Development of polymer putty with biocidal properties using chitosan

Saveliy Lobanov, Andrey Pustovgar and Igor Gulshin
Moscow State University of Civil Engineering, Yaroslavskoe shosse, 26, Moscow, Russia
E-mail: saveliy.lob@gmail.com

Abstract. The article presents the results of studies on the effect of chitosan additives on the biocidal properties of polymer putty for interior decoration.

1. Introduction
The biocidal properties of finishing materials can improve the environmental situation inside the premises and help reduce infectious diseases caused by various types of mold and fungi, so the use of environmentally friendly fungicides is an urgent task. The article systematizes and summarizes experimental data on the effect of chitosan on the construction-technological and biocidal properties of polymer putty.

2. State of the research question and setting of research tasks
The analysis of publications in the field of building materials showed that the main attention is paid to issues related to the technological and physico-mechanical properties, while the environmental component remains poorly understood. Publications statistics in the field of construction materials show that only about 10% of works are related to environmental problems. 189 UN member states signed the Millennium Development Goals (MDGs), In September 2000, in which the seventh goal is related to environmental sustainability [1]. Currently, the problem is considered as an ecological and technological one [2, 3]. For example, in 2000, as a result of mining activities around the world, 6,000 tons of mine waste were processed to produce just 900 tons of raw materials [4]. This means that on average only 0.15% of mine waste is used, which leads to the formation of huge amounts of waste, the disposal of which poses an environmental risk in terms of biodiversity conservation, air pollution and water pollution [1]. Since the environmental safety of building materials includes processing them from renewable raw materials, the use of biopolymers in a closed cycle of building materials production is relevant.

It is known that the vast majority of building materials is subject to biocorrosion and is a favorable environment for the propagation of mold and fungal colonies. In buildings and structures subject to infection by various types of mold and fungi, along with a decrease in the service life of building structures, the sanitary and epidemiological situation is deteriorating. In addition, the problem of biological deterioration is associated primarily with human health, since many of the microorganisms are conditionally pathogenic and can cause diseases [6, 7]. Through the movement of air currents, they enter the lungs of a person, settling on the skin, causing various diseases. According to the European
Medical Society, the smallest doses of mold fungi poison can cause allergies and cancerous tumors [8]. Biological deterioration is caused by various living organisms - microorganisms (bacteria and fungi) and microorganisms (plants and animals). However, the main harm to materials, according to numerous authors, is caused by microscopic organisms. The degree of their destructive impact is determined by physical, chemical, biological and other factors. Under favorable for microbial growth conditions, the destructive processes begin with the migration to the surface, adsorption, education growth microcolony by spread of hyphae and spores, accompanied by release of metabolic products and their accumulation. The most active corrosive bacteria agents are thionic and nitrifying, creating acidic aggressive environments, as well as sulfate-reducing, forming corrosive metabolites (NH3, H2S, CO2, organic acids). Typical metabolites for mycelial fungi are organic acids, redox and hydrolytic enzymes [5]. As a result, building materials are destroyed much faster than estimated standards. Negative effects of microorganisms are prevented in various ways: disinfection of materials surfaces and structures, the introduction of biocidal additives in the composition of composite materials, etc. Dry building mixes (DBM) with biocidal additives can be used in the construction and repair of buildings and structures that are exposed during operation to infection by biological organisms (mycelial fungi, bacteria, actinomycetes), which develop at high humidity and positive temperatures and cause biocorrosion of building structures.

A number of manufacturers refuse to use biocides as chemical agents of strong biological activity because their introduction into the environment causes extremely undesirable environmental shifts (the microorganisms resistant strains formation and active settlement of treated surfaces). An alternative to the biocides use are biostatics that do not cause the development of these phenomena, do not kill microorganisms, but suspend their development. one of the most effective biostatics is chitosan. Natural polycationite chitosan removes the negative charge from the bacteria surface, leads to the membrane structure changes, disrupts its barrier function and slows down the development and reproduction of microorganisms [13].

Chitosan has unique properties, such as: environmental safety, biocompatibility, biodegradability, biocide and others [10, 11], while in contrast to most traditional biocides in terms of toxicity, chitosan belongs to the 4th class and is considered safe [11]. The chitosan functional properties are determined by the features of its structure and depend on the aggregate state, molecular weight, degree of polydispersity, degree of deacetylation, positive charge density, hydrophilic-hydrophobic characteristics, chelating ability, as well as environmental factors - pH, ionic strength, temperature [12]. The chitosan can penetrate the cell wall in fungal cells, and enter DNA, affecting RNA [14]. Based on the study results of the biocidal properties of chitosan, a hypothesis was formed that the chitosan addition will give biocidal properties to polymer putty, the most popular finishing material, used for finishing residential, public and administrative premises.

3. Experimental Studies
In Table 1 the following polymer putty compositions were selected for experimental studies. Polymer putty components are described — fillers and additives used for the production of putty mixtures.

| Component name                  | The components content in the composition of the polymer putty, % |
|---------------------------------|---------------------------------------------------------------|
| Calcium Carbonate 0-50 mcr      | 96.7  96.6  96.2  95.7                                       |
| Redispersible polymer powder Elotex FL2212 | 2  2  2  2                                               |
| Titanium Dioxide Tronox CR-828  | 1  1  1  1                                               |
Sample preparation was carried out in accordance with GOST 31356-2007. The prepared putty was applied to the surface (Figure 1).

![Figure 1. Test samples photo.](image)

An adapted method described in GOST 28206-89 was used to assess the fungicidal potential of the material under study. “Basic test methods for exposure to external factors. Part 2. Tests. J test and manual: Mushroom resistance” [15]. Using this method, the material ability to suppress the mold fungi growth in the most favorable conditions was evaluated.

The technology development for obtaining and optimizing the composition of construction composites with biocidal additives that have increased resistance in biological and chemical aggressive environments, as well as improved physical and mechanical properties, is one of the urgent tasks to be solved in this work.

To achieve this goal, the following tasks were formulated and solved:

• scientifically justify the choice of source materials
• develop a research methodology
• develop the composition and manufacturing technology of a biocidal building mix
• investigate the structure and properties of protective coatings based on the developed mixtures
• find the optimal amount of biocidal additive to be added and examine its effect on the structure and properties of cement composites

The study was aimed at determining the material ability to inhibit the growth of mold fungi and consisted in holding samples infected with a set of mold spores in conditions favorable for their germination and development.

As part of the study, a test was performed in three ways:

Variant 1 - preliminary processing of the samples surface with a nutrient medium before infection with mold spores;

Variant 2 - direct sample infection with mold spores without applying a nutrient medium;

Variant 3 - placing the sample in an environment saturated with molds spores without preliminary application of a nutrient medium and direct infection with spores.

For the study, the following mold fungi cultures were used, classified according to the All-Russian Collection of Industrial Microorganisms FSUE GosNIIgenetika (Russian National Collection of Industrial Microorganisms):

|        | 0 | 0.1 | 0.5 | 1.0 |
|--------|---|----|----|----|
| Chitosan        |   |    |    |    |
| Cellulose ether Walocel MKX 15000PP25 | 0.3 | 0.3 | 0.3 | 0.3 |
1. Stachybotrys chartaum (Russian National Collection of Industrial Microorganisms F-993). According to a number of studies [16, 17], this genus is one of the most common among mushrooms-bio decomposers that affects the interior surfaces of buildings, the engineering equipment of buildings, and elements of building structures. According to the specifications of the Russian National Collection of Industrial Microorganisms, the industrial use of the given strain is the use as a test culture for determining the mushroom resistance of a wide range of materials. Crop culture after revitalization is shown in Figure 2. 

2. Aspergillus sp. An additional genus used as a quality control of the nutrient medium, as one of the most resistant to adverse factors, including inhibitors, building bio decomposers. The strain is derived on a paper filter with a glucose culture medium.

**Figure 2.** Sowing strain Stachybotrys chartaum (Russian National Collection of Industrial Microorganisms F-993).

The Stachybotrys chartaum strains were lyophilized in an ampoule. For sowing and accumulation, potato-glucose agar was used. For application, a potato-glucose broth was used as a nutrient medium on the sample. These media are recommended as the main ones when conducting studies with these genera of moldy fungi [18, 19]. The composition of nutrient media is shown in table 2.

| Ingredients        | Potato and glucose agar | Potato and glucose broth |
|--------------------|-------------------------|--------------------------|
| Potato infusion    | 200.00 g / dm$^3$       | 300.00 g / dm$^3$        |
| Glucose            | 20 g / dm$^3$           | 20 g / dm$^3$            |
| Agar-agar          | 15 g / dm$^3$           | -                        |

The lyophilized strains were revived using a standard method followed by seeding on potato-glucose agar.

After the strains viable cultures accumulation, the samples preparation was performed, which consisted of the following:

1. According to the method described in [15], a spore suspension containing strains of both cultures was prepared. For tests on the first variant, the spore suspension was made on the basis of potato-glucose broth. For tests on the second variant, the spore suspension was made on distilled water. Prepared spore suspensions are shown in figure 3.
2. On the total surface area of each of the samples, a section was conditionally allocated, which was immersed in a container with a spore suspension on potato-glucose broth.
3. In the center of each sample, a section was allocated at which a spore suspension with distilled water was applied using a spray gun.
4. The remaining areas were not processed.

The distribution scheme of the treated areas according to the test variants is shown at figure 4.

![Figure 3. Ready-made spore suspensions. On the left – on distilled water for performing tests, on the right — on potato-glucose broth for the option 2 test.]

![Figure 4. Distribution of areas by options for the option 1 test -option 1, B — option 2, B — option 3.]

After processing, the samples were placed in a wet chamber (a sealed box that maintains a relative humidity of more than 90%). In addition to the samples, test strips were placed in the box, processed according to the 1 and 2 versions of the tests. In this study, the production of negative samples was not provided. The wet chamber was placed in a thermostat WTW TS-606/2-i with a constant temperature of 28 °C.

The placement of the samples and the design of the wet chamber are shown in Figure 5.

The incubation time was 84 days according to the basic procedure [15], since on the 28th day of the study, the appearance of Stachybotrys chartaum colonies was not detected.

Samples were monitored every 7 days. The oxygen saturation procedure of the chamber was performed (opening the box cover for 30 seconds).

Test Checkpoints Table

The results of the study were evaluated visually by direct optical microscopy in reflected light (Zeiss Axio Imager 2 microscope visualized using a BR-5101LM-UF digital camera), stereomicroscopy, and also by scanning electron microscopy (SEM FEI Quanta 250).
Figure 5. Wet chamber placed in the thermostat photo.

4. Results of experimental studies
The samples visual inspection after 7 days of exposure did not show results, although the control strips were completely covered with a mixed culture of moldy fungi (Figure 6).

The first signs of microcolonies growth on the samples were noted on day 12 of the study. Bio — contamination was recorded on sample 3 at the test area for option 1 (with the application of a nutrient medium), as shown in Figure 7. The rest samples remained intact at all areas.

In this case, the affected sample contained mainly Aspergillus sp., As was seen from micrographs (Figure 8).

Then, for 20 days, an extremely low rate of colony growth was observed. Maximum Biopsy of samples was observed on the 48th day of the study (Figure 9). Areas 1 were found to be affected in all samples, while in samples 3 and 2, mold fungi sprouted in areas 2 (to a greater extent on sample 3).
On the 50th day of the study, the final assessment of the bio-lesion of the samples was carried out (the photo of the samples is shown in Figure 9).

As can be seen in the photo, sample 4 was under the least biological effect. Sample 2 (K) showed the maximum specific number of hyphae and conidiophores, which can be seen in microphotographs (Figures 10-29).

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**Figure 8.** Microphotography of culture taken for seeding on day 12 with 3 samples.  
**Figure 9.** Samples for the 50th day of the study.  
**Figure 10.** Sample 1, area 1.  
**Figure 11.** Sample 2(K) – area 1.
Figure 12. Sample 2(K) – area 1.

Figure 13. Sample 2(K) – area 1.

Figure 14. Sample 2(K) – area 2.

Figure 15. Sample 3 – area 1.
As can be seen from microphotographs, most of the mixed culture (up to 95% for conidia) belongs to Aspergillus sp., which is confirmed by photos of crops (Figures 20-21). Sample 4 shows only hyphae without conidiophores, which indicates a possible inhibition of the process of spore formation and maturation.
Examination of samples 2 and 4 with a scanning electron microscope also confirmed the presence of Aspergillus sp. on samples (Figures 22-25). In this case, only hyphae without conidiophores are visible on sample 4.
5. Discussion of research results

As a result of the polymer putty with chitosan additives study, the manifestation of putty fungicidal properties has been established, both in the absence of a nutrient medium (test variants 2 and 3), and when the surface is directly infected with mold spores.

It should be noted that sample 4 showed strong resistance to biodefeat and in the presence of a nutrient medium. The mold culture grown on this specimen did not demonstrate the ability to ripen, as can be seen from the absence of conidiophores.

The maximum specific fouling (the number and length of hyphae, the number of conidiophores to the surface area of the plot) was shown in control sample 2 (K), the fungicidal material content in which was minimal.

The fungicidal characteristics are confirmed indirectly, by the fact that the culture of Stachybotrys chartarum was almost completely displaced by Aspergillus sp. under conditions of initial excess of the substrate. Stachybotrys chartarum is significantly more susceptible to the inhibitory influence of the environment and inhibitors, which is reflected in its certainly lower content on the samples, compared to test strips.

6. Conclusions

The study results showed that the polymer putty modified with chitosan, in comparison with the control samples, has pronounced biocidal properties and has an inhibitory fungicidal effect on Stachybotrys chartarum and Aspergillus sp, both in the absence and in the presence of a nutrient medium.

Research of polymer putty construction-technological and biocidal properties allowed to establish the optimal amount of chitosan additives and determine its influence on the structure and properties of polymer putty and develop nominal compositions of polymer putty.

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