



**NANOTECHNOLOGY**



**NANOMATERIALS**



**NANOPARTICLES**



**NANO-RESEARCH**

# IMPORTANCE & APPLICATIONS OF NANOTECHNOLOGY

# Biosynthesis of Ag and Cu Nanoparticles and their Interactions with Cyanobacteria, Microalgae, and Macroalgae

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## Abstract

This chapter aimed to assess the ability of cyanobacteria, microalgae and macroalgae to biosynthesize silver and copper nanoparticles. Collaboration between engineering and biological sciences has opened new frontiers in the ever-growing domain of nanotechnology aimed at genesis, implementation and use of nanomaterials combining biological systems and research. The biological marine environment is very diverse had shown abundant potential towards nanoscience and nanotechnology in countless ways. It has been acknowledged by the scientific community that nanomaterials exhibit unique chemical, physical, electronic, optical, thermal, mechanical, and biological properties that expressively vary from their bulk counterparts, owing to their small sizes, shapes, and very high surface areas. For example, silver nanoparticles typically measuring 25nm prevents multiplication and growth of bacteria and fungi that cause infection, odour, itchiness and sores and copper nanoparticles smaller than 50 nm behave as an excellent tough material without any comparison towards bulk copper ductility and malleability. Due to their exceptional properties and assorted range of applications, the production of engineered NPs has shown increasing trends in the last two decades. Also projected that the production of nanoparticles doubles every 3 years; this upsurge in the production of nanoparticles for different applications has driven, the entry of new contaminants into our environment, hence there is a need to produce nanoparticles in a more environmentally friendly and sustainable way.

Nanoparticles are synthesized by physical and chemical routes, traditionally, a wet chemistry approach. Considering the current synergistic advances in materials science and biotechnology green methods are a viable way for the significant production of nanomaterials. Biosynthesis of nanoparticles ascends through intracellular and extracel-

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**Keywords:** Nanoparticles; Cyanobacteria; Microalgae; Macroalgae; Interaction; Biosynthesis.

**Abbreviations:** Ag: Silver; Ag Nps: Silver Nanoparticles; Aqu: Aqueous; Au: Gold; Au Nps: Gold Nanoparticles; Ave: Average; Cds Nps: Cadmium Sulfide Nanoparticles; CO Nps: Copper Oxide Nanoparticles; DDA: Disc Diffusion Assay; Dia: Diameter; DPPH: 1,1-Diphenyl-2-Picryl Hydrazyl; ETS: Electron Transport System; FT-IR: Fourier Transform Infra-Red; ICP: Inductively Coupled Plasma; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; M: Molar; MM: Milli Molar; MS: Mass Spectrometry; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; Nm: Nanometer; NP: Nanoparticle; PH: Potential Of Hydrogen; ROS: Reactive Oxygen Species; SEM: Scanning Electron Microscopy; SPR: Surface Plasmon Resonance; TEM: Transmission Electron Microscopy; UV/VIS: Ultraviolet/Visible Spectroscopy; UV-B: Ultraviolet-B; VSM: Vibrating Sample Magnetometry; ZOI: Zone Of Inhibition.

**Symbols:**  $\mu\text{m}$ : Micrometer; cm: Centimeter; -COOH: Carboxyl group; g/l: Gram per litre; hr: Hour; -NH<sub>2</sub>: Amino group; -OH: hydroxyl group; -SO<sub>4</sub>: Sulphate group;  $\lambda_{\text{max}}$ : Wavelength maximum (absorption).

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ular pathways by a variety of microorganisms. In this chapter, the authors focus on the algae-mediated biosynthesis of Ag and Cu nanoparticles and their interactions with cyanobacteria, microalgae, and macroalgae. Alongside biosynthesis, their interactions, also discusses toxicity towards algae, nanoparticles with proteins, the formation of the corona and impacts of protein-nanoparticle interactions on nanoparticles and proteins were discussed. Currently, a wide variety of organisms have been employed for the production of extremely beneficial nanoparticles.

## Introduction

Algal cells are typically surrounded by a rigid cell wall in addition to the plasma membrane. The cell wall maintains the integrity of the algae and constitutes a primary site for interaction with the surrounding environment. Algal cell walls are remarkably diverse among different species in their biochemical composition and structural features. Some algae have cell walls that are similar to the typical terrestrial plant cell walls, which are comprised of networks of cellulose microfibrils and cross-linking glycans. Other algae, for instance, the alga *Chlamydomonas reinhardtii*, do not have cellulose but mainly glycoproteins in their cell walls which are composed of multiple crystalline layers of about 100 nm in thickness.

The Euglenid species (*Euglena gracilis*) are distinguished from other algal species by the lack of a typical cell wall but the possession of a pellicle that is mainly composed of protein, lipid, and carbohydrate. The pellicle has unique surface characteristics such as each pellicle stripe is helically arranged, and cavities are present between two stripes. The algal cell wall composition and structure can undergo dynamic changes during the different stages of cell development. For instance, in the vegetative flagellate *Haemotococcus pluvialis*, the cell wall is mainly composed of carbohydrates and proteins, which are linked to a 35 nm thick layer, while during aplanospore formation, the cell wall contains additionally cellulose and thickens to 2.2  $\mu\text{m}$ . Additionally, the alga, *Ochromonas danica* represents a special algal species that do not possess a cell wall, having a specialized cell membrane as the outer surface instead.

## Interactions

### Interaction of nanoparticles with algae

Unicellular algae are important in nanoecotoxicity studies because they are primary producers and represent the base of aquatic food webs. To evaluate the particle effects and their transfer along the food chain in the aquatic environment, it is necessary to determine the uptake and accumulation of nanoparticles in algae, as well as in other aquatic organisms. However, whether particle internalization is a prerequisite for specific effects is not yet known. For nanoparticles to enter algal cells, they must first pass through the cell wall and subsequently through the plasma membrane via endocytotic processes or passive diffusion. The algal cell wall is semi-permeable, and thus large particles (that are above the size of the pores) might be excluded from passing through the cell wall [1]. The diversity in algal cell wall composition and structure may influence the passage of the particles into, and through the cell wall. Few studies have demonstrated endocytosis in algae. Euglenid species are claimed to acquire particulate nutrients by phagocytosis in the absence of light. Specifically for the alga, *O. danica*, micro-sized blue-green algae were visualized to be internalized using electron microscopy [2]. The permeability of the cells can change

during their life cycles. As mentioned, alga, *H. pluvialis*, some particular molecules were found to be taken up by cells exclusively during cell division. During growth, the cell wall may have an increased porosity, due to the insertion of newly synthesized wall materials. Moreover, the adsorption of nanoparticles to the cell surface, or dissolved metal ions, might cause damage to the cell walls or membranes. It is not known yet whether the changes in cell permeability will facilitate nanoparticle internalization. Internalization of nanoparticles in algae was suggested in only a few studies, all of which used metal-based nanoparticles that tend to release metal ions. For instance, silver nanoparticles (Ag NPs) were visualized inside the cell wall deficient alga *O. danica* using TEM imaging.

More often, nanoparticle uptake was not evidenced in algae [3,4], which emphasizes the role of the algal surface as a barrier against nanoparticle entry to the cells [5]. As shown in a systematic study with the alga *C. reinhardtii* (without a cell wall), neither Ag NP nor cerium dioxide nanoparticles were evidenced to be internalized by the algal cells, as measured by ICP-MS, suggestive of a cell wall and the cell membrane could hamper the particle entry. Using hyperspectral imaging, particulate forms of silver were found to be intracellular in Ag NP-exposed *C. reinhardtii* cells, yet the presence of particles was attributed to the reduction or precipitation of  $\text{Ag}^+$  ions that were released from Ag NP, rather than a direct uptake of Ag NP in the exposure medium. Some studies reported that nanoparticles were clustered onto the algal cell wall. However, it is not clear whether the particles have direct contact with the cells.

### Toxicity of silver nanoparticle to algae

Algae have been examined for their sensitivity to Ag NP and other types of nanoparticles. Toxicity studies have reported inhibitory effects of Ag NP on algal growth, and photosynthesis. The effective concentrations reported in these studies range from  $\mu\text{g}\cdot\text{L}^{-1}$  to  $\text{mg}\cdot\text{L}^{-1}$ . While accepting that the Ag ions were released from Ag NPs is extremely toxic contributing to the effects of algae, it remains unclear to what extent the Ag NP contributes to the total toxicity. For example, the toxicity of Ag NP to the freshwater alga, *C. reinhardtii*, and to a marine diatom *Thalassiosira weissflogii*, was entirely barred due to the presence of thiol ligands, thereby demonstrating that such effects of Ag NP were caused exclusively by Ag ions.

In another study, the addition of thiol ligands reduced the inhibitory effects of Ag NP on freshwater algal growth, *O. Danica*. Further to silver ions, Ag NP also adds to the toxicity. Algae are known to secrete extracellular biomolecules, especially enzymes used for nutrient acquisition. Such enzymes include a variety of hydrolytic and oxidative enzymes (viz: alkaline phosphatase,  $\beta$ -glucosidase, leucine aminopeptidase, and phenoloxidase) which cleave recalcitrant organic matter and produce molecules that are readily transported across the cell membranes. Studies on the interactions of nanoparticles with these extracellular enzymes have reported a decreased enzyme activity upon exposure to the particles. In the case of Ag NP, the effects on extracellular enzymatic activity were attributed to both the silver ions and the particles.

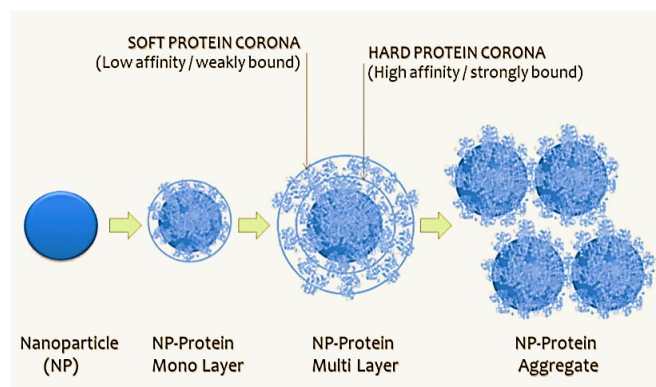
### Interactions of nanoparticles with proteins

The high surface to volume ratio of nanoparticles greatly favours the adsorption of proteins present in the surrounding fluid. Proteins possess different functional groups, such as carboxylate, phosphate, hydroxyl, amine, and sulfhydryl, which offer

a range of active sites to interact and bind with nanoparticles. The adsorbed proteins, 'protein corona', forms single or multiple layers surrounding the nanoparticle surface. The corona determines the fate and interaction of nanoparticles in biological systems. Most of the data regarding the identification and quantification of the protein corona are available from human proteins. Very limited studies have examined the interaction of nanoparticles with yeast and bacterial proteins [6]. No information about the interactions of nanoparticles with proteins in algae exists so far.

### Formation of the protein corona

Interactions of nanoparticles with proteins have been studied with different biological systems, including single selected proteins, extracellular proteins, human plasma, cell extracts, and intact cells. Protein corona is a collective and complex plasma protein corona for all the nanomaterials. The comparative densities of the adsorbed proteins do not associate with their relative abundances in plasma. Therefore, the structure of the protein corona depends on countless parameters and is unique to each nanomaterial. The formation of the protein corona is dynamic in nature (see Figure 1).



**Figure 1:** Hypothetical representation of pathway to nanoparticle associated protein corona formation, with a hard and soft layer of proteins covering the surface of the nanoparticle.

Adsorption of proteins to nanoparticles is driven by colloidal forces and other bio-physicochemical interactions present at the interface, which comprise electrostatic interactions, *Van der Waals* forces, and hydrophobic/hydrophilic interactions. The type of proteins dominating the corona depends on its binding affinity to the particle surfaces and its relative abundance in the surrounding fluid. The corona will be first dominated by abundant proteins, but later by less abundant proteins with a higher affinity. When the equilibrium is reached, the adsorption/desorption of proteins continues at the interface. Depending on the binding affinity, the corona can be classified as a 'hard' corona, composed of high-affinity low exchange-rate proteins, and a 'Soft' corona, composed of low-affinity high-exchange-rate proteins. The binding of proteins to nanoparticle surfaces is influenced by the physicochemical characteristics of particles. It has been shown for gold nanoparticle (Au NP) that the adsorbed protein pattern varied significantly as a function of size, charge and surface coatings of the particles. Also for Ag NP, proteins were found to bind differently to bare surfaces of the particles or to chemically modified surfaces. Knowing the influence of particle physicochemical properties on corona formation may allow the controlled synthesis of nanoparticles with a tunable reactivity with the biological systems, and therefore lower the toxicity of nanoparticles.

## Impacts

### Impacts of protein interactions on nanoparticles

Among the many impacts of protein-nanoparticle interactions on nanoparticles, particle stability is one, which is affected by the adsorption of proteins to the NP surface. The overall charge on the surface of the NP might be either neutralized if proteins that were adsorbed possess the opposite electrical property, or the overall charge might be greater if the adsorbed protein carries the same charge. Changes in surface charge will further affect the stability of nanoparticles. Nanoparticle agglomeration might be driven by molecular forces, like the presence of hydrogen bonding between the particles and proteins. On the other hand, interacting proteins might stabilize the particles, as a result of enhanced electrostatic interactions or steric stabilization. For instance, tungsten carbide nanoparticles quickly agglomerated in the protein-free medium but remained dispersed when the serum protein was supplemented, sterically stabilizing the particles. Moreover, the concentration of proteins was found to affect the stability of nanoparticles, with more agglomerates formed in the presence of a higher concentration of proteins.

### Impacts of protein-nanoparticle interactions

The native conformation of proteins determines their biological functions. During the formation of a protein corona, the proteins undergo a partial loss of structure, which may expose undesired epitopes and render the proteins dysfunctional. Rearrangements of myoglobin structure upon binding to different nanoparticle surfaces have been reported. Using both, experiments and simulations, the destabilization of  $\alpha$ -helix but increased  $\beta$ -sheet was shown in Ag NP adsorbed ubiquitins. In another study, fibrillation of 2-microglobulin (human plasma protein) was found to occur on various types of nanoparticle surfaces, including copolymer nanoparticle,  $\text{CeO}_2$  NP, and carbon nanotubes. The fibrillation process led to the formation of insoluble protein aggregates, which are typically found in many human diseases (e.g. Alzheimer's disease). Also, chemical modifications of proteins, such as carboxylation, might occur upon interactions with nanoparticles. Different kinds of enzymes, including lysozyme, horseradish peroxidase, catalase, and trypsin, were characterized for their interaction with silicon nanoparticles ( $\text{SiO}_2$  NP) and showed that the strong association with the nanoparticles caused conformational changes and significant loss in their enzymatic activities. The sorption of nanoparticles was found to induce alterations of enzyme structure and function in a size-dependent manner. In contrast, the adsorption of luciferase to Ag NP did not induce conformational changes in this enzyme, though reduced enzymatic activity was measured upon interaction with the Ag NP, which was largely accredited to the silver ions that came from particles.

## Mechanisms

### Algal biosynthesis of nanoparticles

In several research investigations, several stages were present in the algal biosynthesis of metal NPs

- The algal extract is prepared either in an aqueous or organic phase - either by heating for a definite period,
- Molar solutions of metallic compounds (ionic state) were prepared and metallic compounds in the ionic state (molar concentrations); algal extracts and were incubated either with or without stirring under measured conditions.

Synthesis of NPs can be done in the intracellular or extracellular way in a dosage-dependent approach depending on the algal type.

- Metallic NPs are formed extracellularly in the algal aqueous phase [7,8,9] is mainly because of the reducing agent's activity, like polysaccharides, proteins, reducing sugars, pigments, peptides or extra reducing elements which reduce metal ions and precipitate the metallic ions (to NPs) [10,11,12].
- Metallic NPs are formed intracellularly is a concern, algal metabolism, a process like respiration and photosynthesis are responsible for reducing metallic ions [13,14].
- Cyanobacteria, the reduction was possible by NADPH or its reductases via reactions that generate electrons either with photosynthetic or Electron Transport System (ETS). To some level, utilizing ETS (respiratory) and to a lesser degree occurring redox reactions at the cell membrane, thylakoid membrane and in the cytoplasm, are crucial for the conversion mechanism of metallic ions to metallic NPs intracellularly.
- It was understood that the enzyme nitrogenase may add to the reduction of gold ions to Au NPs intracellularly. In a distinct study, the isolated chloroplasts/thylakoids from higher plants known to reduce Au(III) to AuNPs via electron transport from H<sub>2</sub>O to Au(III) by photochemical means [15].
- The first synthesis of metallic NPs occurs intracellularly in a dose-dependent manner by internalization of the metallic ions and subsequently internal reduction (via polysaccharides and other macromolecules) to produce stable metal NPs into the extract medium.

Moreover, microalgae are highly accustomed to the production of metallic NPs and bimetallic NPs (nanoalloys), a dosage-dependent routine. In the aqueous phase, physical parameters like pH, temperature, actual concentration and metal type, incubation time, and reducing agent concentration can be controlled to design and produce metal NPs of essential shape and size, besides, prevents agglomeration.

The pH has a reasonable effect on the extracellular synthesis of metallic NPs. In the aqueous phase at <pH, the total reducing capacity of all the functional groups is low due to high H<sup>+</sup> conc., but with an increase in H<sup>+</sup> concentration, the reducing capacity of the functional groups is increased allowing the improved stability of metallic NPs and at the same time preventing their agglomeration. Additionally, for the metal NPs hydrophilic and hydrophobic interactions play a vital role in preventing agglomeration.

In record cases, chromatic variations served as an optical indicator for the confirmation of synthesis of Ag and Au nanometals in the reaction mixture; a colour change to dark brown indicates Ag nanoparticle (Ag NP) formation, and a colour change to dark pink or dark ruby red indicates AuNP availability [16]. Due to surface plasmon resonance metallic NPs display sharp and unique optical characteristics, the aqueous biosynthesis of nanometals is examined in the spectral region (190–1100nm) of absorption spectroscopy. Nanometals have an explicit band of SPR absorption ( $\lambda_{\max}$ ) due to the combined free-electron vibrations coming from metal NPs which are in resonance with the light wave, completely depending on its shape, aspect ratios,

size, and on the metals dielectric constant [17,18]. Au NPs of around ~20 nm exhibit an orange-red colour which slowly shift to blue clearly states the particle size has reached ~100 nm. SPR bandwidth broadening is a clear-cut indicator of nanometal size and polydispersity (Jena et al. 2013). In an aqueous solution, an increase in particle size, bandwidth decreased with increased band intensity; therefore, UV/vis spectroscopy could be a useful tool to determine the metal NPs size. The  $\lambda_{\max}$  appear in the range 324–586 nm (for Ag NPs) and 505–565 nm (for Au NPs) respectively. Diverse biomolecules present in the algal extracts include polysaccharides, peptides, proteins and pigments; responsible for the bioreduction of the metals. Peptides proteins, via-NH<sub>2</sub> groups or cysteine residues and sulfated saccharides (poly) involve in capping and stabilizing the metal nanoparticles in aqueous concentrations. Fourier-Transformed Infrared (FT-IR) investigation reveals the responsible reducing agents for reduction, capping and stabilization of the metal NPs. Several functional groups like -NH<sub>2</sub>-, -C=O-, and -SH- bound to the surface of the nanomaterials have been characterized by FT-IR.

Different algal varieties along with *cyanobacteria*, *microalgae* [19] and *macroalgae* reduced a variety of metals to form nanometals. Ali et al. (2012) observed that the marine *cyanobacterium*, NTDM05 produces nanoparticles of cadmium sulfide (CdSNPs) while mixing CdCl<sub>2</sub> and Na<sub>2</sub>S solutions with cell extracts. Phycobiliprotein C-phycoerythrin produced spherical CdS NPs of ~5 nm size enacting a capping agent. Jena et al. (2014a) established that microalga *Scenedesmus-24* facilitated uptake of Cd, synthesizing intracellular CdS NPs, (150-175 nm); -OH, -NH, -NH<sub>2</sub>, (PO<sub>4</sub>)<sup>3-</sup> and -COOH moieties were associated in the reduction process. Spherical-shaped and extended crystalline copper oxide NPs (CO NPs) were biosynthesized with an average size of 20.6 nm (5–45 nm) using brown alga, *Bifurcaria bifurcata* aqueous extract in 1 mM CuSO<sub>4</sub>, between 110–120 °C while stirring continuously. The blue-coloured solution turned red via a colourless phase, with  $\lambda_{\max}$  at 260 nm with the involvement of -C=O-, -OH-and -C=C- groups have been accredited to the synthesis of CO NPs; also demonstrated antibacterial activity against *S. aureus* and *E. aerogenes*. Iron oxide, Fe<sub>3</sub>O<sub>4</sub> NPs (18 ± 4 nm), cubic-shaped and magnetic were biosynthesized with the brown alga, *S. muticum* aqueous extract. NPs appeared by reducing Fe<sup>3+</sup> in FeCl<sub>3</sub> solution (0.1 M), heated for 90 min at 25 °C using mechanical stirring.

Vibrating Sample Magnetometry (VSM) confirms the superparamagnetic nature of Fe<sub>3</sub>O<sub>4</sub> NPs. The sulfated polysaccharides are the key ingredients act as reducing agents and efficient stabilizers for these Fe<sub>3</sub>O<sub>4</sub> NPs. Lately, spherical monodispersed iron nanoparticles (FeNPs) of 20-45 nm size synthesized with extracts from green microalga, *Chlorococcum sp.* stated in which 0.1 M FeCl<sub>2</sub> solution kept in the dark for 48 h.  $\lambda_{\max}$  was 293 nm and a change in colour from reddish-yellow to yellowish-brown confirms the formation of the FeNPs. These FeNPs further evaluated towards heavy metal remediation, as FeNPs quickly converts 92 % of Cr(VI) to Cr(III), this suggests application towards chromium remediation. Crystalline spherical palladium nanostructures (2 to 15 nm) were also biosynthesized using green microalga, *C. vulgaris*. Reaction mix solution containing algal cells and Na<sub>2</sub>[PdCl<sub>4</sub>] solution (25 mgL<sup>-1</sup>) at 25 °C, with mechanical stirring continuously at 150 rpm thus resulting in the highest yield of Pd nanoparticle (PdNP); this reduction reaction, where Pd(II) converted into Pd(0) NPs primarily accredited to the uptake of microalga, photoautotrophic. The immobilization of PdNPs over chitosan mats in a reaction mixture was further studied; Pd nanocatalysts in Mizoroki-Heck cross-coupling reac-



tion known to have 68% catalytic activity.

Zinc oxide nanoparticles (ZnO NPs, Ave. size of 80 nm) were biosynthesized using *Anabaena strain L31*. Highly crystalline, spherical-shaped ZnO NPs ( $\lambda_{\text{max}}$  was 370 nm; zeta potential of 30.25 mV) were produced after incubating the cell extract in 1 mM ZnNO<sub>3</sub> in the dark at 25 °C for 10-12 h. Conjugating NPs with shinorine, an amino acid-like substance mycosporine exhibited UV-B absorption properties. The ZnO NP-shinorine conjugates (zeta potential, -3.75 mV) forming 75% less reactive oxygen species (ROS) in *Anabaena* in comparison to ZnO NPs show promising application towards possible sunscreen agent. *Scenedesmus obliquus* (green microalga) found to produce (Zn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>) nanoneedles. Azizi et al. (2014) described biosynthesis of hexagonal ZnO NPs (30–57 nm) using dried brown alga powder *S. muticum*, mixed with Zn(O<sub>2</sub>CCH<sub>3</sub>)<sub>2</sub> dehydrate for 3–4 h at 70 °C. Brown to white colour with  $\lambda_{\text{max}}$  at 334 nm indicated the ZnO NP synthesis, accredited to the -SO<sub>4</sub> and -OH moieties present in polysaccharides.

### Biosynthesis of Au NPs using macroalgae

Among the several variants of macroalgae; green algae brown algae and red algae were thoroughly used for Au NPs biosynthesis. *Rhizoclonium fontinale* and *Ulva intestinalis* (green algae) intracellularly produced AuNPs. Thallus colour change to purple confirms the presence of AuNPs, with diverse absorption maxima at varied pH. *Rhizoclonium fontinale* exhibited  $\lambda_{\text{max}}$  at 537 nm (pH 5), 529 nm (pH 7) and 517 nm (pH 9); while *U. intestinalis*, significant peaks were obtained at 542 nm (for both pH 5 and 9) and 541 nm (pH 7). *U. intestinalis*, shapes of the nanoparticles varied from globular to asymmetrical shape with an average particle size of 42.39 nm. The other two green algae, *Pithophora oedogonium* and *Chara zeylanica* could not undergo chemical reduction to produce Au NPs. A maximum yield of monodispersed Au NPs produced from *R. fontinale* while dissolving algal content in 15 mg/L HAuCl<sub>4</sub>.XH<sub>2</sub>O (~pH 9 kept for nearly 72 h). *Prasiola crispa*, a freshwater green alga, produced nearly spherical AuNPs of size (5-25 nm under 12 h) at 25 °C. Biosynthesis of Au NPs is dependent on numerous factors like pH and concentration of the medium and biomass concentrations are responsible for obtaining the Au NPs of variable shapes and sizes.

Extracellular synthesis of AuNPs (size range, 8–12 nm; <12 h) reported with marine brown alga extract, *S. wightii*. The conversion rate is roughly 95 % from Au ions to AuNPs ( $\lambda_{\text{max}}$  at 527 nm). Mata et al., 2009 used algal biomass of *F. vesiculosus* in reducing Au(III) to Au(0) under the effect of varied pH range from 2.0 to 11 (size and shape varied randomly). The optimum pH 7.0 seems good for the Au uptake by algal biomass, perhaps at pH 7, owing to the optimal reactivity of -OH groups and stability of algal cells present in the polysaccharides. The pigment fucoxanthin, rich in -OH groups, suggests being very supportive of this bio-reduction process. The researchers further added that *F. vesiculosus* bio-reduction could be an alternate way for recovering Au from large hydrometallurgical vessels and leachates from electronic industry scraps. Circularly shaped average-sized, 21.56 nm AuNPs (varied range 12.5 to 40 nm; a minor volume fraction with dia. ~10 nm) biosynthesized extracellularly via red marine alga, *Kappaphycus alvarezii*. These nanoparticles were stable for >180 days with slight aggregation. The study revealed that NPs size can be meticulously designed by controlling certain factors like metal concentration, incubation time, and reaction temperature. Sizes of NPs were credited to the large presence of sulfated polysaccharides and at the same time, the

synthesized NPs did not damage any algal cells.

Ganesan et al., (2013) have demonstrated in another study the biosynthesis of Ag NPs using *K. alvarezii*. Aliquots fewer than 10 mL of algal extracts (Aqu.) were added to 190 mL of AgNO<sub>3</sub> (1 mM) while shaking thoroughly for 96 h at room temperature (27 °C). SEM images suggested the average size of these Ag NPs having 75 nm. FT-IR data confirmed the participation of C–O groups in polysaccharides, performs the role of a stabilizing agent. Another species of the brown alga, (*Laminaria japonica*) used to biosynthesize AuNPs; quickly the solution turned to red when diverse algal extracts concentrations were incubated along with HAuCl<sub>4</sub> solution (2.5 mM) at 37 °C. 90–95 % Au<sup>+</sup> was converted intracellularly to AuNPs (size range, 15–25 nm) under 10–25 min. This confirms the polysaccharides, key components for the reduction of Au ions. Rajathi et al. (2012) produced spherical AuNPs (average size, 62.4 nm) using *Stoechospermum marginatum* (brown alga). 1 mM of HAuCl<sub>4</sub> and the algal extract, incubated for 10 min while the solution slowly changed to dark ruby red indicating chemical reduction. The reduction of Au ions was attributed to the -OH groups of algal diterpenoids. Au NPs antibacterial activity against *Klebsiella pneumoniae* and *Enterobacter faecalis* was estimated with Disc Diffusion Assay (DDA).

Using brown alga *Turbinaria conoides* synthesis of triangular, cubic, rectangular and square-shaped Au NPs was reported by Rajesh Kumar et al. (2013). The slow reaction starts after 50 min and was completed under 48 h (ave. size, 60 nm) after mixing 1 mM HAuCl<sub>4</sub> and algal extracts. The so-formed AuNPs showed antimicrobial activity against numerous microbes in the ensuing order:

### *Streptococcus* > *K. pneumoniae* > *B. subtilis*

Vijayan et al. (2014) biosynthesized Ag NPs [20] (2-16 nm) and Au NPs (2-18 nm) from an aqueous solution of *T. Conoides* with its characteristic SPR at 422 and 536 nm, respectively. The cytotoxicity of these Ag nanoparticles against *Artemia salina* with LC<sub>50</sub> being 88.9 µLmL<sup>-1</sup>. The biosynthesized Ag nanoparticles showed potent anti-biofilm activity against some of the marine bacteria [*E. coli* (JN585664), *Salmonella sp.* (JN596113), *Aeromonas hydrophila* (JN561697)] and *S. liquefaciens* (JN5961151). All the species were isolated from Thodi coastal waters, India. Fast biosynthesis of spherical and triangular Au NPs using dried extracts of the brown alga, *Ecklonia cava*, in HAuCl<sub>4</sub> at 75°C under 1 min (ave. size, 30 ± 0.25 nm) [21]. At varying temperatures and pH, another marine brown alga, *P. gymnospora*, bio-synthesized AuNPs extracellularly; optimum synthesis was obtained at pH 10 and 75 °C for 12 h. The size was in the range of 54–66 nm with 60 nm as the max. The height of particle roughness, -OH groups of polysaccharides were suggested to involve in the reduction.

Au nanoparticles synthesis has also been described using aqueous red algal extracts. Abdel-Raouf et al. (2013) described fast and massive biosynthesis of Au nanoparticles using red alga, *Galaxaura elongata* with both aqueous (H<sub>2</sub>O) and ethanolic (C<sub>2</sub>H<sub>5</sub>OH) algal extracts. The reduction of the Au<sup>+</sup> ions was completed in 3 h by the aqueous algal extract, while the ethanolic extract took 2-5 min. In both cases, the solution slowly turned pale-dark red, and the  $\lambda_{\text{max}}$  was observed at 535 (aqueous algal extract) and 536 nm (ethanolic extract) respectively. Sizes also varied with these extracts and were revealed from the zeta potential data; 3.85-77.13 nm size range with shapes like spherical, few triangular, hexagonal, and rod-shaped par-

ticles from TEM images. Compounds like alloaromadendrene oxide, andrographolide, hexadecanoic acid, epigallocatechin, oleic acid, 11-eicosenoic acid, gallic acid catechin, and epicatechin were observed in algal extract act simultaneously as reducing, stabilizing and capping agents. The Au nanoparticles synthesized using ethanolic extract displayed fairly good antibacterial activity against *E. coli*, *K. pneumoniae*, and methicillin-resistant *S. aureus*; while Au nanoparticles synthesized using algal powder was observed to be effective against *P. aeruginosa* and *S. aureus*. *Gracilaria corticata* incubated in 0.001 M H<sub>2</sub>AuCl<sub>4</sub> at 40°C and with stirring continuously at 120 rpm produced Au nanoparticles in the range of 45 - 56 nm in size under 4 h. Au nanoparticles in combination with the antibiotic ciprofloxacin were investigated for their antibacterial activity. The activity was highest against, *E. coli* and *Enterobacter aerogenes*, moderate against *S. aureus*, the lowest activity found against *Enterococcus faecalis*, indicating the effectiveness of the complex against

gram (-)ve bacteria. The Zone Of Inhibition (ZOI) by ciprofloxacin alone was less than that of the nanoparticle-ciprofloxacin conjugate. Properties like the small size, the huge surface areas along the higher penetrating power of AuNPs were credited to these results. Significant results were achieved investigating the antioxidant activity of AuNPs; showed the significant capacity of scavenging with 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH). Sharma et al. (2015) [22] used red alga biomass (dried), *Lemanea fluviatilis*, to biosynthesize AuNPs (5–15 nm) with a SPR around 530 nm while retaining the mixtures red colour while exhibiting the antioxidant activity. The algal proteins are indicative of the reduction and stabilization of the Au nanoparticles. Ramakritinan et al. (2013) engaged *Gracilaria sp.* produced NPs of Au, Ag and even with bimetallic Ag-Au nanoalloys. Thus obtained nanoparticles were colloidal in nature and showed  $\lambda_{max}$  peaks at 536 nm (Au), 419 nm (Ag), 526 nm (Ag/Au, 1:3), 504 nm (Ag/Au, 1:1), and 501 nm (Ag/Au, 3:1).

**Table 1:** List of metal nanoparticles biosynthesized by various classes of cyanobacteria.

Species	Metal/shape of NPs	Size (nm)	Reference
<i>Lyngbya majuscula</i>	Au / spherical	>20	[23]
<i>Nostoc ellipsosporum</i>	Au / nanorods	137–209 (length) 33–69 (diameter)	[80]
<i>Leptolyngbya boryana</i> (as <i>Plectonema boryanum</i> )	Au / nanoparticles	10- 25 nm	[24]
<i>Synechocystis sp.</i>		13±2	[25]
<i>Leptolyngbya boryana</i>	Au/ octahedral	<10 to 6 $\mu$ m	[26]
<i>Leptolyngbya valderiana</i> (as <i>Phormidium valderianum</i> )	Au / spherical	15 (@pH 5)	[69]
	Au / spherical	7.92 (@pH 7)	
	Au / nanorods	411x32 (@pH 5)	
	Au / triangular	24 (@pH 7)	
	Au/hexagonal	13.78 (@pH 9)	
<i>Arthrospira platensis</i>	Au-core-Ag shell / spherical	17 – 25	[27]
	Au-core-Ag shell / spherical	6 – 10	
<i>Arthrospira (Spirulina) platensis</i>	Ag / nanoparticles	11.6	[28]
<i>Anabaena</i>		24.13 ± 2	[44]
<i>Limnothrix sp. 37-2-1</i>		31.86 ± 1	
<i>Synechocystis sp. 48-3</i>		14.64 ± 2	
<i>Leptolyngbya boryana</i>		Ag / spherical & octahedral	10 (IC) 1–200 (EC)
<i>Microcoleus sp.</i>	Ag / spherical	44–79	[30]
<i>Phormidium (Oscillatoria) willei</i>		100–200	[31]
<i>Arthrospira platensis</i>		7–16	[27]
<i>Leptolyngbya boryana</i>		Pt / spherical	<0.3 $\mu$ m
<i>Anabaena sp. L31</i>	ZnO / spherical	80	[38]
<i>Leptolyngbya tenuis</i> (as <i>Phormidium tenue</i> )	CdS / spherical	~5	[33]

**Table 2:** List of metal nanoparticles biosynthesized by various classes of microalgae.

Species	Metal/shape of NP	Size (nm)	Reference
<i>Chlamydomonas reinhardtii</i>	Ag / rounded & rectangular	5–15 in vitro 5–35 in vivo	[34]
<i>Auxenochlorella pyrenoidosa</i>	Ag / spherical	5–10	[35]
<i>Chlorococcum infusionum</i> (as <i>Chlorococcum humicola</i> )		2–16	[36]

<i>Chlorella vulgaris</i>	Ag / nanoplates	28	[37]
	Ag	>15	[42]
		12.62	[38]
	Au / nanoplates	10-20	[39]
[40]			
<i>Chlamydomonas reinhardtii</i>	Bimetallic Ag Au nanoalloy / spherical & round	–	[41]
<i>Nannochloropsis oculata</i>	Ag	>15	[42]
<i>Parachlorella kessleri</i>		9, 14 or 18	[43]
<i>Botryococcus braunii</i>		15.67	[44]
<i>Scenedesmus sp.</i>		15–20	[45]
<i>Auxenochlorella (Chlorella) pyrenoidosa</i>	Au / spherical	25–35	[46]
<i>Klebsormidium flaccidum</i>		9±3.4	[47]
<i>Tetraselmis 'kochinensis'</i>		15	[48]
<i>Cosmarium impressulum, Kirchneriella lunaris</i>		<10 nm	[49]
<i>Tetraselmis suecica</i>		79	[50]
<i>Chlorella vulgaris</i>	Au	40–60	[51]
<i>Eolimna minima</i>		5–100	[52]
<i>Chlorella vulgaris</i>	Pd / spherical	7	[53]
<i>Chlorococcum sp.</i>	Fe / spherical	20–50	[54]
<i>Scenedesmus-24</i>	CdS / spherical	150–175	[55]

**Table 3:** List of metal nanoparticles biosynthesized by various classes of macroalgae.

Species	Type and shape of NPs	Size (nm)	Reference
<i>Caulerpa racemosa</i>	Ag	5–25	[56]
<i>Codium capitatum</i>		3–44	[57]
<i>Sargassum cinereum</i>		45–76	[58]
<i>Sargassum muticum</i>		5–15	[81]
<i>Sargassum vulgare</i>		~10	[59]
<i>Cystophora moniliformis</i>		75–77	[60]
<i>Kappaphycus alvarezii</i>		73	[61]
<i>Sargassum plagiophyllum</i>		20–50	[72]
<i>Gelidiella acerosa</i>	Ag (spherical)	22	[62]
<i>Gracilaria dura</i>		6	[63]
<i>Sargassum plagiophyllum</i>		AgCl	18–42
<i>Bifurcaria bifurcata</i>	Cu	20.6	[65]
<i>Fucus vesiculosus</i>	Au	Several	[66]
<i>Saccharina (Laminaria) japonica</i>		15–20	[67]
<i>Padina gymnospora</i>		53–67	[68]
<i>Rhizoclonium fontinale</i>		16	[69]
<i>Padina tetrastromatica</i>		14	[70]
<i>Padina gymnospora</i>		25–40	[71]
<i>Ulva reticulata</i>		40–50	[72]
<i>Turbinaria conoides</i>		60	[73]
<i>Galaxaura rugosa (as Galaxaura elongata)</i>		3.85–77.13	[74]
<i>Sargassum wightii</i>		8–12	[75]
<i>Gracilaria corticata</i>		45–57	[76]
<i>Lemanea fluviatilis</i>		5–15	[77]
<i>Gracilaria sp.</i>		–	[78]
<i>Kappaphycus alvarezii</i>		12.5–40	[79]



<i>Ulva intestinalis</i>	Au (spherical to irregular)	42.39	[80]
<i>Turbinaria conoides</i>	Ag, Au	2–19, 2–17	[20]
<i>Sargassum muticum</i>	ZnO	30–57	[81]

## Conclusion

Several materials specifically nanomaterials synthesized by various means are playing a key role in our day-to-day life, exclusively in the development of innovative functional materials for numerous applications. Several branches from sciences to engineering allow the growth of nanotechnology and related novel materials to attain their true potential. Nanotechnology is considered a modern advancement in various fields of applications like electronics, medical, pharmaceutical industry etc. Although nanotechnology is advancing in various fields, nanoparticles are found to be hazardous to human health in terms of the size of the particles being synthesized during the process. Owing to their unique optical, magnetic, electronic and catalytic properties with their distinctive feature of size and shape nanoparticles gained interest. Many researchers have biosynthesized using different marine organisms. Diverse marine environments with different biological species have shown abundant potential for the biosynthesis of nanoparticles.

Microalgae are considered as one of the most valuable sources for various applications like phyco-nanotechnology, manufacturing of drugs and food products, etc. Much of the research is also going at a faster pace to make the entire world in the form of nanotechnology. The production of nanotechnological products from various biological sources is being widely studied and is being applied in every field. When it comes to the field of phycology the algae have been considered as the least priority in developing nanotechnological products when compared with other biological sources like plants, bacteria, fungus etc. From the available literature, it is already proven that microalgae are an excellent source not only for the production of biofuels but it is also proven to synthesize the metallic nanoparticles with their diverse applications in various fields like clinical diagnostics, agriculture and other important areas like paints, electronics, coatings and packing. Since the algal growth rate is very high when compared with other sources, approaches like algae mediated metallic NPs synthesis is superior when compared with chemical synthesis. Biosynthesis of metal nanoparticles has been reviewed using cyanobacteria, microalgae and macroalgae were reviewed here at length in this chapter. The interaction of nanoparticles, toxicity towards algae, nanoparticles with proteins, and the formation of the corona were discussed. Currently, a wide variety of organisms have been for the production of highly useful nanoparticles.

Several researchers have reported synthesis/interactions with various species of algae with diverse shapes and sizes being synthesized using various species. Researchers should evaluate which particular species of algae can chemically reduce particular metal with maximum yield. Presently, there are countless species of micro as well as microalgae that can synthesize metallic as well as non-metallic nanoparticles and these have found to have a wide variety of applications in many fields of science. Algae species also can fight against the toxicity of any nanoparticles and make them into a useful form. It was clearly stated that algae hold a promising role in the field of nanotechnology due to its unique varying properties exhibited during the synthesis of various metallic nanoparticles. However, researchers should also study the synthesis process not only with these

metallic nanoparticles but also with the other available materials like more oxide and more chalcogenide-based ones. Future research should focus on these a variety of points and issues to craft nanotechnology a successful function for the upcoming generations to come.

## Conflict of interest

Authors have no conflict of interest and all authors contributed equally to this chapter.

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