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Corynebacterium glutamicum-mediated crystallization of silver ions through sorption and reduction processes

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ABSTRACT

The processes of biosorption and bioreduction can provide important insights for understanding the mechanisms underlying the biosynthesis of nanoparticles. We performed various experiments using active/live and inactive/dead cells of *Corynebacterium glutamicum* to determine the capacity of these cells to adsorb and/or reduce silver ions. The biosorption of silver increased with increases in pH and equilibrium achieved within 30 min. The maximum experimental uptake with an initial silver concentration of 1000 mg/L was found to be 50.1 mg/g for active cells and 52.5 mg/g for inactive cells. After biosorption, we investigated the bioreduction capacity of both active and inactive cells of *C. glutamicum*. Strong plasmon resonance of silver nanoparticles was observed between 400 and 450 nm in the samples obtained from both active and inactive *C. glutamicum*. Transmission electron microscopy (TEM), energy dispersive X-ray (EDX) and X-ray diffraction (XRD) were performed to examine the formation of silver nanoparticles. A significant difference was noted in the formation of nanoparticles by live and inactive cells. A larger amount of reduction occurred on the surfaces of inactive cells. The nanoparticles formed were very irregular in shape and ranged in size from 5 to 50 nm. In the present study, the possibility of nanoparticles formation even in the absence of enzymes and metabolites has been studied. Crystallization of silver ions from aqueous solution by both active and inactive biomass can form a possible platform for a cost effective and eco friendly technique to remove or recover noble metals.

1. Introduction

Silver is used extensively as a raw material in various industries, because of its many advantageous properties such as malleability, ductility, photosensitivity, antimicrobial activity and electrical and thermal conductivity. Therefore, significant amounts of silver are discharged in the effluents from such industries [1]. The current processes used to recover silver can be economical when used on a large scale and if the metal concentration in the effluent is above 100 mg/L [2]. Significant amounts of silver in discharged industrial waste cause environmental toxicity and warrant removal from both the wastes and the environment. There is scope for further improvement in development of cost effective processes for harvesting noble metals from industrial waste. Existing physical and chemical technologies including chemical precipitation, electrocoagulation, membrane filtration and reverse osmosis are either too expensive or not suitable for treatment of dilute solutions [3].

Adsorption technologies using activated carbon appear to be very promising for silver recovery. However, the high cost of preparing the necessary carbon is a major limiting factor [4]. Efforts are being made to recover silver from industrial effluents by means of bioremediation, since microbes contain metal-binding sites that can be used in environmental decontamination [5]. Studies of Ag^+ biosorption using *Thiobacillus* sp. and *Cladosporium* sp. were conducted by Pethkar et al. [6], and similar studies were performed by Gomes et al. [7] using various strains of *Rhodotorula mucilaginosa* as a biosorbent. Though biosorption has been used in bioremediation applications, few studies have analyzed the byproducts of biosorption of metals by microorganisms. In addition, microbes are potent eco-friendly nanomaterial synthesis factories [8]. Nanotechnology is emerging as a cutting-edge technology, which will play a crucial role in this millennium. Unlike bulk materials, nanoparticles exhibit characteristic physical, chemical, optical, magnetic, and thermal properties [8,9]. In context of the current drive to synthesize green chemistry methods for nanomaterials fabrication, biological systems are of significant interest [8]. The biological systems of the microbes provide an ambient chemical system for synthesizing a wide range of inorganic nanomaterials. In particular, many bacterial species are known to reduce the ionic forms of noble metals to the zero valent forms in nanosize [10]. The nanoparticle synthesis occurs intracellularly or extracellularly in organisms [11,12]. Though many general reports about the mechanism of

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metal nanoparticle formation are available, the exact mechanism leading to their formation is not yet completely understood. It has been proven that metals are initially bioadsorbed/bioaccumulated and then reduced to a zero valent form by the microbes as part of a defense mechanism. Numerous studies have confirmed that bioreduction can be caused by two mechanisms: enzymatic catalysis and non-enzymatic reduction [13]. Lin et al. [14] studied non-enzymatic reduction and the results indicated that certain organic functional groups on microbial cell walls could be responsible for the reduction process under certain conditions. Mouxing et al. [15] developed a process for Ag nanoparticle synthesis by bioreduction of $[Ag(NH_3)_2]^+$ to Ag^0 using *Aeromonas* sp. Fu et al. [16,17] demonstrated that dried cells of *Bacillus megaterium* DOI and *Lactobacillus* sp. A09 are capable of reducing silver ions through the interaction between silver ions and the functional groups on the microbial cell wall. *Verticillium* sp. and *Fusarium oxysporum* are also novel organisms for the extracellular synthesis of nanoparticles and have the potential for easy downstream processing [18]. A recent report by Binupriya et al. demonstrated that both live and dead biomass filtrates of *Aspergillus oryzae* var. *viridis* could synthesize gold and silver nanoparticles. They also explained the involvement of organics from dead cells, in crystallization of silver nanoparticles [19,20]. Zhang et al. [13] studied the biosorption and bioreduction of diamine silver complex using live *Corynebacterium* sp. leading to the formation of silver nanocrystals that were 10–15 nm in diameter. They also proved the absence of bioreduction of silver ions of silver nitrate. The studies noted above clearly indicate that the biosorption and bioreduction of metals are linked, but most of the reports do not completely explain the link between the two processes. In addition, there are not many reports available on biosorption-coupled bioreduction processes in active and inactive cells. Therefore, the investigation of biosorption-coupled bioreduction can provide insight into the mechanisms of the enzymatic and non-enzymatic processes involved in the biosynthesis of nanoparticles.

C. glutamicum, a gram-positive bacterium, is being used in a lysine fermentation industry and its waste biomass is generated in a large amount. The waste biomass has been used in studies of dye and metal absorption. Potentiometric titration and Fourier transform infrared spectroscopy (FT-IR) studies have revealed that the cell wall of *C. glutamicum* is comprised mainly of carboxyl, phosphonate and amide groups [21], where negatively charged groups can easily absorb positively charged Ag^+ ions. The main aim of this study is to scrutinize the percentage conversion of biosorbed ions to bioreduced ions. Herein, we for the first time have compared the biosorption-coupled bioreduction capacity of both active and inactive biomasses.

2. Materials and methods

2.1. Bacterial strain and culture conditions

C. glutamicum (ATCC 13032), obtained from Korean culture center of microorganisms, was stored at 4 °C and subcultured once per month on Luria–Bertani (LB) agar plates. A starter culture was developed by transferring one full loop of the organism from the nutrient agar to 100 mL of LB broth in an Erlenmeyer flask and incubated at 30 °C under shaking at 160 rpm. After 24 h, 10 mL of the starter culture was transferred to an Erlenmeyer flask containing 1 L of LB broth for inoculum development and it was incubated for 24 h at 30 °C in an orbital shaker at 170 rpm.

2.2. Preparation of the biosorbent

The active biomass of *C. glutamicum* was centrifuged at 8000 rpm for 15 min in a refrigerated centrifuge after 24 h of incubation. The biomass was washed twice with sterile double distilled water to separate it from the other media components. Active biomass was obtained by pelleting the washed biomass. Inactive biomass was prepared by resuspending the pellets of active cells in sterile double distilled water, followed by autoclaving at 121 °C for 15 min, at 15 psi. Then the heat killed cells were centrifuged at 8000 rpm for 15 min, for obtaining inactive biomass. Freshly prepared biomass was used for each set of experiments.

2.3. Determination of biosorption capacity

2.3.1. Effect of pH on biosorption by active and inactive biomass

Batch experiments were conducted in a series of Erlenmeyer flasks containing 20 mL of 1000 mg/L silver nitrate solution and 0.2 g of wet biomass with an adjusted pH in the range of 1–7 in order to determine the optimum pH level for silver biosorption by active and inactive *C. glutamicum*. The silver nitrate–biosorbent mixture was placed on a rotary shaker at 30 °C and 160 rpm in dark conditions. The pH of the silver ion–biomass suspension was continuously monitored for 6 h and a constant pH level was maintained by the addition of 1 N HNO_3 or NaOH. After 6 h, the samples were centrifuged at 12,000 rpm for 10 min and the supernatant was collected. The remnant Ag^+ concentration in the supernatant was analyzed by inductively coupled plasma spectroscopy (ICP) (Agilent 7500).

2.3.2. Biosorption isotherm of active and inactive biomass

Sorption experiments were carried out using 0.2 g of active and inactive cells in combination with 20 mL $AgNO_3$ solution with concentrations of Ag^+ varying from 100 to 1000 mg/L in order to study the adsorption capacity of the biosorbents. The reaction was allowed to take place 6 h with shaking at 160 rpm at a natural pH (6.04 ± 0.2) level and 30 °C. The supernatant was separated from the cells by centrifugation at 12,000 rpm for 10 min. ICP analysis was then performed to determine the ion concentration of the remnant Ag^+. After ICP analysis, Langmuir and Freundlich equations were used to describe the equilibrium biosorption.

2.3.3. Effect of contact time on biosorption

In order to determine the kinetics of the Ag^+ uptake from the solution, experiments were conducted at an initial concentration of 1000 mg/L for both active and inactive cells. Samples were collected at predetermined intervals and the bacterial cells were promptly separated from the salt solution by centrifugation at 12,000 rpm for 10 min. The supernatant was collected for ICP analysis to determine the residual Ag^+ concentration.

2.3.4. Data evaluations

The amount of metal adsorbed (Q, mg/g) by active and inactive bacterial biomass was calculated using the following equation:

$$Q = \frac{(C_i - C_f)V}{m} \quad (1)$$

where C_i and C_f are the initial and the equilibrium concentrations (mg/L), respectively, V is the working volume of the solution (L) and m is the weight of the biomass (g).

2.4. Determination of bioreduction

Experiments were carried out by suspending 10 g/L of wet, active and inactive biomass in aqueous silver nitrate solutions containing 1000 mg/L of silver. These suspensions were kept in the dark under shaking conditions. Samples were taken at regular intervals and examined using a spectrophotometer to detect the formation of Ag nanoparticles. After bioreduction, the suspension was centrifuged and the sample was subjected to TEM analysis, energy