Comparison of the influence of chemically and biologically synthesized Ag nanoparticles on the algae growth inhibition

MRAŽÍKOVÁ ANNA[1, a)], VELGOSOVÁ OKSANA[1, b)], KAVULIČOVÁ JANA[2, c)], MÁLEK JAROSLAV[3, d)], KRUM STANISLAV[3, e)]
[1]Institute of Materials and Quality Engineering,
[2]Institute of Metallurgy,
Faculty of Materials, Metallurgy and Recycling,
Technical University of Košice
Letná 9, 042 00 Kosice
SLOVAKIA

[3]Department of Materials Engineering,
Faculty of Mechanical Engineering,
Czech Technical University in Prague,
Karlovo náměstí 13, 121 35 Praha 2
CZECH REPUBLIC
[a)]corresponding author: anna.mrazikova@tuke.sk, [b)]oksana.velgosova@tuke.sk
[c)]jana.kavulicova@tuke.sk, [d)]jardamalek@seznam.cz, [e)]s.krum@seznam.cz

Abstract: Unique physicochemical properties of silver nanomaterials dependent not only on the shape and size of silver nanoparticles (AgNPs), but also on the surface capping agent. This study provided comparison of antimicrobial activities of chemically synthesized AgNPs and biosynthesized AgNPs against green algae on agar plates and also in the aqueous environment. The extract of the green freshwater algae *Parachlorella kessleri* acted both as a reducing as well as stabilizing agent during the formation of AgNPs. For chemically synthesized AgNPs, citrate and gelatin were used as a reducing and capping agent, respectively. The formation of AgNPs was confirmed by UV-Vis and TEM measurements. UV-Vis results revealed that both used Bio-AgNPs and Chem-AgNPs exhibited long-term stability. Antimicrobial activities of Bio-AgNPs and Chem-AgNPs were examined against green algae *P. kessleri* using disk diffusion methods. Comparing the antimicrobial activity on agar plates of Bio-AgNPs and Chem-AgNPs according to the zone inhibition around swabs showed stronger toxic effects of Bio-AgNPs. On the other hand, Bio-AgNPs were confirmed to be less toxic in aquatic environments for the growths of green algae *P. kessleri* comparing to Chem-AgNPs where cells growth reduction after 30 h was 60% in the presence of Chem-AgNPs.

Key-Words: silver nanoparticles, antibiofilm activity, biosynthesized nanoparticles, chemically synthesized nanoparticles

1 Introduction

The practical use of silver and its effects have been known since ancient times, and for more than a century, colloidal silver has also been at the forefront of interest [1]. Silver nanoparticles are the most effective form of silver and due to its unique properties they have become one of the most commercially used nanoparticles in consumer products [2]. Moreover, zerovalent silver is considered to be a less toxic and safer antibacterial agent also to higher animals compared to ionic silver. [3].

At present, thanks to nanometer-sized form and due to excellent antimicrobial properties such as: anti-fungal activity, anti-inflammatory activity, anti-viral activity and anti-angiogenic activity nanoparticles are suitable for use in different kinds of industries such as food industry, textile industry, in medical devices and also nanoparticles have found many applications in optics, sensing, painting, cosmetics [4, 5, 6, 7]. Ag nanoparticles show a much higher antibacterial activity even in low concentration compared to ionic Ag^+. The

effect of AgNPs is enhanced by the subsequent release of Ag^+ ions from nanoparticles resulting in a long-lasting antimicrobial effect [8]. Although the mechanism of silver nanoparticles for microorganisms has not been clearly explored yet, it is assumed that nanoparticles cause direct attack of cell walls, what consequently damages cell membrane [9]. According some authors, nanoparticles first enter the cell and then start the release of ionic Ag^+, what they called "Trojan horse" effect [10, 11]. Previously studies confirmed that gram-negative bacteria were more profoundly inhibited by AgNPs due to relative abundance of negative charges on cell walls than that of the gram-positive organisms [12]. Generation of reactive oxygen strains is also next significant antimicrobial activity of silver nanoparticles [9].
A very important factor influencing the effect of silver nanoparticles on the cells is their large surface area to the ratio of particles. Small particles size causes strong reactive interactions also with bacterial intercellular compartments. Nanoparticles can interact with DNA, which seriously damages replication and consequently causes cell death [4, 13]. Nowadays there are many ways Ag nanoparticles can be synthesized (physical, chemical and combination of both). Most commonly, chemical methods are used which use chemical reducing agents to reduce Ag^+ to Ag^0. Since agglomeration of nanoparticles is a main problem in nanoparticles synthesis and stabilization, different stabilizers are used to prevent aggregation and to control particle growth during nanoparticles preparation. But these toxic chemicals cause harmful secondary effects and so chemical synthesis of silver nanoparticles may be associated with environmental toxicity or biological hazards [14, 15, 16].

A serious problem in using nanomaterials is in water conditions, where silver nanoparticles can be easily transported to large aquatic environment. Unfortunately, environmental impact of AgNPs on aquatic system is still unknown [13]. According to Garner and Keller [17], 66.000 metric tons of nanoparticles are released into surface waters every day and also major parts of nanoparticles in the air also contaminate aquatic systems. But contamination of aquatic environment by nanoparticles it is not the only problem. For example, it is estimated, that 30% of silver nanoparticles from an outdoor facade is released into environment and major part of these nanoparticles is sustain in sewage, which is often used as fertilizer [18]. Since silver nanoparticles antimicrobial toxicity is contributed with slow release of Ag^+ to environment, new compounds on silver nanoparticles-based showing antimicrobial effect without releasing toxic chemicals are strong interest for production companies [7].
Biological synthesis of AgNPs preparation has several advantages in comparison with physical and chemical methods. Biological synthesis of Ag nanoparticles utilizes ecologically acceptable reducing agents (algae, plants extracts) and so AgNPs are coated with biological molecules which make nanoparticles more biocompatible. One of the possibilities of green synthesis AgNPs is to use natural extract from green algae. The functional groups present in the cell walls of algae are responsible for nanoparticles formation and simultaneously can serve as capping agents to provide stability of silver nanoparticles [19].

The previous study of freshwater green algae *P. kessleri*, EDS and FTIR analyses revealed that the binding sites on the cell of the freshwater green algae *P. kessleri* as strong acidic groups (phosphoric, sulphonate and carboxylic groups linked to aromatic compound) represent 54.2%, weak acidic groups (carboxylic groups) represent 32.6% and very weak acidic sites (amine groups) represent 13.2% of total acidity. From these groups of the biomass, the Ag^+ ions were attracted to the COO^- group through electrostatic interactions after the addition of $AgNO_3$ solution. According the author, the carbonyl groups could be responsible for Ag^+ reduction and also that the proteins present in the biomass can form coating of nanoparticles which has a positive effect on nanoparticle stability [20].

In the present study, a freshwater green algae *P. kessleri* was used to asses and compare the toxic antialgal effects of biologically synthesized silver nanoparticles (Bio-AgNPs) and chemically synthesized silver nanoparticles (Chem-AgNPs) on agar plates and in aquatic environments against algae *P. kessleri*.

2 Experimental Results

2.1 Algae cultivation

Freshwater green algae strain *P. kessleri* (LARG/1) obtained from the Institute of Botany SAS in Bratislava, was grown in Milieu Bristol medium on

agar plates in Petri dishes at the ambient temperature and lightning interval (12:12).

2.2 Preparation of biomass extract

The algae *P. kessleri* were cultivated on agar plates for 3 weeks. The agar plates were carefully washed with distilled water to remove algae biomass. The biomass was consequently heated to boiling for 10 min using a water-bath.

2.3 Biosynthesis of silver nanoparticles

To prepare silver nanoparticles, 5 ml of extract of algae biomass was transferred into Erlenmeyer flasks containing 250 ml of $AgNO_3$ solution (0.29 mM). The Erlenmayer flasks were stored in lighting condition at the room temperature to allow reducing the silver ions into AgNPs.

2.4 Chemical synthesis of silver nanoparticles

Chemically synthesized AgNPs were performed using chemical reduction method [21] using $AgNO_3$ (0.29 mM) solution, sodium citrate (0.5%) and 0.01% gelatine. First of all, to prepare stock solution of $AgNO_3$, 0.025 g of gelatine was dispersed in 250 ml of 0.29 mM of $AgNO_3$. Silver nanoparticles were prepared by adding drop-wise of 15 ml of 0.5% sodium citrate solution into $AgNO_3$ solution under heating conditions. The resulting solution was stirred for 30 minutes.

2.5 Disk diffusion test

The algae biofilm inhibition after 7 days was performed by standard disk-diffusion method [22]. Milieu Bristol agar plates in Petri dishes were inoculated with 0.5 ml of algal suspension 10^5 CFU/ml and sterile swabs were plated on these agar plates. Swabs were impregnated with 25 µl of silver nanoparticles colloid solution at a concentration of Ag 1mg/l. Agar plates were incubated at the room temperature and lightning interval (12:12).

2.6 Antialgal assay

The antialgal activities of the synthesized silver nanoparticles were assessed against green algae *P. kessleri* in 500 ml of Milieu Bristol medium at a cells concentration of 10^5 CFU/ml where 1 ml of silver nanoparticles colloid solution at a concentration of Ag 1mg/l were added. These inoculated solutions were incubated under continuous shaking at the room temperature and a 12:12 lightning regime. The cells viability was read after 6, 24 and 30 h.

3 Characterizations

The algal cells viability was judged using an EVETM- NanoEnTek. The cells eradication on agar plates was observed using macroscope LEICA WILD M32. An UNICAM UV/vis Spectrometer UV4 was used to analyse AgNPs absorbance. Transmission Electron Microscope (TEM; JEM-2000FX, JEOL) was used for TEM measurements.

4 Results and discussion

The formation of biologically and chemically prepared nanoparticles through reduction method of Ag^+ ions was clearly observed from the colour changes, as depicted Fig.1 The formation of AgNPs was also observed and confirmed by UV-vis spectroscopy (Fig.2(a) and 2(b)), what is very important and reliable method for the characterization of colloidal suspension of nanopartricles. The surface plasmon resonance (SPR) absorption band measured by this method depends on the particle size of AgNPs, dielectric medium, and chemical surroundings and is well documented for various metal nanoparticles with sizes ranging from 2 to 100 nm [23, 24, 25]. The typical maximum of absorption band for silver nanoparticles is in the region of 350-450 nm. The character of SPR bands as a symmetry, position and narrowness can also give an important information about shape, size, agglomeration or oxidation of nanoparticles

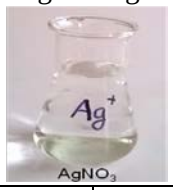
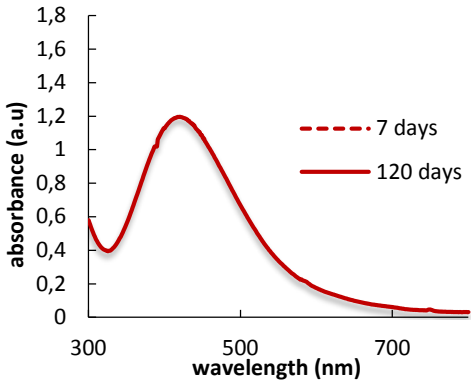
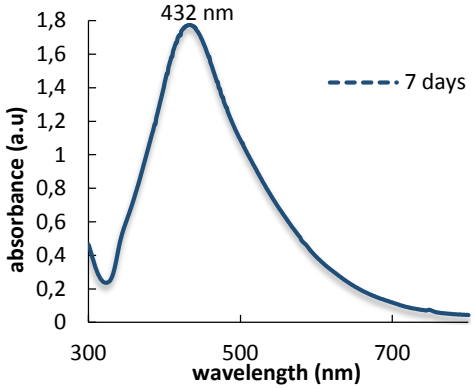
<table>
<tr><th colspan="2">Synthesis of Silver nanoparticles</th></tr>
<tr><td colspan="2">Method:
Reduction of silver salt solution ($AgNO_3$)
$Ag^+ \rightarrow Ag^\circ$
 
</td></tr>
<tr><td>chemically synthesis:
$AgNO_3$
reducing agent: citrate
capping agent: gelatine
 
</td><td>biologically synthesis: $AgNO_3$
reducing an capping agent: extract of *P. kessleri*
 
</td></tr>
</table>

FIGURE 1. The visual observation of silver nanoparticles preparation



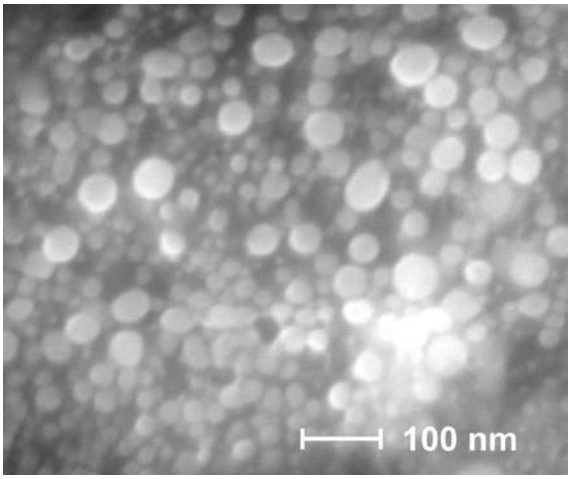
(a)



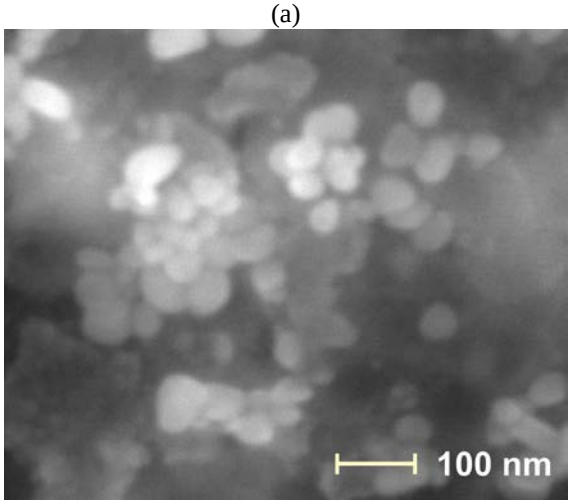
(b)

FIGURE 2. The UV-vis spectroscopy of
a) biologically synthesized silver nanoparticles
b) chemically synthesized silver nanoparticles

[26, 27, 28]. In our work, the stability of AgNPs prepared from biological and chemical methods was observed for more than 150 days. An increase of the absorption peaks located around region of 400 nm from day 7 to 120 was observed, what was indicated the formation of stable AgNPs in both cases of colloidal solutions. Also for Bio-AgNPs and Chem-AgNPs the peaks become sharper with the time (Fig. 2(a) and 2(b)), what was indicated the formation of monodisperse nanoparticles according to literature [29].



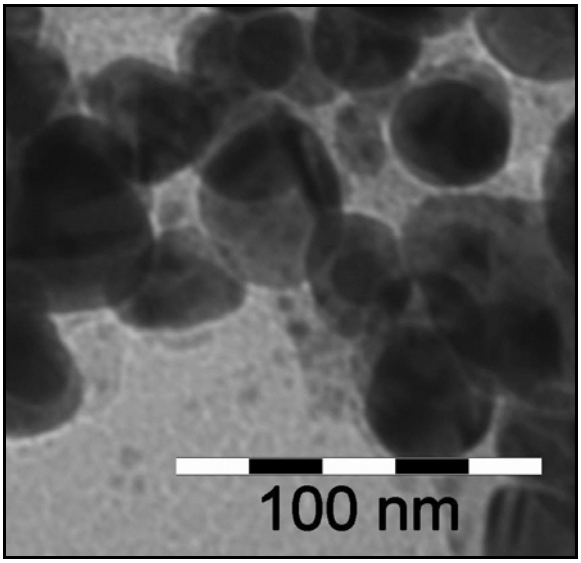
(a)



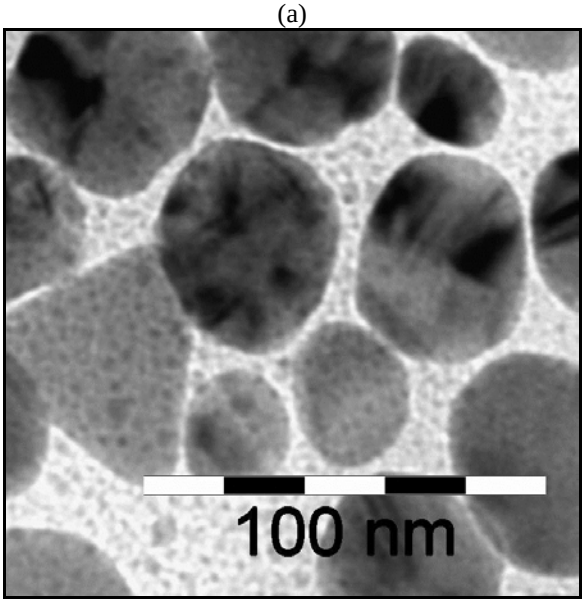
(b)

FIGURE 3. The SEM images of a) biologically synthesized silver nanoparticles
b) chemically synthesized silver nanoparticles

The SEM as a surface imaging method was used for the characterization of nanoparticles shapes, size distribution and also for resolving different particles sizes. Due to provide better spatial resolution of nanoparticles, grain size and morphology, SEM measurements were also expanded by TEM measurements [30]. These micrographs confirmed the formation of smaller spherical Bio-AgNPs with the particles range in size from 3 to 45nm (Fig.3(a) and Fig.4(a)). The results also showed that the Chem-AgNPs were synthesized in various shapes such as spherical and triangle and they were from 4 to 55 nm for (Fig.3(b) and Fig.4(b)). SEM imaging confirmed agglomerates and some dispersed nanoparticles of Chem-AgNPs and more dispersed Bio-AgNPs in colloid solution.

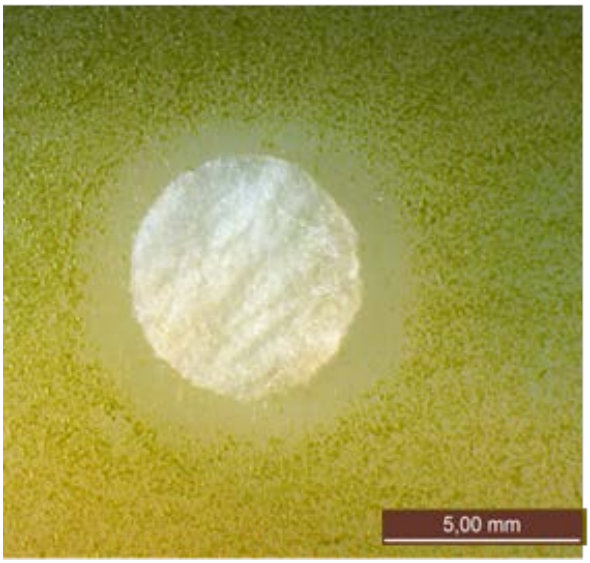


(a)

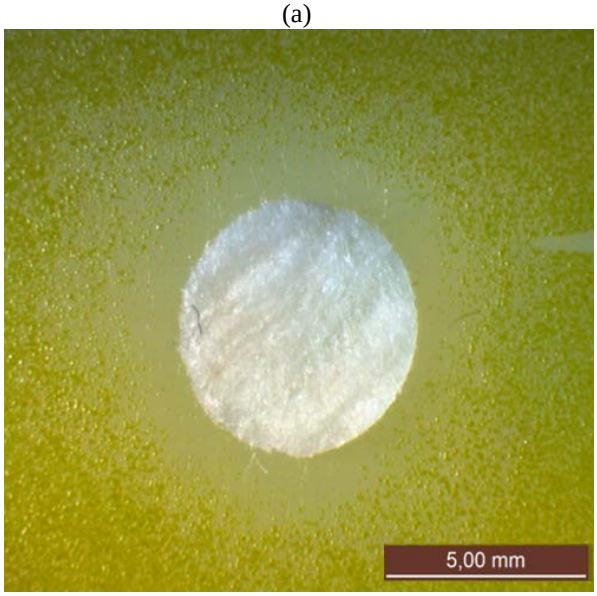


(b)

FIGURE 4. The TEM images of a) biologically synthesized silver nanoparticles
b) chemically synthesized silver nanoparticles



(a)



(b)

FIGURE 5. The antibiofilm effect of
a) biologically synthesized silver nanoparticles
b) chemically synthesized silver nanoparticles

Antialgal effects of biosynthesized and chemically synthesized silver nanoparticles against green algae *P. kessleri* were confirmed by the circular inhibition zone formed around the swabs impregnated with 25 µl of Bio-AgNPs and Chem-AgNPs colloid solution (Fig.5).

The tests of algae *P. kessleri* cells inhibition on agar plates demonstrated that biologically synthesized AgNPs caused strong cells eradication around the swabs impregnated with Bio-AgNPs (Fig. 5(a)). Around the swabs impregnated with Chem-AgNPs, double zone of inhibition was observed (Fig. 5(b)). On the agar plates, direct contact of the nanoparticles with the wall of the cells was allowed, where the size of the AgNPs also played a role in inhibiting the biofilm formation. The partial inhibitory effect – double zone of inhibition of the chemically synthesized nanoparticles is probably due to their bigger nanoparticles sizes, compared to biologically synthesized nanoparticles. In

previous studies, the authors have argued that smaller silver nanoparticles are easier to get into bacterial cells, and so nanosilver can directly interacts with intracellular structures what cause cells damage. [11, 13] have studied the interaction of silver nanoparticles with bacteria cells. They have found that nanoparticles with bigger sizes were not able to pass through pores, but aggregates formation might act as a binding agent between cells and cause inhibition growth of algal cells.
Since microscopic unicellular organisms, like microalgae, are the first target for most of the pollutants present in aquatic, and they whole are damaged [31], we also focused on algal cell´s viability determined in the aquatic environment. Cells of algae *P. kessleri* were exposed for 30 hours in control media, and in media with biologically and chemically synthesized AgNPs (concentration of Ag 1mg/l) what is plotted in Fig. 6.

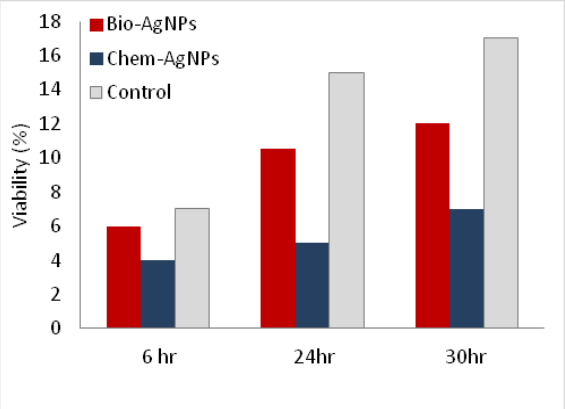


FIGURE 6. Comparison of viability of algae cells (at algal suspension 10^5 CFU/ml) in three growing aquatic systems (*P. kessleri* exposed to biologically synthesized silver nanoparticles, *P. kessleri* exposed to chemically synthesized silver nanoparticles and *P. kessleri* in aquatic medium without AgNPs) for 30h.

As it can be seen, the growths of green algae *P. kessleri* in aqueous media were adversely affected by presence of AgNPs in growing media. The cells growth reduction after 30 h was 30% and 60% for algal exposure to Bio-AgNPs and Chem-AgNPs, respectively. The chemically synthesized silver nanoparticles were significantly more toxic to *P. kessleri* compared to the biologically synthesized nanoparticles. In the aquatic medium where AgNPs were not able to interact directly with the cell walls, biologically synthesized silver nanoparticles showed that they had a better environmental impact on aquatic green algae. It is very likely that due to natural compounds of biomass responsible for the process of AgNPs formation and stabilization biologically synthesized-AgNPs are less toxic and eco-friendlier in aquatic system than chemically synthesized silver nanoparticles [16, 20, 32, 33].
Further studies have explored the major changes that nanoparticles undergo in the environmental and biological media. They pointed, that silver nanoparticles in water media, due to lose some of the coating molecules by water and by other molecules in water media, becomes unstable and undergo aggregation. Later silver atoms on the surface of nanoparticles can be oxidized to silver oxide, which can interact with molecular oxygen and so release Ag ions. Thus nanoparticles as individual particles or as agglomerates can be viewed not only as a source of toxic Ag ions through the slow-release process, but also as a source of surface coating molecules [34, 35, 36].

4 Conclusion

In summary, Bio-AgNPs prepared by reducing method using biomass of algae *P. kessleri* showed, better antibiofilm activity on agar plates than Chem-AgNPs, what was probably caused by direct contact of AgNP cells on agar plates and simultaneously by smaller particle sizes of Bio-AgNPs. At the same time Bio-AgNPs containing biomolecules on their surface proved to be less toxic in the aquatic environment for green algae than Chem-AgNPs. This knowledge confirmed that not only AgNPs cause environmental hazard but also citrate as a reducing agent enhances their biological hazard in aquatic environment. Therefore, the eco-friendly Bio-AgNPs may have precedence option for preparation materials to prevent the biofilm inhibition caused by freshwater algae. It is noteworthy that algae inhibition is depended on the way, how silver nanoparticles were prepared and also what type of surface coating was used.

Acknowledgments

The work was fully supported by the grant from the Slovak National Grant Agency under the VEGA Project 1/0197/15.

References:

[1] B. Nowack, J.F. Ranville, S. Diamond, J.A. Gallego-Urrea, C. Metcalfe, J. Rose, N. Horne, A.A. Koelmans, S.J. Klaine, Potential scenarios for nanomaterial lelease and subsequent alternation in the environment, *Environmental Toxicology and Chemistry*, 31 (1), 2012, pp. 50-59.

[2] M. Chen, Q. Yu, H. Sun, Novel strategies for the prevention and treatment of biofilm related infections, *International Journal of Molecular Sciences*, 14, 2013, pp. 18488-18501.

[3] A. Melayie, W.J. Youngs, Silver and its application as an antimicrobial agent, *Expert Opinion on Therapeutic Patents*, 15, 2005, pp. 125-130.

[4] D. Inbakandan, C. Kumar, L. Stanley Abraham et al., Silver nanoparticles with anti-micro fouling effect: A study against marine biofilm forming bacteria, *Colloids and Surfaces B: Biointerfaces*, 111, 2013, pp. 636-643.

[5] B.Nowack, H.F. Krug, M. Height, 120 years of nanosilver history: implications for policy makers, *Environmental Science and Technology*, 45, 2011, pp. 1177-1183.

[6] J.K. Schluesener, H.J. Schluesener, Nanosilver: application and novel aspects of toxicity, *Archives of Toxicology*, 87, 2013, pp. 569-576.

[7] S.A.H. Jalali and A.R. Allafchian, Assesment of antibacterial properties of novel silver nanocomposite, *Journal of the Taiwan Institute of Chemical Engineers*, 59, 2016, pp.506-513.

[8] A. Gitipour, S. W. Thiel, K. G. Scheckel, T. Tolaymat, Nanosilver as a disinfectant in dental unit waterlines: Assessment of the physicochemical transformations of the AgNPs, *Science of the Total Environment* 557-558, 2016, pp. 363–368.

[9] A.Reidy, A. Haase, A. Luch, K.A. Dawson, I. Lynch, Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications, *Materials*, 6, 2013, pp. 2295-2350.

[10] J.Wang, W.-X. Wang, Low bioavailability of silver nanoparticles presents trophic toxicity to marine medaka *(Oryzias melastigma)*, Environmental Science & Technology, 48, 2014, pp. 8152-8161.

[11] I. Moreno-Gariido, S. Pérez, J. Blasco, Toxicity of silver and gold nanoparticles on marine microalgae, *Marine Environmental Research*, 111, 2015, pp 60- 73.

[12] A.M. Fayaz, K. Bajali, M. Girilal, R. Yadav, P.T. Kalaichelvan, R. Venketesan, Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria, *Nanomedicine and Nanotechnology*, 6, 2010, pp. 103-109.

[13] A. Oukarroum, S. Bras, F. Perreault, R. Popovic, Inhibitory effects of silver nanoparticles in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*, *Ecotoxicology and Environmental Safety*, 78, 2012, pp. 80-85.

[14] OECD WPMN, Dossier development plan: Silver nanoparticles, *ENV/CHEM/NANO, ADD4*, 4, 2009.

[15] C.Mason, S. Vivekanandhan, M. Misra, A.K. Mohanty, Switchgrass (*Panicum virgatum*) extract mediated green synthesis of silver nanoparticles, *World Journal of Nanoscience and Engineering*, 2, 2012, pp. 47-52.

[16] O. S. Oluwafemi, N. Vuyelwa, M. Scriba, S. P. Songca, Green controlled synthesis of monodispersed, stable and smaller sized starch-capped silver nanoparticles, *Materials Letters*, 106, 2013, pp. 332-336.

[17] K.L. Garner and A.A. Keller, Emerging patterns for engineered nanomaterials in the environment: a review of fate and toxicity studies, *Journal of Nanoparticles Research*, 16 (8), 2014, article 2503.

[18] J. Fabrega, R. Zhang, J.C. Renshaw, W.T. Liu, J.R. Lead, Impact of silver nanoparticles on natural marine biofilm bacteria, *Chemosphere*, 85 (6), 2011, pp. 961-966.

[19] D. Sharma, S. Kanchi, K. Bisetty, Biogenic synthesis of nanoparticles: A review, *Arabian Journal of Chemistry*, 2015.

[20] J. Kadukova, Surface Sorption and Nanoparticle Production as a Silver Detoxification Mechanism of the Freshwater Alga *Parachlorella kessleri Bioresource Technology*, 216, 2016, pp. 406-413.

[21] M. Girilal, V. Krishnakumar, P. Poornima et al., A comparative study on biologically and chemically synthesized silver nanoparticles induced Heat Shock Proteins on fresh water fish *Oreochromis niloticus*, *Chemosphere*, 139, 2015, pp. 461-468.

[22] K. Kavita, V.K. Singh, B. Jha, 24-Branched Δ5 sterols from *Laurencia papillosa* red seaweed with antibacterial activity against human pathogenic bacteria, *Microbial Research*, 169(4), 2014, pp. 301-306.

[23] He R., Qian X.F., Yin J., Zhu Z.K., Preparation of polychrome silver nanoparticles in different solvents, *Journal of Materials Chemistry*, 12, 2002. pp. 3783–3786.

[24] M.A. Noginov, G. Zhu, M. Bahoura, J. Adegoke, C. Small, B.A. Ritzo, V.P. Draciiev, V.M. Siialaev, The effect of gain and absorption on surface plasmons in metal nanoparticles, *Applied Physics B*, 86, 2007, pp.455-460.

[25] X.-F. Zhang, Z.-G. Liu, W. Shen, S. Gurunathan, Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches, *International Journal of Molecular Sciences*, 17 (9), 2016: 1534.

[26] Guidance Manual for the Testing of Manufactured Nnomaterials, *ENV/JM/MONO, REV*, 20, 2009.

[27] Sh. Sohrabnezhad, M. Rassa, A. Seifi, Green synthesis of Ag nanoparticles in montmorillonite, *Materials Letters*, 168, 2015, pp. 28–30.

[28] O. Velgosová, A. Mražíková, R. Marcinčáková, Influence of pH on green synthesis of Ag nanoparticles, *Materials letters*, 180, 2016, pp. 336-339.

[29] L. Biao, S. Tan, Y. Wang, X. Guo, et al., Synthesis, characterization and antibacterial study on the chitosan-functionalized Ag nanoparticles, *Materials Science and Engineering C*, 76, 2017, pp. 73–80.

[30] P.C. Lin, S. Lin, P.C. Wang, R. Sridhar Techniques for physicochemical characterization of nanomaterials. Biotechnology Advances, 32, 2014, pp. 711–726.

[31] K. Schrimer, R. Behra, L. Sigg, M.J.-F. Suter, Ecotoxicological aspects of nanomaterials in the aquatic environment, *Safety Aspects of Engineered Nanomaterials*, 2013, pp. 137-158. (ISBN: ISBN 978-981-4364-85-0)

[32] V. Patel, D. Berthold, P. Puranik, M. Gantar, Biofabrication and characterization of silver nanoparticles using aqueous extract of seaweed *Enteromorpha compressa* and its biomedical properties, *Biotechnology Reports*, 5, 2015, pp. 112-119.

[33] P.D. Shankar, S. Shobana, I. Karuppusamy A. Pugazhendhi, V.S. Ramkumar, S. Arvindnaraydan, A review on the biosynthesis of metallic nanoparticles (gold and silver) using bio-components of microalgae: Formation mechanism and applications, *Enzyme and Microbial Technology*, 95, 2016, pp. 28-44.

[34] J.M. Unrine, B.P. Colman, A.J. Bone, A.P. Gondikas and C.W. Matson, Biotic and abiotic interactions in aquatic microcosms determine fate and toxicity of Ag nanoparticles. Part 1. Aggregation and dissolution. *Environmental Science and Technology*, 46, 2012, pp. 6915-6924.

[35] Y. Li, W. Zhang, J. Niu, Surface-coating-dependent dissolution, aggregation, and reactive oxygen species (ROS) generation of silver nanoparticles under different irradiation conditions, *Environmental Science and Technology*, 47, 2013, pp. 10293–10301.

[36] D. McShan, P.C. Ray, H. Yu, Molecular toxicity mechanism of nanosilver, *Journal of food and drug analysis*, 22, 2014, pp. 116-127.