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Microbial Preparation of Gold Nanoparticles by Anaerobic Bacterium

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Certain types of microorganisms present in anaerobic environments have the ability to transfer electrons to Fe(III) and other metals. Such Fe(III)-reducing bacteria are a candidate metal-reducer for preparing precious metal nanoparticles in aqueous media. This paper describes the microbial preparation of gold nanoparticles by the anaerobic bacterium *Shewanella algae*. We found that *S. algae* was capable of precipitating gold nanoparticles by reducing Au(III) ions in aqueous $HAuCl_4$ solutions with molecular hydrogen as the electron donor. In the presence of *S. algae* and hydrogen gas, the Au(III) ions were completely reduced and precipitated from 1 mol/m^3 Au(III) in aqueous solution within 60 min. The formation of gold nanoparticles was also evidenced by the color change of the suspension: the starting solution was initially yellow, whereas the addition of *S. algae* and hydrogen gas to the Au(III) solution resulted in reddish-violet suspension due to the surface plasmon absorption of gold nanoparticles. The TEM observations revealed that the microbially induced gold particles have a diameter of 10 to 20 nm.
Key words: gold nanoparticles, metal-reducing bacteria, bioprecipitation, *Shewanella algae*

1. INTRODUCTION

Nanoparticles of precious metals have been widely applied in various industries. The fairly common applications of precious metal nanoparticles are catalysts in the chemical industries and conductor pastes in electronics. That is because such metal nanoparticles have their catalytic properties due to the large surface area and their specific functions which are different from those bulk metal solids. Many studies have addressed the physical and chemical methods for preparing the precious metal nanoparticles, as reviewed in the literature[1]. In general, the nanoparticles of precious metals are mainly prepared by wet chemical methods such as the reduction of metal ions in aqueous solution with a reductant and a surfactant. Although chemical and physical methods have been extensively developed for the nanoparticles synthesis, another possible synthetic route is the preparation of metal nanoparticles by microbial means. Application of the microbial method to the nanoparticle preparation has the advantage that the microbial preparations offer much saving in reducing agents and energies over the conventional chemical and physical methods.

A candidate metal-reducer in microbial preparation is likely to be the Fe(III)-reducing microorganisms present in anaerobic environments, which have the ability to transfer electrons to Fe(III) and other metals. It is accepted that microbial reduction of metals can influence the fate of metals in aquatic sediments and the geological formation of minerals. Figure 1 shows a conceptual diagram for the microbial reduction of Fe(III) ions to Fe(II) ions. The Fe(III)-reducing bacteria can oxidize lactate to acetate, and the lactate acts as the electron donor. The resulting electron is transferred to Fe(III) as the electron acceptor, so that the Fe(III) ions are reduced to Fe(II) ions. As a result, the Fe(III)-reducing bacteria are capable of growing by the redox reaction. It is likely that the Fe(III)-reducing bacteria are a candidate metal-reducer for accomplishing the preparation of gold nanoparticles in aqueous media, because the reduction potential of Au(III) ions in aqueous chloride solution is almost equal to the reduction potential of Fe(III) ions in the solution:

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad ; \qquad E^0 = 0.77\ \mathrm{V}$$

$$AuCl_4^- + 3e^- \rightarrow Au + 4\,Cl^- ; \quad E^0 = 1.00\ \mathrm{V}$$

This paper describes the microbial preparation of gold nanoparticles, in which Au(III) ions in aqueous solution is microbially reduced and precipitate by using the Fe(III)-reducing microorganism *Shewanella algae*.

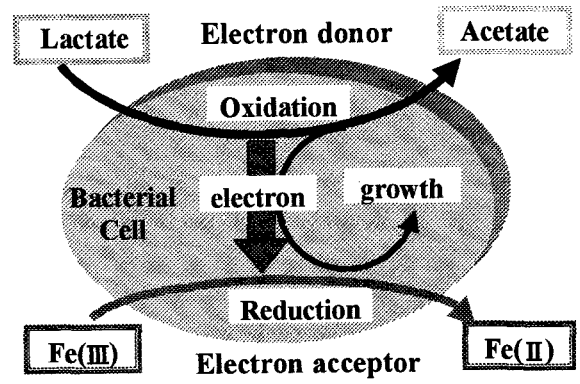

Figure 1. A conceptual diagram for the microbial reduction of Fe(III) ions by the Fe(III)-reducing bacterium.

2. EXPERIMENTAL SECTION

2.1 Microorganism and medium

The Fe(III)-reducing bacterium *S. algae* ATCC 51181 was obtained from the American Type Culture Collection (ATCC), USA. The culture medium used was the ATCC medium 2 (sodium lactate as an electron donor, and iron(III) citrate as an electron acceptor)[2]. For anaerobic incubation, the liquid medium was prepared in a 500 cm^3 screw-cap flask and bubbled with N_2-CO_2 (80:20, vol/vol) for 30 min. The strain was anaerobically subcultured in a screw-cap flask (200 cm^3 of the liquid medium) at 30°C. After 3 to 5 days of batch inoculation, the bacterial cells were harvested anaerobically by centrifugation, resuspended in anaerobic bicarbonate buffer under N_2-CO_2 (80:20, vol/vol), and repellted by centrifugation. This procedure was repeated, and then the washed cells were resuspended in the bicarbonate buffer under N_2-CO_2 (80:20, vol/vol).

2.2 Experimental procedures

In microbial reduction experiments, an aliquot of cell suspension was added to the aqueous $HAuCl_4$ solutions. The initial Au(III) concentrations in the solutions were 0.8 to 2.4 mol/m^3. The cell concentration in the solution was 4×10^{15} cells/m^3 and the experimental temperature was 30°C. Because *S. algae* has a pH optimum for activity at pH 7.0, the solution pH was mainly adjusted to pH 7.0 by using bicarbonate buffer. In some microbial reduction runs, the $HAuCl_4$ solution was used with an unadjusted pH (2.8). The gas-phase was H_2-CO_2 (80:20, vol/vol) when molecular hydrogen was provided as the electron donor. In some runs, the gas phase was N_2-CO_2 (80:20, vol/vol) when lactate was used as the electron donor.

The number of cells in the solution was counted using a Petroff-Hausser counting chamber. The concentration of Au(III) in the aqueous solution was determined by atomic absorption spectroscopy. The precipitates were characterized by electron diffraction (ED) analysis. The particle size and morphology were observed with transmission electron microscopy (TEM).

3. RESULTS AND DISCUSSION

3.1 Microbial reduction of Au(III) ions

Typical reduction behavior of Au(III) ions by *S. algae* is show in Figure 2, where the liquid-phase concentration of Au(III) is plotted against the operating time. In this case, molecular hydrogen was provided as the electron donor for the microbial reduction. In the sterile control containing no cells, the liquid-phase Au(III) concentration did not decrease for 150 min. When the bacterial cells of *S. algae* were added to the aqueous Au(III) solution at pH 7.0, the dissolved Au(III) in the solution was completely reduced and precipitated within 60 min. Thus, it can be concluded that the Fe(III)-reducing bacterium *S. algae* is capable of reducing Au(III) to Au(0) with molecular hydrogen as the electron donor.

Figure 2 also indicates the effect of solution pH on the microbial Au(III) reduction. Even when the solution pH was decreased from 7 to 2.8, *S. algae* had the ability to reduce and precipitate gold. However, the kinetics of reduction and precipitation were markedly affected by the solution pH. At pH 7, the microbial reduction proceeded fast and the liquid-phase Au(III) concentration decreased rapidly from 0.9 mol/m^3 to almost zero for 60 min. When the solution pH was held at low value of pH 2.8, the microbial reduction was comparatively slow and a 50% decrease in the aqueous Au(III) concentration occurred during the first 150 min.

Figure 3 shows the reduction behavior of Au(III) ions by *S. algae* in the presence of different electron donors. When lactate was used as the electron donor, the reducing bacterium *S. algae* did not have the ability to reduce and precipitate gold. Because *S. algae* had the ability to reduce Au(III) ions with molecular hydrogen, the microbial reduction was dependent on two kinds of electron donor. It is likely that the microbial reduction of Au(III) by *S. algae* is an enzymatically catalyzed reaction, which is dependent on the presence of specific electron donor, molecular hydrogen. This result suggests

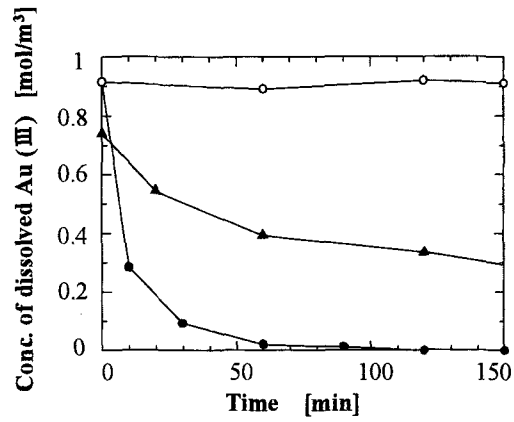

Figure 2. The microbial reduction of Au(III) ions by *S. algae* at different pH values in the presence of H_2 as the electron donor. (●) solution pH 7.0; (▲) solution pH 2.8; (○) sterile control containing no cells at pH 7.0.

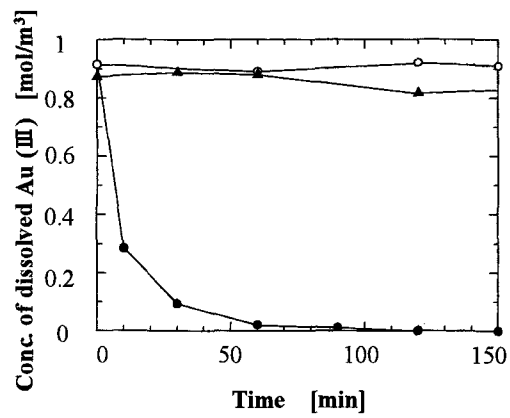

Figure 3. The microbial reduction of Au(III) ions by *S. algae* at pH 7.0 in the presence of different electron donors. (●) H_2 ; (▲) lactate ; (○) H_2 and sterile control containing no cells.

that the microbial Au(III) reduction involves a hydrogenase, which is an enzyme in certain microorganisms that catalyzes the reduction of a substance by molecular hydrogen. However, the mechanism of microbial gold reduction remains to be investigated.

3.2 Characterization of gold nanoparticles

The resulting precipitates were characterized by ED analysis. The observed spacings of lattice planes were consistent with crystalline gold. The formation of gold nanoparticles was also evidenced by the color change of the starting solution. The absorption spectra were determined for the aqueous Au(III) solution before and after the addition of the reducing bacterium *S. algae* and hydrogen gas. The starting solution was initially yellow in the absence of *S. algae* cells. The addition of *S. algae* and hydrogen gas to the starting solution resulted in reddish-violet suspension, and the observed spectrum exhibited an absorption peak at 557 nm, which is assigned to the surface plasmon absorption of gold nanoparticles[3, 4].

Figure 4 shows the TEM observations of *S. algae* cells and resulting precipitates from the aqueous Au(III) solutions. The *S. algae* cell had rod-like shape, and the size is about 1 to 2 μm. When the initial concentration of Au(III) in the starting solution was 1.0 mol/m^3, the microbially induced gold particles had a diameter of 10 to 20 nm. When the initial Au(III) concentration was 2.4 mol/m^3, the gold particles precipitated extracellularly. Much of the gold nanoparticles were attached to the external surface of the *S. algae* cells, and some of the gold nanoparticles were present in the bulk of liquid phase.

3.3 Comparison of the microbial preparation with chemical preparation

In general, one of the promising methods is the chemical synthesis in aqueous solution to prepare gold nanoparticles. The chemical reduction of Au(III) ions to Au(0) was usually achieved by adding citric acid (reductant) with some surfactant. In addition to the use of reducing and dispersion agents, boiling at least for 10 min is necessary for the completion of the chemical reduction. As mentioned above, the gold nanoparticles can be microbially prepared at room temperature for 60 minutes in the presence of reducing bacterium *S. algae*. There are significant differences in the operating conditions between the chemical and microbial methods. It can be concluded that the microbial preparation of gold nanoparticles is carried out at room temperature, thus environmentally friendly in comparison with the conventional chemical production.

4. CONCLUSIONS

The anaerobic microorganism *S. algae* had the very attractive ability to precipitate gold by reducing Au(III) ions to Au(0) with molecular hydrogen as the electron donor. The TEM observations revealed that the microbially induced gold particles have a diameter of 10 to 20 nm. The preparation of gold nanoparticles was a fast process; 1 mol/m^3 Au(III) in the aqueous solution was completely reduced within the first 60 min of exposure to *S. algae* cells.

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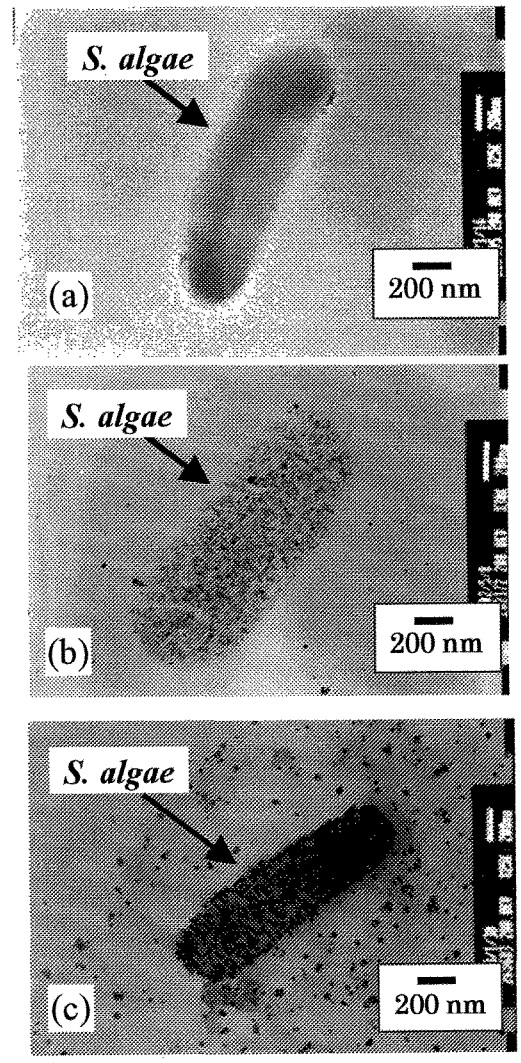

Figure 4. Transmission electron micrographs of (a) *S. algae* cell without gold particles; (b) *S. algae* cell and gold particles at initial Au(III) concentration of 1.0 mol/m^3; (c) *S. algae* cell and gold particles at initial Au(III) concentration of 2.4 mol/m^3.

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