Increasing the Effectiveness of Selecting Bioprotective Drugs to Protect Materials from Bio-Damage

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Abstract. The article presents the results of research in the field of microbiological corrosion of building materials by Russian and foreign specialists. It is shown that microorganisms cause corrosion of composites on polymer and other binding metal and other materials. Bio-damage of plastic masses, as well as other materials, occurs simultaneously with their aging under the influence of external physical and chemical factors (ultraviolet radiation, water, temperature changes, etc.). the biostability of polymer materials is significantly affected by additives, usually always present in polymer compositions (fillers, plasticizers, stabilizers, antioxidants, modifiers, pigments, etc.)

One of the most effective ways to increase the biostability of composite building materials is the introduction of fungicide additives into their composition. However, unfortunately, the selection of biocidal additives currently does not have a scientific basis and is carried out by trial and error. In our study, we first implemented a new approach to the selection of effective means of protection against biological damage based on the application of ecological, physiological and biochemical criteria: environmental criteria includes the fact that you have to work with microorganisms which are the true destructors of a particular construction material; physiological and biochemical criteria includes biochemical testing of fungi on their specific aggressive metabolites undergoing the process materials material; the results of such testing are shown on the example of fungi-destructors of construction composites based on urea and phenol-formaldehyde resins; during the research, Nobles and Bovendamm biochemical tests were used; a significant variety of mold fungi was identified from construction composites, which are able to use their components as a food source, and others are random phases that grow due to external contamination; using the example of urea and phenol-formaldehyde composites, it was found that the studied fungi-active destructors of these materials have a high activity of oxidoreductases (catalase, peroxidase, phenol oxidase). It is shown that when evaluating a comprehensive analysis of the processes of bio-deterioration is required to account for how species of microscopic fungi are destroyers of building materials and micro-organisms which are pathogenic that can cause mycoses, mycotoxicosis and microallergen.
1. Introduction
Intensive studies on the microbiological resistance of materials and products based on metal and wood, concrete of various types based on inorganic and organic binders have been carried out recently both in Russia and abroad [1–17]. Microbiological corrosion is caused by bacteria, mycelial fungi, actinomycetes. The damage caused by microorganisms is estimated at several million dollars annually [3, 15].

2. Relevance and scientific significance of the problem
Increasing the biostability of composites is carried out in various ways, depending on the type of material [18-22].

The stability of cement concretes under the influence of the biological environment is largely determined by the porosity of the material, which increases its permeability to microorganisms and as a result reduces the strength of composites [3]. A sharp increase in resistance is caused by the introduction of a fine filler – waste from the production of ferrosilicon and a polymer additive – an epoxy resin with an aminosilicate hardener into the composition of cement composites. These additives have a positive effect on the structure formation and porosity of the cement stone. Waste from the production of ferrosilicon mainly consists of amorphous silica, which, interacting with calcium hydroxide, contributes to the formation of an additional amount of low-base calcium hydrosilicates, which causes the compaction of the cement stone. The polymer additive, along with reducing the porosity of the cement stone, improves its structure due to the formation of interpenetrating phases of polymer and cement, which increases its stability in aggressive environments of metabolites [3].

Gypsum composites are susceptible to microbial growth. Comparison of compositions on gypsum and phosphogypsum binders shows that samples on phosphogypsum grow slightly more. It was also found that after holding the composites in the biological environment by method 3 for 14 days, the mass content of samples on construction gypsum decreased by 0.056 %, and phosphogypsum materials-by 1.26 % [15]. It was found that under the influence of microscopic fungi, the degradation of gypsum-cement-pozzolanic composites has a heterogeneous character. The obtained data prove that the degree of damage to composites by fungi depends on the content of components. The type and content of the hydraulic additive significantly affect the biological resistance of these composites.

For the manufacture of chemically resistant coatings in construction, the greatest use is found in epoxy binders modified with rubber (compound K-115), as well as diluted with oxidized turpentine and isomerized resin (compound ECR-22) [3]. Comparison of composites biostability based on these binders with CSM on an unmodified binder indicates that these materials have the same activity scores-0 and 3, respectively, according to methods 1 and 3. Comparison of changes in the mass content and the coefficient of resistance of samples allows us to conclude that the performance of composites on the ed-20 resin is better than on its compounds. Fragments of rubber, as well as isomerized resin and oxidized turpentine in the polymer binder grid increase the mass absorption of the compositions and, as a result, lead to a decrease in the coefficient of resistance. At the same time, the compositions on the ECR-22 compound are subject to greater biodegradation [3]. Tests have shown an increase in the activity of fungi in relation to epoxy materials by 1 point when replacing dry quartz sand with wet. The coefficient of resistance of composites on a wet filler after three months of holding in the medium decreased in comparison with the composite on a dry filler by more than 5 %. More resistant composites are materials filled with basalt flour, which in comparison with other fillers has less fouling and improved adhesion to epoxy resins.

3. Theoretical part
Theoretical studies on the assessment of biocorrosion and biological resistance of composite building materials are given in the studies [3, 7, 10, 11, 20, 23].

Cement concrete has a high surface activity and is able to adsorb a variety of substances, including microorganisms. [20, 23, 25-29]. The mechanism of action of microorganisms on concrete can be
represented as follows. When hardening, the concrete is covered with a protective film formed by calcium carbonate. As long as the film is intact, it prevents water from diffusing inside the products and thus protects them from destruction. Thionic bacteria that settle on the surface of the carbonate layer destroy it by changing the pH of the contacting water due to the acid they form. In addition, they are harmful by producing sulfates, since the latter form calcium hydrosulfoaluminate, which accelerates the destruction of the material [3, 20, 25].

It is also proved that nitrifying and butyric acid bacteria contribute to the leaching of calcium from cement and consequently reduce the mechanical strength of the studied cement samples [20,25]. The destruction of cement stone due to the fact that as a result of microorganisms formed of organic and inorganic acids that dissolve protective carbonate film on the surface of the concrete, which opens the sulphate access into the material to form hydrosulphuret of calcium.

Damage to natural and artificial stone, including concrete, in certain conditions can also be associated with the development of mold fungi [3, 20, 25]. Mold formation occurs at different rates depending on the chemical composition of the stone, substrate humidity and ambient temperature. Unlike bacteria, fungi are not capable of independent synthesis of organic substances and exist due to ready-made forms of these compounds, so they are usually found on the contaminated surface of the stone or in the presence of organic additives [30, 31]. According to a number of authors, representatives of the genera Penicillium, Aspergillus, Trichoderma, and Cephalosporium predominate on the surface of stone building materials [32, 33, 34]. Natural and artificial stones are usually more abundantly populated with fungi in conditions of high humidity, which occurs most often in buildings without adequate ventilation and on the North side of stone monuments. Growing on the surface of the building material, fungi form velvety, felt-like and cotton-like deposits of various colors, which cause its functional degradation.

According to a number of authors the destructive effect of micromycetes on stone building materials, including concrete, is due to the aggressive effect of fungal metabolites (organic acids, redox and hydrolytic enzymes) on individual components of materials [35]. Maximum acid production by fungal cultures occurs at high temperatures and low pH values [36].

Synthetic polymers and materials on their basis are significantly higher than on the biological stability of natural polymers. However, polymer materials under certain operating conditions are damaged by biological agents, the main ones being microscopic fungi. Most often they are found Aspergillus flavus, A. versicolor, A. niger, Penicillium funiculosum, P. brevicompactum, P. lanosum, P. commune, P. cyclopium, Paecilomyces varioti, Trichoderma lignorum, Alternaria alternata, Chaetomium globosum, Fusarium oxysporum [5,36,37].

The degree of microbial resistance of polymer materials depends on many factors. The chemical nature of the polymer's macromolecules, its supramolecular structure and composition as well as the physical structure of the material are important. There is a certain relationship between the degree of bio-damage and the chemical structure of the material [37, 38]. The types of bonds C4, R–CH3, R–CH2–R' are considered to be hard to reach and inaccessible to mold fungi. Unsaturated valencies of the type R=CH2, R–CH= R', carbonyl and carboxyl radicals are accessible forms of carbon for microorganisms.

The stability of polymers also depends on their relative molecular weight: the smaller it is the more low-molecular fractions are included in the composition of resins and the less resistant they are to the action of microorganisms [39]. The biostability of polymer materials is significantly affected by additives that are usually always present in polymer compositions (fillers, plasticizers, stabilizers, antioxidants, modifiers, pigments, etc.) [3, 20]. As binders, they use various polymer resins – high-molecular substances that form the basis of any polymer material.

Paint coatings (PCS) for protecting buildings are of great importance in increasing the durability and increasing the service life of some building structures and their function is to protect structures not only from atmospheric influences and other aggressive factors, but also from microbiological corrosion, in particular from mold damage and their metabolites. However, paint-and-lacquer
materials (PLM) and paint coatings (PCS) operated under conditions favorable for the growth and development of mold fungi and bacteria, can themselves undergo microbiological damage [3, 40].

The main agents of microbiological damage to LCP are mold fungi. Bacterial lesions are less common and appear as a colorless or colored mucous plaque. Among the microorganisms that damage LCP, fungi of the genera Aspergillus, Penicillium, Fusarium, Trichoderma, Alternaria, Cephalosporium, as well as bacteria of the genera Pseudomonas, Flavobacteriu.

4. Problem statement
As it could be seen from the above review, currently protection from microbiological damage to building materials is mainly carried out by introducing special biocidal additives into their composition. Unfortunately, the selection of additives currently does not have a scientific basis and is carried out by trial and error. In this regard, protection from bio-damage of building materials is extremely laborious, expensive and time-consuming. For the first time, we have implemented a new approach to the selection of effective means of protection from biological damage based on the application of environmental and physiological-biochemical criteria. The environmental criterion includes the fact that it is necessary to work with fungi which are the true destructors of a particular building material. The physiological and biochemical criteria include biochemical testing of fungi for the presence of specific aggressive metabolites that carry out the process of material biodegradation. It is known that the most common aggressive metabolites of fungi when growing on building materials with organic binders (polymer resins) are exoenzymes from the class of oxidoreductases: catalase, peroxidase, and polyphenol oxidase. In this regard, biochemical testing of true destructors is performed in relation to these enzymes. The results of such testing are shown by us on the example of fungi-destructors of construction composites based on urea and phenolformaldehyde resins.

5. Method of research
In order to determine the spectrum of redox enzymes, fungi-true destructors of building composites were subjected to biochemical tests: the Nobles test to determine the presence of extracellular oxidases; the Bavendamm test to control the presence of phenol oxidase; tests for laccase, tyrosinase and peroxidase activity of microorganisms.

Tests for the presence of extracellular oxidases, laccases, and tyrosinases were performed by adding 1 ml of guaiacol tree resin solution, guaiacol solution, and p-cresol solutions to the tested fungal colonies, respectively. The Bavendamm test was performed using malt agar containing tannic acid (0.5%). Equal amounts of 0.4% hydrogen peroxide solution and 1% pyrogallol solution in water were used to determine peroxidase activity.

6. Results and discussion
The maximum range of selected characteristics was 8 out of 22 fungal strains (Table 1).

| Name of fungus strain | Extracellular oxidase | Phenoloxidase | Laccase | Tyrosin oxidase | Peroxydaza |
|-----------------------|-----------------------|---------------|---------|----------------|------------|
| Alternaria alternata  | –                     | +             | –       | +              | +          |
| Aspergillus carbonarius | +                     | –             | –       | +              | +          |
| Aspergillus flavus    | +                     | +             | –       | +              | +          |
| Aspergillus fumigatus | +                     | +             | –       | +              | +          |
| Aspergillus niger     | +                     | –             | –       | +              | +          |
Aspergillus oryzae - + - + -
Aspergillus penicilloides - - - + -
Aspergillus ustus - - + - +
Botryosporium logibrachiatum + + + - -
Mucor globosus + + - + +
Mucor hiemalis - - - + +
Paecilomyces carneus + + + - -
Paecilomyces variotii + + - + +
Penicillium cyclopium + + - - +
Penicillium funiculosum + + - - +
Penicillium nigricans + + + + -
Penicillium notatum + + - - -
Penicillium palitans + + + - +
Penicillium variabile _ _ + + _
Stemphylium verruculosum + + - + +
Trichoderma koningii + + + + +
Trichoderma lignorum _ _ + + +

+ / _ - positive / negative reaction to biochemical tests.

Aspergillus flavus, A. fumigatus, Mucor globosus, Paecilomyces carneus, Penicillium palitans, Paecilomyces variotii, Stemphylium verruculosum, Trichoderma koningii gave positive results for more than three of the five proposed tests. In particular, each of the eight fungi listed above showed a positive reaction to the Bavenda test, the Nobles test, and the peroxidase cup test. In addition, Aspergillus flavus, Aspergillus fumigatus, Mucor globosus, Paecilomyces variotii, Trichoderma koningii had tyrosinase activity, and Paecilomyces carneus, Penicillium palitans, Stemphylium verruculosum and Trichoderma koningii had laccase activity. Fungi that had a wider spectrum of redox enzymes were more active destructors of building composites. It is important to note that four of the eight fungi listed above turned out to be identical for phenol-formaldehyde and urea SCs: Aspergillus fumigatus, Paecilomyces carneus, Stemphylium verruculosum, Trichoderma koningii.

Thus, a significant variety of types of molds was revealed from building composites, many of which are dangerous for these building materials, because they are able to use their components as a power source.

It is known that polyalkylene guanidine-based compounds (in particular, Teflex, Bior, Troysan) are effective inhibitors of fungal oxidoreductases. Therefore, we further set ourselves the task of finding out whether these compounds will suppress the exooxidoreductases of fungi-destructors of building composites based on phenol-formaldehyde and urea resins. At the first stage, we determined MFCs (minimal fungicidal concentrations) for these compounds in relation to standard fungal cultures.
Table 2 presents the results of studies of fungicidal activity of polyalkylene guanidines (PAG), quaternary ammonium compounds related to ionogen SAW (surface active agents). These compounds are the basis of Teflex biocides. Methyl-phosphonic acid (MPA) was determined in relation to a mixture of 14 fungi — active biodestructors of polymers according to GOST 9.049-50 [414, 415] because microscopic fungi in natural and industrial conditions affect materials and products most often not individually, but in association. In these experiments, PAGs were used in concentrations from 0.1% to 10%.

**Table 2.** Fungicidal activity of polyalkylene guanidino in relation to a mixture of 14 fungi types GOST* 9.049-50.

| №   | Sample Name                          | The concentration of PAGs,% | MFK, cm |
|-----|--------------------------------------|-----------------------------|---------|
| 1   |                                      |                             |         |
| 2   | Polyethylene Guanidine Hydrochloride (A) |                             |         |
| 3   | Polyhexamethylene Guanidine Phosphate |                             |         |
| 4   | Polyethylene Guanidine Hydrochloride (B) |                             |         |
| 5   | Polyethylene guanidine hydrochloride (B) |                             |         |
| 6   | Polyethylene Guanidine Carbonate (A) |                             |         |
| 7   | Polyethylene Guanidine Phosphate |                             |         |
| 8   | Polyethylene Guanidine Carbonate (B) |                             |         |
| 9   | Polyethylene Guanidine Hydrochloride (A) |                             |         |
| 10  | Polyhexamethylene Guanidine Phosphate |                             |         |
| 11  | Polyethylene Guanidine Hydrochloride (B) |                             |         |
| 12  | Polyethylene                          |                             |         |
guanidine hydrochloride (B)

"+" - mold growth was observed
"-" - mold growth not detected

**Table 3.** Determination of the minimum fungicidal activity of PAGs in relation to certain types of mold fungi.

| №№ | Species | PAGs Concentration, % |
|-----|---------|-----------------------|
|     |         | №2  | №5  | №6  | №7  | №12 |
| 1   | Alt. alternata | 0.05 | 0.1 | 0.5 | MFK |     |
| 2   | A. niger | + - | 0.1 + - - - - - 0,5 + - - - - - 0,5 + - - - - - 0,5 + - - - 0,1 |
| 3   | A. oryzae | + - | 0.1 + + - - - 2,0 + + + - - 3,0 + + - - - 1,0 + + - - 0,5 |
| 4   | A. terreus | + + | 0.5 + + + - - 3,0 + + + - - 3,0 + + + - - 2,0 + - - - 0,1 |
| 5   | Ch.globosum | + + | 0.5 + + + - - 2,0 + + + - - 3,0 + + - - - 1,0 + - - - 0,1 |
| 6   | F. moniliforme | + - | 0.1 + - - - - 0,5 + - - - - - 0,5 + - - - - 0,5 + - - - 0,1 |
| 7   | Paec. variotii | + + | 0.5 + + - - - 1,0 + - - - - 0,5 + - - - - 0,5 + - - - 0,1 |
| 8   | P. brevicompactum | + - | 0.1 + - - - - 0,5 + - - - - - 0,5 + - - - - - 0,5 + - - - 0,1 |
| 9   | P. chrysogen. | + + | 0,5+ - - - - 0,5+ + + - - 2,0+ + - - - 1,0+ - - - - 0,1 |
| 10  | P. cyclopium | + - | 0,1+ + - - - 1,0+ + + + - - 3,0+ + + - - - 2,0+ - - - - 0,1 |
| 11  | P. funiculos. | + + | 0,5+ - - - - 0,5+ + + - - 2,0+ + - - - 1,0+ - - - - 0,1 |
| 12  | P. martensii | + - | 0,1+ - - - - 0,5+ - - - - - 0,5+ - - - - - 0,5+ - - - - 0,1 |
| 13  | P. ochro-chl. | + + | 0,5+ + + - - 1,0+ + + - - 2,0+ + - - - 1,0+ + - - - 0,5 |
| 14  | Trich. viride | + + | 0,5+ + + - - 1,0+ + + - - 2,0+ + - - - 1,0+ + - - - 0,5 |
|     |         | + - | 0,1+ + + + + 3,0+ - - - - 0,5+ - - - - 0,5+ - - - 0,1 |

"+" - mold growth
"-" - mold growth absence

The data in Table 2 show that derivatives of polyhexamethylene guanidines more actively inhibit the growth of molds in comparison with derivatives of polyethylene guanidines. Moreover, among polyethylene guanidines, carbonate and phosphate derivatives have fungicidal activity, while the hydrochlorides of these compounds do not inhibit the growth of micromycetes.

PAGs No. 2 and No. 12 (derivatives of polyhexamethylene guanidines) showed the greatest fungicidal activity to the mixture of mushrooms. Their MPA was 0.5%. PAG MPA No. 7 was 2%, and No. 5 and No. 6 - 3% (No. 5 and No. 7 - carbonate; No. 6 - phosphate polyethylene guanidines). The rest of the studied polyalkylene guanidines (Nos. 1,3,4, 8-11) did not exhibit fungicidal properties.

As mentioned above, materials and products are usually affected by a mixture of molds. However, there are also cases where biodeterioration of a material causes one kind of micromycetes. Therefore, it was of interest to identify MPAs in the most effective PAGs No. 2, 5, 6, 7 and 12 in relation to each of the 14 above mentioned types of fungi. For research data PAGs were taken in the following
concentrations from 0.05% to 2.0%; and 3.0% (Table 3). The data in Table 3 show that the MPA for PAG No. 2 was 0.1% with respect to fungi Alternaria alternata, A. niger, Chaetomium globosum, Paec. variotii, P. chrysogenum, P. funiculosum, Trichoderma viride. For the fungi A. oryzae, A. terreus, Fusarium moniliforme, P. brevi-compactum, P. cyclopium, P. martensii, P. ochro-chloron, it was 0.5%. PAG No. 12 (polyhexamethylene guanidine hydrochloride) also proved to be an effective fungicide, in which MPA equal to 0.1% was found for fungi Alternaria alternata, A. oryzae, A. terreus, Chaetomium globosum, Fusarium moniliforme, Paecilomyces variotii, P. brevi -compactum, P. chrysogenum, P. cyclopium, P. funiculosum, Trichoderma viride. For micromycetes A. niger, P. martensii, and P. ochro-chloron, MPA PAG No. 12 was 0.5%. In samples of PAGs Nos. 5, 6, and 7, the lowest MPA was higher than in PAGs No. 2 and 12 and corresponded to a concentration of 0.5%. This MPA in PAG No. 5 was observed in relation to the following fungi: Alternaria alternata, Chaetomium globosum, Paecilomyces variotii, P. brevicompactum, P. funiculosum, P. cyclopium. For fungi Fusarium moniliforme, P. chrysogenum, P. martensii, and P. ochro-chloron, MPA of PAG No. 5 was 1.0%. The most resistant to this PAG were fungi A. oryzae and Trichoderma viride - IFC - 3.0%, as well as A. niger and A. terreus, for which MPA was 2.0%. For PAG No. 6, the MPA for fungi Alternaria alternata, Chaetomium globosum, Fusarium moniliforme, Paecilomyces variotii, P. funiculosum and Trichoderma viride was 0.5%, and for P. brevicompactum, P. cyclopium, P. martensii and P. ochro -chloron - 2.0%. For micromycetes A. niger, A. oryzae, A. terreus, and P. chrysogenum, the MPA was 3.0%. PAG No. 7 had the MPA of 0.5% with respect to the following types of molds: Alternaria alternata, Chaetomium globosum, Fusarium moniliforme, Paecilomyces variotii, P. funiculosum, and Trichoderma viride. A 1% concentration of PAG No. 7 was MPA for fungi A. niger, P. brevi-compactum, P. cyclopium, P. artensii and P. ochro-chloron, and a 2.0% concentration - MPA for A. oryzae and P. chrysogenum.

Summarizing the above, we can say that the MPA of PAGs with respect to the mixture of fungi and to each fungus is separately different. This is due to the different sensitivity of certain types of micromycetes to certain PAGs. The highest value of MPA of PAGs was observed in relation to a mixture of fungi. It coincided with MPA in relation to the species of micromycetes most resistant to these compounds. For example, PAG MPA No. 5 for the mixture of fungi was 3.0%, the same concentration was fungicidal for the fungi A. oryzae and Trichoderma viride. At the same time, such fungi as Alternaria alternata, Chaetomium globosum, Paecilomyces variotii, P. funiculosum, P. brevicompactum, P. cyclopium did not grow on a nutrient medium containing even 0.5% PAG No. 5.

7. Conclusions

1. The article analyzes research in the field of microbiological corrosion of building materials by domestic and foreign experts. It is shown that microorganisms cause corrosion of composite materials on cement, polymer and other binders.

2. Bio-damages of inorganic building materials, which include concrete, are mainly reduced to a violation of the adhesion of the components as a result of exposure to inorganic or organic acids of microbial origin, as well as enzymes and due to chemical reactions between the cement stone of composites and the products of microbial metabolism.

3. Compositions of bituminous composites with different ratios of materials used without inclusion in the composition of fungicides showed non-fungal resistance and non-fungicidal.

4. Artificial polymers and materials on their basis largely exceed the biological stability of natural polymers. However, under certain operating conditions, they are also damaged by biological agents. The biostability of polymer materials is significantly affected by additives that are usually always present in polymer compositions (fillers, plasticizers, stabilizers, antioxidants, modifiers, pigments, etc.).

5. It is shown that the protection of certain building structures (metal products, communications, underground pipelines, and some others) is of great importance in improving the durability and increasing the service life of some building structures.
6. One of the most effective ways to increase the biostability of building materials is the introduction of modifying additives into their composition. Methods of increasing the biostability of cement, gypsum and other composites using various biocidal additives are presented.

7. Unfortunately the selection of biocide additives currently is not scientifically-based, and is done by trial and error.

8. In this paper, a new approach to the selection of effective means of protection from biological damage based on the application of environmental and physiological-biochemical criteria was implemented for the first time.
   • The environmental criterion includes the fact that it is necessary to work with microorganisms that are the true destructors of a particular building material.
   • The physiological and biochemical criteria include biochemical testing of fungi for the presence of specific aggressive metabolites that carry out the process of material biodegradation.
   • The results of such testing are shown on the example of fungi-destructors of construction composites based on urea and phenolformaldehyde resins.
     • When performing studies biochemical tests of Nobles and Bavendamm have been used.
     • A significant variety of mold fungi has been identified from building composites that can use their components as a food source, while others are random phases that grow due to external contamination.
     • On the example of urea and phenol-formaldehyde composites, it was found that the studied fungi-active destructors of these materials have high activity of oxidoreductases (catalase, peroxidase, phenol oxidase).

9. It is shown that when evaluating a comprehensive analysis of the processes of bio-deterioration is required to account for how species of microscopic fungi are destroyers of building materials and micro-organisms which are pathogenic that can cause mycoses, mycotoxicosis and microallergen.

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