

Polyetherguanidines and their microbial degradation

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Film-forming polyetherguanidines with a three-dimensional structure were obtained. The microbial degradation of polyetherguanidine and its compositions with alkyl substituted guanidinium bromide, as well as chemical and physical-mechanical properties of the synthesized materials under the influence of hydrocarbon-oxidizing bacteria (HOB) were studied. Scanning electron microscopy has been used to demonstrate the formation of a biofilm of HOB on the surface of the studied materials. Guanidinium polymers inhibited catalase and lipolytic activity by 1.3-3.7 times compared to the control. According to the data obtained, the destruction of guanidinium polymers was from 2.2% to 6.0%. The physical and mechanical properties of the materials, such as tensile strength and relative elongation, remained practically unchanged over 90 days of the experiment. These results are consistent with the results of infrared spectroscopy, according to which the composition of the materials under study has not changed chemically.

The thermogravimetric analysis showed that the initial decomposition temperature of the studied materials did not decrease, i.e., the polymeric materials did not lose their properties after exposure to the HOB. Based on the data on the destruction of polymeric materials, it can be assumed that under the influence of bacteria, minor surface biodegradation could occur on the surface of these polymers. Thus, the tested polyetherguanidine-based material is promising for protecting various structures from biological damage.

Keywords: polyetherguanidines, microbial degradation, hydrocarbon-oxidizing bacteria, enzymes, IR spectroscopy, thermogravimetry, tensile strength

Поліетергуанідини та їх мікробна деструкція. *М.Я.Вортман, Ж.П.Коптева, Л.О.Білявська, Г.С.Коптева, А.М.Пилипенко, В.М.Лемешко, В.В.Шевченко*

Отримані плівкотвірні поліетергуанідини трьохмірної будови. Вивчено мікробну деструкцію поліетергуанідину та його композиції з алкілзамісним гуанідинійбромідом, а також хімічні і фізико-механічні властивості синтезованих матеріалів за впливу вуглеводеньокиснювальних бактерій (ВОБ). За допомогою сканувальної електронної мікроскопії показано формування біоплівки ВОБ на поверхні досліджених матеріалів. Гуанідинієві полімери пригнічували каталазу і ліполітичну активність в 1,3–3,7 рази відносно контролю. За отриманими даними деструкція гуанідинієвих полімерів – була від 2,2% до 6,0%. Фізико-механічні властивості матеріалів – міцність на розрив та відносне подовження практично не змінились за 90 днів експерименту, склад досліджуваних матеріалів хімічно також не змінився.

Методом термогравіметричного аналізу показано, що початкова температура розкладу досліджених матеріалів не знижувалась. Ґрунтуючись на даних з деструкції полімерних матеріалів, можна припустити, що під впливом бактерій на поверхні цих полімерів, можливо, відбувалась незначна поверхнева біодеструкція. Отже, випробуваний матеріал на основі поліетергуанідину є перспективним для захисту різних конструкцій від біопшкоджень.

1. Introduction

Guanidine derivatives are widely used as antiseptics, insecticides, pharmaceuticals, and preservatives. Today, a number of medicines have been developed on the basis of guanidine, such as biguanidines, sulfaguanidine, and chlorhexidine. The mechanism of biocidal action of polyguanidines is similar to quaternary ammonium compounds and is membrane-toxic in nature [1, 2]. It is known that guanidinium polymers are less toxic than guanidine and belong to the third hazard class. Polyguanidines are characterized by antimicrobial, antiviral, sporicidal, fungicidal, insecticidal, pesticide, algicidal activity, simultaneously affect aerobic and anaerobic microflora, have a prolonged biocidal effect, and are not dangerous for the environment. Polyguanidines are widely used as an active ingredient in many disinfectants used in agricultural production and medicine. The guanidine group in the links of macromolecular chains carries a positive charge; therefore, all these polymers are polycations. The increased reactivity of the guanidine group ensures the ability of polyguanidines to enter into various chemical reactions, which significantly expands the range of polyguanidine compounds and allows for wide variations in their solubility, biocidal, toxic and physicochemical properties. Polyguanidines are readily available, highly effective (more effective than quaternary ammonium compounds and chloractive drugs), do not form toxic products in water, are not inactivated by proteins, and are easily decomposed by the enzyme systems of the human body. The main representatives of polyguanidines are high-molecular salts of polyhexamethyleneguanidine, namely polyhexamethyleneguanidinium chloride (PGMH). As for obtaining net polymers, it is known to obtain acrylate derivatives of PGMHC with subsequent curing by UV-initiated polymerization.

We have studied linear guanidinium oligomers and polymers that are soluble in water and exhibit biocidal properties against bacteria and microscopic fungi. We have previously shown that the introduction of alkyl radicals of different lengths into the chain leads to an increase in the bactericidal and fungicidal effect of the resulting compounds [3]. Alkyl substituted oligomers based on guanidine can be used as disinfectants for indoor disinfection and as additives in polymer compositions to protect them from biological damage. The search for new promising film-forming materials that are

resistant to microorganisms remains timely and relevant. It was considered expedient to obtain film-forming polymer materials that are convenient to use and perform the function of protection against biodamage.

The aim of the work is to obtain net film-forming polyetherguanidines and to investigate the effect of hydrocarbon-oxidizing bacteria (HOB) on the destruction of polyetherguanidines, as well as on the chemical, physical and mechanical properties of these materials.

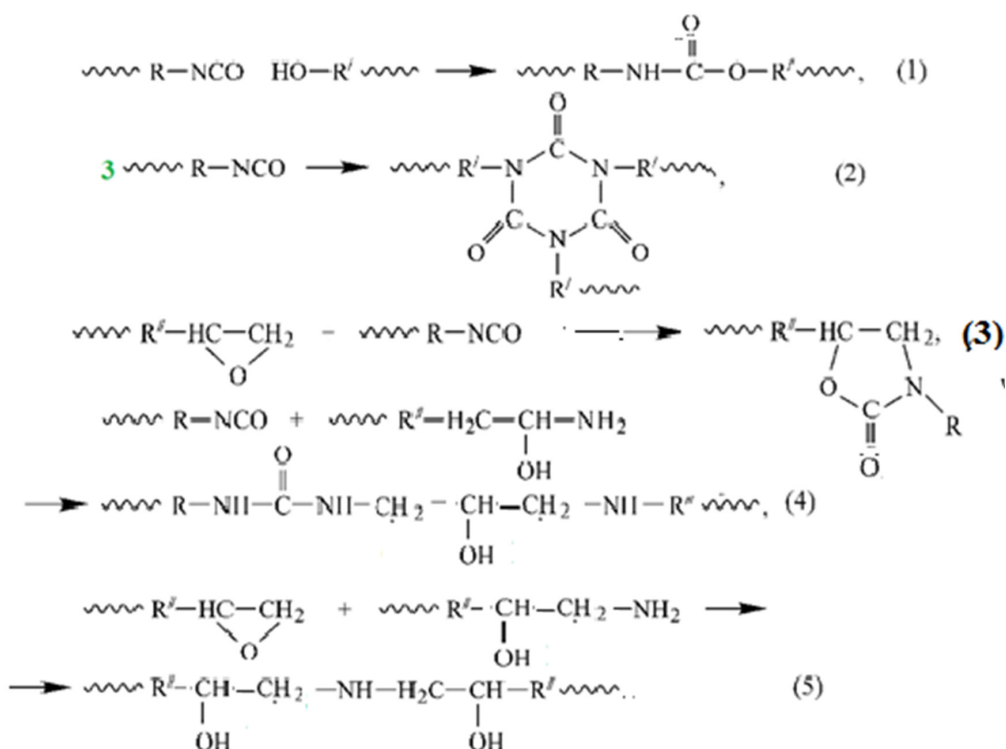
2. Experimental

As starting compounds we used Epycote 828 oligoepoxy (Germany) MW 365 with 25% epoxy groups and epoxidized oligoether Laproxide-703 MW 820 (15% epoxy groups), polyoxypropylene diol (MW 1002), polyoxypropylentriol (MW 503), trimethylolpropane (Aldrich company), toluylenediisocyanate – a mixture of isomers 2,4 and 2,6 (Aldrich company, purity 99.9%) and cyanoguanidine (Aldrich company), which were used without additional purification.

The object of the study was the process of microbial degradation of polymeric materials based on polyetherguanidines. The test cultures used were strains of *Pseudomonas pseudoalcaligenes* 109, *Rhodococcus erythropolis* 102, *Bacillus subtilis* 138, which were isolated from damaged gas pipeline coatings and were stored in the collection of the Department of General and Soil Microbiology of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The materials used in the study were polyetherguanidine and its composition with alkyl substituted guanidinium bromide.

The branched guanidine-containing oligoether was synthesized as follows: 62.5 g (0.1 mol) of epoxidized oligoether Laproxide-703 was placed into a two-necked reactor with a stirrer and ethanol was added to form a 70% solution. 17.9 g (0.3 mol) of cyanoguanidine solution was added to the alcohol solution of the epoxidized oligoether with stirring. The reaction took place at 50-60°C for 2-3 hours. The completeness of the reaction was monitored by IR spectroscopy: the disappearance of epoxy groups in the final product was determined by the disappearance of the absorption band of epoxy groups at 920 cm⁻¹.

The effect of HOB on the tested materials was studied at a temperature of 28 ± 2°C in a Tauson liquid medium which includes the following reagents: [Ca(NO₃)₂ · 4 H₂O] – 1.0 g;



Scheme 1.

KNO₃ – 0.25 g; K₂HPO₄ – 0.25 g; MgSO₄·7H₂O – 0.25 g; FeSO₄·7H₂O; distilled water – 1000 cm³. The pH of the environment is 6.6-6.8. The only source of carbon was the tested materials [4]. An indicator of material degradation was the loss of mass of polyetherguanidine samples under the influence of bacteria, from which the percentage of degradation was calculated [5]. The lipolytic activity was determined spectrophotometrically using a KFC-3-01 device in reaction with *p*-nitrophenyl palmitate; catalase was determined using 0.03% hydrogen peroxide, which formed a stable colored complex with molybdenum salts [6, 7]. Protein was determined in the supernatant using the generally accepted Lowry method. The formation of biofilms on the surface of the studied materials was studied by scanning electron microscopy using a SEM JSM 6060LA microscope at the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine.

Changes in the chemical composition of the investigated materials were studied by FTIR spectroscopy. The spectra were recorded by the method of disturbed total internal reflection on an ATR attachment in the spectral region of 400-4500 cm⁻¹ using a TENSOR-37 spectrophotometer (Bruker Optik, Germany). Tensile strength and relative elongation of materials

were determined by conventional methods: blades 100 mm long with a working part measuring 50 mm were used [4]. Changes in the mass of the material samples as a function of temperature were determined by the thermogravimetric method in air atmosphere; a Q50 thermogravimetric analyzer (TA Instruments, USA) was used in the range from room temperature to 700°C at a heating rate of 20 deg/min. The method is based on a combination of differential thermal analysis and thermogravimetry and studies the chemical and physicochemical processes occurring in a substance under conditions of temperature change [8]. The experiments were repeated three times. Statistical processing was performed using Origin Pro 2016 (ver. b 9.3.226. www.originlab.com).

3. Results and discussion

Synthesis of guanidine-containing polyetherguanidines networks. The composite was obtained by solidifying a composition containing bi- and trifunctional polyether, oligoepoxide, isocyanate adduct, and solvent (material 1). Solidification of the composition was carried out at an elevated temperature by applying the solution to a Teflon substrate until a film of constant mass was formed and the solvent was completely removed.

Table 1 - The amount of HOB in Tauson's medium in the presence of guanidine-containing polymers

The variant of the experiment		Bacterial count
Control (on Tauson's medium)	<i>Pseudomonas pseudoalcaligenes</i> 109	$5.0 \cdot 10^8$
	<i>Rhodococcus erythropolis</i> 102	$1.5 \cdot 10^9$
	<i>Bacillus subtilis</i> 138	$3.6 \cdot 10^8$
Tauson's medium + polyetherguanidine	<i>Pseudomonas pseudoalcaligenes</i> 109	$1.0 \cdot 10^5$
	<i>Rhodococcus erythropolis</i> 102	$1.0 \cdot 10^6$
	<i>Bacillus subtilis</i> 138	$2.0 \cdot 10^5$
Tauson's medium + polyetherguanidine + corrosion inhibitor (alkyl substituted oligomer)	<i>Pseudomonas pseudoalcaligenes</i> 109	$1.0 \cdot 10^2$
	<i>Rhodococcus erythropolis</i> 102	$1.0 \cdot 10^4$
	<i>Bacillus subtilis</i> 138	$2.0 \cdot 10^4$

During the preparation of material 1, the following reactions are possible: formation of urethane, trimerization, formation of oxazolidone, urethane urea, and oligoether.

The composition contains polyethers polyoxypropylene triol (MM 500) and polyoxypropylene diol (MM 1000), the oligoepoxide Epycote 828; as an isocyanate component – an adduct of toluylene diisocyanate and trimethylolpropane with 29- 30% of isocyanate groups; as a guanidine component – a branched oligomer with a mass ratio of components of 1:3:1.8:6:2; as a solvent – ethyl acetate, butyl acetate, cyclohexanone, xylene, and methyl ethyl ketone with a mass ratio of 1:1:2:0.5, respectively (material 1). The gel fraction determined for material 1 in acetone at 60 °C was 92-95 %. Elastic transparent films were obtained.

The structure of the oligomer was studied by IR spectroscopy. There are no absorption bands of isocyanate groups at 2270 cm^{-1} and there is no absorption band of the urea group at 1680 cm^{-1} .

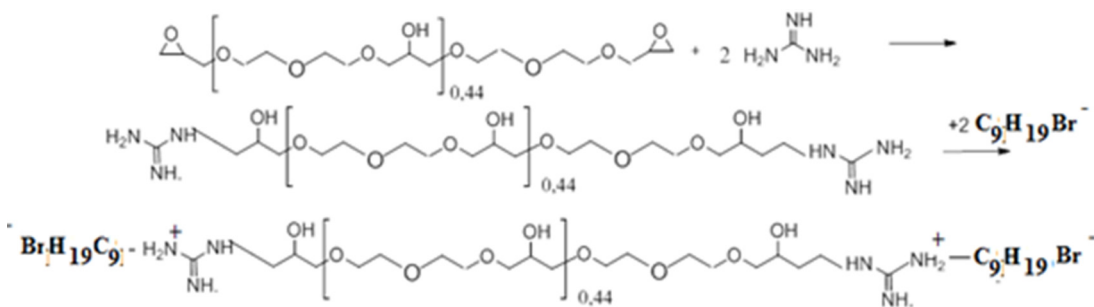
Material 2 was obtained in the same way as material 1; the difference is the addition of a guanidine-containing alkyl substituted oli-

goether that acts as a microbial corrosion inhibitor, it was added in an amount of 6% by weight of the starting components.

The method of obtaining the corrosion inhibitor is described in [3]. The scheme for the preparation of an alkyl-substituted guanidinium-containing oligoether can be presented as follows on Scheme 2:

As a result of the studies, it was noted that over 90 days of the experiment, the amount of HOB decreased by 3-5 orders of magnitude, depending on the strains and materials (Table 1).

In the presence of polyetherguanidine, the number of bacteria was 10^5 to 10^6 cells/ml and in the medium with a corrosion inhibitor – from 10^2 to 10^4 cells/ml. In the control medium (without materials), the bacterial titer ranged from 10^8 to 10^9 cells/ml. Under the influence of an alkyl substituent oligomer in the composition of polyetherguanidine, the bacterial titer decreased by 2-3 orders of magnitude compared to the titer with polyetherguanidine and by 5-6 orders of magnitude compared to the control. Thus, the studied materials inhibited bacterial growth showing antibacterial activity.



Scheme 2.

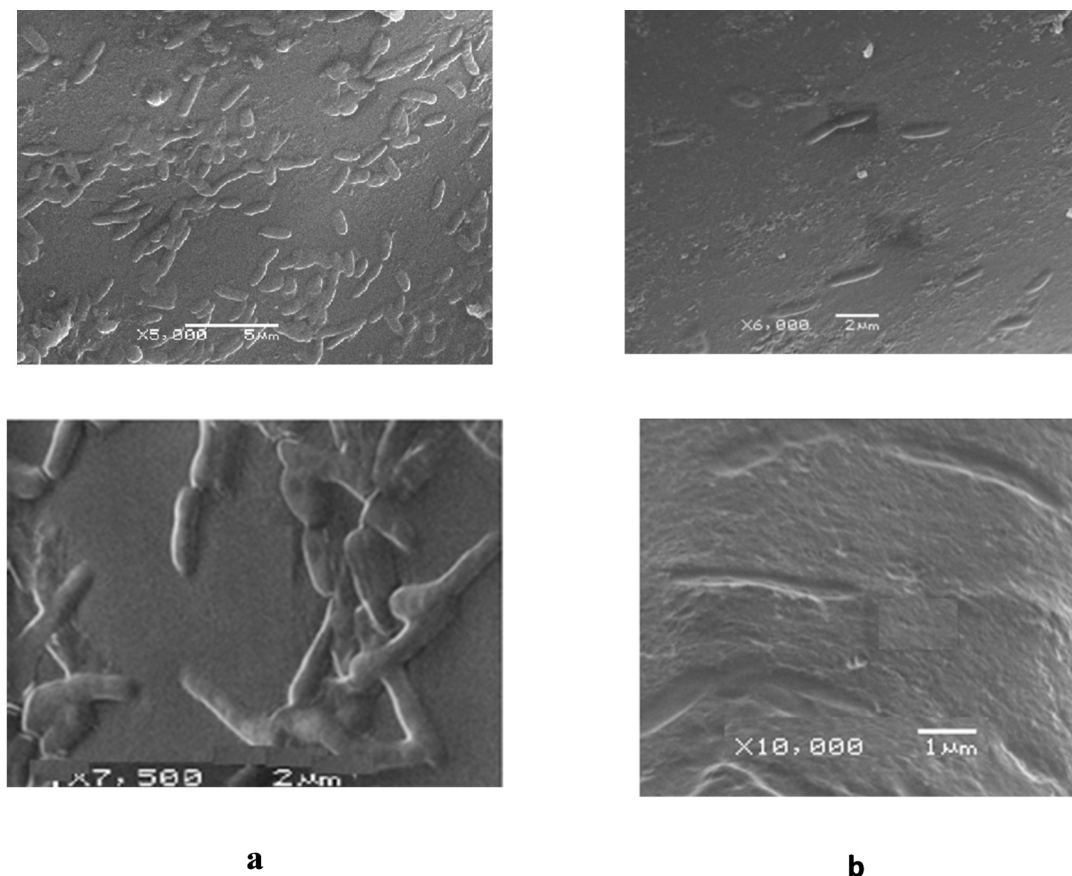


Fig. 1. Scanning electron micrographs of the biofilm: a- *Pseudomonas pseudoalcaligenes* 109 (x 5,000, x 10,000) on the surface of polyetherguanidine; b- *Pseudomonas pseudoalcaligenes* 109 (x6,000, x 10,000) on the surface of polyetherguanidine modified with an alkyl substituted oligomer (corrosion inhibitor).

Earlier we showed that the synthesized oligomer (alkyl substituted guanidinium bromide) combines both biocidal properties and is able to inhibit corrosion on steel caused by sulfate-reducing bacteria. A comparative study of the antimicrobial and anticorrosive properties of the oligomer and the industrial corrosion inhibitors DPX – N-decylpyridinium chloride and Armohib CI-28 showed that the synthesized oligomer is at a high level in terms of its performance [9]. We have tested the bactericidal activity of the newly synthesized alkyl substituted guanidinium-containing oligomers. According to the results of our studies, the obtained compounds exhibit antimicrobial activity against the studied test bacterial cultures at concentrations of 1-3%.

The antimicrobial properties of the synthesized compounds significantly depended on the length of the alkyl radical: as its length increases, the diameter of the bacterial growth inhibition zone increases. A very significant inhibition of the growth of the soil bacteria *Rhodococcus erythropolis* 102 and *Bacillus subtilis* 138 was observed at concentrations of 1-3% oli-

goetherguanidinium bromide (Alk= C₁₀H₂₁Br). The diameter of the zones of bacterial growth inhibition ranged from 20 to 40 mm [3].

The use of electron scanning microscopy made it possible to show that the surface of guanidinium polymers is attached to the HOB (Fig. 1).

In addition, the biofilm formed on the surface of the studied guanidinium polymers also indicates that under extreme conditions (in an environment without organic matter), bacteria attach to the surface of guanidinium polymers as the only sources of carbon, which may be a factor in their bio-damage. Microorganisms forming biofilms have high metabolic activity and are able to damage protective materials as a potential source of energy and nutrition [9, 10, 11]. Biofilms also protect the microbial community from environmental stresses and are a strategy for their survival [9,11, 12].

It is known that microorganisms synthesize a number of enzymes which regulate chemical reactions occurring in bacterial cells. We measured the catalase and lipase activities of HOB to assess their effect on the synthesized mate-

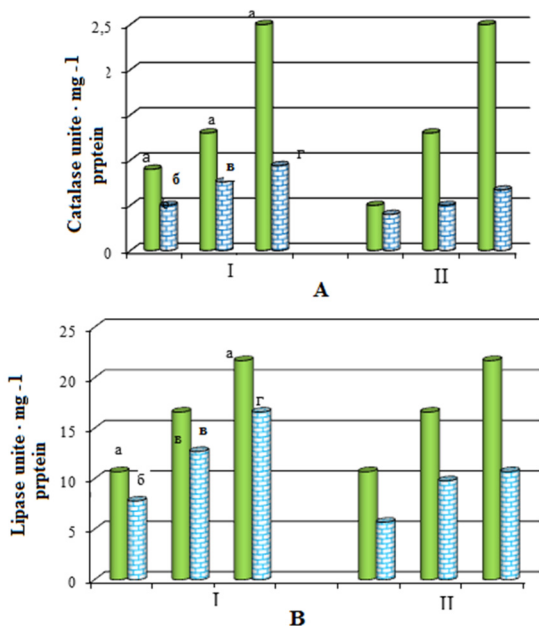


Fig. 2 A - Catalase activity and B - Lipolytic activity of hydrocarbon-oxidizing bacteria in the presence of polymeric materials: I - polyetherguanidine, II - polyetherguanidine+ corrosion inhibitor a - control, b - *Pseudomonas pseudoalcaligenes* 109, c - *Rhodococcus erythropolis* 102, d - *Bacillus subtilis* 138

rials. The catalase activity of bacteria in the control (Tauson’s medium without materials, inoculated with bacteria) was 0.9-2.5 units per mg⁻¹ of protein (Fig. 2 A). In the presence of the studied materials, the activity of bacterial catalase decreased by 1.8-3.7 times. The lipolytic activity of bacteria in the control was 10.7-21.7 units per mg⁻¹ protein (Fig. 2 B). Compared to the control, the addition of polyetherguanidine and corrosion inhibitor to the culture medium inhibited the lipase activity of HOB by 1.3–1.4 and 1.7-2.1 times, respectively. It should be noted that the highest rate of reduction of catalase and lipase activities of bacteria belongs to the corrosion inhibitor (4.9 and 2.1 times, respectively). Among the studied strains, the highest catalase and lipase activity both in the experiment and in the control was observed in

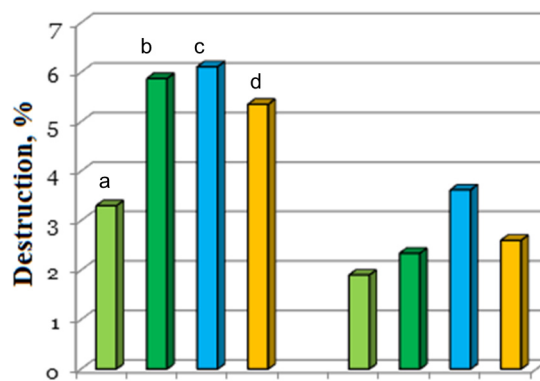


Fig. 3. Destruction of polymeric materials under the influence of hydrocarbon-oxidizing bacteria (%): I - polyetherguanidine, II - corrosion inhibitor, a - control, b - *Pseudomonas pseudoalcaligenes* 109, c - *Rhodococcus erythropolis* 102, d - *Bacillus subtilis* 138

Bacillus subtilis 138. One of the mechanisms of polymer biodegradation is the participation of enzymes in this chemical process. Synthesis of hydrolases by corrosive bacteria, namely lipase, which destroys ether bonds, can lead to the breakage of polymer chains and a decrease in material strength [4, 12, 13].

The study of the physical and mechanical properties of guanidine-containing polymers after exposure to HOB showed that the tensile strength decreased by 15.8-23.4 %; the relative elongation was 1.2–1.4 times higher than that of polyether guanidine (Table 2). In the presence of a corrosion inhibitor, the tensile strength decreased only by 5.0–14.0%, and the relative elongation – by 1.2-2.3 times. The decrease in relative elongation indicates that under the influence of bacteria, this material becomes more rigid and inelastic and can easily lose its strength. In contrast, material 2 is more elastic than material 1. The test cultures of heterotrophic bacteria did not change this quality indicator due to the introduction of an alkyl substituent oligomer that performs a double function as an inhibitor and plasticizer. We can conclude that the synthesized polymeric

Table 2: Physical and mechanical properties of polymeric materials after exposure to HOB

The variant of the experiment	Materials under investigation			
	Polyetherguanidine		Polyetherguanidine + Corrosion inhibitor	
	Tensile strength, MPa	Relative elongation, %	Tensile strength, MPa	Relative elongation, %
Control	47.5±0.7	56,4±0.8	30.35±0.5	107.4±1.7
<i>P. pseudoalcaligenes</i> 109	40.0±0.3	46,4±0.7	27.1±0.3	47.2±1.4
<i>R. erythropolis</i> 102	38.6±0.5	40.6±0.6	28.9±0.3	89.0±1.5
<i>B. subtilis</i> 138	36.4±0.3	44,6±0.7	26.0±0.3	56.0±1.5

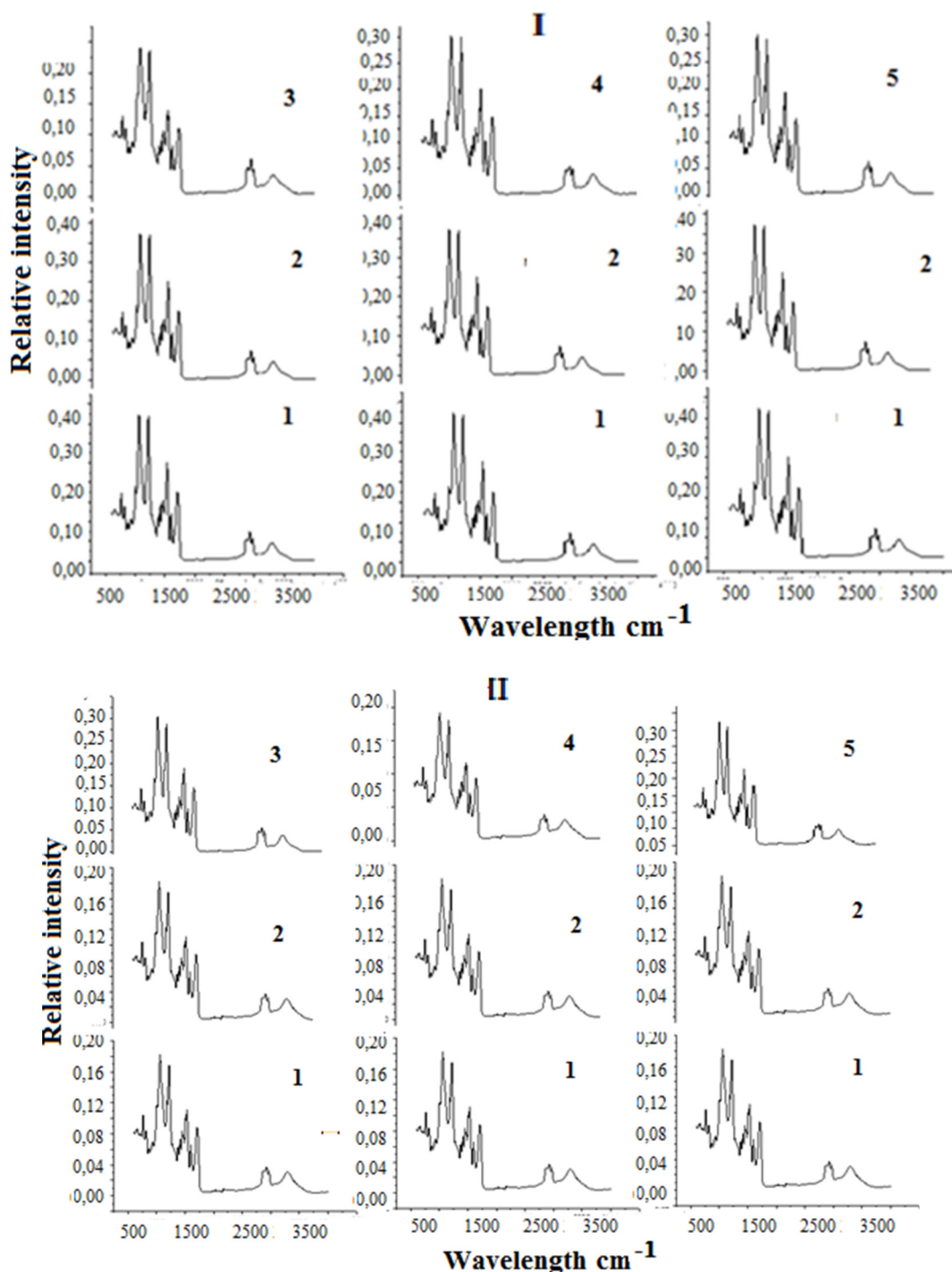


Fig. 4. IR spectra of samples under the influence of HOB: I – polyetherguanidine; II - composition of polyether guanidine + oligomeric alkyl substituted guanidinium bromide; 1 – starting polymer; 2 – control; 3 – under the influence of *Rhodococcus erythropolis* 102; 4 – under the influence of *Pseudomonas pseudoalcaligenes* 109; 5 – under the influence of *Bacillus subtilis* 138

materials did not lose their physical and mechanical properties after exposure to HOB. The data obtained correlate with the IR spectroscopic data and are consistent with the results of the effect of HOB on the strength properties of guanidinium polyurethane [13].

The degree of destruction of the studied materials under the influence of HOB was assessed. According to Fig. 3, polyetherguanidine underwent the greatest destruction (5.35-6.11%). In the control variant (material in Tauson's medium without bacteria), the destruction was 3.3%. In the presence of another

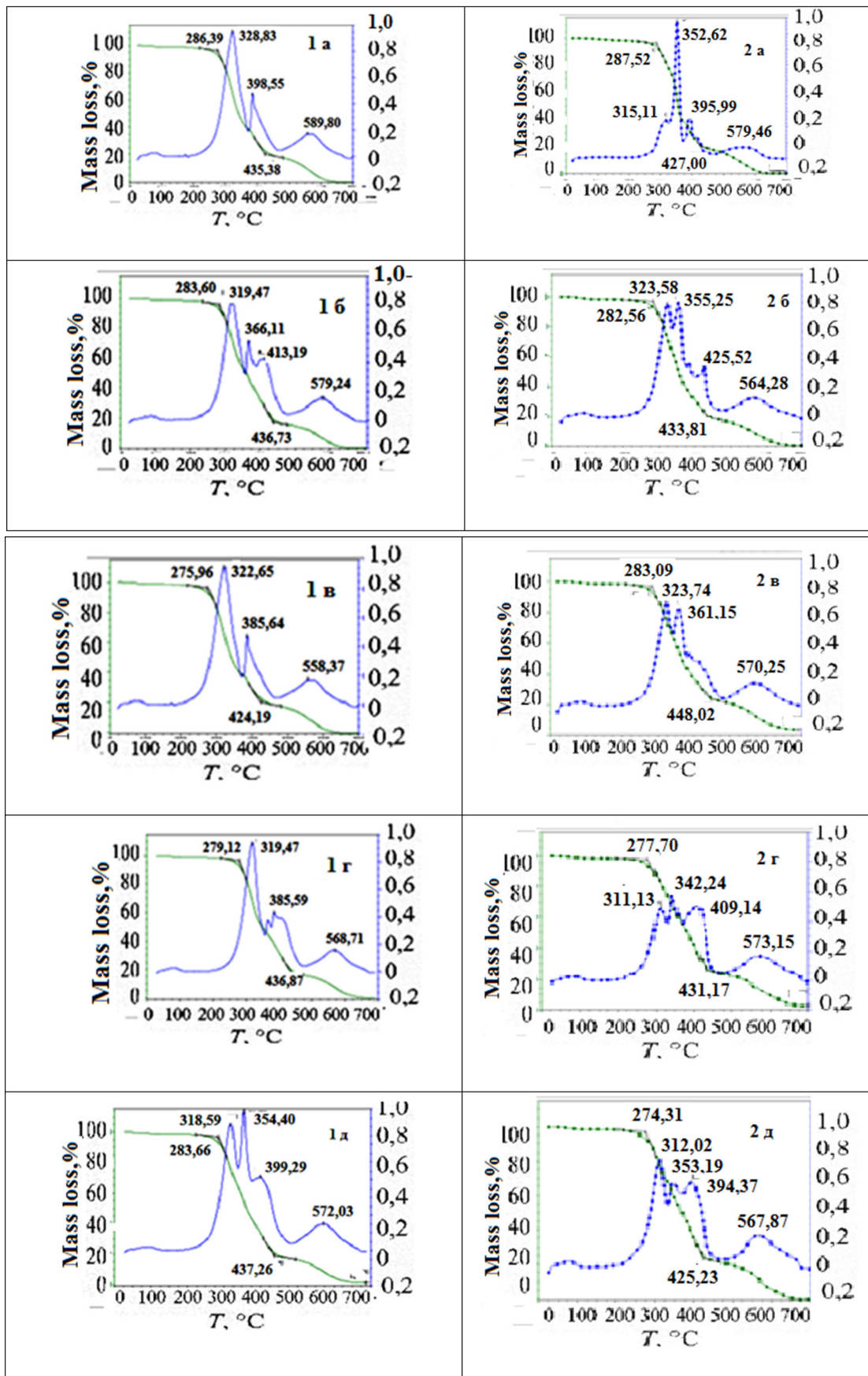


Fig. 5. 1–material 1; 2 – material 2; a – initial materials, b – control, c – action of *R. erythropolis* 102 culture; d – action of *P. pseudoalcaligenes* 109 culture; e – action of *B. subtilis* 138 culture

Table 3 – Degradation temperature of guanidine-containing polyetherguanidines after exposure to HOB for 90 days

The variant of the experiment	Initial and degradation temperature $^{\circ}\text{C}$ at mass loss, %.				
	Material 1				
	Initial	30	70	80	90
Initial	286.39	328.83	398.55	-	589.8
Control	283.60	319.47	366.11	413.19	579.24
<i>R. erythropolis</i> 102	275.96	322.65	385.64	-	558.37
<i>P. pseudoalcaligenes</i> 109	279.12	319.47	385.50	-	568.71
<i>B. subtilis</i> 138	283.66	318.50	399.29	-	572.03
	Material 2				
Initial	287.52	315.11	395.99	-	579.46
Control	282.56	323.58	355.25	425.52	564.28
<i>R. erythropolis</i> 102	283.09	323.74	361.15	-	570.25
<i>P. pseudoalcaligenes</i> 109	277.70	311.13	342.24	409.14	573.15
<i>B. subtilis</i> 138	274.31	312.02	353.19	394.37	567.87

material, alkyl-substituted guanidinium bromide (a corrosion inhibitor), the degradation rate was lower (2.6-3.62%), and in the control -1.9%. The greatest destruction was observed in the tested materials under the influence of *Rhodococcus erythropolis* 102. Thus, the corrosion inhibitor in the composition of polyetherguanidine reduces the percentage of material destruction by 1.7-2.5 times.

Changes in the structure of materials after exposure to HOB were determined by IR spectroscopy. Fig. 4 shows the infrared spectra of polyetherguanidine samples exposed to HOB after 90 days of the experiment in the absence and presence of the inhibitor.

According to the spectral data, the composition of the studied materials exposed to an aggressive environment did not change chemically, i.e., no changes in the absorption bands occurred under the influence of bacteria. Both in the infrared spectra of the control samples of the investigated materials and in the spectra of materials exposed to test cultures of bacteria, the absorption bands of (ν NH, OH) 3156 cm^{-1} , (ν CH₃) 2949 cm^{-1} , (ν CH) 2896 cm^{-1} , (ν CH₂) 2868 cm^{-1} , 1648 cm^{-1} (ν C=N), ($1100\text{--}1300$) cm^{-1} (ν C-O-C) were present (Fig. 4 I, 4 II).

It was also expedient to study changes in the chemical composition of the culture fluids

of HOB in the presence of alkyl-substituted guanidinium bromide. The IR spectra of the culture fluids of HOB in the presence of alkyl-substituted guanidinium bromide were studied. Compared to the control, the absorption band (ν CH) of 2896 cm^{-1} appears in the infrared spectrum of the culture fluids of material 2 with an increase in the band of 1100 cm^{-1} (ν C-O-C) (Fig. 4 III). It can be assumed that under the action of HOB, unreacted initial components are leached into the culture fluid solution. A similar regularity is observed in the IR spectra of the culture liquids of material 1.

The changes in the structure of materials after exposure to HOB were also determined by thermogravimetric analysis (TGA). Fig. 5 and Table 3 show the thermograms of decomposition of polyetherguanidines after exposure to HOB for 90 days of the experiment.

As can be seen from Fig. 5 and Table 3, the initial degradation temperature for materials 1 and 2 slightly decreased or did not change under the influence of HOB. The analysis of the thermogram curves shows that the decomposition process of the studied materials occurred in four stages for material 1 and in five stages for material 2.

Four and five peaks were observed on the decomposition thermograms, respectively. The

initial temperature of decomposition of material 1 after exposure to HOB was more than 275-283°C; at 311-328°C, the decomposition of urethane groups was observed, at 340-398°C – guanidine groups. At 400°C, the deep processes begin, which are associated with the loosening and oxidation of the hydrocarbon skeleton of the polymer, and at 568-589°C, they intensify. The initial decomposition temperature of materials 1 and 2 after exposure to HOB is approximately the same.

Since the initial decomposition temperature for the studied materials decreased slightly and in some cases, increased, it can be assumed that these polymeric materials did not lose their properties after exposure to HOB.

Thus, film-forming polyetherguanidines with a three-dimensional structure were obtained and the influence of hydrocarbon-oxidizing bacteria on their destruction, as well as on the chemical and physical and mechanical properties of these materials, was studied. It is shown that the biodegradation of polymeric materials is a complex heterogeneous process that causes their decomposition. Microorganisms are directly involved in the degradation of synthetic and natural polymers [14–16]. Guanidine-containing polymers introduced into the Tauson medium as a source of carbon reduced the number of hydrocarbon-oxidizing bacteria. Catalase and lipase activities of bacteria in the presence of a corrosion inhibitor in the medium were significantly lower than in the presence of polyetherguanidine. The determination of the physical and mechanical properties of polyetherguanidines showed that the tensile strength and relative elongation change slightly after exposure to HOB. According to the data obtained, polyetherguanidine underwent the greatest destruction. The introduction of a corrosion inhibitor into the composition of the latter material led to a decrease in its destruction. The tested biocidal inhibitors inhibited the vital activity of corrosive bacteria, which may be due to the adsorption of these reagents on the surface of cells and disruption of their metabolism. It is known that a reliable way to protect various coatings from microbial damage is the inclusion of biocides in their composition. It is especially important that they inhibit the processes of denitrification and sulfate reduction; as a result, corrosive metabolites (NO_2 , NH_3 , H_2S) are formed, which reduce the aggressiveness of the environment [15]. The results of IR-spectroscopy on the effect of bacteria on the studied materials under investigation

indicate that oxidation and chain destruction processes do not occur in the chemical composition of polymers.

4. Conclusion

The investigated materials are promising for use as additives in polymer compositions to protect them from biological damage. The obtained microbiological, chemical, physical and mechanical data can also provide fundamental information on the development of promising protective guanidine-containing coatings.

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