International Journal of Agriculture and Food Science Technology (IJAFST)
ISSN No. 2249-3050, Volume 4 No. 2 (2013)

Biological synthesis and characterization of lead sulphide nanoparticles using bacterial isolates from heavy metal rich sites

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ABSTRACT

Lead is highly poisonous heavy metal (regardless if inhaled or swallowed), affecting almost every organ and system in the body. The main target for lead toxicity is the nervous system. Lead poisoning typically results from ingestion of food or water contaminated with lead; but may also occur after accidental ingestion of contaminated soil, dust, or lead-based paint. Bioremediation of lead by bacteria could be an effective measure to remove lead.

This study involves the synthesis of lead sulphide nanoparticle using lead chloride and calcium sulphate salts utilizing bacterial strains isolated from the industrial sites of Allahabad and Kanpur. For synthesis, bacterial cell mass was centrifuged to separate supernatant from pellet. Both supernatant and cell pellet were challenged with lead chloride and calcium sulphate salts and lead sulphide nanoparticles were effectively synthesized. These extracellularly synthesized nanoparticles exhibited an absorbance maximum at 330 nm and were characterized using UV Visible spectrophotometer and Transmission Electron Microscope. The use of these strains seems to be promising and advantageous as it is serving dual purposes of (i) bioremediation and (ii) nanoparticle synthesis. These biologically synthesized nanoparticles could be used in various applications.

Keywords: Bioremediation; Nanoparticle; Transmission Electron Microscope; UV Visible Spectrophotometer

1. INTRODUCTION

In the present date, the applications of semiconductor nanoparticles belonging to group II-IV CdS, PbS, ZnS, CdSe, PbSe extends to wide-ranging areas such as catalysis (Roucoux et al., 2002), biosensing (Niemeyer et al., 2001), drug delivery (Langer et al., 2003), diagnostics (Rosi et al., 2005), solar cell (Hagfeldt et al., 2000), optoelectronics (Jackson et al., 2003), cell labeling and imaging (Parak et al., 2005, Chan et al., 1998), photonic band gap materials (Moran et al. 2004), surface enhanced Raman spectroscopy (Li et al., 2004) etc owing to their unique properties as compared to bulk materials. In order to make practical use of their unique properties, nanoparticles with different sizes, shapes and compositions need to be synthesized. The various chemical and physical methods used for their synthesis suffer from various limitations such as they are mostly toxic and bioincompatible, unstable, uncontrolled crystal growth and aggregation of the nanoparticles. To overcome these limitations, in recent decade attention towards the synthesis of nanoparticles using biological materials is gaining importance as biological methods comprises clean, nontoxic, and environmental friendly procedures for the synthesis and assembly of nanoparticles. Biological method utilizes nature's most efficient machines i.e. living cells for the synthesis.

Amongst other semiconductor nanoparticles, PbS nanoparticles possess unique electrical and optical properties. They exhibit quantum confinement below a certain size threshold called, quantum size effect—that allows their optical and electrical properties to be precisely tuned with size.They are optically active in the near-infrared (NIR) region of the electromagnetic spectrum. They have variety of photonic applications, in single- and multi-junction solar cells (Koleilat et al., 2008, El- Sayed *et al.*, 2004, Kumar *et al.*, 2007), NIR photodetectors for telecommunications (Konstantatos *et al.*, 2006), and NIR light-emitting diodes (LEDs) (Konstantatos *et al.*, 2005).

The chemical, physical and fungal synthesis of lead nanoparticles have been reported much in literature but there is not much work available on bacterial synthesis of lead sulfide nanoparticles. Several researchers have reported the physical and chemical synthesis of lead sulphide nanoparticles**. (**Xusheng *et al.*, 2005) synthesized thiol-capped water-soluble PbS nanocrystals (NCs) stabilized with 1-thioglycerol. (Hassan Karami *et al.*, 2009) synthesized lead dioxide nanoparticles by pulsed current electrochemical method. (Jun-Jie Zhu *et al.*, 2002)

synthesized monodispersed lead selenide nanoparticles through sonochemical route. (Yu Zhaoa *et al*., 2004) synthesized lead sulfide nanocrystals by two novel routes in microwave heating and ultrasonic irradiation, (Junjie Zhu *et al.,* 2002) also synthesized HgS and PbS nanoparticles of about 15 and 20 nm in size by sonochemical irradiation of ethylenediamine solution of elemental sulphur and mercury acetate or lead acetate under air. (Mandeep Singh Bakshi *et al.,* 2007) performed aqueous phase synthesis of lead sulfide (PbS) nanocrystals (NC) at 80˚C by using cationic twin-tail surfactants (TTS) as capping agents in the concentration range from 0.1- to 2 mM. (Sachin Seshadri *et al.,* 2011) reported the fungal synthesis of nanoparticles using *Rhodosporidium diobovatum.* (Anuradha Prakash *et al*., 2010) synthesized extracellular metal nanoparticles (NPs) viz silver, lead and cadmium by using *Bacillus megaterium* strain isolated from fluvial zone of North Bihar.

Since there is relative dearth of work on the synthesis of PbS nanoparticles using bacteria, this paper describes the isolation and use of bacterial strains for the synthesis of PbS nanoparticles along with their characterization.

2. MATERIALS AND METHOD

All the chemicals used in this study were of analytical grade. Nutrient agar was purchased from Hi- media. Nutrient Broth and Luria Broth were procured from Titan Biotech limited Rajasthan. Calcium Sulphate and Lead Chloride were purchased from Sd Fine Chemical Limited Mumbai.

2.1. Collection of Soil Samples To Isolate Lead Resistant Bacteria.

Soil samples were collected from heavy metal waste rich soils of Allahabad and Kanpur in sterilized polythene bags. The industrial sites of SAIL (Steel Authority of India Limited) Allahabad and SAIL Kanpur are expected to be rich in heavy metal waste and therefore these sites were selected for collection of soil samples.

For the isolation of lead resistant bacteria lead chloride salt of 0.5mM was used as lead source in the medium. Collected soil samples were serially diluted and nutrient agar plates of different soil dilutions were prepared.

Plates with 0.5mM lead chloride were taken as test and simple nutrient agar plates of same dilutions as control. All plates were made in duplicate and were incubated overnight at 37ºC in an incubator for 24 hrs. Bacterial colonies were characterized on the basis of colony size, shape, colour, edge, texture, count and elevation, Minimum inhibitory concentration (MIC) test. Pure cultures for all were obtained and preserved in slants.

2.2 Determination of Minimum Inhibitory Concentration (MIC) of Different Cultures

MIC of all the cultures obtained in section 2.1 was determined using microtiter well plate method. In each well 100 µl sample was added. For this a stock solution of 20mM was prepared and from this different concentrations of lead chloride ranging from 0.4mM to 5.6mM was added in each well in duplicate along with a constant volume (10µl) of culture broth and remaining volume makeup was done by using plain broth. Only culture broth without lead chloride was used as control. Plate was incubated for 24 hrs at 37 ºC and absorbance was taken using Tecan ELISA reader at 595 nm. The MIC was taken as that concentration of lead chloride from where a dip in the absorbance (indicative of retardation of bacterial growth) was observed. The bacterial strains showing high tolerance to lead chloride selected using MIC values.

2.3 Preparation of Seed Culture

A loopful of inoculum from the slant was inoculated into the Luria Broth (LB) followed by incubation at 37 ºC and 120 rpm. Twenty-four hour grown culture having OD ˜1.0 at 660 nm using Zenith double beam UV visible spectrophotometer was used as seed culture.

2.4 Synthesis and Characterization of Lead Sulphide Nanoparticles

1.5 lt of LB media was inoculated with 1% seed culture and incubated at 37 ºC with constant shaking at 120 rpm in orbital rotary shaker for 3-4 days to keep the cells in log phase. The bacterial biomass was then harvested from the medium by centrifugation using Remi centrifuge at 7000 rpm for 10 min. The supernatant was

separated and kept for further use. The cell pellet was washed twice with autoclaved double distilled water and divided into two equal parts by weight.

a) To one part of cell pellet, 50 ml solutions of each 1 mM of lead chloride and 1 mM sterilized calcium sulphate in distilled water was added for synthesis of PbS nanoparticles. To remaining part of the cell pellet, 100 ml of distilled water was added and used as control.

b) Similarly 50 ml of supernatant kept above was mixed with 25 ml of 1 mM lead chloride and 25 ml of mM calcium sulphate salt solution. Control for supernatant was prepared without adding salt solutions to it. As lead sulphate is very sparingly soluble in water and hence cannot be used for the synthesis of PbS nanoparticles therefore calcium sulphate was added to provide sulphur ions.

All the flasks were incubated at 37°C for 72 h. A change in the colour of the content of the flasks containing the lead salts gives a preliminary indication of nanoparticles synthesis. The biomass or residue was separated from each flask by centrifugation at 7000 rpm and the supernatant was collected for further characterization. The solution containing lead sulfide nanoparticles were characterized using UV– Vis spectrophotometer and Transmission Electron Microscope (TEM) to confirm the synthesis of PbS nanoparticles.

3. RESULTS AND DISCUSSION

3.1 Isolation of lead resistant bacteria

Bacterial colonies were grown on the nutrient agar medium containing serially diluted soil samples were analysed on the basis of their morphology for different collection sites. Table 1-2, compares the morphological characteristics of bacterial colonies obtained in the control and test plates of different dilutions of soil samples collected from SAIL Allahabad and SAIL Kanpur. On the basis of this comparison eight morphologically different colonies were selected

Table 1: ***Comparison of Morphological Characters of Bacterial Colonies Grown at Various Dilutions of Soil Samples from SAIL Kanpur***

S.No	Soil Used	Dilution	Count	Shape	Size	Colour	Edge	Elevation	Surface Texture
1.	10^{-3}	Control	307 27 4	Round Round Round	Small Large Small	Yellow White Pale yellow	Entire Entire Undulate	Convex Raised Raised	Smooth Smooth Smooth
		Lead Chloride	138 3 19	Round Irregular Round	Small Large Small	**Yellow (NS4)** **Pale yellow(NS7)** White	Entire Undulate Entire	Convex Raised Raised	Smooth Smooth Smooth
2.	10^{-4}	control	160 20	Round Round	Small Large	Yellow White	Entire Entire	Convex Raised	Smooth Smooth
		lead chloride	59 16	Round Round	Small Small	Yellow **White (NS3)**	Entire Entire	Convex Raised	Smooth Smooth
3.	10^{-5}	control	31 1 2 22	Round Round Irregular Round	Small Small Large Large	Dark Yellow Yellow Pale yellow White	Entire Entire Undulate Entire	Convex Convex Raised Raised	Smooth Smooth Smooth Smooth
		lead chloride	118	Round	Small	**Dark Yellow(NS1)**	Entire	Convex	Smooth

Table 2: ***Comparison of Morphological Characters of Bacterial Colonies Grown at Various Dilutions of Soil Samples from SAIL Allahabad***

S.No	Soil Used	Dilution	Count	Shape	Size	Colour	Edge	Elevation	Surface Texture
1.	10^{-3}	Control	40 121 38 41	Round Irregular Round Round	Small Large Small White	Yellow Pale Yellow Lemon Yellow White	Entire Undulate Entire Entire	Convex Raised Convex Raised	Smooth Smooth Smooth Smooth
		Lead Chloride	2 28 15 24	Round Irregular Round Round	Small Large Small Small	Yellow **Pale yellow(NS7)** **Lemon yellow(NS2)** **White(NS8)**	Entire Undulate Entire Entire	Convex Raised Convex Raised	Smooth Smooth Smooth Smooth
2.	10^{-4}	Control	Very densely distributed	Lawn	Large	Yellow	Entire	Convex	Smooth
		Lead Chloride	27 18	Round Round	Small Small	**Dark Yellow(NS5)** White	Lobate Entire	Convex Raised	Smooth Smooth
3.	10^{-5}	Control	158 30 5	Round Round Round	Large Large Large	Yellow Lemon Yellow Pale yellow	Entire Entire Undulate	Raised Convex Raised	Smooth Smooth Smooth
		Lead Chloride	29 3 28	Round Round Round	Large Large Small	Yellow **Lemon yellow (NS2)** **Orange(NS6)**	Entire Entire Entire	Raised Convex Convex	Smooth Smooth Smooth

On the basis of differences in the morphology eight different colonies were selected from the two sites and were used for obtaining pure cultures by repeated streaking on nutrient agar plates as shown by figure 1(a-b). These bacteria were named accordingly as NS1- NS8.

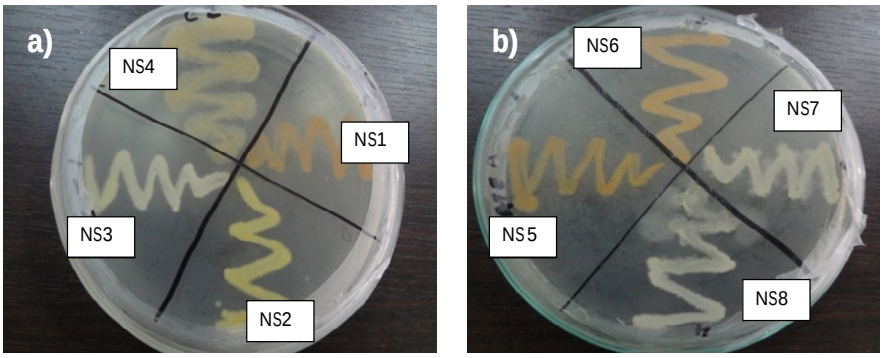


Figure 1 (a-b): *Pure culture of selected bacterial colonies grown using lead chloride in the medium using soil samples collected from different sites of Allahabad and Kanpur*

3.2. Minimum Inhibitory Concentration values of lead chloride for isolated cultures

MIC was calculated for all the isolated bacterial colonies as shown in figure 1 (a-b) using microtiter plate method. On the basis of the MIC results strains with higher metal tolerance were used further for nanoparticle synthesis. Figure 2 shows the MIC result of all isolated bacterial strains each bar of the graph corresponds to the MIC value of the respective bacterial strain. Strain NS2 and Strain NS6 were chosen for lead sulphide nanoparticle synthesis as they show high metal tolerance as per the MIC results in figure 2.

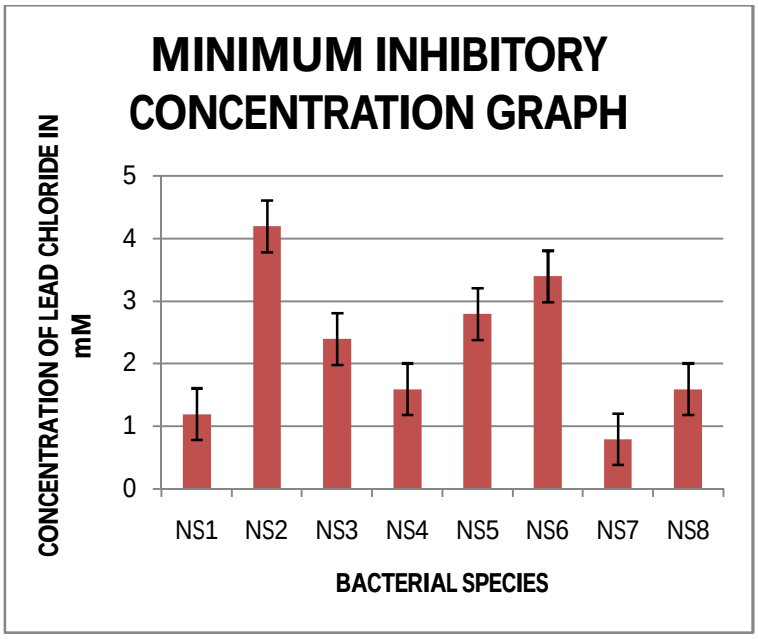


Figure 2: MIC graph showing the minimum concentration of metal tolerance by the bacterial species. Points on the graph indicate the MIC value for each bacterial species

3.3. Synthesis of Lead Sulphide Nanoparticles

For synthesis of lead sulphide nanoparticles the LB media inoculated with the seed culture was centrifuged to separate cell pellet and supernatant. Both bacterial cell pellet and supernatant were used for nanoparticle synthesis as discussed in section 2.2.

After 72 hrs of incubation a deep change in colour was observed in the flask containing lead chloride salt in supernatant while there was no change in colour in the flask having cell pellet and lead chloride as depicted in Figure 3 (a-b) and 4 (a-b). This primarily indicated that nanoparticle synthesis took place in the supernatant and not in the cell pellet.

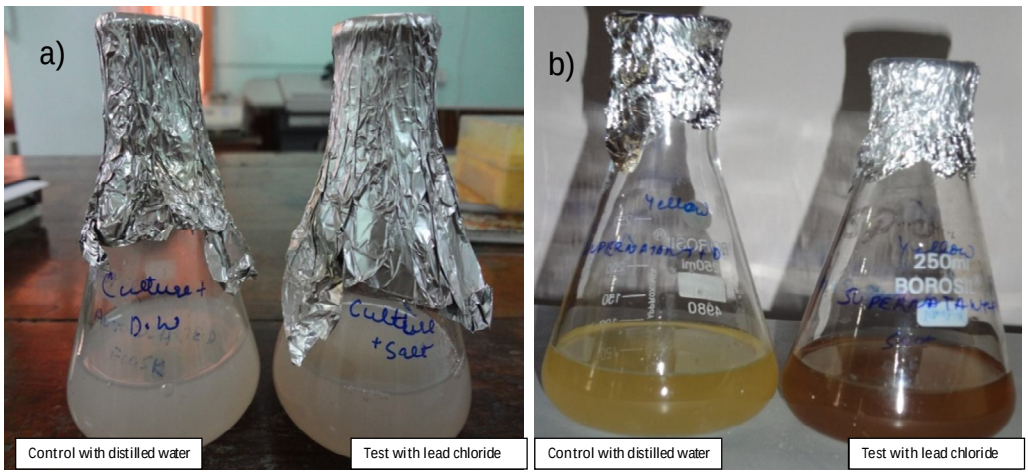


Figure 3 (a-b): *nanoparticle synthesis using bacterial strain NS2 a) flask having cell pellet, no change in colour between control and test b) flask containing supernatant, a change in colour was observed in the test flask and not in the control*

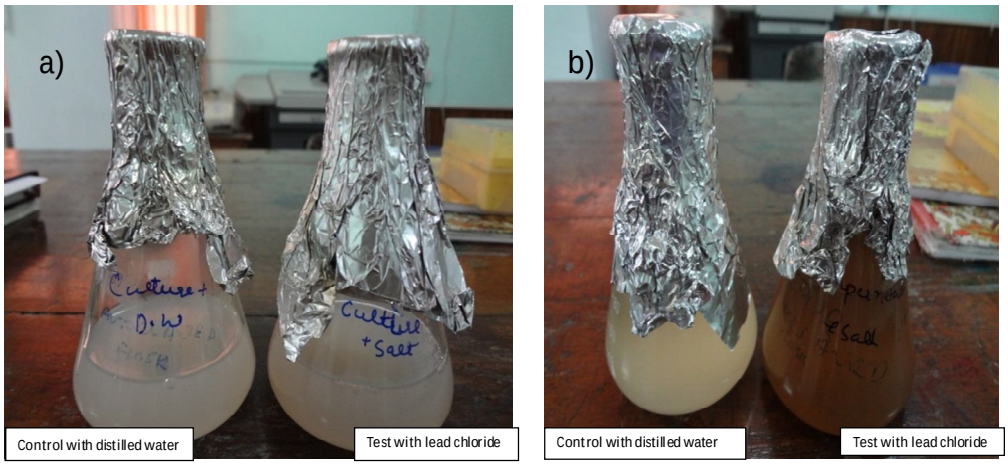


Figure 4 (a-b): *nanoparticle synthesis using bacterial strain NS6 a) flask having cell pellet, no change in colour between control and test b) flask containing supernatant, a change colour was observed in the test flask and not in the control*

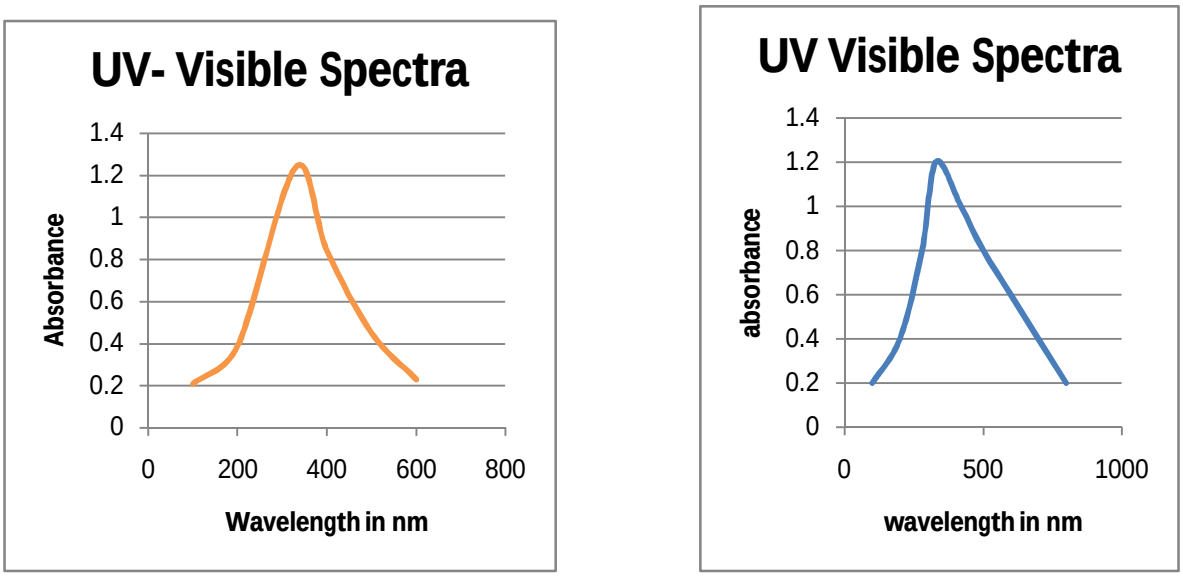
The solution showing nanoparticle synthesis for bacterial strain NS2 and NS6 figure 3(b) and figure 4(b) were centrifuged and their supernatant was used for further characterization and to confirm the synthesis of nanoparticles.

3.4. Characterization of Nanoparticles

The supernatant containing lead sulfide nanoparticles were analyzed by UV– Visible spectroscopy and Transmission Electron Microscope.

3.4.1 UV-Visible Spectrophotometer

The synthesised nanoparticle solution was scanned from 200-700 nm using Zenith double beam UV-Visible spectrophotometer. A sharp peak at 330 nm, characteristic of lead sulphide nanoparticles was observed in the supernatant solutions of both the bacterial strains indicating the synthesis of lead sulphide nanoparticles. Figure 5 (a-b) shows the UV visible graph of supernatant solutions of bacterial strain NS2 and NS6 respectively.

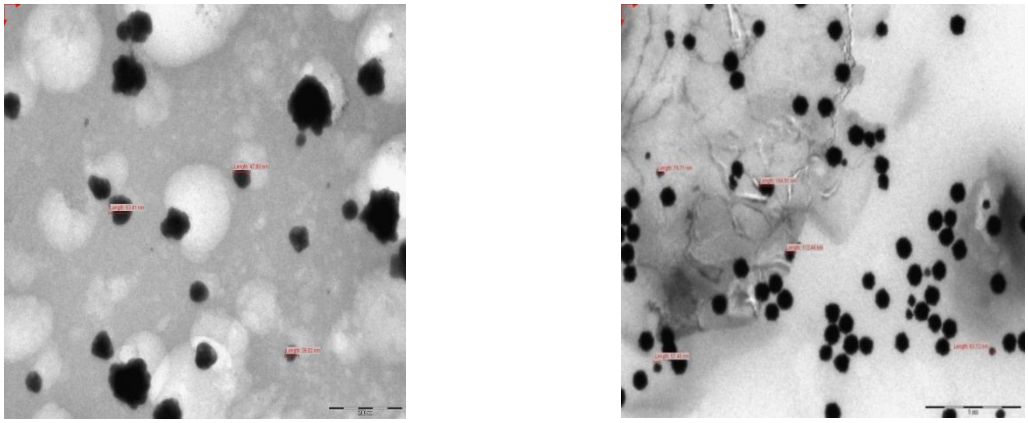


a) b)

Figure 5 (a-b): UV-Vis absorbance Maxima of PbS nanoparticles synthesized by a) bacterial strain NS2 and b) bacterial strain NS6. Peak at 330 nm indicates the PbS nanoparticle synthesis.

3.4.2. Transmission Electron Microscopy

High Resolution Transmission Electron Microscopy (HRTEM) was performed by JEOL JEM 2010 FEF (UHR) electron microscope with an accelerating voltage of 200 kV. Figure 6 (a-b) shows the TEM images formed by supernatant of bacteria NS2 and NS6.



(a) (b)

Figure 6 (a-b) *Transmission electron microscope image of supernatant sample of bacteria a) NS2 particle size ranging between 40- 70 nm were formed b) NS6 particle size ranging between 60- 100 nm were formed*

4. CONCLUSION AND FUTURE PROSPECTS

This study reported the extracellular synthesis of lead sulphide nanoparticles synthesis by two bacterial strains i.e. strain NS2 and NS6 isolated from the heavy metal rich soil samples of Allahabad and Kanpur. The supernatant of these two bacterial species NS2 and NS6 was used for nanoparticle synthesis which shows that the enzymes involved in metal ion reduction are present in the supernatant and not in the cell mass. The UV-Vis spectra of the syntheisized nanoparticles showed a sharp peak at 330 nm which is characteristic for the lead nanoparticles. Further, TEM of the solution confirmed the synthesis of nanoparticles of 40- 70 nm and 60- 120 nm. The nanoparticles formed were extracellular and it is commercially beneficial because steps of intracellular recovery of nanoparticles are eliminated. The biologically synthesized lead nanoparticles may find various applications like Near Infrared Region imaging, optoelectronics, biosensors, semiconductor devices etc. The most important advantage of this study is that the toxic heavy metal lead is been bio-remediated in the form of nanoparticles serving the dual purpose of nanoparticle synthesis and bioremediation without producing any toxic chemicals to environment. These isolated strains can be thoroughly characterized further because they are important as environmental point of view and could be used on large scale for bioremediating lead or synthesis ofnanoparticles.

ACKNOWLEDGEMENT

We gratefully acknowledge Department of Biotechnology, MNNIT Allahabad for support of this work.

5. REFERENCES

1. Anuradha Prakash, Seema Sharma, Naheed Ahmad, Ashok Ghosh and Preety Sinha. **2010**. Bacteria mediated extracellular synthesis of metallic nanoparticles. *International Research Journal of Biotechnology*. 6: 71-79.
2. Chan, W. C. W.; Nie S. M. **1998**. Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection. *Science*. 281: 2016-2018.
3. El-Sayed. **2004**. Small Is Different: Shape-, Size-, and Composition-Dependent Properties of Some Colloidal Semiconductor Nanocrystals . *Acc. Chem. Res*. 37: 326-333.
4. Hagfeldt, A., Graetzel. **2000**. Molecular Photovoltaics. *Acc. Chem. Res*. 33: 269-277.

5. Hassan Karami, Mahboobeh Alipour. **2009**. Synthesis of Lead Dioxide Nanoparticles by the Pulsed Current Electrochemical Method. *International journal of Electrochemical science*. 4: 352-360.
6. Jackson, J. B., Westcott, S. L., Hirsch, L. R., West, J. L., Halas, N. J. **2003**. Controlling the surface enhanced Raman effect via the nanoshell geometry. *Appl. Phys. Lett*. 82: 257-262.
7. Jun-Jie Zhu, Hui Wang, Shu Xu, and Hong-Yuan Chen. **2002**. Sonochemical Method for the Preparation of Monodisperse Spherical and Rectangular Lead Selenide Nanoparticles. *Appl. Phys. Lett*. 86: 274-280.
8. Junjie Zhu, Suwen Liu, O. Palchik, Yuri Koltypin, and A. Gedanken. **2004**. A Novel Sonochemical Method for the Preparation of Nanophasic Sulfides: Synthesis of HgS and PbS Nanoparticles. *Int. J. Nanosci. Nanotech.* 65: 234-241.
9. Koleilat GI, Levina L, et al. **2008.** Efficient, stable infrared photovoltaics based on solution-cast colloidal quantum dots. *ACS Nano*. 2:833–40.
10. Konstantatos G, Howard I. **2006**. Ultrasensitive solution-cast quantum dot photodetectors. *Nature*. 442: 7099-8005.
11. Konstantatos G, Huang C, Levina L, Lu Z, Sargent EH. **2005**. Efficient Infrared Electroluminescent Devices Using Solution-Processed Colloidal Quantum Dots. *Advanced Functional Materials. Nature*. 441: 6003- 6009.
12. Kumar S., Marcus Jones., Shun S. Lo, Gregory D. Scholes. **2007.** Nanorod Heterostructures Showing Photoinduced Charge Separation. *Acc Chem Res.* 9: 1633-1639.
13. Langer, R. **2003**. Where a Pill Won't Reach. *Sci. Am.* 288: 50-57.
14. Li X., Yuanqing He, and Mark T. Swihart. **2004**. Surface Functionalization of Silicon Nanoparticles Produced by Laser-Driven Pyrolysis of Silane followed by HF−HNO_3 Etching. *Langmuir*. 20: 4720-4727.
15. Mandeep Singh Bakshi, Pankaj Thakur, Shweta Sachar, Gurpreet Kaur, Tarlok Singh Banipal, Fred Possmayer, and Nils O. Petersen. **2007**. Aqueous Phase Surfactant Selective Shape Controlled Synthesis of Lead Sulfide Nanocrystals. *J. Nano Lett.* 3: 988- 993.
16. Moran, C. E., Steele, J. M., Halas, N. J. **2004.** Chemical and dielectric manipulation of plasmonic band gap of metallodielectric arrays. *Nano Lett*. 4: 1497-1500.
17. Niemeyer, C. M., **2001**. Nanoparticles, Proteins, and Nucleic Acids: Biotechnology Meets Materials Science. *Angew. Chem. Int. Ed.* 40: 4128-4158.
18. Parak, W. J., Pellegrino, T., Plank. **2005**. Labelling of cells with quantum dots. *Nanotechnology*. 16:1248- 1256.
19. Rosi, N. L., Mirkin, C. **2005**. Nanostructures in biodiagnostics. *A. Chem. Rev*. 105: 1547-1562.
20. Roucoux, A., Schulz, J., Patin. **2002**. Reduced transition metal colloids: a novel family of reusable catalysts. *H. Chem. Rev*. 102: 3757- 3763.
21. Sachin Seshadri, K. Saranya, Meenal Kowshik. **2011**. Green synthesis of lead sulfide nanoparticles by the lead resistant marine yeast, *Rhodosporidium diobovatum. ACS Publications*. 21:1086–1090.
22. Xusheng Zhao, Ivan Gorelikov, Sergei Musikhin, Sam Cauchi, Vlad Sukhovatkin, Edward H. Sargent, and Eugenia Kumacheva. **2005.** Synthesis and Optical Properties of Thiol-Stabilized PbS Nanocrystals. *ACS publications.*21 : 1086–1090.
23. Yu Zhaoa, Xue-Hong Liao b, Jian-Min Honga, Jun-Jie Zhua. **2004**. Synthesis of lead sulfide nanocrystals via microwave and sonochemical methods. *J. E. Chem. Matter*. 22: 6250- 6257.