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ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES

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Abstract

The Microorganisms such as bacteria, yeast and now fungi play an important role in remediation of toxic metals through reduction of the metal ions, this was considered interesting as nanofactories very recently. Using these dissimulatory properties of fungi, the biosynthesis of inorganic nanomaterials using eukaryotic organisms such as fungi may be used to grow nanoparticles of gold and silver intracellularly in *Verticillium* fungal cells. Recently, it was found that aqueous chloroaurate ions may be reduced extracellularly using the fungus *F. oxysporum*, to generate extremely stable gold or silver nanoparticles in water. The study of biosynthesis of nanomaterials offers valuable contribution into materials chemistry. The ability of some microorganisms such as bacteria and fungi to control the synthesis of metallic nanoparticle should be employed in the synthesis of new materials. The biosynthetic methods are investigated as an alternative to chemical and physical ones. It is known that many microorganisms can provide inorganic materials either intra- or extracellularly. For example, bacteria Pseudomonas strutzeri isolated from silver mine is able to reduce Ag+ ions and accumulates silver nanoparticles. The size of such nanoparticle was 16-40 nm, with an average diameter 27 nm. Moreover, silver is occasionally used in the medical field as a topical bactericide. With the progress of nano-technology, many laboratories around the world have investigated silver nanoparticle production as the nanoparticle possesses more surface atoms than a microparticle, which greatly improves the particle's physical and chemical characteristics. However, at present there is no truly efficient method for their large-scale production. Some physical or chemical methods that are currently available for silver nanoparticle production include mechanical smashing, a solid-phase reaction, freeze-drying, spread drying and precipitation (co- and homo-precipitation). In general, these methods consume a lot of energy in order to maintain the high pressures and temperatures that are needed for them to work. In contrast, many bioprocesses occur under normal air pressure and temperature, resulting in vast energy savings.

INTRODUCTION

The Microorganisms such as bacteria, yeast and now fungi play an important role in remediation of toxic metals through reduction of the metal ions, this was considered interesting as nanofactories very recently (Fortin and Beveridge, 2000). Using this dissimilatory properties of fungi, the biosynthesis of inorganic nanomaterials using eukaryotic organisms such as fungi may be used to grow nanoparticles of gold (Mukherjee et al., 2001a) and silver (Mukherjee et al., 2001b) intracellularly in *Verticillium* fungal cells (Sastry et al., 2003). Recently, it was found that aqueous chloroaurate ions may be reduced extracellularly using the fungus *F. oxysporum*, to generate extremely stable gold or silver nanoparticles in water. It is known that many microorganisms can provide inorganic materials either intra- or extracellularly. For example, bacteria Pseudomonas strutzeri isolated from silver mine is able to reduce Ag+ ions and accumulates silver nanoparticles. The size of such nanoparticle was 16-40 nm, with an average diameter 27 nm. The intracellular methods need a special ion transportation system into the microbial cell. Formation of magnetite particles proceeds through sequence of events: the reduction of Fe (III) to Fe (II), precipitation of amorphous oxide and subsequent transformation to magnetite. Also the gold nanoparticles were synthesized in human cells, both cancer and noncancer ones, the scanning microscopic images confirmed they morphology differs significantly. This behavior can have an implication to cancer diagnostics. In contrast, extracellular synthesis of nanoparticles occurs in alkalothermophilic actinomycete, Thermomonospora sp. which reduces gold ion.

There is tremendous current excitement in the study of nanoscale matter (matter having nanometer dimensions, 1 nm = 10–7 cm) with respect to their fundamental properties, organization to form superstructures and applications.

Although it is known that microorganisms such as bacteria, yeast and now fungi play an important role in remediation of toxic metals through reduction of the metal ions, this was considered interesting as nanofactories very recently. Using these dissimilatory properties of fungi, the

biosynthesis of inorganic nanomaterials using eukaryotic organisms such as fungi may be used to grow nanoparticles of gold and silver intracellularly in *Verticillium* fungal cells. Recently, it was found that aqueous chloroaurate ions may be reduced extracellularly using the fungus *F. oxysporum*, to generate extremely stable gold or silver nanoparticles in water. Other process, which was described in the literature, was related to produce silver nanoparticles through oligopeptides catalysis, precipitating the particles with several forms (hexagonal, spherical and triangular). However, in the fungal reduction of Ag ions led colloidal suspension, differently that in the oligopeptides case. Then the mechanistic aspects are still an open question; however this process occurs in the fungal case probably either by reductase action or by electron shuttle quinones or both. Our aims in this research are to compare different strains of *F. oxysporum* in order to understand if the efficiency of the reduction of silver ions is related to a reductase or quinine action.

The silver particle is an important catalyst involved in the following chemical reaction (Force and Bell 1975; Campbell 1985), which is used in the oil industry. Both caesium and chemical chlorine are promoters which improve the selective oxidation of ethylene to ethylene epoxide. Moreover, silver is occasionally used in the medical field as a topical bactericide. With the progress of nano-technology, many laboratories around the world have investigated.

Moreover, silver is occasionally used in the medical field as a topical bactericide. With the progress of nano-technology, many laboratories around the world have investigated silver nanoparticle production as the nanoparticle possesses more surface atoms than a microparticle, which greatly improves the particle's physical and chemical characteristics. However, at present there is no truly efficient method for their large-scale production. Some physical or chemical methods that are currently available for silver nanoparticle production include mechanical smashing, a solid-phase reaction, freeze-drying, spread drying and precipitation (co- and homo-precipitation). In general, these methods consume a lot of energy in order to maintain the high pressures and

temperatures that are needed for them to work. In contrast, many bioprocesses, occur under normal air pressure and temperature, resulting in vast energy savings.

The Microorganisms such as bacteria, yeast and now fungi play an important role in remediation of toxic metals through reduction of the metal ions, this was considered interesting as nanofactories very recently (Fortin and Beveridge, 2000). Thus, to reduce or prevent infections, various antibacterial disinfections techniques have been developed for all types of textiles. Recently, several antibacterial agents of textiles based on metal salt solutions (CuSO4 or ZnSO4) have been developed.

Microorganisms play an important role in toxic metal remediation through reduction of metal ions. These particles can be incorporated in several kinds of materials such as cloths. These cloths with silver nanoparticles are sterile and can be useful in hospitals to prevent or to minimize infection with pathogenic bacteria such as *Staphylococcus aureus*. In this work, the extracellular production of silver nanoparticles by *F. oxysporum* and its antimicrobial effect when incorporated in cotton fabrics against *S. aureus* were studied. In addition, all effluent was bioremediated using treatment with *C. violaceum*. The results showed that cotton fabrics incorporated with silver nanoparticles displayed a significant antibacterial activity against *S. aureus*. The effluent derived from the process was treated with *C. violaceum* and exhibited anefficient reduction in the silver nanoparticles concentration. In conclusion, it was demonstrated the application of biological synthesis to silver nanoparticles production and its incorporation in cloths, providing them sterile properties. Moreover, to avoid any damage to the environment the effluent containing silver nanoparticles can be treated with cyanogenic bacterial strains. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. The use of *Azadirachta indica* (Neem) (Shankar et al., 2004), *Medicago sativa* (Alfalfa) (Gardea – Torresday et al., 2003), *Aloe vera* (Chandran et al., 2006), *Emblica officinalis* (amla, Indian Gooseberry) (Amkamwar et al., 2005), and microorganisms (Duran et

al., 2005; Vigneshwaran et al., 2006; Bhainsa and D'souza, 2006) has already been reported. According to previous reports, the polyol components and the water-soluble heterocyclic components are mainly responsible for the reduction of silver ions and the stabilization of the nanoparticles, respectively. Thereare also reports on reductases (Anil Kumar et al., 2007) and polysaccharides (Huang and Yang, 2004) as factors involved in biosynthesis and stabilization of the nanoparticles, respectively. Our hypothesis is that several factors together determines the nanoparticle synthesis, including the plant source, the organic compounds in the crude leaf extract, the concentration of silver nitrate, the temperature and other than these, even the pigments in the leaf extract. The longtime aim is to identify those compounds and the mechanism in detail. As a preliminary work we screened the following plants: *Basella alba* (*Basellaceae*), *Helianthus annus*, (*Asteraceae*), *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolour* and *Zea mays* (*Poaceae*) and systematic comparative study was carried out to investigate their efficiency to reduce silver ions as well as the formation of silver nanoparticles.

Aim: Antibacterial Activity of Silver Nanoparticles.

Objectives:

- ❖ Isolation & identification of fungus.
- ❖ Synthesis of Nanoparticals.
- ❖ Characterisations of Nanoparticals.
- UV-Visible Spectrophotometric analysis.
- Scanning Electron Microscopy (SEM).
- ❖ Antimicrobial Activity of Nanoparticals.

Material & Methods

Materials:

Soil sample: The soil sample was collected from our college campus .

Methods:

1.Methods used for isolation of fungal culture:

Serial dilution technique was followed for the isolation of fungal culture from the soil. The technique in brief is described below.

The 1 gm of soil sample was added in 9 ml distilled water, from this tube 1 ml sample

is sequencially added to the next tube and so on. The 0.1 ml sample was spread on the plates of Sabourad agar. These plates were incubated at 28^0C for 48-72 hrs.

2. Methods used for the identification of fungal culture:

The identification was done on the basis of cultural, physiological and morphological characteristics and by compairing with known standard strain.

3. Production and separation of fungal Biomass:

Saburouds broth was used for the biomass production of *A. niger and A.terrus.* The spore suspension was inoculated in the Sabourad broth. These flasks were incubated at 24^0C for 48 hrs. Control (without the silver ions) was also run along with the experimental flask.

The biomass was separated by using the filtration technique. The broth was filtered through whatman's filter paper No1. The filtrate was discarded. The mycelia was washed with sterile distilled water to remove the traces of medium. The mycelia growth was resuspended into 250 ml steriledistilledwater. The flask was incubated at 28^0c for 48^0C.

4. Preparation of plant extract:

Crude extracts was prepared by using the fresh leaves of *Neam.* These leaves were throughly washed under running tap water. 20 gm of leaves was crushed in 50 ml distilled water. It was then filtered using Whatman's filter paper No. 1, yielding the crude extract.

5. Silver reduction and its characterisation:

The biomass in the distilled water was filtrated through the whatman's filter paper No.1. The filtrate is collected and the cell mass is discarded. The filtrate or crude extract of leaves were treated with $AgNO_3$ (1×10^3M) solution. The filtrate was incubated for 24-48 hrs. After incubation dark brown colour was developed. It indicates the formation of nanoparticles.

5.1. Characterisation:

5.1.1. UV- Visible spectrophotometer:

The absorbance of reaction solution was measured at various wavelength (200-1000 nm) and at particular time period (0, 24, 48 hrs). (Fig 1, 2 & 3).

6. Antimicrobial activity:

The antimicrobial activity of reaction mixture was tested against pathogen *staphylococcus aureus, Salmonella, Proteus valgaris, Pseudomonas and E. Coli* by well method. 100µl sample was used for inoculation in the well.(Fig. 2 &3)

Result & Discussion

.Methods used for isolation of fungal culture: The isolation of fungal culture was done by using five soil samples. Out of five sample in two sample *A.terrus* was found and in four sample *A.niger* was found.

2. Results of identification of fungal culture: The isolated culture was identified in the basis of following characters.

1. Aspergillus niger:

Macroscopic morphology: Colonies on potato dextrose agar at 25°C are initially white, quickly becoming black with conidial production. Reverse is pale yellow and growth may produce radial fissures in agar.

Microscopic morphology: Hyphae are septate and hyaline. Conidial heads are radiate initially, splitting into columns at maturity. The species is biseriate (vesicles produces sterile cells known as metulae that support the conidiogenous phialides). Conidiophores are long (400-3000 µm), smooth, and hyaline, becoming darker at the apex and terminating in a globose vesicle (30-75 µm in diameter). Metulaeand phialides cover the entire vesicle. Conidia are brown to black, very rough, globose, and measure 4-5 µm in diameter. (Sutton, D. A., 1998., de Hoog, G. S.,).

2. A. terrus:

Macroscopic morphology: Reverse is yellow and yellow soluble pigments are frequently present. Moderate to rapid growth rate. Colonies become finely granular with Colonies on potato dextrose agar at 25°C are beige to buff to cinnamon. conidial production.

Microscopic morphology: Hyphae are septate and hyaline. Conidial heads are biseriate (containing metula that support phialides) and columnar (conidia form in long columns from the upper portion of the vesicle). Conidiophores are smooth-walled and hyaline, 70 to 300µm long, terminating in mostly globose vesicles. Conidia are small (2-2.5 µm), globose, and

smooth. Globose, sessile, hyaline accessory conidia (2-6 µm) frequently produced on submerged hyphae. (Sutton, D. A., 1998., de Hoog, G. S.,).

5. Silver reduction and its characterisation:

The Erlemeyer flask with fungal biomass was a pale yellow colour before the addition of Ag ions and this change to a brownish colour on completion of reaction with Ag ions for 28 hrs the appearance of brownish colour in the solution containing the biomass was a clear indication of formation of silver nanoparticles in the reaction mixture (Sastry et.al., 1998). Upon addition of Ag ions in plant leaf extract or cell free reaction mixture of fungi, change in colour from almost colourless to brown, with intensity increasing during the period of incubation. Control showed no change in colour of the cell filtrate when incubated in the same condition. The formation of colloidal silver particles can be easily followed by changes of UV-Vis absorption.

UV- Visible spectrophotometer:

The UV visible spectrum of the fungal reaction vessel at different times intervals is presented in Table No. 1. In the spectrophotometric analysis the reaction mixture of *Aspergillus niger, Aspergillus terrus & Neam* exhibited a strong absorption between 400, 400 and 500 nm respectively. The absorption spectrum of aqueous silver nitrate only solution exhibited λ max at about 220 nm.

SEM Micrograph of fungus:

The Scanning Electron Microscopy (SEM) showes silver nanoparticles aggregates. In this micrograph it was observed the size spherical nanoparticles was (20-50 nm). The similar results were observed by Z. Sadowski et al., 2008, Nelson Duran et al., 2005. Chen S., 2002 produced elongated (rod-shaped) and truncated triangular silver nanoplates were synthesized by a solution phase method for the large-scale preparation of truncated triangular nanoplates. The size of rod shaped nanoparticles ranges from 5-10 µm. (Fig. 4, 5**).**

Antimicrobial activity:

Antibacterial activity Ag nanoparticles against *E. coli* and *S. aureus.* As the bacteria grew to form a confluent lawn,

the extent of growth inhibition could be measured as the extent of the clear zone surrounding the disk. Bacterial inhibition tests against *E. Coli, Proteus valgaris, Pseudomonas* and *S. aureus* are shown in Figures 1, 2 & 3 Clear zone diameter of the bacterial inhibition zone was correlated to antibiotic activity of silver particles in Tables 2**.** These data are consistent with previously reported studies in which silver ions had effective antimicrobial properties at concentrations of 1×10^{3} M.

SUMMARY

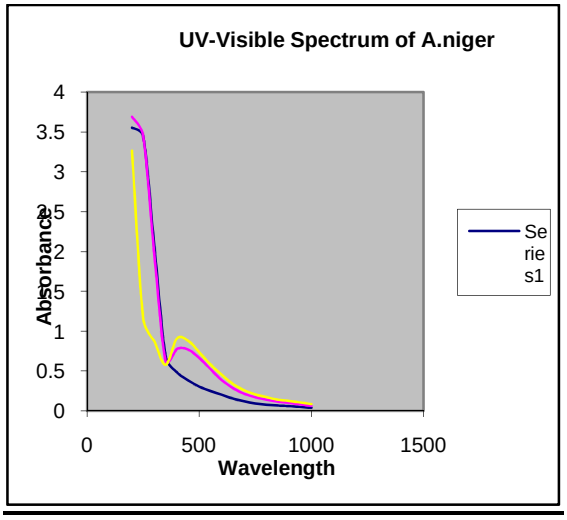
1. Two fungal cultures were isolated from the soil sample obtained from the Krishna Institute of Biotechnology & Bioinformatics, Karad by serial dilution technique using Sabourad agar medium.
2. Cultures were subjected to characterization and were tentatively identified as *Aspergillus niger* and *Aspergillus terrus*.
3. The fungal isolate viz *Aspergillus niger* and *Aspergillus terrus* were subjected to biomass production and subsequent separation of biomass.
4. The aqueous plant extract was prepared from the plant *Neam* by filtration technique.
5. The cultures were subjected for Silver nanoparticals.
6. Antibacterial activity was evaluated against *E. Coli, P. Valgaris, salmonella, Pseudomonas, S. Aurous.*
7. Nanoparticals were characterized with UV-Visible Spectrophotometerically and Scanning Electron Microscope.

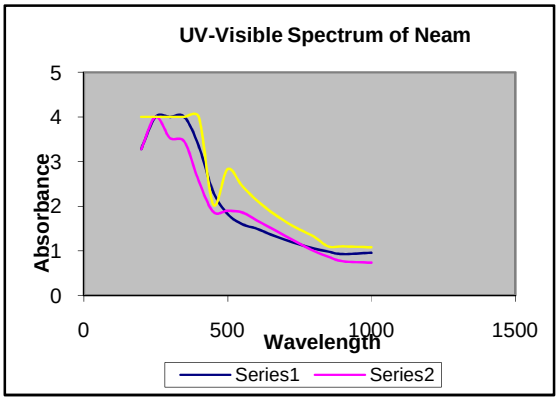
CONCLUSION

Both the fungal isolates obtained from soil have potential to produce Silver nanoparticles which can show activity against *E. Coli, P. Valgaris, salmonella, Pseudomonas, S. Aurous.*

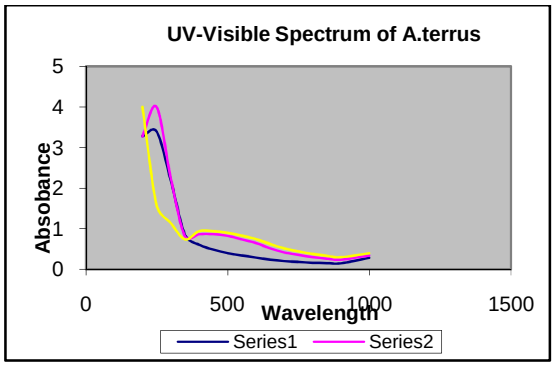
Cultures should therefore be presented for further studies.

Graph 1 UV –Visible Spectrum of reaction mixture of fungi *A.niger* treated with $AgNO_3$.





Graph 2 UV –Visible Spectrum of reactionmixture of fungi *A.terrus* treated with $AgNO_3$.



Graph 3 UV –Visible Spectrum of reaction mixture of fungi *Neam* treated with $AgNO_3$.

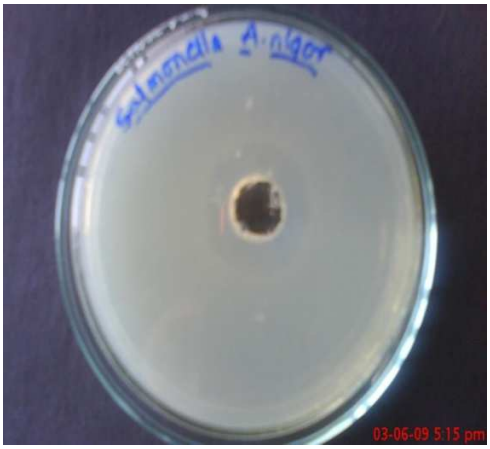


Fig.1 Antibacterial Activity of nanoparticals:

Fig.2 Antibacterial Activity of nanoparticals:

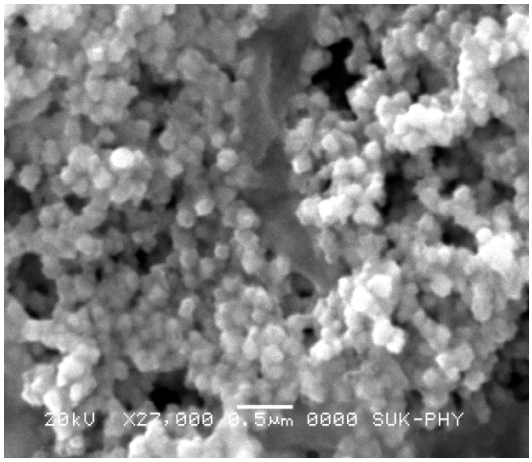


Figure.3. Reduction of Ag^{+} ions

Fig.4 SEM Micrograph of silver nanoparticles:

Table: 1 UV-Vis absorption Spectra during the formation of Silver nanopraticals.

	A. niger			A. terrus			Neam		
OD	o hrs	24 hrs	48 hrs	o hrs	24 hrs	48 hrs	o hrs	24 hrs	48 hrs
200	3.554	3.689	3.265	3.281	3.28	4.000	3.281	3.275	4.00
250	3.447	3.447	1.165	3.395	4	1.577	4.000	4.000	4.00
300	2.063	1.953	0.872	2.186	2.246	1.136	4.000	3.526	4.00
350	0.689	0.625	0.577	0.846	0.81	0.730	4.000	3.446	4.00
400	**0.487**	**0.777**	**0.906**	**0.601**	**0.863**	**0.934**	3.346	2.564	4.00
450	0.386	0.771	0.881	0.479	0.865	0.933	2.325	1.876	2.04
500	0.307	0.665	0.736	0.392	0.822	0.892	**1.827**	**1.897**	**2.83**
550	0.251	0.525	0.582	0.333	0.736	0.825	1.600	1.862	2.46
600	0.204	0.388	0.454	0.281	0.647	0.739	1.498	1.687	2.14
650	0.156	0.288	0.338	0.232	0.514	0.618	1.366	1.516	1.88
700	0.123	0.217	0.26	0.195	0.414	0.508	1.248	1.334	1.66
750	0.094	0.172	0.208	0.175	0.351	0.436	1.145	1.162	1.48
800	0.079	0.142	0.172	0.153	0.299	0.371	1.050	0.997	1.32
850	0.069	0.117	0.141	0.146	0.26	0.326	0.982	0.868	1.10
900	0.062	0.101	0.125	0.140	0.236	0.291	0.926	0.767	1.10
1000	0.041	0.064	0.086	0.276	0.337	0.389	0.958	0.734	1.08

Table: 2 Minimum inhibitory concentration (MIC) results of Ag nonaparticals

Microorganisum used	Cell free extract of organisums	
	A. niger	A.terrus
Proteus vulgaris	-	23
S. aurus	-	14
Pseudomonas	-	14
Salmonella	23	20
E.coli	26	-

REFERNCE:

1. Kolar M, Urbanek K, Latal T. Antibiotic selective pressure and development of bacterial resistance. Int J Antimicrob Ag 2001;17:357–63.

2. Kim JS et al. Antimicrobial effects of silver nanoparticles. Nanomedicine 2007;3:95–101.

3. Lin YE, Vidic RD, Stout JE, Mccartney CA, Yu VL. Inactivation of Mycobacterium avium by copper and silver ions. Water Res 1998;32:1997–2000.

4. Blanc DS, Carrara P, Zanetti G, Francioli P. Water disinfection with ozone, copper and silver ions, and temperature increase to control Legionella: seven years of experience in a university teaching hospital. J Hosp Infect 2005;60:69–72.

5. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, et al. The bactericidal effect of silver nanoparticles. Nanotechnology 2005;16:2346–53.

6. Vaseashta A, Dimova-Malinovska D. Nanostructured and nanoscale devices, sensors and detectors. Sci Technol Adv Mater 2005;6:312–8.

7. Comini E. Metal oxide nano-crystals for gas sensing. Anal Chim Acta 2006;568:28–40.

8. Raveh A, Zukerman I, Shneck R, Avni R, Fried I. Thermal stability of nanostructured superhard coatings: a review. Surf Coat Technol 2007;201:6136–42.

9. Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol 2006;40:4346–52.

10. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for gram-negative bacteria. J Colloid Interf Sci 2004;275:177–82.

11. Siva Kumar V, Nagaraja BM, Shashikala V, Padmasri AH, Madhavendra SS, Raju BD, et al. Highly efficient Ag/C catalyst prepared by electro-chemical deposition method in controlling microorganisms in water. J Mol Catal A Chem 2004;223:313–9.

12. Jain P, Pradeep T. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng 2005;90:59–63.

13. Cho K, Park J, Osaka T, Park S. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim Acta 2005;51:956–60.

14. Cioffi N et al. Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. Chem Mater 2005;17:5255–62.ental Management and Technology",Goa, India, September 21–23, 2006.