Journal of Molecular Liquids 222 (2016) 549–557

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

# Green synthesis of protein capped nano-gold particle: An excellent recyclable nano-catalyst for the reduction of nitro-aromatic pollutants at higher concentration

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ARTICLE INFO

*Article history:*
Received 28 January 2016
Accepted 19 July 2016
Available online 21 July 2016

*Keywords:*
Catalyst
Bioremediation
Waste water treatment
Recyclable nano-gold particle
*Fusarium* sp. MMT1

ABSTRACT

An eco-friendly green technology has been developed to synthesize, protein capped nano-gold particles (NGPs) utilizing culture filtrate of *Fusarium* sp. MMT1 strain. The average diameter of NGPs was 30.61 ± 17 nm. SDS-PAGE analysis revealed the presence of a ~60 kDa protein on NGP surface, rendering higher stability. The fungal based biosynthetic nano-catalyst exhibited excellent recyclable catalytic activity for reduction of toxic p-nitrophenol, o-nitrophenol and o-nitroaniline using sodium borohydride ($NaBH_4$). Interestingly, the nano-catalyst was easily recovered and recycled for next reduction reaction confirming its potential efficiency and good stability. The major significance of our study is the achievement of reusable nano-catalyst efficiently reducing nitroaromatics at a concentration hundred times higher compared to existing reports. This NGP may be utilized as a potential "recyclable nano-catalyst" to eliminate nitroaromatic compounds from environmental effluent efficiently, aiming cleaner environment for the sustainability of development.



## 1. Introduction

Exploration and synthesis of nano-sized particles and their application in industry and medicine are the emerging areas of science in this millennium. Universal necessity for the synthesis of nanoparticles utilizing environmentally benign methods enabled researchers to innovate a green technology. Metallic nanoparticles (MNPs) are of great importance due to their unique properties and application in the fields of catalysis, biological sensors, electronics, magnetic device and also in biomedical fields including imaging, diagnostics, drug delivery, nanomedicine and therapeutics [1–5]. The chemical reduction process to synthesis gold nanoparticle using sodium borohydride or sodium citrate has certain drawbacks, owing to the toxic property of the used chemicals and resulting detrimental effect for the environment. Thus, eco-friendly methods are now coming up for metal nanoparticle synthesis to comply with the requirement [6]. Among other MNPs, nano-gold particles (NGPs) have potential application in biomedical and industrial field because of their pronounced biocompatibility, chemical inertness, nontoxicity, easy functionalization, and characteristic optoelectronic properties [7]. The high surface-to-volume ratio and high surface energy of the MNPs make their surface atoms very active, influencing its chemical reactivity and catalytic activity [8]. Furthermore, gold nanoparticles (GNPs) have acquired much more attention due to their synthesis and the surface modifications [9].

Microbes mediated nanoparticles have the natural propensity for detoxification of metallic ions, where biomolecules secreted by the biomass extracellularly or intracellularly, can act both reducing as well as capping agents during the reaction process finally forming stable nanoparticles, which is more biocompatible [10]. Fungi possess unique property compared to others, because of their wide range of morphological variations, availability in large quantities, metal tolerance property and low cost downstream processing. Fungi secrete large amount of extracellular enzymes during their metabolism; significantly increasing the possibility to large scale production [11].

Nitrophenols are the most toxic and refractory water pollutants disposed into the environment from different industrial sources like pesticides, herbicides, insecticides, and synthetic dyes, pharmaceutical and petrochemical industries [12]. After being discharged into the environment, these anthropogenic compounds create serious public health issues because of their carcinogenic and mutagenic effect in humans [13]. Specifically, *p*-nitrophenol (*p*-NP) has been indexed as a "priority pollutant" by US Environmental Protection Agency (US-EPA) because of its longer stability and nature of solubility in water. Usually, it is a long process to degrade the nitrophenol present in soil and water, posing considerable risk with the chance of being accumulated in deep soil without degradation [14]. So, there is urgent need to develop an efficient method to minimize the toxic compounds efficiently from the environment through catalytic reduction. In the present work,

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http://dx.doi.org/10.1016/j.molliq.2016.07.087
0167-7322/

biosynthesized NGPs were applied for catalytic reduction of *p*-NP, *o*-nitrophenol (*o*-NP) and *o*-nitroaniline (*o*-NA) using $NaBH_4$.

Here, we report simple, one-step sustainable process for biosynthesis of protein capped nano-gold particle in an aqueous solution utilizing culture filtrate (C.F), secreted by *Fusarium* sp. MMT1 strain without addition of any external surfactant, capping agent or template. The $HAuCl_4$ reduction ($Au^{+3}$ to $Au^0$) process occurred at room temperature yielding well dispersed nanoparticles. Synthesized nanoparticles were characterized using UV–vis spectroscopy, dynamic light scattering (DLS), zeta potential measurement (Z-pot), selected-area electron diffraction (SAED), transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS), atomic force microscopy (AFM), fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) study. Biosynthesized NGPs were mostly spherical, but with a distribution of triangular, hexagonal and nanorod shaped particles appearing additionally. Green synthesized NGPs have found significant application in recyclable catalytic degradation of nitro-aromatics (*p*-NP, *o*-NP and *o*-NA) to achieve cleaner environment. This study explores C.F. mediated production of biosynthesized nano-gold particle in cost effective manner to serve as effective nano-catalyst for complete reduction of toxic nitro-aromatic pollutants from environment to detoxify the environment. It was reported that, in the presence of $NaBH_4$ and catalyst *p*-NP, *o*-NP and *o*-NA reduced to less toxic product *p*-AP, *o*-AP and 1,2-benzenediamine respectively. This fungal strain *Fusarium* sp. MMT1, isolated from tannery effluent may be used for future prospect in industrial relevance. Interestingly, the reusable NGPs efficiently reduces nitroaromatics which are much higher in concentration compared to the existing reports; to be more precise, almost hundred times in case of p-NP [15–20] and ten times in case of o-NP [21]. The biosynthesized NGPs acting as potential "recyclable nano-catalyst" for fast degradation of several kinds of nitroaromatic pollutants, transforming them to non-toxic compounds aiming cleaner environment. These NGPs are not only effective in reduction of nitroaromatic compounds at higher concentration, but they also possess an excellent reusable property in catalytic reaction after recycling. This significant reduction and reusability efficiency of NGPs has potential implication in bioremediation of industrial waste utilizing complete green technology to achieve cleaner environment.

## 2. Experimental sections

### 2.1. Reagents and media

Dehydrated microbiological media [potato dextrose broth (PDB) and potato dextrose agar (PDA)] were purchased from HiMedia laboratory, India. All other reagents like $HAuCl_4$, NaOH, SDS, KBr and ethanol were of analytical grade and purchased from Merck, Germany and Sigma, USA. All aqueous solutions were prepared using deionized water from a Milli-Q water system.

### 2.2. Preparation of cultural filtrate from Fusarium sp. MMT1

The fungal strain, *Fusarium* sp. MMT1 was isolated and purified from tannery soil [11]. The strain was grown in 100 mL of PDB medium in 250 mL of Erlenmeyer flasks and was maintained for 72 h in shaking condition at 27 °C temperature. After incubation, the culture filtrate (CF) was separated from fungal biomass by filtration using Whatman no. 1 filter paper and was stored at 4 °C for future use.

### 2.3. Biosynthesis of nano-gold particles

For biosynthesis of nano-gold particles (NGPs) 60 mL of C.F. was mixed with 40 mL of 1 mM $HAuCl_4$ (final concentration of salt being 0.4 mM) in a sterilized Erlenmeyer flask and agitated at 140 rpm in a shaker incubator at 27 °C. Similar experimental set up was maintained to observe any probable synthesis of NGPs with PDB broth as control experiment. Another negative control set up was maintained having only 0.4 mM of $HAuCl_4$ along with this experiment. At a regular time intervals, 2 mL of samples were withdrawn from each of the flasks to check the formation NGPs by visual inspection and UV–vis spectrophotometer (Carry 50, Varian) analysis. Synthesized NGPs were purified by centrifugation at 15,000 rpm for 20 min at room temperature, and the process was repeated for three times.

### 2.4. Optimization of reaction conditions for nano-gold particles

Biosynthesized nano-gold particles were optimized at different reaction conditions e.g. the concentration of $HAuCl_4$ salt (0.2 mM to 0.7 mM), pH of the PDB medium (pH:4 to 7) and C.F. extraction time (24 h, 48 h, 72 h, 96 h, 120 h, and 168 h) for maximum synthesis of nanoparticles. Initial pH of the medium was adjusted by using dilute HCl or NaOH.

### 2.5. Characterization of biosynthesized NGPs

Biosynthesized NGPs were characterized utilizing the following physico-chemical techniques:

### 2.6. Visual observation and UV–vis spectroscopy study

Formation of NGPs was monitored by UV–vis spectrophotometer (Carry 50, Varian) in the wavelength range 400–700 nm with appearance of surface plasmon resonance (SPR) peak of gold nano particles at specific wavelengths.

### 2.7. Dynamic light scattering (DLS) and zeta potential measurement

Hydrodynamic diameter and surface charge of synthesized nano-gold particles were measured by dynamic light scattering equipment (Malvern: Zetasizer Nano Series; Malvern, UK).

### 2.8. HRTEM and energy dispersive spectrometer (EDS) analysis of NGPs

The morphology of biosynthesized NGPs was revealed in terms of size and shape of the nanoparticles by high resolution transmission electron microscope (HRTEM: JEM-2100, JEOL, Japan) operating at an accelerating voltage of 200 kV equipped with EDS (Oxford INCA instruments). The EDS spectrum of NGPs was recorded to confirm the elemental composition of nano-gold particle. Sample was prepared by dropping NGPs suspension on carbon-coated copper grids and was dried at room temperature after discarding the excess amount of solution using filter paper. Simultaneously, surface area electron diffraction (SAED) pattern was also captured on the same grid.

### 2.9. X-ray diffraction (XRD) study

The crystalline structure of the synthesized NGPs was studied by x-ray diffraction (XRD) analysis. The nanoparticle powder was cast on glass substrate and subjected to X-ray diffraction analysis with a Cu K$\alpha$ radiation source ($\lambda = 1.540$ Å) using Bruker AXS D8 Advance (Germany) and the diffraction pattern was recorded in the $2\theta$ range of 20–80°.

### 2.10. Analysis of AFM image

A thin film of colloidal gold nanoparticle was prepared by placing a drop of sample on a cover slip and was left undisturbed until the sample was dried. The particles were analyzed by a multimode AFM (Bruker AXS Pte Ltd., Vecco, Model: Innova) at ambient temperature using silicon probes (RTESPA-CP, Vecco, Santa Barbara, CA) in tapping mode. Long tips (aspect ratio 1:1) cantilever with spring constants ranging from 20 to 80 N/m and resonance frequencies of 276–318 kHz were