Biosynthesis of Au Nanoparticles by a Marine Bacterium and Enhancing Their Catalytic Activity through Metal Ions and Metal Oxides

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The authors report that a marine Shewanella sp. CNZ-1 is capable of producing Au NPs under various conditions. Results showed that initial concentration of Au(III), pH values and electron donors affected nucleation of Au NPs by CNZ-1, resulting in different apparent color of the as-obtained bio-Au NPs, which were further characterized by UV-Vis, TEM, XRD, and XPS analyses. Mechanism studies revealed that Au(III) was first reduced to Au(I) and eventually reduced to EPS-coated Au0 NPs. FTIR and FEEM analyses revealed that some amides and humic acid-like matters were involved in the production of bio-Au NPs through CNZ-1 cells. In addition, the authors also found that the catalytic activity of bio-Au NPs for 4-nitrophenol (4-NP) reduction could be enhanced by various metal ions (Ca2+, Cu2+, Co2+, Fe2+, Fe3+, Ni2+, Sr2+, and Cr3+) and metal oxides (Fe3O4, Al2O3, and SiO2), which is beneficial for their further practical application. The maximum zero-order rate constant $k_1$ and first-order rate constant $k_2$ of all metal ions/oxides supplemented systems can reach 99.65 mg/(L.min) and 2.419 min$^{-1}$, which are 11.3- and 12.6-fold higher than that of control systems, respectively. © 2018 American Institute of Chemical Engineers Biotechnol. Prog., 35: e2727, 2019.

Keywords: biosynthesis, marine bacterium, catalytic reduction, metal ions, metal oxides

Introduction

Noble metal nanoparticles (NPs) have been widely used in chemocatalysis, sensors, biomedicine, hydrogen storage, and fuel cells, and so forth. Noble metals certainly will be in short supply in the near future because of their widespread application and limited reserves. Therefore, to develop noble metal recovery technologies has drawn considerable attention in recent years. Previous studies reported that noble metal NPs recovery through conventional approaches may cause great environmental concern, because they are generally accompanied by high pressures, high temperatures, and the usage of toxic chemicals. Therefore, the environmentally friendly approaches should be developed further for potential large-scale application in noble metal recovery. Recent studies found that various bacteria, including Desulfovibrio, Pseudomonas, Shewanella, Cupriavidus, Paracoccus, and Escherichia genus and so forth, were able to reduce noble metal-containing precursors to inorganic NPs environmentally friendly under mild conditions. Moreover, biomass can recover ppm concentrations of noble metal, which is often below the economic threshold of traditional recovery methods. On basis of these advantages, microbial recovery of noble metals is emerging as a clean alternative to traditional physical and chemical reclaiming treatments.

Gold (Au) is one of the noble metals. In the last few years, Au NPs have received increasing attention because of their unique electrical, optical, and photothermal properties. So far, a number of references reported many biological methods for preparing Au NPs through microbes and the as-prepared biogenic Au NPs (bio-Au NPs) could be mainly used in biomedicine (antimicrobial, drug delivery and cancer treatment, medical imaging and so on) and heterogeneous catalysis (dehalogenation, benzyl alcohol oxidation, propylene epoxidation, 4-NP reduction, organic synthesis and so on). After a comprehensive analysis of previous studies, we believe that there are at least two aspects that need to be further investigated: first, compared to terrestrial microbes, marine bacteria can better adapt to adverse environments (elevated or low temperature, alkaline or acidic pH, high pressure, or high salt concentration and so on) because they are naturally exposed to such unfavorable conditions. However, in spite of great potential, few researches focused on the interaction between marine microbes and Au ions. For marine bacteria, to the best of our knowledge, limited species that capable of producing Au NPs were ever reported. Therefore, more marine bacteria that capable of reducing precious metal ions should be developed and their reduction mechanism needs to be investigated. Secondly, the above mentioned bio-Au NPs are likely to be applied in heterogeneous catalysis. However, the catalytic activity of those Au NPs is much different. The bio-Au NPs
produced in different conditions (microbes, pH values, concentrations of initial Au(III) ions and so on) attain various sizes and shapes,21,22 and the phase and morphology differences in Au NPs often lead to variations in physical and chemical properties.25 It seemed that the catalytic activity of biogenic Au NPs was weaker than those Au NPs that synthesized by chemical methods. Therefore, to improve the prospects of biogenic Au NPs, universal strategies for enhancing the catalytic activity of biogenic Au NPs need to be developed.

In our previous study, a marine electrochemically active bacterium *Shewanella* sp. CNZ-1 was isolated from the sediment of Bohai Straits (China).26 Strain CNZ-1 possesses electrochemical activity and was able to extracellularly reduce various N-substituted aromatic compounds.26 Thus, in view of our previous and other studies, we speculated that CNZ-1, as a member of genus *Shewanella*, could reduce metals and metalloids.26,27 In their published work, the authors found strain CNZ-1 could reduce Pd(II) to Pd0 rapidly.28 In the present study, we studied the capacity of CNZ-1 for Au(III) reduction. Moreover, we also investigated the effects of various metal ions (Ca2+, Cu2+, Co2+, Fe2+, Fe3+, Ni2+, Sr2+, and Cr3+) and metal oxides (Fe3O4, Al2O3, and SiO2) on catalytic performance of bio-Au NPs for 4-nitrophenol reduction, which is beneficial for further practical application of bio-Au NPs.

**Materials and Methods**

**Chemicals**

H4AuCl4, used to prepare Au(III) solution, was purchased from Shanghai Macklin Biochemical Co., Ltd (China). 4-nitrophenol (4-NP) used in this study was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). All other reagents used in this study were of the highest analytical grade.

**Bacterial strain and growth conditions**

*Shewanella* sp. CNZ-1 was isolated from the marine sediment (N 38° 30.29', E 121° 14.10', China) and stored in our lab.26 Luria-Bertani broth (LB) contains (g/L): 10 Peptone, 5 Yeast Extract, 10 NaCl (pH 7.2). The mineral salt medium (MSM) used in this study contains (g/L): 1 NH4Cl, 0.8 Na2HPO4, 0.2 KH2PO4, 0.2 MgCl2-7H2O, 0.1 CaCl2-2H2O, 20 NaCl (pH 7.2).

**Preparation of bio-Au NPs**

Bio-Au NPs were prepared by strain CNZ-1 in the presence of Au(III) ions according to the following steps. First, CNZ-1 cells were cultured for 12 h in 100 mL LB (v/v = 1%) in a rotary incubator shaker (150 rpm, 30°C). Secondly, the cell pellet was harvested by centrifugation (10,000 rpm, 5 min), washed with MSM (a part of subsequent experimental system) twice and then added into the experimental system at a final dry cell weight of 0.5 g cell/L (OD590 = 1.0). The previously mentioned experimental system contained 100 mL deoxygenated sterile MSM, 2 g/L carbon source and 35 mg/L Au(III) in a 135 mL serum bottle. Finally, the cells and Au NPs mixture were collected by centrifugation (10,000 rpm, 5 min) after 12 h of co-incubation. The mixture was treated with ultrasonic processing (30 min), washed with deionized water and dried in a vacuum freeze dryer for 24 h. The obtained dry powder is bio-Au NPs. In addition, effects of various influence factors on Au(III) reduction by CNZ-1, including electron donor (Sodium lactate, glucose, sodium formate, potassium acetate, fructose, lactose), pH (3–10, adjusted by 1 M HCl and NaOH) and initial concentration of Au(III) (17.5–105 mg/L) were studied systematically. Samples were taken simultaneously at 12 h with a sterile needle and a syringe for the analysis of Au species.

**Characterization of bio-Au NPs**

The bio-Au NPs were characterized by ultraviolet-visible spectrometry (UV-Vis, PerkinElmer Lambda 365), transmission electron microscopy (TEM, JEM-1400, Japan), Fourier transform infrared spectroscopy (FTIR, Jasco FT/IR-4100, Japan), X-ray diffraction patterns (XRD, BRUKER D8 ADVANCE, Germany) and X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, England). For TEM analysis, samples were prepared as Wu et al. described.29 For XRD, XPS, and FTIR analyses, CNZ-1 cells were exposed to 35 mg/L Au(III) for 12 h and then separated by centrifugation at 10,000 rpm at 4°C for 5 min. The pellets were washed by deionized water and dried in a vacuum freeze dryer. The supernatant was used for subsequent UV-Vis analysis.

**Extracellular polymeric substances extraction and analysis**

A modified heat extraction method was used to extract the extracellular polymeric substances (EPS) from CNZ-1 cells.30 After 12 h of incubation with MSM in the presence of 2 g/L sodium lactate, CNZ-1 cells (OD600 = 1) were first dewatered by centrifugation in a 50 mL tube (4000 rpm, 5 min). The cell pellet in the tube was then resuspended into 15 mL MSM solution. The mixture was then diluted with the NaCl (0.85%) solution to its original volume of 200 mL. The NaCl solution for dilution was preheated to ~50°C to ensure that the cell suspension reached an immediate warm temperature of 40°C. Without any delay, the cell suspension was then heated at 40°C in a water bath for 60 min, followed by centrifugation at 4000 rpm for 15 min. The organic matter in the supernatant was regarded as the EPS of CNZ-1 cells. The cell pellet was defined as EPS-lacking cells. Both of them were employed further for Au(III) reduction in above mentioned experimental system.

Further, fluorescence excitation emission matrix (FEEM) analyses of EPS that obtained in different reaction systems were performed with a fluorescence spectrophotometer (Hitachi F-4000, Japan). The scan rate and excitation/emission slit band width were 240 nm/min and 2.5 nm, respectively. The scanning field was set as emission spectra from 300 nm to 550 nm and excitation from 220 nm to 400 nm and the contour figures were presented with interval of 0.1. Fluorescence regional integration was used for qualitative analysis of EPS components.31

**The catalytic performance of bio-Au mixture under different conditions**

The catalytic performance of bio-Au mixture was evaluated in a model reaction (4-NP reduction). In brief, the electron donor and electron acceptor were NaBH4 and 4-NP, respectively. The total volume of the reaction mixture was 3 mL, which contained 0–133.3 mg/L metal oxides and metal ions, 8.333–66.66 mg/L bio-Au mixture, 1 g/L NaBH4, and 150 mg/L 4-NP in ultrapure water. Control assays with only CNZ-1 cell powders (drying in a vacuum freeze drier for 24 h) supplemented and without catalysts supplemented were also performed. The mixtures were
incubated at room temperature. Each test was conducted three times and the mean was used for further analyses.

**Analytical methods**

The concentration of Au(III) was determined by inductively coupled plasma source mass spectrometer (ICP-MS). The concentrations of cells and 4-NP were determined by UV-Vis spectrophotometry at 600 nm and 400 nm, respectively. For analyzing reduction products of 4-NP, the supernatant was filtered using 0.22 μm filter membrane and then directly injected into the mass spectrometer. The mobile phase was methanol at 1.0 mL/min.

A zero-order model was applied to describe the kinetics of bio-Au NPs mediated 4-NP reduction in the presence of various metal ions and metal oxides. The zero-order rate constant $k_1$ (mg/L min$^{-1}$) was determined by using Eq. 1.

$$C_i - C_t = k_1 t$$

A pseudo-first-order model was employed to describe the kinetics of bio-Au NPs mediated 4-NP reduction in the presence of various metal ions and metal oxides. The first-order rate constant $k_2$ (min$^{-1}$) was determined by using Eq. 2.

$$\ln\frac{C_i}{C_t} = k_2 t$$

Where $C_i$ (mg/L) and $C_t$ (mg/L) are the initial and residual 4-NP at time zero and t, respectively; t (h) is the reaction time.

**Results and Discussion**

**Au(III) reduction by Shewanella sp. CNZ-1**

The possibility of Au(III) reduction through *Shewanella* sp. CNZ-1 was first examined. After 12 h of co-incubation, the color of Au(III) solution changed from pale yellow to pinkish purple in the presence of CNZ-1 cells. UV-Vis analysis found that a broad peak at ~530 nm (SPR band) appeared, whereas no evidence of a SPR band was recorded in the control sample (Au[III] solution, 35 mg/L), indicating the formation of Au NPs in experimental systems (Figure 1a). TEM images of thin sections of CNZ-1 cells treated with Au(III) solutions were obtained to further clarify the formation of Au NPs. As can be seen in Figure 1b, Au NPs are located on the periplasm of CNZ-1, the size of bio-Au NPs is mainly around ~15 nm (Supporting Information Figure S1).

XRD pattern of the bio-Au NPs was presented in Figure 1c, the diffraction peaks at ~38°, 45° and 65° could be indexed to the (111), (200), and (220) reflections of the fcc Au (JCPDS No.04–0784) structure. In addition, other diffraction peaks of Au NPs might attributed to protein on the surface of CNZ-1 cells. XPS results show the chemical state information and electronic properties of Au NPs. After 12 h of co-incubation with CNZ-1 cells, Au(III) ions were reduced to Au(I) and Au$^0$, indicating the reaction is not a one-step reaction (Figure 1d). As shown in Figure 1d, the binding energies of Au(III) 4f7/2 was appeared at ~87.2 eV. After reduction, the binding energies of Au$^0$ 4f7/2 and Au(I) 4f7/2 were shifted to 83.1 eV and 84.2 eV, respectively, indicating Au(I) was the intermediate species before the complete reduction of Au(III). Above all, we concluded that the Au NPs were produced by CNZ-1 through the following pathway:

$$\text{Au(III)} \rightarrow \text{Au(I)} \rightarrow \text{Au}^0$$

**Effects of initial concentration of Au(III), pH and electron donor**

Effects of initial concentration of Au(III) and pH values on Au(III) reduction and nucleation of Au NPs by strain CNZ-1 in the presence of 2 g/L sodium lactate are shown in Figure 2. As shown in Figure 2a and Table S1, the concentration ratio...
of \(\text{Au}^0/\text{Au(I)}\) reduced gradually and the relative Au content on the surface of CNZ-1 cells increased from 0.02% to 0.11% as the initial concentration of \(\text{Au(III)}\) increased. In addition, XRD analyses illustrated that the \((111)\) reflection of the bio-Au NPs obtained in the system adding 34.93, 52.35, and 69.8 mg/L \(\text{Au(III)}\) was more obvious than that obtained in the reaction systems that supplemented with 17.45 and 104.7 mg/L \(\text{Au(III)}\) (Figure 2b), respectively. XRD results were consistent with the color variation in different reaction systems (data not shown). As the initial concentration of \(\text{Au(III)}\) was increased from 17.45 to 104.7 mg/L, the nucleation of Au NPs were changed correspondingly. When the initial concentration of \(\text{Au(III)}\) was 17.45 mg/L, all \(\text{Au(III)}\) ions were reduce to \(\text{Au}^0\) but nucleation of \(\text{Au}^0\) was not obvious, maybe because CNZ-1 cells offered excess nucleation sites for \(\text{Au(III)}\) absorption. When the initial concentration of \(\text{Au(III)}\) was 104.7 mg/L, most \(\text{Au(III)}\) ions were reduce to \(\text{Au(I)}\) ions instead of \(\text{Au}^0\), resulting in decreased Au NPs nucleation.\(^{33}\)

Therefore, the authors concluded that the relative content of biomass and \(\text{Au(III)}\) in system will affect the nucleation of Au NPs by strain CNZ-1. In the present study, 34.95 mg/L initial \(\text{Au(III)}\) was selected for the following experiments.

As shown in Figure 2c, the concentration ratio of \(\text{Au}^0/\text{Au(I)}\) increased at first when the pH values changed from 3 to 7 and then slightly decreased when the pH values were altered from 9 to 10. Moreover, XPS analyses showed that the relative Au content on the surface of CNZ-1 cells was 0.02, 0.02, 0.05, 0.08, and 0.06% at pH 3, 5, 7, 9, and 10, respectively. Interestingly, the \((111)\) reflection of bio-Au NPs obtained in the systems at pH of 9 and 10 (especially at pH of 9) was very strong (Figure 2d). We speculated that alkaline condition is beneficial for formation of Au NPs through CNZ-1. To further verify our hypothesis, TEM analyses of bio-Au NPs obtained in the systems at pH of 3, 5, and 9 were carried out. As shown in Figure 1b and Supporting Information Figure S2, the amount of Au NPs in alkaline condition (pH 9) was more than that in acid (pH 3 and 5) and neutral conditions (pH 7). A possible explanation is that alkaline conditions can activate the hydroxyl/hemiaceetl/amino groups of EPS (protein, polypeptide, polysaccharide, and so on) secreted by CNZ-1. Meanwhile, EPS or specific enzymes secreted by microbes were proven to be capable of reducing high valence metal ions to corresponding zerовалent metallic states.\(^{10}\) Accordingly, the above-mentioned phenomenon might be attributed to the fact that activated hydroxyl groups have higher activity to reduce \(\text{Au(III)}\) to \(\text{Au}^0\), and more Au NPs were thus formed.\(^{10,34}\)

Another possible explanation is that pH value affects the permeability of the cell membrane and thus has an effect on Au NPs production through CNZ-1. It is remarkable that the valence of Au and nucleation of \(\text{Au}^0\) in the condition of pH 10 were much different from that in the condition of pH 9. In view of the \(\text{Au(III)}\) reduction process by CNZ-1 was very complicated, the authors speculated that excessive \(\text{OH}^-\) ions in the reaction system inhibited the activity of CNZ-1 and thus affected the reduction of \(\text{Au(III)}\) and nucleation of \(\text{Au}^0\).

Figure 3 showed that the effect of electron donor on \(\text{Au(III)}\) removal by CNZ-1 (OD\(_{600} = 1\)) in the presence of 34.5 mg/L initial \(\text{Au(III)}\) and pH of 9. Results showed that the color of reaction system with different electron donor added was much different, which could be attributed to the different size of Au NPs, causing the shift of the SPR band (Figure 3). Previous studies have indicated that NADH- or NADPH-dependent reductases are important factors in the biosynthesis of metal nanoparticles.\(^{35–37}\) Shewanella sp. are known to secrete cofactor NADH- or NADPH-dependent enzymes that may be responsible for the reduction of \(\text{Au(III)}\) to \(\text{Au(I)}\) and the subsequent formation of \(\text{Au}^0\) NPs. Given that the efficient reduction of \(\text{Au(III)}\) by CNZ-1 in the presence of lactate and formate,
lactate/formate dehydrogenase maybe necessary for above process when formate or lactate was served as the electron donor. XPS analyses revealed that both Au(I) and Au(0) could be detected on the surface of CNZ-1 cells (data not shown). UV-Vis analyses further showed that a SPR band could be found at ~550 nm when no electron donor was added in the reaction system, indicating the formation of Au NPs. These findings indicated that Au(III) ions attached on CNZ-1 cells could be reduced to Au(0) NPs through both enzymatic and nonenzymatic process. So far, many studies highlighted the importance of enzymes, such as dehydrogenase, during metal reduction by bacteria, however, limited reports focused on the involvement of nonenzymatic process in metal reduction caused by microorganisms. Thus, FTIR and FEEM analyses were carried out to investigate whether the nonenzymatic process is involved in the Au(III) reduction through CNZ-1.

**Fourier transform infrared spectroscopy analysis**

FTIR spectra can reveal possible physical and chemical interactions between Au(III) and chemical groups on the cell surface. Accordingly, we further investigated Au NPs biosynthesis process under various conditions (different pH values and initial Au(III) concentrations) using FTIR analysis. As shown in Figure 4, some peaks decreased or disappeared after treated with CNZ-1 cells for 12 h. The most obvious changes are centered at ~1540 cm\(^{-1}\) (amide II peak) and ~1250 cm\(^{-1}\) (amide III peak), attributed to N-H in plane bending and C-N stretching of amino groups of cellular proteins. Note that the appearance and disappearance of peaks related to amides were always proportional to the amount of Au(III). As shown in Figure 4a,b, the amide II and III peaks became more and more obvious as the Au(III) concentration increased, and the whole variant trend in different conditions (pH and initial concentration of Au(III)) was consistent with the XPS results (Figure 2a,c). Previous study reported that amino groups on the surface of bacteria cell were involved in Pd(II) reduction, which was proved by using an amino-modified nanomaterial. On the basis of previous studies and the present work, it was inferred that certain EPS containing functional amino groups were involved in Au NPs production through CNZ-1. That’s to say, some structural properties of CNZ-1 cells account for the formation of Au NPs, which have implications for better use of strain CNZ-1.

**EPS and FEEM analyses**

The possible non-enzymatic process that involved in biosynthesis of Au NPs was studied by EPS assays. Figure 5 showed that Au(III) reduction efficiency could be reach 25.5, 66.1, 81.6, and 92.3% at 12 h in the experimental systems supplemented with EPS, EPS-lacking cells, EPS + EPS-lacking cells and untreated cells, respectively. In addition, the Au(III) reduction efficiency was only approximately 2.7% in the system with only HAuCl\(_4\) supplemented. These results further indicated that some contents of EPS were involved in

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**Figure 3.** UV–Vis analyses of bio-Au NPs obtained in different reaction systems with various electron donors.

**Figure 4.** The FTIR spectra of bio-Au NPs obtained in different reaction systems with various initial Au(III) (a) and pH values (b).

**Figure 5.** Effect of EPS on Au(III) removal by CNZ-1.
Au(III) reduction by CNZ-1. To investigate the role of EPS during the synthesis process of Au NPs, FEEM analyses of EPS in different conditions were thus carried out. According to previous literatures, fluorophores in FEEM could be divided into six regions (Supporting Information Figure S3). FEEM analyses revealed that EPS of CNZ-1 under various stressful conditions (including various initial concentration of Au(III), pH value and electron donor) presented different fluorophores at Ex/Em = 220–400/300–550 nm, especially in regions IV, V, and VI (Figure 6). The more Au NPs produced by CNZ-1 (e.g., systems at pH of 9, 10 and systems with 87.25, 104.7 mg/L initial Au(III) supplemented), the weaker intensity of fluorophores at Ex/Em = 220–400/300–550 nm, especially in regions IV, V, and VI were detected. Combined with the results of FEEM (Figure 6), XPS (Figure 2a,c) and XRD (Figure 2b,d), we found that the amounts of Au(III) ions that reduced to Au⁰ have a strong relevance with the signal intensity of fluorophores related to concentration of humic acid substances. Thus, we speculated that the formation of Au NPs through CNZ-1 might have a correlation with humic acid substances, including fulvic acid-like, polyaromatic type humic acid-like and polycarboxylate type humic acid-like matters. Humic acid substances that are capable of adsorbing, complexing and reducing Au(III) play an important role in accumulation of metals in nature. In addition, humic acid substances also could be served as a mediator for metal reduction and electron transfer, which attribute to the electrochemical activity of their quinyl groups and/or amino groups. Tu et al. reported that the addition of humic acid substances (anthraquinone-2,6-disulphonate) could promote the reduction efficiency of Pd(II) or Pt(IV) and enhance the catalytic activities of bio-Pd/Pt NPs. On the basis of these studies, we inferred that some humic acid-like matters were involved in the formation of Au NPs, in a manner of adsorbing/reducing/

![Figure 6](image_url)

Figure 6. The FEEM analyses of EPS obtained in different reaction systems: (a–f) pH = 7, 2 g/L sodium lactate, 17.45–104.7 mg/L initial Au(III); (g–k) 34.95 mg/L initial Au(III), 2 g/L sodium lactate, pH = 3–10; (l–p) 34.95 mg/L initial Au(III), pH = 9, 2 g/L various electron donors.
Enhanced catalytic activity of bio-Au mixture using metal ions and metal oxides

As E0 for 4-nitrophenol/4-aminophenol and H3BO3/BH4⁻ is −0.76 and −1.33 V vs. SHE, respectively, this reaction could not proceed without additional catalyst.50 Thus, this reaction was used to test the catalytic performance of bio-Au mixture (Au(I) and Au0 NPs) in the present study. Results showed that little reduction occurred in control assays during 30 min (<3%) and 4-NP was reduced completely in 30 min with the addition of bio-Au mixture. Zero-order and pseudo-first-order models were applied to describe the kinetics of 4-NP reduction. The kinetic parameters of the zero-order and pseudo-first-order equations were listed in Table S2. In fact, the bio-Au mixture mediated 4-NP reduction process is affected by various factors, including initial concentrations of Au(III) and cells, size of bio-Au NPs, component of EPS and so on. The catalytic performance of the bio-Au mixture obtained at pH of 5 (with 34.93 mg/L initial Au(III) and 2 g/L sodium lactate supplemented) was the best in all conditions (Table S2). A possible explanation is that the proper size of bio-Au NPs obtained at pH of 5 can maximize their catalytic activity, since a previous study found that the size of Au NPs was associated with their catalytic activity.51 Moreover, it is reported that peptide-grafted Au NPs had a regular colorimetric response to metal ions, because metal ions can affect the charge distribution of the peptide interface, which may have a significant influence on the catalytic activity of bio-Au NPs.52 According to the FEEM results, the EPS of CNZ-1 contain some peptide-like matters. We speculated coexisting Au(III) directly or/and assisting CNZ-1 cells to reduce Au(III) to Au(I) and Au0.

Effects of metal ion and metal oxide on catalytic performance of bio-Au mixture

The maximum k1 and k2 values were 49.68 mg/(L·min) and 0.7912 min⁻¹, respectively. These results indicated that the catalytic performance of bio-Au mixture was related to their producing conditions. The reduction product was identified as 4-aminophenol (data not shown). In addition, kinetic analyses found that the experimental data were well explained by the zero-order model in most reaction systems (pH = 3–10, initial concentration of Au(III) = 17.45–68.8 mg/L, R² > 0.93). Only when the initial concentration of Au(III) was 87.25 or 104.7 mg/L (pH = 7), the experimental data were well explained by the pseudo-first-order model (R² > 0.95). In addition, the catalytic activity of these bio-Au mixture was comparable to or even better than those of previously reported Au NPs synthesized by chemical methods.53 These findings indicated that bio-Au mixture obtained in this study have potential application in heterogeneous catalysis, at least for 4-NP reduction.

Effects of metal ion and metal oxide on catalytic performance of bio-Au mixture

The effects of metal ion and metal oxide on catalytic performance of bio-Au mixture were investigated in detail and the results were listed in Tables S3 and S4. Interestingly, the authors found that the catalytic performance of bio-Au mixture for 4-NP reduction could be enhanced by adding some metal ions, including Cu²⁺, Co²⁺, Fe²⁺, Fe³⁺, Ni²⁺, Ca²⁺, Sr²⁺, and Cr³⁺ (Table S3). In the presence of 3.333 mg/L Cu²⁺, the k1 and k2 values of experimental groups were 11.3- and 12.6-fold, respectively, compared to that of control group. Moreau et al.54 reported that the biogenic metal NPs are usually coated by peptides or proteins. These matters are termed as protein corona, which is thought to be important in shaping the surface charges and properties of the coated metal NPs.54 Moreover, it is reported that peptide-grafted Au NPs had a regular colorimetric response to metal ions, because metal ions can affect the charge distribution of the peptide interface, which may have a significant influence on the catalytic activity of bio-Au NPs.55 According to the FEEM results, the EPS of CNZ-1 contain some peptide-like matters. We speculated

![Figure 7](image-url)

Figure 7. Plots of Ct−C0 vs. time (a, b, c) and ln(Ct/C0) vs. time (d, e, f) for the reduction of 4-NP by NaBH4 in the presence of Fe3O4 (a, d), Al2O3 (b, e), and SiO2 (c, f).
that the addition of metal ions might change the electric field distribution around the Au NPs and thus affect the catalytic performance of bio-Au NPs.\textsuperscript{56} Moreover, a recent study showed that special structure of alloy could improve the electron transport and create active sites with high electron density at the surface of noble metal.\textsuperscript{57} Accordingly, the enhanced reduction of 4-NP by bio-Au mixture in the presence of metal ions could be explained as follows: these metal ions promoted the reaction as a Lewis acid or the possible formation of alloy perturbs the reactivity of the Au sites.\textsuperscript{58,59}

Besides, the catalytic activity of bio-Au mixture for 4-NP reduction could also be enhanced by some metal oxides, including Fe\textsubscript{3}O\textsubscript{4}, Al\textsubscript{2}O\textsubscript{3}, and SiO\textsubscript{2} (Table S4 and Figure 7). The catalytic activity for bacterial debrís without Au NPs in the absence/presence of metal ions/oxides were investigated. The results showed that trace 4-NP (<1\%) was reduced in the absence of all tested metal oxides (~133 mg/L) and little 4-NP (<3\%) was reduced in the presence of Fe\textsubscript{3}O\textsubscript{4}, Al\textsubscript{2}O\textsubscript{3}, SiO\textsubscript{2} and TiO\textsubscript{2} (Data not shown). In addition, TiO\textsubscript{2}, Fe\textsubscript{3}O\textsubscript{4}, and Fe\textsubscript{2}O\textsubscript{3} have no effect or slight negative effect on catalytic activity of bio-Au mixture for 4-NP reduction (Table S4). Kinetic analyses showed that \(k_1\) and \(k_2\) values of Fe\textsubscript{3}O\textsubscript{4}, Al\textsubscript{2}O\textsubscript{3}, and SiO\textsubscript{2} supplemental systems were approximately 1.14–2.26 fold and 1.21–2.35 fold, respectively, compared to that of control system (Table S4, Figure 7). Previous studies reported that multiple interfacial reactions can take place between nanopartials and metal oxides after their combination (such as enhanced electron transport). In addition, metal oxides were ever used to enhance lithium storage properties and photocatalytic performance of various nanopartials.\textsuperscript{60–62} In this study, the authors first proved that metal oxides could enhance the catalytic performance of biogenic Au NPs. Our results showed that the bio-Au mixture mediated 4-NP reduction could be enhanced in dose-dependent manner of metal oxides and suitable concentration (33.33 mg/L in the present study) should be selected (Table S4). These findings will benefit future practical application of bio-Au mixture, at least in heterogeneous catalytic field.

**Conclusions**

In this study, the authors have demonstrated that a marine bacterium _Shewanella_ sp. CNZ-1 is able to produce Au NPs under various conditions. This is the first study to investigate the possibility of Au NPs synthesis through halobiotic _Shewanella_ sp. UV-Vis, TEM, XRD, and XPS analyses found that initial Au(III) concentration, pH values and electron donors could affect Au(III) reduction and nucleation of Au NPs by CNZ-1, resulting in different apparent color and size of bio-Au NPs. Mechanism studies showed that Au(III) was first reduced to Au(I) and eventually formed EPS-coated Au NPs, and some amides and humic acid-like matters were involved in the synthesis of Au NPs through strain CNZ-1. In addition, the catalytic activity of bio-Au mixture for 4-NP reduction could be enhanced by some metal ions (Ca\textsuperscript{2+}, Cu\textsuperscript{2+}, Co\textsuperscript{2+}, Fe\textsuperscript{2+}, Fe\textsuperscript{3+}, Ni\textsuperscript{2+}, Sr\textsuperscript{2+}, and Cr\textsuperscript{3+}) and metal oxides (Fe\textsubscript{2}O\textsubscript{3}, Al\textsubscript{2}O\textsubscript{3}, and SiO\textsubscript{2}), which is beneficial for their future practical application. The maximum activity parameters zero-order rate constant \(k_1\) and first-order rate constant \(k_2\) of all metal ions/oxides supplemented systems could reach 99.65 mg/(L·min) and 2.419 min\(^{-1}\), which is 11.3- and 12.6-fold higher than that of control systems, respectively.

**Authors’ contributions**

Haikun Zhang and Xiaoke Hu contributed to the conception of the study. Haikun Zhang performed the data analyses and wrote the manuscript. Xiaoke Hu helped perform the analysis with constructive discussions.

**Competing interests**

We declare we have no competing interests.

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