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Removal, Recovery, and Recycles of Au(III) from Tetrachloroauric Acid Solution Using Immobilized *Pseudomonas* Cells by Biomineralization and Thiourea Oxidation

Takehiko Tsuruta[1, 2, *], Yuki Odajima[1], Kato Marina[1], Ichiro Maeda[2]

[1]Department of Life and Environmental Science, Hachinohe Institute of Technology, Hachinohe, Japan

[2]Department of Mechanical and Biochemical Engineering, Graduate School of Hachinohe Institute of Technology, Hachinohe, Japan

Email address:
tsuruta@hi-tech.ac.jp (T. Tsuruta), g115008@hi-tech.ac.jp (Y. Odajima), g125008@hi-tech.ac.jp (K. Marina), m12102@hi-tech.ac.jp (I. Maeda)

[*]Corresponding author

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Abstract: Removal, recovery, and recycles of gold(III) from aqueous tetrachloroauric acid solution using immobilized *Pseudomonas saccharophila* was investigated. The effect of pH, cell amounts on gold removal, and the time course of gold removal by *P. saccharophila* cells, which removed large amounts of gold from a solution containing hydrogen tetrachloroauric(III) acid were reported in detail. Additionally, to approve the amount of gold recovery much higher, the removal of gold(III) by biosorption and biomineralization from aqueous systems using microbial cells, gold(III) removal by those using microbial cells was investigated. The oxidative recovery of gold after reduced gold(0) by the oxidation using aqueous thiourea solution, and recycles of gold reduction-oxidation cycles were also investigated.

Keywords: Gold(III) Biosorption, Gold(0) Biomineralization, Microorganism, *Pseudomonas saccharophila*, Thiourea, Recycles

1. Introduction

The gold demand has increased significantly as its increasing use in the development of gold-containing drugs and the electrical industry [1]. So, this valuable resource recycling has been interested in a great subject.

Some researchers have reported recovery of gold by microorganisms, such as bacterium [2], fungi [3-5], yeast [6], and algae [7, 8]. However, information on the species of microorganisms that have a high gold removing ability is little.

We previously investigated that some microbial cells remove gold, and checked living 75 microorganisms (25 bacteria, 19 actinomycetes, 14 yeasts, and 17 fungi) from a solution containing tetrachloroauric(III) acid [9]. Tetrachloroauric(III) acid is used for ceramic and medical materials. Of the checked microbial cells, several gram-negative bacteria had gold-removal ability. These microbial cells removed over 330 mol gold per gram of microorganisms (dry wt.) from the tetrachloroauric(III) acid solution within 60 min. The removed gold from tetrachloroauric(III) acid solution using gram-negative bacteria was higher than that removed using actinomicetes, gram-positive bacteria, yeasts, and fungi. These results have quite differences with those reported for the removal of the amount of cadmium [10], lithium [11], rare earth metals [12], thorium [13], and uranium [14], these were removed in much gold amounts by gram-positive bacteria than the gram-negative bacteria, yeasts, and fungi. These results show that gram-positive bacteria can remove much amount of metal ions charged positively, while gram-negative bacteria can remove much amount of

complex ions charged negatively [9-14]. Gold(III) exists as a complex ion charged negatively in acidic solution. The cell surface of negatively charged gram-positive bacteria is higher than that of the gram-negative bacteria, as the amounts of teichoic acid are higher in the before at a neutral pH [15-17]. In other words, the positively charged the cell surfaces of the gram-negative bacteria is higher than that of the gram-positive bacteria. Therefore, gold complex ions charged negatively bond more strongly on the cell surfaces of the gram-negative bacteria charged positively [9].

We investigated the effects of cell amount, external gold concentration, pH, and gold contact time in *Pseudomonas maltophilia*, which removes much gold amounts from a tetrachloroauric(III) acid solution [9].

The research was done to improve removal of gold by biosorption and biomineralization from aqueous solutions using microbial cells in this study. Additionally, to develop a practical approach, the determination of a suitable desorbent for gold adsorbed by immobilized *P. maltophilia* cells and biosorption-desorption cycles was also presented [9]. To approve the amount of gold recovery much higher, the removal of gold(III) by biosorption and biomineralization from aqueous systems using microbial cells, gold(III) removal and recovery recycles using immobilized microbial cells was reported in this paper.

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). 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. 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, 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] were suspended in 100 mL solution containing 50 mg/L (254 μM) gold (pH 3.0) containing tetrachloroauric(III) acid. 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).

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

Fifteen mg of the resting *P. saccharophila* cells (dry wt. basis) were mixed and stirred with 100 mL of the solution (pH 3.0) containing tetrachloroauric(III) acid (254 μM) for 5min - 68 h at 30°C.

2.2.2. pH Influence on the Removal of Gold(III) by P. saccharophila IAM1504

Fifteen mg of the resting *P. saccharophila* cells (dry wt. basis) were mixed and stirred with 100 mL of the solution (pH 1.0-5.0) containing tetrachloroauric(III) acid (254 μM) for 72 h at 30°C.

2.2.3. Cell Amount Influence on the Removal of Gold(III) by P. saccharophila IAM1504

Five to twenty- three mg of the resting *P. saccharophila* cells (dry wt. basis) were mixed and stirred with 100 mL of the solution (pH 4.0) containing tetrachloroauric(III) acid (254 μM) for 72 h at 30°C.

2.2.4. Immobilization of P. saccharophila IAM1504

Precultured *P. saccharophila* cells (5.0 g fresh weight) were suspended in 4.5 ml isotonic sodium chloride solution and 680 mg acrylamide monomer, 34 mg N, N'-methylene-bis(acrylamide), 0.30ml 3-dimethylaminopropionitrile solution (5.0%), and 0.34 ml potassium persulfate solution (2.5%) were added to the suspension. After solidification, the gel was crushed into small pieces (50-100 mesh), washed thoroughly with isotonic sodium chloride solution followed by deionized water, and then used in the following gold removal experiments.

2.2.5. Removal of Gold by Reduction Using P. saccharophila IAM1504 Immobilized Cells

Fifteen mg of the immobilized *P. saccharophila* cells (dry wt. basis) were mixed and stirred with 100 ml of the solution containing gold 50 mg/L (254μM, pH 3.5) as tetrachloroauric(III) acid for 72 h at 30°C.

2.2.6. Oxidative Recovery from Gold Reduced by Immobilized P. saccharophila IAM1504 Using Thiourea Solution

Immobilized microbial cells removed gold was mixed with 100 ml of 0.25M thiourea solution (pH 3.0) for 17 h at 30°C. Treatment after this procedure was same with above mentioned.

2.2.7. Oxidative Recovery from Gold Reduced by Immobilized P. saccharophila IAM1504 Using Thiourea Solution

Recycles of Reductive Gold Removal and Oxidative Recovery Using Immobilized *P. saccharophila* IAM1504.

2.2.8. Time Course of Oxidative Recovery of Gold from Gold Reduced Immobilized P. saccharophila IAM1504 Using Thiourea Solution

Immobilized microbial cells (15 mg dry wt. microbial cell basis) removed gold was mixed with 100 ml of 0.25M

thiourea solution (pH 3.0) for 0.5-18 h at room temperature. Treatment after this procedure was same with above mentioned.

2.2.9. Time Course of Reductive Gold Removal Using Immobilized P. saccharophila IAM1504 at the Second Time

Immobilized microbial cells (15 mg dry wt. microbial cell basis) reduced (72 h) and oxidized (1h) gold one time was remixed within 100 ml of 50 mg/L (254μM) of gold solution (pH 3.5) containing tetrachloroauric(III) acid for 0.5-18 h at room temperature. Treatment after this procedure was same with above mentioned.

2.2.10. Time Course of Reductive Gold Removal Using Immobilized P. saccharophila IAM1504 at the Second Time

Above mentioned reductive removal (for the first time 72h and after second time 1 h, and oxidative recovery of gold for 1h were recycled 5 times.

3. Results and Discussion

3.1. Time Course of Gold Removal by P. saccarophila IAM1504

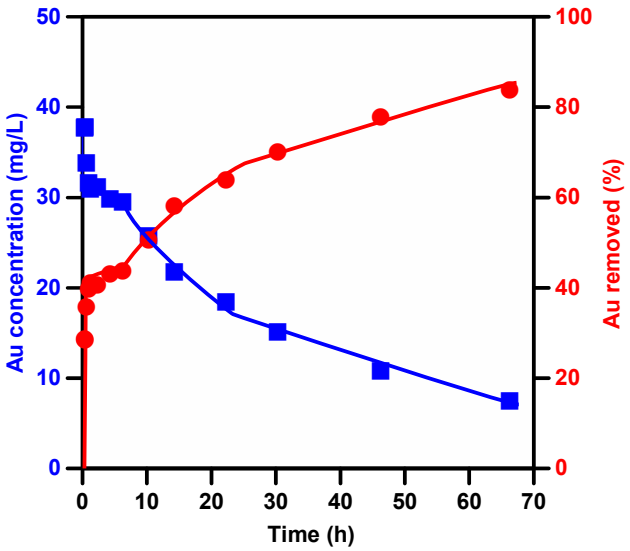

Figure 1. *Time course of gold(III) removed by P. saccharophila cells. Squares: gold concentration in the solution (mg/L), circles: gold removed (%).*

Time course of gold(III) removal by *P. saccharophila* IAM1504 was examined (Figure 1); gold concentration in the solution was decreased with increasing the contact time, whereas gold removed (%) increased. Gold removed reached two different equilibria. The first step of equilibrium state was at about 6 h, and occurred by biosorption. After this, the gold removed (%) amount re-increased, and the solution color changed darker, because of biomineralization. The gold removed amount using *P. saccharophila* IAM1504 cells by the biomineralization was much larger than that by biosorption [9].

3.2. Effect of pH on Gold(III) Removal from Aqueous Gold(III) Using P. saccharophila cells

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

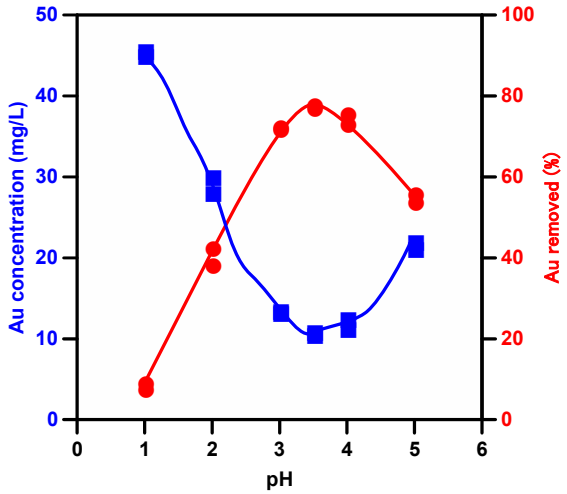

Figure 2. *pH influence on the removal of gold(III) by resting P. saccharophila cells. Squares: gold concentration (mg/L), circles: gold removed (%).*

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 2-3 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:

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

The equilibrium in an acidic solution is driven to the left; thus, suitable pH changed from 2.0-3.0 [9] to 3.5.

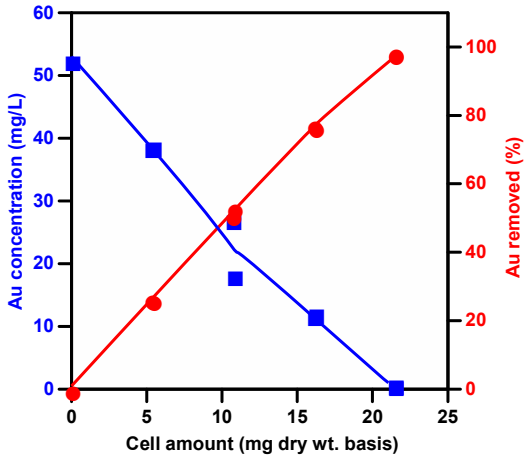

Figure 3. *Influence of cell amounts on the gold(III) removal using P. saccharophila cells. Circles: gold removed (%), squares: gold concentration in the solution (mg/L).*

3.3. 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 increased with an increase in the cell amount (Figure 3). Gold concentration in the solution was decreased with the increasing the cell amount used. Almost all amount of gold was removed by 22.4 mg (dry wt. basis) of the microbial cell used for 72 h contact.

3.4. Recycles of Reductive gold Removal and Oxidative Recovery Using Immobilized P. saccharophila Cells

Recycles of gold removal by biosorption and recovery by desorption using immobilized *P. maltophilia* cells in column system was reported [9]. In this paper, recycles of gold removal by biomineralization and oxidative recovery using immobilized *P. saccharophila* cells by batch system. Removal experiment was proceeded 72h because of the reductive removal was proceeded slowly to violet color (Figure 4a) over 72h [19]. However, after second time, the color of solution was changed to golden color (Figure 4b) rapidly after adding the gold(III) solution. Oxidative recovery was continued one night (17 h), however, color of cell surface was changed to colorless rapidly after adding thiourea solution. As shown in Figure 5, recycles of removal by biomineralization and oxidative recovery can be done 5 times.

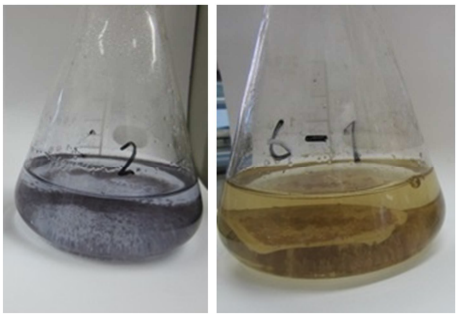

Figure 4. *Color of the reaction mixture.*

a: Immobilized P. saccharophila cells was mixed with Au(III) solution after 72 h (the first time). b: That after 5 min (the second time).

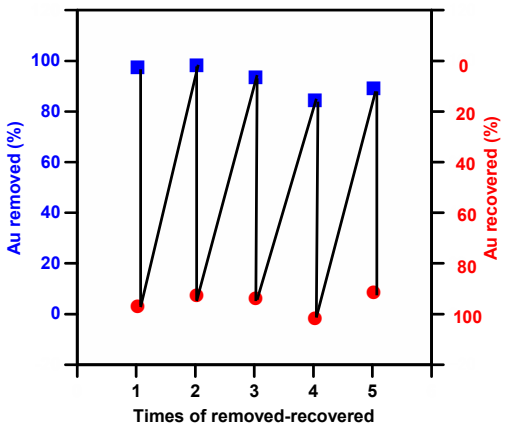

Figure 5. *Recycles gold removal (72h) and recovery of gold (17h) by immobilized P. saccharophila cells. Squares: gold removed (%), circles: gold recovered (%),*

3.5. Time Course of Gold Recovery from the Gold Reduced by Immobilized P. saccharophila Cells Using Thiourea Solution

From the above-mentioned recycled result, the oxidative recovery was proceeded very rapidly. Therefore, time course of gold recovery from the gold reduced by immobilized *P. saccharophila* cells using 100mL of 0.25 M thiourea solution was examined from 0.5-15h. As shown in Figure 6, oxidation of gold(0) was very rapid and over 90% of reduced Au(0) was oxidized and recovered into the solution within 0.5 h.

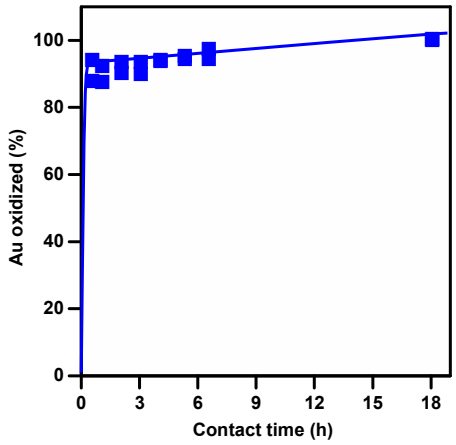

Figure 6. *Time course of gold recovery from the gold reduced by immobilized P. saccharophila cells using 100mL of 0.25 M thiourea solution.*

3.6. Time Course of the Sec. Times Reductive Gold Removal from the Tetrachloroauric(III) Acid Solution Using Immobilized P. saccharophila Cells Used Once

As the color of the solution was rapidly changed after adding the gold solution at the second times of the removal of gold from the tetrachloroauric(III) acid using immobilized *P. saccharophila* cells, time course of second times reductive gold removal from the tetrachloroauraic(III) acid using immobilized *P. saccharophila* cells was examined. As shown in Figure **7**, removal of gold(III) was very rapid and over 90**%** of Au(III) was reduced and removed from the solution within 0.5 h.

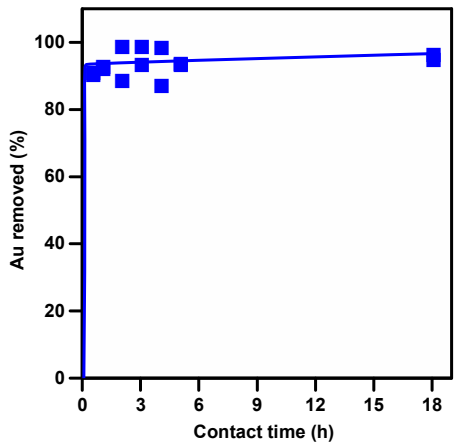

Figure 7. *Time course of the gold removal from the tetrachloroauric(III) acid using by immobilized P. saccharophila cells (at second times).*

3.7. Recycles of Reductive Gold Removal(Improved After Second Times) and Oxidative Recovery Using Immobilized P. saccharophila Cells

Reductive removal of gold was proceeded slowly over 72h by immobilized *P. saccharophila* cells at the first time, however, oxidative recover and the reductive removal after second time was rapidly proceeded within 1 h. Therefore, reductive gold removal by immobilized *P. saccharophila* cells at the first time was done for 72h, followed recycles of oxidative recovery and reductive removal of gold after second times using immobilized *P. saccharophila* cells were done for 1h until total five times. As shown in Figure 8, reductive removal and oxidative recovery of gold were done effectively. Therefore, immobilized *P. saccharophila* cells can remove and recover gold from the solution containing high concentration of tetrachloroauric(III) acid solution.

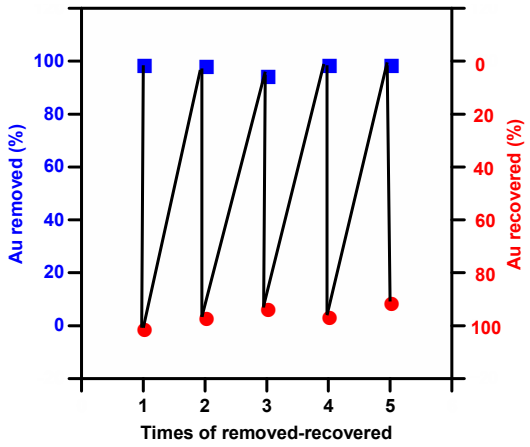

Figure 8. *Recycles gold removal (72h at the first time and 1h after second time) and recovery of gold (1 h) by immobilized P. saccharophila cells.*

4. Conclusion

To optimize gold recovery, we examined some factors affected on the removal of gold(III) from tetrachloroauric(III) acid solution using *P. saccharophila* by biomineralization.

The influences of pH, cell amount, and time course on the removal of gold were investigated. About 50% of the gold(III) was adsorbed from the gold solution by biosorption after a short contact time (1h) and the half remaining gold was reduced from trivalent gold to atomic gold by biomineralization on the *P. saccharophila* cells surface for the long time 72-hour contact.

Gold(III) can be removed using *P. saccharophila* cells by biomineralization effectively. Therefore, the recycles of removal of gold(III) for 72h from gold(III) solution (Au 50 mg/L) using immobilized *P. saccharophila* cells and recovery of removed reduced gold for 17h using 0.25M-thiourea solution was examined 5 times. Most of the gold(III) can be removed and recovered in these cycles. At the first time of removal, gold(III) was reduced slowly and cells became violet color. When recovery of gold was started, the color was rapidly vanished within 1h. At the second cycle of removal, immobilized cells became bright rapidly. Therefore, time course of gold recovery and second cycle of gold removal was examined. Both of oxidative gold recovery and second time reductive gold removal were proceeded rapidly. Therefore, recycles of removal (72h for the first time)-recovery (1h)-removal (1h for after the second time) cycles can be carried out in this system. At the first time of removal, gold(III) was reduced slowly and cells became violet color. When recovery of gold was started, the color was rapidly vanished within 1h. At the second cycle of removal, immobilized cells became more bright gold color rapidly. Therefore, time course of gold recovery and second cycle of gold removal was examined. Both of gold recovery and second time removal of that were proceeded rapidly. Recycles of removal (72h for the first time)-recovery (1h)-removal (1h for after the second time) cycles can be carried out in this system.

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