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Removal of Au(III) from Aqueous Au(III) Solution Using Microbial Cells by Biosorption and Biomineralization

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Abstract

The demand for gold has increased in the medical and industrial fields. Therefore, recycling this element has become essential. Although gold recovery using microbes has been investigated, there is a dearth of these studies on identifying the species that have a high gold recovering ability. Herein, gold (III) removal by microbial cells was investigated to obtain basic information on gold (III) removal from aqueous systems by biosorption and biomineralization. High amounts of gold were removed from the solution containing hydrogen tetrachloroaurate (III) by the tested microbial species, which included bacteria, fungi and yeasts. However, relatively less gold was recovered by biosorption using gram-positive bacteria, fungi, and yeasts than that by gram-negative bacteria. Therefore, we first examined gold (III) removal by biosorption and biomineralization by Pseudomonas saccharophila, which was able to remove the largest amounts of gold (III). Incubation time and other factors affecting gold removal were then examined. P. saccharophila removed about half the amount of gold (III) by biosorption and the remaining half by biomineralization.

Keywords

Gold (III) Biosorption, Gold (0) Biomineralization, Microorganism, *Peudomonas saccharophila*

1. Introduction

The demand for gold has significantly increased because of its increasing use in the electrical industry and the development of gold-containing drugs [1]. Therefore, recycling this valuable resource has become a subject of great interest. Several researchers have investigated gold recovery using microbial cells, such as bacteria [2], fungi [3] [4] [5], yeasts [6], and algae [7] [8]. However, there is little information on the species of microorganisms that have a high gold adsorbing ability.

We previously reported that several microorganisms adsorb gold, and screened resting 75 microbial strains (19 actinomycetes, 25 bacteria, 17 fungi, and 14 yeasts) from a hydrogen tetrachloroaurate (III)-containing solution [9]. Hydrogen tetrachloroaurate (III) is used for medical and ceramic materials. Of the tested microorganisms, some gram-negative bacteria showed gold-adsorption ability. These microorganisms adsorbed over 330 mol gold per gram of microbial cells (dry wt.) from the solution containing hydrogen tetrachloroaurate (III) within 1 h. The gold adsorbed from hydrogen tetrachloroaurate (III) solution by gramnegative bacteria was higher than that adsorbed by gram-positive bacteria, actinomycetes, fungi, and yeasts. These results are in contrast to those reported for the adsorption of the amount of lithium [10], cadmium [11], uranium [12], thorium [13], and rare earth metals [14], these were adsorbed in higher amounts by gram-positive bacteria compared to the gram-negative bacteria, fungi, and yeasts. The results show that gram-positive bacteria can adsorb a large amount of positively-charged metal ions, while gram-negative bacteria can adsorb a large amount of negatively-charged complex ions [9]-[14]. Gold (III) exists as a negatively charged-complex ion in an acidic solution. The negative charge of the gram-positive bacterial cell surface is higher than that of the gram-negative bacteria, because teichoic acid levels are higher in the former at a neutral pH [15] [16] [17]. In other words, the positive charge of the gram-negative bacterial cell surface is higher than that of the gram-positive bacterial cell surface. Accordingly, negatively-charged gold complex ions bond more strongly on the positively-charged gram-negative bacterial cell surface [9].

We investigated the effects of pH, external gold concentration, cell amount, and gold contact time in *Pseudomonas maltophilia*, which adsorbs large amounts of gold from a hydrogen tetrachloroaurate (III) containing solution [9].

In this study, the investigation was performed to improve gold removal by biosorption and biomineralization from aqueous systems using microbial cells.

2. Material and Methods

2.1. Culture of Microorganisms

The strains used in this research were generously donated by the IAM Culture Collection, Center for Cellular and Molecular Research, the Institute of Molecular and Cellular Biosciences, the University of Tokyo (IAM), the Faculty of Engineering, Hiroshima University (HUT), and the Faculty of Agriculture, Hokkaido University (AHU). All chemicals (guaranteed reagents) used in this study were obtained from Nacalai Tesque (Kyoto, Japan).

The bacterial culture medium contained 3 g/L meat extract, 5 g/L peptone, and 5 g/L NaCl in deionized water [9]-[14]. The medium for growing actinomy-

cetes, fungi, and yeasts contained 4 g/L yeast extract, 10 g/L malt extract, and 4 g/L glucose in deionized water with pH 7.1 (for actinomycetes) and pH 5.7 (for fungi and yeasts) [9]-[14]. The microorganisms were maintained on agar slants and grown in 300 mL of the medium in a 500-mL flask with continuous shaking (120 rpm) for 72 h at 30°C. Cells were collected by centrifugation (for bacteria and yeasts) at 15,000 rpm for 3 times, or by filtration through a filter paper (No. 2, Advantec Co. Ltd., Tokyo, Japan) (for actinomycetes and fungi), which is washed thoroughly with deionized water, and then used in gold removal experiments.

2.2. Gold (III) Removal Experiment

Unless otherwise stated, the removal experiments were conducted as follows. Resting microbial cells [15 mg dry weight basis for tetrachloroaurate (III)] were suspended in 100 mL solution containing 50 mg/L (254 µM) gold (pH 3.0) containing hydrogen tetrachloroaurate (III). The suspension was shaken for 72 h at 30°C. The resting microbial cells were then removed by filtration through a membrane filter (0.2 µm pore size). The gold removed by the cells was determined by measuring the gold content in the filtrate with an atomic absorption analysis quantometer (AA-6300, Shimadzu Corporation, Kyoto, Japan). Absorption spectrometry analysis of the filtrate of the gold removal using varying P. saccharophila IAM1504 amounts was measured by UV and visible spectrophotometer (V-650; JASCO Corporation, Tokyo, Japan) at wavelengths ranging from 200 - 870 nm. P. saccarophia and C. krusei cell surfaces were observed via scanning electron microscopy (SEM) and x-ray fluorescence (XRF) analysis (SEM-EDX S-4300, Hitachi High-Tech Corporation, Tokyo, Japan). Gold reduced by each microbial cell was frozen using a freeze dryer (FDU-830, EYELA Corporation, Tokyo, Japan) for 20 h in vacuo. The samples after deposition in vacuo for 24 h were observed SEM and XRF.

2.2.1. Screening of Microorganisms for Gold (III) Removal from the Solution for 72 h

Resting cells (15 mg on a dry wt. basis) were suspended in 100 mL solution (pH 3.0) containing hydrogen tetrachloroaurate (III) (254 μ M, pH 3.0) for 72 h at 30 °C.

2.2.2. Gold Removal as a Function of Time Using *P. saccharophila* IAM1504

Resting cells (15 mg on a dry wt. basis) were suspended in 100 mL solution (pH 3.0) containing hydrogen tetrachloroaurate (III) (254 μ M, pH 3.0) for tenures varying from 5 min to 68 h at 30 °C.

2.2.3. Effect of pH on Gold (III) Removal Using P. saccharophila IAM1504

Resting cells (15 mg on a dry wt. basis) were suspended in a 100 mL solution (pH from 1 to 5) containing hydrogen tetrachloroaurate (III) (254 μ M) for 1 or 72 h at 30 °C.

2.2.4. Effect of Cell Amount on Gold (III) Removal Using *P. saccharophila* IAM1504

Resting cells (from 5 to 23 mg on a dry wt. basis) were suspended in a 100 mL solution (pH 4.0) containing hydrogen tetrachloroaurate (III) (254 μ M) for 1 h or 72 h at 30 °C.

2.2.5. Effect of Gold (III) Concentration on Gold (III) Removal Using *P. saccharophila* IAM1504

Resting cells (15 mg on a dry wt. basis) were suspended in a 100 mL solution (pH 3.0) containing 0 mg/L, 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, or 250 mg/L gold (III) as hydrogen tetrachloroaurate (III) (pH 4.0) for 1 h or 72 h at 30°C.

3. Results and Discussion

3.1. Microorganim Screening for Gold (III) Removal from the Solution for 72 h

To determine the ability of different microbial cells to remove a larger amount of gold (III), 48 microorganism strains (5 actinomycetes, 19 bacteria, 13 fungi, and 11 yeasts) were screened. Gram-negative bacteria, such as *P. maltophilia*, have been reported to remove a large amount of gold (III) from an aqueous hydrogen tetrachloroaurate (III) solution (pH3) in 1 h at 30°C by biosorption [9]. The highest adsorption of gold (III) occurred at pH 3.0 using *P. maltophilia* [9]. Therefore, the pH of the solution was adjusted to 3.0 in this screening process, additionally, we identified some microorganisms that removed a larger amount of gold (III) and reducted the gold in solution to gold (0) in the preliminary experiment.

The ability of the microbial cells to remove gold significantly varied (**Tables 1-5**). Of the tested microorganisms, high gold removal ability was observed in all microorganisms. *Nocardia erythropolis* IAM1399 (gram-positive bacteria), *Eschelichia coli* IAM1264, *P. maltophilia* IAM1554, and *P. saccharophila* IAM1504 (gram-negative bacteria), *Aspergillus niger* IAM2534, *Chaetomium globosum* IAM9272 and IAM9427 (fungi), and *Candida. utilis* IAM4220, *Pichia farinosa* IAM12223, and *Saccharomyces cerevisiae* AHU3818 (yeasts) removed gold > 1200 μmol/g dry cell weight in 72 h at 30°C. In the results of our previous study [9], the amount of gold (III) removed by gram-negative bacteria was higher than that by the gram-positive bacteria, actinomycetes, fungi, and yeasts. The maximum amount of gold (III) removal was 360 μmol/g dry cell weight in 1 h at 30°C. Therefore, many microorganisms were able to remove large amounts of gold (III) after a long incubation time.

As these results were contact time-dependent, we hypothesized that the reaction mechanisms may be different. The solution was almost colorless for 1 h, however, it changed to dark colors like -violet or -dark green after longer removal time. Gold removal within 1 h likely occurred by biosorption, although longer incubation time (72 h) caused the reduction of gold (III) to zero-valent gold.

Table 1. Removal of gold by gram-poitive bacteria.

Species	Au removed (μmo/g dry wt. cells)
Acinetobacter cireus IAM12341	920
A. nicotianae IAM12342	714
Bacillus licheniformis IAM11054	1092
B. megaterium IAM1166	823
B. subtilis IAM1026	740
B. subtilis IAM11060	1106
B. subtilis IAM1633	1145
Brevibacterium helovolum IAM1637	876
Corynebacterium equi IAM1038	1186
C. gutamicum IAM12435	1185
Deinococcus proteolyticus IAM12141	1180
Micrococcus luteus IAM1056	1136
Nocardia erythropolis IAM1399	1340

Table 2. Removal of gold by gram-negative bacteria.

Species	Au removed (µmo/g dry wt. cells)
Citrobacter freundii IAM12471	941
Escherichia coli IAM1264	1230
Pseudomonas aureofaciens IAM12353	1000
Pseudomonas maltophilia IAM1554	1259
P. putida IAM1506	1104
P. saccharophilia IAM1504	1418

Table 3. Removal of gold by actinomycetes.

Species	Au removed (μmo/g dry wt. cells)
Streptomyces albogriseolus HUT6045	1188
S. albus HUT6047	849
S. griseoflavus HUT6153	670
S. hiroshimensis HUT6033	662
S. viridochromogenes HUT6030	991

Table 4. Removal of gold by fungi.

Species	Au removed (μmo/g dry wt. cells)
Aspergillus niger IAM2093	1085
A. nigerIAM2094	1193
A. niger IAM2534	1251
A. niger IAM3020	925
A. niger AHU7120	1000
A. niger AHU7296	888
A. niger var Tieghem IAM2086	920
A. niger var Tieghem var awamori IAM13839	1016
Chaetomium globosum AHU9272	1221
C. globosum AHU9427	1331
Fusarium oxysporum IAM5009	920
Giberella fujikuroi AHU9078	731
Rhizopus japonicus IAM6002	987

Table 5. Removal of gold by yeasts.

Species	Au removed (μmo/g dry wt. cells)
Candida krusei AHU3993	1335
C. utilis AHU3210	1193
C. utilis IAM4220	1251
Cryptococcus albidus AHU3812	925
C. laurentii AHU3671	1000
Debaryomyces hansenii AHU3759	888
Hansenula anomala AHU3702	920
H. saturnus AHU3003	1016
Pichia farinosa IAM12223	1221
Saccharomyces cerevisiae AHU3818	1331
S. cerevisiae IAM4512	920
S. uvarum AHU3978	731
Torulopsis aeria AHU3398	987

Many positively-charged metal ions can be removed at the neutral pH using gram-positive bacteria and actinomycetes [10] [11] [12] [13] [14]. Negatively-charged gold (III) ions can be removed at an acidic pH using gram-negative bacteria [9] through biosorption. However, all metal ions tested can be removed in small amounts using yeasts and fungi [9]-[14]. Therefore, we investigated the removal of gold (III) using *P. saccharophila* IAM1504 in detail because this microorganism removed the largest amount of gold (III) among the tested microorganisms by biomineralization.

3.2. Gold Removal as a Function of Time Using *P. saccarophila* IAM1504

Gold (III) removal as a function of time using *P. saccharophila* IAM1504 was examined (**Figure 1**); the amount of gold removed increased with incubation time. Importantly, gold removal reached two equilibria. The first equilibrium state was at approximately 6 h, and likely occurred by biosorption. Following this, the amount of gold removed increased again, and the solution color became darker, indicating biomineralization. The amount of gold removed using *P. saccharophila* IAM1504 by biosorption was relatively large [9], additionally the amount removed by biomineralization was much larger than biosorption.

3.3. Effect of pH on Gold (III) Removal from Aqueous Gold (III) Using *P. saccharophila* Cells

Gold (III) removal by *P. sacharophila* cells was significantly affected by pH (**Figure 2**). The maximum amount of gold removal occurred at pH 3.0 (for 1 h) or pH 3.5 (for 72 h).

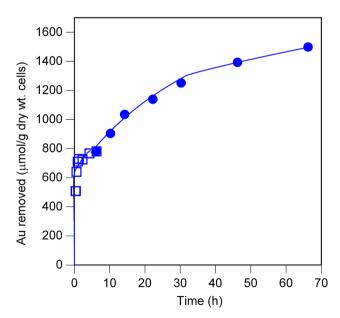


Figure 1. Gold (III) removed by *P. saccharophila* cells with the passage of time. Squares: within 6 h (biosorption); circles: contact for >6 h (biomineralization).

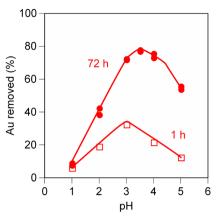


Figure 2. Effect of pH on gold (III) on the removal of gold (III) by *P. saccharophila* cells. Squares: for 1 h (biosorption); circles: contact for 72 h (biomineralization).

These results suggest that longer incubation time may change the reaction mechanism responsible for gold removal. The solution was nearly colorless after 1 h. However, the color changed to violet, green during the 72 h incubation period. Owing to the tetrachloroaurate ion having a negative charge, gold (III) can be effectively removed at pH 3 via biosorption [9]. It can also be reduced to atomic gold (0) by the activity of reductase in the presence of NADH [18] via biomineralization. Reduction occurred as shown in the following equation:

$$2Au^{3+} + 3NADH \rightarrow 2Au + 3NAD^{+} + 3H^{+}$$

The equilibrium in an acidic solution is driven to the left; thus, suitable pH changed from 3.0 to 3.5.

3.4. Effect of Cell Amounts on gold (III) Removal from Aqueous Gold (III) Using *P. saccharophila* Cells

The amount of gold (III) removed (µmol/g dry wt. cells) by *P. saccharophila* cells decreased slightly with an increase in the cell amount (**Figure 3**). However, increasing the cell amount of *P. saccharophila* IAM1504 increased the total gold (III) removal. About 1300 µmol gold/g dry wt. cells were removed using 5.4 mg dry wt. of *P. saccharophila* cells after 72 h incubation. Although the solution color did not change after 1 h incubation, the color changed to violet after 72 h. Therefore, the amount of gold (III) removed by biomineralization was also much larger than that by biosorption.

3.5. Absorption Spectrometry Analysis of Gold Removal Using Varying Cell Amount of *P. saccharophila*

To distinguish between the biosorbed and the biomineralized gold, we analyzed the ionic gold (III) and colloidal atomic gold (0) by absorption spectrometry. The 300 nm absorbance peak decreased with increasing cell amount, while no peak was observed from from 500 nm to 550 nm (**Figure 4**). Broad peak was observed using *C. krusei* on similar experiment [19]. The 525 nm peak was iden-

tified as zero valent gold [20] and the 500 nm - 650 nm peak was small and broad because of the low solubility of gold (0). The amount of biosorbed gold (III) using *P. saccharophilla* cells was relatively large. Therefore, it can be inferred that gold reduction mainly occurred after being biosorbed on the cell surface of *P. saccharophilla* cells. On the other hand, the amount of biosorbed gold (III) using *C. krusei* cells was relatively small, and hence, it can be inferred that gold reduction mainly occurred in solution using *C. krusei* cells.

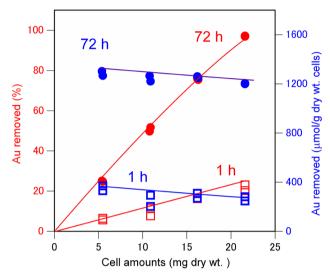


Figure 3. Effect of cell amounts on the removal of gold (III) by *P. saccharophila* cells. Red symbols: gold removed (%); blue symbols: gold removed (μmol/g dry wt. cells); circles: contact 72 h (biomineralization); squares: 1 h (biosorption).

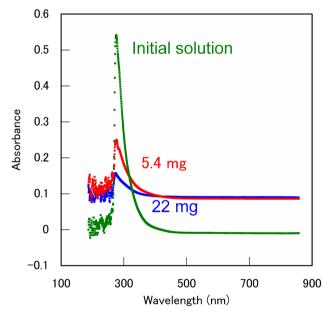


Figure 4. Absorption spectrometry analysis of gold removal using varying *P. saccharophila* IAM1504 amount. The gold removal conditions were identical to that in **Figure 3** (incubation for 72 h). Green line: initial amount of gold (III); red line: incubation with 5.4 mg dry wt. basis cells; blue line: 22 mg dry wt. basis cells.

3.6. Effect of Gold Concentration on Gold (III) Removal from Aqueous Gold (III) Using *P. saccharophila* Cells

To determine the maximum gold (III) removal ability at pH 4.0, we examined the mechanism by which the gold (III) concentration affected the gold removal by *P. saccharophila* cells. The amount of gold removed (μ mol/g dry weight cells) by *P. saccharophila* cells increased with increased gold concentration, whereas the ratio of total amount of gold to the gold concentration decreased (**Figure 5**). For gold (III) concentration of 200 mg/L (1020 μ M), 2500 μ mol gold/g dry cell wt. was observed at pH 4.0.

3.7. SEM and XRF Analyses of Gold Removal Using *P. saccharophila* and *C. krusei*

To confirm the biomineralized gold condition, the cell surfaces of *P. saccharophila* and *C. krusei* were analyzed via SEM and XRF. As shown in **Figure 6**, many particles were observed on the *P. saccharophila* cell surface. It can be inferred that these small particles are reduced gold after adsorption by *P. saccharophila* (**Figure 7**). In contrast, no particles were observed on the cell surfaces of *C. krusei* (**Figure 8**). However, as shown in **Figure 9**, peak was observed for reduced gold in the case of *C. krusei*. It can also be inferred that gold (III) is mainly reduced after being adsorbed on the *P. saccharophila* cells, whereas it is mainly reduced in the solution by *C. krusei* cells. This confirms the observations reported in Section 3.5.

Resting *P. saccharophila* cells (15 mg dry wt. basis) contacted with the hydrogen tetrachloroaurate (III) solution (Au 200 ppm, pH 4.0) for 72 h at 30°C. Magnification times was 20,000x.

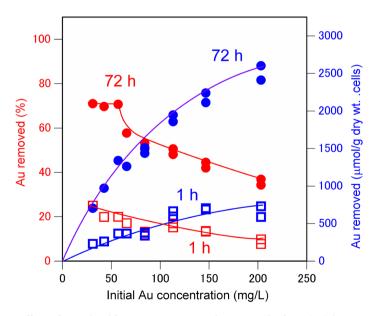


Figure 5. Effect of initial gold concentration on the removal of Au (III) by *P. saccharophila* cells. Red symbols: Au removed (%); blue symbols: Au removed (μmol/g dry wt. cells); circle symbols: contact 72 h (biomineralization); square symbols: 1 h (biosorption).

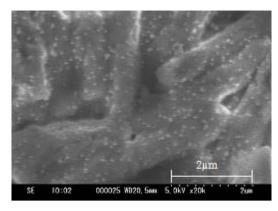


Figure 6. SEM image of *P. saccharophila* cells showing reduced gold particles.

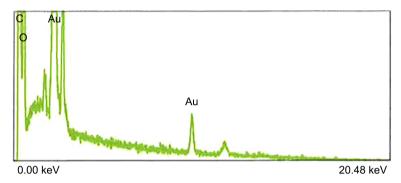


Figure 7. XRF spectrum of gold reduced on *P. saccharophila* cells.

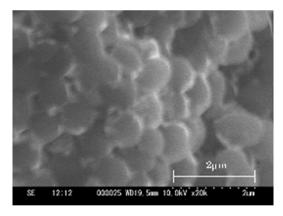


Figure 8. SEM image of *C. krusei* cells showing reduced gold particles.

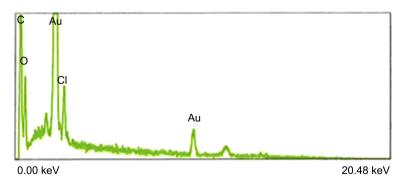


Figure 9. XRF spectrum of gold reduced on *C. krusei* cells.

XRF analysis of dry cell surface of *P. saccharophila* were in contact with the the hydrogen tetrachloroaurate (III) solution (Au 200 ppm, pH 4.0) for 72 h at 30°C.

Resting *C. krusei* cells (15 mg dry wt. basis) contacted with the hydrogen tetrachloroaurate (III) solution (Au 200 ppm, pH 4.0) for 72 h at 30°C. Magnification times was 20,000x.

XRF analysis of dry cell surface of *C. krusei* were in contact with the hydrogen tetrachloroaurate(III) solution (Au 200 ppm, pH 4.0) for 72 h at 30°C.

4. Conclusions

To optimize gold recovery, we first screened microorganisms for gold (III) removal from aqueous hydrogen tetrachloroaurate (III) solution (pH 3.0) after a 72 h incubation period at 30°C. Gold was removed from the solution by all tested microorganisms. *N. erythropolis* IAM1399 among the gram-positive bacteria; *E. coli* IAM1264, *P. maltophilia* IAM1554, and *P. saccharophila* IAM1504 among the gram-negative bacteria; *A. niger* IAM2534, *C. globosum* IAM9272 and IAM9427 among the fungi; *C. krusei* AHU3993, *C. utilis* IAM4220, *P. farinosa* IAM12223, and *S. cerevisiae* AHU3818 among the yeasts removed over 1200 μmol of gold/g dry cell wt.

The effects of incubation time, pH, cell amount, and initial gold concentration on gold removal were analyzed by atomic absorption spectrometry. Absorption spectrometry analysis of the effect of cell amount and gold (III) concentration was also investigated. Additionally, cell surfaces of *P. saccarophia* and *C. krusei* were analyzed via SEM and XRF analysis. We observed that about half the amount of gold (III) was removed from the solution by biosorption after a short incubation time (1 h) and the remaining half was reduced from gold (III) to gold (0) by biomineralization on the cell surface by *P. saccharophila* IAM1504 after 72-hour incubation. Contrarily, small amounts of gold (III) were removed from the solution by biosorption after a short incubation time (1h) and a large amount of gold was reduced from the gold (III) to gold (0) by *C. krusei* AHU3993 in the solution by biomineralization after 72 h incubation.

Of all mentioned in this paper, we think *P. saccharophila* cells can remove the largest amount of gold from the aqueous hydrogen tetrachloroaurate (III) solution. Therefore, we will next examine the removal, recovery, and recycle of gold using immobilized *P. saccharophila* cells.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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