Full Paper

Biosorption and recycling of gold using various microorganisms

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In order to obtain basic information on the biosorption and recycling of gold from aqueous systems using microbial cells, the biosorption of gold by various microorganisms was investigated. Of 75 strains of microorganisms tested (25 bacteria, 19 actinomycetes, 17 fungi and 14 yeasts), high abilities of gold biosorption from a solution containing hydrogen tetrachloroaurate (III) were found in some gram-negative bacterial strains, such as *Acinetobacter calcoaceticus*, *Erwinia herbicola*, *Pseudomonas aeruginosa*, and *P. maltophilia*. Most of the gram-positive bacteria, actinomycetes, fungi and yeasts had a lower ability for gold biosorption than gram-negative bacteria. On the other hand, all of the microorganisms tested adsorbed far smaller amounts of gold from a solution containing gold dicyanoaurate (I). The biosorption of gold from a solution containing hydrogen tetrachloroaurate (III) using *P. maltophilia* having a high adsorbing ability for gold was very rapid and was affected by the pH of the solution, external gold concentration, and cell amounts. *P. maltophilia* cells immobilized with polyacrylamide gel also have a high ability for gold biosorption. The gold adsorbed on the immobilized cells is easily desorbed with 0.1 M thiourea solution. The immobilized *P. maltophilia* cells can be used repeatedly in biosorption-desorption cycles.

Key Words—gold biosorption; gold desorption; gram-negative bacteria; immobilization; *Pseudomonas maltophilia*

Introduction

The demand for gold has increased markedly because of its increasing use in the electrical industry and the development of drugs containing gold. Therefore, the recycling of this valuable resource has become a subject of wide interest.

Recently, some researchers have investigated the recovery of gold using microbial cells, such as fungi (Gomes et al., 1998; Matsumoto and Nishimura, 1992; Pethkar and Paknikar, 1998), yeasts (Karamuchka and

Gadd, 1999), and algae (Hosea et al., 1986; Kuyucak and Volesky, 1989). However, there is little information on which kind of microorganisms has a high gold adsorbing ability.

In this paper, various species and strains of bacteria, actinomycetes, fungi, and yeasts were screened for their ability to adsorb gold from a solution containing hydrogen tetrachloroaurate (III) or sodium dicyanoaurate (I). Hydrogen tetrachloroaurate (III) is used for medical and ceramic materials. On the other hand, dicyanoaurate (I) is used for plating. Both compounds are popular gold compounds and have a different oxidation stage. The effect of pH, external gold concentration, cell amounts on gold biosorption, and the time course of gold biosorption by *Pseudomonas maltophilia* cells, which adsorbed large amounts of gold

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from a solution containing hydrogen tetrachloroaurate (III), were investigated in detail. Additionally, in order 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 investigated.

Materials and Methods

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

Culture of microorganisms. The medium for growing bacteria contained 3 g/L meat extract, 5 g/L peptone, and 5 g/L NaCl in deionized water. The medium for growing the actinomycetes, fungi, and yeasts contained 4 g/L yeast extract, 10 g/L malt extract, and 4 g/L glucose in deionized water, pH 7.1 (for actinomycetes) and pH 5.7 (for fungi and yeasts). 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) or by filtration through filter paper (for actinomycetes and fungi), washed thoroughly with deionized water, and then used in the following biosorption experiments.

Immobilization of microbial cells. 1. Polyacrylamide method: Precultured *Pseudomonas maltophilia* 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), 0.30 ml 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 biosorption experiments.

2. Ca alginate method: Precultured *P. maltophilia* cells (5.0 g fresh weight) were suspended in 20 ml of

sodium alginate (2.0%) solution. The suspension was squeezed into 200 ml of calcium lactate (2.0%) solution using a syringe.

3. Agar method: Precultured *P. maltophilia* cells (3.6 g fresh weight) were suspended in 7.2 ml of agar (4.0%) solution. After solidification of the suspension, the resulting gels were cut into small pieces.

Gold biosorption experiments. Gold solutions were prepared by dissolving hydrogen tetrachloroaurate (III) or sodium dicyanoaurate (I) in deionized water. The pH of the solution was adjusted to the desired value with 0.1 M HCl or 0.1 M NaOH.

1. Batch system: Unless otherwise stated, the accumulation experiments were conducted as follows. Resting microbial cells (15 mg dry wt. basis for tetrachloroaurate (III) or 50 mg for sodium dicyanoaurate (I)) were suspended in 100 ml of 10 mg/L of gold solution (pH 3.0, containing hydrogen tetrachloroaurate (III) or sodium dicyanoaurate (I)), and the suspension was shaken for 1 h at room temperature. The resting microbial cells were then removed by filtration through a membrane filter (pore size 0.2 µm). The amounts of gold adsorbed by the cells were determined by measuring the gold content in the filtrate using an inductively coupled plasma quantimeter (ICPS 8000; Shimadzu, Kyoto, Japan).

2. Column system: Unless otherwise stated, 50 ml of gold solution (10 mg/L gold, HAuCl₄, pH 3.0) was passed through a column (diameter 8 mm, bed volume 5 ml) of immobilized *P. maltophilia* cells at a space velocity (SV) of 20 h⁻¹. The cell concentration of each of the immobilized cells was 0.66 g dry wt. cells/g dry wt. polyacrylamide. The adsorbed gold (III) was desorbed with 50 ml of 0.1 M thiourea solution (pH 3.0) at SV of 10 h⁻¹.

Results and Discussion

Biosorption of gold using various microbial cells

In order to determine the ability of different types of microbial cells to adsorb gold, 75 strains of microorganisms (19 actinomycetes, 25 bacteria, 17 fungi and 14 yeasts) were screened. We reported that the sorption of gold (III) showed a maximum value at pH 3 using immobilized persimmon tannin (Sakaguchi et al., 1995). Therefore, the pH of the solution was adjusted to 3.0 throughout this screening.

As shown in Tables 1–4 and Fig. 1, the ability of the microbial cells to adsorb gold significantly varied. Of

Table 1. Biosorption of gold by bacteria.

Species and strains	Au adsorbed ($\mu\text{mol/g}$ dry wt. cells)		Gram type
	HAuCl ₄ ^a	NaAu(CN) ₂ ^b	
<i>Acinetobacter calcoaceticus</i> IAM 12087	336 $\pm$ 1	19.3 $\pm$ 0.0	–
<i>Arthrobacter simplex</i> IAM 1660	282 $\pm$ 1	5.8 $\pm$ 0.3	+
<i>Arthrobacter tumescens</i> IAM 1447	218 $\pm$ 3	23.9 $\pm$ 2.6	+
<i>Bacillus cereus</i> IAM 1656	306 $\pm$ 6	26.9 $\pm$ 0.5	+
<i>Bacillus licheniformis</i> IAM 11054	266 $\pm$ 9	12.2 $\pm$ 1.0	+
<i>Bacillus megaterium</i> IAM 1166	235 $\pm$ 1	13.2 $\pm$ 0.0	+
<i>Bacillus subtilis</i> IAM 1026	277 $\pm$ 1	10.7 $\pm$ 0.5	+
<i>Bacillus subtilis</i> IAM 1633	276 $\pm$ 0	16.8 $\pm$ 0.6	+
<i>Bacillus subtilis</i> IAM 11060	305 $\pm$ 4	9.4 $\pm$ 0.3	+
<i>Brevibacterium helvolum</i> IAM 1637	279 $\pm$ 0	5.6 $\pm$ 0.0	+
<i>Corynebacterium equi</i> IAM 1038	276 $\pm$ 2	14.5 $\pm$ 0.8	+
<i>Enterobacter aerogenes</i> IAM 1183	308 $\pm$ 0	12.2 $\pm$ 0.0	–
<i>Erwinia herbicola</i> IAM 1562	360 $\pm$ 1	32.0 $\pm$ 0.0	–
<i>Escherichia coli</i> IAM 1264	326 $\pm$ 0	27.7 $\pm$ 0.3	–
<i>Micrococcus luteus</i> IAM 1056	250 $\pm$ 2	5.3 $\pm$ 0.3	+
<i>Nocardia corallina</i> IAM 12121	289 $\pm$ 3	12.7 $\pm$ 0.5	+
<i>Nocardia erythropolis</i> IAM 1399	258 $\pm$ 1	14.7 $\pm$ 0.5	+
<i>Nocardia rugosa</i> kccA0193	295 $\pm$ 0	6.3 $\pm$ 1.3	+
<i>Pseudomonas aeruginosa</i> IAM 1054	340 $\pm$ 5	22.8 $\pm$ 0.6	–
<i>Pseudomonas aeruginosa</i> IAM 1095	314 $\pm$ 6	32.7 $\pm$ 0.8	–
<i>Pseudomonas fluorescens</i> IAM 12022	328 $\pm$ 4	17.8 $\pm$ 0.0	–
<i>Pseudomonas maltophilia</i> IAM 1554	337 $\pm$ 0	37.6 $\pm$ 0.5	–
<i>Pseudomonas putida</i> IAM 1506	326 $\pm$ 1	28.2 $\pm$ 0.3	–
<i>Serratia marcescens</i> IAM 1022	321 $\pm$ 1	20.6 $\pm$ 0.3	–
<i>Thiobacillus novellus</i> IFO 12443	311 $\pm$ 0	22.6 $\pm$ 0.8	–

^a Resting cells (15 mg dry wt. basis) were suspended in 100 ml solution (pH 3.0) containing HAuCl₄ (50.5 μM) for 1 h at room temperature.

^b Resting cells (50 mg dry wt. basis) were suspended in 100 ml solution (pH 3.0) containing NaAu(CN)₂ (50.5 μM) for 1 h at room temperature.

these microorganisms tested, high abilities to adsorb gold from the solution containing tetrachloroaurate (III) were found in some gram-negative bacteria, such as *Acinetobacter calcoaceticus* IAM 12087, *Erwinia herbicola* IAM 1562, *Pseudomonas aeruginosa* IAM 1054, and *P. maltophilia* IAM 1554. These microorganisms could adsorb over 330 μmol of gold per gram dry wt. of microbial cells from the solution containing hydrogen

Table 2. Biosorption of gold by actinomycetes.

Species and strains	Au adsorbed ($\mu\text{mol/g}$ dry wt. cells)	
	HAuCl ₄ ^a	NaAu(CN) ₂ ^b
<i>Micromonospora chalcia</i> kccA0124	254 $\pm$ 4	7.4 $\pm$ 0.8
<i>Streptomyces albus</i> HUT 6047	214 $\pm$ 3	7.1 $\pm$ 0.5
<i>Streptomyces aurantiocogriceus</i> HUT 6201	169 $\pm$ 3	8.9 $\pm$ 0.8
<i>Streptomyces cineroruber</i> HUT 6141	252 $\pm$ 2	10.2 $\pm$ 0.6
<i>Streptomyces cinnamomensis</i> HUT 6050	307 $\pm$ 3	17.8 $\pm$ 0.5
<i>Streptomyces diastaticus</i> HUT 6116	215 $\pm$ 8	9.1 $\pm$ 0.0
<i>Streptomyces echinatus</i> HUT 6090	104 $\pm$ 3	6.1 $\pm$ 1.0
<i>Streptomyces flavoviridis</i> HUT 6147	194 $\pm$ 3	5.6 $\pm$ 0.5
<i>Streptomyces fradiae</i> HUT 6054	257 $\pm$ 7	12.4 $\pm$ 1.3
<i>Streptomyces griseoflavus</i> HUT 6153	179 $\pm$ 1	10.9 $\pm$ 1.3
<i>Streptomyces griseolus</i> HUT 6099	243 $\pm$ 1	6.3 $\pm$ 0.3
<i>Streptomyces hiroshimensis</i> HUT 6033	179 $\pm$ 21	15.0 $\pm$ 0.3
<i>Streptomyces levoris</i> HUT 6156	185 $\pm$ 1	5.3 $\pm$ 0.8
<i>Streptomyces lilacinofulvus</i> HUT 6210	280 $\pm$ 16	15.0 $\pm$ 0.8
<i>Streptomyces olivaceus</i> HUT 6061	165 $\pm$ 3	8.6 $\pm$ 0.0
<i>Streptomyces phaeochromogenes</i> HUT 6013	282 $\pm$ 1	6.6 $\pm$ 0.5
<i>Streptomyces salmonicida</i> HUT 6085	185 $\pm$ 8	11.7 $\pm$ 1.5
<i>Streptomyces viridochromogenes</i> HUT 6030	172 $\pm$ 1	6.1 $\pm$ 0.5
<i>Streptomyces viridochromogenes</i> HUT 6031	195 $\pm$ 6	4.8 $\pm$ 0.3

See footnote of Table 1.

tetrachloroaurate (III) within 1 h. On the other hand, the amounts of gold adsorbed from the solution containing sodium dicyanoaurate (I) were far less than from that containing hydrogen tetrachloroaurate (III). *P. maltophilia* cells could adsorb the highest amounts of gold from the solution containing sodium dicyanoaurate (I) of the microbial cells tested. The amount of gold adsorbed from each solution containing hydrogen tetrachloroaurate (III) or sodium dicyanoaurate (I) by gram-negative bacteria is higher than those by gram-positive bacteria, actinomycetes, fungi and yeasts. These results are in contrast to the case of the amounts of thorium adsorbed by gram-positive bacteria which were higher than those by gram-negative bacteria, fungi and yeasts (Tsuruta, 2003). In addition, the amounts of mercury adsorbed were also higher than with gram-negative bacteria, fungi and yeasts (not published). From these results, gram-positive bacteria can adsorb a large amount of positively charged

Table 3. Biosorption of gold by fungi.

Species and strains	Au adsorbed ($\mu\text{mol/g}$ dry wt. cells)	
	HAuCl ₄ ^a	NaAu(CN) ₂ ^b
<i>Aspergillus niger</i> AHU 7120	193 $\pm$ 12	7.1 $\pm$ 2.5
<i>Aspergillus niger</i> AHU 7296	215 $\pm$ 0	7.9 $\pm$ 0.3
<i>Aspergillus oryzae</i> AHU 7216	208 $\pm$ 26	11.2 $\pm$ 0.0
<i>Chaetomium globosum</i> AHU 9270	139 $\pm$ 3	14.7 $\pm$ 1.5
<i>Chaetomium globosum</i> AHU 9272	228 $\pm$ 9	11.7 $\pm$ 1.0
<i>Chaetomium globosum</i> AHU 9427	110 $\pm$ 4	14.7 $\pm$ 1.0
<i>Fusarium oxysporum</i> IAM 5009	197 $\pm$ 5	14.5 $\pm$ 0.8
<i>Giberella fujikuroi</i> AHU 9078	237 $\pm$ 4	15.0 $\pm$ 0.3
<i>Mucor hiemalis</i> IAM 6088	258 $\pm$ 13	15.0 $\pm$ 0.8
<i>Mucor javanicus</i> IAM 6087	247 $\pm$ 5	11.4 $\pm$ 0.8
<i>Penicillium chrysogenum</i> IAM 7106	201 $\pm$ 10	19.3 $\pm$ 0.0
<i>Penicillium janthinellum</i> AHU 8312	217 $\pm$ 10	9.9 $\pm$ 0.8
<i>Penicillium lilacinum</i> AHU 8357	206 $\pm$ 10	14.2 $\pm$ 0.0
<i>Rhizopus arrhizus</i> AHU 6573	144 $\pm$ 9	10.4 $\pm$ 0.8
<i>Rhizopus japonicus</i> IAM 6002	153 $\pm$ 1	8.6 $\pm$ 1.5
<i>Rhizopus oryzae</i> AHU 6591	121 $\pm$ 0	12.7 $\pm$ 1.0
<i>Trichoderma viride</i> AHU 9503	148 $\pm$ 3	9.9 $\pm$ 1.8

See footnote of Table 1.

metal ions; however, gram-negative bacteria can adsorb a large amount of negatively charged complex ions.

We reported that the ion species of gold (III) in the solution containing hydrogen tetrachloroaurate (III) are mainly Au(OH)Cl₃⁻ and Au(OH)₂Cl₂⁻ at pH 3.0 (Sakaguchi et al., 1995). Gold (III) exists as a negatively charged complex ion in the solution. On the other hand, the negative charge of the gram-positive bacterial cell surface is higher than that of the gram-negative bacterial cell surface because the amount of teichoic acid in gram-positive bacterial cells is higher than that of gram-negative bacterial cells in a neutral pH region (Conn et al., 1987; Fischer et al., 1973a,b). In an acidic pH solution, I tentatively assumed that the amounts of protons used for the neutralization of the negative charge by gram-positive bacterial cells were higher than those by gram-negative bacterial cells. As a result, the positive charge of the gram-negative bacterial cell surface is higher than that of gram-positive bacterial cell surface. Accordingly, the gold complex ions having a negative charge and the positive charge of the gram-negative bacterial cell surface cause stronger bonding than that with the gram-positive bac-

Table 4. Biosorption of gold by yeasts.

Species and strains	Au adsorbed ($\mu\text{mol/g}$ dry wt. cells)	
	HAuCl ₄ ^a	NaAu(CN) ₂ ^b
<i>Candida krusei</i> AHU 3993	229 $\pm$ 1	7.6 $\pm$ 0.5
<i>Candida robusta</i> AHU 3405	216 $\pm$ 0	6.1 $\pm$ 0.5
<i>Candida utilis</i> AHU 3210	243 $\pm$ 5	10.4 $\pm$ 1.3
<i>Cryptococcus albidus</i> AHU 3812	168 $\pm$ 2	4.3 $\pm$ 0.3
<i>Cryptococcus laurentii</i> AHU 3671	208 $\pm$ 1	11.7 $\pm$ 0.5
<i>Debaryomyces hansenii</i> AHU 3759	199 $\pm$ 1	15.0 $\pm$ 0.8
<i>Endomycopsis fibuligera</i> AHU 4113	235 $\pm$ 6	7.9 $\pm$ 0.3
<i>Hansenula anomala</i> AHU 3702	247 $\pm$ 3	6.9 $\pm$ 0.8
<i>Hansenula saturnus</i> AHU 3003	201 $\pm$ 1	9.4 $\pm$ 0.3
<i>Kluyveromyces marxianus</i> IAM 4985	222 $\pm$ 2	3.6 $\pm$ 0.6
<i>Pichia farinosa</i> IAM 12223	269 $\pm$ 1	10.4 $\pm$ 0.3
<i>Saccharomyces cerevisiae</i> AHU 3818	192 $\pm$ 5	6.3 $\pm$ 1.3
<i>Sporobolomyces salmonicolor</i> AHU 4072	275 $\pm$ 18	5.3 $\pm$ 0.3
<i>Torulopsis aeria</i> AHU 3398	147 $\pm$ 1	6.6 $\pm$ 0.5

See footnote of Table 1.

Fig. 1. Biosorption of gold (III) by microorganisms.

Resting cells (15 mg dry wt. basis) were suspended in a 100 ml solution (pH 3.5) containing hydrogen tetrachloroaurate (III) (50.5 μM , pH 3.0) for 1 h at room temperature.

terial cell surface.

Effect of pH on gold biosorption

As shown in Fig. 2, the biosorption of gold (III) by *P. maltophilia* cells was markedly affected by the pH of the solution. Maximum gold biosorption from the solution containing hydrogen tetrachloroaurate (III) was ob-

Fig. 2. Effect of pH on gold (III) biosorption by *P. maltophilia* cells.

Resting cells (15 mg dry wt. basis) were suspended in a 100 ml solution containing hydrogen tetrachloroaurate (III) (50.5 μM) for 1 h at room temperature.

served at pH 2–3, and above and below this pH value, the gold biosorption fell off rapidly.

As described in the above section, the bond between gold (III) and the cell surface depends on the electronic charge of both. The positive charge of the cell surface decreases as the pH increases because of the negative charge of the phosphorous group in the teichoic acid. On the other hand, as the acid dissociation of HAuCl_4 becomes smaller, the non-charged molecular HAuCl_4 concentration becomes greater and causes difficulty in bonding with the positively charged group on the surface of the cells at pH 1. Accordingly, it seems reasonable that the amounts of the gold (III) adsorbed are decreased when the pH values are below pH 2 and above pH 3.

Effect of external gold concentration on gold biosorption

To determine the maximal ability to adsorb gold (III) at pH 3.0, the effect of the external gold (III) concentration on the biosorption of gold (III) by *P. maltophilia* cells was examined. As shown in Fig. 3, the amounts of gold adsorbed by *P. maltophilia* cells (μmol/g dry wt. cells) increased with an increase in the external gold concentration, whereas the ratio of the total amounts of gold adsorbed to the external gold concentration decreased. When the external gold (III) concentration was 1,100 μM (residual gold (III) concentration was 900 μM), a maximum Au (III) biosorption of about

Fig. 3. Effect of external gold (III) concentration on gold biosorption by *P. maltophilia* cells.

Resting cells (15 mg dry wt. basis) were suspended in a 100 ml solution (pH 3.0) containing a desired amount of gold for 1 h at room temperature. Symbols: circles, gold (III) adsorbed (μmol/g dry wt. cells); squares, gold (III) adsorbed (%).

Fig. 4. Equilibrium isotherm of gold biosorption by *P. maltophilia* cells.

C_e , residual gold in the solution (μM); Q , amount of gold adsorbed (μmol/g dry wt. cells).

900 μmol gold (III) per gram dry wt. cells at pH 3.0 was observed.

In the case of the biosorption of the uranium by some bacterial strains, the relationship between the amounts of uranium (Q_U) adsorbed and the residual concentration of uranium in the solution $C_e(U)$ obeys the Langmuir isotherm over the whole concentration tested, $C_e(U)/Q_U = m_U C_e(U) + n_U$, where m_U and n_U are

Table 5. Estimated constants and $Q(\text{Au})_{\max}$ from the Langmuir isotherm.

Initial gold (III) concentration (μM)	Residual gold concentration (μM)	m	n	$Q(\text{Au})_{\max}$ ($\mu\text{mol/g}$ dry cells)
0–535	0–412	1.23×10^{-3}	1.73×10^{-2}	816
535–1,080	412–913	1.01×10^{-3}	8.44×10^{-2}	992
0–1,080	0–913	1.08×10^{-3}	3.78×10^{-2}	928

See the legend of Fig. 3.

constants (Sakaguchi et al., 1996). In the case of gold (III), the following equation is derived and shown in Fig. 4. $C_e(\text{Au})/Q_{\text{Au}} = (m_{\text{Au}} C_e(\text{Au}) + n_{\text{Au}})$, where Q_{Au} indicates the amounts of gold (III) adsorbed ($\mu\text{mol/g}$ dry wt. cells), $C_e(\text{Au})$ is the residual gold (III) concentration in the solution (μM) and m_{Au} and n_{Au} are constants. The dashed line was calculated using under $412 \mu\text{M}$ of residual gold (III) concentration (as the initial gold (III) concentration $535 \mu\text{M}$). On the other hand, the solid line was calculated separately using residual gold (III) concentration under and above $412 \mu\text{M}$ (as the initial gold (III) concentration $535 \mu\text{M}$). It seems that the experimental data does not fit over the whole gold (III) concentration in the solution. Accordingly, the result obtained in the experiment has a dual pattern. When the initial gold (III) concentration increases beyond $535 \mu\text{M}$ (as $412 \mu\text{M}$ of residual gold concentration), the amount of gold (III) accumulated rises to values larger than those calculated on the basis of the relation for a low concentrations range. A similar result was obtained (Epstein, 1966) from the absorption of potassium using barley roots. The estimated m_{Au} , n_{Au} and maximum accumulated gold (III) capacity $Q(\text{Au})_{\max}$ ($=1/m_{\text{Au}}$) are summarized in Table 5. As shown in Table 5, an extremely high $Q(\text{Au})_{\max}$ value of $992 \mu\text{mol/g}$ dry wt. cells is estimated from the high gold (III) concentration region.

Time course of gold biosorption

The time course of the gold (III) biosorption using *P. maltophilia* cells was examined. As shown in Fig. 5, the biosorption of gold (III) by *P. maltophilia* cells was very rapid. The results were fitted to a time-dependent Langmuir adsorption equation (Kondoh et al., 1982). $Q = Q_e[1 - 1/\exp(it + j)]$, where Q is the amount of gold (III) accumulated ($\mu\text{mol/g}$ dry wt. cells), Q_e is the equilibrium biosorption amount, t is the retention time (min), and i and j are constants. The values, i , j and

Fig. 5. Time course of gold (III) biosorption by *P. maltophilia* cells.

Resting cells (15 mg dry wt. basis) were suspended in a 100 ml solution (pH 3.0) containing hydrogen tetrachloroaurate (III) ($50.5 \mu\text{M}$) at room temperature.

Q_e , are estimated to be 0.0201/min, 2.78 and $329 \mu\text{mol/g}$ dry wt. cells, respectively.

Effect of cell amounts on gold biosorption

As shown in Fig. 6, the amounts of gold (III) adsorbed by *P. maltophilia* cells ($\mu\text{mol/g}$ dry wt. cells) decreased as the amount of cells increased, whereas the total amounts of gold (III) adsorbed increased. Twenty milligrams of *P. maltophilia* cells can almost quantitatively adsorb gold (III) in the solution. Based on the results of this experiment, the amount of the *P. maltophilia* cells needed to adsorb gold (III) can be determined for practical cases.

Recovery of gold by the immobilized *P. maltophilia* cells

As shown in the above sections, some gram-negative bacterial cells, such as *P. maltophilia* cells have a

Fig. 6. Effect of cell amounts on gold biosorption by *P. maltophilia* cells.

Desired amounts of resting cells (dry wt. basis) were suspended in a 100 ml solution (pH 3.0) containing hydrogen tetrachloroaurate (III) ($50.5 \mu\text{M}$) for 1 h at room temperature. Symbols: circles, gold (III) adsorbed ($\mu\text{mol/g}$ dry wt. cells); squares, gold (III) adsorbed (%).

Table 6. Effect of immobilization methods of *P. maltophilia* cells on biosorption of gold (III).

Immobilization method	Au adsorbed (%)
Polyacrylamide	99.1
Ca alginate	74.0
Agar	100.0

Fifty milliliters of the gold solution ($50.5 \mu\text{M}$ gold, HAuCl_4 , pH 3.0) were adsorbed on a column (diameter 8 mm, bed volume 5 mm) of the immobilized *P. maltophilia* cells at space velocity of 20 h^{-1} .

high ability to adsorb gold (III) from aqueous solutions. As a step in a practical approach to recover gold (III) using microbial cells, *P. maltophilia* cells, having a high ability to adsorb gold (III), were immobilized by various methods. As shown in Table 6, the *P. maltophilia* cells immobilized with polyacrylamide and agar could almost quantitatively adsorb gold (III) from aqueous solutions.

We reported that uranium adsorbed by some bacterial strains could be desorbed easily by sodium carbonate solution (Sakaguchi et al., 1996). Therefore, $0.1 \text{ M Na}_2\text{CO}_3$ solution was used to desorb gold (III) adsorbed by the immobilized *P. maltophilia* cells with polyacrylamide. However, in contrast to uranium, as

Table 7. Desorbence of gold from immobilized *P. maltophilia* cells.

Desorbent	Au desorbed (%)
$0.1 \text{ M Na}_2\text{CO}_3$	3.8
$0.1 \text{ M thiourea (pH 3.0)}$	97.5
$0.1 \text{ M thiomalic acid (pH 3.0)}$	18.2
$0.1 \text{ M thioglycerin (pH 3.0)}$	11.1

Biosorption experimental conditions were the same as in Table 6. Adsorbed gold was desorbed with 50 ml of each desorbent at SV of 10 h^{-1} .

Fig. 7. Effect of pH on gold desorption by immobilized *P. maltophilia* cells.

Fifty milliliters of the gold solution ($50.5 \mu\text{M}$ HAuCl_4 , pH 3.0) were adsorbed on a column (diameter 8 mm, bed volume 5 mm) of the immobilized *P. maltophilia* cells at space velocity of 20 h^{-1} . Adsorbed gold was desorbed with 50 ml of 0.1 M thiourea solution at SV of 10 h^{-1} .

shown in Table 7, most of the gold (III) adsorbed could not be desorbed. In order to search for a desorbent for the gold (III) adsorbed, some desorbents containing sulfur atoms, which could easily combine with gold, were examined. We reported that the desorption of gold (III) by thiourea solution gave a maximum value at pH 3 using immobilized persimmon tannin (Sakaguchi et al., 1995). Therefore, the desorption experiments were carried out at pH 3.0. As shown in Table 7, of the desorbents tested, 0.1 M thiourea solution desorbed gold from the gold adsorbed by immobilized *P. maltophilia* cells most effectively.

The effect of pH on the desorption of gold (III) by thiourea using immobilized *P. maltophilia* cells with polyacrylamide was also examined. As shown in Fig.

7, thiourea could desorb gold (III) effectively from pH 1–3, especially at pH 3.0, though the amounts of gold desorbed decreased with a pH increase over 4. Thiourea was protonated and desorbed the anionic gold (III) complex ion in the lower pH solution; however, non-protonated thiourea existed in the higher pH solution. Therefore, the amounts of gold (III) desorbed decreased with the increasing pH of the solution.

Repetition test of gold biosorption-desorption cycles using the immobilized P. maltophilia cells

As described above, the *P. maltophilia* cells immobilized with polyacrylamide can adsorb gold (III) with high efficiency, and the gold adsorbed on the immobilized cells is easily desorbed with 0.1 M thiourea solution. Along with these fundamental results, a repetition test of the gold biosorption-desorption cycles using the immobilized *P. maltophilia* cells was conducted in a column system.

As a result, the gold adsorbing ability of the immobilized *P. maltophilia* cells does not decrease after 5 repetitions of the gold biosorption-desorption cycles. Therefore, the immobilized *P. maltophilia* cells provide better mechanical properties and can be used repeatedly in the biosorption-desorption cycles (data not shown).

Conclusion

Some gram-negative bacterial cells, such as *Erwinia herbicola*, *Pseudomonas aeruginosa*, *P. maltophilia* cells, have an excellent ability to adsorb gold from aqueous solutions, and the microbial cells immobilized with polyacrylamide can be applied to recover gold in both batch and column systems. It is considered from these facts that the microbial cells can be used as an adsorbent for gold recovery from aqueous systems.

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