Wastewater treatment by conversion of nitrogen-containing pollution by immobilized microbiocenosis in a biodisk installation

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Abstract. Experimental works are devoted to research the efficiency of deamonization and de-nitrogenation of wastewater during treatment in a biodisk installation. Working hypothesis - immobilization of the microbiocenosis can provide simultaneous deep treatment of wastewater from organic compounds and inorganic nitrogen-containing compounds. The purpose of this work is to assess the efficiency of purification of highly concentrated organic pollutants and mineralized wastewater from nitrogen compounds in a biodisk installation and to determine the main ecological-trophic groups of microorganisms included in the immobilized nitrogen-transforming microbiocenosis. Methods of research of immobilized microbiocenosis - microbiological. physiological, biochemical (inhibitory experiments), natural and model wastewater - hydrochemical. It has been established that in microbiocenosis immobilized on disks conditions that allow heterotrophic and autotrophic microorganisms to actively metabolize organic and inorganic compounds under aerobic and anoxidic conditions are created. The quantitative indicators of the influence on the kinetics of the processes of the concentration of organic substances in the treated wastewater have been determined. On the basis of microbiological, physiological and biochemical studies in the immobilized microbiocenosis, ammonium-acid bacteria (and, possibly, archaea) nitrite-acid and denitrifying bacteria were found, and in the absence of organic substances in the environment - anammox bacteria. Moreover, in the biofilm that was formed in the absence of organic substances in the environment, the activity of anammox bacteria in deamonization significantly exceeded the activity of ammonium oxidizing bacteria and archaea. The obtained results and quantitative requirements were used in the organization of real wastewater treatment in a biodisc plant in industrial conditions.

Keywords: wastewater treatment, nitrogen compounds, immobilized microbiocenosis, deamonization, denitration, nitrification, anammox process, control effects, optimal parameters.

Conventions:
AOB – ammonium oxide bacteria
AOA – ammonium oxide archaea
NOB – nitrite oxide bacteria
COD – chemical oxygen demand
BOD – biochemical oxygen demand
ANAMMOX (ANaerobic AMMonium OXidation) is a process of anaerobic oxidation of ammonium by nitrite.

1. Introduction

The protection of natural waters from eutrophication caused by the discharge of biogenic elements in the composition of insufficiently treated wastewater is an urgent scientific and practical environmental problem. The technologies of biological treatment of wastewater from nitrogen-containing compounds are based on the vital activity of nitrogen-transforming microbiocenoses, which take part in the biogeochemical circulation of nitrogen, and the methods of control and optimization of the process are based on the ecological properties of these microbiocenoses. In the presence of organic on inorganic nitrogen compounds in the treated wastewater, microbiocenoses of biological treatment facilities are theoretically capable of carrying out the main stages of the nitrogen cycle: ammonification, nitrification, anammox process, assimilation and disimilation nigrate reduction (Xiang et al., 2020).

Adsorption technologies are often used to remove nitrogen compounds from wastewater (Malyovanyy et al., 2013; Sakalova et al., 2019), although biological methods are the most widely used. Traditional technologies of deep removal of nitrogen compounds from wastewater by a biological method are based on the use of microbiological nitrification-denitrification (deamonization and de-nitrogenation of the environment). In recent years, the information on the microbiological links of the nitrogen cycle has expanded significantly, which provides prospects for the use of certain innovative solutions for wastewater treatment systems from nitrogen-containing compounds. Removal of ammonium nitrogen (deamonization) from (ter Haseborg et al., 2010) wastewater during biological treatment occurs mainly through its oxidation by chemolithoautotrophic nitrifying microorganisms under aerobic conditions and anammox bacteria under oxygen-free conditions (Wu Y. et al., 2012; Wu L. et al., 2019; Kallistova et al., 2016).

It is with the anammox process – anoxide oxidation of ammonium during the reduction of NO^{2-} to gaseous nitrogen of anammox-planktomycetam – that leading expert in the field of environmental protection associate the possibility of a radical improvement in the quality of water purification from nitrogen compounds due to the environmental and economic advantages of this method. According to modern concepts, 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^- (Ding et al., 2013; Lancaster et al., 2018; Wu L. et al., 2019). One of the main tasks of purification from biogenic elements is the removal of nitrogen (denitrogenation), which is solved mainly due to the processes of chemoautotrophic nitrification (aerobic process) and anammox process (Malovanyy et al., 2014), as well as heterotrophic denitrification (anaerobic process) (Ji et al., 2020) and to a small extent as a result of assimilation of nitrogen-containing compounds with active biomass of wastewater treatment plants.

However, for the active implementation of nitrification, anammox process, as well as heterotrophic denitrification, different environmental conditions (parameters of wastewater treatment) are required: first of all, oxygen regimes and concentrations of organic substances (Gvozdyak & Sapura, 2009; Litty, 2012; Iurchenko et al., 2020). Thus, microbiological deamonization and denitrogenation of wastewater, caused by the vital activity of autotrophic microorganisms, are complicated by the presence in urban and in the vast majority of industrial wastewater of high and ultrahigh concentrations of organic compounds, which cardinally inhibit autotrophic processes. At the same time, denitrification, which in wastewater treatment plants is mainly due to the metabolism of heterotrophic bacteria, is inhibited in the absence of organic substrates. Another problem of deamonizing and de-nitrogenous microbiocenoses is the need to combine highly active microbiological processes in one microbiocenosis, requiring diametrically opposite oxygen regimes (Malovanyy et al., 2018, Malovanyy et al., 2019).

One of the promising directions for solving these problems is the immobilization of microbiocenoses on a solid carrier, which makes it possible to significantly increase the density and biodiversity of active microorganisms in treatment facilities, increase their resistance to stress, and increase the speed and depth of water purification. In the population of anammox bacteria, biofilm formation is regulated by the quorum sensing system (Feng et al., 2014). In addition, the immobilized biofilm spatially distributes microorganisms (Gvozdyak & Sapura, 2009; Litty, 2012) and allows ecological-trophic groups with opposite metabolic properties (heterotrophs and autotrophs) and the attitude to oxygen (aerobic, microaerophiles, anaerobes) to coexist and actively metabolize.

It is known that the use of compact and economical biodisk plants for biological wastewater treatment makes it possible to significantly intensify the removal of organic pollutants from wastewater (Hassard et al., 2015). However, the processes of transformation of nitrogen-containing compounds by immobilized microbiocenoses of these installations and their ecological properties are rather poorly studied. In addition, the methodology for determining the composition of the immobilized nitrogen-transforming microbiocenosis by simple, inexpensive and accessible methods has been little developed; data on the ecology of such microbiocenoses (microbial composition, trophic and spatial relationships, the influence of environmental factors) are limited.

There is also a lack of information (and practical knowledge) on the influence of environmental factors in a biodisk installation; the efficiency of deamonization and de-nitration of highly concentrated (by organic compounds) and mineralized industrial wastewater.

The purpose of this work is to assess the efficiency of purification of highly concentrated organic pollutants and mineralized wastewater from nitrogen compounds in a biodisk installation and to determine the main ecological-trophic groups of microorganisms included in the immobilized nitrogen-transforming microbiocenosis.

2. Materials and methods

The object of the study was immobilized microbiocenoses, which were formed on the disks of a laboratory installation during the treatment of wastewater of various composition: industrial wastewater from a milk processing enterprise and mineralized model wastewater (Table 1).

Table 1. Chemical indicators of the composition of wastewater, in which the biofilm was formed

Wastewater type	COD, mgO/dm^3	BOD_5, mgO_2/dm^3	$N_{general}$. mg/dm^3	$N\text{-}NH_4$ mg/dm^3	$N\text{-}NO_2$, mg/dm^3	$N\text{-}NO_3$, mg/dm^3
Wastewater of a dairy plant	1200	620	68.5	38.07	0.091	3.54
Model wastewater	0	0	Didn't determine	100	100	0

To determine the optimal parameters of de-nitration and deamonization of real and model wastewater by nitrogen-transforming microbiocenosis, a laboratory installation was developed and manufactured (Fig. 1), which consists of two parallel-installed bioreactors that can operate in synchronous or autonomous modes (Tsytlishvili, 2020).

The working volume of one reactor is 28.3 dm^3. The biomass build-up lasted 2 – 4 weeks, depending on the concentration of organic compounds in the treated wastewater. The total concentration of biomass in the installation ranged from 6.7 to 10.2 g/dm^3, and the proportion of immobilized biomass was 62.3 – 75.2%. Experiments on wastewater treatment in a biodisk installation were carried out under contact and flow conditions. When operating in a flow-through mode, the rate of wastewater supply to the bioreactor ensured the duration of wastewater treatment by the immobilized microbiocenosis – 6.6 hours.

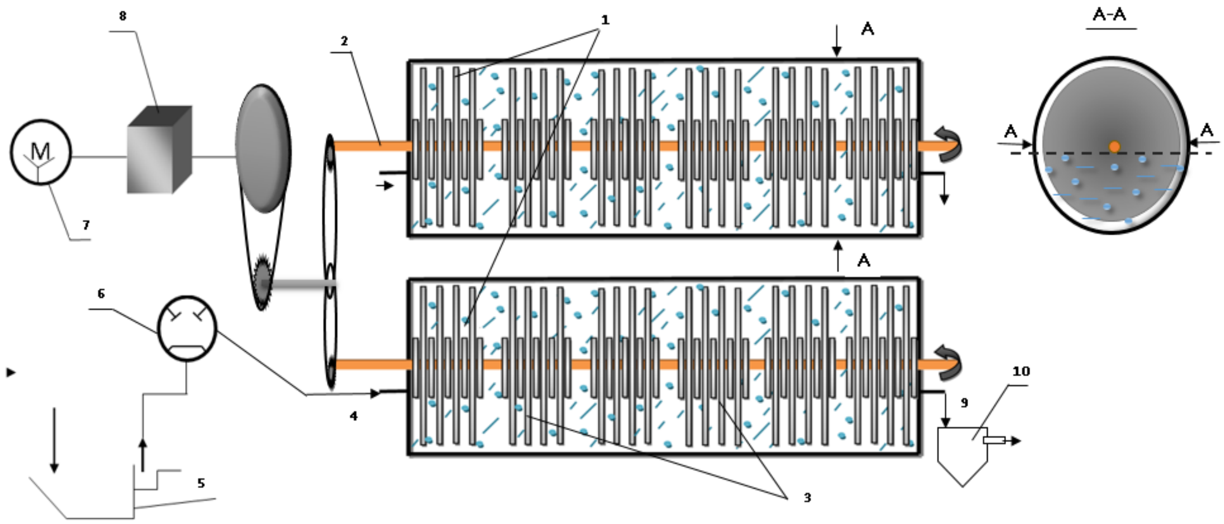


Figure 1. Principle scheme of the laboratory biodisk installation

The unit (Fig. 1) consists of a cylindrical body (1), in which the shaft (2) is located. On it are perpendicularly fixed disks (3) made of polycarbonate, alternating in size. The shaft is driven by a low-speed electric motor (7), the speed of rotation of the disks on the shaft is controlled by a frequency converter (8). Immobilized microbiocenosis is located on the surface of the disks. Waste water is supplied into the bioreactor body through a pipeline (4) from the primary settler (5) by a metering pump (6). After the treatment of the waste liquid by the immobilized biocenosis, the water is supplied through the outlet pipeline (9) to the secondary settling tank (10).

When choosing methods for determining the composition of the immobilized microbiocenosis, it was taken into account that for the scale of treatment facilities and treatment technology, it is practically expedient to have information not about the activity of individual types of bacteria, but the activity of certain ecological and trophic groups and the kinetic indicators of the processes that they perform. Therefore, the study of the composition of the microbiocenosis, which transforms organic and inorganic nitrogen compounds, was aimed at identifying and determing ecological and trophic groups of nitrogen-transforming microorganisms (AOB, AOA, NOB, anammox bacteria and denitrifying microorganisms), primarily those that cause deamonization and de-nitrogenation of wastewater – AOB, AOA, anammox and denitrifying bacteria. Those studies were performed by three independent methods: microbiological, physiological and biochemical (inhibitory analysis).

Microbiological methods were used to determine the concentration of bacteria when inoculating biofilm samples on a solid or liquid elective environment (Shukla, 2017).

Physiological studies of the microbial composition of the immobilized biofilm were performed according to the method (Litty, 2012) by identifying gaseous metabolites formed during wastewater treatment under contact conditions. For this, the immobilized microbiocenosis was removed from the carrier and placed in a selective nutrient environment of the following composition (mg/dm^3): NH_4Cl – 400; $NaNO_2$ – 500; $NaHCO_3$ – 1000; KH_2PO_4 – 450; K_2HPO_4 – 500 and $FeCl_3$ – 20, in vessels with a volume of 5 dm^3 with a sealed lid, a tap and a hose for removing and collecting the gas mixture. Gaseous metabolites were collected in a special device and analyzed on a Nexis GC-2030 Shimadzu gas chromatograph using a TCD thermal conductivity detector. High purity hydrogen was used as a carrier gas. In parallel, the concentration of nitrogen-containing compounds in the aqueous incubation environment was monitored.

In biochemical experiments, an inhibitory analysis based on the action of inhibitors on certain ecological-trophic groups of nitrogen-converting microbiocenosis and their key enzymes was used (Vinogradova & Kozlova, 2012); Lancaster et al., 2018) (Table 2).

Table 2. The effect of inhibitors on microbiological conversions of nitrogen compounds and the activity of some enzymes of nitrogen-transforming microorganisms

Microbiological conversions of nitrogen compounds and enzyme activity	Inhibitors	
	Thiourea	Hydroxylamine
Ammonification	-	+++ [7]
Nitrification of the phase I (AOB)	+++ [9]	! [7]
Nitrification AOA	+++ [9]	Unknown
Anammox	Unknown	+++ [4]
Deamonization	+++ [9]	! [7]
Denitrification	-	+++
Ammonia monooxygenase	+++ [9]	Unknown
Hydroxylamine oxidoreductase	+++ [9]	!
Dehydrogenases of heterotrophic metabolism	-	+++ [7]

Notes: + - weakly suppresses, ++ - actively suppresses, +++ - very actively suppresses, - has no effect,! - enhances.

Inhibitory experiments were carried out with two types of immobilized biofilm. The first type was formed during the treatment of wastewater, which contained organic matter (including nitrogen-containing). The second type was formed in model wastewater without organic matter. The biofilm was washed into flasks with an appropriate incubation environment (when the biomass concentration was brought to 7 g/dm^3): with organic substances (when the COD was brought to 30 mg/dm^3 using waste water) and without them. The concentration of inhibitors in the incubation environment was 10 mg/dm^3. The incubation was performed with constant gentle shaking during 2 – 4 hours. After incubation in an aqueous environment, the concentrations of N-NH_4, N-NO_2, N-NO_3 were determined. Each experiment was performed in three repetitions.

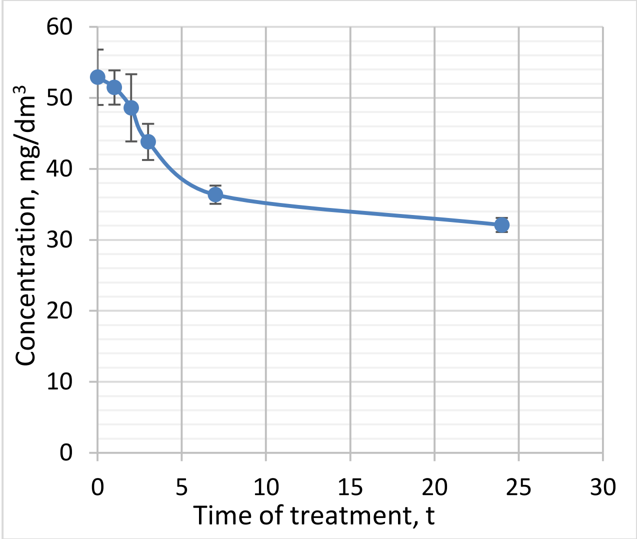
All measurements of the hydrochemical indicators of the composition and properties of liquid environments were carried out according to the certified methods of MVV and KND and according to the methods of scientific research literature (List of methods for performing measurements, 2013). The experimental data were statistically processed using Microsoft Excel.

3. Results and discussion

Presented results of experimental studies of waste water purification from nitrogen compounds in a biodisc reactor. Experimental studies of the dynamics of the total concentration of inorganic nitrogen compounds (Fig. 2a) during the treatment of dairy wastewater under contact conditions showed that after 7 hours of treatment, the concentration of $N_{inorganic}$ – actively decreased, which is associated at the beginning with the assimilation of N-NH_4 (Fig. 2e), denitrification of N-NO_3 (Fig. 2d), and after 3 hours of treatment - with nitrification of N-NH_4 (Fig. 2.b). The rate of removal of $N_{inorganic}$ after 3 hours of treatment (in the absence of organic substances) was 4.7 mg N / (dm^3 h), and the specific velocity (taking into account that the concentration of the ashless biofilm substance in the installation was 2.7 g/dm^3) – 1.8 mg/($g_{without}$ h). The concentration of N-NH_4 (Fig. 2b) slightly increased after the first hour of treatment (the activity of ammonification slightly exceeded the activity of assimilation of N-NH_4). After 3 hours of treatment and removal of organic contaminants (Fig. 2e), deamonization proceeded at a relatively constant rate. The concentration of N-NO_2 remained unchanged during 3 hours of treatment, and during further processing and removal of organic substances began to decrease, which is probably associated with the activation of the phase II of nitrification (Fig. 2c). However, during this period (judging by the dynamics of N-NO_3), the denitrification activity exceeded the nitrification activity of the phase II. The concentration of N-NO_3 (Fig. 2d) began to increase after 7 hours of treatment, and synchronously with a decrease in the concentration of N-NO_2, which turns into N-NO_3.

The balance of the masses - removal (ΔN-NH_4 + ΔN-NO_2) and accumulation of ΔN-NO_3, in the interval after 7 hours of wastewater treatment up to 24 hours (when the organic matter of the wastewater was completely mineralized, and all organic nitrogen was amonificated) practically coincides: $\Delta N\text{-}NH_4 + \Delta N\text{-}NO_2 = 17.7$ mg/dm^3 and, accordingly, $\Delta N\text{-}NO_3 = 16.5$ mg/dm^3. This proves that deamonization predominantly occurs via nitrification, while the annammox process is inactive.

The concentration of nitrogen of inorganic compounds in the studied wastewater at the beginning of treatment with immobilized microbiocenosis was 3 – 3.5 times higher than the concentration of organic nitrogen compounds.



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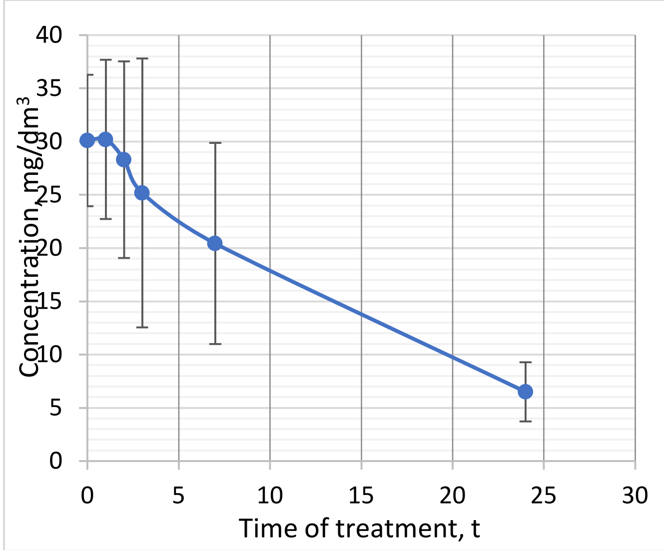

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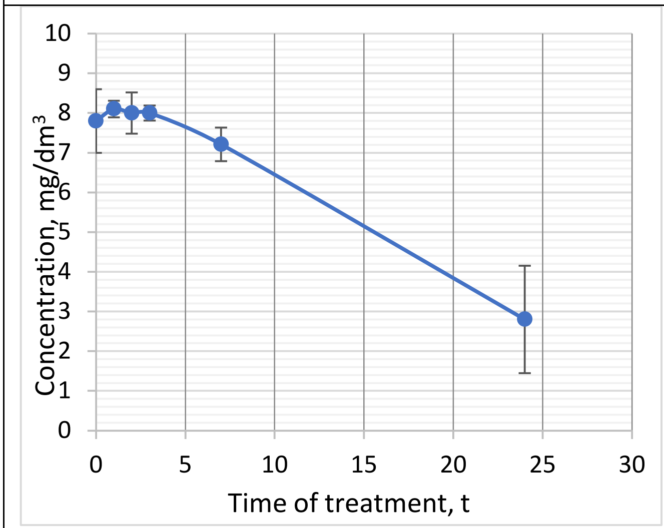

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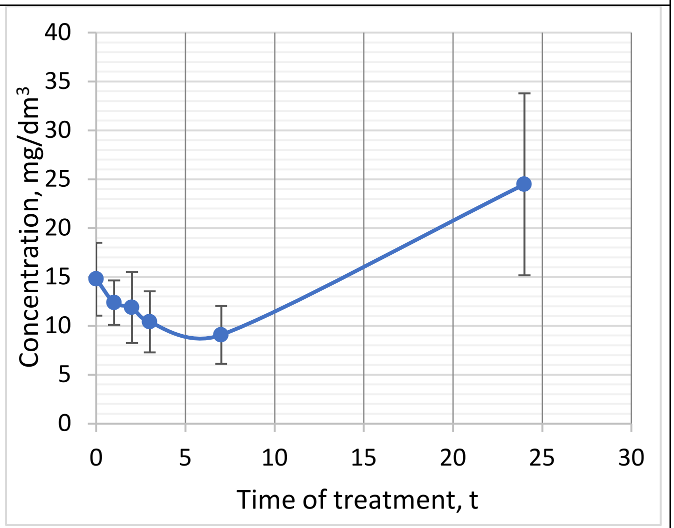

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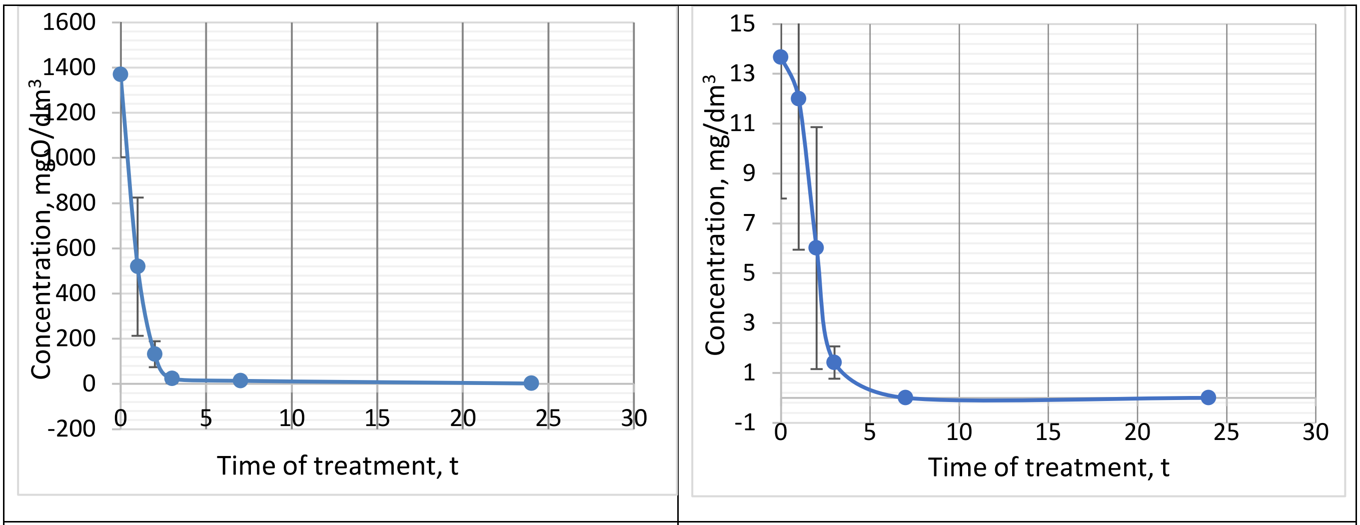

e	f

Figure 2. Concentrations of $N_{inorganic}$ (a), N-NH_4 (b), N-NO_2 (c), N-NO_3 (d), COD (e), N_{org}. (f) in the dynamics of wastewater treatment

The rate of organic nitrogen removal (Fig. 2f) during this period was 4.1 mg N / (dm^3 h). After 7 hours of treatment, there was practically no organic nitrogen in the treated waste water. The overall effect of deamonization (removal of N-NH_4 + N_{org}) during the purification process was 57.6%.

In general, the results of studies in contact conditions have shown a decisive role in the deamonization and denitrogenation of wastewater by immobilization of microbiocenosis the presence and concentration of organic substances as an influential ecological factor in the metabolism of nitrogen-transforming microbiocenosis (Tsytlishvili, 2021).

The results of studies carried out under flowing conditions of wastewater treatment showed that the immobilized microbiocenosis adapted to deamonization of the environment under extreme conditions for autotrophic microflora - extremely high COD (≥ 1000 mgO / dm^3). Probably, in the biofilm immobilized on the disks, such spatial relationships have developed that allowed heterotrophic and autotrophic microorganisms to actively metabolize both organic and inorganic compounds under aerobic and anoxidic conditions.

The study of the efficiency of removal of nitrogen compounds by immobilized microbiocenosis from wastewater of various composition in a flow-through mode was carried out in three treatment modes (Table 3). It was experimentally established that in the 1st mode with an increased COD load (600 – 1300 mgO/dm^3) and ammonium nitrogen (25.0 – 68.1 mg/dm^3), the specific rate of removal of N-NH_4 was 1.30 mg/($g_{ashless\ comp}$ h) at a biomass concentration in the bioreactor of 6.8 g/dm^3. The efficiency of deamonization in the 1st treatment mode reached 98%.

At mode 2 – low COD concentrations (38 – 150 mgO/dm^3) and an increased load of ammonium nitrogen (15.4 – 110.6 mg/dm^3), the specific rate of $N\text{-}NH_4$ removal was 1.57 mg $N\text{-}NH_4$ /($g_{ashless\ comp}$ h) at a biomass concentration in the installation of 7.35 g/dm^3. The efficiency of deamonization at the 2-nd treatment mode reached 97–98%.

In the third mode - an increased load of ammonium nitrogen (26.6 – 143.4 mg/dm^3) and the absence of organic compounds, the specific rate of $N\text{-}NH_4$ removal was 4.34 mg $N\text{-}NH_4$ / ($g_{ashless\ comp}$·h) at a biomass concentration in the installation of 3.4 g/dm^3. The effect of purifying wastewater from nitrogen compounds (denitrogenation) reached 92.5%. Thus, a decrease in the concentration of organic compounds in the treated waste water contributed to an increase in the specific rate of removal of ammonium nitrogen by the immobilized microbiocenosis in the biodisk installation.

Table 3. Indicators of wastewater deamonization in a biodisk installation with a flow-through treatment mode

Treatement modes	Specific rate of removal of $N\text{-}NH_4$, mg $N\text{-}NH_4$ / $g_{ashless\ comp}$·h)	Oxidizing power of the biodisk installation for $N\text{-}NH_4$, mg $N\text{-}NH_4$ / (dm^3 ·day)
1	1.3±0.2	167.0±4.4
2	1.57±0.31	224.0±5.1
3	4.34±0.73	308.0±3.81

Determination of the composition of nitrogen-transforming microbiocenosis immobilized on the disk

The results of studies of the biofilm from the installation, which operated for a month on the waste water of a dairy plant, are presented in the Table 4.

As it can be seen, the biofilm is dominated by ecological-trophic groups according to the heterotrophic type of metabolism (saprophytes, ammonifiers, denitrifiers), which reflects the presence of high concentrations of organic substances in the treated wastewater.

Table 4. Composition of ecological-trophic groups in the immobilized biofilm of a laboratory biodisk installation

Ecological and trophic groups	Bioreactor area	
	Start	Middle
Saprophytes, cells/$g_{dry\ matter}$	$6.6 \cdot 10^9$	$2.6 \cdot 10^5$
Ammonifiers, cells/g	$1.5 \cdot 10^5$	$9.5 \cdot 10^7$
Nitrifiers (AOB), cells/g	$9.5 \cdot 10^5$	$4.5 \cdot 10^4$
Denitrifiers, cells/g	$9.5 \cdot 10^5$	$11.0 \cdot 10^7$

Physiological studies of the dynamics of the concentrations of metabolites of immobilized nitrogen-transforming microbiocenosis dissolved in wastewater (Table 5) indicated the presence in the studied microbiocenosis of such ecological-trophic groups as AOB (possibly AOA), NOB and denitrifiers, since the concentrations of $N\text{-}NH_4$, $N\text{-}NO_2$ and $N\text{-}NO_3$ began to decrease. The analysis of gaseous metabolites showed that the concentration of gaseous N_2 in the gas-air environment increased, reaching 85.7% at the end of the experiment (Table 6). Such an activity of accumulation of gaseous nitrogen could not be due to denitrification, since the activity of this process was inhibited by the absence of organic matter in the environment. In addition, during the entire experiment, against the background of the active formation of gaseous nitrogen, carbon dioxide was present in minimal concentrations. Based on these facts and on the basis of developments (Litty, 2012), it can be concluded that the accumulation of gaseous nitrogen in the studied immobilized microbiocenosis is due to the presence of anammox bacteria.

Table 5. The chemical composition of the aquatic environment in the cultivator in the dynamics of the cultivation of the immobilized biomass

Nitrogen compounds that were controlled	Concentration of nitrogen-containing compounds (mg/dm^3) during the selection period, day					
	0	3	0	7	0	14
$N\text{-}NH_4$	119.7	74.9	120.73	64.4	127.5	25.3
$N\text{-}NO_2$	136.6	0.88	123.9	<0.03	100.1	<0.03
$N\text{-}NO_3$	7.34	2.55	18.81	<0.5	7.02	1.87

Table 6. The composition of biogas in the dynamics of cultivation

Components of the gas mixture	Chemical composition of the gas phase (%) for the selection period, day			
	0	3	7	14
$Ar + O_2$	93.0 + 7.0	6.23	5.93	5.21
N_2	absent	51.33	81.61	85.69
CH_4	absent	42.43	12.21	7.30
CO_2	absent	0.32	0.25	1.80
H_2S	absent	less than 0.001	less than 0.001	less than 0.001

The results of the inhibitory experiment in the incubation environment with an organic substrate (averaged values from three repetitions) are presented in the Table 7. Waste water, added to the incubation environment, created the concentration of organic nitrogen 1.3 – 5.5 mg/dm^3 in it. For each of three parallel experiments, this concentration was determined separately according to the difference between the initial concentration of N-NH_4 and the concentration in the experiment with the addition of thiourea. The withdrawal of N-NH_4 in the assimilation processes (with the production of COD ~ 30 mgO/dm^3) can be neglected. We assume that the denitrification activity in the control variant and with the addition of thiourea had the same activity. The addition of hydroxylamine to the environment increased the concentration of the substrate for the formation of N-NO_2 AOB and AOA and the total content of $N_{inorgenic}$ on ~ 5 mg/ dm^3.

As can be seen from the presented data, in the control variant of the experiment, deamonization of the environment was actively occurring, which was accompanied by a certain increase in the concentration of N-NO_2, which indicates the presence of metabolism of ammonium-oxidizing microorganisms (AOB / AOA).

Table 7. Results of the inhibitory experiment in the incubation environment with organic substrates

Indicators	Change in concentration (mg/dm^3) in the environment after incubation with inhibitors		
	Control (without inhibitors)	Thiourea	Hydroxylamine

$N\text{-}NH_4$	-4.9 ± 2.44	+3.9±1.73	-4.6±0.67
$N\text{-}NO_2$	+1.1 ±0.92	-0.4±0.09	+2.8±2.98
$N\text{-}NO_3$	-0.5±0.15	+0.2±2.84	+3.3±4.91
$N_{inorganic}$	-6.7±0.13	-0.1± 2.17	+0.2± 3.47

The calculation includes loads for organic nitrogen (on average 3.2 mg/dm^3), which, under aerobic conditions, as a result of ammonification, was converted into $N\text{-}NH_4$. Denitrification was recorded. The total de-nitrogenation of the environment was 6.7 mg/dm^3 with deamonization of 4.9 mg/dm^3.

In the variant of the experiment with the addition of thiourea, which inhibits the metabolism of AOB and AOA, the removal of $N\text{-}NH_4$ was not recorded. Therefore, the metabolism of anammox bacteria did not manifest itself. The concentration of $N\text{-}NH_4$ increased slightly due to the ammonification of organic substrates in accordance with the concentration of N_{org}. A certain increase in the concentration of $N\text{-}NO_3$ was observed with a decrease in $N\text{-}NO_2$, probably as a result of the activity of NOB. There was practically no de-nitrogenation of the environment.

In the variant with the addition of hydroxylamine, which suppresses heterotrophic processes (denitrification) and the metabolism of anammox bacteria, active nitrification (active deamonization and an increase in the concentration of $N\text{-}NO_2$) was observed, due to the metabolism of AOB, and, possibly, AOA. Deamonization under these conditions practically coincided with deamonization in the control variant, and practically no de-nitrogenation occurred in comparison with the control variant. Thus, in the first series of inhibitory experiments, which were performed with an immobilized biofilm, formed in the presence of organic substances in the environemt, the activity of AOB (AOA), NOB, and denitrifying bacteria was found in the biofilm.

The second series of inhibitory experiments were performed with an immobilized biofilm formed in a mineral environment in the absence of organic matter (Table 8).

Table 8. Results of the inhibitory experiment in the incubation environment without organic substrates

Indicators	Change in concentration (mg/dm^3) in the environment after incubation with inhibitors		
	Control (without inhibitors)	Thiourea	Hydroxylamine

N-NH_4	-4.1±0.89	-7.9±0.51	-2.8 ±0.56
N-NO_2	-2.7±0.67	+1.6±0.56	-4.5±0.64
N-NO_3	-0.8±0.73	-2.5±0.22	+6.3±0.33
$N_{inorganic}$	-7.6±0.47	-8.8±0.39	-0.4±0.22

Notes: + - increase in concentration - – decrease in concentration.

As can be seen from the presented data, in the control variant, deamonization of the environment occurred, and the concentration of N-NO_2 + N-NO_3 did not increase. It is known that the process of denitrification in the environment without organic substances is significantly suppressed, but the denitrogenation that was observed, was very active, and, mainly (by 90%), due to the removal of N-NH_4 and N-NO_2. A possible reason for this balance of denitrogenation is the presence of anammox process.

When the metabolism of AOB and AOA was suppressed by thiourea, deamonization was even intensified in comparison with the control (the opposite dynamics is comparable with the data in Table 7). Such deamonization in the variant with inhibition of nitrification can only be explained by the presence of the anammox process. The concentration of N-NO_2 slightly increased against the background of an active decrease in the N-NO_3 concentration. Since the nitrification of N-NH_4 to N-NO_2 in this variant is inhibited, a decrease in the concentration of N-NO_2 during oxidation to N-NO_3 and an increase in the concentration of the last one were more expected. In addition, the environmental conditions - the complete absence of organic substrates - inhibit denitrification. Therefore, such dynamics of N-NO_3 and N-NO_2 can be explained by the known ability of anammox bacteria (Anyusheva & Kalyuzhny, 2007) to reduce N-NO_3 to N-NO_2. The total denitrogenation in this variant was even higher than in the control variant.

When anammox bacteria are suppressed by hydroxylamine, the removal of N-NH_4 from the incubation environment is due to nitrification. Accumulation of nitrates was observed, probably as a result of high activity of NOB and inhibition by hydroxylamine of heterotrophic denitrification.

On the whole, as the obtained data show, the anammox process made a greater contribution to the overall effect of deamonization of the environment compared to nitrification, and inhibition of AOB (possibly also AOA) enhanced the anammox process and deamonization and deisotization of the incubation environment by it. This phenomenon is possible due to the formation of nitrifiers of the phase I (AOB and AOA) of hydroxylamine, an inhibitor of the anammox process.

Thus, in the experiments with the immobilized biofilm, formed in the absence of organic substances in the environment, a high activity of anammox bacteria, AOB (possibly AOA), an activity of NOB and a weak activity of denitrifying bacteria were found in it.

4. Conclusions

It was determined that immobilization on biodisks made it possible to obtain a microbiocenosis which actively purified highly concentrated according to the pollution, wastewater from nitrogen-containing compounds. Among the studied environmental factors, the most significant for the deamonization and denitrogenation of wastewater by immobilized microbiocenosis in contact conditions is the concentration of organic matter.

The total effect of removing N-NH_4 (inorganic and in the composition of organic substances - proteins and amino acids) in a biodisk installation with wastewater concentrated according to organic contaminants was 57.6% in the contact mode of treatment, and 98% in the flow mode. The specific rate of removal of N-NH_4 in the flow-through mode of treatment in the presence of organic substances reached 1.6, in the absence – 3.4 mg/($g_{ashless\ comp}$ h) and 4.7 mg/($g_{ashless\ comp}$ h) in contact mode.

In the studied nitrogen-transforming microbiocenosis, formed on biodisks, the following ecological-trophic groups were identified: when adapting in the presence of organic substances - amonifying, AOB (AOA), NOB, denitrifying, when adapting in the absence of organic substances - amonifying, AOB (AOA), NOB, anammox bacteria, denitrifying. The presence of organic substances surpressed the chemoautotrophic microflora (AOB, AOA, NOB, anammox bacteria). During deamonization of the environment by microbiocenosis of the biofilm, formed in the absence of organic substances in the environment, the activity of anammox bacteria significantly exceeded the activity of AOB and AOA. Inhibition of AOB and AOA intensified the anammox process (in the scientific literature such dependence is not covered).

The revealed dependencies are an important tool for the management and intensification of the process of deep purification of wastewater from nitrogen compounds. The obtained quantitative dependences are necessary for forecasting and mathematical modeling of industrial wastewater treatment processes from nitrogen compounds (primarily from ammonium nitrogen - deammonization) while effectively removing organic compounds, to choose the treatment scheme and optimize its technological parameters. The researched biodisk unit has been successfully tested in the treatment of wastewater from a livestock farm from organic and nitrogen-containing contaminants in industrial conditions.

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