Journal of Marine Biosciences Vol 3 (1), 152-160, 2017
ISSN (P) 2454-3519 ISSN(E) 2454-3527

JMBS

Journal of Marine Biosciences

AMET

Biosynthesis of silicon combined gold nanoparticles using marine diatom *Coscinodiscus* spp.

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Abstract

The diatom frustules are naturally made silica nanospheres. These porous silica frustules are well known for its peculiar nanostructures formation properties. Therefore, this study was made attempt for the living marine diatom *Coscinodiscus* spp. are used for synthesizing gold nanoparticles (AuNPs) from tetrachloroaureate salt. The 10 mL of 2-weeks old *Coscinodiscus* spp. culture mixed with 10 mL of gold salt solution (50 mg/mL). The synthesized gold nanoparticles were characterized by X-ray diffraction (XRD), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM). 38.0826, 44.2689, 64.4193, 77.4332 peaks were confirmed by X-ray diffraction (XRD) study. The Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) demonstrated that the nanoparticles were spherical shape and the size ranged from 35 to 61 nm in diameter. The nano-particles coupled with the diatom frustules. On the basis of the XRD, SEM and TEM findings, this study confirmed the synthesis of silicon-gold (Si-Au) nanomaterial. These types of green synthesis of nanocomposites are very important for possible future applications such as biomedical, catalysis and biosensors fields.

Keywords: Diatom, Nanocomposites, nanoparticles, Silica, Gold and AuNPs

INTRODUCTION

Current scenario, outmost research is carried out in biogenic nanoparticles synthesis for various applications. The study of nanomaterial synthesis from biological system was very important in material chemistry (Govindaraju et al., 2008). The gold nanomaterials are predominantly

considered for their extensive range of applications such as antimicrobial activity, catalysis, biocarrier, biomolecular detection, micro electronics and biosensors (Rai et al., 2009, Schultz et al., 2000). A number of methods are successfully used for various metallic nanoparticles synthesis. However they used harmful chemical substance used for the reducing and stabilizing agents, that is could be caused chemical contamination to the environment. Now day eco-friendly methods are developed for nanoparticles synthesis by a natural material for reducing the material toxicity (Anastas and Warner (1998). Some of the biological systems are capability to synthesis biogenic nanomaterials (Simkiss and Wilbur (1989)). Generally the diatoms are produced silica nanospheres for their cell wall (Kroger et al., 1999). The unicellular Diatoms are photosynthesizing microalgae belonging to the brown algae group. It is present both the fresh and marine water bodies all over world. The Diatoms are producing microscale nanosilica frustules with two-dimensional pore arrays (Tian Qin et al., 2008). Diatoms key characteristic is their exoskeletons made to hydrated amorphous silica. The deferent types of shapes and size are unique for every species. The diatoms are used to biotechnologically like monitoring of ecological, production of biofuel and CO2 sequestration due to high carbon concentrating mechanism (Atazadeh and Sharifi 2010, Graham et al., 2012, Xiong et al., 2013) Structure of nanoparticles in occurrence of siliceous frustules possible provides instance for novel bionanocomposite apply. This study is important to practices for biogenic synthesis of AuNPs and silica-gold bionanocomposites by a *Coscinodiscus* spp.

Materials and Methods

Diatom collection

Diatom was collected from Vellar estuary, Tamil Nadu, India. The phytoplankton collecting net was used for diatom collections. That phytoplankton collecting net was made up of bolting silk cloth no 30, mesh size 48 µm and mouth diameter of 0.35 m. the time of diatom collection the net was submerged in the water and towed horizontally from a mechanized boat with an outboard engine at a speed of 01 – 02 knots for half an hour. Collected samples were identified using light microscope by the keys were followed.

Laboratory Culture of Diatom

The culture of diatoms was carried out by the standard methods of Anderson (1975). The *Coscinodiscus* spp. was picked through the assist of micropipette from the F/2 Guillard's medium (Guillard, 1975). Clean auxenic cultures of *Coscinodiscus* spp. were under proscribed conditions [temperature at 25±0.5°C/20±0.5°C, 12/12 day/night cycles; photoperiods of 12 hours light

(fluorescent lamps)] in the algal culture laboratory of Centre of Advanced Study in Marine Biology, Annamalai University of India. The culture was grown-up for around four weeks to achieve a stationary "growth" phase.

Biosynthesis of gold nanoparticles

The study was to examine the marine diatom *Coscinodiscus* spp. using synthesis of AuNPs from aqueous gold salts solution. To begin of the study, 10 mL of tetrachloroaureate solution (HAuCl4; Sigma-Aldrich) (≈ 50 mg/mL Au) was added to 10 mL of 4-weeks old diatom culture in liquid F/2 Guillard's medium (≈ 20 mg dry weight) in 50 mL Falcon flasks. The culture flask was incubating in laboratory conditions for 12 hours.

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

The diatom with AuNPs samples were examined by FEI Tecnai G2T2S-Twin TEM microscope (FEI Tecnai, USA) operating at 200 kV and the SEM microscope JSM-5610LV (JEOL Ltd., Tokyo, Japan). In SEM, The platinum was used for coating material. Analysis of the nanoparticles shape and size using Image analysis tool (JMicrovision: Image Analysis Toolbox) in this study. TEM image of AuNPs underwent the image analyses.

X-ray diffraction (XRD)

X-ray diffraction measurement was done by using Panalytical X'pert PRO powder X-ray diffractometer instrument using Cu Kα radiation and operating at a 15 KVA UPS support.

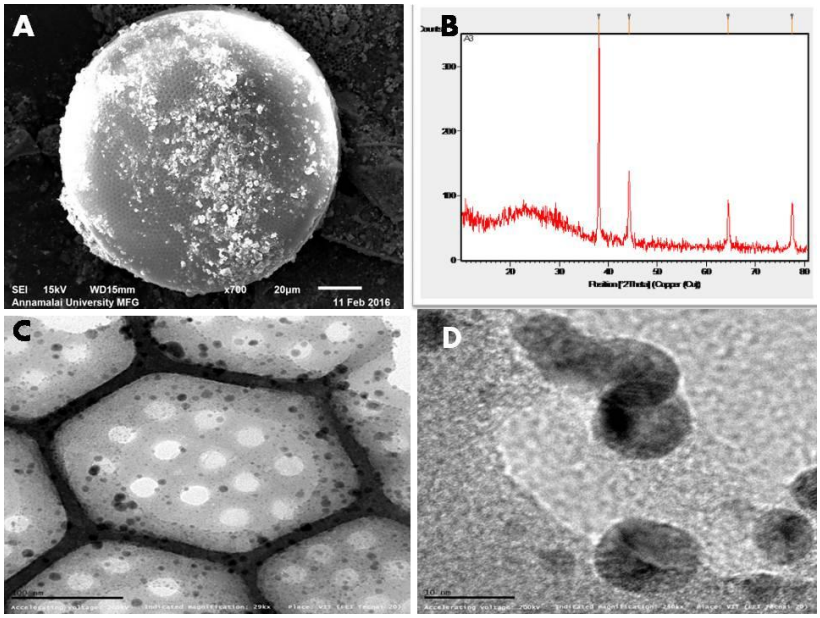

Fig. 1 SEM image showing the AuNPs located on diatom frustules (A), XRD signal showing the AuNPs (B), TEM image shows the AuNPs attaché in the pours of diatom (C), close-up view of the AuNPs (D).

Results

Phytoplankton are collected from Vellar estuary, the *Coscinodiscus* spp. was isolated from this plankton sample and culture in laboratory condition. The culture of diatom *Coscinodiscus* spp. was successfully carried out. After the tow week, the gold salt was added to culture media for biosynthesis of gold nanomaterials. After the gold salt added, approximately 1 hour after the red color was developed in culture media. After the 2 hours the culture media were fully reddish in color. This culture media are incubating for around one day and centrifuged 2000 rpm at 10 minutes. After the centrifuged, the dry sample was reddish in color. The SEM and TEM images are clearly shows the gold nanoparticles are present in diatoms frustule. The spatial distribution of the nanoparticles in the diatom sample was examined using SEM, the attachment of the mass of nanoparticles in diatom was also observed. The SEM photographs (Fig 1.) (Fig. A) evidently showed the shape of the diatom frustules attached with nanoparticles. The detailed TEM pictures (Fig. D) and image analysis confirmed mostly spherical shape of the nanoparticles. Size of the biosynthesized nanoparticles are 8 to 35 nm (Fig. D). AuNPs are confined directly associated with the diatom frustule (Fig.C and D). The XRD clearly confirmed the presence of crystalline gold in the experimental sample. The diffractogram of the sample is shown in Fig. B. All peaks (38.0826, 44.2689, 64.4193 and 77.4332) of the diffractogram are in good agreement with theoretical assumption for cubical gold.

Discussion

More types of methods are used for nanoparticles synthesis. But, it is the simplest, low-cost and less time for production of gold bionanoparticles and silica-gold bionanocombosites compare to others. This types of study also conducted by other micro organism such as fungi, bacteria, green algae, etc. (Vijayakumar and Prasad 2009, Lengke and Southam 2006, Lengke et al. 2006a). Post all the microorganisms study are robustly linked on used biomass, pH, salinity, etc. (Lengke et al. 2007, Brayner et al. 2007). In present study, the given result from the electron microscope images are strongly indicates the biogenic syntheses of gold nanoparticles are attached with the cell wall. The measurements of the UV-VIS spectral are not possible for the nanoparticles synthesis from the diatom culture; the diatoms frustules cause the some changes in the wavelength. AuNPs fixate into the cell structure, the considerable applications for utilization of this nanomaterial for further applications. TEM images are shows the different size of the AuNPs. Also shows the spherical shapes of the AuNPs. The improved potential study of applications of

this types of nanoparticles synthesis from diatoms, it is possibility of produced standed size and the distributions from using different concentrations of tetrachloroaureate, temperature and other parametes are expected (Lengke et al. 2007b). In this study, we are not considerate any viability experiment during this synthesis, the reduction of HAuCl4 occurs in both living and dead biomass. Moreover, the nanoparticles are directly stick on the surface of the siliceous frustule of diatoms (Fig. C & D). Previously similar study discussed in cynobactreria produced nanoparticles, the cyanobactria's EPS role was important in biosynthesis of nanoparticles (Brayner et al. 2007). The role of the EPS was similar for both cynobacteria and diatoms. Basically, they form a shielding layer from the physical and chemical distraction of the cell (Ben-Ari 1999, Flemming et al. 2000, Christensen 1999, Paerl and Pinckney 1996, Allison et al. 2000, Flemming and Windenger 2001, Wimpenny 2000). Commonly the EPS are negatively charged and it have good metal binding capability (Sutherland 2001a, Sutherland 2001b), Still, nanoparticles formation mechanism from the living cells is unclear, so growing number of studies concerned with this issue. From the earlier study with heavy metal recovery from brown algae (Kuyucak and Volesky 1989, Mata et al. 2009,) point out that reduction of Au^{3+} to Au^{0} occur through oxidation of hydroxyl groups to carbonyl groups. the algal pigments rich in hydroxyl groups or other highly reactive functional groups such as sulfhydryl present in the polysaccharides of the cell wall, it is responsible for its brown color – fucoidans (Kuyucak and Volesky 1989)), could be involved in the reduction processes. Greene et al. (1986) determined the importance of these groups in experiments with the green alga *Chlorella vulgaris* (their chemical modification reduced the gold uptake). Last but not least, the role of silaffin polypeptides in the nanoparticles formation should be mentioned. Sillafins are a class of heavily posttranslationally modified proteins responsible for mediating silica deposition at ambient temperature and pressure (Kroger et al. 1999, Davis et al. 1986). Native silaffin polypeptides isolated from a diatom *Cylindrotheca fusiformis* can catalyze the silica precipitation in vitro from a silica precursor under slightly acidic conditions (Kroger et al. 2001)). This process is caused by a self-assembly of the silica due to the silaffins activity resulting into the silica nanoparticle formation (Nam et al. 2009). We expect that the silaffins might play role also in synthesis of other types of nanoparticles such as AuNPs; however additional research beyond the scope of this study would be necessary to

verify this hypothesis. It is well known that gold is an excellent catalyst for many organic oxidation reactions. In fact, current research is focused on the development of gold nanocatalysers for the chemical industry (Hughes et al. 2005). Obtained bionanocomposite appears to be suitable adept for applications in catalysis or further modifications e.g. cell modification by ferrofluids Mosinoewicz-Szablewska et al. 2010).

Conclusion

Biosynthesis of gold nanoparticles has been successfully carried out using *Coscinodiscus spp.* mixed with aqueous $HAuCl_4$ (500 mg/L Au) at laboratory condition. The Shape and sizes, interaction of AuNP with siliceous frustules of the diatom was illustrated by the methods of electron microscopy and X-rays diffraction techniques. Existing method of tetrachloroaurate reduction by diatoms materialize to be meaningful, valuable and economical method of ration.Au-Si bionanocomposites preparation. Moreover, study of the illustrated method is very simple (used organisms generally living in marine worldwide, can be performed at room temperature, and in physiologic pH and salinity) and ecologically friendly compared to other chemical methods that use toxic reagents. Suitable to their outstanding properties, it was also anticipated that silica-gold bionanocomposites have potentially a huge value for a variety of applications such as biomedical, catalysis and biosensors fields.

Acknowledgement

Authors are grateful thanks to Dean and Director of CAS in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai, Tamil Nadu, India for providing facilities and SERB-DST, New Delhi for providing financial support to carry out the work.

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