

# Biosynthesis of silver nanoparticles from *Aspergillus flavus*

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## Abstract

The study aimed to evaluate and optimize the synthesis of silver nanoparticles from the fungus *Aspergillus flavus*. In the present condition researchers use natural process such as biological microorganisms in order to develop eco-friendly methods for the synthesis of nanoparticles. The silver nanoparticles have the antimicrobial properties against pathogen like bacteria, fungi, and plants. *Aspergillus flavus* reacts with silver nitrate solution causes the accumulation of nanoparticles on its surface of the cell wall. Production of metallic products from the fungus is the low cost and the easy process.

**Keywords-** *Aspergillus flavus*, silver nanoparticles, streptomycin, penicillin, PDA slants, UV- visible spectrophotometry.

## INTRODUCTION

Nanoparticles provides great interest in technological and environmental challenges by their controlled size and composition based on its fundamental properties. The synthesis of nanoparticles is highly reactive. The nanoparticles are found to be stable in water for more than 3 months. Recent studies show that synthesis of nanoparticles acts as an antimicrobial agent [Ghassan M Sulaiman *et al*]. Nanoparticles are synthesis by physical, chemical and biological process [ Saeed *et al*]. Nanoparticles possess the increased integrity of optical, chemical, electronic and magnetic properties compared to large particles of bulk materials [ Navin *et al*]. The remediation of toxin metals employs microorganisms such as bacteria and fungi through application of biotechnology. The resistance conferred by bacteria to silver is determined by 'sil' gene in plasmid [ Vigneshwaran *et al*]. High metal ion concentration of microorganisms has the ability to fight against the metal stress. They include oxidation or reduction, bioaccumulation, bio absorption etc. These will be helpful in the field of bioremediation, biomineralization, bioleaching [ Kuber C *et al*]. The nanoparticles react with the cell interior by attaching through its cell wall or cell membrane [ Kim *et al*]. Aflatoxin are the secondary polyketide metabolites mainly produced by the fungus *Aspergillus flavus* [ Mariana *et al*]. It is toxic to the immune system and reduces the function of cell mediated immunity [ Cuilson *et al*]. *Aspergillus flavus* is saprotrophic and pathogenic fungus. It is best known for its colonization of cereal grains, legumes, etc. *Aspergillus flavus* is one of the useful protective agents [ H.B Patil *et al*]. The silver nanoparticles are found to have characteristic adsorption peak at 420 nm and emission peak at 553 nm. Though there are many methods for synthesis of silver nanoparticles researches in this field have turned to biological system for the development of eco-friendly technology [ Kuber C *et al*]. *Aspergillus flavus* when challenged with silver nitrate, the cell wall is accumulated with silver nanoparticles [ H. B. Patil *et al*]. Unless bacteria fungi cannot fix the atmospheric nitrogen. Fungi can easily grow at any medium [ Kamiar *et al*]. They secrete the large amount of the enzymes. The purpose of this review is to provide the outline of the published

studies which uses fungi for the synthesis of silver nanoparticles and for its characteristics.

## Sample

Silver nitrate, penicillin, streptomycin, foetal bovine serum etc.

## Organisms

*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* etc.

## Synthesis of AgNPs

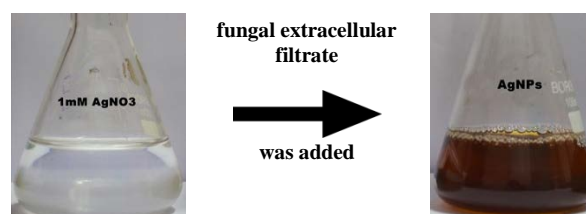
*Aspergillus flavus* was cultured on a saboroud dextrose agar plate. The growth was maintained in Erlenmeyer flask. They are incubated at 150rpm for 96 hours. As a result of incubation, the fungal biomass was separated. The pH of the culture is 6.5. The whole mixture is mixed into a shaker for 72 hours and kept in a dark condition.

## Measurement of antioxidant property

1 millilitres of DPPH were introduced into the different concentration of AgNPs by *aspergillus flavus*. The absorbance was measured against ethanol. The absorbance at low level of reaction indicates the percentage of the activity of the *aspergillus flavus*.

## Evaluation of antimicrobial activity

It is determined by agar disc diffusion method. Using a sterile cotton swab the culture was uniformly swabbed on separate plates. The nanoparticle solutions are added to the mixture. The mixture has the combination of mannitol salt agar and nutrient agar. Incubated at 37-degree C for 24 hours. The zone of inhibition was observed and measured [ Ghassan *et al*].



## Sample

*Aspergillus flavus* is obtained from National Chemical Laboratory culture collection.

### Preparation

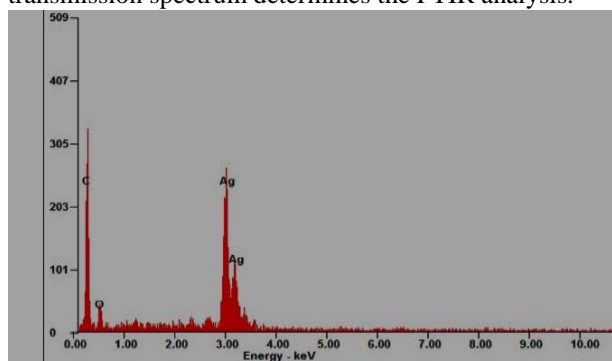
*Aspergillus flavus* is grown on yeast malt broth. The pH is around 6.2. the flask is incubated at 37-degree C. The mycelium is developed. They are separated by deionized water. The sample is then added to the silver nitrate solution. If the result is positive the mycelium growth is controlled, if it is negative the silver growth will be controlled.

### X- ray diffraction analysis

The confined silver nanoparticles and the fungi nanoparticles are made into a fine powder. The spectra were observed in X-ray diffractometer. The intensities ranges from 30 to 80-degree. The fungus sample is also optimized using X-ray diffraction.

### FTIR analysis

The mixture was diluted with potassium bromide in the ratio of 1: 1000 and spectra is recorded. The back-transmission spectrum determines the FTIR analysis.



### Biogenic synthesis of silver nanoparticles

Methods involving the use of organisms like fungi, bacteria, and plants reduces the metal salts. For a clean nontoxic and environmental acceptable condition, the production of nanoparticles must be provided with reduction of metal in biological agents [ Azmath *et al*]. biosynthesis of silver nanoparticles is very easy with fungi. It offers high tolerance to metal and provides more amount of extracellular proteins [ Balaji *et al*]. They do not require additional steps to extract the filtrate. They provide good biomass production [ Gade *et al*]. By maintaining the parameters like temperature, time, pH, biomass the metabolism of fungi cab be manipulated to obtain nanoparticles with desired properties [ Zielonka *et al*].

### Optimization of silver nanoparticles

To get the good stability, and biocompatibility of the particle the parameters must be optimized [ Balakumaran *et al*]. for different parameters the different values will be optimized which raised to give the various physicochemical characteristics of nanoparticles.

### Effects of temperature

he temperature used in synthesis of silver nanoparticles employing fungi can affect parameters such as the speed of the synthesis and the size and the stability of the nanoparticles [ Elamwin *et al*]. Faster rates of synthesis at high temperature leads to the poor quality of nanoparticles.

### Effect of pH

pH is used to control certain characteristics of the nanoparticles [ Nayale *et al*]. The concentration of photon in the reaction medium, the nitrate reductase enzymes could alter. Some studies shown that successful synthesis will occur at neutral alkaline pH.

### Isolation and identification of aspergillus flavus

A fungal strain was obtained from infected cadavers butterfly. The sample was placed in martin rose Bengal agar media, they are incubated at 28-degree C. individual colonies was taken and purified by potato dextrose agar media. The colour, texture of myceli, spore formation patterns are observed.

### Synthesis of AgNPs

*Aspergillus flavus* was maintained on PDA slants for 28-degree C. the stock culture was inoculated in 500 mL sabouraud agar and inoculated at 28-degree C for 7 days. Whatman filter paper seperates the biomass. The reaction mixture was prepared by 10ml of the fungal extract to 90ml of 1mmol AgNO<sub>3</sub> solution in a 250ml conical flask and incubated at 29-degree C for reduction [ Kamiar Zomorodian *et al*].

### Sample

Standard protein molecules weight markers were obtained from MBI Fermentas.

### Isolation and identification of fungal isolate

The fungus was isolated from metal-rich regions. For the serial dilution the soil samples are inoculated and plated on martin rose Bengal agar media. They are incubated at 28-degree C for 5 days. The sub-culturing technique is obtained by picking the fungal colonies. It was done by potato dextrose agar. By the use of protocols genomic DNA is isolated. The primer is used to amplifies the internal space regions. The information is collected, the phylogenetic tree is constructed.

### UV- spectroscopy analysis

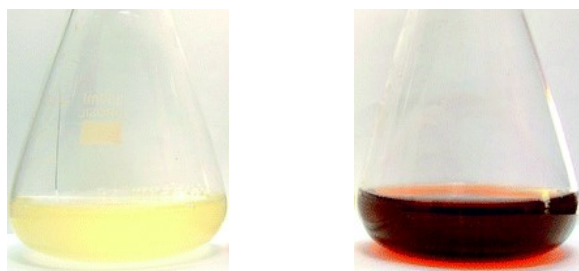
When silver nitrate solution is incubated with aspergillus flavus, the colour changes will occur at the silver nitrate solution. At different time intervals the silver ions reduced as a result of bio reduction. UV- visible spectrophotometers absorbs the absorption measurements. To check the stability of silver nanoparticles the UV- Vis is used.

### EDS analysis

Energy dispersive spectroscopy the samples is prepared in copper substrate by silver nanoparticles drop coating. Single particle analysis was done by Thermo Noran EDS attachment.

### Particle analysis

Dynamic light scattering is used to find the size of silver nanoparticles. Data obtained was evaluated by Zetasizer software [ Navin *et al*].



**WITHOUT SILVER NITRATE      WITH SILVER NITRATE**

**Sample**

The silver nitrate is prepared from sigma.

**Procedure**

*Aspergillus flavus* isolated from Ahar copper mine soli. The process is carried out in SDA slant culture. 1g soil is serially diluted in distilled water. As a result, the concentration is obtained. The volume of 1ml of the sample is transferred to the plates. The growth of fungus will occur after sometimes. *Aspergillus flavus* is isolated by slant culture. The fungus is grown in Erlenmeyer flasks which contains the 100ml of MYPG medium. The medium is composed of yeast extract, glucose, and malt. After, 96 hours of incubation fungi biomass is separated. Centrifuge at 3500 rpm for 20minutes. The fresh biomass is added to the silver nitrate solution. The pH is 6. The sample is scanned by UV- spectroscopy. At the end, the samples are made into powder for the detection of X-ray diffraction [ vigneshwaran *et al*].

**Sample**

*Aspergillus Flavus* is obtained from National Chemical Laboratory, Pune.

**Instruments**

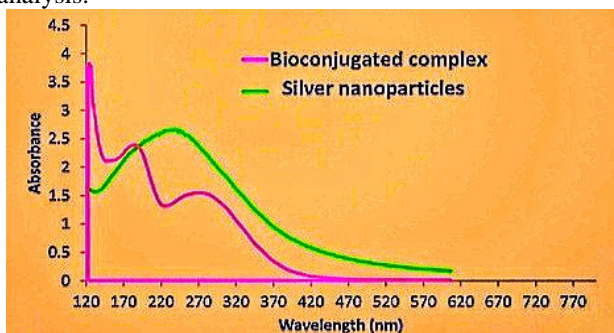
pH meter, UV- visible spectrophotometer, magnetic stirred, electric shaker etc.

**Nutrient medium**

Yeast extract malt dextrose contains yeast extract, malt extract etc. The Hi media is used for the preparation of the experiment.

**Preparation of fungal biomass**

The batch flask was inoculated with fungus. They are incubated at 37-degree C. After incubation the biomass is harvested by the filtration. This are washed with distilled water. 20 gm biomass is adder to 100 ml distilled water for 72 hours at 37-degree C. After incubation the biomass passes through the Whatman filter paper and Millipore filter paper. Prepared samples are subjected to uv- visible analysis.



**RESULT**

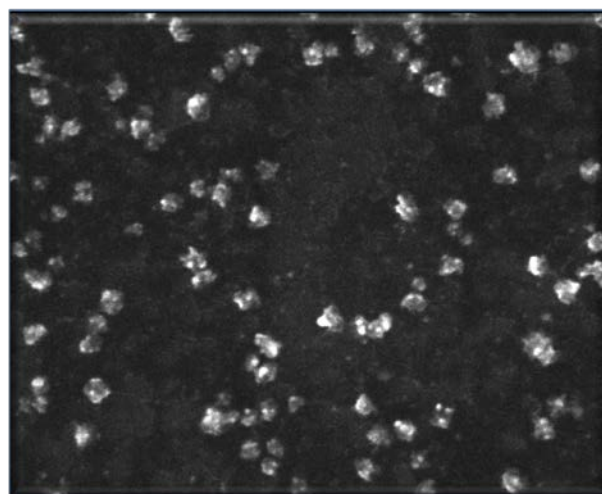
The reduction of Ag<sup>+</sup> ion indicates the synthesis of silver nanoparticles with the supernants. It is absorbed that silver nanoparticles appears brown colour in the water. It is due to the excitation of the surface plasmon resonance in the metal ions. UV- spectroscopic studies as shown that the silver nanoparticles is formed with the frequency width of 420nm. UV- spectroscopy is used to analyse the size and shape of the nanoparticles in aqueous suspensions. Silver nanoparticles exhibits the better antimicrobial agents against the pathogens. The zone of inhibition is increased for all the pathogens. Because of their unique properties it has been extensively used in many industries like nanomedicines, pharmaceuticals etc.

The colour of the filters changed after to intense after 48hours of incubation. The transmission of laser light detects the synthesis of the nanoparticles. *Aspergillus fumigatus* has more intensity compared to the *aspergillus flavus*. So, the biosynthesis of silver nanoparticles is reduced in the *aspergillus flavus*. The size of the silver nanoparticles varies for every different fungus. Most of the fungus were formed to be spherical in shape. Protein is formed in some culture medium plays a vital role in the reduction of nanoparticles.

The fungal mat incubated with deionized water retains its original colour. The fungus treated with the silver particles are turned into dark red. After incubation of the samples the intensity is unchanged at 420nm, results the reduction of the silver ions. The average size of nanoparticle was estimated to be 8.92nm. The stability is determined by the measurement of the intensity.

The biomass that mixing with the aqueous silver ions turns into a brownish colour. UV- visible spectroscope reaches the peak of 425nm in the mixture. Silver nanoparticles involved in the production of mycelial is not found. It is due to the electrostatic reaction with negative charges in the enzymes.

The extracellular synthesis of silver nanoparticles from *aspergillus* species is an effective method. The silver ions are formed after the incubation of 96hours. The SEM micrograph is used to determine the size of the particles with the help of Cottrell's method.



**SEM HV : 20.00 KV ; SEM MAG : 49.97  
ET: SE DETECTOR ; WD : 6.488**

### CONCLUSION

The development of reliable, eco-friendly and environmental aspects for the synthesis of silver nanoparticles plays a vital role in the nanotechnology. Bio absorption mechanism of metal ion by microorganism includes exchange, precipitations and complexation. Though there are several methods for the synthesis of silver nanoparticles. According to my knowledge the production process based on the fungus aspergillus species is considered as the best method. It is the simplest and cheapest method for the production of silver nanoparticles. The incubation time is also less according to the respective species.

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