SPECTROSCOPIC STUDIES OF GOLD(III) BINDING TO ALFALFA BIOMASS

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ABSTRACT

A need to develop environmentally friendly technologies to retrieve precious metals has arisen due to the current mining methods which involve the use of harsh chemicals that endanger the community's health. Alfalfa biomass has shown in previously performed experiments that it has a substantial ability to bind gold(III) from aqueous solutions in a rapid and pH-independent manner. Furthermore, the gold(III) solutions reacted with the alfalfa biomass turned purple, indicating gold(III) reduction to gold(0). Also, time and temperature dependence experiments were conducted to study the gold(III) to gold(0) reduction process at pH 2.0 and pH 5.0. The results obtained show that gold(III) bioreduction is a time, temperature, and pH-dependent process. X-ray adsorption near edge structure (XANES) and extended X-ray adsorption fine structure (EXAFS) data corroborated the reduction of gold(III) to gold(0). We have no knowledge of the actual mechanism of the bioreduction process. However, we are currently performing experiments *in situ* to determine the pathway involved by using XANES and EXAFS techniques.

Key words: *XANES, EXAFS, alfalfa, gold, metal binding, phytofiltration, bioreduction, bioadsorption*

INTRODUCTION

The technology currently implemented for the recovery of gold from its ore has involved the use of harsh chemicals which may threaten the environment, as well as the population's health. These methods include cyanidation, thiourea leaching, and amalgamation (Deschenes et al.,1989; Addison, 1980; Cho and Pitt, 1983). Furthermore, the gold demand keeps increasing due to its vast applications in coinage, jewelry, plating, photography, dentistry, electronics, infrared reflectors, and more (Carson et al., 1986; Lucas, 1985). Therefore, there is a need to develop novel technologies to recover gold which are environmentally friendly and less threatening to the population.

The ability of plants to uptake metal ions has been studied for some time (Lujan et al., 1994; Gardea-Torresdey et al., 1996a; 1996b; 1996c; 1996d). The mechanisms which may be involved in the uptake of metal ions are intracellular accumulation and surface adsorption. The former mechanism is an active process because the plant must be alive to carry it out. On the other hand, surface adsorption is a passive process because the chemical groups attached to the cell walls of the plants can bind metal ions even when the plant is dead. The ability of live plants to uptake metal contaminants has been taken advantage of by phytoremediation technologies. On the other hand, if the chemical groups attached to the cell walls, or binding sites, can adsorb the metal ions, we may use the dead plant tissues to filter such ions out of aqueous solutions. This technology is called phytofiltration.

Accumulation of precious metals in plants has been noticed since the late 1900's (Girling and Peterson, 1980). This information has been used for prospecting purposes (Rapson, 1982). Few biomasses, however, have been studied for their precious metal adsorptive abilities. For example algal biomass *(Persimmon tannin)* and alfalfa are some of the few biomasses that have been studied for gold bioadsorption (Nakajima and Sakaguchi, 1993; Greene et al., 1986; Gardea-Torresdey et al., 1999).

The purpose of this project is to study dead alfalfa plant tissues as a potential phytofilter for the recovery of gold(III) ions. Alfalfa biomass was chosen because it has been studied extensively by Gardea-Torresdey and coworkers, and it has been determined that alfalfa tissues have an appreciable ability to adsorb heavy metal contaminants from aqueous solutions (Gardea-Torresdey et al., 1996c; Tiemann et al., 1999). In addition, previous studies with alfalfa biomass show that it binds an appreciable amount (40 mg of gold/ g of alfalfa) of gold(III) ions in aqueous solutions (Gardea-Torresdey et al., 1999). These studies have shown that gold(III) binds to alfalfa rapidly and in a pH-independent manner from pH 2.0 to pH 6.0 (with a slightly better binding at pH 2.0). Furthermore, Gardea-Torresdey et al. showed that most of the gold adsorbed to the biomass could be recovered (87%) and that silica-immobilized alfalfa columns (studied by X-ray Micro fluorescence) may be recyclable for additional gold(III) binding.

The gold solutions that reacted with the alfalfa biomass in these previous experiments were observed to turn purple. These distinctive purple samples were analyzed by Transmission Electron Microscopy (TEM) and the presence of colloidal gold(0) was confirmed. These results indicated that the biomass was able to reduce gold(III) to gold(0) by a certain mechanism which yielded colloids of different shapes and sizes. The shapes of the colloids included irregular, icosahedral, decahedral, tetrahedral, and hexagonal particles (Gardea-Torresdey et al., 1999).

The objectives of this project were to further characterize the gold(III) reduction mechanism by alfalfa biomass. The effects of temperature on this mechanism were also studied via batch experiments. This experiment will also give us an insight on the type of binding sites involved in the process through the calculation of apparent enthalpies. The time for the reduction (at different pHs) was also studied in batch experiments. In order to determine the oxidation state of the gold bound to the alfalfa biomass, X-ray Absorption Near Edge Structure (XANES) analyses were performed. The coordination environment of the gold adsorbed onto the alfalfa biomass was also determined by extended X-ray Absorption Fine Structure (EXAFS) analyses that were also performed.

EXPERIMENTAL METHODS

Alfalfa Biomass Collection

The Malone population of *Medicago sativa* (alfalfa) was used for this study. The samples were acquired from controlled agricultural field studies at New Mexico State University at Las

Cruces, N.M. The roots were removed from the shoots (stems and leaves) and were oven dried at 90°C for a week. Finally, they were ground with a Wiley mill to pass a 100-mesh screen.

Time Dependence for Gold(III) Reduction

In order to remove any soluble molecules from the alfalfa biomass, which may interact with the metal ions, a 300 mg sample of alfalfa biomass was washed with 0.01 M hydrochloric acid (HC1) twice and with de-ionized (DI) water once. Also, to account for any biomass lost, the washings were collected, dried, and weighed. The biomass was resuspended in 60 ml of DI water to have a 5 mg/ml solution. The solution was adjusted to pH 5.0, using a small amount of dilute sodium hydroxide (NaOH). Clean plastic test tubes were labeled 1, 4, 8, 12, 24, 48, 72, and 96 hrs, respectively. Three different test tubes were labeled at each time interval for statistical purposes. Next, 2.0 ml of the biomass solution were transferred to each of the test tubes to have approximately 10 mg of biomass in each test tube. All of the test tubes were centrifuged at 3000 rpm for 5 minutes and the supernatants were removed in order to leave just the biomass in the test tubes. Then, 2 ml of a 0.3 gold(III) solution (made from the salt of Kau(III)Cl_4) at pH 5.0 were added to each test tube. The tubes were then equilibrated on a rocker at room temperature for their appropriate time interval. Controls of the metal solution alone were also equilibrated. After their respective time intervals, the samples were then centrifuged at 3000 rpm for 5 minutes; the supernatants were removed;and the pellets were saved for analysis. This experiment was repeated in the presence of buffer at pH 5.0 and again at pH 2.0. The experiment with buffer at pH 5.0 was done with the following changes: the initial biomass sample was washed also with 0.01 M sodium acetate, and the gold(III) solution contained 0.01 M sodium acetate buffered to pH 5.0. The experiment at pH 2.0 was performed with the following changes: the initial biomass sample was washed with additional 0.01 M HC1 instead of DI water, and the gold(III) solution was adjusted to pH 2.0. The extent of gold reduction was followed by UV-Visible spectroscopy.

Temperature Dependence for Gold(III) Binding and Reduction

A sample of alfalfa biomass (250 mg) was washed with 0.01M HC1 twice and once with DI water. The washings were saved to account for biomass lost. Fifty ml of DI water were utilized to resuspend the alfalfa biomass. This solution was adjusted to pH 5.0, using dilute sodium hydroxide. Clean plastic test tubes were labeled 4, 25, and 55°C. Three samples per temperature were performed for statical purposes and to assure quality control of the data. Next, 2 ml of biomass solution were put into each test tube. The test tubes were then centrifuged at 3000 rpm for 5 minutes and the supernatants were removed. A 0.3 mM solution of gold(III) was made with potassium tetrachloroaurate salt (K(Au(III)Cl_4)) and the pH was adjusted to 5.0. Before the experiment, all the tubes' separate gold(III) control solutions were equilibrated to their respective temperatures for an hour: the 4°C in a refrigerator, the 25°C on a lab bench top, and the 55°C in an oven. After everything had reached the desired temperature, the respective gold(III) solutions were

transferred to the samples and were equilibrated on a rocker at their specific temperatures for an hour. Also, controls of gold(III) solution and biomass were put to equilibrate. This experiment was repeated in the presence of buffer at pH 5.0 and again at pH 2.0. The experiment with buffer was done with the following changes: the initial biomass sample was also washed with 0.01 M sodium acetate at pH 5.0 and the gold(III) solution contained 0.01 M sodium acetate buffered to pH 5.0. The experiment at pH 2.0 was performed with the following changes: the initial biomass sample was washed with more 0.01 M HC1 instead of DI water, and the gold(III) solution was adjusted to pH 2.0. After an hour, the samples were centrifuged and their supernatants were collected and saved for analysis. The final pH levels of the supernatants were recorded and the analyses for gold content were performed by flame atomic absorption spectroscopy. The extent of gold reduction was followed by UV-Visible spectroscopy.

Van't Hoff's equation

$$\left(\ln\left(\frac{K_2}{K_1}\right)\right)=\left(\Delta H^0\,\frac{(T_2-T_1)}{RT_2T_1}\right)$$

was used in order to calculate the apparent enthalpies. The K values were substituted by distribution ratio values (D) from ion exchange calculations that represent the ratio of the concentration of metal ion adsorbed to the concentration of metal ion in solution at equilibrium (Greene and Darnall, 1988). The use of these ratios is justified by the complexity of the binding sites on the alfalfa tissues.

Flame Atomic Absorption Analyses

A 3110 Perkin Elmer Atomic Absorption Spectrometer with background subtraction was used to determine the amount of gold present in the superenatants of the batch experiments. As specified by the Perkin Elmer Manual, the parameters used were air-to-fuel ration, 2:1; slit, 0.7 nm high; current, 10 mA: and wavelength, 242.8 nm. The instrument was calibrated with known standards and a correlation coefficient for the calibration curve was always 0.98 or greater. Three analyses of each sample were performed and the mean value and relative standard deviation were computed. The samples were diluted when necessary to stay within the calibration range. In order to improve the sensitivity, an impact bead was used. The difference between gold concentration in the control and the supernatants was assumed to be adsorbed to the alfalfa biomass.

UV-Visible Analyses

A double-beam Lambda 14 Perkin Elmer UV-Visible spectrometer was used to determine the amount of gold reduction. Cells with a 1.0 cm path length were used. The pellets were resuspended in glycerine to prevent sedimentation of the colloids. The slit was set to 0.50 nm and the scan speed to 240 nm/min. All the samples were put through one scan cycle. The absorbance at approximately 550 nm, adopted from previous studies, was used to determine the gold colloid formation (Darnall et al., 1986).

Silica-Immobilized Alfalfa Biomass Loading

The process of Malone alfalfa biomass silica-immobilization has been previously reported (Gardea-Torresdey et al., 1996d). A 100 mg sample of immobilized Malone shoots was reacted with 30 ml of a 1000 ppm solution of gold(III) at pH 2.0 and at pH 5.0 (no buffer). The salt utilized for the preparation of the gold(III) solution was potassium tetrachloroaurate ($K(Au(III)Cl_4)$).

Extended X-ray Absorption Fine Structure and X-ray Absorption Near Edge Analyses

The National Synchrotron Light Source line X-18B was used to make the X-ray absorption measurements, which were performed at room temperature. The data was collected with silicon (Si) 111 monochromator crystals and a resolution of ~1-2 electron volts(eV) was obtained. The standard ion detectors were used at the gold (Au) L_{III} edge in transmission mode. The biomass samples were washed with DI water before analysis to remove any unbound gold(III) solution. The ($K(Au(III)Cl_4)$) standard was measured as a solid on tape and the samples as solid powders in 5x15x1 mm cells with tape windows. The inflection point energy for Au foil, 11921 eV, was used to calibrate absolute energy positions. To analyze the EXAFS data, the MacXAFS EXAFS analysis package was used. The E_0 values were determined from the absorption edge inflection point. Nonlinear fits based on the general Extended X-ray Absorption Fine Structure (EXAFS) equation were used to quantitatively compare unknowns and standards. Theoretical simulations were performed with an *ab initio* curved-wave single scattering EXAFS simulation code, FEFF 3.11 (Tiemann et al., 1999).

RESULTS AND DISCUSSION

Figure 1 shows the percent Au(III) bound to the alfalfa biomass with respect to temperature. As is seen in Figure 1, the behavior of the gold(III) binding is greatly affected by temperature. As can be observed in all the experiments performed at the different pHs, the amount of gold bound to the alfalfa biomass increased with temperature. Also seen in this figure, the amount of gold bound follows the hierarchy: pH 2.0 > pH 5.0 unbuffered > pH 5.0 buffered. This may indicate that the buffer (0.1M sodium acetate) is affecting the gold(III)-binding process. The buffer (sodium acetate) may interfere the gold(III) ions in such a way that it is more difficult for the binding sites on the alfalfa to adsorb them. At pH 2.0 there is more binding, so it may be assumed that there are more binding sites available on the surface of the cell walls at this pH.

Table 1 shows the apparent heats of formation for all the experiments performed. The apparent heats of formation can give us insight into the type of ligands that may be involved in the binding of gold(III) ions. The sign (+/-) of the heats of formation is an average of all the individual heats of formation for each binding site. For example, the majority of carboxyl ligands that complex with metal ions have small positive enthalpies. However, the amino and sulfhydryl metal ion complexes have large negative enthalpies (Greene and Darnall, 1988). It may be observed from Table 1 that all of the temperature ranges have positive enthalpies for all the experiments. This indicates that there

might be a greater involvement of carboxyl groups in the binding or reduction of gold(III) by alfalfa biomass. This is consistent with the fact that the higher heats of formation are observed at pH 5.0 which is above the pKa value for most carboxylic groups. Therefore, at pH 5.0 the carboxyl groups should be deprotonated and have a negative charge available for electrostatic binding. In addition, the fact that the apparent heats of formation are lower at pH 2.0 suggests a lesser involvement of carboxyl groups. This could mean that there is a combination of ligands with different pKa values. In addition, if carboxyl groups are involved, the effects of the buffer on the adsorption process could be explained by a competition between higher affinity and lower affinity binding sites. The buffer may be shifting the ligand metal affinity, causing a reduced binding of gold(III) to the biomass.

Figures 2 and 3 show the increase of gold(III) reduction by the alfalfa biomass with temperature (at ~ 550 nm). Figure 2 shows that at pH 5.0 (unbuffered), the amount of colloids increases greatly from 4°C to 25°C, and a smaller increase is observed from 25°C to 55°C. Figure 3 shows that at pH 2.0, the amount of colloids increases slightly from 4°C to 25 °C and a somewhat larger increase is seen from 25°C to 55°C. When this data is compared with the apparent heats of formation and the percent gold bound, we may see there is more gold bound at pH 2.0 but less gold(III) reduction. At pH 5.0 (unbuffered), there is less binding but certainly more gold(III) reduction. In addition, the apparent heats of formation at pH 5.0 (unbuffered) are greater than at pH 2.0. This suggests that there may be a large involvement of carboxylic groups in the reduction process and to a lesser extent of involvement in the binding process.

The data for the reduction as a function of temperature for the pH 5.0 buffered experiment shows an increasing trend. However, it has less colloids present than the data for the pH 5.0 unbuffered experiment. This may indicate that the acetate is also affecting the reducing moieties by affecting the binding sites. This indirectly demonstrates that the gold(III) has to first be bound before reduction occurs. Finally, we may conclude that temperature increases the reduction of gold(III) to gold(0) by the alfalfa biomass.

Something worth noting is the change in trends of the absorbance outside the ~ 550 nm region. This may be due to a probable change in colloid size distribution or shape (or both) as an effect of temperature, which will affect the solution's color. The dependence of the size and shape distribution of gold colloids has already been established for gold(III) reduction to gold(0) by sodium citrate (Chow and Zukoski, 1994). In addition, the reduction of gold(III) to gold(0) by alfalfa biomass may yield five different types of particles with different distributions: Fcc tetrahedral, hexagonal platelet, icosahedral multiple twinned, decahedral multiple twinned, and irregular; the irregular and icosahedral are the most common (Gardea-Torresdey et al., 1998). Furthermore, applications for gold colloids are growing, for example in cell biology and immunocytochemistry, optical microscopy, photoelectron, photon, and fluorescent microscopy. The shape of the colloids may be better for

certain applications than others and the distribution of the colloids may be changed in order to get most of the desired shape by changing pH, and time as well as temperature parameters.

Figures 4 and 5 show the time dependency of the gold(III)-reduction process (at ~550 nm). Figure 4 shows the increase of colloidal gold(0) as a function of time for pH 5.0 (unbuffered). It may be observed from the figure that the reduction keeps increasing at 72 hrs of reaction. Figure 3 shows the increase of colloidal gold(0) as a function of time for pH 2.0. Here, the reduction reaches a maximum by 12 hrs of reaction time. This indicates that as the reaction time increases, the reduction also increases. Furthermore, the reduction at pH 5.0 (unbuffered) is faster and greater than the reduction at pH 2.0.

Figure 6 shows the X-ray Absorption Near Edge Structure (XANES) spectra for the silica-immobilized alfalfa samples reacted with gold(III) solution at different pHs; also included on this spectra are the model compounds. The gold(III) model compound ($K(Au(III)Cl_4)$) edge shows a shift to lower energy compared to gold(0) model compound (gold foil). Furthermore, the alfalfa samples at both pH 2.0 and pH 5.0 show identical inflection points to the gold foil (within experimental error). This proves that the gold(III) reacted with the alfalfa biomass at both pHs. It was reduced to gold(0) and no gold(III) remained on the biomass. In addition, the extended X-ray Absorption Fine Structure (EXAFS) data shown in Figure 7 demonstrates that the near-neighbor environment for the samples is the same as that for the gold foil. This data indicates a lack of ligand coordination and proves that the reduced gold is not bound to the biomass. This further confirms the reduction of gold(III) to gold(0) by alfalfa biomass.

CONCLUSION

The mechanism involved in the reduction of gold(III) to gold(0) by alfalfa biomass is still not fully understood. The experiments performed, however, give insight as to what binding sites may be involved in the binding and reduction process. The temperature dependence showed the possible involvement of carboxylic groups. The time experiments showed that this process is quicker and more efficient at pH 5.0 rather than pH 2.0. Also, x-ray absorption spectroscopic studies reveal that gold(III) is reduced and that gold (0) is plated out of solution. There is a need to perform further studies to follow the reduction of gold(III) as it changes oxidation states. Additional experiments where functional groups are blocked may be performed to follow the reduction of gold(III). With this data we may be able to optimize the potential of alfalfa as a phytofilter to be used in the recuperation and reduction of gold ions from the refining industry and mining effluents.

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Table 1. Apparent heats of formation for gold(III) reacted with alfalfa biomass.

Apparent ΔH° in (kJ/mol)		
	4-25°C	25-55°C
pH 5.0 buffered	8.27	22.34
pH 5.0 unbuffered	16.29	40.74
pH 2.0	3.81	35.83

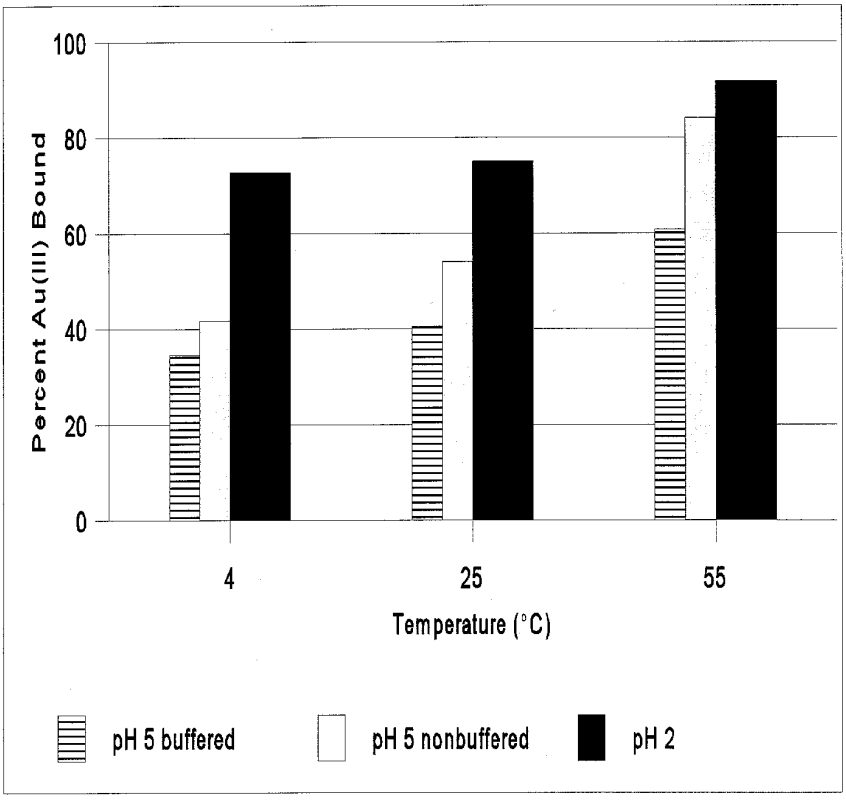

Figure 1. Effect of temperature on gold(III) binding by Malone alfalfa shoots. This experiment was performed at temperatures of 4°C, 25°C, and 55°C with biomass at pH 2.0, pH 5.0 unbuffered, and pH 5.0 buffered.

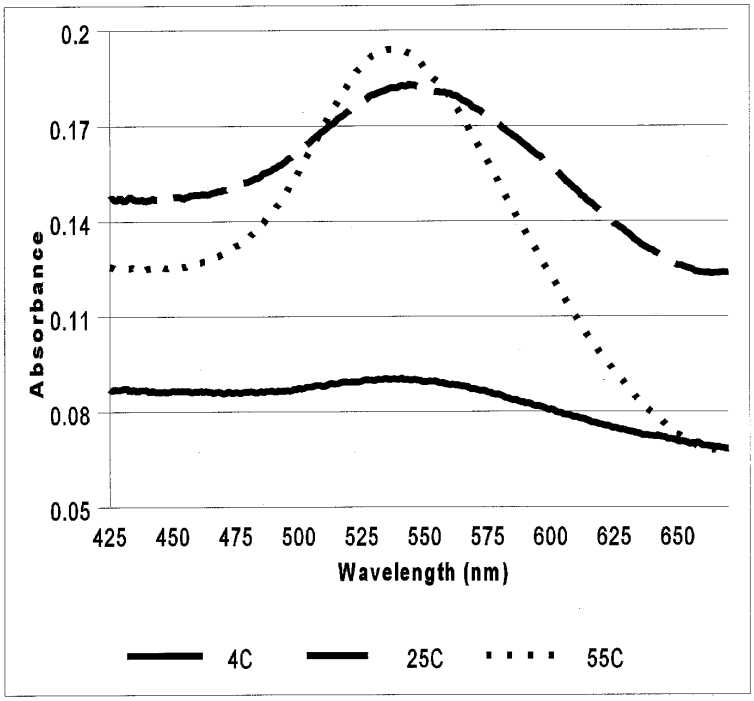

Figure 2. Effect of temperature on gold(III) reduction by Malone alfalfa shoots. This experiment was performed at temperatures of 4°C, 25°C, and 55°C with pH 5.0 unbuffered alfalfa biomass.

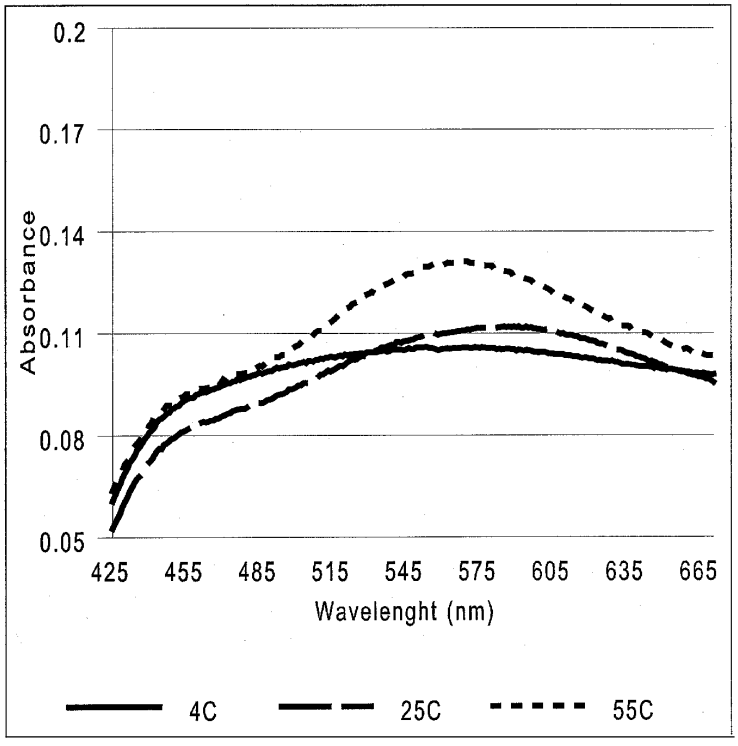

Figure 3. Effect of temperature on gold(III) reduction by alfalfa biomass. This experiment was performed at at temperatues of 4°C, 25°C, and 55°C with pH 2.0 alfalfa biomass.

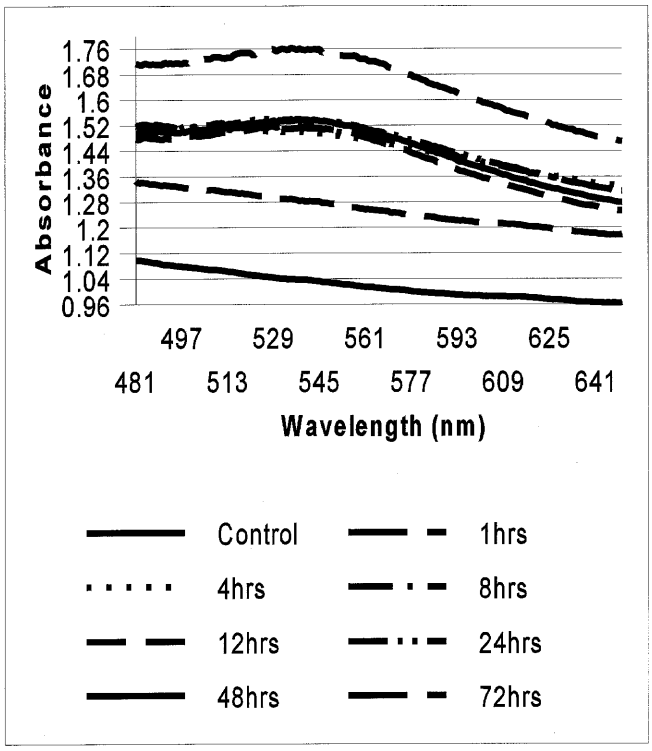

Figure 4. Time dependence of gold(III) reduction by Malone alfalfa shoots. This experiment was performed, at room temperature with pH 5.0 unbuffered alfalfa biomass.

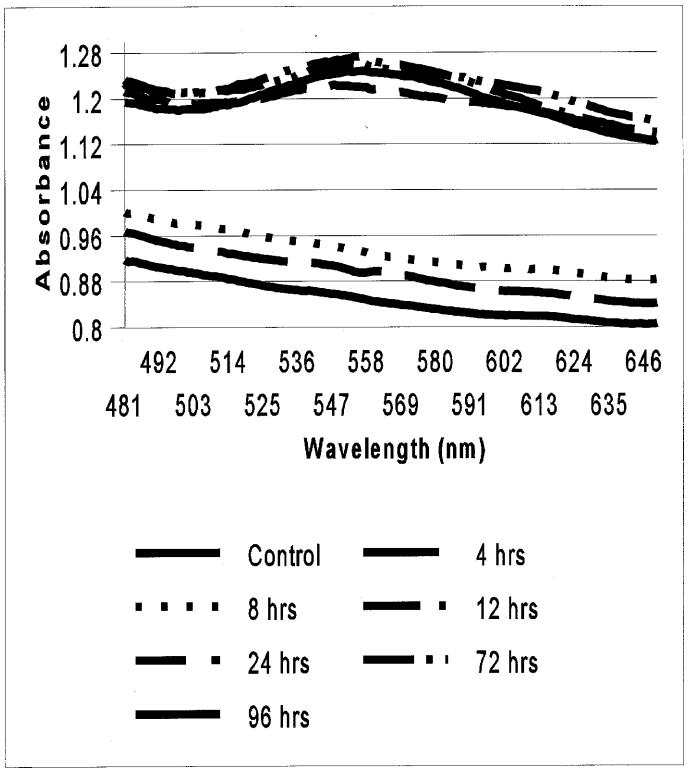

Figure 5. Time dependence of gold(III) reduction by Malone alfalfa shoots. This experiment was performed at room temperature with pH 2.0 alfalfa biomass.

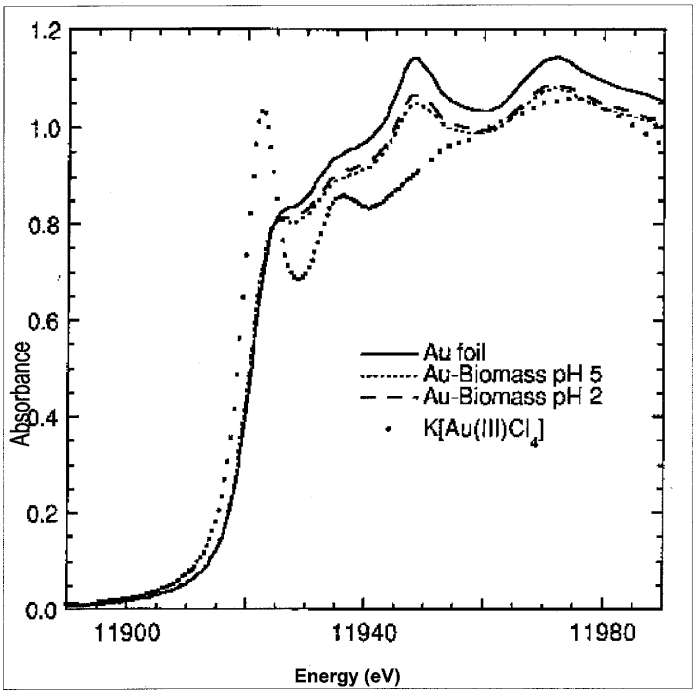

Figure 6. X-ray Absorption Near Edge Structure spectra (XANES) of silica-immobilized alfalfa reacted with gold(III) solutions) and model compounds.

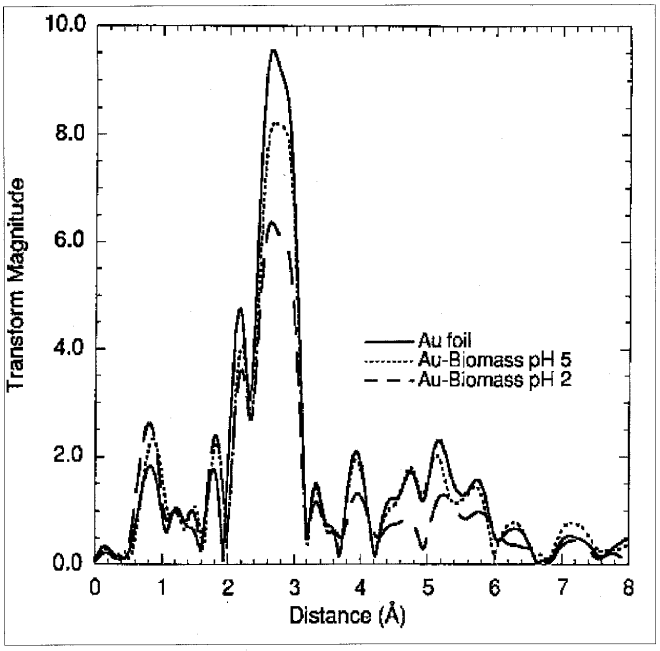

Figure 7. Extended X-ray Absorption Fine Structure spectra (EXAFS) of silica-immobilized alfalfa reacted with gold(III) solutions) and gold foil.