# Gold-cyanide biosorption with L-cysteine

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**Abstract:** L-Cysteine increased gold-cyanide biosorption by protonated *Bacillus subtilis, Penicillium chrysogenum* and *Sargassum fluitans* biomass. At pH 2, the maximum Au uptakes were 20.5 $\mu$mol g$^{-1}$, 14.2 $\mu$mol g$^{-1}$ and 4.7 $\mu$mol g$^{-1}$ of Au, respectively, approximately 148–250% of the biosorption performance in the absence of cysteine. Au biosorption mainly involved anionic AuCN$_2^-$ species adsorbed by ionizable functional groups on cysteine-loaded biomass carrying a positive charge when protonated [(biomass–cysteine–H$^+$)–(AuCN$_2^-$)]. Deposited gold could be eluted from Au-loaded biomass at pH 3–5. The elution efficiencies were higher than 92% at pH 5.0 with the Solid-to-Liquid ratio, S/L, =4. Increasing solution ionic strength (NaNO)$_3$ decreased Au uptake. FTIR analyses indicated that the main functional groups involved in gold biosorption in the presence of L-cysteine are probably N-, S- and O-containing groups. The present results confirm that certain waste microbial biomaterials are capable of effectively removing and concentrating gold from solutions containing residual cyanide if applied under appropriate conditions.
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## INTRODUCTION

Recent experimental results demonstrated that *Bacillus subtilis, Penicillium chrysogenum* and *Sargassum fluitans* biomass could extract Au from cyanide solution.[1] The main mechanism of Au biosorption involved the adsorption of anionic AuCN$_2^-$ species onto N-containing functional groups on biomass through ion-pairing (H$^+$-AuCN$_2^-$). However, the capacities for Au biosorption by *Bacillus subtilis, Penicillium chrysogenum* and *Sargassum fluitans* biomass were not encouraging.

Proteins are known to be capable of complexing with metal ions. Cysteine, which figures prominently in discussions of metal ion binding to proteins, has three possible coordination sites, namely sulfhydryl, amino and carboxylate groups.[2] Hussain and Volet[3] attributed the protection of isolated human lymphocytes from silver toxicity to cysteine through the formation of Ag–thiol complexes.[3] The complexation of Cu–cysteine was ascribed to the complexing of Cu to thiol as well as amino groups.[4] These results showed that cysteine had a tendency to combine well with metals. However, the behaviour of L-cysteine in Au-cyanide complex biosorption has never been examined. As crude cysteine could be derived from food or pharmaceutical industry waste materials, its beneficial uses are worth exploring.

The objectives of this work were to investigate the effect of L-cysteine on Au biosorption from cyanide solution by dead *Bacillus subtilis, Penicillium chrysogenum* and *Sargassum fluitans* biomass. The mechanism of Au-cyanide biosorption under these unconventional conditions was also examined.

## MATERIALS AND METHODS
### Biosorbent preparation

Waste industrial biomass samples of *Bacillus subtilis* and *Penicillium chrysogenum* were collected from Sichuan Pharmaceutical Company, Chengdu, PR China. *Sargassum fluitans* seaweed biomass was collected beach-dried on the Gulf Coast of Florida. Biomass was ground into particles around (0.5–0.85) mm in diameter, then washed with 0.2 mol dm$^{-3}$ HNO$_3$ for 4h and rinsed with distilled water to pH $\sim$4.5. Finally, the biomass was dried in the oven at 50°C for 24h to a constant weight.

### Acidification of gold-cyanide solution

Details of the procedure have been described earlier.[1] The total CN$^-$ was determined by converting all of it to free CN$^-$ using the standard cyanide distillation followed by the titrimetric method for free CN$^-$ determination in the alkaline solution.[5]

### Equilibrium sorption experiments

Dried protonated biomass (approx 40 mg) was combined with 20 cm$^3$ sodium gold-cyanide solution with

or without L-cysteine in $150\,cm^3$ Erlenmeyer flasks, concurrently. The initial cysteine concentration varied from 0 to $0.6\,mmol\,dm^{-3}$ as it was added with the biomass to the gold-containing solution. The initial gold concentrations were less than $20\,mg\,dm^{-3}$ which is in the range of industrial gold-cyanide leach solution. The solution was agitated (orbital shaker, 180 rpm) for 4h to make sure that equilibrium had been reached. Uptakes of gold were determined from the difference of metal concentrations in the initial and final solutions. The pH of the solutions before and during the sorption experiments was adjusted with $0.1\,mol\,dm^{-3}$ NaOH or $HNO_3$. The ionic strength was controlled by adding $NaNO_3$. All reagents were ACS reagent grade quality. Au concentration was determined by a sequential inductively-coupled plasma atomic emission spectrometer (Thermo Jarrell Ash, Trace Scan).

## Cysteine adsorption by biomass

Approximately 40 mg dried protonated biomass was contacted with $20\,cm^3$ cysteine solution of a certain initial cysteine concentration $0-2.0\,mmol\,dm^{-3}$ in $150\,cm^3$ Erlenmeyer flasks. The solution was mixed and left to equilibrate for 4h. The cysteine uptake was determined from the difference of cysteine concentrations in the initial and final solutions. Cysteine was analysed by a UV-visible spectrophotometer (Cary 1).

## Desorption experiments

Au desorption from Au-loaded biomass was examined by first sorbing Au onto biomass at pH 2 in the presence of L-cysteine and then desorbing Au in deionized water at equilibrium pH 3, 4 or 5. The pH level was adjusted with $0.1\,mol\,dm^{-3}$ NaOH. Gold-cysteine pre-loaded *Bacillus* biomass contained $20.5\,\mu mol\,Au\,g^{-1}$ of dry biomass, *Penicillium* biomass contained $14.2\,\mu mol\,g^{-1}$ and *Sargassum* biomass $4.7\,\mu mol\,g^{-1}$, Au–cysteine-loaded biomass (0.02 g) was then contacted with $5\,cm^3$ of the eluent solution for 4h. The percentage of Au recovery, represented by the ratio of the amount of Au released per gram of the biosorbent during desorption to the equilibrium sorption uptake, was calculated for desorption experiments.

## Fourier-transform infrared (FTIR) analysis

FTIR analysis was conducted to investigate the gold form(s) sequestered on biomass and the main functional groups for gold biosorption. The Au-loaded biomass samples were prepared by contacting 40 mg biomass with $20\,cm^3$ of $0.1\,mmol\,dm^{-3}$ Au (Au-cyanide complex) and $0.6\,mmol\,dm^{-3}$ cysteine in the solution at pH 2 for 4h. The biomass was then collected by filtration and washed with distilled water and finally dried in desiccaur with nitrogen gas at room temperature. The cysteine-loaded biomass sample was prepared under the same conditions except for the absence of Au-cyanide. Blanks of protonated biomass and solid L-cysteine samples were also prepared for

spectra comparison. Disks of 100 mg KBr containing 1% (w/v) of finely ground powder of each sample were prepared less than 24h before analysing. Infrared spectra of samples were obtained with a Michelson 100 FTIR spectrophotometer.

# RESULTS AND DISCUSSION

## Effect of L-cysteine on Au biosorption

The effect of L-cysteine on Au biosorption by *Bacillus*, *Penicillium* and *Sargassum* biomass was examined by varying the L-cysteine concentration in the Au-cyanide solution (initial Au concentration of $0.1\,mmol\,dm^{-3}$ Au) from 0 to $0.6\,mmol\,dm^{-3}$ (pH 2.0). No cyanide was released during the process. The results are summarized in Fig 1 where the ratio ($R_{cys}$) of gold uptake in the presence of cysteine to that without cysteine is plotted versus the final cysteine concentration. In the final cysteine concentration range (0–$0.5\,mmol\,dm^{-3}$), the gold uptake of all three biomass types increased, following the sequence: *Bacillus*, *Penicillium* and *Sargassum* biomass. The final cysteine concentration around $0.5\,mmol\,dm^{-3}$ enhanced Au uptakes by *Bacillus*, *Penicillium* and *Sargassum* biomass up to 250% (uptake $0.02\,mmol\,g^{-1}$), 200% ($0.014\,mmol\,g^{-1}$) and 148% ($0.0047\,mmol\,g^{-1}$), respectively.

L-Cysteine biosorption isotherms for *Bacillus, Penicillium* and *Sargassum* biomass in Fig 2 show significal uptake by the *Bacillus* and *Penicillium* biomass, while sorption by *Sargassum* was minimal. Under the experimental conditions, the sequence for the cysteine uptake by the three biomass types was *Bacillus > Penicillium > Sargassum*, the same sequence of increased Au uptake observed in the presence of cysteine.

Enhancement of Au biosorption in the presence of cysteine probably relates to the 'bridging' provided by cysteine between the Au-cyanide complex and biomass. The main active sites on the cysteine molecule
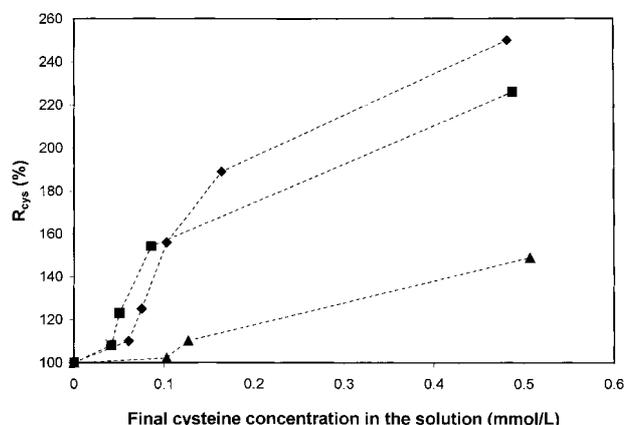


**Figure 1.** L-Cysteine enhancement of Au biosorption uptake by *Bacillus subtilis, Penicillium chrysogenum* and *Sargassum fluitans* from cyanide solution: 0.04 g biomass, initial Au concentration $0.1015\,mmol\,dm^{-3}$, $20\,cm^3$ solution, pH 2.0, incubated for 4h at room temperature. ◆, *Bacillus subtilis*; ■, *Penicillium chrysogenum*; ▲, *Sargassum fluitans*.
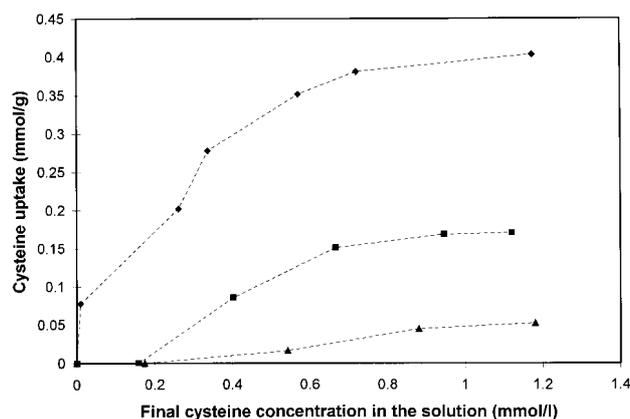
**Figure 2.** L-Cysteine biosorption isotherms for *Bacillus subtilis, Penicillium chrysogenum* and *Sargassum fluitans* biomass: 0.04 g biomass, 20 cm³ solution, pH 2.0, incubated for 4 h at room temperature. ◆, *Bacillus subtilis;* ■, *Penicillium chrysogenum;* ▲, *Sargassum fluitans.*

are amino, sulfhydryl and carboxyl groups.[4] The dissociation constants ($pK_a$) of those groups are, respectively, 10.36, 8.12 and 1.90.[6] At pH 2.0, the cysteine carboxyl group is partially deprotonated and charged negatively which enables it to combine with positively charged groups on biomass. At the same time, the cysteine amino group is protonated and charged positively, which allows for its combination with anionic $AuCN_2^-$.

*Bacillus* cell walls contain as much as 70% of the dry weight as teichoic acid.[7] This polymer (2-D-glucopyranosyl glycerol phosphate) is covalently linked to peptidoglucans[8] which contain weak base groups such as amines. *Penicillium* cell walls contain up to 40% of chitin which is linked to glucan.[9] This complex also contains amine groups. As the $pK$ (proton dissociation constant) of positively charged acetylamine groups in chitin is 3.5, while amine groups of other biomolecules have the $pK$ around 6,[10] almost all amine groups on these two biomass types could be positively charged by protons at pH 2.0. This makes them amenable to combining with the carboxyl moiety on cysteine under acidic conditions.

*Sargassum* biomass contains alginates up to 40% of its dry weight;[11] The active groups in alginates are carboxyl groups. The carboxyl groups of the biomass ($pK_a$ 3.5)[12] should be protonated and therefore carry neutral change at pH 2 and so are less likely to contribute to either cation or anion binding. The low binding of cysteine by *Sargassum* may be a consequence of the smaller amount of phenolic groups present in brown seaweeds.[13] This work confirmed that cysteine increased the Au-cyanide complex uptake by biomass and that increased Au uptake was related to the cysteine uptake by biomass.

## Effect of pH

The effect of pH on Au biosorption in the presence of cysteine was examined by varying the pH of the sorption system from 2 to 6. The initial Au concentration was around 0.1 mmol dm$^{-3}$ Au. The initial ratio

of Au:cysteine was 1:5. During the process of acidifying the Au-cyanide solution and Au biosorption equilibration, there was no cyanide released from the solution. In the case of $AuCN_2^-$ uptake, Au did not dissociate from the cyanide complex at room temperature. Cysteine-aided Au biosorption apparently still involved the uptake of anionic $AuCN_2^-$ complex. Since the pH value tended to increase during the equilibration, 0.1 mol dm$^{-3}$HNO$_3$ was used to stabilise the pH. This observation is opposite to that reported for biosorption of cationic species of Zn, Cd, and Pb(NO$_3$)$_2$ by cysteine alone.[4] Divalent ions of these metals complexed with the cysteine sulfhydryl (—S$^-$) and amino groups. During the adsorption process, the hydrogen of sulfhydryl became dissociated, accounting for the observed decrease in pH values.

The results are summarized in Fig 3 where the ratio ($R_{pH}$) of gold uptake in the presence of cysteine at different pH levels to that at pH 2 is plotted against the final pH. In the presence of cysteine, Au uptake by *Bacillus, Penicillium* or *Sargassum* biomass was strongly affected by pH. The equilibrium uptake of Au at pH 2 was greater than at pH > 2. Similar observations were reported for biosorption of anionic Cr(VI)[14] where lowering of the equilibrium pH from neutral to acidic yielded an increase in Cr uptake by *Sargassum*. This was also the case for $AuCN_2^-$ uptake by *Bacillus, Pencillium* and *Sargassum* biomass in the absence of cysteine addition.[1]

As pH decreased from 6 to 2, weak-base groups either on cysteine or in the biomass become increasingly protonated and many acquire a net positive charge since the $pK$ of positively charged weak base amine groups is around 3.5–6.[10] Carboxyl groups in biomass could also be protonated in their neutral form as the $pK_a$ is around 3–5.[15] In the pH range 2–6, some carboxyl groups on cysteine may still be dissociated as the dissociation constant ($pK$) of the carboxyl group
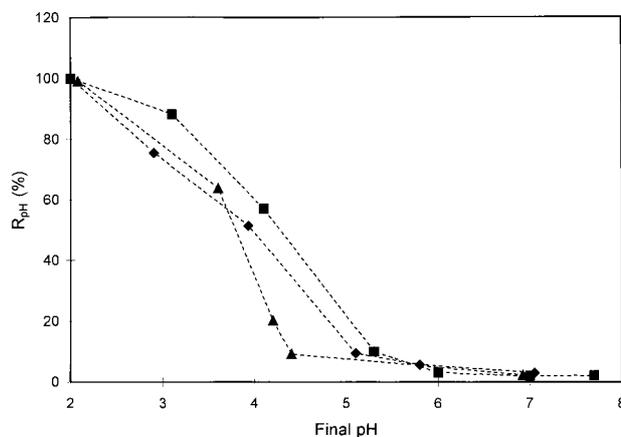


**Figure 3.** The effect of pH on Au uptake by *Bacillus subtilis, Penicillium chrysogenum* and *Sargassum fluitans* from cyanide solution. L-Cysteine was present to enhance biosorption: 0.04 g biomass, 20 cm³ solution, initial Au concentration 0.1015 mmol dm$^{-3}$, initial cysteine concentration 0.6 mmol dm$^{-3}$, incubated for 4 h at room temperature. ◆, *Bacillus subtilis;* ■, *Penicillium chrysogenum;* ▲, *Sargassum fluitans.*

on cysteine is 1.90 whereas the amino group is protonated and with a positive charge. This allows cysteine binding to biomass through the combination of negative cysteine carboxyl groups with some positively charged biomass functional groups. The positively charged cysteine amino groups became available for binding anionic $AuCN_2^-$. Therefore Au could become indirectly sorbed on biomass through cysteine as a bridge: $(BFH_2^+—^-OOC—R—NH_3^+—AuCN_2^-)$, where $BFH_2^+$ represents the biomass functional group bearing a positive charge. The biomass, having higher affinity for cysteine than for Au-cyanide, brought in extra weak base groups for binding $AuCN_2^-$ on biomass resulting in enhanced Au uptake.

While cystine enhanced Au biosorption, uptake was still lower than observed for cation biosorption.[16–18] This is probably because the sites responsible for cation binding are particularly the deprotonated, negatively charged groups which often occur in larger quantities than positively charged groups. A similar phenomenon was found in the anion ion-exchange process whereby a weak base resin would have a relatively low binding capacity with anions indirectly attached onto active sites through proton bridges.[19]

## Ionic strength effect

The Ionic strengths of experimental solutions at pH 2 were adjusted from $0\,mol\,dm^{-3}$ to $0.17\,mol\,dm^{-3}$ using $NaNO_3$. During the process, no cyanide was released from the solution, indicating that the addition of sodium nitrate did not assist in dissociating the gold-cyanide complex. The effect of ionic strength on gold biosorption is shown in Fig 4 where the ratio ($R_{I.S.}$) of the gold uptake to that without $NaNO_3$ addition is plotted versus the solution ionic strength. Increasing ionic strength reduced the Au uptake similar to what was the case in Au-cyanide adsorption by biomass only.[1] As the concentration of $NaNO_3$ increased to

$0.06\,mol\,dm^{-3}$ (ionic strength $0.07\,mol\,dm^{-3}$), the uptake of Au by *Bacillus* and *Penicillium* biomass was reduced to 70% (Au uptake $0.014\,mmol\,g^{-1}$) and 80% ($0.013\,mmol\,g^{-1}$), respectively, of the comparable Au uptake without $NaNO_3$ in the solution. The Au uptake by *Sargassum* decreased to zero already at $0.02\,mol\,dm^{-3}$ $NaNO_3$ concentration (ionic strength $0.03\,mol\,dm^{-3}$). The interfacial potential was affected by changing ionic strength and, therefore, so was the activity of electrolyte ions. In addition, the added $NO_3^-$ could compete with the gold-cyanide complex for the positively charged binding sites on cysteine or biomass as a counterion, thus reducing the Au uptake.

## Desorption of Au-loaded biomass

Au-loaded biomass samples were prepared by contacting biomass with gold-cyanide solution. Then Au-loaded biomass was eluted for 4h in distilled-deionized water which became acidified and required adjustment with NaOH to the desired pH value of 3, 4 or 5. *Bacillus* biomass, initially pre-loaded, contained $20.5\,\mu mol\,Au\,g^{-1}$ of dry biomass, *Penicillium* biomass $14.2\,\mu mol\,Au\,g^{-1}$ and *Sargassum* biomass $4.7\,\mu mol\,Au\,g^{-1}$. The Au elution efficiency (%) represented the ratio of the amount of Au released per gram of the biosorbent during desorption and the equilibrium sorption uptake. Figure 5 shows that 90% of Au was recovered from Au-loaded *Bacillus* biomass ($0.018\,Au\,mmol\,g^{-1}$) and *Penicillium* biomass ($0.015\,Au\,mmol\,g^{-1}$), and 92% from *Sargassum* biomass ($0.004\,Au\,mmol\,g^{-1}$), at pH 5 with the Solid-to-Liquid ratio, $S/L$ ($mg\,cm^{-3}$) = 4 for all of these three biomass types. This clearly indicated that Au binding was reversible. The $AuCN_2^-$ complex was probably bound to the cysteine protonated positively charged amino groups, while cysteine itself was bound on biomass through the negative cysteine carboxyl group and the protonated positively charged biomass amine group. As the pH of the sorption system increased, protons will dissociate from the positively charged



**Figure 4.** The effect of electrolyte concentration on Au uptake by *Bacillus subtilis, Penicillium chrysogenum, Sargassum fluitans* from cyanide solution in the presence of cysteine: 0.4 g biomass, $20\,cm^3$ solution, pH 2.0, initial Au concentration $0.1015\,mmol\,dm^{-3}$, cysteine concentration $0.6\,mmol\,dm^{-3}$, incubated for 4h at room temperature. ◆, *Bacillus subtilis;* ■, *Penicillium chrysogenum;* ▲, *Sargassum fluitans.*



**Figure 5.** The effect of pH on Au elution efficiency: 0.02 g biomass, $5\,cm^3$ solution, initial Au loading $20.5\,\mu mol\,g^{-1}$*Bacillus* biomass, $14.2\,\mu mol\,g^{-1}$*Penicillium* biomass and $4.7\,\mu mol\,g^{-1}$*Sargassum* biomass, incubated for 4h at room temperature. ◆, *Bacillus subtilis;* ■, *Penicillium chrysogenum;* ▲, *Sargassum fluitans.*

acetyl amine groups on the biomass ($pK_a$ 3.5), thereby rendering these groups charge-neutral so that they no longer attract the $COO^-$ of cysteine, therefore the 'bridge' ($BFH_2^+$–$OOC$—$R$—$NH_3^+$—$AuCN_2$) was broken up, and the Au-complex became dissociated from the solid phase.

## Cyanide mass balance

Further investigation of the behaviour of cyanide ($CN^-$) during the adsorption/desorption cycle was done by examing the mass balance of cyanide. The Au-cyanide solution was prepared from $NaAuCN_2$ at pH 11. The initial Au concentration was 0.102 mmol Au $dm^{-3}$ with the ratio of Au: $CN = 1:2$. The control solution of gold-cyanide (without biomass) was subjected to the same AVR procedure where biomass and cysteine was added. Table 1 shows the cyanide mass balance for the Au adsorption and desorption process as well as for the AVR process. No $CN^-$ was recovered from the control gold-cyanide solution in the AVR process, confirming that simple acidification of Au-cyanide solution with nitric acid at room temperature did not dissociate gold-cyanide complex.[1] With biomass and cysteine present in the gold-cyanide solution, the system behaved as for the control Au-cyanide system; no $CN^-$ was recovered in the Au-cyanide biosorption process. After the eluted Au solution was distilled, $CN^-$ was recovered with the ratio of Au: $CN^-$ being around 1.99.

Possible reasons for these observations were: **(a)** $CN^-$ could not be dissociated from the gold complex even though Au adsorption and desorption took place; **(b)** $CN^-$ was dissociated from the gold complex but then it was adsorbed on the cysteine-loaded biomass; **(c)** $CN^-$ was dissociated from the Au-cyanide complex then oxidized by biomass or cysteine and the CN group did not exist any more. Distillate from the eluted Au solution contained $CN^-$ (recovered $CN^-$: Au = 1.99), demonstrating that cyanide was absorbed on the biomass; this finding eliminates the third (c) possibility. If the Au-cyanide complex was dissociated, gold adsorption would involve the Au cation, and a higher pH would better facilitate that process. However, the experimental results indicated the opposite thereby eliminating the second (b) possibility. These results indicated that the presence of biomass and cysteine cannot assist in dissociation of the $AuCN_2^-$ complex.

## FTIR analysis

The investigation of the form of Au sequestered by biomass and of the main functional groups responsible was conducted using Fourier-transform infrared analyses of biomass samples. Au-loaded *Bacillus* biomass was chosen for infrared analysis in order to verify the weak-base amine group involvement in gold binding, as *Bacillus* and *Penicillium* contain an abundance of amino groups in their cell walls. *Sargassum* was more difficult to examine by FTIR because of its low Au uptake. Gold-loaded biomass samples were prepared by adding 0.04 g biomass to the aurocyanide solution containing 20 ml of 0.1 mmol $dm^{-3}$ gold and 0.6 mmol $dm^{-3}$ cysteine at pH 2. When this system reached the sorption equilibrium, the solid biomass phase contained 20.5 µmol Au $g^{-1}$ biomass. Cysteine-loaded biomass was prepared by contacting 0.04 g biomass with 0.6 mmol/$dm^{-3}$ cysteine only (pH2.0), yielding the solid phase with 0.35 mmol cysteine $g^{-1}$ biomass. Table 2 summarizes the characteristic peaks in the FTIR spectra for pure cysteine samples, protonated biomass blank, cysteine-only loaded biomass and Au-cysteine-loaded *Bacillus* biomass, respectively.

The infrared spectral data of blank protonated biomass and cysteine-loaded biomass indicate that the absorbance peak of $v(NH)$ stretching vibrations (2313 $cm^{-1}$) of the spectrum of blank protonated biomass[20] shifted to 2348 $cm^{-1}$ of the spectrum of cysteine-loaded biomass, indicating that the amine group on biomass may be involved in cysteine biosorption. As cysteine was combined on the amine of the biomass material, the stretching vibration of $v(NH)$ on biomass became difficult and therefore the peak was shifted to a higher position.

The spectrum of cysteine showed the absorbance peaks at 1139 $cm^{-1}$, 1595 $cm^{-1}$ and 2568 $cm^{-1}$, which can respectively be ascribed to $NH_3^+$ rocking, —$COO^-$ asymmetric stretch, and SH stretching vibrations.[2]

The peak of $NH_3^+$ rocking shifted from 1139 $cm^{-1}$ of the original cysteine spectrum to 1241 $cm^{-1}$ for the spectrum of cysteine-bound biomass. $NH_3^+$ is hardly combined by functional groups on *Bacillus* biomass. Because the dissociation constants of acidic groups like the carboxyl in *Bacillus* biomass are around 4.5,[16] most of those groups at pH 2.0 were protonated in their neutral form which cannot effectively attract positively charged $NH_3^+$ from the bulk solution to the micro-surface of biomass to combine with them. The

**Table 1.** $Au(CN)_2^-$ mass balance in AVR–biosorption–elution cycle

| Initial $Au(CN)_2^-$ (mmol)[a] | Initial cysteine concentration (mmol) | Final Au in biosorption solution (mmol) | Au in eluted solution (mmol)[b] | $CN^-$ recovered in eluted solution (mmol)[c] | Au: $CN^-$ in eluted solution |
|---|---|---|---|---|---|
| 0.00204 | 0.012 | 0.00122 | 0.00082 | 0.001635 | 1.994 |

[a] Initial $Au(CN)_2$ in solution: 0.102 mmol $dm^{-3}$ 20 $cm^3$ solution, 40 mg biomass.
[b] 20.4 mg biomass containing Au (0.0205 mmol $g^{-1}$) was eluted by 5 $cm^3$ of solution at pH 5.
[c] $CN^-$ was recovered from distillation of eluted Au solution.

| Species | IR bond vibration | | | | |
|---|---|---|---|---|---|
| | $v(CN)$ | $v(NH)$ | $NH_3^+$ rocking | —$COO^-$ asym stretch | $v(SH)$ |
| Protonated biomass blank | / | 2313 | / | / | / |
| Cysteine | / | / | 1139 | 1595 | 2568 |
| Cysteine-loaded biomass | / | 2348 | 1241 | 1651 | 2572 |
| Au–cysteine-loaded biomass | 2280 | 2348 | / | 1642 | 2634 |

**Table 2.** Infrared spectral frequency data (cm$^{-1}$) summary

shift of $NH_3^+$ rocking was most probably because of the spatial structure limitations of bound cysteine which also may bind to other anions, eg to the —$COO^-$ of the next cysteine, forming a chain of cysteine.

Another IR spectrum shift identifiable between the blank cysteine and cysteine-loaded biomass was the peak of carboxyl groups on blank cysteine (1595 cm$^{-1}$) shifting to a higher position (1651 cm$^{-1}$) when cysteine was bound by biomass. As the p$K_a$ of $COO^-$ cysteine was 1.9,[6] when adsorption occurred at pH 2.0, most of protons dissociated from carboxyl groups on cysteine which became mainly negative —$COO^-$ able to combine with the positively charged amine groups on the biomass (eg amine of chitin on biomass, p$K_a$ = 3.5[10]). It could thus be deduced that the carboxyl groups of cysteine may be involved in cysteine adsorption.

The peak of —SH was not identifiably changed (4 cm$^{-1}$) between the blank cysteine and cysteine-bound biomass, indicating that the —SH group on cysteine was not involved binding cysteine to biomass.

It appears that the cysteine binding may involve the combination of carboxyl groups on cysteine and amino groups on biomass. This may explain why *Sargassum* cannot effectively extract cysteine from solution at pH 2.0, as compared with *Bacillus* and *Penicillium*, since it lacks the amino groups, the main component in the *Sargassum* cell wall being alginate. The important active site of alginate is the carboxyl group which is in its neutral form at pH 2, its p$K_a$ being 3.5.[12]

On the spectrum of cysteine-loaded biomass and Au–cysteine-loaded biomass, the peak of (—NH) did not change, indicating that cysteine remained bound to amine groups on the biomass when Au was bound. The peak of $NH_3^+$ rocking on cysteine-loaded biomass disappeared on the spectrum of Au-cysteine-loaded biomass. These results indicate that amine groups of cysteine may be involved in Au adsorption. Amine is typically a weak-base group. In the anion exchange, primary, secondary, and tertiary amine-containing polymers are the principal sorbents used for anion sorption.[19] As the —$NH_3^+$ bound Au, possessing a high atomic weight, the rocking vibration of $NH_3^+$ disappeared.

Another peak of —SH stretching vibrations was found to shift from 2572 cm$^{-1}$ on cysteine-only loaded biomass to 2634 cm$^{-1}$ for Au–cysteine-loaded biomass. Sulfhydryl groups could donate a lone electron pair for the empty orbit of metal ions alone.[4] The cysteine sulfhydryl group probably co-combined $AuCN_2^-$ with the cysteine amine group through a bridging proton whereby the amine lone electron pair becomes shared with that proton to form a relatively stable spatial structure resulting in the shifting of stretching vibrations of —SH to a higher position.

The peak of —$COO^-$ asymmetric stretch on cysteine-loaded biomass did not change significantly (9 cm$^{-1}$) compared with that on Au–cysteine-loaded biomass, indicating that the —$COO^-$ moiety was not effectively involved in Au binding. Furthermore, the spectrum of Au–cysteine-biomass featured a peak at 2280 cm$^{-1}$ which was ascribed to $v(CN)$ of the Au-cyanide complex.[21] There was no peak of the $v(CN)$ vibration of free $CN^-$ on the spectrum of Au–cysteine-loaded biomass which should be located at 2080 cm$^{-1}$.[21] The FTIR results confirmed that cyanide was existing as a $AuCN_2^-$ complex in the Au-loaded biomass.

The results imply that the main functional groups on cysteine involved in Au adsorption probably were amino and sulfhydryl groups. The cysteine binding on biomass most probably resulted from the cysteine carboxyl group combining with the biomass amino group. The results also confirmed that $AuCN_2^-$ is such a stable complex that groups on biomass or cysteine cannot effectively compete for Au with cyanide and displace it during the sorption binding, they can only bind the whole Au-cyanide complex on weak-base groups through a proton ($H^+$) bridge.

## CONCLUSIONS

The following conclusions can be drawn from the results and discussion above:

(1) The presence of L-cysteine enhanced Au biosorption from cyanide solution by *Bacillus, Penicilium* and *Sargassum* biomass.
- Au adsorption in the presence of cysteine is preferred at lower pH. At pH 2, the Au uptake by *Bacillus* biomass increased to 20.5 µmol Au g$^{-1}$, *Penicillium* sequestered 14.2 µmol Au g$^{-1}$, and *Sargassum* 4.7 µmol Au g$^{-1}$.
- The sequence of increased Au uptakes by different biomass types agreed with that of capacity for cysteine adsorption by biomass: *Bacillus* > *Penicillium* > *Sargassum*

(2) The Au uptake significantly decreased with

increasing NaCl concentration from $0.005\,mol\,dm^{-3}$ to $0.15\,mol\,dm^{-3}$.

(3) Au binding is reversible. More than 92% of Au bound to biomass at pH 5 could be eluted with an NaOH solution using the solid-to-liquid ratio of 4 $(mg\,cm^{-3})$.

(4) The FTIR analysis confirmed that the main biomass functional groups involved in gold-cyanide biosorption are probably S-, N-, and O-containing groups on cysteine or on biomass.

(5) Cysteine-enhanced Au binding most probably results from binding the anionic gold-cyanide complex to the cysteine $NH_3^+$ groups while cysteine carboxyls bind to cationic groups in the biomass.

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