

Journal of Hazardous Substance Research

Volume 4

Article 1

1-1-2003

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Herrera, I.; Gardea-Torresdey, J. L.; Tiemann, K. J.; Peralta-Video, J. R.; Armendariz, V.; and Parsons, J. G. (2003) "Binding of Silver(I) Ions by Alfalfa Biomass (*Medicago Sativa*): Batch PH, Time, Temperature, and Ionic Strength Studies," *Journal of Hazardous Substance Research*: Vol. 4. <https://doi.org/10.4148/1090-7025.1026>

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Cover Page Footnote

The authors acknowledge support from the National Institutes of Health (grant S06 GM8012-30) and support of the U.S. Environmental Protection Agency (EPA) through the Center of Environmental Resource Management (CERM). In addition, the authors acknowledge the HBCU/MI Environmental Technology Consortium, which is funded by the Department of Energy

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BINDING OF SILVER(I) IONS BY ALFALFA BIOMASS (*MEDICAGO SATIVA*): BATCH PH, TIME, TEMPERATURE, AND IONIC STRENGTH STUDIES

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ABSTRACT

In this study, the use of alfalfa biomass as a cost-effective and environmentally safe technique to recover Ag(I) ions from aqueous solutions is reported. This investigation consisted of batch pH profile, time, temperature, and ionic strength dependence studies. Results showed that alfalfa biomass presented the highest adsorption of Ag(I) ions in the pH range of 7 to 9 with a maximum adsorption capacity of 27.37 mg Ag•g⁻¹ of dry biomass, evaluated with a solution of 32.4 ppm of Ag(I). Time and temperature studies demonstrated a stable adsorption of Ag(I) ions by the biomass during the first hour of exposure, with a small decrease in adsorption after this period. Temperature experiments showed that Ag(I) adsorption decreases significantly at 50 °C as compared to 4°C and 24 °C. However, the differences between 4°C and 24°C are small. Ionic strength experiments showed that interfering ions (Na and Ca) reduce the adsorption capacity of the biomass. Results of this investigation showed that alfalfa biomass can be effectively used in the recovery process of silver ions from aqueous solutions.

Key words: silver, phytofiltration, ion-exchange resins, alfalfa biomass

INTRODUCTION

Traditional methods utilized in silver extraction employ cyanide solutions, which are hazardous to terrestrial and aquatic life (Addison, 1980; Eisle et al., 1988). In addition, major industries dealing with electronics and photography, for example, release solid and liquid silver wastes into the environment (Purcell and Peters, 1997). Although silver ions are not considered highly toxic to human life, there remains a secondary maximum contaminant level (SMCL) of 0.5 ppm of Ag in drinking water (U.S. Environmental Protection Agency, 1995). In addition, ionic silver (Ag⁺) has been considered to be highly toxic to zooplankton (Hook and Fisher, 2001), aquatic life (Erickson et al., 1998; McGeer et al., 2000; Wood et al., 1996a), and mammals (Warila et al., 2001) when it is supplied as silver nitrate (AgNO₃), a form considered relatively soluble if compared to other silver salts. Although no direct relationship has been found between silver toxicity and photographic wastes containing silver (Hogstrand and Wood, 1998; Wood et al., 1996b), studies have established that other chemicals present in photographic waste are toxic to aquatic life (Edgar, 1995). In

fact, it has been found that algae absorb silver ions from thiosulfate and chlorine complexes (Fortin and Campbell, 2001; Fortin and Campbell, 2000), and some experiments showed that silver thiosulfate complexes enhanced the bioaccumulation of silver in rainbow trout (Hogstrand and Wood, 1998).

Common methods used in the recovery of silver from photographic waste include metallic replacement, electrolysis, and precipitation (U.S. Environmental Protection Agency, 1991). These techniques are usually related with a high initial cost for the equipment, operation, and purification of the final products (Volesky, 2001). Other procedures such as ion exchange (Ajiwe and Anyadiegwu, 2000; Bayer, 1986), and ion flotation (Zouboulis, 1995) have served as alternatives to traditional processes in recovering less concentrated solutions of Ag(I) from pre-treated photographic wastes.

Biological systems that include fungi, algae (Mattuschka et al., 1993; Volesky and Holan, 1995; Mullen et al., 1992; Omar et al., 1997), and the dead biomass of *Datura innoxia* (Drake et al., 1996; Ke et al., 1994) have demonstrated the ability to uptake silver ions from contaminated aqueous solutions. Previous studies have shown that alfalfa biomass has the capability to recover heavy metal ions, such as Cu(II), Cd(II), Zn(II), Pb(II), and Cr(III), from aqueous solutions. In addition, alfalfa biomass is also able to reduce the oxidation state of other metals such as Cr(VI) and Au(III) (Gardea-Torresdey et al., 1996; Tiemann et al., 1999; Gardea-Torresdey et al., 1997; Gardea-Torresdey et al., 2000). Alfalfa, a forage commonly found throughout the U.S., may be an efficient alternative to high-cost silver recuperation processes, and the use of a natural product would make the process more environmentally friendly. The objective of this investigation was to investigate silver(I) binding to alfalfa biomass as an alternative process used in the removal of silver compounds in their most toxic state. Initial experiments were performed to determine the pH, time, ionic strength, and temperature dependence of the binding of silver ion to alfalfa biomass.

METHODOLOGY

Materials and Glassware

In order to avoid silver precipitation, materials and glassware used for the batch experiments were soaked for a 24-h time period in 5 % nitric acid (HNO_3), rinsed twice with de-ionized (D.I.) water, and dried on the lab bench. Silver-nitrate (AgNO_3) salts used for these experiments were reagent grade. Silver solutions were stored in high-density polyethylene (HDPE) amber bottles and amber glass containers. Silver standard solutions were prepared from a 1000 mg/L Ag(I) standard solution, diluted using 5 % solution of HNO_3 , and stored in HDPE amber bottles. The pH measurements were performed using a ROSS semi-micro pH electrode (8115BN ORION) filled with 10% KNO_3 , instead of the recommended 3M KCl solution (ROSS filling solution), in order to avoid further silver precipitation. The pH electrode was calibrated with buffer solutions at pH 4.0 and 7.0 before each experiment, and the correlation value obtained was of 0.98 or better. All the samples were unexposed to light after the experiments were completed. The metal concentrations were determined using a Flame Atomic Absorption Spectrometer (FAAS) Perkin Elmer model 3110 at a wavelength of 338.3nm. The calibration range was up to a concentration of 32.4 mg/L of Ag (0.3mM Ag) with a minimum of three standards spread over this range. The correlation value of the calibration curve was 0.99 ($r^2 = 0.99$) or better. For statistical purposes, a minimum of three samples were run for each experiment performed. Statistical values presented in the results were calculated with a 95% confidence interval.

Alfalfa Collection and Preparation

Alfalfa samples (*Medicago sativa*) were collected at New Mexico State University (NMSU) in an experimental research field. Only the shoots of the plants were used in this study. The biomass of the shoots was oven dried at 90 °C and ground to make it pass a 100-mesh sieve. The alfalfa powder was washed three times with 0.01M HNO_3 to remove insoluble material and debris that might interfere with the experiments, then washed seven more times with de-ionized (D.I.) water to remove acidity, or until supernatants reached a pH of 6-7. The alfalfa biomass pellets were frozen in liquid nitrogen for 30 minutes and lyophilized with a model Labcono freeze-drier for a 48-h period.

pH profile for Alfalfa Biomass and Ion Exchange Resins

Two portions of alfalfa biomass were carefully weighed separately in previously tarred beakers. They were then suspended in D.I. water to obtain a concentration of 5 mg of alfalfa per ml and stirred to form a homogeneous mixture. Suspension of one beaker was designated for pH values 1-4 separated by one pH unit. Suspension of the second beaker was designated for pH values 5 - 9. Three aliquots of 2 ml were transferred from the suspensions to clean test tubes every time the pH value was adjusted to an integer number. Nitric acid and sodium hydroxide solutions were used to adjust the pH of each suspension. The suspension was centrifuged and the supernatants were discarded. A solution of 32.4 mg/L (0.3mM) of AgNO_3 was prepared and separated into individual containers corresponding to each pH value. The pH was then adjusted for each solution. Three, 2-ml aliquots from each solution were transferred to a test tube containing the alfalfa and corresponding to its pH level. Three more were transferred to clean test tubes and set as controls. After one hour, the test tubes were centrifuged and the supernatants were transferred to clean test tubes. Similar procedures were followed for commercially available ion-exchange resins. Each resin contained a different active binding site, which provided an indication of the affinity of silver ions to the functional groups at different pHs. The ion-exchange resins used were SIGMA-Cellulose Phosphate, SIGMA-Dowex 66, SUPELCO-Daion CRB02, SUPELCO-DiaionWTO1S, SUPELCO-Duolite GT-73, and SIGMA-Dowex 50WXZ-100 with phosphate, tertiary amino, glucamino, carboxyl, thiol, and sulphonic functional groups, respectively. The samples were analyzed with FAAS as previously described.

Time-Dependence Studies for Silver(I) Binding to Alfalfa Biomass

A 50-ml solution containing a concentration of 5 mg of alfalfa per ml of suspension was prepared. The suspension was adjusted to pH 7.0, which was the optimal value previously obtained from the pH profile experiment. The solution was centrifuged and the supernatant discarded. The biomass pellet was re-suspended under continuous stirring with 50 ml of a 32.4 ppm Ag(I) solution, previously adjusted to pH 7.0. Three, 2-ml aliquots were taken from the stirred suspension and transferred to clean test tubes at time intervals of 5, 10, 15, 30, 60, and 90 minutes. The

test tube samples were centrifuged and the supernatants were transferred to clean test tubes for analysis with a FAAS. Meanwhile, a separate beaker containing 50 ml of 32.4 mg/L Ag(I) solution was continuously stirred. Three, 2-ml aliquots were taken from this solution at similar times to that of the suspension beaker and transferred to clean test tubes for FAAS analysis. These samples served as controls for each of the reaction times, since it was the same solution and it was never in contact with the biomass.

Silver(I)-Adsorption Capacity by Alfalfa Biomass and Ion-Exchange Resins

Solutions of alfalfa biomass (concentration of 5 mg of alfalfa per ml of D.I. water) and 32.4 mg/L of Ag (as AgNO_3) were adjusted separately to pH 7.0. Three, 2 ml aliquots were taken from the biomass suspension and transferred to clean test tubes. Alfalfa samples were centrifuged and supernatants discarded. Aliquots of 2-ml were taken from the silver solution and transferred to test tubes containing the biomass pellets. These samples were then equilibrated in a rocker for 15 minutes, since in previous experiments it was found that in a time interval lower than one hour, this value provided the maximum binding. The test tubes were centrifuged and supernatants were transferred to clean test tubes for further analysis. This was the end of one capacity cycle. A total of 10 cycles were performed by removing the supernatant from the test tube containing alfalfa, followed by the addition of fresh silver solution and 15 min equilibration. At this point, saturation of the biomass was reached. A similar procedure was followed with the ion-exchange resins for the appropriate number of cycles to achieve the saturation of the binding groups. Silver samples for this reaction were analyzed with FAAS as previously described.

Temperature-Dependence Studies for Silver(I) Binding to Alfalfa Biomass

Alfalfa biomass was exposed to silver ion solutions at temperatures of 4, 24, and 50°C. These temperatures were kept before and during the reaction time. In order to obtain these conditions, experiments were carried out in a refrigerator, on a lab bench, and in a controlled-temperature oven following the procedure described by Greene and Darnall (1988). The pH of the biomass suspension (5mg of alfalfa per ml of D.I. water) was adjusted to 7.0 and separated into three centrifuge tubes. The suspensions were centrifuged and the supernatants were discarded. In order

to adjust the temperature, each centrifuge tube containing the alfalfa pellet and one container with 120 ml of 0.3mM Ag(I), previously adjusted to pH 7.0, were placed at one of the temperatures (4, 24, or 50 °C), one hour prior to reaction. Each biomass pellet was then resuspended with 60 ml of the 0.3 mM silver solution under continuous stirring to obtain a 5 mg of Alfalfa per ml of Ag(I) suspension. The remaining solution of silver was used as a control for this reaction. Beakers were capped using several layers of parafilm to avoid an increase in the concentration of silver ions due to an excessive evaporation of the solvent. Samples for this reaction were taken at intervals of 60, 90, 120, and 180 minutes. Three 2-ml aliquots were taken at the set times from both the reaction and control vessels. The samples were centrifuged and supernatants were transferred to clean test tubes for further analysis with FAAS.

The Van't Hoff equation (1) was used to determine the apparent enthalpies from the data obtained in the temperature-dependence studies, following a method described by Greene and Darnall (1988), where K_n ($n=1,2$) stands for the equilibrium constants at the temperature T_n , ΔH° stands for the change in enthalpy, and R for the perfect gas constant.

$$\ln\left(\frac{K_2}{K_1}\right) = \frac{\Delta H^\circ}{R} \left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad (1)$$

The ratio of heavy metal-adsorbed ions at equilibrium to the weight of the dry biomass (D) was used as a substitute for the equilibrium constants (K), and it only represents a point in an adsorption isotherm. The equation utilized for the calculation of the apparent enthalpies was

$$\Delta H^\circ = \frac{(\ln(D_2 / D_1) \cdot R \cdot T_2 \cdot T_1)}{(T_2 - T_1)} \quad (2)$$

where T is the absolute temperature, R the molar constant for the perfect gases and D_n is the ratio of heavy metal ions to the weight of dry biomass at the temperature T_n ($n=1,2$).

Ionic Strength Dependence and Interference on Silver(I) Binding to Alfalfa Biomass

Solutions containing a total ionic strength (I) of 0.001, 0.01, 0.05, and 0.1 were prepared using NaNO₃ and Ca(NO₃)₂•4H₂O salts. These solutions contained 0.3 mM AgNO₃ (I= 0.0003) and the interfering cation diluted at the concentration necessary to obtain the desired total ionic

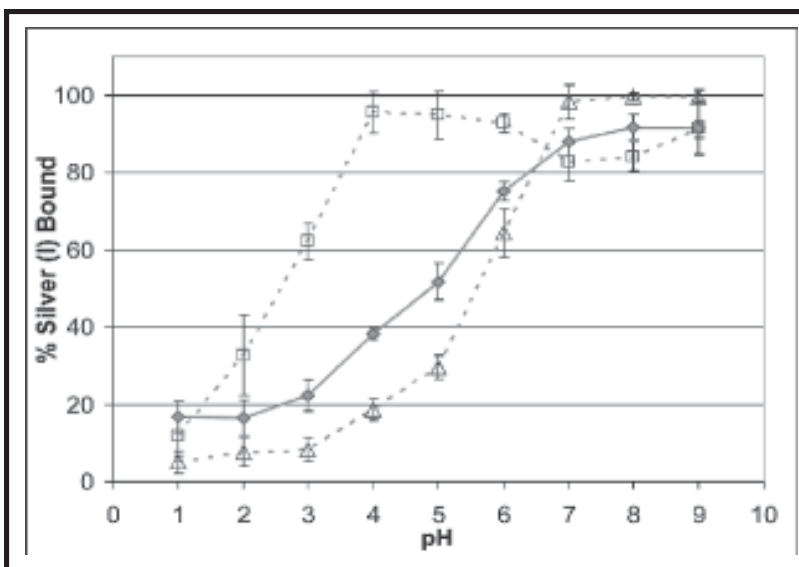


Figure 1. pH profile experiment for Ag (I) binding by alfalfa biomass (◆), weak anion exchange resin containing free amino groups (□), and weak cation exchange resin containing carboxyl groups (△). $I = 0.0003$ for Ag (I) working solution.

strength. A solution mixture of alfalfa biomass (5 mg of dry biomass per ml) was adjusted to pH 7.0 using 0.001 M NaOH. After centrifugation, the supernatants were discarded and the pellets were re-suspended in the appropriate silver solution with the interfering cation. Aliquots of 2 ml were taken at 60, 90, 120, and 180 minutes and transferred to clean test tubes for FAAS analysis. Total ionic strength was calculated from the equation

$$I = \frac{1}{2} \sum_i z_i^2 (b^i / b^0) \quad (3)$$

where b_i is the molality of species i , b^0 is the unit constant to make the formula unit less, and z ($\text{Ag} = +1$, $\text{Na} = +1$, $\text{Mg} = +2$, $\text{NO}_3 = -1$) is the charge of the ion. Molar concentrations were used instead of molal for experimental advantages.

RESULTS AND DISCUSSION

The adsorption of silver ions to alfalfa biomass was found to be pH-dependent with a similar trend to the one presented by a weak cation-exchange resin with carboxyl groups as binding sites (Figure 1). The alfalfa-binding process presented a log phase between pH 3 and pH 7, with an increase of binding from approximately 20 to 90 percent. However, in this range of pH, alfalfa biomass showed a binding capacity 10 percent higher than the previously mentioned carboxyl resin.

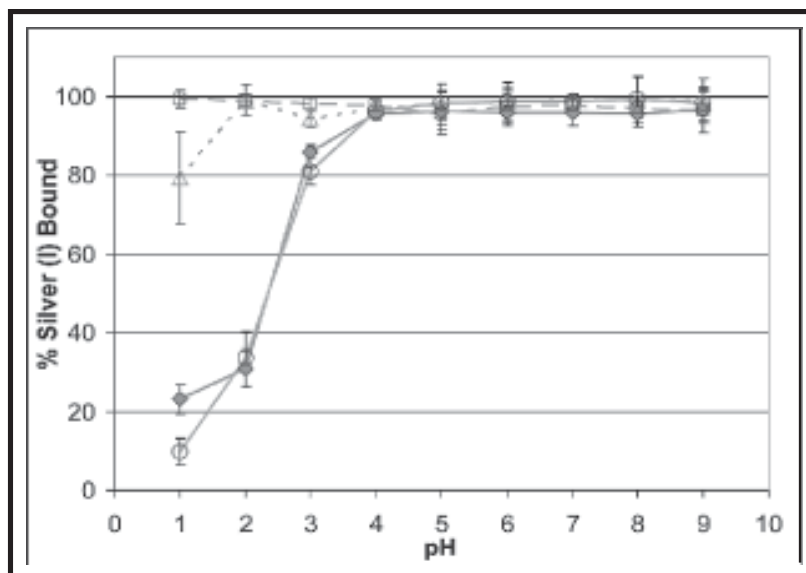


Figure 2. pH profile experiment for Ag(I) binding by strong cation exchange resins containing sulfonic (◆) and phosphate (O) binding groups, and a weak anion exchange resins containing tertiary amine (□) and chelating resin containing thiol (Δ) binding groups.

In addition, between pH 6 and 7, curves for carboxyl resin, amino resin, and alfalfa biomass crossed each other. This decrease in the binding of Ag(I) ions for both the alfalfa biomass and the amino resin in relation to the carboxyl resin indicates the possible involvement of amino groups in the binding process. Presence of a secondary binding group has been previously described by Drake et al. (1996) in the binding of low concentrations of Ag(I) ions. This second group can be an amino group (Diaion CRB02 and Dowex 66, showed in Fig. 2) since the decrease in binding for the alfalfa biomass in relation to the carboxyl resin corresponds to the decrease in binding capacity of the amino resin in the case of the free-amine-containing glucamine groups (Diaion CRB02). The amino-resin-containing tertiary amine groups (Dowex 66) does not present this decrease in the binding capacity at the pH range from 6 to 7, but shows a similar pH profile as the one described by Drake et al. (1996) for low concentration of Ag(I) ions. The thiol resin (Duolite GT-73) presented a pH-independent trend, which indicates the affinity of silver ions to soft-base atoms, which donate pairs of electrons such as nitrogen and sulphur (Figure 2). The phosphate and sulfonic groups presented a silver-binding capacity varying from 30 to 90 percent between pH 2 and 4, binding almost 100% of silver ions present in the solution at pH levels higher than 4. This indicates the strong relative affinity of silver(I) ions to binding groups containing oxygen, which increases the electrostatic forces

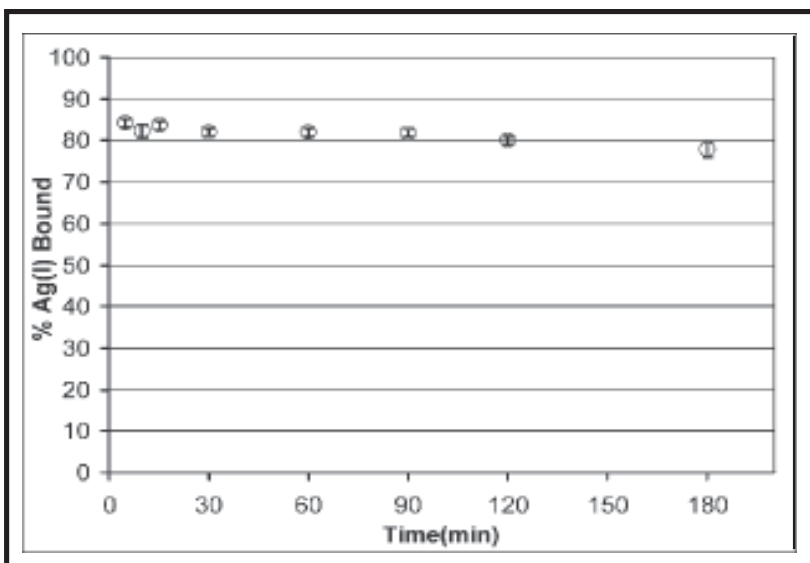


Figure 3. Time dependence study for Ag(I) binding by alfalfa biomass. Percent Ag(I) uptake at time intervals of 5, 10, 15, 30, 60, 90, 120, and 180 minutes.

between the binding group and silver(I) cation (Figure 2). From these results, we can conclude that phosphate and sulfonic groups are not primarily involved in the binding of Ag(I) ions to the alfalfa biomass, since the curve of the pH profiles follow different trends, especially at pHs from 2 to 6; but they can contribute as a secondary binding group if present in the alfalfa biomass. However, the main binding group seems to be the carboxyl group, since the carboxyl-containing resin followed a similar binding trend to the alfalfa biomass.

The binding process of Ag(I) ions to the alfalfa biomass remained almost constant for time intervals lower than one and half hours (Figure 3). A small reduction in the percentage of metal bound was observed after this time interval. This effect might have been caused by a minuscule decrease in the pH of the suspension after periods longer than one and a half hours, since this same effect was observed when adjusting the pH of the alfalfa biomass. However, these changes were never greater than ± 0.10 from the adjusted pH. When exposed to light, the ion-exchange resins containing nitrogen groups presented a characteristic metal coating after being previously exposed to the silver(I) solution. This indicates the reduction of silver ions due to the nature of silver-nitrogen complexes, which can further prove that nitrogen groups are involved as a secondary binding site to the adsorption of Ag(I) ions by alfalfa biomass. Other causes of this reduction in the binding capacity

can be attributed to the bio-precipitation of silver with a sulphur-containing protein present in the biomass. The effect caused by reduction of light can be dismissed since both the controls and the biomass samples were exposed to the same conditions of light and temperature. Therefore, both solutions of Ag(I) were affected in the same proportion, even though the difference in binding capacity can be considered as common variations on experimental procedures.

After 30 saturation cycles of the strong cation-exchange resins (cellulose phosphate and sulfonic acid resins) and 15 cycles for the other cation exchange and chelating resins (carboxylic acid, amino, poly amino, and thiol), the highest binding capacities were found to be 491.57 mg of Ag(I)•g⁻¹ for cellulose phosphate resin, 155.92 mg for Duolite GT-73 resin, and 136 mg for the Dowex 50 WXZ-100 resin (Table 1). From this table, it can be seen that the binding capacity of phosphate resin was more than three times higher than the capacity of the sulfonic acid and thiol resins. Even though the capacity of alfalfa biomass was only 27.37 mg of Ag(I)•g⁻¹ of dry biomass, this amount represents 30% more than the amount of silver ions bound to the polyamino resin. This is indicative of the cost-effective use of alfalfa biomass in the recovery of silver ions from a contaminated solution. Additionally, prices for the cation-exchange resins (approx. \$100 US Dollars/kg) are 600 times higher than the prices for raw alfalfa (approx. \$150 US Dollars/ton). Therefore, the approximate costs to uptake 10 g of Ag(I) will be about 2 dollars with cellulose phosphate and 5.5

Table 1. Adsorption capacities for silver (I) ions to alfalfa biomass and ion exchange/chelating resins.

	Functional Group	Capacity mg Ag/g of Resin	+/-
Dowex 50WXZ-100¹	Sulfonic Acid	136.88	1.79
Cellulose Phosphate¹	Phosphate	491.57	1.97
Duolite GT-73¹	Thiol	155.92	5.32
Diaion WT01S²	Carboxylic Acid	45.00	2.30
Diaion CRB02³	Amine (Glucamine)	15.83	2.44
Dowex 66²	Tertiary Amine	48.44	4.58
Alfalfa¹	Various	27.37	0.39

¹30, ²15, and ³10 cycles were performed to reach saturation.

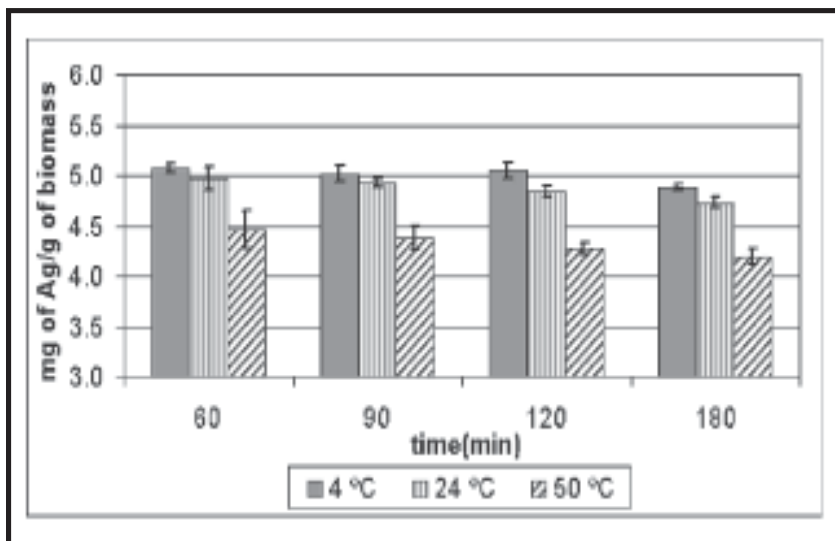


Figure 4. Temperature dependence study of Ag(I) binding to alfalfa biomass at time intervals reacted of 60, 90, 120, and 180 min.

cents with the alfalfa biomass, considering the batch experimental procedure. In order to reduce costs, several pretreatment procedures such as liquid nitrogen freezing and freeze drying can be avoided from the process, and lower concentrations of acids can be used to remove any metals or debris present in the biomass. It is also important to point out that the Ag(I) binding capacity of the alfalfa biomass is similar to that of the carboxyl-containing resin. This corroborates the involvement of carboxyl groups in the Ag(I) binding process.

Effects of temperature on the adsorption process of silver ions to the alfalfa biomass are presented in Figure 4. This figure shows that the silver-binding capacity of the alfalfa biomass decreased on average in amounts of 0.18, 0.24, and 0.28 mg of $\text{Ag} \cdot \text{g}^{-1}$ of biomass for the reaction temperatures of 4, 24, and 50°C, respectively. These results were similar to those found in the time-dependency study and showed that such results were not coincidental but indeed reproducible. The decrease in the adsorption capacity as a function of time and temperature, showed evidence of the reduction process that occurred in the alfalfa biomass and not in the solution. This can be justified since both the reaction vessels and the controls were exposed to the same conditions of time and temperature and the silver control remained unchanged. In addition, the average apparent molar enthalpies were found to be -0.425 ± 0.047 and -1.565 ± 0.127 for the temperature interval

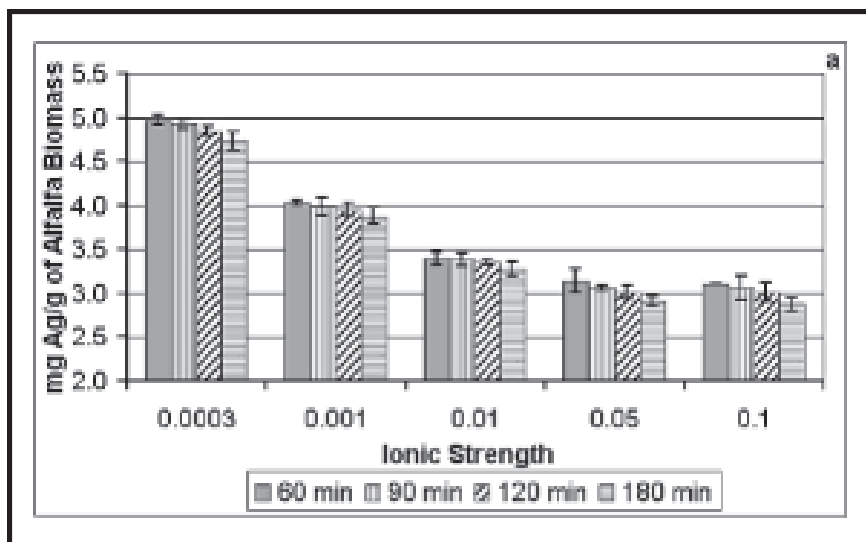


Figure 5a. Ionic strength dependence of Ag(I) binding to alfalfa biomass at time intervals of 60, 90, 120, and 180 min for interfering ion of Na(I).

of 4 to 24°C and from 24 to 50°C, respectively (Table 2). This suggests that the binding process of silver ions to alfalfa biomass is exothermic. Similar results were found by Greene and Darnall (1988) in the bioreduction of Cr(VI) to Cr(III) by *Spirulina* biomass, with the reduction of binding capacity as a function of temperature. Therefore, these results indicate that bioreduction processes are complemented by exothermic processes.

Figure 5 shows the results of the ionic-strength studies at an Ag(I) concentration of 0.3 mM. When sodium (Na^+) was the interfering ion, the binding capacity of the biomass decreased from 4.87 $\text{mg Ag}\cdot\text{g}^{-1}$ of alfalfa, with a total ionic strength of 3.0×10^{-4} , to 3.01 $\text{mg Ag}\cdot\text{g}^{-1}$, with a total

Table 2. Apparent molar enthalpies presented by alfalfa biomass temperature dependence study at temperature intervals of 4-24 °C and 24-50 °C.

Time (min)	* ΔH° 4-24 °C (kJ/mol)	+/-	* ΔH° 24-50 °C (kJ/mol)	+/-
60	-0.316	0.015	-1.414	-0.065
90	-0.279	0.013	-1.587	-0.076
120	-0.620	0.031	-1.656	-0.049
180	-0.484	0.028	-1.604	-0.059
Average	-0.425	0.047	-1.565	0.127

* ΔH° stands for the enthalpy change.

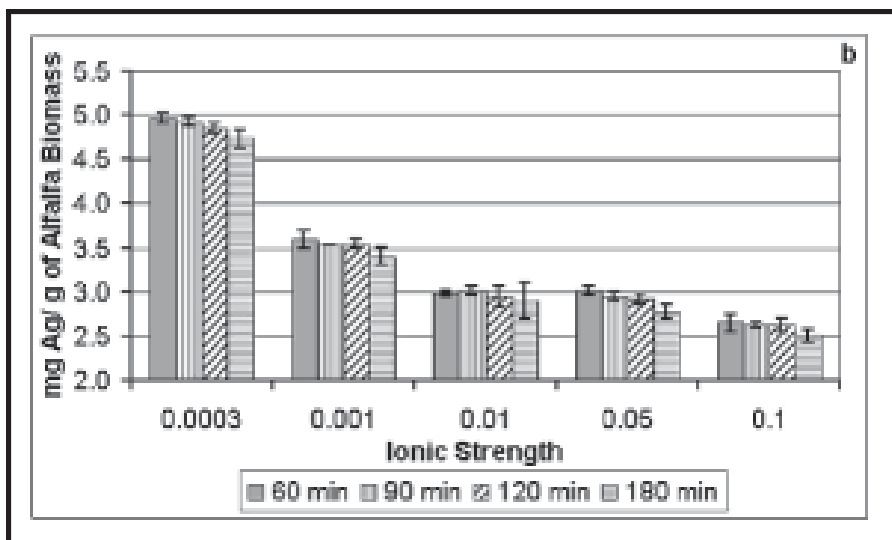


Figure 5b. Ionic strength dependence of Ag(I) binding to alfalfa biomass at time intervals of 60, 90, 120, and 180 min for interfering ion of Mg(II).

ionic strength of 0.1 (Figure 5a). When magnesium (Mg^{2+}) was the interfering ion, adsorption of silver ions to the alfalfa biomass decreased to $2.6 \text{ mg of Ag} \cdot \text{g}^{-1}$ of alfalfa at an ionic strength of 0.1, following a logarithmic trend (Figure 5b). These results indicate that a solution with high ionic strength may desorb silver ions from the alfalfa biomass or that the Na(I) and Mg(II) ions are competing for similar binding sites at higher concentrations. In addition, when considering the carboxyl group as the main ligand responsible for silver (soft acid) adsorption, electrostatic interaction for silver ions was weaker than the one presented by sodium, calcium, or magnesium ions, (hard acids). Therefore, the silver ions can be replaced by calcium and magnesium ions because these two ions have a higher affinity to carboxyl groups than silver ions and this is most likely a cation-exchange mechanism.

CONCLUSIONS

Based on analysis of the results, it was concluded that the binding of silver ions to the alfalfa biomass followed the same trend as a carboxyl, weak cation-exchange resin, which indicates the main involvement of these groups on the binding process. In addition, the binding of Ag(I) ions by alfalfa biomass was enhanced by a secondary group that can be an amino group, since the decrease in alfalfa biomass binding was accompanied by the decrease in binding for the amino resin. Furthermore, it was observed that a reduction process attributed to the light-sensitive complex formed

perhaps by the silver ion and nitrogen donor groups, which is further proof of the involvement of these groups as binding sites. Additionally, x-ray absorption spectroscopy studies have shown that silver is present as Ag(0) in the alfalfa biomass (data not shown), which demonstrate that silver ions are reduced in the alfalfa biomass. Thiol groups can be considered as part of the binding processes, but no clear evidence has been found on this research. The analysis of ion exchange/chelating resins can be of great help toward the study of the binding sites involved in the adsorption of heavy metal ions by biological systems, since it provides a profile of the behavior of binding groups under certain conditions. The adsorption capacity of alfalfa biomass was reduced by both the temperature and ionic strength of the Ag(I) solution. These results indicated that the alfalfa biomass can be effectively used in the recovery process of silver ions from contaminated waters. However, the phosphate-containing resin has a much higher Ag(I)-binding capacity. Further experimentation should be performed in order to determine the nature of the bioreduction process onto the alfalfa biomass and to investigate the binding of Ag(I) under flow conditions.

ACKNOWLEDGMENTS

The authors acknowledge support from the National Institutes of Health (grant S06 GM8012-30) and support of the U.S. Environmental Protection Agency (EPA) through the Center of Environmental Resource Management (CERM). In addition, the authors acknowledge the HBCU/MI Environmental Technology Consortium, which is funded by the Department of Energy.

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Original Manuscript Received: October 1, 2002

Revised Manuscript Received: February 14, 2003