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Algae: A potential source for nanoparticle synthesis

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Abstract
Nanotechnology deals with the particles of diameter size less than 100 nm. Nanoparticles are different from their bulk counterparts due to their distinctive shapes and sizes. They provide larger surface area, essential to carry out different chemical reactions. Other than the physical and chemical methods of nanoparticle synthesis, biological method is getting more popularity, as the process is less toxic, cost effective and eco-friendly. Various biological sources such as algae, plants, bacteria, fungi etc. have been explored for their potential to synthesize nanoparticles. In the literature, algae was found to be more reliable source for the green synthesis of metal nanoparticles as they are readily available in nature and are easy to grow in normal conditions. Metal nanoparticles like gold, silver and iron synthesized from algal source have widespread application in treating environmental pollutants like heavy metal removal, degradation of organic dyes, antimicrobial agents etc.

Keywords: Algae, Bioreduction, Metal nanoparticles, Nanotechnology, Reducing agent

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INTRODUCTION

The prefix “nano” in the term nanotechnology is derived from a Greek word nanos, which means “dwarf”. It relates, to any engineered matter that is one billionth (10^{-9} m) in size or at least one of its dimensions, and is considered nanometer (nm) (Hulkoti and Taranath 2014). Nanoparticles exhibit unique properties like chemical, physical, electronic, optical, thermal, mechanical, and biological properties that significantly differ from their bulk scale counterparts due to its small size, shape and size distribution (Kubik and Sugisaka, 2002; Goodsell *et al.*, 2004; Fawcett *et al*., 2017). Nanoparticles are broadly divided into two groups of organic and inorganic nanoparticles. Organic nanoparticles include carbon nanoparticles (fullerenes) while some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (gold and silver) and semiconductor nanoparticles (e.g. titanium dioxide and zinc oxide etc.). Nanoparticles exhibit distinctive visible properties because they are small enough to confine their electrons and produce quantum effects (Yadav *et al*., 2017). Metallic nanoparticles have possible applications in diverse areas such as electronics, cosmetics, coatings, packaging, and biotechnology. It has the ability to get attached to single strands of DNA without causing any destruction in chain (Thakkar *et al*., 2010).

Nanoparticles can be synthesized by physical and chemical methods which involve the use of hazardous chemicals and harmful radiation that can be life-threatening to human and environment. Apart from physical and chemical methods, biological methods are being explored nowadays for the synthesis of nanoparticles. Green chemistry is the cost-effective, non-toxic and environment friendly approach which involves the use of plant extracts and microorganisms (Abdelghany *et al*., 2017; Bansal *et al*., 2015), where biological components are used as a reducing and stabilizing agents, essential for the synthesis of nanoparticles. Nanoparticle synthesis can be accomplished using two approaches, *viz.* top-down approach and bottom-up approach. The top-down approach is a physical process where the size of the bulk material is reduced using various mechanical techniques to obtain nanoparticles of desired shape and size whereas bottom-up approach is the chemical process where the atoms and molecules are assembled into a larger structure with controlled deposition or reaction parameters (Saif *et al*., 2016; Rotello, 2004; Gottimukkala, 2017). Bottom-up approach is advantageous over top-down approaches as there is greater possibility of obtaining metal nanoparticles with relatively lesser defects and more homogeneous chemical compositions whereas top-down approach causes the imperfection in the surface structure and alters the

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physical properties and surface chemistry due to the high aspect ratio (Thakkar *et al.,* 2010).

Furthermore, diverse group of microorganisms such as algae (Aboelfetoh *et al.,* 2017), bacteria (Shahverdi *et al*., 2007), fungi (Maliszewska *et al.,* 2014) and plant leaf (Ahmed *et al*., 2016) are being used for the green synthesis of nanoparticles. The functional groups containing biological components such as alkaloids, terpenoids, phenolic compounds, polyphenols and proteins act as a reducing and capping agents during the synthesis of nanoparticles. The nanoparticles formed are thus prevented from the further chemical reaction and aggregation which increase their stability (Aboelfetoh *et al.,* 2017; Makarov *et al*., 2014). The main aim of the study is to review the contribution of the researchers in the algae mediated green synthesis of silver, gold and iron metal nanoparticles and their application in day to day environment.

Microalgae facilitated biosynthesis of metal nanoparticles: Phyconanotechnology has been considered as a newer branch of nanoscience which involves the synthesis of nanoparticles using algal extract as they are relatively easy to handle, able to grow at a low temperature and are less toxic in nature (Sharma *et al.,* 2016). Algae are the diverse group of unicellular and multicellular autotrophic organism that carries out the process of photosynthesis in the presence of sunlight. Depending on their size microscopic algae are known as microalgae whereas algae that are macroscopic in nature termed as macro algae. They are widely present in both marine and freshwater ecosystems as well as in terrestrial ecosystems in moist conditions. It helps in absorbing moisture from the atmosphere when in the symbiotic relationship with the lichen on rocks in dry and arid region. Algae is broadly classified into three main divisions viz. chlorophyta (green algae), phaeophyta (brown algae) and rhodophyta (red algae) all of which having chlorophyll *a* in common. In addition to cellulose, the cell wall of chlorophyta, phaeophyta and rhodophyta contains xylans and mannans, alginic acid and fucoidan, xylan and galactans respectively (Davis *et al*. 2003). The rigid cell walls matrices contains several functional groups such as carbonyl, hydroxyl, carboxyl, sulfonate, thiol, amino and amidic groups (Subramaniyam *et al*. 2015; Kuyucak and Volesky, 1989) which play significant role in bulk metal reduction into its elemental form and its accumulation. In the researches, few varieties of both macro and microalgal species have been explored for the green synthesis of metal nanoparticles like gold (Ashokkumar *et al*., 2016), silver (Prasad et al., 2013), palladium (Momeni and Nabipour, 2015) and iron nanoparticles (Subramaniyam *et al.,* 2015). Nanoparticles can be synthesized by extracellular and intracellular mechanism from algal biomass. In extracellular mechanism the bioreduction of metal ion to its nanoparticle takes place on the surface of the algal cell whereas in intracellular mechanism the enzymatic bioreduction process takes place inside the cell wall and cell membrane (Senapati *et al.,* 2012).

Phycosynthesis of metallic nanoparticles:

Gold nanoparticles (AuNPs): Brown algae *Turbinaria conoides* and *Sargassum tenerrimum* were explored for their potential of producing gold nanoparticles. It was concluded that the presence of hydroxyl group in the algal extract facilitated the reduction of Au(III) to elemental gold and act as a capping agent for the synthesized spherical shaped gold nanoparticle of size 5- 57 nm (Ramakrishna*,* 2016). Another species of brown algae, *Cystoseira baccata* were found efficient in producing spherical shaped gold nanoparticle of size 8.4 ± 2.2 nm, polycrystalline in nature. The stability of nanoparticle was confirmed by zeta potential measurement (−30.7 ± 2.0 mV). It was suggested that the polysaccharides and polyphenols contains hydroxyl as a functional groups that helps in the bioreduction of bulk material into its elemental form and presence of protein acts as a capping agent and prevent agglomeration of gold nanoparticles (González-Ballesterosa *et al.,* 2017). Green algae *Pithophora Oedogonia* synthesized spherical shaped gold nanoparticles of 33nm (Li and Zhang, 2016)*.* El-Kassas and El-sheekh (2014), observed the change in the colour of solution from brown to red when aqueous extract of red Seaweed *Corallina officinalis* blended with the gold salt solution that confirmed the formation of gold nanoparticle. The synthesized nanoparticles were of size 14.6±1 nm of spherical shape. It was also concluded that hydroxyl and the carbonyl group assisted in the reduction and stabilization of nanoparticles. The nitrate reductase activity was found responsible for the bioreduction of gold ion to elemental gold (Oza *et al*., 2012). Ghodake and Lee (2011), reported the concentration dependent extracellular synthesis of gold nanoparticle within 20 min at 37°C using different fraction of aqueous extract of brown algae *Laminaria japonica* through a serial dilutions with distilled water i.e. 1:2.5, 1:5, 1:10, and 1:20 denoted as F1, F2, F3, F4 with2mM of chloroauric solution. All of the four fractions showed change in the colour from light yellow to dark red also the completion of the reaction was depended on the concentration of the aqueous extract. The change in the colour of the solution was due to surface Plasmon effect. F1 fraction was used for characterizing the resulted gold nanoparticles. The synthesized nanoparticles were spherical and the size ranged from 15-20nm. The crystalline nature of nanoparticle was confirmed using X-Ray diffraction technique.

Table 1. Algal species and their potential application in metal nanoparticle synthesis.

Algal species	Type of nanoparticle	Size of nano-particles	Applications	References
Caulerpa racemosa	Ag	~ 25 nm	Catalytic degradation of methylene Blue	Edison *et al.,* 2016
Chlorella vulgaris	Pd	2 to 15 nm	Catalytic activity	Eroglu *et al.,* 2013
Codium capitatum	Ag	30 nm	-	Kannan *et al.,* 2013
Hypnea musciformis	Ag	16 to 42 nm	Antibacterial activity	Vadlapudi and Amanchy *et al*., 2017
Laurencia papillosa	Au	3.5 – 53 nm	-	Montasser *et al.,* 2017
Padina gymnospora	Ag	25- 40 nm	Antibacterial activitiy	Shiny *et al.,* 2013
*Padina pavonica (*Linn.)	Ag	10 to 72 nm	Microbicidal activity	Sahayaraj *et al.,* 2012
Rhizoclonium fontinale	Au	~16 nm	-	Parial and Pal, 2015
Sargassum bovinum	Pd	5 to 10 nm	Electrochemical reduction of hydrogen peroxide	Momeni and Nabipour, 2015
Sargassum muticum	Ag	42.30-98.56 nm	Insecticidal activity	Moorthi *et al.,* 2015
Sargassum wightii	Pd	5- 37 nm.	-	Prasad *et al.,* 2015
Spirulina platensis	Ag	~5 nm	Antibacterial activity	Suganya *et al.,* 2015
Turbinaria conoides Sargassum tenerrimum.	Au	27–35 nm.	Catalytic reduction of nitro compounds	Ramakrishna *et al.,* 2016
Turbinaria conoides	Ag Au	2-17 nm 2-19 nm	Antimicrofouling Activity	Vijayan *et al.,* 2014

Further, it was proposed that the electrostatic interaction between the salt solution and functional group chiefly polysaccharides involved in the bioreduction of Au (III) to elemental Au. Fluorescence spectra of Au NPs synthesized by *S. marginatum* biomasses were found to be centered at 550 nm. Therefore, gold nanoparticles exhibit high efficient single photon-induced luminescence, which may be due to their ability to sustain resonating surface Plasmon with minimal damping (Rajathi *et al.,* 2012). Micro algae *Chlorella vulgaris* also studied for the intracellular synthesis of spherical and polyhedral sized gold nanoparticles of size ranged from 40-60 nm (Luangpipat *et al.,* 2011).

In an experiment it was found that *Euglena gracilis* shows maximum growth in mixotrophic condition. Increase in the number of living cells of algae under mixotrophic condition was then found responsible for the quicker formation of more stable gold nanoparticle as compared to the algal species grown under autotrophic condition (Dahoumane *et al.,* 2016). Acidic and alkaline condition of the solution also influences the stability of synthesized nanoparticle. Gold nanoparticles were found stable within the pH range 5-9 whereas agglomeration of nanoparticles were observed at pH 3 due to decrease in the surface charge of the particles and shows no change in the position of the absorbance at 550nm (Namvar *et al*., 2014).

Silver nanoparticles (AgNPs): Silver nanoparticles, due to its unique optical, electronic and catalytic properties have various applications in medical, industrial and commercial sectors. They are widely used as anti-inflammatory, anti-viral, antibacterial and antifungal agents also being used as nano-silver coated surgical instruments and implants and several other purposes (Azizi *et al*., 2013).

Pithophora oedogoni had successfully been explored for the synthesis of silver nanoparticle by showing maximum absorbance at 445 nm. The aqueous algal extract was utilized to reduce silver nitrate solution into silver nanoparticles within few minutes. DLS (dynamic light scattering) and SEM instrumentation were used to confirm the size of the synthesized nanoparticles. The resulting silver nanoparticle size observed was 34.03 nm (Sinha et al., 2015). Aboelfetoh *et al.* (2017), used green marine algae, *Caulerpa serrulata* extract to reduce silver ion to spherical silver nanoparticles of 10 ± 2 nm. Selvam and Shivkumar (2015), reported the biosynthesis of cubic shaped silver nanoparticles from the aqueous extract of red alga *Hypnea musciformis* of size 2- 55.8nm and also stated that the reduction of silver nanoparticles may be due to the presence of peptides.

Similarly, polydispersed type silver nanoparticles were synthesized from Chlorococcalean alga *Chlorella vulgaris* with mean size 12.62 nm and concluded that the presence of functional groups such as carboxylic and aromatic groups acts as a reducing agent and reduces silver ion to silver nanoparticles (Satapathy *et al*., 2015). The aqueous extract of microalga *Scenedesmus* sp. (IMMTCC-25) was used first time for the extracellular as well as intracellular synthesis of silver nanoparticles of size ranged from 5-10 nm and 15 to20 nm respectively. In the experiment, the living cell as well as algal extract was treated separately with 5 mM silver nitrate solution. In extracellular synthesis of silver nanoparticle the raw and the boiled extract of algal biomass was treated with the silver nitrate solution whereas in intracellular synthesis the live cell of algal biomass was extracted from their log phase and treated with silver nitrate solution and the culture were incubated for 72 hrs at 28°C. Formation of silver nanoparticles was observed by change in the colour of the solution from colourless transparent to reddish-yellow colour. It had been suggested that shape, size and conformation of protein molecules present as biological components is responsible for the stabilization of synthesized nanoparticles. The amount of reducing and capping agent in the algal extract was believed to form more stable nanoparticles. Therefore, boiled extract of algal biomass was found more effective in the formation of stable silver nanoparticle as compared to raw algal extract wherein the synthesized nanoparticles resulted in the agglomeration of the particles due to the low concentration of reducing agents (Jena *et al.,* 2012).

Besides, the concentration of reducing and capping agent temperature also plays an important role in controlling the shape and size of the synthesized nanoparticles. Brown algae *Cystophora moniliformis* was found able to synthesized silver nanoparticle of size 75nm at temperature less than 65°C whereas agglomeration of silver nanoparticle resulted due to increases in the size of the nanoparticles with increase in temperature up to 95°C (Prasad *et al.,* 2013). The phytochemical analysis of green alga *Pithophora oedogonia* confirmed the presence of biomolecules namely carbohydrate, steroid, saponins, terpenoids, tannins and proteins which were found responsible for the reduction of Ag^+ ions into its elemental form and prevent agglomeration of synthesized silver nanoparticles (Sinha *et al.,* 2015). Similarly, marine algae *Caulerpa racemosa* was successfully explored for the extracellular synthesis of silver nanoparticle of size ranged 5- 25 nm (Kathiraven *et al.,* 2015).

Iron nanoparticles (FeNPs): Limited researches have been reported in the formation of iron metal nanoparticle from algal species. Subramaniyam *et al.* (2015), explored the possibility of formation of iron nanoparticles for the very first time from soil microalga, *Chlorococcum* sp. MM1, the resulting spherical shaped iron nanoparticles were 20-50 nm in size. The presence of functional group attached with polysaccharides and glycoprotein was confirmed by FTIR analysis and believed to acts as a reducing and capping agent in the process of bioreduction. The aqueous extract of Microalga, *Chlorococcum* sp. was allowed to react with 0.1M iron chloride solution for 48 hr. Carbonyl and amine bonds from polysaccharides and glycoproteins present in the algal cell wall and was responsible for the bioreduction as well as capping of iron nanoparticle.

Application of metal nanoparticles in remediation of environmental pollutants: Metal nanoparticles, such as platinum, silver, and gold are extensively used in products that directly come in contact with the human body, such as shampoos, detergent, soaps, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications (Singh *et al.,* 2013). Silver nanoparticles of size 5–25 nm synthesized using marine algae *Caulerpa racemosa* also been tested for its antimicrobial activity against human pathogen such as *P. mirabilis* and *S. aureus.* It was concluded from the study that silver nanoparticles not only attack the cell membrane but also enter the bacterial cell and affects the respiratory chain cell division which ultimately leads to cell death (Kathiraven *et al.,* 2015). Silver nanoparticles of size 34.03 nm synthesized from aqueous extract of green alga *Pithophora oedogonia* showed maximum zone of inhibition against *Pseudomonas aeruginosa* (MTCC 2581) (17.2 mm) followed by *Escherichia coli* (MTCC 443) (16.8 mm). The antibacterial activity of gold nanoparticles was tested against gram negative and gram positive bacteria (*Pseudomonas aeruginosa, Klebsiella oxytoca, Enterobacter faecalis, Klebsiella pneumoniae, Vibrio parahaemolyticus, Vibrio cholerae, Escherichia coli, Salmonella typhii, Salmonella paratyphi, and Proteus vulgaris*). Maximum zone of inhibition was observed against *E. faecalis* (11 mm) and minimum zone of inhibition was observed against *K. pneumoniae* (6 mm). *E. coli* (0 mm) showed no zone of inhibition. Nanoparticles synthesized by green route are found to be highly effective against gram negative bacteria as compared to gram positive bacteria (Rajathi *et al*., 2012). Gram negative organisms possess a thin cell wall with peptidoglycans whereas gram positive organisms generally have thick cell wall made of peptidoglycans. Thus, an easier permeability could be achieved in the case of Gram negative organisms (Naveena and Prakash, 2013; Subramaniam and Suja, 2012).

Silver nanoparticle synthesized using micro algae, *C. calcitrans, C. salina, I. galbana* and *T. gracilis* showed zone of inhibition against human pathogens *Klebsiella spp., Proteus vulgaris, Pseudomonas aeruginosa* and against *E. coli* and act as a strong antibacterial agent (Merin *et al.,* 2010). Subramaniyam *et al.* (2014), used *Chlorococcum* sp. MM11 to synthesize iron nanoparticles of size 20-50 nm and tested its absorption efficiency. The study showed that the synthesized nanoparticles removed 92% of 4 mg L^{-1} hexavalent chromium to Cr (III) whereas bulk iron only reduced 25% concluding that iron nanoparticle is much more efficient in removing contamination from environment. Silver nanoparticle synthesized from green algal species *Caulerpa serrulata* also found effective in catalysis of Congo red (CR) dye as it provides large surface area for exchange of electron between electron donor and acceptor (Aboelfetoh *et al*., 2017). Besides antibacterial property against gram positive and gram negative bacteria, Silver nanoparticles synthesized from freshwater microalgae *Chlorella pyrenoidosa* has also been tested for its photocatalytic activity against methylene blue dye and further suggested its application in the treatment of effluent containing hazardous dye as a result of chemical processes in the industrial sectors (Aziz *et al.,* 2015). Some of the important work in the field of phyconanotechnology has been shown in Table 1.

Conclusion

The eco- friendly and cost effective biological synthesis of metallic nanoparticles is a promising alternative to the conventional physical and chemical method. Several researchers have explored the biological potential of algae to synthesize nanoparticles of various shapes and sizes under different conditions. It was observed that the biomolecules of algae act as a reducing and capping agent without using any toxic compounds and successfully produced stabilized nanoparticles. Excessive use of toxic organic and inorganic pollutants, degrading the overall quality of environment with time, hence development of eco-friendly techniques is the need of the hour to minimize the adverse effects of pollutants. Phyconanotechnology, is the best technique to remediate the pollutants presents in air, water and soil without having any harmful impact on environment. Despite successful bioreduction of metal salt to its elemental state and its application in various fields, the mechanism involved during synthesis is not known at the molecular level. Also, several other species of algal biomass is still unexplored. Therefore, further researches must be carried out to identify the most suitable and efficient biological source for nanoparticle synthesis of smaller diameter with detailed mechanism involved.

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