

Optimizing Physioculture Conditions for the Synthesis of Silver Nanoparticles from *Aspergillus niger*

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Abstract

The external physiochemical parameters such as fungal biomass quantity, pH, incubation temperature and silver nitrate concentration will not only affect the rate of synthesis of silver nanoparticles from *Aspergillus niger* but also its yield. So in order to enhance product yield optimization of external environment was done to control the above mentioned parameters. A peak at 420 nm was observed for silver nanoparticles by UV visible spectrometry and at 420 nm maximum synthesis of silver nanoparticles were obtained at optimum conditions i.e. 20 g of fungal biomass, alkaline pH of 9.0, incubation temperature of 60°C and 6 mM of AgNO₃ concentration.

Keywords: *Aspergillus niger*; Fungal biomass quantity; pH; Incubation temperature; AgNO₃ concentration

Introduction

Metallic nanoparticles, such as iron, selenium, platinum, lead, silica, titanium, gold, silver and zirconium can be synthesized by different biological organisms [1]. These organisms make use of their biomass or their biomass extracts as the basis of extracellular or intracellular synthesis of nanoparticle by reducing the metal ions. For example iron oxide nanoparticles were synthesized by Bacterias like *Desulfuromonas acetoxidans*, *Shewanella* spp and *Magnetospirillum magnetotacticum* [2]. From aqueous extracts of *Rumex acetosa* (dicotyledonous) and *Hordeum vulgare* (monocotyledonous) plants iron oxide nanoparticles were produced [3]. However exploration of Fungi for the mycofabrication of metallic nanoparticles is the most apt choice because of its high tolerance and bioaccumulation capabilities [4]. Therefore "Fungi" is the right choice of biological organism employed for the large scale mycofabrication of different nanoparticles. Since physiochemical conditions influence the growth and developments of an organism *in vitro* and *in vivo*. The metabolic activity of an organism is thus influenced by the external environment. In case of extracellular synthesis of silver nanoparticle by *Aspergillus niger*, enzyme production is greatly influenced by the condition in which the fungi is cultivated. Therefore optimization studies of the physioculture conditions such as pH, incubation temperature, substrate concentration and fungal biomass etc. was done to determine optimum conditions for silver nanoparticles synthesis.

Materials and Methods

Isolation and identification of the fungus

Aspergillus niger, isolated from soil and maintained on potato dextrose agar (PDA) at 28°C for 4-5 days. The fungus was identified on the basis of morphological characteristics such as color of the colony, texture of the mycelia etc.

Aspergillus niger culturing

Aspergillus niger was inoculated by wire loop method in IL of cezapex Dox broth (glucose (10 g), Sodium nitrate (2 g), ferrous sulphate (0.01 g), yeast extract (1 g), calcium chloride(0.5 g), potassium dihydrogen phosphate(1 g), zinc sulphate (0.01 g) and magnesium sulphate (0.5 g) dissolved in 1000 ml of distilled water and autoclaved at 121°C and 15 psi (pound/square inches) for 20 min at room temperature for 5 days on a rotatory shaker at 150 rpm. Later

the mycelia was harvested using Whatman's filter paper no.1. The cell free filtrate was than centrifuged at 15000 rpm for 10 min to obtain the supernatant.

Mycosynthesis of silver nanoparticles

10 ml of supernatant incubated with 90 ml of 2 mM AgNO₃ at room temperature in dark for 48 hours. Appearance of brown/black color of the media incubated with AgNO₃ indicates mycosynthesis of silver nanoparticles. Control containing freshly prepared CD media with aqueous silver nitrate was run simultaneously with the experimental flasks. Experiment and control were performed in triplicates.

Characterization of silver nanoparticles

UV visible spectrometry (JENWAY 6305) analysis was done by scanning the absorption spectra from 360 nm to 460 nm to find out λ_{max} where maximum optical density was obtained. It is well known that monodispersed nanoparticles show one plasma band in the visible region of the spectrum. Optical density of distill water was used as a control.

Optimization of Reaction Conditions

Fungal biomass quantity

The effect of biomass concentration on the extracellular production of silver nanoparticles was studied by using different wet biomass of the fungus *Aspergillus niger* with a difference of 5 i.e. 5 g, 10 g, 15 g and 20 g grown in cezapex Dox broth. 10 ml of obtained supernatant incubated with 90 ml of 2 mM AgNO₃ at room temperature in dark for 48 hours. Biosynthesis of nanosilver particles at different biomass concentrations was characterized by UV-visible absorption spectroscopy.

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pH

10 ml of supernatant incubated with 90 ml of 2 mM AgNO_3 at room temperature in dark for 48 hours at four different pH i.e. 5, 6, 7, 8 and 9. 1 N Hydrochloric acid and 1 N Sodium hydroxide solutions was used to adjust the pH of the fungal filtrate. Biosynthesis of nanosilver particles at different pH was characterized by UV-visible absorption spectroscopy.

Incubation temperature

10 ml of supernatant incubated with 90 ml of 2 mM AgNO_3 at four different temperature of 20°C, 40°C, and 60 Cand 80°C in dark for 48 hours in an incubator till the synthesis of silver nanoparticles were observed. The reaction mixture was further characterized by UV-visible absorption spectroscopy to determine optimum temperature.

Silver nitrate concentration

Substrate concentration is directly proportional to the rate of product formation. 10 ml of supernatant incubated with 90 ml of AgNO_3 of different mM concentration such as 2 mM, 4 mM, 6 mM and 8 mM in dark for 48 hours at room temperature. The reaction mixture was further characterized by UV-visible absorption spectroscopy to determine optimum substrate concentration.

Results

White cottony mycelia were obtained in cezapex Dox broth after five days of growth.

Mycosynthesis of silver nanoparticle

Appearance of brown/black color of the experimental flask was observed however no change of color was seen in the controls (Figure 1)

Characterization of silver nanoparticles by UV visible spectrometry

UV-Visible Spectroscopy performed on 1 ml of the sample (colloidal silver nanoparticle solution) which was withdrawn after 48 hrs from the experimental flask. A peak at 420 nm was observed which is specific for silver nanoparticles (Figure 2).

Optimization of reaction conditions fungal biomass quantity

Biosynthesis of nanosilver particles at different fungal wet biomass concentrations was characterized by UV- visible spectroscopy at λ_{max} 420 nm. Highest absorbance peak was obtain at 20 g of fungal biomass (Figure 3). Optical density of distill water was measured to be zero.

pH

UV-visible absorption spectroscopy of the samples at different pH gives the absorbance peak at a specific wavelength of 420 nm and the highest absorbance value was obtained at pH 9 (Figure 4). Optical density of distil water was measured to be zero.

Incubation temperature

UV-visible absorption spectroscopy of the samples at different incubation temperature gives the absorbance peak at a specific wavelength of 420 nm and the highest absorbance value was obtained at 60°C (Figure 5). Optical density of distil water was measured to be zero.

UV-visible absorption spectroscopy of the samples incubated with different concentration of AgNO_3 was obtained and the highest absorbance value was obtained at 6 mM of AgNO_3 concentration (Figure 6). Optical density of distil water was measured to be zero.

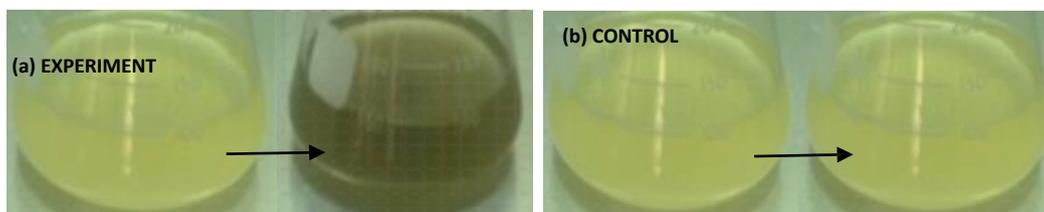


Figure 1: Color of the fungal filtrate after 48 hours of incubation with AgNO_3 (a) pale yellow to brownish black (b) No change in color.

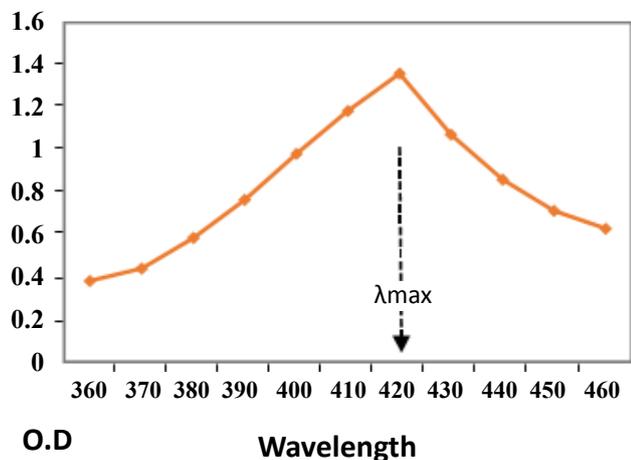


Figure 2: λ_{max} of reaction mixture (fungal filtrate with AgNO_3).

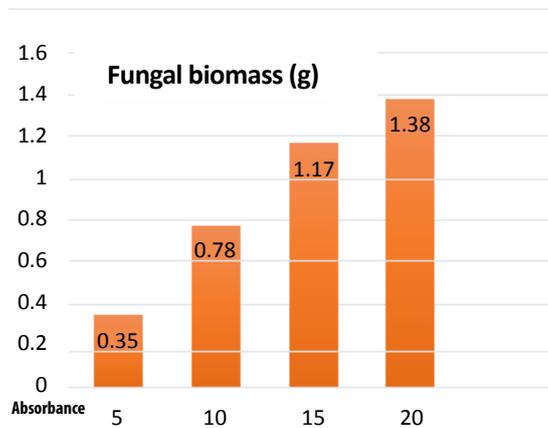


Figure 3: UV-Visible spectrum of silver nanoparticles synthesized at various Fungal Biomass concentration.

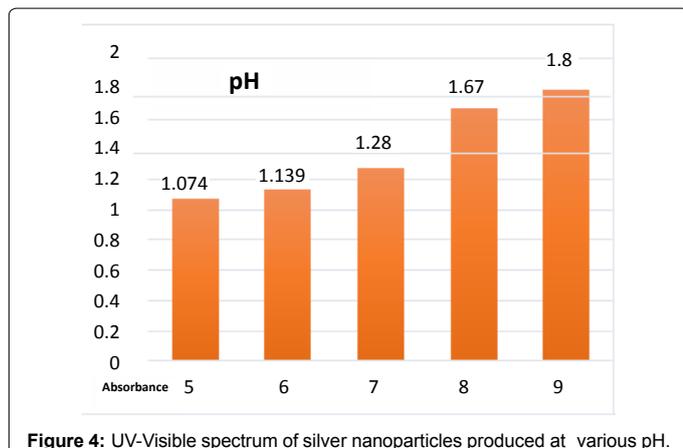


Figure 4: UV-Visible spectrum of silver nanoparticles produced at various pH.

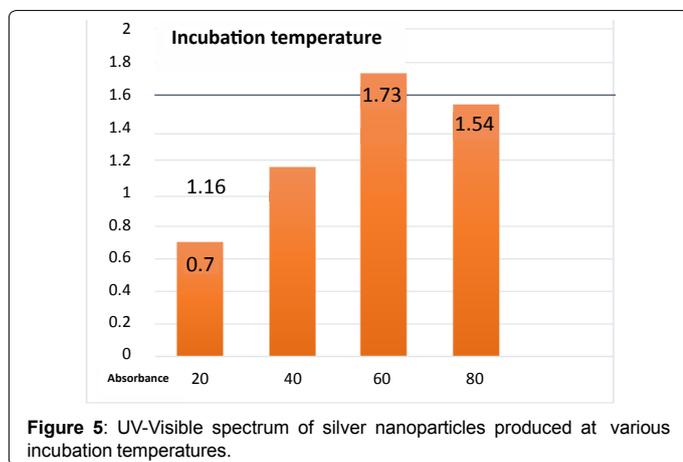


Figure 5: UV-Visible spectrum of silver nanoparticles produced at various incubation temperatures.

Discussion

In this study we investigated the effect of different reaction parameters on the production of silver nanoparticles. The amount of biomass plays a key role in synthesis or complete reduction of Ag^+ to Ag^0 [5]. Greater amount of fungal biomass means increased amount of nitrate reductase in the extracellular media thus increased reduction of silver ions occurs as indicated by the obtained ultra violet visible spectrum depicting increased rate of silver nanoparticle synthesis resulting in greater number of silver nanoparticles in the aqueous solution [6,7]. While studying the effect of incubation temperature it was observed as the temperature increases so does the absorbance but an optimum temperature is required for the functional activity of the enzymes or proteins responsible for silver ion reduction [8]. Similar findings were reported by Darroudi et al. [9] in his study. Beyond this temperature these proteins molecules become denatured and loses its catalytic function as a result bioreduction of metal ion ceases and at elevated temperature of 80°C effects silver nanoparticles stability and resulted in its aggregation [9]. However at lower temperature bioreduction could not be completed resulting in fewer nanoparticles synthesis. The optimum substrate concentration ($AgNO_3$) was found to be 6 mM at a specific wavelength of 420 nm. When the $AgNO_3$ concentration increased to 6 mM the particle size may increase due to increase in substrate concentration available for bioreduction by nitrate reductase to form the final product silver nanoparticle however beyond this concentration the aggregation of large silver nanoparticles occurred as shown by a decline seen in the UV-Vis spectra [10,11]. In case of effect of pH, optimum pH value was found to be 9.0 which is

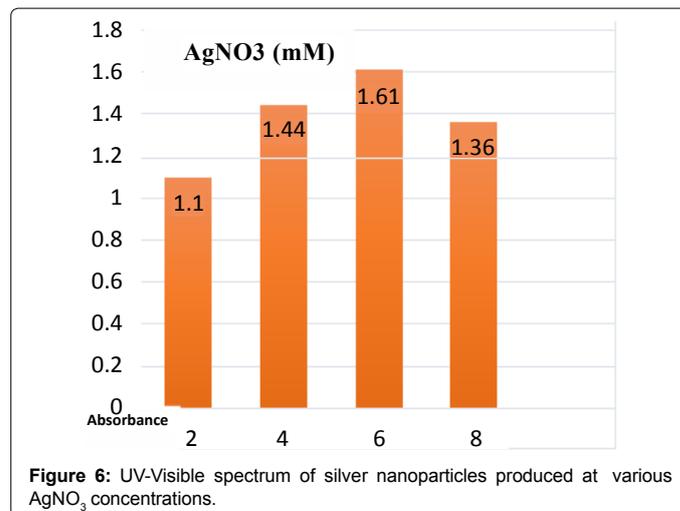


Figure 6: UV-Visible spectrum of silver nanoparticles produced at various $AgNO_3$ concentrations.

an alkaline condition [12]. Similar results were obtained by Chen and Carroll (2004) in their work who stated that silver nanoparticles are quite stable at alkaline conditions as compared to lower pH values where acidic conditions causes aggregation of silver nanoparticles [13-15].

Conclusion

This is evident that microorganisms possess ions reduction capabilities therefore extracellular mycosynthesis of silver nanoparticles could be achieved by the fungal strain, *Aspergillus niger*, a simple biological and cost effective approach for preparation of stable silver nanoparticles. Several types of silver nanoparticles can be produced by monitoring environmental parameters. In current study, optimum parameters for green synthesis of silver nanoparticles from *Aspergillus niger* were found to be at 60°C incubation temperature at pH of 9 and substrate concentration of 6 mM using fungal biomass of 20 grams.

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