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Synthesis and characterization of biomatrixed-gold nanoparticles by the mushroom *Flammulina velutipes* and its heterogeneous catalytic potential

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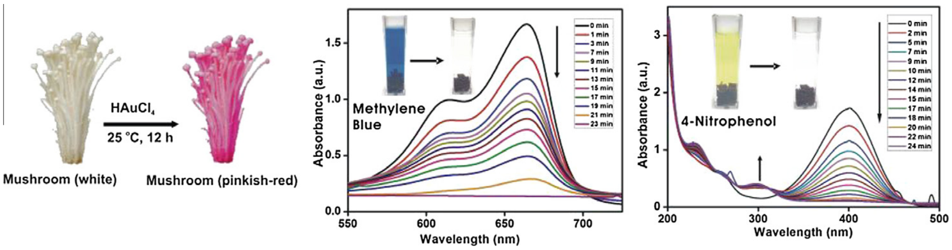
HIGHLIGHTS

- Facile synthesis of intracellular AuNPs by the mushroom *Flammulina velutipes*.
- Biomatrixed AuNPs exhibits heterogeneous catalytic potential.
- It catalytically reduced organic pollutants methylene blue and 4-nitrophenol.
- Biomatrix-embedded metal nanoparticles offers ecofriendly applications.

GRAPHICAL ABSTRACT



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ABSTRACT

Sustainable and greener synthesis of intracellular gold nanoparticles using mushroom *Flammulina velutipes* is reported. Incubation of a mushroom in chloroaurate solution resulted in the synthesis and immobilization of stable gold nanoparticles inside the mushroom mycelia. Transmission electron microscopic (TEM) analysis revealed the presence of gold nanoparticles ($\leqslant$20 nm) inside the mycelia, primarily on the inner surface of the cell membrane. Inductively coupled plasma-optical emission spectrometry (ICP-OES) revealed that the accumulated gold concentration ranged from 64.4 to 330.5 mg kg^{-1} dry weight (DW) in the mushroom mycelia. The reduction of Au^{3+} ions to Au^0 and stabilization of gold nanoparticles occurred within 1 h, and the formation of fcc crystalline gold nanoparticles was confirmed by X-ray diffraction (XRD) analysis. This facile intracellular synthesis of gold nanoparticles by a mushroom without using any toxic chemicals or technologically expensive processes is used as a heterogeneous catalyst in the reduction of organic pollutants methylene blue (MB) and 4-nitrophenol (4NP). The reduction reaction follows pseudo-first order kinetics with a reaction rate constant of 0.0529 min^{-1} and 0.1236 min^{-1} for MB and 4NP, respectively. This biological process of biomatrixing of metal nanoparticles for heterogeneous catalytic reactions is simple, nontoxic, environmentally benign, and economically viable compared to the chemical synthetic routes.

1. Introduction

Metal nanoparticles exhibit unique physicochemical properties when compared to bulk material. Gold nanoparticles show surface plasmon resonance (SPR), Rayleigh scattering and surface-enhanced Raman scattering (SERS), which facilitates its

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applications in the fields of catalysis, optoelectronics, and medicine (Shankar et al., 2004). Although there are several bottom-up strategies for the synthesis of metal nanoparticles, they are mainly synthesized by chemical methods involving toxic compounds such as n-hexadecyltrimethylammonium bromide (HTAB), isopropanol, 2-ethoxyethanol, N-dimethylformamide (DMF), methyl methacrylate and butyl acrylate (Ye et al., 2011). Since the use of toxic chemicals plagues its use in biomedical applications and/or environmentally friendly processes, biological approaches for the synthesis of nanoparticles using microorganisms and plants are preferred. The use of such greener approaches is simple, sustainable and cost-effective and helps to meet the upsurge in demand for environmentally benign processes that is currently occurring worldwide.

Microorganisms normally synthesize metal nanoparticles either intracellularly (*in vivo*) and/or extracellularly (*in vitro*). Despite the fact that extracellular synthesis has more advantages because of its ease of downstream applications, intracellular synthesis, which is very scarce, has some unique applications owing to its added advantages of producing small dimensioned monodispersed nanoparticles with high stability. It is also exploited in the recovery of precious metals from mine wastes and metal leachates. There have been several studies on the use of microbes for various biotechnological applications, but the use of microbial communities for the synthesis of nanomaterials is extremely limited. Intracellularly accumulated metal nanoparticles have various applications in bioremediation, heterogeneous catalysis, sensors, electrocatalysis, fuel cells, optics, and antimicrobial materials (Narayanan and Sakthivel, 2010). Marshall et al. (2007) demonstrated the intracellular accumulation of gold nanoparticles in the plant cells of *Brassica juncea*. The size and the concentration of metal nanoparticles immobilized on the biological matrix can be easily manipulated by changing the growing conditions, incubation time, and the concentration of precursor metal ions. Normally in chemical reactions, these metal nanoparticles, as catalysts, need to be immobilized on supports such as activated carbon, SiO_2, Al_2O_3, TiO_2, and CeO_2 and inert materials such as zeolites and resins for recycling. But, the cost-effective and eco-friendly biomatrixed-metal nanoparticles can be a good solution for the catalyst separation.

Fungi, a special class of heterotrophic eukaryotes are the best for the intracellular accumulation of metals owing to their ability to accumulate and tolerate high levels of metals. For instance, metal-tolerant fungi involve in bioleaching of heavy metals. On the other hand, some fungi such as *Verticillium* sp. (Mukherjee et al., 2001), *Trichothecium* sp. (Ahmad et al., 2005), *V. luteoalbum* (Gericke and Pinches, 2006), *Cylindrocladium floridanum* (Narayanan and Sakthivel, 2011) and *Penicillium* sp. (Du et al., 2011) are reported for the intracellular synthesis of gold nanoparticles. Among fungi, mushrooms are a special group of basidiomycetous fungi, which are becoming more popular nowadays for remediation purposes. Recently, several mushrooms are reported in bioremediation of wastes by the process of biosorption and bioconversion (Kulshreshtha et al., 2013; Kumhomkul and Panich-pat, 2013; Lamrood and Ralegankar, 2013). In particular, edible mushrooms are saprophytic conspicuous fungi, which grow on decaying plant materials. These mushrooms are inexpensive and easy to cultivate, and their anatomical/structural features add merit to their durability in multicellular fungi category. The sustainable and ever-increasing interest on the development of matrix for the immobilization of metal nanoparticles for the catalytic processes of degradation of organic water pollutants have propelled the use of mushroom as a biological entity for the immobilization of metal nanoparticles. Bhat et al. (2013) demonstrated that aqueous extract of edible mushroom *Pleurotus florida* was used for the synthesis of gold nanoparticles. Gurunathan et al. (2014) also demonstrated that the hot aqueous extract of *Ganoderma* spp. was used for the synthesis of biocompatible gold nanoparticles.

Methylene blue (MB) is a water-soluble cationic heterocyclic azo dye and the reduction of MB by reducing agent forms colorless Leuco MB which is reversible. It is used in data storage devices, oxygen detectors, electro-optic devices and textile industry (Galagan and Su, 2008). 4-nitrophenol (4NP) is an anthropogenic pollutant with carcinogenic, mutagenic and embryonic toxicity. So there is an urgent need for devising an efficient method for the degradation of organic pollutants such as MB and 4NP. Silver, gold and copper nanoparticles for the degradation of water pollutants such as dyes and phenols were previously reported (Gangula et al., 2011; Narayanan and Park, 2015). AgBr immobilized on SiO_2-coated Fe_3O_4 nanoparticles (SFN) was used for the degradation of azo dye Acid Orange 7 by visible light driven photocatalytic oxidation (PCO) (Li et al., 2009). Copper oxide nanoparticles (nCuO) catalyze the degradation and debromination of tribromoneopentyl alcohol (TBNPA), and 2,4-dibromophenol (2,4-DBP) (Yecheskel et al., 2013). Although the intracellular synthesis of metal nanoparticles using mushrooms has many advantages, there is no report on this so far. In this study, we demonstrate the synthesis of biologically-immobilized gold nanoparticles using mushroom *Flammulina velutipes*, and investigate its heterogeneous catalytic potential in the reduction of methylene blue (MB) and 4-nitrophenol (4NP) in the presence of sodium borohydride ($NaBH_4$).

2. Materials and methods

2.1. Materials

All the reagents purchased were of analytical grade. Chloroauric acid ($HAuCl_4{\cdot}3H_2O$) was purchased from Alfa Aesar, China. Mushroom, *F. velutipes* (Enoki mushroom) was purchased from Mushland Inc, South Korea. All the aqueous solutions were prepared using Milli-Q water and the experiments were done in triplicates.

2.2. Synthesis of biomatrixed-gold nanoparticles in the mushroom mycelia of F. velutipes

Briefly, 5 g of *F. velutipes* were thoroughly washed with sterile double distilled water and challenged with 10 mL of 5 mM hydrochloroauric acid in aqueous solution and kept in the dark for 12 h at ambient temperature and pH 7.0. The control experiment was done without the addition of chloroaurate ions. After the incubation period, the biotransformation of precursor chloroaurate ions to gold nanoparticles was visually monitored and further characterized.

2.3. Characterization of biomatrixed-gold nanoparticles in the mushroom mycelia of F. velutipes

2.3.1. UV–Vis absorbance spectroscopy

After incubation in the aqueous solution of $AuCl_4^-$ ions, the formation of gold nanoparticles (*extracellular*/*intracellular*) was visually monitored. The UV–Vis absorbance of aqueous solution (*extracellular*) and thin films of mushroom mycelia (*intracellular*) was done using a double-beam UV–Vis spectrophotometer (Shimadzu Scientific Instruments, Model: UV-2600) at a resolution of 1 nm between 300 and 1100 nm.

2.3.2. Powder X-ray diffraction (XRD)

The thin film of mushroom mycelia containing gold nanoparticles was made by washing with sterile distilled water and