



Moulds in biodeterioration of technical materials

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ABSTRACT

Moulds are microorganisms which play the key role in biodeterioration of technical materials which results from their physiological features and metabolism. Technical materials constitute the source of carbon and energy (wood, paper, textiles, fuels, leather) or the surface for fungal growth (bricks, stone, metal, glass). Moulds characterized by a high biodeterioration activity – enzymatic and acidic, belong mainly to the following genera: *Aspergillus*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Paecilomyces* and *Chaetomium*. Members of some taxa (besides the aforementioned also e.g. *Stachybotrys*, *Alternaria*, *Epidermophyton*, *Microsporum*, *Scopulariopsis*, *Trichophyton*) growing on technical substances and producing allergens and mycotoxins cause health hazards. Therefore, basing on the knowledge about conditions for mould development and biodeterioration mechanisms, we should appropriately preserve materials against mould growth. Looking for new disinfection methods safe for technical substances in order to inhibit mould growth is also important. Protective applications of biocides should be limited only to materials most sensitive to biodeterioration (paper, textiles, fuels, paints). On the one hand we should take into consideration environmental protection, on the other production of durable, biodegradable materials ensuring the product life cycle.

KEY WORDS: moulds, biodeterioration, technical materials, biocides, disinfection

Introduction

Biodeterioration of technical materials (destruction caused by the activity of organisms) causes serious economic losses resulting from the disturbance of their properties, disasters and accidents in industry and health hazards when potential and actual pathogens grow on various surfaces.

There are many described examples of microbial accidents during production, storage and usage of materials in the textile, paper, building and other industries (Zyska 2001).

It was estimated that the economic losses caused by microbial deterioration each year amounted to 2% of Gross

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National Product (Zyska & Żakowska 2005). Based on the data of GUS (Central Statistical Office) for Poland in 2013 this value was 3.2×10^{10} PLN. Moreover, it is impossible to estimate the loss of culture heritage when we take into account destruction of historical objects or the health threat to workers and users of materials.

Moulds are dominant and the most important microorganisms in biodeterioration of technical materials considering their ubiquity and the amount of damage and threats. However, we must remember that some technical substances are often exposed to bacteria, algae, insects, higher fungi from lichenized *Ascomycota* and *Basidiomycota*. However, development of actual damaging factors depends on the material composition and environmental conditions. In the case of wood, higher fungi and insects are responsible for its destruction; bricks and

stones are destroyed mainly by algae and cyanobacteria, when they have access to light.

Significance of moulds in biodeterioration of technical materials results from their physiological features and metabolism (Fig. 1). Oligotrophic character and expansion in natural environments are the most important. A wide spectrum of produced enzymes and metabolites enables them to colonize many environments and to use almost every organic substrate. Moreover ease of spreading, different ways of propagation, creation of spores, production of secondary metabolites (antibiotics, toxins) to inhibit the growth of other organisms and high tolerance of unfavorable environmental conditions (UV, disinfection, low pH, humidity) are features which facilitate colonization of technical materials and make fungi difficult to eliminate.

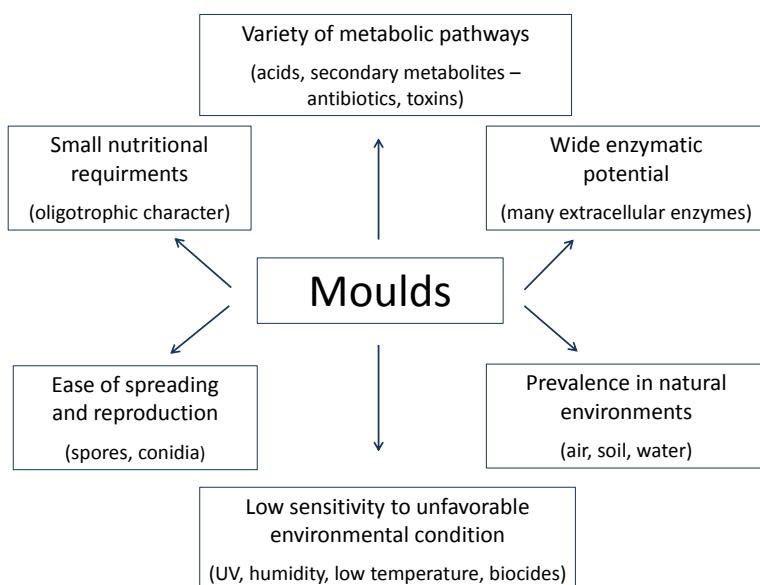


Figure 1. Mould physiological features which allow biodeterioration

High humidity of the environment (optimum $a_w > 0.9$) and access to oxygen (moulds are aerobic organisms growing on surfaces) are factors facilitating their development. However, well-known xerophilic moulds from genera *Aspergillus* and *Penicillium* are able to grow at low substrate humidity ($a_w = 0.8$) and at low relative humidity of the air RH=60%; also production of spores in soil at low oxygen concentration was observed (numerous mould species were isolated from historical objects from tombs).

Mechanisms of mould-induced biodeterioration of technical materials

There are two mechanisms of technical material biodeterioration – biological degradation and biological corrosion (Zyska & Źakowska 2005). Materials of the natural origin - wood, paper, fabrics, leather, petroleum products, oils, fuels, paints, rubber are mould-degradable (biodegradation). Moulds belonging to genera *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Trichoderma* and others are producers of extracellular enzymes which degrade polymers into monomers using them as a source of carbon and energy.

Enzymatic profiles of moulds change according to biochemical induction of environment, in which they grow. The same species growing on carton-gypsum board or on mortar produces different enzymes (Gutarowska 2010). Total mineralization occurs very quickly on organic substances containing cellulose: cotton and paper (e.g. cotton stored in the soil for a few weeks at high humidity becomes degraded by moulds to CO_2 and H_2O). Five-week experiment concerning fuel degradation with *Cladosporium* species showed important changes in the fuel chemical composition leading to the reduction in its octane number (Zyska & Źakowska 2005). Technical materials

In the view of the above facts, possibility of limiting the adverse influence of moulds on technical materials should be considered. This review presents the literature addressing the following issues:

1. which technical materials are most exposed to mould development?
2. which moulds have the greatest biodeterioration potential?
3. whether and when disinfection of mouldy technical materials or antifungal materials should be applied?

like cotton, paper, carton-gypsum board, emulsion paints are most sensitive to mould development and biodegradation processes. Biocorrosion, resulting from chemical reactions between organic acids produced by fungi and technical material is the other mechanism of mould-triggered degradation. This process causes decay of mineral surfaces of building materials (stone, brick, mortars, concrete), metal and glass (Krumbein 1988, May *et al.* 1993). Moulds belonging to *Aspergillus* and *Penicillium* produce significant amounts of organic acids such as citric, oxalic, malic and others which react with elements (Al, Mg, Fe, Si, Mn), cause material demineralization, production of soluble salts or the deposition of insoluble salts forming a layer on surfaces e.g. calcite (Cwalina 2003). Additionally, mycelium damages the material mechanically due to hyphae 3-5 cm penetration into it. All this results in loss of cohesion and increase in porosity, furthermore organic acids buffer the materials, making them susceptible to halophilic bacteria which intensifies destruction. The type of material is crucial, because it stimulates the production of specific organic acids (Gutarowska & Czyżowska 2009).

Among inorganic surfaces stone, brick, mortar are sensitive to mould growth

under high moisture conditions and presence of contaminants.

Moulds involved in the biodeterioration of technical materials

The chemical composition of technical materials determines the development of different moulds (Table 1). Moulds from genera *Aspergillus* and *Penicillium* (called moulds of warehouses) are most common. High air humidity and favorable chemical composition of the material make these fungi not only able to deteriorate many substrates, but also produce secondary metabolites (mycotoxins, volatile compounds) that are toxic to animals (Larssen & Frisvad 1994, Singh 2005). Some allergic aspergilli and penicillia found growing in damp buildings and present in indoor air were also isolated from lung specimens at autopsy (Flannigan & Miller 1994).

Technical materials containing cellulose (wood, paper, cotton, linen) or its derivatives in the form of esters, ethers (emulsion paints) are degraded by fungi belonging to the genera *Aspergillus*, *Penicillium*, *Chaetomium*, *Trichoderma*, *Cladosporium*, *Alternaria* and others.

Cellulose substrates used in buildings readily taking up water from environment (wallpaper, carton-gypsum boards) can provide a source of nutrients necessary for growth of *Stachybotrys chartarum*, which create serious mycotoxin hazard in buildings (Flannigan & Miller 1994). A number of macrocyclic trichothecenes (verrucarins, satratoxins, trichoverrins) were found in colonized materials. Paper and wood are easily degraded by the cellulase enzyme complex; the problems associated with the development of moulds during production of paper in paper mills, as well as during storage of paper in archives and libraries are described in

literature (Zyska & Źakowska 2005). The development of moulds in paper factories begins already during storage of raw material (wood, paper) and production of cellulose pulps. It has been shown that unbleached mass produced from recycled substrates is more contaminated than the unbleached pulp originating from trees (in the bleaching process a biocide, titanium dioxide, is added). The mould growth was also observed on production machinery in the form of biofilms and pulp tanks (Gutarowska & Cichocka 2009). Degradation of paper in archives and libraries mainly by *Aspergillus* and *Penicillium* is a serious problem, especially under the conditions of high humidity of paper/air, such as disaster-related flooding of warehouses or during storage in uncontrolled microclimate (periodical humidity of the air RH<70%). Under such conditions, development of fungi on books is possible; there is also a hypothesis that moulds are involved in the formation of a 'Foxing' phenomenon (orange-brown small spots on paper) common in nineteenth century books (Florian & Manning 2000, Strzelczyk 2001). Numerous examples of mould-induced destruction of historical objects are also described, including paintings, monuments, paper, leather and textiles (Strzelczyk 2001, Strzelczyk *et al.* 2003, Florian 2004). The primary reasons of their biodegradation include mechanical damage, an organic nature, age and the presence of contaminants.

Materials containing proteins (wool, leather) are colonized by *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Acremonium*, *Alternaria* fungi and other taxa with proteolytic properties. What is more, in materials

containing proteins such as keratin and collagen human pathogens such as *Epidermophyton*, *Microsporum*, *Scopulariopsis*, *Trichophyton* can grow. Many species of these genera cause mycoses and are classified to the class 3 of Biosafety Level (BSL) in terms of risk

to human health. They pose a serious threat to people working in tannery, leather and wool product warehouses, as well as technicians, people exposed to fungi in mouldy buildings or users of these materials (Singh 2001).

Table 1. Moulds participating in biodeterioration of technical materials.

Technical material	Fungal taxon*	Reference
Wood	<i>Aspergillus</i> sp. (<i>A. brasiliensis</i>), <i>Chaetomium globosum</i> , <i>Penicillium</i> sp., <i>Trichoderma viride</i>	Fojutowski 2003
Paper	<i>Alternaria</i> sp., <i>Aspergillus</i> sp. (<i>A. brasiliensis</i> , <i>A. versicolor</i>), <i>Chaetomium globosum</i> , <i>Cladosporium</i> sp. (<i>C. herbarum</i>), <i>Geotrichum</i> sp., <i>Mucor</i> sp., <i>Paecilomyces variotii</i> , <i>Penicillium</i> sp., <i>Phoma</i> sp., <i>Stachybotrys</i> sp., <i>Talaromyces funiculosus</i> , <i>Trichoderma</i> sp.	Woźniak & Tymińska 2003, Zyska & Żakowska 2005
Textiles	<i>Alternaria tenuissima</i> , <i>Aspergillus</i> sp. (<i>A. auratus</i> , <i>A. brasiliensis</i> , <i>A. carbonarius</i> , <i>A. fischeri</i> , <i>A. flavus</i> , <i>A. fumigatus</i> , <i>A. nidulans</i> , <i>A. raperi</i> , <i>A. terreus</i> , <i>A. ustus</i> , <i>A. wentii</i>), <i>Chaetomium</i> sp. (<i>Ch. globosum</i> , <i>Ch. cochlioides</i>), <i>Fusarium</i> sp., <i>Penicillium</i> sp. (<i>P. aurantiogriseum</i> , <i>P. canescens</i> , <i>P. citrinum</i> , <i>P. gladioli</i> , <i>P. granulatum</i> , <i>P. simplicissimum</i> , <i>P. wortmannii</i>), <i>Talaromyces funiculosus</i> , <i>Trichoderma viride</i>	Szostak-Kot 2001, 2003, Abdel-Kareem 2009, Błyskal & Syguła-Cholewińska 2001
Leather	<i>Acremonium</i> sp., <i>Alternaria</i> sp. (<i>A. alternata</i>), <i>Aspergillus</i> sp. (<i>A. brasiliensis</i> , <i>A. flavus</i> , <i>A. fumigatus</i> , <i>A. terreus</i> , <i>A. versicolor</i> , <i>A. wentii</i>), <i>Chaetomium globosum</i> , <i>Chrysosporium</i> sp., <i>Cladosporium</i> sp. (<i>C. cladosporioides</i> , <i>C. carrioni</i>), <i>Epidermophyton floccosum</i> , <i>Fusarium</i> sp. (<i>F. oxysporum</i>), <i>Haematonectria haematococca</i> , <i>Microascus brevicaulis</i> , <i>Microsporum canis</i> , <i>Mucor plumbeus</i> , <i>Paecilomyces variotii</i> , <i>Penicillium</i> sp. (<i>P. aurantiogriseum</i> , <i>P. citrinum</i> , <i>P. glabrum</i> , <i>P. ochrochloron</i> , <i>P. purpurogenum</i> , <i>P. verrucosum</i>), <i>Rhizopus stolonifer</i> , <i>Spicaria</i> sp., <i>Scopulariopsis</i> sp., <i>Stemphylium</i> sp., <i>Syncephalastrum racemosum</i> , <i>Talaromyces ruber</i> , <i>Thamnidium elegans</i> , <i>Trichoderma</i> sp., <i>Trichophyton</i> sp. (<i>T. mentagrophytes</i> , <i>T. rubrum</i> , <i>T. tonsurans</i>)	Orlita 2001, Strzelczyk <i>et al.</i> 2003, Perkowski & Goździecki 2003, Falkiewicz-Dulik 2003

* Fungal taxa according to Index Fungorum (www.indexfungorum.org, accessed 22.07.2014)

Technical material	Fungal taxon*	Reference
Petroleum, oil, fuels	<i>Alternaria</i> sp., <i>Amorphotheca resinae</i> , <i>Aspergillus</i> sp. (<i>A. fumigatus</i> , <i>A. versicolor</i> , <i>A. ustus</i>), <i>Fusarium</i> sp., <i>Humicola</i> sp., <i>Paecilomyces variotii</i> , <i>Penicillium</i> sp. (<i>P. canescens</i> , <i>P. spinulosum</i>), <i>Sarocladium strictum</i>	Kwiatkowska 2003
Paints	<i>Aureobasidium pullulans</i> , <i>Paecilomyces variotii</i> ,	Zyska & Źakowska 2005
Rubber, insulation materials	<i>Aspergillus</i> sp., <i>Chaetomium</i> sp., <i>Cladosporium</i> sp., <i>Penicillium</i> sp., <i>Microascus brevicaulis</i> , <i>Trichoderma</i> sp.	Michalski <i>et al.</i> 2001
Plastics	<i>Aspergillus</i> sp. (<i>A. awamori</i> , <i>A. brasiliensis</i> , <i>A. flavus</i> , <i>A. terreus</i>), <i>Paecilomyces variotii</i> , <i>Penicillium</i> sp. (<i>P. expansum</i> , <i>P. ochrochloron</i>), <i>Talaromyces funiculosus</i> , <i>Trichoderma viride</i>	Źakowska <i>et al.</i> 2003, Nowak <i>et al.</i> 2012
Stone, concrete, brick, mortar	<i>Alternaria tenuissima</i> , <i>Aspergillus</i> sp. (<i>A. brasiliensis</i> , <i>A. fumigatus</i>), <i>Aureobasidium pullulans</i> , <i>Cladosporium</i> (<i>C. cladosporioides</i> , <i>C. herbarum</i>), <i>Penicillium</i> sp. (<i>P. chrysogenum</i> , <i>P. rubrum</i>), <i>Phaeospora</i> sp., <i>Phoma putaminum</i> , <i>Purpureocillium lilacinum</i> , <i>Trichoderma</i> sp., <i>Ulocladium consortiale</i>	Cwalina 2003
Glass	<i>Alternaria alternata</i> , <i>Aspergillus</i> sp. (<i>A. brasiliensis</i> , <i>A. versicolor</i>), <i>Talaromyces funiculosus</i>	Sitarz <i>et al.</i> 2003

* Fungal taxa according to Index Fungorum (www.indexfungorum.org, accessed 22.07.2014)

Biodeterioration of building and finishing materials in residential and public buildings is also common. It was estimated that 40% of people in Poland live and work in buildings with high humidity level RH>80% (European Environmental and Health Information System 2006), and in 20% of residential houses there are mould-related problems. They not only reduce the aesthetics of the buildings, but also pose a threat to health of residents and workers. Moulds are capable of growing on most construction, finishing and insulation materials used in buildings (Gutarowska 2010). Moulds from genus *Aspergillus* (*A. brasiliensis*, *A. flavus*, *A. versicolor*), *Penicillium* (*P.*

chrysogenum, *P. expansum*), *Alternaria* and *Cladosporium cladosporioides* are frequently isolated from buildings. It has been shown that the type of building material significantly modifies mould allergenicity and toxicity (Gutarowska 2013).

Growth of moulds with lipolytic properties such as *Cladosporium*, *Acremonium* and *Paecilomyces* in metalworking fluids or oils constitutes a significant threat in industrial factories. Moulds are able to change the chemical composition of oils and to contribute to biocorrosion of metal surfaces, moreover together with bacteria they produce bioaerosols which are harmful for

workers (Cyprowski *et al.* 2007). Some species of *Cladosporium* and *Paecilomyces* are able to grow on polymers, rubber and also in the fuels. The processes of degradation of these materials take several months to several years, however, they are the cause of major economic losses, especially due to the destruction of insulations and fuel tanks (Berryman 1987). Studies conducted in Poland showed the presence of moulds in 50% of samples taken from fuel tanks (Kwiatkowska 2003).

Protection against mould growth and methods of their elimination from technical materials

Similarly as in pharmaceutical, cosmetic and food industries also in plants producing technical materials systems of high quality production have been introduced, including high quality of technological water and raw substrates (paper, paints, building materials), as well as cleanliness of equipment, tanks and warehouses for storing both raw materials and products. The microclimate parameters, which should not exceed RH<60%, 10-15% humidity of materials (depending on the material) and air temperature in warehouses 18-21°C are also monitored.

In the case of textiles, paper, plastics also access to light should be minimized (Abe 2010). However, susceptibility of paper, paints, building materials, wood to harmful impact of microorganisms makes biocide usage during production necessary.

Some examples of biocides and preparations applied to inhibit moulds growth and to produce materials with antifungal properties are presented below (Tab. 2).

Market of chemical substances and preparations (complexes containing biocides and excipients, extending their action) that can be used to protect

Assessment of strength change of external rubber covers of telecommunication cables revealed 80% of mass loss caused by growth of actinomycetes (filamentous bacteria) and moulds (Zyska & Źakowska 2005).

The frequency and scale of the devastation of technical substances and health risks caused by moulds make it necessary to look for new methods of disinfection and protection of materials against biodeterioration.

industrial materials is regulated by 98/8/EC Directive of the European Parliament and of the Polish Council Act of biocidal products (Dz.U.2002 No. 175, poz. 1433) and the Ordinance of the Minister of Health on the categories and types of biocidal products according to their purpose (Dz.U. 2003 No 16 poz 150) (Brycki 2012).

Selection of a biocide suitable to conserve a given technical material is a big challenge for manufacturers. The main feature of a good biocide is its effectiveness in eliminating different forms of microorganisms (in the case of moulds it is essential to eliminate both mycelium and spores). However, we must remember that biocides added to technical materials must meet a number of additional requirements: they must be non-toxic, non-allergic to animals and humans, they cannot influence properties of the material or permanently adhere to it, must be insensitive to the process parameters, cheap and stable during product life cycle. Taking into account these conditions the choice of a biocide requires cooperation of experts, production technicians, chemists and microbiologists. Also it should be noted that this substance must be biodegradable

after usage. Common usage of some biocides (such as nanosilver, Triclosan) to produce antimicrobial materials can cause their accumulation in the environment and in organisms, and subsequently elimination of certain organisms which play a crucial

ecological role, as well as contribute to the appearance of resistant microorganisms. It is therefore prudent to decide whether addition of a biocide to a technical material is indispensable and use it only in justified circumstances.

Table 2. Selected biocides and preparations with antifungal activity used to protect technical materials against biodeterioration.

Technical material	Biocide / preparation	References
Wood	quaternary ammonium salts, triazols, boric acid, propiconazol, ionic liquids, essential oils	Krajewski 2001, Fojutowski 2003, Zabielska-Matejuk <i>et al.</i> 2012
Paper	ethylene oxide, essential oils (thymol), ozone, phenylacetic acid, quaternary ammonium salts, isopropanol, ionic liquids phenylphenol/Irgasan, Nipagin, Rotanox	Woźniak & Tymińska 2003, Karbowska-Berent <i>et al.</i> 2009, Koziróg et al. 2012
Textiles	quaternary ammonium salts, , ethylene oxide, propylene oxide, salicylanilide, o-phenylphenol, 5-chlorophenol, nanosilver and nanocopper / Preventol, Irgasan	Montegut <i>et al.</i> 1991, Szostak-Kot 2003, Abdel-Kareem 2009, Fokszowicz-Flaczyk <i>et al.</i> 2012, Gutarowska & Michalski 2012
Leather	4-chloro-3 methyl phenol, chlorocresol, 2-phenylphenol, phenoxyethanol, 2-thiothiocyanato- methyl-benzothiazole, isothiazoline compounds, diiodomethyltolyl-sulfone, nanosilver, nanozinc and nanocopper / Sanitized	Didato & Yanek 1999, Orlita 2001, Falkiewicz-Dulik 2003; 2012
Petroleum, oil, fuels	Preventol P109 (2-bromo 2-nitro-1,3 propanediol and isothiazolones), MAR 71 (N, N'- methylene-bis-5-methyloxazolidine), Kathon FP1.5 (5-chloro-2-methyl-4 isothiazolin-2-one and 2-methyl-4 isothiazolin-3 -one)	Kwiatkowska 2003

Technical material	Biocide / preparation	References
Paints	Copper alkylbenzenesulphonate, quaternary ammonium salts, titanium dioxide, ionic liquids	Shirakawa <i>et al.</i> 2002, Michałczyk & Cieciecka-Rosłonkiewicz 2009, Markowska-Szczupak <i>et al.</i> 2012
Plastics	nanosilver and nanocopper	Jeziorska <i>et al.</i> 2012
Stone, concrete, brick, mortar	quaternary ammonium salts/ Boramon, Mycetox	Cieciecka-Rosłonkiewicz & Komorowska-Kulik 2003, Piontek and Lechów 2012

Disinfection of technical materials which are already biodeteriorated to stop growth of moulds is another important issue. There is a range of methods used to address this problem: laser used for wood (Piotrowska *et al.* 2003); fumigation with gases (ethylene oxide, propylene oxide), essential oils and nanosilver particles used for paper and textiles (Tymińska 2001, Woźniak & Tymińska 2003, Gutarowska *et al.* 2012); gamma radiation used for textiles, paper and leather (Machnowski *et al.* 2012, Perkowski & Goźdiecki 2003, Woźniak & Tymińska 2003); microwaves for building and finishing materials (Górny *et al.* 2012); photocatalitic ionization (Pietrzak

& Gutarowska 2013) and biocides (Brycki 2012).

Choosing a method of disinfection one should consider not only the inhibition of fungi growth, but also safety for the technical material. This is particularly important for historical objects. Many of the disinfection methods have a negative impact on the treated materials, they change their strength and optical parameters and may also contribute to their faster biodegradation in the future (Machnowski *et al.* 2012, Gutarowska *et al.* 2014). Therefore, it is important to know the effects of disinfection for both microorganisms and for materials to select appropriate process parameters.

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Streszczenie

Pleśnie są mikroorganizmami, które odgrywają kluczową rolę w biodeterioracji materiałów technicznych, co wynika z ich cech fizjologicznych i metabolizmu. Materiał techniczny stanowi dla nich albo źródło węgla i energii (drewno, papier, tekstylia, paliwa, skóra) albo jest podłożem do ich wzrostu (cegły, kamień, metal, szkło). Grzyby charakteryzujące się wysoką aktywnością biodeterioracyjną – enzymatyczną i kwasotwórczą należą głównie do *Aspergillus*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Paecilomyces* i *Chaetomium*. Przedstawiciele niektórych rodzajów grzybów (oprócz wymienionych powyżej, również m.in. *Stachybotrys*, *Alternaria*, *Cladosporium*, *Epidermophyton*, *Microsporum*, *Scopulariopsis*, *Trichophyton*) rosnąc na substancji technicznej oraz wytwarzając alergeny i mykotoksyny stwarzają zagrożenia zdrowotne. Dlatego na podstawie wiedzy na temat warunków rozwoju pleśni i mechanizmów biodeterioracji należy odpowiednio zabezpieczać materiały przed rozwojem grzybów. Istotne jest również poszukiwanie nowych, bezpiecznych dla materiałów technicznych metod dezynfekcji w celu zahamowania rozwoju grzybów. Zastosowanie biocydów w celach ochronnych należy

ograniczyć jedynie do materiałów najbardziej narażonych na biodeteriorację (papier, tekstylia, paliwa, farby), mając na uwadze aspekty ochrony środowiska, a także produkcję materiałów biodegradowalnych, zapewniając cykl życia produktu.