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RESEARCH ARTICLE

Silver Nanobiotechnolgy-Recent Advances

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ABSTRACT

Nanobiotechnology is emerging day by day in the today's technology. Here we are giving the different synthesis procedures of silver nanoparticles and the results of their characterization. All the particles obtained shows nearly similar characteristics. Plant mediated , Algae mediated and fungal mediated are the three different souces taken here. Finally applications of these nanoparticles are mentioned. These nanoparticles are quite impressive and have wide bandwidth of applications.

INTRODUCTION

Nanoparticles have emerged as a new field of research and are quite interesting. Nano-Biotechnology is an interface of nanotechnology and biotechnology which deals with the use of nanotech trends in biotech. Nanoparticles of noble metals such as gold, silver, and platinum, are widely applied inproducts that directly come in contact with the human body such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications. Nanoparticle exhibit completely new or improved properties as compared to the larger particlesof the bulk material that they are composed of based on specific characteristics such as size, distribution, and morphology. Silver is a white and brilliant metal, positioned at 47 in the periodic chart with Ag, meaning "argentum", as its chemical symbol. Silver has beenwidely utilized for thousands of years in human history; its applications include jewels, utensils, currency, dental alloy, photography and explosives.

It is well known that biological entities like microorganisms and living cells are the best examples of machines that possess operating parts at the nanoscale level and perform a number of jobs ranging from generation of energy to extraction of targeted materials at a very high efficiency. The utilization of such microorganisms like bacteria, fungi, herbal extracts and yeasts in the synthesis of nanoparticles is a relatively recent activity. It is known that certain bacteria, yeasts and now fungi play an important role in remediation of toxic metals through reduction of the metal ions so long as they are not toxic in other ways. Biosynthesis of nanoparticles has received considerable attention due to the growing need to develop environmentally benign technologies in material synthesis. Interdisciplinary research has widened the horizons of material research, drawing new inspirations from biological systems. The towering environmental concerns had triggered the researchers to device novel methods of synthesizing the nanomaterials in biological systems such as bacteria, fungi and plants, termed as "green chemistry" approaches. Recently, the utilization of biological systems has emerged as a novel method for the synthesis of nanoparticles. While microorganisms suchas bacteria, actinomycetes and fungi continue to be investigated in metal nanoparticles synthesis, the use of parts of whole plants in similar nanoparticles synthesis methodologies is an exciting possibility that is relatively unexplored and under exploited. Here in this paper synthesis of silver nanoparticles from different biological sources like *Jatropha curcas*, *Cinnamon zeylanicum* bark extract and powder mediated, *Acalypha indica* leaf extracts (plant mediated), *Alfalfa sprouts* (algae) and *Trichoderma Reesei* (fungus). These nanoparticles are characterized and then further tested for their activity over the micro-organism growth. All the particles synthesized from different sources show specific diversity in their rection with the micro-organisms which is described here in detail.

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EXPERIMENTAL

Plant mediated Synthesis

Synthesis via *Jatropha Curcas*

First cut the green stems of *J.Curcas* to obtain the crude latex. Obtained milky white latex was stored at -25^0 C. Another solution of $AgNO_3$ is prepared too [Ref. 1]. All the solutions were prepared using triply distilled deionized water [Ref. 2,3]. In a typical reaction procedure, 1 ml crude latex was diluted to300 ml using triply distilled de-ionized water to make it 3% and 20% of this latex solution was mixed with 20 ml 5 x10^{-3} aqueous silver nitrate solution. Mixture was heated at 85^0 C with constant stirring for 4 hrs in oil bath and silver nanoparticles were obtained gradually.

Synthesis via *Cinnamon zeylanicum* bark extract and powder

C. zeylanicum bark was purchased from a local market, washed to remove any impurities and dried under sunlight for a week to completely remove the moisture [Ref. 4]. The bark

was cut into small pieces, powdered in a mixer and then sieved using a 20-mesh sieve to get uniform size range. The final sieved powder was used for all further studies. For the production of extract, 2.5 g of CBP was added to a 500mL Erlenmeyer flask with 100mL sterile distilled water and then boiled for 5min. For the Ag nanoparticle synthesis from CBP, 100, 500 and 1000mg of CBP was carefully weighed and added to 50mL of 1mM aqueous AgNO3 solution in a 250mL Erlenmeyer flask. The flasks were then incubated in a rotary shaker at 160rpm in the dark at 25 ◦C. For CBPE, 1, 2.5 and 5mL extract was used for the biosynthesis of Ag nanoparticles from 50mL of 1mM aqueous AgNO3 solution [Ref. 5,6].

Synthesis via *Acalypha indica*

Aqueous extract of *A. indica* was prepared using freshly collected leaves (10 g). They were surface cleaned with running tap water, followed by distilled water and boiled with 100 ml of distilled water at 60 ◦C for 5 min [Ref. 7]. This extract was filtered through nylon mesh, followed by Millipore filter (0.45 µm). For synthesis of silver nanoparticles, the Erlenmeyer flask containing 100 ml of AgNO3 (1mM) was reacted with 12 ml of the aqueous extract of *A. indica*. This setup was incubated in dark (to minimize the photoactivation of silver nitrate), at 37 ◦C under static condition. A control setup was also maintained without *A. indica* extract [Ref. 8,9].

Algae mediated synthesis (*Alfalfa sprouts*)

Mesa variety alfalfa seeds were immersed for 30 min in a Captan solution (2 g/L) in order to avoid fungal contamination and then washed three times with sterilized (autoclaved) deionized (DI) water. Under a control air hood (laminar flow hood) and a sterile environment, the seeds were transferred to mason jars containing 75 mL of agar solidified nutrient media The nutrient media contained both the macro-and micronutrients that the plants require to grow [Ref. 10]. The only chloride salts added to the medium were $MnCl_2.4H_2O$ and $FeCl_3$; the other chloride salts were respectively substituted by either their nitrate or sulfate salts to avoid silver chloride precipitation. A 5 g mass of Difcobacto agar was added to every liter of nutrient solution prepared [Ref. 11, 12]. The source of silver ions in this study was supplied as silver nitrate ($AgNO_3$), and the concentrations used were 0, 40, 80, 160, and 320 mg of silver per liter of solution (or parts per million, ppm). Before the addition of agar, the medium was adjusted to pH 5.8 (the physiological pH). After the alfalfa seeds were spread out, all jars were covered with plastic wrap previously treated with formaldehyde (3% v/v) in order to avoid contamination and permit light to pass through it. After planting, all jars were set in a growth chamber for a 12 h light/12 h dark photoperiod at 25 and 18 °C, respectively. Four replicates of each treatment were prepared for quality control and statistical purposes. The alfalfa plants were harvested 9 days after germination. After harvesting, a sample of each treatment was washed three times with DI water and then immersed along with an agar sample in liquid nitrogen for 40 min. Finally, samples were placed in a freeze-dryer (Labconco, Freeze drying system/ Freezone 4.5) for 2 days. The purpose of this was to dehydrate and eventually pulverize the biomass into a fine homogeneous powder using a pestle and mortar [Ref. 13].

Fungi Mediated Synthesis (*Trichoderma Ressei*)

In this study, six different strains of *Trichoderma reesei* were used for experimentation. The fungal inoculates were prepared in potato dextrose agar (PDA) media (a common microbiological media for culturing fungus) at 28°*C* in Petri plates. For the synthesis of nanoparticles, the fungus was grown in 200 *mL* bottles each containing 100 *mL* of GC medium (composed of 0.5 % glucose and 0.4 % casein hydrolysate) and at 25–28°*C* under continuous mixing condition by a magnetic stirrer (rotary shaker IKA KS 260 basic) at 150 *rpm* for 72 hours [Ref. 14]. The reason to use the GC medium is because the growth yield of *Trichoderma reesei* is greater in glucose-casein hydrolysate broth than in other media. Casein hydrolysate is a mixture of amino acids and peptides produced by enzymatic or acid hydrolysis of casein. The mycelial (vegetative part of the fungus) mass was then separated from the culture broth by sterile paper filter, and the settled mycelia were washed thrice with sterile distilled water. The harvested mycelial mass was then used for the synthesis of silver nanoparticles [Ref. 15]. In a typical biosynthesis production scheme of silver nanoparticles, 10*g* of *Trichoderma reesei* fungus wet biomass was mixed with a 100 *ml* aqueous solution of 1 *mM* silver nitrate (*AgNO3*). Then the mixture was placed in a 100 *rpm* rotating shaker at 28 °*C* for 120 hours duration. In this process silver nanoparticles were produced through reduction of the silver ions to metallic silver [Ref. 16].

RESULTS AND ANALYSIS

Plant Mediated Silver Nanoparticles Characterization

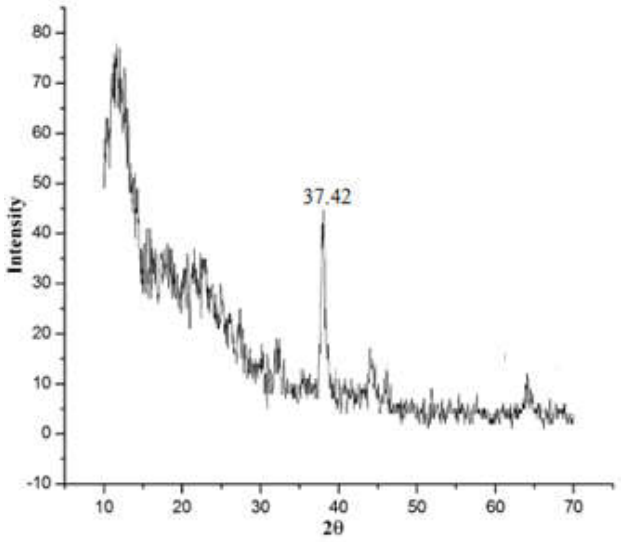

Fig. 1. XRD pattern of Silver Nanoparticles synthesized via *A. indica* Leaf extracts

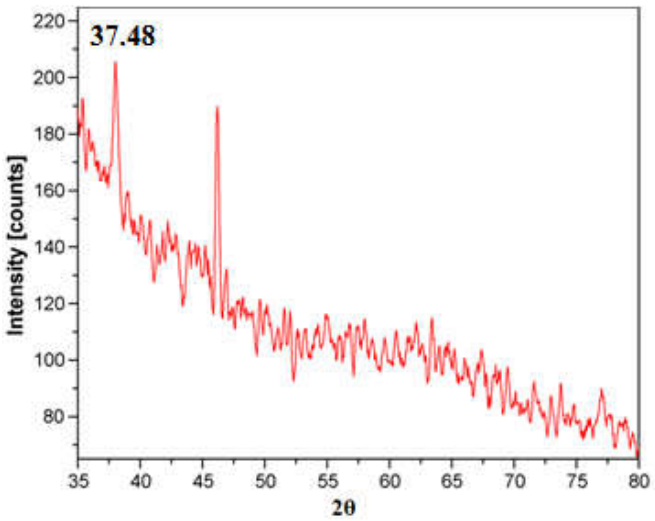

Fig. 2. XRD pattern of silver nanoparticles synthesized via *Jatropha curcas*

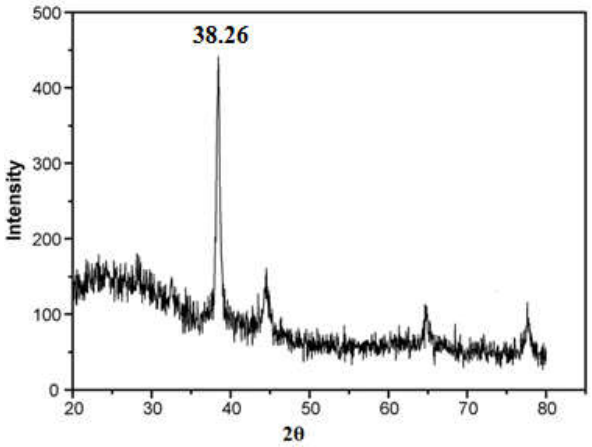

Fig. 3. XRD pattern of silver nanoparticles synthesized via *Cinnamon zeylanicum*

If Fig. 1, Fig. 2 and Fig. 3 are compared then it is quite easily seen that the highest peak of Intensity in all the cases is obtained in between 35^0-40^0. This lead us to the interpretation that all the particles prepared from different sources hereby formed are similar in nature and are the silver nanoparticles. A little variance in the peak angle is there which is due to the use of different sources. Fig. 1 gives peak at 37.42, Fig.2 gives at 37.48 and Fig. 3 at 38.26. Hence the peaks of all the obtained results from different sources are nearby hence the particles obtained are same and a little variation in properties may be observed.

Fungal Mediated (*Trichoderma reesei*)

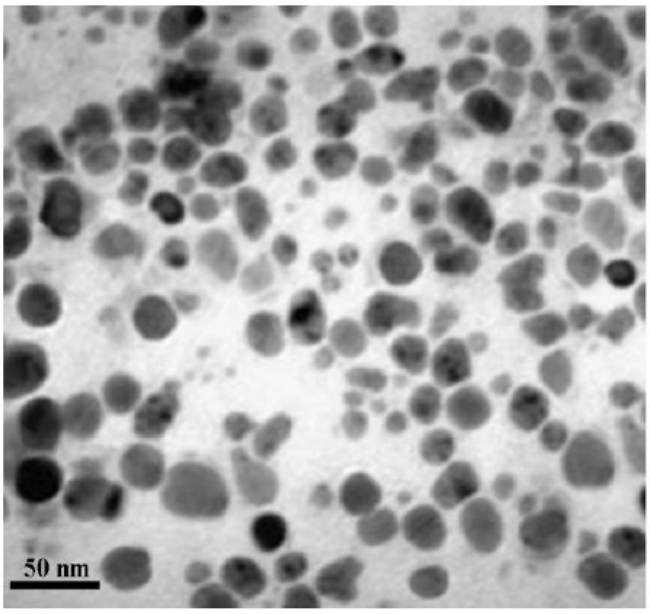

Fig. 5. TEM micrograph recorded from a drop-coated film of an aqueous solution incubated with *Trichoderma reesei* and reacted with Ag+ ions for 72 hours.

In the Fig. 5 a good and clear TEM image of the thin film of silver nanoparticles is seen. This shows the clear visualization of the nanoparticles formed.

Algae mediated

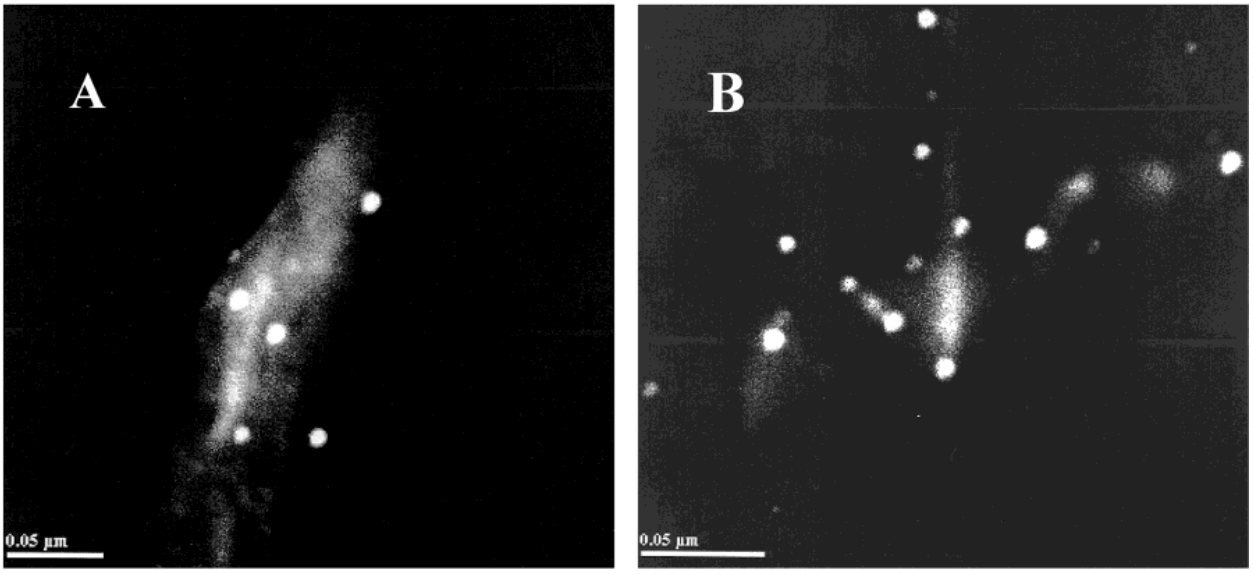

Fig. 4. TEM images obtained. (A) and (B) show silver nanoparticles in a crystalline state as very bright spots being connected, in some cases, by noncrystalline silver nanoparticles or atoms.

Applications

Nanoparticles synthesized can be used in the determination of changes in the membrane permeability of bacterial cells. These can also be used for the determination of respiratory activity of bacterial cells. Moreover the particles obtained are used to monitor the growth process of bacterial cells over a certain agar medium. It gives a clear indication about the reduction in the growth of bacteria by applying the silver nanoparticles. Hence it shows bactericidal effect. Overall these particles have a very great applicative part in the field of biotechnology and hence are supposed to give converging effect which is nano-biotechnology.

Conclusions

Silver Nanoparticles can be obtained from different sources in nature and are quite useful in the field of Biotechnology. Plant-medited source, algal-mediated source and fungal mediated source all are of different origin but gives the silver nanoparticles of same type. These nanoparticles are of high use and applicative in the field of Biotechnolgy. All the synthesis and characterization studies have been done and they all give the appropriate results in the favour of the formation of silver nanoparticles.

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