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Biosynthesis of silver nanoparticles using silver nitrate through biotransformation

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Keywords

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Biological synthesis
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Abstract

The development of appropriate processes for the synthesis of nanoparticles is an important aspect of nanotechnology. In the present study, fungus mediated synthesis of silver nanoparticles using *Fusarium oxysporum* has been investigated. The fungal mat was raised in MGYP broth and were transferred to silver nitrate aqueous solution for 72 hrs and analysed for the appearance of silver nanoparticles. These particles exhibited a new λmax in the visible region as observed from the absorption spectrum. The plasmon peak was observed at 440nm. These nanoparticles were further characterized by FTIR and SEM analysis. There was a shift in peak at 1635 cm^{-1} when compared to the silver nitrate solution. SEM analysis showed that the silver nanoparticles produced in the present study were of varying morphology and ranged in size from 20-70nm. However further studies regarding EDS, XRD, NMR, TEM, AFM are confirmed to strengthen the present findings. Moreover, size controlled production of silver nanoparticles also confirmed further studies regarding the optimization of the medium and culture conditions.

1. Introduction

An important area of research in nanotechnology deals with the synthesis of nanoparticles of different chemical compositions, size and controlled nanodipersity. Currently there is a growing need to develop environmentally begin colloidal nanoparticles synthesis process that do not toxic chemicals in the synthesis protocol which raises great concern for environmental reasons (Sastry *et* al, 2003).Recently, the nanoparticles made from the noble metals like silver , gold, platinum and lead. These metal nanoparticles have been synthesized using a variety of methods including hard, template (Zhou *et* al, 1999), bioreduction (Canizal *et* al, 2001: Mouxing *et al,* 2006; Yu *et* al, 1997).Consequently, researchers have turned to biological synthesis because through this biological synthesis particles obtained have good control on the size distribution rather than the other methods. The nanoparticles could also be stabilized directly in the process by proteins. (Duran *et al*, 2005).

According to national nanotechnology intiative some of the products that benefit from unique properties of nanomaterials include sun screens cosmetics, metal- cutting tools and protective paints etc. Recently, it was found out that aqueous chloroaurate ions may be reduced extra cellularly using the fungus *Fusarium oxysporum*, to generate perfectly stable gold or silver nanoparticles in water (Ahmed *et al*, 2003).Although, it is known that microorganisms such as bacteria, yeast and fungi play an important role in the remediation of toxic metals through reduction of metal ions. Only recently, this approach was considered interesting as nanofactaries.(Fortin and Beveridge, 2000). In addition, the extracts of higher plants could also produce nanoparticles and biosynthesis of nanoparticles by plant extracts is currently under exploitation (Leela and Vivekanandan, 2008). Among the aforesaid nanoparticles, silver nanoparticles play a significant role in the field of biology and medicine. Hence the present study is aimed to synthesis and characterize silver nanoparticles obtained by use of a fungal strain *Fusarium oxysporum* .

2. Materials and Methods

Chemicals: Glucose, Yeast extract, Peptone, Silver nitrate, Malt extract were procured from Himedia, Mumbai. Sterile distilled water was used throughout the experiment.

Fungal Culture Collection and Maintenance: The fungal strain of *Fusarium oxysporum* MTCC 284 was obtained from Microbial Type Culture Collection (MTCC, Chandigargh, India). The strain was maintained at 4°C on malt agar slants and also on PDA medium to produce the mycelium [Fig 1]. Funfal filterate used for biosynthetic experiments were grown aerobically in liquid media containing

malt extract 3g, glucose 10g,yeast 3g and peptone 5g per litre of distilled water. This medium was designated as MGYP and it was autoclaved at 121±1° C for 15 mins. The fungus was grown in 500ml Erlenmeyer flasks each containing 100ml MGYP medium at 25° C±1° C (180 rpm) for 72 hours [Fig 2]. After 72 hours of growth, mycelia were separated from the culture broth by centrifugation (3500 rpm) at 10° C for 20 mins and the mycelia was washed three times with sterile distilled water.

Fig 1 : Profusely branched hyphae of *Fusarium oxysporum* in PDA medium

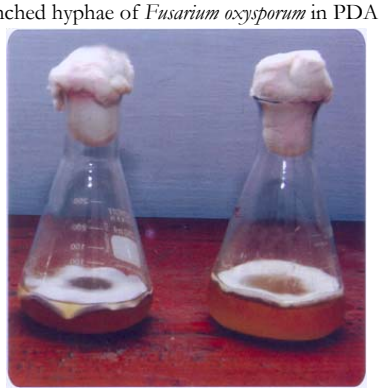
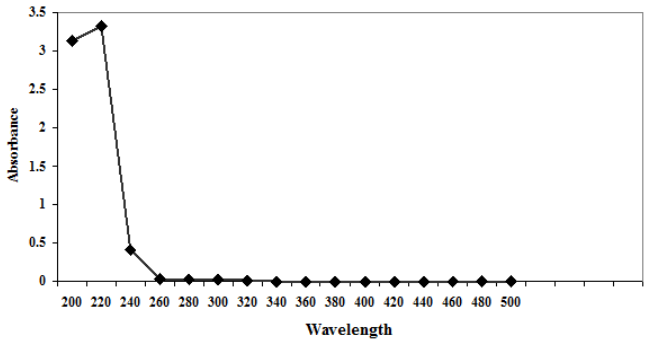
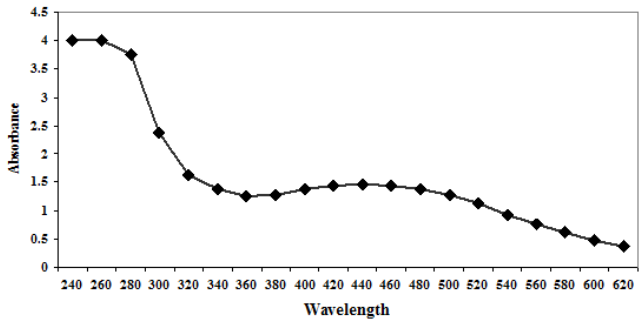


Fig 2 : Mycelial Mat formation of *Fusarium oxysporum* in MGYP Broth

Graph 1: UV Plasmon spectrum of silver nitrate



Graph 2: UV Plasmon spectrum of Silver Nitrate with fungal extract



Synthesis of silver nanoparticles: Fungal mat (20gm) was obtained from the liquid media used for the synthesis of silver nanoparticles. 20 ml of the cell free filterate was brought and transferred to 10^{-3} M concentration silver nitrate Erlen Meyer flask and agitated at 25°C in darker conditions under normal pH. Simultaneously, control without silver ions was also run along with the experimental flasks.

UV visible studies

The reduction of silver ions was monitored by measuring the UV-VIS spectrum of the reaction medium at 24 hrs time interval by drawing 1cm of the samples and their absorbance was recorded at a resolution of 0.5m at 350-800nm using UV-VIS spectrophotometer – UV 2450(Shimadzu).

FTIR Analysis

The chemical bonds present in the analyzed chemicals can be interpreted by FTIR spectrum, by using the kBr pellets with prominent resonance spectra.

The filterate containing the extra cellular proteins secreted by the fungus in the presence of Ag was salted out overnight at 40° C using ammonium sulphate precipitate followed by centrifugation at 5000 rpm for 10 mins. The proteins obtained thereafter was dissolved in the minimal volume of deionized water and dialysed using a 12 kDa cut off dialysis membrane.[BX FT-IR, Perkin – Ermer, Limited].

SEM Analysis

Scanning Electron Microscopic (SEM) analysis was done in SASTRA University, Tanjore, TamilNadu. Thin films of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 mins for emitting characteristic X-rays. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample.

3. Results

Nanosilver formation: The colour change occurred in the cell free extract when challenged with 1mM AgNo3 changed colour from pale yellow (Fig 3) to dark brown colour (Fig 4) in 48 hrs and attained maximum intensity after 72 hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticle. Control without silver ions showed no change in colour of the cell filtrates when incubated under same conditions.

Graph 3 FTIR Spectrum of Aqueous silver nitrate

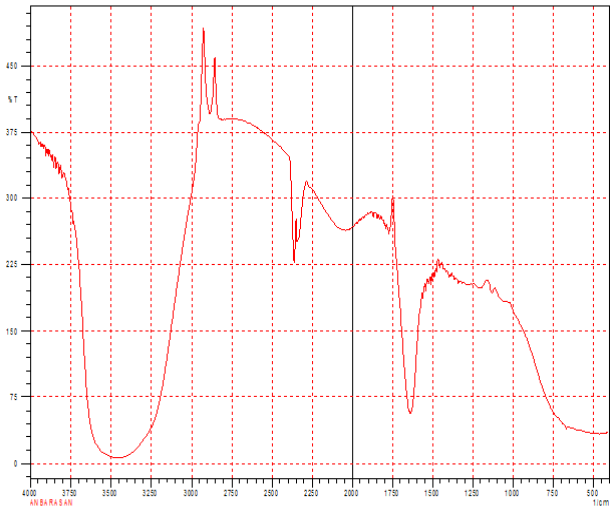


Fig 3. *Fusarium oxysporum* mat in an silver nitrate solution and Gradual appearance of brown colour in silver nitrate solution using *Fusarium oxysporum* mat

Graph 4. FTIR spectrum of Silver nitrate with fungal extract

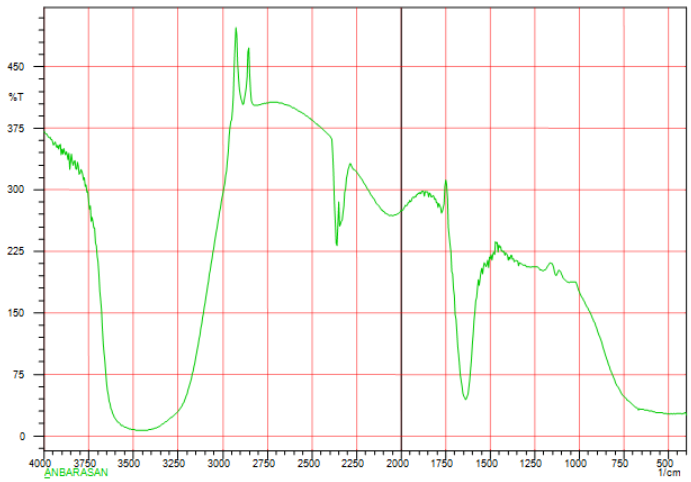


Fig: 4. SEM Micrograph showing 10000 X magnification

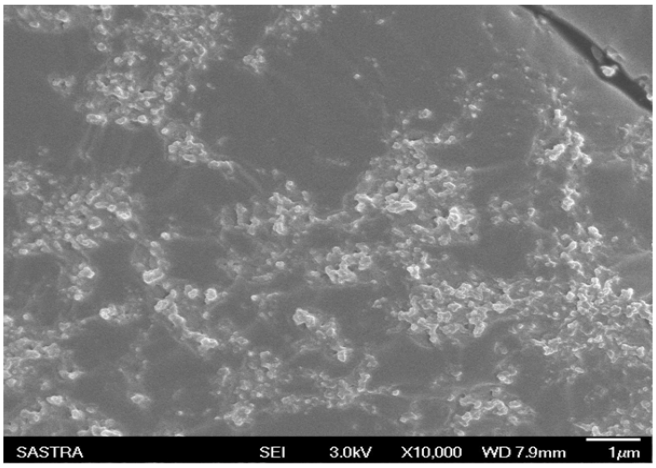


Fig 6. SEM micrograph showing 50000X magnification

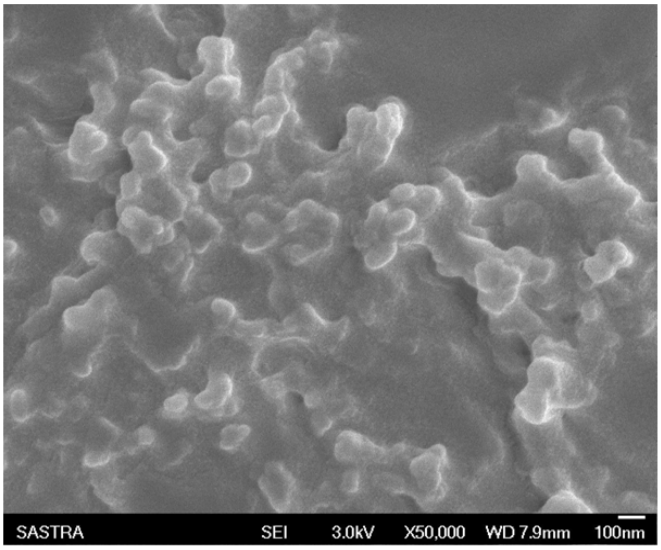
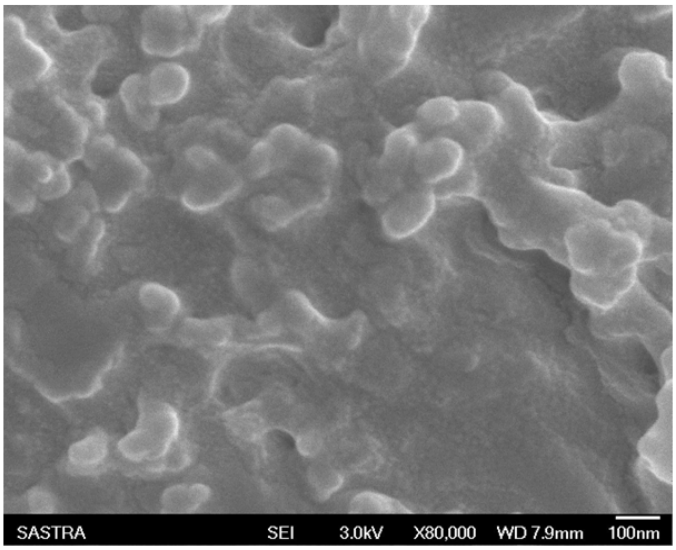


Fig: 7. SEM Micrograph showing 80000 X magnification



UV – VIS spectral studies

Graph 1 (Control) and Graph 2 (Experimental) depicts a series of typical UV- VIS spectra of the reaction solution recorded at an interval between 0 – 72 hrs. Under normal pH 6.0 the change in light absorption profile of the medium and change in intensity of the brown colour during long term incubation (72hrs). In the filtrate obtained from the fungal extract, a new path was observed in the visible region of (440nm) and suggested that the organism reduced the silver nitrate to silver oxide as the growth of the organisms preceeded in the medium.

FTIR analysis

Graph (3) control i.e. Without the fungal extract and Graph (4) experimental i.e with fungal extract depicts a series of FTIR spectra of the reaction solution recorded at intervals of 72 hrs. As a result the peak at 540nm was found to be diminished. Instead, a new peak at 1653.52cm^{-1} was found to have been appeared. These FTIR results are found to line with the finding of UV-VIS spectroscopic studies (Graph 1 and 2). Though the shift in peak confirms that there is a formation of silver oxide by the reduction of silver nitrate, and then it is necessarily to be subjected to SEM analysis to measure the size of the particle.

SEM analysis

The SEM micrographs of the present study were taken at different magnifications. The silver nanoparticles aggregated in Fig. 5 depicts that the SEM image of the Silver nanoparticles at 40000X magnification while the Fig 6 and 7 represents the SEM images of 50000X magnification and 80000X magnifications.

All the plates of SEM images with different magnifications showed that the silver nanoparticles are agglomerated. In these micrographs it was observed that the nanoparticles were in the size ranging from 20-70 nm with a variety of morphology.

4. Discussion

Sawle *et al* (2008) synthesized Au-Ag alloy nanoparticles using *Fusarium semitectum* and observed that the band corresponding to the surface plasmon resonance occurs at 545nm for gold nanoparticles and 443 nm for silver nanoparticles. In the present study *Fusarium oxysporum* was mixed with aqueous solution of the silver ion complex and it started to change the colour form pale yellow to dark brown colour(Fig 1) which indicates the formation of silver nanoparticles. It is generally recognized that UV-Vis spectroscopy could be used to examine the size and shape controlled nanoparticles in aqueous suspensions (Willey *et* al, 2006). FTIR measurements were carried out to identify the possible (protein) biomolecules responsible for the capping and efficient stabilization of the metal nanoparticles synthesized by *Fusarium oxysporum*. Sawle *et al*, (2008) reported that the peak were at 1643, 1543, 1405, and 1045cm^{-1} region is a characteristic of proteins and enzymes that have been found responsible for the reduction of metal ions by the fungal mediated synthesis of metal nanoparticles. In the present study the FTIR spectrum of silver nitrate and fungal extract was observe during 0 and 72 hours, and the gradual deminiton of peak of 540cm^{-1} and gradual appearance of peak at 1635.52cm^{-1} would have been observed, and that may also explain the

gradual reduction of silver nitrate into silver oxide by the fungus.(Fig. 3 and 4). SEM micrographs have confirmed that silver nanoparticles of 20-70nm in the size with various morphology are synthesized from *Fusarium oxysporum* . These silver nanoparticles have been used for antibiotic assay and act as disinfecting filters and coating materials (Singh *et al* (2008).This fungus mediated synthesis of silver nanoparticles would be characterized and further investigations are confirmed pertinent to the techniques like calcinations and Energy Dispersive Spectroscopic studies.

5. Conclusions

In conclusion, the biosynthesis of aqueous Ag+ ions by the fungus extract has been demonstrated. The findings of the present study has been reported as green chemistry approach using a fungus *Fusarium oxysporum* in the synthesis of silver nanoparticles at room temperature without using any harmful reducing agents such as sodium borohydrite or hydroxylamine hydrochloride and any capping or dispersing agent. Thus green synthesis of silver particles has been achieved in the present study using *Fusarium oxysporum.*

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