CHEMICAL PROCESSES INVOLVED IN Au(III) BINDING AND BIOREDUCTION BY ALFALFA BIOMASS

[1]G. Gamez, [1]K. Dokken, [1]I. Herrera, [1]J. G. Parsons, [1]K. J. Tiemann, and [1]J. L. Gardea-Torresdey
[1]Department of Chemistry and Environmental Science and Engineering, PSCI Bldg., University of Texas at El Paso, El Paso, TX 79968; Phone: (915) 747-5359; Fax: (915) 747-5748.

ABSTRACT

Current precious metal recovery technologies that use harsh chemicals may represent a threat to the environment and public health. Thus, there is a necessity to develop environmentally friendly systems to retrieve precious metals. In previous experiments, dead alfalfa biomass has shown to be efficient for gold(III) recovery. Gold(III) binding to alfalfa biomass is rapid, pH independent, and increases with time and temperature. Furthermore, with X-ray absorption spectroscopy, alfalfa was found to reduce gold(III) to gold(0) and produce nanoparticles. Transmission electron micrographs showed nanoparticles of several shapes and sizes. The reduction process was also found to be pH, time, and temperature dependent. In order to resolve the mechanism of both binding and reduction of gold(III) by alfalfa biomass, further experiments were performed. The effect of chemical modification of the alfalfa biomass on gold(III) binding and reduction was determined. Also, further X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectroscopy studies were performed to assess the oxidation state and nearest neighbor of the bound gold as a function of time. The results indicate that gold(III) goes to gold(I) and then to gold(0). Also, EXAFS shows that binding may occur via a nitrogen or oxygen ligand.

Key words: *XANES, EXAFS, alfalfa, gold, phytofiltration, metal binding, chemical modification*

INTRODUCTION

Classical methods to recover gold from its ore, such as cyanidation and thiourea leaching, utilize harmful chemicals that can find their way to the environment(Addison, 1980; Hiskey, 1985; Deschenes, 1998). Thus, they represent a threat to the environment's integrity and also to the health of the public. This brings us to the need to develop new recovery technologies which are environmentally friendly, cost-effective, and efficient.

The ability of plants to uptake gold has been long known (Girling and Peterson, 1980). They may do this either actively, by utilizing the plant metabolism, or passively, by means of the functional groups on their tissues. Thus, we may take advantage of this ability of plants to uptake gold by using their dead tissues and taking advantage of their functional groups to recover gold ions from aqueous solutions.

Gardea-Torresdey et al. have found that dead alfalfa tissues (Medicago sativa) have an appreciable ability to adsorb gold(III) ions from solution (1999a). It was found that gold binding to alfalfa behaved almost pH independently. Also, it was seen to increase with temperature (Gamez et al., 2000). It was also found that the gold reacting with the alfalfa biomass was reduced to elemental gold colloids (Gardea-Torresdey et al., 1999b). The capacity of alfalfa to reduce gold(III) would augment the perfomance of a recovery system for gold from aqueous solutions based on dead alfalfa tissues. In order to enhance such a system, or develop similar ones, the mechanism(s) by which alfalfa uptakes and reduces gold needs to be understood.

The objective of this study is to gain further insight into the chemical processes of uptake and reduction of gold(III) by alfalfa. For this

purpose, the time-dependence behavior of the binding was characterized. Also, esterification of the biomass was performed to asses the role of carboxyl moieties on the mechanism. Furthermore, X-ray absorption spectroscopic analysis was done on several gold(III)-laden samples to see how the gold changed oxidation states and its chemical environment.

MATERIALS AND METHODS

Alfalfa sample collection

The alfalfa samples for this study were acquired from controlled agricultural fields studies at New Mexico State University at Las Cruces, N. M. The harvest conditions were described by Gardea-Torresdey et al., but basically the roots were separated from the shoots, oven dried at 90 °C, and ground to pass a 100-mesh screen (1996). The shoot biomass was used for all the experiments because it showed to have a higher capacity toward gold binding in previous experiments (Gardea-Torresdey et al., 1999a).

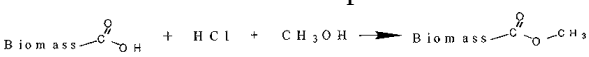
Time dependence

A sample of 2 g of alfalfa biomass was washed twice with 0.01 M HC1 and once with DI water in order to remove any soluble molecules from the alfalfa biomass that may interact with the gold ions. Then, the biomass was resuspended in 200 ml of DI water (10mg/ml), and its pH was adjusted to 5, using very dilute sodium hydroxide (NaOH). Eight ml of biomass mixture was transferred to test tubes labeled 5 min, 1 hr, 3 hr, 4 hr, and 12 hr. These were centrifuged and the biomass pellets were reacted with 8 ml of a gold(III) solution at pH 5.0 made from $KAuCl_4$ (59.1 ppm or 0.3 mM). After their respective times, the tubes were centrifuged and the supernatants analyzed for gold(III) content by flame atomic absorption spectroscopy. To obtain good statistics, the experiment was done in triplicate. The pellets were washed with DI water to remove any unbound gold(III), freeze dried, and saved for XAS.

Chemical modification

Esterification methods were described by Tiemann et al (1999). A Fisher esterification was performed. The biomass was reacted with excess methanol using dilute acid as a catalyst.

The basic reaction is represented below.



Capacity experiments were performed with the modified biomasses as previously decribed (Gardea-Torresdey et al., 1999a) and compared to the native biomass.

X-ray absorption spectroscopic measurements

The X-ray absorption spectra were collected at Stanford Synchrotron Radiation Laboratory, beamline 7-3, for the Au L_{III} edge, and the standard operating conditions were 3 GeV and 60-100 mA beam current. The spectra for the samples were taken in fluorescence mode, but for the model compounds (diluted with boron nitride), transmission mode was used. One-milimiter, path-length aluminum holders with tape windows were used to run the samples as solids. The monochromator had Si(111) crystals, an entrance slit of 1 mm, and was detuned to 50%. Several scans were averaged for each X-ray absorption near-edge

structure(XANES) and extended X-ray absorption fine-structure (EXAFS) spectra (~2 to 4).

The EXAFSPAK software package was used to analyze the X-ray absorption data using standard methods (George and Pickering, 1995; O'Day et al., 1994). The resulting scattering curve from the EXAFS data was weighted by k^3. This was followed by a Fourier transformation to yield a curve similar to a radial structure function, uncorrected for phase shifts, that contains the distance data of the nearest neighbors to the absorbing atom. The FT-EXAFS from model compounds was used to compare to the samples to get structural information.

RESULTS

Time dependence

The behavior of gold(III) adsorption and/or reduction as a function of time is depicted in Figure 1. The process starts fairly rapidly and then slows down. It can be observed that after four hours, practically all the gold is out of the solution. This implies that by this time the gold is either adsorbed or reduced. At 12 hours, we do not see any more gold in solution at trace levels.

Chemical modifications

The gold(III) binding capacities for the differently treated biomasses are shown in Table 1. The esterified biomass capacity is 30.8 mg of gold/g of biomass. This represents a 23.2 % decrease in capacity as compared to the native(untreated) biomass. Thus, carboxyl groups may be involved in the binding of gold(III).

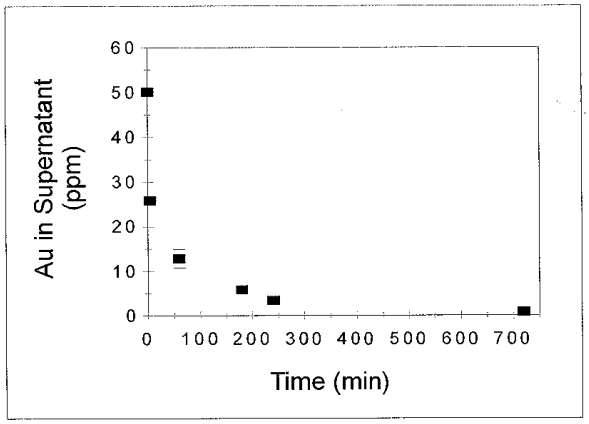


Figure 1. Time-dependence behavior of gold(III) binding to alfalfa biomass. The y-axis shows the amount of gold in the supernatant after the reaction time had taken place.

X-ray absorption spectroscopic experiments

The XANES spectra for selected samples of the time-dependence experiments are shown in Figure 2. It may be observed that the Au-biomass 5-min sample shares the peak around the edge rise (~11922 eV) with the Au(III) acetate model compound but is less intense. This peak is called white line and its high intensity is characteristic of Au(III) compounds (Elder and Eidsness, 1987). Also, the intensity of this line drops even more for the Au-biomass 3-hr sample and its edge features approximate that of the Au(I) sulfide model compound.

Table 1. Gold-binding capacity for the esterified biomass as compared to the unmodified biomass.

	Capacity (mg Au/g biomass)	% Decrease
Unmodified Biomass	40.1	
Esterified Biomass	30.8	23.2

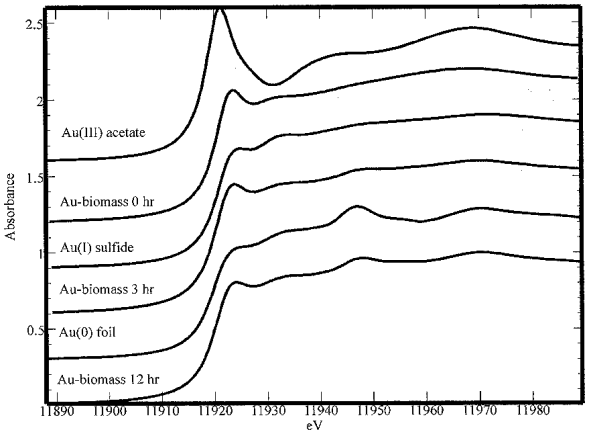


Figure 2. XANES spectra for the time-dependence experiment samples: 5 min, 3 hr, and 12 hr. Included are the absorption edges of the model compounds: Au(III) acetate, Au(I) sulfide, and Au Foil.

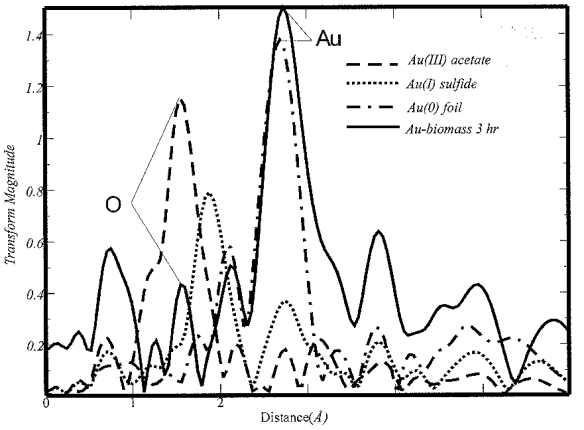


Figure 3. FT-EXAFS of the 3-hr Au biomass sample compared to Au(III) acetate, Au(I) sulfide, and Au foil model compounds.

Finally, the Au-biomass 12-hr sample shows a peak at ~11945 eV and a much lower intensity white line. The peak at ~11945 eV is characteristic of gold metal (Elder and Eidsness, 1987).

Figure 3 shows the EXAFS spectra for the Au-biomass 3-hr sample along with gold-acetate, gold-sulfide, and gold-foil model compounds. It is clear that the sample shares a peak with the gold-acetate model compound. This could represent a gold-oxygen bond length. On the other hand, it does not share a peak with the gold-sulfide model compound. Furthermore, the sample also shares the peak that corresponds to the gold-gold bond seen in the gold-foil model compound.

DISCUSSION

An increase in the removal of gold from the supernatant with time was shown by the time-dependence experiment. Approximately 4 hr passed before practically all the gold in solution had been either adsorbed or reduced. It may be concluded that the uptake of gold(III) by alfalfa biomass should involve a more complex mechanism than ion exchange because these types of reaction are known to occur very rapidly (Nakajima and Sakaguchi, 1993).

A capacity reduction of 23.2% was caused by the esterification of the alfalfa biomass. This suggests that adsorption of gold(III) by alfalfa biomass involves carboxyl groups. Gold tetrachloroaurate is known to exist as the $AuCl_4^-$ square planar anion in solution at pH from ~ 2 to 5.5 (we are working at pH 5) and excess chloride concentrations (Farges et al., 1993). Also, at this pH the carboxyl, groups are mostly deprotonated (negatively charged). Thus, it would be more likely that the carboxyl groups were involved in a ligand exchange mechanism rather than an electrostatic interaction.

The XANES for the 5-min sample of the time-dependence experiment shows a shift of the white-line feature to higher energies, accompanied by a depression of the intensity. The white-line feature in gold is known to shift to

higher energies when the oxidation number decreases (Elder and Eidsness, 1987). In addition, the depression in the white line intensity suggests that the gold is going to lower oxidation states. This is because the white-line arises from 2p to 5d electronic transitions and the electronic configurations of gold(1) and gold(0) should have most 5d states occupied (Pantelouris et al., 1995). This, in conjunction with the absence of a gold(0) peak, suggests that there is a rapid reduction to gold(I). In addition to showing the same trend in the white-line feature, the 3-hr sample shows a peak at ~11945 eV. As mentioned before, this peak is characteristic of gold(0) and can be seen for particles larger than 10 Å (Elder and Eidsness, 1987). Thus, after three hours, some gold(III) has been reduced to gold(0). Furthermore, the 12-hr sample shows a very clear gold(0) peak and almost no signs of a white line. These XANES results suggest that gold(III) goes to gold(I) before going to gold(0), and that the reduction to from gold(I) to gold(0) is much slower than that from gold(III) to gold(I).

The nature of the near-neighbor atoms surrounding the gold can be characterized by EXAFS. This is achieved by probing the backscattering interactions of the ejected photoelectron from the X-ray-absorbing gold atom. Nevertheless, EXAFS cannot distinguish between elements that are close-row neighbors in the periodic table (e.g., O from N) due to their similar phase shifts and scattering potentials (Penner-Hahn, 1999).

The FT-EXAFS of the time-dependence, 3-hr sample shows a gold-oxygen(or nitrogen) peak and a gold-gold peak, but no gold-sulfur peak. These results are consistent with the ones obtained for the chemical modification experiments since they indicate an oxygen-containing ligand. The absence of the gold-sulfur peak was not expected because experiments performed with other biosorbents indicate that the adsorption of gold should involve sulfur-containing moieties. Sulfur-donor binding sites are known to stabilize Au(I) (Sadler, 1976). It has been proposed that sulfur groups are involved in the biosorption of gold(III) by *Chlorella vulgaris,* but they do not account for all the gold binding as amino modification reduced 50% of the binding (Greene et al., 1986; Darnall et al., 1986). Thus, there is also the possibility that amino groups are involved in the mechanism, especially since at low pH, they are positively charged and the gold(III) exists as $AuCl_4^-$.

It is not unreasonable to believe that the majority of the gold goes to oxygen/nitrogen-containing ligands since even though there might be gold-sulfur preferential binding, there might not be enough sulfur groups. It has been known that gold will bind to a low-Z atom such as nitrogen or oxygen if denied access to sulfur; Au(I) will do this in order to achieve two-coordination (Elder and Eidsness, 1987).

On the other hand, the reduction of $AuCl_4^-$ may occur via several mechanisms. The tetrachloroaurate ion could undergo hydrolysis reactions to finally produce elemental gold (Kuyucak and Volesky, 1986). Also, reduction by photolysis, which involves UV radiation, could be occurring (Bronstein et al., 1999). Control solutions do not show gold reduction,

thus these processes might not be playing an appreciable role. This means that the biomass is the major one responsible for the gold reduction. The next step is to try to identify the moiety that gets oxidized on the biomass. It has been demonstrated that proteins containing cysteine and other disulfides are able to reduce gold(III) to the elemental state (Shaw III et al., 1980). Nevertheless, we do not see a gold-sulfur peak in the EXAFS spectra. A possible explanation for this is that sulfur groups are involved in the reduction, but that there are so few gold-sulfur bonds that we cannot see them with the EXAFS technique we employed. On the other hand, hydroxyl groups on sugar balls have been known to reduce Au(III) to Au(0), thus the complex carbohydrates on the alfalfa cell wall could be the ones responsible for the reduction of $AuCl_4^-$ (Kunio et al., 2000).

CONCLUSION

These studies have shown that there could be several mechanisms involved in the binding and reduction of $AuCl_4^-$ by alfalfa biomass. Carboxyl moieties are involved and it is very possible that the hydroxyl groups are responsible for the reduction. More importantly, it was shown that Au(III) goes through Au(I) before going to Au(0). It is possible that $AuCl_4^-$ is involved in ligand exchange, as well as electrostatic interactions with the biomass to make the electron transfer possible. This information should help enhance the ability of alfalfa as a potential phytofilter for the recovery of gold from aqueous solutions, as well as producing gold colloids of useful characteristics.

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