Biological Contamination Study of Epoxy Binders

L A Erofeevskaya¹, A A Kychkin², A K Kychkin², A A Vasileva², S I Sivtsev¹, A R Alexandrov¹

¹Institute of Oil and Gas Problems of the Siberian Branch of the Russian Academy of Sciences, Russia, Yakutsk
²V.P. Larionov Institute of the Physical-Technical Problems of the North of the Siberian Branch of the Russian Academy of Sciences, Russia, Yakutsk

E-mail: lora-07.65@mail.ru¹, kychkinplasma@mail.ru²

Abstract. The results of microbiological studies of epoxy binders for bio-contamination are presented. At least 3 groups of microorganisms were selected from the samples presented for the study: actinobacteria of the genus S. albus (from hardener); spore-forming bacteria of the genus B. atropheus and pathogenic mold fungi of the genus A. fumigatus from a sample of hardened epoxy resin, which indicates the contamination of materials by microflora, which can cause biodeterioration in finished products and polymer composite materials manufacturing process.

1. Introduction

Epoxy binders (resins) are oligomers or monomers that contain at least two epoxy or glycycl groups in a molecule that are capable of being converted into cross-linked polymers.

In epoxy binders, epoxy groups are located in aliphatic chains, at the ends of which glycetyl groups are formed. About 85% of these polymers are used for the manufacture of polymer composite materials (PCM).

Currently, epoxy binders can be obtained in two ways:
1. In the interaction of epichlorohydrin or dichlorohydrin with two and polyhydric phenols, aniline, amines, glycols and other compounds.
2. Direct epoxidation of unsaturated compounds with peracids.

The first method produces diane resins of the ED type, polyepoxy resins based on epoxynolacs of the EN type and polyphenols based on the ETF type (epoxytriphenol) and aliphatic diepoxy resins such as DEG, TEG, MEG.

When curing epoxy binders at moderate temperatures (60-120 °C), the most commonly used:
1. Polyethylene polyamine (PEPA);
2. Hexamethylenediamine (HMDA);
3. Diethyltriamide ((DETA);
4. M-phenylenediamine (MPLA).

At high temperatures (120-200 °C), acidic products are used:
1. Maleic anhydride (MA);
2. Phthalic anhydride (FA);
3. Triethanolaminotitanate (TEAT);
4. Aminophenol formaldehyde novolac resin (SF-314A).

Epoxy binders, like others, artificially prepared and substances of natural origin can be susceptible to corrosion under the influence of various factors.

An important role here is played by microbiological factors.

This is due to the fact that microorganisms quickly adapt to a wide range of materials, begin to intensively develop either due to external pollution, or due to the components of the material itself, which inevitably leads to the destruction of the latter [1].

Epoxy resins, being organic compounds, can be used by various microorganisms as a source of energy and nutrition, causing their bio-infection and biodegradation.

Currently, there is a wide range of laboratory methods for studying PCM biodegradation, which differ in the duration of the experimental studies, the process conditions (temperature, humidity, illumination, etc.), the used cultures of microorganisms (bacteria, fungi, etc.) [2].

A number of well-known standards and research methods take into account the presence of external contaminants on the tested materials, while others do not take into account, which leads to an inadequate assessment when taking into account the test results of the same materials [3-5]. Some of them do not allow to detect biodeterioration of a number of products that may occur as a result of the presence of microorganisms inside the product itself [6].

The purpose of this work was to study the bio-contamination of epoxy binders used for the manufacture of basalt-plastic armature (BPA).

2. Materials

The research material was: samples of liquid and hardened epoxy resin, accelerator and hardener, varnish, basalt fibers.

Research Methods. To isolate and cultivate microorganisms, we used the method of liquid enrichment of cultures in nutrient broth and in a hungry mineral medium of the following composition (g/l):

- KNO\(_3\) – 1,0
- MgSO\(_4\) \(\times\) 7H\(_2\)O – 0,1
- Na\(_2\)HPO\(_4\) – 0,6
- KH\(_2\)PO\(_4\) – 0,14
- NaCl – 1,0
- Water - residual.

Assessment of the viability and purity of the isolated strains was carried out according to the method of Koch and Drigalsky [7].

Identification of the isolated strains was carried out on the basis of studying their morphological, cultural, physiological, and biochemical properties [8], including the analysis of the nucleotide sequences of the 16S rRNA gene.

3. Experimental section

As a result of microbiological studies carried out for special reasons, 3 groups of microorganisms: actinobacteria of the genus *Streptomyces* (*S. albus*, from hardener); spore-forming bacteria of the genus *Bacillus* (*B. atropheus*) and pathogenic mold fungi of the genus *Aspergillus* (*A. fumigatus*) from a sample of hardened resin, which indicates the contamination of materials by microflora, which can cause BPA biodeterioration during their manufacture.

The main differential diagnostic properties of microorganisms isolated from the samples presented in table 1.
Table 1. The main differential diagnostic properties of microorganisms isolated from hardener and hardened evaluation.

| The properties               | Bacillus atropheus | Aspergillus fumigatus | Streptomyces albus |
|-----------------------------|--------------------|-----------------------|--------------------|
| Gram stain                  | +                  | -                     | +                  |
| Cell morphology             | rod-shaped         | -                     | Thin coenocytic hyphae, velvety mycelium in the form of chains |
| Colony morphology           | Large, flat, matte beige | Brown, dusty, growing from cream-colored colonies | The colonies are convex, white, velvety |
| Motility                    | +                  | -                     | -                  |
| Spore availability          | +                  | +                     | +                  |
| Capsule availability        | -                  | -                     | -                  |
| Growth aerobically          | +                  | +                     | +                  |
| Growth anaerobically (+)    | (+)                | (+)                   | (+)                |
| Oxidase                     | -                  | -                     | -                  |
| Catalase Production         | +                  | -                     | -                  |
| Gelatinase Liquefaction     | +                  | -                     | +                  |
| Lecithinase formation       | -                  | +                     | -                  |
| Reduction of NO₃ to NO₂     | +                  | +                     | -                  |
| Hydrolysis of starch        | +                  | +                     | +                  |
| Acid from: glucose          | -                  | +                     | (+)                |
| lactose                     | -                  | +                     | -                  |
| maltose                     | -                  | +                     | -                  |
| mannitol                    | -                  | +                     | -                  |
| xylose                      | -                  | +                     | -                  |
| glycerin                    | -                  | +                     | -                  |
| arabinose                   | -                  | +                     | -                  |
| sorbitol                    | +                  | +                     | +                  |
| sucrose                     | -                  | +                     | -                  |
| Voges-Proskauer reaction   | -                  | -                     | -                  |
| Sodium Citrate Disposal     | -                  | +                     | -                  |
| Disposal of sodium malonate | -                  | +                     | -                  |
| β-galactosidase             | -                  | -                     | -                  |
| Urease formation            | -                  | +                     | -                  |
| Indole formation            | -                  | -                     | -                  |
| Hydrogen sulfide            | -                  | -                     | -                  |
| Lysine                      | -                  | -                     | -                  |
| Ornithine                   | +                  | -                     | -                  |

*+ test positive; - test is negative; (+) weakly positive test

In the framework of this work, studies were performed on the microbial contamination of finished BPA. A wide variety of microorganisms was isolated from BPA fragments and washes from their surface.

The landscape of microbial cultures isolated from BPA samples is presented in table 2.
Table 2. Landscape of isolated microorganisms from finished BPA.

| No. Sample | Sample name | Strain name |
|------------|-------------|-------------|
| 1          | Fragment of BPA (2015) | Streptomyces albus |
| 2          | Fragment of BPA (2015) | Aspergillus niger |
| 3          | Fragment of BPA (2015) | Aspergillus niger |
| 4          | Fragment of BPA (2015) | Bacillus sp. |
| 5          | Fragment of BPA (2015) | Aspergillus albus |
| 6          | Fragment of BPA (2015) | Streptomyces albus, Micrococcus |
| 7          | Fragment of BPA, T-100-0,5% (26.12. 16) | Aspergillus albus |
| 8          | Fragment of BPA, T-100-0,5% (26.12. 16) | Streptomyces kanamyceticus |
| 9          | Fragment of BPA, T-70-0,75% (26.12. 16) | Aspergillus.niger, Rhizopus nigricans, Bacillus sp. |
| 10         | Fragment of BPA ,T-70-0,25% (26.12. 16) | Aspergillus niger |
| 11         | Fragment of BPA (26.12. 16) | Bacillus sp. |
| 12         | Fragment of BPA, T-70-0,25% (26.12. 16) | Aspergillus niger, Aspergillus fumigates, Bacillus sp. |
| 13         | Fragment of BPA (2014-2015) | Strept.kanamyceticus |
| 14         | Fragment of BPA (2014-2015) | Не выделены |
| 15         | Fragment of BPA , Tarkosil T-100-0,75% | Aspergillus niger |
| 16         | Fragment of BPA | Rhodotorula mucilagenosa, Aspergillus albus |
| 17         | Tarkosil based BPA T-100-3,0% | Не выделены |
| 18         | Tarkosil based BPA T-135-0,75% | Rhizopus , Bacillus sp. |
| 19         | Tarkosil based BPA T-70-0,75% | Rhizopus , Aspergillus niger |
| 20         | Tarkosil based BPA T-70-0,75% | Bacillus sp., Mucor |
| 21         | Tarkosil based BPA T-71 | Aspergillus niger, Fusarium culmorum, |
| 22         | Fragment of BPA | Fusarium roseum, Alternaria |

A strain of mold fungus of the genus *A. fumigates* isolated from hardened resin was subsequently used as a test culture for testing BPA for fungal resistance.

Determination of the mushroom resistance of the reinforcement was carried out by the following methods:

1. The samples of the valves were sterilized in an autoclave at a temperature of + 126 ° C (pressure 1.5 atmospheres) for 30 minutes, after which the sterile samples were infected with an aqueous suspension of *A. fumigatus* spores;
2. Non-sterile reinforcement samples were infected with an aqueous suspension of *A. fumigatus* spores;
3. Sterile reinforcing specimens were infected with *A. fumigatus* spore suspension in two versions: a) in the liquid medium of Czapek-Doks;
   b) laying out horizontal sections of reinforcement (disks) on an agarized Chapek-Doks medium with pre-made inoculation of mushroom suspension on the surface of solidified agar.
4. Non-sterile reinforcement samples were infected with a spore suspension of *A. fumigatus* in two versions:
   a) in the liquid medium of Czapek-Doks;
   b) laying out horizontal sections of reinforcement (disks) on an agarized Chapek-Doks medium with pre-made inoculation of mushroom suspension on the surface of solidified agar.

The test results are presented in table 3.
### Table 3. The results of determining the fungus resistance of samples of reinforcement.

| Test method                                                                 | No sample | Points, fungi development intensity                                                                 |
|----------------------------------------------------------------------------|-----------|------------------------------------------------------------------------------------------------------|
| No. 1 - Sterile samples of an infected aqueous suspension of fungal spores | 1/1       | 1 - Germinated spores and slightly developed mycelium are visible under the microscope               |
|                                                                             |           | 2/1 - Germinated spores and slightly developed mycelium are visible under the microscope             |
|                                                                             |           | 3/1 - Germinated spores and slightly developed mycelium are visible under the microscope             |
| No. 2 - Non-sterile samples were infected with an aqueous suspension of fungal spores | 1/2       | 1 - Germinated spores and slightly developed mycelium are visible under the microscope               |
|                                                                             |           | 2/2 - Germinated spores and slightly developed mycelium are visible under the microscope             |
|                                                                             |           | 3/2 - Germinated spores and slightly developed mycelium are visible under the microscope             |
| No. 3 (a) - Sterile samples were infected with a suspension of fungi in the liquid environment of Chapek-Dox | 1/3(a)    | 3 - The mycelium is visible with the naked eye and sporulation is visible                             |
|                                                                             |           | 2/3(a) - The mycelium is visible with the naked eye and sporulation is visible                         |
|                                                                             |           | 3/3(a) - The mycelium is visible with the naked eye and sporulation is visible                         |
| No. 3 (b) - Sterile samples were infected with a suspension of fungi on agar medium Chapek-Doksa | 1/3(b)    | 3 - The mycelium is visible with the naked eye and sporulation is visible                             |
|                                                                             |           | 2/3(b) - The mycelium is visible with the naked eye and sporulation is visible                         |
|                                                                             |           | 3/3(b) - The mycelium is visible with the naked eye and sporulation is visible                         |
| No. 4 (a) - Non-sterile samples were infected with a suspension of fungi in the liquid environment of Chapek-Doks | 1/4(a)    | 3 - The mycelium is visible with the naked eye and sporulation is visible                             |
|                                                                             |           | 2/4(a) - The mycelium is visible with the naked eye and sporulation is visible                         |
|                                                                             |           | 3/4(a) - The mycelium is visible with the naked eye and sporulation is visible                         |
| No. 4 (b) - Non-sterile samples were infected with a suspension of mushrooms on an agar medium Chapek-Doksa | 1/4(b)    | 5 - With the naked eye, the development of fungi is clearly visible, covering more than 25% of the test surface |
|                                                                             |           | 2/4(b) - With the naked eye, the development of fungi is clearly visible, covering more than 25% of the test surface |
|                                                                             |           | 3/4(b) - With the naked eye, the development of fungi is clearly visible, covering more than 25% of the test surface |
| No. 5 - Sowing a sterile sample of reinforcement on sterile nutrient agar as a control of the purity of the experiment | 5         | 0 - no growth of microscopic fungi and other microflora                                               |
As a control, sterile reinforcement samples on a sterile nutrient medium without inoculating a mushroom suspension were used.

Armature samples infected with fungal spores were exposed in a thermostat at a constant temperature (+25 °C) for 28 days.

The mushroom resistance of the samples was evaluated on a 6-point scale in accordance with GOST 9.048-89 [9]. Non-sterile reinforcement specimens infected with fungal spores on the Chapek-Doks agar medium showed the highest activity for colonization of products by A. fumigates culture. In the experience with the naked eye, the development of fungi is clearly visible, covering more than 25% of the test surface (table 2 (sample 4 (b)).

Simultaneously with the test for the mushroom resistance of BPA in laboratory experiments, the growth rate of A. fumigates in the variant with liquid nutrient medium was studied by taking into account the colonies of the forming units (CFU / cm3) and on agarized Chapek-Doks medium with sucrose to study the radial growth of strains, which allowed for processing the obtained results, establish the cause of the reduced activity of the selected strain and establish the main phases of the development of A. fumigatus on a nutrient medium.

To infect reinforcing fragments with A. fumigatus culture, a concentration of microbial cells in the amount of 1 billion CFU / cm3 of microbial suspension was used. The exposure was 28 days. Cultivation took place in a thermostat at constant humidity and temperature plus 25 °C. The control seeding of the microbial suspension was carried out 1 time per week.

The peak of the total number of CFU of the studied strain falls on the first week of cultivation. The growth of microbial cells sharply decreased after two weeks, which is probably caused by toxic metabolic products of the strain itself. Despite this, a complete halt in the development of molds did not occur, which makes the resulting A. fumigatus culture promising for use as a destructor of PCM-based products.

4. Conclusion

Three groups of microorganisms were isolated from hardener and hardened resin: actinobacteria of the genus Streptomyces albus (from hardener); spore-forming bacteria of the genus Bacillus atropheus and pathogenic mold fungi of the genus Aspergillus fumigatus from a sample of hardened resin, which indicates the contamination of materials by microflora, which can cause PCM biodeterioration during their manufacture and operation.

The results of the test to determine the mushroom resistance of BPA samples made it possible to use the selected A. fumigates strain isolated from the hardened epoxy resin sample to colonize the finished products as one of the components of which epoxy resin was used.

The highest activity for PCM colonization with A. fumigates fungi was shown by non-sterile BPA samples infected with a fungal suspension on Chapek-Doks agar medium. This can be explained by the use of micromycetes as an additional source of carbon nutrition - sucrose, which is part of the nutrient medium, as well as the symbiotic relationships of microorganisms colonizing a non-sterile sample of the finished product, which exhibit mutually beneficial relationships in the association.

5. References

[1] Krivushina A A, Bobyreva T V, Goryashnik Yu S, Bukharev G M 2019 The study of microorganisms-destructors of functional polymer materials in a simulated tropical climate Material testing Proceedings All Russian Scientific Research Institute of Aviation Materials, 7(79) pp 76-83

[2] Belik E S, Rudakova L V, Kulikova Yu V, Burmistrova M V, Slyusar N N 2017 Evaluation of the effectiveness of biodegradation of polymer composite materials Ehkologiya i prirodopol'zovanie. Vestnik NVGU 4 pp 111-118

[3] Kryazhev D V, Smirnov V F, Smirnova O N, Zakharova E A, Anikina N A 2013 Analysis of methods for assessing the biostability of industrial materials (criteria, approaches) Vestnik Nizhegorodskogo universiteta im. N.I. Lobachevskogo 2(1) pp 118–124
Acknowledgements

This work was funded by the RFBR Project No. 18-29-05012 "Development of the scientific basis for the creation of new composite materials under the influence of abiogenic and biogenic factors in the Arctic and subarctic zones of the Republic of Sakha (Yakutia)".