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Study of Microorganisms on Polymer Composite Materials in Frigid Climate Conditions

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***Abstract* – The paper presents research findings on extraction and identification of bacterial and mycelial forms of microorganisms on polymer composite materials under the influence of extreme frigid climate for further study of the destruction of these materials. The study made it possible to take wipe samples and scrapes from basalt plastic fittings, basalt- and fiber-glass plastic on open stands in frigid climate, as well as basalt plastic pipes used for water disposal in the Arctic zone of Yakutia. The following fungal species were identified from microorganisms-destructors of polymer composite materials via biochemical tests: Aspergillus fumigatus, Aspergillus niger, Aspergillus albus, Trichoderma viridans, Penicillium sp., Fusarium roseum. At least two species of spore-forming bacillary strains Bacillus atropheus and Bacillus subtilis were defined from the bacterial group.**

Keywords – composite material; basalt fiber reinforced polymer; climatic factor; microorganisms-destructors; identification; bacillary strains.

I. INTRODUCTION

Polymer composite materials (PCM) belong to a new class of materials, which due to a variety of essential advantages over metal materials, are widely used in different engineering products. Under the influence of numerous climatic factors (temperature, humidity, sunlight, etc.) polymer materials can considerably change their strength properties, which shall be considered in the design of various critical structures and engineering items intended for use in all climatic conditions [1]. With regard to PCM, the characteristics of such materials are specified to designers with a particular practical focus on materials, which strength properties during operation within 25-30 and more years would decrease by not more than 20-25%.

The work [2] presents the comparison tests of epoxy-fiber glass conducted under laboratory temperature and humidity influences and after their natural exposition in various climatic zones.

The work [3] describes the study of the influence of moderate thermal and warm humid climate on climatic resistance of PCM on epoxy binders ST-2227M, GKM-1M, VPS-31, ST-69N-15(P). The following mechanical property indicators $\sigma_{v.i.}$, $\sigma_{v.comp}$, τ_v, E defining material efficiency in operational conditions served the criterion for performance assessment.

The work [4] studies the influence of climatic factors of moderately warm and warm humid climate on climatic resistance of structural PCM on epoxy binders. It is found that it is possible to forecast strength characteristics through laboratory tests in case of full moisture saturation of a material. Thus we get the lower boundary of possible changes of material properties. The analysis of obtained data was confirmed by the results of the study [3].

The synergism demonstrated through the influence of humid conditions and mechanical loads is shown in works [5, 6].

The works [7, 8] devoted to PCM on the basis of epoxy binders were studying the influence of climatic factors through accelerated hygrothermal and natural climatic tests at static and cyclic loads on the change of residual strength of a material via bending, moisture content and structural transformations in the glass transition range of a material. It is revealed that the static load increases the moisture content, reduces the bending strength and glass transition temperature, as well as the glass-transition range.

However, such tests are followed by a variety of other problems, some of which are formulated in work [9]. In particular, it refers to insufficient study of physical and chemical changes in the material in the course of aging, lack of

information on the change of properties under the influence of static and dynamic loads, study of microbiological stability of materials.

At present there are more than 200 various methods to test the biostability of different industrial and construction materials. It is related to the fact that besides the international standards there are national and industry standards of certain countries. Industrial companies of our country use the standards of the Unified System of Corrosion and Aging Protection (GOST 9) that includes 123 standards to test biostability and the influence of biodestructors on materials. The variety of methods is caused, on the one hand, by a wide range of organisms acting as agents of biodeterioration, and on the other hand – by a big range of tested and newly created materials.

In recent years the study of the destruction of polymer composite materials (PCM) under the influence of bacterial and filamentous forms of microorganisms has been holding a special place. Getting into a composite a microbial cell interacts with any important compound components by means of enzymes. As a result of the influence of extracellular enzymes and metabolites the polymer material is transferred into a soluble state thus forming low-molecular decay products, which are available to microorganisms as energy and feed sources. Most often microbiological damages occur under the influence of microscopic fungi changing color and structure of polymers. In thin films – tightness and strength.

In work [10] the quantitative assessment of micromycete growth was carried out via vertical photometry and mathematical modeling. The standard equipment (a LINKEY-photometer, a PC) was used. The main methods of structural analysis of construction and industrial materials include infrared and electronic microscopy, X-ray analysis, double refraction. The microscopic methods measure the radiation absorption spectra in visible, ultra-violet and infrared regions. The experimental results and IR spectra reference make it possible to conclude on structural transformations under aggressive influence, i.e. microorganisms and their metabolites [11].

To study the biostability of composite construction materials the authors [12] used different combinations of inorganic and organic acids of low concentration, which impact on various materials would provide for accurate modeling of biological corrosion under the influence of metabolism products of microorganisms on the material. Acetic acid (0.01-1.0%), oxalic acid (0.01-1.0%), citric acid (0.01-1.0%) were used as probable initiators of biological corrosion.

The valid reason for the discrepancy of assessment of destruction test results under the influence of microorganisms of one and the same materials, which were carried out in laboratory and natural conditions, was not their aging.

Thus, under the influence of climatic aging factors the polymers are exposed to changes of their chemical composition and structure. Being exposed to such chemical changes, after a while they may become compounds with different chemical composition and structure compared to initial samples, thus, at some point being initially funginert these materials are exposed to destruction by certain fungi species with the corresponding set of metabolites.

Thus, standard test methods may also include the assessment of biostability of products and elements taking into account their performance under the influence of various types of biological fouling, products of not only living, but also dead fragments of microorganisms on constructional elements: spores, part of mycelium, as well as climatic aging of materials.

The purpose of the given work is to study and identify microorganisms on the structure of polymer composite materials in extremely frigid climate.

II. METHODS AND MATERIALS

The study used wipes and scrapes from basalt plastic fittings (BPF), basalt- and fiber-glass plastic on open stands in frigid climate and fragments of basalt plastic pipes used for water disposal in the Arctic zone of Yakutia within 4 years (Tiksi village, Republic of Sakha, Yakutia).

BPF samples were placed at the test site of the Center of Collective Use of the V.P. Larionov Institute of Physical-Technical Problems of the North of the Siberian Branch of the Russian Academy of Sciences according to GOST 9.708-83. The samples were exposed to natural climatic factors within 50 months.

The following elements were taken for microbiological tests of air, soil, binder components: hardener, accelerator, ED-22 epoxy resin (liquid and solid forms) and basalt fiber.

Microbiological studies were conducted via classical methods.

Sequencing was performed on AE3000 automatic sequencer. Specialized phylogenetic computer programs were used in sequencing analysis.

III. RESULTS

Figure 2 shows the exhibited samples of basalt composite fittings of various diameters placed at the test site of the Center of Collective Use of the V.P. Larionov Institute of Physical-Technical Problems of the North of the Siberian Branch of the Russian Academy of Sciences in Yakutsk to test their climatic resistance.

A great variety of spore-forming anaerobic bacteria and microscopic fungi were identified among test samples. The following fungal species were identified from microorganisms-destructors of polymer composite materials via biochemical tests: Aspergillus fumigatus, Aspergillus niger, Aspergillus albus, Trichoderma viridans, Penicillium sp., Fusarium roseum. At least two species of spore-forming bacillary strains Bacillus atropheus and Bacillus subtilis were defined from the bacterial group.

The Penicillium fungi were wiped from a fragment of a basalt plastic pipe (Fig. 2, a) used for water disposal in the Arctic zone of Yakutia within 4 years (Tiksi village).

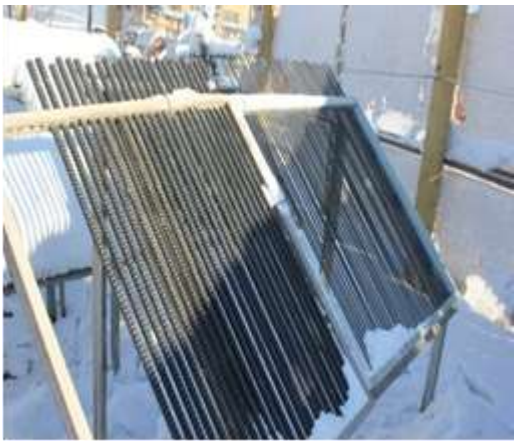
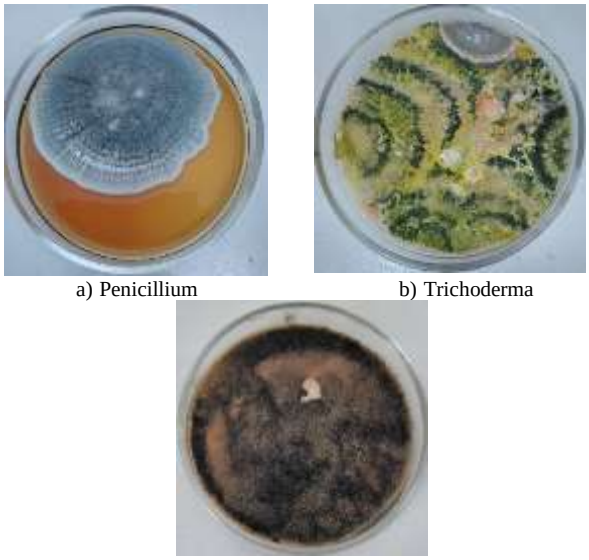


Fig. 1. Samples of basalt composite fittings placed at the test site of the Center of Collective Use of the V.P. Larionov Institute of Physical-Technical Problems of the North of the Siberian Branch of the Russian Academy of Sciences

Penicillium forms downy green colonies on solid nutrient mediums. The myriads of its spores are found everywhere. The main place of its habitat is soil. In the course of metabolism, they emit enzymes that quickly and effectively turn cellulose into soluble sugar. The penicillium mycelium is multicellular, consists of branching filaments divided by partitions into cells. The fruiting body resembles brushes located at the edges of mycelium filaments.



a) Penicillium b) Trichoderma

c) Aspergillus

Fig. 2. Identified microorganisms

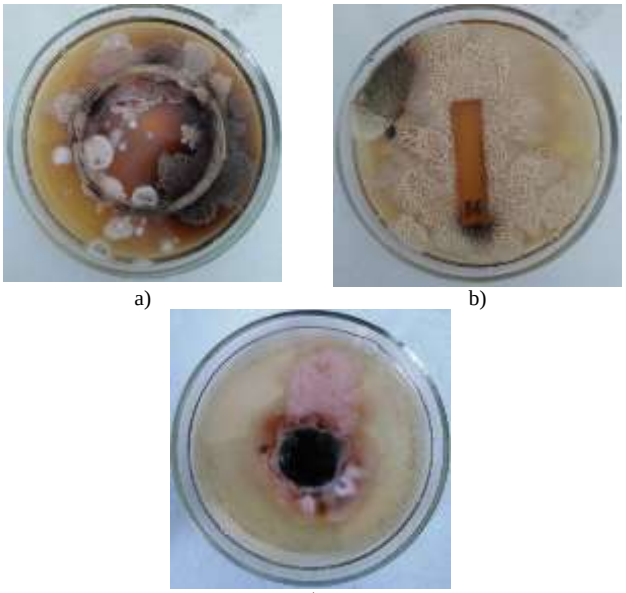
The Trichoderma fungi (Fig. 2, b) is identified from the exhibited samples of basalt plastic fittings placed at the test site of the Center of Collective Use of the V.P. Larionov Institute of Physical-Technical Problems of the North of the Siberian Branch of the Russian Academy of Sciences in Yakutsk.

At the beginning of its growth Trichoderma forms colonies with white mycelial film, with age they become hairy due to the formation of poor air hyphae. Conidial clusters (zones of conidial sporulation of a colony) change their size from 2-5 mm to 1-3 cm and more (accrete spots), in the early days the clusters are pulvinated, velvet-like white and whitish, but with the growth they turn into crowns and mucedinous clusters – greenish, blue, dark bluish, emerald and dark green with white or yellow downy edge. The reversum (a reverse side of fungi colony at its cultivation on solid agarized media) is colorless. The reproduction is asexual.

Aspergillus fungi (Fig. 2, c) is identified from a scrape of a basalt plastic pipe fragment.

Aspergillus species form downy colonies of different color; they have segmented mycelium and unicellular conidiophore. During the microscopic study of aspergilli, the arrangement of exospores resembles water streamlets of a watering pot.

Figure 3 shows the fouling stages by native microflora of polymer materials in vitro.



a) b)

c)

Fig. 3. Fouling of polymer materials by native microflora

Figure 3 a) shows the sample of basalt plastic pipe fouling in vitro by native microflora, spore-forming bacteria of Bacillus and Aspergillus fungi.

Figure 3 b) shows the fragment of fouling of a cured binder sample on the basis of ED-22 epoxy resin by native microflora with spore-forming bacteria of Bacillus and Aspergillus fungi.

Figure 3 c) shows the fragment of fouling of BPF sample by native microflora with Fusarium fungi.

Almost all mold fungi identified from the studied samples belong to pathogenic and opportunistic species.

However, the possibility of using mold fungi as sanitary indicator microorganisms is scarcely considered. There are no standards for the frequency of mold fungi findings on external objects yet.

The study showed that molds are permanently present in the environment. We also identified pathogenic species of mold fungi from air and soil samples taken at the climatic test site,

which can easily serve as the reason for biocontamination of the studied samples.

Despite the fact that sometimes molds serve for the benefit of environmental sanitation, nevertheless their wide-spread occurrence inevitably leads to the damage of materials and objects of the environment. This defines the need for further analysis of theoretical and practical experience in order to collect factual evidence, to develop methods to fight against biocontamination, as well as to assess the real importance of mycological study and to define the scope of its objects.

We shall consider in more detail the bacterial strains of Bacillus species, which were identified from finished products (fragments of pipes), from a compound component of polymer composite materials (cured binder) and from environmental objects (air and soil samples taken from the climatic test site). At least two strains of B. atropheus and B. subtilis are identified. One of bacillary strains is able to lyse microscopic fungi, which were also identified from a surface of finished goods (fragments of pipes, fittings).

According to its studied properties, the strain with lyse capacity belongs to gram-positive spore bacteria. The sporulation capacity allows bacilli withstanding adverse environmental factors, including, low temperatures.

Cultural character. On beef-extract agar-agar (wt. %): enzymatic peptone – 1.0; sodium chloride – 0.5; agar – 1.0; meat water – the rest, pH – 7.0-7.2, forms yellow dryish colonies.

On nutrient agar-agar on the basis fish-flour hydrolysate (wt. %): fish-flour hydrolysate – 1.2; enzymatic peptone – 1.2; sodium chloride – 0.6; agar – 1.0; distilled water – the rest; pH – 7.1-7.5, forms similar yellow colonies.

On beef-extract broth (wt. %): enzymatic peptone – 1.0, sodium chloride – 0.5, meat water– the rest; pH – 7.0-7.2, creates turbidity and film on broth surface.

Physiological and biochemical character.

The aerobic strain grows at a temperature from +5 to +40 °C, more intensively at +20 … +30 °C. Grows at pH 5.5-9.0. Optimum pH 6.0-8.0. Grows in salt broth with addition of 0.1-2.0% of NaCl.

The used carbon sources: sorbite.

The checked unused carbon sources: lactose, glucose, sodium citrate, sodium malonate. The strain is indole-negative. The Voges-Proskauer reaction is negative. Phenylalanine Desoxaminase is negative. Does not ferment beta-galactosidase. Does not utilize inositol. The Voges-Proskauer reaction is a product synthesized by a strain: surfactants able to destroy hydrocarbon mixes with various molecular weight and some other chemical compounds. Uses hydrocarbons as a power source.

The strain can be maintained by regular subcultures (once every 10-14 days) on slant beef-extract agar-agar or kept in lyophilized state in ampoules at 4 °C.

Sequencing was performed on AE3000 automatic sequencer. Specialized phylogenetic computer programs were used in sequencing analysis.

When sequencing variable sites 16S rDNA the following nucleotide sequence for the studied strain is received:

AGTCGAACGAACTCTTCGGAGTTAGTGGCGGACG
GGTGAGTAACACGTGGGAACGTGCCTTTTGGTTCGG
AATAACTCAGGGAAACTTGTGCTAATACCGAATGTG
CCCTTCGGGGGAAAGATTTATCGCCATTAGAGCGGC
CCGCGTCTGATTAGCTAGTTGGTGAGGTAAAAGCTC
ACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATG
ACCAGCCACACTGGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATCTTGCGCAATGG
GCGAAAGCCTGACGCAGCCATGCCGCGTGAATGATG
AAGGTCTTAGGATTGTAAAATTCTTTCACCGGGGACG
ATAATGACGGTACCCGGAGAAGAAGCCCCGGCTAAC
TTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTA
GCGTTGCTCGGAATTACTGGGCGTAAAGGGCGCGTA
GGCGGATCGTTAAGTCAGAGGTGAAATCCCAGGGCT
CAACCCTGGAACTGCCTTTGATACTGGCGATCTTGAG
TATGAGAGAGGTATGTGGAACTCCGAGTGTAGAGGT
GAAATTCGTAGATATTCGGAAGAACACCAGTGGCGA
AGGCGACATACTGGCTCATTACTGACGCTAAGGCGC
GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGATGATTGCTAGTTGTCGGG
CTGCATGCAGTTCAGTGACGCAGCTAACGCATTAAG
CAATCCGCCTGGGGAGTACAGTCGC

Fig. 4. PCR analysis of the studied strain using species-specific primers: PCR fragment when using secYsubF and secYsub – 1; Marker O'GeneRuler 1 kb DNA Ladder (250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000, 10000 bps, from top to bottom) – 2.

Primary screening performed according to the GenBank and RDP-II database showed that the studied strain belongs to the following systematic Bacteria groups; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus, and the homology with some Bacillus species makes 99%.

The sequences were leveled with the corresponding sequences of the next bacterial species from the GenBank database.

Thus, on the basis of cultural, morphological, differential-diagnostic and phylogenetic features it is found that in terms of its scientific classification the identified strain belongs to the following systematic groups:

Domain: Bacteria
Type: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Sort: Bacillus

The obtained strain is perspective for biotechnological application, namely for biological treatment of polymer composite materials against microscopic fungi participating in biocontamination of finished products.

IV. CONCLUSION

A great variety of spore-forming anaerobic bacteria and microscopic fungi were identified among test samples. The following fungal species were identified from microorganisms-destructors of polymer composite materials via biochemical tests: Aspergillus fumigatus, Aspergillus niger, Aspergillus albus, Trichoderma viridans, Penicillium sp., Fusarium roseum. At least two species of spore-forming bacillary strains Bacillus atropheus and Bacillus subtilis were defined from the bacterial group.

Almost all mold fungi identified from the studied samples belong to pathogenic and opportunistic species.

The spore-forming strain of the Bacillus species is identified, which is perspective for biotechnological application, namely for biological treatment of polymer composite materials against microscopic fungi participating in biocontamination of finished products.

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