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BIOTECHNOLOGY OF GROUNDWATER PURIFICATION FOR WATER SUPPLY SYSTEMS, USING *GALLIONELLA* AND *LEPTHOTHRIX* FERROBACTERIA

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Aim. The aim of the study was to consider the possibility of using the consortia of chemolithoautotrophic ferrobacteria from *Gallionella* genus and heterotrophic bacteria from *Lepthothri*x genus for the biological method of groundwater purification. **Methods**. The photocolorimetric method to determine the concentrations of ammonium and iron ions, the titrimetric method to determine the hydrocarbon and total alkalinity, the method of determining the permanganate oxidizability using the Kubel method, the potentiometric method to determine the values of pH and Eh, the electronic microscopy using the X-ray spectral analysis of matrix structures of bio-minerals, microbiological and statistical methods. **Results**. The main technological parameters of the water deironing process were defined as follows: the filtration velocity of the bioreactor – 7–11 m/h, and of the filters – 3.5–5 m/h; the filter-cycle duration – 48 h. It was found that the application of the two-stage technology of biological deironing in the bioreactor and filters provided for the possible removal of Fe^{2+} compounds up to 5.0 mg/cdm, ammonium nitrogen — up to 1.5 mg/cdm, soluble organic substances by PO – up to 6.0 mg O_2/cdm. It was determined that the optimal parameters for the process of biological purification of neutral groundwaters, containing increased concentrations of Fe^{2+} cations were as follows: pH 7.0–7.2; hydrocarbon alkalinity 2.5–2.2 mmol/cdm; content of soluble oxygen – 1.5–2.0 mg/cdm. The ability of concentrated (D_{os} 200 mg/cdm) matrix structures of *Gallionella* and *Lepthothrix* ferrobacteria to remove Cr^{6+} ions from natural groundwaters was determined. The study found no considerable differences in the efficiency of applying disinfectants, produced using polyhexamethylene guanidine chloride (PHMGchl) or polyhexamethylene biguanidine chloride (PHMBchl). In concentrations of 0.25–0.5 %, they effectively disinfect pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The same was found true for the bacteria, most common in the systems of technical reverse water supply, the systems of water circulation, plant watering, and fire tanks, including *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Pseudomonas sp.*, *E. coli*, *Flavobacterium columnare*, *Micrococcus lysodeikticus*. **Conclusions.** This was the first study on the possibility of applying the biotechnology of groundwater purification from excessive amounts of iron in conditions of uneven hydraulic burden, notable for the water supply systems of rural areas and most agricultural enterprises in the north-western and northern regions of Ukraine. The specificities and perspectives of PHMGchl application in the systems of water preparation were studied. It was found that from the standpoint of safety and efficiency, the use of the water deironing processes involving iron bacteria was reasonable in the systems of water consumption and technical water supply, and the disinfection of water using PHMG was possible only in the second case, due to some toxicity of the preparation. The optimal parameters for the process of biological purification of neutral groundwaters, containing increased concentrations of Fe^{2+} cations were determined.

Key words: water purification, ferrobacteria, matrix structures of bio-minerals, bioreactor, water disinfection, guanidine derivatives.

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INTRODUCTION

Water is the foundation of life and development of modern technogenic society. The existence and development of agricultural production and rural settlements are impossible without this extremely valuable natural resource. The provision of consumers with a sufficient amount of water is obligatorily related to the need for strict compliance with the norms regarding its quality, which is especially urgent in the times of war (Drechsel et al, 2023; Kravchenko et al, 2022; 2023; National Report, 2022).

The situation with centralized water supply in Ukrainian rural areas is usually much worse than in the cities. For instance, according to the data in the National Report on the quality of drinking water and the state of water for consumption in Ukraine (National Report, 2022), in 2021, the share of the drinking water samples, taken from the sources of centralized water supply, which did not meet the standard by sanitary-chemical and microbiological indices, was 18.2 and 5.1 % (in 2020, 16.8 and 4.7 %), respectively, for community water pipes, while for rural water pipes, these indices were almost twice higher – 28.9 and 11.9 % (in 2020, 26.9 and 13.8 %), respectively. Among the total number of studied rural water pipes, the share of the ones, whose results of laboratory water analyses did not meet the norms, was 38.3 %.

In the Rivne region, the situation is no better; in 2023, the specialists of the state enterprise Rivne OTsKPKh of the Ministry of Health (the Rivne Regional Center for Control and Prevention of Diseases of the Ministry of Health of Ukraine) studied 1,443 samples of the well water to define their sanitary and chemical indices, and 841 samples – to study the microbiological indices. The results of this study demonstrated that 34.3 % of samples did not meet the sanitary and chemical standards due to the excessive content of different substances, including nitrates, ammonium, total iron, and total hardness, and 25.2 % of samples had microbiological deviations (https://rv.cdc.gov.ua/articles/yakist vody-tsentralizovanogo-ta-detsentralizovanogo-vodopostachannya-za-2023-rik).

The reasons for this phenomenon are complicated, but one of the critical components is the need to apply effective modern technologies for groundwater deironing (Khomutetska et al, 2023).

The primary water consumers in rural areas are the communal sector, animal farms, and the enterprises of agroindustrial complex; water is also used for vegetables, gardens, and vineyards. It should be noted that the enterprises of the agroindustrial complex mainly use drinking water for their technological purposes, water after special preparation and stabilization treatment – for cooling systems, and water, kept in special fire tanks, which requires treatment with special disinfectants to prevent the development of bacteria – for firefighting systems (Khomutetska et al, 2023).

At present, about 30 % of all the drinking water in Ukraine is collected from underground wells. In case of agricultural water supply, this figure goes up to 90 %. However, only 26 % of the rural population uses the services of centralized water supply (National Report, 2005). All other consumers use local wells for their drinking purposes; usually, these are pit wells, and the water in most of them is in an unsatisfactory state due to the pollution of water-bearing horizons (National Report, 2005; National Report, 2022).

The main kinds of industrial pollution of groundwaters are insufficiently purified runoffs and infiltration waters from waste ponds and slurry slumps. Among the most dangerous elements of technogenic pollution of natural waters are heavy metal ions, including hexavalent chromium compounds. Usually, Cr^{6+}, which has toxic properties, is present in groundwaters in the form of hydrogen chromate ion ($HCrO_4$) or chromate anion (CrO_4^{2}). The reduction of these components to the safe form of Cr^{3+} under neutral pH values in natural conditions is very complicated. Chromium (IV) contributes to gastric and duodenal ulcers, and its carcinogenic and mutagenic effects have also been proven. The MAC of Cr^{6+} in drinking water is 0.05 mg/cdm; the limiting indicators of harmfulness are sanitary and toxicological.

The agricultural pollution of groundwater is related to removing poisonous chemicals and mineral fertilizers (nitrogen, phosphorus, and potassium-based) from the soil with rain or irrigation waters, and the nitrogen compounds are of particular relevance here. The sources of nitrogen compounds may also be the sewage waters of animal complexes, poultry farms, and household runoffs. When nitrogen compounds penetrate the soil, the ammonium nitrogen NH^{4+} gets oxidized, at first to nitrites, then to nitrates. Due to their high solubility and absence of hydrochemical barriers, nitrates are the most common pollutants of groundwaters in rural areas.

In the areas, fertilized with peat and swamped, the decrease in the level of groundwater occurs along with the decomposition of organic matter in the layers, which promotes the increase in the content of nitrogen-containing and highly molecular organic compounds of iron in the water.

The progressing pollution of groundwater-bearing horizons is also affected by their insufficient natural protection. According to (National Report, 2005), the degree of protection of the main water-bearing horizon of Ukraine is as follows: 39.6 % protected; 24.2 % relatively protected; and 36.2 % unprotected groundwaters.

The study of the quality composition of artesian waters in the active water collectors (National Report, 2022; Kvartenko, 2023) demonstrated a tendency towards their gradual deterioration in terms of such main parameters as the degree of aggressiveness, the content of the compounds of iron, manganese, ammonium nitrogen, humic complexes, general mineralization, microbiological indices, seasonal occurrence of phenols in some water-bearing horizons. For instance, in Volyn and Rivne regions, where the percentage of protected groundwater is 7.8 and 3.4 %, respectively, in two recent decades, we have noticed a tendency towards the pollution of groundwater-bearing horizons with the ammonium nitrogen compounds (**Table 1**) and iron (**Table 2**) (Kvartenko, 2023).

Most current Ukrainian stations of groundwater purification were designed by the technology of filtration with simplified aeration, which has some limitations in terms of the quality of the obtained water: Fe^{2+} < 10 mg/cdm; H_2S < 0.5 mg/cdm; pH ≥ 6.7; permanganate oxidizability up to 5 mg O_2/cdm (DBN V.2.5:2013, item 10.21.14), and does not envisage the complex purification from the abovementioned ingredients (Kvartenko, 2023).

The analysis of traditional reagent-free technologies (Kvartenko, 2023) for the purification of slightly acid and neutral groundwaters in the presence of dissolved organic substances demonstrated insufficient effectiveness of their action and the aggressiveness of the filtrate.

The application of methods involving reagents requires additional technological equipment, which complicates the exploitation, raises the cost of the purified water, and does not exclude the possibility of forming organochloride substances, products of ozonolysis. This approach provides for efficient deironing of water, but its implementation is accompanied with the formation of a large amount of precipitate, which requires further disposal (Trus et al, 2021).

The use of the schemes with active catalytic load is reasonable only if there are no dissolved organic compounds, hydrogen sulfide, and microorganisms in the groundwater.

The application of modern baromembrane technologies is possible only at the initial pressure starting with 20–40 bar and further process of the filtrate remineralization.

One of the possible ways to solve the issue of complex purification of groundwaters is the application of biotechnologies using the natural biocenosis, typical for water-bearing layers of specific areas, in the bioreactors (Kvartenko, 2023). The most common micro-

Table 1. The results of the monitoring study, conducted at 67 water collectors in Rivne and Volyn regions to check the content of ammonium nitrogen (Kvartenko, 2023)

Content of ammonium nitrogen, mg/cdm	up to 0.05	0.06–0.5	**0.51–1.5**	**>1.5**
Rivne region				
% of settlements	16	30	**24**	**30**
Volyn region				
% of settlements	–	19	**31**	**50**

Table 2. The results of the monitoring study, conducted at 49 water collectors in Rivne and Volyn regions to check the content of iron compounds (Kvartenko, 2023)

Content of ammonium nitrogen, mg/cdm	up to 3.0	3.0–5.0	from 5.0 to 10.0	from 10 to 30
Rivne region				
% of settlements	45	24.5	14.2	16
Volyn region				
% of settlements	52	18	24	6

organisms in the global underground iron-containing waters are different groups of ferrobacteria (obligate aerobes, facultative and obligate anaerobes), each of them having its own areas of activity that depend on the acidity (pH) and redox potential (Eh) of the aqueous medium (Kvartenko, 2017a).

The study of physiology, morphology, taxonomy, and genome of ferrobacteria is described in rather many scientific publications. For instance, the article (Straub et al, 2001) describes anaerobic dissimilative bacteria, deoxygenating bivalent iron and bacteria, oxidizing bivalent iron, obtaining energy via deoxygenation or oxidation of iron minerals. The publication (Johnson & Hallberg, 2008) is dedicated to the review of interrelations between the transformation of carbon, ferrum, and sulphur by acidophilic microorganisms and the fact that these bacteria are relevant for both industrial and ecologic context. The publications (Emerson et al, 2010, 2013) presented the results of the study on ecologic and genomic aspects of ferrobacteria, capable of catalyzing dissimilative oxidation of iron at pH, close to the neutral one, in microaerobic and anaerobic media.

Our study considered the possibility of using the consortia of chemolithoautotrophic ferrobacteria from *Gallionella* genus and heterotrophic bacteria from *Lepthothrix* genus for the biological method of groundwater purification from Fe(II) and Cr(VI) compounds.

Unearthed ferrobacteria of *Gallionella* genus were first found by Ehrenberg in ochreous mineral resources in 1836 (Ehrenberg, 1836). The first studies that presented the description of morphology and cycle of the development of these microorganisms were published in 1924 by M.H. Cholodny (Cholodny, 1924). Further on, the study of physiology, genomics, and the processes of mass exchange of *Gallionella* ferrobacteria was described in the publications (Hallbeck & Pedersen, 1991; de Vet et al, 2011; Emerson et al, 2013). The bacteria develop in microaerophilic conditions with the concentration of the dissolved oxygen from 0.1 to 3.0 mg/cdm, t = 8–16 ºC, pH 5.5–7.5 and Eh = –100–300 mV, and the concentrations of Fe^{2+} from 1.0 to 30 mg/cdm. The microorganisms of *Lepthothrix* genera are heterotrophs, which use organic substances as a carbon source. The area of the development of microorganisms of *Lepthothrix* genus is located in the range of values of pH 7.0–8.0 and Eh = –200…300 mV.

The velocity of Fe (II) oxidation in the medium with ferrobacteria exceeds the velocity of their oxidation with air oxygen. The publication (Nedkov et al, 2016) presents the results of the study on nanostructured matrices formed by the bacteria of *Leptothrix* genus due to the biochemical oxidation of iron (II). The results, presented by the authors on comparative studies on the kinetics of Fe (II) oxidation by *Leptothrix* ferrobacteria in Adler medium and in the control sample without bacteria, demonstrate a considerable difference in the efficiency of these processes. For instance, while cultivating bacteria in Adler medium, Fe (II) oxidation mainly occurred in the first weeks, it was 75 %, while only 2.5 % got oxidated in the medium without bacteria (Nedkov et al, 2016).

There are also promising microbiological methods of removing heavy metal ions from the solutions using different taxonomic groups of microorganisms (Javanbakht et al, 2014; Jeyakumar et al, 2023). For instance, the cells of *Thiobacilus ferrooxidans* remove the ions of Cd(II), Co(II), Cu(II), Cr(VI), Ni(II) (Liu et al, 2004) from the solutions.

The first data about the possibility of using ferrobacteria in water purification stations were presented by Tanimoto in 1952, who described the process of Fe (II) removal on slow sand filters in Tadotsuco, Kagawa, Japan (Tanimoto et al, 1952). The first modern systems of biological deironing of groundwater in Europe using fast sand filters were developed and introduced in Alsace in the 1980s (Mouchet, 1992). Later, this method was introduced in the purification facilities in more than 100 settlements of France, with the routine of 20 to 2,200 cubic meters/h. The first stations of biological deironing in Great Britain and the USA were made in 1987 and 1997, respectively (Cameron & Bourgine, 1999). In Ukraine, the work at the investigation and introduction of the biological method of water deironing started in the 1970s at the Chair of Water Supply and Boring of the Ukrainian Institute of Water Management (now the National University of Water and Environmental Engineering), headed by Prof. M.A. Safonov. However, this issue still retains its urgency even now, not only for Ukraine but also for the European countries, the USA, and Canada (Kvartenko, 2023; Khomutetska, 2023; Tekerlekopoulou et al, 2008; Lin et al, 2012; Hushchuk et al, 2022).

The aim of the study: to conduct experimental industrial testing of the groundwater purification biotechnology for the system of non-centralized water supply for a rural settlement, an agricultural enterprise under conditions of uneven relative hydraulic load in the course of 24 h and to determine its main technological parameters; to determine the optimal parameters for the process of biological purification of underground neutral waters with the increased content of cations Fe^{2+}; to study the effect of low (10–2 % (vol %)) concentrations of the polyhexamethylene guanidine preparation on the processes of biological purification of water from Fe^{2+} compounds in the suspended layer of matrix structures of ferrobacteria; to study the ability of concentrated matrix structures to remove Cr (IV) cations, to study disinfecting properties of different guanidine derivatives regarding the bacteria which may occur in fire tanks and the systems of reverse water supply.

MATERIALS AND METHODS

The major part of the study was conducted in 2004–2019 in the water supply objects of Rivne region.

Experiment 1. Selecting the optimal technological scheme of biological groundwater purification with pH values, close to the neutral ones.

The study was conducted from 2012 to 2016 at a biological deironing station with a performance of up to 2,000 cubic meters/day, including the reactor with the 2.8 m diameter, a height of 8 m, and three gravitation-expanded polystyrene filters of 2.0 × 2.0 × 4.3 m which were equipped with the hydroautomatic washing system (**Fig. 1**).

To analyze changes in water quality parameters in the mode of uneven hydraulic load, samples of the obtained water were taken after the bioreactor and filters every two hours. For a typical filter-cycle, their number amounted to 20 samples respectively to determine the concentration of Fe_{total} in the obtained water after the bioreactor and filters; 18 more samples to determine the parameters of permanganate oxidizability, and 20 more to determine the concentration of NH_4^+ cations after filters. All the samplings were done in three repeats.

The concentrations of Fe^{2+} ions were evaluated by the colorimetric method with potassium rhodonite on the photoelectrocolorimeter KFK-3 with a wavelength of 490 nm (EPA Method 3500 Fe). The mass concentrations of NH^{4+} ions were determined by the photocolorimetric method with Nessler's reagent using the photoelectrocolorimeter KFK-3 with a wavelength of 440 nm (ISO 15923-1:2013. Water quality). The mass concentrations of chromium ions were determined by the extraction-photocolorimetric method with diphenylcarbazide using the method MVV 081/12-0114-03 (at a wavelength of 540 nm). The pH values were determined by the potentiometric method according to EPA Method 150.1 using the universal ion meter EV-74 with the application of a glass /ESL-43-07/ and platinum /EPZ-1/ electrodes at the constant temperature of 25.0 ± 0.2 °C. The silver-chlorine half-cell /EVL-1M1/, filled with KCl solution, was used as the comparison electrode. The hydrocarbon alkalinity was determined by the titrimetric method (DSTU ISO 9963-1:2007).

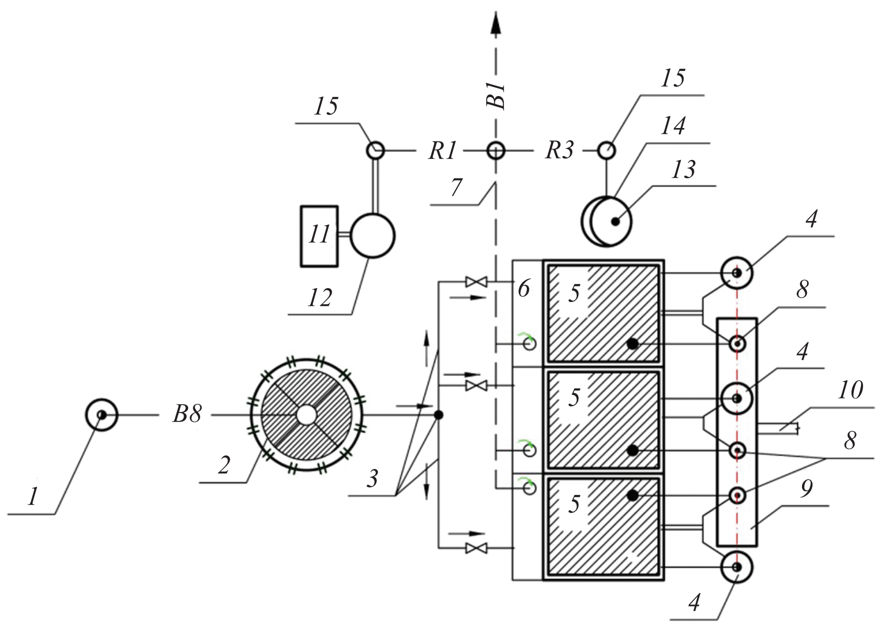

Fig. 1. The technological scheme of biological purification of neutral groundwaters: 1 – a bore-well; 2 – a bioreactor; 4 – air separators; 5 – filters; 6 – filtrate collection socket; 8 – hydrorobot; 9 – channel for collection of washing waters; 10 – pipe for removal of waste wash-out waters; 11 – electrolyzer; 12, 14 – tanks for the solutions of sodium hypochlorite and $Ca(OH)_2$; 13 – mixer; 15 – dispensing pumps; 3, 7 – technological pipes (our own elaboration)

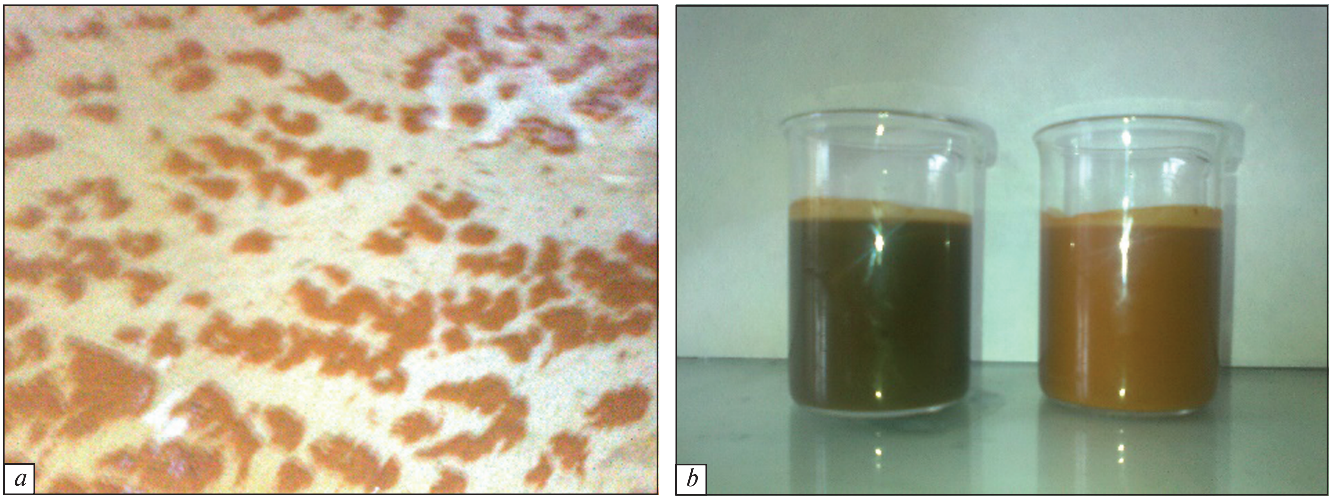

Fig. 2. The photo of the precipitate, obtained after washing the filters of the biological groundwater deironing station: *a* – agglomerates of concentrated matrix structures; *b* – condensed precipitate

Experiment 2. The elaboration of the pilot unit for the study of the oxidation process of Fe(II) in the weighed precipitation layer with matrix structures of bio-minerals and ferrobacteria.

To study the oxidation process of Fe(II) in the weighed precipitation layer with matrix structures of bio-minerals and ferrobacteria, the pilot unit of our own elaboration (Kvartenko, 2017b) was used.

The unit worked as follows: Kamovsky pump was used to pump air out of the system of initial water volumes – the reaction tank – the filter. The volume for the initial water was filled with the groundwater with the subsequent mixing. Using Kamovsky pump, 500 ml of the obtained solution was pumped into the reaction tank via the system of technological hoses. During the entire experiment period, the solution was mixed in the reaction tank with the gradual selection of samples in 5, 15, 30, 40, and 60 min and the filtration through the replaceable paper filters, "blue ribbon". The content of Fe^{2+} and Fe_{total} was checked for each investigated sample simultaneously in the filtrate and on the paper filter, "blue ribbon", dissolving it after the filtration of the corresponding sample volume in the Petri dish 1 : 1 with HCl solution.

The variants of the investigations with variable indices of water quality are presented in Table 5. They include the study on kinetics and efficiency of groundwater purification in the suspended layer of matrix structures of ferrobacteria depending on the time of the contact in the following cases: 1 – a change in the quality parameters of the incoming water; 2 – a change in the concentration of oxygen, dissolved in the solution; 3 – a presence of the activator and inhibitor of the biochemical oxygenation process. A total of 125 samples were taken and analyzed to determine Fe^{2+}.

The study of the ferrobacteria precipitate structure with the X-ray spectral analysis of their matrix structures was conducted using the scanning electron microscope (SEM) Quanta 200.

Experiment 3. The determination of the kinetics of purifying groundwaters, containing cations Cr^{6+}, using the matrix structures of *Gallionella* and *Lepthothrix* ferrobacteria.

To determine the kinetics of purifying groundwaters, containing cations Cr^{6+}, using the matrix structures of *Gallionella* and *Lepthothrix* ferrobacteria, the volume of reactor 2 (**Fig. 2**) was filled with 500 ml of groundwaters, containing 0.75–0.8 mg/cdm of cations Cr^{6+} with the addition of different amounts of precipitate from the washing of the filters of biological deironing station. The precipitate concentrations (C) in each series of experiments were 40, 100, and 200 mg/cdm, respectively. The mixture was stirred using the magnetic mixer. The samples were taken after 10, 30, 45, and 60 minutes and filtered through replaceable paper filters, "blue ribbon", determining the concentrations of the dissolved Cr^{6+}.

Experiment 4. The determination of disinfecting properties of different guanidine derivatives and the prospects of their further application in the role of disinfectants to decrease the biogenic activity of water. The studies involved the use of the polyhexamethylene guanidine chloride (PHMGchl) and polyhexamethylene biguanidine chloride (PHMBchl), manufactured by PF Termit, Rivne (https://www.termitua.com/uk/by-guanydyny).

In this experiment, the antimicrobial properties of preparations were tested by standard methods (Morozova, 2008) using the test strains of microorganisms: gram-negative bacteria *Escherichia coli* ATCC No. 25922 and *Pseudomonas aeruginosa* ATCC No. 27853 F 51, and gram-positive bacteria *Staphylococcus aureus* ATCC No. 25923 F 49 (the strains of the Volyn regional laboratory of veterinary medicine, Lutsk).

The 0.01–1.0 % solutions of PHMGchl and PHMBchl were prepared using distilled water with pH 5.5–6.5. Cambric test-objects, contaminated with *E. coli*, *S. aureus*, *B. cereus*, were immersed into the working solutions. 15, 30, and 60 minutes after the immersion of the test-objects into the disinfecting solution, two test-objects at a time were taken using the loop, and after double washing in the water, they were sown into the meat-and-peptone broth and agar (MPB and MPA). The inoculations were incubated in the thermostat at 37 °C. The results were registered daily for 6–7 days.

Experiment 5. The study of the effect of PHMBchl on saprophytic and relatively pathogenic bacteria of the aqueous medium.

In cooperation with the Institute of Fisheries, the NAAS, (Kyiv), we checked the effect of PHMBchl in the concentrations from 0.1 to 1.0 % using field strains of the following microorganisms: *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Pseudomonas sp*., *E. coli*, *Flavobacterium columnare*, *Micrococcus lysodeikticus* (the strains of the Institute of Fisheries, the NAAS, Kyiv). The mentioned microorganisms belong to the group of saprophytic and relatively pathogenic bacteria of the aqueous medium. The study method, the culture media, and the conditions of cultivating microorganisms were chosen as described in (Kryvko et al, 2021).

The degree of activity was evaluated by the value of the growth inhibition zones of microorganisms according to the following parameters: 1 – a growth delay zone, with a diameter of up to 10 mm, or the absence thereof demonstrated that microorganisms were not sensitive to the preparation, introduced into the vial; 2 – a growth delay zone with 11–15 mm diameter demonstrated poor sensitivity of the culture; 3 – a growth delay zone with 15–25 mm diameter was considered the sensitivity indicator of microorganisms; 4 – a growth delay zone with the diameter more than 25 mm demonstrated high sensitivity of microbes (Determination of the sensitivity of microorganisms …, 2007).

RESULTS

The results of Experiments 1 and 2 were previously analyzed by us and are currently presented in the Discussion section.

Experiment 3. The determination of the kinetics of purifying groundwaters, containing cations Cr^{6+}, using the matrix structures of *Gallionella* and Lepthothrix ferrobacteria (Fig. 2) demonstrated that the highest purification effect was observed in the series of experiments with the precipitate concentration of C =

Table 3. The efficiency of removing cations Cr^{6+} from groundwaters using the matrix structures of *Gallionella* and *Lepthothrix* ferrobacteria

Contact duration $t_к$, minutes	10	30	45	60
The concentration of Cr^{6+} in the obtained water, 0.8 mg/cdm; $C_{precipitate}$ = 40 mg/cdm				
$Cr^{6+}_{filtrate}$, mg/cdm E, %	0.7 12 %	0.57 40 %	0.57 40 %	0.57 40 %
The concentration of Cr^{6+} in the obtained water, 0.75 mg/cdm; $C_{precipitate}$ = 100 mg/cdm				
$Cr^{6+}_{filtrate}$, mg/cdm E, %	0.25 67 %	0.24 68 %	0.24 68 %	0.22 71 %
The concentration of Cr^{6+} in the obtained water, 0.8 mg/cdm; $C_{precipitate}$ = 200 mg/cdm				
t_k, min $Cr^{6+}_{filtrate}$, mg/cdm E, %	10 absent 100 %	30 absent –	45 absent –	60 absent –

Source: our own results.

Table 4. The effect of PHMBchl on the control strains of microorganisms, n = 3

Test culture	Preparation concentration, %			
	1	0.5	0.25	0.1
	diameter of the delayed growth zone, mm (±0.5 mm)			
A. salmonicida	14	11	10	8
E. coli	13	12	10	10
Pseudomonas sp.	15	13	12	11
A. hydrophila	16	14	13	12
F. columnare	42	35	30	22
M. lysodeikticus	40	35	32	22

= 200 mg/cdm, and the lowest – for the concentration of C = 40 mg/cdm (**Table 3**).

Experiment 4. The comparison of the bactericide activity of PHMHchl and PHMBchl.

For *E. coli*, the MMC of PHMGchl was 0.05 % at the 30 min exposure, and weak growth was still observed after 15 min of exposure. As for PHMBchl, the MMC after 15 min of the exposure was 0.02 %. A similar situation was found for *P. aeruginosa* – after the exposure of 5 min the MMC of PHMGchl was also 0.05 % and that of PHMBchl – 0.02 %. For *S. aureus*, the MMC of PHMGchl was 0.04 % after the exposure for 15 min, and for PHMBchl, after 15 min of the exposure, the MMC was also 0.02 %.

Further studies demonstrated that in case of a protein load, for instance, blood serum or biofilms of microorganisms, formed in the internal surfaces of the water supply system, some advantages of PHMBchl almost vanished, and no considerable differences were observed in the effect of both preparations. Thus, both PHMGchl and PHMBchl in concentrations of 0.25–0.50 % can be used effectively to disinfect water in the systems of technical water preparation and technical water supply.

Experiment 5. In this series of experiments, we checked the effect of PHMBchl on saprophytic and relatively pathogenic bacteria of the aqueous medium (**Table 4**).

According to the obtained results, *M. lysodeikticus* and *F. columnare* were found to be the most sensitive to the effect of PHMBchl. 0.1 % of the preparation concentration was enough to inhibit their growth. Bacteria of *A. salmonicida* were found practically insensitive to the concentration of 0.1 % of PHMBchl and *E. coli* – weakly sensitive; this concentration had a bacteriostatic effect on the remaining strains. Thus, it is reasonable to use several times higher concentrations of PHMBchl, about 0.25–0.5 %.

DISCUSSION

Selecting the optimal technological scheme for biological purification of groundwaters with pH values close to neutral ones (Experiment 1). The results of the filtration cycle of 48 h after washing the bioreactor and filter No. 3 are presented in **Fig. 3.** The parameters of the initial water quality: Fe_{total} 1.95–2.15 mg/cdm, hydrocarbon alkalinity 3.5 mmol/cdm, permanganate oxidizability 1.7–1.84 mg O_2/cdm, the content of ions NH^{4+} 0.65 mg/cdm, pH 7.1–7.15. The hydraulic load of the bioreactor was 11–12 m/h, and that of the filters – 5–5.5 m/h.

The presented data demonstrated that the efficiency of the bioreactor work was affected by switching off the water supply by the bore-well pumps, which was related to uneven water consumption. After the water supply was restored at the velocity of 11–12 m/h, there was an expansion of the lower loaded layer, accompanied with the removal of some precipitate, captured before, from the interporiferous space of the bioreactor to the space under the filter. The presence of three filters at the station with the ascending filtration direction provided for retaining this precipitate most of the time and obtaining a filtrate, complying with the norms (DSanPiN 2.2.4-171-10).

In the second half of the filtration cycle (Fig. 3, from noon till the end of the filtration cycle), there was stabilization of the bioreactor work, characterized by enhanced efficiency of water purification by iron cations (to 47–55 %), a decrease in concentrations of dissolved organic substances (from 37 to 70 % by PO) and ammonium nitrogen from 0.65 mg/cdm to the traces which, at pH 7.15–7.20, demonstrated intense origin of the biological oxidation processes. The biological oxidation processes take place on the surface of the con-

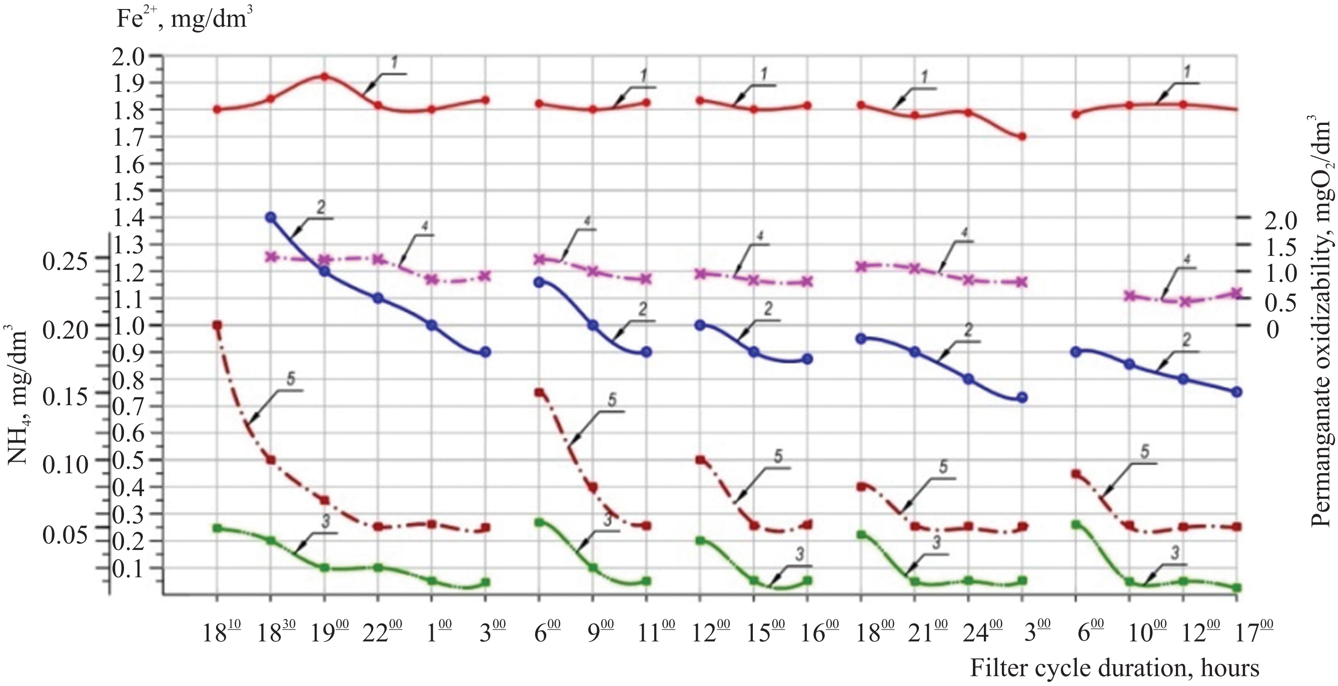

Fig. 3. The change in water quality parameters during the filtration cycle of 48 h from May 14 till May 16, 2012, after washing the bioreactor and filter No. 3: 1 – Fe_{total} in the initial water; 2 – Fe_{total} after the bioreactor; 3 – Fe_{total} after the filter; 4 – permanganate oxidizability after the filter; 5 – content of NH^{4+} cations after the filter. (Source: our own results)

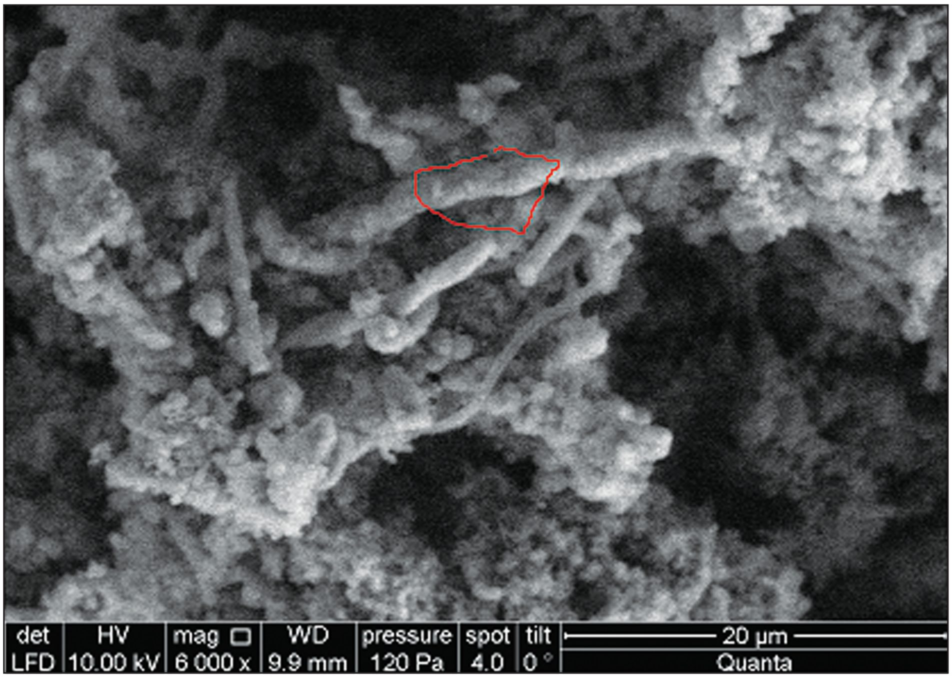

Fig. 4. The electronic image of the matrix structures of *Gallionella* from the interporiferous space of the contact load, × 6,000. (Source: our own results)

tact load granules due to the formation of the active catalytic surface out of cells and matrix structures and in the interporiferous space (**Fig. 4**). The process takes place due to the adhesion of bacteria with the negative charge on the primary catalytic film. The duration of "charging" depends on the water quality parameters: pH values, hydrocarbon alkalinity, the concentration of Fe(II) ions, and the presence of ferrobacteria. The

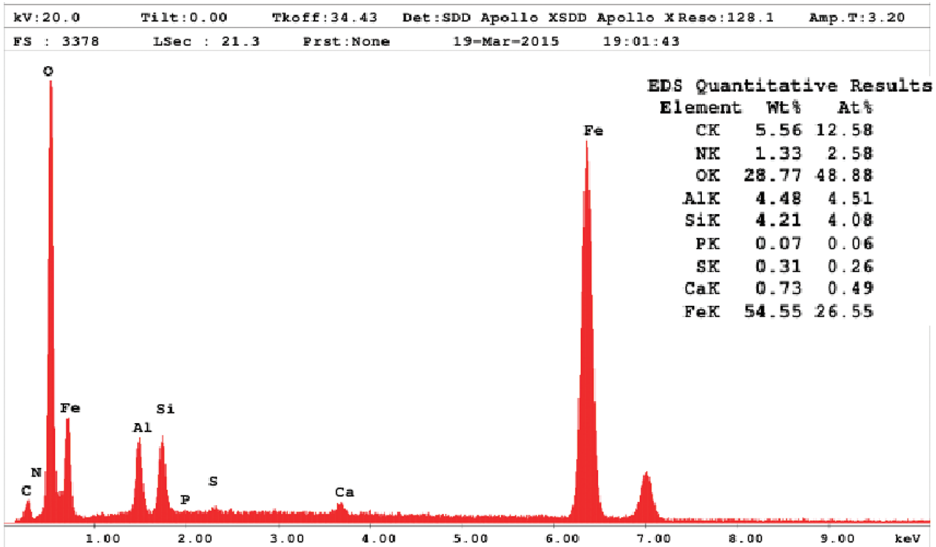

Fig. 5. The X-ray spectral analysis of the matrix structures of *Gallionella* bacteria from the interporiferous space of the bioreactor contact load. (Source: our own results)

formation of the initial matrix structure takes place during this period. New concentrations of bacteria come to the bioreactor with each portion of the initial water, increasing their total number.

During their life activity, *Gallionella* bacteria create matrix coil-like structures that fill the entire interporiferous space over time. The electronic microscopy of the matrix structure samples was conducted using the X-ray spectral analysis on the scanning electron microscope (SEM) Quanta 200 (**Fig. 5**). The increase in the volume of biomineral matrix structures in the interporiferous space led to their gradual migration to the lower layers with the descending flow of water with their gradual calmatation. The filters were set into the washing mode once every two days.

The results of the second series of experiments (Expe-riment 2) demonstrate that the best purification effect was observed in the series of experiments with hydrocarbon alkalinity of 2.5 mmol/cdm, pH 7, the concentration of the dissolved oxygen was ≈2.0 mg/cdm and amounted to, respectively: in the first 15 min – 78 %; 30 min later – 90 %; 45 min later – 98 % (**Fig. 6**, curve 1).

The determination of the kinetics of the purification of iron-containing groundwaters, neutral in their acidity, was conducted in the suspended layer of matrix structures of ferrobacteria depending on the time of contact and the quality parameters of the initial water (**Table 5**).

Practically the same results were obtained in the series of experiments with the hydrocarbon alkalinity of 2.2 mmol/cdm, pH 7.2; the concentration of the dissolved oxygen was ≈1.5 mg/cdm (**Fig. 6**, curve 5). When the hydrocarbon alkalinity was decreased down to 1.8 mmol/cdm, there was inconsiderable inhibition of the process of biochemical oxidation of Fe^{2+} cations, respectively: in the first 15 min – 63%; 30 min later – 80%; 45 min later – 90% (Fig. 6, curve 2). After pH value was decreased down to 6.5, the concentration of the dissolved oxygen – to ≈1.0 mg/cdm and the hydrocarbon alkalinity – to 1.3 mmol/cdm, the purification efficiency on average decreased from 10 to 15% (Fig. 6, curve 3). Therefore, the main factors, inhibiting the process of biochemical oxidation of Fe^{2+} cations in the weighed precipitate layer with matrix structures of bio-minerals and ferrobacteria, were the values of hydrocarbon alkalinity and the content of the dissolved oxygen. However, the most significant factor, inhibiting the process, was the presence of 10^{-2} % (vol %) of the preparation of PHMGchl in the water to disinfect it.

After the introduction of PHMGchl into the reaction zone, even at optimal values of the bicarbonate alkalinity of 2.2–2.5 mmol/cdm, in the neutral medium (pH 7), in the presence of the activator (40 mg/cdm of the solution of Na_3CO_3), the process of oxidation of Fe^{2+} ions with ferrobacteria was inhibited (Fig. 6, curve 4). The purification efficiency in the first 15 min was 40 %; after 30 min of the contact – 48%; after 45 min – 60 %. Thus, as compared to the previous series of experiments, the total effect of the purification decreased down to 38–40 %. It may be explained by the process of inhibiting the bacteria activity by the prep-

Table 5. The quality parameters of the investigated neutral imitation water solutions with pH 6.5–7.5, n = 5

No. of Chart in Fig. 6	Parameters of solution quality					
	pH	Fe^{2+}, mg/cdm	Alkalinity, mmol/cdm	O_2, mg/cdm	Na_2CO_3, mg/cdm	PHMG, mg/cdm
No. 1	7.0	37.0	2.5	≈2.0	–	–
No. 2	6.9	37.0	1.8	≈1.5	–	–
No. 3	6.5	43.8	1.3	≈1.0	–	–
No. 4	7.0	39.0	2.2	≈1.0	40.0	0.01
No. 5	7.2	38.0	2.2	1.5	–	

Source: our own results.

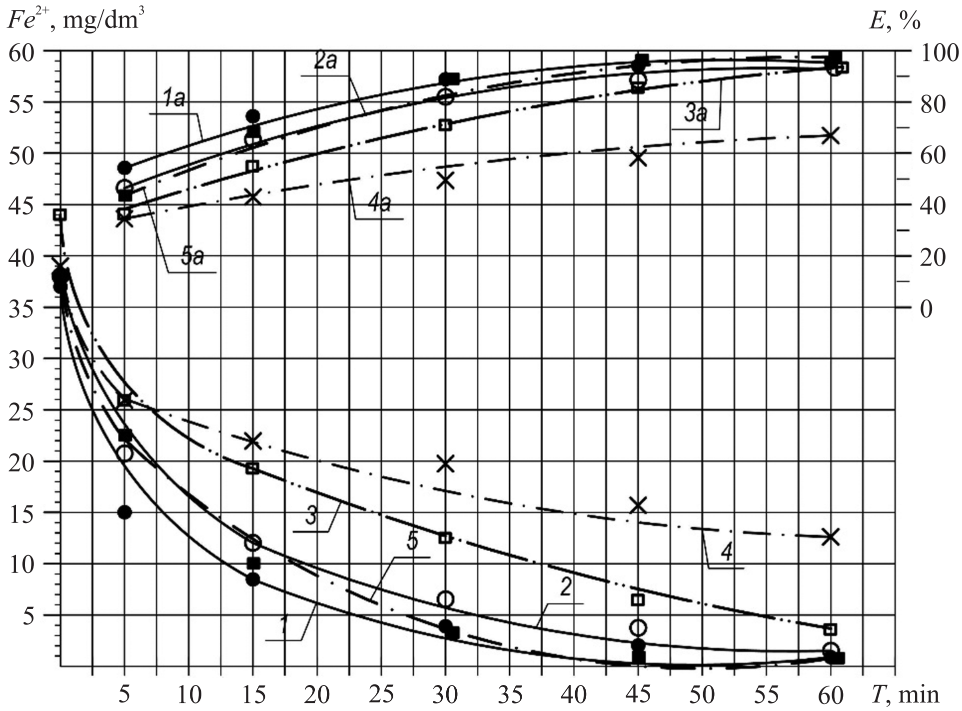

Fig. 6. The kinetics and efficiency of the purification of neutral iron-containing groundwaters in the suspended layer of matrix structures of ferrobacteria depending on the time of contact and the quality parameters of the initial water. The numbers of charts correspond to the quality parameters of the water, presented in Table 5. (Source: our own results)

aration due to its bactericidal effect (Lysytsya et al, 2015) and the absorption of polycations on the surface of matrix structures of ferrobacteria which displace Fe^{2+} cations and thus inhibit the transfer of electrons inside the cells, onto the breathing chain.

Therefore, it is not reasonable to use PHMGchl on the first stage of purification (deironing) to disinfect water. At the same time, during the purification of the systems of technical reverse water supply, and technical water of fire tanks, etc, polymer derivatives of guanidine (including PHMGchl) were found rather efficient (Kvartenko et al, 2021).

Thus, the application of the biological method provides for the increase in the filtration velocity up to 10–30 m/h (Mouchet, 1995; Søgaard et al, 2001; Kvartenko, 2023) as compared to 5–8 m/h while using the traditional technologies of deironing. The ability to capture and retain filth in the filtration load increases 4–5-fold (Mouchet, 1992), and there is a higher possibility of using the system in a larger number of filtration cycles.

Depending on the parameters of groundwater quality (pH-Eh; the content of dissolved organic substances, carbon dioxide, Fe(II) compounds), both chemolithoautotrophic bacteria of *Gallionella* genus and heterotrophic bacteria of *Lepthothrix* genus may exist in them, although in different ratios. The application of such consortia of bacteria, which have different mechanisms of oxidizing Fe(II) compounds provides for a stable work of bioreactors under conditions of seasonal changes in the water quality parameters. According to the data of (Cholodny, 1924; Starkey, 1945; Hallbeck & Pedersen, 1991), the bacteria of *Gallionella* genus use the energy of the iron carbonate oxidation to assimilate carbon dioxide, which acts as a carbon source as per the following equation:

$$2FeCO_3 + 3H_2O + 1/2O_2 = 2Fe(OH)_3 + 2CO_2 + 29 \text{ calories}$$

This reaction is accompanied with the energy release in the amount of 125 g-cal per 1 g of the oxidized salt $FeCO_3$. This is the energy used in the cells of *Gallionella* for all the work, required for the breakage of carbon (IV) oxide (CO_2) and the synthesis of organic matter in the cell (Starkey, 1945; Hallbeck & Pedersen, 1991). The process of enzymatic oxidation of Fe^{2+} ions occurs due to the transfer and integration of the electron into the breathing chain of the bacteria. The synthesis of 1 g of the cellular biomass of the microorganism requires the enzymatic oxidation of 276 g of Fe^{2+} ions with the release of 500 g of ferric hydroxide (Starkey, 1945), which, as a result of the biochemical transformation of $Fe^{3+} \rightarrow \gamma FeOOH$, creates the matrix structures of biominerals.

The mechanism of Fe^{2+} ion oxidation by the bacteria of *Lepthothrix* genera occurs by the peroxide pathway and appears in the capsules and on the surface of the cellular wall:

$$2Fe^{2+} + H_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + 2H_2O$$

It was found that while using the biological purification method, there is a possibility of removing ferric-humic complexes, ammonium nitrogen (to 1.5 mg/cdm), and easily oxidized organic substances (by PO to 6.0 mg O_2/cdm) from the groundwaters. In the molecular form, the oxidation of the ferric-humic complexes (IGC) by the activity products of the ferrobacteria from *Lepthothrix*, *Crenothrix* genera can be shown by the following equation of the chemical reaction:

$$Fe^{2+} - IGC + |O^{\bullet}| + 2H_2O \rightarrow Fe(OH)_3 - IGC + H^+$$

In the groundwaters with neutral or close to neutral values of pH, in the presence of chemolithoautotrophic bacteria of *Gallionella* genus, the removal of ammonium nitrogen in the bioreactors can be presented by the process of NH^{4+} ion sorption on the surface of the matrix structures of bio-minerals (Fig. 5), and on the cells of the very bacteria, due to the presence of the following functional groups on their surface: PO_4^{-3}, COO^-, OH^-.

The use of the two-stage scheme, as compared to the known schemes of biological deironing (Mouchet, 1992; 1995; Søgaard et al, 2001), provides for the application of the equipment under conditions of uneven hydraulic loads, notable for the water supply systems of rural settlements. The exploitation filtration velocities in this case were 7–11 m/h for the bioreactor, and 3.5–6 m/h for the filters.

The prolongation of the filtration cycle duration to 48 h became possible due to applying the biochemical purification method instead of the traditional physical-chemical one.

The study on the kinetics of the removal of Fe^{2+} cations from the neutral iron-containing ferrobacteria in the suspended layer of the matrix structures helped determine the optimal parameters of the initial water quality by the following values: pH 7.0–7.2; hydrocarbon alkalinity 2.5–2.2 mmol/cdm; content of soluble oxygen – 1.5–2.0 mg/cdm.

The results of the studies also demonstrated that the presence of even insignificant concentration of PHMG (10^{-2} % (vol %)) in the water led to the inhibition of the processes of biological oxidation of Fe^{2+} cations by ferrobacteria which may be used in preventing the biological growth in the pipes of the reverse water supply systems for the agroindustrial enterprises. These results may be conditioned by the process of inhibiting the bacteria activity by the preparation due to its bactericidal effect and the absorption of polycations on the surface of matrix structures of ferrobacteria, which displace Fe^{2+} cations and thus inhibit the transfer of electrons inside the cells, onto the breathing chain.

The analysis of the kinetics of removing Cr^{6+} ions (Table 3) leads to the conclusion that its efficiency depends on the precipitate concentration and the contact duration.

The comparative studies on the disinfecting properties of different guanidine derivatives demonstrated that both PHMG and PHMB would have the same effect on the colonies of microorganisms and biofilm in the reverse water supply systems or water storage tanks. For instance, PHMB showed relatively high activity regarding the biofilms, formed by *Prototheca spp*. (Fidelis et al, 2022). It is related both to the general bactericidal activity of the polymer derivatives of guanidine and their ability to affect intercellular interactions, proteins, and information exchange between the cells. This effect is known at least for eukaryotic cells (Kim et al, 2023). It is also relevant for the application of PHMG that, contrary to free guanidine, it is poorly broken by guanidine hydrolases of cyanobacteria and heterokont algae (Funck et al, 2022) and can ensure a long-term protective effect. At the same time, if it concerns the irrigation systems (watering of plants), their disinfection using PHMG does not have a negative effect on plants (Lysytsya, 2017). On the contrary, in the concentrations usually used for disinfection (≈0.01–0.1 %), it has a positive effect on the growth and development of plants, enhancing their stress resistance (Lyoshyna et al, 2020). It should be noted that, as compared to other disinfecting means, PHMG has the following advantages: biocidal effect regarding a broad spectrum of microorganisms, prolonged action, low toxicity for macroorganisms, absence of unpleasant smell, notable for many traditional disinfectants, stability and chemical inertness of their aqueous solutions, in particular, they do not damage surfaces made of metal, plastic, or resin (Lysytsya et al, 2015).

CONCLUSION

This was the first study on groundwater purification biotechnology under uneven hydraulic burden, notable for the water supply systems of rural settlements. The optimal technological parameters of the process were selected. For instance, it was determined that the filtration velocity of the bioreactor should be 7–11 m/h, for the filters – 3.5–5 m/h; the recommended filtration cycle duration – 48 h.

It was found that the application of the two-stage technology of biological deironing in the bioreactor and filters provided for the possible removal of Fe^{2+} compounds up to 5.0 mg/cdm, ammonium nitrogen – up to 1.5 mg/cdm, soluble organic substances by PO – up to 6.0 mg O_2/cdm.

It was determined that the optimal parameters for the process of biological purification of neutral groundwaters, containing increased concentrations of Fe^{2+} cations were as follows: pH 7.0–7.2; hydrocarbon alkalinity 2.5–2.2 mmol/cdm; content of soluble oxygen – 1.5–2.0 mg/cdm.

The ability of concentrated (C = 200 mg/cdm) matrix structures of *Gallionella* and *Lepthothrix* ferrobacteria to remove Cr^{6+} ions from natural groundwaters was first studied.

This was the first study on the effect of low (from 10^{-2} %) concentrations of PHMG on the processes of biological water purification from Fe^{2+} substances, the results of which demonstrated that this preparation inhibits the processes of biological oxidation of Fe^{2+} cations by ferrobacteria. It was confirmed that the systems of drinking water supply for consumers should use the following technological scheme: bioreactor – filter – disinfecting unit, using sodium hypochlorite or UV-radiation, allowed for such systems (Guidelines for drinking water quality, 2022). To decrease the biogenic and corrosion activity of the reverse water in the systems of technical water supply, it is possible to use the solution of PHMGchl.

Our comparative study on the disinfecting properties of different guanidine derivatives demonstrated that their efficiency did not change considerably when disinfectants manufactured based on PHMGchl or PHMBchl were used. In the concentrations of 0.02–0.05 %, they effectively disinfected most pathogenic microorganisms in the course of 15–30 min. The same was found true for the bacteria, most common in the systems of technical reverse water supply, the systems of water circulation, plant watering, and fire tanks, including *M. lysodeikticus*, *F. columnare*, *A. salmonicida*, etc. In this case, similar to the situation with protein load, it is reasonable to use PHMGchl and PHMBchl in the concentrations of 0.25–0.50 %.

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Біотехнологія очистки підземних вод для систем водопостачання з використанням феробактерій *Gallionella* та *Lepthothrix*

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Мета. Розглянути можливість використання консорціумів хемолітоавтотрофних феробактерій роду *Gallionella* та гетеротрофних бактерій роду *Lepthothrix* для біологічного методу очистки підземних вод. **Методи.** Фотоколориметричний метод для визначення концентрацій іонів амонію, феруму, титрометричний метод для визначення гідрокарбонатної та загальної лужності, а також перманганатної окисності за методом Кубеля, потенціометричний метод для визначення величин pH і Eh, електронну мікроскопію з проведенням рентгеноспектрального аналізу матриксних структур bio-мінералів, мікробіологічні та статистичні методи. **Результати.** Визначено основні технологічні параметри процесу знезалізнення води: швидкості фільтрування для біореактора 7–11 м/год, для фільтрів 3,5–5 м/год; тривалість фільтроциклу 48 годин. Встановлено, що в результаті застосування двоступеневої технології біологічного знезалізнення в біореакторі та фільтрах можливе видалення сполук Fe^{2+} до 5,0 мг/дм^3, амонійного нітрогену до 1,5 мг/дм^3, розчинених органічних речовин за ПО до 6,0 мг O_2/дм^3. З'ясовано, що оптимальними параметрами процесу біологічного очищення підземних нейтральних вод, які містять підвищені концентрації катіонів Fe^{2+} є: pH 7,0–7,2; гідрокарбонатна лужності 2,5–2,2 ммоль/дм^3; вміст розчиненого кисню 1,5–2,0 мг/дм^3. Встановлена можливість концентрованих ($Д_{ос}$ 200 мг/дм^3) матриксних структур феробактерій *Gallionella* та *Lepthothrix* вилучати іони Cr^{6+} із природних підземних вод. Встановлено, що при застосуванні дезінфектантів виготовлених на основі полігексаметиленгуанідину хлориду (ПГМГхл) або полігексаметиленбігуанідину (ПГМБхл) істотних відмінностей в їх ефективності немає. В концентраціях 0,25–0,5 % вони ефективно знешкоджують патогенні мікроорганізми, зокрема *Staphylococcus aureus*, *Esherichia coli* і *Pseudomonas aeruginosa*. Це стосується зокрема й бактерій найбільш поширених у системах технічного оборотного водопостачання, системах циркуляції води, поливу рослин і в протипожежних резервуарах, у тому числі *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Pseudomonas sp.*, *E. coli*, *Flavobacterium columnare*, *Micrococcus lysodeikticus*. **Результати**. Вперше проведено дослідження щодо можливості застосування біотехнології очищення підземних вод від надлишкової кількості заліза в умовах нерівномірності гідравлічних навантажень, які характерні для систем водопостачання сільських населених пунктів і більшості агропідприємств північно-західної та північної частин України. Вивчено особливості та перспективи застосування ПГМГхл в системах водопідготовки. З'ясовано, що процеси знезалізнення води за допомогою залізобактерій з точки зору безпеки й ефективності доцільно використовувати як у системах господарсько-питного так і технічного водопостачання, а знезараження води з використанням ПГМГ, через певну токсичність препарату, можливо лише в другому випадку. Визначено оптимальні параметри процесу біологічного очищення підземних нейтральних вод, які містять підвищені концентрації заліза.

Ключові слова: водоочищення, феробактерії, матриксні структури bio-мінералів, біореактор, знезараження води, похідні гуанідину.

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