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Original Research Article

EXAMINATION OF DETERIOGENIC BIOFILMS ON BUILDING FACADES WITH SCANNING ELECTRON MICROSCOPY

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Abstract

Destruction of facades is a complex process in which technical material changes its properties, and which is caused by depositing biological agents. The examination of biofilms from building facades is difficult because sampling for tests may result in the damage to the structure of the facade's material. Also biological analysis of the material obtained from a biofilm is arduous. Some species of microorganisms are impossible to be isolated and their pure cultures cannot be cultivated in laboratory conditions. It is multispecies cultures that most frequently develop on the surfaces of the facade's technical material. Clustered in a group, they cooperate with each other and reveal different features than single cells. It is essential to identify organisms present in the biofilms, since they may initiate deterioration processes. The aim of the research was the observation of the biofilm, collected from two facades, in a micrometer scale with the use of a scanning electron microscope.

Keywords: biofilm, scanning electron microscope (SEM), facade, biocorrosion, biodeterioration

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1. DETERIORATION OF BUILDING FACADES DUE TO BIOLOGICAL AGENTS

The term - deterioration involves a wide spectrum of phenomena causing weakening, loss of quality and destruction. All technical materials used on facades, are exposed to depositing of biological agents, and consequently susceptible to the development of biological corrosion. The technical state of a facade, its material properties and the environmental conditions are of great importance in the progression of the destruction [3, 5, 7, 8, 12, 16]. Biocorrosion refers to facades of both old and new buildings, facades with insulation and the ones with thin-layer plasters. This phenomenon leads to a weakening of the functional characteristics of the technical material such as: cracks, detachment, crushing and worsening of the visual aesthetics (Fig. 1.).

Fig. 1. Detail of the facade of the building with clearly visible biocorrosion

The most important factor in initiating the formation and development of biocorrosion is moisture. In its presence, it is possible for microorganisms to develop and multiplicate. Biodegradation is a consequence of acidic products secreted by microorganisms in respiration and photosynthesis processes. Biological factors penetrate, with moisture, the facade material through defects in its structure causing its stresses and, consequently, physical damage [7, 8, 16]. Moisture content is influenced by: adsorption and absorption, surface tension, condensation on the surface of the material, capillary transport,

diffusion, water vapour condensation inside partitions and their efficiency of drying up [8, 13]. Essential factors contributing to the development of biological corrosion are as follows: climatic conditions, especially temperature and humidity, wind speed and direction, sunshine and vegetation vicinity increasing biological pollution emissions [5, 7, 16]. Microorganisms which colonize a facade feed on nutrients from the dirt on its surface, and then if possible, feed on the compounds contained in the material it is made of, causing its deterioration.

2. SKANNING ELECTRON MICROSCOPE

The use of a Scanning Electron Microscope (SEM) allows observations in a nano-sized magnification. The SEM uses a focused beam of electrons the wavelength of which is much smaller than that of visible-light used in the light microscope.

Fig. 2 . Scanning electron microscope JEOL JSM SEM-7600F at the Faculty of Mechanical Engineering, University of Zielona Góra

Scanning electron microscopes are widely used due to their high magnification power, high resolution and due to the depth of field allowing three-dimensional visualization of a sample. They are also valued for the possibility of carrying out non-destructive testing of samples which, in the case of solid materials, are easy to prepare [15]. Pictures taken with SEM highlight the topography and morphology of the sample. They allow recording the shape, size and distribution of the particles that make up the examined surface,

and identification of any defects or chemical composition. The essence of the scanning electron microscope is the use of the electron optical microscope for scanning of the tested sample with an electron beam formed by a system of magnetic lenses. The basis of the electromagnetic lens design is the solenoid which produces an electromagnetic field after electric current has flown through it. Electrons are very sensitive to magnetic fields, and thus can be controlled by changing the current flowing through the lens. An electron beam of high energy is concentrated in the area of the influence of electromagnetic lenses and reaches the sample, thereby generating signals on its surface. This results in the transmission of, inter alia, backscattered electrons, Auger electrons, secondary electrons, characteristic X-rays, cathodoluminescence and other photons of different energies. They are received by a detector, consisting of a photomultiplier and a scintillator. The main task of the scintillator is to transform signals emitted from the sample into electrical pulses which are next amplified by the photomultiplier. The signal from the detector reaches the electronic circuits that control the image on the computer screen [4, 9, 10].
The main limitation of the use of the SEM is a high vacuum generated in the electron-optical column and in the chamber in which test samples are placed. This excludes the imaging of the preparations with a high degree of hydration. At the same time, it creates the need to ensure electrical conductivity of the sample to prevent the accumulation of electrical charge on its surface. In the case of hydrated biological materials, the sample preparation procedure should follow some measures which need to be purposefully undertaken to dehydrate, fix and to protect the samples against the destructive influence of the electron beam.

3. PREPARATION OF BIOLOGICAL SAMPLES FOR SEM OBSERVATION

The selection of the method of biological samples preparation for SEM observation is determined by a number of factors e.g.: the type and size of the observed sample, its degree of hydration, or the availability of materials and equipment used in the sample dehydration process.
Subsequent stages of the preparation of biological samples for scanning electron microscope observations are as follows [6]:

- sample pretreatment: cutting, cleaning the surface,
- fixation,
- rinsing,
- dehydration,
- drying

- sputtering a layer of conductive material.

The aim of the fixation (or stabilization) stage is to strengthen the structure of the sample, while maintaining its chemical identity and to increase its resistance to radiation. This is most frequently performed with the use of a solution of glutaraldehyde in a phosphate buffer. The fixation conditions should be separately adapted for each case due to differences in the sample sizes, water content, permeability, physiological condition, osmolarity, or pH. Fixation time depends mainly on the thickness and composition of the sample. After the fixation step, the sample is rinsed to remove excess of fixative. The process is performed in a buffer which has previously been used as a solvent for the fixative. The gradual dehydration of the sample in a series of dilution of ethanol or acetone solutions minimizes the impact of the surface tension. Next, the sample is dried and then a layer of conductive material is sputtered onto it [1, 6].

4. METHODS AND MATERIALS

4.1. Sampling location

Material for SEM observation has been derived from visible biological films on northern façades, in places adjacent to the ground, of the following buildings:

1. from an approx. 30-year-old, non-insulated building covered with a rough cement-lime plaster (Fig. 3a.)
2. from an approx. 14-year-old building insulated with styrofoam, covered with a porous thin-layer plaster (Fig. 3b.).

Fig. 3. Parts of the facade adjacent to the ground which the biological film for SEM observation was derived from:, a - 30-year-old, non-insulated facade, very porous, b- 14-year-old facade insulated with styrofoam, covered with a thin-layer plaster

The biofilm was scraped from the facade with the use of sterile scalpels into sterile tubes, and then was subjected to further processing in a laboratory.

4.2. Sample preparation

Biological material, collected from the facade was fixed with glutaraldehyde in phosphate buffer (for 1 h), then samples were washed in phosphate buffer three times for 15 minutes. The dehydration process was repeated in increasing concentrations of acetone (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 2x100%). These operations were performed at room temperature. The samples were dried in open air, and then sprayed with a layer of chromium with the use of a modular vacuum sputtering system with a turbomolecular pump Q150T S. The samples were observed in scanning electron microscope JEOL JSM-7600F.

5. RESULTS AND DISCUSSION

In total, 102 pictures of samples were taken in the micrometer scale, including the ones presented below, (Fig. 4-6). All images revealed the presence of biological agents. The identification of individual species was difficult because majority of them had integrated with the entire structure. The colour of the biofilm on facade "a" in the above picture indicates superiority of aerofitycznych algae, which is confirmed by the SEM images of the sample (Fig. 4 A). Also, numerous colonies of spherical bacteria are visible in the images (Fig. 4 B) as well as growing lichens (Fig. 5 A).

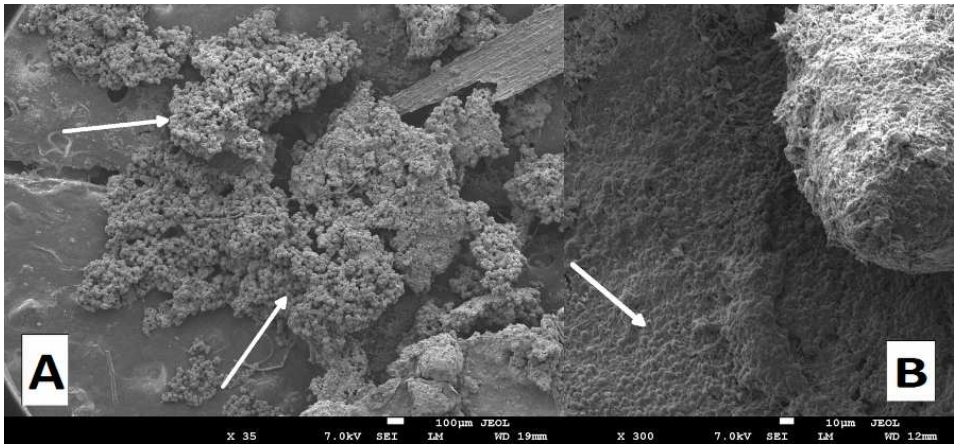

Fig. 4. SEM image of a sample obtained from the biofilm on facade "a", in Part A, the arrows indicate agglomeration of unicellular cyanobacteria or algae; in part B, the arrows indicate numerous spherical bacteria, blended with the structure of the biofilm

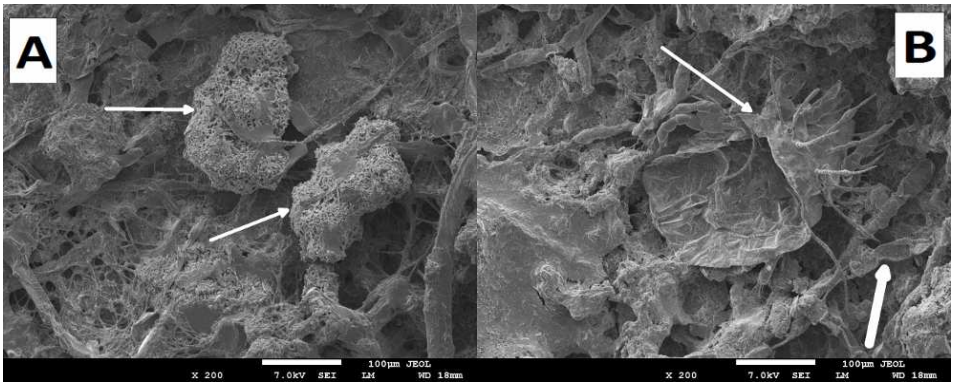

Fig. 5. SEM images of biofilm samples; in part A - derived from facade "a" (arrows indicate lichens) and in part B - from facade "b" (the thinner arrow indicates dead mites and the thicker one - fungal hyphae)

Fungal hyphae are visible in the dark-grey biofilm obtained from facade "b" (Fig. 5 B). The image also shows a representative of microfauna (a dead mite) (Fig. 5 B).

Images in micrometer magnification revealed considerable salting on the facades, which evidences the deterioration of the technical material (Fig. 6 A). They illustrate a complex, multi-layered structure of the biofilm on both facades, which is stabilised by polymeric substances produced by microorganisms, so-called EPS (extracellular Polymeric substances) (Fig. 6 B).

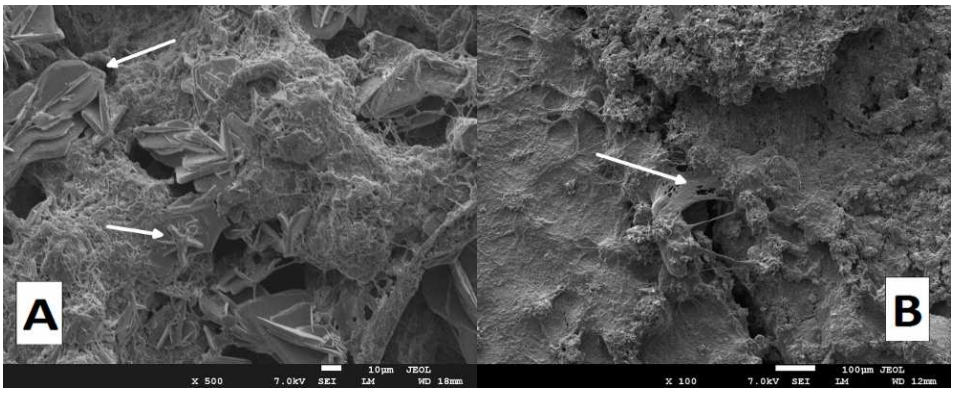

Fig. 6. A SEM image of the biofilm from the facade: part A - the arrows indicate exemplary salting, part B - the arrow indicates an exemplary place with a polymeric substance which stabilizes its structure

Technical materials used on facades are most frequently colonized by bacteria, cyanobacteria, actinomycetes, fungi, algae, mosses, lichens and protozoa and rotifers [2, 3, 7, 11, 12, 14, 16]. Groups of microorganisms contribute to the

deterioration of facades in many different ways. Frequently the reasons for the destruction of facade are acids produced by biological organisms, which form the biofilm, in the process of respiration and photosynthesis as a by-product. This form of biodegradation is characteristic for such bacteria as e.g. sulphur bacteria which, oxidise sulphur-containing nutrients to sulphuric acid which, in turn, decomposes stone, or nitrifying bacteria which produces corrosive nitric acid [16]. Actinomycetes decompose organic compounds with the use of extracellular enzymes. Slow growth of actinomycetes often contributes to the fact that facade materials are attacked with a delay (in further stages), leading to more serious damages than those which are caused by moulds. These, in turn, secrete acid metabolic products (eg. oxalic acid, citric acid) to a substrate, causing changes in the structure of the material, for example crushing of bricks and mortar. The growth of bacteria and fungi enhances the presence of cyanobacteria which accumulate adsorbed inorganic compounds, thus facilitating the adhesion of solid particles from the air. Bacteria, fungi, cyanobacteria and algae contribute to the discolouration on the walls. The biomass of algae, cyanobacteria and fungi facilitates the growth of moss and lichen, which can exacerbate the damage to the surface as a result of interference of thallus in the structure of the material. In the metabolic process, lichens produce biogenic organic acids and other chelating agents which results in the occurrence of voids, cracks or cavities [8, 12, 16].

6. CONCLUSIONS

The SEM images revealed the presence of biological agents in the facade biofilm. The consequence of their presence is a significant deterioration in aesthetics (coloured coating) and the initiation of degrading processes in the technical material (salting presented in pictures). A complex structure of the biofilm was presented. The isolation of particular organisms was difficult, though. Scanning electron microscopy is a technique that is not easy. There is a high risk of error. Sample preparation of the tested material involves a number of steps. A single fault at one of the stages influences, and may even prevent, obtaining the correct images. Furthermore, the resulting image, in a very high magnification, requires a lot of experience at the interpretation stage, since it differs a lot from the images acquired for example with an optical microscope. Despite the risk, the SEM technique has been more and more frequently used for imaging materials surfaces of for many years. Images obtained with this technique are not only fascinating, but provide valuable information on the structure and biodiversity of biological films on a facade and the degree of its biodeterioration.

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BADANIE DETERIOGENNYCH NALOTÓW BIOLOGICZNYCH NA ELEWACJACH BUDYNKÓW METODĄ ELEKTRONOWEJ MIKROSKOPII SKANINGOWEJ

S t r e s z c z e n i e

Niszczenie elewacji to proces złożony, w którym należy brać pod uwagę zmiany właściwości materiału technicznego, wywołane przez osadzające się czynniki biologiczne. Badania nalotu biologicznego są trudne nie tylko ze względu na pobór materiału, który wiąże się z możliwością uszkodzenia struktury elewacji, ale również pod względem analizy biologicznej. Niektóre gatunki mikroorganizmów nie udaje się wyizolować i wyhodować w czystych kulturach w warunkach laboratoryjnych. Na powierzchniach materiału technicznego tworzą skupiska, najczęściej wielogatunkowe. W grupie współpracują i wykazują odmienne cechy, niż komórki żyjące w pojedynczej, w wolnej postaci. Kluczowe znaczenie ma oznaczenie organizmów, obecnych w nalotach, które mogą inicjować deterioracje. Celem badań była obserwacja zebranego nalotu biologicznego z dwóch elewacji w skali mikrometrycznej przez zastosowanie mikroskopu skaningowego.

Słowa kluczowe: nalot biologiczny, elektronowa mikroskopia skaningowa (SEM), elewacje, biokorozja, biodeterioracja

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