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SYNTHESIS OF COPPER NANOPARTICLES BY *ASPERGILLUS* SPECIES

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Abstract

Recent developments in the biosynthesis of nanomaterials have demonstrated the important role of microorganisms in nanotechnology. The organisms show a unique potential in environmentally friendly production and accumulation of nanoparticles with different shapes and sizes. The present study proposed a green process for synthesis of copper nanoparticles using Aspergillus species. Syntheses of copper nanoparticles were characterized by UV-visible spectroscopy. The extracellular synthesis of copper nanoparticles was characterized by scanning electron microscopy and Transmission electron microscopy.

Keywords *Copper nanoparticles, Aspergillus species, Extra cellular synthesis, Scanning Electron Microscopy, Transmission Electron Microscopy.*

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Introduction

Nanotechnology is an emerging field of science which involves synthesis and development of various nanomaterials. The nanoparticles possess unique physico-chemical, optical and biological properties and are used in various fields such as optical devices [1] , catalytic [2], bactericidal [3], electronic [4] sensor technology [5] , biological labeling [6]. Copper nanoparticles, due to their unique physical and chemical properties have been of great interest recently. Copper nanoparticles have potential industrial use such as gas sensors, catalytic processes, high temperature superconductors, solar cells [7]. In the last decade, application of nanomaterial has been extensively increased and the high demands lead to the bulk production of the nanomaterial. Biosynthesis of nanoparticles using bacteria [8], fungus and plants [9 , 10] has emerged as a simple and viable alternative to more complex physical and chemical synthetic procedures to obtain nanomaterials. The ability of some microorganisms such as bacteria and fungi to control the synthesis of metallic nanoparticles, should be employed in the search for new materials. Synthesis of nanoparticles either intracellularly or extracellularly was reported [11,12] previously for heavy metals like Pb, Ag, Au, cd, but very limited studies have been reported using fungi for nanoparticles biogenesis. Fungi, due to their tolerance and bioaccumulation ability of metals, are taking the centre-stage of studies on biological metal nanoparticle generation. Two different genera of fungi, *Fusarium oxysporum* sp. [13] and *Aspergillus terreus* [14], when exposed to aqueous gold and silver ions, reduced the metal ions intracellularly. But in the case of *Aspergillus fumigatus* [15] and *Trichoderma reesei* [16] , reduction of the silver ions occurred extracellularly. [17] reported biological synthesis of copper oxide nanoparticles using *Pseudomonas* strain with variable sizes and shapes. Since copper costs significantly less than silver and gold it is economically attractive in various fields. Keeping the lacunae in view the present study attempted to examine the biogenesis of copper nanoparticles using *Aspergillus species.*

Experiment Details

Aspergillus species (BankIt ID: 1526725) isolated from the soil samples collected from Hyderabad Metal Plating Industry, I.D.A, Balanagar, Hyderabad, A.P. were preserved at 4ºC in a refrigerator.

Extracellular Synthesis of copper Nanoparticles UV-Vis studies. Extracellular synthesis & stability of copper nanoparticles was determined by using UV-Vis Spectroscopy. The fungus strain was grown in 1.0 mmol/L $CuSO_4$ for 96 hrs at pH-5, 25^{o}C in orbital shaking incubator. Control (With out copper) was also run along with the experimental flasks. Nanoparticles in the medium were analyzed by filtering the media intermittently at 24, 48, 72 and 96 hrs separately and all the samples were analyzed immediately after the filtration of media at different absorbance using UV-Vis spectrophotometer.

SEM studies. The Presence of extracellular copper nanoparticles were analyzed by using scanning electron microscopy. The fungus was grown in 1.0mmol $CuSO_4$ and after 48 hrs the filtrate embedded with zinc nanoparticles was dried under vacuum and subjected to SEM studies.

TEM Studies. The fungus was grown in 1.0mmol $CuSO_4$ and after 48 hrs the medium is centrifuged and fungal pellet is collected. The fungal pellet is used for TEM Studies to identify copper nanoparticles.

Results and Discussions

During the present study, the capacity of *Aspergillus species* to synthesize copper nanoparticles was observed.

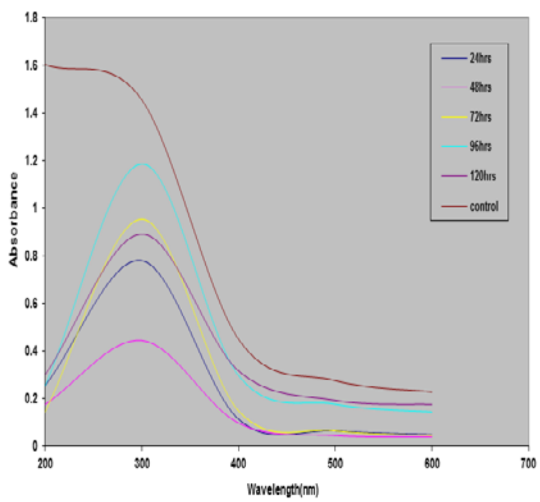

Figure 1: *UV-Vis spectrum of aqueous medium during synthesis of copper nanoparticles*

Stability of the nanoparticles formed in the broth was confirmed by using UV-Vis spectroscopic analysis. When the filtered broth sample was analyzed in the wavelength range between 200nm to 600nm, the maximum absorbance was observed at 300nm. When the spectral analysis was performed after 24hrs, 48hrs, 72hrs, and 96 hrs of incubation, the maximum absorbance was observed at the same wavelength for all incubation time periods as shown in the (fig.1) with wavelength on x-axis and absorbance on y-axis and the absorbance above 400nm was found to be negative.UV-VIS spectroscopy has provided confirmation for the presence of nanoparticles extracellularly and their stability.

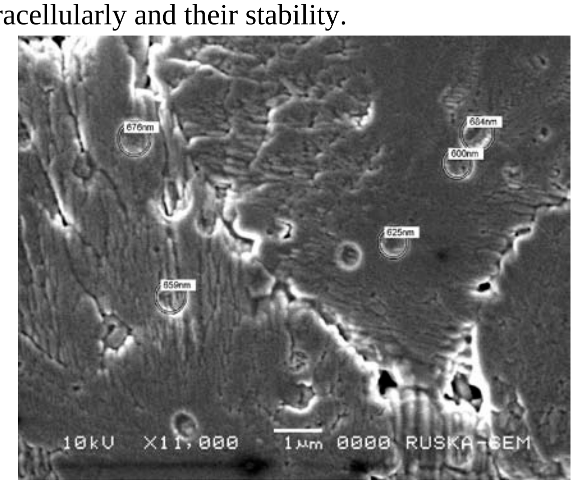

Figure 2: *SEM image of Copper nanoparticles formed by Aspergillus species 3800X.*

The spectroscopic analysis showed maximum peak at 300nm indicating the presence of nanoparticles in broth, as no variation was observed at maximum absorbance the copper nanoparticles were confirmed to be stable. Ahmed et.al, reported that CdS nanoparticles formation was due to the release of sulphate reductases enzymes by *Fusarium Oxysporum* [18]. Scanning Electron Microscopy has provided further insight into the morphology and size details of the synthesized nanoparticles.SEM micrograph revealed the formation of polydispersed nanoparticles with 600nm – 684 nm sizes range and are spherical in shape. Nanoparticles are formed in aggregates may be to minimize the toxicity by formation of insoluble complexes with metal ions. Also when copper nanoparticles are exposed to air, surface oxidation occurs and ultimately aggregation appears in a short time. The size and dispersity control may be due to interaction of different proteins with metal nanoparticles.

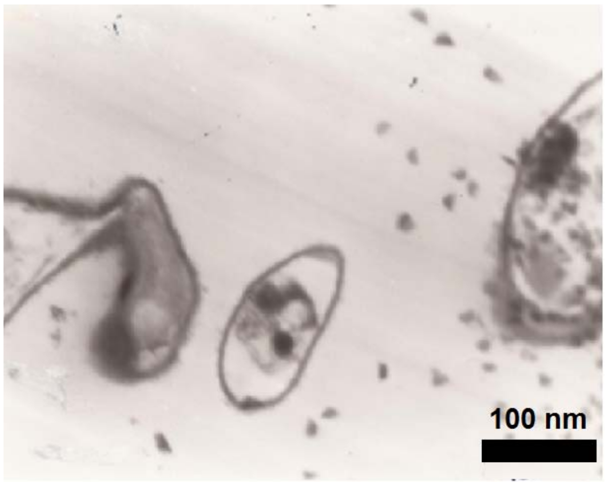

Figure 3: *TEM image of Copper nanoparticles formed by Aspergillus species 3800X.*

SEM studies clearly establish that the reduction of Cu ions occurs extracellularly, it would be important to identify the reducing agents responsible for this reaction. Previous studies [19] have indicated that NADH and NADH dependent enzymes are important factors in the biosynthesis of metal nanoparticles.It is also reported that *Penicillium citrinum, Penicillium waksmanii and Penicilliumaurantiogriseum* produced spherical copper nanoparticles of size 90- 295 nm [20]. S*treptomyces sps* extracellularly produced copper nanoparticles in the size range of 100-150 nm [21]. TEM studies revealed the presence of copper nanoparticles on the surface of the mycelia and inside the cell. The average size of the nanoparticles is approximately 500nm. The same species synthesised zinc nanoparticles of size 5-20nm [22]. The SEM and TEM data clearly indicates that the sample exposure to air is affecting the size of the copper nanoparticles. We speculate that since the nanoparticles are formed in the media and on the surface of the mycelia and in the cytoplasm, the first step involves the reduction of copper ions by the enzymes secreted extracellularly by the fungus and in the second step copper nanoparticles are attached to the fungal cell wall. To the best of our knowledge, this is the first report on the biogenesis of copper nanoparticles using *Apergillus* species.

Conclusions

Fungal mediated green chemistry approach towards synthesizes of nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, possibility of covering large surface area by suitable growth of mycelia etc., Extracellular synthesis of nanoparticles could be highly advantageous from the point of view of synthesis in large quantities and easier downstream processing as compared to the intracellular synthesis. In future it would be important to achieve better control oversize and polydispersity of the nanoparticles and to understand biochemical and molecular mechanism of the synthesis of the nanoparticles. The study also exemplies the application of metal- tolerant fungi for the metal biotransformation.

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