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Biological synthesis of silver nanoparticles by *Aspergillus flavus*, isolated from soil of Ahar copper mine

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Abstract

In this study, silver nanoparticles were synthesized using the fungus, *Aspergillus flavus* at high yield with an aqueous solution of $AgNo_3$. The formation of silver nanoparticles was confirmed using UV-visible spectrophotometer and their presence was displayed by XRD studies. The size and place formation of silver nanoparticles were investigated using Scanning Electron Microscopy (SEM).

Keywords: Silver nanoparticles, Fungi, *Aspergillus flavus*, Biosynthesis.

Introduction

Nanotechnology involves the production manipulation and use of materials ranging in size from less than a micron to that of individual atoms (Badri Narayanan & Natarajan, 2010; Gajendran, 2007). One of the most important criteria of nanotechnology is that of the development of clean, nontoxic and eco friendly green chemistry procedures (Sharma & Yangard, 2009). Silver nano particles have found potential application in many fields such as, antibacterial effect, biological sensors, drug delivery, textile, and filters (Elechiguerra *et al*., 2005; Gajendran, 2007). Nanoparticles can be synthesized by physical, chemical and biological methods (Kathiresan, 2009). Synthesis of nanoparticles employing microorganisms has attracted much due to their usual optical, chemical, photoelectron chemical and electronic properties and many biological organisms, such as bacteria, fungus, yeasts and plants either intra or extracellular (Castro-Longoria *et al*., 2010) which are of higher production yields and with low expenses. Fungi are ideal candidates in the synthesis of metal nanoparticles, because of their ability to secrete large amount of enzymes (Kathiresan *et al*., 2009). In this regard, we report the use a fungi *Aspergillus flavas* in synthesis of extra cellular of silver nanoparticles.

Material and methods

All chemical agents including $AgNo_3$ were prepared from sigma. The *Aspergillus flavus* fungus isolated from Ahar (Iran) copper mine soli and was cultured in SDA slant. Soil sample was remained in 4° C for next experiments. One gram of soil sample was serially diluted in sterilized distilled water to get a concentration range from 10^{-1} to 10^{-6}. A volume of 0/1 ml of each dilution was transferred aseptically to plates. Then fungus growth after 48 h. any one of fungus was cultured in new SAD plates until perfect isolation of fungus. Then *A. flavus* was isolated by slide culture method for examination. For syntheses of silver nanoparticles from the biomass, *Aspergillus flavus* was growth in 250 ml Erlenmeyer flask containing 100ml MYPG medium which composed of malt extract (3gr/L) peptone(5gr/L), glucose (10 gr/L) and yeast extract (3gr/L) (Ahmad et al., 2003). This culture was incubated on orbital shaker with 150-rpm agitation at 28 °C for 96 h. After 96 h incubation, fungal biomass was Separated from MYPG broth by centrifuge 3500 rpm at 10°C for 20minutes and washed with distilled water to remove any medium components. Fresh and clean biomass was exposed in 100 ml of 1 Mm aqueous $AgNo_3$ solution by pH 6.5 in 250 ml Erlenmeyer flask. The whole mixture was put into a shaker at 28 °C for 72h in dark. The sample was scanned in the range of 350 to 750 by UV-visible spectrophotometer (Bhainsa & D'Souza, 2006). Sample was powdered and prepared for X-Ray diffraction at the end step. Sample was fixated by glutaraldehyde, dehydrated by alcohol for SEM analysis (Vigneshwaran *et al*., 2007; Talebia *et al*., 2010).

Fig. 1. Change of biomass color from colorless (Right) to brownish (Left)

Result and discussion

The biomass that mixing with the aqueous solution of Ag ions, the color of the biomass changed from colorless to brownish (Fig.1) usually after 72 incubation that was the first symptom of nanosilver production. UV-Visible spectrophotometer results showed a peak at 425 nm (Fig. 2). X-RD pattern was compared with standard pattern and evidenced for production of nanosilver crystals (Fig.3). In final step, prepared a SEM picture and showed silver nanoparticles in size rang 7 nm (Fig. 4). In earlier

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research, production of silver nanoparticles in fungi with a number of these particles trapped on the mycelia (Mukherjee *et al*., 2001). Our results then showed similar silver nanoparticles that were involved on the mycelia and not found in solution. This may be caused by electrostatic reaction among Ag^+ of $AgNo_3$ and groups with negative charged (carboxylate groups) in enzyme present in the cell wall of the mycelia.

Fig. 2. UV-visible spectrophotometer scans in the range of 350 to 750

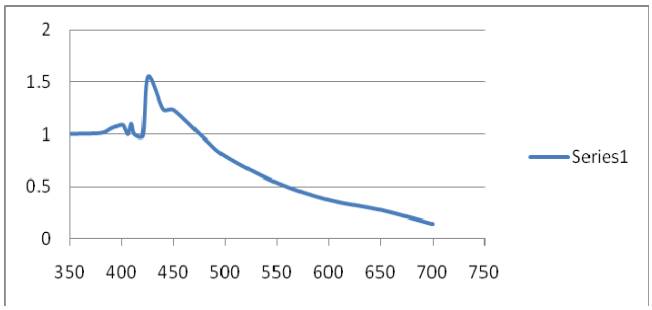


Fig. 3. X-Ray diffraction of fungi powder

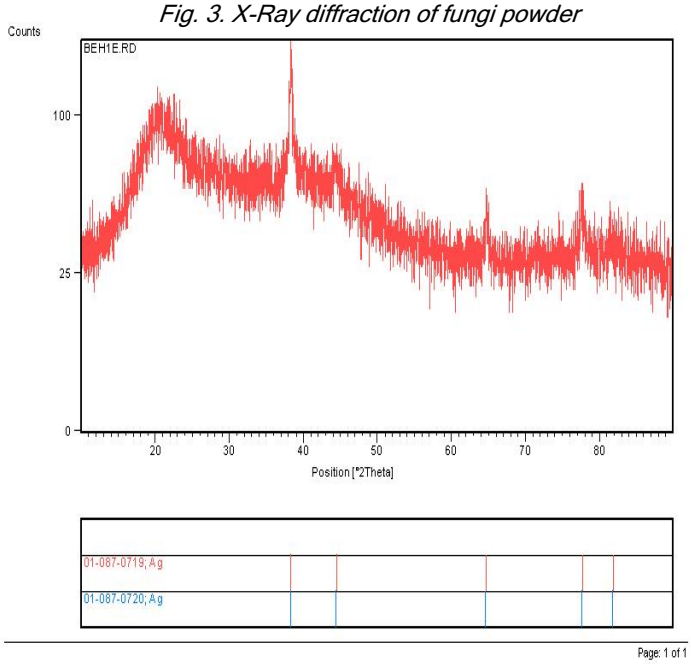
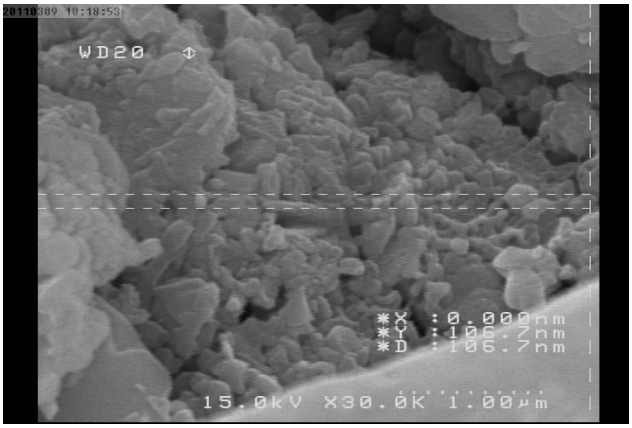


Fig. 4. SEM picture of silver nanoparticles in the Aspergillus flavus



Conclusion

A green chemistry synthetic route has been used for silver nanoparticles. Microorganisms have been employed for syntheses of metallic nanoparticles. In next investigation we can used fungi that isolated from metallic mines for synthesis of other metallic nanoparticles, as the microorganisms are resistant to metallic stresses.

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