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Mycosynthesis of Gold Nanoparticles from Gold Salt using *Aspergillus niger*

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Abstract—*Gold nanoparticles have found prominence due to their inert nature and versatile applications. Mycosynthesis of noble metal nanoparticles has received profound interest owing to their potential to be produced in a less stringent and eco-friendly method, unlike physical and chemical methods. This report depicts economically viable synthesis of gold nanoparticles (GNPs), mediated by Aspergillus niger NCIM 1025. Growth patterns of the strains confirmed that for the purpose of nanoparticle formation, a more efficient approach would be to first grow the biomass in non-limiting conditions and then add gold salt solution for reduction. Cyclic Voltammetry studies were conducted to show the varying reducing capacities of these strains. The process of metal reduction can only take place if the mediators formed by the microorganisms have lower reduction potential than the metal ions present in the solution. The present work focuses on the production of nontoxic, biocompatible gold nanoparticles by Aspergillus niger as a reducing agent. An efficient approach for fungal growth was discussed wherein the gold salt solution was added to the biomass after growing under non-limiting conditions. Production of gold nanoparticles has been successfully achieved using Aspergillus niger and the significant change of biomass color from colorless to purple was achieved within 4 hours of incubation. Cyclic Voltammetry studies were conducted to show to the reduction in the efficiency of the strain. UV-Vis spectroscopy studies of blank Czapek Dox media did not exhibit any characteristic peak near the 540 nm range indicating no extracellular synthesis of GNPs (as per no change in media color) and only intracellular gold nanoparticle formation. Aspergillus niger NCIM 1025 gave promising results under optimum conditions. Further, characterization and application of gold nanoparticles will be checked for wound healing purposes.*

Keywords*: Gold nanoparticles; Bioreduction; Cyclic voltammetry; Wound healing.*

1. INTRODUCTION

The synthesis of nanoparticles has great interest due to their unusual chemical [1], physical [2], electronic [3] and optical properties [4]. And there are significant amount of researchers working in the nanotechnology industry due to the wide range of applications these nanoparticles provide. The properties of nanoparticles are dependent on the parameters like size, shape, and composition. Although the physical and chemical methods used to produce the metal nanoparticles are popular they also limit the biomedical applications. So, there is a need to emphasize on the production of metal nanoparticles using a reliable and eco-friendly method. Although the quantity of nanoparticles synthesized using the biogenic method is less but is eliminating the use of outdated methods and production of hazardous chemicals in this process. A variety of nanoparticles can be synthesized using different types of microorganisms like bacteria, fungi and other plant derivatives [5]. The nanoparticles generated using this biogenic approach have better catalytic activity and an improved contact between the metal salt and enzyme [6]. And the microbes have the potential to produce these NPs either intracellularly or extracelluarly. It has been reported that variation in physical parameters like pH and temperature affects the formation of NPs [7, 8, 9]. The usage of fungal strains for the production of NPs is highly advised due to the ease of scaling up, downstream processing and the presence of mycelia give an increased amount of surface area [10]. Mukherjee et al. also suggested the usage of fungus over bacteria because the fungi secrete higher amounts of protein than bacteria which amplifies the production of nanoparticles. Our work here is limited to the production of gold nanoparticles (AuNPs). Based on published literature, it is almost not possible to compare the performance of bacteria and fungi in terms of their metal salt reduction ability. In fact, the experimental conditions adopted (e.g. temperature, initial metal concentration, pH) are not homogeneous. This issue needs to be addressed by developing a general model for the production of AuNPs in viable microorganisms. Repeatable biosynthetic experiments at the same biomass and metal concentration will provide a sound basis to assess metal nanoparticle biosynthesis across bacteria and fungi. Here, we chose to work with the Aspergillus species is known to produce the AuNPs both intracellularly and extracellularly. And the work focuses on the biogenic reduction of the gold chloride salt. Although in recent times several other organisms have been investigated for the fabrication of nanoparticles, the mechanism is still not well understood. This review, therefore, focuses on the biogenic synthesis of AuNPs by fungi and attempts to explore the areas where the produced AuNPs can be put to use.

Fig. 1: Fungal Biomass (0.2 mM)

2. MATERIAL AND METHODS

2.1 Media Components

Sucrose, yeast extract, agar, malt extract, peptone for bacteriology and auric chloride AR grade were purchased from Central Drug House (P) Limited, New Delhi.

2.2 Microorganisms and culture conditions

The fungus *Aspergillus niger* NCIM 1025 was obtained for the National Collection of Industrial Microorganisms (NCIM), NCL, Pune and maintained on the potato-dextrose agar slants. Stock cultures were maintained by subculturing at regular intervals. The fungus was grown at 28C for 3 days.

2.3 Production of the gold nanoparticles

Aspergillus Niger was used to prepare the fungal biomass. The fungal biomass was allowed to grow for three days and then separated from the media using a filter paper. This obtained biomass was then washed with distilled water thoroughly to remove any media components left. The biomass was brought in contact with gold salt solutions of different concentrations (0.1 mM and 0.2 mM).

2.4 Characterizations

To determine the growth kinetics, the biomass (with the gold salt and without the gold salt) was dried at 60C for overnight and the dry weight was measured. Absorbance was measured using the UV-Vis spectrophotometer. We used Shimadzu-1800 in the wavelength range of 200-800 nm and distilled water was used as blank.

3. RESULTS AND DISCUSSION

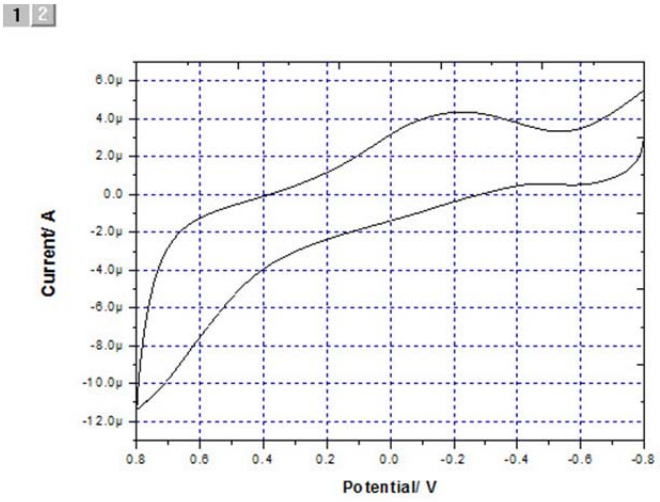
3.1 Nanoparticle formation

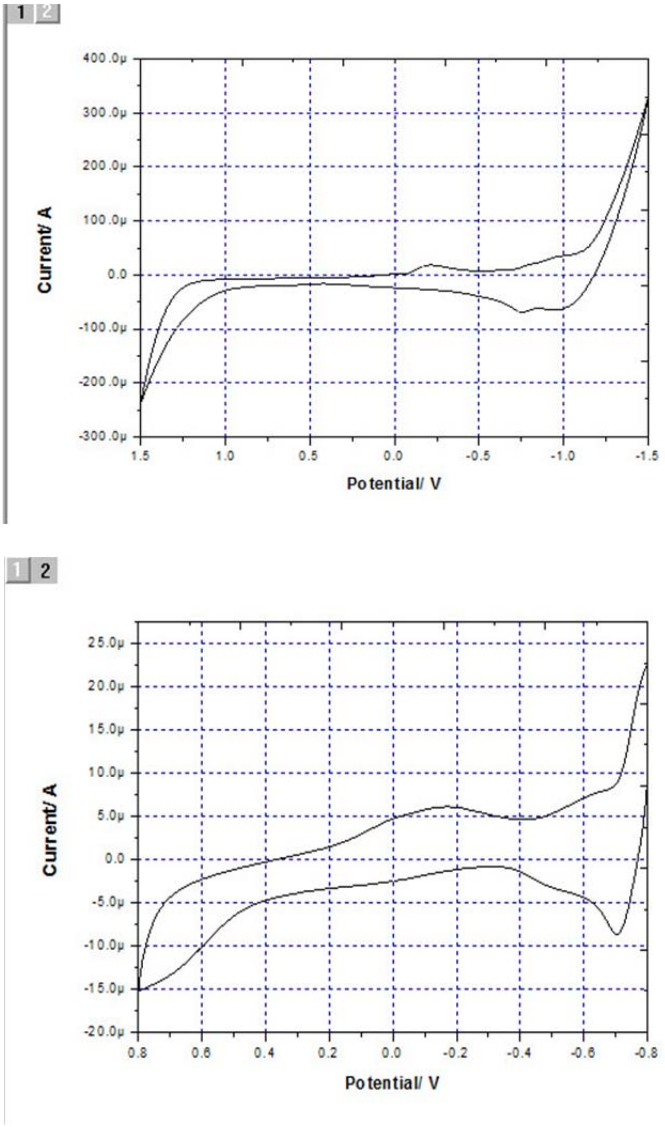
After the conversion using this biogenic approach, we observed the significant change in the color of the broth. This change in color varied as the concentration of gold salt in the broth varied. It is also observed that the intensity of color varied with the time. It was observed that after 4 hours of incubation the color of biomass changed into purple. (Salt concentration was 0.2 mM)

4. CHARACTERIZATION OF FUNGAL BIOMASS

4.1 Cyclic Voltammetry analysis

The fungal strain showed excellent pellet formation at the end of aerobic growth phase. Cyclic Voltammetry (CV) of cell free filtrate, gold cell mass and filtrate along with cells was performed. This suggests that the cell free filtrate does not contain any charge as such. When CV analysis of fungal biomass with media broth was performed, notable peaks were observed and also the $E_{mediator}$ value calculated was 0.8V. This suggests that the cell mass has the reducing power of its own and have tendency towards mediator to release when soluble electron acceptor species are present in the media. CV analysis of fungal biomass was conducted and significant peaks were observed. The $E_{mediator}$ value was then calculated to be 0.750 V. Therefore it shows that the fungal biomass has a good reducing property and the fungal biomass as a whole supports the intracellular synthesis of AuNPs. Cell biomass grown in gold chloride supplemented media showed peaks in the positive cycle. This suggests that the strains have natural tendency towards mediator release when soluble acceptor electron are present in the media. The $E_{mediator}$ value of fungal biomass supplemented with gold turned out to be 0.7850 which is less than the $E_{mediator}$ value of fungal biomass itself rendering fungal biomass to be strong reducing agent as compared to all the other samples.





Figures: Cyclic voltammetry analysis of *Aspergillus niger* NCIM 1025 (a) gold cell mass (b) Cell mass and filtrate (c) gold filtrate

4.2 Growth characterization

Growth pattern of the fungal strain was studied in normal nutrient media with/ without gold chloride solution to establish the effect of the gold salt on the growth of the microorganism. Normal growth pattern was observed in fungal culture grown in nutrient media (Czapek Dox). However, growth was limited in case of culture grown in presence of gold chloride, indicating toxicity of the salt towards the strain. In addition, the fungal biomass acquired a purple colour, while the media acquired a paler tint. This suggests that though the presence of gold chloride in inhibitory to fungal growth, the limited growth is associated with reduction of the salt. Inoculation and the growth of the fungal strain in gold chloride containing media limited growth and also hampered the reduction of gold chloride. Growth associated nanoparticle reduction is not very efficient. Hence, for the formation of nanoparticles, the efficient approach would be to first grow the fungal biomass in normal non-limiting condition, followed by addition of the gold chloride solution. Specific growth rate was calculated as 0.01803 h^{-1} for biomass without gold chloride and 0.00302 h^{-1} for biomass with gold. This means that some inhibitory effect was caused due to addition of gold chloride solution.

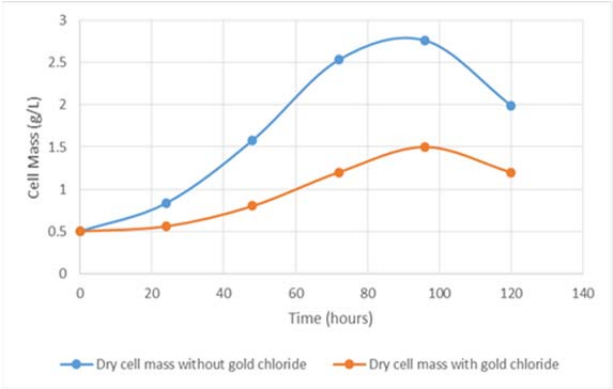


Fig. : Growth Characteristics of *Aspergillus niger* in absence and presence of gold chloride. Experiment-with gold chloride solution)

5. CHARACTERIZATION OF NANOPARTICLES

5.1 UV-Vis Spectroscopy

The culture in which the fungus was grown was exposed to the gold chloride salt for 72 hours and were tested for the UV-Vis spectroscopy. No characteristic peaks were observed suggesting the intracellular formation of gold nanoparticles.

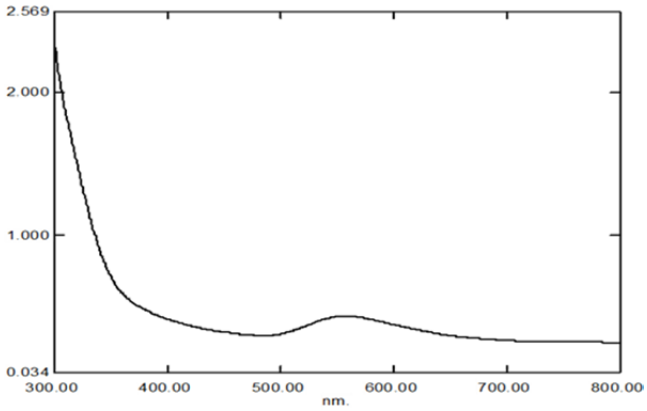


Figure: UV-Visible Spectrophotometric Characteristic Peak of Gold Nanoparticle produced by *Aspergillus niger NCIM* 1025

6. CONCLUSION

The production of nanoparticles has been carried out successfully using the fungus *Aspergillus niger* NCIM 1025. The change in the color of biomass indicated the production of

gold nanoparticles. The controlled parameters played an important role in making the production intracellularly. UV-Vis spectrophotometric studies of the blank media showed no significant peaks which indicates no extracellular growth. Therefore, it was found that *Aspergillus niger* NCIM 1025 can be used for the production of gold nanoparticles. These synthesized will be further characterized and assessed for potential applications like biocompatibility and wound healing.

7. ACKNOWLEDGEMENT

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