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POSSIBILITIES OF PHYSICAL METHODS IN DEVELOPMENT OF MICROBIAL NANOTECHNOLOGY

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Abstract: We provide the findings of practical research in nanobiotechnology that several groups of Georgian scientists have conducted using various physical and chemical methodologies. To create the silver and gold nanoparticles, several novel terrestrial actinomycete strains were used, together with the blue-green algae Spirulina platensis, which had been previously discovered in Georgian soils and rocks. To characterise the synthesised nanoparticles and find the best conditions for synthesis, a range of spectral and analytical approaches were used. There is a detailed description of the research methodologies employed and the outcomes that were produced using them. The methods are compared based on their benefits and their ability to describe the nanoparticle manufacturing process.

Keywords: microbial synthesis, nanoparticle, gold, silver, nanotechnology, biotechnology

Introduction

One innovative biotechnological strategy that has evolved in recent years is the manufacture of nanosized materials using the physical characteristics and metabolic activities of microbial cells.1 The production of highly structured metallic nanoparticles has been made possible by a wide variety of microorganisms, including yeasts, fungi, and bacteria. To create nanoparticles, some cellular processes in microbes allow surface functional groups (peptides, proteins, nucleic acids) to interact with metal ions in water-based solutions.2- 4. Electronics, IT, catalysis, medicine, pharmacology, sensing, and photonics are just few of the many uses for gold and silver nanoparticles. Oncology, cardiology, immunology, neurology, and endocrinology are among of the medical fields that have shown therapeutic promise with these.5- 7 It is critical to find novel, simple, and environmentally safe ways to hunt for efficient microbial strains that can produce nanoparticles of gold and silver. Actinomycetes and the blue green algae Spirulina platensis are two examples of the microorganisms that have attracted a lot of attention because of the medicinal possibilities they provide. This article describes and discusses the potential outcomes of the collaborative research that Georgian scientists have conducted over many years in an effort to create biotechnological approaches.

Materials and methods

Materials

A great deal of microbiological and plant biodiversity may be found in some regions of Georgia. Microbes are of particular interest because of the enormous biotechnological potential they provide for use in medicine. Several groups of Georgian scientists worked together to examine several microorganisms that are typical of the Georgian environment. Researchers in Georgia looked at the distribution of terrestrial actinomycetes strains in a variety of rock types, soil types, and the rhizosphere. In order to create strategies for nanoparticle production, researchers looked at several groups of newly identified microbes from Georgia. Gold and silver nanoparticles for pharmaceutical and medicinal applications were also produced from the blue-green algae Spirulina platensis. Below, in Table 1, you can see the microorganisms that were investigated. You may find a detailed description of the procedures used to cultivate the examined bacterial culture and produce biomass using gold and silver nanoparticles elsewhere [14–18].

Methods

A variety of spectral and analytical methods was used to characterize the synthesized gold and silver nanoparticles.

Table 1. Studied microorganisms

Names of bacteria	Species of bacteria	Site of bacteria isolation
Arthrobacter genera	*Arthrobacter globiformis* 151B *Arthrobacter oxydans* 61B	Isolated from the Kazreti region in Georgia
Streptomyces genera	*Streptomyces glaucus* 71MD *Streptomyces sp.* 211A	Isolated from the rhizosphere of soybeans in Georgia Isolated from the Cinnamonic calcareous soil of Sagarejo region in Georgia
Extremophile bacteria	*Streptosporangium spp*. 94A	Isolated from the Black soil of Shiraki Valley in Georgia
Thermophilic actinomycetes	*Thermoactinomycete spp*. 44Th *Thermomonospora spp*. 67Th	Isolated from the red soil of Adjara Region Isolated from the cinnamonic calcareous soil of Tetritskaro region in Georgia
Blue-green alga	*Spirulina platensis*	Strain IPPAS B-256

A spectrophotometer "Cintra 10" (GBC Scientific Equipment Pty Ltd, Australia) with a wavelength range of 190 - 1100 nm was used to record the UV-vis spectra of the samples. We used a Dron-2.0 diffractometer to measure the X-ray diffraction (XRD). The radiation was produced by the BCV-23 X-ray tube that had a Cu anode (CuKα: λ = 1.54178 Å).A JEOL SX-100 (Japan) TEM system running at 100 kV was used for the transmission electron microscopy (TEM). A drop of solution containing gold or silver nanoparticles was placed on carbon-coated transmission electron microscopy grids to prepare the samples. System for Microscopy and Analysis (Moscow, Russia)/Quanta 3D FEG (United States of America) scanning electron microscopes were used for the examination. The experimental microscope's operational characteristics were a voltage range of 1–30 kV and a magnification range of 100–200000 ×. An energy-dispersive X-ray analysis spectrometer (EDAX, USA) was used to perform microprobe examination on clusters of gold and silver nanoparticles.19 The experimental samples were analysed for gold and silver using flame atomic absorption spectrometry (AAS) using "Analyst-800" and "Beckman-495" spectrometers.

The neutron activation analysis (NAA) at the reactor IBR-2 of the Frank Laboratory of Neutron Physics of the Joint Institute for Nuclear Research (Dubna, Russia) was used to assess the elemental composition of samples, concentrations of gold and silver, and other similar parameters. The experimental setup and sample irradiation protocols are detailed in another section.20 Genie 2000 was used to analyse the NAA data and determine the element concentrations.21 The biosorption process on bacterial cells was studied using equilibrium dialysis with atomic-absorption analysis, which was also used during nanoparticle synthesis.To enhance the processes involved in the formation of nanoparticles, the bacterial biomass was subjected to sonication using an ultrasonic generator at a frequency of 35 kHz for a duration of 10 to 30 minutes.

Results and discussion

The biomolecules, proteins, and enzymes found in bacterial cells undergo a reduction of metal ions during the microbial creation of metal nanoparticles in a solution of a metal compound. In an interaction between a silver nitrate (AgNO3) aqueous solution and a bacterial suspension, for instance, silver ions are reduced from Ag (I) to Ag (0), leading to the aggregation of silver nanoparticles. Similarly, in a chloroauric acid (HAuCl4) water solution, the reduction of Au(III) ions to Au(0) is the process that forms the gold nanoparticles. Detection of nanoparticles and assessment of experimental conditions for nanoparticle formation by bacterial cells were the primary uses of ultraviolet-visible UV-vis spectrometry.Nanoparticles' spectral extinction peaks are sharp because of localised surface plasmon resonances in the visible and near-infrared bands.22 When metallic nanoparticles interact strongly with incoming electromagnetic radiation, it leads to the collective excitation of conducting electrons, which in turn causes extinction.23 Absorption spectra measured using

ultraviolet-visible surface plasmon resonances showed that gold had a peak at 530 nm and silver at 425 nm. Nanoparticles were formed at concentrations ranging from 10-2 to 10-4 M in each instance to determine the best concentrations of AgNO3 and HAuCl4 in aqueous solutions. Figure 1 illustrates the results of an experiment that was conducted on Spirulina platensis biomass to produce gold nanoparticles (a) and silver nanoparticles (b) with respect to absorbance maximums against the dosage of AgNO3. The ideal concentration for bacterial manufacture of nanoparticles was determined to be 10-3 M in every instance. One thing to keep in mind is that compared to the silver nanoparticles, the spectra acquired from the gold ones are obviously more prominent.

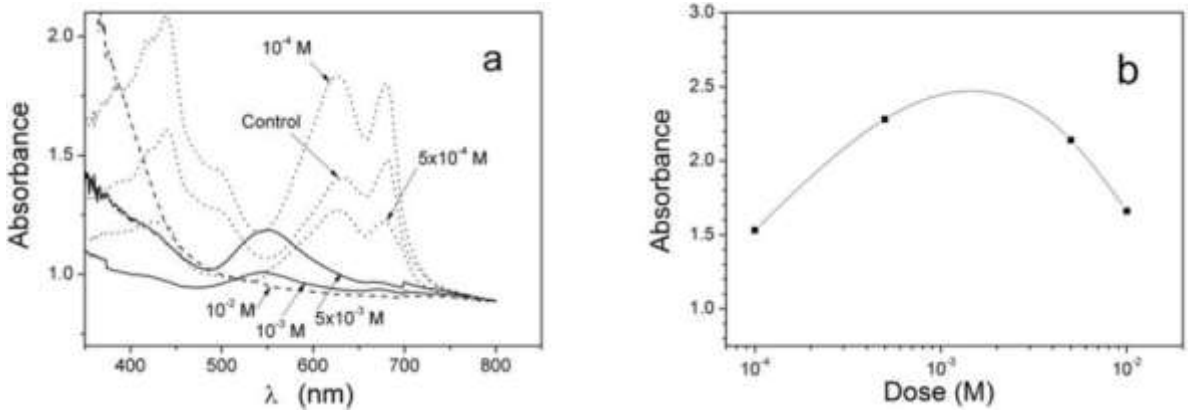

Figure 1. UV-vis spectra of gold nanoparticles in *Spirulina platensis* biomass obtained for different $HAuCl_4$ doses (a) and silver nanoparticles absorbance maximums versus the dose of $AgNO_3$ (b).

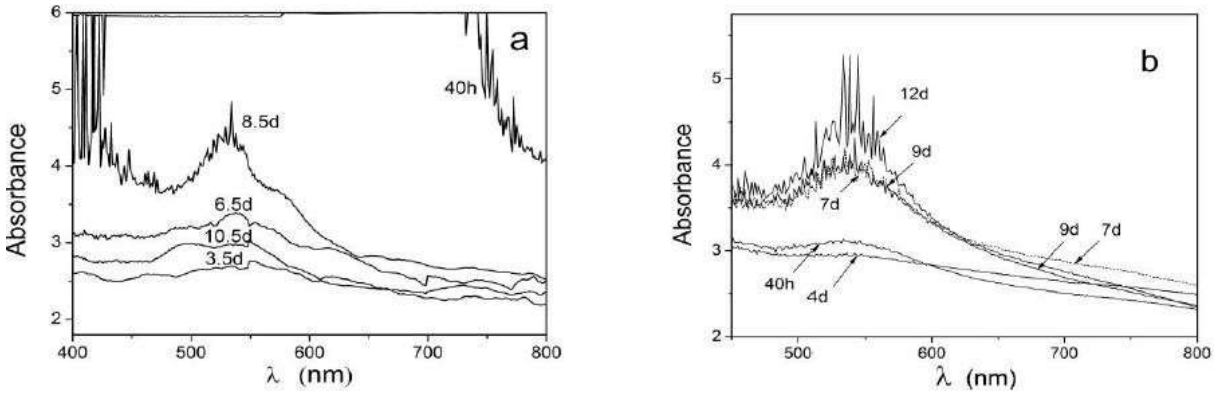

Figure 2. The absorption spectra of Au nanoparticles detected in the suspension of *Streptomyces glaucus* 71MD (a) and *Arthrobacter oxydans* 61B (b) at different time reaction with $HAuCl_4$ 10^{-3} M water solution.

The UV-vis spectra of the actinomycetes Streptomyces glaucus 71MD (a) and Arthrobacter oxydans 61B (b) in suspension were obtained at various reaction periods with a HAuCl4 10-3 M water solution, as shown in Figure 2. An absorption peak at 530 nm, corresponding to Au, appears in the given spectra and its strength grows with reaction time. Gold nanoparticles mostly have spherical forms, as seen by the shapes of these peaks. tests using biomass Streptomyces glaucus 71MD showed an optimal reaction time of hours for Au nanoparticle formation, but tests with Arthrobacter oxydans 61B showed a favourable reaction time of days.

Figure 3a shows the X-ray diffraction (XRD) spectra of gold nanoparticles in Arthrobacter oxydans 61B biomass following a 12-day reaction with 10-3 M HAuCl4 (chloroauric acid), and Figure 3b shows the XRD spectra of silver nanoparticles in Spirulina platensis biomass following a 1-day reaction with AgNO3 (silver nitrate). The four distinctive peaks (111), (200), (220), and (311) may be seen in Figure 3, which corresponds to a face centred cubic (fcc) structure of gold (or silver). Crystalline gold nanoparticles were produced by reducing Au (III) and Ag (I) ions in Spirulina platensis and Arthrobacter oxydans 61B cells, according to the

findings. The Sherrer's formula[24,25] was applied for evaluating sizes of the gold nanoparticles on the basis one of the peaks in the diffractogram for different samples:

$$d = \frac{K\lambda}{\beta \cos\theta}$$

where K is the dimensionless shape factor, for cubic crystals it is $0.9 - 1$; λ is the X-ray wavelength, for Cu K_α = 1.54178 Å; β is the line width at half the maximum intensity in radians, θ is the Bragg angle, and d is the size of nanoparticles in nm.

Grain sizes below 100 nm may be modelled using the Sherrer's formula. We used the (111) interferential maximum to get an approximation of the nanoparticle size. We have θ = 38o here. Only instrumental broadening of β (≈0.3°) was included in the calculations, and the impact of crystal defects on the form of the interferential maximum was not evaluated. This technique found that the gold nanoparticles in Arthrobacter oxydans 61B biomass had a size of around 22 nanometers. Findings from other methodologies are consistent with this one.

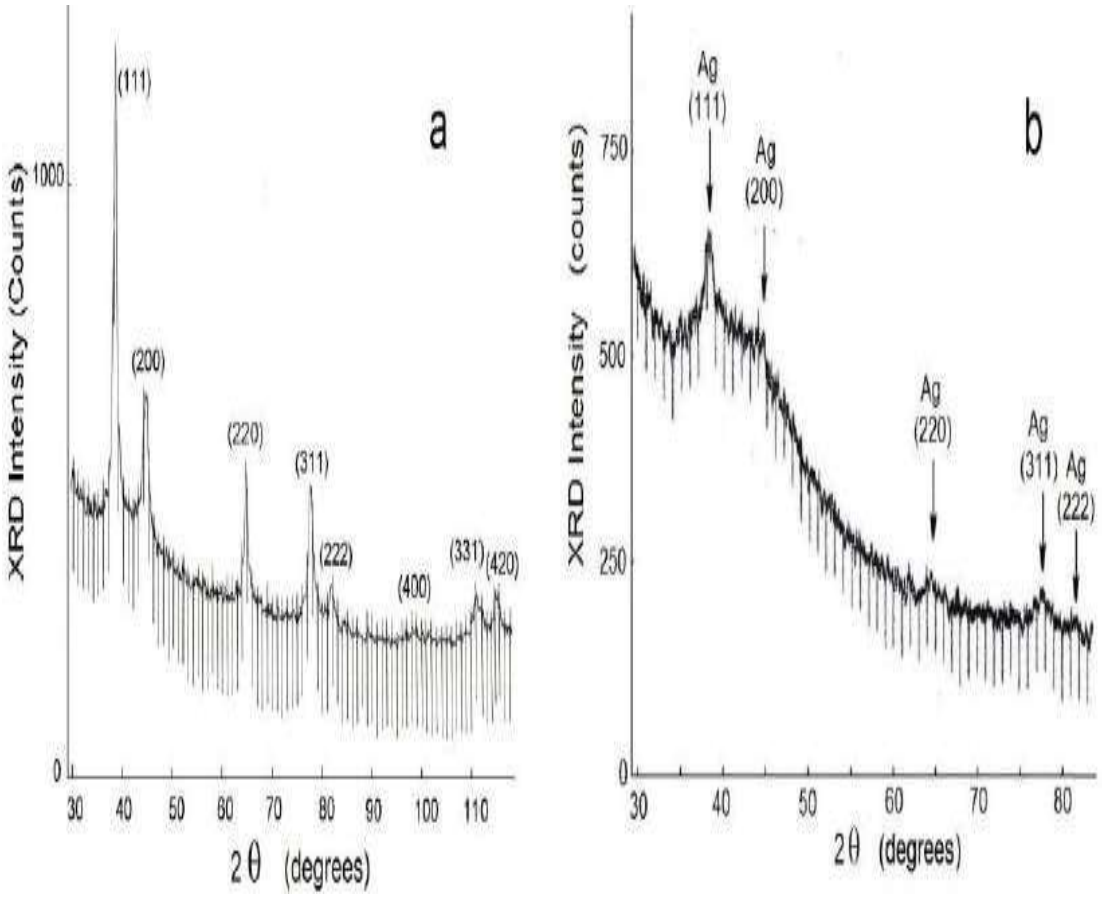

Figure 3. The XRD diffractogram for gold nanoparticles synthesized by *Arthrobacter oxydans* 61B treated with chloroauric acid for 12 days (a) and silver nanoparticles synthesized by *Spirulina platensis* treated with silver nitrate for 1 day (b).

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were carried out for visualization and approximate assessment of sizes of the formed nanoparticles.

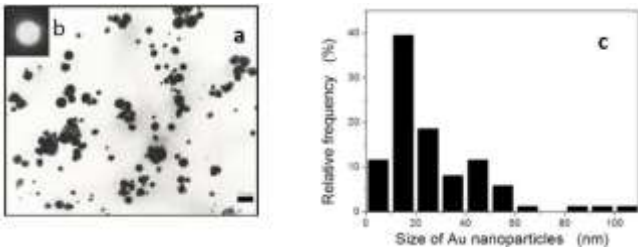

Figure 4. TEM micrographs recorded from drop-cast films of gold nanoparticles formed by the reaction of the $HAuCl_4$ solution with biomass *Thermoactinomycete spp.* 44Th after 6 days (a) the diffraction pattern of the selected area recorded from the gold nanoparticles (b) and Au nanoparticle sizes distribution (c).

The transmission electron micrograph (TEM) picture captured from the drop-cast film of gold nanoparticles synthesised after 6 days of reacting the chloroauric acid solution with 44Th biomass of Thermoactinomycete spp. is shown in Figure 4a. Figure 4b shows that the face-centered cubic (fcc) structure of gold nanoparticles is consistent with the diffraction pattern in the chosen region. With an average size of 20 nm, the particle size histogram derived from this picture (Figure 4c) reveals that the gold nanoparticles' sizes vary between 5 nm and 60 nm.Figure 5 shows scanning electron micrographs (SEMs) of silver nanoparticles (a) and gold nanoparticles (b) produced by the actinomycete Streptosporangium spp. 94A and the actinomycete Streptomices spp. 211A, respectively. The scanning electron micrographs show that the particles are mostly round and do not aggregate into large structures.The provided EDAX spectra show the energy as a function of the relative counts of the detected X-rays. The existence of silver nanoparticles in the Streptomices spp. 211A biomass (a) and gold nanoparticles in the Arthrobacter globiformis 151B biomass (b) were confirmed by the spectra shown in Figure 6. Figure 6a shows that Streptomyces spp. 211A has four distinct Ag peaks. Proteins and enzymes found in biomass also contribute to the X-ray emission signals from C, O, and P. recorded. Arthrobacter globiformis 151B exhibits many Au peaks along with signals from C, O, K, P, and Ca (Figure 6b).

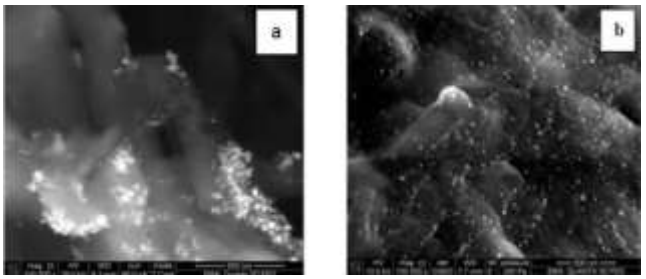

Figure 5. SEM image of silver nanoparticles formed on the surface of actinomycete *Streptomices spp.* 211A (a) and gold nanoparticles formed on the surface of actinomycete *Streptosporangium spp.* 94A (b).

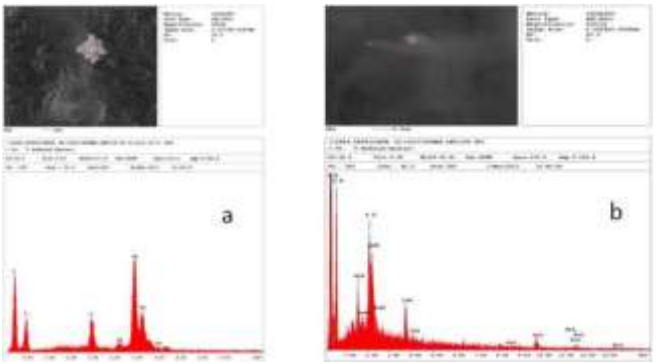

Figure 6. EDAX spectra recorded from *Streptomices spp*. 211A biomass with silver nanoparticles (a) and from *Arthrobacter globiformis* 151B biomass with gold nanoparticles (b).

Using equilibrium dialysis and atomic absorption analysis, researchers investigated the biosorption process on bacterial cells during nanoparticle synthesis. Since the sorption is dependent on the type and content of the cell wall, the Freundlich equation, which predicts that there are heterogeneous sorption sites on bacterial surfaces, was followed by the concentrations of the metal adsorbed by the bacteria in the solution at equilibrium dialysis.
Freundlich adsorption isotherms describe the adsorbent's capacity as well as the equilibrium connection between the adsorbent and adsorbate:

where Cb represents the adsorbed metal concentration, Ct the equilibrium metal ion concentration in the solution, K the biosorption constant, and n the sorptive capacity, the empirical constants in this context. Gold nanoparticles in Streptomyces spp. 211A biomass were studied using Freundlich adsorption linearized isotherms (A for homogenised and B for particulate homogenised), as shown in Figure 7.

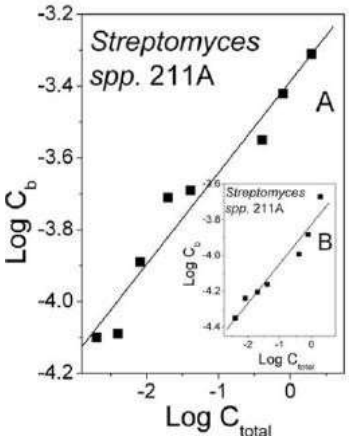

Figure 7. Freundlich adsorption isotherms for Au nanoparticles in *Streptomyces spp*. 211A biomass (A – for homogenized and B – for particulate homogenized).

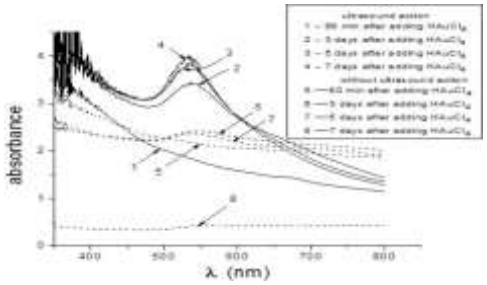

Figure 8. UV-vis spectra recorded as a function of time of reaction aqueous solution of $HAuCl_4$ with *Spirulina platensis* (after and without sonication).

The blue-green algal biomass containing Au nanoparticles was sonicated for 10 minutes at 35 kHz using an ultrasonic generator in order to investigate the potential for intensifying nanoparticle formation. The optical microscopy analysis reveals that the Spirulina platensis biomass broke down into tiny pieces. Afterwards, a 10-3 M concentration of HAuCl4 aqueous solution was added to the suspension in order to produce Au nanoparticles. Figure 8 shows the results of measuring the UV-vis spectra for the reaction at various time intervals.Figure 8 shows that compared to the alga treated without sonication, the absorption peak of Au nanoparticles is four times greater after sonication. One possible explanation is that sonication increases the overall surface area of the tiny bacterial pieces, lending credence to the idea that nanoparticles grow on their outsides.

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Figure 9a shows a transmission electron micrograph of gold nanoparticles that were produced when the algae was subjected to sonication. Figure 9b illustrates the size distribution of the Au nanoparticles, and the mean size is around 15 nm, while it was 25 nm before sonication [17]. So, during sonication, the creation of nanoparticles is enhanced and their sizes are reduced.

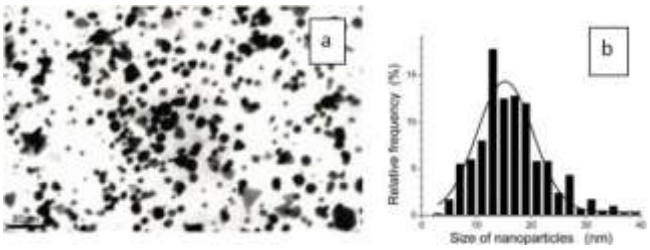

Figure 9. The TEM image of Au nanoparticles formed in the reaction of $HAuCl_4$ solution with *Spirulina platensis* biomass subjected to sonication (a) and Au nanoparticles size distribution histogram (b).

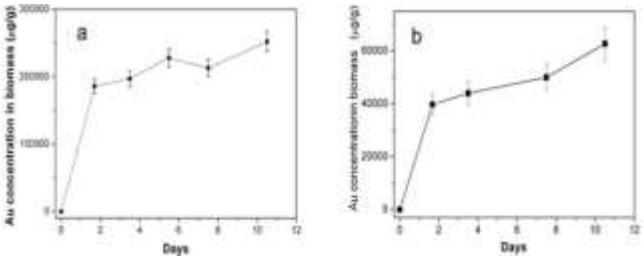

Figure 10. The total gold concentrations in biomass *Streptomyces spp*. 211A determined by NAA (a) and by AAS (b).

We determined the total Au and Ag content in the biomass of the bacteria we tested using the analytical procedures of neutron activation analysis (NAA) and atomic absorption spectrometry (AAS). Figure 10 shows examples of analytical determinations of total concentrations of gold in the bacterial biomass of Streptomyces spp. 211A using NAA (a) and AAS (b).Total metal buildup followed the same analogous dynamic in all instances: concentrations of metal rose sharply in the first few hours before gradually levelling out. The first step included the primary adsorption of metal ions onto the extracellular surface of bacterial cells. Subsequently, metal ions were brought into cells and built up inside them.Considering the potential medicinal use of the produced biomass with Au and Ag nanoparticles, NAA was also used to investigate the multi-elemental composition of the bacterial samples. In Figure 11, we can see the example for The glaucomatous streptomyces bacteria (71MD). The NAA findings indicate that the biomass was not too polluted with harmful elements, allowing for the potential industrial, medicinal, and pharmaceutical applications of materials synthesised using Au and Ag nanoparticles.

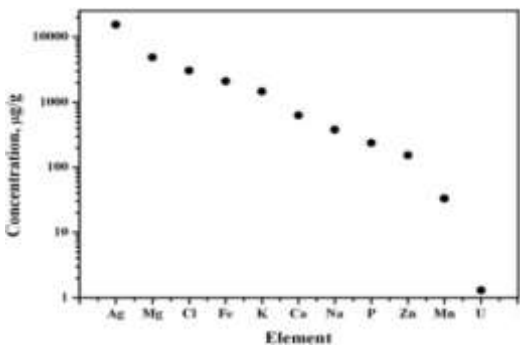

Figure 11. The distribution of elements in *Streptomyces glaucus* 71MD sample.

Conclusions

Based on the results of the experiments, the microorganisms that were studied can interact with 10-3 M aqueous solutions of chloroauric acid (HAuCl4) and silver nitrate (AgNO3) to produce gold and silver nanoparticles. These microorganisms include new strains of actinomycetes from the Arthrobacter genera (Arthrobacter globiformis 151B and Arthrobacter sp.61B), extremeophiles Streptomyces spp. 211A and Streptomyces glaucus 71MD, thermophiles Thermoactinomycete spp. 44Th and Thermomonospora spp. 67Th, and the blue-green algae Spirulina platensis.The majority of the gold and silver nanoparticles produced by bacterial biomass are extracellular and have a crystalline structure. Bacteria typically have spherical forms and diameters ranging from 5 to 60 nanometers, with an average size of 15 to 35 nanometers among various strains. Atomic adsorption spectroscopy (AAS), neutron activation analysis (NAA), scanning electron microscopy (SEM) with energy dispersive X-ray diffraction (EDAX), and ultraviolet-visible and X-ray diffraction (UV-vis) spectrometry are all potent tools for studying Au and Ag nanoparticles in bacterial biomass and determining how they are formed. Investigating the surface biosorption process during nanoparticle production by the microorganisms under research required a series of experiments using equilibrium dialysis techniques coupled with AAS.According to the results of the research, the microorganisms under study have enormous medical and industrial potential as a source of possible new technologies for the production of gold and silver nanoparticles that are clean, simple, nontoxic, and ecologically acceptable.

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