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Nanocomposite complex ZnO in combination with a triazoloazepinium derivative for inhibition of microbial steel corrosion

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We have established the possibility of using ZnO nanoparticles to inhibit microbial corrosion with simultaneous inhibition of growth and sulfate-reducing activity of bacteria of the corrosive active group. The search for ways to increase the antibacterial and anticorrosive effect of ZnO nanoparticles makes it possible to combine them with substances, in particular, inhibitors-biocides of microbial steel corrosion.

The nanocomposite complex of ZnO nanoparticles and cationic heterocyclic compound (triazoloazepinium derivative) was studied as a biocide and inhibitor of microbial corrosion on steel. The component concentrations are 3 mg/mL and 1 mg/mL accordingly. The experiments were carried out by using a microbiological and corrosion control methods. It has been established that the proposed nanocomposite complex affects the number of bacteria in the plankton and biofilm. In its presence, there is a complete inhibition of the growth of sulfate-reducing bacteria (the most aggressive component of the microbial community) in plankton. Also, the number of denitrifying bacteria and iron-reducing bacteria in plankton decreases by 3 and 4 orders, respectively. The biofilm formed in the presence of the nanocomposite in the culture medium is less dense in terms of the number of bacterial cells of the sulfidogenic community.

Keywords: ZnO nanoparticles, biocorrosion, sulfate-reducing bacteria.

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Introduction

In recent years, there has been a rapid development of nanotechnology in various areas of human activity. Nanoparticles (NPs) of metals and their oxides are increasingly being used to create new useful materials and substances. For example, substances based on silver nanoparticles demonstrate high antimicrobial properties and are used in medicine and biotechnology [1-4]. Therefore, they are considered an alternative to antimicrobials.

For example, the authors [5] showed the antimicrobial effect of ZnO, CuO, and Fe_2O_3 nanoparticles on gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. It was identified that the nanoparticle-based substances had a greater antimicrobial effect on gram-positive than gram-negative microorganisms. It was also discovered that the antibacterial activity decreases in the ZnO - CuO - Fe_2O_3 series.

Recent advances in the field of nanotechnology, in particular, the possibility of obtaining highly ordered metal nanoparticles of any size and shape, have led to the development of new approaches to the creation of biocidal agents, used also for the prevention of microbial metal corrosion [5-7]. The antimicrobial effect on corrosive sulfate-reducing bacteria (SRB) was found for CuO and Ag nanoparticles. It has been shown that silver nanoparticles stabilized with sodium alginate contribute to the formation of a less dense biofilm under conditions of microbial steel corrosion initiated by sulfate-reducing

bacteria of the genus *Desulfomicrobium*. Meanwhile, bacteria of the genus *Desulfovibrio* proved to be resistant to silver nanoparticles [8]. CuO nanoparticles, obtained by biosynthesis with *Shewanellaindica*, are able to inhibit microbial corrosion induced by *Desulfovibrio marinisedimins* [1].

Researchers pay special attention to ZnO nanoparticles, since zinc ions can participate in ligand formation processes with organic molecules [2]. At the same time, zinc as a complexing agent has advantages over iron, silver, and copper, as it is characterized by relative biosafety and lack of oxidizing properties.

In [9], ZnO nanoparticles were studied and proven to be effective as part of composites for inhibiting acid steel corrosion. Thus, a composite containing 3-((3-acetylphenyl)imino)indolin-2-one and ZnO nanoparticles in 1M hydrochloric acid provides corrosion inhibition efficiency of up to 92%.

ZnO nanoparticles obtained by mycosynthesis using an aqueous extract of *Agarius bisporus* have anticorrosive properties for copper [10]. The use of such an inhibitor significantly reduces the thickness of the biofilm on the copper surface, which is formed mainly by the highly aggressive bacteria *Bacillus thuringiensis* EN2, *Terribacillus aidingensis* EN3, and *Bacillus oleronius* EN.

The mechanisms of corrosion protection can be different, but mostly nanoparticles are able to fill in defects on the surface of materials, covering cracks and crevices, thereby protecting the metal from aggressive agents [5, 7, 9].

The advantage of nanotechnology-based inhibitors is their environmental friendliness and low effective concentrations compared to traditional inhibitors.

We have established the possibility of using ZnO nanoparticles to inhibit microbial corrosion with simultaneous inhibition of growth and sulfate-reducing activity of bacteria of the corrosive active group [11]. The search for ways to increase the antibacterial and anticorrosive effect of ZnO nanoparticles makes it possible to combine them with substances, in particular, inhibitors-biocides of microbial steel corrosion, in one solution. The most promising and effective biocide inhibitors of microbial steel corrosion, induced by sulfate-reducing bacteria, are triazoloazepinium derivatives [12].

The aim of this paper is to investigate the effect of a nanocomposite complex of "ZnO nanoparticles - cationic heterocyclic compound (CHC)" on the components of the sulfidogenic microbial community under conditions of biocorrosion of structural steel and to evaluate the effectiveness of its corrosion protection compared to individual components.

I. Materials and methods

The nanocomposite complex of ZnO nanoparticles and cationic heterocyclic compound (triazoloazepinium derivative) was studied as a biocide and inhibitor of microbial corrosion on steel. The component concentrations are 3 mg/mL and 1 mg/mL accordingly.

ZnO nanoparticles were prepared by the electropulse ablation method and provided for research [13]. The ZnO preparation consists of individual nanoparticles with triangular, oval, and irregular shapes, along with their aggregates. This was determined through transmission electron microscopy. The triangular nanoparticles have a side length of 30-50 nm, while the oval and irregular nanoparticles are 30-60 nm in size. To achieve a uniform colloidal system, the nanoparticles were resuspended in bidistilled water using ultrasound. For the study, a newly prepared suspension was utilized.

As cationic heterocyclic compound was used 1-[2-(4-bromphenyl)-2-oxoethyl]-3-(3-chloro-2-methyl-anylinmethil)-6,7,8,9-tetrahydro-5H-[1,2,4]triazol[4,5-a]-azpinium bromide [12].

The experiments were carried out by using a microbiological [14] and corrosion control [15] methods.

In microbiological studies were used 5-day cultures of sulfidogenic microbial community (SMC) and strain *Desulfovibrio* M-4.1. Cultivation was done by using Postgate "B" media, which is optimal for the growth of sulfate-reducing bacteria and does not hinder the development of other components, in anaerobic jars at 28±2°C. The number of bacteria (in cultural liquid, in biofilm, which appeared on the metal samples surface) was calculated using the method of decimal serial dilution during the bacteria seeding to the correspondent liquid selective mediums: sulfate reducing bacteria – to the Postgate "B" medium [16], iron reducing bacteria (IRB) – to the Kalinenko medium, denitrifying (DNB) – Giltay medium. The biofilm cells, which appeared on the surface of steel samples during tests, were gathered into the fixed volume of (20 ml) 0.1N of phosphate buffer (pH=7) with the help of ultrasound with a frequency of 25 kHz (30s) twice with the 60s interval using UZM-003/n.

Strain *Desulfovibrio* sp. M-4.1 was isolated by us from natural sulfidogenic microbial community and described by morphological and cultural characteristics and identified by polymerase chain reaction method with the use of universal primers to the fragments of 16S rRNA genes. When conducting a comparative analysis of DNA sequencer which encodes 16S rRNA gene of tested bacteria with the strains of similar sequences from the GenBank database, sulfate reducing bacteria strain M-4.1 belonging to Desulfovibrio genus has been confirmed [17].

The antibacterial properties of the cationic active heterocyclic compound were determined by the method of wells into which alcohol solutions with a concentration of the studied salt of 0.2% and 2% were introduced.

The sensitivity of sulfate-reducing bacteria to CHC was determined by measuring the diameter of their growth retardation zone. Electron-microscopic studies were conducted, to investigate the effect of ZnO nanoparticles on the morphology of bacterial strains. The studies were carried out on an electron microscope Tesla BS 540, with an instrumental magnification of 22000 and an accelerating voltage of 75 kV. The preparations were made on copper grids with a collodion film that was fixed by carbon sputtering after application. The samples were not contrasted.

The corrosion study was conducted in sealed glass containers with samples of construction St3ps steel (surface area plate 19 sm^2) in sterile medium Postgate "B" inoculated with sulphidogenic microbial community: SRB - 10^6 cell/mL, IRB - 10^6 cell/mL, DNB – 10^7 cell/mL.

Samples soaking time was 14 day at 28 ± 2°C.

Using weight lost of samples were calculated corrosion rate (K_m, g/(m^2×h)), corrosion inhibition coefficient ($\gamma_m=K_m/K_m'$, where K_m та K_m' – corrosion rate with and without the inhibitor) and the inhibition efficiency ($Z_m=(1-1/\gamma_m)\times100$ %).

The concentration of biogenic hydrogen sulfide was measured with iodometric titration. The following rules were followed: hermetically sealed crusts, minimum time for sample collection, front drainage of the container and mixing of the suspension with a sterile pipette.The degree of influence (S, %) on bacteria sulfate reduction was calculated using the formula: S = ((C-C′)/C)×100%, where C and C′ are the average hydrogen sulphide concentration with and without the inhibitor accordingly, mg/l.

The concentration of Zn^{2+} in cultural liquid and samples of biofilm was measured with inversion voltammetry on the voltammetry analyzer TA-Lab (Research and Development Enterprise “Tomanalit”) in a three-electrode electrochemical cell. As an indicator electrode amalgam electrode was used. As an auxiliary electrode - KCl electrode.

The analysis was performed on background electrolyte, which contains 200 mcL of concentrated formic acid (chemically pure), on the following conditions: electrochemical purification of the indicator electrode at potential +0.05. During 10 seconds, metals accumulation on the surface of the indicator electrode at potential 1.50 V. During 10 seconds, solution calming at potential 1.30 V during 5 seconds, anodic oxidation at a liner speed of scanning the potential with speed 80 mV·s^{-1}.

Zinc ions definition was performed by the method of additives using standard solution, which contains 0.015 mM of Zn^{2+}. Solution was prepared on the base of state standard samples and double distilled water. Calculation of the zinc concentration was performed with the help of method of one addition that is stopped up by the manufacturer of device TA - Lab (specialized computer program, version 3.6.10).

Statistical analysis of experimental data for the reliability level 95% was conducted with with signs of normal t-distribution. The experiment was conducted three times. Relative error does not increase 10%.

Computer calculation was done using the computer software ChemOffice (PerkinElmter Informatics Inc.).

II. Results and discussion

Microbiological and corrosion indicators of microbial corrosion of structural steel, induced by sulfidogenic microbial communities (SMC), in the presence of ZnO NPs and a nanocomposite complex of "ZnO nanoparticles - cationic heterocyclic compound" are presented in Fig. 1 and Table1.

It has been established that the proposed nanocomposite complex affects the number of bacteria in the plankton and biofilm. In presence of nanocomposite complex there is a complete inhibition of the growth of sulfate-reducing bacteria (the most aggressive component of the microbial community) in plankton. Also, the number of denitrifying bacteria and iron-reducing bacteria in plankton decreases by 3 and 4 orders, respectively. The biofilm formed in the presence of the nanocomposite in the culture medium is less dense in terms of the number of bacterial cells of the sulfidogenic community.

A significant (5 orders) decrease in the number of SRB and 2 orders in the number of IRB were observed. The number of DNB in the biofilm in the presence of the complex remains at 10^5 cell/ml. Thus, ZnO nanoparticles in combination with HCC prevent the adhesion of bacteria on a metal surface, demonstrating antibiofilm properties. Our results are consistent with the data obtained during the study of the ZnO/RAM composition, which ensures the formation of a less aggressive biofilms [18].

In the presence of the proposed nanocomposite

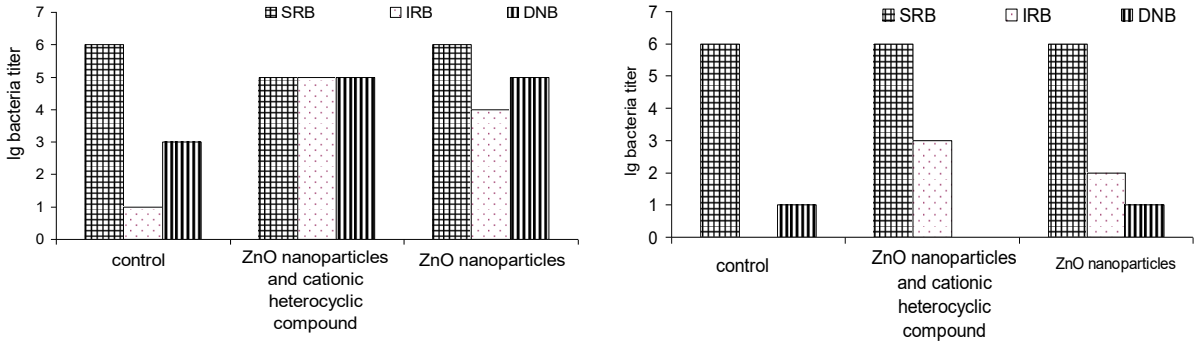


Fig. 1. Microbiological indicators of microbial steel corrosion, induced by sulfidogenic microbial community: a) in biofilm, b) in corrosive medium.

Table 1.

Corrosion indicators of microbial steel corrosion, induced by sulfidogenic microbial community

Postgate "B" medium	K_m, g/(m^2×h)	Content H_2S, mM	Zn^{2+} concentration in biofilm, mM
SMC (control)	15.89×10^{-3}	15.56	-
SMC + NPs+ CHC	0.36×10^{-3}	4.30	0.075
SMC + NPs	1.07×10^{-3}	3.50	0.051

«-» - the indicator was not determined

complex in the Postgate "B" medium, the concentration of biogenic hydrogen sulfide was 3.6 times lower than the control, which corresponds to a degree of influence on bacterial sulfate reduction at the level of 72 %. This determines the high anticorrosive effect of the proposed composite, with a protective effect of 98 %.

It should be noted that ZnO nanoparticles also exhibit antimicrobial properties on the components of the sulfidogenic microbial community, as shown by us earlier [11]. In particular, free-floating bacteria (plankton) are most affected: the number of SRB and IRB decreases by 5 orders, and DNB were not detected at all in the presence of NPs (Fig. 1). Of all biofilm, the greatest impact of nanoparticles is experienced by SRB, the number of which decreases by 3 orders. The biofilm DNBs remain insensitive to ZnO nanoparticles, and the number of IRBs decreases only by 1 order. The degree of influence on bacterial sulfate reduction is 78 %, and the protective effect is 94 %.

The cationic active CHC, used to prepare the nanocomposite, is characterized by a biocidal effect on SRB. Thus, the diameter of the inhibition zone of their growth at a solution concentration of 0.2 % and 2 % is 31±1.4 mm and 36±1.8 mm respectively. The compound has no effect on the corrosion rate in the sterile Postgate "B" environment, and under conditions of microbial corrosion, it has a protective effect of 76 %. This is due to its impact on the microbiological factor (Fig. 2). The number of planktonic bacteria in the community decreases under the influence of CHC. Thus, there is a decrease in the number of SRB by 4 orders, IRB - by 3 orders, DNB - by 1 order compared to the control.

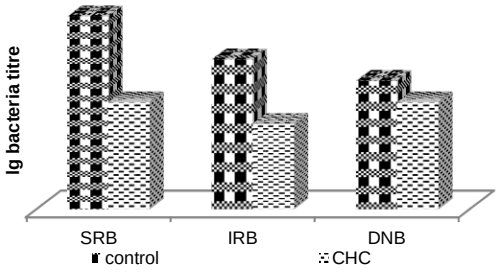
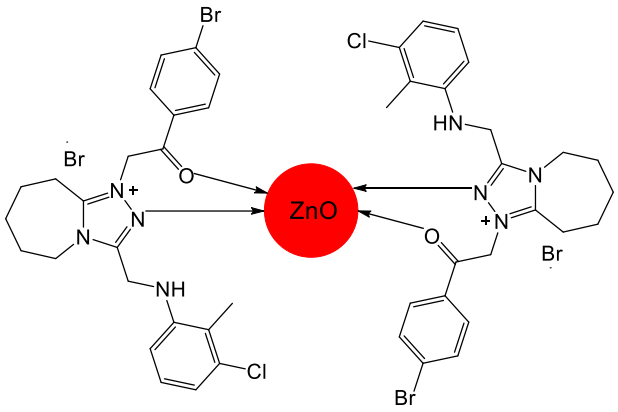


Fig. 2. The number of bacterial cells of the sulfidogenic microbial community in plankton without (control) and in the presence of CHC under conditions of microbial steel corrosion.

Therefore, the nanocomposite complex of ZnO nanoparticles and cationic heterocyclic compound is characterized by a greater effect on the components of the sulfidogenic microbial community and greater efficiency of corrosion protection. This can be explained by the ability of ZnO nanoparticles to participate in the processes of ligand formation with organic molecules [2]. In this case, the formation of a chelate complex is possible with the participation of undivided pairs of electrons of the Oxygen from carbonyl group and the Nitrogen of the CHC triazole cycle, not involved in the formation of chemical bonds. Quantum-chemical calculations of the effective charges (Table 2) revealed that these atoms are characterized by an increased electron density.

The difference in the energy values of the higher occupied molecular orbital and the lower vacant molecular orbital (E_{HOMO} - E_{LUMO}) is 4.861 eV and indicates a high reactivity of the compound.

The structure of the chelate complex can be represented by the following scheme:



The formed complex is able to interact with bacterial cells of the biofilm better than individual nanoparticles. It was established (Table 1) that the amount of zinc ions in the biofilm formed in the presence of the nanocomposite complex is 48 % higher than in the biofilm formed only in the presence of NPs. This can be explained by the fact that the ZnO nanoparticles of the chelate complex have a larger surface area, leading to the formation of more Zn^{2+}, which limits the functioning of corrosive bacteria. As a result, the adhesion of bacteria to the metal surface of the sample and corrosion under the biofilm is effectively suppressed (Table 1, Table 3).

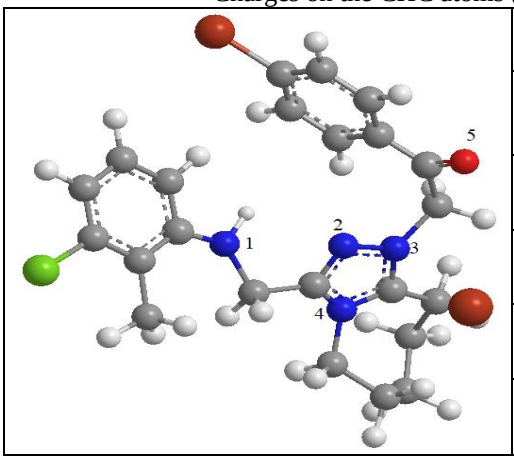
In further studies, the effect of ZnO nanoparticles and a nanocomposite complex of "ZnO nanoparticles - cationic heterocyclic compound" on the functioning of sulfate-reducing bacteria of the strain *Desulfovibrio sp.*M-4.1 under conditions of microbial steel corrosion was evaluated (Table 3). It has been shown that introducing ZnO nanoparticles and nanocomposite into the corrosive medium leads to a decrease in the number of sulfate-reducing bacteria cells in plankton and biofilm.

At the same time, bacteria in plankton were not detected during the exposure, and inhibition of sulfate-reducing activity of bacteria was observed: the concentration of biogenic hydrogen sulfide decreased by 2.5-13.0 times compared to the control (Table 3). Thus, both ZnO NPs and the nanocomposite inhibit bacterial cell division in plankton and their physiological activity. In the presence of substances in the corrosive medium (NPs, NPs+CHC), the number of bacterial cells in the biofilm decreases by 4-5 orders, which leads to the inability of bacteria of the *Desulfovibrio sp.*M-4.1 strain to form a dense biofilm on a metal surface. The corrosion rate is inhibited by 1.8-4.7 times. Simultaneously, the highest protective effect (Z_m = 79 %) and the most pronounced antibiofilm properties under conditions of microbial steel corrosion, induced by bacteria of the *Desulfovibrio sp.*M-4.1 strain, were discovered for the nanocomposite.

Thus, it was established that under the conditions of microbial corrosion, ZnO nanoparticles and NPs in the composition with CHC have an antibacterial effect on

Table 2.

Charges on the CHC atoms (Chem3D)



Atom (number)	Charge
N(1)	0.19300
N(2)	-0.39600
N(3)	0.62198
N(4)	0.49282
O(5)	-0.59096

Table 3.

Indicators of microbial steel corrosion, induced by sulfate-reducing bacteria of the strain *Desulfovibrio* sp. M-4.1

Postgate "B" media with *Desulfovibrio* sp. M-4.1	$K_m \times 10^3$, g/(m^2×h)	γ_m	C(Zn^{2+}) in biofilm, mM	C(H_2S), mM	Bacteria titer, cell/mL	
					plankton	biofilm
Control	16.8	-	-	9.94	10^5	10^6
ZnO	9.28	1.8	0.09	0.77	0	10^2
ZnO and heterocyclic compound	3.57	4.7	0.10	3.90	0	10^1

«-» - the indicator was not determined

a) b) c)

Fig. 3. Morphology of cells of sulfate-reducing bacteria strain *Desulfovibrio sp.*M-4.1 without (a) and under the influence of ZnO nanoparticles; (b) and ZnO nanoparticles in combination with CHC (c), electron microscopy (×22000).

sulfate-reducing bacteria of the *Desulfovibrio sp.*M-4.1 strain.

The mechanism of antimicrobial activity of ZnO nanoparticles may be related to the violation of the integrity of the bacterial cell membrane, reduction of the hydrophobicity of the cell surface, and inhibition of transcription of oxidative stress resistance genes in bacteria. The inhibition of sulfate-reducing bacteria growth may also be affected by the penetration of nanoparticles (in the form of Zn^{2+} ions) into the bacterial membrane and further interaction with intracellular components. Fig. 3b shows that under the action of ZnO nanoparticles, cytoplasmic content leakage (reduction in cell size) occurs, indicating a strong interaction of zinc oxide nanoparticles with the outer surface of the cell and intracellular components, which leads to cell death and destruction of biofilms on the metal surface.

Significant surface binding of zinc oxide nanoparticles with subsequent intracellular absorption disrupts cell morphology and causes significant damage to the cell membrane (Fig. 3 b).

Under the action of the nanocomposite complex, the morphology of the cells of sulfate-reducing bacteria strain *Desulfovibrio sp.*M-4.1. differs from that of the ZnO NPs: the cell size did not change (Fig. 3c). It can be assumed that in the composition with CHC there is a change in the physical characteristics of ZnO nanoparticles (structure, surface, size), which leads to a mutual enhancement of antibacterial and anticorrosive effects. In this case, the mechanism of the antibacterial activity of the proposed nanocomposite is possibly due to its electrostatic interaction with the negatively charged outer membrane of gram-negative bacterial cells, in particular the sulfate-reducing strain *Desulfovibrio sp.*M-4.1. This leads to a breach of the composition of the outer membrane and limits the supply of essential nutrients to the bacterial cell for normal functioning.

Conclusion

Thus, the obtained data indicate an increase in the antibacterial and corrosion activity under conditions of microbial corrosion of steel with ZnO nanoparticles in combination with a cationic heterocyclic compound of a number of triazoloazepinium derivatives. The proposed composition can reduce bacteria's sulfate reduction by 72.4 % and protect structural steel by 98 %, and can be recommended for retarding corrosion initiated by sulfate-reducing bacteria.

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Нанокомпозитний комплекс ZnO в комбінації з похідною триазолоазепінію для інгібування мікробної корозії сталі

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Встановлено можливість використання наночастинок ZnO для інгібування мікробної корозії з одночасним пригніченням росту та сульфатвідновлювальної активності бактерій корозійно-активного угруповання. Пошук шляхів підвищення антибактеріальної та антикорозійної дії наночастинок ZnO дає можливість комбінувати їх з речовинами, зокрема, інгібіторами-біоцидами мікробної корозії сталі. Нанокомпозитний комплекс наночастинок ZnO та катіонної гетероциклічної сполуки (похідного триазолоазепінію) досліджували як біоцид та інгібітор мікробної корозії сталі. Концентрації компонентів становили 3 мг/мл та 1 мг/мл відповідно. Дослідження проводили з використанням мікробіологічних та корозійних методів. Встановлено, що запропонований нанокомпозитний комплекс впливає на кількість бактерій у планктоні та біоплівці. За його присутності спостерігається повне пригнічення росту сульфатвідновлювальних бактерій (найагресивнішого компонента мікробного угруповання) в планктоні. Також кількість денітрифікуючих бактерій та залізовідновлюючих бактерій у планктоні зменшується на 3 та 4 порядки відповідно. Біоплівка, що утворюється за присутності нанокомпозиту в поживному середовищі, є менш щільною за кількістю бактеріальних клітин сульфідогенного угруповання.

Ключові слова: наночастинки ZnO, біокорозія, сульфатвідновлювальні бактерії.