Microalga *Scenedesmus* sp.: A Potential Low-Cost Green Machine for Silver Nanoparticle Synthesis

Jayashree Jena$^{1,2,*}$, Nilotpala Pradhan$^1$, Rati Ranjan Nayak$^3$, Bishnu P. Dash$^2$, Lala Behari Sukla$^1$, Prasanna K. Panda$^1$, and Barada K. Mishra$^1$

$^1$CSIR - Institute of Minerals and Materials Technology, Bhubaneswar 751013, India  
$^2$Fakir Mohan University, Balasore, Odisha, India  
$^3$CSIR - Indian Institute of Chemical Technology, Hyderabad 500 007, India

**Introduction**

In recent years, nanoscale materials have attracted considerable attention due to their structure (high surface area) and unique optical, electrical, and catalytic properties that differ significantly from bulk materials [32, 42]. The synthesis of nanomaterials of specific composition and size and their self assembly is one of the most challenging areas of nanotechnology as it is strongly influenced by experimental conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of the stabilizing agent with metal nanoparticles [9, 34, 39]. Among the nanoparticles, preparation of silver nanoparticles (SNPs) has attracted particular attention owing to their diverse properties and uses, such as magnetic and optical polarizability, electrical use due to the highest electrical and thermal conductivity among metals, use as catalysts, in biological sensing, and in surface-enhanced Raman scattering [25]. Among all, the silver ions and silver-based compounds with their well-known activities, SNPs proved to be the material of choice as an antimicrobial agent even at a very low concentration and they inhibit the growth of antibiotic-

Bionanotechnology has revolutionized nanomaterial synthesis by providing a green synthetic platform using biological systems. Among such biological systems, microalgae have tremendous potential to take up metal ions and produce nanoparticles by a detoxification process. The present study explores the intracellular and extracellular biogenic synthesis of silver nanoparticles (SNPs) using the unicellular green microalga *Scenedesmus* sp. Biosynthesized SNPs were characterized by AAS, UV-Vis spectroscopy, TEM, XRD, FTIR, DLS, and TGA studies and finally checked for antibacterial activity. Intracellular nanoparticle biosynthesis was initiated by a high rate of Ag$^+$ ion accumulation in the microalgal biomass and subsequent formation of spherical crystalline SNPs (average size, 15–20 nm) due to the biochemical reduction of Ag$^+$ ions. The synthesized nanoparticles were intracellular, as confirmed by the UV-Vis spectra of the outside medium. Furthermore, extracellular synthesis using boiled extract showed the formation of well scattered, highly stable, spherical SNPs with an average size of 5–10 nm. The size and morphology of the nanoparticles were confirmed by TEM. The crystalline nature of the SNPs was evident from the diffraction peaks of XRD and bright circular ring pattern of SAED. FTIR and UV-Vis spectra showed that biomolecules, proteins and peptides, are mainly responsible for the formation and stabilization of SNPs. Furthermore, the synthesized nanoparticles exhibited high antimicrobial activity against pathogenic gram-negative and gram-positive bacteria. Use of such a microalgal system provides a simple, cost-effective alternative template for the biosynthesis of nanomaterials in a large-scale system that could be of great use in biomedical applications.

**Keywords:** *Scenedesmus* sp., silver nanoparticle, biosynthesis, antimicrobial activity
resistant bacteria. SNPs interact with bacterial membrane proteins and DNA, which possess sulfur and phosphorous compounds that have a high affinity towards silver ions [26]. One gram of SNPs is known to impart antibacterial properties to hundreds of square meters of substrate material [22].

In the last decade, owing to a phenomenal increase in industrialization and urbanization, high demands for nanoparticles have led to their large-scale production. A wide range of chemical and physical methods are available that make use of hazardous toxic chemicals such as reducing agents, organic solvents, or non-biodegradable stabilizing agents, which are potentially dangerous and have a deleterious effect on the environment and biological systems. In recent years, many attempts have been made to develop clean, non-toxic, and environmentally benign methods using natural biological resources, an approach known as “green chemistry” that consists of an environmentally acceptable solvent system and eco-friendly reducing and capping agents [23, 37, 40, 41].

The biological approach to nanomaterials synthesis using bacteria [15, 16, 31], fungi [1, 9, 23], plants [33, 42], and algae [40, 41] has been reported by several researchers. Bacteria and microalgae show the potential to take up metal ions and produce metal nanoparticles during detoxification. Microalgae are prokaryotic or simple eukaryotic photosynthetic microorganisms that are present in aquatic and terrestrial ecosystems, representing a wide variety of species. They can grow rapidly with a minimum growth requirement of sunlight, atmospheric CO$_2$, and a few common mineral salts to reproduce their biomass very rapidly. Microalgae can potentially be used for a large number of biotechnological areas, including cosmetics, pharmaceuticals, nutrition, food additives, aquaculture, and pollution control such as wastewater treatment [2, 21]. Besides the above potential, the use of microalgae for biofuel production and carbon dioxide sequestration on an industrial scale is an important area of research. Methods for the commercial production of algal biomass on a large scale have already been reported.

Among the blue-green algae (cyanobacteria), *Anabaena, Calothrix, Leptolyngbya,* and *Nostoc ellipsosporum* have been reported to synthesize intracellular gold, silver, palladium, and platinum nanoparticles [5, 27]. The green microalga *C. vulgaris* has also been reported to produce gold, platinum, palladium, ruthenium, rhodium, and iridium nanoparticles intracellularly [20], whereas the cell-free extract efficiently produces gold and silver nanoplates [39, 40]. Extracellular SNP synthesis has been reported for the boiled extract of the brown seaweed *Padina tetraspermatica* [12].

Despite these many reports and considering the enormous diversity and chemical richness of algal species, more research is needed to explore and provide a better platform for microalgal nanoparticle production. To the best of our knowledge, *Scenedesmus* sp. has not yet been reported to possess nanomaterial biosynthesis ability, although it has been proved to be a potential source for large-scale biodiesel production due to its high oil content and biomass productivity [10, 13, 42, 36].

The present study reports for the first time the use of the green oilseogenous microalga *Scenedesmus* sp. (IMMTCC-25) for intracellular as well as extracellular biosyntheses of SNPs. The synthesized SNPs were characterized by different techniques and tested for antibacterial activity against pathogenic bacteria.

Materials and Methods

Chemicals, Organism, and Culture Conditions

The brackish water microalga *Scenedesmus* sp. (IMMTCC-25) was obtained from the Culture Collection Centre and Repository of the Bioresources Engineering Department, CSIR-Institute of Minerals and Materials Technology, Bhubaneswar, India. The culture was grown in modified Bold Basal medium composed of $(mg/l)$ KH$_2$PO$_4$ (175), CaCl$_2$·2H$_2$O (25), MgSO$_4$·7H$_2$O (75), NaNO$_3$ (250), K$_2$HPO$_4$ (75), NaCl (25), Na$_2$EDTA (50), KOH (31), FeSO$_4$·7H$_2$O (4.98), H$_2$SO$_4$ (1 µl), H$_2$O (11), MnCl$_2$·4H$_2$O (1.81), ZnSO$_4$·7H$_2$O (0.22), NaMoO$_4$·5H$_2$O (0.39), CuSO$_4$·5H$_2$O (0.079), and Co(NO$_3$)$_2$·6H$_2$O (0.0494) at an ambient temperature of 28 ± 2°C in a 16/8 h light/dark cycle under cool fluorescent light with an intensity of 3,500lux in an incubator shaker (INNOVA 44R) agitated at 126 rpm. All the chemicals used in this study were of analytical grade.

Intracellular Synthesis of SNPs Using Whole Algal Cells

Silver nitrate (AgNO$_3$) was dissolved in double-distilled water to prepare a 100 mM stock solution. Healthy microalgal cultures were harvested in the logarithmic phase by centrifugation at 4,000 rpm for 20 min at 4°C. The culture filtrate was removed and the pelleted biomass was washed with sterile deionized water to remove absorbed impurities. The washing process was repeated five times and the washed biomass was resuspended in distilled water. A stock solution of AgNO$_3$ (100 mM) was added to the biomass to obtain a final concentration of 5 mM AgNO$_3$. In the control setup, no AgNO$_3$ was added to the algal biomass. The cultures were incubated at 28°C for 72 h under similar conditions as described above. Experiments were carried out in triplicate. After completion of the reaction, the biomass was separated by centrifugation, washed with distilled water to remove unbound silver ions, if any, and stored at −20°C until further characterization.
Extracellular Synthesis of SNPs

Extracellular synthesis of SNPs by Scenedesmus sp. was carried out using two types of algal extract, that is, raw algal extract and boiled algal extract.

Preparation of Raw and Boiled Algal Extract

Typically, 3.0 g (wet weight) of algal biomass was suspended in 20 ml of deionized water for 5 days. The supernatant (raw algal extract) was then separated by centrifugation at 7,000 rpm for 20 min. AgNO₃ was added to the raw algal extract to get a final concentration of 5 mM to start the reaction.

Similarly, 3.0 g (wet weight) of algal biomass was suspended in 20 ml of deionized water and heated for 20 min at 100°C in an Erlenmeyer flask. After boiling, the volume was made up. The mixture was cooled and centrifuged at 12,000 rpm for 15 min. The SNP synthesis reaction was started by adding AgNO₃ to get a final concentration of 5 mM at room temperature. The synthesis of SNPs was indicated by a change in the color of the reaction mixture.

Characterization of SNPs

Bioreduction of precursor silver ions was monitored by sampling an aliquot of 3 ml of supernatant at different time intervals. Absorption measurements were carried out on a UV-Visible spectrophotometer between 190 and 1,100 nm. The concentration of silver in the solution was analyzed by atomic absorption spectroscopy (AAS; Perkin Elmer Model AA-400) after appropriate dilution of the sample.

Transmission electron microscopy (TEM) images of both intracellularly and extracellularly synthesized SNPs were obtained using a FEI TECHNAI G2 TEM operating at an accelerating voltage of 200 kV. In order to obtain TEM micrographs, samples were prepared by placing a drop of sample on the carbon-coated copper grid and dried under infrared lamp prior to examination. The nature of the observed crystalline structures was confirmed by the selected area electron diffraction (SAED) method. The elemental composition of the sample was determined by EDAX attached to TEM.

X-ray diffraction analysis (XRD) was carried out by using an X-ray powder diffractometer (Philips X'pert Pro, Panalytical) having CuKa (λ = 1.54 Å) radiation and a programmable divergence slit. The voltage and current of the X-ray source were 40 kV and 20 mA, respectively. A thick coat of sample on the silica plate was obtained by applying many layers with intermittent drying.

For Fourier transform infrared spectroscopy (FTIR), freeze-dried algal biomass was used. A small amount of dried biomass was grinded with potassium bromide (KBr). The FTIR spectrum was recorded on a Perkin Elmer FT-IR system Spectrum GX model. All measurements were carried out in the range of 400–4,000 cm⁻¹ at a resolution of 4 cm⁻¹.

Dynamic light scattering (DLS) of SNPs formed in boiled algal extract was performed using a Dawn Heleos II (Wyatt) system operating at a wavelength of 658 nm.

Thermogravimetric analysis (TGA) was performed using a Mettler TGA/SDTA. The dried algal biomass was subjected to TGA. Ten to twenty-five milligrams of both silver-exposed and unexposed biomass was heated to 1,000°C at a rate of 20°C/min individually in a nitrogen atmosphere. A constant flow of nitrogen (99.9 wt % purity) was maintained at 40 ml/min to remove air and avoid any oxidation reaction.

Biosynthesized SNPs were analyzed for their antimicrobial activity against the gram-negative bacterium Escherichia coli and gram-positive bacterium Streptococcus mutans. The test bacterial strains used in this investigation were stock cultures of the respective bacterial strain grown separately in liquid nutrient broth medium (containing NaCl (5 g/l), peptone (5 g/l), and beef extract (3 g/l)) at 35°C for 24 h. Nutrient agar plates were spread-plated with an individual bacterial culture for all experiments. For the preparation of algal disks, the algal culture was filtered through 0.2 μm filter paper using vacuum filtration and the paper was allowed to dry at 50°C in a hot air oven. Paper containing both exposed and unexposed dried biomass was cut into 2 mm diameter disks and put on a freshly applied lawn of individual bacteria. An unexposed biomass disk was taken as a control. To liberate the intracellular SNPs, the exposed biomass was sonicated for 20 min. To check the antimicrobial activity of different concentrations of liberated SNPs, wells were dug into the agar plate spread with the test bacterium E. coli and filled with 10, 20, 30, or 40 μl of ruptured algal culture (corresponding to 0.066, 0.132, 0.198, or 0.264 mg of biomass). The zone of inhibition was measured after 24 h of incubation at 35°C. Similarly, the antimicrobial activity of biosynthesized SNPs in boiled extract was tested against E. coli by the addition of 10 μl of synthesized SNPs into the wells dug in the nutrient agar plates.

Results

Intracellular Synthesis of SNPs

The test organism Scenedesmus sp. (IMMTCC-25) is a brackish water alga with an oval-shaped body, belonging to the class Chlorophyceae. It is a small, nonmotile colonial green photosynthetic microalga. The colonies most often have two or four cells arranged linearly and are occasionally unicellular. In the present work, the algal biomass when exposed to silver ions showed a change in color of the algal biomass from the inherent bright green to brown, indicating biosorption of metal) compared with the control biomass, which retained its original green color. A fast intracellular accumulation and reduction of silver ions was observed during exposure of algal biomass suspended in the aqueous medium. The silver ion concentration was measured by AAS. The kinetics of Ag⁺ uptake by the algae as a function of time is shown in Fig. 1A. Biosorption of metal started from the
moment Ag$^+$ ions were added and reached about 25% after 1 h of exposure. The rate of silver uptake increased logarithmically and reached about 80% within 24 h of incubation and then slowed down. By the end of 72 h, a net accumulation of about 93% was observed.

During 72 h of biomass exposure to Ag$^+$, no change in the outside medium was observed, indicating the absence of extracellular formation of SNPs. For further confirmation, the outside medium was subjected to UV-Vis spectroscopy after different exposure times (24, 48, and 72 h). No characteristic absorption peak for SNPs at 420 nm was observed even after 72 h of incubation (Fig. 1B). This suggests that the formed SNPs were completely intracellular. To investigate the morphology and size of synthesized nanoparticles, TEM analysis of the exposed biomass was carried out. The TEM image reveals that the cells were oval in shape with a dimension of about 10 µm (length) × 5 µm (breadth). The unexposed algal cells showed some cellular organelles through the transparent cytoplasm and the cell membrane, but they did not exhibit any metal deposition (Fig. 2A). Algal cells were intact even after 72 h of exposure to silver nitrate (Fig. 2B). However, silver-exposed cells showed well-dispersed metal nanoparticles deposited throughout, as shown in Figs. 2B and 2C. The maximum numbers of intracellularly deposited particles were found

Fig. 1. (A) Kinetics of silver accumulation in *Scenedesmus* cells on exposure to silver nitrate solution, and (B) UV-Vis spectra of the outside medium of silver-exposed *Scenedesmus* cells (after 24, 48, and 72 h of exposure).

Fig. 2. TEM images of (A) unexposed *Scenedesmus* cells, (B, C) silver-exposed *Scenedesmus* cells with entrapped SNPs, (D) particle size distribution, (E) EDAX, and (F) SAED profiles of SNPs produced by *Scenedesmus* sp.
towards the compact interior area of microalgal cells (Fig. 2C). When observed under higher magnification, SNP clusters were well separated, spherical, and highly crystalline. The intracellular SNPs were in the size range of 3–35 nm with a maximum size of 15–20 nm (Fig. 2D). EDAX of typical biomass-SNP complexes showed strong signals for silver with additional peaks for C, N, O, P, Mg, S, Ca, Cl, and Cu (Fig. 2E). All of the peaks except Cu may be attributed to proteins and other biomolecules associated with the biomass. Conspicuous peaks for C and Cu were due to the carbon-coated grids used for TEM analysis. Furthermore, the crystalline nature of the particles was confirmed by SAED analysis (Fig. 2F). The bright spots on SAED correspond to the face-centered cubic (fcc) structure of elemental silver and silver chloride nanoparticles. The elemental SNPs were preferentially directed towards (111), (200), (220), and (311) planes. Along with SNPs, some amount of silver chloride nanomaterial was also present and preferentially directed towards (111), (200), (220), and (311) planes.

FTIR analysis of untreated biomass showed bands for cell components such as proteins, lipids, and carbohydrates (Fig. 3A). Several peaks located at about 2,847, 1,739, 1,666, 1,525, 1,450, 1,412, 1,370, 1,244, 1,155, 1,070, 1,030, 932, 783, and 704 cm⁻¹ were observed. In the silver-exposed biomass, shifts in peak positions were observed along with some additional peaks. A peak at 1,525 cm⁻¹ corresponds to amide II bonds involving carbonyl and N-H stretching of proteins [8]. A peak at 2,847 cm⁻¹ may be assigned to -CH₂ groups characteristic of lipids and proteins, whereas a peak at 1,739 cm⁻¹ may be due to –C=O stretching of lipids and fatty acid esters [17]. A peak at 1,155 cm⁻¹ may be due to C-O-C stretching of carbohydrates and polysaccharides. Similarly, peaks at 1,030 and 1,244 cm⁻¹ remained unchanged and may be assigned to the P=O bond of phospholipids, DNA, and RNA. Again, a peak due to CH₃ and CH₂ groups of proteins at 1,450 cm⁻¹ remained unchanged, but an additional peak appeared at 1,348 cm⁻¹, which may be due to C-N stretching vibration of aromatic amines [8, 11]. An additional peak appearing at 1,588 cm⁻¹ in treated

Fig. 3. (A) FTIR, (B) XRD, and (C) TGA patterns of unexposed and silver-exposed Scenedesmus biomass.
biomass may be assigned to aromatic C-C skeletal vibration in the ring structure of phenylalanine side chains of proteins [8]. Similarly, a peak at 1,348 cm\(^{-1}\) may be assigned to C-N stretching vibration of aromatic amines. Peaks at 3,360 cm\(^{-1}\) may be assigned to –OH stretching of hydroxyl groups and the N-H stretching mode arising from different macromolecules that include carbohydrates, proteins, and lipids [4, 17], but in the present case, it may be assigned to proteins, as other bonds assigned to carbohydrates and lipids remain unchanged. This result shows that the protein molecules may be involved in SNP formation and subsequent stabilization.

The exact nature of the biosynthesized SNPs was further confirmed from the XRD spectrum (Fig. 3B). The XRD patterns of a drop cast film of the exposed biomass shows a number of sharp diffraction lines at low angles (20° to 80°). The Bragg reflections at 2θ = 38.48°, 44°, 64.74°, and 77.4° can be indexed to the fcc structure of the SNPs corresponding to (111), (200), (220), and (311) lattice planes, which matched with the standard JCPDS card No. 89-3722. The XRD pattern thus clearly shows that the SNPs were of a crystalline nature. Additional diffraction peaks appearing at 2θ values of 27.95°, 32.35°, and 57.98° correspond to the (111), (200), (220), and (222) crystal planes of the fcc AgCl (JCPDS file: 31-1238). The obtained XRD spectrum of the nanoparticles was in good agreement with the SAED results.

TGA of the biomass before and after silver exposure showed different patterns (Fig. 3C). TGA and DTG curves of Scenedesmus sp. biomass with and without SNPs were obtained at a heating rate of 20°C/min. Weight loss was more prominent in the case of unexposed biomass than in the silver-exposed biomass. Chlorella biomass has been reported to show weight loss at three different stages [28]. Initial weight loss below 200°C is assigned to the loss of free water and water loosely bound to biomolecules and represents the first stage. The second step is reported to lie between 180°C and 400°C, which corresponds to the decomposition of protein and carbohydrate. Similarly, the third step is reported to occur in the range of 400–760°C, which is assigned to the complete oxidation of organic matter. Peng et al. [28] reported that Chlorella showed maximum weight loss in the second step at 180–400°C. In the present study, the Scenedesmus biomass showed a different pattern. The initial weight losses at 320°C and 465°C were comparatively small compared with the weight loss at 600°C, which may be due to the oxidation of organic matter. The presence of a higher lipid concentration (25% (w/w)) in the biomass may be the cause of the higher weight loss at 600°C. Another peak at 690°C may be due to the inorganic materials present in the biomass. The exposed Scenedesmus biomass bound with SNPs showed an altogether different peak pattern. Peaks at 270°C and 340°C may be assigned to the decomposition of carbohydrates and proteins. When compared with unexposed biomass, the peak at 320°C was shifted to 340°C in silver-exposed biomass. The peaks at 320°C and 340°C in unexposed and exposed biomass, respectively, may be due to a protein that was shifted to a higher temperature. The shift is a result of SNPs binding to the protein molecules. The peak at 465°C disappeared in the SNP-bound biomass. The major weight loss peak at 600°C of unexposed biomass shifted to 610°C in SNP-bound biomass but the peak size was smaller. The most interesting finding was the continued weight loss in the SNP-exposed biomass beyond 600°C. This may be due to the presence of metallic SNP ions that resist weight loss even at such a high temperature. Inorganic materials such as metals show slower weight loss. The appearance of a major peak at 660°C instead of a smaller peak at 690°C may be due to the SNP particles. The TGA curve also showed more ash for the SNP-bound biomass compared with the unexposed biomass.

**Extracellular Synthesis of SNPs Using Raw and Boiled Algal Extract**

Raw algal extract and boiled algal extract were treated with 5 mM AgNO\(_3\) solution and the formation of extracellular SNPs was investigated by UV-Vis spectroscopy. The raw algal extract was colorless and exhibited a peak at 260–280 nm. UV-Vis spectral analysis of silver-exposed raw algal extract showed the formation of a broad hump at nearly 430 nm, suggesting the initiation of SNP formation. With an increase in reaction time, the intensity of the peak increased. A broadening of the peak along with a red shift with increased incubation time suggests the formation of polydispersed and aggregated SNPs in the raw algal extract (Fig. 4A). The UV-Vis spectral result was further confirmed by TEM analysis. Fig. 4 (B, C, and D) shows the TEM image of biosynthesized SNPs in the raw algal extract after 3, 5, and 7 days of incubation, respectively. Polydispersed SNPs of size 30–150 nm were obtained after 3 days as is evident from the TEM image. Maximum particles were in the range of 50–60 nm (Fig. 4B). With the increase in reaction time, particle size increased owing to the agglomeration of particles (Fig. 4C). After 7 days, larger SNPs of different shapes with less stability were observed (Fig. 4D). In addition, a very clean ring in the diffraction pattern obtained by SAED analysis indicates that the particles were highly crystalline in nature (Fig. 4E). 

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five bright diffraction rings obtained by SAED correspond to the (111), (200), (220), (311), and (331) planes of elemental silver. EDAX analysis showed a strong peak for elemental silver (Fig. 4F). Although the synthesized SNPs in the raw algal extract were highly crystalline in nature, they were less stable owing to the rapid agglomeration of SNPs within a short period. Hence, the system may not suitable for biogenic synthesis of SNPs.

As the concentration of reducing agents in the reaction mixture are known to play an important role in the formation of nucleation points and subsequent control of the size of SNPs, this might be the reason for the less stable SNPs in the raw extract. Hence, another experiment was performed to increase the concentration of such agents in the reaction mixture. The biomass was boiled in water to get more reducing and stabilizing agents and this showed a better result. The initial light yellow color of the boiled extract turned brown on treatment with silver nitrate solution, indicating formation of SNPs. With an increase in reaction time, the color of the reaction mixture turned darker until 10 days. No further change in color was observed after 10 days of incubation, indicating the complete conversion of silver ion to SNPs. The formation of SNPs was further proved by the UV-Vis spectra, which showed a characteristic surface plasmon resonance (SPR) band for SNPs. Fig. 5A depicts a series of UV-Vis spectra of the reaction mixture recorded at various time intervals. Boiled algal extract exhibited a peak only at 260–280 nm, which might be due to the presence of peptides. On exposure to silver ions, a steady increase in the intensity of the peak centered at 420 nm was observed as a function of time without any shift in peak wavelength. A sharp SPR band at 420 nm is indicative of relatively small monodispersed and spherical SNPs. However, neither the negative control (pure silver nitrate solution without cell-free boiled extract) nor a positive control (cell-free boiled extract without silver nitrate) showed the characteristic change in color of the solution nor a characteristic SNP peak, indicating that the synthesis is not a thermal and temporal process. As a function of reaction time, a shift in peak position towards the low wavelength region (blue shift) in the protein/peptide band (260–280 nm) occurred, which may due to the interaction between protein residues and biosynthesized SNPs [30].

Fig. 4. Extracellular synthesis of SNPs using raw algal extract. (A) UV-Vis spectra, (B, C, D) TEM images of SNPs after 3, 5, and 7 days, respectively, (E) SAED and (F) EDAX profiles of SNPs.
The TEM micrograph of the synthesized nanoparticles in boiled algal extract confirmed a proper dispersion of the SNPs (Figs. 5B and 5C). The morphology of the synthesized nanoparticles were found to be spherical with a crystalline nature and in the size range of 3 to 22 nm, with an average size in the range of 5–10 nm (Fig. 5B). The particle size distribution histogram determined from TEM suggests that the synthesized SNPs were well scattered and evenly distributed (Fig. 5D). The stability of biosynthesized SNPs using boiled algal extract was observed over a period of 3 months at room temperature by measuring the intensity at 420 nm using a UV-Vis spectrophotometer. No observable variation was seen in the optical properties of the nanoparticle solution even after 3 months of synthesis, confirming that the nanoparticles are highly stable, which was further confirmed by TEM. Even after 3 months of synthesis, highly dispersed SNPs without any aggregation or flocculation were observed (Fig. 5C). This might be due to the presence of some stabilizing agents in the algal extract [22, 24].

Furthermore, the SAED spots corresponded to the (111), (200), (220), and (311) planes of fcc structure of elemental silver, confirming the presence of crystalline SNPs (Fig. 5E). EDAX analysis also showed a heavy peak in the silver regions, which further confirms the formation of SNPs (Fig. 5F). The DLS study supported the presence of small

![Figure 5](image_url)

**Fig. 5.** Extracellular synthesis of SNPs using boiled algal extract. (A) UV-Vis spectra, (B, C) TEM images of SNPs after 10 days and 3 months, respectively, (D) particle size distribution, (E) SAED, and (F) EDAXs profile of SNPs.

![Figure 6](image_url)

**Fig. 6.** DLS pattern of biosynthesized SNPs in boiled algal extract.
colloidal SNPs (Fig. 6). The DLS result indicates that the particles were in the range of 12 to 45 nm, with a hydrodynamic radius in the range of 6 to 23 nm, which agrees well with the TEM results.

**Antibacterial Activity of SNPs**

Biosynthesized SNPs in the intracellular system were analyzed for their antimicrobial activity against the gram-positive bacterium *S. mutans* (Fig. 7B) and gram-negative bacterium *E. coli* (Fig. 7D). The antimicrobial activity of biomass incorporated with SNPs was observed to be quite low (less clear zone) against both the tested bacteria (Figs. 7B and 7D). Among the two bacterial strains, a good antibacterial effect was observed against *E. coli* (Fig. 7D), whereas in the case of silver-unexposed algal cells (control) confluent growth of both test bacteria was observed (Figs. 7A and 7C) without any clear zone. Out of curiosity, a fraction of the biomass entrapped with nanoparticles was weighed and sonicated to rupture the biomass and liberate the trapped SNPs. The same amount of both intact and ruptured silver-exposed biomass was tested against *E. coli*. As expected, the intact exposed cells showed a much smaller zone of inhibition (Fig. 7E), whereas a very clear and prominent zone of inhibition was observed in the ruptured fraction, indicating greater antibacterial activity due to the release of SNPs in the ruptured fraction (Fig. 7F).

Moreover, on increasing the amount of ruptured algal suspension, the zone of inhibition also increased gradually from 13 to 17 mm (Fig. 7G). In contrast, in the case of intact cells, there was no such increase in the zone of inhibition with an increase in the amount of biomass (data not shown), as the synthesized SNPs were completely intracellular. Hence, the trapped SNPs were not capable of diffusing freely into the medium and, as a result, a smaller amount of silver was available to act against the bacteria. This could be the reason for the very limited antimicrobial activity of the intact exposed biomass as compared with the ruptured biomass. Furthermore, the SNPs synthesized in the boiled extract were tested against *E. coli* (Figs. 7H and 7I). The study clearly indicated that the synthesized SNPs using the boiled extract of *Scenedesmus* sp. have a good antibacterial effect against the test organism, exhibiting a very clear and prominent zone of inhibition of 15 mm even at a very low concentration (Fig. 7I) and no such zone of inhibition in the case of the control algal extract (Fig. 7H).

**Discussion**

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for the synthesis of metallic nanoparticles. As a result, research in the field of nanomaterial synthesis has turned towards biological systems to achieve desired quantities of nanoparticles in a completely green way. Several microorganisms possess tremendous ability for the bioremediation of toxic and precious metals through chelation, chemical transformation, and metal accumulation in the form of nanoparticles. Microalgae preferentially develop peptides capable of binding heavy metals and can produce organometallic complexes in the cytoplasm, thereby preventing a toxic effect, making them a potential candidate for heavy metal detoxification. Although the microalgae *Scenedesmus* sp. has been reported to detoxify several heavy metals (uranium, cadmium, copper, and zinc) on an industrial scale [29], the use of this microalgal strain for silver metal accumulation has been unexplored. This aroused our interest in the present investigation, which reports a detailed account of the intracellular and extracellular syntheses of SNPs through the indigenous microalgae *Scenedesmus* sp.

On exposure of biomass to silver nitrate, 80% of Ag⁺ was taken up by the algal biomass within 24 h, with concomitant formation of SNPs. The relatively high intracellular accumulation and formation of metal particles may be due to several possible mechanisms involved in the process.
Metal accumulation by biological species occurs via two processes; that is adsorption or biosorption (metabolism independent) on the cell surface and absorption to organelles/cytoplasmic ligands (metabolism dependent) [7]. In the present study, intracellular synthesis of SNPs by the organism Scenedesmus sp. was very fast, as indicated by a change in the color of the biomass. Algae cells possess pores 3–5 nm wide, which permit the passage of low molecular weight substances such as water, inorganic ions, gases, and other small nutrient substances required for growth and metabolism [38] and are essentially impermeable to large molecules or macromolecules. TEM analysis of the exposed biomass revealed that the synthesized SNPs were 4–35 nm in size, which is quite a bit larger than the size of pores present in the algal cell wall. Hence, it is proposed that at the first step, the metal ions (Ag+) are trapped on the surface of the algal cell, possibly via electrostatic interaction between the negatively charged functional groups present in the cell surface, followed by the reduction of metal ions by several enzymes, leading to the formation of nuclei in the cytoplasm. Simultaneously, growth and accumulation of these nuclei occur by the subsequent reduction of metal ions to metal particles [3, 7, 38].

As observed from the TEM images, synthesized SNPs were of a spherical shape with no agglomeration. SNPs were widely distributed throughout the cytoplasm and more nanoparticles were localized towards the compact region, which constitutes different organelles. In previous studies, it has been reported that maximum accumulation of metal occurs inside the cytoplasm, periplasm, nucleus, and pyrenoid in organisms such as Chlamydomonas (Ag), Chlorella (Au, Pd, Ru, Rh), and Shewanella (Au, Pt) [3, 20]. Upon metal stress, the photosynthetic machinery (chloroplast) responds first to the metal by the overexpression of proteins: ATP synthase, RUBP carboxylase, and oxygen-evolving enhancer protein. The enhanced activity of these enzymes may lead to the cell-mediated biosynthesis of SNPs [3]. This could possibly explain the reason for the synthesis and accumulation of a larger number of SNPs towards the compact region of the cell. Further FTIR spectroscopic studies confirmed that amino acid residues of the protein moiety have a strong binding ability with metals, suggesting the formation of a layer surrounding the metal nanoparticles and acting as a capping agent to prevent agglomeration, thereby providing high stability to the metal nanoparticles. These results confirm the presence of possible proteins/peptides acting as reducing and stabilizing agents of SNPs [35]. The SAED image showed that along with SNPs, silver chloride nanoparticle are also formed, which was further confirmed by the EDAX analysis (presence of a small chloride peak). The above reason, along with the UV-Vis spectra of the outside medium, suggests the complete intracellular biosynthesis of SNPs and is in good agreement with the earlier reports [27].

In comparison with whole-cell-mediated SNP biosynthesis, the size of the extracellularly synthesized SNPs using raw algal extract was larger (more than 50 nm) and less stable, as agglomeration occurred within a short period of time. The synthesis process was also very slow. To overcome this problem, the boiled extract of algae was used. The biosynthesis of SNPs using boiled algal extract proved to be suitable owing to the high rate of SNP formation along with the high stability of biosynthesized SNPs. The synthesized SNPs were smaller in size (5–10 nm), with a spherical shape. The current finding suggests that the intracellular synthesis of SNPs using whole cells is faster than the extracellular SNP synthesis using raw and boiled algal extract, which may be due to the presence of active biomolecules in the live cell [3]. The variation in SNP formation in the above three systems may be due to the variation in the reducing agent, primarily proteins/peptides. For confirmation, the total protein concentration in the systems was estimated by the Folin Lowry method using bovine serum albumin as a standard. This showed that the algal biomass, when suspended in water over an extended time, does not release a significant amount of proteins (0.12 mg/ml) in suspended medium (raw algal extract). Moreover, in the case of intracellular SNP formation, when biomass was incubated in water along with silver ions, the release of protein was quite low (0.3 mg/ml). However, in this case, 80% of the silver ions were taken up by the algal biomass within 24 h with concomitant formation of SNPs, indicating the presence of intracellular reducing and capping agents (i.e., the active biomolecules). To achieve higher SNP formation in the extracellular liquid, the biomass was boiled with water first to enhance the release of metabolites and peptides/proteins into the aqueous medium. The boiling of biomass released about 4 mg/ml of peptides/proteins into the medium. The subsequent addition of silver ions gave very good formation of stable SNPs. The formation of comparatively less-stable, large-sized SNPs in the raw extract, where the concentration of proteins/peptides was low, and uniform, small-sized, highly stable SNPs in boiled extract, where the concentration of proteins/peptides was high, indicates the role of reductants/peptides in SNP formation. Hence, from all these observations, it is clear that the concentration of proteins/peptides plays a vital role in SNP formation and stabilization. The present
result is in good agreement with a previous report by Barwal et al. [3], where the protein-depleted fraction of Chlamydomonas cell extract treated with silver nitrate solution resulted in the formation of large-sized SNPs in comparison with a whole-cell extract. The above finding suggests that the protein concentration is directly proportional to the rate of particle formation and inversely proportional to the size of the particles [3]. Hence, the size, shape, and stability of SNPs biosynthesized within a short period of time in boiled Scenedesmus sp. extract make the strain a suitable candidate for extracellular SNP formation.

The green chemistry approach of microalgal nanoparticle synthesis could be considered technically feasible owing to its economic viability and the possibility of scale-up at a low cost. The present study thus offers a simple, novel, and environmentally benign bioreduction method for the biosynthesis of SNPs using whole cells of the microalga Scenedesmus sp., as well as the boiled algal extract. In view of the high rate of silver accumulation and its ability to form a large amount of intracellular nano-silver, this microalga may become a viable, cost-effective system for intracellular SNP synthesis and may be exploited as a potential source for phytomining of silver from industrial wastes in large-scale systems. Besides this potential, the extracellular synthesis of highly stable SNPs using a boiled extract of Scenedesmus biomass possesses a big advantage over the other currently used biological methods. Owing to their excellent antimicrobial activity, biosynthesized SNPs could be considered as an effective antimicrobial agent for various biomedical applications. Because of to the minimal growth supplements required for its growth, such as sea water, sunlight, and commercial fertilizers, and its high biomass productivity, the microalga Scenedesmus sp. can become a potential low-cost green cell factory for intracellular and extracellular SNP syntheses.

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