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Research Article

# Biosynthesis and Characterization of Manganese and Zinc Nanoparticles

[1]Waghmare, S. S., [2]Deshmukh, A. M., [3]Kulkarni S. W.and [4]Oswaldo, L. A.

[1]Department of Microbiology, Fergusson College, Pune-413 004,(M.S.) India
[2]Department of Microbiology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, subcampus, Osmanabad-413 501, (M.S.) India.
[3]Department of Microbiology, S.B.Z.College, Barshi, Dist-Solapur (M.S.) India
[4]LAQES, Institute of Chemistry UNICAMP, Brazil.
**Corresponding author: shivajiwaghmare@gmail.com**

## Abstract

**The biosynthesis of metal nanoparticles is an expanding research area due to the potential applications for the ecofriendly development of novel technologies. Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. Present study reported a fast, convenient and intracellular method for the synthesis of manganese and zinc nanoparticles by reducing Manganese sulphate and Zinc sulphate with the help of *Streptomyces sp.* HBUM171191. The characterization of nanoparticles was done by using UV- Vis Spectrophotometer. The morphology of silver nanoparticles was confirmed by Transmission Electron microscopy (TEM). The size of $MnSO_4$ and $ZnSO_4$ was ranges from 10 to 20nm.**

**Keywords:** Biosynthesis nanoparticles, Characterization, Manganese and Zinc

## 1. Introduction

For the synthesis of clean, nontoxic and environmentally benign synthetic technologies the biosynthetic method widely used. Microbial resistance against heavy metal ions has been exploited for biological metal recovery via reduction of the metal ions or formation of metal sulfides (Stephen *et al*., 1999). So the attractive procedure is using microorganisms such as bacteria and fungi to synthesize gold nanoparticles recently. An earlier study found that *Bacillus subtilis* (Beveridge and Murray, 1980) were able to reduce $Au^{3+}$ ions to gold nanoparticles with a size range of 5-25 nm inside the cell walls. *Shewanella algae* were found to reduce $Au^{3+}$ ions forming 10-20 nm gold nanoparticles extracellularly with the assistance of hydrogen gas (Konish *et al*., 2004). Fungi (*Verticillium* sp (Mukherjee *et al*., 2001) and *Fusarium oxysporum* (Mukherjee, 2002) and actinomycete, *Thermomonospora* sp. (Ahmad *et al*., 2003) and *Rhodococcus* sp. (Ahmad *et al*., 2003a) were also used to synthesize nanoparticles intra or extracellularly. The bacteria produce their nanomaterials by a process called biomineralisation. This is achieved by coupling the oxidation of organics or hydrogen to the reduction of a whole array of electron acceptors including sulfur, selenium and tellurium oxyanions, along with iron oxyhydroxides, resulting in the production of elemental (e.g. SeO), mixed oxide (e.g. magnetite $Fe_3O_4$) or precipitated (e.g. ZnS) nanomaterials.

Application of nano scale material and structures are usually ranging from 1-100 nm and is emerging area of nanoscience and nanotechnology. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms; have been studied extensively because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties (Catauro *et al*., 2004; Crabtree *et al*., 2003; Krolikowska *et al*., 2003; Zhao and Stevens, 1998). Synthesis of noble nanoparticles for the applications such as catalysis, electronics, environmental and biotechnology is an area of constant interest (Hussain *et al*., 2006 and Sharma *et al*., 2009).

## 2. Materials and Methods

### *2.1 Preparation of Biomass*

Actinomycetes were grown in 500 ml Erlenmeyer flask containing 100 ml sterile Malt extract glucose yeast extract peptone (MGYP) broth supplemented with griseofulvin antibiotic 50 µg/ml on shaker (200 rpm) at 35 $^0$C for four days. The flasks were removed from the shaker and kept at steady condition at 5 to 10 $^0$C, so that mycelial biomass could get settled. The supernatant was discarded and 100 ml of sterile distilled water was added for washing the cells. The flasks were kept at 5 to 10 $^0$C to settle the biomass, for 30 minutes. Supernatant was discarded slowly, 100 ml of sterile distilled water was again added in the flask, this procedure was repeated for three times. The mycelial mass was then separated from the sterile distilled water by centrifugation (1500 rpm) for 10 minutes, mycelial pellets were weighed and used for synthesis of zinc and manganese nanoparticles.

### *2.2 Exposure of Biomass to Metal Solutions*

Five gram of actinomycetal wet biomass was exposed to 50 ml of an sterilized aqueous solution of metals at various dilutions as given above in 250 ml Erlenmeyer flaks and the flaks was kept on shaker at 200 rpm and 35$^0$C for 4 days.

### *2.3 Characterization of Metal Nanoparticles*

#### *2.3.1 Visual Observations*

Reaction mixtures in the flasks used for biosynthesis of nanoparticles were observed for visual colour change after 12, 24, 48 and 72 h. The change in colour pale yellow to brownish indicates the formation of silver nanoparticles, pale yellow to pinkish colour indicates the formation of gold nanoparticles and the formation of whitish yellow to yellow colour indicates the formation of manganese and zinc nanoparticles.

### *2.4 U.V. Visible Spectroscopy*

Biosynthesis of metal ions was studied by taking 2 ml of reaction mixture at different time intervals and centrifuging it at 5000 rpm for 10 min. The centrifuged biomass was washed with

double distilled water thrice and biofilm was prepared. The biofilm was dried in oven at 45 $^0$C for one hour and observed for spectroscopic analysis at 12, 24, 48 and 72 h time intervals. The analysis was carried out by using SL 159 U. V. visible spectrophotometer at (300 to 800 nm). The growth in the flasks showing desired color change was used for further studies.

### *2.5 Transmission Electron Microscopy (TEM)*

The biomass was dispersed in water, left for five minutes in ultrasonicator and then left to rest for 10 minutes. One drop of suspension was placed on a grid of copper coated with 300 mesh palladium and carbon. The grid with the dispersion was examined in the microscope Zeiss CEM902 at 80kV.

## 3. Results and Discussion

The change in biomass brown in colour indicates the intracellular formation of silver nanoparticles. It was also observed that after 72 h treatment the $AgNO_3$ solution was colorless, thereby indicating that the extracellular reduction of the $AgNO_3$ ions has not occurred. It was found that the yellowish colour of biomass *Streptomyces sp.* HBUM171191 was changed gradually and after 72 h it was dark yellow in colour when treatment with aqueous solution of ($10^{-3}$ mM) $MnSO_4$ and ($10^{-3}$ mM) $ZnSO_4$ (Plate and Plate ).

U.V. visible spectroscopy of $MnSO_4$ and $ZnSO_4$ treated *Streptomyces* sp. HBUM171191 biofilm was carried out after 12, 24, 48 and 72 h exposure at different wavelength (fig. 3 and fig. 4). The $MnSO_4$ and $ZnSO_4$ treated *Streptomyces* sp. HBUM171191 showed maximum absorption at critical wavelength, corresponding to the absorption maxima of $MnSO_4$ and $ZnSO_4$ nanoparticles that is 350 nm and 380 nm after 72 h exposure respectively.


**Fig. 1: *Streptomyces* sp. HBUM171191 biomass after exposure to $MnSO_4$ solution (A, B, C, D, and E) to various concentration of $MnSO_4$ solution for 72 h**

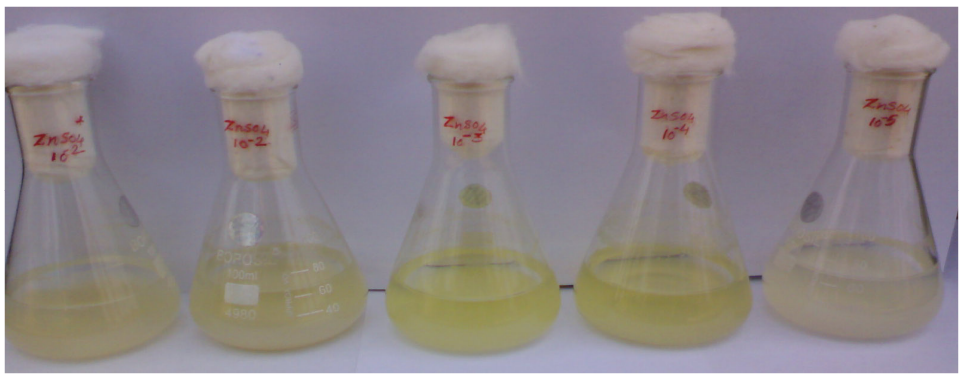



**Fig. 2: *Streptomyces* sp. HBUM171191 biomass after exposure to $ZnSO_4$ solution (A, B, C, D and E) to various concentration of $ZnSO_4$ solution for 72 h**

In visual observation studies also, it was observed that increase in exposure time was accompanied by increase in the color intensity. The colour of *Streptomyces* sp. HBUM171191 biomass was gradually turned to faint pink at 12, pinkish at 24, pink at 48 h and finally violet in colour after 72 h exposure. Senapati (2005) reported that a weak absorption edge was observed in the U.V.Visible spectrum of *Fusarium oxysporum* MnSO4 reaction mixture. The appearance of absorption edge at 350 nm is a clear indication of the formation of MnS nanoparticles. A broad emission band was observed in the fluorescence spectrum of the same solution and was attributed to deep trap emission (Landes, 2002). Senapati (2005) reported that *Fusarium oxysporum* secretes sulfate reductase enzymes when exposed to aqueous MSO4 (where M = Cd, Pb, Mn and Zn) solution and results in the formation of extremely stable metal sulfide nanoparticles in solution.

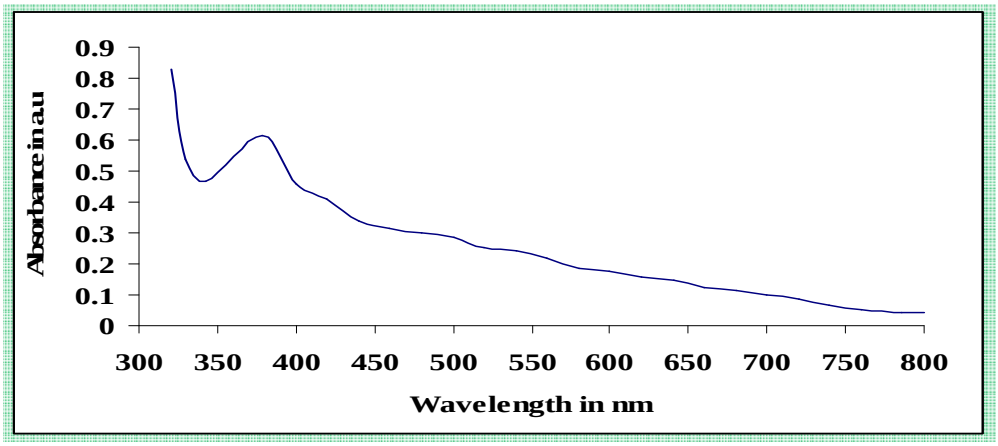



**Fig. 3: U. V. visible absorbance of $MnSO_4$ ($10^{-3}$ mM) treated *Streptomyces* sp. HBUM171191 biofilm after 72 h.**

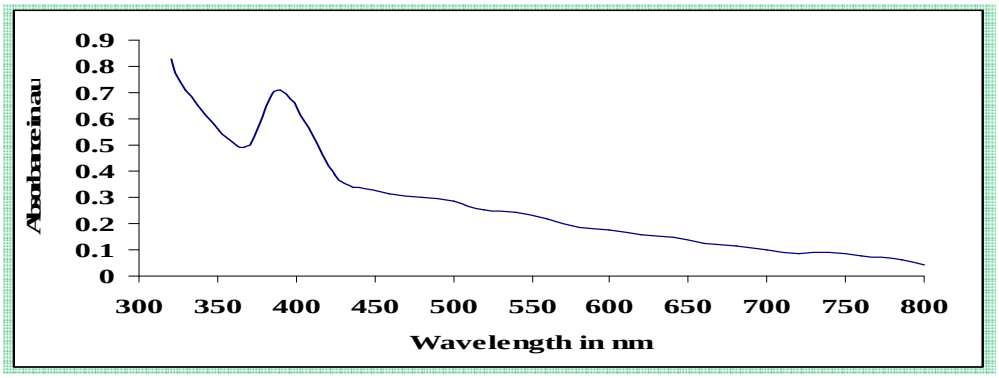
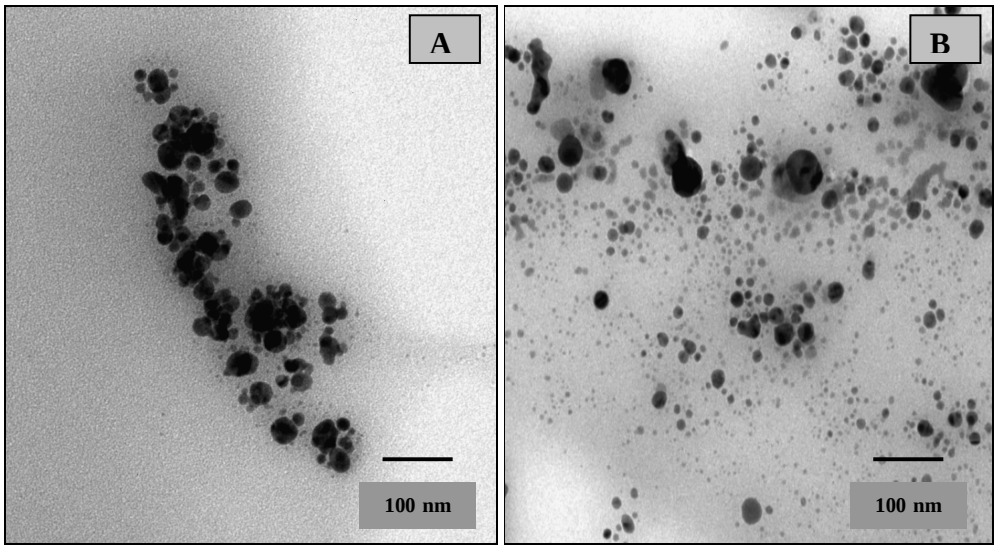



**Fig. 4: U. V. visible absorbance of $ZnSO_4$ ($10^{-3}$ mM) treated *Streptomyces* sp. HBUM171191 biofilm after 72 h**



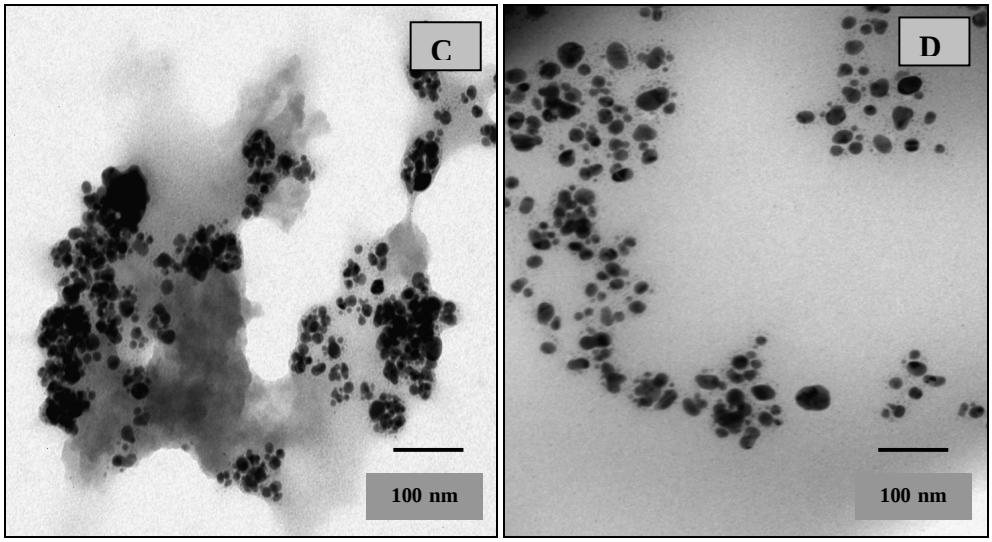



**Fig. 5: Characterization of Nanoparticles by Transmission Electron Microscopy (A, B, C and D)**

TEM image showing the well dispersed polymorphic Manganese nanoparticles (A) and (B), Zinc nanoparticles (C) and (D) before exposure to $10^{-3}$ mM aqueous solution MnSO4 and $ZnSO_4$ respectively synthesized by using *Streptomyces* sp. HBUM171191. Ahmad *et al.* (2002) found that *Fusarium oxysporum*, synthesize quite important semiconductor metal nanoparticles extracellularly by a purely enzymatic process. They also observed that *Fusarium oxysporum* secretes sulfate reductase enzymes when exposed to aqueous $MnSO_4$, $CdSO_4$, $PbSO_4$ and $ZnSO_4$ solution and results in the formation of extremely stable metal sulfide nanoparticles in solution.

## 4. Conclusion

The Manganese and Zink nanoparticles could be successfully synthesized by using *Streptomyces* sp. HBUM171191. The U.V. visible spectroscopy and Transmission electron microscopy results clearly show the polymorphic nanoparticles with 10 to 20nm. Actinomycetes are well known to secrete much higher amounts of proteins, thereby significantly increasing the productivity of this biosynthetic approach. So, the actinomycete is good source for the synthesis of nanoparticles with polymorphic size.

## 5. Acknowledgement

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