Accumulation of gold by microorganisms

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Summary

Thirty species of microorganisms (8 bacteria, 9 actinomycetes, 8 fungi and 5 yeasts) were screened for maximal gold accumulation. Extremely high abilities to accumulate gold from the solution containing hydrogen tetrachloroaurate(III) were found in bacterial strains, such as *Escherichia coli* and *Pseudomonas maltophilia*. Most of actinomycetes, fungi and yeasts have less ability to accumulate gold in comparison with bacteria. Some of microorganisms can accumulate similar amounts of gold from the solution containing sodium gold(I) thiomalate as those from gold(III) solution. However, most of microorganisms tested accumulate far lesser amounts of gold from the solution containing sodium dicyanoaurate(I) than from the other two gold solutions. The accumulation of gold from the solution containing hydrogen tetrachloroaurate(III) by *Pseudomonas maltophilia* was very rapid, was affected by the pH of the solution, and obeyed the Langmuir adsorption isotherm. The *Pseudomonas maltophilia* cells immobilized with polyacrylamide gel adsorbed gold effectively from the solution containing hydrogen tetrachloroaurate(III). The gold adsorbed on the cells was easily desorbed with 0.1 M thiourea solution. The immobilized *Pseudomonas* cells could be used repeatedly in the adsorption-desorption cycle using 0.1 M thiourea solution as desorbent.

Introduction

The metal recovery technique using microorganisms will be cheap in cost and have little disposal problems, so that its development should be worthwhile to study (Volesky, 1999). Recently, the recovery of gold from aqueous systems has been actively studied from the standpoints of the gold recovery from industrial resources, such as electronics parts and plating materials. However, most of studies have been restricted to the limited species of microorganisms, such as algae (Hosea *et al*. 1986; Kuyucak & Volesky, 1989a), fungi (Matsumoto & Nishimura, 1992; Pethkar & Paknikar, 1998; Gomes *et a*l., 1998), and yeasts (Karamuchka & Gadd, 1999). Thus, the present investigation was undertaken to screen microorganisms having highest ability to accumulate gold and to obtain fundamental information on the recovery of gold from aqueous systems. Firstly, to determine microorganisms for maximal accumulation of gold, 30 species of microorganisms (8 bacteria, 9 actinomycetes, 8 fungi and 5 yeasts) were screened. Secondly, the basic features of gold accumulation and some factors affecting the accumulation by *Pseudomonas maltophilia*, having the highest accumulating ability for gold, were examined. Practical conditions for gold recovery from aqueous systems were also determined by using the immobilized *Pseudomonas* cells.

Materials and Methods

Strains, media and growth conditions

Strains used in this study were generously donated by the Faculty of Agriculture, Hokkaido University (AHU), the Faculty of Engineering, Hiroshima University (HUT),

and the IAM Culture Collection, Center for Cellular and Molecular Research, Institute of Molecular and Cellular Biosciences, The University of Tokyo (IAM). Media for growing microbial strains contained the following (in grams per liter of deionized water): meat extract 3 g, peptone 5 g, sodium chloride 5 g, pH 6.5 for bacteria; yeast extract 4 g, malt extract 10 g, glucose 4 g, pH 7.1 for actinomycetes; yeast extract 4 g, malt extract 10 g, glucose 4 g, pH 5.7 for yeasts and fungi. The pH of the medium was adjusted using 0.1 N NaOH and 0.1 N HCl solutions. Microbial cells were grown in 300 ml medium in a 500 ml culture flask with continuous shaking (130 rpm) at 30°C. Cells in linearly growing phase were collected by centrifugation (18000 × g) or filtration, washed thoroughly with isotonic sodium chloride solution, and then used for gold accumulation experiments. Heat-killed cells were prepared by boiling the fresh cells for 10 min (Horikoshi *et al*., 1981).

Immobilization of microbial cells

Precultured fresh *Pseudomonas maltophilia* cells (5 g fresh weight) were suspended in 5.4 ml of an isotonic sodium chloride solution. Six hundreds and eighty milligrams of acrylamide monomer, 34 mg of N,N'-methylene-bisacrylamide, 0.3 ml of 3-(dimethylamino)propionitrile solution (5 %, w/v) and 0.34 ml of potassium persulphate solution (5 %, w/v) were added to the suspension. The suspension was mixed and allowed to stand for 10 min at 25°C. The resulted gels were crushed into small pieces (50 -100 mesh), washed thoroughly with isotonic sodium chloride solution followed by deionized water, and then used for gold adsorption experiments. The cell concentration of the gels was 65.9 %. Precultured fresh *Pseudomonas maltophilia* cells (4.5 g fresh weight) were suspended in 18 ml of 2 % sodium alginate solution. The suspension was squeezed into 200 ml of 2 % calcium lactate solution using a syringe. The cell

concentration of the gels was 84.0 %. Precultured fresh *Pseudomonas maltophilia* cells (3.6 g fresh weight) were suspended in 7.2 ml of 4 % agar solution. After solidified the suspension, resulted gels were cut into small pieces. The cell concentration of the gels was 72.8 %. Microbial cells in each gels grew again in medium, suggesting most of microbial cells were still alive after immobilization.

Gold accumulation experiments

Hydrogen tetrachloroaurate(III) tetrahydrate and potassium dicyanoaurate(I) were obtained from Nacarai Tesque, Inc. (Kyoto, Japan), and gold(I) thiomalate, from Shionogi & Co. Ltd.

Batch system. Fifteen milligrams of fresh microbial cells were suspended in 100 ml of the solution (pH 3) containing 50 μM of gold, and the suspension was shaken for 1 h at 30°C. Gold was supplied as hydrogen tetrachloroaurate(III), sodium gold(I) thiomalate and sodium dicyanoaurate(I). Preliminary experiment showed that the maximum amounts of gold taken up by microbial cells were found at pH 3 from the solutions containing hydrogen tetrachloroaurate(III) and sodium dicyanoaurate(I), and at pH 2 from the solution containing sodium gold(I) thiomalate. Thus, the pH of the solution was adjusted to optimal values, respectively, with 0.1 N HCl or 0.1 N NaOH solution. After take up of gold, microbial cells were centrifuged at 18000 × g, and then remaining gold in the supernatant was determined by using the inductively coupled plasma quantometer (Shimadzu ICPQ-1000II). Sorption experiments for the time course of the gold adsorption were conducted as follows: 15 mg (dry weight basis) of fresh microbial cells were suspended in 100 ml of a solution (pH 3) containing 50 μM of hydrogen tetrachloroaurate(III) and the suspension was stirred for 5 min - 2 h at 30 °C. Sorption

experiments for the gold sorption isotherm was conducted as follows: 15 mg (dry weight basis) of fresh microbial cells were suspended in 100 ml of solutions containing 50 - 1000 μM of hydrogen tetrachloroaurate(III). Before pH adjustment, pH of gold(II) solution was around 3. Thus, 0.1 N HCl was used for the pH adjustment below pH 3. As the solution pH was preserved in the range from 3.00 to 3.04 during the adsorption experiments, the solution pH was not controlled. The gold adsorption tests using the immobilized *Pseudomonas* cells were conducted in similar manner as described above. The experiments were conducted three times and averaged.

Column system. Fifty milliliters of the gold solution (pH 3) were passed through a column (diameter 8 mm, bed volume 2 ml) of the immobilized *Pseudomonas* cells (58 mg dry weight basis) at a velocity of 20 ml h^{-1}. The gold adsorbed was desorbed with 50 ml of 0.1 M thiourea solution (pH 3) at the same velocity. Contributions for gold adsorptions by gels without microbial cells were 3.3 % (polyacrylamide), 0.2 % (calcium alginate), and 0.9 % (agar), respectively.

Results and Discussion

Accumulation of gold by microorganisms from solutions containing different chemical species of gold

Thirty species of microorganisms (8 bacteria, 9 actinomycetes, 8 fungi, and 5 yeasts) were screened for maximal gold accumulation from a solution containing hydrogen tetrachloroaurate(III). Uptake tests were also conducted using gold solutions containing 3 different chemical species of gold, viz., hydrogen tetrachloroaurate(III), sodium gold(I) thiomalate and sodium dicyanoaurate(I), respectively. Preliminary experiment showed

that the maximum amounts of gold taken up by microbial cells were found at pH 3 from the solutions containing hydrogen tetrachloroaurate(III) and sodium dicyanoaurate(I), and at pH 2 from the solution containing sodium gold(I) thiomalate. Thus, the pH values of the solutions were adjusted to be optimal values, respectively. The results on the gold accumulation by microorganisms were listed in Table 1. Of microorganisms tested, extremely high abilities to accumulate gold from the solution containing hydrogen tetrachloroaurate(III) was found in bacterial group, such as *Escherichia coli* and *Pseudomonas maltophilia*. Actinomycetes, fungi and yeasts accumulated smaller amounts of gold than bacteria. As shown in Table 1, some microorganisms, such as *Corynebacterium equi, Neurospora sitophila* and *Pseudomonas maltophilia*, could accumulate similar amounts of gold from the solution containing sodium gold(I) thiomalate as those from gold(III) solution. Most of microorganisms tested accumulate far smaller amounts of gold from the solution containing sodium dicyanoaurate(I) than from other two gold solution. The amounts of gold taken up in the microbial cells from the solution containing sodium dicyanoaurate(I) are less than one-fifth of those from the solution containing hydrogen tetrachloroaurate(III). Similar small amounts of gold accumulation were found in microalgae (Greene *et al*., 1986). As tetrachloroaurate(III) ion easily released chloride ion in the aqueous solution, the resulting chloride-free gold(III) ion combined with microbial cells. However, dicyanoaurate(I) ion, being such a stable complex anion, is hardly dissociated, which resulted very small amounts of gold(I) accumulated. As gold(I) thiomalate ion, having intermediate stability, will be partially dissociated, and some microorganisms could accumulate the gold(I) ion. When the mixture of gold(III) ion solutions and microbial cells were stood for 24 hrs, the color of some microorganisms tested were changed to purple with an absorption peak of 530 nm. Greene *et al*. (1986) reported that the color of microalgae changed to purple color

after adsorption of gold caused by gold(III) reduction to gold(0) in the cells. Our results also indicated similar gold(III) reduction to gold(0) by fungi and yeasts.

Basic features of gold accumulation by Pseudomonas maltophilia

As described in the previous section, some microorganisms, such as *Pseudomonas maltophilia*, can accumulate gold from the solution containing hydrogen tetrachloroaurate(III) with high efficiency. To search for appropriate conditions of gold recovery from aqueous solution, some factors affecting gold accumulation from the solution containing hydrogen tetrachloroaurate(III) by *Pseudomonas maltophilia* were examined.

As shown in Fig. 1, the accumulation of gold was markedly affected by the pH of the solution. Maximum gold accumulation from the solution containing hydrogen tetrachloroaurate(III) was observed at pH 3, and above and below this pH region, the gold accumulation fell off rapidly. Table 2 indicates the chemical species of gold(III) ion in the solution containing hydrogen tetrachloroaurate(III) calculated using the formation constants listed by Sillen and Martell (1971). As shown in Table 2, five complex ions exist in the solution, which ratios alternate with pH of the solution. In the pH region below 2, stable complex ion, $AuCl_4^-$, is dominant. As increase of the solution pH, chloride ion in $AuCl_4^-$ is displaced one by one with OH^- ion. Finally, $Au(OH)_4^-$ becomes dominant above pH 6. When microbial cells are placed in the solution, ligand groups in microbial cells combine with Au(III) in place of Cl^- ions at the pH from 2 to 6. Thus, gold accumulation by microbial cells may occur in the intermediate pH region. Though similar a pH profile was obtained for algal biomass, the clear decrease below pH 3 was not presented for gold(III) solution because of large cell amounts in the solution (Greene *et al*., 1986).

As shown in Fig. 2, the accumulation of gold(III) by *Pseudomonas maltophilia* was very rapid. Eighty-seven percent of gold supplied was taken up in the first 5 min, followed by a gradual increase of gold amounts. Though similar rapid uptake was observed for algal biomass, clear time-dependency was not presented because of large cell amounts in the solution (Greene *et al*., 1986). Rapid metal uptake was also observed in uranium (Nakajima, 2002a) and copper (Nakajima *et al*., 2001; Nakajima, 2002b) biosorptions by bacteria.

As shown in Fig. 3, the accumulation of gold by *Pseudomonas* cells obeys the Langmuir isotherm, Qe = k Qm Ce / (1 + k Ce), where Qe is the equilibrium amount of gold taken up (μmol/g dry cells), Qm, the maximum gold amounts taken up (μmol/g dry cells), Ce, the equilibrium gold concentration (μM), and k, the constant (M^{-1}). The values of Qm and k were estimated to be 939 μmol/g dry cells and 2.62×10^4 M^{-1}, respectively. Kuyucak and Volesky (1989a) reported that most of fungi and red algae tested showed 700-900 μmol/g dry cells, though a brown alga, *Sargassum natans*, had an extremely high gold accumulation ability, 2 mmol/g dry cells. Unfortunately, they did not consider equilibrium isotherms. Our previous results gave Qm = 611 μmol/g dry cells and $k = 1.04 \times 10^4$ M^{-1} for uranium biosorption by *Micrococcus luteus* (Nakajima, 2002b). Values of Qm = 327,527, 361, 603 μmol/g dry cells and k = 1.14, 1.10, 2.14, 17.6×10^4 M^{-1} were also given for copper biosorption of *Bacillus subtilis*, *Micrococcus luteus*, *Pseudomonas stutzeri* and *Arthrobacter nicotianae*, respectively (Nakajima *et al*., 2001; Nakajima, 2002a). The values Qm and k for gold were almost in similar range of those for other metal ions.

As shown in Table 3, the amounts of gold(III) accumulated by heat-treated *Pseudomonas* cells were larger than those by fresh cells, which is similar as that found in uranium biosorption (Nakajima *et al*., 1981; Horikoshi *et al*., 1981). These results

suggested that heat-treatment of the cells made it easy to incorporate gold(III) ion into inner cells.

The results on the time course, the gold accumulation isotherm, and the heat-treatment suggested that the mechanism for the gold accumulation by *Pseudomonas* cells should be similar as those for ordinal biosorption of metal ions through physico-chemical processes, such as the ion-exchange reaction. In some of microorganisms, gold(III) was reduced to gold(0) after being taken up in the cells.

Recovery of gold by the immobilized Pseudomonas maltophilia *cells*

As described in above sections, some microorganisms, such as *Pseudomonas maltophilia*, have an extremely high ability to accumulate gold from aqueous systems. As a step of the practical approach to recover gold by using microorganisms, *Pseudomonas maltophilia* cells were immobilized with various procedures listed in Table 4. As shown in Table 4, the *Pseudomonas* cells immobilized with both polyacrylamide and agar had very high abilities to adsorb gold from a solution containing hydrogen tetrachloroaurate(III). As gold(III) ion is classified into a soft acid according to the HSAB idea (Pearson, 1963; Hancock and Martell, 1989), they can combine strongly with sulfur-containing compounds, such as thiourea, thiomalic acid and thioglycerin. Previously, we have shown that the gold adsorbed on the adsorbent are easily desorbed with 0.1 M thiourea solution (Nakajima & Sakaguchi, 1993). Kuyucak and Volesky (1989b) showed that thiourea solution was effective for gold elution from algal cells. Thus, 0.1 M thiourea solution was used as a desorbent for gold in this experiment. As shown in Table 4, the gold adsorbed on the immobilized *Pseudomonas* cells with both polyacrylamide and agar was quantitatively desorbed with 0.1 M thiourea solution. The

Pseudomonas cells immobilized with polyacrylamide were utilized for the following experiments, because of their mechanical stability compared with agar immobilization.

As mentioned above, the *Pseudomonas* cells immobilized with polyacrylamide can adsorb gold with high efficiency and the gold adsorbed on the cells is easily desorbed with 0.1 M thiourea solution. Along with this fundamental result, a repetition test of gold adsorption-desorption cycle by the immobilized *Pseudomonas* cells was conducted in a column system. The gold adsorption ability of the adsorbent maintained for at least five adsorption-desorption cycle. Thus, the immobilized *Pseudomonas* cells acquire better mechanical properties and can be used repeatedly in the adsorption-desorption cycle.

Conclusion

Thirty species of microorganisms were screened for gold accumulation from aqueous systems. Extremely high abilities to accumulate gold from the solution containing hydrogen tetrachloroaurate(III) was found in bacterial group, while actinomycetes, fungi and yeasts have fewer abilities to accumulate gold compared with bacteria group. Microorganisms tested accumulated gold far readily from hydrogen tetrachloroaurate(III) solution than from sodium dicyanoaurate(I) solution. As tetrachloroaurate(III) ion easily released chloride ion in the aqueous solution, the resulting chloride-free gold(III) ion easily combined with microbial cells. However, dicyanoaurate(I) ion, being so stable complex anion, is little dissociated in aqueous solution, which resulted the very few amounts of gold(I) accumulated. The accumulation of gold by *Pseudomonas maltophilia*, having the highest accumulation ability of gold, was rapid, was affected by the solution pH, and obeyed the Langmuir isotherm. The amounts of gold accumulated

by the cells were increased by the heat-treated. Summarizing these results, the mechanism of the gold accumulation by microbial cells will be similar as those of ordinal biosorption of other metal ions. However, gold(III) ion taken up by some microorganisms should be reduced to gold(0). The *Pseudomonas* cells immobilized with polyacrylamide could adsorb gold sufficiently. The gold adsorbed on the immobilized cells could be easily desorbed with 0.1 M thiourea solution. As gold(I) and gold(III) ions are classified into soft acids according to the HSAB idea (Pearson, 1963; Hancock and Martell, 1989), they can combine with soft bases such as sulfur-containing compounds strongly. The immobilized *Pseudomonas maltophilia* cells could be used repeatedly in the adsorption-desorption cycle using 0.1 M thiourea as desorbent. Thus, the immobilized *Pseudomonas* cells can be applicable to recover gold in both batch and column systems.

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Table 1. Accumulation of gold from solutions containing different chemical species of gold by microorganisms.

Species	Au sorbed (μmol/g cells)		
	$HAuCl_4$ [a]	$NaAu(CN)_2$ [b]	NaAu-thiomalate [c]
Bacteria			
Arthrobacter tumescens IAM 1447	218 ± 9	23.9 ± 4.0	217 ± 5
Bacillus subtilis IAM 1026	277 ± 11	10.7 ± 2.0	235 ± 11
Brevibacterium helovolum IAM 1637	279 ± 10	5.6 ± 3.0	223 ± 9
Corynebacterium equi IAM 1038	277 ± 9	14.7 ± 3.5	309 ± 11
Deinococcus proteolyticus IAM 1214	269 ± 12	11.7 ± 2.5	250 ± 9
Escherichia coli IAM 1264	326 ± 14	27.9 ± 5.0	106 ± 8
Pseudomonas maltophilia IAM 1554	337 ± 16	37.6 ± 6.0	322 ± 9
Zooglea ramigera IAM 12136	240 ± 10	13.7 ± 4.0	135 ± 7
Actinomycetes			
Actinomyces flavoviridis HUT 6147	194 ± 9	5.6 ± 1.5	164 ± 8
Streptomyces albus HUT 6047	214 ± 11	7.1 ± 1.5	182 ± 9
Streptomyces cinnamonensis HUT 6050	307 ± 15	17.8 ± 4.5	165 ± 8
Streptomyces echinatus HUT 6090	104 ± 8	6.1 ± 2.0	85 ± 7
Streptomyces fradiae HUT 6054	257 ± 14	12.7 ± 3.5	240 ± 9
Streptomyces lilacinofulvus HUT 6210	280 ± 16	15.2 ± 4.0	270 ± 8
Streptomyces olivaceus HUT 6061	165 ± 11	8.6 ± 2.0	232 ± 11
Streptomyces phaeochromogenus HUT 6013	282 ± 16	6.6 ± 1.5	286 ± 12
Streptomyces viridochromogenes HUT 6031	195 ± 15	5.1 ± 1.0	208 ± 12
Fungi			
Aspergillus niger AHU 7296	215 ± 13	8.1 ± 2.0	209 ± 11
Chaetomium globosum AHU 9427	110 ± 7	14.7 ± 3.0	109 ± 7
Fusarium oxysporum IAM 5009	198 ± 13	14.7 ± 4.5	118 ± 8
Gibberella fujikuroi AHU 9078	237 ± 9	15.2 ± 4.5	121 ± 10
Mucor javanicus IAM 6087	247 ± 15	11.7 ± 2.5	188 ± 10
Neurospora sitophila AHU 9213	259 ± 12	19.7 ± 3.5	311 ± 12
Penicillium chrysogenum IAM 7106	201 ± 14	19.3 ± 4.5	169 ± 8
Rhizopus arrhizus AHU 6573	195 ± 12	7.1 ± 1.5	194 ± 9
Yeasts			
Candida utilis AHU 3210	243 ± 13	10.7 ± 2.0	10 ± 3
Debaryomyces hansenii AHU 3759	199 ± 15	15.2 ± 2.5	29 ± 6
Endomycopsis fibuligera AHU 4113	236 ± 15	8.1 ± 1.5	71 ± 7
Saccharomyces cerevisiae AHU 3818	192 ± 15	6.6 ± 1.5	47 ± 6
Sporobolomyces salmonicolor AHU 4072	275 ± 11	5.6 ± 1.0	66 ± 8

a) 15 mg of precultured cells were suspended in 100 ml of $HAuCl_4$ solution (50 μM Au, pH 3) for 1 h at 30°C.

b) 50 mg of precultured cells were suspended in 100 ml of $NaAu(CN)_2$ solution (50 μM Au, pH 2).

c) 15 mg of precultured cells were suspended in 100 ml of sodium gold thiomalate solution (50 μM Au, pH 2).

Sorption amounts represent mean ± standard deviation of triplicate.

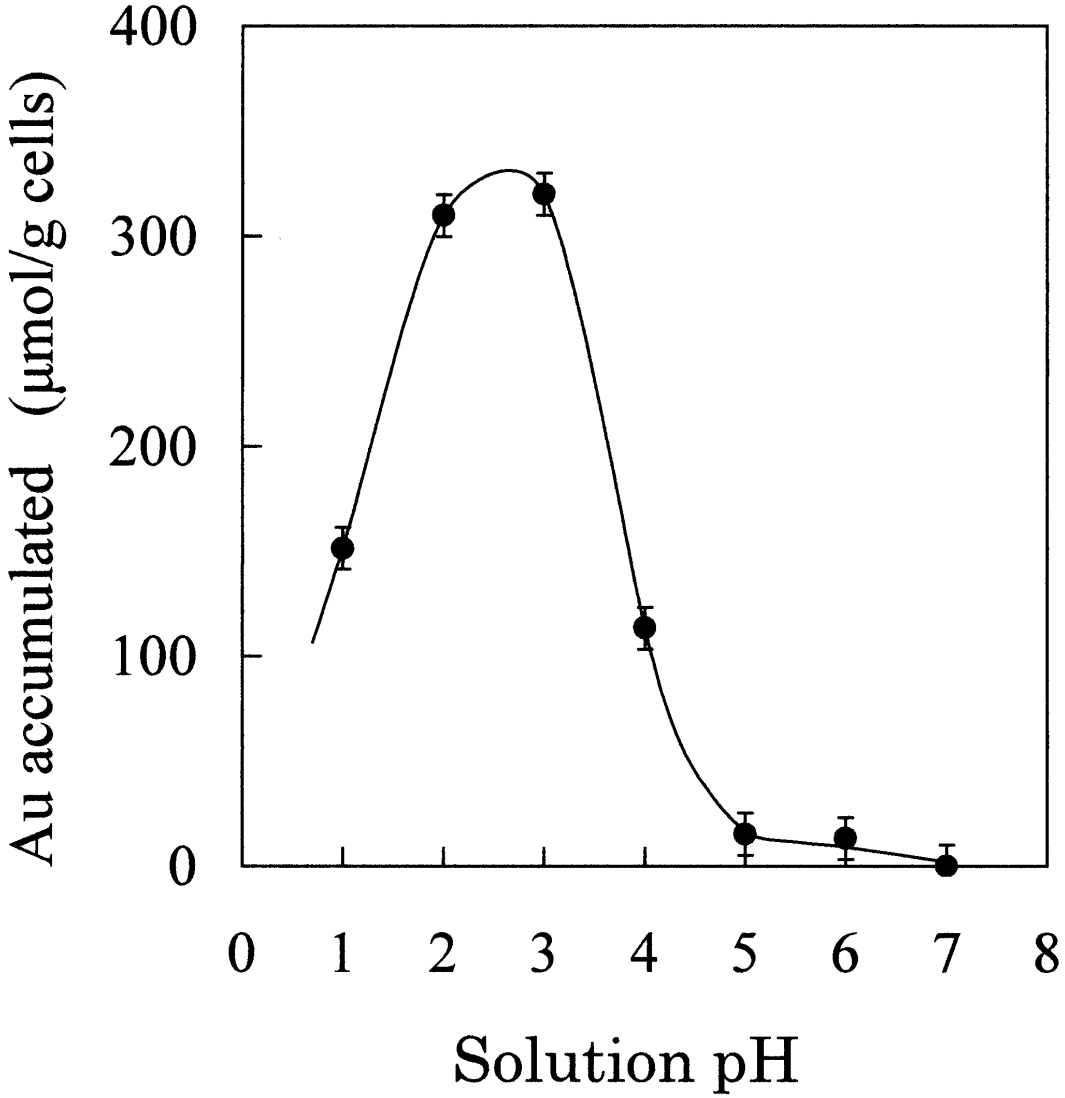

Fig. 1. Effect of pH on the gold accumulation by *Pseudomonas maltophilia*.

Fifteen milligrams of the precultured cells were suspended in 100 ml of the solution containing 50 μM of gold supplied as $HAuCl_4$ for 1h at 30°C. The pH of the solution was adjusted with 0.1 N HCl and 0.1 N NaOH solutions. Each point represents mean ± standard deviation of triplicates.

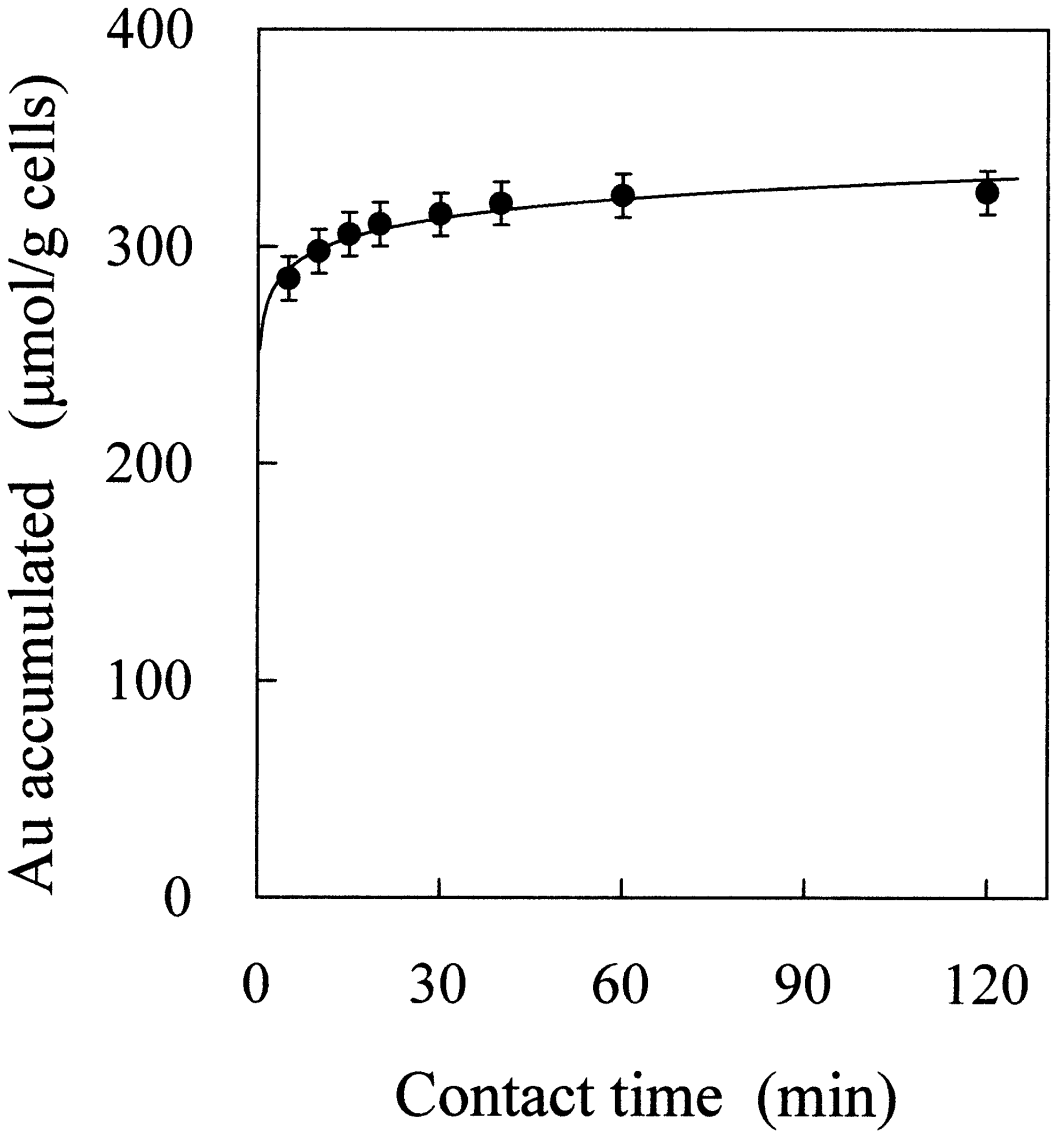

Fig. 2. Time course of the gold accumulation by *Pseudomonas maltophilia*.

Fifteen milligrams of the precultured cells were suspended in 100 ml of the solution (pH 3) containing 50 μM of gold supplied as $HAuCl_4$ at 30°C. Each point represents mean ± standard deviation of triplicates.

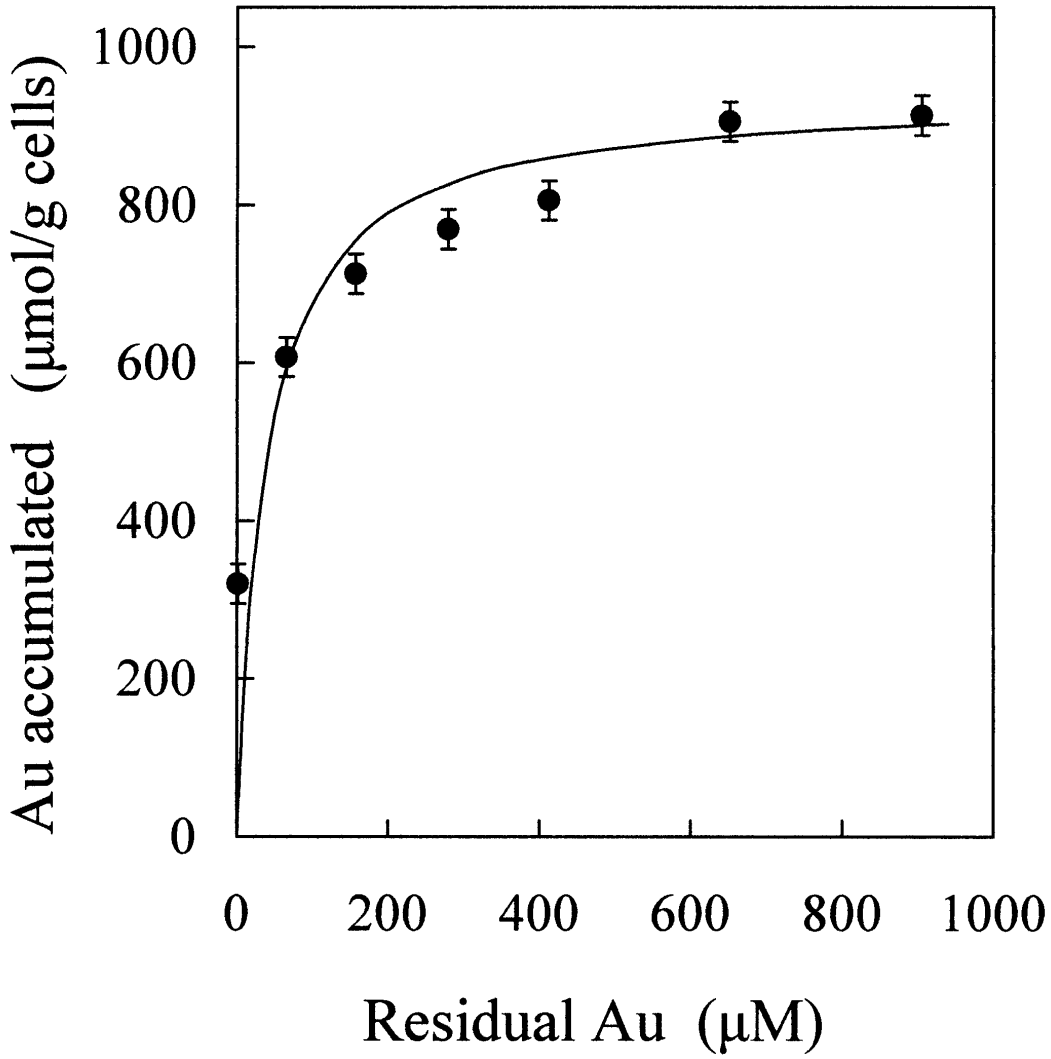

Fig. 3. Gold accumulation equilibrium of *Pseudomonas maltophilia*.

Fifteen milligrams of the precultured cells were suspended in 100 ml of the solution (pH 3) containing 50 - 1000 μM of gold supplied as $HAuCl_4$ for 1 h at 30°C.
Each point represents mean ± standard deviation of triplicates. Solid curve indicates the Langmuir isotherm, $Q = Qm\ k\ Ce / [\ 1 + k\ Ce\ ]$ with Qm = 939 μmol/g cells and $k = 2.62 \times 10^4\ M^{-1}$.