VILNIUS TECH
Vilnius Gediminas Technical University

12th International Conference
ENVIRONMENTAL ENGINEERING
April 27–28, 2023, Vilnius, LITHUANIA

eISSN 2029-7092
eISBN 978-609-476-342-7
Article ID: enviro.2023.851
https://doi.org/10.3846/enviro.2023.851

PURIFICATION OF THE WATER ENVIRONMENT FROM AMMONIUM NITROGEN DURING NITRIFICATION IN NATURAL RESERVOIRS AND IN WATER USE FACILITIES

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Received 16 January 2023; accepted 20 February 2023

Abstract. Nitrification are two unique reactions of sequential oxidation of ammonium nitrogen, carried out by chemolithoautotrophic bacteria and archaea. Establishing the main source of nitrification in aquatic ecosystems is necessary to manage this process. In experimental researches it has been established that in natural water bodies with a low technogenic load, nitrification is caused by processes in bottom sediments, in areas of water bodies after wastewater discharge – by processes in the water column. In technogenic environments (water use facilities) nitrification is caused by processes in solid phases (filter fillings and activated sludge). Nitrification activity of activated sludge in treatment facilities with deep biological treatment is high and the discharge of deeply purified wastewater into natural water bodies leads to an increase in the processes of nitrification and the activity of self-purification from nitrogen compounds in them.

Keywords: nitrification, phases of the aquatic ecosystem, natural reservoirs, water treatment systems, biological treatment facilities.

Introduction

The removal of ammonium nitrogen from aquatic environments (deamonization) occurs mainly through its oxidation by chemolithoautotrophic nitrifying microorganisms under aerobic conditions and by anammox bacteria under anoxic conditions (Wu et al., 2019; Yu et al., 2020; Mai et al., 2021; Wu et al., 2012). Nitrification is a crucial step in the nitrogen cycle in the biosphere. In natural water bodies, nitrification causes their self-purification from nitrogen compounds (Shiozaki et al., 2016; Iurchenko et al., 2020; Zlyvko et al., 2014; Raimonet et al., 2015), and in the technosphere, the activity of deep wastewater treatment (How et al., 2018; Di Capua et al., 2022; Gnida et al., 2016; Iurchenko et al., 2022). The nitrification process takes place in two successive phases (Ward, 2013):

$$NH_3 + O_2 + CO_2 \rightarrow HNO_2 + [CH_2O]; \quad (1)$$

$$HNO_2 + O_2 + CO_2 \rightarrow HNO_3 + [CH_2O]. \quad (2)$$

Nitrifying microorganisms include: ammonium oxide bacteria and archaea (AOB and AOA), which carry out 6-electron oxidation of NH_3 to NO_2^- (phase I nitrifiers), and nitrite oxide bacteria (NOB, phase II nitrifiers), which perform 2-electron oxidation NO_2^- to NO_3^-, as well as "complete NH_3 oxidizers" (comammox) bacteria, which carry out the 8-electron oxidation of NH_3 to NO_3^- (Lancaster et al., 2018; Koch et al., 2019; Bartelme et al., 2017).

1. Analysis of recent research and publications

The ecology of microbiocenoses reveals the diversity of nitrogen-transforming microorganisms and their interaction in the natural and technogenic environment, and also determines their activity in their habitat. Aquatic microorganisms (including nitrifiers), which carry out closed cycles of basic elements in a natural water body, are present in plankton and benthos, preferring immobilization on floating particles and bottom sediments. In technogenic water bodies (water use facilities), microorganisms are also prone to immobilization both in controlled biotechnological and spontaneous processes (Iurchenko et al., 2022; Bartelme et al., 2017; Henze, 2002, pp. 246–270).

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Self-purification of natural water systems is due to many natural and man-made processes: hydrological, hydrochemical and hydrobiological. At the same time, aerobic microbiological processes occur mainly in the upper layers of the reservoir and the surface of bottom sediments and fouling, while anaerobic processes occur in the thickness of the bottom sediments of the reservoir, where dissolved oxygen is practically absent. According to research literature (Zlyvko et al., 2014; Raimonet et al., 2015; Telfer, 2014), the main contribution to nitrification in natural water bodies is made by the vital activity of nitrifying bacteria immobilized in the upper layer of bottom sediments. However, it was established (Raimonet et al., 2015), that the nitrifying microflora carried out from treatment facilities with discharged wastewater intensifies nitrification in natural water bodies. Moreover, the increase in the activity of the nitrification process occurs precisely in the water column. Emission of bacteria from on-site wastewater treatment plants after the discharge of treated wastewater affects the nitrogen cycle in these ecosystems. The noted properties somewhat change the existing ideas about the distribution of nitrification activities in natural aquatic ecosystems – the water column and bottom sediments and the influence of certain environmental factors on them.

In the technosphere – water use facilities, microbiological nitrification is also observed: in water treatment facilities spontaneous (Telfer, 2014; Yang & Cheng, 2007), in biological wastewater treatment facilities, nitrification as a directed biotechnological process determines the activity of deep removal of nitrogen compounds (Wu et al., 2019; Mai et al., 2021; How et al., 2018; Di Capua et al., 2022; Iurchenko et al., 2022). In sewage treatment plants, this microbiological process solves one of the most urgent problems of modern technologies for biological wastewater treatment in Ukraine and abroad as well. This is a deep removal of nutrients to protect natural water bodies from eutrophication. For reasonable management of this process, it is necessary to establish the main source of nitrification (solid or liquid phase) in aquatic natural and technogenic ecosystems.

The purpose of the work is to develop a methodological approach to the study of nitrification processes, which includes an assessment of the activity of this process in the liquid and solid phases of natural and technogenic ecosystems.

2. The main part of the study

2.1. Objects and research methods

7 sections of natural and technogenic water bodies were examined: 5 sections of rivers (the river Siverskyi Donets, the river Udy) and 2 sections of water use enterprises (drinking water treatment facilities and urban biological sewage treatment facilities).

In natural water bodies, the assessment of nitrification included: in the liquid phase – the calculation of the nitrification index according to the data of hydrochemical analysis and the calculation of biokinetic constants based on the data of a laboratory experiment, in the solid phase (bottom sediments) – monitoring the activity of the key enzyme of the first phase of nitrification hydroxylamine oxidoreductase.

In water treatment facilities: in the liquid phase the indices of nitrification of water were determined before and after treatment, and in the solid phase (the filling of filters) the activity of hydroxylamine oxidoreductase was determined. In the facilities for biological wastewater treatment: in the aqueous phase the nitrification indices were controlled before and after water treatment, the dynamics of nitrogen compounds were controlled during treatment, and in activated sludge the activity of hydroxylamine oxidoreductase and the biokinetic constants of nitrification were controlled. In the treated natural and waste water and activated sludge, the concentrations of nitrifying bacteria of the first phase of nitrification were determined.

The water nitrification index (NI), which is the ratio of the product of the second phase of nitrification (nitrate concentration) to the sum of the components of its 2 phases (nitrite nitrogen concentration, nitrate nitrogen and ammonium nitrogen), was determined by the formula recommended by the scientific literature (Zlyvko et al., 2014):

$$NI = C_{NO_3} / (C_{NH_4} + C_{NO_2} + C_{NO_3}), \tag{3}$$

where C_{NH_4}, C_{NO_2}, C_{NO_3} – concentration of nitrogen, nitrates, ammonium and nitrites, respectively.

For laboratory experiments to determine the kinetic constants of nitrification in the studied reservoirs, water samples of 2.5 dm^3 were taken. The exposure of the experimental variants was carried out in a dark place at a temperature of 19 °C in loosely sealed vessels to provide oxygen access for up to 36 days. After 1–2 days, samples were taken from each option and the concentration of inorganic nitrogen compounds was determined.

The value of biokinetic constants (Michaelis constant – K_m and maximum rate of biochemical reaction – V_{max}) was determined by linearization of the obtained experimental data by the Walker-Schmidt method (Vinnov & Dolganova, 2013).

The nitrifying activity and rate of nitrification in bottom sediments collected from rivers in the studied areas, in backfills of rapid filters at the water treatment complex and in activated sludge of sewage treatment plants were determined by the biochemical method (Iurchenko, 2007, pp. 272–282) by the activity of the enzyme that catalyzes the reaction of chemolithotrophic oxidation of ammonium – hydroxylamine oxidoreductase (HAO).

The potential rate of the first phase of nitrification was calculated according to the determined values of the HAO in the solid phases of the investigated aquatic ecosystems according to the formula (Radionov, 2021, p. 36):

$$y = 0.0011x + 0.006, \tag{4}$$

where: y – the rate of the first phase of nitrification, mg $N\text{-}NH_4(g_{dry\ m.}\cdot h)^{-1}$; x – activity of hydroxylamine oxidoreductase, μg of formazan $(g_{dry\ m.}\cdot h)^{-1}$.

The nitrifying activity of the upper layer of bottom sediments (V, mg $N\text{-}NH_4$ $(m^3\ day)^{-1}$, which leads to the oxidation of ammonium nitrogen in the water layer above the bottom sediments (1 m^2 of the bottom surface), was calculated by the formula:

$$V = \frac{y \cdot m_b \cdot 24 \cdot 1000}{W_w}, \tag{5}$$

where: m_b – weight of the upper layer of bottom sediments on an area of 1 m^2; 24 – conversion factor, hours/day; 1000 – conversion factor, kg/t; W_w – the volume of water above bottom sediments, m^3 (2).

$$m_b = d_b \cdot V_b, \tag{6}$$

where: d_b – specific gravity of sand sediments, t/m^3 (2.7); V_b – the volume of the upper layer of bottom sediments in which nitrification is possible on the area 1 m^2.

$$V_b = k \cdot S, \tag{7}$$

where: k – the thickness of the layer of bottom sediments in which nitrification can occur, m (0.001); S – the area of bottom sediments, 1 m^2.

The concentration of nitrifying bacteria of the first phase of nitrification in water was determined by the microbiological method of limiting dilutions on the Soriano and Walker medium (Vinogradova & Trofimenko, 2019, pp. 56–59).

Hydrochemical analysis of aqueous media was carried out according to standard methods in accordance with the requirements of Ukrainian regulatory documents (Governing Normative Document [GND] 211.1.4.030-95, 1995; GND 211.1.4.023-95, 1995; GND 211.1.4.027-95, 1995). Determined:

- ammonium nitrogen – colorimetrically with Nesler's reagent;
- nitrogen of nitrites – colorimetrically with α-naphthylamine;
- nitrogen of nitrates – colorimetrically with sodium salicylate;
- dry matter of activated sludge, bottom sediments and filter fillings – gravimetrically after drying at 105 °C.

2.2. Results and discussion

In the Table 1 shows the values of the average annual concentrations of $N\text{-}NH_4$ and nitrification indices in water at the studied sites.

The evidence varies significantly, which can be explained by higher levels of water pollution (including $N\text{-}NH_4$) in the lower reaches of the river. It is the increased concentration of $N\text{-}NH_4$ that can explain the significant increase in the maximum nitrification rate and the Michaelis constant in this area. The river Udy in the studied areas experienced a significantly greater anthropogenic load compared to the studied areas of the river Siv. Donets, as evidenced by higher concentrations of ammonium nitrogen in the water. In addition, during the period of determining the biokinetic constant, the concentration of ammonium nitrogen in the river water was somewhere 3–5 times higher than the average annual. On the example of the site after the discharge of wastewater in the river Siv. Donets, we see the inhibition of the rate of nitrification, which indicates a low depth of treatment of these wastewaters. This assumption is confirmed by the hydrochemical analysis of these wastewaters. In contrast, in Udy River, the nitrification index and the maximum rate of this process increase significantly after the discharge of municipal wastewater. This is due to the deep purification of these wastewater from nitrification, which is confirmed by their hydrochemical analysis.

Table 1. Results of calculations of the nitrification index and biokinetic constants of nitrification in the water phase of natural reservoirs

Object	Concentration $N\text{-}NH_4$, mg/dm^3	Nitrification index	Michaelis constanta, mg/dm^3	V_{max} nitrification of the first phase, mg $N\text{-}NH_4$ $(m^3\ day)^{-1}$
The river Siv. Donets (upstream)	0.16–0.17	0.58–0.65	0.03	50
The river Siv. Donets (50 km downstream) before the discharge of untreated sewage	1.37–1.45	0.12–0.44	0.1	220
The river Siv. Donets after discharge of untreated wastewater	1.09–1.56	0.16–0.30	0.12	70
The river Udy before the discharge of treated wastewater	1.58–1.63	0.56–0.68	1.7	480
The river Udy after discharge of treated wastewater	1.49–1.55	0.63–0.76	0.17	1290

Table 2. Results of determining the nitrifying capacity of bottom sediments in natural and artificial reservoirs

Object	Activity of HAO, μg of formazan $(g_{dry\ m.} \cdot h)^{-1}$	The rate of the first phase of nitrification, mg $N\text{-}NH_4(g_{dry\ m.} \cdot h)^{-1}$	The calculated rate of $N\text{-}NH_4$ oxidation in the water layer above bottom sediments, mg $N\text{-}NH_4$ $(m^3\ day)^{-1}$
The river Siv. Donets (upstream)	1.69	0.025	1620
The river Udy before the discharge of treated wastewater	0.05	0.006	388
The river Udy after discharge of treated wastewater	0.05	0.006	388

Analyzing the nitrifying activity of bottom sediments in the research areas of the rivers (see Table 2), it can be noted that this activity reacts negatively to increased pollution of the aquatic environment (including organic compounds). In aquatic environments with a greater anthropogenic load (the river Udy), the nitrifying capacity of bottom sediments is much lower than in slightly polluted waters. When comparing the data in Table 1 and Table 2, it can be seen that nitrification in the area of the river Siv. Donets (upstream) is due to the activity of bottom sediments, as noted in (Zlyvko et al., 2014; Telfer, 2014). And in the polluted areas of water in the river Udy, nitrification of water is due to nitrifying activity in accordance with the data (Raimonet et al., 2015).

As evidenced by the presented data (see Table 3), when the natural water passes through water treatment facilities (drinking water supply), the nitrification index steadily increases, which indicates the presence of spontaneous microbiological nitrification at this facility. No nitrifying bacteria were found in the water treated at these facilities. And with biological wastewater treatment, the nitrification index increases by almost 100 times. This indicates an extremely high activity of nitrification processes in the studied biological treatment facilities.

Table 3. The water nitrification indices before and after treatment in water use facilities

Object	Nitrification index	
	Before processing	After processing
Water in water treatment facilities	0.58–0.66	0.74–0.80
Wastewater at sewage facilities	0.004–0.007	0.68–0.77

A biochemical research backfilling of filters at water treatment facilities (see Table 4) revealed nitrification activity, indicating immobilization of nitrifying microflora on these backfilling materials. Moreover, the backfill of zeolite had a slightly higher nitrifying activity than the backfill of quartz sand, which is also confirmed by the works of other authors.

Activated sludge in the studied sewage treatment plants (see Table 4) had a relatively high nitrifying activity.

Table 4. Determination of the nitrifying activity of backfilling filters at water treatment facilities and activated sludge at sewage treatment facilities (by biochemical and biokinetic indicators)

Object	Activity of HAO, μg of formazan $(g_{dry\ m.} \cdot h)^{-1}$	The rate of the first phase of nitrification, mg $N\text{-}NH_4$ $(g_{dry\ m.} \cdot h)^{-1}$
Backfilling of fast filters at water treatment facilities:		
quartz sand	7.2	0.085
zeolite	7.8	0.092
Activated sludge of sewage treatment facilities	15–160	0.17–1.82

It was confirmed by the dynamics of the concentration of ammonium nitrogen in the treatment process – a decrease by an average of 92.0% to 2 mg/dm^3 and an increase in the concentration of nitrate nitrogen from 0.2 to 7.5 mg/dm^3. This assumption is confirmed by the microbiological analysis of activated sludge, which found that the concentration of nitrifying bacteria in it is 10^6–10^8 cells/dry things, which leads to an average concentration of nitrifying bacteria in the sludge liquid at the level of 10^7 cells/dm^3. While in the treated wastewater the concentration of nitrifying bacteria is only 10^4 cells/dm^3.

To recap the main point, at the investigated water use facilities, the main role in the nitrification of the aquatic environment belonged to the immobilized microflora: in water treatment facilities – on filter fillings, in biological wastewater treatment facilities – on activated sludge flakes.

Conclusions

Microbiological oxidation of ammonium nitrogen to nitrates (nitrification) in aquatic ecosystems of natural and technogenic origin is carried out by nitrifying bacteria and archaea. This microflora develops in the water column and during immobilization on solid substrates as well (in natural reservoirs on bottom sediments and higher aquatic vegetation, in technical media for filter fillings and activated sludge flakes). In the scientific and technical literature, there is no unanimous opinion about which of the nitrifying microbiocenoses (free-floating or immobilized) makes the main contribution to the nitrification of ammonium nitrogen contained in the aquatic environment. And the identification of the main source of nitrification in aquatic ecosystems is necessary for making technical decisions when managing this process in technical objects and for solving environmental problems in natural water bodies. A methodology is proposed for assessing the contribution of free-floating and immobilized nitrifying microflora to water nitrification in the ecosystem under study, which includes laboratory experimentation to assess the activity of free-floating microflora, biochemical analysis of microflora immobilized on solid substrates, and calculation of the contribution of each of the microbiocenoses to nitrification of the aquatic environment. Using this technique, it was found that:

– in natural reservoirs with a low technogenic load and a low level of pollution by ammonium nitrogen, microbiological nitrification of water is due to processes in bottom sediments;
– in areas of natural reservoirs with increased pollution by ammonium nitrogen, including after the discharge of wastewater, water nitrification is caused by processes in the water column;
– in technogenic environments (water disposal facilities) microbiological nitrification of water is caused by the processes carried out by the microflora immobilized on the solid phase of the system (filter fillings);
– in biological wastewater treatment facilities, nitrification is due to the metabolism of nitrifying bacteria immobilized on activated sludge flakes. The discharge of deeply purified wastewater (containing nitrifying microorganisms) into natural water bodies leads to an increase in the processes of nitrification and the activity of self-purification from nitrogen compounds in them.

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