

Biosynthesis and Characterization of Silver Nanoparticles with Bacterial Isolate from Gangetic-Alluvial Soil

Amar Kumar and Ashok Ghosh

Dept. Of EWM, A. N. College, Patna-13.

Abstract

The biosynthesis of metal nanoparticles from biological processes is now become an expanding research area due to their multifarious and potential applications for the eco-friendly expansion of novel technologies. Usually, nanoparticles are synthesized by variety of chemicals which are quite toxic, flammable in nature and their modes of working are unethical to the environment. This study deals with more convenient and environmental friendly method for the synthesis of silver nanoparticles from Silver Nitrate with the help of *Bacillus* sp-***Brevibacillus borstelensis*_MTCC10642**.

The formation and characterization of Silver nanoparticles were confirmed by UV-Vis Spectroscopy, X-Ray Diffraction (XRD), Fourier Transform Infra Red Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM).

Keywords: Nanoparticles, Silver Nitrate, *Bacillus*, UV-Vis Spectroscopy, X-Ray Diffraction, SEM.

Introduction:

Today world is at the cutting edge of environment paucity. Everyday scientists are developing new tools and techniques to save our planet and the improvement of eco-friendly technologies in material synthesis is one of the vital connotations to expand their biological applications. With the advent of Nano many novel avenues has opened the door to frenetic search of new nanomaterials and methods and a variety of inorganic nanoparticles with well-defined chemical composition, size, and morphology have been synthesized by using different microorganisms, and their applications in many avant-garde technological areas have been explored. [**Gericke Marickie and Pinches Anthony; 2006**]

Nanoparticles—particles having one or more dimensions of the order of 100 nm or less—have ensnared great concentration due to their unusual and fascinating

properties, and applications gainfully prevail over their bulk counterparts. Nanoparticles fabricated by a biogenic enzymatic process are far superior, in several ways, to those particles produced by chemical methods. Despite that the latter methods are able to produce large quantities of nanoparticles with a defined size and shape in a relatively short time, they are complicated, outdated, costly, and inefficient and produce hazardous toxic wastes that are harmful, not only to the environment but also to human health. With an enzymatic process, the use of expensive chemicals is eliminated, and the more acceptable “green” route is not as energy intensive as the chemical method and is also environment friendly. The “biogenic” approach is further supported by the fact that the majority of the bacteria inhabit ambient conditions of varying temperature, pH, and pressure. The particles generated by these processes have higher catalytic reactivity, greater specific surface area, and an improved contact between the enzyme and metal salt in question due to the bacterial carrier matrix. **[Bootharaju and Pradeep;2010]**

Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the element metal through enzymes generated by the cell activities. **[Frontasyeva, Marina Vladimirovna; et al. ;2013]**. It can be classified into intracellular and extracellular synthesis according to the location where nanoparticles are formed. **[Mukherjee et. al, 2001]**

Silver has a long and intriguing history as an antibiotic in human health care. The antimicrobial properties of silver have been known to cultures all around the world for many centuries. The silver ion is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes. It is fact that Microbes secrete more amounts of proteins which directly translate to higher productivity of nanoparticle formation and the parameters like pH, temperature, time, size and shape of the nanoparticle can be strictly controlled **[Prabhu & Poulouse ;2012]** The possible mechanism of silver nanoparticle formation through nitrate reductase, characterization and the antibacterial activity of the silver nanoparticles are reported in the present investigation.

Biosynthesis of Silver nanoparticles using microorganisms like Bacteria, Fungi, and Yeast has been already exploited. Further, synthesis of Silver nanoparticles using extract of various plants like Aloe vera, Cinnamon zeylanicum, Stevia rebaudiana, Papaya, Bamboo were also reported. Synthesis of Gold and Silver **[Mukherjee et. al, 2001]** nanoparticles by eukaryotic cells such as fungi has been reported. Synthesis of Gold nanoparticles by Shwanella algae **[Konish et. al, 2004]** and Silver nanoparticles by fungus Verticillium, Fusarium viz Fusarium oxysporum **[Duran et al;2005]**, Aspergillus fumigates and Aspergillus flavus **[Vighneshwaran et al;2007]** were also reported. Bacteria mediated extracellular synthesis of Silver nanoparticles has been also reported. **[Ghosh et. al, 2010]**

This paper provides a brief overview of the current research activities that centre on the biological synthesis of metallic nanoparticles, the paper concludes with discussions on the current limitations and prospects of nanoparticle synthesis by microorganisms.

Experimental:

Materials-The bacterial isolate was collected from Microbiological lab, Department of Environment and Water Management, A. N. College, Patna-13, Bihar which was identified from IMTECH, Chandigarh. The strain was identified as *Bacillus sp-Brevibacillus borstelensis MTCC10642*. Patna Chemicals, Mithapur, Patna, Bihar was the supplier of Silver nitrate. Throughout the experiment the bacteria were cultured in Nutrient Broth media and Millipore distillation unit was used for distilled water.

Preparation of Bacterial Culture extract-To prepare the extract of Nutrient Broth chemicals like Peptone-5, Beef extract-3, NaCl-5, respectively in g/ litre were taken as a fix parameter for 500 ml Erlenmeyer flask. The flasks were pre-incubated at 37.5 degree Celsius in incubator for 72 hrs.

Biosynthesis of Silver Nanoparticles-Silver nitrate was added in different concentration ranging from 500, 1000, 1200, 1500, 2000, 2500, 3000, 4000, and 5000 ppm respectively. After 72 hours this culture was filtered through Whatmann filter paper no. 1 and the cell free supernatant was observed on UV-Vis Spectrophotometer with wavelength ranging from 200-600 nm. The culture was then centrifuged at 10000 rpm for 10 minutes to recover the synthesized Nanoparticles in the aliquot.

Characterization of Silver Nanoparticles-

1. **Observed value-**The synthesis of Silver nanoparticles were observed by naked eyes. The colour of the mixed solution changed to deep orange after 24 hrs of agitation with silver nitrate which further blackened in 72 hours. The black colour of the culture confirmed the reduction of Silver salt to Silver nanoparticles, however the bacterial strain treated with ionized water retained its original colour. (Fig 1.)
2. **UV-Vis Spectral analysis-**The biosynthesized Silver nanoparticles was confirmed by sampling the aquatic component of different time intervals and the absorption maxima was scanned by UV-Vis Spectrophotometer at the wavelength of 300-800 nm on Cole-Parmer Spectrophotometer.
3. **FTIR Spectral analysis-**The dried samples were grinded with Potassium-Bromide and make pellets used for Fourier Transform Infra Red measurements. The spectrum was recorded in the range of 4000-400 /cm using Spectrum Two-FTIR Spectrophotometer.
4. **XRD analysis-**The X-Ray Diffraction pattern was measured by powder diffractometer and the same was employed with X-ray diffractometer of characteristic Co-K alpha radiation in the range of 20-80 degree at a scan rate of 0.02 degree per minute with the time constant of 2 second.
5. **SEM analysis-**Morphology and size of the Silver nanoparticles were investigated by SEM images using Phillips instrument.

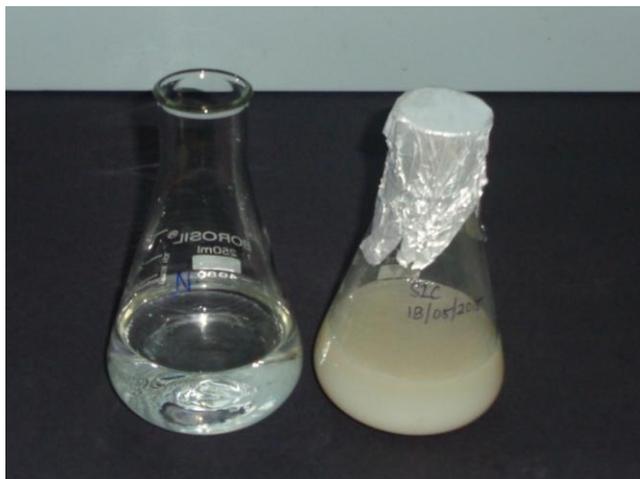


Figure 1: Change of colour after bacterial culture.

Results and Discussion-

UV-Vis Spectroscopy is an indirect method to examine the bio-reduction of AgNPs from aqueous silver nitrate solution. It was found that the biosynthesized AgNPs were very stable in the solution, even one week after their synthesis. The UV-Vis absorption spectra of biosynthesized AgNPs using bacterial extracts have shown that the surface Plasmon band occurred at around 430 nm indicating the presence of AgNPs. (Fig 2.)

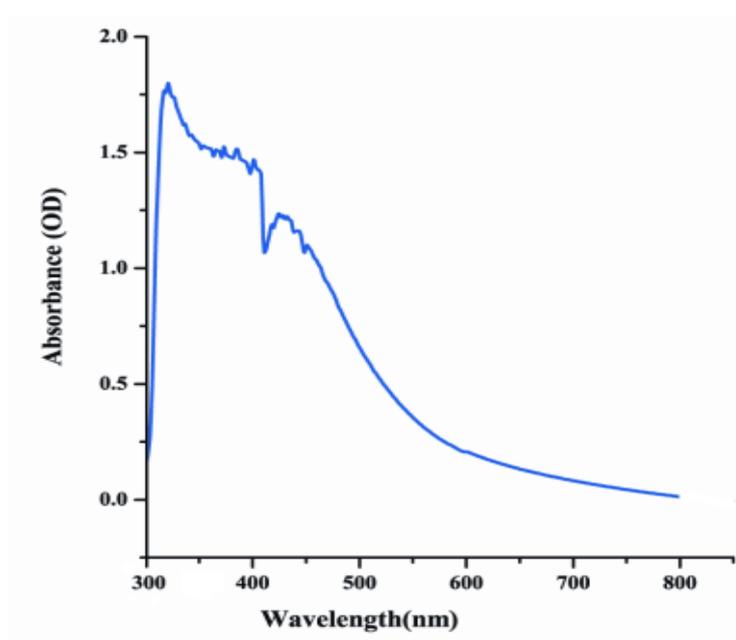


Figure 2: UV-Vis Absorbance maxima of AgNPs after 72 hrs of agitation.

Mechanism of Formation of AgNPs by FTIR-

FTIR measurements were performed to identify the potential bio-molecules in the bacterial extract responsible for the reduction. The peak was centered at 1345/cm which indicated the presence of Nitrate in the residual solution. The band at 3626/cm corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 3072/cm corresponds O-H stretch Carboxylic acids. The stretch at 1651/cm corresponds to N-H bond primary amines. The peak at 1410/cm corresponds to C-N stretch of Aromatic amine group and the bands observed at 631/cm corresponds to C-N stretching esters.

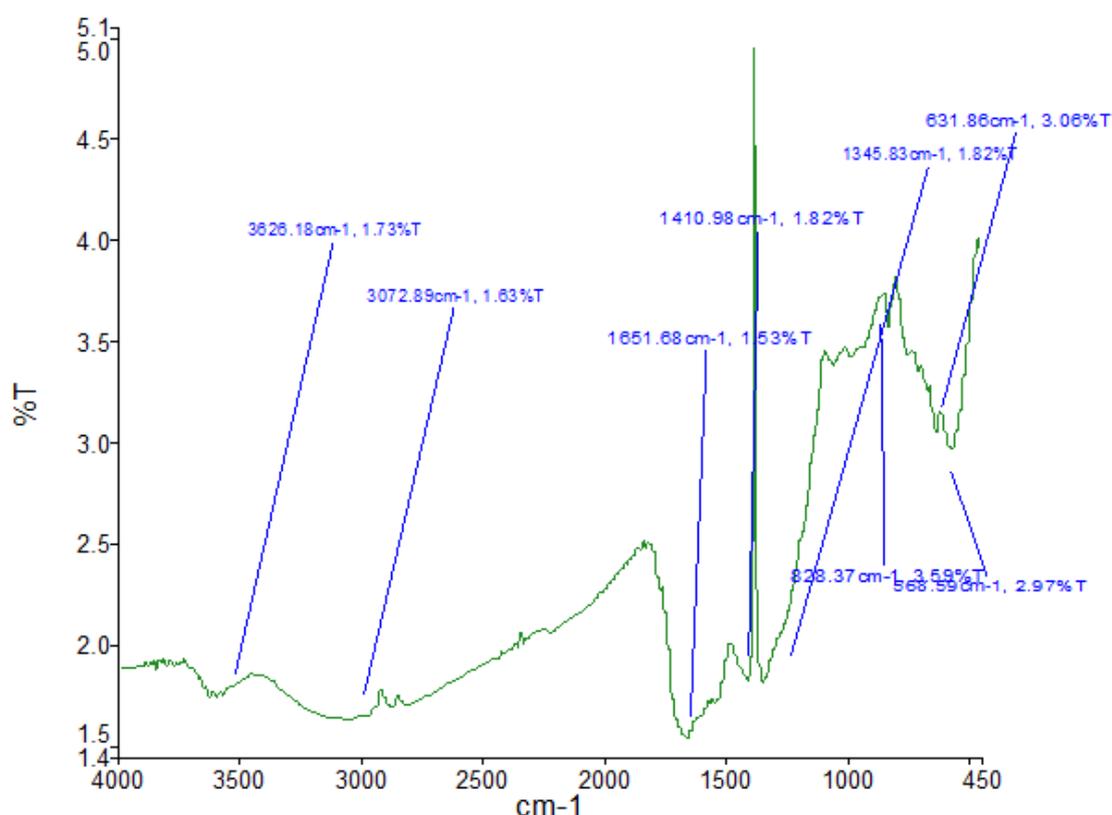


Figure: Showing FTIR Spectra of AgNPs.

Confirmation of AgNPs by XRD-

XRD is a very important technique that is commonly used to analyse the characteristic and structural details of Nanoparticles. The XRD patterns were obtained by measuring the angles at which X-ray beam is diffracted by the crystalline phases in the specimen. The XRD pattern of biosynthesized AgNPs is shown in fig. Where four prominent peaks at 32⁰(2 theta), 46⁰(2 theta), 57⁰(2 theta), 76⁰(2 theta) indicated the presence of (111), (200), (220), and (311) sets of lattice planes and accordingly could be indexed as Fcc structures of AgNPs. Hence, from the XRD pattern it is clear that AgNPs formed using bacterial extracts were essentially crystalline in nature. (Fig-3. and 4.)

Peak list						
2-theta (deg)	d (ang.)	Height (cps)	Int. I(cps/deg)	FWHM(deg)	Size	Phase name
17.3(2)	5.12(6)	6(5)	6.6(15)	1.01(18)	83(15)	Unknown
32.74(7)	2.733(6)	31(10)	30(2)	0.82(6)	105(7)	Unknown
46.07(5)	1.9687(19)	27(10)	25.3(13)	0.82(4)	111(6)	Unknown
57.26(15)	1.608(4)	9(6)	10.5(16)	1.06(13)	89(11)	Unknown
76.23(8)	1.2479(11)	7(5)	15.7(13)	1.83(19)	58(6)	Unknown

Figure 3: Four Data parameters of Prominent Peak.

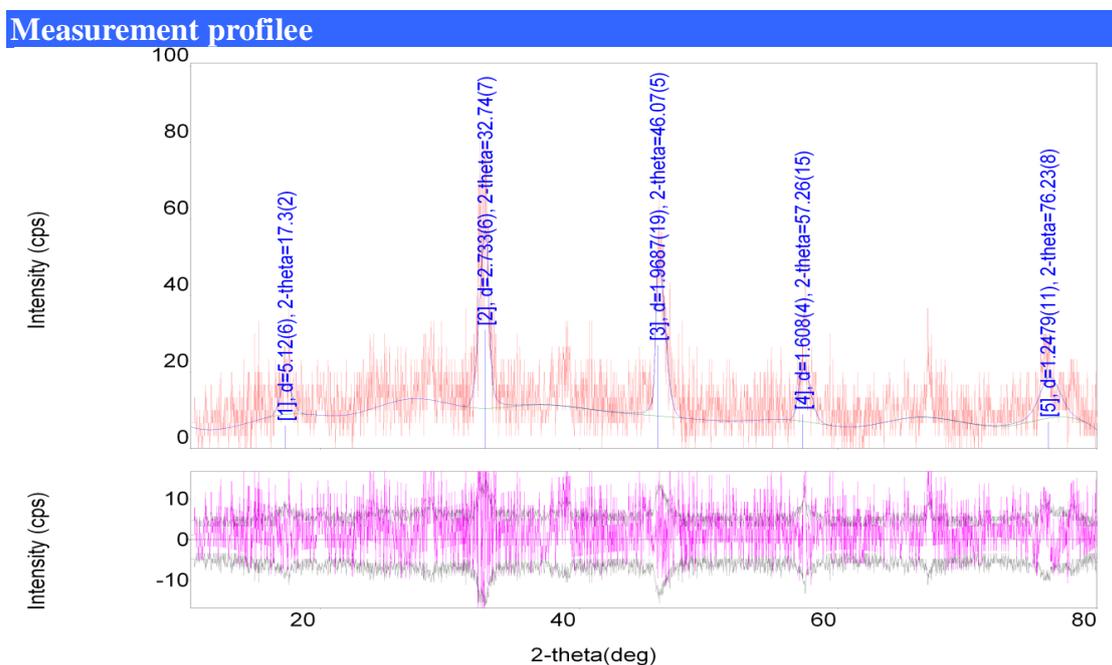


Figure 4: Showing XRD pattern of AgNPs.

Quantitative Assessment by SEM-

SEM visualization allows the measurement of the size and shape of the synthesized nanoparticles. The SEM images of biologically synthesized AgNPs using bacterial extract confirm that the obtained AgNPs are cubical in shape with diameter ranging from 5 nm to 15 nm. (Fig. 5.)

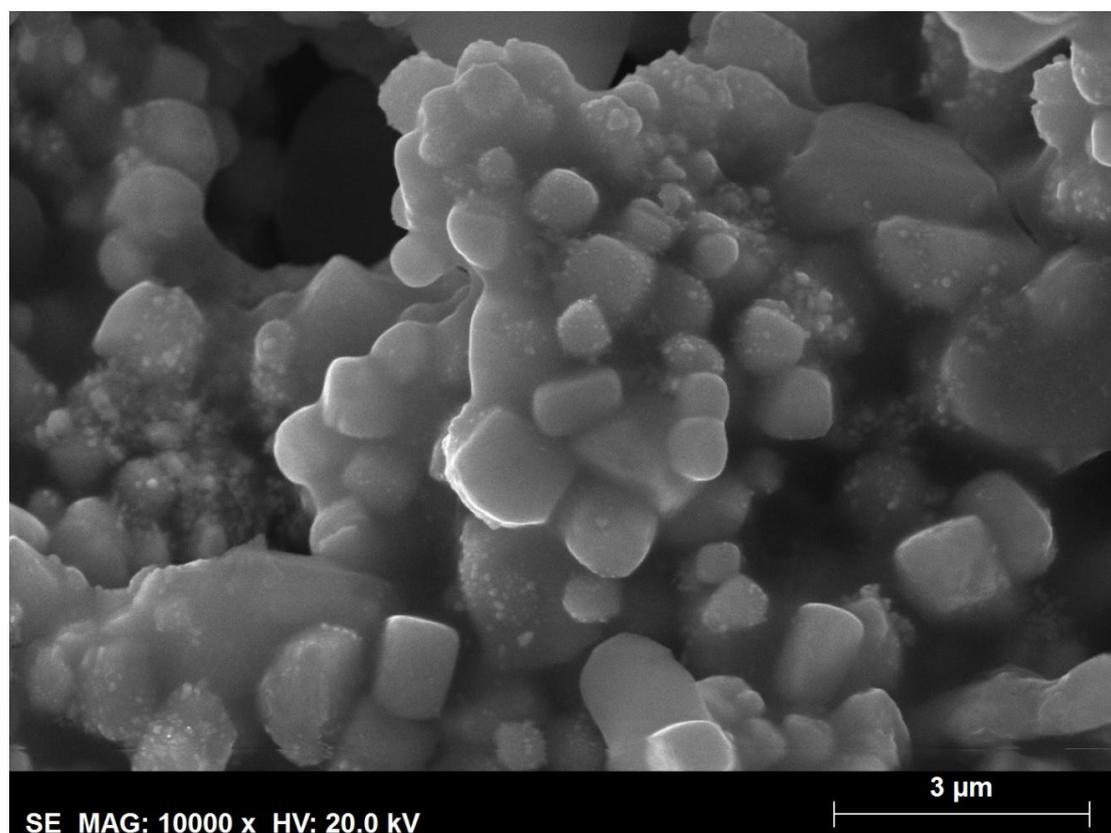


Figure 5: Showing SEM micrograph of AgNPs.

Conclusion-

It was found that biological synthesis of metal nanoparticles is much reliable and trustworthy when we follow eco-friendly protocols and regulations. Biosynthesis of silver nanoparticles using culture supernatant of newly isolated *Bacillus sp.* was demonstrated. Mono-dispersed silver nanoparticles in the range of 5 to 15 nm was synthesised through extracellular mode. The extracellular synthesis offers great advantages over intracellular synthesis from the application point of view. In future, it would be important to understand the biochemical and molecular mechanism of the synthesis of the nanoparticles by the cell filtrate. Results conclude that isolated *Bacillus sp.* is a prominent producer of silver nanoparticles and current study also reveals that the *Bacillus* bacterial extract is quiet capable of producing Silver nanoparticles at room temperature.

Acknowledgement-

I am thankful to Dr. Abha Sharan, H. O. D-Department of Physics, Magadh Mahila College, Patna University, Patna for her kind support in FTIR Analysis, Dr. Amrendra Narayan, Department of Physics, Science College, Patna for XRD analysis and Mr. Raju kumar, INESC Microsystems & Nanotechnologies, Lisbon, Portugal for SEM

analysis. I am also thankful to senior researchers and colleagues for their moral support in completing my research work.

This work is supported and funded by Rajiv Gandhi National Fellowship, UGC, New Delhi, India, Which we gratefully acknowledge.

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