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Nanosilver as a microbicide for disinfection of technical materials

The doctoral dissertation is thematically coherent set of seven articles in scientific journals (International Biodeterioration and Biodegradation – 2 publications, Metallomics, Acta Biochimica Polonica, Textile Research Journal, Current Nanoscience, Journal of Cultural Heritage) having total 5-year Impact Factor of 14.674. The percentage share in the collected publications ranges from 56.7% to 80.0%.

The lack of research on the mechanism of action of silver nanoparticles (AgNPs) on moulds and the need to find a new alternative to the existing ones disinfection method of historical objects, were the basis of the purpose of doctoral dissertation, which included the determination of the sensitivity of microorganisms isolated from museums, library and archive on silver nanoparticles and the establishment of mechanism of their action on moulds. Moreover, the research aimed to optimize the silver nanoparticles misting and its use for the disinfection of technical materials and historical objects.

In the first stage of research, the sensitivity range of microorganisms isolated from museums, archive and library on silver nanoparticles was determined (from the most sensitive): bacteria from genera *Escherichia*, *Sphingomonas* and *Nocardia*, yeasts *Candida* sp. and *Rhodotorula* sp., moulds from genera *Alternaria*, *Cladosporium* and *Mucor*, bacteria *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp. and moulds from genera *Aspergillus*, *Penicillium* and *Rhizopus*. It was found that the AgNPs disinfection is more effective against vegetative form of microorganisms than spores of bacteria and moulds. Microorganisms isolated from museum environment were characterized by lower sensitivity to AgNPs, compared to microorganisms originated from the collection of pure cultures. Moulds, which are responsible for the biodeterioration of historical objects belong to microorganisms sensitive to nanosilver. The time required to inhibit their growth, as a result of AgNPs action (concentration of 45 ppm, φ 10-80 nm), is shorter by 3-5 hours compared to bacteria.

In the second stage, studies were carried out on the impact of silver nanoparticles on moulds. Significant morphological changes, under the influence of AgNPs, were shown, including plasmolysis, shortening of hyphae and multidirectional changes in the cellular ultrastructure: vacuolization, thickening of membranous structures, accumulation of lipid material, deformation and disintegration of organelles, including nucleus, condensation and fragmentation of chromatin, formation of apoptotic bodies and internal cell membrane. Based on the study of the metabolome, the formation of silver adducts with many compounds in mould cells was noticed, including with coenzyme A, phenylglycine, peptide LeuSerAlaLeuGlu, monoglycerides, glycerophospholipids and the elimination or reduction of the activity of many metabolites. Moreover, AgNPs limited the production of organic acids (e.g. citric, oxalic, succinic and malic) and mycotoxins (e.g. Fumonisin B1, Aflatoxin B1, B2, G1, G2 and Ochratoxin A) and also changed the profile of extracellular enzymes and decreased the total cytotoxicity of tested moulds.

In the third stage of the research, the optimization of the conditions of silver nanoparticles misting disinfection of technical materials was performed. It has been shown that the

disinfection process using silver nanoparticles misting (φ 10-80 nm) in the designed disinfection chamber is the most effective with the parameters: time of 8 hours, concentration of 90 ppm of the preparation and the horizontal - vertical setting of spray nozzles. It is also recommended that disinfected material should be preconditioned at elevated relative humidity for several hours to activate microbial spores. Amount of silver nanoparticles deposited during the disinfection process depends on the type of the technical material and ranges from 1.2 to 7.0 ppm. The highest efficiency of AgNPs disinfection (65-92%) can be obtained on cellulose materials (linen, cotton, pine and spruce paper), lowest (62-67%) is obtained on wood, leather, silk and wool. The increase of the mass humidity of technical materials to the level of 84% for textiles and 40% for paper, significantly raise the effectiveness of AgNPs disinfection. Above these values, the increase of the process efficiency is not observed. Silver nanoparticles misting protects technical materials against microbial growth for at least 3 months. After this time, the number of microorganisms on the AgNPs protected material was lower by 32-93%, compared to the untreated surface.

The research on the safety of silver nanoparticles misting process for the environment and workers showed that the process of AgNPs misting in the disinfection chamber should be carried out with caution and the use of personal protective equipment during an 8-hour working time is recommended, due to exceeding of the concentrations limit of carbon nanoparticles in the air, established by the USA Occupational Safety and Health Administration (OSHA).

The fourth stage of the study, concerned the determination of the effect of the silver nanoparticles misting on the changes in mechanical and optical parameters of various technical materials. It allowed to state that AgNPs disinfection does not cause significant changes in the mechanical strength (elongation, breaking strength, tensile and tear indices, breaking strength) and colour parameters (R457 whiteness, CIE L*a*b*, ΔE) of tested technical materials (paper, wood, cotton, wool). AgNPs disinfection is not recommended for materials such as silk, leather and spruce paper, in particular artificially aged and historical, due to changes in colour parameters.

In the last, fifth stage of research, AgNPs disinfection of historical objects (fragments of wooden floor, paper map, parchment, linen selvedge of painting and pre-Columbian textiles made of wool, cotton and sisal) was performed, showing that the process has inhibitory effect on the growth of bacteria and fungi (30.1-99.9%) and does not cause changes in the chemical structure and pH of textile materials.

Developed within the doctoral dissertation silver nanoparticles misting method of technical materials, including selected historical materials, in the future may be an alternative to current disinfection methods, due to the high efficiency of microbial growth inhibition and lack of negative impact on the mechanical properties of the material and colour.