ISSN 0976 – 3333

Available Online at www.ijpba.info

**International Journal of Pharmaceutical & Biological Archives 2012; 3(6):1418-1423**

**ORIGINAL RESEARCH ARTICLE**

# Extracellular Synthesis of Silver Nanoparticles by Marine Thermophilic Bacteria

**P. Dhandapani and N. Supraja***

*Department of Corrosion Protection Division, Central Electrochemical Research Institute (CECRI), Karaikudi-630006, Tamil Nadu, India*

Received 03 Sep 2012; Revised 23 Nov 2012; Accepted 01 Dec 2012

**ABSTRACT**

The thermophilic *Bacillus* sp have a silver reduction competent of this isolated from marine environment soil. This bacterium was identified by biochemical test. The bio reductant of silver ions and effect of temperatures were monitored by UV-visible spectroscopy at various time intervals. Bio-organic functional groups interact with nano particles were characterized by FTIR. The morphology and crystalline phase of the nanocrystals were determined by Transmission electron microscopy (TEM) and X-Ray diffraction (XRD). The results shows that combined effect of biological activities and extreme conditions to generate unusual size and shape of silver nanoparticles.

**Key words:** Marine thermophilic bacteria, Bioreductant, silver nanoparticles, spherical shape.

## 1. INTRODUCTION

The use of micro organisms for the synthesis of nano sized materials has recently emerged as a novel approach and designed for the production of heavy metals and metal nanoparticles by fungi [1, 2]. The biosynthesis of silver nanoparticles was carried out by using bio-reducing agent like bacteria, fungi and plant extracts [3]. The advantages of biological generation of nanoparticles are three fold; the bio-species can act as a template, reducing and even as capping agent for nanoparticles. It is well explained for the formation of biogenic silver nanoparticles based on the intra and extra cellular activities of microbes, such as *Pseudomonas sp, Klebsiella sp, Escherichia coli, Vibrio cholera, Bacillus sp, Salmonella typhus* and *Staphylococcus aureus* [4]. The metal reducing bacterial species were isolated from various soil environments like pond water sediment, marine fungus [5], ore, mine, inorganic pollutant areas and rare earth element environments. Marine habited micro organisms effortlessly adapted with heavy metals and it can produce unusual size and shape of inorganic nanoparticles via, intra or extra cellular mechanisms. This section describe the production of various types of metallic nanoparticles including Au, Ag, Pt, Pd, Cu, [6] Bi-metallic alloys (Au-Ag, Au-Pt, Ag-Pt); metal sulphide nanoparticles (ZnS, PbS, CdS) and other oxide nanoparticles consisting of magnetic, nonmagnetic oxide, $TiO_2$, ZnO, $SiO_2$ [7] . The biogenic approach is further supported by the fact that the majority of the bacteria inhabit various ambient conditions of varying temperature, pH and pressure. It is an advantage of the biosynthesis of nanoparticles, because of combine effects of biological activities and extreme conditions to generated unpredictable [8] sizes and shape of particles. [3]Have reported the extra cellular biosynthesis of nano particles by marine fungi Fusarium oxysporum, [9] Green synthesis of silver nanoparticles using latex of *Jatropha curcas*, monodisperse gold nanoparticles by a novel extremophilic actinomycete and *Thermomonospora* sp [10]. To our best knowledge, extra cellular synthesis of nanoparticles by marine thermophilic bacteria has been rarely reported. It is possible that these microbes' generated nanomaterials can be exploiting to industry applications with increase in their efficiency. Bio-genic metal nanoparticles were suspended in a solution or coated on supporting materials which are expected to be used as functional materials because of their unusual optical, electrochemical, photo catalytic properties [11]. The integration of nanoparticles with biological molecules has lead to the development of diagnostic devices like contrast agents, quantum fluorescent biomarkers,

***Corresponding Author:** N. Supraja, **Email:**biocorrcecri@gmail.com

cell labelling agents and important tools in cancer therapy, DNA sequencing labelling and broad spectrum of antibacterial agent [12]. In the present contribution was to synthesize and characterize silver nanoparticles obtained by use of marine thermophilic bacteria isolated from marine water. This bacterium species was growth on nitrate broth at 50°C and pH 8.0. The combine effects of temperature and alkaline pH during synthesis of silver nanoparticles was monitored by UV-Visible spectroscopy. The morphology and crystalline phase of the bioreducant nanoparticles were characterized TEM and XRD. This is the first report on the extra cellular synthesis of silver nanoparticles using marine microorganisms. Presented below are details of the investigation. However control over temperature, for the synthesis of silver nanoparticles using micro organism have been rarely reported.

## 2.0. Materials and methods

### 2.1. Selection of bacterial species

In this study, the marine thermophilic *Bacillus* sp was isolated from marine environment in Mandapam (Ramanathapuram District). This bacterial species was identified by biochemical test. This bacterium species was used as synthesis of silver nanoparticles at various temperature levels.

### 2.2. Synthesis of silver nanoparticles

The marine thermophilic bacteria was grown in 250ml Erlenmeyer flasks containing 100ml of nitrate broth which is composed of (g/L) peptone-5, beef extract-3 and potassium nitrate-1. The pH should be maintained constantly at 8.0. The culture was grown in the water bath at 50ºC for 48hrs incubation. The grown bacteria were separated from the culture broth by centrifugation (6000 rpm) for 30min. The bacterial cells were washed thrice with 0.1M phosphate buffer pH (8.0) under sterile conditions. The cultured bacteria were then resuspended in 100ml of $10^{-3}$ aqueous silver nitrate solution in 250ml Erlenmeyer flasks at pH 8.0. The whole mixture was kept in a water bath at 50ºC and the experiment was carried out at various temperatures like (10ºC, 27ºC & 50ºC) and maintained in dark conditions for 24 hrs. After the formation of brown colour solution it was subjected to ultrasonication bath for 30min in order to separate nanoparticles from bio-organic complex with nanoparticles. This medium was filtered by using Whatman NO.1 filter paper. The filtrate solution was centrifuged for 45 min at 12,000 rpm to collect bioreductant nanoparticles.

### 2.3. Characterization of silver nanoparticles

#### 2.3.1. UV – Visible spectrum:

The bio reduction of the $Ag^+$ ions was carried out by marine thermophilic bacteria at different temperature levels. The bioreductant silver ions were monitored by UV–visible spectrum at various time intervals. The UV – Visible spectra of this solution was recorded in spectra 50 ANALYTIKJENA Spectrophotometer, from 250 to 800 nm.

#### 2.3.2. FTIR Analysis

The bioreductant nanoparticles were harvested and characterized by FTIR. The FTIR spectrum was taken in the mid IR region of 400–4000 $cm^{-1}$. The spectrum was recorded using ATR (attenuated total reflectance) technique. The sample was directly placed in the KBr crystal and the spectrum was recorded in the transmittance mode.

#### 2.3.3. Crystal phase and morphological analysis of nanoparticles

The bioreductant nanoparticles were harvested and characterized by XRD and TEM. The XRD pattern was recorded using computer controlled XRD-system, JEOL, and Model: JPX-8030 with CuK radiation (Ni filtered = 13418 $A^o$) at the range of 40kV, 20A. The 'peak search' and 'search match' program built in software (syn master 7935) was used to identify the peak table and ultimately for the identification of XRD peak. The morphology of the nanoparticles was characterization by Transmission electron microscope (TEM), Model: Tecnai 20G2, FEI Company, Eindhoven, and the Netherland.

## 3. RESULTS AND DISCUSSION

### 3.1. Selection of marine thermophilic bacteria and synthesis of silver nanoparticles

Marine extreme environment bacterial species have unusual biological activities depending upon the metabolisms under temperature, pH and pressure. It is well- known that silver nanoparticles exhibit brown colour, arising due to excitation of surface Plasmon vibrations in the silver nanoparticles. **(Fig 1)** shows the effect of pH on marine thermophilic bacteria at different temperatures (27°C and 50°C). pH under goes changes when compare to first day (7.2) and on tenth day the pH raises up to 8.2 at 50°C, when compared to 27°C system. **(Fig 2)** shows the bacterial growth rate at different temperatures. It shows that marine bacterium growth rate gradually increases in 50°C, whereas 27°C system to observe that there is no bacterial growth rate. It

clearly indicates that marine thermophilic bacterium is temperature dependent bacterial growth rate at 50°C. **(Plate 1)** shows the optical microscopic observation of marine thermophilic bacterium in simple staining. It is long rod shaped and endospore-forming organism. This bacterium species was identified by biochemical tests shows that *Bacillus* sp (not shown in data).The physiological activity on bio reduction of silver ion, was carried out at different temperatures (27ºC, 50ºC) followed by incubation with 1 mM $AgNO_3$ solution without NaCl in the dark condition, the two solutions changed as brown colour from colour less medium. The brown colour was primarily due to the surface Plasmon resonance of deposited silver nanoparticles. The silver nanoparticles were produced at 50ºC where the solution was dark brown colour. Room temperatures (27ºC) generated silver nanoparticles where partially brown colour was noticed. The colour variation indicates that silver ions reduction on the medium depend upon the biological activities under different temperature conditions. The characterization analysis of silver nanoparticles gave partial yield at 50°C which were characterised by UV, FTIR and TEM. In present study, thermophilic *Bacillus* sp [13] has the ability to grow at 50ºC because the bacterial protein is dependent on the temperature. It is one of causative factor or accelerated for the silver ions reduction to silver nanoparticles on the medium to be appeared dark brown colour when compared to another temperatures system. The present results support the work of the previous investigators.

### 3.2. UV-Visible spectral analysis

UV-Visible spectroscopy was employed to understand the time dependent kinetics of biosynthesis of silver nanoparticles by marine thermophilic bacteria at different temperature levels. **(Fig 3a)** shows the UV-Visible adsorption spectra of silver nanoparticles after 48hrs incubation with different temperatures (27°C and 50°C) at different time intervals. **(Fig 3b)** shows the pictures of bioreducant silver nanoparticles at different temperatures. The spectrum shows peaks at 420 - 460 nm. But the maximum absorbance peak is observed at 450nm under 50°C in 48hrs; the peak observed at 472nm under 27°C shows less absorption which clearly indicates increase in silver nanoparticles size. The short time enough for the silver ions to silver nanoparticles at 50ºC temperature. Whereas, room temperature shows the silver reduction absorbance peak intensity was lower. Silver reduction capacity was observed under UV-Visible adsorption spectroscopy. This shows UV-Vis spectra obtained from solution at different reaction temperature at 8.0 pH. The overall observations suggest that silver nanoparticles generated by marine thermophillic *Bacillus* sp [14] is temperature dependant. Besides, the marine thermophillic *Bacillus* sp is activated only at 50ºC temperature and it enhances the production of silver nanoparticles. The bio reduction of (silver ions) $Ag^{+}$ to $Ag^{(0)}$ was confirmed by UV-Visible spectroscopy at various temperatures **(Plate 2)**. Marine thermophilic *Bacillus* sp growth on nitrate broth at 50°C temperature enhances the production of silver nanoparticles because of bacterial protein activation. The production of silver nanoparticles was lower at 27°C

### 3.3. FTIR Analysis

FTIR Spectrum for nanoparticles synthesized by bacteria at two different temperatures (27°C and50°C) is represented in **(Fig 4)**. The interface between bio-organic functional groups and metal nanoparticles were illustrated by FTIR spectrum. The peaks at 3856, 3743 and 3429 $cm^{-1}$ were assigned to the stretching vibrations of primary, secondary amines and hydroxyl group respectively. These peaks were corresponding to protein enzymes or polysaccharide components from cell biomass. The peaks at 1605, 1385, 1312 $cm^{-1}$ corresponded to C=O, C-N stretching vibrations of aromatic and aliphatic amines, respectively. The two peaks were observed at 1078 and 1016 $cm^{-1}$ which can be assigned to the (C=O) groups. In addition to this band at 733 and 530 $cm^{-1}$ corresponds to metal binding carboxylic (M↔C≡O) groups. Whereas bioreductant silver nanoparticles at 27ºC, shows the same bio-organic functional groups peaks. But one peak at 1078 to 1119 $cm^{-1}$ shows that chemical transformation occurs during synthesis of silver ions to nanoparticles at 50ºC. The nanoparticles are bound to the functional organic groups (carboxyl and amine) from the bacterial content of protein .This functional group May acts template, reducing and capping of nanocrystals. Which with the observation made by FTIR results. It clearly indicates that biosynthesis of silver nanoparticles and capping agent was activated at 50ºC by marine thermophilic bacteria. Proposed that the proteins can bind with nanoparticles either through free amine groups or crystalline residues in the proteins. The carboxylic groups are known to coordinate with metal ions which may act as a

nucleation site for nanoparticles formation. The overall peak from FTIR observation confirms the presence of protein moiety in the samples of silver nanoparticles. It can be assumed that protein molecules or peptide chains may act as template nucleation site of silver atom clusters and reduce silver ions to form silver nanoparticles. This clearly suggests that in addition to extra cellular proteins bacterial physiology also plays a significant role toward the synthesis and shape control of silver nanoparticles.

### 3.4. TGA analysis

The purity of the bioredutcant silver nanoparticles was characterized by TGA and presented in **(Fig 5)**. From this data, it can be observed that three distinct slopes of weight loss process from places at 99°C, 200°C and 445°C. The curve shows 200°C OH groups. Below 200°C in the biomass 445°C was assigned to carbon contents are present in the nanoparticles. The TGA shows that metal surface desorption of bio-organic substance (4.20%) is present in the sample. It is negligible for the bio-impurities present in the sample. The impurities can act as nucleation sites and capping agent for nanoparticles. Since, biomass has large number of carbohydrate, lipid and protein component, protein is the responsible molecule for synthesis of nanoparticles. TGA result shows that the purity of silver nanoparticles was 95% from biosynthesis of silver colloidal medium carried out by ultrasonication method. This technique eliminates the bio-organic content and separates the nanoparticles from bio-complex substances.

### 3.5. Crystal phase and morphological analysis of silver nanoparticles

**(Fig 6)** Shows XRD patterns of marine thermophilic bacteria mediated synthesis of silver nanoparticles at 50ºC. The peaks were assigned to diffraction signals of (111), (200), (220), and (311) plane for face centred cubic (FCC) silver. The lattice constant calculated from this pattern was 4.0869Aº a value in agreement with literature report (4.0855 Aº) JSPCDS file no 89-3722. The microbial and enzymes mediated synthesized Ag $^{(0)}$ were mostly observed in FCC crystals type [14]. The crystal phase analysis of face centred cubic (FCC) and then high intensity of 111 plane structure supports the SAED (Selected area electron diffraction) observation results.

### 3.6. TEM

The size and shape of bioreductant nanoparticles were characterized and shown by the TEM micrograph of silver nanoparticles. The spherical nanoparticles in the range between 5-20 nm were noticed. The scale bar corresponds to 20nm. Selected area electron diffraction pattern form the single spherical shaped silver nanoparticles. The set of spots with the strongest intensity could be indexed to 111 and 110 reflections which indicates that Ag are single crystals with a (111) lattice plane as the basal plane **(Fig 7)**. The SAED pattern indicates that silver nanoparticles are spherical where (111) lattice plane for the face centred cubic crystal structures was confirmed.

TEM images suggested that particles are mostly spherical in shape and average particles size range is 10-100nm.The nanoparticles are bound to the functional organic groups (carboxyl and amine) from the bacterial content of protein .This functional group May acts template, reducing and capping of nanocrystals.

This study shows the temperature dependent biosynthesis of silver nanoparitcles by marine thermophilic *Bacillus* sp. The characterization analysis of silver nanoparticles [15] gave partial yield at 50°C. In the present study, thermophilic *Bacillus* sp has the ability to grow at 50ºC because the bacterial protein is dependent on the temperature [16]. It is one of causative factors for acceleration of silver ions reduction to silver nanoparticles the medium present at 50°C appeared dark brown colour when compared to another temperature system. The overall discussion to be concluded is that the bio-species generated different size and shape of nanoparticles which depend upon the biological activities and physiological factors. There was rarely reported that the marine thermophilic bacteria sp at 50°C for synthesis of silver nanoparticles. In the present study marine strains at 50°C were selected for the synthesis of nanoparticles.

**Fig 1: Effect of pH on marine thermophilic bacteria at two different temperatures (27°Cand 50°C)**

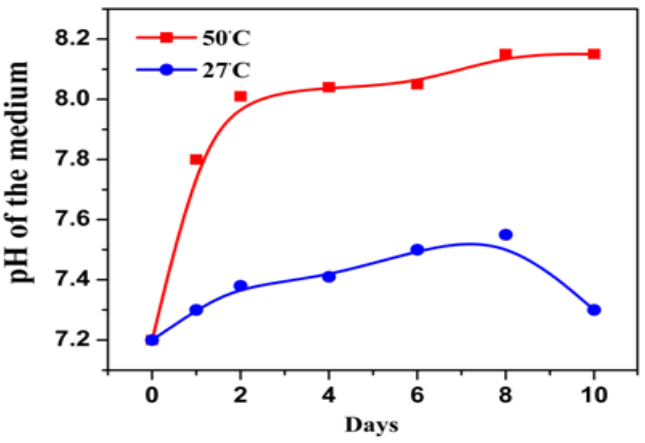


**Fig 2: Effect of temperature on marine thermophilic bacteria at two different Temperatures (27°C and 50°C)**

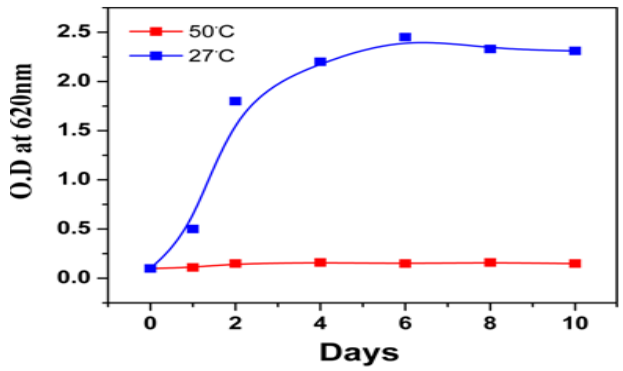



**Fig 3a, 3b, 3c: shows UV-Visible adsorption spectra of Silver nanoparticles after 48hrs of reaction at different Reaction temperature (27°C and 50°C) under different Time intervals**

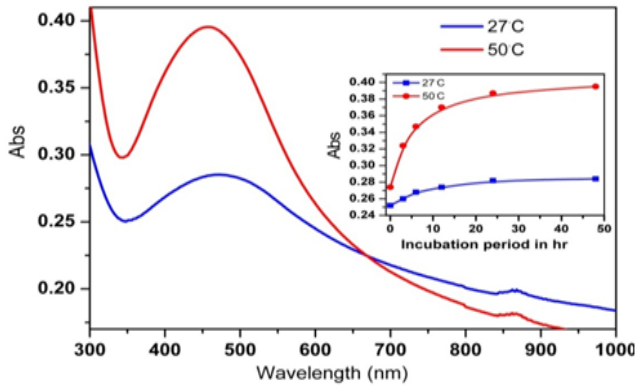



**Fig 4: FTIR Spectrum recorded by making KBr pellet with synthesized silver nanoparticles at two different temperatures (27°C and 50°C)**

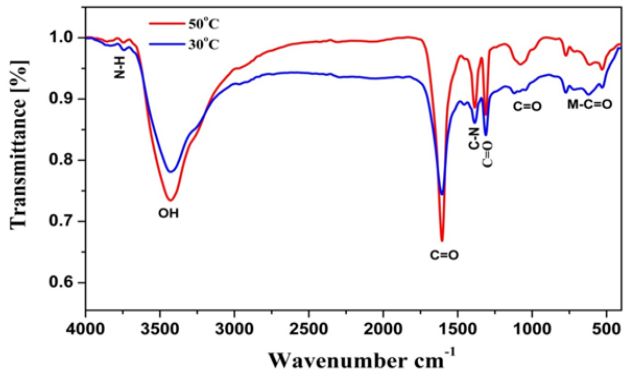



**Fig 5: Represents TGA spectra of bio reductant silver nanoparticles were carried out by marine thermophilic bacteria at 50°C**

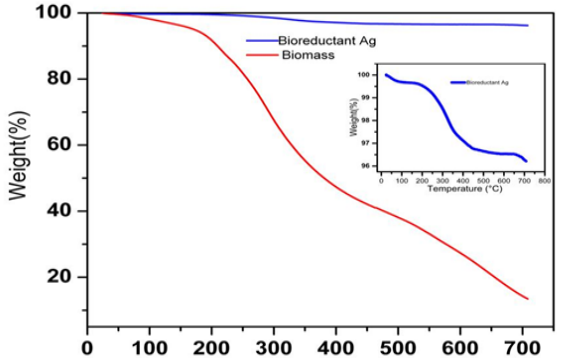



**Fig 6: Represents XRD analyses of silver nanoparticles were carried out by marine thermophilic bacteria at 50°C**

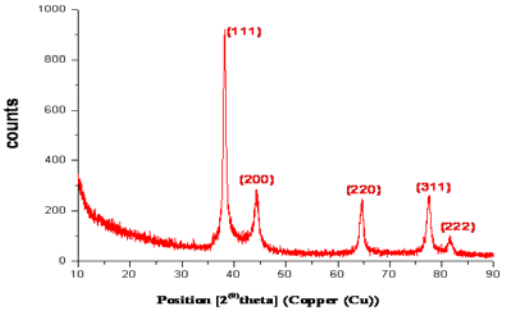



**Fig 7: TEM studies for silver nanoparticles formation collected from marine thermophilic bacteria at 50°C**

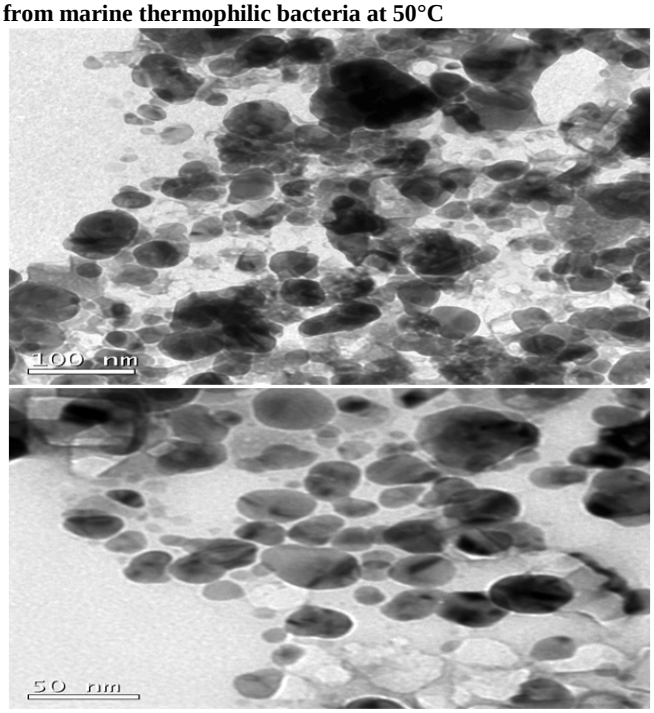



**Plate1: Optical microscope shows the rod shaped marine thermophilic bacteria at 50°C**

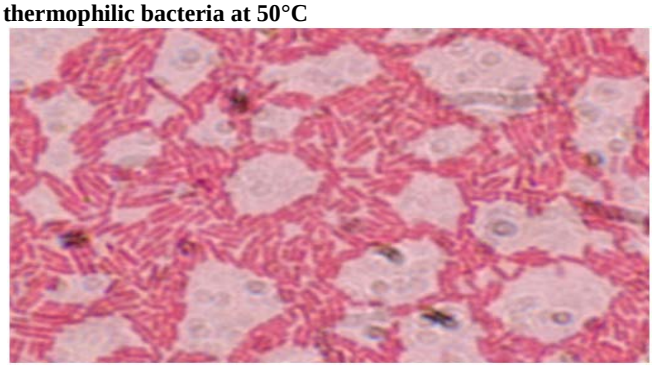
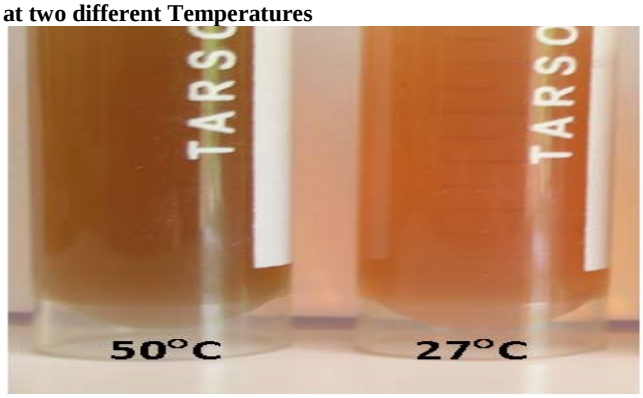


**Plate 2: This picture indicates two tubes containing the marine thermophilic bacterial *sp* in aqueous solution of 10-3M AgNo3 at two different Temperatures**



## REFERENCES


1. Bae, W., W. Chen, A. Mulchandani and R.K. Mehra. 2000. Enhanced bioaccumulation of heavy metals by bacterial cells displaying synthetic phytochelatins. *Biotechnol. Bioeng: 70: 518–524.*
2. Sastry, M., A. Ahmad, M.I. Khan and Kumar. R. 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 85:162–170
3. Ahmad, A., P. Mukherjee, P. Senapati, D. Mandal, M. Islam Khan and R. Kumar. 2003. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. *Colloid Surf B*: 28:313–8.
4. Basavaraja, S., S.D. Balaji, A. Legashetty, A.H. Rasab and A. Venkatraman. 2008. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. *Mater Res Bull.* 43:1164–1170.
5. Kathiresan, K., S. Manivannan, M.A Nabeel and B. Dhivya. 2009. Studies on silver nanoparticles synthesized by a marine fungus, penicillium fellutanum isolated from coastal mangrove sediment. *Colloids Surf.* B: Bio interfaces. 71: 133-137**.**
6. Shankar, SS., A. Rai, A. Ahmad and M.J. Sastry. 2004. Rapid synthesis of Au, Ag and bimetallic Au shell nanoparticles using Neem. *J Colloid Interf Sci:* 275:496–502.
7. Silver, S. 2003. Bacterial silver resistance: molecular biology anduses and misuses of silver compounds. FEMS *Microbiol Rev*. 27:341–353.
8. Adams, L.K., D.Y. Lyon and P.J.J. Alvarez. 2006. Comparative eco-toxicity of nanoscale $TiO_2$, $SiO_2$, and ZnO water suspensions. *Water Res*: 40:3527–32.
9. Hare Krishna, B., K. Dipak, P. Sankar and Ajay Misra. 2009. Green synthesis of silver nanoparticles using latex of *Jatropha curcas* .*Colloids Surf.* A: 339 134–139.
10. Deendayal, A. And D. Mandal. 2006. Extra cellular biosynthesis of mono disperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora sp. Langmair* 19: 3550-3553.
11. Armelao, L., D. Barreca, G. Bottaro, A. Gasparotto, C. Maccato and C. Maragno. 2007. Photocatalytic and antibacterial activity of $TiO_2$ and $Au/TiO_2$ nanosystems. Nanotechnology: 18:375709 7 pp.
12. Mulvaney, C.R. 1991. Nanotechnology: Convergence with modern biology and medicine. *Curr opn Biotechnol* 14: 337-346.
13. Magudapathy, P., P. Gangopadhyay, B.K. Panigrahi, K.G.M. Nair and S. Dhara. 2001. Biosynthesis of silver nanocrystals by *Bacillus licheniformis.* Phys. B 299: 142.
14. Kalimuthu, T., R. Joerger, E. Olsson and C. G. Granqvist. 2008. Silver-based crystalline nanoparticles, microbially fabricated. *Proc Natl Acad Sci USA*. 96: 13611–13614.
15. Klaus, T., C.G. Granqvist, R. Joerger and E. Olsson. 1999. Silver-based crystalline nanoparticles, microbially fabricated. *Proc Natl Acad Sci* 96: 13611–13614.
16. Mohammed Fayaz, A., k. Balaji, P.T. Kalaichelvan and R. Venkatesan. 2009. Fungal based synthesis of silver nanoparticles – An effect of temperature on the size of particles. *Colloids Surf.*B: Bio interfaces 74: 123-126.