Green synthesis of metallic nanoparticles using algae and microalgae

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ABSTRACT

Biosynthesis of nanoparticles is considered to be an environment-friendly and cost-effective process. Ions (typically positively charged/anionic moieties) are absorbed to the surface of microorganisms due to the presence of negative charge on the cell wall resulting in the formation of metal nanoparticles (usually nanoparticles) on the surface of these cells. In this entry, some important aspects of nanoparticle formation by the employment of microalgae and cyanobacteria have been reviewed. After a short definition of nanoparticles and their biosynthesis, different types of nanoparticles produced by cyanobacteria and microalgae are presented. The ability of the marine microorganisms to form nanoparticles with favorable physicochemical and morphological properties in ideal sizes has opened up a novel approach to nanoparticle synthesis.

Keywords: biosynthesis; microalgae; nanoparticles.

1. INTRODUCTION

Nanotechnology involves application and synthesis of particles with sizes ranging from 1 to 100 nm [1, 2]. Different types of nanoparticles can be synthesized by biological organisms such as plant, fungi, algae, cyanobacteria, bacteria as well as by the employment of biomolecules [3, 4]. Nanoparticles can be produced by diverse groups of the plant kingdom including algae. Algae are eukaryotic, aquatic and oxygenic photoautotrophs, and some of them are capable of accumulating different heavy metals. However, there are very few reports about the biological synthesis of noble metal nanoparticles employing algae [5, 6]. Several chemical or physical methods of synthesis of nanoparticles are also reported. However, they often require expensive and complex procedures. To address these problems, biological synthesis of nanoparticles and their efficient characterization is considered to be a breakthrough. Moreover, scientists are trying to find out the suitable biological entity for the synthesis in terms of availability, less time-consuming and novelty [7].

Algae, as a "bio-factory", are also being employed for the synthesis of metallic nanoparticles. Among different types of bioreducers, seaweeds have well-defined benefits due to their high metal uptake capacity, in addition to their macroscopic morphology and being readily available [8]. The synthesis of silver, gold, platinum and palladium nanoparticles using the freshwater cyanobacterium, Plectonema boryanum (UTEX 485) has been already reported [9-12]. On the other hand, several microalgae are in GRAS list and many reports indicate their health beneficial impact on human following their consumption. Spirulina in food causes unique therapeutic effects including immunomodulation, anticancer, antioxidant, antiviral and antibacterial, metalloprotective [13-15]. In this context, Arthrosira platensis (A. platensis) is the most available and commonly utilized genus and has been the subject of many studies in the food and pharmaceutical industries [3, 16, 17]. Brown seaweeds, such as Sargassum wightii, Turbinaria conoides, and Laminaria japonica can rapidly produce Au nanoparticles through extracellular biosynthesis [18, 19]. Among the key advantages which the biological approach has over traditional chemical and physical nanoparticle synthesis methods, is the biological capacity to catalyze reactions in aqueous media at the ambient temperature and pressure. Production in aqueous media under the standard conditions leads to many economical advantages, with respect of both equipment and operating expenses, especially in the purchase and disposal of solvents and other consumable reagents. Biosynthesis can be implemented in almost any setting and at any small or large / industrial scale [20]. The properties of the biosynthesized materials may vary depending on their preparation methods. Biosynthesis can result in the formation of nanostructures (such as alloys and wires), which are difficult to make using other techniques. Biosynthesized nanoparticles (NPs) can also have enhanced stability and biocompatibility and reduced toxicity. This is mainly due to their coating with biogenic surfactants or capping agents. The extensive range of sizes, shapes, morphologies and compositions of the biosynthesized NPs can be utilized in a broad range of existing and new nanomaterial applications [2]. Table 1 shows different size of nanoparticles produced by different cyanobacteria and microalgae. Variables including seaweed extract, metal ion concentration, and pH, as well as temperature and reaction time
influence on the bioremoval efficiency. *S. myriocystum* can synthesize zinc oxide nanoparticles which can be observed through the change of the colour and UV-visible spectrophotometry assay [20]. Singaravelu et al. [21] and Rajasulochana et al. [22] reported the synthesis of extracellular metal bionanoparticles using *Sargassum wightii* and *Kappaphycus alvarezii*, respectively. Also, Senapati et al. [23] reported the production of gold nanoparticles in the cells by Tetraselmis kochinensis. Also, bioreduction of Au(III)–Au(0) by brown alga *Fucus vesiculosus* has been reported [24]. *C. crispus* is a small purplish-red seaweed (up to 22 cm long), which can be found on rocky shores and in pools. It has been reported that the cell wall polysaccharide carrageenan can bind heavy metals [25]. Authors suggested that differences in the levels of sulphation in carrageenans may account for their different abilities to bind heavy metals. This red alga has been tested because of the well-known ability of its sulphur content to cap and stabilize gold nanoparticles [25, 26].

### 1.1. Example of produced nanoparticles by using algae.

*Chlorella vulgaris* can bind to tetrachloroaurate ions, and subsequently reduce it to Au (88% in metallic state). Gold crystals can be observed and collected in the inner and outer cell membrane with decahedral, tetrahedral, and icosahedral structures [16].

*Spirulina platensis* is an edible blue–green alga which its dried biomass can be used for the extracellular synthesis of gold, silver and Au/Ag bimetallic nanoparticles [27].

Table 2 shows lists of reported marine organisms capable for producing of nanoparticles. Naveena et al report on the extracellular biosynthesis of gold nanoparticles from the marine alga *Gracilaria corticata* for its antimicrobial and antioxidant activity. *Gracilaria corticata* is a prevailing red microalga species found in coastal regions of Indian subcontinent, belonging to the family Gracilariacea [28].

The brown seaweed *Sargassum wightii* is can produce gold nanoparticles (8 and 12 nm) [8], which produced nanoparticles are quite stable [29]. Also, *Fucus vesiculosus* can biosorbed gold for further reduction, which can be considered as an echo-friendly bioremoval process for recovering gold from dilute hydrometallurgical solutions and leachates of electronic scraps. Finally gold nanoparticles will be synthesis in different size and shape [24]. Similarly, the extracellular synthesis of silver nanoparticles by the brown seaweed *Sargassum wightii* and their antibacterial effects are registered [28]. In addition to their antibacterial activities, the nanoparticles synthesized by seaweed extracts possess a stabilizing effect on cotton fabrics [30].

### 2. BIOSYNTHESIS OF AgNPS BY ALGAE

*Nannochloropsis oculata*, *Dunaliella salina* and *Chlorella vulgaris* were employed in a similar method for monitoring of nanoparticle. Three replicates of each algal culture were considered in 1000 ml Erlenmeyer flasks as following:

1. N. oculata culture was fulfilled approximately 120h in basal medium comprising 0.1% (v/v) Valea medium nutrients, 0.1% (v/v) trace metal solution, and 1.7 µM urea as a nitrogen source, in artificial seawater (A.S.W).

2. SK medium (Gladue and Maxey 1994) was used for D. salina culture.

3. Finally *C. vulgaris* was cultured in M8 medium (Mandalam and Palsson 1998) and were exposed to a light level of 1500 lx, in 24±2°C under a photoperiod of 16.8. After addition of AgNO₃ solutions for 24 and 48 h, biomass was harvested. *N. oculata* and *C. vulgaris* and produced AgNPs [34].

The initial pH value of the solution have significant impact on the gold nanoparticle size and shape (particularly for *C. crispus*) [8].

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**Table 1.** The size of produced nanoparticles produced by various photosynthetic microorganism: cyanobacteria and microalgae.

| No | Photosynthetic microorganism | Shapes of nanoparticles | Size |
|----|-------------------------------|-------------------------|------|
| 1. | *Plectonema boryanum* UTEX 485 | Octahedral, gold platelets | <10nm |
| 2. | *Anabaena* Sp. | Spherical | |
| 3. | *Calothrix* Sp. | Hexagonal triangular | <100nm |
| 4. | *Leptolyngbya* Sp. | Spherical | |
| 5. | *Spirulina platensis* | Rhombic | <6-10nm |
| 6. | *Spirulina subsalsa* | Spherical | <20nm |
| 7. | *Lyngbya majuscula* | Spherical | <100nm |
| 8. | *Phormidium valderianum* | Hexagonal triangular | <100nm |
| 9. | *Phormidium* Sp. | Lrregular | |
| 10. | *Microcoleus chthonoplates* | Hexagonal triangular | 25nm |
| 11. | *Phormidium willei* | Triangles | |

**Microalgae (Eukaryotic)**

| No | Photosynthetic microorganism | Shapes of nanoparticles | Size |
|----|-------------------------------|-------------------------|------|
| 12. | *Rhizoglonium hieroglyphicum* | Spherical | <20nm |
| 13. | *Navicula minima* | Spherical | |
| 14. | *Rhizoglonium riparium* | Spherical | <100nm |
| 15. | *Ulva intestinalis* | Spherical | |
| 16. | *Rhizoclonium fontanale* | Spherical | |
| 17. | *Chlorella* Sp. | Spherical | |
| 18. | *Tetraselmis suecica* | Spherical | 79nm |
| 19. | *Chlorella vulgaris* | Spherical | 40-60nm |
| 20. | *Tetraselmis kochinensis* | Spherical | 5-35nm |
| 21. | *Coelatrella* Sp. | Triangles | 30nm |

* reference (9).
Spirogyra insignis can synthesize silver and gold nanoparticles in specific condition and feasibility of metal recovery by absorption on the biomass surface is very important [27].

3. SYNTHESIS OF GOLD NANOPARTICLES BY DIFFERENT KINDS OF ALGA

The nanoparticles synthesis and accumulation reactions were started after the algae were introduced into 1 mM aqueous chloroauric acid (HAuCl₄) solution. Incubation occurred in dark room condition then the pale-yellow color reaction mixture was turned to colorless which confirmed the synthesis of gold nanoparticles. The color change was occurred due to the reduction of the gold metal ions into gold nanoparticles that the active molecules present in the photosynthetic microorganism do it. The incubation time of nanoparticles synthesis directly has effect on intensity of the color change. It is due to the excitation of surface plasmon resonance (SPR) and reduction of AuCl₄. The accumulation of gold nanoparticles inside the microalgal cells with ruby red color was observed. These types of intracellular changes were found the cells of algae. Experimentally, cyanophycean and chlorophycean biomass treated with 1 mM of HAuCl₄ solution started to turn purple within an hour of exposure, in opposite of the control sets which remained the primary color. A better understanding of the mechanisms of gold biosynthesis of bacteria and cyanobacteria is needed to go forward our ability to detect anomalously rich gold deposits in the natural environment and to optimize gold recovery from aqueous solutions [9, 35-39].

4. PRODUCED GOLD NANOPARTICLES BY THE BLUE-GREEN ALGAE SPIRULINA PLATENSIS

Blue-green algae Spirulina platensis, one of the many popular microorganisms, synthesis gold nanoparticles too [40]. The complex of optical and analytical methods was applied for survey of experiential samples after exposure to chloroaurate (HAuCU) solution at different doses and for several time intervals. To specify formed gold nanoparticles UV-vis, TEM, SEM, ED AX, and XRD were used. Neutron activation analysis and atomic absorption spectrometry were employed to determine gold concentrations in the Spirulina platensis. Concentration of gold nanoparticles increased rapidly at the start, followed by some increase for the next few days. The biomass can be applied for different applications in medicine, pharmaceutics, and technology [41].

In another study, 50 ml of freshly grown algal culture was centrifuged at 10,000 rpm for 5 min, and then pellet was re-suspended in HEPES buffer, shook for 2 min again centrifuged at 10,000 rpm for 5 min. Finally, the pellet was re-suspended in double distilled water in order to eliminate ions and salts present on the surface of the cell. 500 mg of algal culture was introduced into 100 ml of 1 mM aqueous HAuCl₄ for synthesis of gold nanoparticles. The reaction mixture was holed for an hour in dark condition. After the incubation period, reaction volume was assessed and centrifuged at 6000 rpm for 7 min. The same procedure was also followed for cyanobacterial mediated synthesis [9, 42].

In order to formation of AuNP, mixing 50 ml of aqueous solution of tetrachloroaurate with the different types of algae and variant initial pH values (2–10) along with stirring at room temperature were prepared. For the synthesis of gold nanoparticles, 0.25 g of alga was added to solutions. Various samples were collected at different times of reaction for analysis. The biomass was picked up from the reaction mixture by filtration using nylon membrane filters 0.2 μm from Whatman [27, 37].

5. SIZE AND SHAPE

Table 2. Biosynthesis of nanoparticles by marine organism*§.

| Organism          | Name of the species | Types of nanoparticles | Size (nm) |
|-------------------|---------------------|------------------------|-----------|
| Marine microbes:  |                     |                        |           |
| Cyanobacteria     | Spirulina platensis | Silver                 | 7-16      |
|                   | Oscillatoria willei | Gold                   | 6-10      |
|                   | Phormidium tenuis   | Bio-metallic           | 17-25     |
|                   | Stauronitis Sp.     | Silver                 | 100-200   |
|                   | Phormidium tenuis   | Cadmium                | S         |
| Marine algae      | Stauronitis Sp.     | Silicon, Germanium     |           |
|                   | Stauronitis wightii | Gold                   | 8-12      |
|                   | Stauronitis wightii | Silver                 | –         |
|                   | Turbinaria conoides | Silver                 | –         |
|                   | Gelidium acerosa    | Silver                 | 22        |
|                   | Ulva fasciata      | Silver                 | 28-41     |
|                   | Fucus vesiculosa   | Gold                   | –         |
|                   | Cladosiphon okamuranus, | Gold                  | 854-1074  |
|                   | Kjellmaniella crassifolia |                |           |

Modified from Reference [31, 35]; § Biological activities of some of these nanoparticles includes antibacterial, antifungal, bio-sorption and strengthening.

through changing key parameters. In purpose to control the size and shape of the metallic nanoparticles, the effect of parameters such as pH or time exposed to metallic precursors using different biomasses was investigated [27].
6. ANTIBACTERIAL ACTIVITY

Antibacterial activity of the gold nanoparticles were found out using the agar well diffusion method against pathogenic gram negative and gram positive bacteria (Pseudomonas aeruginosa, Klebsiella oxytoca, Enterobacter faecalis, Klebsiella pneumoniae, Vibrio parahaemolyticus, Vibrio cholera, Escherichia coli, Salmonella typhi, Salmonella paratyphi, and Proteus vulgaris) [18, 42, 43]. The inoculums suspensions were swabbed identical in different plates. Cavities were made in each plate using a well-cutter and it was filled with gold nanoparticle solution (100 mL) and then incubated at 37°C. [43] Hydrogen tetrachloroaurate was used as negative control and Tetracyclines of 0.25 mg/mL concentration were applied as a control antimicrobial agent. The antibacterial activity was indicated by a clear halo around the cavity [18].

7. CONCLUSIONS

Antibiotic resistance of different pathogens urges scientists to find new strategies for the inhibition of microbial growth and disinfection purposes. Biosynthesis of nanoparticles by microalgae can be considered as an ecofriendly approach and green technology, for the production of nanoparticles due to its low energy requirement, environmental compatibility, reduced costs of manufacture, scalability, and nanoparticle stabilization compared to the chemical synthesis procedures. Due to their enormous diversity, microalgae possess great potential for the synthesis of nanoparticles and they can be regarded as potential biofactories for the synthesis of metallic nanostructures. For commercialization purposes, it would be advantageous to have a nonpathogenic and eco-friendly biological system with the capability of producing metallic nanoparticles.

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