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EXISTENCE OF MOULD SPORES IN BIOFILM ON THE BUILDING FASADE OF THE INSTITUTE OF ENVIRONMENTAL ENGINEERING, UNIVERSITY OF ZIELONA GÓRA

A b s t r a c t

The aim of this study was to estimate the qualitative diversity of mould spores on the biofilm that has grown on the western facade of the building of the Institute of Environmental Engineering, University of Zielona Góra. Scientific literature describes instances where mould spores lead and contribute to the process of biodegradation of technical materials. A part of the material was subjected to analysis performed with the use of an scanning electron microscope. The material from the remaining part was inoculated directly into Petri plates onto medium to grow fungal cultures. Identified spores belonging to 9 species of moulds: Aureobasidium pullulans (De Bary) Amund, Cladosporium cladosporioides (Fres.)de Vries, Cladosporium herbarum (Pers.) Link, Cladosporium macrocarpum Preuss, Epicoccum nigrum Link, Penicillum brevicompactum Dierckx, Penicillium chrysogenum Thom, Scopulariopsis brevicaulis (Sacc.) Bain, Ulocladium chartarum (Preuss) Simmons.

Key words: moulds, fungi, biocorrosion, biodeterioration, biofilm, exterior facades

INTRODUCTION

The prevalence of moulds is conditioned by the production of a considerable number of spores and insignificant food requirements. Due to these facts, they may grow on virtually any surface. They occur at both low and high temperatures in the presence of insignificant, or even periodic moisture. They grow particularly well on slightly acidic substrates. They may inhabit external facades due to water retention and organic material (dust) accumulated in any cavities or irregularities in facades, which foster their growth and proliferation [Warscheid and Braams 2000, Piontek 2004, Karyś 2010, Piontek and Bednar 2011, Piontek

and Lechów 2013, Piontek et al. 2013]. Finishing materials used in construction support the development and prevalence of fungi in the environment, since their ingredients often include all the mineral and organic substances required for fungi growth. These, in turn, enter into chemical reactions with the substrate and cause irreversible changes in its structure, enabling technical material to be deeply penetrated. In metabolic processes, moulds produce alkaline or aggressive acids (sulfuric, nitric and other) and surface-active substances [Piontek 2004]. They are chemoorganotrophs. They use a variety of carbon sources: amino acids, organic acids, simple and complex carbohydrates and their derivatives, alcohols and other [Piontek 2004, Samson et al. 2004, Piontek and Bednar 2011]. For growth, in addition to carbon, they need mainly hydrogen, oxygen, nitrogen, phosphorus and sulphur. They most frequently occur in symbiosis with algae forming lichens on inorganic substrates [Karyś 2010]. In the process of respiration, they produce carbon dioxide, which in combination with water, causes the formation of acidic calcium carbonate ($CaCO_3$). The produced organic acids contribute to the mortar and bricks crumbling, causing the deterioration of many materials (marble, limestone, basalt and granite). Despite the application of biocides in elevation materials, the ability to inhibit the development of biocorrosion ceases in time. In practice, there is no such material that would have the ability to inhibit the growth of biocorrosion for an unlimited period of time [Wójcik 2008].

The intensity of the deposition of mould spores on facades is conditioned by: the porosity of and moisture content in the technical material, humidity, wind speed and direction, sunlight as well as neighbouring vegetation which increases the emissions of biological pollution. Also, the physical-chemical composition of the finishing surface is important, as well as its pH, its condition and protection against biocorrosion (the use of biocides) [Warscheid and Braams 2000, Karyś 2010, Piontek and Lechów 2013]. The most important factors for growth and mycotoxin production are temperature, water activity (a_w) and oxygen. The opimal temperature is 25-30 °C for most *Penicilla* and 30-40 °C for most *Aspergilli*. If the temperature in the material is not uniform, the water activity can rise locally due to migration moistrure to the cold areas, thus leading to fungal growth. The terms water activity or equilibrium – relative humidity (ERH) is a measure of the unbound water in a material that is availabe for chemical and biological reactions. Water activity is defined as the ratio of water vapor pressure of a substance (p) to the vapor pressure of pure water (p_o) at the same temperature (i.e., $a_w = p/ p_o$). Therefore, ERH can be defined as water activity expressed as a percentage (ERH = a_w x 100). Water activity is not the same as moisture content, although materials that have high moisture are most likely to have greater water activity as compared to dry ones [Clontz 2009]. The moisture requirement for mould growth was studies by Grant et al. He found that the lowest water activity level recorded for growth on malt extra

agar was 0.76 while for building materials such painted woodchip wallpaper it was 0.79. He also observed that increasing the temperature and the amount of nutrients led to reduction in the water activity in building materials should be maintained below 0.80 [Altamirano-Medina et al 2009]. Considering the minimum rate of hygroscopic balance (a_w - min.) expresses the demand of mould moisture needed for germination and growth can be divided into three groups [Samson et al. 2004]:

- colonizers primary able to grow on and in less than 0.8 (and <0.8). These include species of: *Wallemia*, *Penicillium*, *Aspergillus*, and telemorfic form *Eurotium*,
- secondary midwayers colonizers for which the a_w min is in the range 0.8-0.9 (mold fungi of the species: *Cladosporium*, *Phoma*, and *Alternaria ulocladium*),
- colonizers tertiary (hydrophile), and requiring at least 0.9, (and> 0.9). These are the species, as: *Stachybotrys*, *Trichoderma*, *Chaetomium* and *Aureobasidium* [Grant et al 1989, Piontek 2004]. Oxygen is usually necessary for the growth of fungi, but certain species can also grow under anaerobic conditions with the formation of ethanol and organic acids.

The aim of this study was to estimate the qualitative diversity of moulds spores on the biofilm that has grown on the western facade of the building of the Institute of Environmental Engineering, University of Zielona Góra.

METHODS AND MATERIALS

The sampling place

The biofilm that had formed on the north-west elevation of the building of the Institute of Environmental Engineering at the University of Zielona Góra was tested for the presence of mould spores (Phot. 1). A 15-year-old facade is covered with a rough acrylic plaster. It is shaded for the better part of the day. At a distance of approx. 3 m, there are trees which hinder the exposure to the sun while providing an additional source of emission of biological agents on the elevation surface.

Methodology of collection and cultivation of the biological material

The biofilm on the facade was gently scraped with a sterile scalpel in May 2014. A part of the obtained material was collected in a sterile container and sent for the analysis performed with the use of an scanning electron microscope in the Electron Microscopy Laboratory at the Wrocław University of Environmental and Life Sciences. The other part was inoculated directly into Petri

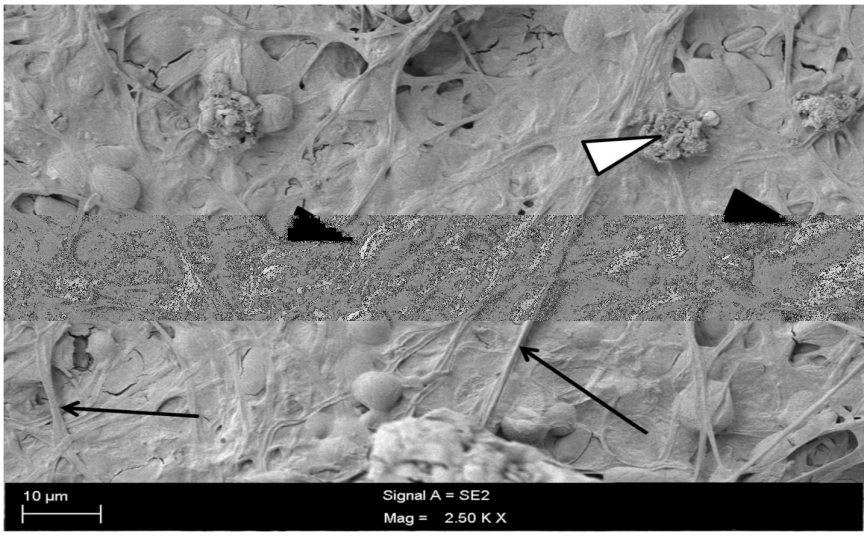
plates onto malt agar (mix malt for brewing 100 ml with 20 g agar and 1000 ml distilled water- sterillize at 121 °C for 15 min) intended for fungal culture. The material was incubated in a growth chamber at room temperature ranging from 18-22 °C while maintaining a circadian rhythm synchronized with the day and night cycle. After 5 days of cultivation on the agar plates, the fungal colonies were observed to have grown. In order to identify the species of the microorganisms, individual colonies were passaged according to their colour and morphology. Individual microorganisms forming a colony were isolated in a sterile room purposefully intended for inoculation of microorganisms on microbiological medium. The average time of culture growth was 7 days. Several colonies required more than one inoculation, in order to obtain a pure culture. The images of moulds spores were photographed with the use of a Nikon light microscope Eclipce 200 with a digital camera.

Phot. 1. View of the north-west facade of the building of the Institute of Environmental Engineering at the University of Zielona Góra, covered with the biofilm [Lechów 2014]
Fot. 1. Widok na zachodnio-północną elewację budynku Instytutu Inżynierii Środowiska Uniwersytetu Zielonogórskiego z nalotem biologicznym [Lechów 2014]

RESULTS

Image analysis of the biological material

With the use of a scanning electron microscope confirmed the presence of mould spores in the biofilm formed on the external elevation of the building (Phot. 2).



Phot. 2. A micrograph of the material collected from the western facade of the building of the Institute of Environmental Engineering. Hyphae (black arrows), spherical formations (black arrowheads) and fragments of plaster (white arrowheads); original magnification 2500x

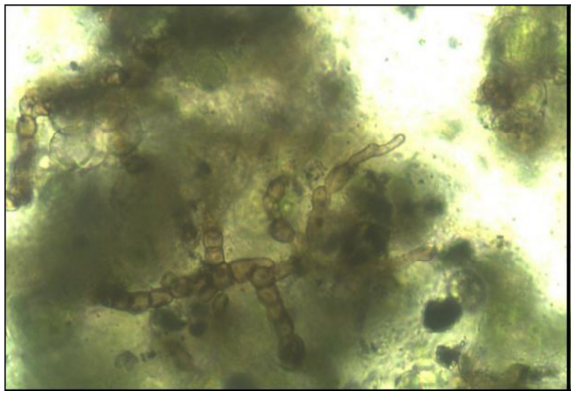
Fot. 2. Mikrografia pobranego materiału z zachodniej elewacji budynku instytutu inżynierii środowiska przedstawiająca strzępki grzyba (czarne strzałki), kuliste twory (czarne groty) oraz fragmenty tynku (białe groty); oryginalne powiększenie 2500x

Identification of fungi

The species of mould were determined macroscopically and microscopically on the basis of morphological and physiological features with the use of the key [Piontek 1999, Samson et al. 2004]. The analysis of the biological material led to the identification 9 species of moulds (Phot. 3-11).

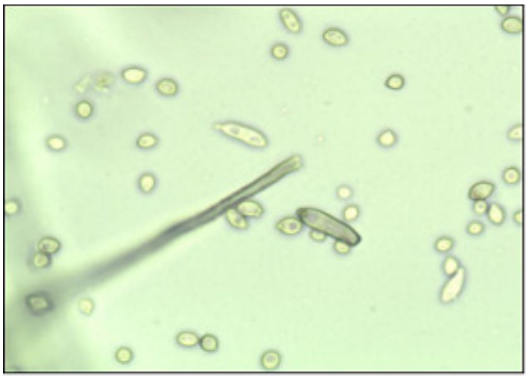
- *Aureobasidium pullulans* (De Bary) Arnaud (Phot. 3) Colonies smooth, soon covered with a slimy mass of spores, yellow, cream, pink, brown or black. Aerial mycelium sometimes formed, scanty, thinly floccose. Hyphae in diam,

hyaline, smooth- and thin-walled. In older cultures dark-brown, thick-walled hyphae may be formed. These thick-walled hyphae may act as chlamydospores or often fall apart into separate cells (arthroconidia). Conidiogenous cells undifferentiated, intercalary or terminal on subhyaline hyphae or arising as short lateral branches. Blastic conidia produced synchronously in dense groups from indistinct scars or denticles. Conidia one-celled, hyaline, smooth-walled. Temperature range for growth 2-35 °C: optimum 25 °C; maximum 35 °C for growth 0.88 [Samson et al. 2004].



Phot. 3. Aureobasidium pullulans (De Bary) Arnaud, a microscope photo 100x
Fot. 3. Aureobasidium pullulans (De Bary) Arnaud, fotografia z mikroskopu 800x

- *Cladosporium cladosporioides* (Fres.) de Vries (Phot. 4) – together with *Cladosporium herbarum*, it is one of the most common fungi of the *Cladosporium*-type growing on dead organic matter. Conidiophres arising laterally or sometimes terminally from the hyphae, without sympodial elongations and swellings, bearing branched conidial chains, pale to mid-olivaceous-brown, smooth-walled or verruculose. Ramoconidia towards the base of chain, more or less cylindrical, brown or greenish-brown, smooth-walled or sometimes minutely verruculose. Conidia in acropetal branched chains, mostly one-celled, ellipsoidal to lemon-shaped, brown or greenish-brown, mostly smooth-walled, sometimes verruculose. Temperature for growth: optimum 20-28 °C; minimum -6 °C to -10 °C. Minimal water activities (a_w) for germination and growth 0.86-0.88 [Samson et al. 2004].



Phot. 4. Cladosporium cladosporioides (Fres.) de Vries a microscope photo 400x
Fot. 4. Cladosporium cladosporioides (Fres.) de Vries, fotografia z mikroskopu 400x

- *Cladosporium herbarum* (Pers.) Link (Phot. 5) A fungus from saprophytic mould. It occurs in all climate zones. It is most frequently represented in air (of approx. 90%) of all spores of moulds. Common species with a world-wide disribution, especially abundant in temperate regions on dead or dying plant substrates and other orgnic matter. Isolated from air, soil, foodstuffs, stored friut, cereal grains (especially wheat), groundnuts, paint, textiles etc. Colonies on MEA olivaceous-green to olivaceous-brown. Reverse greenish-black. Conidia in long, often branched chains, ellipsoidal to cylindrical with rounded ends, rather frequently 2 or more celled. Temperature for growth: optimum 18-28 °C; minimum -6 °C; maximum 28-32 °C. Minimal water activities (a_w) for growth 0.85-0.88 [Samson et al. 2004].

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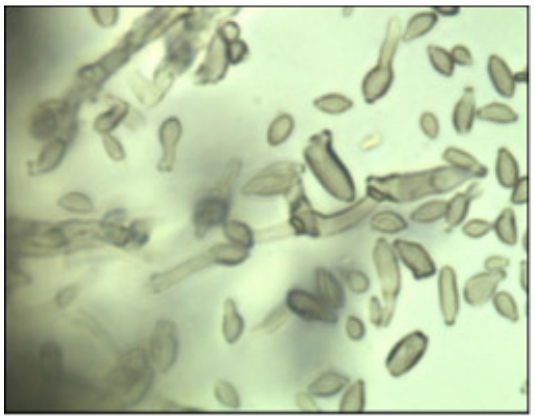
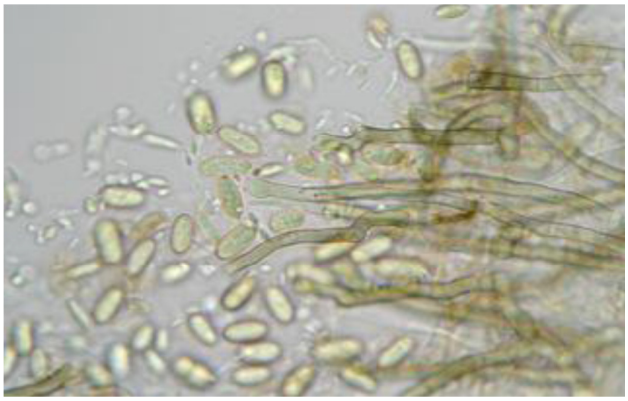


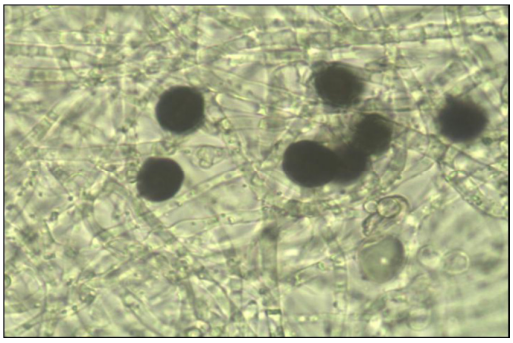
Phot. 5. Cladosporium herbarum (Pers.) Link, a microscope photo 400x
Fot. 5. Cladosporium herbarum (Pers.) Link, fotografia z mikroskopu 400x

- *Cladosporium macrocarpum* Preuss (Phot. 6) Common species with a world-wide disribution. Colonies on MEA often covered with greyish aerial mycelium, olive-green. Reverse greenish-black. Conidiohores arising laterally from the hyphae, often becoming geniculate and nodose by sympodial elongation, terminal and intercalary swellings, pale to mid-brown or olivaceous-brown, smooth or partly verruculose. Conidia usually in rether short chains, 0-3-septate, ellipsoidal, one-celled mostly, pale to mid-brown or olivaceous brown, densely verruculose [Samson et al. 2004].



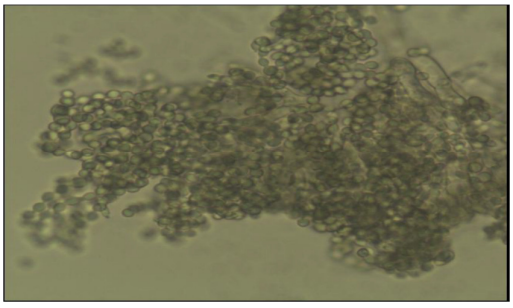
Phot. 6. Cladosporium macrocarpum Preuss, a microscope photo 400x
Fot. 6. Cladosporium macrocarpum Preuss, fotografia z mikroskopu 400x

- *Epicoccum nigrum* Link (Phot. 7) Colonies on MEA lanose to felty, yellow-orange, red or brown, sometimes greenish. Reverse similar, often more intensely coloured. Pulvinate sporodochia appearing in 8-10 days, visible as black spots. Conidiophores in clusters, straight or more or less flexuous, terminating inmore or less isodiametric conidiogenous cells, colourless to pale brown. (blasto)condia formed singly, globose to pyriform with funnel-shapedbase and broad attachment scar, often with pale protuberant basal stalk cell, dark golden brown, verrucose, obscuring the septa which divide the conidia into up to 15 cells. World-wide distribution (food, indoor). Temperature for growth: optimum 23-28 °C; minimum (-3)-4 °C; maximum 45 °C. Minimal water activities (a_w) for growth 0.86-0.90 [Samson et al. 2004].



Phot. 7. Epicoccum nigrum Link, a microscope photo 400x
Fot. 7. Epicoccum nigrum Link, fotografia z mikroskopu 400x

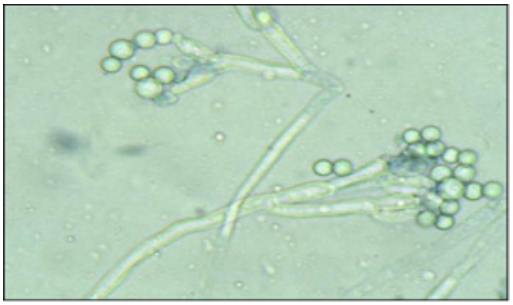
– *Penicillum brevicompactum* Dierckx, (Phot. 8). This species characterized by its restricted growth and the large compact penicillin. Using the dissecting microscope the conidiophores resemble those of *Aspergillus.*Colonies consisting of a dense felt of large, grey-green to yellow-green. Conidiophores consisting of a smooth, large, wide stipe terminating in a compact penicillus, terverticillate to quatervertililate. Conidia globose to subglobose, smooth or slightly roughened. Important (toxic) metabolites: botryodiploidin, mycophenolic acid, brevianamide A, met O. Minimal water activities (a_w) for growth 0.78-0.82 [Samson et al. 2004].



Phot. 8. Penicillum brevicompactum Dierckx, a microscope photo 100x
Fot. 8. Penicillum brevicompactum Dierckx, fotografia z mikroskopu 100x

Penicillium chrysogenum Thom (Phot. 9) is a fungus widespread in a moderate climate. It occurs in forest and meadow soils as well as in arable and fallow soils. Colonies yellow-green or pale green-blue, becoming darker at age. Exudate typically produced as yellow drops, sometimes hyaline or absent. Odour usually aromatic, fruity. Conidiophores arising from the substrate, mononematous, usually ter- to quaterverticillate, in some strains varying from bi-

to terverticillate to more complex branched. Conidia at first subglobose to ellipsoidal, remaining so or later becoming globose, hyaline or slightly greenish, smooth-walled, usually produced in loose columns. Important (toxic) metabolites: roquefortine C, meleagrin, penicillin. Very common on various food products, also products with low a_w. Also frequently occurring in indoor environments. Minimal water activities (a_w) for growth 0.78-0.81 [Samson et al. 2004].

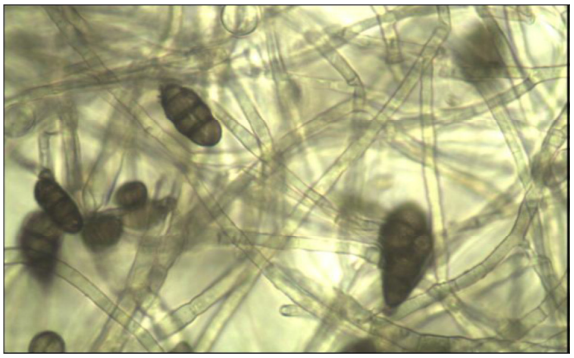


Phot.9. Penicillium chrysogenum Thom, a microscope photo 400x
Fot.9. Penicillium chrysogenum Thom, fotografia z mikroskopu 400x

- *Scopulariopsis brevicaulis* (Sacc.) Bain (Phot.10.) World-wide distribution. Colonies on MEA whitish becomoming brownish-rose (vinaceous-buff), more or less funiculose at first, becoming powdery with a prominent central tuft. Reverse in cream to brownish shades. Conidiogenous cells ammellate, borne singly on aerial hyphae or terminally on once or twince branched conidiophores, cylindrical, often with a more or less swollen base. Conidia globose to ovoid with a distinctly truncate base, apex sometimes more or less pointed, roughwalled, rose-brown (avellaneous) in mass. Temperature for growth: optimum 24-30 °C; minimum 5 °C ; maximum 37 °C. Minimal water activities (a_w) for growth [Samson et al. 2004].

Phot. 10. Scopulariopsis brevicaulis (Sacc.) Bain, a microscope photo 400x
Fot.10. Scopulariopsis brevicaulis (Sacc.) Bain, fotografia z mikroskopu 400x

- *Ulocladium chartarum* (Preuss) Simmons (Phot.11.) widely widespread. Commonly occurs as a saprophyte on rotting leaves. Conidia on MEA black to olivaceous-black. Conidiophores simple or sometimes branched, golden brown, smooth-walled, geniculate with one or more conidiogenous sites. Conidia obovoid, short ellipsoid, base conical when young, becoming rounder with age, smooth-walled to verrucose. Conidia solitary or commonly in chains of apical production of short secondary conidiophores. *Ulocladium* species are often misidentified as *Alternaria*, however the conidia of *Ulocladium* remain obovoid unless a secondary conidiophores is produced. [Samson et al. 2004].



Phot. 11. Ulocladium chartarum (Preuss) Simmons, a microscope photo 400x
Fot.11. Ulocladium chartarum (Preuss) Simmons, fotografia z mikroskopu 400x

CONCLUSIONS

It is sometimes possible to notice a biofilm which reminds of dirt on a plaster surface of facades. Dust, accumulating in cavities and irregularities of facades, as well as water retention foster sedimentation and proliferation of biological agents, including mould spores. The phenomenon is negative, not only in terms of aesthetics. The presence of deteriogenic biological agents, including fungi, on the facade surface poses a risk of changes to the original properties of the technical material, and consequently of its damage. Scientific literature describes instances where moulds produce corrosive metabolites and draw nutrients from the substrate. The prevalence of mould spores in biofilms heightens the possibility of biodeterioration.

Pictures taken with a scanning microscope revealed the presence of hyphae of moulds in the collected material.

The analysis of the biofilm on facades contain moulds spores belonging to 9 species: *Aureobasidium pullulans* (De Bary) Amund, *Cladosporium cladosporioides* (Fres.)de Vries, *Cladosporium herbarum* (Pers.) Link,

Cladosporium macrocarpum Preuss, *Epicoccum nigrum* Link, *Penicillum brevicompactum* Dierckx, *Penicillium chrysogenum* Thom, *Scopulariopsis brevicaulis* (Sacc.) Bain, *Ulocladium chartarum* (Preuss) Simmons.

Biofilm on the facades contain moulds spores belonging to various species, however, no observed growth of colonies of mould the surface of the building facade. No growth of colonies is probably due to the structure of the biofilm, which includes microorganisms belonging to various species (bacteria, cyanobacteria, actinomycetes, fungi, algae and other) and changing climate raid [Weischred and Braams 2000, Gaylard and Gaylard 2005, Gaylard et al. 2011, Piontek and Lechów 2012]. Among cooperate and have different characteristics, than a single living cells, free form. Important role of creative biofilm played by cyanobacteria, which produce extracellular polysaccharides. In the temperate climate zone, the greater part of the biofilm are the algae [Gaylarde i Gaylarde 2005, Wójcik 2008, Kielar 2009]. The coexistence of several species in the biofilm and environmental conditions prevent the increase of mould colonies on facade.

REFERENCES

1. ALTAMIRANO-MEDINA H; DAVIES M; RIDLEY I; MUMOVIC D; ORESZCZYN T.; 2009. Guidelines to avoid mould growth in buildings. Advances in Building Energy Research , 3 (1), 221-236.
2. CLONTZ L.; 2009. Microbial limit and bioburden tests: validation approaches and global requirements. 2nd ed. CRC Press Taylor& Francis Group, Boca Ration London New York.
3. GAYLARDE C.C., GAYLARDE P.M.; 2005. A comparative study of the major microbial biomass of biofilms on exteriors of buildings in Europe and Latin America.International Biodeterioration & Biodegradation 55, 131-139.
4. GAYLARDE C.C., MORTON L.H.G.; LOH K., SHIRAKAWA M.A.; 2011. Biodeterioration of external architectural paint films - A review. International Biodeterioration & Biodegradation , 3, 1189-1198.
5. GRANT C., HUNTER C.A., FLANNIGAN B., BRAVERY A.F. 1989. Water activity requirements of moulds isolated from domestic dwellings. International Biodeterioration 25, 259-284.
6. KARYŚ J.; 2010. Różnorodne formy korozji biologicznej występujące na elewacjach budynku. Ochrona przed korozją, 38-39.
7. KIELAR P.; 2009. Tynki cienkowarstwowe – Artykuł Sponsorowany [Dokument elektroniczny] Dostępny w World Wide Web: http://inzynierbudownictwa.pl/technika,materialy_i_technologie,artykul,tynki_cienkowarstwowe_-_artykul_sponsorowany,pp. 2664.

8. PIONTEK M.; 1999. Grzyby pleśniowe: Atlas. Wydawnictwo Politechniki Zielonogórskiej, Zielona Góra.
9. PIONTEK M.; 2004. Grzyby pleśniowe i ocena zagrożenia mikotoksycznego w budownictwie mieszkaniowym. Wydawnictwo Uniwersytet Zielonogórski, Zielona Góra.
10. PIONTEK M.; LECHÓW H.; 2013. Deterioracja elewacji zewnętrznych wywołana biofilmem. Zeszyty Naukowe Uniwersytetu Zielonogórskiego, 151, 79-87.
11. PIONTEK M.; LECHÓW H., ŁUSZCZYŃSKA K.; 2013. Zastosowanie płytek kontaktowych do określenia zagrożenia mikologicznego na elewacjach zewnętrznych w budownictwie (jtk /100 cm2, cfu/ cm2).Ochrona budynków przed wilgocią i korozją biologiczną. Polskie Stowarzyszenie Mykologów Budownictwa, Monografia ; 9), 241-250.
12. PIONTEK M., BEDNAR K.; 2011. Grzyby pleśniowe przyczyną biodeterioracji powłok syntetycznych stosowanych w nowoczesnym budownictwie mieszkaniowym. Ochrona przed korozją, nr 1, 8-13.
13. PIONTEK M., JASIEWICZ M., BEDNAR K.: Biodeterioracja pleśniowa wywołana występowaniem wad technologicznych w budownictwie. Ochrona przed korozją, 1, 15-18.
14. SAMSON R.A., HOEKSTRA E.S., FRISVAD J.C., FILTENBORG O.; 2004. Introduction to Food- and Airborne Fungi. 6th Ed.Utrecht, Centraalbureau voor Schimmelcultures - Utrecht, The Netherlands.
15. WARSCHEID T.; BRAAMS J.; 2000. Biodeterioration of stone: a review. International Biodeterioration & Biodegradation, 46, 343-368.
16. WÓJCIK R.; 2008. Odporność pocienionych wypraw elewacyjnych na zawilgocenie i porastanie glonami. Materiały Budowlane, Nr 3, pp.38.

OBECNOŚĆ ZARODNIKÓW GRZYBÓW PLEŚNIOWYCH W NALOCIE NA ELEWACJI BUDYNKU INSYSTUTU INŻYNIERII ŚRODOWISKA UNIWERSYTUTU ZIELONOGÓRSKIEGO

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

W pracy badaniu poddano nalot biologiczny z zachodniej elewacji budynku Instytutu Inżynierii Środowiska Uniwersytetu Zielonogórskiego pod kątem obecności zarodników grzybów pleśniowych. Z literatury naukowej wiadomo, że zarodniki grzybów pleśniowych mogą prowadzić i biorą udział w procesach biodegradacyjnych materiałów technicznych. Część materiału poddano analizie z wykorzystaniem elektronicznego mikroskopu skaningowego. Drugą część posiano bezpośrednio do szalek Petriego z podłożem biologicznym, przeznaczonym do hodowli i namnażania grzy-

bów pleśniowych.. Z analizy mikroskopowej zidentyfikowano zarodniki należące do 9 rodzajów grzybów pleśniowych: Aureobasidium pullulans (De Bary) Amund, Cladosporium cladosporioides (Fres.) de Vries, Cladosporium herbarum (Pers.) Link, Cladosporium macrocarpum Preuss, Epicoccum nigrum Link, Penicillum brevicompactum Dierckx, Penicillium chrysogenum Thom, Scopulariopsis brevicaulis (Sacc.) Bain, Ulocladium chartarum (Preuss) Simmons.

Słowa kluczowe: grzyby pleśniowe, biokorozja, biodeterioracja, nalot biologiczny, elewacje zewnętrzne