



## Diversity of Aurum bioreduction by *Rhodobacter capsulatus*

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### ABSTRACT

An environmentally friendly method using *Rhodobacter capsulatus* is proposed to deposit Aurum nanoparticles. Experiments of Au (III) bioreduction were conducted. There were three kinds of locations found of deposited Aurum nanoparticles, outside, inside and on the plasma membrane of the cell, which showed the diversified ability of *R. capsulatus* to reduce heavy metal. The mechanism for each of them had been discussed. The methods by which *R. capsulatus* reduced heavy metal could be more diverse than those of other microbes reported before. The capping agent was also found to stabilize the reduced Aurum in supernate. In conclusion PNSB has the powerful and diversified ability of high valent Aurum reduction.

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### 1. Introduction

Because of the increasing demands for gold in the fields of electronics, catalysis and nanotechnology [1–3], exploration for new gold deposits in the natural environment has become very important. Meantime the recovery of gold in waste solutions from industrial processes, e.g., gold mining and gold electroplating effluents, is also of significance. Although chemical methods have been extensively developed for exploring and recovering gold from the aqueous solutions [4], none of them is eco-friendly. Therefore, there is a substantial need to develop cost-effective and environmentally friendly alternative to deposit gold.

Purple non-sulfur bacteria (PNSB), inhabiting anaerobic environments, have been applied in the field of environmental protection, such as the treatment of sewage and wastewaters [5], and the bioremediation of environment polluted by organic matters [6]. Because PNSB are known to have light-dependent metal (metalloid)-oxidizing or -reducing capability, this ability has attracted many researchers [7–9]. But few researches have been done on Au (III) reduction of PNSB comprehensively.

The previous work [10] had revealed the phenomena of bioreduction of Au (III) by *Rhodobacter capsulatus*, a typical PNSB, at room temperature. However, more information was needed to concern the characterization of gold deposit reduced. In this research, experiments had been conducted to investigate the extracellular and intracellular bioreduction of Aurum using *R. capsulatus*.

### 2. Materials and methods

#### 2.1. Experiment of Au (III) bioreduction

The cells in Logarithmic growth phase, when bacteria multiplies exponentially, were harvested and washed twice by sterile physiological solution. Bacterial concentrations were adjusted to  $10^9$  cells/ml measured by MPN (most probable number) method [11]. Experiments were carried out with 50 ml cell-HAuCl<sub>4</sub> solution at different initial concentrations on 40, 80 and 160 mg/L. The solution was incubated for 20 h on a rotary shaker at 150 rpm and 25 °C under continuous illumination. The headspace was replaced by N<sub>2</sub> gas and lactate is the hydrogen donor. Each experiment was repeated for three times.

#### 2.2. Analytical techniques

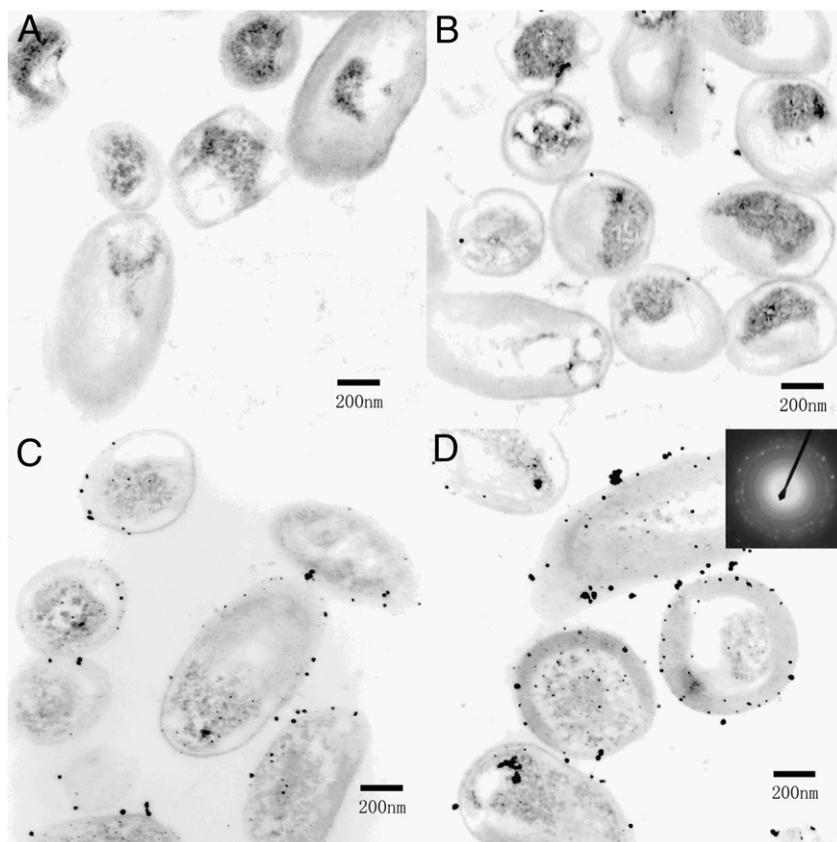
The cells of *R. capsulatus* with different initial Au (III) concentration were analyzed by TEM (transmission electron microscopy) (JEOL, JEM-200EX) and the resulting particles were characterized by SAED (selected area electron diffraction) (ED, JEOL, JEM-200EX). UV/Vis spectrum, SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and FTIR (Fourier transform infrared spectroscopy) studies were conducted to investigate the proteins involved in reducing and capping Au (III) extracellularly.

### 3. Results and discussion

#### 3.1. Effect of initial Au (III) concentration on Au (III) bioreduction

Initial HAuCl<sub>4</sub> concentration had the great impact on the removing ability of PNSB [10]. The amount of removed Aurum increased with higher concentration of HAuCl<sub>4</sub>. Therefore the different initial Au (III) concentrations, 40, 80 and 160 mg/L, were chosen to study the Au (III) bioreduction.

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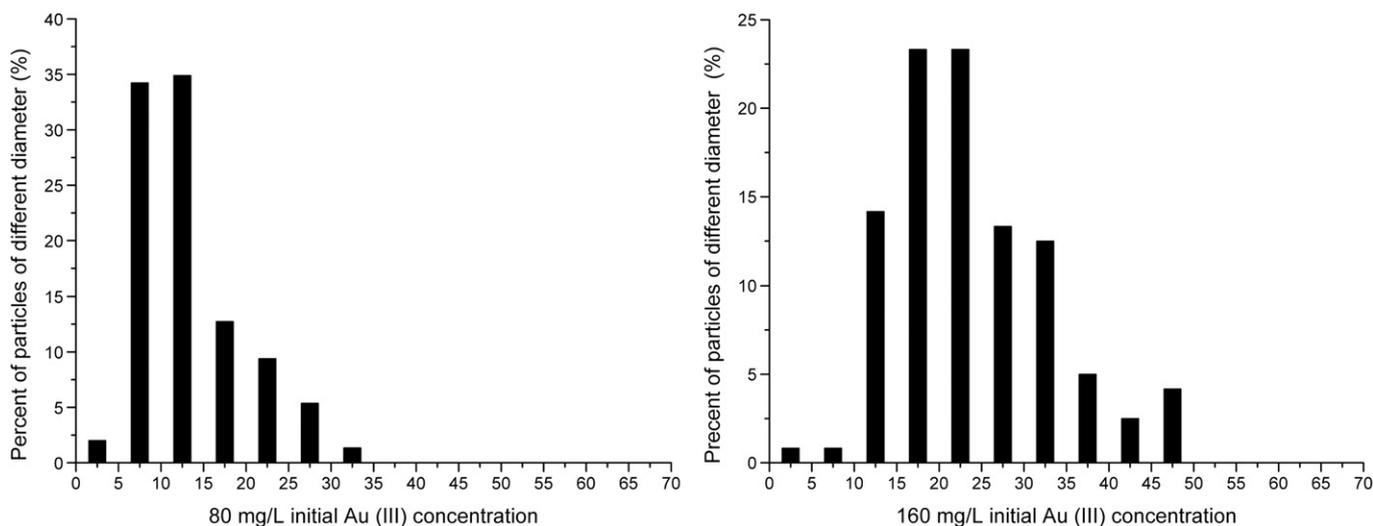
**Fig. 1.** TEM of Aurum on the *R. capsulatus* cell surface at different initial Au (III) concentration: (A) cell suspension without Au (III); (B) 40 mg/L initial Au (III) concentration; (C) 80 mg/L initial Au (III) concentration; (D) 160 mg/L initial Au (III) concentration. The inset shows a typical SAED pattern of Aurum nanoparticles.

### 3.2. Membrane-bound and intracellular bioreduction of Au (III)

To demonstrate the existence of Aurum particles and figure out the locations of them, ultrathin sectioning technique, TEM and SAED were used to analyze the cells after incubation. Representative TEM images of *R. capsulatus* cells were shown in Fig. 1. SAED pattern and the observed ring pattern were consistent with the reference to face centered cubic (FCC) gold, indicating that the biogenic nanoparticles seen in the TEM images are crystalline Aurum. With the increase of the initial  $\text{HAuCl}_4$  concentration, the amount of Aurum nanoparticles bound on cell wall increased. Meantime the sizes of particles also had the trends to become larger with higher initial Au (III) concentration, as shown in Fig. 2. At the 80 mg/L initial Au (III) concentration the sizes of the most populated particles on cell wall were around

10 nm, however the ones at 160 mg/L were near 20 nm and the largest reached 48 nm. What's more, comparing the Fig. 1C and D the aggregation of Aurum nanoparticles increased with higher Au (III) concentration.

Majority of the particles deposited were found on plasma membrane. Carotenoid and proteins of electron transfer chain are embedded in plasma membrane, which could take main responsibility for depositing Aurum on plasma membrane. Carotenoid with antioxidant properties plays important role in protecting organisms against peroxidative reactions [12,13]. The rapid reduction of Au (III) by carotenoid was observed [10]. Because the liberation of electron from organic matter is complex [14], the proteins of electron transfer chain could function tardily. As the electron donor, lactate offers electrons to proteins of electron transfer chain [15]. Then these proteins have the ability to reduce Au (III) to Au (0).



**Fig. 2.** Distribution of particles of different sizes at different initial Au (III) concentration: 80 mg/L (left); 160 mg/L (right).

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