Journal of Environmental Management 218 (2018) 442–450

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Biosynthesis of cobalt oxide nanoparticles using endophytic fungus *Aspergillus nidulans*

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ARTICLE INFO

Article history:
Received 23 September 2017
Received in revised form
28 March 2018
Accepted 6 April 2018

Keywords:
Cobalt oxide nanoparticles
Endophytes
Ligand
Sulfur-bearing proteins
Surface plasmon resonance

ABSTRACT

Metallic oxide nanoparticles have profound applications in electrochemical devices, supercapacitors, biosensors and batteries. Though four fungi were isolated from *Nothapodytes foetida*, *Aspergillus nidulans* was found to be suitable for synthesis of cobalt oxide nanoparticles, as it has proficient tolerance towards metal under study. The broth containing precursor solution and organism *Aspergillus nidulans* had changed from pink to orange indicating the formation of nanoparticles. Characterization by x-ray diffraction analysis (XRD), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and energy dispersive x-ray analysis (EDX) confirmed the formation of spinel cobalt oxide nanoparticles at an average size of 20.29 nm in spherical shape with sulfur-bearing proteins acting as a capping agent for the synthesized nanoparticles. The nanoparticles could be applied in energy storage, as a specific capacitance of 389 F/g showed competence. The study was a greener attempt to synthesize cobalt oxide nanoparticles using endophytic fungus.

1. Introduction

Nanomaterials are materials having dimensions on a nanoscale. They are the materials in the size of 10^{-9} m. When compared to their bulk material counterparts, nanoparticles illustrate peculiar physical and chemical properties (Ullah et al., 2014). In addition, they reveal superior electric, mechanical, thermal, catalytic and magnetic properties than macrostructures due to the greater surface area to volume ratio. The predominant types of nanomaterials are carbon-based nanomaterials, metal-based nanomaterials, semiconductor nanoparticles, fullerenes, dendrimers and composites. They are synthesized by both top-down and bottom-up methods. In top-down methods (lithography, photolithography, micromachining, laser machining), macroscopic initial structures are reduced to nano-scale structures. While in bottom-up methods (atomic layer deposition, inert-gas expansion, inert-gas condensation, ultrasonic dispersion), self-assembly of miniature compounds are performed. Cobalt oxide is one of the metallic oxides, being cobalt as a transition metal. Its nanoparticle counterparts are considered reliable in the field of nanotechnology as they have erected favorable applications in optoelectronics (Salavati-Niasari et al., 2009).

In chemical methods of synthesis of nanoparticles, separate capping and stabilization agents are required to stabilize and control the growth of nanoparticles and prevent agglomeration. Further, extraction and purification problems exist (Seabra and Durán, 2015). Nevertheless, chemical methods deliver generous yield in relatively short times, they employ toxic chemicals and exercise stringent protocols (Yadav et al., 2015), that engage severe reaction conditions like decomposition and combustion. They also embroil more than one step and involve processes that are highly energy intensive (Loo et al., 2012). The instance of contamination is inevitable affecting the quality of the nanomaterials obtained. Owing to these disadvantages, biological methods have gained importance recently, as they are clean, non-toxic, pollution free, economically viable and eco-friendly, even though the yield is less. The proteins secreted by microorganisms can act as both capping and stabilizing agent to control the size of nanoparticles, thus yielding biocompatible particles of lesser toxicity. Some of the microorganisms used for the synthesis of nanoparticles are given in Table 1.

Generally, biological synthesis involves synthesis using bacteria, yeast, plant extract and fungi. In plants, the extracts from leaves, flowers and fruits have been used for the synthesis. Lactic acid bacteria synthesize nanoparticles through non-enzymatical methods. In case of microorganisms like fungus, it is easy to

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https://doi.org/10.1016/j.jenvman.2018.04.032
0301-4797/

Table 1
Microorganisms involved in synthesis of nanoparticles (Ingale and Chaudhari, 2013).

Microorganisms	Nanoparticle type and size (nm)
Bacillus cereus (bacterium)	Ag 5
Bacillus thuringiensis (bacterium)	Ag 10/20
Escherichia coli (bacterium)	Ag 30/50
Aspergillus niger (fungus)	Ag 20
Aspergillus oryzae (fungus)	Ag 5-50
Fusarium oxysporum (fungus)	Ag 1/5
Silver-tolerant strain MKY3 (yeast)	Ag 2/20
Candida glabrata (yeast)	CdS 50/150
Schizosaccharomyce pombe (yeast)	CdS 50/150

isolate and culture and the yield of biomass is more which has a direct relevance in the production of nanoparticles (Saxena et al., 2014). Endophytic fungi secrete a significant number of bioactive metabolites and antimicrobial compounds that are responsible for the synthesis of nanoparticles in presence of precursor compounds via bioactive principles tailoring the elemental compositions (Baker and Satish, 2012). The endophytic fungus is fancied over bacterium as it is noticeable, providing high yield of proteins, large amount of biomass to be handled (Ingale and Chaudhari, 2013) and optimum growth of mycelium with large surface area (Tomar et al., 2015). Fungal biomass along with the supernatant serves as a reduction medium for the formation of the nanoparticles from the precursor solution (Gupta and Bector, 2013). Biosynthesis techniques involving fungi are usually free from toxic chemicals (Kashyap et al., 2013). *Nothapodytes foetida* has been chosen to isolate the endophytic fungi, which is a medicinal plant distributed in the Western Ghats, used in the treatment of cancer and bacterial infections and classified as vulnerable species (Musavi and Balakrishnan, 2014). It is a producer of camptothecin, which is an alkaloid topoisomerase I-DNA inhibitor (Namdeo and Sharma, 2012). It is reported to house more than 170 species of endophytes and the dominant ones are highly tolerant to toxic metals even at high concentrations and harsh conditions (Musavi and Balakrishnan, 2013). Moreover, their adaptability is one of the major reasons to be used to produce metal-oxide nanoparticles.

The biological methods used are given in Table 2. The reactors for the synthesis process are usually beakers or smaller conical flasks kept in an incubator shaker (Kora and Rastogi, 2016; Rahmatpour et al., 2017). Disposal of cobalt oxide nanoparticles has yet to be reported. In life cycle assessment of magnetite nanoparticles to outweigh the environmental benefits to costs, nanoparticles from used solution were recovered (Sashukan et al., 2017). Silver nanoparticles were removed by electrocoagulation (Matias et al., 2015). Cobalt oxide nanoparticles are primarily obtained by acidic treatment of chemical precursor sources in the solvent environment. Still, endophytic fungi are yet to be explored for the synthesis of cobalt oxide nanoparticles. Hence, synthesis can be bidden using potential microorganisms, possibly endophytic fungi that are relatively tolerant to these metals and respond quickly. The current study illustrates an attempt made to biosynthesize and characterize cobalt oxide nanoparticles via an endophytic fungus *Aspergillus nidulans* isolated from *Nothapodytes foetida*.

2. Experimental

2.1. Materials

Samples of plant *Nothapodytes foetida* were collected from Agumbe forest located in the Western Ghats (13°30′N, 75°02′E), Shimoga District, Thirthahalli taluk in the Malnad region of Karnataka, India. Fresh and healthy parts of the plant like stem, seed and leaves were cut with a sterile knife and refrigerated for further use. Potato dextrose agar (PDA) was purchased from Sisco Research Laboratories, Mumbai, India and potato dextrose broth (PDB) and chloramphenicol were acquired from Himedia Chemicals Pvt. Ltd., Mumbai, India. Cobalt (II) acetylacetonate (Sigma Aldrich, Mumbai) was used as salt source and rhodamine 6G (Loba Chemie, Mumbai) was taken as reference in photoluminescence studies. For fungal identification, 23S rRNA sequencing was performed by Yaazh Xenomics, Coimbatore.

2.2. Isolation of endophytic fungi

Isolation of endophytic fungi was carried out by the method described in Wang et al. (2007) with modifications in process time and antibiotic. Explants (leaves, stems, flowers and fruits) samples were surface sterilized with 75% ethanol for 1 min and 2.5% sodium hypochlorite solution for 3 min, followed by repeated rinsing and washing with sterile distilled water. Leaves, stems and flowers were dried and further cut into small pieces (approximately 0.5 cm) and spread on sterile PDA media supplemented with a pinch of chloramphenicol in petri plates. These petriplates were kept for incubation at room temperature for 3 days. Subculturing of the fungal inoculum was done repeatedly by streaking the colonies on the media in petri plates, until pure isolates were obtained.

2.3. Tolerance studies of isolated fungi

The fungal isolates were subjected to tolerance studies using 500 ppm and 1000 ppm concentrations of cobalt acetylacetonate salt amended on PDA plates and isolates on PDA without salt solution were used as control. Thus, isolates that showed higher tolerance to these metal concentrations and successfully produced enough colonies in comparison with the control were selected for synthesis of nanoparticles. Careful observations on the growth of isolates were done by measuring the colony diameter in comparison with the control at certain time intervals and expressed as tolerance index (Anahid et al., 2011). The strain with the greatest tolerance index was chosen for further studies.

2.4. Synthesis of cobalt oxide nanoparticles

The synthesis of nanoparticles was done using the successful isolate from the tolerance studies. 5 hoops of fungal inoculum from the agar plate were taken and inoculated into a conical flask containing 100 mL of PDB medium. The flask was incubated in a rotary shaker at constant room temperature, (30 °C ± 2 °C) and at 115 rpm for three days. Thereafter, the solution was filtered by Whatman

Table 2
Biological methods of synthesis of cobalt oxide nanoparticles reported.

S.No.	Authors	Precursor	Method	Nanoparticle size/diameter
1	Kumar et al., 2008	Cobalt acetate	Bacterial (*Brevibacterium casei*)	5-7 nm
2	Shim et al., 2011	Cobalt chloride hexahydrate	Bacterial (*Bacillus subtilis*) and chemical	2-5 nm
3	Diallo et al., 2015	Cobalt nitrate hexahydrate	Plant extract (*Aspalathus linearis*)	3.5 nm
4	Khalil et al., 2017	Cobalt acetate	Plant extract (*Sageritia thea*)	20 nm
5	Bibi et al., 2017	Cobalt nitrate	Plant extract (*Punica granatum*)	40-80 nm