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Optimization Studies of Silver Nanoparticle Synthesis by *Aspergillus terreus*

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Abstract

Myconanotechnology encompasses fabrication of metallic nano-sized particles using eukaryotic fungi. It is an inter-disciplinary field that combines nanotechnology with mycology with substantial prospective, relatively due to abundance and diversity of fungi. In case of synthesis of silver nanoparticle by *Aspergillus terreus* optimization studies revealed that the optimum incubation time was 55 h using fungal biomass of 20 g to reduce the available substrate, i.e., Silver nitrate of 6 mM at pH of 9.0. Thus using these reaction conditions, maximum yield of silver nanoparticles could be obtained.

Keywords: *Aspergillus terreus*; Incubation time; AgNPs; Mycosynthesis

Introduction

Mycotechnology is the assembly of metallic nanoparticles by the eukaryotic fungi. Using fungi, especially their biomass or biomass extracts, for the synthesis of AgNPs can be beneficial compared with other bio-based processes because fungi resources, being abundant in nature and can easily be isolated by plating, serial dilutions and hyphal extraction. Since they are totipotent therefore spores or hyphae can be used to grow fungus to obtain pure isolate after sub culturing [1].

Besides high tolerance toward metal nanoparticles concentration in the medium, easy optimization of the reaction conditions could be achieved. Thus making them appropriate biological candidates for the production of different metallic nanocrystals that have diverse applications in different disciplines. For example Shahi and Patra depicted the synthesis of usnic acid nanoparticles from the fungus *Usnea longissima*. These nanoparticles were uniform in shape and showed the potential for curing dermatophytic infections in humans by the formation of nanoemulsion [2].

Bansal et al. on the other hand had reported the synthesis of extracellular Zirconia nanoparticles from *Fusarium oxysporum* by the reaction of fungal extract with an aqueous zirconium. The size of these nanoparticles was between 3 and 11 nm and were spherical in shape [3]. Similarly extracellular silica and Titania nanoparticles were produced by the reaction of *Fusarium oxysporum* fungal cell filtrate with aqueous solutions of di-potassium silicon and titanium hexafluoride respectively. The silica nanocrystals were quasispherical with diameter range between 5–15 nm and Titania nanoparticles were 6–13 nm in diameter with spherical morphology [4]. White rot fungus *C. versicolar* on the other hand has been reported to be involved in the formation of silver nanoparticles [5].

Since physiochemical conditions are known to play an important role in the growth and developments of an organism in *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 terreus*, silver nanoparticles production is greatly influenced by the condition in which the fungus is cultivated. The concentration of the enzyme produced has a direct impact on the rate of silver ions reduction therefore affecting the concentration of the silver nanoparticle being synthesized. The external physiochemical parameters such as incubation time, pH, silver nitrate concentration, biomass concentration, etc. will not only affect the rate of synthesis of silver nanoparticle but also its yield [6,7].

So in order to enhance product yield in the current study optimization of external environment of the above mentioned parameters was done to achieve optimum conditions where maximum synthesis of silver nanoparticles could be achieved.

Materials and Methods

Aspergillus terreus cultures were obtained from Yeast and Fungal Biotechnology Lab, Department of Microbiology, Faculty of life sciences, BUITEMS. *Aspergillus terreus* was grown aerobically in yeast-malt extract (YM) broth which consists of glucose 10 g, malt extract 3 g, peptone 5 g and yeast extract 3 g dissolved in 1 L and sterilized by autoclaving at 121°C for 20 min at 15 psi (pound/square inches).

Inoculation of the media with *Aspergillus terreus* was done by wire loop method under laminar flow cabinet. The inoculated media was placed on an orbital shaker at room temperature at 140 rpm for five days.

Harvesting of fungal biomass was achieved by simple filtration using Whatman's filter paper no. 1 to obtain cell free fungal filtrate. The fungal filtrate was then centrifuged at 10000 rpm for 20 min. Control containing freshly prepared YM broth with silver nitrate solution was run simultaneously as standard with the experimental flask (experiment and control were performed in triplicates). 10 ml of centrifuged fungal filtrate incubated with 90 ml of one millimolar $AgNO_3$ solution at room temperature for 24 h in dark [8].

AgNPs synthesis was confirmed by change of fungal filtrate colors after the addition of silver nitrate solution and by means of UV visible spectrophotometer analysis which was done for 400 to 500 nm to determine optimum wavelength.

Optimization studies with respect to pH, incubation time, silver nitrate concentration and fungal wet weight was done using four different values of each parameters such as for PH (6.0, 7.0, 8.0 and 9.0),

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incubation time (45 h, 55 h, 65 h and 75 h), silver nitrate concentration (2 mM, 4 mM, 6 mM and 8 mM) and fungal wet weight (5 g, 10 g, 15 g and 20 g). 0.1 N HCl and 0.1 N NaoH was used to change the pH of the extracellular aqueous media. UV visible spectrophotometer analysis at λ max was used to obtain optimum conditions.

Results and Discussion

Synthesis of AgNPs was confirmed by change in the colour of the filtrate from yellow to light brown. However no change was observed in the controls (containing freshly prepared CD broth with silver nitrate solution). Change in colour is due to the reduction of silver ions [9]. The exact mechanism of silver ion reduction is unknown however numerous evidences depicted the importance of nitrate reductase in the synthesis of silver nanoparticle. For example, Srivastava et al. confirmed the role of nitrate reductase during the synthesis of AgNPs from *Halococcus salifodiane* [10]. Evidence was provided by *Trichoderma virens* mediated silver nanoparticles synthesis which involves nitrate reductase mediated silver ion reduction [11]. Reduction of silver ions by nitrate reductase in the culture supernatant of *Nocardiopsis* sp was also documented [12] (Figure 1).

UV visible spectrophotometer analysis which was done for 400 to 500 nm and optimum wavelength was found to be 450 nm (Figure 2).

A result of the optimization studies revealed that optimum pH was found to be 9.0.which is an alkaline medium (Figure 3). On the other hand at acidic pH of 5, enzymes responsible for reduction gets denatured which affects its catalytic activity as a result bioreduction of silver metal ions is reduced [13].Therefore silver nanoparticles were found to be stable at alkaline pH 9.0 [14] (Figure 3).

Figure 1: Change in color of fungal filtrate (a) before $AgNO_3$ (b) after addition of $AgNO_3$.

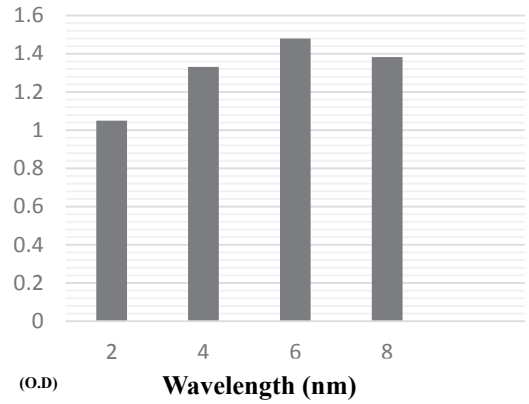

Figure 2: UV-visible spectrum depicting optimum wavelength, i.e., 450 nm.

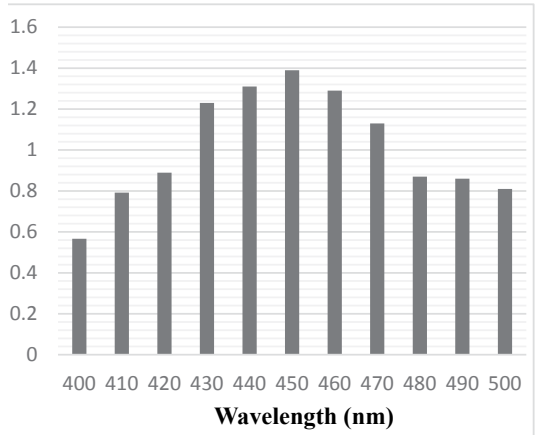

Figure 3: UV-visible spectrum depicting optimum pH of 9.0 at 450 nm.

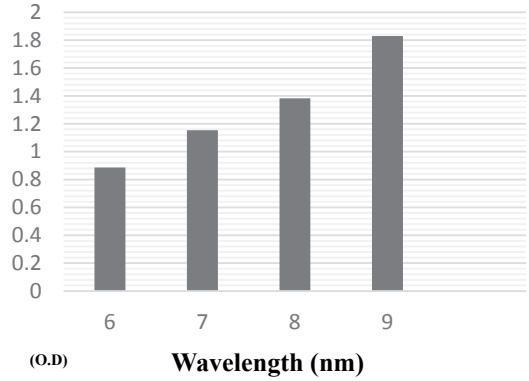

Figure 4: UV-visible spectrum depicting optimum silver nitrate concentration of 6 mM at 450 nm.

Optimum silver nitrate concentration was found to be 6 mM indication synthesis of silver nanoparticles at maximum rate as the concentration of substrate is increased [15]. But beyond this optimum concentration the amount of substrate exceeds the amount of proteins available in the fungal filtrate required for silver nanoparticles synthesis. As a result rate of silver nanoparticles synthesis is decreased (Figure 4).

Optimum fungal wet weight was found to be 20 g. It means greater

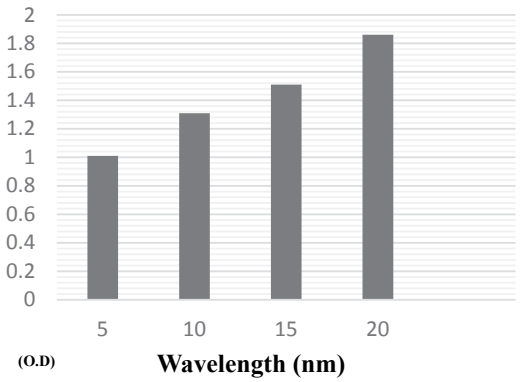

Figure 5: UV-visible spectrum depicting optimum fungal wet weight of 20 g at 450 nm.

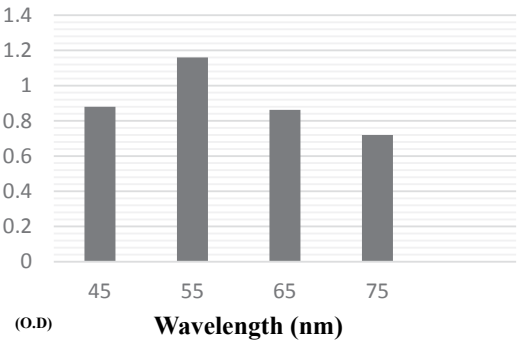

Figure 6: UV-visible spectrum depicting optimum incubation time of 55 h at 450 nm.

the amount of fungal biomass more will be the concentration of enzymes nitrate reductase in the external media thus greater will the concentration of silver nanoparticles because reduction of silver metal ions will be rapid (Figure 5).

Optimum incubation time of 55 h at 450 nm was depicted by the UV-visible spectrum (Figure 6).

An incubation period of 55 h was found to be optimum as enough reaction time is there for the interaction of proteins with aqueous silver metal ions to form its corresponding silver nanoparticles. Beyond this time period stability of the synthesized silver nanoaprticles was affected as a result aggregation was observed marked by low optical density value.

Conclusion

Optimization studies of silver nanoparticle synthesis revealed that the optimum incubation time was 55 h using fungal biomass of 20g to reduce the available substrate, i.e., Silver nitrate of 6mM at pH of 9.0. Thus maximum yield of silver nanoparticles could be obtained.

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