INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-3: July-Sept -2011 IJABPT *ISSN 0976-4550*

A NOVEL APPROACH FOR THE MICROBIAL PRODUCTION AND OPTIMIZATION OF GOLD NANOPARTICLES- AN ALTERNATE APPROACH TO CHEMICAL SYNTHESIS

S.Venkatesan[1], S.Karthiga[1], M.Kirithika[1], R.Ganesan[2] and K.Muthuchelian[3]

[1]Research Department of Microbiology, VHNSN College, Virudhunagar, Tamil Nadu, India.
[2]Department of Microbiology, P.K.N College, Thirumangalam, Tamil Nadu, India.
[3]Department of Energy Sciences, MKU, Madurai, Tamil Nadu, India.

ABSTRACT : Nanotechnology is the application of science to control matter at the molecular level. A nanometer (nm) is one billionth of a meter, or roughly the length of three atoms side by side. A nanoparticle is a microscopic particle with atleast one dimension less than 100 nm. The applilcation of nanoscale materials and structures, usually ranging from 1 to 100 nanometer are an emerging area of nanoscience and nanotechnology. Gold nanoparticles, in particular, are of interest, due to their stability under atmospheric conditions, resistance to oxidation and biocompatibility. Microorganisms as possible eco–friendly nanofactories can exert control over size, morphology, composition and crystallographic orientation of the as–prepared metal nanostructured particles. In the area of nanotechnology, the development of techniques for the controlled synthesis of metal nanoparticles of well defined size, shape and composition is a big challenge. This work was highly focused on the Gold nanoparticles production from *Pseudomonas* sp. and concentrating the work towards the production at controlled size by means of various parameter optimization studies like pH, temperature and concentration. The optimization work was characterized by means of TEM. With the results of TEM, the small sized nanoparticle of about 10nm was observed at pH of 9 and 12nm was produced at the pH of 3. Low temperature of 25ºC favored the small size nanoparticle at a concentration of 250 mg/l using $HAuCl_4$.

Key words: Metal nanoparticles, TEM, $HAuCl_4$, Optimization, *Pseudomonas* sp.

INTRODUCTION

Nanotechnology is the application of science to control matter at the molecular level. It has been well known that living cells are the best examples of machines that operate at the nano level and perform a number of jobs ranging from generation of energy to extraction of targeted materials at very high efficiency (Good well, 2004). The term nano is adapted from the Greek word meaning "Dwarf" when used as a prefix, it implies 10^{-9}. A nanometer (nm) is one billionth of a meter, or roughly the length of three atoms side by side. A nanoparticle is a microscopic particle with atleast one dimension less than 100 nm (Duan *et al*., 1999). Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. The applilcation of nanoscale materials and structures, usually ranging from 1 to 100 nanometer is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment (Dahl *et al.,* 2007).

Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macroscale counterparts. Synthesis of noble metal nanoparticles is an area of constant interest. Gold, silver and copper have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in the areas such as photography, catalysis, biological labelling, photonics, optoelectronics and Surface–Enhanced Raman Scattering (SERS) detection (Hussian *et al*., 2003). Gold nanoparticles, in particular, are of interest, due to their stability under atmospheric conditions, resistance to oxidation and biocompatibility (Corti *et al.,* 2004 and Hung *et al.,* 2005).

Additionally, metal nanoparticles have a surface plasmon resonance absorption in the UV–Visible region. The surface plasmon band arises from the coherent existence of free electrons in the conduction band due to the small particle size. The band shift is dependent on the particle size, chemical surrounding, absorbed species on the surface and dielectric constant. A unique characteristic of these synthesized metal particles is that a change in the absorbance or wavelength gives a measure of the particle size, shape and interparticle properties. Moreover, functionalized, biocompatible and inert nanomaterials have potential applications in cancer diagnosis and therapy. The target delivery of anticancer drugs has been done using nanomaterials with the use of fluorescent and magnetic nanocrystals, the detection and monitoring of tumor biomarkers have been demonstrated (Mulvaney *et al.,* 1996).

In the area of nanotechnology, the development of techniques for the controlled synthesis of metal nanoparticles of well defined size, shape and composition is a big challenge. If you keep cutting until the gold pieces are in the nanoscale range, they don't look gold anymore. They look RED! In fact, depending on size, they can turn red, blue, yellow, and other colors. Different thicknesses of materials reflect and absorb light differently. Generally, metal nanoparticles can be stabilized by physical and chemical methods; the chemical approach such as chemical reduction, electrochemical techniques, and petrochemical reduction is most widely used (Sengupta *et al.,* 2005). A typical procedure involves growing nanoparticles in a liquid medium containing various reactants, in particular reducing agents eg. sodium borohydride (Kim *et al*., 2007) or potassium bitartrate (Tan *et al.,* 2003) or methoxy polyethylene glycol (Mallick *et al*., 2004) or hydrazine (Li *et al.,* 1999). To prevent the agglomeration of metallic nanoparticles, a stabilizing agent such as sodium dodecyl benzyl sulfate (Li *et al*., 1999) or polyvinyl pyrrolidone (Tan *et al*., 2003) is also added to the reaction mixture generally the chemical methods are low–cost for high volume; however, their drawbacks include contamination from precursor chemicals, use of toxic solvents, and generation of hazardous by–products.

The physical methods of synthesizing metallic nanoparticles include attrition, and pyrolysis. In attrition, macroscale or microscale particles are ground by a size– reducing mechanism. The resulting particles are subsequently air classified to recover oxidized nanoparticles. In pyrolysis, an organic precursor is forced through an orifice at high pressure and burned. The resulting ash is air classified to recover oxidized nanoparticles (Chen *et al*., 2003). Studies have shown that the size, morphology, stability and properties (chemical and physical) of metal nanoparticles are strongly influenced by the experimental conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of stabilizing agent with metal nanoparticles. Hence, the design of a synthesis method in which the size, morphology, stability and properties are controlled has become a major field of interest (Wiley *et al*., 2007). Notably, the production rate of a forementioned "physical" procedures for achieving synthesis of metallic nanoparticles is quite low, and importantly, the expense is very high (Li *et al*., 1999). A further drawback of "physical" approaches is enormous consumption of energy to maintain the high pressure and temperature used in the synthesis procedures. In contrast, most bioprocesses occur under normal air pressure and temperature, resulting in vast energy savings (Chen *et al*., 2003).

There is an increasing need to develop high–yield, low–cost, non toxic and environmentally benign procedures for synthesis of metallic nanoparticles. Therefore, the biological approach for synthesis of nanoparticles becomes important. A vast array of biological resources available in nature including plants and plant products, algae, fungi, yeast, bacteria and viruses could all be employed for synthesis of nanoparticles. Microorganisms as possible eco–friendly nanofactories can exert control over size, morphology, composition and crystallographic orientation of the as–prepared metal nanostructured particles (Shankar *et al*., 2004). It is well known that biological systems can provide many examples of specifically tailored nanostructures with highly optimized properties and characteristics including magnetotactic bacteria synthesizing intracellularly magnetic nanocrystals in magnetosomes, diatom cell walls regarded as a paradigm for controlled production of nanostructured silica, bacterial S –layer as templates for the formation of regularly arranged nanoparticles and nanobacteria also known as calcifying nanoparticles (Pickson *et al*., 1999) the synthesis of gold nanoparticles with various size and shapes in the nanometer range by fungal species such as *Verticillium* and *Fusarium* (Mukherjee *et al.,* 2001 and Ahmad *et al*., 2003), the production of silver nanoparticles within the periplasmic space of *Pseudomonas stutzeri* (Klaus *et al*., 1999), synthesis of nanoscale, semiconducting CdS crystals in the yeast *Schizosaccharomyces pombe* (Kowshik *et al.,* 2003) and the formation of palladium nanoparticles using sulphate reducing bacteria in the presence of an exogenous electron donor (Yong *et al.,* 2002).

The ability of bacteria, fungi, actinomycetes (Nakajima *et al.,* 2003), yeast (Korobushkina *et al.,* 1989), algae (Kuyucak *et al*., 1989) and plants (Armendariz *et al.,* 2004) to accumulate gold ions from solution has been reported and the synthesis of gold nanoparticles has been successfully demonstrated in a range of organisms including *Bacillus* sp. fungal species such as *Verticillium* and *Fusarium* (Mukherjee *et al.,* 2001 and Ahmad *et al.,* 2003), actinomycete such as *Rhodococcus* and *Thermomonospora* (Ahmad *et al*., 2003) and lactic acid bacteria (Nair *et al*., 2002). The interest also extends to the synthesis of nanostructures such as nanowires and the assembly of nanoparticles using biological templates such as DNA, proteins, S-layers and viruses (Wahl *et al*., 2001 and Blum *et al*., 2004).

MATERIALS AND METHODS

Sample collection

The soil sample was collected from V.H.N.S.N. College campus. The soil sample was serially diluted and it was plated on nutrient agar plates and incubated for 24h. Individual colonies were picked and streaked on Pseudomonas isolation agar plates. The individual colonies were further purified by sub–culturing on agar slants.

Identification of Bacteria

In order to identify the microorganism the culture was subjected to various biochemical tests and Gram staining.

Screening of cultures for Gold Accumulation and Nanoparticle synthesis

Culture was grown up in conical flasks containing 10ml nutrient broth in shaker incubators at 28^{o}C. After 24-48h. incubation the biomass was separated from the medium by centrifugation at 7500 rpm for 10 min and washed three times in sterile distilled water to remove any nutrient media that might interact with gold ions.

The biomass was resuspended in 10 ml distilled water and the pH was adjusted between 5 and 6 with 0.2M NaOH. $AuCl_4$ was added to give an overall Au-concentration of 250 mg/l. The accumulation and reduction of gold were followed by visual observation of the biomass turning purple, an indication of the formation of gold nanoparticles (Mariekie Gericke, 2006).

Effect of Growth parameters on nanopartilcle production

Time

The organism was inoculated and incubated at 28ºC for 24, 48 and 72h. After the incubation, the biomass was separated from the medium and it separated from the medium by centrifugation at 7500 rpm for 10min and the pH was adjusted between 5 and 6. $HAuCl_4$ was added to give an overall Au concentration of 250 mg/1. The samples were incubated at 28ºC for 24h. The accumulation and the reduction of gold were followed by visual observation of the biomass turning purple (Mariekie Gericke, 2006).

pH

The organism was inoculated in the nutrient broth and incubated at 28ºC for 24h. After the incubation, the biomass was separated from the medium by centrifugation of 7500 rpm for 10min and washed three times in sterile distilled water.

The effect of pH on nanoparticle formation was evaluated by resuspending 100 mg biomass in 10 ml distilled water and adjusting the pH to 3, 5, 7 and 9 respectively. $HAuCl_4$ was added to give an overall Au concentration of 250 mg/1. The sample was incubated at 28ºC for a further incubation of 24h. The accumulation and reduction of gold were followed by visual observation of the biomass turning purple (Mariekie Gericke, 2006).

Temperature

The organism was inoculated in the nutrient broth and incubated at 28ºC for 24h. After incubation, the biomass was separated by centrifugation at 7500 rpm for 10 min. The effect of temperature on nanoparticle formation was evaluated by re suspending 100 mg of biomass in 10ml distilled water and adjust the pH between 5 to 6. $HAuCl_4$ was added to give an overall Au concentration of 250 mg/l. The samples were incubated at 25ºC, 35ºC and 50ºC for a further 24h. The accumulation and reduction of gold were followed by visual observation of the biomass turning purple (Mariekie Gericke, 2006).

Concentration

Culture was grown up as described above. After 24h. incubation, the biomass was separated from the medium by centrifugation at 7500 rpm for 10min and washed three times in sterile distilled water and adjusting the pH between 5 to 6. Biomass exposed to gold concentration of 250, 500 and 2500 mg/l were used to determine the effect of varying the $AuCl_4$ concentration in the medium on nanoparticle formation. There cell suspensions were incubated at 28ºC (Mariekie Gericke, 2006).

Antibacterial activity of Gold nanoparticle

The gold nanoparticle was tested for their antibacterial activity by the agar diffusion method. Muller–Hinton agar plates were prepared. The inoculum suspension of *Pseudomonas, Vibrio parahaemolyticus, Serratia, Escherichia coli, Salmonella, Bacillus, Shigella, Staphylococcus aureus, Streptococcus* were swabbed uniformly in different plates.

Cavities were made in each plate using a well-cutter and it was filled with gold nanoparticle solution (100µl) and then incubated at 37 ºC. The formation of a clear zone around the cavity is an indication of antibacterial activity (Cappucino, 2006).

Characterisation of the nanoparticles

Samples were prepared for TEM analysis by separating the biomass from the liquor by centrifugation and washed twice in sterile distilled water. The samples were fixed for 1 hour in 2.5% gluteraldehyde in 0.075 M phosphate buffer (pH7.4), followed by 3 washes in 0.075 M phosphate buffer. After a second fixation step of 1 h in 1% osmium tetroxide, the cells were washed in distilled water. The cell pellet was subjected to dehydration with 30, 50, 70% ethanol, followed by three dehydration steps in 100% ethanol. Infiltration of the resin was carried out by placing the pellet in 30% Quetol in ethanol for 1 hour, followed by 1 hour in 60% Quetol. After centrifugation, the pellet was resuspended in 100% Quetol for 4h before polymerisation at 65°C for 24 hours. Ultrathin sections were not stained prior to analysis, due to possible interference of the stain with the gold particles. TEM analyses were done on a Philips 301 transmission electron microscope.

For TEM analysis of the cell-free extract, a drop of the sample was placed onto a carbon-coated copper grid. After about a minute, the extra solution was removed using blotting paper and the grid air-dried before analysis.

RESULTS

The isolated colonies were observed on the Pseudomonas isolation agar plates (Plate 1). The colonies were analysed both microscopically and biochemically. When Gram stained, the isolated colonies showed pink colour, this indicated that the colonies were gram negative.

Pseudomonas sp. on Pseudomonas isolation agar plates

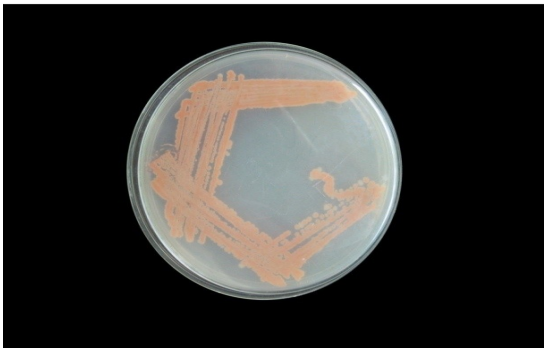

Plate 1: *Pseudomonas* sp. on Pseudomonas isolation agar

Biochemical Examination

When the colonies were inoculated in SIM broth, the tube showed no coloration on top of the medium. This indicated that it was indole negative.When inoculated and incubated the colonies in MR–VP medium, it showed no red coloration while the addition of methyl red indicator. This indicated it was confirmed that it was VP negative. Simmon's citrate agar changed to blue colour after the inoculation of medium in agar stants. This indicated that they were citrate positive. No coloration was observed in urea broth and this indicated that the organism was urease negative.

Brisk effervescence was observed when the culture was in contact with hydrogen peroxide. This showed that it was catalase positive. Oxidase disc got changed to purple was observed when the addition of culture on the disc, which indicated that it was oxidase positive. While testing the carbohydrate fermentation, the acid and gas production was not observed and the medium remained red after the incubation period. Nitrate reduction was occurred and it was identified when the culture was inoculated in nitrate broth and it was tested by adding α-naphthol and sulfanilic acid. When inoculated the colonies on gelatin stab, it liquefied the gelatin. It was confirmed that the organism was positive for gelatin hydrolysis.

From the above results, it was confirmed that the organism may be *Pseudomonas* and the result were tabulated in table 1.

Table 1. Microscopic and Biochemical Examination

S.No	Biochemical test	Result
1.	Gram staining	Gram negative
2.	Indole production test	Negative
3.	Methyl red test	Negative
4.	Voges–Proskauer test	Negative
5.	Citrate Utilization test	Positive
6.	Urease test	Negative
7.	Catalase test	Positive
8.	Oxidase test	Positive
9.	Carbohydrate Fermentation	Negative
10.	Nitrate reduction test	Positive
11.	Gelatin liquefaction	Positive

Screening of culture for gold accumulation and nanoparticle synthesis

The accumulation and reduction of gold were followed by the visual observation of the biomass turned to purple (Plate 2). This indicated that the isolated culture has the ability to produce gold nanoparticles.

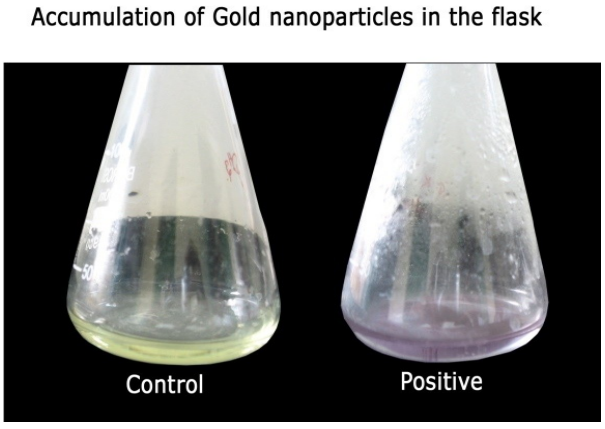

Plate 2: Screening of culture for gold accumulation and nanoparticle synthesis

Effect of growth parameters on nanoparticle production

Time

Gold nanoparticles production was tested with various time intervals such as 24h., 48h., and 72h. It was observed that biomass become purple even at the end of 24h. and also found that it was no effect on further incubation time. So 24h. was preferable for the production of gold nanoparticles. The result was tabulated in table 2.

Table 2. Time–Effect of Growth parameters on nanopartilce formation

S.No	Time	Result
1	24h.	Purple coloration
2	48h.	Purple coloration
3	72h.	Purple coloration

pH

Gold nanoparticle production was practiced with respect to various pH like 3, 5, 7 and 9. From the results of TEM, it was inferred that the low acidic and high alkaline pH produced the small nanosized particle of size 12nm. The result was shown in table 3 and plate 3-6.

Table 3. pH – Effect of growth parameter on Nanoparticle formation

S.No	pH	Result	Size (nm)
1	3	Red coloration	12
2	5	Purple coloration	200
3	7	Purple coloration	150
4	9	Red coloration	10

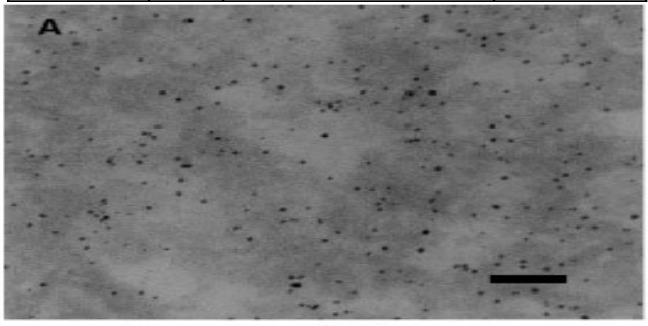

Plate 3: Effect of pH 3 on nanoparticle Production

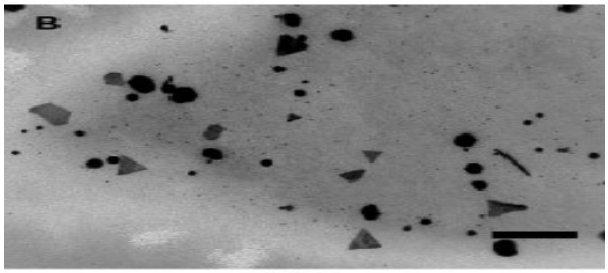

Plate 4: Effect of pH 5 on nanoparticle Production

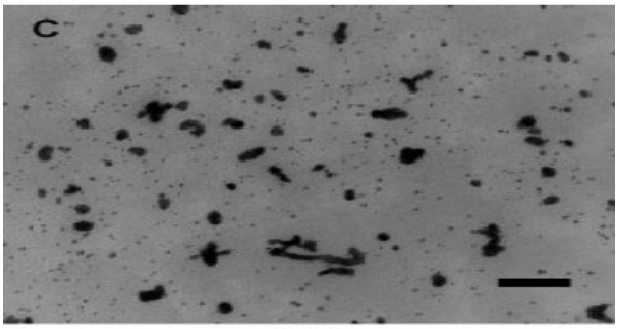

Plate 5: Effect of pH 7 on nanoparticle Production

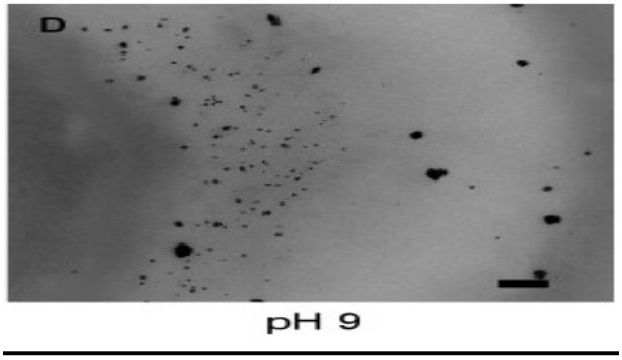

Plate 6: Effect of pH 9 on nanoparticle Production

Temperature

Various temperatures were set to determine the gold nanoparticle production such as 25ºC, 35ºC and 50ºC. It was found to be Red, Maroon, purple coloration respectively at 25ºC, 35ºC and 50ºC. From the results of TEM, it was also inferred that the small sized nanoparticle was better found at the temperature of 25ºC, 35ºC and then 50ºC. The production was well observed in 35ºC. The result was shown in table 4 and plate 7-9.

Table 4. Temperature–Effect of growth parameters on Nanoparticle formation

S.No	Temperature	Result
1	25ºC	Red coloration
2	35ºC	Maroon coloration
3	50ºC	Purple coloration

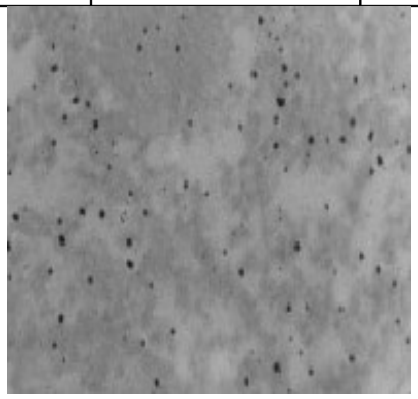


Plate 7: Effect of Temperature 25ºC on nanoparticle Production

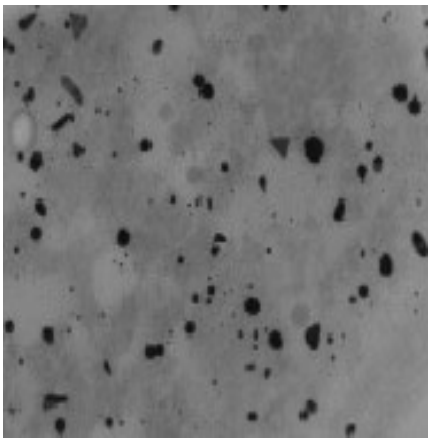


Plate 8: Effect of Temperature 35ºC on nanoparticle Production

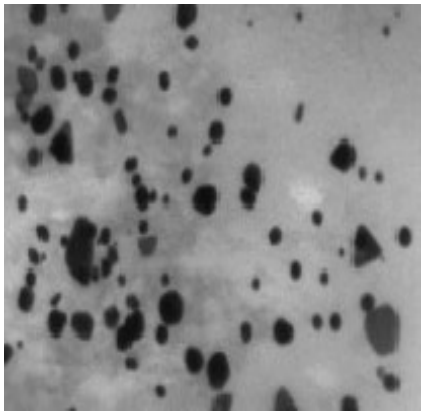


Plate 9: Effect of Temperature 50ºC on nanoparticle Production

Concentration

While increasing the concentration upto 2500 mg/l, there was no purple coloration of the biomass. But both the concentration of 250 and 500 mg/l favored the nanoparticle production. The result was tabulated in table 5.

Table 5. Concentration – Effect of Growth parameters on nanoparticle formation

S.No	Concentration (mg/ l)	Result
1	250	Purple coloration
2	500	Purple coloration
3	2500	No colour change

Antibacterial activity of gold nanoparticle

The gold nanoparticles were tested against various organisms and the observed result was tabulated in table 6. From the table, it was confirmed that gold nanoparticle has most of its activity against gram negative organism.

Table 6. Antibacterial activity of gold nanoparticle

S.No	Bacteria	Zone of inhibition (mm)
1	Pseudomonas	8
2	Vibrio parahaemolyticus	6
3	Serratia	5
4	E.coli	10
5	Salmonella	4
6	Bacillus	2
7	Shigella	4
8	Staphylococcus	4
9	Streptococcus	2

DISCUSSION

This study was intended to bring the alternate remedy for the production of gold nanoparticle by chemical means of synthesis and this study has got succeeded in the microbial production of gold nanoparticles.

The ability of bacteria, fungi, actinomycetes (Nakajima *et al*., 2003), yeast (Korobushkina *et al*., 1989), algae (Kuyucak *et al*., 1989) to accumulate gold ions from solution has been reported and the synthesis of gold nanoparticles has been successfully demonstrated using various microorganisms. I have done the synthesis of gold nanoparticle using the microorganism, *Pseudomonas* sp.

Shiying *et al*., 2007 showed the bacteria *Rhodopseudomonas capsulata* produces gold nanoparticles of different size and shape. He incubated *R.Capsulata* biomass into aqueous chlorauric acid, pH ranging from 4 to 7. They found that at pH 7, gold nanoparticles of nanosize level were formed. Husseiney *et al*., 2007 have performed a series of studies on biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa.*

There is an increasing need to develop high–yield, low–cost, non toxic and environmentally benign procedures for synthesis of metallic nanoparticles. Therefore, the biological approach for synthesis of nanoparticles becomes important. A vast array of biological resources available in nature including plants and plant products, algae, fungi, yeast, bacteria and viruses could all be employed for synthesis of nanoparticles. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment (Dahl *et al.,* 2007).

In the area of nanotechnology, the development of techniques for the controlled synthesis of metal nanoparticles of well defined size, shape and composition is a big challenge. The gold nanoparticle production was at the pH of 3, 5, 7 and 9.But small size observed at 3 and 9. The small sized gold nanoparticle production was good at the temperature of 25, then 35°C and 50°C. The gold nanoparticle production was well at the lowest concentration of 250 mg/L and 500 mg/L. The produced gold nanoparticle was tested for the antimicrobial activity and was found good for gram negative microorganisms.

REFERENCES

A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar, M. Sastry (2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum.* Colloids surf B. Vol. **28** 313 -8.

A. Ahmad, S. Senapati, M.I. Khan, R. Ramani, V. Srinivas, M. Sastry (2003). Intracellular synthesis of gold nanoparticles by a novel *Rhodococcus* sp. Vol. **14** 824-8.

A. Ahmad, S. Senapati, M.I. Khan, R. Ramani, M. Sastry (2003). Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora* sp. Langmuir. **19:** 3550 – 3.

P.T. Anstas, J.C. Warner (1988). Green chemistry. Theory and practice. Newyork.oxford university press. Inc.

V. Armendariz, I. Herrara, J.R. Peralta – videa, M. Jole – Yacaman, H. Troiani, P. Santiago, J.L. Gardea – Torresdey (2004). Size controlled gold nanoparticle formation by *Avena sativa* biomass: Use of plants in nanobiotechnology. J Nanoparticle Res. **6:** 377 – 82.

T.J. Beveridge, M.N. Hughes, H. Lee, K.T. Leuncy, R.K.Poole, I. Savvaids, S. Silver, J.T. Trevors (1997). Advances in Microbial physiology. Vol. **38** 177 – 243.

T.J. Beveridge, R.G.E. Murray RGE (1980). J Bacteriol. Vol.**141** 876.

A.S. Blum, M. Soto, C.D. Wilson, J.D. Cole, M. Kim, B. Gnade, A. Chatterji, W.F. Ochoa, T. Lin, J.E. Johnson, B.R. Ratna (2004). Nanoletters. Vol. **4** 867.

M. Brust, C.J. Kiely (2002). Colloids and surfaces A. Physicochemical and engineering Aspects. Vol. **202** 175 – 186.

G. Cappuccino and Natalie Sherman (1992). Microbiology A laboratory manual. Third Ed**.** 31 – 36, 127 – 178.

S.P. Chandran, M. Chaudhary , R. Pasricha, A. Ahmad, M. Sastry (2006). Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol Prog. Vol. **22** 577 – 83.

J.C. Chen, Z.H. Lin, X. Ma (2003). Evidence of the production of silver nanoparticles via pretreatment of *phoma* sp. 3. 2883 with silver nitrate. Lett.Appl.Microbiol. vol. **37** 105 – 8.

C.W. Costi, R.J. Holiday, P.T. Thompson (2002). Gold Bulletin**. Vol. 35** 111

R.A. Cross, B. Kalra (2002) Science. Vol. **297** 803.

J.M. Desimone (2002). Science. Vol. **297** 799.

H. Huang, X.Yang (2005). Colloids and surfaces A. Physicochemical and Engineering Aspects. Vol. **255** 11 – 17.

M.I. Husseiney, M. Abd El – Aziz, Y. Badr, M.A. Mahmoud (2007). Biosynthesis of gold nanoparticles using *pseudomonas aeruginosa.* Spectrochin Acta. Vol. **67** 1003– 6.

H. Jiale, L. Qingbiau, S. Daohua, L. Xinghua, S. Yuanbo, Y. Xin (2007). Biosynthesis of silver and gold nanoparticles by novel sun dried *Cinnamomum Camphora* leaf. Nano technology. Vol. **18** 105104 – 15.

X. Jianping, Y.L. Jim, I.C.Q. Daniel, P.I. Yen PI (2007). Identification of active biomolecules in the high–yield synthesis of single–crystalline gold nanoplates in algal solutions.Vol. **3 (4)** 668 – 72.

J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee (2007). Antimicrobial effects of silver nanoparticles. Nanomedicine. NBM. Vol. **3** 95 – 101.

T. Klaus–Joerger, R. Joerger, E. Olsson, C.G. Granqvist (2001). Trends in Biotechnology. Vol. **19** 15 – 20.

T. Klaus, R. Joerger, E. Olsson, C.G. Granqvist (1999). Proceedings of the National Academy of Sciences. Vol. **96** 13611 -13614.

Y. Konishi, K. Ohno, N. Saitoh, T. Nomura (2004). Microbial synthesis of gold nanoparticles by metal reducing bacterium. Trans mater Res soc Jpn.
Vol. **29** 2341 – 3.

E.D. Korobushkina, V.I. Biryuzova, I.M. Korobushkin, G.I. Karavaiko (1989). Dokl. Akad, Nauk. SSSR. Vol. **304** 431.

M. Kowshik, S. Ashtaputre, S. Kharrazi, W. Vogel, J. Urban, S.K. Kulkami, K.M. Paknikar, Nanotechnology. Vol. **14** 95 – 100.

M. Kowshik, N. Doshmukh, W. Vogel, J. Urban, Kulkarnisk, K.M. Paknikar (2003). Biotechnology and Bioengineering.Vol. **78:** 583 – 588.

N. Kuyucak, B. Volesky (1989). Biorecovery. Vol. **1:** 189.

C. Li, W. Cai, C. Kan, G. Fu, L. Zhang, (2004). Materials Letters. Vol. **58** 196 -199.

Y. Li, X. Duan, Y. Qian, Y. Li, H. Liao (1999). Nanocrystalline silver particles: synthesis, agglomeration, and sputtering induced by electron beam. J colloid Interface Sci. 209.

S. Mandal, S. Phadtare, M. Sastry (2005). Current Applied physics: Vol.**5**1127 – 1218.

Marieke Gericke and Anthony pinches (2006). Microbial production of Gold nanoparticles. Gold Bulletin. **Vol. 1** 39.

D. Mukherjee, A. Ahmad, P. Mandal, S. Senapati, S. Sainkar, M.I. Khan, R. Ramani, R. Parischa, P.V. Ajayakumar, M. Alam, M. Sastry, R. Kumar (2001). Angewandle chemie International Edition. **Vol. 40** 3585 – 3588.

D. Mukherjee, A. Ahmad, P. Mandal, S. Senapati, S. Sainkar, M.I. Khan (2001). Fungus mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix : a novel biological approach to nanoparticle synthesis. Nano Lett. Vol. **1:** 515 – 9.

B. Nair, T. Pradeep (2002). Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains. Cryst Growth Des Vol. **2** 293 – 298.

A. Nakajima, J. World (2003). Microbiol Biotechnol. Vol. **19** 369.

J.A. Pahl, B.L.S. Maddux, J.E. Hutchison (2007). Chem Rev. **107** 2228.

P. Raveendran, J. Fu, S.L. Wallen (2003). J Am chem. Soc. Vol. **125** 13940.

Y. Roh, R.J. Lauf, A.D. McMillan, C. Zhang, C.J. Rawan, J. Bai, T.J. Phelps (2001). Solid state communications Vol **118** 529 – 534.

S.S. Shankar, A. Rai, A. Ahmad, M. Sastry (2004). Journal of colloid and Interface Science. Vol. **275** 496 – 502.

H. Shiying, S. Zhirui, Y. Zhanga, S. Zhanga, J. Wanga, G. Ning (2007). Biosythesis of nano gold by *Pseudomonas capsulata*. Mater Lett. Vol. **61** 3984 – 7.

G. Singaravelu, J. Arockiamary, K. Ganesh, K. Govindaraju (2007). A novel extracellucar synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii*. Colloids Surf B Biointerfaces. Vol. **57** 97-101.

Y. Tan, Y. Dai, Y. Li, D. Zhua (2003). Preparation of gold, platinum, palladium and silver nanoparticles by the reduction of their salts with a weak reduction – potassium bitartrate. I mater chem. Vol. **13** 1069 – 75.

Tanja Klaus, Ralph joeger, Eva olsson, and claes-Goran Granqvist (1999). Silver-based crystalline nanoparticles. Vol. **24** 13611 – 13614.

R. Wahl, M. Mertig, J. Raff, J. Selenska-Pobell, W. Pombe (2001). Advanced Materials. Vol.**13** 736.