

## GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING MICROBES

\* Khadeeja Yasmeen<sup>1</sup>, A. Mohamed Sikkander<sup>2</sup>

<sup>1</sup>Department of Biotechnology, North East Frontier Technical University, Arunachal Pradesh

<sup>2</sup>Department of Chemistry, Velammal Engineering College, Chennai, India

\* 1, 2 Corresponding Authors: khadeejaowais@gmail.com, ams240868@gmail.com

### ABSTRACT

*For the synthesis of nanoparticle, scientists used microorganisms and then plant extracts; it is a cheaper way for nanoparticles synthesis. Nature has devised various processes for the synthesis of nano length scaled inorganic materials<sup>[1]</sup> which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials. Synthesis of metal nanoparticles assisted by biological agents provides advantages over chemical and physical methods due to the fact that it is cost effective, environmentally friendly and easily scalable for large-scale synthesis and obviates the need for high pressure, energy, temperature, and toxic chemicals. However, research on this field has been focused mainly on lead and zinc and many applications of these metallic nanostructures have been sufficiently explored. In the case of heavy metals such as lead and zinc, traditional biological procedures are employed to synthesize nanoparticles.*

**KEYWORDS:** Nano sized materials; Non toxic material; Microbial enzymes; Capping agents; Pharmaceutical applications; Anti oxidant

### INTRODUCTION

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with anti oxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles. The three main steps in the preparation of nanoparticles that should be evaluated from a green chemistry perspective are the choice of the solvent medium used for the synthesis, the choice of an environmentally benign reducing agent and the choice of a non toxic material for the stabilization of the nanoparticles. Most of the synthetic methods reported to date rely heavily on organic solvents. This is mainly due to the hydrophobicity of the capping agents used<sup>[2]</sup>. Synthesis using bio-organisms is compatible with the green chemistry principles: the bio-organism is eco-friendly, the reducing agent employed and the capping agent in the reaction<sup>[3]</sup>. Parashar *et al.* (2009) found that often chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. This is not an issue when it comes to biosynthesized nanoparticles as they are eco friendly and

biocompatible for pharmaceutical applications<sup>[4]</sup>.

#### **Usage of microorganism to synthesize nanoparticles**

The use of microbial cells for the synthesis of nano sized materials has emerged as a novel approach for the synthesis of metal nanoparticles. The interactions between microorganisms and metals have been well documented and the ability of microorganisms to extract and/or accumulate metals is employed in commercial biotechnological processes such as bioleaching and bioremediation<sup>[5]</sup>. Bacteria are known to produce inorganic materials either intra cellularly or extra cellularly.

Microbial synthesis of nanomaterials utilizes of biological components, primarily prokaryotes and eukaryotes. Microbes play direct or indirect roles in several biological activities. Metals and non-metals present on earth are in constant association with biological components. The most abundant organisms in our biosphere are bacteria. Slight climate changes can potentially be disastrous to the life processes of bacteria; this can result in the prolific advantage for the production of nanoparticles. Synthesis of gold and silver nanoparticles by eukaryotic cells such as fungi is

reported. Synthesis of gold nanoparticles by *Shewanella algae*<sup>[6]</sup> and silver nanoparticles by fungus *Verticillium* were also reported. Several strains of *Fusarium* viz *Fusarium oxysporum*, *Aspergillus fumigatus* and *Aspergillus flavus* were used for successful production of metal nanoparticles. Recently white rot fungus *Coriolus versicolor* has also been used for the synthesis of stable silver nanoparticles. Biologically synthesized nanoparticles have wide application viz., biosensors biolabelling, in cancer therapeutics and in coating of medical appliances<sup>[7]</sup>. Some well known examples of bacteria synthesizing inorganic materials include magnetotactic bacteria (synthesizing magnetic nanoparticles) and S layer bacteria which produce gypsum and calcium carbonate layers<sup>[8]</sup>. Some microorganisms can survive and grow even at high metal ion concentration due to their resistance to the metal. The mechanisms involve: efflux systems, alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extra cellular complexation or precipitation of metals and lack of specific metal transport systems. *Pseudomonas stutzeri* AG 259 isolated from silver mines has been shown to produce silver nanoparticles<sup>[11]</sup>.

Among the noble metals, silver (Ag) is the metal of choice in the field of biological system, living organisms and medicine. Green synthesis of nanoparticles is an emerging branch of nanotechnology. In the present study an attempt was made to prepare silver nanoparticles of various concentrations as these particles are fast and specific in their target towards the applications where they are used<sup>[9]</sup> and evaluate their antimicrobial activity. The most important application of metal nanoparticles is in medical industry such as typical ointments to prevent infection against burnt and open wounds. Silver, zinc, lead nanoparticles have diverse applications both *in vitro* and *in vivo* conditions<sup>[10]</sup>. A number of approaches such as reduction of solution, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical and microwave assisted process and recently via green chemistry route are available for the synthesis of silver nanoparticles.

Silver is a nontoxic, safe inorganic antibacterial

agent used for centuries and it has the capability of killing different type of diseases causing microorganisms. Silver has been known to be a potent antibacterial, antifungal and antiviral agent, but in recent years, the use of silver as a biocide in solution, suspension, and especially in nanoparticulate form has experienced a dramatic revival. Due to the properties of silver at the nano level<sup>[11]</sup>, nanosilver is currently used in an increasing number of consumer and medical products. The remarkably strong antimicrobial activity is a major reason for the recent increase in the development of products that contain nanosilver. The main challenge in nanomaterials synthesis is the control of their physical properties such as obtaining uniform particle size distribution, identical shape, morphology, chemical composition and crystal structure.

Actinomycetes are microorganisms that share important characteristics of fungi and prokaryotes such as bacteria. Even though they are classified as prokaryotes, they were originally designated as ray fungi. Focus on actinomycetes has primarily centred on their exceptional ability to produce secondary metabolites such as antibiotics. It has been observed that a novel alkalothermophilic actinomycete, *Thermomonospora sp.* synthesized gold nanoparticles extracellularly when exposed to gold ions under alkaline conditions<sup>[12]</sup>. In an effort to elucidate the mechanism or the processes favouring the formation of nanoparticles with desired features, Ahmad *et al.*<sup>[13]</sup> studied the formation of monodisperse gold nanoparticles by *Thermomonospora sp.* and concluded that extreme biological conditions such as alkaline and slightly elevated temperature conditions were favourable for the formation of monodisperse particles. Based on this hypothesis, alkalotolerant actinomycete *Rhodococcus sp.* has been used for the intracellular synthesis of monodisperse gold nanoparticles. In this study it was observed that the concentration of nanoparticles were more on the cytoplasmic membrane. This could have been due to the reduction of metal ions by the enzymes present in the cell wall and on the cytoplasmic membrane but not in the cytosol. The metal ions were also found to be non toxic to the cells which continued to multiply even after the formation of the nanoparticles.

Compared to bacteria, fungi could be used as a source for the production of large amount of nanoparticles. This is due to the fact that fungi secrete more amounts of proteins which directly translate to higher productivity of nanoparticle formation. Extracellular secretion of the microorganisms offers the advantage of obtaining large quantities in a relatively pure state, free from other cellular proteins associated with the organism with relatively simpler downstream processing. Mycelia free spent medium of the fungus, *Cladosporium cladosporioides* was used to synthesise silver nanoparticles extracellularly<sup>[14]</sup>. It was hypothesized that proteins, polysaccharides and organic acids released by the fungus were able to differentiate different crystal shapes and were able to direct their growth into extended spherical crystals.

#### Usage of plant to synthesize nanoparticles

Plant biotechnology has opened up unexpected new ways for finding new way for trapping their potential resources. The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction. A number of plants are being currently investigated for their role in the synthesis of nanoparticles. Gold nanoparticles with a size range of 2- 20 nm have been synthesized using the live alfa alfa plants [15]. Nanoparticles of silver, nickel, cobalt, zinc and copper have also been synthesized inside the live plants of *Brassica juncea* (Indian mustard), *Medicago sativa* (Alfa alfa) and *Heliantus annus* (Sunflower). Certain plants are known to accumulate higher concentrations of metals compared to others and such plants are termed as hyperaccumulators. Of the plants investigated, *Brassica juncea* had better metal accumulating ability and later assimilating<sup>[16]</sup> it as nanoparticles.

Recently much work has been done with regard to plant assisted reduction of metal nanoparticles and the respective role of phytochemicals. The main phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids in the light of IR spectroscopic studies. The main water soluble phytochemicals are flavones, organic acids and quinones which are responsible for immediate reduction. The

phytochemicals present in *Bryophyllum sp.* (Xerophytes), *Cyprus sp.* (Mesophytes) and *Hydrilla sp.* (Hydrophytes) were studied for their role in the synthesis of silver nanoparticles. The Xerophytes were found to contain emodin, an anthraquinone which could undergo redial tautomerization leading to the formation of silver nanoparticles. The Mesophyte studied contained three types of benzoquinones, namely, cyperoquinone, dietchequinone and remirin. It was suggested that gentle warming followed by subsequent incubation resulted in the activation of quinones leading to particle size reduction. Catechol and protocatechaldehyde were reported in the hydrophyte studied along with other phytochemicals. It was reported that catechol under alkaline conditions gets transformed into protocatechaldehyde and finally into protocatecheuic acid. Both these processes<sup>[17]</sup> liberated hydrogen and it was suggested that it played a role in the synthesis of the nanoparticles. The size of the nanoparticles synthesized using xerophytes, mesophytes and hydrophytes were in the range of 2- 5nm.

Recently gold nanoparticles have been synthesized using the extracts of *Magnolia kobus* and *Diopyros kaki* leaf extracts. The effect of temperature on nanoparticle formation was investigated and it was reported that polydisperse particles with a size range of 5- 300 nm was obtained at lower temperature while a higher temperature supported the formation of smaller and spherical particles. Nanoparticles due to their smaller size and large surface to volume ratio exhibit remarkable novel properties and methodical applications in the field of biotechnology, sensors, medical, catalysis, optical devices, DNA labeling, drug delivery<sup>[18]</sup> and they are rewardingly treated as a bridge between bulk material and atomic and molecular structures. Approaches such as simple solution-based methods, chemical precipitation, sol-gel, solvothermal electrochemical and photochemical reduction techniques are more widely used. Chemical method leads to the presence of some toxic chemicals adsorbed on the surface that may have adverse effects. Increasing awareness towards green chemistry and biological processes has led to the development of an eco-friendly approach for the synthesis of nanoparticles. The use of

environmentally benign microbes for the synthesis of zinc oxide and lead nitrate nanoparticles offers copious profit of eco-friendliness where toxic chemicals are not used. ZnO is non toxic. It can be used as photocatalytic degradation materials of environmental pollutants.

## RESEARCH AND METHODOLOGY

### Materials and reagents

LB broth (1.5%): weighed 1.5 gm LB broth medium and dissolved in 100ml distilled water, autoclaved and stored at RT, PDB, Chemical salts, 1 M NaOH and HNO<sub>3</sub>, Microbes.

### Equipment

Electronic balance scale, Sterilized glass equipments (Erlenmeyer flask), Incubator, Autoclave, Rotary shaker, Laminar air flow, Nichrome inoculating loop.

### Selection of Microbial and chemical

#### Microbes are

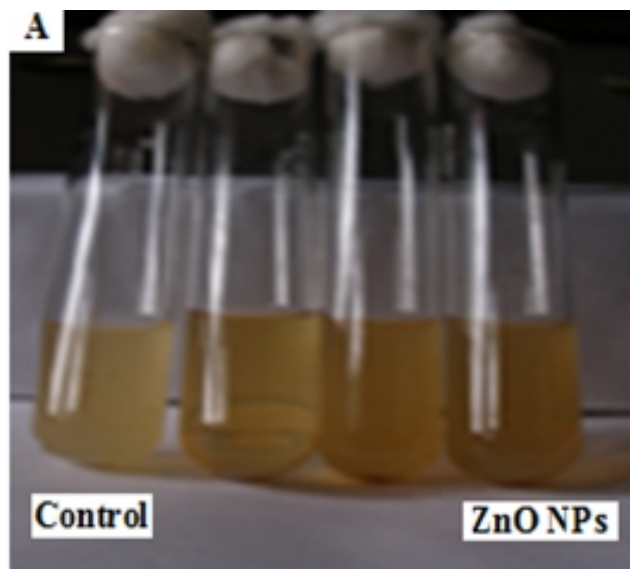
- *Escherichia coli* (DH5a), (bacteria)
- *Aspergillus niger* (fungus)

### Chemical salt

#### · Zinc oxide (ZnO)

Selection of microbes in order to synthesize metal oxide nanoparticles are *Escherichia coli* (bacteria) and *Aspergillus niger* (fungus). Heavy metals at relatively low concentration are considerably hard to remove through conventional techniques. A feasible alternative to realize this kind of removal can be through the biosorption application by bacterial and fungal biomass. This research was carried out to estimate the removal of lead nitrate and zinc oxide from an aqueous solution by biomass of *Escherichia coli* and *Aspergillus niger*. Essential chemical salt used in the protocol is Zinc oxide (ZnO). Zinc is a transition metal, some time may be harmful (regardless of it swallowed or inhaled), affecting almost every system and organ in the body. Bioremediation of zinc through microbes could be a functional measure to remove zinc

(Figure 1) .z



**Figure 1: Nanoparticle synthesis using bacterial strain *E.Coli* (DH5a) (A) Synthesis of ZnO nanoparticles**

Use of micro-organisms for the production of nanostructured materials emerges as an exciting as well as eco-friendly approaches. Researchers generally prefer a biological synthesis method because of the particles' distribution control can be obtained through this method is better than other methods<sup>[19]</sup>. In addition, biological method also

involves no environmental toxicity that is generally accompanied with the use of other chemical methods. Microbial synthesis of nanostructured particles makes use of biological materials, primarily prokaryotes or / and eukaryotes. Microorganisms perform direct / indirect roles in various biological activities. The various physical and chemical methods used for nanoparticles production suffer from several limitations. The limitations of this method include, they are mostly

bioincompatible and toxic, uncontrolled, unstable crystal growth and aggregation of nanosized particles. To overcome these limitations, in current decade consideration towards the production of nanoparticles utilizing biological components is gaining attention as biological methods includes environmental friendly, nontoxic and clean procedures for the production and assembly of nanomaterials. Biological method uses most efficient machines of nature i.e., living cells for the production. For the introduction of growth medium, following procedure is used, The bacterial strain *E.Coli (DH5a)* used in the experiment was purchased from Pks, Infra Engineers Pvt Ltd, Ghaziabad. Broth formulations like LB (Luria Bertani) medium which contain transition metal salt ZnO was prepared with desired concentration.

1. **LB media preparation** – (for 1000 ml solution)

Peptone	5 gm
Beaf extract	3 gm
NaCl	5 gm
pH	7.2

LB media suitable for bacterial culture.

2. **Chemical salts**                      **Molecular weight**

ZnO	81.38 g/mol
-----	-------------

Salts obtained by Sigma Company

**Procedure**

For rapid growth of the bacterial cells, LB medium was prepared and sterilized for each experiment. Obtained bacterial strain *Escherichia coli (DH5a)* was cultured in LB medium to produce the biomass for biosynthesis. Inoculation of bacterial culture was carried out in Luria Bertani (LB) medium using the shake flask method<sup>[20]</sup>.

- 1.5% of LB broth was prepared in Erlenmeyer flask (250 ml) by mixing of 1.5g of LB broth powder in 100 ml distilled water. Autoclaved it and allowed the medium to cool up to 40-45 °C.
- Flamed the neck of the flask, picked a single colony by sterile loop from culture plate and put that loop in to the flask. Labeled the flask to indicate date and strain no.
- Once inoculated the flasks were incubated overnight in an incubator shaker at 250 rpm speed, 37 °C.

Use a three set of 25ml test tube was also sterilized before experimentation. 10ml of LB medium was

transferred to all test tubes. Various concentrations of (1.5mM) chemical salt of ZnO were carefully placed into all test tube, leaving one as a control to track the normal growth of the microbial cells without salt after that autoclaved it. Cool it (45-50 °C) at normal room temperature in laminar air chamber. Each test tube was then inoculated with 50ul of *E.Coli (DH5a)* grown in liquid LB medium. Each test tube was shaken using a rotary shaker at 180 rpm at 37°C for 72 h.

A colour changing obtained in the content of the test tubes containing chemical salts gave a preliminary indication of nanoparticles synthesis. The obtained residue / biomass were separated from all test tubes through centrifugation that was carried out at 7000 rpm and collected the supernatant for further characterization. Produced nanoparticles were characterized through UV– Vis spectrophotometer where optical density measurements from each test tube were done for monitoring the reduction of synthesized nanoparticles and Electron Microscope (TEM, SEM) to confirm the synthesis of ZnO nanoparticles.

The fungus *Aspergillus niger* used in the experiment was purchased from Pks, Infra Engineers Pvt Ltd, Ghaziabad. Potato dextrose broth / PDB which contains transition metal salt ZnO was prepared with two different (low and high) concentration.

1. **PDB media preparation** – (for 1000 ml solution)

Potato	200 gm
Dextrose	20 gm
pH	5.6

PDB suitable for fungus culture.

2. **Chemical salts**                      **Molecular weight**

ZnO	81.38 g/mol
-----	-------------

Salts obtained by Sigma Company

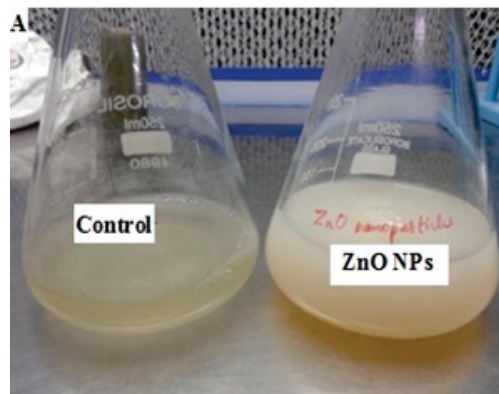
**Procedure**

Biomass of fungus *A.niger* (purchased) was cultivated in potato dextrose broth (PDB), using the shake flask method as described in section 3.4.1 with respect of PDB. Through the use of loop, fungus culture was transferred in to an Erlenmeyer flask (250 ml) containing 50 ml PDB. After inoculation, flasks were shaken at 150 rpm speed on a rotary shaker at 30±2 °C for 3 days.

The culture grew like discrete pellicles. Biomass harvesting was carried out through filtering and then washing the biomass, known as “viable biomass”. The viable biomass of pellet was utilized in the salt uptake studies. The salt tolerance of mycelia growth was investigated through weighing the dry biomass of pellet after 3 days incubation at 150 rpm speed on a rotary shaker at  $30 \pm 2^\circ\text{C}$  in the potato dextrose broth with desired concentration of chemical salts. Weighted dry biomass of fungus in 100 ml distilled water + ZnO at the concentration range of 10mg, as compared with that was without salt used as control. Usually 10gm of wet weighted biomass were introduced in to the contact of sterile doubled distilled water of 100 ml in an Erlenmeyer

flask at  $27^\circ\text{C}$  for 48 hours that agitated at 150 rpm. After incubation, Whatman filter paper no. 1 was used to filtered the cell filtrate. Once filtered, the pH of observed cell filtrate was adjusted at 7.2, through the use of 1 M NaOH and  $\text{HNO}_3$ . In to the 50 ml of cell filtrate, a carefully weighed quantity of chemical salt, ZnO was added to the Erlenmeyer flask, in one flask that is filtrate + ZnO and incubated at normal room temperature in dark. Control, use in this protocol, containing cell free filtrate without any salt concentration was run simultaneously as standard with the experimental flask. According to Pimpa W et al., (2004) , nanoparticles were concentrated through shaken at 125 rpm at  $30 \pm 2^\circ\text{C}$  on a rotary shaker for 2 hours<sup>[21]</sup>

(Figure 2).

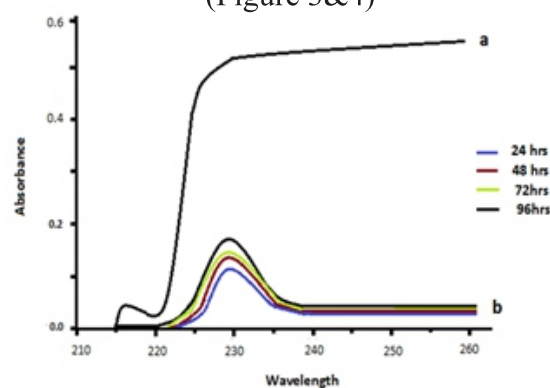


### Figure 2: Nanoparticle synthesis using fungal extract *Aspergillus niger* A) Synthesis of ZnO nanoparticles

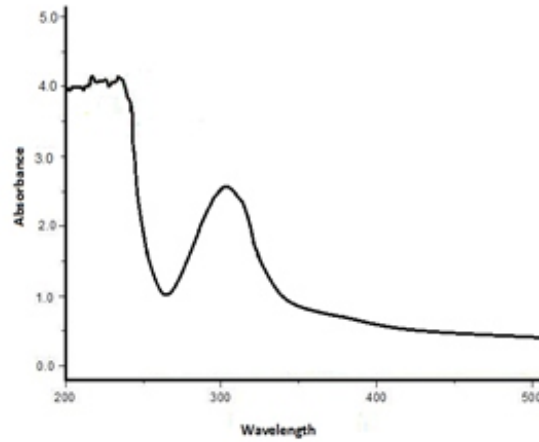
A colour changing obtained in the content of the flasks containing chemical salts gave a preliminary indication of nanoparticles synthesis. The obtained biomass was separated from all flasks through centrifugation which carried out at

7000 rpm and collected the supernatant for further characterization. Produced nanoparticles were characterized through UV– Vis spectrophotometer where optical density measurements from each flask were done for monitoring the reduction of nanoparticles were taken every 24 hrs and record the fungus growth

(Figure 3&4)

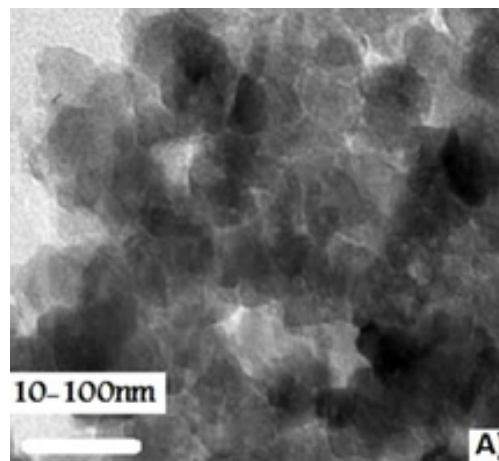


**Figure: 3. Synthesis of ZnO nanoparticle by fungus (*A.Niger*)**

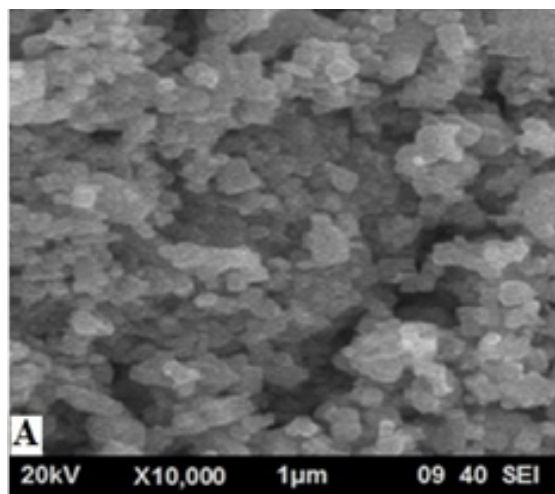


**Figure 4: Synthesis of ZnO (zinc oxide) nanoparticles using *E.coli***

Electron Microscope (TEM, SEM) was used to confirm the synthesis of ZnO nanoparticles (Figure 5&6).



**Figure 5: TEM micrograph of (A) ZnO**



**Figure 6: SEM micrograph of (A) ZnO**

## CONCLUSION

A concise overview of the microorganisms' uses such as bacteria and fungi to biosynthesize metal NPs has been described, with shake flask methods currently in vogue. The production of metal oxide nanoparticles through bacteria (*E.coli*) and fungus (*A. niger*) has a considerable potential over traditional methods of synthesis. For the biological synthesis of nanomaterials, technology has to be scaled up to check the cost effectiveness. The process of synthesis is eco-friendly, rapid, followed biological approach mechanism. *E. coli* bacteria have a high growth rate and are relatively cheap to cultivate in comparison of other biological systems. Bacteria have few advantages over fungi because it can be manipulated easily, make them as the chassis of choice for the near term bio-production of nanoparticles. Alternatively, fungus *A. niger* have the advantage of producing very high yields of secreted proteins, which may increase nanoparticle synthesis rate.

The micro-organism fungi have mycelia which provide an increased surface area to fungus species in comparison of bacteria. Thus this increased area could be helpful to support the interaction of fungal reducing agent with metal ions and enhancing the conversion of ions to metal oxide nanoparticles. Fungi also have the advantage of ease of downstream processing when nanoparticles are produced, allowing for a more efficient way of extracting nanoparticles from them. Scalability, another factor for consideration in the content of commercial production of nanoparticles, gives fungi the edge as the chassis of choice in order of long term development as they can be used more easily in large-scale reactors than bacteria. Since fungus *A. niger* has ability to secrete much larger amounts of proteins than bacteria *E. coli*; thereby considerably enhancing the productivity of biosynthetic approach of production and

characterization of metal oxide nanoparticles.

The area of biological production of metal oxide nanoparticle is relatively novel and underexplored; however it shows great potential in the biotechnology sector. There are many aspects of these biological methods to be discovered, and later manipulated, as the technology emerges.

Use of microbes for the production of metal oxide nanoparticles is a reliable and with eco-friendly protocol. The characterization of metal ion (zinc oxide) exposed to microbial strain and the reduction of these metal ions to respective nanomaterials was confirmed by UV-Vis Spectrophotometer. After the addition of lead nitrate and zinc oxide to the bacterial culture, the synthesized nanoparticle solution was scanned through UV-Visible spectrophotometer. The ZnO NPs has sharp absorbance with the highest peak at 300nm respectively.

Similarly, after the addition of zinc oxide and lead nitrate to the fungal culture, the synthesized nanoparticle solution was scanned UV-Visible spectrophotometer. The ZnO NPs has sharp absorbance with the highest peak at 230nm respectively. The characteristic of metal oxide nanoparticles was more clearly observed in the supernatant solutions of fungal strains indicating the synthesis of NPs. It was observed that a greater reduction of metal oxide nanoparticles in the growth medium of fungus *A. niger* than bacteria *E. coli* during the 24 hour incubation, decrease in the intensity of radiation directly proportional to the concentration of the solution. *A. niger* have the advantage of producing very high yields of secreted proteins as well as an increased surface area, which may increase nanoparticle synthesis rate. Thus compared to bacteria, *A. niger* have significantly increase the productivity of biosynthetic approach of metal oxide nanoparticles.

## REFERENCES

1. Mohanpuria P., Rana N. K., Yadav S. K., 2008. Bio-synthesis of nanoparticles: technological concepts and future applications. J. Nanopart. Res., 10: 507–517.
2. Raveendran P. F., Wallen J., 2003. Completely “Green” Synthesis and Stabilization of metal nanoparticles. J American Chemical Society, 125(46): 13940-13941.
3. Li S. S., Xie Y., Yu A., Qui X., Zhang L., 2007. Green synthesis of silver nanoparticles using *Capsicum annum* L. extract, Green Chemistry, 9: 852-858.
4. Parashar U. K. S., Srivastava S.P., 2009. Bioinspired synthesis of silver nanoparticles. j nanomaterials biostructures, 4(1): 159- 166.

5. Gericke M., Pinches A., 2006. Biological synthesis of metal nanoparticles. *Hydrometallurgy, J Natural Science*, 83: 132-140.
6. Ogi T., Saitoh N., Nomura T., 2010. Room-temperature synthesis of gold nanoparticles and nanoplates using *Shewanella* algae cell extract, *Journal of Nanoparticle Research*, 12 (7): 2531–2539.
7. Rasooly A., Jacobson J., 2006. Development of biosensors for cancer clinical testing, *Biosens Bioelectron*, 21(10): 1851–1858.
8. Shankar S.S., Rai A., Ahmad A., Sastry M., 2004. Rapid synthesis of Au, Ag and bimetallic Au core Ag shell nanoparticles using neem (*Azadirachta indica*), *J colloid and interface science*, 275: 496-502.
9. Muhammad Q., Nopphadol U., Jihyun C., 2018. Antimicrobial activity of silver nanoparticles encapsulated in poly-N-isopropylacrylamide-based polymeric nanoparticles, *Int J Nanomedicine*, 13: 235–249.
10. Boisseau P., Loubaton B., 2011. Nanomedicine, nanotechnology in medicine. *Comptes Rendus Physique*, 12: 620-636.
11. Auffan M., Rose J., Bottero J., Lowry G., 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective, *Nature Nanotechnol*, 4:634–641.
12. Ahmad A., Senapati S., Khan M. I., Kumar R., Sastry M., 2003. Extracellular Biosynthesis of Monodisperse Gold Nanoparticles by a Novel Extremophilic Actinomycete, *Thermomonospora* sp., *Biochemical Sciences*, 19(8): 3550–3553.
13. Ahmad A., Mukherjee P., Senapati S., 2001. Fungus-Mediated Syntheses of Silver Nanoparticles and Their Immobilization in the Mycelial Matrix, *Nano Letters*, 1: 515-519.
14. Balaji D. S., Basavaraja S., Mahesh D. B., Venkataraman A., 2009. Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus, *J biointerfaces*, 68: 88-92.
15. Torresday J. L. G., Parsons, J. G., Gomez E., Yacaman M. J., 2002. Formation and growth of Au nanoparticles inside live alfalfa plants. *J Nanoletters*, 2(4): 397-401.
16. Bali R., Razak N., Lumb A., 2006. The synthesis of metal nanoparticles inside live plants, *Nanosci. Nanotechnol. International Conference*.
17. Jha A. K., Prasad K., Prasad K., 2009. Plant system: Nature's nanofactory, *J Biointerfaces*, 73(2): 219-23.
18. Wang N. X., Recum H. A., 2011. "Affinity-Based Drug Delivery", *Macromol Biosci*. 11 (3): 321–332.
19. Duran N., Marcato P. D., 2007. Antibacterial effect of nanoparticles produced by fungal process, *J Biomed Nanotechnol*, 3: 203–208,
20. Baka J., Comer E. A., 2008. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound, *J Pharmaceutical and Biomedical*, 46(2): 335-341.
21. Pimpa W., Netpae T., 2004. Use of pelleted biomass of *Aspergillus oryzae* for lead removal, *Thai. Environ. Eng. J.*, 18(1): 21-28.