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RESEARCH NOTES

Rapid Preparation Process of Silver Nanoparticles by Bioreduction and Their Characterizations*

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Abstract Bioreduction as a novel nanoparticle synthesizing technology attracts increasing attention. Dried cells of the bacterium *Aeromonas* sp. SH10 rapidly reduced $[Ag(NH_3)_2]^+$ to Ag^0 in the solution into which some amount of OH^- was introduced. The surface plasmon resonance centered at 425 nm on the UV-vis spectra and five broad Bragg reflections on the XRD pattern showed that stable silver nanoparticles were formed during the bioreduction process. TEM and SEM observations suggested that the silver nanoparticles were uniform in size and well dispersed on the cells and in the solution. Therefore, silver nanoparticles could be prepared rapidly by this bioreduction technology.
Keywords bioreduction, silver, nanoparticle, bacterium, hydroxide

1 INTRODUCTION

Nanoparticle synthesis has received considerable attention in recent years as a result of their optical, electronic, magnetic, and chemical properties and their potential applications in subsequent technology development[1]. Silver nanoparticles can be used in areas such as integrate circuit[2], cell electrode[3], antimicrobial deodorant fibre[4], catalysis[5] and chemical analysis[6]. Several techniques to manufacture silver nanoparticles are in use. However, atomistic, molecular and particulate processing in a vacuum or in a liquid medium is usually employed. Most of the techniques are capital intensive, as well as inefficient in materials and energy use[3]. It is well known that biological systems can provide a number of metal or metal-containing particles in the nanometer size range. The synthesis of magnetite nanoparticles by magnetotactic bacteria[7], siliceous materials by diatoms[8] and gypsum and calcium carbonate layers by S-layer bacteria[9] are some of the examples. Consequently, increasing attention has been paid to bioreduction as an efficient and green chemistry approach. For silver bioreduction, examples include the fungus *Verticillium* sp. and *Fusarium oxysporum*, which were able to reduce the metal ions into silver nanoparticles intracellularly and extracellularly, respectively[10, 11]. It was believed that the enzymes of the organisms played an important role in the reduction. However, some studies proved that dried cells of some microorganisms could also reduce silver ions, where the processes of reduction were probably non-enzymatic. For example, Fu *et al*. showed that dried cells of *Bacillus megaterium* D01, *Lactobacillus* sp. A09, were capable of reducing silver ions through the interaction between silver ions and groups on microbial cell wall [12, 13]. Our group also developed some study of silver bioreduction. Silver nanoparticles in the size range of 10—15nm were produced by treating dried cells of *Corynebacterium* sp. SH09 with diammine silver complex[14]. The ionized carboxyl of amino acid residues and the amide of peptide chains were the main groups trapping $[Ag(NH_3)_2]^+$ onto the cell wall and some reducing groups, such as aldehyde and ketone, were involved in subsequent bioreduction[14]. However, it was found in our previous study that the bioreduction reaction progressed too slowly. Further investigation herein demonstrated that the bioreduction could be considerably accelerated in the presence of OH^-. The characterizations of the nanoparticles rapidly prepared *via* the OH^- assisting bioreduction process are presented in detail in this paper.

2 EXPERIMENTAL

2.1 Strain and reagent

Aeromonas sp. SH10 was isolated from Shanghang silver mine, Longyan, Fujian, China. Diammine

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silver complex was prepared by adding some amount of ammonia into silver nitrate solution.

2.2 Biomass preparation

For inoculum preparation, a loop of spore suspension of the bacterial culture was transferred from the agar slant into 50ml sterile medium containing final concentrations of 1% soya peptone and 0.5% beef extract in 250ml Erlenmeyer flask. The flask was incubated at 30℃ for 18h on a rotary shaker at 150r·min^{-1}. Then several milliliters of late-log phase culture were inoculated into 250ml Erlenmeyer flasks containing 50ml medium. The inoculated flasks were incubated at 30℃, shaken at 150r·min^{-1} again for 16h and then the cells were harvested by centrifugation (3500r·min^{-1}, 15min at room temperature) and washed three times with Millipore water. Finally, the wet biomass was dried in the oven at 60℃ for more than 24h till constant mass, and then ground into fine particles.

2.3 Nanoparticle preparation

In a typical procedure for the nanoparticle preparation, dried biomass of known mass was well dispersed into Millipore water of controlled volume. Then the whole suspension was transferred into a tightly stoppered Erlenmeyer flask of 50ml, and then some amount of NaOH and $[Ag(NH_3)_2]^+$ solution was added to the flask in sequence and the bioreduction system was adjusted to the volume of 25ml by adding Millipore water. The flask was shaken on the rotary shaker at 125r·min^{-1} in dark at 60℃ during the bioreduction.

2.4 Nanoparticle characterizations

Nanoclusters were characterized by UV-vis, XRD, TEM and SEM-EDS. During the reduction, samples of the mixture (no more than 0.1ml) were taken and then diluted for several times with Millipore water. After dilution, they were centrifuged at 8000r·min^{-1} for 5min. The supernatants were scanned by UV-300 spectrophotometers (UNICAM) for UV-vis spectra. After bioreduction, the mixtures were sampled for TEM observation on H-600 Electron Microscope (Hitachi) at a voltage of 120kV. The mixtures were dried in a 60℃ oven and then ground into powder for the assay of powder X-ray diffraction scans on an X'Pert Pro X-ray Diffractometer (PANalytical B. V.) at 40kV and a current of 30mA with Cu K_α radiation, and for SEM-EDS observation on a LEO-1530 (LEO) at 20kV.

3 RESULTS AND DISCUSSION

3.1 Rapid preparation of silver nanoparticles

In our previous study, fabrication of silver nanoparticles was proved to be time-consuming by the bioreduction in which $[Ag(NH_3)_2]^+$ was simply reduced by the dried cell. The result was almost the same with the bioreduction of $[Ag(NH_3)_2]^+$ by *Corynebacterium* sp. SH09[14]. It was reported that some amount of OH^- tended to promote the reduction of silver ions in some chemical methods[15]. With this understanding, silver nanoparticles were successfully prepared in a timesaving way by adding some quantity of NaOH to the bioreduction system in this study. The reaction time was shortened from a long period, *e.g.* nearly one month, to a couple of hours. As the reaction went on, the color of the bioreduction system changed from pale yellow to dark yellow. The appearance of the yellow color indicated the formation of silver nanoparticles in the reaction mixture, as it is well-known that silver nanoparticles exhibit striking colours (light yellow to brown) due to excitation of surface plasmon vibrations in the particles[16]. Note that precipitates of AgOH emerged when excess OH^- was introduced, but they disappeared gradually and a generated yellow color deepened with time, which indicated that increasing silver particles formed. It was speculated that $[Ag(NH_3)_2]^+$ first reacted with OH^- to form Ag_2O, which was then metabolized independently and reduced into silver nanoparticles by the biomass. The mechanism of the OH^- assisting bioreduction was being investigated in detail.

3.2 Silver nanoparticle characterizations

3.2.1 *UV-vis spectrum*

UV-vis spectrum is quite sensitive to the formation of silver nanoparticles. Fig.1 shows the spectra recorded at different time of reaction. It is observed that the strong surface plasmon resonance centered at ca. 425nm clearly increases in intensity with time, stabilizing after ca. 4h quickly, which is an indication of rapid preparation of silver nanoparticles. Moreover, no obvious shift of the absorption peak suggests little aggregation of the silver particles in solution. The stability of the silver nanoparticles can be seen more clearly by the inset graph in Fig.1, which shows the intensity of the absorbance at 425nm corresponding to the reaction time. It was observed that the solution containing the nanoparticles remained stable for more than six months, with no signs of aggregation or precipitate.

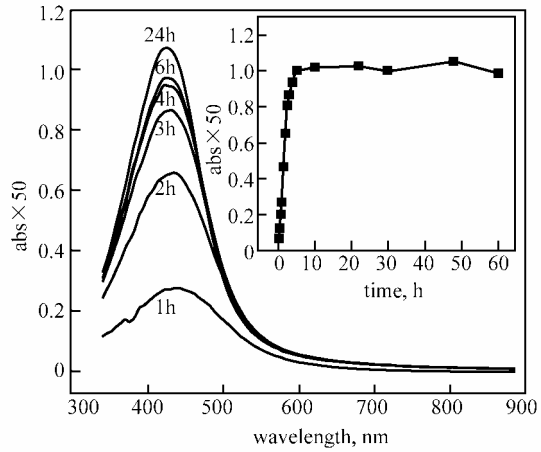


Figure 1 UV-vis spectra recorded as a function of reaction time (The inset shows the intensity of the absorbance at 425nm corresponding to the reaction time. $c_i = c_b = 1 g \cdot L^{-1}$, $[OH^-] = 0.02 mol \cdot L^{-1}$)

3.2.2 *XRD*

An XRD pattern obtained for the silver nanoparticles is shown in Fig.2. A number of Bragg reflections corresponding to (111), (200), (220), (311) and (222) sets of lattice planes are observed, which may be indexed based on the fcc structure of silver. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature. Furthermore, the average diameter of the silver nanoparticles is figured out at ca. 6.4nm by the Scherrer equation.

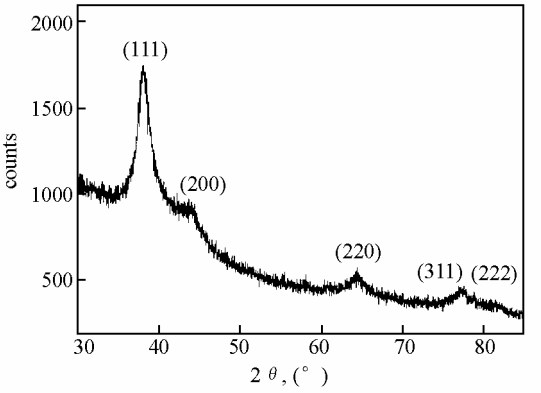


Figure 2 XRD pattern of silver nanoparticles synthesized by treating dried biomass of SH10 with $[Ag(NH_3)_2]^+$ for 24h ($c_i = c_b = 1 g \cdot L^{-1}$, $[OH^-] = 0.02 mol \cdot L^{-1}$)

3.2.3 *TEM*

A representative TEM image of the bacterium SH10 after reaction of 48h is given in Fig.3. It seems that the bacteria are hollowed leaving distinct contours and numerous electron opaque nanoparticles are present inside and outside the cells, which are considered to be silver nanoparticles. The formation of the nanoparticles outside the cells seems to be arisen by the biomass released from the cells. This was also testified by the fact that reduction took place using the extractive of the cells by boiling water instead of the dried cells. Moreover, it is observed that the silver nanoparticles are monodisperse and uniform in size without distinct aggregation which may attribute to the well protection from the biomass.

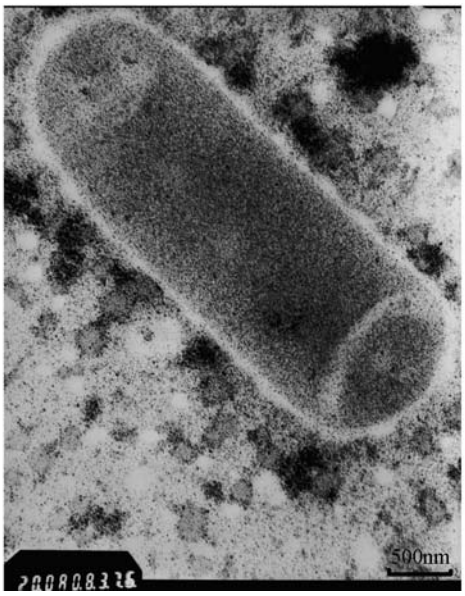
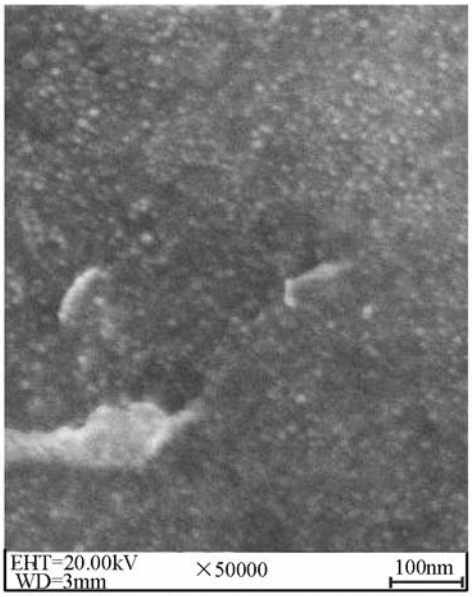


Figure 3 TEM image of the bacterium SH10 after bioreduction of 48h ($c_i = c_b = 1 g \cdot L^{-1}$, $[OH^-] = 0.02 mol \cdot L^{-1}$)

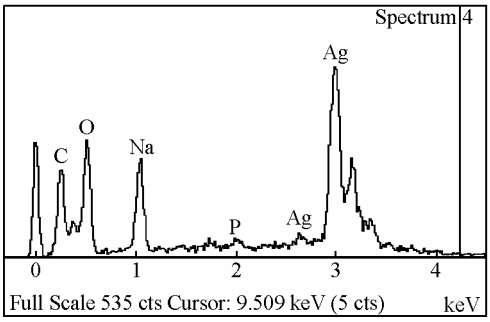
3.2.4 *SEM-EDS*

Figure 4(a) shows an SEM image of the cells of SH10 after exposure to $10 g \cdot L^{-1}$ aqueous $[Ag(NH_3)_2]^+$ solution for 48h. Uniformly distributed silver nanoparticles on the surface of the cells are observed. However, it does not indicate that all the nanoparticles are bound to the surface of the cells, because those dispersing in the solution may also deposit onto the surface of the cells during the drying process which is a necessary step before SEM observation. The uniform size of the silver nanoparticles suggests that the particles on the cells and in the solution may have the same size. There was no obvious growth of the nanoparticles during the drying process at 60℃ as a result of the well protection from the biomass. Furthermore, Fig.4(b) shows the EDS spectrum recorded in the spot-profile mode. Strong signals from the silver atoms are observed (31.23% in mass), while weaker signals from C, O, P and Na atoms are also recorded. Those weaker signals are likely to be due to X-ray emission from the organisms and NaOH which was introduced to the reduction

system as described above.



(a)



(b)

Figure 4 (a) SEM image of the SH10 cells after bioreduction of 48h and (b) EDS spectrum recorded from one of the films (c_i=c_b=10g·L^{-1}, [OH$^-$]=0.1mol·L^{-1})

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NOMENCLATURE

c_b mass content of biomass, g·L^{-1}

c_i initial mass content of diammine silver complex, g (Ag)·L^{-1}

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