Chemical Engineering Journal 225 (2013) 857–864

Contents lists available at SciVerse ScienceDirect

Chemical Engineering Journal

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

Catalytic gold nanoparticles immobilized on yeast: From biosorption to bioreduction

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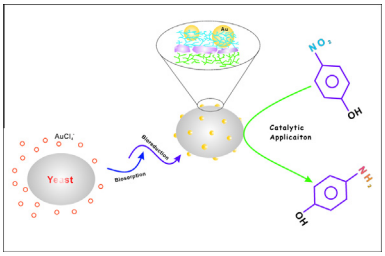
HIGHLIGHTS

- AuNPs can be immobilized on dead yeast cell by reacting *Pichia pastoris* with $HAuCl_4$.
- $-NH_2$ and —OH were demonstrated to play a crucial role in the biosorption process.
- Mannocarolose may be responsible for the bioreduction of $[AuCl_4]^-$.
- Au/*P. pastoris* exhibited good catalytic activity for the reduction of 4-NP.
- The catalytic activity is closely related to the interaction between AuNPs and yeast.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 December 2012
Received in revised form 22 March 2013
Accepted 2 April 2013
Available online 11 April 2013

Keywords:
Pichia pastoris
AuNPs
Catalysts
4-Nitrophenol

ABSTRACT

In this study, gold nanoparticles (AuNPs) were biologically synthesized and bound to the cell surface of yeast *Pichia pastoris* (*P. pastoris*), which exhibited high affinity for Au(III) species in aqueous solution. With the assistance of TEM, FTIR and XRD, the biosynthesis of the AuNPs was investigated by chemical modification of the cell surface, KBr desorption of Au(III) species from the cell surface, and reduction of Au(III) species with mannocarolose extracted from the yeast. Furthermore, the results showed that $[AuCl_4]^-$ ions were rapidly absorbed and reduced into Au(I) ions by $-NH_2$, —OH, and other functional groups on the cell surface. Further, the Au(I) ions were reduced into Au(0) to form AuNPs immobilized on the cells. Mannocarolose played an important role in the reduction process. And the catalytic application of AuNPs/*P. pastoris* to the reduction of 4-nitrophenol was studied. Two other catalysts were prepared by introducing an extraneous reductant to clarify the importance of the AuNPs-yeast involvement in the reduction of 4-nitrophenol. The AuNPs/*P. pastoris* composites exhibited the best catalytic activity, and their catalytic activity can remain stable even after several rounds of reuse. This work demonstrates the fabrication of a catalyst, through the immobilization of AuNPs onto yeast by extending biosorption to biosynthesis, thus highlighting their potential application as environmentally benign catalysts.

1. Introduction

Metal nanostructures have been widely applied in different fields, such as electronics, defense, aerospace, life sciences, and medicine owing to their unique properties, compared with bulk

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http://dx.doi.org/10.1016/j.cej.2013.04.003

metals. Traditional physical and chemical methods have been explored to synthesize application-oriented nanostructures. However, these methods are energy intensive and/or not environmentally benign, making it necessary to develop cleaner and non-toxic technologies for synthesizing nanostructures. In this regard, the preparation of nanoparticles using some organisms or microbial biomass has emerged as one of the research highlights in the field of nanotechnology in recent years [1]. Bacteria, fungi, actinomycetes, and genetically engineered bacteria have been used to prepare metal nanoparticles as Ag [2–7], Au [8–15] and Pd [16–18]. In the case of live microorganisms, it has been demonstrated that enzymes can transfer the electrons of a reductant to metal ions, which are thus reduced into metal atoms to form nanoparticles [19,20]. However, the main drawback of enzyme-catalyzed reduction is that the as-synthesized nanoparticles are in the cell periplasm, which poses difficulties in subsequent processing and applications. Only few studies using dead microorganisms to synthesize nanoparticles have been reported [21,22]. In contrast to enzyme-catalyzed reduction, as metabolic processes are not viable in dead cells, most metal ions would be uptaken by the cell wall and prohibited from penetrating the intracellular membrane, thus leading to the formation of nanoparticles on the cell surface. Our group previously demonstrated that *Corynebacterium* SH09 and *Aeromonas* SH10 could strongly bind silver complex and that silver nanoparticles (AgNPs) were formed on the cell surface [23,24].

Stripping nanoparticles from the cell surface is usually necessary to realize subsequent applications. During the past few decades, solid inorganic (e.g., metal oxides and activated carbon) and organic (e.g., polymers) materials have been used as catalytic supports [25–28]. In recent years, some researchers demonstrated that microorganisms not only can act as reductive matrix but also may serve as support structures. For example, Pd nanoparticles that were loaded on the cell periplasm and surface could be used to catalyze the dechlorination process of polychlorinated biphenyls and the reduction process of Cr(VI) to Cr(III) [18,29–34]. Very recently, Kannan and Natarajan used *Cylindrocladium floridanum* (*C. floridanum*) to synthesize AgNPs and AuNPs and later used them as heterogeneous catalyst [35,36]. All of these reports demonstrated that microorganisms can be used as novel support structures in catalysts and hold promising prospects for application.

Gold catalysts have been receiving much attention in recent years as gold had been thought to be catalytically inert for a long time [37]. Compared with the non-supported catalyst, researchers seem more interested in supported catalyst as it presents several advantages. For example, the support can be used to stabilize the nanoparticles and prevent the nanoparticles from aggregation. The interactions between the support and nanoparticles are believed to account for the catalytic activity [38]. Moreover, the supported catalyst can be readily recycled. 4-nitrophenol is one of the most common organic pollutants in industrial and agricultural wastewaters. One of the effective treatment is to reduce it into 4-aminophenol in the presence of a catalyst. Although there are a lot of reports about supported gold catalysts such as Au/TiO_2, Au/Al_2O_3, Au/polymer and etc. which can be used to catalyze the reduction of 4-nitrophenol [39–44]. However, the preparation of these catalysts usually involves complicated processes or harsh reaction conditions. The synthesis of environmentally friendly catalysts is of great interest. In this study, we present a novel yeast-immobilized AuNPs (Au/*Pichia pastoris*), in which yeast cells not only served as support, but also used as adsorbent and reducing agent. There are several advantages to utilize such Au/*P. pastoris* nanocomposite as a catalyst. On one hand, the catalyst can be readily prepared by one-step green synthesis. On the other hand, such a synthetic protocol gives rise to the strong interaction between the AuNPs and the yeast, which endows the Au/*P. pastoris* catalyst with high stability. This work exemplifies the fabrication of catalytic AuNPs immobilized on yeast by extending biosorption to biosynthesis, thus pointing to their potential application as environmental catalysts.

2. Materials and methods

2.1. Chemicals and reagents

Chloroauric acid ($HAuCl_4{\cdot}3H_2O$), hydrochloric acid, sodium hydroxide, peptone, yeast extract powder, glucose, 4-nitrophenol, sodium borohydride ($NaBH_4$), and other agents used in the experiment were purchased from Sinopharm Chemical Reagent Co., Ltd. All the chemicals and reagents were used as received without further purification.

2.2. Cultivation of the microorganisms

P. pastoris was purchased from Invitrogen. The cells were incubated in the medium containing 10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose at 30 °C for 48 h. Then, they were crushed after being harvested through centrifugation, rinsed with deionized water and dried at 70 °C for at least 16 h to kill the cells. Later on, they were stored in a dessicator prior to use.

2.3. Biosorption and bioreduction of $[AuCl_4]^-$

Experiments were conducted in 250-mL Erlenmeyer flasks containing 100 mL of 1 mM $HAuCl_4$ and 0.4 g of dry biomass of *P. pastoris* (untreated unless specially mentioned) to investigate the biosorption and bioreduction process of $[AuCl_4]^-$ by yeast. The flasks were shaken on a shaker at a rotation rate of 180 rpm in the dark at 30 °C. Samples were taken at regular intervals for analysis.

2.4. Chemical modification of P. pastoris

The yeast were treated with different methods and then subjected to biosorption using the process as in the experiment mentioned above to identify the functional groups involved in the biosorption process.

2.4.1. Esterification of carboxyl of P. pastoris

Yeast powder (1 g) was mixed with 50 mL of methanol and 0.5 mL of concentrated nitric acid (68%) in a 100-mL Erlenmeyer flask that was placed in a water bath at 30 °C for 6 h. Thereafter, the yeast powder was separated from the methanol by centrifugation and washed with deionized water thrice to remove the residual methanol and nitric acid. Subsequently, the yeast was dried in an incubator at 60 °C and ground into powder.

2.4.2. Methylation of amino of P. pastoris

Yeast powder (1 g) was mixed with 40 mL of formic acid and 20 mL of formaldehyde in the flask. The flask was placed in a water bath at 30 °C for 6 h. Then the yeast was separated, washed, and dried as described in Section 2.4.1.

2.4.3. Acetylation of P. pastoris

Yeast powder (1 g) was mixed with 100 mL of acetic anhydride in a 250-mL round-bottom flask. The flask was then placed in an oil bath adjusted to 80 °C for 12 h with magnetic stirring and reflux. The yeast was then separated, washed, and dried as described in Section 2.4.1.