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PAINT-DEGRADING MICROORGANISMS

MIKROORGANIZMY ZDOLNE DO DEGRADACJI POWŁOK MALARSKICH

Abstract

The paper presents a literature survey of research on the biodeterioration and biodegradation of paint coatings, as well as study findings on the biodegradation of varnish coatings and agents used for their removal.

Keywords: biodeterioration, biodegradation, paints, microorganisms

Streszczenie

W artykule przedstawiono przegląd literaturowy dotyczący biodeterioracji i biodegradacji powłok malarskich. Przedstawiono również badania dotyczące biodegradacji substancji używanych do usuwania powłok lakierniczych.

Słowa kluczowe: kluczowe: biodeterioracja, biodegradacja, powłoka malarska, mikroorganizmy

1. Introduction

Paint and varnish coatings are used for protective and/or decorative purposes, and their properties largely depend on their ingredients, i.e. on the film-forming substances employed. The latter primarily include macromolecular organic substances or substances that turn into macromolecular compounds by way of reactions that accompany the formation of a varnish layer. These substances also determine the possible uses and applications of paint coatings. Film-forming substances consist of resins and other accessory substances. In order to invest coatings with specific properties a mix of resins is also often used [1].

The literature distinguishes between the processes of biodeterioration and biodegradation. Biodeterioration involves the destruction of an economically important substance by microorganisms. The term is often used to refer to a deterioration in the properties of substances that are normally immune to microbiological attack, such as metals, plastics, medicines, cosmetics, paints and varnishes, fuels, oils, and other objects. Biodeterioration is thus an adverse phenomenon. In contrast, biodegradation is viewed as beneficial. The biodeterioration of varnish coatings caused by microorganisms is often accompanied by the deterioration of their protective and decorative properties. The proliferation of microorganisms on and inside paint may cause it to peel. The growth of fungi, in turn, may lead to discolorations [2, 3].

The microbiological resistance of resins used in varnishes can be measured on the Wasserbauer scale, which assesses the proliferation of microorganisms on a scale from 0 to 3, where 3 indicates robust growth. Varnishes with the index of 2 or more are described as non-resistant to microorganisms. The values of microbiological resistance for selected synthetic resins are as follows: acrylic resin 0.51; phenol formaldehyde resin 0.73; urea-formaldehyde resin 0.17; polyester resin 1.20; epoxy resin 0.19; polyvinyl resin 0.51 [4].

2. Biodeterioration and biodegradation of paint films

2.1. Biodeterioration

Only water-based paints are susceptible to biodeterioration. The cellulose ethers they contain as thickening agents are often attacked by enzymes produced by fungi and bacteria, which enter the paint at the manufacturing stage together with contaminated ingredients [3]. Orehek et al. studied the biodeterioration of carboxymethyl cellulose by *Bacillus subtilis* subsp. *subtilis* NCIB 3610. The bacteria turned the original pseudo-plastic non-Newtonian fluid that contained carboxymethyl cellulose into a Newtonian fluid. In addition, the fluid's viscosity dropped from 10 to 1.4 mPas and the concentration of reducing sugars increased eighteen-fold. *Bacillus subtilis* subsp. *subtilis* NCIB 3610 was also shown to synthesize cellulases that effectively break down chemical bonds in carboxymethyl cellulose; however, it is not able to reutilize the end products of hydrolysis [5].

Obidi et al. [2009] studied the microbiological contamination of painting products. Fresh samples were taken to isolate the following bacterial strains: *Bacillus brevis*, *Bacillus laterosporus*,

Bacillus polymyxa, *Lactobacillus gasseri*, *Lactobacillus brevis*, *Proteus mirabilis*, *Escherichia coli* and fungi *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium citrinum*. Monitored over a period of 10 months, the population of isolated microorganisms increased. For bacteria, the population grew from $1.6 \cdot 10^1$ cfu·cm⁻³ in a fresh sample to $4.7 \cdot 10^5$ cfu·cm⁻³ 10 months later. The corresponding figures for fungi were $1.0 \cdot 10^1$ and $5.5 \cdot 10^3$ cfu·cm⁻³, respectively [6]. In addition, the researchers took samples of water-based paints to isolate a strain of *Pseudomonas aeruginosa* [7].

Shirakawa et al. (2011) studied microorganisms living in four different acrylic paints exposed to atmospheric conditions for seven years in Sao Paulo and the coastal town of Ubatuba in Brazil. They identified bacteria from the cyanobacteria group. The main strains present on the surface of all four types of paint belonged to *Gloeocapsa* and *Scytonema*. In addition, the following genera were detected: *Synechocystis*, *Synechococcus*, *Xenococcus*, *Chroococcidiopsis*, *Myxosarcina*, *Oscillatoria*, *Leptolyngbya*, *Chlorella*, *Stichococcus*, *Coccoid chlorophyte*, *Klebsormidium*. The researchers also observed certain differences depending on the location from which the samples were obtained. Paint degradation related to the presence of biofilm was shown to be very low, but progressed faster in Ubatuba [8]. Other researchers also isolated cyanobacteria from painted buildings in Latin America [9-11]. *Stenotrophomonas maltophilia* were also detected in water-based acrylic paints [12].

De Souza and Gaylarde (2002) isolated microorganisms from varnished wood panels exposed to atmospheric conditions for two years in South Brazil. The following bacteria were isolated from the samples: *Corynebacterium* sp., *Bacillus* sp., as well as bacteria from the *Enterobacteriaceae* family. Yeasts were also detected: *Rhodotorula lactosa*, *Exophiala jeanselmei*, as well as fungi: *Aureobasidium* sp., *Penicillium* sp., *Paecilomyces* sp. [13].

Pangallo et al. (2015) investigated the biodeterioration of epoxy resins. The most frequently isolated bacteria present on surfaces coated with epoxy resins belonged to the Firmicutes phylum, especially the genus of *Bacillus*. γ -Proteobacteria, and particularly *Pseudomonas*, were also frequently detected [14]. Similar results were obtained by Cappitelli et al. [15], who studied surfaces coated with epoxy resins and isolated Gram-positive bacteria of the *Bacillus*, *Brevibacillus*, *Micrococcus*, *Staphylococcus* and *Kocuria* genus, as well as Gram-negative bacteria from the genus of *Pseudomonas*, *Agrobacterium* and *Ochrobacter* [15]. In addition, researchers [14] identified several species of black yeast: *Friedmanniomyces endolithicus*, *Pseudotaeniolina globosa*, *Phaeococcomyces catenatus* and *Catenulostroma germanicum*.

The authors of paper [16] listed the following species of microorganisms: *Aspergillus versicolor*, *Aspergillus niger*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, *Engyodontium album*, *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, *Trichoderma longibrachiatum*, *Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, *Bacillus subtilis*, *Bacillus licheniformis* and *Rhodococcus fascians*, all isolated from paints [16].

Bacteria present in paint on stone walls were studied by Gurtner et al. [17]. The researchers analysed mural paintings in the chapel of an Austrian castle and a German church. The following bacteria were isolated: bacteria from the phylum of Actinobacteria, such as *Arthrobacter* sp., *Actinobispora* sp., *Amycolata* sp., *Asiosporangium* sp., *Frankia* sp., *Geodermatophilus* sp., *Nocardioidea* sp., *Promicromonospora* sp., *Pseudonocardia* sp., *Rubrobacter* sp., *Streptomonospora* sp., *Saccharopolyspora* sp., *Sphaerobacter* sp. and *Thermocrisum* sp., bacteria from the Proteobacteria

phylum, such as *Aquaspirillum* sp., *Chromohalobacter* sp., *Deleya* sp., *Erythrobacter* sp., *Halomonas* sp., *Porphyrobacter* sp., *Pseudomonas* sp., *Rhizobium* sp., *Salmonella* sp. In addition, the researchers isolated bacteria from the genera of *Bacillus*, *Paenibacillus*, *Micrococcus*, *Staphylococcus*, *Methylobacterium*, and *Halomonas* [17]. Similar results were obtained by the authors of articles [18, 19], who studied mural paintings in Spain and Italy. Apart from the phyla mentioned above, researchers who wrote papers [20, 21] also isolated *Cyanobacteria* (*Chlorogloea microcystoides*, *Chroococcus lithophilus*, *Gloeocapsa* spp., *Gloeotheca rupestris*, *Pseudocapsa dubia*). Bacteria from the *Actinobacteria* phylum of the *Pseudonocardia*, *Streptomyces*, *Nocardia*, *Rhodococcus*, *Nocardioidea*, *Amycolatopsis*, *Saccharothrix*, *Brevibacterium*, *Microbacterium* genus and *Bacillus*, *B. megaterium* were also isolated in Paleolithic cave paintings in southern Spain [22–24].

Apart from bacteria, wall paintings also contained fungi from the genus of *Cladosporium*, *Penicillium*, *Nectria* and yeast from the genus of *Rhodotorula* [25].

Okunye et al. [26] studied species of fungi capable of causing the degradation of paint. Thirty six samples obtained from various locations in Southeastern Nigeria helped isolate *Rhizopus* and *Aspergillus* spp., which were the most frequently present. Other genera of fungi as: *Absidia*, *Monilia*, *Alternaria*, *Fusarium* and *Penicillium* were also detected. [26]. *Aspergillus*, *Penicillium*, *Botrytis*, *Acrodictys*, *Mucor*, *Absidia*, *Alternaria*, *Cladosporium*, *Rhizopus*, *Cephalosporium*, *Fusarium*, *Helminthosporium*, *Trichotermia*, *Acremonium*, *Pullularia*, *Monilia*, *Epicoccum* and *Udeniomyces* were likewise isolated genera from the painted surfaces investigated in studies [27–29]. Likewise, species of fungi *Aspergillus niger* and *Cladosporium* sp. were isolated from paint coatings [30, 31]. Fungi such as *Aureobasidium* sp. and *Cladosporium* sp. [9, 11, 14, 15, 32–36] were also frequently found. In addition, Lugauskas et al. (2003) identified fungi from the genera of *Aureobasidium pullulans*, *Aspergillus niger*, *Aspergillus versicolor*, *Cladosporium cladosporioides*, *Paecilomyces sulphurellus*, *Trichoderma viride*, *Ulocladium atrum* as capable of degrading polymer materials [37].

The following fungi species: *Acremonium charticola*, *A. strictum*, *A. kiliense*, *Acremonium* spp., *Aspergillus sydowii*, *Aureobasidium pullulans*, *Beauveria* sp., *Cladosporium* sp., *Cladosporium sphaerospermum*, *Chrysosporium* sp., *Engyodontium album*, *Mycelia sterilia*, *Scopulariopsis brevicaulus*, *Verticillium lecanii*, *Verticillium suchlasporium*, *Verticillium* sp. were isolated from walls painted in antiquity [38]. The same species were isolated and described in study [39], which also isolated species from the genera of *Arthrobacter* and *Bacillus* [39]. Researchers studying similar subjects isolated the following genera of fungi and bacteria: *Pseudomonas alcaligenes*, *Nocardia asteroides*, *Arthrobacter* spp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptococcus* spp., *Pseudomonas fluorescens*, *Micrococcus roseus*, *Bacillus pumilus*, *Streptomyces* spp., *Micrococcus luteus*, *Alternaria alternata*, *Aspergillus versicolor*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Aspergillus terreus*, *Aspergillus oryzae*, *Aureobasidium* spp., *Penicillium* spp. and *Penicillium stoloniferum* [40].

Studies carried out by Romero-Noguera et al [41, 42] looked into the biodeterioration of turpentine varnishes. Based on their findings, researchers identified the following microorganisms capable of degrading Venetian turpentine coatings: *Chrysonilia sitophila*, *Streptomyces celluloflavus*, *Bacillus amyloliquefaciens*, and *Arthrobacter oxydans* [41]. Moreover they identified microorganisms capable of degrading Sandarac coatings (*Chrysonilia sitophila*, *Penicillium chrysogenum*, *Rhizopus oryzae*, *Mucor indicus*) and Manila Copal coatings

(*Aspergillus niger*, *Aureobasidium pullulans*, *Chrysonilia sitophila*, *Penicillium chrysogenum*, *Pleospora herbarum*, *Rhizopus oryzae*) [42].

Polish studies also looked into the presence of microorganisms in paint coatings. Scientists isolated fungi present in paint inside residential buildings and public utility buildings. The following genera of fungi were isolated in residential buildings: *Acremonium stictum*, *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium cyclopium*, *Penicillium janthinellum*, *Penicillium thomii*, *Penicillium viridicatum*, *Rhizopus nigricans*, *Trichoderma viride*. Researchers also studied fungi in the Registry Office, an office building, a university, a polytechnic, a primary school, a hospital, and a hotel in the Silesian Voivodship. 20 species of fungi were isolated, including: *Acremonium stictum*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus ustus*, *Aspergillus versicolor*, *Cladosporium cladosporides*, *Chateomium globosum*, *Gliocladium catenulatum*, *Gliocladium roseum*, *Memnoniella echinata*, *Paecilomyces variotii*, *Penicillium albidum*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium rugulosum*, *Penicillium terrestre*, *Penicillium variabile*, *Scopulariopsis brevicaulis*, *Stachybotrys chartarum*, *Trichoderma viride* [4].

Research was also conducted on fungi present in paint coatings in food industry plants, breweries, dairies, and fruit preserve factories.

Alternaria sp., *Cladosporium herbarum*, *Leptographium* sp., *Phoma violacea*, *Trichothecium* sp., *Trichoderma koningii* were isolated in food industry plants, *Aspergillus flavus*, *Botrytis cinerea*, *Acremonium* sp., *Chalaropsis* sp. in breweries, and *Geotrichum* sp. and *Fusarium* sp. in dairies. Paint in fruit preserve factories was shown to contain the following species of fungi: *Aspergillus nidulans*, *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus versicolor*, *Penicillium glabrum*, *Penicillium italicum*, *Penicillium spinulosum*, *Botrytis cinerea*, *Aureobasidium pullulans*, *Cladosporium elatum*, *Cladosporium herbarum* and *Alternaria tenuis*, as well as various species of *Fusarium* sp. In chocolate-processing factories, the following species of fungi were detected: *Mucor hiemalis*, *M. racemosus*, *M. spinosus*, *Rhizopus nigricans*, *Aspergillus ochraceus*, *Aspergillus versicolor*, *Penicillium cyclopium*, *Penicillium expansum*, *Penicillium globrum* and *Penicillium brevicompactum*. The following fungi were isolated in a juice factory: *Cladosporium sphaerospermum*, *Chaetophoma* sp. and *Acremonium strictum* [4].

Table 1. Microorganisms isolated from paint exposed to atmospheric conditions [2, 43]

Microorganism	Oil paint	Emulsion paint	Microorganism	Oil paint	Emulsion paint
Fungi			Bacteria		
<i>Alternaria dianthicola</i>	+	+	<i>Alcaligenes recti</i>	+	
<i>Aureobasidium pullulans</i>	+	+	<i>Alcaligenes</i> sp.	+	
<i>Aspergillus flavus</i>	+	+	<i>Bacillus cereus</i>	+	
<i>Botryodiplodia malorum</i>	+		<i>Bacillus mycoides</i>	+	+
<i>Botrytis cinerea</i>	+		<i>Bacillus sphericus</i>	+	

Microorganism	Oil paint	Emulsion paint	Microorganism	Oil paint	Emulsion paint
<i>Cephalosporium carpogenum</i>	+	+	<i>Bacillus</i> sp.	+	
<i>Cladosporium sphaerospermum</i>	+	+	<i>Flavobacterium invisibile</i>	+	
<i>Cladosporium</i> sp.	+	+	<i>Flavobacterium marinum</i>	+	+
<i>Fusarium flocciferum</i>	+		<i>Micrococcus albus</i>	+	
<i>Helminthosporium spiciferum</i>	+	+	<i>Micrococcus candidus</i>	+	
<i>Paecilomyces variotii</i>	+		<i>Micrococcus ureae</i>	+	
<i>Penicillium oxalicum</i>	+	+	<i>Sarcina flava</i>	+	+
<i>Phoma glomerata</i>	+	+	<i>Pseudomonas cepacia</i>	+	
<i>Stemphylium consortiale</i>	+	+	<i>Pseudomonas maltophilia</i>	+	
<i>Torula nigra</i>		+			

2.2. Biodegradation

Giacomucci et al. [44] studied the biodegradation of nitrocellulose paint by the *Desulfovibrio desulfuricans* bacterium. The study measured changes in the concentration of nitrates, nitrites, and the concentration of ammonia in a culture medium. In order to determine the chemical changes in the paint, infrared spectroscopy was performed, as well as colorimetric assays designed to identify the changes in colour caused by microorganisms. Microscopic measurements showed that bacteria were able to adhere to the surface of the paint and alter its adhesive properties. The study determined that *Desulfovibrio desulfuricans* was capable of degrading nitrocellulose used as a binder in the products mentioned [44].

Ishfaq et al. [45], who authored a study, researched the biodegradation of paint coatings by fungi and bacteria. The researchers detected fungi such as *Aspergillus* sp., *Phanerochaete chrysosporium* sp. and *Rhizopus* sp. on an agar culture medium that contained paint. The study also used bacteria from the *Bacillus* sp. and *Pseudomonas* sp. group. Results showed that, under stress conditions, the selected species of fungi and bacteria were capable of adhering to the surface of the paint and causing its degradation. It was also observed that the selected fungi showed a greater ability to degrade paint coatings than bacteria [45].

Study [46] identified bacteria present in waste produced by the varnish industry. The following bacteria were identified as able to cause the biodegradation of the sludge: *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus megaterium*, and *Pseudomonas fluorescens*.

In addition, the authors of the study determined bacterial generation time under growth conditions. These were, respectively, 44.67 minutes for *Pseudomonas fluorescens*; 45.04 minutes for *Bacillus subtilis*; 35 minutes for *Bacillus licheniformis*, 18 minutes for *Bacillus cereus*, 19 minutes for *Bacillus megaterium*, and 53 minutes for mixed bacterial cultures.

2.3. Biodegradation of polyurethane coatings

The study conducted by Wojturska [47] concerned the susceptibility of polyurethane coatings to enzymatic degradation. The coatings were obtained as the result of polyaddition of polycarbonate diol or polyesterether polyols of different degrees of branching, 1,4-butanediol to 2,2,4-trimethyl-1,6-hexamethylene diisocyanate (TMPD). Polyurethane coatings were subjected to enzymatic degradation by lipases isolated from *Candida antarctica* type A (Novozym 735), type B (CALB L), *Aspergillus* sp. (Novozym 51032), lipase isolated from *Rhizomucor miehei* (Pallatase 20000 L) or lipase from *Thermomyces lanuginosus* (Lipozyme TL 100 L). Samples were subjected to enzymatic activity for 6 weeks, each at its optimum temperature, i.e. $T = 50^{\circ}\text{C}$ for Novozym CALB L and 36°C for all the rest. Studies showed that lipase type B isolated from *Candida antarctica* (Novozym CALB L) has the greatest affinity to the synthesized PUR coatings; the smallest change was caused by the lipase isolated from *Thermomyces lanuginosus* (Lipozyme TL 100 L).

The authors of [48] studied the growth of bacteria in a culture medium, using polyurethane coatings as the sole source of carbon. They used the following bacteria: *Acinetobacter calcoaceticus*, *Pseudomonas* sp. and *Arthrobacter globiformis*. All the microorganisms in the study showed an ability to grow using polyurethane coatings as the only source of carbon [48]. In addition, the following bacteria were also shown to degrade polyurethane coatings: *Pseudomonas chlororaphis*, *Pseudomonas putida*, *Pseudomonas cepacia*, *Pseudomonas aeruginosa*, *Corynebacterium* sp., *Acinetobacter calcoaceticus*, *Arthrobacter globiformis* [49–51].

3. Biodegradation of varnish removal agents

Studies were also conducted to investigate the biodegradation of waste produced in the process of varnish removal. Arquiga et al. [52] studied the decomposition of paint stripping wastewater, which contained methylene chloride at the concentration of $5000\text{ mg}\cdot\text{dm}^{-3}$, phenol at the concentration of $1800\text{ mg}/\text{dm}^3$, and other organic ingredients, such as paraffin waxes, cellulose derivatives, petroleum sulfonates, and naphthalene at the total concentration of $2200\text{ mg}\cdot\text{dm}^{-3}$. Active sludge was used. The study identified bacteria present in active sludge and their ability to grow in the presence of phenol and varnish stripping wastewater. In addition, researchers investigated whether these substances can serve as a source of carbon for the bacteria [52]. They isolated such bacteria as *Enterobacter* sp., *Klebsiella* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Bacillus* sp., *Acinetobacter* sp.. The bacteria most frequently isolated from the wastewater were Gram-negative, with the majority belonging to the *Pseudomonas* genus. Gram-positive bacteria, represented by the genus *Bacillus*, were less

numerous. Studies showed that most of the isolated bacteria can biodegrade phenol and paint stripping wastewater. However, the most important role in the process is played by bacteria from the *Pseudomonas* and *Bacillus* genus [52].

Vanderberg-Twary et al. [53] researched the biodegradation of solvents used in varnish removal agents, focusing on the biodegradation of typical agents of this kind. They prepared two kinds of agents: one contained toluene, acetone, and dichloromethane, the other – dichloromethane, isopropyl alcohol, and methanol. The study used two types of bacteria: *Hyphomicrobium* sp. and *Rhodococcus rhodochrous* sp. [53].

The study showed that the selected strains of bacteria are able to degrade solvents present in paint stripping agents [53].

In patent US8202424, Almadidy et al. [54] described the biodegradation process of paint stripping waste containing high levels of organic substances. Their study used two kinds of bacteria: *Pseudomonas fluorescens* and *Bacillus subtilis* and one type of fungus, *Cunningham elegans*. They tested the changes in the COD of waste samples subjected to microbial activity. The study showed that it is possible to achieve as much as 47% decrease in COD with the use of the selected bacterial strains [54].

4. Conclusions

As evidenced by the above literature survey, there is a great diversity of microorganisms capable of growing on and in paint coatings. The most frequently observed bacteria belong to the genus *Bacillus* and *Pseudomonas*. The most frequently detected of fungi were *Penicillium* sp., *Aspergillus* sp. and *Cladosporium* sp. Studies on the biodegradation of varnishes and their stripping agents show that some of these microorganisms can be used for the deliberate degradation of old varnish coatings.

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