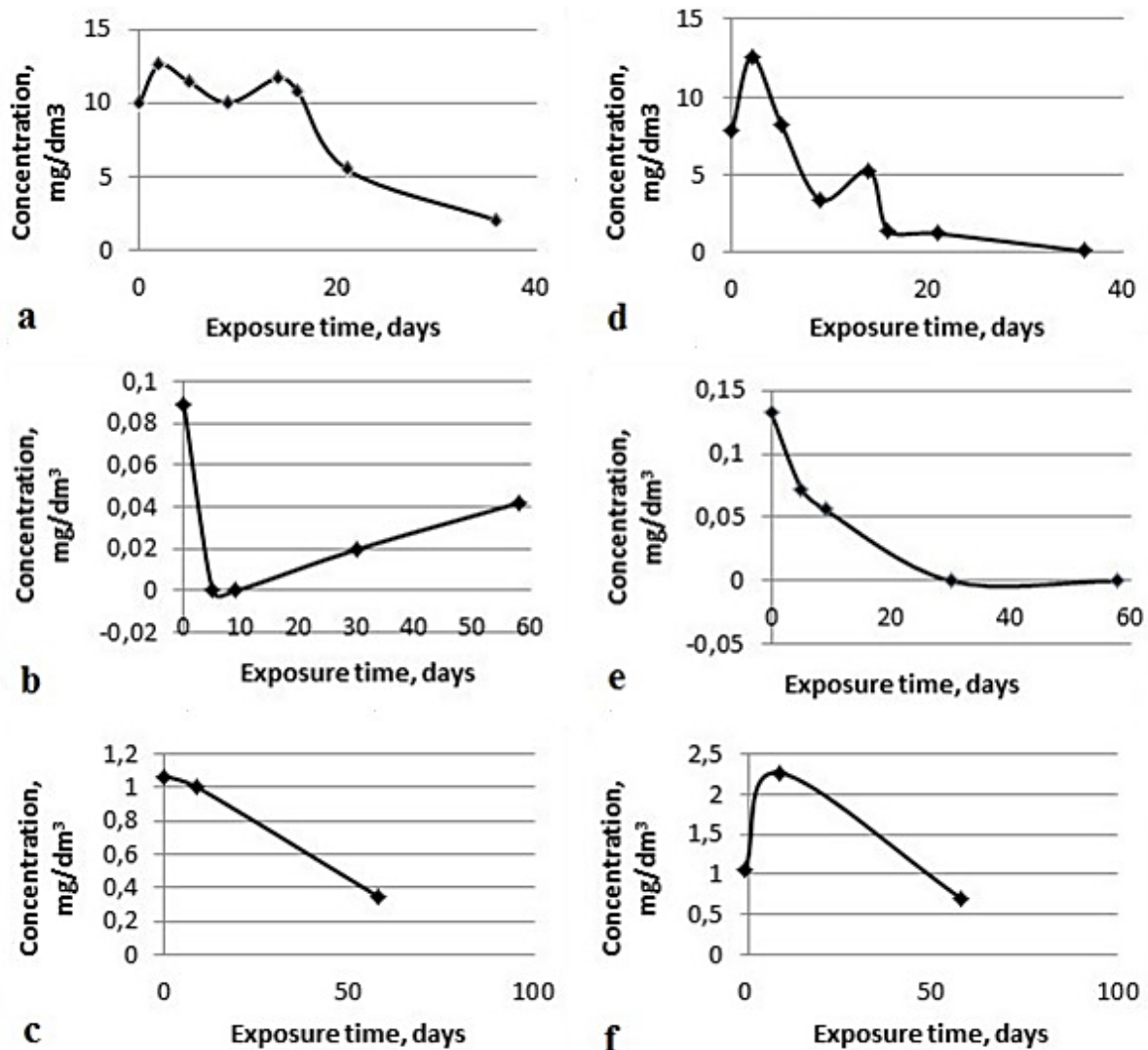
Figure 4. Dynamics of concentration of N-NH$_4$ (a, d), N-NO$_2$ (b, e) and N-NO$_3$ (c, f) in the water from the Udy river, sampled 500 m before the discharge (a, b, c) and 500 m after the discharge (d, e, f) of treated wastewater

area after the discharge of wastewater, it is much more active than in the area before the discharge.

During the exposure of the nitrites nitrogen in the variant with water before the wastewater discharge after a significant decrease in the concentration of N-NH$_4$ increase steadily, which is a clear sign of passing the I phase of nitrification. However, the absence of a decrease in the concentration of N-NO$_2$ and a steady decrease in the concentration of N-NO$_3$ in the dynamics of further cultivation, indicates low activity of phase II of nitrification. The decrease in the concentration of N-NO$_3$ may also indicate the predominance of denitrification rate over the rate of phase II of nitrification in this version of the experiment. The dynamics of the N-NO$_2$ concentration in the experiment with water from

the site after wastewater discharge shows no accumulation of nitrites, i.e. high rate of phase II of nitrification; therefore, it constitutes an environmentally safer option for nitrification than in the experiment with water before wastewater discharge. However, the high rate of N-NO$_2$ removal was not accompanied by the accumulation of N-NO$_3$, which is probably due to the high denitrification activity.

The sequence of biochemical transformations of nitrogen compounds studied in laboratory experiments can be represented as follows:

$$N_{opг} \overset{k_1}{\to} NH_4^+ \overset{k_2}{\to} NO_2^- \overset{k_3}{\to} NO_3^+ \qquad (2)$$
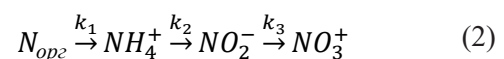
where: k_1 is the rate constant of ammonification,
k_2 is the rate constant of the first phase of nitrification,

k_3 is the rate constant of the second phase of nitrification.

In order to calculate the rate constants of ammonification reactions and the first phase of nitrification, the authors used the method (Ryzhakov, 2012), which takes into account that these reactions are consistent and have the first order. This system of two equations is as follows:

$$[N_{N-NH_4}]_{max} - [N_{N-NH_4}]_0 =$$

$$= [N_{org}]_0 \left(\frac{k_2}{k_1}\right)^{\frac{k_2}{k_1-k_2}} \qquad (3)$$

$$t_{max} = \frac{ln\frac{k_2}{k_1}}{k_2-k_1},$$

where: $[N_{орг}]_0$ is the initial concentration of organic nitrogen, mg/dm³;

$[N_{ам}]_{max}$ – maximum concentration of N-NH₄, mg/dm³;

$[N_{ам}]_0$ – initial concentration of N-NH₄, mg/dm³;

t_{max} – time to reach the maximum concentration of N-NH₄, days.

The rate constant of the second phase of nitrification was calculated by means of the formula:

$$[N - NO_2^-]_{max} - [NO_2^-]_0 =$$

$$= k_1 k_2 [N_{org}]_0 \left[\frac{e^{k_1 t}}{(k_1 - k_2)(k_3 - k_1)} \frac{e^{k_2 t}}{(k_1 - k_2)(k_3 - k_2)}\right] \qquad (4)$$

where: $[NO_2^-]_{макс}$ is the maximum concentration of N-NO₂, mg/dm³;

$[NO_2^-]_0$ – initial concentration of N-NO₂, mg/dm³,

t – time to reach the maximum concentration of N-NO₂, days.

As can be seen from table 1, the value of the constant k_1 (ammonification rate constant) is the same in both areas. The nitrification rate constants k_2 and k_3 in the area after wastewater discharge are slightly higher than in the area before discharge. This indicates an increase in the nitrification activity in the water of the Udy river after the wastewater discharge. In the water samples both

before and after the discharge of treated effluents, k_2 is several times higher than k_1, and k_3 is significantly higher than k_2, which allows us to assume a minimum probability of nitrite accumulation in the river. In general, the values of k_1, k_2 and k_3 established in the experiments correspond to the data of studies on the nitrification processes in northern natural reservoirs, presented in the scientific and technical literature (Ryzhakov, 2012).

In order to determine the biokinetic constants by linearization of experimental data, a line was built in the coordinates of auxiliary variables: $\frac{P}{\ln\frac{S_0}{S_0 - P}}$ (abscissa axis) and $\frac{t}{\ln\frac{S_0}{S_0 - P}}$ (ordinate axis), where S_0 is the initial substrate concentration (ammonium nitrogen), S_t is the substrate concentration at time t, P is the concentration of biochemical reaction products (S_0-S_t), t is the measurement time. The tangent of the angle of inclination of this line is equal to $\frac{1}{V}$, where V is the maximum rate of biochemical reaction. The segment truncated on the y-axis is equal to $\frac{K_S}{V}$, where K is the Michaelis constant.

Linearization of the experimental data for determination of K_S and V_{max} at the site 500 m before and 500 m after the discharge of treated wastewater is graphically presented in Fig. 5, and the calculated values of these biokinetic constants – in table 2.

The calculations of biokinetic parameters (Table 2) showed that the obtained data correspond to the data of studies of other natural reservoirs and biological treatment facilities (Ryzhakov, 2012). Before the discharge of treated wastewater, K_s is an order of magnitude higher than after the discharge, which indicates a much lower affinity of nitrification enzymes to ammonium nitrogen in the area before the discharge. The rate of nitrification at the site after wastewater discharge is more than twice this figure before discharge.

Thus, the discharge of deeply treated wastewater in the Udy river increases the nitrifying capacity of the aquatic environment. Such

Table 1. Kinetic characteristics of ammonification and nitrification in water of the Udy river at the sites 500 m before and 500 m after discharge of treated wastewater

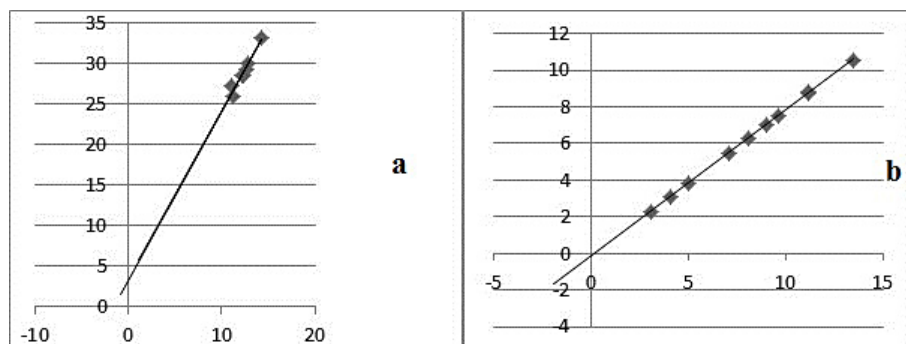| Reservoir	Rate constants of ammonification, I and II phases of nitrification (k_1, k_2, k_3), day⁻¹		
	k_1	k_2	k_3
Udy river (500 m before the discharge)	0.22	0.9	1.3
Udy river (500 m after the discharge)	0.22	1.0	1.4

Figure 5. Linearization of experimental data with water from the Udy river at the site 500 m before discharge of treated wastewater (a) and 500 m after discharge (b). The designations of the coordinate axes are given above

Table 2. Biokinetic indicators of nitrification in the Udy river and concentration of nitrifying bacteria of phase I in the aqueous medium at the sites before and after the discharge of treated wastewater

Nitrifying microbiocenosis	K_S, mg/dm³	V_{max} of nitrification of phase I, mg N-NH₄/(dm³ day)	Concentration of nitrifying bacteria of phase I, cells/cm³
Udy river at the site 500 m before the discharge of treated wastewater	1.7	0.48	$2.9 \cdot 10^6$
Udy river at the site 500 m after the discharge of treated wastewater	0.17	1.29	$7.9 \cdot 10^6$

a phenomenon at the site after the discharge of treated wastewater was noted by foreign scientists (Raimonet et al., 2015).

According to the established rate of nitrification, the concentration of nitrifying (ammonia-oxidizing) bacteria (kinetic determination) (Table 2) can be calculated based on data of (Carini et al., 2008, Gnida et al., 2016): the rate of oxidation of NH_3 by one cell of ammonia-oxidizing bacteria reaches 484 *f*mol of NH_3/year. The obtained results taking into account the concentration of $N-NH_4$ in the used aqueous media correspond to the data of other researchers (Carini et.al., 2008).

Determination of nitrifying capacity of bottom deposits in the Udy river

According to the research literature (Ryzhakov, 2012), the main contribution to nitrification in natural reservoirs is made by the activity of nitrifying bacteria immobilized in the upper layer of bottom deposits. The results of determining the nitrifying ability of the upper layer of bottom deposits according to biochemical analysis are presented in Table 3.

According to the obtained data, the activity of the enzyme hydroxylamine oxidoreductase in the bottom deposits before and after the discharge has the same order of values, and therefore the rate of nitrification in these bottom deposits is almost the same. Thus, increasing the activity of nitrification in the Udy river, which was established according to long-term control of nitrogen concentrations, pH, nitrification index at the sites before and after wastewater discharge, is due to the intensifying effect of treated wastewater discharge on these indicators in the water column, rather than on the nitrification indicators in deposits. This conclusion confirms the data of French scientists obtained in the study of nitrification in the Seine river (Raimonet et al., 2015).

Determination of indicators of nitrifying capacity of the active sludge of the municipal treatment facilities complex No. 2

The averaged data for regular control of the concentration of $N-NH_4$, $N-NO_2$ and $N-NO_3$ in wastewater at the inlet and outlet of urban treatment facilities No. 2, which discharge

Table 3. Biochemical characteristics of bottom deposits in the Udy river

Bottom deposits	Activity of hydroxylamine oxidoreductase, µg of formazan/(g $_{suhr}$·min.)	Rate of nitrification of phase I, mg N-NH₄/ (g $_{suhr}$ hour)
Before the wastewater discharge	3.1	0.03
After the wastewater discharge	3.2	0.03
Active sludge [Beliakov, 2014]	22–50	0.35–0.77

treated wastewater into the Udy river, are presented in Figure 6.

As it can be seen, the concentration of ammonium nitrogen after treatment significantly decreases (up to 92.5%), and the concentration of nitrites and nitrates increases, which is a clear sign, firstly, of deep biological wastewater treatment, and, secondly, the passage complete nitrification process (phases I and II).

Biological wastewater treatment at municipal treatment facilities takes place in four corridor aeration tanks with concentrated supply of activated sludge and dispersed supply of wastewater to the first half of the second corridor. The dynamics of the concentration of $N-NH_4$ in wastewater during its treatment in the displacing aeration tank is presented in Figure 7.

As it can be seen, the process of active nitrification of $N-NH_4$ occurs in the fourth corridor of the aeration tank after a significant reduction in the CSA of wastewater (Table 4). Relatively weak nitrification is manifested in the third corridor, as evidenced by the dynamics of $N-NO_3$ (Fig. 7b). It is not reflected by the dynamics of $N-NH_4$ (Fig. 7a), probably because in the third corridor the rate of consumption of $N-NH_4$ by the nitrification and assimilation processes is balanced by the rate of its formation in the process of ammonification of nitrogen-containing organic substances.

Compared with bottom sediments, the rate of nitrification carried out by activated sludge is an order of magnitude higher (Table 3). The data from direct microbiological determination of the concentration of nitrifying bacteria in the
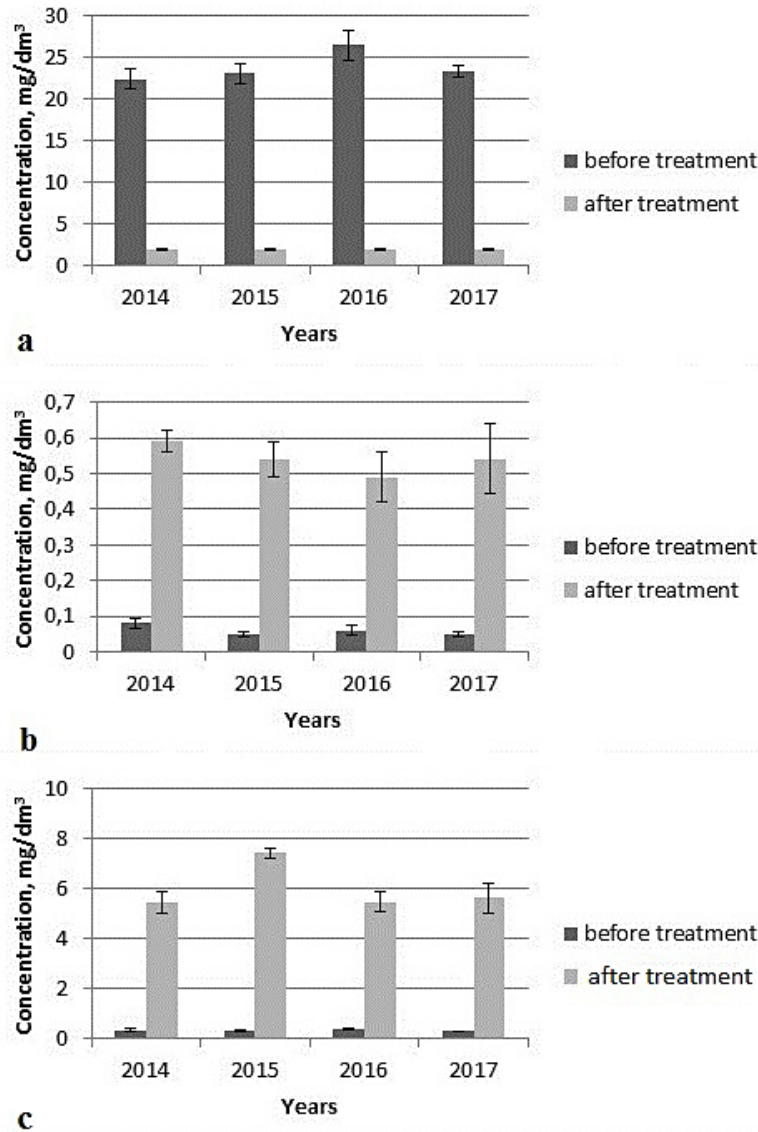

Figure 6. Dynamics of average annual nitrogen concentration: ammonium (a), nitrogen nitrites (b) and nitrates (c) in wastewater
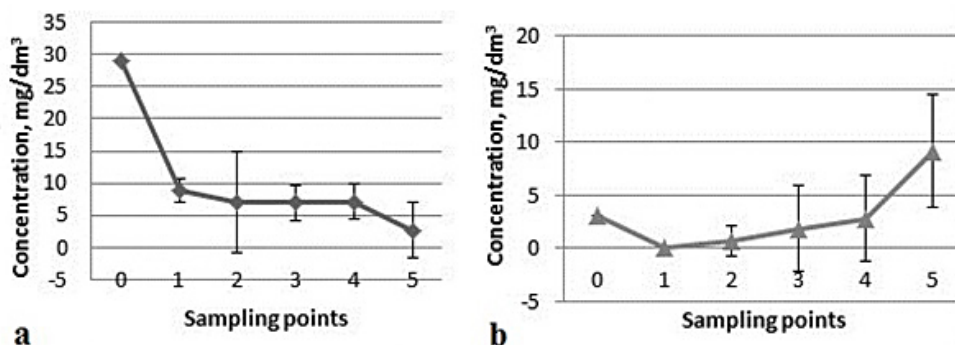
Figure 7. Concentration of N-NH$_4$ (a) and N-NO$_3$(b) in wastewater in the areas of the displacing aeration tank: 0 – influent wastewater, 1 – the middle of the second corridor, 2 – entrance to the third corridor, 3 – exit from the third corridor, 4 – entrance to the fourth corridor, 5 – exit from the fourth corridor

Table 4. Microbiological characteristics of the nitrifying capacity of activated sludge of urban treatment facilities №2

Sludge sampling	CSA, mg/dm^3	Concentration of N-NH$_4$, mg/dm^3	Concentration of nitrifying bacteria of phase I, cells/g
Beginning of the displacing aeration tank	110–160	16.7–19.1	10^6–10^7
End of the displacing aeration tank	30–52	1.8–2.3	10^6–10^8

activated sludge of biological treatment facilities and its nitrifying activity are presented in Table 4.

According to the presented data, the concentration of nitrifying bacteria in the activated sludge of treatment facilities at the end of the displacing aeration tank increases due to the passage in this zone of active nitrification and reaches 10^6–10^8 cells/g of dry weight of sludge. Taking into account the concentration of suspended solids in wastewater during discharge (≤ 15 mg/dm^3) and the volume of discharge (180 thousand m^3/day), the daily emission of nitrifying bacteria from treatment facilities in the Udy river can reach 2.7 ($10^{15} - 10^{17}$) cells/day.

CONCLUSIONS

1. According to the long-term observations of nitrogen-containing compounds, water pH and nitrification index, the wastewater discharge from the treatment facilities that perform deep biological treatment with nitrification increases the activity of nitrification processes in natural reservoirs, and hence the activity of "self-purification" of reservoirs from ammonium of autochthonal and allochthonal origin.

2. Nitrifying microflora, which is removed from the treatment facilities with discharged

wastewater, intensifies nitrification in natural reservoirs. While using the process that occurs in the water layer, the activity of denitrification in the Udy river at the site after the discharge of treated wastewater decreases slightly compared to the site before the discharge.

3. Calculations of biokinetic constants of nitrification showed that the rate of the nitrification process at the site after discharge of treated wastewater exceeds the figure at the site before discharge more than twice. The Michaelis constant in river water before discharge is much higher than after discharge. This shows a lower affinity of nitrification enzymes to ammonium nitrogen at the site after discharge and possibly changes the species composition of nitrifying microbiocenoses in the water of the Udy river.

4. Nitrifying microflora, which is removed from wastewater treatment facilities, enhances the process of nitrification in a natural reservoir. Moreover, this process occurs only in water. The denitrification activity in the Udy river at the site after the discharge of treated wastewater is slightly reduced compared to the site before the discharge.

5. Daily emission of nitrifying bacteria from treatment facilities in the Udy river can reach 2.7 ($10^{15} - 10^{17}$) cells/day.

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