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Mechanism of resistance of microorganisms to nickel compounds

Nikiforova Lidiya Osipovna [1,*] and Mousse Smail Rauf [2]

[1] *Russian Geological Prospecting University, Faculty of Hydrogeology, Department of Construction of Water Supply and Sanitation Systems, Moscow, Russia.*
[2] *Moscow State University of Food Production, Faculty of Biotechnology, Department of Technosphere Safety, Moscow, Russia.*

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Abstract

The purpose of the research was to accumulate results proving the ability of biocenosis in natural water bodies and biological treatment facilities to maintain stability at nickel concentrations above the MPC up to 30% without reducing biomass growth. Studies have shown that microbial communities in the aquatic environment synchronize natural defense systems and are able to change the mechanisms of resistance. The complex formation of nickel with inorganic compounds and mixed-ligand complexes of conjugated bases of amino acids makes it possible to obtain three-dimensional spatial structures near the silt particle that bind nickel ions and thereby reduce its toxic effect.

Keywords: Microorganisms; Nickel compounds; Toxicity; Complex formation

1. Introduction

Large consumption of various nickel products by the population inevitably leads to environmental pollution [1]. A large amount of medical research has made it possible to prove the toxicity of nickel compounds both in the form of aqueous solutions and in the form of fine dust suspensions and the occurrence of a number of diseases in the population in contact with these systems [2, 3, 4]. Therefore, the International Agency for Research on Cancer (IARC) in 1990 classified all nickel compounds as Group 1 human carcinogens [5].

Natural water systems tend to contain negligible concentrations of nickel compounds. An increase in the concentration of nickel ions in open water bodies is always associated with the discharge of industrial wastewater that has not undergone a complex purification process at local treatment facilities. Therefore, pollution of the aquatic environment with heavy metals is constantly increasing. The dissolution of heavy metals leads to an increase in their concentrations in the water and soils of the surrounding biotopes [6]. As a result of the entry of heavy metals into water bodies, constant changes are observed in microbes, that is, a mutation is recorded. As a result of mutation, microorganisms acquire new properties. Therefore, studies are underway both to assess the concentrations of heavy metals necessary for the life of microorganisms as trace elements, and these metals are considered as energy sources and electron acceptors. Ecological regulation of maximum permissible concentrations of heavy metals in water bodies provides for elucidation of the influence of concentrations that can cause the destruction of microbial communities, the death of certain types of microorganisms, as well as disrupt the processes of microbial biodegradation of pollutants.

The purpose of this study was to accumulate results proving the ability of microbial communities in natural water bodies and biological treatment facilities to maintain stability in the presence of nickel compounds at concentrations above the MPC without reducing biomass growth using metabolic products.

* Corresponding author: Nikiforova Lidiya Osipovna

Understanding these processes will allow in the future solving a number of environmental problems associated with the transformation of heavy metal compounds and detoxification of the natural environment.

2. Material and methods

2.1. Cultures of microorganisms

In the work, we use bacterial cultures obtained from the Russian Collection of Microorganisms in Push chino (Russia).

2.1.1. Bacteria that oxidize lactate to acetate and CO_2*(group A)*

- *Desulfovibrio desulfuricans BKM 1388;*
- *Desulfovibrio desulfuricans BKM 1799;*
- *Desulfovibrio vulgaris BKM 1760;*
- *Desulfovibrio gigas BKM 1382;*
- *Desulfotomaculum nigrificans BKM 1492;*
- *Desulfotomaculum orientis BKM 1628;*
- *Desulfomicrobium baculatus BKM 1378;*
- *Desulfobacterium macestii BKM 1598;*
- *Thermodesulfobacterium mobile BKM 1128.*

2.1.2. Bacteria that oxidize lactate to CO_2 *(group B)*

- *Desulfobacter postgatei BKM 1643;*
- *Desulfotomaculum kuznetsovii BKM 1805.*

The following cultures of bacteria were also used in the research. The genera *Acidithiobacillus* and *Pseudomonas* belong to close groups, so *Pseudomonas putida BS394* and *P. putida KT2442* were used in plasmid transfer experiments. *Pseudomonas* strains are aerobes and grow on standard nutrient media. For growth, it is necessary to maintain a pH of 6.0 – 8.0.

2.2. Cultivation

Bacterial cultures were grown on an agar medium and washed off with a liquid medium of the same composition. Then it was poured into test tubes containing different concentrations of nickel salts. Cultivated at 24°C and constant stirring on an orbital shaker for 12 days. Maintained 180 rpm. The concentrations of $NiCl_2$ and $NiSO_4$ from 0.1 to 2.0 mg/dm^3 were used. In studies with the transfer of nickel resistance plasmids, bacteria were grown on Luria – Bertani medium [7]. For endogenous isolation of plasmids, nickel salts and 100 CFU/ml of fresh cells of the acceptor strain were placed in test tubes with Evans medium and placed in a thermostat at 28°C for 3 days. After this time, inoculations were carried out in dishes with Luria – Bertani medium and with the addition of $NiCl_2$ or $NiSO_4$. Exogenous isolation of plasmids was carried out in dishes with Luria – Bertani agar medium with and without addition of nickel compounds. A solution of a certain concentration of nickel was placed in the center of the Petri dish, and then a fresh bacterial suspension of the recipient with a concentration of 1010 CFU/ml was added.

2.3. Quantification of bacteria

Quantitative counting of bacteria was carried out by direct counting of cells during microscopy in a Goryaeva chamber, and for pseudomonads, counting was carried out according to the number of CFU.

2.4. Tests used to determine wastewater toxicity

To determine the toxic concentrations of nickel compounds in the aquatic environment, the methods of biological tests were used, which characterized the biological reactions of microorganisms. To do this, we first studied the reaction of a "pure culture" of a certain type of aquatic organisms to the addition of a toxicant (nickel solution) and determined changes in some characteristic, accurately measured indicator, which integrally reflects violations of important vital functions. When conducting research, such indicators were: 1) release or absorption of oxygen, 2) assimilation and absorption of carbon dioxide, 3) growth rates and other manifestations of the vital activity of the microorganism. The

data obtained in the control system was taken as 100%. Changes in indicators compared to the control system of more than 10% were accepted as significant and were evaluated as a toxic effect of nickel compounds. Microbiological tests are sensitive qualitative and quantitative indicators for heavy metal compounds in wastewater. *Daphnia magna* was also used for bio testing. Microbiological tests characterized the response of the test object to the damaging effect of different concentrations of nickel compounds.

The possibility of increasing resistance to nickel compounds was tested on a model object, a potential intermediate host for the transfer of resistance plasmids, representatives of the genus *Pseudomonas*. Aboriginal bacteria of nickel sulfide ores were used as starting material for isolation of plasmids of resistance to nickel compounds. This was due to the fact that a number of native bacteria are carriers of heavy metal resistance plasmids [8]. Native thionic bacteria in these experiments were considered as donors of plasmids with genes for resistance to nickel ions. And the strains of *Pseudomonas putida BS394* and *P. putida KT2442* were used as recipients. *Pseudomonas putida BS394* is a cysteine marker characterizing resistance to gentamicin.

P. putida KT2442 – markers – resistance to kanamycin, fluorescence of the *Gfp* protein, accompanied by a green glow under UV irradiation with λ = 254 nm on Luria – Bertani medium. If it was not possible to isolate and further cultivate plasmid – containing cultures, then endogenous and exogenous isolation of plasmids was performed. During exogenous isolation, the natural substrate and the recipient strain were mixed with native microorganisms. Next, the mixture was cultivated on a nutrient medium, after which trans conjugated clones of the recipient strain were isolated and selected. In the case of endogenous isolation of plasmids, the recipient strain was directly introduced into the natural substrate. After incubation, it was isolated and trans conjugated clones of the recipient were selected. In the course of experiments on endogenous isolation of plasmids from the microflora of nickel ores, there was no growth of colonies of acceptor strains on agar media. This indicated the absence of transfer and expression of plasmids with genes for resistance to nickel ions in these strains.

2.5. Research methods

The research methods were pH and spectrophotometry in combination with mathematical modeling, IR spectroscopy, and quantum chemical calculations. pH values were determined in aqueous solutions and titration was performed using a pH meter Unipractic (pH measurement accuracy on average ± 0.05 log units) and on a Metrohm Basic Titrino 794 automatic titrated with a Metrohm 6.0228.000 glass electrode (pH measurement accuracy in average ± 0.001 log. units) under thermostat ting conditions – 25°C with an error of ±0.1°C. The optical density of solutions was determined on a PerkinElmer Lambda–35 spectrophotometer with an accuracy of ±0.001 units, in a quartz cuvette 1.0 cm thick.

Modeling of the parameters of complex formation equilibria and calculation of the spectral characteristics recorded in solutions were carried out using the programs CPESSP (Complex Formation Parameters of Equilibrium in Solutions with Solid Phases), и STALABS [9]. The images were recorded on an electron microscope (Hitachi S-4800, Japan) in accordance with the manufacturer's instructions.

2.6. Experimental part

All solutions were prepared in distilled water with the addition of KNO_3 to maintain a constant ionic strength of the solution. The total concentration of nickel (II) in an aqueous solution was determined by titration [10]. Amino acids were purified by recrystallization from distillate. Working solutions were prepared by accurately weighing the preparations. Complexation of nickel with amino acids was studied by potentiometric titration using a combined glass electrode. The potential measurement accuracy was 0.01 mV. The temperature of the potentiometric cell and electrodes was maintained with an accuracy of ±0.1°C using a thermostat. The concentration of amino acids in the cell was 0.001 mg/dm^3. Three parallel measurements were carried out. The stability constants of complex compounds were calculated using the New DALSFEK program [11].

2.7. Statistical analysis

The data obtained are presented as mean ± standard errors (S.E.) for n=10 experiments. Significant differences were determined using R software or Student's t–test. Significance was established at $p \leq 0.05$.

3. Results and discussion

Nickel ions can be in the aqueous medium in the form of various complex particles, and the ratio between them can be any. To characterize the depth of complexation, Bjerrum introduced the concept of average coordination number ($\overline{n}$). The depth of complex formation, characterized by the average coordination number, is only a function of the equilibrium

concentration of the ligand. To calculate the distribution of nickel over various complex forms, it is necessary to have data on the stepwise formation constants and the equilibrium concentration of the ligand.

The stability constants of the complexes for different amino acids differ slightly [12]. Calculation of the stability constants of pure ligands and binary complexes showed that mixed ligand complexes have increased stability [13]. Literature data show that the mixed ligand complexes of nickel with glycine and phenylalanine are the most stable [14]. For amino acids in which an additional donor group has an average acidity ($pK_a < pK_{ADG} < pK_b$), there is a possibility of different combinations of nickel ion binding [15, 16]. It is impossible to clearly predict the complexing ability of nickel in natural and waste waters due to the fact that the processes of complex formation are associated with differences in the degree of oxidation of both inorganic ligands and the distribution of electron density in amino acids, which depends on the chemical composition of the aqueous medium.

In wastewater, the presence of inorganic compounds of heavy metals promotes the formation of ligands in the form of a suspension. Nickel compounds $[Ni(H_2O)_6]^{2+}$ in wastewater at values of 6.0< pH >8.0 are green; cobalt compounds are pink at concentrations up to 0.1 mg/dm^3 (figure 1).

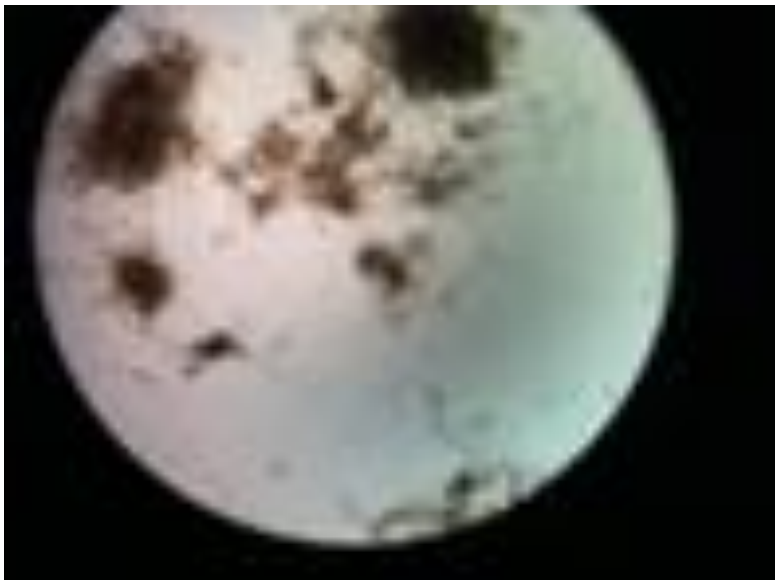

Figure 1 Phase-contrast microscopy of the aquatic environment of the bioreactor containing compounds of nickel and cobalt

Any toxic substance in threshold and subthreshold concentrations simultaneously disrupts various aspects of cell metabolism. Penetration of nickel ions into cells is initially compensated by the natural ability of microbes to compensatory-adaptive reactions that ensure the regulation of energy balance. Therefore, for practical purposes, it is necessary to determine the concentrations of substances that cause the toxic effect of nickel ions on the kinetics of biochemical processes of oxidation of organic compounds and the rate of biomass growth.

3.1. Physiological and biochemical parameters of microorganisms under the action of nickel compounds

The changes that occur in the cells of microorganisms are primarily associated with the morphology of the cells. When cultivating microorganisms on media in the presence of high concentrations of heavy metals, an increase in cell size is observed, similar to the effects that are observed when unfavorable chemical and physical factors act on microorganisms. In most cases, these disorders are associated with dissociation of the processes of growth and cell division.

The conducted studies showed that an increase in the concentration of nickel ions in water up to 0.1 mg/dm^3 had no effect on the growth of bacteria. The number of cells in the control sample and in samples with different concentrations of nickel ions from 0.01 to 0.1 mg/dm^3 after 10 days of cultivation practically did not differ (table 1). A further increase in the concentration of nickel ions in the medium to 0.2 mg/dm^3 led to a slowdown in the growth of bacteria in comparison with the growth in the control sample. Changes in the pH value of the liquid medium occurred the higher, the greater the concentration of nickel ions.

Table 1 Number of bacteria at various concentrations of nickel ions in the medium (initial number 6.3 · 10^8cells/ml)

Nickel ion concentration, mg/dm^3	Number of cells in 1 ml after 10 days	Dynamics of pH change in the environment	
			ΔpH
0.000	2.45 x 10^9	6.00	0.00
0.010	2.43 x 10^9	5.96	0.04
0.025	2.31 x 10^9	5.91	0.09
0.050	2.28 x 10^9	5.87	0.13
0.075	2.15 x 10^9	5.83	0.17
0.100	2.03 x 10^9	5.70	0.30
0.125	8.90 x 10^8	5.43	0.57
0.200	7.20 x 10^8	5.36	0.64

In this case, acidification of the medium was observed due to an increase in the concentrations of H^+ ions. This is explained by the fact that the $NiSO_4$ salt was used, which undergoes hydrolysis in an aqueous medium.

To increase the resistance of bacteria to nickel compounds, studies were carried out on representatives of the genus *Pseudomonas* on the transfer of resistance plasmids of a potential intermediate host. Aboriginal bacteria of nickel sulfide ores, which are carriers of resistance plasmids to a fairly large amount of heavy metals, were used as starting material for the isolation of plasmids of resistance to nickel ions [17, 18]. Native thionic bacteria served as potential donors of plasmids with resistance genes, and *Pseudomonas putida BS394* and *P. putida KT2442* strains were used as recipients. Endogenous and exogenous isolation of plasmids was carried out, and then plasmid-containing cultures were cultivated.

During exogenous isolation, the recipient strain and natural substrate were mixed with native microorganisms, and the mixture was cultivated on Bertani agar medium (at a concentration of 1 mM for *P. putida BS394* and 5 mM for *P. putida KT2442*) [19].

Then, the isolation and selection of trans conjugated clones of the recipient strain *KT2442* was carried out in the case of the introduction of nickel ores $CanT - A$ and $CanT - C$, which were cleared on the Luria Bertani medium with a high content of nickel chloride. During further studies, *KT2442* trans conjugants were obtained, which retained the ability to grow up to a nickel ion concentration of 0.4 mg/dm^3. Therefore, due to exogenous isolation of natural plasmids of resistance to nickel ions, it is possible to increase the resistance of the *Pseudomonas* strain by 4 times.

Studies on the endogenous isolation of plasmids from the microflora of nickel ores have shown that there is no increase in the growth of colonies of acceptor strains on agar media. Thus, in these strains, there is no transfer and expression of plasmids with genes for resistance to nickel ions.

3.2. Investigation of the survival of microbial populations in biological treatment facilities in the presence of higher concentrations of nickel compounds than MPC values

The efficiency of biological wastewater treatment depends on the totality of bio sorption processes and the rate of biochemical oxidation of pollutants by microorganisms. The complexity of the associative relationships of structure – forming bacterial cultures of activated sludge largely depends on the structure and level of development of microbial particles. The bacterial composition of silts is usually represented by *p.p. Pseudomonas, Bacterium, Bacillus, Sarcina, Micrococcus, Corenebacterium, Mycobacterium, Actinomyces, Nocardia*. Also, in addition to heterotrophic bacteria, activated sludge always contains autotrophic bacteria.

As a result, chemical compounds are always present near the surface of activated sludge particles, which are metabolic products and which are absent in the aquatic environment. These compounds are usually classified as chemically active substances.

Nickel ions are among the trace elements necessary for the development of microorganisms, as they take part in enzymatic reactions and oxidative processes. Therefore, they easily form complexes with amino acids. Nickel ions readily form complexes with highly hydrophilic amino acids [20, 21, 22, 23, 24]. In complex compounds, anions of amino acids: arginine, histidine, lysine, serine, aspartic acid, glutamic acid and ATP in aqueous solutions are bidentate cyclic ligands and are coordinated due to the nitrogen of the amino group and the oxygen of the carboxyl group. These are the "LX" ligands. They almost always occupy a position on the outer surface of the protein molecule. As a result, a five – membered chelate ring is formed.

As a rule, activated sludge is usually resistant to the action of various chemicals contained in wastewater, since the biocenosis of aquatic organisms is formed based on the availability of available nutrients, and microorganisms adapt to the specific composition of wastewater treatment plants.

The concentration of nickel compounds in water up to 0.1 mg/dm^3 does not have a toxic effect on the biocenosis of aeration structures. Increasing the concentration to 0.2 mg/dm^3 leads to a decrease in the specific oxidation rate, this reduces the oxidizing power of aeration structures (table 2). Consequently, nickel ions replace cations in the active centers of enzymes and inhibit the enzymatic processes of oxidation of organic pollutants in an open reservoir or in an bioreactor. As a result, in order for the purified water to have the same values of controlled indicators, it is necessary to increase the duration of aeration. And this leads to an increase in the volume of structures and an increase in construction and operating costs. Oxidation capacity of the aeration structure

Table 2 Dependence of work efficiency aeration structure from the concentration of nickel compounds in the aquatic environment

Nickel ion concentration, mg/dm^3	**Specific oxidation rate, mg BOD/g_{silt} · h**	**Oxidizing power of the bioreactor, g/m^3 · day**	**Aeration duration, h**
0.00	38	700	5.0
0.05	35	750	5.0
0.10	32	730	5.0
0.12	30	680	5.4
0.15	25	670	6.0
0.20	18	580	6.2

3.3. The mechanism of resistance of microbial communities to nickel compounds, due to the peculiarities of its chemical structure

A feature of group VIII atoms, including nickel, is *d* – compression due to the penetration of electrons to the pre external *d* – sublevel. The electronic configuration of the nickel atom is:$1s^2 2s^2 2p^6 3s^2 3p^6 3d^8 4s^2$. In accordance with the rules for filling levels and sublevels, the nickel atom has 2 unpaired electrons at the pre external 3*d* – sublevel.

As a result, changes in chemical properties are observed, since d-metals and their ions have unpaired electrons. This helps, when absorbing energy, to move from the main energy levels and sublevels to higher levels, that is, to an excited state. As a result, the reducing properties of metals increase and the oxidizing properties decrease. In biochemical reactions, nickel ions act as complexing metals. Ligands in this case are biologically active substances, both organic compounds and anions of inorganic acids.

The spatial volume of these aqua complexes reduces the ability of nickel ions to penetrate into cells, but increases the probability of their adsorption on the surface of the microbial community agglomerate. As a result of the formation of such an adsorption layer, the rate of dissolved oxygen supply to the surface of microorganisms decreases. An automated system of devices with an increase in the concentration of nickel compounds in the aquatic environment records a decrease in the rate of oxygen consumption for endogenous respiration (table 3).

Table 3 Effect of nickel ion concentration on the rate of endogenous respiration

The concentration of nickel ions in the aquatic environment, mg/dm^3	**The rate of endogenous respiration, mg O/g_{silt} · h**
0.00	7.30
0.05	7.46
0.10	7.52
0.12	7.64
0.15	7.0
0.20	4.9

The decrease in cell permeability to nickel ions is also due to the fact that bulky aqua complexes formed near the surface of the microbial community (activated sludge particles) have low values of the chemical index «Solubility Product» (SP/PR). It is these poorly soluble agglomerates that perform protective functions against the penetration of free nickel ions into cells with an increase in their concentration, since they act as an adsorbent. Thus, they play the role of a biochemical barrier.

3.4. Comparison of the effect of nickel and cobalt compounds on the structure of aqua complexes

In natural ecosystems, nickel compounds are often combined with cobalt compounds [25].

Compounds of nickel and cobalt are essential trace elements for the maintenance of vital activity of microorganisms as energy sources or electron acceptors. Forming complexes with biomolecules in biochemical reactions, they also play a catalytic role. Nickel and cobalt concentrations influence such reactions as oxygen and nitrate respiration, one-electron catalysis.

If the aqueous medium contains not only nickel compounds, but also cobalt compounds, then it is the value of the solubility product of the resulting aqua complexes that is of decisive importance. The lower the PR value, the more stable and denser the bulk structure is formed near the surface of the activated sludge particles, which protects the further penetration of the heavy metal into the cells (Table 4).

Table 4 Values of PR for nickel and cobalt compounds

Nickel compounds		**Cobalt compounds**	
Substance	**Solubility product**	**Substance**	**Solubility product**
$Ni(OH)_2$	$(2.0 \cdot 10^{-15}) - (6.3 \cdot 10^{-18})$	$Co(OH)_2$	$(1.6 \cdot 10^{-15}) - (2.0 \cdot 10^{-16})$
$Ni\,S$	$9.3 \cdot 10^{-22}$	$Co\,S$	$1.8 \cdot 10^{-20}$
$NiCO_3$	$1.3 \cdot 10^{-7}$	$CoCO_3$	$1.05 \cdot 10^{-10}$
$[Ni(NH_3)_6]^{2+}$	$9.8 \cdot 10^{-9}$	$[Co(NH_3)_6]^{2+}$	$1.3 \cdot 10^{-5}$
$Ni_3\,(PO_4)_2$	$4.74 \cdot 10^{-32}$	$Co_3\,(PO_4)_2$	$2.05 \cdot 10^{-35}$

Figure 2 shows that nickel aqua complexes are concentrated near the surface of particles of the microbial community, while cobalt compounds are in solution.

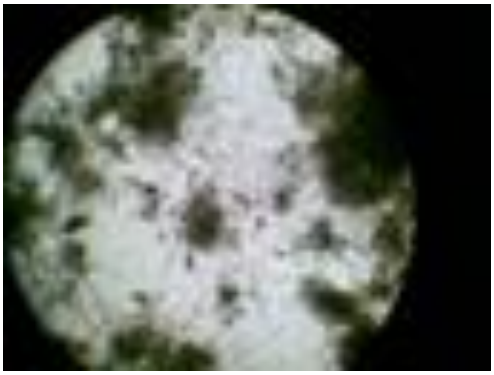

Figure 2 Influence of the value of the solubility product on the spatial formation of aqua complexes near the surface of the microbial community

These compounds are characterized by a low solubility constant, which makes it possible to partially reduce the toxicity of the aquatic environment when excess activated sludge is removed from the system.

Enzymatic metal detoxification promotes the transfer of nickel ions from a soluble form to a less insoluble one. In urban wastewater, the predominant method in aeration facilities was the formation of complex compounds in the sludge mixture of the type:

$$Ni^{2+} + NH_3 + H_2O \rightarrow \lfloor Ni(H_2O)_2(NH_3)_4 \rfloor (OH)_2$$

$$Ni^{2+} + NH_3 + H_2O \rightarrow \lfloor Ni(NH_3)_6 \rfloor (OH)_2$$

In denitrifies, nickel salts $Ni(NO_3)_2$ are hydrolyzed by denitrifying silt sludge to form:

$$Ni(NO_3)_2 + HOH \rightarrow Ni(OH)NO_3 + HNO_3$$

In nitrifies, the nitrifying biomass promotes the acceleration of the complex formation reaction of nickel compounds near the surface of membranes with stronger ligands:

$$[Ni(H_2O)_6]SO_4 + 4\,(NH_3 + H_2O) \rightarrow [Ni(H_2O)_2\,(NH_3)_4]SO_4 + 8H_2O$$

As a result, the toxicity of nickel compounds in biological treatment plants is reduced.

But this structure of the aqua complex performs protective functions up to certain concentrations of free nickel ions (Figure 3).

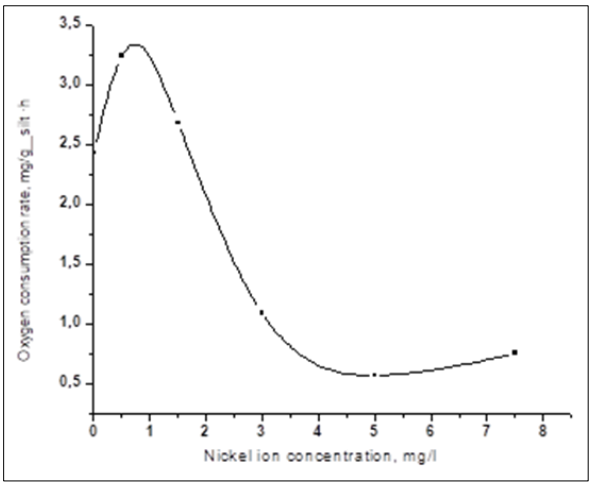

Figure 3 Influence of the concentration of nickel ions on the rate of oxygen consumption in the bioreactor

The results obtained show that an increase in the concentration of nickel ions in the aquatic environment up to 1.0 mg/dm^3 promotes the activation of the formed microbial community. This is the main difference between the stability of the biocenosis of bioreactor and the biocenosis of natural water bodies. A further increase in nickel ions in water to 2.0 mg/dm^3 leads to a decrease in the rate of oxygen consumption by 20% from the initial value of 2.5 mg/g $_{silt}$ h. An increase in nickel ions to 3.0 mg/dm^3 leads to a sharp decrease in the consumption of dissolved oxygen. The authors explain this by the fact that complex structures begin to form in water, including metabolic products of the microbial community. A further increase in the nickel ion concentration in the solution leads to compaction of the structure of the aqua complex. This dense structure leads to a drastic reduction in the supply of nutrients for the entire microbial community. As a result, after 130 minutes of contact of the microbial community with an aqueous medium containing nickel compounds, a decrease in the rate of kinetic reactions is recorded (Figure 4).

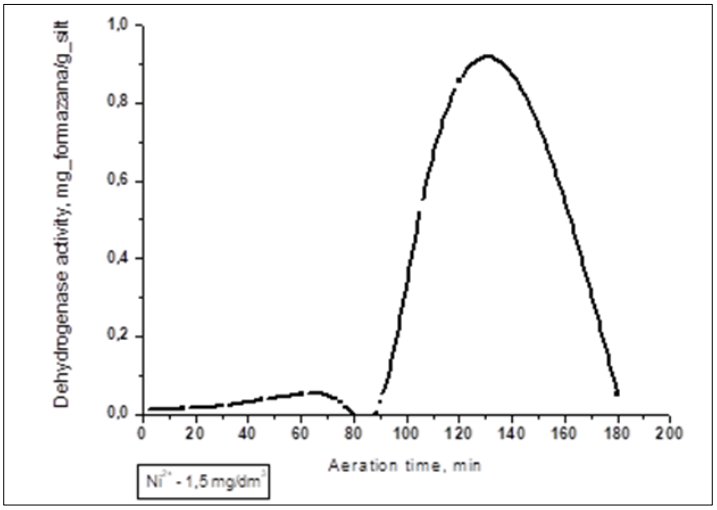

Figure 4 Changes in the total value of the dehydrogenase activity of the microbial community in the presence of the maximum concentration of nickel ions C=1.5 mg/dm^3

The presence of nitrogen and phosphorus compounds in water contributes to the acceleration of complex formation (figure 5). The $[Ni(H_2O)_2(NH_3)_4](OH)_2$ and $NiCO_3$ aqua complex formed in the presence of ammonium ions and carbon dioxide perform a protective function and reduce the rate of penetration of free nickel ions to the cell surface to 30 %.

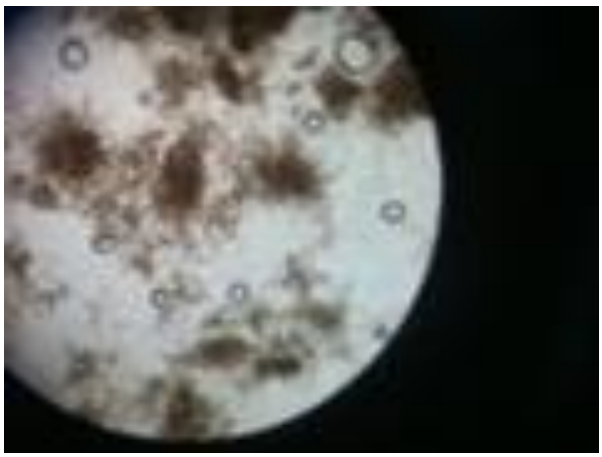

Figure 5 Emission of carbon dioxide and molecular nitrogen in the sludge mixture at biological treatment facilities at a nickel ion concentration of 1.5 mg/dm^3

As a result of the competitive complex formation reaction, the ammonia complex of nickel in an aqueous medium can be destroyed in the presence of active organic compounds and metabolic products. The new structure of the resulting complex is stronger due to the presence of five and six – membered cyclic configurations.

3.5. Influence of Nickel compounds on Evolutionary Processes in the Aquatic Environment

Studies have shown that microbial communities remain viable at higher nickel concentrations than the MPC values approved at the legislative level. This is due to the fact that MPC values are determined by the bio testing method, which imitates natural water bodies in which there is no long-term stay of the same biocenosis in waters with elevated concentrations of nickel compounds. In the biological treatment facilities, the biocenosis stays from 5 to 40 days. Therefore, microbial communities have time to balance the natural defense systems characteristic of each strain into a single step structure of resistance that uses all the products of cellular metabolism. In addition to organic compounds, inorganic substances are used to build aqua complexes with limited solubility in water.

The formed spatial structure of the aqua complex makes it possible to actively use the adsorption properties of each strain, the patterns of their growth to protect the microbial population of the aeration tank as a whole under conditions of high concentrations of heavy metal compounds.

It has been established that the presence of two heavy metals in an aqueous medium causes a shift in the chemical equilibrium of the substances of one of the metals at the stage of dissociation of complex compounds. Metal compounds having a lower water solubility have advantages in adsorption on the cell surface, as they prevent the penetration of ionic forms of the same metal and another metal ion.

The release of the gas phase as a result of biochemical reactions contributes to the formation of more complex compounds.

Increasing the resistance of bacteria to nickel concentrations up to 1.0 mg/dm^3 can be achieved by exogenous isolation of plasmids containing resistance genes, which made it possible to obtain improved strains in biological treatment facilities. The obtained stability constants of complex forms, determined at 25°C, had values for nickel complexes $5.37 < \log \beta > 6.72$, for cobalt complexes $4.75 < \log \beta > 5.2$. This explains the higher concentration of nickel complexes near the surface of the microbial community than the concentration of cobalt complexes.

The results obtained made it possible to establish a mechanism for increasing the resistance of bacteria to nickel ion concentrations exceeding the established MPC in water. The ionic composition of the aquatic environment and metabolic products contribute to the formation of aqua complexes that provide protection for biocenosis particles at higher concentrations than MPC values. Microbial communities develop a single line of defense, which makes it possible to maintain the viability of all strains even at concentrations exceeding the MPC by 10–15 times.

The degree of inhibition of microbial communities by nickel compounds determined in these experiments confirmed the ecological hazard of this metal for the biocenosis of natural aquatic environments in cases of discharge of untreated industrial wastewater into open water bodies.

Denial of responsibility

The results and opinions expressed in the article are those of the authors and do not reflect any policy.

4. Conclusions

The values of the maximum permissible concentrations of nickel compounds in open water bodies are determined by the values that cause the death of certain types of microorganisms or the destruction of microbial communities. Environmental regulation reveals a violation of the processes of microbial biodegradation of substances. In the process of evolution, each form of life was forced to develop a certain system of homeostasis in order to provide tight control over the concentrations of nickel ions inside the cells. Nickel ions form complexes with hydroxyl, phosphate and amino groups. Also covalent bonds with sulfhydryl groups of proteins. As a result, nickel ions are able to combine with proteins, nucleotides, coenzymes, and phospholipids. This can have a toxic effect on cell metabolism. Thus, interacting with the active sites of microbial enzymes or substituting individual ions in them, nickel ions can cause both an increase and a decrease in their activity.

Inorganic nickel compounds are more toxic than complex compounds with inorganic and organic ligands. The conducted studies have shown that microbial communities in the aquatic environment, in contrast to experiments on sterile media with pure cultures, synchronize the natural defense systems and are able to change the resistance mechanisms. This allows you to maintain the viability of all strains in a particle of activated sludge or the entire complex of cell agglomerate in an open reservoir. This developed natural combination of protection systems in the interests of

the entire microbial community in a given area of the water body allows maintaining the viability of all strains in the presence of higher concentrations of nickel compounds than MPC values. Studies of microbial communities in natural water bodies and in biological wastewater treatment facilities have shown that exceeding the concentrations of nickel compounds up to 30% of the MPC values slows down the growth of biomass, but does not reduce the total viability of the biocenosis.

Complexation of nickel with inorganic compounds and mixed-ligand complexes of conjugated amino acid bases makes it possible to obtain three-dimensional spatial structures near the silt particles that bind nickel ions and thereby reduce the toxic effect of nickel compounds. Thus, microbial communities in the aquatic environment synchronously develop mechanisms that protect them from the effects of heavy metals.

The results presented in the article show that different concentrations of nickel ions can both increase the enzymatic activity of cells and cause cell death. That is, with the correct selection of nickel ion concentrations, it becomes possible to restore cellular mechanisms. Therefore, it is possible to obtain drugs to accelerate the growth of the skin in cases where a person receives burns over a large area of the body.

Knowledge of this regularity of the influence of nickel ions in certain concentrations on the processes of cell resistance and the possibility of suppressing cell growth can be used in the synthesis of drugs to suppress the growth of cancer cells.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest between the authors. Complete understanding.

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