

Microbial Synthesis of Silver Nanoparticles Using *Aspergillus flavus* and Their Characterization

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1 Introduction

Nanotechnology is the design, characterization, production and applications of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale that produces structures, devices, and systems with at least one novel/superior characteristics or property [1]. Nanoparticles are the fundamental units of the nanotechnology. Nanoparticles are typically in the range from 1 to 100 nm and can be tuned in various sizes and shapes, each having different quantum mechanical properties and hence different exploitation. The size of the nanoparticles is the most important quality of nanoparticles.

Silver nanoparticles have potential applications in the field of photonics [2], electronics [3], optical receptor [4], Biolabeling [5] and antimicrobial agents [6], water filters and also used in solar energy applications. Several methods like physical and chemical routes are employed to prepare silver nanoparticles of various shapes and sizes which are found to be expensive and involve the use of toxic chemicals. There is a growing need to develop cost effective and environmentally friendly technique for the synthesis of silver nanoparticles. Exploitation of microbial cells is a novel approach for the synthesis of metal nanoparticles [7]. The biological route is the reliable protocol for the synthesis of nanoparticles over a range of chemical composition, sizes, and high monodispersity. Biological synthesis takes place within living organisms and is catalyzed by enzymes.

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Attempts were made to prepare silver nanoparticles using the fungus *Aspergillus flavus* by optimizing the conditions and the corresponding surface Plasmon resonance was studied.

2 Materials and Methods

2.1 Preparation of Cell Filtrate and Synthesis of Silver Nanoparticles

The fungus culture *Aspergillus flavus* (NCIM650) was obtained from National Chemical Laboratory culture collection center, Pune, India. It was sub-cultured and maintained in the Microbiology Lab of R.C. Patel College, Shirpur. The biomass of *Aspergillus flavus* has grown aerobically in potato dextrose broth for seven days at 30 °C. After seven days the mycelia was separated, washed thoroughly with distilled water three times to remove any media components. Fresh 5 g of biomass was re-suspended in 100 ml distilled water. The flask was again incubated at 37 °C at 120 rpm. After 72 h, biomass was separated by filtering through Whatman filter paper, and the cell filtrate was used for the synthesis of silver nanoparticles [8, 9].

For the synthesis of silver, nanoparticles-cell filtrate was challenged with the equal amount of 1 mM silver nitrate solution at optimized pH value, and the flask was incubated at 37 °C at 120 rpm for 96 h. A control containing cell filtrate without silver nitrate solution was run simultaneously as standard with the experimental flask.

2.2 UV-Visible Characterization

Change in color of the cell filtrate incubated with silver nitrate solution was visually observed over a period. The bio-reduction of precursor silver ions was monitored at different time intervals. UV-visible spectra were also carried for three months to check the stability of the synthesized silver nanoparticles.

2.3 XRD Measurements

The prepared silver nanoparticles solution was centrifuged at 8000 rpm for half an hour to obtain purified silver nanoparticles which were then re-suspended in deionized water and again centrifuged under same conditions. The collected pellets were air dried at room temperature for obtaining the powder form. X-ray diffraction technique was used to analyze the metallic nature of the nanoparticles after bio-reduction.

2.4 FTIR Measurements

The presence of proteins and potential biomolecules responsible for the synthesis of silver nanoparticles was analyzed by FTIR spectroscopy.

2.5 Scanning Electron Microscopy

The solution of silver nanoparticles was obtained in powder form for scanning electron microscopy.

2.6 TEM Measurements

A drop of colloidal silver nanoparticles solution was placed on the carbon-coated copper grid and dried by allowing water to evaporate at room temperature. Electron micrographs were obtained using Philips CM 200 transmission electron microscopy.

3 Result and Discussion

3.1 Biosynthesis of Silver Nanoparticles

The filtrate was pale yellow in color before the addition of silver nitrate solution and this change to brown in color on completion of the reaction with Ag^+ ions after 48 h (Fig. 1). The appearance of the brown color of the solution containing the cell

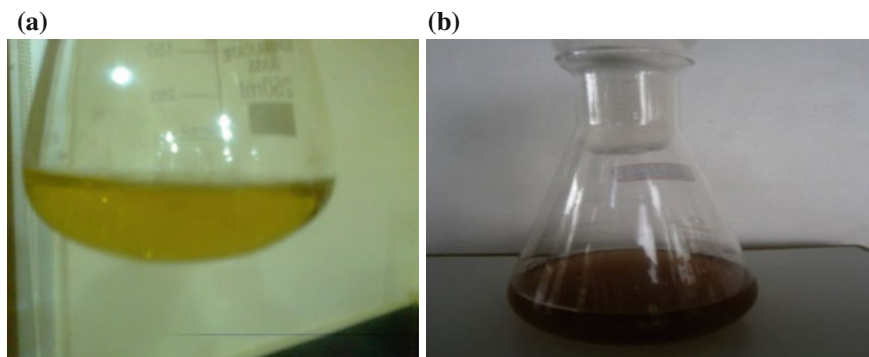


Fig. 1 a Flavus filtrate before treatment of AgNO_3 b after treatment

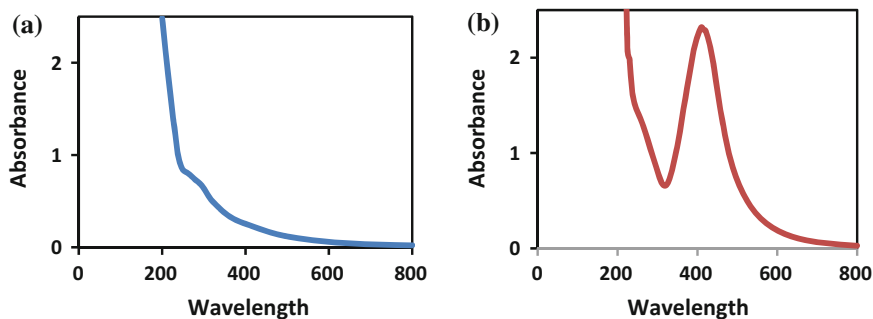


Fig. 2 UV-visible spectra of *Aspergillus flavus* filtrate (a) and filtrate treated with silver nitrate (b)

filtrate indicates the formation of silver nanoparticles in the reaction mixture [10]. After 48 h no significant change in the color was observed.

3.2 UV-Visible Spectra

The UV-visible spectra of only *Aspergillus flavus* filtrate and filtrate treated with silver nitrate solution is depicted in Fig. 2a, b respectively. Figure 2a does not show any absorbance band while in Fig. 2b the absorbance peak was observed at 409 nm at 12 h and attained the maximum intensity after 48 h. After 48 h no change in the maximum intensity was observed indicating the complete reduction of silver ions. The absorbance peak at 409 nm is due to surface Plasmon resonance band occurring due to collective oscillation of free electrons in metal nanoparticles in resonance with the light wave [11, 12]. The result correlates with Shivaraj Ninganaogouda's result [9].

3.3 XRD Analysis

XRD analysis of biosynthesized silver nanoparticles was used to study the crystalline nature of the particles. XRD pattern of the prepared sample is shown in Fig. 3. The diffracted intensities were recorded from 10° to 80° through 2θ angles, and it reveals that particles were crystalline in nature with FCC structure.

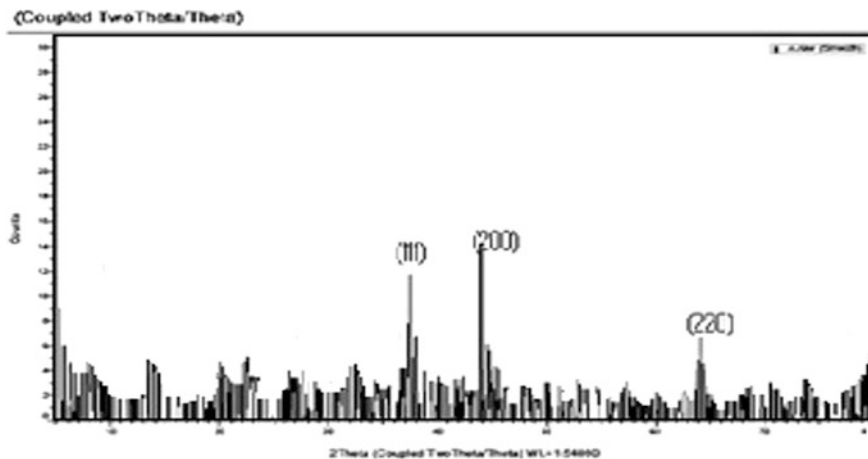


Fig. 3 XRD spectrum of synthesized silver nanoparticles

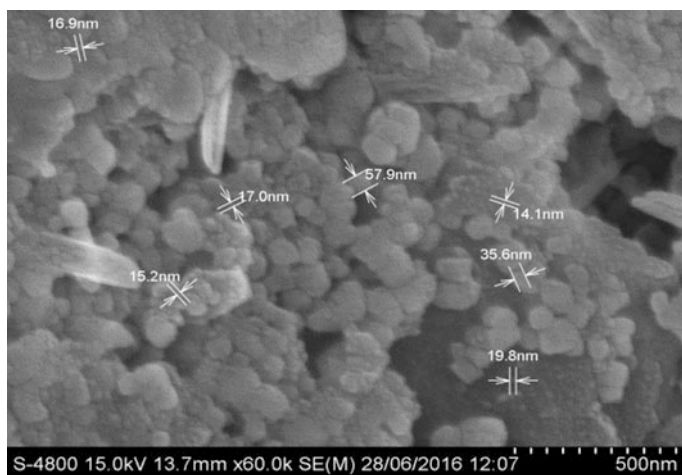


Fig. 4 Scanning electron micrograph of the silver nanoparticles

3.4 SEM

The scanning electron micrograph of silver nanoparticles synthesized by treating cell filtrate with 1 mM silver nitrate solution is depicted in Fig. 4. Which clearly shows surface deposited silver nanoparticles and some agglomeration was also observed.

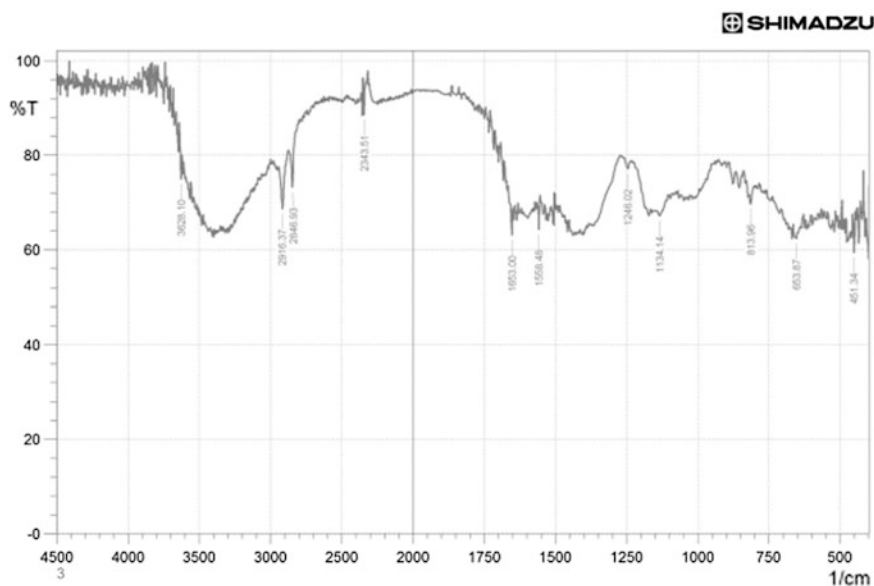


Fig. 5 FTIR spectra of silver nanoparticles

3.5 FTIR Measurements

FTIR spectrum of the prepared sample of silver nanoparticles is shown in Fig. 5. The study of FTIR spectra confirms the presence of proteins in the samples of silver nanoparticles.

3.6 TEM

TEM image of the drop coated film of silver nanoparticles showed distinct shape and size of the nanoparticle (Fig. 6). The particles were spherical in shape with mean of 7.13 nm. Silver nanoparticles are uniformly distributed with some agglomeration was noticed. The selected area diffraction pattern confirms the crystalline FCC structure of metallic silver.

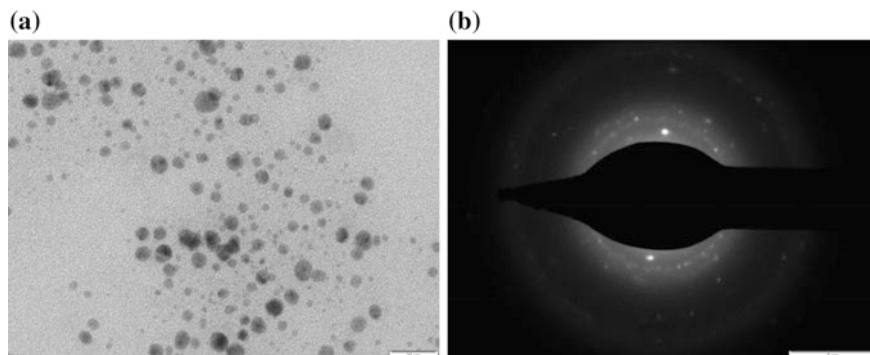


Fig. 6 **a** TEM of silver nanoparticles, **b** diffraction pattern of silver nanoparticles

4 Conclusions

The fungus *Aspergillus* has a potential to synthesize silver nanoparticles extracellularly and is the developing nano factories because of downstream processing and handling of biomass would be simple. UV-visible spectra show the maximum absorption at 409 nm with a single SPR band confirm that the particles are spherical. SEM observations show that the particles synthesized in the range between 10 and 55 nm with spherical in size. FTIR analysis confirms the presence of the proteins in the cell filtrate solution of silver nanoparticles. The synthesized silver nanoparticles were found to stable over a period of three months. TEM reveals that the particles were spherical with the average size of 7.13 nm and diffraction pattern exhibit the FCC structure.

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