



Green microalgae for inorganic nanoparticles preparation

D. Zerrouki ^{a*} 

^a Lab. Dynamic Interactions and Reactivity of Systems, Kasdi Merbah University, Ouargla 30000, Algeria.

ARTICLE INFO

Article history:

Received: 07 November 2018

Revised: 06 December 2018

Accepted: 19 December 2018

Published: 20 December 2018

Keywords:

Microalgae

Nanoparticle

Biosynthesis

Inorganic

Green

Eco-friendly

Microorganism

ABSTRACT

The biosynthesis of nanoparticles (NPs) by microorganism is a green and eco-friendly technology. This review focuses on the use of diverse green microalgae strain for the synthesis of inorganic NPs such as silver, gold, platinum, zirconium, palladium, iron, cadmium and metal oxides such as titanium oxide, zinc oxide, etc. The synthesis of NPs may be intracellular or extracellular. The presence of biomolecules such as carboxyl group, primary and secondary amines, amide I, II, and III bands could serve as bio reducing and capping agents, and guarantee a high stability to particles by preventing agglomeration and growth. The size and morphology of the NPs vary with the organism used and various operation conditions during the preparation including the pH, temperature and substrate concentration. The microorganisms provide diverse environment for biosynthesis of NPs. These particles are safe and eco-friendly with many applications in medicine, agriculture, cosmetic industry, drug delivery and biochemical sensors. The challenges for redressal include optimal production and minimal time to obtain desired size and shape, to enhance the stability of NPs and optimization of specific microorganisms for specific application.

© 2018 mbmscience.com. All rights reserved.

Introduction

Nanotechnology has brought revolution in many industry sectors such as information technology, medicine, transportation, energy, food, environment, sensors, and detection (Maynard and Pui, 2007; Sharma et al., 2015). The benefits of depend on the fact that it is possible to shape the structure of materials at extremely small scales to achieve specific properties, at this scale the ratio surface to volume is the most important feature. Large chemical diversity of NPs could be find in form of metals, metal oxides, semiconductors, polymers, carbon materials, organics or biological. With various morphology such as spheres, cylinders, disks, platelets, hollow spheres and tubes.

Various chemical and physical procedures have been employed to fabricate organic and inorganic nanoparticles. at a liquid phase the nanoparticles synthesized using precipitation process in homogeneous solution or in surfactant based system; sol gel and hydrothermal process, in gaseous phase based on aerosol process (flame hydrolysis, spray pyrolysis) most of these techniques are costly, as well as inefficient in materials and energy use. The synthesized NPs have to be surface modified in most cases, in order to passivate and stabilize them since their NPs scale renders them chemically very reactive and/or physically aggregative. The NPs are also surface functionalized in order to meet the needs of specific applications (Bagwe et al., 2006).

Inorganic NPs can be synthesized by physical, chemical and biological method. Although the physical and chemical methods produce pure, well-defined particles, these methods are not cost effective or ecofriendly. One of the most important criteria of nanotechnology is that of the development of clean, nontoxic and eco-friendly green chemistry procedures. Hence, currently, there is a growing need to develop environmentally caring NPs synthesis processes that do not use toxic chemicals in the synthesis protocol. Green chemistry procedures for synthesis of NPs not only provides advancement over chemical and physical methods as it is environment friendly but also it is a cost effective and in this method there is no need to use high-pressure, energy, temperature and toxic chemicals.

The use of environmentally benevolent materials like plant leaf extract, bacteria and fungi for the synthesis of NPs offers numerous benefits (Li et al., 2011). During recent times, several successes been achieved in the synthesis of silver, gold, and palladium nanoparticles. using extracts obtained from unicellular organisms like bacteria and fungi, as well as extracts from plant parts (Hassaan and Hosny, 2018; Li et al., 2011).

The spectacular success in this field has opened up the prospect of developing bio-inspired methods of synthesis of metal NPs with tailor-made structural properties. In this review, we focus on the use of green microalgae for NPs preparation and present overview various used procedure.

✉ * Corresponding author: Djamel Zerrouki
djamel.zerrouki@gmail.com

Green microalgae for green Nanotechnology

Microalgae have recently attracted considerable interest worldwide, due to their extensive application potential in the NPs synthesis as renewable, sustainable, and economical sources, microalgae are rich on bioactive products (Grif et al., 2016; Hassaan and Hosny, 2018). Several microalgae species have been investigated for their potential for inorganic NPs preparation with remarkable pharmacological and biological qualities (Hassaan and Hosny, 2018). They are a perfect substitute to toxic solvent.

Microalgae are a rich source of carbon compounds, which can be utilized in biofuels, health supplements, pharmaceuticals, and cosmetics (Spolaore et al., 2006). They also have applications in wastewater treatment and atmospheric CO₂ mitigation. Microalgae produce a wide range of bioproducts, including polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants (Rawat et al., 2011). Microalgae are photosynthetic organisms naturally capable of reducing metals due to the constant exposure to them in their natural environment; due to this characteristic, algae are often used for water bioremediation and sorption of toxic and radioactive metal ions.

Mechanism and protocol

In most of the studies, the steps involved in the algae-mediated biosynthesis of metal NPs are the following (Hassaan and Hosny, 2018):

- (i) preparation of algal extract in water or in an organic solvent by heating or boiling it for a certain duration,
- (ii) preparation of molar solutions of ionic metallic compounds
- (iii) incubation of algal solutions and molar solutions of ionic metallic compounds followed either by continuous stirring or without stirring for a certain duration under controlled conditions. Depending upon the type of algae, synthesis of NPs can be performed by either an extracellular or an intracellular fashion in a dose-dependent manner.

The extracellular formation of the metallic NPs has been inferred due to the activity of the reducing agents present in the algal aqueous phase such as polysaccharides, reducing sugars, peptides, proteins, pigments or other reducing factors, which may reduce metal ions and precipitate the metallic ions in the form of NPs.

As far as intracellular formation of metallic NPs is concerned, algal metabolism, i.e. photosynthesis and respiration, might be liable for the reduction of metallic ions.

Physical parameters such as temperature, pH, initial concentration and type of the metals, duration of the exposure, type and concentration of the reducing agents in the aqueous phase can be altered selectively to determine the desirable shape and size of the metal NPs as well as to prevent the aggregation and agglomeration.

Biosynthesis of NPs Using green Microalgae

Chlorella vulgaris

Chlorella vulgaris have been exploited for green NPs Synthesis, is one of the most exploited strain for NPs preparation, various type of NPs have been prepared using extracellular or intracellular process.

Xie et al.(2007) reported the production of a triangular and hexagonal Au NPs plates by incubating 1 mL of HAuCl₄ solu-

tion (10 mM) and 10 mL of algal extract for 48 h. The protein was identified as the bioreducing agent of gold ions as well as being responsible for the shape regulation, However, Mohseniazar et al. (2011) demonstrated that *C. vulgaris*, have the potential of NPs silver production in a culture medium containing 0.001 M of AgNO₃ within 24 hours.

Annamalai and Nallamuth. (2015) accomplished self-assembled of gold NPs (GNPs) using an aqueous extract. *C. vulgaris* cell-free extract was diluted through a series of dilutions with 1 mM HAuCl₄. The reaction mixtures (10 mL) were put aside at 37 °C and the reduction of the gold ions started at 1 mM HAuCl₄.

Satapathy et al. (2015) investigated the production in a continuously stirred non-aerated culture of silver NPs, and find that *C. vulgaris* can reduce the silver ions and remains viable at 10⁻³ M silver nitrate concentration. The obtained NPs were poly-disperse with a moderate stability of NPs in the environment.

Chlorella pyrenoidosa

Many studies are focused on the biosynthesis of NPs using *C. pyrenoidosa*. Sari et al. (2018) synthesized NPs silver (AgNPs) on *C. pyrenoidosa* in higher concentration of silver using agitation treatment. The microalgae grown in waive media, 100ml of microalgae extract was added to 250mL AgNO₃ 2mM solution under agitation for 6 hours at room temperature, a spherical and cubical shape have been observed using SEM. However, Oza et al. (2012). Demonstrated the preparation of a stable gold AuNPs using *C. pyrenoidosa* extract. Fog's medium used for cell culture, the biomass harvested at the stationary phase by centrifugation at 6000 g for 15 min, dried to make a fine powder and then washed with HCl and deionized water. Algae extract was prepared by adding 2 g of powder to 100 ml of deionized water and the mixture aged for 2 days followed by immediately adding of HAuCl₄ precursor, the pH adjusted by adding NaOH or HCl the reaction carried under vigorous stirring at room temperature for 48h. A spherical and icosahedral structure of AuNPs have been obtained, the most important parameter for the synthesis was the pH and the temperature .

The presence of reductases and dehydrogenase help in NADH dependent extracellular reduction and formation of GNPs. Nitrate reductases are considered to be most efficient. NADH-dependent enzyme acting as a nucleating as well as capping agent for gold NPs synthesis.

Chlorococcum Humicola

Pradhan et al. (2013), reported the utilization of *Chlorococcum humicola* for AgNPs preparation. The culture grown in a modified BB medium, for in vitro synthesis the cell free extract was prepared by boiling a dry biomass for 30 min and then the supernatant was used, the AgNPs prepared by incubating an AgNO₃, and however, in the in vivo synthesis the complete cell was used instead of extract. In the case of in vivo synthesis, the NPs stick at the algae cell surface.

Chlamydomonas reinhardtii

In the literature, only AgNPs and ZnSNPs have been synthesized using *Chlamydomonas reinhardtii*.

Barwal et al. (2011), reported the use of cell free extract (in vitro) and cells mediated synthesis (in vivo) of silver NPs re-

Table 1. Outline of work on biosynthesis of NPs using green microalgae.

Microorganism strain	Shape and type of NPs	Size (nm)	Ref
<i>Chlorococcum humicola</i>	silver spherical	2-16nm	(Pradhan et al., 2013)
<i>Chlamydomonas reinhardtii</i>	silver	5 - 15 nm 5-35 nm	(Barwal et al., 2011)
<i>Chlamydomonas reinhardtii</i> .	zinc sulfide Spherical	8–12 nm	(Divya and Gautam, 2016)
<i>Parachlorella kessleri</i>	silver and silver chloride	N.R.	(Moussa et al., 2016).
<i>Botryococcus braunii</i>	silver	N.R.	(Arévalo-gallegos et al., 2018)
<i>Chlorella pyrenoidosa</i>	Au (spherical)	25–35nm	(Oza et al., 2012)
<i>chlorella pyrenoidosa</i>	Ag (spherical)	5–10nm	(Sharma et al., 2015)
<i>Chlorella vulgaris</i>	gold NPspates	1.7µm	(Xie et al., 2007)
<i>Chlorella vulgaris</i>	Ag (NPs plates))	28 nm	Sharma et al. (2009b)
<i>Chlorella vulgaris</i>	Ag	>15nm	(Mohseniazar et al., 2011)
<i>Chlorella vulgaris</i>	Ag	12 nm	(Satapathy et al., 2015)
<i>Chlorococcum sp.</i>	Fe (spherical)	20–50 nm	Subramaniam et al. (2015)
<i>Tetraselmis suecica</i>	Au(spherical)	79	Shakibaie et al. (2010)
<i>Nannochloropsis oculata</i>	Ag	15	Mohseniazar et al. (2011)

veals NPs of size range 5 ± 1 to 15 ± 2 nm and 5 ± 1 to 35 ± 5 nm respectively with rounded/rectangular morphology. The in vivo process is much faster compared to the in vitro, this due to the presence of active biomolecule in the live cell than in the cell extract.

In vivo synthesized NPs are localized in cytoplasm, periplasm, nucleus, pyrenoids, and also in the flagella, the proteins have a direct control on the synthesis process. However, Ahmadi et al. (2016) synthesized a 10 nm AgNPs using *Chlamydomonas reinhardtii* cell compared to the result reported by Barwal et al. (2011) the process was faster (48h). The Ag reduction was attributed to the cellular reductases as an extra cellular enzyme; moreover, the adsorption of ion into the cell surface can be reduced by reductase enzyme. However, Divya and Gautam. (2016) reported the use of *Chlamydomonas reinhardtii* in the synthesis of zinc sulfide (ZnS) NPs using cell extract. A spherical particle measuring 8–12 nm has been obtained under optimal condition (pH, temperature and extract concentration). The involvement of protein on the biosynthesis processes was revealed using FTIR spectroscopy, where numerous oxidative protein was identified using SDS PAGE.

Parachlorella kessleri

The utilization of *Parachlorella kessleri* for NPs synthesis was reported by Moussa et al. (2016) the production of Ag/AgCl-NPs using aqueous extract was achieved with a cubic predominant morphology with a size range from 10 to 47 nm. However, the protein molecules were identified as the responsible for the stability and the reduction of the NPs.

Botryococcus braunii

To the best of our knowledge the use of *Botryococcus braunii* for AgNPs was reported only recently by Arévalo-gallegos et al. (2018). two method was presented based on extra-cellular and intercellular process, B braunni cultured in Bold 3N medium and harvested in the lag phase (23 days), AgNO₃ solution added to 5g of wet biomass in 100ml of water, the mixture incubated for 48hours at room temperature under stirring and illumination, for extracellular synthesis a cell free extract was used. Various shapes were obtained including NPs silver flakes, cubic structures depending on AgNO₃ concentration and pH value. In the case of intracellular synthesis the molecules responsible for the reduction and stability of the biosynthesized AgNPs were identified as proteins/peptides and carbohydrates, however, in extracellular synthesis the exopolysaccharide (EPS) indicated as the most responsible for the NPs production.

Tetraselmis suecica

AuNPs synthesis using marine microalgae *Tetraselmis suecica* carried out by Shakibaie et al. (2010) cell free extract was prepared by harvesting during lag phase a culture of *T. suecica* in f/2 seawater-medium. The formation of AuNPs was investigated by adding various amounts of *T. suecica* cell extract to 4 ml of aq. 10^{-3} MHAuCl₄ and incubated at different temperatures 30 and 90°C. Particle sizes of 79 nm was obtained after 5 min incubation at 90°C, substances such as polyols and water-soluble heterocyclic compounds have been proved

to be responsible for the reduction of silver or chloroaurate ions and stabilization of NPs respectively.

Nannochloropsis oculata and *Dunaliella salina*

N. oculata and *D. salina* cultured in Basal and Sk medium, respectively was used to synthesis of AgNPs by Mohseniazar et al. (2011). AgNO₃ solution added directly to the culture under illumination and incubated for 24h, the author has showed that there is no influence of the incubation time, the size was less than 15 nm, the reduction and the stability of the NPs was attributed to secreted protein.

Scenedesmus sp.

Microalgae *Scenedesmus* sp. for intracellular as well as extracellular biosyntheses of AgNPs was reported by Panda and Mishra (2014), the biomass harvested from a culture in modified Bold Basal medium, the interacellular synthesis was achieved by incubation at 28°C for 72 h under agitation and illumination microalgae cell and AgNO₃ solution. A spherical, and highly crystalline AgNPs with a maximum size of 20 nm was obtained the result shows that the protein molecules may be involved in NPs formation and subsequent stabilization. Extracellular synthesis used raw and Boiled *Scenedesmus* sp. Biomass, using boiled extract give a high regular shape and faster process compared to the raw extract (more than 3 days), The variation in NPs formation in the above three systems may be due to the variation in the reducing agent, primarily proteins/peptides.

Conclusion

There is always a search for reliable and environmentally friendly processes to manufacture metal and metal oxide NPs minimizing or even eliminating the use of hazardous chemicals. The only way to develop these “green” processes is to adapt benign synthesis approaches that use mild reaction conditions and non-toxic reaction precursors.

Through the paper we attempted to summarize the previous work related to green microalgae-mediated approaches for the synthesis of metallic NPs, a variety of metallic NPs with several sizes, shapes using green microalgae has been carried out in the research laboratory. Further, investigation needed to unveil the underlying reaction mechanisms and to characterize the compounds implicated in the reduction process. In addition, evaluated which particular green microalgae to sue for reduce a particular metal type with a higher yield.

References

Ahmadi, E.S., Tanhaeian, A., Pirkohi, M.H., 2016. Biosynthesis of Silver Nanoparticles Using *Chlamydomonas reinhardtii* and its Inhibitory Effect on Growth and Virulence of *Listeria monocytogenes* 14, 3–8.

Annamalai, J., Nallamuthu, T., 2015. Characterization of biosynthesized gold nanoparticles from aqueous extract of *Chlorella vulgaris* and their anti-pathogenic properties 603–607.

Arévalo-gallegos, A., Garcia-perez, J.S., Carrillo-nieves, D., 2018. *Botryococcus braunii* as a bioreactor for the production of nanoparticles with antimicrobial potentialities 5591–5604.

Surendiran, A.; Sandhiya, S.; Pradhan, S. C.; Adithan, C., 2009. Novel applications of nanotechnology in medicine. *Indian Journal of Medical Research* 130, 689–701.

Bagwe, R.P., Hilliard, L.R., Tan, W., 2006. Surface modification of silica nanoparticles to reduce aggregation and nonspecific binding. *Langmuir* 22, 4357–62.

Barwal, I., Ranjan, P., Kateriya, S., Yadav, S.C., 2011. Cellular oxido-reductive proteins of *Chlamydomonas reinhardtii* control the biosynthesis of silver nanoparticles 1–12.

Divya, R.M., Gautam, P., 2016. Facile bio-inspired synthesis of zinc sulfide nanoparticles using *Chlamydomonas reinhardtii* cell free extract: optimization, characterization and optical properties. *Green Process. Synth.* 5, 379–388.

Grif, M., Harrison, S.T.L., Smit, M., 2016. *Algae Biotechnology*. Springer International Publishing Switzerland 2016, *Algae Biotechnology*, Green Energy and Technology.

Hassaan, M.A., Hosny, S., 2018. Green Synthesis of Ag and Au Nanoparticles from Micro and Macro Algae - Review 2, 10–22.

Li, X., Xu, H., Chen, Z., Chen, G., 2011. Biosynthesis of Nanoparticles by Microorganisms and Their Applications. *Journal of Nanomaterials* 2011.

Maynard, A.D., Pui, D.Y.H., 2007. *Nanotechnology and occupational health*. Springer.

Mohseniazar, M., Barin, M., Zarredar, H., Alizadeh, S., Shanehbandi, D., 2011. Potential of Microalgae and *Lactobacilli* in Biosynthesis of Silver Nanoparticles 1, 149–152.

Moussa, S., Abdel-raouf, N., Borie, I., Ibraheem, M., 2016. Biosynthesis of silver and silver chloride nanoparticles by *Parachlorella kessleri* SAG 211-11 and evaluation of its nematicidal potential against the root-knot nematode; *Meloidogyne incognita*. *Australian Journal of Basic and Applied Sciences* 10, 354–364.

Oza, G., Mewada, A., Kalita, G., 2012. Facile biosynthesis of gold nanoparticles exploiting optimum pH and temperature of fresh water algae *Chlorella pyrenoidosa*. *Adv Appl Sci Res* 3, 1405–1412.

Panda, P.K., Mishra, B.K., 2014. Microalga *Scenedesmus* sp.: A Potential Low-Cost Green Machine for Silver Nanoparticle Synthesis 24, 522–533.

Pradhan, N., Sukla, L.B., Panda, P., 2013. Biosynthesis and Characterization of silver nanoparticles using microalga *Chlorococcum humicola* and its antibacterial activity. *Int J Nanomater Biostruct* 3, 1–8.

Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2011. Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Appl. Energy* 88, 3411–3424.

Sari, Y.W., Sanders, J.P.M., Bruins, M.E., 2018. Nanosilver microalgae biosynthesis : cell appearance based on SEM and EDX methods Nanosilver microalgae biosynthesis : cell appearance based on SEM and EDX methods. In *Journal of Physics: Conference Series* 1025, 012084.

Satapathy, S., Shukla, S.P., Sandeep, K.P., Singh, A.R., Sharma, N., 2015. Evaluation of the performance of an algal bioreactor for silver nanoparticle production. *J. Appl. Phycol.* 27, 285–291.

Shakibaie, M., Forootanfar, H., Mollazadeh-moghadam, K., Bagherzadeh, Z., Nafissi-varcheh, N., Shahverdi, A.R., Faramarzi, M.A., 2010. Green synthesis of gold nanoparticles by the marine microalga *Tetraselmis suecica*. *Biotechnology*

and applied biochemistry 75, 71–75.

Sharma, A., Sharma, S., Sharma, K., 2015. Algae as crucial organisms in advancing nanotechnology : a systematic review. *J. Appl. Phycol.* 28, 1759-1774.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. *J Biosci Bioeng* 101, 87-96.

Xie, J., Lee, J.Y., Wang, D.I.C., Ting, Y.P., 2007. full papers Identification of Active Biomolecules in the High-Yield Synthesis of Single-Crystalline Gold Nanoplates in Algal Solutions. *small* 3, 672-682.

Conflicts of interest

Authors declare no conflict of interests.

Notes

The authors declare no competing financial interest.

Materials & Biomaterials Science

This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

How to cite this article

D. Zerrouki. Green microalgae for inorganic nanoparticles preparation. *Materials and Biomaterials Science* 01 (2018) 041–045.