Removal of Pathogenic Bacteria from Wastewater using Silver Fungal Nanoparticles

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Abstract

Nanotechnology is rapidly progressing and has been proven to be more successful than traditional water treatment methods, allowing for the safe use of unusual water sources. Fungi are more versatile in terms of proliferation and metal tolerance than bacteria. The goal of this study is to show that silver nanoparticles may be synthesised outside of the cell. *Penicillium Citreonigum Dierck* and *Scopulaniopsos brumptii Salvanet-Duval* are two filamentous fungi found in Lake Michigan. Burullus uses UV–vis spectroscopy and transmission electron microscopy to study biosynthesized nano-silver particles (TEM). Fourier transform infrared (FTIR) analysis was used to identify a functional group of protein molecules surrounding AgNPs. At two doses (550.7 and 676.9 mg/l), test the bactericidal effectiveness of biosynthesized silver nanoparticles, as well as their long-term interactions with bacteria (15, 60 and 120 min). To eliminate dangerous microorganisms in contaminated water, polyurethane foam was employed as a silver transporter and nano-silver solution. The AgNPs have exceptional antibacterial efficacy against both Gram-positive and Gram-negative bacteria.

Keywords :- AGNPs, fungi, nanoparticles, Antibacterial activity, silver, bacteria, contaminated water.

Introduction

The arrangement of safe drinking water is presently a high need to compassionate objectives and stays a fabulous worldwide challenge to the 21st century [1]. There is restricted chance of an increment in the stock of new water due to contending requests of different monetary areas. Because of a scarcity of fresh water, several countries are focusing on creating unconventional water sources such as desalination, precipitation water collection, waste water treatment, and abrasive water use [2]. *Shigella spp., Salmonella spp., Vibrio spp., E. coli,* and *Cryptosporidium spp.* are among the bacteria that can cause sickness when discovered in water. (i.e., loose stools, gastrointestinal sickness) in water assets [3]. According to the World Health Organization, all drinking water should be free of non-recognizable trash and coliform levels [4]. The World Health Organization (WHO) has shown that organochlorine sanitization procedures currently utilised in drinking water treatment may effectively control microbiological germs, but has requested that elective cycles be tracked down to effectively accomplish water sterilisation and contamination removal [5]. One of the most common applications of nanotechnology is as a quick, low-cost, free of dangerous synthetic chemicals, and a green science approach for mixing nanoparticles that is environmentally friendly [6]. The creation of materials with a thickness of less than 100 nanometres is referred to as nanotechnology [7]. Due to increased water demand, traditional water/wastewater treatment improvements are still unable of supplying ideal and safe water, there is a pressing need to employ high-level and new applications water refinement advancements such as nanotechnology to provide reasonable solutions to the challenges faced by water, to give the chance to combat new water shortages by utilising unusual water sources to boost water supply, as well as to deliver wastewater medications [1]. Because of their charge limit, nano-materials such as silver, titanium, and zinc have been readied for use in the sanitization of various watery infections caused by microbes for a long time [8]. Natural methods, as opposed to traditional made compound and actual procedures, are now a superior arrangement of the union of nano-materials [9]. Chemical techniques, on the other hand, relied on the well-known borohydride and citrate reduction processes, which are both toxic to the environment and promote nanoparticle aggregation quickly, as a result, large particles with low monodispersity are produced [10]. Biological tactics for nanoparticle formation, in contrast to earlier procedures, have attracted a lot of interest since they are safe (they don't supply harmful combinations during the cycle), cost-effective, take the least amount of time, and are close to natural norms [11]. Various live organic things, such as microorganisms, organisms, yeasts, green growth, and higher plants, are used in the biomineralization process for the production of nanoparticles. Silver, gold, platinum, manganese, selenium, iron, and other nanoparticles are accelerated using these organisms [12]. *Aspergillus flavus*, a parasitic fungus, was created to aggregate extracellular Ag nanoparticles [13]. As a result, analysts have viewed silver nanoparticles as a powerful antimicrobial against both gramme positive and gramme negative microorganisms, infections, and other creatures, which is primarily reliant on a high surface area to volume proportion with interesting physical, substance, and organic properties. It may also be readily stored on durable materials (for example, Ag/sand, Ag/zeolite, and Ag/fibre) [14,15,16,17,4]. Because of their ability to release a huge range of substances, parasites are the best up-and-comer in the metal nanoparticle combination, financial decency, leniency, and metal bioaccumulation ability; release a huge number of proteins downstream; Furthermore, different species develop fast and get polished as a result, and maintaining them in the lab is quite straightforward [18]. A (NADH) subordinate reductase protein generated as an optional metabolite is linked to the reduction of Ag+ to silver nanoparticles Ag. *Fusarium oxysporum* generated silver nanoparticles in a simple protein test [19], although the results were inconclusive. The current evaluation tries to accomplish four objectives. (1) The extracellular union of silver nanoparticles was demonstrated using two filamentous organisms from wastewater, *Penicillium Citreonigum Dierck* and *Scopulaniopsos brumptii Salvanet-Duval*. (2) UV–vis spectroscopy and Fourier change infrared spectroscopy were used to examine biosynthesized nano-silver particles (FTIR). (3) In several focal areas, investigate the antibacterial activity of biosynthesized silver nanoparticles. (4) Using polyurethane foam as silver transporters and nano-silver solutions, remove hazardous microorganisms from contaminated water.

Nanoparticle Synthesis by Bacteria

The use of prokaryotes as a source of metallic nanoparticles has been widely investigated. Bacteria are abundant in the environment and have the capacity to adapt to a variety of situations. They're also easy to cultivate and maintain, simple to nurture and manage. Temperature, for example, affects the rate of growth. Controlling oxygenation and incubation time is simple. According to research, by changing the pH of the growth medium was found by He et al. During incubation, nanoparticles of various sizes are produced. [20] It's crucial to be able to control such attributes because they might change over time.

Nanoparticles of various sizes are required for many purposes, including optics, catalysts, and antimicrobials are just a few examples, Palladium with zero valent has just been discovered. *Bacillus licheniformis* has been discovered to generate AgNPs found within cells [21]. The culture's colour following the inclusion of the presence of AgNPs caused silver ions to become a dark brown colour [21].

Kalimuthu and colleagues demonstrated that the nanoparticles were formed of Ag and were well diffused in solution. However, as a result of the intracellular creation of the nanoparticles, Kalimuthu & al. they had to add an additional extraction stage to their procedure. Pugazhenthiran and colleagues when they tried to make biological organisms, they got a similar outcome. *Bacillus sp*. was revealed to create intracellular AgNPs, were sub-cultured in medium containing $AgNO_3$. The reaction was gradual, and it took 7 days to complete [22].

Nanoparticle Synthesis by Fungi

Fungi have sparked a lot of interest in manufacturing metallic nanoparticles since they have several benefits over bacteria when it comes to nanoparticle creation. The simplicity with which scaling and downstream processing may be accomplished, as well as the economic feasibility and presence of mycelia, are all factors to consider, which gives a bigger surface area, are all important factors to consider [23]. Mukherjee et al. further suggested that because fungi produce far more proteins than bacteria, nanoparticle manufacturing productivity will rise [24].

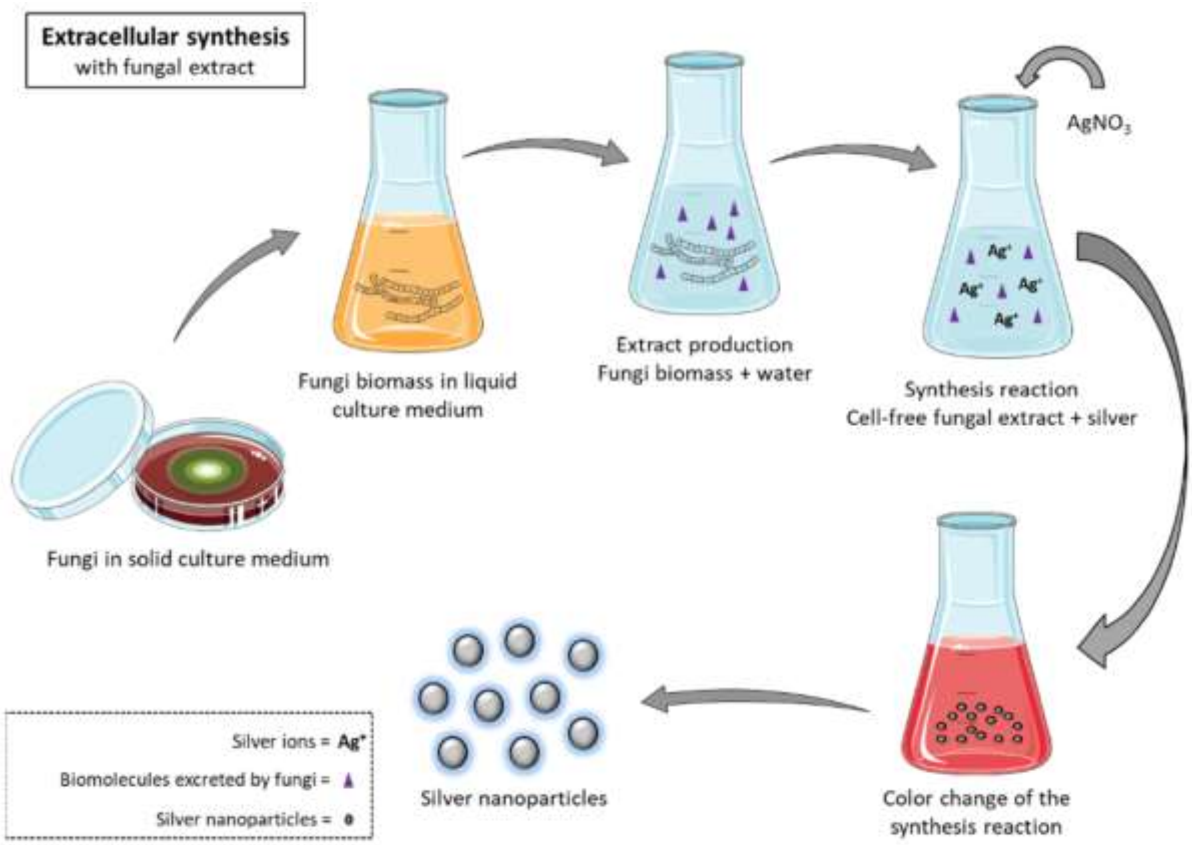

Fig.1 Extracellular Synthesis of Fungi by AgNPs

Silver nanoparticles (AgNPs)

Bacteria are known to produce both extracellular and intracellular inorganic components that can be utilised to make silver nanoparticles [25]. The *Pseudomonas stutzeri AG259* strain obtained from a silver mine provided the first evidence for AgNP synthesis by bacteria [26]. AgNPs are made by combining bacterial biomass with silver nitrate solution (AgNO3) under certain pressure and temperature conditions. The method includes bacterial bio-reduction, in which reductase enzymes convert silver ions to AgNPs either intracellularly or extracellularly, depending on where the silver ions are reduced. It has to do with the NADH-dependent reductase enzyme receiving electrons from NADH while also reducing Ag ions to AgNPs [27].

For biofilm-mediated production of proteins, both intracellular and extracellular mechanisms are applicable. A metal-reducing bacteria, *Geobacter sulfurreducens*, uses it as both a terminal electron acceptor and a metal-consuming capacity. For the purpose of breathing Electrons are delivered by C-type cytochromes, periplasm to the cell wall's outer membrane. As a result of the reduction, nucleation, and development of reduced atoms, in the production of nanoparticles [28].

Toxicity of AgNPs

AgNPs are a kind of metal nanoparticle that may be found in a wide range of applications. Because of their broad-spectrum bactericidal, fungicidal, and antiviral action, they are used in a variety of consumer items as well as medicinal applications. AgNPs have been demonstrated to penetrate through biological membranes and enter cells due to their tiny size, producing toxicity at various amounts depending on the organisms impacted. Shape, size, concentration, aggregation, chemical coating, surface charge, and the procedures utilised for their production (biological, physical, or chemical approaches) will all have a direct impact on AgNP toxicity [29, 30]. The toxicity of AgNPs is also affected by the number of targeted live creatures or test species, which is linked to the organism's defensive systems for removing unwanted substances. Furthermore, the culture medium to which the organisms are exposed will alter the response in toxicity testing [31].

Both AgNPs and Ag+ released from the nanoparticles are thought to cause toxicity [32] by causing membrane damage, reactive oxygen species (ROS) production, protein oxidation and denaturation, mitochondrial malfunction, DNA damage, and cell proliferation suppression [33,34]. Because silver and sulphur have a high affinity, McShan et al. [35] suggest that the interaction of AgNPs with proteins or other sulfur-containing macromolecules is a major toxicity mechanism, during the investigation [36].

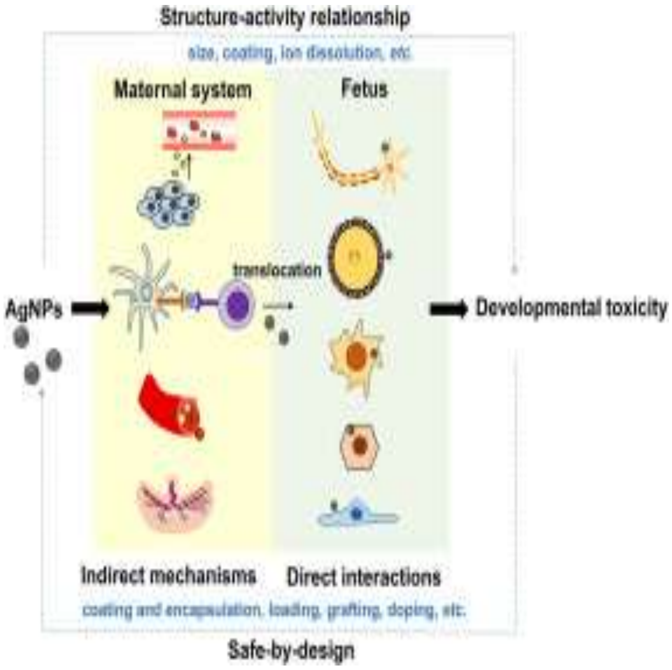

Fig. 2 Toxicity of AgNPs

Antimicrobial effect of silver Nanoparticles

Microbial species have become more prevalent as a result of their emergence and growth. With the continued focus on health-care costs and resistance to various antibiotics, several researchers have attempted to discover new, efficient antibacterial reagents that are both resistant and inexpensive. As a result of such issues and requirements, the revival in the usage of antiseptics based on silver that might be harmful connected to broad-spectrum activity and a far decreased likelihood to commit suicide.

Antibiotics are less likely to cause microbial resistance [37]. Antimicrobial surface coatings of Ag nanoparticles with amphiphilic hyperbranched macromolecules have been shown to be effective antibacterial agents by Mocking4 and colleagues [38]. Nanomaterials can also be changed for improved performance [39], efficiency in order to ease their use in many domains' biology and medicine, for example. Our research in this study, a group of specialists from several fields has looked into the antimicrobials.

Collection of samples

For this experiment, fungi cultures were employed. To generate silver nanoparticles, two fungal species (*Scopulaniopsos brumptii Salvanet-Duval* and *Penicillium citreonigum Dierck*) were grown in Sabouraud dextrose agar (SDA) media (Khan et al., 2014) [40].

Source of organism and media for growth

From Lake Burullus, two fungal strains were isolated, Individual fungal colonies were chosen and purified further by subculture on Sabouraud dextrose agar (SDA) and incubation at 28ºC. The isolates were discovered at Ain-Shams University's Faculty of Agriculture in Cairo, Egypt.

Preparation of $AgNO_3$ solutions with a concentration of 5 M

To prevent auto-oxidation of silver, 0.081 gm AgNO3 was dissolved to provide a precise solution of 5 M silver nitrate (Chemicals used were of Sigma grade, USA) in 100 ml of double sterile distilled water and storing in a Yellow (amber) coloured container [41].

Biological extracellular synthesis of Silver Nanoparticles

The preparation of biomass for *Penicillium Citreonigum Dierck* and *Scopulaniopsos brumptii Salvanet-Duval* were cultivated in a fluid medium comprising (in grammes per litre) KH_2PO_4 7.0, K_2HPO_4 2.0, $MgSO_4$ $7H_2O$ 0.1, $(NH_4)_2SO_4$ 1.0, yeast extract 0.6, and glucose 10.0. At 28 °C, a flask containing medium was incubated for eight days. The biomass was extracted and cleaned with distilled water after incubation. 6 g biomass was mixed with 100 ml deionized water and incubated for 72 hours. After incubation, the suspension was filtered using Whatman filter paper No. 1 to get the supernatant[42].

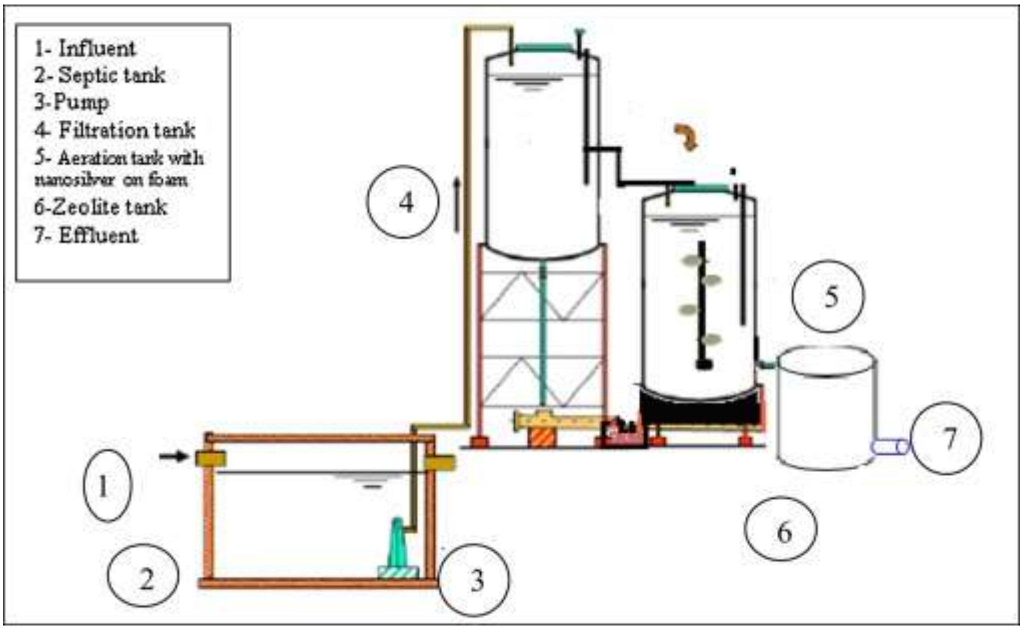

Fig. 3 Schematic diagram of the pilot-scale treatment system

Conclusion

It may be argued that one of the most significant aspects of nanotechnology is the development of dependable, environmentally acceptable procedures for the production of nanomaterials. *Penicillium Citreonigum Dierck* and *Scopulaiopsos brumptii Salvanet* were used to synthesise silver nanoparticles, which were found to be quite affordable, devoid of harmful chemicals, safe, and environmentally friendly. For remote populations, this technology can provide full antimicrobial treatments. The number of bacteria must be reduced to zero at a concentration of 676.9 mg/l and contact durations of 15 and 120 minutes. All sorts of bacteria, including gramme positive and gramme negative bacteria, may be destroyed using nano-silver coated foam.

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Conflicts of interest

The authors declare no conflicts of interest.

References

[1] Qu, X.L., Brame, J., Li, Q., Alvarez, J.J.P., 2013. Nanotechnology for a safe and sustainable water supply: enabling integrated water treatment and reuse. Acc. Chem. Res. 46 (3)

[2] Gikas, P., Angelakis, A.N., 2009. Water resources management in Crete and in the Aegean Islands, with emphasis on the utilization of nonconventional water sources. Desalination 248 (1–3), 1049–1064

[3] Lukhele, l.P., Krause, R., Mamba, B., Momba, M., 2010. Synthesis of silver impregnated carbon nanotubes and cyclodextrin polyurethanes for the disinfection of Water Res. Comm. 36 (4), 4.

[4] Jain, P., Pradeep, T., 2005. Potential of silver nanoparticle-coated polyurethane from as an antibacterial water filter. Biotechnol. Bioeng. 90, 59-63.

[5] World Health Organization (WHO), 2008. Water Quality, Incorporating 1st and 2nd addenda, 1 (3).

[6] Prashant, S., Raja, R.B., 2011. Biological synthesis and characterization of silver nanoparticles using the fungus Trichoderma harzianum. Asian J Exp. Biol. Sci. 2, 600-605.

[7] Narayanan, B.K., Natarajan, S., 2010. Biological synthesis of metal nanoparticles by microbes. Adv. Colloid Interface Sci. 156, 1-13.

[8] Amin, M.T., Alazba, A.A., Manzoor, U., 2014. A removal of pollutants from water/wastewater using different types of nanomaterials. J. Adv. Mater. Sci Eng., 24, Article ID 825910.

[9] Parashar, U, K., Preeti, S.S., Anchal, S., 2009. Bioinspired synthesis of silver nanoparticles. Dig. J. Nanomater. Biostruct. 4 (1), 159-166.

[10] Mukherjee, P., Roy, M., Mandal, B.P., Dey, G.K., Mukherjee, P.K., Ghatak, J., Tyagi, A.K., Kale, S.P., 2008. Green synthesis of highly stabilized nanocrystalline silver particles by anon-pathogenic and agriculturally important fungus T. asperellum. IOP Publ. Nanotechnol. J. 9, 075103 (7 pp).

[11] Arya, V.M 2010. Living systems eco-friendly nanofactories. Dig J. Nanomater. Biostruct. 5 (1), 9-21.

[12] Saifuddin, N., Wong, C.W., Yasumira, A.A.N., 2009. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave-irradiation. E.J. Chem. 6 (1), 61-70.

[13] Vighneshwaan, N., Kathe, A.A., Vardadarajan, P.V., Nbachane, R.P., Bala Subramanian, R.H., 2007. Silver-protein (core-shell) nanoparticle production using spent mushroom substrate. Langmuir 23, 7113-7117.

[14] Duran, N., Marcato, P.D., De Conti, R., Alves, O.L., Costa, F.T.M., Brocchi, M., 2010. Potential use of silver nanoparticles on pathogenic bacteria, and their toxicity and possible mechanisms of action. J. Braz. Chem. Soc. 21, 949-959.

[15] Nair, A.S., Pradeep, T., 2007. Extraction of chlorpyrifos and malathion from water by metal nanoparticles. J. Nanosci. Nanotechnol. 7, 1871-1877.

[16] Nangmenyi, G., Yue, Z., Mehrabi, S., Mintz, E., Economy, J., 2009. Synthesis and characterization of Ag nanoparticles impregnated fiberglass and utility in water disinfection. J. Nanotechnology 20, 495-505.

[17] Piksova, K., Weiserova, M., Jedlickova, A., Fojitik, A., 2009. Silver nanoparticles and their bactericidal effect. J. Nano Con. 10, 20-22.

[18] Sastry, M., Ahmad, A., Islam Khan, M., Kumar, R., 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. J. Curr. Sci. 85, 162-170. Castro-Longoria, E., Moreno-Velasquez, S.D., Vilchis-Nestor, Arenas-Berumen, E., Avalos-Borjra, M., 2012. Production of platinum nanoparticles and nanoaggregates using Neurospora crassa. J. Microbiol. Biotechnol. 22, 1000-1004.

[19] Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, Kumar, R, Sastry, M., 2003a. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids Surf. B: Biointerfaces 27, 313. Duran, N., Marcato, P.D.Alves, O.L., de Souza, G.I.H., Esposito, E., 2005. Mechanistic aspects of biosynthesis of silver nanoparticles by several a strain. J. Nanobiotechnology. 3.

[20] He S, Guo Z, Zhang Y, Zhang S, Wang J, et al. (2007) Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata. Materials Letters 61: 3984-3987.

[21] Kalimuthu K, Suresh Babu R, Venkataraman D, Bilal M, Gurunathan S (2008). Biosynthesis of silver nanocrystals by Bacillus licheniformis. Colloids Surf B Biointerfaces 65: 150-153.

[22] Pugazhenthiran N, Anandan S, Kathiravan G, Udaya Prakash NK, Crawford S, et al. (2009) Microbial synthesis of silver nanoparticles by Bacillus sp. Journal of Nanoparticle Research 11: 1811-1815.

[23] Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar S, et al. (2001) Fungus Mediated Synthesis of Silver Nanoparticles and Their Immobilization in the Mycelial Matrix- A Novel Biological Approach to Nanoparticle Synthesis. Nano Letters 1: 515-519.

[24] Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27: 76-83.

[25] Rafique, M., Sadaf, I., Rafique, M.S., Tahir, M.B., 2016. A review on green synthesis of silver nanoparticles and their applications. Artif. Cells. Nanomed. Biotechnol. 45 (7), 1272e1291.

[26] Jorge de Souza, T.A., Rosa Souza, L.R., Franchi, L.P., 2019. Silver nanoparticles: an integrated view of green synthesis methods, transformation in the environment, and toxicity. Ecotoxicol. Environ. Saf. 171, 691e700.

[27] Javaid, A., Oloketuyi, S.F., Khan, M.M., Khan, F., 2018. Diversity of bacterial synthesis of silver nanoparticles. Bionanoscience 8, 43e59. BioNanoScience 8 (1).

[28] Tanzil, A.H., Sultana, S.T., Saunders, S.R., Dohnalkova, A.C., Shi, L., Davenport, E. Ha, P., Beyenal, H., 2016. Production of gold nanoparticles by

electrode-respiring Geobacter sulfurreducens biofilms. Enzyme. Microbe. Technol. 95, 69e75.

[29] *N. Durán, M. Durán, C.E. de Souza, Silver and Silver Chloride Nanoparticles and their Anti-Tick Activity: a Mini Review, J. Braz. Chem. Soc. 28 (2017) 927-932.*

[30] *M. Akter, M.d.T. Sikder, M.d.M. Rahman, A.K.M.A. Ullah, K.F.B. Hossain, S. Banik, T. Hosokawa, T. Saito, M. Kurasaki, A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives, Adv. Res. 9 (2018) 9: 1–16.*

[31] *S. Lekamge, A. Miranda, A. Abraham, V. Li, R. Shukla, V. Bansal, D. Nugegoda, The Toxicity of Silver Nanoparticles (AgNPs) to Three Freshwater Invertebrates with Different Life Strategies: Hydra vulgaris, Daphnia carinata, and Paratya australiensis. Frontiers in Environmental Science, 6 (2018).*

[32] *N. Duran, M. Duran, M.B. Jesus, A B. Seabra, W. J. Favaro, G. Nakazato, Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity, Nanomedicine: NBM 12 (2016) 789–799.*

[33] *C. Liao, Y. Li, S. Tjong, Bactericidal and Cytotoxic Properties of Silver Nanoparticles, Int. J. Mol. Sci, 20 (2019) 449.*

[34] *T. Zhang, L. Wang, Q. Chen, C. Chen, Yonsei, Cytotoxic Potential of Silver Nanoparticles, Med. J. 55 (2014) 283-291.*

[35] *D. McShan, P. Ray, H. Yu, Molecular toxicity mechanism of nanosilver. J. Food Drug. Anal, 22 (2014), 116–127.*

[36] *W. Rolim, J. Pieretti, D. Reno, B. Lima, M. Nascimento, F. Ambrosio, C. Lombello, M. Brocchi, ACS. de Souza, A.B. Seabra, Antimicrobial activity and cytotoxicity to tumor cells of nitric oxide donor and silver nanoparticles containing PVA/PEG films for topical applications, ACS Appl. Mater. Interfaces, 11 (2019) 6589-6604.*

[37] *Jones SA, Bowler PG, Walker M, Parsons D. Controlling wound bioburden with a novel silver-containing Hydrofiber dressing. Wound Repair Regen 2004;12(3):288 - 94.*

[38] *Aymonier C, Schlotterbeck U, Antonietti L, Zacharias P, Thomann R, Tiller JC, et al. Hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibiting antimicrobial properties. Chem Commun (Camb) 2002; 24:3018 - 9.*

[39] *Mirkin CA, Taton TA. Semiconductors meet biology. Nature 2000; 405:626 - 7.*

[40] *Khan, R.H., Yasmeen, K., Kishor, K., 2014. Biological synthesis and characterization of silver nanoparticles from Fusarium oxysporum. Pelagia Research Library. Der Pharm. Sin. 5, 112–117.*

[41] *Maliszewska, I., Puzio, M., 2009. Extracellular biosynthesis and antimicrobial activity of silver nanoparticles. Proceedings of the III National Conference on Nanotechnology vol. 116.*

[42] *Maliszewska, I., and M. Puzio. "Extracellular biosynthesis and antimicrobial activity of silver nanoparticles." Acta Physica Polonica A 116 (2009): S160-S162.*