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# Biological Approaches for Synthesis of Silver Nanoparticles for Environmental Applications - A Review

Shreya*
Centre of Research for Development, Parul University, GJ, India
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*shreyamodi20@gmail.com

## ABSTRACT

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology. Over the past few decades, nanoparticles of noble metals such as silver exhibited significantly distinct physical, chemical and biological properties from their bulk counterparts. Silver nanoparticles are particles of silver that are in the range of 1 to 100 nm in size. Several products of colloidal silver are already available in the market. The major methods used for silver nanoparticle synthesis are the physical and chemical methods, which are expensive and can also have toxic substances absorbed onto them. To overcome this, the biological method provides a reliable, economic, eco-friendly and feasible alternative. The major biological systems involved are bacteria, fungi and plant extracts. Silver nanoparticles have broad-spectrum applications due to their advanced properties such as high surface area to volume ratio and smaller size than bulk silver. Using such nanoparticles, it is possible to solve the environmental pollution problem like drinking water, wastewater, removal of pathogenic microorganisms, heavy metal removal, textile dye removal, pesticide mineralization and food preservation and they can be used as disinfectants. Apart from these, silver nanoparticles are very efficient sensors to detect heavy metals from the environment. This review article provides some information about different modes of silver nanoparticles synthesis using a biological system, and also its fascinating environmental applications.

**Keywords:** Nanoparticle applications; Nano-Bioremediation; Silver nano remediation; Nano science; Nanotechnology; Silver nanoparticles.

## 1. INTRODUCTION

Nanotechnology plays an increasingly crucial role in many key technologies of the new millennium. (Mandal *et al.* 2006). Study of nanoscale materials and devices, with dimensions ranging from 1 to 100 nanometers, is an emerging field of nanoscience and nanotechnology. In the fields of solar energy conversion, catalysis, medicine and water treatment, nanomaterials can provide solutions to technological and environmental challenges (Sharma *et al.* 2009). Nano materials often show unique and considerably changed physical, chemical and biological properties compared to their macro-scaled counterparts (Sharma *et al.* 2009). Metal nanoparticles are intensely studied due to their unique optical, electrical and catalytic properties. To utilize and optimize chemical or physical properties of nano-sized metal particles, a large spectrum of research has been carried out; especially, to control the size and shape of nanoparticles, which is crucial in tuning their physical, chemical and optical properties (Alivisatos *et al.* 1996; Bruchez *et al.* 1998; Coe *et al.* 2002). Various techniques, including chemical and physical means have been developed to prepare metal nanoparticles, such as chemical reduction (Petit *et al.* 1993; Vorobyova *et al.* 1999; Yu *et al.* 2007; Textor *et al.* 2010), electrochemical reduction (Kéki *et al.* 2000; Liu *et al.* 2004), photochemical reduction (Bae *et al.* 2002; Smetana *et al.* 2005) and heat evaporation. However, these methods have certain disadvantages due to involvement of toxic chemicals and radiation. Therefore, research is shifting towards biological methods to synthesize metal nanoparticles. Biological synthesis process provides a wide range of rapid, cost effective, eco-friendly and environmentally acceptable methodology. At the same time the biologically synthesized silver nanoparticles has many applications including usage as catalysts in chemical reactions (Królikowska *et al.* 2003), bio labeling, antimicrobial agent, electrical batteries (Klaus-Joerger *et al.* 2001) and optical receptors. Microbial source to produce the silver nanoparticles shows the great interest towards the precipitation of nanoparticles due to its metabolic activity. Definitely the precipitation of nanoparticles in external environment of a cell indicates the extracellular activity of organism.

There are three major sources of synthesizing silver nanoparticles using biological approach: Microbial, enzyme/protein and using different plant parts. Biosynthesis of silver nanoparticles is a bottom-up approach that mostly involves reduction/oxidation

reactions. It is majorly the microbial enzymes or the plant phytochemicals with antioxidant or reducing properties that act on the respective compounds and give the desired nanoparticles. The three major components involved in the synthesis of nanoparticles by biological approaches are: (i) a solvent medium for synthesis, (ii) an environment-friendly reducing agent and (iii) a non-toxic stabilizing agent. The general aspect of nanoparticles is that the small size of nanoparticles provides for a larger surface area for the particle and hence increases the effect. The nano-size of the particles also increases the penetration potential of the silver particles, hence again aiding in better utilization of the metal properties. (Kalimuthu *et al.* 2008). The bio-reduction of metal ions through a mixture of biomolecules present in the extracts of some species (e.g., enzymes/proteins, amino acids, polysaccharides, vitamins) is a biologically complicated process that is environment-friendly. Many experiments have shown that species (microorganisms and biological systems) can successfully synthesize silver nanoparticles (Iravani *et al.* 2011).

## 2. METHODOLOGY

### 2.1 Silver Nanoparticle Synthesis Using Bacteria

Husseiny *et al.* (2007) used the *Pseudomonas stutzeri* AG259 strain isolated from a silver mine to reveal the first reports of bacteria synthesizing silver nanoparticles. Some microorganisms can live and expand in high metal ion concentrations, and this is due to their resistance to the metal. Efflux processes, changes in solubility and toxicity due to reduction or oxidation, biosorption, bioaccumulation, metal extracellular complex forming or precipitation, and a lack of unique metal transport systems are all involved in resistance. Researchers have been focusing on developing effective green chemistry methods that use natural reducing, capping and stabilizing agents to prepare silver nanoparticles of desired morphology and scale in recent years. Silver nanoparticles can be synthesized using biological processes rather than harsh, harmful and costly chemical compounds.

While these species can thrive at lower concentrations, they can become poisonous when exposed to higher concentrations of metal ions. The involvement of the nitrate reductase enzyme is the most commonly known pathway for silver biosynthesis. Nitrate is converted to nitrite by the enzyme. The existence of alpha-nicotinamide adenine dinucleotide phosphate reduced form (NADPH) based nitrate reductase in *in-vitro* silver synthesis using bacteria will eliminate the downstream processing stage that is needed in other cases. During the reduction, nitrate is converted into nitrite and the electron is transferred to the silver ion; hence, the silver ion is reduced to silver ($Ag^+$ to $Ag^0$). This has been said to be observed in *Bacillus licheniformis* which is known to secrete NADPH and NADPH-dependent enzymes like nitrate reductase that effectively converts $Ag^+$ to $Ag^0$ (Vaidyanathan *et al.* 2010). The mechanism was further confirmed by using purified nitrate reductase from *Fusarium oxysporum* and silver nitrate along with NADPH in a test tube, and the change in the color of the reaction mixture to brown and further analysis confirmed that silver nanoparticles were obtained. There are also cases which indicate that there are other ways to biosynthesize silver nanoparticles without the presence of enzymes. It was found that dried cells of *Lactobacillus sp.* A09 can reduce silver ions by the interaction of the silver ions with the groups on the microbial cell wall.

The synthesis of silver nanoparticles is done recently through *E. coli*. which generates silver nanoparticles as a part of its metabolism, when encountered by silver ions from nature. The silver ions are then converted into silver atoms. It is also revealed that temperature also play an important role in nanoparticles size. Larger nanoparticles are produced at lower temperature, and the size decreases at higher temperatures. The nanoparticles synthesis from *E. coli* varies with the change in $AgNO_3$ concentration. 1 mM concentration is the patented one. Silver, when in lower concentration, helps in inducing the organism to synthesize nanoparticles, whereas at higher concentration induces cell death. Silver nanoparticle synthesis from Metal-tolerant Marine Bacteria *Pseudomonas aerogenosa* was described by (Sondi *et al.* 2004). The synthesized nanoparticles then characterized by UV-Vis., SEM, XRD and FTIR measurements. On treating the bacteria with 1 mM $AgNO_3$, it was found to have the ability to form silver nanoparticles at room temperature within 24 hours. AgNPs were characterized by UV-Visible spectrophotometer, X-ray diffraction (XRD) and Scanning electron microscopy (SEM). UV-Visible absorption scan of a 48 h culture exposed to 5mM silver nitrate revealed a broad peak at 450 nm indicative of the Surface Plasmon resonance of SNPs. This was confirmed by the visual observation and UV-Vis. absorption at 450 nm. The nanoparticles proved excellent antimicrobial activity. Hence, the biological approach appears to be a cost-effective alternative to conventional physical and chemical methods of silver nanoparticles synthesis and would be suitable for developing a biological process for commercial large scale-production.

It has been reported that the silver ions in nature are highly toxic for the bacterial cells. Hence, their cellular machinery helps in the conversion of reactive silver ions into stable silver atoms. Further, temperature and pH play important roles in their production. At room temperature, the size of nanoparticles is 50 nm; at higher temperature, i.e., at 60 °C, the size of nanoparticles reduces to 15 nm. This indicates that with the increase in temperature size decreases. Under alkaline conditions,

nanoparticles synthesis by the microbe is more as compared to the acidic conditions. But, after pH 10 cell deaths occur. Silver-resistant bacteria have been found repeatedly in environments where silver toxicity might be expected to be selected for resistance, in particular from the burn wards of hospitals (Larkin Mchugh *et al.* 1975; Monafo *et al.* 1987; Jr. *et al.* 1998; Klasen *et al.* 2000a; Klasen *et al.* 2000b). Apart from this, silver also found in polluted soil around mines, water catchment associated with photographic film production and processing, institutional water distribution systems where metal compounds are used for control of infectious agents such as Legionella, and as presumably beneficial components of health food supplements (Monafo *et al.* 1987; Jr. *et al.* 1998; Tian *et al.* 2007). It is reported that some bacteria contain plasmid with *sil* genes which are responsible for silver resistance of bacteria (Larkin Mchugh *et al.* 1975). The bacteria which are capable to synthesize silver nanoparticles are presented in Table 1.

## 2.2 Synthesis of Silver Nanoparticles using *Actinomycetes*

The species of *Actinomycetes* can be used to synthesize silver nanoparticles (Abdeen, 2014). The comparison of biological approach was made to chemical method. The obtained silver nanoparticles in their study were characterized using UV-Vis. spectroscopy and TEM. TEM images of microbially synthesized silver nanoparticles were of smaller size (10-20 nm) compared to chemical methods (60-80 nm). The biologically synthesized silver nanoparticles using *Actinomycetes* were found to be highly toxic to bacteria and found that smaller silver nanoparticles synthesized by microbial route had a greater antibacterial activity when compared to their chemical moieties.

**Table 1. Synthesis of Silver Nanoparticles by Bacteria**

| S. No. | Bacteria | Size(nm) | Reference |
|---|---|---|---|
| 1 | *Bacillus megaterium* | 42–92 | Das *et al.* 2014 |
| 2 | *Plectonema boryanum* | 1-200 | Lengke *et al.* 2007 |
| 3 | *Enterobacter cloacae* Ism26 | 7-25 | El-Baghdady *et al.* 2018 |
| 4 | *Escherichia coli* | 5-25 | El-Shanshoury *et al.* 2011 |
| 5 | *B. subtilis* | 5-60 | Saifuddin *et al.* 2009 |
| 6 | *Lactobacillus fermentum* | 11.2 | Sintubin *et al.* 2009 |
| 7 | *Klebsiella pneumonia* | 50 | Mokhtari *et al.* 2009 |
| 8 | *Proteus mirabilis* | 10-20 | Samadi *et al.* 2009 |
| 9 | *Brevibacterium casei* | 50 | Kalishwaralal *et al.* 2010 |
| 10 | *Staphylococcus aureus* | 150 | Feng *et al.* 2008 |
| 11 | *Ureibacillus thermosphaericus* | 1-100 | Juibari *et al.* 2011 |
| 12 | *Bacillus thuringiensis* | 10-20 | Jain *et al.* 2010 |
| 13 | *Geobacter sulfurreducens* | 200 | Law *et al.* 2008 |
| 14 | *Corynebacterium* SH09 | 10-15 | Zhang *et al.* 2005 |
| 15 | *Acinetobacter calcoaceticus* | 8-12 | Singh *et al.* 2015 |
| 16 | *S. typhimurium* (Flagella) | 3-11 | Gopinathan *et al.* 2013 |
| 17 | Halococcus salifodinae BK3 | 12, 22 | Srivastava *et al.* 2013 |
| 18 | *Myxococcus virescens* | 7-50 | Drzewiecka1 *et al.* 2014 |
| 19 | *P. Antarctica* | 6-13 | Shivaji *et al.* 2011 |
| 20 | *Clostridium versicolor* | 20-23 | Sanghi *et al.* 2009 |
| 21 | *Streptomyces Coelicolor* klmp33 | 50 | Manikprabhu *et al.* 2013 |
| 22 | *Bacillus flexus* | 12-65 | Priyadarshini *et al.* 2013 |
| 23 | *Bacillus amyloliquefaciens* | 14.6 | (Wei *et al.* 2012) |
| 24 | *Corynebacterium sp.* | 15 | Gowramma *et al.* 2015 |

## 2.3 Silver Nanoparticle Synthesis using Fungi

Fungi, in contrast to bacteria, can create more nanoparticles so they can secrete more proteins or enzymes, which directly translates to increased nanoparticle productivity (Mohanpuria *et al.* 2008). The following steps are said to be involved in the processing of silver nanoparticles by fungi: trapping of Ag+ ions on the surface of the fungal cells, followed by reduction of the silver ions by enzymes present in the fungal system (Mukherjee *et al.* 2001). Extracellular enzymes such as naphthoquinones and anthraquinones are thought to help in the reduction process. The NADPH-dependent nitrate reductase and a shuttle quinine extracellular mechanism are thought to be responsible for the production of nanoparticles in F. oxysporum (Durán *et al.* 2005). Though the exact mechanism underlying the formation of silver nanoparticles by fungi is unknown, it is thought that the above-mentioned phenomenon is to be blamed. Silver nanoparticles have been synthesized through the reduction of silver ions by the fungus *Aspergillus flavus*. This is one of the simplest and cheapest processes for obtaining silver nanoparticles. UV spectroscopy revealed the surface plasmon property, while XRD analysis and SEM images revealed the nano-nature of the prepared samples. Average size estimated from above studies is 44 nm. These silver nanoparticles are found to have characteristic absorption peak at 420 nm and emission peak at 553 nm (Patil *et al.* 2011). Numbers of studies have been reported for silver nanoparticles synthesis by fungi (Table 2).

## 2.4 Silver Nanoparticle Synthesis using Plants

The main benefit of using plant extracts for silver nanoparticle synthesis is that they are readily available, stable and nontoxic in most situations; they contain a wide range of metabolites that can help in the removal of silver ions, and are faster in the synthesis than microbes. Plant-assisted reduction due to phytochemicals is the key mechanism considered for the process.

**Table 2. Synthesis of Silver Nanoparticles by Different Fungi**

| S. No. | Fungi | Size (nm) | Reference |
|---|---|---|---|
| 1. | *Verticillium. Sp* | 25 | Mukherjee *et al.* 2001 |
| 2. | *Phoma sp.* 3.2883 | 70 | Gade *et al.* 2013 |
| 3. | *F. oxysporum* | 20-50 | Durán *et al.* 2005 |
| 4. | *Aspergillus fumigates* | 7.19 | Navazi *et al.* 2010 |
| 5. | *Aspergillus flavus* | 7-10 | Vigneshwaran *et al.* 2007 |
| 6. | *Fusarium semitectum* | 10-60 | Basavaraja *et al.* 2008 |
| 7. | *Coriolus versicolor* | 350-600 | Sanghi *et al.* 2009 |
| 8. | *Fusarium solani* | 5-35 | Ingle *et al.* 2009 |
| 9. | *Phanerochaete chrysosporium* | 100 | Huang *et al.* 2004 |
| 10. | *Cladosporium cladosporioides* | 10-100 | Balaji *et al.* 2009 |
| 11. | *Penicillium brevicompactum* WA 2315 | 23-105 | Shaligram *et al.* 2009 |
| 12. | *Fusariumacum inatum* | 5-40 | Ingle *et al.* 2008 |
| 13. | *Aspergillus clavatus* | 10-25 | Verma *et al.* 2011 |
| 14. | *Penicillium fellutanum* | 1-100 | Kathiresan *et al.* 2009 |
| 15. | *Trichoderma harzianum* | 30-50 | Singh *et al.* 2011 |
| 16. | *Fusarium acuminatum* | 5-40 | Ingle *et al.* 2008 |
| 17. | *Neurospora crassa* | 20-50 | Castro-Longoria *et al.* 2011 |
| 18. | *Phoma glomerata* | 60-80 | Birla *et al.* 2009 |
| 19 | *Trichoderma viride* | 2-4 | Mohammed Fayaz *et al.* 2009 |
| 20 | *Trichoderma Reesei* | 5-50 | Vahabi *et al.* 2011 |
| 21 | *Epicoccum nigrum* | 1-22 | Qian *et al.* 2013 |
| 22 | *Bryophilous Rhizoctonia sp.* | 25-50 | Raudabaugh *et al.* 2013 |
| 23 | *Trichoderma asperellum* | 13-18 | Mukherjee *et al.* 2008 |
| 24 | *Alternaria alternate* | 20-60 | Gajbhiye *et al.* 2009 |
| 25 | *Bipolaris nodulosa* | 10-60 | Saha *et al.* 2010 |
| 26 | *Pleurotus ostreatus* | 50 | Devika *et al.* 2012 |
| 27 | *Trametes versicolor* | 200 | Durán *et al.* 2014 |
| 28 | *Phytophthora infestans* | 210 | Thirumurugan *et al.* 2011 |

Terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids are the major phytochemicals involved. Water-soluble phytochemicals such as flavones, organic acids and quinones are responsible for the immediate reduction of ions. Xerophytes include emodin, an anthraquinone that undergoes tautomerization, resulting in the formation of silver nanoparticles, according to research. Mesophytes were discovered to possess three different forms of benzoquinones: cyperoquinone, dietchequinone and remirin. It has been proposed that phytochemicals are specifically involved in ion reduction and the formation of silver nanoparticles (Jha *et al.* 2009). Table 3 shows the results of an analysis on the synthesis of silver nanoparticles using various plants.

**Table 3. Synthesis of Silver Nanoparticles Using Different Plants**

| S. No. | Plant | Size, nm | References |
|---|---|---|---|
| 1 | *Medicago sativa* | 2–20 | Gardea-Torresdey *et al.* 2003 |
| 2 | *Phyllanthu samaras* | 18–38 | Kasthuri *et al.* 2009 |
| 3 | *Emblica officinalis* | 10–20 | Ankamwar *et al.* 2005 |
| 4 | *Azadirachta indica* | 5–35 | Shankar *et al.* 2003 |
| 5 | *Cinnamom mumcamphora* | 55–80 | Huang *et al.* 2007 |
| 6 | *Coriandrum sativum leaf extract* | 26 | Sathyavathi *et al.* 2010 |
| 7 | *Carica papaya* | 60–80 | Mude *et al.* 2009 |
| 8 | *Jatropha curcas* | 10–20 | Bar *et al.* 2009 |
| 9 | *Gliricidia sepium* | 10–50 | W. *et al.* 2009 |
| 10 | *Aloe vera* | 15 to 20 | Chandran *et al.* 2006 |
| 11 | *Desmodiumtri florum* | 5 to 20 | Ahmad *et al.* 2011 |
| 12 | *Alfalfa sprouts* | 2-20 | Gardea-Torresdey *et al.* 2003 |
| 13 | *Geranium leaves plant extract* | 16-40 | Shankar *et al.* 2003 |
| 14 | *Ginger root extract* | 20 | Ojha *et al.* 2012 |
| 15 | *Orange Peel Extract* | 20-50 | Devabharathi *et al.* 2014 |
| 16 | *Carob leaf extract* | 5-40 | Awwad *et al.* 2013 |
| 17 | *Olive leaf extract* | 20-25 | Khalil *et al.* 2014 |
| 18 | *Mangosteen leaf extract* | 35 | Veerasamy *et al.* 2011 |
| 19 | *Pterocarpus santalinus l* | 20-50 | Gopinath *et al.* 2013 |
| 20 | *Cinnamomum camphora leaf* | 55 to 80 | Huang *et al.* 2007 |
| 21 | *Raspberry leaf extract* | <100 | Kandasamy *et al.* 2014 |
| 22 | *Dioscorea bulbifera tuber* | 8-20 | Chopade *et al.* 2012 |
| 23 | *Coleus aromaticus leaf extract* | 44 | Vanaja *et al.* 2013 |
| 24 | *Svensonia Hyderabadensis Leaf extract* | 45 | Linga Rao *et al.* 2011 |
| 25 | *Argemone mexicana leaf extract* | 30 | Singh *et al.* 2010 |
| 26 | *Onion (Allium cepa) extract* | 33.65 | Saxena *et al.* 2012 |
| 27 | *Ficus benghalensis leaf extract* | 16 | Saxena *et al.* 2012 |
| 28 | *Crossandra infundibuliformis leaf* | 38 | Kaviya *et al.* 2012 |
| 29 | *Avicennia marina mangrove plant* | 71-110 | Gnanadesigan *et al.* 2012 |
| 30 | *Murraya koenigii* | 10-25 | Christensen *et al.* 2011 |
| 31 | *Cycas circinalis* | 2-6 | Jha *et al.* 2010 |
| 32 | *Ipomea carnea* | 30-130 | Daniel *et al.* 2014 |
| 33 | *Bacopa monniera* | 15-120 | Mahitha *et al.* 2011 |
| 34 | *Phyllanthus maderaspatensis* | 59-76 | Annamalai *et al.* 2014 |
| 35 | *Nicotina tobaccum* | 8 | Kumar *et al.* 2011 |
| 36 | *Memecylonedule leaf extract* | 50-90 | Elavazhagan *et al.* 2011 |
| 37 | *Saraca indica* | 13-50 | Tripathi *et al.* 2013 |
| 38 | *Syzgium cumini leaf extract* | 100-160 | Jha *et al.* 2010 |
| 39 | *Syzgium cumini bark* | 20-60 | Prasad & Swamy 2013 |
| 40 | *Rhizophora apiculata leaf extract* | 13-19 | Antony *et al.* 2011 |
| 41 | *Santalum album* | 80-200 | Ram, Prasad; Samy *et al.* 2012 |
| 42 | *Phyllanthus emblica* | 19.8-92.8 | Masum *et al.* 2019 |
| 43 | *Salvia spinosa* | 19-125 | Pirtarighat *et al.* 2019 |
| 44 | *Impatiens balsamina* &<br>*Lantana camara* | 12<br>15 | Aritonang *et al.* 2019 |

### 2.5 Silver Nanoparticle Synthesis using Cyanobacteria

Cyanobacteria isolated from Muthupet mangrove, India, includes *Aphanothece sp, Oscillatoria sp, Microcoleus sp, Aphanocapsasp, Phormidium sp, Lyngbya sp, Gleocapsa sp, Synechococcus sp, Spirulina sp* which were set in compliance with their cellular mechanism of nano silver creation, and were investigated by UV-Vis. spectrophotometer, EDX and SEM. Silver nanoparticles were spherical-shaped and well-distributed without aggregation in solution with an average size of about 40-80 nm. *Plectonema boryanum*, UTEX 485, a filamentous cyanobacterium, was used to effectively biosynthesize silver nanoparticles by reacting with aqueous $AgNO_3$ solutions (560 mg/L Ag) at 25-100 ºC for up to 28 days. In solutions, the association of cyanobacteria with aqueous $AgNO_3$ facilitated the precipitation of spherical silver nanoparticles and octahedral silver platelets (up to 200 nm). Silver nanoparticles which were absorbed by cyanobacteria by metabolic processes such as nitrate utilization at 25°C and organics released from dead cyanobacteria at 25-100 ºC (Lengke *et al.* 2007).

### 2.6 Silver Nanoparticle Synthesis using Yeast

Ag-NPs have been fabricated extracellularly using an Ag-tolerant yeast strain MKY3, a silver-tolerant yeast species; when challenged with soluble silver in the log phase of growth, majority of silver precipitate extracellularly as elemental nanoparticles. Differential thawing methods for the sample and for separation of the metallic nanoparticles from the medium was used. Studies are still being carried on to search further for diverse groups of beneficial yeasts (Kowshik *et al.* 2003). Comparative study for nanoparticle synthesis using chemical and biological approach was carried out by Mishra *et al.* 2011. Silver nanoparticles were synthesized using *Candida guilliermondii.* The synthesis of silver nanoparticles was confirmed by color change and UV-Visible spectroscopic technique. Subsequently, the size, shape and morphology were characterized by XRD and microscopic techniques. The size range of gold nanoparticles was observed from 50 to 70 nm and for silver nanoparticles from 10 to 20 nm. The highest efficiency of antimicrobial activity of bio-synthesized silver nanoparticles against pathogenic bacteria *Staphylococcus aureus* was also demonstrated.




**Fig. 1: General outline to synthesize silver nanoparticles using plants.**

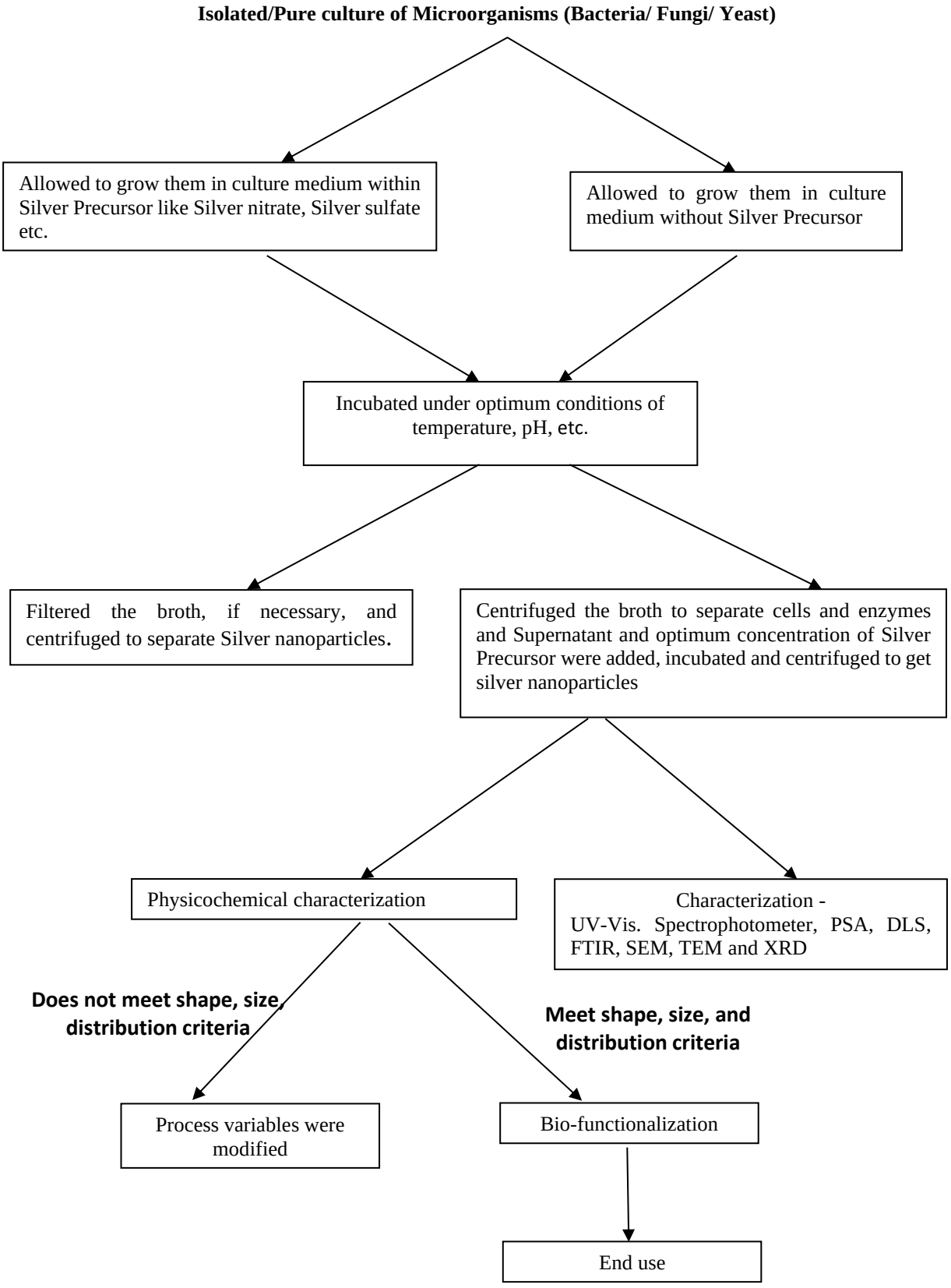



**Fig. 2: General flow chart for silver nanoparticles synthesis.**

### 2.7 Silver Nanoparticle Synthesis using Algae

Algae are diverse group in plant kingdom that is being explored for applications in nanotechnology. Besides the production of NPs, algae are also being explored for determining its nutritional value, efficacy in bio-diesel improvement as well as its vast potential for therapeutic application. Extracellular synthesis of silver nanoparticles by a brown seaweed, *Sargassum wightii*, was reported by Govindaraju *et al.* 2009. The antibacterial effect against bacteria isolated from the infected silkworm was found more potent when compared to the chemically synthesized silver nanoparticles and it is expected to be biocompatible. In another study, *Nannochloropsis oculata* and *Chlorella vulgaris* have the potential of nano-silver production in a culture medium containing 1 mM of $AgNO_3$ within 24 h. The size range of particles was approximately less than 15 nm.

## 3. CHARACTERIZATION OF SILVER NANOPARTICLES

After the nanoparticle synthesis, sophisticated instruments are required to be used to characterize size, shape and morphology of nanoparticles. A straight forward technique may simply detect the presence of nanoparticles; others may give the quantity, the size distribution or the surface area of the nanoparticles. Different techniques will suit different types of samples. For example, some techniques require the sample to be as an aerosol and others will use a suspension or liquid sample. There may be a sample protocol to be followed for the collection of the sample for analysis by a certain technique. There are techniques for *in situ* measurements of samples and others require treatment of the sample before analysis. Sometimes samples may not be able to withstand the required treatment and decompose or react. The amount of sample required can also vary and restrict the choice of technique. The particle size and size distribution of nanoparticles can be determined using numerous commercially available instruments. Instrument scan be used for the analysis of dry powders and powders dispersed in suspension. In general, there are two basic methods of defining particle size. The first method is to inspect the particles and make actual measurements of their dimensions. Microscopic techniques, for example, measure many dimensional parameters from particle images **(**Burleson *et al.* 2004). Since different techniques provide different information and accuracy, efforts have and will be made to standardize the way nanoparticles are measured to assess occupational exposure, health risks from products and environment risk. Table 4 indicates some of them.

**Table 4. Instrumentation for characterization of silver nanoparticles.**

| S. No. | Parameters | Methods / Instruments |
|---|---|---|
| 1 | Reduction to Nano size (primary) | UV- Visible Spectrophotometer |
| 2 | Particle Size distribution | TEM, SEM, XRD, Zeta Nano size Analyzer, DLS, AFM |
| 3 | Shape and Morphology | TEM, SEM, AFM |
| 4 | Surface area | BET |
| 6 | Composition/Functional Group | Mass Spectrometry, Spectroscopy (UV Vis, Raman, IR, NMR) |
| 7 | Hydrophobicity | MATH |
| 8 | Surface charge | Suspension/Solution Zeta potential |
| 9 | Crystal structure | TEM, XRD |
| 10 | Porosity | MIP |
| 11 | ROS generation capacity | DTT, Furfuryl alcohol. assay, Nano sensors |

Apart from these, offline character tools like SMPS, TDMA, APS, O NSAM, etc. are available for characterization of nanoparticles. (Aerosol and Air Quality Research Lab)

## 4. ENVIRONMENTAL APPLICATION OF SILVER NANOPARTICLES

Silver nanoparticles synthesized using several methods have gained special attention because they have potential applications in many fields such as antimicrobial agent, as catalyst, sensors, disinfectant and so on. Apart from these, silver nanoparticles represent excellent biocompatibility and low toxicity. Nanoscience and nanoparticles are thus found to have new approaches to solve environmental problems.

### ➢ As Anti-Microbial Agent

The silver nanoparticles show very efficient antimicrobial characteristics as compared to other salts and bulk silver due to their advanced properties like extremely large surface area, smaller size, higher reactivity, which provide better contact with microorganisms and destruct their structure (Rai *et al.* 2009). In the 17$^{th}$ century silver nitrate was used for the

treatment of fistulae from salivary glands, veneral diseases and bone and perianal abscesses (Klasen *et al.* 2000a; Klasen *et al.* 2000b). In the 19th century silver nitrate was used to remove granulation tissues to allow epithelization and promote crust formation on the surface of wounds. Till now varying concentrations of silver nitrate was used to treat burns (Klasen *et al.* 2000a; Klasen *et al.* 2000b; Castellano *et al.* 2007). In 1881, Carl S. F. Crede treated *Opthalmia neonatorum* using silver nitrate eye drops; had designed silver impregnated dressings for skin grafting (Klasen *et al.* 2000a; Klasen *et al.* 2000b). After the 1940s, the use of silver for the treatment of bacterial infections minimized due to discovery of penicillin antibiotic (Chopra *et al.* 2007). But in the 1960s silver again came in picture when Moyer introduced the use of 0.5% silver nitrate for the treatment of burns. He proposed that this solution does not interfere with normal tissues and epidermal proliferation and possess antibacterial property against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Moyer *et al.* 1965; Bellinger *et al.* 1970). The combined activity of silver nitrate with sulfonamide to form silver sulfadazine cream, which served as a broad-spectrum antibacterial agent, was used for the treatment of burns. The effectiveness of silver against bacteria like *E. coli, S. aureus, Klebsiella sp.* and *Pseudomonas sp.* was also found. It also possesses some antifungal and antiviral activities (Fox *et al.* 1974). Nowadays, due to the emergence of antibiotic-resistant bacteria and limitations of the use of antibiotics, the clinicians have returned to silver wound dressings containing varying level of silver (Gemmell *et al.* 2006; Chopra *et al.* 2007).

The exact mechanism of silver nanoparticles as the antimicrobial agent is not clearly known. There are however various theories on the action of silver nanoparticles on microbes to cause the microbiocidal effect. Silver nanoparticles have the ability to bind to the bacterial cell wall and penetrate it, thus by causing structural changes in the cell membrane they change the permeability of the cell membrane and lead to death of the cell (Sondi *et al.* 2004). Silver nanoparticles are also able to form free radicals by which the cells die. This study was carried out using electron spin resonance spectroscopy. It is also suggested that these free radicals have the ability to damage the cell membrane and make it porous which can also ultimately lead to cell death (Danilczuk *et al.* 2006). It has also been proposed that of the nanoparticles release some silver ions (Feng *et al.* 2000), and these ions can interact with the thiol groups of many vital enzymes and lead to inactivation of the enzymes and thus destroy the function of bacterial cell (Matsumura *et al.* 2003). Silver can act as soft acid, and there is a natural tendency of an acid to react with a base; in this case, a soft acid react with a soft base (Morones *et al.* 2005). The cells are majorly made up of sulfur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the adverse reaction and subsequently cause the cell death. It is also a fact that the DNA has sulfur and phosphorus. Silver nanoparticles also interfere in the process of DNA replication of the bacteria and thus terminate the microbes. It has also been suggested that the nanoparticles can modulate the signal transduction in bacteria. It is a fact that phosphorylation of protein substrates in bacteria affects the bacterial signal transduction. Dephosphorylation is noted only in the tyrosine residues of gram-negative bacteria. The phosphor-tyrosine profile of bacterial peptides is altered by the nanoparticles. It was reported that the silver nanoparticles show dephosphorylation activity to the peptide substrates on tyrosine residues, which leads to signal transduction inhibition and thus they stop the growth of bacterial cell. Further research is required on the topic to thoroughly establish the claims (Hatchett *et al.* 1996; Shrivastava *et al.* 2007).

### ➢ As Anti-Fungal Agent

Various forms of silver ions and silver nanoparticles were tested to examine the antifungal activity on two plant-pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea*. They were carried out in *in vitro* petri dish assays which indicated that silver ions and nanoparticles had a significant effect on the colony formation of these two pathogens. They have also found that effective concentrations of the silver compounds inhibiting colony formation by 50% (EC50) were higher for *B. sorokiniana* than for *M. grisea* and the inhibitory effect on colony formation significantly diminished after silver cations were neutralized with chloride ions. Growth chamber inoculation assays further confirmed that both ionic and nanoparticle silver significantly reduced these two fungal diseases on perennial ryegrass (*Lolium perenne*). Several types of plant pathogenic fungi develop Sclerotia, which is asexual, resting and melanized. In Sclerotia hyphae become interwoven, aggregated, dehydrate and can be observed on infected plant tissues. To inhibit such sclerotia hyphal growth, Min *et al.* 2009 have obtained silver nanoparticles from BioPlus Co. Ltd. (Korea) of average size of 4 to 8 nm. Three different sclerotium-forming fungal species namely *Rhizoctonia solani*, *Sclerotinia sclerotiorum and S. minor*, have been selected for analysis. Analysis demonstrated that silver nanoparticles remarkably inhibit the hyphal growth in a dose-dependent manner. Different antimicrobial efficiency of the silver nanoparticle was observed among the fungi on their hyphal growth in the following order:

*R. solani* > *S. sclerotiorum* > *S. minor*. Tests for the sclerotial germination growth revealed that the nanoparticles showed significant inhibition effectiveness. Researchers have also reported the sclerotial germination growth of *S. sclerotiorum* was most effectively inhibited at low concentrations of silver nanoparticles. Silver nanoparticles of size 60 nm was synthesized using raspberry extract. The antifungal activity of silver nanoparticles was demonstrated against plant pathogenic fungi such as *Cladosporium cladosporioides and Aspergillus niger*. The efficiency of silver nanoparticles was proved even at very low concentration. It has been found that suspension having 50 ppm concentration of silver nanopartilces inhibits the growth of *Cladosporium cladosporioides* by 90% and the same concentration causes 70% growth inhibition of *Aspergillus niger* (Pulit *et al.* 2013).

### ➢ For Water Purification

Silver nanoparticles are probably used for disinfection. It is applied in over 100 consumer products such as commercial home water purification systems, including Aquapure, Kinetice and QSI-Nano as an antimicrobial agent. Researchers had synthesized silver nanoparticles having the size of 11.2 nm using *Lactobacillus fermentum*. The antiviral properties of biologically synthesized silver nanoparticles were also demonstrated on the murine norovirus 1 (MNV-1), a model organism for human noroviruses. Silver nanoparticles were applied to an electropositive cartridge filter (Nano Ceram) to evaluate its capacity for continuous disinfection (De Gusseme *et al.* 2010). Silver nanoparticle embedded porous ceramic nanocomposite was developed by exposing the colloid solution of silver nanoparticles to a porous ceramic modified by a aminosilane coupling agent, 3-amino-propyltriethoxysilane (APTES), overnight. To increase the fixation of silver nanoparticles, the developed nanocomposite was immersed in ultrasonic bath for 15 minutes. There is no much loss of silver nanoparticle occurred after immersion. It was reported that thus developed silver nanoparticle decorated with porous ceramic demonstrated effectiveness in prohibiting the growth of *Escherichia. Coli* and other pathogenic bacteria (Lv *et al.* 2009).

### ➢ For Surface Disinfection

The bactericidal coating on surfaces has attracted increasing interest to protect human health and the environment. Among them, Ag-NPs-embedded paints are of particular interest due to their potential bactericidal activity. An environmental-friendly approach was reported to synthesize metal nanoparticle-embedded (like silver nanoparticle-embedded) paint, in a single step, from common household paint. No any external reducing or stabilizing agents was used during the nanoparticle synthesis. Naturally occurring oxidative drying process was used in oils, which involves free-radical exchange for reducing metal salts and dispersing metal NPs in the oil media. These well-dispersed metal NPs-in-oil dispersions can be used directly on different surfaces such as wood, glass, steel and different polymers. The results found that the surfaces coated with silver-nanoparticle paint showed excellent antimicrobial properties by killing both gram-positive human pathogens (*S. aureus*) and gram-negative bacteria (*E. coli*) (Carlson *et al.* 2008).

### ➢ As Environmental Sensors

The determination of hydrogen peroxide ($H_2O_2$) in trace concentrations is a very important and necessary task, because $H_2O_2$ is the product of reactions catalysed by a large number of oxidase enzymes. Silver nanoparticles have been synthesized with average diameter 14.4±3.3 nm using ultrasound-mediated reduction of silver nitrate by D-glucose. These silver nanoparticles exhibit a catalytic activity in the reduction of hydrogen peroxide ($H_2O_2$). The degradation of silver nanoparticles, induced by the catalytic decomposition of hydrogen peroxide, causes a considerable change in the absorbance strength of localized surface plasmon resonance band depending on the $H_2O_2$ concentration. On the basis of this mechanism, a LSPR-based sensor for hydrogen peroxide is proposed. This sensor has a very good sensitivity and a linear response over the wide concentration range of $10^{-1}$ to $10^{-6}$ mol/L $H_2O_2$. Furthermore, the quantification limit of this sensor is found to be 0.9M $H_2O_2$, which is lower than certain enzyme-based biosensors. This LSPR-based optical sensor for hydrogen peroxide has the potential to be applied for the determination of other reactive oxygen species as well (Vasileva *et al.* 2011).

Green synthesized silver nanoparticles were used for colorimetric sensing of toxic metal cation such as $Hg^{+2}$ and $Pb^{+2}$. Silver nanoparticles have been synthesized using fresh neem extract, neem bark as well as dried neem leaf extract during their study. Researchers have synthesized AgNPs using fresh neem leaf extracts (NF-AgNPs). Fresh neem leaf extracts was found to detect $Hg^{2+}$ selectively, whereas sun-dried neem leaf extract-based AgNPs (ND-AgNPs) were observed to detect $Hg^{2+}$ and $Pb^{2+}$ at micro-molar concentrations. Neem bark extract-based AgNPs exhibited selective colorimetric sensing of $Hg^{2+}$ and $Zn^{2+}$. Similarly, AgNPs

synthesized from mango leaves (fresh, MF-AgNPs; sun-dried, MD-AgNPs) and green tea extracts (GT-AgNPs) showed selective colorimetric sensing of $Hg^{2+}$ and $Pb^{2+}$ ions. AgNPs obtained using pepper seed extracts were found to detect $Hg^{2+}$, $Pb^{2+}$ and $Zn^{2+}$ selective colorimetric sensor properties. Importantly these green synthesized AgNPs selectively detected the presence of hazardous metal ions in aqueous solutions across a wide range of pH values (2.0–11), which is a highly desirable attribute from the perspective of different sources of water pollution. The selective colorimetric sensing of $Hg^{2+}$, $Pb^{2+}$ and $Zn^{2+}$ ions by green synthesized AgNPs over a wide pH range demonstrates the multi-functional utility of plant extracts in green nanotechnology and environmental sensor applications (Karthiga *et al.* 2013). In another study, silver nanoparticles have been applied to detect copper from waste water without any surface modification. They have used neem leaf extract to synthesize silver nanoparticles. The uniform size of silver nanoparticles with an average diameter of 15−20 nm along with the mono-dispersion was confirmed using TEM. Bio-mediated synthesized silver nanoparticles were found to be successful in detecting even the minimal amount of heavy metal copper (II) ion and exhibited excellent specific metal ion selectivity.

Recently, researchers have developed silver nanoparticle films as hydrogen sulphide ($H_2S$) sensors. They have found that the reaction between AgNP film and $H_2S$ gas determine the $H_2S$ gas emission from degraded materials' concentration under ambient conditions (Chen *et al.* 2014). Ammonia gas is widely used in many human and industrial activities. Ammonia is corrosive, colourless toxic gas with a sharp odour, and exposure to ammonia is extremely irritating to the skin, eyes and lungs. Due to such reasons ammonia monitoring is very important. To solve such problems, researchers have developed sensors based on silver nanoparticles. Different concentration of silver was taken to synthesize silver nanoparticles by UV irradiation method and poly-methacrylic acid (PMA). AgNPs/PMA samples were loaded with multi-walled carbon nanotubes (MWCNTs) and the production of conductive films was allowed which was suitable for sensing applications. According to the analysis, the developed silver nano-sensors are found to be active at low temperature, exhibiting fast response/recovery times in a wide range of detection levels from ppm to v/v (%) (Cannilla *et al.* 2014). In another study nano sensor based on silver nanofilm have been designed during their research to detect the hydrogen sulfide gas. Hydrogen sulfide gas is found naturally and during industrial processes. It is a highly toxic gas and high concentration also causes death; hence, it is very essential to detect the presence of gas. Peak intensity of surface Plasmon resonance (LSPR) absorption of silver nanoparticles was monitored as the sensor responded to hydrogen sulfide gas ($H_2S$). The fabricated AgNP films were found to be very sensitive to the presence of $H_2S$ with a gas concentration below 1 part per million by volume (ppmv) range. The intensity of Localized Surface Plasmon resonance peak of the AgNP films was found to decrease rapidly upon exposure of hydrogen sulfide gas (Chen *et al.* 2013).

### ➢ For Food Preservation

In addition to the antimicrobial coatings for household paints, medicinal and therapeutic fields mentioned above, AgNPs-coated paper may be useful for preventing microbial growth for longer periods in food preservation by acting as a reservoir for gradual ionic silver release from the surface to the bulk while also preventing growth on the surface. The sono-chemical coating is a basic technique for developing a colloidal silver coating on paper using ultrasonic radiation (Arora *et al.* 2009). It was revealed that by varying the precursor concentrations and reaction times, the thickness of the AgNPs coating and the particle size can therefore be controlled to a great extent. Moreover, these AgNPs-coated papers have been shown to possess microbiocidal properties against the gram-negative *E. coli* as well as against the gram-positive *S. aureus* bacteria. The findings revealed that Ag-NPs-coated paper with a long shelf life and antifouling properties could be used as a packaging medium in the food industry. The colloidal silver nanoparticle-coated paper was created using the ultrasound radiation technique. Uniform coatings ranging from 90 to 150 nm in thickness have been obtained using the ultrasound process, depending on the precursor concentrations and ultra-sonication duration. It has been shown that the silver coatings obtained are extremely durable, with very little leakage of silver from the coated surfaces, which is critical for the active lifespan of such coated papers. The coated paper has a high level of antibacterial activity against both gram-positive and gram-negative bacteria, implying that it could be used as a food storage material to extend shelf life and avoid cross contamination. The conductivity of the standardized coating of metallic silver could be used to build paper-based biosensors for rapid and compact biochemical detection procedures (Gottesman *et al.* 2011).

### ➢ For Dye Removal from Waste Water

Researchers have found that use of nanotechnology and nanomaterials has better result than other techniques used in waste water treatment because of its smaller size, high surface area and higher reactivity than bulk materials. Researchers have reported the decolorization of the Congo red dye by silver

nanoparticle which were synthesized using *Aspergillus niger* having the size of 80 nm in diameter and its comparison with plain culture of *Aspergillus niger*. These particles were then checked for their efficiency to decolorize the dye. It was found that the nanoparticle efficiently decolorized the dye within 48 hours of incubation whereas the plain culture (Control) partial decolorization continued upto 78 hours. In another Congo red dye removal study, Nithya *et al.* (2011) had concluded that the silver nanoparticle decolorize Congo red dye better than the plain culture of microorganisms. They find out the *Pleurotus sajor caju* silver nanoparticle effectively decolorized 78% of the dye in 24-hour incubation and the dye was fully decolorized after 35-hour incubation. Whereas the plain culture (*Pleurotus sajor caju*) was able to degrade only 67% of the dye under same incubation conditions and complete decolorization was observed after 48-hour incubation. The development of such particles may be considered a breakthrough in the field for the efficient cleanup of the dyes on large scale process since they are easy to synthesize on large scale and cost effective.

The application of silver nanoparticles have been reported to remove Congo red dye contamination as well as application as antimicrobial agent against the pathogenic strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* - ATCC 25923, *Salmonella typhi* - ATCC 6539 and *Klebsiella pneumoniae* - NCIM 2883. Silver nanoparticles of size in the range of 20 – 80 nm were successfully synthesized using the pods of *Phaseolus vulgaris*. They were found that the silver nanoparticles were able to decolorize Congo red dye up to 50%, while Mordant Black 17 was minimally decolorized which may be due to their complex structure. The catalytic activity of silver nanoparticles was studied to remove methyl orange dye in the presence of solar light. Silver nanoparticles were synthesized using *Hypnea musciformis* (Wulfen) *J.V. Lamouroux*. Silver nanoparticles were found within the range of 2 to 55.5 nm in size confirmed by atomic force microscope. Photocatalytic degradation of methyl orange was measured spectrophotometrically by silver nanoparticles under visible light illumination. The results proved that biosynthesized silver nanoparticles using *H. musciformis* was found to be impressive in degrading methyl orange (Ganapathy Selvam *et al.* 2015).

### ➢ As Catalyst

Silver nanoparticles also show the catalytic activity. Catalytic activities of silver nanoparticles differ from the chemical properties of the bulk silver. For instance, the research work have been stated on the bleaching of the organic dyes by application of potassium peroxodisulphate in aqueous solution at room temperature is enhanced strongly by the application of silver containing nanoparticles (Köhler *et al.* 2008). Apart from this, AgNPs was found to catalyze the chemiluminescence from luminol–hydrogen peroxide system with catalytic activity better than Au and Pt colloid (Guo *et al.* 2008). The silver nanoparticles synthesized by microwave assisted method in the presence of hexamine as reducing agent was reported. The biopolymer pectin was used for silver nanoparticles stabilization. Spherical shape with an average diameter of 18.84 nm was confirmed by TEM analysis. The pectin stabilized silver nanoparticles showed advanced catalytic activity for the reduction of 4-nitrophenol to 4-aminophenol by $NaBH_4$. The rate of the reduction of 4-nitrophenol to 4-aminophenol was found to increase with increasing temperature and the activation energy was found to be 47.3 kJ $mol^{-1}$ . Moreover, silver nanoparticles supported halloysite nanotubes (Ag/HNTs), with Ag content of about 11% was used to catalyze the reduction of 4-nitrophenol with $NaBH_4$ in alkaline aqueous solutions (Liu *et al.* 2009). The discovery and development of efficient catalysts for carbon dioxide ($CO_2$) electro-reduction is one of the grand challenges to energy department. Recently, scientists have reported the rate of conversion of $CO_2$ to Carbon monoxide (CO) per unit surface area is about 10 times higher on 5 nm silver nanoparticles than on bulk silver. Even though measurements on single crystal catalysts show much smaller variations in rate, they have also found that the catalytic activity increases with decreasing particle size until a certain particle size, here, 5 nm, and that the activity drops when going to even smaller nanoparticles size up to 1 nm (Salehi-Khojin *et al.* 2013)**.** Recently, researchers at University of Saskatchewan, Canada have found the efficiency of silver nanoparticles as catalyst for borohydride-induced reductive degeneration of Eosin-Y, a dye that has been classified as a Class 3 carcinogen by the International Agency for Research on Cancer. They have reported that silver nanoparticles having size from 15.7±6.1 nm to 3.7±0.8 nm catalyzed degradation of EY dye in the presence of $LiBH_4$ in a $PR_4Cl$ IL/THF medium, increase almost tenfold in rate as compared to the uncatalyzed counterpart of the reaction (Banerjee *et al.* 2014). In another research, silver nanoparticles have been synthesized using starch by autoclaving method. α-amylase was immobilized on silver nanoparticles to reduce the starch. An increase of 4.7-fold in reducing sugar formation and 1.5 times faster enzyme activity confirmed the catalytic activity of silver nanoparticles as a nanocatalyst. This study represents that the nanoparticles may have a significant role in the field of nanocatalysis, promising potential use in starch industries for rapid degradation of the complex molecule

to simpler ones by immobilizing the enzymes onto the surface of nanoparticles (Ernest *et al.* 2012). All above research work proves the efficiency of silver nanoparticles to remove pollutants from the environment and to solve the energy problems.

### ➢ To Remove Pesticides

In present day scenario, pesticides are widely used for pest control in agriculture, because of which, the drinking water sources (both surface and ground water) gets contaminated. Majority of the pesticides are very difficult to biodegradation and they are found to be carcinogenic in nature even at very trace levels. Till now, various conventional methods employed for removal of pesticide are surface adsorption, photo-catalysis, membrane separation and biodegradation. As these methods have several disadvantages such as time consumption and expensive, they are not feasible. Elimination of toxic pesticide from environment is major problem. To solve such problems, researchers had carried out their study to estimate the mineralization potential of silver nanoparticles supported on polyurethrene foam (PUF). They have synthesized silver nanoparticles (60-90 nm in size) using tri-sodium citrate as reducing agent. The pesticide concentration found to be directly proportional to mineralization time. However, it was found that the concentration of pesticide affect the mineralization activity of silver nanoparticles (Manimegalai *et al.* 2011). Recently, researchers have found the silver nanoparticles enhance the adsorption efficiency of cellulose acetate membrane for chlorpyrifos pesticide removal. Silver nanoparticles were synthesized using glucose as a reducing agent was found in the range of 60–75 nm and incorporated on cellulose acetate membrane. It is observed that the silver nanoparticles can effectively mineralize the pesticides, and the concentration of nanoparticles enhances the rate of mineralization. While concentration of pesticide increase mineralization time (Manimegalai *et al.* 2014).

Adsorption technology is widely used to remove toxic pollutants from waste water and environment. Chitosan as a non-toxic and biodegradable natural polymer was embedded with silver nanoparticles to prepare silver nanocomposite. Nanocomposite was prepared to investigate the adsorption and release behaviors of the pesticide, Atrazine. The varying concentration from 1 to 25 ppm and time dependencies of adsorption for the pesticide removal were studied. They have observed that at 2.0 g dosage of nanocomposite, 98% of pesticide having the 1 ppm concentration was removed. They have offered a convenient and cost effective means of removing pesticides from water (Saifuddin *et al.* 2011).

### ➢ Silver Nanocomposites

To eliminate toxic dyes from textile waste effluents, researchers have developed silver nanocomposite. Firstly, silver nanoparticles were synthesized using *Ocimum tenuiflorum* (Black Tulsi) leaf extract. According to characterization data by size was found between 20 to 40 nm. To prepare nanocomposite they have used pure soil and solution of silver nanoparticles was added slowly, then agitated the flask for 24 hours to allow the coating of nanoparticles to soil particles. Then supernatant was discarded and composite mixture was dried and used for dye decolorization. This nanocomposite was determined to possess higher adsorption efficiency in comparison to soil for the removal of reactive turquoise blue dye under same experimental conditions. Moreover using these Ag-nanocomposites as adsorbent helped in achieving about 96.8% removal of reactive turquoise blue dye from effluent solution. Same research work was also done to remove the Crystal violet dye contamination from the waste water. Silver nanoparticles have been synthesized using leaf extracts of *Azadirachta indica*. Ag-nanocomposite as adsorbent helped in achieving about 97.2 % removal of crystal violet dye from the effluent solution. Researchers have found that green algae capture sun-light (Satapathy *et al.* 2015).

### ➢ Silver- Zeolite Nanocomposite

Nanocomposite can be formed by a bulk matrix in the form of micro particles and nanostructure/nanoparticles material which is incorporated to form the composite material. The combination of nanostructured material and micro structured material results into the improvement of their basic physico-chemical properties such as mechanical properties. To eliminate the load of microorganisms from the waste water, the composite material was prepared, based on zeolite incorporated with silver nanoparticles. Silver nanoparticles have been synthesized using Tollens process with the average diameter of approx. 30 nm. Then silver nanoparticles were adsorbed on the surface of several types of synthetic zeolites. Researchers have demonstrated the enhanced antibacterial activity of nanocomposite (Mpenyana-Monyatsi *et al.* 2012). In another study, the novel properties of silver silica (zeolite) nanocomposite material were studied as an antimicrobial agent. The efficiency of silver nitrate and silver zeolite nanocomposite was compared as antimicrobial agent. The silver silica nanocomposite

material was synthesized using an industrial flame spray pyrolysis process. The silver nanoparticles embedded on silica matrix were characterized and size was found in the range of 1 to 10 nm. They have concluded that developed silver-silica nanocomposite material exhibited very good antimicrobial activity against a wide range of microorganisms (Egger *et al.* 2009).

### ➢ To Remove Air Pollutants

Petrol-driven vehicles exhaust large amounts of toxic gases like $SO_2$ and $NO_2$. Living beings are facing the problems of such toxic gases. To overcome such problems, researchers have developed a catalytic tube which consists of AgNPs for controlling air pollutant $SO_2$ caused by petrol-driven vehicles. They have synthesized silver by *Ficus elastica* using the alternative energy source of microwave irradiation. The formation of silver nanoparticles was confirmed by SEM. Investigation carried out on adsorption studies revealed that it is following pseudo-second order kinetics. Optimum dosage for 98% removal of $SO_2$ is equal to eco-friendly nontoxic energy saving method employed in the synthesis of nanoparticles. In another investigation the researchers had found that the silver nanoparticles synthesized using leaf extract of *Vinca rosea* could eliminate 98% sulphur dioxide from air**.**

### ➢ To Solve Energy Issues

Due to explosion of population and technology, energy generation is becoming a great demand. To fulfill the energy demand research is moving towards the development of bio fuel. Some bacteria and algae are reported which produce fatty acids and glycerin. Microbial enzymes convert fatty acid to biofuel. Algae require very specific range of visible light, namely blue-violet spectrum. They have found that silver nanoparticle suspension sped up the algal growth by 30 percent by increasing the visible light's blue range. They have proposed that nanoparticle size and concentration affect the algal growth.

Development of clean energy sources is necessary to mitigate coming climate change and shortage of readily available fuel. Among all the possible fuel sources, hydrogen offers potential alternative as a clean, renewable energy source. Among all, dark fermentative bi-hydrogen production method is reliable method for hydrogen production. To investigate the efficiency of silver nanoparticles over the fermentation ability of bacteria, silver nanoparticles were added into anaerobic batch reactor to enhance microbial activity for the fermentative hydrogen production. Sodium citrate was used as reducing agent for silver nanoparticle synthesis obtained nanoparticles were approximately 15 nm in size. The varying concentration of silver nanoparticles from 0 to 200 nmol $L^{-1}$ and inorganic nitrogen 0 to 4.125 g $L^{-1}$ were supplied to glucose-fed mixed bacteria. It was observed that the yield of acetic acid increased and yield of ethanol decreased. They have demonstrated that silver nanoparticles increase the hydrogen yield and reduce the lag phage for the hydrogen production (Zhao *et al.* 2013). Moreover, an anatase titanium dioxide thin film decorated with silver nanoparticles was developed. Silver nanoparticles embedded film was used to cleave water to produce hydrogen gas in the presence of methanol as a hole scavenger. They have demonstrated that $Ag/TiO_2$ nanocomposite film showed the high efficiency for hydrogen gas production. The developed nanocomposite film was having with the advantage of regeneration and reuse. It was studied that without the silver nanoparticles, hydrogen production decreased dramatically to 4.65 µmol/h/g at room temperature (Alenzi *et al.* 2010). Formic acid (HCOOH) contributes high energy density and non-toxicity and it is safe to handle. Formic acid (HCOOH) is a potential source of hydrogen for fuel cells. It requires catalyst for production. Researchers have reported that silver nanoparticles coated with a thin layer of palladium atoms can enhance the hydrogen gas production from formic acid at ambient temperature. It was demonstrated that silver nanoparticles promote Pd electronically and enhance the catalytic properties (Tedsree *et al.* 2011). The study on energy saving electrolysis of sodium carbonate to sodium bicarbonate and sodium hydroxide for alumina production has been carried out. During the research work silver nanoparticles was employed to catalyze oxygen reduction cathode to substitute conventional hydrogen evolution method. Silver nanoparticles ranging from 15 to 50 nm have been synthesized. It has been demonstrated that the application of AgNPs ORC allows reduction of cell voltage and electric energy consumption of up to 39.8% at 100 mA $cm^{-2}$ (Tang *et al.* 2013). Researchers have proposed a technique incorporated with nanoparticles which help to improve the efficiency of photovoltaic and photo catalysis. They have found that metallic nanoparticles such as silver nanoparticles and gold nanoparticles enhance the power efficiency of both solar cells and photo catalytic thin film electrodes. They have reported that the surface plasmon resonance of nanoparticles play a crucial role to effect the energy conversion process.

## 5. CONCLUSION

The unique physical and chemical properties of silver nanoparticles only increase the efficacy than bulk silver. Generation of silver nanoparticles by physical-chemical methods is very tedious, costly and lead to second pollution; whereas, their synthesis through microbes such as silver resistant bacteria, fungi and plants is a fast, reliable and an eco-friendly approach. Silver resistant bacteria indicate their survival in presence of silver, but researchers have to find out whether they convert silver to nanoparticle or not. This is a new approach to synthesize silver nanoparticles. Analytical techniques, such as ultraviolet-visible spectroscopy, X-ray powder diffraction, transmission electron microscopy, scanning electron microscopy and zeta potential measurements are applied to confirm the synthesis and to characterize the nanoparticle size and morphology. Silver has always been an excellent antimicrobial and has been used for the purpose for ages. Apart from this, silver nanoparticles are also applied to solve some environmental pollution problems like removal of toxic dyes and heavy metals, water purification, environmental sensor, solving energy problems and so on. In some studies, due to advanced properties of silver nanoparticles like smaller size and higher surface area to volume ratio, a new technology has been developed known as nano-bioremediation, which enhances the process of bioremediation. Hence, this current review concludes with a hope and prayer that there would be mechanisms devised to nullify any toxicity caused by nano silver to humans and the environment so that the unique properties of this substance can be put to greater use for human betterment and to address the environmental issues.

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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