

## Biological Synthesis of Zinc Nanoparticles by *Aspergillus niger*

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Nanoparticles have gained interest due to its unique properties and benefits that can upgrade the future technologies. However, the production of nanoparticles has adopted physical and chemical methods which have been proven as non-eco friendly and produces toxic by-products. The present study proposes an eco-friendly synthesis of zinc nanoparticles using microorganisms. The synthesis of zinc nanoparticles using *Aspergillus niger*, and the effect of the biomass ratio to the salt solution and effect of different type of salt solutions are presented in this paper. The fungus is grown in a suitable medium and biomass was challenged with varying concentration of zinc sulphate solution and zinc nitrate solution for 72 hours. The formations of nanoparticles were characterized using UV-Vis spectrophotometer and Scanning Electron Microscope (SEM) to ascertain the size and polydispersity of the nanoparticles produced. The synthesis shows that lower ratio of biomass used in the synthesis give faster indication of nanoparticle formation and tolerance of the fungi mycelia are dependent on the concentration and type of salt solutions.

**Key words:** Biological Synthesis; Zinc Nanoparticles; Fungus Species; *Aspergillus niger*.

Production of nanoparticles (NPs) can be approached in two ways either top down and bottom up by using physical and chemical methods<sup>1,2</sup> respectively. High energy wet ball milling and electrospaying which can produce nano-sized powder form<sup>1</sup> are the examples of the physical method while chemical methods such as solution plasma<sup>2</sup> involve scaling up the size of the material from atom structure into nanosized material<sup>3</sup>.

However, physical and chemical methods rely on the use of large amount of toxic chemicals, relatively high production cost and non-environmental friendly. Therefore, there is strong

demand in adopting the biological synthesis method which offer cheaper alternatives for NPs production and expansion of application especially in biomedical industries<sup>5</sup>. Current researches have shown the ability of microorganisms such as bacteria and fungi in producing various type of metal nanoparticles such as gold NPs by *Verticillium* sp.<sup>6</sup>, silver NPs by *Trichoderma reesei*<sup>7</sup>, and zinc nanoparticles by *Streptomyces* sp.<sup>5</sup>.

Zinc nanoparticles (ZnNPs) have been used in the industry for its efficacious as a catalyzer for carbinol synthesis with carbon dioxide and hydrogen and the like<sup>8</sup>. Zinc NPs are also used as an anti corrosive coating for hot dip galvanization of iron and steel, and there are recent attempts to use this nanoparticles in preparation of pharmaceutical and cosmetology industries. However the current methods for the production of zinc nanoparticles are adopting either the physical or chemical methods which are quite expensive.

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The aim of this study was to demonstrate the ability of the fungus *Aspergillus niger* to produce zinc nanoparticles. The effects of various experimental parameters on the formation of ZnNPs were also analyzed.

## MATERIALS AND METHODS

Potato Dextrose Agar and Potato Dextrose Broth (pH of  $5.6 \pm 0.2$ ) was purchased from Sigma Aldrich (St.Louis, USA). Zinc sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) and zinc nitrate ( $\text{ZnNO}_3 \cdot 6\text{H}_2\text{O}$ ) were purchased from R & M chemicals. All chemicals used in this study were of analytical grade.

### Culture

The selected strain of *Aspergillus niger* was collected from the laboratory stock of Environmental Biotechnology Engineering Lab, International Islamic University Malaysia, Gombak. Strain was sub-cultured into Potato Dextrose Agar for 5 days and kept for culture stock in the 4°C chiller.

### Mycelia Preparation

*A. niger* was sub-cultured on Potato Dextrose Agar (pH of  $5.6 \pm 0.2$ ) for 5 days. After reaching maturation, the spores were taken and grown on Potato Dextrose Broth for 3 days. The flasks were incubated in a shaker at 150 rpm and 37°C.

### Synthesis of Nanoparticles

After 5 days of incubation, the mycelium was separated by filtration (Sartorius Grade 292) and washed thrice with sterile distilled water. The washed mycelium (fresh wet weight 0.1 to 5 g  $\pm$  0.01) was challenged with 50 ml of zinc sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) and zinc nitrate ( $\text{ZnNO}_3 \cdot 6\text{H}_2\text{O}$ ) solution at various concentrations (1, 5 and 10mM) and incubated in shaker at 150 rpm in 27°C for 72 hours. Simultaneously, a positive control of the fungus mycelium with deionized water was incubated under the same condition.

### UV-Visible spectral analysis

Change in color was observed in the zinc salt solution incubated with *A.niger*. The UV-visible spectra of this solution was recorded in SECOMAM UviLine 9400 Spectrophotometer, from 300nm to 700nm at different time intervals (24 hr, 48 hr, 72 hr and 96 hrs). Surface plasmon resonance of zinc nanoparticles were detected in the range of 350 to 400 nm.

### One Factor at Time Studies (OFAT)

The effect of biomass weight, metal salt concentration and the temperatures on the formation of the nanoparticles, were studied.

### FE-SEM and EDX

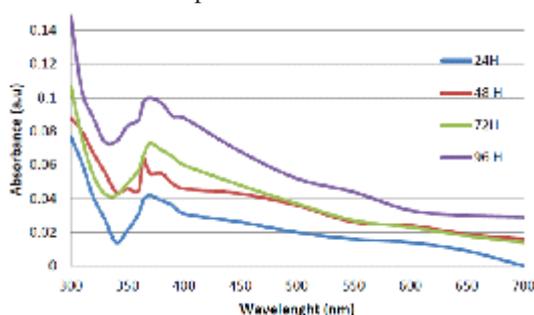
Samples of incubated fungal mycelia with distilled water, and incubated fungal mycelia with zinc sulphate solution were centrifuged under 10,000 rpm for 30 min and the washed thrice with sterile distilled water. The resultant mass was dispersed in 95% ethanol and dried in ambient temperature. The samples were analyzed using Field Emission Scanning Electron Microscope (JEOL JSM-6700F) and Energy Dispersive X-Ray spectrophotometer (JEOL JSM-6700F).

## RESULTS AND DISCUSSION

In this work *A. niger* was used for the synthesis of stable zinc nanoparticles. While the fungal mycelia incubated with distilled water (positive control) retained its original color, the zinc sulphate treated with fungus mycelia turned to light yellow after 72 hr due to the deposition of the zinc nanoparticles. Fig. 1 shows the UV-visible spectra of the sample when zinc salt solutions were challenged with *A. niger* biomass. A characteristic surface plasmon absorption band in the range of 350-400 nm was observed for every 24 hr. The absorbance values of the ZnNPs were found to increase as a function of time.

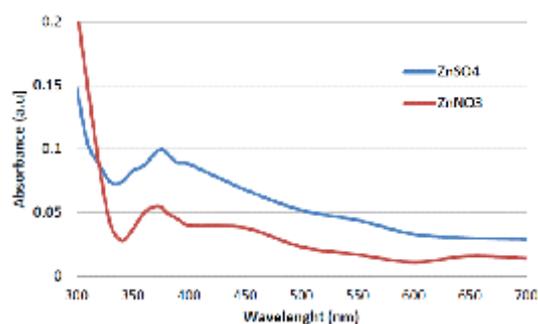
### Effect of different anions on the formation of the nanoparticles

Zinc sulphate and zinc nitrate solutions



**Fig. 1.** UV-visible spectra of *A.niger* biomass as function of time. Curve corresponds to that of incubation with zinc sulphate salt solution (5 mM) after 72 hr at 37°C. The peak at ~376 nm corresponds to the surface plasmon resonance of zinc nanoparticles.

were used in this study. Both salt solutions were found to form NPs when incubated with *A. niger*. Fungi have different tolerance towards sulphate and nitrate ions due to the differences in the capabilities of the microbial secretions to carry out the reduction of these ions. The UV-Vis spectra of ZnNPs formed from zinc sulphate and zinc nitrate salt solutions are shown in Fig. 2. Zinc nanoparticles formed from zinc sulphate solution shows much larger absorption values, implying that the zinc sulphate is more preferred compared to zinc nitrate for the formation of ZnNPs. Hence only zinc sulphate solution was used in all further investigations.

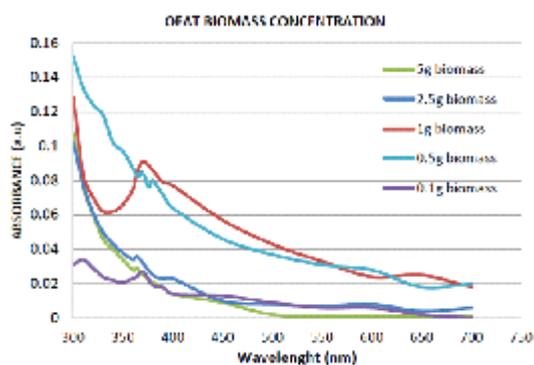


**Fig. 2.** UV-visible spectra of *A. niger* biomass as a function of different salt solution. Curve at ~376 nm corresponds to that of incubation with zinc sulphate and zinc nitrate salt solution (5 nM) after 72 hr with 1 g of fresh wet weight of biomass.

#### Effect of biomass weight on the formation of the nanoparticles

Different biomass weight (0.1 g to 0.5 g) had been used in this work to study the effect of it on the formation of the zinc nanoparticles when challenged with zinc sulphate solution. Fig. 3 shows the UV-visible absorption when the zinc salt solutions were challenged with the different quantity of the biomass with 50 ml of zinc sulphate solution after 72 hr. The increase in biomass concentration appears to increase the nanoparticles production enhances the reaction rate of the nanoparticles synthesis<sup>10</sup>. Higher the biomass concentration, more amounts of enzymes required for the reduction of the metal ions will become available, and hence the nanoparticles formation

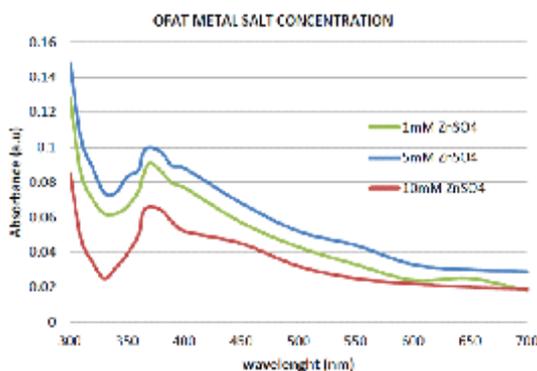
is expected to increase. Tam<sup>9</sup> have made similar observation in the formation of selenium nanoparticles by *Shewanella sp.* HN-41. After reaching optimum conditions, the formation of NPs no longer depend on the biomass concentration, and lowering of the Surface Plasmon Resonance (SPR) of the zinc nanoparticles at biomass concentration higher than 1 g might due to different nanoparticles in size and shape as SPR depends on various factors such as size, mono dispersity and shape of the nanoparticles as well as the composition of the surrounding media and interactions between nanoparticles with the stabilizing ligands<sup>11</sup>.



**Fig. 3.** UV-visible spectra of *A. niger* biomass as a function of biomass weight. Curve at 376 nm corresponds to that of incubation with zinc sulphate salt solution (5 nM) after 72 hr with different range of fresh wet weight biomass (0.1g to 5g).

#### Effect of metal salt concentration on the formation of the nanoparticles

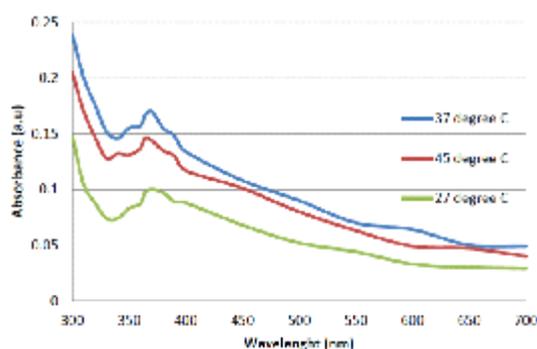
One gram of biomass is challenged with ZnSO<sub>4</sub> solutions in the concentrations of 1 mM - 10 mM at 27 °C, and agitation speed of 150 rpm (Fig. 4). It is found that 5 mM of metal salt solution provides the optimum condition for the nanoparticles production. Initial small increases in concentration of metal salt solution will increase the reduction of the metals ions to metal nanoparticles<sup>12</sup>. However, further increase in metal salt concentration decreases nanoparticles production due to the intolerance of the fungi to the metal salt concentration.



**Fig. 4.** UV-visible spectra of *A. niger* biomass as a function of different metal salt concentration (1, 5, 10 mM) with 1 g of biomass after 72 hr at 27 °C and agitation of 150 rpm.

#### Effect of temperature on the formation of the nanoparticles

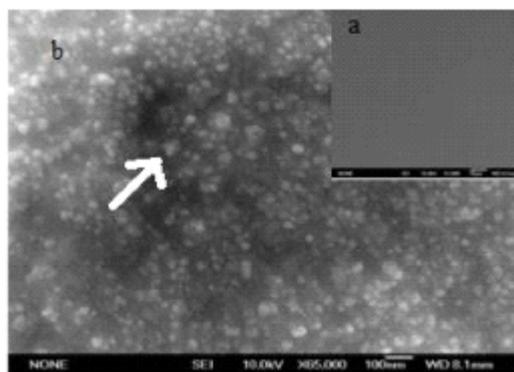
The effect of temperature on the formation of ZnNPs was carried out in the temperature range of 27°C to 45°C, and the results are shown in Fig. 5. Studies show optimum temperature at 37°C. Increase in temperature from 27°C to 37°C leads to increase in the production of ZnNPs. Further increase in the temperature to 45°C, slows down the production of the nanoparticles. This could be most probably due to the fungi intolerance at higher temperatures. According to Venkatesan<sup>12</sup>, changes in temperature not only influence the production of the nanoparticles, but also in the size and morphology of the nanoparticles.



**Fig. 5.** UV-visible spectra of *A. niger* biomass as a function of different temperature range (27 - 45 °C) after incubation of 5 mM metal salt solution with 1g biomass for 72 hr at agitation speed of 150rpm.

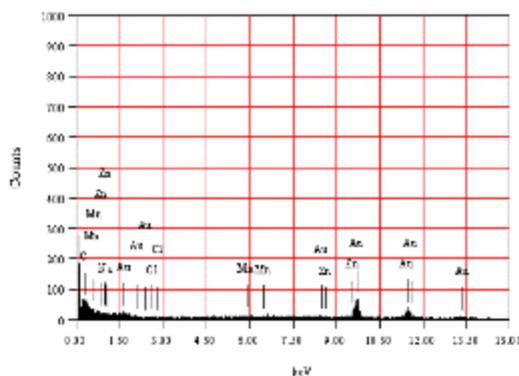
#### EDX and FE-SEM

Fig. 6a shows the scanning electron micrograph of the fungal mycelium treated as positive control while Fig. 6b shows the fungal mycelium treated with 1.0 mM zinc sulphate solution for 72 h. Fig. 6a shows no deposition of zinc nanoparticles as shown in Fig. 6b (indicated by the white arrow). From the figure it can be estimated that the size of the ZnNPs are in the range of 20-50 nm.



**Fig. 6.** Scanning electron micrographs of: (a) fungal mycelium incubated with deionized water and (b) fungal mycelium incubated with 1.0 mM zinc sulphate solution for 72 hr. Scale bar corresponds to 100 nm using 65000 times resolution.

Fig. 7 shows the metal composition of the fungal mycelia treated with zinc sulphate solution samples by using EDX. Zinc element in the samples contains about 98% mass of the samples (data not shown). The presence of the Au metal inside Fig. 7



**Fig. 7.** Energy Dispersive X-ray of zinc sulphate solution treated with fungal mycelia after 72 hr.

was due to the gold coating applied to avoid electron reflection on the sample, in case the sample was not electron conducting.

### CONCLUSIONS

Reduction of metal ions by fungi species may be the process by which the microorganisms protect themselves from the toxic effects of the metal ions. This study shows the reduction of the zinc ions into zinc nanoparticles by *Aspergillus niger*. Studies need to be done to examine the enzymes that play the role in this mechanism. The average size of the nanoparticles was estimated to be in the range of 20-50 nm. These zinc nanoparticles are found to have characteristic absorbance peak at the range of 350 to 400 nm (~370 nm). This process of nanoparticles production is eco-friendly as it is free from any excessive solvent or toxic chemicals and the method is also attractive for the large scale production.

### ACKNOWLEDGEMENTS

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