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FORMATION OF SILVER NANOPARTICLES USING BIOMASS OF BACTERIUM *ACTINOMYCES SPP.* NSX-333

Abstract. *In this article, we studied biosynthesis of silver nanoparticles using biomass (cells) of Actinomyces spp. NSX-333, isolated from the Azerbaijan soil. $AgNO_3$ solution was added to the cell culture and reaction mixture was incubated at 28 ^{0}C for 24 hours in the dark condition. Derived silver nanoparticles were characterized using UV-visible spectroscopy, Scanning Electron Microscopy (SEM) micrographs and Energy Dispersive Analysis (EDS) methods.*
Keywords: *Actinomyces spp., biomass, biosynthesis, silver nanoparticles.*

Introduction. Currently, nanoparticles find a wide application in medicine (synthesis, delivery and disposal of drugs, cancer treatment), biology (immune research, use of nanoparticles as biomarkers in the study of intracellular processes) and technology (electronics, information technology, obtaining new materials with improved properties) [4, 6]. There are a lot of publications about the biological synthesis of gold, silver, selenium, platinum, quartz and other compounds with bacteria, antinomycetes, fungi and yeasts, as an active search of effective bioobjects is conducted for obtaining nanoparticles of different chemical nature [1, 2, 5, 7]. However, despite the stability, biologically produced nanoparticles are not homogeneous and the synthesis is rather slow. To overcome these problems, a comprehensive study of all the factors affecting this process is necessary [6, 8].

A lot of physico-chemical methods have been developed for the synthesis of nanoparticles. However, despite their successful application, these methods often remain expensive and require the use of hazardous chemical compounds. Therefore, for the synthesis of various nanoparticles using microorganisms,there is a need to develop effective methods safe for the environment and human [9-11].

We have previously shown silver nanoparticles formation using the cell-free culture fluid of *Actinomyces spp*.NSX-333[3]. The purpose of this work was to obtain silver nanoparticles using the biomass of *Actinomyces spp.*NSX-333.

Materials and methods. The strain *Actinomyces spp.* NSX-333, isolated from the soil, was used as an object. The pure culture of actinomycete was stored in a microbial culture collection of Baku State University.

To study the ability of the actinomycete to form silver nanoparticles, the culture was grown on the Gauze liquid mineral medium with the following composition (g /l): soluble starch -20; K_2HPO_4-0.05; KNO_3-0.1; $FeSO_4$-0.005; $MgSO_4$-0.05; agar-20.The liquid media was inoculated with the actinomycete culture and incubated at 28 °C for 7-14 days. The biomass was separated from the culture medium by centrifugation and to remove traces of nutrients, it was washed three times with sterile distilled water. After washing, the biomass was resuspended in 100 ml of sterile distilled water and incubated at 28 °C for 24 hours. Then, the suspension was filtered and obtained biomass was subsequently used for the synthesis of nanoparticles.

To synthesize silver nanoparticles, 10 g of wet biomass was mixed with 100 ml of an aqueous solution of 1 mM silver nitrate ($AgNO_3$) and incubated in a thermostat at 28 °C under dark conditions for 7, 14, and 28 days.The control without $AgNO_3$ was kept under the same conditions as described above.

At the end of the incubation, the biomass was separated by filtration and liquid was analyzed on a SPECORD 250 plus spectrophotometer (Germany). The size and morphological characteristics of silver nanoparticles were examined on a scanning electron microscope JEOLJSM-7600F (Japan). EDS analysis of the silver nanoparticles was also carried out.

Results. The formation of silver nanoparticles in the reaction mixture can first be visually determined from the color change of the solution, inoculated with biomass. Color change was observed 15-20 days later. The color of the suspension, which does not contain silver ions, was remained pale yellow. The reaction mixture changes its color to dark brown in the presence of silver ions (Fig. 1). The color change during the reaction from pale yellow to dark brown confirms the cellular (biomass) formation of nanoparticles.

Spectrophotometric analysis of the biomass filtrate of *Actinomyces spp*. NSX-333 showed weak absorption at a wavelength of 420 nm, for a culture incubated 7, 14 and 28 days (Fig. 2).

Analysis of the sample on a scanning electron microscope showed that silver nanoparticles have a spherical shape with a size of 22.4 nm. Agglomerization of nanoparticles is observed (Fig.3).

*Fig.1. Color change of reaction mixture during formation of silver nanoparticles with the biomass of Actinomyces spp.*NSX-333: *a-experiment; b-control*

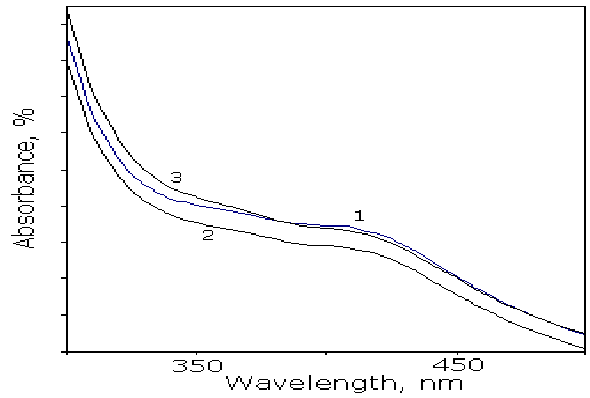

Fig. 2. UV-spectr of silver nanoparticles synthesized with the biomass of Actinomyces spp. NSX-333. 1- 28 days; 2- 14 days; 3- 7 days

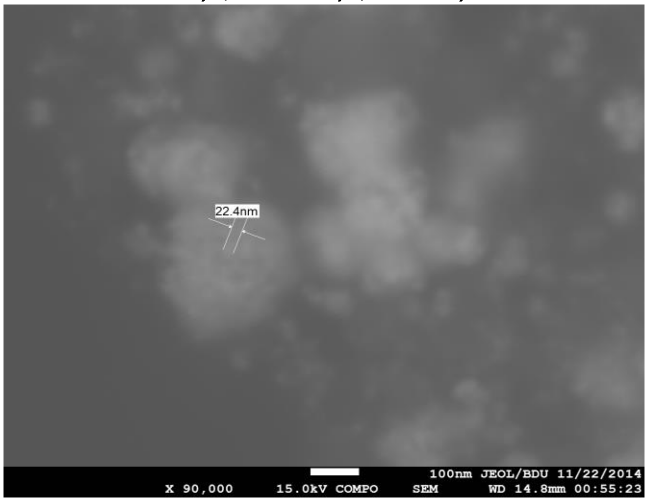

Fig.3. SEM images of silver nanopartiles, synthesized with the biomass of Actinomyces spp.NSX-333

Energy-dispersive X-ray spectroscopy of the sample showed a characteristic peak (AgLa1), indicating the presence of silver nanoparticles (Fig.4).

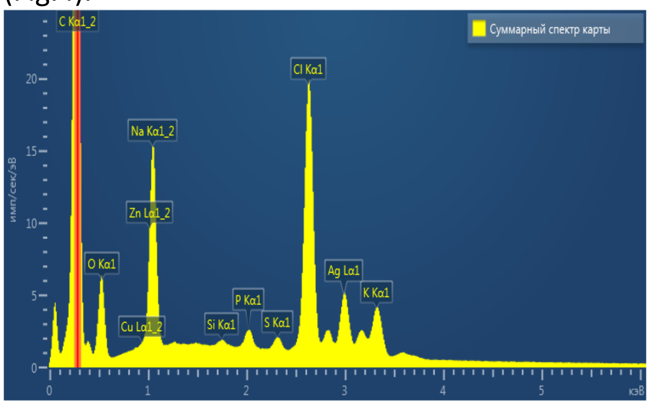

Fig.4. EDS-spectr of nanoparticles, synthesized with the biomass of Actinomyces spp.NSX-333

Discussion. It should be noted that this culture is able to form silver nanoparticles by extracellular (culture liquid). The silver nanoparticles formed by the biomass of the culture differ from the extracellularly formed silver nanoparticles by the spherical shape and stable size. So that, extracellularly formed silver nanoparticles are irregular in shape and their sizes vary from 20 to 130 nm [5].

Conclusion. Thus, spherical silver nanoparticles with a size of 22.4 nm. are formed in the presence of biomass (cells) *Actinomyces spp*. NSX-333, whereas nanoparticles of an irregular shape and with the size of 20-130 nm. are formed in the presence of a cell-free culture liquid. Futher research on the role of temperature, acidity (pH), concentration of $AgNO_3$ and other parameters will be carried out.

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