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Synthesis of Gold Nanoparticles by Blue-Green Algae Spirulina Platensis

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The synthesis of gold nanoparticles by one of the many popular microorganisms – blue-green algae Spirulina platensis was studied. The complex of optical and analytical methods was applied for investigation of experimental samples after exposure to chloroaurate ($HAuCl_4$) solution at different doses and for different time intervals. To characterize formed gold nanoparticles UV-vis Spectrometry, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Energy-dispersive analysis of X-rays (EDAX) were used. It was shown that after 1.5 – 2 days of exposure the extracellular formation of nanoparticles of spherical form and the distribution peak within the interval of 20-30 nm took place. To determine gold concentrations in the Spirulina platensis biomass neutron activation analysis (NAA) was applied.

Keywords: Gold Nanoparticles, Microorganisms, *Spirulina Platensis*, Biotechnology, Optical and Analytical Methods.

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1. INTRODUCTION

Biosynthesis of nanoparticles using microorganisms as an emerging bionanotechnology has received considerable attention due to a growing need to develop environment-friendly technologies in materials synthesis [1]. Biological production systems are of special interest due to their effectiveness and flexibility. Nanoparticles produced by a biogenic enzymatic process are far superior in biomedical applications to those produced by chemical methods [2, 3].

Gold nanoparticles play their significant role in nanotechnology due to their potential utilization in industry and medicine [4]. Blue-green microalgae *S. platensis* is widely used as a matrix for pharmaceuticals and also as a biologically active food additive for humans and animals. The ability to biotransform and endogenously add the desired essential elements (Se, I, Cr, and others) producing complexes easily assimilated by a human organism is a distinctive feature of *S. platensis*. In the present work the results of studies of Au nanoparticles synthesis by biomass of blue-green algae *S. platensis* are reported.

2. METHODS OF SAMPLE PREPARATION AND ANALYSIS

2.1 Sample preparation

A In the experiments the strain of *S. platensis* IPPAS B-256 from A. K. Timiriazev Institute for Plant Physiology of the Russian Academy of Sciences was used. The conditions of cultivation *S. platensis* cells in standard Zaroukh water-salt nutrient medium are described elsewhere[21, 23]. All chemicals used in the experiment were ACS-reagent grade, produced by Sigma (St. Louis, MO, USA).

The bacterial cells were harvested after 5-6 days cultivation and then were washed twice in distilled water. In the first series of experiments the dose dependency of the gold nanoparticles formation was study. The wet biomass of *S. platensis* (1 g) was resuspended in 250 ml Erlenmeyer flasks with 100 ml aqueous $HAuCl_4$ (gold chloroaurate) solution with different concentrations ($10^{-2} - 10^{-4}$ M) and incubated at the room temperature for 5 days being shaken continuously.

In the second series of experiments the temporal dependency of Au nanoparticles formation was studied. Again 1 g of wet biomass was resuspended in the same flasks with 100 ml 10^{-3} M aqueous $HAuCl_4$ solution and incubated at room temperature, shaking for different time intervals (1 – 6 days).

2.2 Methods

UV-vis Spectrometry

The UV-visual spectra of the samples were recorded by a spectrophotometer "Cintra 10" (GBC Scientific Equipment Pty Ltd, Australia) with digital data acquisition system, wavelength range 190 – 1100 nm.

Transmission Electron Microscopy (TEM)

TEM was performed using the JEOL SX-100 equipment (Japan) operating at 100 kV. The TEM studies were done at 50 000x magnification. Samples were prepared by placing a drop of solution with the gold nanoparticles on carbon-coated TEM grids. The films on the TEM grids were allowed to dry at room temperature before analysis.

Scanning Electron Microscopy (SEM)

The FEI Quanta 3D FEG, USA / Systems for Microscopy and Analysis, (Moscow, Russia) high-performance instrument with three modes was used for

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sample visualization.

SEM was carried out using the SDB (small dual-beam) FEI Quanta 3D FEG with the EDAX Genesis system with the resolution 1.2 nm. Operational features of the microscope used in the experiment: magnification 5000 – 150000x; voltage 1 – 30 kV.

Energy-dispersive X-rays analysis (EDAX)

Microprobe analysis of gold nanoparticles clusters was conducted with the energy-dispersive X-ray analysis spectrometer (EDAX, USA). The acquisition time ranged from 60 to 100 s, and the accelerating voltage was 20 kV.

Neutron activation analysis (NAA)

The gold concentration in *S. platensis* samples was determined by neutron activation analysis (NAA) at the nuclear research reactor SAFARI-1 of the NECSA (Nuclear Energy Corporation of South Africa), Pelindaba, South Africa. The samples were irradiated for 8 s by a neutron flux density of approximately 10^{14} n·cm^{-2}·s^{-1}. Their activities were measured three times, after cooling for 3 and 30 hours and after 7 days, respectively. The gold content was determined on the 411.8 keV γ-line of ^{198}Au.

The elemental content of *S. platensis* samples was determined using NAA at the reactor IBR–2 of FLNP, JINR, Dubna, Russia. The concentrations of elements based on short half-life radionuclides were determined by irradiation for 60 s under a thermal neutron flux density of approximately 1.6×10^{13} n·cm^{-2}·s^{-1}. The NAA data processing and determination of element concentrations were performed using Genie 2000 software.

3. RESULTS ANS DISCUSSION

The UV-vis absorption spectra of the suspension of *S. platensis* after addition of the gold chloroaurate solution in different concentrations (the dose dependence) are shown in Fig. 1. As it seen from the figure, one broad peak of gold surface plasmon resonance (SPR) appears at 530 nm for the concentrations of 10^{-3} – 10^{-4} M. The presence of the gold (SPR) peak at ~ 530 nm confirms the gold ion reduction from Au(III) to Au(0) by biomolecules/proteins and enzymes on the surface of *S. platensis* cells and aggregation of the gold nanoparticles in the solution.

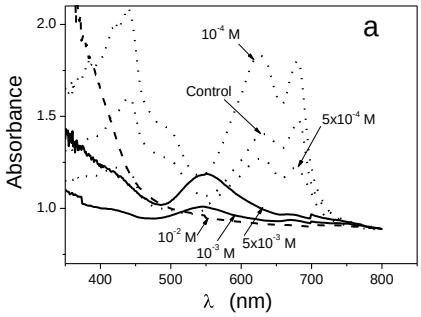


Fig. 1 – UV-vis spectra of *S. platensis* suspension for different doses of chloroaurate

Fig. 2 shows the TEM image recorded from the drop-cast film of gold nanoparticles synthesized after reaction of the chloroauric acid $5\cdot10^{-3}$ M solution with *S. platensis* biomass for 5 days. In the picture the nanoparticles of spherical and other shapes are observed.

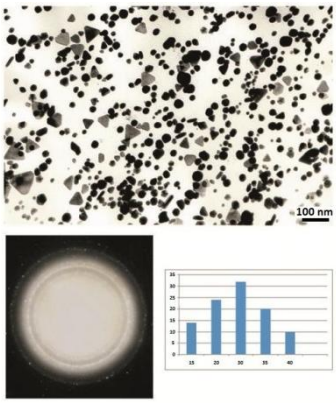
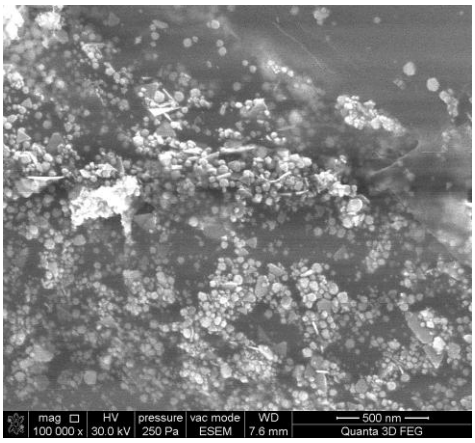


Fig. 2 – TEM image, selected area diffraction pattern and size histogram of Au nanoparticles in biomass of *S. platensis*

Fig. 3 presents the SEM image of *S. platensis* cells after interacting with gold chloroaurat at different concentrations: $5\cdot10^{-3}$ M and 10^{-2} M for 5 days. The mode of the natural environment (ESEM) allows studying moist and non-conducting samples. Since *Spirulina* cells are non-conducting, they were visualized at this mode. The SEM images for concentration $5\cdot10^{-3}$ M illustrate that most of the particles are spherical and do not create big agglomerates whereas at concentration 10^{-2} M the size of formed particles is not in a nanoscale range.



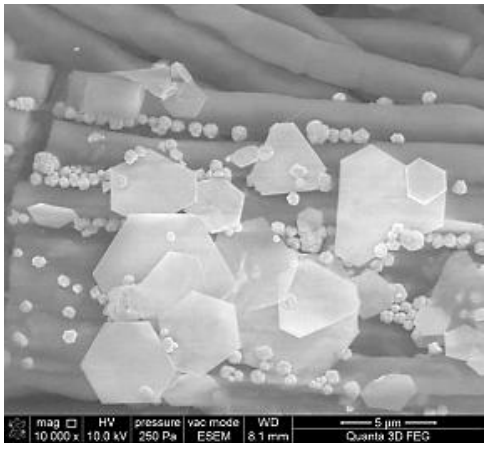


Fig. 3 – SEM pictures of gold nanoparticles in S. platensis under the different gold chloroaurate concentrations: $5\cdot10^{-3}$ M (a) and 10^{-2} M (b)

The results of NAA for Au obtained at the nuclear research reactor SAFARI-1 are presented in Fig.4a and Fig.4b. The total concentrations of gold in *S. platensis* biomass for different doses of chloroaurate are presented in Fig. 8a. Fig. 8b show that the total concentration of gold in samples (extracellular and intracellular) is rapidly increasing in the beginning and then does not change over time significantly. The other elements in the given experiment in the presence of high Au concentrations were not determined. The concentrations of the matrix elements Mg, Mn, Cl, Ca, P and the traces of U in the biomass of *S. platensis* were determined at the reactor IBR-2 based on short half-life radionuclides.

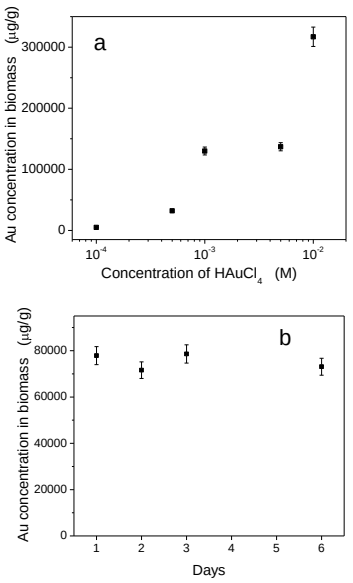


Fig. 4 – The gold concentrations in biomass of S. platensis versus the concentration of gold chloroaurate (a) and the time of ex posure to gold chloroaurate (b) determined by NAA

CONCLUSIONS

The results of the presented studies using the complex of optical and analytical methods show that *S. platensis* is capable of producing gold nanoparticles extracellularly when exposed to the gold chloroaurate solution. The shape of the majority of the nanoparticles is spherical and on average their sizes are in the range 20–30 nm. The "green route" of biosynthesis of gold nanoparticles in *Spirulina* is simple, economically viable and an eco-friendly process which offers a great advantage over an intracellular process of synthesis from the point of view of applications in medicine, pharmacology and other branch of technology.

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