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Recovery of gold from thiourea solutions using microorganisms

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The recovery of gold from gold-thiourea solutions using various types of waste biomass was investigated. All organisms tested, namely, *Saccharomyces cerevisiae*, *Spirulina platensis* and *Streptomyces erythraeus* removed gold rapidly from gold–thiourea solutions. The process of gold accumulation was pH-dependent for *Saccharomyces ceresvisiae* and *Streptomyces erythraeus* and independent of pH in the case of *Spirulina platensis*. Of all strains of microorganisms examined, *Spirulina platensis* had the highest affinity and capacity for gold even at low pH values. Thus, all three microorganisms tested for their ability to recover gold from gold–thiourea solutions can therefore be used in biotechnological applications, especially *Spirulina platensis* which has the highest binding capacity for gold at low pH values.

Keywords: biosorption, gold bioleaching, metals bioremediation, thiourea

Introduction

Gold is a precious metal always in high demand by the industry. Because of this demand and fluctuating prices of this metal, processing of lower grade ores, waste-rock dump materials, and scrap materials are receiving considerable attention. It has been demonstrated that certain types of microbial biomass have a high potential to bind and concentrate metal ions from aqueous solutions even when the cells are not living (Volesky 1987). In fact, several studies suggest that biomass, which can be produced at low cost, might be a valuable aid in the treatment of contaminated waters and recovery of metal ions in mining operations (Brierley *et al*. 1989, Gadd & White 1993). Relatively few reports, however, appear on the interaction of gold or other precious metals with microorganisms (Darnall *et al*. 1986, Savvaidis *et al*. 1997).

The use of thiourea for the recovery of gold from ores as an alternative to the traditional process involving cyanide has been suggested (Hiskey 1984, Kai *et al*. 1992). The biooxidation of refractory gold-bearing ores and concentrates is conducted under acid conditions, so that to extract the gold by cyanidation it is necessary to raise the pH of the bioleachate to around 10 by adding lime to prevent the formation of HCN. It would be very advantageous to be able to extract gold under acidic conditions by using thiourea. In the presence of a suitable oxidizing agent, such as O_2, H_2O_2 or Fe, thiourea will solubilize gold to give a gold(I) species (Equation 1):

$$Au + 2CS(NH_2)_2 \longrightarrow Au[CS(NH_2)_2]^{2+} \qquad (1)$$

Irrespective of whether cyanide or thiourea is used to form a soluble complex, the metal must subsequently be extracted from solution. Methods for the recovery of gold from solutions generated by bioleaching or conventional extraction include activated carbon adsorption, ion-exchange, solvent extraction, extraction by SO_2 or reduction using zinc. The use of living or dead microorganisms for precious metal recovery from solutions provides a potential alternative to these physical and chemical treatments and the applicability of this technology is currently assessed. The aim of this work was to study the recovery of gold from gold–thiourea solutions using waste biomass.

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Materials and methods

Most of the biomass types used for the recovery of gold from gold–thiourea solutions, were grown in the Department of Microbiology, King's College London, UK, and these were obtained as fermentation waste products. The cyanobacterium *Spirulina platensis* was supplied by Professor S.J. Pirt, the bacterium *Streptomyces erythraeus* by Dr A. Trilli, and the yeast *Saccharomyces cerevisiae* was purchased from Holland & Barrett, London, UK and used as a dry powder.

Culture media

Spirulina platensis was grown to a steady state using a loop reactor under constant illumination, constant temperature (30 °C) and fixed dilution rate ($D = 0.002$ h^{-1}), where $D = f\backslash v$ (f is the flow rate in ml h^{-1} through the chemostat and v is the working volume in ml). The working volume of the vessel used was 1.0l. The chemically defined medium used to grow *Spirulina platensis* was made up from three solutions which were autoclaved separately. Part A contained (g l^{-1}) NaCl (20.0), $NaNO_3$ (6.1), K_2SO_4 (1.0), $MgSO_4.7H_2O$ (0.4) and $CaCl_2.2H_2O$ (0.04). Part B contained $NaHCO_3$ (8.4) and K_2HPO_4 (0.46), adjusted to pH 9.5 with 5 M NaOH. The trace elements solution (Part C) contained $FeSO_4.7H_2O$ (0.01), H_3BO_3 (0.029), $MnCl_2.4H_2O$ (0.0004), $CuSO_2.5H_2O$ (0.0001), $Na_2MoO_4.2H_2O$ (0.001), $NiSO_4.7H_2O$ (0.0001), $Co(NO_3)_2.6H_2O$ (0.001), $NaVO_3$ (0.0005), KI (0.0005), Na_2SeO_3 (0.0005) and $ZnSO_4.5H_2O$ (0.004). The trace elements solution, Part C, was made up as 100× strength in 0.1 M H_2SO_4. Part C (10 ml) was first added to part A (700 ml) followed by part B (200 ml), and finally the medium was made up to 1 l with distilled water.

Streptomyces erythraeus (GS/Z 1205) was grown in continuous culture under phosphate-limited conditions at a dilution rate $D = 0.005$ h^{-1}. No phosphate was detected using a phosphomolybdate assay. The growth medium had the following composition (g l^{-1}): asparagine (10.0), $MgSO_4$ (0.6), betaine HCl (0.5), $CaCl_2.2H_2O$ (0.11), NaCl (5.0), glucose (20.0), KH_2PO_4 (0.25), and mineral solution A (6 ml). Solution A consisted of (g l^{-1}): $Na_2EDTA.2H_2O$ (6.6), $FeSO_4.7H_2O$ (2.0), $ZnSO_4.7H_2O$ (1.0), $CuSO_4.5H_2O$ (0.5), $MnSO_4.7H_2O$ (0.37), $CoCl_2.6H_2O$ (0.1) and distilled water (1 l). The pH of the medium was automatically controlled by addition of 2N NaOH 1N HCl, and was finally adjusted to pH 7.0. Glucose, asparagine and KH_2PO_4 were sterilized separately and added separately to the medium.

Preparation of the gold–thiourea solutions

Solutions of the gold–thiourea complex were prepared as follows: 10 ml of the gold(III) solution ($HAuCl_4$, 1000 μg ml^{-1} of Au, pH 0.55) was transferred to a 50 ml beaker and stirred magnetically, while the pH was adjusted to pH 2.0 with 10 M KOH solution. Thiourea solution (about 5 ml, 0.5 M) was then added to the gold(III) solution dropwise until the gold–thiourea complex was formed. This was indicated by a colour change as the yellow colour of Au(III) disappeared, giving colourless Au(I). The gold–thiourea solution was then made up to 25 ml with distilled water to give a concentration of 400 μg ml^{-1}.

Determination of metal-binding

Culture (1 l) was centrifuged using a Beckmann centrifuge, model J2-21 and a JA 10 rotor at 9000 r.p.m (12500*g*) for 10 min at 4 °C and the cells washed once with distilled water (or for *Spirulina platensis* with 0.1 M NH_4HCO_3 to remove excess salt), and then resuspended in 0.2 M KCl–0.2 M HCl solution (pH 2.0). All biomass suspensions were adjusted to give equal concentrations (dry weight ml^{-1}) by addition of 0.2 M KCl–0.2 M HCl solution. Cell suspension (4 ml) was then transferred into Corex glass tubes, and 5 ml of gold–thiourea solution (10–190 μg ml^{-1}) was added. The metal–cell suspension was then allowed to stand, usually for 5 min at room temperature, after which biomass was separated from the gold–thiourea solution by centrifugation using the JA 20 rotor and rubber adapter cups for a Beckman (model J2-21) centrifuge as described previously (in some cases, the cells were separated using a microcentrifuge 13500 r.p.m., 1 min). The supernatant liquid was carefully transferred into volumetric flasks, made up to volume with 0.2 M KCl–0.2 M HCl solution and kept for analysis for gold. The cell pellets were further washed with 5 ml of 0.2 M KCl–0.2 M HCl solution, recentrifuged, and the supernatant liquid treated as before and kept for analysis for gold. The cell pellets were then allowed to dry overnight at 105 °C. Cell digestion involved the use of a mixture of concentrated sulfuric and nitric acids. Concentrated sulfuric acid (100 μl) was added to the cell pellets, and the mixture was heated to 120 °C, while 0.5 ml of concentrated nitric acid was added dropwise. The mixture was then heated to 140 °C until complete dissolution of the cell pellet had occurred. The clear solutions were cooled to room temperature and 2 ml of aqua regia (HCl/HNO_3; 3:1 (v/v)) was added. These solutions were made up to known volumes with distilled water and analysed for gold by atomic absorption spectroscopy.

Atomic absorption spectroscopy

Gold concentrations were determined using a Perkin-Elmer 2380 atomic absorption spectrometer at 242.8 nm using an air acetylene flame. Gold standards in the range 0.1–10 μg ml^{-1} were made up to 100 ml in 1% HCl and stored in volumetric flasks in the dark to minimize decomposition and loss of gold to the surfaces. Standard gold solutions were freshly prepared on a regular basis. A careful comparison of the new standards with those to be discarded showed that decomposition of the standards was not taking place over the normal period of use. The extent of gold uptake by biomass was determined on samples digested using the procedure described previously.

Determination of biomass

Optical density measurements were made using a Pye-Unicam SP6-450 spectrophotometer in cuvettes of 1 cm pathlength at 600 nm for *Str. erythraeus* and *Sac. cerevisiae* and at 680 nm for *S. platensis*. Biomass dry weight was also determined using preweighed membrane filters, 0.45 μm (Nyflow, Oxoid Ltd., Basingstoke, England). Duplicate 5 ml samples were filtered under vacuum and the filters washed twice with 10 ml distilled water (except for *S. platensis*, where 0.1 M NH_4HCO_3 solution was used to remove excess salt) and the preweighed filters were then dried to constant weight at 105 °C.

Results

The pH-dependence of the binding of the cationic gold–thiourea complex to cell surfaces was investigated. Figure 1 shows the effect of pH on gold uptake using *S. platensis*, *Str. erythraeus* and *Sac. cerevisiae*. The uptake of gold by *S. platensis* was nearly independent of pH in the range 2.0–7.0. The pH-independence of gold uptake by *S. platensis* from gold–thiourea solutions is similar to that found by Darnall *et al.* (1986) from $AuCl_4^-$ solutions. In all other cases, gold uptake was dependent on the pH of the solution. The uptake of gold by *Sac. cerevisiae* was constant in the pH range 1.0–4.0, rising to an optimum value of pH 5.0, and finally decreasing to smaller values at pH 6.0–7.0. Gold uptake by *Str. erythraeus* did not change significantly over the pH range 1.0–3.0, but increased at pH 4.0, and finally decreased in the pH range 4.0–7.0. It is interesting to note the similarity in the pattern of the gold uptake plots of *Sac. cerevisiae* and *Str. erythraeus*, but the dependence on pH looks unusual.

Figure 2 shows the dependence on time of gold uptake from gold–thiourea solutions by *S. platensis*, *Str. erythraeus* and *Sac. cerevisiae*. Gold uptake from gold–thiourea solutions by *S. platensis* was the

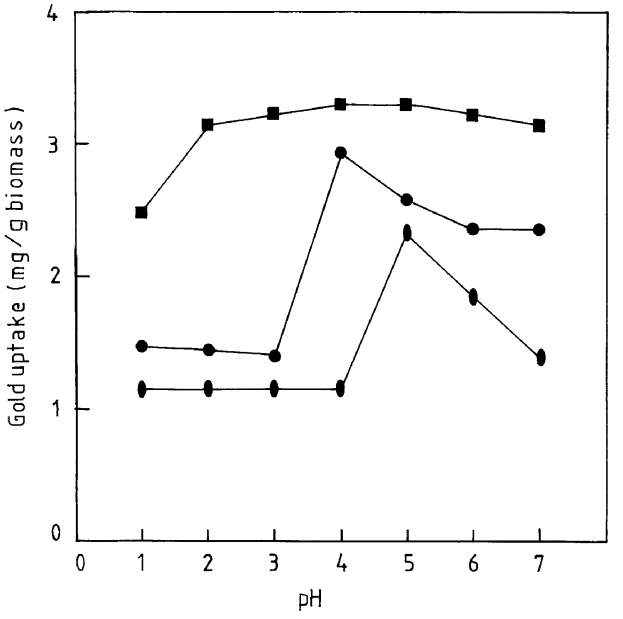

Figure 1. Effect of pH on gold uptake from gold–thiourea solutions using *Sac. cerevisiae*, *Str. erythraeus* and *S. platensis*. Gold–thiourea solution (0.75 ml, 27 μg ml^{-1}), was added to 0.75 ml cell suspensions of *Sac. cerevisiae* (●), dry weight (3.8 mg ml^{-1}), *Str. erythraeus* (●), dry weight (3.6 mg ml^{-1}) and *S. platensis* (■), dry weight (3.9 mg ml^{-1}) to give a final concentration of gold of 13–5 mg ml^{-1} in 0.2 M KCl–0.2 M HCl solution at pH 1–2 or in 0.1 M citrate–0.2 M phosphate solution at pH 3–7 in Eppendorf tubes. The cells were agitated using a vortex-mixer, and after 15 min of incubation were separated from gold–thiourea solutions by centrifugation. The supernatant solutions were then analysed for gold by atomic absorption spectroscopy.

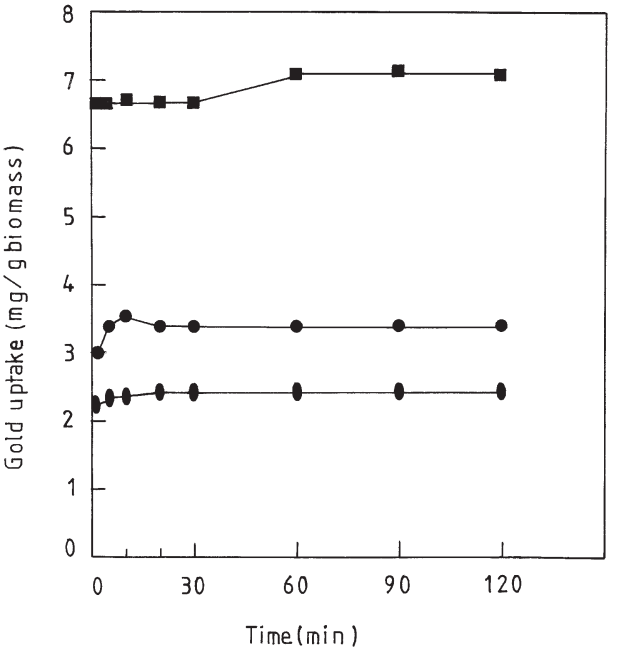

Figure 2. Effect of time on gold uptake from gold–thiourea solutions using *Sac. cerevisiae*, *Str. erythraeus* and *S. platensis*. Gold–thiourea solution (4 ml, 206 μg ml^{-1}) was added to cell suspensions (36 ml) of *Sac. cerevisiae* (●), dry weight (6.02 mg ml^{-1}), *Str. erythraeus* (●), dry weight (2.42 mg ml^{-1}) and *S. platensis* (■), dry weight (2.07 mg ml^{-1}) to give a final concentration of gold of 20.6 μg ml^{-1} in 0.2 M KCl–0.2 M HCl solution at pH 2.0 with stirring. Aliquots (1 ml) were withdrawn at time intervals (1–120 min) and the cells were separated from the gold–thiourea solutions by centrifugation using a microcentrifuge (13500 r.p.m., 1 min). The supernatant solutions were then analysed for gold by atomic absorption spectroscopy.

highest of the three types of biomass used. The rate of gold uptake was rapid, reaching almost immediately 90% of the final level attained within one hour. In the case of *Str. erythraeus* and *Sac. cerevisiae*, the uptake of gold was also rapid but complete within the first 5 min of contact time.

Table 1 summarizes data obtained on the uptake of gold from gold–thiourea solutions using *S. platensis* over a range of gold concentrations up to 190 μg ml^{-1}. The total gold recovered in the analysis as a percentage of that added as gold–thiourea solution is generally good, being in the range 95–104%, but usually close to 100%. Similar data were also obtained in the uptake of gold from gold–thiourea solutions for *Sac. cerevisiae* and *Str. erythraeus*.

Incubation of the three types of biomass in the gold–thiourea solutions resulted in biosorption of the metal as shown by the presence of gold in cell digests (Figure 3). In all cases, the uptake of gold in solution increased up to 30 μg ml^{-1}, and then started to level off. The cyanobacterium *S. platensis* was found to have the highest accumulation of gold from solutions containing 10–50 μg ml^{-1} compared to those exhibited by *Str. erythraeus* and by *Sac. cerevisiae*, although the differences were not considerable. The yeast, *Sac cerevisiae* had the lowest gold accumulation capacity at low gold concentrations 5.8 μg ml^{-1} whereas it achieved the highest value of gold accumulation at the highest gold concentration 104 μg ml^{-1} of all types of biomass used.

Table 1. Gold uptake by *S. platensis* from gold–thiourea solutions. Gold solutions (5 ml) in the range 10–190 μg ml^{-1} were added to 4 ml cell suspensions (*S. platensis*, dry weight 7.45 mg ml^{-1}), and the mixtures were incubated for 5 min at room temperature. Gold uptake was measured in the supernatant solutions and cells (digests) by atomic absorption spectroscopy

Gold concn (μg ml^{-1})	Gold added (μg)	Gold (s/n) (μg)	Gold(digest) (μg)	Gold recd (%)	Gold uptake (μg g^{-1})
10.4	52.2	9.9	44.5	104	1495
20.4	102	28.9	70.3	97	2361
40.4	202	99.7	92.5	95	3104
49.9	250	143	108	100	3641
96	480	369	133	104	4455
187	936	793	153	101	5134

Notes: conc: concentration, s/n: supernatant, recd: recovered.

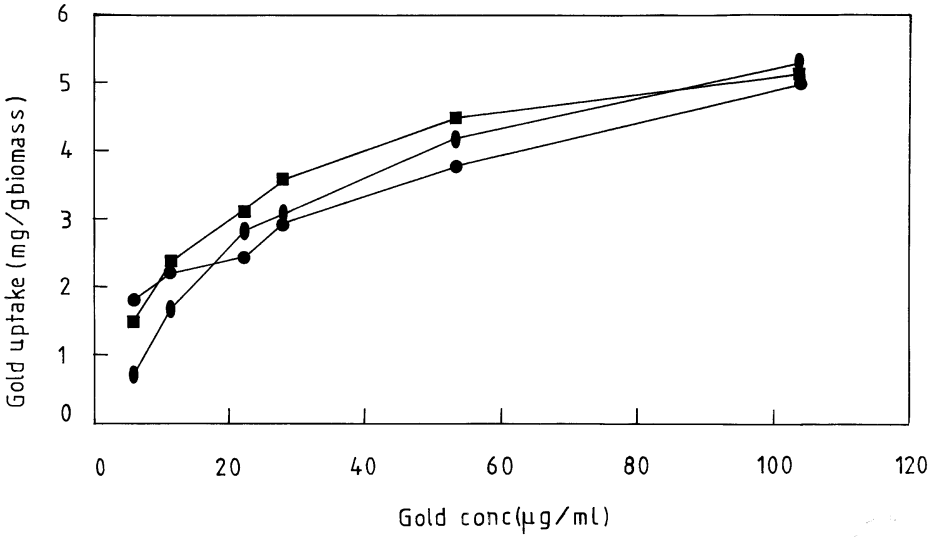

Figure 3. Effect of gold concentration on gold uptake by *Sac. cerevisiae*, *Str. erythraeus* and *S. platensis* from gold–thiourea solutions. Gold solutions (5 ml) in the range 10–190 μg ml^{-1} were added to cell suspensions (4 ml) of *Sac. cerevisiae* (●), dry weight (7.5 mg ml^{-1}), *Str. erythraeus* (●), dry weight (5.66 mg ml^{-1}) and *S. platensis* (■), dry weight (7.45 mg ml^{-1}) to give final concentrations in the range 5.8–104 μg ml^{-1} in 0.2 M KCl–0.2 M HCl solution at pH 2.0. The mixtures were incubated for 5 min at room temperature and gold uptake was measured in the supernatant solutions and in the cells by atomic absorption spectroscopy.

Langmuir plots of gold adsorption by *S. platensis*, *Sac. cerevisiae* and *Str. erythraeus* are shown in Figure 4. The Langmuir Equation is given in Equation 2 (Langmuir 1918):

$$\frac{1}{q_e} = \frac{1}{Q^o} + \frac{1}{bQ^oC} \quad (2)$$

where b (binding affinity) is a constant related to the energy or net enthalpy of adsorption, Q^o (binding capacity) is the number of moles of solute adsorbed per unit weight of adsorbent in forming a complete monolayer on the surface, q_e is the amount of the metal adsorbed per unit weight of biomass and C is the metal concentration in solution at equilibrium. Data given in Table 1 (results are shown here only for *S. platensis*) and similarly for the other two microorganisms, were used for the determination of q_e and C. In constructing the Langmuir plots, the results obtained at the lowest gold concentrations used were disregarded as these suffered from greater errors in overall gold recoveries. The errors are substantial relative to the gold bound to the biomass. It can be seen that the adsorption of gold by *Sac. cerevisiae*, *S. platensis* and *Str. erythraeus* conforms to the Langmuir model (Figure 4). The binding capacities and binding affinities of *Str. erythraeus*, *S. platensis* and *Sac. cerevisiae* and the gold–thiourea complex were determined using the Langmuir plots (Figure 4) as described previously. These values are summarized in Table 2. The capacities of *Sac. cerevisiae* and *S. platensis* are similar and slightly lower than *Str. erythraeus* which achieved the highest value of gold accumulation of all types of biomass tested. The affinities of these organisms for gold are in the order *S. platensis* > *Str. erythraeus* > *Sac. cerevisiae*.

The biosorption capacities for gold from various gold-containing solutions using various types of microorganisms are also tabulated in Table 3.

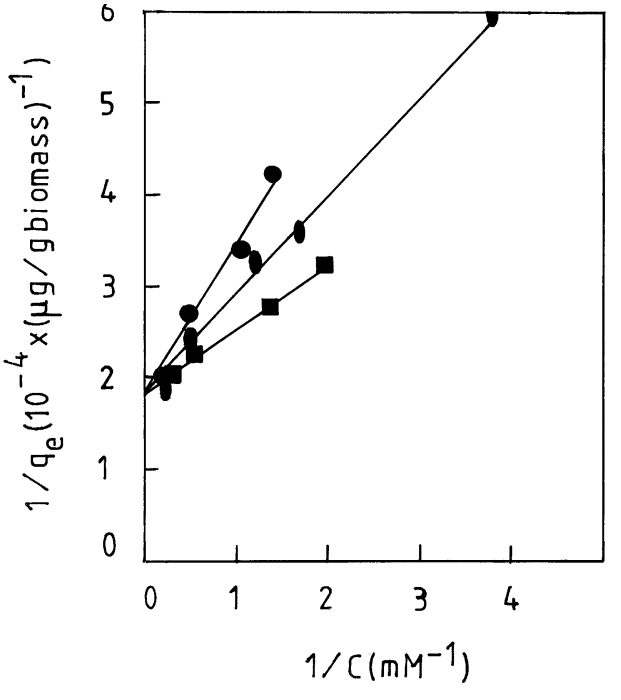

Figure 4. Langmuir plots of gold adsorption by *Sac. cerevisiae* (●), *Str. erythraeus* (●) and *S. platensis* (■).

Discussion

The uptake of gold from gold–thiourea solutions by *Sac. cerevisiae* and *Str. erythraeus* was dependent upon pH. In contrast, gold bisorption by *Spirulina platensis* was almost independent of pH (Figure 1). At pH values above the isoelectric point of the cells there will be an overall negative charge on the cells. Potential ligands such as carboxyl, phosphate, imidazole and amino groups will be ionized and available for gold and other metal ions. As the pH is lowered, the overall surface charge on the cells will eventually become positive, which will inhibit the approach of positively charged metal cations. It is likely that protons will compete with gold ions for the cell surface donors, thus decreasing the interaction of gold ions. The lack of pH-dependence on the

Table 2. Binding capacities and binding affinities of *Sac. cerevisiae*, *S. platensis*, *Str. erythraeus* and gold–thiourea complex

Microorganism	Binding capacity (10^3 μg g^{-1})	Binding affinity (mM^{-1})
Sac. cerevisiae	5.55	0.51
S. platensis	5.55	2.54
Str. erythraeus	6.00	0.93

Table 3. The biosorption capacities for gold from various gold-containing solutions using different types of biomass

Biomass	Solution	Capacity (mmol g^{-1})	Reference
S. natans	aqueous	2.1	Kuyucak & Volesky (1988)
Unknown	cyanide	0.9	Brierley *et al.* (1985a,b)
C. vulgaris	aqueous	0.5	Darnall *et al.* (1986)
S. platensis	thiourea	0.026	This work

biosorption of gold by *Spirulina platensis* suggests that the interaction of gold with the surface ligands involves covalent bond formation. This is in contrast to the behaviour of *Chlorella vulgaris* for the gold–thiourea complex $Au(CSN_2H_4)^{2+}$ where binding by the cells increased with pH and was highest at pH values 7–8 (Greene *et al*. 1986). In all other cases, because the binding of gold to *Saccharomyces cerevisiae* and *Streptomyces erythraeus* was pH-dependent, the binding of the gold to the cells could be related to interactions between the gold ions and the negatively charged groups on the cell surface. The cyanobacterium *Spirulina platensis* exhibited a higher capacity for gold biosorption at all pH values tested (Figure 1) than the other two organisms. The pH-independence of gold binding to *S. platensis* suggests that it may be possible to recover selectively individual metal ions from a mixture of metals by means of pH variation, provided the other metals have an affinity that varies with pH. The uptake of gold from gold–thiourea solutions by all organisms, namely, *S. platensis*, *Sac. cerevisiae* and *Str. erythraeus* was rapid and complete within 5 min of contact time. Biosorption of gold in all cases appears to involve binding to one type of adsorption sites, as shown by the linearity of the Langmuir plots (Figure 4). It is likely that differences in the binding affinity values for gold amongst the types of biomass used may be attributed to the cell wall composition and especially to differing proportions of the wall components. In the case of *Sac. cerevisiae*, a variety of potential accumulation sites are available in the cell wall including phosphate, carboxyl, amine and hydroxyl groups as well as components like chitin and chitosan. The cell wall of the cyanobacterium *Spirulina platensis* resembles that of the Gram-negative bacteria (Darnall *et al*. 1988). The principal components are peptidoglycans, muramic and diaminopimelic acids and N-acetyl glucosamine residues. The cell walls of *Streptomyces erythraeus* are typical of the Gram-positive bacteria with peptidoglycan as the major component of the wall and teichoic acids (which are absent from the walls of Gram-negative bacteria). The peptidoglycan of the Gram-positive bacterium *Bacillus subtilis* 168 has been found to be the primary component for metal deposition (including Au(III)) via its carboxylate group (Beveridge & Murray 1976, 1980). It is, therefore, possible that the binding of gold to *S. platensis* and *Str. erythraeus* may occur to similar adsorption sites present in the cell wall components.

The mode of gold binding to the types of organisms used in the present study can be speculated upon. It could possibly involve the adsorption of gold–thiourea complex $Au[CS(NH_2)_2]^{2+}$ by the cell wall binding groups, or there is a possibility of ligand exchange reactions leading to the formation of bonds between gold(I) and sulfur or nitrogen ligands on the cell surface, as in the case of the binding of gold to *Chlorella vulgaris* (Watkins *et al*. 1987). If gold is bound to the cells as Au(I) then it could be possibly reduced to Au(0) by the cells, as in the case of *Chlorella vulgaris* which rapidly reduces tetrachloroaurate(III) to gold(I), and then slowly reduces gold(I) to gold(0) (Greene *et al*. 1986). The possibility of some gold binding intracellularly should also be considered, as in the case of the biosorption of gold by *Sargassum natans*, where the cell wall was the main location of the gold deposition, and with also small amounts of gold found in the cell interior at longer time contacts (Kuyucak & Volesky 1988).

A comparative evaluation of the capacity of *Spirulina platensis* to adsorb gold from thiourea solutions is not possible at present, since other reports have used aqueous or cyanide solutions (Table 3). Furthermore, the binding of gold in other cases was studied using different forms of biomass i.e. immobilized biomass (Darnall *et al*. 1986) or granulated microbial product (Brierley *et al*. 1985a,b). However, to compare and assess the capacity for gold biosorption of *S. platensis* with the capacities obtained by others (Table 3), an experiment was carried out under conditions used by Kuyucak & Volesky (1988) in the recovery of gold(III) from aqueous solutions of $HAuCl_4$ by *S. platensis*. The biosorption of gold (III) was apparently independent of time over the periods studied and complete within 15 min, in marked contrast to the biosorption of gold(III) by the brown alga *S. natans* which was time-dependent and complete in 16 h (Kuyucak & Volesky 1988). From this experiment the gold biosorption capacity of *Spirulina platensis* was found to be 0.3 mmol (g biomass)$^{-1}$. This value is almost 12 times higher than that obtained in the binding of gold from gold–thiourea solutions. It also compares quite well to value quoted by Darnall *et al*. (1986), 0.5 mmol (g biomass)$^{-1}$, but is probably more promising since the experiment in the present study did not involve immobilization of the biomass.

Of all organisms used in the present study of gold uptake from gold–thiourea solutions, *Spirulina platensis* seems to be the best biosorbent for gold from gold–thiourea solutions, as it has the highest affinity for gold, the highest gold capacity even at low pH values and also binds gold very rapidly. However, the poor recovery of gold from the

gold–thiourea complex by *S. platensis* is surprising as the cationic complex $Au[CS(NH_2)_2]^{2+}$ would be expected to bind better to cell surfaces compared to anionic gold cyanide complex $Au(CN)_2^-$.

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