DETERMINATION OF TRACE-LEVEL GOLD(III) BINDING TO ALFALFA BIOMASS USING GFAAS WITH ZEEMAN BACKGROUND CORRECTION

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ABSTRACT

Due to current technologies used to extract gold, increasing environmental pollution has occurred. Existing technologies for the extraction and recovery of gold include cyanidation, which uses toxic cyanide. Because of the potential health threat and waste generated by the cyanidation process, an increasing need to develop new technologies to extract and recover gold from aqueous solution has arisen. Previous experiments performed have shown that alfalfa has the ability to bind and subsequently to reduce gold(III) to gold(0). These experiments have also shown that gold(III) at high concentrations is adsorbed by the alfalfa biomass almost independently of pH. Batch laboratory pH profile experiments have been performed at trace levels for both native biomass and chemically modified biomass using a 100 ppb gold(III) aqueous solution. However, at trace gold(III) concentrations, the adsorption of gold(III) has been observed to be pH dependent. The pH profiles performed for both the native and esterified biomass were between pH 2.0 and pH 5.0. The observed trend in the binding by the native alfalfa biomass was highest at pH 3.0 (97%) and decreased to (approximately 50%) pH 4.0 and pH 5.0. However, the esterified biomass had an opposite binding trend, a low binding at pH 2.0 and 3.0 (50%) and a sharp increase in binding at pH 4.0 with a maximum binding at pH 5.0 (90%). To explore the effects of hard cations on the trace-level gold(III) binding, calcium, magnesium, and sodium were used. In addition, experiments were performed using all three cations combined in solution to further explore the effects of multiple hard cations on the trace-level gold(III) binding to the alfalfa biomass. All experiments showed that under optimal conditions, the binding was not greatly affected by the presence of the hard cations.

Key words: *alfalfa, gold, Zeeman effect, GFAAS bioremedation, phytoremedation*

INTRODUCTION

In the past few decades, both heavy metal contamination and by-products from metal production have become a large concern. Metal processing has created large environmental problems, such as acid mine drainage and the leaking of toxic heavy metals into the environment (Winterbourn et al., 2000). The processing and production of metals such as gold and silver use cyanidation methods, which provide an easy route for cyanide to enter the environment.

Current technologies to extract metals from wastewater, such as ion exchange, precipitation, coagulation, activated carbon, and membranes have many problems associated with their use. Ion exchange technologies use resins, which are produced by a polymerization process and usually have leachates or toxic chemicals contained within them (either by-products from polymerization or unreacted reactants such as trichloro ethane) (Petruzzelli et al., 1997). Another problem with ion exchange resins is their inability to work in hard waters, where they become quickly exhausted. The use of either precipitation or coagulation requires harsh chemicals and is costly (Ayres, 1997). Membrane technologies such as reverse osmosis are usually very expensive to operate and maintain (Rautenbach et al., 2000). Therefore, there is a need to develop more cost-effective methods to clean heavy metals from the environment in an environmentally friendly manner.

Recently, phytofiltration has been investigated as an alternative to the aforementioned techniques to remove heavy metal ions from wastewater. Phytofiltration is a method that offers an inexpensive and effective alternative to current water treatment methods. The technique of phytofiltration has been proven to work effectively at the laboratory scale for both single-metal and multi-metal solutions at high concentrations (ppm levels) (Gardea-Torresdey et al., 1999). The actual premise behind phytofiltation is to use dead plant biomass as a sorbent for heavy metals. For example, alfalfa (*Medico sativa*) has shown great promise as a biosorbent for copper(II), lead(II), zinc(II), chromium(III), and chromium(VI) (Gardea-Torresdey et al., 1999). Another biomass that has been studied is peat moss which has also shown the ability to removal copper(II) from aqueous solution (Gardea-Torresdey et al., 1996). The cresote bush has also shown excellent potential to sorb chromium(III), chromium(IV), lead(II), nickel(II), and zinc(II) (Gardea-Torredey et al., 1997). Many other biomasses have been found to be effective biosorbents for heavy metals such as algal biomasses, seaweeds, and bacteria (Kratochvil and Volesky, 1998). For phytofiltration, there are two main processes used; the biomass alone is added to a heavy metal solution to adsorb the metals or the biomass is immobilized into a polymer matrix such as a silica polymer (Kratochvil and Volesky, 1998).

Although phytofiltration has been proven to work effectively with high metal concentrations, very little data exists for the extraction of trace-level metal ion concentrations (ppb levels) from solutions with this method. To achieve trace-level metal determination, several analytical methods can be employed for analysis of metal ions in solution at sub ppm (parts per million) concentrations. Three of these methods are inductively coupled plasma mass spectroscopy (ICP/MS), inductively coupled plasma atomic emission spectroscopy (ICP/AES), and/or graphite furnace atomic absorption spectroscopy (GFAAS). Of these methods, GFAAS is one of the best to determine trace-level metal concentrations because of its proven long use in the analytical field and the effectiveness of the Zeeman effect for background correction, which allows for better detection limits and better sensitivity.

GFAAS uses four basic steps drying, ashing, atomization, and clean out. The first step, drying, is used to eliminate the solution part of the matrix and to eliminate spattering (which may cause loss of the analyte). The second step, ashing, is used to remove the matrix components (non-solution components such as ions and salts). The third step, atomization, is where the analyte is put into the gaseous phase as an exited atom which absorbs the analytical line emitted from the source. The fourth step, clean out, is used to remove any residuals of the analyte or matrix from the graphite tube (Gardea-Torresdey et al., 1995). An important part of the drying, ashing, and atomization steps are matrix modifiers. The matrix modifiers have two purposes in GFAAS. The first is to make the matrix more volatile (thus reducing any spattering that may occur),

and the second purpose of the matrix modifier is to make the analyte less volatile (allowing the atomization to occur at higher temperature). Thus, there is less loss of the analyte in pre-atomization stages, providing a cleaner atomization (Gardea-Torresdey et al., 1995).

The objectives of this study were to develop a method for determining trace-level gold(III) (as tetrachloroaurate) in solution using GFAAS and to determine the ability of alfalfa biomass to bind trace levels of gold(III). In addition, this study was designed to determine the effect of pH on the binding of trace levels of gold(III) (100 ppb) to the alfalfa biomass and the time required for gold(III) sorption to take place on the alfalfa biomass. In addition, it was necessary to explore the effects of varying concentrations of hard cations (calcium, magnesium, and sodium, and all three cations combined together) on the sorption of trace levels of gold(III) to the alfalfa biomass, with cation concentrations varying from 0.1 ppm to 1000 ppm.

METHODOLOGY

Determination of the Furnace Parameters

The furnace was optimized for maximum sensitivity by varying the ratios of the matrix modifiers and the atomization temperature. In this study, it was found that the modifiers (magnesium nitrate and palladium nitrate) were inhibiting the atomization of the gold. The ashing temperature was dropped to 180 ^{0}C because of this problem, and the atomization temperature was varied. A wavelength of 242.8 nm was used for all the gold analysis (refer to Table 1 for the furnace parameters for gold analysis). Calibration of the instrument was performed in the linear working range for the analysis of gold up to a concentration of 100 ppb. The correlation coefficient of the calibration curve was 0.99 or greater for all the analyses performed. Known standards were periodically checked to ensure the quality of the data.

Alfalfa Collection

The alfalfa was collected from controlled field studies at New Mexico State University as previously described by Gardea-Torresdey et al. (1998). The plants were removed from the soil and washed throughly to remove large particles and debris from them. After washing, the shoots (stems and leaves) were oven dried at 90^{0}C for one week. The dried biomass

Table 1. The furnace program used for the gold analysis.

Temperature (^{O}C)	110	130	180	1800	2400
Read	off	off	off	on	off
Ramp Time (S)	1	5	10	0	1
Hold Time (S)	20	30	20	5	2
Argon Flow (mL/S)	250	250	250	0	250

samples were then ground using a Wiley Mill to pass through a 100-mesh sieve.

PH Profile for Gold(III) Binding

Batch laboratory experiments were performed for the pH studies. A 200-mg sample of alfalfa was washed three times with 0.01 M nitric acid (HNO_3) and twice with deionized (DI) water. The washing was performed to remove any soluble material or debris that may have interacted with the metal ions in solution. The biomass was resuspended in 40 mL of DI water to create a solution of 5 mg of biomass per mL of solution. The biomass suspension was adjusted to a pH of 2.0 and 2.0 mL of the biomass solution was transferred to a 5-mL plastic tube. This was performed in triplicate for statistical purposes. The pH of the biomass was adjusted to the following: 3.0, 4.0, 5.0, and 6.0 and allowed to equilibrate at each respective pH. The adjustment of the pH of the biomass was done with dilute HNO_3 or dilute sodium hydroxide (NaOH), and 2.0 mL of each pH-adjusted biomass was transferred to 5-mL plastic tubes (in triplicate). The biomass solution was then centrifuged at 3,000 rpm for five minutes and the supernatants were discarded. The gold(III) 100-ppb solution was made from tetrachloroaurate in DI water and adjusted to the appropriate pH using either dilute HNO_3 or dilute NaOH. To the pH-adjusted biomass pellet, 2.0 mL of the pH-adjusted gold(III) solution was added to the respective pH-adjusted biomass. At each pH, control solutions of gold(III) were set up and all tubes were equilibrated for one hour on a rocker. After equilibration, the biomass solutions were centrifuged at 3,000 rpm for five minutes and the supernatants were collected and analyzed for metal content. The final pH of the solutions was measured and gold analysis was performed with a Perkin Elmer 4100ZL GFAAS with Zeeman background collection. Calibration of the instrument was performed in the range of analysis up to a gold concentration of 100 ppb. The correlation coefficient of the calibration curve was 0.99 for all analyses performed. The difference between the concentration of the control and the biomass solution was assumed to have been the amount of gold sorbed by the biomass.

Chemical Modification of the Alfalfa Biomass

This experiment was performed similarly to one previously described by Gardea-Torresdey et al.(1996). A 3.0-g sample of biomass was added to a 1,000-mL, three-neck flask followed by 300 mL of HPLC-grade methanol, to make a biomass solution of 0.04 mg/mL. To this solution, 2.5 mL of concentrated hydrochloric acid was added to make a 0.1-M acid solution, which was used to catalyze the reaction. Next, the reaction vessel was heated to 60 ^{o}C and kept at a constant temperature for six hours using a condenser cooled with water, while the sample was continually stirred. The sample was then washed three times with DI water and centrifuged as described earlier. The samples were then placed in liquid nitrogen for 45 minutes until completely frozen and subsequently lyophilized (in a Labconco Freeze Dry System/ Freezone 4.5) . The chemically modified biomass was reacted with gold(III) solution at

different pH values, ranging between 2.0 and 5.0 by the method previously described in the pH profile study. All gold(III) supernatants resulting from the controls and reactions were analyzed using GFAAS.

Time-Dependency Experiments

Batch experiments were performed with the alfalfa biomass for the time-dependency study. A 200-mg sample of alfalfa was washed three times, once with 0.01 M nitric acid and twice with DI water. The biomass was resuspended in 40 mL of DI water to create a solution of 5 mg of biomass per mL. When observing the gold(III) time dependency for binding, the biomass and gold(III) were adjusted to a pH of 3 (pH 3 was the observed optimal pH for gold(III) binding to the alfalfa biomass). In the gold(III) time-dependency studies, 2.0 mL of the biomass solution was transferred to a 5-mL plastic tube. This was performed in triplicate for statistical purposes. The adjustment of the pH of the biomass was performed as described earlier. Tubes were equilibrated at the following times: 5, 10, 15, 30, 45, and 60 minutes. The equilibrated solutions were centrifuged as described earlier, and the gold analysis was performed with graphite furnace atomic absorption.

Interference-Binding Studies

Batch experiments were performed with the biomass for the binding-interference study. A 200-mg sample of alfalfa biomass was washed three times, once with 0.01 M nitric acid and twice with DI water. The biomass was resuspended in 50 mL of DI water to create a solution of 5 mg of biomass per mL. The biomass was adjusted to a pH of 3.0 (the observed maximum binding pH for gold(III) and the alfalfa biomass), and 2.0 mL of the biomass solution was transferred to a 5-mL plastic tube (this was performed in triplicate for statistical purposes). The pH-adjusted biomass was then centrifuged at 3,000 rpm for five minutes and the supernatants were discarded. Gold(III) solutions (100 ppb) with the following hard cation concentrations were made for the interference-binding study: 0.1 ppm, 1.0 ppm, 10.0 ppm, 100 ppm, and 1,000 ppm for each of the interferences of either calcium, magnesium, or sodium. Another study performed was to observe the effect of all three interfering ions together in solution at the same concentrations (0.1 ppm of calcium, magnesium, and sodium combined together in solution, which was increased up to 1,000 ppm). The pH of each solution was adjusted to pH 3.0 using dilute NaOH or HNO_3 (as mentioned earlier, pH 3 was the optimal pH for gold(III) binding to the alfalfa biomass). Control gold(III) solutions containing the same concentrations of the hard cations as the reactions were also set up as described previously. An aliquot of 2.0 mL of each solution was added to three different tubes, and the tubes were equilibrated on a rocker for one hour. After equillibration, the samples were centrifuged at 3,000 rpm for five minutes, and the supernatants for both the reactions and controls were collected for gold analysis. The gold analysis was performed using GFAAS as described earlier.

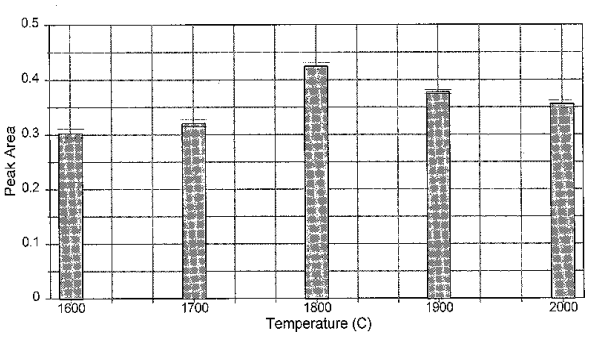

Figure 1. The effect of temperature on the atomization of gold in the furnace.

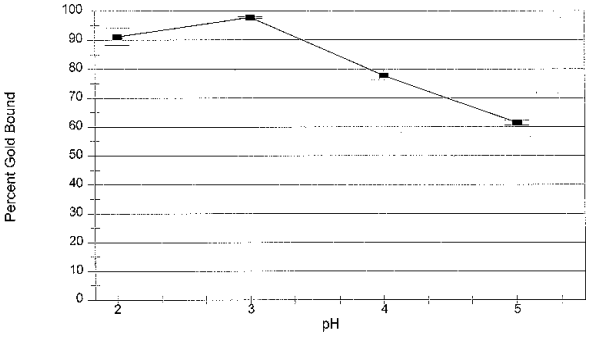

Figure 2. The effect of pH on the binding of tetrachloroaurate (gold(III)) to alfalfa biomass from aqueous solution at trace levels.

RESULTS AND DISCUSSION

To optimize the graphite furnace, parameters for the analysis of metals involved varying the ratios of matrix modifiers, ashing temperature, and atomization temperature. However, for the analysis of gold in this study, it was found that the modifiers were either inhibiting the atomization of gold from the graphite platform, or the modifiers were making the gold more volatile. Therefore, use of the matrix modifiers was eliminated from the furnace program. To elevate some of the problems associated with not using matrix modifiers, the ashing temperature was lowered to 180 ^{O}C. The best atomization temperature was determined by varying the atomization temperature and observing the peak area of the analyte or gold in this study. As can be seen in Figure 1, the optimal temperature for gold atomization under the defined furnace parameters was 1,800^{O}C. This temperature was chosen because the observed peak area of the gold deviated the least from the ideal peak area of 0.500 A-s (absorbance seconds). All gold analysis was performed using the parameters displayed in Table 1.

In all of the experiments, except for the time-dependency ones, the samples were reacted with the gold for one hour and the difference between the controls and the reacted samples was assumed to be the amount bound by the alfalfa biomass.

Figure 2 shows the pH dependency for the binding of trace levels of tetrachloroaurate to the alfalfa biomass. In Figure 2, it can be seen that at trace concentrations gold binds to the alfalfa biomass in a pH-dependent manner. The maximum binding of the gold(III) to the alfalfa biomass occurred at pH 3.0 and decreased almost linearly to pH 5. However, the binding of gold(III) to the alfalfa biomass at high concentrations shows a different trend, as can be observed in Figure 3. This difference in binding at trace and high levels may be an artifact of the presence of high affinity ligands in or on the alfalfa biomass (ligands such as sulfur and amino groups). At high gold(III) concentrations, the gold may be forced to bind to low-affinity ligands, but at trace levels the binding may occur through the high-affinity ligands only. In addition, the difference may be due to a charge

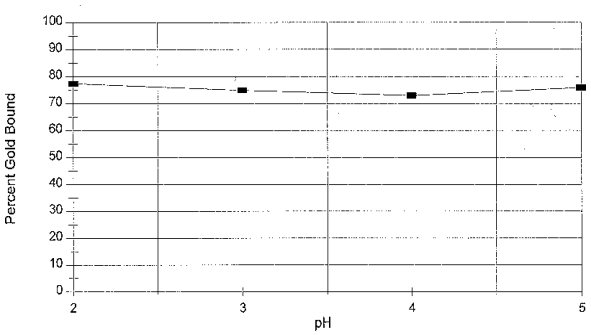

Figure 3. The effect of pH on the binding of gold(III) to alfalfa biomass at high concentrations.

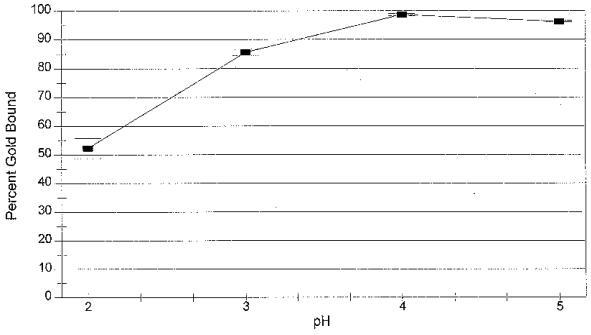

Figure 4. The effect of pH on the binding of gold(III) to chemically modified (esterified) alfalfa biomass at trace levels.

repulsion of the tetrachloroaurate and the surface of the alfalfa biomass. The tetrachloroaurate is present in solution as a negatively charged complex, and at above pH 4, the surface charge on the biomass should be negative. This would explain the observed reduction in the binding of the tetrachloroaurate to the alfalfa biomass with increasing pH.

By using the Fisher esterification method, the carboxyl groups can be blocked or changed to a methyl ester. By blocking the carboxyl group, a different trend in the binding of the trace gold(III) levels to the alfalfa biomass is observed. As can be seen in Figure 4, the binding becomes directly dependent on the pH. However, at low pH, the observed binding of the alfalfa biomass is decreased by 40 percent. Nevertheless, by blocking the carboxyl group, at higher pH values the surface charge on the alfalfa biomass is changed from a negative to a neutral charge. This change in the surface charge of the alfalfa biomass may allow the gold(III) ions to get closer to the alfalfa biomass at higher pH and allow an increase in the binding to occur as was observed by the esterified biomass in Figure 4. The binding of the gold(III) to the esterified alfalfa biomass is further evidence that the carboxyl group is not responsible for the binding of the gold(III) to the alfalfa biomass.

The results from the time-dependency study for the binding of trace-level gold(III) to the alfalfa biomass are shown in Figure 5. As can be seen in Figure 5, the majority of the binding occurs within the first five minutes of contact; however, the binding does not become constant until after 20 minutes of contact of the biomass and gold(III) solution. It has been observed by Gardea-Torresdey et al. that the binding of many positively charged metal ions from solution is independent of time, meaning that sorption of the metal ions occurs within the first five minutes and remains constant up to 60 minutes (1998). This may indicate that the binding of the gold(III) ions to the alfalfa biomass is occurring through a different process. It has been shown that the binding mechanism of tetrachloroaurate to different sorbents occurs through a reduction mechanism. The mechanism of gold(III) adsorption to algal biomass involves

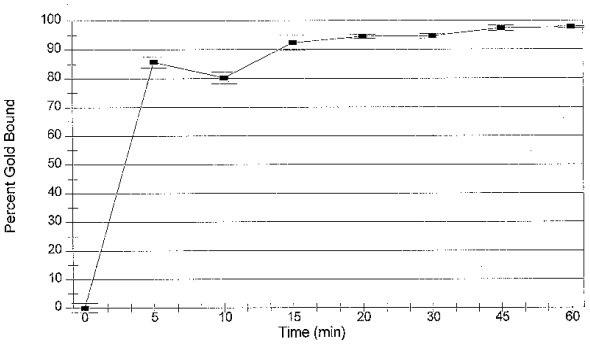

Figure 5. The effect of time on the sorption of gold(III) at trace levels to the alfalfa biomass from aqueous solution at pH 3.

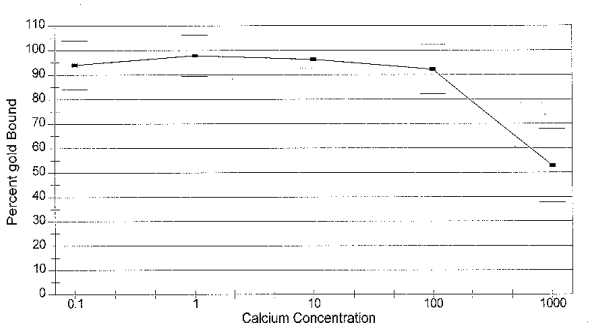

Figure 6. The effect of calcium on the binding of gold(III) to alfalfa biomass at trace levels from aqueous solution at pH 3.

the reduction of gold(III) to gold(I) (which involves the loss of two chlorine molecules) and further reduction to gold(0) (Greene et al., 1986). So the observed results for time dependency of the sorption of the gold(III) to the alfalfa biomass may be indicating that at trace levels, this reduction mechanism is occurring, thus slowing the binding of the tetrachloraurate to the alfalfa biomass and causing a time dependency for gold(III) adsorption to the alfalfa biomass. It was determined from this experiment that a reaction time of one hour was sufficient for the reaction of tetrachloroaurate with the alfalfa biomass of the experiments performed.

The final part of this study was to determine the effects of hard cations (calcium, magnesium, and sodium) on the binding of the gold(III) ions to the alfalfa biomass. These reactions were performed as described in the methodology section. Figure 6 shows the results for the effect of calcium on the binding of the gold(III) ions to the alfalfa biomass. As is shown in Figure 6, calcium had little to no effect on the binding of trace levels of gold(III) to the alfalfa biomass. However, there is a decrease in the binding of the gold(III) to the alfalfa biomass at a calcium concentration of 1,000 ppm. Nevertheless, if the calcium were binding to the alfalfa biomass at a concentration of 1000 ppm calcium and 100 ppb of gold(III), there should be no binding of the gold(III) to the alfalfa biomass. The calcium should overwhelm the high- and low-affinity binding sites for the gold(III), and as seen from Figure 6, this is not the case. The results of the reaction of magnesium and gold(III) with the alfalfa biomass are displayed in Figure 7. As with the calcium interferent, there is little to no observed effect on the binding of the trace levels of gold(III) to the alfalfa biomass. In Figure 7 there is no observed decrease in the binding of the gold(III) to the alfalfa biomass with increasing magnesium concentration. The interference of sodium was also studied for its effect on the binding of the gold(III) to the alfalfa biomass. The results of this study are displayed in Figure 8, where it can be seen that the binding of the gold(III) to the alfalfa biomass is independent of the sodium concentration in solution. For the individual

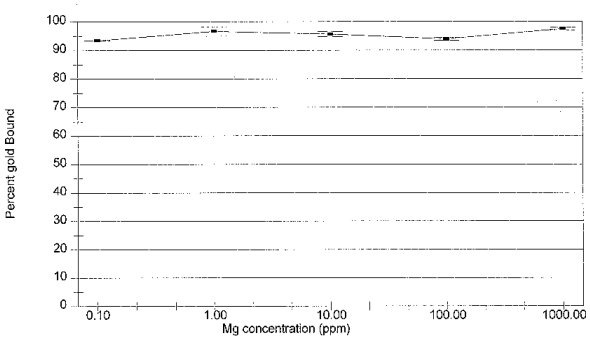

Figure 7. The effect of magnesium on the binding of gold(III) to the alfalfa biomass at trace levels from aqueous solution at pH 3.

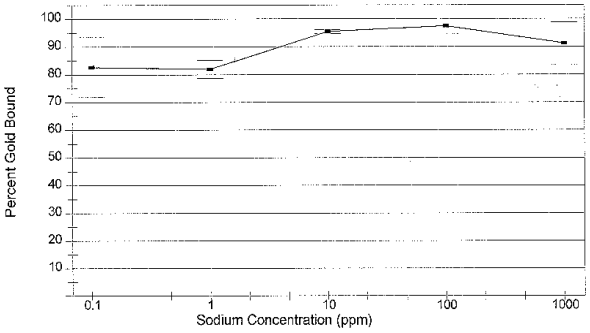

Figure 8. The effect of sodium on the binding of gold(III) to the alfalfa biomass at trace levels from aqueous solution at pH 3.

cations in solution, there is no apparent competition of the hard cations and the gold(III) for binding sites on the alfalfa biomass. However suppression of the binding may be due to ionic strength of the solution since there are 10,000 times the parts per million solution concentration of the hard cations than the gold(III). Figure 9 displays the effect of all three hard cations combined together in solution on the binding of trace levels of gold(III) to the alfalfa biomass. As with the experiments performed with the individual hard cations, there was little to no effect on the binding of gold(III) to the alfalfa biomass. If the calcium, magnesium, and sodium were binding to the alfalfa biomass, there should be no binding of the gold(III) to the alfalfa with concentrations of 1,000 ppm of calcium, 1,000 ppm magnesium, and 1,000 ppm of sodium combined together in solution. This selectivity of the alfalfa biomass of gold(III) over calcium, magnesium, and sodium means that this may be a potential method to remove gold(III) from hard waters. Any depression of the binding of the trace-level gold(III) to the alfalfa biomass may be due to the ionic strength of the solution. In this case there were 30,000 times the parts per million concentration of calcium, magnesium, and sodium in solution than gold(III). The gold is present as a negatively charged complex in solution and it may be more difficult to bind the gold(III) in the presence of such a high ionic strength. However, binding of the gold(III) still occurs, showing selectivity of the alfalfa biomass.

CONCLUSION

It was found that pH is very important to the binding of trace levels of gold(III) to the alfalfa biomass, for both the native biomass and the chemically modified (esterified) biomass. But, the pH dependency for the binding of the gold(III) to the native alfalfa biomass was different than that observed for the esterified biomass. This trend in dependence on pH for binding trace-level gold(III) was not observed at high-concentration gold(III) reactions with the alfalfa biomass, which may be due to an expression of the high-affinity ligands in the alfalfa biomass for gold(III). At high gold(III) concentrations, the low-affinity ligands in the biomass may be available to bind the gold(III) from solution. However, at trace concentrations, only the high-affinity ligands would bind the

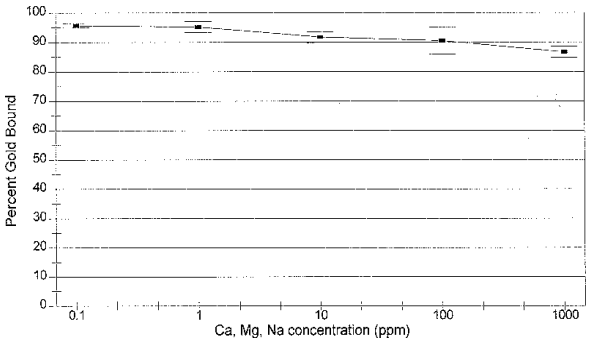

Figure 9. The effect of all three hard cations on the binding of gold(III) to the alfalfa biomass at trace levels from aqueous solution at pH 3.

gold(III). Also, determined in the studies was that time is important for the sorption of trace levels of gold(III) to the alfalfa biomass. The interference studies showed that the alfalfa biomass is a potential method for the recovery of gold(III) from hard waters. This was shown in the selectivity of the alfalfa biomass for gold(III) over calcium, magnesium, and sodium ions in solution.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the Center for Environmental Resource Management (CERM) at University of Texas at El Paso through funding from the HBCU/MI Environmental Technology Consortium. The HBCU/MI is funded by the Department of Energy and the Office of Exploratory Research of the U.S. EPA (Cooperative Agreement CR-819849-01-04). The authors also acknowledge financial support from the Bioremedation Education Science and Technology (BEST) program, the Department of Defense, and the Army Corps of Engineers.

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