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Bactericidal properties of two-dimensional ordered linear-chain carbon

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Laboratory tests of the bactericidal properties of stainless steel samples with a protective nanolayer of linear-chain carbon of various modifications were carried out in a closed volume of air at atmospheric pressure, 100 % humidity and room temperature. After testing, it was shown that with respect to spore-forming gram-positive bacteria of the *Bacillus pumilus* species, the coatings showed bacteriostatic activity, which did not allow these microorganisms to multiply, and with respect to vegetative forms of Gram-negative bacteria *Pseudomonas aeruginosa*, all tested samples showed bactericidal activity, expressed in almost complete suppression of their viability on all types of coatings. For fungi, only one type of coating doped with lithium atoms had antifungal properties when there was a complete absence of fungal growth and the absence of viable micromycete units. For the remaining samples, there was a slight growth of fungi compared to the control samples.

Keywords: stainless steel, two-dimensionally ordered linear-chain carbon, protective nanolayer, micromycetes, bacteria.

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Introduction

Currently, carbon and new carbon-based composite materials are of great practical and scientific interest in all branches of human activity. For a long time, only two allotropic modifications of carbon – graphite and diamond – were actively studied. At present, the polytypes of diamond and graphite have been thoroughly investigated and described [1–3]. If we consider diamond (a three-dimensional carbon polymer) as the limiting case of the paraffin series, and graphite (a planar polymer) as the limiting case of the aromatic series, then it is logical to assume that the next form will be the limiting case of the unsaturated series, which can be represented as a linear polymer whose molecules are built from polyynes or cumulene chains [4]. The possibility

of the existence of linear carbon polymers with *sp*-hybridization was considered as early as 1885 by Baeyer. He attempted to synthesize a chain carbon – polyyne. Attempts to obtain one-dimensional polyyne chains from tetraacetylene were unsuccessful due to the instability of tetraacetylene [5]. As a result of long-term research, Baeyer postulated the impossibility of obtaining linear chain carbon. This work ceased for a long time. However, in 1960, the A. N. Nesmeyanov Institute of Organoelement Compounds of the Russian Academy of Sciences (INEOS), discovered the third crystalline allotropic form of carbon and named it carbyne [6]. Subsequently, various methods for obtaining carbyne were developed [5, 7, 8, 9]. The next step was the modification of surface properties by creating thin carbon films or layered

film structures on them, with thicknesses ranging from microns to fractions of a micron. The orientation of carbon molecular chains can be controlled while the structures are growing [10–12]. Such film structures serve as the basis for building the elemental base of modern micro- and nanoelectronics. Thin films can also perform functional tasks such as surface hardening or changing wettability and the friction coefficient. When deposited onto a solid surface, carbon atoms can form various allotropic modifications differing in hybridization type: sp^3 and sp^2 , which correspond to the stable modifications of diamond and graphite, sp^1 and sp^0 , which are these are metastable modifications of linear chain carbon (LCC) and face-centered (FC) carbon. Upon condensation on the surface, the chains cross-link to form a graphite-like condensate. Under certain condensation conditions of carbon vapor on the surface, films of two-dimensionally ordered linear chain carbon (2DO LCC), which consist of linear carbon chains oriented normal to the surface and forming ordered ensembles can be formed [10–12]. 2DO LCC films are ordered ensembles of quantum wires spaced $0.4\div 0.5$ nm apart, and therefore weakly interacting with each other and possessing unique electrophysical properties. Integration of additional atoms into carbon chains or the cross-linking of polymer chains of linear carbon allows modifying both their physical properties and biological activity.

Biomedical studies of implants coated with linear chain carbon have shown the absence of protein denaturation on their surfaces and an extremely low blood coagulation potential. The application of 2DO LCC coatings on the surface imparts exceptional thromboresistance and improves the biocompatibility of medical implants and devices, reducing the risk of thrombosis, implant rejection, and inflammation [13]. According to prior studies, carbyne in the form of nanopowders exhibits biocidal activity against microorganisms of various species, in particular against representatives of human automicroflora and opportunistic pathogenic microorganisms [14].

The aim of this paper is to obtain experimental data on the effect of coatings made from various modifications of 2DO LCC on the viability of airborne microorganisms at atmospheric pressure, 100% humidity, and room temperature.

Experimental Section

For the purpose of the research, sixteen samples of 12X18H10T stainless steel, each measuring $50\times 25\times 1$ mm, were produced. On the surface of 12 samples, a layer of two-dimensionally ordered linear chain carbon (2DO LCC) approximately 200 nm thick was synthesized by ion-beam deposition [10]. On the surface of 4 samples, the layer was deposited in the presence of hydrogen in the residual atmosphere (2DO LCC + H); on 4 samples, the layer was deposited in the presence of nitrogen (2DO LCC + N); on 4 samples, the layer was doped with lithium atoms in an inert atmosphere (2DO LCC + Li); and 4 samples were used as controls without coating. The biocidal properties of the fabricated samples were tested using representatives of bacterial and fungal flora. The bacterial test cultures used were representatives of Gram-negative flora – bacteria of the species *Pseudomonas aeruginosa* and spore-forming Gram-positive bacilli of the species *Bacillus pumilus*. As micromycete cultures, the following fungal species from the All-Russian Collection of Microorganisms (VKM) were used:

Aspergillus niger van Tieghem – VKM F – 1119;
Aspergillus terreus Thom VKM F-1025;
Aspergillus oryzae (Ahlburg) Cohn VKM F – 55;
Chaetomium globosum Kunze VKM F – 109;
Paecilomyces varioti Bainier VKM F – 378;
Penicillium funiculosum Thom VKM F – 1115;
Penicillium chrysogenum Thom VKM F – 245;
Penicillium cyclopium Westling VKM F – 265;
Trichoderma viride Pers. ex Fr. VKM F – 426.

On two samples from each group, microdroplets of the prepared bacterial suspension with a bacterial count ranging from 1×10^6 to 4×10^6 CFU were applied. On two samples from each group, droplets of a fungal spore suspension were applied. The suspension was prepared by mixing equal parts of the suspensions of each fungal species. After that, all samples with the applied suspension were placed in a closed sealed volume at atmospheric air pressure, room temperature, and 100% humidity. A stainless steel chamber with an inner radius of 60 mm and a height of 140 mm was used to create the sealed volume (Fig. 1). The test samples were placed inside the chamber on an aluminum rack-type holder. To achieve the required humidity of the gas environment, a recess with a volume of 2 cm^3 was made in the upper part of the holder and filled with distilled

water (Fig. 1). The test duration was 14 days for bacterial cultures and 28 days for fungal cultures.

After the bacterial tests were finished, the samples were subjected to microbiological analysis to identify differences in the quantitative level of viable microorganisms on the test and control samples. The analyses were carried out by standard methods [15]. The results of the antimicrobial property tests for samples with different coating types are presented in Table 1.

As shown in Table 1, on unprotected stainless steel samples, approximately 4×10^2 CFU/sample of *Pseudomonas aeruginosa* were found, while on the protected samples, only single cells (0 to 9) were detected, corresponding

to 97.75% to 100% protection efficiency. Comparative tests of protected and unprotected steel samples show that spore-forming microorganisms of the species *Bacillus pumilus* remain viable, but their quantitative level was not found to increase above that on the control samples in any case.

After the mycological tests were finished, the material samples were inspected in diffuse light at $10\times$ magnification using a Stemi 2000 stereomicroscope. Fungal resistance was evaluated by the intensity of fungal growth on the samples using a 6-point scale as per GOST 9.048-75 [16], as shown in Table 2. Table 3 shows the sample surface inspection results in points.

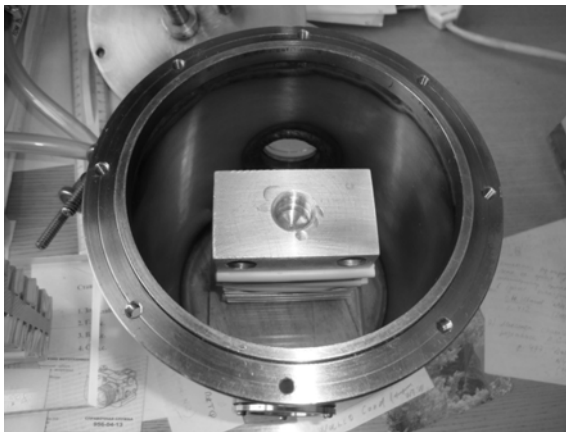


Fig. 1. Chamber with the holder installed Top view

Table 1

**Test results of antimicrobial properties of the coatings against Gram-negative microorganisms
Pseudomonas aeruginosa and *Bacillus pumilus***

Sample and coating type	<i>Pseudomonas aeruginosa</i> , CFU/sample	<i>Bacillus pumilus</i> , CFU/sample
Stainless steel (control)	4.0×10^2	6.0×10^4
Stainless steel + 2DO LCC + H	0–9	3.6×10^4
Stainless steel + 2DO LCC + N	0–8	3.0×10^4
Stainless steel + 2DO LCC + Li	0	4.5×10^4

Table 2

Fungal growth evaluation

Point	Point description
0	No fungal growth is visible under a microscope.
1	Germinated spores and a slightly developed mycelium in the form of unbranched hyphae are visible under a microscope.
2	A mycelium in the form of branched hyphae and sporulation structures are visible under a microscope.
3	Fungal growth is barely visible to the naked eye, but is clearly visible under a microscope.
4	Fungal growth is clearly visible to the naked eye, with the fungi covering less than 25% of the tested surface.
5	Fungal growth is clearly visible to the naked eye, with the fungi covering more than 25% of the tested surface.

Table 3

Evaluation of fungal growth on sample surfaces after exposure

Material	Fungal growth score (points)	Evaluation results
Stainless steel (control)	2	The gas environment contains chemical compounds used by fungi for slight growth.
Stainless steel + 2DO LCC + H	2–3	The gas environment and the coating contain chemical compounds used by fungi for greater growth than in the control.
Stainless steel + 2DO LCC + N	2–3	The gas environment and the coating contain chemical compounds used by fungi for greater growth than in the control.
Stainless steel + 2DO LCC + Li	0	The material surface has a coating that prevents fungal growth.

As can be seen from the presented data, the air environment contains compounds that micromycetes are able to use for their anabolism. This is confirmed by the growth of micromycetes on the control samples. On the test coatings, fungal growth in the form of branched hyphae and weak sporulation was observed (Fig. 2). The exception was the stainless steel samples with the 2DO LCC + Li coating, on which no fungal growth was detected (Fig. 3). After culturing the washings from these samples, no viable micromycete CFU were found. Thus, according to the results of the microbiological tests conducted, only the coating deposited on the stainless steel substrate and consisting of 2DO LCC doped with lithium atoms exhibited antifungal properties.

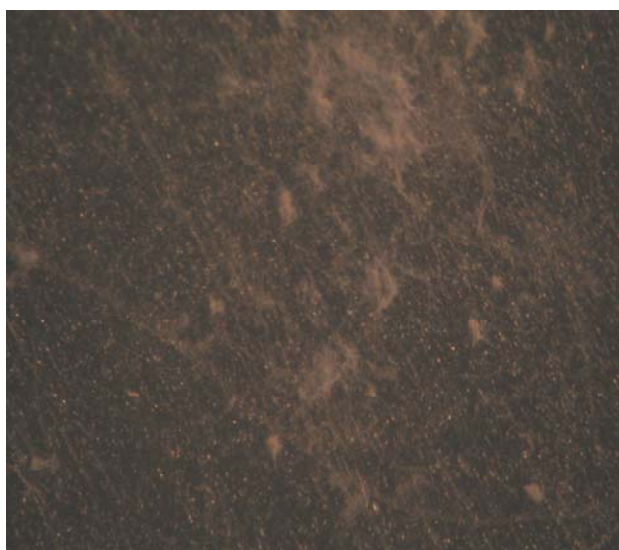


Fig. 2. Surface of the sample with the deposited 2DO LCC + N layer after testing. Fungal growth is observed. Magnification – $\times 10$



Fig. 3. Surface of the sample with the deposited 2DO LCC + Li layer after testing. No fungal growth is observed. Magnification – $\times 10$

Conclusion

All tested 2DO LCC nanocoatings exhibited bactericidal activity against vegetative forms of Gram-negative microorganisms *Pseudomonas aeruginosa*, which was expressed in the almost complete suppression of their viability. Against spore-forming Gram-positive bacteria of the species *Bacillus pumilus*, the coatings exhibited bacteriostatic activity that prevents these microorganisms from reproducing and increasing in number on the protected samples. According to the results of the microbiological tests against fungi, one type of coating, 2DO LCC + Li, exhibited antifungal properties, with a complete absence of fungal growth and no viable micromycete CFU

observed. For the remaining samples, slight fungal growth was observed compared to the control samples.

2DO LCC nanocoatings are promising for preventing fungal growth and suppressing bacterial viability. The differences in the biological properties of the protective nanocoatings of two-dimensionally ordered linear chain carbon require further investigation of their

surface properties and the mechanisms of microbial growth suppression.

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