Optimization of Reaction Parameters for Silver Nanoparticles Synthesis from *Fusarium Oxysporum* and Determination of Silver Nanoparticles Concentration

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Abstract

A number of physical and chemical methods available for the production of silver nanoparticles however these methods are quite costly and make use of poisonous chemicals. Thus use of biological organism as bionanofactories offers a clean and cost effective alternative process for the fabrication of silver nanoparticles. Extracellular synthesis of silver nanocrystals from *Fusarium oxysporum* was accomplished. Data obtained from Ultra violet visible spectrometry was used to calculate the concentration of silver nanoparticles at optimum conditions. The optimum conditions where the concentration of silver nanoparticles maximum was found to be 20 g of fungal biomass incubated at 40°C at pH 8.0 using substrate concentration of 2 mM.

Keywords: Mycosynthesis; *Fusarium oxysporum*; Silver nanoparticles; Ultra violet visible spectroscopy

Introduction

Biological organisms are known to involve in the synthesis of different metallic nanoparticles. For example extremophilic *Ureobacillus thermophilaericus* was explored by Ijuhari to have potential to produce AgNPs at raised temperatures and high Ag ion concentrations [1]. Extracellular synthesis of AgNPs size ranging from 16-40 nm was produced by *Pseudomonas stratureri* (bacterium) [2]. Mann reported the synthesis of magnetite (Fe₃O₄) or greigite (Fe₃S₄) nanoparticles by magnetotactic bacteria and the extracellular formation of siliceous material was documented in diatoms [3]. Another example reported by Ahmad et al. was the synthesis of CdS nanoparticles by the fungus *Fusarium oxysporum* extracellularly [4]. Not only microorganisms but plants can also be employed for nanoparticle synthesis as described by Shankar S that the synthesis of pure metallic silver and gold particles was achieved by the interaction between the Neem (*Azadirachta indica*) leaf broth with aqueous solution of silver nitrate or chloroauric respectively outside the plant cell [5]. Several species of *Fusarium oxysporum* [4,6,7] such as *Fusarium acuminatum* [8], *Fusarium solani* [9,10], *Fusarium semitectum* [11], *Penicillium brevicompactum* [12], *Penicillium fellutanum* [13], *Pleurotus sajor-caju* [14], *Phoma glomerata* [15], *Alternaria alternate* [16], *Aspergillus clavatus* [17] and *Aspergillus flavus* [18] have been known to synthesize AgNPs. In order to obtain silver nanoparticles of definite shape and size, optimization of the reaction parameters such as temperature, pH, silver nitrate concentration and fungal biomass was done.

Materials and Methods

The fungus culture of *Fusarium oxysporum* was obtained from Yeast and Fungal Biotechnology Lab, BIITEMS. Fungal biomass was obtained on one liter of CD (cezapex dox) broth. Cezapex dox broth consists of the following.

- Ferrous sulphate (0.01g)
- Calcium chloride (0.5 g)
- Magnesium sulphate (0.5 g)
- Sodium nitrate (2 g)

- Yeast extract (1 g)
- Glucose (10 g)
- zinc sulphate (0.01 g)
- Potassium dihydrogen phosphate (1 g)

The culture medium was autoclaved for 15 min at 121°C at 15 psi (pound/square inches). Inoculation was done in sterile air under Laminar flow cabinet. The cultured flasks were then incubated at room temperature on a rotatory shaker at 150 rpm for 120 hrs. Fungal mycelia were harvested after 120 hours of growth using Whatman’s filter paper no.1 to obtain fungal filtrate. The filtrate was centrifuged at 15000 rpm for 15 min. 20 ml of centrifuged filtrate (supernatant) was brought in contact with 150 ml of AgNO₃ solution (1 mM) [19]. Control containing freshly prepared CD broth with aqueous AgNO₃ was run as standard.

Confirmation of silver nitrate formation

Silver nanoparticle formation was visually observed by the gradual change in color of the experimental flasks containing fungal filtrate with AgNO₃ solution incubated for a specific period of time. UV visible absorption analysis was done to obtain optimum wavelength. Optimization of external environment is important in order to control reaction parameters to achieve optimum conditions where maximum product yield could be obtained [20].

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Effect of incubation temperature

Optimization was performed with temperature ranging from 20°C to 50°C with difference of 10°C on fungus, *Fusarium oxysporum* for AgNPs production. The sample was subjected to ultra violet visible spectrometry to study further effect of temperature on the rate of synthesis of silver nanoparticle.

Effect of pH

pH range from 5.0 to 8.0 was used with the difference of 1.0 to see the effect of pH on AgNPs formation. 1N Hydrochloric acid and 1N Sodium hydroxide was used to change the pH of the extracellular aqueous media.

Effect of AgNO₃ concentration

In this case different concentration of AgNO₃ from 0.5 to 2.0 mM was studied with a difference of 0.5 mM. The optimum concentration for the synthesis of nanosilver is confirmed by UV-visible absorption spectroscopy.

Effect of biomass (wet weight) concentration

Effect of fungal biomass concentration was studied by using 5 to 20 g of wet biomass with a difference of 5 of fungi *Fusarium oxysporum*. Biosynthesis of nanosilver particles at different biomass concentrations was characterized by UV-visible absorption spectroscopy.

AgNPs concentration calculation

Concentration of silver nanoparticles for each of the optimized parameter was calculated using UV-visible absorption data by applying Beer-lambert law:

\[ A = \varepsilon l c \]

Where,

C=concentration of AgNPs

A=absorbance at specific wavelength

\( \varepsilon = \)molar absorptivity constant

L=path length

Results and Discussion

Silver nanoparticle formation was visually observed when the appearance of brownish black color was seen in the experimental (Figure 1). Presence of protein nitrate reductase in fungal filtrate is accountable for silver ion reduction causing the appearance of brownish black color [8,21,22]. UV visible absorption analysis revealed a characteristic peak at 430 nm which is specific for silver nanoparticles (Figure 2). Silver nanoparticle yields extremely high absorption values within the UV/visible range [23,24]. Optimization studies with respect to temperature revealed that maximum synthesis of silver nanoparticles occurred at 40°C (Figure 3). At this high temperature enzymatic activity of nitrate reductases was maximum resulting in increased concentration of silver nanoparticles as confirmed by the measured concentration calculated from UV absorption data by using Beer-lambert law (Table 1). Mitra B et al. also stated that improved AgNPs synthesis occurred at high temperature [25]. Thus temperature greatly influences formation of silver nanoparticles in terms of concentration [26]. Optimum pH

![Figure 1: Color change (a) before AgNO₃ addition (b) after AgNO₃ addition.](image1.png)

![Figure 2: UV visible absorption spectrum.](image2.png)

![Figure 3: UV visible absorption spectrum showing optimum incubation temperature.](image3.png)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Incubation Temperature (°C)</th>
<th>Concentration (mol/lit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>9.46*10⁻⁶</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>1.28*10⁻⁵</td>
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<td>3</td>
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<td>1.58*10⁻⁵</td>
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<tr>
<td>4</td>
<td>40</td>
<td>2.07*10⁻⁵</td>
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Table 1: Concentration of AgNPs at different incubation temperatures.

<table>
<thead>
<tr>
<th>S.no</th>
<th>pH</th>
<th>Concentration (mol/lit)</th>
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</thead>
<tbody>
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<tr>
<td>4</td>
<td>8</td>
<td>1.86*10⁻⁴</td>
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Table 2: Concentration of AgNPs at different pH.
was found to be 8.0. The results suggested that an alkaline medium is more suitable for the synthesis of silver nitrate than a low acidic pH as rate of reduction of silver ions were higher at pH 8.0 (Figure 4 and Table 2).

Rate of bioreduction is directly proportional to the substrate concentration [27]. In this case the optimum concentration was found to be 2 mM of silver nitrate. Reaction kinetics and morphology of nanoparticle is affected by precursor solution (silver nitrate) [28-30] (Figure 5 and Table 3). Optimum fungal biomass was found to be 20 g. It seems that the biocatalysts were agents responsible for the amalgamation of nanoparticles. The enzyme reductase is an NADH dependent enzyme associated with the bioreduction of silver ions in case of fungi [8] (Figure 6 and Table 4).

**Conclusion**

Numerous synthetic methods were available for the synthesis of silver nanoparticles but these methods were quite cost ineffective and uses chemicals that were toxic in nature. Therefore employing biological organism such as fungi, plant etc. offers a clean and cheap alternative process for the amalgamation of silver nanoparticles. Among these microorganisms’ fungi is the most suitable biological entity for nanoparticle fabrication because it not only offers simple downstream processing for product recovery but makes the handling of biomass quiet easy. Besides, optimization of the reaction parameters can easily be achieved to obtain maximum concentration of silver nanoparticles of unique morphology.

**References**


