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# 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 method 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 siver nanoparticles at optimum conditions. The optimum conditions where the concentration of silver nanoaprticles were 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 *Ureibacillus thermosphaericus* was explored by Juibari 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 strutzeri* (bacterium) [2]. Mann reported the synthesis of magnetite ($Fe_3O_4$) or greigite ($Fe_3S_4$) 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 sajorcaju* [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, BUITEMS. 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 $AgNO_3$ solution (1 mM) [19]. Control containing freshly prepared CD broth with aqueous $AgNO_3$ 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 $AgNO_3$ solution incubated for a specific period of time. UV visible absorbtion 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 $AgNO_3$ concentration

In this case different concentration of $AgNO_3$ 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 UVvisible absorbtion data by applying Beer-lambert law:

$$A = \varepsilon lc$$

Where,

C=concentration of AgNPs

A=absorbance at specific wavelength

Ɛ=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 absorbtion 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 absorbtion 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

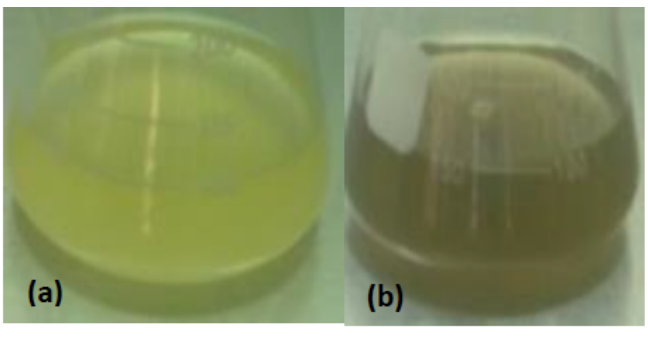


**Figure 1:** Color change (a) before $AgNO_3$ addition (b) after $AgNO_3$ addition.

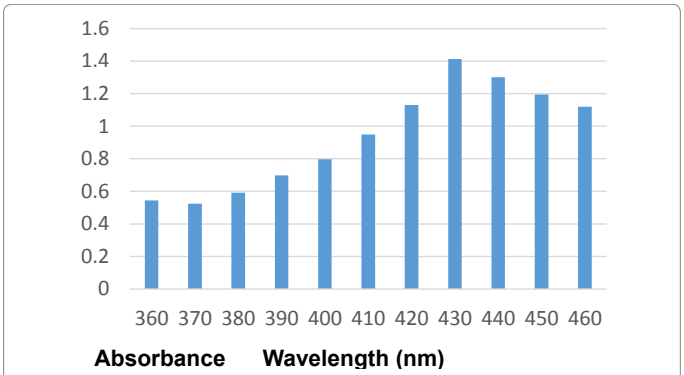


**Figure 2:** UV visible absorbtion spectrum.

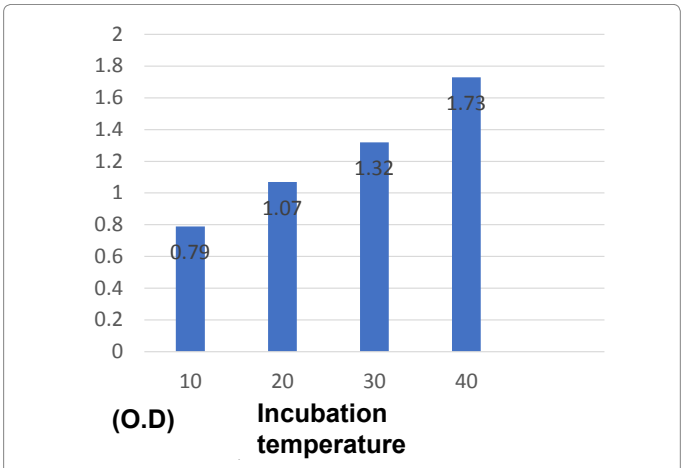


**Figure 3:** UV visible absorption spectrum showing optimum incubation temperature.

| S.No | Incubation Temperature (°C) | Concentration (mol/lit) |
|---|---|---|
| 1 | 10 | $9.46 * 10^{-6}$ |
| 2 | 20 | $1.28 * 10^{-5}$ |
| 3 | 30 | $1.58 * 10^{-5}$ |
| 4 | 40 | $2.07 * 10^{-5}$ |

**Table 1:** Concentration of AgNPs at different incubation temperatures.

| S.no | pH | Concentration (mol/lit) |
|---|---|---|
| 1 | 5 | $1.44 * 10^{-5}$ |
| 2 | 6 | $1.47 * 10^{-5}$ |
| 3 | 7 | $1.4 * 10^{-5}$ |
| 4 | 8 | $1.86 * 10^{-5}$ |

**Table 2:** Concentration of $AgNP_S$ 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).

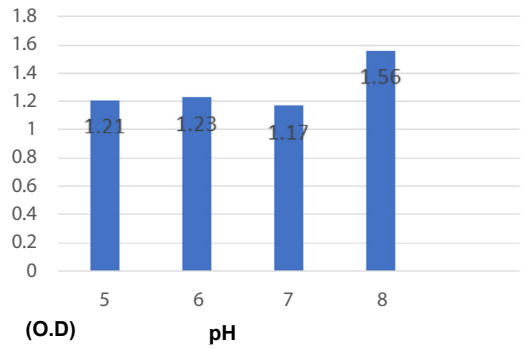


**Figure 4:** UV visible absorption spectrum showing optimum pH.

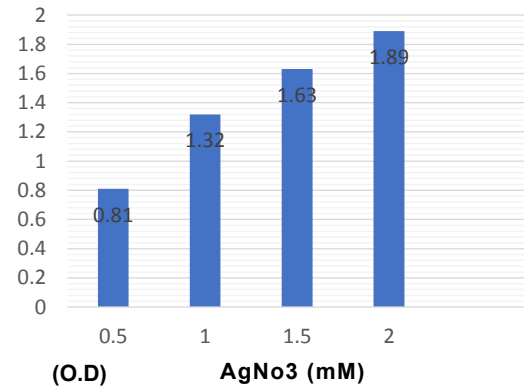


**Figure 5:** UV visible absorption spectrum showing optimum $AgNO_3$ concentration.

| S.no | $AgNO_3$ Concentration | Concentration (mol/lit) |
|---|---|---|
| 1 | 0.5 | $2.85*10^{-6}$ |
| 2 | 1 | $1.58*10^{-5}$ |
| 3 | 1.5 | $2.5*10^{-5}$ |
| 4 | 2 | $3.38*10^{-5}$ |

**Table 3:** Concentration of AgNPs at different $AgNO_3$ concentration.

| S.no | Fungal Biomass (g) | Concentration (mol/lit) |
|---|---|---|
| 1 | 5 | $1.04*10^{-6}$ |
| 2 | 10 | $1.44*10^{-5}$ |
| 3 | 15 | $1.83*10^{-5}$ |
| 4 | 20 | $2.33*10^{-5}$ |

**Table 4:** Concentration of AgNPs at different fungal biomass.

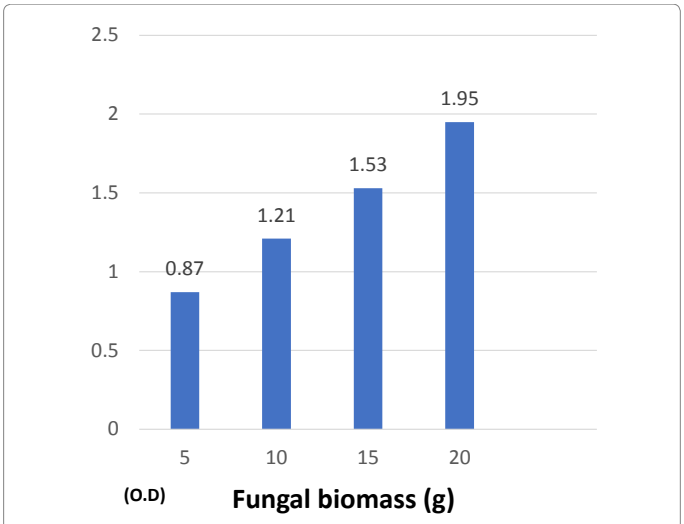


**Figure 6:** UV visible absorption spectrum showing optimum fungal biomass concentration.

## 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

1. Juibari MM, Abbasalizadehb S, Jouzanib GS, Noruzic M (2011) Intensified biosynthesis of silver nanoparticles using a native extremophilic Ureibacillus thermosphaericus strain. Mater Lett 65: 1014-1017.
2. Jeorger K, Jeorger R, Granqvist O (2001) Bacteria as workers in the living factory: metal accumulating bacteria and their potential for material science. Trends Biotechnol 19: 15-20.
3. Mann S (2001) Biomineralization: principles and concepts in bioinorganic materials chemistry. Oxford University Press, Oxford.
4. Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, et al. (2002) Enzyme mediated extracellular biosynthesis of CdS nanoparticles by the fungus Fusarium oxysporum. J Am Chem Soc 124: 12108-12109.
5. Shankar SS, Rai A, Ahmad A, Sastry M (2004) Rapid synthesis of Au, Ag and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci 275: 496-502.
6. Durán N, Marcato PD, Alves OL, de Souza GIH, Esposito E (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. J Nanobiotechnology 3: 1-8.
7. Karbasian M, Atyabi SM, Siadat SD, Momem SB, Norouzian D (2008) Optimizing nano-silver formation by F. oxysporum (PTCC 5115) employing response surface methodology. Am J Agric Biol Sci 3: 433-437.
8. Ingle A, Gade A, Pierrat S, Sonnichsen C, Rai M (2008) Mycosynthesis of silver nanoparticles using the fungus F. acuminatum and its activity against some human pathogenic bacteria. Curr Nanosci 4: 141-144.
9. Ingle A, Gade A, Bawaskar M, Rai MK (2009) Fusarium solani: a novel biological agent for the extracellular synthesis of silver nanoparticles. J Nanopart Res 11: 2079-2085.

10. El-Rafie MH, Mohamed AA, Shaheen THI, Hebeish A (2010) Antimicrobial effect of silver nanoparticles produced by fungal process on cotton fabrics. Carbohydr Polym 80: 779-782.
11. Bhainsa KC, D Souza SF (2006) Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. Colloids Surf B Biointerfaces 47: 160-164.
12. Ahamed M, Alsalhi MS, Siddiqui MKJ (2010) Silver nanoparticle applications and human health. Clin Chim Acta 411: 1841-1848.
13. Kathiresan K, Manivannan S, Nabeel AM, Dhivya B (2009) Studies on silver nanoparticles synthesized by a marine fungus Penicillum fellutanum isolated from coastal mangrove sediment. Colloids Surf B 71: 133-137.
14. Nithya R, Ragunathan R (2009) Synthesis of silver nanoparticle using Pleurotus sajor caju and its antimicrobial study. Dig J Nanomater Bios 4: 623-629.
15. Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP, et al. (2009) Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. Lett Appl Microbiol 27: 76-83.
16. Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M (2009) Fungus mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomed Nanotechnol Biol Med 5: 382-386.
17. Verma VC, Kharwar RN, Gange AC (2010) Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus Aspergillus clavatus. Nanomedicine 5: 33-40.
18. Vigneshwaran N, Ashtaputre M, Nachane RP, Paralikar KM, Balasubramanya H (2007) Biological synthesis of silver nanoparticles using the fungus Aspergillus flavus. Mater Lett 61: 1413-1418.
19. Basavaraja S, Balaji SD, Lagashetty A, Rajasab AH, Venkataraman A (2007) Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. Mater Res Bull 43: 1164-1170.
20. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, et al. (2009) Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids Surf B Biointerfaces 74: 328-335.
21. Hamedi S, Shojaosadati SA, Shokrollahzadeh S, Hashemi-Najafabadi S (2014) Extracellular biosynthesis of silver nanoparticles using a novel and non-pathogenic fungus, Neurospora intermedia: controlled synthesis and antibacterial activity. World J Microbiol Biotechnol 30: 693-704.
22. Gholami-Shabani M, Akbarzadeh A, Norouzian D, Amini A, Gholami-Shabani Z, et al. (2014) Antimicrobial activity and physical characterization of silver nanoparticles green synthesized using nitrate reductase from F. oxysporum. Appl Biochem Biotechnol 172: 4084-4098.
23. Wilcoxon J (2009) Optical absorption properties of dispersed gold and silver alloy nanoparticles. J Phys Chem B 113: 2647- 2656.
24. Jain PK, Lee KS, El-Sayed IH, El-Sayed MA (2006) Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: Applications in biological imaging and biomedicine. J Phys Chem B 110: 7238-7248.
25. Mitra B, Vishnudas D, Sant SB, Annamalai A (2012) Green synthesis and characterization of silver nanoparticles by aqueous leaf extracts of Cardiospermum helicacabum leaves. Drug invent today 4: 340-344.
26. Chen JC, Lin ZH, Ma XX (2003) Evidence of the production of silver nanoparticles via pretreatment of Phoma sp 32883 with silver nitrate. Lett Appl Microbiol 37: 105-108.
27. Christensen L, Vivekanandhan S, Misra M, Mohanty AK (2011) Biosynthesis of silver nanoparticles using Murraya koenigii (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size. Adv Mat Lett 2: 429-434.
28. Khan M, Khan M, Adil SF, Tahir MN, Tremel W, et al. (2013) Green synthesis of silver nanoparticles mediated by Pulicaria glutinosa extract. Int J Nanomedicine 8: 1507-1516.
29. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M (2006) Synthesis of gold nanotriangles and silver nanoparticles using Aloe Vera plant extract. Biotechnol Prog 22: 577-583.
30. Singh AK, Talat M, Singh DP, Srivastava ON (2010) Biosynthesis of gold and silver nanoparticles by natural precursor clove and their functionalization with amine group. J Nanopart Res 12: 1667-1675.