Prospects for the Use of Spore-forming Bacteria to Combat the Destruction of Polymeric Composite Materials

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Abstract. In order to develop an environmentally and economically sound method of dealing with biological damage of polymer composite materials (PCM), studies of environmental objects (EO) and finished products placed at the site of climatic tests in the city of Yakutsk were carried out. A strain of aerobic spore-forming psychrotolerant bacteria of the genus Bacillus was isolated from a fragment of basalt plastic reinforcement. Its biochemical properties and morphological characteristics were studied. It was found that the resulting strain has the ability to destroy fungi. This property of the Bacillus can be used to combat the bio-contamination of PCM. Biogenic factors of contamination of finished products with pathogenic fungi have been studied. It is established that the soil cover and atmospheric air in the area of the climatic test site contain similar types of microscopic fungi that were isolated from the surfaces of the prototypes. The results of studies on the microbiological characteristics of OOS it is possible to predict bizarrerie and, as a consequence, the biodegradation of PKM, as in the finished product, in contact with the aerial environment and soil cover of the landfill environmental tests found the same species (A. niger and A. fumigatus) with similar biochemical properties, which requires additional research

1. Introduction
The composition of microorganisms capable of contaminating and causing biological damage to PCM is not as diverse as, for example, biodestructors of materials and substances of natural origin. The most common biological damage of polymer composites is associated with exposure to microscopic fungi from the genera Alternaria, Aspergillus, Chaetomium, Cladosporium, Mucor, Fusarium, Penicillium, Rhizopus, Scopulariopsis, Trichoderma, etc. [1, 2].

Under favorable conditions (temperature, humidity, pH), the fungal mycelium is able to penetrate into microcracks, accumulate its biomass there, resulting in expansion and branching of cracks. In the process of life mushrooms secrete metabolic products that cause damage and corrosion of materials.
Today, the problem of combating the infection of PCM with mold fungi is very urgent, because synthetic polymers are the most common components for the production of various construction, medical, household goods and products.

It is known that in the world practice as a test cultures use strains of fungi, which most often and significantly destroy the materials in their operation and storage in different climatic zones. Given the presence of fungi high adaptive capacity, set the test cultures for laboratory test methods and biological stability necessary to continuously improve based on specific climatic conditions where you use a particular material or product. In this regard, the study of bio-contamination of PCM should go in parallel with the study of the influence of environmental factors on the processes of damage caused by microorganisms in different climatic conditions. This is necessary for the development of effective application of microbial technologies aimed at long-term operation of PCM. Insufficient study of the influence of abiotic and ekologicheskoi factors on microorganisms–biodestruktorov polymeric materials reduce the efficiency of methods for their protection biosurgery and biological damage.

The aim of this work is to find environmentally and economically feasible ways to combat biological infections of PCM mold.

2. Materials
The material for the research was fragments of ready PCM.

Sampling and sample preparation were performed according to generally accepted procedures [3, 4].

To isolate, the method of accumulation cultures on the mineral environment Muntz [5, 6, 7].

Specific identification of the isolated microorganisms was carried out using the Bergey’s guide [8], using the analysis of the nucleotide sequences of the 16S rRNA gene [9, 10, 11, 12, 13].

The emulsifying activity of the isolated cultures was determined by the Cooper method [14].

Investigation of the sorption properties of materials was using the methods of Anufrieva et al [15].

Environments for cultivation and storage of cultures of mushrooms and tests prepared according to the standard [16].

Established test methods were used to assess the fungal resistance and degradation of polymeric materials [16, 17, 18, 19, 20, 21].

3. Experimental part
Three strains isolated from the surface of the basalt-plastic reinforcement fragment were used in the experiment.

The microorganisms selected (according to the principle of antagonistic action): two mycelia of the strain A. niger and A. fumigates– as potential agents of biosurgery and the biodegradation of PKM and spore-forming strain of bacteria of the genus Bacillus as a biodegrader selected fungi.

To assess the ability of PCM to destruction under the influence of the obtained micellar strains, a method corresponding to the real conditions of degradation in the environment was used, placing the prototypes at the climatic test sites in Tiksi (BULUN district, North-Western group of ulus of the Republic of Sakha (Yakutia)) and in Yakutsk (Central Yakutia). Infected 24 non-sterile sample of rebar with a length of 1 m each.

For option 1, pure cultures aged at room temperature for 14 days from the date of sowing on the
Chapex-Dox nutrient medium were used. Spore suspension for inoculation of samples of rebar were prepared by the method of washout of the culture in distilled water. For each culture, a separate flask was prepared, after which the collected suspensions from the prepared cultures of fungi were mixed in a sterile container in a ratio of 1:1. The resulting suspension from the spores of the two obtained cultures was thoroughly mixed by vigorous shaking until all the lumps of spores were crushed. The concentration of microbial cells in the culture fluid was adjusted to the number of at least $1 \times 10^9$ cells per 1 cm$^3$ of the microbial suspension according to the Tarasevich turbidity standard. The suspension was defended for 30 minutes, then filtered from pieces of mycelium, agar and lumps of spores. Infection of samples with mold spores was carried out in the bath by immersion for 5 days. After that, the reinforcement samples were dried and placed on the site of climatic tests in Yakutsk and Tiksi.

4. Results and discussion

A strain of aerobic spore-forming bacteria of the genus Bacillus was isolated from a fragment of basalt plastic reinforcement. Its biochemical properties and morphological characteristics were studied.

The resulting strain is characterized by the following features.

It is a gram-positive rod bacterium, measuring 1.2 µm. In the smear it is placed in pairs and chains. It doesn’t form capsules, forms spores.

It is a facultative anaerobe.

The resulting strain forms a wet muddy-yellow colonies of round shape in a meat-peptone agar (mass. %: enzymatic peptone – 1.0; sodium chloride - 0.5; agar – 1.0; water meat is rest, pH 7.0 to 7.2). The relief of the colonies is changing after 1-2 days. The colonies are turning dry, the edge of the colony becomes rough.

In the Saburo medium (wt.%: hydrolyzed fish meal – 1.0; pancreatic hydrolysate of casein - 1.0; yeast extract - 0.2; dehydrogenated sodium phosphate – 0.2; D-glucose – 4.0; agar – 1.0; distilled water – the rest; pH of 6.0±3) it forms large colonies with yellowish color, the maximum diameter of which is 0.5 cm.

In meat-peptone broth (wt. %: enzymatic peptone – 1.0, sodium chloride - 0.5, water meat – rest; pH 7.0 to 7.2) it causes diffuse opacity and dry film.

In the mineral Muntz medium of the following composition: (wt.%: KNO3 – 0.4; MgSO4 •7H2O – 0.08; NaCl – 0.1; -0.14 K2 HPO4; KH2 PO4, or 0.06; agar-2.0; distilled water – the rest, pH 7.2) the strain grows in the form of lusterless non-transparent yellow colonies with a diameter of 0.3 cm.

Physiological and biochemical characteristics.

The strain grows at temperatures from +4 to +37°C. Under aerobic conditions it grows better. In anaerobic conditions, the strain does not die immediately, but develops slower. The optimum growth is under conditions of +30°C, pH should be from 5.0 to 9.0.

The strain grows in a salt broth with the addition of 2.0-6.0% NaCl.

The strain is catalysis positive and oxidize positive.

It has metabolism of oxidative type. Biochemical activity of the strain is weak. It is not able to assimilate polyhydric alcohols.

Glucose and sucrose is poorly fermented by the strain, other carbohydrates are not fermented at all.

The strain does not assimilate lysine, assimilate ornithine. It doesn’t apply citrate and malonate sodium.

It does hydrolyze gelatin, decompose starch. The strain is Indole negative. Reaction Voges - Proskauer is negative. Phenylalanine Dezoksaminaza is negative. It can does not ferment beta galactosidase.

Genetic characteristics.

Antagonistic properties of the obtained strain were studied. It was found that the strain Bacillus isolated from the reinforcement fragment has suppressive qualities in relation to pathogenic fungi of the genus Aspergillus (studied by A. niger, A. fumigatus). Bactericidal ability of Bacillus strain in relation to the studied fungi is associated with the production of biologically active substances in the metabolism. Thus, the fungicidal activity of Bacillus was revealed, and the strain itself is considered as a promising biological agent for the creation of a biological product to protect PCM from biological damage.
At the next stage, the growth rate of the obtained mold fungi in the version with a liquid nutrient medium was studied by taking into account the colonies of forming units (CFU/cm$^3$). The peak of the total number of CFU for the consortium on the basis of the two studied fungi falls on the first week of cultivation. The growth of microbial cells decreased sharply after two weeks, which is probably caused by toxic products of metabolism of microorganisms themselves. Despite this, there was no complete stop in the development of micromycetes, which makes the resulting consortium promising for use as a biological destructor of products based on PCM to activate the processes of decomposition of plastic products and materials at landfills.

However, approximately on the 20-th day of cultivation, the process of life activity of the selected strains followed the path of catabolism and was most likely associated with the formation of toxic products of metabolic decay of the studied fungi, as evidenced by the results of biotesting for phytotoxicity.

As a test plant, seeds of Ovena sativa (oat) were used, which were pre-soaked in pre-prepared mushroom suspensions. For this purpose, the mycelium of the studied 4-week strains was washed away with 0.15 M NaCl (pH 6.8-7.0). The obtained suspension was filtered using cloth filters to be exempt from mycelial fragments. Filters with the obtained spores were diluted to obtain a suspension corresponding to 5 UNITS of the optical turbidity standard of L. A. Tarasevich. Then, 10 seeds of test plants were put into test tubes and the resulting suspensions were poured (three tubes for each strain and a consortium of these strains).

Soaked seeds were kept for 20 hours. Then placed on Petri dishes with 10 ml of distilled water. Non-infected seeds of Ovena sativa were used as control.

According to the results of biotesting it was found that seed germination in the control variant was 90%; in the variant with suspension of A. fumigates – 50%; in the variants with suspension of A. niger and the consortium of both strains – 40%; which indicates the toxic effect of the obtained strains on higher plants. Thus, further research is needed to determine the feasibility of using the resulting strains for biotechnological production.

5. Conclusion
As a result of microbiological studies 52 cultures of microorganisms capable of colonizing products based on PCM (valves, pipes, plates) were identified. The beginning of the formation of a collection of strains-biodestructors PCM.

At this stage of the work, from the diversity of the microbial cultures selected for the study are selected (according to the principle of antagonistic action) of two mycelial strain of A. niger and A. fumigates as potential agents of biosurgery and the biodegradation of PKM and spore-forming strain of bacteria of the genus Bacillus as a biodegrader selected fungi.

Studies on the fungicidal and bactericidal properties of the Bacillus strain continue.

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