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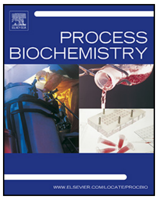

Development of gold nanoparticle-fungal hybrid based heterogeneous interface for catalytic applications

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ABSTRACT

Unsupported and free gold nanoparticles (Au NPs) represent great potential in the field of catalysis. However, shortcomings like agglomeration and loss of the precious catalyst has encouraged the development of supported Au NPs as catalyst with increased activity, selectivity, ease of separation from the reaction mixture and recyclability. The present work demonstrates an eco-friendly, rapid and facile synthesis of catalytically active bio-supported Au NPs using a soil fungus, *Aspergillus japonicus* AJP01. The dual role of the fungal isolate in synthesis as well as immobilization of Au NPs is the remarkable feature of the study. The fungus successfully reduced Au(III) into Au NPs containing principally Au(0) with a small percentage of Au(I) as revealed by X-ray photoelectron spectroscopy. The particles were spherical in shape and well distributed on fungal mycelia with size ranging predominantly between 15 and 20 nm. The as-synthesized nanoparticle-fungal hybrid was found to be highly efficient in catalyzing sodium borohydride mediated reduction reactions of 4-nitrophenol and hexacyanoferrate(III). The versatility of the bionanocatalyst was further demonstrated by catalyzing the A^3 coupling reactions for the synthesis of propargylamines.

1. Introduction

Gold is considered as the metal of beauty, commerce and pride since antiquity. In addition, gold is being used in various scientific applications viz. advanced electronics, fuel cells, coatings, dentistry and biomedicine due to its unique properties like malleability, inertness, heat and electrical conductivity [1]. Breaking the ancient viewpoint wherein gold was considered as an inert metal, later part of the 20th century witnessed the rise of gold based catalysis reactions. The stoichiometry of gold has now been employed in various chemical processes like oxidation of alcohols and aldehydes, epoxidation of propylene, hydrochlorination of ethyne, carbon–carbon bond formation and many other reactions [2]. Presently, gold in its nanoform as “gold nanoparticles (Au NPs)” is extensively used in diverse applications like electronics, sensors, photo-thermal and photo-dynamic therapy, drug and gene delivery, probe fabrication, diagnostics, cosmetics and lubricants [3]. Besides these applications, Au NPs have also become the preferred catalyst for a wide variety of chemical reactions [4]. Compared to its bulk form, Au NPs outperform as catalyst due to their increased surface area, presence of more active sites and improved reaction selectivity [5,6].

Shortcomings like agglomeration and loss of precious catalyst during harsh reaction conditions have compromised the applicability of unsupported and free Au NPs. This has encouraged researchers for the development of supported Au NPs as catalytic system. As catalysts, supported Au NPs exhibit multiple improvements over free Au NPs like increased activity, ease of separation from the reaction mixture and recyclability [7]. The on-cell synthesis of nanoparticles using microbes is an exciting eco-friendly approach which provides a low-cost nanoparticle support system and a smart heterogeneous interface for the catalytic reactions [8]. Various properties of nanoparticles including their size, shape, surface structure and composition contribute significantly to the catalytic activity, efficiency and selectivity [3]. Biological systems maintain strict control over these properties and immobilize nanoparticles on their surface either by entanglement to the cell wall or by embedment within the cell wall leading to marginal metal leaching and thus retains excellent recyclability [9,10].

Use of Au NPs in reduction reactions mediated by sodium borohydride ($NaBH_4$) has been reported by several researchers [11–14]. The reduction of 4-nitrophenol to 4-aminophenol as well as hexacyanoferrate(III) to hexacyanoferrate(II) are redox catalysed reactions wherein Au NPs transfer electrons from borohydride ion

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to the reducing entity. As no chemical reaction occurs between the reactants and nanoparticles, the catalytic property and colloidal stability of participating Au NPs is retained even after reaction completion [13,14]. Considering the importance of simple separation and reusability of Au NPs, researchers have also turned to explore their use in catalyzing three component coupling reactions known as A^3 coupling which were earlier mostly reported to be catalysed by the ionic form of gold [15–19]. A^3 coupling reactions involve conversion of aldehyde, amine and alkyne for the synthesis of propargylamines which acts as a building block and skeleton for the synthesis of biologically active compounds [20].

The present study reports an eco-friendly and rapid synthesis of catalytically active bio-supported Au NPs using a soil fungus, *Aspergillus japonicus* AJP01. The fungal biomass played a dual role as it simultaneously synthesized and immobilized the Au NPs on its surface. The nanoparticles were characterized by scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), UV–vis spectroscopy and transmission electron microscopy (TEM) measurements. The versatile catalytic property of the as-synthesized bionanocatalyst has also been comprehensively discussed.

2. Experimental

2.1. Chemicals

All the chemicals used were of analytical grade and procured from Sigma-Aldrich or Merck Chemicals unless otherwise stated. The culture media components were purchased from HiMedia Laboratories. Milli-Q water was obtained from a Milli-Q Biocel water purification system manufactured by Merck Millipore.

2.2. Mycosynthesis of bionanocatalyst

Soil fungus *A. japonicus* AJP01 was used for the synthesis of Au NPs. The complete details of isolation and molecular characterization of the fungal isolate can be obtained from our previous report [21]. Stock culture of the fungal isolate was maintained on potato dextrose agar (PDA) slants (pH 5.6) by repeated sub-culturing on fresh media at 28 °C. From an actively growing culture, a loopful of spore was inoculated in 100 mL of MGYP medium (0.3% malt extract, 1.0% glucose, 0.3% yeast extract, 0.5% peptone; pH 7.0) in 250 mL Erlenmeyer flask. The inoculated flask was incubated at 28 °C for 72 h on a rotary shaker (120 rpm) under dark conditions. After the incubation period, fungal biomass was separated from the culture medium by centrifugation (5000 rpm, 15 min, 4 °C) and washed thrice with autoclaved Milli-Q water to remove all traces of media from the biomass surface. Typically, 20 g (wet weight) of biomass was resuspended in 100 mL of 1.0 mM gold(III) chloride trihydrate solution in 250 mL Erlenmeyer flask and incubated for 72 h under the similar conditions described above. Biomass in autoclaved Milli-Q water (without gold(III) chloride solution) as positive control and pure gold(III) chloride solution (without biomass) as negative control were also incubated simultaneously at 28 °C for 72 h on a rotary shaker (120 rpm) under dark conditions along with the experimental flasks in three replicates. After incubation, the biomass was separated by filtration using Whatman grade 1 filter paper and washed thrice with autoclaved Milli-Q water before further characterization and use in catalytic application.

2.3. Characterization of bionanocatalyst

The amount of Au(III) ions remaining in the solution after exposure to *A. japonicus* AJP01 as a function of time was monitored by measuring the UV–vis absorption spectrum of culture supernatant using a Jasco V-630 UV–vis spectrophotometer operated at a scan speed of 400 nm min^{-1}. The synthesis of Au NPs on the fungal biomass surface was visually validated and further confirmed by SEM imaging. Sample for SEM was prepared by fixing the fungal biomass bearing Au NPs in 2.5% (v/v) glutaraldehyde (prepared in 0.1 M sodium phosphate buffer, pH 7.2) at 4 °C for overnight. After fixation, the specimen was rinsed with phosphate buffer (pH 7.2) and dehydrated in a series of 30–100% ethanol followed by drying in a vacuum desiccator. SEM micrographs were obtained by imaging the specimen on a Hitachi S-3400N SEM instrument. EDS measurements of specimen were carried out using a Thermo Noran System SIX EDS attached with the SEM instrument. XRD measurement of the freeze dried biomass was carried out to authenticate crystallinity of Au NPs using an X'Pert PRO X-ray diffractometer operated at a voltage of 40 kV and current of 30 mA with CuK_α radiation. The crystal phase was determined by comparing the calculated values of interplanar spacing and corresponding intensities of diffraction peaks with theoretical values from the Joint Committee on Powder Diffraction Standards-International Centre for Diffraction Data (JCPDS-ICCD) database. Scherrer's equation was applied to determine the size of the Au NPs using the data acquired by XRD analysis [22].

The chemical state of gold species within the Au NPs trapped on the surface of fungal biomass was determined by XPS using an Omicron EA 125 instrument equipped with a seven-channel detection system. The sample was fixed on a small piece of conducting carbon tape and dried overnight under an ultra-high vacuum condition. Monochromatic light of Al-$K\alpha$ radiation of energy 1486.7 eV was used as X-ray source. Wide scale and high resolution XPS spectrums of Au 4f were obtained using energy steps of 0.1 and 1.0 eV, respectively. The emission current of 15 mA and chamber base pressure below 10^{-9} mbar was used throughout the XPS measurement.

In order to extract the trapped Au NPs, 0.1 g (wet weight) of fungal biomass was suspended in 1.0 mL Milli-Q water and sonicated for 3 h in an ultrasonic cleaner. After sonication, the fungal biomass was separated by centrifugation (3000 rpm, 5 min) and the supernatant containing extracted Au NPs was analysed by UV–vis spectroscopy and TEM. Sample for TEM was prepared by placing a drop of nanoparticle solution on to a carbon coated copper grid. The extra solution was removed using an absorbent paper and the grid was incubated overnight in vacuum desiccator prior to measurement. TEM micrographs were captured on a JEOL JEM-2100 instrument at an operating voltage of 200 kV.

2.4. Catalytic activity of bionanocatalyst

2.4.1. Reduction of 4-nitrophenol and hexacyanoferrate(III)

The reduction reaction of 4-nitrophenol as well as hexacyanoferrate(III) was performed using $NaBH_4$ in presence of bionanocatalyst. The progress of reaction was monitored by recording the absorption spectrum at one minute interval. Briefly, the reduction reaction of 4-nitrophenol was performed by adding 2885 μL of water, 15 μL of 4-nitrophenol (0.01 M) and 100 μL of aqueous $NaBH_4$ (0.1 M) to a 3 mL quartz cuvette containing 6 mg of bionanocatalyst. Similarly, reduction reaction of hexacyanoferrate(III) was performed by adding 2670 μL of water, 30 μL of potassium ferricyanide (8.33×10^{-2} M) and 300 μL of aqueous $NaBH_4$ (0.1 M) to a 3 mL quartz cuvette containing 6 mg of bionanocatalyst. In order to rule out the possibility of reduction reaction by fungal mycelia, the biomass without Au NPs was used as negative control in both these reactions.

2.4.2. Synthesis of propargylamines

Scheme 1 represents the synthesis of propargylamines by A^3 coupling of variety of aromatic aldehydes (R = H, CH_3, Cl), secondary amine and alkyne under inert nitrogen atmosphere [19].