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Research Article

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Comparison Studies on the Synthesis of Selenium Nanoparticles by Various Micro-organisms

Santanu Sasidharan* and R. Balakrishnaraja
Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam,
Erode Dt., Tamil Nadu, India
*Corresponding Author E-mail: santanu.sasidharan@gmail.com

ABSTRACT

Nanoparticles have been synthesized chemically for the past few decades. The chemical methods of production are too costly to be implemented in industrial scale and the stability of the nanoparticles then produced is also an unanswered question. The organic synthesis of nanoparticles which includes the synthesis of nanoparticles using micro-organisms and plant parts opens a window into the production of low cost and efficient nanoparticles and the production of stable encapsulated nanoparticles.
The present study involves the review of synthesis of selenium nanoparticles using the various organisms and the characterization study of selenium nanoparticles that is produced by the same method. Microorganisms have been observed to have separate pathways for reducing property and the same mechanism is employed here. Selenium has been known to be analogous to sulphur in its structure and this has many pharmaceutical and industrial applications as pesticides and algaecides. The review shows the effect of concentration, temperature and light intensity and the optimization of the selenium production. The characterization of selenium nanospheres has been done by UV-spectrophotometer, X-ray diffraction and by atomic force microscopy. The study further puts an insight into the usage of selenium nanoparticles as food additives and as various other analogue mechanisms for sulphur.

Keywords: *Nanoparticles, nanospheres, selenium*

INTRODUCTION

An important area of research in nanotechnology is the synthesis of nanoparticles of different chemical compositions,sizes, shapes, and controlled dispersities. Currently,there is a growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocol.Even though many biotechnological applications such as remediation of toxic metals employ microorganisms such as bacteria and yeast (the detoxification often occurring via reduction of the metal ions/formation of metal sulfides), it is only relatively recently that material scientists have been viewing with interest such microorganisms as possible eco-friendly nanofactories[1]. Beveridge and co-workers have demonstrated that gold particles of nanoscale dimensions may be readily precipitated within bacterial cells by incubation of the cells with Au^{3+} ions[2]. Klaus-Joerger and co-workers have shown that the bacteria *Pseudomonas stutzeri* AG259 isolated from a silver mine when placed in a concentrated aqueous solution of $AgNO_3$ resulted in the reduction of the Ag^{+}ions and formation of silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria[6]. Nair and Pradeep have synthesizednanocrystals of gold, silver, and their alloys by reaction of the corresponding metal ions within cells of lactic acid bacteria present in buttermilk. Very recently[3], Jose-Yacaman and co-workers have shown that gold nanoparticles may be synthesized in live alfalfa plants by gold uptake from solid media. In a break from tradition, which has hitherto relied on the use of prokaryotes such as bacteria in the intracellular synthesis of nanoparticles,we have recently shown that eukaryotic organisms such as fungi may be used to grow nanoparticles of different chemical compositions and sizes. A number of

different genera of fungi have been investigated in this effort, and it has been shown that fungi are extremely good candidates in the synthesis of gold, silver and, indeed, quantum dots of the technologically important CdS by enzymatic processes. The use of fungi is potentially exciting, since they secrete large amounts of enzymes and are simpler to deal with in the laboratory. However, the possible drawback to this approach could be that genetic manipulation of eukaryotic organisms as a means of over expressing specific enzymes identified in nanomaterial synthesis would be much more difficult than that in prokaryotes. As can be seen from the above, the use of biological organisms in the deliberate and controlled synthesis of nanoparticles is a relatively new and exciting area of research with considerable potential for development.

MATERIAL AND METHODS

Strain

The following bacterial strains were chosen for the*Lactobacillus casei*; *Streptococcus thermophilus; Bifidobacterium;Lactobacillus acidophilus*; *Lactobacillus helveticus* and *Klebsiella pneumoniae.*

Culture Media

The growth of all the bacteria primarily depends on carbon, nitrogen, inorganic ions and organic materials. The broth should be rich in vitamin complexes to facilitate the growth of *Lactobacillus sp.* and the yeast extract is added in appropriate proportions to other components. Skimmed milk with 5% fat content can also be used as a substrate for the growth and production of selenium nanoparticles. The bacteria such as *Streptococcus thermophilus* and *Bifidobacterium* have been grown successfully in MRS broth before and the same procedure is followed in this process. The media should also contain antifoaming and buffer agents. The *Klebsiella pneumoniae* is to be grown separately since the activity of the species is higher in Triptic Soy broth. The bacteria are to be characterized for pure culture and any contamination is to be removed as and then all the cultures are stored at 4ºC

Production and Recovery

Lactobacillus sp.

[4]480 ml of milk containing 5% fat content is sterilized at 120ºC for 15 minutes. As selenium source we use sodium hydrogen selenite(NaHSeO3) in a 10.000 mg/l stock solution. To this, we add 10 ml of this stock solution to 490 ml of the sterilized milk, it meansaround 200mg/l concentration. From the fresh bacterium culture (previously stored at 4ºC for maximum 3-4 days)we add 10ml to 500 ml of selenite containing milk.For the inoculation we choose the following strains of *Lactobacillus acidophilus* and *Lactobacillus casei.* It is not very essential to adjust the pH of the culture medium, but usually it remains around 7-8 in the beginning and decreases to around 3-4 to the end of the fermentation process. This is due to the production of lactic acid by the *Lactobacillus sp.*[4]After this, the culture is placed into the shaking incubator for 36-48 hours at 37ºC (optimum temperature for lactic acid bacteria full reproduction cycle). At the end of the fermentation process, the culture medium becomes red,because of the produced elemental selenium. Elemental selenium is generally red in color and the production of red color confirms the reduction of the sodium hydrogen selenite to elemental selenium. Next step is to centrifuge the medium at 10.000 rpm for 10-15 minutes and the supernatant is discarded. The pellets are suspended in purified water. The formation mechanism of elemental selenium is mainly intracellular in lactic acid bacterium, that is why the cells have to be digested because of their very resistant cell wall. The most effective and money saving method is to use high concentration of hydrochloric acid (37% HCl). Enzyme hydrolysis is too costly to be taken up as a unit process. So, to this we add around 1.5x acid to the nanoselenium sample in 1.5:1 ratio (i.e.) for every 100 ml 150 ml of acid is to be added. This acidic hydrolysis takes an approximate offive days at room temperature. After this step, we have to get rid of the acid; accordingly we centrifuge the sample at 10,000rpm for 15minutes. The sample are centrifuged and washed many times with purified water until its pH returns to neutral. The samples are then ultrasonicated for 10-15 minutes in order to disintegrate the cohesive selenium spheres. As a last step we use vacuum filter to get rid of the rest of the bacteria cell wall. The separation is done by one plastic filter layer and two paper layers. In the end, the quality is checked by visual or laser controlling whether they meet the requirements.

Good quality samples have typical, nano-particle like light diffraction properties. For further utilization, the samples are stored at 4°C.

Bifidobacterium sp.

[4]480 ml of MRS broth is sterilized at 120ºC for 15 minute and the broth is kept in a sterilized environment. The sodium hydrogen selenite(NaHSeO3) is made in a 10.000 mg/l stock solution. To this, we add 10mlof this stock solution to 490 ml of the sterilized MRS broth medium, it means around 200mg/l concentration. The same procedure performed for Lactobacillus sp. is performed for Bifidobacterium sp. (previously stored at 4ºC for maximum 3-4 days). [4]The Bifidobacterium sp. is added in 10ml concentration to 500 ml of selenite containing MRS broth. The fermentation bottle is maintained at 37ºC and 140 rpm and for 36-48 hrs. The bacterial cells are subjected to lysis using French press and the sample is centrifuged at 10000 rpm for 15 minutes. The supernatant sample is ultrasonicated to disintegrate the spheres of selenium. The samples are then filtered in vacuum and they are characterized for light diffraction properties.

Klebsiella pneumoniae

[5]A uniform inoculum was prepared by aseptically transferring a loopful of *K. pneumoniae* from a Triptic Soy Agar (TSA) plate to 500 ml of sterile Triptic Soy Broth (TSB) and growing theculture. This solution constituted the inoculum at pH 7.2 was prepared, sterilized, and supplemented with a 200 mg/l Se+4 solutions (the source being sodium hydrogen selenite). [5]Subsequently, 1% (v/v) of the inoculum was added to selenium containing TSB, and the culture flask was incubated at 37 °C at 140 rpm for 24 hours. After incubation, the reduction of the Se+4 ions in the solution was determined by the presence of red color (elemental selenium). From this aqueous sample, 6 ml was removed and centrifuged at 14,000 rpm for 15 minutes.[5]Next, this supernatant was assayed using the titrimetric analysis previously described. In the next step, *K. pneumoniae* cells containing the red selenium particles were disrupted using a wet heat sterilization process in a laboratory autoclave at121°C, 17 psi for 20 minutes. The released selenium nanoparticles were centrifuged at 14000 rpm for15 minutes and washed three times with distilled water. The washed sample was sonicated for 10 minutes and characterized by transmission electronmicroscopy and UV-Visible spectroscopy.

RESULTS

Characterization of Selenium Nanoparticles

Lactobacillus sp.

The nanoparticles were measured and the particle size and distribution of the purified selenium spheres produced by *Lactobacillus sp.* bacteria were determined between 50-500nm. The analysis showed that the distribution varies with the pH, because the cohesion of the spheres depends on the pH of the liquid medium. [4]At pH up to 10, particles, the nanoparticles stick to each other, but pH above 10, disintegrates the coherence of the particles. The consumed elemental nano-Se in anorganic system continually transforms to selenite (SeO_3^{2-}). The selenite concentration change in the supernatant for a few days was analyzed and a graphical representation was made.[4]Purified elemental selenium nano-spheres under electron microscopic picture of 250 nm sized selenium nanospheres were synthesized.

Fig. 1

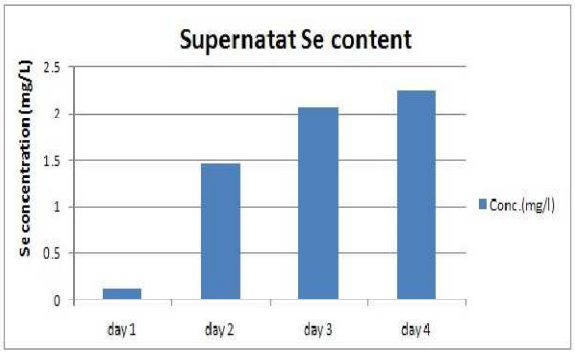


Fig. 2

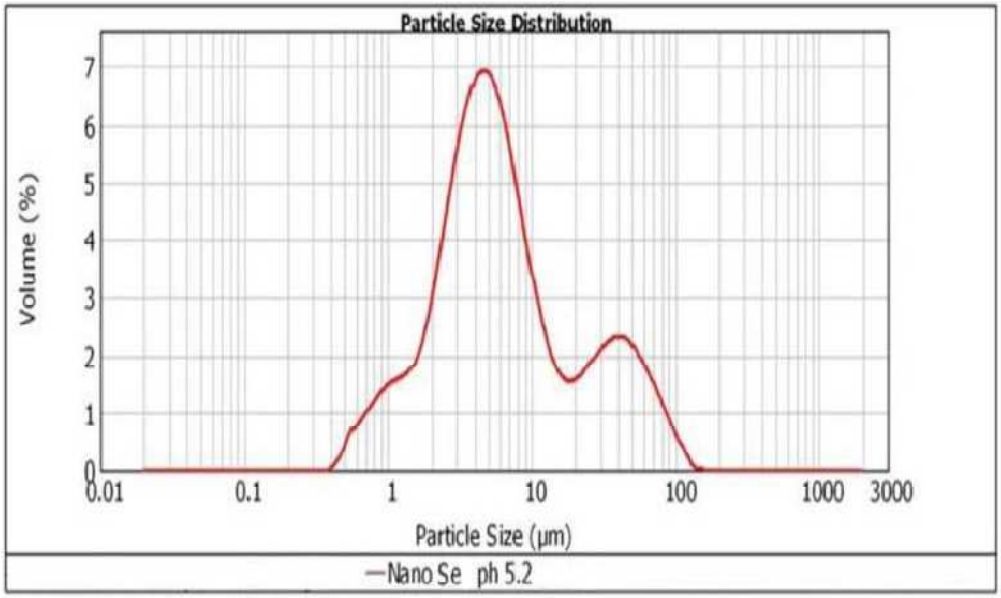


Fig. 3

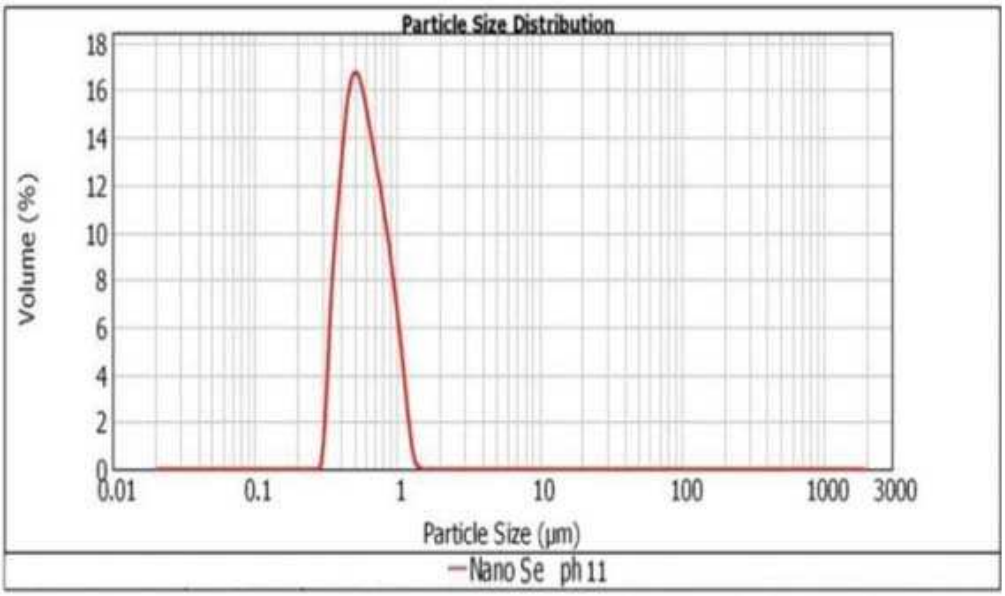
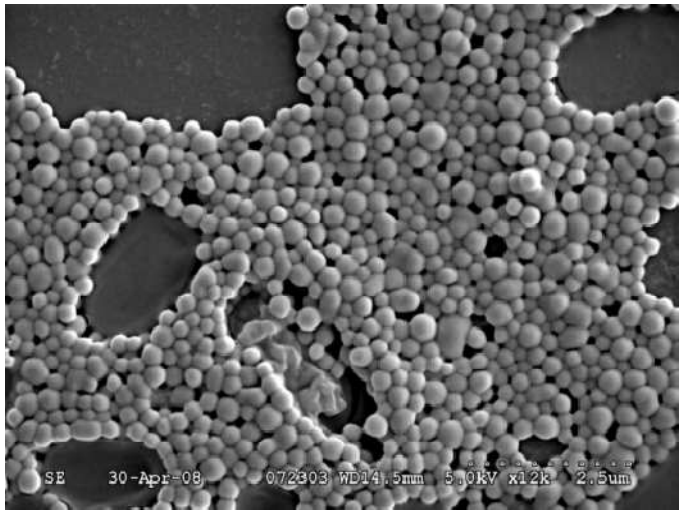


Fig. 4



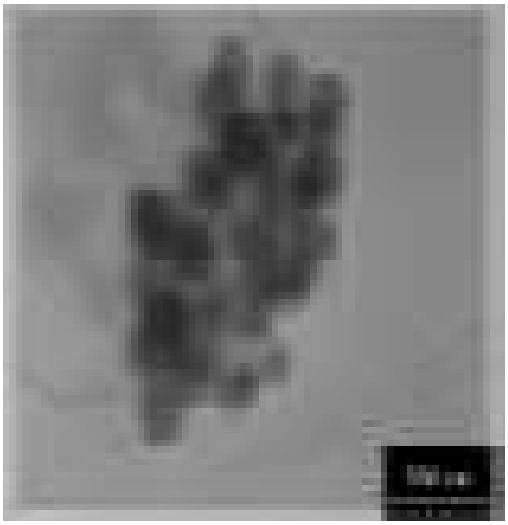
Bifidobacterium sp.

The nanoparticles were characterized and the size distribution varied from 400-500 nm. [4]The size of the nanoparticles did not show any significant change with respect to pH. The selenium remained elemental even after the 7-10 days. The reduction of selenium to selinate or selenite firms was not significantly seen and this may be due to the encapsulation of the selenium nanoparticles using certain chemically synthesized coatings.

Klebsiella pneumoniae

[5]Selenium nanoparticles were further characterized by UV–visible spectroscopy. The technique proved tobe very useful for the analysis of nanoparticles. As in procedure, the spectrum absorption bands with a maximum (218, and 248 nm) located between 200nm and 300nm was observed due to formation of selenium. Biosynthesis of selenium nanoparticles produced during reduction of selenium ions(Se^{+4}) was confirmed. The wet heat sterilization process was used for disrupting the bacterial cells containing the selenium particles. The TEM images of the selenium nanoparticles synthesized by *K. pneumoniae* and released after sterilization process[5]. The released nanoparticles showed nanoparticles in the range of 100—550 nm, with an average size of 245 nm. These nanoparticles were chemically stable theduring sterilization process, suggesting a possible utilization ofwet heat sterilization for recovering selenium nanoparticles from the cell mass of bacteria and for recovering other intracellular metal nanoparticles generated by microorganisms. The nanoparticles confirmed thereduction of selenium ions to elemental selenium and its chemical stability during cell disruption using the sterilization process.

Fig. 5



DISCUSSION

Simplicity and rapidity of the technology and safety of the employed strains makes this a significant progress over the art in producing selenium nanospheres. Homogeneity and quality of the shape and size of elemental selenium nanoparticles produced chemically by other technology used in the art does not come close to the characteristics of the material prepared by the fermentation technology.

Table .1

No.	Species	Size(nm)
1.	*Lactobacillus acidophilus*	50-500
2.	*Lactobacillus casei*	50-500
3.	*Bifidobacterium sp.*	400-500
4.	*Klebsiella pneumoniae*	100-550

A novel technology for producing selenium nanospheres in homogeneous in form and size within a short period of time (12-48 hours) has been developed. Elements with metallic properties have not been previously produced by fermentation using non-toxic aerobic bacteria,advantageously using bacteria permitted for use in the food industry. Selenium produced this way is exceptionally good raw material for forming nanosurfaces because of its homogeneous particle size distribution and regular, spherical shape. The technology developed is a manufacturing process which enables forming of a suspension as well as a powder containing valuable selenium spheres having unique characteristics.

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