UDC 541.49:546.791.6 +546.73 https://doi.org/10.15407/biotech13.04.060

BIODEGRADATION AND ANTIMICROBIAL ACTIVITY OF GUANIDINE-CONTAINING POLYETHYLENE OXIDE HYDROGEL

G. O. Iutynska[2]
M. Ya. Vortman[1]
D. R. Abdulina[2]
Zh. P. Kopteva[2]
A. Ye. Kopteva[2]
A. V. Rudenko[3]
V. V. Tretyak[3]
V. N. Lemeshko[1]
V. V. Shevchenko[1]

[1]Institute of Macromolecular Chemistry of the National Academy of Sciences of Ukraine

[2]Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine

[3]Institute of Urology of the National Academy of Medical Sciences of Ukraine

E-mail: vmar1962@i.ua

Received 11.05.2020
Revised 19.07.2020
Accepted 31.07.2020

The aim of the study was to determine the biodegradation of guanidine-containing polyethylene oxide hydrogel under the action of bacteria and to study its antimicrobial properties. The antimicrobial activity of newly synthesized polyethylene oxide hydrogel was studied by a disco-diffusion method. The enzymatic activities of bacteria were determined by spectrophotometry. To study the resistance of guanidine-containing polyethylene oxide hydrogel to microbial destruction, bacteria were grown in Tauson's liquid medium with the addition of meat peptone broth at a temperature of 28±2 °C. Changes in the chemical composition of the studied materials were analyzed by infrared Fourier spectroscopy and 1H NMR spectroscopy.

Guanidine-containing polyethylene oxide hydrogels were obtained by reacting oligooxyethylene glycol MW 6000 with toluene diisocyanate and guanidine-containing oligomer, which acted as a crosslinking, ion-containing and antibacterial-agent. The synthesized hydrogel showed antimicrobial activity against both gram-positive and gram-negative bacteria. The biodegradation of hydrogels under the action of various bacterial strains and enzymes that they synthesize was studied. The presence of the tested materials lead to a decrease in the enzymatic activity of bacteria in 2.5 times compared with the control.

Synthesized guanidine-containing polyethylene oxide hydrogel showed antimicrobial activity against the studied test cultures. The introduction of the studied materials into Tauson's environment as additional sources of carbon and energy helped to reduce the catalase and lipase activities of hydrocarbon-oxidizing bacteria. Under the influence of the studied bacteria, the hydrogels were destroyed up to 88.4% from the initial value.

Key words: Guanidine-containing polyethylene oxide hydrogel, antibacterial properties, gram-positive and gram-negative bacteria, catalase and lipase activities, biodegradation, IR spectra.

Biodegradation of various materials in the environment is important in terms of their protection because a lot of synthetic materials are stable for a long period. However, at the end of the life cycle, the materials must be involved in the natural cycle and decomposed. Expired materials and products are exposed to aggressive microorganisms. This confirms the need to replace such materials on biodegradable ones [1–3]. Under natural conditions, the microbial attack on biodegradable material includes not one species, but the association of microorganisms both anaerobes and aerobes interact with each other and produce enzymes that are involved in the material destruction [4–6]. These microorganisms' activity is

influenced by environmental conditions, as well as the composition and chemical structure of the material. The bacteria of *Cytophaga, Bacillus, Rhodococcus, Streptomyces, Mycobacterium, Pseudomonas* genera and filamentous fungi of *Aspergillus, Alternaria, Penicillium, Trichoderma* genera, and others play the greatest role in the microbial destruction of materials [3].

In the current literature, there are a lack of data about enzymatic activity of polymer-destroying bacteria and mainly about on the activity of redox and hydrolytic enzymes in micromycetes that damage building materials, foams, rubbers, etc. [7, 8]. However, the lipolytic and catalase activities of bacteria, which result in microbial destruction of materials, reduce their strength and elasticity, remain poorly studied [7, 8].

In modern polymer chemistry, one of the promising areas is the obtaining and studying the properties of hydrogel systems, among which the polyethylene oxide hydrogels take a significant place. They are widely used in various fields of science and technology due to non-toxicity, high degree of swelling [9–11], as well as the ability to biodegradation [12–16]. One method of polyethylene oxide hydrogels producing is based on the reaction of urethane formation in the presence of a crosslinking agent. Particular importance is attached to the construction of pH-sensitive hydrogels, which are carried out by introducing a controlled number of ionic groups by copolymerization of acrylic derivatives of oligooxyethylene glycols with acrylic monomers containing such groups, or the introduction of ionic additives in the urethane formation reaction. The obtaining of hydrogels using derivatives of such a highly basic compound as guanidine, which has antimicrobial activity is of particular interest. Among guanidinium-containing polymers, polyhexamethylene guanidinium chloride attracts attention due to its biocide properties, which are important for practical use [17, 18]. It belongs to the fourth security class. The high reactivity of the guanidine fragment stimulated research in the direction of chemical modification of polyhexamethylene guanidine chloride in order to provide it with additional functionality. Its derivatives with methylene, acrylate groups are used for obtaining pH-sensitive hydrogels [19]. Guanidine-containing polyethylene oxide hydrogels have not yet been described in the literature. It can be assumed that such hydrogels should have antibacterial and biodegradable properties as well as polyhexamethylene guanidine chloride will be low-toxic.

The aim of this work was to determine the antimicrobial properties of materials against bacteria isolated from pathogenic material of sick people and biodegradation of guanidine-containing polyethylene oxide hydrogel under the action of bacteria previously isolated and identified from damaged protective coatings of gas pipelines.

Materials and Methods

Materials. Diane epoxy oligomer DER-331 (DOW Chemical Company, Germany), mass fraction of epoxy groups 23.5% with a content of 0.6% hydroxyl groups was dehydrated by heating in vacuum for 2–6 hours at 80–90 °C and a final pressure of 266.6 pa. Guanidine hydrochloride (GD) (Sigma-Aldrich, USA, degree of purity 99.9%), medical ethanol rectification (96%), oligooxyethylene glycol (OEG) MW 6000 (Sigma-Aldrich, USA, 99.9%) and toluene diisocyanate (TDI) — a mixture of isomers 2.4 and 2.6 (Sigma-Aldrich, USA, 99.9%) were used without further purification. Dimethylformamide (DMF) was purified by distillation.

Guanidine-containing oligomer (GO) was obtained at a temperature of 50 °C for two hours by reaction of 0.1 M of epoxy oligomer in 70% ethanol solution while stirring with an alcoholic solution of 0.2 M guanidine obtained immediately before the reaction. The yield of the final product was 95%.

Obtaining polyethylene oxide hydrogel was performed by melting oligooxyethylene glycol MW 6 000 and mixing it with guanidine-containing oligomer at 85 °C to homogenize the mixture at a molar ratio of the original components of OEG:GO-1.5:(1–1.5), then mixing with TDI at a molar ratio of the original components of 1.5:(1–1.5):2. The formation of the hydrogel was performed at 25 °C for one hour on a substrate, curing — at 80 °C for 4 hours. Two types of hydrogel are obtained, which differ in the molar content of guanidine-containing oligomer: hydrogel 1 — ratio of the initial components of OEG:GO:TDI (1.5:1:2) and hydrogel 2 — ratio of the initial components OEG:GO:TDI (1.5:1.5:2). The degree of crosslinking of the obtained hydrogel was determined by the content of the gel fraction in the Soxhlet apparatus in 93–95% acetone solution.

Disco-diffusion method was used to determine the bactericidal activity of the

original guanidine-containing oligomer and polyethylene oxide hydrogels against the bacteria *Staphylococcus aureus* 451, *Escherichia coli* 475, *Pseudomonas mirabilis* 494. These strains have been isolated from biomaterial of urogenital system of sick people and stored in the Laboratory of Bacteriological Research of the Institute of Urology of the National Academy of Medical Sciences of Ukraine. Also, hydrocarbon-oxidizing bacteria *Pseudomonas pseudoalcaligenes* 109, *Rhodococcus erythropolis* 102, *Bacillus subtilis* 138, which were previously isolated and identified by us from damaged protective coatings of gas pipelines [4, 6] and stored in the Collection of the Department of General and Soil Microbiology of the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine were used. Guanidine-containing oligomer in the form of a 0.1 ml aqueous solution was used with standard paper disks with a diameter of 6 mm on the surface of meat-peptone agar inoculated with the appropriate bacterial test-culture. Polyethylene oxide hydrogels have been obtained based on guanidine-containing oligomer in the form of a film with a size of 10x10x0.5 mm. They were applied to the surface of meat-peptone agar inoculated with the appropriate test culture of bacteria. Incubation was performed for 18 hours at 28 ± 2 °C. Antimicrobial activity was calculated in diameter (mm) of the zones of growth inhibition of microorganisms. Antimicrobial activity was estimated by the following scale: zone 0–10 mm — non sensitive, 15–25 mm — sensitive, up to 25 mm — high sensitive.

Microbial degradation of guanidine-containing polyethylene oxidehydrogel was studied with the use of hydrocarbonoxidizing bacteria *P. pseudoalcaligenes* 109, *R. erythropolis* 102 and *B. subtilis* 138 strains. Bacteria had grown in Tauson's liquid medium at 28 ± 2 °C [5] with the addition of 20% meat-peptone broth (MPB) as a source of Nitrogen and Carbon Test materials' samples with the sizes of 10×10×0.5 mm were weighed on electronic scales (ANG-200, AXIS) and sterilized with 72% solution of ethyl alcohol (30 min) and with UV rays with a wavelength of 256 nm (15 min) and were put in Tauson medium, inoculated with one of the above-mentioned bacterial strains. The amount of cells were 10^6 cells/ml calculated with serial dilution method. Variants with Tauson's medium. with materials without the addition of bacteria were used as control. The term of the experiment was 60 days. The destruction of the samples was determined gravimetrically by loss of their mass. To perform this, after the exposure, the material samples were removed from the cultural liquid, air-dried, then weighed and the weight loss of control and test samples was determined. The amount of bacteria in the cultural liquid was determined with serial dilution method [20].

Enzymatic activity. To determine the effect of materials on the enzymatic activity of bacteria, cultural liquid was centrifuged for 20 min at 8 000 rp/min (2 000 g) with the use of Eppendorf 5810R centrifuge (Germany). Lipolytic activity was determined by reaction with *p*-nitrophenylpalmitate [21], catalase activity was determined using 0.03% hydrogen peroxide, which formed a stable colored complex with 4% molybdenum phosphate solution [22]. Enzymatic activity was determined in the supernatant spectrophotometrically on KFK-3 (Russian Federation). Total protein concentration in the cultural liquid was determined by the Lowry method. The specific activity of the studied enzymes was calculated according to the formulas specified in [18, 19] and was expressed in u·mg^{-1} protein.

IR spectra of the investigated materials with Fourier transform were obtained on a spectrophotometer TENSOR 37 (BrukerOptik GmbH, Germany) in the spectral range of 6 000–400 cm^{-1} in KBr tablets.

^1H *NMR spectra* were recorded on a Varian VXR-400 MHz instrument in $CDCI_3$ solution.

The degree of swelling of the obtained polyethylene oxide hydrogels in water for injections at pH 4.0, 7.0 and 9.8 at room temperature to constant values was measured by weighing on the torsion scales. The degree of swelling of the samples was calculated with the formula: $\alpha = (m - m_0)/m_0$, where m_0 is the mass of the sample before swelling, m is the mass of the swollen sample.

Statistical analysis of the obtained results was performed using the software package MS Excel 2010 and OriginPro 2016 (ver. B 9.3.226. www.originlab.com/). The obtained data were represented as $M \pm n$ ($n = 3$, at $P \leq 0.05$).

Results and Discussion

To obtain a polyethylene oxide hydrogel, a guanidine-containing oligomer of linear structure with terminal guanidine fragments has been synthesized by the reaction between a bifunctional aromatic oligoepoxide (MW 365) and guanidine at a molar ratio of 1:2 (Fig. 1).

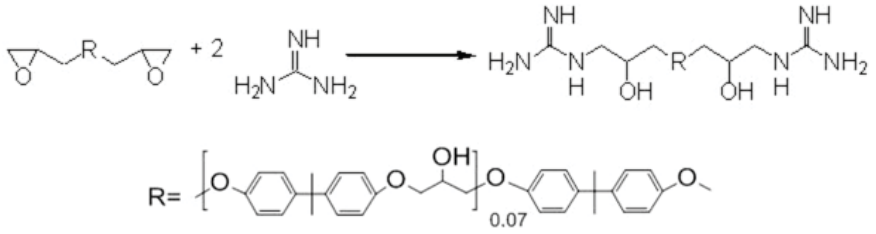

Fig. 1. **Scheme of synthesis of guanidine-containing oligomer**

The structure of the synthesized guanidine-containing oligomer of linear structure was confirmed by IR and ^{1}H NMR spectrometry by the following data:

IR (KBr) (νNH, OH), 3 156 cm^{-1} (δNH), 2 929 cm^{-1} (νCH), 2 963 cm^{-1} (νCH_2), 2 869 cm^{-1}(νCH_3), 1 648 cm^{-1}(νC=Nandν NH), 1 450–1 650 cm^{-1}(νC=C C_6H_4), 1 100–1 300 cm^{-1}(νC-O-C), the absorption bands of epoxy groups at 920 cm^{-1} were disappeared. In ^{1}H NMR ($CDCI_3$) spectra of guanidine-containing oligomer there are proton signals at 1.72 ppm (t, 3H, $-CH_3$), 2.73 ppm –NH ($NHCH_2$)), 2.58 ppm $-CH_2$ (CH_2CHOH), 3.58 ppm –OH (CH–OH)), 3.96 ppm –CH (CH–OH)), 6.8 ppm and 7.2 ppm –CH benzene ring, 8.4 ppm and 8.6 ppm –NH ($-NH_2$ groups).

The synthesized guanidinium-containing oligoether with terminal guanidine moieties was used for the synthesis of polyethylene oxide hydrogels (1 and 2) as a crosslinking and antimicrobial agent. According to the results of microbiological studies, the obtained guanidine-containing oligoethers showed antimicrobial activity (Table 1; Fig. 2) against the studied bacterial test-cultures *P. mirabilis* 494, *E. coli* 475, *P. pseudoalcaligenes* 109 and *S. aureus* 451.

The diameters of inhibition zone of bacterial growth were in the range of 13–20 mm. The largest zones of growth inhibition were observed for strains of *R. erythropolis* 102 and *B. subtilis* 138 (24–26 mm) under the influence of hydrogels 1 and 2. The obtained two variants of polyethylene oxide hydrogels showed antimicrobial activity against the studied test-cultures. The diameters of the zone growth inhibition for strains *E. coli* 475, *P. mirabilis* 494, *S. aureus* 451, *P. pseudoalcaligenes* 109 were in the range 10–11 and 15–16 mm for hydrogel 1 and hydrogel 2, respectively. Hydrocarbon-oxidizing bacteria *R. erythropolis* 102 and *B. subtilis*138 were more sensitive to synthesized hydrogels because inhibition zones were 15–19 and 20–24 mm for hydrogel 1 and 2, respectively. Hydrogel 1 had low antibacterial activity against the studied test cultures due to lower content of guanidine-containing oligoether in hydrogel 1, which acts as a crosslinking and antibacterial agent (Table 1). The scheme of synthesis of polyethylene oxide hydrogel is shown on Fig. 3.

The structure of the obtained hydrogels was confirmed by IR spectroscopy (Fig. 4).

IR spectra consists of (KBr) (νNH, OH) 3 156 cm^{-1}, 2 949 (νCH), 2 896 cm^{-1} (νCH_2), 2 868 cm^{-1} (νCH_2), 1 648 cm^{-1} (δNH), (νC = N), (1 450–1 650) cm^{-1} (νC_6H_5), (1 100–1 300)) cm^{-1} (νCOC).

The difference between the IR spectra of hydrogels 1 and 2 is the different ratio of the intensity of the absorption bands of the valence vibrations of the –CH bonds to the valence vibrations of the hydroxyl (–OH) and amino(–NH) groups. There was an increase in the absorption band of NH groups, because the molar content of GO in the hydrogel 2 was in 1.5 times higher than in hydrogel 1.

The degree of swelling of the polyethylene oxide hydrogels were obtained for various conditions, i.e. pH, temperature and swelling duration (Fig. 5).

The degree of swelling under different pH was in the range 8.05–11.5 (Fig. 5, *a*). The data show that both hydrogels are highly swellable and this parameter depends on the pH of the medium, the high degree of swelling 10.2–11.1 is observed under pH 4.0. The dependence of the degree of swelling of polyethylene oxide hydrogel 2 in water on temperature (Fig. 5, *b*) showed that high values 14 is observed at 56 °C.The study of the duration of swelling of hydrogel 2 (Fig. 5, c) showed that under pH 4.0 and pH 7.0 during 180–210 min there was complete swelling and values were 11.1 and 9.0, respectively.

The hydrocarbon-oxidizing bacteria *P. pseudoalcaligenes* 109, *R. erythropolis* 102 and *B. subtilis* 138 produced extracellular poly- and lipopolysaccharides, due to which these bacteria adhere to the surface of protective materials, ensuring their rapid reproduction and promoting survival of bacterial population

Table 1. **Antimicrobial properties of the original oligomer and obtained polyethylene oxide hydrogels**

Bacterial strains	Growth inhibition zone diameter, mm		
	Guanidine-containing oligoether	Guanidine-containing hydrogel 1	Guanidine-containin ghydrogel 2
Gram-negative bacteria			
Escherichia coli 475	14 ± 0.20	10 ± 0.08	16 ± 0.20
Pseudomonas mirabilis 494	13 ± 0.15	11 ± 0.13	16 ± 0.15
P. pseudoalcaligenes 109	15 ± 0.17	10 ± 0.10	15 ± 0.17
Gram-positive bacteria			
Rhodococcus erythropolis 102	24 ± 0.32	15 ± 0.15	20 ± 0.32
Bacillus subtilis 138	26 ± 0.25	19 ± 0.20	24 ± 0.25
Staphylococcus aureus 451	20 ± 0.08	11 ± 0.17	15 ± 0.08

Notes: 0–10 mm non sensitive microorganism; 15–25 mm — sensitive, up to 25 mm — high sensitive (n = 3)

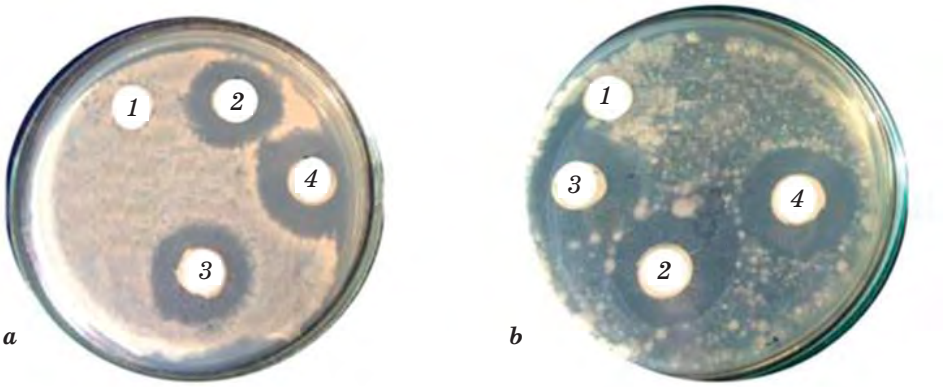

Fig. 2. **Growth inhibition zone diameter of the *Rhodococcus erythropolis* 102 (*a*) and *Bacillus subtilis* 138 (*b*) under the influence of polyethylene oxide hydrogels**
1 — control (distilled water); *2* — hydrogel 1; *3* — hydrogel 2; *4* — initial guanidine-containing oligomer

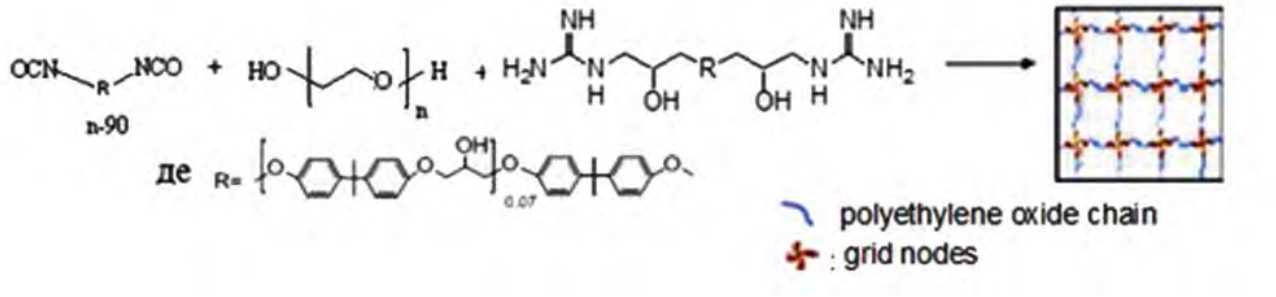

Fig 3. **Scheme of polyethylene oxide hydrogel synthesis**

in extreme conditions. The identified exopolysaccharides differed in monosaccharide composition and fatty acid content. Analysis of the fatty acid composition of the exopolymer complex of bacteria revealed the presence of saturated, unsaturated fatty acids and hydroxyl- acids with a carbon chain length of 11 to 18 carbon atoms [7].

In Tauson's liquid medium (with the addition of MPB, inoculated with bacterial strains and without hydrogels (control), increasing the number of hydrocarbon-oxidizing bacteria to 10^9 cells/ml over 60 days of the experiment was observed. In the experimental variants in the presence of the samples of studied hydrogels it was observed

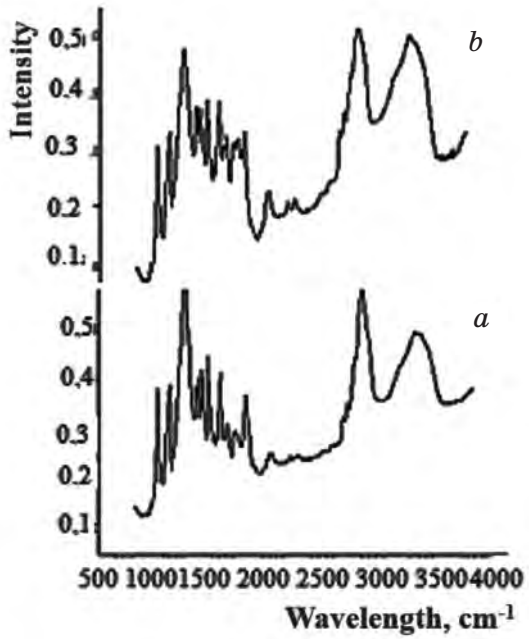

Fig. 4. **IR spectra of polyethylene oxide hydrogels : hydrogel 1 (*a*) and hydrogel 2 (*b*)**

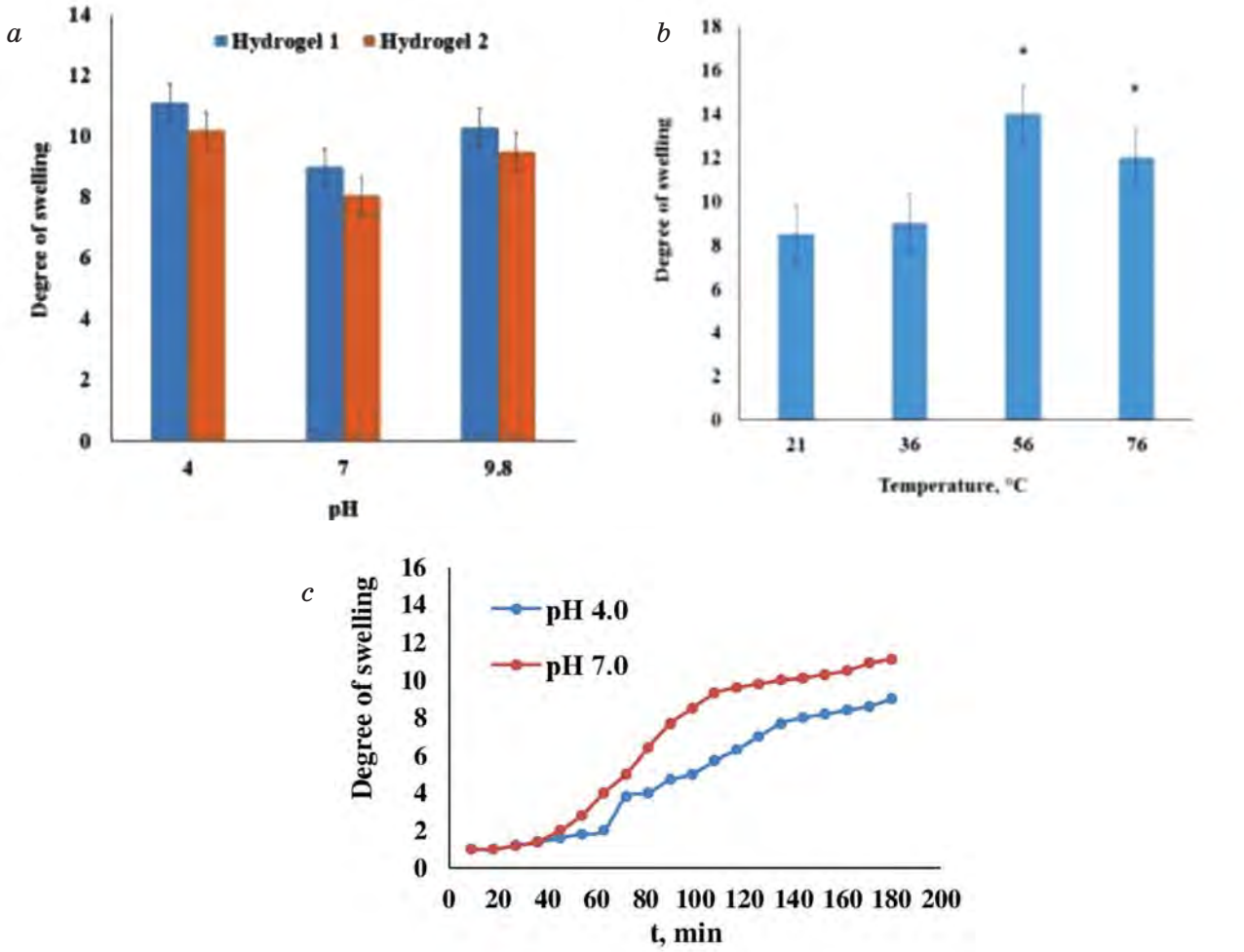

Fig. 5. **The degree of swelling of polyethylene oxide hydrogels in water depends on pH (*a*), temperature (*b*) and duration of swelling under different pH (*c*):**
$n = 3$, data marked with * are statistically significant ($P \leq 0.05$)

the reduced number of bacteria in 2–3 times compared to the initial titer.

Thus, we studied the influence of obtained hydrogels on the enzymatic activity of bacteria. Bacterial cultures in the presence of the studied hydrogels had different enzymatic activity depending on the hydrogels (Fig. 6).

The specific catalase activity in the control (nutrient media with bacteria and without the hydrogels) was higher than in presence of bacteria and materials. The highest specific catalase activity was in *B. subtilis* 138 and *R. erythropolis* 102 (5.3 ± 0.5 and 5.2 ± 0.4 u·mg^{-1} protein, respectively), but in the presence of the studied hydrogels enzymatic activity was decreased in 1.1–1.9 times for different bacterial cultures. Catalase activity of *P. pseudoalcaligenes* 109 in the presence of hydrogels 1 and 2 was significantly lower from 2.4 to 5.0 times, respectively (Fig. 6, *a*). In Tauson's medium without the introduction of materials, higher lipase as well as catalase activities were found in *B. subtilis*138 (19.25 ± 2.1 u·mg^{-1} protein) and *R. erythropolis* 102 (18.6 ± 1.9 u·mg^{-1} protein), compared with other bacterial cultures. In the presence of the studied hydrogels 1 and 2 lipase activities decreased in 1.4–1.8 times, respectively. The specific lipase activity of *P. pseudoalcaligenes* 109 with guanidine-containing hydrogels in cultural liquid was decreased in 1.3–1.9 times compared to control. In the presence of hydrogel 1, compared to hydrogel 2 the lipase activity of bacterial test-cultures was lower (7.8 ± 1.4–12.7 ± 2.3 u·mg^{-1} protein).

It is known from literature data that changes in catalase activity are one of the indicators of processes intensity of organic matter oxidation [23, 24]. There is a close correlation between the reduction degree of catalase activity of destructive strains and the efficiency of oil consumption. Active destructive bacteria have characterized in increasing their amount and decreasing catalase activity in contact with the petroleum products [25]. According to several reports, hydrocarbon-oxidizing bacteria activate lipase activity of soils, while in parallel with the activation of lipolysis, there is an increase in the number of hydrocarbon-oxidizing bacteria and a decrease of petroleum products [25].

The utilization of different materials after their use is an important aspect of the problem of materials biodamages. Significant indicator of material degradation is loss of the sample mass due to bacterial action (Table 2).

After 60 days of the experiment, the largest weight loss 0.35 ± 0.1 g (85.4% from the initial mass) of hydrogel 1 samples was observed under the influence of *B. subtilis* 138. In the variant of the experiment with hydrogel 2, the weight loss of the samples under the influence of bacteria was from 0.12 ± 0.01 to 0.22 ± 0.05 g (59.5–88.4%). In the control, the weight loss of hydrogel 1 was 0.06 ± 0.01 g (17%).

Degradation of guanidine-containing hydrogel materials under the influence of heterotrophic bacteria was confirmed by IR spectroscopy. As in the control hydrogels, and in the IR spectra of the hydrogels under the influence of bacterial test-cultures there

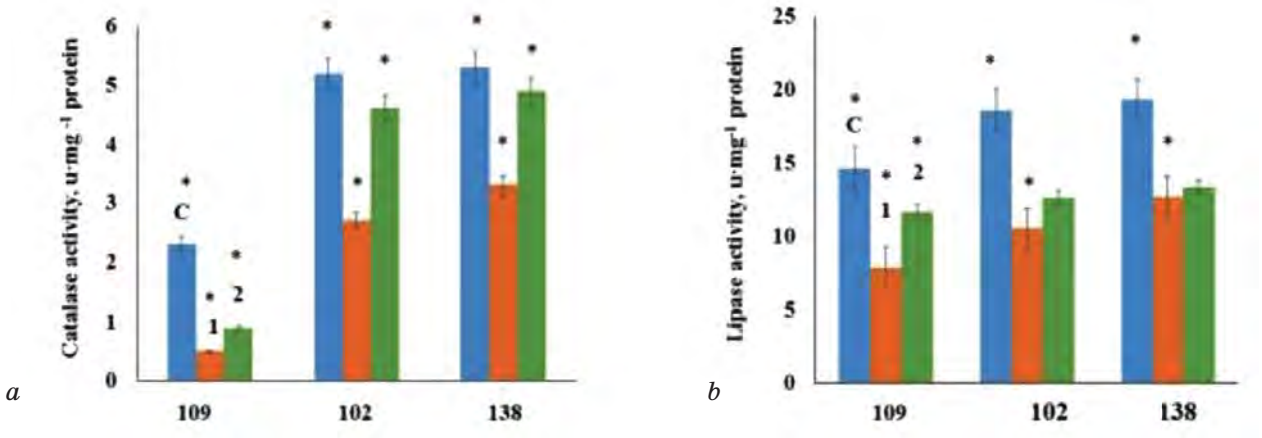

Fig. 6. **Specific catalase (*a*) and lipase (*b*) activity of hydrocarbon-oxidizing bacteria in the presence of guanidine-containing hydrogels**

C — control (without the introduction of materials), *1* — hydrogel 1; *2* — hydrogel 2; 109 — *P. pseudoalcaligenes* 109; 102 — *R. erythropolis* 102; 138 — *B. subtilis* 138; $n = 3$; data marked with * are statistically significant ($P \leq 0.05$).

Table 2. Changes **in the mass of samples of guanidine-containing polyethylene oxide hydrogels in the presence of hydrocarbon-oxidizing bacteria**

Experiment variant, g		Loss of sample mass	
		% from the initial mass	
Hydrogel 1	*P. pseudoalcaligenes* 109	0.26 ± 0.09	75.0
	R. erythropolis 102	0.19 ± 0.04	63.1
	B. subtilis 138	0.35 ± 0.10	85.4
	Control without bacteria and with material	0.06 ± 0.01	17.0
Hydrogel 2	*P. pseudoalcaligenes* 109	0.12 ± 0.01	59.5
	R. erythropolis 102	0.22 ± 0.05	88.4
	B. subtilis 138	0.24 ± 0.06	80.0
	Control without bacteria and with material	0.03 ± 0.01	38.5

Notes: $n = 3$, $P \leq 0.05$.

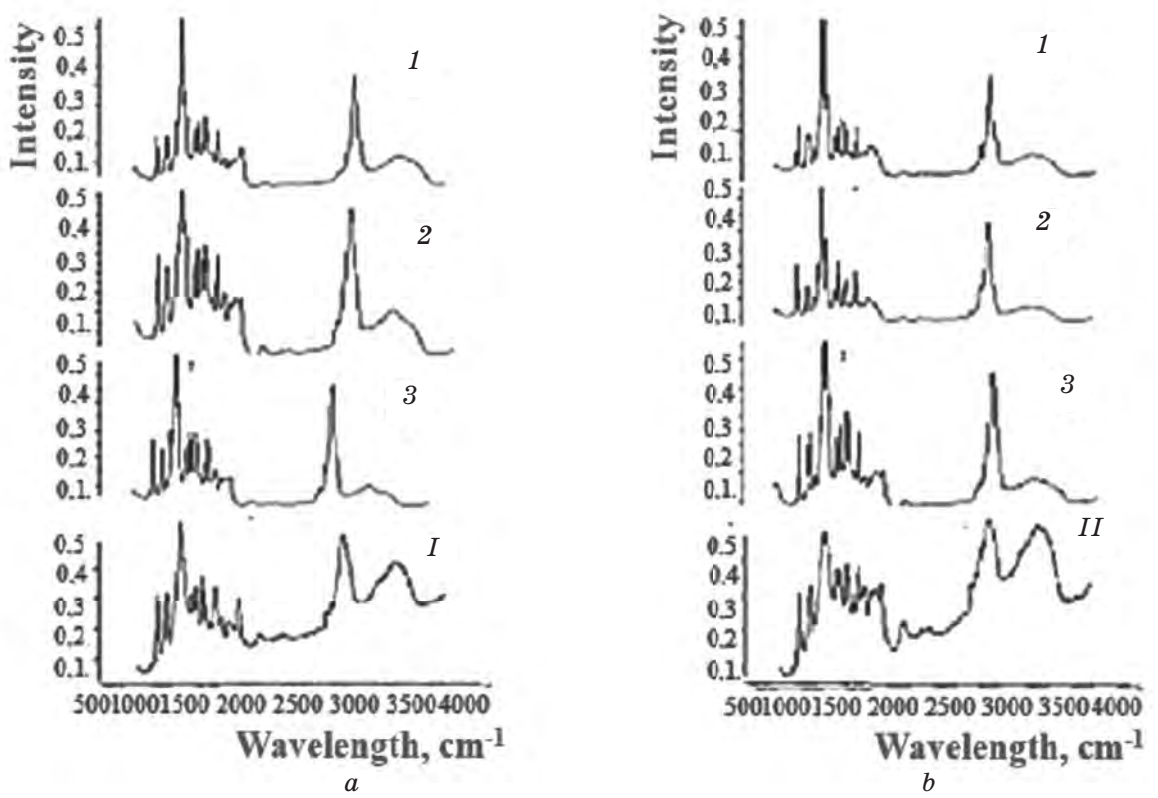

Fig. 7. **IR spectra of polyethylene oxide hydrogels samples under the influence of hydrocarbon-oxidizing bacteria:**
a — hydrogel 1; *b* — hydrogel 2; *I* — initial hydrogel 1; *II* — initial hydrogel 2; *1* — control; *2* — *B. subtilis* 138; *3* — *R. erythropolis* 102

appeared next absorption bands (νNH, OH), 3 156 cm^{-1} (δNH), 2 949 cm^{-1}(νCH), 2 896 cm^{-1} (νCH_2), 2 868 cm^{-1} (νCH_2), 1 648 cm^{-1} (νC = N), (1 450–1 650) cm^{-1} (νC_6H_5), 1 100–1 300 cm^{-1} (νCOC) (Fig. 7).

The difference was that for hydrogels 1 and 2 under the influence of *R. erythropolis* 102 and *B. subtilis* 138 after 60 days, decreased the absorption band of 3500 cm^{-1} decreased, which was responsible for the valence fluctuations of hydroxyl (–OH) and amino (–NH) groups (Fig. 5). Because crosslinking of polyethylene oxide hydrogels occurs through the formation of amide bonds that are resistant to hydrolysis and biodegradation, it can be assumed that the presence of a significant number of secondary amino groups in the crosslinking agent contributes as a catalyst to biodegradation of hydrogels, especially under influence of the *R. erythropolis* 102.

The mechanism of biocidal action of polyguanidines is similar to quaternary ammonium compounds and is of membrane-toxic nature: guanidine polycations are adsorbed on the negatively charged surface of bacterial cells; diffuse through the cell wall; bind to acidic phospholipids, proteins of the cytoplasm membrane, which causes its breakage. As a result, the microbial cell dies. The increase in the biocidal activity of PHMGC as compared to the low-molecular biocides is due to the cooperative interaction of the adjacent guanidine polycation group links with the microbial cell. The increase in the activity of polyguanidines as compared to quaternary ammonium salts (QAS) is also due to the peculiarities of the guanidine group structure: unlike QAS cation, in which a large positive charge is localized on a single nitrogen atom, guanidine cation has a positive charge distributed between three nitrogen atoms, and additionally delocalized according to the σ-links system. It is well known that guanidinium polymers are less toxic than guanidine and belong to the third class of hazard [17, 18].

The antimicrobial properties and biodegradation of newly synthesized guanidine-containing polyethylene oxide hydrogels, in which the linear guanidine-containing oligomer simultaneously acts as a crosslinking, ion-containing and antimicrobial agent, were studied. The synthesized hydrogel showed antimicrobial activity against the studied test cultures of bacteria. The largest zones of growth inhibition were observed for *S. aureus* 451, *R. erythropolis* 102, *B. subtilis* 138. The introduction of the studied materials into the Tauson's medium as additional sources of Carbon and energy, contributed to the reduction of catalase and lipase activities of hydrocarbon-oxidizing bacteria. Under the influence of the studied bacteria, destruction of hydrogels, which is appeared in a decreasing of the studied samples mass, was observed. Infrared spectroscopy showed that the degradation of guanidine-containing hydrogels is affected by the presence of a significant number of secondary amino groups in the crosslinking agent, which can serve as catalysts for the biodegradation of hydrogels.

The research was carried out within the framework of the projects of the National Academy of Sciences of Ukraine "Functional activity and prospects of use in biotechnology soil microorganisms from natural, man-caused and agro systems" (2016–2019, State registration number GDR 0116U006319) and "Development of methods of synthesis of monomers containing perfluorated and highly basic nitrogenic and other functional groups and fragments" (2018–2022, GDR0118U002055).

REFERENCES

1. *Shtilman M. I.* Biodegradation of Polymers. *J. Sib. Federal Un-ty. Biology 2.* 2015, N 8, P. 113–130. (In Russian). https://doi.org/10.17516/1997-1389-2015-8-2-113-130/
2. *Kuznetsov A. E., Gradova N. B., Lushnikov S. V.* Applied ecobiotechnology: textbook: in 2 vols. V.1. *Moskva: Binom.* 2012, 629 p. (In Russian). ISBN 978-5-9963-2631-0
3. *Vinogradova O. N., Prudnikova S. V., Zobova N. V., Kolesnikova V. L.* Microbial Degradation of Poly-3-Hydroxybutyrate in Samples of Agrogenically Changed Soils. *J. Sib. Federal Un-ty. Biology 2.* 2015, N 8, P. 199–209. (In Russian). https://doi.org/10.17516/1997-1389-2015-8-2-199-209

4. *Andreyuk K. I., Kozlova I. P., Kopteva Zh. P., Pilyashenko-Novokhatny A. I., Zanina V. V., Purish L. M.* Microbial corrosion of underground structures. *Kyiv: Nauk. dumka*. 2005, 258 p. (In Ukrainian). ISSN 0201-8462
5. *Kopteva Zh. P., Zanina V. V., Boretskaya M. A., Kopteva A. E., Kozlova I. A.* Influence of lipolytic and catalase activity of heterotrophic bacteria on physical and mechanical properties of Poliken 980-25 coating. *Mikrobiol. Zh*. 2013, 75 (1), 41–47. (In Russian).
6. *Teeraphatpormchai T., Nakajima-Kambe T., Shigeno-Akutsu Y., Nakayama M. L.* Isolation and characterization of a bacterium that degrades various polyester–based biodegradable plastics. *Biotechnol. Lett*. 2003, 25 (1), 283–284. https://doi.org/10.1023/a:1021713711160
7. *Kireeva N. A., Tarasenko E. M., Shamaeva A. A., Novoselova E. N.* The oil products on the activity of lipase of the gray forest soil. *Soil Sci.* 2006, N 8, P. 1005–1011. (In Russian).
8. *Tugay T. I., Zhdanova N. M., Buzarova O. I.* Influence of ionizing radiationof low intensity on activity of catalase and superoxide dismutase of *Hormoconis resine*. *Mikrobiol. Zh*. 2009, 71 (1), 16–21 (In Ukrainian).
9. *Graham N., Zultigar M.* Interaction of Polyethylene oxide with solvents. Synthesis and swelling in water of cross-linked polyethylene glycol urethane networks. *Polymer*. 1989, V. 30, P. 2130–2135. https://doi.org/10.1016/0032-3861(89)90305-4
10. *Gnanou Y., Hild H., Rempp P.* Hydrophylic polyurethane networks based on polyethyleneoxide. Synthesis, characterization and properties. Potential Applications and Biomaterials. *Amer. Chem. Soc*. 1984, V. 17, P. 945–952. https://doi.org/10.1021/ma00134a069
11. *Burke G.,Cao Z., Devine M.*. Preparation of biodegradable polyethylene glycol dimethacrylate hydrogels. *Polymers*. 2019, V. 11, P. 1339–1345. https://doi.org/10.3390/polym11081339
12. *Silviya P., Jennie Z., Leach B.* Hydrolytically degradable poly(ethylene glycol) hydrogel scaffolds with tunable degradation and mechanical properties. *Biomacromolecules*. 2010, V. 11, P. 1348–1357. https://doi.org/10.1021/bm100137q
13. *Saez-Martinez V., Olalde B., Martinez-Redondo D.* Degradable poly(ethylene glycol)-based hydrogels: Synthesis, physico-chemical properties and in vitro characterization. *J. Bioactive Compatible Polymers: Biomed. Appl*. 2014, V. 29, P. 270–276. https://doi.org/10.1177/0883911514528597
14. *Deshmukh M., Singh Y., Gunaseelan S.* Biodegradable poly(ethylene glycol) hydrogels based on a self-elimination degradation mechanism. *Biomaterials*. 2010, 31 (26), 6675–6684. https://doi.org/10.1016/j.biomaterials.2010.05.021
15. *Jain E., Sheth S. Polito K*. Storage stability of biodegradable polyethylene glycol microspheres. *Mater. Res. Express*. 2017, 4 (10), 123–126. https://doi.org/10.1088/2053-1591/aa8e37
16. *Im S., Choi Y., Subramanyam E.* Synthesis and characterization of biodegradable elastic hydrogels based on poly(ethylene glycol) and poly(ε-caprolactone) blocks. *Macrom. Res*. 2007,15 (4), 363–369.
17. *Vointseva I. I., Gembitsky P. A.* Polyguanidine disinfectants and multifunctional additives in composite materials. *Moskva: Lakokr. prom*. 2009, 300 p. (In Russian). https://doi.org/10.15372/KhUR20160610
18. *Zhang C., Yin Z., Luo Q.* Poly(hexamethylene guanidine)-based hydrogels with long lasting antimicrobial activity and low toxicity. *J. Polym. Chem*. 2017, 55 (12), 2027–2035. https://doi.org/10.1002/pola.28581
19. *Ochirov O. S, Mognonov D. M, Stel'makh S. A.* Polymeric hydrogels based on polyhexamethylene guanidine hydrochloride and formaldehyde. *J. Appl. Chem*. 2015, 88 (2), 331–334. (In Russian). https://doi.org/10.1134/S1070427215020238
20. Workshop on microbiology. *Ed. by A. I. Netrusov. Moskva: Publishing Center "Academy"*. 2005, 608 p. (In Russian).
21. *Aisenberg V. L., Karpel V. I., Syrchin S. A.* Approbation of a quantitative method for determining lipolytic activity using a chromogenic substrate. *Mikrobiol. Zh.* 1995, 57 (5), 84–89. (In Russian).
22. *Korolyuk M. A., Ivanova L. I., Mayorov I. G.* Method for determining catalase activity. *Lab. Case*. 1988, N 1, P. 16–18. (In Russian).
23. *Abdulina D. R., Kopteva Zh. P., Kopteva A. E.. Vortman M. Ya.* Influence of polymeric and rubber materials on hydrocarbon-oxidizing bacteria. *Microbiol. Biotechnol.* 2019, 46 (2), 51–64. (In Russian). http://dx.doi.org/10.18524/2307-4663.2019.2(46).169092/
24. *Gogoleva O. A.* Catalase activity of hydrocarbon-oxidizing bacteria. Author's ref. Dissertation of Cand. Biol. Sci. *Orenburg*. 2012, 18 p. (In Russian).
25. *Kireeva N. A., Tarasenko E. M., Shamaeva A. A.* Influence of oil and oil products on lipase activity of gray forest soil. *Soil Sci.* 2006, N 8, P. 1005–1011. (In Russian).

БІОДЕГРАДАЦІЯ ТА АНТИМІКРОБНА АКТИВНІСТЬ ГУАНІДИНВМІСНОГО ПОЛІЕТИЛЕНОКСИДНОГО ГІДРОГЕЛЮ

Г. О. Іутинська[2], М. Я. Вортман[1], Д. Р. Абдуліна[2], Ж. П. Коптєва[2], Г. Є. Коптєва[2], А. В. Руденко[3], В. В. Третяк[3], В. М. Лемешко[1], В. В. Шевченко[1]

[1]Інститут хімії високомолекулярних сполук НАН України
[2]Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, Київ
[3]Інститут урології НАМН України, Київ

E-mail: vmar1962@i.ua

Метою роботи було визначити біорозкладання гуанідинвмісного поліетиленоксидного гідрогелю під дією бактерій та вивчити його антимікробні властивості.

Антимікробну активність новосинтезованих поліетиленоксидних гідрогелів вивчали диско-дифузійним методом. Ензиматичну активність бактерій визначали спектрофотометрично. Стійкість гуанідинвмісного поліетиленоксидного гідрогелю до мікробної деструкції перевіряли в рідкому середовищі Таусона з тест-культурами бактерій з додаванням м'ясо-пептонного бульйону за температури 28±2 °С. Зміни хімічного складу досліджених матеріалів вивчали методом інфрачервоної та ^{1}H ЯМР-спектроскопії.

Гуанідинвмісний поліетиленоксидний гідрогель було отримано шляхом взаємодії олігооксіетиленгліколю ММ 6000 з толуїлендіізоцианатом і гуанідинвмісним олігомером, який відіграє роль зшиваючого, іонвмісного та протимікробного агента. Синтезований гідрогель виявляв антимікробну активність щодо грампозитивних та грамнегативних бактерій.

Внесення в середовище Таусона досліджуваних матеріалів як додаткових джерел вуглецю та енергії призводило до зниження каталазної та ліпазної активності вуглеводньоокиснювальних бактерій. За впливу досліджуваних бактерій відбувалась деструкція гідрогелів до 88,4% від початкового значення.

Ключові слова: гуанідинвмісний поліетиленоксидний гідрогель, антибактеріальні властивості, грампозитивні та грамнегативні бактерії, каталазна та ліпазна активність, біодеградація, ІЧ-спектри.

БИОДЕГРАДАЦИЯ И АНТИМИКРОБНАЯ АКТИВНОСТЬ ГУАНИДИНСОДЕРЖАЩЕГО ПОЛИЭТИЛЕНОКСИДНОГО ГИДРОГЕЛЯ

Г. А. Иутинская[2], М. Я. Вортман[1], Д. Р. Абдулина[2], Ж. П. Коптева[2], Г. Е. Коптева[2], А. В. Руденко[3], В. В. Третяк[3], В. Н. Лемешко[1], В. В. Шевченко[1]

[1]Институт химии высокомолекулярных соединений НАН Украины, Киев, Украина
[2]Институт микробиологии и вирусологииим. Д. К. Заболотного НАН Украины, Киев
[3]Институт урологии НАМН Украины, Киев

E-mail: vmar1962@i.ua

Целью данной работы было определение биоразложения гуанидинсодержащего полиэтиленоксидного гидрогеля под действием бактерий и изучение его антимикробных свойств.

Антимикробную активность новосинтезированного полиэтиленоксидного гидрогеля изучали диско-диффузионным методом. Энзиматическую активность бактерий определяли спектрофотометрически. Устойчивость гуанидинсодержащего полиэтиленоксидного гидрогеля к микробной деструкции проверяли в жидкой среде Таусона с тест-культурами бактерий с добавлением мясопептонного бульона при температуре 28 ± 2 °С. Изменения химического состава исследованных материалов изучали методом инфракрасной Фурье- и ^{1}H ЯМР-спектроскопии.

Гуанидинсодержащий полиэтиленоксидный гидрогель был получен путем взаимодействия олигооксиэтиленгликоля ММ 6000 с толуилендиизоцианатом и гуанидинсодержащим олигомером, который выступает как сшивающий, ионсодержащий и противомикробный агент. Синтезированный гидрогель проявлял антимикробную активность в отношении грамположительных и грамотрицательных бактерий.

Внесение в среду Таусона исследуемых материалов как дополнительных источников углерода и энергии снижало каталазную и липазную активность углеводородоокисляющих бактерий. Под влиянием исследуемых бактерий происходила деструкция гидрогелей до 88,4% от первоначального значения.

Ключевые слова: гуанидинсодержащий полиэтиленоксидный гидрогель, антибактериальные свойства, грамположительные и грамотрицательные бактерии, каталазная и липазные активности, биодеградация, ИК-спектры.